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Can algae-based technologies be an affordable green process for biofuel production and wastewater remediation?

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Abstract

Algae is a well-known organism that its characteristic is prominent for biofuel production and wastewater remediation. This critical review aims to present the applicability of algae with in-depth discussion regarding three key aspects: (i) characterization of algae for its applications; (ii) the technical approaches and their strengths and drawbacks; and (iii) future perspectives of algae-based technologies. The process optimization and combinations with other chemical and biological processes have generated efficiency, in which bio-oil yield is up to 41.1%. Through life cycle assessment, algae bio-energy achieves high energy return than fossil fuel. Thus, the algae-based technologies can reasonably be considered as green approaches. Although selling price of algae bio-oil is still high (about \$2 L^{-1}) compared to fossil fuel's price of \$1 L^{-1}, it is expected that the algae bio-oil's price will become acceptable in the next coming decades and potentially dominate 75% of the market.

Keywords: Algae, Biofuel Production, Wastewater Remediation, Nutrient Removal

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1. Introduction

Algae is a well-known organism which is closely linked to the earth's long history. It is one of the most common floras found in the biosphere and exists everywhere. Generally, it is plant-like and has photosynthetic functions; however, there are no true developed roots, stems, leaves, flowers and vascular system (Mata et al., 2010). Unlike plants, algae is mostly found in aquatic environments (i.e. fresh water, marine) while a few species have been identified in terrestrial habitats. Simply, algae can be divided respectively into microalgae and macroalgae.

Algae is currently associated with environmental problems and one of the effects is algae bloom (eutrophication). This causes tremendous devastation in social, economic and environments. It changes water chemistry and reduces wastewater treatment efficiency (Wallace et al., 2016). However, algae does have practical benefits. The common applications of algae are agar, alginates, energy feedstock, fertilizer, nutrition, pollution bioremediation, natural pigments (carotenoids and chlorophylls) and stabilizing substances (Mata et al., 2010; Spolaore et al., 2006). Also, algae plays a huge role in biotechnology industry, such as glycerol, enzyme and other relevant products' extraction (Varshney et al., 2015); while the close relationship between algae and microbiology has been stated elsewhere (Ramanan et al., 2016).

Algae has been witnessed its prominent characteristics, in terms of green technology, in biofuel production and wastewater remediation. It contains abundant pigments (i.e. lipid, fatty acid and carbohydrate) and possesses biosorption ability (Sudhakar et al., 2017; Xu et al., 2014). The initial application of algae biomass in anaerobic digestion process to produce biomethane gas was conducted during the 1950s, by Golueke et al. (1957). At the same time, wastewater remediation was proposed by Oswald and

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Golueke (1960). Since then, algae applications have been magnifying significantly with the blossom of advanced techniques. Each technique exhibits typical performance in biofuel production and wastewater remediation, coupling own merits and demerits. Unfortunately, there is rare systematic review of technical approaches and comparison of these techniques; whereas the detail of future development is also insufficient, especially in commercialization and industrial scale applications. This critical review study aims to present the applicability of algae with in-depth discussion regarding three key aspects: (i) characterization of algae for its applications; (ii) the technical approaches and their strengths and drawbacks; and (iii) future perspectives of algae-based technologies.

2. Characteristics for biofuel production and wastewater remediation

Algae has many applicable characteristics for biofuel production and wastewater remediation. This generate from its biochemical identities. Thus, this section describes understanding of algae's biochemical origin to elucidate theirs implications for biofuel production and wastewater remediation.

Algae stores energy in cells via photosynthesis process, and these energy sources are extracted as feedstock for biofuel production. The quantity and quality of feedstock vary with algae species, cultivation techniques and extract technologies. Algae generally contains lipids, carbohydrates and proteins. However, the level of these constituents depends considerably on algae strains and culture conditions (Chia et al., 2017). Some species contain more lipid fractions while others have dominant carbohydrate profile.

The lipid content of microalgae varies from 2% to 75% and is shown in Table 1. *Chlorella emersonii*, *Chlorella vulgaris*, *Dunaliella* sp., *Nannochloris* sp. and *Phaeodactylum tricornutum* achieved lipid content more than 50%, and the

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maximum lipid productivity reached 116 mg L^{-1} d^{-1} (Mata et al., 2010). *Chorella* sp. and *Dunaliella* sp. were noticeable candidates for biofuel production. However, it is recommended to further consider lipid productivity, since high lipid content algae might have moderate productivity capacity. For instance, *Botryococcus braunii* and *Dunaliella tertiolecta* have lipid content above 70%; yet, their respective lipid productivity was modest.

Macroalgae, however, consists of lower lipid content compared to microalgae. The red and brown algae contained less than 5% lipid (Ross et al., 2008). Yet, carbohydrate was a dominant constituent from 60-70% in the form of cellulose and starch (Bucholc et al., 2014; Roesijadi et al., 2010). Therefore, macroalgae was more appropriate to produce alcoholic energy (i.e. bioethanol and biobutanol) with carbohydrate-based feedstock (Fernand et al., 2017).

Many studies have been undertaken to maximize lipid accumulation of algae, especially microalgae (Kotchoni et al., 2016; Vitova et al., 2015; Zhang et al., 2016). The environmental stresses (i.e. temperature, nutrient limit) were applied to activate defensive mechanism, resulting in the enhancement of lipid content (25-46%).

Table 1. Typical lipid profiles of algae species

[Insert Table 1]

Regarding fatty acid composition, Bigelow et al. (2011) also found that it differed from algae species. The *Cyanophyceae* sp. contained significant amount of palmitic acid (C16:0), oleic acid (C18:1n9c) and linoleic acid (C18:2n6) (Sahu et al., 2013). Moreover, *Nannochloropsis oculata* was a promising candidate for biofuel production without C22:6 (polar lipid) (Chia et al., 2017).

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3. Feedstock for bioenergy generation

3.1 Overall the application of algae as competitive feedstock for biofuel production

Thanks to its plentiful lipid and carbohydrate profile, algae has been employed as feedstock for biofuel production. Currently, several techniques are applied to produce biofuel and variety of products have been achieved. This section briefly describes the overall concept and technical feasibility of algae application in biofuel production. However, the combustion principle, which is not relevant to the scope of this review, is not discussed.

In biofuel production, algae serves as substrate to produce ethanol, butanol, biodiesel, hydrogen, methane and other products. Hossain et al. (2015) confirmed the possibility of producing ethanol from *Spirulina* sp. and this was strongly congruent in other reviews (Bibi et al., 2017; Patel et al., 2017; Vassilev and Vassileva, 2016).

