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Microbial conversion of organic residues into acid rich process liquids and their use in bio-electrochemical systems

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Microbial conversion of organic residues into acid rich process liquids and their use in bio-electrochemical systems

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Content

Abbreviations

AD	anaerobic digestion
AF	anaerobic filter
BES	bio-electrochemical systems
CHP	combined heat and power
CH_4	methane
COD	chemical oxygen demand
CO_2	carbon dioxide
g	gram
kg	kilogram
kW	kilowatt
L	liter
MEC	microbial electrolytic
MET	microbial electrochemical technology
MFC	microbial fuel cell
m^2	square meter
m^3	cubic meter
mA	milliamp
mW	milliwatt
oDM	organic dry matter
OFMSW	organic fraction of municipal solid waste
ORP	oxidation reduction potential
VFA	volatile fatty acids
TC	total carbon
TN	total nitrogen
TOC	total organic carbon
TWh	terawatt hour
W	watt

1 General introduction

1.1 Waste availabilities

Worldwide, 2.01 billion tons of municipal solid waste had been generated in 2016, which amounts to an average waste generation of 0.74 kg/day per person. The national waste volumes vary widely from 0.11 to 4.54 kg/day per person [1]. However, the global waste is expected to grow to 3.40 billion tons by 2050 [1]. There is a strong positive general correlation between waste generation and income level, where 34% of the world's waste is generated by high-income countries [2]. Predicting that by 2050, daily per capita waste generation is to be increased by 19% in high-income countries and approximately 40% or more in low-income and middle-income countries [1]. Worldwide, most of the waste is currently disposed or dumped of in some form of a landfill. Roughly 37% of waste is disposed in a landfill, 8% of which is disposed of in sanitary landfills with landfill gas collection systems. Open dumping accounts for about 33% of waste, 19% recovered through recycling and composting and 11% is incinerated for final disposal [1].

Based on the generated volume of waste, its composition, and the way it is managed, it is estimated that 1.6 billion tons of carbon dioxide (CO_2) equivalent greenhouse gas emissions were generated from solid waste treatment and disposal in 2016 [1]. This is about 5% of global greenhouse gas emissions, mainly driven by food waste and improper waste management by open dumping and disposal in landfills without gas capture systems. Solid waste related emissions are anticipated to increase to 2.6 billion tons of CO_2-equivalent per year by 2050 if no improvement steps are made in the sector [1]. Even basic systems improvements of waste management can reduce these emissions by 25% or more [1]. The greenhouse gas emissions can be mitigated through improved waste collection, waste reduction, reuse of products, recycling, organic waste management, and energy recovery [1].

1.2 Sustainable waste management

There are many ways to treat the organic fraction of municipal solid waste (OFMSW) and extract energy out of it as an alternative to fossil fuels. A sustainable waste management system first requires a reduction in the amount of waste generated and a separate collection of the organic fractions for composting and waste to energy concepts [2, 3]. The best example for this is Germany, where about 15.6 million tons of biowaste were treated biologically in 2016 and the volume of separately collected biodegradable waste increased further in 2017 [4].

This OFMSW in Germany mainly included biowaste and green waste from households, municipal solid organic waste from park and lawn maintenance as well as food waste. A large proportion (app. 51.3%) of the biogenic waste has been composted, but the energy it contains cannot be used [4]. Composting of OFMSW is resulting in 4.4 million tons of compost per year. Also, the share of biowaste which is used energetically via anaerobic digestion has been increasing for some years, thus leading to 3.6 million tons of fermentation residues used as fertilizer in agriculture [4]. There are currently around 9,523 biogas plants in Germany as of July 2019 (204 more than in 2018) [5]. By 2019, in Germany biowaste has been used as feedstock in 400 waste digestion plants, and in 2019 alone, two million tons of source-separated organic waste from households were utilized by 135 of these plants [6]. In 2018, around 51.3 TWh of electricity was provided from biomass in Germany [7]. The most important factors for electricity generation from biomass include biogas (29.5 TWh), solid biomass (10.7 TWh) and the biogenic share of waste (6.2 TWh) [7]. In Germany, 12.8% of electricity generated from renewable energies in 2018 was from biogas [7]. The anaerobic digestion (AD) systems operated with biodegradable wastes including energy crops in Germany produced 626 million m^3 of biogas in 2017 [4].

In the European Union (EU), around 110 million tons of biowaste are produced every year of which only around 20% is collected and recycled separately [8]. The EU has defined that waste to energy technology is able to create synergies with the EU energy and climate policy, without compromising the achievement of higher reuse and recycling rates [9]. In many countries of the world, however, organic waste is still disposed of in landfills or incinerated together with other combustible municipal wastes. However, these two approaches are facing more and more economic and environmental stresses. Due to its organic- and nutrient-rich composition, especially food waste can be utilized easily as a useful resource for production of biofuel through various fermentation processes targeting different forms of end products [10].

1.3 Waste to bioenergy production

There is a variety of ways of turning the OFMSW into energy [11]. Wastes are convertible to useful energy forms like hydrogen (biohydrogen), biogas, bioalcohol, biodiesel, bioethanol, bioelectricity etc., through waste-to-energy technologies [4, 11–15]. These conversion methods involve biological treatment, thermal treatment of waste, landfill gas utilization and biorefineries and the intense research that has been conducted in the last decade has resulted in improved yields of product or energy formation together with decreased environmental impact [15, 16].

AD is one of the important renewable energy technologies for OFMSW treatment aiming the production of biogas [17]. Two different process concepts based on anaerobic microbial conversion of biomass are used to recycle biowaste: single stage AD or two-stage AD [14, 18]. In single stage AD systems all the biochemical reactions take place in one reactor while in two-stage AD systems the hydrolysis/acidification and acetogenesis/methanogenesis processes are separated to different reactors [19]. Problems with biological process stability and control in conventional single-stage applications have led researchers to find new solutions [20]. Pohland and Ghosh [20] firstly proposed the physical separation of acid-formers and methane-formers in two separate reactors, where optimum environmental conditions for each group of organisms would be provided to enhance the overall process stability and control, thus increasing operation flexibility and enabling the use of a wide variety of substrates [21]. Additionally, two-stage hydrogen/methane fermentation has significantly greater potential for recovering energy than methane-only fermentation and is a very suitable process for producing hydrogen and methane simultaneously [22, 23]. Biogas from an anaerobic bio-digestion process can produce heat in a boiler, heat and electricity in combined heat and power (CHP) or vehicle fuel after upgrading and compressing [16].

In addition to these "traditional" microbial conversion systems, microbial electrochemical technology (MET) have become prominent and bio-electrochemical systems (BES) are described in literature as new systems for the conversion of renewable biomass and wastewaters into biohydrogen and bioelectricity. BES are broadly classified to microbial fuel cell (MFC), which focus on the production of electricity and microbial electrolytic cells for the production of fuels and chemicals (MEC) [24–28]. BES are characterized by the fact that microorganisms interact with electrodes and, in the course of their metabolic processes, take up electrons from or transfer them to electrodes. Until now, production of hydrogen peroxide, hydrogen, methane, ethanol, electricity and value added chemicals in BES have been described in the research [29–36]. Furthermore, bio-electrochemical systems can be used as biosensors or denitrification of wastewater [26, 36]. This new conversion technology is a potential source to produce renewable energy, which has not yet been exploited to its full potential. Significant progress has recently been made to increase the power output of MFC systems designed to convert organic wastes to electricity, but substantial additional optimization will be required for large-scale electricity production [29].

Furthermore, with BES systems production of some valuable biochemicals directly from organic wastes is the new research with high interest [37]. BESs are capable of energy and valuable organic and inorganic chemical production by removing wastewater, these systems are considered promising sustainable waste-to-energy/industrial chemical platforms. [38, 39]. Research shows that efficient hydrogen peroxide generation from organic matter in a bioelectrochemical system, which is a strong oxidant used for bleaching and a life-cycle analysis suggests that production of hydrogen peroxide in MECs is more sustainable than presently used routes [39-41]. The BES is able to perform a variety of value-added element conversion reactions, including production of electric energy from organic carbon, synthesis of chemicals from carbon dioxide, oxidation of sulfide into element sulfur, reduction of nitrate/nitrite into nitrous oxide and reduction of metal ions into solid metals and/or metal oxides [42].

1.4 Acidification of substrates

To make waste treatment possible in BES systems, it is necessary to disintegrate OFMSW to liquids rich in organics. Therefore, organic residues like lignocellulosic biomass has to be converted to soluble monosaccharides, volatile fatty acids (VFA) or other low-molecular-weight components prior to the addition into MFCs or MECs [43]. VFA are valuable intermediate products produced by AD of various substrates including waste streams such as primary sludge, waste activated sludge and food waste [44].

Hydrolysis, acidogenesis, acetogenesis and methanogenesis are the four major steps involved in the AD process [44–47]. In the two-stage AD procedure described by Pohland and Ghosh [20], the first stage features the formation of VFA, while the conversion of VFA to methane and carbon dioxide takes place in the second stage [48, 49]. pH is one of the most critical parameters that affects the VFA concentration and composition in the first stage since it influences both acidogenic process and hydrolysis rate [50, 51]. Anaerobic batch tests using food waste from a university canteen revealed that, in the acidic range of pH tested (4-6), improved VFA production was obtained at pH 6 [52, 53]. As a pretreatment step, the hydrolysate enriched with VFA achieved from waste in the first stage of two-stage AD can be fed either to an anaerobic filter (AF) for methane production or to BES for hydrogen, methane or bioelectricity production.

1.5 Membrane filtration

In addition to dissolved sugar compounds, alcohols and VFA, the hydrolysates also contain particles and fiber components. which can lead to technical problems. When using the process fluids in BES, these solid fibers can cause several problems (e.g. clogging) in long-term operation making a solid-/liquid separation necessary. Membrane filtration is one of the optimal methods to separate small particles from the liquid. Membranes have gained an important place in chemical technology and are used in a broad range of applications. Its basic principle is to allow one component of a mixture to permeate through the membrane freely, while hindering the permeation of other components [54]. The developed industrial membrane separation processes are microfiltration, ultrafiltration, nanofiltration, reverse osmosis, and electro dialysis. Although reverse osmosis, ultrafiltration, microfiltration and nanofiltration are conceptually similar processes, they differ in pore diameter of the membrane. Microfiltration porous membranes with diameters between 0.1 and 10 μm are used for the filtration processes to separate suspended particles [54]. The most widely used process designs are the dead-end or in-line filtration and cross-filtration method. In the dead-end method, the entire fluid flow is forced through the membrane under pressure. As particles accumulate on the membrane surface or in its interior, the pressure required to maintain the required flow increases, until at some point the membrane must be replaced or cleaned [54]. In cross-flow systems, the feed solution is circulated across the surface of the filter, producing two streams: a clean particle-free permeate and a retentate (also known as concentrate) containing the particles. The equipment required for cross-flow filtration is more complex, but the membrane lifetime is longer than dead-end filtration [54].

The filtration of hydrolysates can also be advantageous in two-stage AD systems since microfiltration enhances the methane production and protects the fixed bed of the methane reactor against blockages by particles [55]. According to Tuczinski et al. 2018 [56], for an undisturbed operation of two-stage high-pressure fermentation up to 100 bar, a particle-free hydrolysate called permeate appears to be necessary and this is even more important if the second stage, i.e., the methane reactor, is designed as pressurized fixed bed. Tuczinski et al. 2018 [56] investigations show that solid-liquid separation of hydrolysate from first-stage into permeate and concentrate with ceramic microfiltration membranes can be a reliable tool to avoid clogging and blocking in a fixed bed methane reactor. Furthermore, Tuczinski et al. 2018 achieved a sustainable membrane flux of up to 33 L/ (m^2 h) to produce permeate through crossflow filtration operated at thermophilic temperature [56].

1.6 Bio-electrochemical conversion

BES like MECs and MFCs are an emerging technology for converting organic matter into hydrogen, methane and other value-added products [57]. A basic BES is typically designed as a two-chamber system consisting of an anode and a cathode chamber separated by a proton exchange membrane (PEM) [58]. The provided substrate is anaerobically degraded by the microorganisms present in the anode chamber and the generated hydrogen protons pass through the PEM to the cathode chamber [59]. An MFC is a device that converts chemical energy into electrical energy as a result of oxidation of complex organic carbon sources which are utilized as substrates by microorganisms [60]. Further it was found that the production of electrical power is not necessarily the only outcome of a BES. By adding power to the system, it was shown that hydrogen could also be produced at the cathode, which is referred as a MEC [27]. Despite of hydrogen and bioelectricity being the important products of BES, novel research findings with new reactor concepts aim to achieve high calorific biogas by providing surplus CO_2 at the cathode chamber [61, 62].

BESs have proved to be a robust technology able to deal with a wide range of organic substrates. For instance, according to Liu et al. 2005 [63] simple VFA such as acetate, butyrate and propionate are evidently preferable substrates in BES. Solid waste degradation and electric current generation at the same time are achieved when MFCs are integrated with anaerobic digestion/fermentation or composting of agricultural by-products so that waste hydrolysate obtained after hydrolysis can be used as substrate [64].

Microorganisms that can couple the oxidation of organic compounds to electron transfer to electrodes offer the promise of self-sustaining systems that can effectively convert waste organic matter and renewable biomass into electricity [32]. At the microbial level, this is an anaerobic treatment technology and bacteria must be grown in an anaerobic environment in order to produce electricity [65]. In BES either pure culture or mixed culture of microorganisms could be used for inoculation. Power densities of 148 mW/m^2 and 5.6 mW/m^2 and current densities of 65 mA/m^2 were achieved using specific microorganisms like *Shewanella oneidensis* MR-1, *Paracoccus denitrificans* PS-1, *Shewanella Putrefaciens* PS-2 and *Geobacter sulfurreducens* to operate the BES with pure culture systems [66–69]. According to Varanasi et al. 2016 [70] and Ungerfeld et al. [71] there are several disadvantages of pure culture systems including their high cost effecting requirements on the substrates like sterilization and stringent anaerobic conditions thus leading to low long-term stability or the necessity of adding chemical inhibitors to suppress the simultaneous undesired degradation pathways.

Contradictory to the pure cultures, research done by Nimje et al. 2012 [66], Kiely et al. 2010 [67] and Watson and Logan, 2010 [68] showed that, mixed culture BES systems achieved higher power densities of 150 mW/m^2, 10.1 mW/m^2 and 858 mW/m^2 respectively. Similarly, Venkata Mohan et al. 2008 [72] research achieved high current densities of 747.96 mA/m^2 and 862.85 mA/m^2. By comparing mixed culture with pure culture setups in BES, Logan, 2005 [65] found that pure cultures were not needed. Furthermore, he stated that when using bacteria present in ordinary wastewater as a microbial source, high power levels could be achieved dependent on reactor configuration and operation and not on the inoculum. The review done by He et al. 2017 [73] also concluded that the performances of MFCs with mixed inoculation are better than those with pure culture inoculation in both power density and removal efficiency. Chen et al. 2019 [74] and Nimje at al. 2012 [66] stated in their research that mixed culture BES systems are advantageous for their robustness due to nutrient adaptability, easy substrate handling, long term stability, stress resistance and general tendency to produce higher current densities. Additionally, different operational parameters such as temperature variation, pH variation and external resistance play a crucial role in the performance of a BES system [75].

In early studies the amount of current generated in microbial fuel cells was very low, but in the past few years there have been substantial increases in power generation [65]. Scientific research has advanced on different BES technologies in the laboratory at an amazing pace with power densities having reached over 1 kW/m^3 (reactor volume) and to 6.9 W/m^2 (anode area) under optimal conditions [76]. Current research studies are still restricted at bench scale, in spite of some pilot scale studies with capacities ranging from 20 to 1000 L, which are unstable and performance limited due to several obstacles like solution leakage, influent oscillation, low power yield and adverse products [76, 77]. The main challenge is to bring these technologies out of the laboratory and engineer practical systems for bioenergy production at larger scales [76]. Substantially, the scaling-up of MFCs to cubic meter volume is crucial in order to accomplish the practical operation, with the possible integration of the bioreactor with existing infrastructure [78].

In most of the above discussed literature, BES systems are often fed with wastewater streams or synthetic substrates. Hence, this research study is a preliminary step in taking this upscaling of BES fed with the organic fraction of municipal solid waste to the next level of development. Therefore, as a primary pretreatment step it is necessary to efficiently convert different waste residues to process liquid like hydrolysate rich with high organic acids.

Since undissolved particles and fibers may cause technical problems and blockages in the following process stages, filtration of the hydrolysate is unavoidable. Consequently, to improve the quality of the hydrolysate, a next treatment step called membrane filtration has to be conducted. Within the study, a ceramic membrane microfiltration should be used to gain a particle-free process liquid called permeate with high VFA concentrations. The process liquids from hydrolysis and membrane filtration are to be further treated in BES reactors. The results of this research study are used as a ground work for upscaling the BES reactors with innovative construction and biogas production concepts.

1.7 Objectives of the study

The main objective of this research study was to make the treatment of different organic wastes in bio-electrochemical systems (BES) possible. For this, it is primarily necessary to convert these waste residues to liquid hydrolysates extracting high organic acids through acidification process in a two-stage AD system. In order to avoid any hindrances like biofouling, blockage and lower microbial degradability in the further treatment process, a ceramic membrane filtration step should be integrated to remove inert particles from the hydrolysate. Finally, the achieved process liquids from the first two treatment steps must be treated successfully in the BES systems.

To make this research work possible, the experimental studies and the reactors necessary were constructed and installed at the State Institute of Agricultural Engineering and Bioenergy. The working objectives of this research is further divided as follows:

1.3.1 How to sustainably extract the organics from vegetable waste residues so that they are made feasible to be further treated either in AF or in BES reactor?

2.3.1 How to prevent the hindrances like lower microbial degradability and blockage caused by the inert particles present in hydrolysate during further treatment processes in AF or in BES reactor, keeping the organic concentrations intact?

3.3.1 How to efficiently convert the produced process liquids in the BES targeting high degradation rates, thus leading to high current densities and other value added products?

1.8 Structure of the thesis

The framework of this thesis is subdivided to three steps, first, the vegetable waste substrate was converted to hydrolysate rich with VFA in the acidification reactor of the two-stage AD system at high pH values 5.5 and 6 and then followed by the simultaneous treatment of the hydrolysate in the AF to produce biogas [Publication 1: Effects of target pH-value on organic acids and methane production in two-stage anaerobic digestion of vegetable waste]. Since the inert particles present in the hydrolysate caused low microbial degradability in AF, therefore the hydrolysate was subjected to ceramic cross-flow filtration to produce particle-free permeate with high VFA concentrations in the second step [Publication 2: Integration of membrane filtration in two-stage anaerobic digestion system: Specific methane yield potentials of hydrolysate and permeate]. Finally, the produced process liquids hydrolysate and permeate were fed to two chamber BES with three electrode setup aiming high organic degradation rates and current densities. In addition, these results are used as a pre-requisite research step for the consequent upscaling and continuously operating the large scale flat panel reactor construction [Publication 3: Utilization of untreated process liquid substrates with high organic loads in bioelectrochemical systems: organic degradation rates & current densities] and further producing other value added platform chemicals by treating vegetable waste hydrolysate in BES [Publication 4: Development of a production chain from vegetable biowaste to platform chemicals].

2 Publication 1: Effects of target pH-value on organic acids and methane production in two-stage anaerobic digestion of vegetable waste

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Abstract

Vegetable waste is one of the major organic residues available for sustainable bioenergy production. The aim of this work is to study the influence of pH-value on process stability, hydrolysis, degradation degree and methane production in two-stage anaerobic system. A mixture of vegetable wastes with carrot mousse, carrots, celery, cabbage and potatoes was treated in two-stage system at target pH-values 5.5 and 6 in acidification reactor (AR). At pH 6, high concentrations of organic acids were recorded whereas high amount of hydrolysate was produced at pH 5.5. The chemical oxygen demand (COD) concentration in the hydrolysate produced in AR was 21.85% higher at pH 6 compared to pH 5.5, whereas the overall specific methane yield was slightly higher at pH 5.5 (354.35 ± 31.95 and 326.79 ± 41.42 L kg−1 oDMadded, respectively). It could be shown, that the described two-stage system is well suited for manure-free digestion of vegetable waste.

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ABSTRACT

Vegetable waste is one of the major organic residues available for sustainable bioenergy production. The aim of this work is to study the influence of pH-value on process stability, hydrolysis, degradation degree and methane production in two-stage anaerobic system. A mixture of vegetable wastes with carrot mousse, carrots, celery, cabbage and potatoes was treated in two-stage system at target pH-values 5.5 and 6 in acidification reactor (AR). At pH 6, high concentrations of organic acids were recorded whereas high amount of hydrolysate was produced at pH 5.5. The chemical oxygen demand (COD) concentration in the hydrolysate produced in AR was 21.85% higher at pH 6 compared to pH 5.5, whereas the overall specific methane yield was slightly higher at pH 5.5 (354.35 ± 31.95 and 326.79 ± 41.42 L kg^{-1} oDM_{added}, respectively). It could be shown, that the described two-stage system is well suited for manure-free digestion of vegetable waste.

1. Introduction

Organic residues such as biowaste, food waste, fruit and vegetable waste are generated throughout the world in large quantities. It's reported that about one third of supplied food for human consumption is being wasted globally (Gustavsson et al., 2011). In developing countries like India, 50 million tons of vegetable waste are produced, which is 30% of its total production and large part of it is disposed by dumping it on the outskirts of the city (Singh et al., 2012; Verma et al., 2011; Srilatha et al., 1995). In developed countries like Germany, about 8.3 million tons of biowaste from households and trade are collected per year, of which 7.1 million tons are used for composting and 1.2 million tons treated in anaerobic digestion plants (Weiland, 2000).

However, research has shown that anaerobic digestion of organic residues to produce biogas is a more promising option than composting (Bouallagui et al., 2004a; Gallert et al., 2003; Bouallagui et al., 2003; Mata-Alvarez et al., 1992). In recent years, the global bio-energy research is being focused on evaluating the potential and to address the relevant research challenges of anaerobic digestion (Appels et al., 2011). Therefore, different substrates such as energy crops, residues and wastes are being tested for this process, aiming a sustainable energy production and a reduction of greenhouse gas emissions (Jaiganesh et al., 2014; Rao et al., 2010; Weiland, 2010). Studies conducted in Germany showed that the treatment of biowaste generated biogas yields of 120 m^3 per ton of waste substrate (Weiland, 2010; Weiland, 2000). This confirms the robustness of anaerobic digestion process and its efficient application for organic waste treatment.

Different anaerobic processes, such as batch or continuous one-stage and continuous two-stage systems have been applied for the treatment of fruit and vegetable waste (Bouallagui et al., 2005). The two-stage anaerobic digestion system is a complex microbial conversion process, which involves four major steps (hydrolysis, acidogenesis, acetogenesis and methanogenesis). These steps are further grouped into acidification (hydrolysis and acidogenesis) and methanation (acetogenesis and methanogenesis) to achieve optimal conditions for each group of microorganism. The two-stage process appears to be a highly efficient technology for digestion of fruit and vegetable waste, which is mainly due to process stability and substantial biogas productivity (Veeken et al., 2000; Lindner et al., 2015a; Bouallagui et al., 2004b). This is achieved through the spatio-temporal separation of degradation steps (acidification and methanogenesis) which ensures optimal growth conditions for different microbial groups (Merkle et al., 2017; Lemmer et al., 2015a; Lindner et al., 2015a).

Currently, two-stage anaerobic digestion involving anaerobic leach bed reactors in combination with anaerobic filters are an active field of research. However, its application is not yet fully investigated for substrates such as vegetable waste (Lemmer et al., 2015b; Schönberg and Linke, 2012; Zielonka et al., 2010). Leach bed reactors are batch systems sequentially filled with substrates (e.g. energy crops), where hydrolysis and acidogenesis take place. The produced organic acids are washed out of the substrate by percolation in the reactor and transferred to the anaerobic filter for methanogenesis (Zielonka et al., 2010).

In recently published studies, continuously fed CST-reactors with an

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internal solid/liquid separation system were used as AR in a two stage fermentation system (Lindner et al., 2015a). Lindner et al. (2015a) examined the digestibility of different energy crops (hay/straw mixture, maize and sugar beet silage) in this innovative two stage system. They found out, that the environmental conditions during acidification influenced the biodegradability of the substrates significantly. In these studies, the pH in AR was regulated precisely by exchange of liquids between AR and anaerobic filter (AF) without using any additional chemical additives and high process efficiency for maize silage in two-stage system was achieved with in pH range 5.5–6 in AR (Lindner et al., 2015a). The pH-value from 5.2 to 6.5 plays a crucial role in the acidogenesis step in a two-stage system, because it promotes favorable conditions for the growth of acidogenic microorganisms (Demirer and Chen, 2004). Further studies showed, that reducing the organic load rate (OLR) (< 2.6 g VS/L/d) and controlling the pH can significantly improve the overall performance of the system (Zuo et al., 2013; Romano and Zhang, 2011; Mata-Alvarez et al., 2000). Therefore, it is of high importance to optimize and adapt the process parameter for the fermentative conversion of waste in two-stage system in order to achieve high process efficiency.

In current study vegetable waste was investigated in the continuously operated laboratory based two-stage anaerobic digestion system with automatically controlled pH-regulation in AR. The first objective was to maintain the pH-value stable in AR throughout the process without adding any buffering substances or acids whilst fermentation of vegetable waste in order to investigate the influence of this parameter on the process efficiency. The second objective of this experiment was to evaluate the influence of different pH-values in AR on different parameters like the production of hydrolysate, intermediate compositions of volatiles fatty acids (VFA's) as well as concentrations (hydrolysate and effluent), gas compositions, degradation degree and specific methane yields. The comparison between the specific methane yield of the two-stage system and substrate specific methane yields measured in the batch system (Hohenheim Biogas Yield Test) are also part of this study.

2. Materials & methods

2.1. Set up of the test facility

All experiments were performed in the biogas laboratory of the State Institute of Agricultural Engineering and Bioenergy at the University of Hohenheim, Stuttgart. The two-stage anaerobic digestion plant is equipped with a continuously stirred horizontal acidification reactor with 124 L capacity and a fixed bed anaerobic filter as shown in Fig. 1. AR is installed with a reel agitator with 12 stirring rods for mixing. A stainless steel sieve of 100-µm is fixed in between two metal screening plates to separate the solid phase and liquid phase in the reactor. Two brushes coupled with the stirring rod cleaned the sieve on the solid phase side permanently. Eight electric heating foils (H1, Thermo GmbH, Rohrbach, Germany) were used for heating the AR (60 ± 0.5 °C). A pH sensor (type Orbipac CPF81D, Endress + Messtechnik GmbH + CO, KG, Stuttgart, Germany) and temperature sensor (type Screw-in RTD Pt100 Temperature probe with Form B Terminal Head Jumo, Fulda, Germany) is installed in the liquid phase of the AR. Lindner et al. (2015a) and Lindner et al. (2016) explained the detailed construction of the AR.

The AF is a cylindrical reactor filled with 20 L of fixed bed consisting of sintered glass fillers (Sera Siporax, Heinsberg, Germany) to provide surface area (surface area 270 $m^2 L^{-1}$) for the growth of microorganisms and heated up to 37 °C with heating foil (480 W, Thermo GmbH, Rohrbach, Germany). There is a gas chamber at the headspace of the reactor accounting 15% of reactors volume. A pH sensor (type Orbisint CPF11D, Endress + Messtechnik GmbH + CO, KG, Stuttgart, Germany), a temperature sensor (GTF 103 Pt100, Greisinger, Regenstauf, Germany) and three Level sensors (Liquicap T FMI21, Endress + Hauser, Reinach, Switzerland) are installed in the AF. A control valve (Bürket 2712, Ingelfingen, Germany) and a pressure sensor (Ceraphant T PTC31, Endress + Hauser, Reinach, Switzerland) controlled the whole process. Lemmer et al. (2015b) and Chen et al. (2014) explained the detailed construction of the AF reactor (Fig. 1).

