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Advances in environmental bioprocess technology for an effective transition to a green circular economy

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12.1 Introduction

The greatest environmental challenge of the 21st century is global warming. Over-industrialization, urbanization, and rapid population growth have caused an enormous increase in energy and materials necessity as well as carbon dioxide (CO_2) emissions [1]. Several agreements and protocols (e.g., the Paris Agreement and the Kyoto Protocol) target to restrain global warming by decreasing the production of greenhouse gas (GHG) emissions [2]; however, CO_2 emissions continue to increase [3]. New strategies are required to achieve the Paris Agreement's primary objective: limiting the global temperature rise below 1.5°C by reducing carbon emissions. Most importantly, petroleum-based production (chemical, energy, and feedstock) needs to be transformed into environmentally friendly and sustainable production methods. As Zeschmar-Lahl evoked that "*waste is raw materials at the wrong time in the wrong place*" [4], the recovery of valuable resources from waste streams should be considered as a unique opportunity to reach biobased, sustainable, environmentally friendly, and commercially relevant production methods.

Traditional wastewater treatment plants (WWTP) focus on removing carbon, nutrients, metals, solid particles and pathogens to recover water. Shortage of material and energy sources with adverse environmental effects enforce a shift from traditional WWTPs to next genera-tion WWTPs, namely water resource recovery facilities (WRRFs) [5] focusing on the recovery of carbon, energy, nutrients from wastewater. The recovery of these products requires reli-able, efficient, sustainable, environmentally friendly technologies being cost-competitive with existing treatment processes [6]. Furthermore, the novel technology needs to reach a mature status. According to Parker [7], the development of treatment technologies consists of several
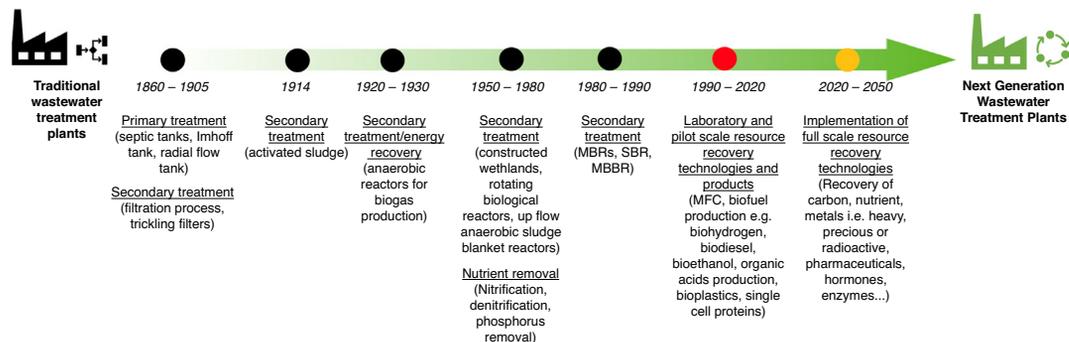
FIG. 12.1 Transformation of traditional WWTPs into the next generation WWTPs (adapted from Lofrano and Brown, 2010). AS, activated sludge; CW, constructed wetlands; RBC, rotating biological reactors; UASB, upward-flow anaerobic sludge blanket; MBRs, membrane biological reactors; SBR, sequencing batch reactors; MBBR, moving bed biofilm reactors [133].

stages: (1) technology development, (2) pilot to demonstration scale, (3) first scale applications and (4) mature technology stage. The adaptation of resource recovery technologies into next generation WWTPs is expected to take approximately 20–40 years from the development stage to mature state, depending on the process type [8]. Fig. 12.1 illustrates the historical transformation of WWTPs including a selection of treatment processes and products. Despite that resource recovery technologies from waste streams have been studied for decades, far-reaching improvements are necessary to fulfill full-scale application requirements.

Besides the development of the suitable technologies/processes, an effective implementation of resource recovery depends on several drivers including national/international agreements, policies and regulations. Currently, many countries, particularly EU states, are taking steps toward carbon neutral WWTPs: numerous EU funded projects address the development of resource recovery technologies [9–12]. The 2030 Agenda for Sustainable Development, adopted by all United Nations member states in 2015, intends to improve health and education, reduce inequality, and spur economic growth—while tackling climate change and preserving oceans and forests [13]. Specifically, Sustainable Development Goal (SDG) 6 (Clean Water and Sanitation), SDG 7 (Affordable and Clean Energy), SDG 11 (Sustainable cities and communities), SDG 12 (Responsible Consumption and Production), and SDG 13 (Climate Action) target resource recovery technologies to reach sustainable, environmentally friendly, and carbon-neutral production. In line with the objectives of the UN SGDs, the EU has introduced multiple policies and targets to implement resource recovery from waste streams via approaching zero carbon emission and targeting circular economy objectives. One cornerstone of the European Green Deal [14] is the new Circular Economy Action Plan for a cleaner and more competitive Europe, which was published in March 2020 by the EU Commission. Its main aim is to reduce the total environmental and resource footprint of production and consumption while providing strong incentives for innovation, sustainable business and markets for climate-neutral and nontoxic circular products via highlighting the strong synergies between climate action and the circular economy, in particular in energy and carbon-intensive industries and calls

for the establishment of an EU-level target for resource efficiency [14]. Furthermore, the EU Climate Action put forward a plan to (1) further cut emissions by at least 55% by 2030 as well as to become the world's first climate-neutral continent by 2050, (2) to encourage international partners to increase their ambition to limit the rise of global temperature to 1.5°C to avoid the most severe consequences of climate change [15]. In line with these targets, the Roadmap to a Resource Efficient Europe and the Circular Economy Package address the transformation of the EU's economy until 2050 by providing a future-oriented agenda to achieve a cleaner and more competitive EU to fully contribute to climate neutrality [16].

