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Review

Integrating micro-algae into wastewater treatment: A review

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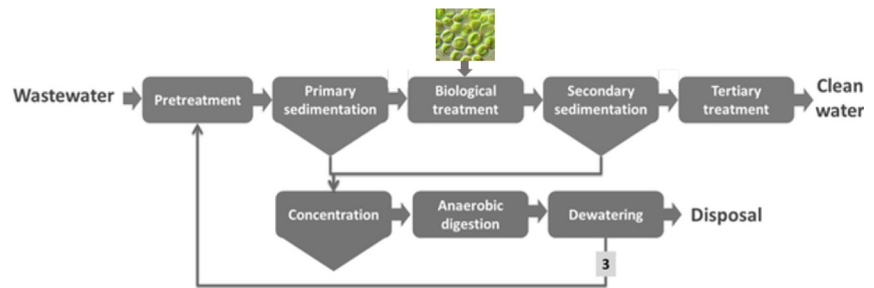


HIGHLIGHTS

- A critical overview of the role of micro-algae cultivation for wastewater treatment.
- Efficient reduction of N, P, and COD by micro-algae in wastewater treatment discussed.
- The energy demand of conventional biological treatment systems compared to micro-algae cultivation.
- Economic challenges of microalgal cultivation in wastewater treatment reviewed.
- Various abiotic and biotic factors influencing micro-algae discussed.

GRAPHICAL ABSTRACT

Wastewater treatment dates back to the 1800s when the first municipal water treatment plant was built in Scotland, and since then the process has become established throughout the world for treatment of municipal and other sewage. In addition to any preceding physical and mechanical treatment operations, the process fundamentally relies on the biological breakdown of organic matter and pollutants, driven by bacterial consortia. In recent years, mixotrophic micro-algae have received increased interest in implementing them as part of wastewater treatment. This is based on their ability to utilise organic and inorganic carbon, as well as inorganic nitrogen (N) and phosphorous (P) in wastewater for their growth, with the desired results of a reduction in the concentration of these substances in the water. The aim of this review is to provide a critical account of micro-algae as an important step in wastewater treatment for enhancing the reduction of N, P and the chemical oxygen demand (COD) in wastewater, whilst utilising a fraction of the energy demand of conventional biological treatment systems. Here, we begin with an overview of the various steps in the treatment process, followed by a review of the cellular and metabolic mechanisms that micro-algae use to reduce N, P and COD of wastewater with identification of when the process may potentially be most effective. We also describe the various abiotic and biotic factors influencing micro-algae wastewater treatment, together with a review of bioreactor configuration and design. Furthermore, a detailed overview is provided of the current state-of-the-art in the use of micro-algae in wastewater treatment. This review is intended to be a source of information and references for both experts and those who are new to this field, with the hope also that it will garner significant interest towards integrating micro-algae for the enhanced and cost-effective treatment of wastewater.



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ABSTRACT

Improving the ecological status of water sources is a growing focus for many developed and developing nations, in particular with reducing nitrogen and phosphorus in wastewater effluent. In recent years, mixotrophic micro-algae have received increased interest in implementing them as part of wastewater treatment. This is based on their ability to utilise organic and inorganic carbon, as well as inorganic nitrogen (N) and phosphorous (P) in wastewater for their growth, with the desired results of a reduction in the concentration of these substances in the water. The aim of this review is to provide a critical account of micro-algae as an important step in

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1. Introduction

The main aim of wastewater treatment is to significantly reduce the quantity of carbonaceous (organic; predominantly determined as biological oxygen demand (BOD)) materials and, where sensitive waters are involved, nitrogen (N) and phosphorus (P) compounds prior to being discharged into receiving systems (Gray, 2004; Grady et al., 2011). This is because the presence of these materials in large concentrations can have deleterious effects on dissolved oxygen (O_2) concentration levels, the trophic state and ultimately the well-being of the fauna and flora in the water (UN-Water, 2015). Achieving improved ecological status of water sources is a growing focus for many developed and developing nations, in particular with reducing N and P in wastewater effluent (European Commission, 2016; UN-Water, 2017). Characterised by the increase in phytoplankton growth, blooms of toxic and non-toxic algae associated with eutrophication reduce water transparency resulting in attenuated light levels to submerged aquatic vegetation and hence reduced dissolved O_2 generation via photosynthesis (Bricker et al., 2008; Heisler et al., 2008; Granéli and Turner, 2006). The concentration of dissolved O_2 is further reduced during the decay of the formed biomass following nutrient deprivation, as heterotrophic bacteria digest the biodegradable organic matter (i.e. dead phytoplankton). It is estimated that the organic material of phytoplankton biomass produced from the discharge of 1 kg of P can exert 100 kg of O_2 demand, while that produced from the discharge of 1 kg of N can exert 14 kg of O_2 demand (Grady et al., 2011). Consequently, hypoxic or anoxic conditions form and that can adversely affect the indigenous fauna and flora, causing loss of species diversity and ecosystem function in water bodies.

In Europe the Urban Wastewater Treatment Directive (UWTD) sets effluent discharge limits for chemical oxygen demand (COD) at 125 mg L^{-1}

O_2 , and for total phosphorus (TP) at 1 or 2 mg L^{-1} and total nitrogen (TN) at 10 or 15 mg L^{-1} for population equivalence (PE) of $>100 \text{ k}$ or $<100 \text{ k}$, respectively (European Commission, 2016). Conventional wastewater treatment systems subject the wastewater to two main treatment phases: primary and secondary treatment. The provision of O_2 is essential at this stage to enable the microorganisms to digest and mineralise the materials into a form that is resistant to further biological activity. Biodegradable carbonaceous matter in wastewater is estimated to have an O_2 demand in the order of $2 \text{ kg } O_2 \text{ kg}^{-1} \text{ COD}$ (Grady et al., 2011). Maintaining this level of dissolved O_2 concentration during conventional activated sludge secondary wastewater treatment is energy intensive and hence expensive. To achieve TN and TP concentrations in wastewater effluent that is in compliance with the provisions of the UWTD, biological nutrient removal (BNR) systems are extensively used based on the processes of autotrophic nitrification, heterotrophic denitrification and enhanced biological phosphorus removal – performed in, for example, an anaerobic-anoxic-oxic reactor, a Bardenpho sequence batch reactor or a DEPHANOX reactor configuration (Gray, 2004; Grady et al., 2011). Nitrification is also an aerobic process providing the required O_2 in conventional systems is equally expensive.

Despite these systems achieving significant reductions in carbonaceous, nitrogenous and phosphorus materials, there is growing concern that set discharge concentrations are not inadequate to limit the effects of eutrophication, especially in small inland rivers. There are also the additional problems of blue baby syndrome (methemoglobinemia) and increased dose of chlorine during disinfection in drinking water caused by the presence of oxidised nitrogen compounds. Wastewater effluent is estimated to hold N and/or P concentrations three orders of magnitude, or more, than receiving systems (Mainstone and Parr, 2002; Carey and Migliaccio, 2009). For example, Andersen et al. (2004) reported

considerably higher nitrate (NO_3) and soluble reactive phosphorus (SRP) concentrations in a South Carolina stream downstream from the discharge point of two wastewater treatment facilities ($\text{NO}_3\text{-N}$: 50.5 mg L^{-1} and SRP: 3.7 mg L^{-1}) compared to the ambient concentrations measured upstream ($\text{NO}_3\text{-N}$: 1.6 mg L^{-1} and SRP: 0.3 mg L^{-1}). This is not surprising given that the two effluent discharges combined accounted for over 70% of the total measured flow at the downstream river location. Chambers et al. (2012) evaluated the threshold of TN and TP concentration at which eutrophication in streams occurs to range between 0.21 and 1.2 mg L^{-1} and 0.01 and 0.1 mg L^{-1} , respectively. With regards to regulation concerning water quality, considerations are being put forward to lower the required TN and TP concentrations in the effluent before the water can be discharged, with P the main focus (European Commission, 2007; Hendriks and Langeveld, 2017; Ahn et al., 2010). In most ecosystems, P is the rate-limiting nutrient for phytoplankton growth; therefore, reducing inputs of P to receiving systems is considered key to reducing eutrophication (Hendriks and Langeveld, 2017; Schindler et al., 2008). In this situation, a holistic approach is applied to determine effluent P concentrations that reflect the natural ecological P concentration of the water body, which include an account of the site's alkalinity and altitude. In other countries, more stringent effluent P standards are set to all point source discharges regardless of population numbers served. For example, in Denmark a TP effluent concentration of 0.3 mg L^{-1} is applied to all municipal treatment facilities, whereas in Sweden a 90% reduction is required (compared to 80% reduction in relation to the load of the influent stated by the UWTD) (Swedish EPA, 2008).

In view of achieving more stringent effluent standards to improve water quality, concern has grown over the sustainability of conventional wastewater treatment systems in terms of economic feasibility and environmental impact. Energy consumption and greenhouse gas emissions from wastewater treatment are amongst the aspects that have become key factors concerning the overall performance of a wastewater treatment system (Longo et al., 2016; Wan et al., 2016). It is estimated that 0.6 to 3% of the total electricity generated in developed nations is expended on treating wastewater, and depending on the source of energy, the associated carbon emissions can be substantial (Chae and Kang, 2013; Plappally and Lienhard, 2012; Wang et al., 2016). For example, the per annum CO_2 emission from electricity consumed for wastewater treatment in Germany was estimated to be 2.2 million tonnes, approximately 2.1 million tonnes in the United Kingdom, and approximately 11.5 million tonnes in the United States (Rothausen and Conway, 2011; US Department of Energy, 2016; Chisholm, 2013). Of the energy consumed, it is estimated that 50% or more is expended on the O_2 transfer equipment in the biological secondary stage of the wastewater treatment train (Chae and Kang, 2013; Plappally and Lienhard, 2012; McCarty et al., 2011; Pabi et al., 2013).

Regarding N and P, the complexity of the process through which removal is achieved increases the energy requirements substantially resulting in an increase in the overall cost of treatment. For example, in the anaerobic system the wastewater transitions between anaerobic, anoxic and aerobic environments in sequence. Removal of N, P and carbonaceous materials is accomplished in the separate environments: inorganic N is removed by nitrifiers and denitrifiers in, respectively, the aerobic and anoxic environments; inorganic P in the anaerobic and anoxic environments by phosphate-accumulating organisms; and carbonaceous material in the aerobic and anoxic environments by, respectively, heterotrophic and denitrifying organisms (Grady et al., 2011). The separation of the different environments in space and time increases the complexity of the treatment process, while a higher quantity of O_2 is consumed for inorganic P and N removal by the respective organisms to facilitate assimilation or conversion. Meta-analysis from 50 wastewater treatment plants based across Germany, Spain and Italy ranging between 1000 and 100,000 PE capacity, reported average energy consumption of $0.49 \text{ kWh kg}^{-1} \text{ COD}$, while the removal of TN and TP to permissible discharge concentrations amounted to $6.74 \text{ kWh kg}^{-1} \text{ N}$ and 8.26 kWh

$\text{kg}^{-1} \text{ P}$, respectively. While improving effluent quality is essential to safeguard water sources for future use as it is clear that lowering discharge standards drastically increases energy consumption and unless sourced from renewable sources, also a direct increase in carbon emissions. Based on an electricity generation carbon footprint of $0.421 \text{ kg CO}_2\text{eq kWh}^{-1}$ (global OECD emission factor), the energy consumed to remove 1 kg N and P from the wastewater would generate 2.8 and 3.4 kg CO_2 equivalent respectively (International Energy Agency, 2016).

Other gases that are emitted from wastewater which contribute to the greenhouse effect are methane and hydrogen sulphide in the sewers and nitrous oxide (N_2O) in the treatment process (Guisasola et al., 2008; Zhang et al., 2008; Wunderlin et al., 2012). The discharge of N_2O is of a concern as it has an approximate 320-fold stronger effect than CO_2 , and therefore even low emission levels are undesirable (Intergovernmental Panel on Climate Change, 2007). Nitrogen oxides catalytically react with ozone of the stratosphere, reducing the ozone layer by generating O_2 (Ravishankara et al., 2009). The Intergovernmental Panel on Climate Change (IPCC) reports that N_2O emissions from wastewater treatment account for approximately 2.8% of the total anthropogenic sources, and are expected to increase by approximately 13% between 2005 and 2020 (Intergovernmental Panel on Climate Change, 2007; Law et al., 2012).

During the biological nitrification reaction, ammonia (NH_3) is oxidised to nitrate and nitrite (NO_3 and NO_2) and in the denitrification reaction the formed NO_2 is reduced to N_2 (Grady et al., 2011; Kampschreur et al., 2009). Whilst during these biological reactions N_2O is formed as an intermediate, incomplete oxidation to NO_2 or reduction to N_2 is caused by non-optimal cultivation conditions (e.g. dissolved O_2 concentration, pH and temperature) that inhibit the completion of the reaction (Law et al., 2012; Massara et al., 2017). Overall, it is estimated that conventional wastewater treatment systems contribute approximately 3% to the total global anthropogenic greenhouse gas emissions (Intergovernmental Panel on Climate Change, 2007; Bogner et al., 2008).

A further drawback of conventional wastewater treatment systems, as shown in Fig. 1, especially the activated sludge technology, is the high production of sludge. Between 2006 and 2007, the total quantity of sludge produced by 27 member states of the European Union was estimated at 10.1 million tonnes of dry solids – an amount which is expected to rise to 13 million tonnes by 2020 (Milieu, 2003). In the United States it is approximated that a total of 13.8 million tonnes of dry solids are generated annually from the estimated 15,000 public-owned treatment works alone (Seiple et al., 2017). The handling and disposal of sewage sludge not only presents a significant challenge in wastewater management, but further adds to direct and indirect emissions of greenhouse gases and environmental problems. Although the disposal of sludge by direct application to land (agricultural use) is a feasible option, as the high N and P content serve as a fertiliser, the introduction of various regulations has made this an unacceptable operation in dealing with sludge. High concentrations of toxic metals and persistent chemicals (e.g. polychlorinated biophenyls) that accumulate in the sludge can restrict the application on agriculture land, whilst to reduce the risks of contamination from residual pathogens, the sludge must be itself treated before being applied to soil in which crops are grown (Reilly, 2001; Singh and Agrawal, 2008). Furthermore, application of sewage sludge or ash (after incineration) to landfills can cause secondary pollution by the leaching of toxic metals and organic pollutants into surrounding soil and surface or groundwater systems (Pathak et al., 2009).

