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The Current Methods for the Biomass Production of the Microalgae from Wastewaters: An Overview

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Abstract: Different kinds of technologies are now adopted to treat the industrial and agricultural wastewaters by chemical, physical and biological means. Research on microalgae as a probable solution for the wastewater treatment has gained importance in the recent years since; the microalgae have the potential to utilize inexpensive substrates in the wastewater for their growth and sustenance. Moreover, the ability of microalgae to treat wastewater and produce biofuels makes it an economically viable and environmental friendly feedstock. The present review deals with the treatment of wastewaters using micro algal cultivation systems such as open and closed systems and the relative effectiveness of the various strategies of harvesting micro algal like filtration, sedimentation and centrifugation are addressed. Currently, there are various approaches used by industries and companies taking pure water from laboratory to pilot scale; nevertheless, large scale production systems for controlled microalgae cultivation and biomass harvesting for wastewater treatment application and subsequent processing for biofuels production are lacking. Searching methods on large scale algae cultivation and harvesting for biofuels production are necessary for further development. The viability of each application is evaluated on the basis of its algae cultivation nutrient removal efficiency, biomass harvesting, economic feasibility and future perspectives are also discussed in this review.

Key words: Wastewater treatment · Microalgae biomass · Cultivation · Bioreactor · Harvesting · Biofuel

INTRODUCTION

Water pollution has become a serious problem to the humankind owing to rapid industrialization. Industrial waste waters without proper treatment released into the water bodies such as rivers, lakes and oceans interrupt the natural process i.e. respiration, photosynthesis, nitrogen fixation, precipitation and evaporation. Therefore, wastewater treatment is key to the restoration of a healthy environment. Secondary treatment of the industrial and domestic wastewater still releases a high amount of nitrogen and phosphorus into the environment. Physical, chemical and biological methods are available today to remove pollutants from the wastewater. The main aim of wastewater treatment board is the protection of the ecosystem and the public health by protecting the water bodies. Biological treatment by the use of bacteria and fungi to remove contaminants by absorbing them has long been a stronghold of wastewater treatment in many industries. Because they are effective and widely used, many biological treatment options are available today in which microalgae use the wastewater as substrate for their growth. The advantage is that while microalgae remove the nutrients from the wastewater, the biomass can be utilised for producing other useful by-products. The use of various microalgae for treating the domestic wastewaters has been revealed and the efficiency of this method is believed to be promising [1-3]. The levels of metals, nitrates and phosphate [4, 5] in the wastewater are greatly lowered due to microalgal growth for some time. These observations reveal the possibility that the microalgae could be utilized for the tertiary wastewater

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treatment. Recently, microalgae have received a great deal of interest because their biomass has become a source of renewable energy. Some of the main characteristics which set the microalgae suitable for biomass production are that microalgae do not require agricultural land, freshwater is not essential and nutrients can be provided through the wastewater [6].

Therefore the aim of this review paper summarizes the current trends in terms of wastewater treatment and biomass production by the role of microalgae. Growth condition of microalgae, recent algae cultivation systems as well as identifies the biomass harvesting methods are discussed in detail.

Nutrients From Wastewater Streams: Generally, wastewater contains large amount of nutrients, several microorganisms, as well as various toxic materials. The aim of wastewater management is the environment protection in a manner adequate with social and economic concerns. Wastewater can be characterized by its physical, chemical and biological components. Temperature, colour, conductivity and odour are some of the physical characteristics of wastewater. Table 1 shows the different levels of untreated wastewater composition. Temperature of the wastewater is crucial because it affects chemical and biological reactions in wastewater. Temperature is also important in determining the other factors such as conductivity, pH and saturation level of the gases. In general, the colour of wastewater acts as an indicator of its age. Odour of the wastewater is caused by the dissolved impurities produced by the decaying aquatic microorganisms besides evaporation of the gases [7].

Combination of carbon, oxygen, hydrogen and the other elements such as phosphorus, sulphur, ammonia and iron make up the organic matter [8]. Presence of ammonia is considered as a chemical evidence for organic pollution. On the other hand, the sewage wastewater contains high amount of nitrogenous material and it is considered as inorganic pollution when the wastewater is released into the water bodies.

Wastewaters naturally support the growth of large amount of macro and microorganisms. Determining of the type of bio-treatment to be carried out depends on the quantity of the species of macro and microorganism and the other aquatic life in the receiving water body. Within the treatment amenities, wastewater offers an ideal growth medium for prospective microbial cells irrespective of being aerobic or anaerobic conditions.

Table 1: Composition of untreated wastewater [7]

	Concentration (mg/L)		
Parameters	Low	Medium	High
Total solids (TS)	350	720	1200
Total dissolved solids (TDS)	250	500	850
Fixed	145	300	525
Volatile	105	200	325
Suspended solids	100	220	350
Fixed	20	55	75
Volatile	80	165	275
Settle able solids	5	10	20
BOD_5 (Biological oxygen demand), 20°C	110	220	400
TOC (Total organic carbon)	80	160	290
COD (Chemical Oxygen demand)	250	500	1000
Nitrogen (total as N)	20	40	85
Organic	8	15	35
Free ammonia	12	25	50
Nitrites	0	0	0
Nitrates	0	0	0
Phosphorus (total as P)	4	8	15
Organic	1	3	5
Inorganic	3	5	10
Chlorides	30	50	100
Sulphate	20	30	50
Alkalinity (as $CaCO_3$)	50	100	200
Oil and Grease	50	100	150
Volatile organic compound	<100	100-400	>400

The Factors at Play: To ensure that the wastewater can be treated by microalgae successfully, good algal growth and the factors that affect their growth must be determined. The growth rate of microalgae is influenced by various physical, chemical and biological factors. Several authors observed that the microalgae have a large potential in the wastewater treatment in view of their ability to adapt to wastewater and utilize its nutrients such as nitrogen, sulphate and other nutrients for their growth. Besides the nutrients, microalgal growth also depends on certain other physical factors such as light, temperature and pH of the medium [9]. Therefore, nutrients and the physical factors play a crucial role in culturing the microalgae especially in the case of wastewater treatment.

