Biowaste valorisation through biorefinery system according to Circular Economy strategy

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Francesca Demichelis

Turin, April 6, 2021

I would like to dedicate this thesis to my loving parents

I would like to dedicate this work and the whole path to my mum, daddy, sister Roberta and grandmother Maddalena, who always encourage me with strong love. They are my umbrella under the rain, and they bring me toward the rainbow and happiness.
I would say thank you to Matilda and Oliver the sweetest friends.
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Introduction to thesis:

The dependency on non-renewable resources represents an environmental, economic and social problem, globally affecting the planet Moncada et al.(2016) with emissions of greenhouse gases (GHGs) and resulting in increased fuel extraction costs (Chu and Majumdar, 2012). Currently, more than 90% of the organic chemical compounds and 80% of the energy produced derive from non-renewable fossil resources (Maity, 2015 I; Parajuli et al., 2015). In 2018, Europe produced 46% of the energy consumed and imported 54% of the total energy consumed (Santos et al., 2017). The fact that Europe is not energy independent, and still strongly relies on energy imports, often from non-renewable sources and from non-EU countries, represents a problem for three main reasons (PBL,2017): 1) EU energy security: imports of fossil fuels come from countries with limited geopolitical stability, 2) EU competitiveness: strong dependence on extra-EU imports exposes the continent to the risk of possible fuel price shocks, which affects (among other) electricity prices and 3) EU environmental sustainability: fossil fuel consumption has a significant impact on greenhouse gas (GHG) emissions (CO_2, CH_4, N_2O, etc.).

The dependence on fossil non-renewable fuels is bound to increase, unless something changes, due to the increased demand for energy and chemicals induced by the fast world population growth (Eurostat, 2019). According to Pelkmans and Fritsche (2019) only a paradigm change, focusing for example on high-added value bio-products and bioenergy, allowing the transition from high to low greenhouse gases (GHG), can limit global warming below 2°C by 2100.. The production of biobased products and bioenergy should be considered as a unit of an integrated value chain of processes in the overall green bioeconomy.

The EU Green Deal (EU-Green Deal, 2019) is a European political initiative promoting a new growth strategy aimed at transforming the EU into a fair and resource-efficient economy, where emissions of greenhouse gases in 2050 decrease

of 30% and economic development is decoupled from resource use. Furthermore, the Green Deal enhances the EU's natural capital and protect the health and well-being of citizens from environment-related risks and impacts. The bioeconomy**,** a circular economy powered by nature and emerging from nature, is based on renewable biological resources and sustainable biobased solutions. Bioeconomy is fundamental for moving towards a carbon neutral EU reality and fossil free material and energy scenarios. This change of perspective promotes the shift from a linear to a circular structure of industrial processes, where by-products and wastes can become new secondary raw materials. Moreover, under this viewpoint, the whole economic system is boosted to pass from linear to circular structure, exploiting and valorising renewable biological resources, like agro-wastes, municipal solid wastes, industrial organic wastes and forest resources. Biodiversity is a milestone for bioeconomy in order to have a sustainable and resilient structure able to achieve the targets set by the Green Deal.

To implement this transformative policy, bioeconomy promotes the concept and realisation of biorefinery. Biorefinery can represent the catalyst for systemic change to tackle holistically the social, economic and environmental perspectives. The biorefinery builds a new and synergistic relationship between technology and nature, between ecology and economy growth and belongs to Green Chemistry or Sustainable Chemistry.

To face these problems and challenges, bioeconomy has a fundamental role. Bioeconomy faces the main environmental challenges in the world: climate change, depletion of resources and consequent loss of biodiversity (Global Resource, 2019; Green New Deal, 2020).

Based on new biotechnological approaches, the bioeconomy maximizes the use of waste and resources, both biological, terrestrial and marine, as well as non-biological, CO_2 and fossil waste streams, as inputs for industrial and energy production, implementing a circular logic management to maximise opportunities of reuse, recycling and recovery (OECD, 2020).

In Europe, the bioeconomy has an annual turnover of more than 2 trillion €, employing 18 million people (10% of THE EU's employment) (EU, Bioeconomy, 2018).

In Italy, the bioeconomy has 2 million job positions with an annual turnover of more than 330 billion €, of which around 17 % related to agricultural production and 42% to food industrial production (BIT, 2019).

In Europe, the production of biobased materials is 4.7 Mt/year, about 3% of total production, with a 21% increase for 2025 (WEF, 2020).

Recently, Europe joined the 2030 Agenda for Sustainable Development Goals (SDGs, 2019), which established new targets in climate change and energy-production to ensure greater competitiveness, safety and stability of energy systems. The target defined by 2030 Agenda for Sustainable Development are: 1) GHG reduction equal to 40% of the levels of 1990, 2) at least 27% of the used energy must come from renewable energy and 3) 27% energy savings compared to current situation (Unrich, 2018). To achieve these targets, biorefinery system plays a key role. Biorefinery enables the realization of Green Chemistry at the full scale, optimizing the supply chains of enhancement of biomass, ad hoc and waste, CO_2 and fossil waste stream, in local contexts developing integrated technology platforms and cascading use schemes. Biorefinery has several definitions, but among them the worldwide official recognised are here reported:

- "biorefinery is a sustainable processing of biomass into a spectrum of marketable products (food, feed, materials and chemicals) and energy (fuel and power heat)" Sonnermberg et al. (2007);
- "biorefinery is the integral upstream, midstream, and downstream processing of all types of biomass into a range products and energy with volume and prices market competitive" Sonnermberg et al.(2007)
- "biorefinery is an integrated bio-based industry using a variety of technology to make products such as chemicals, biofuels, food and feed ingredients, bio-materials, fibres and heat and power, aiming at maximizing the added value along the three pillars of sustainability: environment, economy and society" Andiappan et al. (2015).

These three definitions underlined the three fundamental units of biorefinery: 1) biomass, 2) process and 3) product.

Biomass supplies energy for 12% of global status of renewable energy inputs ranging between 40 to 50 % in developing countries. According to Task 42, biorefinery system is classified according its main features: 1) feedstock (starch, lipids, lignocellulosic), 2) processes (chemical, thermos-chemical, biological), 3) platform chemical (intermediate C5-C6 carbohydrates, syngas, lignin, pyrolytic liquid) and 4) product (chemical material and energy).

The biorefinery process is like the petrochemical refining, but the crucial difference is the nature of the starting material; because for biorefinery is biomass, a renewable matter, for the petrochemical refinery is coal and petroleum, namely fossil resources.

Biorefinery is classified on the ground of biomass origin in first generation (1G), second (2G) and third (3G) generation biorefinery, respectively feed with ad

hoc biomasses, waste biomasses and algae. This thesis focuses the attention on 2G-biorefinery for ethical, environmental, economic and social reasons.

In the present thesis two processes are considered: fermentation for lactic acid (LA) production and anaerobic digestion (AD) for biogas production

EU Commission legislations are boosting the valorisation of biowaste. Anaerobic digestion is one of the most adopted technology to valorise biowaste, from 2017 the AD plant in EU28 increase of 1.94 billion m^3 (European Biogas Association. 2019) which resulted in treating 5% of total biowaste generated in EU.

The present thesis has the following structure:

Chapter1: investigates 2G-biorefinery system and its three fundamental units: the starting biomass, the corresponding process and the resulting products. The aim was the realization of three data inventories: 1) biomass available in EU28, 2) process technical-economic-environmental feasibility and 3) generable high-added value products. The study combined bottom up and top down approaches, aimed respectively to evaluate how the fundamental units are interlaced and influenced each other and to define a sustainable biorefinery system.

Chapter 2 develops a methodology for the technical and environmental assessment of biowaste valorisation in 2G-biorefineries in Italy.

Italy was chosen as case study, considering years 2016-2019. Italian context was evaluated through the following key parameters: 1) Gross domestic power, 2) climate, 3) demography and 4) population density distribution. The evaluations of geo-localisation and quantitative availability of biowaste amounts aimed to define the dimension and localisation of the biorefinery plant to optimise the supply and transport chains, while the qualitative characteristic aimed to evaluate the most promising process among two different biorefineries systems: thermo-valorisation (TH) and anaerobic digestion (AD).

Chapter 3 investigates the simultaneous saccharification and fermentation (SSF) and separated hydrolysis and fermentation (SHF) to produce L (+)-lactic acid (LA) from the organic fraction of municipal solid waste (OFMSW). The aim of Chapter 3 is the optimisation of SSF and SHF. In detail, for SFF the analysis includes 1) the identification of the most suitable LA strain producers: three types of *Lactobacillus sp*. and one type of *Streptococcus sp*. strains, 2) the evaluation of the necessity of autoclavation of the OFMSW and 3) the production of market value L (+)- LA. For SHF the analysis includes: 1) type and loading of enzyme and 2) solid to liquid ratios.

OFMSW is employed as source of carbon and nitrogen to carry out SSF by using for L (+)-LA production.

In SHF two enzymes were tested: Stargen and Fermgen to hydrolyze starch and proteins. Hydrolytic performance was investigated according to different solid-to-liquid ratios.

Downstream processing including micro- and nanofiltration, electrodialysis, chromatography and distillation produced a pure 702 g/L of L (+)-LA formulation with an optical purity (OP) of 97%.

Chapter 4 investigates the acid-enzymatic hydrolysis and fermentation of L (+)-lactic acid (LA) with *Bacillus Coagulans* from spent coffee ground (SCGC). SCGC, a lignocellulose residue from coffee production consisted of 34.26 ± 2.67% cellulose, 7.31% ± 2.54% hemicellulose and 24.88 ± 0.11% of lignin. Sequential and combined acid-enzymatic hydrolysis were carried out respectively, at 121°C for 15 min with 1%v/v H_2SO_4 and 14.5% SCG wet and at 52°C for 24h with 0.25 mL Accellerase 1500 per gram of dry SCG, achieving a total sugar extraction efficiency of 41.24 ± 4.53%.

Fermentations were carried out both at the laboratory (2L) and technical (50L) scales and no scale effect was observed.

At 50L scale, LA yield per gram of sugar consumed and per dry gram of SCG were 0.956 ± 0.015, 0.18 ± 0.63 respectively. Downstream processing resulted in 786.70 gLA/L and 99.5% optical purity.

Chapter5 concerns the investigation of the sequential production of L(+)-lactic acid (LA) and biogas from organic fraction municipal solid waste (OFMW).

LA was produced from OFMW using a *Streptococcus sp.* strain A620 (optimized in Chapter 3) by means of two fermentative pathways: separate enzymatic hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF). Via SHF a yield of 0.33 g_{LA}/g_{FW} (productivity 3.38 $g_{LA}/L \cdot h$) and via SSF 0.29 g_{LA}/g_{FW} (productivity 2.08 $g_{LA}/L \cdot h$) was reached. Fermentation residues and OFMSW were tested as feedstocks for anaerobic digestion (AD) (3 wt% TS). The following biogas yields were achieved: 0.71, 0.74 and 0.90 Nm^3/kg_{VS} for OFMSW and residues from SFF and SHF respectively.

The innovation of the approach consists in considering the conversion of OFMSW into two different sequential products through a biorefinery system, therefore making economically feasible LA production and valorising its fermentative residues.

Chapter6 evaluates the economic and energy assessments of a singular and integrated biorefinery system for sequential production of fermentative lactic acid (LA) and biogas from organic fraction municipal solid waste (OFMSW) and spent coffee grounds (SCG). Four scenarios were evaluated and compared: **Scenario IA** exclusive fermentative production of LA by means of simultaneous saccharification

and fermentation (SSF) (explained in Chapter 3), **Scenario IB** LA production carried out with separated hydrolysis and fermentation (SHF) (explained in Chapter3), Scenario II exclusive biogas production by means of anaerobic digestion. **Scenario III A-B** for sequential fermentative LA production and biogas by means of SSF and SHF from OFMSW. **Scenario IV** LA production by means of SHF from SCG. The integrated biorefinery process was compared to single processes for either lactic acid or biogas production. The economic evaluation, considering catchment areas from 2000 to 1 million inhabitants, was based on data from real biorefinery plants and carried out using SuperPro Designer® 8.0. The consistency of the approach was assessed through a set of composite indicators. The integrated biorefinery system was investigated from three main perspectives: 1) economic feasibility of producing LA and biogas, 2) the effect of process scale and 3) energy consumption/requirement. The present study proved that an integrated biorefinery system contributes more to optimal use of energy and material flows than single processes both for the sequential production of two market value products and optimisation of waste management. Profitability was achieved for catchment areas bigger than 20,000-50,000 inhabitants.

Chapter 7 evaluates the key role of inoculum in mesophilic anaerobic digestion (AD) of organic fraction municipal solid waste (OFMW). Two inocula were tested, one coming from the mesophilic digestate of wastewater activated sludge (WAS) and the other one from the mesophilic digestate of cow-agriculture sludge (CAS). Both inocula were anaerobically cultivated for three different periods: 0, 5 and 10 days and then inoculated in OFMW considering three substrate-inoculum ratios (S:I) 1:2; 1:1; 2:1. First order kinetics and Gompertz modified model were applied to define disintegration rate, lag phase and maximum biogas yields. Energy sustainability index was calculated to define which configurations were suitable to be scaled-up. Then multi criteria decision aid was performed to outranking the AD configurations tested. The AD configurations with the best performances were: AD performed with S:I=2:1 with CAS cultivated for 5 days, AD performed with S:I=1:1 and 2:1with CAS cultivated for 10 days and AD performed with S:I=2:1 WAS cultivated for 10 days

References

1) Communication from the commission to the European parliament, the European council, the council, the european economic and social committee and the committee of the regions. 2019. The European Green Deal

2) Sustainable development gooals,2019. https://www.un.org/sustainabledevelopment/development-agenda/ (last acces 2/07/2020)

Chapter 1: The strategic role of biomass in second generation biorefinery

- Based on the paper: The pivoting role of biomass in biorefinery system. Demichelis F., Fiore S. in preparation. 2020

Abstract

Chapter 1 investigates 2G-biorebinery system and its three fundamental units: the starting biomass, the corresponding process and the resulting products. The aim was the realization of three data inventories: 1) biomass available in EU28, 2) process technical-economic-environmental feasibility and 3) generable high-added value products. The study combined bottom up and top down approaches, aimed respectively to evaluate how the fundamental units are interlaced and influenced each other and to define a sustainable biorefinery system. Biomass plays a pivoting role in 2G-biorefinery, since process and obtainable products depends on biomass quantity and quality. Biomasses are currently considered social, economic, environmental problems, whereas 2G-biorefinery provides a new concept of biomass. Biomasses, containing more than 40-50%w/w of Carbon, were classified as carbohydrate, lipid and lignocellulose rich substrates suitable for platform chemicals and energy productions, facing social, environmental and economic needs, according to Circular and Bio-Based Economy.

Abbreviation

AD= anaerobic digestion
AFF= Waste from agriculture, forestry and fishing activities
BAT= best available technology
EoWC= End-of-Waste Criteria
GDP= Gross Domestic Product
HLV= heating low value
IA= Itaconic acid
IFB= Waste from manufacturing of food and beverage products

LA= Lactic acid
KET= Key Enabling Techniques
MA= malic acid
MSW= Municipal solid waste
pc= pro capita
RQ= research question
SHF= separate hydrolysis and fermentation
SSF= simultaneous saccharification and fermentation
TRL= technical readiness level
WF= water footprint
WSS= Wastewater and sewage sludge

1.1 Introduction

The research questions (RQ) of the Chapter 1 aim to realise data inventories referred to European (EU 28) contests for:

- RQ1 - Biomass: biomass geo-localisation, available quantity and composition
- RQ2 - Process: according to biorefinery process classification, considering pros and cons from technical economic and environmental perspectives
- RQ3: Product generable by biorefinery systems able to face market requirements as purity grade, market size and value.
- RQ4: Correlation of biomass-process-product to evaluate the most promising biorefinery system configuration, in terms of maximum biomass conversion, minimum waste production and product generation.
- RQ5: Evaluation of biorefinery system sustainability at design level.

The novelty of the proposed approach is the critical evaluation of the singular fundamental biorefinery units with correlations of biomass-process, process-product, biomass-product and biomass-process-product, to evaluate the technical-economical-environmental feasibility and the implementation at full scale.

Currently, the scientific literature focuses on biomasses classification De Corato et al. (2017); Maity, (2015 II), biomass database referred to IEA, task42 (Black et al., 2016), processes Maity (2015I), generable product Koutinas et al., (2014), sustainability Azarpagic et al. (2014); Parada et al. (2017), and biorefinery design Moncada et al. (2016).

Chapter 1 develops a combined approach: first a bottom-up approach to study the biorefinery system split into its three fundamental units (biomass, process and product) to identify their mutual influences and then a top-down approach is adopted to assess the sustainability of biorefinery system.

1.2 Methodology

The evaluation of the available quantity and quality of biomass in EU 28 is carried out to design biorefinery processes and obtainable products assessing biorefinery system sustainability. Chapter1 includes the review of 297 papers, belonging to the 2002-2019 period, except for 2 papers belonging to 1991-1994 and 2 patents.

1.2.1 Biomass

In biorefinery system, biomass plays a key role, because the types of generable product and employable processes depend on the starting biomass.

Biomass evaluation is based on 107 papers (48% of them are review papers). In the present screening study, biomass evaluation is performed in Europe 28 (EU28) and it consists in: 1) origin, 2) available quantity and geo-localisation, 3) collection and transport costs, 4) water footprint, 5) chemical-physical composition, 6) biomass current management.

The origin and composition of biomass define the biorefinery categories: biorefinery of first (1G), second (2G) and third (3G) generations.

The available quantity of feedstocks is fundamental to define the size of the biorefinery process and the number of generable products. The quantitative analysis of biomass is based on Eurostat and FAO database (FAO, 2018).

In Chapter 1, according to EU Commission Decision 2000/532/EC and Eurostat database, are considered the four biomass classes:

1) wastewater and sewage sludge (WSS);
2) municipal solid waste (MSW);
3) waste from agriculture, farming and fishering activities (AFF);
4) waste from industrial food and beverage activities (IFB).

The biomass quantity is expressed as average and deviation standard of the latest period (three-four years) according to Eurostat data availability. The map of EU 28 with available quantity of biomass is drawn with Data-wrapper (open source software of Google).

Collection and transport costs are evaluated from technical, economic and environmental perspectives.

Water footprint (WF) is an environmental indicator measuring the amount of water used to produce goods and services. The WF study, developed according to Water Footprint Network and ISO14046, aims to quantify the amount of water reduction through the valorisation of the biomasses according to environmental sustainability and resource efficiency. WF is a study depending on georeferenced and boundary conditions, but in the present research, data is used to provide an order of magnitude of WF of agricultural biomasses and residual agricultural biomasses. WF is calculated only for residual agricultural biomasses, since data is not available for the other biomasses considered in Chapter 1.

The biomass qualitative analysis consisted in elemental and macro-components biomass evaluations. The qualitative evaluation is performed by CHNS elemental

analysis, total solids (TS) and volatile (VS). Furthermore, biomass is evaluated in terms of their macro-constituents, as carbohydrate, oils/lipids and lignocellulose to define biomass feedstock biorefinery category according to IEA (Task 42).

The qualitative analysis is carried on 14 biomasses, defined as the most representative of the four above mentioned categories: 1) wastewater and sewage sludge, 2) organic fraction municipal solid waste, 3) rice waste, 4) animal waste, 5) milking waste, 6) corn and wheat waste, 7) fruit and vegetables agrowaste, 8) winery waste, 9) dairy waste, 10) slaughter waste, 11) processed candy waste, 12) olives and oil waste, 13) processed fruit and vegetables and 14)spent ground coffee.

To conclude, the current EU28 biomass management situation is provided for 1) WSS, 2) MSW, 3) AFF and 4) IFB

1.2.2 Process

The process is the second fundamental unit of biorefinery system. According to IEA (Task 42) biorefinery processes are classified in three main categories: thermo-chemical, chemical and biological processes. The aim of this section is the critical analysis of waste biomass process-conversion from environmental engineering perspective combined with integrated waste management systems. Hence, the analysis focuses on the whole process according to the following criteria:1) type of process, 2) work conditions, 3) technical-environmental and economic pros and cons 4) technical readiness level (TRL) and 5) correlation of biomass- process and process-products. In detail, the analysed processes were:

- Thermochemical: thermo-valorisation, gasification, pyrolysis and liquefaction
- Chemicals: trans-esterification, acid/alkali/thermal hydrolysis
- Biological: enzymatic hydrolysis, simultaneous saccharification and fermentation, separate hydrolysis and fermentation, anaerobic digestion and dark fermentation.

The evaluation of process design is performed, considering hierarchical, sequential and integrated designs.

1.2.3 Products

2G-biorefineries convert biomasses into high added-value products: platform chemical and energy. In this section, the hierarchical production, first platform chemicals and then bioenergy and biofuels, was discussed by technical, environmental, economic and social standpoints. The analysed platform chemicals are ethanol, lactic acid, propionic acid, 1,2 propandiol acid, 2,3 butanediol acid, succinic acid, malic acid, butirric acid, fumaric acid, itaconic acid and xylitol, while the energies and biofuels are: biogas, hydrogen, syngas, bio-oil, biodiesel and methanol. Products, both platform chemical and bioenergy, are described one by one according to: 1) chemical-physical characteristics, 2) feed biomass, 3) process

conversion, 4) yield and productivity, 5) market size, application and value To complete the study, the correlations biomass-product, process-product and biomass-process-product are carried out. Biomass-product correlation is sorted into biomass-platform chemical and biomass-energy.

The biomass- platform chemical correlation is performed considering the maximum theoretical biomass stoichiometric carbon conversion into product. The carbon content in the dry biomass fraction and in product are depicted in Figure1-7A-B and respectively expressed as g C/g dry biomass and g C/g product.

The biomass-energy correlation is based on electricity production by means of biogas from anaerobic digestion (AD) and power from thermo-valorisation. The biogas production is calculated by Buswell and Neave equation (Bonomo, 2014). The correlation process-product considers: type and cost of the process and market size, application and value of the product. Biomass-process-product correlation aims to evaluate: 1) biomass valorisation with consequentially reduction of waste generation, 2) most promising process and 3) market product size satisfaction. Finally, biomass valorisation is evaluated both as biomass percentage conversion to produce platform chemicals and bio-energies and the percentage of satisfied market size. This evaluation is based on biomass yield conversions reached by lab-pilot-technical tests from scientific literature (available in Table 1-12) and current market size (available in Table1-11). The calculation of biomass valorisation and market size satisfaction is not performed from economic perspective, since production costs are not available for all the 2G- biorefinery processes and products

1.2.4 Sustainability

Sustainability analysis is performed through top down approach to evaluate the whole biorefinery system from environmental, economic and social viewpoints. Sustainability is considered as synergy of three pillars (3P): Planet (environment), Profit (economy) and People (society). Sustainability was studied through mono, bi and three-dimensional indicators. Mono-dimensional (1D) evaluates one by one environmental, economic and social perspectives. Bi-dimensional (2D) involves environmental-economic and environmental-social perspectives. Three-dimensional (3D) is the combination of environmental, economic and social perspectives.

To conclude, sustainability is studied as key factor in 2G- biorefinery system design, considering the following parameters: 1) potential displacement of fossil fuels and materials, 2) mitigation of environmental impacts, 3) renewability, 4) economic feasibility, 5) preservation of biodiversity and 6) social responsibility. The quantitative evaluation of sustainability is not performed, since sustainability is geo-rereferred and case-specific boundary dependent and consequently generalization should be meaningless.

1.3 Results

In this section the salient results are discussed. We analyse the biomass, the process and the products. Concerning biomass, we evaluate the quantity and quality, the collection and transport, the water footprint (WF) and the current management.

Concerning process, we analyse type (thermal, chemical and biological process), and design.

Concerning products, we investigate the platform chemicals and energies according to market size, application and values.

To conclude, we state the correlation between the starting biomass, the corresponding process and the resulting products

1.3.1 Biomass

European directive 2009/28/EC defines biomass as "*the biodegradable fraction of products, waste, residues from biological origin from agriculture (including vegetal and animal substances), forestry, and related industries including fisheries and aquaculture, as well as the biodegradable fraction of industrial and municipal waste*". In biorefinery system, biomass plays a strategic role, because of process and products are defined on the ground on the feed biomass.

There are three types of origins of biomass, which defined respectively three biorefinery categories: first (1G) second (2G) and third (3G) biorefinery generations. First-generation biomass (1G) includes edible crops as edible vegetables oils, sugar-cane, rice, wheat and corn, while second-generation biomass (2G) consists in waste biomasses coming out from municipal, agricultural and industrial contests and the third-generation biomass (3G) employs algae and microalgae (IEA, task42).

1G biomasses employs ad hoc biomass, which faced social, environmental and economic challenges, since the mono-cultivation consumed arable land reducing biodiversity, edible-food production and boosting climatic change Azapagic, (2014).

2G biomasses overcome the dilemma products-food and represents a valid solution to improve the current waste disposal Moncada et al. (2016).

3G biomasses exhibit advantages as microalgae low-cost culture, high energy, eco-friendly and completely renewable processes (Pleissner and Rumpold, 2018).

Among these three types of biorefinery categories, 2G-biorefinery could 1) produce high added value products valorising wastes, 2) reduce GHG emission, 3) achieve zero waste landfill, 4) convert economic system from liner to circular structure enabling Circular Economy principles Azapagic, (2014).

From Chapter 2 to Chapter6, the biorefinery system analysis concerns with 2G-biorefinery, which employs as feedstock waste biomasses available in Europe 28 (EU28).

2G-biomasses are a programmable resource in the short and long periods and their use for chemicals and energy productions are a consolidated reality in EU28 (Mossman, 2018). In the world, the produced 2G-biomass is about 50 billion tonnes

per year, of which 1.3 billion of food waste (FW) Alexandri and Venus, (2017). A sustainable supply of biomass is the key to guaranty biobased value chains, including high added value bioproduct and bioenergy Pelkmans and Fritsche, (2019). The concept of sustainability is included from the early stage of projection to the final disposal of products.

2G-biorefinery biomass valorisation-management agrees with the policies of Circular Economy (CE). The Circular Economy, in its 114 definitions Kirchher et al.(2017), promotes self-regenerative biorefinery systems in which the flows of matter are partly restored in the biosphere and partly destined to a new manufacture system, reaching the goal of "zero waste" with valorisation of the whole biomass (Ellen McArthur Foundation, 2015). In detail, CE promotes the Green Economic Growth, which improved economic growth, social wellness and environmental sustainability boosting investments and innovations, which can reinforce sustainable development and allow the passage from Linear to Circular Economy Song et al. (2019).

1.3.1.1. Biomass quantity in EU 28

The study of biomass is carried out in EU 28. To assess the quantitative and qualitative biomass inventory, EU 28 contest is investigated through three complementary perspectives:

1) Geographical division to understand how climate affects the seasonality, quantity and quality of biomass,

2) Demographic distribution to understand how much and in which areas the biomass is produced

3) Economic development to correlate the economic situation and produced biomass (in this way, waste production become a key factor to evaluate and compare EU28 countries)

EU28 is conventionally divided according to cardinal points in: Northern (Denmark, Finland, Iceland, Latvia, Lithuania, Estonia, Norway and Sweden) with predominantly continental clime, Western (Austria, United Kingdom, Belgium, France, Germany, Ireland, Luxembourg, Liechtenstein, Holland and Switzerland) with predominantly oceanic climate, Eastern (Belarus, Bulgaria, Moldova, Poland, Czech Republic, Romania, European Russia, Slovakia, Ukraine, Hungary and Cyprus) with predominantly mid-continental climate and Southern (Albania , Bosnia and Herzegovina, Croatia, Greece, Italy, Macedonia, Malta, Montenegro, Spain, Portugal, San Marino, Serbia, Slovenia and Turkey) with Mediterranean climate.

In January 2019, the population of EU28 is estimated at 511.8 million people, with an average population density of 155 inhabitants per km^2 (Eurostat, 2020). The demographic distribution (Figure1-1) is expressed as mean value plus standard deviation performed for the individual cities and/or districts of each EU nation, according to NUTS2 statistical method of the EUROSTAT database. The percentage ratio between standard deviation and mean value, (representing the

degree of heterogeneity of population density in each EU28 country) is around 50% and it underlines the living differences between cities and countryside, which influences the biomass collection system.

The Gross Domestic Product (GDP) (Figure1-2), measuring the economic activity of EU28 country, is calculated as the value of all produced goods and services minus the value of each employed product and/or service in their realization. The EU28 GDP is around $565 \cdot 10^{6}$€, with the highest and lowest values for Norway ($171 \cdot 10^{6}$€), and Bosnia-Herzegovina ($28 \cdot 10^{6}$€), respectively (Eurostat, 2020).

Figure 1-1: Population density, avarage from 2007-2018 (Eurostat, 2020)

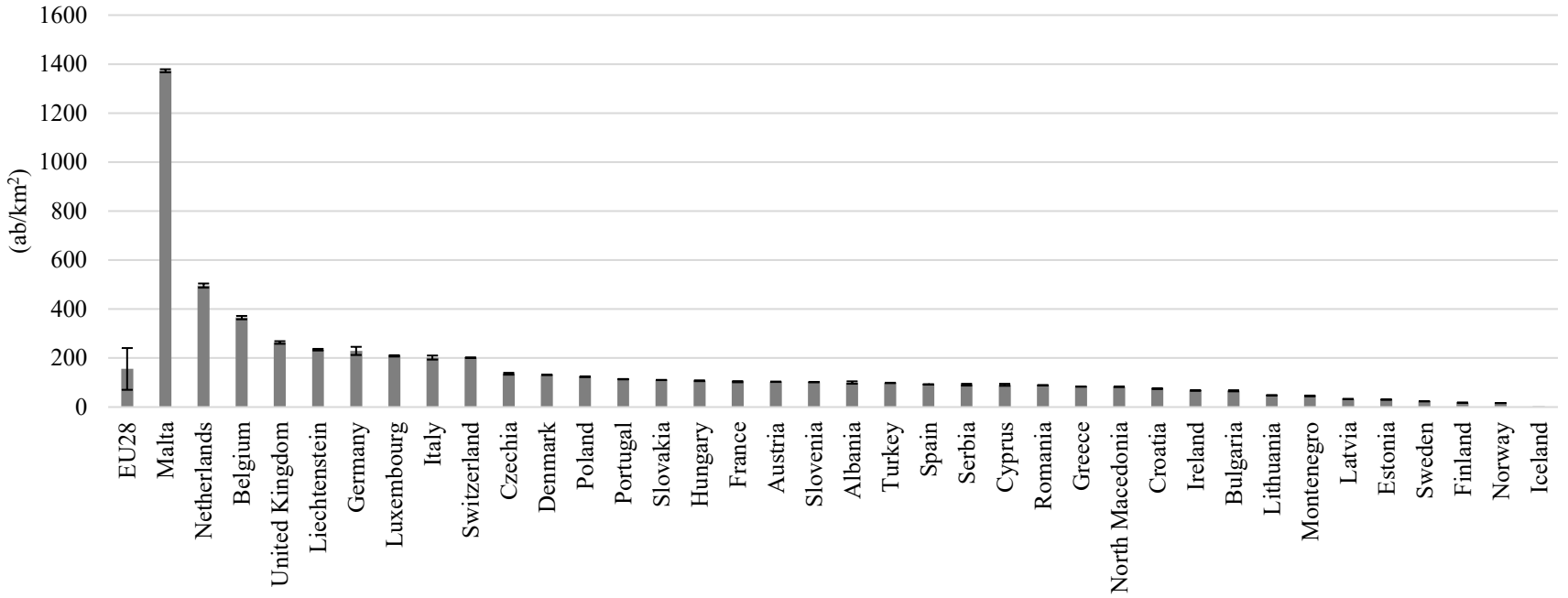
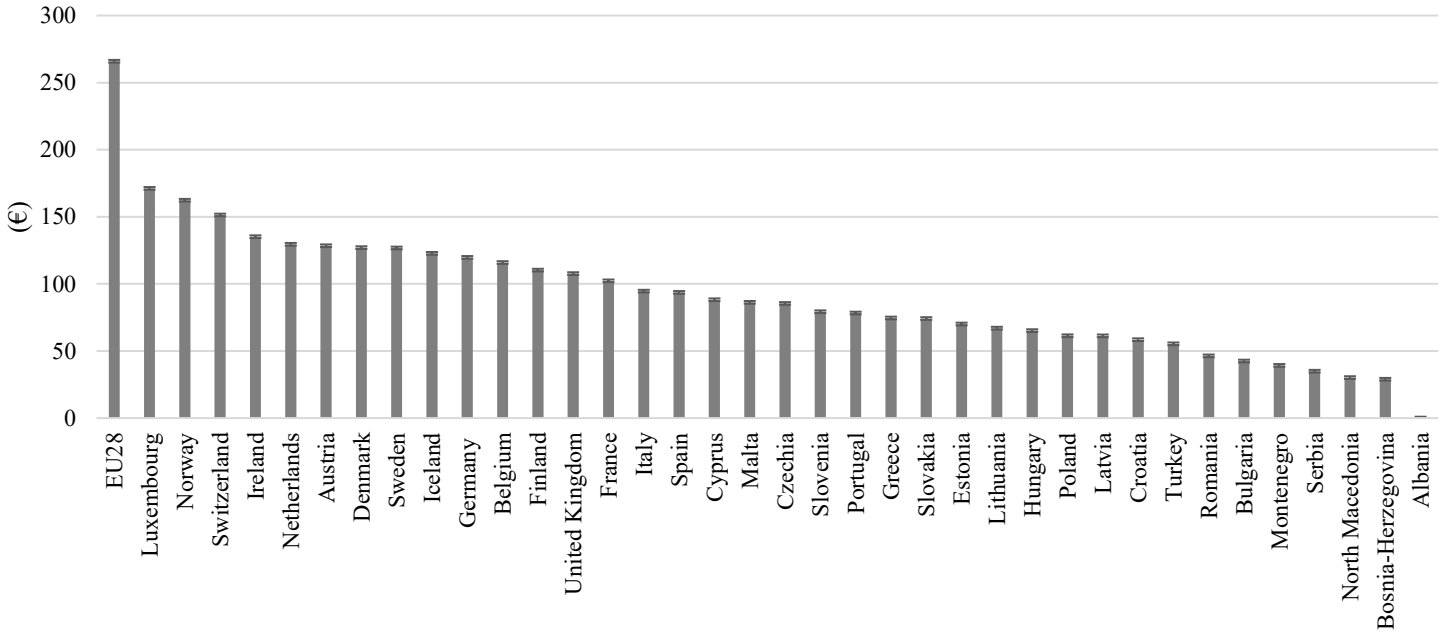


Figure 1-2: Gross domestic product (GDP) average from 2007-2018 (Eurostat, 2020)



Biomass has different characteristics based on the origin. In Europe, the Commission Decision 2000/532/EC and Technical Guidance on the classification of waste (2018/C 124/01) (EU Commission, 2018) classify waste and biomass according to the activity from which they are produced, in detail: 1) Residential, 2) Commercial, 3) Public, 4) Construction (construction and demolition), 5) Public services, 6) Treatment plants, 7) Industrial and 8) Agriculture.

In EUROSTAT database, the above waste classification is provided into four categories:

1)**Wastewater and sewage sludg**e (WSS) corresponding to category 6 of the EU legislations;

2)**Municipal solid waste** (MSW) corresponding to categories 1, 2, 3, and 5, respectively residential, commercial, and public services defined by EU legislations;

3)**Waste from agriculture, forestry and fishing activities (AFF)**: corresponding to category 8, agricultural activities defined by EU legislations

4)**Waste from manufacturing of food and beverage products (IFB)** corresponding to category 7, industrial activity defined by EU legislations.

With wastewaters we refer to wastewater from households, towns and public buildings Asano et al.(2014). In the world, wastewaters production is around $330 \cdot 10^3 m^3$ /y Asano et al.(2014). Sewage sludge refers to separate solids during urban wastewater treatment. In EU28, Wastewater and sewage sludge (WSS) average production, expressed in ($10^3 Mt$), refers to years 2000-2018 (Figure 1-3A). The minimum value is recorded in Eastern countries about 6.33·Mt, where the collection system is not really implemented and population density is low, while the highest is recorded in Germany with $1.8 \cdot 10^3$ Mt (Eurostat, 2020).

Municipal solid waste (MSW) is defined as waste generated by households which may also include similar waste generated by small businesses and public institutions and wastes not collected by the municipality. This part of municipal waste may vary from municipality to municipality and from country to country, depending on the local waste management system and human habits. For areas not covered by a municipal waste collection system, the amount of waste generated is not available. MSW production average of years 2000-2018 (Figure1-3B), are expressed as (kg/pc) In EU28, MSW has a maximum value in Denmark with 758 kg/pc and a minimum value in Kosovo with 213 kg/pc (Eurostat, 2020). The produced MSW depends on the level of economic activity and welfare of country, since it reflects production and consumption (EU, 2020). FAO reports (FAO, 2019) shows that Eastern European countries produce less waste because they have a lower GDP than other EU 28 countries. In EU28, 30-50% of MSW is organic fraction (OFMSW) (Mossman, 2018), thus the range of OFMSW available in EU28 is 173-64 kg/pc.

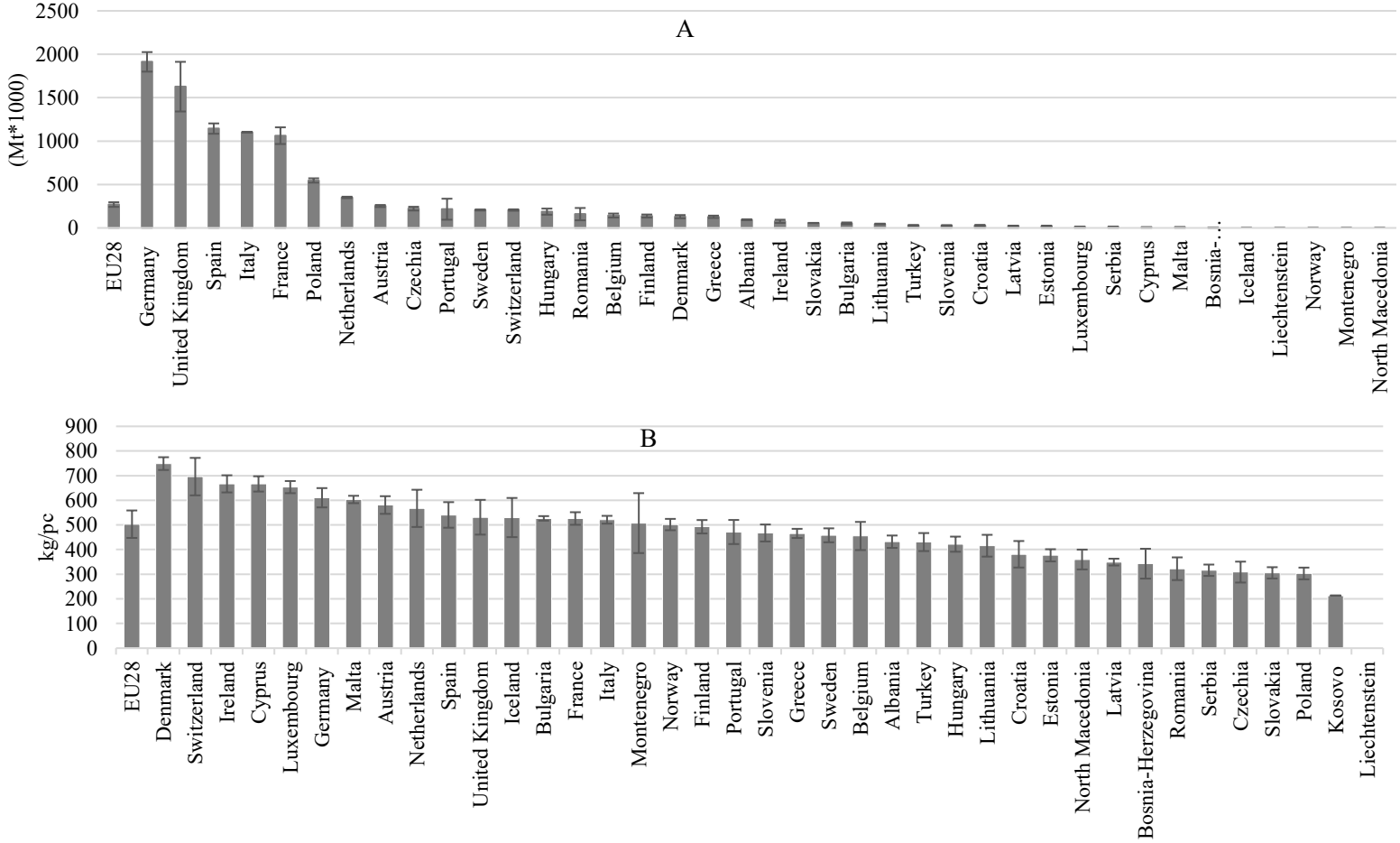
Agriculture, forestry and fishing category (AFF) consider the exploitation of vegetal and animal natural resources, including the activities of growing of crops, raising and breeding of animals, harvesting, animals or animal products from a farm or their natural habitats. The Eurostat database reports these wastes in the

agricultural, farming and fishing activities in the categories W091, WO92 and W093 (Eurostat, 2020).

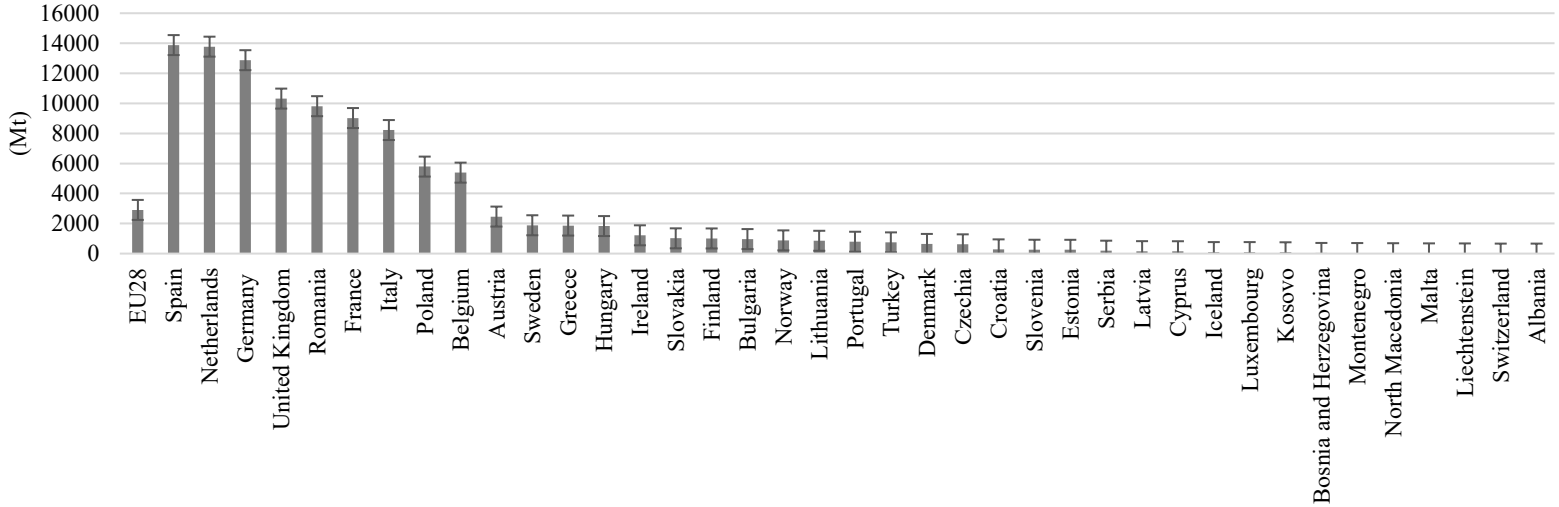
In EU28, AFF (Figure1-3C) production in 2004-2016 is 2908Mt. The highest AFF production values are recorded in Spain and the Netherlands, 5 and 4 Mt respectively, while the lowest values are recorded in Switzerland and Albania, with values of 1239 and 1880 t. The percentage (Figure 1-4A) contribution of each type of waste produced in AFF category highlighted that animal waste are the most produced waste with a variable contribution from 50 to 70%.

Waste from manufacturing of food and beverage products (IFB), including processing waste from manufacturing of food and beverage products are considered and calculated from classes C10: Manufacture of food products and C11: Manufacture of beverages of Eurostat database dealing with the following subcategories:10.1 Processing and preserving of meat and production of meat products, 10.2 Processing and preserving of fish, crustaceans and mollusks, 10.3 Processing and preserving of fruit and vegetables, 10.4 Manufacture of vegetable and animal oils and fats, 10.5 Manufacture of dairy products, 10.6 Manufacture of grain mill products, starches and starch products,10.7 Manufacture of bakery and farinaceous products, 10.8 Manufacture of other food products, 10.9 Manufacture of prepared animal feeds. The EU28 IFB waste production for average period of 2004-2016 is around 8000 Mt. The highest percentage contribute (Figure1-4) coming out from manufacture of other food products (16-50%), processing and preserving of fruit and vegetables (14-30%), manufacture of vegetable and animal oils and fats (3-53%), manufacture of dairy products (4-18%), grain mill products (3-29%), starches and starch products of bakery and farinaceous products (3-35%) (Eurostat, 2020). Geographical distribution of the four biomass categories, elaborated with Data-wrapper and based on Eurostat database 2020 are represented in Figure 1-5.

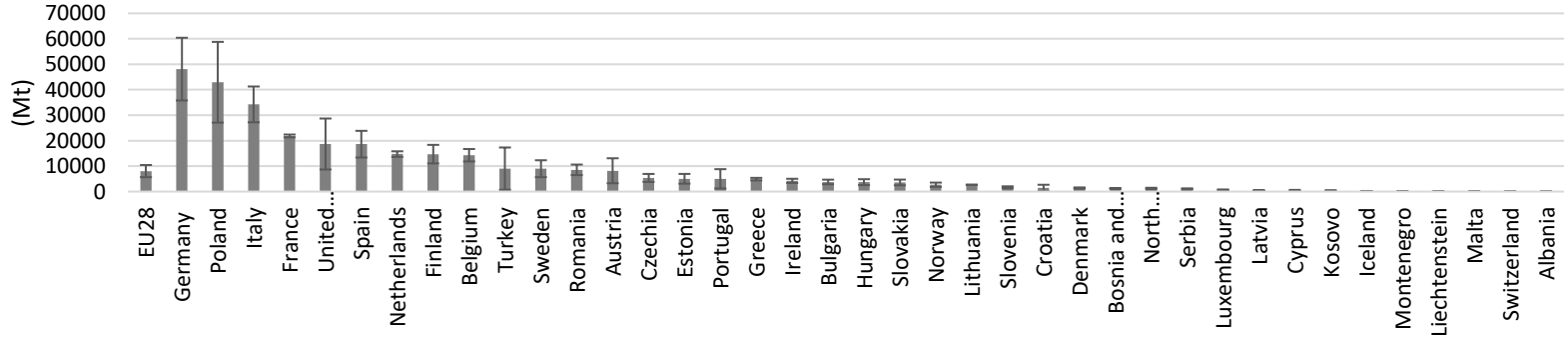
Figure 1-3:A) Wastewater and sewage sludge (WSS) production average: 2000-2018 expressed as Mega tons per 10^3, **B)** Municipal solid waste (MSW) production average: 2000-2018 expressed as kg per capita, **C)** Agriculture, forestry and fishing category (AFF) production average: 2004-2016, expressed as mega tons and **D)** Waste from manufacturing of food and beverage products (IFB) average 2007-2016, expressed as mega tons (Eurostat,2020)



C


(Mt)
16000
14000
12000
10000
8000
6000
4000
2000
0
EU28
Spain
Netherlands
Germany
United Kingdom
Romania
France
Italy
Poland
Belgium
Austria
Sweden
Greece
Hungary
Ireland
Slovakia
Finland
Bulgaria
Norway
Lithuania
Portugal
Turkey
Denmark
Czechia
Croatia
Slovenia
Estonia
Serbia
Latvia
Cyprus
Iceland
Luxembourg
Kosovo
Bosnia and Herzegovina
Montenegro
North Macedonia
Malta
Liechtenstein
Switzerland
Albania

D


(Mt)
70000
60000
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EU28
Germany
Poland
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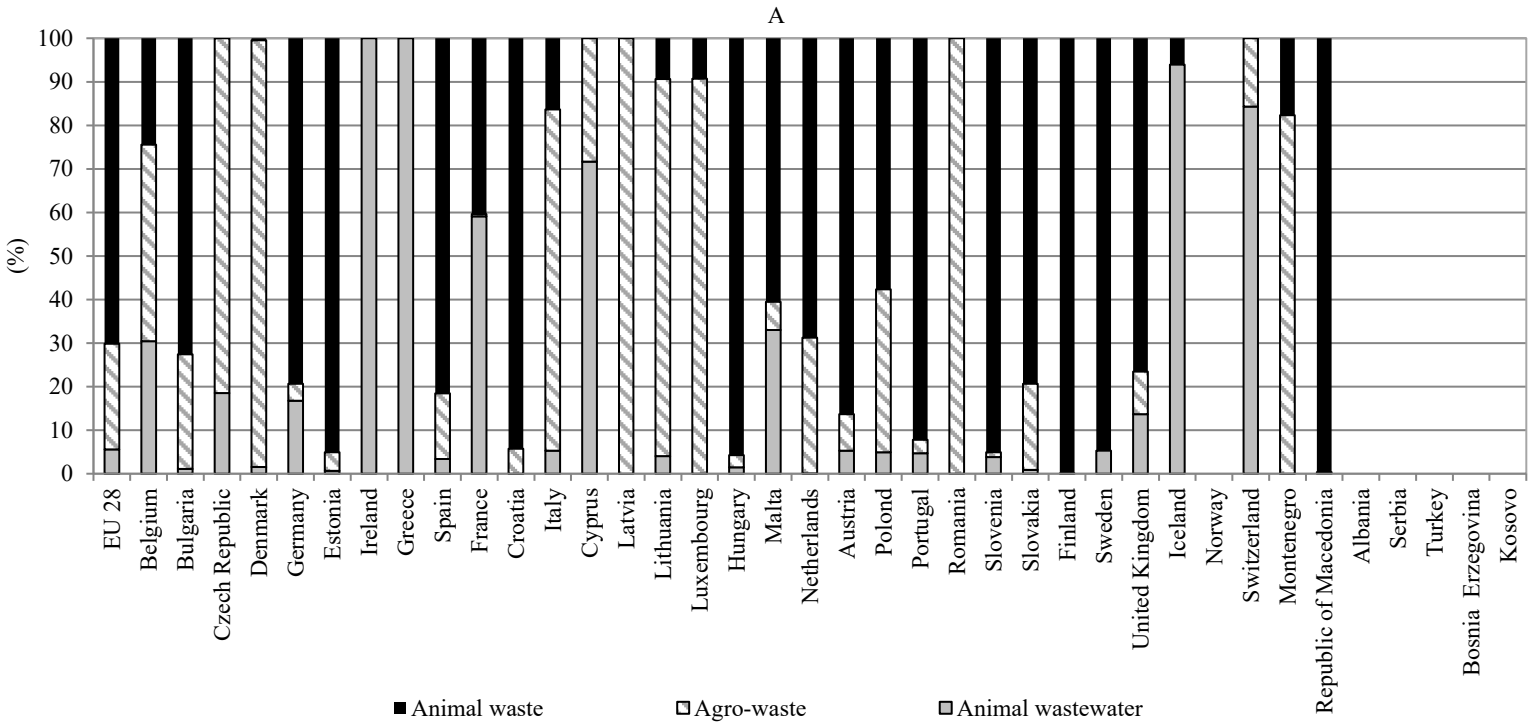
Netherlands
Finland
Belgium
Turkey
Sweden
Romania
Austria
Czechia
Estonia
Portugal
Greece
Ireland
Bulgaria
Hungary
Slovakia
Norway
Lithuania
Slovenia
Croatia
Denmark
Bosnia and...
North...
Serbia
Luxembourg
Latvia
Cyprus
Kosovo
Iceland
Montenegro
Liechtenstein
Malta
Switzerland
Albania

Figure 1-4: Percentage contribution of each type of waste produced in AFF category (A) and Percentage contribution of each type of waste produced in IFB category(B) (Eurostat, 2020)



B

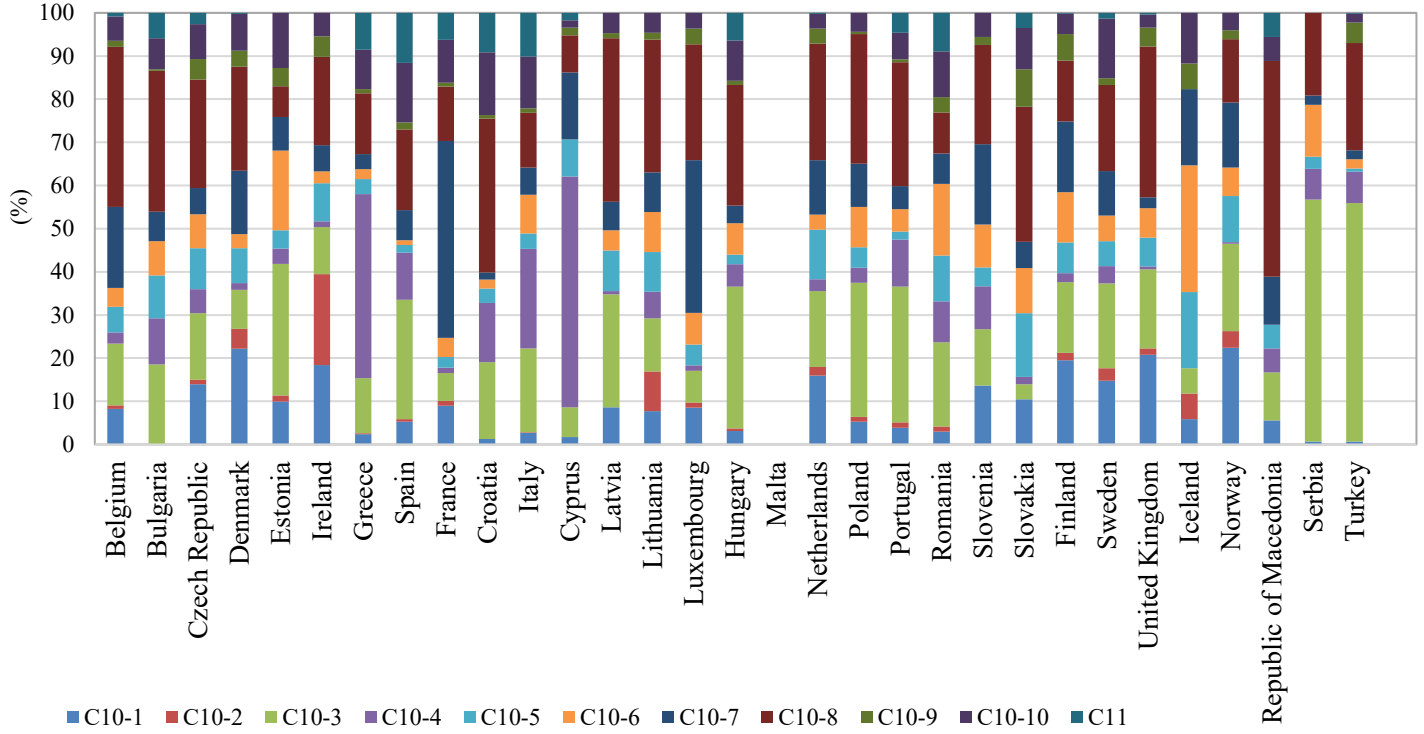
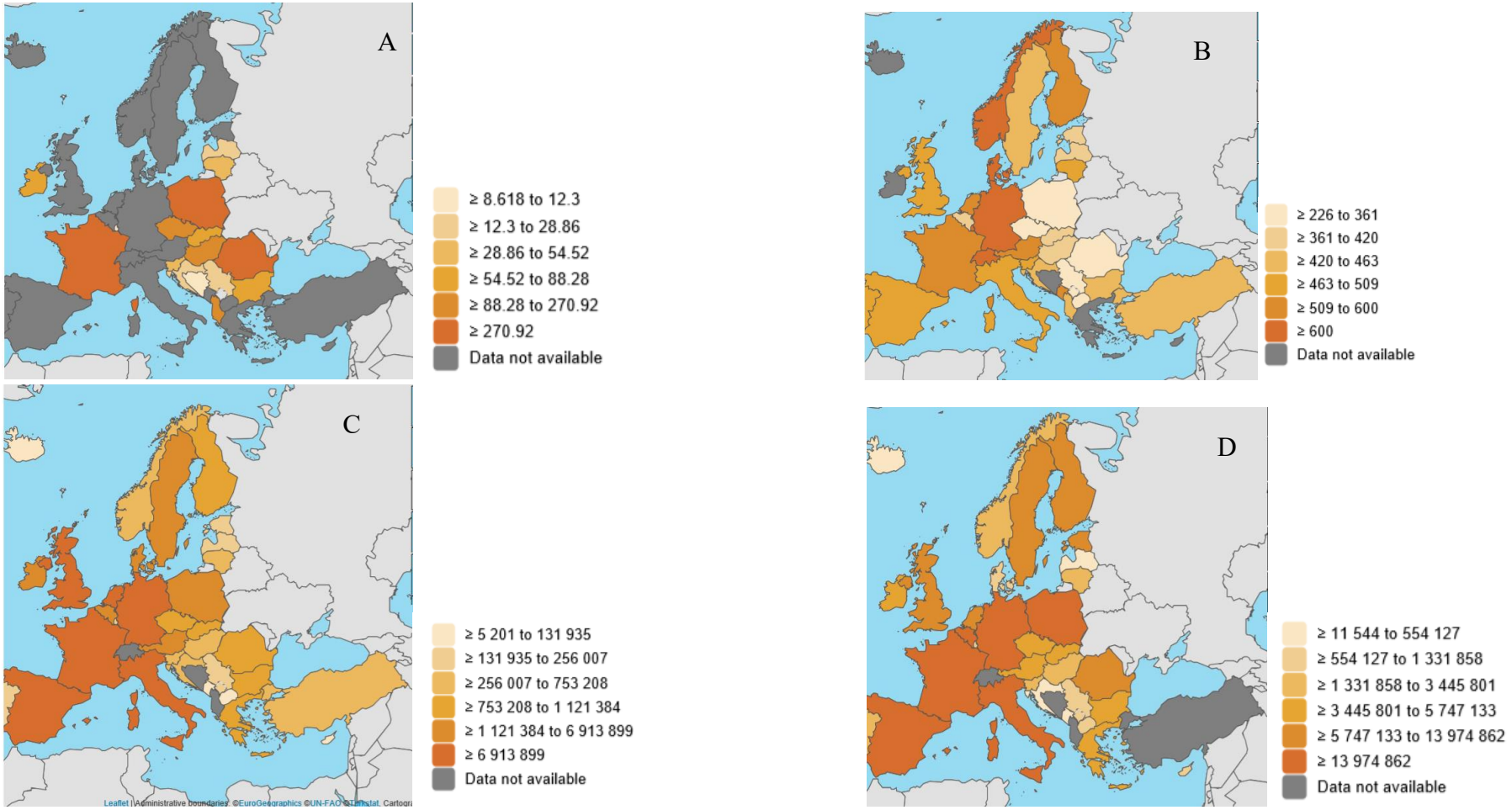

100
90
80
70
60
50
40
30
20
10
0
(%)
Belgium
Bulgaria
Czech Republic
Denmark
Estonia
Ireland
Greece
Spain
France
Croatia
Italy
Cyprus
Latvia
Lithuania
Luxembourg
Hungary
Malta
Netherlands
Poland
Portugal
Romania
Slovenia
Slovakia
Finland
Sweden
United Kingdom
Iceland
Norway
Republic of Macedonia
Serbia
Turkey
C10-1
C10-2
C10-3
C10-4
C10-5
C10-6
C10-7
C10-8
C10-9
C10-10
C11

Figure 1-5: A) Geographical distribution of biomasses (t/y) of the four categories: waste water and sewage sludge (WSS) (2018), **B)** municipal solid waste(MSW) (2018), **C)** waste from agriculture, forestry and fishing activities(AFF) (2016) and **D)** waste from manufacturing and beverage sector.



1.3.1.2. Biomass collection and transport

The potential of biomass for energy production ranges between 33-1135 EJ/y Hoogwijk (2003). This value is equal to 5-185 billion barrels of oil, able to satisfy the world energy necessity of 820 EJ/y by 2040 (EIA, 2020). For these reasons, biomasses are a touchable and concreate reality to produce energy with no carbon fossil resources. Biomasses are not a free resource since there has: 1) production costs, 2) collection costs, 3) transportation costs and 4) conversion costs. Biomass production costs includes growing and harvesting, but these costs are equal to zero in the case of 2G biorefineries, which employs waste biomass. Biomass collection costs depends on waste management collection defined by singular towns and countries. Biomass transportation costs is one of the biggest bottlenecks of biorefinery system. Several studies consider biomass transportation costs independently by location, assuming uniform spatial distribution of biomass and road structure without considering biorefinery plant size. Recent studies prove that biomass yield density (t/ha·y) [1]varied with biomass supply distance (km) from biorefinery plant location. In detail Golencha and Gan (2016) study states a mutual influence and dependency between biomass yield density (t/ha·y), supply distance (km) and tortuosity road factor. Tortuosity factor is defined considering real road network and it is smoothed with the increase of ran distance. Golencha and Gan (2016) develop a Taylor polynomial series approximated at 1°order to express biomass yield density (t/ha·y) and tortuosity road factor to linear function of supply distance (km) from biorefinery plant. Based on the Taylor series, Golencha and Gan (2016) define a biomass transport costs model able to depict the transport cost of different biomasses. In this section, the Golencha and Gan (2016) study is analysed to understand the optimal biorefinery size and biomass supply radius considering biomass yield density, tortuosity road factor and biomass radius. Studies such as the one by Sultana et al. (2014) state that biomass availability (t/y) increases exponentially with an increase in supply radius R (km) which defines the biorefinery plant capacity, while Golencha and Gan (2016) state that supply radius increases ranging between 50-75 km for biorefinery size of 500 kt/y. In detail, biomass transport costs include fixed costs per trip multiply per number of trips to satisfy biorefinery capacity plus biomass transport cost per unit of mass and distance from biomass field to biorefinery. According to Golencha and Gan (2016), a transport amplification factor, which is a ratio of real and theoretical biomass transport costs per unit of biomass, must be consider The real biomass transport cost per unit of biomass take into account a variation between biomass yield density and tortuosity factor based on literature data Maung et al (2013); Leduc et al. (2010) Zhang et al (2011). Several analyses, published in the literature (Golencha and Gan, 2016; Sultane et al. (2014); Leduc et al. (2010) establishing that 1) without considering biomass yield density, biorefinery plant sizes higher than 600 kt/y has an increase of more than 50% difference of biomass supply radius, 2) for biomass

[1] t/ha y means tons per hectare per yea

yield density of 0.5 t/ha and biorefinery plant of 400 kt/y amplification transport factor is 1.25, 3) increasing the transport distance, the tortuosity factor decreases and consequently the transport amplification factor decreases, 4) for biorefinery plant of 1000 kt /y and biomass yield density of 150 t/ha conventional transport had biomass supply radius of 50 km with transport costs of 10.46 €/t, while transport with transport amplification factor has biomass supply radius of 70 km with transport costs of 11.96 €/t, 5) costs of transport per unit of biomass rises drastically increasing biorefinery plant size leading to let up of scaling biorefinery system in our society. Biomass transport might be analysed from environmental and economic perspectives. Environmental and economic transport costs vary according to feedstocks moisture content and kilometers run. Increasing water content, transport cost ranges between 0.41-1.2 €/t Ramli and Epplin, (2017). Transport over 100 km is judged unsustainable for feedstock with water content higher than 30% w/w Bahera et al. (2014). From sustainability criteria, collection and transport of 2G biomass must carry out 40-60% GHG reduction comparing the biorefinery outcomes to analogous products deriving from no renewable resources Budzianowski et al. (2017). Another important parameter affecting waste biomass collection and transport is the seasonality variation of the available biomass Budzianowski et al. (2017).

1.3.1.3. Biomass water footprint

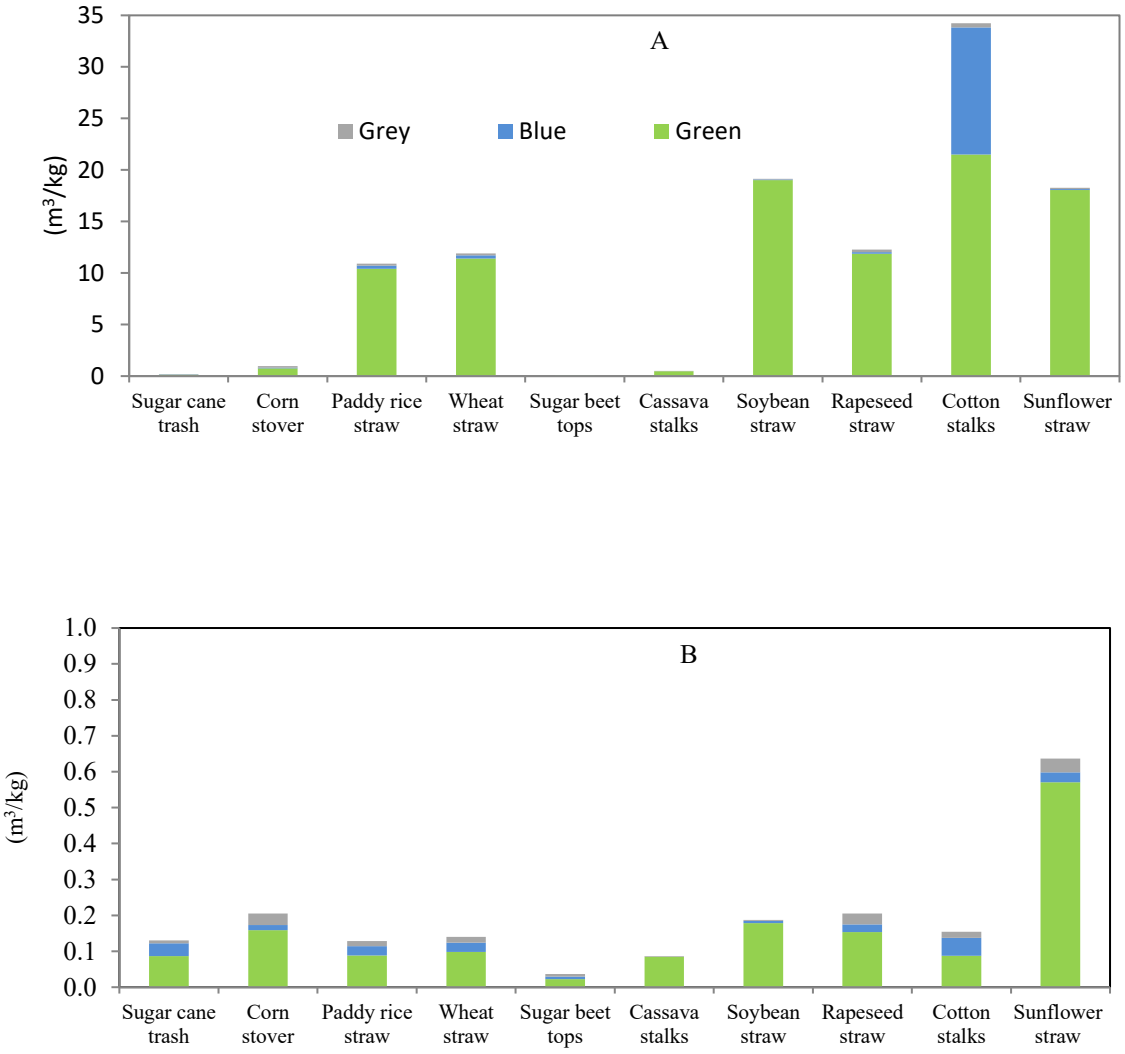
Water footprint (WF) quantifies the water employed to produce goods and services and it is composed of three contributes: green, blue and grey waters according to Water Footprint Network and ISO14046. Here, WF is not considered as final environmental indicator to assess the sustainability of the waste biomasses, but it sets the importance to consider 2G-biomasses as resource in 2G-biorefinery system.

In Italy more than 20% of agricultural area is irrigated, which means that 26 Gm^3/y [2] of water is used for irrigation/agricultural purposes, representing 49% of total Italian water demand Antonelli and Greco (2013).Typically, WF studies depend on geo-rereferred and assessed boundary conditions, but in the present study, data from different papers is considered and averaged to provide an order of magnitude of WF of agricultural biomasses and residual agricultural biomasses.

According to the studies of Hoekestra et al. (2010) and Mekomen et al. (2011), the WF of residual biomass is two orders of magnitude lower than WF of biomass as well (Figure 1-6), which proved the efficient water use through valorisation of waste biomasses, according to (FAO, 2015) principles for water use reduction and agrowaste prevention.

[2] Gt/y means Giga tonnes per year

Figure 1-6: Water footprint of agro- biomasses (A) and residual agro-biomasses (B)



1.3.1.4. Biomass qualitative analysis

The qualitative analysis of biomass defines which biorefinery process could be adopted and which platform chemicals and bioenergy could be produced.

The qualitative analysis consisted in elemental composition referring to volatile solids (VS) (Table1-1): 1) to describe the biomass through a chemical formula and in macro-constituents (Table1-2) and 2) to classify the type of biorefinery based on feed biomass: carbohydrate, lipids/oil and lignocellulosic biomasses. The analysed biomasses are the most representative of the four Eurostat categories. In total, 14 biomasses are selected

Sewage sludge and the organic fraction of municipal solid waste (OFMSW) are respectively taken into consideration to represent the first and second Eurostat categories, WSS and MSW, respectively.

For agriculture, forestry and fishing (AFF) category the following biomasses are considered: rice cultivation waste, farming waste, milking waste, corn and wheat waste and fruit and vegetable agrowaste.

For waste from manufacturing of food and beverage products (IFB) category the following 2G-biomasses are considered: winery, dairy, slaughter, processed candy, olives and oil, processed fruit and vegetable and spent ground coffee wastes. Based on Eurostat data base and data in scientific literature, the available quantity of the 14 considered biomasses are: wastewater and sludge 6696 Mt/y, OFMSW 750 Mt/y, rice waste 1380 Mt/y, farming waste 561 Mt/y, milking waste 459 Mt/y, corn and wheat waste 1492 Mt/y, fruit and vegetable agrowaste 857.9 Mt/y Lam et al. (2016), winery waste 130.30 Mt/y, slaughter waste 34 Mt/y, dairy waste 1.5 Mt/y Abd-alla et al. (2017), processed candy waste 482.11 Mt/y, olives and oil waste 300Mt/hay Santos et al. (2017), processed fruit and vegetable waste 1824 Mt/y and spent ground coffee 15 Mt/y Neu et al.(2016). All the 14 considered biomasses have carbon content higher than 40-50% w/w (Table1-1), and for this reason 2G-biomass is a value feedstock for 2G biorefinery processes, especially for biological ones. The carbon content is also provided in Figure1-7 in terms of Carbon per molecular weight, both in percentage (Figure 1-7A) and grams per grams (Figure 1-7B). Table 2 shows the biomass macro-constituents and the following statements can be assessed:

- 43% of the considered biomass consists mainly of carbohydrates. OFMS, agrowaste as rice, fruit and vegetables, industrial processed of fruits and vegetables have a high content of carbohydrates ranging between 40-87% w/w
- 36% of the considered 2G-biomass consists mainly of lipids and oils. farming and milking waste. Waste from the dairy industry and processing wine and olives and oils have a high content of lipids and oils ranging between 36.5-68 % w/w
- 14% of the considered biomass consists mainly in lignocellulose. Corn and wheat waste and spent ground coffee have a high content of lignocellulose ranging between 14-44 % w/w

Not enough data is available for the WSS category and it is not possible to characterize biomass. In Table1-2, the considered biomasses could be sorted out as carbohydrate, lipids/oil and lignocellulosic biomasses, according to the IEA biorefinery classification: carbohydrate, triglycerides and lignocellulosic feedstock biorefinery (IEA, Task 42). The knowledge of biomass chemistry is fundamental to define the process and obtainable products. The most abundant waste biomasses are carbohydrate (75%), followed by oil/lipids (20%) and lignocellulosic (5%) Corma et al. (2007) (Table1-3). Among these three types of biomasses, lignocellulosic is the most employed one, but it required pre-treatments before to be converted. In detail, lignocellulosic pre-treatments are aimed to disrupt the lignocellulose structure, to make available sugars for enzymes and micro-organism avoiding sugar degradation products or formation of inhibitory components for the subsequent hydrolysis and fermentation of substrates into products Agbor et al. (2011).

Table 1-1: Elemental composition and brute formula of biomasses available in EU28

	Biomass	C (%)	N(%)	S (%)	H (%)	O (%)	ST (%)	SV /ST (%)	Chemical formula	Refernces
WSS	Sludge+ wastewater	50	3	1.9	8.6	36.5	2	65	$C_{19}NH_{40}O_{11}$	Van Lier et al., 2008
MSW	OFMSW	49.66 ± 8.23	2.64 ± 0.34	0.436 ± 0.27	6.026 ± 1.03	35.982 ± 6.52	18 ± 0.29	45	$C_{22}NH_3O_{12}$	Venus et al., 2018 Cerda et al., 2018 Schaneset al., 2018
AFF	Rice	37.17	7.24	18.43	5	32.16	88	98	$C_6NS_1H_{11}O_6$	Chung et al., 2018
	Farming waste	40.96	1.48	0	5.2	33.14	19	98	$C_{32}NH_{49}O_{20}$	Nečemer et al., 2016
	Milking waste	46.5	4.9	5.62	8.43	34.55	13	98	$C_{11}NSH_{24}O_7$	Vidal et al., 2000 Nečemer et al., 2016
	Corn and wheat waste	43.6	0.6	5	8	42.8	89	94	$C_{85}NS_4H_{187}O_{65}$	Lam et al., 2016; Banerjee et al., 2017
	Fruit and vegetable agro-waste	41.3 ± 5.57	1.2 ± 1.17	0	5.65 ± 0.39	51.85 ± 5.14	21	95	$C_{40}NH_{66}O_{37}$	Link et al., 2018
IFB	Winery waste	49.8	2	0	5.8	42.4	85	81	$C_{29}NH_{41}O_{38}$	Borone et al., 2018 Nečemer et al., 2016
	Dairy waste	46.9	4.9	5.62	9.03	33.55	43	97	$C_{11}NSH_{26}O_7$	Borone et al., 2018; Nečemer et al., 2016
	Slaughter waste	54.9	5.9	1	8.5	29.7	90	80	$C_{11}NSH_{20}O_4$	Shahzad et al., 2017;;Kokossis and al., 2012
	Processed Candies waste	47.6	2.57	0	7.3	42.53	77	98	$C_{22}NH_{40}O_{14}$	Haque et al., 2017Gustavsson et al., 2011; Lin et al., 2013
	Olives and oil waste	49	10.4	0	10.3	30.3	56	99	$C_5NH_{14}O_3$	Innangi et al., 2017
	Processed fruit and vegetable waste	43.2	0.15	0	6.22	50.43	66	99	$C_{345}NH_{596}O_{302}$	Ruffino et al., 2017; Ruffino et al., 2015
	Spent ground coffee	47.5	1.18	0	6.01	44.86	86.8 ± 6.3	90.7 ± 3.0	$C_{47}NH_{71}O_{33}$	Woiciechowski et al., 2000; Pleissner et al, 2016 ; Oliveira et al., 2018;Pujol et al., 2013

Figure 1-7: Gram of carbon content per gram of biomass (grey) and carbon content per gram of dry biomass (black)

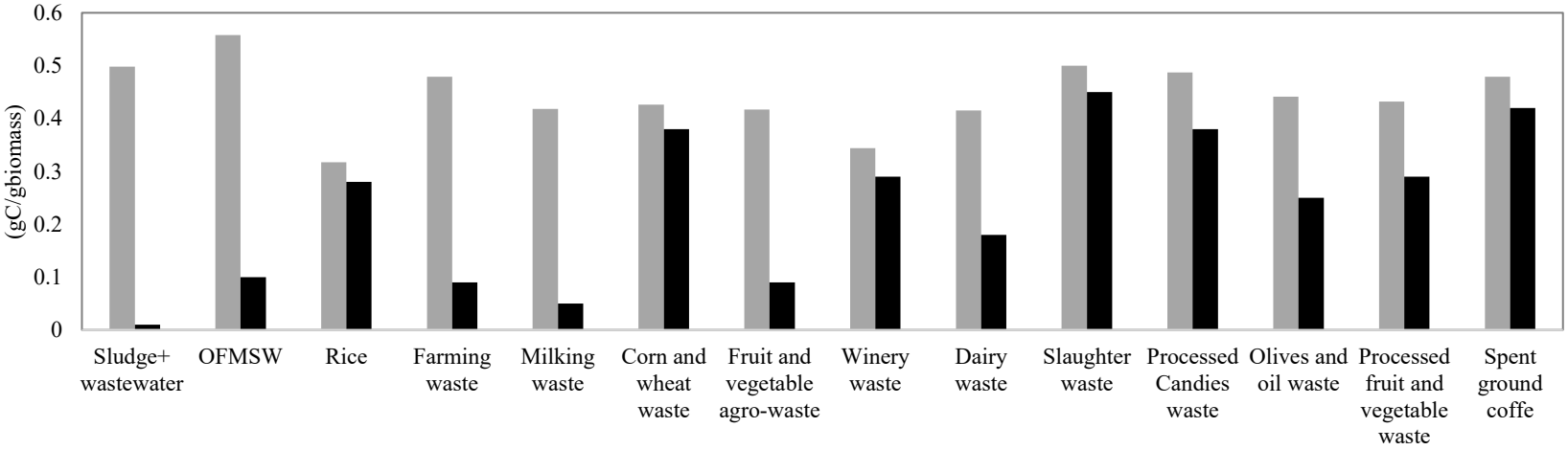


Table 1-2:Percentages of carbohydrates, proteins, fats and lignocellulose constituting the considered biomasses. n.d = no detected

	Biomass	Carbohydrate (%)	Protein (%)	Oil/fat/lipid (%)	Cellulose (%)	Hemicellulose (%)	Lignine (%)	References
WSS	WSS	13.00±8.49	43.7 ±26.45	n.d	n.d	n.d	n.d	Van Lier et al., 2008, Gonzalez et al., 2018
MSW	OFMSW	55.25 ± 13.08	13.00± 3.54	3,60± 0,85	4.8	3.1	0.1	Venus et al., 2018; Cerda et al., 2018; Schaneset al., 2018; Pleissner, et al., 2017
AFF	Rice	86.1	7.3	1.1	n.d	n.d	n.d	Chung et al., 2018; Kokossis and al., 2012
	Farming waste	7.5	57.1	35.4	n.d	n.d	n.d	Jeon et al., 2013
	Milking waste	17.89	46.71	35.4	n.d	n.d	n.d	Vidal et al., 2000; Nečemer et al., 2016
	Corn and wheat waste	64.5	15.5	4.2	44.4±0.4	27.8 ±0.3	19.6+0.2	Xu et al., 2018; Kokossis and al., 2012 Apostolis et al., 2014
	Fruit and vegetable agro-waste	46.13 ±12,91	9.33 ± 6.4	5.33 ± 3,4	n.d	n.d	n.d	Lam et al., 2016; Banerjee et al., 2017
IFB	Winery waste	4.1 (sugars)	15.10	5.40	n.d	n.d	n.d	Link et al., 2018
	Dairy waste	39	25	26	n.d	n.d	n.d	Borone et al., 2018; Nečemer et al., 2016
	Slaughter waste	n.d	45.9 ± 14.49	11.00 ± 2.37	n.d	n.d	n.d	Shahzad et al., 2017; Jeon et al., 2013 Kokossis and al., 2012
	Processed Candies waste	39.5	4.3	1.5	n.d	n.d	n.d	Haque et al., 2017; Gustavsson et al., 2011; Lin et al., 2013
	Olives and oil waste	7	25	68	n.d	n.d	n.d	Innangi et al., 2017
	Processed ruit and vegetable waste	46.13 ±12.91	9.33 ± 6.4	5.33 ± 3.4	n.d	n.d	n.d	Ruffino et al., 2017; Ruffino et al., 2015
	Spent ground coffe	7.8± 4.11	10± 0.71	9.5± 10.61	14.7 ± 1.6	10.2±0.4	10.1±3.7	Pleissner et al, 2016; Oliveira et al., 2018; Pujol et al., 2013

1.3.1.5. Biomass current management and policy

Landfill Directive 1999/31/EC obliges member states to reduce the amount of landfilled biodegradable municipal waste to 45% of 1995 levels by 2021, without prescribing specific waste treatment technology.

