Cultivation of microalgae on liquid anaerobic digestate for depollution, biofuels and cosmetics: a review

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Received: 16 June 2022 / Accepted: 29 June 2022 / Published online: 26 July 2022
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Abstract
Solid wastes from domestic, industrial and agricultural sectors cause acute economic and environmental problems. These issues can be partly solved by anaerobic digestion of wastes, yet this process is incomplete and generates abundant byproducts as digestate. Therefore, cultivating mixotrophic algae on anaerobic digestate appears as a promising solution for nutrient recovery, pollutant removal and biofuel production. Here we review mixotrophic algal cultivation on anaerobic waste digestate with focus on digestate types and characterization, issues of recycling digestate in agriculture, removal of contaminants, and production of biofuels such as biogas, bioethanol, biodiesel and dihydrogen. We also discuss applications in cosmetics and economical aspects. Mixotrophic algal cultivation completely removes ammonium, phosphorus, 17β-estradiol from diluted digestate, and removes 62% of zinc, 84% of manganese, 74% of cadmium and 99% of copper.

Keywords Anaerobic digestate · Heavy metals · Pollutants · Circular economy · Mixotrophic algal cultivation · Nutrients · Biorefinery

Introduction
Anaerobic digestion of solid waste, particularly animal, sludge, household and lignocellulose materials, has gained considerable attention due to its efficiency and bioenergy yield. However, liquid anaerobic digestate is produced, which is predominantly rich in heavy pollutants, nutrients and occasionally pathogens. The digestate is mainly a mixture of microbial biomass, undigested substrates, and various metabolites. Anaerobic digestate contains excessive amounts of phosphorous, nitrogen and high stability carbon content (Osman et al. 2022). Diverse types of liquid anaerobic digestate were treated with monocultures or co-cultures of microalgae/ bacteria (Yu et al., 2019) and fungi (Massa et al. 2017; Kumsiri et al. 2018). Rich in nitrogen compounds and phosphorous, the liquid anaerobic digestate promotes the growth of microalga cells. Microalgae are freshwater photosynthetic eukaryotic microorganisms with a size of 1–10 mm. Through photosynthesis, microalgal cells convert carbon dioxide and water into organic compounds (glucose) and oxygen. Cyanobacteria and microalgae share similar characteristics. Microalgae can be categorized as heterotrophic, photoautotrophic, or mixotrophic based on their cultivation methods.

The biomass accumulation during photoautotrophic algal cultivation results in self-shading phenomena with lower algal biomass yield and productivity. Heterotrophic algae are more bearable for contamination than mixotrophic algae (Dragone 2022). Because of its higher biomass productivity and the dual role of heterotrophy and photo-autotrophy in
using organic and inorganic matter, mixotrophic is excellent for anaerobic digestate valorization. As shown in Fig. 1, the bacteria-microalgae relationship maximizes nutrient removal and biomass productivity. Carbon dioxide produced by aerobic bacteria is consumed during the algal photosynthesis process. Desmodesmus sp. EJ9-6 completely eliminated ammonium and total phosphorus from diluted piggery digestate (Ji et al. 2014). Likely, nutrients were removed by 90% using Chlorella vulgaris cultivation fed with highly diluted anaerobic piggery digestate (Franchino et al. 2016). The immobilization of Microcystis aeruginosa on activated carbon treating piggery digestate removed more than 90% of the total nitrogen and phosphorous (Gong et al. 2020).

Fortunately, microalgae can convert the nutrient-rich digestate into valuable biomass for biofuels and biodiesel production (Katiyar et al. 2017). Chlorella PY-ZU1 used undiluted digested manure supplemented with phosphorus and 15% carbon dioxide to produce 4.81 g/L biomass, which contained 42, 19.5 and 13% proteins, lipids and carbohydrates, respectively (Cheng et al. 2015). Chlorella vulgaris cultivation on 1.5-fold diluted digested sludge from the pulp and paper industry yielded 2.91 g volatile solids/L biomass with complete removal of phosphate and ammonia (Tao et al. 2017a). When cultivated with pre-treated swine manure digestate, combined Chlorella vulgaris and activated sludge produced 2.3 g/L biomass and removed 97.2% of the chemical oxygen demand, as well as 94% nitrogen and 99.7% phosphorus (Wang et al. 2019a).

Mixotrophic growth of Chlorella vulgaris was cultivated for anaerobic digestate of municipal wastewater with 2.0 g/L glycerol resulting in biomass and lipid productivity of 0.064 g/L.d and 20.4 mg/L.d, respectively (Ge et al. 2018). The total phosphorus removal was 74.7% achieved by Chlorella sp. grown in digestate of dairy manure and increased up to 87.2% from a mixture of starch digestate and alcohol wastewater, at a dilution ratio of 15:1 by Chlorella pyrenoidosa (Wang et al. 2010a; Tan et al. 2018). Coexisting active bacteria in the algal culture medium will increase the biomass productivity and the nutrients removal due to the synergistic effect of algal/bacteria interaction (Rada-Ariza et al. 2017; Wang et al. 2020).

Therefore, the current review aims to provide a comprehensive and critical discussion for digestate types, sources and characterization. Pathogens, heavy metals, volatile fatty acids, phenolic compounds, antibiotic resistance genes, antibiotics, and emerging pollutants are reviewed as impediments to the liquid anaerobic digestate’s utilization in agricultural applications. Microalgae cultivation as an innovative method for treating liquid digestate is extensively discussed. Emerging pollutants, heavy metals and nutrient removal mechanisms by mixotrophic algal cells are discussed. The valorization of algal cells for producing fatty acid methyl ester, biogas, hydrogen, biodiesel, bio-oil, and bioethanol is evaluated. To bridge the gap between waste generation and zero waste discharge, economic aspects of algal cell cultivation productivity and anaerobic digestate utilization are examined.

**Digestate types, sources and characterization**

Solid wastes are originally derived from domestic, industrial and agricultural sectors (Tawfik et al. 2021b; Chen et al. 2022). Those wastes are abundant in developing countries and cause severe economic and environmental problems.
The allocated finances for solid management in developing countries are quite low, creating serious health and ecosystem problems (Tawfik et al. 2021c). Fortunately, the anaerobic digestion process of solid waste produces energy and is suitable for application in such countries (Ali et al. 2019b). The anaerobic bacteria transformed the biodegradable organics into useful energy, i.e., hydrogen and methane (Meky et al. 2021). However, the digestate resulting from anaerobic digestion still contains biodegradable organics, nitrogen components, phosphorous, fatty acids, and metabolites by-products which need further treatment prior to disposal and/or reuse (Bonetta et al. 2014; Eraky et al. 2021). The digestate resulting from the anaerobic digestion of industrial solid waste could contain toxic and refractory compounds, which negatively affect the subsequent treatment process (Tawfik et al. 2021d).

Digestate can be produced from anaerobic co-digestion of different feedstocks such as organic fractions of municipal solid wastes, agricultural residues and cattle manure (Amon et al. 2007; Macias-Corral et al. 2008), sewage sludge and organic solid wastes (Murto et al. 2004), municipal solid wastes, paper mill sludge and gelatins solid waste (Elsamadony and Tawfik 2015). Digestate products could be easily utilized for agricultural economic crops due to its richness in nutrients and minerals (Ai et al. 2020; Jin et al. 2022). However, the digestate still has a high concentration of nutrients, heavy metals, elements, emerging pollutants and pathogenic bacteria, which need to be removed for safe reuse (Nasr et al. 2021) (Fig. 2). The liquid digestate resulting from anaerobic digestion contains solids content not exceeding 15% (Nagarajan et al. 2019). The solid contents are negatively affected by the growth of microalgal cells and need to be diluted to mitigate the inhibition effect of high turbidity. The digestate has a pH of 6.7–9.2 and is mildly alkaline or neutral (Solé-Bundó et al. 2019b). These pH values are suitable for the cultivation of microalgal cells. The liquid digestate is rich with nutrients in terms of nitrogen and phosphorous. The total nitrogen varied from 139 to 3456 mg/L, and total phosphorous ranged from 7 to 381 mg/L (Ali et al. 2019a).

