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Anaerobic digestate as a low-cost nutrient source for sustainable microalgae cultivation: A way forward through waste valorization approach

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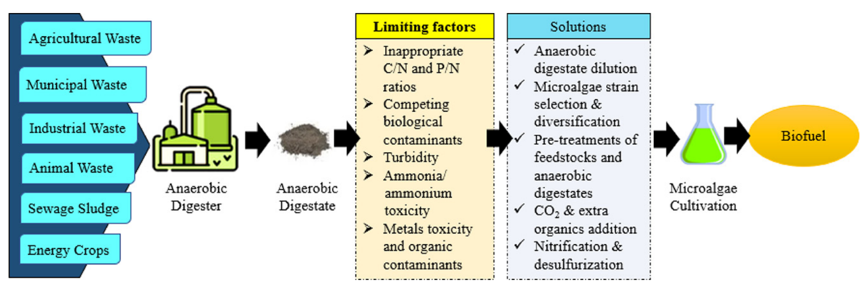
HIGHLIGHTS

- Microalgae is a sustainable source for future biofuel production.
- Digestate from anaerobic digestions is an alternative nutrient to grow microalgae.
- The effect of digestate properties towards the growth of microalgae are discussed.
- Potential ways to enhance the microalgae growth using digestate are recommended.

GRAPHICAL ABSTRACT



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ABSTRACT

To suffice the escalating global energy demand, microalgae are deemed as high potential surrogate feedstocks for liquid fuels. The major encumbrance for the commercialization of microalgae cultivation is due to the high costs of nutrients such as carbon, phosphorous, and nitrogen. Meanwhile, the organic-rich anaerobic digestate which is difficult to be purified by conventional techniques is appropriate to be used as a low-cost nutrient source for the economic viability and sustainability of microalgae production. This option is also beneficial in terms of reutilize the organic fraction of solid waste instead of discarded as zero-value waste. Anaerobic digestate is the side product of biogas production during anaerobic digestion process, where optimum nutrients are needed to satisfy the physiological needs to grow microalgae. Besides, the turbidity, competing biological contaminants, ammonia and metal toxicity of the digestate are also potentially contributing to the inhibition of microalgae growth. Thus, this review is aimed to explicate the feasibility of utilizing the anaerobic digestate to cultivate microalgae by evaluating their potential challenges and solutions. The proposed potential solutions (digestate dilution and pretreatment, microalgae strain selection, extra organics addition, nitrification and desulfurization) corresponding to the state-of-the-art challenges are applicable as future directions of the research.

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Contents

1. Introduction

Today, the solid organic waste accumulation has raised public health apprehensions and environmental consciousness, therefore sustainable management is needed to maintain the overall equilibrium in the ecosystem and to reduce environmental burdens. The organic fraction of solid waste has been recognized as promising anaerobic digestion feedstock for biogas production as this valuable resource can be transmuted into functional products through various microbial transformations (Lesteur et al., 2010). In the absence of oxygen, organic biomass spontaneously decomposes by anaerobic microorganisms during anaerobic digestion to produce biogas, digested water and solid digested water (slurry) (Abomohra et al., 2020). Apparently, various types of anaerobic digestate arose from different solid organic wastes and animal by-products, such as animal manure, food waste, agricultural residues, municipal solid waste, and other feedstocks have been documented in published literature. Digestate is the residual organic material produced as the co-product of biogas; its managements are highly dependent on the quality, characteristics of the feedstocks and digester operating conditions. Some of the digestate induces environmental problems such as intensive transportation needs, greenhouse gases emission during storage, and nutrients leaching during land application (Baral et al., 2018). With respect to this, a critical bottleneck has been faced by digestate management and different digestate valorization routes have been proposed such as composting or pyrolysis for the bio-oil, syngas, and biochar production. Besides, the high nutrient and largely stabilized digestate can also be adopted as bio-fertilizer, soil conditioner (Duan et al., 2020), and the primary nutrients required for microalgal growth (Jimenez et al., 2020).

Microalgae cultivation is recognized as one of the promising solutions for the nutrient recovery in the digestate. Due to its advantages in minimizing the cost to input scarce resources such as fertilizer, freshwater and promoting sustainable biofuels production, microalgae cultivation coupled with anaerobic digestate management has attracted immense attention recently. As aquatic eukaryotic photosynthetic microorganisms, microalgae are capable of generating biomass via photosynthesis for the development of biochemicals and biofuels by utilizing digestive nutrients and harvesting solar energy (Abu Hajar et al., 2016). There are many benefits of cultivating microalgae such as it has a large amount of storable lipids and CO_2 absorption (Zuliani et al., 2016), high growth rate, high photosynthetic efficiency, no competition with food, arable land, and edible feedstocks (Chen et al., 2018). Additionally, the microalgae-derived biofuel is renewable and sustainable with low CO and no SO_x emissions (Abu Hajar et al., 2016), therefore the high dependence on fossil fuels can be reduced gradually. In addition to light and CO_2, the availability of nutrients along with various microelements is a key factor behind the high cost of biomass derived from algae, thus restricting the industrial cultivation of these species (Zuliani et al., 2016). Therein, the appropriate utilization of the nutrient content in the anaerobic digestate may resolve the high-cost issue for microalgae cultivation.

Nevertheless, the usage of the anaerobic digestate to grow microalgae possesses several barriers including the inappropriate concentrations of nutrients, high turbidity, presence of competing biological contaminants, ammonia toxicity, and metal toxicity. Depending on the origins of the waste, anaerobic digestate can be especially toxic and pernicious to microalgae when the culture strains have not previously been exposed to such conditions. By predicting ammonia inhibition and nutrient deficiency, the carbon to nitrogen (C/N) ratio of the anaerobic digestate is a significant parameter where the ideal C/N ratio for algal growth ranges from 4 to 8 (Ward et al., 2014). The existence of the biological contaminants in the anaerobic digestate such as bacteria, zooplankton, pathogens, viruses, and foreign algae that may potentially establish ecological competition with the microalgae (Xia and Murphy, 2016). High turbidity of the anaerobic digestate caused by high dissolved, ammonia content, and suspended materials can inhibit the algal growth by obstructing the light transmission through the water (Marcilhac et al., 2014). The high concentration of metal concentration in digestate, especially when it is mixed with industrial effluents may also cause toxic effects for microalgae cultivation.

Several potential solutions have been proposed to combat the challenges stated above for optimizing the utilization of anaerobic digestate in microalgae cultivation. Diluting the anaerobic digestate may become necessary before microalgae inoculation to avoid inhibition from excessive amounts of nutrients and inhibitors. It is also important to select microalgae strains that can withstand digestate's toxic nutrient concentrations to optimize the algae's light usage and photosynthetic efficiencies (Wang et al., 2013). Pre-treatment methods such as filtration, autoclaving, hydrogen peroxide (H_2O_2) oxidation, dewatering, supernatant extraction, chemical, thermobaric, and thermochemical treatment, etc. can be applied to digestate prior to microalgae cultivation for reducing the suspended solids concentration and prevent intrusion from other inhibitors (Abu Hajar et al., 2016). Extra organic carbon and inorganic carbon can be supplied to low carbon digestate. Nitrification technology can stabilize the nitrogen and reduce ammonium toxicity of the digestate by reducing the concentrations of organic compounds and improving phosphorus to nitrogen (P/N) ratio. Owing to the dearth of reports on the feasibility of using anaerobic digestate as an alternative low-cost nutrient for microalgae cultivation, this review article aims to disclose a perspective on the recent trends of microalgae grown on the digestate. The challenges, common endeavours to improve the mentioned problems, and potential solutions correlated with such a process are discussed in the following sections.

2. Anaerobic digestate

2.1. Sources of feedstocks

Anaerobic digestate is known as the prevalent residual organic matter produced as side product of biogas production during the anaerobic digestion process of organic matter. During the anaerobic digestion process, anaerobic micro-organisms are prone to decompose short chain hydrocarbons, such as sugars than the longer chain hydrocarbons, such as hemicelluloses and celluloses (Cheng et al., 2021a). Meanwhile, the feedstocks that consist of high amount of lignin (long-chain hydrocarbon) such as woody materials are hardly to be decomposed by anaerobic micro-organisms. Therefore, the feedstocks of these digestates are ranged from manures (pig, cow, horse, poultry, etc), sewage sludges, agricultural crops (corn, millet, white sweet clover, maize, etc), household waste, industrial wastes (food/beverage processing, dairy, starch, sugar, slaughterhouse, sugar industry waste, etc), municipal solid waste (coffee and tea filters, food waste, fruit and vegetable waste, other organic leftovers, etc) and many others as shown in Fig. 1. For anaerobic digestion, feedstocks with <60% dry matter organic content are not promising; thus, 70%-95% is suggested to be the ideal organic content of the feedstocks. The most conventional anaerobic digestion feedstocks are animal manure and sewage sludge; sewage sludge is often not being considered as high-quality substrate as they are not favourable for biogas production. In this regard, industrial, municipal, and household solid wastes have grabbed considerable attention as alternative feedstocks that can contribute to high biogas production. These feedstocks require appropriate pre-treatments prior to the anaerobic digestion, owing to their possible presence of toxicity and contaminants. Instead of disposing the wastes to landfilling, the introduction of novel pre-treatment technologies can eventually recover the energy from these wastes and reutilize them. The ideal nutrient ratio, C/N ratio and carbon, nitrogen and phosphorus (C/N/P) ratio for anaerobic digestion is recommended to be from 20:1–30:1 and 100:5:1, respectively (Bedoic et al., 2019). The moisture content of feedstocks is also crucial for the practicability of the anaerobic digestion, where the optimum total solid concentration is proposed in the range of 6–10% (Steffen et al., 1998). Too high solid content in the feedstocks leads to the mixing, solid settling, and clogging issues, whilst high water content in the feedstocks require high processing cost to supply higher heat input and higher digester volume.

