ROLE OF PHYCOREMEDIATION FOR NUTRIENT REMOVAL FROM WASTEWATERS: A REVIEW

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Abstract. The presence of high concentrations of chemical oxygen demand (COD), biochemical oxygen demand (BOD) and nutrients in wastewater generated industrially or domestically has resulted in significant water pollution situations and subsequently is leading to adverse health problems. Algae have been used in various applications in environmental biotechnology especially for phycoremediation as a tertiary wastewater treatment strategy through assimilation of high concentration of nitrogen and phosphorus for their growth, thus reducing potential eutrophication problems. This article discusses the role of phycoremediation to remove COD, BOD and nutrients from wastewater. The mechanism for nutrient removal from wastewater, challenges to process development and current commercial-scale algae-based wastewater treatment are reviewed too. It appears that phycoremediation plays a vital role to treat wastewaters efficiently.
Keywords: *microalgae, wastewater pollution, COD, BOD, efficiency*

Introduction

In the last few decades, the rapid population growth, industrial revolution, and urbanization have led to various forms of environmental pollution. The disposal of untreated wastewater (e.g. industrial, municipal, palm oil mill effluent, amongst others) directly into water bodies such as rivers, lakes, and oceans is considered a simple and cheap discharge method in communities where wastewater disposal is not well regulated (Chan et al., 2009). This contributes significantly to water shortage. In addition, the potential of high concentrations of toxic pollutants moves into human and animal food chain could result in significant health problems (Barakat, 2011).

The quality of water will degrade when untreated wastewater discharged into the receiving water body (e.g. rivers, streams, lakes) and lead to the problem of clean water for human consumption. Besides that, the discharged of wastewater containing the excessive amount of nutrients (e.g. nitrogen, phosphorus) into the receiving water body can also lead to another problem like eutrophication resulted in the depletion of oxygen level in the water (Lau et al., 1997). Generally, phosphorus in the form of orthophosphates is known as the limiting nutrient in the freshwater system. However, runoff wastewater containing extremely concentration of phosphorus can lead to the eutrophication (Cai et al., 2013). The abnormally low level of oxygen in the water body can harm aquatic life by inducing the reduction of aquatic animals (e.g. fishes, prawns, among others) and microorganisms (e.g. bacteria, fungi, algae) population (Sperling and Chernicharo, 2005).

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According to Driscoll et al. (2003) and Smith (2003), the eutrophication in fresh and coastal or marine ecosystems also leads to some problems such as water discoloration and foaming and increasing in blooms of toxic algal species and their biomass, mortality rate of aquatic species, sedimentation of organic particles as well as decreasing in water transparency.

Nitrate occurs naturally in water; however, it is undesirable substance in public water because of its high concentration in drinking water may either cause serious health problems like methemoglobinemia (blue-baby syndrome) or source of nitrosamines after its reduction to nitrite (Schoeman and Steyn, 2003; Abdel-Raouf et al., 2012). According to Abdel-Raouf et al. (2012) also stated that the purpose of removing phosphate from wastewater is to protect water from eutrophication.

Treated wastewaters obtained from conventional treatments remain undesirable for discharge because of their characteristics still not able to meet the standards set by local authorities (Loh et al., 2013). Therefore, more effective treatment technologies are required in order to reduce the exposure of toxic chemicals to natural ecosystems. The use of microalgae to treat wastewater and hazardous contaminants is currently of global interest due to the effective photosynthetic uptake of high concentrations of minerals, ionogens, and organics by microalgae, and the capacity to simultaneously utilize carbon dioxide (CO_2) (Mohan et al., 2001; Zeng et al., 2012). Microalgae cells have the ability to remove nutrients such as phosphorus, nitrogen, ammonium as well as heavy metals in wastewater (Phang and Ong, 1988; Aziz and Ng, 1992; Sydney et al., 2011; Abdel-Raouf et al., 2012). A life cycle economic assessment has shown that, for microalgae cultivation, the growth media formulation and composition contribute significantly to the operating cost and is a major consideration for scale-up design (Clarens et al., 2009; Lam and Lee, 2011). Hence there is the need to look into cheaper nutrients sources, and wastewaters containing the right nutrients compositions could be a viable alternative. This will also reduce the cost of microalgae biomass generation for the production of biofuels, animal feed, and essential oils amongst others. Meanwhile, the microalgae biomass production after treatment of heavy metal from wastewaters can be used as a potential feedstock for biochar, charcoal, biofuel and biogas production (Safonova et al., 2004; Chinnasamy et al., 2010; Poo et al., 2018). Therefore, the aims of this paper are to comprehensively review the current use of both free cells and immobilized algae in treating wastewaters to obtain some new ideas to deal with wastewaters without having a negative effect on the environment.

Microalgae and macroalgae

Algae are aquatic plant-like organisms (phytoplankton) with various shapes. They lack roots, stems, and leaves, with cell walls made of cellulose. Algae cells are divided into macroalgae and microalgae. Macroalgae are multicellular organisms with size up to several meters while microalgae are small organisms (unicellular) with their size in the range 0.2-100 μm (Bhatt et al., 2014).

Sharma et al. (2011) categorized microalgae into several groups: (i) prokaryotic blue-green (cyanobacteria); (ii) eukaryotic green (*Chlorophyceae*); (iii) eukaryotic brown (*Phaeophyceae*); (iv) eukaryotic red (*Rhodophyceae*); and (v) eukaryotic diatoms (*Bacillariophceae*) as shown in *Table 1*.

According to Brennan and Owende (2010) and Mata et al. (2010), algae are photosynthetic prokaryotic or eukaryotic microorganisms that can grow rapidly and

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have the ability to adapt to harsh environments due to their unicellular or simple multicellular structure. They are thallophytes containing chlorophyll as their main photosynthetic pigment. In fact, microalgae have more effective access to carbon dioxide, water (H_2O) and nutrients due to their simple cellular structure compared to terrestrial plants. As a result, they are known to have very high carbon capturing and photosynthetic efficiencies with the ability to convert solar energy into useful biomass and reduce CO_2 concentrations in the atmosphere more efficiently than terrestrial plants (Packer, 2009; Kumar et al., 2013).

Table 1. *Classification of microalgae and related species (Packer, 2009)*

Algae group	Microalgae species
Prokaryotic blue-green (*Cyanobacteria*)	*Arthrospira, Gloeocapsa, Microcystis, Oscillatoria*, etc.
Eukaryotic green (*Chlorophyceae*)	*Botryococcus, Chlamydomonas, Chlorella, Scenedesmus*, etc.
Eukaryotic brown (*Phaeophyceae*)	*Dinobryon, Mallamonas, Ochromonas, Synura, Uroglena* etc.
Eukaryotic red (*Rhodophyceae*)	*Porphyridium*
Eukaryotic diatoms (*Bacillariophyceae*)	*Asterionella, Cyclotella, Fragilaria, Surirella,* etc.

Metabolism of microalgae

Generally, the growth of microalgae biomass depends on carbon source and photons to perform photosynthesis (Costa and de Morais, 2013). Microalgae can modify their internal structure by both biochemical and physiological acclimations. Externally, they can also excrete various compounds to other cells, supply nutrients or limit the growth of competitors.

