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Dark fermentation as an environmentally sustainable WIN-WIN solution for bioenergy production

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ABSTRACT

The current energy and environmental crisis, linked to increasing industrialisation, has progressively driven the adoption of solutions focused on circular life cycle thinking, such as waste management with resource recovery and decarbonization of technologies. In this context, this study was built to quantify the environmental performance of a two-stage wastewater treatment process (dark fermentation with anaerobic digestion) in which three feedstocks (sugar beet molasses, cheese whey and wine vinasses mixed with wastewater treatment plant sludge) from the food industry were valorized as hydrogen. In this regard, several environmental profiles were created using the Life Cycle Assessment methodology with two system boundaries (cradle-to-gate and gate-to-gate) and two methods (ReCiPe Midpoint and Endpoint). Furthermore, this research was synergistically complemented with an energy analysis including indicators and input-output flow balances to provide a win-win solution for food waste utilization. The results have taken different directions depending on the methodological assumptions considered but, in general terms, the sugar beet molasses scenario can be claimed in all cases as the energetically sustainable process with the best environmental profile. With an energy surplus of 155%, the cradle-to-gate scenario recorded the best environmental impact in 4/8 midpoint categories and an overall reduction of 67% and 94% (excluding co-products) for the ReCiPe damage single score compared to the wine vinasses and wastewater treatment plant sludge and cheese whey scenarios, respectively. In this sense, the viability and competitiveness of these two scenarios is compromised by the lack of energy self-sufficiency (there is a 53% deficiency in the wine vinasses and wastewater treatment sludge scenario) and the lack of climate-neutral outcomes (a result of 5510 mPt/Nm^3 H_2 shows that the cheese whey scenario is far from being a zero-emission process).

1. Introduction

It is axiomatic that energy availability is a critical pre-condition for the development of economic activities (Månsson, 2014). However, nowadays energy resources are not evenly distributed around the globe and therefore many energy-importing regions remain vulnerable to the external intervention of the most energy-rich countries (Gökçe et al., 2021). As a result, the competitiveness to gain access to this precious commodity increases and, consequently, market prices fluctuate. It is not surprising then that political tensions between nations grow and the risk of conflict increases. For this reason, many countries have adopted active political strategies to diversify their energy mix and increase their production (Zavadskas et al., 2021). This is the solution so far adopted by the European Union and addressed by the Renewable Energy Directive 2018/2001/EU. In addition, other sustainable approaches have been encouraged and promoted with the development of technologies focusing on the renewability of feedstocks, as in the EU Waste Management Directive 2018/851 (Prieto-Sandoval et al., 2018).

Hydrogen is regarded as a viable energy vector to solve most of these political-economic problems, but it also presents other significant advantages such as portability and environmental friendliness when used (zero Greenhouse Gas (GHG) emissions) (Staffell et al., 2019). Unfortunately, despite its mitigating effects on the global warming potential, further research is still required to improve the environmentally sustainability of the manufacturing stage (Acar and Dincer, 2019). The most critical issue is that H_2 is mainly produced from fossil fuels and, therefore, requires a paradigm shift in feedstock. Therefore, the goal is to increase its production from renewable materials beyond the current 8% (Mishra et al., 2019). The most direct response to this dilemma is the

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Nomenclature

An-MBR	Anaerobic membrane bioreactor
A/O-MBR	Anoxic/oxic sequence membrane bioreactor
CC	Climate Change
C/N	Carbon nitrogen ratio
COD	Chemical oxygen demand
CW	Cheese Whey scenario
FE	Freshwater eutrophication
FRS	Fossil Resource Scarcity
GHG	Greenhouse Gas
LCA	Life Cycle Assessment
LCI	Life Cycle Inventory
LCIA	Life Cycle Impact Assessment
LU	Land Use
MDEA	Methyl diethanolamine
ME	Marine eutrophication
NACE	Statistical classification of economic activities in the European Community
SBM	Sugar Beet Molasses scenario
SOFC	Solid Oxide Fuel Cell
TA	Terrestrial Acidification
TE	Terrestrial Ecotoxicity
TN	Total Nitrogen
TP	Total Phosphorus
TS	Total solids
UASB	Up-flow Anaerobic Sludge Blanket
VFA	Volatile Fatty Acids
VS	Volatile Solids
WC	Water Consumption
WVS	Wine vinasses and WWTP sludge scenario

creation of a causal relationship between sustainable energy policies and zero waste goals.

In this context, food waste stands out as one of the most interesting wastes for valorization. Its massive generation (32.2 Mt from primary production, 30.6 Mt from manufacturing, 6.7 Mt from distribution and 59.9 Mt from consumption in Europe), low-cost and reliable composition (75–90% moisture content, 19–346 g/L chemical oxygen demand (COD), and 14–37 C/N ratio) are some of the main reasons (Antonopoulou et al., 2008; Caldeira et al., 2019; Habashy et al., 2021). Although the consumption stage of the food production chain seems to be the most wasteful, attention should also be paid to other parts of the food chain, such as the food processing industry. The proportions of food waste in this sector are variable (solid waste or wastewater) and depend on the food products manufactured. Some examples are the wastewater streams coming from the brewery, confectionery and dairy industry, as well as others from the livestock and agriculture sectors such as cattle manure (Rajesh Banu et al., 2020).

Due to this diversity, the selection of treatment technologies must be adapted to the waste to be recovered. A total of 19 hydrogen production processes were compared by Nnabuife et al. (2022) but only two of them can use several sources of organic biomass as feedstock (dark fermentation and photo-fermentation). The others were only fed with natural gas, coal, woody biomass, algae and water (reliance on a single source). Apart from dark fermentation and photo-fermentation, bio-photolysis, enzymatic processes, and microbial electrolysis are also promising biological technologies for H_2 production (Aziz et al., 2021). However, microbial electrolysis seems to be the only alternative to dark fermentation and photo-fermentation that requires organic waste as substrate (Ferraren-De Cagalitan and Abundo, 2021).

The selection of one of these systems is not an easy task given the numerous advantages and disadvantages of each of them. Dark fermentation is capable of treating a wide range of substrates with a very simple reactor configuration and a high production rate under light-independent operating conditions (Ding et al., 2016). However, the yield is low due to the accumulation of volatile fatty acids and therefore the effluent must undergo additional treatment (Patel et al., 2018). To cope with both weaknesses, the dark fermentation is coupled with other treatment technologies such as photo-fermentation, microbial electrolysis cells, microbial fuel cells or anaerobic digestion (Tapia-Venegas et al., 2015). Among them, anaerobic digestion is currently the most technically developed, the cheapest and the easiest to implement on an industrial scale (specially compared to electrochemical systems) (Cavinato et al., 2012). Moreover, and unlike photo-fermentation, it is not as sensitive to other factors such as the availability of sunlight or the concentration of free ammonia (Sivagurunathan et al., 2018). For instance, Meky et al. (2021) kept the ammonia concentration below 0.48 mg/L to prevent the suppression of photosynthetic bacteria. In contrast, Zhang et al. (2018), proposed a much higher value for anaerobic digestion, 5000 mg/L.

When implemented together, dark fermentation and anaerobic digestion are aimed to produce two well differentiated products (hydrogen and biogas) in separate reactors connected in series (Albini et al., 2019b). Both energy streams are the result of the biological activity involved during the acidogenesis (for dark fermentation) and methanogenesis (for anaerobic digestion) stages (Nagarajan et al., 2022). The optimal requirements of these stages differ from each other, and thus the operational conditions within each reactor (such as the pH) (Dareioti et al., 2022). That is why the selection of the most appropriate treatment and operational conditions is not a straightforward decision since a multiple criteria assessment should be performed considering the scale, composition of the feedstock, location of the facility (which affects light availability), legislation, performance variables, budget and a better environmental profile (Vassilev et al., 2010).

