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Upcycling the anaerobic digestion streams in a bioeconomy approach: A review

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ABSTRACT

Gaseous and liquid anaerobic digestion (AD) streams, currently are at best used for electricity and heat production or simply spreading at the fields, respectively. However, electricity and heat are economically produced from other renewables and advanced fertilizers are needed to avoid leaching and boost nutrients capture. Hence, AD seeks new opportunities to support circular bioeconomy. The overall objective of this review is to present state-of-the-art resource recovery routes for upcycling the AD streams to reduce carbon footprint and formulate alternative products to increase sustainability. Technical barriers and integrated systems to upcycle AD streams through biological means are presented. New technologies and methods to capture CH_4, CO_2 and nutrients from the digested residual resources are presented, as a) methanotrophs cultivation to be used as feed ingredients; b) CO_2 conversion and micro-nutrients capturing from microalgae to be valorized for a wide range of applications (e.g. biofuels, food and feed, fertilizers, bioactive compounds); c) CO_2 transformation to biodegradable plastics precursors (e.g. Polybutylene succinate, Polyhydroxyalkanoate); d) digestate valorization for biochar production to support efficient agricultural usage. Moreover, the environmental factors and life cycle assessment perspectives of the novel biorefinery routes are revised highlighting the need for regionalized models or assessments that can reveal the most sustainable routes based on local conditions and requirements. Despite AD poses some positive characteristics related to environmental benefit and emissions reduction, the present work reveals that the novel routes can further enhance sustainability metrics supporting circular bioeconomy.

1. Introduction

Arable lands and seas are over-utilized due to intensified industrial activities. However, the overexploitation of natural resources is strongly contradictory to the United Nations' sustainable development goals (SDGs); for example, SDG 12: Sustainable consumption and production and SDG 1: Climate action. Besides the issue of overexploited natural resources, the global municipal solid waste production is projected to be 3.40 billion tons by 2050 [1]. Considering that organic waste can represent 70% of municipal waste, enormous amounts of degradable residues are annually generated [2].

Focusing on organic waste, anaerobic digestion (AD) is the most established and widespread technology to safely manage agro-urban wastes for energy and nutrient recovery. Despite AD process is a well-established technology, novel approaches are still needed to enhance process metrics of biogas production facilities. Focusing on upstream strategies, the addition of enzymes, inoculation with microbes and micro-aerobic treatments appear as innovative approaches having the potential to enhance substrates' biodegradability and boost biological conversion toward higher biogas production [3]. Regarding mainstream and downstream strategies, biological methods to remove impurities as CO_2 and H_2S are gaining increased attention owing to increasingly stringent environmental regulations [4].The most prevalent technology at downstream of AD is heat and electricity production from the generated biogas/biomethane and in parallel, spreading the digestate with/without treatment on agricultural lands. Within the frame of SDGs, AD majorly contributes to ensuring affordable and sustainable energy

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Abbreviations

Ammonia monooxygenase AMO
Anaerobic digestion AD
1,4-butanediol BDO
Candida antarctica CA
Candida antarctica lipase B CALB
Carboxymethylcellulose CMC
Combined Heat and Power CHP
Dimethyl succinate DMS
Dry cell weigh DCW
European Union EU
Greenhouse gas GHG
High density polyethylene HD-PE
Hydrothermal carbonization HTC
Long-chain-length PHAs LCL-PHAs
Low density polyethylene LD-PE
Medium-chain-length PHAs MCL-PHAs
Methane-oxidizing bacteria MOB
Methanol dehydrogenase MDH
Molecular weight distribution $\overline{M}_w/\overline{M}_n$
Particulate monooxygenase pMMO
Photobioreactor PBR
Polybutylene succinate PBS
Polycyclic aromatic hydrocarbons PAHs
Polydimethylsiloxane PDMS
Polyhydroxyalkanoate PHA
Polyhydroxybutyrate PHB
Polypropylene PP
Short-chain-length PHAs SCL-PHA
Single cell protein SCP
Six in One biogas system SIOBS
Soluble monooxygenase sMMO
Succinic acid SA
Sustainable development goal SDG
Titanium (IV) butoxide TBT
Tricarboxylic acid TCA
Volumetric mass-transfer coefficient K_La
Weight-average molecular weight $\overline{M}_w$

(SDG 7) while also contributes to more targets (SDG 6, 9, 13, 15). Nevertheless, the uncertainty triggered by forthcoming reduced governmental subsidies has lately forced the biogas plants to seek alternative products in order to be economically sustainable and accelerate the green transition. Due to the fact that electricity and heat are more economically produced from other renewable energy sources (e.g. wind, and sun) compared to AD and that more efficient fertilizers are needed to avoid leaching and nutrients capture from the plant, the proper utilization of gas and liquid AD streams is recently re-evaluated.

Concerning sustainable development and having SDGs as a benchmark, it is also an urgent need to ensure the existence of an adequate amount of proteins to avoid starving of the increasing population (SDG 2). Different types of microorganisms (fungi, algae, yeast, bacteria) can be used as a source of single cell protein (SCP), with bacteria posing unique characteristics as low mass doubling time and high protein content [5]. Among them, methane-oxidizing bacteria (MOB) could accumulate high amounts of protein and thus, appear as an attractive solution to help on the forthcoming global protein scarcity. Furthermore, feeding biogas to methanotrophs to produce feedstuff is considered a promising alternative [6], as CH_4 from biogas can provide the needed carbon for biomass assimilation alleviating also the dependencies on fossil resources (i.e. natural gas). On the other hand, nitrogen-rich digestates can provide the most essential nutrient for microbial protein production [7]. In addition, digestates can supply the needed phosphorus to substitute phosphate salts and release from the pressure of mining rocks phosphates. Through such valorization route, residual resources upcycling can significantly contribute towards the replacement of traditional meal proteins which have high environmental costs related to their production [8]. In parallel, land, water, and natural resources could be alleviated from intense exploitation to produce feed for aqua- and agri-culture. For example, more than 300 m^2 of land are needed to supply the agriculture with 1 ton potato protein and the production of 300–400 kg high-quality fishmeal needs 1 ton of fish to be grown. In contrast, the production of microbial protein from residual streams (*i.e.* biogas and digestate) does not rely on the dependence of the limited natural resources. However, there is lack of literature on the production of microbial protein from AD streams as the majority of reviews are focused on conventional C and N sources (Table 1).

Cultivating microalgae using AD streams could potentially capture the CO_2 contained in biogas and the micro- and macro-nutrients in

Table 1
Summary or literature findings and advances of present article.

Utilization of 1st generation resources (i.e. natural gas, high grade chemicals)			Utilization of 2nd generation resources (i.e. biogas, residual nutrients)			Conventional and thermal applications of digestate	Alternative applications of digestate	Reference
Microbial feed	Microalgae	Bioplastics	Microbial feed	Microalgae	Bioplastics			
✓	×	×	×	×	×	×	×	[21]
✓	×	×	×	×	×	×	×	[22]
×	✓	×	×	×	×	×	×	[23]
×	✓	×	×	×	×	×	×	[24]
×	✓	×	×	×	×	×	×	[25]
×	×	✓	×	×	×	×	×	[26]
×	×	✓	×	×	×	×	×	[27]
×	×	✓	×	×	✓	×	×	[28]
×	×	×	×	×	×	✓	×	[29]
×	×	×	×	×	×	✓	×	[30]
×	×	×	×	×	×	✓	×	[31]
×	×	×	×	×	×	✓	×	[32]
×	×	×	×	×	×	✓	×	[33]
×	×	×	✓	✓	✓	×	✓	This review

√: included.
×: not included.

digestate converting them into valuable biomass. Considering the carbon fixation during the cultivation and the possibilities for various applications, the valorization of microalgae grown on AD streams can markedly contribute to multiple SDGs including SDG 2, 6, 7, and 13. The versatile microalgal metabolism provides a group of high value biochemicals such as phycobiliproteins, long-chain polyunsaturated fatty acids, pigments, and other antioxidants [9]. Despite the availability of such compounds can significantly increase product's economic value, the impact of AD streams on microalgae cultivation is not deeply discussed in the literature (Table 1).

Carbon dioxide (CO_2) in biogas can also be used in microbial processes for the production of bio-based polymers as a strategy to replace the production of petroleum-based plastics contributing to SDG 12. In particular, polybutylene succinate (PBS) and polyhydroxyalkanoate (PHA) which can be used in bio-based plastics formulations with high biodegradability can be produced using CO_2 as feedstock and be used in biobased plastics formulations with high biodegradability. PBS can be produced by transesterification, direct polymerization, and condensation polymerization reactions between succinic acid (SA) and 1,4-butanediol (BDO), which in turn can be synthesized either by chemical processes or fermentation routes. The fermentative process for SA production makes use of renewable resources and CO_2, consumes less energy, and has a better performance in terms of GHG emissions compared to chemical processes [10]. The bio-based process for BDO production involves the use of glucose to produce SA followed by a chemical reduction to yield butanediol. Glucose can be obtained from organic wastes and/or residual streams from biorefinery facilities rather than using dedicated feedstocks such as corn or sugarcane for its production. Polyhydroxyalkanoates (PHAs) can be produced directly by bacterial fermentation, and depending on the strain PHAs can be synthesized *via* heterotrophic, autotrophic, or photoautotrophic conditions or combinations of them. A clear benefit of using biogas during the production of these bio-based polymers is that methane is also obtained, which can further improve the economy and sustainability metrics of the process. Production of succinic acid (SA) from different organic carbon sources *via* fermentation has been thoroughly reviewed in several studies [11–14]. On the contrary, processes and strategies for the production of SA to PBS and PHA from CO_2 and/or a gas mixture containing CO_2 is not addressed in the literature (Table 1).

Inevitably, AD leads to huge amounts of digestate which should be treated properly to avoid causing environmental pollution due to the high content of nutrients [15]. Digestates have long been traditionally applied to agricultural farms as soil amendments to substitute chemical fertilizers, because nitrogen, phosphorus, potassium, and other essential trace elements are available in the digestate and can be uptaken by plants [16]. Apart from the problems such as requiring high storage capacity, transportation of such bulky materials, and application costs [17], concerns over the oversupply of digestate or its application in improper time [18] as well as high mobility of the nutrients, which results in low utilization of the nutrients, have raised considerable debates [19]. For these reasons, direct application of digestate on farmlands as a soil amendment might not be the most effective way to use the contained nutrients, and therefore, increased attention is expected to be given to the alternatives for digestate use. Among alternatives, digestate could be upcycled into nursery substrate for plant seedlings cultivation, feed for earthworm engineering, or dissimilar biochar products (e.g. for soil fertilization, soil remediation, compost amendment, nutrients recovery) contributing to several SDGs (i.e. 2, 3, 6, 13, 15).

As presented above, several novel bioprocessing routes can be established for the valorization of AD effluents into a vast number of value-added products. The sustainability of such bioprocessing routes depends on the environmental costs of the final products and the created revenue compared to the conventional counterparts; especially those from petrochemical routes. More specifically, environmental performance is a function of the environmental loads created in the foreground and background systems as well as the environmental impacts of the competing products. Accordingly, to investigate how sustainable a novel route is, the production process of the specific product including all the required up- and downstream processes along with the environmental performance of the competing products must be taken into account to reach a realistic conclusion [20]. Biorefinery approach can potentially make synergies among various bioprocessing routes to achieve the highest environmental and economic benefits, decrease the amount of waste streams, and increase the number of final marketable products. Under combined integrated systems, technical barriers to holistically upcycle AD streams can be overcome and sustainability goals can be attained (SDG 8, 9).

Efficient resource recovery routes are needed to reduce carbon footprint, formulate alternative products, and upcycle the gaseous and liquid AD streams. There is a large number of potential routes from biogas streams. However, the present work is focusing beyond the state-of-the-art (Table 1) on topics as: a) biogas and digestate valorization for single cell protein (SCP) production; b) CO_2 and micro-nutrients capturing from microalgae to be valorized for a wide range of applications (e.g. biofuels, food and feed, fertilizers, and source of bioactive compounds); c) biodegradable plastics precursors (i.e. PBS and PHA) production from CO_2; d) digestate valorization for efficient agricultural usage; e) environmental factors and LCA perspectives of the novel routes; f) biorefinery approaches focused on the circular economy.

2. Microbial protein *via* biogas and digestate upcycling

MOB are a subset of methylotrophs that uptake CH_4 as a sole carbon and energy source to produce a vast platform of products including among others PHB, glycogen, methanol, formaldehyde, organic acids, sucrose, ectoine, lipids, enzymes, pharmaceutical and antimicrobial proteins [34]. Among alternatives, the production of microbial protein triggers the interest for commercial applications as MOB can store approximately 70% protein in their biomass [22,39]. The proteinaceous bacterial mass can be a suitable feed supplement in monogastric animals' diet (e.g. pigs, chicken, mink, salmon) according to amino acid profile which is comparable to fishmeal and soybean meal, digestibility and also, livestock health and performance [40]. MOB are classified as Group I (γ-proteobacteria mainly using the RuMP cycle), II (α-proteobacteria mainly using the Serine cycle), and III (Verrucomicrobia using the CBB cycle) [41]. Nevertheless, Deltaproteobacteria are also predicted to aerobically utilize CH_4 *via* the CBB cycle [42]. Methane monooxygenase (MMO) enzymes, either particulate (pMMO) or soluble (sMMO), are responsible to catalyze the first step of MOB metabolism which is the oxidation of CH_4 to CH_3OH (Fig. 1). Between the two MMOs, sMMO has more diverse applicability [43] while pMMO has a higher affinity for CH_4 [44].