Thus, although conventional wastewater treatment systems have been applied with relative success, their application has been described as problem shifting by way of leading to secondary pollution because of high-energy consumption and the production of waste sludge and greenhouse gases (Wan et al., 2016). In order to reduce the environmental impact of wastewater treatment, it is therefore necessary to develop and adapt processes with a substantial reduction in energy consumption and sludge production. Key criteria to achieving lower energy consumption are reducing aeration requirements and operation

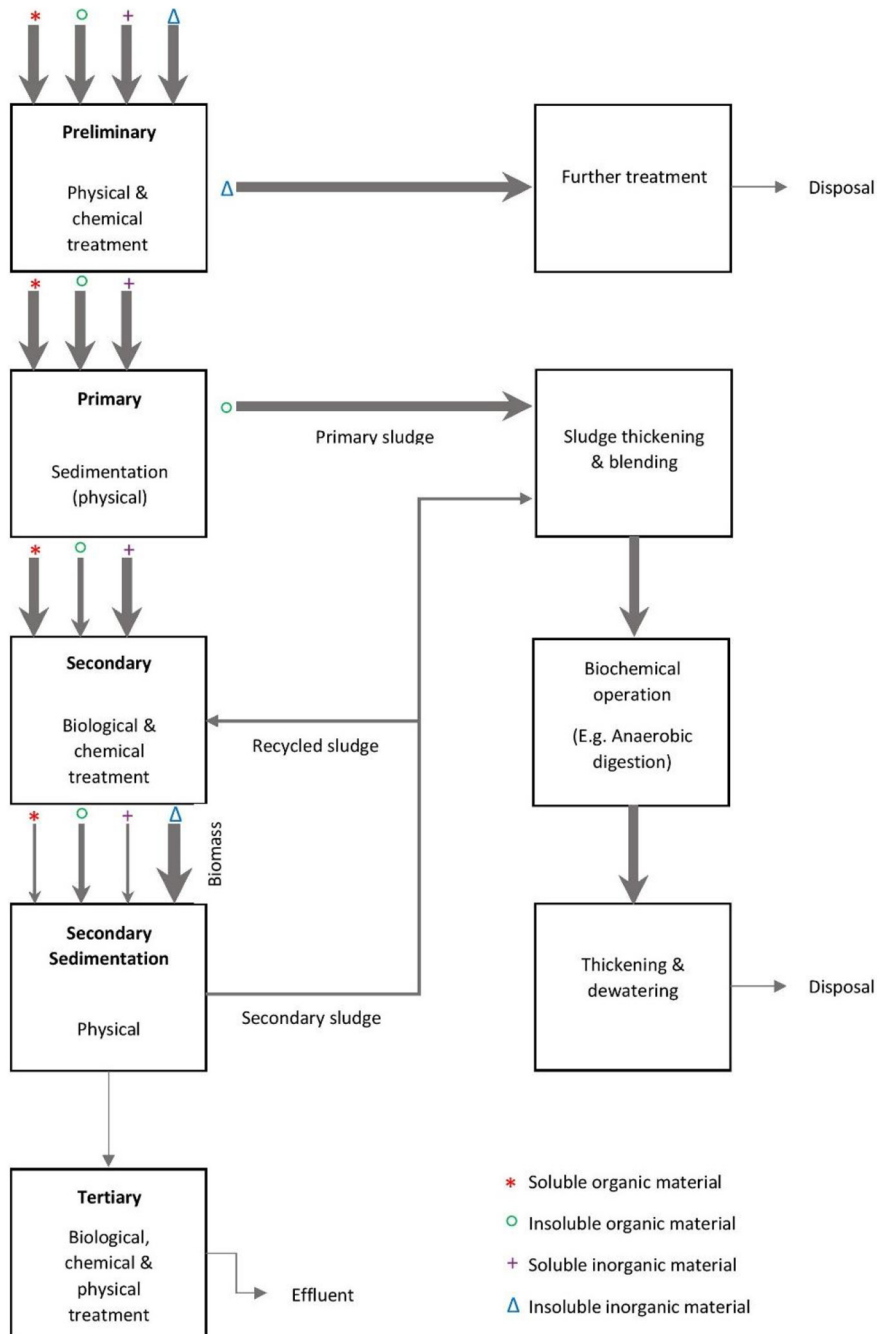


Fig. 1. Schematic diagram of conventional wastewater treatment systems.

complexity without affecting performance with respect to meeting mandated effluent standards.

2. Why micro-algae?

Micro-algae, including eukaryotic algae and cyanobacteria, have demonstrated to be an environmentally friendly and sustainable alternative to energy-intensive and conventional biological treatment processes that are widely used today (Singh et al., 2015; Oswald, 2003).

In addition to being a renewable source for biomass, the use of micro-algae in wastewater treatment is a cost effective and feasible method for bio-fixation of CO_2 (Almomani et al., 2019). The rationale behind the use of mixotrophic micro-algae to treat wastewater lies in their ability to utilise organic and inorganic carbon, as well as inorganic N and P in wastewater for their growth, resulting in reductions in the

concentration of these substances in the water. The principal advantage of incorporating micro-algae into wastewater treatment is the generation of O_2 through photosynthesis, necessary for heterotrophic bacteria to biodegrade carbonaceous materials.

Although it is difficult to compare the effect of algal culture in wastewater treatment, various studies have demonstrated that algal formation can support nutrient removal in wastewater (Chawla et al., 2020). The use of algae granules in synthetic wastewater has been reported to be highly efficient for the removal of phosphorus and its recovery and reuse from the obtained P-rich algae biomass (Cai et al., 2019). In addition, to being efficient for CO_2 capture and nutrient removal from wastewater, microalgae have shown to be a potential source of energy generation (Arun et al., 2020). Micro-algae can utilise both organic nitrogen (such as urea) and inorganic nitrogen (in the form of ammonium/ammonia) as well as nitrite and nitrates (Ross et al., 2018). The

emission of N₂O in the treatment process of wastewater is a consequence of the environmental conditions under which N-removal proceeds (Arun et al., 2020).

Furthermore, wastewater treated by an algal-bacterial co-culture does not need to transition between different operating environments to facilitate inorganic N and P removal, requiring only a single-step treatment stage and thereby reducing the complexity and energy of the treatment process (Sturm and Lamer, 2011; Gouveia et al., 2016). This is because micro-algae assimilate ammonia (NH₃) and phosphate (PO₄) directly and in concert for cell growth and metabolic function (Falkowski and Raven, 2007; Borowitzka et al., 2016). As a result, micro-algae treatment processes have a lower greenhouse gas emission rate; for instance, the majority of N is assimilated by the micro-algae instead of being converted to oxides of nitrogen. Various studies have reported on the negligible emission of N₂O caused by micro-algae in conjunction with associated microorganisms in wastewater treatment (Guieysse et al., 2013; Fagerstone et al., 2011). Based on the analysis of Alcántara et al. (2015), a micro-algae wastewater treatment process is estimated to have an emission factor of 0.0047% g N₂O-N g⁻¹ N-input. Overall, furnishing wastewater with dissolved O₂ through micro-algae photosynthesis is a sure bet for significant savings in energy demand and reductions in associated greenhouse gas emissions.

3. Economic challenges

Despite these advantages, several practical and economic challenges still hinder the implementation of micro-algae to treat wastewater and would need to be addressed in order for it to reach industrial application. One such challenge relates to the energy consumed in the cultivation process. As with most conventional wastewater treatment operations, aeration and pumping systems are often used in micro-algae culturing to generate turbulent flow that improve the exchange of O₂ and CO₂ to maintain an optimal environment for their performance. A techno-economic assessment of microalgal technology implementation in the Arabian Gulf based on a combined flue gas biofixation and wastewater treatment reported a promising financial benefit for emerging economies without a mineral oil-based economy (Al Ketife et al., 2019). The break-even selling price (BESP) of the generated biocrude (typically the selling price of the product) was reported to be \$0.544 per kg biomass, corresponding to \$0.9 L⁻¹ for the extracted biocrude, covering the operating expenditure (OPEX).

High rated algae ponds (HRAP) have shown to have great potential for the treatment of municipal wastewater in locations with sufficient solar radiation. A recent study on life-cycle sustainability assessment of algae and bacteria-based wastewater treatment systems indicated that HRAP is more beneficial both environmentally and economically (0.18 €/m³ versus 0.26 €/m³), contributing to CO₂ sequestration and eutrophication potentials (146.27 vs. 458.27 × 10⁻³ kg CO₂ equiv./m³; 126.14 vs. 158.01 × 10⁻⁶ kg PO₄ equiv./m³) (Kohlheb et al., 2020). Another study examined the potential of wastewater treatment HRAP for production of low-cost biofuel, reporting 800–1400 GJ/ha/year energy produced in the form of harvested algal biomass (Mehrabadi et al., 2017).

Life-cycle analyses by Stephenson et al. (2010) and Jorquera et al. (2010) on micro-algae biomass production determined that the majority of the operational energy was consumed in the cultivation stage. The results suggest that mixing in photobioreactors (PBR) by means of pumping and/or aeration required approximately 10 times more energy than mixing by paddlewheels in high rate algae ponds (HRAP). In a case study carried out in Almeria, Spain, analysing the cost of operating a 30 m³ PBR plant found that the use of recirculation pumps and aeration pumps to be, respectively, the first and second highest energy exponders in the operation (Acien et al., 2012). The study also showed that the recorded power consumption of the recirculation pumps and aeration pumps per unit were 24 and 96 kWh d⁻¹ respectively; the reason the recirculation pumps accounted for higher energy consumption is because

ten units were employed but only one aeration pump. The overall rate of energy consumption was 15 kWh m⁻³, which is 100-fold higher in the energy consumption rate compared to mechanical and/or aerated mixing in conventional wastewater treatment systems (between 0.15 and 0.62 kWh m⁻³; (Plappally and Lienhard, 2012). A similar conclusion was drawn by Gouveia et al. (2016) when analysing the cost for micro-algae wastewater treatment in a PBR. The authors estimated the cost to treat 1 m³ of wastewater at approximately €95 under continuous operation (14 days), with the energy consumption (as electricity) the highest cost factor. This approximation does not compare favourably against the treatment cost by conventional wastewater treatment systems of between 0.1 and 0.2 € m⁻³ (Cashman et al., 2014).

The principal reason for aeration in the cultivation of micro-algae is to supply carbon in the form of CO₂ to the algae, an important nutrient required for growth and to facilitate the assimilation of inorganic N and P (Liu et al., 2020). However, the energy required to compress the air (enriched or not with CO₂) is an energy-intensive process and is one of the main factors that account for the high operation cost (Davis et al., 2016). A life cycle assessment conducted by Kadam (2002) calculated the electrical consumption of CO₂ injection required in a 1000 ha sized HRAP to be 22.2 kWh t⁻¹ CO₂. In this scenario, 680 t of CO₂ were injected into the system per day to ensure a micro-algae productivity rate of 45 g m⁻² d⁻¹ consuming 15.1 MWh of electricity at an estimate expense of 1760 € d⁻¹ – this figure is based on the average 2016 electricity price of 0.1668 € kWh⁻¹ for industrial consumers (Eurostat); prices are from the first half of the year (January to June) and exclude VAT and other recoverable taxes and levies. Furthermore, aeration inevitably results in CO₂ loss from the suspension to the atmosphere by outgassing and is a major constraint in ensuring a sufficient concentration of carbon for micro-algae use (Lee et al., 2016; de Godos et al., 2014). An alternative approach to overcome the operational cost and inefficiencies associated with carbon supply via aeration is to supplement the medium directly with dissolved carbon, such as inorganic carbon salts (i.e. bicarbonate) or organic substrates (i.e. glucose) (Evans et al., 2017; Kesaano et al., 2015; Gupta et al., 2016; Perez-Garcia et al., 2011a). The premise of this approach theoretically ensures the complete utilisation of the added carbon by the micro-algae. Additionally, by incorporating waste streams rich in bioavailable carbon to augment the supply, the treatment of wastewater by micro-algae would have wider environmental benefits through resource recovery and reduced material costs, and in so doing align to the concept of a circular economy model.

A further influence on the economic feasibility of implementing micro-algae to treat wastewater is the stage of the treatment train the process is introduced. The application of micro-algae in wastewater has customarily been applied to polishing secondary treatment effluent – i.e. in tertiary treatment after the energy intensive secondary treatment stage, to further reduce the inorganic N and P concentrations. Consequently, the introduction of micro-algae at this stage of the treatment train would not result in the much-desired reduction in overall energy demands of wastewater treatment. As described above, this is largely a direct result of additional mixing and aeration provided. A more effective treatment process would be to integrate the micro-algae into the treatment train as the secondary biological treatment phase, applied to treat primary settled wastewater directly.

A novel study for microalgal bio-fuel production investigated the integration of wastewater treatment and hydrothermal liquefaction (HTL) of biomass into bio-oil production. During the HTL process, the bio-oil yield (29.37% wt) was increased by solid catalysts. Bio-oil can be used in bio-refinery due to its high calorific value (19.6 ± 0.8 MJ/Kg) and it can be enriched further into liquid transportation fuels. In this study a liquid-liquid extraction process was used to enrich HTL bio-oil and resulted in 18.2% wt yield with purity of 92.85% (Arun et al., 2018).

An additional aspect that should be taken into consideration is the efficiency and reliability of the process performance. Multiple studies have evaluated different micro-algae species in treating wastewater; however, these were mostly performed independent of one another

under varying environmental and cultivation conditions. As such, a direct comparison in the treatment performance of a microalgal strain to a wastewater source cannot be made definitively.

4. Micro-algae wastewater treatment

The investigation into the biological removal of carbonaceous, nitrogenous and phosphorus material via micro-algae in wastewater effluents has been evaluated by several studies. This has been performed with various microalgal species on a range of wastewater types, including municipal, agricultural, brewery, refinery, and industrial effluents with varying efficiencies in treatment performance and micro-algae growth (Cai et al., 2013; Gentili, 2014; Wu et al., 2014; Chiu et al., 2015). The strain *Scenedesmus obliquus* has been demonstrated to successfully remove nutrients (carbon, N and P) from piggery wastewater (Ji et al., 2013; Prandini et al., 2016), while *Chlorella pyrenoidosa* successfully grew in dairy production effluent (Kothari et al., 2012). Other *Chlorella* species, including *Chlorella vulgaris*, have been reported to be suitable candidates in the remediation of N and P from municipal wastewater effluent at the primary stage ($\text{PO}_4^{3-}\text{-P}$: 8 to 3 mg L⁻¹; NH_4^+ : 119 to 37 mg L⁻¹), secondary stage ($\text{PO}_4^{3-}\text{-P}$: 6.1 to 0.5 mg L⁻¹; $\text{NH}_4^+\text{-N}$: 6.9 to 0.8 mg L⁻¹) and from centrate (TP: 215 to 40 mg L⁻¹; TN: 116 to 12 mg L⁻¹) (Gouveia et al., 2016; Li et al., 2011a). Choi (2016) reported 88% BOD, 82% TN and 54% TP removal from initial concentrations in brewery effluent by *C. vulgaris*. Other micro-algae species examined for their bioremediation potential include *Chlamydomonas* sp., *Nanochloropsis* sp., *Dunaliella* sp., *Spirulina* sp. and *Botryococcus* sp. (Cuellar-Bermudez et al., 2015; Gonçalves et al., 2017).