Autotrophic microalgae use inorganic compounds and sunlight as a carbon and energy source. In the presence of light, these autotrophic microalgae are referred to as photoautotrophic since they use light photons as an energy source to generate chemical energy by photosynthesis (Amaro et al., 2011). During photosynthesis by autotrophic algae, CO_2 and water are converted into carbohydrate (glucose) and further metabolized to yield energy which drives the formation of adenosine triphosphate (ATP) from adenosine diphosphate (ADP). The energy in ATP is then used to drive various processes in the cells, and in doing so is converted back to ADP ready to pick up more energy to enable growth (Brennan and Owende, 2010). Heterotrophic microalgae use solely organic compounds and exogenous nutrients as a source of carbon and energy for growth in dark conditions (Amaro et al., 2011). According to Huang et al. (2010), cultivation of heterotrophic microalgae overcomes problems associated with limited light photons that affect the attainment of high cells densities during photosynthesis. Some microalgae are mixotrophic, with the capacity to exist as autotrophic or heterotrophic depending on the concentration of organic compound and also the availability of light (Chojnacka and Noworyta, 2004).

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Phycoremediation

Microalgae have been used in various applications of environmental biotechnology especially for bioremediation (e.g. phycoremediation). Bioremediation is the part of environmental biotechnology that uses a biological process to treat contaminants (Boopathy, 2000). Gani et al. (2015a), Rao et al. (2011), and Olguin (2003) defined phycoremediation as the use of algae to remove or transform pollutants, including nutrients and toxic chemicals from wastewater and CO_2 from waste air together with biomass production. Wastewaters treatment by microalgae can be performed in the form of suspended free-cells culture and immobilized cells. The suspended free-cells culture is the condition of microalgae living cells move independently within the bottles containing medium under a condition to ensure uniform cells distribution (Katarzyna et al., 2015). Meanwhile, the immobilized cells is the condition of microalgae living cells be prevented from flow freely from its original location to all parts of the medium. This approach can be performed by keeping the microalgae living cells in the carriers such as NaCS-PDMDAAC capsules (Zeng et al., 2012), alginate (Sumithrabhai et al., 2016) and chitosan beads (Fierro et al., 2008).

The use of suspended free-microalgae cells culture to treat wastewater was first studied by Oswald et al. (1957). The process involves the removal of nitrogen and phosphorus from wastewater whilst simultaneously providing oxygen (O_2) for aerobic bacteria coexisting in the culture. Biologically treating wastewater using microalgae is a reliable process due to the high photosynthetic efficiency and growth rates. Furthermore, microalgae have effective nutrient uptake capacity with the potential to achieve great removal of nitrogen and phosphorus as well as heavy metals from wastewater (Hernandez et al., 2006; Hameed, 2007; Sengar et al., 2011; Abdel-Raouf et al., 2012).

Microalgae require significant amounts of phosphorus and nitrogen for proteins synthesis (45-60% microalgae dry weight), nucleic acids and phospholipids for their growth (Rao et al., 2011). In this respect, nutrient removal using microalgae presents major prospects for tertiary wastewater treatment aimed at removing ammonia, nitrate, and phosphate (Rawat et al., 2011; Abdel-Raouf et al., 2012; Gani et al., 2015b). After nutrient uptake by microalgae during wastewater treatment, the purified water can be decanted to harvest the free cells microalgae (Abdel-Raouf et al., 2012).

Phycoremediation has been used in various applications: (i) removal of nutrients from organic matter-rich wastewater; (ii) removal of nutrients and xenobiotic compounds using algae-derived sorbents; (iii) treatment of heavy metal-rich wastewater; (iv) sequestration of CO_2; (v) transformation and degradation of xenobiotic; and (vi) detection of toxic compounds using algae-based biosensors. Application of phycoremediation for wastewater treatment has significant benefits (Eroglu et al., 2012; Sivakumar and Rajendran, 2013; Whangchenchom et al., 2014; Gani et al., 2015a). The following are key characteristics of the process.

i. It is cost-effective, eco-friendly and safe.
ii. Microalgae used are non-pathogenic photosynthetic organisms and produce non-toxic substances.
iii. It efficiently reduces nutrient load and leads to a reduction in total dissolved solid.
iv. It detoxifies and removes pollutants (e.g. heavy metals) from toxic waste-rich sludge more effectively than conventional chemical treatment technologies.

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v. It increases dissolved oxygen (DO) levels via photosynthetic activity.
vi. Microalgae use CO_2 fixation from the atmosphere as a source of carbon for growth thus reduce greenhouse gasses (GHG).
vii. Production of high-value products derived from nutrient-rich microalgae biomass for bio-fertilizer production and as feed for animals and aquaculture.
viii. Simple operation and maintenance.
ix. Construction and operation costs are cheaper than mechanical treatment plants such as activated sludge and sequencing batch reactors.
x. Sustainable treatment solution with significant potential for energy and nutrient recovery.

Figure 1 shows the schematics of mixed microbial community-based treatment of wastewater exploiting the metabolic relationship between microalgae and bacteria. First, the bacteria proliferate and produce CO_2 for microalgae growth. The CO_2 is used by the microalgae during photosynthesis in the presence of light to produce O_2 which is assimilated by the bacteria for growth. According to Sharma and Khan (2013), microalgae produce oxygen from water as a by-product of photosynthesis and bacteria use the oxygen to oxidize organic compounds. During photosynthesis, the end product of bio-oxidation of organic compounds namely, carbon dioxide, is further fixed into cells carbon by microalgae. As a result, the pollutants level in wastewater are reduced to undetectable or acceptable limits set by local authorities.

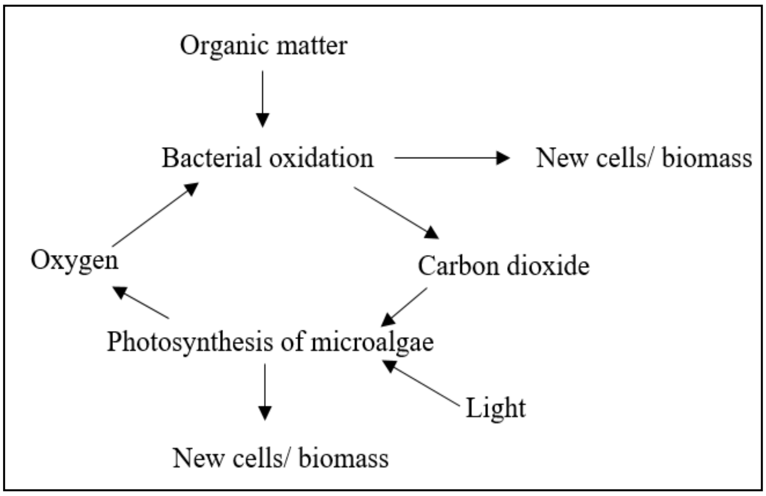

Figure 1. *BOD removal through the photosynthetic oxygenation approach (Gani et al., 2015a)*

Generally, the generated microalgae biomass from wastewater treatment process is used for agriculture (fertilizer and soil conditioners) and biofuels industries (Eroglu et al., 2012). In this respect, the cultivation of microalgae using wastewater serves a dual role of pollutants load reduction and production of various valuable products.

Mechanisms of carbon, nitrogen and phosphorus removal in phycoremediation

Elements like carbon, nitrogen, phosphorus and, sulfur together with small amounts of trace metals (e.g. sodium, calcium, iron etc.) are required for algae growth. Amongst

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these elements, uptake of nitrogen and phosphorus is critical for algal growth (Cai et al., 2013).

Autotrophic microalgae fix carbon (in the form of CO_2) biologically from the atmosphere by photosynthesis. Microalgae can also use carbon in the form of soluble carbonates for their growth, either by direct uptake or conversion of carbonate to free CO_2 through a carboanhydrase activity (Cai et al., 2013).