The response of EU member states since 1990s is the implementation of mechanical biological treatment, anaerobic digestion and composting processes. For the next 20 years, it will be mandatory to improve the management of bio-waste by supporting technical solutions aimed to added value products generation. The current EU28 waste treatment situation (Eurostat, 2020) is:

- for wastewater and sewage sludge, the main management systems consist in recycling, 30-40% incineration with energy recovery and landfill
- for municipal solid waste, the main management systems consist in 30-65% recycling, incineration with energy recovery in the northern Europe and landfill in Eastern Europe
- for agriculture, forestry and fishing category, the main management systems consist in recycling, 17-60% incineration with energy recovery more in the northern than eastern Europe and landfill in Eastern Europe
- for IFB 2G-biomass data are not available.

A fundamental problem, for the full use of waste biomass in the perspective of the Circular Economy, is the correct application of the concept of End-of-Waste Criteria (EoWC). EoWC is the whole and controlled process that allows the waste to play a useful role as a product, increasing the circularity factor.

The excessive bureaucratic process and the uncertainties of the definitions of waste and by-products of new products represented the greatest obstacles to the development and application of 2G-biomass in the 2G biorefinery. A deep change in the authorization process is therefore indispensable, which guarantees operators of the sector a few clear and effective rules.

The main European targets are landfilling no more than 10% by 2035; preparation for the reuse and recycling of urban waste at least 50% for paper, metals, plastics and glass by 2020. The targets for the re-use and recycling of urban waste to be achieved are 55% by 2025, 60% in 2030, 65% in 2035 (ISTAT, 2020)

1.3.2 Process

Biorefinery process is the second fundamental unit of biorefinery system. Biorefinery process allows the conversion of the biomass into spectrum of high added value products: platform chemical and energy. Biorefinery processes were classified in three main categories: thermo-chemical, chemical and biological processes. The aim of this section is the critical analysis of waste process from environmental engineering perspective combined with integrated waste management system.

1.3.2.1. Thermal process

Thermal waste process is defined as a process of converting solid waste into gaseous, liquid and solid products with the simultaneous release of heat and energy. Thermal processes are classified according to air demand. Thermo-valorisation process occurs under stoichiometric oxygen condition, while gasification and pyrolysis processes occur respectively under sub- stoichiometric conditions and in the absence of oxygen. Gasification and pyrolysis are indirect thermal conversion processes, because of waste were converted into an intermediate liquid or gaseous product

In waste management, **thermo-valorisation** is employed as waste disposal process, which produces gaseous effluent, ash and powders as end products. In the last decades, the heat developed during the waste- incineration is generally recovered and used to produce steam, electricity and heat. Waste incineration without energy recovery causes net emission of 181 kg CO_2 equivalents per ton of MSW; the adoption of an electricity recovery technology produces a positive balance of 10 kg CO_2 equivalents per ton of waste and the adoption of electricity and heat recovery provide a benefit of 348 kg CO_2 equivalents per ton of waste (Adam et al, 2001). These results agree with Kyoto Protocol (1998), boosting a CO_2 emission reduction from 7 to 9 million t of CO_2. and the EU 28 target (by 2025) of 27% of renewable energy from waste thermo-valorisation. Thermo-valorisation process is carried out in four main phases: 1) feed: high heating value feedstock was required to support the thermos-reaction, thus lignocellulosic biomass was the most suitable feedstock Maity, (2015), 2) feedstock combustion was carried out at atmosphere pressure at temperature ranges between 800-1450°C. The reaction is exothermic and mixture of CO_2, water vapour, O_2 and N_2, slag, and heat are released. 3) Electricity and heat generation and 4) slag extraction and vapours treatment. Thermo-valorisation of waste has two main objectives: 1) reduction of the amount of waste to dispose around 10 % in volume and 30 % in mass and 2) sanitation of waste Binieck et al. (2005). The sub-products of thermal processes consist of slag and ash which can be used for road foundations and/ or building materials. The waste-to-energy plants are widely implemented at industrial scale especially in Northern Europe and Japan. In Norther Europe, 71% of MSWis incinerated and 1770 MW of electricity are generated Makarichi et al. (2018).

Pyrolysis is an endothermic dissociation of organic substances carried out in absence of oxygen or other oxidants, eventually supported by catalysts at temperatures between 400-900 °C. Through pyrolysis, the chemical bonds of organic molecules such as sugars, celluloses, fats and proteins are broken, and the volatile substances are distilled with sequential recombination in the form of organic molecules simpler than the starting ones. From pyrolysis, three main products are obtained: 1) gaseous fraction the syngas, which contains hydrogen, methane, carbon monoxide, carbon dioxide and other gases such as hydrocarbons

and alcohols, 2) liquid or tar, a fuel oil containing acetic acid, acetone, methanol and oxygenated complex hydrocarbons; and 3) solid fraction or char with almost pure carbon and inert materials originally present in the waste. Temperature and dimension of the waste plays a key role in pyrolysis.

For temperatures lower than 500°C waste with size smaller than 1 cm can be treated successfully, for higher temperatures 500 °C pyrolysis is fast, and the limiting factors are heat transfer and product diffusion.

The passage from 480 °C to 925 °C determined an increase of percentage production of gas from 12.33% to 24.36%. Therefore, increasing the working temperature, the energy of the waste was mainly converted into the gaseous product and there was an increase in the hydrogen content with a simultaneous decrease of carbon dioxide percentage, respectively from 5.56% -32.48 and 44.77% to 18.31%.

In general, 1 kg of waste through pyrolysis produces 0.125-0.185 m^3 of syngas with a heating low value of 3000 kcal/m^3. The liquid fraction, named tar, represents about 50-60% of the dry waste feed and it decreased with the increase of the working temperature. The quantity and quality of the solid matter, named char, respectively, decreased and increased with the increase of the working process temperature. Char produced 480 °C and 925 °C are similar respectively to bituminous coal and anthracite matter. The calorific value of char decreases increasing the working process temperature reaching a maximum heating low value of 6700 kcal / kg Cao et al. (2014)

Depending on temperature and residence time, pyrolysis is divided into 4 types: 1) slow pyrolysis with a temperature lower than 500 ° C, long residence times and char production equal to 30% of food waste, 2) conventional pyrolysis: temperature lower 600 ° C, moderate residence times, syngas, tar and char are produced in equal percentage based on volume of waste feed, 3) fast pyrolysis with temperature in the range of 500 °C -600 °C, contact times 1-6 seconds, there is no formation of intermediary products and tar production around 70-80% of the waste fed and 4) flash pyrolysis with temperature higher than 700° C contact times lower than 1s tar production up to 80% of the waste fed. The reactors used for pyrolysis are like the incineration reactors, they can be horizontal, vertical, rotating and fluidized Maity, (2015 I). The quality of the pyrolysis process is assessed by two parameters: conversion efficiency (mass flow rate of produced gas per mass flow rate of feed waste) and carbon conversion efficiency (carbon flow rate converted into gaseous products per carbon flow rate of provided fuel). These parameters evaluated the amount of product obtained and the waste to be disposed of Sirini et al. (2015)

Gasification is a high temperature process consisting of the pre-oxidation of a solid or liquid fuel to generate combustible rich in hydrogen, carbon monoxide (CO) and methane (CH_4).

The gasification consists of three main steps: 1) exothermic oxidation reaction, 2) endothermic pyrolysis reaction and 3) gasification reaction in the carbon was converted into gas.

Gasification could be carried out in three main ways Sirini et al. (2015):

1) Partial oxidation with air: a gas diluted with atmospheric nitrogen (up to 60%) was produced. Air gasification eliminates the costs of oxygen planting which

have an impact on both the initial capital and the maintenance costs. The gas produced has heating low value: 5.5-7.5 MJ / Nm^3.

2) Partial oxidation with oxygen: the gas produced is free of nitrogen with heating low value between 8.0-14.0 MJ / Nm^3. The quality of syngas increased but the operating costs were expensive and sustainable only for big plants.

3) Gasification with steam: the process is endothermic, and the gas has heating low value (hlv)between14.0 -20 MJ/Nm^3.

The gasification process consisted of two combined and sequential reactions: from 0 to 160 ° C the phase is physical and after 160°C is chemical. The chemical phase consists of two steps 1) chemical degradation of the solid fuel with formation of gas, char and tar. 2) gasification and combustion of char.

The yields and percentages of gas, tar, char and H_2 in the syngas depended on chemical composition and properties of the 2g-biomass, type of reactor, type of gasification medium and use of catalysts Sirini et al. (2015):The main gasification control parameters are equivalent ratio, steam/fuel ratio and efficiency of carbon conversion. Gasification reactors are selected based on the gas-solid contact and type are divided into fluid and fixed bed gasifier.

Gasification solid residue, in the form of ash, is around 160-3330 kg/t gasifier waste. The main treatments for ashes consist of 1) physical-chemical separation by dimensional separation, magnetic separation, chemical extraction, chemical precipitation and adsorption, 2) solidification or stabilization by solidification/ stabilization with hydraulic binders, chemical stabilization and aging and 3) heat treatment by sintering, fusion and vitrification Maity, (2015 II)

Liquefaction is also known as hydrothermal processing (HTC), hydrothermal pyrolysis, depolymerisation and direct liquefaction.

The liquefaction is carried out at temperatures between 300b° C-400b°C and pressure of 5-20 MPa. The liquefaction process is initially built with the aim of transforming coal into liquid fuel, but recently this technology is implementing to convert lignocellulose biomasses such as waste from forests and agrowaste into bio-oil, by means of a series of reactions for a total time of 0.2-1 h (Mortensen et al., 2011).The bio-oil was upgraded to liquid hydrocarbon fuels by means of three main technologies: 1) by catalytic hydro-deoxygenation with high hydrogen pressure 75-300 bar at 796 ° C-1446 °C Demirbas et al.(2011); Taarming et al. (2011) 2) by hydrocarbons/aromatics employing zeolite upgrading technique with atmospheric pressure at 846 ° C- 1146 ° C Jacobson et al. (2013), Graca et al.(2013;) and 3) by steam reforming Ayalur Chattanathan et al. (2012). Liquefaction process requires higher pressure, longer time reaction, more expensive investment cost and lower bio-oil rate than flash pyrolysis. On the other side, liquefaction can be performed on biomass with high levels of moisture contents, which means no initial dried pre-treatment and therefore money saving Maity, (2015 I). The pyrolysis oil quality is lower than liquefaction bio-oil's one, since the amount of carbon and high heating values were respectively: 54-58% and 16-19 MJ/kg and 72.58% and 36.05 MJ/kg Xiu et al. (2011). Recent studies Maity et al.(2015); Xiu et al (2011) show that liquefaction of animal waste, OFMSW and sewage sludge produce bio-oil with higher yields (5% increase) and higher heating value compared to lignocellulosic

biomasses and agricultural waste and more over the process released less amount of waste and pollutants. Currently liquefaction is not implemented at industrial scale, since deeper knowledge of the reaction kinetics was required to scale up the process Xiu et al (2011).

1.3.2.2. Chemical process

Transesterification is a chemical process able to produce biodiesel and glycerol from oil-fat biomass. The transesterification is carried out at temperatures between 50-80 °C and in the presence of a catalyst, which boosts the conversion of triglycerides into 90% w/w biodiesel and 10% w/w glycerol Sirini et al. (2015). Methanol is the main reactant employed for transesterification, since it is cheap and has ideal chemical and physical properties. Sodium hydroxide is the main catalyst employed because it is the cheapest and the most reactive Maity, (2015 I). To reduce process costs, oil and lipids biomass was pre-treated to remove fatty acids from triglycerides and reagents must be anhydrous Maity, (2015 II). Non-edible oil crops, animal fats and cooking waste oils, which are free fatty acids rich substrate, must be pre-treated before to undergone to transesterification to produce biodiesel. Transesterification main advantage is the limited process time, but conversely the main drawback is the necessity of anhydrous reactants and free fatty acid biomasses Kubička et al. (2018). Currently the process of transesterification for biodiesel production is implemented at industrial scale.

Hydrolysis is a chemical process through which the chemical bonds of the molecules are split into two or more parts due to the addition of water to degrade the macro-molecules in their elementary constituents. Hydrolysis converts carbons contained in sugar, starch and lignocellulose biomasses into pentose and hexose sugars (C5 and C6) to provide products with high added value. There are four types of hydrolysis: three types are chemical, while the last one is biological, which is described in Chapter 3. The acid hydrolysis can be performed with addition of sulphuric acid diluted (1.5% H_2SO_4 and temperatures between 200° -240°C) or concentrated (30-70% H_2SO_4 at 40°C) Maity, (2015). For lignocellulosic biomass, the acid hydrolysis was carried out in two phases: 1) hydrolysis of hemicellulose at temperature lower than 200°C and 2) hydrolysis of cellulose at temperature higher than 220°C Westensee et al., (2018). The acid hydrolysis has fast kinetic reaction, but it caused corrosion on the instruments and generates inhibitory by-products. Furthermore, from economic point of view, the recovery of the acid solution and the removal of degradation products were expensive Maity, (2015). Acid hydrolysis reaches the following results: the hemicelluloses was realised around 80–100% in form of acetic acid and other acids and lignin was removed with formation of inhibitory compounds Brodeur et al (2011). Alkaline hydrolysis was carried out with sodium hydroxide (NaOH) or potassium hydroxide (KOH) at room temperature, atmospheric pressure and under anaerobic conditions. The main drawback of alkaline hydrolysis was the possible production of by-products able to inhibit the subsequent fermentation process David et al. (2016).Thermal hydrolysis is generally performed at two temperature ranges known as low and high

temperatures 50-90°C respectively, and 160° -180°C with a pressure of 6-8 bar for reaction time between 15min to 9h. Thermal hydrolysis was often carried out before anaerobic digestion to improve solubilisation of the biomass solid phase and consequently increased biogas production. The choice of thermal hydrolysis as pre-treatment, however, strongly depended on the type of 2G-biomass Carrere et al., (2016). Lignocellulosic biomass pre-treated with thermal hydrolysis, reaches the following targets: 40-60% of biomass is hydrolysed with 4–22% of cellulose, 100% of hemicellulose and 35–60% of lignin with low formation of inhibitors Sukumaran et al. (2010). All three types of hydrolysis are implemented at industrial scale.

1.3.2.3. Biological process

Enzymatic hydrolysis is widely employed to pre-treat lignocellulosic compounds. Enzymes are essential macromolecular catalysts, produced by living organisms, able to interact with organic substrates. Enzymes accelerate chemical reactions by providing an alternative reaction pathway with lower energy demand. All enzymes are proteins, composed by a long, specific string of amino acids, highly selective and able to work only with a specific substance; for this reason, there are different kinds of enzymes to treat carbohydrates, proteins and cellulose. Reactions with enzymes need specific setting of physical parameters, such as temperature and pH. Increasing the temperature, high degradation rates are observed.

Perfect temperatures of reactions for most of enzymes are around 37° C Sirini et al. (2015). Usually, enzymes work in a specific range of pH; however, too high or too low pH values can be harmful for these molecules. Finally, the rate of the catalysed reaction depends also on the enzyme concentration; generally, if their concentration increases, degradation performances augment, until steady state conditions are reached. The concentration of enzyme represents a economic costs and for this reason concentration of enzyme is a process parameter which needs to be optimised. It is important to evaluate the costs-benefit ratio to consider the perfect amount of enzymes needed in the process and the reached conversion yield. Enzymes could be classified in three big categories, depending on their function and on the employed substrate, they can degrade: glucoamylase, protease and cellulase are analysed. Glucoamylase is used for saccharification of starchy material to glucose to produce a feedstock, enhancing biological fermentation processes and eventually producing a valuable material as ethanol or lactic acid. Pavezzi et al., (2008). Different microorganisms produce glucoamylase; for industrial production mainly, fungi were exploited. Aspergillus *awamori*, *Aspergillus niger* and *Rhizopus oryzae* were considered the most important (Pavezzi et al., (2008). Protease enzymes were able to break peptide bonds and release free amino nitrogen Neitzel, (2010). Cellulase could work with cellulose by breaking β-1,4-glucosidic bonds and by releasing glucose. It was mainly produced by fungi and bacteria; in particular, the most used fungi for cellulase production were *Trichoderma ressei* and *Trichoderma viride*8. Due to the hydrolysing lignocellulosic material difficulties,

the degradation of cellulose and hemicellulose often required pre-treatments of the substrate Yang et al. (2011). Commercial cellulase formulations are Sun and Cheng (2002): 1) Endoglucanase which was responsible for realinig free chain-ends by adding a molecule of water in regions of low crystallinity and so by cleaving cellulose in its internal regions; 2) Exoglucanase or cellobiohydrolase, which could release cellobiose found in the free chain-ends and 3) β-glucosidase that finally converted cellobiose in glucose by cleaving the intramolecular bonds.

To reach higher conversion degrees from hemicellulose and cellulose into glucose, it would be preferable to pre-treat the material with physical, physical-chemical, chemical or biological processes Carrere et al. (2016).

Fermentation is a biological process aimed at biomass conversion into product, based on chemical or biotechnological path, involving microorganisms and enzymes, belongs respectively to Green Chemistry and White Biotechnology domains. Fermentation required specific ratio of carbon nutrient, pH around 6-7, specific temperature ranges, available engineered and selected micro-organisms, and depth know-how of metabolic and fermentative paths. The main drawbacks of biological processes were: 1) general low yield and productivity, 2) expensive downstream separation and upstream process and 3) large amount of by-products generation. The downstream efficiency separation depends on fermentation optimisation in terms of low amount of generated by-products, high product generation and low concentration of feed nutrients Carrere et al. (2016). The quality of upstream process depends on nutrients for effective micro-organism growth, such as specific carbon source, organic nitrogen, phosphorous and minerals. Fermentative process is carried out in three different ranges of temperatures according to the micro-organism employed: psychrophilic, mesophilic and thermophilic ranges. pH influenced the performance of the whole process and in general, most micro-organisms worked at pH around 6.5-8.0, under anaerobic conditions and atmospheric pressure. Fermentation requires low investment and operational costs, but expensive 2G-biomass pre-treatment and product purification steps respectively called up and down-stream. Costs of the downstream process ares around 50-60 % of the whole process cost Demichelis et al., (2018); Venus et al. (2018). In Chapter1, four fermentative processes are analysed: simultaneous saccharification and fermentation and separate hydrolysis and fermentation for platform chemical production and anaerobic digestion and dark fermentation for energies production. Fermentation is performed on carbohydrate, protein, lipid and pre-treated lignocellulosic biomass with micro-organisms belonging to bacteria, yeasts and fungi group Loaces et al., (2017); Pleissner, (2016); Dahnum (2015). Fermentation for chemical compound production could be carried out by Simultaneous Saccharification and Fermentation (SSF) and Separate Hydrolysis and Fermentation (SHF). The main differences are in process time, reactor and operational condition. SHF is carried out in two distinct phases: 1) (chemical and enzymatic) hydrolysis converted the biomass into simple sugars by breaking the bonds of these macromolecules, and subsequently fermentation (bacterial or with yeasts) can transform sugars into a wide range of products. SSF was carried out in a single phase and in a single reactor and the temperature and pH conditions are the

same for saccharification (hydrolysis) and fermentation, which mean low time process and only one operation of set up. To compare the SSF and SHF the following observation can be stated Loaces et al. (2017):

- SHF allows the completely hydrolysation of the feed biomass increasing the realise of sugars for the sequential fermentation. Excessive release of sugar could inhibit the process
- SSF since is a one-step reaction, it is time shorter and capital cost investment cheaper than separate hydrolysis and fermentation. There is not risk of inhibitory product formation.

Both SSF and SHF were implemented at the industrial scale.

Anaerobic digestion (AD) is a consolidate technology implemented at industrial scale. AD produces two main products: biogas, a valuable energy key source and digestate. employed as soil amendment and addition of mineral fertilisers. In EU28, AD is a common practice to manage the organic wastes from urban and industrial production, because of the requirements of European Union member states to reduce the organic wastes in landfill (European Environmental Agency, 2009). AD is carried out for two main reasons: 1) reduction of waste amount combined with organic matter stabilisation by energy production, 2) AD of substrate with 80% w/w of water content achieved COD reduction between 60-65% (Hagman et al., 2018). As proven in Demichelis et al. (2018); Hagman et al (2018) AD is a process able to make sustainable an integrated biorefinery system both from economic and environmental perspectives, since it represents an energy provider and waste reduction process. AD is a four sequential steps process, in which the previous one influences the rates of degradation of the single steps: solubilisation, acidogenesis, acetogenesis and methanogenesis Van Lier et al. (2008).

During solubilisation molecules are first solubilised from the solid substrate into the liquid phase. Then hydrolysis is the most limiting step Angelidaki, (2009) aimed at conversion of polymeric complex substances (i.e. polysaccharides, proteins, lipids) into monomers (i.e. sugars and amino acids). Acidogenesis converts simple monomers into volatile fatty acids (VFA). Then Acetogenesis converts VFA into acetic acid, carbon dioxide and hydrogen. Finally, methanogenesis is the last step of the process, in which acetates were converted into methane and carbon dioxide, while hydrogen was consumed. Different group of bacteria take part in AD processes according to their specific phase. The most common hydrolytic bacteria were *Bacteriocides, Clostridia, and Bifidobacteria* (Weiland, 2010). Obligate hydrogen-producing acetogenic bacteria work on high volatile fatty acids converting them into acetate and hydrogen. Unluckily hydrogen-producing acetogenic bacteria are not well characterised. Typical acetogenic bacteria are *Acetobacterium woodii and Clostridium aceticum* Weiland, (2010). Two different groups of methanogenic bacteria produced methane from acetate or hydrogen and carbon dioxide, at the end of the degradation process. These types of bacteria belong to anaerobic group and they require a lower redox potential for growth than most other anaerobic bacteria Weiland, (2010). Species able to convert acetate into methane and carbon dioxide were not so common. The most known bacteria of this group were Methanosarcina barkeri, Metanonococcus mazei and

Methanotrix soehngenii Van Lier et al. (2008). Macro and micro-nutrients are required for the growth and survival of microorganisms. The main macronutrients were: carbon, phosphorus, and sulphur and their ratio was set as $C{:}N{:}P{:}S$ = 600:15:5:1 Weiland, (2010), Angelidaky (2009). AD is carried out with different ratio of substrate to inoculum from 1/3 to 2/1 with the aim to increase the biogas production (more inoculum than substrate) and increase the amount of waste substrate treated (more s substrate than inoculum) Demichelis et al. (2018); Parra-Orobio et al. (2018). Another important parameter is the solid content. The optimum is between 4% - 12% of organic total solids (OTS). Less than 4% OTS, the energy content of the digested substrate is too low. Over 12% OTS, the digested substrates couldn't be longer pumped, because it has limited flow properties Deublein, (2008). AD can be performed in batch, feed batch and continuous batch mode, for one organic substrate or a combination of substrates (co-digestion). Temperature ranges influenced the kinetics, higher temperature means higher kinetic speed and consequentially a reduction of the volume reactor. AD can be carried out in one and two stages. In two stage AD systems, the physical separation enables optimal conditions for the acidogenic and the methanogenic bacterial biomass, optimising specific metabolic activities and maximising methane generation Schievano et al., (2014) plus hydrogen formation. Among biofuel, hydrogen is the fuel with the highest energy content, 142 MJ/kg and sequential H_2 and CH_4 production was maximised with two stage AD. Furthermore, the sequential H_2 and CH_4 production enhances the content of CH_4 in the biogas around 15-20% Voelklein et al. (2016).H_2 recovery through dark fermentation of organic substrates is not yet considered neither technically reliable nor commercially attractive.

Currently, **dark fermentation** is implemented at technical scale. Considering the increased energy recovery and the enrichment of CH_4 yields of biogas, two stage AD could greatly contribute to the affirmation of fermentative H_2 production as a viable process De Gioannis et al. (2017). In Table1-3 all the main features of the above described process are reported and summarised.

Table 1-3:Biorefinery process conditions, generable products, technical-economic and environmental pros and cons and technical readiness level (TRL)

	Process	Process conditions	Products	Pros	Cons	TRL
Thermo-chemical	Gasification	T= 500°-900°C, high pressure	Syngas Bio-oil	Reduction of waste volume	High cost for syngas upgrading	6: Technical-industrial scale
	Pyrolysis	T= 450°-500°C, dry feedstock	Syngas, Bio-oil Bio-char	High yield =80% w/w of dry biomass low capital cost	Bio-oil quality lower than bio-oil quality by liquefaction	7: Industrial scale
	Liquefaction	T=300° -400 ° C p=5-20 MPa	Thermal and electric energy	High waste volume reduction: 70-90% w/w dry	Big plant size	7: Industrial scale
	Thermo-valorisation	T= 300°-400°C, p= 5-20 MPa, t= 0.2-1h	Bio-oil	High quality bio-oil	High capital cost Lower bio-oil rate d than pyrolysis	7: Industrial scale
Chemical	Trans-esterification	T= 50°-80°C, NaOH= catalyzator	Biodiesel	Fast process	Anhydrous reagents	7: Industrial scale
	Acid hydrolysis	T=40-240°C H_2SO_4 = 1.5-70%	Sugars (C_5 - C_6)	High yield of sugars release	High process cost corrosion	7: Industrial scale
	Alkali hydrolysis	T=40-240°C NaOH = 1.5-70%				7: Industrial scale
	Thermal hydrolysis	T= 30-300°C			High energy requirement	7: Industrial scale
Biological	Enzymatic hydrolysis	T=35-55°C, pH=4-7 t=30min-1d	Sugars (C_5 - C_6)	High realise of sugar	High costs to maintain pH and possible inhibory formation	7: Industrial scale
	Fermentation with SSF, SHF	T=35-55°C, pH = 6.5-9, anaerobic condition t=1d-6d	Variety of platform chemicals acccording selective micro-organism or fungi	Low investment cost	Expensive downstream cost	7: Industrial scale
	Anaerobic digestion	T=5-65°C, pH= 6.5-8	Biogas o bio-methane	Energy saving, organic waste stabilisation	T and pH conditions have to be controlled Inhibitory agents release	7: Industrial scale
	Dark fermentation	T=10-55°C, pH=4-5	Bio-hydrogen	Energy saving	T and pH conditions have to be controlled Inhibitory agents release	6: Technical-industrial scale

1.3.2.4 Biorefinery design

Biorefinery design is a hot and open topic. Due to feedstocks, hierarchy of products and process technologies, referring to social, economic, environmental and legislation aspects, several design configurations could be estimated. Biorefinery process has to be designed according to Key Enabling Technologies (KET) and Best Available Techniques (BAT), to guarantee and promote process technical feasibility and maximal feedstock conversion minimizing the waste and by product generation (IEA, Task42). Biorefinery design consisted of three main phases: 1) definition of the goal, 2) definition of the conceptual and detailed process diagram and 3) implementation of the diagram considering the available technologies and evaluating process cost and profitability.

Biorefinery design drivers are Kamee et al. (2017):

1) Enhancement of feedstock conversion into high-added value products,

2) Minimization of waste generation in biorefinery system

3) Reduction of economic cost and environmental efforts.

Biorefinery design, based on hierarchical, sequential and integrate approaches Moncada et al. (2016), aims to overcome the complexity and it realizes a real-life-integrated biorefinery system. Hierarchical deconstruction of biorefinery considered the relation between biomasses and products. Therefore, the process structure is modeled on feedstocks composition and final product application requiring a specific grade of purity to be suitable for market demand. Hierarchical approach identifies the main process bottleneck and it limites their negative effect interlinking biorefinery system elements each other (Moncada et al. (2014), The evaluation of the products considered six different categories: 1) biofuels (related to liquid fuels), 2) bioenergy, 3) biochemical, 4) biomaterials, 5) foods and 6) bio fertilizer Moncada et al. (2013). The range of products obtainable through a biorefinery process is much wider than the one produced through the petrochemical-refinery. However, the goal of the hierarchical approach is not to increase the range of products, but to fully exploit the secondary raw material (waste biomass) to increase productivity and minimize waste. For example, in thermo-chemical biorefinery, hierarchical system underlines that the limiting step is the pretreatment in the case of waste of lignocellulosic matrix rather than the process conversion Luque et al. (2014). Sequential approach defines logical link between process technology and products. Feedstock conversion path and technologies are designed according to the desire products quality. Biorefinery chain adopts sequential approach, since it promotes first the assembly of products with the highest purity restriction and then the others one. Based on sequential principles the well-established biorefinery systems are: sequential production of anti-oxidize, pectin and ethanol from orange waste through chemical process Moncada et al. (2012) and sequential lactic acid production and biogas from organic fraction municipal solid waste (OFMSW) by biological conversion Demichelis et al., (2018), Pleissner et al. (2017), Kim et al (2016). Integration approach consistes in integration of different biorefinery categories based on sequential process design

and hierarchical decomposition of feedstocks, product and technologies. Integration principles drove towards the following design solutions:

1) Integration of feedstocks: different feedstocks can be exploited in the same process to reduce raw material input, biomass season variability, transport cost and waste generation.

2) Integration of products: sequential manufacture of market-able products from the same feedstock maximizing revenues and minimize waste generation among the biorefinery system Sy et al. (2018).

3) Technologies integration to ensure maximal yield conversion of feedstocks into products. Integration of technologies consists of integration of two or more tools or processes in the same plant exchanging waste and products with other biorefineries and symbiotic relation with existing industries Budzianowski et al. (2016); Azapargic, (2014).

1.3.2.5 Correlation biomass-process

Since all the three process categories, thermo-chemical, chemical and biological, produce both platform-chemicals and biofuel-energy, the choice of the type of process depends on the feeding 2G-biomass. According to 2G-biomass category defined in Table1-2 corn and wheat waste and spent ground coffee, made up of hemicelluloses, cellulose and lignin, are resistant feedstock more suitable for chemical and thermal biorefineries than biological ones. Feedstocks from agricultural and organic wastes have water content around 60-80 %w/w, thus biological biorefinery are more suitable than thermal one. Food and beverage industrial wastes have high organic content (COD = 140 g/L), which is not readily available as witnessed by total COD/soluble COD ratio ranging between 5-15% Demichelis et al, (2018); Maragkaki et al. (2018). Hence, industrial waste need to be pre-treated and then employed as feedstocks in all the three biorefinery categories. To sum up the following statements can be assessed for the correlation biomass-process (Table1-4):

- carbohydrate biomasses are mainly converted through chemical and biological processes
- lipid and oil biomasses are converted by means of chemical and thermo-chemical processes.
- lignocellulosic biomasses are the most versatile and valorised in all three types of process. Lignocellulosic biomass can’t be employed in the transesterification.

Table 1-4: Correlation biomass-process

	Process	Biomass		
		Carbohydrate	**Lipids/oil**	**Lignocellulosic**
Thermo-chemical	Gasification			X
	Pyrolysis		X	X
	Liquefaction (HTC)			X
	Thermo-valorisation			X
Chemical	Trans-esterification		X	
	Acid hydrolysis	X		X
	Alkali hydrolysis	X		X
	Thermal hydrolysis	X		X
Biological	Enzymatic hydrolysis	X		X
	Fermentation SSF, SHF	X		X
	Anaerobic digestion	X		X
	Dark fermentation	X		X

1.3.3 Products

Biorefinery system could produce one product, platform chemical or energy, or multi-products as sequential production of chemical and energy. Sequential production of chemical compound and energy is the most adopted biorefinery system design, because of legal policy, technical feasibility and environmental, economic and social sustainability. From legal policy, the production of chemical compound before energy agrees with Waste Framework Directive 2008/98/EC, recommending a hierarchical waste management: waste prevention, re-use, re-cycle, energy recovery and disposal. Platform chemical production represents material re-use and re-cycle, while energy production satisfied energy recovery.
From technical feasibility perspective, the sequential production of chemical compound and energy had twice folds:1) production of double high added value products and 2) maximal valorisation of the feed biomass with reduction of waste production. Chemical compounds as lactic acid, succinic acid and others are mainly produced from sugar-rich substrates, while energy can be produced both from sugars and fats, therefore energy production before platform chemical production would make impossible the production of platform chemicals.
From environmental perspective, the sequential production of platform chemical and energy guaranties the maximal valorisation of the feed biomass, which means reduction of waste disposal and zero or at least minimisation and neutralisation of CO_2 emissions. From economic perspectives, sequential production of chemical compound and energy has three main advantages: 1) double incomes and furthermore the market values of platform chemicals were higher than energy's ones, 2) waste disposal cost reduction and 3) the produced energy can make self-energy sufficient the biorefinery process From social perspective, the sequential production of platform chemical and energy provides up to 90000 new job position in 2030 and contributes to the bio-economy Satchatippavarn et al. (2016). Benefits of sequential production of platform chemical compound and energy are discussed

and critical analysed according to increase of catchment area size in Demichelis et al. (2018). The growing energy demand, the depletion of fossil fuels and the sequential increase of the price of crude oil were the main reasons for the exploration of renewable resources for the sustainable production of electricity, heat, fuels, organic chemicals and polymers. In the following section chemical compounds and energies were described and analysed according to chemical and physical properties, technical process feasibility, biomass yield conversion, market application and value.

1.3.3.1 Platform chemicals

In this section, platform chemical generable from 2G-biorefiney are described.

Ethanol (C2) is a linear alkyl chain alcohol in the form of a colourless, volatile and extremely flammable liquid, completely soluble in water and other organic solvents such as acetone, chloroform, diethyl ether Koutinas et al. (2014).Ethanol is used in several sectors, such as: 1) food and beverage sector, since it is naturally produced by sugars fermentation and it is added in a variable percentage in alcoholic beverages as beers, wines, liqueurs and distillates 2) resins, for the preparation of paints and also in many household cleaning products as a disinfectant, antifreeze and defrosters, 3) cosmetic sector for perfume and deodorant production and 4) in medical sector for medicine, wipe, disinfecting gel and antiseptic production Koutinas et al. (2014).Ethanol is both a final product and a starting compound to produce ethylene and ethylene glycol, which are used to produce bio-polymers such as polyethylene (PE) and polyethylene terephthalate (PET). European ethanol production is about 5.67 million L, which represent the 5% of the world ethanol production. Thammasittirong et al. (2017). In 2015, the world production of ethanol is $97.2 \cdot 10^9$ L and several studies indicated that the global market is strongly growing, due to the increase of ethanol demand as biofuel. The United States was the world's largest producer of ethanol, and together with Brazil, produce about 85% of the world's ethanol Pardo-Planas et al. (2017). Ethanol market value is around 0.68-0.81 €/kg Koutinas et al. (2014) for ethanol as chemical compound and around 0.42-0.53 €/L Mag Doris (2016) for ethanol as energy. Ethanol could be produced by three main pathways: 1) thermal processes as pyrolysis and gasification, 2) fermentation of carbon-rich organic matter and 3) thermal fermentation. Theoretical yield of ethanol produced from glucose is 0.511 g/g Song et al. (2018). Pre-treating the biomass with acid washing, the anhydrous-sugars concentration in pyrolysis oil increases up to the 75% w/w suitable for the sequential conversion into ethanol with a yield ranges between 0.45-0.50 g/g glucose in pyrolitic oil Luque et al. (2014). In gasification 0.78 t syngas/t of dry biomass can be further converted into 0.44 m^3 ethanol/t syngas Taylor-de-Lima et al. (2018). In thermo-chemical processes, 60% of total ethanol production cost is operational cost, in which energy requirement represented 80% of total operational item costs Taylor-de-Lima et al. (2018). Ethanol fermentation, carried out by micro-organisms on starchy, sugar, sugar-beet, corn and lignocellulosic materials, is a

consolidate process and implemented at full scale. In ethanol fermentation, the most adopted micro-organism ethanol producers are, *Z mobilis* and *S. Cerviase. Z. mobilis* was better than *S. Cerviase* in ethanol yield conversion, respectively around 0.48-0.46 g/g of glucose Koutinas et al. (2014). Fermentation process, performed at 30-35C at pH around neutrality, can be divided into 3 steps: 1) glycolysis phase, in which the glucose was transformed into pyruvate, 2) pyruvate reduction to acetaldehyde (pyruvate-decarbolsylase phase) and 3) acetaldehyde conversion into ethanol by means of micro-organisms. After fermentation, the fermentative broth is undergone to downstream process consisting in adsorption, distillation and dehydration Onuki et al. (2008). New optimised ethanol process consists in ethanol production from combined gasification and syngas fermentation of sugar-cane and lignocellulosic biomasses. *Clostridium carboxidivorans* Phillips et al. (2017), *Alkalibaculum bacchi* Liu et al.(2012) and *Clostridium ragsdalei* Devarapalli et al. (2017), are the best micro-organism to produce organic acids especially ethanol via syngas fermentation. This process, reaching 0.31-041 m^3ethanol/biomass, is a three step process made up of: 1) gasification, 2) fermentation of syngas with cell/water recycling and fermentative syngas condensation and 3) distillation and drying process to obtain ethanol with 85-97% optical purity as required by the market Roy et al. (2015) estimate ethanol production costs from gasification and syngas fermentation from 906 €/m^3 to 1046 €/m^3.

Research on ethanol production is focused on the development of new processes and micro-organisms to increase the conversion of sugars with C-5 and C-6 carbon atoms from lignocellulosic biomass. For this type of biomass, pre-treatments are necessary to realise the lignin and hemicellulose component which had a recalcitrant / non-edible structure and thus facilitate the hydrolysis process. The pre-treatments can be physical (fragmentation, grinding, milling), physico-chemical (auto-hydrolysis, steam explosion) or chemical (acid or alkaline hydrolysis) (Koutinas et al., 2014; De Madoires et al, 2017).

Lactic acid (C3) (LA) known as 2-hydroxypropanoic acid, belongs to Alpha Hydroxy Acids (AHAs) and it is a white-yellow liquid at room temperature, water soluble and widely distributed in nature. LA conjugate base is called lactate ($CH3CH(OH)CO-$) and it took part to several biochemical processes. LA is chiral molecule with two optical isomers: L-lactic acid and D-lactic acid and a mixture of these two isomers in equal parts is called DL- lactic acid. Among the two isomers, L-lactic acid has the highest market and technical value. LA is used in different sectors such as: in food and beverage, pharmaceutical, cosmetic and chemical: it is used to produce biopolymers as polylactic acids – PLA, which are biodegradable polyesters. In 2013, 800 000 t of LA are produced and currently the largest consumer in the world is the USA, which uses 31% of the total Koutina et al., (2014). The market value of LA depends on its optical purity, in detail 1.18€/kg for purity grade between 50-88% and 1.35 €/kg for purity grade over 90% (Eurostat, 2018) LA is produced by chemical and biological routes. Chemical synthesis consists in the hydrolysis of lactonitrile, which is the output of the reaction of acetaldehyde with hydrogen cyanide. In general, chemical path required a lot of chemicals such as base-catalyzed for sugar degradation and oxidative reaction as:

oxidation of propylene glycol, hydrolysis of chloropropionic acid, nitric acid reaction of acetaldehyde, carbon monoxide and expensive working parameters as: water at high temperatures and pressures Gao et al. (2011). LA chemical synthesis is not technically and economically feasible Gao et al. (2011). Furthermore, the costs of LA chemical synthesis dependents on no-renewable feedstocks and by-products are produced. The chemical LA production yield is 0.84 g/g of glucose Gao et al. (2011). Fermentative LA production could be performed by separate hydrolysis and fermentation (SHF) and simultaneous hydrolysis and fermentation (SFF) Pleissner et al. (2016,) Pliessner et al. (2017). Fermentative LA production is carried out in batch and feed batch configuration, but. LA concentration in feed batch mode, is lower than in batch configuration (Bonk et al. (2017), The most employed L-LA producers are: *L. helveticus* Wang et al.(2015) employing as substrate whey, *L. Casei* Buyukkileci and Harsa, (2004) employing as substrate molass and *L.delbrueckii* Surendran et al (2005) employing as substrate camel and cow milk. In fermentative LA production, the key factors are presence of available carbon source for glucose release, proper amount of nitrogen for micro-orgnaism growth, pH around neutrality for fermentative step and temperature ranging from psychrophilic to thermophilic range according to the employed micro-organisms Pleissner et al. (2017). The cost of nutrients is one of the main weakness points for the competitive biotechnological production of lactic acid. The lactic acid can be produced using sugary biomass of different origins such as starch (cassava, potatoes, food waste), lignocellulose (pineapple wood, spent ground coffee), residues and secondary products from agro-industrial activities (waste from processing of cotton, corn, wheat, etc.) Pleissner, (2017), Demichelis et al. (2017). LA yield is reached respectively by means of SHF and SSF are around: 0.34-0.60 g/g of xylo-oligosaccharides waste from corncob Zhang et al.,(2015), 0.65-089 g/g of wheat corn Li et al. (2017) and 0.29-0.33 g/g of mixed food waste Pleissner, (2017), Demichelis et al. (2017). From economic perspective, the highest item costs for fermentative LA production are downstream processing, carried out separately from fermentative step and waste disposal. Downstream process, aimed to achieve the optical purity required by the market (80% for food grade and 90 % for pharmaceutical), includes filtration, dialysis, chromatography and distillation (Venus et al., 2018). Downstream processes may represent up to 41% of the costs of a conventional fermentation process Wang et al. (2016), around 1.44–1.74 €/kg lactic acid Joglekar et al. (2006). A large amount of waste occurring during the fermentation and downstream processing, which could make around 85% of the total solids of the feedstock Pleissner et al. (2017). According to (Demichelis et al., 2018) the costs of downstream and waste management disposal decrease with increasing the catchment area size and moreover the waste from LA recovery can be valorised by anaerobic digestion to make energy self-sufficient lactic acid production.

Propionic acid (C3) is a monocarboxylic acid and its consumption accounted for almost 80% in preservation of animal feed, grain, and food (calcium and sodium propionates). Around 51% of world propionic acid consumption is addressed to animal feed and grain preservation, while about 29% is employed in the production

of calcium and sodium propionates, for food and feed industry application (ICIS, 2018; ICIS, 2018). Other significant markets are herbicides and diethyl ketone. Other applications include cellulose acetate propionate, pharmaceuticals, solvent esters, flavours and fragrances, plasticizers, dyes, and textile, leather, and rubber auxiliaries. Propionic acid is produced both by chemical and fermentative processes. In 2016, propionic acid production was 349000 t in the world. Chemical propionic acid production is an oxidative process that begins by reacting ethylene and carbon monoxide. The obtained intermediate product is propionaldehyde, which is further oxidized to obtain propionic acid Koutinas et al. (2014). Chemical propionic acid production can also be performed as a secondary product during the production of acetic acid, but due to acetic process conditions the quantity produced is limited. The expected growth of the propionic acid market requires a more sustainable production based on non-renewable resources. Fermentative propionic acid is carried out with propionic bacteria under anaerobic conditions. Propionic bacteria are facultative anaerobic, gram-positive and rod-shaped bacteria which are able to exploit dicarboxylic acid pathway to produce propionic acid as main product and acetate and succinate as by-products from sugars, glycerol and lactate as the carbon source Wang et al. (2013). Strong inhibition of propionic acid occurs during the fermentative production, which limited product titre, yield, and productivity and consequently the implementation at the industrial scale of the fermentative propionic acid production. Inhibition of propionic acid can be solved from process perspectives 1) using high density culture via cell recycle Wang et al. (2015a.) 2) immobilization Dishisha et al., (2015) 3) product recovery to ease product inhibition Wang et al. (2012), while from bacteria perspective developing metabolic and engineered bacteria to increase propionic acid production Guan et al., (2015), Guan et al. (2016); Wang et al.(2015b).The most adopted propionic acid bacteria are *Escherichia coli Propianicbacterium freudenreichi, Propianicbacterium acidipropionici*, with a propionic acid yield ranging between 0.36-0.53 g/g of substrate. Among these propionic acid bacteria, the best propionic acid producer is *P. acidipropionici*, which can produce propionic acid yield of 0.5 g/g substrate consumed and productivity of 0.32–2.1 g/L h, depending on the process conditions Wang et al. (2013). Fermentative propionic acid production is carried out both in batch and fed batch reactors, both via cell recycle and immobilization Dishisha et al., (2015); Wang et al., (2015a). with pH buffer between 5.5.-6.5 respectively with pure sugars and residues as carbon source and carbon-nitron ratio equal to 2/1. In detail, fermentations with pure sugars requires pH around 5.5 and yeast extract (10 g/L) and Trypticase (5 g/L) as nitrogen source, while fermentations with soy molasses residues and hydrolysate requires pH around 6.5, 30–50 g/L glucose, 10 g/L yeast extract and corn step liqueur powder (30 g/L) as the nitrogen source (Yang et al.,(2018). The carbon source is generally glucose, molasses coming from sugar cane or glycerol and the yield was around. Glycerol was less expensive carbon source than glucose and glycerol productivity was 2-4 times higher than that obtained using glucose. The main drawback of using glycerol as carbon source is the release of acid able to drop the pH and inhibits the growth of propionic bacteria, reducing the productivity and efficiency of the propionic acid fermentative

production. Recently, other carbon sources are under investigation, soy molasse residues, made of 28% carbohydrate in forms of starch and sucrose,21% lipid and 2% protein. One of the main cons of soy molasses is the difficult digestibility for bacteria of raffinose-family oligosaccharides and sucrose generated in the production of soy protein concentrate Yang et al.(2018). Propionic acid fermentative production has been tested on different substrate, ad hock substrate as glucose feedstock Liu et al., (2016); Wang et al., (2015a) and secondary raw materials as waste glycerol Dishisha et al., (2015); Zhang et al., (2015), cane molasses Feng et al. (2011), cheese whey Yang et al. (1995), corn meal Huang et al. (2002), wheat flour Kagliwal et al. (2013), sugarcane bagasse Zhu et al.(2012), corncob molasses Liu et al. (2012a), and corn stover Wang et al. (2016). Although good fermentation performance in terms of propionic acid titre (up to 106 g/L), productivity (> 2.0 g/L h), and yield (~0.5 g/g) are reported. The substrate employed as feedstock plays a key role, because of ad hoc glucose is an expensive fermentation substrate, the other ones are cheaper than glucose, but they require expensive pre-treatments and enzymatic hydrolysis. However, further improvement in product yield and costs reduction are required to make fermentative production competitive with petrochemical processes Tufvesson et al. (2013). The raw materials mainly sugar and nitrogen sources used in fermentation, accounted for a large portion (more than 30%) of the product cost for the biobased propionic acid Tufvesson et al. (2013). Efforts have thus focused on using low-cost carbon and nitrogen sources to replace the more expensive sugar (glucose) and yeast extract. General fermentative propionic acid production consists in residual feedstock pre-treatment, fermentation, cell separation or immobilization, evaporation with optional water recycling and drying process Ahmadi et al. (2017). According to the study of Yang et al. (2018) the production costs are respectively: 2.81 €/kg for pure sugar, 2.19 €/kg for corn, and 2.07 €/kg for soy molasses. These values are characterised by a different percentage breakdown of the raw and operational costs, in details the transition from sugar to secondary raw material as corn and soy molasses lead to decrease of feedstock costs from 46.89% to 17.60 %, while operational costs increase from 30% to 60 % of the total costs. Propionic acid selling price is 2.60 €/kg Yang et al. (2018).

1,3-Propanediol (C3) is a three-carbon diol employed in several chemical reactions. In 2013, the global demand for 1,3-propanediol is 140500 t. Due to the increasing use of polyesters in various industrial applications, 1,3-propanediol production will increase significantly between 2018 and 2022, with an annual rate of 10.8%. The market value is around 34.2-45.5 €/kg (Eurostat, 2018)

1,3-propanediol is employed as: 1) solvent and as antifreeze due to its low melting temperature, it is added to many industrial products to improve particular properties 2) reagent in many chemical reactions for the production of polyesters, polyethers and polyurethanes and 3) building block molecule for the synthesis of polymethylphenethetraftalate, a new polymer with characteristics similar to those of nylon and used in carpets and textile fibres. 1,3-propanediol is produced both by chemical and biological routes. The chemical synthesis is carried out with two processes: Degussa process in which propylene is oxidised to acrolein and

subsequently hydrolysed under high pressure to form 1,3-propanediol and Shell process in which ethylene is oxidised and then hydroformylation reaction at high pressure occurred to form1,3-propanediol. The chemical 1,3-propanediol conversion yield with Degussa and Shell processes are 65% and 80%, respectively Koutinas et al. (2014). Biological 1,3-propanediol production consisted in a fermentation carried out by wild-type of micro-organisms exploiting glycerol as exclusive carbon source. The micro-organisms belong d to Clostridium genus and the best producer in terms of product concentration, productivity and yield are: *Klebsiella pneumonia*, *Citrobacter freundii*. Some lactic acid bacteria could convert glycerol into 1,3-propanediol only in presence of other carbon source as fructose, maltose, glucose etc. 1,3-propanediol yield conversion from glycerol ranged between 0.39-0.56 g/g Metsoviti et al. (2012); Wilkens et al. (2012). The bioconversion is more environmentally friendly and more attractive in terms of yield than chemical process.

Biological 1,3-propanediol fermentation is carried out in batch and feed batch configuration, with single step, two-step and multi-stage fermentation, sole and co-substrate fermentation, co-culture, and microbial consortium Zhu et al. (2016). Fermentations of glycerol or glucose as single substrate has been reviewed by many researchers and improved as co-fermentation of glycerol with cheap secondary raw carbon source substrates such as glucose Xiu (2007): Su et al., (2010) xylose (Jin et al., (2011), sucrose Yang et al., (2007) hemicellulosic hydrolysates Xin et al., (2016) and cassava Apiwatanapiwat et al. (2016). The co-fermentation increases the production of 1,3-propanediol. Recently, a two-step fermentation has been investigated and improved recombining two microorganisms of *S. cerevisiae strain HC42* and *Clostridium acetobutylicum DG1* can convert glucose and molasses into 1,3-PD (Suma et al., 2018; Mendes et al., 2011). The1,3-propanediol fermentative production was strongly influenced by1) fermentation mode, 2) purity grade of the feed glycerol, 3) employed sugar substrates, 4) shape of the reactor and 6) initial substrate conditions (Mayti, 2015 I). Since pure or crude glycerol was the mandatory carbon source for 1,3-propanediol fermentative production, the cost of the substrate accounts at least 50% of the entire production cost Zeng et al.(2011). For this reason, some studies are testing crude glycerol from biodiesel production and hydrolysates of lignocelluloses as alternative cheaper substrate.

2,3-Butanediol (C4) is an organic transparent and odourless liquid at room temperature. 2,3-butanediol has three stereo-isomers: two enantiomers optically active: levo D (-) and dextro L (+) and the third is a meso-form optically inactive. The L (+) isomer has a low melting point (-60 ° C) which can be used as an antifreeze, while both enantiomers are used in the pharmaceutical, agro-chemical and food sectors. The 2,3-butanediol can also be used as a starting compound to produce synthetic rubber, polyester and polyurethane. In 2012, global production of 2,3-butanediol was around 61800 t and recent market studies suggested a market growth with an annual rate of 3.2% between 2015 and 2020. The market value varies between 0.2-0.26 €/kg (Eurostat, 2018). 2,3-butanediol production is performed both by petrochemical and fermentative routes. Fermentative 2,3-butanediol is a mixed acid fermentation pathway occurring during anaerobic or

micro-aerobic growth of different wild-type microorganisms belonging to micro-orgnaism group as, *Klebsiella pneumoniae, Enterobacter aerogenes, K. oxytoca and to the species Paenibacillus polymyxa, Serratia marcescens and Bacillus amyloliquefaciens*. Sheldon (2016), Ocha-Gomez et al. (2015). Fermentative 2,3-butanediol production is already optimised employing different carbon-source biomass as, pure glucose, and waste as fruit and vegetable, starchy substances, molasses, whey, glycerol, wood residues and corn. Lignocellulosic Li et al. (2014), Li et al. (2015) fruit and vegetable Liakou et al. (2018) biomasses are considered as a cheaper carbon source and used for 2,3-butanediol production. The yield of 2,3-butanediol biological production range is 0.31-0.53 g/g of waste sugar rich biomass Petrov et al. (2010). 2,3 butanediol fermentative production is carried out in batch and feed batch configuration with pH around 4.5-6.5 and 35° C. BDO production is influenced by the following 5 factors: 1) dissolved oxygen, since the synthesis of 2,3 butanediol was enhanced under low dissolved oxygen tensions), pH slightly acidic conditions and incubation temperature Koutina et al.(2014). 2,3 butanediol fermentative production required a recovery and purification steps, since the purity required at industrial scale ranged between 97.99.9% Li et al. (2012). Purification step, consisting mainly in solvent extraction followed by vaccum distillation, can reach 98.7 % of optical purity Dai et al. (2015). The purity of 2,3 butanediol increases by enhancing the fermentative condition as in study: the purity of the (*R,R*)-stereoisomer by *P. polymyxa* reaches only around 98% by the end of the culture, with 2% corresponding to the *meso*-2,3-BD form, reducing of 20-40% the cost of downstream process in the total production cost Yanjun et al. (2016).

Succinic acid (C4), also known as butanedioic acid, is a saturated di-carboxylic acid with a linear structure and odourless white crystals form at room temperature. Succinic acid played a significant role in biological metabolism and it is mainly used in three sectors: food: as a flavouring agent in foods and beverages; pharmaceutical sector for the production of vitamin A and in the production of various medicines and in industrial sector as a building block for the production of different chemical compounds such as butanediol and biopolymers such as PBS and PBST. Succinic acid annual global production varies between 30000-50000 t Koutinas et al. (2014) and recent market forecasts indicated that by 2020 production will grow significantly with an annual rate of 32.9% and will reach 700,000 t (Market Research, 2018; Marketsand Markets2012). Succinic acid market value ranges between 1.96-2.45 €/kg according with the increase of purity degree Efe et al.(2013). Succinic acid production is carried out both by chemical and biological routes. Most of the succinic acid derives from petrochemical processes through the reduction of maleic anhydride and a more limited quantity derives from fermentation which employs as carbon source biomasses such as: sugars, starch, lignocellulose and wheat. Recent studies predicts that in the coming years the production of succinic acid by means of biomass will prevail on chemical path due to the high production costs and the price variability of oil. Fermentative succinic acid production is implemented at industrial scale by companies as: Bioamber (Bioamber, USA 2018), Reverdia (Reverdia Netherlands, 2010) and Myriant (Myriant Canadsa, 2012). Micro-organisms, able to convert carbon biomass into

succinic acid are isolated from bovine rumen Koutinas et al., (2014) The most studied and high producer succinic acid bacteria are: *Anaerobiospirillum succiniciproducens, Actinobacillus succinogenes, Mannheimia succiniciproducens and Escherichia coli.* Leung et al., (2012) These bacteria metabolise C5-C6 sugars from lingocellulosic material, agricultural residue and organic waste. In biological succinic acid production phosphoenolpyruvate and dissolved CO_2 playesa key role. Phosphoenolpyruvate, the main branch metabolite, drives carbon towards C4 instead of C3 pathways to guaranty the production of succinic acid, while the quantity and quality of succinic acid depend on the availability of dissolved CO_2 and electron donors in the cultural media and broth Wang et al., (2012)

Succinic acid fermentative production ranges between 0.57 to 1.16 g/g respectively of wheat and waste bread Leung et al. (2012) while 1.13-1.16 g/g of pure glucose Wang et al. (2012). Succinic acid fermentation is carried out in batch and feed batch reactor with pH=6 and T=37°C, followed by downstream process and succinic acid recovery. Succinic acid recovery and purification from fermentation broth is multi-step and expensive process depending on the micro-organism, feedstock, employed for production, nutrients supplied, acids and bases added to correct pH, solubility of the intermediates and final product titre. Recovery step consists in removal of cells and insoluble solids by means of filtration and centrifugation. purified by the cation exchange chromatography Schröder et al., (2015); Krawczyk et al. (2016) or evaporative crystallization step in which 95 wt %of succinic acid was recovered as a solid, with the remaining succinic acid in the starting broth recycled to the evaporation step Dunuwila et al. (2012); Fruchey et al. (2012) The fermentative succinic acid production with the available technology employed by Bioamber, Reverdia and Myriant is cheaper than petrochemical production, even in the worst-case scenario, the fermentative production cost of biological succinic acid is only 41% of the production cost of petrochemical-based succinic acid :1.36 €/kg vs. 3.32 €/kg Nghiem et al. (2017)

Butyric acid (C4) was a four-carbon aliphatic organic acid addressing different sectors: cosmetic, polymer, chemical, food and pharmaceutical manufacturing, textile fibres, photographic film and eyeglasses frames manufacturing. Butyric acid market is around $8 \cdot 10^5$ t/y with a selling price 0.4-0.51 €/kg Wang et al. (2016)

Butyric acid could be produced from petrochemical resources and biomasses. Petrochemical production of butyric acid is based on butyraldehyde chemical synthesis Maity (2015 II). Butyric acid from biomass can be produced by means of fermentative process carried out by engineered micro-organism such as *Clostridium, Butyribacterium, Butyrvibrio,, fusobacterium* Jha et al. (2014); Zhang et al. (2009b); Zigová and Šturdík, (2000). Butyric acid is used as precursor for bio-butanol production in liquid biofuel in acetone-butanol-ethanol (ABE) fermentation by solventogenic clostridia Luo et al. (2015); Luo et al. (2017); Richter et al. (2012); Tashiro et al. (2004). At industrial scale, butyric acid is produced through chemical synthesis of crude-oil, since fermentative butyric acid production is economically more expensive than chemical synthesis route, due to the low productivity, and high production cost Luo et al. (2018). The main bottleneck of the fermentative production of butyric acid is the cost of fermentative substrate, fermentative

equipment and the operation cost concerning downstream process for the separation and purification steps Luo et al. (2018). Substrate fermentative cost can be reduced employing waste biomasses at the place of ad hoc biomass, but waste biomass fermentation required higher downstream cost than ad hoc biomass. On the other side, fermentative butyric acid production cost can be balanced with enhancement of butyric acid purity, yield and productivity Varrone et al. (2017). Butyric acid fermentative process is carried out both in batch, feed batch and continuous configurations under pH control range: 5.5.-7.0 Huang et al. (2016b,) Varrone et al. (2017). Feed-batch configuration reached usually higher butyric acid yield and productivity than batch feeding Varrone et al. (2017). Butyric acid can be produced both from carbohydrate (food processing waste, starch, etc) and lignocellulosic (agro-waste, corn- fibers, etc) biomasses Luo et al. (2018). Both the types of biomasses are pre-treated before fermentation. Carbohydrates are hydrolysed to convert long chain-sugars into mono-saccharides: glucose and xylose, while lignocellulosic biomasses are physical-chemical pre-treated to disrupt the close components. Inhibitory compounds can occur in pre-treatments of lignocellulosic biomass, such as lignocellulosic acid hydrolysis releases 5-hydroxymethylfurfural, formic, acetic, ferulic and p-coumaric acids Baral and Shah, (2014) able to inhibit micro-organisms as *C. Acetobutylicum* Baral and Shah, (2014). Other waste biomasses for fermentative butyric acid production were syngas, by-products of food processing industrial waste, starchy biomass, lignocellulosic biomass and biodiesel industry with whey and glycerol. High-efficient fermentation strategies consisted in multi-culture metabolic engineering microbial Zhou et al. (2014) and cell immobilisation fermentative process Abdel-Rahman et al. (2013)

Malic acid (C4) is a dicarboxilic acid with asymmetric carbon, having two isomeric structures: D(-) and L(+) malic acid and mixture of DL malic acid, which have different application sectors, since it is a precursor of many industrially chemicals in the food, chemicals and pharmaceutical sectors. DL-Malic acid accounted in food sector for 52% as acidulant and taste enhancer in candy, beverages (liquid and powder and mainly in fruit-flavoured beverages) and 38% as confection and food, while 10 % in non-food sector as cosmetic, pharmaceutical, metal cleaning and plastic production (Chemical Economics Handbook, 2018). Malic acid production worldwide is around 40000–60000 t/y of with a 4 % annually growth rate with a market price around 2.03 €/kg (Deng et al., 2016). Malic acid production is carried out both via chemical synthesis and fermentation. Malic acid via chemical synthesis can be carried out with two paths: 1) hydration of maleic or fumaric acid to obtain DL racemic mix or 2) addition of immobilised or isolated fumarase enzymes to obtain malic acid L (+) from fumaric acid. Chemical synthesis is based on carbon fossil raw material and both chemical paths, hydration and enzymatic process have drawbacks. In details, hydration process required high temperature and pressure conditions, which make the process economically unsustainable for high operational cost and environmental unfriendly for GHG emissions. Fumarase enzymatic process exhibits a strong sensitivity to temperature and inhibitory substrate effect, which reduce the large-scale production of L (+) malic acid. Whereas, fermentative path can synthesize pure L-malic acid from

renewable feedstocks as lignocellulose, such as soybean hull and corn and soy molasses, reducing petroleum-based feedstock dependency and depletion (Dai et al., 2018). Since 1990s, several micro-organisms have been engineered and selected for malic acid production, such as *Aspergillus spp*., *Zygosaccharomyces rouxii Penicillium spp*. and *Ustilago trichophora* Schroder et al. (2015).

L-malic acid can be either directly converted from pyruvic acid by one-step conversion, or several steps through reductive or oxidative pathways with micro-organism Dai et al. (2018). The most adopted malic acid pathways production, using oxaloacetic acid and/or acetyl-CoA as precursors are reductive tricarboxylic acid, tricarboxylic acid cycle and glyoxylate cycle pathways. In reductive tricarboxylic acid pathway, pyruvic acid is immediately carboxylsed to oxaloacetic acid, followed by the reduction of oxaloacetic acid to malic acid in the cytosol. Theoretically,1 mol CO_2 is fixed with formation of 1 mol of malic acid, reaching a maximal theoretical yield of 2 mol/mol glucose. Anyway, in the real case, reductive tricarboxylic acid pathway is carried out in the cytosol and requires two steps of enzymatic conversion from pyruvic acid performed by pyruvate carboxylase. Malic acid production through tricarboxylic acid cycle pathway consists in catalyse of oxaloacetic acid and acetyl-CoA to citric acid, followed by multi-steps oxidative reactions in the mitochondria. With tricarboxylic acid cycle pathway, the conversion of glucose to malic acid releases 2 moles of CO_2, which will reduce the maximum theoretical malic acid yield to 1 mol/mol glucose. The third metabolic pathway for malic acid production is glyoxylate pathway reaching a maximum malic acid yield equal to 1 mol/mol glucose because of the carbon loss taking place in the oxidative decarboxylation reaction.

Fermentative malic acid production from different sugar forms are tested in Cheng et al. (2017) achieving the following results: 0.51 ± 0.05 g MA/g fructose, 0.61±0.02 g MA/g glucose, 0.56 ± 0.01 g MA/g galactose and 0.57± 0.20 g MA/g xylose. The fermentative production of malic acid from sugars demonstrates that glucose is the best fermentable sugar with addition of carbonate salt when the nitrogen concentration is limiting in the medium. The carbonate salt plays double key role: source of CO_2 and neutralizing agent Dai et al. (2018). Fermentative malic acid production, carried out with the above cited micro-organisms, requires the following set up conditions: 10% v/v of reactor volume filled with glucose (carbon source) 60-80 mg/L carbonate salts with a working time process ranged from 60 to 198 h in a temperature range between 25-55° C Dai et al. (2018). To improve malic acid yield, engineered micro-organism are studied and the most promising were: 1) *Aspergillus spp A. Oryzae* (eukaryote) able to employ simultaneously glucose and xylose derived from lignocellulosic feedstocks Begum and Alimon, (2011); Duarte and Costaferreira, (1994); Prathumpai et al. (2003), achieving 0.68-1.1 g MA/g glucose Liu et al. (2017); Knuf et al. (2014), 2) *S. Cerevisiae* (eukaryote) had a robust tolerance to high substrate concentration, less sensitive to metal ions, able to provide better process control, preventing mould formation, capability of utilizing a wider range of carbon sources and safety in food industry and 3) E. Coli (prokaryote) with high stress condition tolerance suitable for different sugar forms and achieving 0.35-0.58 g MA/g glucose Dong et al. (2017); Gao et al. (2017)

From economic perspective, the microbial fermentative of malic acid production, achieving low yield because of the by-product formations, like fumaric acid, citric acid and the loss of CO_2, is not yet optimised for a real implementation at industrial scale. Fermentative malic acid production from lab to pilot scale was carried out through three fundamental steps: pre-treatment of substrate, such as enzymatic hydrolysis, fermentation and final downstream to separate malic acid liquid from microbial broth performed with sequential concentration, separation, hydrolysis and drying. Currently more than 50% of total cost of fermentative malic acid production are due to separation and purification Dai et al. (2018).

Fumaric acid (C4) is a dicarboxylic acid with a chemical structure containing a double bond and it was one of the top 12 building-block chemicals. Fumaric acid is a maleic acid isomer, but compared to maleic acid, fumaric acid is not toxic. Fumaric acid is mainly used as a stabiliser and acidity regulator in food production, in the pharmaceutical sector and chemical industry to produce resins, plastics and other products.

In 2012 global fumaric acid production is 225200 t and it is expected that by 2020 the market for fumaric acid will grow due to demand increase in the food sector, due to the use of pre-cocked food and ready-made beverages. Fumaric acid market value is about 64.5 €/kg (Eurostat, 2018). Fumaric acid is produced both by chemical and biological processes. The greater amount of fumaric acid is currently produced by chemical synthesis exploiting fossil fuel and petro-chemistry path via the catalytic isomerization of maleic acid. The fumaric acid biological production was a fermentation carried out by *Rhizopus arrhizus* and *Rhizopus oryzae* fungi from sugar biomass rich of glucose, sucrose, molasses. Koutinas et al. (2013). Fumaric acid fermentation was performed at pH= 5, 35°C and dissolved oxygen around 50-60% for both the growth of biofilm for the micro-organisms and production phases Naude et al. (2018). The most adopted reactor configurations are batch, feed-batch and batch for the growth of biofilm combined with feed-batch for fumaric acid production Naude et al. (2018). The filamentous fungus *Rhizopus oryzae (ATCC 20344*) is recognised as the best microbial fumaric acid producer Roa Engel et al. (2008); Xu et al. (2012); Jang et al. (2012) exploiting several substrates such as glucose, xylose and plant hydrolysates Xu et al. (2010). Fermentative fumaric acid production, optimised for glucose and xylose substrates in batch condition, is 46.78 g/L using 80 g/L glucose by *Rhizopus arrhizus RH 7-13-9#,* the optimum glucose/xylose ratio and carbon/nitrogen ratio in co-fermentation is estimated respectively 75/25 (w/w) and 800/1 (w/w) Huan et al., (2011). In combined batch and feed-batch, nitrogen concentration is a key parameter for fermentative fumaric acid production by *R. oryzae*, since the increase of the feed nitrogen, also in form of urea, from 0.625 to 1.875 mg/L h, lead an increase of 25% of fumaric acid reaching 0.81-096 g/g of glucose.Liu et al., (2017); Fu et al. (2010b) The costs of the carbon source for fumaric acid production represent the 50% of the total cost, thus alternative cheaper carbon sources as starch and lignocellulose is investigated Xu et al. (2012). The bio-conversion of starch material into fumaric acid is more effective with the sequential enzymatic hydrolysis and fermentation of the starch by micro-organism *Niger* reaching

fumaric acid yield around 0.75 g/g Zhang et al. (2007). Currently, lignocellulose is enzymatically digested to obtain a glucose-rich liquid used for fumaric acid production. reaching a yield of 0.35 g/g Xu et al. (2012). At the end of fermentative process, downstream including in situ recovery and precipitation, is carried out to obtain fumaric acid with the purity required by the market. Downstream process represents 40-50% of the total cost for fumaric acid production Xu et al. (2012).

Itaconic acid (C5) is a unsatured decarboxylic acid essential as building block in the chemical industry to replace petrochemical-based monomers as acrylic and methacrylic acid for production of polyesters Robert et al. (2016); Magalhães et al., (2016). Itaconic acid addresses wide range of business sector applications like agricultural, pharmaceutical and medical fields. Worldwide, more than 80 000 t/y of IA is produced and sold at a price of 2.02€/kg Okabe et al. (2009). At the industrial scale, itaconic acid is produced through fermentation of glucose and sucrose by means of *Aspergillus terreus,* a filamentous fungus. Generally, Itaconic acid is produce from pure and ad hoc glucose and sucrose, since *Aspergillus terreus* was very sensitive to substrate impurities, in fact a manganese concentration over 3 ppb negatively influences the structure of *Aspergillus terreus* and the itaconic acid yield decreases Karaffa et al. (2015). However, waste biomasses as feedstocks for itaconic acidic production are under evaluation, specifically starch-based hydrolysates Dwiarti et al., (2015) and lignocellulose-based feedstocks. Inhibitory compounds like weak acids, phenolic components and furan derivates can occur during pre-treatments for high temperatures or low pH-conditions Palmqvist et al. (2000). For itaconic acid production Tippkotter et al. (2014) and *Aspergillus terreus* tolerance Pedroso et al. (2017), these inhibitory components are removed with purification and detoxification Li et al. (2016). Itaconic acid can be produced both through separate enzymatic hydrolysis and fermentation and (SHF) or a simultaneous saccharification and fermentation (SSF) Lind et al. (2002). *Aspergillus terreus* first grew with glucose media and typical fluffy pellets were formed. Afterwards, all monosaccharides are consumed to produce itaconic acid, except rhamnose after 7-9 days. After hydrolysis, the hydrolysed substrate is purified and detoxed via activated carbon or ion-exchanger.to remove inhibitory components McCartney et al., (2006). Itaconic yields by means of SHF from wheat hydrolysate, after removal of inhibitory components was around 0.27 g/g – 0.53 g/g Tippkotter et al, (2014); McCartney et al., (2006). Higher itaconic acid yields are achieved after higher efficiency detoxification and purification steps. Itaconic acid biochemical production from sugar industry is technical feasible, but the high production costs limited this technology. The current strategy to make viable biological itaconic acid production is the exploitation of cheaper substrates as waste sugars and lignocellulosic feedstocks together with reaching higher product yield. Furthermore, another adopted strategy was the sequential production of itaconic acid and electricity from sugarcane bagasse and trash lignocellulosic feedstocks through integrated biorefinery system Krull et al., (2018).Three biorefinery scenarios for combined production itaconic acid and electricity are designed and simulated with Aspen Plus®.Nieder-Heitmann et al. (2018) Scenario 1: Itaconic acid and electricity from lignocellulose, Scenario 2: Itaconic acid from glucose and

electricity from lignocellulose and Scenario 3: Itaconic acid from lignocellulose and electricity from coal. The subsequent economic analyses indicates that lignocellulose waste feedstocks reduces the itaconic acid production cost from 1.74 €/kg for glucose to 0.70 €/kg for lignocellulose, but coal addition was necessary to enhance the production cost to 0.72€/kg for a competitive itaconic acid selling value of 2.02 €/kg, in comparison with 2.09 €/kg market price. Anyway, addition of coal is not favourable for an energy self-sufficient biorefinery system, because energy operational cost is higher than IA market price Nieder-Heitmann et al. (2018).

Xylitol (C5) is an organic compound with five carbon atoms and with a white and odourless solid crystalline structure at room temperature. Xylitol is large employed as a sweetener in many foods since it is an ideal sugar for diabetic people because its metabolism is insulin-independent, it is used in the pharmaceutical industry to prevent ear infections and formation of caries in toothpaste and mouthwashes. Thanks to the growing sensitivity of public opinion towards health issues and the maintenance of adequate body weight, the demand for xylitol to produce low-calorie products is rapidly increasing. In 2016, 190900 tonnes of xylitol are produced, and the market is expected to reach 266500 tonnes by 2022, with an annual growth rate of 5.7% Mayti, (2015 II). Xylitol market value is around 4.9-5.7 €/kg (Eurostat, 2018). Xylitol is both produced by chemical and biological routes. The chemical pathway is performed by means of catalytic reduction of pure D-xylose under high temperatures and pressures. The chemical process is expensive, since considerable amounts of energy was used and up and down-stream processes were required to achieve the commercial purity grade. Therefore, biological processes are viable alternatives, since they are economically more sustainable than chemical ones. Fermentation. reaches high quality xylitol without requiring further expensive or specific downstream processes. The xylitol fermentative production employs bacteria, fungi and yeasts exploiting as carbon base substrate hydrolysed xylose and hemicellulose. Xylose is metabolized to xylitol by specific microbial strains in a sequential catalytic activity of xylose reductase and xylitol dehydrogenase enzymes. Among yeast the most engineered and studied is *Candida* strains. Among micro-organisms the most adopted were *C. tropicalis* and *Kluyveromyces marxianus* reaching respectively xylitol at a conversion yield of 0.85 g/g of xylose and 0.49–0.63 g/ hemicellulose Zhang et al., (2013). Xylitol fermentation is carried out in batch and feed-batch configuration generally in mesophilic range, but the enhancement of working temperature up to thermophilic range offers advantages like lower energy input, less chance of contamination and faster sugar conversion Alff-Tuomala et al (2016). The pH managed the transport and maintenance of the protonation state of XR to guaranty its catalytic activity Dasgupta et al. (2016). The biological production of xylitol is limited by the following factors: 1) temperature, 2) PH, 3) agitation, 4) aeration and 5) cellular inhibitors. From the economic perspective the most expensive items are the cost of the starting biomass and the large demand for water. Fermentation xylitol yield can be enhanced by enzyme addition, but it represents additional cost. Comparing chemical and biological routes the main differences are: 1) upstream process: purification and detoxification respectively for chemical and biological

paths, 2) downstream process: crystallisation and centrifugation downstream respectively for chemical and biological. Chemical path requires more expensive upstream and downstream processes than biological one Dasgupta et al. (2017). The current and open challenge for fermentative xylitol production was the validation of bioprocess including feed preparation and product down streaming starting from waste carbon biomass as lignocellulose at industrial scale. Further improvement must be performed in the design of modified genetically microorganism to fit the specific demand of bio-catalysis fermentative process, and in the development of utilization of whole cell biocatalysts under energy benign conditions Kang, et al. (2016). Anyway, fermentative xylitol production from lingo-cellulosic biomass is proven as a cost competitive process and low environmental impact Dasgupta et al. (2017).

1.3.3.2 Bio-energies

In EU 28, over 80% of energy comes from non-fossil resources Maity, (2015 I) for the following uses: 33.1% for transport, 25.4% for household, 25.3% for industry, 13.6% for public services and 2.2 % for agriculture and forestry (Eurostat, 2018). More than a third of the produced energy is consumed in the transport sector and with an average annual increasing rate of 1.4% up to 2040 (IEA in 2016). The production of biofuels can improve the sustainability of the transport sector from an economic, environmental and social perspectives, reducing the dependence on fossil fuels at global scale. Biofuels are all the liquid and gaseous fuels which were produced from biomass. These fuels are used both in the transport sector and to produce electricity and heat. In the following section, biofuels implemented at technical and industrial scale are described and analysed.

One of the fundamental next generation transport fuel is the **ethanol** Xiu et al. (2011) described in the 5.2.1 section.

Bio-oil is a complex mixture of volatile hydrocarbons, alcohols, organic acids, aldehydes, ketones, furans, phenols, and other non-volatile compounds. It is in the form of a brown viscous liquid and it has a pH between 2-4 with corrosive power. Bio-oil cannot be directly used as fuel dues to high viscosity, high water and ash content, low heating power; instability of some of its components and high corrosivity. Bio-oil dues to its immiscibility, does not allow the creation of mixtures as in the case of diesel-ethanol. The market value of bio-oil is 14.70€/L (Eurostat, 2018). Bio-oil is produced by thermo-chemical processes as liquefaction, gasification and pyrolysis from different carbon biomasses as oil-cake, crop, fruit and agro-waste Luque et al., (2014). Bio-oil has three main applications: 1) boiler fuel, 2) fuel in combustion engines and 3) raw material to produce of chemical and bio-products as ethanol.

Currently, one of the main limiting factors for industrial scale implementation of bio-oil application is the poor quality and low production rate of bio-oil. According to the feed biomass and working temperature, the quality and quantity of bio-oil changes. The quality of bio-oil from pyrolysis decreases from 61.8 %to 58.9 % with the increase of the temperature from 480 ° C to 925 ° C David et al.,

(2018). Acid washing of feedstock is required to increase the concentration of anhydrous-sugar into bio-oil, with consequential enhancement of the quality of bio-oil Luque et al (2014). At technical and industrial scale, bio-oil is produced by means of flash pyrolysis and liquefaction. The main differences between flash pyrolysis and liquefaction are: flash pyrolysis required around 1 s gas residence time (short time), atmospheric pressure and temperature between 450-500 °C, but the feed biomass has to be dried, while liquefaction requires lower temperatures 300-400 °C, but longer residence times around 0.2-1.0 hr and relatively high operating pressure (5-20 Mpa), but the biomass could be used without drying pre-treatment. Bio-oil yields respectively with flash pyrolysis and liquefaction are up to 80% on dry feed; and 20-60% on the dry feed. Before application as fuel, bio-oil requires an upgrading process, consisting in: steam reforming, hydrodeoxygenation or zeolite upgrading. Hydrodeoxygenation is considered the best method since it provides bio-oil with characteristics very closed to crude oil. However, it is a very expensive process because it involves the use of high-pressure hydrogen Maity, (2014). Pyrolysis oil production requires lower capital and operational cost than liquefaction. The pyrolysis oil quality is lower than liquefaction bio-oil, since the amount of carbon and heating high heating values are respectively: 54-58 % and 16-19 MJ/kg and 72.58 % and 36.05 MJ/kg Gan et al (2010); Xiu et al. (2010)

Syngas is a mixture of gas essentially consisting of carbon monoxide and hydrogen coming out from gasification and pyrolysis. Syngas is used to produce 1) hydrogen in the chemical industry, 2) biofuels, chemical products through and the Fischer-Tropsch synthesis process (FT-synthesis) and 3) electricity and heat (Maity II, 2014). In 2014, 116600 MWth of syngas are produced and production is expected to grow with an annual rate of 9.5% to reach 213100 MW_{th} in 2020 Marketsandmarkets, (2018). The market value of syngas is around 0.27 - 1.28 € / l (Eurostat, 2018).

In detail, syngas is employed to produce methanol, dimethyl ethere, hydrogen and methane. Syngas can be employed as intermediate compounds to be converted into acetate by means of anaerobic digestion (AD). In detail, mesophilic condition is suitable for syngas conversion to acetate by anaerobic mixed cultures, reaching an acetate yield of 75.8% in batch mode under pH equal to 6.5 and 7.5. It is important to underline that lower or higher pH resulted in the production of butyrate and ethanol Anzola-Rojas et al. (2016); Dudyński et al. (2012)

The presence of tar and methane in the mixture limited the use of syngas to produce biofuels because of the presence of tar blocks gasification process, while methane causes problems during the Fischer-Tropsch synthesis process. However, it has been shown that the presence of these two substances in the syngas can be reduced by using a catalyst during the process, generally dolomite. Syngas can be produced using fossil sources, such as natural gas, coal, natural gas or from fat biomass, which contains triglycerides, or lignocellulosic. The fat biomass is subjected to a steam / dry reforming process, while the lignocellulosic process can be subjected to a flash pyrolysis process or gasifier with air under sub-stoichiometric conditions and at high temperatures (800-900 ° C).