Several review and perspective papers address the transformation of traditional WWTPs into WRRFs and the concept of resource recovery in next-generation treatment plants [17–20]. This chapter focuses on the most promising bioproducts that have great untapped potential for resource recovery and strategies to enhance resource recovery by microbial communities in biological wastewater treatment.

12.2 Promising biobased products for resource recovery at WWTPs

The resource recovery approach from wastewater has been studied since the 1990s [18–20]. Although several newly developed technologies in the biological wastewater treatment process enable certain aspects of resource recovery (carbon and nutrient recovery), these methods have been used primarily for the purpose of efficient, sustainable, and environmentally friendly wastewater treatment. One of the well-known resource recovery approaches from WWTPs relies on anaerobic digestion technology for energy recovery through biogas production [21]. However, anaerobic digestion technology started to exceed energy recovery as a consequence of the great interest in the carboxylate platform [22]. Currently, short chain and medium chain fatty acids are the most promising products from waste streams via anaerobic digestion in the carboxylate platform.

12.2.1 Short-chain and medium-chain fatty acids

Short-chain and medium-chain fatty acids—intermediate products of anaerobic digestion—and their derivatives are one of the most promising biobased products in carboxylate platforms [23]. Particularly, short-chain fatty acids (SCFA, fatty acids with 2–6 carbons), also called volatile fatty acids (i.e., acetic acid, propionic acid, butyric acid, etc.), are used in upstream processes as feedstock in several industries (i.e., chemical, pharmaceutical, agricultural, food, textile, etc.) [24] and downstream processes as a carbon source for the production of various biobased materials (such as biogas, biohydrogen, biopolymers, medium and long-chain fatty acids) [25,26]. Because of their wide usage area, the global market demand of SCFA is increasing year by year: the *global annual acetic acid, propionic acid and butyric acid productions are estimated to reach 20.3 million tons by 2024* [27], *550 kilotons in 2026* [28] *and 120 kilotons in 2026* [29], respectively. The market size for these SCFAs ranges at around USD 2.1 billion for acetic acid, USD 288 million for propionic acid and USD 97.5 million for butyric acid, respectively [24].

Nevertheless, approximately 98% of this production is supplied synthetically from petrochemical derivatives [30]. The cheap manufacturing cost of petroleum based SCFA and challenges in biobased SCFA production (such as low production efficiency, unstable SCFA composition for upstream and poststream applications, complications in purification and separation of the end products, postprocess requirements, high substrate cost, etc.) limit industrialization of biobased SCFA production.

Various studies address the enhancement of biobased SCFA production efficiency by optimizing operational and environmental conditions [31–36]. These studies identified several parameters such as pH, temperature, retention time, loading rate, reactor type and mixing as key parameters for efficient biobased SCFA production. The effects of operational and environmental conditions on SCFA production and composition according to a selection of recent studies are summarized in Table 12.1. The effects of operational and/or environmental parameters on SCFA production efficiency and product profile depend on the specific scenario. For instance, the SCFA production efficiency from the fermentation of microalgae biomass, cheese whey and maize silage under alkali pH conditions were relatively similar [37]. On the other hand, fermentation of (agro-industrial cucumber, tomato, and lettuce waste) resulted in different SCFA production efficiencies [38]. Furthermore, the increase in temperature from 35°C to 55°C had no significant effect on SCFA production (max. production was 27.9 gCOD/L at 35°C; 27.3 gCOD/L at 55°C), whereas it resulted in a sharp decrease at 70°C (max. production was 24 gCOD/L) [39]. Additionally, the temperature change did not affect the product profile [39]. It should also be noted that pH has a significant effect on both SCFA production efficiency and composition; however, this effect is closely connected with the type of substrate and inoculum. For example, the highest SCFA concentration from potato peel waste fermentation was obtained under pH 7 [40]. In contrast, alkali pH (pH 10) significantly promoted SCFA production efficiency in sewage sludge fermentation [41].

SCFA can be converted to medium chain fatty acids (MCFA, fatty acids with <6 carbons, i.e., caproic acid, heptylic acid, caprylic acid, etc.,) via chain elongation (reverse β-oxidation) [42]. The physical and chemical properties of MCFA (e.g., high hydrophobicity and energy content) allow for easier separation and purification and versatile application areas. MCFA has been extensively used to manufacture various products (drugs, personal care products, lubricants, detergents, etc.) [43]. Caproic acid, which is the most well-known MCFA, is currently produced from coconut and palm oils, despite that these oils include only 1–2% caproic acid [44]. Although caproic acid can be gained via chain elongation through anaerobic digestion, this process requires the external addition of an e-acceptor (mainly butyric acid or lactic acid) and an e-donor (mostly ethanol). The majority of these chemicals (e-acceptor and e-donor) are obtained by unrenewable and environmentally hazardous petroleum-based production [43], even though they can alternatively be produced by anaerobic digestion in a biobased way. Reddy et al. (2018) conducted a study to enhance chain elongation. For this purpose, anaerobic mixed culture was fed by synthetic SCFA (8 g/L acetate, 1 g/L propionate and 1 g/L butyrate) serving as an e-acceptor and ethanol (10 g/L) was used as e-donor under pH 7 at 37°C for 12 days. The results showed that the highest total production yield of SCFA and MCFA was obtained under methane inhibited condition, and it was mainly comprised of butyrate (8.2 g/L), caproate (8.6 g g/L), heptanoate (0.7 g/L), and caprylate (0.23 g/L) [45]. Similarly, Grootscholten et al. (2013) evaluated

Table 12.1 SCFA production efficiency with product profile under various operational and environmental conditions.