The need to provide nutrients and the other growth requirements for algal growth is essential. Many microalgae species are able to successfully grow in the wastewater habitats by way of their ability to utilise inorganic nitrogen and phosphorus as well as organic carbon available in the wastewater. The application of microalgae in the wastewater treatment has been in vogue for long [10]. Microalgae are proficient in removing nitrogen, phosphorus and the other toxic metals from the wastewater [11, 12] and hence, have the great potential for bioremediation. Algae are capable of synthesizing their own food from inorganic substances by utilizing solar energy and CO_2 by photosynthesis. The most common elements of algal cell are $C_{106}H_{181}O_{45}N_{16}P$ and for optimal growth of the microalgae, medium should provide them in this proportion. Vonshak, [13] summarised the requirement of nutrients for microalgal growth as follows; (i) the total content of salt, (ii) composition of cells in terms of the major ionic elements i.e. K^+, Mg^{2+}, Na^+, Ca^{2+}, SO_4^- or HCO_3^-; (iii) the source of nitrogen particularly nitrate, (iv) the source of carbon either CO_2 or HCO_3^-, (v) pH, (vi) Some chelating agent and trace elements i.e. EDTA and (vii) vitamins.

Carbon: The supply of inorganic carbon such as CO_2 and HCO_3- is essential for high rate autotrophic algal production [4]. Dispersion rates for CO_2 from the atmosphere into the open ponds can at the major productivities approximately 10g (dw)$m^{-2}d^{-1}$. Generally, the CO_2-H_2CO_3-HCO_3—CO_3^{2-} structure is the imperative buffer available in the freshwaters and it is the greatest means to maintain and control particular pH ranges that are suitable for algal cultivation.

Carbon dioxide is provided by the buffer system of bicarbonate-carbonate for photosynthesis according to the following reactions:

$$2HCO_3^- \leftrightarrow CO_3^{2-} + H_2O + CO_2$$
$$HCO_3^- \leftrightarrow CO_2 + OH^-$$
$$CO_2 + 2OH^- \leftrightarrow CO_3^{2-} + H_2O$$

These reactions occur during photosynthetic CO_2 fixation. A gradual rise of pH could be because of the OH^- accumulation in the growth medium. Richmond and Grobbelaar, [14] reported that there has been a high pH ranges as high as 11 in high microalgal production with no extra CO_2 being provided. CO_2 sparing into the culture media is the most convenient method for pH control. In photosynthesis, microalgae absorb inorganic carbon by utilising solar energy and convert to chemical energy and oxygen (O_2) as a side product. Later, the chemical energy is used to assimilate CO_2 to convert into sugar. CO_2 from the atmosphere can be supplied to the medium culture by aeration. Nevertheless, CO_2 only consists about 0.033% of gases in the atmosphere and may not affect the growth of algae much. To overcome this problem, extra carbon supply is needed and this can be accomplished by providing 1-5% CO_2 [15] air enriched medium. Stoichiometric formula for photosynthesis is shown by the following reaction:

$$6\,H_2O + 6\,CO_2 + light \leftrightarrow C_6H_{12}O_6 + 6\,O_2$$

Besides using the inorganic carbon, there are some algal species that have the ability to use organic carbon for their carbon requirements. This heterotrophic metabolism is vital in waste-accumulated ponds where standing algal biomass is very high and the carbon dioxide may be exhausted. Almost 25 - 50% of the microalgal carbon in high rate of algal pond is obtained from the heterotrophic consumption of organic carbon [16].

Nitrogen: Nitrogen is the second important nutrient for algae that contributes to the biomass production. Mostly nitrogen is supplied in the nitrate (NO_3^-) and ammonium (NH_4^+) form [16]. However, ammonia nitrogen is preferred as the nitrogen source by the algae [17]. The absorption of either NO_3^- or NH_4^+ is directly related to the growth medium pH. When ammonia is utilised as nitrogen source for algal growth, pH could decrease during active growth period because of releasing of H^+ ions. On the other hand, pH could increase when nitrate is provided as the only nitrogen source. In addition to ammonium and nitrate, the other nitrogen compounds such as urea ($CO(NH_2)_2$) and nitrite (NO_2^-) can be utilised as N-source, but at a higher concentration, it is toxic and inhibits the algal growth [18]. Gonzalez *et al*. [19] studied the efficiency of ammonia uptake by *Chlorella vulgaris* and *Scenedesmus dimorphus* from the agroindustrial wastewater and showed that almost 80% of NH_3 was removed after 216 hours of incubation. Various cyanobacteria are also able to utilizing nitrogen by the decrement of N_2 to NH_4^+, a process catalysed through the nitrogenase enzyme [5]. Although this process occurs in the ecosystems, the quantities are too low and not appropriate for large scale production of microalgae.