Biogas is a colourless and odourless gas in standard conditions. Biogas is mainly composed by 55-70% of methane (CH_4,) 30-45% v/v of carbon dioxide (CO_2,) 0-0.5% v/v of hydrogen sulfide (H_2S), 0-5% of nitrogen (N_2,) 0-0.5% v/v of ammonia (NH_3) and 1-5% v/v of water Chengyuan et al. (2011). The CO_2 presented in the biogas is neutral about greenhouse effect Morita, (2012). Nowadays, biogas playes a key role in the emerging market for renewable energy production since 25% of UE-28 renewable energy target by 2020 will be met by biogas. Lau et al. (2011). For this reason, the global capacity for power generation from biogas technology will be more than twice over the next decade, increasing from14.5 GW in 2012 to 29.5 GW in 2022 Sun et al. (2015). The market value of biogas and methane were respectively: 0.12 €/m^3 and 0.14-0.24 €/m^3(RNR market research, 2018). Biogas is produced by anaerobic digestion (AD)of organic materials in a sealed reactor with batch, feed batch and continuous feeding mode. Organic bio-degradable materials such as agro-waste, fruits, vegetable, animal manures, sludge from wastewater treatment and etc, were converted into biogas by methane-producing bacteria under pH around 6.5-8, through a biological process that occurs in psychrophilic, mesophilic and thermophilic conditions. Biogas yield depended on the type of feed biomass, temperature range and feed mode. In detail, the biogas yields and methane contents, generated by different substrates, are due to the relative abundance of carbohydrates (about 0.8 Nm^3 biogas/kg_{TS}, with an average methane content of 50%), proteins (about 0.7 Nm^3 biogas/kg_{TS}, with an average methane content of 70%) and lipids (about 1.2 Nm^3 biogas/kg_{TS}, with an average methane content of 68%) Weiland, (2010); Raposo et al. (2008). Biogas production is influenced by different factors, such as organic loading rate, pH, temperature, liquid phase components, ammonia, carbon to nitrogen ratio (optimum 20/1), volatile fatty acid, solid content, substrate composition, hydraulic retention time and sludge retention time. Biogas is directly adopted for: electricity and heat generation in full- scale facilities and in combined heat and power generations (CHPs) as gas. Biogas cannot be directly used as a combustible because of the presence of some impurities and low heating value (lhv) around 18.8 -21.6 MJ/Nm^3. Currently, Italy and Brazil are the countries with the highest number of CH_4-cars more than 2.4 million (Market RFA; 2018). Many cars are also in Germany, USA and Sweden (IEA, task 42). Biogas can be used as bio-fuels after the bio-methane upgrading process, during which the undesirable substances such as water H_2S, CO_2 and NH_3 are removed, increasing the CH_4 percentage up to 70%. Bio-methane upgrading is performed with several technologies and the ones implemented at technical and full scale are: water scrubbing Ali et al. (2013), cryogenic separation Allengue et al. (2012), physical and chemical absorption Deng et al. (2010); Li et al. (2012), membrane technology Weiland. (2010). Among them, cryogenic separation reaches the highest clean efficiency 96.00 % with the highest energy consumption 1275MJ/ton CO_2 the other technologies ranged between 85-94 % of clean efficiency and 0.45 kJ/ton CO_2 – 466 MJ/ton CO_2 Braguglia et al. (2018). Methane upgrading process is expensive both from economic and environmental perspectives. Water scrubbing ranges between 0.47–0.53 €/ kWh Sun et al.(2015), cryogenic separation around 4.80-7.10 €/ kWh, physical adsorption 1.05-1.42 €/

kWh Sun et al.(2015), and membrane technology 6.70 €/ kWh Tajima et al.m, (2004).To enrich bio-methane content in biogas, two stage anaerobic digestion is carried out. In detail, two stage anaerobic digestion allows the production of both hydrogen and biogas with an enhancement of methane content of 35% De Gioannis et al. (2017). Biogas represents a solution to improve the sustainability of biorefinery systems, in term of energy self-sufficient process Hagman et al. (2018)

Bio-hydrogen has a high energy content around 143MJ/kg and its production is free of CO_2 emissions. For these reasons, bio-hydrogen was one of the best alternatives to the use of fossil fuels. Bio-hydrogen market value varies between 0.43-1.51 €/L (Eurostat, 2018). Bio-hydrogen is conventionally produced through processes such steam reforming of natural gas, partial oxidation of hydrocarbons and the gasification of coal using fossil fuels. However, all these processes are economical expensive, with high environmental impact due to the large quantities of greenhouse gases emissions. Currently, bio-hydrogen is produced from non-fossil resources such as carbohydrate, fatty and lignocellulosic biomass through chemical and biological processes. The chemical process is very similar to the one employed to produce hydrogen using fossil fuels. The production of bio-hydrogen from sugar biomass is carried out by chemical process. Bio-hydrogen is obtained by reforming the aqueous phase containing the C6 sugars, obtained through enzymatic hydrolysis of the feed biomass Anzola-Rojas et al. (2016). The production of bio-hydrogen from fat biomass is carried out by chemical process. The triglycerides contained within the vegetable oils and/or animal fats are transformed into syngas (mixture of CO and H_2) through a process of stream reforming followed by the process of water-gas shift in which the gas is transformed into hydrogen. The production of bio-hydrogen by lignocellulosic biomass was performed by biological process consisting in dark fermentation of pre-treated lignocellulose, to make cellulose and hemicellulose available for conversion into H_2. Recent studies have shown that the production of bio-hydrogen through AD is technically feasible process, but fermentative production has not yet been implemented at industrial scale. They inhibited the process and the unsustainable costs. Yield and productivity of AD vary according to carbon content of the starting biomass. The yield is 91.03 ml/g Khan et al. (2016). The storage of hydrogen is complicated from a safety point of view. The problems to be considered are: 1) the low stocking density makes difficult the preservation of hydrogen (it will be necessary to find a material to realize the storage tanks able to meet the volumetric needs and to withstand high pressures) 2) materials in contact with hydrogen become brittle, because hydrogen atoms spread in the metal structure and tend to lead to material rupture and 3) the storage system can lose up to 2-3% w/w of hydrogen per day. This not only contributed to an increase in the cost and frequency of supplies, but also creates safety-related problems when the storage system is in confined environments.

Biodiesel together with bioethanol, is considered one of the most promising biofuels for new generation transport. Biodiesel is a light amber yellow liquid with a viscosity like diesel oil, not flammable and not explosive. Biofuel, in terms of physical and chemical structure and energy content is like conventional diesel

coming from petrochemical resources. Biodiesel as ethanol are large employed, but biodiesel is easier to pump, store and manage than ethanol, since biodiesel employs the same infrastructure, devices and procedure used for conventional diesel because biodiesel does not produce explosive vapours with relatively high flash point close to 150°C. Biodiesel is generally mixed together with diesel from fossil fuels and any diesel engine can use biodiesel with mix percentages of 5% by volume (B5) or less. Low-level biodiesel blends with B2 and B5 are popular fuels in the trucking industry because of biodiesel has excellent lubricating properties, so mixtures can benefit from engine performance. In 2009, the annual production of biodiesel is 15.7 million m^3, and market studies predict that production will triple to reach 45.3 million t in 2020. (ChemPub, 2018) However, biodiesel production cost is high. In biodiesel production, the most expensive items are the cost of biomass, catalyst, methanol addition and labours. The cost also varies depending on the geographical area, the seasonal availability of biomass and the price of crude oil. The market value of biodiesel, produced from palm oil, is around 658-674 €/t (Eurostat, 2018). Biodiesel is mainly produced by transesterification of fats. Transesterification is a chemical process during which triglycerides react with an alcohol and are transformed, in the presence of an alkaline catalyst, into fatty acids and glycerol. Yuan et al. (2007). The catalyst which is used to improve the reaction rate and efficiency is generally sodium or potassium methoxide, while methanol is used as alcohol. During the biodiesel transesterification production; glycerol is produced about 10% by weight of biodiesel Ocfmia et al (2006). The production of glycerol supports and makes more sustainable the economy of biodiesel production

1.3.3.3 Biomass-product and process-products correlations

After the evaluation of the single fundamental units of the biorefinery system, biomass-product and process-product correlations are evaluated. The correlation biomass-product is based on theoretical calculation and it is sorted into biomass-platform chemical and biomass-energy. The biomass- platform chemical correlation is performed considering the maximum theoretical stoichiometric biomass Carbon content of the dry biomass fraction and product, expressed as gC/g dry biomass and gC/g product (Table1-5 and Figure1-8),

Table 1-5 correlates biomasses and products showing the percentages of carbon conversion expressed as g dry biomass/ g product. The average carbon contents in biomass and product are respectively 31.7-52.8 % and 35.82-52.87 w/w. In Table1-6, analysing the percentage conversion yields based on the ratio between the grams of carbon present in the products per gram of carbon of dry biomass, it is possible to conclude that:

- wastewater and sewage sludge have the lowest C-conversion due to the high-water content

- lignocellulosic biomasses as corn and wheat and spent ground coffee reach the highest C-conversion for all the platform chemicals, confirming the strong versatility of lignocellulosic biomasses.
- among the carbohydrate biomasses, the processed candy waste, followed by processed fruit and vegetable waste and fruit and vegetal agrowaste reach the highest C-conversion.
- Among oil-lipids biomasses, dairy waste followed by milking waste reach the highest C-conversion. Dairy and milking waste reach the highest C-conversion for malic acid production.

As proven in Table1-5, the Carbon content of biomass is not completely consumed with the generation of one platform chemical, thus, biomass can be undergone to sequential biorefinery process to maximise the valorisation and reducing the generable amount of waste. The correlation process-product (Table1-6), considers type and cost of the process and market size, application and value of the product. Considering process-product correlation (Table1-9), the following statement can be assessed:

- ethanol is the only product generable by thermo-chemical, chemical and biological process from waste biomasses. The other products have only the thermochemical or biological process based on waste-biomasses.
- products, both the platform chemicals and bio-energies are still produced from petrochemical resources but 2G-Biorefinery processes exhibit potential and viable alternatives
- production costs are not available for all the products, but the general trend witnessed that 2G-biorefinery production cost is higher than bio-product selling price.

All the products have a large and growing market with several applications, thus investment to improve the 2G-biorefinery process is fundamental

Figure 1-8: Gram of Carbon content per gram of products

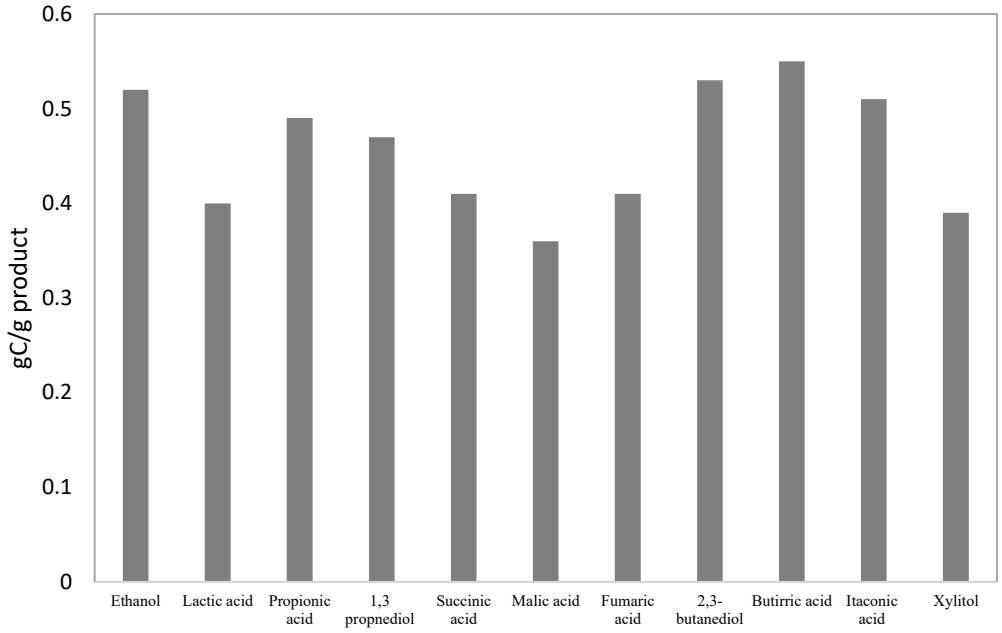


Table 1-5:Carbon content consumed in the biomass to generate the product (g C dry biomass/g C product)

	Wastewater and sewage sludge	OFMSW	Rice waste	Farming waste	Milking waste	Corn and wheat waste	Fruit and vegetable agro-waste	Winery waste	Dairy waste	Slaughter waste	Processed Candies waste	Olives and oil waste	Processed fruit and vegetable waste	Spent ground coffe
Ethanol	1.91	19.26	53.50	17.46	10.41	72.59	16.77	56.08	34.21	86.25	71.89	47.35	53.50	79.72
Lactic acid	2.49	25.12	69.78	22.77	13.58	94.68	21.88	73.15	44.62	82.50	93.76	61.76	69.78	56.98
Propionic acid	2.05	20.65	57.37	18.72	11.16	77.85	17.99	60.14	36.69	92.50	77.09	50.78	57.37	65.50
1,3 propanediol	2.10	21.21	58.93	19.23	11.46	79.95	18.47	61.77	37.68	95.00	79.18	52.16	58.93	67.81
Succinic acid	2.45	24.70	68.62	22.39	13.35	93.10	21.51	71.93	43.88	60.63	92.20	60.74	68.62	102.25
Malic acid	2.78	28.05	77.92	25.43	15.16	85.73	24.43	81.68	49.83	75.63	90.70	68.97	77.92	65.11
Fumaric acid	2.41	24.28	67.45	22.01	13.12	91.52	21.15	70.71	43.14	78.75	90.64	59.71	67.45	76.52
2,3- butanediol	1.87	18.84	52.33	17.08	10.18	71.01	16.41	54.86	33.47	84.38	70.32	46.32	52.33	77.99
Butirric acic	1.83	18.42	51.17	16.70	9.96	69.43	16.04	53.64	32.72	82.50	68.76	45.29	51.17	76.25
Itaconic acid	1.96	19.76	54.89	17.91	10.68	74.48	17.21	57.54	35.10	88.50	73.76	48.59	54.89	81.80
Xylitol	2.52	25.45	70.71	23.08	13.76	95.94	22.17	74.12	45.22	64.00	95.01	62.59	70.71	85.37

Table 1-6:Correlation of product-process considering product market size application and price and process cost

Product		Market size	Market sector	Market value	Process	Process cost
Chemical compound	Ethanol	147 ·10^6 gal	Ethanol chemical compound: food, pharmaceutical and cosmetic. ethanol bioenergy: biofuel	0.68-0.81(€/kg)	1) thermo-chemical: thermos hydrolysis of carbohydrate and lignocellulosic biomass 2) biological: fermentation of carbohydrate biomass	906 €/m3 to 1046 €/m3. from gassification process
	Lactic acid food grade 50-98%	800000 t/y	Food, pharmaceutical and cosmetic	1.18-1.35 (€/kg)	1) chemical 2)biological: fermentation of carbohydrate biomass	1.44–1.74 €/kg downstream
	Propionic acid	3490000 t/y	Agriculture, food, pharmaceutical and cosmetic	2.6 (€/kg)	1) chemical: oxidation of ethylene 2) biological: fermentation of glycerol and carbohydrate biomasses	2.81 €/kg for pure sugar, 2.19 €/kg for corn, and 2.07 €/kg for soy molasses.
	1.3- propandiol (88-99 %)	140500 t/y	Solvent and polymer industrial production	34.2-45.5 (€/kg)	1) chemical: oxidation of ethylene or hydration of acroylina 2)biological: fermentation of glycerol	substrate accounts at least 50% of total production cost
	2,3-butanediol	61800 t/y	Chemical industry	0.2-0.26 (€/kg)	1) chemical: from petro-chemialc source 2) biological: fermentation of carbohydrate biomasses	na
	Succinic acid	700000 t/y	Industrial, pharmaceutical and cosmetic	1.96-2.45 (€/kg)	1) chemical: reduction of maleic anidride 2) biological: fermentation of glucose and glycerol	1.38- 3.32 €/kg
	Malic acid	40-60000 t/y	Food, pharmaceutical and cosmetic	2.03 (€/kg)	1) chemical: hydration of fumaric and/or maleic acid 2) biological: fermentation of carbohydratic biomasses	downstream accounts more than 50% of total production cost
	Butyric acid	800000 t/y	Food, pharmaceutical, cosmetic and chemical industry	0.4-0.51 (€/kg)	1) chemical: oxidation of buatanale 2) Biological: fermentation of carbohydrate and lignocellulosic biomasses	na
	Fumaric acid	90000 t/y	Food, pharmaceutical and chemical industry	64.50 (€/kg)	1) chemical: oxidation of maleic acid 2) Biological: fermentation of carbohydrate biomasses	na

	Acid itaconic	80000 t/y	Chemical industry	2.02 (€/kg)	1) chemical: oxidation of buatanale2) Biological: fermentation of carbohydrate biomasses	1.74 €/kg for glucose to 0.70 €/kg for lignocellulose
	xylitol	190900 t/y	Food and pharmaceutical	4.9-5.7 €/kg	1) chemical: reduction of xylose 2) Biological: fermentation of xylose and/or hemicellulosic hydrolyzate	na
Bio-energy	biogas	29.5 GW /y	Transport and biofuel, electricity and heat	0.10-012 (€/MWh) for biogas and 0.20-0.24 (€/MWh) for upgraded biogas	1) Biological: fermentation of carbohydrate, lipid and lignocellulosic biomasses	0.47-0.53 €/kWh
	bio-oil		biofuel	14.68 (€/L)	1)thermo-chemical: pyrolysis, liquefaction and gasification of carbohydrate and lignocellulosic biomasses	na
	syngas	116600 MWth/y	Transport and biofuel, electricity and heat	0.27 - 1.28 (€/L)	1)thermo-chemical: gasification of lignocellulosic biomass	na
	bio-deisel	na	Transport	0.63 (€/L)	1) chemical: transesterification of triglycerids	na
	bio-idrogeno	na	Transport	0.43-1.51 (€/L)	1)thermo-chemical 2) biologica: two-step anaerobic digestion and dark fermentation	na

1.3.3.4 Correlation biomass-process-products to evaluate market size satisfaction

The correlation between biomass-process-products (Table1-7) is based on technical feasibility, since data from economic and environmental perspectives were not available for all product and process.

Correlation between biomass-process-products considers: 1) biomass used as feedstock, 2) process type and working conditions as yield, productivity and reactor condition and 3) products with the purity grade required by market. Ethanol, lactic acid, propionic acid, succinic acid are the platform chemicals with 1) the highest yield and productivity, by means of biological processes, according with Agenda 2030 and green-white chemistry. After the assessment of biomass-process-product correlation, the quantification of the amount of obtainable product according to 1) process technical feasibility, 2) biomass conversion yield and market size was provided in Table1-8. In detail, the percentage of satisfied market demand, the contribution of both process and biomass were estimated. For this analysis, 1,3 propanediol was not considered since it was currently produced from pure glycerol at industrial scale.

2G-biorefinery contributed positively to satisfy product market demands in a range between 14-57.22 %, except for biogas production, for which a surplus of 11% is estimated. The biogas trend confirmed the fundamental role of biogas to support biorefinery energy self-sufficiency and to satisfy social need, according to Hagman et al. (2018). Referring to biomass percentage contribute lignocellulosic biomasses were the most versatile and employed. It is worth to underline, that lignocellulosic biomasses were the first type of biomasses employed in biorefinery system (1G-biorefinery are mainly based on crops) and consequently lignocellulosic biomasses are the most studied and optimised for the different processes until now.

Table 1-7:Biomass-process-product correlation

Product	Process type	Micro-organism	Biomass	Reactor	Yield (g/g)	Productivity (g/Lh)	References
Ethanol	Fermentation	*Z. mobilis*	Glucose	Batch	0.5		Koutinas et al, 2014
		E. Coli or S. cerevisiae	Xilose	batch	0.46-0.48		Koutinas et al, 2014
		Sch.shehatae TTC79	Sugar cane	batch	0.44-046	0.26-0.30	Koutinas et al, 2014
	Pyrolysis		Lignocellulosic (wood pine, crops)	fed batch	0.45-0.50	n.a	Luque et al., 2014
Lactic acid	Chemical		from Lactate	batch	0.84	n.a	Goel et al., 2011
	Fermentation	*SHF+ Fungi*	Xylo-oligosaccharides waste from corncob	batch	0.34	n.a	Zhang et al., 2015
		SFF+ Fungi		batch	0.6	n.a	
		SHF+ Bacteria	Mixed food waste	batch	0.29	2.08	Pleissner et al., 2017; Kwan et al 2015
		SFF+ Bacteria		batch	0.33	3.38	Demichelis et al., 2017
		SHF+ Bacteria	Wheat corn	batch	0.89	1.94	Liu et al., 2017
		SFF+ Bacteria		batch	0.65	0.81	
Propionic acid	Chemical	Reppe syntesys:	na	na	na	na	na
		Aerobic oxidation	na	na	na	na	na
	Fermentation	*Propionibact*	Glycerol	Fed-batch	0.54–0.71	0.2	Zhang et al, 2009 43
			Glucose	Batch	0.35	n.a	Zhang et al, 2009
				Fed-batch	0.53	0.07	Wang et al, 2015a
			Sugar cane molasses	batch	0.45	0.06	Wang et al, 2015b
		Propionibacterium freudenreichii	Glucose	Fed-batch	0.46	0.36	Wang et al, 2013
			Hydrolised Sugar cane molasses	Fed-batch	0.5	0.57	Wang et al, 2013
1, 3Propandiol	Chemical syntesys	Shell process	Acrolein	na	65%	n.a	Koutinas 2014
			Ethilene	na	80%	n.a	Koutinas 2014
	Fermentation	*C. butyricum*	Glycerol	batch	0.51-0.53	n.a	Suma et al., 2018;
				Continuous	0.55	n.a	Mendes et al., 2011
				Fed-batch	0.51-0.55	n.a	Metsoviti et al., 2012;
		K. Pneumoniae		Fed-batch	0.4	n.a	Wilkens et al., 2012
				Fed-batch	0.45	n.a	Mendes et al., 2011
Succinic acid	Chemical	na	na	na	na	na	na
	Fermentation	*E.Coli*	Glucose	Fed-batch	1.13	1.55	Koutinas et al., 2014

		A.succinogenes	Wheat hydrolysates	Batch	0.81	1.19	Krawczyk et al.,2016
			Cane molasses	Batch	-	1.15	Krawczyk et al.,2016
			Corn stalks	Batch	0.66	0.56	Efe et al, 2013
			Waste bread	Batch	1.16	1.12	Li et al, 2018
			Chease waste	Batch	0.57	0.44	Koutinas et al., 2014
Malic acid	Chemical production	Hydratation	n.a	n.a	n.a	n.a	na
	Fermentation	T. Fusca	Corn stover	Batch 55°C	0.43	n.a	kahn et al 2014
		R. delemar HF-119	Caorn straw	batch 30°C	0.48-097	n.a	Li et al 2014
		C. ljungdahli DSM 13528-A. oryzae DSM 1863 25	Syngas (plant biomass)	batch 25°C	0.17	0.02	Oswal et al., 2016
		Aspergillus oryzae	120 g/L glucose		0.51	0.16	Ochsenreither et al. (2014)
			45 g/L glucose		0.67	0.64	Knuf et al. (2013)
		Aspergillus flavus ATCC 13,697	120 g/L glucose		0.94	0.59	Battat et al. (1991)
		E.coli	65 g/L glucose		0.55	0.58	Gao et al. (2017)
Fumaric acid	Fermentation	Thermobifida fusca muC-16	100 g/L cellulose		0.63	0.51	Deng et al. (2016)
			50 g/L milled corn stover		0.43	0.18	Deng et al. (2016)
2,3-butandiolo	Fermentation	*K. pneumoniae*	pure glucosecommercial-industrial glucose	Fed batch	0.43		Koutinas et al, 2014
		Serratia marcescens	sucrose	Fed batch	0.41		Koutinas et al, 2014
		K.oxytoca	molasses	Continuous	0.42		Ocha-Gomez et al, 2015
		E. aerogens	waste glycerol	Shake flaske	0.4		Sheldon 2016,
butyric acid	Fermentation	*C. tyrobutyricum*	Sugar--> Glucose	batch, feed batch, pH=6	0.42-0.46	0.62-1.1	110, (Huang et al.,2016)
			Corn fibers hydrolisate		0.47	2.91	Koutinas et al, 2014
			Glucose		0.47	0.37	Koutinas et al, 2014
			Cane molasses		0.46	3.22	Koutinas et al, 2014
			Sugar-->Sucrose	feed batch pH=5.5	0.35	0.3	(Dwidar et al., 2013)
			lignocellulosic--> corn fiber	batch, feed batch, pH6	0.42	2.91	(Zhu et al., 2002)
			lignocellulosic--> oil seed rape	batch, feed batch, pH=6	0.37-0.43	0.93-2.46	(Huang et al.,2016c)
		C. beijerinckii	cheese waste -->	batch, pH=5.5	0.23	0.08	(Alam et al.,1988)
		Microbial community	food--waste	continuous, pH=7	0.52	0.09	(Stein et al., 2017)
			Glycerol	continuous pH=5.5	0.46	0.1	(Varrone et al.,2017)

Itaconic acid	Fermentation	Aspergillus terreus	Glucose from ad hoc biomass		0.51	0.22	(Krull et al., 20017)
			Crude whaet chaff	Enzymatic hydrolysis (Cellulase)+ SHF	0.27	0.16	(McCartney et al., 2006)
			Wood	enzymatic hydrolysis (Cellulase)+ multi-stage detoxification+ SHF	0.53	0.22	Tippkotter et al, 2014
			Wheat hydrolysates	enzymatic hydrolysis (Cellulase)+ zeolite, anion and cation-exchanger detoxification+ SHF	0.3	0.1	Palmqvist et al., 2000
			Wheat	enzymatic hydrolysis (Cellulase)+ detoxification+ SHF	0.41	0.19	Dwiarti et al., 2015
			Artifical wheat chaf hydrolysate	removal of metals	0.49	0.26	Karaffa et al., 2015
Xylitolo		S.Cerviase	glicerol as co-substrate	batch	0.96	1.11	Oh et al.,2013
		C.tropicalis	high xilose yield+ co-substrate required	batch	0.98	3.2	Ko et al., 2016
						Heating value (MJ/kg)	
Bio-oil	liquefaction		oil cake		20-60%	n.a	Xiu et al., 2011
			woods	T=277-377°C	28%	28.3-33.9	Karagoz et al., 2005
			Rice straw	T=260-350°C	13-38.35	27.6-35.8	Yuan et al., 2007
			Swine manrue	T=285-305°C	2.8-53.3	25.2-33.1	Ocfemia et al., 2006
	flash pyrolisis		oil-cake	n.a	80%	n.a	Xiu et al., 2011
Syngas	Pyrolis, gassification	na	na	na	na	na	na
Biogas			mixed food waste	batch	0.71,CH_4=55% w/w		Pleissner et al, 2017
			pre-treted food waste with SHF and SFF	batch	0.9 CH_4=65% w/w		Demichelis et al., 2017
			vegetable mixed	eed batch	0.554 ± 0.038 CH_4=54.9%		Ruffino et al., 2015
			vegetable mixed	continuous batch	0.50±0.029 CH_4=44.0%		Ruffino et al., 2016
			animal manure	feed batch	0.78 Nm^3/kg/VS		(Hagman et al., 2018)
			sludge	feed batch	0.15 Nm^3/kgVS		Raposo, 2008
Bio-diesel			rice	Feed batch	0.45 Nm^3/kgVS		Weiland 2010
Bio-H2	Dark fermentation		carboydrate		0.09 L/g	n.a	Khan et al., 2017

Table1-8:quantification of market demand satisfaction and percentage contribute of the biomasses.

Ethanol	Gasification (Mt/y)	1658.1	44.9% from wheat and corn 55.1% spent ground cofee
	Fermentation (Mt/y)	1588.7	26.06% rice, 14.16% OFMSW,16.20 %fruit and vegetable agrowaste, 0.03% dairy waste, 9.10% processed candies waste, 34.45% processed fruit and vegetable waste, 15% spent ground coffee
	Tot (Mt/y)	3246.8	51.07 %from gasification 48.9 % from fermentation
	Market satisfaction (%)	**57.22**	
Lactic acid	Fermentation (Mt/y)	2727.17	16.69% rice, 35.5 %OFMSW,16.20% corn and wheat waste, 10.38 fruit and vegetable agro-waste, 0.01% dairy waste, 5.38% processed candies waste, 22.07 % processed fruit and vegetable waste, 0.36% spent ground coffee
	Market satisfaction (%)	**34.09**	
Propionic acid	Fermentation (Mt/y)	2118.32	14.16 % OFMSW, 26.06% Rice, 16.20%fruit and vegetable agro-waste,0.03% dairy waste, 9.10 % processed candies waste, 34.45%processed fruit and vegetable waste
	Market satisfaction (%)	**14.12**	
Succinic acid	Fermentation (Mt/y)	2773.78	31.36% OFMSW, 9.95% rice, 35.5% corn and wheat waste, 0.01% dairy waste, 3.48% processed candy waste, 13.15% Processed fruit and vegetable waste, 0.36% spent ground coffee
	Market satisfaction (%)	**39.63**	
2,3- butandiolo	Fermentation (Mt/y)	2789.15	11.02% OFMSW, 20.29% rice, 21.93% corn and wheat waste, 0.02% dairy waste, 7.09 % processed candieswaste,26.82% Processed fruit and vegetable waste, 0.22% spent ground cofee
	Market satisfaction (%)	**45.13**	
Butyric acid	Fermentation (Mt/y)	2425.77	10.20 % OFMSW, 18.77% rice, 27.68% corn and wheat waste, 11.67% fruit and vegetable agrowaste, 0.02% dairy waste, 6.56 % processed candy waste, 24.82%processed fruit and vegetable waste, 0.28% spent ground coffee
	Market satisfaction (%)	30.32	
Itaconic acid	Fermentation (Mt/y)	452.10	99% corn and wheat waste, 1% spent ground coffee
	Market satisfaction [%]	**56.51**	
Xylitol	Fermentation (Mt/y)	518.99	14.6 % OFMSW, 26.06% rice, 16.20% fruit and vegetable agro-waste, 0.03% dairy waste, 9.10 % processed candy waste, 34.45%processed fruit and vegetable waste
	Market satisfaction (%)	**27.30**	
Bio-oil	Liquefaction (Mt/y)	1022.97	17.54% rice,21.94% animal waste, 17.95% milking waste, 40.84 corn and wheat waste,16.20% fruit and vegetable agro-waste, 1.33% Slaughter waste, 0.41 %spent ground coffe
Syngas	n.a	n.a	n.a
Biogas	Fermentation (Mt/y)	637.11	3.15% sludege and wastewater, 47.89% rice,2.91% animal waste, 1.19% milking waste, 17.77%corn and wheat waste,3.70 % fruit and vegetable agro-waste, 0.34% winery waste, 0.09% Slaughter waste ,2.08% processed candies wat, 0.78%olives and oil waste 0.53 %spent ground coffe
	Market satisfaction (%)	**111.22**	

1.3.4. Sustainability

Sustainability evaluation of the whole biorefinery system is carried out by means of top down approach. The use of waste as feedstock in 2G-biorefinery was a necessary but non-enough condition to reach the sustainability. In 2G biorefinery, valorization of waste biomass as feedstock was a valid and proven solution for waste management, but biomass collection, transport, conversion and delivered reducing the environmental, economic and social benefits. Sustainability was the synergic sum of three pillars: environment, economy and society, also expressed with the 3P concept: planet, profit and people. 2G-biorefinery, designed on the concept of 3R: Reuse Recovery and Recycle of waste according to European Waste Management Directive, Circular Economy, bio-based economy and 2030 Agenda Development, was able to reach sustainability. In detail, 2G-biorefinery realizes the link between 3R- 3P concept. Sustainability is made up of two quantitative pillars environment and economy and one qualitative society. In the present study, the three pillars are evaluated through: Mono- (1D), bi- (2D) and three- (3D) dimensional indicators (Dahiya et al.,2018). Environmental 1D indicator consisted of two tools. The first is Carbon Footprint evaluation (CFP), which measured the CO_2 emission both in the single phase and in the whole process. The second is Life Cycle Assessment (LCA) for the evaluation of GHG emission referring to equivalent CO_2, Ozone depletion and Eutrophication at midpoint and DALY (Disability Adjusted Life Year) and PDF (Potentially Disappeared Fraction) at the endpoint. Based on the available LCA study, the most adopted approaches were cradle-to-grave considering products and wastes generation and cradle-to-gate until the production step Wernet et al., (2016). 2G-biorefinery designed as restorative and auto-regenerative system must be evaluated with cradle-to-cradle approach including waste biomasses collection, transport, conversion, reuse, recovery, recycle and integration of products and technologies Silvestre et al. (2014).

The limits of CFP and LCA are the dependency on geographical contests and on definition of boundary conditions consequently CFP and LCA are geo-referred and case study specific evaluations Shonnard et al. (2015). Between 2015-2018, 219 LCA studies for ethanol production from lignocellulose are published witnessing CO_2 emission reduction for conversion process and CO_2 emission production for biomass transport and product delivers Loukia et al. (2018); Parada et al. (2017). Economic 1D indicator considered capital and operational costs and revenues from product market. Capital costs included fixed capital investment (FCI) and working capital cost. FCI includes: the purchase of equipment and facilities to build up the plants and the installation cost. Operational costs consisted of raw materials collection and transport, equipment maintenance, utilities and labor. To evaluate the process economic profitability, the following parameters are employed: a composite indicator made up of net present values (NPV), payback time, return of interest (ROI) and Euro spent and gained respectively per ton of feedstock and generated products, had to be designed. Economic indicator was the classical tool adopted by industrial chemical process design, but it is not enough to

assess the sustainability. Social 1D indicator is Human Development Index (HDI) a qualitative metric ranging between 1-10 (DeVries et al., 2009). Social indicator evaluation is fundamantal, since 2030 Agenda Sustainable Developping Goals policies drove economic growth, job position creation and new job figures conception Timmis et al. (2017). Referring to bi-dimensional indicators the most important are economic-environmental and socio-environmental indicators. Economic-environmental indicator consists in Process Cost and Environmental Impact (PCEI) (Cheali et al., 2015) based on process indicator combination in an integrated and optimised systems.

Environmental-social indicators are: Crop Sustainability Index (CSI), EcoIndicator99(EI99) and WAR. CSI is an impact category able to quantify water use efficiency, pollutants emissions and social growth Golberg et al., (2014). EcoIndicator99 (EI99) (Ministry of Housing and the Environment 2000), evaluated pros and cons in part or in the whole biorefinery systems including environmental and social perspectives. EI99 is an endpoint method of LCA analysis Zanghelini et al., (2018). The output of EI99 is the sum of all impacts normalized on a common standard EU value. The third indicator was WAR algorithm methodology (Young and Cabezas, (1999), which considers ecological and human toxicity based on aquatic-terrestrial toxicity and ingestion-inhalation-dermal contacts. The unit of measur of these impact categories are: LC50, LD 50, OSHA PEL Li et al. (2011). The final output from WAR algorithm methodology was the sum of all impacts previously normalized per their own reference values. A complete sustainability assessment was carried out with 3D indicator including the simultaneous evaluation of environmental compatibility, economic profitability and social benefit. 3D indicator refers to Institution of Chemical Engineers (IChemE)'s estimation based on the following three statements:1) environment: quantification of land uses, GHG and other pollutants emission, request of fossil raw materials, 2) economy: cost-benefit analysis and 3) society: creation of new job positions and figures for the valorization of human capital. Mono-Bi and Three-dimensional indicators had to be considered since the earlier stage of biorefinery design. At the design level, the assessment of biorefinery system was based on the performance comparison of different scenario to figure out the best scenario based on multi-criteria analysis, considering stakeholders and social interests, or LCA Mata et al., (2013). Whereas, at the design level, sustainability had to be evaluated with 6 main categories Sacramanto-Rivero et al. (2016); Parada et al. (2017):

1) potential displacement of fossil fuels and materials,
2) mitigation of environmental impacts,
3) renewability,
4) economic feasibility,
5) preservation of biodiversity
6) social responsibility.

Potential displacement of fossil fuels and materials consists of three sub-categories: non-renewable energy share, fresh-water use reduction and raw-materials cost ratio, which quantify the potential of biorefinery system to replace a reference fossil system providing the same quality and quantity of product Martinez-Hernandez et

al. (2015). Mitigation of environmental impacts is based on GHG emission reductions compared to emissions due to fossil reference system Martinez-Hernandez et al. (2015). Biorefinery renewability consists of biotechnological-valorisation potential and raw-materials consumption to quantify the capacity of the biorefinery system to satisfy products generation Ou et al. (2015). Economic feasibility provided estimation of biorefinery profitability. Biodiversity preservation provided an estimation of loss biodiversity due to land use change. Community commitment included employment extent and community development investment to quantify local community benefits from the biorefinery in terms of job position creation due to biorefinery system (OECD, 2015). All the above-mentioned sustainability methodologies and indicators must be incorporated and optimised design. All the above analysed methodologies and indicators measure the feasibility of biorefinery system, since biorefinery system can be managed only if it was measurable

All these indicators must be referred to a critical value for the sustainability normalized scales Parajul et al. (2015), based on world, European and/or national regulations

1.5 Conclusions

This study investigated 2G-biorebinery system in EU28. The aim is the evaluation of the fundamental biorefinery units: biomass, process and product and their correlation. According to the European Technical Guidance waste classification (2018/C 124/01) and Eurostat database, four biomass categories are evaluated: wastewaters and sewage sludge, municipal solid waste, waste from agriculture, forestry and fishing activities and waste from manufacturing of food and beverage products.

2G-biorefinery faces social, economic, environmental and technical problems due to the huge amount of biomasses, considering biomass as secondary raw material to valorize through platform chemical and energy production. This chapter investigates 14 biomasses, the most representative of the four biomass categories, which have carbon content over 50% w/w and belong to carbohydrate, lipids and lignocellulose feedstock groups respectively for 43 %, 36% and 14%. The correlation biomass-process stated that lignocellulose biomasses are suitable for thermochemical, chemical and biological processes, while carbohydrate and lipid biomass are respectively suitable for biological and chemical processes. The correlation biomass-process-product assesses that among the 11 analysed platform chemicals, ethanol, propionic acid, lactic acid and succinic acid have the highest yield through biological processes, allowing 14-57.22 %. market size satisfaction and 9% to 36%, biomass valorisation with consequentially waste reduction. Among the 5 considered bio-energies, biogas is the only one able to satisfy completely the market size with a surplus of 11%. The achieved results prove: 1) the fundamental contribution of biomass to chemical and energy sectors and 2) biogas fundamental role in biorefinery system.

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Chapter 2: Biowaste management in Italy: challenges and perspective.

The main findings of the current study are already published in:

- F. Demichelis, F. Piovano, S.Fiore, (2018) Biowaste management in Italy: challenges and perspectives (IF=2.80) 11(15), 4213; https://doi.org/10.3390/su11154213

Abstract

Chapter 2 developed a methodology for the technical and environmental assessment of biowaste valorisation in 2G-biorefineries in Italy.

Italy was chosen as case study, considering the years 2016-2018. The Italian context was evaluated through the following key parameters: 1) Gross domestic power, 2) climate, 3) demography and 4) population density distribution. The four most abundant biowaste categories were defined through their amounts and geo-localisation: 1) wastewater and sewage sludge (WSS, 4.06 Mt/y), 2) organic fraction of municipal solid waste (OFMSW, 1.7 Mt/y), 3) agricultural livestock waste (AFF, 5.7 Mt/y) and 4) waste deriving from the food industry (IFB, 2.6 Mt/y). The evaluations of geo-localisation and quantitative availability of biowaste amounts aimed to define the dimension and localisation of the biorefinery plant to optimise the supply and transport chains, while the qualitative characteristic aimed to evaluate the most promising process among two different biorefineries systems: thermo-valorisation (TH) and anaerobic digestion (AD). All considered biowaste were suitable for biorefinery systems, since carbon content was more than 40 % and carbon-nitrogen ratio was between 10 and 30. The achieved results established that AD produced more energy with lower CO_2 emission release than TH.

The primary energy production of AD and TH for WSS, OFMSW, AFF and IFB were respectively: 7.89 *vs.* 2.4 kWh/kg; 8.7 *vs.* 2.6 kWh/kg; 10.85 *vs.* 5.5 kWh/kg and 12.5 *vs.* 7.8 kWh/kg. The main findings of this work were: 1) the adoption of AD was technically more efficient than TH and AD increased the avoided CO_2 emissions of 10 - 89.9 % depending on the considered biowaste category.

1.1 Introduction

Considering the importance of clean energy production and GHG emissions reduction in SDGs, Chapter 2 evaluated the technical feasibility and the environmental impacts of two biorefinery systems for biowaste valorisation: anaerobic digestion (AD) and thermo-valorisation (TH).

The aim of the work reported in this Chapter was the proposal of a methodology to establish biowaste potential in 2G-biorefinery systems in Italy. The bio-waste categories and flows defined in Chapter 1 for EU 28 were investigated in Italy.
Italy was chosen as case study considering its geography, which covers different climates and social distributions, specular to the EU 28 situation.
The investigation period considered the years 2016 – 2018 (this period was chosen because of the availability of data). According to the classification of biomasses in EU-28, studied in Chapter1, four biowaste categories were analysed:

1) wastewater and sewage sludge (WSS),
2) organic fraction of municipal solid waste (OFMSW),
3) agricultural - livestock waste (AFF)
4) waste deriving from the food industry (IFB).

The **novelty** of the proposed methodology consisted in the combination of technical and environmental assessments into the following seven sequential phases: 1) definition of the case study framework; 2) quantitative evaluation and geo-localisation of available biowaste; 3) physical and chemical evaluation of biowaste; 4) analysis of present biowaste management; 5) quantitative assessment of the valorisation of biowaste through two biorefinery processes: anaerobic digestion (AD) and thermal valorisation (TH); 6) comparison between present and proposed biowaste management perspectives on the grounds of CO_2 emissions; 7) analysis of 2G-full-scale biorefinery systems in Italy.
In Chapter 2, the attention was focused on energy production, since transport and heat sectors were strongly influenced by the local economic framework (De Jong 2015; Dahiya et al, 2018).

Biorefinery and biowaste valorisation were crucial topics in the scientific literature and industrial reality. From 2015 to 2020, Science Direct listed: 1585 research articles, 261 book chapters, 274 review articles and 13 encyclopaedia items, with a percentage enhancement of publications from 2016 to 2020 of 51%. Works available in the literature focused on the valorisation of biowaste of different origins and structure commonly at the lab and or pilot scale, concerning for 25% on generable products, 30% on specific biowaste stream and 45% lab process parameter control.
The novelty and consistency of the study of Chapter 2 was the creation of a bridge between scientific literature achievements and real biorefinery case to scale up process, product and biorefinery systems.
Chapter 2 concerned the theoretical study of two mature and well established biorefinery systems: thermo-valorisation (TH) and anaerobic digestion (AD), adopting the scientific upgrade to study, analyse and improve the real biorefinery system available in Italy. To evaluate pros and cons of these two biorefinery systems, TH and AD yields and efficiencies were calculated, both from technical and environmental perspectives, referring to biowaste, which can be collectable and available according to Italian region and season. Truthfully, the geo-localisation and quantitative evaluations of the available biowaste amounts pointed at defining the dimension and localisation of the biorefinery plant and at optimising supply and transport chains, while qualitative evaluation measured the most promising process

among TH and AD. The present work was the first to quantitatively assess the perspectives for the adoption of Circular Economy strategies in Italy about biowaste management through two biorefinery systems aimed at energy production. These strategies must be applied both in urban and in industrial systems, converting biowaste concept, which were mostly perceived as underestimated low-value streams, into more valuable product and energy. The generation of energy and various commodities in a combined approach addressing sustainability was the challenge and key-driver for EU28 (Istat, Ambiente e energia, 2017).

2.1. Materials and methods

2.1.1 Case study framework

The present study was carried out in Italy, which was analysed under three complementary perspectives: 1) geographical and climate area divisions, by means of Köppen classification (Istat, Popolazione e famiglie, 2019); 2) demographic distribution, described through national reference statistics (Istat, 2020. Report e Statistiche dei Conti Regionali, 2019) based on years 2016 – 2018; and 3) economic development, considering gross domestic product (GDP), which measured the income and output for a given country's economy and it was equal to the total expenditures for all final goods and services produced within the country in a defined period of time, usually 1 year Dahiya et al. (2018); (Eurostat, 2019).
The maps in Figure 1 were drawn with Data-Wrapper, an open source map-creator software.

2.1.2 Biowaste quantitative analysis and geo-localisation

The available quantity of biowaste was the key parameter to define the size of the biorefinery plant and the amount of obtainable products. The quantitative analysis of biowaste was based on national and EU databases referring to years 2016 - 2018 (Eurostat, 2020; Ispra, 2019. Rapporto Rifiuti Speciali Ed.2020; Ispra 2019 Rapporto rifiuti urbani,). Four bio-waste categories, defined according to EU Commission Decision 2000/532/EC and Eurostat – ISPRA (Istituto Superiore per la Protezione e per la Ricerca Ambientale) databases classification, were selected: 1) wastewater and sewage sludge (WSS), 2) organic fraction of municipal solid waste (OFMSW), 3) agricultural - livestock waste (AFF) and 4) waste deriving from the food industry (IFB).
Collection and transport costs were evaluated from technical, economic and environmental perspectives.

2.1.3 Biowaste qualitative analysis: physic-chemical features

The qualitative description of biowaste categories was performed through elemental analysis, total solids (TS) and volatile solids (VS) (Table 2).
The qualitative analysis concerned 14 biowaste, which were defined the most representative of the four considered categories according to the biowaste considered for EU28 in Chapter1:
1.wastewater and sewage sludge (WSS);
2. municipal solid waste (MSW);
   i. Organic fraction municipal solid waste (OFMSW);
3. waste from agriculture, farming and fishering activities (AFF);
   i. rice waste,
   ii. animal manure,
   iii. corn and wheat waste,
   iv. fruit and vegetable from
4. waste from industrial food and beverage activities (IFB).
   i. winery waste,
   ii. milking waste from animal,
   iii. dairy waste,
   iv. slaughter waste,
   v. processed candies waste,
   vi. olives and oil waste,
   vii. processed fruit and vegetable waste
   viii. spent coffee grounds

2.1.4 Biowaste qualitative analysis: physic-chemical features

The current management and disposal of the four categories of biowaste was analysed considering national (Ispra, 2019. Rapporto Rifiuti Urbani Edizione 2019, Ispra 2018.Rapporto Rifiuti Urbani Edizione 2018; Ispra, Rapporto Rifiuti Speciali Edizione 2017; EEA, 2018) and international (FAO, 2018) database.

2.1.5 Technical and environmental analysis of biowaste valorisation through processes

The technical assessment consisted in the evaluation of the amount of primary energy theoretically produced from stoichiometric evaluations, while the environmental assessment consisted in the evaluation of CO_2 equivalent and avoided CO_2 emissions, referring to 1 kg of biowaste. About AD, the specific biogas production (SBP) and methane (CH_4) were calculated by means of Buswell and Neave equations, since based on the elementary composition of the biowaste fed in the AD process, it is possible to determine the maximum theoretical production of biogas and methane.

It was assumed that 1 Nm^3 of methane produced 10.5 kWh of primary energy.

While for TH, the lower heating values (LHV) of the 14 biowaste were considered. CO_2 equivalent emissions were calculated directly from AD and TH processes, modeled with stoichiometric reactions, while the avoided CO_2 emissions were calculated through the conversion factor of 0.44 t CO_2 emitted per MWh produced, defined for Italy and central EU-28 (ENEL, 2018)

2.1.6 Full-scale biorefinery system in Italy

The most important five Italian biorefinery systems were described and geo-referred: a chemical biorefinery system (Betarenewable, 2020): two biological biorefinery systems (Acea Pinerolese, 2020; TRM, 2020); and two thermo-chemical biorefinery systems (ENI, 2020; Enea, 2020.). The data was derived from the Internet sites and public sustainability reports of the above-mentioned companies.

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2.3 Results and discussion

2.3.1 Case study framework

Italy is a peninsula extending between the 36th and 47th north parallels, divided into three-macro areas: North, Centre and South, with a total of 20 regions (Figure 2-1A). The regions are located as follows: 8 in the North (Liguria, Piemonte, Lombardia, Valle d'Aosta, Emilia Romagna, Friuli-Venezia-Giulia, Trentino-Alto-Adige and Veneto), 4 in the Centre (Lazio, Marche, Toscana and Umbria) and 6 in the South (Abruzzo, Campania, Calabria, Basilicata, Molise, Puglia), plus 2 major islands (Sicilia and Sardegna). According to the Köppen classification, ten climate categories were identified for Italy (Figure 2-1B) and the general climate trends are: humid temperate in the North of Italy, and Mediterranean climate with dry summer period in the Centre, South and the Islands.

In 2017, Italian population is equal to 60.6 M inhabitants, with the following partition: 48.6 % male and 51.4 % female (Istat, 2018. Report e Statistiche dei Conti Regionali). The most and the least populated regions were Lombardia and Valle d'Aosta, respectively with 10 M and 127 k inhabitants (Istat, 2019. Report e Statistiche dei Conti Regionali) (Figure 2-1C). Italy is the 5th most populated country of EU 28, accounting an average of 201 inhabitants/ km^2. The most and least density populated regions were Lombardia and Valle d'Aosta, with respectively 420 inhabitant/km^2 and 39 inhabitant /km^2 (Istat, 2019. Report e Statistiche dei Conti Regionali).

The economic situation was evaluated (Figure 2- 1D) in 2017, the Italian GDP was 115.6 k€, with the partitions: North-West 34.2 k€, North-East 33.3 k€, Centre 29.9 k€ and South plus the Islands 18.2 k€ Dahiya et al. (2018)

Figure 2-1: Case study framework description: A. geographical distribution (region); B. climate areas (type of clime); C. population density (inhabitant /km2); D. GDP (euro) in Italy in 2016 - 2018

2.3.2 Biowaste quantitative analysis and geo-localisation

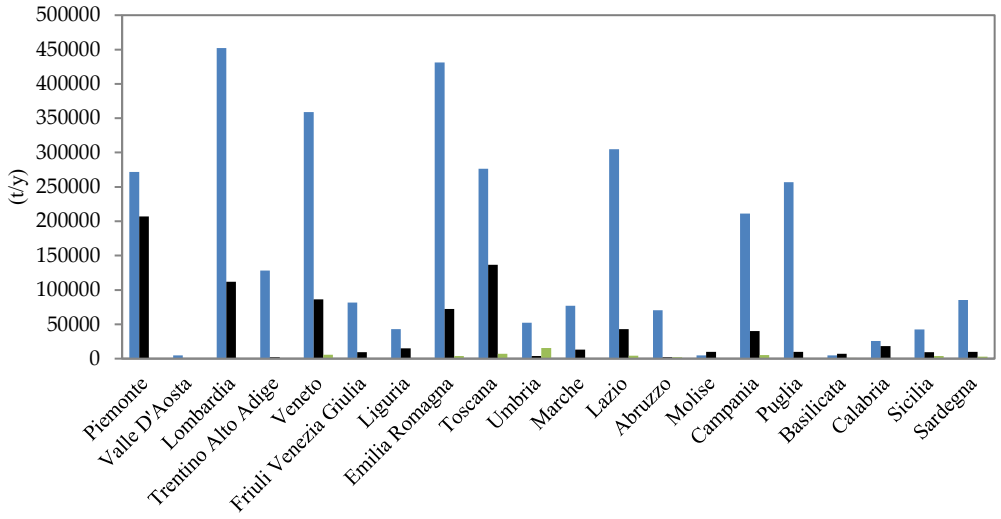
The geo-localisation and quantitative evaluations of the available biowaste amounts might define the dimension and localisation of the biorefinery plant and at optimise the supply and transport chains. In Italy and EU-28, the Commission Decision 2000/532/EC and Technical Guidance on the classification of waste (2018/C 124/01) sorted out waste on the grounds of the activity from which they were produced: residential, commercial, public, construction and demolition, public services, treatment plants, industrial and agriculture. The adopted methodology defined four main biowaste categories, according to EU Waste Directive and European Policies, Eurostat and ISPRA databases, as defined in section 2.2.2

WSS enclosed 3 types of waste flows: WSS from urban wastewater treatment plants (CER 190805); not hazardous WSS from industrial wastewater treatment plants (CER 190812 and 190814); hazardous WSS from industrial wastewater treatment plants (CER 190811* and 190813*) (Figure 2-2). The biowaste amounts were expressed as average value plus/minus standard deviation, considering of the considered period 2016-2018.

In 2018, WSS production in Italy was 4.06 Mt/y (Ispra 2019, Rapporto Rifiuti Speciali Edizione 2019, Corte dei Conti, 2019). All regions exhibited the same trend: the highest production concerned WSS from urban wastewater, followed by not hazardous WSS and then hazardous WSS (Figure 2-2), respectively with 3.2 Mt/y, 0.81 Mt/y and 0.07 Mt/y. These trends were consistent with EU-28 WSS production of middle – high income countries (Eurostat, 2018. Sewage sludge production and disposal; US EPA, 2017) witnessing both the significance of Italy as case study, and the social-economic development for wastewater management system, related to SDG no. 6.

In Italy, the highest and lowest productions of WSS were recorded in Lombardia (1.14 Mt/y) and in Valle d'Aosta (0.008 Mt/y), respectively. The different amounts of produced WSS were due to fact that Valle d'Aosta had lower dimension and density population compared to other Italian regions. Valle d'Aosta had trends comparable to Luxemburg (Eurostat, 2018; Ispra, 2016. Rapporto Rifiuti Speciali Ed.2019).

Figure 2-2: WSS production in Italy in 2016-2018 (Ispra, Rapporto Rifiuti Speciali Edizione 2019, Corte dei Conti, 2019): CER 190805 (in blue), CER 190812 and CER 190814 (in black)WSS production in Italy in 2016-2018 CER 190805 (in blue), CER 190812 and CER 190814 (in black) and CER 190811* and 190813* (in grey)



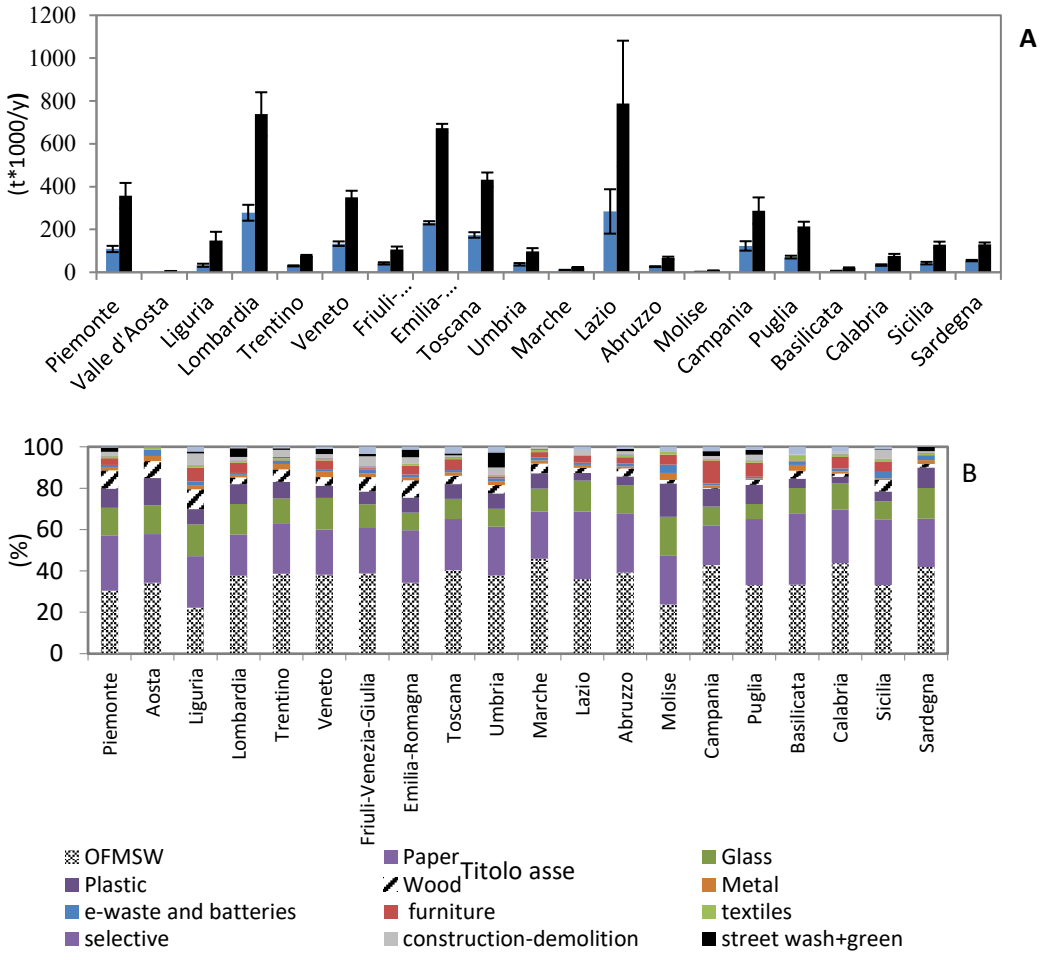
OFMSW was a sub-category of municipal solid waste (MSW). According to US Environmental Protection Agency (EPA), MSW was composed by plastic (product packaging), grass clippings and OFMSW, large furniture, clothing, glass (bottles and cans), paper, appliances, e-waste and batteries. MSW derive from households, hospitals, schools, small business activities and bar/restaurants (Sultana et al., 2014). EPA's definition of MSW did not include sludge from municipal wastewater treatment, waste from industrial processes, end-of-life vehicles, ash from MSW combustion, construction and demolition waste. In 2018, the total amount of OFMSW in Italy was 1.7 ± 0.25 Mt/y (Ispra, Rapporto Rifiuti Speciali Edizione 2019). The calculation of biodegradable matter in MSW and furthermore the perspectives to exploit this fraction together with OFMSW were based on the amounts of MSW and OFMSW (Figure 2-3A) and the percentages of specific waste streams (Figure 2-3B). Lazio reached the highest production of MSW and OFMSW (283.95 · 10^3 t/y ± 103.63), followed by Lombardia (277.87 · 10^3 t/y ± 37.12) and Emilia-Romagna (230.92 · 10^3 t/y ± 7.61), showing trends similar to Germany, Denmark and Belgium (Ispra, 2019. Rapporto Rifiuti Speciali Ed.2019). These data can be explained by the high GDP per capita, which leads to higher consumption and therefore greater production of OFMSW and MSW. Figure 3A evidences a strong standard deviation, related to the heterogeneity of OFMSW and MSW in the different parts of each region, emphasizing the importance of efficient waste collection systems (Istat. Ambiente e energia, 2019)

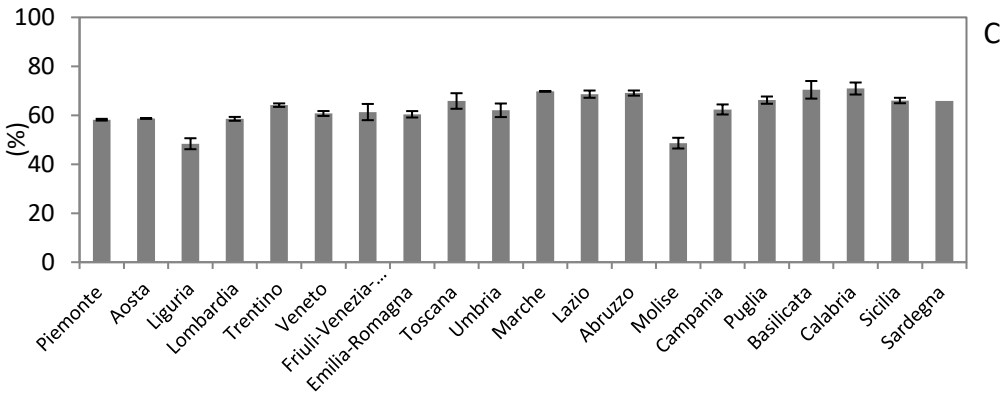
In details, in 2015-2018, the collection rate of OFMSW (i.e. kitchen waste, wet waste, waste from the maintenance of gardens and parks), increased of 7.3% (Ispra 2019, Rapporto Rifiuti Speciali Edizione 2019). OFMSW total amount in 2018 was equal to 1.7 ± 0.27 Mt/y and it enclosed the fractions destined to domestic composting (about 0.22 Mt/y) (Ispra, Rapporto Rifiuti Speciali Edizione 2019). Considering the three Italian macro-areas, (North, Centre and South plus Islands), the separate collection from 2015 to 2018 increased more in the central and

Southern regions (about 10 %), and less in the North (5.4 %). However, in terms of collection per capita the values were in the opposite ranking: 129 kg / inhabitant per year in the North, 111 kg / inhabitant per year in the Centre and 77 kg / inhabitant per year in the South plus the Islands, with a national average of 108 kg / inhabitant per year (Ispra 2019. Rapporto Rifiuti Speciali Edizione 2019). These trends agreed with central EU countries as Germany, Netherlands and Austria (Eurostat, 2019; Ispra, 2019. Rapporto Rifiuti Speciali)

From 2015 to 2018, the separate collection of the cellulosic fraction (paper and cardboard) increased in the North, Centre and South respectively of: 2.2 %, 2.6 % and 0.3 % Ispra, Rapporto Rifiuti Speciali Edizione 2019). The total biodegradable fraction of MSW that could be valorised in biorefinery processes could increase from 30 to 50 %v/v depending on the regions, considering the amounts of OFMSW, paper and clothes, (Figure 2-3B). Data coming from the update version of Eurostat database of 2017-2018, ISPRA database 2017-2018, but these databases reported data of previous years such as 2015-2016

Figure 2-3: 3A) Amounts of MSW (in black) and OFMSW (in blue); **3B)** percentage distribution of waste streams in MSW; **3C)** biodegradable fraction of MSW.





AFF concerned waste from agricultural activity (i.e. corn, wheat, fruit, vegetables, rice, pomace, olive wastes) and livestock waste as animal manure and milking wastes. The total amount of AFF produced in Italy in 2018 was 5.7 ± 0.23 Mt/y. The main streams of AFF were reported in Table 2-1 and Figure 2-4. The wide standard deviation range (50 – 103 %) witnesses the high heterogeneity of the availability of AFF in each Italian region (Table 2-1). The region with the highest AFF production were Lombardia (0.33 ± 0.30 Mt/y), Puglia (0.24 ± 0.33 Mt/y) and Piemonte (0.29 ± 0.27 Mt/y), representing respectively 15 %, 10% and 9 % of total Italian production. The reason of these trends were: in Piedmont the soil was efficient exploited thanks to the adoption of efficient irrigation systems and use of modern agricultural machinery; anyway, Lombardia had a higher productivity thanks to the abundance of irrigation, the adoption of modern techniques, good fertilizers and to the presence of large rationally organized companies (Ispra, 2019. Rapporto Rifiuti Urbani, 2019)

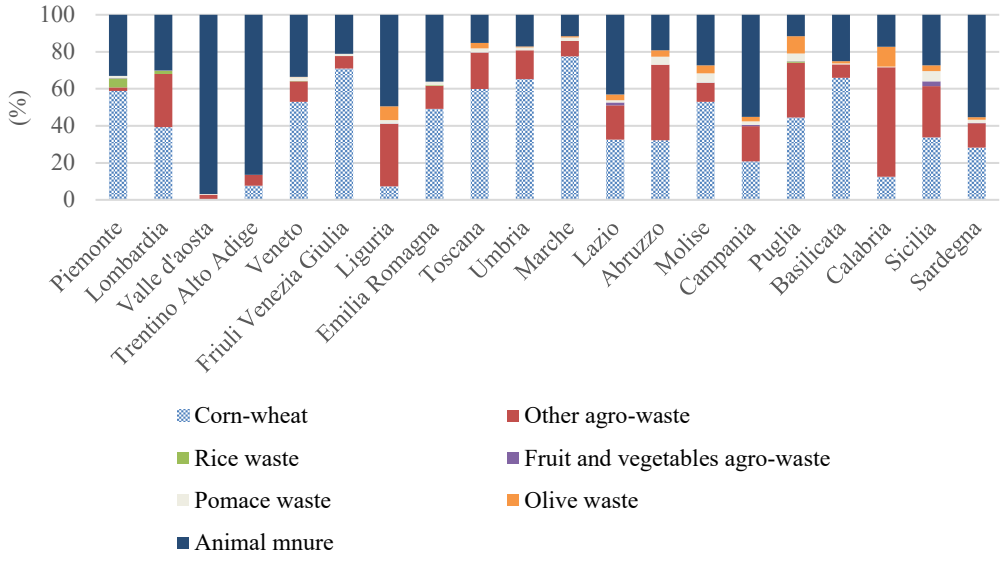
Considering rice waste (Ispra, 2019. Rapporto Rifiuti Urbani Edizione 2019), the highest production was observed in Piemonte (0.024 ± 0.043 Mt/y) and Lombardia (0.014 ± 0.036 Mt/y). These data may be explained by the fact that the Italian rice fields were mainly located in the Novara and Vercelli provinces (in Piemonte, near the border with Lombardia).

About fruit and vegetable waste (Ispra, 2019. Rapporto Rifiuti Urbani Edizione 2019) the highest production was registered in Sicilia (6.1 ± 4.9 Mt/y), Lazio (0.004 ± 0.008.6 Mt/y) and Campania (0.002 ± 0.002.3 Mt/y). Finally, considering pomace waste, the chief producing regions were Puglia (0.022 ± 0.002 Mt/y), Sicilia (0.014 ± 0.02 Mt/y) and Veneto (0.011 ± 0.011 Mt/y). These data can be explained by the very favourable climate present in these regions. For the olive waste the major producing regions were Puglia (0.052 ± 0.034 Mt/y), followed by Calabria (0.037 ± 0.028 Mt/y). Finally, the highest productions of animal manure were observed in Lombardia (0.253 ± 0.68.1 Mt/y) and Campania (0.167 ± 0.171 Mt/y). These data could be explained through the high number of cattle and pig farms present in these regions. For the aim of this study, it was important to know the percentage production of the individual agricultural biomasses present in each region (Figure 2-4).

Table 2-1:Biowaste streams of AFF category exspressed in (t·1000)(Ispra, 2019)

	corn and wheat	other agro-waste	rice waste	fruit and vegetables waste	pomace waste	olives waste	animal manure	Total
Piemonte	295.7±273.0	10.0±16.9	24.4±43.5	0.8±1.5	6.1±7.6	0.0±0.0	166.2±269	503.1
Lombardia	330.1±303.7	242.0±789.7	13.7±36.1	0.2±0.6	1.5±3.1	0.0±0.0	253.4±268.1	840.9
Valle d'Aosta	0.2±0	1.7±0.0	0.0±0.0	0.0±0.0	0.3±0.0	0.0±0.0	67.8±0.0	70.0
Trentino Alto Adige	0.8±0.4	0.6±0.2	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	8.6±8.5	10.0
Veneto	249.2± 134.0	52.4±52.6	0.5±0.9	0.0±0.0	10.5±10.7	0.2±0.0	158.4±101.7	471.3
Friuli Venezia Giulia	148.2±180.2	14.1±10.8	0.0±0.0	0.0±0.0	2.8±2.1	0.0±0.0	44.0±47.3	209.1
Liguria	1.1±1.1	4.8±3.0	0.0±0.0	0.0±0.0	0.3±0.2	1.1±0.0	7.1±1.2	14.4
Emilia Romagna	173.0±107.9	44.3±33.8	0.9±2.6	0.0±0.0	6.9±6.7	0.0±0.0	127.0±122.5	352.1
Toscana	72.4±65.8	23.8±20.0	0.0±0.0	0.0±0.0	2.8±3.3	3.6±2.9	18.3±15.5	120.9
Umbria	215.0±233.2	50.9±38.5	0.0±0.0	0.0±0.0	5.5±1.2	1.3±1.4	56.6±45.3	329.5
Marche	134.8±12.6	14.5±11.2	0.0±0.0	0.0±0.0	3.3±2.6	1.0±0.5	20.2±15.6	173.8
Lazio	87.4±52.3	49.6±28.5	0.0±0.0	4.0±8.6	3.3±3.6	8.1±3.2	115.5±49.9	267.8
Abruzzo	57.3±51.8	72.6±92.7	0.0±0.0	0.0±0.0	7.5±10.9	6.3±7.2	34.1±13.5	177.7
Molise	81.7±98.2	15.7±16.1	0.0±0.0	0.1±0.01	7.7±8.9	6.8±7.2	42.0±14.4	154.2
Campania	63.4±60.5	57.3±14.2	0.0±0.0	2.4±2.3	5.7±4.3	7.4±8.0	167.5±171.2	303.7
Puglia	243.9±332.6	162.8±84.1	3.4±7.6	1.2±1.2	22.3± 18.3	51.7±33.6	63.4±47.2	548.6
Basilicata	226.0±37.1	25.0±26.2	0.0±0.0	0.0±0.0	1.8±0.3	4.0±0.2	86.3±58.1	343.1
Calabria	42.4±34.5	202.4±165.8	0.0±0.0	0.0±0.0	1.3±0.9	36.7±28.2	59.0±41.5	341.8
Sicilia	81.3±68.0	66.4±36.7	0.0±0.0	6.1±4.9	13.5±20.7	7.2±3.9	65.9±48.1	240.4
Sardegna	65.0±36.3	30.2±19.5	0.0±0.0	0.1±0.2	4.1±2.4	3.1±1.1	127.3±54.6	229.8

Figure 2-04:Agricultural and livestock waste percentage distribution (Ispra, 2019)



IFB comprised food and beverage manufacturing waste. Data was taken from the economic activity identified as ATECO 10 - 11 (Ispra, 2019. Rapporto Rifiuti Urbani Edizione 2019). The biowaste from IFB was calculated through mass and energy balances per unit of product and the total amount was 2.6 Mt/y for 2016 (Ispra, 2019. Rapporto Rifiuti Urbani Edizione 2019).

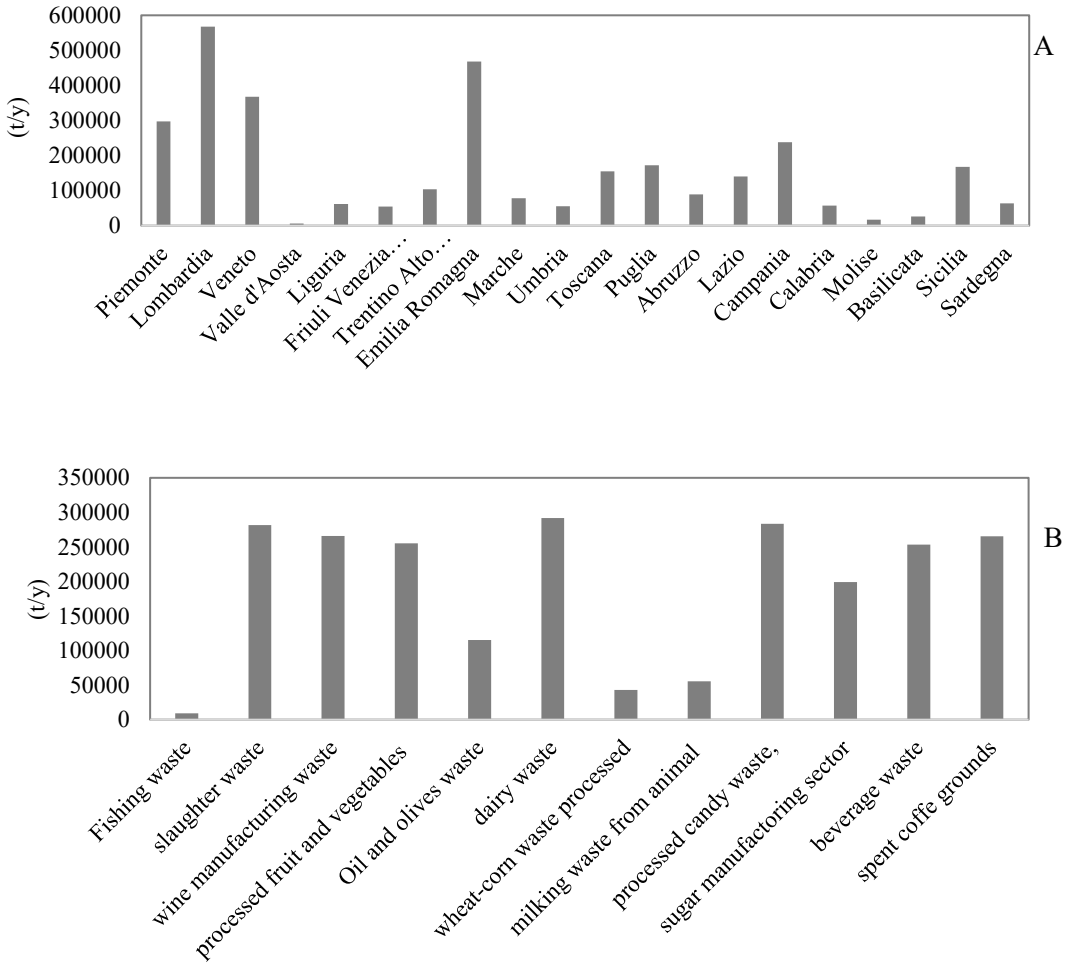
In Figure 2-5A, the amount of IFB was reported for each region for the year 2018. The regions with the highest IFB production were Lombardia (0.56 Mt/y), Emilia Romagna (0.47 Mt/y) and Veneto (0.36 Mt/y), which represented respectively 19 %, 17 % and 13 % of total national production (Ispra, 2019. Rapporto Rifiuti Urbani, 2019). Lombardia, Emilia Romagna and Veneto were characterised by high number of food manufacturing enterprises and high GDP (Dahiya et al 2018; Istat, 2019. Report e Statistiche dei Conti Regionali). The data of the principal food manufacturing streams (Figure 2-5B) referred to the whole Italy, because the data for the single regions and all food manufacturing sectors were not available. The considered main food manufacturing waste streams derive from: fishing, slaughter, wine manufacturing, processed fruit and vegetables, oil and olives, dairy, wheat-corn processing, milking, candy and sugar manufacturing, beverage production and spent coffee ground. Among these specific waste streams, the most abundant ones were dairy waste (12.6 % of the total IFB in 2016), processed candy waste (12.2 %), slaughter waste (12.1 %), and spent coffee ground (11.5 %). Based on Ispra, 2018. Rapporto Rifiuti Urbani ,2018), the trend of IFB production in Italy in 2017 for macro-areas was: Nord East 36.4 %, Nord-West 25.5 %, South and Islands 29.3 % and Centre 8.8 %, witnessing the representativeness of Italy as case study for EU-28 biowaste stream production. Comparing ISPRA and EUROSTAT database, IFB production of Nord East of Italy was like Germany, while South and Islands with Spanish

and France (Eurostat, 2020. Generation of municipal waste per capita; Eurostat). The main findings among the four studied biowaste categories were:

- the order of biowaste production was: AFF (5.7 ± 0.23 Mt/y), WSS (4.06 Mt/y), IFB (2.6 Mt/y) and OFMSW (1.7 ± 0.25 Mt/y), leading to a total amount of 14 Mt/y.
- IFB production was lower than AFF and WSS, since food industry manufacturing processes were usually optimised to maximise the production and minimise waste production (Mossman et al., 2018)

Nevertheless, biowaste was not a free resource since there were costs connected to collection, transport, pre-treatments and biomass conversion. The highly heterogeneous geo-localisation of biowaste in each region (48 - 70%) made collection/logistics systems quite complicate, reducing the efficiency of the collection system and maximal biowaste valorisation Golecha et al. (2016). Transport cost was affected by tortuosity factor, biowaste density, collection site-plant distance and type of vehicle (oil, gas, small-medium-big sizes). In general, the cost of transport Ramli et al. (2017) had to be analysed from economic and environmental viewpoints. Biowaste transport cost ranged between 0.41 and 1.2 €/t (Behera et al. (2014) but over 100 km distance and water content over 30 %, the transport was considered unsustainable Budzianowski et al. (2015). From an environmental point of view, collection and transport of biowaste were sustainable if there was a reduction of GHG of 40 - 60 % compared to current situation (Pleissner 2016). Another important parameter affecting waste biomass collection and transport was the seasonal variation of the available biomass (Pleissner 2016).

Figure 2-5: A) Food and beverage waste for each regions, **B)** main food beverage waste streams (Ispra, 2019)



2.3.3 Biowaste qualitative analysis: physic-chemical features

The biowaste were chemically and physically characterised to identify the most suitable biorefinery process among AD and TH and consequently the amount of energy that can be produced.

The qualitative biowaste features such as carbon content, carbon/nitrogen ratio, water content and biowaste structure, were crucial to define the suitability of the biowaste to be processed in biorefinery process to obtain the desired products. For AD process the most important qualitative features were Carbon content, C/N ratio, pH and VS/TS, while for TH process were water and Carbon contents. The qualitative analysis referred to the 14 biowaste already described in Table 1-2 of Chapter 1.

All the considered waste flows exhibited at least 40 % carbon content and carbon - nitrogen ratio was between 10 and 30, which made them suitable feedstocks for 2G

biorefinery systems, according to different sources, e.g. FAO (2018) and Pleissner (2016).The high carbon content of these biowaste flows represented an important renewable carbon resource, which could be valorised and exploited to produce both platform chemicals and bioenergy, significantly contributing to the reduction of the use of non-renewable resources. The main drawback of the employment of biowaste as carbon source was the high-water content 60 – 90 % according to (Demichelis. et al., *in preparation*). On the other side, the organic matter expressed as VS/TS was higher than 80 % w/w for all the considered biowastes, thus thermo-chemical and biological processes were both suitable to maximise biowaste conversion into high added-value products Van Lier, et al. (2008). Biowaste could be integrated into plants and processes, thus producing added value in terms of jobs, investment costs and growth of the bioresource product market Koutinas et al. (2014).

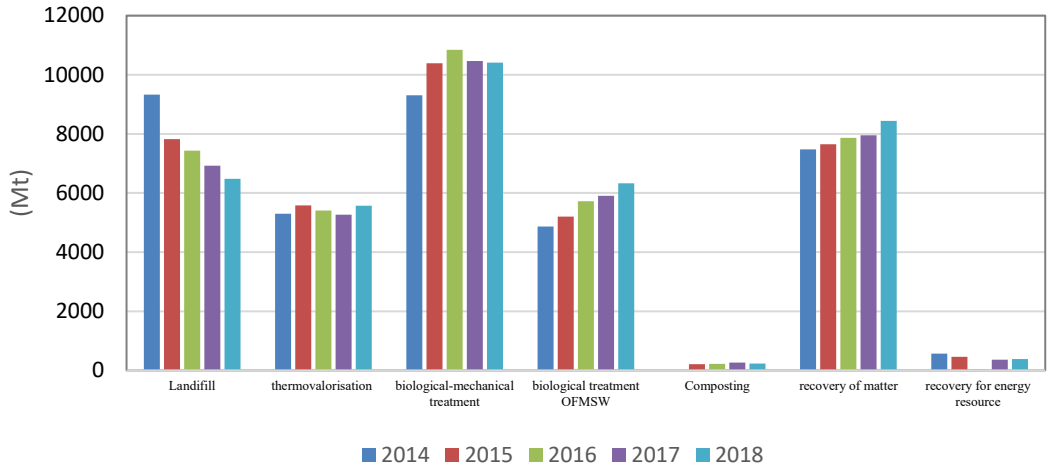
2.3.4 Current biowaste management

Italy's current policies of waste management was based on the 2008/98/EC European Directive, aimed to reduce waste production and its environmental impact, transforming them from cost to economic resource. The priority hierarchy ranges from prevention, preparation for reuse, recycling, recovery (as energy) to disposal.
Another crucial principle applied to waste management were the extension of producer responsibility, which forces the polluter to economically cover the costs of prevention, control and reclamation. The current management and disposal operations referred to the four considered biowaste categories was analysed considering the national reference database (Ispra, 2019) In Italy there were:

- 21 AD plants (90 % in the North) treating 0.7 Mt/y of biowaste;
- 31 facilities combining anaerobic and aerobic processes (89 % in the North) treating around 4 Mt/y of biowaste; 5.4 Mt/y of waste (i.e. MSW and the dry fraction selected by mechanical-biological plants).
- 274 composting facilities (61 % in the North) treating over 4.1 Mt/y of biowaste;

WSS management consisted mainly in landfilling (24 – 62 %), followed by incineration (32 - 50 %); OFMSW management consisted in mechanical biological treatment (38 – 72 %), incineration with energy recovery (16 – 52 %) and AD (7 – 32 %). In Valle d'Aosta 100 % OFMSW underwent composting.
AFF and IFB management consisted mainly in energy recovery (20 - 57 %).
The Italian biowaste scenario was consistent with current EU28 waste management (Eurostat, 2018): for WSS 30 – 40 % landfilling, followed by incineration with energy recovery; for MSW, in general mostly recycling (30 – 65 %), for AFF, mainly incineration with energy recovery (17 - 60 %).
Data for IFB management were not available. The Italian waste management for the period 2014-2018 was reported in detail in Figure 2-6 (Ispra, 2019)

Figure 2-6:Waste management in 2014-2018 (Ispra, 2019)



2.3.5 Technical and environmental assessment of biowaste valorisation through biorefinery processes.

The potentiality of energy production of biowaste ranges between 33 and 1135 EJ/y, which corrsponds to 5 - 185 billion barrels of oil, able to satisfy the energy world necessity of 820 EJ/y by 2040 (FAO,2018) Biowaste-derived energy and liquid transportation fuels boosted the mitigation of climate change and the dependency on fossil fuels. In EU28, over 80 % of energy comes from non-fossil resources Maity S, (PartI, 2015) for the following uses (Sirini et al., 2015):

- transport (33.1 %),
- households (25.4 %),
- industrial sector (25.3 %),
- public services (13.6 %)
- agriculture and forestry (2.2 %)

with the aim to produce bioenergy, the technical feasibility of AD and TH were scrutinised for all the 14 considered biowaste flows (Table2-2).