Those nutrients are necessary for the growth of microalgal cells. Ammonia represents 65–98% of the total nitrogen. Phosphate is essential for building up deoxyribonucleic acid and nucleic acids of the algal cells and represents 82–90% of total phosphorus. The chemical oxygen demand of the digestate highly fluctuated from 210 to 6900 mg/L, including volatile fatty acids (Ayre et al. 2021). The inorganic carbon sources are mainly bicarbonate (939–1353 mg/L) in the digestate. The liquid anaerobic digestate also contains macronutrients (potassium, manganese, sulfur) and micronutrients (iron, nickel, cobalt, zinc, and cupper) (Nasr et al. 2021).

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**Fig. 2** Origin and characterization of the digestate. Biodegradable solid wastes are originally derived from agricultural, domestic and industrial sectors. The anaerobic digestion process of solid waste produces energy. The digestate resulting from the anaerobic digestion process still contains biodegradable organics, nitrogen components, phosphorous, fatty acids, and metabolites by-products. The digestate has a high concentration of nutrients, heavy metals, elements, emerging pollutants and pathogenic bacteria, which need to be removed before reusing in agriculture.
In conclusion, anaerobic digestate types, sources, and characterization are the main important parameters for the valorization of such digestate. The digestate resulting from the anaerobic digestion process still contains organics, nitrogen components, phosphorous, fatty acids, and metabolites by-products that need further treatment prior to disposal and/or reuse.

**Barriers to the use of liquid anaerobic digestate in agriculture**

Anaerobic digestion of solid waste generates energy (biogas) (Mostafa et al. 2017; Elsamadony and Tawfik 2018). Nevertheless, wet anaerobic digestion produces liquid digestate containing amino acids, vitamins, humic acids, micronutrients (zinc, iron, calcium, magnesium and copper) and hydrolytic enzymes (Eich-Greatorex et al. 2018; Huang et al. 2020). Heavy metals, antibiotic resistance genes and antibiotics were highly presented in the liquid digestate resulting from the anaerobic fermentation of chicken manure (Riaz et al. 2011), which is not allowed for land applications based on the standards and guidelines. Escherichia coli, Enterococci was accounted for 3.6 and 5.2 log10, respectively, in the digestate product of co-digestion of organic fraction of municipal solid waste, sludge and cattle slurries (Venglovsky et al. 2006).

*Enterobacter* and *Listeria*, parasites (*Ascaris, Cryptosporidium* and *Giardia*), viruses (*Norovirus, Hepatitis A virus, enterovirus, and rotavirus*) and fungi (*Candida, Trichophyton and Aspergillus*) (Venglovsky et al. 2006; Sidhu and Toze 2009), as shown in Fig. 3. Unfortunately, the majority of pathogens remain in the digestate product (Mahmoud et al. 2011), which is not allowed for land applications based on food waste. Although the European Food Safety Authority reported that 5–8 log10 colony-forming units per gram of the food source are sufficient to cause food-borne diseases, low concentrations of 3–4 log10 colony-forming units per gram have been recorded in some food poisoning cases (EFSA 2005). *Bacillus cereus* was reported in three anaerobic digestate based on food wastes in a risky concentration of 3.3–4.8 log10 CFU/g (Golovko et al. 2022). Likely, *Clostridium perfringens* and helminthes eggs were detected in the digestate, indicating the existence of other spore-forming pathogenic bacteria. Bagge et al. (2005) and Bonetta et al. (2011) identified the presence of *Clostridium perfringens* in the digestate. The helminthes eggs were highly counted in digested bio-solids in industrialized countries (Rubio-Loza and Noyola 2010). *Salmonella enterica subsp. arizonae* (33%) and *Salmonella enterica* (67%) were detected in the digested sludge and organic fraction of municipal solid waste.

**Pathogens**

The application of anaerobic digestate, particularly from the domestic origin (sludge) in the agricultural sector without disinfection, could potentially spread dangerous pathogens in the farm, causing health problems and crop contamination. Wastes originally arise from animals, and human contains pathogenic bacteria (*Salmonellae, Clostridia, Enterobacter* and *Listeria*), parasites (*Ascaris, Cryptosporidium* and *Giardia*), viruses (*Norovirus, Hepatitis A virus, enterovirus, and rotavirus*) and fungi (*Candida, Trichophyton and Aspergillus*) (Venglovsky et al. 2006; Sidhu and Toze 2009), as shown in Fig. 3. Unfortunately, the majority of pathogens remain in the digestate product (Mahmoud et al. 2011), which is not allowed for land applications based on the standards and guidelines. Escherichia coli, Enterococci was accounted for 3.6 and 5.2 log10, respectively, in the digestate product of co-digestion of organic fraction of municipal solid waste, sludge and cattle slurries (Venglovsky et al. 2006).

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waste samples (Dahab and Surampalli 2002; Sahlström et al. 2004; Sidhu and Toze 2009). The presence of those bacterium species in the organic fractions of municipal solid wastes is originally derived from the sludge co-substrate. *Listeria monocytogenes* were detected in the digested sludge and OFMSW (Sidhu and Toze 2009). The digested sludge and OFMSW contained up to 10 and 100 *Cryptosporidium* oocysts and *Giardia* cysts per gram (Macias-Corral et al. 2008).

In summary, after applying the digestate in agriculture as fertilizers, pathogens can transfer from the soil into the plants and then into the animals and humans, causing serious diseases (Subirats et al. 2022). Thus, removing pathogens from the digestate is necessary to avoid health problems.

**Heavy metals**

The anaerobic digestate originally derived from the chemical industry contains considerable quantities of heavy metals. On another side, the accumulation of heavy metals in anaerobic digestate originating from the animal manure is reported due to the routine adding of the microelements to the animal feed to improve the animal productivity. Traces of these microelements, such as iron, nickel, molybdenum, and copper, are essential for some enzymatic reactions as cofactors during the anaerobic digestion process (Chen et al. 2014; Zhang et al. 2015; Yan et al. 2018). The presence of significant amounts of heavy metals in the digestate is a great concern due to its negative impact on soil fertility and crop productivity. Table 1 presents the concentrations of heavy metals in digestate originating from different feedstocks. Heavy metals were detected in the digestate of municipal sludge, organic fraction of municipal solid waste and cattle manure (Dong et al. 2010; Uysal et al. 2010; Tulayakul et al. 2011). Bonetta et al. (2014) found significant quantities of copper, nickel and zinc in the digestate of organic wastes, including sludge, organic fraction of municipal solid waste and manure. The values of copper, zinc, lead, nickel and cadmium exceeded the limits for reuse in agriculture production.

Moreover, the anaerobic digestate could be rich in aluminum, zinc and copper, which will certainly affect the microalgal culture in the subsequent process. The digestate of anaerobic digestion of swine manure contained 394.00 μg/L for zinc, 70.08 μg/L for copper, 35.87 μg/L for nickel, 0.88 μg/L for cadmium, 13.97 μg/L for arsenic and 1.06 μg/L for lead (Zheng et al. 2020). Xu et al., (2019) found that the cadmium concentration in the undiluted digestate was 12.3 μg/L while the maximum accepted level of cadmium was 10 μg/L. This high concentration limits the utilization of the digestate as fertilizers because of cadmium uptake by plants causing hazardous health risks for animals and humans (Table 2). Although the heavy metals

| Table 1 | Concentration of heavy metals (mg/kg) in anaerobic digestate originated from the different feedstock |
|---------|--------------------------------------------------------------------------------------------------|
| Feedstock | Manganese | Zinc | Copper | Nickel | Arsenic | Cadmium | Lead | Chromium | Iron | Cobalt | Mercury |
| Chicken manure | 1131.1 | 900.2 | 135.5 | 12.4 | 0.3 | 4.6 | 25 | | | | | |
| Chicken manure and corn Stover | 540 | 100 | 7.2 | 7.4 | 0.34 | 2.7 | 13 | | | | | |
| Food waste + manure+ slaughterhouse water | 180 | 41 | 11 | 0.37 | 74 | 20 | | | | | |
| Food waste + organic byproducts + industrial waste | 220 | 67 | 15 | 0.37 | 74 | 20 | | | | | |
| Energy grass | 0.137 | 0.016 | 0.016 | 0.0036 | 0.01 | 0.013 | 0.19 | | | | |
| Pig manure | 1.592 | 1.29 | 14.57 | 0.97 | 0.006 | 1.9 | 0.0068 | | | | |

Heavy metals were detected in the feedstock of solid waste. Zinc, Copper, Nickel, Cadmium, Lead, and Chromium was the majority of abundance heavy metals. Pig manure is the most solid waste rich in Zinc and Copper. Chicken manure is the second largest waste containing heavy metals. Food waste contains low quantities of heavy metals derived mainly from fruits and vegetables.
concentrations may inhibit the microalgal growth and affect their ability to treat wastewater, some studies reported the bioremediation capacity of microalgae for heavy metals removal (Chipasa 2003; Xia and Murphy 2016; Sayed et al. 2019).