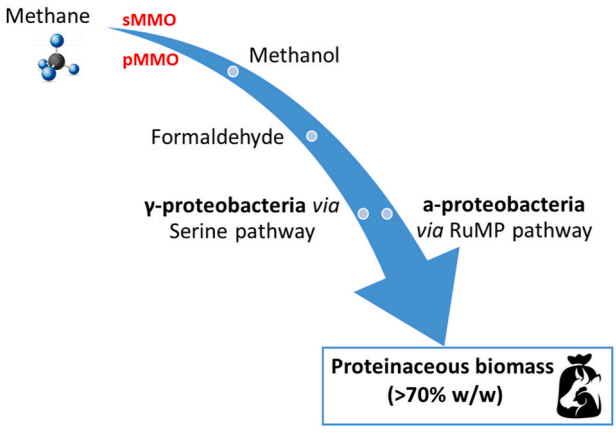

Fig. 1. Methanotrophic CH_4 assimilation.

2.1. Nutritional needs of methanotrophs

Copper and iron are the most critical micro-nutrients metals for the mediation of MMO expression. Especially, 'copper switch' mainly regulates the biosynthesis of pMMO and sMMO in MOB which can express both MMO-forms [45]. Specifically, sMMO biosynthesis is predominant at low Cu to biomass concentrations while the pMMO activity is mainly detected at ratios higher than 5 μmol Cu/$g_{biomass}$ [41,46]. On the contrary, an excess of iron could stimulate the expression of sMMO [47]. Nevertheless, the provision of the correct amount of trace elements is crucial to achieving a high rate of CH_4 to CH_3OH conversion and subsequently, efficient overall metabolism. The growth of mixed MOB culture can be markedly eliminated at Cu scarcity and *vice versa*, inhibited at Cu excess using digested municipal biowaste for nutrients provision [48]. In the cited study, the digestate was highly diluted to reach the desired N levels leading to Cu deficiency. As alternative, the adaptation of the community to higher N levels would have been a solution to reduce the need for high dilution (decreasing the water footprint) and also, decrease the risk of micro-nutrients scarcity.

Regarding macro-nutrients, upcycling nitrogen into protein is gaining increased attention because it can favour sustainable development within the framework of residual resources recovery [49]. Nitrogen is essential to synthesize amino acids, the building blocks for protein synthesis. While methanotrophy is possible with different nitrogen forms (i.e. nitrate, NO_3^- or ammonium, NH_4^+), NH_4^+ which is the dominant *N*-form in a digested organic waste can be stimulant for CH_4 oxidation compared to NO_3^- [44,50]. On the other hand, the usage of NH_4^+ can lead to inhibition or growth cease due to either competition with CH_4 as the active sites of pMMO and ammonia monooxygenase (AMO) are similar or accumulation of produced toxicants as hydroxylamine and nitrite [51]. According to Hu and Lu [52], NO_3^- stimulated type I and II MOB while NH_4^+ promoted the presence of type I. On the contrary, the affinity for CH_4 of type I MOB was found to be higher at 80 than 4 mM NH_4^+ and type I could be enriched at NH_4^+ rich environment [44]. Different N sources and their impact on MOB growth from literature are summarized in Table 2. Overall, the impact of NH_4^+ varies among MOB with regards to optimum and inhibitory thresholds and is always advised to be defined for each pure or mixed culture at certain conditions (i.e. temperature, pressure, and N, O_2, and CH_4 levels) [41].

2.2. Biogas as a carbon source

Despite biogas has been previously used to grow MOB (e.g. *Methylosinus sporium*, *Methylomicrobium alcaliphilum, Methylococcus capsulatus Methylosinus trichosporium*) [53,54], the researchers' attention was mainly given to the production of other molecules (e.g. methanol, lactic acid) and not protein quality. Hence, the accurate effect of biogas on the quality of produced microbial protein is not deeply defined. Nevertheless, the composition of raw biogas can highly affect MOB growth since CH_4 is not the sole compound in the gaseous stream. For example, CO_2 is the second biggest fraction (*i.e.* 25-50%) in raw biogas. Despite CO_2 can reduce growth due to the formation of carbonates, a few MOB of Group I (e.g. *Methylococcus*) and II (e.g. *Methyloferula*) can uptake CO_2 [42]. CH_4 in biogas can be still used as carbon source to build MOB biomass, while CO_2 is captured and methanol is enzymatically produced [21]. Thus, MOB are perfect candidates for sequestration of C1 compounds and conversion into feed. Moreover, biogas contains H_2S which can reach up to 20,000 ppmv based on feedstock. H_2S is toxic and corrosive and has to be carefully taken into consideration [55]. Specifically, a *Methylophilus* and *Methylonomas* rich MOB culture was inhibited by 38% due to the existence of approximately 1000 ppm H_2S in raw biogas [6] (Table 2). Similarly, *Methylosinus trichosporium* OB3b seems to be incapable of utilizing biogas due to H_2S toxicity [56]. On the other hand, the product yield of a *Methylocaldum* isolate grown on biogas to form methanol was increased due to the supplementation of methanol dehydrogenase (MDH) inhibitors and formate as an electron donor [57]. Similarly, the product yield of *M. sporium* on raw biogas containing 0.13% H_2S was increased by approximately 40%, *via* the addition of H_2 as electron donor and covalent immobilization of the cells on chitosan [53]. A recent study examined the effect of S on the cultivation of *Methylocapsa acidiphila* and shown that crude protein was significantly reduced while the content of other molecules (e.g. extracellular polysaccharides) was increased at biomass forming agglomerates [58]. Mitigation actions to overcome low product yields could be the gradual acclimatization to toxicants [59], application of biogas upgrading to remove CO_2 [60], addition of extra electron donors, or inoculation with tolerant to inhibitors MOB (i.e. *Methylocaldum* for H_2S, members that assimilate CO_2 *via* the CBB cycle) [61,62].

While the industrial application of methanotrophic single-cell protein production was not achieved yet, a few SMEs are using CH_4 to grow MOB. For example, UNIBIO is supplied with CH_4 from the Danish gas grid to grow a *M. capsulatus* dominant culture, UniProtein®. Nevertheless, in Denmark, clean biogas free of H_2S is also injected into the gas grid and mixed with natural gas. Hence, UNIBIO's culture can be occasionally grown on stream containing biogas and, in this framework, UNIBIO is continuously involved in projects exploiting biogas as a sole CH_4 source to fully develop the concept and improve the environmental footprint [63,64]. On this topic, the complete replacement of natural gas with biogas could reduce by 70% of the lifecycle emissions associated with the production of FeedKind® by Calysta [65]. Focusing on targeted markets, Uniprotein® targets all animal and fish species, while Calysta provides three products (i.e. FeedKind® aqua, terra, and pet) based on the nutritional need of species. Nevertheless, both SMEs can supply the market with a high proteinaceous product (~70% crude protein) to be used as animal feeds [22,65]. Before market implementation, biomass nutritional value as protein ingredient should be explored at targeted animal species to reveal potentially adverse impacts that are originated from residual resources utilization. For instance, examination of

Table 2
Cultivation of MOB for microbial protein production using liquid and gaseous AD streams.

Community	C source	N source	Biomass concentration, g CDW/L	Protein content, % CDW	Reactor	Reference
Methylomonas dominated	a) Biologically upgraded biogas b) Biologically upgraded biogas	a) NH_4Cl b) Electrochemical extracted NH_4^+ from digested biowaste	a) 0.60 b) 2.32	a) -b) 50	a) Batch b) CSTR	[48]
Methylophilus dominated	a) Raw biogas b) Pure CH_4	a) NH_4Cl b) Digested biowaste	a) 0.61 b) 0.60	a) -b) 41	a) Batch b) CSTR	[6]
Enriched MOB	Electrochemically upgraded biogas	NH_4Cl	0.59	66	Batch	[73]
Enriched MOB	Biologically upgraded biogas	Electrochemical extracted NH_4^+ from digested biowaste	0.49	50	Batch	[49]
M. acidiphila	Raw biogas	NH_4^+ and NO_3^-	0.07–0.09	33–58	Batch	[58]

different inclusion levels and feed formulations should take place on performance trials. To define product's suitability, the animals should be evaluated in terms of production performance, health, body weight gain, feed intake, feed efficiency and other relevant parameters. On this topic, it was previously found that methane protein has no adverse impact on the growth of pig and trout and on the contrary, the quality of pork was enhanced [66]. Similar studies should be conducted in other animal species (e.g. poultry) before performing feed formulations. Furthermore, the final product should always meet the highest safety standards and be free of pathogens, heavy metals and contaminants before provided in livestock. Hence, full compliance of the product with the existing regulatory governance framework at national and international level should be met for all parts of the value chain.

Despite the usage of biogas and recovered nutrients from residual resources that can improve the economic viability of the process, few challenges need to be overcome in order to improve yields and production rates and roar the protein production from MOB [39,40]. For example, UNIBIO targets to increase their current productivity (i.e. 3-4 kg/m^3/hr) by 50% *via* optimization algorithms, BioOptimizer, and pressure/pressureless system at the patented U-loop technology [67].

Also, the high pKa of CH_4 (~50) settles it extremely resistant to dissociation and mass transfer limitations make it difficult to provide as a substrate for the submersed bacteria. Bubble-free membrane reactors, gas lift reactors to form suspension and granulations, or the addition of paraffin oil as a water-immiscible solvent could also be explored as means to enhance cell growth and cell density [39,68,69].

Another challenge is that bacterial cells usually have a high nucleic acid content (~16%) that reduces the commercial basis to only animal feed for livestock with short life spans [70]. To expand MOB to the food market, enzymatic treatment *via* ribonucleases, thermal treatment at 60–90 °C, alkaline hydrolysis and chemical extraction have been already applied [22].

Moreover, downstream optimization is also needed to achieve a financially viable alternative process. Despite the energy-intensive spray driers are typically used to produce the final dry MOB powder, forward osmosis technology was lately examined to partially dewater MOB [71]. Among draw solutions, wastewater brine was also tested as a cheap solution in the circular economy framework. However, low water fluxes were achieved indicating that only brines with high salt content can be used. On the contrary, deep eutectic solvents are shown as a green, sustainable, efficient, and recyclable energy resource that can be used for concentrating proteins [72]. Moreover, the final market product should be optimized towards increased fat and protein digestibility and absorption. Ultra-heat treatment process can be also explored to secure optimal homogenization and retain protein quality and functionality.

3. Microalgae cultivation with biogas and digestate

3.1. Capturing carbon dioxide from biogas

Microalgae can grow autotrophically in bubble column reactor using CO_2 in biogas as a carbon source. Recent research suggested that the CO_2 removal efficiency could theoretically reach to 100% by adjusting crucial parameters such as pH and liquid/gas ratio of a bubble column reactor [74]. However, the O_2 produced during photosynthesis would potentially lead to safety issues due to the explosive gas mixture (O_2 and CH_4). Studies have shown that injecting biogas into microalgae cultivation vessels results in 10%–24% O_2 concentration in CH_4 [75]. Thus, the biggest technical challenge of using biogas-CO_2 for microalgae cultivation minimize O_2 concentration, by continuously removing it from the gas to avoid reaching explosive CH_4–O_2 mixtures [76]. Several approaches have been proposed to capture/remove O_2 from the gas. One approach to achieve this is to firstly upgrade biogas to biomethane, while the captured CO_2 is separately provided for microalgal cultivation. In this way, CH_4 is not injected to a reactor filled with microalgae and thereby contact of CH_4 with the O_2 generated by microalgae is avoided.

Another possibility to address the dangerous CH_4–O_2 challenge is to establish co-culture, where the O_2 produced by microalgal photosynthesis is consumed by fungal respiration [77,78]. Another approach was to co-cultivate microalgae with MOB in the same pot, having microalgae consume CO_2 while the produced O_2 was instantly taken up from the MOB producing two sources of proteinaceous biomass, namely microalgal and MOB biomass, and at the same time avoiding explosive mixtures of gas [79]. However, these concepts have only been demonstrated at lab scale and therefore validation at a larger scale is needed.

An example of decoupling the CO_2 capture from microalgae cultivation, which significantly shortens the contact time between biogas and O_2-producing cultures (Fig. 2). The microalgae biogas upgrading system has been extensively investigated at lab scale, with a few pilot-scale demonstrations (Table 3). In such a system, an external absorption column can be used to capture biogas-CO_2 into the liquid phase, whereas the microalgae in the cultivation unit use the CO_2-rich liquid for biomass accumulation. Bahr et al. [80] have demonstrated a proof-of-concept study using a packed bed column as the absorption column and a pilot high rate algal pond as the cultivation unit. The system removed 100% H_2S, 90% CO_2 from the biogas with less than 0.2% O_2 remained in upgraded biomethane. Following the proof of concept, several studies are dedicated to optimizing the operational strategies by adjusting parameters such as biogas flow rate, retention time, and operation temperature [76,81,82]. Recently, a control system was developed to maintain biomethanation quality by adjusting the recycling liquid flowrate in the absorption column [83]. This automatic control system managed to achieve high-quality biomethane (negligible H_2S, $CO_2 \leq 2.5\%$, $O_2 \leq 1\%$) biogas flowrate from 60 to 150 mL min^{-1}. The control system was also proved to be robust and could restore biomethane quality in less than 2 h after the system shut down [84]. Besides, optimizing the operation of the absorption column, the application of polydimethylsiloxane (PDMS) gas-liquid membrane and biogas scrubbing at high pressure also proved to have a positive effect on removing N_2 and O_2 from microalgae-upgraded biomethane [85].