Inoculum	Substrate	Environmental and operational conditions	Reactor type	SCFA production	SCFA composition	References	Notes
Rumen fluid	Apple pomace (AP: 41.24%*), Grape pomace (GP: 27.87%*) and Citrus pomace (CP: 52.59%*), separately.	39 °C without pH control	Anaerobic batch reactor (125 mL)	123.42 mM from AP; 104.48 mM from GP, 131.51 mM from CT**	Acetic/Propionic/Butyric/Valeric/Caproic acids for AP: 71.08/37.83/3.19/1.52/9.80; GP: 63.30/24.51/2.54/0.85/11.29; CT: 79.85/38.23/2.51/1.24/9.68**	[128]	*Nonfiber carbohydrates; **Fermentation by the fresh inoculum
Anaerobic digester sludge	Potato peel waste (carbon content %35.36)	pH 5.0, pH 7.0, pH 11.0, and uncontrolled pH at 37°C	Continuous stirred-tank reactor (5L)	VFA yield (mgCOD/gVS): uncontrolled pH: 309.5; pH 5: 272.6; pH 7: 632.2; pH 11: 31.4	Acetic/Propionic/Butyric/Hexavnoic acids yield (mgCOD/gVS): Uncontrolled pH: 69.3/3.7/157.2/78.3; pH 5: 46.6/6.8/128.1/89.4; pH 7: 289.5/180.2/153.0/0; pH 11: 28.4/1.9/1.1/0	[40]	
Anaerobic digester sludge	Household biowaste (20%TS and 17.3%VS)	pH 5.0; pH 5.5; pH 6.0	Anaerobic batch reactor (20L)	Total VFA (g/L): pH 5.0: 11.51; pH 5.5: 13.51; pH 6.0: 20.54*	Acetic/Propionic/Butyric/Valeric (mass based%): pH 5.0: 63.2/28.6/8.2/0; pH 5.5: 57.2/29.7/11.9/1.3; pH 6.5: 5.8/0.8/40.5/53.0*	[129]	*72-h process duration
Substrate was used as an inoculum	Municipal biowaste (OFMSW)	Without pH adjustment (initial pH 6.6) at 35°C; 55°C and 70°C	Anaerobic batch reactor (250 mL)	Max. VFA (gCOD/L): at 35°C: 27.9; at 55°C: 27.3; at 70°C: 24.0	Acetic/Propionic/Butyric/Valeric/Caproic/Heptanoic acids (%): at 35°C: 28/22/25/14/9/3; at 55°C: 30/23/29/11/5/2; at 70°C: 33/23/25/12/5/2	[39]	
Substrate was used as an inoculum	Food waste (22466 mgSCOD/L)	pH 4.5, pH 5.4, pH 6.5, and uncontrolled pH at 50°C	Pilot scale anaerobic batch reactor (100L)	Max. total VFA (mgCOD/L): 8419 at pH uncontrolled; 7625 at pH 4.5; 12034 at pH 5.5; 15048 at pH 6.5	The total VFA mainly composed of acetic and butyric acid at uncontrolled pH/pH 4.5/pH 5.5, whereas, butyric acid was dominant at pH 6.5	[130]	

(Continued)

Table 12.1   *(Cont'd)*

Inoculum	Substrate	Environmental and operational conditions	Reactor type	SCFA production	SCFA composition	References	Notes
Granular sludge was sourced from an industrial up-flow anaerobic sludge bed (UASB)	Leachate from thermal diluted acid hydrolysis of brewery spent grain (Total carbonhydrate 15 g/L)	pH 5.85–6.0 at 37°C	Expanded granular sludge bed (EGSB) volume of 2.27 L; working volume of 1.78 L)	120.5 mmol/L	Acetic/Butyric/Propionic (mmol/L): 74.4/36.6/9.5	[131]	
The mixed culture of marine microorganisms from saline beach sediment and microorganisms from yogurt, kefir, and cheese.	Mixture of cheese whey and brewery wastewater (107 gCOD/L)	Under neutral pH at 40°C and 55°C	Anaerobic batch reactor (500 mL)	max. total carboxylic acid (g/L): 78 at 40°C; 66 at 55°C	In both sets, 89% of the carboxylic acid composed of lactic acid.	[132]	
Mixture of a conventional anaerobic digester sludge (50%) and a psychrophilic digester sludge (50%)	Agroindustrial waste (Cucumber: 30 gSCOD/L; Tomato: 45gSCOD/L; Lettuce 35.7 gSCOD/L)	Under pH 5.5–6.0 at 25°C	CSTR with 1L working volume	VFAs (gCOD/L): 25.3 for cucumber; 29.8 for tomato; 25.0 for lettuce	Acetic/Propionic/Isobutyric/Butyric/Valeric/Caproic acids (gCOD/L): Cucumber: 3.9/1.0/0.6/11.8/0/2.4/5.7; Tomato: 5.4/0.9/5.4/8.0/0/0.9/9.7; Lettuce: 5.5/1.9/0.7/10.6/0.5/3.0/3.3	[38]	