The whole system was operated by adjusting pH to targeted values (pH value-5.5 and 6) and temperature of the AR (60 °C) and the AF (37 °C). Substrate mixture was fed every day to the AR where acidogenic microbes hydrolysate the substrate and produce organic acids and alcohols. The sieved process liquid enriched in organic acids and alcohols was referred to as "hydrolysate", which was pH-dependent removed from the liquid phase compartment of the reactor and pumped to the buffer tank-1. Subsequently, this hydrolysate was fed to the AF (1.5 bar pressure) based on COD concentration of the hydrolysate, aiming stable OLR in the AF throughout the experimental phase. Syntrophic co-cultures of secondary fermenting bacteria and methanogenic microorganisms further metabolized the organic components of the hydrolysate to methane, carbon dioxide and water. Thereafter, effluent from the AF was pumped to the buffer tank-2.

To maintain pH in the AR at the target pH value (5.5 and 6), 1 L of hydrolysate was pumped from the AR to tank-1 when target pH value dropped below the target value. Automatically, the removed liquid was

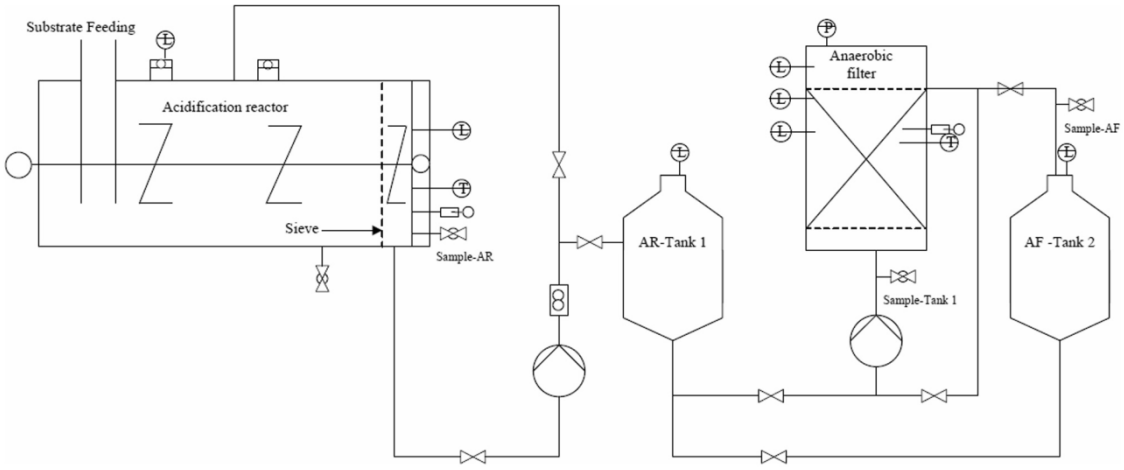

Fig. 1. Piping and instrument schematic diagram of two-stage anaerobic digestion system.

replaced by effluent from the AF stored in the buffer tank-2. The pumped hydrolysate volumes from the AR to tank-1 and tank-2 to the AR was determined by a flow meter (Type Promag 53P, Endress + Messtechnik GmbH + CO, KG, Stuttgart, Germany). Temperature, pH values and pumping volumes of hydrolysate and effluent were recorded in database (Fig. 1).

The gases produced in AR and AF were collected in gasbags and measured both quantitatively and qualitatively (AR thrice per day and AF four times per day). A gas analyser (SICK MAIHACK S710; SICK Vertriebs-GmbH, Düsseldorf, Germany) was used to detect the gas qualities of produced methane, carbon dioxide and hydrogen. Gas volumes produced were measured using a drum gas meter (TG 20/5, Dr.-Ing. RITTER Apparatebau GmbH & Co. KG, Bochum, Germany). The gas volumes produced were corrected to standard conditions (1013 hpa, 273.15 K) (Lindner et al., 2016).

2.2. Experimental procedure

Vegetable waste used for the experiments was collected from BAYHA GEMÜSE, a farm in Filderstadt, Germany. This vegetable waste includes a constant mixture of carrot mousse, carrots, celery, cabbage and potatoes. The feeding amounts were prepared depending on the dry matter (DM) and organic dry matter (oDM) contents of the respective vegetable as shown in Table 1A.

Prior to feeding, the vegetable waste was pretreated mechanically. Therefore, all vegetable wastes were cut in a grinding machine for one minute, except carrot mousse (Robot Coupe R8, Saarbrücken, Germany). However, potatoes were washed first to remove the mud. To stabilize the two-stage anaerobic digestion at different pH-values in the AR, the OLR had to be adapted to the process phases (5.4 kg m^{-3} d^{-1} at pH-value 5.5 and 6.1 kg m^{-3} d^{-1} at pH 6.0, respectively) (Table 2A). Once a week, daily feeding bags were prepared as shown in Table 1A, stored at 4 °C and supplemented by 1 g of trace element mixture (Schaumann Bioenergy, Pinneberg, Germany) per kg of fresh material according to Vintiloiu et al. (2012).

At the beginning of each experimental phase, AR was initially started by adding 80 kg of water, 20 kg of separated liquid digestate collected from the biogas plant at "Unterer Lindenhof" (Lemmer et al., 2013), 2 kg maize silage, 1 kg of digestate from past experiments, 0.24 kg acetic acid and 0.13 kg lactic acid.

The planned experiments were run at two different pH values 5.5 and 6 in the AR and 1.5 bar pressure in the AF. The vegetable waste experiments included four different phases. Beginning with starting phase (60 days) and secondly followed by the experimental phase one (pH 5.5) that ran for about 21 days continuously. The third phase is the change phase (75 days) where pH in the AR was regulated to 6. Finally, followed by the experimental phase two (pH 6) lasting for a duration of 21 days.

2.3. Sample collection & laboratory analysis

The fresh vegetable materials were individually examined for the contents of DM (%) and oDM (%) on a weekly basis by drying at 105 °C for 24 h followed by incineration at 550 °C for 8 h (VDI-Society Energy and Environment, 2006). Thrice a week, samples were collected from AR, tank 1 and AF for further laboratory analysis, like COD using Hach Lange's cuvette test (Hach Lange Type LCK 014) which includes a sensor array photometer (Hach Lange Type LASA 20) and a high temperature thermostat (Hach Lange Type HT 200S). Gas chromatography (GC) for measuring VFA's (GC with 2100Plus with a FID-detector and a capillary column WCOT Fused Silica, Shimadzu, Germany) and high performance liquid chromatography (HPLC with RI-detector, BioRad Aminex HPLC column HPX-87H, BioRad-precolumn HPX-87H, BISCHOFF Analysentechnik und -geräte GmbH, Leonberg, Germany) was used for analyzing other organic acids, sugars and alcohols. Total carbon (TC), total organic carbon (TOC), inorganic carbon (IC) and total nitrogen (TN) were determined using the TOC/TNb analyzer (Analytik Jena AG Type multi N/C®, Jena, Germany). Table 1B shows the contents of crude ash (XA), crude protein (XP), crude fat (XI) and crude fibre (XY) of vegetable waste substrates determined by "Weender Feed Analysis" meeting European regulations and using standard methods of the federation of German Agricultural Investigation and Research Institutes. According to the same regulations, the neutral detergent fiber (NDF) was also determined. Lindner et al. (2016), Lindner et al. (2015a) as well as Stockl and Oechsner (2012) described all these laboratory methods in detail.

2.4. Batch digestion test

Hohenheim Biogas yield test (HBT) was performed with all the vegetable waste to determine the biogas and methane yield potential for all substrates individually. This test was executed under the regulation of VDI Guideline 4630 and explained in detail by Lindner et al. (2015b) and Mittweg et al. (2012). With the substrate specific methane yield of individual substrates and considering the proportion to the substrates in the fed mixture, the specific methane yield of the feeding mixture ($SMY\text{-}HBT_{calculated}$) is calculated.

2.5. Calculations and statistical analysis

For both stages, process parameters like temperature, pH-value and pressure (only in AF) were monitored and recorded in real time with one-minute intervals. In addition, the pumped volumes of hydrolysate and effluent as well as the measured gas volumes were logged. The retrieved data were saved in a MySQL database (Toad for MySQL 7.0.0.2038). For data calculations, the "R Studio" statistical software was used.

Similar to the approach of Lindner et al. (2015a) and Krümpel et al. (2016), the description of the OLR in the AR was related to oDM of the vegetable waste fed. Whereas, the OLR in context to AF was related to the COD of the hydrolysate obtained from hydrolysis step in the AR.

Solid retention time (SRT) is the ratio of fermenter volume (114.72) to the volume of fresh matter fed ($\dot{v}_{fm}$) (Eq. (1)).

$$SRT = \frac{114.72}{\dot{v}_{fm}} \quad (1)$$

The ratio of fermenter volume (114.72) to the exchange volume ($\dot{v}$) is defined as recirculation time (RT_{AR}) (Eq. (2)).

Table 1A
Feeding mixtures & DM/oDM (%) of substrates fed to AR.

Substrate	Amount (g)	Average DM (%)	Average oDM (%)	Amount (g)	Average DM (%)	Average oDM (%)
		pH-5.5			pH-6	
Carrot Mousse	400	6.15 ± 0.37	5.74 ± 0.91	500	2.79 ± 0.67	2.57 ± 0.63
Carrot	1000	10.52 ± 0.18	9.39 ± 0.22	1250	9.72 ± 0.33	8.74 ± 0.33
Celery	600	10.28 ± 1.28	9.13 ± 1.13	750	8.93 ± 1.23	7.59 ± 1.10
Cabbage	500	10.15 ± 0.28	9.14 ± 0.18	650	6.38 ± 1.53	5.67 ± 1.42
Potato	1700	20.67 ± 1.11	19.33 ± 1.23	2150	21.49 ± 2.52	20.05 ± 2.29
Total	4200			5300		

Table 1B
Nutritional values of vegetable waste determined by Weender/vanSoest.

Substrate	Water (%)	Crude Ash (%)	Crude Protein (%)	Crude Fat (%)	Crude Fiber (%)	aNDF (%)	ADF (%)	ADL (%)
Carrot Mousse	9.85 ± 0.64	6.50 ± 0.57	5.30 ± 0.14	1.30 ± 0.28	18.90 ± 0.28	31.20 ± 0.71	34.4 ± 0.28	1.50 ± 0.00
Carrot	10.20 ± 1.27	9.25 ± 0.78	9.85 ± 0.78	1.50 ± 0.14	9.05 ± 0.92	16.95 ± 0.49	17.15 ± 1.34	1.15 ± 0.21
Celery	8. 85 ± 1.20	10.65 ± 0.07	12.15 ± 1.34	1.30 ± 0.14	9.50 ± 0.28	19.85 ± 0.49	22.50 ± 0.85	4.90 ± 0.99
Cabbage	11.90 ± 0.57	9.15 ± 0.64	15.50 ± 0.85	1.30 ± 0.14	10.45 ± 0.21	17.75 ± 0.92	16.85 ± 0.21	< 0.5 ± 0.00
Potato	6.00 ± 0.71	6.30 ± 0.42	10.45 ± 1.34	0.62 ± 0.01	2.50 ± 0.14	10.75 ± 4.45	4.30 ± 0.85	0.80 ± 0.28

Table 2A
Effect of target pH-value on SRT, RRT and hydrolysate produced in AR.

Parameter	pH-5.5	pH-6
OLR_{AR} (kg m^{-3} d^{-1})	5.4 ± 0.18	6.1 ± 0.51
SRT (d^{-1})	27.31	21.65
RT_{AR} (d^{-1})	4.16	6.18
Hydrolysate (L d^{-1})	27.55	18.56
Hydrolysate (L kg^{-1} ODM)	50.55	30.33

$$RT_{AR} = \frac{114.72}{\dot{v}} \quad (2)$$

The specific methane yield of AR, AF and the complete system were related to oDM_{added}, COD_{added} and oDM_{added} respectively (Lindner et al., 2015a). The oDM degradation degree for AR and the complete system was calculated, by dividing the produced mass of biogas with the oDM fed as Lindner et al. (2015a) explained the calculation in detail. The COD-degradation degree in AF was calculated as the ratio of $COD_{degraded}$ to COD_{added}.

3. Results and discussion

3.1. Measured pH-value fluctuations in the AR and AF at different target pH-values in the AR

The measured pH-values during the two experimental phases in AR and AF are presented in Fig. 2. At target pH-values 5.5 and 6 in the AR, the measured mean pH-value in AR was 5.45 ± 0.09 and 5.96 ± 0.13, respectively. The median value signifies process stability confined in between 25% and 75% quantiles in the pH-value boxplot. Both the target pH-values in the AR were consistently stable with lowest standard deviations. The pH-value in AR was maintained constant without using additives, instead pumping the effluent from the AF when there was a pH drop after feeding fresh substrate (Fig. 2).

The measured mean pH-value in the AF during the two experimental phases was 7.21 ± 0.09 and 7.42 ± 0.04 at target pH-values 5.5 and 6 in AR respectively, showing no significant difference. No influence of different target pH-values in AR on AF's pH-value was noticed. These results lead to the conclusion that, stable processes were obtained in the experimental phase without any biological disturbances. It could be shown, that the described two-stage biogas system is particularly well-suited for the digestion of vegetable waste under consistently stable conditions without adding any chemicals for pH-adjustment. These results are in line with the findings of Lindner et al. (2016) and Lindner et al. (2015a) (Fig. 2).

3.2. Effects of pH-value in the AR on chemical characteristics and compositions of hydrolysate and effluent

The influence of the pH-value on hydrolysate produced, SRT, RT_{AR} and chemical compositions were inspected. The absolute amount of hydrolysate produced was 32.6% higher at pH 5.5 (27.55 L d^{-1}) than pH 6 (18.56 L d^{-1}). Similarly, the specific hydrolysate production related to the oDM_{added} to the AR was 40% higher at pH 5.5 compared to pH 6 as shown in Table 2A. The SRT at pH 5.5 was 27.31 days and 21.65 days for pH 6, which is 20.7% less than pH 5.5. In contrast to SRT, the RT_{AR} of pH 6 was 32.8% higher than pH 5.5 as shown in Table 2A.

In Table 2B, the chemical characteristics and compositions of produced hydrolysate and effluent at different pH-values are described. In contrary to the hydrolysate produced, the COD-concentration in AR was 21.8% higher at pH 6 compared to the experimental phase with pH 5.5. The organic acid concentrations in the AR showed distinctive differences at target pH-values. The acetic acid equivalent was higher at pH 6 (12.2 ± 0.61 g kg^{-1}) than pH 5.5 (5.9 ± 0.47 g kg^{-1}). Acetic acid, propionic acid and *n*-butyric acid produced was higher at pH 6 by 51.4%, 57.8% and 46.3% respectively. However, caproic acid produced was 77.2% lower at pH 6 than pH 5.5. Insignificant differences in concentrations of iso-butyric acid, iso-valeric acid, n-valeric acid and ethanol at pH 6 are measured (Table 2B). For both the target pH-values,

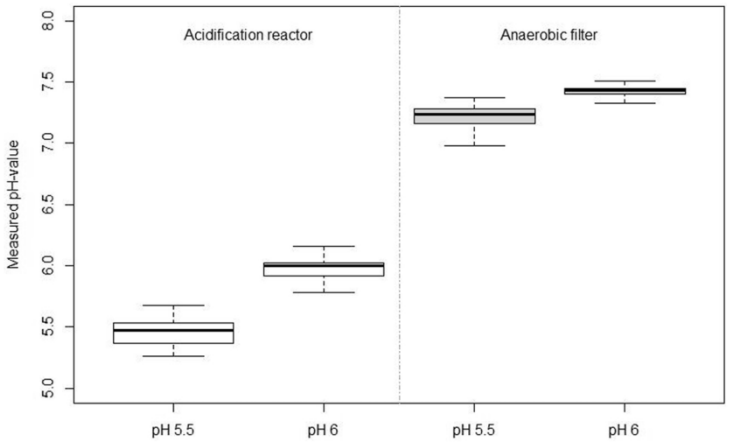

Fig. 2. Distribution of measured pH-values at different experimental phases in AR and AF.

Table 2B
Effects of target pH-values on intermediate concentrations and compositions of hydrolysate (AR) and effluent (AF).

Parameters	pH 5.5		pH 6	
	AR	AF	AR	AF
Acetic acid (g kg^{-1})	4.463 ± 0.30	0.894 ± 0.29	9.190 ± 0.97	0.933 ± 0.34
Propionic acid (g kg^{-1})	0.522 ± 0.06	0.122 ± 0.03	1.236 ± 0.20	0.138 ± 0.06
iso-Butyric acid (g kg^{-1})	0.043 ± 0.01	0.020 ± 0.01	0.142 ± 0.03	0.024 ± 0.01
n-Butyric acid (g kg^{-1})	1.166 ± 0.21	0.214 ± 0.09	2.172 ± 0.17	0.161 ± 0.05
iso-Valeric acid (g kg^{-1})	0.051 ± 0.00	0.014 ± 0.00	0.143 ± 0.01	0.019 ± 0.01
n-Valeric acid (g kg^{-1})	0.120 ± 0.05	0.014 ± 0.01	0.253 ± 0.11	0.027 ± 0.01
Caproic acid (g kg^{-1})	0.381 ± 0.21	0.035 ± 0.05	0.087 ± 0.98	0.014 ± 0.08
Ethanol (g kg^{-1})	0.100 ± 0.13	0	0.350 ± 0.08	0
TOC (g L^{-1})	8.280 ± 1.44	4.890 ± 0.54	10.840 ± 0.85	4.520 ± 0.96
IC (g L^{-1})	0.078 ± 0.33	1.025 ± 0.09	0.091 ± 0.11	2.185 ± 0.13
TC (g L^{-1})	8.560 ± 1.40	6.010 ± 0.54	11.055 ± 0.81	6.670 ± 0.96
TN (g L^{-1})	1.280 ± 0.13	1.400 ± 0.08	2.245 ± 0.19	2.440 ± 0.17
COD (g L^{-1})	22.92 ± 3.06	14.84 ± 1.51	29.33 ± 2.42	13.31 ± 2.64

no sugars and no other alcohols were detected. At pH 6, measured concentrations of TOC, TC and TN were higher by 23.6%, 22.6% and 43% respectively. Whereas, the IC concentration did not vary significantly for both target pH-values.

Organic acids produced in the AR specified in Table 2C in relation to oDM_{added} showed huge variations between both target pH-values. Acetic acid, propionic acid and *n*-butyric acid produced was higher at pH 6 by 39%, 83.6% and 31% respectively. Yet, caproic acid produced was 86.6% lower at pH 6 than pH 5.5. The total organic acids produced were 38% higher at pH 6 (347.75 g kg^{-1} oDM_{added}) than pH 5.5 (215.34 g kg^{-1} oDM_{added}).

In context to the effluent from AF, COD-concentration in AF was 14.84 ± 1.51 g L^{-1} at pH 5.5 and 13.31 ± 2.64 g L^{-1} at pH 6 (Table 2B). There were very little amounts of organic acids measured which were almost negligible amounts under 0.2 g kg^{-1} for both experimental phases. In the AF, there were no sugars and alcohols witnessed at both pH-values. TOC and TC concentrations in the AF were similar at both experimental phases but, when compared to concentrations in the AR, there was a decrease of about 40.9% and 58.3% TOC (pH 5.5 & 6) and 29.8% and 39.7% TC (pH 5.5 & 6). In contrary, the IC and TN concentrations in AF were 53.1% and 42.6% higher at pH 6 than pH 5.5. While, in comparison to the AR concentrations the TN values showed negligible differences in the AF, which were under 1 g L^{-1}.

These experiments showed evident variations in hydrolysate production, SRT and RRT in the AR with increasing pH-value. Organic acids concentrations increased at target pH-value 6 which is relatable to research done by Lindner et al. (2015a) in the past. It can be assumed, that the pH 6 is optimum for certain microbial growth rates (Horiuchi et al., 1999). Obtained results of this research are relevant to several research study findings. The produced fractions of acetic acid, propionic acid and *n*-butyric acid were highly dominating acids of all organic acids. The reason for this result is an effect of pH-value in the AR prompting acidogenesis process (Lindner et al., 2015a; Horiuchi et al., 2001; Elefsiniotis and Oldham, 1994; Zoetemeyer et al., 1982). In contrast to this, pH-value influenced insignificantly the remaining fractions of the other organic acids and alcohols (Lindner et al., 2015a; Yu and Fang, 2003). The small standard deviations from the measured organic acids also showed that there is a very high process stability.

Table 2C
Organic acids produced per ODM_{added} at different at different target pH-values in AR.

Organic acids produced (g kg^{-1} ODM_{added})	pH 5.5	pH 6
Acetic acid	146.24	240.59
Propionic acid	5.26	31.99
iso-Butyric acid	1.16	2.19
n-Butyric acid	41.55	60.30
iso-Valeric acid	0.45	3.72
n-Valeric acid	4.35	6.78
Caproic acid	16.33	2.19
Total organic acids	215.34	347.75

3.3. Effects of target pH-values on gas qualities and quantities in the AR and AF

In Fig. 3, the gas qualities in the AR and AF are presented. The methane content measured in the AR is slightly higher at pH 6 (25.2 ± 7.4%) than pH 5.5 (18.6 ± 6.1%). Quite the reverse, the carbon dioxide measured was higher at pH 5.5 (75% and 77%, respectively). While the hydrogen content reached highest at pH 5.5 (4.9 ± 4.7%) and decreased by 66.5% at pH 6. This result is similar to the effects of pH-value on gas qualities as shown in several literature (Lindner et al., 2016; Lindner et al., 2015a; Yu and Fang, 2003). The experiments showed, that even slight changes of the pH value during the acidification of vegetable waste has a significant influence on the composition of the gas produced in the first reactor of the two-stage biogas system. Lower pH-values lead to raising hydrogen and carbon dioxide contents in the gas. Simultaneously, lower pH-values result in a reduced metabolic activity of the methanogenic microorganisms in this stage, which is in accordance to the findings of Lindner et al. (2016), Lindner et al. (2015a) and Yu and Fang (2003) (Fig. 3).

In the anaerobic filter, the added organic acids were converted efficiently to biogas. In both experimental phases, the hydrogen content amounted to approx. 1% for both target pH-values. The carbon dioxide content measured was ranging from 26% to 34% for pH 6 and pH 5.5 respectively. The two-stage anaerobic digestion process leads to a separation of the produced gases, resulting in high methane contents in the gas produced by the AF. The described varying composition of the hydrolysate gained at different pH-values resulted in higher methane contents 73 ± 1.5% of the AF-gas (experimental phase pH 6) compared to 65.7 ± 1.1% (experimental phase pH 5.5). Other studies using the two-stage system for the treatment of vegetable waste reported lower methane concentrations (60%) (Zuo et al., 2013). The results obtained are similar to the research done by Lindner et al. (2015a), using a comparable system for the digestion of energy crops (Fig. 3).

The methane quantities measured in the AR were 40% higher at pH 6 (38.2 ± 14.3 L d^{-1}) than pH 5.5 (22.9 ± 6.4 L d^{-1}). In contrary, the quantity of hydrogen produced was 53.1% higher at pH 5.5 (5.8 ± 7.4 L d^{-1}) than pH 6. The amount of carbon dioxide measured ranged from 92.6 ± 15.9 L d^{-1} (pH 5.5) to 110.9 ± 16.6 L d^{-1} (pH 6).

Regarding the gas quantities in the AF, the hydrogen measured was less

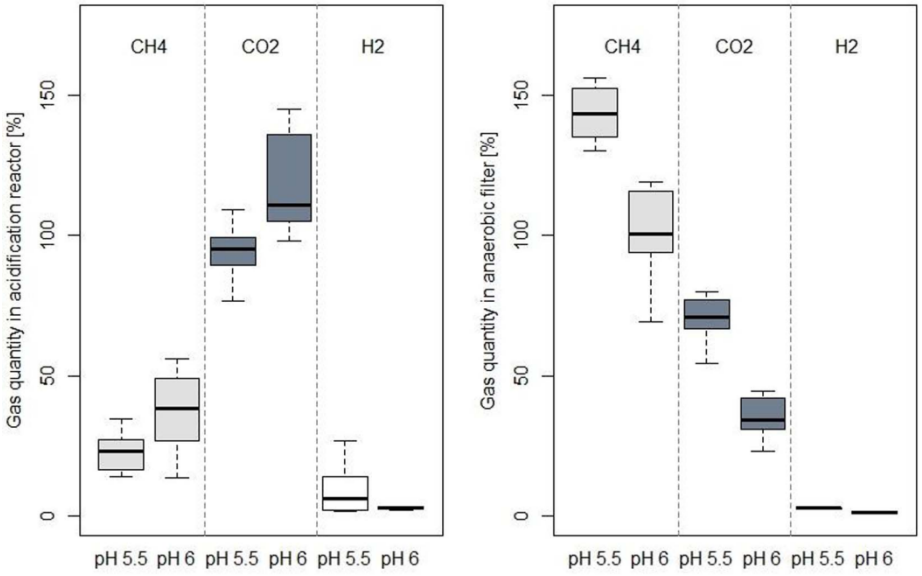

Fig. 3. Effect of target pH-values in AR and AF on methane, carbon dioxide and hydrogen concentrations.

Table 3
Effect of different target pH-values on specific methane yields (AR, AF, complete system), oDM-degradation degree (AR, complete system) and COD-degradation degree (AF).

	pH-5.5	pH-6
SMY-AR (L kg^{-1} oDM_{added})	42.37 ± 11.93	62.34 ± 23.26
SMY-AF (L kg^{-1} COD_{added})	233.97 ± 20.00	259.03 ± 33.91
SMY-$HBT_{Calculated}$ (L kg^{-1} oDM_{added})	331.44 ± 0.52	332.64 ± 0.13
SMY-Complete system (L kg^{-1} oDM_{added})	354.35 ± 31.95	326.79 ± 41.24
oDM-degradation $degree_{AR}$ (%)	26.14 ± 4.06	29.32 ± 4.85
oDM-degradation $degree_{Complete\ system}$ (%)	47.57 ± 6.82	44.70 ± 4.78
COD-degradation $degree_{AF}$ (%)	35.32 ± 5.90	57.12 ± 8.85

than 3 L d^{-1} for both experimental phases. Whereas, the carbon dioxide quantity was 70.9 ± 11.9 L d^{-1} and 34 ± 6.8 L d^{-1} for pH 5.5 and pH 6 respectively. The methane amounts measured were 22.4% higher at pH 5.5 (143.3 ± 21.9 L d^{-1}) than pH 6 (111.2 ± 21.9 L d^{-1}) (Fig. 3).

3.4. Effects of target pH-values on specific methane yields and the substrate degradation degree

As shown in Table 3, the specific methane yields were affected by the target pH-values in the AR. The specific methane yield (SMY) in the AR, signifies the relation of methane produced per kg oDM_{added}. The highest SMY-AR was observed at pH 6 (62.34 ± 23.26 L kg^{-1} oDM_{added}) by 32% than pH 5.5 (42.37 ± 11.9 L kg^{-1} oDM_{added}). Similarly, the SMY-AF related to the COD_{added} to the AF showed 9.7% higher value at pH 6 (259.03 ± 33.91 L kg^{-1} COD_{added})) than pH 5.5. Batch test (HBT) results of vegetable waste showed that the SMY of celery, cabbage, carrot mousse, potato and carrot were 303, 317, 325, 335 and 343 (L kg^{-1} oDM_{added}) respectively. For cabbage and carrot, Appels et al. (2011) and Gunaseelan (2004) showed almost similar results in their biochemical methane potential research. The SMY values calculated for the feeding mixture from the batch test did not show a significant difference with yields achieved (SMY-Complete system) at both the experimental phases pH 5.5 (331.44 ± 0.52 L kg^{-1} oDM_{added}) and pH 6 (332.64 ± 0.13 L kg^{-1} oDM_{added}). In comparison to SMY-$HBT_{calculated}$, SMY for the complete two-stage system in relation to oDM_{added} was almost achieved at pH 6 (326.79 ± 41.42 L kg^{-1} oDM_{added}) with a difference of 1.8%. While, SMY for the complete system at pH 5.5 (354.35 ± 31.95 L kg^{-1} oDM_{added}) showed 6.5% higher yield than SMY-$HBT_{calculated}$. Because the batch test (SMY-$HBT_{calculated}$) and experimental phases (SMY-Complete system) showed similar results, both the experimental phases appeared to be good enough in volatile substance degradation by easy degradable substrates as explained by Lindner et al. (2016).

The oDM degradation related to the whole system showed a quite similar degradation $degree_{complete\ system}$ of 47.57 ± 6.83% (pH 5.5) and 44.70 ± 4.78% (pH 6). The COD-degradation degree in the AF was 38.2% higher at pH 6(57.12 ± 8.85%) than pH 5.5 (35.32 ± 5.90%). Lindner et al. (2015a) and Zverlov et al. (2010) showed similar results like, oDM-degradation degree in the AR and methane yields raised as the pH-value in the AR increased.