In addition to efficient CO_2 capture, a well-performed cultivation unit is essential to further valorize the CO_2 in biogas. Microalgae cultivation can be influenced by many parameters including temperature, pH, nutrients availability, light, etc. To ensure an efficient CO_2 capture, the recirculated liquid in adsorption columns typically has a pH value higher than 9. In the case, significant challenges are posed to the sequential microalgae cultivation unit, as it is required to grow under high pH and inorganic carbon concentration. Currently, most of the research addressing these challenges has been focused on improving the microalgae cultivation by finding functional microalgae strains. Granada-Moreno et al. [86] identified a special alkali-tolerant microalgae consortium in a microalgae biogas upgrading system composed mainly of *Picochlorum* sp. and *Scenedesmus* sp. Nevertheless, Bose et al. [87] concluded that *Anabaena cylindrica*, *Chlorella sorokiniana*, *Scenedesmus obliquus*, *Spirulina platensis*, and *Synechococcus* sp. fit best for biogas upgrading considering the ability for mixotrophic growth, high pH tolerance, external carbonic anhydrase activity, high CO_2 tolerance, and ease of harvesting. However, the overall operational parameters could also be holistically considered for the absorption column and the microalgae cultivation unit. The media retention time in absorption column should be efficient for CO_2 fixation and results into a proper CO_2 concentration for microalgae cultivation.

3.2. Digestate as a cultivation medium

Although microalgae can sometimes survive in sediment or soil, microalgae grow the best in aquatic environments such as freshwater and marine systems. Thus, liquid digestate from wet AD (total solids content in digester below 15%) is suitable for microalgae cultivation. AD effluent typically contains sufficient macro- and micro-nutrients that can support autotrophic, heterotrophic, and mixotrophic growth of microalgae. Depending on the operational conditions of the AD process, the

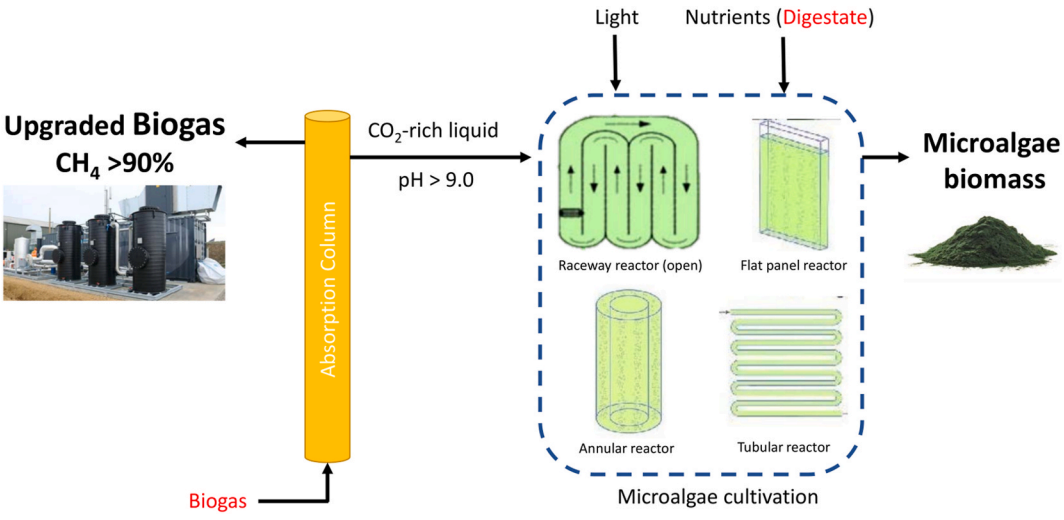

Fig. 2. Combined biogas upgrading and microalgae cultivation systems.

Table 3
Pilot-scale demonstration of microalgal biogas upgrading system.

Absorption column	CO_2 removal	Nutrients	Reactor size	Reactor type	Microalgae species	Biomass yields	Reference
45 L	91%	Agriculture wastewater	12 m^3	Tubular photobioreactor	*Chlorella vulgaris*, *Stigeoclonium tenue*, *Nitzschia closterium*, and *Navicula amphora*	n.a	[88]
150 L	99%	Domestic wastewater	9.6 m^3	High rate algal pond	Algal-bacterial	n.a	[84]
0.35 L	89–94%	Media	25 L	High rate algal pond	Natural algal community	11 mg/L	[86]
30 L	89–93%	Media	50 L	Open-photobioreactor	*Chlorella sorokiniana*	0.6 g/L	[89]

digestate may also contain a high amount of indigestible particles, active bacteria, dark colour substance, and unpleasant odours, which will hinder its application as microalgae cultivation media.

3.2.1. Digestate treatment

In pursuit of optimal growth of microalgae, three main factors need to be adjusted, namely: 1) removing the indigestible particles and dark colour which will hinder the light penetration during microalgae cultivation; 2) controlling the indigenous bacteria which potentially will compete with microalgae for nutrients; 3) adjust ratio of C, N, P for microalgae growth. For factors 1) and 2), centrifugation and/or autoclavation are extensively used for lab investigations. However, upscale such pretreatment to the commercial-scale is economically challenging. On the other hand, dilution, settlement, and membrane filtration are found more promising for large-scale applications [90]. Especially, membrane filtration gained extensive attention in recent years because it can remove the particles and microorganisms (e.g. protozoa, bacteria) in a one-step process [91].

3.2.2. Alleviation of ammonia inhibition

Digestate is typically rich in ammonia-N, which can serve as a nitrogen source for microalgae growth. However, high free ammonia concentration can also inhibit the growth of microalgae damaging important protein complex used for photosynthesis [92] and interfering with microalgal intercellular pH regulation [93]. Thus, acclimatizing microalgae to high ammonia concentration is important for maintaining high biomass productivity. Besides diluting the digestate, screening of ammonia tolerant microalgal strain is an effective strategy to establish an efficient microalgae consortium using the liquid effluent of AD as a cultivation medium. Chuka-Ogwude [94] recently reported that *Oocystis* sp. was capable of proliferation in up to 600 mg L−1 NH_3–N concentration in digestate. In addition, combining the nitrification process with microalgae cultivation also significantly alleviates ammonia inhibition to microalgae growth [95].

3.3. Integrated carbon dioxide and nutrients recovery

While biogas upgrading could be also achieved applying novel and efficient chemical methods using for example deep eutectic solvents with high economic potential compared to ionic liquid and amine [96], the biological CO_2 capturing *via* microalgae cultivation provides an alternative technology due to the advantage of simultaneous nutrients recovery. Many studies are dedicated to developing an integrated system combining both CO_2 and nutrients capture [97–99]. An LCA study showed that an artificial lighting system for microalgae cultivation contributes to over 90% of energy demand and the biomass recovery step (harvesting and drying processes) exhibited the highest environmental impacts [100]. Nevertheless, the study might have underestimated the digestate pretreatment steps, which could significantly contribute to environmental impacts.

Currently, microalgae can be cultivated in both closed photobioreactors (PBR) and open pond systems. The former requires high construction and operational costs for significantly higher biomass quality and yield compared to the latter. The closed PBR are suitable for the production of value-added chemicals; whereas the biomass produced from the open pond is normally used for the production of biofuels or biofertilizers. Up to now, the lab and pilot investigation showed that the AD-streams microalgae cultivation system could achieve biomass yields comparable to standard cultivation media [75,95,101]. However, the AD-streams-based microalgae cultivation systems also face similar upscaling challenges as any other microalgae system, such as sufficient light penetration and the difficulties in harvest and downstream process [102]. Thus, further implementation depends on the development of more cost-effective microalgae cultivation and

valorization systems.

4. Carbon dioxide into biodegradable polymers

4.1. Synthesis of polybutylene succinate

4.1.1. Precursors for polybutylene succinate production

Succinic acid and its derivatives diamines and diols can be used as monomer units for the synthesis of a variety of plastics, such as polyesters, polyamides, and polyesters amides. Among them, poly (1,4-butylene succinate) (PBS), a linear aliphatic polyester with excellent thermo-mechanical properties and biodegradability can be synthesized from succinic acid and 1,4-butanediol (BDO). Both precursors can be produced either from renewable feedstocks such as glucose, and sucrose *via* fermentation [11–14,103–109] or petroleum-based feedstock. However, the production of glucose is responsible for half of all GHG emissions of SA production when it is used as substrate indicating that the utilization of waste co-substates has a high potential for mitigating GHG emissions. Moreover, the fermentative production of SA has been estimated to emit 12–55% CO_2-eq. less than the petrochemical-based route [10].

The advantage of the succinic acid fermentative route *via* the TCA reductive pathway is that consumes CO_2 compared to the petrochemical process [12]. In addition to the extensive research performed on the use of engineered strain or variants of the wild types [110–112], alternative low-cost carbon sources [14,113–115], improvement of downstream process technology [116,117] to make the fermentative production of succinic acid cost-effective, there have been also some attempts at lab scale to use biogas as a low-cost CO_2 source (Table 4). The advantage of using biogas in succinic acid production *via* fermentation is that high purity CH_4 can also be produced as demonstrated by Gunnarsson et al. [118], who developed a concept to convert the CO_2 in biogas to succinic acid through a biological process. The microorganism used was the strain *Actinobacillus succinogenes* 130Z (DSM22257) known as one of the most efficient natural producers of succinic acid. The bacterial strain upgrades the biogas to vehicle fuel/gas grid quality by consuming the CO_2 required for the fermentative production of SA. The effect of the pressure and the gas-liquid ratio on the system were investigated. As a result of a slight increase in the pressure (from 101.3 to 140 kPa) during fermentation higher CO_2 saturation level in the broth was attained, thereby increasing the CO_2 consumption rate by 16.4% and the SA titer and yield by 6.2 and 13.8%, respectively. The final methane content attained under these conditions corresponded to 95.4% (v/v). In this sense, the CO_2 availability to the cell should influence SA productivity as CO_2 is essential for the reductive TCA cycle flux and PEP carboxykinase activity, the key enzyme responsible for CO_2 fixation. Affinity for CO_2 of the enzymes responsible for its fixation is low and thus, high CO_2 partial pressures are required to drive the metabolic flux towards SA production [14]. Babaei et al. [119] investigated the same concept using the organic fraction of household kitchen waste as substrate, raw biogas as CO_2 source, and *Basfia succiniciporducens* as the fermentative strain. Though SA titer and yield were low, the outcome of the study proves the ability of *B. succiniciproducens* to be used as an alternative bacterium capable of converting CO_2 present in biogas into SA.

In 2019, NEOSUCCESS a European project was launched [120], in order to commercialize the concept developed by Gunnarsson et al. [118]. The objective of the NEOSUCCESS project is to make for the first time commercially available the technology to produce 2nd generation succinic acid from organic wastes and biogas. On the other hand, since 2012 Genomatica has commercialized the technology to produce BDO from sugars under the trade name GENO BDO™ and has been licensed for commercial plants by both BASF and Novamont [103].

4.1.2. Polybutylene succinate production methods

Once succinic acid and BDO have been synthesized, PBS can be produced by one of the following processes: transesterification-polymerization, direct polymerization, polymerization-condensation, and enzyme-catalyzed.

Briefly, the transesterification-polymerization process involves two steps: the first step is the transesterification of dimethyl succinate (DMS) and BDO to obtain oligomers (Eq. (1)), and the second step is the polycondensation of these oligomers to obtain high-molecular-weight PBS (Eq. (3)). In the transesterification reaction, the temperature of the system is increased until the acid component is completely melted and the temperature is maintained in a range of 150–190 °C under a gas nitrogen atmosphere. Then polycondensation reaction further proceeds under high vacuum at high temperatures of 220–240 °C. Different catalysts can be employed for the synthesis method, one of the most common ones is titanium (IV) butoxide (TBT) [121–123].

$$HO(CH_2)_4OH + H_3COOC(CH_2)_2COOCH_3 \rightleftharpoons H[O(CH_2)_4OOC(CH_2)_2CO]_nO(CH_2)_4OH + CH_3OH \quad (1)$$

$$HO(CH_2)_4OH + HOOC(CH_2)_2COOH \rightleftharpoons H[O(CH_2)_4OOC(CH_2)_2CO]_mOH + H_2O \quad (2)$$

$$H[O(CH_2)_4OOC(CH_2)_2CO]_nO(CH_2)_4OH + H[O(CH_2)_4OOC(CH_2)_2CO]_mOH \rightleftharpoons H[O(CH_2)_4OOC(CH_2)_2CO]_{n+m}OH + HO(CH_2)_4OH \quad (3)$$

In the direct melt polymerization method, the esterification reaction takes place between succinic acid and BDO (Eq. (2)) to further proceed with the second step the polycondensation reaction (Eq. (3)). Both reactions should take place in a nitrogen atmosphere to prevent oxidation in the presence of catalysts such as titanium tetrabutoxide and titanium (IV) isopropoxide [121]. In the polymerization-condensation method, a chain extender is used, to improve the molecular weight and certain physical properties of PBS such as toughness, viscosity, and thermal stability. However, one disadvantage of adding a chain extender is that will reduce the biosafety and will affect the biodegradability properties of the PBS obtained by this method, and thus, chain-extended PBS cannot be used as a food-contacting material. Some of the chain extenders used in PBS synthesis *via* the condensation polymerization method are acid anhydride, diisocyanate, oxazoline, aziridine, di-epoxide [124]. In the process developed by Showa Denko to produce Bionolle™ −the high molecular weight PBS *via* polycondensation reaction of BDO with succinic acid, −hexamethiylene diisocyanate is used as a chain extender which resulted in an increase of the molecular weight of PBS up to about 33,000 sufficient for practical use [124]. More recently Ferreira et al. [125] used rutin as a chain extender in the synthesis of PBS *via* direct polymerization-polycondensation of succinic acid and BDO in a ratio 1.0:1.1. The authors reported that the use of a small amount of rutin (1 wt%) increased the viscosity of PBS by around 100%. Also, the molecular weight was increased by 36% and the crystallinity presented a reduction of 7%.