Considering optimal algae strains for biofuel production, Hou et al. (2016) insisted *Golenkinia* sp. SDEC-16 generated electricity up to 170 mV in microbial fuel cells amongst five algae species. Aziz (2016) developed an integrated energy generation system, using macro algae (*Fucus* sp.). This system exhibited far greater power generation efficiency of 60%. Ripoll et al. (2017) produced hydrogen from combusting natural gas-air mixture with three types of algae. The hydrogen concentration from *Lessonia trabeculata* was essentially higher than *Lessonia nigrescens* and *Ulva lactuca*, achieving 9.56%.

An innovative strategy of using spent algae biomass from other industrial processes was initiated. Prajapati et al. (2014b) developed a “closed loop process” for biomethane production. Algae biomass, cultivating by effluent of anaerobic digestion process, was

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reused as substrate for biomethane production in that exact anaerobic process. Similarly, industrial spent biomass was applied for ethanol production after pigment extraction processes (Sudhakar et al., 2017). This was a co-producing concept as suggested by Song et al. (2015).

Xu et al. (2016) compared the performance of algae-based biofuel and second generation diesel fuel through combustion rate and other thermal properties. The burning rate of biofuel was comparable to diesel fuel, even higher while comparing droplet flames. Consequently, algae-based biofuel was suggested as an alternative for second generation diesel fuel.

3.2 Approaches and technologies used in biofuel production

3.2.1 Chemical reaction

The most common chemical reaction technique in biofuel production is transesterification. Oil is extracted from algae by alcohol chemical groups (i.e. methanol, ethanol), and converted into biodiesel with the involvement of catalysts (i.e. acid, alkaline and enzyme) and other relevant conditions (i.e. temperature, pH and retention time). The esterification process is less popular than transesterification technique (Fasahati and Liu, 2016; Ferreira et al., 2012). The optimization of operating parameters is studied critically.

Viêgas et al. (2015) conducted the *in-situ* transesterification of *Chlorella* sp., using sulfuric acid catalyst (5–20 wt%), at 60 and 100°C. The maximum ester yield was 96-98% while the oxidative and thermal stability were slightly lower than soybean-based biodiesel. Narula et al. (2017) optimized the transesterification process of Jatropha and algae oil blend through low temperature scenario (50°C). The biodiesel yield was

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81.98%, coupled with the methanol/oil volumetric ratio (3:5), KOH as catalyst (0.9% w/w) in a 180-min operational time at 50°C temperature. The author suggested the application of Jatropha and algae oil blend created economic benefits for industry.

Regarding an appropriate catalyst, Jin et al. (2014) studied the performance of Lewis acid catalysts from $SnCl_2$, $FeCl_3$, $ZnCl_2$, $AlCl_3$ and $NbCl_5$ for in-situ esterification/transesterification process. The catalyst of $ZnCl_2$ performed promisingly to produce crude biodiesel which fatty acid contain reached approximately 53%. However, initial moisture of material had significant drawback in the yield and characteristic of crude biodiesel. Lipolase was also an effective enzyme catalyst (96.9% oil yield) which oil products satisfied European standards (EN 14214) (Makareviciene et al., 2017). The optimal conditions were temperature of 30^oC, lipase amount of 10%, ethanol to oil molar ratio of 3:1 and reaction time of 26 hours. Besides, enzyme concentration and reaction time were actively determined oil yield; whereas molar ratio and temperature had negatively impact. In industrial scale, alkaline catalyst was preferred thanks to high reaction rate and low cost (Berrios et al., 2010). Temperature could accelerate reaction rate three times; yet, the quality of fatty acids was not influenced. The methanol and molar ratio was reported with modest effect on fatty acid contents if the ratio was higher than 6:1.

The Bligh-Dyer extraction method was also widely applied in chemical reaction approach. Specifically, the pretreatment application by free nitrous acid, under the Bligh-Dyer method, could augment extraction yield (Bai et al., 2014). The lipid content was found to increase during pretreatment time (up to 48 h) and with free nitrous acid concentration of 2.19 mg NO_2^--N L^{-1}. Likewise, Santillan-Jimenez et al. (2016) employed Bligh-Dyer method to reduce solvent:biomass ratio while oil yield (12.6 ± 0.8

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%) was higher than transesterification (6.2 ± 0.8 %). The Bligh-Dyer extraction method was fast but traditional Foch method witnessed more accurate results, employing to lipid content of algae higher than 2% (Iverson et al., 2001).

3.2.2 Biochemical conversion

i) Fermentation

Prior to fermentation, saccharification process was used as pretreatment method to break down the long chain carbohydrate compounds into monomers. This augmented the efficiency of latter processes, such as fermentation. For instance, saccharification coupled with fermentation process achieved more than 30% bioethanol yield (Lee and Lee, 2016). Furthermore, saccharification of spent algae biomass could combine with mild acid and/or marine bacterial consortia, followed by fermentation (Sudhakar et al., 2017). Although the spent biomass lost amounts of sugar contents (i.e. total carbohydrate, cellulose) in the pigments extraction process, its saccharification achieved more reducing sugar than the fresh algae. This was because of the complex sugar contents (i.e cellulose, hemicellulose) in fresh algae which hindered the microbial activity. The ethanol productivities of spent biomass from agar and alginate industries were recorded of 2.34 and 2.60 g L^{-1}, respectively.

In order to improve fermentation efficiency, Xia et al. (2016) co-fermented macroalgae (*Laminaria digitate*) and microalgae (*Arthrospira platensis*). The optimal mixing ratio of microalgae and macro algae was 90:10 based on C/N ratio. The pretreatment with acid and temperature were also applied to favor the formation of easy-consuming reducing sugars. The products of hydrogen (5.7%), ethanol (15.6%), acetic acid (9.6%) and butyric acid (18.5%) were received, corresponding with energy conversion efficiency of 54.5%.

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It can be seen the pretreatment process is critical which determines the efficiency of fermentation process. The long-chain complex compounds are degraded and formed monomers, such as glucose, fucose, mannitol, glycerol, enhancing the fermentation yield.

ii) Anaerobic digestion

The potential of anaerobic digestion in renewable energy sector has been stated elsewhere (Khan et al., 2017). Similar to fermentation, the pretreatment process was also important to anaerobic digestion. Thermal pretreatment, using *Nanochloropsis oculata* as substrate, increased net biogas up to 0.44 L biogas g^{-1} volatile solid (VS) according to heating time function of $90^{o}C$, but not at 30 and $60^{o}C$ (Marsolek et al., 2014). However, this thermal pretreatment brought minus energy balance. In industrial application, the excess heat from other processes is required to compensate for this thermal pretreatment. In term of operating conditions, Capson-Tojo et al. (2017) assessed the influence of temperature on anaerobic digestion, which were mesophilic (35 °C) and thermophilic (55 °C) conditions. Only the thermophilic temperature enhanced hydrolysis process and the soluble fraction in thermophilic reactor was 2.5 - 4 g L^{-1}, being higher than mesophilic reactor of 1.5 g L^{-1}. However, methane yield was almost similar in both conditions due to the shortage of acetogenic, methanogenic activities or the obstruction of toxic compounds.