Nitrogen in wastewater is present in the form of NH_4^+ (ammonia), NO_2^- (nitrite) and NO_3^- (nitrate) (Hadiyanto et al., 2013). The conversion of inorganic nitrogen into organic forms can be carried out by eukaryotic microalgae via assimilation (Cai et al., 2013). *Figure 2* shows the steps involved in the conversion of inorganic nitrogen into organic forms. Firstly, translocation of inorganic nitrogen takes places across the plasma membrane of the algae cells with subsequent reduction to nitrate and nitrite by nitrate and nitrite reductase, respectively. The next step is the conversion of ammonium into amino acids (glutamine). Nitrate reductase utilizes the reduced form of nicotinamide adenine dinucleotide (NADH) to transfer two electrons in the reaction for the conversion of nitrate into nitrite. Next, nitrite is further reduced to ammonium by nitrite reductase and ferredoxin (Fd) to transfer six electrons in the reaction. All inorganic forms of nitrogen are reduced to ammonium before being incorporated into amino acids within the intracellular fluid. Finally, glutamine synthase using glutamate (Glu) and adenosine triphosphate (ATP) facilitates the incorporation ammonium into amino acids (glutamine) (Cai et al., 2013).

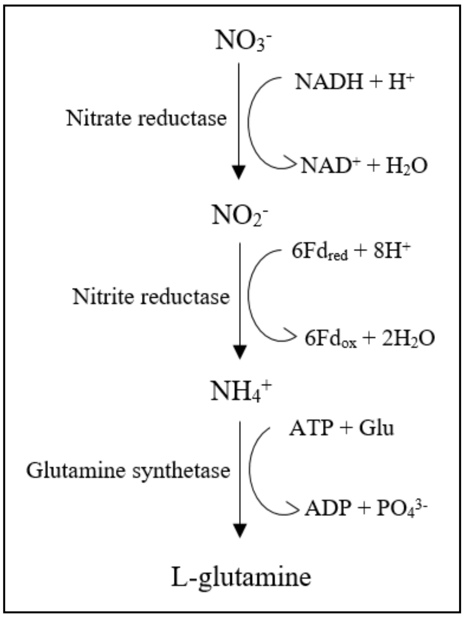

Figure 2. *Schematic of the conversion of inorganic nitrogen to its organic form via assimilation (Cai et al., 2013)*

The phosphorus present in lipids, nucleic acids, and proteins as well as intermediates of carbohydrate metabolism is a result of phosphorus uptake. Inorganic phosphorus in the form of phosphates plays a crucial role in the growth of algae cells and also their energy metabolism. According to Martínez et al. (1999), algae metabolism relies mostly on inorganic phosphorus in the forms of hydrogen phosphate (HPO_4^{2-}) and dihydrogen

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phosphate (H_2PO_4) which is then incorporated into organic compounds through a phosphorylation process involving the production of ADP-derived ATP together with energy input. The oxidation of respiratory substrates, electron transport system of mitochondria, or light (photosynthesis) is all sources of energy input. Phosphates are transferred across the plasma membrane of the algal cells for utilization.

The nitrate and phosphate in the wastewater are adsorbed through the matrix pore surface of microcapsules membrane. After that, the uptake and assimilation of nitrate and phosphate ion by immobilized microalgae cells for growth occur and result in the reduction of N and P content in wastewater and further improves the quality of wastewater for discharge (Zeng et al., 2012).

Selection of microalgae species for phycoremediation

The selection of microalgae species for wastewater treatment is a critical consideration. According to Shi et al. (2007) and Olguin (2003), the selection of microalgae species for wastewater treatment should consider its robustness against wastewater pollutants, the capability to grow well (high growth rates), and their efficiency in assimilating nutrient from wastewater. A lot of studies focusing on various species of microalgae cultivated in wastewater for the removal of nitrogen and phosphorus have been reported. *Scenedesmus*, *Chlorella*, and *Botryococcus* are commonly used microalgae for removing nutrients (nitrogen and phosphorus), COD and BOD as shown in *Table 2*.

Table 2. *Comparison of phycoremediation efficiency by different microalgae species (suspended free-cells microalgae cultures) grown in the various wastewater effluent*

Microalgae species	Source of the wastewater (ww)	Parameter	Removal efficiency (%)	Ref.
Chlorella sp.	Synthetic aquaculture wastewater	COD	COD = 15%	Udom et al., (2013)
		TN	TN = 91%	
		NH_4^+	NH_4^+ = 100%	
		TP	TP = 93%	
	Domestic wastewater before the primary settling	COD	COD = 50.90%	Wang et al., (2010)
		TN	TN = 68.40%	
		NH_4^+	NH_4^+ = 82.40%	
		PO_4^{3-}	PO_4^{3-} = 83.20%	
Chlorella vulgaris	Sewage wastewater collected from the treatment plant	BOD	BOD = 70%	Abou-Shanab et al., (2013)
		COD	COD = 66%	
		TN	TN = 71%	
		TP	TP = 67%	
	Treated piggery wastewater	TN	TN = 49%	Abou-Shanab et al., (2013)
		TP	TP = 18%	
	Sewage wastewater collected from the various drains	BOD	BOD= 98.70%	Ahmad et al., (2013)
		COD	COD = 98.30%	
		TKN	TKN = 93.10%	
		NO_3^-	NO_3^- = 98.30%	
		TP	TP = 98%	

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Microalgae species	Source of the wastewater (ww)	Parameter	Removal efficiency (%)	Ref.
		PO_4^+	PO_4^+ = 98.60%	
	Drainage solution from the commercial green production	TN	TN = 20.70%	Hultberg et al., (2013)
		TP	TP = 99.70%	
	Sewage wastewater collected from the treatment plant	BOD	BOD = 70.91%	Kshirsagar, (2013)
		COD	COD = 80.64%	
		NO_3	NO_3 = 78.08%	
		PO_4^+	PO_4^+ = 79.66%	
	Sewage wastewater collected from the 2nd clarifier treatment plant	NO_3^-	NO_3^- = 99%	Su et al., (2012)
		NO_2^-	NO_2^- = 99%	
		NH_4^+	NH_4^+ = 99%	
		PO_4^{3-}	PO_4^{3-} = 99%	
	Sewage wastewater collected from the primary settling tank	NH_4^+	NH_4^+ = 50%	Lau et al., (1997)
		PO_4^{3-}	PO_4^{3-} = 50%	
	Synthetic sewage	NO_3^-	NO_3^- = 87%	Eroglu et al., (2012)
	Leather processing collected from the manufacturing facility	BOD	BOD = 22%	Rao et al., (2011)
		COD	COD = 38%	
		TKN	TKN = 73%	
		NO_3^-	NO_3^- = 91.49%	
		NO_2^-	NO_2^- = 89%	
		NH_4^+	NH_4^+ = 80%	
		PO_4	PO_4 = 94%	
	Textile wastewater (garment factory) collected from the holding tank	COD	COD = 62.30%	Lim et al., (2010)
		NH_4^+	NH_4^+ = 45.10%	
		PO_4^+	PO_4^+ = 33.30%	
	Chemical (based products) wastewater collected from the Periyor	NO_3^-	NO_3^- = 84%	Dominic et al., (2009)
		NO_2^-	NO_2^- = 100%	
		PO_4^{3-}	PO_4^{3-} = 69.23%	
	Sewage wastewater	COD	COD = 78%	Kumar et al., (2018)
		NO_3^-	NO_3^- = 75%	
Chlorella sakina	Tannery wastewater	NO_3^-	NO_3^- = 9.11%	Jaysudha and Sampathkumar, (2014)
		NH_4^+	NH_4^+ = 62.04%	
		PO_4^{3-}	PO_4^{3-} = 81.94%	
Chlorella pyrenoidosa	Dairy wastewater collected from the farm	BOD	BOD = 88%	Yadavalli et al., (2013)
		COD	COD = 85%)	
		NH_4^+	NH_4^+ = 98%	
		PO_4^{3-}	PO_4^{3-} = 98%	
Chlorella zofingiensis	Piggery wastewater collected from the private farm	COD	COD = 79.84%	Zhu et al., (2013)
		TN	TN = 82.70%	
		TP	TP = 98.17%	