In summary, the presence of high levels of heavy metals in the anaerobic digestate is a great concern due to its negative impact on soil fertility and crop productivity.

**Volatile fatty acids**

Anaerobic digestate is composed of volatile fatty acids, mainly propionic and butyric acid, which significantly inhibit algal growth (Wang et al. 2019b). More than 5.0 g/L of volatile fatty acids in the growth media significantly inhibit all freshwater microalgae (Turon et al. 2016). The half-maximal effective concentration of propionate and butyrate amounted to 450 mg/L, which affected the digestate treatment (Franke-Whittle et al. 2014). Furthermore, the digestate contains long-chain fatty acids resulting from lipid hydrolysis during anaerobic digestion (Sousa et al. 2013). These long-chain fatty acids were lethal to *Chlorella* (Wu et al. 2006). Food waste and waste cooking oil are rich with lipids and generate high quantities of chain fatty acids in the digestate, causing algal inhibition. A portion of phenolic compounds was determined in the digestate of lignocellulosic wastes (Hecht and Griebl 2009), and algal species were highly inhibited due to the presence of phenolic compounds (Wang et al. 2016).

Moreover, the digestate contained inorganic molecules such as hydrogen sulfide and several volatile organics such as amine and benzene homologues. These toxic volatile organics are responsible for the stinking odors of the digestate (Wang et al. 2013b). These odors compounds may disrupt human respiration, interfere with the physiological functioning of the liver and kidneys, impair judgment and memory, and even cause cancer (Cheng 2009).

In conclusion, the digestate contains long and short-chain fatty acids resulting from organics hydrolysis during the anaerobic digestion process. These fatty acids could be utilized for microbial and algal growth for energy harvesting to minimize the discharge risks of the digestate in the environment.

**Antibiotic resistance genes and antibiotics**

Animal, pig and chicken feed is supplemented with antibiotics for growth promotion and infection resistance (Cui et al. 2016). Huge quantities of animal, pig and chicken manure are generated, containing unwanted antibiotics and their corresponding antibiotic resistance genes. The latter was highly detected in chicken manure (Zhou et al. 2013; Fang et al. 2014), causing human pathogens (Christou et al. 2017). The European Union and the United States prohibited the use of fatal antibiotics to support the growth of livestock (Brown et al. 2017), but the livestock sector still consumes 200,235 tons of antibiotics annually (Van Boeckel et al. 2017). Unfortunately, anaerobic digestion partially removed the antibiotics from the manure by adsorption and entrapment on the anaerobes' biomass (Riaz et al. 2020).

In conclusion, considerable quantities of antibiotics remain in the liquid anaerobic digestate, which should be removed prior to reuse for agricultural purposes.
Emerging pollutants

Digestates have previously been found to include anthropogenic toxins and dangerous chemicals. These toxins may exist in the substrate or be produced through the anaerobic digestion process and affect the function and metabolism activities of microorganisms during the anaerobic digestion process (Ali et al. 2019a). Most solid wastes contain emerging pollutants which are partially removed by the anaerobic digestion process. The anaerobic digestate contains quantities of emerging pollutants that make a barrier to land application (Table 3). Pharmaceuticals (Ibuprofen, Diazepam and Iopromide), detergents (Triclosan), pesticides (m-Cresol), and fragrances (Galaxolide) are partially removed by anaerobes. Linear alkyl benzene sulfonates, synthetic musks, nonylphenols, ampicillin and estrogens (Estrone) are strongly resistant to anaerobic degradation (Mitchell et al. 2013) and are released in the anaerobic digestate causing endocrine disruption in aquatic and terrestrial ecological receptors.

In conclusion, emerging pollutants in the digestate can be transferred to humans directly from air, water and soil, affecting people's health. The anaerobic digestion process partially removed emerging pollutants from wastes. The remaining emerging pollutants should be removed to avoid environmental risk problems.

Turbidity

The high turbidity of digestate product is caused mainly by high suspended maters, which affect the subsequent treatment process. Turbidity is caused by impurities such as clay, silt, finely dispersed inorganic and organic debris, soluble colored organic compounds, and other microorganisms associated with suspended particles or contaminants that interfere with the water clarity. Raw anaerobic digestate has high turbidity, which reduces light penetration and thus hinders algal photosynthesis (Tawfik et al. 2021a). Furthermore, the presence of particles in industrial effluent might lead to flock formation and culture settlement (Wang et al. 2010a, 2019b; Pawar 2016). Filtration (Yang et al. 2015b), precipitation (Serna-García et al. 2021) and centrifugation (Muller et al. 2017) methods were employed for the removal of suspended particles of the digestate to reduce the turbidity and allow the light penetration and algal photosynthesis (Huang et al. 2022).

In conclusion, the turbidity degree is affected by the digestate content. Because of the prevention of light penetration, increased turbidity inhibits microalgae production. The microalgae's photosynthetic process is disrupted.

| Emerging pollutants | Formula | Anaerobic digestion and land application | References |
|----------------------|---------|----------------------------------------|------------|
| Antibiotic (Ampicillin) | C₁₆H₁₉N₃O₄S | Not biodegradable, inhibitory to anaerobic digestion at high concentrations | Mitchell et al. (2013) |
| Medicine (Bezafibrate) | C₁₉H₂₀C₂NO₂ | Moderately biodegraded in anaerobic digestion, unknown end-product | Martín de Vidales et al. (2013) |
| Resins (Bisphenol A) | C₁₅H₁₆O₂ | Moderately biodegraded in anaerobic digestion | Samaras et al. (2014) |
| Plastics (Bis (2-ethylhexyl) Phthalate) | C₂₄H₃₈O₄ | Not assessed in anaerobic digestion, probably biodegradable in soil | Ejlertsson and Svensson (1996) |
| Solvent (Butanone) | C₄H₈O | Not assessed in anaerobic digestion, probably volatile in soil | Itakura et al. (2003) |
| Pesticide (m-Cresol) | C₇H₈O | Not assessed in anaerobic digestion, unknown end-product; inhibited soil ammonia-oxidizing bacteria | Muller et al. (2010) |
| Hormone (Estrone) | C₁₈H₂₂O₂ | Poorly biodegraded in anaerobic digestion, End-product unclear; persistent in soil | Carballa et al. (2007a); Chase et al. (2012) |
| Fragrance (Galaxolide) | C₁₈H₃₈O | Moderately biodegraded in anaerobic digestion, unclear end-product; no crops uptake | Carballa et al. (2007b), Gottschall et al. (2012) |
| Medicine (Naproxen) | C₁₄H₁₄O₃ | Moderately biodegraded in anaerobic digestion | Samaras et al. (2014) |
| Solvent, fuel (Toluene) | C₇H₈ | Completely biodegraded in anaerobic digestion | Enright et al. (2007) |
| Detergent (Triclosan) | C₁₂H₂₄Cl₂O₂ | Moderately biodegraded in anaerobic digestion, unclear end-product; rapidly dissipated in Soil | Lozano et al. (2010), Narumiya et al. (2013) |

Antibiotics are the inhibitor pollutants for anaerobes. Pesticides and detergents are moderately anaerobically biodegraded. The biodegradability of solvents such as butanone could be examined by anaerobic degrading recalcitrant compounds.
Microalgae prefer free ammonia/ammonium ion over other types of nitrogen; however, excessive ammonia levels are likely to impede a variety of algal species. High quantities of total ammonia nitrogen (1000–3000 mg/L) can be found in anaerobic digestate, while concentrations greater than 100 mg/L are harmful to most microalgal strains (Dębowski et al. 2017). Ammonia nitrogen concentration in the digestate affects the next treatment step causing an inhibition of the biological process. Belkin and Boussiba (1991) assumed that 50 mg/L of total ammonia nitrogen is an inhibitory threshold, and 100 mg/L causes toxicity for algal growing species. The inhibitory effect of ammonia depends on the microalgal species. Chen et al. (2014) found that total ammonium nitrogen of 260 mg/L in the digestate was not toxic for the cultivated Scenedesmus sp. Likely, total ammonium nitrogen at 100 mg/L has not suppressed the growth of Scenedesmus sp. (Santos-Ballardo et al. 2016). Total ammonium nitrogen exceeding 500 mg/L caused a drop in the growth of algal cells by a value of 30%. Only 35% of algal growth was recorded at a total ammonium nitrogen concentration of 1000 mg/L, as reported earlier by (Sniffen et al. 2016). The main reason for the total ammonium nitrogen inhibition is the presence of a fraction of free ammonia (NH₃) in the anaerobic digestate.