For all the considered biowaste streams, AD reached the highest primary energy production. Even if these values came from stoichiometric calculations, AD resulted more efficiencient for these type of biowaste, because AD required a strict regulation of control paramenters (i.e. pH, temperature, TS and VS), with a more flexible ranges than TH. AD was a suitable process also for biowaste stream with high water content as WSS, more than 97%w/w.

TH required less flexible operational conditions than AD, like: feedstock having LHV of at least 2,000 kcal/kg, water content below 40 %, 30 % w/w mass reduction and patogens stabilisation Angelidaki, (2009). Considering the feasibility of AD, the highest performances were achieved by dairy waste with 13.6 kWh/kg, milking waste with 12.9 kWh/kg and olive and oil waste with 11.6 kWh/kg.

This trend was due to biowaste composition, in fact the high amount of proteins and lipids could increase methane production and consequently energy production Agriregionieuropa (2017).

The environmental assessement (Table2-3) compared AD, TH and current biowaste mangement in Italy in terms of CO_2 equivalent emissions and CO_2 avoided emissions. To achieve a coherence and robust results, a period of four years (2012 - 2016) was considered Alexandri, (2017). For all biowaste streams, the environmental benefit was reached by AD since it was a CO_2 neutral process.

However, among the four biowaste categories, the highest CO_2 emissions derived from AFF, due to the large use of fertilisers and pesticides Sirini et al. (2015).

The main findings of the technical and environmental assessments were the following: compared TH and AD:

- AD achieved the best performances in terms of produced energy and avoided CO_2 emissions.
- The average values of the primary energy production of AD and TH for the four bio-waste categories (expressed as average and standard deviation of the 14 biowaste streams) were: for WSS 4.6 vs. 2.4 kWh/kg; for OFMSW 6.7 vs. 2.6 kWh/kg; for AFF 7.95 ± 0.42 vs. 3.43 ± 0.25 kWh/kg and for IFB 8.9 ± 0.98 vs. 6.31 ± 0.72 kWh/kg.
- The avoided CO_2 emissions increased in a range between 10 and 89.9 %, according to waste biomass categories, with the adoption of AD instead of TH.
- AD, compared to present biowaste management, may reduce CO_2 emissions in a range between 72.2 and 98.9 % depending on the biowaste category.

Hence, to sum up, the present study proved AD as a promising process both by technical and by environmenal persectives.

Table2-2 Technical assessment of the feasibility of anaerobic digestion (AD) and thermo-chemical (TH) biorefinery processes on the considered biowaste flows (SBP: specific biogas production; LHV: lower heating value)

	Biowaste	**Formula**		**AD**			**TH**	
			C/N	SBP (Nm^3/kgsv)	CH_4 (%)	Primary Energy (kWh/kg)	LHV (kcal/kg)	Primary Energy (kWh/kg)
WSS	wastewater and sewer sludge	$C_{19}NH_{40}O_{11}$	16.7	1.0	60.0	4.6	2118	2.46
MSW	OFMSW	$C_{22}NH_3O_{12}$	18.8	1.0	53.0	6.7	1912	2.22
AFF	rice waste	$C_6NS_1H_{11}O_6$	5.1	0.7	44.0	5.9	3570	4.14
	animal manure	$C_{32}NH_{49}O_{20}$	27.7	1.0	53.0	2.52	2000	2.32
	corn-wheat-waste	$C_{85}NS_4H_{187}O_{65}$	72.7	0.9	55.0	4.8	4017	4.66
	fruit and vegetable from agro-waste	$C_{40}NH_{66}O_{37}$	34.4	0.8	46.0	18.9	2261	2.62
IFB	winery waste	$C_{29}NH_{41}O_{38}$	24.9	0.9	70.0	8.2	8092	7.39
	milking waste	$C_{11}NSH_{24}O_7$	9.5	0.9	58.0	12.9	4760	5.52
	dairy waste	$C_{11}NSH_{26}O_7$	9.6	0.9	61.0	13.6	4284	4.97
	slaughter waste	$C_{11}NSH_{20}O_4$	9.3	1.0	60.0	9.0	6182	7.17
	processed candies waste	$C_{22}NH_{40}O_{14}$	295.9	0.9	65.0	6.4	2618	3.04
	olive and oil waste	$C_5NH_{14}O_3$	14.1	0.9	63.0	11.6	9996	10.60
	processed fruit and vegetable waste	$C_{345}NH_{596}O_{302}$	18.5	0.9	55.0	6.7	3570	4.14
	spent coffee ground	$C_{47}NH_{71}O_{33}$	40.3	1.1	57.0	8.4	4046	4.69

Table 2-3: Environmental assessment of the feasibility of anaerobic digestion (AD) and thermo-chemical (TH) biorefinery processes on the considered biowaste flows and comparison with current biowaste management in Italy.

	Biowaste	Formula	AD		TH		AD vs TH $kgCO_2$ avoided/kg biowaste	Current management 2012-2016 (Ispra, 2017)
			kg CO_2 emitted/kg bio-waste	kg CO_2 avoided/kg biowaste	kg CO_2 emitted/kg biowaste	$kgCO_2$ avoided/kg bio-waste	[%]	kg CO_2 emitted/kg biowaste
WSS	wastewater and sewer sludge	$C_{19}NH_{40}O_{11}$	0.09	2.02	1.83	1.08	46.5	10.1 ± 1.5
MSW	OFMSW	$C_{22}NH_3O_{12}$	0.074	2.95	1.82	0.98	66.9	3.6 ± 0.1
AFF	rice waste	$C_6NS_1H_{11}O_6$	0.46	2.60	1.38	1.82	29.8	103.4 ± 12.1
	animal manure	$C_{32}NH_{49}O_{20}$	0.16	1.11	1.5	1.02	7.9	
	corn-wheat-waste	$C_{85}NS_4H_{187}O_{65}$	0.65	2.11	1.7	2.05	2.9	
	fruit and vegetable from agro-waste	$C_{40}NH_{66}O_{37}$	0.16	8.32	1.54	1.15	86.1	
IFB	winery waste	$C_{29}NH_{41}O_{38}$	0.59	3.61	1.83	3.25	9.9	11.1 ± 0.6
	milking waste	$C_{11}NSH_{24}O_7$	0.09	5.68	1.71	2.43	57.2	
	dairy waste	$C_{11}NSH_{26}O_7$	0.3	5.98	1.37	2.19	63.5	
	slaughter waste	$C_{11}NSH_{20}O_4$	0.6	3.96	2.01	3.15	20.3	
	processed candy waste	$C_{22}NH_{40}O_{14}$	0.53	2.82	1.58	1.34	52.5	
	olive and oil waste	$C_5NH_{14}O_3$	0.37	5.10	1.79	4.66	8.6	
	processed fruit and vegetable waste	$C_{345}NH_{596}O_{302}$	0.61	2.95	1.75	1.82	38.2	
	spent coffee ground	$C_{47}NH_{71}O_{33}$	3.3	3.70	1.74	2.06	44.2	

2.3.6 Full-scale biorefinery systems in Italy

This section presents five examples of full-scale 2G biorefinery systems in Italy: Novamont, a chemical biorefinery system; Betarenewable and Acea, two biological biorefinery systems; TRM and ENI, two thermo-chemical biorefinery systems.

Novamont is a chemical company founded in 1990 by Montedison Group and based in Novara (Piemonte, North of Italy), while the headquarter is in Terni (Umbria, Centre of Italy) (Novamont, 2018). Novamont works in the bio-plastics sector, both in Italy and in Europe, with the goal of combining chemistry and agriculture in an integrated biorefinery system, thus boosting social, economic and environmental benefits. Novamont manufactures three products from agricultural biowaste: Mater-Bi, Matrol-Bi and Celus-B. Mater-Bi is a thermoplastic biopolymer produced from corn starch, cellulose and vegetable oils. Mater-Bi is biodegradable and compostable according to the European standard UNI EN 13432 and it was employed to produce biodegradable bags for shopping and for OFMSW separate collection. Matrol-Bi is a bio-lubricant oil produced from renewable agricultural feedstocks; it has technical features comparable to fossil fuels and it was biodegradable. CELUS-BI is an ingredient employed in the personal care and cosmetic industries, made up of biodegradable micro-granules; it is used, for example, to produce biodegradable moisturizers, shampoo, foundation cream and lipsticks.

Beta Renewables is a joint venture between Biochemtex, an engineering company of the Mossi Ghisolfi group, the American fund TPG (Texas Pacific Group) and the Danish Novozymes, a leader in bioinnovation. Betarenewable is a 2G biorefinery system located in Crescentino (Piemonte, North of Italy) able to convert agricultural waste from a 70 km radius catchment area into bio-ethanol (Betawrenewable, 2018). The plant produces 40,000 t/y of bio-ethanol for the European market. The project is supported by the European Commission under the VII Framework Program for Research and Development. The technology used to obtain the bio-ethanol involves the integration and collaboration of PROESA® (ethanol production from biomass) which, combined with Cellic® enzymes produced by Novozymes, converted sugars present in the lignocellulosic biomass to obtain alcohol, fuels and other chemicals with lower GHG emissions and competitive costs compared to fossil fuels. PROESA® also produces biofuels that ensure a reduction in GHG emissions close to 90 % compared to the use of fossil fuels; a considerably higher reduction compared to that achieved by first-generation biofuels.

TRM (Metropolitan Waste Treatment), belonging to Iren Group, is a waste-to-energy plant dedicated to the treatment of MSW (TRM, 2018) TRM plant can operate in an electric or cogeneration arrangement, supplying energy for district heating in Turin city: in the first case, the plant produced the energy corresponding to the annual needs of about 175,000 families of three people; in the second case

the plant produced the thermal energy for the annual needs of 17,000 houses of 100 m^2 and the electricity consumed by about 160,000 families. Energy recovery from MSW in TRM plant saves about 70,000 t/y of fossil fuel.

Acea Pinerolese is a multi-utility located in Pinerolo (Piemonte) (Acea Pinerolese, 2018) The Ecological Pole is internationally recognized as a model for OFMSW valorisation (around 60,000 t/y). It was made of five process units: a 75,000 people equivalent wastewater treatment plant (with tertiary treatment in the water line and mesophilic AD in the sludge line), a pre-selection line (to separate the impurities from OFMSW), an anaerobic digestion unit for the OFMSW, a composting facility and a MSW landfill. AD of the OFMSW was a thermophilic process fed with 14 % TS. In the composting plant, the digestate deriving from AD of the OFMSW was mixed with green waste and after three months it became quality compost (Florawiwa®), sold to farmers and floriculturists. The water necessary for AD derived from the wastewater treatment facility. The biogas stored in a gasometer derives from the flows: AD, the sludge line of the wastewater treatment facility and the MSW landfill. A CHP plant, powered by biogas, produced heat partly used for the operation of the plant and partly for the district heating of part of the City of Pinerolo and renewable electricity used by the Ecological Pole and partly sold on the network. Since 2014 part of the biogas is transformed into bio-methane. Acea Pinerolese collaborates with FCA Group's Fiat Research Center, with which it has already completed several projects, including the development of the Biomethair Panda fed with biomethane and bio-hydrogen mixtures obtained from the AD of OFMSW. The ecological pole treats 60,000 t/y of OMSW and 20,000 t/ y of green waste, producing about 6,000 t/y of compost. In 2016, the biogas production was 10,241.50 Nm^3/y, yielding 17.1 GWh /y of electrical energy and 18.8 GWh/year of thermal energy available for district heating and internal heating. With the biogas produced in one year by the Pole it is possible to heat around 2,500 homes and produce electricity for about 5,700 households.

Eni developed waste to fuel processes able to convert OFMSW into bio-oils to be used as fillers in the refining cycle to obtain biofuels (Eni, 2017) Compared to alternative solutions for waste treatment, such as composting or AD, the waste to fuel technology developed and patented by Eni allowed greater energy recovery, up to 80 % of the energy contained in waste, reducing odour emissions and the occupation of areas. Eni had a pilot photo-bioreactor plant at the Ragusa Oil Center (Sicilia, Southern Italy) for the use of CO_2 produced in the field of hydrocarbon extraction through micro-algae, in order to produce 3G- bio-oil and Omega 3. The experimental plant module can capture and exploit about 80 t/y of CO_2 contained in the gas associated with the extraction of oil from wells. The plant, which exploited the process of growth, reproduction and photosynthesis of selected algal strains favoured by concentrated sunlight, was launched on April 2017 and it was currently undergoing biological ramp-up. Eni's Green Refinery project in Porto Marghera, Venezia (Veneto, North-East Italy) was the first case in the world for the conversion of a conventional petrochemical refinery into 2G- biorefinery, able to transform palm oil, spent fried oil and vegetable oil into high quality biofuels. The 2G-biorefinery in Porto Margherita produces four types of fuels: 1) green diesel, 2)

green naphtha, 3) liquid petroleum gas and 4) jet fuel. The technology adopted for the 2G-biorefinery in Porto Margherita was based on the Ecofining^{TM} project, a system developed in San Donato Milanese (Milano, Lombardia) laboratories in collaboration with Honeywell-UOP and then applied to the catalytic hydrodesulphurization section of the Venice refinery. The analysis of petrol and diesel samples have shown that Eni's biodiesel, thanks to 15 % of renewable components, reduces pollutant emissions: up to 40 % compared to conventional un-burnt hydrocarbons and carbon monoxide. Moreover, thanks to a more sustainable production cycle, it contributed to reduce CO_2 emissions by an average of 5 %.

2.4. Conclusions

The aim of this study was the definition of a methodology for the quantitatively and qualitatively assessment of biowaste potential in 2G biorefinery systems in Italy according to Circular Economy strategies. Italy, chosen as representative case study of EU-28 reality, was described through the following key parameters: gross domestic power, climate, demography and density distribution. To evaluate the dimension and localisation of necessary 2G-biorefinery plants, the amounts and geo-localisation of four biowaste categories were estimated: WSS, 4.06 Mt/y, OFMSW, 1.7 ± 0.25Mt/y, AFF, 5.7± 0.23 Mt/y and IFB, 2.6 Mt/y. Physic-chemical features of the considered biowaste streams were suitable for biorefinery processes, since carbon content was more than 40 % and carbon-nitrogen ratio ranged between 10 and 30. Compared to TH, AD achieved the best performances for the production of energy and avoidance of CO_2 emissions.

The main findings of the present study were primary energy production of AD and TH for WSS, OFMSW, AFF and IFB were 7.89 *vs.* 2.4 kWh/kg, 8.7 *vs.* 2.6 kWh/kg, 10.85 *vs.* 5.5 kWh/kg and 12.5 *vs.* 7.8 kWh/kg, respectively.

The avoided CO_2 emissions were increased between 10 and 89.9 %, according to biowaste categories, with the adoption of AD instead of TH.

Compared to current waste biomass management, AD may reduce CO_2 emissions between 72.2 and 98.9 % among the four biowaste considered.

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Chapter 3: Experimental evaluation of lactic acid production from Organic Municipal Solid Waste

The main findings of the current study are already published in:

1. D.Pleissner, F.Demichelis, S.Mariano, S.Fiore, I.Gutiérrez, R.Schneider, J.Venus (2017) Direct production of lactic acid based on simultaneous saccharification and fermentation of mixed restaurant food waste. Journal of Cleaner Production, Vol143, pp 615-623.
2. F.Demichelis D.Pleissner, S.Fiore, S.Mariano, I.Gutiérrez, R.Schneider, J.Venus (2017) Investigation of food waste valorization through sequential lactic acid fermentative production and anaerobic digestion of fermentation residues. Bioresource Technology, Vol 241, pp 508-516.

Part of data came from the following thesis:

1. Simultaneous saccharification and fermentation and anaerobic digestion for production of lactic acid and biogas from food waste, F.Demichelis. Relatori S.Fiore and D.Pleissner. Politecnico di Torino
2. Separate hydrolysis and fermentation and anaerobic digestion for production of lactic acid and biogas from food waste, S. Mariano. Relatori S.Fiore and D.Pleissner. Politecnico di Torino
3.

Abstract

Chapter 3 investigates the simultaneous saccharification and fermentation (SSF) and separated hydrolysis and fermentation (SHF) to produce L (+)-lactic acid (LA) from the organic fraction of municipal solid waste (OFMSW). The aim of Chapter 3 is the optimisation of SSF and SHF. In detail, for SSF the analysis includes 1) the identification of the most suitable LA strain producers: three types of *Lactobacillus sp*. and one type of *Streptococcus sp*. strains, 2) the evaluation of the necessity of autoclavation of the OFMSW and 3) the production of market value L (+)- LA. For SHF the analysis includes: 1) type and loading of enzyme and 2) solid to liquid ratios.

OFMSW is employed as source of carbon and nitrogen to carry out SSF by using for L (+)-LA production.

OFMSW consists of (w/w) 33.5% starch, 14.8% proteins, 12.9% fat and 8.5% free sugars.

In SSF, *Lactobacillus sp*. strains reached a productivity of 0.27-0.53 g/Lh and a yield of 0.07-0.14 g/g of theoretically available sugars, while *Streptococcus sp*. degraded OFMSW more efficiently, achieving a LA production at a maximum rate of 2.16 g/Lh and a yield of 0.81 g/g.

In SHF two enzymes were tested: Stargen and Fermgen to hydrolyze starch and proteins. Hydrolytic performance was investigated according to different solid-to-liquid ratios: 11, 12.5, 20 and 25%, w/w, while enzyme loading investigations were tested only at a solid-to-liquid ratio of 20% (w/w).

Via SHF a yield of 0.33 g_{LA}/g_{FW} (productivity 3.38 $g_{LA}/L{\cdot}h$) was reached with enzyme load equal to 0.32 μg/L of Stergen and fermentation performed with *Streptococcus sp.*

When *Streptococcus sp*. was employed, LA concentration and solid to liquid ratio exhibited a linear relationship. Statistically, per 20g dry OFMSW 52.4 g/L of LA may be produced. Experimentally, per 20% (w/w) solid to liquid ratio of OFMSW (which was the highest solid-to-liquid ratio that could be treated using the applied equipment) 58 g/L of LA was achieved.

Among the investigated four strains, *Streptococcus sp*. efficiently liquefied OFMSW and converted the released nutrients directly into LA without considerable production of other organic acids.

Downstream processing including micro- and nanofiltration, electrodialysis, chromatography and distillation produced a pure 702 g/L of L (+)-LA formulation with an optical purity (OP) of 97%.

3.0 Lactic acid

3.0.1 Lactic acid physical and chemical properties

Carl W. Scheele discovered LA in 1780 in sour milk in 1857, Pasteur's studies defined LA as a metabolic product of lactobacteria. The industrial production of LA started in the United States in 1881, after its first fermentative production performed by the French scientist Frémy Castillo Martinez, (2013).

Lactic acid (LA), chemically known as 2-hydroxypropanoic acid, belongs to Alpha Hydroxy Acids (AHAs) and has formula:

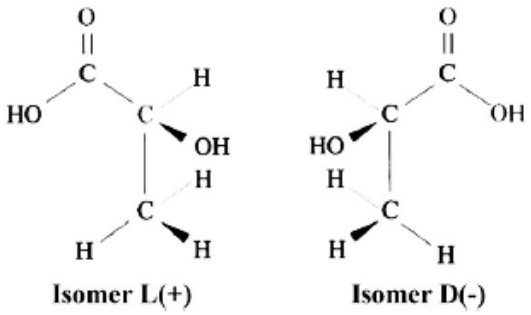
$$CH_3CH(OH)CO_2H$$

It is a white-yellow liquid at room temperature, water soluble and widely distributed in nature. Its conjugate base is the lactate ($CH_3CH(OH)CO-$) and it is employed in biochemical processes.

LA is a chiral molecule and it has two optical isomers, known as L-lactic acid and D-lactic acid (Figure1). The mixture of the two isomers in equal parts was called DL- lactic acid and it is miscible with water and ethanol above its melting

point which is around 17-18° C. L-lactic acid has a higher market-value than D and DL lactic acids

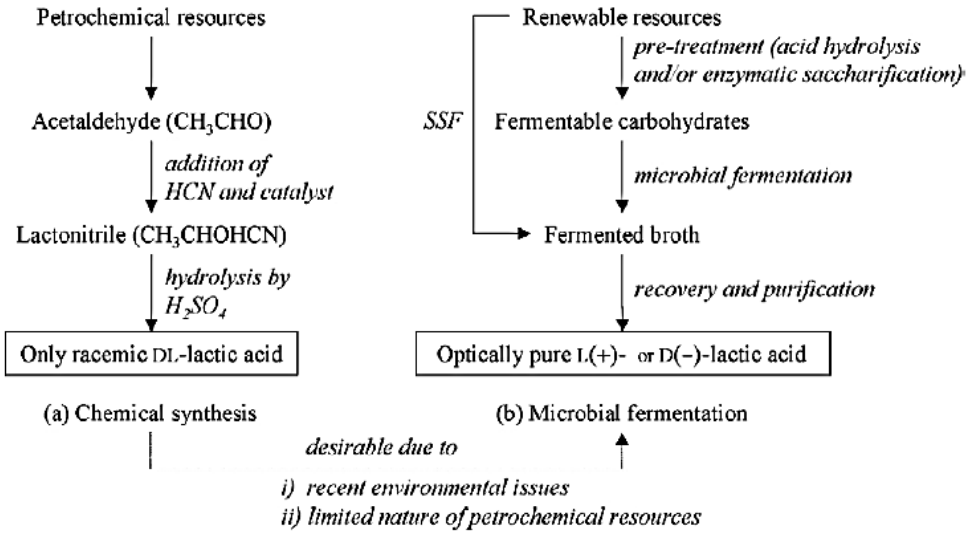
Figure 3-1:Structure of D(-) and L(+) isomers of lactic acid (Castillo Martinez, 2013)



3.0.2 Lactic acid production

LA is a high value compound and it may be produced by microbial fermentation (fermentation of carbohydrates) and chemical method from petrochemical resources Ferreira, (2015) (Figure 3-2).

Figure 3-2:overview of the two manufacturing methods of la:(a) chemical synthesis and (b) microbial fermentation. SSF represents simultaneous saccharification and fermentation (Wee et al., 2006)



Microbial LA fermentation belonged to anaerobic biological process performed with the combination of nutrients, sugars and selected micro-organisms. LA may be produced from renewable resources that can be pre-treated to obtain fermentable carbohydrates. Microbial fermentation has the advantage that by choosing strains, it is possible to produce only one of the two isomers with the market-required optical purity (OP).

LA fermentation must be performed under well-defined conditions of substrate, selected microorganism, temperature ranges, pH, aeration and agitation. However, compared with chemical synthesis of LA, fermentative LA production provides the following advantages Pandey, (2007): 1) low cost of substrates; 2) LA production at low temperature; 3) low energy consumption; 4) high product– L and D lactic acid; and 5) high yields.

3.0.3 Micro-organisms

LA producers are divided in two main groups: bacteria and fungi. In the present study, the attention is only focused on bacteria (Lactic Acid Bacteria, LAB). LAB are the most promising and studied micro-organisms within biorefinery processes, as they can convert waste biomasses into high added value products with high market value (Abdel, 2013).

LAB are named according to their ability to produce LA as the main product of sugar fermentation Holzapfel W. J., (1995), Holzapfel W. J (1997). They are few micro-meters long and their shapes can range from spherical to rod-like and spiral-like. LAB belong to a group of gram-positive anaerobic bacteria, non-sporing, aero-tolerant, acid tolerant, organotrophic and a strictly fermentative rod or coccus and they require amino acid and vitamins for their growth Axelsson, (2004).

LAB are classified about temperature ranges, raw materials used in the LA production and capabilities to produce one specific stereoisomer of the lactic acid, L or D or a mixture of them in various proportions. Temperature ranges, in which LAB can work, are:

- *psicrophilic:* 2°-20°C
- *mesophilic:* 10°C-40°C
- *thermophilic*: 40°-80° C
- *iperthermophilic:*65°- >90°C

According to Kandler and Weiss (1986) LAB can be subdivided about their metabolic attitude into three groups:

- Group I, obligate homo-fermentative;
- Group II, facultative hetero-fermentative;
- Group III, obligate hetero-fermentative.

These subdivisions are based on the principal saccharolytic pathways employed by the species Surendran, (2005), as discussed in the following section.

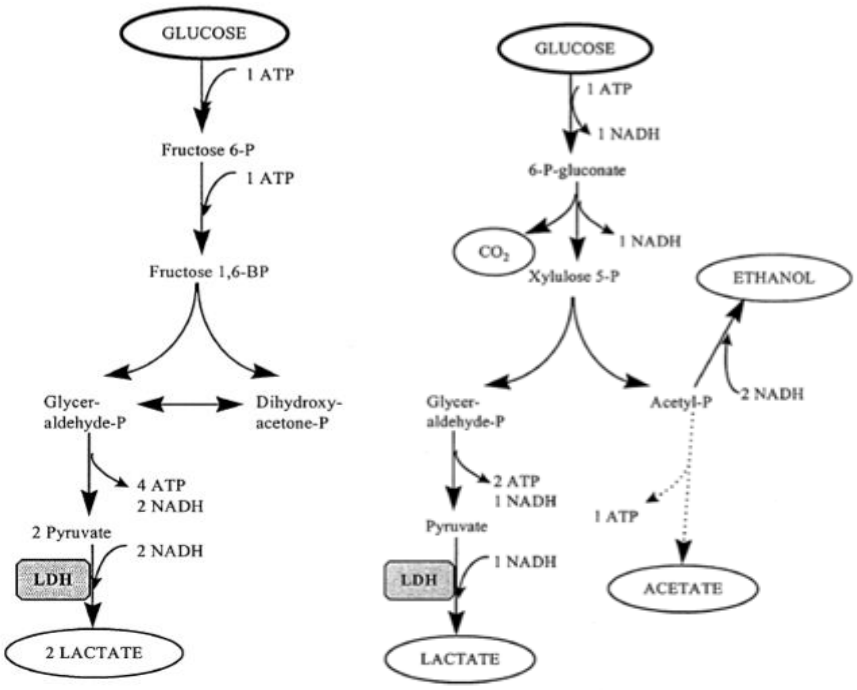
3.0.4 Homo-fermentative and Hetero-fermentative lactic acid fermentation

Homo-fermentative LA fermentation consists in two consecutive steps. In the first step, the glycolysis, glucose is transformed into pyruvic acid. In the second step, pyruvic acid is reduced to LA by the reducing power previously produced in the form of nicotinamide adenine dinucleotide hydrolysed (NADH). The chemical reaction is:

$$Glucose \rightarrow 2Lactic\ Acid + 2\ ATP$$

Micro-organisms that use only this path for the consumption of carbohydrates are called obligatory homo-fermentative. Theoretically homo-fermentative LA fermentation (Figure3-3A) releases two moles of LA per mole of consumed glucose with a theoretical yield of 1g of product per 1g of substrate, but the experimental applications achieved lower yields, because a part of the carbon source is used by the biomass for its production Castillo Martinez E. M.,(2013).

Figure3-3:Scheme of homo-fermentative pathway of glucose(A) and hetero-fermentative pathway of glucose (Hofvendahl & Hahn-Hagerdal, 2000)



A B

The hetero-fermentative LA fermentation produced co-products like CO_2, ethanol and/or acetic acid (Figure3-3B). The first step of the process is called pentose phosphate pathway: the glucose degradation leads to glyceraldehyde 3-phosphate, acetyl-phosphate and CO_2.

Glyceraldehyde 3-phosphate enters the glycolysis through which it was transformed into LA, while acetyl-phosphate was converted into acetic acid and/or ethanol according to the following reactions Castillo Martinez E. M., (2013):

$$Glucose \rightarrow Lactic\ Acid + CO_2 + Ethanol + ATP$$
$$Glucose \rightarrow Lactic\ Acid + CO_2 + Acetic\ acid + 2ATP + 2\ NADH$$

The relation between the amounts of acetic acid and ethanol, which reduced the theoretical LA yield to 0.50 g/g of substrate, changed with the capability of the micro-organism to re-oxidise the NADH, generated in the early stages of the process. Micro-organisms, that use only this metabolic pathway for the consumption of carbohydrates, are the obligatory hetero-fermentative. Main features of LAB are schematically represented in Table3-1

Table 3-1:Main features of microorganism used in LA production

Name	Genus and shape	Substrate	Carbon source	LA isomer	Glucose fermentative	References
L. Casei	Lactobacillus rod	Molasses	Saccharose	L- LA	Homo-fermentative	Buyukkileci & Harsa, 2004; Hujanen, Linko, Linko, & Leisola, 2001;
L.delbrueckii	Lactobacillus rod	Camel milk, cow milk	Lactose	L- LA	Homo-fermentative	Sukumaran et al, 2007;

L. coryniformis	Lactobacillus rod	Corrugated	Glucose	D-lLA	Homo-fermentative	Bustos, Alonso, & Vazquez, 2004;
L. brevis	Lactobacillus rod	Wheat straw	Xylose	L- LA	Hetero-fermentative	Wu-Tai, Driehuis, & Van Wikselaar, 2003
L. helveticus	Lactobacillus rod	Whey	Lactose	L-D LA	Homo-fermentative	Thibault, & Lacroix, 2002
L. plantarum	Lactobacillus rod	Soy fiber	Glucose	L-D LA	Homo-fermentative	Hofvendahl & Hahn-Hagerda, 2000; Yoshida et al. 2011
Lactobacilllus salivarius UCO_979C	Lactobacillus rod	Chemically defined medium13	Glucose	L- LA	Facultatively hetero-lactic	(Valenzuela, Pinuer, Cancino, & Yáñez, 2015

3.0.5 Factors affecting lactic acid fermentation

The most important factors affecting LA fermentations are: 1) substrates, 2) carbon- and nitrogen-source, 3) nutrients, 4) pH, 5) temperature, 6) substrates/products inhibition and 7) fermentation mode.

About substrates, nowadays scientific studies are looking for low-cost feedstocks to be used in LA production through fermentation, to promote the scale up and industrial application of fermentative LA production (Castillo Martinez E. M., (2013).

The main features of alternative less expensive substrates may be low levels of contaminants, rapid production rate, high yield, fermentation with little or no pre-treatment, limited by-product formation, and year-round availability.

About carbon- and nitrogen-source, LAB had complex nutritional requirements Young-Jung Wee, (2006) and they need to be cultured under specific conditions Akaoa,(2007). Nitrogen compounds play an important role in fermentative process. Ammonium ions are a nitrogen source for bacterial growth, and they have an influence on the metabolism of some amino-acids and yeast during fermentation Chen et al. (2020). They are present as amino acids, peptides and inorganic compounds that can be added to the culture media as peptone, yeast extract, urea or ammonium sulphate. The cost of nutrients is one of the main bottlenecks for the competitive bio-technological production of LA. During the fermentative LA production, substrate inhibition may occur.

Substrate inhibition can be due to two main factors: carbon source and microorganism. High concentration of carbon source can inhibit two main activities: LA fermentative production and LAB growth Gonçalves, (1991). Inhibition frequently took place in batch conditions performing separate hydrolysis and fermentation (SHF).

The substrates usually adopted for LA production are divided in two groups: monosaccharide/disaccharides and polymeric substrates such as whey, molasses, starch and lignocellulose Castillo Martinez E. M., (2013).

Starch is present in foods such as potatoes, wheat, maize, rice and cassava and it has amylose and amylopectin from 20% to 25% and from 75% to 80% by weight respectively (Brown, 2005).

From starch, it is possible to produce glucose thanks to a preliminary liquefaction of the material Castillo Martinez E. M., (2013).

Whey is a by-product of the dairy industry, which contains about 5% (w/v) lactose, 1% protein, 0.4% fat, mineral salts, water-soluble vitamins and other essential nutrients for micro-organism growth Koller, (2007).

Molasse, easily available and cheap, is a by-product of the refining of sugarcane and sugar beet into sugar, which was composed by water, 50% of sugars (sucrose, glucose, fructose and raffinose), nitrogen compounds, organic acids and amino acids Kotzamanidis, (2012).

Lignocellulose is present in wood, paper waste and plant material. It was mainly composed of cellulose, hemicellulose and lignin, which form 90% of the total dry matter Harmsen, (2013). Lignocellulose requires enzymatic hydrolysis to realize the sugars used in fermentation Abdel-Rahman, (2011).

LA production entails decrease of pH and acidification of the medium, which consequentially inhibited the fermentation process, thus pH was kept around 5.5-6.5 by addition of base substances such as NaOH. The optimal working pH is generally chosen according to the kind of LAB used.

Temperature is one of the most influencing parameters in fermentation processes. The most adopted temperatures are: 35°C for mesophilic bacteria and 52°C for thermophilic ones. Fermentation processes performed at thermophilic temperatures prevented cost efficient sterilisation phases Jiang et al. (2019).

During LA fermentation, specifically in hetero-fermentative lactic acid fermentations, secondary products, as formic acid and acetic acid, can be generated and they can halt microorganism activity. Secondary products may cause the drop of pH towards acid values, not suitable for bacteria work conditions. Furthermore, LA itself could represent an inhibitory product over certain concentration Loubiere, (1997). Fermentative technologies are currently optimised to remove inhibitory products from the medium at the same time in which they are released, to prevent inhibition problems Komesu et al. (2018)

Fermentation mode is another important factor that influences the fermentative LA production. The possible configurations are batch, fed-batch and continuous fermentations. The main difference among the three kinds of reactors concerns the inflow and outflow systems. Batch fermentation was adopted in the present study, employing a partially closed system in which the required material was feed into the reactor before the process starts and it was removed at the end of the process. The only mass flows in/out during a batch fermentation were the gas exchanged and eventually pH control solutions.

Batch system is therefore an unsteady-state system, although a well-mixed reactor, whose features are supposed to be uniform throughout the reactor at any instant time.

In a batch reactor, bacteria growth is characterized by four phases Stanbury, (2000)

a) Lag phase: a period of adaptation of the micro-organisms to their new environment with a minimal increase in cell density. It may be absent in some fermentations;
b) Exponential phase: it was also known as logarithmic phase. In this step cells had adjusted to their new environment and they were dividing at a constant rate resulting in an exponential increase of their number. This was known as the specific growth rate and it was mathematically represented by a first order kinetic model:

$$\frac{dx}{dt} = (\mu - k_d) \cdot x$$

where x was the cells concentration, μ was the cell growth rate and k_d was the cell death rate. The cell death rate was sometimes neglected if it was considerably smaller than cell growth rate. Cell growth rate was substrate limited and it followed Monod kinetic.

During cell growth the following problems may occur:

- Substrate inhibition: in batch fermentation, this can occur during the initial growth phase when substrate concentration was high. To overcome this problem, continuous or fed-batch should be employed.
- Products inhibition

c) Stationary phase: It occurs when the number of cells dividing and dying was in equilibrium and can be the result of the following:
    - Depletion of one or more essential growth nutrients;
    - Accumulation of toxic growth associated by-products;
    - Stress associated with the induction of a recombinant gene.
d) Death phase: it was also called decline phase and it is expressed through a first order kinetic:

$$\frac{dx}{dt} = -k_d \cdot x$$

The biggest two advantages of batch fermentation mode are:

1) Batch is a close system which can prevent the risk of contamination of substrate
2) LA concentration obtained in batch mode was higher than the LA concentration obtained in continuous and feed-batch mode Halm-Hagerdal, (2000).

However, the two main disadvantages of batch process are the presence of down time and the low cell concentration. Unproductive time was due to operations like loading, sterilization, discharge, second sterilization to prevent unhealthy environmental condition- cleaning of the system and re-start process (Ferreira, (2015). The limited amounts of nutrients may reduce cell concentration boosting the formation of inhibitors Abdel, (2013). Usually a fermentation process required mechanical mixing, which is essential to achieve an optimal contact between substrate and bacteria and to enhance heat and mass transfer in the medium.

The last important factor affecting LA fermentation is sterilisation, which eliminated all the pathogenic and undesirable micro-organism presented in the medium. There are different sterilisation procedures, such as moist heat, dry heat, irradiation, filtration and chemical methods. In the present study moist heat sterilisation was performed.

3.0.6Separate hydrolysis and fermentation and simultaneous saccharification and fermentation

LA production can be studied adopting two different biological pathways: Separate Hydrolysis and Fermentation-(SHF) and Simultaneous Saccharification and Fermentation (SSF) (Figure3-4 and Table3-2).

SHF was performed in two separated steps: hydrolysis and fermentation. Hydrolysis consisted in the breakdown of chemical bonds by the addition of water and this kind of reaction modifies the pH of the environment in which it occurred. The process through which carbohydrates are broken into sugar molecules by hydrolysis, is called saccharification.

There are different kinds of chemical hydrolysis: salt hydrolysis, basic hydrolysis, acid hydrolysis and enzymatic hydrolysis.

Enzymes are macromolecular biological catalyst and usually most of them are proteins. They are added to accelerate or catalyse the reaction, because they can improve the rate of the process by lowering its activation energy. In the present Chapter and following Chapters 4,5,6 acid and enzymatic hydrolysis are studied.

Enzymatic hydrolysis consisted of the addition to the medium of enzymes (Glucoamylase, Cellulase and Protease) to degrade the substrate and produce glucose and free amino nitrogen. Then, the glucose released was used by LAB during the fermentation process to convert glucose into LA. In SHF, hydrolysis and fermentation processes required different temperatures and pH conditions, so the complete process (hydrolysis plus fermentation) need to be set up twice.

Enzymes are essential macromolecular catalysts, produced by living organisms, able to interact with substrate and speed up its degradation, because they accelerate chemical reactions by providing an alternative reaction pathway of lower activation energy. All enzymes are proteins, composed by a long, specific string of amino acids. Their peculiar tri-dimensional shape, which was called active site, made them highly selective and able to treat specific matter; thus, there were different kinds of enzymes.

Reactions with enzymes need specific setting of physical parameters, such as pH, temperature and enzyme concentration.

Usually, each enzyme can work in a specific range of pH; however, too high or too low pH is harmful for them Temperature increase results in high degradation rates, but if temperature is too high, the structure of the enzyme breaks down. Generally, the optimal temperatures for enzyme actions is around 35°- 37°C. Enzyme concentration influences the rate of catalyst reaction, depending also on

enzyme concentrations. If the concentration of enzyme increases, the degradation performances are augmented, until the achievement of steady state conditions and from that moment on, the addition of enzymes is not necessary anymore. Enzyme addition increases the operational cost of SHF, thus, it was necessary to evaluate the costs-benefits ratio to consider the ideal amount of enzyme to be added in the process.

As already mentioned, in nature there is a huge variety of enzymes and each of them are different from the others according to its peculiar string of amino acids.

However, enzymes can be grouped in three big categories, depending on their function and on the substrate they can degrade.

In Chapter 3,4,5 Glucoamylase, Protease and Cellulase were analysed.

Glucoamylase is used for saccharification of starchy material to glucose for creating a feedstock, enhancing biological fermentation processes and eventually producing a valuable material as i.e. ethanol or LA. These enzymes can hydrolyse the glycosidic bonds inside the starch molecules. Glucoamylase hydrolyses the α-1,4 glycosidic bonds of the single glucose units from non-reduced ends of starchy molecules Pavezzi, (2008). Different micro-organisms can produce glucoamylase; for industrial production mainly, fungi were exploited. Ones of the most employed are *Aspergillus awamori*, *Aspergillus niger* and *Rhizopus oryzae*, which were considered Pavezzi, (2008). Glucoamylase is formed by two components: GA1 and GA2. The former contained both starch-binding domain that let glucoamylase be absorbed into starch material and the active site in which hydrolysis took place (Nyamful, 2013).

Protease defines a class of enzymes able to break peptide bonds and release free amino nitrogen

Catalysis occurs according to different mechanisms and proteases was divided in five classes: 1) theorine, 2) aspartic, 3) cysteine,4) metallo and 5) serine proteases (Neitzel, 2010). Considering the latter one, during hydrolysis, a serine group was used as an active site to break, in two following steps, the peptide bonds. A molecule of water is added in order to break the bond and to obtain one peptide group and one amino acid group.

Cellulase works with cellulose by breaking β-1,4-glucosidic bonds and by releasing glucose. Cellulase was mainly produced by fungi and bacteria; and the most used fungi for cellulase production were *Trichoderma ressei* and *Trichoderma viride*. Due to the difficulties in hydrolysing lignocellulosic material, the cellulose and hemicellulose degradations required pre-treatments of the substrate (Young-Jung Wee et al., 2006)

Commercial cellulase formulations are generally composed of several enzymes and the most important is Young-Jung Wee et al. (2006) Endoglucanase, which is responsible for the production of free chain-ends (by adding a molecule of water in regions of low crystallinity and so by cleaving cellulose in its internal regions). It is also worth mentioning:

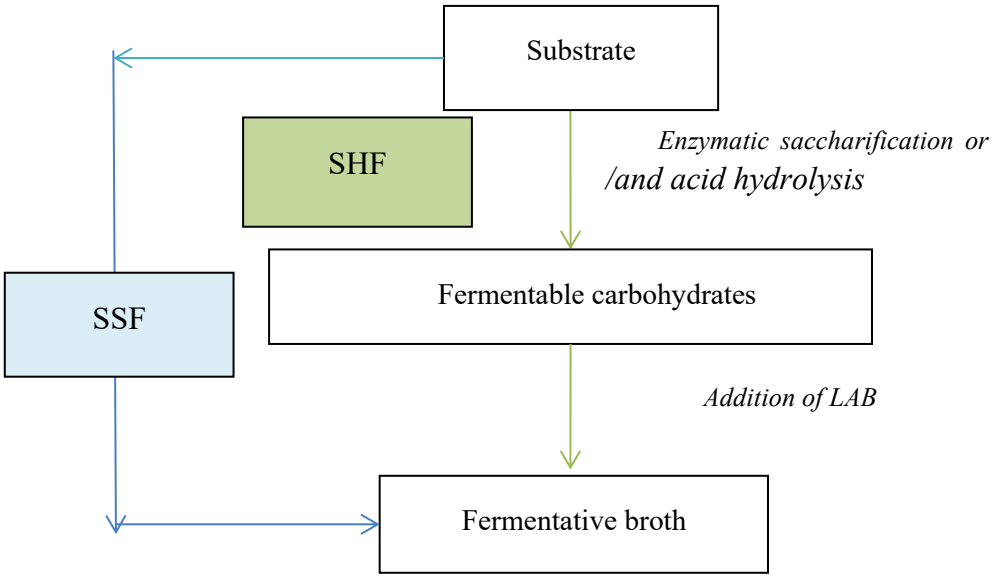
1) Exoglucanase or Cellobiohydrolase, which released cellobiose found in the free chain-ends

2) β-glucosidase that finally converted cellobiose in glucose by cleaving the intramolecular bonds.

To reach higher conversion degrees from hemicellulose and cellulose into glucose, it would be preferable to pre-treat the material with physical, physic-chemical, chemical or biological processes.

Otherwise, SSF process consists in a single step LA production from polysaccharide material. SSF was composed by a preliminary hydrolysis of the substrate to monosaccharides (saccharification) and then fermentation. In SSF saccharification and fermentation occured under the same operational conditions in a single reactor, reducing the investment cost for processing plants and for the same reason less energy and cooling water are required Lee, (2004). SSF partially solved the problems concerning inhibition substrate, because microorganism consumed glucose at the same rate it was formed, which reduces the substrate inhibition and, consequently, the enzyme loading and the risk of external contamination. Castillo Martinez E. M. (2013); Rygielska, (2015)

Figure 3-4:Scheme of SHF and SFF process



The most common kinds of SSF employed the following operations Ramalingam, (2015):

a) saccharifying enzymes and a fermenting microbe were added simultaneously to the substrates. This was adopted in the present study.
b) a saccharifying microbe and a fermenting microbe were added simultaneously to the substrates.
c) a single microbe was used. It can saccharify the substrate and ferment the intermediate sugars. This was the procedure adopted in the present study.

Table 3-2:Comparison between SHF and SSF

	SHF	SSF
Process steps	Two steps process: hydrolysis plus fermentation	One step process: hydrolysis and fermentation take place in the same reactor
Set up conditions	-Temperature and pH for hydrolysis; -Temperature and pH for fermentation The process requires more time, but it is easier work at different temperature for different phases, because each phase works under its optimal condition.	-Temperature and pH are the same for hydrolysis and fermentation Less time is required to set parameters, but it is more difficult find the suitable temperature for two different processes
Inhibition of substrate	Completely hydrolysation of substrate, so the sugar concentration is very high	Not completely hydrolysation of substrate, so inhibition is avoided. Glucose is realized at the same time lactic acid is produced
Energy and costs	Energy and costs are related to two separated steps	One step process reduces energy cost and capital cost for the reactor

At the end of fermentation, the fermentative broth must be purified to separate out LA and to eliminate impurities such as salts, residual sugars, color, nutrients, bacteria and other organic acids. Purification and recovery processes (conventionally named downstream processes) depended on the desired quality of the final product. LA is commercially available at different levels of quality: ranged between 20–90 %, with food-grade, pharmaceutical-grade and plastic-grade products at the top values. Vijayakumar, (2007). Purification process had many drawbacks, because it represented more than 50% of the LA production costs and the use of chemicals caused environmental pollution Demichelis et al (2018).

Purification is a multi-step process: reactive extraction, membrane separation, ion exchange, electrodialysis and distillation were accomplished in a conventional downstream process. Reactive extraction is performed using aliphatic ammines. They are used to extract LA as an acid-amine complex and then they are stripped.

Membrane separations could replace the conventional solvent extraction, offering three main advantages: no back mixing, no direct exposure of microbes to extraction reagents (thereby ensuring biocompatibility), no need for mixing and potentially high efficiency Unrean, (2018) Wasevar, (2005). Polymeric and ceramic membranes may be employed, requiring high pressure in both cases. Polymeric membrane was cheaper than ceramic one, but the latter was preferable at high temperatures.

Ion exchange technique employed resins to remove ions and particles which color the LA on the grounds of the physical principle of adsorption.

Electrodialysis was a process where ion exchange membranes removed ions from an aqueous solution under the driving force of an electrical field. It was applied to remove salts from solutions or to concentrate ionic substances Unrean, (2018) Wasevar, (2005). Usually, distillation was used as a last step to separate LA and the demineralized water added during the downstream process.

3.0.7 Commercial applications of Lactic acid

The current applications of LA involve five categories: food, cosmetic, pharmaceutical, chemical industries and chemical feedstocks. The optically pure LA was increasingly used as a renewable bio-based product to replace petroleum-based plastics Castillo Martinez E. M., (2013).

LA is the monomer in the production of biodegradable PLA, which is well-known as a bioplastic. The worldwide demand for LA is estimated roughly to be 130,000 to 150,000 metric tonnes per year Zhou et al., (2016) Naveena, (2005) and it is expected to increase rapidly. PLA had several applications, for instance protective clothing, food packaging, mulch film, trash bags, rigid containers, shrink wrap, and short shelf-life trays Young-Jung Wee, (2006) Vink, (2003); Jong, (2011).

3.1 Introduction

The aim of Chapter 3 is the development and optimisation of process for the direct conversion of organic municipal solid waste (OFMSW) into lactic acid (LA) by means of simultaneous saccharification and fermentation (SSF) and separated hydrolysis and fermentation (SHF).

For this purpose, three thermophilic *Lactobacillus sp.* strains and one mesophilic *Streptococcus sp. strain*, all isolated from various substrates at the Leibniz Institute of Agricultural Engineering and Bioeconomy Potsdam, Germany, were tested. The preliminary study of microorganisms was performed in 500 mL Erlenmeyer shaking flasks, to degrade organic material and to produce L (+)-LA. Furthermore, different solid-to-liquid ratios (5, 10, 15, 20 %w/w) of OFMSW were tested at laboratory scale (2 L) to identify its effect on LA production. No sterile OFMSW undergone to 2L SSF test to evaluate the difference of LA production under sterile and not sterile conditions. Furthermore, SSF of OFMSW was tested at technical scale (50 L) test a possible industrial application and scale up. Finally, to obtain a LA formulation with market value, downstream processing, including filtration, electrodialysis, ion-exchange and distillation, was carried out for pure.

In SHF process was optimised the hydrolysis step, in details two enzymes were tested Fergen and Stargen at different loading rate, respectively from 0.11-3.50 µL/g and 0.32-5.00 µL/g under four solid to liquid ratios from 10-25%w/w

The novelty of the approach adopted in the present study was the conversion of OFMSW into LA through two different fermentative pathways SSF and SHF

3.2 Material and methods

3.2.1. Organic fraction municipal solid waste

Organic fraction municipal solid waste (OFMS) was daily collected for 15 days at the canteen of the Leibniz Institute of Agricultural Engineering and Bioeconomy Potsdam and it contained noodles, potatoes, vegetables, rice, fruits, meat and sauce. Immediately after collection, the wasted food was homogenized using a kitchen blender and the blend stored at −20°C until used in experiments. All food waste was pooled and homogenized

3.2.2 Simultaneous saccharification and fermentation

3.2.2.1 Micro-organisms

Four micro-organisms were tested: three thermophilic *Lactobacillus* sp. strains: A28a, A59 and A211 isolated from straw hydrolysate, rye corn and rye biomass, respectively, and one mesophilic *Streptococcus* sp. strain: A620 (internal labels) isolated from tapioca starch were employed in experiments. Classification was carried out by the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany).

All the four strains were produced in 300 mL Erlenmeyer shaking flasks, containing 60 mL of MRS broth (Merck, Germany) and 0.67 g Everzit Dol (Evers, Germany) dolomite as buffer. Autoclavation of Erlenmeyer shaking flasks containing MRS broth was carried out at 118°C for 15 min. Thermophilic strains were incubated at 52°C for 14–16h, while the mesophilic strain was incubated at 35 C for 24h. The initial pH in all flasks was 6. Flasks were shaken at 100 rpm in an orbital shaker (CERTOMAT® H incubation shaker B-Braun Biotech, Germany).

3.2.2.2 Laboratory scale SSF

For all laboratory SSF a 2 L BIOSTAT bioreactor (Sartorius AG, Germany) containing 1L of blended OFMSW was employed. The blended OFMSW was autoclaved at 118 C for 15min. SSF was respectively carried out at 35°C and 52°C for the mesophilic and thermophilic strains and at pH 6. Stirring occurred at 200 rpm using a double Rushton turbine. Regulation of pH was carried out by adding 20% (w/w) NaOH. A 6% (v/v) inoculum was used in all fermentations, as standard procedure at Leibniz Institute of Agricultural Engineering and Bioeconomy Potsdam.

To compare the performances of strain, SSF was carried out using blended OFMSW with a solid-to-liquid ratio of 10% (w/w). After the evaluation of the most

suitable strain, the mesophilic *Streptococcus* sp. strain A620, different solid to liquid ratio were tested: 5, 10, 15 and 20% (w/w). Solid-to-liquid ratio was obtained by adding demineralized water to the blended OFMSW. Finally, SSF was investigated in duplicate under non-sterile conditions at a solid-to-liquid ratio of 20% (w/w) using *Streptococcus* sp. strain A620. Samples were taken regularly for the analysis of sugar (glucose, fructose and sucrose), lactic and acetic acids concentrations. Samples were inactivated by heating at 95°C for 20 min. After inactivation, samples were stored at−20°C until used in analysis. Mean values are presented for all fermentations carried out in duplicate.

3.2.2.3 Technical scale SSF

SSF using *Streptococcus* sp. strain A620 was carried out at technical scale in a 50 L BIOSTAT UD bioreactor (B-Braun Biotech, Germany) containing 40 kg of sterilized and blended food waste with a solid-to-liquid ratio of 20% (w/w). Fermentation was carried out at 35 C and pH 6. Stirring occurred at 400 rpm using a double Rushton turbine. Regulation of pH was carried out by adding 20% (w/w) NaOH. A 5% (v/v) inoculum was used, according to Leibniz Institute of Agricultural Engineering and Bioeconomy Potsdam.

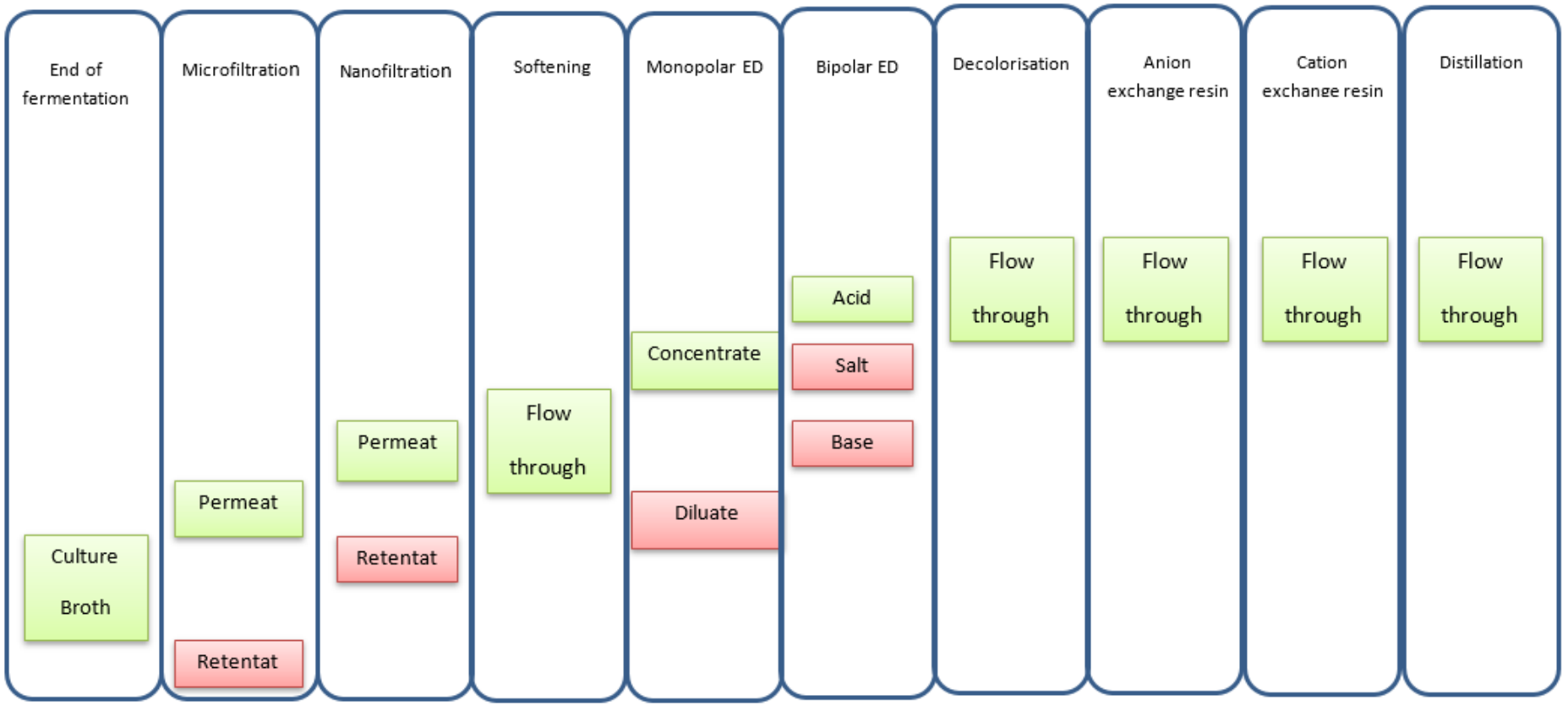
The inoculum was grown for 17h in a 5L fermentation vessel containing 2L of medium consisting of 66 g/L dextrose monohydrate and 15 g/L yeast extract inoculated with 120 mL MRS culture.

Samples were taken regularly and treated as described in Section 3.2.2.2. After fermentation, culture broth was inactivated at 85°C for 30min and stored at−20°C until used in downstream processing.

3.2.2.4 Downstream processing

Downstream processing included micro- and nanofiltrations, softening, mono- and bipolar electrodialysis, purification through anion- and cation-exchange resins, and distillation. The methods were explained in detail in Figure3-5.

Figure 3-5: Standard downstream processing procedure. The first columns represent the process step starting from left to right. ed (electrodialysis). last column represents the outcome of the process step and indicates which component was used in the following step.



3.2.3 Separate hydrolysis and fermentation

3.2.3.1 Enzymatic hydrolysis

Enzymatic hydrolysis tests were carried out without repetitions in presence of 1 L OFMSW in a 2 L BIOSTAT bioreactor (Sartorius AG, Germany). Stargen and Fermgen (Genencor International, The Netherlands) were employed to hydrolyze starch and proteins at 59°C and pH 4.5 for one hour, respectively. Hydrolytic performance was investigated according to different solid-to-liquid ratios: 11, 12.5, 20 and 25%, w/w and enzyme loading Enzyme loading investigations were tested only at a solid-to-liquid ratio of 20% (w/w). Mixing was set between 400 and 800 rpm depending on viscosity of the OFMSW. Samples were withdrawn, then inactivated at 95°C for 20 minutes, centrifuged at 5000 RPM for 10 minutes and the supernatant was stored at -20°C until used in analyses.

Yields of glucose and Free Ammino Acids (FAN) per gram of dry food waste (Y, g/g) was calculated with Eq 1

$$Y = P / FW, \tag{1}$$

where P (g)is the release in glucose or FAN

OFMSW the amount of food waste applied (g).

3.2.3.2 Lactic acid fermentation

LA fermentation was carried out in duplicate using a 2 L BIOSTAT bioreactor (Sartorius AG, Germany) containing 1 L of OFMSW with a 20% (w/w) solid-to-liquid ratio. After enzymatic hydrolysis (section 2.3), the reaction parameters were changed from 59°C to 35°C and from pH 4.0 to pH 6.0.

A 6% (v/v) *Streptococcus* sp. strain A620 inoculum was used. Samples were analysed for sugars (glucose, fructose and sucrose) and LA concentrations. Results are presented as mean values of two replicates plus the standard deviation. After LA fermentation, solids and the oily phase were separated through centrifugation, and the supernatant was afterwards inactivated at 95°C for 20 minutes and stored at -20°C. The residual solids were mixed with the oily fraction floating on the supernatant and employed as secondary raw feedstock for anaerobic digestion (AD) tests.

3.2.4 Analytics

Total number of cells was determined using a THOMA cell chamber (Glaswarenfabrik Karl Hecht GmbH & Co KG, Germany) and number of living cells was determined as colony forming units counted on a plate containing Nutrient Agar (Merck, Germany) after 24 h of incubation at 52 C for the thermophilic *Lactobacillus* sp. strains and 35 C for the mesophilic *Streptococcus* sp. strain.

To determine the dry matter of blended food waste, a certain amount was weighed and dried at 105 C until constant weight. Afterwards a certain amount of dried blended food waste was weighed and combusted at 550 C for 5h in a muffle furnace. The weight of remaining ash was subtracted from the dry matter in order to obtain the organic fraction of dry matter.

Lactic acid and sugar concentrations in fermentation samples were analyzed by high performance liquid chromatography (DIONEX, USA): 10µL of sample volume was added on a Eurokat H column (300mm×8mm×10µm, Knauer, Germany) and eluted isocratically with 0.8mL/min of 5mM H_2SO_4. Detection was carried out by a refractive index detector (RI-71, SHODEX, Japan). Each analysis was carried out in duplicate and peak areas and retention times were compared to analyses of known concentrations of pure lactic acid, glucose, fructose and sucrose.

Cat- and anion concentrations in fermentation samples were analyzed by ion chromatography (DIONEX, USA). For quantification of cations, 25 µL of sample volume was added on an IonPac CS 16 column (250mm × 4µm, DIONEX, USA) and eluted isocratically with 1.0 mL/min of 30mM CH_3SO_3H at 40°C. For quantification of anions, 25µL of sample volume was added on an IonPac AS9-HC column (250 mm×4 µm, DIONEX, USA) and eluted isocratically with 1.2 mL/min of 9 mM Na_2CO_3 at room temperature. Detection of cat- and anions was carried out by a conductivity cell. Each analysis was carried in duplicate and peak areas were compared to analyses of known concentrations of salt-solutions consisting of cat- and anions of interest.

The ratio of the optical isomers in the lactic acid formulation was checked using HPLC (KNAUER, Germany) coupled with a Chiralpak®MA(+) column (DAICEL, Japan, 50 mm × 4.6mm×3µm) and an ultraviolet detector. The mobile phase was 2mM $CuSO_4$ and the flow rate 0.8 mL/min.

Fat analysis was performed by means of ANKOM Technology (USA) according to the ANKOM Technology Method 2, 01-30-09: Determination of Oil/Fat Utilizing High Temperature Solvent Extraction (ANKOM, 2009).

Sugar content determination was carried out by cold water extraction. To 3–5g of dried blended food waste 50mL of demineralized water was added, and the mixture shaken for 30min. Afterwards 2mL of a 30% (w/w) $ZnSO_4$ solution and 2mL of a 15% (w/w) $C_6N_6FeK_4$ solution were added. After shaking, the mixture was filtrated, and the clear filtrate analyzed by HPLC as described above.

The theoretical amount of sugar was calculated from the sugar content of the blended food waste and the starch content. A conversion factor of 1.111 g glucose per g starch (obtained by dividing the molar mass of glucose by the molar mass of one starch unit, 180.16 g/mol-162.16 g/mol) was used.

Kjeldahl-nitrogen (Kjeldahl-N) content of blended food waste was determined according to the DIN-EN-25663 standard method. Protein content was calculated by multiplying the Kjeldahl-N content with 5.7 (Leung et al., 2012).

Free amino nitrogen (FAN) concentration was measured using the ninhydrin reaction method described earlier (Lie, 1973). Glycine was used as standard.

3.2.5 Elaboration data analysis: evaluation of LA

Productivity (P), yield (Y) and turnover (T) were the main parameters used to describe the fermentation processes. The evaluation of the quality of the produced LA concerned the optical purity for L (+)-lactic acid (OPL/D).

The fermentation volume was considered as the liquid phase of the fermentation broth. The liquid phase consisted of homogenised OFMSW, inoculum and base addition to control the pH.

Productivity was calculated for the total fermentation process (P_{tot}) Eq.1

$$P_{tot}\left(\frac{g}{Lh}\right) = \frac{C(LA)_{end} - C(LA)_{start}}{\Delta t_{tot}} \tag{1}$$

where:

$P_{tot}\left(\frac{g}{Lh}\right)$is the total volumetric productivity;

$C(LA)_{start}$ $\left(\frac{g}{L}\right)$is the concentration of the LA at the starting point of fermentation t=0 s

$C(LA)_{end}\left(\frac{g}{L}\right)$is the LA concentration at the end of fermentation.

$\Delta t_{tot}[h]$ is the duration of fermentative process $t_{end} - t_{start}$.

For the calculation of LA yield (Y), the adopted equation considered the total amount of sugars present in the fermentative broth and calculated by HPLC test:

$$Y\left(\frac{g}{g}\right) = \frac{(C(LA)_{end}) - (C(LA)_{start})}{(C(S)_{start}) - (C(S)_{end})} \tag{2}$$

where:

$Y\left(\frac{g}{g}\right)$ was the total volumetric productivity;

$C(LA)_{start}$ $\left(\frac{g}{L}\right)$ was the concentration of the LA at the starting point of fermentation t=0 s

$C(LA)_{end}$ $\left(\frac{g}{L}\right)$was the LA concentration at the end of fermentation.

$C(S)_{start}$ $\left(\frac{g}{L}\right)$was the concentration of sugar at the starting point of fermentation t=0 s

$C(LA)_{end}$ $\left(\frac{g}{L}\right)$was the concentration of sugar at the end of fermentation.

To evaluate the concentration of sugars, the initial amount of starch and sugars in the substrate was considered. The performed analyses defined that the percentage of starch was 33.94% w/w $_{DM}$ and sugar was 8.5 % w/w.$_{DM}$.

The conversion factor for the transformation of starch to glucose was 1.1 due to the hydrolysis of starch to glucose by the addition of one molecule of water (all

referred to molecular weights of starch and glucose; starch 180 ($\frac{g}{mol}$)and glucose 162 ($\frac{g}{mol}$)

Thus, the theoretical initial amount of sugar was calculated as (the percentages are referred to the DM of the food waste analysed) Eq.3

$$m_{Th} = C(S)_{start} = DM_{fW} \cdot \%\, starch \cdot 1{,}1 + DM_{fW} \cdot \%\, glucose \quad (3)$$

where:

m_{Th} was the theoretical amount of food sugar;

% starch was the amount of starch in the substrate

%glucose was the amount of glucose in the substrate.

30 μl of Glucoamylase enzyme were added in the last sample of each SSF process, to evaluate the total amount of sugars present in at the end of SSF processes. Thereafter, the sample was incubated for 24 hours in a vertical shaking incubator SM 25B-Swip Orbital Shaker (Edmund buhler GmbH, Germany) at 52°C.

The consumption of sugars was calculated in order to define the amount of sugar used during the fermentation process, according to Eq. 4:

$$T(\%) = \frac{(C(S)_{start} \cdot v_{start}) - (C(S)_{end} \cdot V_{end})}{(C(S)_{start} \cdot v_{start})} \cdot 100 \quad (4)$$

where:

$T(\%)$ was the total volumetric productivity;

$C(S)_{start}$ ($\frac{g}{L}$) was the concentration of sugar at the starting point of fermentation t=0 s

$C(LA)_{end}$ ($\frac{g}{L}$) was the concentration of sugar at the end of fermentation.

v_{start}[(L) was the start volume of the process

v_{end} (L) was the end volume of the process

The different downstream flows were evaluated through LA recovery (RLA), which was based on the LA amount present in the former process step (Eq.5), and purity (PLA) compared to present ions concentration Eq 6.

$$R_{LA}(\%) = \frac{C(L{\sim}LA)_2 \cdot V_2}{C(L{\sim}LA)_1 \cdot V_1} \cdot 100 \quad (5)$$

where:

$R_{LA}(\%)\ was$ recovery of lactic acid of flow2

$C(L{\sim}LA)_2(\frac{g}{L})$ was the LA concentration in flow 2

$C(D{\sim}LA)_1(\frac{g}{L})$ was the LA concentration in flow 1

$V_2(L)$ was the volume of flow2

$V_1(L)$ was the volume of flow1

$$P_{LA}(\%) = \frac{C(LA)_1 \cdot V_1}{C(LA)_1 \cdot V_1 + C(ion)_1 \cdot V_1} \cdot 100 \quad (6)$$

where:

$R_{LA}(\%)$ was purity of lactic acid of flow1

$C(LA)_1(\frac{g}{L})$ was the LA concentration in flow 1

$C(ion)_1(\%)$ was the ion concentration in flow 1

$V_1\ (L)$was the volume of flow1

3.2.6 Statistical analysis

To measure the statistical difference of LA production of those fermentations carried out in duplicate using *Streptococcus* sp. strain A620 and different solid-to-liquid ratios, and under sterile and non-sterile conditions a *t*-test was performed. Statistically significant difference in median values was accepted for $P<0.05$.

3.3 Results and discussion

3.3.1 Simultaneous saccharification and fermentation

3.3.1.1. Evaluation of micro-organisms

The dry matter (TS) and organic dry matter (VS/TS) of blended OFMSW were 18.1% and 93.2% (w/w), respectively. It consisted of (w/w) 33.5% starch, 14.8% proteins, 12.9% fat and 8.5% free sugars. The composition of OFMSW was known to be highly variable, but German food usually contains potatoes and noodles, and thus the predominant fraction was most likely starch.

Four bacterial strains were evaluated for LA production from OFMSW: three Lactobacillus sp. with the internal labels: A28a, A59 and A211, and one Streptococcus sp. A620, all suitable to degrade organic material.

The test was carried out in 500 mL Erlenmeyer shaking, as preliminary test, before to perform the scale up, in order to verify the capability of the abovementioned micro-organisms to degrade OFMSW by means of SSF and to form LA from the released nutrients.

Lactic acid bacteria require not only carbon to produce LA, but also nitrogen, as proven by the study of Amrane and Prigent, (1998) Jiang et al., (2019)on *L. helveticus*. Hence, nitrogen sources were essential to keep cells growing and forming LA.

In the present experimental study nitrogen was supplied in form of proteins and free amino nitrogen (FAN) and carbon in form of starch and free sugars.

The performance of all the four micro-organisms for LA production and sugar consumption in SSF of blended OFMSW with a solid-to-liquid ratio of 10% (w/w) was reported in Figure6. All four strains produced lactic acid. However, different concentrations, yields and productivities were obtained.

The comparison of productivity is usually based on exponential growth phase.

However, in the present study, it was difficult recognise clearly the experimental growth, therefore, the calculation of productivity was based on the whole fermentation of 28 hours. In all fermentation, free sugars were analysed in forms of glucose, fructose and sucrose. The concentration of free sugars ranged from 5 to 17 g L-1 (Figure3-6). The variation in sugar concentration was caused by the complexity of the OFMSW and the autoclavation performed before to SSF.

Strain A28a produced 7.4 g/L LA within 28 hours resulting in a productivity of 0.26 g/Lh

(Figure 3-6A and Table3-3). The yield was 0.07 g /g dry OFMSW. Based on starch content and theoretically obtainable sugars, yields were 0.22 and 0.14 g/g, respectively.

The strains A59 and A211 showed a slightly better performance than strain A28a (Figure3-6B and 3-6C, Table3-3). However, a LA concentration of 10-15 g/L was still low, and one may conclude that only the free sugars were converted, but no starch. This is an interesting finding since it was known that bacteria from the genus Lactobacillus were able to produce extracellular amylases in order to make starch as carbon source available Champ et al. (1983); Jiang et al. (2019)

The *Streptococcus sp*. strain A620 behaved differently compared to the Lactobacillus sp. strains, because, in SSF performed with Lactobacillus, the LA concentration level off after 10 hours, while SSF performed with *Streptococcus sp*. strain A620 reached steadily state conditions after 28 hours with: LA concentration of 36.80 g/L, productivity of1.32 g/Lh (Figure3-6D), yields based on dry food waste material, starch and theoretically obtainable sugars equal to 0.37, 1.10 and 0.67 g/g respectively. Hence, Streptococcus sp. not only converted free sugars, but also starch.

However, also acetic acid has been produced and concentrations ranged between 2 and 3 g/L in all fermentation broths. Even when the acetic acid concentration was rather low compared to LA, its formation may complicate downstream processing and further extra separation steps, such as simulated moving bed Lee et al., (2004), might be necessary, in order to reach the target of a pure LA formulation.

Nevertheless, due to the good performance regarding conversion of OFMSW into LA, Streptococcus sp. strain A620 was defined the most suitable strain and the further investigation will be performed with Streptococcus sp. strain A620

Figure 3-6: Strain comparison. consumption of glucose (closed circle), fructose (open triangle), sucrose (open square) and production of acetic acid (open star) and lactic acid (open circle) concentrations, during SSF using lactobacillus sp. strains A28a (a), A59 or A211 (c) and using streptococcus sp. strain A620 (d) at a solid-to-liquid ratio of 10% (w/w).

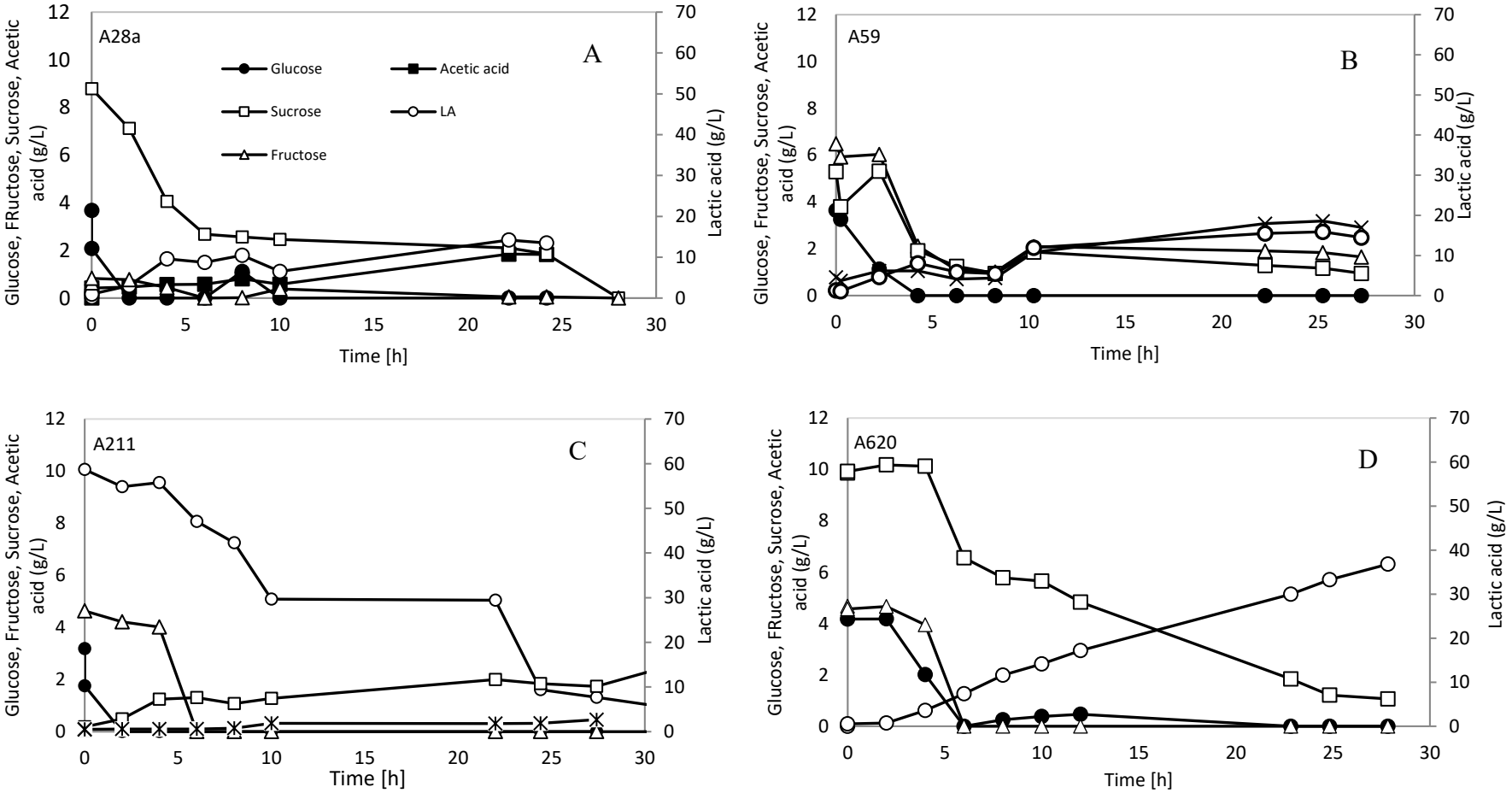


Table 3-3: Lactic acid productivity within 28 hours of cultivation time (P), yield of lactic acid per gram of dry OFMSW (YOFMW), per gram of starch (YST) and per gram of sugars theoretically present (YSU) of SSFs carried out at laboratory scale using the four different strains

Batch	P (g/Lh)	Y_{OFMSW} (g/g)	Y_{ST} (g/g)	Y_{SU} (g/g)
Lactobacillus sp. strains A28a	0.27	0.07	0.22	0.14
Lactobacillus sp. strains A59	0.53	0.14	0.43	0.29
Lactobacillus sp. strains A211	0.37	0.14	0.41	0.24
Streptococcus sp. strain A620	1.32	0.37	1.10	0.67

3.3.1.2 SSF carried out at different solid to liquid ratio

After the identification of the most suitable LA strain producer, A620, the solid to liquid ratio of OFMSW was investigated, since it was hypothesised that the concentration of LA could dependent on the solid-to-liquid ratio.

With Streptococcus sp. strain A620, SSFs was performed in duplicate at four solid to liquid ratios (s/l w/w): 5%, 10%, 15% and 20% (Figure 7). Increasing the solid-to-liquid ratio (Figure3- 7A, B, C and D) the LA concentration increased.

A regression analysis revealed that LA concentration increased linearly with increasing solid-to-liquid ratio (Figure3- 8).

However, due to a high viscosity of OFMSW, solid- to-liquid ratio over 20% (w/w) could not be carried out for no appropriate mixed and therefore not further increase were performed. Nevertheless, s/l equal to 20% (w/w) was enough to produce 58 g /L LA (Figures 3 and 4). The high concentrations of free glucose, fructose and sucrose additionally contributed to this high product formation (Figure 7D).

Generally, free sugar concentration was dependent on solid-to-liquid ratio employed. However, the major part of sugars exploited by Streptococcus sp. A620 to produce LA, came from starch, since the concentration of free sugar was not enough to reach the LA concentrations obtained.

Productivity and yield of fermentations carried out at different solid-to-liquid ratios were reported in Table3- 2. At 20% (w/w), productivity and yield were 2.08 g/Lh and 0.63 g per gram of theoretically obtainable sugars, respectively. Even though the LA concentration slowly levelled off after 28 hours of cultivation, it is expected that higher titre and eventually a better yield can be obtained when fermentation duration is protracted.

A better example to illustrate the performance of Streptococcus sp. A620 was the SSF

carried out at 5% (w/w). Here the potential of OFMSW as source of nutrients could be fully exploited within 28 hours and yields per gram of dry food waste, starch and theoretically obtainable sugars were 0.39, 1.15 and 0.81 g. The obtained results can be compared to the study of Kwan et al. (2016) based on OFMSW hydrolysis and utilization of hydrolysate in LA fermentation Kwan et al. (2016); Komesu et al. (2017) first recovered 85% of available sugars from mixed food waste and bakery waste by fungal hydrolysis and afterwards converted the sugars recovered at a yield of 0.94 g/g using *L. Casei Shirota* into LA.

Hence the overall yield was 0.80 g/g, which is near identical to the yield of 0.81g/g- obtained in the present study.

With similar mixed OFMSW composition, Kwan et al. (2016) reached lower yield per gram of dry OFMSW than the one reached in the present study, respectively: 0.23-0.27 g (Kwan et al., 2016), and 0.39 g (Table3-4), while Kwan et al. (2016) reached a higher productivity than the one of the present study, respectively:2.61 g/Lh Kwan et al. (2016) and 0.69-2.08 g/Lh (Table3-4) according to the increasing of solid to liquid ratio (from 5 to 20 (w/w)). The reason of this trends can be the necessity of cells in SSF to degrade the organic material prior to conversion in LA.

The important achievement of the present experimental study was the efficient and direct conversion of OFMSW, the organic substrate, into LA, skipping the hydrolysis step.

The FAN concentration was not affected to the same extent by the solid-to-liquid ratio as the concentration of free sugars. Even though the FAN concentration increased from 179 to 350 mg/L with an increase in the solid-to-liquid ratio from 5 to 10% (w/w), no further rise was observed at higher solid-to-liquid ratios.