4.1. Carbon, N and P ratios in different waste streams

A significant influence to the microalgal treatment performance is the composition of the wastewater. In order to grow and function, micro-algae require three primary nutrients: carbon, N and P (Falkowski and Raven, 2007). The assimilation of these nutrients is strongly affected by the overall composition of nutrients that are

available in the cultivation medium (Kapdan and Aslan, 2008). Nutrient utilisation rates by micro-algae are closely associated with their growth, and a limited supply of a primary nutrient can significantly reduce their growth rate (Xin et al., 2010a, 2010b; Al Ketife et al., 2017). In this context, to ensure optimal nutrient removal efficiency from the cultivation medium, an optimal ratio of nutrients that is reflective of the micro-algal elemental stoichiometry needs must be present. Further to this, trace amounts of micronutrients, such as calcium, magnesium, potassium, manganese, silica, zinc, iron and others are essential and generally abundantly available in wastewater (Falkowski and Raven, 2007; Borowitzka and Moheimani, 2013).

Within a conventional municipal wastewater treatment train, two different wastewater streams are identified as potential points at which to integrate a micro-algae treatment process; either to treat PSW or secondary treatment effluent (STE). Indeed, a more economical and environmentally sustainable treatment process would be to integrate micro-algae as the secondary treatment phase, directly treating PSW to effluent standards. In addition, PSW exhibits a more optimum nutrient ratio and hospitable microbial community to support micro-algae growth compared to STE (detailed below). When comparing the carbon, N and P quantity in PSW and STE, it can be concluded that they are relatively similar in nutrient composition, but differences exist in their concentrations. Tables 1 and 2 summarise the N, P and carbon concentrations of municipal wastewater from PSW and STE respectively, as reported in recent studies on micro-algae cultivation.

In STE the concentration of N, P and carbon (represented as the COD) were in the range of 0.63 and 50 mg L⁻¹ N, 0.1 and 26 mg L⁻¹ P, 11 to 340 mg L⁻¹ O₂, respectively. In PSW the concentrations were higher, with N in the range of 23 to 93 mg L⁻¹, P in the range of 1.5 and 33 mg L⁻¹, and COD in the range of 93 and 400 mg L⁻¹ O₂. By comparing the average C/N/P ratio of the different wastewater effluents with the proximate composition of freshwater micro-algae, it can be observed that PSW more closely matches the stoichiometric ratio. With an average C/N/P ratio of 100/34/7, STE contains either an excess ratio of N to P or, conversely, is limited in carbon (Table 2). In STE, nearly all of the pollutants that could be a source of bioavailable carbon are degraded

Table 1
Carbon, N and P ratios in PSW used in microalgal-based wastewater.

Microalgae species	N	P	C	Ratio (C:N:P)	Reference
<i>Chlorella</i> sp.	38.9 ^b	6.9 ^e	224	100/17/3	Wang et al., 2010
Algae consortium & bacteria	45 ^a	6.5 ^e	400	100/11/1.6	Valigore et al., 2012
Algae consortium & bacteria	93 ^c	33 ^f	176 ^g	100/53/18	García et al., 2017
<i>Desmodesmus communis</i> & bacteria	33.6 ^c	1.54 ^e	-	-	Samorì et al., 2013
<i>Scenedesmus</i> sp. ZTY1 & bacteria	41 ^b	8.4 ^e	235	100/17/3.5	Zhang et al., 2014
Microalgae screening	36.1 ^{b,s}	4 ^{e,s}	93 ^s	100/39/4	Wang et al., 2014
<i>Desmodesmus communis</i> & bacteria	32.4 ^c	2.4 ^f	-	-	Samorì et al., 2014
<i>Chlorella protothecoides</i>	37.4 ^b	2.6 ^e	-	-	Sforza et al., 2014
<i>Chlorella vulgaris</i>	43.3 ^d	0.6 ^f	256	100/17/1	Ebrahimian et al., 2014
<i>Neochloris oleoabundans</i>	40.8 ^d	10 ^e	242	100/17/4	Almomani and Örmeci, 2016
<i>Chaetomorpha linum</i>	24.5 ^d	2.4 ^e	307	100/8/1	Ge and Champagne, 2017
Microalgae screening	23 ^b	8.6 ^e	270	100/8.5/3	Bohutskyi et al., 2016
<i>Chlorella vulgaris</i>	36.3 ^d	4.2 ^f	317	100/11/1.3	Mahdy et al., 2016
Microalgae screening	41 ^b	4.7 ^e	70	100/58/7	Mehrabadi et al., 2017
<i>Chlorella vulgaris</i> (WWTP 1)	84 ^{b,s}	6 ^{e,s}	150 ^s	100/56/4	Cabanelas et al., 2013
<i>Chlorella vulgaris</i> (WWTP 2)	42 ^{b,s}	5.9 ^{e,s}	180 ^s	100/23/3	Cabanelas et al., 2013
<i>Chlorella protothecoides</i> & bacteria	44.4 ^d	8 ^f	130	100/34/6	Ramos Tercero et al., 2014
Algae consortium & bacteria	18.9 ^d	3.8 ^e	140	100/20/3.5	Su et al., 2011
Average	31	5.6	158	100/19/3	

Unless otherwise stated, carbon was measured as COD.

^a Total Kjeldahl Nitrogen.

^b TN.

^c NH₃-N.

^d NH₄⁺-N.

^e TP.

^f PO₄-P.

^g Total organic carbon.

^s Soluble fraction.

Table 2
Nutrient ratios in STE used in microalgal-based wastewater treatment studies.

Microalgae species	N	P	C	Ratio (C:N:P)	Reference
<i>Chlorella</i> sp.	19.1 ^a	0.3 ^d	42	100/45/0.7	Wang et al., 2010
<i>Haematococcus pluvialis</i>	42.4 ^c	2.6 ^e	22	100/193/12	Kang et al., 2006
<i>Scenedesmus</i> sp. ZTY1 & bacteria	11 ^a	1.9 ^d	41	100/27/4	Zhang et al., 2014
<i>Desmodesmus communis</i> & bacteria	1.47 ^a	0.1 ^d	–	–	Samori et al., 2014
<i>Chlorella vulgaris</i>	0.63 ^b	0.6 ^e	96	100/0.6/0.6	Ebrahimian et al., 2014
<i>Neochloris oleoabundans</i>	44 ^b	26 ^d	59	100/75/44	Almomani and Örmeci, 2016
<i>Chaetomorpha linum</i>	17.9 ^b	0.5 ^d	30	100/60/2	Ge and Champagne, 2017
Microalgae screening	7 ^a	1.6 ^d	38	100/18/4.2	Bohutskyi et al., 2016
Microalgae consortium	50 ^a	15 ^d	63	100/79/24	Shayan et al., 2016
Microalgae consortium	17 ^a	1.9 ^e	34	100/50/5	Soydemir et al., 2016
<i>Scenedesmus dimorphus</i>	15.8 ^a	0.8 ^d	32	100/49/2.5	Zhang et al., 2015
Microalgae consortium	16.5 ^a	1.5 ^d	11	100/150/13	Yu et al., 2015
<i>Chlorella</i> sp.	18.9 ^a	1.7 ^d	11	100/171/15	Cho et al., 2011
<i>Neochloris oleoabundans</i>	12.3 ^b	3 ^e	340	100/3.6/1	Wang and Lan, 2011
<i>Botryococcus braunii</i>	11.9 ^a	11.5 ^e	50	100/24/23	Órpez et al., 2009
<i>Chlorella vulgaris</i> (WWTP 2)	65.6 ^{a,s}	7.5 ^{d,s}	90 ^s	100/73/8	Cabanelas et al., 2013
<i>Chlorella vulgaris</i> (WWTP 1)	36 ^{a,s}	2.4 ^{d,s}	90 ^s	100/40/3	Cabanelas et al., 2013
Average	22.8	4.6	66	100/34/7	

Unless otherwise stated, carbon was measured as COD.

^a TN.

^b NH₄⁺-N.

^c NO₃-N.

^d TP.

^e PO₄-P.

^s Soluble fraction.

in the biological treatment stage with the remaining carbon material being composed of complex polymers that are either recalcitrant or only partially digestible (Gray, 2004; Katsoyiannis and Samara, 2007). In STE the ratio of biodegradable dissolved organic carbon to dissolved organic carbon (DOC) has been reported to range between 0.21:1 and 0.28:1, with a concentration of DOC as low as 7.8 mg L⁻¹ (Li et al., 2006).

The discrepancy in carbon, N and P concentrations between the different wastewater streams has been demonstrated to affect micro-algal removal efficiencies. In a comparative study, Wang et al. (2010) found that a *Chlorella* sp. had a higher average specific growth rate with a concomitant improved efficiency in inorganic N and P removal from PSW, compared to STE. The removal capacity of the micro-alga from PSW was 68.5% TN and 90.6% TP, and from STE 50.8% TN and 4.96% TP. Moreover, a 56.5% decline in COD was recorded from the PSW, while in the STE an increase of 22.7% was reported, indicating that oxidisable carbon matter was being excreted by the micro-algae. In a study by Cabanelas et al. (2013), a similar effect in treatment efficiency with the micro-alga *C. vulgaris* strain SAG211–12 was observed across the two types of wastewater streams. Higher TN, TP and COD removal rates were recorded when cultured in PSW compared to STE, with experiments for each wastewater stream conducted on samples from two independent wastewater treatment plants. Higher *C. vulgaris* growth rates were recorded in the PSW samples, varying from 111 to 125 mg L⁻¹ d⁻¹ compared to 63 to 68 mg L⁻¹ d⁻¹ in the STE samples.

In respect to the ratio of bioavailable N and P, various studies have demonstrated the ability of micro-algae to grow and effectively treat wastewater under ratios that deviate from the canonical N and P stoichiometry of freshwater micro-algae (Borowitzka et al., 2016; Xin et al., 2010a; Klausmeier et al., 2004). Kapdan and Aslan (2008), for example, reported a lower residual NH₄-N concentration when treating synthetic wastewater with *C. vulgaris* after an optimum N:P ratio was established for the species. In this study, effluent NH₄-N concentrations decreased from 5.1 to 2 mg L⁻¹ when the N:P ratio was increased from 4:1 to 8:1, with a significant decline in removal efficiency occurring with increasing ratios. Al Ketife et al. (2017) reported a slightly higher optimal N:P ratio for a different *C. vulgaris* strain, with complete N and P removal achieved at a ratio of 10:1. Arbib et al. (2013a) examined the

removal efficiency of *S. obliquus* under varying N:P ratios, and concluded that for an efficient simultaneous nutrient removal the ratio should be between 9:1 and 13:1. In general, an N:P ratio of 30:1 suggests a deficit in P availability and a 5:1 ratio a deficit in N availability for micro-algae (Larsdotter, 2006).

Numerous studies employing different culturing techniques have demonstrated the success of micro-algae in treating PSW, albeit with varying degrees of efficiency (Table 3 and references therein). For example, from unsterilized PSW using *C. vulgaris* cultured in a micro-algal membrane bioreactor, up to 96.6% of TN and 92.7% of TP was removed in addition to 96.9% of COD (Choi, 2015). In a different study, the micro-alga *Chlorella protothecoides* was capable of removing NH₃-N and PO₄-P from PSW with an efficiency of 94% and 62%, respectively (Ramos Tercero et al., 2014). However, the authors state that the organic matter concentration in PSW remained constant, a possible result of CO₂ sparging which promoted autotrophic metabolism over heterotrophic metabolism. Almomani and Örmeci (2016) demonstrated removal efficiencies of 63.2% NH₄⁺-N, 32.4% total dissolved P, and 64.9% COD from PSW employing a native micro-algal consortium isolated from the secondary wastewater basin of a treatment plant. Although the depuration of the nutrients from the wastewater sample mediated by the micro-algae consortium is far lower than in the other two studies, it must be noted that the cultures were treated under near static conditions, which would have lowered the mass transfer rates of substances (e.g. O₂ and CO₂) and optimal growth conditions. The difference in the autochthonous flora of the wastewater between PSW and STE is shown to have an effect on micro-algal growth and treatment performance. Ramos Tercero et al. (2014) reported that aerobic bacteria from the activated sludge that were present in the final effluent had strongly competed with algal growth, indicating that sterilization of the STE was necessary. By comparison, *C. protothecoides* seemed to be resistant to competition with the autochthonous microbial community of PSW. In a study by Sforza et al. (2014), no difference in *C. protothecoides* growth was detected between unsterilised and sterilised PSW, corroborating the reported observation that the autochthonous microbial community of the PSW may not negatively affect algal growth. Thus, in a proposed micro-algal wastewater treatment process, to ensure efficient treatment and minimise the potential negative effects of bacteria competing with micro-algae, it would be more appropriate to integrate the micro-algae after the primary settling stage.

Table 3
Carbon, nitrogen and phosphorus removal capacities from municipal wastewater by microalgae in different bioreactor types as reported in independent studies. Concentration values are in mg L⁻¹ (C_i and C_f; % removal percentage).