This profile is representative of the environmental performance of a product/process, which reflects the effects of the resource consumption and emissions over the natural environment (Gallego-álvarez et al., 2014; Schultze and Trommer, 2012). However, the environmental performance of dark fermentation technologies (both single and two stage) have not been widely measured as of today with the Life Cycle Assessment (LCA) methodology. In this regard, a literature review performed within this study proved that only 13 articles have been located since 2011. Hydrogen is the key product in 8 of them, although it was also a co-product in the production of cellulose nanocrystalline and as intermediate product in the manufacturing process of other commodities such as urea (Katakojwala and Venkata Mohan, 2022; Ghavam et al., 2021). The combination of fermentation and anaerobic digestion systems were addressed in 4 of the studies reviewed: Masilela and Pradhan (2021) compared both technologies separately with different substrates (brewery wastewater, municipal solid waste, and cattle manure), Sarkar et al. (2021) analyzed the two-stage dark fermentation-anaerobic digestion system with one substrate (food waste), Francini et al. (2019) provided three scenarios (anaerobic digestion, anaerobic co-digestion and dark fermentation followed by anaerobic digestion) for the treatment of municipal organic waste and/with sewage sludge and Escamilla-Alvarado and Poggi-Varaldo (2017) produced hydrogen and enzyme co-products from one feedstock (municipal organic waste). On the other side, Djomo and Blumberga (2011) have analyzed the environmental profile of a two-stage system comparing three feedstocks but, in their study, the second stage was photo-fermentation. Sun et al. (2019) has also determined the environmental performance of a two-stage dark-fermentation and anaerobic digestion processes for microalgae and food waste but their assessment focused on one impact category (global warming potential).

Based on the gaps in the current state of the art, the main objective of this study was to provide the environmental performance of a hybrid dark fermentation-anaerobic digestion system for the separate treatment of three different wastewater streams from the food industry: wine molasses mixed with wastewater treatment plant sludge, cheese whey and sugar beet molasses. The environmental contributions of the processes fed by each feedstock were analyzed with changes in the system boundaries (defined as cradle-to-gate and gate-to-gate for the waste stream) and in the selection of the method (MidPoint and EndPoint). All of them were then compared to define which one was more environmentally friendly under different methodological assumptions. In addition to the environmental profile constructed with the LCA methodology, the study was completed with an analysis of the primary energy input and output flows of the facility for each raw material in order to determine its self-sustainability. This term was defined in this research as the ability of the process to maintain both indefinite self-sufficiency and prosperity to future generations through energy use from renewable sources. Thus, it integrates the concepts of source renewability by origin (waste) and the process energy integration.

Because of this, the present study allows decision-makers to understand the most appropriate wastes to be recovered based on the environmental and energy performances of the processes. In this regard, it provides insight about focal points of sustainability which may lead to investment decisions. Besides, knowledge is provided about the weaknesses and thus, improvement strategies can be withdrawn to enhance the least profitable processes.

2. Materials and methods

2.1. State-of-the art review

The state-of-the art has been studied through a bibliometric analysis performed in July 2022 on the SCOPUS database for the period of 2011–2022. A total of 33 combinations of 10 selected keywords (Table 1 of the Supplementary material) were formed based on the specific characteristics of the study: "LCA" or "Life Cycle Assessment", "Anaerobic digestion", "Dark fermentation", "Hydrogen" or "Biohydrogen", "Sludge", "Vinasses", "Molasses" and "Whey".

Searches yielded results ranging from no publications to 12,880 ("Anaerobic digestion AND "sludge"), a very dissimilar figure compared to the 1221 found for other generic searches ("Life Cycle Assessment AND hydrogen") more focused on environmental impact studies using the LCA methodology. On the other hand, this number was drastically reduced when the keywords "Dark fermentation" and "Biohydrogen" were introduced. Then, ten of the 20 LCA-related combinations did not exceed 100 publications and all of them total 69 after elimination of repeats (56.5% are original research articles, 18.8% reviews, 14.5% books or book chapters, 8.7% conferences and 1.4% were listed in other categories). In addition, approximately half of them (51.2%) were published since 2019.

Table 1
Composition of the waste streams handled in the biohydrogen production process.

Parameter	Vinasses:Sludge 50:50	Sugar beet molasses	Cheese whey	Unit
pH	5.35[a]	4.50[d]	6.23[h]	–
TS	35.70[b]	103.00[d]	8.69[h]	g/L
VS	28.66[b]	78.40[d]	8.09[h]	g/L
COD	52.91[a]	101.00[d]	60.50[h]	g/L
VFA	1.31[a]	7.24[c]	0.62[l]	g/L
Carbohydrates	24.50[a]	49.00[d]	43.80[h]	g/L
Saccharose	–	20.40[f]	–	g/L
Lactose	–	–	38.2[h]	g/L
Proteins	6.10[jl]	91.50[d]	7.00[h]	g/L
Fats	–	–	1.00[h]	g/L
TN	969.0[c]	2800.00[g]	826[l]	mg/L
TP	445.80[c]	983.30[e]	289.00[h]	mg/L
C/N ratio	25.30[a]	17.50[d]	53.00[h]	–

Acronyms: TS: Total solids; VS: Volatile solids; COD: Chemical oxygen demand; VFA: Volatile fatty acids; TN: Total nitrogen; TP: Total phosphorus; C/N: Ratio carbon-nitrogen.
References: [a] (Tena et al., 2020); [b] (Tena et al., 2021); [c] (Battista et al., 2020); [d] (Vatsala et al., 2008); [e] (Park et al., 2010); [f] (Detman et al., 2017); [g] (Balachandar et al., 2020); [h] (Venetsaneas et al., 2009); [i] (Antonopoulou et al., 2008); [j] (Vlyssides et al., 2010); [k] (Supaporn et al., 2019); [l] (Inoue et al., 1996).

The information from the 39 selected articles was reviewed to check that all of them used the life cycle assessment methodology and could therefore show some of the following aspects: software, method, system boundaries, functional unit, type (attributional or consequential) and life cycle inventory. Compliance with these guidelines and multi-criteria decision-making reduced the number of articles to 19 (2 were eliminated for not providing methodological information, 6 for not following a multi-criteria assessment with more than one impact category and others for not presenting any LCA analysis). Table 2 of the Supplementary material shows these 19 articles and the related information. Finally, out of the 69 publications from the keywords entered in SCOPUS, only 13 articles can be framed as LCA studies with dark fermentation technologies.

Almost all the articles related to dark fermentation show a complete inventory (except 2) and most of them use the SimaPro software (10) to support the calculations. The functional units are very varied, although the most representative is the one that reflects the amount of hydrogen produced (46.2%). On the other hand, the database has not recorded results for the keywords relating the LCA methodology to dark fermentation for substrates such as molasses, vinasses and whey.

2.2. Feedstock selection

The organic-rich waste streams selected to be treated in the dark fermentation-anaerobic digestion hybrid process were a mixture of wine vinasses and sludge, sugar beet molasses and cheese whey. They were chosen on the basis of a combination of the following criteria: availability of biomass waste, suitability of composition and accessibility of technical data.

The first indicator was assessed based on the information gathered from the Spanish National Institute of Statistics and the Statistical Office

Table 2
LCA inventory of the biohydrogen production process from wine vinasses and WWTP sludge per functional unit (1 Nm^3 hydrogen).