4. Conclusion

The investigated two-stage anaerobic system with automatically controlled pH-value in AR was well suited for mono-digestion of vegetable waste. Organic residues added were efficiently converted to organic acids and subsequently to biogas. Two-stage process led to the separation of gases during production, thus resulting in methane contents up to 75% in AF. Compared to the methane yield potentials of substrates, high methane yields could be achieved in two-stage system. Hence, making food processing waste treatment in combination with energy production possible. Furthermore, lint-free hydrolysate produced in AR enables multiple applications, like organic acids extraction for bulk chemical production or as feedstock for microbial fuel cells.

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Corrigendum

Corrigendum to "Effects of target pH-value on organic acids and methane production in two-stage anaerobic digestion of vegetable waste" [Bioresour. Technol. 247 (2018) 96–102]

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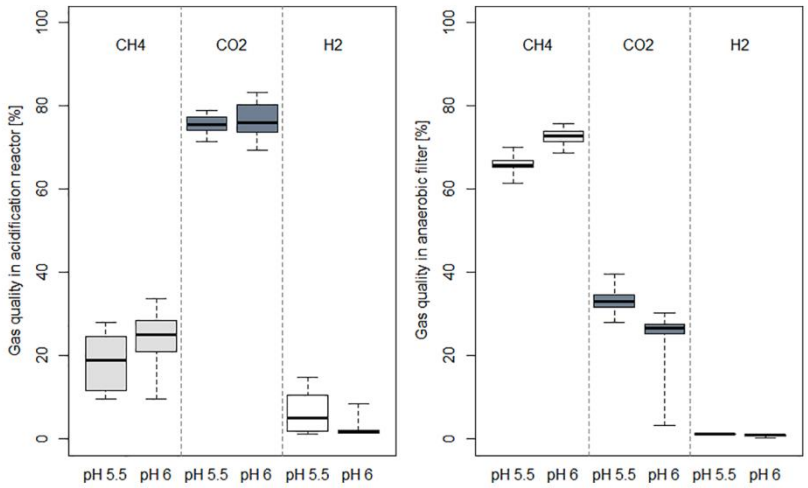
The authors regret that < for the inaccuracy happened with figure 3 as gas quantities graph was used instead of gas qualities > .



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3 Publication 2: Integration of membrane filtration in two-stage anaerobic digestion system: Specific methane yield potentials of hydrolysate and permeate

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Abstract

Two-stage biogas systems consisting of a CSTR-acidification reactor (AR) and an anaerobic filter (AF) were frequently described for microbial conversion of food and agricultural wastes to biogas. The aim of this study is to investigate the integration of a membrane filtration step in two-stage systems to remove inert particles from hydrolysate produced in AR in order to increase the efficiency of the subsequent AF. Hydrolysates from vegetable waste (VW) and grass/maize silage (G/M) were treated in cross-flow ceramic membrane filtration system (pore size 0.2 μm). Organic acids were extracted efficiently through filtration of hydrolysate. For both the substrates, membrane permeability was stable and high (46.6–49.3 Lm-2h-1bar-1). Filtration process effectively improved the specific methane yield of permeate by 40% (VW) and 24.5% (G/M) compared to hydrolysate. It could be shown that, the filtration-step increased hydrolysate's degradability, which lead to higher conversion efficiency in the following AF.

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Integration of membrane filtration in two-stage anaerobic digestion system: Specific methane yield potentials of hydrolysate and permeate

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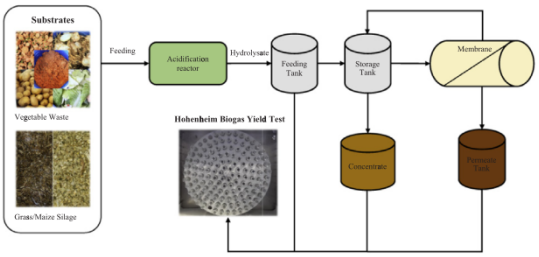
GRAPHICAL ABSTRACT



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ABSTRACT

Two-stage biogas systems consisting of a CSTR-acidification reactor (AR) and an anaerobic filter (AF) were frequently described for microbial conversion of food and agricultural wastes to biogas. The aim of this study is to investigate the integration of a membrane filtration step in two-stage systems to remove inert particles from hydrolysate produced in AR in order to increase the efficiency of the subsequent AF. Hydrolysates from vegetable waste (VW) and grass/maize silage (G/M) were treated in cross-flow ceramic membrane filtration system (pore size 0.2 µm). Organic acids were extracted efficiently through filtration of hydrolysate. For both the substrates, membrane permeability was stable and high (46.6–49.3 $L\,m^{-2}\,h^{-1}\,bar^{-1}$). Filtration process effectively improved the specific methane yield of permeate by 40% (VW) and 24.5% (G/M) compared to hydrolysate. It could be shown that, the filtration-step increased hydrolysate's degradability, which lead to higher conversion efficiency in the following AF.

1. Introduction

Bioenergy is a propitious, never-ending sustainable source to overcome the environmental problems caused by depleting fossil fuels (Singh et al., 2012). Anaerobic digestion is a very efficient process to convert biomass like energy crops, organic residues and wastes into the secondary energy source biogas. Therefore, this process gains increasing interest in order to reduce greenhouse gas emissions and to facilitate a sustainable development of energy supply (Weiland, 2010). Anaerobic digestion is a microbiological process in which the complex organics are degraded via four microbial steps (hydrolysis, acidogenesis, acetogenesis and methanogenesis) and converted to methane gas in the absence of oxygen (Monnet, 2003; Chang et al., 1994; Khanal, 2008).

In almost all full-scale biogas plants, these four steps run simultaneously in one reactor. In order to provide optimal conditions for each group of microorganisms, Pohland and Ghosh, (1971) suggested a process optimization by spatially dividing the acidogenic stage and

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methanogenic stage in two different reactors. In the first reactor of a two-stage system, the formation of volatile fatty acids (VFA) takes place, while in the second reactor, the methanogenic microorganisms convert the VFA to methane and carbon dioxide (Ueno et al., 2007; Azbar et al., 2001). The spatial separation of the degradation steps ensures the optimum growth conditions for different group of microorganisms (Merkle et al., 2017; Lemmer et al., 2015; Lindner et al., 2015a). Two-stage anaerobic digestion systems are often considered to be advantageous compared to a one-stage processes (Lindner et al., 2016). As the stage separation showed increased process stability with optimum conditions like pH value, temperature and organic loading rate (OLR) in each phase (Fox and Pohland, 1994; Cohen et al., 1980).

Different combinations of reactors are described in literature for two-stage anaerobic digestion systems. Recently published studies are mainly focusing on different arrangements of CSTR as acidification reactor (AR) in combination with following anaerobic filters (AF), thus enabling an efficient overall performance and high process stability (Merkle et al., 2017; Lemmer et al., 2015; Lindner et al., 2015a). Although these systems guarantee a very high biological process stability, their technical implementation is challenging since AF can only be operated only with fiber-free, liquid substrates, which makes a filtration step prior to AF necessary. Furthermore, the research done by Bär et al. (2018) prove that inert particles of the hydrolysate are leading to the formation of thick and inefficient biofilms or can even clog high-loaded fixed-bed methane reactors of the second process stage even after a filtration of the hydrolysate with a conventional sieve. Similarly, for an undisturbed operation of two-stage high-pressure fermentation up to 100 bar, particle free hydrolysate is necessary (Tuczinski et al., 2018). The effect of incorporating microfiltration of the feeding stream showed improved biogas quality and process stability of a continuously operated lab scale high-pressure fermentation (Bär et al., 2018). Therefore, an effective separation method for getting rid of inorganic particle substances from the hydrolysate is needed. The filtered permeate could be fed not only to the AF and high pressure AF but also might be used in bioelectrochemical systems like microbial fuel cell or microbial electrolytic cell. In particular, recently published, novel process combinations, in which the hydrolysate obtained in the AR was used as a feeding substrate in bioelectrochemical systems (Schmidt et al., 2018).

One optimal method for the separation of small particles from liquids is the membrane filtration. In previous studies, membrane filtration systems were used to remove the organic compounds, suspended solids, colour and turbidity from anaerobically treated dairy wastewater and results indicating higher chemical oxygen demand (COD) removal (Zielińska and Galik, 2017). Membrane filtration has several advantages like the operation at ambient temperatures without adding any chemicals to the substrate solutions. They are made of various materials which can resist high temperatures and high acidic environment and after the filtration process both permeate and concentrate can be utilized (Strathmann et al., 2006; Wagner, 2001). Pressure-driven membrane filtration is the most well-developed filtration process, which is widely used in almost all kinds of chemical, pharmaceutical, food and dairy industries for removing interfering components (Søren Prip Beier, 2007).

There are four different types of pressure-driven membranes for aqueous solutions called microfiltration, ultrafiltration, nanofiltration and reverse osmosis based on the pore sizes of the membranes (Baker, 2004). Microfiltration refers to filtration processes that use porous membranes to separate suspended particles with diameters between 0.1 and 10 μm (Baker, 2004). The membrane filtration process can basically be operated in two flow configurations: dead-end and cross-flow method. In dead-end mode, the entire feed flows perpendicular to the membrane, therefore, most of the retained particles accumulate on the membrane surface. While for a cross-flow system, the feed stream flows parallel to the membrane surface so that only a small fraction of feed passes through the membrane under driving pressure and the concentrate can be continuously swept off from the membrane surface to support further down flows (Wang et al., 2011). Previous studies showed that cross-flow filtration is a viable option for removing suspended contaminants or particles from aqueous solutions and waste water treatment (Jeison and van Lier, 2007; Sondhi et al., 2000).

According to several publications, many optimal operational parameters can be adjusted for enhancing a membrane filtration process. Better filtration performance was observed when an anaerobic membrane reactor for sewage sludge treatment was operated at thermophilic temperature in the studies conducted by Meabe et al. (2013) and also the combination of thermophilic digester and membrane filtration appeared very promising. Álvarez et al. (2000) research showed that a pressure higher than 1.5 bar did not result in a higher permeability while producing apple juice and apple aroma concentrate with an integrated membrane process.

In these experiments a continuously operating acidification reactor (AR) of a two-stage system was combined with a ceramic cross-flow membrane filtration unit for the first time. The primary objective of this investigation was, to study the influence of two different substrates on the operational parameters in AR and in the membrane filtration system. Followed by the secondary objective, which was to distinguish the influence of membrane filtration on organic acids, total carbon (TC) and COD concentrations before (hydrolysate) and after filtration (permeate and concentrate). In addition to this, the permeability of the ceramic membrane was determined for both the substrates to evaluate the membrane performance during filtration. The final objective was to determine and compare the specific methane yields of hydrolysate, permeate and concentrate obtained from Hohenheim Biogas Yield Test (HBT) for both the substrates.

2. Materials and methods

2.1. Integration of the test facility

The laboratory research plant in this study is consisting of a continuously stirring AR with the volume of 124 L and a ceramic membrane filtration unit. The AR is equipped with a stainless steel sieve of 100 μm. While the dissolved organic compounds that pass through the sieve are taken from the reactor as “hydrolysate”, whereas the undissolved solids remain in the reactor. In this way, the retention time of the non-degraded solids is decoupled from the retention time of the circulating process liquid. A reel agitator with 12 stirring rods is fixed inside the reactor for mixing and the stirring rod is equipped with two brushes to clean the sieve on the side of solid phase continuously (Fig. 1). Heating foils are utilized to heat the AR (60 ± 0.5 °C). A temperature sensor and pH sensor is installed on the liquid phase side of the AR. The detailed construction of the AR is explained in several literatures (Ravi et al., 2018; Lindner et al., 2016; Lindner et al., 2015a).

A ceramic cross-flow membrane filtration system was installed to the AR to remove the inert particles from the hydrolysate produced in the AR. The cross-flow system has an 18 L storage tank to store the hydrolysate, a stainless steel tubular pressure vessel for the ceramic membrane, a permeate tank to collect the produced permeate and a cross-flow pump. The ceramic membrane (Al_2O_3, inopor GmbH, Veilsdorf, Germany) used has a pore size of 0.2 μm, a length of 250 mm and an area of 0.033 m^2. For the integration of a membrane unit in a two-stage anaerobic digestion system ceramic membranes were proved to be a better option for filtration due to their chemical and temperature resistance (Tuczinski et al., 2018).

The AR is operated at 60 °C temperature and is fed every day with the substrate feeding mixture where the hydrolysis and acidogenesis process takes place leading to the production of hydrolysate, which contains high concentrations of organic acids. This hydrolysate is sieved inside the AR and pumped to the buffer Tank 1. Simultaneously, the produced hydrolysate is then pumped to the storage tank of the cross-flow system. Then, it is further pumped to the ceramic membrane to

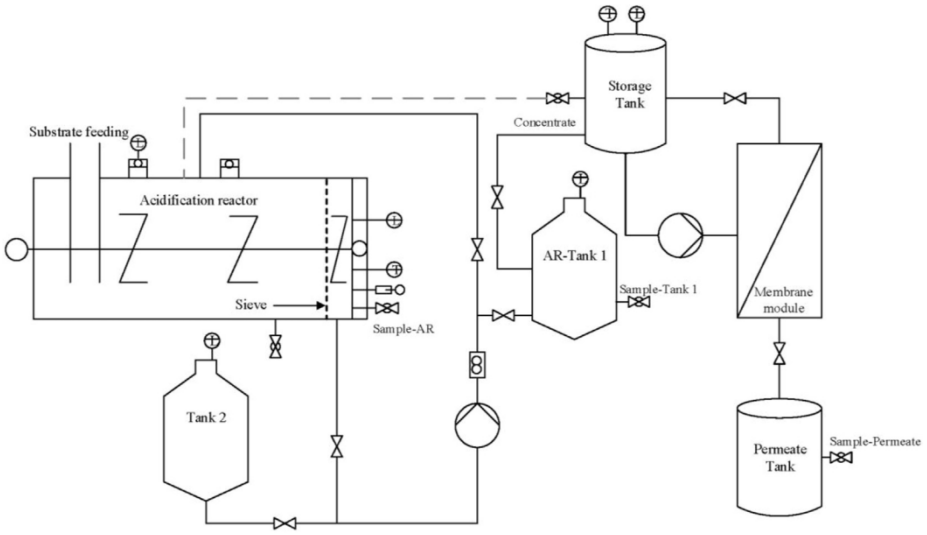

Fig. 1. Piping and instrument schematic diagram of integrated membrane filtration setup in two-stage anaerobic digestion system.

filter the hydrolysate and remove the particles in it. The liquid which passed through the membrane, the so-called "permeate", is collected in the permeate tank and the remaining liquid with particles, the so-called "concentrate", is circulated back into the storage tank. For further improvement of the complete system it is required to pump this concentrate back in to the AR, to ensure that there would be no losses of remaining organic acids and simultaneously increasing the solid retention time in the AR (Fig. 1).

The permeate produced through filtration could be fed to an AF for further processing either in pressurized AF or AF without pressure. However, in this research we did not use any AF to feed the permeate, instead we simulated an AF with the HBT to distinguish the methane production potential of the permeate and hydrolysate.

The AR was operated at the target pH 5.75 by pH-dependent removal of the acid-rich hydrolysate. This pH 5.75 was chosen based on the results obtained by Lindner et al. (2015a) where the target pH value 5.5 and 6 produced hydrolysate rich with high organic acid concentrations. The effluent from AF to regulate the pH in the AR was simulated because the hydrolysate was not fed to an AF. Therefore, a dilution of the liquid manure with water at 1:5 ratio was prepared and stored in the Tank 2. Every time when the pH dropped in the AR below the target value due to the formation of organic acids, 1 L of hydrolysate from the AR was pumped to Tank 1, and 1 L of diluted liquid manure was pumped back to the AR from Tank 2 in order to maintain the pH value stable. The pumping volumes of hydrolysate and the effluent was determined by a flow meter (Type Promag 53P, Endress + Messtechnik GmbH + CO, KG, Stuttgart, Germany). The measured pH value, temperature and pumping volumes were logged in the database.

The gas produced in the AR was collected and stored in gasbags and was measured qualitatively and quantitatively thrice per day. Gas volumes produced were measured using a drum gas meter (TG 20/5, Dr.-Ing. RITTE Apparatebau GmbH & Co. KG, Bochum, Germany). The gas qualities of produced methane, carbon dioxide and hydrogen were detected by a gas analyzer (SICK MAIHACK S710; SICK Vertriebs-GmbH, Düsseldorf, Germany). The gas volumes produced were corrected to standard conditions (1013 hpa, 273.15 K) (Lindner et al., 2016). This research work was executed in the biogas laboratory of the State Institute of Agricultural Engineering and Bioenergy at the University of Hohenheim, Stuttgart.

2.2. Experimental procedure

The experiments were initiated by adding 80 kg of water, 20 kg of separated liquid digestate collected from the biogas plant at "Unterer Lindenhof" (Lemmer et al., 2013), 2 kg maize silage, 1 kg of digestate from past experiments, 0.24 kg acetic acid and 0.13 kg lactic acid to the AR.

For the planned experiments, two different substrates vegetable waste (VW) and grass/maize (G/M) silage were used for feeding the AR. G/M silage used for the experiments was collected from the biogas station "Unterer Lindenhof" and the VW from BAYHA GEMÜSE, a vegetable growing and processing farm in Filderstadt, Germany. These two different substrates were chosen to investigate the influence of different substrates on the performance of the newly integrated membrane filtration unit in two-stage system. The VW included a mixture of carrot mousse, carrots, celery, cabbage and potatoes. The feeding amounts were prepared depending on the dry matter (DM) and organic dry matter (oDM) contents of the respective vegetable as shown in Table 1. The VW pretreatment and their feeding bag preparation was similar to the process described by Ravi et al. (2018). Feeding bags and G/M silage were stored at 4 °C and supplemented by 1 g of trace element mixture (Schaumann Bioenergy, Pinneberg, Germany) per kg of fresh material according to Vintiloiu et al. (2012).

To run the two-stage anaerobic digestion at the target pH value in the AR, the OLR had to be adapted to the process phases ($7.60 \pm 0.33\ kg\ m^{-3}\ d^{-1}$ for VW and $8.74 \pm 0.44\ kg\ m^{-3}\ d^{-1}$ for G/

Table 1
Feeding amounts and DM/oDM (%) of two different substrates fed to the AR.

Substrate	Amount (g)	Average DM (%)	Average oDM (%)
VW			
Carrot Mousse	500	2.67 ± 0.22	2.43 ± 0.20
Carrot	1250	9.02 ± 0.26	7.89 ± 0.30
Celery	750	9.44 ± 1.11	8.11 ± 1.12
Cabbage	650	6.69 ± 0.44	5.52 ± 0.92
Potato	2150	27.22 ± 1.30	25.91 ± 1.39
Total	5300	15.58 ± 0.58	14.43 ± 0.62
G/M silage			
Grass silage	1510	37.28 ± 3.08	33.45 ± 2.67
Maize silage	1080	35.61 ± 2.18	34.27 ± 2.18
Total	2590	35.84 ± 2.84	33.07 ± 2.35

Table 2
Significant parameters measured in the AR and Membrane system for two different substrates.

Parameter	VW	G/M silage
OLR_{AR} (kg m^{-3} d^{-1})	7.60 ± 0.33	8.74 ± 0.44
pH_{AR}	5.76 ± 0.10	5.81 ± 0.05
$Temperature_{AR}$ (°C)	60.34 ± 1.14	60.26 ± 1.36
SRT (d^{-1})	21.65	44.29
RT_{AR} (d^{-1})	4.93	3.24
Hydrolysate (L d^{-1})	23.25	35.44
Hydrolysate (L kg^{-1} FM)	4.39	13.68
Hydrolysate (L kg^{-1} oDM)	30.59	40.55
$Temperature_{Membrane\ system}$ (°C)	47.38 ± 3.03	50.08 ± 2.99
Working $Pressure_{Membrane\ system}$ (bar)	0.64 ± 0.14	0.66 ± 0.14
$Permeate_{Membrane\ system}$ (L d^{-1})	29.36	28.92

M silage at pH 5.75, respectively) (Table 2). The system was fed daily with 5.30 kg and 2.59 kg fresh matter (FM) for VW and G/M silage experiments respectively. These experiments were run stable for about 18 days of short span continuously for both the substrates. For each substrate a new membrane was used during the experimental run. The samples were collected daily from the AR-Tank 1, Permeate Tank and concentrate from Storage Tank.

2.3. Lab analysis

The collected samples hydrolysate, permeate and concentrate were analyzed in the laboratory to determine COD, VFAs, alcohols, sugar contents, total organic carbon (TOC), total carbon (TC), inorganic carbon (IC), total nitrogen (TN). The fresh substrate materials were individually tested for the contents of DM (%) and oDM (%) twice per week by drying at 105 °C for 24 h followed by incineration at 550 °C for 8 h (VDI-Society Energy and Environment, 2006). Hach Lange's cuvette test (Hach Lange Type LCK 014) was used to determine the COD content of liquids. The testing system includes a high temperature thermostat (Hach Lange Type HT 200 S) and a sensor array photometer (Hach Lange Type LASA 20). The concentrations of VFAs (acetic acid, propionic acid, n- and iso-butyric acid, n- and iso-valeric acid and caproic acid) were determined by gas chromatography (GC with 2100Plus with a FID-detector and a capillary column WCOT Fused Silica, Shimadzu, Germany). Other organic acids (D/L-lactic acid and formic acid), sugars like glucose and sucrose and alcohols like propylene glycol and ethanol were determined by high performance liquid chromatography (HPLC with RI-detector, BioRad Aminex HPLC column HPX-87H, BioRad-precolumn HPX-87H, BISCHOFF Analysentechnik und -geräte GmbH, Leonberg, Germany). The TOC/TNb analyzer (Analytik Jena AG Type multi N/C®, Jena, Germany) was used to determine the TC, TOC, IC and TN. These methods are well described by Lindner et al. (2015a) and Stockl and Oechsner (2012).

2.4. Batch digestion test

Hohenheim Biogas Yield Test (HBT) was performed to determine the biogas and methane yields of hydrolysate, permeate and concentrate produced from the two different substrates during the experiments. The process of HBT was described in detail by Mittweg et al. (2012). This test was executed under the regulation of VDI Guideline 4630 and explained in detail by Lindner et al. (2015b) and Mittweg et al. (2012).

2.5. Calculated parameters

In AR, the parameters influencing the process like temperature, pH and hydrolysate pumped were monitored and recorded in real time. The recorded data was saved in a "MySQL database" (Toad for MySQL 7.3.1.3290). According to several literature work, the OLR in the AR was related to oDM of the substrates fed and additionally, the solid retention time (SRT) and recirculation time (RT_{AR}) were calculated as explained in literature (Ravi et al., 2018; Lindner et al., 2015a; Krümpel et al., 2016). The pressure and temperature values of the membrane system were recorded and saved as csv files. The data retrieved was investigated and calculated by means of "R Studio" (Version 1.0.143) statistical software.

Solid retention time (SRT) is the ratio of fermenter volume (114.72) to the volume of fresh matter fed ($\dot{v}_{fm}$) (Ravi et al., 2018) (Eq. (1)).

$$SRT = \frac{114.72}{\dot{v}_{fm}} \quad (1)$$

The ratio of fermenter volume (114.72) to the exchange volume ($\dot{v}$) is defined as recirculation time (RT_{AR}) (Ravi et al., 2018) (Eq. (2)).

$$RT_{AR} = \frac{114.72}{\dot{v}} \quad (2)$$

The permeabiltiy $[L{\cdot}h^{-1}{\cdot}m^{-2}{\cdot}bar^{-1}]$ of the ceramic membrane was calculated as (Eq. (3)).

$$Permeabilty\ \ [Lh^{-1}m^{-2}bar^{-1}] = \frac{P_f}{O_p\ \times Ms_a} \quad (3)$$

Where $P_f\,(L/h)$ is permeate flow, O_p is operating pressure (bar) and Ms_a is surface area of membrane (m^2).

3. Results and discussion

3.1. Variance in gas qualities and quantities in the AR for 2 different substrates

The targeted pH value and temperature in the AR was maintained very stable during the experimental run for both the substrates (Table 2). The SRT of G/M silage (44.29 days) was 51.12% higher than VW substrate (21.65 days). On contrary to this the RT_{AR} of VW (4.93 days) was 34.28% higher than G/M silage (3.24 days).

The gas concentrations produced in the AR for two different substrates are shown in Fig. 2. At target pH value 5.75, the methane content of the produced gas in the AR is slightly higher for G/M silage (30.93 ± 3.47%) than VW (24.76 ± 1.52%). In contrary to this, the hydrogen content produced was higher for VW (2.80 ± 1.13%) than G/M silage (0.88 ± 0.16%). Carbon dioxide produced was 72.66 ± 1.97% and 63.82 ± 8.70% for VW and G/M silage, respectively.

The quantities of methane produced in relation with OLR for G/M silage (49 ± 11.49 L kg^{-1} oDM) was higher than the VW (42.38 ± 5.76 L kg^{-1} oDM). Whereas the hydrogen quantity produced for VW (4.65 ± 1.40 L kg^{-1} oDM) was higher than for G/M silage (1.39 ± 0.35 L kg^{-1} oDM). Carbon dioxide quantities produced were 124.59 ± 17.54 L kg^{-1} oDM and 99.63 ± 18.06 L kg^{-1} oDM for VW and G/M silage, respectively.

Overall, the composition of the product gas in the AR showed only minor differences between the two substrates. The hydrogen content was produced slightly higher for VW than G/M silage while easily degradable substrates like VW led to a decrease of the pH value below the target pH of 5.75, resulting in an increased hydrogen production (Lindner et al., 2016). In contrast, methane concentration was higher for G/M silage than VW. Declining pH value in the AR leads to an inhibition of methane formation and further resulting in the increase of hydrogen production (Lindner et al., 2016, 2015a; Ravi et al., 2018). Similar to the research done by Lindner et al. (2016) the methane concentration produced showed significant difference with initial substrate fed to the reactor. The gas quantities of methane and hydrogen showed the same behavior as gas concentrations elevation. The research done by Wijekoon et al. (2011) witnessed that with increasing OLR, the methane quantities produced per day (L d^{-1}) also increased. The results of the gas concentrations and quantities of VW at pH value

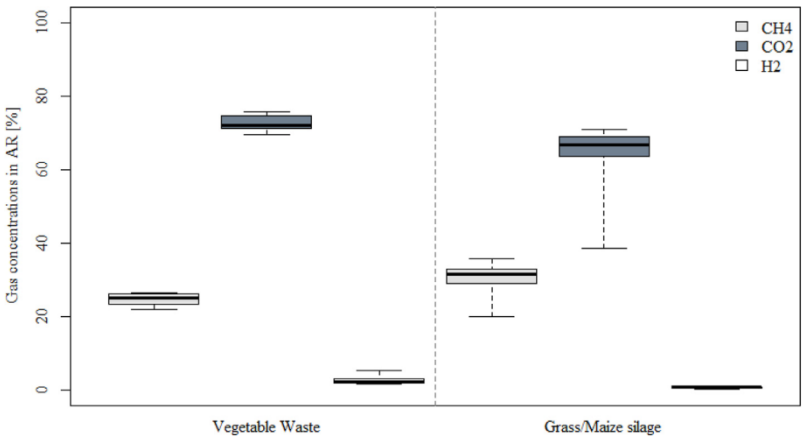

Fig. 2. Difference in the gas concentrations measured in the AR for two different substrates.

5.75 are highly relevant to the experiments done by Ravi et al. (2018).

3.2. Influence of substrates on significant parameters of the AR & membrane filtration

In AR, the amount of hydrolysate produced was higher for G/M silage than VW substrate as shown in Table 2. Likewise, the hydrolysate produced in relation to the FM fed to the AR was 13.68 (L kg^{-1} FM) and 4.39 (L kg^{-1} FM) for G/M silage and VW, respectively. Similarly, the specific hydrolysate produced in relation to the added oDM was 40.55 (L kg^{-1} oDM) for G/M silage and 30.59 (L kg^{-1} oDM) for VW. In both the cases the produced hydrolysate is higher for G/M silage.