PBS can also be synthesized *via* enzymatic catalysis using *Candida antarctica* lipase B (CALB) as biocatalyst [126–130]. Ring-opening

Table 4
Main Overview of the process for succinic acid production using alternative CO_2 sources.

Microorganisms	Organic substrate	CO_2 source	Operation mode and process conditions	Succinic acid titer (g/L)	Succinic acid yield (g/g_{sugars})	Succinic acid productivity g/L·h	Final CH_4 content (% v/v)	Reference
Actinobacillus succinogenes 130Z	Organic wastes	Raw biogas	*NeoSuccess* project is ongoing and is expected a production capacity ranging from 10,000 to 100,000 Nm^3/year of biomethane and 35 to 350 t/year of bioSA					https://neosuccess-project.eu/
Basfia succiniciproducens	Organic fraction of household kitchen waste hydrolysate (15 g/L of glucose and 2 g/L of xylose)	Raw biogas	Batch, lab-scale, 37 °C, pH = 6.75, 140 kPa	3.8	0.25	Not reported	4.7% (v/v) increase respect to CH_4 content in raw biogas	[119]
Actinobacillus succinogenes 130Z	Glucose (30 g/L)	Synthetic biogas (40% CO_2/60% CH_4)	Batch, lab-scale, 37 °C, pH = 6.75, 140 kPa, gas-liquid ratio: 5:1	13.53	0.63	0.56	95.4	[118]

polymerization and polycondensation are two common strategies in the lipase-catalyzed synthesis of polymers. Sugihara et al. [127] reported the direct polycondensation of BDO with DMS using immobilize lipase CA (40 wt.-%) from *Candida antarctica* in toluene as reaction solvent at 100 °C for 24 h. The weight-average molecular weight ($\overline{M}_w$) and molecular weight distribution, ($\overline{M}_w/\overline{M}_n$) obtained by this method were 45,000 and 3.7 respectively. The same authors also reported the two-step synthesis of PBS *via* cyclic oligomerization of diol BDO and the ester DMS with the subsequent ring-opening polymerization of the cyclic oligomer. The resulting PBS presented an $\overline{M}_w$ of 130,000 which is significantly higher compared to the one produced by the direct polycondensation method (45,000) and by the conventional chemical catalyst. Azim et al. [126] investigated the effect of temperature at 60, 70, 80, and 90 °C on the *Candida antarctica* lipase B catalyzed PBS synthesis from DMS and BDO in diphenyl ether as the reaction solvent. Polymerization reaction at 80 °C resulted in the PBS precipitation at 5–10 h, which limited the growth of polymeric chains. Increasing temperature from 80 to 95 °C resulted in a monophasic reaction mixture but also an increase in the PBS weight-average molecular weight to $\overline{M}_w$ = 38,000 ($\overline{M}_w/\overline{M}_n$= 1.39). One drawback of the lipase-catalyzed method is that the PBS obtained presents a lower molecular weight when compared to the one synthesized by the chemical methods and thus future research should focus on the improvement of the molecular weight [122].

4.2. Synthesis of polyhydroxyalkanoates

PHAs are a family of linear polyesters with the general structure shown in Fig. 3a. The monomeric chain HA presents a side chain ***R***, which can be a saturated alkyl, unsaturated alkyl, branched alkyl, or substituted alkyl groups, respectively. PHAs can be classified based on the number of carbons in this side chain as short-chain-length PHAs (SCL-PHAs) with less than five carbons, medium-chain-length PHAs (MCL-PHAs) which consist of 6–14 carbons in the side-chain (Fig. 3b), and long-chain-length PHAs (LCL-PHAs) with more than 14 carbons in the side chain [27,131].

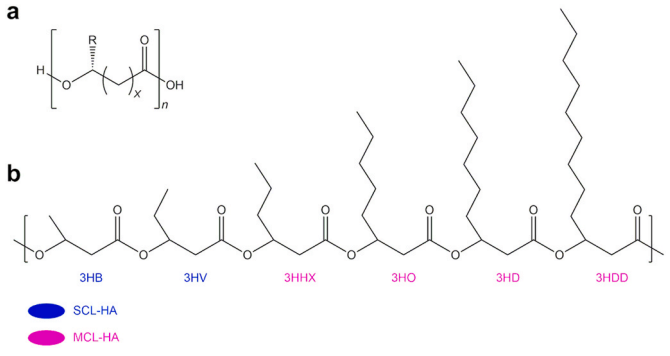

Fig. 3. (a) Structure of PHAs (b) short-chain length PHA monomers and middle-chain length PHA monomers.

PHAs can be synthesized *via* microbial fermentation, enzymatic, and chemical processes, respectively. PHA production *via* fermentation has been reported to save 44, 36, and 22% in GHG emissions with respect to its fossil counterparts PP, LD-PE, and HD-PE, respectively [132]. By fermentation, these polymers can be synthesized by numerous bacteria of the genera such as *Alcaligenes*, *Pseudomonas*, *Enterobacter*, *Necator*, *Rhodobacter*, *Ralstonia*, and *Cupriavidus* as intracellular carbon and energy reserve [133]. At present, the main carbon source for commercial PHAs production is still food-based glucose and vegetable oils [26], which conflicts with the food chain supply. Due to the large impact of the carbon feedstock on the PHAs production cost; research efforts have been devoted to exploring the use of low-cost feedstocks such as industrial wastes mainly from food production and processing plants, and agricultural residues [134,135]. Another possibility is to use CO_2-rich gaseous streams as an alternative low-cost feedstock, which implies a clear benefit in the reduction of GHG emissions. Sources of CO_2 include biogas plants, biomass gasification plants, bioethanol production facilities and breweries, and any biological and chemical process that emits/produces CO_2.

4.2.1. Carbon dioxide rich streams as a low-cost feedstock for polyhydroxyalkanoates production

Extensive research has been conducted on the use of CO_2 as the ultimate feedstock for PHA production. In this direction, some microorganisms can accumulate PHA inside the cell in specialized storage granules under nutrient-limited conditions. Depending on the strain, PHA accumulation can take place under heterotrophic conditions (using organic compounds as carbon and energy source) and autotrophic conditions (using CO_2 as carbon source and H_2 as energy source). Based on these, two cultivation strategies for the autotrophic production of PHAs can be performed. The first one (*i.e.* autotrophic-autotrophic) uses a gas mixture of CO_2, H_2, and O_2 for both biomass growth and PHA accumulation, whilst the second one (*i.e.* heterotrophic-autotrophic) uses an organic carbon source for heterotrophic biomass growth followed by autotrophic PHA production on CO_2, H_2, and O_2. Regardless of the strategy to be applied high cell-density culture with high PHA accumulation and productivity while keeping the O_2 concentration in the gas phase below the lower explosion limit (LEL) of O_2 in H_2 (4.0–6.9% (v/v)) have to be achieved [136,137]. Despite a gas explosion is avoided by decreasing the O_2 concentration in the gas phase below the LEL, this could lead to gas mass transfer limitations and ultimately decreasing biomass concentration and productivity.

Garcia-Gonzalez et al. [137] investigated the technical feasibility of

Table 5
Overview of selected studies for PHA's production from CO_2 rich streams.

Microorganism	Process configuration	Culture condition	PHA composition	PHA content % DCW	Cell biomass concentration gDCW/L	PHA concentration g/L	Productivity g/L·h	Reference
Cupriavidus necator DSM 545	Two-stage heterotrophic-autotrophic cultivation system	First stage: heterotrophic with glucose (5*, 15†, 40‡g/L of RCC) and waste-glycerol (5*, 15†, 40‡g/L of RCC), respectively	PHB	glucose 74* 41† 0.3‡	 21* 27† 29‡	 16* 11† 0.1‡	 0.252* 0.116† –	[137]
		Second stage: autotrophic with H_2, O_2, and CO_2 with nitrogen and oxygen limitation		waste glycerol 72* 61† 3‡	 18* 44† 31‡	 13* 28† 0.9‡	 0.187* 0.168† –	
	Two-stage heterotrophic-autotrophic cultivation system	First stage: heterotrophic with glucose Second stage: autotrophic with CO_2 –rich off gaseous streams with nitrogen limitation	PHB	73	21	15.3	0.227	[138]
Acetobacterium woodii + *Ralstonia eutropha* H16	Two-stage autotrophic-heterotrophic cultivation system	First stage: autotrophic with CO_2:H_2 mix to produce acetic acid Second stage: heterotrophic with acetic acid and nitrogen limitation	PHB	33.3	n.r.	0.5	n.r.	[139]
Synechocystis sp. *PCC6803*	One-stage photoautotrophic cultivation system	Photoautotrophic, 30 °C, CO_2 carbon source, 120 μE/m^2 s, BG11 medium	3HB	n.r.	n.r.	533.4[a]	25.4[b]	[140]
Synechocystis salina CCALA192	One-stage photoautotrophic cultivation system	Photoautotrophic, CO_2 from a coal power plant as carbon source, optimized BG11 medium	PHB	4.8–9.0	0.9–2.1	n.r.	n.r.	[141]
Synechocystis sp. PCC6803	One-stage photoautotrophic cultivation system	Photoautotrophic, CO_2 carbon source, 150 μE/m^2·s, nitrate limitation	PHB	8.0	n.r.	n.r.	n.r.	[142]
Synechocystis	One-stage photoautotrophic cultivation system	Photoautotrophic, 5% (v/v) CO_2 carbon source, 100–300 μE/m^2·s, BG11 medium	3HB	n.r.	n.r.	1.84	263[b]	[143]v

[a] mg/L·h.
[b] mg/L: n.r. – not reported.

Cupriavidus necator DSM 545 for the autotrophic production of PHB from CO_2 in a two-stage cultivation system. In the first stage, cells were grown heterotrophically using two different substrates, glucose, and waste glycerol, respectively. In the second phase, PHB synthesis was triggered by nitrogen and oxygen limitation under autotrophic conditions using a gas mixture of H_2, O_2, and CO_2 at an overpressure of 40 mbar. Autotrophic PHB production on glucose-grown cells was triggered after 20 h of heterotrophic cell growth at 5 g/L of RCC attaining the maximal PHB content, concentration, and productivity (Table 5). Delaying nutrient limitation (after c.a. 30 h of heterotrophic growth) at 15 g/L of RCC, reduced the fermentation performance and when shifting to PHB production phase at 40 g/L of RCC, PHB accumulation did not occur. Similar trends were observed for the autotrophic PHB production on waste glycerol-grown cells at 5 and 15 g/L of RCC reaching the maximal PHB content, concentration, and productivity at the former RCC (Table 5). PHB accumulation at high cell density (35 g/L RCC) was not observed even after 92 h of autotrophic cultivation, however after dilution of the cell mass concentration to 9 g/L PHB accumulation started to take place. In this regard, the authors asserted that the key enzymes machinery for the autotrophic metabolism were formed during the heterotrophic phase but the accumulation of PHB was restricted by mass transfer limitation of O_2. Thus, to be competitive with the current heterotrophic cultivation system, the O_2 mass transfer ought to be optimized to enhance the PHB productivity.

. Garcia-Gonzalez et al. [138] investigated the same system but using CO_2-rich off gaseous streams. In the first stage, cells were grown heterotrophically on glucose followed by autotrophic PHB accumulation using industrial CO_2-rich off-gas streams from a biogas plant equipped with an amine scrubber to recover and enrich the CO_2 from biogas. The gas stream sampled at the biogas plant was indeed an enriched CO_2 stream coming from the amine scrubber treatment applied on the biogas to separate CH_4 from CO_2. This allowed obtaining a stream with a 97.8% (v/v) of CO_2 which is preferred to avoid dilution of the CO_2 concentration in the final gas mixture required during fermentation. The main outcome of the study was that the use of real off-gaseous streams in PHB production did not affect the bacterial performance achieving high PHB content of up to 73% DCW and productivities of up to 0.227 g/L·h. Despite the authors pointed out that the results of the study showed a trade-off between productivity and PHB concentration and content, a thorough techno-economic assessment must be performed to determine the optimal process conditions.