the effects of ethanol addition on chain elongation during acidification of organic fraction of municipal solid waste (OFMSW). Despite the production of MCFA (caproate, heptanoate and caprylate increased to 2.7 g/L, 1.5 g/L and 0.5 g/L, respectively) after ethanol addition, the hydrolysis rate and the carboxylic acid yield in reactors were decreased by ethanol addition [46]. Furthermore, De Leeuw et al. (2019) showed that the enriched microbial community (inoculum mixture from ethanol-based chain elongation reactor and methanol-based chain elongation to produce iso-butyrate reactor) to gain iso-caproate from iso-butyrate (44 ± 6 mmol C/L) [47]. Although several methods have been employed to improve chain elongation for MCFA production, further studies are required to (1) achieve control of the terminal product length and (2) obtain high production efficiency with a stable product profile. SCFA and MCFA possess a wide usage area in up-stream and down-stream applications, as illustrated in Fig. 12.2.

12.2.1.1 Up-stream applications

SCFA and MCFA are used as feedstock in several industries after separation and purification in the up-stream processes. However, product recovery including separation and purification from the effluent are the most significant bottlenecks for commercializing SCFA and MFCA production. The three main difficulties that challenge the development of SCFA recovery from the effluent are (1) low concentration of SCFA in the effluent, (2) the molecular structure of
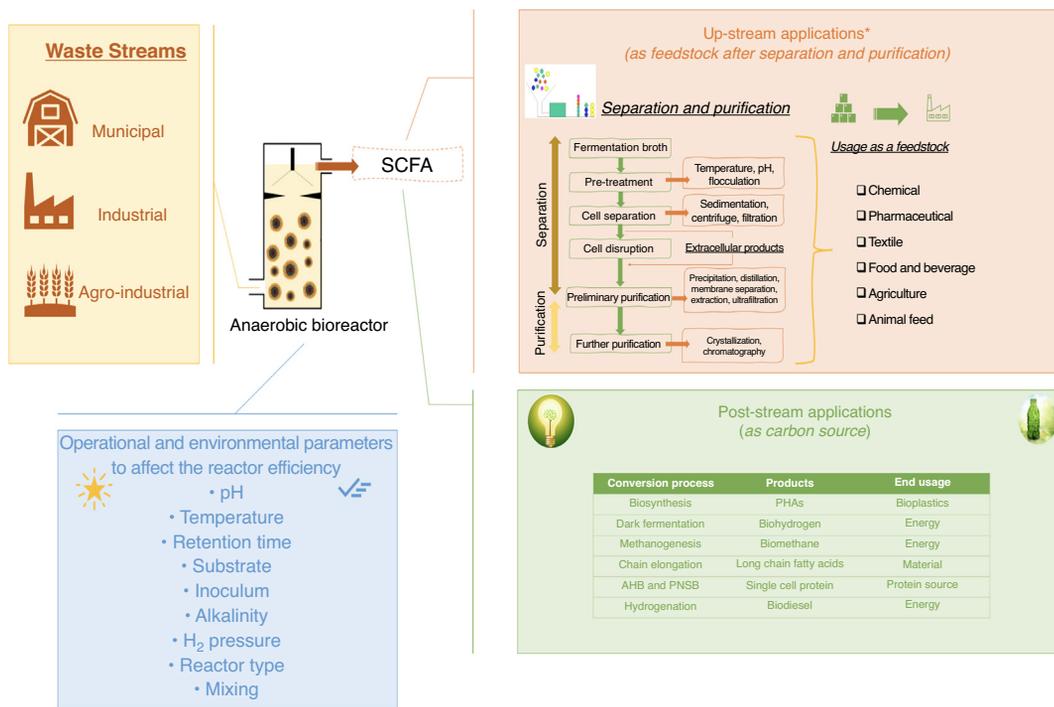

FIG. 12.2 Upstream and downstream applications of SCFA and MCFA (adapted from Li et al. (2016) [134]).

SCFAs and (3) a considerable amount of other dissolved compounds in the effluent [48–51]. Many recovery methods for SCFA/MCFA separation and purification have been developed; cf. Ramos-Suarez et al. (2021) for a comprehensive review on SCFA recovery techniques [49]. Many recovery methods have been used for SCFA recovery from the effluent; the process has to be selected and developed for the targeted end product instead of the SCFA mixture [52].

The most common recovery methods include absorption by different materials, adsorption, solvent extraction, electrocoagulation, esterification, distillation, gas stripping, membrane processes such as nanofiltration, electrodialysis, microfiltration, ultrafiltration, membrane contactor and reverse osmosis and in-line recovery [24,48,49,51,53]. Each of these methods has specific limitations regarding economic aspects, sustainability perspectives or their applicability in full-scale operations. Besides these limitations, the efficiency of the recovery method depends on several environmental and operational factors such as pH, temperature, the composition of effluent, type of substrate, etc. Furthermore, each method has its design and optimization approaches. For instance, the type of resin and desorption chemical, their capacity and contact time play an essential role for the efficiency of SCFA recovery in the adsorption process [54]. In addition, electrodialysis—one of the most performed SCFA recovery methods—is usually applied to centrifuged/filtered fermentation broth. Therefore, the composition of the fermentation broth significantly affects the electrodialysis efficiency [55]. In a similar manner, the recovery efficiency by membrane separation, which is the most promising SCFA recovery method, strictly depends on the configuration and type of the membrane [56].