Inputs from the Technosphere			Outputs from the Technosphere		
Materials			Products		
Vinasses	0.44	m^3	Biohydrogen	1.00	m^3
Sludge from WWTP	0.44	m^3			
NaOH (50%)	2.01	L	**Avoided co-products**		
Polyelectrolyte	0.089	kg	Electricity	12.36	kWh
MDEA (49.5%)	0.62	L	Concentrated fertilizer	60.03	kg
Air	38.20	m^3	Liquid fertilizer	0.81	m^3
Transport			**Emissions to air**		
Vinasses	18.15	t·km	CO_2	5.56	m^3
Sludge from WWTP	17.85	t·km	H_2O	0.01	m^3
NaOH	$2.14·10^{-3}$	t·km	MDEA	3.08·10-4	m^3
Polyelectrolyte	$8.88·10^{-5}$	t·km	O_2	1.34	m^3
MDEA	0.25	t·km	N_2	30.18	m^3
			H_2	0.05	m^3
Energy			CH_4	0.03	m^3
Steam	42.96	kWh			

of the European Union. According to these data sources, 2.33 tons of non-dangerous waste were produced in 2018 in Spain while manufacturing food products, beverages and tobacco products. Although the volume of municipal waste is much higher (16.47%), the valorization of industrial food waste (1.69% of total NACE waste) can be considered as a complementary strategy for food waste management. In fact, the largest volume of waste comes mainly from three categories: animal waste and mixed food products (33.85%), vegetable waste (31.29%) and common sludge (6.01%) (National Statistics, 2022). In terms of wastewater production, 370,410 m^3/d were produced in the food-processing industry in Spain for the same year. Given the limitations found in the databases in terms of the disaggregation of the volume of waste/wastewater produced by economic activity, two other aspects were considered for the assessment of this type of biomass availability: the monetary value of production and the expected waste percentage of commodities. While the manufacture of meat and meat products seems to be the most profitable activity in the country (23.58% of the results), its residues seem to have limitations to be used in dark fermentation due to their composition (high nitrogen and fat content) (Cieciura-Włoch and Borowski, 2019; Boni et al., 2013; Eurostat, 2022). In addition, other waste sources such as bakery waste (7.31%) were not taken into account due to their high concentration of total solids (more than 10%) or technical data deficiencies (Govindaraju et al., 2021). As stated by Ghimire et al. (2018), it seems that the hydrogen production yield is higher in wet fermentations than in those operating under solid-state conditions. Therefore, only residues from four of the NACE activities (dairy, fruit and vegetables, beverages and other food products) seem to meet the selected criteria regarding to moisture, nitrogen and fat content.

Within this context, cheese whey and wine vinasses belong to economical profitable sectors (7.88% and 5.27% of the outcomes of the food and beverages industry) and sugar beet molasses are the residues (as roots and tubers commodities) with the largest expected waste percentage generation during agricultural production (20%), postharvest handling and storage (9%), and processing and packaging (15%) (Gustavsson et al., 2011). Finally, the selection of these feedstock was based on technical data accessibility. As stated in section 2.1, the SCOPUS database has not collected LCA studies with dark fermentation and feedstocks like vinasses, molasses and whey. However, techno-experimental studies were found for these substrates. Lin et al. (2012), Liu et al. (2013) and Łukajtis et al. (2018) are examples of reviews compiling technical information for different feedstocks. Table 1 summarizes the influent composition of the selected waste streams.

2.3. LCA: goal and scope definition

The objective of this manuscript is the quantification and benchmarking of the environmental profile of bio-hydrogen production from different organic-rich waste streams (mixture of wine vinasses and sewage sludge, sugar beet molasses and cheese whey). For this purpose, a facility with a feedstock flowrate capacity of 30 m^3/d has been modelled and the four stages of an LCA, according to ISO standards 14,040–14044:2006, has been followed. Although the main function of the process is the production of biohydrogen, other value-added products such as electricity, heat, struvite and solid and liquid fertilizers are obtained. The process presents a typical behavior of a biorefinery and therefore, the system was analyzed in terms of its multifunctionality. Furthermore, the study was developed according to an attributional approach and under the framework of two possible system boundaries: cradle-to-gate and gate-to-gate, differentiated from each other in the upstream processes considered.

While cradle-to-gate includes upstream processes such as extraction of raw materials, energy and chemicals, gate-to-gate perspective has not considered loads from raw materials. The idea is that the construction of a new design facility for the valorization of organic waste streams prevents environmental impacts related to waste treatment and disposal (Olofsson and Börjesson, 2018). As a consequence, indirect impacts were only related to the demand for chemicals and energy for the operation of the facility.

The general scheme of the process, including its subsystems, the stages involved, and the main input and output flows is depicted in Fig. 1. The characteristics of the organic waste feedstocks used will determine the particularities (operational and equipment) of the process, whose diagrams can be checked in the Supplementary material. In addition, only the environmental impacts arising from the operation of the process have been analyzed. The impacts related to infrastructure and construction were not incorporated in this manuscript and the geographical scope was restricted to European level.

In this regard, three possible scenarios were designed for the production of hydrogen (**WVS** or Wine vinasses and WWTP sludge scenario, **SBM** of Sugar Beet Molasses scenario and **CW** or Cheese Whey scenario). All of them were subsequently divided into three sub-systems: S.1-Biohydrogen production, S.2-Energy valorization and S.3-Effluent treatment.

Subsystem S.1 includes the fermentation and hydrogen purification processes. The dark fermentation stage has been modelled to operate at thermophilic temperatures (55 °C) with pH between 5.5 and 7.0, which is controlled with solutions of sodium hydroxide or sodium bicarbonate depending on the characteristics of the feedstock. The type of reactor has been selected based on the concentration of solids in the stream to be valorized (Rajesh Banu et al., 2020). Based on this, stirred tank configurations (54 and 37 m^3 respectively) were implemented in WVS and SBM (the feed is between 2 and 12%) while a UASB reactor (Up-flow Anaerobic Sludge Blanket) of 39 m^3 has been considered for cheese whey (CW) (<2%) (Liu et al., 2013). Because of the high carbon dioxide concentration of the hydrogen obtained from the dark fermentation, a purification system by chemical absorption with amine solutions was used for all the scenarios (Ljunggren and Zacchi, 2010; O-Thong et al., 2018).

Subsystem S.2 is composed of the anaerobic digestion and cogeneration process. The anaerobic digestion stage was performed with mesophilic conditions (35 °C) maintaining pH between 6.5 and 7.8 (Majd et al., 2017; Tena et al., 2021). The effluent from the dark fermentation stage of the scenarios WVS and SBM was then valorized in stirred tank reactors with a capacity of 358 and 392 m^3, while the CW scenario used an UASB of 322 m^3. The main product from this stage was the biogas, which was transformed in a cogeneration system into electricity and heat. As Solid Oxide Fuel Cells (SOFC) can directly use methane instead of hydrogen, the fuel cell generation was chosen over internal combustion generators due to a number of advantages, namely reduction of conversion stages, transformation of the fuel into energy in a single stage and a high efficiency of electrical conversion (Guilera et al., 2020). Considering this, 53% electrical efficiency and 33% thermal efficiency was assumed in this study for the SOFC system incorporated in the scenarios (Rillo et al., 2017).