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Microalgae species	Source of the wastewater (ww)	Parameter	Removal efficiency (%)	Ref.
Chlorella minutissima	Primary treated domestic wastewater	BOD	BOD = 95%	Sharma and Khan, (2013)
		COD	COD = 90%	
		NO_3^-	NO_3^- = 91.49%	
		NH_4^+	NH_4^+ = 90%	
		PO_4^{3-}	PO_4^{3-} = 74.27%	
Chlorella sorokoniana	Synthetic municipal wastewater	PO_4^{3-}	PO_4^{3-} = 69%	Hernandez et al., (2006)
	Municipal wastewater collected from the aerobic activated sludge	PO_4^{3-}	PO_4^{3-} = 72%	
Chlorella sp.	Mixed wastewaters from piggery and winery	TN	TN = 89%	Ganeshkumar et al., (2018)
		TP	TP = 49%	
Chlamydomonas sp.	POME collected from the facultative pond	COD	COD = 29.l3%	Ding et al., (2016)
		TN	TN = 72.97%	
		NH_4^+	$NH4^+$ = 100%	
		TP	TP = 63.53%	
Scenedesmus sp.	Noodle processing - MLSS (aeration tank)	COD	COD = 71.85%	Whangchenchom et al., (2014)
	Noodle processing - effluent (final sedimentation tanks)	COD	COD = 39.89%	
	Primary treated domestic wastewater	NH_4	NH_4 = 90%	Sharma and Khan, (2013)
	Synthetic 2f medium with 44 mg/L nitrate and 6 mg/L phosphate	NO_3^-	NO_3^- = 20%	Fierro et al., (2008)
		PO_4^{3-}	PO_4^{3-} = 30%	
Scenedesmus obliquus	Treated piggery wastewater	TN	TN = 58%	Abou-Shanab et al., (2013)
		TP	TP = 24%	
		TN	TN = 60%	Jimenez-Perez et al., (2004)
		NO_3^-	NO_3^- = 84%	
		NH_4^+	NH_4^+ = 57%	
		TP	TP = 83%	
	Synthetic brewery effluent	COD	COD = 57.50%	Mata et al., (2012)
		TN	TN = 20.80%	
Scenedesmus acutus	Municipal wastewater effluent was collected from the conventional activated sludge plant	COD	COD = 77.30%	Sacristán de Alva et al., (2013)
		NO_3^-	NO_3^- = 71.10%	
		NH_4^+	NH_4^+ = 93.60%	
		PO_4^{3-}	PO_4^{3-} = 66.20%	
Scenedesmus quadricauda	Domestic wastewater collected from the sewage wastewater treatment plant	BOD	BOD = 89.21%	Kshirsagar, (2013)
		COD	COD = 70.97%	
		NO_3^-	NO_3^- = 70.32%	
		PO_4^{3-}	PO_4^{3-} = 81.34%	
Scenedesmus rubescens	Municipal wastewater collected from the secondary treatment plant	TP	TP = 11.40%	Aravantinou et al., (2013)
	Sewage wastewater collected from the 2nd clarifier treatment plant	NO_3^-	NO_3^- = 99%	Su et al., (2012)
		NO_2^-	NO_2^- = 99%	
		NH_4^+	NH_4^+ = 99%	
		PO_4^{3-}	PO_4^{3-} = 99%	
Scenedesmus	POME	BOD	BOD =	Rajkumar and

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Microalgae species	Source of the wastewater (ww)	Parameter	Removal efficiency (%)	Ref.
dimorphus			71.50%	Takriff, (2015)
		COD	COD = 79%	
		TN	TN = 87.50%	
		NH_4	NH_4 = 88.50%	
		TP	TP = 92.50%	
Chlamydomonas incerta	POME	COD	COD = 3.8%	Kamyab et al., (2017)
		NO_3^-	NO_3^- = 12.5%	
		NH_3^-	NH_3^- = 3.7%	
		PO_4	PO_4 = 70%	
		COD	COD = 67.35%	Kamyab et al., (2015)
Chlamydomonas mexicana	Treated piggery wastewater	TN	TN = 62%	Abou-Shanab et al., (2013)
		TP	TP = 30%	
Chlamydomonas polypyrenoideum	Dairy wastewater collected from the oxidation pond	NO_3^-	NO_3^- = 90%	Kothari et al., (2013)
		NO_2^-	NO_2^- = 74%	
		NH_4^+	NH_4^+ = 90%	
		PO_4^{3-}	PO_4^{3-} = 70%	
Chlamydomonas reinhardtii	Sewage wastewater collected from the 2nd clarifier treatment plant	NO_3^-	NO_3^- = 99%	Su et al., (2012)
		NO_2^-	NO_2^- = 99%	
		NH_4^+	NH_4^+ = 99%	
Chlamydomonas sp.	Leachates	NH_4^+	NH_4^+ = 70%	Paskuliakova et al., (2018a)
		NH_4^+	NH_4^+ = 83%	Paskuliakova et al., (2018b)
Botryococcus sp.	Diary wastewater collected from the goat breeding farm	BOD	BOD = 73.30%	Gani et al., (2015c)
		COD	COD = 48.80%	
		TN	TN = 48.28%	
		PO_4^{3-}	PO_4^{3-} = 62.71%	
	Greywater from the residential area	BOD	BOD = 82%	Gani et al., (2015b)
		COD	COD = 88%	
		TN	TN = 52%	
		PO_4^{3-}	PO_4^{3-} = 37.50%	
Botryococcus braunii	Domestic wastewater collected from the Adyar river	BOD	BOD = 66.67%	Raj, (2015)
		COD	COD = 71.21%	
		NH_4^+	NH_4^+ = 82.94%	
	Greywater collected from the hostel	TP	TP = 97.59%	Gokulan et al., (2013)
		BOD	BOD = 76.13%	
		COD	COD = 91.32%	
		NO_3^-	NO_3^- = 69.58%	
		NH_4^+	NH_4^+ = 97.82%	
	Municipal wastewater collected from the primary settling tank	NO_3^-	NO_3^- = 60.31%	Can et al., (2013)
		NO_2^-	NO_2^- = 53.50%	