Aktij et al. (2020) performed a comprehensive review of the feasibility of membrane processes for the recovery of SCFA, highlighting the advantages and limitations of each method [56]. They concluded that the hybrid membrane process is a promising SCFA recovery approach to increase the overall product recovery efficiency and improve product purity [56]. However, besides overall SCFA recovery efficiency, the selected recovery method must be applicable for the separation and purification of individual acids (such as acetic acid, propionic acid, butyric acid, etc.) from the SCFA mixture and needs to be cost-effective for their upstream applications.

12.2.1.2 Poststream applications

SCFA and MCFA are valuable carbon sources for the production of different bioproducts such as bioenergy (microbial fuel cells, alcohols including bioethanol, biobutanol, biohexanol, biohydrogen, biodiesel, biogas, biohythane), biopolymers (polyhydroxyalkanoates) and single-cell proteins. Furthermore, SCFA is ideal for the efficient biological nutrient removal (BNR) processes. Most of these processes require advanced technologies: e.g., different reactor configurations under optimized operational and environmental conditions with suitable inoculum sources. There are several comprehensive literature reviews on the production processes of these products and technologies [24–26,57,58]. The main focus of this section lies on SCFA/MCFA usage as a carbon source in the production of these biobased compounds.

Biopolymers

Biopolymers came into prominence as a green alternative for petroleum-based plastics due to their unique properties, i.e., their full degradability and compatibility. Bioplastics are polyesters produced by microorganisms via accumulation under various operating and environmental

conditions [59]. Based on their composition, biopolymers are obtained from (1) agro-industrial sources, (2) microbial processes, (3) chemical synthesis, (4) renewable resources with synthetic monomers [60]. Biopolymers derived from microbial processes have gained enormous attention in the last decade [61] because of their comparable properties.

Depending on the type of microorganisms and the physicochemical properties of the accumulated material, the monomer composition and structure differ [62]. Bioplastics can be classified by different criteria such as biosynthetic origin, chemical nature, number and size of the monomers and the polyester type to accumulate [62]. The most known and widely produced microbial bioplastics are polyhydroxyalkanoates (PHA), and particularly polyhydroxybutyrate (PHB) [63].

PHAs are biodegradable polymers that contain either medium or long-chain length monomers [62]. The biodegradable, biocompatible, thermoplastic and environmental sustainability properties make them competitors to replace traditional plastics in several market sectors, such as packaging and biomedical applications [64].

Many literature reviews discuss the properties, production processes, configurations, advantages and limitations of PHAs [62–67]. Despite that PHA production is not economically feasible yet—due to high substrate cost, low production yield, challenges in extraction and purification processes [18]—the developments on the process configurations of PHA production and new regulations are expected to result in their commercialization.

PHA is mainly produced in three steps: (a) SCFA/MCFA production by fermentation of waste streams, (b) enrichment of the biomass for high PHA storage capacity, and (c) PHA accumulation by the enriched biomass, which is fed by previously produced SCFA/MCFA [61,68–70]. Herein, SCFA/MCFA has a significant role in PHA production: the type of SCFA/MCFA (such as acetate, propionate, butyrate, and caproate) influences the physical and chemical properties of the PHA. For instance, the mixture of glucose and propionic acid as carbon sources resulted in 78.3% PHA content as P(3HB-co3HV) [71]. On the other hand, P(3HB) with 31.9% PHA content and mcl-PHA with around 40% PHA content were separately produced from butyric acid in [72,73].

Single-cell protein

The current global protein production is not sustainable, yet the worldwide population is expected to reach 10 billion in 2050, and the global demand for protein will then be doubled [74]. Single-cell protein (SCP), also called microbial protein, is one of the most promising and sustainable protein sources because of its high protein content (the average dry weight protein content is 40–60% for algae; 30–70% for fungi, and 50–83% for bacteria) and the possibility to use different feedstock sources. Protein production via SCP has unique advantages: independence of climate, soil characterization and weather, decrease of greenhouse gas emissions, nitrogen losses, rapid production with a small footprint, high protein, carbohydrates, vitamin, mineral contents and nucleic acids [75,76]. On the other hand, high nucleic acid content, especially RNA, causes health disorders (e.g., kidney stone and gout) [77] that limits SCP usage for human consumption [76]. Therefore, RNA content from SCP should be removed by suitable methods (e.g., heat treatment, alkaline hydrolysis, RNA degrading enzymes and other chemical extraction methods) before human consumption [76,78].

At the same time, SCP has the potential to close the global protein gap while achieving the ambitious goal of sustainable food production as stated by UN SDGs [76]. One of the main limitations is substrate cost: although waste streams are attractive substrate sources for SCP production, contamination by pathogens is a major problem. Using methane and off-gas as feedstocks promotes SCP production technologies, while the process requires the external addition of nutrients, vitamins, and trace elements [79]. Phototrophic SCP production is another promising way; however, it depends on an external light source [80] and the investment/operation costs are higher than those for heterotrophic reactors. On the other hand, organic acid rich fermentation broth prevents contaminations by pathogens due to acidity. It provides rich substrate—including a high amount of nutrients, vitamins, and trace elements—for economically competitive heterotrophic SCP production. Furthermore, a different organic acid profile can steer the produced protein type [81]. SCFAs are one of the most advantageous organic acids used for efficient and stable SCP production [81]. Besides many advantages of SCFA usage for SCP production, only a few studies have focused on heterotrophic SCP production from SCFAs [82,83].