The most important parameters which played key role in the membrane filtration process were temperature, pressure and the amount of permeate produced per day. Contradictory to the effect of substrates on the parameters of AR, the substrates showed no distinctive differences with respect to the membrane system performance. The working temperature (°C) of the membrane system could be kept stable throughout the experimental phase 47.38 ± 3.03 and 50.06 ± 2.99 for VW and G/M silage, respectively. The working pressure on the membrane was similar for both substrates too, as shown in the Table 2. For both the substrates VW and G/M silage the membrane filtration process worked identically which is reflected in the identical values of the produced permeate (29.36 L d^{-1} for VW and 28.92 L d^{-1} for G/M silage).

In the current experiments, the working temperature and pressure were significantly maintained stable leading to no distinctive difference in the amounts of permeate produced for both the substrates (Abadi et al., 2011; Scholz and Fuchs, 2000).

3.3. Intermediate compositions of hydrolysate, permeate and concentrate produced for two different substrates

The intermediate compositions of produced hydrolysate (AR-Tank 1), permeate and concentrate (membrane system) with VW and G/M silage substrates are shown in Table 3. The acetic acid and n-butyric acid concentrations produced in the hydrolysate (AR-Tank 1) were 4.05 ± 1.32 g L^{-1} and 1.72 ± 0.32 g L^{-1} for VW substrate respectively, while the G/M silage produced 3.86 ± 0.27 g L^{-1} acetic acid and 0.54 ± 0.06 g L^{-1} n-butyric acid. Differing to this, the propionic acid produced was 0.62 ± 0.08 g L^{-1} for G/M silage and 0.43 ± 0.06 g L^{-1} for VW. Whereas the concentrations of iso-butyric acid, iso-valeric acid, n-valeric acid and caproic acid showed insignificant differences for both the substrates by producing small negligible fractions under 0.1 g kg^{-1} (Table 3). The TC, TOC and TN concentrations of hydrolysate are 8.30 ± 1.29 g L^{-1}, 8.20 ± 1.29 g L^{-1} and 1.30 ± 0.08 g L^{-1} for VW and 6.28 ± 0.59 g L^{-1}, 5.98 ± 0.36 g L^{-1} and 1.07 ± 0.06 g L^{-1} for G/M silage, respectively. The IC concentration of hydrolysate was 0.31 ± 0.25 g L^{-1} for G/M silage and 0.10 ± 0.16 g L^{-1} for VW. The COD concentration of the hydrolysate was 22.10 ± 2.40 g L^{-1} for VW substrate and 15.03 ± 1.69 g L^{-1} for G/M silage (Table 3).

The organic acid concentrations from the hydrolysate could almost be recovered entirely after the membrane filtration step, since permeate and concentrate showed only minor differences for VW. The acetic acid concentration recovered for VW through the membrane filtration step was 4.13 ± 0.63 g kg^{-1} in the permeate and 3.86 ± 0.83 g kg^{-1} in the concentrate, respectively. The recovered amount of n-butyric acid was 1.66 ± 0.32 g kg^{-1} and 1.52 ± 0.34 g kg^{-1} in the permeate and concentrate individually. All other fractions of organic acids remained unaffected showing no significant differences through filtration. Contradictory to this, only 41.81% of TC and 40.98% of TOC in the hydrolysate was extracted through filtration in the permeate where the TOC is measured as dissolved organic carbon (DOC) when filtered with ≤ 0.45 µm pore size (Tuczinski et al., 2018). Similarly, only 38.19% COD concentration was recovered in the permeate (8.44 ± 0.86 g L^{-1}). The TC, TOC and COD in the concentrate was measured (Table 3).

For G/M silage the organic acid concentrations from the hydrolysate recovered after the membrane filtration step in the permeate and concentrate showed significant difference. The acetic acid concentration retrieved through membrane filtration step was 1.92 ± 1.12 g kg^{-1} in the permeate and 1.75 ± 0.01 g kg^{-1} in concentrate, respectively. All other fractions of organic acids remained unaffected showing no significant differences through filtration. Contrarily, only 39.49% of TC and 34.95% of TOC concentration in the hydrolysate was extracted through filtration in the permeate, which is DOC as mentioned above, and the TC, TOC and COD measured in the concentrate was in the form of particles. Similarly, only 33.13% COD concentration was recovered in the permeate (4.98 ± 1.05 g L^{-1}) (Table 3).

When permeate with diluted COD fed to the AF, the hydraulic retention time will be reduced which could be a problem for normal AF. However, the COD related biogas produced with permeate is increased even with lower COD. But for high pressure AF high amounts of permeate can be fed and CO_2 in gaseous phase is reduced due to higher amounts of liquid.

3.4. Permeability of the ceramic membrane for two different substrates

The permeability effect of the ceramic membrane (0.2 µm) on different substrates VW and G/M silage is represented in the form of

Table 3
Intermediate concentrations and compositions of hydrolysate (AR-Tank 1), permeate and concentrate (Membrane system) for two different substrates.

Parameters	pH 5.75					
	VW			G/M silage		
	Hydrolysate	Permeate	Concentrate	Hydrolysate	Permeate	Concentrate
Acetic acid (g kg^{-1})	4.05 ± 1.32	4.13 ± 0.63	3.86 ± 0.83	3.25 ± 0.27	1.92 ± 1.12	1.75 ± 0.01
Propionic acid (g kg^{-1})	0.43 ± 0.06	0.53 ± 0.08	0.54 ± 1.12	0.62 ± 0.08	0.65 ± 0.06	0.70 ± 0.08
iso-Butyric acid (g kg^{-1})	0.01 ± 0.02	0.02 ± 0.02	0.02 ± 0.02	0.03 ± 0.02	0.04 ± 0.01	0.04 ± 0.01
n-Butyric acid (g kg^{-1})	1.72 ± 0.32	1.66 ± 0.32	1.52 ± 0.34	0.54 ± 0.06	0.51 ± 0.09	0.54 ± 0.09
iso-Valeric acid (g kg^{-1})	0.00 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.03 ± 0.02	0.05 ± 0.01	0.06 ± 0.01
n-Valeric acid (g kg^{-1})	0.03 ± 0.04	0.06 ± 0.03	0.06 ± 0.03	0.05 ± 0.02	0.05 ± 0.03	0.05 ± 0.02
Caproic acid (g kg^{-1})	0.04 ± 0.07	0.05 ± 0.05	0.05 ± 0.05	0.00 ± 0.01	0.02 ± 0.02	0.02 ± 0.03
TC (g L^{-1})	8.30 ± 1.29	3.47 ± 0.35	19.81 ± 1.92	6.28 ± 0.59	2.48 ± 0.36	18.96 ± 2.37
TOC (g L^{-1})	8.20 ± 1.39	3.36 ± 0.38	19.61 ± 1.99	5.98 ± 0.36	2.09 ± 0.61	18.49 ± 2.39
IC (g L^{-1})	0.10 ± 0.16	0.11 ± 0.13	0.19 ± 0.16	0.31 ± 0.25	0.38 ± 0.29	0.46 ± 0.31
TN (g L^{-1})	1.30 ± 0.08	0.88 ± 0.08	2.29 ± 0.41	1.07 ± 0.06	1.33 ± 1.95	2.21 ± 0.30
COD (g L^{-1})	22.10 ± 2.40	08.44 ± 0.86	49.30 ± 5.16	15.03 ± 1.69	04.98 ± 1.05	50.72 ± 4.64

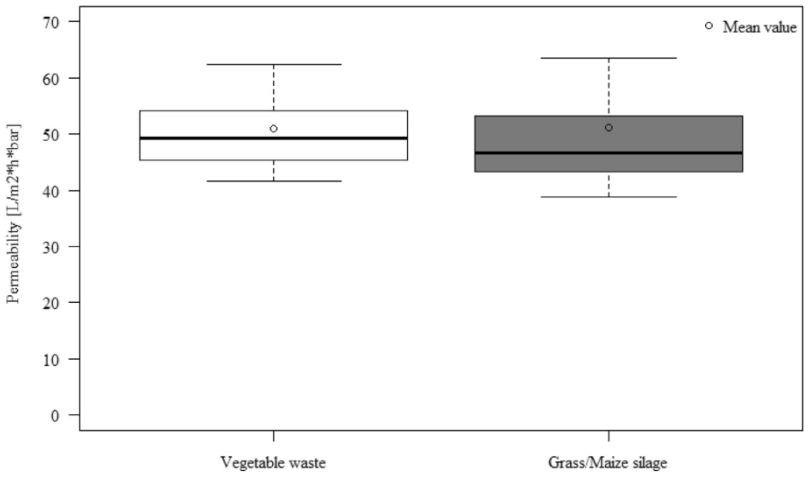

Fig. 3. Permeability of the ceramic membrane measured for two different substrates.

boxplot in Fig. 3. The permeabiltiy of the membrane showed no distinguishable difference with the substrates used at a cross-flow velocity (v_{cf}) > 1.5 m·s^{-1}. The median value of the permeabiltiy was 49.25 L m^{-2} h^{-1} bar^{-1} for VW and 46.63 L m^{-2} h^{-1} bar^{-1} for G/M silage. During the experimental run, no significant membrane blockage due to cake formation or membrane fouling was observed. As Martinez-Sosa et al. (2011) showed, higher fouling rates were measures at lower temperatures. In addition, the preliminary tests conducted by Jeison and van Lier (2007) showed that cross-flow operation may be a feasible alternative to reduce particle deposition, in case only if the high shear stress does not negatively affect the physical properties of the sludge.

3.5. Substrate specific methane yields of hydrolysate, permeate and concentrate produced for two different substrates

The HBT tests were performed to investigate the methane yield potentials of hydrolysate, permeate and concentrate to evaluate the effect of a filtration step. HBT results of substrate specific methane yields (L kg^{-1} COD_{input}) of produced hydrolysate (AR-tank1), permeate and concentrate (membrane system) for VW and G/M silage substrates based on COD_{input} are shown in the Fig. 4. The specific methane yield of the hydrolysate was 158.25 L kg^{-1} COD_{input} (VW) and 171.42 L kg^{-1} COD_{input} (G/M silage). While the specific methane yield of permeate was 264.01 L kg^{-1} COD_{input} and 227.15 L kg^{-1} COD_{input} for VW and G/M silage, respectively. Similarly, the specific methane yield of concentrate was 166.12 L kg^{-1} COD_{input} for VW and 89.98 L kg^{-1} COD_{input} for G/M silage.

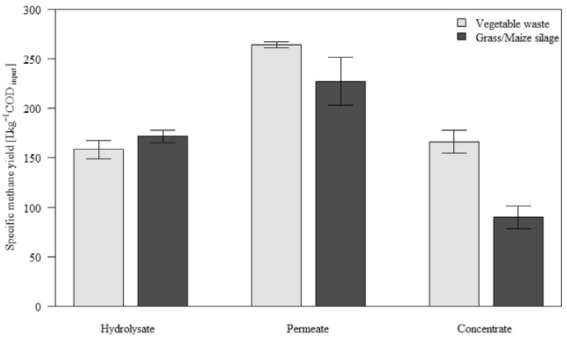

Fig. 4. Substrate specific methane yields of hydrolysate, permeate and concentrate obtained from two different substrates.

For VW substrate, the specific methane yield of permeate was 40% higher than that of hydrolysate. Similar to VW results, the specific methane yield of the permeate for G/M silage was also 24.53% higher than hydrolysate.

Through microfiltration step the COD concentration in the permeate

could be reduced in comparison to the hydrolysate while the organic acid concentrations remained stable. The reduction in COD concentration is due to the filtration of inert particles left behind in the concentrate. By filtration of these inert particles which cannot be converted into biogas, the higher specific methane yield of the permeate than hydrolysate can be explained, also shown by results from Tuczinski et al. (2018). The inert particles are accumulated in the concentrate leading to higher COD concentrations. While the organic acid concentrations remain constant but are adhered on the surface of fibrous substances and particles making it not possible to pass through the microfiltration and also hardly accessible for microorganisms. Because of this, the COD related specific methane yield of concentrate is much lower in comparison to hydrolysate and permeate especially for the G/M silage. However, for both the substrates, the permeate has a higher specific methane yield than the yield of hydrolysate, which shows that after the removal of inert particles from the hydrolysate through membrane filtration process the COD_{input} degradability of the permeate is much higher.

4. Conclusion

High biological process stability of two-stage anaerobic digestion processes are interesting approaches for fermentation of organic residues, especially when methane reactors are designed as AF. Therefore, the hydrolysate from first stage must be free of particles. This investigation proves that by integrating cross-flow membrane filtration between the two stages, inert particles can be separated from hydrolysate so that AF clogging or inefficient biofilm formation is prevented. Furthermore, membrane filtration enables efficient extraction of organic acids from hydrolysate, leading to increased specific methane yields. Overall, the performance of expensive AF can be significantly increased by integrating membrane filtration in two-stage system.

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4 Publication 3: Utilization of process liquids with high organic loads in bioelectrochemical systems: organic degradation rates & current densities

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Abstract

This study presents investigations on an enlarged lab-scale bioelectrochemical systems (BES), which are necessary for a technical upscaling of the processes. Maize silage was first hydrolysed and the process liquids hydrolysate and a membrane filtered permeate was subsequently fed to the BES anode chamber. Aim was to investigate the suitability of mixed-culture BES for utilization of process liquids obtained. In all runs, organic acids were completely degraded. Permeate showed 87% and 88% of COD and TOC degradation. Average current density of 470 $\mu A/cm^2$ was attained for hydrolysate with added acetic acid. Altering the pH value of used process liquids from 5.75 to 7 led to significantly higher current production and degradation rates. Experiments revealed a positive correlation between generated current and ΔORP between anode and cathode chamber. In future, high purity methane or hydrogen production systems can be developed by combining the fermentative biomass digestion with the BES.

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Utilization of process liquids with high organic loads in bioelectrochemical systems: Organic degradation rates & current densities

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ABSTRACT

This study presents investigations on an enlarged lab-scale bioelectrochemical systems (BES), which are necessary for a technical upscaling of the processes. Maize silage was first hydrolysed and the process liquids hydrolysate and a membrane filtered permeate was subsequently fed to the BES anode chamber. Aim was to investigate the suitability of mixed-culture BES for utilization of process liquids obtained and the substrate influence on the BES performance in terms of organic compounds removal. In all runs, organic acids were completely degraded. Permeate showed 87% and 88% of chemical oxygen demand and total organic carbon removal. Average current density of 4.7 A/m^2 was attained for hydrolysate with added acetic acid. Altering the pH value of used process liquids from 5.75 to 7 led to significantly higher current production and degradation rates. Experiments revealed a positive correlation between generated current and difference in redox potential between anode and cathode chamber.

1. Introduction

One of the major global concerns in recent years is the increasing waste production, which negatively impacts the environment, when the organic residues are not recycled or treated sustainably. Globally, waste management practices like landfills, incineration and composting lead to air, water and soil pollution and greenhouse gas emissions (Hoornweg and Bhada-Tata, 2012). Solid organic wastes are abundant resources that can be used for conversion to different biofuels (Zhang et al., 2019). The most common sustainable way of converting organic waste residues to renewable bioenergy is by anaerobic digestion and the production of biogas (Hassanein et al., 2017). The alternative novel sustainable technology with increasing research interest is to treat waste residues in Bioelectrochemical Systems (BES) like Microbial Fuel Cell (MFC) or Microbial Electrolysis Cell (MEC) (Santoro et al., 2017). However, these novel processes are not suitable for the recycling of solid organic residues, as they can only be charged with liquids.

Previous research studies showed that different microbial communities either pure or mixed cultures are involved in the process of current generation (Schmidt et al., 2018; Zhang et al., 2009; Logan et al., 2006). Nimje et al. (2012), Kiely et al. (2010), Watson and Logan (2010) and Bond and Lovley (2003) described BES operated with pure culture systems. Problems with pure culture systems include the low long-term stability, high requirements on the substrates like sterilization, strict anoxic conditions and application of chemical inhibitors suppresses the simultaneous undesired degradation pathways (Schmidt et al., 2018). Therefore, Nimje et al. (2012), Kiely et al. (2010), Watson and Logan (2010), Venkata Mohan et al. (2008) and Bond and Lovley (2003) suggested mixed culture BES systems. These mixed culture BES systems are advantageous for their long-term stability, easy substrate handling, robustness due to nutrient adaptability, stress resistance and general tendency to produce higher current densities (Chen et al., 2019; Nimje et al., 2012).

In addition to microbial cultures, there are other important factors that are crucial for an ideal BES performance. Operational parameters including anode potential, pH value, temperature, external resistance and feeding mode greatly influence BES performance and thus the resulting current generation (Jadhav and Ghangrekar, 2009; Pham et al., 2009). Research done by Jadhav and Ghangrekar (2009) showed that MFC fed with influent anodic pH maintained at 7.5, 7.0, 6.5, 6.0 and 5.5 affected the performance and the internal resistance. According to He et al. (2008), bacteria require a pH close to neutral for their optimal growth. Similarly, Ren et al. (2007) found a significant decrease of the power production when the final pH dropped to 5.2 due to the acidic

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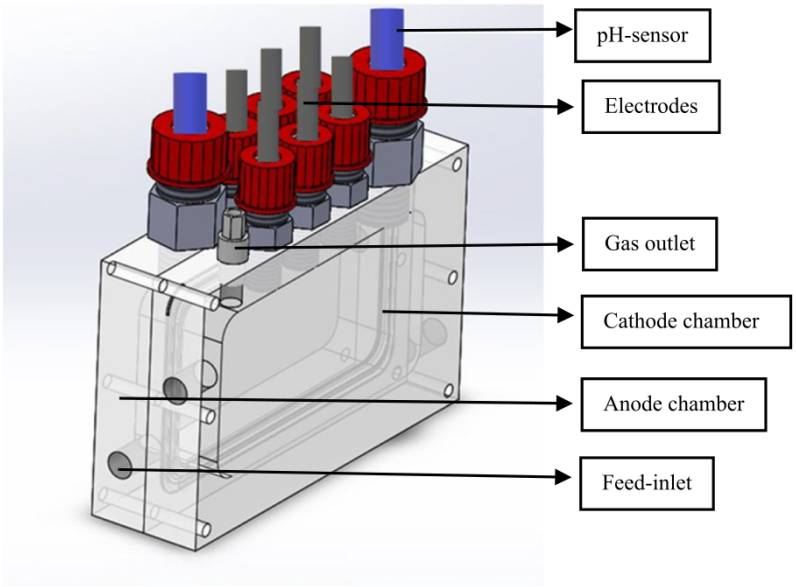

Fig. 1. Two chamber BES detailed construction with all the ports.

products of fermentation and resumed quickly when the pH was recovered to 7.0.

Microbial conversion of organic residues in BES may become an interesting technical application in the future because exoelectrogens can convert a wide range of soluble or dissolved complex organic wastes and renewable biomass. Different kinds of substrates can be digested anaerobically by exoelectrogens in BES like vegetable waste, potatoes, food waste, lignocellulosic biomass and also vegetable waste, vegetable waste in the form of hydrolysate mainly containing acetate, butyrate and propionate were oxidized and treated in BES (Schmidt et al., 2018; Hassanein et al., 2017; Santoro et al., 2017; Tharali et al., 2016; Du and Li, 2016; Kaur et al., 2013; Venkata Mohan et al., 2010; Zeng et al., 2010; Pant et al., 2010; Logan et al., 2008). Furthermore, synthetic waste water streams are also used as a substrate to evaluate the performance of BES because it is easy to control the parameters like pH and loading rates (Pant et al., 2010; Jadhav and Ghangrekar, 2009; Venkata Mohan et al., 2008). However, according to Ren et al. (2007) lignocellulosic biomass cannot be directly utilized by microorganisms in MFCs for electricity generation. It must be converted to monosaccharides or other low-molecular-weight compounds. Also the results of Catal et al. (2019) and Catal et al. (2008) demonstrated that all the monosaccharides in a hydrolysate from acid hydrolysis of lignocellulosic materials like pinewood flour and hazelnut leaves could be used for electricity and hydrogen generation. Moreover, the prominence of present waste management is on reuse and recovery of energy, which has led to new views on how these streams can be dealt with (Pant et al., 2010).

The novelty of this research work is that it is a prerequisite study to upscale the real time waste residues treatment in a continuously run BES. Different solid organic waste residues available for the treatment are not feasible to be fed directly to a BES reactor. Therefore, primarily the waste residues are treated and converted to an untreated process liquid called hydrolysate rich with organic acids in the acidification reactor of a two-stage anaerobic system (Ravi et al., 2018). Additionally, unlike in an anaerobic filter, it is very important to avoid problems caused by inert particles including blockage, biofouling and low microbial degradability in subsequent processing of hydrolysate in BES. Therefore, ceramic membrane filtration of the hydrolysate is performed to attain inert particle free process liquid called permeate (Ravi et al., 2019). The acquired untreated process liquids with the pre-process steps are then fed to the BES to see if there is any difference in the results obtained between particle rich hydrolysate and particle free permeate. The hypothesis of the present research is that permeate and mixed bacterial culture as a suitable substrate for BES than hydrolysate which would be reasonable for application in continuous operation of upscaled BES. Thus, practical implementation of BES requires pre-treatment of organic wastes and application of sustainable bacterial cultures able to stay efficient beyond special laboratory conditions. In the course of our research special attention was also paid to change in oxidation-reduction potential (ORP) in relation to current generation. From this point of view, regulation of pH and resulted in change in ORP could be also one more factor determining performance of BES.

The main objective of this study was to investigate the performance of mixed culture BES when fed with two different untreated process liquid substrates like maize silage hydrolysate and permeate rich with organic acids in batch tests. The influence of these substrates on the performance of BES was evaluated by the measured and calculated consumption and degradation rates of the organic acids, chemical oxygen demand (COD) and total carbon/total organic carbon (TC/TOC) over the period of time for all the runs. Furthermore, the resulted current generation over the period of time was correlated with the dominant organic acids degradation of acetic acid, butyric acid and propionic acid. Additionally, the coulombic efficiencies were determined in two ways: in relation to COD degradation and in relation to organic acids degradation. Important performance parameters – pH value and redox potentials – were measured and evaluated during the experiments. Further, the developed electroactive biofilm was evaluated, and a correlation coefficient was determined between current and difference of redox potentials in the anode and cathode chambers (ΔORP).

2. Materials & methods

2.1. Two chamber BES construction

The BES reactors were constructed in the biogas laboratory at the

State Institute of Agricultural Engineering and Bioenergy at the University of Hohenheim, Stuttgart, Germany. Two similar reactors were made of Plexiglas, which were flexible to run either as a single chamber BES without a proton exchange membrane (PEM) separation or a two chamber BES with a membrane. For this research they were used as a two chamber BES separated with a cation exchange membrane (fumasep® Ultrex™ CMI-7000, Fumatech BWT GmbH, Bietigheim-Bissingen, Germany) to an anode and a cathode chamber. The total volume of the reactor was 400 mL, with an anode chamber of 220 mL and a cathode chamber of 180 mL. The two chamber BES was constructed as shown in Fig. 1. The cells had ten ports in total, with five ports per chamber on the top of the reactor. For the anode chamber the five ports were allocated for pH sensor, reference electrode, gas outlet and two electrodes connected together as a common working electrode. Whereas for the cathode chamber the ports were for pH sensor, gas outlet and three electrodes connected together as a common counter electrode. The reason for application of multiple electrodes in both chambers was to increase the surface area of the electrodes. In addition, each chamber had two ports on either side of the chamber as inlet and outlet ports installed with a septum to collect the samples with syringes.

The electrodes used in both chambers were graphite rods (CP-Graphitprodukte GmbH, Wachtberg, Germany) with an anode surface area of 37.7 cm^2 and a cathode surface area of 56.6 cm^2 in total. The reference electrode used to apply the potential was Ag/AgCl electrode (Gamry Instruments, Warminster, USA). The pH values and redox potentials (ORP) were measured during the experimental period by combined redox-pH sensors (GMH 3511-set, GHM Messtechnik GmbH — Greisinger, Regenstauf, Germany) in both chambers. Redox potential in both reactors was measured as real-time signal vs. Ag/AgCl reference electrode. The gas outlets from both chambers were connected to their respective gas bags. The anode, cathode and reference electrodes of each reactor were connected to the respective potentiostats (Interface 1010 Potentiostat 12012, Interface 1010 Potentiostat 12013, Gamry Instruments, Warminster, USA). The two BES reactors were placed in a climate chamber to maintain a constant temperature of 30 °C.

The working electrodes in the anode chamber were poised to 0 mV versus standard hydrogen electrode (SHE) and the current produced was measured by the potentiostat. Four pH sensors were connected to the computer through a serial interface converter (GRS 3105, GHM Messtechnik GmbH — Greisinger, Regenstauf, Germany). Two potentiostats were connected to the same computer as well. The pH values and redox potentials were measured throughout the experimental period, and measurement data was acquired with the help of data logger EASYBUS-Software (EBS 20 M, GHM Messtechnik GmbH — Greisinger, Regenstauf, Germany). The applied voltage and the current produced were measured and recorded in the Gamry Framework™ and further analyzed in the Echem analyst™ (Gamry Instruments, Warminster, USA).

2.2. Feeding substrates and microorganisms

In this research the feeding substrates in the anode chamber were hydrolysate and permeate produced from maize silage. Maize silage was chosen as a raw material for hydrolysate because it is the most popular source of substrate for biogas plants. Aiming on practical implementation of emerging BES technologies it is reasonable to follow conventional technologies of utilization of organic wastes. Therefore, a continuously operated acidification reactor (AR) was fed with maize silage at 60 °C and pH 5.75, where hydrolysis and acidogenesis took place producing the hydrolysate rich with organic acids, as described in detail in (Ravi et al., 2019; Ravi et al., 2018). The permeate was produced by filtering the hydrolysate at a 45–50 °C temperature in a cross-flow ceramic membrane filtration system with a membrane pore size of 0.2 µm. The filtration step was applied to remove the inert particles from the hydrolysate and to extract the organic acids efficiently (Ravi et al., 2019). The hydrolysis in the acidification reactor and the ceramic membrane filtration processes were explained in detail in the research by Ravi et al. (2019) and Ravi et al. (2018). The cathode chamber was fed with the effluent taken from a biological hydrogen methanation plant (BHM) (Ullrich and Lemmer, 2019; Lemmer and Ullrich, 2018) in order to promote efficient reduction of gaseous hydrogen in the close proximity to the cathode's surface and its further consumption by hydrogenotrophic microorganisms. At the same time, BHM effluent did not contain any organic acids which availability could lead toward development of acetoclastic microorganisms.

No specific microbial cultures were added to the reactors. Instead, mixed microbial communities from the feeding substrates were allowed to develop on the electrodes surface under applied potential. As microbial sources the mixed cultures already present in the feeding substrates originating from the AR were used in the anode chambers and in the cathode chambers the mixed culture from BHM plant was used. Under the influence of applied potential on anode, a self-established biofilm was formed on the surface of the electrode. The mixed microbial culture of AR was preferred for the availability of electrogenic microorganisms in the substrate and their tolerance toward negative environmental factors like aerobic conditions.

Hydrolysate used as a substrate for anode chambers was produced in the acidification reactor of the two-stage anaerobic digestion system. As explained in detail by Ravi et al. (2018), the AR was initially started by adding 20 kg of separated liquid digestate collected from the biogas plant at “Unterer Lindenhof” (Lemmer et al., 2013) and 1 kg of digestate from the past experiments as microbial inoculum for the experimental run. While the effluent from the BHM plant used as a substrate for cathode chambers was produced by using the effluent from the anaerobic filter of lab-scale two-stage system's to provide the necessary microbes and nutrients and was sprinkled over the fixed bed and a trickled bed reactor. This process was also explained in detail by Ullrich and Lemmer (2019) and Lemmer and Ullrich (2018).

Table 1
Detailed over view of the planned experimental Runs 1, 2 and 3 with different operational parameters.