The anaerobic digestion is modified as co-digestion concept to improve efficiency. Methane production is enhanced significantly compared to the digestion of single substrate. The problem of low C/N ratio of single substrate, which discouraged bacteria development, is overcome (Ward et al., 2014). Zhong et al. (2013) tested the co-digestion possibility of blue algae (Taihu lake, China) with corn straw. The single

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feedstock of blue algae received 108 to 160 mL CH_4 g^{-1} VS; however, there was a decline in methane production due to the elevation of ammonia concentration. The methane productivity of co-digestion was elevated 64% with productivity of 234 mL CH_4 g^{-1} VS. The optimal ratio of blue algae and corn straw was 20:1. Likewise, the optimal ratio of blue algae in Taihu lake and swine manure in co-substrate was 2:1 with methane yield of 212.7 mL g^{-1} VS (Miao et al., 2014). The co-digestion of *Microcystis* sp. (blue green algae), cannery seafood wastewater and glycerol waste achieved 291 mL CH_4 g^{-1} VS, corresponding to the respective mixing ratio of 94:1:5 (Panpong et al., 2015). It can be seen that the methane yield of co-digestion process was likely higher than 200 mL g^{-1} VS; whereas the single substrate's yield was rarely reached that amount.

The methane generation, pH, inorganic nitrogen and other alcohol products (i.e. acetate, propionate, butyrate, and valerate) could be estimated by anaerobic digestion model no.1 (Yuan et al., 2014). The operational parameters of anaerobic digestion are summarized in Table 2.

Table 2. Summary of anaerobic digestion process operations.

[Insert Table 2]

3.2.3 Thermal conversion

i) Hydrothermal liquefaction process

Hydrothermal liquefaction is a direct liquefaction process, producing bio-oil in the relative oxygen absence state by pressurizing inert gases (i.e. N_2 or He) or reducing gases (i.e. H_2 or CO) at high temperature (200–380°C) and pressure (5–28 MPa). HTL has substantial advantages compared to traditional bio-oil production methods,

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including rapid reaction, using high moisture content feedstock with no lipid content restriction (Tian et al., 2014). Moreover, HTL does not require intensive amount of energy for the drying stages.

Reddy et al. (2014) applied microwave assisted heating and conventional heating to extract lipid from wet algae (subcritical condition). The conventional heating and microwave assisted heating performed maximum extraction efficiency at 220^{o}C and 205^{o}C, respectively. The microwave assisted heating could completely extract lipid ingredient, whereas conventional heating only reached 70%. The microwave assisted heating saved 2-8 times energy consumption than traditional extraction. Yet, the industrial application of microwave assisted technology was not available due to the insufficient penetration depth of microwave at large scale. Regarding solvents, alcoholic solvents, such as methanol and ethanol, achieved 22.8 and 23.8% bio-oil yield, respectively, which was observed higher bio-oil yield from water solvent of 16.33% (Biswas et al., 2017). Likewise, catalysts (i.e. platinum, ruthenium, nickel and cobalt) could further improve heating value of biocrude through the formation of hydrocarbons (Wang et al., 2016). Cobalt and nickel favored the formation of octadecane; and platinum and ruthenium elevated octadecane and hexadecane of 698% and 228%, respectively. Costanzo et al. (2016) pretreated algae in 225^{o}C and 15 min, coupled with 5% Ru/C catalyst, resulted in 15-22% high quality oil yield. Xu and Savage (2017) concluded that temperature and catalyst were able to control bio-oil quantity and quality. Rising of temperature (350^{0}C to 400^{o}C) favored insoluble biocrude yield (38.1–42.5 wt%), while diminishing soluble biocrude efficiency (6.6-2.5 wt%). Conclusively, several authors agreed that temperature and catalysts are the key factors in enhance bio-oil production in HTL process (Shakya et al., 2015).

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The composition of algae species also determined HTL yield. The twice larger carbohydrate composition of algae species in the study of Singh et al. (2015), compared to Shakya et al. (2015), resulted in double bio-oil yield. The maximum bio-oil yield of *Ulva fasciata* was 12% and lowest bio-oil yield of 7% was produced by *Enteromorpha* sp.

By-products of the HTL process have tremendous potential for further applications. The aqueous phase from HTL process contained substantial amounts of nutrient for recovery. Tommaso et al. (2015) reused aqueous phase of HTL process for anaerobic degradation while Maddi et al. (2016) recommended the aqueous phase for liquid fuel and chemical production in industrial scale. Interestingly, Shanmugam et al. (2017) recovered 99% phosphorus and 40-100% ammonia from these aqueous feedstock by struvite formation. These nutrients could be recirculated to the algae cultivation process to reduce the economic burden.

ii) *Pyrolysis*

Pyrolysis of algae is complied with temperature from 300 to 700^{o}C or beyond, while there is a definite absence of oxygen. The main products are solid form (biochar), liquid form (bio-oil) and gas form (pyrogas) (Chiaramonti et al., 2017).

Microwave-assisted technology and catalysts were extensively applied in pyrolysis process. Li et al. (2013) assessed the influence of microwave power, metal oxides (CuO and MgO) and metal salts in the pyrolysis process. The maximum bio-oil yield (20.63%) was achieved at microwave operation of 1500 W without adding catalysts. While mixing algae with metal salts (5% $MgCl_2$, $ZnCl_2$ and NaH_2PO_3), bio-oil yield increased 6.3%, 16.92% and 0.71%, respectively. The authors suggested MgO is the best microwave absorber. Zhang et al. (2016) insisted that 10% H_2/Ar gases assisted

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extensive conversion of long chain fatty acids into hydrocarbons with the highest energy yield of bio-oil (236.9%). The Mg-Al catalysts with double oxide/ZSM-5 composites (MgAl-LDO/ZSM-5) were witnessed significantly higher bio-oil yield than pyrolysis without catalysts (Gao et al., 2017). The optimal conditions were temperature of 823 K, catalyst/algae ratio of 0.75 and heating rate of 10 K min^{-1}, receiving 41.1% bio-oil yield. The high heating value of 37.164 MJ kg^{-1} was higher than the one without catalyst (21.243 MJ kg^{-1}).

Similar to anaerobic digestion, Yan et al. (2017) received higher saturated carbon (85%) products when co-pyrolysis micro/macro algae with used engine oil, compared to the bio-oil from algae pyrolysis only. The drawbacks of single substrate pyrolysis were curtailed by significantly decreasing the N, S and O contents and improving energy recovery.