Phosphorus: Phosphorus is the essential nutrient for growth and several cellular functions. The preferred form of phosphorus for algae is orthophosphate (PO_4^{3-}). Phosphorus is often one of the significant growth-limiting chemical factors in algal biology because it is easily bound to the other ions such as CO_3^{2-} and iron. Supplementation of phosphorus affects the composition of the produced algae biomass particularly the lipid and carbohydrate substance [20]. Therefore, algae cultivated in the wastewater may have large amount of phosphorus.

Other Nutrients: As stated above, besides carbon, nitrogen and phosphorus, other macro nutrients such as potassium, calcium, magnesium and micro-nutrients manganese, nickel, boron, zinc, molybdenum, copper, iron, chloride and trace element are important for microalgal cultivation [21]. Many of these elements are involved essentially for enzyme reactions and synthesis of various compounds. Addition of a metal chelator such as EDTA prevents the macro and micronutrients from bonding with phosphorus that will result in the precipitation and consequently renders them unavailable. In addition, certain algae need special elements such as silicon, iodine and vanadium.

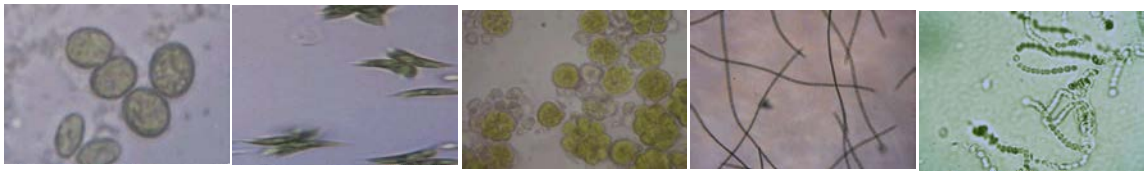
Heavy Metals: Microalgae can also be grown in the wastewater containing heavy metals. Industrial processes and the agricultural practices always result in the discharge of several heavy metals into the environment. Heavy metals are stable and cannot be degraded and because of that, natural uptake of heavy metals by microalgae shows the potential in them for the application of the bioremediation processes. Microalgae have a notable ability to accumulate heavy metals from their neighbouring environment. Moreover, it is abundant in the natural environment. Various studies showed that microalgae have the capability to sequester several metal ions such as cadmium, copper, nickel, chromium and gold [22, 23]. Maria Lourdes *et al*. [24] showed that in a laboratory batch culture system, *Chlorococcum* sp. removed copper (Cu) up to 43-75% with the highest removal at 10 mgL^{-1} initial concentration.

Physical Factors: Many physical factors influence the uptake of nutrient rates for microalgae species, including pH, temperature and light intensity. Of these parameters, light intensity has impacts directly on removing nutrients. Other parameters have been involved indirectly by light intensity or alteration of nutrient concentration. Each of the factors documented above is discussed in the following subsections.

Temperature: Temperature plays a major role in algal growth. The temperature factor could affect the biochemical reactions and eventually it may affect the biochemical composition of algae [25]. Increased temperature stimulates algal growth until an optimal temperature is attained. Further increase in temperature may cause stress and affect growth resulting in the decrease of growth rate. Microalgae growth has an optimum temperature and their growth rate dependent on temperature differs from species to species. Some species of microalgae can cultivate at high temperatures whereas other species can tolerate at low temperatures. It can grow a range of temperatures and their behaviour to temperature differences can influence the following (i) nature and rates metabolism (ii) requirement of nutritional level and (iii) cell compositions [26]. Generally, temperature up to 15°C is the most favoured temperature for algal cultivation [22, 26].

Light: Light is essential for microalgae because they convert it into chemical energy through photosynthesis. However, there are some microalgae classified as the heterotrophs which are able to obtain energy from organic compounds like carbon as their source of energy. When cultivated in wastewater, shading effect due to high content of particulate matter may prevent microalgae that are not floating from getting the optimum light for energy build-up. To minimize this turbulence can be created in the culture so as to facilitate the cells getting exposed to the light at least for a brief period to enhance productivity. The other way to counter this problem is by limiting the depth of culture vessel to ensure that the light can penetrate up to the bottom of the culture vessel. Generally, a vessel depth between 15 and 50 cm is recommended [27]. Friedman *et al*. [28] observed that high light intensities tend to increase polysaccharide production in the algal cell. Tredici *et al*. [29] also reported that *Spirulina platensis* grown under outdoor conditions was significantly higher in productivity on sunny days than on the cloudy days. Light intensity is also a key factor affecting nutrient uptakes of microalgae since it provides energy to the cells. The carbon and phosphorus removal efficiency by heterotrophic *Chlorella kessleri* was reported to be higher under a12h light/12h dark lighting than that under constant lighting, while the nitrate removal efficiency under constant lighting scheme was higher than that under diurnal lighting illumination [30].

pH: pH is also an important factor that affects growth of the microalgae. In microalgal cultivation, pH usually rises due to photosynthetic CO_2 assimilation. pH affects the availability of inorganic carbon [31]. Absorption of nitrogen by microalgae also affects the pH of the medium. Assimilation of nitrate ions may increase the pH. Higher pH may cause precipitation of phosphate in the medium but this can be avoided by reducing the pH by the process of respiration where CO_2 assimilation does not



(a) (b) (c) (d) (e)

Fig. 1: Light microscopic picture of frequently used microalgae in wastewater treatment application. (a) *Chlorella vulgaris*; (b) *Scenedesmus* sp.; (c) *Botryococcus braunii*; (d) *Spirulina* sp.; (e) *Phormidium* sp.

happen. Studying the influence of pH on the microalgae in wastewater, Hodaifa *et al*. [32] found that the growth rate of microalga, *Scenedesmus obliquus* was optimum when the medium pH was constant.