Al Rowaihi et al. [139] investigated a two-stage biological gas-to-liquid process to convert CO_2 into PHB. In the first stage, acetic acid was produced by *Acetobacterium woodii* from a gas mixture of CO_2:H_2 (15:85 v/v) under elevated pressure (≥2.0 bar) to increase the H_2 solubility in the culture broth. Although with this approach the problem of gas explosion is avoided, gas-liquid mass transfer of H_2 is the main limitation. During this phase, a concentration of 3.2 g/L of acetic acid was obtained. In the second phase, acetic acid was converted to PHB (3 g/L acetate into 0.5 g/L PHB) by *Ralstonia eutropha* H16 obtaining a PHB content of 33.3% (percentage ratio of PHB concentration to cell concentration) after 217 h.

Another possibility to produce PHA is through photoautotrophic bacterial systems using cyanobacteria. Some cyanobacteria species have exhibited considerable potential for PHA accumulation, as they are capable of using sunlight and CO_2 as energy and carbon sources,

respectively. This is a clear advantage over the chemoheterotrophic bacterial systems, which makes use of an organic carbon source (e.g. glucose, fructose) representing 30–40% of the total production cost [26].

Troschl et al. [141] reported the operation of a 200 L pilot tubular glass PBR for PHB production by *Synechocystis salina* CCALA192 using the flue gases from a coal power plant containing 11–13% (v/v) CO_2. The flue gas stream was washed to remove residual NO_x and SO_x and to increase the CO_2 content. Different cultivation conditions such as nutrient solution, cultivation time, illumination, acetate addition, were examined with the cyanobacterial CCALA192 strain. Overall, final biomass concentration and PHB accumulation in the range of 0.9–2.1 g/L and 4.8–9.0% DCW were reported, respectively. Digestate from a biogas reactor was also tested as an alternative source of nutrients attaining a final biomass concentration and PHB accumulation of 1.6 g/L and 5.5% DCW, respectively.

Carpine et al. [142] investigated the photoautotrophic accumulation of PHB by *Synechocystis* sp. PCC6803 from CO_2 under light/dark cycles (16 h light/8 h dark at 150 μE/m^2 s) and nitrate concentration reduced to half of the optimal cell growth concentration in a 0.8 L inclined bubble column PBR. A gas stream −2.0% (v/v) CO_2 in air at 4 vol/-vol/min −was sparged at the bottoms of the PBR's. Under these conditions, the PCC6803 strain was able to accumulate PHB up to 8.0% DCW.

PHB content, titers, and productivities reported for cultures performed under photoautotrophic conditions using solely CO_2 as carbon source are lower than that reported for cultures under heterotrophic-autotrophic conditions (Table 5). In this respect, genetically engineered strains of cyanobacteria could be a promising door to enhance PHB accumulation and productivities under photoautotrophic conditions. Wang et al. [140] reported a titer of 533.4 mg/L 3HB after photoautotrophic cultivation of the engineered cyanobacterium *Synechocystis* sp. PCC6803 with average productivity of 25.4 mg/L·d. The engineered cyanobacterium *Synechocystis* was able to produce up to 1.84 g/L 3HB after photosynthetic cultivation with peak productivity of 263 mg/L·d [143]. It is clear that spite of these efforts, titers, and productivities are still very low and further improvements are necessary to attain economic feasibility.

One common bottleneck to the biochemical routes described before is the gas-liquid mass transfer limitation since the biocatalyst is active in the aqueous phase and at least one of the reactants is in the gas phase. Moreover, the PHA autotrophic production process with O_2 as a terminal electron acceptor faces the risk of explosion of H_2/O_2 mixture. The problem associated with the gas-liquid mass transfer limitation can be solved by increasing the pressure in the fermentation to increase the solubility of the gas reactants in the aqueous phase. This was already demonstrated by Gunnarsson et al. (2014) for succinic acid production (1.013–1.4 bar) and in other studies at a range of 5–10 bar [144]. In the case of PHA production, Yu et al. [145] demonstrated that PHA production could be significantly enhanced by increasing the pressure in the bioreactor resulting in a higher growth rate, cell density, and gas uptake rate. (Table 5). Alternatively, the mass transfer rate can be increased by decreasing the size of bubbles, increasing the gas hold-up time, or the addition of chemicals to the fermentation media. On the other hand, a potential solution to the problem associated with the H_2/O_2 mixture in the PHA production could be the application of other terminal electron acceptors (e.g. NO_3^-) rather than O_2 [146]. This could potentially permit the direct use of biogas in the autotrophic cultivation process obtaining in this way not only PHA but also upgraded methane.

5. Digestate application in agricultural framework

5.1. Nursery substrate and growth media for seedlings

Digestate contains considerable amounts of various macro- and micro-nutrients, nitrogen, phosphorus, potassium, trace elements, as well as organic matters, and therefore, it can be regarded as a nutrient supplier and/or nursery substrate for plant seedlings cultivation. Ronga et al. [147] comparatively studied the use of liquid digestate, solid digestate, and standard nutrient solution to cultivate seedlings of leaf lettuce. Results suggested that both methods can be alternatively employed as a sustainable growing medium.

The digestate remaining after AD can be valorized into nutrient-enriched soil which can be widely employed in nurseries. Traditionally, residues obtained from AD are directly applied on the farmlands as a soil amendment which besides the provision of nutrients has the intention to improve soil physicochemical properties. In some cases, digestate in a mixture with agricultural residues is composted, to prepare a stable biofertilizer as nursery substrate with enough nutrients for seedlings cultivation. For example, a mixture of digestate, pig manure, and spent mushroom substrate (at volume ratios of 1:1:1) was composted to be used as a growth medium for tomato and pepper seedlings [148] . The produced compost showed a high quality as a good alternative to peat allowing 100% replacement; while 20%–50% replacement resulted in tomato and pepper seedlings with higher morphological growth and lower *Fusarium* concentrations. Moreover, Meng et al. [149] assessed the physicochemical properties and maturity of the produced compost during 118 days of co-composting digestate and spent mushroom substrate. Total organic carbon, available phosphorus, and ammonium decreased along with the composting process during the thermophilic period (>50 °C) lasted for 52 days; while the content of total potassium, total phosphorus, and nitrate increased. In contrast to the control (peat: perlite mixture at a volumetric ratio of 5:1 with and without fertilizer), the treatments with compost addition showed better tomato seedling growth.

Although composting can eliminate most of the pathogenic bacteria and weed seeds, alleviate heavy metals and drug chemicals toxicity, the process suffers some drawbacks including high moisture and difficulty in separation which limit the efficiency of aerobic composting [150,151].

It is worth mentioning that digestate can be also a valuable cultivation medium for hydroponic systems in which soil-less growing medium supplemented with nutrient solution are used to cultivate various plants [152,153]. What should be considered herein is the microbiological changes in the plants cultivated with digestate. Ronga et al. [147] showed a microbiological change in baby leaf lettuce caused by digestate application as cultivation medium. However, the authors claimed that the washing operations were found as an effective method to make the baby leaf lettuce ready-to-eat, pointing out the possible use of the digestate for the hydroponic cultivation of this vegetable. Instead of direct use of digestate for hydroponic cultivation humic-like substances can be extracted from the digestate for further use in plants cultivation [154]. The intrinsic lack of standards for the quality of humic-like substances from digestate is one of the major problems of this sector for a wider agriculture adoption. Furthermore, the lack of consensus on application doses for agricultural use has hindered its widespread use [155].

5.2. Feed for earthworm engineering

Digestate is often processed as feed for organisms such as earthworms due to its high content of amino acids and vitamins and the slightly alkaline pH. Koblenz et al. [156] compared the growth of earthworms by feeding digestate as well as organic and chemical fertilizers (i.e., cattle and pig slurry, chemical fertilizers) under short and long-term experiments. They noticed that applying digestate and slurry induced a positive impact on earthworm density and the produced biomass. In a 4-month short-term experiment, the highest earthworm density was observed where cattle and pig slurry had been used. In contrast, the quality and quantity of the earthworm biomass grown with chemical fertilization or without any additives as control were significantly lower. Under a 3-year experiment, higher earthworm biomass was attained when they were fed with slurry and digestate compared with those grown on chemical fertilizer. The result indicated that the

feeding of slurry and digestate benefit earthworm population increase. Thus, this approach can be further used for the management and recycling of the digestate.

Vermicomposting is another eco-friendly technology that converts a vast range of waste materials into stable organic products through breeding earthworms with organic wastes. Sun et al. [157] showed that vermicomposting is also an effective way to remove heavy metals such as Cd by earthworms (*Eisenia fetida*) during digestate vermicomposting and suggested the optimum addition of earthworm hydrolysates for the production of *Trichoderma guizhouense* spores. Under optimum conditions, the hydrolysis rate of earthworms was ~97% and the removal efficiency of Cd was up to 93%. Furthermore, the addition of 20% of earthworm hydrolysate promoted the largest production of *Trichoderma sporulation* (~3 × 108 CFU/g straw), implying the ability of earthworm hydrolysates to promote the growth of *Trichoderma guizhouense*. More importantly, applying digestate to raise earthworms is conducive to reduce the feed cost and increase the economic benefits because of organic waste disposal and valuable fertilizer production [158]. Although feeding earthworms or vermicomposting with digestate may create less economic benefit compared with other strategies discussed, it has been shown as an promising treatment method under a biorefinery platform based on the creation of a chain including putrescible waste, in vertebrates and biofuel/proteins [159].

5.3. Valorization into biochar

To promote digestate recycling, separation and pelletization are often applied. The former process results in separated fractions of the liquid and solid stream. On the other hand, the pelletization produces a solid energy carrier with high density for further agricultural, energy, and environmental use. Among potential production routes, biochar production has gained much interest since it is a carbon-rich solid material with unique chemical, physical and biological properties such as aromatized carbon matrix, large specific surface area, high pore volume, enriched surface functional groups, and high mineral content [160]. It can be used for various agricultural purposes, such as a compost additive, soil remediation, and as adsorbent for nutrients recovery.

5.3.1. Biochar for soil fertilization

Despite high degradation efficiencies succeeded in AD process, the effluent typically contains recalcitrant organic materials (e.g. cellulose and lignin) as well as considerable amounts of nutrients. These components can enrich digestate fertility in farmlands [161,162]. However, toxic and harmful chemicals or drugs, pathogens, weed seeds, antibiotic resistance genes -that can potentially contaminate the food chain-might be found in digestate [15]. Hence, in these cases, alternative methods of digestate recycling will benefit for biochar safe use in farmland. A food waste digestate biochar, as an example, can contain up to 61% volatile solids, 45% C, and 6% N [163].

Pyrolysis for biochar production has been proposed to convert the remaining organic components into stable aromatized carbon, retaining most of the nutrients in the biochar matrix and eliminate toxic and harmful chemicals, pathogenic bacteria, and weed seeds [164]. As a new type of carbon-based fertilizer, biochar can improve soil physical and chemical properties, increase soil buffer acid and alkali ability, and fertilizer performance [165].

Inyang et al. [166] determined the physicochemical properties of the digested bagasse biochar and undigested bagasse biochar and noticed that the AD-derived product had higher pH, surface area, cation, and anion exchange capacity, hydrophobicity, and increased negative surface charge. All these properties are generally desirable for soil amelioration and fertilization use. Later, Ma et al. [167] investigated the characterization and potential use of biochar produced from anaerobically digested dairy manure under different processing temperatures (300, 600, and 1000 °C). The researchers demonstrated that the pyrolysis process could transform the biomass waste into high-value biochar enhancing soil fertilization and reducing pollution. They also suggested that biochar from digestate can be an economically and environmentally production route in the agricultural sector due to its positive impact on crop growth, yield, soil nutrient status, and enzyme activity [168,169].

One of the obstacles for promoting biochar for field application is its high market price. The recommended application rate for biochar varies depending on the biochar characteristics from 5 to more than 100 ton/ha. Having considered biochar prices ranging from 500 to 1000 €/t, it cannot be a promising agricultural input in many developing countries since farmers cannot afford to use it [163]. Therefore, attempts need to be made herein to decrease the final price of digestate biochar providing a higher market demand for such a valuable product.

5.3.2. Soil remediation

Biochar from digestate has great potential to be used in multiple applications. Stefaniuk et al. [170] analysed the content of heavy metals and polycyclic aromatic hydrocarbons (PAHs) in biochar produced from digestate pyrolysis finding that despite the higher pH, surface area, and carbon content, the biochar showed low levels of heavy metals (Cr, Cu, Pb, and Mn) and PAHs. Huang et al. [171] evaluated the potential of digestate for preparing biochar at a broad temperature range of 300–900 °C. Results demonstrated that the porous solid residue-based biochar might be used as a soil amendment due to the mesoporosity and highly abundant active adsorption sites, developed pore structure, active minerals, and functional groups. Tao et al. [172] studied the production of biochars using digested corn straw silage at pyrolysis temperature in the range of 300–700 °C for adsorptive stabilization of Cd^{2+}. Higher temperature resulted in a significant increase of surface area from 4.24 to 56.58 m^2/g and increased densities of oxygen-containing functional group and mineral components such as $CaCO_3$ and KCl content. Moreover, increased Cd^{2+} adsorptive stabilization was observed and it was concluded that at temperatures above 600 °C effective Cd adsorptive stabilization can be achieved. Besides Cd^{2+}, the immobilization and bioavailability elimination of other cations (Cu^{2+}, Pb^{2+}, Zn^{2+}, and Hg^{2+}, etc.) could also occur through physical adsorption, cation exchange, surface complexation, precipitation, and electrostatic interactions [173,174]. While reduction and complexation can be useful for metal oxyanion compounds (CrO_4^{2-}, AsO_4^{3-} and AsO_3^{2-}, etc.) [175] and adsorption and degradation for organic concomitants in soil [176].