3.3 Comparison of algae-based technologies

In terms of biofuel quality and yield, HTL and pyrolysis produced similar carbon distribution products. The carbon chains of C_4 to C_{20} were generated by pyrolysis process, and HTL was preferred with C_4 to C_{22} carbon chains, including major of C_6, C_{16} and C_{18} (Yang et al., 2016). This was noticeable that high lipid content was not important in these two technologies. Referring to energy generation, algae-based biofuel does have potential benefits. Liu et al. (2013), through life cycle assessment process, revealed that algae bio-energy generating from HTL process resulted in high energy return than petroleum fuel. Therefore, the benefit was achievable from algae-based biofuel with industry support (i.e. CO_2 supply as nutrient).

Although the advantages of these technologies are evident, the restraints are also considerable (Table 3). Firstly, the characteristics of feedstock may not meet the

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requirement for biofuel production. The macro algae contains unfavorable compositions for anaerobic digestion (i.e. polyphenols, cellulosic fibres and lignin). The activity of micro-organism, therefore, is limited and it resulted in insufficient digestibility and gas production. The sulfide content of macro algae also caused difficulties in the anaerobic digestion process (Ward et al., 2014). However, the high ash and metal content, associated with low heat value, made macro-algae more appropriate for hydrous pyrolysis and anaerobic digestion (Ghadiryanfar et al., 2016). Brownbridge et al. (2014) qualitatively evaluated biodiesel production yield by esterification as the following order: algae oil content > algae annual productivity per unit area > plant production capacity > carbon price increase rate. Clearly, the identity of algae feedstock is the most important criterion in bio-energy production. As previously mentioned, temperature and catalysts also play an important role. Catalysts, such as metals, metal oxides and metal salts, were preferable in hydrothermal liquefaction and pyrolysis technologies; on the other hand, temperature was involved critically in all technologies.

Some micro-algae species contain low C/N ratio so they were co-fermented with macro-algae (high C/N ratio) in hydrogen fermentation process (Xia et al., 2016). The high ash content in wastewater algae which affects the HTL process was also considered by Chen et al. (2014). Thus, pretreatments by centrifugation and ultrasonication are recommended which can improve bio-oil from 30 to 55%. Additionally, the low lignin and high polysaccharides compositions made macroalgae a proper choice for liquid biofuel production through the fermentation, and biogas production through anaerobic digestion (Ghadiryanfar et al., 2016).

The high energy consumption and cost of feedstocks in the production process also attract attention. Fasahati et al. (2017) examined heat and power production from

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anaerobic digestion process. The anaerobic digestion unit used the most energy with 14% of total consumption. Yet, the HTL process did not need feedstock drying process; thus, energy consumption was less (Ghadiryanfar et al., 2016). Roesijadi et al. (2010) compared fermentation and HTL process. The HTL process likely brought more revenue. The market prices of fermentation (ethanol product) and HTL (biodiesel product) were \$2.2 and \$2.8 gallon^{-1}, respectively. This was due to the better productivity of fermentation process. Cerón et al. (2008) applied the concept of using wet biomass for transesterification, like the HTL process, but the product yield fell by 20%.

Table 3. Advantages and disadvantages of technologies

[Insert table 3]

4. Application of algae in wastewater remediation

4.1 Overall wastewater remediation by algae

Generally, algae is able to remove nutrient mainly via biosorption (assimilation) mechanism, to generate biomass. The nutrient in wastewater sources serves as substrate for biomass formation. The main macro nutrients are carbon, nitrogen and phosphorus, although the role of micro nutrients (Ca, Fe, Zn, Mg) are also recognized (Zhao et al., 2016). It must be noted that different types of algae species have different nutrient removal capacities. For this reason, some authors screened out algae species to find the best one (Ge and Champagne, 2017; Hou et al., 2016). Also, the effect of operating conditions (i.e. retention time, initial nutrient, recycling ratio) and seasonal conditions (i.e. temperature, light period) strongly affected the outcomes (Sindelar et al., 2015). Therefore, the optimization of these conditions is critically needed.

Algae contributes to nutrient removal via different mechanisms (i.e. precipitation, volatization, biosorption, nitrification, denitrification). This section explores the influences of these processes in nutrients removal. It also looks at lab scale to full scale scenarios, with various algae species, operating conditions, wastewater sources and synergistic cooperation with microbial consortium.

4.2 Approaches of algae-based technologies in wastewater remediation

Through lab scale experiments, the performance of different algae species was examined. Prajapati et al. (2014a) used *Chroococcus* sp.1 to remove beyond 80% pollutants, such as NH_4^+-N, NO_3^--N, Total Phosphorus (TP), and produced the highest amount of biomass and best nutrient recycling from livestock wastewater. In municipal wastewater, macroalgae (*Chaetomorpha linum*) treated respective nitrogen and phosphorus of 86.8 ± 1.1% and 92.6 ± 0.2% (Ge and Champagne, 2017). Also, *Golenkinia* sp. SDEC-16 was 3-fold better than S*elenastrum capricornutum* in terms of chemical oxygen demand (COD) and total nitrogen (TN) removal (Hou et al., 2016). In aqueous effluent of anaerobic digestion, *Arthrospira maxima* and *Tetradesmus obliquus* could remove nitrogen at 98.9-99.8%, whereas *Phaeodactylum tricornutum* and *Botryococcus braunii* treated 79.0-88.5% phosphorus (Massa et al., 2017).

Varieties of operating conditions to optimize nutrient removal were also conducted. In algae scrubber, the higher inflow resulted in higher algae productivity but the pulsed inlet condition brought no benefit compared to constant inflow. The TP-Ca correlation was independent of algae species and relied on pH value (Sindelar et al., 2015). Some authors enhanced nutrient removal efficiency by introducing additional compounds. For example, Zhao et al. (2016) added ion Fe^{3+}, iron and $CaCO_3$ powder to fix iron deficiency and balance carbonate and bicarbonate system. Consequently, biochemical oxygen demand (BOD_5), TN and TP removal efficiency increased 28%, 8.9% and 22%, respectively. However, the authors insisted the low temperature concept should be further investigated. The agricultural phytohormones (i.e. gibberellin, indole-3-acetic acid, and brassinolide) could also boost biomass growth (Pei et al., 2017). However, the authors did not document the nutrient removal results.