Due to the majority of the energy contents of animal manure and sewage sludge are consumed after the digestion process; therefore, recent advances have been shifted towards the intermixing and digesting together the other feedstocks with them in order to enhance the biogas production. This process is recognized as co-digestion, where two or more feedstocks are blended and digested at the same time in an anaerobic digester. The selections of the co-digestion feedstocks have to fulfil the requirements of presence of high amount of micro/macro nutrients, easily biodegradable, high potential of biogas production, absence of any limiting factors for the process, easily available, and reasonable price. Apart from primary feedstock of manures or sewage sludges, one or more types of energy-rich feedstocks such as food processing waste, organic fraction of household waste/municipal solid waste, agricultural crops, slaughterhouse waste, fats, oils and grease, etc. can be added during co-digestion. Among these energy-rich co-feedstocks, organic fraction of the municipal waste and food processing waste are more promising owing to their high energy recovery potential and biogas production. Food waste with greater biodegradability has three times the production capacity of methane (CH_4) than biosolids, but only a small amount of final sludge is produced. The co-digestion of these waste feedstocks in an anaerobic digester instead of landfilling can reduce CH_4 emission to the atmosphere, but approximately three times higher CH_4 production can be achieved when these wastes are anaerobically digested. Generally, anaerobic digestion of these wastes can maximize nutrient recycling, recover valuable energy, and enhance renewable energy generation. The high organics content of

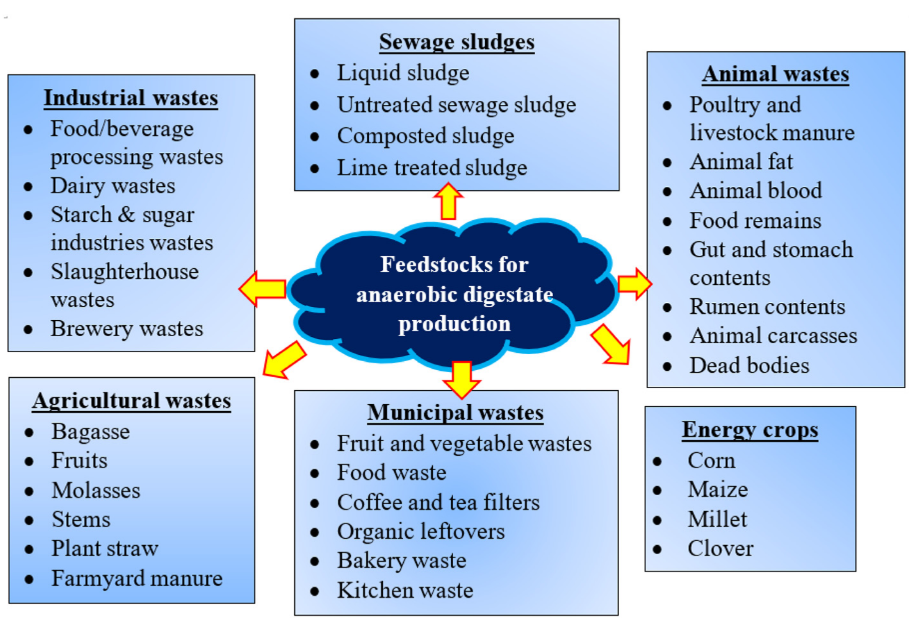

Fig. 1. Classification of feedstocks for anaerobic digestate production.

anaerobic digestates can also be alternative low-cost nutrient sources for microalgal growth to partially replace the conventionally used CO_2, nitrogen and phosphorous. Zuliani et al. (2016) cultivated *Chlorella vulgaris* microalgal strains by using anaerobic digestates collected from sewage sludge, municipal wastewater, and agro-waste treatment plants as nutrient sources. The outcomes of the study reported on the 300% increment in the lipid production and an intense increase in carotenoid accumulation per volume in *Chlorella vulgaris* cultures has been achieved when using anaerobic digestates from sewage sludge treatment and agro-waste. The findings here showed the possibility of enhancing the biomass accumulation or the lipids development by using various sources of anaerobic digestates.

To conclude, the feedstock type is not the sole factor that affecting the type and even the characteristics of anaerobic digestates since the anaerobic digestion is a complex biological process that is highly dependent on several process parameters, such as pH (with/without periodical/continuous pH control), temperature (mesophilic and thermophilic), microbes (mesophilic/thermophilic strain, indigenous/foreign microbes, genetic engineered/non-genetic engineered strain, and isolated strain or mixed consortium), pre-process acclimatization (supplementation of various organic/inorganic nutrients), the extent of anaerobic condition (N_2 purging prior to or after the microbial inoculation, facultative or strict anaerobic condition), and etc.

2.2. Types of anaerobic digestates

Anaerobic digestion of organic matter consists mainly of four phases: enzymatic hydrolysis (break down of large polymers to smaller molecules), acidogenesis (acid formation), acetogenesis (acetic acid production), and methanogenesis (CH_4 production). However, in the context of anaerobic digestion, most researchers preferred to highlight both the acidogenic (for production of volatile fatty acids) and methanogenic (for biogas production) stages. Digestate is produced both by acidogenesis and methanogenesis and each has different characteristics. The acidogenesis and methanogenesis processes differ in terms of liquid separation and in the way the microorganisms were retained in the reactors. As the acidogenesis is the early stage of anaerobic digestion, most of the organics have not been fully degraded and mainly retained in the solid phase, hence acidogenic digestate is commonly referred as the solid digestate. Meanwhile, the methanogenesis is the final stage of anaerobic digestion, most of the organics have been broken down and degraded, so the methanogenic digestate is referred as liquid digestate. Thus, there are generally three types of anaerobic digestate: fibrous digestate (solid fraction), liquor digestate (liquid fraction), or whole digestate (combination of the two fractions in sludge form) as shown in Fig. 2 and their respective characteristics have been tabulated in Table 1 (Peng and Pivato, 2019). The solid fraction can be separated from the whole digestate sludge with <15% dry matter by using screw-press separators, slope screens, or rotary drum thickeners. As compared, the fibrous acidogenic digestate is relatively stable, having high moisture content, low nutrient content, consists of a high amount of lignin, cellulose-rich organic material, a small amount of phosphorus, and some mineral components. The solid acidogenic digestate also contains dry matter content ˃15% and retains most of the digestate phosphorus. This type of digestate is very suitable to be used as feedstock for ethanol production or low-grade building products (Cesaro and Belgiorno, 2015). The liquid methanogenic digestate is rich in nutrients such as ammoniums and potassium, which has been always used as fertilizer and possess high potential as a low-cost nutrient source for algal growth. The liquid methanogenic digestate also composes of 90% of the volume digestate, particles <1.2 mm in size, 2%–6% of dry matter, and preserves most of the soluble nitrogen and potassium. The nutrients are divided between solid and liquid fractions for the whole digestate, where the liquid part contains 70%-80% of nitrogen while the remaining content solid part contains 55%–65% of phosphorous content (Makádi et al., 2012). It is also estimated that the remaining 20%–30% of the total NH_4^+-N are distributed in solid fraction and the remaining total phosphorus (35%–45%) is found in the liquid fraction (Peng and Pivato, 2019). Overall, the nutrients in the anaerobic digestate such as nitrogen, phosphorous, and

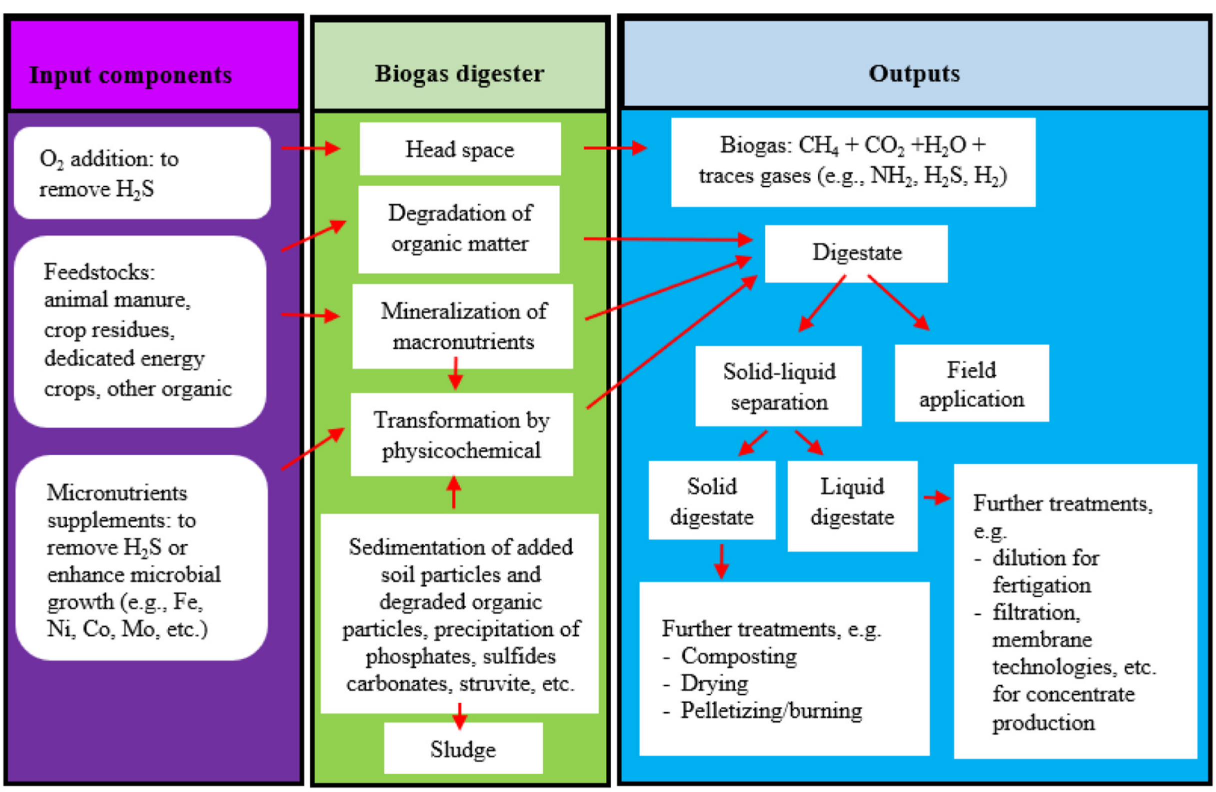

Fig. 2. Overview of the processes flow during the anaerobic digestion (Möller and Müller, 2012).