Viability of Microalgae for Wastewater Treatment: Algae worldwide include thousands of diverse strains that, combined with the recent advances in bioremediation, offer a good starting point for the improvement of algal cultivating systems based on the treatment of wastewaters. Strain selection and adaptation of strains with desirable characters that allocate the use of water chattels of varying water quality also play important roles in the outlook of using microalgae. Industrial wastewaters are widely varied and the microalgae involved to treat these wastewaters also vary accordingly. Some of the algae strains which are used most frequently are listed: *Chlorella vulgaris, Scenedesmus dimorphus*, *Botryococcus braunii, Spirulina* sp and *Phormidium* sp seem to be the ideal species due to their good growth ability and good tolerance of different environmental conditions (Fig. 1a-e).

Future algal strain development will explore methodologies for waste water treatment to screen and improve new strains that yield high growth and high value added products. Most of the algae research on the treatment of wastewater has come from the assessment of laboratory basis pilot pond scale and small scale cultures and from the experimental high-rate algal ponds. A lot of studies have focussed on the growth of microalgae under different wastewater environment, mainly growth in the agricultural manure wastewater and municipal sewage wastewater. Kamarudin *et al*. [33] successfully cultivated *Scenedesmus dimorphus* in anaerobically digested palm oil mill effluent. The maximum reported nutrient removal abilities in their experiment were 99.5% NH_3-N; 98.8% Phosphorus; 86% COD and 86.5% BOD in anaerobically digested palm oil mill effluent. Similarly, Zainal *et al*. [34] investigated nutrient removal by role of *Spirulina platensis* in removal of 87% NH_3-N; 80% Total Phosphorus; 90% COD and 86.5% BOD in anaerobically digested palm oil mill effluent. Many microalgal species are able to metabolize various types of nutrients and their prospective application in the wastewater treatment has been investigated by several authors [35, 36]. It revealed that the success of the experiment should be based on selection of algal strains and wastewater type and also minimum processing requirements. Most of the studies on the use of microalgae for wastewater treatment are based on the use of a monoculture to remove a specific nutrient and only a few studies have focussed on the use of mixed algal cultures for wastewater treatment [3]. Some of the earlier studies involving microalgae grown in various types of wastewaters along with their relevant nutrient removal efficiency are tabulated (Table 2).

Wastewater contains a variety of nutrients and it is difficult to get a single strain that can concurrently remove all the nutrients from the wastewater. In both activated sludge and oxidation pond processes, complex mixtures of the natural populations of microorganisms are involved and the composition and relative proportions of these microorganisms vary depending on the composition of the wastewater and also the stage of treatment. Microalgae vary in their nutritional requirements and it is possible to select a good combination of microalgae for simultaneous removal of diverse nutrients from the wastewater. Development of an efficient system for treating highly polluted organic wastewater with microalgae can be developed for diverse applications.

The nutrient removal from the synthetic wastewater by various microalgae was evaluated together with the feasibility of using a mixed culture of these phototrophs for simultaneous removal of various nutrients from the wastewater. It is very important to select a wastewater with balanced nutrient accessibility. It has been revealed that high content of COD has synergistic effect on microalgal growth only if nitrogen and phosphorus are presented in sufficient amount [39, 42, 43]. While the

Table 2: Microalgae used for the treatment of wastewaters [37]

Microalgae species	Wastewater (WW)	% Removal rationutrients	Reference
Chlorella vulgaris	Rubber latexconcentrate processing	93.39% COD79.33% TKN	[38]
Spirulina platensis	Sago starchy WW (AD-treated)	98.0% COD99.9% NH3-N99.4% phosphate	[39]
Chlamydomonas, Chlorella,Scenedesmus spp.	WW containing 85-90%carpet industry effluents	>96% nutrient removal	[40, 3]
Chlamydomonas reinhardtii	Industry effluent (undiluted)	83.0% TKN14.45% phosphorus	[41]
Chlorella sp. 227	Effluent after secondaryTreatment (Domestic WW)	92% TN86% TP	[42]
Chlorella sp.	Centrate (MunicipalWW)	70%,COD61%,TKN61% phosphorus	[43]
Co-culture of Chlorella vulgaris			
Planktothrix isothrix	MunicipalWW	80% NH4-N100% phosphorus	[44]
Unknown	Municipal WW(Lawrence WWTP)	61.4% TN removal90.6% TP removal	[45]
Chroococcus sp.	Grey water	89-98% TP98% NO3-N100% NH3-N	[46]
Mixture of green algae collected fromlocal			
municipal/winery WWT pond	Dairy WW	96% NH4 -NN99% orthophosphate	[47]
Algal-bacterial consortium	Pretreated piggery WW	56 ± 31% COD98 ± 1% NH4+ removal	[48]
Neochloris oleoabundans	Anaerobically digested dairy manure (1:50 dilution)	90-95% N removal	[49]
Scenedesmus dimorphus	Anaerobically digested palm oil mill effluent	99.5% NH3-N; P 98.8% ; 86% COD; 86.5% BOD	[33]
Spirulina platensis	Anaerobically digested palm oil mill effluent	87% NH3-N; 80% TP; 90.4% COD ; 86.5% BOD	[34]

actual wastewater may not have the ultimate nutrient input, appropriate recipe of high nutrient wastewater may be working for good productivities.