Experiments	Experimental plan			
	Anode feed	Cathode feed	Inoculation	Applied voltage
Run 1	• Hydrolysate-maize silage • pH elevated to 6.8 • Acetic acid added up to 20 g kg^{-1}	Effluent (BHM plant)	Mixed culture (Anode: AR/cathode: BHM plant)	−200 mV Vs Ag/AgCl
Run 2	• Permeate-maize silage • pH elevated to 6.8 • No acetic acid added	Effluent (BHM plant)	Mixed culture (Anode: AR/cathode: BHM plant)	−200 mV Vs Ag/AgCl
Run 3	• Hydrolysate • pH 5.75 no elevation • No acetic acid added	Effluent (BHM plant)	Mixed culture (Anode: AR/cathode: BHM plant)	−200 mV Vs Ag/AgCl

2.3. Experimental setup & electrochemical techniques

The experiments were planned and divided into three different experimental runs (Run 1, Run 2 and Run 3) depending on the varying feeding substrate in the anode chamber as shown in Table 1. All three experimental runs were batch experiments and executed with two identical reactors in parallel to evaluate reproducibility and repeatability. Each experimental run lasted for a duration of 30 days. In Run 1 the anode chamber was filled in with maize silage hydrolysate to which surplus acetic acid was added in order to reach the concentration up to 20 g kg^{-1} acetic acid equivalent in order to investigate whether the increased concentration of acetic acid is completely degradable in the BES or not. Furthermore, the pH value of the hydrolysate was elevated to 6.8 by adding 3-M sodium hydroxide. Whereas the cathode chamber was filled in with effluent with surplus acetic acid up to 3 g kg^{-1} to provide initial food for the microbial growth. And in Run 2 the anode chamber was filled in with maize silage permeate with no added acetic acid but with adjusted pH value at 6.8 by adding 3-M sodium hydroxide. Finally, in Run 3 the anode chamber was filled in with maize silage hydrolysate without added acetic acid and no pH alteration. But for both the experimental runs 2 and 3 the cathode chamber was always filled with the effluent with no alterations. The chemical reaction occurred in anode and cathode chambers are described by Logan et al. (2008).

2.4. Laboratory analysis

The liquid samples from the anode and cathode chambers of both reactors were collected three times per week. In order to determine the concentration of volatile fatty acids (VFAs), chemical oxygen demand (COD), total organic carbon (TOC), total carbon (TC), inorganic carbon (IC) and total nitrogen (TN), samples were analyzed in the laboratory. Additionally, conductivity and salinity tests of the feeding samples were performed with EC300 (VWR International GmbH, Darmstadt, Germany). The concentrations of VFAs (acetic acid, propionic acid, n- and isobutyric acid, n- and isovaleric acid and caproic acid) were determined by gas chromatography (GC with 2100Plus with a FID-detector and a capillary column WCOT Fused Silica, Shimadzu, Germany). Hach Lange's cuvette test (Hach Lange Type LCK 014) was used to measure the COD content of the liquids, which included a high temperature thermostat (Hach Lange Type HT 200 S) and a sensor array photometer (Hach Lange Type LASA 20). Furthermore, to quantify the TC, TOC, IC and TN, a TOC/TNb analyzer (Analytik Jena AG Type multi N/C®, Jena, Germany) was utilized.

2.5. Calculated parameters

To measure the performance of the system, degradation rates of COD and TC/TOC for all the runs were calculated by using the equation Eq. (1) (Di Lorenzo et al., 2010):

$$\text{Degradation rate } [\%] = \frac{\text{Concentration}_{\text{Input}} - \text{Concentration}_{\text{Output}}}{\text{Concentration}_{\text{Input}}} * 100 \quad (1)$$

Furthermore, in order to assess the acetic acid's mass removal from the substrate, the Gompertz function was applied using equation Eq. (2) as follows:

$$m\,[g] = m_0(1 - a \bullet e^{-b \bullet e^{-c \bullet t}}) \quad (2)$$

where m_0 is the initial mass of acetic acid in the substrate (g), a is the coefficient determining Y-coordinate of the asymptote for the function, b is the coefficient determined by the lag phase of bacterial growth and c is the coefficient setting intensive bacterial growth during the second phase (current generation) (day^{-1}).

In order to find the day when the maximal mass degradation rate was achieved, the 1st derivative of the expression (Eq. (2)) was taken:

$$\frac{dm}{dt}[g/day] = -m_0 \bullet a \bullet b \bullet c \bullet e^{-b \bullet e^{-c \bullet t} - c \bullet t} \quad (3)$$

To evaluate the conversion efficiency of organic waste substrates to energy as current in the BES, it is important to calculate the current density. Current densities were always calculated by relating the current produced to the surface area of the anode by using the equation Eq. (4):

$$\text{Current density } [A/m^2] = \frac{\text{Current produced}}{\text{Surface area of electrode}} \quad (4)$$

Coulombic efficiency is a ratio of electric charge measured during the experiment to the maximum possible electric charge which could be released by chemical reactions of the molecules in the substrate. It indicates the extent to which the produced electrons contribute to the desired end product (Stager et al., 2017). The actual charge transferred by the oxidation of the substrate is determined by integrating the current produced over the experimental time:

$$Q\,[C] = \int_{t_{start}}^{t_{end}} I dt \quad (5)$$

The coulombic efficiency (CE) of the system was calculated in two ways in order to investigate the significant differences in the results. As the first option, CE was calculated by considering the removal of organic acids in the substrates. In our case, we have considered the removal of three organic acids contributing significantly to current generation: acetic, propionic and butyric acids. The calculation of CE was done according to Eqs. (5) and (6) (Kaur et al., 2013; Schröder, 2007):

$$\text{Coulombic efficiency } [\%] = \frac{Q}{F * V(8 * \Delta C_{\text{acetate}} + 20 * \Delta C_{\text{Butyrate}} + 12 * \Delta C_{\text{Propinonate}})} * 100 \quad (6)$$

The alternative way of the CE calculation is considering the COD removal during the experiment with Eqs. (5) and (7) (Logan et al., 2006):

$$\text{Coulombic efficiency } [\%] = \frac{8.\,Q}{F * V(\Delta COD)} * 100 \quad (7)$$

where Q is the transferred charge, F is Faraday's constant (96,485 C/mol), V is the volume of the anode chamber (L), ΔC is the difference in VFA concentrations between the beginning and the end of the experiment (mM/L), 8, 20 and 12 in Eq. (6) are the number of moles of electrons produced per mole of acetate, butyrate and propionate, respectively, 8 in Eq. (7) is the mass of oxygen per electron exchanged, ΔCOD is the removed COD and I is the current produced (A) (Du and Li, 2016; Logan et al., 2006). Calculation of CE was performed in two ways in order to facilitate comparison of our results and those ones provided in publications of other authors.

In the process of further analysis of the data acquired during this experimental work, the correlation between two important measured parameters were investigated. To inspect the relationship or connection between current and ORP, the important statistical formula called Pearson correlation coefficient was used (Asuero et al., 2007).

2.6. Microorganisms community analysis

The microbial communities used in these research experiments were mixed cultures, so it was interesting to look into the number of active dominant microbial colonies grown in the BES reactor during the experiments. Therefore, at the end of the experiments, the leftover substrate feed in the BES reactors were collected and plated by surfacing with a spreader without any further dilution by pipetting 100 µL of the solution on the Lysogeny broth (LB) plates in anaerobic conditions. Further to investigate the microbial biofilm on both anodes and cathodes, the microbial biofilm was collected by wiping the electrode surface with a swab and preserving the swab in the test tube with the

Table 2.A
Intermediate concentrations and compositions of feeding substrates in anode chamber at the beginning and end of the different experimental Runs 1, 2 and 3.

Experiments	Run 1		Run 2		Run 3	
Parameters	Start	End	Start	End	Start	End
Acetic acid (g kg^{-1})	20.62 ± 0.31	0.01 ± 0.01	8.39 ± 0.00	0.01 ± 0.02	8.99 ± 0.07	0.23 ± 0.33
Propionic acid (g kg^{-1})	0.73 ± 0.01	0.04 ± 0.05	0.98 ± 0.00	0.30 ± 0.24	1.21 ± 0.01	0.85 ± 0.33
Isobutyric acid (g kg^{-1})	0.07 ± 0.00	0	0.06 ± 0.00	0	0.08 ± 0.001	0.07 ± 0.09
n-Butyric acid (g kg^{-1})	1.05 ± 0.01	0	3.12 ± 0.00	0	3.85 ± 0.01	0
Isovaleric acid (g kg^{-1})	0.13 ± 0.00	0	0.14 ± 0.00	0.01 ± 0.01	0.23 ± 0.00	0.13 ± 0.04
n-Valeric acid (g kg^{-1})	0.03 ± 0.00	0	0.25 ± 0.00	0	0.04 ± 0.00	0
Caproic acid (g kg^{-1})	0.03 ± 0.00	0	1.06 ± 0.00	0	0.03 ± 0.00	0
HAC (g kg^{-1})	22.08 ± 0.34	0.24 ± 0.27	12.13 ± 0.00	0.27 ± 0.21	12.82 ± 0.08	1.05 ± 0.67
TC (g L^{-1})	15.83 ± 0.13	4.87 ± 0.04	8.58 ± 0.00	1.95 ± 0.22	16.30 ± 0.19	4.77 ± 0.51
TOC (g L^{-1})	15.47 ± 0.07	2.98 ± 0.38	8.52 ± 0.00	1.03 ± 0.13	16.29 ± 0.18	3.50 ± 0.29
IC (g L^{-1})	0.36 ± 0.05	1.89 ± 0.04	0.06 ± 0.00	0.92 ± 0.09	0	1.28 ± 0.22
TN (g L^{-1})	2.29 ± 0.04	0.85 ± 0.03	1.55 ± 0.00	0.55 ± 0.05	2.47 ± 0.01	1.08 ± 0.05
COD (g L^{-1})	37.38 ± 0.26	8.58 ± 0.30	22.64 ± 0.00	2.99 ± 0.37	42.17 ± 0.49	10.45 ± 0.67

medium (Swabs and test tube with culture medium, Meus S.r.l, Piove di Sacco (PD), Italy). It was further T-streaked on LB plates to isolate, identify, and study the number of microbial colonies anaerobically. The inoculated LB plates were placed in the incubator at a temperature of 30 °C.

3. Results and discussion

3.1. Intermediate compositions, concentrations and its degradation

3.1.1. Organic acid degradation

The intermediate compositions like organic acids, COD and TC/TOC of the substrates at the beginning and the end of all the chronoamperometric experimental runs in the BES reactor are presented in Table 2.A. Almost all the organic acids were completely degraded in Run 1. Whereas, with the exception of propionic acid in Run 2 (70% degraded) and Run 3 (30% degraded), all the other organic acids were completely degraded. For Run 1 and Run 3 with hydrolysate, all the organic acids were effectively degraded from the 6th to the 27th day whereas in Run 2 with permeate they were degraded faster — between the 6th and 20th day. Because of the faster and complete degradation, the experiments were stopped at the 26th day for Run 2. The most dominant organic acids in the feeding substrates were acetic acid, n-butyric acid and propionic acid. Degradation of these dominant acids in the BES over the period of time for all the experimental Runs 1, 2 and 3 are shown in Fig. 2. Irrespective of the increasing acetic acid concentrations provided in the feeding substrates as in Run 1 (20 g kg^{-1}), the mixed cultures in the reactor could completely degrade it by the end of the experiments in all the runs. Both of the identical reactors running simultaneously showed similar results with very low standard deviations — less than 0.8%. Similar behaviour of microbial adaptation and organic acid degradation was found in research work done by Schmidt et al. (2018). With optimal conditions like pH regulation to 7 and an applied voltage of −200 mV vs. Ag/AgCl reference electrode (0 mV vs. SHE), even higher concentrations of organic acids were degraded successfully. Furthermore, as the acetic acid was the most dominant and altered acid in the substrate solution, all the further analysis is related only to the mass of acetic acid. Modified Gompertz function was used to describe the acetic acid degradation in the substrate solution. Coefficients a, b and c control different parameters of the model as explained above, the calculated values for these coefficients were defined in accordance to the least square method for all three Runs as shown in Table 2.B. Having the analytical expression describing the mass degradation, it is possible to take the first derivative of this expression to define the maximal acetic acid degradation rate (g/day) and the day when this degradation was achieved (Table 2.B). Moreover, from the Table 2.B the acetic acid degradation rates of Run 1 and Run 2 are nearly equal, irrespective of the initial mass of acetic acid provided.

The investigations were designed to determine whether microfiltration separates inert particles so that the degree of degradation of the process liquid supplied can be increased. The achieved COD and TC/TOC removal rates for all the three experimental runs are shown in Table 2.C. After 30 experimental days COD degradation rates from 75 to 87% were reached for all these experiments (initial and final values are provided in the Table 2.A). The COD removal rate for Run 2 with filtered permeate as the substrate was 10–12% higher when compared to Run 1 and Run 3. The TOC removal rates for all the three experimental runs ranged from 78 to 88%. Similar to the COD removal rate, the removal degree of TOC for Run 2 was 7–10% higher when compared to Run 1 and Run 3. TC removal rates from 69 to 77% were reached for all the runs. Particularly in the experiments with permeate (Run 2), the COD and TC/TOC were removed more efficiently as there were no inert particles causing poor microbial biodegradability (Ravi et al., 2019). The TOC removal achieved with hydrolysate in this study was 80.77%, which is nearly comparable to the 79.3% TOC removal obtained in research work done by Schmidt et al. (2018) involving the feeding of vegetable waste hydrolysate in a BES. But with permeate as the substrate, the TOC removal achieved was 87.92%, which is higher when compared to the TOC removal achieved for Run 1 with hydrolysate and 79.3% TOC removal achieved by Schmidt et al. (2018) with hydrolysate. The COD removal rates achieved with the permeate in Run 2 are comparable and similar to the research work done by Du and Li (2016), where the performance of the MFC was investigated with cooked potato waste. The investigations show that microfiltration significantly increases the degradability of the process liquid in terms of COD or TC.

3.2. Current densities produced from substrates

Further investigations should answer questions on whether the acid concentration and the pH value of the process liquid used influenced the measured current densities. The current density values achieved over the period of time for all the different experimental runs are shown in Fig. 2. Maximum average current densities of 4.70, 1.31 and 0.71 A/m^2 were reached for Run 1, Run 2 and Run 3, respectively. The magnitude of current density achieved in Run 1 is in line with outcomes obtained in research work of Hari et al. (2017). The standard deviation between the two identical reactors for all the runs was less than 0.1%. For Run 1 the current density started to rise slowly from the 6th day with a decreasing organic acid concentration. The current produced increased and reached its maximum value of 17.72 mA between the 17th and 19th day. Similar to Run 1, in Run 2 the current density had risen from the 6th day until reaching its maximum value of 4.94 mA between the 15th and 19th day. However, in contradictory to the

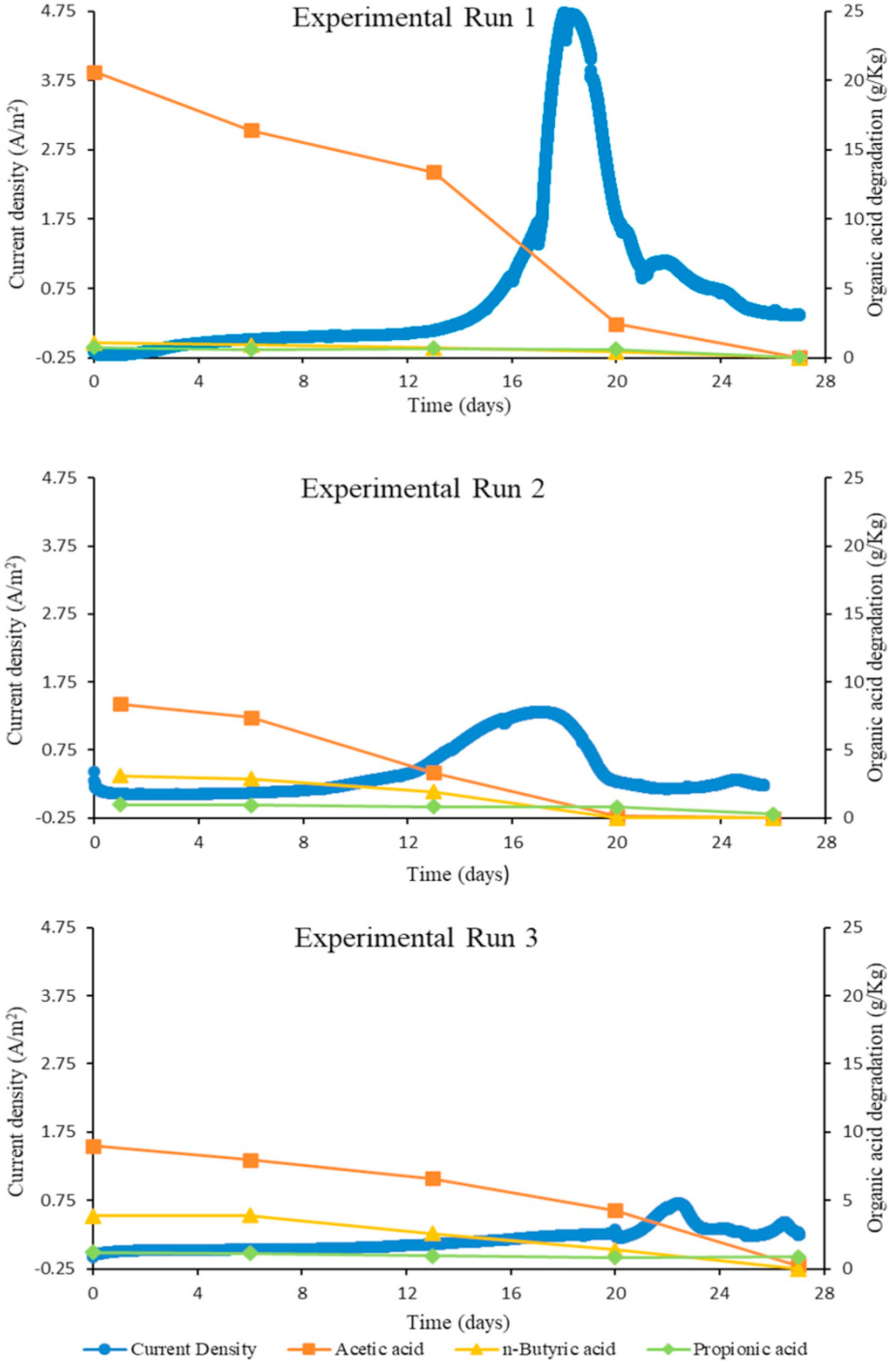

Fig. 2. Simultaneous organic acid degradation and measured current density over time during Run 1, Run 2 and Run 3.

results of Run 1 and Run 2, in Run 3 the current density had started to increase slowly from the 11th day, and the current reached its maximum of 2.67 mA between the 22nd and 23rd day.

The results show that raising the pH value to 6.8 significantly shortens the "lag phase" at the beginning of the experiment until the current density increases because pH value close to neutral provides favorable conditions for biofilm growth. The lower pH of the substrate in Run 3 resulted in a longer adaption of the microbial community and

Table 2.B
Coefficients for expression (Eq. (2)): coefficients are calculated with 95% confidence bounds.

Experiments	Values of the coefficients for best fit curve			Day of maximal degradation rate	Maximal degradation rate (g/day)
	a	b	c		
Run 1	1.2990	3.9930	0.1102	13	−0.2389
Run 2	1.0080	9.5980	0.2983	8	−0.2027
Run 3*	6.7450	5.1750	0.0377	43.5*	–

* Bad value was excluded from initial experimental data.

Table 2.C
COD and TC/TOC removal rates attained during the experimental Runs 1, 2 and 3.

Experiments	COD (%)	TOC (%)	TC (%)
Run 1	77.05 ± 0.68	80.77 ± 0.09	69.27 ± 0.02
Run 2	86.78 ± 0.58	87.92 ± 1.07	77.33 ± 1.81
Run 3	75.22 ± 1.33	78.55 ± 1.09	70.74 ± 1.97

a retarded formation of the biofilm on the anode electrodes. The maximum current density produced can be correlated to the concentration and to the degradation of acetate, butyrate and propionate (Schmidt et al., 2018). Run 1 with hydrolysate containing a high acetate concentration produced the highest current density. Projecting that when the anode mixed microbial communities are provided with adaptable pH 7 and higher amounts of acetate, they are capable of producing a higher current density. The gradually decreasing values of current density and organic acid concentration happened simultaneously, showing the interdependency between the two processes (Schmidt et al., 2018).

3.3. Coulombic efficiencies

The coulombic efficiency of the processes is calculated in two different ways: first by quantifying the removal efficiency of the organic acids followed by the removal efficiency of COD by bacterial anode respiration. The coulombic efficiency of the different experimental runs is presented in Table 3. When compared to Run 3, both Run 1 and Run 2 showed higher coulombic efficiencies in both the calculated cases, showing minor insignificant differences. The coulombic efficiency achieved in this research work with permeate and hydrolysate ranged between 8.49 (Run 2) and 9.24% (Run 1) which is comparable to the coulombic efficiency results shown by Thygesen et al. (2011), where a range of 9–12% was achieved with wheat straw hydrolysate and synthetic media as the feeding substrates. Furthermore, the research work done by Schmidt et al. (2018) achieved a 13.3% coulombic efficiency, indicating the presence of a methanogenesis process. However, when 2-bromoethanesulfonate was added as a methanogenesis inhibitor, the coulombic efficiency increased to 30%. The overall low average values of coulombic efficiencies either ways implicate that there are other competing processes where the majority of electrons had been involved

Table 3
Accomplished coulombic efficiencies in relation to volatile fatty acids degradation and COD degradation for Runs 1, 2 and 3.

Experiments	Coulombic efficiencies (%)	
	Coulombic efficiency$_{VFA\text{-}degradation}$	Coulombic efficiency$_{COD\text{-}degradation}$
Run 1	9.24 ± 0.87	7.99 ± 0.72
Run 2	8.49 ± 1.39	6.69 ± 0.76
Run 3	3.28 ± 1.06	1.74 ± 0.40

in other chemical reactions than current generation. One of the main competing processes is likely to be methanogenesis, which is initiated by the inoculation of mixed microbial cultures in the BES reactors during the experiments (Schmidt et al., 2018; Zhang et al., 2009). It is quite interesting that Run 3 showed the lowest coulombic efficiency since the low pH-value of the hydrolysate used there should inhibit the methane formation, too. The delayed development of the biofilm in Run 3 and the delayed increase in current density compared to Run 1 and Run 2 increased the competitiveness of alternative processes. Current curves together with mass degradation rates of acetic acid are graphically represented in the Fig. 3 for Run 1. It can be clearly seen in the graph that, the declining of mass degradation rate curve corresponds to the negligible current generation due to simultaneous competing reactions like methanogenesis and activity of non-exoelectrogenic bacterial species. This fact supports the low coulombic efficiency achieved: the sufficient share of acetic acid was consumed before the exoelectrogenic biofilm was established on the electrodes and then started to produce the current.

3.4. Performance parameters

Parameters like temperature, pH value and redox potentials were monitored and measured throughout the experiments. Measured pH values and redox potentials in both anode and cathode chambers for all the experimental runs are shown in Fig. 4. The pH values in the anode chamber increased from 6.84 and 6.83 to 8.04 and 7.73 at the end of the experiments for Run 1 and Run 2, respectively. While for experimental Run 3 with non-altered pH of hydrolysate, the pH increased from 5.78 to 7.42 at the end. Whereas the pH value in the cathode chamber rose from 7.39, 7.59 and 7.73 to 9.22, 8.64 and 8.27 at the end of the experiments for Run 1, Run 2 and Run 3, respectively. The pH value started to increase over the period of time with a decrease in the organic acid concentrations and current production. The increase in pH value in both the chambers over the course of the experiments is similar to the research work done by Jadhav and Ghangrekar (2009) by exposing the MFC to variations in operating parameters like pH value and temperature.

3.5. Microbial community screening

The Lysogeny broth (LB) plates were taken from the incubator and grown colonies were counted manually. The undiluted hydrolysate and effluent from anode and cathode chambers showed enormous growth of different colonies on the LB plates. The LB plate was divided into four different sections making the counting of densely grown colonies easier. For Run 1 approximately 2.89 × 103 and 1.1 × 103 colonies were grown from the liquid sample taken from the anode and cathode chamber respectively. This growth of microbes shows that the feeding substrate solutions consist of many active mixed cultures of microbe's present.

The results of the swabbing test of the microbial biofilm from the surface of the anode and cathode electrodes showed different colonies distinguished on the basis of their form, size, surface, opacity and color. The anode electrodes biofilm swab test showed an average of 5 ± 0.89 different colonies and 42 ± 2.79 colonies in total. Whereas the cathode electrodes biofilm showed an average of 6 ± 1.80 different colonies and 39 ± 2.94 colonies in total. These growth of different colonies is a sign of a very well developed biofilm over the experimental period. However, further investigations of screening the microbial cultures would be of high research interest in the study of the influence of the mixed communities in BES. According to the research done by Schmidt et al. (2018) the transcriptomic analysis of hydrolysate with the same inoculum from the AR revealed huge diversity of microorganisms. Similarly, in the study done by Hu et al. (2019) 16S rRNA pyrosequencing technology was applied to analyze the microbial community, and demonstrated multiple syntrophic interactions

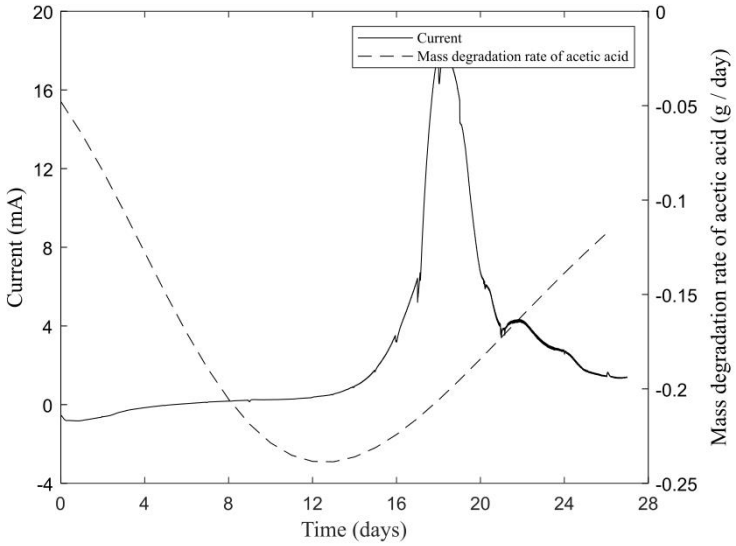

Fig. 3. Current generation curve and mass degradation of acetic acid occurred over time for Run 1.

between different bacterial species.

3.6. Correlation between electrical current and redox potential

During the experiments a correlation between current (mA) and redox potential (mV) was observed. Over the experimental period the redox potential was steadily changing in both the anode and the cathode chambers, and as a parameter requiring assessment, the difference between redox potentials of anode and cathode substrates mentioned as ΔORP was taken. Interestingly, as soon as current production in the reactors was stabilized, ΔORP tended to follow current curves in all three runs with different substrates fed in the anode chamber of the experiments as shown in Fig. 5. The plots of Run 1 and Run 2 show that the current peak and ΔORP peak are clearly interlinked. However, for Run 3 with a non-altered hydrolysate, the ΔORP peak more or less tends to repeat the current curve. A correlation coefficient was calculated to assess the similarity of both parameters. Calculated results of the correlation coefficient for the whole time interval (from the 1st experimental day to the 27th experimental day) and for the interval of stable current production (from 10th experimental day to the 27th experimental day) are presented in Table 4.

Values of correlation coefficients for experimental phases with altered hydrolysate (Run 1) and for permeate (Run 2) prove similar behavior of two parameters – current and ΔORP – which is also reasonable from the point of view of electrical engineering and its basic laws (e.g. Ohm's law) even in the case of non-linear correlation of the mentioned parameters. Non-linearity can be explained by the complexity of processes occurring within a second-class conductor (e. g. formation of

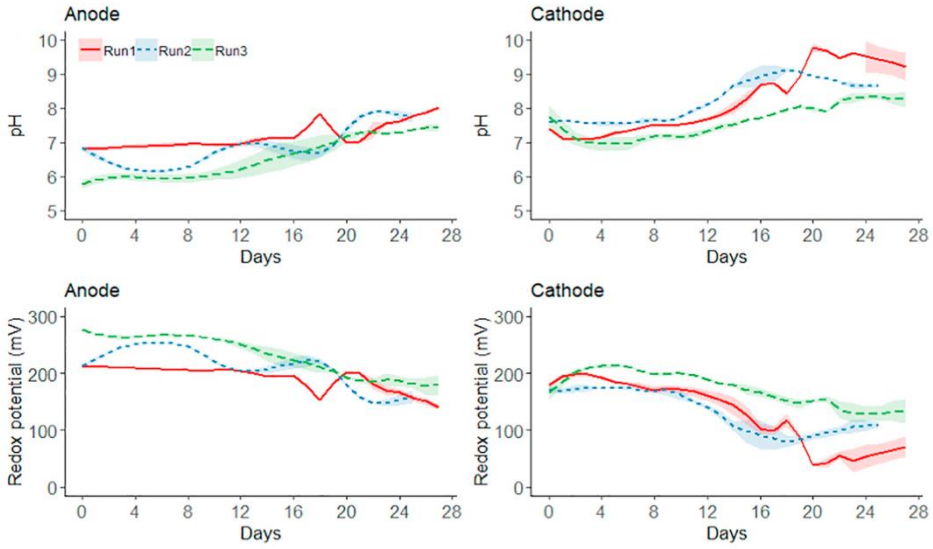

Fig. 4. Measured pH and redox potentials over time in anode and cathode chambers for experimental Runs 1, 2 and 3 with the shadows representing the standard deviations.