Table 1
Types of anaerobic digestate and their properties.

Types of anaerobic digestate	Acidogenic digestate	Methanogenic digestate	Whole digestate
Physical state	Fibrous digestate (solid fraction)	Liquor digestate (liquid fraction)	A sludge-based combination of the two fractions
Nutrient level	Low	High	High
Total solids	High	Low	Medium
Dry matter	>15%	2–6%	<15%
Lignin, cellulose-rich organic material	High	Low	Low
Major constituent	Phosphorus	Nitrogen and potassium	Liquid: 70%–80% nitrogen; solid: 55%–65% phosphorous

potassium are conserved and transformed to a more organic form during the anaerobic digestion. This shows that the total mass of nutrients entering and leaving the digester are the same. There are two ways that nitrogen reaches the digester, which are ammonium and organic nitrogen. The amount of ammonium in the anaerobic digester is comparatively higher than the substrate feed due to the conversion of organic nitrogen to ammonium during protein degradation. Meanwhile, some phosphorous is converted to soluble orthophosphorous inside the digester (Logan and Visvanathan, 2019). It is of particular interest that anaerobic digestion treatment methods minimize the loss of nutrients through volatilization and achieve more than 2–3 log pathogen reduction in the digestate.

2.3. Characteristic of anaerobic digestate

The chemical and biochemical characteristics of the anaerobic digestate are subjected to the characteristics of the feedstocks (carbon-nitrogen ratio (C/N), pH, chemical composition, particle size), digestion process parameters (hydraulic retention time, inoculum, temperature, organic loading rate, microbial community, etc.), technology process (wet/dry process, stripping, etc.), treatment options (pre- and post-digestion), and configuration of anaerobic digestion system (mesophilic vs. thermophilic temperature conditions, batch vs. continuous process mode,). The characteristics of the anaerobic digestate from different sources have been tabulated in Table 2. Generally, the anaerobic digestate should exhibit well-balanced nutrients, such as optimal C/N ratio to attain physiological requirements for microalgae growth. Neither too high nor too low C/N are favourable to grow microalgae as excessive C/N supply will cause restriction effect on the growth of microalgae, while low C/N will lead to ammonia inhibition (Gao et al., 2019). By decomposing readily degradable carbon compounds in the digesters, the quantities of carbon content and organic dry matter of the digestate are decreased. The high amount of mineral nitrogen present in the digestate in the form of ammonium which is favourable for plants and microalgae.

The use of anaerobic digestates as a nutrients source in microalgae growth can save non-renewable sources of energy for more sustainable production but needs further treatment to improve its properties. Digestates within the C/N range of 15–20 are considered safe without further treatment for agricultural land application (Zeshan and Visvanathan, 2014). The anaerobic digestate contains a high number of macronutrients and micronutrients. The chemical oxygen demand (COD), biological oxygen demand (BOD), and suspended solids (SS) of anaerobic digestate from anaerobic ponds, upflow anaerobic sludge blanket (UASB), Imhoff tank and septic tanks treating sewage have been reported to differ from 60 to 150; 100–200, and 50–100 mg/L, respectively (Chernicharo, 2006; Foresti et al., 2006). COD concentration in the liquid digestate which includes volatile fatty acids such as acetate is in the range 210–6900 mg/L (Xia and Murphy, 2016). In anaerobic sewage treatment systems, as organic phosphorous and nitrogen hydrolysed to phosphate and ammonia, respectively, insignificant or negligible nutrient removal can be anticipated (Moawad et al., 2009). The concentration of these nutrients increases in the liquid phase as they are not removed by anaerobic processes. Ammonia nitrogen and phosphorus concentrations were reported to be 30–50 and 10–17 mg/L, respectively, in anaerobically treated sewage (Foresti et al., 2006).

Anaerobic digestates based on food waste produce comparable levels of total ammonia nitrogen with digestate of cattle and piggery slurry but greater total ammonia nitrogen content than anaerobic digestate of wastewater sludge and dairy waste. The total ammonia nitrogen and nitrogen to phosphorus (N/P) ratio in majority of the food wastes-based anaerobic digestates are higher than other digestates. The pH of the anaerobic digestate typically varies from 6.7–8.4 due to the processing of ammonia and the degradation of volatile fatty acids (Tampio et al., 2016). This alkaline pH of digestate is beneficial to combat with the worldwide problem of the soil acidification. For freshwater microalgae and alkaliphilic microalgae cultivations, the ideal pH range are 6–8 and 8.5–10, respectively (Zhu, 2015; Markou and Georgakakis, 2011). The NH_4 content of the digestate is approximately 60–80% to its total N content. Generally, the concentration of NH_4-N is rich in the feedstocks such as diary by-products, kitchen food wastes and slaughterhouse waste (Menardo et al., 2011). The pH and NH_4^+-N of the digestate through anaerobic digestion not only affects the solubility of phosphate (PO_4^{3-}), but also increases the digestate's polluting capacity during storage and land distribution (Möller et al., 2008). High pH value of >10 causes precipitate PO_4^{3-} into magnesium or calcium phosphates ($Mg_3(PO_4)_2$ or $Ca_3(PO_4)_2$); low pH value increases the solubility of phosphates.

3. Limiting factors of using anaerobic digestate for microalgae growth

The following are the possible limiting factors for the cultivation of microalgae when using anaerobic digestate as an alternative nutrient source.

3.1. Inappropriate C/N and P/N ratios

Anaerobic digestate is rich in numerous types of bioactive substances such as monosaccharides, vitamins, fulvic acid, phytohormones, free amino acids, nucleic acids, etc. that stimulate microalgae growth and boost the tolerance to abiotic and biotic stress. Besides, microalgae as photosynthetic organisms also assimilate phosphorus (P) and nitrogen (N) in the digestate during their growth, while their biomass produced can be transformed after proper processing into additional raw materials or energy (Ometto et al., 2014). Therefore, the C/N ratio of the anaerobic digestate can be useful tools for accessing the nutrient scarcity by relating to the relationship amidst the amount of carbon and nitrogen (Hartmann et al., 2005), as it will affect the microalgae growth and the quality of product in terms of the available nutrients and stabilization degree (Chang and Hsu, 2008). The C/N ratio has been known to have a linearly influence on the production of biomass in algal cultures (Sutherland et al., 2015), where higher C/N ratios yielding higher biomass. The rapid absorption of nitrogen by methanogens refers to a high C/N ratio. It was found that anaerobic digestate effluent has low C/N ratios of 1.53 (Shefali and Themelis, 2002), whereas the C/N ratios of 4–8 are optimal for algal growth (Ward et al., 2014). A low C/N ratio refers to the ammonia accumulation (free inhibitory ammonia dominates than ammonium ion (NH_4)) and an increase in pH values to >8.5 is very detrimental to methanogenic bacteria (Shefali and Themelis, 2002). The bacteria can adapt to this high ammonia concentration only if it is given sufficient time for the gradual

Table 2
Characteristics of anaerobic digestate from different sources.

Source	Type of digestion process	Reactor[a]	pH of the digestate	Total-N (g/L)	Carbon content (%)	NH_4-N (g/L)	Total-P (g/L)	Total-S (g/L)	Total-K (g/L)	TS (g/L)	VS (g/L)	C/N ratio	Ref.
Swine manure	Mesophilic	–	8.1	2.93	–	2.23	0.93	–	1.37	–	–	–	Loria et al. (2007)
Wet hydrolyzed dissolved air flotation sludge and stockyard waste	Mesophilic	UASB	7.46	3.61	29.66	–	–	0.795	–	–	–	8.23	Okoro et al. (2018)
Calf manure and food waste (corn silage and olive oil waste)	–	–	8.3	–	–	1.06	0.46	–	–			–	Siciliano and Rosa (2014)
Palm oil mill effluent	–	–	7.64	–	–	0.342	0.242	–	–			–	Zafisah et al. (2018)
Municipal waste activated sludge	Mesophilic	CSTR	7.2	0.8	–	0.543	0.208	–	–	6.4	3.8	–	Leite et al. (2017)
Swine manure, wasted fruits and vegetables	Mesophilic	Bioreactor	7.62	10.8	24.88	5.48	0.54	0.10	–	–	–	–	(Simeonov et al., 2012)
Municipal waste	Thermophilic	Sequential batch reactors	8.05	15.9 g /100 g TS	–	–	0.233	0.952	–	–	–	–	Botheju et al. (2010)
Food and vegetable waste	–	–	8.42	10.1	–	–	3.99 g/kg	–	4.46 g/kg	–	–	18.7	Krishnasamy et al. (2014)
Food waste	Thermophilic	–	–	2.43				0.49		18.5	73.1	17.6	Svensson et al. (2018)
Domestic wastewater sludge	Mesophilic	–	–	4.70	–	–	–	1.95	–	21.8	68.4	7.0	Svensson et al. (2018)
Hygenized food waste	Mesophilic	CSTR	3.9	–	–	0.388	0.477	–	–	13.4	90	–	Svensson et al. (2018)
Hygenized sludge	Mesophilic	CSTR	6.0	–	–	0.363	0.781	–	–	6.9	79	–	Svensson et al. (2018)
Swine manure	Mesophilic	CSTR	8.1	5.3	–	3.9	–	–	–	54 g/kg	37.6 g/kg	–	Timmerman et al. (2015)
Dairy cattle manure	Mesophilic	CSTR	7.8	3.5	–	2.0	–	–	–	53.2 g/kg	40 g/kg	–	Timmerman et al. (2015)
Energetic crops, cow slurry, agro-industrial waste, and the organic fraction of municipal solid waste	Thermophilic	CSTR	8.3	110 g/kg	–	2.427	11.79 g/kg	–	–	36 g/kg	684 g/kg	3.43	Tambone et al. (2009)
Energy crops, cow manure slurry and agro-industrial waste	Thermophilic	CSTR	8.7	105 g/kg	–	2.499	10.92 g/kg	–	–	–	–	–	Tambone et al. (2009)
Liquid cattle slurry	Mesophilic	–	7.77	4.27	–	52.9	0.66	–	4.71	–	–	8.4	Möller and Stinner (2009)
Animal wastes (whole digestate)	Mesophilic	CSTR	7.18	3.075	42.35	–	–	–	–	6.01	77.63	–	Zhong et al. (2016)
Animal wastes (liquid digestate)	Mesophilic	CSTR	7.56	1.90	–	–	–	–	–	1.83	–	–	(Zhong et al., 2016)
Animal wastes (solid digestate)	Mesophilic	CSTR	–	–	41.97	–	–	–	–	30.60	89.18	–	Zhong et al. (2016)
Cow dung	Mesophilic	Batch reactor	–	–	–	–	–	–	–	77.83%	40.86%	13.02	Chomini et al. (2015)
Poultry manure	Mesophilic	Batch reactor	–	–	–	–	–	–	–	84.49%	39.05%	12.45	Chomini et al. (2015)
Cow dung + poultry manure	Mesophilic	Batch reactor	–	–	–	–	–	–	–	71.82%	26.79%	7.93	Chomini et al. (2015)
Dairy wastewater	Mesophilic	CPBR	6.9	0.46	–	0.41	0.031	–	–	–	–	–	Debowski et al. (2017)
Municipal wastewater sludge	Mesophilic	CPBR	7.0	0.596	–	0.47	0.029	–	–	–	–	–	Debowski et al. (2017)
Maize silage and swine slurry	Mesophilic	CPBR	6.8	1.4	–	1.15	0.074	–	–	–	–	–	Debowski et al. (2017)
Cattle manure	Mesophilic	CPBR	7.0	1.16	–	0.91	0.061	–	–	–	–	–	Debowski et al. (2017)