Finally, subsystem S.3 comprises the separation of the liquid and solid fraction of the effluent from the anaerobic digestion stage and the treatment of the liquid effluent. Although the evaluation of the content of pathogens (and other parameters) is essential for the use of the solid digestate in agriculture, this study has considered the possibility of recovering it through its direct application. This assumption has been deemed to be valid based on the conclusions reached by Lloret et al. (2013), Riau et al. (2010), Rubio-Loza and Noyola (2010) and Tena et al. (2021) in terms of *E. coli* and *Salmonella* content in two-stage systems with dark fermentation and anaerobic digestion under thermophilic and mesophilic conditions. The liquid fraction of the digestate still has a remarkable concentration of nutrients that can be exploited before direct emission to the environment. For this reason and depending on its characteristics, a two-fold strategy for treatment could be followed. The effluent of WVS is still highly concentrated in organic matter, the effluent of SBM has a high nutrient concentration and the characteristics for the CW scenario, on the other hand, are similar to urban wastewater.

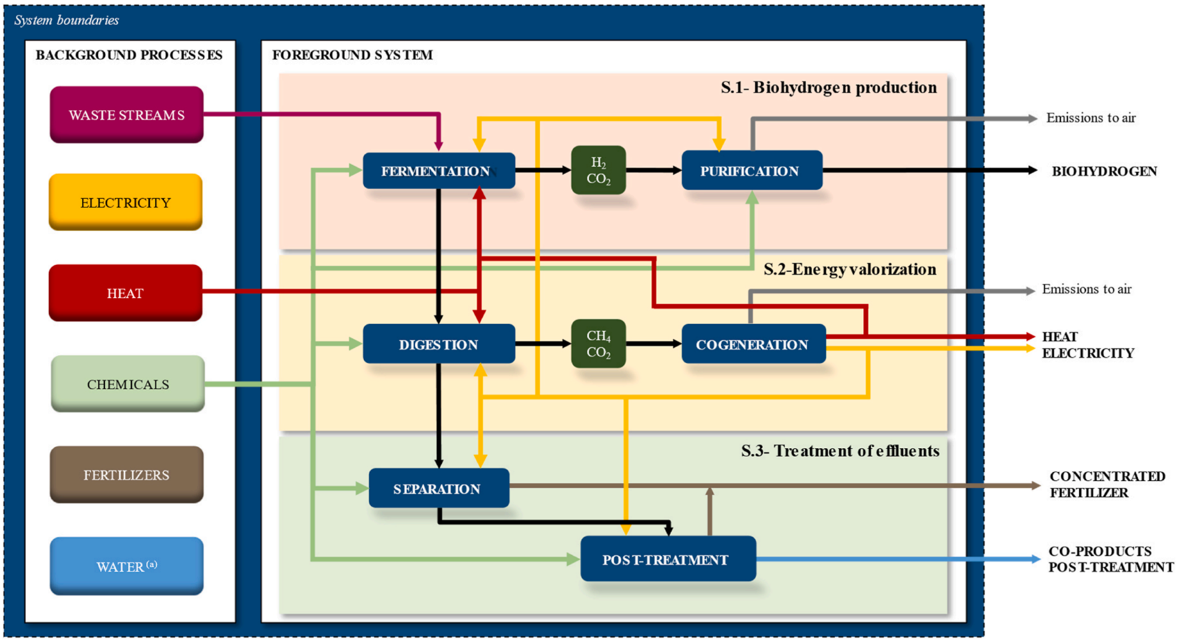

Fig. 1. Definition of system boundaries and sub-systems. (a) Water is a co-product but is also a raw material of the process.

Thus, the following technologies have been selected respectively for each scenario: anaerobic membrane bioreactor (An-MBR), struvite precipitation unit and anoxic/oxic sequence membrane bioreactor (A/O-MBR) and centralized conventional wastewater treatment. The An-MBR converts the influent into biogas and nutrients (N and P) while generating a high-quality effluent that can be reused in agriculture. Its total energy consumption is 0.2 kWh/m^3. Although their main goal is also the recovery and removal of compounds from wastewater, the struvite precipitation followed by an A/O-MBR reactor is more energy intensive (2.95 kWh/m^3).

Given the objective (hydrogen production), the multifunctionality of the system and the diversity of co-products, the allocation of the impacts was applied with system expansion through the substitution of the co-products with others widely implemented in the market (Moretti et al., 2020). This impact avoidance facilitates the integration of the products (both expressed in energy and mass units) with the selected functional unit. The avoided allocation of biofertilizers was made considering their N and P composition (their main nutrients) and the substitution value of mineral fertilizers (68% for solid fertilizer and 62% for liquid fertilizer) (Rigamonti et al., 2013; Pedizzi et al., 2018), respectively.

Bioenergy-based co-products were assumed to be equivalent to others from non-renewable production. Therefore, the environmental loads were all allocated to hydrogen and the functional unit was subjected to the hydrogen production target: 1 Nm^3 biohydrogen at 99% purity under normal operating conditions (1 bar and 293 K).

2.4. Description of scenarios and system boundaries

2.4.1. Wine vinasses and WWTP sludge (WVS)

Wine vinasse generated as a by-product in the wine distillation process (10–15 L produced per L of alcohol) was mixed in a 50:50 ratio with sewage sludge. Ripoll et al. (2020) and Tena et al. (2021) have demonstrated the considerable increase (up to 40%) in process efficiency when both waste are combined. In addition, this approach would solve the problems related to the final treatment of waste before disposal and reduce the volume of organic waste ending up in landfills.

This biodegradable mixture was first introduced into a dark fermentation reactor where the organic matter was transformed into a gaseous stream consisting mainly of hydrogen (55%), carbon dioxide (44%) and methane (1%). This biohydrogen is then purified by chemical absorption to 99% for commercialization. The effluent from the dark fermentation stage, given the metabolic pathways of the organic matter in the acidogenic process, is too concentrated in volatile fatty acids (VFA) to be discharged directly into the environment. Therefore, anaerobic digestion has been proposed as a subsequent treatment. There, the VFA are degraded into biogas (70% CH_4 and 30% CO_2), which is further valorized into electricity and heat in the SOFC. The recovered heat provides enough energy to produce 19.2% of the heat needed in the process. As for the solid digestate from the anaerobic digestion, the solid and liquid fractions were segregated. Dewatering of this effluent in a filter press resulted in a solid fertilizer. Further treatment of the liquid fraction in an anaerobic membrane bioreactor resulted into more biogas and a liquid fertilizer. Chemicals such as sodium hydroxide and methyldiethanolamine (MDEA) were also consumed during the operation of the process. Their goal was to keep the pH constant between 5.5 and 7.0 in the dark fermentation and to remove carbon dioxide and possible trace of sulfur compounds in the hydrogen purification. Figure S1 shows the block diagram of the process described above in the Supplementary material.

2.4.2. Sugar beet molasses (SBM)

In the food industry, 7 t sugar beet is processed into 1 t sugar and 0.25–0.35 t molasses, which have 10–15% of the sucrose of raw sugar beet (Schmid et al., 2019). Due to their high sucrose concentration, organic matter and market availability, they have a high potential for valorization through dark fermentation (Park et al., 2010). The treatment process is very similar to the one presented above for wine vinasses and sludge, with some minor differences (Figure 2). The expected purity of the biohydrogen generated in the dark fermentation step is slightly lower (52% H_2, 47% CO_2 and 1% CH_4) and the composition of the biogas produced in the anaerobic digestion is richer in methane (81% CH_4 and 19% CO_2). The post-treatment of the liquid fraction of the anaerobic digestion is the section of the plant where the WVS and SBM processes differ the most. In this case, the treatment consists of two steps: precipitation and carbon-nutrient removal. Nitrogen and phosphorus are first recovered in the form of struvite (fertilizer) and then part of the remaining organic matter and nitrogen are degraded and released into the atmosphere. The composition of the effluent is still very rich in nutrients but complies with the European legislation. Therefore, the