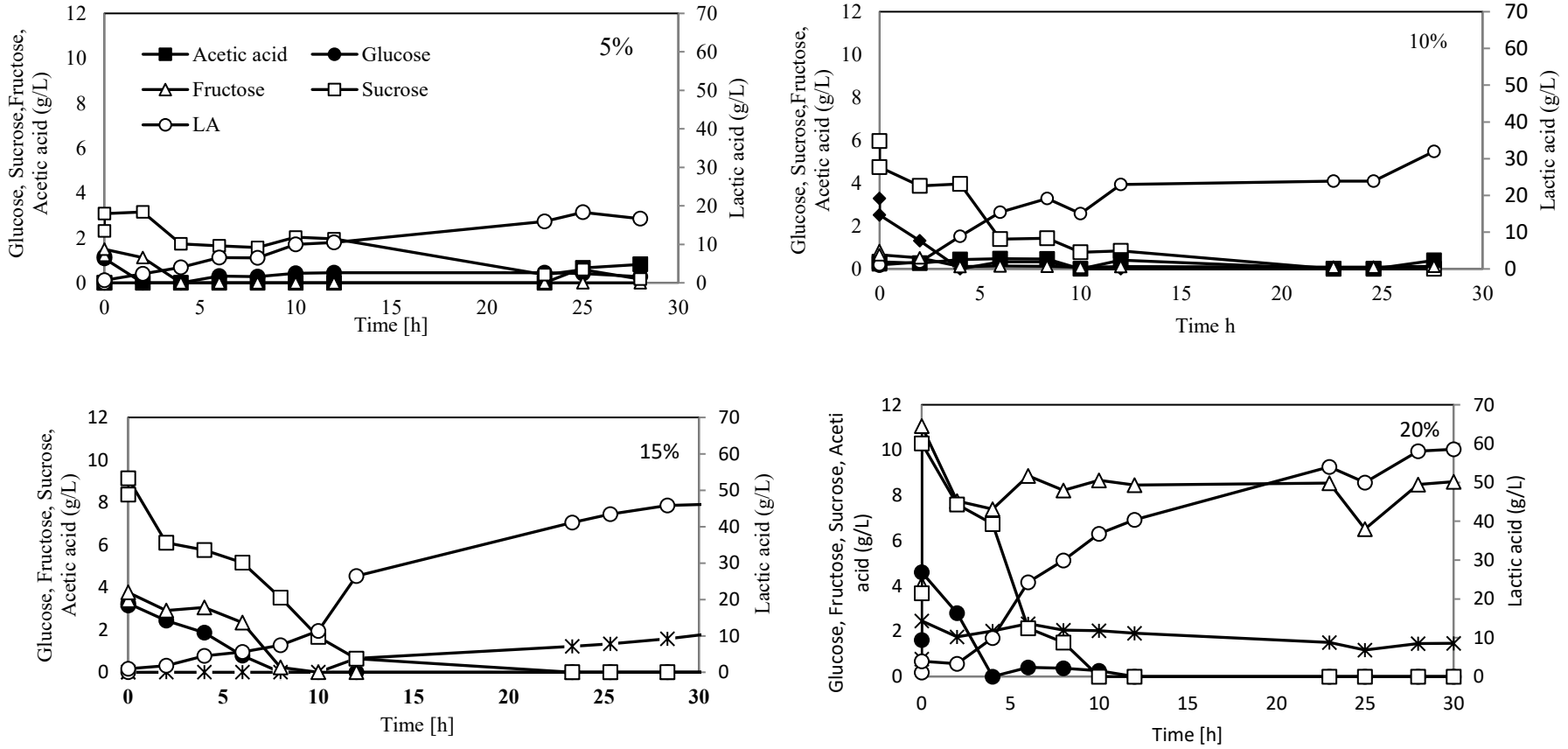
Not enough data were collected to calculate the exponential growth rate, but growth was obviously fast in all cultures during the first 2 to 5 hours and levelled off afterwards (Figure 3). This was also the time-period were FAN was consumed.

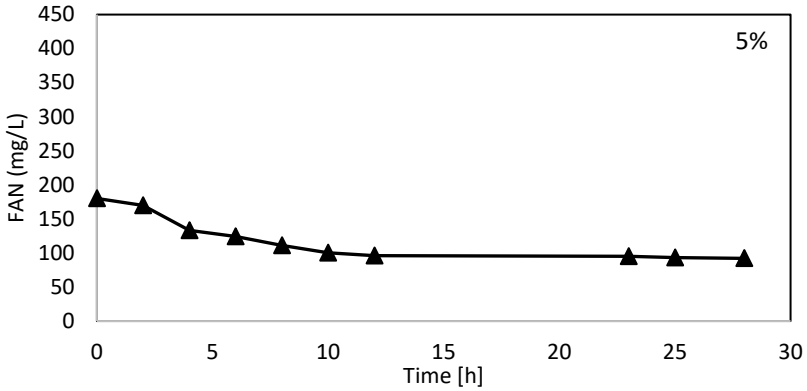
Compared to the studies of Plessner et Neu et al. (2016); Pleissner et al. (2016) carried out with *Bacillus*

Coagulans, the number of total and living cells in all fermentations did not decrease after growth stopped.

This trend indicates that enough nitrogen was available to keep a predominant fraction of cells alive, which caused eventually a continuous production of LA (Figures 3-7 and3-8).

Figure 3-7: Influence of solid-to-liquid ratio. Change of glucose (closed circle), fructose (open triangle), sucrose (open square), FAN (closed triangle), acetic acid (open star) and lactic acid (open circle) concentrations during SSF using Streptococcus sp. Strain.




5%
FAN (mg/L)
Time [h]
0
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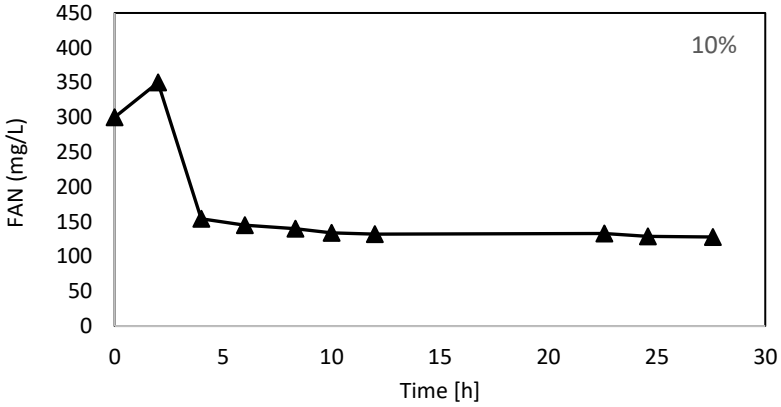
150
200
250
300
350
400
450
0
5
10
15
20
25
30


10%
FAN (mg/L)
Time [h]
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200
250
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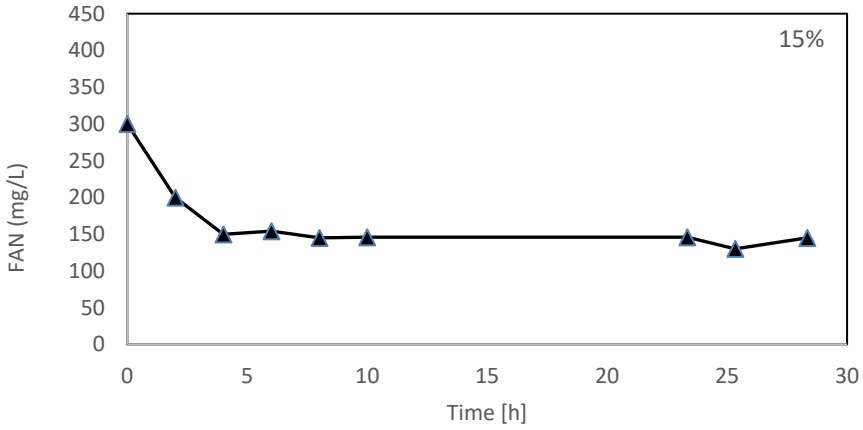
15%
FAN (mg/L)
Time [h]
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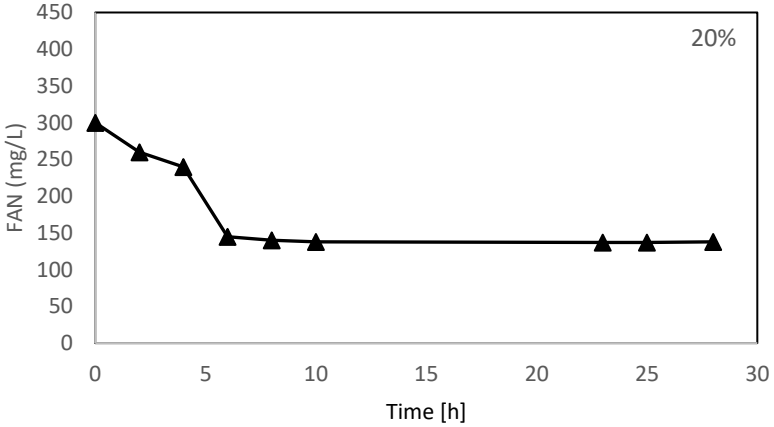
20%
FAN (mg/L)
Time [h]
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Figure3-8:Relationship between LA titre and solid to liquid ratio

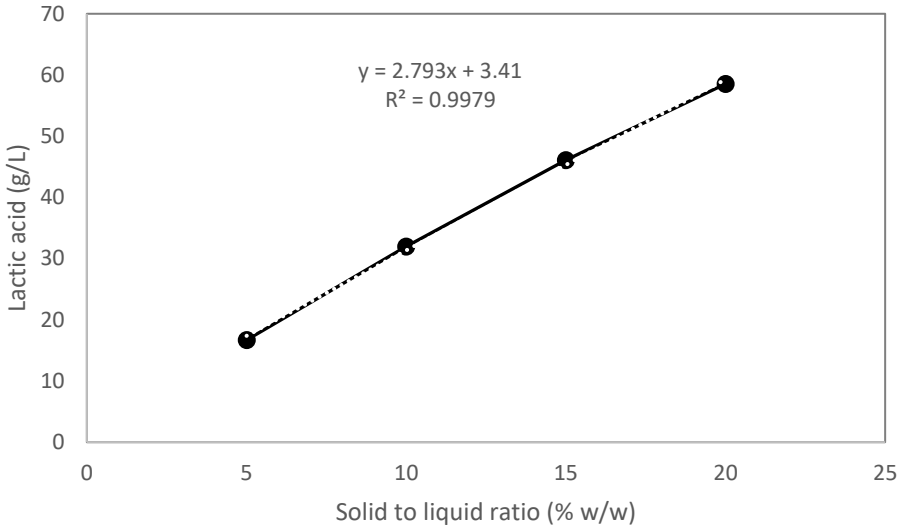


Table 3-4::LA productivity within 28h of cultivation time (P), yields of LA per g of dry OFMSW (Y_{OFMW}), per g of starch (Y_{ST}) and per g of sugars theoretically present (Ysu) of SSFs carried out at laboratory scale at different solid to liquid ratio using Streptoco

S/l (%w/w)	P (g/Lh)	Y_{OFMSW} (g/g)	Y_{ST} (g/g)	Y_{SU} (g/g)
5	0.69	0.39	1.15	0.81
10	1.25	0.35	1.04	0.73
15	1.67	0.31	0.94	0.67
20	2.08	0.29	0.88	0.63

3.3.1.3 SSF under non-sterile conditions

The previous experiments were carried out under sterile conditions to systematically investigate SSF.

In this section SSF was performed under no-sterile conditions, since autoclavation was an energy intensive processes, which make it hardly economically feasible running at industrial scale according to Li et al. (2014).

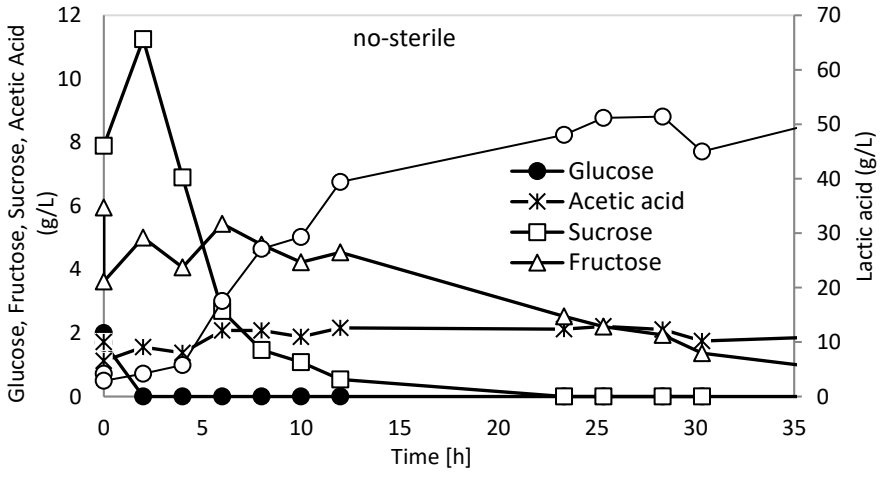
Therefore, SSF was carried out under non-sterile condition at a solid-to-liquid ratio of 20% (w/w). There was obviously no significant difference in productivity and yields compared to sterile SSF (Figures3-6 and 3-7, and Tables3-3 and3-4). LA concentration increased within 28 hours to 55 g/L (Figure 3-9). Free glucose, fructose and sucrose were detected at concentrations of 1.8 g/L, 6.3 g/L and 9.3 g/L, respectively, and used for LA production.

Under no-sterile condition, it was detected that the acetic acid concentration remained despite non-sterile conditions below 2 g/L as proven in the study of Tang et al. (2016) which investigated the conversion of food waste into LA employing an indigenous microbial community. In their study, beside a high concentration of LA, around 40 g/L, also acetic, propionic and butyric acid at around 10 g/L were produced.

However, this was not the case in the fermentation shown in Figure 5. Nevertheless, it may also be concluded that *Streptococcus sp.* A620 outcompeted a possibly present indigenous microbial community. The fact that no sterilization and hydrolysis were needed make SSF for LA production a simple process that can be implemented relatively fast at locations where OFMSW occurred in large amounts, such as in densely populated urban areas and food industries.

The simplicity of the process was comparable to the process of AD for biogas production, but the conversion of carbon into LA was more efficient and no CO_2 was produced by microbial activity.

Figure 3-9: SSF under no-sterile condition. Change of Glucose (closed circle), Fructose (open triangle), Sucrose (open square), Acetic acid (open star), Lactic acid (open circle), FAN (closed triangle) concnetration during SSF using Streptococcus sp.strain A620 carried out at a solid to liquid ratio of 20% (w/w) under non sterile conditions



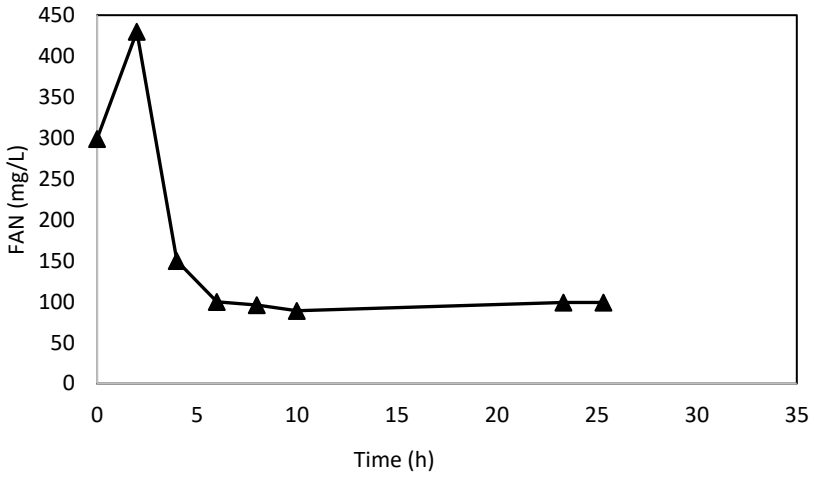


Table 3-5: Productivity within 28h of cultivation time (P), yields of LA per g of dry OFMSW (Y_{OFMW}), per g of starch (Yst) and per g of sugars theoretically present (Ysu) of SSFs carried out at laboratory scale under no sterile conditions and at the technical scale under sterile conditions using Streptococcus sp.strain A620 and a solid to liquid ratio of 20 % (w/w)

Batch	P (g/Lh)	Y_{OFMSW} (g/g)	Y_{ST} (g/g)	Y_{SU} (g(g)
Non sterile conditions	2.12	0.27	0.79	0.58
Technical scale	2.16	0.25	0.75	0.64

3.3.1.4 SSF carried out at technical scale and downstream processing

SSF has been carried out at technical scale of 50L, using Streptococcus sp. strain A620 and a solid-to-liquid ratio of 20% (w/w), to evaluate a scale-up of OFMSW valorisation processes for L (+)-LA production. The concentration of LA reached 60.5 g/L within 28 hours with a productivity of 2.16 g/ Lh.

Yields of lactic acid per gram of dry OFMSW, starch and sugars theoretically obtainable were 0.25, 0.75 and 0.64 g, respectively. The results achieved at the technical scale were comparable to the observations made at laboratory scale (Tables 3-4 and 3-5).

At the end of SFF, performed at technical scale, no remaining free sugars and acetic acid were present in the fermentation broth which certainly eases downstream processing.

However, advanced techniques were still needed to separate impurities and salts introduced by the OFMSW, and acids and base used for pH regulation. Downstream processing included micro- and nanofiltrations, softening, mono- and bipolar electro-dialyses, purification via anion- and cation-exchange resins, and distillation. In Figure 10 was depicted the concentrations of salt ions and LA during the downstream processing.

In the 48L of fermentation broth obtained from technical scale SSF, most ions were made of sodium, potassium and chloride with concentrations of 16.1 g/L, 1.1

g/L and 3.6 g/L, respectively. The LA concentration was 60.5 g/L. After the SSF, the fermentation broth was micro- and nano-filtrated and most of ions was made of 12.8 g/L sodium, 0.9 g/L potassium and 3.0 g/L chloride. The LA concentration decreased due to dilution to 45.1 g/L.

Mono- and bipolar electrodialysis were carried out to concentrate LA and to separate it from salts. After electrodialysis the LA concentration increased to 171 g/L.

The concentration of ions was still high, in fact sodium, potassium and chloride were 2.7 g/L, 0.3 g/L and 11.6 g/L, respectively.

Hence, anion- and cation-exchange was carried out which decreased the concentration of all salt ions to less than 0.01 g/L.

However, due to a strong dilution the LA concentration also decreased to 54.1 g/L.

To concentrate the LA, as final step of downstream processing, the water was evaporated and the final L (+)-LA formulation had a volume of 1.6 L and a concentration of 702 g/L, and thus 38% of the initial LA could be recovered from fermentation broth. The loss of 62% of LA represented a big drawback of the current downstream processing, nevertheless the pro was that conventional downstream technique can be applied on complex nutrient source as OFMSW, after fermentation process. Pleissner et al. (2016b) employed the same downstream technique, but they included an ion-exchange chromatography after microfiltration, which was carried out with the resin Amberlite FPA53 and 12.5 mn H2SO4 as eluent. In this way, Pleissner et al. (2016b) recovered 90% of initial LA, comparable with literature data Min et al. (2011), but the drawback was the precipitation of $CaSO_4$

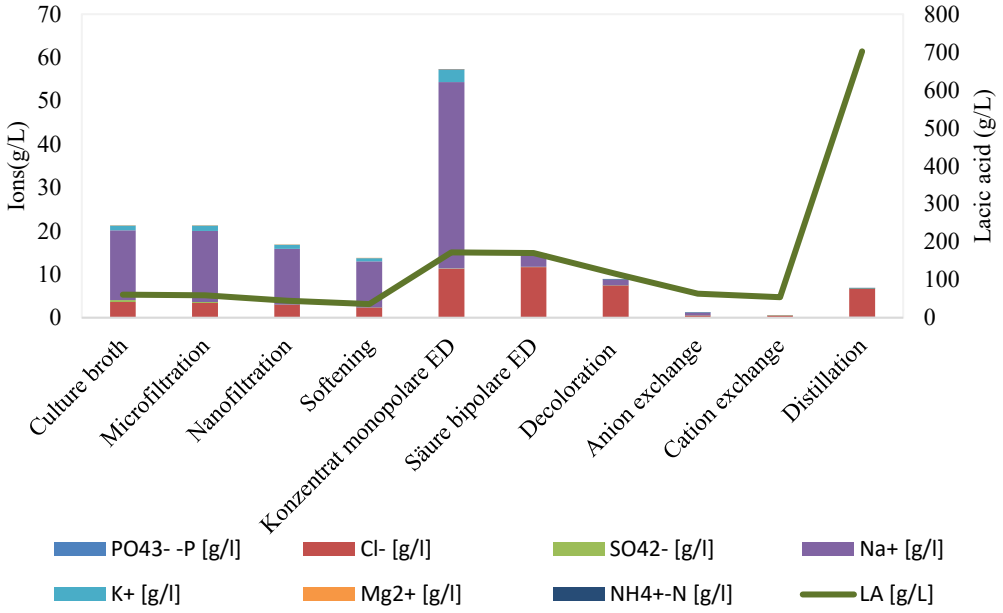
The optical purity of the obtained L (+)-LA formulation was 99.7%.

Inkinen et al. (2011) reviewed the quality requirements of LA formulation used in PLA synthesis and stated that the impurities should be below 0.05 mol %. In the present experimental study, the major source of impurities in the obtained L (+)-LA formulation were chloride-ions.

However, the concentration found was 5 g/L and thus below 0.05 mol % in accordance with Komesu et al. (2017).

The low LA recovery rate did not indicate an economic un-feasible process, but the intention of the present study was to evaluate whether LA can be purified, and a pure formulation produced when OFMSW was used as substrate.

Figure 3-10:Downstream processing ions and LA concentration during different downstream processing steps.



3.3.2 Separate hydrolysis and fermentation

3.3.2.1 Enzymatic hydrolysis: solid to liquid ratio evaluation

The efficient recovery of nutrients from OFMSW depends on the activity of the employed enzymes Chen, (2020) attested that the efficiency of an enzyme in hydrolysis of pretreated barley straw decreases when the viscosity of the slurry gets too high. Different solid-to-liquid ratio and enzyme loadings were investigated. To evaluate the effect on OFMSW and to reduce the amount of enzyme needed to effectively hydrolysis OFMSW, which represent an economic cost, and to recover glucose and FAN

Glucose recovery was strongly dependent on the solid-to-liquid ratio (Figure 11A). After 5-10 hours glucose concentration leveled off and 54.2 g/L was obtained when solid to liquid ratio of 11% (w/w) was employed. Glucose concentration steadily increased to 80.9 g/L when 25% (w/w) was employed. A 33.5% (w/w) starch content and a 25% (w/w) solid-to-liquid ratio accounts to a starch loading of 83.8 g. The theoretical conversion of starch into glucose is 0.9 according to Zhou, (2016); Rajendran et al. (2016), and so 94.4 g/L can be theoretically recovered. The obtained glucose concentration was 80.9 g/L, which implied a recovery of 85%. Theoretically, 41.8 g/L of glucose can be obtained at a solid-to-liquid ratio of 11% (w/w). The obtained glucose concentration of 54.2 g/L, however, indicated the presence of a remarkable amount of free glucose. Table3-6 exhibited that the yield of glucose per gram of OFMSW decreased with increasing solid-to-liquid ratio. It is assumed that better mixing conditions achieved at 11% (w/w) contributed to a

better hydrolytic performance, and thus to a higher yield (0.49 g/g_{FW}), while at 25% (w/w) a yield equal to 0.33 g/g_{FW} was obtained.

Contrarily, even when the solid-to-liquid ratio was increased, the amount of recovered FAN remained relatively constant (Figure 3-11B). Even though the concentration increased from 0.23 g/L to 0.29 g/L within 24 hours with increasing solid-to-liquid ratio, this trend is not comparable to the results shown in Figure 1A. The complete digestion of 14.3% (w/w) proteins in OFMSW had certainly an effect on FAN concentration. However, it might be concluded that proteases used are not appropriate for the digestion of proteins in OFMSW. The yield of FAN (see Table 4) decreased by increasing solid-to-liquid ratio. While 2.04 mg/g of dry FW was obtained at 11% (w/w), only 1.15 mg/g was obtained at 25% (w/w).

Figure 3-0-11: Solid-to-liquid ratio and enzyme loading. Recovery of glucose (A) and FAN (B) when enzymatic hydrolysis of blended food waste was carried out in presence of 350 µl Stargen and 700 µL Fermgen at different solid-to-liquid ratios (w/w): 11.1% (open circle), 12.5% (closed circle), 20% (open triangle) or 25% (closed triangle). Recovery of glucose (C) and FAN (D) when enzymatic hydrolysis was carried out at a solid-to-liquid ratio of 20% (w/w) at different specific enzyme loadings: 3.5 µL/g Stargen and 5 µL/g Fermgen (open circle), 1.75 µL/g Stargen and 2.5 µL/g Fermgen (closed circle), 0.88 µL/g Stargen and 1.25 µL/g Fermgen (open triangle), 0.44 µL/g Stargen and 0.63 µL/g Fermgen (closed triangle) or 0.11 µL/g Stargen and 0.32 µL/g Fermgen (open square). Results are based on single measurements.

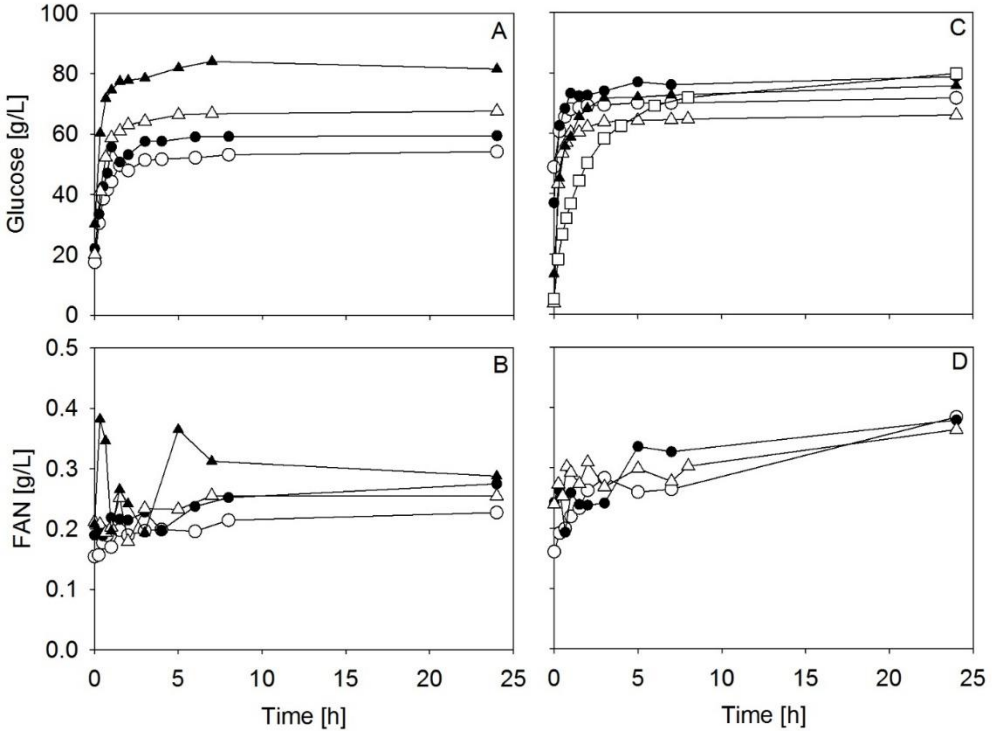


Table 3-6: Yields of glucose (Yglc/OFMSW) and fan (Yfan/OFMSW) per gram of dry food waste when enzymatic hydrolysis was carried out at different solid-to-liquid ratios.

Solid-to-liquid ratio (%, w/w)	$Y_{Glc/OFMSW}$ (g/g)	$Y_{FAN/OFMSW}$ (mg/g)
11.1	0.49	2.04
12.5	0.48	2.20
20	0.34	1.27
25	0.33	1.15

3.3.2.2 Enzyme concentration

To determine the lowest specific enzyme loading for glucose and FAN recovery different specific enzyme loadings were tested (Table3-7). Contrarily to the solid-to-liquid ratio, the specific enzyme loading had no remarkable effect on glucose and FAN recovery (Figures 3-1C and D). Yields were between 0.33 and 0.39 g glucose and between 1.82 and 1.92 g FAN per gram of dry OFMSW (Table3- 7).

Table 3-7: Yields of glucose (Yglc/FW) and fan (YFAN/FW) per gram of dry food waste when enzymatic hydrolysis was carried out at a solid-to-liquid ratio of 20% (w/w) and different enzyme concentrations of Stargen and Fermgen per gram of dry food waste (n. a. = not analysed

Enzyme concentration (µL/g)		$Y_{Glc/OFMSW}$ (g/g)	$Y_{FAN/OFMSW}$ (mg/g)
Stargen	**Fermgen**		
3.50	5.00	0.36	1.92
1.75	2.50	0.39	1.92
0.88	1.25	0.33	1.82
0.44	0.63	0.38	1.61
0.11	0.32	0.39	n. a.

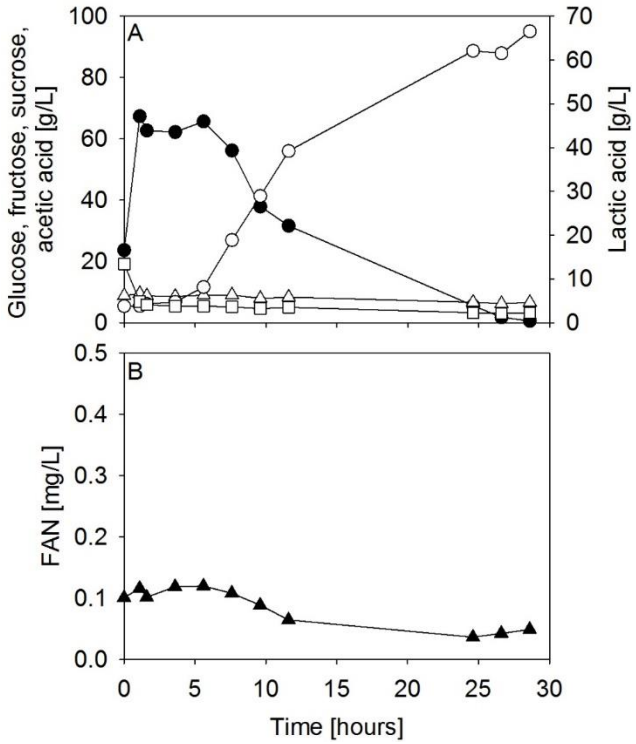
3.3.2.3 Separated hydrolysis and fermentation: SHF

Due to the previously mentioned viscosity problems, 20% (w/w) solid-to-liquid ratio was chosen for LA fermentation. OFMSW hydrolysis with Stargen kept short for only one hour as it was demonstrated in Figure 8, where the release of glucose occurs quickly. After one hour 67.3 g/L of glucose were obtained which was in accordance with Figure 8. The hydrolyzed substrate was then inoculated with *Streptococcus* sp. strain A620 and the fermentation was carried out for 29 hours. Immediately after inoculation, LA concentration increased exponentially, reaching 39.2 g/L after 11 hours. Afterwards, it further increased linearly to 66.5 g/L until fermentation was stopped (Figure3-12). Glucose was completely consumed, but traces of sucrose and fructose, available as additional carbon sources, were still available. The first 11 hours was also the period where most of the FAN was consumed (Figure 3-12B). Fermentation was carried out in duplicate and no statistical difference (P=0.637) was found for LA formation between repetitions. The yield obtained in the present study adopting SHF, considering LA concentration after 29 hours, was 0.33 g_{LA} /g dry OFMSW with a productivity of 3.38 $g_{LA}/L{\cdot}h$.

SSF performed on same OFMSW reached a yield of 0.29 g_{LA}/g_{OFMSW} and a productivity of 2.08 $g_{LA}/L{\cdot}h$ after 28 hours Pleissner et al. (2017), thus SHF resulted in higher yield and productivity. Yields ($g_{LA}/g_{dry\ FW}$) available in scientific literature were 0.27 Kwan et al. (2016) and 0.99 Kitpreechavanich et al. (2016) for SHF processes; 0.85 Kwan et al. (2016) and 0.46 Tang et al., (2016) are accounted for SSF processes. However, the yield strongly depends on feedstock composition and

on the strain. Productivity, defined as mass of LA generated per volume of fermentation broth in a unit of time, is therefore a more reliable criterion to assess the performance of a fermentation process. During exponential phase 3.38 $g_{LA}/L \cdot h$ was produced in the present study, which is remarkably higher than productivity values in literature. It is known that *Streptococcus* sp. strain A620 Pleissner et al., (2017) can degrade food waste, and thus this capability may additionally contribute to the release of glucose. Lowest productivity of 0.28 $g_{LA}/L \cdot h$ was found when FW was converted with an indigenous microbial consortium Tang et al. (2016). This is not surprising, as the microbial consortium is not specialized to form only LA, but a mixture of different organic acids. The study of Kim et al. (2016) is of particular relevance for FW utilization approaches as it illustrates how FW can be utilized in repeated batch cultures over a long period of time. Even though a higher productivity was obtained in the present study and by Kwan et al. (2016) when FW was first enzymatically pretreated, the simplicity of processes presented by Pleissner et al. (2017), Kim et al. (2016) and Tang et al. (2016) clearly shows that the process steps can be reduced to a minimum.

Figure 3-12: Lactic acid fermentation. Change of glucose (closed circle), fructose (open triangle), sucrose (open square), FAN (closed triangle) and lactic acid (open circle) concentrations during enzymatic pre-treatment of food waste with 700 µL Stargen and subsequentially carried out lactic acid fermentation using *Streptococcuus sp*. strain A620m (A,B). Fermentation were carried out in duplicate and mean value as are shown. No statistical difference P= 0.637 was found between replicates



3.4. Conclusions

LA was produced from blended OFMSW through SSF and SHF at the laboratory (2L) and technical scales (50L). *Lactobacillus sp.* strains did not show

an efficient conversion of OFMSW into LA. Whereas, *Streptococcus sp*., liquefied the material and produced LA.

For SSF process the maximum productivity of 2.16 g/Lh was achieved at technical scale, while the highest yield of 0.81g/g of theoretically present sugars was obtained in SSF carried out at solid to liquid ratio of 5w/w.

The LA concentration achieved from 20%w/w of bended OFMSW was 58g/L. Both under sterile and not sterile conditions SSF carried out with *Streptococcus sp* A620 directly converted OFMSW into LA without considerable production of other acids.

For SHF process the hydrolysis was carried out for 1h with Stargen and sequential LA concentration after 29 hours, was 0.33 g_{LA} /g dry OFMSW with a productivity of 3.38 $g_{LA}/L \cdot h$.

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Chapter4: Fermentative production of L(+)lactic acid from spent coffee grounds

The main findings of the current study are under revision in: Fermentative production of lactic acid from spent coffee ground: critical evaluation of upstream and downstream processes. F.Demichelis, S.Fiore*, A. Nardi, J. Venus and D. Pleissner

Part of data came from the following thesis:
Biological valorisation of spent coffee grounds through production of lactic acid. A. Nardi. S.Fiore and D.Pleissner. Politecnico di Torino

Abstract

Chapter 4 investigates the acid-enzymatic hydrolysis and fermentation of L (+)-lactic acid (LA) with *Bacillus Coagulans* from spent coffee ground (SCGC). SCGC, a lignocellulose residue from coffee production consisted of 34.26 ± 2.67% cellulose, 7.31% ± 2.54% hemicellulose and 24.88 ± 0.11% of lignin. Sequential and combined acid-enzymatic hydrolysis were carried out respectively, at 121°C for 15 min with 1%v/v H_2SO_4 and 14.5% SCG wet and at 52°C for 24h with 0.25 mL Accellerase 1500 per gram of dry SCG, achieving a total sugar extraction efficiency of 41.24 ± 4.53%.

Fermentations were carried out both at the laboratory (2L) and technical (72L) scales and no scale effect was observed.

At 50L scale, LA yield per gram of sugar consumed and per dry gram of SCG were 0.956 ± 0.015, 0.18 ± 0.63 respectively. Downstream processing resulted in 786.70 gLA/L and 99.5% optical purity.

4.1 Introduction

In this Chapter the evaluation of fermentative production of LA from SCG is discussed. In the present work, LA production consisted in 1) acid-enzymatic hydrolysis, 2) fermentation and 3) downstream processing. Acid and enzymatic hydrolyses, were tested at 0.5-5L scales to define the optimal acid concentration,

retention time, solid to liquid ratio and hydrolysate separation options between centrifugation and micro-filtration. Fermentation processes were carried out at 52°C employing and *Bacillus Coagulans* at pH 6, with batch feed at two scales: laboratory and technical, 2L and 50L, respectively. Concerning downstream processes, micro- and nano-filtration, ion exchange, electrodialysis and vacuum concentration were sequentially performed on the fermentation broth coming from the technical scale test

The approach consisted in experimental evaluation and optimisation of each step of fermentative LA production. After having assessed the optimised LA production configuration, scale-up issues were also investigated.

The novelty of the present study is the employment of SCG as LA feedstock, optimising all process parameters from up-to-down-stream processing, minimising possible bottlenecks to carry out the scale up of the process and evaluate the minimum amount of SCG to make LA production from lignocellulose matter economic profitable and energetic sustainable. The optimisation of all parameters was aimed to boost the process, from laboratory scale to industrial applications.

4.2. Materials and Methods

4.2.1. Spent Coffee Ground (SCG)

Cenicafè, a Colombian coffee research institute, supplied two SCG samples deriving from Arabica coffee brew preparation. SCG samples were analysed for dry matter, ashes, CHNS analysis, fibres, proteins, hemicelluloses, cellulose and lignin contents. Before hydrolysis and fermentation, a mechanical pre-treatment was performed to reduce particle size of SCG approximately to 1 mm, by means of knife mill GRINDOMIX GM 200 for one minute at 9000 rpm.

4.2.2. Acid-enzymatic hydrolysis

Acid, enzymatic and combined acid-enzymatic hydrolyses were performed in 500 mL Erlenmeyer shaking flasks to define the optimal:

1. H_2SO_4 concentration, for different solid to liquid ratios (s/l) of SCG; the goal here is to achieve the maximal sugar release (Cs) (Table4-1, configuration 1-4A),
2. Residence time-H_2SO_4 concentration on the highest investigated S:L of SCG (Table4-1, configuration IA-IVB);
3. Comparison of exclusive acid pre-treatment and combined acid pre-treatment and enzymatic hydrolysis (Table4-1, configuration IIB1-IIB2-IIIB-IIIB2).

The acid hydrolysis was performed in VARIOKLAV® 75 S (H+P Labortechnik AG, Germany) autoclave at 121°C, testing three H_2SO_4 concentrations (0%, 0.5%

and 1% [v/v] referring to SCG as well) on four solid to liquid ratio (s/l) (9.6%, 14.5%, 19.3% and 28.9%) for four residence times (0,15,30, 60 min) (Table4-1).

The enzymatic hydrolysis was carried out in the Erlenmeyer flasks at 52°C and 150 rpm. for 24h with 0.25 ml/g SCG ACCELERASE® 1500 enzyme (GENENCOR®), under pH= 6.0, in CERTOMAT® H incubation shaker (B-Braun Biotech, Germany). The hydrolysate separation was performed with SIGMA 4K15 centrifuge (DJB Labcare Ltd, UK), set-up at 5000 rpm for 15 minutes.

Acid, enzymatic and combined acid-enzymatic hydrolyses were evaluated in terms of sugar release (C_s) and sugar extraction efficiency (E). C_s was determined by HPLC instrument, the global efficiency, E, is the sum of glucose and mannose efficiencies and was evaluated through the following equations

$$E_g(\%) = \frac{m\ glucose\ released}{m\ glucose\ theoretical} \qquad m\ glucose\ theoretical = \frac{m_{SCG} \cdot y_c}{0.9} \tag{1}$$

$$E_m(\%) = \frac{m\ mannose\ released}{m\ mannose\ theoretical} \qquad m\ mannose\ theoretical = \frac{m_{SCG} \cdot y_m}{0.9} \tag{2}$$

where:

- m_{SCG} was the initial dry biomass,
- y_c and y_m were fractions of cellulose in biomass (% dry biomass)

mannose in biomass (% dry biomass), respectively, while 0.9 is the conversion factor between cellulose and glucose and mannan and mannose (Wyman et al., 2004).

Table 4-1:Evaluation of acid hydrolysis, acid concentration- residence time and benefits of exclusive acid pre-treatment and combined acid pre-treatment and enzymatic hydrolysis. all configurations were performed in 100 ml of distilled water and SCG dry matter

Configuration	S:L (%)	Time (min)	H_2SO_4 (%v/v)	SCG as well (g)	H_2SO_4 (mL)	Enzymatic hydrolysis
1	9.6	15	0	10.45	0	NO
2	14.5	15	0	15.67	0	NO
3	19.3	15	0	20.89	0	NO
4	28.9	15	0	31.34	0	NO
1A	9.6	15	0.5	10.45	0.55	NO
2A	14.5	15	0.5	15.67	0.57	NO
3A	19.3	15	0.5	20.89	0.60	NO
4A	28.9	15	0.5	31.34	0.64	NO
1B	9.6	15	1	10.45	1.10	NO
2B	14.5	15	1	15.67	1.14	NO

3B	19.3	15	1	20.89	1.19	NO
4B	28.9	15	1	31.34	1.28	NO
IA	28.9	15	0.5	31.34	1.28	Yes
IB	28.9	15	1	31.34	1.28	Yes
IIA	28.9	30	0.5	31.34	1.28	Yes
IIB	28.9	30	1	31.34	1.28	Yes
IIB1	28.9	30	1	31.34	1.28	Yes
IIB2	28.9	30	1	31.34	1.28	NO
IIIB	28.9	60	1	31.34	1.28	Yes
IIIB2	28.9	60	1	31.34	1.28	NO
IV	28.9	0	0	31.34	1.28	Yes
IVB	28.9	0	1	31.34	1.28	Yes

.4.2.3 Separate Hydrolysis and Fermentation (SHF)

SHFs were performed to evaluate the following two process parameters:

1. Optimal hydrolysate separation systems comparing centrifuge and microfiltration with a solid-to-liquid (S:L) ratio of SCG of 9.6% w/w,
2. Fermentation efficiency after exclusive enzymatic hydrolysis and combined acid and enzymatic hydrolysis considering S:L ratio of SCGs of 14.5%, 19.5% w/w and 28.9% w/w (Table2).

4.2.4 Hydrolysis at laboratory scale

The acid hydrolysis was performed at 121°C for 15 min in a 3L bottle. Then, enzymatic hydrolysis was carried out in a BIOSTAT® B 5L stirrer fermenter (B-Braun Biotech, Germany) for 24h with 0.25 ml ACCELERASE® 1500 enzyme (GENENCOR®)/g SCG, under pH = 6.0, with a stirrer system - double Rushton turbine (6-blade disk impeller) at 300 rpm. At the end of enzymatic hydrolysis, a sample was collected and frozen at -20°C. Two aliquots of the hydrolysed were compared about separation modes (centrifugation and microfiltration) in terms of sugar release (C_s) (Eq.1), sugar extraction efficiency (E) (Eq.2) and separation efficiency (SE) (Eq.3)

$$\mathrm{SE(v/v)} = \frac{V\ liquid\ obtained}{V\ liquid\ total} \cdot 100$$

(3)

where

- V liquid obtained was the volume (mL) of separated/centrifuged solution
- V liquid total (mL) was the hydrolysed as well.

Centrifugation was carried out using a SIGMA 4K15 centrifuge (DJB Labcare Ltd, UK) at 5000 rpm for 15 min at 6°C. Filtration was performed through a cross-flow filtration system equipped with four INSIDE CéRAM ™ membranes (0.2 µm) (TAMI Industries, France) and a PERICOR® SF70 peristaltic pump (Verder, Germany) operating at 0.8 bar.

4.2.5 Fermentation at laboratory scale

4.2.5.1. Microorganisms and media culture

Bacillus Coagulans A166, a thermophilic homo-fermentative bacterium isolated by Leibniz Institute for Agriculture Engineering Potsdam – Bornim (ATB) from fresh hemp, was employed. MRS (de Man Rogosa and Sharpe, Merk KGaA, Germany) medium broth and agar were used for cultivation and stock cultures. Stock cultures were maintained at 7°C in 5 ml vials containing MRS agar and $CaCO_3$ before use. *B. Coagulans A166* cells were transferred from stock cultures to a 250 ml flask containing 60 ml MRS broth and 0.67 g EVERZIT® Dol (0.5–2.5 mm). Inoculum was incubated at 52°C for 16 h at 100 rpm in a CERTOMAT® H incubation shaker (B-Braun Biotech, Germany) before inoculation at 2% v/v into the fermenter.

4.2.5.2 Fermentation

Fermentation tests were performed at 1L-scale in BIOSTAT® B 2L stirrer fermenter (B-Braun Biotech, Germany), on 14.5 %, 19.5% and 28.9% w/w SCG at 52°C and pH 6.0, with no aeration. Nitrogen was supplied within 10 g/L yeast extract powder (Deutsche Hefewerke GmbH, Germany). Fermentation performances were evaluated though LA concentration (Eq.4), LA maximal and total productivity (Pmax and Ptot) (Eq 5,6), LA yield (Y) (Eq.7) and optical purity (OP). The equation adopted were the same ones reported in Chapter 3 section 3.2.5

Table 4-2: SHF configurations in 5l reactor with working volume of 3 l.

Configuration	S:L (%)	SCG as well (g)	H_2SO_4 (mL)	Accellerase (mL)	Hydrolysate separation system
1	9.6	288	32.88	72	microfiltration
2	9.6	288	32.88	72	centrifuge
3	14.5	435	34.35	108.75	centrifuge
4	14.5	435	0	108.75	centrifuge
5	19.5	585	35.85	146.25	centrifuge
6	19.5	585	0	146.25	centrifuge

7	28.9	867	38.67	216.75	centrifuge

4.2.6 SHF at technical scale and downstream processing

The scale up of the SHF process was carried out with H_2SO_4 1%v/v and enzymatic hydrolysis 0.25 ml/g SCG ACCELERASE® 1500 enzyme (GENENCOR®) on 14.5% SCG. The acid pre-treatment was performed in 10L bottle in the VARIOKLAV® 75 S steam autoclave for 15 min at 121°C. SHF was carried out in 72L BIOSTAT® UD bioreactor (B-Braun Biotech, Germany) containing 45 L working volume with 14.5% (S/L ratio) of SCG. As regards the inoculums (*Bacillus Coagulans A166*) preparation, a BIOSTAT® B 2L stirrer fermenter was inoculated with 20 ml from an incubation flask which was prepared as described in section 4.2.5.2. The culture vessel was loaded with 1L of distilled water containing 80 g/l of glucose and 10 g/l of yeast extract. The fermenter – filled with 700 ml of distilled water and glucose - was sterilized for 15 minutes at 121°C into the VARIOKLAV® 75 S steam autoclave, while 300 ml of distilled water, mixed with yeast extract, were sterilized in a separate bottle during the same sterilization cycle and then pumped into the bioreactor under sterile conditions.

After 16 hours of growth, the inoculum (*Bacillus Coagulans A166*) was pumped from the culture vessel into the fermenter under sterile conditions. At the end of SHF (22h), the fermentation broth was inactivated by setting the thermostat system of the fermenter to 80°C for 30 min. Downstream processing included micro- and nanofiltrations, softening, mono- and bipolar electrodialysis, purification through anion- and cation-exchange resins, and distillation. The methods are explained in detail in another work (Neu et al., 2016)

4.3. Results and discussion

4.3.1 Spent coffee grounds

The two SCG samples, SCG1 and SCG2, employed in the present study exhibited similar compositions (Table4-3). The average SCG composition had 94.13% ±2.79 dry matter and it consisted mainly in fibres, cellulose, lignin and hemicelluloses.

Elemental composition, proteins, ashes and lignin values agreed with literature data, while cellulose and hemicelluloses were different Mussatto et al. (2010) Ballesteros et al. (2014) (see Table4-3). Chemical composition of SCG can vary significantly according to (Jooste et al., 2013) due to the influence of the coffee bean origin and process conditions applied during roasting and water treatment for soluble solids extraction. Comparing three types of SCGs coming from different process conditions: SGC from brew coffee preparation (present study), SCG from soluble coffee production Mussatto et al. (2011) Ballesteros et al. (2014) and SCGs

from instant coffee production through thermal water extraction from roasted coffee beans, the cellulose and hemicelluloses contents varied significantly. In details, SGC from brew coffee preparation and SCGs from instant coffee production through thermal water extraction from roasted coffee beans had similar compositions, since more cellulose (around 48% dry matter) than hemicellulose (around 40% dry matter) was detected, while SGC from brew coffee preparation revelled cellulose higher and hemicelluloses contents lower than the ones of SCG from soluble coffee production, respectively +69.30% ± 7.78 and -80.81% ± 3.43. According to Zabed et al. (2017) cellulose, lignin and hemicelluloses represented around 70% of SCG dry matter.. Among waste biomasses (sugars, starchy and lignocellulosic biomasses), lignocellulosic biomass is the most abundant and complex matrix. The complex, hydrophobic and recalcitrant structure of lignin, makes lignocellulose resistant to mechanical, thermo-physical and biological degradation limiting the employment of lignocellulosic feedstocks in fermentative processes Hassan et al. (2020). Furthermore, cellulose has strong physic-chemical interactions with the hemicelluloses and lignin stabilized by strong intermolecular hydrogen bonds between hydroxyl groups of the adjacent molecule Saini et al., (2019). Complete hydrolysis of cellulose results in glucose, whereas hydrolysis of hemicelluloses provides pentoses as xylose and arabinose, which are not ready fermentable, hexoses, as mannose and glucose and co-product as acetic acid Mata et al. (2018). The following steps are required and studied: breaking down the complex structure of the lignocellulosic matrix by means of chemical hydrolysis and depolymerisation of cellulose and hemicellulose into their monomers through enzymatic hydrolysis.

Table 4-3:Physic-chemical features of SCG (data are expressed as % dry matter).

	this study									
	SCG1	**SCG2**	**Average**	**(Mussatto et al., 2011)**	**(Pujol et al., 2013)**	**(Ballesteros et al., 2014)**	**(Ruffino et al., 2015)**	**(Karmee, 2018)**	**(Kourmentza et al., 2018)**	**(Zabaniotou & Kamaterou, 2019)**
Dry matter	96.1	92.16	94.1±2.8					88.3		
N kjeldhal	1.5	1.	1.5±0.03						2.8±0.10	
NDF	66.4	66.4	66.4±0.03							
ADF	60.9	57.3	59.1±2.57							
ADL	24.8	25.0	24.9±0.11							
C	61.9				57.2 -59.8		51.7	53.0		52.5±0.4
N	1.7				1.2-1.3-		2.7	1.5		3.5±0.01
S	0.2						0.1	0.1		0.10±0.00
H	7.2				7.2-7.6		6.7	7.1		7.0±0.03
Cellulose	36.1	32.4	34.3±2.67	8.6		12.4			12.4±0.79	
Hemicellulose	5.5	9.1	7.3±2.54	36.7		39.1			39.1±1.94	
Lignin	24.8	25.0	24.9±0.11			23.9			23.9±1.70	
Arabinose				1.7		3.6			3.6±0.52	
Galactose				13.8		16.4			16.4±1.66	
Mannose				21.2		19.1			19.1±0.85	
Proteins	9.3	9.6	9.5±0.19	13.6		17.4			17.4±0.10	
Ashes	0.8	0.4	0.6±0.33	1.6		1.3			1.3±0.10	

4.3.2. Hydrolysis

4.3.2.1 Acid-enzymatic hydrolysis

The Erlenmeyer shaking flask experiments were performed to optimise the role of acid hydrolysis in the release of fermentable sugars from SCG, both with direct hydrolysis of hemicellulose and with the action on lignocellulose structure to enhance the subsequent enzyme action. Currently, lignocellulosic biomass can be pre-treated with four pre-treatment methods: physical, physico-chemical, biological and chemical. Nevertheless, in the present study only chemical pre-treatment was considered, since according to literature reviews: physical, physicochemical and biological pre-treatments are not yet ready for the commercial scale Hassan et al., (2020). Furthermore, physical pre-treatments are energy consuming and environmentally unfriendly Hassan et al. (2020), biological pre-treatments are characterized by low energy requirement, but very low rate and long residence times (Zabed et al., 2019) while physico-chemical ones require harsh conditions to achieve rapid treatment rate Hassan et al. (2020). Chemical pre-treatments are the most effective pre-treatment, recommended for industrial applications and with rapid treatment rate Artola et al. (2019). Hence, this preliminary study concerned the amount of H_2SO_4 according to four S:L ratios of SCG evaluating separation efficiency (SE), concentration of fermentable sugar release (C) and sugar extraction efficiency release (E).

SE, performed by means of centrifuge, decreased with the increase of s/l ratio of SCG, in detail SE decreased from -13.3% ± 0.77 to -58.0 %± 0.4 increasing the s/l concentration from 9.6% to 28.9% (Table 4-4). Liquid loss in autoclave did not depend on acid hydrolysis, but only on the S:L ratio of SCG, in detail the S:L from 9.6% to 28.9% leaded to a percentage liquid loss increase equal to 6.9% ± 0.48 (Table4-4). The sugar release (C_s) and sugar extraction efficiency (E_g E_m) were evaluated for total sugars (C_s, E_s), glucose (C_g, E_g), and mannose (C_m, E_m). Without considering the S:L ratio of SCG, but only referring to untreated and pre-treated SCG matter, the addition of 0.5 %v/v and 1%v/v of H_2SO_4 increased the mannose extraction respectively of 37.87% ± 4.65 and 52.98% ± 4.65. Hence, the acid hydrolysis achieved the target to enhance the availability of mannose extraction from hemicellulose compound. Evaluating H_2SO_4 dose and S:L ratio of SCG (Figure2), the total fermentable sugars percentage increased by adding 0.5-1% v/v H_2SO_4 increase for all s/l tested, and in detail the highest percentage increase release was obtained on 14.5% (Figure4-2, configuration 2A-2B) equal to 22.45% ± 1.90. Hence, considering the achieved results of Cs and E (Table4- 4, Figure4- 1), the most promising combination of H_2SO_4-SCG, minimising the acid addition and maximizing the SCG employments was: 1%v/v of H_2SO_4 and 14.5% SCG as well. The achieved result agreed with Mata et al. (2018) study concerning acid pre-treatments of SCG. The achieved result proved that H_2SO_4 pre-treatment can change the intrinsic properties of lignocellulosic materials and break up the linkage among cellulose, hemicellulose, and lignin to prepare the biomass for enzymatic

degradation Zabed et al. (2019). In detail, acid pre-treatments can reduce the particle size and crystallinity of cellulose, increase the solubilisation of hemicellulose and lignin, and to enhance the accessibility of cellulose - in term of surface area - to the enzyme in the following enzymatic hydrolysis step (Mohapatra et al., 2017).

The second parameter evaluated for acid hydrolysis was the residence time. Although, Table 4 depicted that 14.5%of S:Lratio for SCG reached the most efficient release of fermentable sugars, the optimization of acid hydrolysis was carried out on 28.9% SCG, the highest initial concentration, with the aim to make the process economically promising using as much feedstock as possible. Furthermore, the configuration 1% v/v of H_2SO_4 and 28.9 % of SCG made the acid pre-treatment similar to dilute acid concentration, (acid concentration below 4 % referring to dry matter) according to Zabed et al (2017), which were wider performed in scientific literature and more comparisons could be performed. Linear correlation test was performed to prove the significance of the retention time-acid concentration experiments and it was possible to state that the sugar release (C) and sugar extraction efficiency (E) improved with significant linear positive correlation ($r=0.943$ and $p<0.05$)) respectively with the addition of 0.5%v/v to 1%v/v of H_2SO_4, while the increase of retention time didn't exhibit a significant linear positive correlation ($p>0.05$) for 15 min, 30 min and 60 min. In detail, increasing H_2SO_4 from 0.5%v/v to 1%v/v, the glucose and mannose concentrations increased respectively from 78 mg/g to 94 mg/g and from 14 mg/g to 27 mg/g as regards a residence time of 15 min and from approximately 94 mg/g to 111 mg/g and from 13mg/g to 30 mg/g, concerning a residence time of 30 min, in accordance with Kovalcik et al.(2018). Further experimental analysis concerning the effect of pH adjustment for configuration IIB (after cooling phase overnight) and IIB1 (after few hours after acid pre-treatment) proved no influence in the release of mannose, since configuration IIB and IIB1 achieved the same mannose release, respectively: 17.2 mg/mg and 18.64 mg/mg with $p>0.05$ (Table4-4). Hence, for energetic reason, the acid hydrolysis was set to residence time of 15 min at 121°C with and the dose of 1%v/v of H_2SO_4 was confirmed (Table 4-4). The result achieved represents a hybrid acid pre-treatment configuration, since acid pre-treatment is generally performed at high temperature (>180° C) for short time (1-15min) or at low temperature (80-120°C) for long time (30-1h) (Zabed et al., 2017). To confirm the benefit of acid hydrolysis for mannose release from hemicellulose matter, the configuration IV (without H_2SO_4 addition) didn't release mannose. In configurations IV and IVB, the autoclavation was not performed, which means no thermal treatment, and the concentration of fermentable sugars substantially dropped around -20%, proving the fundamental role of temperature, according to Peng et al. (2012) Chu-ky et al. (2015) The last evaluation concerned the contribute of exclusive acid hydrolysis and combined acid and enzymatic hydrolysis, in configurations IIB-IIB2 and IIIB-IIIB2 (Figure 4- 2A-B). The results witnessed that for both configurations, with different residence time (30 and 60 min) the biggest amount of mannose detected at the end of enzymatic hydrolysis was released during acid hydrolysis, proving once more the effect on saccharification of hemicelluloses in mannose release.

Based on Table4-4, referring to the total amount, the percentage of mannose released during acid pre-treatment was 80.8 %± 0.32% for IIB-IIB2 and 98.3%± 0.45% for IIIB-IIIB2. Concerning the other fermentable sugar, the glucose released during acid hydrolysis was only in trace if compared to the total normalized concentration observed after the action of enzyme since, as already mentioned, cellulose hydrolysis during acid pre-treatment requires more severe conditions in terms of temperature and reaction time Somnuk et al. (2017). In the present study, the performance achieved by enzymatic hydrolysis agreed with literature data for SCG matrix, employing Accelerase as enzymes Jooste et al. (2013); Procentese and Rehmann, (2018). Indeed, glucose extraction efficiencies were very low for both residence time, i.e. around 0.4% and 1.3% for 30 minutes and 60 minutes, respectively, in accordance with acid hydrolysis of SCG carried out by (Mussatto et al. (2011); López-Garzón & Straathof, (2014). According to (Mcnutt and He, (2019), to increase the extraction efficiencies, the lipid extraction or defatting can be performed, but the free fatty acids and triglycerides releases made slow the hydrolysis of total sugars

Table 4-4: Evaluation of liquid loss in autoclave, separation efficiency (SE)concentration of sugar release (Cs) in total and separated phase and sugar extraction efficiency (E)

	Separation		Cs in liquid phase total			Cs in liquid phase separated			Sugar extraction efficiency		
	Liquid loss (%)	SE (%w/w)	Glucose (mg/g)	Mannose (mg/g)	Tot sugars (mg/g)	Glucose (mg/g)	Mannose (mg/g)	Tot. sugars (mg/g)	Eg (%)]	Em (%)	Etot (%)
1	5.74±1.53	75.5±0.14	58.19±13.44	0.96±1.35	59.15±14.79	45.95±10.66	0.75±1.07	46.70±11.73	15.29±3.53	2.04±2.89	13.83±2.83
2	5.69±0.01	66.91±0.01	63.83±0.23	0.00±0.00	63.83±0.23	46.70±0.27	0.00±0.00	46.70±0.27	16.77±0.06	0.00±0.00	14.93±0.05
3	5.94±0.81	58.02±2.01	58.73±3.65	0.90±1.27	59.63±4.92	36.65±6.38	0.60±0.85	37.25±7.23	15.43±0.96	1.92±2.72	13.95±1.15
4	6.17±0.17	47.3±1.76	67.64±7.04	0.96±1.36	68.60±8.4	33.77±1.54	0.50±0.71	34.27±2.25	17.77±1.85	2.04±2.89	16.04±1.33
1A	3.81±0.41	77.15±0.40	96.30±9.00	15.99±0.52	112.29±9.52	78.69±7.78	13.06±0.36	91.75±8.14	25.30±2.36	34.10±1.12	26.26±1.98
2A	5.1±0.25	67.59±0.30	79.47±5.85	11.88±0.02	91.35±5.87	58.44±4.38	8.74±0.00	67.18±4.38	20.88±1.54	25.33±0.04	21.36±1.36
3A	3.29±0.26	61.61±1.00	96.35±13.00	13.95±0.68	110.30±13.68	66.11±9.59	9.56±0.37	75.67±9.96	25.31±3.42	29.76±1.44	25.80±2.88
4A	4.21±0.47	47.83±1.00	83.28±6.14	12.14±0.24	95.42±6.38	45.44±4.03	6.62±0.23	52.06±4.26	21.88±1.61	25.89±0.51	22.32±1.49
1B	3.53±0.06	78.42±0.01	113.00±10.33	31.52±0.7	144.52±11.03	89.99±8.28	25.10±0.57	115.09±8.85	29.68±2.71	67.22±1.49	33.80±2.58
2B	4.96±0.01	69.49±0.24	100.52±1.27	26.03±0.97	126.55±2.24	75.36±1.19	19.51±0.67	94.87±1.86	26.41±0.33	55.51±2.07	29.60±0.07
3B	3.33±0.02	62.67±1.12	98.48±8.91	28.09±0.5	126.57±9.41	66.35±6.54	18.92±0.49	85.27±7.03	25.87±2.34	59.91±1.06	29.60±2.20
4B	3.38±0.61	51.23±1.51	109.46±10.11	24.82±0.64	134.28±10.75	61.57±8.03	13.93±0.17	75.50±8.20	28.76±2.66	52.93±1.37	31.41±2.22
IA	6.15±0.36	48.31±0.42	42.10±3.34	7.53±0.33	49.63±3.67	77.87±6.29	13.94±0.64	91.81±6.93	20.46±1.65	29.73±1.36	21.47±1.62
IB	6.14±0.26	49.87±0.79	53.17±6.07	15.21±0.99	68.38±7.06	93.98±9.33	26.90±1.34	120.88±10.67	24.69±2.45	57.36±2.86	28.27±2.50
IIA	3.75±0.32	50.78±0.82	55.29±5.67	7.54±1.76	62.83±7.43	93.79±8.36	12.81±3.16	106.6±11.52	24.64±2.20	27.33±6.74	24.93±1.22
IIB	2.96±0.58	52.01±0.24	67.07±0.38	18.20±0.91	85.27±1.29	110.67±1.05	30.04±1.61	140.71±2.66	29.07±0.27	64.06±3.43	32.91±0.62

IIB1	3.10±0.35	51.21±0.39	62.74±1.87	17.64±0.57	80.38±2.44	105.70±1.95	29.73±1.29	135.43±3.24	27.77±0.51	63.40±2.75	31.67±0.15
IIB2	3.21±0.45	37.50±0.37	0.64±0.03	9.65±0.54	10.29±0.57	1.61±0.09	24.28±1.28	25.89±1.37	0.423±0.02	51.78±2.73	/
IIIB	6.28±0.02	46.76±0.18	56.66±2.23	21.82±0.72	78.48±2.95	109.44±4.29	42.15±1.39	151.59±5.68	28.75±1.13	89.90±2.96	35.46±1.33
IIIB2	6.48±0.03	36.46±0.04	1.92±0.09	16.41±0.28	18.33±0.37	4.85±0.32	41.42±0.01	46.27±0.33	1.27±0.08	88.34±0.03	/
IV	0.00±0.00	48.78±0.24	30.04±1.45	0.00±0.00	30.04±1.45	53.34±2.59	0.00±0.00	53.34±2.59	14.01±0.68	0.00±0.00	12.47±0.61
IVB	0.00±0.00	49.73±0.79	32.94±4.16	0.00±0.00	32.94±4.16	60.24±9.07	0.00±0.00	60.24±9.07	15.83±2.38	0.00±0.00	14.09±2.12

Figure4-1:Evaluation of percentage increase of total fermentable sugars by H_2SO_4 addition

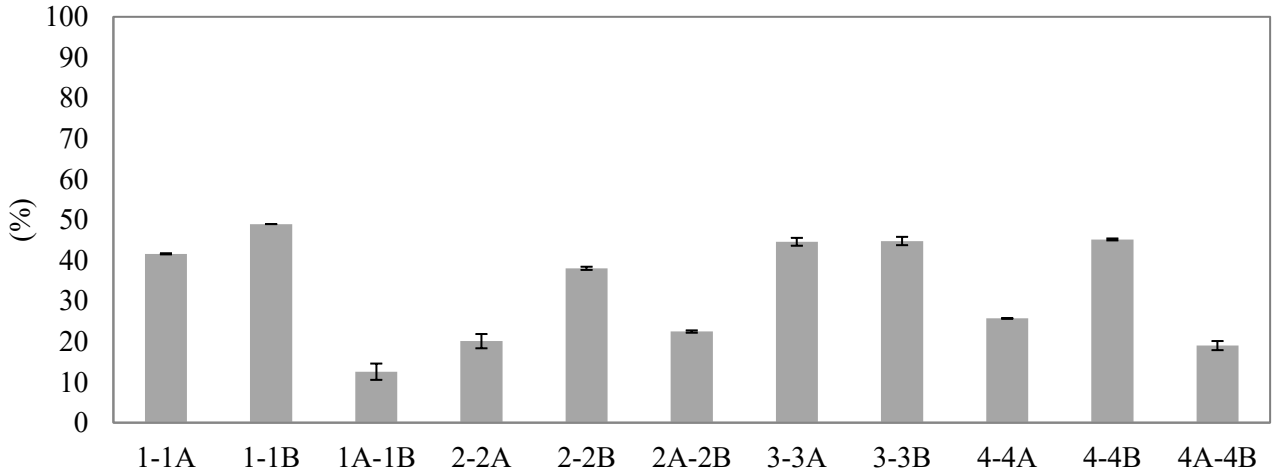
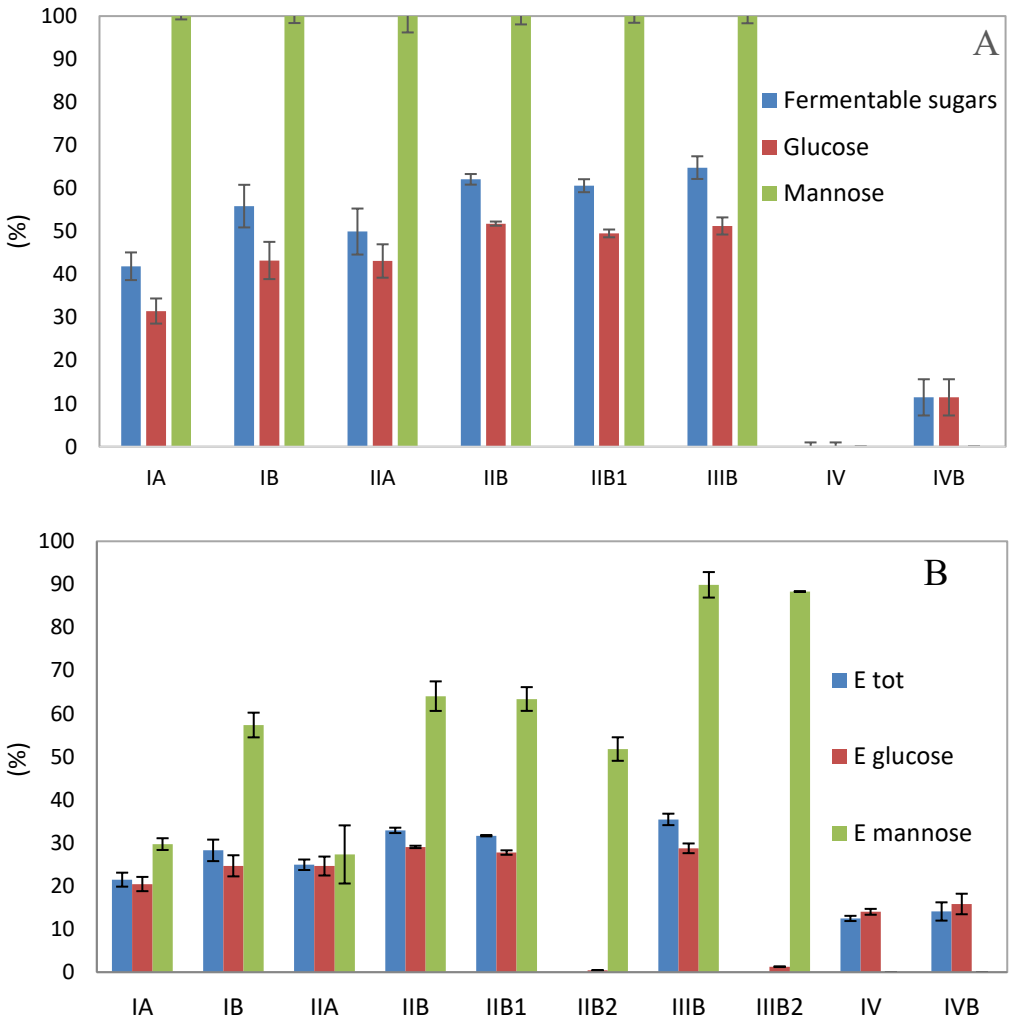


Figure 4-2: Evaluation of retention time-H_2SO_4 dose as percentage increment of sugars release (%) referring to the sample without acid pre-treatment and autoclavation. b) sugar extraction efficiency (e)



4.3.2.2 Separate Hydrolysis and Fermentation at laboratory scale

SHFs were performed to evaluate the following three process parameters: 1) optimal hydrolysate separation systems comparing centrifuge and microfiltration with s/l ratio of SCG of 9.6% w/w, 2) SHF considering fermentation efficiency after exclusive enzymatic hydrolysis and combined acid -enzymatic hydrolysis and 3) s/l ratio of SCGs of 14.5%, 19.5% w/w and 28.9% w/w.

Combined H_2SO_4 and enzymatic hydrolysis was performed on S:L ratio of 9.6 % SCG, since the target was the evaluation of separation systems and not the dependency of separation on the amount of substrate employed. The following concentrations of fermentable sugars (C) and sugar extraction efficiencies (E) in the hydrolysate were achieved: 127.1 mg/g and 33.4±2.6 % for glucose and 14.5 mg/g ±3 and 1.4±3.6 %. for mannose. As depicted in Table 4-7, the centrifuge reached

the highest separation efficiency (SE) and minimal fermentable sugar losses. In details, considering the same volume of hydrolysate (1.5 L), for centrifuge and filtration SEs were 68% and 63%, respectively, while for centrifuge and filtration the losses of total fermentable sugars were 31.89 %±0.3% and 42.59 %±0.1 respectively (John et al., 2009). The fermentations of the centrifuged and microfiltred hydrolysates reached respectively the LA concentrations of 93.35 ± 0.04 mg LA/gSCG dry and 77.96 ± 0.03 mg LA/gSCG dry after 25 hours (Table4-5, Figure4- 3A1-A2). The highest amount of fermentable sugars after centrifuge separation lead to higher LA production during fermentative process in agreement with Zeng, (2019). Hence, centrifugation was chosen as hydrolysate separation system.

The second investigated parameter was the effect of acid pre-treatment on fermentation efficiency on two S:L ratios: 14.5% and 19.5 % SCGs (Table4-6). The presence of mannose was observed only in the case of H_2SO_4 addition (Figure 4-3-B1, Figure4- 3-C1), as expected since mannose is released from hemicellulose during the acid hydrolysis, and it was completely consumed during biological fermentation for both S:L ratios investigated, 14.5% and 19.5% respectively. Actually, this confirms the preference of *Bacillus coagulans* in fermenting glucose and mannose before other sugars released from hemicellulose extracts, already investigated by De Paula et al., (2019); Zabed et al. (2019). Increasing the s/l ratio from 14.5% to 19.5%, the collection of samples during fermentation, by using the sample system of the fermenter was impossible, even after the action of enzyme, thus only initial and final concentration of fermentable sugars and LA are available for 19.5% SCG and no living cell counts can be performed.

Comparing SHF3-4 (Fig4-3B1-B2) and SHF 5-6 (Figure4- 3C1-C2) the performance of H_2SO_4 hydrolysis combined with enzymatic hydrolysis increased more than 3-fold the concentration of fermentable sugars suitable for the fermentative strain in comparison with the process in which only enzymatic hydrolysis was performed. In detail, the fermentable sugars increased from 57.0 mg/g to 176.31 mg/g from SHF4 to SHF3, and from 39.0 mg/g to 127.0 mg/g from SHF5 to SHF6, in accordance with the studies of Abdel-Rahman et al.(2013) Satlewal et al., (2018)

More in details, two considerations can be done (Figure4-4): 1) in the present study, only the performances of acid hydrolysis allowed the release of mannose, in SHF3 was 25.28 ± 0.30 mg/g with E_m equal to53.92% ±19.38and in SHF5 was 23.0 ± 0.28 mg/g with Em equal to 48.46%±17.12% and 2) the combination of acid-enzymatic hydrolysis boosted the release of glucose and disaccharideres. The release of glucose in SHF3 was 65.56% higher than in SHF4 and in SHF5 was 62.5% higher rather than in SHF6. The action of acid allowed the hydrolysis of hemicellulose in its simple sugars as well as the disruption of lignocellulosic structure, enhancing the accessibility of cellulose to the enzyme in the following enzymatic hydrolysis improving the global release of fermentable sugar Satlewal et al.(2018).

The higher concentration of fermentable sugars after combined acid-enzymatic hydrolysis lead to longer lag phase in LA fermentative production (Figure3 B1-B2),

in fact the lag phase of SHF3 was 6h longer than in SHF4. The fermentative consumption of high concentration of sugars can produce by products as furfural and HMF, which can inhibit cell growth and production of LA, although by-products should not be produced due to mild conditions of temperature and acid concentration of H_2SO_4 employed in the present study. A higher concentration of fermentable sugars may have exerted an inhibitory effect on adaptation of bacteria to the new environment. Low concentrations of acetic acid (AA) can be observed during fermentation without acid hydrolysis. AA can be a co-product of fermentation processes, when pentose sugars are consumed through the phosphoketolase pathway, but *Bacillus coagulans* is a homofermentative strain Pessôa et al. (2019); Y. Wang et al. (2018). Moreover, AA can be produced during acid pre-treatment – already under mild conditions - in which the acetyl group of hemicellulose linked to the lignin is released and reacted in acid form Bosco et al., (2014); Kourmentza et al., (2018).

Among the seven studied SHF configurations, SHF 3 achieved the highest LA yield and optical purity (Table4-6) witnessed the benefit of combined acid-enzymatic- hydrolysis. In detail, for SHF3 the LA concentration, yield and productivity were respectively 177 mg/g, 90% and 6.0 g/l h, respectively, while, SHF4 reached LA concentration, yield and productivity of 48mg/g, 92% and 2.2 g l-1 h-1, respectively. Beneficial of H_2SO_4 pre-treatment was confirmed by SHF5-6, thus the necessity of acid hydrolysis for SCG matter was proven (Table4-5),

The third and last evaluated parameters in acid-enzymatic separated hydrolysis and fermentation was the S:L of SCG to achieve the highest LA production (Table 6, Figure 3 B1, C1, D1 and Figure 4). Considering the release of fermentative sugars, LA concentration, LA yield and LA-optical purity the highest performance was achieved by SHF3. In detail, the sugar release of SHF3 was respectively 28.0% and 48.8% higher than SHF5 and SHF7 while the LA concentration of SHF3 was respectively 28.12% and 55.5% higher than SHF5 and SHF7.

To sum up the SHF configuration to scale up was the one corresponding to a S:L ration of 14.5% in SCG with combined acid-enzymatic hydrolysis.