A predictive and quantification tool was developed by Zimmo et al. (2004) to estimate nitrogen removal and balance in algae pond. Generally, high temperature favored nitrogen removal while the effect of organic loading was insignificant. Interestingly, the authors elucidated the contribution of each removal mechanism (i.e. sedimentation, volatilization and denitrification) in overall nitrogen removal. The denitrification in algae pond contributed more than 7-37%, while the ammonia volatized was lower than 1.1% of total nitrogen removal. Unfortunately, the authors did not quantify nitrogen biosorption by algae. The model for N removal was constructed in Eq (1):

$$\text{Overall N-removal rate} = [N_{denit}\ \text{rate} + N_{sed}\ \text{rate}] + [N_{AV}\ \text{rate}] = [-317.8 + 15.5\lambda_{S,N} + 0.26\lambda_{S,BOD} + 2.87T] + [3.3NH_3 + 4.9] \qquad \text{Eq (1)}$$

Whereas

N_{denit} : Denitrification nitrogen mg-N m^{-2} d^{-1}

N_{sed} : Sediment nitrogen mg-N m^{-2} d^{-1}

N_{AV} : Volatile nitrogen mg-N m^{-2} d^{-1}

T: Temperature

$\lambda_{S,N}$: Nitrogen loading rate (kg N ha^{-1} d^{-1})

$\lambda_{S,BOD}$: Organic loading rate (kg BOD ha^{-1} d^{-1})

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The most common type of full scale algae-based nutrient removal system is High Rate Algae Pond (HRAP) (Figure 1a) thanks to its low cost and less operation requirement. The HRAP attracted interest in combining with other processes as pretreatment or post-treatment options. Park et al. (2013) reported the recycling of algae resulted in higher biomass (more than 20%) productivity in HRAP. The recycling activity enhanced the reproduction of *Pediastrum boryanum*. HRAP could remove 51-57% of phosphorus and beyond 85% of NH_4^+-N in anaerobic digested wastewater, whereas TN removal was moderate from 51% to 62% (de Godos et al., 2016). This finding confirmed the contribution of biosorption (or assimilation) in TN removal which varied seasonally from 17%-28% in winter and 37%-57% in summer. Typically in manure wastewater, the nitrogen uptake rate in HRAP was 1.10 g N m^{-2} d^{-1} during winter; whereas the phosphorus uptake rate was 0.16 g P m^{-2} d^{-1} (Mark Ibekwe et al., 2017). In HRAP, the dominant micro-organism species included *Cyanobacteria*, *Alpha*-, *Beta*-, *Gamma*-, *Epsilon*-, and *Delta-proteobacteria*, *Bacteroidetes*, *Firmicutes*, and *Planctomycetes*. The existence of *Chlamydomonadaceae* favored H_2 production in fermentation process whereas *Cyanobacteria* generated photosynthetic oxygen. It can be seen all the authors agreed on the significant contribution of microbial community on nutrient removal in HRAP.

Another emerging system concerns algae-based photo/membrane bioreactors (Figure 1b&c), which attract interest thanks to the significant nutrient removal efficiency. Zhu et al. (2013) cultivated microalgae *Chlorella zofingiensis* in photobioreactors with piggery wastewater. As a result, COD, TN and TP were removed 65.81% - 79.84%, 70.88% - 82.70% and 98.17% - 100%, respectively. Xu et al. (2014) applied an algae-based membrane bioreactor for nutrient removal. The system was able to remove 66 ± 9% total P. Yet, the contribution of algae in nutrient removal was not discussed. Also,

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Hu et al. (2015) combined nano TiO_2 modified hollow fiber membranes in an algae reactor for wastewater polishing. While 78% phosphorus was removed, the precipitation of P was detected on algae surface, without any P biosorption into algae's cells. According to all authors, the algae-based membrane bioreactors were reported with clogging problems due to the flocculation of algae and filamentous species. The extracellular polymeric substances generated by micro-organisms could be one factor responsible for clogging; however, it was less important. Alternatively, the embedded nano TiO_2 hollow fiber allowed elevating the surface hydrophilicity and membrane porosity, resulting in reducing membrane's clogging (Hu et al., 2015).

Algal Turf Scrubber (ATS) model is a water filtering system which allows the penetration of sunlight to nourish algae, coupled with the removal of undesired chemical substances (Figure 1d). In specific, nitrogen removal efficiencies in synthetic and horticultural wastewater were 59-99% and 20-86%, respectively. However, there was a significant difference in phosphorus removal due to chemical precipitation of phosphorus in horticultural wastewater (Liu et al., 2016). Bohutskyi et al. (2016) remove nutrients in agricultural storm water by ATS. The TN, TP and BOD_5 removal efficiencies were 6%, 22% and 21%, respectively. The poor nitrogen removal efficiency was due to the limited P source.

[Insert Figure 1]

Figure 1. The HRAP (1a); Photobioreactor (1b); Algae-Based Membrane Bioreactor (1c) and Algae Scrubber (1d)

Nutrient removal efficiency was boosted by the assistance of microbial activity (Ramanan et al., 2016). The synergistic support of microbial community and algae could increase COD and TP removal up to 93.01% and 98.78%, respectively (Liu et al.,

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2017). In contrast, Huang et al. (2015) found that nitrogen and phosphorus removal of algae-bacteria symbiosis in sequencing batch reactor (SBR) contributed 40.7% - 45.5% in total N and 44% in total P removal, respectively. This was lower in the SBR system without algae. The authors explained the involvement of algae delayed the development of the microbial community, especially *Nitrospiraceae* and *Nitrosomonadaceae*. Alternatively, the short anaerobic digestion of activated sludge was applied as pretreatment to increase microbial community, before adding algae and activated sludge (Li et al., 2016). Noticeably, Liu et al. (2016) quantified the contribution of each algae species (% N and % P removal efficiency) in overall nutrient removal. A summary of nutrient removal by algae is documented in Table 4.

Table 4. Summary of algae species' nutrient removal performance

[Insert table 4]

4.3 Comparison of algae-based technologies

Algae biomass is a key factor in contaminants remediation and it is true that the more biomass achieves, the more pollutants are removed. Importantly, these processes have their own advantages and disadvantages.

Regarding algae ponds, theirs benefits have been stated and emphasized elsewhere (Mark Ibekwe et al., 2017) with less N and P residuals, less threatening to surface and ground water quality. It made cultivation of sufficient biomass possible thanks to the vast area available. The nitrogen accumulation in sediment was an important removal mechanism, but depending on temperature and organic loading rate (Zimmo et al., 2004). Through the sedimentation process, the algae-based pond removed nitrogen more than 46% compared to duckweed-based pond; furthermore, it was also 7 to 37%

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higher via denitrification process. Although the algae-based pond had higher alkaline environment than the duckweed-based pond; the reason of difference was not elucidated in nitrogen removal efficiency.

In HRAP, the challenge posed by inadequate sunlight and nutrient level was tackled by Park et al. (2013) by recycling algae to improve contact time. The recycling concept further enlarged the microbial community. The capital cost of HRAP was also attractive, calculated by Posadas et al. (2017), to be €8.5 m^{-2}.