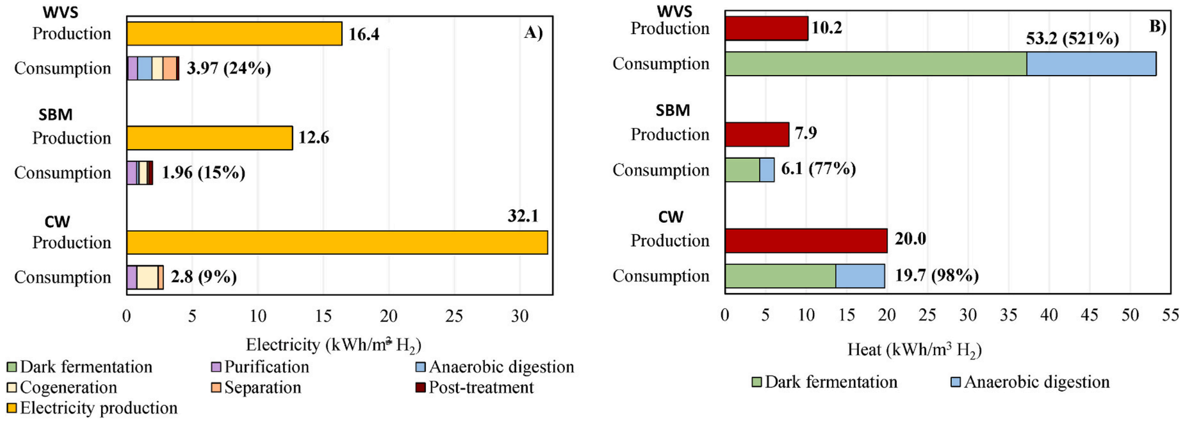

Fig. 2. Energy benchmark of scenarios (kWh/m^3 H_2). A) Electricity balance; B) Heat flow balance. CW: Cheese Whey scenario; SBM: Sugar Beet Molasses scenario and WVS: Wine vinasses and sludge from wastewater treatment scenario.

Table 3
LCA inventory of the biohydrogen production process from sugar beet molasses per functional unit (1 Nm^3 hydrogen).

Inputs from the Technosphere			Outputs from the Technosphere		
Materials			Products		
Molasses	0.10	m^3	Biohydrogen	1.00	m^3
NaOH (50%)	0.63	L			
Polyelectrolyte	0.01	kg	**Avoided co-products**		
MDEA (49.5%)	0.70	L	Electricity	10.68	kWh
Air	26.90	m^3	Heat	1.80	kWh
$MgCl_2$ (30%)	0.05	L	Concentrated fertilizer	4.62	kg
			Liquid fertilizer	0.07	m^3
Transport			Struvite	0.03	kg
Molasses	4.03	t·km			
NaOH	$6.67 \cdot 10^{-4}$	t·km	**Emissions to air**		
Polyelectrolyte	$8.83 \cdot 10^{-6}$	t·km	CO_2	3.84	m^3
MDEA	0.29	t·km	H_2O	$4.14 \cdot 10^{-3}$	m^3
$MgCl_2$	$5.86 \cdot 10^{-5}$	t·km	MDEA	$3.48 \cdot 10^{-4}$	m^3
			O_2	0.94	m^3
			N_2	21.28	m^3
			H_2	$5.21 \cdot 10^{-2}$	m^3
			CH_4	0.12	m^3

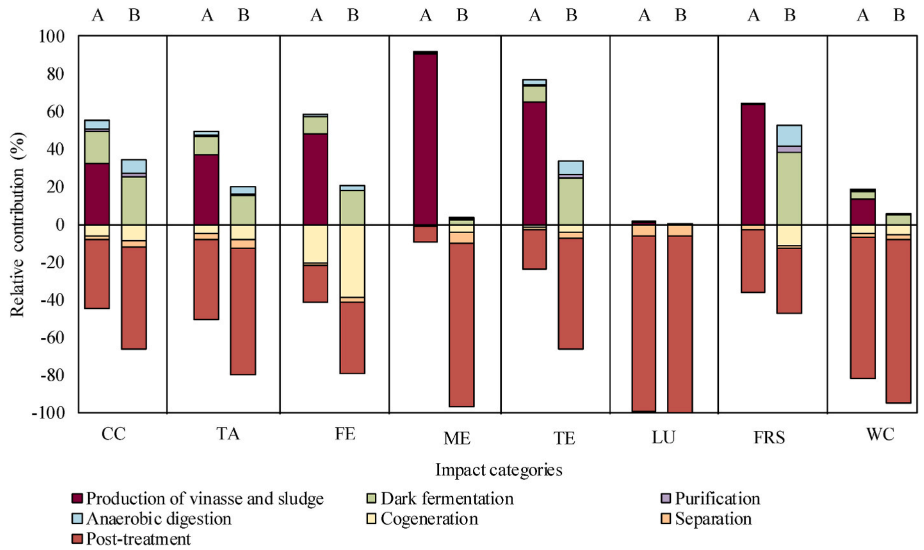

Fig. 3. Relative environmental profile of the WVS scenario per 1 Nm^3 of purified hydrogen for the cradle-to-gate (A) and gate-to-gate (B) approaches. CC: Climate change; FE: Freshwater eutrophication; FRS: Fossil resource scarcity; LU: Land use; ME: Marine eutrophication; TA: Terrestrial acidification; TE: Terrestrial ecotoxicity; WC: Water consumption.

stream can be valorized as liquid fertilizer for irrigation of surrounding areas.

2.4.3. *Cheese whey (CW)*

This scenario transforms cheese whey into biohydrogen, electricity, heat and solid biofertilizer (Figure 3). The differences from the previous scenarios can be listed as follows: the biohydrogen generated in the dark fermentation is mainly composed of 54.5% H_2, 43.5% CO_2, and 2% CH_4 and the biogas from the anaerobic digestion is 74.9% CH_4 and 25.1% CO_2, the pH control of the dark fermentation was carried out with the addition of sodium bicarbonate and the post-treatment of the liquid fraction (due to its characteristics and high fat content) was carried out in a centralized treatment plant.

2.5. *LCA data collection*

Life Cycle Inventory (LCI) is the LCA stage where the quantified input and output flows of the three scenarios (WVS, SBM and CW) were compiled in Tables 2–4 (Davis et al., 2017; Li et al., 2012). For that purpose, the data gathering procedure involved process modelling built on secondary data from other scientific publications, from computational engineering software such as Aspen Hysys and databases such as

Table 4
LCA inventory of the biohydrogen production process from cheese whey per functional unit (1 Nm^3 hydrogen).

Inputs from the Technosphere			Outputs from the Technosphere		
Materials			Products		
Cheese whey	0.32	m^3	Biohydrogen	1.00	m^3
$NaHCO_3$	3.23	kg			
Polyelectrolyte	0.02	kg	**Avoided co-products**		
MDEA (49.5%)	0.62	L	Electricity	29.36	kWh
Air	68.48	m^3	Heat	0.30	kWh
			Concentrated fertilizer	5.67	kg
Transport					
Molasses	13.36	t·km	**Emissions to air**		
$NaHCO_3$	$3.23 \cdot 10^{-3}$	t·km	CO_2	8.83	m^3
Polyelectrolyte	$1.63 \cdot 10^{-6}$	t·km	H_2O	$9.95 \cdot 10^{-3}$	m^3
MDEA	0.25	t·km	MDEA	$3.07 \cdot 10^{-4}$	m^3
			O_2	2.40	m^3
			N_2	54.11	m^3
			H_2	$5.21 \cdot 10^{-2}$	m^3
			CH_4	$3.06 \cdot 10^{-2}$	m^3
			Wastewater treatment		
			Liquid digestate	0.31	m^3

Ecoinvent 3.6.