5.3.3. Compost amendment

In addition to the above-mentioned utilization methods, digestate can be also used as a raw material in composting. Due to the high operating temperature, aerobic composting is a safe and effective way to treat organic residues including digestate [177]. For example, Meng et al. [178] explored the feasibility of a full-scale composting process to dispose of digestate and evaluate compost quality. Digestate reached the thermophilic stage (>50 °C) for 20 days. Ammonium and total organic matter contents decreased along with the composting process, while the contents of total potassium, phosphorus, and nitrite increased accordingly. The final compost product showed acceptable phytotoxicity and maturity. During the composting process, *Anaerolineaceae* and *Limnochordaceae* were the main bacteria involved in the composting process, and *Chaetomium* was the major fungi representative.

In contrast to the traditional processing method which leads to the additions of chemical pesticides, hormones, and potential ammonia oxidation-inhibiting substances; the biological conversion of digestate into a stable compost fertilizer is a promising alternative method [166,178]. Besides previous research has demonstrated that biochar can be also used as an additive in composting improvement [179], it is also proved that co-composting of biochar with organic waste can lead to high value-added organic fertilizer. While only a few studies are reporting the usage of biochar from digestate in composting process, co-composting of biochar derived from biogas slurry with organic waste such as crop residue, animal manure, and sewage sludge has great

potential on organic waste management and recycling. It has however to be remarked that composting is requiring high dry matter of biomass for permitting temperature increase. Therefore, digestates that have usually low dry matter contents cannot be composted untreated. The digestate can be concentrated by centrifugation or screw pressure to remove the extensive water content. Moreover, the digestate cannot be composted alone and additions of other dry biomass fractions that have a high content of organic matter ready to be oxidized for increasing the temperature and stabilize the material, have to be mixed.

5.3.4. Nutrients recovery

Although many studies have already concluded that biochar can be used for soil amelioration, the needed amount to provide the necessary nutrients to the soil would be relatively large due to low-nutrient content compared with chemical fertilizer [180]. Biochar can have a highly porous structure with large specific surface area, high pore volume, enriched surface functional groups, and acceptable adsorption performance [175]; and thus, researchers previously exploited it as an adsorbent for nutrients recovery from wastewater as a means to improve the elemental composition of biochar [173,181]. For example, Alghashm et al. [182] evaluated biochars for phosphorus recovery and soil amendment applications. Experimental results suggested that the amount of adsorbed phosphorus is increasing along with temperature increase for biochar derived from digestate. Specifically, the amount of phosphate adsorbed onto the biochar at 900 °C was larger compared to biochars derived at lower temperatures. The growth of cabbage was significantly improved in pot experiments due to improved water retention capacity of the soil and simultaneously nutrients solubilization in the soil. To examine the potential of biochar on phosphate adsorption recovery, Yao et al. [183] studied biochar samples prepared from digested sugar beet tailings at 600 °C through slow pyrolysis revealing a large amount of colloidal and nano-sized MgO particles on biochar surface. The digested sugar beet tailing biochar showed the highest phosphate adsorptive removal ability with more than 70% removal efficiency. The product could be directly applied to agricultural fields as a slow-release phosphate fertilizer to improve soil fertility and sequester soil carbon.

In addition to adsorbing phosphate, digestate derived biochar has also a good adsorption effect on nitrogen contained compounds. For instance, Zheng et al. [19] investigated the ammonia adsorption of biochar prepared from distillers' grains digestate with different pyrolysis temperatures. The higher the pyrolysis temperatures, higher aromaticity, specific surface area, and pore volume, as well as decreased biochar polarity, was observed, which ultimately promoted the adsorption capacities for NH_4^+. Similarly, Pan et al. [184] reported that the maximum ammonium adsorption capacities of biochars from digested pig manure and straw reached 37–49 mg/g and 21–29 mg/g in artificial and real wastewaters, respectively. All these results indicated that the ash component (minerals contain Mg, Ca, Fe, and Al, etc.) in the biochar played an important role in ammonium and/or phosphate adsorption through mechanisms including physical adsorption, surface complexation, precipitation, and electrostatic interactions [175,181,183]. The biochar produced from digestate not only can act as an alternative waste management approach but also increases the utilization potential of biochar in agriculture.

6. Life cycle assessment perspectives of the novel routes

The valorization of biomethane and digestate into proteinaceous microbial biomass, so-called single-cell protein or microbial protein, has aroused much interest recently because such products can substitute commercial proteinaceous feed such as soybean meal with intrinsic considerable environmental footprints [185,186]. The use of methanotrophs, as an example, for upcycling digestate and biogas/biomethane into feed grade biomass has recently been broadly investigated and promoted by many researchers [187,188]; It is because the produced biomass has a high protein content and amino acid profile comparable with commercial animal feed sources [6]. However, the sustainability of such integrated systems (Fig. 4) has not been widely investigated. Digestate contains nitrogen, mostly in form of ammonium, which can be assimilated by methane or hydrogen oxidizing bacteria and transformed into protein. Methanotrophs in form of mixed culture can reportedly be directly cultivated in digestate following centrifugation, filtration, and pasteurization (70 °C for 1 h) steps to remove particles and deactivate all the potential pathogens [187], however, the direct cultivation can undermine the safety of the final product as animal feed. The other possibility is to recover nitrogen from digestate using electrochemical reactors [189,190] and then, cultivate the microbial biomass into nutrient-supplemented media [49,191]. Such pretreatments would increase the energy demand of the process and hence, raise the environmental footprints caused at the background system for heat and electricity production. The results of an LCA study exhibited that the

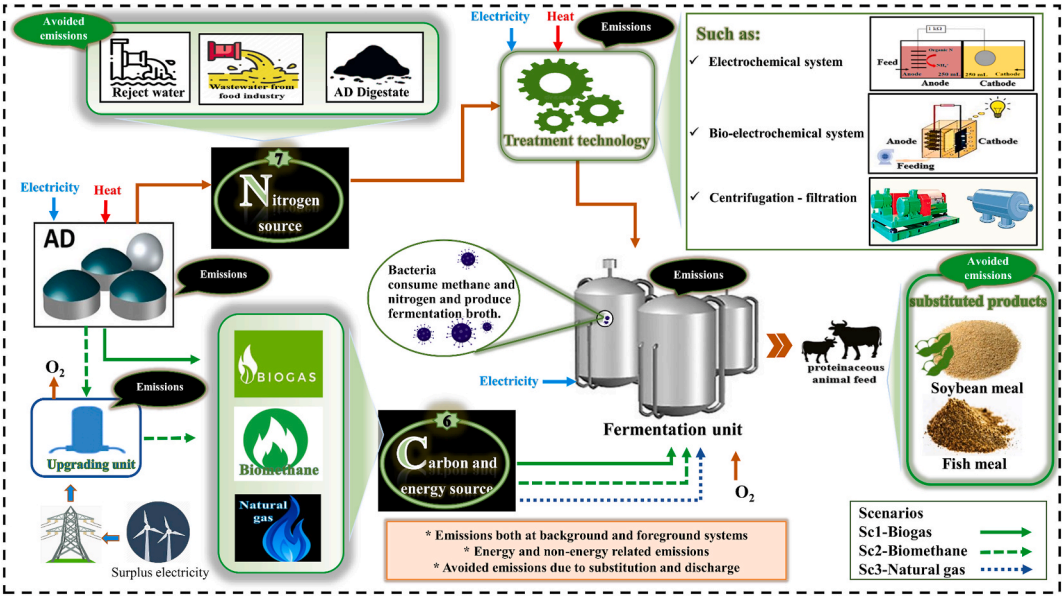

Fig. 4. The associated system boundary describing the life cycle assessment of single-cell protein production from AD effluents.

cultivation of methanotrophs in the centrifuged filtered digestate (i.e., anaerobically digested organic fraction of municipal solid waste) as nitrogen source as well as using biogas and biologically upgraded biogas as carbon source would be an environmentally friendly alternative to soybean meal as a common livestock feed [192]. Results showed that under a biorefinery approach that integrates biogas plant and single-cell protein production facility, the use of biologically upgraded biogas for microbial protein production led to better environmental impacts in four damage categories namely, Climate change, Human health, Ecosystem quality, and Resources. Such biorefinery approach led to an avoided impacts of −58 to −147 kg $CO_{2,eq}$ per ton of treated biowaste which is much promising compared to a commercial product growing on natural gas with a reportedly 2.229 kg $CO_{2,eq}$ per kg protein [193]. The prospective studies in this context should assess and compare the environmental impacts of direct cultivation strategies and various nitrogen recovery methods. Furthermore, different nitrogen rich residual streams should be taken into account to find the most environmental friendly nitrogen rich substrate for valorizing biogas/biomethane into proteinaceous feed.

Sustainability assessment without considering economic aspect of the system under consideration is meaningless. The economic aspect of microbial protein production has not widely studied yet. Verbeeck et al. [194] assessed the economic performance of up-cycling recovered resources from anaerobic digestion through microbial protein production. They used a practical case digester which co-digested pig manure (70%) and other organic waste (30%). Their results demonstrated that producing microbial protein using both methanotrophs and hydrogen oxidizing bacterial would appear economically and technically feasible within the current range of market prices existing for high-quality protein. Although Verbeeck et al. [194] claimed microbial protein production is economically feasible, there is a big scientific gap in this context which needs further attention. Furthermore, it is highly suggested other aspects of sustainability such as exergetic performance and exergoenvironmetal performance to be considered as prospective work [195,196].

The production of microalgae is among the most popular bioprocessing routes for the upcycling of AD effluents because, on the one hand, the CO_2 content of biogas can be used as C-source needed for biomass growth, and on the other hand, the nutrient content of digestate can be recycled/removed when used as cultivation media [18]. Such a concept has risen great interest for simultaneous nutrient removal and biogas upgrading [197]. The valorization of CO_2 can be performed either as simultaneous upgrading and microalgae cultivation within photobioreactors [198] or in separate units after removing the CO_2 from biogas and its injection to raceway ponds as a carbon source for microalgae growth [199]. Most of the LCA studies investigating the environmental performance of microalgae cultivation, unanimously reached the conclusion that the cultivation of algal biomass and its further dewatering and processing are energy extensive and regarded as the hotspot in terms of environmental footprints (Table 6). According to the results of the previous LCA studies, pumping systems, compressors, paddlewheels, centrifuges, and dryers are among the most energy-demanding equipment in algal cultivation units [18,200]. The use of artificial light for biomass growth [198] and the high heating demand needed to regulate the temperature of cultivation media in winter [201] can also notably increase both electrical and thermal energy demand within the plants. Depending on the source of energy, e.g., coal-fired power plant, natural gas-fired power plant, the magnitude of environmental impacts reportedly varied among LCA studies. Hence, increasing the energy use efficiency and the use of renewable electricity, i.e., solar and wind electricity, to supply plants' energy demand have been introduced as practical solutions to decrease the environmental footprints of microalgae cultivation [202]. Having an optimized cultivation system with the maximum biomass yield can to some extent improve the environmental performance under different impact and damage categories [18]. CO_2 and CH_4 tolerance is a species-specific parameter and different species would have different yield, productivity, and compositions under various dosages of CO_2 and biomethane [203]. Moreover, sulfide is a toxic element for microalgae, hence the concentration of H_2S in biogas is a determinant factor during simultaneous upgrading and algal biomass production [98]. However, the impact of such parameters on the overall environmental performance of microalgae production facilities has not been scrutinized yet.

The cultivated algae/microalgae using biogas effluents can be further recycled back into the biogas plants for energy production *via* anaerobic digestion. Such recycling can be implemented with/without biomass pretreatment which can lead to different energy/exergy balance within the system. Such pretreatment, on the one hand can increase the energy recovery, but on the other hand, enhance the energy demand of the system. Having applied exergy analysis, Xiao et al. [204] compared three different routes for biogas production from microalgae: without pretreatment, with hydrothermal pretreatment, and with solar-driven hydrothermal pretreatment. Their results approved that the exergy efficiency of the system would be higher with solar-driven hydrothermal pretreatment (40.8%) and hydrothermal pretreatment (35.9%). Furthermore, they showed that the maximum exergy loss could be caused by biogas residue, ranging from 35 to 60%, approving that energy and exergy flow analysis should be given more attention in the sustainability assessment such integrated systems [205].

The integration of surplus electricity from renewable resources such as wind turbines to biogas facilities for either biological biogas upgrading [192] or biogas-based methanol production [206] could bring about significant impacts on the sustainability of overall biorefinery. Although such integration for biological biogas upgrading has shown better environmental performance compared to other upgrading technologies [207], unresolved questions have remained in the field which need further investigations (Fig. 5). The safety and high cost of hydrogen storage is a serious challenge which undermine the economic feasibility of power-to-gas technologies. A capital expenditure of 33–44 €/m3 hydrogen storage capacity is estimated for hydrogen storage facilities depending on the storage pressure i.e., 50–200 bar [208]. The surplus electricity has an intermittent nature and is not always accessible through the year which undermines the sustainability of the whole process. Some electrolyzers cannot efficiently work under variable loads, thus intermittent surplus electricity would increase the overall process costs and decrease the efficiency of the system. Therefore, under the current level of knowledge and technology, a complete transition from commercial biogas upgrading technologies to biological biogas upgrading still suffers uncertainty and needs more detailed analysis before commercialization.