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Microalgae species	Source of the wastewater (ww)	Parameter	Removal efficiency (%)	Ref.
		NH_4^+	NH_4^+ = 100%	
		PO_4^{3-}	PO_4^{3-} = 99%	
Oscillatoria sp.	Municipal wastewater collected from the Pune Corporation	NO_3^-	NO_3^- = 97%	Azarpira et al., (2014)
		PO_4^{3-}	PO_4^{3-} = 93%	
Nostoc commune		NO_3^-	NO_3^- = 96%	
		PO_4^{3-}	PO_4^{3-} = 84%	
Nostoc sp.	Primary treated domestic wastewater	NO_3^-	NO_3^- = 45.68%	Sharma and Khan, (2013)
		NH_4^+	NH_4^+ = 90%	
	Dairy wastewater collected from the treatment plant	BOD	BOD = 40.44%	Kotteswari et al., (2012)
		PO_4^{3-}	PO_4^{3-}= 21.08%	
Desmodesmus sp.	Sewage wastewater collected from the facultative lagoon treatment plant	TN	TN = 80%	Komolafe et al., (2014)
		PO_4^{3-}	PO_4^{3-} = 38.70%	
	Synthetic industrial wastewater	TP	TP = 94%	Rugnini et al., (2018)
Tetraselmis suecica	Aquaculture wastewater collected from the fish farm	TN	TN = 95.70%	Michels et al., (2014)
		TP	TP = 99.70%	
Tetraselmis chuii	Aquaculture – recirculation aquaculture system (RAS)	TN	TN = 69.50%	Sirakov and Velichkova, (2014)
		NO_2^-	NO_2^-= 79.17%	
		PO_4^{3-}	PO_4^{3-} = 64.70%	
Neochloris vigensis	Municipal wastewater collected from the secondary treatment plant	TP	TP = 53.40%	Aravantinou et al., (2013)
Chlorococcum spec.		TP	TP = 25.10%	
Spirulina sp.	Synthetic dairy wastewater	BOD	BOD = 81%	Sumithrabhai et al., (2016)
		COD	COD = 83%	
		TN	TN = 77%	
		TP	TP = 69%	
	Dairy wastewater collected from the factory	COD	COD = 77%	Ahmed, (2014)
		NO_3^-	NO_3^- = 80%	
		PO_4^{3-}	PO_4^{3-} = 72%	
	POME collected from the anaerobic fourth pond	COD	COD = 50%	Hadiyanto et al., (2014)
		TN	TN = 40%	
		TP	TP = 40%	
Spirulina platensis	POME collected from the anaerobic fourth pond	COD	COD = 50.79%	Hadiyanto et al., (2013)
		TN	TN = 96.50%	
		TP	TP = 85.92%	
	POME	BOD	BOD = 78.30%	Rajkumar and Takriff, (2015)
		COD	COD = 84.90%	
		TN	TN = 91%	
		NH_4-N	NH_4-N = 93.80%	
		TP	TP = 96.80%	
Auxenochlorella protothecoides	Municipal wastewater collected from the treatment plant	COD	COD = 88.99%	Zhou et al., (2012)

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Microalgae species	Source of the wastewater (ww)	Parameter	Removal efficiency (%)	Ref.
		TN	TN = 59.70%	
		TP	TP = 81.52%	
Oocystis sp.	Fish processing wastewater collected from the fish farm	COD	COD = 71.10%	Riano et al., (2011)
		NH_4^+	NH_4^+ = 95%	
		TP	TP = 74.10	
Euglena viridis	Sewage wastewater collected from the drain opens into river, Yamuna	BOD	BOD = 96.20%	Sengar et al., (2011)
Gloeocapsa gelatinosa		COD	COD = 82%	
Synedra affinis		NO_3^-	NO_3^- = 100%	
		NO_2^-	NO_2^- = 100%	
		PO_4^{3-}	PO_4^{3-} = 100%	
Gloeocapsa gelatinosa	Chemical (based products) wastewater collected from the Periyor	NO_3^-	NO_3^- = 80.90%	Dominic et al., (2009)
		NO_2^-	NO_2^- = 100%	
		PO_4^{3-}	PO_4^{3-} = 75%	
Synechocystis salina		NO_3^-	NO_3^- = 82.50%	
		NO_2^-	NO_2^- = 96.23%	
		PO_4^{3-}	PO_4^{3-} = 64.52	
Pithopora sp.	Thermal wastewater collected from the power station	BOD	BOD = 88.23%	Murugesan and Dhamotharan, (2009)
		COD	COD = 87.75%	
		NO_3^-	NO_3^- = 23.07%	
		PO_4^{3-}	PO_4^{3-} = 89.37%	
Nannochloris oculata	Aquaculture wastewater -recirculation aquaculture system (RAS)	TN	TN = 78.40%	Sirakov and Velichkova, (2014)
		NO_2^-	NO_2^- = 84.38%	
		PO_4^{3-}	PO_4^{3-} = 14.70%	
Characium sp.	POME collected from the anaerobic pond	COD	COD = 45.41%	Selvam et al., (2015)
		TN	TN = 88.60%	
		NH_3^-	NH_3^- = 90.35%	
		NH_4^+	NH_4^+ = 87%	
		TP	TP = 99.5.0%	
		PO_4^{3-}	PO_4^{3-} = 99.10%	
Micro algal mixture	POME collected from the final pond	COD	COD = 71.16%	Kamyab et al., (2014)
	Textile wastewater	TN	TN = 70.10%	Huy et al., (2018)
		TP	TP = 100%	
	Urban wastewater	BOD COD TN TP	BOD = 51% COD = 91% TN = 95.10% TP = 88.9%	Marella et al., (2018)
Algal-bacterial culture	Municipal wastewater collected from the 2nd clarifier treatment plant	COD	COD = 98.2%	Su et al., (2011)
		TKN	TKN = 88.3%	
		PO_4^{3-}	PO_4^{3-} = 64.8%	

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Microalgae species	Source of the wastewater (ww)	Parameter	Removal efficiency (%)	Ref.
	Synthetic wastewater	COD	COD = 65.62%	Ji et al., (2018)
		TN	TN = 21.56%	
		TP	TP = 70.82%	
	Municipal wastewater	TN	TN = 83%	Delgadillo-Mirquez et al., (2016)
		TP	TP = 100%	
Algal biofilm	Artificial municipal wastewater	TP	TP = 97%	Sukačova et al., (2015)

Application of phycoremediation in treating wastewater using suspended-free cells of microalgae

Microorganisms especially microalgae have received significant attention in wastewater treatment. This is due to their capability to take up and assimilate plant nutrients, pesticides, organic and inorganic pollutants in their unicellular structure (Sahu, 2014). According to Pittman et al. (2011), a lot of microalgae species thrive in wastewater containing high concentration of nitrogen and phosphorus and use them as a vital source of energy for their growth. This leads to significant uptake and reduction of nutrient concentrations.

Cultivation of microalgae in wastewater treatment system offers several advantages. The process is simple, economical and sustainable. Zhou et al. (2012) reported that growing microalgae in wastewater is probably the most promising approach to reduce costs of production in term of nutrients and clean water supply. Furthermore, Rawat et al. (2011) reported that readily available municipal wastewater can be used as a growth media to cultivate microalgae together with the added benefits of bioremediation (e.g. phycoremediation).

Chlorella sp. reduced COD (15%), total nitrogen (TN) (91%), ammonium (NH_4^+) (100%) and total phosphorus (TP) (93%) from synthetic aquaculture for 22 days (Komolafe et al., 2014). It has been shown that *C. vulgaris* grown in sewage wastewater accomplished removal of BOD (98.70%), COD (98.30%), total kjeldahl nitrogen (TKN) (93.10%), nitrate (NO_3^-) (98.30%), TP (98%) and (phosphate) PO_4^{3-} (98.60%) (Wang et al., 2010). As Whangchenchom et al., (2014) reported that *Scenedesmus sp.* was capable of removing 73.37% of COD from wastewaters in Thailand. Suspended free-cell of *Scenedesmus sp.*, cultivated in synthetic 2f medium reduced NO_3^- and PO_4^{3-} by 20% and 30%, respectively (Fierro et al., 2008). Removal of TN, NO_3^-, NH_4^+, and TP from piggery wastewater using *Scenedesmus obliquus* was achieved at 60, 84, 57 and 83%, respectively (Ji et al., 2013). Sacristán de Alva et al., (2013) and Su et al. (2012) used others strains of *Scenedesmus* species to treat wastewater. High removal of NO_3^-, (nitrite) NO_2^-, NH_4^+, and PO_4^{3-} were achieved as compared to Kothari et al. (2013) using *Chlamydomonas polypyrenoideum*. Gokulan et al. (2013) evaluated the nutrients removal efficiency of *Botryococcus braunii* in greywater samples collected from a hostel. They reported that *B. braunii* removed 76.13%, 91.32%, 69.58%, 97.82%, 97.59% of BOD, COD, NO_3^-, NH_4^+ and TP, respectively. Ganeshkumar et al. (2018) studied the potential of *Chlorella sp.* to treat mixed wastewater from piggery and winery in India. The treatment process was conducted for 10 days in at 23°C in an orbital shaker. The initial concentration of TN and TP of 284 mg/L and 11 mg/L was

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reduced to 30.22 mg/L and 4.78 mg/L, respectively. Therefore, the study achieved good removal of TN and TP up to 89.36% and 56.56%, respectively. The removal efficiency of nutrients, COD and BOD from various wastewaters for other studies were summarized in *Table 2*.