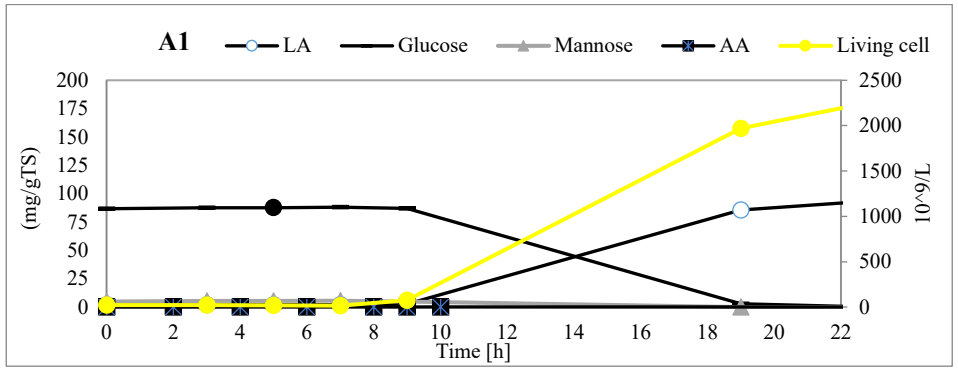
Table 4-5: Evaluation of centrifuge and microfiltration separation

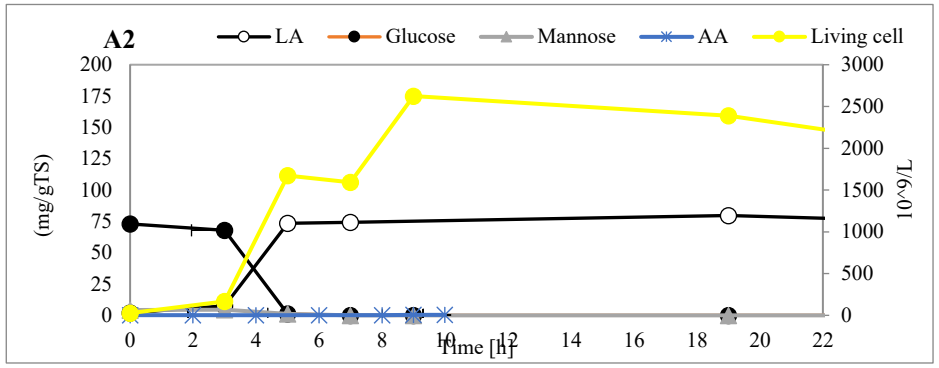
	Glucose	Xylose	Fermentable sugars	Separation efficiency
	(mg/g)	(mg/g)	(mg/g)	(%)
At the end of hydrolysis	127.05±0.02	7.41±0.01	134.47±0.05	
Centrifuge	86.85±0.01	4.74±0.09	91.59±0.11	68
Loss in centrifuge [%]	31.65	36.03	31.89 ±0.3	
Filtration	72.95±0.13	4.25±0.1	77.19±0.2	63
Loss in filtration [%]	42.58±0.07	42.69±0.09	42.59 ±0.1	

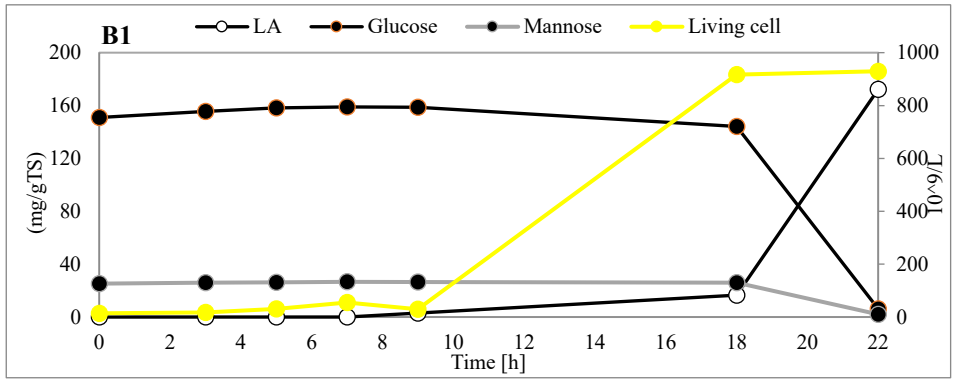
Table 4-6:Evaluation of SHF detecting hydrolysis and fermentation on s/l ratio of 9.6%, 14.5%, 19.5 and 28.9% of SCG. na=not available

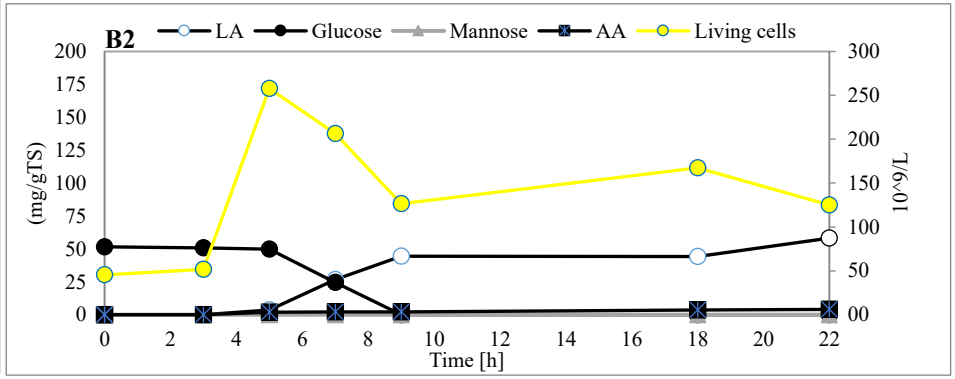
		Hydrolysis			Fermentation				
		E g (%)	**E m** (%)	**E f. s** (%)	**P max** (gLA/L·h)	**P tot** (gLA/L·h)	**LA** (mgLA/g SCG TS)	**Y** (mgLA/mg fs)	**OP** (%)
SHF 1	acid + enzymatic hydrolysis: microfiltration 9.6%	33.37±2.6	15.8± 2.5	31.45± 3.6	6.72 ±0.06	0.62±0.09	77.96 ± 0.00	0.98±0.6	31.45± 3.6
SHF2	acid + enzymatic hydrolysis: centrifugation 9.6%	33.37±2.6	15.8± 2.5	31.45± 3.6	6.30±0.06	0.69±0.00	93.35±0.04	0.99±0.4	99.60
SHF3	acid + enzymatic hydrolysis: 14.5 %	39.68±3.12	53.92±19.38	41.24±4.53	6±0.09	1.13±0.07	176.80±0.01	0.94±	99.66
SHF4	enzymatic hydrolysis: 14.5 %	13.54±1.06	0.00±13.20	12.06±1.46	2.2±0.05	0.30±0.07	47.57±0.06	0.88±	94.90
SHF5	acid + enzymatic hydrolysis: 19.5 %	27.29±2.15	48.46±17.12	29.61±3.23	na	na	127.07±0.48	0.93±	99.60
SHF6	enzymatic hydrolysis: 19.5 %	10.27±0.80	4.85±1.75	9.67±1.12	na	na	42.00±0.23	0.96±	94.30
SHF7	acid pre-treat + enzymatic hydrolysis: 28.9 %	31.92±0.90	75.24±1.80	36.67±0.99	5.60±0.07	1.24±0.08	78.57±0.08	0.83±	na

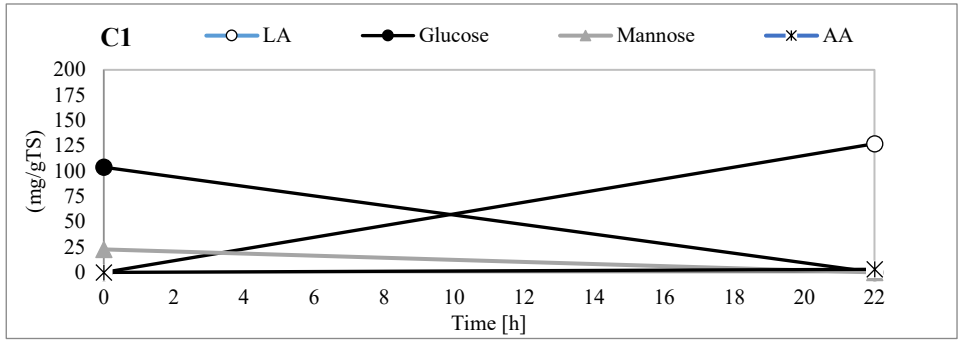
Figure 4-3: Evaluation of fermenatable sugars, LA and AA of: centrifuged SHF1 (A1) and microfiltred SHF2 (A2), hydrolysated with S:L ratio of 9.6%SCG, acid enzymatic SHF3 (B1) and enzymatic SHF4 (B2) with S:L ratio of 14.5 % SCG, acid+ enzymatic SHF (C1) and enzymatic SHF6 (C2) with S:L ratio of 19.5% SCG and acid+enzymatic hydrolysis SHF6 (D1) with S:L ratio equal to 28.9%

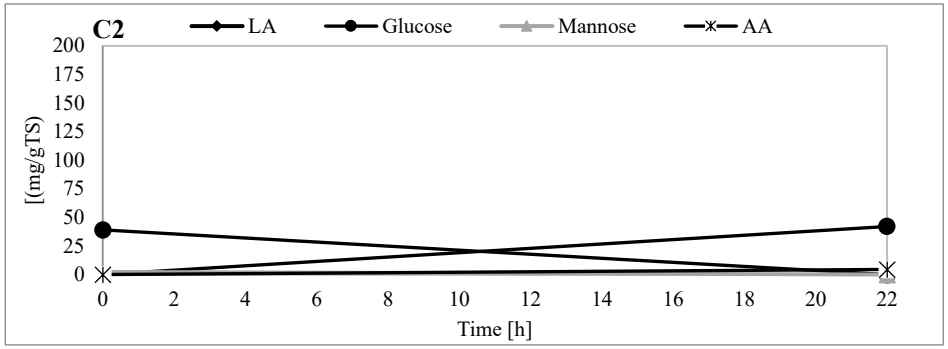









C1
LA
Glucose
Mannose
AA
(mg/gTS)
Time [h]


C2
LA
Glucose
Mannose
AA
[(mg/gTS)
Time [h]

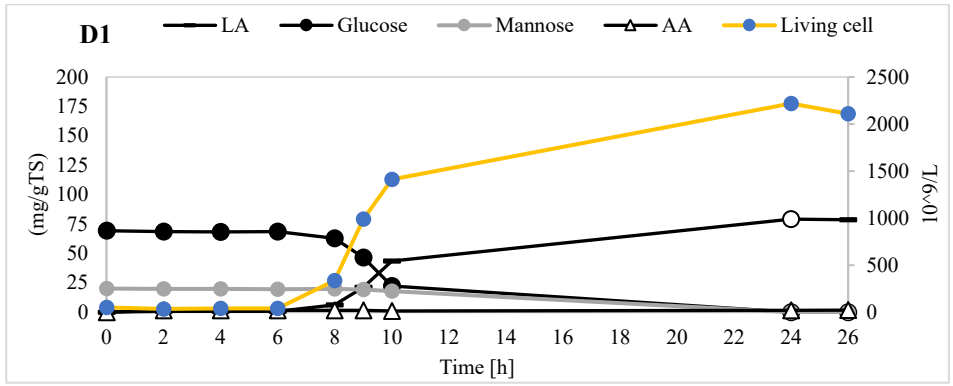
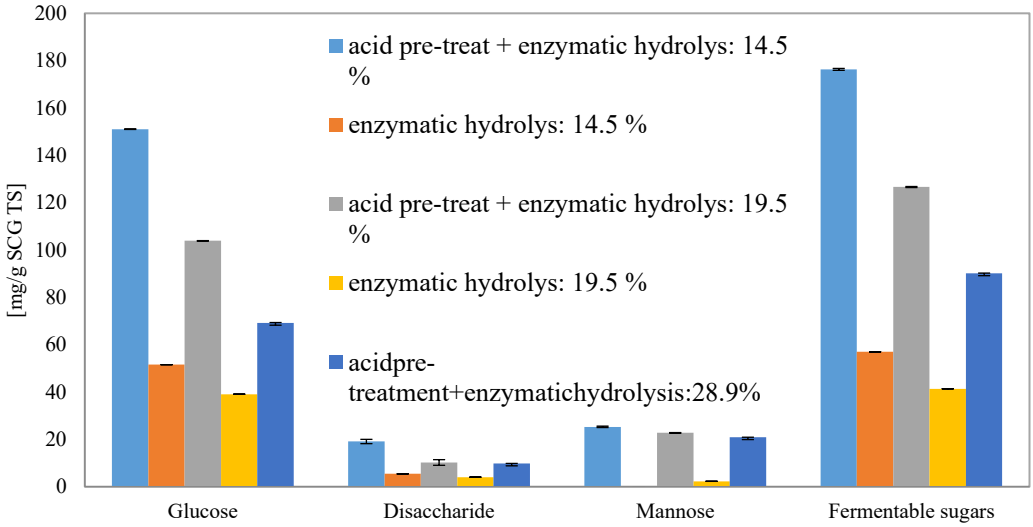

D1
LA
Glucose
Mannose
AA
Living cell
(mg/gTS)
10^9/L
Time [h]

Figure 4-4:Fermentative sugar release (cs) with acid and without acid hydrolysis at 14.5%,19.5% and 28.9%



4.3.3 SHF at technical scale and downstream processing

According to SHF performed at laboratory scale, the concentration of fermentable sugars remains approximately constant for 10 hours, reflecting a slow phase of adaptation by the side of *Bacillus coagulans*. Then, during the exponential phase, glucose was firstly consumed and afterwards mannose was completely fermented. Indeed, from an initial concentration of fermentable sugars around 180 mg/g dry, no glucose or mannose were detected after 24 hours of process time, producing a final LA concentration of 182.65 ± 0.63 mg/gTS with LA yield equal to 95.6 ± 1.5%. and exponential productivity of 5.6 g/l h. Furthermore, no AA was detected in 22h fermentation. Table7 witnessed that only 0.6% of the total glucose measured at the end of enzymatic hydrolysis can be released during acid hydrolysis, since cellulose hydrolysis during acid hydrolysis requires more severe conditions in terms of temperature and reaction time Mussatto, et al. (2011a). So, glucose extraction efficiency associated to acid pre-treatment was only around 0.6% which is in accordance with acid hydrolysis of SCG performed by Mussatto, et al. (2011a).

In Table4-8 downstream processing is reported. In total 47.2 L of pre-filtered culture broth with a LA concentration of 21.31 g /L was collected and used in micro-filtration to remove remaining SCG fibres and bacteria cells. After micro-filtration, 25.2 L of permeate with a LA concentration of 22.23g /L achieved a recovery of 55.7%, while the retentate (22 L) contained 19.62 g LA /L.

Nano-filtration was carried out to eliminate high and low molecular weights compounds from the permeate. The addition of 5 L of purified water were necessary to enhance the performance of the nano-filtration system. Nano-filtration resulted in 26.0 L permeate and 4 L retentate with LA concentration of 19.29 g/L and 8.73 g/L, respectively. Only permeate was used in the next down-stream processing steps, which resulted in a loss of 34.92 g LA.

A loss of around 20% and more after nano-filtration is not unusual in lactic acid down-stream processing Demichelis et al. (2018). After filtration steps, softening was carried out to remove cations from permeate with addition of 2.5 L of purified water, with a final volume of 30.4 L and concentration of 16.16 gLA/L.

After softening, according to the dialysis downstream step was performed to remove organic impurities, separate salt ions and convert sodium lactate into lactic acid

Monopolar electro-dialysis achieved a concentrate and diluate containing 66.44 g LA/L and 0.45 gLA/L, respectively. The recovery of lactic acid in the concentrate was 44.21% from the initial LA amount in the pre-filtered broth

The concentrate of the monopolar electro-dialysis was undergone to bipolar electro-dialysis and three streams were obtained: acid, base and salt. In bipolar electro-dialysis LA was concentrated predominantly in 5L acid stream with a concentration of 80.45 g LA/L and recovery of LA was 39.9% from the initial LA amount in the pre-filtered broth.

To reduce the staining of LA and the concentration of salt ions, decolorization, an- and cation-exchange chromatography were performed. After ion-exchange, 12.7 L of a 25.33 gLA/ L solution were reached obtained, which contained only traces of cat- and anions.

Final step of downstream processing was distillation to concentrate the LA solution, using vacuum distillation. Distillation reached resulting in 0.37 L of a 786.7 gLA/ L of pure LA solution with an optical purity of 99.8%. Through the whole process, the recovery rate starting from fermentation broth was 28.93% (Figure 24)

Considering the same downstream processing, the achieved LA recovery rate was lower than the ones achieved by Neu et al. (2016) with 38.9% with mucillagine coffee and Demichelis et al. (2017) with 90% with food waste. Comparing the present study with Neu et al., (2016); Demichelis et al., (2017) the SCG have higher amount of lignocellulosic matter and dry matter which make more difficult LA fermentation and downstream processing efficiency.

The high amount of waste coming out from fermentative broth and downstream processing represented a technical limiting factor for further or possible scale up of TRL (technical readiness level) of SCG conversion into LA. As depicted in Table 9, the main waste streams were: 10% retentate of pre-filtration and 50% from filtration steps. The retentate of pre-filtration, characterised by 38.5% dry matter (DM), 18.7%$_{DM}$ lignin, 17.2%$_{DM}$ cellulose and 4%$_{DM}$ hemicellulose, witnessed two factors: 1) biodegradation occurred from starting SCG: -24.82% DM lignin, -49.80%DM cellulose and -45.24% DM hemicellulose, 2) it could be still valorised in other sequential biological processes.

Table 4-7: SHF evaluation at 72L

	Hydrolysis						
	Glucose	**Disaccharide**	**Mannose**	**Fermentable sugars**	**E glucose**	**E mannose**	**E fermentable sugars**
	(mg/gSCG TS)	(mg/gSCG TS)	(mg/gSCG TS)	(mg/gSCG TS)	(%)	(%)	(%)
Acid-hydrolysis	1.00	3.17	6.05	7.05	0.26	12.91	1.65
Enzymatic-hydrolysis	170.51	16.82	9.41	179.91	44.79	20.06	42.08
Δ[%]	99.41	81.17	35.66	96.08			
	Fermentation						
	Pmax (gLA/L*h)	P tot (gLA/L*h)	LA (mgLA/g SCG TS)	Y (mgLA/mg free sugars)	OP (%)		
Fermentation	5.6±0.004	0.80±0.004	182.65 ± 0.63	0.956 ± 0.015	99.5%.		

Figure 4--5: SHF at 72L scale.

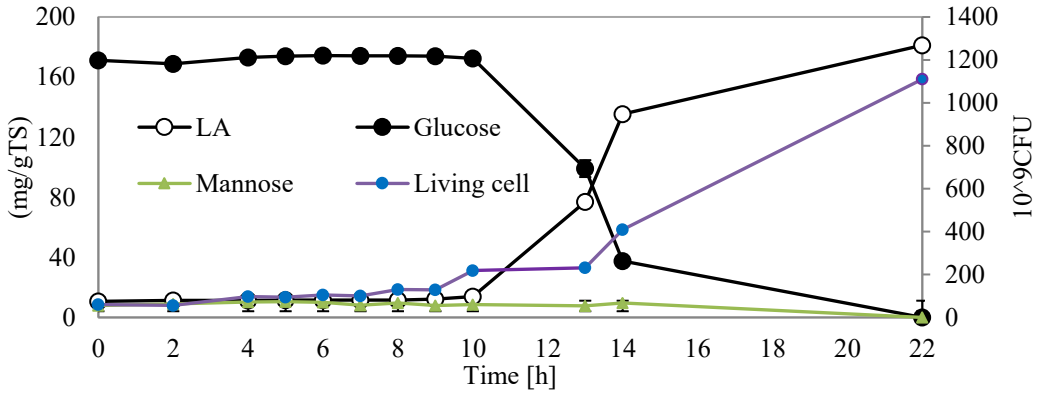


Table 4-8: downstream processing of 50 l SCG fermentation broth including volume of each fraction (v), volume of water added (h2o added), lactic acid, acetic acid, conductivity, pH, sulphate, sodium concentrations, concentrations of anions and other cations.

Process		V (L)	Water addition (L)	Lactic acid[3] (g/L)	Acetic acid (g/L)	Conductivity (mS/cm)	pH	SO_4^{2-} (mg/L)	Na^+ (mg/L)	Other anions* (mg/L)	Other cations**(mg/L)
Pre-filtration	Permeate	47.2	0	21.3	0.0	23.8	6.1	15357.9	13196.5	78.1	514.4
Microfiltration	Permeate	25.2	0	22.2	0.0	29.7	6.1	14862.	12809.4	75.01	471.1
	Retentate	22		19.6	0.0	/	/	/	/	/	/
Nanofiltration	Permeate	26	5	19.3	0.0	13.8	6.3	1318.0	5916.0	63,7	177.6
	Retentate	4		8.8	0.00	55.9	5.8	53548.2	27088.5	78.1	1090.5
	Softening (S950)	30.4	2.5	15.2	0.00	12.50	10.29	1014.00	12044.43	57.62	141.83
Monopolar ED	Concentrate	6.7	4	66.4	0.00	39.30	10.71	10156.41	46653.29	369.03	530.77
	Dilute	28		0.5	0.00	4.71	7.94	9.91	157.50	1.62	3.14
Bipolar ED	Acid	5.0	4.0	80.4	0.00	10.58	2.77	3846.00	4057.00	337.09	162.44
	Base	6.6	7.0	1.1	0.00	127.50	12.49	110.40	20503.3	0.38	247.67
	Salt	5.7	0.0	1.4	0.00	4.81	5.20	2036.00	1409.00	13.39	20.81
	Decolorization (MN502)	7.3	3.0	56.9	0.00	6.98	2.13	2644.00	1146.00	221,41	14.26
	Anion Exchange Resin (EXA 133)	9.5	3.0	35.1	0.0	2.8	2.80	21.14	879.90	0.47	8.78
	Cation Exchange Resin (EXC 08)	12.7	4.0	25.3	0.00	2.28	2.30	12.84	0.32	0.30	0.53

Distillation	Lactic acid	0.4	0.0	786.7	0.00	0,30	0.95	412.80	63.76	25.96	24.49
	Condensate	12.1	0.0	0.2	0.28	0.29	3.35	10.39	9.59	1.98	6.36

Figure 4-6: Downstream processing ions and la concentration during different downstream processing steps

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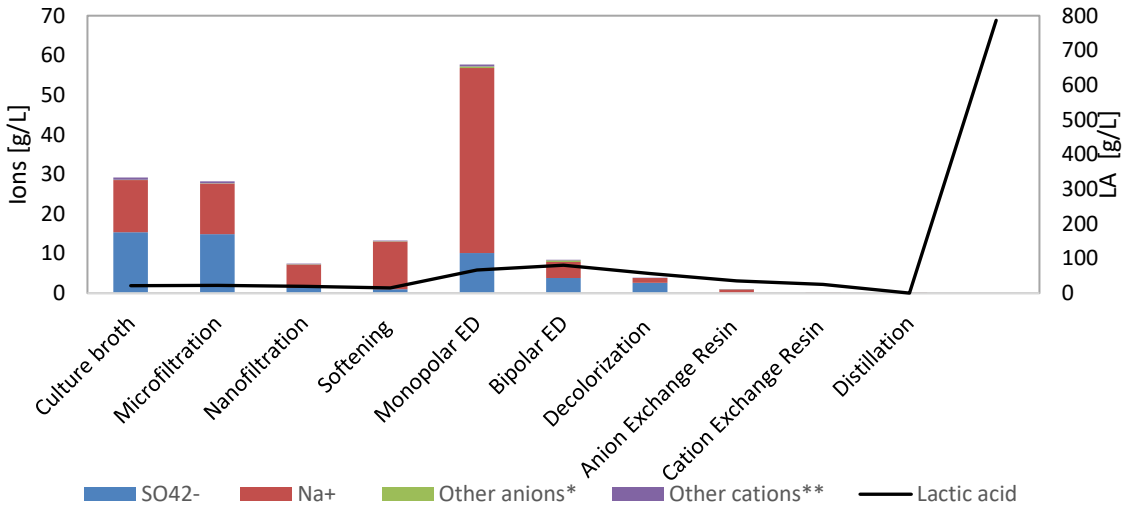

4.4. Conclusions

The present study investigated the acid-enzymatic hydrolysis and fermentation with *Bacillus Coagulans* of L(+)-lactic acid (LA) from spent coffee ground (SCG).

SCG, a lignocellulose residue from coffee production consisted of 34.26 ± 2.67% cellulose, 7.31% ± 2.54% hemicellulose and 24.88 ± 0.11% of lignin. Sequential and combined acid-enzymatic hydrolysis were carried out respectively, at 121°C for 15 min with 1%v/v H_2SO_4 and 14.5% SCG wet and at 52°C for 24h with 0.25 mL Accellerase 1500 per gram of dry SCG, achieving a total sugar extraction efficiency of 41.24 ± 4.53%. Fermentations carried out at laboratory (2 L) and technical (72 L) scales did not exhibit scale effects. At the 72L scale, LA yield per gram of sugar consumed and per dry gram of SCG were 0.956 ± 0.015, 0.18 ± 0.63 respectively. Downstream processing reached 786.70 gLA/L with 99.5% optical purity.

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Chapter 5: Investigation of Organic Fraction Municipal Solid Waste (OFMSW) valorisation through sequential lactic acid fermentative production and anaerobic digestion of fermentation residues

The main findings of the current study are already published in:

F.Demichelis D.Pleissner, S.Fiore, S.Mariano, I.Gutiérrez, R.Schneider, J.Venus (2017) Investigation of food waste valorization through sequential lactic acid fermentative production and anaerobic digestion of fermentation residues. Bioresource Technology (IF=5.6), Vol 241, pp 508-516.

Part of data came from the following thesis:

1. Simultaneous saccharification and fermentation and anaerobic digestion for production of lactic acid and biogas from food waste, F.Demichelis. Relatori S.Fiore and D.Pleissner. Politecnico di Torino
2. Separate hydrolysis and fermentation and anaerobic digestion for production of lactic acid and biogas from food waste, S. Mariano. Relatori S.Fiore and D.Pleissner. Politecnico di Torino

Abstract

Chapter 5 concerns the investigation of the sequential production of lactic acid (LA) and biogas from organic fraction municipal solid waste (OFMW).

LA was produced from OFMW using a *Streptococcus sp.* strain A620 (optimized in Chapter 3) by means of two fermentative pathways: separate enzymatic hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF). Via SHF a yield of 0.33 g_{LA}/g_{FW} (productivity 3.38 $g_{LA}/L{\cdot}h$) and via SSF 0.29 g_{LA}/g_{FW} (productivity 2.08 $g_{LA}/L{\cdot}h$) was reached. Fermentation residues and OFMSW were tested as feedstocks for anaerobic digestion (AD) (3 wt% TS). Achieved biogas yields were 0.71, 0.74 and 0.90 Nm^3/kg_{VS} for OFMSW and residues from SFF and SHF respectively.

The innovation of the approach consists in considering the conversion of OFMSW into two different sequential products through a biorefinery system, therefore making economically feasible LA production and valorising its fermentative residues. Finally, a mass balance of three different outlines is presented

5.0 Anaerobic digestion

5.0.1. Biogas properties

Biogas was biological produced by anaerobic digestion (AD) of organic substrates in a sealed fermenter. In standard conditions, biogas is a colourless and odourless gas. Its boiling point is 162° C at a pressure of 1 atm and it is flammable only over a narrow range of concentrations (5–15%) in air. Biogas is mainly composed by 55-70% of methane CH_4, 30-45% v/v of carbon dioxide CO_2, 0-0.5% v/v of hydrogen sulphide (H_2S), 0-5% of nitrogen N_2, 0-0.5% v/v of ammonia NH_3 and 1-5% v/v of water Labatut et al. (2011). The CO_2 presented in the biogas is neutral as far as the greenhouse effect is concerned Labatut et al.(2011).

5.0.2 Process structure

AD is multi-stages process, made up of hydrolysis, acidogenesis, acetogenesis and methanogenesis, which are generally carried out by different kinds of microorganisms such as: hydrolytic, acetogens, acidogens and methanogens bacteria. Since AD is a multi-stages process, each stage influences the next one and it is, in turn, influenced by the previous one (Figure5-1)

Hydrolysis is the first step of AD, in which the conversion of complex molecules takes place. In detail, complex biopolymers as carbohydrates, lipids and proteins are converted by hydrolytic bacteria into soluble monomers as sugar, fatty acids and amino acid. The most studied and employed bacteria are: *Streptococci, Bacteriocides, Clostridia, Enterobacteriaceae* and *Bifidobacteria*, which can release hydrolytic enzymes as cellulase, amylase, lipase, protease and xylanase to break the complex compounds into soluble organic substances which can be used by bacteria to perform the next step of acidogenesis. However, cell walls containing crosslinked glycan strands by peptide chains oppose a resistance in the hydrolysis phase (Kiran et al 2015) thus for this reason, hydrolysis is defined as the limiting rate-step. To overcome this problem, pre-treatments can be performed.

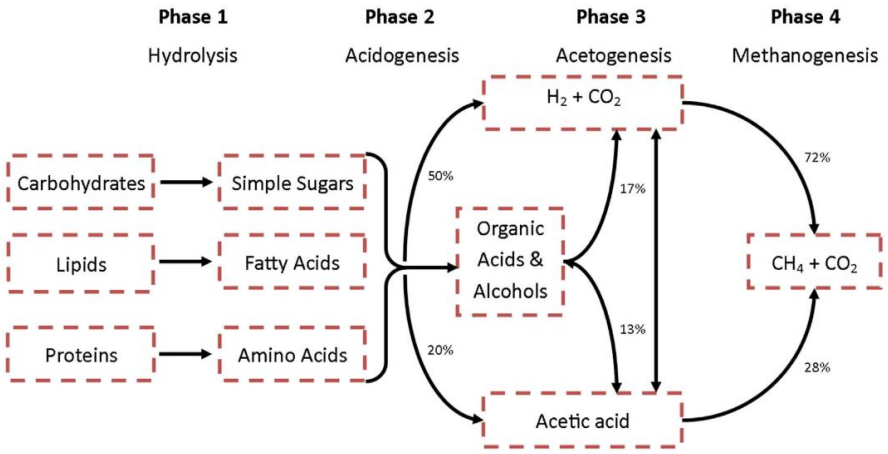
Acidogenesis is the second step of AD, in which sugar, fatty acids and amino acids are converted by acidogens bacteria into carbonic, alcohols and volatile fatty acids as propionic and butyric acids.

Acetogenesis is the third stage of AD, in which of the products of acidogenesis are converted by homo-acetogens into acetate, H_2 and CO_2.

Methanogenesis is the fourth and last step of AD in which biogas, gas mainly constituted by CH_4 and CO_2, is produced by two groups of methanogens. In detail, from acetate by acetoclastic bacteria which are acetate consumers and from carbon dioxide and hydrogen by methanogens, known as carbon dioxide reducing methanogens. Among microbial groups involved in AD, methanogens have the slowest growth rate, hence growth of methanogens is generally considered the rate-limiting step in the AD process. However, in the case of lignocellulosic substances hydrolysis can also be the rate-limiting step.

Acetoclastic methanogens convert acetate into CH_4 and CO_2, while hydrogen utilizing methanogens produce CH_4 exploiting CO_2 (electron acceptor) and H_2 (electron donator). The most adopted acetoclastic bacteria were *Methanosarcina barkeri, Metanonococcus mazei* and *Methanotrix soehngenii* Weiland, (2010)

Figure5-1:Anaerobic digestion pathway (Kouas 2020)



5.0.3 Parameters affecting anaerobic digestion

The most important operative parameters investigated in this Chapter were: 1) Total Solids (TS) and Volatile Solids (VS) feed, 2) feed modality, 3) working temperature ranges, 4) pH, 5) biogas composition and percentages, 6) organic loading rate (OLR) and hydraulic retention time (HRT), 7) Carbon-Nitrogen ratio, 8) inoculum-substrate ratio and 9) inhibitory substances formation as Volatile fatty acids (VFA) concentration. According to the amount of TS feed, AD was classified in dry and wet AD. If TS was major or minor 15%, AD was defined dry and wet, respectively. Usually, wet AD has major reaction intensity and shorter hydraulic retention times (HRT) compared to dry AD, whereas dry AD had lower reactor capacity and energy necessity than wet AD. Furthermore, dry AD had easier control of the floating of lignocellulosic (Figure2)

AD have three feed modalities: batch, feed-batch and continuous. According to Farghali et al. (2020) batch mode reaches higher CH_4 content than the other

ones, but the it was not possible the control of pH and removal of inhibitory elements Zhao et al.(2019) Kouas et al. (2019).

According to the employed micro-organisms AD can work in three temperature ranges:

- Psychrophilic: T <20°C
- Mesophilic 25°C <T<37°C
- Thermophilic >50°C

It is of fundamental importance to keep temperature under control, to prevent possible fluctuations negatively affecting the biogas production rate (Figure5-2).

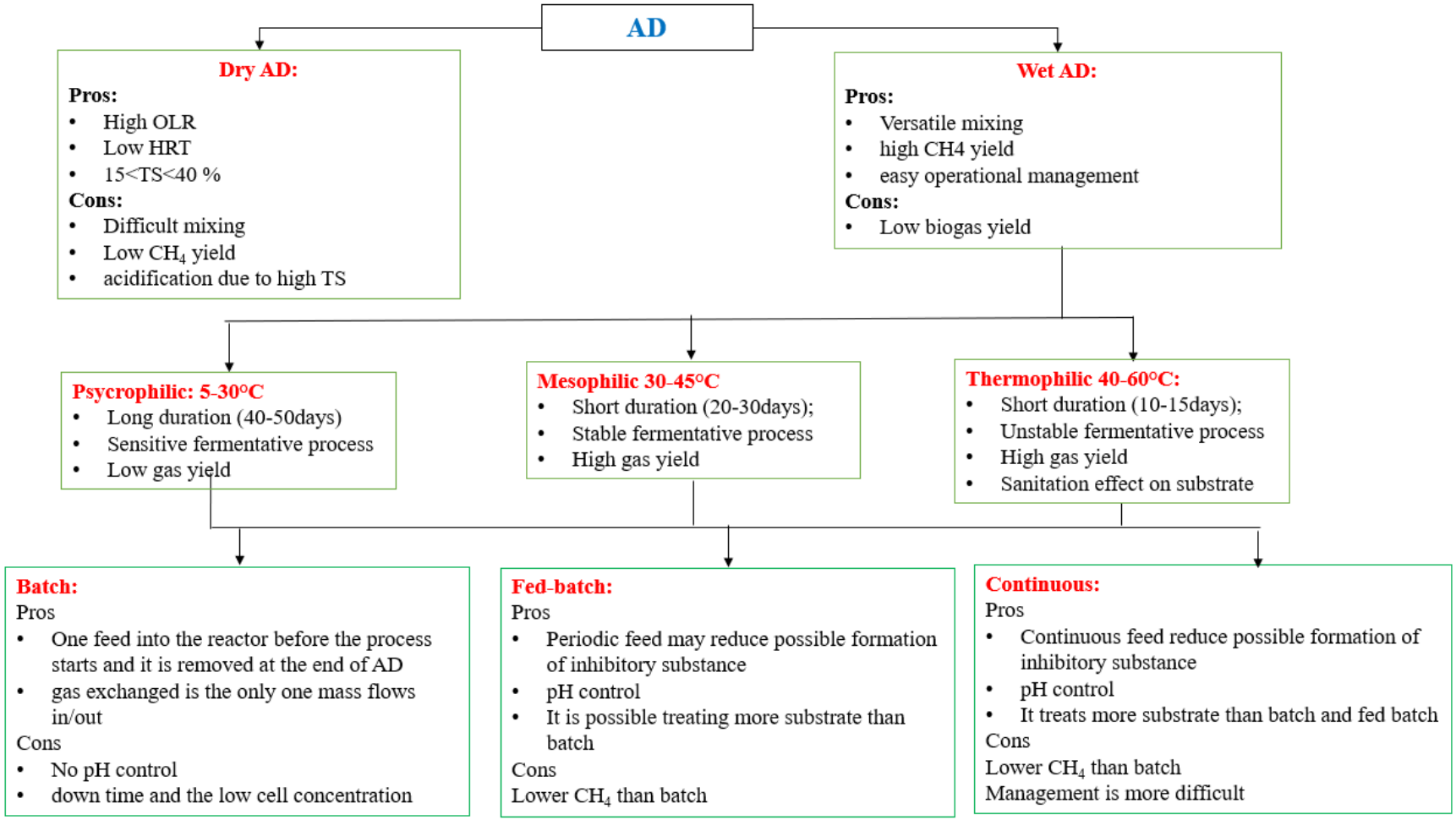
Thermophilic AD was more sensitive to temperature changes than mesophilic/psychrophilic ones, since high temperature system required longer time of adaptation to a new temperature. Whereas, mesophilic bacteria can tolerate fluctuations of temperature of about +/- 3°C Weiland, (2010).

Most of the biogas processes are carried out in mesophilic conditions, because psychrophilic operation is considered difficult for its slower degradation rates and long hydraulic HRT and sludge retention times (SRT) Kiran et al. (2015).

At thermophilic temperatures, the AD process is faster and more efficient, because the growth rate of methanogenic bacteria increases. However, at temperatures higher than thermophilic ones were achieved, biogas production decrease, due to the drop and damage of nucleic acid, proteins and other cellular components and inhibition of micro-organisms activity. Mesophilic bacteria, instead, show a better stability and required less energy to heat up the reactor than at thermophilic conditions. Furthermore, in mesophilic condition, ammonia inhibition can be controlled to reach higher methanogenic rates.

Currently a new configuration of AD was under evaluation, the combination of thermophilic mesophilic temperatures, named temperature phased AD, to exploit the higher hydrolysis and conversion rate of thermophilic AD and the process stability of mesophilic AD Panigrahi and Dubey, (2019). The AD classification is depicted and summarized in Figure5-2

Figure 5-2:Classification of the AD process



Methane formation take place in a restricted pH range (6.5 - 8.5) and the optimum interval was between 7.0 and 8.0. The process can be inhibited if the pH goes outside of these limits. Furthermore, pH depends on the optimum working condition of the bacteria; for hydrolysis and acid-producing bacterial the optimum pH range is between 5.00 and 6.00, instead for methane-producing bacteria is 6.5-7.8 (Kafle, 2013). The accumulation of VFA decreases the pH value Weiland, (2010), whereas pH increase may be due to NH_3 accumulation during the degradation of proteins.

Another fundamental parameter of AD is the carbon/nitrogen (C:N) ratio, since carbon is the energy source for anaerobic micro-organism activity and nitrogen increases the microbial community.

C:N is an indirect control of 1) the total ammonia nitrogen (TAN) in the substrate, 2) nutrient level of substrate and VFA concentration in the digestate.

The C:N ratio ranges between 20:1-30:1, with optimal 25:1 Panigrahi and Dubey, (2019)

If C:N is higher low protein solubilisation occurs which leads to VFA concentration, whereas low C:N

HRT and OLR depends on the type of substrate and process parameters, but HRT could not be lower 2-4d for methanogens micro-organisms Kainthola et al., (2019).

Mixing is a crucial parameter of AD and especially in dry AD. It is generally performed by mechanical tools and with liquid (digestate) or gas (biogas) re-circulation Panigrahi and Dubey, (2019). Other important parameters are the macro- and micro-nutrients, required for the growth and survival of microorganisms. The main macronutrients were: carbon, phosphorus, and sulphur and their ratio was set as $C{:}N{:}\ P{:}S$ = 600:15:5:1 Weiland, (2010). HRT and OLR depend on the type of substrate and process parameters, but HRT could not be lower than 2-4 days for methanogens micro-organisms Kainthola et al. (2019).

AD feed substrate must contain carbohydrates, proteins, fats, cellulose, and hemicellulose as main components. The biomasses with the following features are employed in AD Dinuccio,(2010):

- The content of VS should be appropriate and ranging between 55-100% $_{v/v}$
- The substrate should be free of pathogens and other organisms which may be dangerous for AD;
- The content of harmful substances should be low to allow ADs to take place smoothly and producing a biogas without dangerous substances as H_2S.
- The composition of the digestate should be made of substance employable as fertilizer.

The most common substrates adopted are residues from the agroindustry, cattle manure, agricultural residues from grass clippings, spent grains, sludge from wastewater treatment plant and slaughterhouse wastes Deublein, (2008).

VFA were one of the most important parameters for the control of biogas production. The process of conversion of VFA into methane is one of the limiting steps in methane generation Fantozzi, (2011). The increase of VFA concentration

lead to a drop of the pH and consequently inhibition of methanogenesis Wyman (2004). Optimal concentration values of VFA may be under 200 mg/L Nkemka, (2010).

To summarise in Table5-1 was reported the optimal range of operational parameters of AD.

Table 5-1:Optimal range of operational parameters of AD.

Parameters		Optimal condition
Temperature	Psycrofilic	5-30°C optimum 10°C
	Mesophilic	30-45°C optimum 35°C
	Thermophilic	45-65°C optimum 55°C
pH	Acidogens	4-8.5
	Methanogens	6.5-7.5
C:N ratio		25
HRT		Should be not less than 2-4, but it changed according feedstocks and temperature
SRT		Depending on Feed mode
TS	Wet AD	10-15%
	Dry AD	25-40%
ORP		-200:350 mV
Free NH_3		600-800 g/L
Headspace pressure		Up to 20

5.0.4 Anaerobic digestion limiting factors: Hydrogen, Carbon-Nitrogen and Ammonia

According to the AD operational parameters of section 5.0.2, the main limiting factors for AD were: Hydrogen, Carbon-Nitrogen and Ammonia concentrations.

Hydrogen can be an inhibitory substance for acetogenic bacteria, H_2-producing bacteria and methanogens community; hence it was fundamental to keep it under low partial pressure.

The C:N ratio depends on the feedstocks and it influenced biogas yields and composition Dioha, (2003). Micro-organisms need a 20-30:1 ratio of carbon to nitrogen, with the largest percentage of carbon being readily degradable. A correct ratio of C:N sources was requested in for enhancing the efficiency and yield of the process at maximum levels.

As before mentioned, ammonia was another inhibitory substance. The risk of ammonia inhibition was mainly referred to thermophilic conditions, because ammonia toxicity increases with the increment of temperatures

5.0.5 Biogas application:

Nowadays, biogas plays a key role in the emerging market for renewable energy production since 25% of UE-28 renewable energy target by 2020 will be

met by biogas. Lau et al. (2011). For this reason, the global capacity for power generation from biogas technology will be more than twice over the next decade, increasing from14.5 GW in 2012 to 29.5 GW in 2022 Sun et al. (2015). The market value of biogas and methane are respectively: 0.12 €/m^3 and 0.14-0.24 €/m^3(RNR market research, 2018). The production of biogas from bio-waste may provide economic and environmental benefits for the society, since it was a clean material and it is produced by renewable feedstocks. AD feed with bio-waste has two main benefits: 1) exploit by valorising the waste, which usually are incinerated or landfilled and 2) AD is a neutral CO_2 process.

In the world of renewable energy source- wind, solar, etc- biogas was the most used one. Biogas is directly adopted for: electricity and heat generation in full- scale facilities and in combined heat and power generations (CHPs) as gas.

Biogas cannot be directly used as a combustible because of the presence of some impurities and low heating value around 18.8 -21.6 MJ/Nm^3. Currently, Italy and Brazil are the countries with the highest number of CH_4-cars more than 2.4 million (Market RFA; 2018).

Biogas can be used as bio-fuels after the bio-methane upgrading process, during which the undesirable substances such as water H_2S, CO_2 and NH_3 were removed, increasing the CH_4 percentage up to 70%.

Bio-methane upgrading is performed with several technologies and the ones implemented at technical and full scale are: water scrubbing Ali et al. (2013), cryogenic separation Allengue et al. (2012), physical and chemical absorption Deng et al.(2010) Li et al. (2012), membrane technology Weiland., (2010). Among them, cryogenic separation reaches the highest clean efficiency 96.00 % with the highest energy consumption 1275MJ/tonCO_2 the other technologies range between 85-94% of clean efficiency and 0.45 kJ/tonCO_2 – 466 MJ/tonCO_2 Braguglia et al. (2018). AD also produced another material called digestate, a valuable fertiliser, because of its high nutrients content and it can be used as fertiliser in agriculture system.

5.1 Introduction

The aim of Chapter 5 is the investigation of the sequential production of lactic acid (LA) and biogas from organic fraction municipal solid waste (OFMW).

The novelty of the approach was considering AD as process to make and increase the sustainability of LA fermentative production reducing the amount of generable waste. In this Chapter, AD was not the first process to treat OFMSW, whereas it is considered as secondary process in a cascade biorefinery system. Two main advantages were identified: production of two high added products; LA and biogas production and increase of biogas yield thanks LA fermentation, which may be considered as biological pre-treatment for AD.

5.2 Materials and methods

5.2.1 Organic fraction municipal solid waste

Organic Fraction Municipal Solid Waste (OFMSW) was the same employed to carry out the experiments in Chapter 3 for Lactic acid (LA) production.

5.2.2. Anaerobic digestion

Anaerobic digestion (AD) was performed on three substrates: homogenized OFMSW and fermentation residues from SHF and SSF processes.

AD was carried out with batch feeding at 37°C using 3% (w/w) total solids (TS) in 2 L (1.5 L working volume) SCHOTT glass bottles. Substrate-to-inoculum ratio was 2:1. Digesters were manually shaken once a day. Each bottle was connected by 4/6 mm Teflon tubes (PTFE, Germany) to 3 L sampling tubes containing a saturated saline solution acidified with some drops of concentrate sulphuric acid. Biogas volume and composition were daily measured through water displacement and a gas analyser, respectively. Each AD test was carried out in triplicate and expressed as mean value plus standard deviation. Moreover, controls using inoculum and cellulose, and only inoculum called blanks were carried out in triplicate.

AD tests finished when marginal biogas production was below 1%. Of the total biogas production, up to that time.

Solubilisation (made of disintegration and hydrolysis*)* was considered the most rate-limiting step during AD of complex substrates rich in suspended solids (Van Lier et al., 2008). The disintegration constant (k_d) values were calculated according to Angelidaki et al. (2009) expressed in Eq.2. Assuming a first order kinetic model, the disintegration rate can be achieved through the first part of the cumulative biogas curve obtained from AD tests, according to:

$$B(t) = B_{exp}(1 - e^{-k_{dis}t}) \quad (2)$$

where:

B(t) represented the cumulative biogas/methane production at a given time

B_{exp} was the ultimate biogas/methane potential yield of the substrate

k_{dis} was the first order disintegration rate (1/d)

t was the time (d).

5.2.3 Statistical analysis

Analysis of variance was carried out in Microsoft Excel and was used to measure the statistical difference of LA formation between repetitions. Statistically significant difference in median values was accepted for $P < 0.05$.

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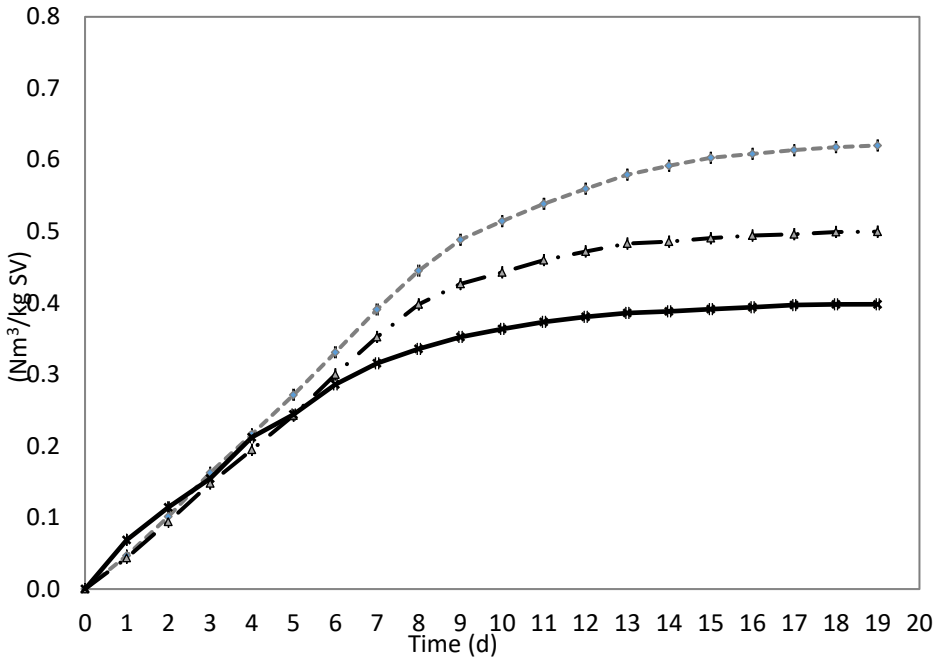
5.3. Results

5.3.1. Anaerobic digestion

AD tests lasted 20 days and resulted in following yields (Figure5-3): OFMW 0.710±0.02 Nm^3/kg_{VS} biogas, 0.398±0.035 Nm^3/kg_{VS} methane (56.35% v/v); fermentative residues from SSF: 0.743±0.01 Nm^3/kg_{VS} biogas, 0.499±0.008 Nm^3/kg_{VS} methane (67.19% v/v); fermentative residues from SHF: 0.90±0.016 Nm^3/kg_{VS} biogas, 0.62±0.013 Nm^3/kg_{VS} methane (68.8% v/v). Biogas and methane yields obtained from fermentation residues were higher than the ones achieved from OFMSW, because of the differences among the 3 substrates in relative abundance of carbohydrates, proteins and lipids. In detail, fermentative residues were rich in proteins and lipids, since their carbohydrate fraction was mostly already exploited in LA fermentation. Hence biogas and methane yields of fermentative residues were like pure proteins (0.7 Nm^3/kg_{VS} biogas, with an average methane content equal to 70%, v/v) and lipids (1.2 Nm^3/kg_{VS} biogas with an average methane content equal to 68%, v/v) (Weiland, 2010). OFMSW was made of carbohydrates, proteins and lipids, but carbohydrates are the most abundant fractions, and thus biogas and methane trends were comparable to carbohydrates typical values (0.8 Nm^3/kg_{VS} biogas, with an average methane content of 50%, v/v) (Weiland, 2010).

Both SSF and SHF demonstrated two accomplishments: generation of a value-added product (LA) and enhancement of biogas and methane yields. In a certain way, SSF and SHF had on AD the effect of a highly effective biological pre-treatment resulting in an improvement of methane production. In fact, the main purpose of AD pre-treatments is breaking the structure of substrate particles and transforming them in easily biodegradable liquefied products (Kafle, 2013). Considering the results achieved in the present research, it is possible to affirm that LA fermentation exploited carbohydrate (mainly) and protein (partly) fractions, leaving the lipids almost unaltered for the consequently carried out AD process (see Table 3) and boosting the kinetics of methane production. This assumption was confirmed by the values of the disintegration constant (k_d), calculated according to Angelidaki Angelidaki, (2009), which were equal to 0.43 1/d for FW, 0.35 1/d for SSF residues and 0.33 1/d for SHF residues. These values are of the same order of magnitude of the ones obtained in other studies Wang et al. (2016); Ruffino et al.(2015) using rice bran and husk (0.38 L/d), coffee dust and peel (0.31 1/d), mixed vegetable waste (0.38 L/d) and pesto sauce waste (0.25 L/d). Other Authors obtained 0.15-0.29 1/d for fruit pulp Gali et al. (2009), 0.34 1/d and 0.26 1/d for onion and potato respectively Giuliano et al. (2013), and 0.14-0.35 1/d for mixed food waste Alibardi and Cossu, (2015). However, the trend of k_d values obtained in this study (OFMW>SSF>SHF) was expected because, as before mentioned, both fermentative residues were deprived from the readily digestible carbohydrate fraction, with a higher efficiency of enzymatic hydrolysis.

Figure 5-3:Specific methane production from organic fraction municipal solid waste (OFMSW) (continuous line), SFF fermentative residues (triangle-dot line) and SHF fermentative residues (dotted line) through anaerobic digestion.



5.3.2 Mass balance

A mass balance was evaluated (Figures 4-6) for three different scenarios with the aim to assess the amount of LA and biogas that may be produced considering different scenarios.

In detail, LA production by means of SHF or SSF (Scenario1); biogas generation through anaerobic digestion (Scenario2); sequential production of LA from OFMW and of biogas from fermentative residues (Scenario3). The mass balance starts with a theoretical amount of 1000 kg dry OFMW made of 335 kg of starch, 148 kg proteins 129 kg fat and 85 kg free sugars. About LA production, downstream processes were considered according to the process scheme usually adopted at Leibniz Institute for Agricultural Engineering and Bioeconomy in Potsdam. In detail, a sequence of micro- and nanofiltration, softening, mono- and bipolar electro dialysis, decolourisation, anion and cation exchange and distillation was considered.

Considering Scenario1, 148.2 kg of LA and 851.8 kg of wastes (residual solids plus LA lost in downstream process) and 149 kg of LA and 851.1 kg of wastes (residual solids plus LA lost in downstream process) were produced respectively through SSF and SHF. Using Scenario2, 260.49 Nm^3 of CH_4 and consequentially 2604.9 kWh of primary energy could be produced. Taking into account Scenario3, combined SSF and AD produced 148.2 kg LA and 236.5 Nm^3 of CH_4 and therefore 2365 kWh of primary energy and 417 kg of digestate; while coupling SHF and AD produced 149 kg LA and 269.64 Nm^3 of CH_4 and therefore 2696.4 kWh of primary

energy and 408.52 kg of digestate. Wastes generated within the three scenarios, residual solids generated by Scenario1, as well as digestate deriving from Scenarios 2 and 3 could be valorised in a composting process.

The mass balance of Scenario 1 (Figure4) underlines that the main bottleneck of LA fermentation is the huge amount of wastes produced after fermentation and downstream processes. In Scenario 3(Figure5-6), this drawback was partially resolved by the consecutive AD. Anyway, downstream processes were usually highly complex and expensive, and they require a careful optimization Komesu et al.(2017).

Figure 5-4: Mass balance from food waste to lactic acid: Scenario1 represents the L(+)-lactic acid production through separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF). Mass balance is based on dry weight. OFMSW: organic fraction of municipal solids wastes

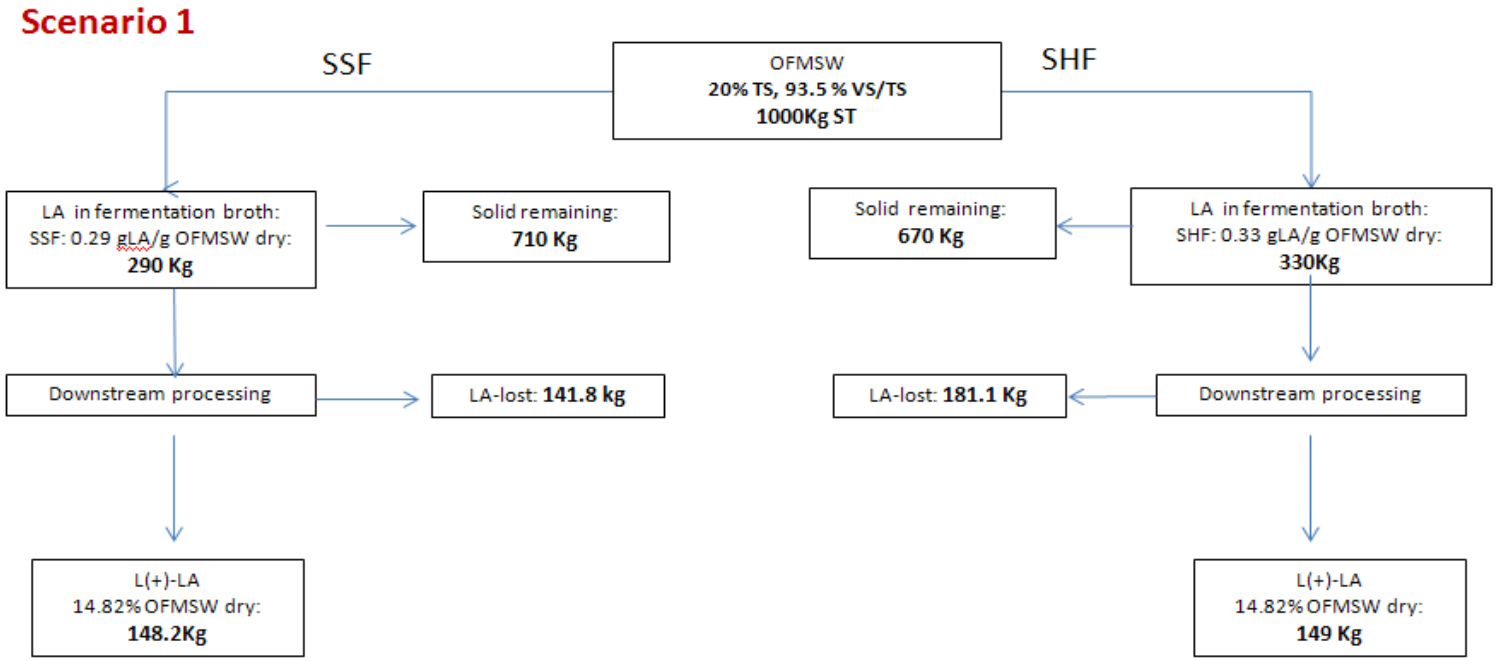


Figure 5-5: Mass balance from food waste to biogas: Scenario 2 represents biogas and methane production through anaerobic digestion (AD). Mass balance is based on dry weight. OFMSW: organic fraction of municipal solids wastes

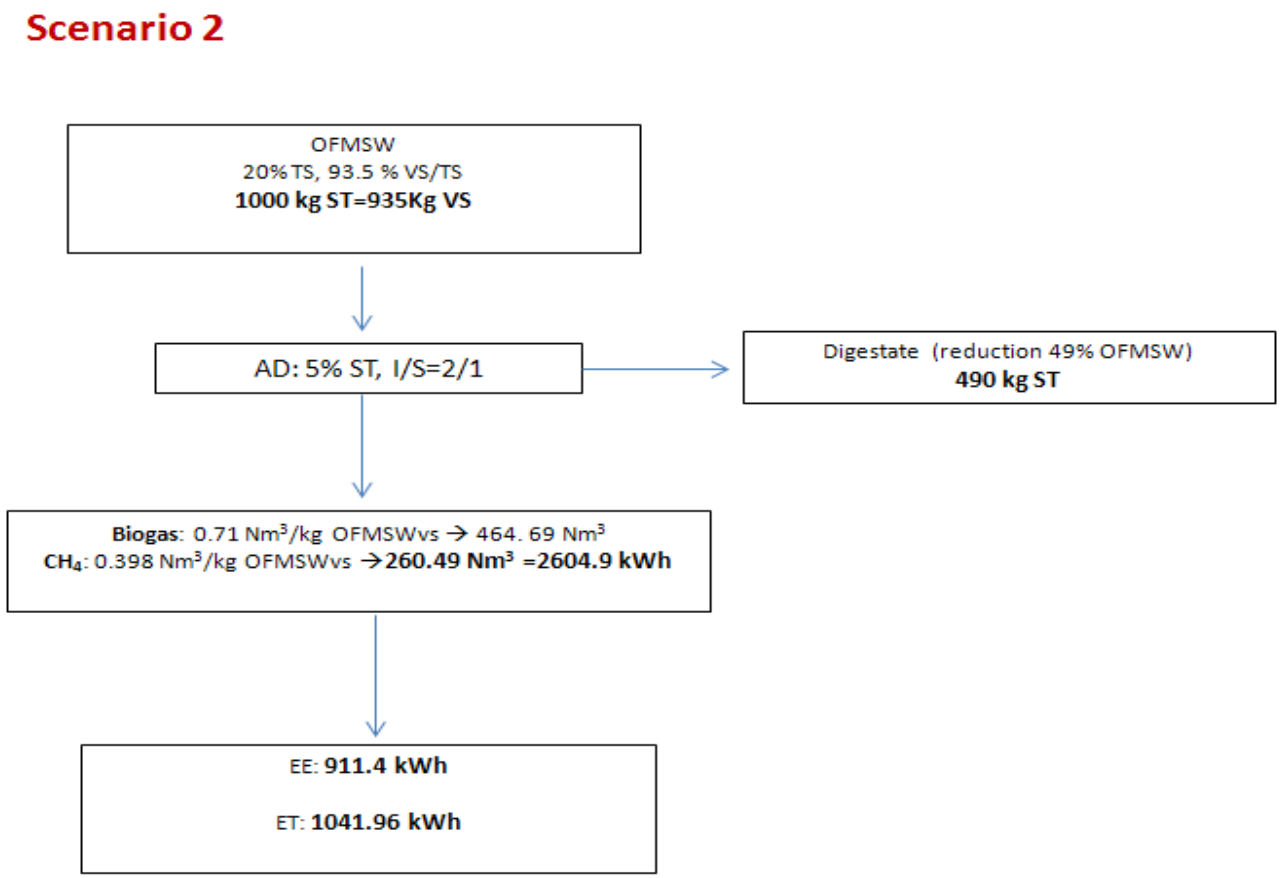
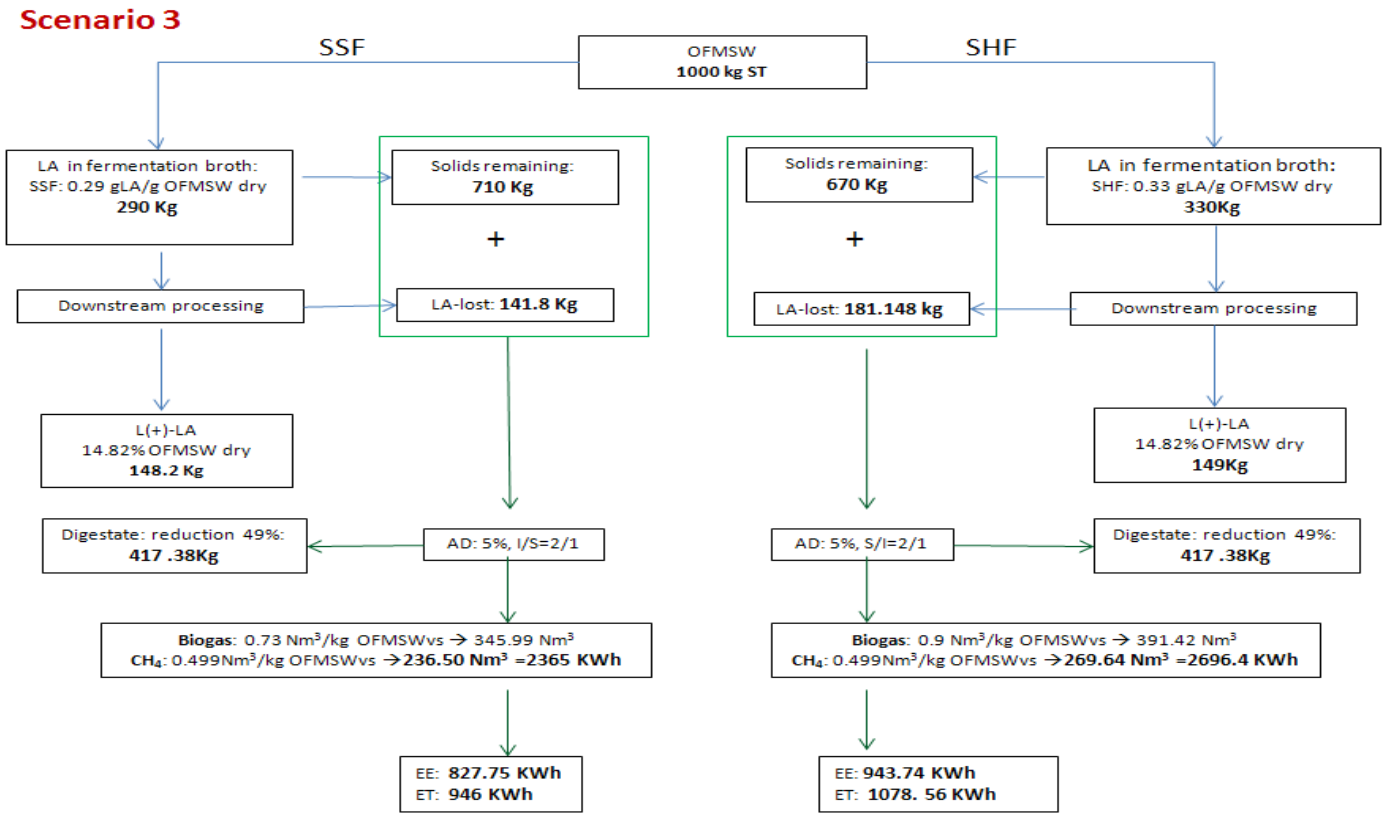


Figure 5-6: Mass balance from food waste to lactic acid and biogas scenario 3 represents combined L(+)-lactic acid and biogas production. mass balance is based on dry weight. OFMSW: organic fraction of municipal solids wastes



5.4. Conclusions

The present study investigated the technical feasibility of a sequential biorefinery systems for the sequential production of LA and biogas from OFMSW via either SHF or SSF. The main findings of the research were that SHF achieved higher yield and productivity than SSF, lasting one hour more than SSF. Sequential LA and biogas production moved forward from biomass conventional management and showed two profits: first, AD reduced and valorised the fermentative residues generated from LA fermentation; second, SSF and SHF determined an effective enhancement of biogas and methane yields with respect of OFMSW.

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Chapter 6: Technical and economic assessment of Organic Fraction Municipal Solid waste and Spent Coffee Grounds valorisation through a biorefinery chain.

The main findings of current study are already published in:
F.Demichelis, S. Fiore, D. Pleissner,J. Venus (2018). Technical and economic assessment of food waste valorization through a biorefinery chain. Renewable and Sustainable Energy Reviews (IF= 9.18), Vol. 94, pp.38-48

Part of data come from:
Biological valorisation of spent coffee grounds through production of lactic acid. Nardi A., Fiore S., Genon G.

Abstract

Chapter 6 evaluates the economic and energy assessments of a singular and integrated biorefinery system for sequential production of fermentative lactic acid (LA) and biogas from Organic Fraction Municipal Solid Waste (OFMSW) and Spent Coffee Grounds (SCG). Four scenarios were evaluated and compared: **Scenario IA** exclusive fermentative production of LA by means of simultaneous saccharification and fermentation (SSF) (explained in Chapter 3), **Scenario IB** LA production carried out with separated hydrolysis and fermentation (SHF) (explained in Chapter3), Scenario II exclusive biogas production by means of anaerobic digestion (explained in Chapter 5). **Scenario III A-B** for sequential fermentative LA production and biogas by means of SSF and SHF from OFMSW (explained in Chapter 5). **Scenario IV** LA production by means of SHF from SCG (explained in Chapter 4). The integrated biorefinery process was compared to single processes for either lactic acid or biogas productions. The economic evaluation, considering catchment areas from 2000 to 1 million inhabitants, was based on data from real biorefinery plants and carried out using SuperPro Designer® 8.0. The consistency of the approach was assessed through a set of composite indicators. The integrated biorefinery system was investigated from three main perspectives: 1) economic feasibility of producing LA and biogas, 2) the effect of process scale and 3) energy consumption/requirement. The present study proved that an integrated biorefinery system contributes more to optimal use of energy and material flows

than single processes both for the sequential production of two market value products and optimisation of waste management. Profitability was achieved for catchment areas bigger than 20,000-50,000 inhabitants.

6.1. Introduction

The aim of the present Chapter was to compare four different scenarios to evaluate pros and cons of LA and biogas productions from OFMSW and SCG from technical-economic-environmental perspectives. Productions were considered as single processes or as a two-step chain to investigate the hierarchical and sequential design approaches Moncada et al. (2016). The chosen scenarios were: 1) Scenario I A-B: LA fermentation from OFMSW, 2) Scenario II biogas production, 3) Scenario III A-B sequential LA fermentation and biogas production from fermentation residues from Scenario I A-B and 4) Scenario IV LA fermentation Scenario IV from SCG.

These four scenarios aimed to be representative for the whole EU, thus scenarios were not geo-referred, and the environmental evaluation considered only the energy balance and no Life cycle assessment) was included.

The novelty of the presented approach was the economic and energy analyses based on experimental data from existing biorefinery plants. Those data were investigated using SuperPro Designer® 8.0 software. In scientific literature world, previous studies concerned economic and energy assessments of biorefineries, like fungal hydrolysis and LA fermentation, LA production, biogas and other bio-based products generation, based on software simulations through SuperPro Designer® 8.0 Kwan et al.,(2015); Kwan et al., (2018) and Aspen Plus® Daful et al. (2017); Wingren et al. (2003), and on stochastic models as Monte Carlo statistical simulation Akeberg and Zacchi, (2000). Compared to the above-cited scientific literature, exclusively focused on the economic analysis of a single process and on the market value of the products, the present Chapter considered a two-step chain biorefinery system: sequential production of a platform chemical and biogas, according to the general hierarchy assumed by biorefineries De Jong et al. (2015) and the management of the waste proving the crucial and versatile role of biogas in single and sequential biorefinery processes Hagman et al. (2018). The consistency of the present study was assessed through: 1) Implementing the four above-mentioned scenarios in catchment areas from 2000 to 1 million inhabitants, 2) a final evaluation through composite indicators of the most promising scenario based on net-incomes after 5 years of amortization, NPV, ROI, payback time and economic incomes per ton of treated FW and 3) multi criteria decision ranking to define which of the four Scenario was the most sustainable from technical-economic-environmental perspectives.

6.2 Materials and methods

6.2.1 Modelling approach and boundary conditions

The biorefinery system of the four scenarios were studied and designed by an empirical model rather than a stochastic one. A stochastic model provides as output probable distribution with uncertainty based on the chosen random variables and model, which generally must infer from prior experimental data collected from real plants Donosco-Bravo et al. (2011). Contrarily, an empirical model described exactly how processes work under different boundary conditions and is validated through simulation models Khoshnevisan et al. (2018). The empirical data were taken from Pleissner et al. (2017); Demichelis eta al., (2017), (Demichelis et al, in preparation) explained in Chapter 3-5 and simulated using SuperPro Designer® 8.0 to evaluate mass and energy balances and costs. To perform the mass balance data, 0.8 conservative factor was adopted to full-scale plants according to Chemical Engineering Plant Cost Index and so to scale up the studies of Pleissner et al., (2017); Demichelis et al. (2017) and explained in Chapter 4-5. Economic and energy data were taken from literature and existing plants Piotrowski et al (2013). Batch and continuous operations were considered for LA and biogas production, respectively, with a 300 days per year operation time and 90% working capacity. Extra time was accounted for filling, emptying and cleaning of the fermenter in batch mode. According to previous studies Kwan et al (2015); Shahzad et al. (2015) a plant lifetime of 20 years was assumed.

The four scenarios were not geo-referred, because of the chore of the present Chapter was the evaluation of process profitability and sustainability according to catchment area between 2000 and 1 million inhabitants without any influence of location.

6.2.2 Biorefinery processes description

Productions were considered as single processes, exclusive LA or biogas fermentative productions, or as a two-step chain to investigate the hierarchical and sequential design approaches Moncada et al. (2016):

- Scenario I:
    - Scenario IA: Production of LA from OFMSW through simultaneous saccharification and fermentation (SSF);
    - Scenario IB: Production of LA from OFMSW through separate hydrolysis and fermentation (SHF)
- Scenario II: Generation of biogas from OFMSW by means of mesophilic anaerobic digestion;
- Scenario III:

    - Scenario III A: Sequential LA production from OFMSW through SSF and biogas generation from LA fermentative broth and downstream residues through mesophilic AD.
    - Scenario III B: Sequential LA production from OFMSW through SHF and biogas generation from LA fermentative broth and downstream residues through mesophilic AD.
- Scenario IV: Production of LA from OFMSW through separate hydrolysis and fermentation (SHF)

The three scenarios were based on processes already investigated at laboratory (2 L) and technical scales (72 L) Pleissner et al. (2017); Demichelis et al. (2017). In accordance to (Tchobanoglous, 2009) OFMSW was considered equal to 30%-wt of EU-28 average municipal solid waste (MSW) production (Eurostat, 2016).

6.2.2.1 Scenario I

In Scenario I the production of LA from blended OFMSW (20%-wt TS) through two alternative fermentative routes: SSF (Scenario IA) or SSH (Scenario IB) was considered. SSF was carried out at 35°C and pH 6 for 29 h reaching 0.29 gram of LA per gram of dry substrate with the employment of *Streptococcus* sp. A620, a mesophilic strain isolated from tapioca starch (Scenario I A, see Figure6-1A).

In SHF, hydrolysis was carried out with 3.5 µL Stargen enzyme formulation per gram of dry substrate for 1 h at 59°C and pH 4.5. Then, the hydrolyzed substrate was inoculated with *Streptococcus* sp. A620 and the fermentation was carried out at 35°C and pH 6 for 29 hours reaching 0.33 of LA per gram of dry substrate (Scenario IB, see Figure6-1B)

In both fermentative routes, downstream processing included centrifugation, microfiltration, ultrafiltration, electrodialysis and concentration of LA by vacuum distillation. The downstream processing was defined according to Pleissner et al., (2017) Demichelis et al. (2017) and the downstream scheme available at Leibnitz Institute, Potstdam, Germany. Downstream processing resulted in a L(+)-LA solution with 90% optical grade purity, which consistent with the downstream processing performance presented elsewhere (Vijayakumar, 2008) and with L(+)-LA with consistent market value.

6.2.2.2 Scenario II

Scenario II depicted the AD of OFMSW at 35°C with 2:1 substrate-to-inoculum ratio and 10 %-wt TS (Figure6-2). OFMSW was fed to a blender together with a respective amount of water and afterwards to a continuous stirred tank reactor (CSTR). Biogas was collected in a gasometer and transferred in a combined heat and power (CHP) system consisting of a normal pressure biomass boiler. The

simultaneous production of electricity and heat contributes to the principle of cascade use and saves primary energy. The digestate, (the residues of AD) can possibly be converted into compost. Biogas and methane yields were 0.71 Nm^3/kg_{VS} and 0.39 $Nm^3/kg_{VS,}$ respectively according to Demichelis et al (2017) and explained in Chapter 5

6.2.2.3 Scenario III

Scenario III evaluated the sequential production of LA and biogas. In detail, LA was produced from OFMSW through SSF or SHF, and biogas was generated from fermentation residues. Two configurations were considered for Scenario III:

- Scenario III A: SSF and AD (Figure 3A);
- Scenario III B: SHF and AD (Figure 3B).

6.2.2.4 Scenario IV

Scenario IV (Figure 4) depicted the acid-enzymatic hydrolysis and fermentation with *Bacillus Coagulans* of L(+)-lactic acid (LA) from spent coffee ground (SCGC).

Sequential and combined acid-enzymatic hydrolysis were carried out respectively, at 121°C for 15 min with 1%v/v H_2SO_4 and 14.5% SCG wet and at 52°C for 24h with 0.25 mL Accellerase 1500 per gram of dry SCG, achieving a total sugar extraction efficiency of 41.24 ± 4.53%. Fermentations reached LA yield per gram of sugar consumed and per dry gram of SCG were 0.956 ± 0.015, 0.18 ± 0.63 respectively. Downstream processing reached 786.70 gLA/L with 99.5% optical purity.

Figure 6-1: Process scheme of the Scenario IA (1A), and Scenario IB (1B)

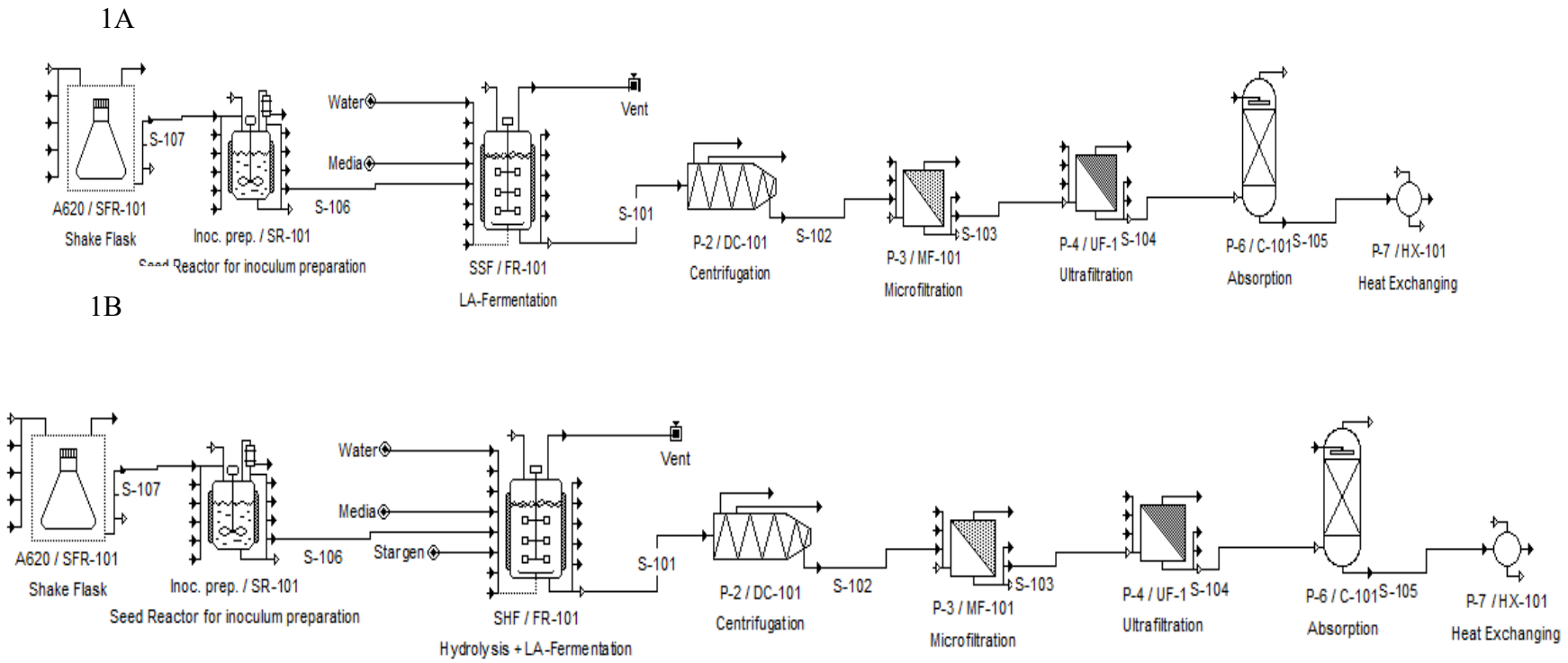


Figure 6-2: Process scheme of the Scenario II.

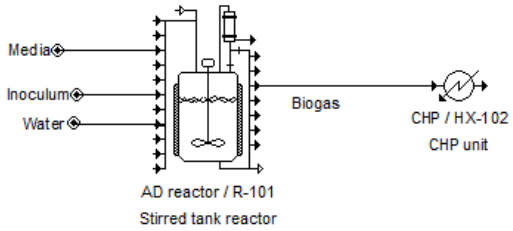
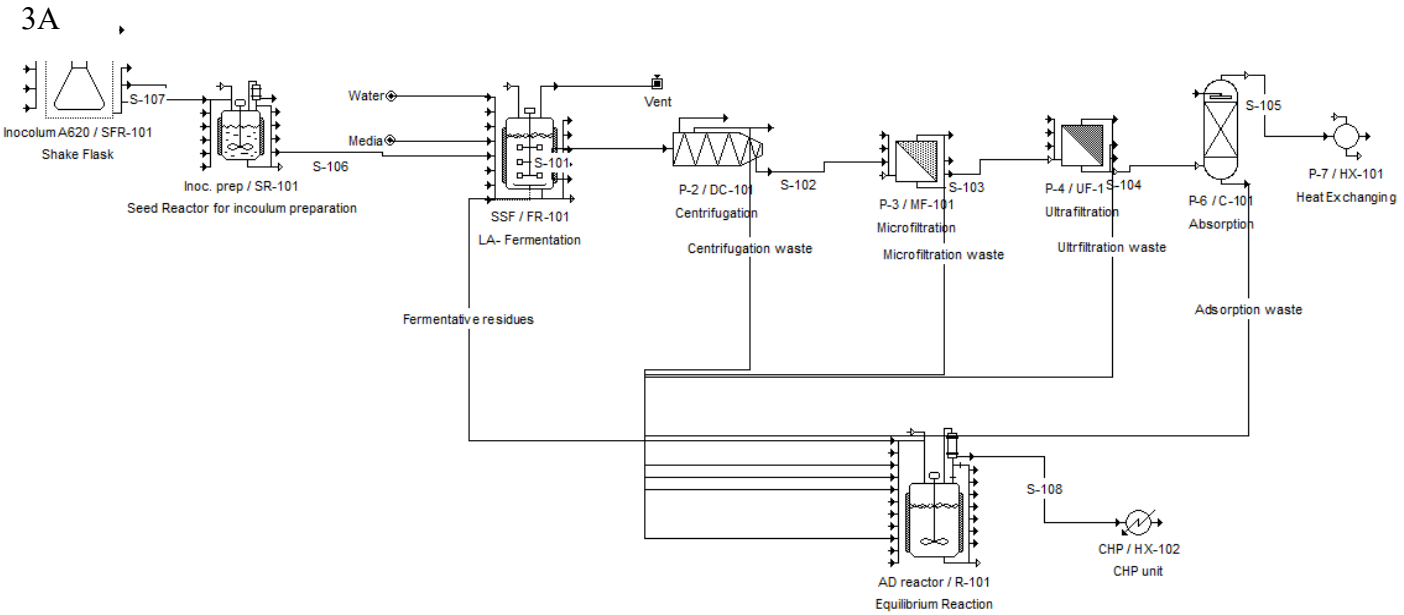


Figure 6-3: Process scheme of the Scenario III A (3A), and Scenario III B (3B).



3B

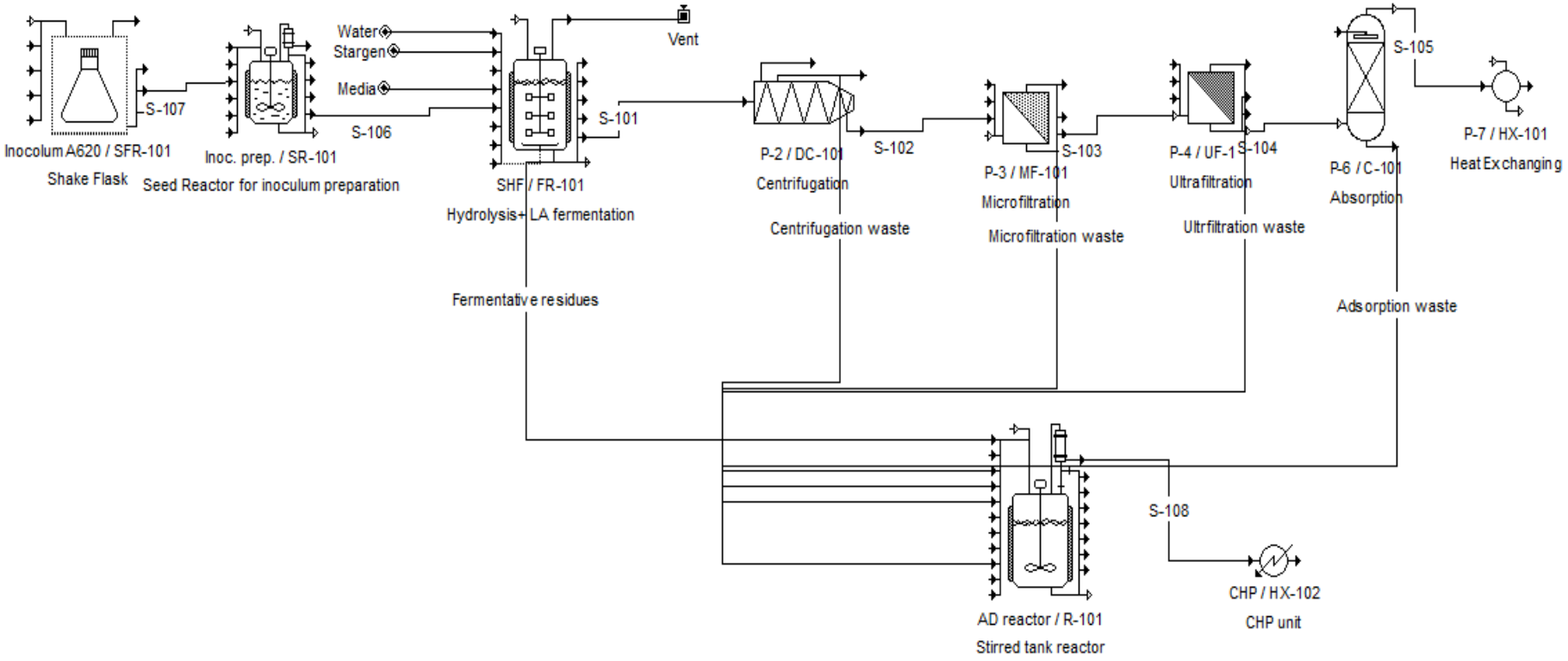
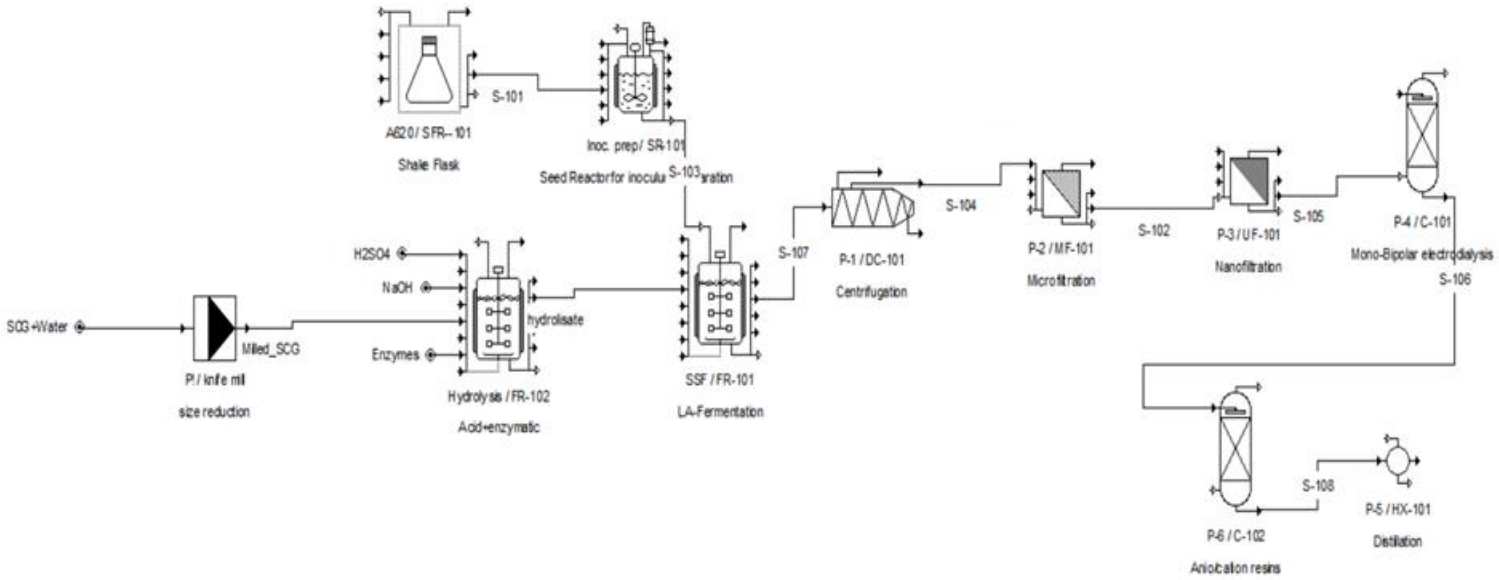

Inocolum A620 / SFR-101
Shake Flask
S-107
Inoc. prep. / SR-101
Seed Reactor for inoculum preparation
Water
Stargen
Media
S-106
SHF / FR-101
Hydrolysis+ LA fermentation
Vent
S-101
P-2 / DC-101
Centrifugation
S-102
Centrifugation waste
P-3 / MF-101
Microfiltration
S-103
Microfiltration waste
P-4 / UF-1
Ultrafiltration
S-104
Ultrfiltration waste
P-6 / C-101
Absorption
S-105
Adsorption waste
P-7 / HX-101
Heat Exchanging
Fermentative residues
AD reactor / R-101
Stirred tank reactor
S-108
CHP / HX-102
CHP unit

Figure 6-4: Process scheme of the Scenario IV



6.2.3 Energy balance

The energy balance was modeled according to the model presented in Mehr et al. (2017):

- Atmospheric air is made of 79% $_{v/v}$ N_2 and 21% $_{v/v}$ O_2.
- Ideal gas law is applied and gas leaks from the components and connecting pipes are negligible.
- The analysis is carried out under thermodynamic equilibrium and steady state conditions.