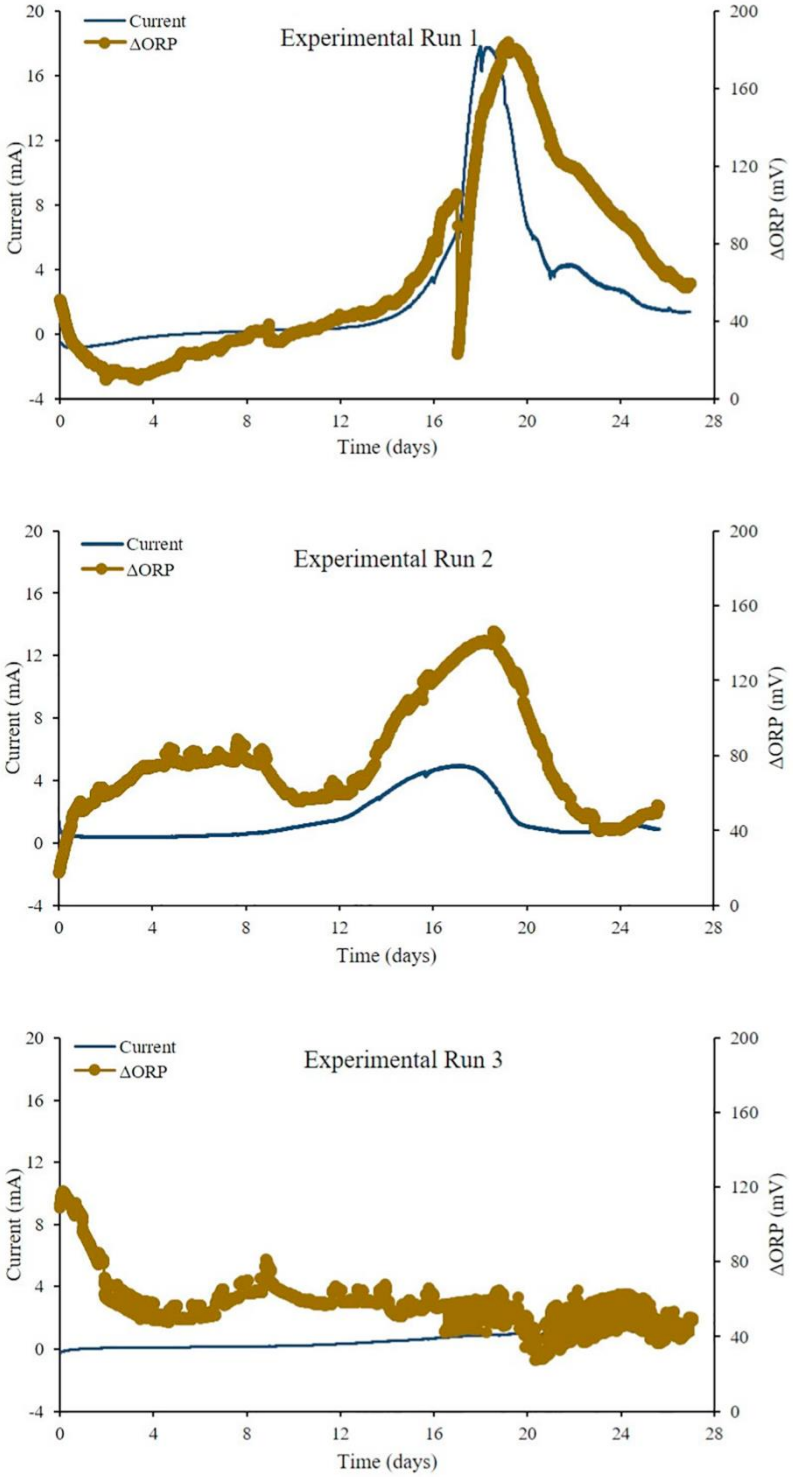

Fig. 5. Current and ΔORP measured over time during A. Run 1, B. Run 2 and C. Run 3.

Table 4
Correlation coefficients of current and ΔORP for experimental Runs 1, 2 and 3.

Experiments	Correlation coefficient	
	From 1st day	From 10th day
Run 1	0.8137	0.9106
Run 2	0.7687	0.8287
Run 3	−0.5267	−0.5514

capacitive double layer around electrodes, conductivity of biofilm, metabolic activity of microorganisms, etc.). The results from Run 3 have only a limited informative value, since the alternative processes dominate here.

3.7. Outlook

The results obtained from this research study helps as a ground work in upscaling the BES systems and their application in practice The BES reactors were very efficient in treating organic process liquids with high COD content which may be obtained in future through liquefying solid organic waste by a hydrolysis step. With the achieved results, it could be seen that further upscaling of the reactors volume and continuously fed experiments with permeate as substrate would lead to stable current production. Moreover, the upscaled BES reactors could be constructed in such a way where the hydrogen producing cathode chamber is provided with CO_2 which is further reduced by methanogens to produce high calorific methane. Additional gas measurements from both the chambers would be an interesting research aspect. The correlation found between current produced and ΔORP needs further detailed investigation. In future, high purity methane or hydrogen production systems can be developed by combining the fermentative biomass digestion with the BES.

4. Conclusion

The influence of different substrates with pH and organic acid concentration variations were evaluated on BES performance. Efficient total organic acids degradation for different feeding substrates was achieved. For BES fed with permeate, higher COD (87%) and TC/TOC (88%) removal rates were achieved. While, BES fed with hydrolysate with altered pH and added acetic acid produced maximum current (4.70 A/m^2). High concentrations of organic acids can be effectively and efficiently converted to bioelectricity in BES if the solution pH is altered to neutral. This study proves that the BES systems are reliable in conversion of different substrates to sustainable bioenergy.

Author contributions

Padma Priya Ravi: conceptualization, design of the study, methodology, resources, execution, data curation, evaluation and interpretation of results, visualization concept and writing- original draft preparation and literature review.

Anastasia Oskina: conceptualization, formal analysis, evaluation and interpretation of results, supervision: writing - review & editing.

Wolfgang Merkle: supervision: mentorship and reviewing and editing.

Thorben Schilling: supervision: microbiology lab tests.

Ludwig E. Hölzle: supervision: microbiology lab tests.

Andreas Lemmer: validation, supervision: oversight, mentorship and reviewing and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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5 Publication 4: Development of a production chain from vegetable biowaste to platform chemicals

Annemarie Schmidt[1], Gunnar Sturm[1], Christian Jonas Lapp[1], Daniel Siebert[2], Florencia Saravia[3], Harald Horn[3], Padma Priya Ravi[4], Andreas Lemmer[4] and Johannes Gescher[1,5]

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Abstract

Background: A future bioeconomy relies on the development of technologies to convert waste into valuable compounds. We present here an attempt to design a biotechnological cascade for the conversion of vegetable waste into acetoin and electrical energy.

Results: A vegetable waste dark fermentation effluent containing mainly acetate, butyrate and propionate was oxidized in a bioelectrochemical system. The achieved average current at a constant anode potential of 0 mV against standard hydrogen electrode was 177.5 ± 52.5 μA/cm2. During this step, acetate and butyrate were removed from the effluent while propionate was the major remaining component of the total organic carbon content comprising on average 75.6%. The key players with regard to carbon oxidation and electrode reduction were revealed using amplicon sequencing and metatranscriptomic analysis. Using nanofiltration, it was possible to concentrate the propionate in the effluent. The effluent was revealed to be a suitable medium for biotechnological production strains. As a proof of principle, the propionate in the effluent of the bioelectrochemical system was converted into the platform chemical acetoin with a carbon recovery of 86%.

Conclusions: To the best of our knowledge this is the first report on a full biotechnological production chain leading from vegetable waste to the production of a single valuable platform chemical that integrates carbon elimination steps leading to the production of the valuable side product electrical energy.

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Microbial Cell Factories

RESEARCH

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Development of a production chain from vegetable biowaste to platform chemicals

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Abstract

Background: A future bioeconomy relies on the development of technologies to convert waste into valuable compounds. We present here an attempt to design a biotechnological cascade for the conversion of vegetable waste into acetoin and electrical energy.

Results: A vegetable waste dark fermentation effluent containing mainly acetate, butyrate and propionate was oxidized in a bioelectrochemical system. The achieved average current at a constant anode potential of 0 mV against standard hydrogen electrode was 177.5 ± 52.5 µA/cm^2. During this step, acetate and butyrate were removed from the effluent while propionate was the major remaining component of the total organic carbon content comprising on average 75.6%. The key players with regard to carbon oxidation and electrode reduction were revealed using amplicon sequencing and metatranscriptomic analysis. Using nanofiltration, it was possible to concentrate the propionate in the effluent. The effluent was revealed to be a suitable medium for biotechnological production strains. As a proof of principle, the propionate in the effluent of the bioelectrochemical system was converted into the platform chemical acetoin with a carbon recovery of 86%.

Conclusions: To the best of our knowledge this is the first report on a full biotechnological production chain leading from vegetable waste to the production of a single valuable platform chemical that integrates carbon elimination steps leading to the production of the valuable side product electrical energy.

Keywords: Biowaste, Vegetable waste, Bioelectrochemical system, Acetoin, Propionate, Organic acids

Background

In the last decades, the amount of food waste increased steadily. Globally, 1.3 billion tons of food are discarded per year. Hence, approximately one-third of all edibles is not used for consumption [1]. Globally, these organic residues are disposed to a large extend via landfills, thus leading to groundwater pollution, greenhouse gas emissions and spreading of pathogenic microorganisms [2, 3]. In Europe, biowaste is mostly degraded by composting and only minor shares are digested in biogas plants. Especially in composting facilities, a high amount of energy is lost in the form of heat and easily degradable carbon is lost in the form of carbon dioxide [4]. A favorable alternative to composting could be to use the organic waste streams for the production of bioenergy. This energy is preferably produced in the form of biogas. However, the quality of this biogas (55–65% methane content) does usually not comply with the regulations regarding the purity of natural gas (90–95% methane content) [5]. Hence, most often biogas is fed directly into a combined heat and power unit, since the purification of the gas is not economically viable for most of the biogas plant owners [6]. Moreover, methane is at least currently a rather cheap end-product and subventions are necessary to render the process economically viable for the producer. Another utilization of mixed biomass waste streams could be its direct application as substrate for the production of platform chemicals. Still, it is most likely the mixture of many different carbon sources in the waste streams that has

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so far hampered this approach [7, 8]. To solve this limitation, a fermentation routine consisting of a dark fermentation step followed by a bioelectrochemical oxidation is presented in this study that enables the stepwise conversion of biomass to a highly enriched single carbon source.

In a dark fermentation process, biomass is often fermented under slightly acidic and most often under high temperature conditions. Acidification leads to an inhibition of methanogenesis [9] and the degradation of organic matter stops at the acidogenesis step with the production of carbon dioxide, short- and middle-chain volatile fatty acids (VFAs) and hydrogen [10]. The main VFAs comprised in the final solution are acetic, propionic, butyric and valeric acid [10, 11]. Usually, the effluent of dark fermentation plants is neutralized and thereafter used for biogas production, or the main aim of the process is the production of hydrogen [12–15]. Generally, the profit from biomass based hydrogen production increases if the remaining constituents of the dark fermentation percolate are used as substrate for further processes [12, 13, 15–18].

The production of hydrogen is a consequence of prevailing acetate-butyrate fermentation, while higher propionate concentration in the fermentation vessel is associated with lower hydrogen yields. This is due to the overall stoichiometry of propionate fermentation that is accompanied by the consumption of electrons according to: glucose $+ 2\ H_2 \rightarrow 2$ propionate $+ 2\ H_2O$ [19].

Regarding the energy release under standard state conditions, propionic acid fermentation has higher $\Delta G^{0\prime}$ values compared to typical other fermentation processes (e.g. butyrate fermentation with -247 kJ per mole glucose versus -279.4 kJ per mole glucose for propionate fermentation). Hence, if hydrogen is available, organisms thriving via propionic acid fermentation could have a selective advantage and the concentration of propionic acid should increase.

Interestingly, propionate seems to be a rather recalcitrant substance under anoxic conditions. This might be due to the oxidation of succinate to fumarate in the tricarboxylic acid (TCA) cycle which is part of the propionate metabolism and commonly ubiquinol dependent. This ubiquinol dependence necessitates the presence of electron acceptors with rather high redox potentials. Furthermore, the toxicity of intermediates of propionic acid production seems to have selected for regulatory routines that prohibit propionate oxidation in some organisms even in the presence of alternative electron acceptors [20]. Certainly, there are organisms that consume propionate as carbon and electron source under anoxic conditions, but the mechanisms used by other organisms to strictly prevent anaerobic propionate consumption suggest that special adaptations are needed for its efficient anaerobic consumption.

Previous studies revealed that dark fermentation effluents can be used as substrate for bioelectrochemical systems [15–17, 21]. In these systems microorganisms couple the oxidation of an electron donor to the transfer of respiratory electrons to an anode surface [22]. Hence, the oxidation of organic compounds is directly coupled to the production of an electrical current [22, 23].

In this study, we report on the selective oxidation of acetate and butyrate from dark fermentation effluents in bioelectrochemical systems. Via amplicon sequencing and metatranscriptomics, we could identify potential biocatalysts for the oxidation of these substances on the anode surface. Different membrane systems were screened to concentrate the remaining propionate in the effluent. Finally, we show that this effluent is a proper carbon source for typical production organisms and present data for the aerobic biotechnological production of acetoin from biomass based propionate.

Methods

Chemicals

Chemicals and biochemicals were acquired from Sigma-Aldrich (Munich, Germany), Roth (Karlsruhe, Germany) and Promega (Mannheim, Germany). Enzymes were purchased from New England Biolabs (Frankfurt am Main, Germany).

Bioelectrochemical system

The bioelectrochemical system (BES) used in this study was described by Sturm-Richter et al. [24]. The system is based on a two-chamber reactor of 270 ml volume including a three-electrode-setup. A carbon felt with 35 cm^2 surface area served as working electrode, while a platinum net of 1.25 cm^2 surface was used as the counter electrode. The reference of the system was a Ag/AgCl electrode. The electrodes in the two chambers were separated by a proton exchange membrane (Fumapem F-950, Fumatech, Germany). The working electrode was poised to 0 mV versus standard hydrogen electrode and the electrical current was measured by a potentiostat (uniscan instruments, PG8850RM). The electron acceptor of the cathode compartment was oxygen. The substrate of the system was changed under strictly anoxic conditions in an anoxic glove box. Coulombic efficiencies of the chronoamperometric experiments were calculated as described previously [25, 26].

Substrate for the bioelectrochemical system

For all experiments, a slightly acidic hydrolysate was used as substrate (pH 5.5–6). The hydrolysate was produced from vegetable waste by dark fermentation at the

University of Hohenheim [27] and was received either fresh or frozen. The hydrolysate was 100 µm filtered and contained mainly acetate (47.3–85.7 mM), butyrate (9.14–22.7 mM) and propionate (8.4–15.4 mM). The concentrations of the acids varied depending on the individual batch. The hydrolysate pH was adjusted to pH 7 with sodium hydroxide and it was purged with nitrogen gas to gain an anoxic substrate. The prepared hydrolysate was applied to the BES also under anoxic conditions. Over the whole experimental phase, the reactor was purged with a gas mixture of N_2/CO_2 (80%/20%). The pH was not further adjusted and samples were taken every 2–3 days.

Bacterial strains and culturing conditions

A starter biofilm was developed on the anodes before the addition of the percolate to accelerate the initiation of the carbon oxidation process. The microorganisms used in this study are listed in Table 1 and were partly isolated from different waste water streams as ferric citrate reducing organisms (Epple et al. unpublished). All strains except *Geobacter sulfurreducens* were incubated in LB medium overnight. *Geobacter sulfurreducens* was pre-cultured for 2–3 days at 30 °C in a minimal medium according to Dolch et al. [28]. As electron donor, sodium acetate (10 mM) was added while 40 mM sodium fumarate served as electron acceptor.

Subsequently, all strains were used for inoculating the BES with a starting OD_{600} of 0.5. The strains were incubated in the system for 4 days in the above described medium without the addition of sodium fumarate, as the anode served as electron acceptor. After the pre-incubation, the medium was changed to the vegetable hydrolysate.

Sample analysis and measurements

Samples were collected every 2–3 days for pH measurements as well as for quantifying the concentration of volatile fatty acids (VFAs) and total organic carbon (TOC). The amount of VFAs was determined via HPLC analysis (UltiMate3000; Thermo Scientific) using an Aminex HPX-87H column. Total organic carbon was measured via a Total organic carbon/Total nitrogen analyzer from Analytic Jena (multi N/C 2100). All samples were filtered through a 0.2 µm filter prior to analysis.

Bioinformatic analysis

Genomic DNA as well as total RNA samples were obtained from anode biofilms using the Wizard Genomic DNA Purification Kit (Promega, Mannheim) and the RNA PowerSoil® Total RNA Isolation Kit (MoBio/Qiagen, Hilden). 16S Illumina MiSeq sequencing (paired-end, 2 × 250 bp reads), rRNA depletion as well as Illumina RNA TruSeq sequencing (paired end, 2 × 150 bp) were conducted at IMGM Laboratories (Martinsried, Munich). The primers for the 16S Illumina MiSeq were Bakt_341F/Bakt_805R for bacteria and A519F/A906R for archaea (see Additional file 1: Table S1). 16S data analysis was conducted using the CLC Genomic workbench software (Qiagen, Hilden).

Metatranscriptome raw data was analysed using the software diamond v0.9.10.111 [31]. In total, 192 million reads were obtained from sequencing of eight lanes. Alignment of paired end Illumina reads was performed using the BLASTX algorithm with an e-value threshold of 10^{-6}. 18 million reads matched with sequences of the NCBI nr-database. mRNAs corresponding to acetate kinase, phosphate acetyltransferase and acetyl-CoA synthetase encoding genes were used

Table 1 Bacterial strains and plasmids used in this study

	Genotype	Source
Strains for BES		
Geobacter sulfurreducens barcode strain	Synthetic sequence; 453226::kan barcode	[25]
Shewanella oneidensis barcode strain	Synthetic sequence; 71982::barcode	[25]
Escherichia coli		unpublished
Enterococcus faecium		unpublished
Shewanella putrefaciens		unpublished
Strains for production		
E. coli K12 Δrnr (JW5741-1)	F^-, *Δ(araD-araB)567*, Δ *lacZ4787*(::rrnB-3), λ^-, *rph-1*, *Δ(rhaD-rhaB)568*, *Δrnr-729::kan*, *hsdR514*	[29]
Corynebacterium glutamicum (ATCC® 13032™)		
Plasmids		
pMAL_*alsSD*	Amp^R, P_{lac}, *alsSD*	[30]

The barcode strains contain a short synthetic DNA sequence integrated in their genome that allows for the specific quantification of these organisms in mixed species communities [25]. The unpublished *E. coli*, *E. faecium* and *S. putrefaciens* strains were isolated previously from waste water as ferric iron reducing organisms (unpublished results)

to assign transcriptomic data to putative acetate oxidizing microorganisms. Similarly, butyrate kinase and butyryl-CoA:acetate CoA-transferase were chosen for the metabolism of butyrate. Transcripts for *c*-type cytochromes were used to identify potential anode reducing microorganisms.

Crossflow filtration and nanofiltration

After the BES step, the effluent was centrifuged (30 min up to 1 h, 30,000×*g*) and crossflow-filtered with a 0.2 µm or a 1 kDa crossflow module (mPES MidiKros® Filter Modules D02-E20U-05-N or D02-E001-05-S in a KR2*i* TFF-System, SpectrumLabs, Breda, Netherlands). With the usage of the 1 kDa-Membrane instead of the 0.2 µm Membrane, parts of the dark brown colour could be filtered off in the crossflow system without affecting propionate concentrations. The subsequent nanofiltration was conducted under a nitrogen pressure of 4–4.5 bar, and with a nanofiltration membrane (Dow Filmtec, either NF90 or NF270, Sterlitech, USA) in a 350 ml stirring cell (Amicon, Merck Millipore, Germany), to achieve a concentration of propionate.

Production of platform chemicals

The filtered and sterilized effluent from the BES was used as medium for an acetoin producing *Escherichia coli* strain as well as for *Corynebacterium glutamicum* (compare Table 1) to conduct simple growth experiments. HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) was added to the fermentate to an end concentration of 50 mM to avoid pH fluctuation, and the pH was set to 7 before the fermentate was autoclaved.

Corynebacterium glutamicum and the *E. coli Δrnr* strain containing the pMAL_*alsSD* plasmid were subsequently used in cell suspension assays. It could already be shown before that another *E. coli* strain could produce acetoin with the here used pMAL_*alsSD* construct from glucose [30]. The production of acetoin, catalyzed by AlsS and AlsD, branches off at pyruvate, which is also the end-product of the 2-methylcitrate cycle used by *E. coli* to metabolize propionate.

Corynebacterium glutamicum is known for its biotechnological potential in amino acid production and could deliver interesting possibilities for the production of valuable chemicals from the here available propionate.

The cells were pre-incubated in LB-medium at 37 °C overnight and washed once before usage. Cells were added to the filtrated and sterilized fermentation broth to an OD_{600} of 2.5. Induction of the pMAL plasmid was achieved by addition of IPTG (50 µM). Induction of *C. glutamicum* for the release of glutamate to the cell medium was achieved by addition of Penicillin G (0.75 U/ml). Concentrations of acetoin in the liquid phase were measured using the Voges–Proskauer (VP) test according to Bursac et al. [32], while concentrations of glutamate were measured with the L-glutamic acid assay kit (Megazyme, Ireland).

Results and discussion

Current development from vegetable waste percolates

A vegetable fermentate originating from a biologically catalyzed acidic hydrolysis step was used as substrate for the BES without further filtration and after pH adjustment to pH 7. The BES were operated in batch mode and were pre-incubated with the laboratory model organisms *G. sulfurreducens* and *Shewanella oneidensis* as well as three isolates with 16S rRNA sequences that are most similar to *Shewanella putrefaciens*, *E. coli* and *Enterococcus faecium*. The preincubation was supposed to steer the oxidation process in the reactors towards anode reduction and to accelerate the anode reduction process. In previous experiments, it could be shown that, under certain process conditions, the preincubation can lead to stable biofilms that remain on the anode even under non-axenic conditions [25]. In Fig. 1, a representative experiment from the overall 10 bioelectrochemical experiments conducted in duplicate reactors was chosen to display the results of anode-assisted percolate oxidation. In other words, two independent reactors were fed 10 times each with percolate and we recorded the 20 experiment with regards to carbon consumption and current production. After a delay of 5 days, current density increased and reached its maximum value of 261.5 µA/cm² between day 12 and 13. The length of the initial lag-phase decreased

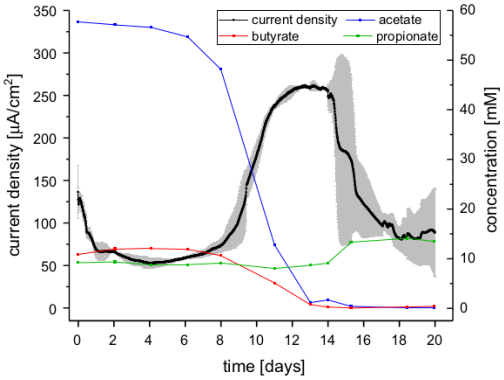

Fig. 1 Representative graph of current density over time on the primary y-axis and concentrations of organic acids over time on the secondary y-axis. Current density is given in µA/cm², concentrations in mM, while time is given in days. The grey area represents the standard deviation. This graph is representative for all 10 individual experimental phases

Table 2 Current density in µA/cm² for all BES experiments

Experiment	Current density (µA/cm²)		
	Average	Maximum	Minimum
1	71.8 ± 39.5	189.7 ± 82.6	16.2 ± 1.6
2	119.9 ± 19.3	261.5 ± 0.8	50.0 ± 61.7
3	82.2 ± 50.1	158.5 ± 47.3	27.1 ± 35.7
4	94.8 ± 48.7	252.2 ± 86.0	53.7 ± 27.3
5	116.1 ± 76.0	151.2 ± 4.7	69.5 ± 71.0
6	80.2 ± 76.8	140.4 ± 86.5	27.5 ± 25.7
7	84.0 ± 40.9	106.1 ± 59.5	52.4 ± 2.3
8	117.5 ± 39.1	151.1 ± 4.7	50.9 ± 7.2
9	96.8 ± 22.1	225.7 ± 30.4	37.5 ± 3.5
10	106.1 ± 27.6	139.0 ± 34.7	59.3 ± 9.6
ø	96.9 ± 17.3	177.5 ± 52.5	44.4 ± 16.7

The same BES reactors were fed 10 times with fresh substrate and the current was measured via a potentiostat. From these measurements, the average, maximum and minimum values of current density were calculated

Table 3 Starting and end values of total organic carbon (TOC) for all individual experimental runs as well as the TOC elimination in g/h m² and in %

Experiment	TOC *start* (g/l)	TOC *end* (g/l)	Decrease (g/h m²)	Decrease (%)
1	4.50 ± 0	0.67 ± 0.35	1.79	85.0
2	3.64 ± 0.18	0.39 ± 0.22	1.61	89.2
3	4.57 ± 0.28	0.72 ± 0.3	1.76	84.3
4	2.99 ± 0.04	0.67 ± 0.06	1.95	77.5
5	4.61 ± 0.20	0.75 ± 0.08	2.54	83.8
6	10.52 ± 0.27	6.16 ± 6.8	12.43	42.2
7	3.21 ± 0.07	0.55 ± 0.21	2.25	82.8
8	3.64 ± 0.02	0.30 ± 0.14	2.00	91.6
9	3.46 ± 0.04	0.39 ± 0.24	1.78	88.7
10	3.42 ± 0.11	1.1 ± 0.28	1.78	68.3
ø	4.46 ± 2.20	1.20 ± 1.76	2.99	79.3

The decrease in % is given for the whole experiment time

with the number of transfers to 1 or 2 days, indicating an adaptation of the anode community to the process of vegetable fermentate oxidation. The average maximum current density was 177.5 µA/cm² (see Table 2). This value is comparable to BES that were operated with a mixture of boiled and raw potatoes [33, 34]. In the here presented experimental set conducted with dark fermentation percolates, the maximum in current could be correlated to the degradation of acetate and butyrate. In the beginning of the experiment, the dominant VFA was acetate, which accounted on average for 34% of the initial TOC, while butyrate and propionate comprised 19.4 and 9.6%, respectively. At the end of the bioelectrochemical batch conversions, acetate and butyrate were almost completely consumed, while the propionate content remained relatively stable or even increased. The depletion of the fermentate in acetate and butyrate went along with decreasing current densities, indicating that these acids were at least partly or indirectly consumed by anode respiring microorganisms. Overall, 79.3% of the initial TOC content were averagely eliminated (see Table 3). Since the general TOC content decreased and propionate was not consumed, its share of the overall TOC content (1.2 g/l) increased on average to 75.6%. Therefore, 25% of the residual TOC, corresponding to about 0.3 g/l, comprised a mixture of other compounds which corresponds to previous results [35]. Those are conceivably humic or fulvic acids, as the fermentate possesses a dark brownish color.

The efficiency of the organic carbon removal by anode respiration was quantified by calculating the coulombic efficiency of the process. The values for the individual experiments can be depicted from Fig. 2. The average value of 13.3% indicates the presence of competing processes like methanogenesis. Therefore, a 16S rRNA gene based phylogenetic as well as a metatranscriptomic analysis were conducted to investigate which processes might prevail on the anode surface and which organisms were the key biocatalysts of the biocenosis.