Biomethane is a multifunctional energy carrier that can be used to supply heat and/or power *via* combustion in biogas engines, can be employed as transportation fuel, or can be stored in the natural gas grid, where it can be transported for use in other locations than it is produced. The conversion of biogas into other forms of energy carriers such Fischer-Tropsch diesel could be regarded as another valorization pathway for exploiting biogas because it would increase the energy density of the final product and facilitate its distribution (Table 7). Fischer-Tropsch is a number of chemical reactions takes place at temperatures of 150–300 °C and both low and high pressure to convert a mixture of carbon monoxide and hydrogen into liquid hydrocarbons at the presence of catalysts. Steam reforming breaks down the biogas molecules into syngas which is the primary feedstock for Fischer-Tropsch diesel production from AD effluent [209,210]. Having analysed through exergy analysis, dry reforming and power generation have identified as the most inefficient subsystems of biogas dry reforming. An overall exergy efficiency of 54.7% has been reported for biogas reforming with reformer and turbines as the main source of irreversibility [211]. In another study led by Minutillo et al., hydrogen separation and compression accounted for 16–18% of exergy losses within biogas reforming systems [212]. Only a few studies have investigated the liquefaction of biogas/biomethane from LCA points of view [209,

Table 6
A summary of LCA results of algal biomass production under different scenarios and scopes.

Species	Scale	Cultivation method	CO_2 source	Nutrient	Hotspots	Future improvements	LCIA	FU	Downstream use	Selected Impact (Unit/FU)	Ref.
Phaeodactylum tricornutum	ISI	PhB	Synthetic *vs* biogas	–	Cultivation and freeze-drying	Algal productivity, electricity source, nutrients culture medium, and cleaning solutions	ILCD handbook midpoint impact category	1 kg dried biomass	–	GWP = 257; WRD = 175; FAETP = 27; FETP = 2.36E-03 kg P eq; HTnc = 1.54E-06	[198]
Chlorella vulgaris	IP	ORP; TPhB; HCF; ORP	Synthetic	–	High energy demand during cultivation and dewatering	Biomass cultivation on hydrolyzed food	IMPACT 2002+ V2.11	1- 1 kg biomass (85–90% of moisture content) 2- Whole dried biomass 3- 1 kg bulk proteins	Food and feed	0.5 to 9.8 mPt (Weighted result)	[217]
Chlorella 1067	SSU	ORP	Synthetic	Pig manure AD digestate	Digestate storage, transportation	Sealed storage system, fast-continuous downstream, lowered distance	IMPACT 2002+ V2.11	1 tonne of pig manure	AD of microalgae	HH = 3.77E-05; EQ = 1.74 E + 01; CC = 3.55 E + 01; RU = −1.61 E + 03	[18]
Chlorella vulgaris	VS	ORP	Flue gas CO_2	Chemicals	–	Change in the algae composition	Selected midpoints	1 kg biodiesel	Biodiesel production	GWP = 0.26–0.42	[218]
Chlorella vulgaris	VS	ORP	Coal-fired flue gases	Chemicals	Biomass cultivation, harvest, and energy conversion	Elevated lipid content for higher energy recovery	CML method	1 MJ biofuel	Biofuel products	GWP = −61 to −173	[219]
Chlorella vulgaris	VS	ORP	Not specified	AD digestate	Feed production, on-farm emissions	Energy saving paddlewheel motor	ReCiPe version 1.13	1000 kg live weight pig	–	Relatively comparison	[202]
Nannochloropsis	PS	ORP; TPhB	Synthetic	Synthetic media	Energy consumption for temperature regulation, Production of supplementary nutrients	Optimized temperature regulation systems	CML	1 kg dried biomass	–	EQ = ~500 Pt; HH = ~150 Pt	[201]
Chlorella spp., *Scenedesmus* spp. *and Chlamydomonas* spp	PS	ORP	CHP flue-gas	AD digestate	High electricity demand, On site air emissions (ammonia volatilization)	Increasing biomass yield, Strict pH control	ILCD 2011	1000 m^3 influent wastewater	AD of microalgae	GWP = −0.45; WRD = −4.64E-3; FETP = −42.38; HTPc = 2.52E-8;	[220]
Not specified	PS	ORP	Not specified	AD digestate	Artificial illumination under indoor cultivation, harvesting, and drying processes	Outdoor cultivation, Process optimization, Renewable energy for drying and harvesting	ReCiPe V1.13	MJ $CH_{4,\ produced}$	–	Relatively comparison	[100]

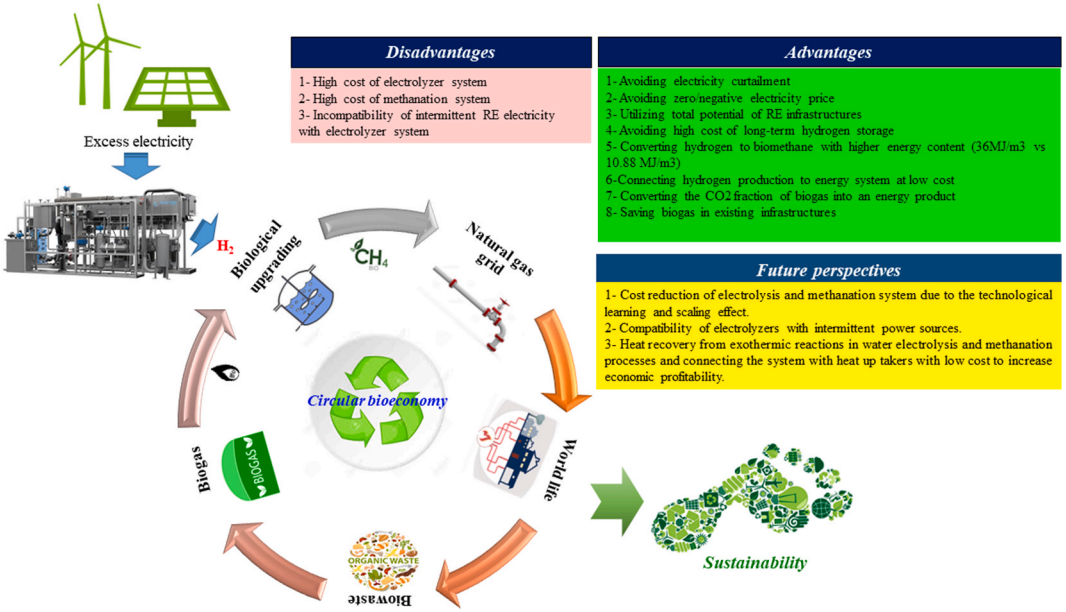

Fig. 5. The unresolved problems in sustainability of power-to-gas technologies and the future perspective.

Table 7
Advantages and disadvantages of liquified transportation fuel generated from biogas.

Item	Advantage	Disadvantage	Production pathway
Fischer–Tropsch Diesel	Interchangeable with conventional diesel; compatible with existing diesel engines; high cetane number; not contain sulfur or nitrogen; blending with diesel; reduction of exhaust emissions		Biogas/biomethane → syngas → catalytic synthesis to Fischer–Tropsch diesel
Methanol	Efficient combustion and ease of distribution; used directly as fuel or blended with petrol; converted to dimethyl ether as a diesel replacement; used in the biodiesel production process; a high-octane fuel;	Toxic; has an affinity to water; has half the energy content of petrol on a volumetric basis; corrosive to certain materials; modifications in engines before fueled with methanol	Steam reforming of methane to synthesis gas →high-pressure catalytic conversion of the synthesis gas to methanol
Dimethyl ether	Limited formation of PM and NOx emissions during combustion; combustion does not produce soot;	Stored under pressure as a liquid	CH3Br from oxidative bromination reaction of methane in the presence of HBr and oxygen over a Rh–SiO2 catalyst → CH3Br hydrolyzed to DME over silica-supported metal chloride catalyst

213,214]. The results of an LCA study comparing five downstream processes for exploiting biogas/biomethane (i.e., biogas compression, biogas liquefaction, methanol production, dimethyl ether production, and Fischer-Tropsch diesel production) demonstrated that compressed biogas, methanol, and dimethyl ether had the highest specific fuel productivity. However, at longer distribution distances dimethyl ether, methanol, and liquified biogas had the best energy balance [215]. Having considered Fischer-Tropsch diesel, methanol, and dimethyl ether, the syngas/fuel synthesis unit found to be the most pollutant stage in terms of global warming potential with 449, 127, and 156 g CO_2-eq./Nm^3 raw biogas, respectively. Researchers also found that the choice of electricity mix (i.e., renewable vs fossil-based electricity) could have a large impact on global warming potential.

The prospective research on biogas effluent upcycling can focus on the sustainable integration of biogas plant with different configuration of methanol production facilities and the associated economic and environmental impacts that such integration can bring. However, the establishment of such approach seems far from sustainable commercialization since it suffers much uncertainty which originates from the reference systems to which such comparison is made as well as the substitution of co-products. Methanol production from biogas or biomethane as discussed above would have lower environmental footprints when compared with conventional methanol production from fossil resources [216], however the environmental profile highly depends on the upstream and downstream processes and can be highly site-dependent. For instance, Navas-Anguita et al. reported that synthetic fuels produced *via* biogas dry reforming and Fischer-Tropsch synthesis would not be an environmentally friendly alternative for conventional diesel fuel [209]. Their results demonstrated that biogas production and direct emissions to the air from the biogas-to-liquid plant raised as the main sources of impact which necessitates future studies for process optimization and pollution reduction.

Last but not least, finding the most environmentally friendly pathway for valorizing AD effluents into value added products and bioenergy needs a comprehensive and comparative studies. Regionalized models or assessment are needed to find the most sustainable platform based on local conditions and requirements. However, no comparison has been made among various valorization pathways such biogas-based methanol (i.e., thermo-catalytically conversion of methane to methanol) production and other application of biogas such as combined heat and power (CHP) production or microbial protein

production. Apart from the technological improvements, future works in this field need to consider how to bring it into a circular concept, decrease the production and environmental costs, and increase synergy with other upstream and downstream technologies [206,216].

SI= Semi-industrial – IP = industail pilot scale – SSU = simulated scaled up – VS = virtual system – PS = pilot scale – PhB = photobioreactor – ORP = Open raceway pond – TPhB = photobioreactor – HCF = heterotrophic closed fermenters – WRD = Water resource depletion – GWP = Global Warming Potentia (kg CO2 eq.) l – FAETP = Freshwater Aquatic Ecotoxicity Potential – FETP = Freshwater Eutrophication Potential (kg P eq) – HTnc = Human toxicity, non-cancer effects (CTUh) – HH = Human health (DALY) – EQ = Ecosystem quality (PDF/m2.yr) – CC = Climate change (kg CO2 eq.) – RU = Resource use (MJ primary).

7. Biorefinery approach to achieve circular economy of the different anaerobic digestion streams

7.1. From linear to circular economy

In the past centuries, human society has been lived with a linear life pattern of resource utilization, i.e. “take-make-use-dispose” or “extract-produce-use-dump”, which is not sustainable since it is created based on the assumption of infinite resources and energy [221]. At the same time, it creates a massive number of wastes and brings about extreme environmental issues. Hence, a circular economy concept has been proposed to adopt a “closing the loop” approach to achieve a sustainable development model through good process design to promote circular economy processes, foster sustainable consumption, and increase the lifespan of resources [222]. Recently, in March 2020, the European Union (EU) also announced A new Circular Economy Action Plan, which will be used as a future-oriented agenda to achieve a cleaner and more competitive Europe [223]. In this plan, recycling food, water, and nutrients are highlighted that are of utmost importance to adopt circular approaches. Hence, waste management holds a central role particularly in how to inject the wasted materials/resources back into the market as new raw materials closing the loops and ensuring circularity.

Among the many waste disposal technologies, AD is the widely used method in tackling organic waste for many years due to its process simplicity and robustness, as well as the wide waste acceptance range. The two direct output streams from AD are 1) biogas consisting of mainly methane and carbon dioxide, and 2) nutrient-rich digestate containing non-degraded recalcitrant lignocellulosic waste and cells. From a circular economy perspective, the two streams, even though originated from waste, are not the end life of the inherent nutrients/value of the waste. How to efficiently utilize the two streams draws the attention of researchers, policy-makers, and relevant stakeholders. Biorefinery, as a process integrating bio-based materials/chemicals and biofuels production from different biomass [224], provides potential ideas to circulate the AD streams, while minimizing environmental damage and maximize the resources efficiency at the same time.