Due to the conceptual process designed on the basis of information from other research publications, data quality is one of the main sources of uncertainty associated with this study. Therefore, the results obtained are subject to the combined effects of data variability. In addition to parameter uncertainty, this variability is also related to two other categories: methodological and model uncertainty (Bamber et al., 2020). The first is related to the LCA assessment assumptions adopted, such as the choice of system boundaries or functional unit. The other comes from the mathematical relationships followed during scenario design (including scale, technology and feedstock selection) or the characterization factors chosen at the Life Cycle Impact Assessment (LCIA) stage (Brandão et al., 2022). In this sense, it is important for readers to realize that the results provided are directly related to the assumptions made in the study and that significant changes in the process could lead to different results.

2.6. *Life cycle environmental assessment method*

The environmental analysis described in this manuscript is based on the application of ReCiPe 2016 v1.1 Midpoint (H) methodology. Moreover, the ReCiPe 2016 EndPoint (H/H) can also report a single environmental damage score for each of the scenarios and thus facilitate benchmarking. Once the assessment method was selected, the LCI input-output flows were multiplied by characterization factors according to the chosen impact categories (JRC-IES, 2012). The selected impact categories were climate change (CC), terrestrial acidification (TA), freshwater eutrophication (FE), marine eutrophication (ME), terrestrial ecotoxicity (TE), land use (LU), fossil resource scarcity (FRS) and water consumption (WC). All of them were chosen based on the recommendations provided by the LCA guidelines for the prevention, valorization, and treatment of wastewater in the food industry (Davis et al., 2017).

2.7. *Energy analysis*

One of the most recurring hotspots while performing the environmental assessment of technologies and facilities comes from energy consumption, which is an extremely resource demanding upstream process (Leme et al., 2014). Each energy source is associated with an environmental impact and, as consequence, may become a critical selection in LCA studies (Arvidsson and Avanström, 2015). Therefore, the focus of the scenarios analyzed in this study should not only be on the elimination of the environmental burdens associated with the raw materials as waste but should also focus on the energy self-sustainability of the processes. In this way, the energy consumed at each stage of the hydrogen manufacturing process can be offset by the on-site recovery of some of its co-products. The performance of the facility was then assessed by considering two procedures: (1) energy input-output flows by type (heat and electricity) and (2) energy indicators. The two selected indicators are ratios calculated from energy balance data that provide information on the energy surplus or deficiency of a process. Ruggeri et al. (2013) defined their ratio as the excess energy produced by the process and the energy required for its operation while Djomo and Blumberga (2011) approach it as the energy content of hydrogen (main product) and the energy demanded by the process with or without co-products.

3. Results and discussion

3.1. *Environmental impacts of WVS*

Within the scope of this study, the environmental impacts attributed to an integrated dark fermentation-anaerobic digestion process fed by different biodegradable feedstock have been identified. The ReCiPe MidPoind and EndPoint methods were followed. Fig. 3 shows the relative environmental profile of the WVS scenario for a cradle-to-gate (A) and a gate-to-gate (B) perspective. The results were displayed for the 8 midpoint impact categories selected.

The comparison of both cradle-to-gate and gate-to-gate approaches provides information related to the share of impacts associated with the raw materials, as this is the only scenario whose environmental impacts were not directly taken from the *Ecoinvent* 3.6 database. The proportional impacts from the production of wine vinasses were estimated considering the viticulture and winemaking process studied by Cortés et al. (2019) and those from the sewage sludge from a WWTP modelled by Lorenzo-Toja et al. (2016). In this regard, the relative contribution of the blended feedstock considered varied between 13% (CC) and 90% (ME). The marine eutrophication, which is the most affected category, is mainly influenced by the application of nitrogen fertilizers to the vineyards (both production and emissions). The dark fermentation (included in subsystem S.1) follows as the most polluting stage with impacts fluctuating between 4% (CC) and 21.5% (FRS). On the other hand, the co-products offset the negative impacts from the process. For example, the liquid fertilizer counteracts this effect more than 8% in all cases. Moreover, the heat supply also becomes relevant for CC (19%), TE (12%) and FRS (26%).

3.2. *Environmental impacts of SBM*

As in the previous scenario, the main environmental impact from SBM is related to the generation of the sugar beet molasses (feedstock). Fig. 4 identifies ME (97%), TA (87%), TE (84%) and WC (79%) as the categories with the largest drawbacks over the environmental footprint.

The production of molasses is a background process from the *Ecoinvent 3.6* database which considers the proportional impacts from activities such as cultivation and harvest of sugar beet, conditioning, sugar extraction, purification and concentration of the juice and crystallization. ME, the most concerning category, is adversely affected by the application of fertilizers during the growth of the crops. TA and CC are also dependent on farming activities (cropping and irrigation) while TE relies on emissions from electricity, heat, and fuel consumption. The sections of the process associated with the cogeneration and separation have, instead, offset the global environmental impact because of the production of electricity, heat and organic concentrated fertilizer. The electricity production has reported to improve the environmental profile in all categories more than 2% (minimum value expected for the marine eutrophication) except in LU. The enhancement in this case is given by

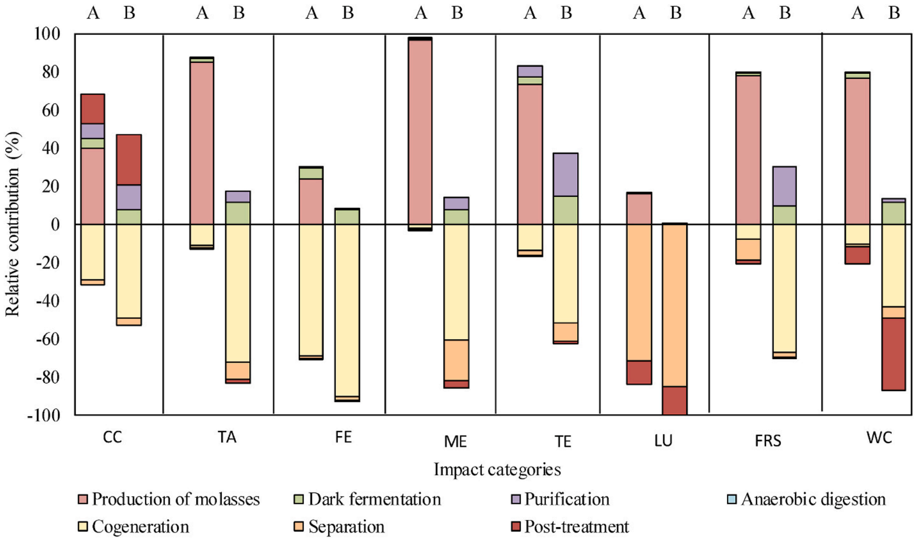

Fig. 4. Relative environmental profile of the SBM scenario per 1 Nm^3 of purified hydrogen for the cradle-to-gate (A) and gate-to-gate (B) approaches. CC: Climate change; FE: Freshwater eutrophication; FRS: Fossil resource scarcity; LU: Land use; ME: Marine eutrophication; TA: Terrestrial acidification; TE: Terrestrial ecotoxicity; WC: Water consumption.

the production of the solid fertilizer (84%).

On the other hand, the gate-to-gate analysis highlighted the dark fermentation and biohydrogen purification (subsystem S.1) as the stages with the greater environmental relevancy on the process. Their maximum contribution can be found in water consumption (13%) and terrestrial ecotoxicity (37%), respectively.