Algae	Waste water type	Treatment conditions and reactor	HRT	Treatment time (days)	Nitrogen			Phosphorus			Carbon			Reference
					C _i	C _f	%	C _i	C _f	%	C _i	C _f	%	
Immobilised - passive														
Centrate wastewater native algal-bacterial consortium	PSW	Algal biofilm reactor, fixed, V = 31 L, 0.5 m ² , artificial illumination, 21.9 °C; pH 7.7	10	40	91 TN	27.3	70	7 PO ₄ ³⁻ -P	0.05	85	181 TOC	18.1	90	Posadas et al., 2013
Consortia of <i>Chlorella</i> and <i>Phormidium</i> sp.	Gray water	Algal biofilm reactor, fixed, V = 3 L, 630 cm ² , natural sunlight, pH 7.3	6	-	29.8 TAN	1.7	94	24.5 TDP	2.4	90	235 COD	71	69	Choudhary et al., 2017
Consortium of <i>Woronichinia</i> sp., <i>Actuodesmus</i> sp., <i>Aulacoseira</i> sp., <i>Desmodesmus quadricaudatus</i> , <i>Nitzschia</i> sp., <i>Limnothrix redekei</i> and <i>Gomphonema parvulum</i>	PSW	Algal biofilm reactor, fixed, V = 31 L, 0.5 m ² , artificial illumination, 21.7 °C; pH 8.3	10	40	86 TN	6.8	92	12 PO ₄ ³⁻ -P	0.96	96	167 TOC	18.3	89	Posadas et al., 2014
	PSW	Algal tubular biofilm reactor, fixed, V = 31 L, 0.5 m ² , artificial illumination	10	40	86 TN	17.2	80	12 PO ₄ ³⁻ -P	3.84	68	167 TOC	25.0	85	Posadas et al., 2014
Consortium of <i>Scenedesmus</i> , <i>Chlorella</i> , Cyanobacteria, <i>Oocystis</i> , <i>Ankistrodesmus</i> and <i>Synura</i>	STE	Rotating algal biofilm disk, fixed, V = 8 L, artificial illumination, 21 to 25 °C, pH 8.5 to 9	6	21	46.5 TN	8.7	81	15.1 TP	0.07	99	63.1 COD	-	-	Shayan et al., 2016
Predominant strain was <i>Halochlorella rubescens</i>	STE	Twin-Layer PBR biofilm, fixed, V = 55 L, 3 x 2m ² modules, artificial illumination, 18 to 32 °C, pH 8.4	1	8	7.5 NO ₃ -N	1.3	83	0.61	0.17	73	-	-	-	Shi et al., 2014
<i>Scenedesmus</i> sp. and natural bacteria population	STE	Algal biofilm reactor, fixed, V = 96 L, artificial illumination, 20 to 22 °C, pH 7.76	2	91	18.5 TN	11.8	36	1.32 TP	<0.5	62	60 COD	39	35	He and Xue, 2010
<i>Chlorella vulgaris</i>	Treated municipal waste water	Suspended carrier, suspended V = 20 L, aerated, artificial illumination, 25 to 30 °C; pH 8.2 to 9	0.1	37	17.4 DIN	6.7	61	3.07 TP	0.8	71	21 COD	-	-	Tao et al., 2017
<i>Chlorella</i> sp., <i>Scenedesmus</i> , <i>Pediastrum</i> , <i>Nitzschia</i> , <i>Navicula</i> , <i>Crucigenia</i> , <i>Synedra</i> and bacteria	Waste water Lagoon effluent	Rotating algal biofilm disk, fixed, V = 535 L, natural sunlight, 9.6 to 19.2 °C; pH 8 to 10	0.25	20	4.5 TN	1.1	75	2.1 TP	1.6	23	-	-	-	Christenson and Sims, 2012
<i>Nitzschia</i> sp. and other green filamentous microorganisms	Municipal waste water	Algal biofilm, fixed, 1.8 m ² , artificial illumination, 22 °C; pH 7	0.7	10	5.57 NO ₃ -N	2.2	60	0.97 PO ₄ ³⁻ -P	0.2	88	-	-	-	Boelee et al., 2011
<i>Scenedesmus obliquus</i> and bacteria	STE	Biofilm in a twin wall polycarbonate sheet, fixed, V = 5 L, 0.5 m ² , natural illumination, 21.7 °C; pH 7.6	1	130	32 TAN	1.6	95	1.7 TP	0.1	94	61 COD	37	39	Zamalloa et al., 2013
Immobilised - active														
<i>Scenedesmus obliquus</i>	STE	Sodium alginate beads, suspended, V = 2.5 L, aerated, batch mode, artificial illumination, 25 °C; pH 9 to 9.5	-	2	34 NH ₄ ⁺ -N	1.2	96	2.5 PO ₄ ³⁻ -P	1.12	55	-	-	-	Ruiz-Marin et al., 2010
<i>Chlorella vulgaris</i>	BNR treatment effluent	Alginate beads, V = 5 L, agitated, artificial illumination, 30 °C; pH 6.7	-	6	8.73 TN	0.1	99	0.8 TN	0.32	60	-	-	-	Filippino et al., 2015
<i>Chlorella vulgaris</i>	PSW	Sodium alginate - medium concentration, V = 1.6 L, aerated, artificial illumination, 25 °C; pH 6.5 to 7.2	-	2	42 NH ₃ -N	0.4	99	12 PO ₄ ³⁻ -P	0.62	94	-	-	-	Hameed, 2007
Consortium of algae and bacteria; main algae were <i>Scenedesmus</i> and <i>Chlorella</i>	STE	Alginate beads, V = 2.5 L, no mixing, artificial illumination, 23 °C; pH 8.05 to 9	-	10	36 NH ₄ ⁺ -N	3.6	90	0.86 TP	0.03	97	49 COD	-	-	Solé and Matamoros, 2016
Suspended - PBR														
Consortium with the predominate strains <i>Actinastrum</i> , <i>Scenedesmus</i> , <i>Chlorella</i> , <i>Spirogyra</i> .	PSW	Semi-continuous mode, V = 1 L, aerated, artificial illumination, 23 to 25 °C; pH 7 to 8	3	10	39 NH ₄ ⁺ -N	6.1	84	2.1 PO ₄ ³⁻ -P	<0.1	99	-	-	-	Woertz et al., 2009
Prevalent microalgae species was	STE	Batch, V = 15 L, pump	-	1	36	0.1	99	2.56	0.03	98	-	-	-	Di Termini

Table 3 (continued)

Algae	Waste water type	Treatment conditions and reactor	HRT	Treatment time (days)	Nitrogen			Phosphorus			Carbon			Reference
					C _i	C _f	%	C _i	C _f	%	C _i	C _f	%	
<i>Scenedesmus</i>		mixed, artificial illumination, 20 °C; pH 7.2 to 8.5			NH ₄ ⁺ -N			PO ₄ ³⁻ -P						et al., 2011
Prevalent microalgae species was <i>Scenedesmus</i>	STE	Batch, closed, V = 15 L, pump mixed, natural illumination, 4 to 28 °C	-	7	21	4.6	79	1.49	0.44	70	-	-	-	Di Termini et al., 2011
<i>Scenedesmus obliquus</i> and wastewater microbial community	STE	Batch, flat panel PBR, V = 4.5 L, aerated, artificial illumination, 20 °C; pH 7	1.1	-	19.7	2	89	1.75	0.09	84	-	-	-	Ruiz et al., 2013
Consortium of chlorococcales and cyanobacteria as well as natural wastewater microbial community	Anaerobic wastewater effluent	Batch, flat panel PBR, V = 8 L, aerated CO ₂ , artificial illumination, 28 to 32 °C; pH 7.2	2	-	59	-	67	-	-	97	51	-	-	Ruiz-Martinez et al., 2012
<i>Scenedesmus obliquus</i> and wastewater microbial community	STE	Semi-continuous, tubular air lift reactor, V = 330 L, natural illumination, 13 °C; pH 8.72	5	110	26.16	3.4	86	1.77	0.21	88	76.6	-	24	Arbib et al., 2013a, 2013b
<i>Scenedesmus obliquus</i>	STE	Batch, V = 2.5 L, aerated, artificial illumination, 25 °C; pH 9 to 9.5	-	2	34	0.1	99	2.5	0.42	83	-	-	-	Ruiz-Marin et al., 2010
<i>Chlamydomonas reinhardtii</i>	STE	Batch, V = 5 L, mixed, artificial illumination, pH 7 to 10	-	4	25	0.1	99	1.7	<0.1	98	30.2	-	-	Su et al., 2012
<i>Chlorella vulgaris</i>	Tertiary wastewater	Batch, V = 0.2 L, mixed, artificial illumination, 27 °C; pH 7.3 to 5.7	-	4	8.7	0.1	99	1.71	<0.1	99	22.6	-	-	Ji et al., 2013
<i>Chlorella vulgaris</i> and natural wastewater microbial community	Municipal wastewater	Membrane photobioreactor, closed, V = 10 L, aerated, artificial illumination, 25 °C; pH <9	2.5	-	8.3	3.6	56	1.24	<0.3	82	55.6	-	-	Gao et al., 2014
<i>Chlorella vulgaris</i> and natural wastewater microbial community	Pre-PSW	Optical panel membrane PBR, closed, V = 40 L, aerated, artificial illumination, 25 °C; pH 7.2	3.4	150	40.2	11	70	9.24	4.37	52	209	86	58	Choi, 2015
<i>Chlorella</i> sp. ADE4 and natural wastewater microbial community	Treated sewage effluent	Membrane PBR, closed, V = 7 L, aerated, artificial illumination, 25 °C; pH 7.5 to 8.5	2	18	18.8	6.3	66	1.01	<0.1	94	10.5	-	-	Boonchai and Seo, 2015
Suspended - HRAP														
<i>Scenedesmus obliquus</i> and wastewater microbial community	STE	HRAP, V = 533 L, mixed, natural illumination, 13 °C; pH 9.32	10	110	26.16	11	55	1.77	0.64	64	76.6	-	12	Arbib et al., 2013a, 2013b
<i>Chlorella pyrenoidosa</i> and wastewater microbial community	STE	HRAP, V = 165 L, mixed, natural illumination, 31 to 6 °C; pH 7.8 to 9.3	-	18	46	2.1	95	3.22	0.59	84	426	90	78	Dahmani et al., 2016
Consortium of <i>Chlorella</i> , <i>Nitzschia</i> sp., <i>Navicula</i> sp., <i>Stigeoclonium</i> sp., ciliate, protozoa and bacteria	PSW	HRAP, V = 470 L, mixed, natural illumination, 23.7 °C	6	-	36	0.3	99	-	-	-	318	64	80	Gutiérrez et al., 2016
Unspecified algae and microorganisms	PSW	HRAP, mixed, natural illumination, 13 to 19 °C; pH 7.4 to 8.9	8	-	51.2	14	72	8.5	4.8	43	260	170	34	García et al., 2006
<i>Micractinium pusillum</i> , <i>Desmodesmus communis</i> , <i>D. opliensis</i> , <i>Pediastrum boryanum</i> , <i>Actinastrum hantzshii</i> , <i>Closterium</i> and natural bacteria	PSW	HRAP (Spring), V = 4375 m ³ , mixed, natural illumination, 13 °C; pH 9.7	7	-	22	4	79	1.8	1.6	22	-	-	-	Sutherland et al., 2014
Prevalent organisms <i>Coelastrum</i> amongst others	USAB effluent	HRAP, (Spring L-CO ₂), V = 9600 m ³ , mixed, natural illumination, pH 7.9 to 8.1	7	-	48	2.9	94	7.8	3.2	58	167	63	62	de Godos et al., 2016
Chlorophyta	PSW	Experiment 1, V = 15 L, mixed, natural illumination, 23 °C; pH 7.5	4	16	29.1	15	48	4.1	3.56	13	-	-	-	Sutherland et al., 2015

COD, chemical oxygen demand; DIN, dissolved inorganic nitrogen; DRP, dissolved reactive phosphorus; NH₄⁺-N, ammonium-nitrogen; NO₃-N, nitrate-nitrogen; PO₄³⁻-P, phosphate-phosphorus; TAN, total ammonia nitrogen; TC, total carbon; TDP, total dissolved phosphorus; TN, total nitrogen; TOC, total organic carbon; TP, total phosphorus.

4.2. Carbon, N and P removal mechanism by micro-algae

4.2.1. Carbon

In photoautotrophic mode, micro-algae can utilise inorganic carbon, predominantly CO_2 , as their primary carbon source (Falkowski and Raven, 2007). In aqueous solutions, gaseous CO_2 dissociates into bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions depending on the pH, with the precise equilibrium subject to the temperature of the environment, cation concentration and salinity (Hill et al., 2014). As a result of the non-polar nature of CO_2 , it can easily diffuse across the plasma membrane of micro-algal cells, whereas HCO_3^- requires active transport mechanisms (Fig. 2) (Falkowski and Raven, 2007; Borowitzka et al., 2016). In the chloroplast, HCO_3^- is rapidly catalysed to CO_2 through the enzymatic action of carbonic anhydrase to facilitate the fixing of inorganic carbon (Falkowski and Raven, 2007; Colman et al., 2002) (Falkowski and Raven, 2007; Colman et al., 2002). Most micro-algae have adapted carbon concentration mechanisms to minimise the loss

of photosynthetic activity in order to improve CO_2 accumulation rate within the chloroplast because of the low CO_2 concentration in aquatic environments (Raven et al., 2008).

Micro-algae convert inorganic carbon to organic carbon via the Calvin cycle by utilising the reductant NADPH (nicotinamide adenine dinucleotide phosphate oxidised) and energy from ATP hydrolysis produced in the photosynthetic electron transport chain (Falkowski and Raven, 2007). Inorganic carbon, as CO_2 , is fixed to ribulose-1,5-bisphosphate (RuBP), the acceptor molecule, yielding two molecules of 3-phosphoglycerate (3-PGA) in a reaction catalysed by the enzyme ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCo) (Fig. 2). The carboxyl carbon on each 3-PGA molecule is subsequently phosphorylated to form 1,3-bisphosphoglycerate (3-bisPGA) and is successively reduced to glyceraldehyde-3-phosphate (G3P). In this reaction, for every three molecules of CO_2 fixed, four molecules of RuBP are produced with only three remaining in the cycle. The additional G3P is transferred into storage or metabolised further to pyruvate through the glycolytic