Concerning the algae reactor, the treatment in outdoor reactors with proper conditions (ambient temperature and sunlight), likely bringing more biomass and nutrient removal efficiency than the control environment in lab conditions. The microbes consortium in outdoor condition shifted biomass yield (Prajapati et al., 2014a). Moreover, the high ammonia removal efficiency was attributed to the accumulation and adsorption of organic carbon on the algae and bacteria surface, and aeration causing volatilization and shifting of pH.

The common bottle neck in algae-based remediation was the unbalance of C:N:P ratio and additional compounds (Hou et al., 2016; Zhao et al., 2016). This resulted in the insufficient COD, TN or TP removal efficiency. The loss of nitrogen in the nitrification and volatilization process (5-33%) shortened the nitrogen supply for algae cells. Moreover, some compounds (i.e. EDTA) which were able to diminish the P precipitation should be noted (Liu et al., 2016). This could be altered by diluting the influent or adding more substrates, such as CO_2, Fe^{3+}, $CaCO_3$ (de Godos et al., 2016; Zhao et al., 2016). The addition of CO_2 could double the biomass production per surface unit. Also, the carbonate-bicarbonate and Fe^{3+} amended the physico-chemical balance

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and enhanced interaction of algae and micro-organism. The optimal C:N:P ratio was suggested as 106:16:1 (Redfield ratio), and recently modified to 117:14:1.

5. Future perspectives on algae application in biofuel production and wastewater remediation

The technical feasibility of algae-based biofuel production as third generation feedstock is definitely clear (Ho et al., 2014; Hossain et al., 2015). The common technical approaches are optimizing the operation processes, and combining with other chemical-based, biological-based processes to enhance removal efficiency and reduce costs (Figure 2).

[Insert Figure 2]

Figure 2. Overall approaches of algae-based technology in energy production and wastewater remediation

However, economic feasibility is still not able to fulfill the sustainable commercialization's demand. Most researches agreed on the modest economic benefit of algae-based biofuel production (Gendy and El-Temtamy, 2013; Song et al., 2015). One of the reasons was the low ethanol percentage (0.85-1%) (Hossain et al., 2015). The Return of Investment (ROI) was mostly based on the algae oil content, while the assumption of longer ROI has resulted in higher uncertainty results. Moreover, larger plant capacity could decrease production costs. For example, the 10,000 tons year^{-1} plant had 50% probability of producing biofuel with a cost greater than £1.3 per kg, whereas the 100,000 tons year^{-1} plant had 20% probability of producing biofuel at a cost less than £1.0 per kg (Brownbridge et al., 2014).

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Chia et al. (2017) suggested the economic and commercialization advantage of biogas compared to biofuel. The biogas production consumed all of the algae biomass while biofuel needed part of the algae biomass (i.e. lipid, carbohydrate). Furthermore, the biogas product offered more energy security than biofuel (Campbell et al., 2009). Thus, the public support for the right policy is strongly needed if this environmentally friendly fuel is widely commercialized (Amanor-Boadu et al., 2014). This is one of the critical factor configuring the success of algae biofuel's commercialization.

Unlike biofuel production, the techno-economic feasibility of wastewater remediation was not sufficiently established. The estimation of algae pollution permit was R2.25 and R111 g^{-1} algae (R as rand, South African currency) (de Lange et al., 2016). The integration of wastewater remediation and biofuel production system could also produce revenue (Zeraatkar et al., 2016). Similar to biofuel production, the public support that encouraged the growth of this market is also strongly needed.

The selling cost of algae oil was more than \$2 per L, whereas the fossil fuel price is below \$1 per L (Judd et al., 2017). A 35-86% price reduction was achieved when recycled products were coupled with CO_2, nutrients remediation. However, the selling price was still uncompetitive with fossil fuel's price in most circumstances, and this could be tackled by the following alternatives:

- Properly installation of algae-based technologies near wastewater treatment and CO_2 abatement sources to reduce transportation cost.
- Taking advantage of geographical and climate conditions in algae cultivation. For instance, the region from Middle East to East Asia had sufficient natural illumination intensity and temperature. The artificial light further burdened \$25 kg^{-1} dry weight of biomass.

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- Enhancing algae's lipid content and productivity. The increase of lipid content (25-50%) has cut off 39% price, and productivity of 25 to 50 g m^{-2} d^{-1} has reduced 19% price.
- Reusing extracted algae which value was from \$0.27 kg^{-1} to \$1.8 kg^{-1}.
- Employing highly efficient feeding sources, such as municipal wastewater. This could concurrently lessened the wastewater treatment price and diminished biofuel price of \$0.55-0.59 L^{-1}.

In the future, Gambelli et al. (2017) predicted the development of an algae biofuel market that would be in place by the year 2030 and dominate 75% of market share. Ruiz et al. (2016) admitted that the algae-based biofuel could not generate large revenues in the short-term, but they did believe that in the next decade this trend would change thanks to improvements in R&D activities.

6. Conclusion

In conclusion, technical improvements in algae-based biofuel production and wastewater remediation have been magnified in recent years. Nutrients were also removed to a satisfying degree. Yet, the algae-based technology does have certain disadvantages in commercialization and industrial scale application. The high energy and capital cost demands limit the profits according to most authors. Furthermore, there is a lack of adequately public support. However, it is believed that more R&D work will reduce the production costs and increase revenue in the coming decades.

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[Figure captions]

Figure 1. The HRAP (1a), Photobioreactor (1b), Algae-Based Membrane Bioreactor (1c) and Algae Scrubber (1d)

Figure 2. Overall approaches of algae-based technology in energy production and wastewater remediation