[a] UASB: upflow anaerobic sludge blanket, CSTR: continuous stirred-tank reactor, CPBR: closed photobioreactor.

increment in the ammonia concentrations. The high pH could inhibit the activity of the microorganisms by influencing the chemical balance of H_2S, NH_3, and volatile fatty acids (VFAs). It has been stated that the optimal pH range for anaerobic digestate for algae growth is between 6.8 and 7.4 (Khalid et al., 2011). Low C/N ratio of the digestate can theoretically solved by the addition of CO_2 sourced from flue gas or other sources.

The relatively low phosphorus to nitrogen (P/N) ratio is another disadvantage of the anaerobic digestate, which does not satisfy the stoichiometric phosphorus requirement of the microalgae (Praveen and Loh, 2016). Phosphate is known as the dominant component of total phosphorus (82–90%) in the digestate. Phosphorus is indispensable for energy transfer, cellular processes in the microalgal growth and it is preferably in the phosphate form (Cheng et al., 2021b). For each microalgae strain, the optimum P/N ratio range is different, whereas the P/N ratio of 7–16 has been suggested as adequate balanced nutrients for algae (Redfield, 1934), where low P/N ratio represents the excess nitrogen in the form of ammonia.

Indeed, the nutrient structure of the digestate is entirely relative to the digestate source (feedstocks for the anaerobic digestion) and their respective operating conditions. The issues of unbalanced C/N and P/N ratios can be solved either by modifying the feedstocks for anaerobic digestion, by combining different feedstock materials with low C/N ratios (e.g., animal manure or sewage) or high C/N ratios (e.g., organic solid waste) or to achieve an optimal C/N ratio level and to supplement nutrient inadequacies for the microalgae cultivation (Zeng et al., 2017). The technique of combining different organic feedstock materials for anaerobic digestion process is known as co-digestion. Applicability of co-digestion was affirmed and reported by Malolan et al. (2020) and Shamurad et al. (2019). A primary benefit of co-digestion is that it can combine feedstock with different composition of C and N effectively; where the methane production is likely to be increased by the balanced C/N ratio of feedstocks. The combination of two or more substrates will produce a synergistic effect by relieving the nutrient imbalance and, in turn, attenuating the inhibition effects of the individual substrate (Khalid et al., 2011). The impacts of the C/N and P/N ratios on the lipid and biomass productivities of the microalgae cultivation have been acknowledged. According to Liang et al. (2009), mixotrophic growth and lipid content of *Chlorella vulgaris* were influenced by the C/N proportion. In previous studies, the optimal C/N ratio for cultivating *Chlorella vulgaris* mixotrophically was 5–15 (Silaban et al., 2014). For example, sodium acetate mixotrophic cultures (C/N: 15/1) demonstrated in a high mean biomass productivity (156 $g\,m^{-3}\cdot d^{-1}$) and neutral lipid productivity, respectively (24.07 $g\,m^{-3}\cdot d^{-1}$) (Silaban et al., 2014). It is also shown that the *Chlorella vulgaris* accumulated lipid as high as 35.3% and 36.5% for TOC/TN ratios of 24 and 30, respectively. The further increment of TOC/TN ratio from 24 to 30 did not resulted in significant lipid accumulation in the microalgae (Gao et al., 2019).

3.2. Competing biological contaminants

The biological status of the anaerobic digestate is related to the presence of pathogens, bacteria, zooplankton, viruses, fungi, and foreign algae (Fig. 3) that may potentially establish ecological competition for the microalgae (Xia and Murphy, 2016). Since biological contaminants are capable of inhibiting the growth of the target microalgae species, their control strategies are essential for future microalgae mass cultivation. The existence of these biological pollutants depends on the nature of the feedstock input and the anaerobic digestion process parameters, such as pre-treatment techniques, pH, temperature, hydraulic retention period, etc. The risk is higher when manure is included as the feedstocks for anaerobic digestion, owing to the presence of pathogenic protozoa *Cryptosporidium*, *Giardia* and bacteria such as *Salmonella, Campylobacter, E. coli, Yersinia* (Hutchison et al., 2006). During the anaerobic digestion process, the presence of these protozoa and bacteria in the digestate is not removed and can form spores in animal manure. Phytoplankton-lytic bacteria are known to affect the growth of microalgae by two mechanisms: direct attack based on cell-to-cell touch or indirect attack mediated by extracellular compounds (Kang et al., 2005). When bacteria-rich digestate is used, unavoidable bacterial contamination offers either a beneficial symbiotic relationship (e.g., vitamins, CO_2 production, ammonium, intake of O_2) or a negative impact (e.g., competition for nutrients or micronutrients) in the cultivation of microalgae (Monlau et al., 2015). Bacteria may cause the death of the microalgae by disrupting the integrity of microalgae cell wall, breaking the double helix structure of DNA in microalgae, and spilling the intracellular material (Wang et al., 2013).

Meanwhile, zooplanktons (e.g., ciliate, cladocerans, and rotifers) can be regarded as the predator for mass cultivation microalgae, where the algal population and concentration can be decreased drastically within just a few days (Lam and Lee, 2012). The high specificity of microalgal virus infection causes changes in the structure and succession of algal cells and can also reduce the amount of algae in culture rapidly (Kagami et al., 2007). When the anaerobic digestate used for microalgae

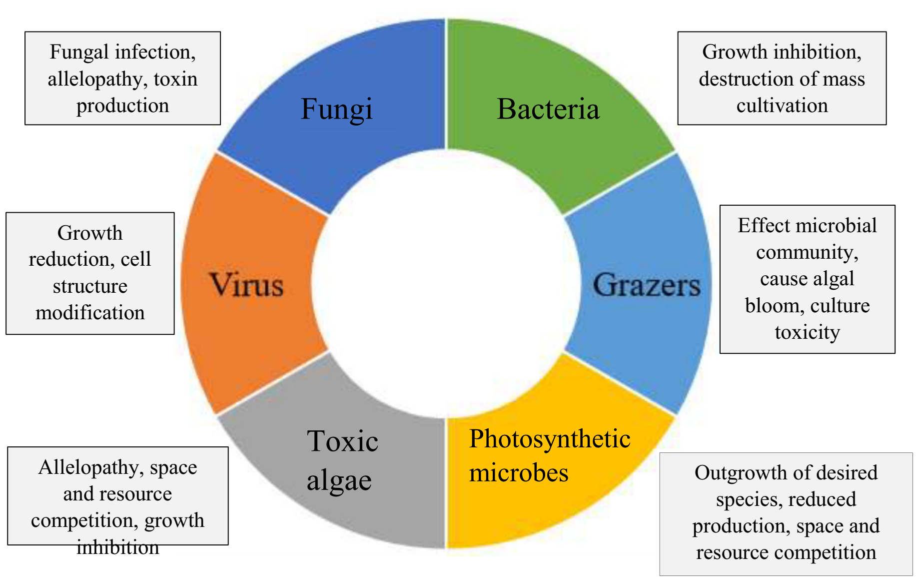

Fig. 3. Biological contaminants and their effect on microalgae cultivation (Shahid et al., 2019).

cultivation consists of foreign algae, the foreign algae are more likely to over-grow than the desired microalgae strain due to the resource competition and allelopathy (Wang et al., 2013). This can be explained by the availability of CO_2 and nutrient is less than the potential biological requirement, the competition between different algae species will be favoured the microalgae species with larger nutrient intake capability. Allelopathy refers to the biological phenomenon where a microalgae species creates biochemicals that can affect the development and reproduction of other algae species (Cheng et al., 2021a).

Owing to the sterilisation process not favourable for mass cultivation systems of microalgae monoculture, chemical addition and filtration are promising techniques to inhibit biological contaminants and to remove large organisms during microalgal cultivation (Wang et al., 2013). However, the chemical addition technique possesses drawbacks such as the chemicals may damage the growth of target microalgae and additional treatment process is needed after the chemical addition. High energy consumption of the filtration process is required as the liquid microalgae needs to be continuously filtered over a period of 3–4 days in order to thoroughly remove the big biological contaminations. With regards to this, the selection of microalgal strains species with strong resistance/non-susceptibility to biological contamination and the control of cultivation process parameters play a significant role for their mass cultivation. In general, monitoring the biological contamination of anaerobic digestate in microalgal mass cultivation is necessary in order to gain an understanding of the interactions between different species before identifying effective and economical methods to preserve microalgal dominance.