The free ammonia can pass through the cell membrane of microorganisms, rupture and lyses the cells (Akizuki et al. 2019). A 77% reduction of the algal growth rate occurred for Scenedesmus sp. at increasing the free ammonia nitrogen levels in the digestate from 9 to 34 mg/L (Serna-García et al. 2021). Photosynthesis process rates of Dunaliella tertiolecta and S. obliquus were reduced by 50% at a free ammonia nitrogen concentration of 17 mg/L (Chuka-ogwude et al. 2020). Dilution of the digestate product was one of the methods to mitigate the inhibition effect of free ammonia nitrogen; however, the growth of algal cells was reduced due to insufficient nutrients (Shanthakumar et al. 2018). Furthermore, the consumption of huge quantities of fresh water for the dilution process makes the total process less attractive for application in the industry. Insufficient phosphorus in the digestate product would affect microalgal cultivation (Hirooka et al. 2003; Nagarajan et al. 2019). The nitrogen/phosphorous ratio was optimized for microalgal growth at a level of 7 (Wang et al. 2017). The algal growth was doubled by supplying phosphate into the liquid digestate (Tao et al. 2017b).

In summary, ammonia and phosphorous exceeding the limits in the liquid digestate causes severe problems for land application. However, ammonia and phosphorous could be successfully utilized for algal biomass production.

Microalgal cultivation

Microalgal biomass productivity from treating an anaerobic digestate is a promising approach to economic and environmental costs. The biowaste is utilized in bioenergy and intermediates by-products suitable for the growth of algal biomass. The latter is dually used for the treatment of the digestate and could be utilized for bioenergy productivity (Table 4). The required nutrients for the growth of algal cells for the treatment of anaerobic digestate are presented in Fig. 4 (a and b). Food waste is rich in organic nitrogen, which can be easily mineralized by the anaerobic digestion process into ammonium forms (Free and ionic forms). The digestate from anaerobic digestion of food waste containing inorganic nutrients was employed for microalgal biofuel productivity. The biomass productivity of Dunaliella tertiolecta and Cyanobacterium aponinum was 0.88 and 0.34 g/L/day, respectively. Nutrients were removed by 80–98.99% for total nitrogen and 65% for total phosphorus (Wu et al. 2020). However, microalgal cultivation and biomass productivity was destructively affected at a high loading of the digestate, i.e., the accumulation of organic matters and coarse suspended solid in the biological reaction medium (Zhang et al. 2016). The leachate (digestate) resulted from anaerobic digestion of kitchen waste and was utilized as a nutrient source for Chlorella vulgaris cultivation (Fernandes et al. 2020). Chlorella vulgaris successfully grew for 28 days achieving a growth rate of 0.62 g/d and dry weight of 0.86 g/L. However, culture growth declined beyond 28 days due to heavy metals and ammonium accumulation in the reaction medium. Hu et al. (2021) found that the growth of microalgae with biomass productivity of 441 mg/L.d was feasible in liquid digestate of anaerobic co-digestion of pig manure and filamentous algae. The nutrients (nitrogen and phosphorus) removal rates were 31.4 mg/L.d and 3.0 mg/L.d, respectively, and Chlorella, Scenedesmus, and Desmodesmus were dominant.

The integrated anoxic-aerobic microalgal-bacterial system was employed to treat the digestate of food waste at a hydraulic retention time of 10 days (Torres-Franco et al. 2021). The module achieved removal efficiency of 85–96% for total organic carbon and 85–96% for total nitrogen. Nitrification/denitrification was optimized at digestate fractions of 25% and 50%. The dominated microalgal community was Cryptomonas sp., Pseudoanaabaena sp. and Chlorella vulgaris. Cultivation of microalgae (Ankistrodesmus falcatus var. acicularis) in two identical sequencing batch reactors treating the liquor of anaerobic digestion was assessed by (Wang et al. 2020c). The modules achieved nutrient removal of 96% and dissolved organic carbon 86%. The anaerobic swine digestate
| Anaerobic digestate type | Algal species treatment | Dilution fold and pretreatment | Biomass productivity / concentration | Nitrogen and phosphorus removal efficiency (%) | Dry weight (DW) biomass content | References |
|-------------------------|-------------------------|-------------------------------|------------------------------------|---------------------------------------------|-------------------------------|------------------|
| Poultry litter | *Chlorella sorokiniana*, *Chlorella minutissima*, *Scenedesmus bijuga* | | 71–75 (mg dry weight / L. d) | Total nitrogen (80–100%), total phosphorous (70–75%) | Lipid (3.5–9.6%) | Singh et al. (2011) |
| Dairy manure | *Chlorella sp.* | | | Total nitrogen (76–83%), total phosphorous (63–75%) | Lipid (9.0–13.7%) | Wang et al. (2010a) |
| Vegetable, cow, swine, algae waste | *Scenedesmus sp. AMDD* | | | Total nitrogen (23–100%), total phosphorous (13–99%) | | Bjornsson et al. (2013) |
| Swine manure | *Chlorella sp.* UMN271 | | 0.04–0.08 mg dry weight / L. d | Total nitrogen (22–34%), total phosphorous (23–71%) | Lipid (7.5–10.9%) | Hu et al. (2012) |
| Wastewater sludge | *Chlorella sp.* And microbial consortia | | 30 mg dry weight / L. d | Total nitrogen (89%), TP (17%) | | Yuan et al. (2012) |
| Livestock waste | *Scenedesmus sp.* | | 46–57 mg dry weight / L. d | Total nitrogen (40%), total phosphorous (55%) | | Park et al. (2010) |
| Wastewater sludge | *Nannochloropsis salina* | | 68–92 mg dry weight / L. d | Total nitrogen (87–100%), total phosphorous (98–100%) | Lipid (21–36%) | Cai et al. (2013a) |
| Synechocystis sp. | | | 41.3–150.9 | Total nitrogen (71.2–100%), total phosphorous (99–100%) | Lipid (11.8–13.5%) | Cai et al. (2013b) |
| Dairy manure | *Chlorella sp.* | | 22.8 mg dry weight / L. d | Total nitrogen (72–98%), total phosphorous (55–58%) | Lipid (9.3–10.8%) | Chen et al. (2012) |
| Mixed green algae culture | | | 17.5 mg dry weight / L. d | Total nitrogen (96%), total phosphorous (98%) | Lipid (10–29%) | Woertz et al. (2009) |
| Pig waste | *Spirulina platensis* | | 19.9 mg dry weight / L. d | Total nitrogen (80%), total phosphorous (30%) | Lipid (4.2%) | Franchino et al. (2016) |
| Swine digestate | *Chlorella vulgaris* | | 2.56 g/L | Free ammonia (99.13%) Carbohydrates (991.01 mg/L) | | Ran et al. (2021) |
| Dairy digestate | *Chlorella sp.* | | 1.88 g/L | Free ammonia (100%) | | Wang et al. (2010a) |
| Biogas slurry | *Chlorella vulgaris* | | 1.36 g/L | Free ammonia (29.43%) Carbohydrates (836.40 mg/L) | | Tan et al. (2016a) |
| Anaerobic digestate | *Chlorella sorokiniana* | | 0.506 g/L | Free ammonia (41%) Carbohydrates (113.34 mg/L) | | Singh et al. (2011) |
| Municipal wastewater | *Chlorella vulgaris* | | 1.86 g/L | Carbohydrates (837 mg /L) | | He et al. (2013) |
| Cattle manure digestate | *Chlorella sorokiniana* | | 0.280 g/L | Free ammonia (74.7%) Carbohydrates (57.92 mg /L) | | Kobayashi et al. (2013) |
### Table 4 (continued)