16S rRNA sequencing and bioinformatic analysis

The phylogenetic diversity of the community was assessed using sequencing of amplicons derived via archaea and bacteria specific primer pairs (see Additional file 1: Table S1). The distribution for archaea is depicted in Fig. 3a. The family *Methanocorpusculaceae* comprised 88.9% of the archaea in the community. Members of this family can utilize H_2/CO_2, formate, 2-propanol/CO_2 and 2-butanol/CO_2 as substrates for methane production. Growth on acetate could not be observed [36]. The *Methanosarcinaceae* were the second most abundant family (9.2%). Members of this family are known to be able to use a wide range of substrates, such as methylated amines, methanol, H_2/CO_2, acetate, dimethyl sulfide, methanethiol and carbon monoxide [37]. Hence, the low coulombic efficiency could be due to prevailing methanogenesis that could be sustained by hydrogen, CO_2 and acetate producing primary and secondary fermentative organisms (see below).

The bacterial community (see Fig. 3b) was comprised mainly out of typical fermentative organisms, amongst others belonging to the *Chloroflexi* (24.2%), *Bacteroidetes* (31.3%) and *Clostridia* (25.6%), showing similarities to other anaerobic digester communities [38, 39]. Members of these phyla are capable of degrading cellulose. The *Anaerolineaceae* are the most abundant family in

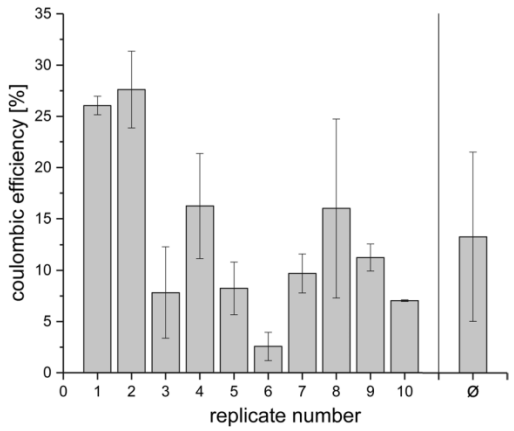

Fig. 2 Calculated coulombic efficiency of the BES reactors over all individual experimental phases. The coulombic efficiency was calculated from the integral of the current curve and the quantity of electrons available from the amount of the degraded total organic carbon. In the last column, the average of all experimental phases is given

the here described community (24.2%) which correlates to other studies describing anaerobic digester or biogas communities [40–42]. Members of this family can hydrolyze cellulose and are frequently encountered in *n*-alkane degrading microbial communities [43]. Members of the Bacteroidetes are typically able to hydrolyze cellulose and to degrade proteins and amino acids to acetate and ammonia [44].

The frequency of detected 16S rRNA genes that are most similar to the *Coriobacteriaceae* (12.7%) was not expected. These organisms are gut bacteria, normally not belonging to typical anaerobic digester communities. They can thrive either via fermentation or anoxic respiration. Nevertheless, there is also evidence that organisms belonging to this family are electroactive, as they comprised a large fraction of a biocathode community. Moreover, the genome of the recently sequenced strain EMTCatB1 contains 18 putatively *c*-type cytochrome encoding genes, which could also be involved in the transfer of electrons onto anodes, possibly explaining the presence in this study [45].

We observed a rapid depletion of butyrate and acetate in our study. As already mentioned, methanogenesis could represent a process involved in the consumption of a portion of the available acetate. Additionally, methanogenesis could be involved in butyrate consumption if synthrophic organisms catalyze the intermediate step from butyrate to acetate and hydrogen. This oxidation of butyrate is known to be accomplished by acetogens of the genera *Syntrophus* and *Syntrophomonas* [46]. The family *Syntrophomonadaceae,* which includes both genera, comprised on average 12.4% of the bacterial 16S rRNA genes and is one of the main families of the bacterial community.

Although the anodes of the system were pre-incubated with *G. sulfurreducens*, a model organism for extracellular electron transfer, it was detectable in the 16S rRNA analysis by only 0.6%.

Transcriptomic analysis

A transcriptomic analysis was used to assign acetate and butyrate degradation as well as electron transfer to microbial taxa within the anode biofilm. For acetate degradation, reads for acetate kinase/phosphate acetyltransferase as well as acetyl-CoA synthetase were assigned to archaeal as well as bacterial families.

Most of the reads for bacterial acetyl-CoA synthetase (see Fig. 4b) seem to be derived from members of the *Anaerolineaceae* (32.4%), which, as already described, are at least partly able to degrade cellulose and n-alkanes. They were further observed to produce acetate and provide it, for example, to acetoclastic methanogens [43], which can also be observed in the here studied community. In this case, acetyl-CoA synthetase would be responsible for acetate production and not for its degradation. Also, *Ruminococcaceae* (14.9%) were described as cellulolytic organisms, that produce acetate and formate or succinate [47, 48]. Moreover, also the *Porphyromonadaceae* (10.8%), the third most abundant group to which acetyl-CoA synthetase reads could be assigned, seems to contain primarily acetate producing organisms [49].

77.7% of the reads for acetate kinases could be assigned to members of the family *Methanosarcinaceae*, which is known to be acetoclastic. The other 22.3% for acetate kinases could be assigned to bacterial families (see Fig. 4a). A very similar distribution can be found for phosphate acetyltransferase (83.5% *Methanosarcinaceae*, 16.5% bacterial phyla), which catalyzes the step from acetate to acetyl phosphate. The latter can then be further processed to acetyl-CoA by acetate kinase. Also, the bacterial taxa assigned to produce acetate kinase and phosphate acetyltransferase are similar (see Fig. 4a, b).

The largest bacterial group to which acetate kinase reads could be assigned are the *Syntrophomonadaceae* with 81.7%, a family which could also be detected in the 16S analysis results. Although acetate kinase catalyzes a reversible reaction, organisms belonging to this family catalyze rather the production of acetate than its consumption [50].

Following, with 3.7 and 2.6% of bacterial acetate kinases respectively, are the *Clostridiaceae* and *Synergistaceae*. The latter could also be found in the 16S analysis, while

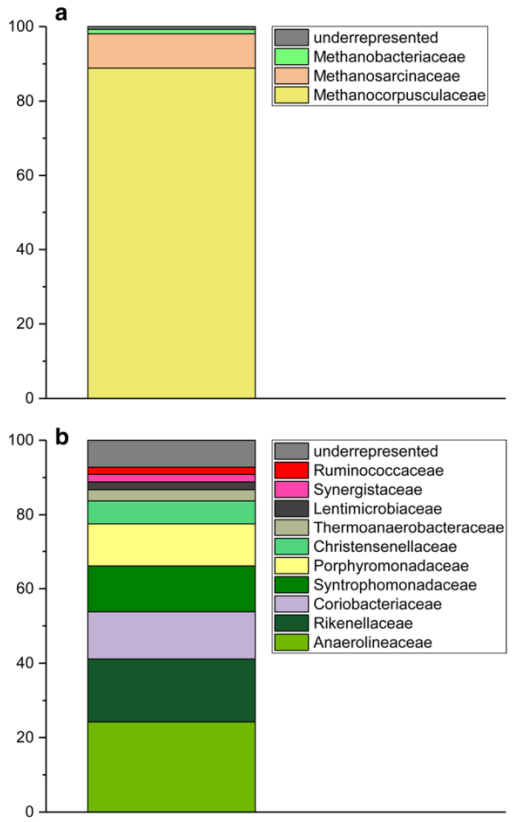

Fig. 3 16S analysis. Samples were isolated from anodic biofilms of the BES reactors and used as template for a 16S Illumina MiSeq sequencing (paired-end, 2 × 250 bp reads). The primers for the 16S Illumina MiSeq were Bakt_341F/Bakt_805R for bacteria (**a**) and A519F/A906R for archaea (**b**) (see Additional file 1: Table S1)

Clostridaceae could not be detected (however, other families of the order *Clostridiales*).

Interestingly, 1.48% of the bacterial acetate kinase reads could be assigned to the family *Geobacteraceae*, a model family for exoelectrogenic organisms [51], which was also detected in the 16S analysis. Therefore, we can conclude that organisms of this family take part in acetate degradation. However, this family seems more important regarding the distribution of *c*-type cytochromes, proteins known to have an important function in extracellular electron transfer reactions [52] (Fig. 4f). Over 90% of the reads for *c*-type cytochromes could be designated to the family *Geobacteraceae*, indicating that organisms belonging to this family are involved in the extracellular electron transfer to the electrode. Of note, although the number of detected and assignable reads for bacterial acetate kinases and *c*-type cytochromes is rather similar (4337 versus 3797), members of the *Geobacteraceae* account for a 61-fold higher percentage of *c*-type cytochrome reads compared to acetate-kinase reads. Multiple c-type cytochromes are necessary for the transfer of electrons to the cell surface and this could be the reason for the observed acetate kinase to *c*-type cytochromes read-ratio. Nevertheless, it is also conceivable that acetate might not be the only electron donor used by members of the *Geobacteraceae*. Other potential electron sources could be hydrogen or direct interspecies electron transfer [53–55]. Still, in the latter case, *Geobacter* cells would most likely only operate as a cable to the anode and would not be able to use the redox potential difference between adjacent organisms and the anode for the production of cellular energy. Energy production would only be possible if the inward electron transfer pathway from the outer membrane through the periplasm and into the cytoplasm would be insulated from the outward electron transport chain to the cell surface.

Butyrate degradation seems to be conducted by syntrophic organisms of the family *Syntrophomonadaceae* and the order *Syntrophobacterales*. Butyrate degradation starts by activation of butyrate to butyryl-CoA, under usage of acetyl-CoA. This reaction is catalyzed by the butyryl-CoA:acetate CoA-transferase [56]. The read count for this enzyme is comparable to that of acetate kinase and c-type cytochromes, while reads for butyrate kinases, acetyl-CoA synthetases and phosphate butyryl transferases could be detected only in minor quantities. Therefore, we proposed for the here described BES, that the first step of acetate degradation is conducted by an acetate kinase rather than by acetyl-CoA synthetase and that the first step of butyrate degradation is catalyzed by a butyryl-CoA:acetate CoA-transferase rather than by a butyrate kinase.

The phylogenetic distribution of bacterial mRNAs for butyryl-CoA:acetate CoA-transferases is depicted in Fig. 4e. Similar to the results for acetate kinases, the major fraction of reads could be designated to members of the *Syntrophomonadaceae* (45.9%). With 18.5%, *Ruminococcaceae* represent the second largest group for this enzyme, and *Lachnospiraceae*, with 7.6%, the third most abundant group. The latter two families are known as butyrate-producing gut bacteria, often found in the human intestine [57–59].

In conclusion, a possible physiological model is, that the family *Syntrophomonadaceae* is mainly responsible for the conversion of butyrate to acetate via secondary fermentation. The produced acetate is mainly used by members of the *Methanosarcinaceae* but is also a main substrate for members belonging to the *Geobacteraceae*.

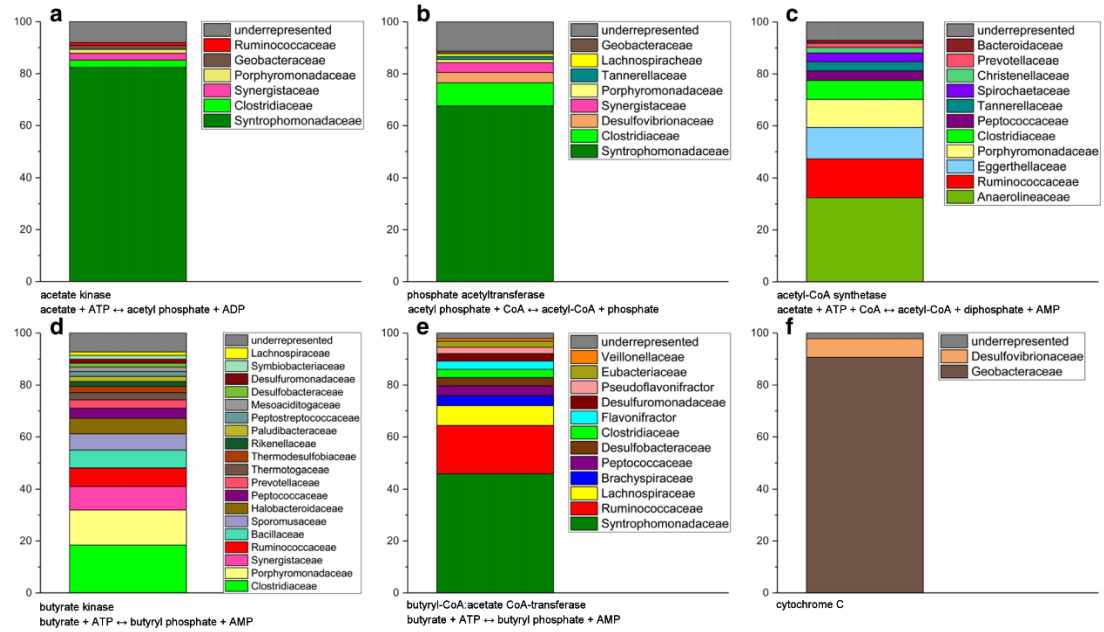

Fig. 4 Transcriptomic analysis. Samples were isolated from anodic biofilms of the BES reactors and used as template for rRNA depletion as well as Illumina RNA TruSeq sequencing (paired end, 2 × 150 bp). Key genes of potential anaerobic metabolic pathways were phylogenetically binned: **a** acetate kinase, **b** phosphate acetyltransferase, **c** acetyl-CoA synthetase, **d** butyrate kinase, **e** butyryl-CoA:acetate CoA-transferase, **f** cytochrome C

Inhibition of methanogenesis

A further experiment with vegetable waste was conducted in the BES to reveal whether indeed methanogenesis can be accounted as the major reason for the loss of electrons in the reactors. 2-Bromoethanesulfonate is known as common inhibitor for methanogenesis as it represents a structural analogue of coenzyme M, which is required for methyl transfer [60]. Previous experiments revealed, that inhibition of methanogenesis by 2-bromoethanesulfonate could increase the coulombic efficiency from 35 up to 70%, even at low inhibitor concentrations of 0.27 mM [61].

Hence, the BES were started without 2-bromoethanesulfonate and ran for 7 days. Thereafter 2-bromoethanesulfonate was added to a final concentration of 50 mM. Concentrations of organic acids as well as TOC were determined before and after addition of 2-bromoethanesulfonate. With these values, coulombic efficiencies were calculated and can be extracted from Fig. 5.

As expected, the coulombic efficiency increased 4 to 4.5-fold after addition of bromoethanesulfonate, which supports the hypothesis that methanogenesis is the major competing factor in the BES. Consequently, it was also observed that the concentration of acetate as well as butyrate was more stable compared to the inhibitor-free

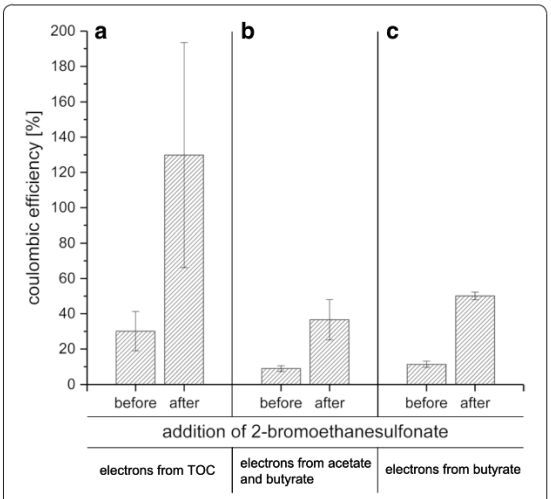

Fig. 5 Calculated coulombic efficiency of the BES before and after the addition of 2-bromoethanesulfonate. The coulombic efficiency was calculated from the integral of the current curve and the quantity of electrons available from the amount of the degraded total organic carbon in the liquid phase (A) and from the amount of the degraded acetate and butyrate (B) or only butyrate (C)

control systems, where both acids were nearly completely oxidized. In the inhibitor-containing system, only 20% of the available acetate was degraded, while butyrate degradation was less restricted (72.7% of the available butyrate was degraded).

Nanofiltration

Propionate was the major remaining end-product of the anode-assisted anoxic conversions. Propionate itself was presented as valuable platform chemical and could be used by other organisms as substrate for biotechnological reactions that could lead to more valuable end-products [62]. As propionate is metabolized via pyruvate, platform chemicals with metabolic pathways branching off at this intermediate should be especially suited for propionate based consumption.

First it was tested whether a higher concentration of propionate in the effluent of the BES could be achieved using nanofiltration membranes. For this purpose, the performance of two different membranes was compared in a stirring cell (see Table 4). Centrifuged and pre-filtered fermentate was concentrated with these membranes under nitrogen pressure. The NF90 membrane is supposed to have a tighter pore structure compared to the NF270 membrane. This could be corroborated by the filtration time, as the filtration process took about four to five times longer with NF90 compared to the NF270 membrane. Nevertheless, the better filtration result was achieved with the NF270 membrane, as the concentration factor for propionate was approx. 14.5% higher (see Table 4) and almost no propionate was found in the permeate.

Production of platform chemicals

In a first set of experiments, we tested whether the propionate containing effluent from the BES could be used as substrate for potential biotechnological production organisms. Hence, *C. glutamicum* and an *E. coli* strain were used for simple growth experiments (see Fig. 6). The *E. coli* strain contained a deletion of the *rnr* gene, which was previously presented to accelerate propionate consumption, especially under anoxic conditions [20]. As depicted in Fig. 6, both organisms grew under oxic conditions with the fermentate as only carbon and electron source. The addition of further supplements was not necessary. The doubling time for the *E. coli* strain was rather slow with 6.5 h, while *C. glutamicum* grew distinctly faster with a doubling time of 2.1 h. Also, *C. glutamicum* consumed the contained propionate distinctly faster than the *E. coli* strain.

As it could be shown that both strains are able to grow in the filtered fermentate and metabolize the contained propionate, it was of interest if these strains could produce any interesting chemicals out of the propionate.

Unfortunately, cell suspension assays with *C. glutamicum* lead to no detectable amounts of glutamate in the

Table 4 Conditions used for the nanofiltration process and the calculated concentration factor

Membrane type	Volume (ml)		Propionate (mM)		Concentration factor
	Start	End	Start	End	
Filmtec™ NF270	200	100	7.7	15.3	1.99
Filmtec™ NF90	200	100	10.5	17.8	1.70

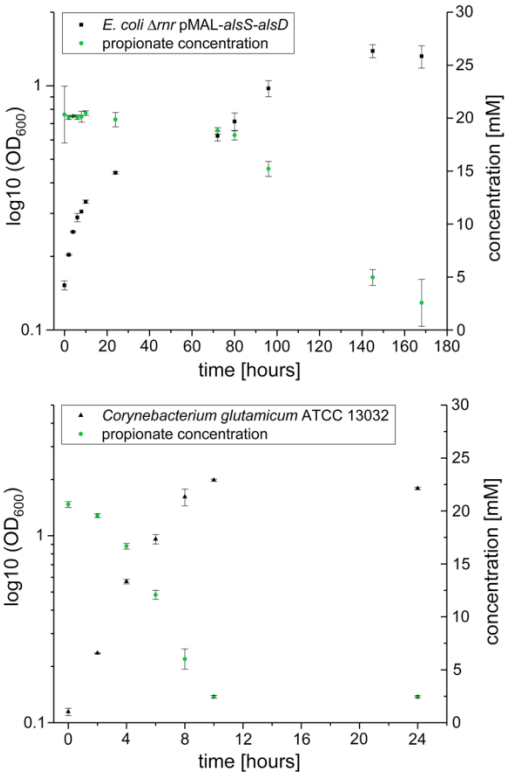

Fig. 6 Growth experiments with *E. coli* Δ*rnr* pMAL-*alsS-alsD* (top) and *Corynebacterium glutamicum* (bottom). OD_{600} is shown in semi-log mode over time in hours. Fermentate from the BES without prior 2-bromoethanesulfonate addition, centrifuged and filtered with the crossflow system, was used as medium for both strains and pH fluctuations were prevented by addition of HEPES (50 mM). Both growth curves were conducted under oxic conditions. Propionate concentrations are given in mM, measured at same time points as OD_{600}

supernatant. Further examination will be necessary to investigate production of glutamate from propionate in *C. glutamicum.* Also, Penicillin G addition should be further characterized in terms of appropriate concentration, incubation time and OD_{600} at induction time point.

The cell suspension assay was also chosen to examine whether it could be possible to produce acetoin from the propionate of the fermentate. Therefore, the *E. coli* Δ*rnr* strain containing a plasmid for the heterologous expression of the *alsSD* genes (catalyzing the two metabolic steps from pyruvate to acetoin) was used. The cells were prepared as described, and the OD_{600} was adjusted to 2.5. At the end of the experiment, at 24 h, 3.12 mM of acetoin could be measured in the fermentate. Overall, 7.25 mM propionate were consumed in this timeframe. This ratio corresponds to a conversion rate of 86% of the theoretical maximum.

Conclusion

This study reveals that vegetable waste can be a suitable substrate for the biotechnological production of platform chemicals. By the employed methods, namely dark fermentation combined with a bioelectrochemical system, a nanofiltration and, as last step, a biotechnological conversion, vegetable wastes could be converted to valuable platform chemicals and electrical energy. Our intention was to use the different organic acids from the hydrolysis step according to their biotechnological potential. Butyrate is metabolized via acetate and then fed into the citric acid cycle. Hence, using acetate or butyrate for the production of compounds branching of from pyruvate does not seem to be an efficient strategy as it would involve the energy consuming reaction to pyruvate first. This step is usually used for anabolic purposes only. In contrary, the propionate metabolism has pyruvate as its end product. Therefore, we believe that the production of electrons from acetate and butyrate in a bioelectrochemical system and platform chemicals from propionate is the best way of efficient usage of percolates from the hydrolysis reactor. Currently, the efficiency of the bioelectrochemical conversion of butyrate and acetate to carbon dioxide and electrons is hampered by methanogenesis. Nevertheless, this seems to be a problem that can be tackled by the design of reactors with higher surface to volume ratios since the bioelectrochemical oxidation of organic acids is thermodynamically more favorable compared to methanogensis. In other words, the currently used reactors provide a niche for methanogens by their high volume and low anode surface area, that can be omitted by the design of new reactors that favor productive biofilms. Our proof of principle experiments revealed the suitability of BES effluents as medium for biotechnological conversion or upcycling of propionate. Future steps will entail the development of production strains that are characterized by faster conversion rates.

Additional file

Additional file 1: Table S1. Primer sequences and amplicon size for the 16S Illumina MiSeq.

Authors' contributions
AS generated the data and analyzed and interpreted it. GS and CJL did the bioinformatical analyses of the DNA and RNA data, interpretation was done by AS. DS provided the *Corynebacterium glutamicum* strain and helped with the cultivation. FS and HH provided information and know-how for the filtration process. PPR and AL provided the vegetable fermentate for the experiments. JG supervised all experiments and was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

Availability of data and materials
All data generated or analyzed during this study are included in this published article. All DNA and RNA sequences that were retrieved for this study are publicly available through NCBI BioProject PRJNA445223. BioSample accessions: SAMN08773685, SAMN08773686.

Consent for publication
Not applicable.

Ethics approval and consent to participate
Not applicable.

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6 General discussion

Concepts for sustainable treatment of the OFMSW are mainly focused on anaerobic digestion, but recently the research field of bio- electrochemical systems (BES) is emerging, as these new technologies enable other end products (e.g. hydrogen, high calorific biogas, platform chemicals). At present the upscaling of controlled laboratory BES to process engineering applications is the subject of research. However, in order to use solid organic residues or energy crops in BES, these must be first degraded by fermentation to soluble organic compounds (e.g. volatile fatty acids (VFA)) in a so-called acidification reactor (AR). In this study different substrates like vegetable waste, grass/maize silage and maize silage were treated in the AR of the continuously run two-stage anaerobic digestion system (AD) in the laboratory to convert them to hydrolysate enriched with high concentrations of VFA. First, these organic acids were converted into biogas in the fixed- bed methane reactor (so-called anaerobic filters (AF)) of a two-stage AD process in order to obtain a benchmark for the subsequent investigations in the BES. During this experimental process, lower microbial degradability caused by the inert particles of the hydrolysate in the AF was observed. Hence, a cross-flow ceramic membrane filtration step was integrated to the AR of the two-stage AD system to improve the quality of the VFA-rich hydrolysate. This membrane filtration step resulted in a particle-free hydrolysate known as permeate. A lab-scale two- chamber BES reactor was constructed for further simultaneous treatment of the permeate and the unfiltered hydrolysate. Parameters influencing the achieved current densities and degradation rates could be identified and new approaches for the future production of platform chemicals could be developed. Furthermore, this research is a first step towards the upscaling of the BES reactors and their innovative construction and continuous operation. These three experimental concepts were demonstrated in detail in publications 1-4.

6.1 VFA and biogas production in two-stage AD

The first aim of this research work was to convert vegetables waste mixture of carrot mousse, carrots, celery, cabbage and potatoes in a continuously operated two-stage AD system into a hydrolysate rich in VFA, as shown in publication 1. Since the pH-value in the AR significantly influences the production of organic acids and their concentrations, two different target pH-values (pH 5.5 & 6.0) had been tested [79, 80]. Lindner et al. 2015 [81] investigated the intermediate concentration of the hydrolysate in the AR and showed that pH 5.5 and 6.0 resulted in a higher organic acid production compared to higher or lower pH-levels. This is

because at this pH range, the primary fermenting microorganisms in the acidogenesis step can grow optimally [82]. In order to achieve controlled process conditions in the AR, it must be combined with a second process stage where the VFA are degraded. In the first trial phase, a fixed-bed methane reactor was used for this purpose. The target pH-values in the AR were maintained consistently stable throughout the experimental phase by pumping the effluent from the AF avoiding the use of additives.

The target pH-value in the AR showed a significant effect on the organic acid concentrations and different process parameters. The dominant VFA produced from vegetable waste were mainly acetic acid, n-butyric acid and propionic acid. In comparison to pH 5.5, the hydrolysate produced at the target pH-value 6.0 contained higher concentrations of organic acids. The acetic acid, n-butyric acid and propionic acid produced were 9.19 ± 0.97 g kg^{-1}, 2.17 ± 0.17 g kg^{-1} and 1.24 ± 0.20 g kg^{-1}, respectively, for pH 6.0, which is 51%, 58% and 46% higher when compared to pH 5.5. This high VFA production increase at pH 6.0 is similar to the results of the research presented by Lindner et al. 2015 [81]. In contrast, no significant differences were found in the concentrations of other VFA. This proves that at pH 6.0, the acidogenic bacteria efficiently degrades the vegetable waste to valuable volatile fatty acids [52, 53]. Related to the added organic dry matter (oDM_{added}), the produced organic acid concentrations exhibited large differences in total organic acids produced between both the target pH-values 5.5 (215.34 g kg^{-1} oDM_{added}) and 6.0 (347.75 g kg^{-1} oDM_{added}). Moreover, the COD, TOC, TC and TN concentration measured were also higher at pH 6.0 than 5.5. This study reveals that, as an effect of pH 6.0 in the AR, the acidogenesis process excels in effective digestion of vegetable waste [79, 81].

During the acidogenesis process of the vegetable waste in the AR, the produced gases were measured. At pH 6.0, the produced methane content measured was higher (25.2 ± 7.4 %) than for pH 5.5 (18.6 ± 6.1 %) in the AR. In contrary, the hydrogen content produced was highest at pH 5.5 (4.9 ± 4.7 %). In accordance with our own investigations, other publications also report that hydrogen formation occurs in the AR at pH-values below 6.0 [83]. In the AR, 75% to 77% of carbon dioxide was measured at both target pH-values. These gas quality results are similar to the research work shown in literature [49, 81]. This experimental work showed that a minor variation in the pH-value in the AR leads to significant consequences on the gas and VFA compositions in the first stage of a two-stage AD system. Lower pH-values resulted in an increase of hydrogen and a decrease in methane content as the metabolic activity of methanogenic microorganisms in the AR was diminished [49, 81].