Generally, the ideal microalgal strains for wastewater treatment and valuable biomass production must have: (a) high tolerance to a wide range in ecological variations such as temperature, light, etc; (b) Maximum lipid/protein/carbohydrate productivity and content; (c) superior settle ability to harvest; (d) rapid growth ability and easy to cultivate; (e) high ammonia nitrogen tolerance; (f) high O_2 generation rates and high CO_2 sequestration ability; (g) ability to grow dominantly in open environment; (h) good tolerance to grow in toxic pollutants [50]. However, no known algal strains could promise all these requirements and the selection of suitable algae strains is mainly dependent on the purposes. In future, genetic modification is one possible option would develop genetically modified algae to improve the algal function and hence to recognize the overall optimization. The modification of microalgal antenna size by molecular techniques could enhance the algal photosynthetic rates and biomass productivity [51]. Lundquist *et al*. [36] also stated that genetic modification of the microalgae can deliver both high biomass productivity and high oil content. Furthermore, along with the content of nutrients in wastewater, the design of cultivation system also affects the yield of algae biomass. Bioreactor for Wastewater Nutrient Removal: The design of an efficient algae cultivation system requires careful attention for removing nutrients successfully and accumulating biomass sufficiently. There are several methods for algae culture systems that can be used for the use of nutrients removal from wastewaters with concurrent microalgae biomass proliferation such as photobioreactors, open raceway ponds, open maturation or oxidation ponds, polybags and vertical tank reactors [52-54]. There are various pros and cons with these cultivation systems (Table 3).

In this way, various microalgae strains have been selected and tested for their potentials in wastewater treatment and microalgal biomass production under various experimental conditions [3, 41, 55-61] (Table 4). Nevertheless, these results are difficult to evaluate as they are obtained under different environmental conditions with various bioreactors configurations.

The open system cultivation is more suitable because it is cheaper and simpler to develop. However, the open system stands exposed to the environmental factors such as temperature, light intensity and air and therefore it may get polluted faster. Closed system cultivation is more complex but allows a controlled environment for cultivation. There are many advantages and disadvantages for both systems as summarized in table 3. Nevertheless, the design of efficient microalgal bioreactor for removing nutrients and producing biomass is an ongoing exercise [62].

Open System Cultivation: The open system cultivation is a preferable method in view of its low cost and can be performed on a large scale and is also easier to manage. Moreover, it is more durable than the large closed reactors. The open system cultivation can be carried out in the natural or artificial lakes and ponds. There are many types of ponds designed and experimented earlier for optimum cultivation of the microalgae. Despite many types of open system (ponds) have been proposed in the past, only three major designs have been activated on a large scale. The three main designs are raceway ponds,

Table 3: Advantages and disadvantages of open ponds and closed photobioreactors

Cultivation Methods	Advantages	Disadvantages
Open raceway pond	Relatively cheap to construct and operate; Low energy inputs; Easy to clean.	Minimum biomass productivity; Poor mixing and light utilization; Required large land area; Contamination and high pollution risks; Limited to only a few species; Poor dispersion of CO2 to the environment.
Closed photobioreactors	Process conditions (pH and temperature etc.,) controlled; Low evaporation losses; Less sensitive to contamination; Required small area; More suitable for sensitive strains and mono-cultivation; Easier to harvest due to high cell mass productivities; Low hydrodynamic pressure.	Very difficult and expensive to construct and operate; Difficult to clean the reactors.

Table 4: Different types of cultivation systems used for the wastewater treatment applications

Strains of microalgae	Types of wastewater	Cultivation systems	Reference
Chlorella vulgaris	Textile wastewater	High Rates Algae Ponds (HRAP)	[58]
Chlorella sorokinia	Piggery wastewater	Photobioreactor	[59]
Neochloris oleoabundans	Municipal wastewater	Cylinder flasks	[61]
Scenedesmus accuminatus	Piggery wastewater	Photobioreactor	[60]
Chlamydomonas reinhardtii	Municipal wastewater	Biocoil photobioreator	[41]
Spirulina arthrospira	Piggery wastewater	Raceway ponds	[57]
Phaeodactylum triconutum	Municipal wastewater	Corrugated raceway	[55]
Chlamydomonas globosa,			
Scenedesmus bijuga, Chlorella minutissima,	Carpet industry wastewater,	Raceway ponds, vertical tank reactors,	[3]
Scenedesmus obliquus.	Autoclaved municipal wastewater	polybags, Photobioreactor	[56]

Table 5: Comparison of microalgae biomass and lipid productivities grown on various wastewater [6].