The total system thermal load (*Qs*), expressed in kW, was calculated considering the seasonal variations (Eq. 1):

$$Q_s = Q_{sub} + Q_{loss} + Q_p \quad (1)$$

$$Q_{sub} = m_{sub} \cdot c_p \cdot (T_{in} - T_{reac}) \quad (2)$$

where

- Q_{sub} is the thermal power required to heat the substrate from inlet temperature (5-26°C depending on the season) to 35°C (see Eq. 2).

In Eq. 2,

- m_{sub} represents the mass substrate flow rate,
- T_{in} and T_{reac} are the inlet and reactor temperatures, respectively
- c_p is the specific heat capacity (here considered equal to the value of water, as OFMSW dry matter is equal to 20%-wt).
- Q_{loss} is the heat loss through the reactor walls and calculated using Eq. 3:

$$Q_{loss} = U_{ug} \cdot A_{ug} \cdot (T_{reac} - T_{gr}) + U_{ext} \cdot A_{ext} \cdot (T_{reac} - T_{ext}) \quad (3)$$

where

- U_{ug} and U_{ext} are the heat transfer coefficients for underground walls and non-ground walls, respectively,
- $A_{ug}$and A_{ext} are the areas of underground walls and partial walls, respectively, and roof;
- T_{gr} and T_{ext} are the temperatures of underground walls and partial walls, respectively.

Qp is the heat loss through piping and it is calculated using Eq. 4:

$$Q_p = \%_p \cdot (Q_{sub} + Q_{loss}) \quad (4)$$

European temperature average was taken as inlet, underground and partial walls temperatures (IPCC, 2018).

6.2.4 Economic analysis

Economic feasibility of the four investigated scenarios was evaluated considering: total capital investment, total operational costs, profitability of the processes, ROI,

Euro paid and gained per tons of OFMSW or SCG treated and Euro paid per tons of LA and methane produced.

6.2.4.1 Capital cost evaluation

Capital cost included fixed capital investment (FCI) and working capital cost. FCI enclosed the purchase of equipment and facilities for construction and installation. The working capital was assumed to be 6.5% of FCI Peters et al. (2003). The details of capital cost estimation are reported in Table6-1. Costs adjustment for the different catching area sizes was performed according to Chemical Engineering Plant Cost Index. The cost of land was not considered, as the study is not geo-referred. A 5-years amortization with a 2% interest was assumed for the investment cost and calculated using Eq. 5:

$$A(Euro) = C_0 \cdot \frac{i \cdot (1+i)^n}{(1+i)^n - 1} \tag{5}$$

where:

- A was the amortization cost,
- C_o was the initial capital,
- i was the interest
- n the number of years considered for amortization.

Table 6-1: Detail of capital costs

Technique	Unit	Cost (€/unit)	References
Grinder	kg/s	2323.3	Akeberg and Zacchi, 2000
Reactor	m^3	2514.7	Kumar et al., 2012
Stirrer	kW	46465.3	Akeberg and Zacchi, 2000
Centrifuge	kg/s	116163.2	Akeberg and Zacchi, 2000
Microfilter membrane and module	m^2	24637.8	Akeberg and Zacchi, 2000
Ultrafilter membrane and module/	m^2	6164.1	Akeberg and Zacchi, 2000
Dryer	No. of drums	1152.3	Akeberg and Zacchi, 2000
Electrodialysis unit	No. of cells	6576.3	Akeberg and Zacchi, 2000
Electrode	No. of cells	3288.2	Akeberg and Zacchi, 2000
Heat exchanger	m^2	889.96	Akeberg and Zacchi, 2000

6.2.4.2 Operational cost evaluation

The operational costs were related to raw materials, equipment maintenance, troubleshooting service, utilities and labor. Raw material costs involved collection and transport of OFMSW supported by local solid waste management service. Details about the utility costs, necessary to run the processes (i.e. fuel, steam, cooling water, process air, process water and electricity), are scheduled in Table6-2.

Table 6-2: Detail of operational costs

Technique	Operation	Unit	Cost (€/unit)	References
Raw material	Collection cost of OFMSW	Euro/t	0.21	Arpa, 2017
Fermentation unit	Enzymes	Euro /L	4.10	Lam et al. 2014
	Inoculum	Euro/m^3	4.10	Wingren et al. 2003
	NaOH	Euro /kg	0.27	Akeberg and Zacchi, 2000
Downstream	Ultrafiltration membrane	Euro/m^2 year	114.29	Akeberg and Zacchi, 2000
	Softened	Euro/m^3	0.12	Lam et al. 2014
	Cleaning of membrane	Euro/m^3 permeate	0.12	Akeberg and Zacchi, 2000
	Electrodialysis	Euro/cell year	411.26	Akeberg and Zacchi, 2000
	Demineralisation	Euro/m^3	0.46	Wingren et al. 2003
	Condensation	Euro/m^3	0.95	Wingren et al. 2003
	Distillation	Euro/m^3	0.97	Lam et al. 2014
Water and energy consumption	Process water cooling	Euro/m^3	0.13	Akeberg and Zacchi, 2000
	Power	Euro/kWh	0.034	Akeberg and Zacchi, 2000
	Electric power	Euro/MW	5.24	Wingren et al. 2003
	Steam boiler	Euro/MW	72.80	Wingren et al. 2003
	Steam for AD process	Euro/kg	0.02	Akeberg and Zacchi, 2000
	Waste disposal	Euro/t	40.00	Arpa, 2017
	Labor	Euro/year	44966.40	Eurostat, 2016

6.2.4.3 Revenue

The market value of L(+)-LA is around 1360 Euro/t (ICIS, 2016), while electric energy has a value of 0.20 Euro/KWh and thermal energy of 0.201 Euro/KWh (Eurostat, 2016). The annual profit was evaluated as the difference between the revenue and the total production costs, including operational costs and amortization for the firsts 5 years.

6.2.4.4 Evaluation of profitability through composite indicators

A set of composite indicators based on: NPV, payback time, ROI and costs expressed as Euro per ton of feedstock or products, was defined to evaluate the profitability of the investigated processes.
NPV (Eq. 6) represents the profitability for the plant lifetime (20 years) considering a 5% discount on the future cash flows to the present value, according to Pleissner et al. (2015). If NPV > 0 the process is profitable.

$$NPV\ (Euro) = \sum_{t-1}^{T} \frac{C_t}{(1+d)^t} - C_0 \tag{6}$$

where:

- t was the plant lifetime,
- C_t was the net cash flow during period t,
- C_0 was the initial capital investment;
- d was the discount rate.

Payback time was the time required to regain the investment cost. *ROI* was defined as shown in in Eq 7:

$$ROI\ [\%] = \frac{Annual\ net\ profit}{Initial\ total\ investment} \cdot 100 \tag{7}$$

Specifically, *ROI* is the annual net profit referred to the annual net profit after 5 years of amortization.
Three different composite indicators (expressed in Euro per ton of feedstock or products) were defined: $P_{feedstock}$, $P_{product}$ and P_{net} referred to annual operational costs per ton of treated OFMSW (Eq. 8), annual operational costs per ton of generated products (Eq. 9) and Euro gained per ton of treated OFMSW (Eq. 10), respectively:

$$P_{feedstock}\ (\frac{Euro}{t}) = \frac{annual\ operational\ cost}{annual\ OFMSW\ treated} \tag{8}$$

$$P_{product}\ (\frac{Euro}{t}) = \frac{annual\ operational\ cost}{high-added\ value\ products\ (\ LA-CH4)} \tag{9}$$

$$P_{net}\ (\frac{Euro}{t}) = \frac{net\ profit\ after\ 5\ years\ of\ amortisation}{annual\ OFMSW\ treated} \tag{10}$$

6.2.5. Multi criteria Decision Aid

Multi Criteria Decision Aid (MCDA) and the Outranking methods analysis was performed with ELECTRE II (ELimination Et Choix Traduisant la REalité; Roy, 1968). The MCDA was performed for ranking the four Scenarios from technical, economic and energy and combined technical-economic-energy perspectives.

Technical perspective (Table6-3) considered the following criteria with the following weights: productivity (g/Lh) 0.15, LA yields (gLA/g substrate) 0.2, LA yield per consumed sugars (gLA/g sugars consumed) 0.05, Optical purity (%)0.2, waste production (kg waste/kg substrate) 0.2 and number of biological steps (N) 0.2 .

Economic perspective (Table6-3) considered the following criteria with the following weights: net present value NPV (euro) 0.10. payback time (y) 0.05, Return of interest (ROI) (%) 0.05, Euro paid per kg of substrate (euro/kg) 0.25, Euro paid per kg of product (euro/kg) 0.25 and Euro gained per kg of substrate treated (euro/kg) 0.3.

Technical-economic-energy perspectives (Table6-3) had the same weight 0.33 and the above-mentioned criteria for technical and economic were considered, for energy the criterium was energy required per treated substrate (kWh/kg $_{substrate}$).

Table 6-3:Multi criteria decision aids template

Technical		Productivity (gLA/Lh)	LA yield (gLA/gdry substrate)	LA yield (gLA/g sugars)	Optical purity (%)	Waste (kg waste/kg substrate)	N° process step
		0.15	**0.2**	**0.05**	**0.2**	**0.2**	**0.2**
Economic		NPV (€)	Payback time (y)	ROI (%)	Euro paid/kg of biomass	Euro paid/kg of product	Euro gained/kg of biomass
		0.1	**0.05**	**0.05**	**0.25**	**0.25**	**0.3**
Technical-economic-energetic	Technical: **0.33**	Productivity (gLA/Lh)	LA yield (gLA/gdry substrate)	LA yield (gLA/g sugars)	Optical purity (%)	Waste (kg waste/kg substrate)	N° process step
		0.0.495	**0.066**	**0.0165**	**0.066**	**0.066**	**0.066**
	Economic **0.33**	NPV (€)	Payback time (y)	ROI (%)	Euro paid/kg of biomass	Euro paid/kg of product	Euro gained/kg of biomass
		0.033	**0.0165**	**0.0165**	**0.0825**	**0.0825**	**0.099**
	Energetic **0.33**	Qtot (kW/t biomass)					
		0.33					

6.3 Results and discussion

Previous works of the authors Pleissner et al. (2017); Demichelis et al. (2017) reported in Chapter 3-5, respectively, assessed the technical feasibility of the four following scenarios:

- **Scenario I**:
    - Scenario IA: Production of LA from OFMSW through simultaneous saccharification and fermentation (SSF);
    - Scenario IB: Production of LA from OFMSW through separate hydrolysis and fermentation (SHF)
- **Scenario II**: Generation of biogas from OFMSW by means of mesophilic anaerobic digestion;
- **Scenario III**:
    - Scenario III A: Sequential LA production from OFMSW through SSF and biogas generation from LA fermentative broth and downstream residues through mesophilic AD.
    - Scenario III B: Sequential LA production from OFMSW through SHF and biogas generation from LA fermentative broth and downstream residues through mesophilic AD.
- **Scenario IV**: Production of LA from OFMSW through separate hydrolysis and fermentation (SHF)

LA fermentation was carried out in a batch reactor, while biogas production was performed in a continuous stirred tank reactor (CSTR).

Usually, LA fermentation was carried out as batch process, but some continuous processes were also described Kim et al. (2016). LA concentration obtained in continuous mode was lower than in batch configuration Bonk et al. (2017), and so the present study considered the latter. The same batch reactor for LA production by means of SSF and SHF was employed in Scenarios I A and B as well as in Scenarios IIIA and B.

Considering the reactor volume two characteristics were investigated: 1) comparison of volumes of batch reactor and CSTR (Scenario I A or B vs. Scenario II, and Scenario III A or B vs. Scenario II) and 2) volume of CSTR (for Scenario II vs. Scenario III A or B). The comparison of batch reactor and CSTR revealed that batch reactor volume (Scenarios I A and B and Scenarios IIIA and B) represented 7.8% of CSTR volume (Scenario II). Batch mode was superior from technical and economic viewpoints compared to a continuous process, as proven by other studies Akeberg and Zacchi, (2000).

Comparison CSTR volumes of AD in Scenarios II and III revealed that reactor volume in Scenario III was equal to 73.2% of Scenario II. This was due to LA fermentation carried out prior to biogas production, which reduced the amount of feedstock in the digester increasing methane yield. In integrated sequential LA and biogas productions, LA fermentation played a key role, because of it contributed to 1) biological pre-treatment of the feedstock for AD Croce et al. (2016); Maity et al. (2015) and 2) volume reduction of the digester.

The estimation of waste production was crucial to assess the economic feasibility (Table6- 5). For ScenariosIA-B and ScenarioIV, wet waste consisted of fermentation residues and residues from downstream processing. The amount of fermentation residues (83.35 %-wt of total waste) produced by Scenario I A was slightly higher than in Scenario IB (78.82 %-wt of total waste) due to a slightly higher LA yield in Scenario I B (0.33 compared to 0.29 g_{LA}/g_{OFMSW}). The amount of downstream processing waste (21.2 %-wt of total waste) produced by Scenario IB was higher than in Scenario I A (16.65 %wt of total waste).

For Scenario IV, waste production represented 97% of LA production.

Scenarios I A and B produced the highest amount of waste was produced by, followed by Scenario II and lastly by Scenarios III A and B (Table 6-4).

AD played a key role, since it is a technology able to reduce the amount of waste (Kosseva, 2011), providing energy. Moreover, the present study demonstrated that the combination of LA and biogas generation accomplished the highest reduction of waste, with energy production.

The main findings of technical analysis related to Scenarios III A and B were:

1) Production of valuable products (LA and methane),

2) reduction of digester volume;

3) reduction of the amount of waste generated.

The decrease of the volume of reactors and waste amount represents important benefits both from technical and economic point of views.

Waste minimization and optimization of reactor volumes were defined as key parameters to prove the readiness of biorefinery system scale up at the industrial scale Pommeret et al. (2017); (Li and Hu, 2016). The main objective of a biorefinery is the optimisation the use of resources minimizing wastes, and maximizing benefits and profitability Kokossis et al. (2015)

Table 6-4: Technical assessments of Scenarios I, II and III for catchment areas of different sizes (AD: anaerobic digestion, k: thousand, M: million, *represents the amount of wet waste generated during fermentation and downstream processing).

Scenario		**2k**	**5k**	**10k**	**20k**	**50k**	**100k**	**200k**	**500k**	**1M**
	OFMSW produced in 2015 (t)	286.2	715.5	1431	2862	7155	14310	28620	71550	143100
I A	Reactor volume for LA (m^3)	6.8	17	33.9	67.8	169.6	339.2	678.4	1.696.00	3.392.00
	LA unclean produced (t)	16.6	41.5	83	166	415	830	1660	4149.9	8299.8
	(L+)-LA produced by SSF (t)	8.5	21.2	42.4	84.8	212.1	424.1	848.3	2120.7	4241.5
	Total waste* (t)	243.8	609.5	1218.9	2437.8	6094.6	12189.3	24378.5	60946.3	121892.6
I B	Reactor volume for LA (m^3)	6.8	17	33.9	67.8	169.6	339.2	678.4	1.696.00	3.392.00
	LA unclean produced by SHF (t)	18.9	47.2	94.4	188.9	472.2	944.5	1888.9	4722.3	9444.6
	(L+)-LA produced by SSF (t)	8.6	21.5	42.9	85.9	214.6	429.3	858.6	2146.5	4293
	Total waste* (t)	243.3	586.7	1173.4	2346.8	5867.1	11734.2	23468.4	58671	117342
II	Reactor volume for AD (m^3)	86.5	216.2	432.3	864.6	2161.4	4322.8	8645.6	21614.1	43228.1
	Methane (Nm^3)	11688.6	29221.6	58443.2	116886.4	292215.9	584431.8	1168863.7	2922159.2	5844318.4
	Total waste* (t)	140.2	350.6	701.2	1402.4	3505.9	7011.9	14023.8	35059.5	70119
III A	Reactor volume for LA (m^3)	6.8	17	33.9	67.8	169.6	339.2	678.4	1.696.00	3.392.00
	Reactor for AD (m^3)	66.7	166.7	333.3	666.7	1666.7	3333.4	6666.7	16666.9	33333.7
	LA unclean produced (t)	16.6	41.5	83	166	415	829.9	1659.9	4149.9	8299.8
	(L+)-LA produced by SSF (t)	8.5	21.2	42.4	84.8	212.1	424.1	848.3	2120.7	4241.5
	Methane (Nm^3)	13537.8	33844.5	67689	135378.1	338445.2	676890.4	1353780.8	3384451.9	6768903.9
	Total waste* (t)	124.33	310.8	621.7	1243.3	3108.3	6216.5	12433	31082.6	62165.2
III B	Reactor volume for LA (m^3)	6.8	17	33.9	67.8	169.6	339.2	678.4	1.696.00	3.392.00

	Reactor for AD (m^3)	63.3	152.7	305.5	610.9	1527.3	3054.6	6109.2	15272.9	30545.8
	LA unclean produced (t)	18.9	47.2	94.5	188.9	472.2	944.5	1888.9	4722.3	9444.6
	(L+)-LA produced by SSF (t)	8.6	21.5	42.9	85.9	214.6	429.3	858.6	2146.5	4293
	Methane (Nm^3)	15425.1	37201.8	74403.5	148807	372017.5	744035.1	1488070.2	3720175.6	7440351.1
	Total waste* (t)	116.8	281.6	563.2	1126.5	2816.2	5632.4	11264.8	28162.1	56324.2
	SCG produced in 2019 (t)	21.6	54	108	216	540	1080	2160	5400	10800
	Reactor volume for LA (m^3)	0.21	0.60	1.20	2.39	5.98	11.95	23.90	59.75	119.50
	LA unclean produced (t)	1.2	3.1	6.2	12.5	31.1	62.3	124.5	311.4	622.7
	(L+)-LA produced by SSF (t)	2.0	5.7	11.3	22.6	56.6	113.1	226.3	565.7	1.131.4
	Total waste* (t)	74.0	205.6	411.2	822.4	2.056.0	4111.9	8223.8	20559.5	41119.0

Table 6-5 Comparison of reduction of wet waste for scenarios i a and b, scenario ii and scenarios iii a and b.

	Waste reduction (%)
II vs I a	42.5
II vs I b	42.3
III a vs I a	49.0
III b vs I b	52.0
III a vs II	11.3
III b vs II	20.1

6.3.2 Energy balance

The seasonal thermal load value calculated for Scenarios I, II, III and IV were depicted in Figure6-5. The four scenarios showed the same trend: in winter and fall the energy requirement was higher than in spring and summer. The four scenarios were compared in catchment areas of different sizes (defined by the number of inhabitants): 2000-10,000, 20,000-100,000 and 200,000-1 million. Scenarios I A and B and Scenarios III A and B were simply defined as Scenario I and Scenario III, because the volume of reactors as well as the inflow and outflow temperatures were similar for the LA fermentation steps

For all the four ranges of catchment areas, Scenario II and Scenario I exhibited the highest and the lowest energy demands, respectively. These trends were mainly due to 1) plant structure and 2) reactor volume. In fact, Scenario I involved a batch process, while Scenario II a continuous process, thus the working hours and volumes employed in Scenario II were higher than in Scenario I.

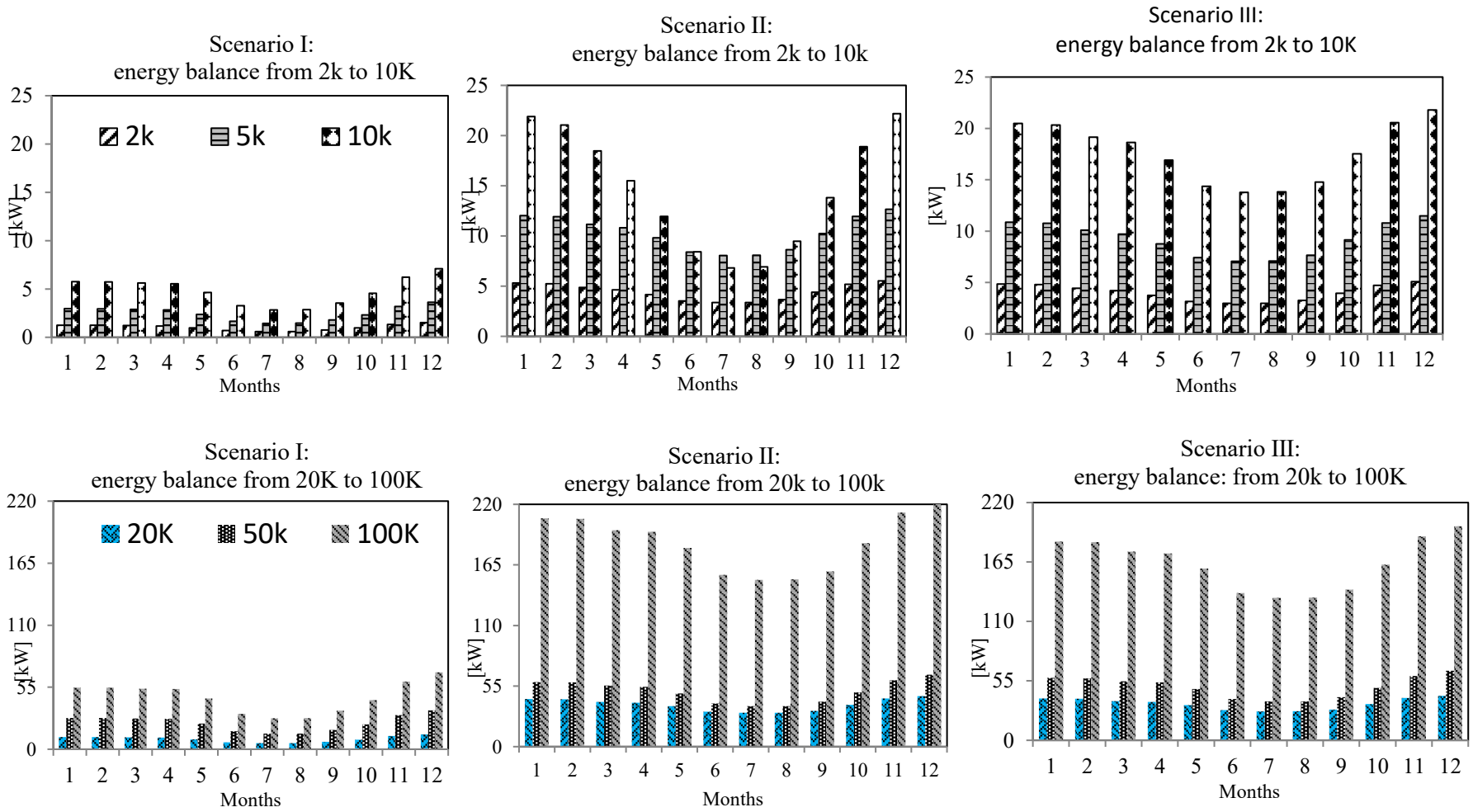
From a technical perspective, Scenario III represented the combination of Scenario I and Scenario II, but from an energy balance perspective Scenario III was not the average of the energy demands of the two scenarios, but rather an optimization of them.

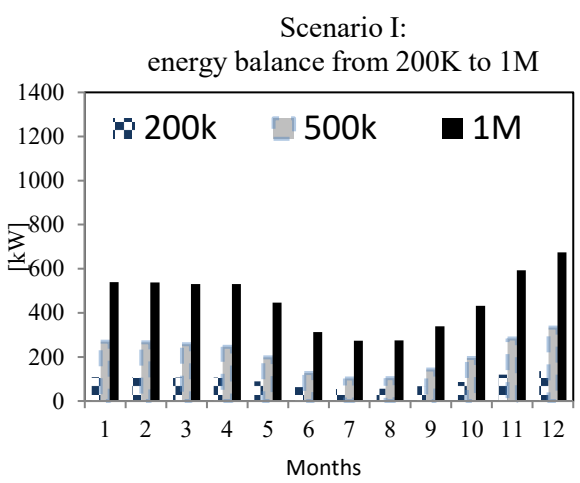
The energy balance of Scenario II was consistent with the studies of Mehr et al. (2017); Feiz et al. (2017). Literature data about the energy balance for Scenario I and IV were not available, Energy requirement of Scenario I was higher than Scenario IV, because the amount of OFMSW to treat were higher than the amount of SCG treated.

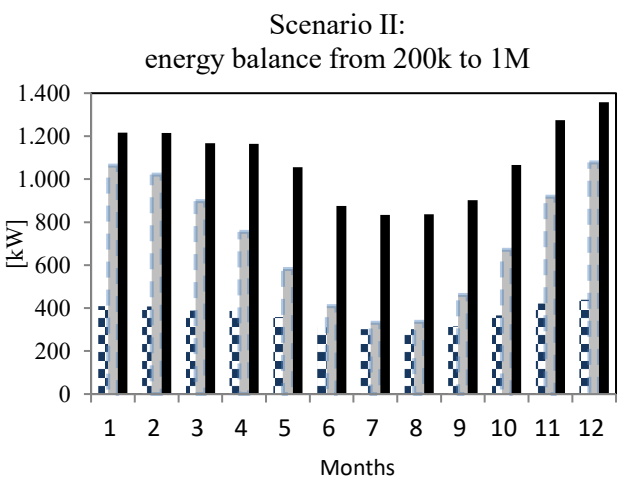
Table 6-6 showed the details of annual energy demand in terms of energy consumed to heat the OFMSW (Scenarios I-II-III) and SCG (Scenario IV) in the reactor and heat losses through reactor walls and pipes. Q_s represented the highest item in energy requirement followed by $Q_{loss.}$

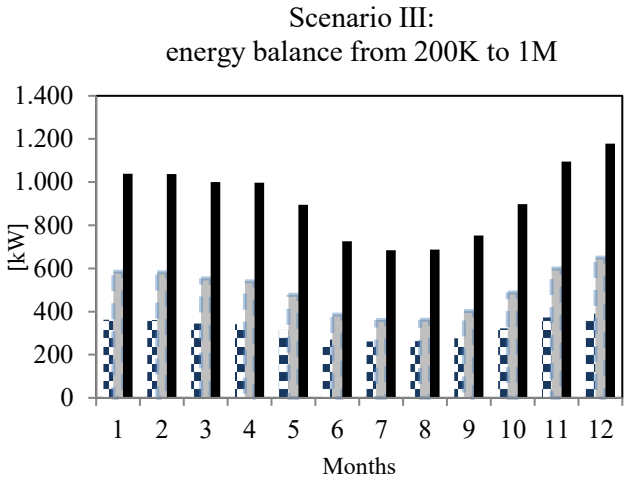
Increasing the catchment area size, the contribute of Q_{los} to the total energy balance for Scenario I, II and III and IV decreased from 32 to 24%, from 81to 65 % from 79 to 60 %, 70 to 64%, respectively.

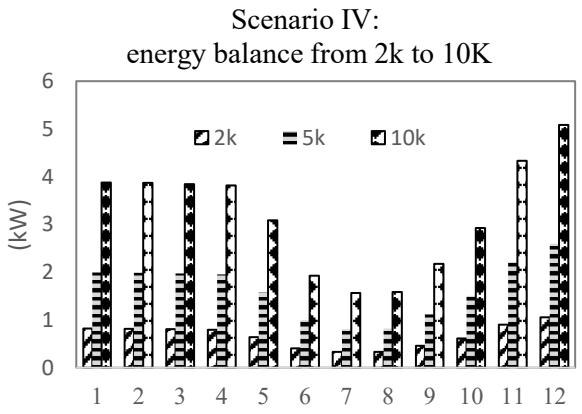
Figure 6-5: Energy balance of Scenarios I a and b, Scenario II and Scenarios II a and b (k: thousand, M: million).

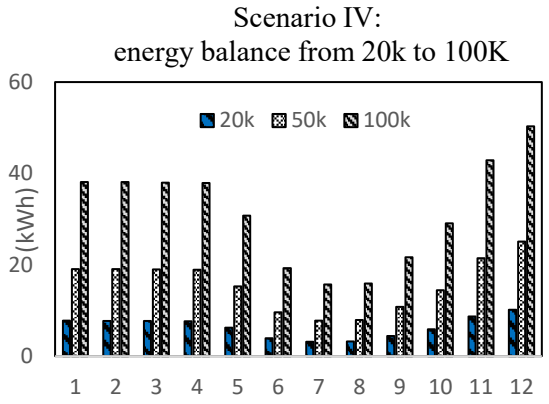



Scenario I:
energy balance from 200K to 1M
200k
500k
1M
[kW]
Months


Scenario II:
energy balance from 200k to 1M
[kW]
Months


Scenario III:
energy balance from 200K to 1M
[kW]
Months


Scenario IV:
energy balance from 2k to 10K
2k
5k
10k
(kW)


Scenario IV:
energy balance from 20k to 100K
20k
50k
100k
(kWh)

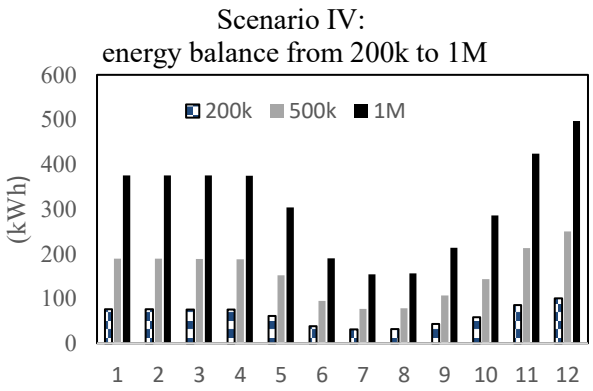

Scenario IV:
energy balance from 200k to 1M
200k
500k
1M
(kWh)

Table 6-6: Energy balance for the Scenarios I, II, III and IV for catchment areas of different sizes (k: thousand, M: million).

Scenario		**2k**	**5k**	**10k**	**20k**	**50k**	**100k**	**200k**	**500k**	**1M**
Ia-b	Q_s (kW)	7.76	19.41	38.82	72.75	194.09	388.19	776.37	1940.93	3889.99
	Q_{los} (kW)	3.97	8.67	16.08	30.37	71.97	139.95	274.25	464.36	1333.6
	Q_{pipe} (kW)	0.59	1.4	2.74	5.16	13.3	26.41	52.53	120.26	261.18
	Q_{tot} (kW)	12.32	29.48	57.64	108.28	279.37	554.54	1103.16	2525.55	5484.77
II	Q_s (kW)	7.76	19.41	38.82	72.75	194.09	388.19	776.37	1940.93	3889.99
	Q_{los} (kW)	42.94	98.42	128.25	362.91	380.79	1729.53	3418.75	6178.4	8457.48
	Q_{pipe} (kW)	2.54	5.89	8.35	21.78	28.74	105.89	209.76	405.97	617.37
	Q_{tot} (kW)	53.24	123.72	175.42	457.45	603.63	2223.6	4404.88	8525.29	12964.85
III a-b	Q_s (kW)	7.76	19.41	38.82	72.75	194.09	388.19	776.37	1940.93	3889.99
	Q_{los} (kW)	38.13	86.21	163.24	313.51	370.81	1480.97	2920.91	3761.41	6576.87
	Q_{pipe} (kW)	2.29	5.28	10.1	19.31	28.25	93.46	184.86	285.12	523.34
	Q_{tot} (kW)	48.18	110.9	212.17	405.58	593.15	1962.62	3882.14	5987.45	10990.2
IV	Q_s (kW)	7.031	17.578	35.156	70.313	175.782	351.564	703.128	1757.820	3515.640
	Q_{los} (kW)	0.567	1.039	1.121	2.536	3.477	8.210	14.301	23.693	31.050
	Q_{pipe} (kW)	0.380	0.931	1.814	3.642	8.963	17.989	35.871	89.076	177.334
	Q_{tot} (kW)	7.979	19.548	38.091	76.492	188.222	377.763	753.300	1870.589	3724.024

6.3.3 Economic analysis

6.3.3.1 Capital costs evaluation

Table6-7 depict capital costs with and without 5-year amortisation and the percentage of cost items contributing to total costs of Scenarios I A and B, Scenario II and Scenarios III A and B and Scenario IV, for catchment areas from 2000 to 1million inhabitants. Scenarios I A and B had the same capital costs setting. In Scenarios I A-B and Scenario IV, downstream capital costs decreased with increasing catchment area. Specifically, downstream processing represented the highest item of capital costs from 2000 to 200,000 inhabitants, in a range from 98 to 73%.

The lowest contribution, equal to 38% was found for 1million inhabitants. Several authors, such as Wang et al. (2016); Pommeret et al. (2017), stated that downstream processing costs were more than 41% of the costs of a conventional fermentative process. However, downstream processing is fundamental and crucial to obtain LA with a market purity more than 80%.

In Scenarios I A-B and IV, capital costs for 2000 inhabitant agreed with literature data for a batch plant configuration fed with 1 t/d of bakery waste (Lam et al. (2014).

In Scenario II capital costs of the digester increased, while CHP unit costs decreased by increasing the size of catchment area. The reactor cost was around 3/4 of the whole equipment purchased Varrone et al. (2013). Capital costs of Scenario II for catchment area over 200,000 inhabitants were consistent with capital costs estimated by Gerssen-Gondelach et al.(2014), which were around 500 Euro/kW produced biogas.

In Scenarios III A and B downstream processing costs decreased, while LA fermentation, biogas production and CHP unit costs increased with the increase of catchment area. The main differences between Scenarios III A and B were the percentage of cost items of digester and CHP unit contributing to total costs.

In Scenario III B, the capital costs of the AD digester ranged between 2.8-56.8% and it was lower than in Scenario III in which it ranged between 3.2-69.7 %, as the CSTR in Scenario III B was 5% smaller than in Scenario III A.

CHP unit capital costs in Scenario III B, ranging between 0.7-14.2%, was higher than in Scenario III A ranging between 0.4-7.7%, as the methane yield achieved in Scenarios III A and B were 0.49 and 0.62 Nm^3/kg_{vs}, respectively.

Scenario I A-B and Scenario IV had the trend, thus the capital cost depended on process and not on substrate treated.

Table 6-7: Investment costs and amortisation of the Scenarios I, II and III for catchment areas of different sizes, and percentage of cost items contributing to total costs

Scenario		2k	5k	10k	20k	50k	100k	200k	500k	1M
I a-b	Investment costs *1000 (Euro)	1399.24	1406.26	1461.65	1485.06	1692.19	1718.33	1952.66	2743.83	3939.53
	Amortisation*1000 (Euro)	296.86	298.35	310.1	315.07	359.01	364.56	414.27	582.13	835.8
	Reactor for fermentation (%)	2.1	2.51	4.99	6.48	9.85	16.52	26.54	45.38	61.96
	Downstream processing (%)	97.9	97.49	95.01	93.52	90.15	83.48	73.46	54.62	38.04
II	Investment costs*1000 (Euro)	62.54	121.51	243.01	374.04	865.41	1192.99	2011.94	4141.21	6644.53
	Amortisation*1000 (Euro)	13.27	25.79	51.56	79.36	183605.1	253.1	426.85	878.59	1409.69
	Anaerobic digester (%)	50.28	64.7	63.89	70.06	75.7	76.88	78.13	79.1	80.85
	CHP unit (%)	49.72	35.3	36.1	29.94	24.3	23.12	21.87	20.9	19.5
III a	Investment costs*1000 (Euro)	1450.89	1498.26	1645.65	1803.56	2414.22	3112.91	4692.34	9518.82	17489.5
	Amortisation*1000 (Euro)	307.82	317867	349.14	382.64	512.2	660.43	995.52	2019.5	3710.54
	Reactor for LA fermentation (%)	2	2.4	4.4	5.3	6.9	9.1	11	13.1	14
	Downstream processing (%)	94.4	91.6	84.4	77	63.2	46.1	30.6	15.7	8.6
	Anaerobic digester (%)	3.2	5.5	10.1	15.9	26.9	40.3	52.5	64.1	69.7
	CHP unit (%)	0.4	0.5	1.1	1.8	3	4.5	5.9	7.1	7.7
III b	Investment costs*1000 (Euro)	1449.54	1510.9	1634.4	1781.06	2357.98	3000.42	4467.34	8956.32	16364.51
	Amortisation*1000 (Euro)	307.53	320.55	346.75	377.87	500.26	636.56	947.78	1900.16	3471.87
	Reactor for LA fermentation (%)	2	2.5	4.5	5.4	7.1	9.5	11.6	18	19.8
	Downstream processing (%)	94.7	91.8	85	78	64.7	47.8	32.1	16.7	9.2
	Anaerobic digester (%)	2.91	5	7.36	11.12	16.05	17.79	19.98	21.54	21.56
	CHP unit (%)	0.39	0.7	3.14	5.48	12.15	24.91	36.32	43.76	49.44
IV	Investment costs *1000 (Euro)	1.370.96	1.414.06	1.456.77	1.503.54	1.509.77	1.527.66	1.553.69	1.655.36	1.759.21
	Amortisation*1000 (Euro)	290.86	300.00	309.07	318.99	320.31	324.11	329.63	351.20	373.23
	Reactor for fermentation (%)	0.83	17.89	35.10	35.67	39.65	45.99	58.59	92.63	85.38
	Downstream processing (%)	99.17	82.11	64.90	64.32	60.34	54.01	41.41	7.37	14.62

6.3.3.2 Operational costs evaluation

The annual operation costs and the percentage of cost items contributing to total costs are reported in Table 6-8. Operational cost of Scenarios I A-B and Scenario IV exhibited similar trends: fermentation, downstream processing and waste management costs increased, while labour costs decreased with increasing catchment areas.

In detail, fermentation costs in Scenario I A, ranging between 0.11-0.91 %, was lower than in Scenario I B ranging between 1.1-7.69 %, due to the addition of enzymes and HCl for hydrolysis in SHF. On the other side, downstream operational costs in Scenario I B was lower, ranging from 2.51-to 7.32 % than in Scenario I A, ranging from 3.80 to 11.64 %, because of LA fermentation yield was higher in Scenario I B than in Scenario I B.

In Scenarios I A-B , operational costs for 2000 inhabitant agreed with literature data concerning batch plant configuration fed with 1 t/d of bakery waste (Leung et al. (2012) and for 5000 inhabitants Pommeret et al.(2017). Operational costs of Scenario I A-B and Scenario IV had the same trends, but operational costs of Scenario IV was lower than Scenario IA-B ones, since the treated SCG was lower than the treated OFMSW. Operational costs of Scenario II increased for AD digester, CHP and waste management, while labour cost decreased with increasing catchment area. Operational costs in Scenario II were consistent with literature data Arnò et al.(2017); Stürmer, 2018). In detail, according to Skovsgaard et al.(2017), who investigated AD for catchment areas between 500,000 and 1 million inhabitants, economies of scale for operational expenditures reduces unit costs. In detail, Operational costs decreased with the increment of catchment area size.

Operational costs of Scenarios III A and B proved qualitatively similar but quantitatively different trends: downstream processing, LA fermentation, digestion process and waste management costs decreased, while labor costs increased with increasing catchment area. Specifically, the percentage share of LA fermentation and downstream processing in Scenario III B were higher than in Scenario III A due to the already mentioned addition of enzymes and HCl and the higher amount of LA produced in SHF. For Scenario III A, AD, due to maintenance of digester and CHP unit, represented the highest operational process cost item, while LA fermentation, including reactor and downstream system maintenance, represented the highest operational process cost item for Scenario III B. To conclude, the operational costs analysis of the three scenarios proved that increasing the catchment area size, increases production and purification of LA, and biogas as well as waste management costs, and decreases labor costs. Labor is a turning point cost item in economic feasibility assessment of the of a process Wan et al. (2014); Pommeret et al., (2017), and in all scenarios it ranged between 85% and 5% according to the increase of the catchment area size. At the same time, the use of a cheap substrate, such as OFMSW, as feedstock in a biorefinery system implies beneficial from the environmental (use of a waste biomass instead of pure or ad hoc raw materials) and economic viewpoints Kim et al., (2016) Hafid et al.(2017).

Table 6-8: Operational costs of Scenarios I,II III and IV for catchment areas of different sizes. data are expressed as % weight of operational item costs (k: thousand, m: million).

Scenario		2k	5k	10k	20k	50k	100k	200k	500k	1M
I a	Operational costs (Euro) *1000	103.85	118.45	191.94	248.1	416.59	742.83	1304.44	2989.81	5986.64
	Reactor for LA fermentation (%)	0.11	0.23	0.28	0.44	0.65	0.73	0.84	0.91	0.91
	Downstream processing (%)	3.8	3.62	4.03	5.88	8.44	9.41	10.62	11.53	11.64
	Raw material (%)	0.06	0.13	0.16	0.24	0.36	0.4	0.46	0.5	0.5
	Waste (%)	9.43	20.5	25.4	39.3	58.52	65.64	74.76	81.54	81.44
	Labor (%)	86.97	75.64	70.28	54.37	32.38	24.21	13.79	6.02	6.01
Ib	Operational costs (Euro) *1000	104.77	119.25	192.65	249.52	420.14	749.94	1318.67	3025.37	6049.03
	Reactor for LA fermentation (%)	1.11	1.95	2.41	3.73	5.53	6.2	7.05	7.69	7.69
	Downstream processing (%)	2.51	2.55	2.7	3.81	5.35	5.94	6.65	7.2	7.32
	Raw material (%)	0.09	0.13	0.16	0.24	0.36	0.4	0.46	0.5	0.5
	Waste management (%)	9.3	19.68	24.36	37.62	55.86	62.59	71.19	77.57	77.59
	Labor (%)	86.74	75.42	70.02	54.06	32.11	23.98	13.64	5.95	5.95
II	Operational costs (Euro) *1000	103.97	125.02	205.07	275.24	485.76	881.59	1583.32	3688.5	7197.13
	Digester (%)	3.97	8.26	10.07	15	21.25	23.42	26.08	27.99	28.69
	CHP unit (%)	4.13	8.59	10.47	15.61	22.11	24.37	27.13	29.12	29.85
	Raw material (%)	0.06	0.12	0.15	0.22	0.31	0.34	0.38	0.41	0.42
	Waste management (%)	5.4	11.22	13.68	20.38	28.87	31.81	35.43	38.02	38.97
	Labor (%)	86.5	71.94	65.78	49.01	27.77	20.4	11.36	4.88	2.5
III a	Operational costs (Euro) *1000	105.58	122.78	200.59	265.4	459.84	829.33	1477.45	3422.33	6851.67
	Reactor for LA fermentation (%)	0.1	0.22	0.27	0.41	0.59	0.66	0.74	0.8	0.8
	Downstream processing (%)	3.44	3.5	3.86	5.5	7.65	8.43	9.38	10.08	10.17
	Digester (%)	2.81	5.99	7.36	11.12	16.05	17.79	19.98	21.56	21.54
	CHP unit (%)	3.38	7.22	8.87	13.4	19.34	21.44	24.07	25.98	25.96

	Waste management (%)	4.73	10.09	12.4	18.74	27.04	29.98	33.66	36.33	36.29
	Labor (%)	85.54	72.99	67.25	50.83	29.34	21.69	12.17	5.26	5.25
III b	Operational costs (Euro) *1000	106.07	122.39	198.93	262.07	451.51	812.68	1444.15	3339.07	6676.43
	Reactor for LA fermentation (%)	0.87	1.88	2.32	3.52	5.11	5.68	6.39	6.91	6.91
	Downstream processing (%)	3.41	3.52	3.89	5.57	7.79	8.61	9.59	10.33	10.43
	Digester (%)	2.55	5.34	6.57	9.97	14.47	16.08	18.1	19.57	19.57
	CHP unit (%)	3.98	6.57	8.08	12.27	17.8	19.78	22.27	24.08	23.95
	Waste management (%)	4.4	9.2	11.33	17.19	24.95	27.72	31.2	33.74	33.75
	Labor (%)	84.78	73.48	67.81	51.47	29.88	22.13	12.45	5.39	5.39
IV	Operational costs (Euro) *1000	92.02	95.72	145.90	156.61	187.55	284.07	432.13	741.34	1.391.82
	Reactor for LA fermentation (%)	0.27	0.64	0.84	1.57	3.28	4.33	5.70	8.30	8.84
	Downstream processing (%)	0.45	1.28	1.28	2.20	3.72	4.53	5.70	8.07	8.53
	Raw material (%)	.001.6	.003.9	.005.2	.009.7	.020.2	.026.6	.035.0	.051.0	.054.3
	Waste (%)	1.55	4.13	5.42	10.09	21.07	27.82	36.58	53.30	56.78
	Labor (%)	97.74	93.95	92.46	86.13	71.93	63.32	52.03	30.33	25.85

6.3.3.3 Revenues

Table6-9 listed the revenues of LA and biogas trade and incomes (difference between revenues and capital and operational costs) before and after 5 years of amortization. Referring to catchment areas from 2000 to 1 million inhabitants, the yearly net-incomes for the four scenarios are:

- Scenarios IA-B were unprofitable below 200,000 inhabitants and cost-effective from 200,000 to 1 million inhabitants after 5 years of amortization. Incomes of Scenarios I A and B for a catchment area of 500,000 inhabitants was consistent with the net revenues achieved by Kwan et al. (2018) from LA production through fungal hydrolysis and fermentation of OFMSW powder.
- Scenario II was unprofitable up to 50,000 inhabitants, cost-effective for 50,000 inhabitants after 5 years of amortization and immediately profitable over 100,000 inhabitants. Profitability of Scenario II could be improved for catchment area below 10,000 inhabitants when other waste streams are co-digested, as suggested by (Dennehey et al., *in press)* Sen et al. (2016). Scenario II carried out in a catchment area over 100,000 inhabitants was advantageous regarding cost-efficiency, biogas production and investments in production facilities (Feiz et al.(2017).
- Scenarios III A-B were unprofitable below 20,000 inhabitants, cost-effective between 20,000 and 50,000 inhabitants after 5 years of amortization and immediately profitable between 200,000 and 1 million inhabitants. Integrated waste biorefinery processes: 1) combining multiple process flows, 2) enhance the production of valuable products and 3) at the same time minimizing waste generation and management cost Galik, (2015).
- Scenarios IV was unprofitable below 500,000 inhabitants and cost-effective from 200,000 to 1 million inhabitants after 5 years of amortization. Incomes of Scenarios IV for a catchment area of 500,000 inhabitants was consistent with the net revenues achieved by Kwan et al. (2018) from LA production through fungal hydrolysis and fermentation of OFMSW powder

Among the three scenarios, Scenarios III A and B implemented in a catchment area of 20,000 and more inhabitants provides the highest economic revenues. It was due to the cascade use of OFMSW, first for LA production and second for energy generation. According to (Hagman et al, 2018), biogas production represented a key solution in single and integrated biorefineries, which made processes more versatile and resilient, and improves the value of the product portfolio. Table6-10 evidenced that profitability did not exhibit a direct proportional increment with the enhancement of the catchment area. After the evaluation of cost items and revenues for the single scenarios, an economic profitability increment analysis was performed among scenario configurations A and B. Scenarios I B and III B were more cost-effective than Scenarios I A and III A. In detail, when Scenario I B was compared to Scenario I A highest economic percentage increase, equal to 4.1% was achieved at 200,000 inhabitants. When Scenario III B was compared to Scenario III A best economic benefit increment, equal to 15.1% was achieved at 50,000

inhabitants. In catchment areas of 100,000 to 1 million inhabitants the economic profitability declined from 13.7 to 12.0%.

Table 6-9: Revenues and net incomes of Scenarios I, II and III for catchment areas of different sizes (k: thousand, M: million)

Scenario		2k	5k	10k	20k	50k	100k	200k	500k	1M
I a	Revenue (Euro)*1000	13.57	33.93	67.86	135.73	339.32	678.64	1357.27	3393.19	6786.37
	Net incomes (Euro)*1000	-387.14	-382.87	-434.18	-427.44	-436.28	-428.75	-361.44	-178.75	-36.07
	Net incomes after 5 years (Euro)*1000	-90.28	-84.52	-124.08	-112.37	-77.27	-64.19	52.83	403.38	799.74
I b	Revenue (Euro)*1000	13.74	34.34	68.69	137.38	343.44	686.88	1373.76	3434.4	6868.8
	Net incomes (Euro)*1000	-387.9	-387.13	-434.07	-427.22	-435.71	-427.62	-359.18	-173.1	-16.04
	Net incomes after 5 years (Euro)*1000	-91.04	-84.9	-123.96	-112.15	-76.7	-63.06	55.09	409.03	819.77
II	Revenue (Euro)*1000	23.55	58.87	117.73	235.47	588.67	1177.33	2354.67	5886.67	11773.33
	Net incomes (Euro)*1000	-93.69	-91.93	-138.9	-119.13	-80.7	42.64	344.5	1319.57	3166.51
	Net incomes after 5 years (Euro)*1000	-80.42	-66.15	-87.34	-39.78	102.9	295.74	771.35	2198.17	4576.2
III a	Revenue (Euro)*1000	32.92	82.29	164.58	329.16	822.89	1645.79	3291.57	8228.94	16457.87
	Net incomes (Euro)*1000	-380.48	-358.36	-385.15	-318.89	-149.14	156.03	818.61	2787.11	5895.66
	Net incomes after 5 years (Euro)*1000	-72.66	-40.49	-36.01	63.76	363.06	816.46	1814.13	4806.61	9606.21
III b	Revenue (Euro)*1000	36.04	87.94	175.88	351.77	879.41	1758.83	3517.66	8794.14	17588.28
	Net incomes (Euro)*1000	-377.57	-354.99	-369.79	-288.17	-72.36	309.58	1125.73	3554.91	7439.99
	Net incomes after 5 years (Euro)*1000	-70.04	-34.44	-23.04	89.69	427.9	946.15	2073.51	5455.07	10911.86
IV	Revenue (Euro)*1000	2.769	7.694	15.387	30.774	76.936	153.871	307.743	769.357	1.538.714
	Net incomes (Euro)*1000	-380.106	-388.033	-439.579	-444.828	-430.924	-454.302	-454.014	-323.179	-226.341
	Net incomes after 5 years (Euro)*1000	-89.247	-88.028	-130.514	-125.841	-110.614	-130.195	-124.387	28.019	146.890

Table 6-10: Comparison of economic incomes obtained from different scenarios for catchment areas of different sizes (k: thousand, M: million, n.p: non profitable). In-comes are based on LA yield obtained in simultaneous saccharification and fermentation (SSF), and separate hydrolysis and fermentation (SHF) as well as methane formation.

Comparison		**2k**	**5k**	**10k**	**20k**	**50k**	**100k**	**200k**	**500k**	**1M**
I b/I a (%)	Scenario I a: LA-SSF= 0.29 g/g Scenario I b: LA-SHF = 0.33 g/g	n.p	n.p	n.p	n.p	n.p	n.p	4.1	1.4	2.4
III b/III a (%)	Scenario III a: LA-SSF= 0.29 g/g CH_4=0.49 Nm^3/kg VS Scenario III b: LA-SHF= 0.33 g/g CH_4=0.62 Nm^3/kg VS	n.p	n.p	n.p	n.p	15.1	13.7	12.5	11.9	12

6.3.3.4 Evaluation of profitability through composite indicators

To complete the economic assessment, the process profitability and the most effective scenario configuration according to the size of catchment area were calculated through a set of composite indicators (Table 6-11)

Profitability was considered when all parameters of the composite indicators were:

- ROI >0,
- NPV>0,
- Payback time <20 (20 years plant lifetime was hypothesized according to (Kwan et al, 2015; Shahzad et al., 2015),
- Euro gained per tons of OFMSW >0
- Euro spent per tons of OFMSW treated as well as lowest amount of waste generated.

Scenarios I A and B achieved profitability after 500,000 inhabitants, Scenario II after 100,000 inhabitants and Scenario III after 50,000 inhabitants. Configuration B reached the highest profitability, as it had the highest LA and biogas yields. The higher productivity counterbalances the costs of downstream processing, which were estimated as 1.57-1.62 Euro/kg_{LA} according to Joglekar et al. (2006).

ROI and NPV values of Scenarios I A and B agreed with Kwan et al. (2015) and those of Scenario II agreed with Yasar et al., (2017). For Scenario III no data for comparison was found. Among the three investigated scenarios, Scenario III reached the highest NPV values from 20,000 to 1 million inhabitants, proving the efficiency of the investment and integrated design of process Kalmykovaa et al, in press; Sacchi-Homric et al., (2018).

Production costs of all three scenarios ranged between 0.05-0.36 Euro/tons of OFMSW, which agreed with earlier reported costs Kwan et al. (2015). Euro paid per ton of OFMSW and Euro paid per ton of LA and methane increased with decreasing catchment area from 1 million to 2000 inhabitants. In all proposed configurations, Scenarios III A and B reached the highest value as Euro gained per ton of OFMSW from 100,000 to 1 million inhabitants, which was in accordance to the 55 Euro/t_{OFMSW} defined by Kim et al. (2016). Whereas, Scenarios I A and B achieved the lowest value from 200,000 to 1 million inhabitants, which agreed with the 1.8 €/t_{OFMSW} evaluated by Kwan et al. (2015). Scenario II showed a value from 14-32 €/t_{OFMSW} (50,000 to 1 million inhabitants), which ws close to earlier reported 24 €/t_{OFMSW} by Kim et al. (2016). According to Van Deal et al. (2013) it was economically more attractive investing an integrated chain biorefinery, as Scenarios III A and B, than two separate systems, as Scenarios I A and B and II.

The main findings from composite indicators assessment were:

1) Technical benefits of an integrated and sequential biorefinery process (Scenario III) were confirmed both from technical and economic perspective;

2) Scenarios III A and B were cost-effective for a catching area from 50,000 inhabitants, while Scenarios II as well as I A and B reached the profitability above 100,000 and 500,000 inhabitants, respectively;

3) Scenarios III A and B achieved the highest profitability among the three investigated scenarios in terms of ROI, NPV, payback time and Euro gained per tons of OFMSW.

4) For Scenario IV the whole economic assessment based on capital and operational costs, revenues, ROI, NPV and payback time, proved that the minimum plant size to achieve economic profitability after 5-years amortisation with positive ROI and NPV was equal to the 25% of the world SCG production, about 1,669.5 t/y, but the payback time was higher than the 20 years of designed plant life. The minimum plant size to achieve a payback time shorter than 20 years, was the size equal to 50% of the world SCG production, 3,339 t/y with 16 y.

To conclude the techno-economic assessment, Scenario III represented a techno-economic solution to the challenge of developing economically feasible biorefineries system for waste valorization by coupling high-value bio-products and energy productions Budzianowski (2017); Maity et al. (2015). According to Budzianowski et al. (2016); Kurian et al. (2013), integrated biorefinery processes minimize waste generation and the sequential fermentative processes enhanced products formation counterbalancing capital and operational costs.

Table 6-11: ROI evaluation for the four scenarios for catchment areas of different sizes (k: thousand, M: million). all data are expressed in years.

Scenario		2k	5k	10k	20k	50k	100k	200k	500k	1M
I a	ROI (%)	<0	<0	<0	<0	<0	<0	2.7	14.7	20.3
	NPV *1000 (euro)	<0	<0	<0	<0	<0	<0	<0	218.93	301.31
	Payback time (year)	>20	>20	>20	>20	>20	>20	>20	19	14
	Euro paid/kg_{OFMSW}	0.36	0.17	0.13	0.09	0.06	0.05	0.05	0.04	0.04
	Euro paid/$kg_{products}$	12.24	5.59	4.53	2.92	1.96	1.75	1.54	1.41	1.41
	Euro gained/t_{OFMSW}	< 0	< 0	< 0	< 0	< 0	< 0	1.85	5.64	5.59
I b	ROI (%)	<0	<0	<0	<0	<0	<0	2.8	14.9	20.8
	NPV *1000 (euro)	<0	<0	<0	<0	<0	<0	<0	2657.9	3000.8
	Payback time (year)	>20	>20	>20	>20	>20	>20	>20	18	13
	Euro paid/kg_{OFMSW}	0.37	0.17	0.13	0.09	0.06	0.05	0.05	0.04	0.04
	Euro paid/$kg_{products}$	12.2	5.5	4.4	2.9	1.9	1.7	1.5	1.3	1.3
	Euro gained/t_{OFMSW}	< 0	< 0	< 0	< 0	< 0	< 0	1.9	5.7	5.7
II	ROI (%)	<0	<0	<0	<0	<0	24.8	38.3	53.1	68.9
	NPV *1000 (euro)	<0	<0	<0	<0	<0	1396.7	5752.7	19448.9	44281.8
	Payback time (year)	>20	>20	>20	>20	>20	11	6	4	3
	Euro paid/kg_{OFMSW}	0.36	0.17	0.14	0.1	0.07	0.06	0.06	0.05	0.05
	Euro paid/$kg_{products}$	6.97	3.35	2.75	1.85	1.3	1.18	1.06	0.99	0.97
	Euro gained/t_{OFMSW}	< 0	< 0	< 0	< 0	14.38	20.6	26.9	30.7	31.9
III a	ROI (%)	<0	<0	<0	<0	<0	26.2	38.7	50.5	54.9
	NPV *1000 (euro)	<0	<0	<0	<0	611	4202.6	13605.6	41638.8	86160.3
	Payback time (year)	>20	>20	>20	>20	18	12	6	4	4
	Euro paid/kg_{OFMSW}	0.37	0.17	0.14	0.09	0.06	0.06	0.05	0.05	0.05

	Euro paid/$kg_{products}$	4.79	2.23	1.82	1.21	0.84	0.75	0.67	0.62	0.62
	Euro gained/t_{OFMSW}	< 0	< 0	< 0	< 0	22.3	50.7	57	63.4	67.2
III b	ROI (%)	<0	<0	<0	5	18.1	31.5	46.4	60.9	66.7
	NPV *1000 (euro)	<0	<0	<0	<0	808.8	6034.7	17269.8	50799.2	104590
	Payback time (year)	>20	>20	>20	>20	16	10	5	3	3
	Euro paid/kg_{OFMSW}	0.37	0.17	0.14	0.09	0.06	0.06	0.05	0.05	0.05
	Euro paid/$kg_{products}$	4.4	2.1	1	1.1	0.8	0.7	0.62	0.57	0.57
	Euro gained/t_{OFMSW}	< 0	< 0	< 0	31.3	59.8	66.1	72.4	76.2	76.2
V	ROI (%)	-6.51	-6.23	-8.96	-8.37	-7.33	-8.52	-8.01	1.69	8.35
	NPV *1000 (euro)	< 0	< 0	< 0	< 0	< 0	< 0	< 0	< 0	135231.95
	Payback time (year)	>20	>20	>20	>20	>20	>20	>20	>20	14
	Euro paid/kg_{SCG}	59.60	24.59	12.67	6.54	2.63	1.33	0.68	0.29	0.15
	Euro paid/$kg_{products}$	45.20	16.92	12.90	6.92	3.32	2.51	1.91	1.31	1.23
	Euro gained/t_{SCG}	<0	<0	<0	<0	<0	<0	<0	0.01	0.01

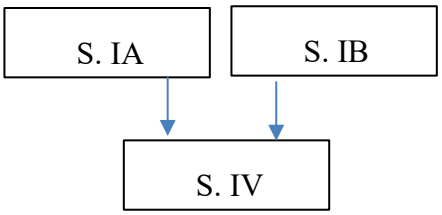
6.3.3.5. Multicriteria aids out-ranking

Multi Criteria Decision Aid (MCDA) and the Outranking methods analysis was performed with ELECTRE II for technical, economic and technical-economic-environmental perspectives for the all four Scenarios.

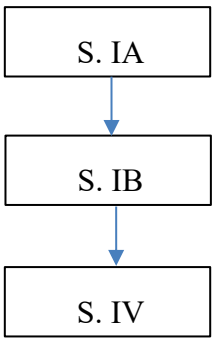
Considering the technical perspective the production of exclusive L(+)-LA (Figure6-6A) was more efficient from OFMSW (Scenario IA-B) than SCG (Scenario IV), because of the LA yield, waste production and number of process steps Table6-12.

Considering the economic perspective the production of exclusive L(+)-LA was more profitable for Scenario IB, IA and IV, because of SHF in Scenario I had more convenient NPV and payback time than SSF.

Figure 6-6: Out rank (MDA) for exclusive L(+) production from technical (A) and economic(B) production perspectives



A



B

Table 612: Outrank (MDA) for exclusive production of L(+)LA production from technical (A) and economic (B) perspectives

A

Scenario		Productivity P (g/Lh)	Y LA (gLA /g dry OFMSW)	Y (gLA/g sugar)	Optical purity OP (%)	Waste (kg waste /kg biomass)	N° step process
	weight	**0.15**	**0.2**	**0.05**	**0.2**	**0.2**	**0.2**
Scenario I A	SSF of FW	3.38	0.33	0.64	99.6	0.8511	6
Scenario I B	SHF of FW	2.16	0.29	0.8	99.7	0.8518	3
Scenario IV	SHF of SCG	0.8	0.18	0.95	99.5	5.02	9

B

Scenarios		NPV [€]*1000	Payback time [y]	ROI [%]	Euro paid/kg of biomass	Euro paid/kg of product	Euro gained/kg of biomass
	weight	**0.1**	**0.05**	**0.05**	**0.25**	**0.25**	**0.3**
Scenario I A	SSF of FW	3.7	19.0	14.7	0.04	1.37	5.64
Scenario I B	SHF of FW	3.9	18.0	14.9	0.04	1.40	5.7
Scenario IV	SHF of SCG	0.1	16.0	17.2	0.090	1.10	2.7

Considering the technical-economic-environmental perspective of exclusive L(+)LA production (Table6-13, Figure6-7) the outrank was Scenario IA-B followed by Scenario IV.

Considering these outputs, the study of biowaste valorisation was focusing exclusive on OFMSW.

Figure 6-7: Out rank (MDA) for exclusive L(+) production from technical-economic-environmental perspectives

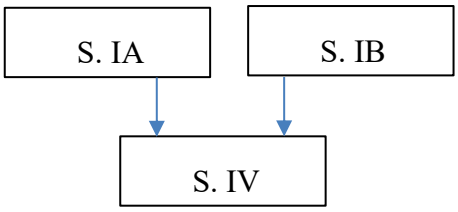


Table 6-13: Out rank (MDA) for exclusive L(+) production from technical-economic-environmental perspectives

		Technical perspective w=0.33					
Scenarios		Productivity P (g/L h)	Y LA(gLA /g dry OFMSW)	Y per sugars (gLA/g sugar)	Optical purity OP(%)	Waste (kg waste/kg biomass)	N° step process
	weight	**0.0495**	**0.066**	**0.0165**	**0.066**	**0.066**	**0.0666**
Scenario I A	SSF	2.16	0.29	0.8	99.7	0.8518	3
Scenario I B	SHF	3.38	0.33	0.64	99.6	0.8511	6
Scenario IV	SHF	0.8	0.18	0.95	99.5	5.02	8
		Economic perspectives w=0.33					
		NPV (€)*1000	Payback time (y)	ROI (%)	Euro paid/kg of biomass	Euro paid/kg of product	Euro gained/kg of biomass
	weight	**0.033**	**0.0165**	**0.0165**	**0.0825**	**0.0825**	**0.099**
Scenario I A	SSF	3.7	19.0	14.7	0.04	1.37	5.64
Scenario I B	SHF	3.9	18.0	14.9	0.04	1.4	5.7
Scenario IV	SHF	0.08	16	17.2	0.09	1.14	2.7
		Environmental perspective w=0.33					
		Q tot (kW/t biomass)					

	weight	**0.33**					
Scenario I A	SSF of FW	0.035					
Scenario I B	SHF of FW	0.035					
Scenario IV	SHF of SCG	0.037					

Further MDA was performed to evaluate single and integrated biorefinery systems feed with OFMSW, in details Scenario IA-B, Scenario II and Scenario IIIA-B were evaluated (Figure6-8A-B, Table6-14) Figure 8A proved the efficiency of OFMSW valorisation by means of integrated and sequential production of L(+)LA and biogas(Scenario III), followed by exclusive production of biogas(Scenario II), and L(+) lactic acid(Scenario I).

From economic perspective the outrank (Figure6-8B, Table6-14) set at the same level (second position) AD biorefinery and integrated biorefinery for LA and biogas productions by means of SSF.

In Scenario IIIB, LA and biogas productions were higher than in Scenario IIIA and higher enough to cover the higher operational cost due to enzyme addition in SHF.

Figure 6-8: Out rank (MDA) for biorefinery systems feed with OFMSW from technical (A) and -economic (B) perspectives.

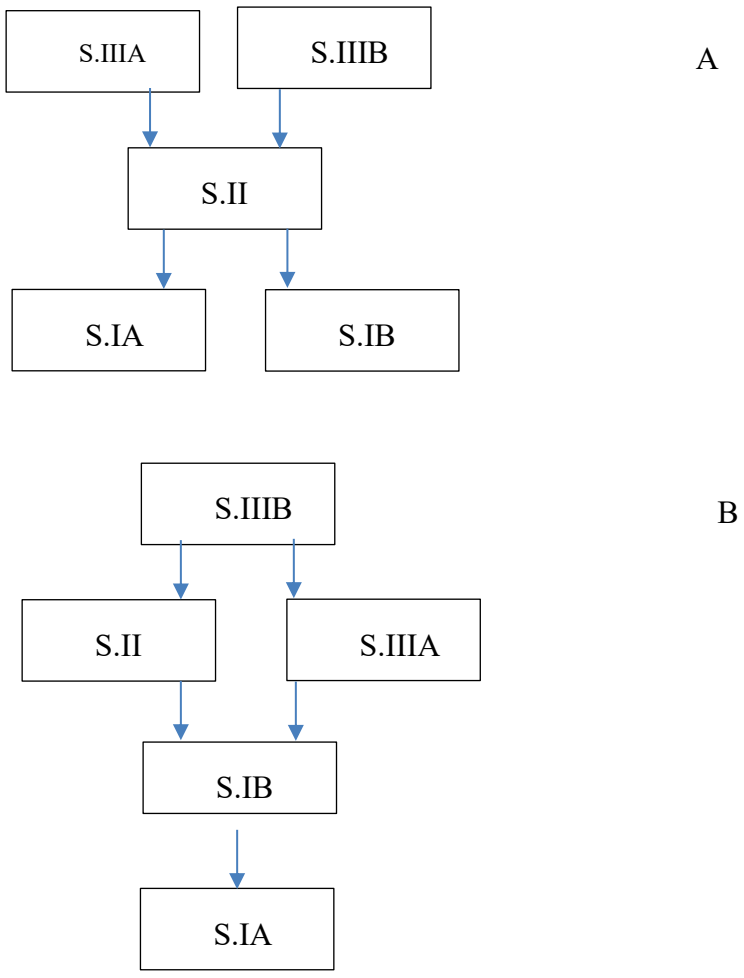


Table 6-14: Out rank (MDA) for biorefinery systems feed with OFMSW from technical (A) and -economic (B) perspectives.

A

	Productivity P (g/Lh)	Y LA (gLA /g dry OFMSW)	Optical purity OP (%)	fermentative residues: CH4 (Nm^3/kg_{VS})	kd (1/d)	Waste (kg waste /kg biomass)	N° step process
Scenarios	0.1	0.2	0.1	0.2	0.1	0.2	0.1
I A	2.16	0.29	99.7	/	/	0.8518	3
I B	3.38	0.33	99.60	/	/	0.8511	6
II	/	/	/	0.39	0.35	0.49	2
IIIA	2.16	0.29	99.70	0.62	0.43	0.39	5
III B	3.38	0.33	99.60	0.43	0.33	0.43	8

B

	NPV (Euro)*1000	Payback time (y)	ROI (%)	Euro paid/kg of biomass	Euro paid/kg of product	Euro gained/kg of biomass
Scenarios	0.1	0.05	0.05	0.25	0.25	0.3
I A	3.71	19.00	14.70	0.04	1.30	5.64
IB	3.06	18.00	14.90	0.04	1.41	5.70
II	97.60	11.00	24.80	0.02	0.90	1.18
IIIA	293.68	12.00	26.20	0.06	0.75	50.70
IIIB	421.66	10.00	31.50	0.06	0.70	66.10

To conclude, from combined technical-economic-environmental perspectives (Figure6-9 and Table6-15) the outrank was Scenario IIIB, Scenario II-Scenario IIIA, Scenario IB, Scenario IA.

Figure 6-9: Out rank (MDA) for biorefinery systems feed with OFMSW from technical-economic-environmental perspective.

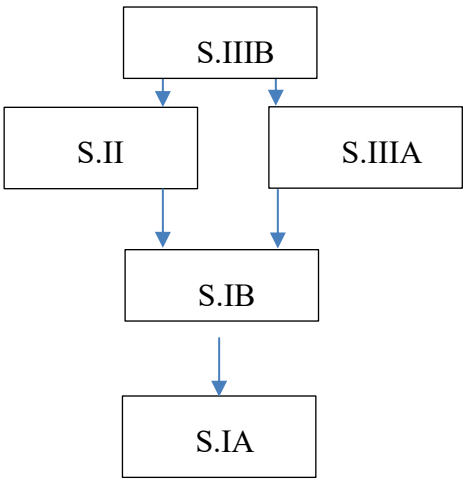


Table 6-15: Out rank (MDA) for biorefinery systems feed with OFMSW from technical (A) and -economic (B) perspectives.

Technical perspectives w=0.33							
	Productivity P (g/Lh)	Y LA (gLA /g dry OFMSW)	Optical purity OP(%)	CH_4 (Nm^3/kg VS)	kd (1/d)	Waste (kg waste /kg biomass)	N° step process
Scenario	**0.033**	**0.066**	**0.033**	**0.066**	**0.033**	**0.066**	**0.033**
IA	2.16	0.29	99.7	/	/	0.85	3
IB	3.38	0.33	99.6	/	/	0.85	6
II	/	/	/	0.39	0.35	0.49	2
IIIA	2.16	0.29	99.7	0.49	0.33	0.434	5
IIIB	3.38	0.33	99.6	0.62	0.43	0.39	8
Economic perspectives w=0.33							
		NPV (€)*1000	Payback time (y)	ROI (%)	Euro paid/kg of biomass	Euro paid/kg of product	Euro gained/kg of biomass
Scenario		**0.033**	**0.0165**	**0.0165**	**0.0825**	**0.0825**	**0.099**
IA		3.71	19.00	14.70	0.04	1.30	5.64
IB		3.06	18.00	14.90	0.04	1.41	5.70
II		97.60	11.00	24.80	0.02	0.90	1.18
IIIA		293.68	12.00	26.20	0.06	0.75	50.70
IIIB		421.66	10.00	31.50	0.06	0.70	66.10
Environmental perspectives w=0.33							
	Q tot (kW/t biomass)						
	0.33						
IA	0.035						
IB	0.035						
II	0.155						
IIIA	0.137						
IIIB	0.137						

6.4 Conclusion

The present Chapter proves the technical-economic-environmental profitability and feasibility of sequential LA and biogas productions from OFMSW compared to the exclusive LA and biogas fermentative productions from OFMSW and SCG, carried out as single processes.

The assessed integrated biorefinery resulted in a reduced amount of produced waste, reduced digester volume for biogas production and consequently minimized the energy demand as well as improved the production of market valuable products.

The economic assessment through composite indicators of the four scenarios based on the catchment area size revealed that Scenarios I achieved profitability after 500,000 inhabitants, Scenario II after 100,000 inhabitants, Scenario III after 50,000 inhabitants and Scenario IV after 500,000 inhabitants. Scenario III represented the opportunity to pass from linear to circular bio-economy by

supporting a regenerative and restorative system through waste prevention and economic profitability.

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Chapter 7: Effect of inoculum origin and substrate-inoculum ratio to enhance the anaerobic digestion of Organic Fraction Municipal Solid Waste

The main findings of current study are under review in:

DemichelisF., Tommasi T., Deorsola F., Marchisio D, Fino D. Effect of inoculum origin and substrate-inoculum ratio to enhance the anaerobic digestion of organic fraction municipal solid waste. Under review.

Abstract

Chapter 7 evaluates the key role of inoculum in mesophilic anaerobic digestion (AD) of organic fraction municipal solid waste (OFMW). Two inocula were tested, one coming from the mesophilic digestate of wastewater activated sludge (WAS) and the other one from the mesophilic digestate of cow-agriculture sludge (CAS). Both inocula were anaerobically cultivated for three different periods: 0, 5 and 10 days and then inoculated in OFMW considering three substrate-inoculum ratios (S:I) 1:2; 1:1; 2:1. First order kinetics and Gompertz modified model were applied to define disintegration rate, lag phase and maximum biogas yields. Energy sustainability index was calculated to define which configurations were suitable to be scaled-up. Then multi criteria decision aid was performed to outranking the AD configurations tested. The AD configurations with the best performances were: AD performed with S:I=2:1 with CAS cultivated for 5 days, AD performed with S:I=1:1 and 2:1with CAS cultivated for 10 days and AD performed with S:I=2:1 WAS cultivated for 10 days.

Abbreviation
AD: Anaerobic digestion
CAS: Cow Agriculture Sludge
COD: Chemical Oxygen demand
ESI: Energy Sustainable Index
GHGs: Green House Gas emissions
OFMSW: Organic Faction Municipal Solid Waste
TS: Total Solids

TOC: Total Organic Carbon
VS: Volatile Solids
VFA: Volatile Fatty Acids
WAS: Waste Activated Sludge

7.1 Introduction

Considering the results of the previous Chapters, the substrate considered for further analysis and valorisation was OFMSW, we neglected Spent ground Coffee (SCG). From Chapter 5 and 6, anaerobic digestion (AD) resulted a process with considerable technical-economic-environmental potentially.

The aim of Chapter7 was the investigation of the key role of inoculum to optimise AD of OFMW.

Two inocula were tested; the first inoculum came from the mesophilic digestate of wastewater sludge (WAS) and the second inoculum came from the mesophilic digestate of cow-pig manure (CAW). Both inocula were anaerobically cultivated for three different periods and then they were inoculated in OFMW considering three substrate-inoculum ratios (S:I) 1:2; 1:1; 2:1. Inocula with different origins were considered to evaluate how different biomasses can enhance biogas yield. Three inocula cultivation periods were tested to determinate if the growth of methanogens microorganisms improved days after days, with the benefit to enhance the biogas production reducing lag phase and consequently AD process time. Three S:I ratios were tested, a conventional one; S:I=1:2 Panigrahi et al.(2020); Wu et al., (2019), and two unconventional S:I=1:1 Harun et al.(2019) Pramanik et al (2019a) and 2:1Pramanik et al.(2019b) , with the aim to treat as much OFMW quantity as possible. The novelty of the present study was the investigation of all these three features: inocula origin, cultivation time and S:I ratio in experimental laboratory tests and model with Angelidaki first order kinetic and Gompertz modified model. Generally, the largest part of research on AD of OFMSW focused the attention on AD design Srisowmeya et al.(2020) and operation as focuses on feedstock compositions Mu et al. (2020) chemical, physical and biological pre-treatments Tao et al. (2020) and co-digestion of different organic substrates Kainthola et al. (2020) to enhance biogas production. Here, the present study optimised the biogas yield and methane content through the analysis of inoculum, which generally comes from digestate of wastewater treatment plant. Moreover, in the present study, the OFMSW come from a real selection system, while in literature a lot of research used synthetic OFMSW Teigiserova et al(2020) or in general food waste (FW) non edible Carmona-Cabello et al. (2020).

The most promising configurations were the AD configuration with inoculum cultivation periods between 5 and 10days with S:I equal to 1: and 2:1, in details: AT5_3_CAS, AT10_2_CAS, AT10_3_CAS and AT_10_3_WAS

7.2. Materials and methods

7.2.1 Substrate and inoculum characterisations

Anaerobic digestion (AD) was performed on organic fraction municipal solid waste (OFMW), provided by a food processing company, San Carlo S.p.A (Fossano, Italy). Two inocula were tested: the first inoculum came from the mesophilic digestate of wastewater activated sludge (WAS) and the second inoculum came from the mesophilic digestate of cow-agriculture sludge (CAS). The choice of these inocula origins was based on a scientific literature survey of the most used inocula (Liu et al., 2019).

Both inocula were anaerobically cultivated for three different periods: 0, 5 and 10 days and then the anaerobic digestate were inoculated in OFMW considering three substrate-inoculum ratios weight by weight (S:I) 1:2; 1:1; 2:1 based on volatile solids (VS). The experiment configuration and the relative codes used hereafter are reported in Table 7-1. To be more clear, the experiment names were codified as TX_Y_ZZZ, where X can be 0, 5 or 10 and represents the cultivation period (0, 5 or 10 days), Y represents the S:I ratio, specifically 1 for S:I=1:2, 2 for S:I=1:1 and 3 for S:I=2:1, and ZZZ indicates the origin of the inoculum (WAS or CAS).