3.3. *Environmental impacts of CW*

The cheese whey manufacturing is the most notorious environmental impact of the scenario (around 98.7%) and includes activities such as the production of coagulum, separation of the curds from the whey and manipulation of the curds (as indicated in the *Ecoinvent* database). Its burden ranges between relative shares as low as a 35% (FE) and as high as 94% (TE) (Fig. 5). The dark fermentation stage ranks secondly with a participation to the profile from 5% (CC) to 10% (WC). The remaining stages of the scenario have not surpassed 5% in all cases.

On the other hand, the excess electricity produced was exported to the grid and provides benefits to the profile beyond 2% (ME) except for the LU. The enhance for this category is given entirely by the avoided burden of the production of the solid fertilizer (around 13%).

A further examination of the valorization process with the gate-to-gate perspective allowed the identification of the influence of the stages of the process over the profile but also of the materials. Regarding the energy sustainability of the process, the most impactful consumable was the $NaHCO_3$ with shares between 3% (ME) and 40% (TE). Moreover, the wastewater treatment also highlights as impactful stage in the marine eutrophication category (62%).

3.4. *Comparison between scenarios*

The SBM is the scenario with the best environmental profile when a comparative analysis is performed considering the mid-point impact categories of the ReCiPe method and a cradle-to-gate approach. As

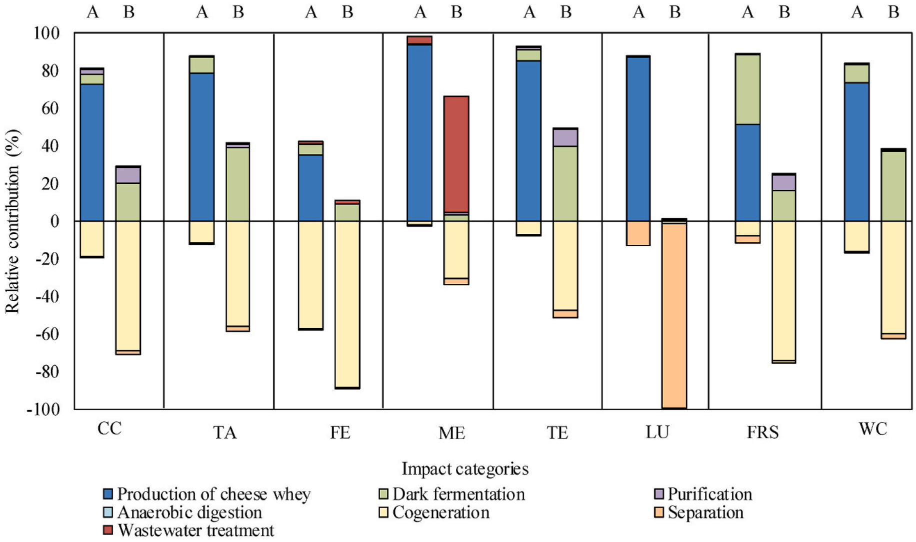

Fig. 5. Relative environmental profile of the CW scenario per 1 Nm^3 of purified hydrogen for the cradle-to-gate (A) and gate-to-gate (B) approaches. CC: Climate change; FE: Freshwater eutrophication; FRS: Fossil resource scarcity; LU: Land use; ME: Marine eutrophication; TA: Terrestrial acidification; TE: Terrestrial ecotoxicity; WC: Water consumption.

shown in Fig. 6, its relative contribution is lower than that of the other scenarios in 4 of the 8 categories analyzed (CC, ME, TE and FRS). VWS follows with 3 impact categories (TA, LU and WC) and CW only performs better in FE. In terms of GHG emissions (carbon footprint), the cradle-to-gate approach for SBM, CW and WVS scenarios reports absolute values of 3.56, 39.74 and 9.13 kg CO_2/Nm^3 H_2 which considerably differs from those of the gate-to-gate perspective (−0.65, −7.33 and −18.40 kg CO_2/Nm^3 H_2). Despite the large influence of the feedstock environmental impact, the SBM and WVS are competitive scenarios compared to the hydrogen production from a steam reforming process (12.08 kg CO_2/Nm^3 H_2) (Djomo and Blumberga, 2011).

According to the midpoint assessment, the environmental profile of the sugar beet molasses scenario shows the most convenient environmental profile. However, the results of the single score of the endpoint characterization method, collected in Table 5, point to a different assessment. WVS (−67110 mPt) ranks first, while CW (5510 mPt) has reported a higher environmental impact than SBM (−770 mPt). The avoided impacts of the products are the origin of the negative values and of the overall result obtained. For example, the major environmental benefits (−61530 mPt) in the WVS scenario comes from the recovery of the nitrogen in the form of concentrated and liquid fertilizers.

The quantification of the single score is calculated by integrating the Human health (HH), Ecosystem quality (EQ) and Resource scarcity (RS) damage indicators. The first two (HH and EQ) accounted for the largest contribution in all cases, around 35% and 53% respectively. However, the impact on HH has been reduced to 24%–85% (CW-WVS) when the effect of avoided impacts of co-products has been considered. EQ was the indicator that most defines the final single score of the scenarios because it is extremely affected by the type and quantity of co-products and RS was the least significant damage indicator.

The environmental impacts resulting from foreground and background process activities without products scored positive values (adverse effects on the environment): 1268 mPt for WVS, 6576 mPt for CW and 412 mPt for SBM. This environmental impact is reduced by approximately 76%, 99% and 83% when considering a gate-to-gate approach. In this context, the raw material production route contributes significantly to the environmental profile. Moncada et al. (2018) and Bello et al. (2018) have come up to similar conclusions for a C6 sugar production process from spruce and corn. In each case the relative contribution of the feedstock was around 10% and 15%, respectively.

Table 5
Summary of the single score Endpoint results per scenario (expressed in mPt per FU).

Scenarios	WVS	SBM	CW
Cradle-to-gate			
Hydrogen*	1268	412	6576
Coproducts	−68,378	−1182	−1066
Net value	**−67,110**	**−770**	**5510**
Gate-to-gate			
Hydrogen*	306	70	85
Coproducts	−68,378	−1182	−1066
Net value	**−68,072**	**−1112**	**−981**

* Hydrogen production without considering avoided impacts of the coproducts.

As shown in Table 5, the best performance of the process (gate-to-gate without considering the co-products) corresponds to the feedstock with the highest COD concentration (sugar beet molasses). Along with pH and residence time, the feed concentration is one of the key parameters affecting hydrogen productivity in a dark fermentation process, but its effect is negligible over the hydrogen yield (Moussa et al., 2022). In this latter case, the influence comes from the carbohydrate content instead of the COD concentration of the substrate (Alibardi and Cossu, 2015). Considering that the LCA has a product-based functional unit (1 Nm^3 of biohydrogen), a larger productivity of the process should be associated to a lower environmental impact. Therefore, there is an inverse relationship for the studied scenarios in the following order: SBM-CW-WVS. The higher hydrogen productivity corresponds to SBM (7.19 m^3 $H_2/d{\cdot}m^3_{reactor}$). However, this correlation is not direct because hydrogen is not the only obtained product and the energy integration modelled for the manufacturing processes also contribute to their environmental profile. The biogas valorized with such purpose came from the anaerobic digestion stage, whose productivity is also dependent on its feed composition (Simonetti et al., 2020). Based on this, the process performance is associated to the characteristics of the feedstock and thus, can be identified as one source of uncertainty of the obtained results (Amaya-Santos et al., 2021).