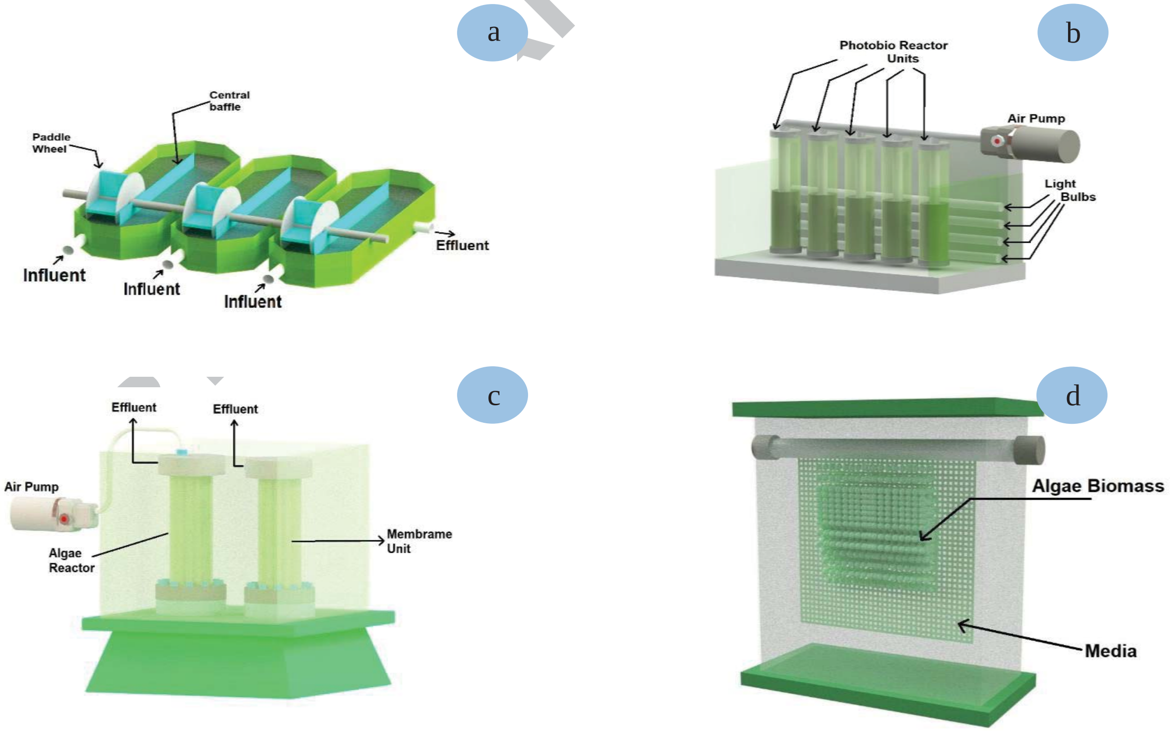

Figure 1. The HRAP (1a), Photobioreactor (1b), Algae-Based Membrane Bioreactor (1c), and Algae Scrubber (1d)

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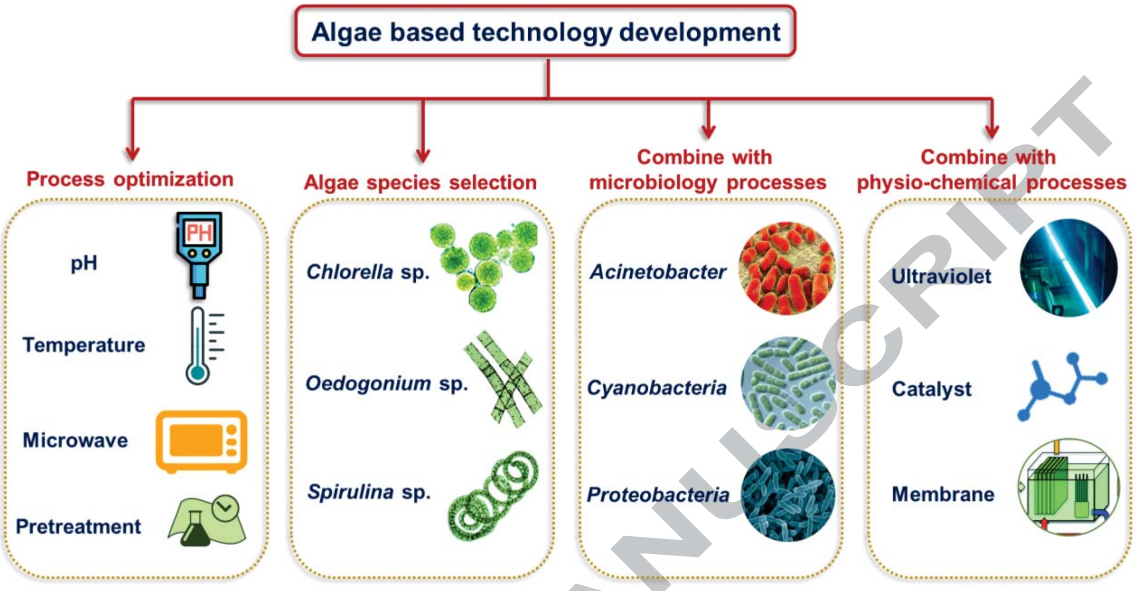

Figure 2. Overall approaches of algae-based technology in energy production and wastewater remediation

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Tables Captions

Table 1. Typical lipid profiles of algae species

Table 2. Summary of anaerobic digestion process operations

Table 3. Merits and demerits of technologies

Table 4. Summary of algae species' nutrient removal performance

Table 1. Typical lipid profiles of algae species

Algae species	Lipid content (%)	Reference	Algae species	Lipid content (%)	Reference
Anabaena cylindrica	4–7	Becker (2007)	*Chlorella zofingiensis*	34-45	Zhu et al. (2013)
Aphanizomenon flos-aquae	3		*Ulva fasciata*	1.83 ± 0.21	Singh et al. (2015)
Chlamydomonas rheinhardii	21		*Enteromorpha sp.*	5.6 ± 0.2	
Chlorella pyrenoidosa	2		*Sargassum tenerrimum*	2.03 ± 0.3	

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Chlorella vulgaris	14-22		*Nannochloropsis*	18.12	Shakya et al. (2015)
Dunaliella salina	6		*Pavlova*	13.88	
Euglena gracilis	14-20		*Isochrysis*	18.98	
Porphyridium cruentum	9-14		*Chaetomorpha linum*	12-18*	Ge and Champagne (2017)
Scenedesmus obliquus	12-14		*Golenkinia* SDEC-16	36-38*	Massa et al. (2017)
Spirogyra sp.	11-21		*Chlorella vulgaris*	27-30*	
Arthrospira maxima	6-7		*Selenastrum capricornutum*	30-33*	
Spirulina platensis	4-9		*Scenedesmus* SDEC-8	29-30*	
Synechococcus sp.	11		*Scenedesmus* SDEC-13	27-28*	
Spirulina platensis	7.75 ± 0.06	Sahu et al. (2013)	*Nannochloropsis oculata*	15.31	Chia et al. (2017)
Oscillatoria acuta	4.47 ± 0.06		*Chlorella vulgaris*	16.41	
Calothrix sp.	3.42 ± 0.05		*Nannochloropsis* sp.	59.9	
Lyngbya sp.	2.52 ± 0.03		*Porphyridium cruentum*	8	
Leptolyngbya sp.	3.23 ± 0.07		*Scenedesmus obliquus* CNW-N	22.4	
Synechococcus sp.	4.20 ± 0.06		*Dunaliella tertiolecta* ATCC 30929	70.6-71.4	

Note: *Retrieved from graph

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Table 2. Summary of anaerobic digestion process operations

Algae species	Digestion concept	Pretreatment	Organic loading rate (g VS L^{-1} d^{-1})	Incubation Temperature (°C)	Digestion time	Methane production (mL CH_4 g^{-1} VS)	References
Microcystis spp. (blue algae)	Co-digestion with corn straw	-	4.00	35	10 days	234	Zhong et al. (2013)
Microcystis, *Cyclotella*, *Cryptomonas* and *Scenedesmus*	Swine manure	-	86.68 ± 1.47 to 89.86 ± 2.15	35	22 days	212.7	Miao et al. (2014)
Microcystis sp. (blue green algae)	*Cannery seafood wastewater and glycerol waste*	-	4.48 - 24.91	35	64 days	291	Panpong et al. (2015)
Nanochloropsis oculata	-	Heating (90°C)	44.8 ± 2.2 g soluble COD L^{-1} algae	37	12 days	0.41, 0.43, and 0.44 L biogas g^{-1} VS corresponding 1, 3.5, and 12 h preheated	Marsolek et al. (2014)
Nannochloropsis gaditana	-	-	0.5 g COD·L^{-1}·d^{-}	35 (Mesophilic)	30	400 - 450*	Capson-Tojo et al.