| Anaerobic digestate type | Algal species treatment | Dilution fold and pretreatment | Biomass productivity / concentration | Nitrogen and phosphorous removal efficiency (%) | Dry weight (DW) biomass content | References |
|--------------------------|-------------------------|--------------------------------|-------------------------------------|-----------------------------------------------|--------------------------------|------------|
| Anaerobic digestion      | *Chlorella sp*           | 0                              | 0.41 g/L                            | Free ammonia (63.47%)                        | –                              | Ouyang et al. (2015) |
| Anaerobically digested sludge | *Chlorella pyrenoidosa* | 1.5                            | 2.26 g/L                            | Free ammonia (75.9%)                        | –                              | Tan et al. (2016b) |
| Starch processing wastewater | *Chlorella pyrenoidosa* | Filtration, Sterilization, Mixed with alcohol wastewater | 3.01 g/L | Total nitrogen (91.6%), total phosphorous (90.7%), chemical oxygen demand (75.8%) | –                              | Yang et al. (2015b) |
|                           | *Chlorella pyrenoidosa*  | Precipitation, Filtration      | 2.05 g/L                            | Total nitrogen (83.1%), total phosphorous (97.0%), chemical oxygen demand (66.0%) | –                              | Tan et al. (2014) |
| Swine manure and sewage   | *Chlorella PY-ZU1*       | Centrifugation, Autoclave      | 4.81 g/L                            | Total ammonia (73%), total phosphorous (95%), chemical oxygen demand (79%) | -                              | Cheng et al. (2015) |
| Dairy manure              | *Chlorella sp.*          | Dilution, Filtration           | 1.71 g/L                            | Total nitrogen (82.5%), total ammonia (100%), total phosphorous (74.5%), chemical oxygen demand (38.4%) | –                              | Wang et al. (2010a) |
| Wastewater sludge         | *Chlorella sp.*          | Mixed with wastewater treatment plant effluent | 2.11 g/L | Total nitrogen (83.7%), total phosphorous (94.2%), chemical oxygen demand (86.3%) | –                              | Åkerström et al. (2014) |
| Dairy manure              | *Chlorella vulgaris*     | Dilution                        | -                                  | Total nitrogen (93.6%), total ammonia (100%), total phosphorous (89.2%), chemical oxygen demand (55.4%) | –                              | Wang et al. (2010b) |
| Poultry litter            | *Chlorella minutissima, Chlorella sorokiniana and Scenedesmus bijuga* | Centrifugation, Dilution         | 0.612 g/L                           | Total nitrogen (60%), total phosphorous (80%) | –                              | Hollinshead et al. (2014) |
| Livestock waste           | *Chlorella vulgaris, Scenedesmus obliquus, and Neochloris Oleo-abundans* | Ultraviolet, Filtration          | -                                  | Total nitrogen (76.0%), total phosphorous (63.2%), chemical oxygen demand (63.1%), carbon dioxide (62%) | –                              | Zhao et al. (2015) |
| Anaerobic digestate type | Algal species treatment | Dilution fold and pretreatment | Biomass productivity / concentration | Nitrogen and phosphorous removal efficiency (%) | Dry weight (DW) biomass content | References |
|--------------------------|-------------------------|--------------------------------|------------------------------------|-----------------------------------------------|-------------------------------|-------------|
| Vinasse                  | *Chlorella vulgaris and nitrifying–denitrifying activated sludge* | Dilution 0.6 g/L | - | - | - | Serejo et al. (2015) |
| Cattle slurry and raw cheese whey | *Chlorella vulgaris, Neochloris oleoabundans, and Scenedesmus Obliquus* | Dilution 0.26 g/L.d | total ammonia (99.9%), total phosphorous (97.3%) | - | Franchino et al. (2013) |
| Pig manure               | *Desmodesmus sp.*      | Filtration, Dilution 0.385 g/L | - | - | - | Ji et al. (2015) |
| Municipal Wastewater digestate | *Nannochloropsis Salina* | Mixed with artificial seawater 0.92 g/L | total nitrogen (100%), total phosphorous 100% | - | Scherson et al. (2014) |
| Livestock waste          | *Scenedesmus Obliquus* | Filtration, Autoclave, Dilution 0.311 g/L.d | total nitrogen (74.6%), total phosphorous (88.8%), chemical oxygen demand (75.3%), carbon dioxide (73.8%) | - | Stiles et al. (2018) |

The highest lipids biomass content is recorded for *Nannochloropsis salina* treating the digestate of anaerobic digestion of sludge. *Chlorella vulgaris* treating Swine digestate produces biomass with high content of carbohydrates. *Synechocystis sp.* highly removed total nitrogen and total phosphorous from the digestate of anaerobically pretreated sludge. Pretreatment of the anaerobic digestate improves the algal growth and pollutants removal at high levels.
containing high ammonia concentrations was treated using *Chlorella vulgaris* at a dilution ratio of 1:7 biomass concentration of 1.33 g/L (Ran et al. 2021). The ammonia was removed by 66%, and total phosphorus reached 99%. Microalgae demonstrated a strong potential to bio-absorb heavy metals from the anaerobic digestate (Papadimitriou et al. 2008; Kropat et al. 2015). Toxicity could occur due to metal accumulation in microalgae, which would severely impair cell growth and development (Mehta and Gaur 2005; Al-Rub et al. 2006; Zhou et al. 2012). Magnesium plays a big role in microalgal growth. Magnesium is an important chelating agent in the chlorophyll complex and affects microalgae growth and development (Park et al. 2010). The magnesium deficiency in the anaerobic digestate declined the microalgae growth cultivated on digestate from pig slurry (Bjornsson et al. 2013). The bacteria are important for organic and nitrogen compounds from the digestate. However, the bacteria degrading pollutants compete with algal cells (*Chlorella vulgaris*) for nutrients which decline the growth rate and produce algicidal compounds causing microalgal cell lyses (Mayali and Azam 2004). Bacteria had a high contribution to the re-mineralization of ammonia and improved the bioremediation efficiency of microalgae (Sniffen et al. 2016; Luo et al. 2017; Hu et al. 2019). The subsequent microalgal remediation by *Dunaliella tertiolecta* and *Cyanobacterium aponinum* boosted nitrogen and phosphorus removal from the digestate produced after anaerobic bacterial ammonification of bio-waste. Microagal cultivation treating liquid digestate is sustainable green biotechnology for further biofuel productivity and valorization of biowaste materials.

In conclusion, mixotrophic cultivation is an excellent treatment process for digestate valorization where the bacteria and algae have a high contribution to nutrient removal. The heterotrophic bacteria produce carbon dioxide from the degradation of organics required for algal growth. The algal species are efficient for the reduction of ammonia and phosphorus to lower levels. However, dilution and/or pretreatment of the anaerobic digestate are needed.

**Microalgal cultivation for removing contaminants**

The anaerobic liquid digestate is rich in macro- and micro-nutrients, which play a role in algal growth. However, the high strength of digestate will suppress the growth of algae and reduce their biodegradation efficiency. Therefore pretreatment processes, including dilutions, are recommended to mitigate the inhibition effect of the pollutants on the algal growing process. Dilution of 10–30 fold was ideally utilized to alleviate the inhibition effect of anaerobic digestate (Franchino et al. 2016). Pre-aeration of the digestate efficiently promoted the algal growth and treatment activity on the anaerobic liquid digestate (Wang et al. 2019b).