3.3. Turbidity

Regardless of the cultivation method, the main limiting factor of any community of microalgae growth is the quality and quantity of light. At light saturation point, maximum algal growth is achieved as photosynthesis correlates with an increase in irradiance (Morales et al., 2015). The quality and quantity of light for microalgae cultivation will be affected by the high turbidity of the digestate caused by the presence of dark color, high dissolved, suspended materials and ammonia content in the medium (Zhang et al., 2010). The turbidity of liquid digestates can be measured with a TN-100/T-100 turbidimeter and its values may vary from 2960 to 51,400 NTU for the liquid fraction of manure digestates as recorded from 11 digestion plants (Akhiar et al., 2017). The impurities that cause high turbidity include microorganisms, clay, soluble coloured organic compounds, silt, and finely divided organic and inorganic matter (Chuka-Ogwude et al., 2020). Turbidity is a measure of the dispersion and absorption of light-induced in a fluid by the presence of foreign particles, where the insoluble suspended particles in the anaerobic digestate are the main contributor to the high turbidity when added to cultivation media. Although the digestate with the presence of suspended organic compounds is susceptible to bacterial invasion, the resulting turbidity can decrease nutrient removal, photosynthetic ability, and productivity of biomass (Marcilhac et al., 2014). As the suspended materials scatter and absorb light, the suspended materials in the anaerobic digestate effluent increase the attenuation of light and then interfere with the transparency of the liquid medium (Wang et al., 2010). This phenomenon has resulted in the light is unable to transmitted in straight lines through the water sample, thus inhibit the microalgae growth.

The previous study showed that the availability of photosynthetically active radiation (PAR) basically limits microalgae growth, so any dissolved or suspended matter growing light absorption between 400 and 700 nm will significantly decrease the yield of microalgal growth (Monlau et al., 2015). Pre-treatment of the anaerobic digestion feedstocks such as filtration, centrifugation, adsorption, and precipitation can be used to adjust the turbidity of the digestate by removing the suspended solids and impurities. However, these techniques require high treatment cost, which hindered their wide application for pre-treatment of the feedstocks (Cheng et al., 2015). The energy-intensive method of centrifugation can restrict its applicability to the anaerobic digestate as a pre-treatment level (Sturm and Lamer, 2011). Supernatant extraction was found to be the simplest and most effective way to reduce the turbidity and COD of the diluted anaerobic digestate while maintaining adequate nutrients (particularly nitrogen) for the cultivation of microalgae.

Another promising technique to reduce the turbidity of the digestate is by dilution using freshwater or different sources of wastewater (domestic, urban, municipal, and aquaculture wastewater). In order to minimize the overall environmental and economic practicability, it is recommended to substitute part of the freshwater by wastewaters in future works. Owing to the high turbidity and high chromaticity of effluent and digestate impede and weaken the growth of microalgae (Wang et al., 2010), immense efforts have been contributed by the researcher and demonstrated the better algae growth and nutrient removal upon reduced turbidity in the anaerobic digestate (Wang et al., 2010; Torres Franco et al., 2018; Uggetti et al., 2014). Wang et al. (2010) cultivated *Chlorella vulgaris* species in a dairy manure based digestate with turbidity of 1800-1900 NTU under various dilution factors (10, 15, 20 and 25-fold) for 21 days. The outcome of the study demonstrated the inverse association between specific algal growth rates and turbidity, suggesting the high turbidity can limit the microalgal growth by resulting the poor light penetration. To date, only a few papers have recorded the impact of the turbidity of the medium on the microalgae cultivation, therefore it is much difficult to compare the findings (e.g., the microalgae biomass production and the efficiency of nutrients removal) with other studies with similar medium turbidity. Therein, it is highly suggested that future work prospects can be directed to the turbidity study on microalgae growth to guarantee the high feasibility of the utilization of waste anaerobic digestate in it.

3.4. Ammonia/ammonium toxicity

The toxicity of high ammonia-nitrogen concentration in the anaerobic digestate is another significant issue with their use for microalgae cultivation (Chuka-Ogwude et al., 2020; Nwoba et al., 2016), especially when wastewater or anaerobically digested wastewater are used as feedstocks for anaerobic digestate. In aqueous solutions, total inorganic nitrogen is present in the forms of the protonised cation ammonium (NH_4^+) and the more reactive gaseous form ammonia (NH_3). As less energy is required and the absence of redox reaction, NH_4^+ has been shown to be the more preferred nitrogen source for microalgae (Markou et al., 2016), but it may be detrimental to the microalgae growth at a high concentration. NH_3-N is often known as the more toxic and common inhibitors to microalgae and its inhibition may take place at a wide range of concentrations. Several studies have reported ammonia inhibition with total inorganic nitrogen between 1400 and 17,000 mg N/L (Chen et al., 2008). Anaerobic digestate food effluent with an NH_3-N concentration of 1000–6000 mg/L and NH_4^+-N concentration >100 mg/L have been demonstrated to show inhibitory effects on microalgae development in the anaerobic digestate effluent (Uggetti et al., 2014; Park et al., 2010). On another study, Xia and Murphy (2016) reported that NH_3-N concentrations ranging from 50 to 260 mg/L can inhibit microalgae growth (Xia and Murphy, 2016). Levine et al. indicated that the 50 mg/L of NH_4^+-N concentration is the inhibitory threshold for *Neochloris oleoabundans* (Levine et al., 2011). When the NH_3-N concentration increased from 9 to 34 mg/L, the growth rate of *Scenedesmus* sp. was found to decrease by 77% (Uggetti et al., 2014). Park et al. (2010) and Uggetti et al. (2014) found that a total NH_4^+-N level of 100 mg/L and 260 mg/L, respectively did not suppress the growth for a mixed microalgal *Scenedesmus* sp. culture. The hydrophobic NH_4^+ diffuses passively across the cell membrane in microalgae and inhibits cell functioning; thus, its absorption must be regulated to prevent too large levels of ammonia in the cell membrane (Boussiba and Gibson, 1991).

The ammonia toxicity to the microalgae involves uncoupling the electron transport from photophosphorylation (Crofts, 1966), disrupting the equilibrium of protons and potassium within the cell (Kayhanian, 1999), disrupting the photosynthesis system of microalgae (Drath et al., 2008). This inhibition can cause intermediate digestion products such as volatile fatty acids (VFA) to become imbalanced and accumulate, which can lead to digester acidification. Phytotoxic reactions linked to organic acid and NH_4^+-N concentrations have also been identified by some of the researchers (Franchino et al., 2016). The balance of the two nitrogen forms is easily depicted by the pH of the digestate, where the corresponding proportion of total inorganic nitrogen at normal pH is the equal form of both NH_3 and NH_4^+. NH_3 dominates at pH > 9 and at pH < 8 is dominated by NH_4^+. In addition, during its application for microalgae development, high digestate pH will lead to a large amount of NH_3 not being assimilated but released by NH_3 volatilization and leaching of nitrate (NO_3^-) to the atmosphere (Ayre et al., 2017). Akhiar et al. (2017) recorded a NH_4^+ proportion ranging from 16 to 72% of the total inorganic nitrogen in several agro-industrial digestate (Akhiar et al., 2017). Researchers attempted dilution technique to dilute the NH_3-N level to 50–100 mg/L to mitigate the inhibitory effects of the high nutrient levels in microalgae cultures (Franchino et al., 2016). Indeed, microalgae can withstand higher NH_4^+ concentrations than those typically measured if long enough adaptation period is given. While high NH_4^+ concentrations are likely to be inhibitory for a number of species of microalgae, it does not seem to be a big issue for nutrient-tolerant *Chlorella* and *Scenedesmus* species as long as the pH is regulated (Ayre et al., 2017). In general, while NH_4^+ is a preferred type for the microalgae, high levels of total NH_3-N can result in microalgae growth inhibition. It has been recommended to control the high NH_3 concentrations to less than 2.3 μM for favourable microalgae growth (Cho et al., 2013). The inhibitory thresholds differ significantly with the cultivation conditions and species of microalgae.

3.5. Metals toxicity and organic contaminants

The inhibitory influences of heavy metals such as cobalt (Co), nickel (Ni), copper (Cu), cadmium (Cd), aluminium (Al), zinc (Zn), iron (Fe), manganese (Mn), etc. in anaerobic digestate have been known for several decades, especially when it is mixed with industrial effluents (Kupper et al., 2014). A study conducted by Zhu et al. (2014) reported that the pseudo-total content of heavy metals concentration in anaerobic digestates comprise of Cu, Zn, Pb, Ni, Cr, Cd, Mn, and As with the concentration of 1131.64 mg/kg, 2741.23 mg/kg, 225.15 mg/kg, 74.31 mg/kg, 63.53 mg/kg, 10.22 mg/kg,1738.88 mg/kg, and 25.79 mg/kg, respectively. During the anaerobic digestion, the rise in heavy metal concentration may be due to the use of metallic chemicals, microbial mineralization, pipeline corrosion, loss of volatile solids, bleaching, and neutralization (Ciavatta et al., 1993; Reza and Singh, 2010).

In microalgae cultivation, the stimulatory, inhibitory or even toxic effects of the heavy metals are highly dependent on their concentration. By inducing the morphological alternations in the size and shape of microalgae cells, heavy metals could hinder the growth of microalgae, thus influencing their microbial photosynthesis. The high concentration, non-biodegradable properties, and their high NH_3 emission of these metals serve high potential to jeopardize the human health and environment, potential sanitary impact, cause cellular toxicity and accumulation (Cheng et al., 2021b).

Even though some metals such as Ni, Fe, Cu, Zn are also micronutrients for microalgae growth, however they will become inhibitory factor or toxic when their concentrations are above the threshold values. Pig and cattle slurry based digestates are particularly rich in micronutrients such as Zn and Cu (Alburquerque et al., 2012). On the other hand, some of the anaerobic digestates also consist of detrimental nitrous and sulphur oxides (NO_x and SO_x) for microalgal growth (Muñoz and Guieysse, 2006), thus their concentrations must be reduced by certain treatments to appropriate levels. Due to personal care products and pharmaceutical residues, anaerobic digestives may also contain pronounced traces of organic contaminants such as including humic substances, dye, surfactants, phenolic compounds, pesticides, and pharmaceuticals, contributing to a potentially adverse effect on the growth of microalgae.