3.5. Energy efficiency and sustainability

SBM is the scenario with the largest absolute electrical production (3767.9 kWh/d) from the biogas generated in the anaerobic digestion stage, while WSV and CW present much lower values (557.1 and 2987.6

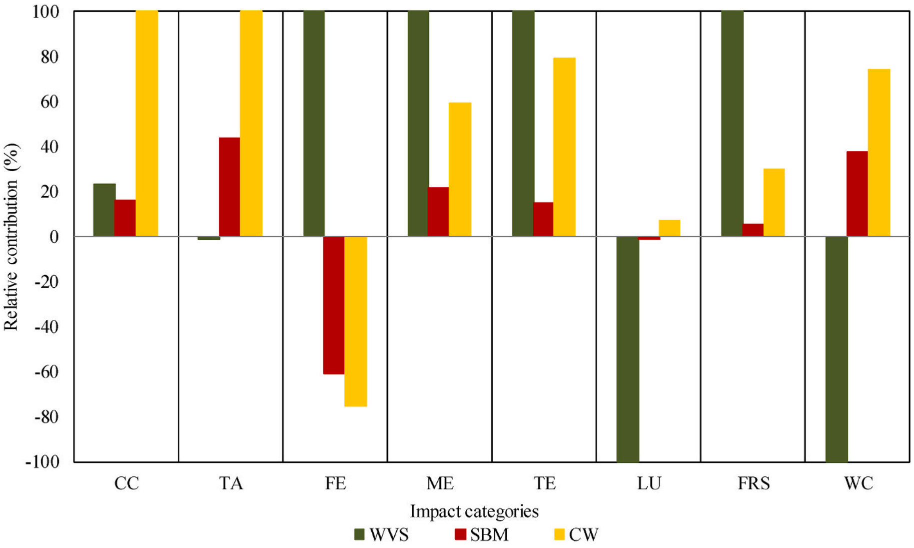

Fig. 6. Comparative environmental analysis of the WVS, SBM and CW scenarios per 1 Nm^3 of purified hydrogen. CC: Climate change; CW: Cheese Whey; FE: Freshwater eutrophication; FRS: Fossil resource scarcity; LU: Land use; ME: Marine eutrophication; SBM: Sugar beet molasses; TA: Terrestrial acidification; TE: Terrestrial ecotoxicity; WC: Water consumption; WVS: Wine vinasses-sludge.

kWh/d, respectively). Organic matter concentration is not the only parameter interconnected with energy production but there is also a strong influence from the removal efficiencies in both dark fermentation and anaerobic digestion. Cheese whey and the mixture of wine vinasses and sludge seem to provide similar concentrations of COD, but their energy production is significantly different. In this case, the higher electricity production would be related to a lower removal efficiency in dark fermentation (4.1% of COD for CW compared to 42.8% of COD for WVS) and a higher one in anaerobic digestion (98.3% of COD for CW and 76% of COD for WVS). However, it should be pointed out that a reduced degradation in dark fermentation, result in a lower hydrogen production. Therefore, the electricity generation in the process for the different scenarios were depicted in Fig. 2A considering the production of 1 Nm^3 of biohydrogen (functional unit of the LCA). When the main goal of the processes is the manufacturing of hydrogen, their benchmarking rearranges to CW > WSV > SBM in decreasing order.

Therefore, CW is the most efficient process for electricity production (32 kWh/m^3 hydrogen). Moreover, the electricity self-demand represents only 9% of the electricity produced. As shown in Fig. 2A, all of them seem to be very sustainable processes because they are able to operate independently from the centralized electrical network. Although SBM produces more biohydrogen, 3.2 times more hydrogen than CW and 8.7 than WSV, the net electricity balance has reported the worst results of the three scenarios. In this context, the integration of the anaerobic digestion step is proven to be favorable for the overall energy balance of the facility (Albini et al., 2019a).

Heat is also needed maintain the temperature of the reactors at 55 °C (dark fermentation) and 35 °C (anaerobic digestion). Therefore, a similar analysis has also been carried out for the heat demand and, as before, the estimates have only included the direct consumptions related to the operation of the installation. Fig. 2B depicts the energy balance related to heat consumed and produced in the processes. The heat demand is closely related to the temperature in the reactors, which has been set the same for all three scenarios. Thus, similar absolute results have been obtained for all of them (around 1260 kWh/d). Fig. 2B shows that the functional unit penalizes the scenario with the lowest hydrogen production (WVS) and benefits the highest producer (SBM). The heat consumption for SBM, CW and WSV accounted for about 77%, 98% and 521% of the heat produced. As in the case of the electricity balance, the order changes to CW > WSV > SBM when the results are expressed per functional unit.

SBM and CW scenarios appear to be energetically sustainable when a separate analysis is performed for electricity and heat, but WSV scenario is not sustainable in terms of heat demand because its consumption is 5.21 times higher than its production. The WVS scenario stands at 0.47 for the ratio proposed by Ruggeri et al. (2013), which is less than 1 and thus, not sustainable. SBM and CW have reported values of 2.55 and 2.32, respectively, since they present an energy surplus of 155% and 132%. The Djomo and Blumberga (2011) indicator was also used to determine the effect of the co-products over the energy sustainability. When co-products are not taken into account and exclusively dark fermentation and purification (single stage process) are considered for WVS, SBM and CW, the results were 0.08, 0.60 and 0.21. These values are much higher for the two-stage system, where the co-products are included (WVS, SBM and CW ratios increase to 0.52, 2.96 and 2.47, respectively). Djomo and Blumberga (2011) reported values between 1.40 and 2.67 for bio-based fermentation processes with co-products but they have also published results of this hydrogen/process energetic ratio for other chemical pathways. For example, steam reforming attains a ratio of 1.19, which means that there is a 19% energy surplus. The coupling of the dark fermentation with the anaerobic digestion seems to increase the energy outcomes of the process but the selection of the feedstock is also relevant to ensure that an environmentally friendly system is also energetically sustainable.

4. Conclusions and future perspectives

This study has demonstrated the relevancy of the selection of the feedstock to be valorized with a two-stage dark fermentation-anaerobic digestion system given that the main hotspot is the impact associated to the wastewater coming from the food-industry as background process. The reduction of the system boundaries initially chosen (from cradle-to-gate to gate-to-gate) suppressed the impact of this environmental profile stream, leading to a change in direction of the results when combined with two LCA methods (MidPoint and EndPoint).

The results of the Endpoint analysis with avoided co-products (both cradle-to-gate and gate-to-gate) seemed to favor the process fed by wine-vinasses and WWTP sludge while the Midpoint analysis with a cradle-to-gate boundary highlighted the sugar beet molasses as the key feedstock. The discrepancy between methods is solved when the co-products are not assumed to be an avoided burden (environmental offsetting) and thus, the best environmental performance results from the valorization of the sugar beet molasses. In contrast with the environmental research, the energy sustainability analysis has always revealed better achievement for the sugar beet molasses feedstock than for the others.

Future research should mainly focus on the validation of the environmental and energy profiles built for the modelled scenarios analyzed in this study with on-field large-scale experimental data. On the other hand, other research opportunities were found in relation to the following weaknesses: current databases do not provide specific information on the source of the waste, the treatment of waste from the bakery industry has not been sufficiently addressed in dark fermentation and little guidance was provided on the inhibitory effects of ammonia for other emerging processes such as bioelectrochemical systems.

CRediT authorship contribution statement

Claudia Irene Camacho: Methodology, Formal analysis, Investigation. **Sofía Estévez:** Writing – original draft, Visualization, Validation, Writing – review & editing, Supervision. **Julio J. Conde:** Writing – review & editing, Supervision. **Gumersindo Feijoo:** Validation, Writing – review & editing, Supervision. **María Teresa Moreira:** Conceptualization, Validation, Writing – review & editing, Supervision.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.

org/10.1016/j.jclepro.2022.134026.

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