**Removal of emerging pollutants**

Algae can efficiently bio-sorb emerging pollutants due to their unique chemical composition of the cell wall (cellulose, alginate, chitin and glycan), which provide necessary sites for pollutants sorption (de Wilt et al. 2016). The presence of carboxyl, amine, and phosphoryl functional groups is responsible for the negative charge of the algal cell wall. This charge induces cation biosorption and biodegradation. The extracellular bio-adsorption process mainly depends on chemical structure, hydrophobicity of the pollutants’ functional groups and algal species types (Ghernaout 2014). The major portions of cationic groups of pharmaceutical compounds are removed by bio-adsorption due to the electrostatic interactions (Ayangbenro and Babalola 2017). Bio-accumulation is another removal mechanism of emerging...
pollutants by algae. The n-octanol/water partition coefficient ($K_{ow}$) of the emerging pollutants is the main factor affecting their toxicity and bioaccumulation potential. This highly explained the fate of emerging pollutants inside the algal cells and in the environment (Hermens et al. 2013; Harris and Logan 2014).

Mixotrophic algae are not efficient only for the removal of inorganic and organic pollutants (Pacheco et al. 2020; Reddy et al. 2021) but also are highly removing emerging contaminants (pesticides, nanoparticles, hormones, pharmaceuticals and flame retardants (Tolboom et al. 2019). Emerging organic pollutants are mainly assimilated and/or degraded via phago-trophy and/or osmo-trophy process by algal cells (Subashchandrabose et al. 2013). The mixotrophic algae have a high capability to tolerate extreme environmental circumstances and have the ability to utilize organic contaminants in the liquid digestate as carbon substrate. This ideally makes them excellent candidates for bioremediation of emerging pollutants over autotrophic and heterotrophic microorganisms.

Pharmaceutical compounds are fully removed by monocultures of algal species (Bai and Acharya 2017; Yu et al. 2017), Chlorella sorokiniana highly removed (60–100%) from selected pharmaceuticals (diclofenac, ibuprofen, metoprolol and paracetamol) (de Wilt et al. 2016). Matamoros et al., (2016) found that 99% of tributyl phosphate, galaxolide was removed by Chlorella sp and Scenedesmus sp. via volatilization and 40% of ibuprofen was eliminated by biodegradation. Microalgae highly removed recalcitrant carbamazepine and sulfamethoxazole (Xiong et al. 2016; Bai and Acharya 2017; He et al. 2020). Carbamazepine at an initial concentration of 200 mg/L was removed by 35% and 28% by Chlamydomonas mexicana and Scenedesmus obliquus due to the biodegradation process (Xiong et al. 2016). He et al., (2020) reported that carbamazepine was highly removed compared with the mixture of pharmaceuticals containing carbamazepine, atenolol, ibuprofen and naproxen by Navicula sp. The major emerging pollutants removal mechanisms by microalgal cells, particularly Navicula sp. were due to bioaccumulation for carbamazepine (He et al. 2020). Steroid hormones were highly biodegraded by microalgal cells (Zhang et al. 2014; Hom-Diaz et al. 2015; Sami and Fatma 2019). The removal mechanism of norgestrel and progesterone was due to bio-adsorption, biodegradation and bioaccumulation by Scenedesmus obliquus and Chlorella pyrenoidosa (Zhang et al. 2014). Scenedesmus obliquus biodegraded ‘95% of progesterone and reduced up to 60% Chlorella pyrenoidosa.

The removal of antibiotics clarithromycin, erythromycin, sulfamethoxazole, levofloxacin, ciprofloxacin (Bai and Acharya, 2017), amoxicillin and cefradine was assessed by microalgal species. Microalgal-bacterial consortia cultivated in a stirred-tank photo-bioreactor achieved complete removal of aspirin, 20% of ketoprofen and 80% of paracetamol at a hydraulic retention time of 3–4 days and an initial concentration of 0.5 mM of each drug (Ismail et al. 2017). 68% of 17α-ethinylestradiol was bio-transformed due to the presence of nutrients by Desmodesmus subspicatus within 72 h of cultivation (Maes et al. 2014; Lauritano et al. 2019). Pilot-scale high-rate algal ponds cultured with Chlorella vulgaris removed 90% of caffeine, acetaminophen and ibuprofen (Matamoros et al. 2016; Solé-Bundó et al. 2019a). Anti-inflammatory drugs were highly biodegraded by Chlamydomonas reinhardtii cultured in a pilot-scale photo-bioreactor (Hom-Diaz et al. 2017).

In summary, the cultivation of microalgal-bacterial consortia efficiently removed the emerging pollutants from liquid digestate by adsorption, bio-sorption and bioaccumulation process. However, some of the recalcitrant emerging pollutants need advanced oxidation processing prior the algal cultivation to minimize the toxicity of such contaminants.

### Removal of heavy metals

The mechanism of removal of heavy metals by algae from anaerobic liquid digestate is due to bio sorption and bioaccumulation (Sayed et al. 2019). In microalgae, there was a gradient of selective bio sorption for specific metal ions. Through ion exchange, coordination, complex formation, chelation, and micro-precipitation, carboxyl (-COOH), hydroxyl (-OH), and amidogen (-NH$_2$) in microalgal cell walls and external metabolites (polysaccharide and mucus) might chemically adsorb substantial amounts of metal ions. The adsorption (Passive absorption) is the trapping of heavy metals in the cell wall surfaces and then adsorbed onto the cell wall binding site (Fig. 5). When they enter the cell, heavy metals are exposed to the microalgal metabolism through cell growth. This process is termed active absorption (Xu et al. 2019; Sun et al. 2020). The higher pH of the microalgal growth media induces the hydroxylation of heavy metals to form insoluble metal hydroxides, which can be removed from the digestate (Xu et al. 2019). Moreover, the growth of algal cells required heavy metals (manganese, nickel, copper, molybdenum, iron and zinc as micronutrients. However, exceeding the limits of those heavy metals in the liquid digestate will affect the phycoremediation process negatively.

Other metals (Tin, gold, cadmium, lead, strontium, titanium, and mercury) are not essential for the biological activities of algal cells and cause severe toxicity (Jais et al. 2017). Aluminum and copper strongly inhibited the growth of algae (Wong et al. 1994), which was quite low in the anaerobic digestate. The dilution and filtration of anaerobic liquid digestate can greatly alleviate heavy metals’ toxicity and turbidity (Wang et al. 2019b).
and calcium were removed by *Spirulina sp.* growing in municipal wastewater (Al-Homaidan et al. 2015). *Chlorella minutissima* achieved removal efficiency of 62% for zinc, 84% for manganese, 74% for cadmium and 84% for copper (Yang et al. 2015a). 99% copper and 85% zinc were adsorbed and removed by *Cladophora fracta* (Mahdavi et al. 2012). Pre-aeration of the anaerobic digestate mitigate the inhibition effect of the heavy metals on the treatment process by *Chlorella sorokiniana* (Wang et al. 2019b). Similarly, (Xu et al. 2019) treated the diluted liquid digestate (1:1 with tap water) with *Scenedesmus sp.* The removal efficiency of chromium, lead and cadmium were 50%, 60.7% and 59.7%.

The piggery wastewater generated from anaerobic digestion of pig manure was treated with mono-cultivated microalgae, co-cultivated microalgae with fungi (*Ganoderma lucidum*) and with activated sludge (as a source of nitrifying-denitrifying bacteria). The microalgal strains were *Chlorella vulgaris*, *Scenedesmus obliquus* and *Neochloris oleoabundans*. Under different initial inoculum concentrations of microalgae, fungi and bacteria (C1, C2 and C3 were 62.06 mg/L, 121 mg/L, and 180 mg/L, respectively), zinc removal efficiency was shown in Fig. 6. Although co-cultivation of microalgae with fungi improved the zinc removal efficiency, co-cultivation with bacteria had the highest removal efficiency. *Chlorella sp.* and activated sludge combination had the best removal efficiency of Zinc which was more than 51% (Guo et al. 2020).

In conclusion, biosorption and bioaccumulation are the main heavy metals removal mechanisms by the mixotrophic
carnation process from anaerobic liquid digestate. A portion of heavy metals is utilized by microorganisms as micronutrients.