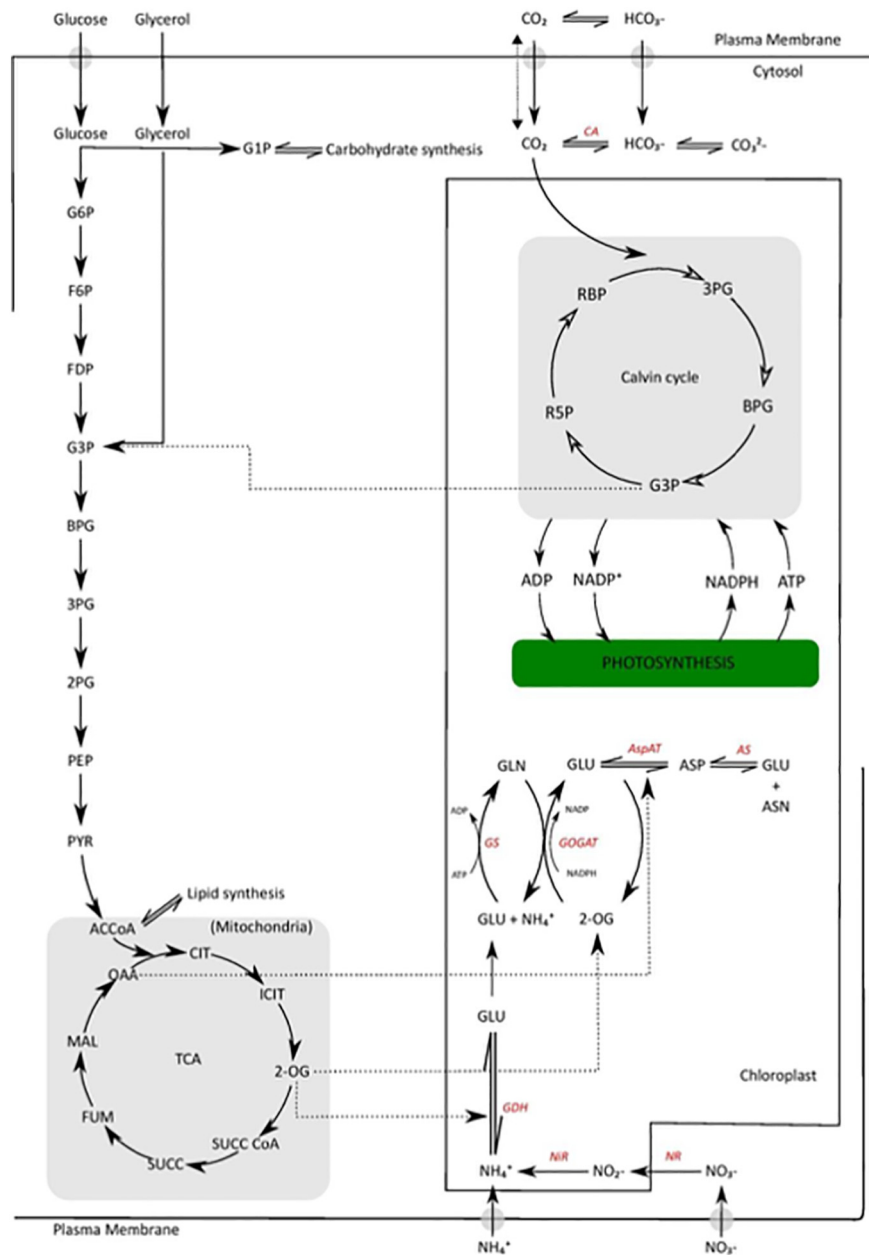


Fig. 2. Schematic of metabolic pathways for assimilation of carbon and nitrogen in the production of energy and amino acids in photoautotrophic and heterotrophic cultivation mode of micro-algae (adapted from Inokuchi et al., 2002; Perez-Garcia et al., 2011b).

pathway and subsequently into the tricarboxylic acid cycle (TCA). The Calvin cycle provides the carbon skeletons necessary for other metabolic reactions to produce amino acids and lipids in micro-algae (Falkowski and Raven, 2007).

Previous studies have reported that, other than light, the quantity of carbon in wastewater is one of the principal rate-limiting factors for micro-algal growth (Arias et al., 2017). Low availability in carbon, in particular inorganic carbon, can limit micro-algal growth and directly the quantity of N and P assimilated by micro-algae. To increase the availability of carbon in the wastewater medium, and exogenous supply, in the form of CO₂ or bicarbonate salts, is commonly used (Kesaano et al., 2015; Razzak et al., 2013; Cragg, et al., 2011). The effect is a significant improvement in micro-algal growth and remediation of N and P from wastewater, with the efficiency dependent on the CO₂ concentration and injection period. Shen et al. (2015a) reported on the remediation of TN from artificial wastewater by *S. obliquus* at CO₂-to-air ratios of 1%, 5%, 10% and 15%. In this treatment, a 99.6% removal efficiency of TN occurred within 2 days at 5% CO₂, with the concentration decreasing from an initial value of 25.0 to 0.08 mg L⁻¹. In comparison, 1%, 10%, 15% CO₂ or ambient air were only capable of reducing the TN concentration to, respectively, 3.55, 3.0, 5.5 and 6.15 mg L⁻¹ within 3 days.

A similar effect has been reported by other studies, with the supply of CO₂ in the range of 1 to 6% described as optimum to promote micro-algal growth and nutrient removal (Yao et al., 2015; Qi et al., 2017; Hu et al., 2012). Concentrations above this range have been found to reduce the beneficial effect of CO₂, with reported inhibitory effects on micro-algal respiration (Sforza et al., 2012). It must be noted that the tolerance to CO₂ is strain dependent, with certain species capable of acclimating to elevated CO₂ concentrations up to 100% (Wang et al., 2008; Zhao and Su, 2014).

While the strategy of CO₂ injection is a viable option to augment carbon availability for micro-algae in wastewater, its provision is energetically expensive. Furthermore, the supply of CO₂ may reduce the potential of the micro-algae to use and therefore reduce the carbonaceous material in wastewater. Hu et al. (2012) reported the occurrence of this effect, with the rate in COD reduction inversely related to the CO₂ concentration supplied. A potential strategy that may mitigate this is through intermittently supplying micro-algae with CO₂, promoting autotrophic growth followed by heterotrophic consumption of the carbonaceous material. Indeed, it was observed that intermittent sparging for a specific period of time minimised carbon losses to micro-algae when cultured in a raceway reactor, with higher CO₂ concentrations in the gas necessitating a lower gas flow (Duarte-Santos et al., 2016).

Alternatively, certain micro-algae can be cultivated on organic carbon substrates, in theory utilising the carbonaceous material in wastewater as a source. Some photosynthetic micro-algae are facultative heterotrophs, able to metabolise organic carbon compounds, either in a mixotrophic mode with CO₂ and light or in a strict heterotrophic mode (without light) (Perez-Garcia et al., 2011b). However, the complexity of the carbonaceous material in wastewater may limit its availability as a viable carbon source. Carbonaceous material in municipal wastewater is extremely heterogeneous with compounds ranging from simple low-molecular-weight compounds, like butyric acid, to more complex compounds such as polycyclic aromatic hydrocarbons and synthetic polymers (Huang et al., 2010). For example, Devi et al. (2012) reported a COD reduction of only 18.3% with a final concentration of 328 mg L⁻¹ O₂ in sterile municipal wastewater only when treated by a micro-algae consortium under strict heterotrophic conditions.

Analysis of municipal wastewater has identified the majority of biological carbonaceous material to be composed of fibers and proteins, while sugars account for only a total 10% or less (Huang et al., 2010). It has been suggested that in wastewater treatment the decomposition of complex organic carbon compounds by heterotrophic microorganisms (i.e. bacteria and fungi) is necessary to facilitate the conversion of the carbonaceous material to a suitable substrate in order to be a viable

carbon source for micro-algae (González et al., 2008; Lowrey et al., 2015). Corroborating evidence by He et al. (2013) showed no substantial reduction in BOD or DOC concentration from sterile secondary wastewater treated by *C. vulgaris* under mixotrophic conditions, whereas an average 90% BOD removal efficiency was recorded in unsterilized secondary wastewater under the same conditions. A faster reduction with a lower final NH₄⁺-N and PO₄-P concentration was recorded in the unsterilized wastewater micro-algae treatment.

However, the capacity of micro-algae to assimilate and metabolise carbonaceous material from wastewater may also be highly dependent on the composition of the wastewater itself, and not only the culture conditions. For example, Sacristán de Alva et al. (2013) recorded 77.3% COD removal efficiency from sterile PSW treated by *S. obliquus*, decreasing from 782 to 177 mg L⁻¹ O₂ under mixotrophic mode. In two independent studies treating sterile centrate wastewater, Li et al. (2011b) reported a consistent COD removal efficiency (>80%) when treated by *C. vulgaris* strain UTEX 25 in either autotrophic, heterotrophic and mixotrophic mode, while Hu et al. (2012) reported a similar COD removal efficiency (78.9%) when treated by *Auxenochlorella protothecoides* under mixotrophic conditions (5% CO₂). It is clear from the reported experimental evidence that the ability of micro-algae to grow on and simultaneously reduce the carbonaceous material from wastewater is dependent on its composition, in addition to species and culture conditions. However, a paucity of information exists on the precise nature and mechanisms by which micro-algae are capable of digesting and assimilating more complex carbon compounds from their aquatic environment (Lee, 2001).

To improve the treatment efficiency of wastewater by micro-algae, which may be limited by a labile source of carbon and without adopting CO₂ injection, supplementation with a source of readily biodegradable carbon has been examined. Addition of organic carbon to micro-algae cultures has predominantly focused on substrates such as glucose, glycerol, acetate or ethanol known to directly enter into the glyoxylate or glycolytic pathways (Perez-Garcia et al., 2011b; Bhatnagar et al., 2011; Yee, 2015; Abreu et al., 2012) (Fig. 2). Other carbon sources include mono- and di-saccharides, such as fructose, sucrose and lactose (Lee, 2001). Chandra et al. (2014) reported an improved efficiency in NO₃-N and PO₄-P removal from synthetic wastewater enriched with glucose using a natural microalgal consortium. In the treatment without amendment with glucose, the concentration of NO₃-N and PO₄-P decreased by 33% and 9.9%, respectively, whereas the glucose supplemented treatments (at concentrations of 0.5 to 3 g L⁻¹) effected a removal efficiency between 36% and 55% for NO₃-N, and 54% to 55% for PO₄-P. Interestingly, the authors observed a decrease in COD removal efficiency with an increase in glucose concentration. Perez-Garcia et al. (2011a) reported a higher rate of NH₄⁺-N removal from both synthetic and real municipal wastewater when treated with *C. vulgaris* supplemented with either glucose or acetate. Although enrichment with organic carbon could be a strategy to improve the treatment efficiency of a micro-algal wastewater treatment process, supplementing organic compounds increases production costs. Low-cost or waste organic carbon substrates have been researched mainly to improve biomass yield of micro-algae, including food waste (e.g. dairy waste and cane molasses), polysaccharide hydrolysate (produced from starch or straw) and high strength domestic or livestock wastewater (centrate) (Xu et al., 2006; Cheng et al., 2009; Wei et al., 2009; Gélinas et al., 2015).

4.2.2. Nitrogen

Micro-algae are able to utilise N from a variety of inorganic (e.g. NH₄⁺, NO₃⁻ and NO₂⁻) and organic sources (e.g. amino acids, urea, purines and nucleosides) (Ross et al., 2018; Cai et al., 2013). In regard to inorganic N, micro-algae express a clear preference for NH₄⁺ if available because its assimilation and incorporation is energetically more efficient (Perez-Garcia et al., 2011b). Ruiz-Marin et al. (2010) demonstrated preference for NH₃ as an N source from wastewater to any other N

source by species of *S. obliquus* and *C. vulgaris*. Ammonium is assimilated by a group of membrane transporter proteins belonging to the ammonium transporter family, an evolutionarily common protein expressed in bacteria, yeast, algae and higher plants (Wilhelm et al., 2006). Once translocated across the membrane, NH_4^+ can directly be incorporated into amino acids necessary for growth and other metabolic functions (described below). In contrast, NO_3 and NO_2 must be reduced to NH_4^+ ; a reaction catalysed by the enzymes nitrate reductase and nitrite reductase, which respectively require the reductants NADH and ferredoxin (Falkowski and Raven, 2007). Moreover, the transport of NO_3 into the cell is an energy-dependent process directly consuming ATP.

Although a decrease of NH_4^+ mediated by nitrification can be viewed as a benefit, from an operational viewpoint of a micro-algae wastewater treatment process, the generation of NO_x is undesired as it is not eliminated by micro-algae in the presence of NH_4^+ . Therefore, in an algae-bacteria process in which nitrification occurs, either a denitrification step in the treatment train needs to be included or a sufficient long hydraulic retention period is necessary for the micro-algae to effectively reduce the NH_4^+ and then NO_3 in order to meet the required total N discharge limits. Both approaches have the disadvantage of increasing operational cost and complexity. Furthermore, nitrification may induce N-limited conditions, with micro-algal growth rates reduced because of their competition for nutrients (Meseck et al., 2007). In a steady state algae-bacteria process, various authors have reported that an approximate 60 to 85% of NH_3 in the medium is oxidised to NO_3 with only 13 to 40% assimilated by the micro-algae (Karya et al., 2013; Vargas et al., 2016).

Inorganic N assimilation in micro-algae is inter-connected with their carbon metabolism, requiring carbon skeletons in the form of keto-acids to incorporate N into organic compounds (Falkowski and Raven, 2007) (Fig. 2). Anabolism of amino acids in micro-algae requires inorganic N in the form of NH_4^+ as the primary N donor molecule. The integration of N is catalysed by the sequential action of the evolutionary conserved enzymes glutamine synthetase (GS) and glutamine 2-oxoglutarate amino transferase (GOGAT) (Inokuchi et al., 2002; Lu et al., 2005) (Fig. 2). GS fixes NH_4^+ on a glutamate molecule to yield glutamine, and the added amino group then can act as the N donor to 2-oxoglutarate in the NADPH dependent conversion to yield two glutamate compounds catalysed by GOGAT (Inokuchi et al., 2002). The assimilated N can then be further distributed to form other amino acids via transamination reactions. For example, aspartate aminotransferase (AspAT) transfers the amino group of glutamate to oxaloacetate yielding aspartate and 2-oxoglutarate, whereas asparagine synthetase (AS) transfers the amino group of glutamine to aspartate to form asparagine, with both reactions being reversible (Inokuchi et al., 2002). Consequently, glutamine, glutamate, aspartate and asparagine are precursor substrates for the synthesis of organic N compounds, such as amino acids, nucleotides, chlorophylls, polyamines and alkaloids (Inokuchi et al., 2002; Coruzzi, 2003).

An auxiliary pathway in the regulation of NH_4^+ assimilation into amino acids was identified as the reversible reductive amination of 2-oxoglutarate regulated by the enzyme glutamate dehydrogenase (GDH) (Mifflin and Habash, 2002). Although the pathway is highly conserved between micro-algae species it is not thought to have a significant part in the formation of amino acids (Inokuchi et al., 2002). In fact, evidence suggests its main role is to catabolise glutamate, which returns the carbon from the amino acid (Lea and Mifflin, 2003). The activity of GDH is believed to be active under conditions of stress, particularly carbon shortage, and thus provides a feedback of necessary carbon skeletons to the TCA cycle in the mitochondria ensuring that energy production is not impaired (Lu et al., 2005; Lea and Mifflin, 2003).

In photoautotrophic mode, the inorganic carbon fixed in the Calvin cycle can enter the glycolytic pathway (also known as the Embden-Meyerhof pathway) as G3P, in which it becomes metabolised into pyruvate (Perez-Garcia et al., 2011b; Johnson and Alric, 2013) (Fig. 2). The generated pyruvate is then transported to the mitochondria upon

which it enters the TCA cycle following its conversion to Acetyl-CoA. Through the TCA cycle, Acetyl-CoA is further metabolised to yield CO_2 , reducing equivalents, ATP and carbon skeletons, including 2-oxoglutarate oxaloacetate for biosynthesis and further respiration as recycled substrates in the cycle (Voet and Voet, 2011) (Fig. 2).