7.2. Conventional circular economy mode of anaerobic digestion streams

The biogas from AD has a high heating value while the digestate is rich in nutrients, such as N, P, K. Thus, the current most common practice for a biogas plant, as shown in Fig. 6, is the direct use of the output streams [38,207,225] to generate heat and electricity *via* a CHP unit, and to apply the digestate as biofertilizer at agricultural lands; following regional or national legislation (e.g. Nitrates Directive 91/676/EEC). This is a relatively mature route to recover energy and nutrients fitting into the circular economy concept because it can further

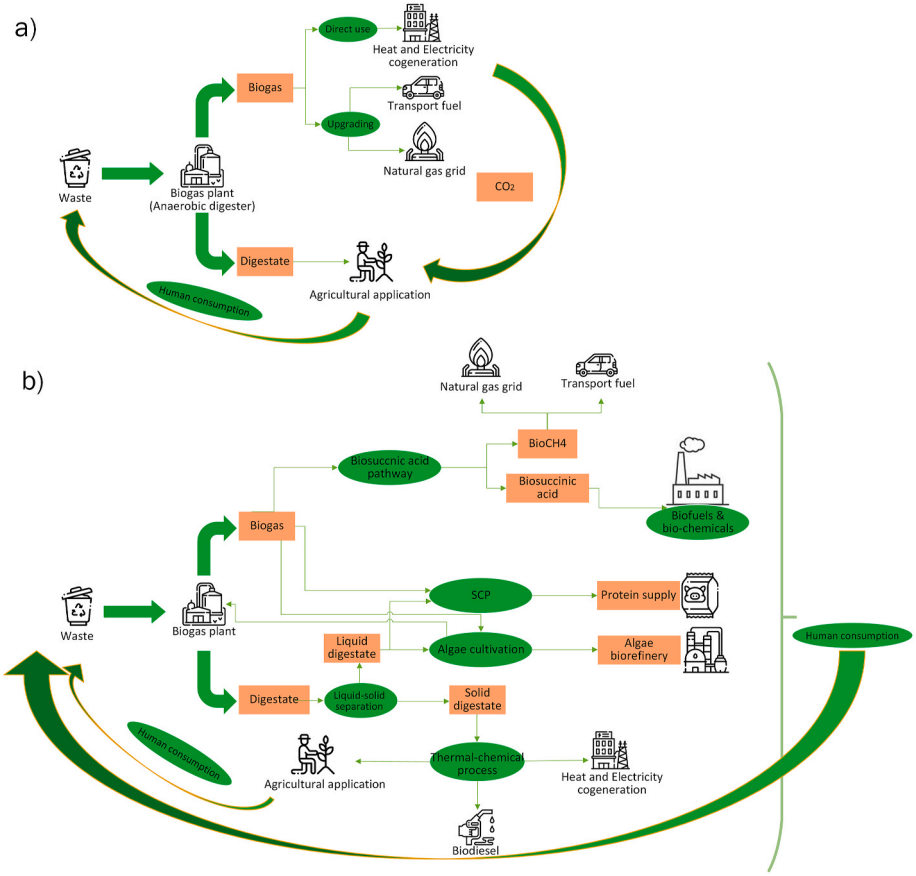

Fig. 6. The comparison of a) the conventional circular economy mode and b) the proposed novel circular economy mode of different AD streams.

recover renewable energy and extend the lifetime of the nutrients. Vaneeckhaute et al. [226] evaluated the conventional process from a sustainability (environmental, economic, and social) view taking Southern Sweden as an example, and highlighted the negative environmental impacts, the positive net present economic value, and the acceptable attitude of the stakeholders. Specifically, the authors demonstrated that by treating 12,689 tons of mixed organic residues annually to produce heat, electricity, and biofertilizer, the biogas plants can achieve around 1000 tons of GHG emission reduction and avoid $6*10^6$ MJ in fossil resources depletion. Moreover, the economic internal rate of return after 20 years of the biogas plant application is estimated as 23.6%. The fertilizer quality assurance is identified as a key issue from social acceptance perspective.

To diversify the utilization of biogas, reduce the environmental burdens, and maximize profitability, other than the direct combustion in the CHP unit, different technologies have also been explored. For example, direct usage of biogas for cooking is widely reported in Asian countries, such as China, India, Malaysia, etc. [227,228]. Besides, upgrading the biogas into biomethane (>96% CH_4 concentration) and followed by either injecting the biomethane into the natural gas grid or compressing it as transportation fuel, are often practiced in Europe [229,230]. Regarding the digestate, technologies have also been proposed to ensure a more secure and economical biofertilizer application. For example, liquid-solid separation followed by composting or struvite precipitation to form solidified fertilizer resulted in better environmental performance by less nutrients leaching and emissions [231,232]. Except for the full-scale centralized big biogas plants, there are also a lot of small-scale household digesters (6–10 m^3) in Asian countries, particularly in China's rural areas where there are more than 40 million biogas digesters. Chen et al. [233] studied the so-called Six in One biogas system (SIOBS) consisting of the main digester, cropping, pig breeding, fruit cultivation, vegetable growing, and agricultural processing, targeting to use the biogas for cooking, lighting, and hot water, while to use the digestate as fertilizer, feed additive, and seed soaking. The life cycle results demonstrate a significant contribution to GHG emission (i.e. reduction of 0.3 kg CO_2-eq/MJ energy output) and fossil fuel saving (i.e. output/input energy efficiency of 173%) [233].

Even though the abovementioned traditional and classic practice contributes to a certain extent in a circular economy, the demand for large agricultural land due to the continuous production of a large quantity of digestate is still a challenge for land-limited counties. Thus, an oversupply of digestate and/or the necessity of long transportation distance of digestate has been observed [234]. At the same time, it is also reported that bio-based materials/chemicals actually can result in much higher economic returns compared to biofuels (biogas in this case) [235,236]. There are still opportunities/spaces to improve/enhance the conventional circular economy model. Hence, more and more advanced technologies with higher value-added products have been proposed in recent years, targeting to boost the circularity of the AD output flows.

7.3. Newly proposed circular economy mode of anaerobic digestion streams

As mentioned above microbial protein, plays an important role in addressing the protein scarcity concern. Therefore, instead of directly burning methane in the biogas, the renewable methane can be used by methanotrophs, such as *Methylococcus capsulatus* and *Methylocapsa acidiphila*, to produce SCP [58,187]. Both upgraded biogas (biomethane) and raw biogas have been proved to be suitable for SCP production. Moreover, the advantage of using the upgraded biogas is that CO_2 can be coupled with H_2 to form CH_4 during biological upgrading technology [60] further increasing the carbon circularity. Another advantage of integrating AD with SCP production is that the nutrients in the digestate, particularly the N sources, can be utilized efficiently as an inexpensive medium by the microorganisms to synthesize SCP [237]. In the end, the produced SCP can be used as animal feed while the animal waste can be fed into the AD system, where a closed loop is formed to meet the circular economy requirement.

Another novel circular economy concept of AD streams is the integration of AD with algae cultivation, which is driven by the lower cost of recycling CO_2 and nutrients. The different micro/macro nutrients in the digestate can meet part of the algae cultivation medium requirement. Different types of algae have been reported to be cultivated successfully after an adjustment/treatment of the digestate [238,239]. Importantly, the CO_2 present in the raw biogas (around 30–40%) can be utilized by the algae without the necessity of external CO_2 supply, which further enhances carbon utilization and promotes the whole loop closure. The full-scale case study of the AD and microalgal integration system was also reported in Sweden with a 9.4% annual biomethane increase compared to AD standalone [240]. After the cultivation of the algae, it can be either fed back into the AD system as feedstock [241] or injected into the algae biorefinery platform after further extraction and purification for high-value-added chemicals production [242].

To further capture and utilize the high CO_2 content in the raw biogas, biosuccinic acid production has also been proposed as a novel technology to achieve biogas upgrading and CO_2 utilization at the same time, where methane content can be increased to above 95% with a biosuccinic acid production rate of 0.56 g/L/h [118,119]. Recently, a LCA study also demonstrated that biosuccinic acid production integrated with CO_2 capture from raw biogas brings about environmental benefits to mitigate climate change [192]. The succinic acid is an important building block for many other products, such as bioplastic, pharmaceuticals, cosmetics, and inks, etc [38]. Beyond succinic acid production, biogas has also been tested to directly produce bioplastic material [243], namely PHA. Due to the current high cost of the raw materials (mainly carbon substrates) to synthensize PHA, biogas provided a relatively cheap carbon source for PHA production at about 8.6–8.8 €/kg PHA in a medium-size biogas plant, and can further optimize the process to lower the price to 4.2–4.6 €/kg PHA [244], which offered a novel and high value-add downstream choice.

To valorize the digestate stream, particularly the solid part after liquid/solid separation or drying, thermal-chemical processes [234] have been discussed intensively, namely hydrothermal carbonization (HTC), pyrolysis, and gasification. The driven initiative is that the recovered heat from the biogas CHP unit can cover the energy needed for the drying process. The main output from the thermal-chemical process is syngas, bio-oil, and biochar. The yield of the three products can vary significantly, depending on the different downstream purposes, by adjusting the reaction condition, such as temperature, pressure, carrier gases, etc [245]. The produced bio-oil and/or syngas can improve the overall energy output performance of the integrated system by up to 60% [37,246,247], while the syngas can be further used for other liquid biofuel production, such as biodiesel *via* Fischer–Tropsch process and methanol. The produced biochar can be used as a soil amendment and bio-adsorbent to remove toxicant compounds [248,249], and at the same time serve as carbon sequestration to mitigate GHG emissions. Moreover, studies also demonstrated that the biochar produced from the solid digestate mixed with the liquid digestate can further improve the growth of the plants, such as wheat and maize, compared to either sole soil or liquid digestate alone [250].

7.4. Challenges and future perspective to achieve a novel circular economy

For a long time, human relies on fossil fuel as the main energy source and uses derivatives from petroleum for daily life. However, it brings server problems such as climate change, air/water pollution, fossil fuel depletion, etc. Hence, biorefinery based biowaste, aiming to circular current resources and mitigate carbon emission, should play a central role moving forward. First of all, the novel biorefinery approach aims to compete with the current petroleum refinery, thus developing cost-effective biotechnologies is of utmost importance, other than just

propose the biorefinery concept. For example, the biomethane from AD is incompetent compared to natural gas in terms of cost when injecting the biomethane into natural gas pipe without governmental policy support [251], thereafter reducing the cost of the biogas upgrading and associated infrastructure by optimizing the technology is important. Moreover compared to the petroleum based plastics, such as Polypropylene, Polyvinyl chloride, Polyethyleneterephtalate, etc., which has a production price lower than €1/kg [252], the biogas based bioplastic, namely PHA, seems not competitive due to its much higher cost of 4.2–4.6 €/kg [244]. Secondly, even though the lab test has demonstrated the feasibility of using biogas as SCP carbon source, large-scale industrial production based on biogas has never been reported. Additionally, the concept of biosuccinic acid production from biogas was lately proved but the process efficiency still needs to be improved *via* optimization or integration with other technologies to make this pathway competitive [119]. Hence, more R&D activities and pilot-scale testing are needed to make these novel biorefinery pathways more efficient and economically feasible. Thirdly, most of the studies only focused on the conventional circular economy concept when analysing the economic feasibility, such as biogas production cost and selling price [253], and cost comparison of different waste treatment technologies (i.e. AD, incineration, landfill, etc.) [254], but the technical-economic analysis focusing on novel circular economy concept, based on biorefinery is seldom reported, particularly from the whole life cycle process [49]. Holistic assessment from both environmental and economic perspective, to identify the most suitable biorefinery pathways is urgently needed in the near future. Finally, the circular economy model cannot be achieved only from scientific communities, policy-makers and industry stakeholders are encouraged to take part of the responsibility to invest and join the technologies development. For example, Hussain et al. [255] demonstrated the important role of small and medium enterprises (SMEs) in speeding up the process efficiency towards zero waste under the circular economy of AD. Besides, and the whole society shall try to change the current "take-make-use-dispose" life mode and keep the circular economy in mind and start from contributing waste segregation for easier downstream circular technologies as well.

8. Conclusions and future directions

AD process is a proven technology for converting all types of organic matter to methane and carbon dioxide. Despite biogas has been traditionally used for heat and electricity production and digestate as fertilizer, AD streams can better support on the establishment of circular bioeconomy:

- Upcycling gas and liquid streams of AD for the cultivation of proteinaceous biomass can be a competitive alternative to the conventional agricultural-based feed sources
- Bringing digestate into agricultural framework has been proven as a promising approach for upcycling AD effluents
- Polymers formation as PBS through succinic acid production or direct production of PHAs is technically feasible using CO_2-rich streams
- The production of value-added products under biorefinery concept has considerable environmental improvements compared to their counterparts from fossil routes

Research and development actions and governmental incentives are needed to roar the upcycling of AD streams and establish their role in circular bioeconomy:

- Presence of pathogens, heavy metals and contaminants should be avoided to ensure the suitability of the proteinaceous biomass before feed applications
- Cultivation conditions of microalgae should be improved to enhance CO_2 utilization without O_2 contamination, ensure production at high pH due to digestate valorization and alleviate from high dissolved CO_2 concentration
- Photoautotrophic cultivation systems for PHA production are still far away to attain economic feasibility since titers and productivities are very low
- O_2 gas-liquid mass transfer rate must be enhanced for the autotrophic systems to compete with the current PHA heterotrophic production system
- The processing cost for digestates, lack of supportive regulations and incentives, and high market prices should be considered to lead in a widespread use

All above-discussed technologies can have significant environmental and economic implications directly affecting the establishment of circular economy. Specifically, their environmental benefits deriving from the increased carbon capture and decreased GHG emissions and the economic benefits deriving from the introduction into the market of new and green industrial applications are great advantages. The promising research-based results can positively influence the implementation of green policies supporting the circular bioeconomy. Subsequently, the applied models have the potential to alleviate high dependencies on fossil reserves and overcome regional limitations that undermine the sustainability aspects.

Credit author statement

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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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