Wastewater type	Microalgae species	Biomass productivity (mgL^{-1} day^{-1})	Lipid content (%)	Lipid productivity (mgL^{-1} day^{-1})	Reference
Municipal (primary treated)	nd	25[a]	nd	nd	[85]
Municipal (centrate)	*Chlamydomonas reinhardtii* (biocoil-grown)	2000	25.25	505	[41]
Municipal (secondary treated)	*Scenedesmus obliquus*	26[b]	31.4[i]	8[i]	[86]
Municipal (secondary treated)	*Botryococcus braunii*	345.6[c]	17.85	62	[87]
Municipal (primary treated + CO_2)	Mix of *Chlorella* sp. *Micractinium* sp. *Actinastrum* sp.	270.7[d]	9	24.4	[35]
Agricultural (piggery manure with high NO_3-N)	*B. braunii*	700[e]	nd	69	[88]
Agricultural (dairy manure with polystyrene foam support)	*Chlorella* sp.	2.6 gm^{-2} day^{-1}	9[i]	230[i] $mg\ m^{-2}$ day^{-1}	[89]
Agricultural (fermented swine urine)	*Scenedesmus* sp.	6[f]	0.9[i]	0.54[i]	[90]
Agricultural (anaerobically digested dairy manure)	Mix of *Microspora willeana*, *Ulothrix zonata, Ulothrix aequalis, Rhizoclonium hieroglyphicum, Oedogonium* sp.	5.5 gm^{-2} day^{-1}	nd	nd	[91]
Agricultural (swine effluent, maximum manure loading rate)	*R. hieroglyphicum*	10.7 gm^{-2} day^{-1}	0.7[i]	72[i] $mg\ m^{-2}$ day^{-1}	[92]
Agricultural (daily effluent + CO_2. maximum manure loading rate)	*R. hieroglyphicum*	17.9 gm^{-2} day^{-1}	1.2[i]	210[i] $mg\ m^{-2}$ day^{-1}	[92]
Agricultural (digested dairy manure, 20× dilution)	*Chlorella* sp.	81.4g	13.6[i]	11[i]	[93]
Agricultural (dairy wastewater, 25% dilution)	Mix of *Chlorella* sp. *Micractinium* sp. *Actinastrum* sp.	59[h]	29	17	[35]
Industrial (carpet mill, untreated)	*B. braunii*	34	13.20	4.5	[3]
Industrial (carpet mill, untreated)	*Chlorella saccharophila*	23	18.10	4.2	[3]
Industrial (carpet mill, untreated)	*Dunaliella tertiolecta*	28	15.20	4.3	[3]
Industrial (carpet mill, untreated)	*Pleurochrysis carterae*	33	12. 00	4. 0	[3]
Artificial wastewater	*Scenedesmus* sp.	126.54	12.8	16.2	[94]

nd - not determined; DW - dry weight.

[a] Estimated from biomass value of ~1000 mg L^{-1} after 40 days.

[b] Estimated From biomass value of 1.1 mgL^{-1} h^{-1}.

[c] Estimated from biomass value of 14.4 mg L^{-1} h^{-1}.

[d] Estimated from biomass value of 812 mg L^{-1} after 3 days.

[e] Estimated from biomass value of 7 g L^{-1} after 10 days.

[f] Estimated from biomass value of 197 mg L^{-1} after 31 days.

[g] Estimated from biomass value of 1.71 g L^{-1} after 21 days.

[h] Estimated from lipid productivity and lipid content value.

[i] Fatty acid content and productivity determined rather than total lipid.

inclined systems and the circular ponds. In this review, we highlighted the raceway pond cultivation method alone because it is the efficient method used for wastewater treatment and biomass production [14, 63].

Raceway Ponds: Open culture pond is the most common cultivation method for mass cultivation of microalgae because it is simpler and cost-effective. In this system, water level is kept at not less than 15 cm and the algae are cultured according to the optimum growth conditions by supplying the necessary nutrients. The pond is built in a raceway make-up, in which a paddlewheel rotates and mixes the algal cells with the nutrient [50]. The raceways are prepared as of poured concrete to prevent the ground from sopping up the liquid condition. The fresh feed containing all kinds of nutrients is supplied in front side of the paddle wheel and the algal cells are harvested from behind the paddle wheel. Although the open system is cost-effective, it has some disadvantages. Among the disadvantages, it requires large land areas for a considerable biomass yield. Moreover, this cultivation technology is carried out in the open air, the water level can be affected by evaporation and rainfall. Besides, biomass productivity could also be curtailed due to contamination by the undesirable algal species and organisms that feed on these algae. Several studies revealed that certain microalgae such as *Dunaliella* sp. *Spirulina* sp. *Chlorella* sp. could not be cultured in the open ponds due to the harsh culture conditions [64]. Fig. 2 shows schematic open raceway pond design for wastewater treatment applications.

Despite the salient drawbacks of this cultivation system, the following processes can be used for efficient wastewater treatment and sufficient biomass production: (i) the benefit of this method is that offered wastewater could be used as media for the cultivation of microalgae with the added advantage of nutrient removing, (ii) if the open raceway pond system is located near an industrial power plant, inexpensively available released flue gas can be used to fast up the photosynthetic growth rates in the open pond [65], (iii) the open raceway pond can be sheltered similar to the agricultural greenhouse model. Maximum sunlight intensity can be enter to this shelter in order to avoid debris and rainfall contaminations, (iv) For sufficient light penetration, the depth of water in the open raceway pond should not be allow a maximum level of 30 cm [52, 66], (v) In order to examine the physiological function of the algal cells, the analytical parameters such as cell density, pH, temperature, conductivity, light intensity, salinity, evaporation rates, dissolved oxygen, dissolved carbon dioxide, Total dissolved solids (TDS), nitrate and phosphate levels should be carefully monitored in the raceway pond [67].

Fig. 2: Schematic open raceway pond design for wastewater treatment applications

Closed System Cultivation: Closed cultivation system of microalgae is done in a controlled environment. This cultivation method can maximize the yield of biomass and reduce the possibility of contamination during the cultivation but it is not cost-effective. The most common cultivation technology for the closed system cultivation is the photobioreactor (PBR). Typical closed reactors include tubular and flat plate photobioreactors. It made up of glass or plastic are the two types of closed systems and only the former type is used at large scale [50]. They are arranged in a vertical, horizontal, helical or inclined manner. An airlift system or mechanical pump is used to permit CO_2 and O_2 to be substitute as well as to supply a mechanical mixing [68]. PBR is more effective as compared to the open system cultivation because it allows better light penetration which is usually less than 30 mm and enhances productivity within a short period of retention time [69]. But owing to its complexity the PBR is more expensive and requires much more energy and the system also requires an expert monitoring. By employing visible pipes for cultivation, the inner shadowing effect among the algal cells is minimized and penetration of light is better when compared to the open system cultivation. The light refraction creates shaded spaces in the tubes though and enough turbulence is consequently needed to be provided [70]. The production of algae biomass for closed PBR commonly range from 20-40 $g/m^2/d$ and are higher than those of open ponds [71]. According to wastewater treatment, that photobioreactors are not perfect for large scale due to the high amounts requiring remediation. Nevertheless, they are essential for small scale cultivation of microalgae [72]. Microalgae based cultivation systems can decrease considerable amount of organic material and nutrients in piggery wastewaters at low energy cost in simple solar-powered photobioreactors at small scale process [73].