It is indisputably that waste sources, such as industrial waste generate digestate with a higher concentration of organic contaminants and heavy metals than digestate produced from properly separated organic fractions of organic solid waste. Organic solid waste is also known as bio-waste, which includes green waste from restaurants, retail premises, households, caterers or food processing plants. In this regard, it has been demonstrated that the origin of the input material for anaerobic digestion could be decisive due to the heavy metal aerial deposition. The input material selection season is particularly crucial as the heavy metal aerial deposition tends to reach its highest point during the winter period (Cercasov and Wulfmeyer, 2008). Generally, the presence of a high concentration of heavy metals and organic contaminants can jeopardize the microalgae growth, therefore the balanced nutrient availability in conjugation with ideal growth condition is indispensable for the healthy growth of microalgae.

4. Potential solutions

Previous section has mentioned on limiting factors of anaerobic digestate for microalgal growth. Therefore, several potential solutions have been proposed and discussed in detail in this section. Table 3 compares the advantages and disadvantages of the potential solutions on limiting factors when using anaerobic digestate for microalgal growth.

4.1. Anaerobic digestate dilution

Compounds such as nitrogen, phosphorus and heavy metals in the anaerobic digestate vary with the various operating conditions during the anaerobic digestion process. The presence of high concentration toxic compounds in the digestate that can inhibit the growth of algal cells, thus dilution appears to be a preferred scheme to dilute the raw digestate with tap water, secondary/tertiary wastewater, saline groundwater, synthetic culture medium or seawater. This technique not only avoiding inhibition from excessive amounts of nutrients and inhibitors or from self-shading, but also allow a suitable amount of nutrients in the culture medium. Previous literature had reported on the feasibility of this method to minimize turbidity, alleviate inhibition of NH_4^+-N, and increase the P/N ratio of the nutrient medium (Praveen et al., 2018), the initial algal growth rates were subsequently marginally higher than the chemical control medium.

Several studies have shown the ability of growing *Neochloris oleoabundans* with diluted anaerobic digestate at different dilution ranges. Levine et al. (2011) cultivated *Neochloris oleoabundans* in batch culture experiments using 50-, 100-, and 200-fold diluted dairy-derived anaerobic digestate to determine how light attenuation may be influenced by nutrient concentrations, suspended solids, and endogenous microorganisms. The outcome of the study elucidated the 50-fold dilution (2.6 mg P/L phosphorus; 42 mg N/L ammonia, and 60 mg N/L total nitrogen) produces the highest algal growth rates. Abu Hajar et al., 2016 also inoculated *Neochloris oleoabundans* by using multiple times diluted anaerobic digestate, where the highest growth of the microalgae was achieved in the 2.29% diluted supernatant (total nitrogen concentration: 100 mg N/L) as compared to the filtered digestate and other dilutions. Franchino et al. (2013) cultivated *Chlorella vulgaris, Scenedesmus obliquus*, and *Neochloris oleoabundans* on agro-zootechnical digestate at various tap water dilutions ratios (10-, 15-, 20-, 25-folds). These dilution ratios were chosen after the preliminary test confirmed that the *C. vulgaris* could not survive at a dilution ratio of 1:1 and 1:2, possibly due to the medium's high turbidity. At a dilution

Table 3
Comparison of advantages and disadvantages of the potential solutions on limiting factors when using anaerobic digestate for microalgal growth.

Potential Solutions	Limiting factors	Advantages	Disadvantages
Anaerobic digestate dilution	• Turbidity • Metals toxicity and organic contaminants • P/N ratio • Ammonia/ • ammonium toxicity	• Simple • Reuse secondary/tertiary wastewater, saline groundwater, synthetic culture medium or seawater • Readily available water supply • Avoid inhibition from excessive amounts of nutrients • Minimize turbidity, alleviate inhibition of NH_4^+-N, and increase the P/N ratio	• Nutrients supply for microalgal will be diluted at the same ratio • Selection of appropriate dilution ratio is necessary at the preliminary study stage • The installation of underground pipes inflict the loss of riparian vegetation and wildlife habitat. • Only certain microalgae species can survive in saline groundwater and seawater
Microalgae strain selection and diversification	• Competing biological contaminants • Ammonia/ • ammonium toxicity • Metals toxicity and organic contaminants	• Proper starin selection has resistance/non-susceptibility to the invasion and contamination of a wide range of pollutants, high alkalinity tolerance, and the high lipid content. • Mixed culture systems are more stable than monocultures, less vulnerable to invasion, cheaper, easier to operate.	• Pure monoculture in open ponds: not robust enough to prevent cross-contamination or infection by biological pollutants. • Key challenge in mixed consortia and mixotrophic systems: ensure the bacteria do not dominate the consortium system.
Pre-treatments of feedstocks and anaerobic digestates	• Turbidity • Metals toxicity and organic contaminants	• Reduce the suspended solids in the nutrient medium • Mitigate possible toxicity caused by high inorganic and organic matter concentrations • Reduce turbidity • Avoid interference by other microorganisms such as protozoa or bacteria	• Costly as additional steps are required. • Thermochemical treatment- high energy and chemical consumption • Hydrogen peroxide (H_2O_2) oxidation (chemical) – handling of harmful oxidative chemical, high cost • Autoclaving (thermobaric) – not practical as involve high temperature pressure. More suitable for lab scale investigation. • Filtration – Costly membrane
CO_2 and extra organics addition	• C/N ratios	• Possibility of sourcing waste source of CO_2 like flue gas • Flue gas: more environmentally friendly, lower carbon footprint • Can achieve the high biomass demands to promote the growth of microalgae • Possibbility of using dissolved carboxylic acids, volatile fatty acids such as propionic, acetic, and *n*-butyric acids from waste sludge to replace commercial organic carbon sources	• Low natural CO_2 dissolution rate from the air into water • Pure CO_2 is expensive • High cost of commercial organic carbon sources • Flue gas: require pretreatment like desulphurization or denitrification
Nitrification and desulfurization	• Ammonia/ • ammonium toxicity • Metals toxicity	• Reduce the accumulation of NH_4^+ • Nitrified digestate has superior quality (less toxic metal content). • Remove highly toxic and corrosive hydrogen sulfide (H_2S) in biogas	• High contamination risk • Time-consuming with separate biological stages • Dilution-free with minimal freshwater intake

rate 1:10, specific growth rate of 0.64, 0.49, 0.27 d^{-1} were observed for *Chlorella vulgaris*, *Scenedesmus obliquus*, and *Neochloris oleoabundans*, respectively. *Chlorella vulgaris* and *Scenedesmus obliquus* demonstrated the highest ammonium elimination capacity for 1:10 dilution rate of the digestate sample, while *Neochloris oleoabundans* species grew faster in 20- and 25-folds diluted substrates.

The digestate collected from a municipal wastewater treatment plant was diluted by Cho et al. (2013) to cultivate microalgae at several dilution ratios of tap water (1:2, 1:3.5, 1:5, 1:10, 1:20). In their preliminary experiment, the high ammonia toxicity of the raw digestate had inhibited the microalgal growth; 10-folds dilution was identified as the optimal dilution ratio of the digestate for optimal growth of microalgae. They also found that when the digestate was mixed with wastewater expelled from sludge concentrate tanks (10:90, *v*/v), the highest biomass production (3.01 g dry cell weight/L) of *Chlorella vulgaris* was demonstrated. Yang et al. (2011) also found that the biomass growth rate was the highest at 50-fold diluted ratio as compared to other dilutions for most of the waste categories. Despite the large potential of this dilution approach for enhancing the feasibility of anaerobic digestate for microalgae growth, yet there are some drawbacks pointed out by the researchers to doubt its practicability, especially when a large amount of freshwater is needed during the dilution process. This phenomenon has limited the application of this technique in industrial scale; thus, it is suggested to replace the usage of part of tap water/ freshwater by wastewater and seawater to maximize the process's overall environmental and economic viability.

The anaerobic digestate to be used to cultivate microalgae can also undergo some pre-treatment steps to alleviate its toxicity to reduce the dilution ratio needed, eventually decrease the overall amount of water needed. Another major concern of the dilution technique is that the nutrients supply needed for microalgal growth would be diluted at the same ratio as the inhibitors, resulting to a reduction in the overall microalgal biomass concentrations (Uggetti et al., 2014). Therefore, the selection of appropriate dilution ratio is necessary at the preliminary study stage as it may vary with many decisive factors, such as the quality of the digestate, types of microalga strains, experimental conditions, etc. To date, the data in the literature regarding the diluted anaerobic digestate a substitution nutrient source for microalgae are not comparable due to different variables used in the different studies, however, it can be concluded that a suitable dilution ratio can dilute the inhibitory compounds and maximize the algal growth.

4.2. Microalgae strain selection and diversification

Proper microalgae strain selection and diversification are most likely a feasible approach to address the issues of competing biological contaminants, ammonia, metal, and organic toxicity when using anaerobic digestate for microalgae cultivation. These undesirable species release toxins or other inhibitors for microalgae growth, prey on microalgae, and/or compete for nutrients and light. Thus, the commercialization of microalgae cultivation has been burdened by the additional costs associated with the identification and control of pollution. There are several criteria to be considered during the potent strain selection for mass cultivation such as the resistance/non-susceptibility to the invasion and contamination of a wide range of pollutants (Wang et al., 2013), high alkalinity tolerance (Zhang et al., 2019), and the high lipid content. The suitability of the microalgae oil for biodiesel production should be considered as well. The genera *Desmodesmus*, *Chlorella*, and *Scenedesmus* are among the few species considered as robust survivors in wastewater or in digestate, especially *Chlorella vulgaris* and *Scenedesmus obliquus* (Stiles et al., 2018). *Picochlorum oklahomensis* with high lipid and proteins contents can resist salinity (NaCl) of up to 140 g/L, thus it is commonly known as salt-tolerant microalgae (Henley et al., 2002).