In heterotrophic mode, organic carbon substrates, as in the example for glucose, would be actively transported into the cytosol by the hexose/ H^+ symporter system together with H^+ ions at a stoichiometry of 1:1 with the energy provided for this by the hydrolysis of one ATP molecule (Tanner, 2000). In the cytosol, glucose becomes metabolically active through the glycolytic pathway, which transforms one glucose molecule into pyruvate (Perez-Garcia et al., 2011b). Glucose may also be metabolised in the pentose phosphate pathway (PPP) producing ribose-5-phosphate and erythrose-4-phosphate, which are precursor substrates in nucleic acid and amino acid synthesis respectively (Kruger and von Schaewen, 2003). The function of both pathways are considered anabolic and anaerobic because no O_2 is consumed and because ATP and reducing equivalents are required which are generated in alternative aerobic pathways, mainly from the mitochondria electron transport chain and oxidative phosphorylation. The main difference between the two pathways is the condition under which they are activated; PPP generally has a high rate of activity under dark conditions, while glycolysis mainly takes place in light conditions (Yang et al., 2000). Glycerol, as an alternative carbon substrate, can translocate across the membrane by passive diffusion into the cytosol of micro-algae upon which it becomes sequentially phosphorylated and reduced to G3P and glycerate (Perez-Garcia et al., 2011b). It is, however, impossible to precisely determine which substrate is preferred by any given micro-algae. Overall, the carbon and N cycles in micro-algae are integrally connected, with as much as 35% of carbon coupled to the incorporation of N in micro-algae (Falkowski and Raven, 2007; Perez-Garcia et al., 2011b).

4.2.3. Phosphorus

In micro-algae, P is an important element involved in innumerable metabolic pathways as well as a structural component of phospholipids, nucleotides and integral to the biological energy currency, ATP (Borowitzka et al., 2016). Inorganic P in wastewater exists in several ionic states and like inorganic carbon the specific species is dependent on pH (H_3PO_4 , <2.15; H_2PO_4^- , 2.15 to 7.20; HPO_4^{2-} , 7.20 to 12.33; and PO_4^{3-} , >12.33) (Shen et al., 2015b). Inorganic P is generally regarded as the most bioavailable form of P, with micro-algae reported to preferentially assimilate HPO_4^{2-} and H_2PO_4^- (Falkowski and Raven, 2007; Silva et al., 2015). In algae, PO_4^{3-} enters the cell by means of active transport through a symporter channel with H^+ or Na^+ ions providing the driving force, established by a plasma membrane H^+ -ATPase pump (Falkowski and Raven, 2007). It is increasingly recognised that soluble organic P compounds are a critical source of bioavailable P (Borowitzka et al., 2016; Li and Brett, 2013). These are made accessible to the micro-algae by the expression of extracellular membrane-bound as well as free phosphatases, which non-specifically hydrolyse bound PO_4^{3-} groups (Borowitzka et al., 2016; Hoppe, 2003).

Phosphorous is incorporated into organic compounds following phosphorylation of adenosine diphosphate (ADP). This is an endergonic reaction with the energy input obtained from either the oxidation of respiratory substrates or the photosynthetic electron transport chain (Borowitzka et al., 2016; Gonçalves et al., 2017). The produced ATP permits the transfer of the PO_4^{3-} group to organic compounds at the substrate level, as for example in the conversion of glucose to glucose-6-phosphate in the glycolytic pathway (Falkowski and Raven, 2007; Voet and Voet, 2011). Furthermore, in P-rich environments, micro-algae can accumulate P in excess of their metabolic needs and store it as acid-insoluble polyphosphate granules – a mechanism termed ‘luxury uptake’ which only occurs without a prior starvation period (Eixler et al., 2006).

4.3. Abiotic and biotic factors influencing micro-algae wastewater treatment

4.3.1. Bacteria

Extensive research in wastewater treatment has been performed with single micro-algal species or a consortium of different species. In reality, the presence of other microorganisms (e.g. bacteria and fungi) is unavoidable in a micro-algal wastewater treatment system, as it is not feasible to previously sterilise the water because of the enormous volumes to be processed. In these conditions, the dynamics in community structure are generally a function of operational and environmental conditions, as well as the composition of wastewater being processed (Posadas et al., 2014; Ferrero et al., 2012). With regards to bacteria, only a few studies report on community dynamics in algal-bacterial co-culture treatment processes. Su et al. (2011) treating PSW with a micro-algal consortium, reported the enrichment of certain bacterial species and which stabilised over the course of a semi-continuous treatment system. Notably, the bacterial community became dominated by members of the classes *Bacteroidia* (50%), *Flavobacteria* (25%), *Betaproteobacteria* (12.5%) and *Gammaproteobacteria*. In a subsequent study where different inoculation ratios of micro-algae to sludge were investigated for their removal efficiency of contaminants from PSW, variations in the bacterial community composition occurred between the treatments of different inoculation ratios (Su et al., 2012). Bacterial species that were not detected in the original inoculum became enriched to varying degrees during operation, which may have contributed to the difference in removal efficiency between the treatments. When cultured in digestate, the microbial community was dominated by *Gammaproteobacteria*, mainly *Pseudomonas stutzeri*, followed by members of the class *Alphaproteobacteria* (Vasseur et al., 2012). Conversely, in pig manure 54% of the community was represented by members belonging to the phylum *Verrucomicrobium*, with also high representation by *Gammaproteobacteria* and members of the phylum *Firmicutes* (Ferrero et al., 2012). Overall, micro-algae have a significant effect on the microbial community and were found to reduce the diversity of bacteria present (Lee et al., 2013a, 2013b).

With regard to the treatment of wastewater, bacteria are necessary and indeed can be beneficial to micro-algae. Bacteria may support the photoautotrophic growth of micro-algae by providing CO₂ through their heterotrophic metabolism of organic matter, mineralising it to inorganic compounds that can be consumed directly by the micro-algae, including NH₄⁺ and PO₄³⁻ (Bordel et al., 2009; de Godos et al., 2010). In return, micro-algae provide O₂ generated via photosynthesis, required by the heterotrophic bacteria to degrade the organic matter, and required back by the micro-algae during dark respiration (Falkowski and Raven, 2007). In fact, photosynthetic oxygenation has the potential to meet dissolved O₂ needs to a treatment system without the use of mechanical aeration or mixing, thereby reducing the energy demands for the treatment process. To exemplify, Karya et al. (2013) employed a sequence batch design with *Scenedesmus* sp. and nitrifying bacteria isolated from activated sludge to evaluate whether this co-culture system can support nitrification. Without mechanical aeration, the process was shown successful in reducing 81 to 85% of NH₄⁺-N through its conversion to NO₃-N by nitrification, for which the O₂ for this process had been generated by the micro-alga. Similarly, Wang et al. (2015) reported that photosynthesis by a micro-algal consortium (predominantly *Chlorella* sp.) generated a sufficient quantity of dissolved O₂ to support nitrification in a photo-sequence batch reactor. In this process, centrate from anaerobically digested swine manure was cycled in the reactor between light and dark conditions, with the micro-algae under illumination providing enough O₂ for complete nitrification, while in the dark condition denitrification occurred with the addition of acetate as a carbon source. Overall, 80% of the N was removed through nitrification and denitrification from an influent that was not aerated and had a mean NH₄⁺-N concentration of 297 mg L⁻¹. González et al. (2008) reported that the micro-alga *C. sorokiniana* was capable of providing a sufficient dissolved O₂ concentration for heterotrophic degradation of

swine slurry medium when diluted 4 and 8 times, with O₂ concentrations reaching 2.5 mg L⁻¹.

The interaction between bacteria and micro-algae is more complex than the exchange of just nutrients. Certain bacteria can promote micro-algal growth by excreting growth-promoting compounds or vitamins (e.g. thiamine, biotin, etc.) (Droop, 2007; Higgins et al., 2016; Ramanan et al., 2016). De-Bashan et al. (2004) found that the bacterium *Azospirillum brasilense* (strain Cd) promoted the growth and nutrient uptake rate of *C. vulgaris* and *C. sorokiniana* when co-immobilised in alginate beads. The algal-bacterial co-culture was capable of removing 100% NH₄⁺-N, 15% NO₃-N and 36% PO₄-P from municipal wastewater, while a corresponding culture with only micro-algae achieved 75% NH₄⁺-N, 6% NO₃-N and 19% PO₄-P removal within 6 days. Micro-algae can promote bacterial growth through micro-algal exudates that either stimulate their growth directly or can be assimilated as a source of carbon (Mandal et al., 2011; Fouilland, 2012). Hulatt and Thomas (2010) quantified the amount of DOC excreted by micro-algae in polythene photobioreactors, demonstrating a significant increase in bacterial population in micro-algal-bacterial co-cultures as a result when compared to control cultures with only the bacteria. The authors of this study also showed that the DOC released by *C. vulgaris* and *Dunaliella tertiolectra* accounted for a maximum 6.4% and 17.3% of the total organic carbon in the culture, respectively. Conversely, metabolites presenting either bactericidal or fungicidal activity excreted by micro-algae have been reported, including activity against the bacterium *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, as well as the fungus *Candida albicans* (DellaGreca et al., 2010; Najdenski et al., 2013). Similarly, certain species of bacteria have been found able to excrete algicidal compounds (Natrah et al., 2014).

4.3.2. Industrial contaminants

Micro-algae have shown great potential in removal of industrial contaminants, such as pharmaceutical pollutants and heavy metals. A study on the efficiency of HRAP for the removal of pharmaceuticals in urban wastewater indicated that HRAP was more efficient eliminating some pharmaceuticals that are of environmental concern, such as a diclofenac and antibiotics, while the most commonly used analgesics and anti-inflammatories, such as ibuprofen and paracetamol, were slightly better removed in the conventional WWTP (Villar-Navarro et al., 2020). Presently, various studies have demonstrated wastewater treatment by micro-algae to be technically possible at laboratory scale (Mennaa et al., 2017), pilot and demo scale (Arbib et al., 2017). However, the economic feasibility of this technology needs to be justified in comparison to conventional wastewater treatment methods.

4.3.3. pH

Several studies have reported on abiotic mechanisms by which bacteria and micro-algae adversely affect each other. For example, an increase in pH and dissolved O₂ concentration observed in micro-algae cultures can have a detrimental effect on bacterial activity (Schumacher et al., 2003; Ansa et al., 2011; Sousa, 2013). Assimilation of inorganic carbon by micro-algae, if not replenished at an equivalent rate of consumption, can cause the pH to increase in the medium leading to an alkaline environment (pH >9) [67, 146]. Under these conditions, the benefit provided by aerobic and facultative bacteria in wastewater may be reduced as their growth and function becomes impaired. A strong correlation between heterotrophic bacteria abundance and pH is reported by other studies, which demonstrate an increased "inactivation" of bacteria with increasing pH (Ansa et al., 2011; Awuah et al., 2001; Ansa et al., 2012). Reduction in coliform bacteria and other pathogenic microorganisms is reported to occur at pH 8.5 with pH 9.5 resulting in the highest elimination of the wastewater bacterial community (Metcalf and Eddy, 2003; Awuah, 2006). The effects are mediated through several different, potentially co-occurring mechanisms, such as conformational changes in bacterial membrane structure, respiratory chain damage and increased susceptibility to exogenous factors such as light (Bossard et al., 2010).

Consequently, the reduced abundance of the microbial community in wastewater treated by micro-algae will lead to a lower rate of CO₂ release via respiration that would otherwise serve the micro-algae with an alternative source for photosynthesis (Subashchandrabose et al., 2011; Muñoz and Guieysse, 2006). The optimal pH range for the majority of freshwater micro-algae species is reported to be between 7 and 9 (Richmond, 2003; Pandey et al., 2013; Kumar et al., 2010). Environments outside of the optimum range for a particular species or consortium may adversely affect their growth rate and limit their capacity to remediate nutrients from the medium. From two independent experimental runs, Sutherland et al. (2015) reported a decrease in removal efficiency of dissolved inorganic nitrogen (DIN), with increasing pH from PSW treated with a natural consortium of micro-algae. In this study, pH 6.5 and 7 resulted in an approximate 62% DIN removal, whereas at pH 7.5 to 8 resulted in the removal of approximately 50% DIN. Martínez et al. (2000) observed that cell rupture of *S. obliquus* was associated with the point at which the pH of the medium reached its highest value (>11) when treating municipal STE. Overall, establishing an optimal environment for micro-algal cultivation in wastewater for the purpose of nutrient remediation can be a critical step in preserving species dominance. These conditions are, however, highly dependent on the micro-algal species and the cultivation method employed. Therefore, a suitable strategy might be to allow a natural species to acclimate to the subsequent processing conditions that naturally develop or are expected.

4.3.4. Temperature and light

As nutrients (e.g. N and P) become limiting, the autochthonous microbial community in wastewater may compete with exogenous micro-algae (supplemented into the wastewater) for resources (Lee et al., 2013a, 2013b; Grover, 2000). It is therefore essential to establish an environment which promotes the growth of the micro-algae above that of bacteria and fungi. In this context, temperature and light have a significant influence. In regard to light, its availability is fundamental for normal micro-algal functioning. Energy captured from light drives the process of O₂ evolution and generates ATP and reducing agents necessary for fixing CO₂ into organic carbon (Falkowski and Raven, 2007; Williams and Laurens, 2010) (Fig. 2). Below the light saturation point, the rate of photosynthetic activity is proportional to the irradiance intensity, with intensities above this point causing photo-inhibition as receptor systems become damaged (Falkowski and Raven, 2007; Williams and Laurens, 2010). The illumination intensity at which saturation occurs may differ depending on the micro-algal species and temperature. In general, the illumination saturation point for freshwater micro-algae is reported to lie between 200 and 400 μE m⁻² s⁻¹ (Muñoz and Guieysse, 2006; Cheirsilp and Torpee, 2012; Singh and Singh, 2015). Maintaining an algal culture at or below the saturation point has a practical component because excess light is not utilised by the algae, and which would be otherwise be a waste of energy expenditure in the form of excess electricity usage.