Harvesting Techniques: Microalgae harvesting is an important process for the efficient nutrient removal from wastewater and the valuable biomass production. The choice of harvesting method is the significant to the economics of bioenergy production as harvesting can make up 20-30% production of the total cost. The method of harvesting mainly depends upon the characteristics of the culture cultivated [50]. Microalgae that are suitable for wastewater treatment of and biofuels production tend to be of unicellular structure of low density. It makes difficult for economical biomass harvesting and significant for cost of biomass recovery [6, 50, 53, 74]. Methods of harvesting include filtration, centrifugation, sedimentation and flocculation and floatation [67]. In this review, we focus on three types of harvesting methods of the microalgae filtration, sedimentation and centrifugation.

Filtration: Filtration is the most simple and cost-effective harvesting method [75]. Filtration can be carried out either on a small or large scale. Filtration can be done by using the filter paper on laboratory scale or by a coarse screening on a large scale harvesting of the microalgae. Besides, Cyanobacteria such as *Spirulina* can be harvested by using the basic principal of filtration called microstraining. This process consists of a rotating fine-mesh screen and backwash to harvest the microalgae. According to Benemann *et al*. [76] by the microstraining method of harvesting microalgae, almost 20-folds of concentration and more can be achieved. Mohn, [77] reported that the filtration harvesting method, rotary vacuum and the chamber filter emerge to be the usually employed type of filter for the large size of microalgae. The advantages of these filters are that they can be used in a continuous operation and are useful even when sterility and contamination occurs. However, filtration is suitable for harvesting the microalgae and not for separating certain microalgae like *Scenedesmus*, *Dunaliella* or C*hlorella* [77]. The disadvantage of this method is to be costly, regular membrane replacements and energy intensive and forcing of algae biomass [6].

Sedimentation: In general, sedimentation without the addition of any chemicals is the most popular method in full-scale facilities, but to facilitate sedimentation, prior flocculation is more desirable because the microalgae have the tendency to float on the surface of water to harness the light. Sedimentation combined with flocculation is reported to be cost-effective because of the use of gravity for biomass settling and its minimal power consumption [52, 75, 76]. Sedimentation cultivation method depends on the density of the microalgae. Edzwald, [78] observed that low density microalgae do not settle fast. Flocculation of algae some times causes secondary pollution due to the use of the flocculating agents like $FeCl_3$, or $Ca(OH)_2$. However, this problem can be overcome by the use of chitosan and certain potato starch derivatives [79].

Centrifugation: Centrifugation method can be applied to almost every type of microalgae for biomass harvesting as it is rapid, efficient and universal [67]. Centrifugation works on the principle of sedimentation with enhanced gravitational force to increase the sedimentation rate. There are some types of centrifugation harvesting method and it is depending on the particle size. Tubular bowl centrifugation provides the most efficient method for harvesting but its capacity is very limited. This method is more preferably applied on a small laboratory scale. For slurries and high content of biomass (5-80% v/v), decanter bowl discharge centrifuge is more suitable [80]. However, there are some disadvantages like cell wall damage to high gravitational and shear forces [81]. It is not economically viable for harvesting at large scale due to it practice being greatly energy intensive [6].

Immobilization: Immobilization systems as effective harvesting techniques have been widely used and tested for water treatment. Immobilised system can be defined as a system where the algal cell is trapped in a solid medium and is prevented from moving independently. This system can solve the harvesting problem [82]. It has been divided into 6 different methods: affinity immobilization, adsorption, covalent coupling and confinement in liquid-liquid emulsion, capture behind semi-permeable membranes and entrapment in polymers [11, 83]. Among these, entrapment-based immobilization method is one of the most frequently used methods particularly in wastewater treatment using microalgae [6].

To immobilise the algae, cells are trapped in alginate or a synthetic polymer which allows the substances in the wastewater to diffuse through the cell. The mixture of the algae-medium is often formed as beads but can even to wrap the screens. The immobilised systems have been tested against several wastewater treatments and previous research revealed that the entrapped algae are able to remove the nitrogen and phosphorus in the secondary effluent and be considered a tertiary wastewater treatment [84]. It should be mentioned here that most of the immobilized algal cell are employed only on a small scale in the laboratory and our knowledge on their performance on a large scale remains to be understood. Therefore, harvested algae biomass from the wastewater treatment cultivation systems using the above suitable technique could be converted through various pathways to biofuels.

The Potential of Biofuel Production Using Wastewaters: The microalgae have the good ability to grow well in certain wastewater conditions, as explained above, has designated the potential of wastewater resources as favour growth medium for biofuel feedstock. The application of microalgae for bioremediation and biomass production is a feasible option. It is expected to create a situation where the water quality gets improved through the elimination of nitrogen and phosphorus while a renewable energy is produced from the algae to generate various valuable products. In this part we will briefly highlight that how efficient wastewaters are in giving considerable amount of microalgal biomass and whether this biomass can make high contents of lipids for biodiesel production.