**Removal of organics**

The organics removal by bacteria is important to produce the required carbon dioxide for algal growth and photosynthesis. However, the anaerobic digestate contains big portions of organics in terms of chemical oxygen demand and total organic carbon, which should be diluted and/or pretreated to enhance the algal growth. Microalgae *Scenedesmus obliquus* (FACHB-31) was cultivated in the diluted liquid digestate of piggery waste attaining chemical oxygen demand levels of 200, 2200, 1600, 1200, 800, and 400 mg/L, respectively. The chemical oxygen demand and carbon dioxide removal were maximized at 61.58–75.29% and 54.26–73.81%, respectively.

**Removal of nutrients**

The anaerobic digestate is rich in nutrients which encourage the cultivation of algal cells for further utilization. Nitrogen and phosphorus are removed and proportionally related to algal growth. 33% and 52% of ammonium removal from the anaerobic digestate of poultry litter was achieved by green algae of *Auxenochlorella protothecoides* and *Chlorella sorokiniana* (Bankston and Higgins 2020). The nitrite and nitrate levels were unaffected and non-indicating the removal of ammonia was due to algal uptake and/or volatilization rather than the nitrification process by nitrifiers (Cai et al. 2013a). Ammonium uptake by algal cells refers to the ability of microorganisms to assimilate the extracellular into intracellular nitrogen that can be easily metabolized and utilized for synthesizing amino acids and other metabolic processes (Wang et al. 2013a). 48% and 52% of the ammonium was up-taken by *Auxenochlorella protothecoides* cultures from anaerobic digestate of poultry litter, and cellular uptake of ammonium by *Chlorella sorokiniana* cultures was highly varied from 52 to 73% (Bankston and Higgins 2020). Moreover, *Auxenochlorella protothecoides* cultures removed phosphate by 30%. *Auxenochlorella protothecoides* were used for nutrient removal from diluted (1:2) agricultural liquid digestate resulting in nitrogen and phosphorus removal of 79.45% and 78.4%, respectively (Krziemińska et al. 2019). *Scenedesmus obliquus* (FACHB-31) cultivation in diluted piggery liquid anaerobic digestate was investigated by (Xu et al. 2015). Total nitrogen and total phosphorous were removed by values of 58.39–74.63 and 70.09–88.79, respectively. *Chlorella zofingiensis* cultivated in the digestate of pig manure resulted in total nitrogen removal efficiency of 68.96–82.70% (Zhao et al. 2013).

In summary, the nutrients in the liquid digestate are efficiently utilized by algal cultivation. However, digestate dilution is required to reduce the organics and ammonia content to the levels acceptable for the metabolism process.

**Valorization of algal cells**

**Fatty acid methyl ester productivity from microalgal cells fed with digestate-rich nutrients**

The microalgal species treating the anaerobic digestate of bio waste are rich in lipids, mainly fatty acid methyl ester (Fig. 7). Volatile fatty acids of the digestate can be consumed by microalgae as a carbon source. Although acetate was the most preferred volatile fatty acid for the microalgae, butyrate has an inhibition effect on this acetate utilization (Turon et al. 2015; Patel et al. 2021). *Cyanobacterium aponinum* treating the anaerobic digestate of food waste contained a high content of unsaturated eicosatrienoic, i.e., 52.11% fatty acids of C20:3n6. *Dunaliella tertiolecta* genera rich in fatty acids 19.45% of saturated stearic C18 and 33.02% of unsaturated docosahexaenoic C22:6n3 (Wu et al. 2020). The lipids productivity from microalgal cells is mainly affected by the phosphorus and nitrogen contents in the feedstock (Breuer et al. 2013). Those polyunsaturated lipids by micro-algal strains can be utilized as feedstock to blend with diesel to form biodiesel achieving better performance in the combustion process.

In summary, volatile fatty acids contents of the anaerobic digestate were consumed by microalgal cells as a carbon source. The microalgal cells can produce and accumulate lipids that may be used for biodiesel production.

**Biogas productivity**

The growth of algal cells is quite high in the anaerobic digestate resulting in biomass-rich proteins, lipids and carbohydrates (Osman et al. 2021b). This biomass could be easily utilized for biogas production via an anaerobic digestion process to close the loop. This approach will create a zero-waste discharge. Valorization of *Scenedesmus obliquus* via anaerobic digestion for biogas productivity was examined by (Adamietz et al. 2019). The methane yield was 213.2 L/kg of volatile solids. A combined macro-algae *Laminaria*-based hydrochar and microalgae *Chlorella*-based hydrochar was anaerobically digested and yielded methane productivity of 222.9 ± 1.6 mL/g chemical oxygen demand for *Chlorella*-based hydrochar and 234.8 ± 9.5 mL/g chemical oxygen demand for *Laminaria*-based hydrochar (Wang et al. 2020a). Methane
generation from algae is quite low and slow due to the poor biodegradability and solubility.

Wang et al., (2019a, b, c) found that 240–530 mg ammonia for 24 h is efficient pretreatment for algal cells to enhance biodegradability and solubility. The methane productivity increased to 140–154 L/kg of total chemical oxygen demand. Energy production from food waste was improved using algae-based microbial fuel cell (Hou et al. 2020). The anodic chamber was algal cells that yielded 2.9 L of methane which doubled the biogas productivity from classical anaerobic digestion. Xie et al., (2019) found that the addition of activated carbon (0.5 g/L) during anaerobic digestion of algae increased the volatile fatty acids productivity from 2026 to 4875 mg/L. The addition of activated carbon increased the hydrolysis and solubilization of algae and enhanced acid-forming bacteria due to the electron transfer among acidogenesis. Methane yield from anaerobic digestion of Scenedesmus dimorphus was 267 mL/g volatile solids and increased to 392 mL/g volatile solids for co-digestion with 75% of sludge (Peng and Colosi 2015; Bohutskyi et al. 2019).

In conclusion, the microalgal cultivation on the anaerobic digestate produces algal biomass. This biomass can be further digested anaerobically to generate biogas. Algal-based microbial fuel cells were used to enhance biogas productivity. Furthermore, pretreatment of the algal biomass with ammonia or the addition of activated carbon increased the biodegradability of the algal biomass and the biogas productivity.

Hydrogen productivity

Dihydrogen (H₂) harvesting from lignocellulosic matrix has gained great attention recently due to a depletion of fossil fuels (Osman et al. 2021c). Algal biomass represents a low-cost substrate for hydrogen productivity as it is rich in protein, carbohydrates and lipids. Co-fermentation of algae and sewage sludge was improved by the addition of 600 mg Fe²⁺/L and produced hydrogen of 28 mL/100 mL (14.8 mL H₂/g volatile solids added) (Yin et al. 2021). Those values were higher than those for hydrogen fermentation of algal biomass only by 2.0 times. The Clostridium sensu stricto, Clostridium tertium and Terrisporobacter were dominant due to the addition of iron in the fermentation medium. Moreover, the hydrogenase enzymes were quite high with iron supplementation. Co-hydrothermal gasification of sludge and macroalgae Kappaphycus alvarezii produced a maximum bio-hydrogen yield of 36.1% for sludge: algae ratio of 2:1 at a temperature of 360 °C (Jayaraman et al. 2021). Nano zero-valent iron (10 mg/g total solids) improved the hydrogen productivity by 29.20% from co-digestion of algae and food waste (Zhao et al. 2020). In conclusion, dihydrogen production from algal biomass represents is cost-effective. Supplementation with iron ions or zero-valent nanoparticles may improve hydrogen productivity.

Biodiesel productivity

The industrial community needs low-cost alternative energy sources from renewables to substitute conventional
fossil fuels. This motivates policymakers and researchers to develop innovative and new research approaches toward non-conventional energy resources with high caloric value. Biodiesel is the most valuable renewable fuel and can be easily produced from microalgae. Microwave-mediated heating proved effective for extracting algal lipids. The lipids extraction efficiencies were 70% and 100% at 220 and 205 °C using conventional and microwave-assisted heating, respectively (Reddy et al. 2014). The required energy for lipid extraction from algal biomass was highly reduced by 2–8 folds microwave-assisted heating compared to the classical solvent extraction. Moreover, sugars, protein-rich algae and omega-3 fatty acids were harvested during the lipids extraction process.