Under high concentration of bicarbonate, *Neochloris oleoabundans* is a promising candidate for lipid production. Green marine microalgae *Dunaliella tertiolecta* strain can grow in a high concentration of NaCl (Elenkov et al., 1996) with an oil content of 36-42% of dry cell weight (Tsukahara and Sawayama, 2005). Both of the *Parachlorella hussii* and *Chlorella luteoviridis* are verified to be able to withstand high toxicity from the dewatering of the activated sludge, such as centrate liquor (Osundeko and Pittman, 2014). In a study of keeping the microalgal domination in the photosynthetic bioreactors, Bongiorno et al. (2020) reported that the obligate anaerobes (*C. perfringens*) deriving from digestate could not survive in the oxygen-rich microalgal culture upon intense photosynthesis by the microalgae. Despite the harsh environmental circumstances (high pH and salinity due to high water loss), Chlorophyta *Mychonastes homosphaera* remained dominant in a consortium of cyanobacteria/microalgae (Toledo-Cervantes et al., 2016). Previous literature indicated that cyanobacteria prefers pH range from 8 to 9 (Unrein et al., 2010); green algae *Scenedesmus* sp. outcompeted cyanobacteria when the pH value was approximately 10 (Arias et al., 2017).

Eukaryotic algal strains offer the advantages such that their cell walls are having greater structural and composition complexity as compared to wild-type prokaryotic cyanobacteria (blue-green algae) (Wensel, 2018). Freshwater algae such as *Scenedesmus* sp. and *Chlorella vulgaris* cultivation are superior to marine algae (*Chlorella salina*) as gases such as CO_2 is more soluble in freshwater than in saltwater. and impurities of salts are devoid in the freshwater (Wensel, 2018). Wensel (2018) also suggested that small spherical types of algae are better than large filamentous algae, owing to their favourable morphology that is able to enhance algal light, increase bubble-algal adhesion rates during the dissolved air-flotation-based harvesting methods, and boost the mass transfer of dissolved nutrients across the cellular wall.

On the other hand, it is elucidated that using pure monoculture in open ponds during the cultivation of microalgae for a few months is very troublesome because these are not robust enough to prevent cross-contamination or infection by biological pollutants (Rodolfi et al., 2009). The key challenge in mixed consortia and mixotrophic systems is to ensure that the bacteria do not dominate the consortium system where there is presence of dissolved carbon source. Since microalgae cultivation is a complex symbiotic microalgae-bacterial-zooplankton system, thus proper selection of microalgae strains is extremely essential. Microalgal consortia (mixed culture systems) of higher diversity are known are more stable than monocultures, less vulnerable to invasion (Chuka-ogwude et al., 2020), cheaper, easier to operate, allows precipitation of phosphorus (Kang et al., 2018) and nutrient retention upon the usage of consortia species with different metabolic capacities or strains with different spectrums of light absorption (Ogbonna, 2015). These advantages explain why the algal-bacteria consortia or algal consortia are more appropriate for mass microalgae cultivation on anaerobic digestate than monocultures, with higher growth rates were achieved under mixotrophic conditions than autotrophic or heterotrophic conditions.

Qin et al. (2016) found that 91–96% total phosphorus and 57–63% COD were removed from the microalgae consortia as compared to the *Chlorella* sp. monoculture (87% total phosphorus and 45% COD removals). The lipids yielded from the consortium of microalgae are very suitable for processing biodiesel due to its high content of saturated fatty acids. As the composition of the commercial culture media differs significantly from what was observed in anaerobic digestate, the variations in the growth environment will therefore limit the performance of microalgae and require long-term recovery (Hu et al., 2019a). Some of the anaerobic digestate can be particularly toxic to microalgae if those habitats are not previously exposed to it. In such complex conditions, survival skills of the microalgae strains should include the ability to adapt to and withstand stress rapidly. To help microalgae better adapt to the culture medium by promoting the growth of native organisms, an acclimation stage can be added to an environment similar to the anaerobic digestate (Lau et al., 1996). Additionally, wastewater-acclimated strains have the ability to have improved efficiencies for the nitrogen (N) and phosphorus (P) removals (Vats et al., 2020). Acclimated *Scenedesmus obliquus*, *C. vulgaris* and *Chlorella sorokiniana* reported by Hu et al. (2019a) adapted better to the environment in wastewater and marked pronouncedly higher biomass production than unacclimated species.

4.3. Pre-treatments of feedstocks and anaerobic digestates

Pre-treatment of the anaerobic digestate refers to the treatment of the feedstocks at the upstream before emerges from the digester such as filtration, autoclaving (thermobaric), hydrogen peroxide (H_2O_2) oxidation, dewatering, supernatant extraction, and thermochemical treatment, etc. The pre-treatment results are also highly contingent on the initial biodegradability of the digestate and the operating conditions of the anaerobic digestion process that generated it. The pre-treatment of feedstocks can facilitate the breakdown of complex organics into the soluble and bioavailable substrates during the anaerobic digestion, thus play a vital role in affecting the physicochemical properties of anaerobic digestates despite indirect upstream relation. Appropriate pre-treatment methods are capable of reducing the suspended solids in the nutrient medium, mitigating possible toxicity caused by high inorganic and organic matter concentrations, reducing turbidity and the demand for chemical oxygen (COD), as well as retaining adequate nutrients for the microalgae cultivation (Abu Hajar et al., 2016). Pre-treatment of the different substrates is also primarily used to increase the hydrolysis rate and improve the surface area to volume ratio of the substrates during the anaerobic digestion processes (Vats et al., 2020). Pre-treatment methods such as filtration, autoclaving, or other techniques are frequently applied to wastewaters and anaerobic digestate before the microalgae cultivation to avoid interference by other microorganisms such as protozoa or bacteria and to minimize the concentration of suspended solids. Plastic, glass, metal, rubber, sand, ceramics, cellulose materials, stones, etc. are the most common physical impurities in the organic fraction of solid waste.

For filtration method, the digestate can be filtered using <10 μm welded polyester filter bags. Meanwhile, hydrogen peroxide (H_2O_2) can be used either alone as a potent oxidant that can be used in the anaerobic digestate to oxidize the COD and ammonia or in conjunction with other oxidation techniques such as ozone and UV light or with an iron catalyst (Ksibi, 2006). However, the H_2O_2 pre-treatment is not an effective technique for anaerobic digestate with high alkalinity, as the presence of bicarbonate as a radical scavenger will decrease the efficiency of advanced oxidation processes (Park et al., 2010). Supernatant extraction is more effective and less costly in decreasing the COD and TSS content of the digestate compared to H_2O_2 treatment and filtration. By steam treating the feedstocks at constant pressure and temperature, an autoclave may be used to pre-treat digester feedstocks to clean, pasteurize, and breakdown organic matter inside the feedstocks. Since the inorganic material and contaminants are removed and organic matter is broken down, therefore the digestate quality produced from digestion process is higher. Tanaka et al. (Tanaka et al., 1997) investigated different pre-treatment techniques on the domestic waste activated sludge by heating (thermal), NaOH addition (chemical), and by heating with NaOH addition (thermochemical). The thermochemical pre-treatment was the best among the three methods, with 40–50% of volatile suspended solid was solubilized and the soluble COD fraction increased from 16.3%–50.7%. This was indicative of the hydrolysis and subsequent solubilization of organic residues in the sludge. Thermobaric pre-treatment is another applicable approach in an industrial context to increase the hydrolysis rate by utilizing heat exchange and substrate dewatering (Harris et al., 2017).

Pre-treatment of the anaerobic digestates harvest during anaerobic digestion process to alter its physicochemical properties (i.e., reduction of turbidity, suspended solids, soluble COD, ammoniacal nitrogen, particulate, sulphides, pathogens, phosphorus, and toxins), thereby ensure the growth, survival, and even dominance of the desired microalgae. Similar pre-treatment techniques of feedstocks may also be used for pre-treatment of anaerobic digestate, depending on the starting feedstock and intended digestate form. The size reduction and sanitation of the anaerobic digestate can also be involved in this. Nutrient concentrates can be produced due to the pre-treatment of digestate, so liquid and solid digestate fractions could be conditioned to a standardized microalgae nutrient source. Enhance marketability, minimize the storage, handling, and transportation costs are the primary advantages of digestate pre-treatment.

4.4. CO_2 and extra organics addition

Supplementary organics must be supplied to sustain microalgae mixotrophic growth, since anaerobic digestate is having low C/N ratio. The carbon sources in anaerobic digestate for microalgae growth can be divided into organic carbon (saccharides and volatile fatty acids) and inorganic carbon (carbon dioxide (CO_2) and bicarbonate (HCO_3^-)). Microalgal growth in the digestate can be stimulated by external carbon source, which can be done by adding extra organics and inorganics carbon into the culture medium. Along with sunlight, water and nutrients, CO_2 is one of the essential components needed to grow algae. By increasing biomass and controlling pH, CO_2 would optimize the anaerobic digestate effluent utilization process in the microalgae cultivation (Sutherland et al., 2015). The pH of the medium will be decreased when the carbonic acid (H_2CO_3) if formed upon CO_2 dissolution, but CO_2 is absorbed from the medium to become alkaline pH when the photosynthesis rate and microalgae biomass increase (Moheimani and Borowitzka, 2006). Based on the algal biomass average chemical composition, 1 t of biomass requires approximately 1.8 t of CO_2 to grow. Previous study indicated that maintaining a pH of 8 for the culture medium by regulating the CO_2 addition to the ponds can increase the chlorophyll content of the microalgae consortium (Ayre et al., 2017).

Due to the relatively low atmospheric CO_2 content and high-water surface tension, the natural CO_2 dissolution rate from the air into water is low, which is one of the keys limiting factors in the cultivation of microalgae (He et al., 2012). This could be improved by direct bubbling air into microalgae culture if modifications have been done to either increase retention time, increase the diffusivity, and/or reduce the gas bubble size to minimize atmospheric release of CO_2. There is also a possibility of sourcing waste source of CO_2 like flue gas from a boiler as carbon source for microalgae cultivation as using pure CO_2 is rather expensive. This option can facilitate the simultaneous microalgae cultivation on anaerobic digestate and biogas upgradation to produce 'bio-methane'. Flue gas is free and usually consists of ~4–15% of CO_2 or even generates revenue if a greenhouse gas emission prevention financial scheme is available (Songolzadeh et al., 2014). Recent literature has reported on no harmful effects have been found when flue gas is used to grow microalgae. Generally, the addition of supplementary CO_2 can achieve the high biomass demands to promote the growth of microalgae, yet the main obstacle for cost-effective microalgae biofuels is the high cost of commercial carbon sources.