The illumination period and intensity to which a algal-bacterial consortium is exposed to can significantly affect the ratio of bacteria to algae, and consequently the efficiency of carbon, N and P removal in wastewater. Under prolonged dark conditions, Lee et al. (2015) reported a reduced capacity in N and P removal from municipal wastewater when treated with a algal-bacterial consortium. After 12 days of operation, the total dissolved nitrogen (TDN) concentration was reduced to 4.8, 14.0 and 25.6 mg L⁻¹, and the total dissolved phosphorus (TDP) concentration reduced to 0.6, 1.7 and 3.0 mg L⁻¹ in photobioreactors under 12:12 h, 36:12 h and 60:12 h dark-light cycles, respectively. Conversely, the soluble COD concentrations were reduced to 72, 56 and 35 mg L⁻¹ O₂, respectively. A significant shift in the bacteria to micro-algae ratio was observed following quantification by qPCR assay. Under prolonged dark conditions, a higher ratio of bacteria to micro-algae was recorded, with the lowest microbial biomass in terms of dry weight and chlorophyll *a* in the 60:12 h dark-light cycle treatment. González-Camejo et al. (2017) examined the treatment response of a algal-bacterial

consortium cultured in effluent from an anaerobic membrane system under varying light intensity. At the lowest set light intensity (40 μE m⁻² s⁻¹) a higher activity of nitrifying bacteria was observed causing increased concentrations of NO₃ and NO₂ in the effluent with only 73.9% of NH₃ reduction credited to micro-algae assimilation, and consequently the TN concentrations exceeded the permissible discharge standard (i.e. 10 mg L⁻¹ TN). In comparison, light intensities of 85 and 125 μE m⁻² s⁻¹ favoured micro-algae growth over nitrifying bacteria, with recorded NH₃ removal efficiencies by the micro-algae of 98.3% and 99.3%, respectively. Conversely, between the different light intensities examined, no difference in P removal efficiency was recorded (98.6, 99.2 and 99.5% at 40, 85 and 125 μE m⁻² s⁻¹, respectively). These observations indicated that the illumination period and intensity have a strong influence on the population dynamics in a algal-bacterial wastewater treatment system. Thus, in order to promote the growth of the micro-algae above bacterial growth and to ensure an adequate response in treatment, these parameters must be adjusted accordingly.

The environmental temperature also has a significant influence on micro-algal productivity and treatment efficiency in wastewater. Ruiz-Martínez et al. (2015) assessed the NH₄⁺-N removal rate by *Scenedesmus* sp. at various temperatures from effluent of a pilot scale submerged anaerobic membrane bioreactor; at a higher temperature the removal rate of NH₄⁺-N increased with 15 °C, 18 °C, 26 °C and 34 °C, demonstrating a rate of 4.3, 6.7, 15.7 and 17 mg N L⁻¹ d⁻¹, respectively. However, the optimal temperature has been shown to vary depending on the microalgal species and their acclimation to a particular environment. For instance, Filippino et al. (2015) reported a high efficiency in nutrient removal within a shorter cultivation period by *C. vulgaris* at a lower temperature. A 90% reduction in TDN and PO₄-P was achieved within 4 days of cultivation at 15 °C versus 12 days at 25 °C. Similarly, Sforza et al. (2014) reported a lower NH₄⁺-N concentration in the effluent of treated PSW by *C. protothecoides* at lower temperatures (15 °C) compared to temperate conditions (23 °C to 30 °C). Interestingly, the authors reported that specific growth rate, based on the parameter of cell number, was positively correlated with temperature, while total biomass (measured as total suspended solids (TSS)) tended to increase with decreasing temperature.

In general, most micro-algae are capable of surviving at temperatures between 10 °C to 30 °C, with the optimal temperature within a more narrow range, often between 15 °C and 25 °C (Singh and Singh, 2015). Although higher temperatures are generally associated with higher growth rates and increased nutrient uptake rates because of higher metabolic activity, these conditions are not always compatible with the conditions for wastewater treatment. Maintaining an optimum temperature in a micro-algal wastewater treatment process through artificial heating is not feasible given the excessive volumes, and thus huge energy input required. Therefore, the micro-algal species or consortium employed to treat the wastewater should be selected on their ability to thrive under the environmental conditions that are frequented at the treatment plant. The temperature of wastewater for mid-latitude climates has been reported to range between 3 °C to 27 °C (Metcalf and Eddy, 2003).

As photosynthetic carbon assimilation (i.e. Calvin cycle) is enzymatically mediated, the rate of the reaction is temperature-dependent with a lower reaction rate recorded at lower temperatures (Falkowski and Raven, 2007). To compensate for the imbalance of more light being adsorbed than can be used for carbon fixation, micro-algae respond by reducing their chlorophyll concentration at lower temperatures compared to cells at temperate conditions under the same illumination intensity. The reduction in chlorophyll is accompanied with an increase in the carotenoid xanthophyll (Christov et al., 2001). Xanthophyll forms part of the light harvesting antenna complex of photosystem II (PS II) and is proposed to modulate the transition of the complex to a dissipative photo-protective state, protecting the complex against damage from light saturation (Ruban, 2009). Therefore, cultivation at low temperature may require lower light intensities to minimise light saturation and photo-inhibition and, hence, may reduce power consumption

associated with the provision of illumination. A further benefit of a low operating temperature is the improved solubility of O₂ and reduced growth rates of indigenous microorganisms (Gray, 2004; Metcalf and Eddy, 2003; Christov et al., 2001).

4.4. Micro-algae bioreactor configuration for wastewater treatment

Wastewater treatment by micro-algae faces several challenges that range from varying wastewater composition to the large volumes that need to be treated. Different micro-algae cultivation techniques have been studied to ensure optimal micro-algae productivity, high effectiveness in the removal of nutrients or contaminants, and to accommodate the large volumes of wastewater (Hoh et al., 2016). The design and configuration of the reactor has a large effect on the treatment performance, with control over light and temperature influencing growth and in turn the assimilation and removal of contaminants from the wastewater (Mata et al., 2010). The different micro-algal cultivation techniques can be broadly categorised as either suspended or immobilised systems (Christenson and Sims, 2011). These systems are further sub-categorised as being either open to the environment or enclosed. The main performance consideration of the bioreactor is its economic cost, with examples for a micro-algae wastewater treatment system including (but not limited to) PBR, HRAP, matrix-immobilised micro-algae and attached micro-algal biofilms systems (Borowitzka and Moheimani, 2013). The efficiency of the wastewater treatment and biomass productivity varies considerably based on different types of reactors (Hoh et al., 2016).

4.4.1. Immobilised cells

The immobilisation of micro-algae can be achieved through the self-attachment (passive) to a bedding material, which is either completely or partially submerged to support biofilm development (i.e. flat panel or rotating algal biofilm reactor), or through entrapment (active) in gel matrices that can be induced or mediated by flocculent or chemical agents (Ting et al., 2017; Kesaano and Kesaano, 2015; de-Bashan and Bashan, 2010; Moreno-Garrido, 2008).

Biofilm formation initially occurs because cations, inorganic and organic compounds adhere to the surface of the bedding material, in effect increasing the concentration relative to the aqueous phase and creating a favourable environment for microbial growth (Stephens et al., 2015; Qureshi et al., 2005). Once colonised onto the surface, micro-algae and bacteria secrete extracellular substances composed of nucleic acids, proteins, polysaccharides and phospholipids which serve to improve adherence to the bedding material but also to entrap and concentrate nutrients necessary for cell growth (Qureshi et al., 2005). As micro-algae cells rely on photosynthesis for their growth and metabolism, factors such as light transmission, carbon dioxide and oxygen levels play a key role in designing the biofilm reactor (Hoh et al., 2016). Several studies have demonstrated and developed algal biofilm reactors for the efficient consumption of N and P from the wastewater and converting them into biomass (Yu et al., 2020; Yang et al., 2018). A variety of materials, such as stainless steel, natural fibers and nylon, have been reported to support the formation of algal biofilm (Hoh et al., 2016). In a study on the influence of algal biofilm on the photobioreactor wall, it was demonstrated that the formation of algal biofilm caused a significant reduction in phosphorus and nitrogen content of the wastewater (Su et al., 2016).

In general, micro-algal biofilms are restricted to a single plane because of the need for light and gas exchange, with biofilm thickness between 0.052 and 2 mm for optimal performance (Irving and Allen, 2011; Boelee et al., 2014). In the case of active immobilisation, the most widely used technique is the encapsulation of micro-algae into polymer matrices made of artificial (e.g. acrylamide) or natural materials (e.g. carrageenans or alginates) (Mallick, 2002; de-Bashan and Bashan, 2010). Manufactured to form beads, the micro-algae are entrapped in a suspended form within the pores of the polymer matrix that are smaller than the cells, retaining them while allowing the

diffusion of water and substances for their metabolisms and growth (Cohen, 2001).

The principal advantage of immobilised micro-algae systems is that they eliminate or reduce the processing cost associated with separating the algal biomass from the treated water before discharge (Christenson and Sims, 2012; Moreno-Garrido, 2008). Furthermore, by immobilising micro-algae a higher concentration of cells relative to free suspended systems can be maintained in the water. Up to 3.3 g L⁻¹ dry weight (DW) (Whitton et al., 2015) compared to 1.5 to 1.7 g L⁻¹ DW and 0.25 to 1 g L⁻¹ DW in suspended tubular and raceway ponds respectively, has been reported (Christenson and Sims, 2011). It is thought that the high concentration of active biomass within biofilms or other matrices allows for an increased rate of biodegradation activity and therefore improved removal efficiency (Cohen, 2001). This effect could also be attributed to the fact that particulate, organic and inorganic compounds attach to the surface of the immobilising polymers or biofilms, increasing and sustaining a high concentration of these substances to the proximity of the micro-algae and other microorganisms, in effect facilitating their biodegradation. However, no study has directly examined this occurrence to any great extent. Similarly, the close proximity of co-immobilised micro-algae and bacteria, which generate O₂ and CO₂ respectively, can avoid gas diffusion problems inside the medium or immobilising matrix (Stephens et al., 2015; Muñoz and Guieysse, 2006; Gonzalez and Bashan, 2000). Conversely, Jiménez-Pérez et al. (2004) found the N and P uptake rates of the micro-alga *S. intermedius* and *Nannochloris* sp. to be slightly higher when cultured in suspension compared to when immobilised. The authors argued this to be because of the additional resistance of nutrient diffusion across the polymer and impeded light penetration caused by the dense growth of cells within the inner surface of the beads, thereby reducing the photosynthetic activity.

The performance of immobilised micro-algae systems to treat wastewater has been well documented (Table 3). However, despite being effective at removing contaminants from wastewater, aspects of this technology still limit its commercial application. In active immobilisation, the polymers used to form the matrices are vulnerable to degradation over time, which can result in cells leaching (Mallick, 2002; Serp et al., 2000). Furthermore, the technical knowledge necessary for the manufacturing and high cost associated with the materials can prohibit their application, especially when the aim is to treat large volumes of wastewater (Gonçalves et al., 2017; Cohen, 2001). On the other hand, micro-algal biofilms require a large surface area. A theoretical analysis estimated 0.32 to 2.1 m² PE⁻¹ is required to accommodate a micro-algae biofilm treatment process in addition to the 0.2 to 0.3 m² PE⁻¹ of the conventional wastewater treatment system when employed as a post-treatment process (i.e. tertiary) (Acien et al., 2016; Boelee et al., 2012). Functioning as the primary biological treatment process for municipal wastewater, an estimated 0.76 m² PE⁻¹ is required (Boelee et al., 2012). The aerial requirement compromises the environmental sustainability of this technology. Also, when exposed to the natural elements, fluctuations in both irradiance and temperature affect the performance, with low irradiance leading to low micro-algal growth and O₂ generation in the biofilm and, hence, a reduced efficiency in nutrient removal (Boelee et al., 2011; Gross and Wen, 2014).

Attempts to optimize light utilisation in algal biofilm-based systems have been directed to bioreactor design modification, typically designed with high surface area to volume ratio (Muñoz et al., 2009). A rotating algal biofilm was designed and operated by Christenson and Sims (2012) to allow periodic exposure of the biofilm between the medium and light. Posadas et al. (2014) compared the treatment of domestic wastewater by two micro-algae biofilm systems, one grown on an open surface and the other enclosed in clear tubes. Overall, the open surface algal-bacteria biofilm had higher efficiency in inorganic carbon, N and P removal compared to the enclosed biofilm reactor. The main hypothesis put forward to explain the difference in efficiency between the two biofilm systems was the location of the active micro-algal

population in respect to the light source. In the enclosed system, photo-synthetic O_2 originated at the tubular surface and needed to diffuse to the centre of the tube in order for it to be utilised via heterotrophic metabolism; on the other hand, in the open biofilm O_2 originated in close contact to the contaminants at the biofilm-wastewater interface. Microalgal biofilms are also prone to sloughing, defined by the detachment of micro-algae and other particulate matter from the matrix surface in the course of treatment. For example, Boelee et al. (2011) noted an average suspended solids concentration of 3.2 mg L^{-1} in the final effluent, containing a high proportion of micro-algae biomass. This corresponded to an average concentration of 0.13 mg L^{-1} N and 0.07 mg L^{-1} P. Taking this into account, under continuous operation the biomass requires separation from the water prior to discharge to minimise the input of these captured nutrients into receiving systems, effectively negating the main advantage of micro-algae immobilisation (Mallick, 2002; Qureshi et al., 2005). When managed incorrectly, micro-algae biomass can account for a considerable proportion of the suspended solids content, contributing substantially to the effluent BOD (Grady et al., 2011).

4.4.2. Suspended cultures

Suspended cultivation of micro-algae allows the cells to move freely in the aqueous phase and is amongst the most commonly applied algal cultivation technique for treating wastewater (Borowitzka and Moheimani, 2013; Pires et al., 2013; Chisti, 2007). Open suspended systems can be categorised into natural ponds, such as facultative ponds and lagoons, or artificial containers such as raceway ponds. In facultative ponds, different environments naturally form as a result of the greater depths (over 1 m) and with minimal mixing as provided solely by wind, natural convection currents and water flow (Butler et al., 2017). Consequently, stratification occurs as aerobic conditions form at the surface of the water because of micro-algae photosynthesis, while anaerobic conditions form towards the bottom (Butler et al., 2017). However, improved treatment efficiency as a result of the stratification has been reported, as it has allowed different microbial communities with opposing roles in the treatment to become established (Meneses et al., 2005). In practice, high BOD, NH_3 and PO_4 removal rates and micro-algal growth have been reported in facultative ponds with minimal operation cost and maintenance required (Butler et al., 2017; Steinmann et al., 2003; Wallace et al., 2016). HRAP can be considered as an improvement to the design of facultative ponds, with added operational control over mixing and culture conditions (Chisti, 2007). Generally designed with depths of 0.2 to 0.5 m, HRAP are configured as a closed single canal, or meandering canal divided by central walls (Butler et al., 2017; Park et al., 2011). To prevent sedimentation of the micro-algae and to ensure periodic exposure to light, mixing is provided by means of a paddlewheel that is normally operated at velocities between 10 and 30 cm s^{-1} , while a CO_2 inlet can provide control over pH (Lee et al., 2016; Sutherland et al., 2015; Rawat et al., 2011).