It can be summarized that the phycoremediation technology has the ability to remove nutrients from wastewaters up to certain removal efficiency. However, the removal efficiency of nutrients depends on the types of wastewaters and microalgae species to be treated and be used as bioremediation agent, respectively. Besides that, some microalgae species shown great removal efficiency of nitrogen removal from wastewaters. An explanation for this was due to the role of nitrogen to build microalgae cells through anabolism pathway. Protein, chlorophyll, amino acids and also genetic materials are major made up of nitrogen (McElwee et al., 2006). Thus, the phycoremediation process has a potential to reduce the nitrogen to the lowest concentration from wastewaters.

Rock minerals, soil erosion and animal waste decomposition are the natural sources of phosphorus in the aquatic system. Phosphorus removal from wastewaters is vital to avoid eutrophication problem. This problem could be achieved through phycoremediation process which exhibited great removal efficiency of phosphorus as shown in *Table 2*. *Table 2* also shows the phycoremediation process for various wastewaters using different types of microalgae species.

Algae immobilization

De-Bashan and Bashan (2010) defined immobilized cells as living cells by which natural or artificial methods have been used to restrict independent movement from its original position to all part of an aqueous phase. Immobilization of microalgae in polymers can overcome problems associated with biomass harvesting from suspended free-cells cultivated in wastewater. Although solid-liquid separation technologies such as centrifugation and filtration can be used to separate free cells. There are several types of immobilization: (i) covalent coupling (ii) affinity immobilization (iii) adsorption (iv) confinement in liquid-liquid emulsion (v) capture behind semi-permeable membrane; and (vi) entrapment in polymers (Malik, 2002; Eroglu et al., 2015). They can be further categorized into "passive" (immobilization onto natural or synthetic gel-like carriers) and "active" (using flocculants, chemical attachment, and gel encapsulation) (Moreno-Garrido, 2008).

Immobilization of microalgae by entrapment using gel polymers is the most common method in wastewater treatment applications (Eroglu et al., 2015). There is a physical separation between the microorganisms and the treated wastewater in polymeric immobilization, and this is similar to biofiltration. The microalgae cells are immobilized and entrapped alive in the polymer gel matrix. The gel pores are smaller in size than the microalgae. The wastewater fluid flows through the pores of the polymer and sustains microalgae metabolism and growth (Cohen, 2001). The wastewater diffuses through the polymer pores, resulting in uptake of nutrients by the entrapped microalgae cells. Compared to suspended free-cells microalgae cultures cells, the following are some advantages of immobilized microalgae in treating wastewater: (i) provides stability to the photobioreactor (PBR) system design (ii) enhance operational stability (iii) easy to regenerate immobilized microalgae (iv) avoids cell washout (v) facilitates the cultivation of microalgae and easy of harvesting of their biomass (vi) high and rapid

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uptake of nutrient plus shorter retention time (vii) allows bioprocess with better light utilization efficiency per area and higher cell densities (viii) yields significant metabolite concentrations (ix) high tolerance against harsh environments like extreme pH, temperature, ultraviolet radiation and toxic compounds (x) protects aging cultures against the harmful effects of photoinhibition (xi) rotects microalgae cells from being consumed by wild zooplankton (xii) enhances the capacity of biosorption and bioactivity of the biomass; and (xiii) allow immobilization of more than one microorganism (usually microalgae co-immobilized with bacteria species) (de-Bashan and Bashan, 2010; Eroglu et al., 2015; Vasilieva et al., 2016).

Successful entrapment allows microalgae cells to move freely within the space of beads with the optimal pore size that facilitates diffusion of wastewater and metabolic products into and/or out of the polymer system (Malik, 2002). According to Eroglu et al. (2015), the dual effect of enhanced photosynthetic rate and ionic exchange between the nutrient ions and the immobilized matrix results in efficient removal of nutrients from wastewater. Anionic gels (such as carrageenan and alginate) and cationic gels (such as chitosan) adsorb cations (e.g. NH_4^+) and anions (PO_4^{3-}, NO_3^-, NO_2^-) with high efficiency. In addition, PO_4^{3-} is removed efficiently from wastewater via precipitation by calcium ions of alginate or chitosan gels.

Immobilizing materials or carriers can be put into two categories; synthetic and natural polymer (Eroglu et al., 2015; Vasilieva et al., 2016). Examples of synthetic polymers for wastewater treatment include polyacrylamide, polyurethane, polyvinyl, polypropylene, and polystyrene, polysulfone, epoxy resins, and filter papers. Natural polymers can be derived from plant polysaccharides. These include agar, cellulose, alginate, carrageenan, and chitosan. The immobilizing materials possess hydrophilic properties for enhanced diffusion of wastewater into the beads.

Natural polymers such as alginate, carrageenan, and chitosan are the most commonly used immobilizing materials in wastewater treatment (Shi et al., 2007; Zhang et al., 2008; Moreno-Garrido, 2008; Eroglu et al., 2015; Sumithrabhai et al., 2016), and this due to the following advantages. They are (i) non-toxic, easy to process, and cost-effective; (ii) transparent and permeable; (iii) hydrophilic and have higher nutrient/product diffusion rates than synthetic polymers; (iv) more environmentally friendly and produces less hazardous waste following treatment; and (v) bio-compatible. The use of these natural polymers in wastewater treatment also poses some disadvantages. They are (i) less stable as they dissolve slightly in highly contaminated wastewater; (ii) do not retain their polymeric structure in the presence of high concentration of phosphate and some cations (e.g. calcium and magnesium); and (iii) susceptible to microbial degradation. However, the degradation of the natural polymer in highly contaminated wastewater can be minimized by the composite assembly of polymers. For example, the stability of carrageenan gels can be enhanced by mixing the carrageenan with polyacrylamide (Eroglu et al., 2015).

There is a generic method for immobilizing microalgae onto polymers. Briefly, the microbial suspension is mixed with the macromolecular monomers of the selected polymer (e.g. alginate, carrageenan, chitosan solution) to form polymeric gels (e.g. spherical beads produced via the small orifice of syringe) after solidification. The monomers cross-link to each other with di- and multi-valent cations such as calcium chloride to produce polymers with entrapped microbes within the matrix. Generally, as the concentration of monomers and cross-linking agents' increases, the mechanical strength of the polymer increases, resulting in pore size reduction (de-Bashan and

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Bashan, 2010). *Table 3* shows the removal efficiency of pollutants from wastewater using different immobilizing materials.