**Bio-oil productivity**

Algal biomass research has gained great attention for bio-oils productivity. Single-step process achieving considerable high valuable products from algal community. Hydrothermal liquefaction is an efficient and promising method for converting algal biomass containing high moisture content into bio-oil and does not consume intensive energy for drying steps. Macroalgae of *Ulva fasciata*, *Enteromorpha sp.* and *Sargassum tenerrimum* were converted by a value of 81% into bio-oil by hydrothermal liquefaction method (280 °C for 15 min) and biomass/water ratio of 1:6 (Singh et al. 2015). Water-soluble fraction, ether soluble fraction (bio-oil), gaseous fraction and solids residue were produced from liquefaction of algal biomass. A higher portion of bio-oil was produced from the liquefaction of *Ulva fasciata* due to the high content of carbohydrates. Furthermore, nuclear magnetic resonance and Fourier-transform infrared spectroscopy spectra showed that bio-oils contained a high percentage of aliphatic functional groups.

**Bioethanol**

The macroalgal cells are preferable for bioethanol productivity due to their easily harvesting and richness with high carbohydrate content. The bioethanol productivity from algal cells is carried out by hydrolysis and fermentation processes. The macroalgal biomass cells produced 0.1 g bioethanol/g algae and 0.12 g bioethanol/g reducing sugars using acid hydrolysis, followed by yeast fermentation with ethanol productivity (36.6 g/L). The ethanol theoretical conversion efficiency amounted to 75% (Alfonsín et al. 2019). Fungal pretreatment of marine algae was employed for bioethanol productivity (Sulfahri et al. 2020). The fungal pretreatment substantially increased sugar yields by 2.3 fold compared to raw algae, and the ethanol yields were improved by 38.23%. Fungal pretreatment increased the enzyme hydrolysis of algae and, subsequently, the fermentation process.

Bioethanol productivity amounted to 1.296 kg/s from algal cells (Martín and Grossmann 2014). Industrial-scale bioethanol productivity (400,000 tons/year) from brown algae was economically assessed by (Fasahati et al. 2015).

**Cosmetics**

Polysaccharides extracted from brown algae (*Saccharina japonica*) are absorbed and retain skin moisture. Likely, the extraction of polysaccharides from *Saccharina japonica* provided better moisturization, indicating that algaed-derived polysaccharides efficiently could be utilized as an additive in cosmetics compared to other algal species (Wang et al. 2015). Red algae (*Chondrus crispus*) is highly rich in polysaccharides and minerals, which have moisturizing, therapeutic and hydrating effects. The extract from green algae, i.e., *Codium tomentosum*, can efficiently regulate water distribution in the body skin avoiding skin dryness. Furthermore, deoxyribonucleic acid extraction from algae can be used to moisturise and protect the skin. The extracts from *Polysiphonia lanosa*, *Undaria pinnatifida*, *Ascophyllum nodosum*, *Cladosiphon okamuranus*, *Durvillaea antarctica*, and *Pediastrum duplex* is abundant and widely used for skin moisturization and protection. Overall, the extract from macroalgal cells (*Chlorella vulgaris*) is promising biomass for use in skin care products, where it supports skin tissue and boosts the synthesis of collagen and thereby reducing wrinkles.

**Economy**

The anaerobic digestion technology provides cost-effective solutions to the energy demand and is considered an eco-friendly method of waste valorization; however, the generating digestate byproducts still need sustainable technologies for good management and transformation into value-added products to meet the circular economy principal (Ran et al. 2022; Lamolinara et al. 2022). Kaza et al. (2018) assumed that the anaerobic digestion of one-ton feedstock produces 850 to 900 kg digestate. Microalgae culture has been identified as a possible solution for nutrient recovery from digestate. Microalgae cultivation combined with anaerobic digestate management has lately gotten much interest because of its advantages in lowering the cost of precious resources like fertilizer and fresh water while also encouraging sustainable biofuels generation (Chong et al. 2022).

As shown in Fig. 8, the liquid fraction of the digestate can be used as media for microalgal cultivation due to its nutrient composition required for microalgal growth. Photo-bioreactors can maintain the light intensity and all optimum conditions required for microalgal cultivation and high biomass productivity (Nagarajan et al. 2019). The
bioconversion of the microalgal biomass into energy products such as biodiesel, bioethanol, bio-hydrogen and bio-oil, in addition to several value-added products such as animal feed, bio-fertilizers, bio-plastic, cosmetics and pharmaceutical products (Levine et al. 2011; Koutra et al. 2018; Chukagwude et al. 2020; Qasim et al. 2021). Microalgal cultivation can emphasize the Zero waste principle and direct the economy into a circular economy instead of a linear one (De Bhowmick et al. 2019). After extraction of its lipid contents, the microalgal biomass residue can be recycled and digested or co-digested for more biogas productivity (González-González et al. 2018). Microalgal cultivation can increase the efficiency of the biogas production technology through biogas upgrading, and microalgae have the ability to capture the carbon dioxide contents of the biogas, increasing the biomethane purity (Nagarajan et al. 2019; Osman et al. 2021a).

In another word, the integration between anaerobic digestion and microalgal cultivation technologies could maximize the revenues and minimize the environmental impacts (Chong et al. 2022).

Various economic analyses of the treatment and valorization of anaerobic digestate employing microalgae have reached some similar findings. Techno-economic analysis of microalgae cultivation on the anaerobic digestate demonstrates that if done correctly, the technique can be economically viable. In large-scale microalgae farming, daily algal output (productivity), pond area, nutrient-depleted water for recycling, total capital costs, total operational expenses, and algae production cost are all key considerations (Chukagwude et al. 2020; Gengiah et al. 2022).

Total capital costs refer to the fixed investment capital that is amortized over 15 years. This cost does not cover taxes, interest, or land upkeep, which are not amortized because they do not diminish with time or usage, while each process' total operational expenses include labor, energy, raw materials, utilities, and wastewater treatment, and consumables. Maintenance, operational supplies, contingencies, and overheads were also addressed in total operational expenses (Vázquez-Romero et al. 2022).

In conclusion, microalgal cultivation can integrate the anaerobic digestion technology. Not only for valorizing the recalcitrant generated digestate but also for bioenergy and value-added products production. Economically, microalgal cultivation decreases the processing cost of the anaerobic digestate and maximizes the processing revenue.

**Conclusion**

The anaerobic digestion technology provides cost-effective solutions to the energy demand and is considered an eco-friendly method of waste valorization; however, the generating digestate byproducts still need sustainable technologies for good management and transformation into value-added products to meet the circular economy principal. The anaerobic digestion fed with one-ton feedstock produces 900 to 850 kg digestate. Microalgae culture is a promising approach for nutrient recovery from digestate. Furthermore, microalgal cultivation combined with anaerobic digestate management highly reduces the cost of precious resources like fertilizer and fresh water while also encouraging sustainable biofuels generation. Photo-bioreactors can be employed in full-scale applications for microalgal cultivation and high biomass productivity that are further used for bioenergy.
and biofertilizers harvesting. The bioconversion of the microalgal biomass into energy products such as biodiesel, bioethanol, biohydrogen and bio-oil are value-added products, i.e., animal feed, bio-fertilizers, bio-plastic, cosmetics and pharmaceutical products. Microalgal cultivation can emphasize the zero waste principal and direct the economy into a circular economy instead of a linear one. Furthermore, microalgal cultivation can capture carbon dioxide to mitigate greenhouse gas emissions. In other words, integrating anaerobic digestion and microalgal cultivation technologies could maximize revenues and minimize environmental impacts.

Acknowledgements The first author acknowledged the Science Technology Innovation Funding Agency (STIFA) –Egypt (Project ID: 41591) for fully financially supporting this research and bilateral research project between academy of Scientific Research and Technology (ASRT)-Egypt and National Natural Science Foundation of China. The first author is grateful to the National Research Centre-Egypt for fully financially supporting this research and bilateral project (Project ID VA5048). The Bryden Centre project is supported by the European Union’s INTERREG VA Programme, managed by the Special EU Programmes Body (SEUPB).

Funding This work was funded by STIFA and SEUPB.

Declarations

Conflict of interest The authors declare no conflict of interest.

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