Bioenergy

The most established way of energy recovery from anaerobic digestion is biogas production, which can be used for heating and electricity production. Raw biogas consists of 60–65% CH_4, 30–35% of CO_2, and small percentages of water vapor, H_2, and H_2S. After purification to remove CO_2, H_2S, and other impurities, the upgraded, pipeline quality biogas is used as a natural gas substitute [21]. Biogas production has been used as an energy source for a long time: it is a worldwide accepted mature technology and widely applied to organic waste valorization. Due to short supply and expensive energy costs, anaerobic digestion has great relevance. The main advantages of biogas production are (1) low energy consumption during degradation of waste, (2) all biodegradable organic matter in biomass is converted to biogas and (3) even low biomass yield result in high methane production [84]. However, there exist certain limitations for biogas production. Methane can escape into the atmosphere if handled inadequately, and the global warming potential of methane is twenty times higher compared to carbon dioxide [2]. In addition, more than 50% of methane can be dissolved in the bioreactor effluent at low-strength wastewater with low temperatures [85]. Therefore, anaerobic digestion technology has started to go beyond biogas production. Nevertheless, biobased energy sources are still required.

According to the World Energy Resources report (2016), the reduction of the carbon emissions from fossil fuels and their substitution is the major driver for the development of bioenergy [135]. The Paris Agreement is providing much-needed motivation in shifting from fossil fuels to renewables to focus on renewables drives investments in bioenergy [135]. The carbon tax is another driver to reduce fossil fuel use and increase energy efficiency by leading to a more sustainable and greener lifestyle [135]. Many countries, for example, Sweden, have successfully implemented the carbon tax leading to increased use of bioenergy. Swedish Climate Act aims for zero GHG emissions up to 2035. Therefore, they have been started to shift biogas usage in public transportation with renewable energy sources [86]. In conclusion, although biogas

production is the most common way of recovering energy from waste streams, it is likely to be replaced by other options in the near future.

Besides biogas production, several other promising bioproducts derived from SCFA/MCFA have been successfully used as an energy source. One of them is biodiesel, which is mono-alkyl esters of fatty acids derived from vegetable oil, animal fats, algal lipids, or waste grease [136]. Biodiesel has been commercially produced and used as petrol diesel. Rudolf Diesel invented biodiesel in the 1970s as an alternative to fossil fuels [58]. Initially he used peanut oil as biodiesel source; meanwhile there are several feedstocks to produce biodiesel, such as vegetable oils (camelina, canola, castor bean, coconut, jatropha, palm, peanut, rapeseed, soybean, sunflower, and tung), algal lipids, animal fats (beef tallow, pork lard, chicken fat, etc.), and yellow grease (used vegetable oil) [87]. In general, biodiesel is classified into four generations regarding their feedstock and production methods. The first generation is produced from edible oil seeds, which accounts for more than 95% of the global biodiesel production. However, this generation has to replace with next generations due to edible oilseed production needs great fertile lands and water resources which is caused by food shortages and GHG crisis. Second-generation biodiesel production depends on nonedible resources or biomass which includes waste oil, grease, animal fats, nonedible energy crops. It is a suitable solution for waste oil disposal, second-generation biodiesel production has limitations, i.e., land requirement, extensive water usage, and increase GHG emissions and carbon footprint. In third-generation biodiesel production algae are used as a feedstock. It has unique advantages over other generations such as less GHG emissions and more sustainability. However, the fourth generation of biodiesel production developed, due to the low production yield of biodiesel from algae [58,88,89]. Genetically engineered crops and microalgae are used as feedstock for biodiesel production with high oil yields. Even though the next generation biodiesel production methods have great advantages, there are still some problems before, during and after biodiesel production. Feedstock selection is the most important part of the appropriate cost of biodiesel. In all methods, the feedstock cost should not be more than 50% of the production cost to cope with petrol-based methods [90].

Biodiesel is produced by means of the transesterification process of triglyceride: 90% of biodiesel and 10% of glycerol are produced as an end product of the process [91]. SCFA has an important role in transesterification process: acetic acid has been used as subcritical material to convert substrate into fatty acids and triacetin for biodiesel production [92]. Although waste derived acetic acid can reduce the production cost there are limiting factors for industrial scale biodiesel production: (1) requirement of pretreatment and purification, (2) reaching the quality/standards of biodiesel, (3) public acceptance and (4) the sustainability problem including issues concerning feedstock, land consumption, and the impact of biodiesel industry on the environment [87].

On the other hand, biodiesel has a promising future due to its cleaner emission profile, production simplicity, ease of use, and cost competitiveness. It will have a global energy market substantially after economically producing renewable feedstocks such as vegetable oils and algal lipids in sustainable manners. Herein, SCFA and MCFA tender great potential for sustainable and environmentally friendly biodiesel production via fermentation: land-independent production, higher product yield, easy application without external enzyme and sterilization requirement [93].

Besides biogas and biodiesel, SCFA has been used for other bioenergy production in various forms such as electricity via microbial fuel cells, fuel via catalytic upgrading to sustainable aviation fuel (SAF). For instance, Huq et al. (2021) showed that the SAF derived from SCFA could be produced for $0.30/kg, while a Fast-Track fuel is produced for a minimum of $2.50/kg [94]. SFCA derived SAF has the potential to reduce greenhouse gas emissions up to 165% compared to the conventional production methods [94].