Table 3. *Comparison of phycoremediation efficiency by immobilized algae grown in the various wastewaters*

Microalgae Species	Immobilizing material	Source of the wastewater	Parameter	Removal efficiency (%)	Ref.
Chlorella vulgaris	Chitosan nanofiber mats	Synthetic sewage effluent	NO_3^-	NO_3^- = 87%	Eroglu et al., (2012)
	Twin-layer system	Synthetic secondary wastewater	NO_3^-	NO_3^- = 93%	Shi et al., (2007)
			NH_4^+	NH_4^+ = 94%	
			PO_4^{3-}	PO_4^{3-} = 89%	
		Municipal wastewater	NO_3^-	NO_3^- = 98%	Shi et al., (2007)
	Calcium alginate beads	Domestic primary treated wastewater	NO_3^-	NO_3^- = 96.40%	Hameed, (2007)
			NH_4^+	NH_4^+ = 100%	
			PO_4^{3-}	PO_4^{3-} = 95%	
		Synthetic primary settled domestic wastewater	NH_4^+	NH_4^+ = 100%	Tam and Wong, (2000)
			PO_4^{3-}	PO_4^{3-} = 95%	
	Carrageenan beads	Sewage wastewater collected from the primary settling tank	NH_4^+	NH_4^+ = 95%	Lau et al., (1997)
			PO_4^{3-}	PO_4^{3-} = 99%	
Chlorella vulgaris and *Azospirillum brasilense*)	Alginate beads	Municipal wastewater collected from the stream of wastewater after the initial aerobic activated sludge treatment	NO_3^-	NO_3^- = 15%	De-Bashan et al., (2004)
			NH_4^+	NH_4^+ = 100%	
			PO_4^{3-}	PO_4^{3-} = 36%	
Chlorella sorokiniana and *Azospirillum brasilense*			PO_4^{3-}	PO_4^{3-} = 72%	Hernandez et al., (2006)
Chlorella pyrenoidosa and activated sludge	Polyvinyl alcohol (PVA) – sulfate gel	Synthetic wastewater	NO_3^-	NO_3^- = 80%	Huang and Wang, (2003)
			PO_4^{3-}	PO_4^{-3} = 88%	
Chlorella salina	Sodium alginate beads	Tannery wastewater	NO_3^-	NO_3 = 98.71%	Jaysudha and Sampathkumar, (2014)
			NH_4^+	NH_4 = 98.54%	
			PO_4^{3-}	PO_4 = 99.39%	
Spirulina maxima		Synthetic dairy effluent	BOD	BOD = 81.05%	Sumithrabhai et al., (2016)
			COD	COD = 82.86%	
			NO_3^-	NO_3^- = 77%	
			PO_4^{3-}	PO_4^{3-} = 69.05%	
Scenedesmus rubescens	Twin-layer system	Synthetic secondary wastewater	NO_3^-	NO_3^- = 95%	Shi et al., (2007)
			NH_4^+	NH_4^+ = 96%	
			PO_4^{3-}	PO_4^{3-} = 89%	
		Municipal wastewater	NO_3^-	NO_3^- = 96%	Shi et al., (2007)
Scenedesmus	Chitosan	Synthetic 2f medium with 44	NO_3^-	NO_3^- = 70%	Fierro et al.,

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Microalgae Species	Immobilizing material	Source of the wastewater	Parameter	Removal efficiency (%)	Ref.
sp.	beads	mg/L nitrate and 6 mg/L phosphate	PO_4^{3-}	PO_4^{3-} = 94%	(2008)
	Sodium alginate sheets	Domestic secondary effluent	NH_4^+	NH_4^+ = 100%	Zhang et al. (2008)
			PO_4^{3-}	PO_4^{3-} = 100%	

Factors affecting nutrients removal from wastewater in immobilized systems

There are several factors that influence nutrients removal from wastewater in immobilized systems. These include the thickness of immobilized media, concentration of microalgae, and amount of beads.

The thickness of immobilized media

Zhang et al. (2008) investigated the effect of the thickness of *Scenedesmus sp.* immobilized gel on the uptake of nitrogen and phosphorus. They found out that gel thickness up to 3 mm is removed nutrients efficiently with higher algal biomass. Another studied by Hameed (2007) reported that small size beads with about 2.8 mm in diameter demonstrated higher removal efficiency for nitrate and phosphate than others beads (4 and 6 mm in diameter) in 48 h. Low thickness media facilitate convective transport of nutrients between the gel media and the nutrient environment.

The concentration of immobilized algae

Too high cell density in gel results in low removal efficiency (Zhang et al., 2008). Sodium alginate sheets (3 mm thickness) which containing microalgae with 2 x 10^8 cells achieved higher nutrient removal compared to other sheets with 1.33 x 10^8 and 3 x 10^8 cell counts. Compared to low (3.5 x 10^5 cells) and high (3.10 x 10^6 cells/bead) cell stocking, calcium alginate beads containing microalgae cells (1.5×10^6 cells/bead) demonstrated a greater capacity to effectively remove NH_4^+ and PO_4^{3-} from primary treated domestic wastewater (Hameed, 2007). They revealed that increasing cells stocking in beads causes leakage problems and affects the removal efficiency of target pollutants from wastewater. According to Jimenez-Perez et al. (2004), super-concentrated cells stockings may restrict to some extent the nutrient diffusion through the gel pores.

The quantity of beads in wastewater

The removal of target pollutants from wastewater is influenced by the quantity of beads containing algae cells used in the wastewater treatment process. A more effective removal of nitrate and phosphate from wastewater was achieved by 11 beads of algae than 16, 32, and 64 beads (Hameed, 2007). Tam and Wong (2000) accomplished a great removal of NH_4^+ and PO_4^{3-} from synthetic primary treated domestic wastewater within 24 h in bioreactors having an optimal algal bead concentration of 12 beads/mL equivalent to 1:3 algal beads: wastewater v/v. NH_4^+ removal was significantly lower with 15 beads/mL algal concentration. They discussed that excessive increase in the quantity of beads results in high-density beads structure that hinders light penetration into cells and subsequently enhances self-shading effects, which limits the metabolic activities of microalgae. Moreover, the high concentration of beads results in the

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settling of beads at the bottom of the reactor due to ineffective air distribution to fluidize them.

Current commercial-scale in wastewater treatment systems using suspended free-cells of microalgae

There are several companies used algae for wastewater treatment practices worldwide for the commercial and industrial application. The companies that involve in algae-based energy (biogas) and fuel (biofuel and biodiesel) research are Algae Enterprises (Australia), Aquanos Energy Ltd. (Israel) and Fcc Aqualia (Spain). Adequate mass cultivation and harvesting of algae for algae biomass in wastewater is important to the aforementioned companies to achieve commercial viability. This is because the biomass can be used as feedstock for any biogas, biofuel, biodiesel or biofertilizer. According to the Website of these companies, they have developed different systems to treat wastewater using algae.

Algae Enterprises collaborated with the main shareholder, namely Sustainability Ventures Group have developed a technology, known as Photoluminescent Algae System (PAS) comprises of thin plastics embedded with fluorescent dyes in Australia (http://www.algaeenterprises.com/wastewater-treatment). Based on this system, the growth of algae is improved by fine-tuning the colors and wavelengths of incoming abundant sunlight that reaches the algae inside. This sustainable technology uses alga to remove pollutants including excessive nutrients from dairy wastewater in the presence of sunlight. In addition, this technology also integrates with the anaerobic digester to generate electricity through anaerobic digestion of harvested algae biomass to produce biogas with methane as the main component which can be used as a renewable source. Nutrient-rich residue materials (fertilizer product) resulting from the biogas generation are used for agriculture to produce even more algae. Meanwhile, the treated waste streams are recycled for irrigation and other purposes on the dairy farm.