On the other hand, microalgae mixotrophic culture is found to be able to increase the growth rates dramatically and to accumulate more lipids in microalgal cells by shortening the growth cycles and generating higher biomass (Pang et al., 2019). Mixotrophic cultivation growth mode of microalgae is referring to its simultaneously use of different sources and combinations of organic carbon and inorganic CO_2 in the presence of light; therefore, heterotrophy and photo-autotrophy happen concurrently (Wang et al., 2014). It is noted that only certain microalgae species can grow mixotrophically, such as freshwater *Rhodomonas reticulate, Nannochloropsis* spp., *Scenedesmus obliquus*, *Brachiomonas submarina*, *Ochromonas minima*, *Chlorococcum* sp., *Chlorella* spp., *Cyclotella cryptica*, *Nitzschia* sp., *Phaeodactylum tricornutum*, *Navicula saprophila*, *Euglena gracilis*, *Haematococcus pluvialis*, etc. (Bassi et al., 2013). Among the chemicals, previous studies have indicated the suitability of several commercial organic compounds such as ethanol, glucose, organic acids, fructose, and glycerinum could be used as organic carbon sources for microalgal mixotrophic growth (Deng et al., 2019). However, the high cost of artificial chemicals addition urged the application of dissolved carboxylic acids, volatile fatty acids such as propionic, acetic, and *n*-butyric acids from waste sludge anaerobic fermentation for algal mixotrophic growth while simultaneously reduce waste volume (Luo et al., 2018). Under mixotrophic conditions, the addition of acetate as a source of carbon *in Scenedesmus obliquus* was found to increase its growth rate as compared to the autotrophic one (Chalima et al., 2017).

Heterotrophic microalga *Crypthecodinium cohnii* is the most typical example of heterotrophic growth on acetate, as acetic acid is able to control the pH (Ratledge et al., 2001). The feasibility of four types of volatile organic acids (propionate, acetic, isovaleric acid, and *n*-butyric acids) for cultivating mixotrophic freshwater green algae *Chlorella pyrenoidosa* in anaerobically digested waste sludge has been tested by Luo et al. (2018). The outcomes of the study elucidated no noticeable increase in biomass production after the addition of propionate and isovaleric acid as algal growth was somehow inhibited at their low concentrations. Meanwhile, the addition of *n*-butyric acid and acetic to the digested waste sludge with initial C/N of 10 increased the biomass production by1.9–2.4 times in comparison with the blank culture. Other genera of microalgae such as *Chlamydomonas* and *Chlorella* can also grow well with no major inhibitory effects on volatile fatty acids (Ratledge et al., 2001). Tan et al. (2020) attempted a new approach for algal mixotrophic culture in anaerobic digestates by using acidified starch wastewater as waste organic carbon source. The optimal dosage of wastewater to anaerobic digestate ratio was 1:1 *v*/v, where the lipid and biomass production increased by 4.2-fold and 0.5-fold, respectively. The addition of wastewater also promoted the higher saturation quality of biodiesel produced from microalgae. However, the optimal selection of volatile fatty acids and dosages specifically for different species of the microalgae in anaerobic digestate have rarely been reported, therefore attempts on further research are highly encouraged. Generally, it is an ideal technique to incorporate optimal volume of volatile organic acids from wasted sources, as it is efficient to increase the nutrients recycling and the production of algal biodiesel from anaerobically digested waste sludge.

4.5. Nitrification and desulfurization

The two forms of ammonia nitrogen species; NH_3 and NH_4^+ are having strong equilibrium relations with pH and temperature (Benabdallah et al., 2009). Due to the abundant unionized NH_3 type, untreated digestate typically have a pH of about 7.5 to 8.5, therefore using NH_3 as the sole source of nitrogen for algae growth will lead to acidification in the root region (Botheju et al., 2010). Nitrification has been proposed to reduce the accumulation of NH_4^+ by introducing nitrifying bacteria, thereby avoid the NH_4^+ toxicity that potentially inhibits the microalgae growth. The anaerobic digestate nitrified by bacteria is very promising as a microalgae culture medium to prevent the microalgae from encountering NH_4^+-N toxicity due to the conversion of NH_4^+-N to harmless NO_3^-. The anaerobic digestate nitrified by bacteria is very promising as a microalgae culture medium to prevent the microalgae from encountering NH_4^+-N toxicity due to the conversion of NH_4^+-N to harmless NO_3^- (Svehla et al., 2017). The conversion of total ammonia nitrogen to NO_3^- via biological nitrification is expected to allow the direct use of ADE in microalgal cultivation without dilution requirements.

Previously, digestate nitrification coupled with microalgae cultivation had been applied by Praveen et al. (2018) in both batch and continuous mode of experiments. This technique is very appropriate for digestate nutrient recovery (97% of the efficiency of nitrogen removal has been achieved), although it would require separating anaerobic bacteria from microalgae. By operating the MBR with aerobic heterotrophic micro-organisms in conjugation with a membrane photobioreactor (MPBR) under autotrophic conditions, the use of membrane bioreactors (MBR) will simplify these processes (Praveen and Loh, 2016). Membrane filtration will avoid microorganism mixing, thus enabling activity with high biomass retention at lower HRTs. For this process, they diluted the digestate with municipal wastewater for 3-20-fold; however, the suitability of nitrified, undiluted digestate for microalgae cultivation remains unknown. The nitrification lowers the pH to <5.0, where the surplus ammonia is present as >99% NH_4^+ (Botheju et al., 2010). In the nitrified liquid digestate, the toxic metal content is much smaller than that of the initial digestate. As a nearly transparent and odourless liquid, the nitrified digestate also achieved superior quality.

On the other hand, biogas generated during the anaerobic digestion process consists of highly toxic and corrosive hydrogen sulfide (H_2S), thus biogas desulfurization treatment by physicochemical or biological methods is highly required. The transformation of sulphur-containing protein produces H_2S, and its concentrations in raw biogas typically range from 200 to 2000 ppm (0.02–0.2%) (Miltner et al., 2012). Since the growth conditions for nitrifying bacteria and sulphur oxidizing bacteria are both similar (Muñoz et al., 2015), therefore some researchers combined the anaerobic digestate nitrification with biogas desulfurization. The main concept of this technology is to convert the H_2S in the biogas and total ammonium nitrogen in the digestate simultaneously to harmless SO_4^{2-} and NO_3^- by sulphur-oxidizing bacteria and nitrifying bacteria, respectively by using O_2 produced in the algal reactor. Sekine et al. (2020) cultivated *Chlorella sorokiniana* microalgae over nitrification-desulfurization treated undiluted anaerobic digestate. In the study, a sequencing batch reactor (effective volume: 2.1 L) was used as the nitrification-desulfurization reactor and operated with a 24-h cycle and NaOH was used to adjust the reactor pH as it was decreased by nitrification and desulfurization. The study revealed that 50% of metal concentrations of the anaerobic digestate had been decreased, while the salinity of the digestate increased from 0.35% to 0.66% upon the nitrification-desulfurization treatment. The microalgal growth using nitrification-desulfurization-treated undiluted anaerobic digestate was almost the same with synthetic medium, suggesting that this dilution-free anaerobic digestate-treated nitrification-desulfurization is useful for microalgal cultivation with minimal freshwater intake.

5. Conclusion and future prospect

The development of biogas through anaerobic digestion has grown rapidly in recent years, where the digestate is generated as primary by-product. Traditional land application of the digestate creates environmental concerns and requires substantial energy inputs. Alternatively, microalgae can effectively use the digested waste nutrients to generate high-value biomass and minimize the process inputs by offsetting the expenses of nutrients and anaerobic digestate management. Different works have been carried out on the usage of anaerobic digestate for the microalgae cultivation from different sources. The main inhibitions of the usage of anaerobic digestate for microalgae cultivation is its detrimental nutrients concentrations, competing biological contaminants, high turbidity, ammonia and metal toxicity. By mitigating the NH_4^+-N inhibition, reducing turbidity, and improving the P/N ratio, anaerobic digestate dilution with secondary, tertiary wastewater or synthetic culture medium can be effective. Proper microalgal strain selection such as the resistance/non-susceptibility to the invasion and contamination of a wide range of pollutants, contamination, and high alkalinity tolerance is also important. In view of limited robust microalgal strains that can outcompete cyanobacteria and other predators, it is also recommended as future works that the researchers or biologists focus on discovering, crossbreeding, or even genetic engineering for more robust microalgae species. Appropriate pretreatment methods are capable of reducing the suspended solids in the nutrient medium, mitigating possible toxicity, reducing turbidity and retaining adequate nutrients for the cultivation of microalgae. Extra organics saccharides and volatile fatty acids and inorganic CO_2 can be supplied to low C/N ratio digestate. In order to promote simultaneous biogas upgradation and digestate treatment, there is a possibility of sourcing CO2 from biogas as a carbon source. Nitrification and simultaneous nitrification-desulfurization technologies can stabilize the nitrogen, reduce NH_4^+ toxicity, and changing the nitrogen oxidation state to a more amenable nitrate form. It can be anticipated that large-scale biofuel production from microalgae fuelled by waste anaerobic digestate would be a reality in the foreseeable future, with extensive research and development currently underway in these regions. Microalgae cultivation coupled with anaerobic digestate management is deemed as a win-win strategy for sustainable waste management and sustainable production of renewable microalgae-derived biofuel. Since the microalgae cultivation using anaerobic digestate is still underdeveloped, it is worthwhile to further investigate the influence of various reactor configurations, separation and purification technologies, and downstream processing for multitude microalgal products on the overall process feasibility.

CRediT authorship contribution statement

Chi Cheng Chong: Writing - original draft, Conceptualization, Manuscript writing, Review & editing, Visualization. **Yoke Wang Cheng:** Conceptualization, Visualization, Manuscript writing, Review & editing. **Syukriyah Ishak:** Manuscript writing, Review & editing. **Man Kee Lam:** Resources; Writing - review & editing, Project administration, Supervision. **Jun Wei Lim:** Conceptualization, Writing - review & editing. **Inn Shi Tan:** Writing - review & editing. **Pau Loke Show:** Writing - review & editing. **Keat Teong Lee:** Writing - review & editing. All authors read and approved the final manuscript.

Declaration of competing interest

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

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