In addition, when the hydrolysate produced in the AR was fed to the AF of the two-stage AD system for further conversion of these organic acids to methane, the produced methane content measured was 73.0 ± 1.5 % for pH 6.0 and 65.7 ± 1.1 % for pH 5.5 in the AF. In contrast, the research done by Ueno et al. 2007 [48] involving the production of methane and hydrogen by phase-separation of the AD process showed only 60% of methane in the produced gas at pH 6.0. In single-stage biogas plants, on the other hand, only methane contents of 50- 60% are reached when carbohydrate-rich substrates are processed [84]. In order to assess the efficiency of the two-stage process approach, the substrate-specific methane yields measured were compared with the methane yield potentials of the substrates determined using the Hohenheim Biogas Yield Test (HBT). The measured overall specific methane yield (SMY) of the complete system (326.79 ± 41.24 L kg^{-1} oDM_{added}) and calculated SMY (332.64 ± 0.13 L kg^{-1} oDM_{added}) through the HBT showed nearly similar results. Therefore, it was demonstrated that the organic residues like vegetable waste can be efficiently treated in a two-stage AD system to organic acids and successively to biogas.

In conventional two-stage biogas plants, the organic acids formed are used in fixed-bed methane reactors (AF). The innovative alternative to AF in treatment of hydrolysate produced from the vegetable waste is bio-electrochemical conversion in BES reactors (publication 3). It could be shown that the hydrolysate gained from vegetable waste is a suitable substrate for BES reactors. The suitability of the hydrolysate for utilization in BES was demonstrated in the measured high current densities and high degradation rates of the added COD. If the cathode chamber of BES is operated under anaerobic conditions, either hydrogen or high calorific biogas can be produced, depending on the construction and operation of the reactor. In the BES studies carried out so far, the majority of the research is based on various kinds of artificial and real wastewaters [36]. In contrast, we were able to test a process approach in which solid biomass, such as OFMSW, can also be used in BES by fermentative pre-digestion at low pH- values. “Real” solid vegetable waste without any pre-treatment steps was used for this purpose, in difference to other studies [36, 43]. Furthermore, exoelectrogenic microorganisms can efficiently oxidize acetate in the anode chamber of BES [36, 85]. In our investigations, acetate was the dominant acid in the hydrolysate from the AR, demonstrating the suitability of this disintegration method to convert solid substrates into process liquids (publication 3 & publication 4) rich with high organic loads, thus making them highly feasible for further treatment process in BES [36].

6.2 Integrated membrane filtration step

The anaerobic digestion of vegetable waste in the two-stage system (publication 1) resulted only up to 48% of oDM degradation degree for the complete system and a COD degradation degree of 57% in the AF. These degradation rates achieved are quite low, although pre- filtration step with 100-µm sieve in the AR (publication 1) was used to separate the liquid fraction from solids. This is because of the inert particles of hydrolysate accumulating in the AF leading to poor microbial degradability caused by thick inefficient biofilm formation and even clogging of fixed bed in the AF [55, 86]. In order to overcome this problem, the integration of a ceramic membrane cross-flow filtration step to the AR of the two-stage AD process and its influence on organic acids, COD, TOC and TC concentrations after filtration was investigated (publication 2). Furthermore, the performance of a membrane filtration system when fed with two different substrates was investigated (vegetable waste and grass/maize silage). The results obtained in this study showed that for vegetable waste substrates, organic acids of hydrolysate could be completely recovered in the permeate after filtration. The concentration of acetic acid (4.05 ± 1.32 g L^{-1}) and n-butyric acid (1.72 ± 0.32 g L^{-1}) in hydrolysate showed no significant difference to the concentration of acetic acid (4.13 ± 0.63 g L^{-1}) and n-butyric acid (1.66 ± 0.32 g L^{-1}) in permeate after filtration. In contrast, the membrane filtration of hydrolysate gained from a mixture of grass/maize silage fed to the AR led to significant differences in the concentrations of acetic acid in hydrolysate compared to permeate (hydrolysate: 3.86 ± 0.27 g L^{-1}; permeate: 1.92 ± 1.12 g L^{-1}). Although, all other fractions of organic acids for both substrates indicated no significant variance and remained unaffected by the filtration process. This study results prove that the integrated microfiltration of hydrolysate from the AR is successful and efficient in recovering organic acids in permeate.

Contradictory to organic acid concentrations, the COD, TOC and TC concentrations showed significantly different concentrations after filtration. Only 38.19% and 33.13% COD concentration was recovered in the permeate for vegetable waste and grass/maize silage substrates, respectively. Only 40.98% of TOC and 41.81% of TC for vegetable waste and 34.95% of TOC and 39.49% of TC for grass/maize silage were extracted through the filtration in the permeate from the hydrolysate. COD, TOC and TC measured in the concentrate can be considered as the inert particle fraction left behind after filtration. So, the microfiltration step led to the decrease of the COD concentration in permeate compared to hydrolysate but enabled a stable and efficient extraction of the organic acids simultaneously.

In accordance to our findings, research done by Bär et al. 2018 [55] revealed that the crossflow filtration of hydrolysate in a two-stage AD process reduced the inert part of the COD which could not be converted to biogas. Moreover, the methane content in the biogas generated in the AF increased when permeate was used instead of hydrolysate. The membrane filtration of hydrolysate was demonstrated to be the best remarkable process for removing the inert particles fraction, which further leads to high conversion efficiencies of permeate in an AF or in a BES.

The principal advantages of the filtered permeate compared to the hydrolysate for the subsequent conversion steps could already be shown in the context of the investigations during utilization in the AF. A reduction of hydraulic retention time in the AF can be possible when it is fed with permeate which contains only diluted COD. Yet, using permeate, the substrate specific biogas yield in relation to the COD added increased compared to hydrolysate. To further evaluate the influence of the filtration step, permeate and hydrolysate were investigated for their specific methane formation potential with the HBT system. For the vegetable waste substrate, the SMY of permeate (264.01 L kg^{-1} COD_{input}) was 40% higher than that of hydrolysate (158.25 L kg^{-1} COD_{input}). Similarly, the SMY of permeate (227.15 L kg^{-1} CODinput) for grass/maize silage was also 24.5% higher than hydrolysate (171.42 L kg^{-1} COD_{input}). These results verify that the ceramic filtration successfully reduced inert particles which were left behind in the concentrate, thus leading to an increased SMY of permeate compared to hydrolysate. Similar results were shown in the research done by Tuczinski et al. 2018 [56]. The hydrolysate with higher concentration of COD and similar organic acid concentrations like permeate showed lower SMY because the inert particles in hydrolysate were only partially degradable by microorganisms. It could be shown from this research study that the integration of a cross-flow ceramic membrane filtration step into the AR increased the hydrolysate´s degradability, which resulted in higher conversion efficiency in the following treatment in AF. The process liquids achieved through the above acidification and simultaneous membrane filtration of different substrates are further used as potential substrates in a BES reactor (publication 3 & publication 4).

6.3 Bio-electrochemical conversion of VFA

In the process of technical upscaling the research study of publication 3 was necessary for understanding the operational insights needed for planning continuously operated BES reactors in future. In this study, the process liquids “hydrolysate” and “permeate” produced through acidification and subsequent membrane filtration of maize silage was fed to the mixed culture BES reactors during batch tests for bio-electrochemical conversion.

The research experiments of this study were segregated into three different experimental Runs: 1. (hydrolysate with altered pH and added acetic acid), 2. (permeate with altered pH and no acetic acid added) and 3. (pure hydrolysate without alterations) depending on the varying substrate fed to the anode chamber (publication 3). The main objective of this research was to investigate the suitability of mixed-culture BES reactors for the treatment of process liquids gained from solid substrates by pre-fermentation in AR. Furthermore, the degradation of organic acids, TOC, COD and current densities achieved over time were evaluated to analyse the efficiency of the BES systems. Additionally, important parameters like temperature, pH and redox potentials were measured and monitored during the experiments.

6.3.1 Organic degradation

Acetic acid, n-butyric acid and propionic acid were the dominant VFA in the feeding liquid substrates. The measured concentrations of organic acids, TC/TOC and COD at the beginning and the end of chronoamperometry experimental runs showed that for Run 1 almost all the organic acids were completely degraded. While for Run 2 and Run 3, a complete degradation of all organic acids took place with an exception of propionic acid with only 70% and 30% degradation rates achieved. Furthermore, all the organic acids were effectively degraded in between the 6^{th} and 27^{th} day of experimental Run 1 and Run 2 fed with hydrolysate, however, the degradation happened much faster in between the 6^{th} and 20^{th} day for permeate as feed in Run 2. However, it must be noted that the reactor load in Run 1 was significantly higher than in Run 2 due to the higher acid concentration. However, due to the identical lag phase in the first two runs, biological overloading of the reactor in Run 1 can be excluded. The acetic acid was completely degraded by the mixed cultures in the reactors for all the experiments, regardless of the increased acetic acid concentration supplied in the substrate for Run 1 (20 g kg^{-2}). The mass degradation of acetic acid has been described with the help of the Gompertz function to see the maximal degradation rate (-0.239 and -0.203 g/day for Run 1 and Run 2, respectively), since acetic acid had been the dominant VFA in all the process liquids used in BES experiments. Appling the Gompertz function, also the day of maximal degradation rate could be calculated (13^{th} and 8^{th} day for Run 1 and Run 2, respectively) revealing the positive effect of membrane filtration on biodegradability of process liquids in BES. For all the experimental runs in publication 3, degradation rates from 78 to 88% (TOC), 69 to 77% (TC) and 75 to 87% (COD) were achieved. The degradation rates of filtered permeate in Run 2 was 10-12% higher for COD and 7-10% higher for TOC in comparison to Run 1 and Run 3.

The investigations prove that the degradation of organic acids is significantly influenced by the pH-value of the process liquid. Even higher concentrations of organic acids were successfully degraded when the initial pH-value of the hydrolysate (pH 5.5-6.0) was raised to 7.0 and optimal conditions at the electrode like an applied voltage of -200 mV vs. Ag/AgCl were provided. The research work done in publication 4 also showed similar organic acid degradation and microbial adaptation behaviour. Du and Li, 2016 [87] investigated the performance of an MFC fed with cooked potato waste. They achieved similar COD degradation rates ranging in between 83-87%. The 80.77% of TOC degradation achieved with hydrolysate in publication 3 almost corresponds to the results presented in publication 4, where TOC degradation results of 79.3% were achieved in a BES fed with vegetable waste hydrolysate although completely different technical set-ups of the BES were used.

6.3.2 Current densities

The influence of different substrates on the performance of the BES reactor was also investigated within the study. The reactor performance was mainly evaluated by two parameters: the current density over time and the coulombic efficiency. The maximum average current densities produced for Run 1, Run 2 and Run 3 were 470, 131 and 71 µA/cm² respectively. For Run 1 and Run 2, with decreasing organic acid concentration, the current density started to rise slowly from the 6th day. The current produced increased and reached to its maximum value of 17.73 mA between the 17th and 19th day for Run 1 and to 4.94 mA between the 15th and 19th day for Run 2. But on the contrary, the current density for Run 3 increased very slowly from the 11th day and reached its maximum current of 2.67 mA between the 22nd and 23rd day. The results achieved from this study show that the “lag phase” of the microbes can be reduced significantly when the pH of the substrate is raised from 5.5 or 6.0 to at least 6.8. Whereas for Run 3, the lower pH of the substrate led to the longer adaptation of the microbial community and a poor biofilm formation on the anode electrodes surface. Correlating the maximum current density produced to the concentration and degradation of dominant acids acetate, butyrate and propionate is possible (publication 3 & publication 4). The highest current density was achieved for Run 1 with hydrolysate containing high acetate concentration, showing that providing adaptable pH 7 and higher amounts of acetate to the anode mixed microbial communities, they are capable of producing a higher current density. The results of this study show a definite interdependency between the current density and organic acid concentration which is similar to the results of publication 4.

6.3.3 Coulombic efficiencies and pH

The coulombic efficiency of the process is further calculated to analyse the relation between the amount of electrons released during the oxidation of the organic acids and the amount of electrons "captured" by the anode [88]. The coulombic efficiencies achieved varied between 8.49% to 9.24% with permeate (Run 2) and hydrolysate (Run 1) in this study. Whereas Run 3 showed the lowest coulombic efficiency caused by the low pH-value of the hydrolysate thus leading to a prolonged lag phase at the beginning of the experiments enhancing alterative "electron consuming" processes. The relatively low coulombic efficiency is typical for batch investigations in BES systems in which a biofilm must first establish itself on the electrodes. During this start-up phase, however, planktonic microorganisms present in the process liquids can already partially metabolize the dissolved organic acids.

Accordingly, similar coulombic efficiencies were achieved with vegetable waste hydrolysate (13.3%) in publication 4, although the BES set-up used was completely different to the systems used in the investigations for publication 3. Thygesen et al. 2011 [89]. used wheat straw hydrolysate in batch-BES and reached also similar coulombic efficiencies (9 -12%). The lower coulombic efficiency yields during the start-up phase at the beginning of each Run, indicates the occurrence of other competing processes like methanogenesis where the majority of the electrons had been consumed. This happens mostly when a BES reactor is inoculated with mixed microbial cultures, where the methanogenesis is one of the main competing processes [90].

Additionally, important parameters like temperature, pH and redox potentials were measured and monitored during the experiments in both anode and cathode chambers. The pH in the anode chamber increased by the end of the experiments to 8.04, 7.73 and 7.42 from 6.84, 6.83 and 5.78 for Run 1, Run 2 and Run 3, respectively. While, the cathode chamber pH also rose from 7.39, 7.59 and 7.73 to 9.22, 8.64 and 8.27 at the end of the experiments Run 1, Run 2 and Run 3, respectively. With a gradual decrease in the organic acid concentrations and current production, the pH started to increase over the time period. Research done by Jadhav and Ghangrekar, 2009 [75] by applying different operational parameters like temperature and pH in MFC showed that pH increases in both the anode and cathode chambers over the experimental period.

6.3.4 Correlation between current and redox potential

During the experimental period, current and redox potentials were measured and interestingly a correlation between them was observed. Similar to the pH, the redox potential(ORP) in the anode and cathode chambers was steadily changing. As an important parameter requiring assessment, the difference between redox potentials of anode and cathode substrates was taken into account as ΔORP. Once the current production became stable, interestingly the ΔORP curve tended to follow the current curve for all the Runs. Specially for Run 1 and Run 2 a clear interlink was observed between ΔORP peak and current peak. Furthermore, to assess the similarity of both the parameters, a correlation coefficient was calculated for the whole experimental period (1^{st}-27^{th} day) and for the time interval of stable current production (10^{th}- 27^{th} day). For Run 1 (altered hydrolysate) and Run 2 (permeate), the two parameters ΔORP and current exhibited similar behaviour, which is reasonable even though it is a case of non- linear correlation of the mentioned parameters. This non-linearity could be explained by all the complex processes occurring with a second-class conductor leading to several factors like metabolic activity of microorganisms, biofilm conductivity and capacitive double layer formation around the electrodes.

6.4 Conclusions and outlook

In this study, different waste residues like vegetable waste, grass/maize silage and maize silage were first hydrolyzed in the AR of a two-stage AD system into liquid hydrolysates rich in VFA. Secondly, to further improve the quality of the process liquid gained and to avoid problems like low microbial degradability or blockages caused by inert solid particles in subsequent process steps, a ceramic membrane filtration step was integrated and tested. As a result, a particle-free liquid called permeate was produced with equal concentrations in organic acids compared to the initial hydrolysate. Finally, the process liquids hydrolysate and permeate were further treated in BES reactors to produce current and to achieve high degradation rates of the organics added. The results of this research study can be summarized in the following conclusions:

- Organic residues like vegetable waste were efficiently converted to hydrolysate rich in volatile organic acids (mainly acetic acid) in the first reactor of a two-stage AD system. The investigated pH-value in the acidification of 6.0 proved to be advantageous compared to pH 5.5, as higher acid concentrations and higher overall degradation degrees were achieved.

- The integrated cross-flow membrane filtration step in between the two stages of AD to produce particle-free hydrolysate (permeate) showed that the inert particles can be successfully removed from the hydrolysate. Therefore, the formation of inefficient biofilms, clogging, reduced microbial degradability and biofouling could be prevented during further treatment of hydrolysate either in an AF or in BES reactors. Furthermore, the membrane filtration step enabled the efficient extraction of organic acids from the hydrolysate to produce organic acid rich permeate for both vegetable waste and grass/maize silage substrates.

- The treatment of the process liquids hydrolysate and permeate with different concentrations of organic acids and pH-values in BES systems showed that all the organic acids were completely degraded. Though, with permeate as substrate, higher COD and TC/TOC degradation rates were achieved, whereas maximum current was produced by hydrolysate with altered pH and added acetic acid. This study confirms that a BES reactor is capable of producing bioelectricity by efficiently converting the high concentrations of organic acids fed when the substrates pH is altered to 6.8. However, the mixed-culture approach in combination with the batch-setup of the testing system led to coulombic efficiencies lower than 10%. However, the mixed-cultures used proved to be ideal in practical applications for their efficient substrate degradation, current production and they are easy to handle. This study concludes that the conversion of different substrates to bioenergy and to produce value added platform chemicals in BES systems may become an interesting and reliable process.

The results obtained from this study are significantly helpful in the groundwork of technically upscaling the BES systems for their application in practice. BES reactors were very efficient in treating the organic process liquids with high COD contents, gained via acidification of solid wastes in the AR. Based on the results achieved, it could be perceived that permeate would be an ideal substrate for the upscaled BES reactors with high volume and when operated and fed continuously, it would produce stable current. Besides, high calorific methane could be produced when the upscaled BES reactors are constructed in a way that allows the feeding of CO_2 to the cathode chamber, thus enabling hydrogenotrophic methanogens to convert hydrogen and CO_2 into methane. Additional detailed insights of gas measurements in both anode and cathode chambers would be an impactful aspect of future research. Furthermore, a detailed investigation of the correlation found between current produced and ΔORP between the electrodes is necessary.

7 Summary

In 2016, 2.01 billion tonnes of solid waste were generated worldwide. The volume of waste is expected to grow to 3.40 billion tonnes by 2050. Worldwide, most solid waste is disposed of in landfills or dumps. Due to improper treatment and disposal of solid waste, nearly 1.6 billion tonnes of CO2 equivalents of greenhouse gas emissions were generated worldwide in 2016. This amount is expected to rise to 2.6 billion tonnes of CO2 equivalents per year by 2050.

It will therefore become increasingly important in the future not only to treat waste sustainably, but also to use it as an alternative to fossil fuels. Different waste-to-energy concepts are used, particularly for the treatment of OFMSW. As an alternative to the previously dominant biogas production, intensive research is currently being carried out into technologies for the recycling of organic residual materials, including so- called bio-electric systems (BES). In contrast to biogas production, this technology enables the treatment of a wide range of wastes to produce different end products, e.g. electrical energy, hydrogen or methane, can be preferred in BES depending on the selected process parameters. Despite numerous advances in research, considerable additional optimization is still required in order to be able to use the systems in large-scale power generation.

In order to use solid organic waste in BES systems, fermentative digestion is required to convert the organic components into dissolved short-chain organic acids (Volatile Fatty Acids (VFA)) and alcohols. In the course of the investigations, the solid waste residues were first digested to acid-rich hydrolysate in a hydrolysis reactor at pH-values of 5.5 and 6.0. However, this hydrolysate also contains particles that are inert to a subsequent degradation step leading to technical process disturbances. These inert particles can be removed by means of a membrane filtration step; a particle-free permeate is produced, which can be fed to the BES reactors. Within the scope of the present work, the basics of the utilization of OFMSW via microbial digestion, membrane filtration and utilization in BES should be investigated. Lab-scale BES reactors were developed and batch tests were carried out. The work was subdivided into the following three subtasks:

1. Fermentative conversion of vegetable waste into an acid-enriched hydrolysate in a hydrolysis stage of a two-stage biogas plant,

2. integration of membrane filtration to reduce inert particles in the hydrolysate and improve degradation of organic matter in the AF or in a BES reactor,

3. studies on the use of process liquids for acid degradation in mixed-culture BES systems in batch operation.

The vegetable waste residues from hydrolysis could be efficiently converted into hydrolysate. At a pH value of 6.0, higher organic acid concentrations were achieved than at pH 5.5. At pH 6.0, based on the added organic dry matter, these were approx. 350 g kg-1 (oDMadded) and at pH 5.5 approx. 215 g kg-1 oDMadded. Likewise, the concentration of chemical oxygen demand (COD) of the hydrolysate at pH 6.0 was 21.85 % higher than at pH 5.5. However, the COD degradation rates in the AF used were insufficient because the inert particles present in the hydrolysate could not be completely microbially degraded.

The subsequent integration of ceramic cross-flow membrane filtration into the two-stage system produced a particle-free permeate and significantly the increased microbial degradability. Clear differences could be shown depending on the substrate used (plant waste and grass/maize silage). The filtration step resulted in a significant improvement of the specific methane yield of permeate by 40% (vegetable waste) and 24.5% (grass/maize silage) compared to hydrolysate; proof that inert particles were separated efficiently. Finally, the process liquids hydrolysate and permeate produced by the hydrolysis of maize silage and the subsequent membrane filtration were fed to the anode chamber of two mixed-culture BES reactors. The investigations showed that all organic acids in both process liquids could be completely degraded in the BES. The highest COD (87%) and TOC degradation rates (88%) were achieved with permeate. However, the hydrolysate with added acetic acid yielded the highest current density of 470 µA/cm². Increasing the pH-value of the process liquids from 5.75 to 6.8 also significantly improved the current production and degradation rates. In this batch studies, relatively low Coulomb efficiencies of less than 10% were achieved due to the use of a mixed cultures.

The promising results show that at high pH-values (pH 6.0) in hydrolysis organic residues can be efficiently converted into a hydrolysate with high concentrations of organic acids and that the system can be further optimized by coupling membrane filtration. The utilization of the permeate in BES enables, a sustainable production of bioenergy and platform chemicals with permeate enables, depending on the BES reactor configuration. In summary, it was described for the first time that the combination of the fermentative biomass degradation process with filtration via ceramic membranes and the use of permeate in BES systems is possible.

8 Zusammenfassung

Im Jahr 2016 fielen weltweit 2,01 Milliarden Tonnen an festen Abfällen an. Es wird erwartet, dass das Abfallaufkommen bis 2050 auf 3,40 Milliarden Tonnen anwächst. Weltweit werden die festen Abfälle überwiegend über Deponien oder Müllhalden entsorgt. Aufgrund unsachgemäßer Behandlung und Entsorgung wurden weltweit in 2016 nahezu 1,6 Milliarden Tonnen CO_2-Äquivalente an Treibhausgasemissionen verursacht, mit weiter steigender Tendenz.

Die nachhaltige Verwertung der Abfälle und deren Nutzung zur Energiegewinnung wird in Zukunft weiter an Bedeutung gewinnen. Insbesondere zur Behandlung der organischen Bestandteile des Hausmülls (Organic Fraction of Municipal Solid Waste (OFMSW)) kommen unterschiedliche Waste-to-Energy-Konzepte zum Einsatz. Alternativ zur bisher dominierenden Biogasgewinnung wird derzeit intensiv an neuen Technologien geforscht, u.a. an sogenannten bio-elektrischen Systemen (BES). In BES interagieren exo-elektrogene Mikroorganismen, die als Biofilm auf Elektroden aufwachsen, mit diesen und ermöglichen die Konversion gelöster organischer Verbindungen zu unterschiedlichen Endprodukten, z.B. elektrische Energie, Wasserstoff oder Methan. Trotz zahlreicher Fortschritte in der Forschung besteht noch erheblicher zusätzlicher Optimierungsbedarf, um die Systeme im technischen Maßstab einsetzen zu können.

Um feste organische Abfälle in BES-Systemen einsetzten zu können, bedarf es zunächst eines fermentativen Aufschlusses zur Überführung der organischen Bestandteile in gelöste kurzkettige organische Säuren (VFA) und Alkohole. Dieses Hydrolysat enthält jedoch auch Partikel, die gegen einen nachfolgenden Abbauschritt inert sind und zu technischen Prozessstörungen führen können. Diese inerten Partikel können mithilfe eines Membranfiltrationsschritts entfernt werden; es entsteht ein partikelfreies Permeat, welches den BES-Reaktoren zugeführt werden kann. Im Rahmen der vorliegenden Arbeit sollten die Grundlagen der Verwertung von OFMSW über mikrobiellen Aufschluss, Membranfiltration und Verwertung in BES untersucht werden. Dazu wurden BES-Reaktoren im Labormaßstab entwickelt und Batch-Tests durchgeführt. Die Arbeit wurde dabei in die folgenden drei Teilaufgaben unterteilt:

1. Fermentativer Aufschluss von pflanzlichen Abfällen zur Gewinnung eines säurereichen Hydrolysats in einer Hydrolysestufe einer zweistufigen Biogasanlage,

2. Integration einer Membranfiltration zur Reduktion der inerten Partikel im Hydrolysat und Verbesserung der biologischen Abbaubarkeit,

3. Untersuchungen zum Einsatz dieser Prozessflüssigkeiten auf den Säureabbau in Mischkultur-BES im Batch-Betrieb.

Die pflanzlichen Reststoffe konnten fermentativ effizient in Hydrolysat überführt werden. Bei einem pH-Wert von 6,0 wurde höhere organischen Säurekonzentrationen und -erträge erreicht als bei pH 5,5. Die gebildete Gesamtmasse der kurzkettigen organischen Säuren betrugen bei pH 6,0, bezogen auf die zugeführte organische Trockenmasse, ca. 350 g kg^{-1} (oDM_{added}) und bei pH 5,5 ca. 215 g kg^{-1} oDM_{added}. Ebenso war die Konzentration an Chemischem Sauerstoffbedarfs (CSB) des Hydrolysats bei pH 6,0 um 21,85 % höher als bei pH 5,5. Die CSB-Abbauraten im genutzten Festbettreaktor waren jedoch unzureichend, da die im Hydolysat enthaltenen inerten Partikel nicht vollständig mikrobiell abgebaut werden konnten. Durch die anschließende Integration einer keramischen Cross-Flow- Membranfiltration in das zweistufige System konnte ein partikelfreies Permeat erzeugt werden und die mikrobielle Abbaubarkeit erheblich gesteigert werden. Dabei konnten deutliche Unterschiede in Abhängigkeit des eingesetzten Substrats (Pflanzenabfälle und Gras-/Maissilage) aufgezeigt werden. Durch den Filtrationsschritt konnte eine signifikante Verbesserung der spezifischen Methanausbeute von Permeat um 40% (pflanzliche Abfälle) und 24,5% (Gras-/Maissilage) im Vergleich zu Hydrolysat erreicht werden; ein Beleg dafür, dass inerte Partikel effizient abgetrennt wurden. Schließlich wurden die aus Maissilage erzeugte Prozessflüssigkeit Hydrolysat und dass durch die Membranfiltration gewonnene Permeat der Anodenkammer zweier BES-Reaktoren, die mit einer Mischkultur angeimpft waren, zugeführt. Die Untersuchungen zeigten, dass alle organischen Säuren in beiden Prozessflüssigkeiten im BES vollständig abgebaut werden können. Die höchsten CSB- (87%) und TOC-Abbauraten (88%) wurden mit Permeat erreicht. Das Hydrolysat mit zugesetzter Essigsäure ergab hingegen die höchste Stromdichte von 470 µA/cm². Die Erhöhung des pH-Wertes der Prozessflüssigkeiten von 5,75 auf 6,8 verbesserte auch die Produktions- und Abbauraten deutlich. In diesen Batchstudien wurden aufgrund der Verwendung von Mischkulturen relativ niedrige Coulomb-Wirkungsgrade von weniger als 10% erreicht.

Die vielversprechenden Ergebnisse zeigen, dass bei hohen pH-Werten von 6,0 in der Hydrolyse organische Reststoffe effizient in ein Hydrolysat mit hohen Konzentrationen an organischen Säuren überführt werden können und mit der Kopplung einer Membranfiltration das System weiter optimiert werden kann. Die Verwertung des Permeats in BES ermöglicht, je nach Konfiguration der Reaktoren, eine nachhaltige Erzeugung von Bioenergie- und Plattformchemikalien. Zusammenfassend wurde erstmalig beschrieben, dass die Kombination des fermentativen Biomasse-Abbauprozesses mit der Filtration über keramische Membranen und Nutzung des Permeats in BES-Systemen möglich ist.

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