Table7-1: Anaerobic digestion configurations description by means incubation time of inoculum, substrate- inoculum ratio (S:I) and identification code

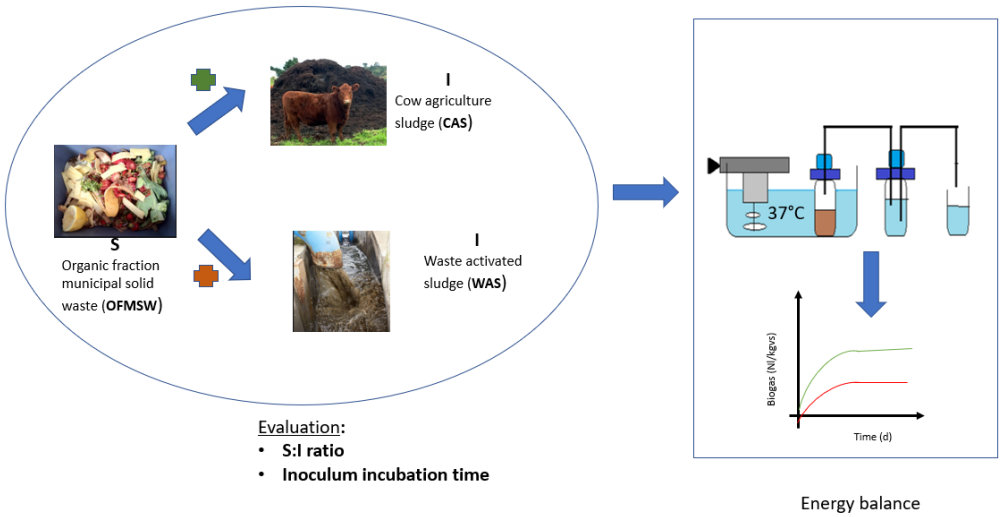
Description	Inoculum incubation time (d)	S:I	Code
mesophilic digestate of wastewater sludge	/		WAS
mesophilic digestate of cow-agriculture sludge	/		CAS
WAS at day 0 (T0) at S:I=1:2 (1)	0	1:2	T0_1_WAS
WAS at day 0 (T0) at S:I=1:1 (2)	0	1:1	T0_2_WAS
WAS at day 0 (T0) at SI=:2:1 (3)	0	2:1	T0_3_WAS
CAS at day 0 (T0) at S:I=1:2 (1)	0	1:2	T0_1_CAS
CAS at day 0 (T0) at S:I=1:1 (2)	0	1:1	T0_2_CAS
CAS at day 0 (T0) at S:I=2:1 (3)	0	2:1	T0_3_CAS
WAS at day5 (T5) at S:I=1:2 (1)	5	1:2	T5_1_WAS
WAS at day 5(T5) at S:I=1:1 (2)	5	1:1	T5_2_WAS
WAS at day 5 (T5) at S:I=2:1 (3)	5	2:1	T5_3_WAS
CAS at day 5 (T5) at S:I=1:2 (1)	5	1:2	T5_1_CAS
CAS at day 5 (T5) at S:I=1:1 (2)	5	1:1	T5_2_CAS
CAS at day 5 (T5) at S:I=2:1 (3)	5	2:1	T5_3_CAS
WAS at day 0 (T10) at S:I=1:2 (1)	10	1:2	T10_1_WAS
WAS at day 0 (T10) at S:I=1:1 (2)	10	1:1	T10_2_WAS
WAS at day 0 (T10) at S:I=2:1 (3)	10	2:1	T10_3_WAS
CAS at day 0 (T10) at S:i=1:2 (1)	10	1:2	T10_1_CAS
CAS at day 0 (T10) at S:I=1:1 (2)	10	1:1	T10_2_CAS
CAS at day 0 (T10) at S:I=2:1 (3)	10	2:1	T10_3_CAS

7.2.2Anaerobic digestion set up

Anaerobic digestion (AD) was carried out in a batch mode feeding 6% TS of OMFW. AD tests were performed in 0.5 L Pyrex glass bottles (Duran, Germany). AD was performed in mesophilic condition, 37 °C, in a 55 L thermostatic water-bath (Julabo-Corio-C). The temperature was established according to literature researches, evaluating the energetical and operation benefits of working in mesophilic conditions (Kovalovszki et al., 2020). Each digestor was manually shaken four times per day. AD tests were stopped when the daily biogas rate fell below 1 % of the total volume of biogas produced up to that time Angelidaki, (2011). Each configuration was tested in replicates in order to assess the uncertainty associated with the experiments.

A total of 40 digestors were manged: 4 for the blank (inoculum), 36 for the biogas measurement. Each digestor was connected by 6 mm Teflon tubes (PTFE, Germany) to a 0.5 L Pyrex glass bottles (Duran, Germany) containing distilled water, acting as gasholder. The measurement of the biogas volume was performed through the water displacement method. The digestor was connected by a plastic tube of 4mm in diameter to a gasholder, filled with demineralised water. The biogas flow exerted a pressure on the water, which flowed out from the gasholder, consequently the amount of water displaced was equal to the flow of biogas produced referred to normal condition of temperature and pressure. The scheme of the biogas plant is depicted in Figure**7-1**.

Figure 7-1: Scheme of anaerobic digestion set up, with the description of the studied process: Substrate S the Organic Fraction of Municipal Solid Waste (OFMSW), with two inocula Waste Activated Sludge (WAS) and Cow Agriculture Sludge (CAS), the evaluation of Substrate Inoculum (S:I) ratio and inoculum incubation time.



The biogas composition was monitored by means of a Biogas-Analyser (GA5000, GMBH, gas-analyser). Elemental composition of OFMW was provided by San Carlo S.p.A. The biogas production, COD and TOC were calculated by using to Buswell and Neave equations (Rodrigues et al., 2019) neglecting biomass

growth and degradation products and they read as follows**.** In order to complete the evaluation of the AD tests, mass and carbon balance were respectively calculated by using Eq. (2) and Eqs. (3), (4) and (5):

$$C_a\,H_b\,O_c\,N_d + \left(a - \frac{b}{4} - \frac{c}{2} + \frac{3d}{4}\right) \cdot H_2O \rightarrow \left(\frac{4a + b - 2c - 3d}{8}\right) \cdot CH_4 + \left(\frac{4a - b + 2c + 3d}{8}\right) \cdot CO_2 + dNH_3 \quad (1a)$$

$$COD_t = \frac{8(4a + \mathrm{b} - 2\mathrm{c} - 3\mathrm{d})}{(12a + b + 16c + 14d)} \quad (1b)$$

$$TOC_t = \frac{12a}{(12a + b + 16c + 14d)} \quad (1c)$$

In order to complete the evaluation of the AD tests, mass and carbon balance were respectively calculated by using Eq. (2) and Eqs. (3), (4) and (5):

$$weight_{in} = weight_{end} + removed\ weight \quad (2)$$

$$C_{in} = C_{out} \quad (3)$$

$$C_{in} = C_{inoculum} + C_{OMW} \quad (4)$$

$$C_{out} = C_{digestate} + C_{CO2} + C_{CH4} \quad (5)$$

The samples were filtered with 1.2 µm filter to evaluate pH, FOS (Flüchtigen Organischen Säuren, organic volatile fatty acids) and TAC (Totales Anorganisches Carbonat, inorganic total carbonate). The pH was measured through the DIN 38404 C5 methodology, while FOS/TAC were measured by titration with a 1:10 diluted sample by adding sulphuric acid 0.1N to reach a pH value of 5 (P_0) and then of 4.3 (P_1). The volume of acid added to obtain P_0 and P_1 was V_0 and V_1, respectively. FOS /TAC were calculated as follows:

$$FOS = (V_1 \cdot 1.66 - 0.15) \cdot 500 \quad (6)$$

$$TAC = V_0 \cdot 250 \quad (7)$$

The pH, FOS /TAC measurements were carried out with a pH340 WTW-pH-meter. The total organic carbon (TOC) was measured to evaluate the carbon balance of the AD tests.

7.2.3 Kinetic study

In order to evaluate the kinetics of the AD, a disintegration rate (k_d) was considered by assuming a first-order kinetic model calculated through the first part of the cumulative methane curve by using Eq. (8) Angelidaki et al., (2009):

$$B(t) = B_{exp}(1 - e - k_{dis}t) \tag{8}$$

where, $B(t)$ represents the cumulative methane production at a given time t, B_{exp} is the ultimate methane potential yield at the 5th day, k_{dis} is the first-order disintegration rate (1/d) and t is the time (expressed in days). To complete the investigation of the AD process, the Gompertz-modified model Frunzo et al., (2019) was employed as a deterministic function, based on non-linear regression. Non-linear regression method was performed with the Solver Tool-Pak of Microsoft Excel. Gompertz-modified model allows to estimate the lag-phase, maximum biogas production rate and maximum biogas yield potential (Nguyen et al. (2016) by using Eq (9):

$$y_t = y_m \exp\left\{-\exp\left[\frac{R_m e}{y_m} \cdot (\lambda - 1) + 1\right]\right\} \tag{9}$$

where, y_t is the predicted biogas yield (L/kg$_{VS}$) obtainable after time t, y_m is the maximum biogas yield potential (L/kg$_{VS}$), R_m represents the maximum biogas production rate (L/kg$_{VS}$ d) and λ is the lag phase (d).

7.2.4 Energy evaluation

The energy balance was modeled considering the following assumptions Mehr et al. (2017):

- atmospheric air composed by 79% v/v N_2 and 21% v/v O_2;
- application of ideal gas law
- steady state conditions and thermodynamic equilibrium
- possible gas leaks from the connecting pipes not considered.

The total system thermal load (Q_s) is expressed in kWh (Eq. 10):

$$Q_s = Q_{sub} + Q_{loss} + Q_p \tag{10}$$

$$Q_{sub} = m_{sub} \cdot c_p \cdot (T_{in} - T_{reac})$$

(11)

where Q_{sub} is the thermal power required for heating the substrate from an inlet temperature of 20 °C to 37 °C (see Eq.11). In Eq. (11) m_{sub} represents the mass substrate flow rate, while T_{in} and T_{reac} are the inlet and reactor temperatures, respectively, and c_p is the specific heat capacity, considered as equal to that of water, since OFMSW dry matter was equal to 11%-wt. Q_{loss} is the heat loss from the reactor walls and it was calculated by using Eq. (12):

$$Q_{sub} = U_{ug} \cdot A_{ug} \cdot (T_{reac} - T_{gr}) + U_{ext} \cdot A_{ext} \cdot (T_{reac} - T_{ext})$$

(12)

where U_{ug} and U_{ext} are the heat transfer coefficients for underground walls and non-ground walls, respectively; $A_{ug}$and A_{ext} are the areas of underground walls and partial walls, respectively, and top; T_{gr} and T_{ext} are the temperatures of underground walls and partial walls, respectively.

Q_p is the heat loss through the tube and it was calculated using Eq. (13):

$$Q_p = \%_p \cdot (Q_{sub} + Q_{loss})$$

(13)

The laboratory temperature was taken as inlet, underground and partial walls temperatures.

In order to evaluate the energetic sustainability of the AD configuration, the energy sustainable index (ESI) was calculated according to Eq. (14):

$$ESI = \frac{Q_{pro}}{Q_s}$$

(14)

where Q_{pro} is the energy produced from AD tests considering the low heating CH_4 equal to 9.4 KWh/m^3. The energy evaluation was proved by Super Pro design model simulation (Figure 1, Appendix A)

7.2.5 Sensitivity analysis

The statistical analysis of the AD tests was carried out by using data analysis extension of Excel 2016. In detail, Pearson and ANOVA (Analysis-Of-Variance) tests were performed to evaluate linear correlations among the different configurations of AD and considering significant only the ones with values minor of 0.05p.

Multi Criteria Decision Aid (MCDA) to perform outranking methods analysis was performed by means of ELECTRE II (ELimination Et Choix Traduisant la REalité; Roy, 1968). The MCDA was performed for ranking the 18 AD configurations from the best to the worst. The set-up of MCDA was reported in Table 7-2. For MCDA, three main criteria were defined: Yields, Energy Sustainability (if the system is energetically self-sufficient) and Time optimisation

with the following weights: 0.4, 0.4 and 0.2. Yield was divided into four sub-criteria: biogas yield, CH_4 content, TOC removal and weight removal with the following weights: 0.133, 0.133, 0.67 and 0.67. Sustainability included ESI with weight equal to 0.40. Great importance was given to ESI, since energy sustainability is a key parameter to define the AD configuration to be scaled-up. Time optimisation was dived into two sub-criteria: first-order constant (k_{dis}) and lag phase (λ), both weighted 0.1.

Table 7-2:ELECTRE II analysis

	Yields				**Sustainability**	**Time optimisation**	
Weight	**0.4**				**0.4**	**0.2**	
	Biogas	**CH4**	**TOC removal**	**Weight removal**	**ESI**	**kd**	**λ**
	(NL/kgvs)	(%)	(%)	(%)	(-)	(1/d)	(d)
Weight	**0.133**	**0.133**	**0.067**	**0.067**	**0.4**	**0.100**	**0.100**
Range	[700-1000]	[60-80]	[80-100]	[6-12]	[0;1]	[0.05-0.11]	[3-12]
Preference	800						
Veto	900	65	85	8		0.075	5

7.3 Results and discussion

7.3.1 Substrate and inoculum characterisations

Physical and chemical characteristics of the substrate, that is the organic fraction of the municipal solid waste (OFMW), and of the two inocula, namely the mesophilic digestate of wastewater sludge (WAS) and the mesophilic digestate of cow-pig manure (CAS), are reported in Table 7-3. VS and TOC contents higher than 70% and 8000 mg/kg, respectively, proved the abundance of organic matters suitable to be anaerobic digested, according to Zhang et al. (2019a)Zhang et al., (2019b) Mirmohamadsadeghi et al.(2019). The TOC values of OFMSW was in accordance with the general TOC trends of the European OFMSW mainly composed by lignocellulosic matter (Harun et al. (2019) and the difference between experimental (TOC_{exp}) and theoretical (TOC_{th}) ranged between 0.1-0.5%.

Table 7-3:Physical and chemicals characteristics of the substrate, organic municipal solid waste (OFMSW) and the two inocula, mesophilic digestate of wastewater sludge (WAS) and mesophilic digestate of cow-pig manure (CAS).

	WAS	CAS	OFMSW
TS (%)	5.00	5.88	11.00
VS/TS (%)	70.00	71.05	97.00
C (%)	35.00	40.96	44.50
N (%)	4.50	3.80	3.90
S (%)	0.00	0.00	0.00
H_2 (%)	3.00	3.00	1.00
O (%)	57.50	52.24	50.60
C/N (-)	7.78	10.78	11.41
TOC th (mg/kg)	9775.30	12298.99	48333045.24
TOC exp(mg/kg)	9432.50	12158.09	46057055.00
TOC ex/TOC th	0.04	0.01	0.05
COD th (mg/kg)	2496.08	10010.96	44594.93
pH (-)	7.00	7.82	6.70

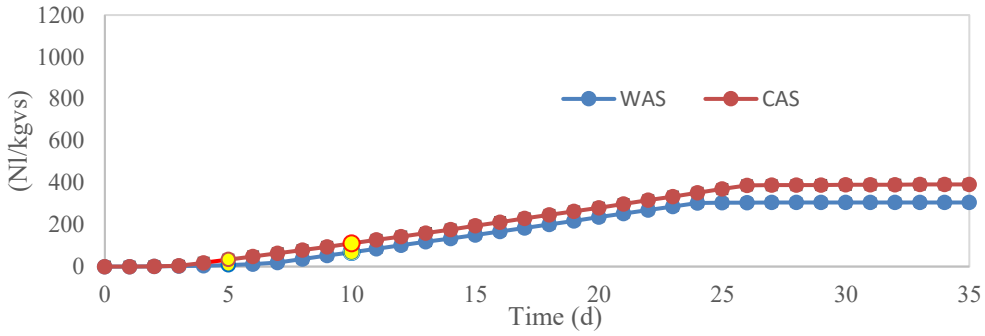
7.3.2 Anaerobic digestion

The AD tests were performed to enhance the biogas yield and methane content, focusing the attention on the key role played by the inoculum. The key role of the inoculum was investigated by three viewpoints: 1) origin (WAS and CAS), 2) incubation time (T) and 3) substrate inoculum ratio (S:I).

According to a literature work Li et al. (2020), the S:I ratio influences the efficiency of acidogenic performance and the origin of the inoculum is a crucial biological factor affecting the metabolic pathways Wang et al. (2014). In Figure7-2, the biogas cumulate production of the two inocula was depicted. The AD of both inocula, WAS and CAS, lasted 26 day, before reaching the highest biogas yield, 305 NL_{biogas}/kg_{VS} and 392.23 NL_{biogas}/kg_{VS}, respectively. The biogas yield of CAS was 22% higher than the biogas yield of WAS. This trend was not confirmed by the scientific literature, because the potential biogas yield of animal manure is usually inhibited by the low and imbalanced carbon to nitrogen (C/N) ratio Neshat et al. (2017).

However, CAS was recently adopted as possible inoculum for AD process with C/N correction with the addition of a carbon source Lavergne et al.(2020). In the present study, the biogas yield reached by CAS agreed with Kalamaras et al. (2020). In Figure 7-2, the biogas production at days 5 and 10 were yellow coloured in order to point out that 5 and 10 days were the cultivation period of inocula to carry out T5 and T10 (see Table 7-1) anaerobic digestion. The cultivation period of inocula was set considering the trend of experimental biogas production of WAS and CAS (the blanks) in order to find the best time in which the biomass of the inocula were more suitable for biogas and methane production.

Figure 7-2: Biogas yield obtained by AD test performed on inocula: WAS (blue line) and CAS (red line)



7.3.2.1Analysis of cultivation period of inoculum

In Figure 7-3 the net biogas cumulate production of AD carried out on OFMSW with WAS (Figure 7-3 A, B and C) and CAS (Figure 7-3 D, E and F) are depicted. The S:I ratio was maintained constant and the effect of the three cultivation periods of the inoculum (0, 5 and 10 days) was considered. Results show that the net biogas yields increased by increasing the cultivation periods of the inoculum from 0 to 10 days. In detail, by using the WAS inoculum and a S:I=1:2 the increases of the net biogas yield after 5 and 10 days were 7% and 10%, respectively, with S:I=1:1 the increases were 13% and 19% after 5 and 10 days, respectively, and with S:I=2:1 the biogas yield augmented by 24% and 29% after 5 and 10 days respectively. Analogously for the CAS inoculum, the biogas increase after 5 and 10 days of cultivation was 7% and 8% with S:I=1:2, 12 and 15% with S:I=1:1 and 21 and 25% with S:I=2:1.

By comparing the biogas yield after 10 days with that obtained after 5 days, the percentage increases with S:I equal to 1:2, 1:1 and 2:1 were 4%, 6% and 6.3%, respectively, by using the WAS inoculum while they were 1%, 4% and 5%, respectively, by employing the CAS inoculum.

Considering the statistical analysis, the net biogas yield of anaerobic digestion performed with the inoculum incubated for 5 and 10 day at S:I equal to 2:1 respectively with CAS and WAS (T5_3_CAS and T10_3_WAS) did not show not significant differences. Increasing the cultivation period of the inoculum, the S:I ratio can be increased and this trend was confirmed by other scholars J. Zhang et al. (2019a), which tested three inoculum cultivation periods (0, 20 and 50 days) and reached the highest biogas yield (545.5 L_{biogas}/kg vs) with an inoculum cultivation period of 50 days, S:1 = 2:1 by performing AD on agricultural waste at 37°C. Furthermore, Yan et al. (2019) proved that the cultivated inoculum was able to develop microorganisms tolerant to the ammonia content of substrate thus offering an efficient way to manage ammonia inhibition during AD. Usually, the degradation of OFMSW produces ammonia and by-products from the catabolism of proteins, which can inhibit the AD process, resulting in operational instability and low methane production Tian et al. (2018). In the present study, the cultivation

of inoculum warded off the ammonia and by products generations. Furthermore, increasing the incubation time of the inocula, the lag phase was reduced and the kinetic of the anaerobic digestion (AD) was improved. These trends agreed with Calicioglu et al. (2018) since during incubation, the inoculum became more suitable to produce biogas and methane. This assertation was further proven by C:N ratio improvement. Increasing the inoculum incubation time, the C:N ratio was between 15-20, falling within the 10-30 range, which is the optimal for AD processes according the literature Da Silva et al. (2017).

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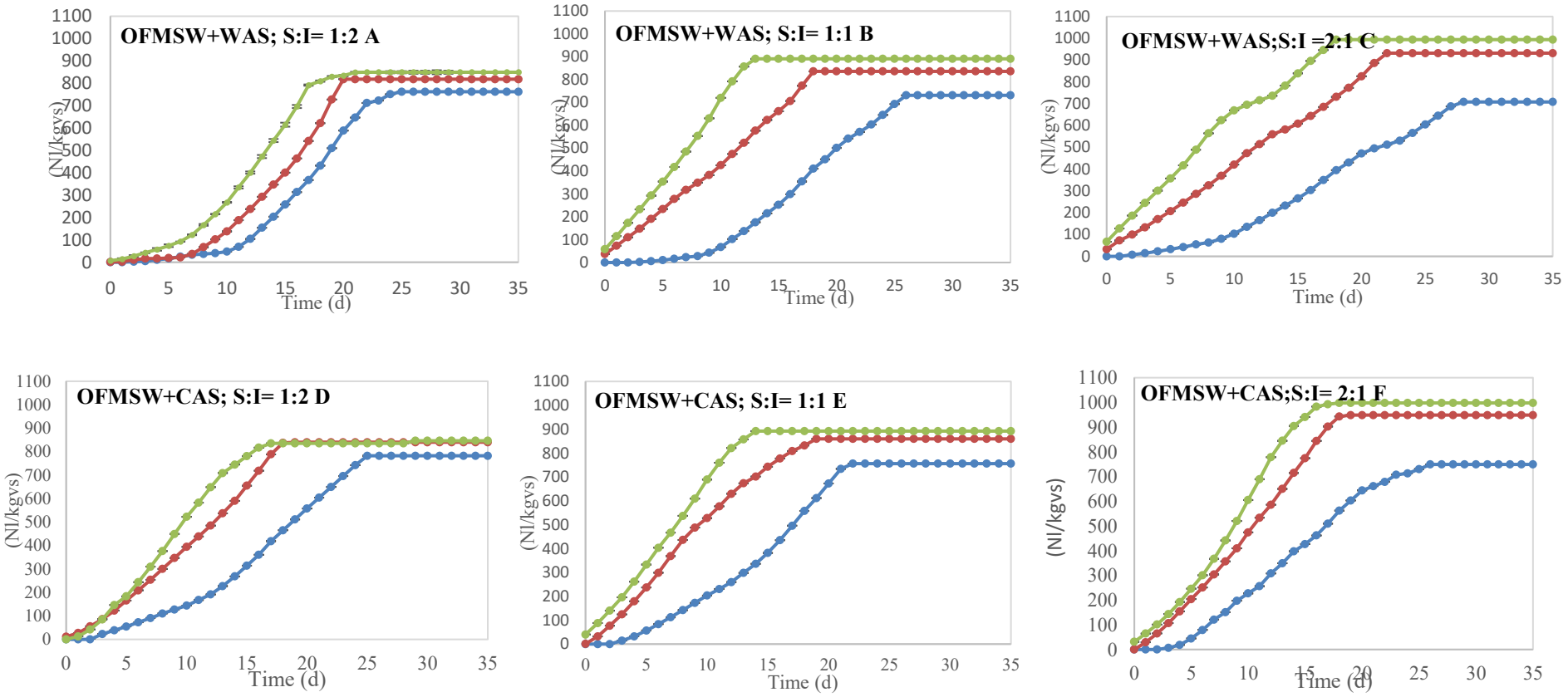
Figure 7-3: Net biogas yield obtained by AD test performed on OFMSW with WAS at S:I= 1:2; 1:1 and 2:1.for incubation time 0d (blue line), 5d (red line) and 10d (green line) under three



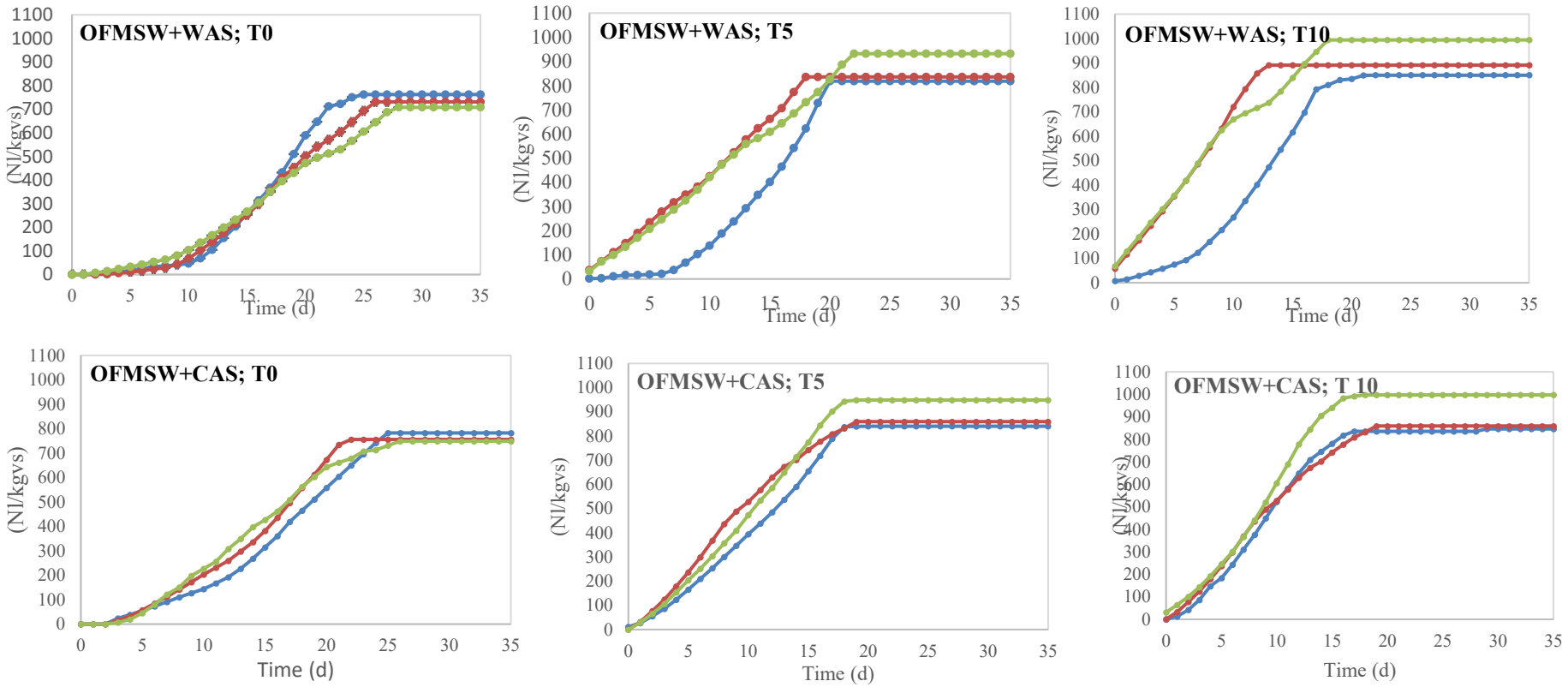
7.3.2.2. Analysis of substrate inoculum (S:I) ratio

In Figure 7-4, the net biogas productions are presented by considering the effect of the S:I ratio keeping constant the inoculum cultivation period. By employing the inocula without cultivation period (0 d), the AD on OFMSW performed with both WAS and CAS reached the highest biogas productions for S:I=1:2, followed by S:I=1:1 and S:I= 2:1. This trend agreed with most of values reported in the literature, i.e. 750.0 NL_{biogas}/kg_{VS} with S:I=1:2 on corn strew Li et al. (2020), 631.0 NL_{biogas}/kg_{VS} for S:I =2:1 and 462.0 NL_{biogas}/kg_{VS} for S:I=4:1 on agricultural waste (Latifi et al., 2019).

The net biogas yields with S:I=1:2 at cultivation time 0 d by using the WAS and CAS inocula were 762.5 NL_{biogas}/kg_{VS} and 781.53 $NL_{biogas}/kg_{VS,}$ respectively. There were not statistical differences among these two configurations. Anaerobic digestion (AD) performed with inoculum incubation time of 0 d and with S:I ratio equal to 1:2 (T0_1) is the most common AD configuration adopted in AD process, and the results achieved in the present study agreed with Donoso-Bravo et al.(2019) and Edwiges et al (2018) which reached 790 L_{biogas}/kg_{vs}, 780.77 L_{biogas}/kg_{vs}, respectively. For cultivation time of 5 (T5) and 10 (T10) days, the net biogas production was increased by increasing the S:I ratio. In detail, the highest net biogas productions were reached with S:I=2:1 for both inocula: 994.20 NL_{biogas}/kg_{VS} for WAS and 997.81 NL_{biogas}/kg_{VS} for CAS. It is worth to notice that there were statistical differences between T5_1_WAS and T5_2_WAS, T5_1_CAS and T5_2_CAS, T10_1_CAS and T10_2_CAS. The biogas yields reached with the T5 and T10 anaerobic digestion configurations and S:I equal to 1:1 and 2:1 were in line with the biogas yield of AD performed on pre-treated substrates, as confirmed by the studies of Deepanraj et al (2017),Mahmoodi et al. (2018) and Dehkordi et al., (2020) performing enzymatic, hydrothermal and mechanical pre-treatments on food and fruits wastes, respectively.

In the literature, the S:I ratio equal to 2:1 was not encouraged, because of the inhibition of AD occurs. However, in the present study high performance of biogas yield was achieved with S:I=2:1 thanks to the increase of the inoculum cultivation period. This trend was confirmed by J. Zhang et al. (2019). Performing statistical tests, the cultivation period and S:I was proven to be significantly correlated. To conclude this section, it is possible to assert that inoculum incubation can be considered as an innovative form of pre-treatment which allows to increase the S:I ratios. This trend agrees with Circular Economy aims to treat as much as possible amount of waste (as OFMSW) by valorisation and production of high added values and energy as biogas Schoggl et al.(2020).

Figure 7-4: Net biogas yield obtained by AD test performed on OFMSW for incubation time 0d (T0), 5d(T5) and 10d (T10) under three S:I= 1:2(blue line),; 1:1(red line) and 2:1 (green line).



7.3.2.3 Analysis of inoculum origin

Considering the origins of the two inocula, no statistical differences were observed between:

anaerobic digestion performed with inoculum incubated 5 days with S:I equal to 1:1 respectively with WAS and CAS (T5_2_WAS and T5_2_CAS),

anaerobic digestion performed with inoculum incubated 5 days with S:I equal to 2:1 respectively with WAS and CAS (T5_3_WAS and T5_3_CAS),

anaerobic digestion performed with inoculum incubated 10 days with S:I equal to 1:1 respectively with WAS and CAS T10_2_WAS and T10_2_CAS,

anaerobic digestion performed with inoculum incubated 10 days with S:I equal to 2:1 respectively with WAS and CAS (T10_3_WAS and T10_3_CAS).

The performed AD tests proved that the increase of the cultivation inoculum time from 0 to 10 d brought to the increase of the S:I ratio from 1:2 to 2:1, reaching the highest net biogas productions. However, the origin of the inoculum for the highest cultivation time and S:I had not significant influence.

Through Buswell and Neave equations (see Eq. 1b), the theoretical biogas production was calculated to evaluate the maximum biogas yields keeping into account the OFMSW composition. The theoretical biogas yields for WAS, CAS and OFMSW were: 457.7 NL_{biogas}/kgvs, 543.6 NL_{biogas}/kgvs and 1010.8 NL_{biogas}/kgvs, respectively. The difference between theoretical and experimental biogas yields agreed with the BMP tests performed by Donoso-Bravo et al. (2019) and Da Silva et al. (2018). The differences between experimental and theoretical biogas yields was around 20-30% and the reason may be due to the complexity of the three substrates, in particular the degradation of lignocellulosic matter in the first step of AD by solubilization and hydrolysis, according to Kainthola et al. (2019).

In order to evaluate the quality of AD, TOC removal, FOS/TAC and pH were monitored every 5 days and they are depicted in Figure 2, Figure 3 and Figure 4 in Appendix B. By increasing the S:I ratio with the not-cultivated inoculum (T0) the biogas production was inhibited due to the production of volatile fatty acid, which decreased the pH (Figure 4 of Appendix B) and increased of FOS/TAC values (Figure3, Appendix B). According to Calicioglu et al (2018) FOS/TAC values must range between 0.3-0.4.

The complete evaluation of the all tested AD configurations is reported in Table 4. All the AD configurations reached a CH_4 content higher than 60%$_{v/v}$, in particular the highest CH_4 contents were reached according to the following order: T10_3_CAS (70%$_{v/v}$) > T10_2_CAS (69.85%$_{v/v}$) > T10_3_WAS (69%$_{v/v}$) > T10_1_CAS (68.31%$_{v/v}$) > T10_2_WAS (68.2%$_{v/v}$) > T10_1_WAS (67.9%$_{v/v}$) > T5_3_CAS (67.57%$_{v/v}$). The CH_4 yield (Table 3) of T0_1 performed with both inocula WAS and CAS was in line with the CH_4 yield reached by Koch et al., (2017) with inocula coming from digestates from wastewater (447 NL_{biogas}/kgvs) and agricultural manure (440 NL_{biogas}/kgvs). Moreover, the CH_4 yields of T5 and T10 agreed with AD performed on pre-treated substrate as proven in the (Latifi et al.,

2019) study. The TOC removal was higher than 80% for all the AD configurations, proving high performances of AD and biogas production. In details, the higher TOC removals were achieved in the following ranking: T10_3_CAS (89.96%) > T10_3_WAS (89.98%) > T5_3-CAS (89.80%) > T5_3-WAS (89.49%) > T10_2_WAS (89.33%) > T5_2_WAS (88.15%) > T10_2_CAS (87.35%) > T5_2_CAS (87.31%) > T10_1_CAS (86.38%). The achieved TOC removal values agreed with TOC removal of AD performed on pre-treated substrates, as TOC removal equal to 90% was obtained with thermal pre-treated corn straw (Brémond et al., 2018).

Table 7-4:Evaluation of AD tests considering biogas yields and compositions, pH initial and final, FOS/TAC, TOC, TOC and weight removals

	Biogas	**CH_4**	**CH_4**	**CO_2**	**O_2**	**CO**	**H_2S**	**Balance**	**pHin**	**pHfin**	**FOS/TAC**	**TOCfin**	**TOC removal**	**Weight reduction**
	(NL/kgvs)	(NL/kgvs)	(%)	(%)	(%)	(ppm)	(ppm)	(%)	(-)	(-)	(-)	(mg/kg)	(%)	(%)
WAS	305.15	184.45	60.446	30	4.1		/	5.454	7.14	7.5	/	1658.13	80.47	/
CAS	393.23	252.87	64.306	30	4		/	1.694	7.82	8	/	1918.40	82.85	/
T0_1_WAS	762.5	493.08	64.666	30	2	37.38	/	3.334	7.39	7	0.35	1109190.78	84.95	10.25
T0_2_WAS	731.12	458.82	62.756	31	3	44.26	/	3.244	6.81	7.2	0.36	1960706.20	82.27	8.5
T0_3_WAS	708.12	442.76	62.526	32.86	3	36.53	/	1.614	6.62	7.01	0.45	2782886.64	80.69	7.75
T0_1_CAS	782.58	516.55	66.006	31.8	1.8	35.09	/	0.394	7.2	7.3	0.47	981022.60	85.73	7.5
T0_2_CAS	755.89	490.09	64.836	31.47	2.5	48.46	/	1.194	7	7.19	0.43	1873071.16	83.44	6.5
T0_3_CAS	748.89	462.33	61.736	35	3	49.1	/	0.264	6.5	7	0.45	2576427.56	82.53	9
T5_1_WAS	818.52	505.85	61.8	30	2.7	46.76	/	5.5	7.41	7.9	0.45	1107305.67	84.98	8.5
T5_2_WAS	835.95	524.98	62.8	31.9	3.7	43.36	/	1.6	7.34	7.6	0.37	1309618.76	88.15	10.25
T5_3_WAS	932.06	601.18	64.5	30.96	0	42.26	/	4.54	7.3	7.12	0.44	1496524.07	89.49	11
T5_1_CAS	840.49	536.40	63.82	35	0	35.36	/	1.18	7.3	7.3	0.48	1281703.76	82.62	9
T5_2_CAS	859.49	558.67	65	29	0	36.4	/	6	7	7	0.38	1403361.13	87.31	10.5
T5_3_CAS	948.68	641.02	67.57	32.08	0	35.36	/	0.35	6.9	8	0.41	1452442.57	89.80	11.5
T10_1_WAS	810	549.99	67.9	31.3	0	39.93	/	0.8	6.4	6.4	0.47	1080333.22	85.67	9.5
T10_2_WAS	890.96	607.63	68.2	31.5	0	42.6	/	0.3	6.29	7.56	0.37	1179055.18	89.33	10.5
T10_3_WAS	994.2	686.00	69	30.3	0	37.6	/	0.7	6.03	6	0.39	1410470.37	89.98	11.85
T10_1_CAS	835.23	570.55	68.31	31	0	33.6	/	0.69	7.11	7.4	0.45	1004441.64	86.38	9.75
T10_2_CAS	892.2	623.20	69.85	30.02	0	34.64	/	0.13	7	8	0.37	1398578.93	87.35	11.13
T10_3_CAS	997.81	698.47	70	29	0	35.9	/	1	6.9	7.9	0.44	1396306.75	89.96	11.75

7.3.3 Kinetic analysis

In order to analyze the AD tests, a kinetic study was performed considering two models: first-order kinetic and Gompertz modified model (Table 7-5). The graphic of Gompertz modified model is reported in Figure 5A and 5B of Appendix. The first-order kinetic Angelidaki et al.(2009) defines the disintegration rate (k_{dis}). The highest k_{dis} values are obtained in the following order: T10_3_CAS (k_{dis}=0.108) > T10_3_WAS (k_{dis}=0.107) > T10_2_WAS (k_{dis}=0.103) > T10_2_CAS (k_{dis}=0.09). It is worth to notice that for T0 (inoculum cultivation period equal to 0 d) performed with both inocula, WAS and CAS, the k_{dis} values decreased by increasing the S:I ratio. Otherwise, for T5 and T10 (inoculum cultivation periods respectively equal to 5 and 10 d) the k_{dis} values increased by increasing the S:I ratio. Cultivated inocula gave the possibility to raise the S:I ratio, preventing possible inhibitory effects J. Zhang et al.(2019b). In the present study, the highest k_{dis} values reached agreed with the k_{dis} values of thermal pretreated agricultural waste with S:I=1:2 of (Pellera and Gidarakos, 2016).

Considering Gompertz modified model, the lag phase and maximal net biogas yield were defined. This model proved that by increasing the cultivation period of the inoculum and the S:I ratio the lag phase was reduced from 7-8 d to 3-4 d, which meant a reduction of the AD process time. AD in mesophilic condition is generally carried out for 25-30 d, whereas in the present study it has been proved that the AD can be performed for 14-18 d, in detail the following values are obtained (inoculum cultivation period of 5 and 10d and S:I ratios equal to 1:1 and 2:1): T10_2_WAS (14 d) and T10_3_WAS (18 d) T5_2_CAS (19 d), T5_3_CAS (18 d), T10_2_CAS (14 d) and T10_3_CAS (18 d).

These results allowed to reduce the energy consumption (see section 7.3.4) and to perform more run of AD. In the literature, Gompertz modified models for cultivated inocula are not available, but only for AD performed with not cultivated inocula at different S:I ratio. The Gompertz modified model for configuration T0 was confirmed by Frunzo et al., (2019) and Da Silva et al.(2018): for not-cultivated inocula the lag phase increased by increasing the S:I ratio.

Table 7-5: Evaluation of kinetics of all AD configurations.

	1°kinetic		**Gompertz modified model**				
	k_{dis} (1/d)	**R^2**	**λ (d)**	**Biogas th (NL/kg VS)**	**Biogas exp (NL/kg VS)**	**Dev.s t.t**	**Difference (%)**
WAS	0.053	0.998	6	362.49	305.15	0.001	15.82
CAS	0.08	0.989	7	516.19	393.23	0.01	23.82
T0_1_WAS	0.087	0.999	12	789.67	762.5	0.01	3.44

T0_2_WAS	0.045	0.989	8	925.15	731.12	0.01	20.97
T0_3_WAS	0.044	0.999	6	871.19	708.12	0.02	18.72
T0_1_CAS	0.088	0.984	8	858.81	782.58	0.02	8.88
T0_2_CAS	0.064	0.988	4	837.8	755.89	0.003	9.78
T0_3_CAS	0.08	0.998	4	889.34	748.89	0.002	15.79
T5_1_WAS	0.081	0.999	7	877.94	818.52	0.001	6.77
T5_2_WAS	0.05	0.999	4	951.69	835.95	0.003	12.16
T5_3_WAS	0.074	0.989	3	1007.66	932.06	0.04	7.5
T5_1_CAS	0.078	0.987	5	1015.32	840.49	0.004	17.22
T5_2_CAS	0.045	0.999	4	991.26	859.49	0.001	13.29
T5_3_CAS	0.055	0.999	3	1027.85	948.68	0.01	7.7
T10_1_WAS	0.083	0.999	4	973.86	810	0.001	16.83
T10_2_WAS	0.103	0.999	3	964.85	890.96	0.005	7.66
T10_3_WAS	0.107	0.987	3	1028.01	994.2	0.002	3.29
T10_1_CAS	0.044	0.989	4	905.15	835.23	0.004	7.72
T10_2_CAS	0.099	0.987	4	931.45	892.2	0.006	4.21
T10_3_CAS	0.108	0.998	3	1125.33	997.81	0	11.33

7.3.4 Energy Evaluation

To complete the study of AD tests, the energy evaluation was carried out and the energy sustainability index (ESI) was calculated and reported in Table 7-6.

The configuration energetically sustainable had ESI value equal o higher than one and are depicted in Table 7-6. Thus, the AD configurations energetically sustainable were: T10_3_CAS (ESI=1. 83) T10_2_WAS (ESI=1.82), T5_3_CAS (ESI=1.74), T10_2_CAS (ESI=1.58), T10_2_WAS (ESI=1.57), T5_3_WAS (ESI=1.46), T5_2_CAS (ESI=1.12). The energy produced by T5 and T10 were in line with the energy production of thermophilic AD of agricultural waste (Strübing et al., 2017) and (Strübing et al., 2018). This trend proved the benefit of inoculum incubation, which can represent an innovative form of pre-treatment. Conventionally pre-treatments were performed on the organic substrate undergone to AD, in the present study, the pre-treatment was the inoculum incubation.

Table 7-6: Energy evaluation in terms of Qs, Qp and energy sustainability index ESI

	Qp (kWh)	Qs (kWh)	ESI
T0_1_WAS	33.20	66.00	0.50
T0_2_WAS	47.76	68.64	0.70
T0_3_WAS	61.69	73.92	0.83
T0_1_CAS	34.08	66.00	0.52
T0_2_CAS	49.37	58.08	0.85
T0_3_CAS	65.24	68.64	0.95
T5_1_WAS	35.64	55.44	0.64
T5_2_WAS	54.60	66.00	0.83
T5_3_WAS	81.19	55.44	1.46
T5_1_CAS	36.60	50.16	0.73
T5_2_CAS	56.14	50.16	1.12
T5_3_CAS	82.64	47.52	1.74
T10_1_WAS	37.00	58.08	0.64
T10_2_WAS	58.19	36.96	1.57
T10_3_WAS	86.61	47.52	1.82
T10_1_CAS	36.85	44.88	0.82
T10_2_CAS	58.27	36.96	1.58
T10_3_CAS	86.92	47.52	1.83

7.3.5 Sensitivity analysis

The statistical analysis of the AD tests was carried out by using data analysis extension of Excel 2016; in detail, Pearson and ANOVA (Analysis-Of-Variance)

tests were performed to evaluate linear correlation among the different configuration of AD and considering significant the ones with 0.05.p

Multi Criteria Decision Aid (MCDA) analysis was performed with ELECTRE II for ranking the 18 AD configurations from the best to the worst and the graphical outranking is reported in Figure 7-5.

The following configurations were equally ranked in the first position: T5_3_CAS, T10_3_WAS, T10_2_CAS and T10_3_CAS. They were followed in the second position by T10_2_WAS. The MCDA analysis confirmed that higher cultivation period of inoculum allowed to reach the best performances in AD mesophilic process.

The highest weight in the MCDA analysis was given by the ESI values since energy sustainability established the cut off to scale up the tested AD at the industrial level.

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Figure 7-5: Outranking of AD configuration performed by ELECTRE II

Rank	Configuration
1	T5_3_CAS
	T10_3_WAS
	T10_2_CAS
	T10_3_CAS
2	T10_2_WAS
3	T5_3_WAS
4	T10_1_WAS
	T10_1_CAS
5	T5_2_CAS
6	T5_2_WAS
7	T0_1_CAS
8	T5_1_WAS
9	T0_1_WAS
	T5_1_CAS
10	T0_2_CAS
11	T0_2_WAS
12	T0_3_WAS
	T0_3_CAS

7.4 Conclusions

AD was performed on OFMSW with two inocula, coming from mesophilic digestate: wastewater sludge (WAS) and cow-pig manure (CAS). Both inocula were anaerobically cultivated for three periods: 0, 5 and 10 days and then inoculated in OFMW at three S:I ratio: 1:2; 1:1; 2:1. Increasing cultivation period of inocula (WAS and CAS), S:I ratio can increase reaching biogas yields ranged 818.0-997.4NL_{biogas}/kgvs, with ESI higher than 1. The most promising AD configurations

were: AD performed with S:I=2:1 with CAS cultivated for 5day, AD performed with S:I=1:1 and 2:1with CAS cultivated for 10day and AD performed with S:I=2:1 WAS cultivated for 10day

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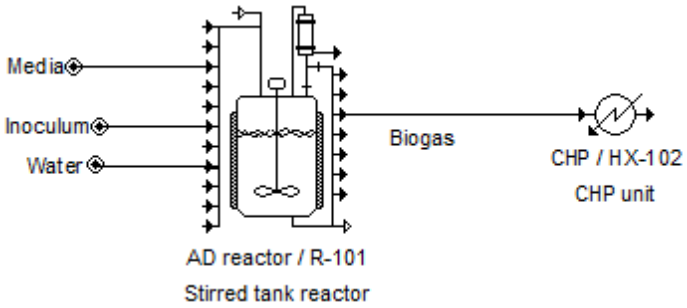
Appendix A

Figure1: Anaerobic digestion modeled with Super Prodesign **(**CHP=cogeneration heat and power)



Appendix B

Figure 2: TOC removal during AD_WAS (a), AD_CAS(b) and AD of the two inocula WAS and CAS (c)

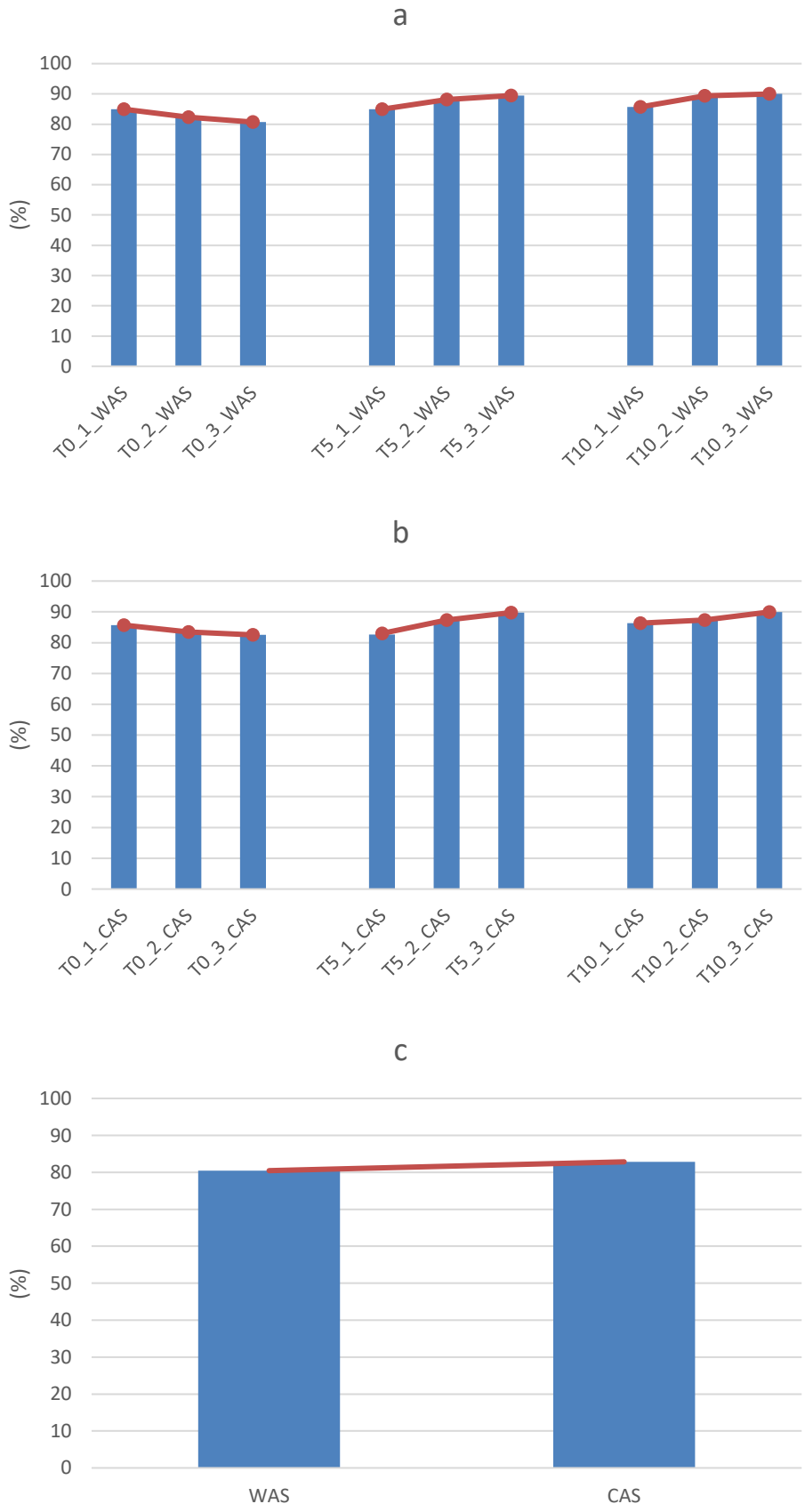


Figure3: Evaluation of FOS/TAC trends od AD_WAS (a) and AD_CAS(b)

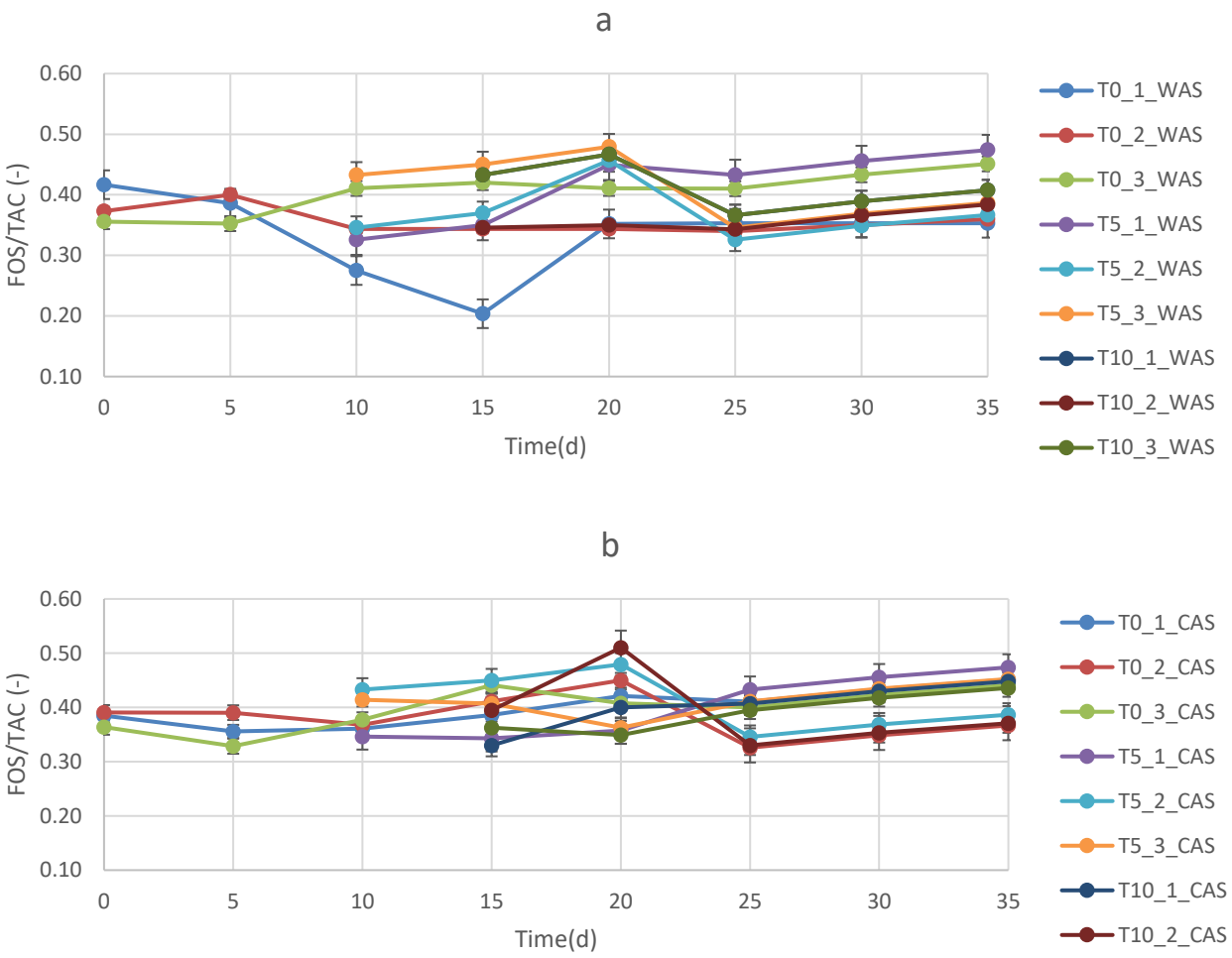


Figure4: Evaluation of pH trends od AD_WAS (a) and AD_CAS(b)

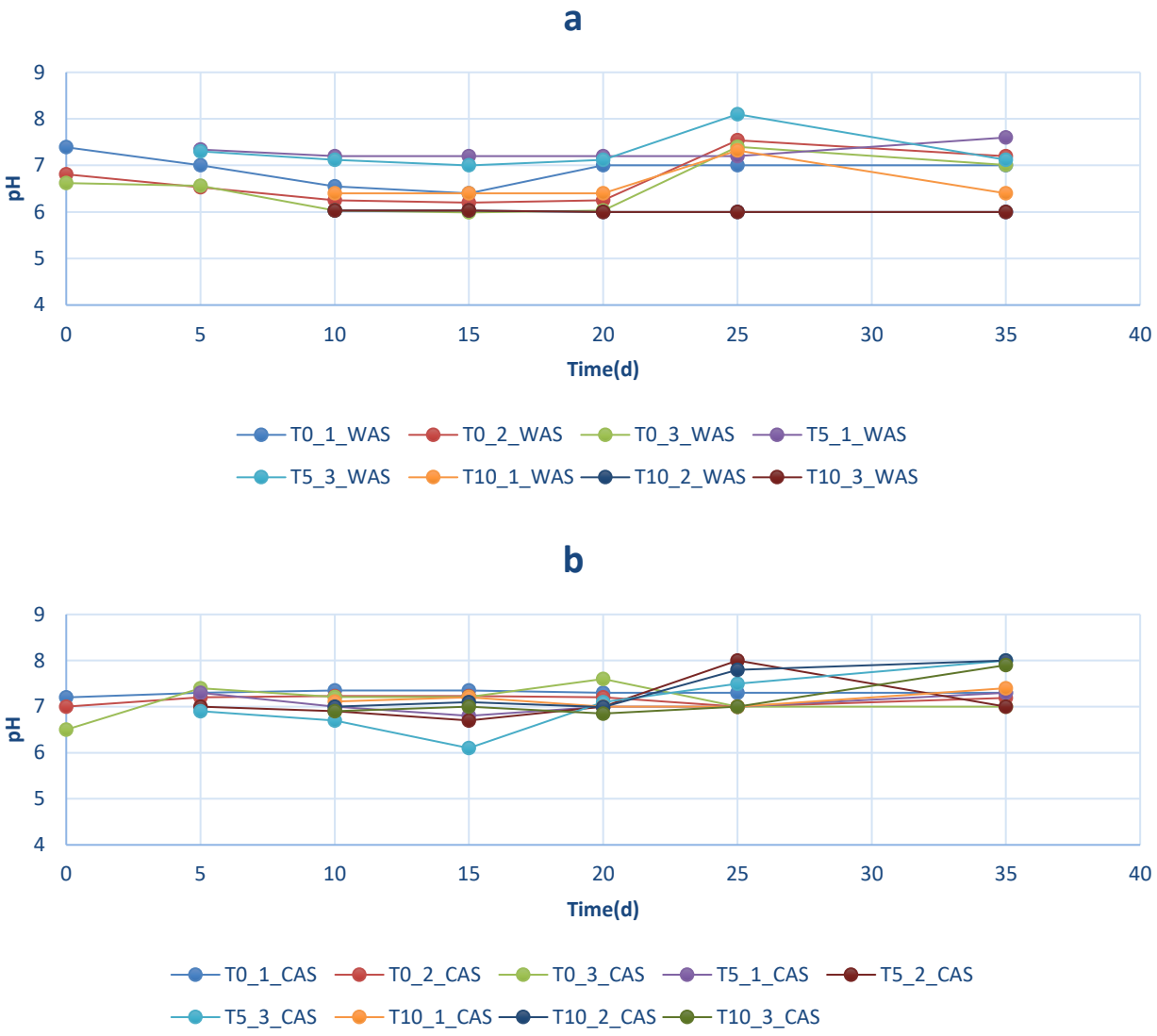
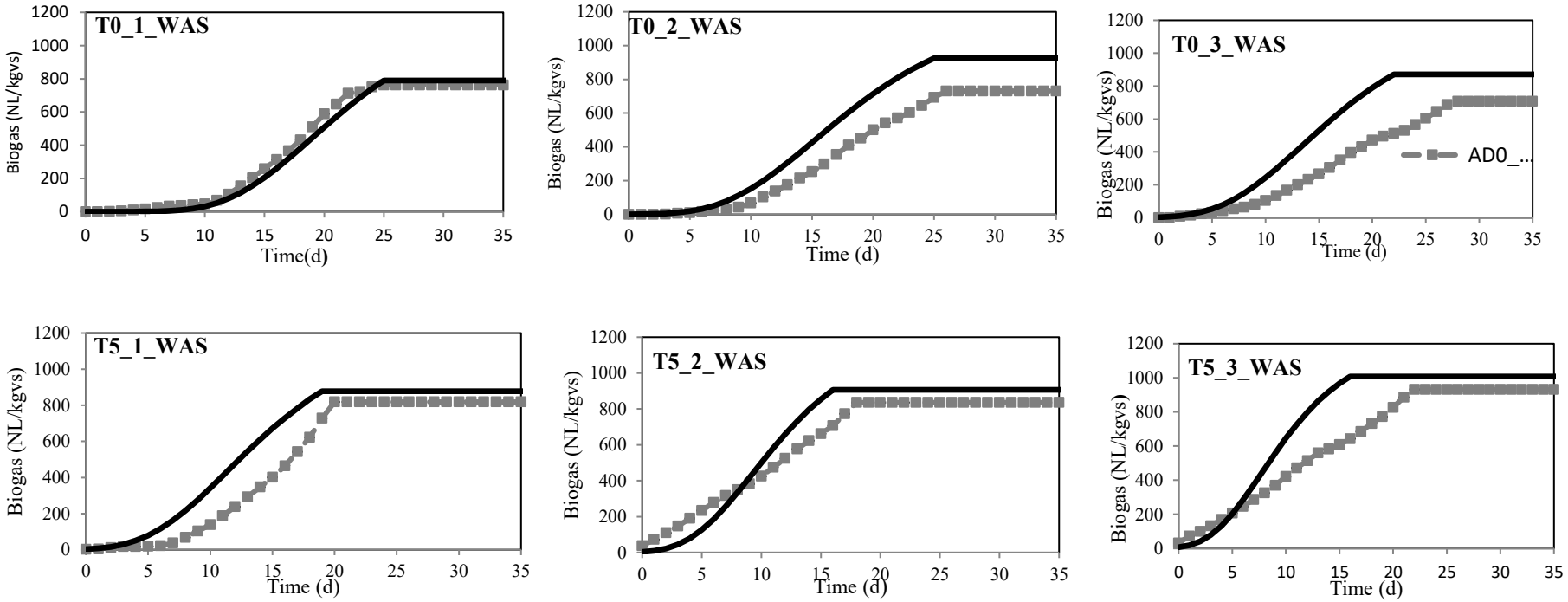
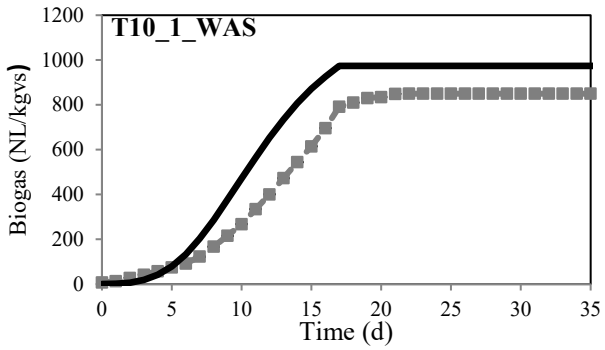
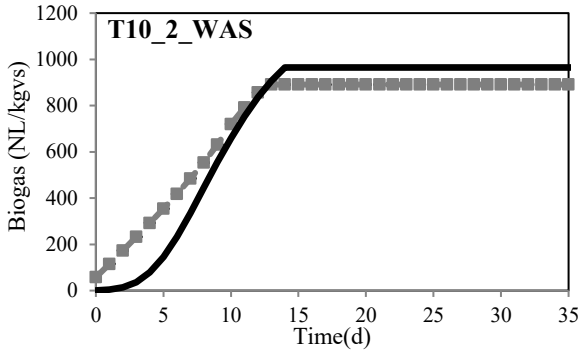


Figure5a: Gompertz modified model to evaluate the lag phase and net biogas yield (black line) compared to experimental net biogas yield (dot-line) of all the AD configurations tested with inoculum WAS




T10_1_WAS
Biogas (NL/kgvs)
Time (d)
0
200
400
600
800
1000
1200
0
5
10
15
20
25
30
35


T10_2_WAS
Biogas (NL/kgvs)
Time(d)
0
200
400
600
800
1000
1200
0
5
10
15
20
25
30
35

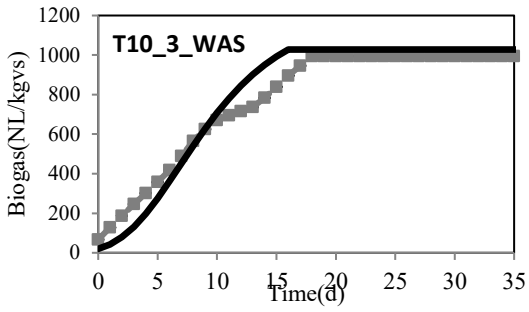
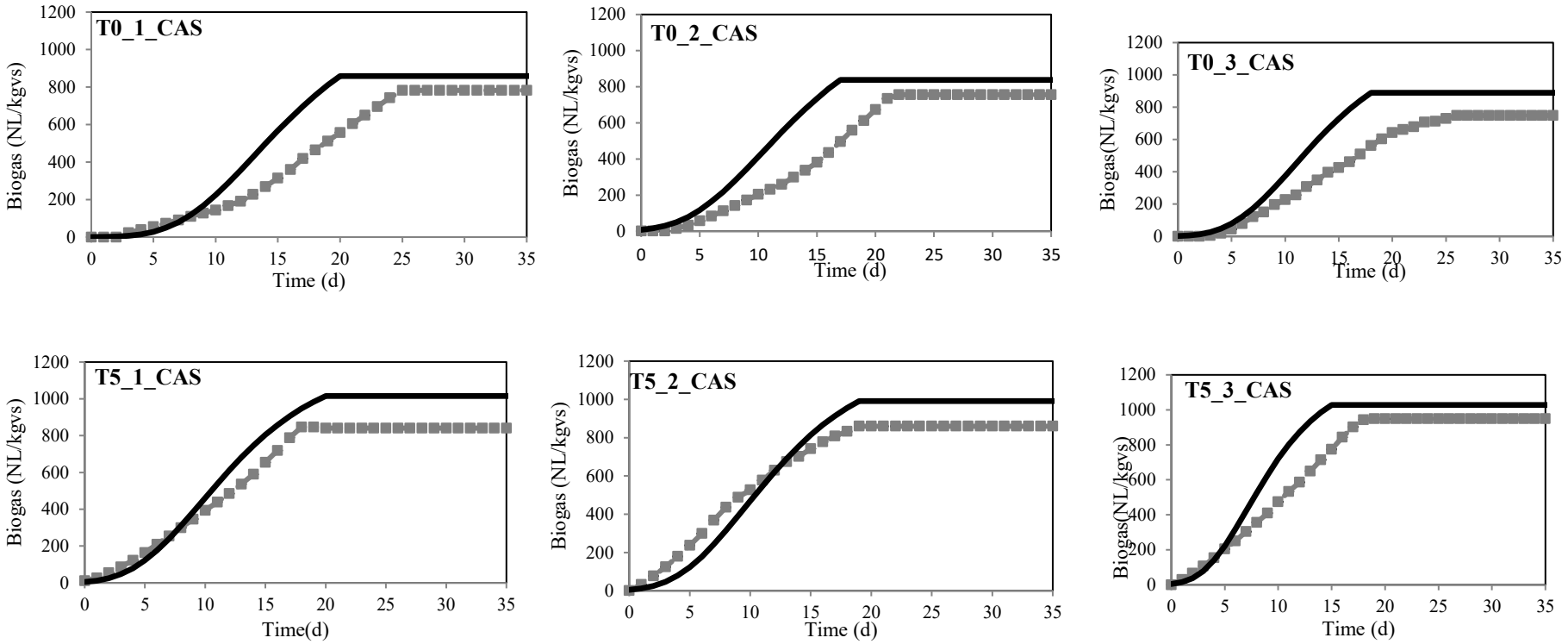
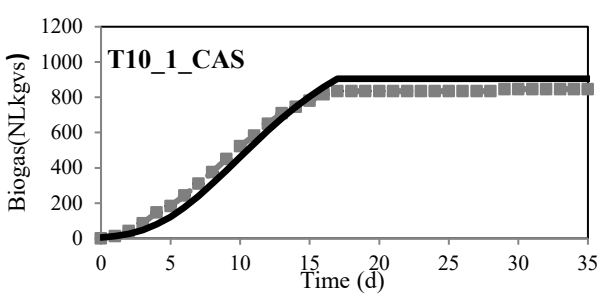
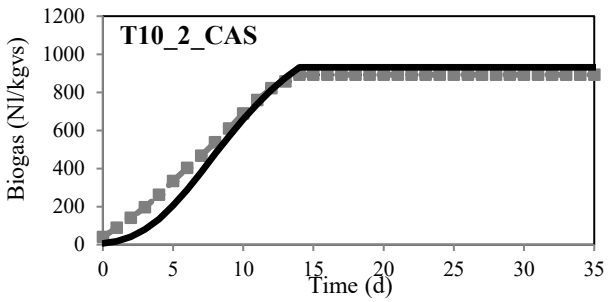
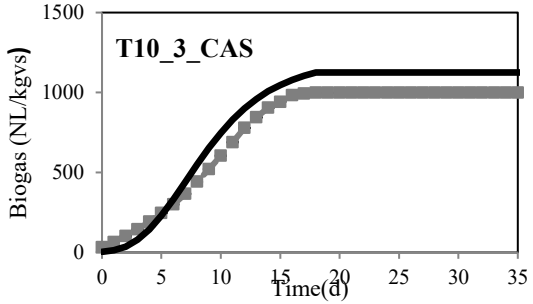

T10_3_WAS
Biogas(NL/kgvs)
Time(d)
0
200
400
600
800
1000
1200
0
5
10
15
20
25
30
35

Figure5b: Gompertz modified model to evaluate the lag phase and net biogas yield (black line) compared to experimental net biogas yield (dot-line) of all the AD configurations tested with inoculum CAS




T10_1_CAS
Biogas(NL/kgvs)
Time (d)
0
200
400
600
800
1000
1200
0
5
10
15
20
25
30
35


T10_2_CAS
Biogas (Nl/kgvs)
Time (d)
0
200
400
600
800
1000
1200
0
5
10
15
20
25
30
35


T10_3_CAS
Biogas (NL/kgvs)
Time(d)
0
500
1000
1500
0
5
10
15
20
25
30
35

Conclusions:

The dependency on non-renewable resources represents an environmental, economic and social problem, globally affecting the planet with emissions of greenhouse gases (GHGs) and resulting in increased fuel extraction costs. The dependence on fossil non-renewable fuels is bound to increase, unless something changes, due to the increased demand for energy and chemicals induced by the fast world population growth. The production of biobased products and bioenergy should be considered as a unit of an integrated value chain of processes in the overall green bioeconomy.

The EU Green Deal (EU-Green Deal, 2019) is a European political initiative promoting a new growth strategy aimed at transforming the EU into a fair and resource-efficient economy, where emissions of greenhouse gases in 2050 decrease of 30% and economic development is decoupled from resource use. Furthermore, the Green Deal enhances the EU's natural capital and protect the health and well-being of citizens from environment-related risks and impacts. The bioeconomy, a circular economy powered by nature and emerging from nature, is based on renewable biological resources and sustainable biobased solutions. Bioeconomy is fundamental for moving towards a carbon neutral EU reality and fossil free material and energy scenarios. This change of perspective promotes the shift from a Linear to a Circular structure of industrial processes, where by-products and wastes can become new secondary raw materials. To implement this transformative policy, bioeconomy promotes the concept and realisation of biorefinery. Biorefinery can represent the catalyst for systemic change to tackle holistically the social, economic and environmental perspectives. The biorefinery builds a new and synergistic relationship between technology and nature, between ecology and economy growth and belongs to Green Chemistry or Sustainable Chemistry.

Based on new biotechnological approaches, the bioeconomy maximizes the use of waste and resources, both biological, terrestrial and marine, as well as non-biological, CO_2 and fossil waste streams, as inputs for industrial and energy production, implementing a circular logic management to maximise opportunities of reuse, recycling and recovery (OECD, 2020).

Recently, Europe joined the 2030 Agenda for Sustainable Development Goals (SDGs, 2019), which established new targets in climate change and energy-production to ensure greater competitiveness, safety and stability of energy systems. The target defined by 2030 Agenda for Sustainable Development are: 1) GHG reduction equal to 40% of the levels of 1990, 2) at least 27% of the used energy must come from renewable energy and 3) 27% energy savings compared to current situation. To achieve these targets, biorefinery system plays a key role. Biorefinery enables the realization of Green Chemistry at the full scale, optimizing the supply chains of enhancement of biomass, ad hoc and waste, CO_2 and fossil

waste stream, in local contexts developing integrated technology platforms and cascading use schemes.
The biorefinery process is like the petrochemical refining, but the crucial difference is the nature of the starting material; because for biorefinery is biomass, a renewable matter, for the petrochemical refinery is coal and petroleum, namely fossil resources.
Biorefinery is classified on the ground of biomass origin in first generation (1G), second (2G) and third (3G) generation biorefinery, respectively feed with ad hoc biomasses, waste biomasses and algae. This thesis focuses the attention on 2G-biorefinery for ethical, environmental, economic and social reasons.

In the present thesis two processes are considered: fermentation for L(+) Lactic acid (LA) production and anaerobic digestion (AD) for biogas production.

The study starts with the analysis of 2G-biorefinery system in EU28 and its three fundamental units: the feed biomass, the corresponding process and the resulting products. The aim is the realization of three data inventories: 1) biomass available in EU28, 2) process technical-economic-environmental feasibility and 3) generable high-added value products. The study combines bottom up and top down approaches, aimed respectively to evaluate how the fundamental units are interlaced and influenced each other and to define a sustainable biorefinery system.

According to the European Technical Guidance waste classification (2018/C 124/01) and Eurostat database, four biomass categories are evaluated: wastewaters and sewage sludge, municipal solid waste, waste from agriculture, forestry and fishing activities and waste from manufacturing of food and beverage products. 2G-biorefinery faces social, economic, environmental and technical problems due to the huge amount of biomasses, considering biomass as secondary raw material to valorize through platform chemical and energy production. 14 biomasses are studied and these 14 biomasses are the most representative of the four biomass categories, which have carbon content over 50% w/w and belong to carbohydrate, lipids and lignocellulose feedstock groups respectively for 43 %, 36% and 14%. The correlation biomass-process stated that lignocellulose biomasses are suitable for thermochemical, chemical and biological processes, while carbohydrate and lipid biomass are respectively suitable for biological and chemical processes. The correlation biomass-process-product assesses that among the 11 analysed platform chemicals, ethanol, propionic acid, lactic acid and succinic acid have the highest yield through biological processes, allowing 14-57.22 %. market size satisfaction and 9% to 36%, biomass valorisation with consequentially waste reduction. Among the 5 considered bio-energies, biogas is the only one able to satisfy completely the market size with a surplus of 11%. The achieved results prove: 1) the fundamental contribution of biomass to chemical and energy sectors and 2) biogas fundamental role in biorefinery system. Thus, the present study focuses the attention on Lactic acid (LA) and biogas production by means of biological routes, fermentation and anaerobic digestion respectively. Before starting the analysis of LA and biogas productions, a focus on biowaste management in Italy is investigated and described. In particular, a methodology for the technical and environmental assessment of biowaste valorisation in 2G-biorefineries in Italy. Italy is chosen as case study, considering years 2016-2019. Italian context is evaluated through the following key parameters: 1) gross domestic power, 2)

climate, 3) demography and 4) population density distribution. The evaluations of geo-localisation and quantitative availability of biowaste amounts aimed to define the dimension and localisation of the biorefinery plant to optimise the supply and transport chains, while the qualitative characteristic aimed to evaluate the most promising process among two different biorefineries systems: thermo-valorisation (TH) and anaerobic digestion (AD). The main finding of the study witness that AD is more sustainable energetically than TH.

Then, the thesis investigates two process to produce L (+)Lactic acid (LA) from the organic fraction of municipal solid waste (OFMSW): the simultaneous saccharification and fermentation (SSF) and separated hydrolysis and fermentation (SHF). The study was carried out at labour and technical scale, with the support of modelling by SuperPro Designer® 8.0. The aim of the study is the optimisation of SSF and SHF. In detail, for SFF the analysis includes 1) the identification of the most suitable LA strain producers: three types of *Lactobacillus sp*. and one type of *Streptococcus sp*. strains, 2) the evaluation of the necessity of autoclavation of the OFMSW and 3) the production of market value L (+)- LA. For SHF the analysis includes: 1) type and loading of enzyme and 2) solid to liquid ratios.

OFMSW is employed as source of carbon and nitrogen to carry out SSF by using for L (+)-LA production.

In SHF two enzymes are tested: Stargen and Fermgen to hydrolyze starch and proteins. Hydrolytic performance is investigated according to different solid-to-liquid ratios.

Lactobacillus sp. strains does not show an efficient conversion of OFMSW into LA. Whereas*, Streptococcus sp*., liquefies the material and produced LA.

For SSF process the maximum productivity of 2.16 g/Lh is achieved at technical scale, while the highest yield of 0.81g/g of theoretically present sugars is obtained in SSF carried out at solid to liquid ratio of 5w/w.

The LA concentration achieved from 20%w/w of bended OFMSW is 58g/L. Both under sterile and not sterile conditions SSF carried out with *Streptococcus sp* A620 directly convertes OFMSW into LA without considerable production of other acids. At technical scale (72L) SSF is implemented and the downstream processing including micro- and nanofiltration, electrodialysis, chromatography and distillation produced a pure 702 g/L of L (+)-LA formulation with an optical purity (OP) of 97%.

For SHF process the hydrolysis is carried out for 1h with Stargen and sequential LA concentration after 29 hours, is 0.33 g_{LA} /g dry OFMSW with a productivity of 3.38 $g_{LA}/L \cdot h$

Furthermore, L(+) Lactic acid production is investigated from spent coffee ground (SCGC). In detail, the acid-enzymatic hydrolysis and fermentation of L (+)-lactic acid (LA) with *Bacillus Coagulans* from spent coffee ground (SCGC) is studied. SCGC, a lignocellulose residue from coffee production consists of 34.26 ± 2.67% cellulose, 7.31% ± 2.54% hemicellulose and 24.88 ± 0.11% of lignin. Sequential and combined acid-enzymatic hydrolysis are carried out respectively, at 121°C for 15 min with 1%v/v H_2SO_4 and 14.5% SCG wet and at 52°C for 24h with 0.25 mL Accellerase 1500 per gram of dry SCG, achieving a total sugar extraction efficiency of 41.24 ± 4.53%.

Fermentations are carried out both at the laboratory (2L) and technical (50L) scales and no scale effect is observed.

At 72L scale, LA yield per gram of sugar consumed and per dry gram of SCG were 0.956 ± 0.015, 0.18 ± 0.63 respectively. Downstream processing results in 786.70 gLA/L and 99.5% optical purity.
After the evaluation of L(+)Lactic acid from OFMSW and SCCG carried out by fermentation route, the thesis investigates the sequential production of L(+)-LA and biogas from organic fraction municipal solid waste (OFMW).
LA is produced from OFMW using a *Streptococcus sp.* strain A620 (optimized at the begging of the study in this thesis) by means of two fermentative pathways: separate enzymatic hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF). Via SHF a yield of 0.33 g_{LA}/g_{FW} (productivity 3.38 $g_{LA}/L{\cdot}h$) and via SSF 0.29 g_{LA}/g_{FW} (productivity 2.08 $g_{LA}/L{\cdot}h$) are reached. Fermentation residues and OFMSW are tested as feedstocks for anaerobic digestion (AD) (3 wt% TS). The following biogas yields are achieved: 0.71, 0.74 and 0.90 Nm^3/kg_{VS} for OFMSW and residues from SFF and SHF respectively.

The innovation of the approach consists in considering the conversion of OFMSW into two different sequential products through a biorefinery system, therefore making economically feasible L(+)-LA production and valorising its fermentative residues.
A economic and energy analysis is performed to complete the technical study of L(+)-LA and biogas productions in singular and combined process from OFMSW and SCG
Four scenarios are evaluated and compared: **Scenario IA** exclusive fermentative production of LA by means of simultaneous saccharification and fermentation (SSF), **Scenario IB** LA production carried out with separated hydrolysis and fermentation (SHF), **Scenario II** exclusive biogas production by means of anaerobic digestion. **Scenario III A-B** for sequential fermentative LA production and biogas by means of SSF and SHF from OFMSW. **Scenario IV** LA production by means of SHF from SCG. The integrated biorefinery process is compared to single processes for either L(+)-LA or biogas production. The economic evaluation, considering catchment areas from 2000 to 1 million inhabitants, is based on data from real biorefinery plants and carried out using SuperPro Designer® 8.0. The consistency of the approach is assessed through a set of composite indicators. The integrated biorefinery system is investigated from three main perspectives: 1) economic feasibility of producing LA and biogas, 2) the effect of process scale and 3) energy consumption/requirement. The present study proved that an integrated biorefinery system contributes more to optimal use of energy and material flows than single processes both for the sequential production of two market value products and optimisation of waste management. Profitability was achieved for catchment areas bigger than 20,000-50,000 inhabitants.

Finally, the present thesis focused the attention on the optimisation of AD. I detail, the key role of inoculum in mesophilic anaerobic digestion (AD) of organic fraction municipal solid waste (OFMW) was studied. Two inocula are tested, one coming from the mesophilic digestate of wastewater activated sludge (WAS) and

the other one from the mesophilic digestate of cow-agriculture sludge (CAS). Both inocula are anaerobically cultivated for three different periods: 0, 5 and 10 days and then inoculated in OFMW considering three substrate-inoculum ratios (S:I) 1:2; 1:1; 2:1. First order kinetics and Gompertz modified model are applied to define disintegration rate, lag phase and maximum biogas yields. Energy sustainability index was calculated to define which configurations were suitable to be scaled-up. Then multi criteria decision aid was performed to outranking the AD configurations tested. The AD configurations with the best performances are: AD performed with S:I=2:1 with CAS cultivated for 5 days, AD performed with S:I=1:1 and 2:1with CAS cultivated for 10 days and AD performed with S:I=2:1 WAS cultivated for 10 days

The present thesis is developed according the Circular Economy pillars: technical feasibility environmental sustainability and economic profitability and according to SGDs goals to promote the passage from Linear to Circular Economy. The main finding of the present study is the valorization of organic waste, from negative concept of waste to second renewable source to produce high added value product as L(+) Lactic acid and bioenergy as biogas.