Aquonos Energy Ltd developed a novel algae-based wastewater treatment system in Israel (https://finder.startupnationcentral.org/company_page/aquanos). The Aquanos system is characterized by lower energy consumption than conventional wastewater treatment system. Basically, this system consists of three distinct but interrelated processes namely, anaerobic treatment, aerobic treatment and separation of solid (algae) from treated effluent. The first stage is the anaerobic treatment of the incoming wastewater. The purpose of anaerobic treatment is to reduce the organic load to the downstream aerobic processes and at the same time to produce biogas for energy recovery as well as to produce CO_2. The second stage is the aerobic treatment of anaerobic effluent which takes place in the fix film aerobic system. The aerobic system is aerated by a stream of oxygen-rich algae which are grown in the separate raceway pond. Based on this system, the algae are grown in the raceway pond to produce high dissolved oxygen in the liquid. Then, this liquid recirculates through the fix film system supplying oxygen for bacterial decomposition of organic pollutants. At the end of the process, namely solid separation stage is where the excessive algae and excessive biomass are separated from treated effluent. The treated effluent is discharged into the environment and reused for agriculture, while the excessive biomass is returned to the anaerobic stage to produce additional biogas. The end product is high-quality effluent produce using less energy than the conventional system as well as resource harvesting through the production of high quality and high-level algae by-product.

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Fcc Aqualia in Spain partnered with University of Southampton (England), BDI (Austria), Frounhofer Society (German), HyGear (Netherlands) and Volkswagen (German) companies under All-Gas project for developing a new pond system to cultivate algae using nutrients present in the wastewater at wastewater treatment plant to produce biodiesel and biogas (methane) made from biomass to power vehicles (https://www.power-technology.com/uncategorised/newsaqualias-biofuel-project-produces-first-algae-biomass-in-spain/). Basically, they have two different processes of the sequential order under this project, namely All-Gas Alternative 1 (post lipid extraction) and All-Gas Alternative 2 (pre lipid extraction) to obtain biodiesel, biomethane, and biogas. For All-Gas Alternative 1, the incoming raw wastewater is pre-treated and the effluent is discharged into raceway pond containing algae culture. After that, the dense algae culture is harvested and the treated wastewater is discharged to the environment, while the harvested algae biomass undergoes the anaerobic process in anaerobic digester. The biogas is produced during anaerobic treatment and the further undergo pre-treatment and upgrading process to obtain pure methane and CO_2, respectively. The biomethane are stored in the refueling station, while the CO_2 is supplied to the algae culture grown in the aforementioned raceway pond to promote high growth rate and yield of algae. In addition, the remaining residue after anaerobic treatment in anaerobic digester undergoes dewatering method to concentrate prior to the extraction process to produce biodiesel and biofertilizer (remain residue). The All-Gas Alternative 2 has the same process of sequential order as All-Gas Alternative 1 but the lipid extraction process occurs directly upon harvesting. They produce approximately 200000 L of biodiesel and 600000 m^3 of biomethane per year from approximately 3000 kg of dry algae with 20 percent of oil content after grown in ponds of 10 hectares. They claim that the Volkswagen vehicles that have been power using algae-based biogas emit zero emissions. The European Commission contributed about 12 million euro ($15.9 million USD) for this project with the target of at least 10% renewable energy used in their transport sectors by 2020. So far, there is none company use immobilized algae to treat wastewaters commercially.

Wastewater treatment challenges using suspended free-cells of microalgae

To date, limited investments have been pushed into the development of commercial-scale algal wastewater treatment plant, and this has been the result of technical challenges relating to scale-up feasibility, harvesting, and dewatering of biomass. Practical application of current emerging technologies is still in its infancy, with most of the technologies validated only at the laboratory scale.

Most algae are cultivated in closed PBRs for phycoremediation and biomass production (Kamarudin et al., 2015; Lage et al., 2018). The expensive culture system with high capital cost and energy requirements for mixing and gas exchange together with the cost of harvesting to achieve feasible algal solid concentration has constrained the integration of microalgal system with wastewater treatment at large-scale levels. This can partially be addressed through the use of other systems such as open raceway pond. However, environmental factors such as temperature fluctuation, weather influence, and light penetration can affect the efficiency of phycoremediation and productivity of biomass.

Algae require sufficient amount of CO_2 for growth. Thus, low-cost approach using flue gas from power plants as carbon source can be applied. However, the high

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concentration of substances like nitrogen oxide, sulfur oxide, and heavy metals presents in the flue gas causes the medium very acidic for algae cultivation. As a result, algae cultivation is prone to contamination and inhibition, resulting in low wastewater treatment efficiency and low biomass production. Therefore, the flue gas can be pre-treated to minimize contamination before exposure to the microalgae cells. The pre-treatment process could significantly increase the total operation cost.

Kamarudin et al. (2015) propose the following for consideration in algal POME treatment: (i) pre-treatment of wastewater to remove growth inhibitors; (ii) feasible and economical method for algae cultivation and biomass harvesting; and (iii) selection of suitable microalgae strains.

High concentration of nutrients such as ammonium can inhibit the growth of algae and lead to poor wastewater treatment efficiency. According to Cai et al. (2013), nitrogen in the form of ammonium is the most preferred source for effective and rapid growth of algae due to effective redox reaction during nitrogen assimilation and the less energy requirement for the reaction to occur.

The selection of suitable algae for effective phycoremediation and CO_2 fixation is critical to the process development as microalgae cells have different tolerance to the range of pollutants, CO_2 concentration, and also the culture condition. According to Choul-gyun (2002), algal strain such *Chlorella kessleri* is capable of removing various concentration of nitrogen up to 1400 mg/L indicating that this algal strain has a high tolerance to nitrogen.

Numerous studies have been conducted by researchers around the world on the application of algae for phycoremediation and biomass production for sustainable bio-products production. With further research and development targeted at addressing some of the above-mentioned challenges, commercial-scale wastewater treatment using algae can be achieved.

Wastewater treatment challenges using immobilized microalgae

There are some challenges with the use of polymer immobilized microalgae systems according to Cai et al. (2013). These are (i) the capability of the system to remove pollutants present in wastewater effectively; (ii) cost of polymer and subsequent immobilization process; (iii) chemical forces and interactions between the immobilization matrix and the cell wall may result in abiotic stresses (iv) limited diffusion of substrate or fluid like wastewater, metabolic products, oxygen and CO_2 to and from the cells in the polymeric matrix; (v) sufficient light penetration into the polymer matrix containing algal cells; and (vi) the metabolism of microalgae is affected by its confinement in a limited space. However, these issues can be addressed by combining optimized immobilization matrices with smart bioreactor designs.

Conclusion

Effective wastewater treatment is vital in order to improve the quality of wastewaters effluent which must be met the regulations standards set by local authorities before discharge. Thus, the use of suspended free-cells microalgae culture emerges as a viable option in future and can be explored to treat wastewaters containing nutrients sources with simultaneous CO_2 capture which are required for growth and support microalgae cultivation. For that reason, the use of biochemical abilities of suspended free-cells

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microalgae culture is a popular approach to be used as a tertiary treatment in conventional wastewater treatment which can remove nutrient and BOD efficiently in the engineered system like high rate algal ponds. Most recent studies have highlighted the various advantages of immobilized microalgae in carriers as compared to suspended free-cells microalgae culture for removal of nutrients, BOD and COD from wastewaters. For instance, application of immobilized algae to treat wastewaters capable to reduce the cost of all process by circumventing the need for downstream processes using dewatering and harvesting methods to separate the biomass and treated effluent. In contrast, a very costly dewatering and harvesting methods are required to obtain biomass derived from free-cells microalgae culture due to their dilute nature and small in size. Therefore, the immobilized microalgae in capsules not only ease the harvesting process but also increase the efficiency of wastewaters treatment with CO_2 sequestration and bioproduct generation derived from microalgae biomass. The removal of nutrient, BOD and COD from wastewaters using immobilized microalgae cells has been one of the major interesting research subjects carried out by researchers globally due to its environment-friendly approach for sustainable development in the future.

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