In addition to the carbon recovery from WWTPs, several other products have great potential for resource recovery. Previous research primarily focused on the removal of nutrients (nitrogen and phosphorus), metals (heavy, precious, radioactive), pharmaceuticals, hormones, enzymes from wastewater, while several more recent studies address their recovery from wastewater as well. One of them is enzyme recovery from wastewater.

12.2.2 Enzyme recovery

Enzyme recovery from activated sludge has been already investigated [95,96]; however, it is an entirely new resource recovery approach for WWTPs. The comprehensive literature review on enzyme recovery from biological wastewater stated that several enzymes (phosphatase, protease, lipase, amylase, lipase, and glucosidase) have been detected in activated sludge at very significant amounts [97]. Processes including centrifugation, ultrasonication and sonication with TX100 have the potential to purify these enzymes as 19–51 m^3 from a WWTP that has a flow rate of 50,000 m^3/day [98]. The recovered enzymes can be used for several purposes: wastewater treatment as improving hydrolysis of complex substrates [99], degradation of pharmaceutically active compounds [100], industrial applications, etc.

Even though enzymes have been extensively used in many different processes at WWTPs, and integration of enzyme recovery processes into WWTPs is technically viable, neither the process is economically competitive—due to installation and operational costs—nor is the implementation of enzyme recovery technologies into existing facilities practical. However, integration of enzyme recovery into the next generation WWTPs will pursue the overall sustainability of wastewater treatment via contributing circular economy.

12.3 Manipulation of microbial community performance for resource recovery

One of the pillars to achieve the transformation of the traditional WWTPs into the next generation WWTPs is the microbial community's performance. Manipulation of the microbial community can ensure stable and efficient performance in treatment processes and product recovery by: (1) obtaining the desired end product profile, (2) enabling easier adaptation to operational and environmental conditions, (3) ensuring less sensitivity to inhibitory and toxic conditions, (4) providing cost-efficient operation by decreasing additional additives and energy requirement, (5) enabling easier separation of end products from the effluent. These and further advantages make the microbial community the key to reach sustainable, environmentally friendly, and economically competitive resource recovery facilities. The enhancement of

microbial community performance has been extensively studied. One of the most promising strategies to manipulate microbial community activities is bioaugmentation.

12.3.1 Bioaugmentation

Bioaugmentation is a method to enrich the existing microbial community by adding external pure culture, coculture or mixed culture to achieve optimized target activity [101]. The microbial community structure in bioreactors is highly complex. Only a minority of the microbial community has been studied and described in detail [102], and each species may have different functions [103]. The activity of these functions can be improved by the addition of the selected species to help the degradation of inhibitory or toxic compounds, to increase product yield and treatment efficiency, to obtain target product composition, etc.

Bioaugmentation has been used to improve biogas production—via increasing organic loading rate [104], enhancing degradation of lignocellulosic materials [105], preventing ammonia and organic acid inhibition [106]—and biohydrogen production [107–109]. Furthermore, the method has been applied to increase the production of SCFA/MCFA [110–114] within the development of the carboxylate platform. Dams et al. (2018) bioaugmented the mixed microbial culture by *Clostridium acetobutylicum* to enhance chain elongation [110]. Their results indicated that bioaugmentation by *C. acetobutylicum* with the addition of 100 mM ethanol increased caproic acid concentration from 1.61 g/L to 3.82 g/L; caprylic acid concentration from 0.22 g/L to 1.72 g/L; butyric acid concentration from 0.52 g/L to 0.73 g/L, respectively [110].

In previous studies, the mixed culture was bioaugmented by different pure cultures to separately enhance acetic acid, propionic acid and butyric acid production in the VFA mixture. For instance, compared to the control reactor, the bioaugmented mixed culture by *Clostridium butyricum* increased butyric acid production more than 11-fold [112]; the bioaugmented mixed culture by *Clostridium aceticum* enhanced acetic acid production 9.6-fold [115]; the bioaugmented mixed culture by *Propionibacterium acidipropionici* increased propionic acid production almost fourfold [114]. Besides the significantly improved concentrations of the targeted acids in the VFA mixture, the production of other intermediate and end products was enhanced as well. The dominant acid type in the VFA mixture shifted only in the bioaugmented mixed culture by *C. butyricum.* However, there was no significant difference in the concentration of the butyric and propionic acids.

Furthermore, the bioaugmentation by *C. aceticum* did not affect propionic acid dominancy. The bioaugmented mixed culture by *P. acidipropionici* increased propionic acid concentration slightly; it also enhanced valeric and isovaleric acid concentrations. Interestingly, these products are not produced in the metabolic pathway of *P. acidipropionici* [116].

Consequently, these results indicate enhanced syntropy/competition in the bioaugmented microbial community and/or biochemical processes such as chain elongation. Moreover, the same studies showed that the bioaugmentation of mixed culture by selected pure cultures changed the bacterial community profile via enhancing certain unknown syntrophic relations and corresponding metabolic pathways. Therefore, it is crucial to gain a deeper understanding of bioaugmentation effects on the microbial community, particularly to establish its profile,

functions and interactions in the mixed culture for resource recovery. Furthermore, identifying metabolic networks for each acid production is essential for a comprehensive view of bioaugmentation effects.