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			1	55 (Thermophilic)		350 -400*	(2017)
Caulerpa prolifera *Gracilaria bursa-pastoris* *Colpomenia sinuosa*	-	Heating at 105 °C in 24 h	9% TS	35	40	86.35 74.68 24.53	El Asri et al. (2017)
Saccharina japonica (brown algae)	-	-	29 wt% TS (sediment) 1 wt% TS (algae)	37	110	180.3 ± 11.7	Miura et al. (2015)
Saccharina japonica	-	-	2.00 2.87 1.74	37	39 28 46	358 335 346	Miura et al. (2016)

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Table 3. Merits and demerits of technologies

	Transesterification/*In-situ* transesterification/Esterification	Fermentation	Anaerobic Digestion	Hydro Thermal Liquefaction	Pyrolysis
Merits	High yield bio-oil Simple concept Relative low temperature No by-products	High density product (H_2) as 97% High energy density by mass (142 MJ kg^{-1}) and clean combustion product (H_2O)	Provided excessive nutrient recovery and biogas to generate revenue High methane yield (higher 200 mL g^{-1} VS) of co-digestion of macro and microalgae	Using wet biomass Rapid reaction (10-120 mins) Dependent on lipid content of feedstock Contain energy density twice pyrolysis	Considered to be intermediate energy carrier Fast reaction time Nutrient in aqueous phase can be recovered
Demerits	Prefer dry biomass High energy requirement through the long lipid extraction stage Require extraction solvent Highly depend on the lipid content of feedstock Time-consuming	Depend on characteristics of feedstock Require pretreatment to accelerate hydrolysis	Process can be inhibited by ammonia, saline and sulfur Require pre-treatment process Much energy required for drying and processing biomass Low carbon to nitrogen ratio	High ash content reduces bio-oil yield Prefer organic solvent and catalyst	Depend on feedstock characteristic Prefer catalyst to improve oil quality High temperature requirement (300 to 700^{o}C or beyond) High energy consumption
Reference	Narula et al. (2017) Jin et al. (2014)	Lee and Lee (2016) Xia et al. (2016)	Ward et al. (2014) Panpong et al. (2015)	Tian et al. (2014) Bridgwater and Peacocke (2000)	Chiaramonti et al. (2017) Gao et al. (2017)

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Table 4. Summary of algae species' nutrient removal performance

No.	Type of algae	Type of wastewater	Initial concentration (mg L^{-1})						Removal efficiency (%)						Note	Reference
			pH	NH_4^+-N	NO_2^--N	NO_3^--N	TP	COD	NH_4^+-N	NO_2^--N	NO_3^--N	TN	TP	COD		
1	Macroalgae *Chaetomorpha linum*	Primary wastewater	7.28-7.54	8.9-24.5	<0.1	0-0.83	0.75-2.35	102.4-307.6	-	-	-	97.6±0.8	79.1±2.5	-	-	Ge and Champagne (2017)
		Secondary wastewater	6.72-7.13	0-0.85	<0.1	9.56-17.98	0.13-0.54	14.5-29.6	-	-	-	98.6±0.3	72.7±0.8	-	-	
		Centrate wastewater	7.88-8.35	632-896	0-0.09	0-0.15	15.5-20.6	304-446	-	-	-	86.8±1.1	92.6±0.2	-	-	
2	*Chroococcu*	Dairy	7.80 ±	160.67 ±	-	74.67	201.67 ±	2965.00 ±	98	-	83	-	84.5	76.	-	Prajap

	s sp.1	cattle based livestock wastewater	0.56	2.73		± 1.37	6.83	20.49			.7			6		ati et al. (2014a)
	Chroococcus sp.2								73.7	-	82.3	-	71.1	77.4	-	
	Chlorella pyrenoidosa								92.9	-	78.8	-	63.6	78.2	-	
	Chlorella vulgaris								93.2	-	81.2	-	52.3	68.8	-	
3	*Golenkinia* sp. SDEC-16	Kitchen waste anaerobically digested effluent	8.31	1172.38	-	0.24	11.69	4258.28	-	-	-	37.39	98	43.59	-	Hou et al. (2016)
	C. vulgaris								-	-	-	22.12	98	20	-	
	S. capricornutum								-	-	-	12.3	97	13.51	-	

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	Scenedesmus SDEC-8								-	-	-	12.30	98	26.92	-	
	Scenedesmus SDEC-13								-	-	-	21.24	97	36.71	-	
4	*Diatom (82%)*, *Oscillatoria* (0.5 %) and various species	Street wastewater	7.55 ± 0.13	-	-	4.52 ± 0.96	1.39 ± 1.01	-	-	-	-	-	10 - 50 %	-	-	Sindelar et al. (2015)
5	*Chlorella* sp.	Contaminated	-	0.75 ± 0.2	-	2 ± 0.5	3.25 ± 0.5	-	-	-	-	99.98	77.71	91	-	Zhao et al. (20

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		surface water														16)
6	Mainly genera *Coelastrum*	Anaerobically digested wastewater	-	48.8 ± 12.3	-	-	8.4 ± 1.4	167 ± 20	65-85	-	-	-	51-57	-	Spring	de Godos et al. (2016)
			-	46.1 ± 9.7	-	-	9.2 ± 2.3	178 ± 11		-	-	-		-	Summer	
			-	53.5 ± 12	-	-	8.9 ± 1.6	140 ± 39		-	-	-		-	Autumn	
			-	58.1 ± 9.7	-	-	8.1 ± 1.9	159 ± 34		-	-	-		-	Winter	
7	*Chlorella zofingiensis*	Piggery wastewater	6.1 ± 0.1	162.0 ± 8.0 (TN)			209.0 ± 5.5	3700 ± 51	-	-	-	70-80*	>85*	65-80*	-	Zhu et al. (2013)
8	*Chlorella*	Syntheti	-	-	-	-	5.0 ± 0.	-	-	-	-	-	66 ±	-	-	Xu et

emersonii	c wastewater	1	9	al. (2014)

*Retrieved from graph

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