Photobioreactors are enclosed suspended cultivation systems, designed as an enclosed system composed of transparent plastic or glass materials which hold the algal biomass and growth medium within a confined boundary (Lee et al., 2016; Chisti, 2007). As a cultivation method, PBR have the benefit of better control over the culture environment. Temperature is easily controlled by heating or cooling the tubing, fluctuations in the pH are minimised through direct CO_2 injection or acid addition, and evaporation or contamination is greatly reduced because of the sealed system limiting the exposure of the culture environment (Mata et al., 2010). The main advantage of PBRs is the improved light utilisation rate with a higher surface area to volume ratio compared to open pond systems (Jorquera et al., 2010; Carvalho et al., 2006). The increased irradiance to which the micro-algae are exposed to promotes higher photosynthetic rates and cell densities. However, the use of PBRs for large-scale application is likely to be limited because of the high economic cost for materials, construction and operation (Jorquera et al., 2010; Ación et al., 2013; Gupta et al., 2015).

Both facultative and HRAPs are open cultivation systems and thus dependent on sunlight as the primary source of irradiance. As such, variations in effluent quality will occur between seasonal cycles, with the most effective period being the summer months (Gross and Wen, 2014; Park et al., 2011). Other factors that affect the performance of open reactors are temperature, evaporation and potentially inorganic carbon deficiencies. Evaporation helps maintain a stable temperature (during the day), however, the loss of water from the system can result in significant change in the ionic composition which can directly affect microalgal growth (Pulz, 2001). Likewise, CO_2 diffusion to the atmosphere can reduce the biodegradation activity and growth of the micro-algae, leading to a less efficient treatment performance (de Godos et al., 2014). Open culturing systems (i.e. HRAP) are also susceptible to contamination by protozoa and zooplankton, which can reduce the algal concentration within a few days (Wang et al., 2013).

A further disadvantage to micro-algal pond systems is the large surface area required because of the shallow depths that are necessary for facilitated light exposure to the micro-algae (Lee et al., 2016). Craggs et al. (2003) reported that the surface area of HRAP at a depth of 0.45 and 0.3 m operating at a volume of 37.5 m^3 would, respectively, occupy an area of 85 and 128.1 m^2 . Under the proposed loading rate in the study, the surface area required to treat $1 \text{ m}^3 \text{ d}^{-1}$ of wastewater was 17 and 25.6 m^2 based on the depth of the pond. In a study by Wang et al. (2015), the authors estimated the surface area occupied by a HRAP using data from a laboratory pilot experiment. Depending on the N load the system required between 12 and 60 m^2 to treat $1 \text{ m}^3 \text{ d}^{-1}$ of centrate wastewater from anaerobically digested swine manure. In comparison, PBRs have inherent limitations associated to their design, such as high dissolved O_2 accumulation that can reduce photosynthetic activity, and biofouling with microbial films forming on the internal surfaces of the reactors which can adversely affect light penetration. PBRs placed outdoors are also susceptible to the seasonal variation in illumination intensity. Molina et al. (2001) designed an outdoor tubular PBR with a working volume of 200 L, with vertical tubes made of plexi-glass connected to a 4 m tall airlift and degasser section to examine the pilot-scale production of the micro-alga *Phaeodactylum tricorutum*. In this reactor, a maximum biomass productivity of $1.9 \text{ g L}^{-1} \text{ d}^{-1}$ was obtained with a decline to $1.2 \text{ g L}^{-1} \text{ d}^{-1}$ in the spring cultivation period.

4.4.3. Treatment performance and duration

The COD, N and P concentration in the effluent and duration of the treatment are key criteria in assessing the performance of a bioreactor system of a micro-algal wastewater treatment process. The performance of the treatment process must be able to meet current mandatory effluent concentrations, as set in Europe by the UWTD, with the prospect of achieving lower set standards (Swedish EPA, 2008). Furthermore, the addition or integration of a micro-algal biological treatment process within a conventional wastewater treatment train must complement the upstream and downstream processes by achieving a constant output and flow. With regards to hydraulic retention time, the shorter the time the smaller the reactor system necessary, which has benefits to capital costs and also surface area requirements (Metcalf and Eddy, 2003; Ruiz et al., 2013). Table 3 lists the remediation data by micro-algae reported from independent studies treating municipal wastewater cultured either by matrix-immobilised (active), biofilm-immobilised (passive), PBR suspended, or HRAP suspended systems.

When comparing the N and P removal efficiency for a micro-algal cultivation system, a vast difference is noted, both between and within the different cultivation systems (Table 4). Between immobilised and suspended cultivation systems, a consistently high N and P removal efficiency over the shortest treatment duration is noted for PBR suspended systems, despite the vast differences in operating parameters (i.e. biomass inoculation concentration, temperature and irradiance). In PBR suspended systems, an average 87.3% N and 82.9% P removal efficiency was achieved, within an average of 3.1 days (or HRT). Of all the collated

Table 4
Average N and P removal efficiency in different culturing conditions (data from studies in Table 3).

Factor	No. of studies	P % removal	N % removal
Biofilm immobilised (passive)	11	77.4	79.3
Matrix immobilised (active)	8	76.9	94.4
HRAP Suspended	7	47.4	81.2
PBR Suspended	13	87.3	82.9

studies in this category, with the exception of that by Choi (2015), a final N concentration below 10 mg L⁻¹ and P concentration below 1 mg L⁻¹ was reported, with the majority of studies reporting P concentrations below 0.5 mg L⁻¹ (Table 3). The dominant species of micro-algae used were of the family *Chlorophyceae*, which is well known for their N and P remediation abilities, including *Chlorella* sp. and *Scenedesmus* sp. A similar consistent rate of N and P removal, but at a lower efficiency, is noted in the biofilm-immobilised systems, at a respective 77.4% and 79.3% efficiency, taking an average treatment time of 4.3 days (or HRT). Matrix-immobilised cultivation systems were operated for treatment duration of 4.1 days (or HRT), during which the highest N removal efficiency was achieved compared to all other systems. The HRAP-suspended systems did not perform as well, achieving the lowest P removal efficiency and requiring a longer treatment time with an average 8.6 days (or HRT).

With respect to N removal, the high efficiency recorded in the matrix-immobilised systems cannot be completely attributed to the function of the micro-algae. In part, the ionic interactions of the N cations and anions (i.e. NH₄⁺ and NO₃⁻) with the polymer used as the matrix would contribute to their reduction. For example, Fierro et al. (2008) reported a higher inorganic N and P removal efficiency from synthetic wastewater by *Scenedesmus* sp. immobilised in chitosan (PO₄-P: 94%; NO₃-N: 70%) compared to that in a free-living suspended state (PO₄-P: 20%; NO₃-N: 30%). Despite the vast difference in removal efficiency, no statistical difference between the treatments was computed following the removal efficiencies adjustment of the immobilised treatment with the control treatment, the latter of which constituted the chitosan beads only (i.e. without micro-algae). In the control experiment, a 60% PO₄-P and 20% NO₃-N reduction was recorded, suggesting that the net removal efficiency contributed by micro-algal assimilation was only 34% PO₄-P and 50% NO₃-N. The higher PO₄-P removal in the immobilised treatments was attributed to the release of calcium ions from the polymer that contributed to its precipitation rate (Song et al., 2002). The same effect was reported by Ruiz-Marin et al. (2010) when comparing NH₄⁺-N and PO₄-P removal from urban wastewater by both *C. vulgaris* and *S. obliquus*, with each micro-alga cultured either immobilised in sodium alginate or in free-living suspended state. A higher NH₄⁺-N uptake rate and growth rate was recorded in the immobilised micro-algae treatments, with *S. obliquus* more effective in removing the inorganic nutrients within the 2-day cultivation period.

The discrepancy in N and P removal efficiency between open and enclosed micro-algal systems is mainly a result of the different environments that may form (i.e. nitrification and/or denitrification) and surface to volume ratios. In a comparative study, Molinuevo-Salces et al. (2010) assessed the performance of a micro-algal consortium treatment in an anaerobically digested swine slurry in an open HRAP and enclosed PBR. Depuration of NH₄⁺-N was recorded in both bioreactor types; however, in the open HRAP, NH₃ volatilisation was the dominant mechanism of removal, whereas in the enclosed PBR nitrification and denitrification became dominant. A higher N concentration was recorded in the biomass of the enclosed PBR, but interestingly a higher P concentration was recorded in biomass of the open HRAP. In a similar study, Arbib et al. (2013b) compared the treatment performance of a mesocosm HRAP (530 L) and airlift tubular-PBR (380 L) run in parallel under continuous operation fed with secondary treatment effluent. A statistically

significant average TN and TP removal efficiency was recorded in both systems with a respective 65% and 58% removal in the HRAP, and 89% and 86% in the tubular PBR over the course of the treatment duration (157 days). The majority of inorganic N and P removal was attributed to assimilation by the micro-algae and other microorganisms in the wastewater, with only a small fraction through chemical volatilisation or precipitation. The main reason for the better efficiency in the tubular-PBR was the higher surface-to-volume ratio of the system, which facilitated a greater photosynthetic rate, and which in turn promoted higher growth of the micro-algae. Comparing both systems, a maximum suspended solids concentration of 733 mg L⁻¹ was recorded in the tubular PBR, whereas an average 188 mg L⁻¹ was recorded in the HRAP. Furthermore, the input of atmospheric air to the tubular PBR helped maintain a stable pH of the wastewater, with the elevated pH in the HRAP affecting the performance of the micro-algae and other microorganism.

5. Conclusion and future directions

The sustainable development of a wastewater treatment system needs to be technologically feasible, environmentally friendly and economically viable. The current evidence is that integrating micro-algae as an alternative biological wastewater treatment option is technologically and environmentally feasible. It is also economically competitive if it is borne in mind that the huge cost associated with the cultivation of a micro-algae plant discussed here can be regarded as “installation” cost, but conventional systems also have installation costs. As regards operational costs, micro-algae systems incur little or no operational costs, which altogether makes them much more sustainable than conventional systems. Consequently, the use of micro-algae to reduce nitrogenous, phosphorous and carbonaceous material does have the potential to operate at a lower footprint in terms of energy consumption and greenhouse gas generation compared to conventional biological wastewater treatment processes. Another major challenge that still limits the application of micro-algae to treat wastewater is the non-sterile environment associated with the process. Researchers should be encouraged on the co-culturing of natural community(ies) of micro-algae and other microorganisms such as yeasts, fungi and bacteria in order to create stable communities that perform in a predictable manner, whilst filling all ecological niches to limit the potential for contamination and culture crashes. Therefore, future work should assess the organic carbon enriched wastewater treatment strategy on a naturally formed micro-algal-bacterial consortium, both in regards to its treatment performance and settling characteristics, in order to further reduce operating cost and improve treatment efficiency.

Furthermore, by using an established community of microorganisms, including the micro-algae, for wastewater treatment, known transcription factor(s) expressed by certain microorganisms in the consortium could be used as indicators of community health in response to variations in wastewater composition. To monitor the health of a micro-algal-bacterial consortium, a quantitative PCR assay could routinely be used to monitor the expression of known genes involved in oxidative stress response, such as for example the antioxidant enzyme ascorbate peroxidase in algal cells. This would be beneficial to the treatment process as it would permit relatively quick changes in operating parameters, such as light intensity, HRT, STR and aeration, which may alleviate against the acute change in wastewater composition and aid in stabilising the treatment performance. Adoption of such an assay would require further understanding and identification of genes and their transcription factor(s) involved in the response mechanism to known compounds ubiquitous to wastewater, for example pharmaceuticals and personal care products.

The use of a micro-algal-bacterial consortia with good settling characteristics may not be practical under static culture conditions since keeping the cells in suspension positively influences their operation in the treatment of the wastewater. Intermittent aeration is thus advised in the operation of static systems as it reduces the energy consumption

for aeration. Thus, further development should be carried out in a reactor configuration that is aerated intermittently, potentially coupled with CO₂ injection. This would have a dual benefit of providing a means of maintaining micro-algal-bacterial flocs in suspension during the treatment phase of the process and provide an economic and effective means of pH control. As has been noted, a major limitation to the inference of its suitability as a process can be the lack of pH control, which is an important culture parameter. In general, it is highly recommended that subsequent experiments assess the effects of various parameters such as osmotic potential, pH, O₂ concentration and temperature on the treatment efficiency and biomass productivity to obtain the optimal conditions for industrial scale cultivation. Furthermore, long term studies of a static micro-algae treatment process in various reactor configurations designed with internalised lights could offer a better insight towards optimising culture performance and, indirectly, treatment efficiency, especially in regards to substantiating the effects of biofilm growth inside the reactors.

The treatment performance of a micro-algal-bacteria consortium in PSW enriched with organic carbon sources could potentially improve the removal of N and P. Various food industry by-product streams, including industrial by-product streams from the fruit processing industry, dairy industry and brewing industry including molasses streams, contain high concentrations of saccharides that could be explored in this respect. It is important that such by-product streams not contain toxic compounds (e.g. copper) or exhibit an unbalanced concentration of inorganic N or P, which could interfere with the physiology, growth and metabolism of the micro-algal-bacterial consortium. This particular aspect is poorly explored, so future work could examine alternative by-product streams containing high concentrations of organic carbon in regard to how they are utilised by micro-algal cells. This would be beneficial in respect to optimising the process through establishing suitable micro-algal-bacterial communities. This strategy, however, would require establishing an appropriate community of relevant and suitable bacteria which can associate in a beneficial manner with the algae. Therefore, in future, the use of alternative food industry by-product streams as a source of organic carbon for enrichment in PSW treated by a micro-algal-bacterial consortium requires additional work to analysing the bioconversion of algal indigestible carbon.

A final route of future investigation would be to conduct a life cycle assessment (LCA) of static micro-algae wastewater treatment process, ideally using experimental data on contaminate depuration using a naturally formed micro-algal-bacterial consortium. The use of LCA would provide insight on the overall sustainability of a static micro-algal-bacterial treatment process (or intermittently aerated process) by considering the processing method, its energy investment and environmental impact compared to conventional secondary biological treatment process. Downstream biomass processing for methane gas generation, or as a source of fertiliser following further processing, such as curing the biomass, would be invaluable towards improving the overall sustainability and environmental impact of the treatment process by contributing to a circular economy model.

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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