Recent laboratory-based studies have accounted reasonable lipid synthesis in wastewater-cultivated microalgae, varying from low (<10% DW) to modest (25-30% DW) lipid amount and in a few studies this can convert to relatively high lipid yield when together to high biomass (Table 5).

Microalgae cultivated on the untreated carpet mill wastewater were examined for lipid content. The level of total lipid content varied from 12% to 18.1% per cell DW based on species of microalgae. In view of the account, the calculated algae biomass (varied from 23 to 34 mg L^{-1} day^{-1}) and the quantity of wastewater produced every year, this resource was calculated to produce biomass varied from 16.1 to 28.1 tons ha^{-1} $year^{-1}$ and lipid productivity ranging from 3260 to 3830 L ha^{-1} $year^{-1}$ [3], suggesting that energy productivity from this kind of wastewater have future viable option. Similarly, *Chlamydomonas reinhardtii* grown on municipal wastewater was produced the total content of lipid 16.6% DW [41]. In addition, this lipid yield could be combined with better nutrient removal efficiency [41]. Interestingly, Orpez *et al*. [87] also observed the total lipid content (17.85% DW) of *B. braunii* cultivated in treated secondary municipal wastewater and these contents were higher compared to the microalgae was cultivated in synthetic growth medium (yielding 10.96% DW) suggesting that the wastewater stress conditions may be inducing the lipid production. A number of recent studies have indicated the potential of biofuel production of algae cultivation on dairy manure. For example, Woertz *et al*. [35] revealed the algae lipid value from mixed cultures cultivated in dairy manure (anaerobically digested) in outdoor batch cultures. The content of lipid reached up to 14% - 29% DW depending on the concentration of wastewater used, producing estimated lipid yield of 2.8 g m^{-2} day^{-1} on after 6 days growth. Likewise, Wang *et al*. [93] and Johnson and Wen, [95] assessed microalgae culture (*Chlorella* sp.) on anaerobically digested dairy manure and carry out a detailed analysis of the lipid profile of the algae cells. A critical evaluation as to the potential of microalgal biofuel production using the resources of wastewaters is whether the process will grant a positive energy outcome which will also evaluate the economic feasibility of the process.

Further, microalgae biomass can be converted to biofuel by different kind of methods. Extraction of lipid from algae for the production of biodiesel is one of the most viable options, specifically if the remaining residual biomass is used for biogas production [96]. After oil extraction the pending algal biomass can also be reused to produce methane, ethanol, livestock feed and can be used as organic fertilizer in view of its high N:P ratio, or is simply burned for energy cogeneration [2]. The algal biomass can be an excellent source for many useful metabolites [97], energy sources such as oil [98]. Depending on both experimental and commercial scale studies, the algal biofuel production cost for a highly productive system was found to be mostly related to 77% growth rate, 12% harvesting and 7.9% extraction [99]. It was expected that the usage of wastewater as alternative for commercial algae nutrients medium would extensively reduce the algae cultivation operational cost. However, analysis of different cultivation systems to grow an algal consortium for wastewater treatment and biofuel productions has not received much attention [3].

Need for Research and Development: In recent years, several papers have been published in the area of wastewater treatment using various species of microalgae. In this review article we present a partial overview of how microalgae could contribute to the wastewater treatment and some of the factors that call for due consideration to sustain this practice. It is better to expend money on avoiding contaminants from entering the water than on removing them at a later stage. Wastewater treatment is only one of the many uses of microalgae. The know-how gained by those interested in the other uses of microalgal systems should be practicable, at least in part, in the waste water treatment. Before such systems are implemented, more studies are required as to the biology of algae, their culture conditions and their ability to metabolize the wastes etc. According to the algal physiology, some attempt should be directed to heterotrophy and mixotrophy cultivation systems. Most effluents contain organic wastes and it is necessary to study the ability of the various species to thrive on such toxic materials with little demand for intense light. Microalgae are known for their ability to sequester certain heavy metals. Microalgae should therefore be studied for their ability to use or remove these heavy metals. Not all algae are endowed with the ability to detoxify the polluted waters, for their abilities vary from species to species. Therefore, any conclusions should be drawn only after thorough investigations. Recommendations could be made only after sufficient trials on the waste waters. Microalgae also differ in their ability to uptake specific nutrients. Thus, a thorough study on the individual species is imperative for the removal of the nutrients from the effluents.

A microalgal consortium could also be put to use to treat highly polluted waste waters. A consortium could be the best-bet. There is another possibility that the genetically manipulated microalgae, which are being used to increase the lipid content [100]. Likewise, it could be used to increase biomass yield and or lipid yield under wastewater nutrient conditions. This new technical approach has the market value to further increase microalgae lipid yield and therefore enhance the biodiesel production. In addition, microalgae grown on organic carbon-rich wastewater is might be an ideal means to enhance the lipid yield.

CONCLUSIONS

Most of the recent technologies adopted for algal cultivation and for the generation of various products are economically feasible. Dual use of microalgal cultivation for the treatment wastewaters combined with useful product generation has therefore been an attractive option in terms of minimizing the production cost, green house gas emissions, nutrient and freshwater supply costs etc. The high biomass production of wastewater-reared microalgae advocates that this cultivation method suggests the real potential as a feasible means for the generation of sustainable and renewable energy. Thus, the aim of this article is to present an overview and discussion on the current trends on the dual aspects of the potential of microalgae for the treatment of wastewaters and their cultivation for the purpose of extracting certain metabolite of commercial value.

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