12.3.2 Enrichment

Enrichment is another method to enhance microbial community by stimulating the target group of the microorganisms' growth by amenable manipulation of the operational and environmental conditions [84]. Furthermore, microbial community can be enriched by using a sequential dilution method to improve their specific activity [117]. Lins et al. (2010) used an acetate degrading enrichment culture to enhance biogas production via reducing accumulated VFAs [118]. After enrichment, maximum biogas production reached up to 0.53 L/day total gas and 0.45 L/day CH_4, while the accumulated VFAs were consumed rapidly [118]. Hao et al. (2017) evaluated two different enriched cultures (acetate-hydrolysate enriched mixed culture and valerate-hydrolysate enriched mixed culture) for PHA production [119]. Their results revealed that valerate-hydrolysate enriched mixed culture has excellent capability to synthesize 3HV and 3H2MV monomers [119].

Candry et al. (2020) enhanced chain elongation by six enriched inocula with acetic acid and ethanol under pH 5.5 and pH 7 [120]. After 12 sequential transfers, caproic acid became dominant acid type in pH 7 sets; nevertheless, acid composition varied at pH 5.5 depending on the inoculum source [120]. Their results indicate that the success of the enrichment strategy closely depends on substrate type, inoculum source and pH [120].

Furthermore, Zagrodnik et al. (2020) compared caproic acid production performance by the bioaugmented anaerobic mixed culture by *Clostridium kluyveri* and the caproate enriched anaerobic mixed culture from a synthetic mixed substrate (lactose, lactate, acetate, and ethanol) [121]. The most interesting result is that the enriched mixed culture produced 11.44 ± 0.47 g/L, whereas the bioaugmented mixed culture produced only 4.10 ± 0.3 g/L caproic acid. Furthermore, they indicate that the complexity of the mixed culture decreased by the enrichment method [121].

Besides bioaugmentation and enrichment strategies, cell immobilization techniques offer tremendous advantages for next generation WWTPs. Cell immobilization techniques have been used mainly for the removal of toxic compounds (e.g., heavy metals, dyes, etc.) and/or nutrients (nitrogen and phosphorus) in biological wastewater treatment processes [122,123]. These techniques provide high biomass concentration, physical strength and high resistance to toxic chemicals [123]. Although cell mobilization techniques include several methods such as adsorption, covalent bonding, cell to cell cross-linking, entrapment, and encapsulation [124], encapsulation has gained distinctive attention for the resource recovery approach [125].

12.3.3 Encapsulation technology

Encapsulation technology is a physicochemical process to entrap a substance/cell/enzyme into a specified material (shell or coating) for protection against adverse conditions [126]. Organic (natural and synthetic) and inorganic materials have been used as encapsulation host matrices based on the selected application (i.e., spray drying, emulsification, extrusion, spray coating,

etc.) [126]. Despite that encapsulation technology have been used in biomedical, food, agriculture, and wastewater treatment, it is a relatively new approach for resource recovery methods. Chen et al. (2021) carried out an extensive literature review on encapsulation technologies for resource recovery by highlighting significant research opportunities and drawbacks [125]. The main advantages of the encapsulation technology are (1) providing the ability to encapsulate specific bacteria, (2) to decrease the sensitivity of the microbial community against adverse environmental conditions and shock loads and (3) the enhancement of microbial activity [125]. Besides these advantages, the application of encapsulation technology lead to a significant increase in biohydrogen and biomethane production compared to the free cells [127]. Nevertheless, there is a limited number of studies on the application of encapsulation technology for resource recovery. Further studies and developments on encapsulation technology will lead to sustainable, efficient and economically competitive resource recovery applications.

12.4 Conclusions and perspectives

With regard to the reduction of both greenhouse gas (GHG) emission and the dependence on nonrenewable and fossil raw materials, the resource recovery approach is broadly accepted as a pioneering step to transform wastewater treatment plants into water resource recovery facilities—the next generation biorefineries. As stated in *1.Introduction,* (1) reducing GHG emission by at least 40% compared to 1990 levels is aimed within the EU 2030 Climate and Energy Framework as part of A European Green Deal; (2) developing new biorefining technologies to sustainably transform renewable natural resources into biobased products and materials is targeted in the EU 2030 Agenda for Sustainable Development; (3) enhancing biobased sectors to contribute on achieving the goals of the circular economy regarding the development of capacities to turn organic waste streams into valuable biobased products is stated within the EU Updated Biobased Strategy. Toward the objectives of the EU, several EU countries have established their national road map to reach sustainable and environmentally friendly industries.

In line with the European and global sustainable development objectives, the transformation of the traditional WWTPs into the next generation WWTPs come into prominence. In this regard, anaerobic digestion possesses excellent potential for biobased materials production from waste streams. Specifically, short chain and medium chain fatty acids (intermediate products of anaerobic digestion) gain importance because of their versatile usage area and high market demand. As discussed in Section 12.2. *Promising biobased products for resource recovery at WWTPs,* the production methods of these biobased compounds (material and energy) require research-intensive technologies. At the same time, recent developments have ensured that achieving sustainable, environmentally friendly, and economically competitive biobased production via the next generation WWTPs is possible. Especially strategies to enhance microbial community activities as discussed in *3. Manipulation of microbial community performance for resource recovery* provides a great opportunity for this purpose. A successful implementation of this transformation must be supported by authorities (e.g., government) via legislation, law enforcement and public awareness. (e.g., preference for biobased products, awareness on for environmental issues, reduced consumption of petroleum-based products, etc.).

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