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Utilization of Microalgae for Integrated Biomass Production and Phycoremediation of Wastewater

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Abstract

Increasing population and Industrialization are key pollutant contributors in water bodies. These wastes are highly hazardous for humansand ecosystem and require a comprehensive, effective treatment before discharge into water bodies. Over the years, many up gradations have been introduced in traditional water treatment methods which were expensive and ineffective especially for removal of heavy metals. Micro alga has been gaining attention due to its mutual benefit in wastewater treatment and for valuable algae biomass production. Waste water especially sewage and industrial effluents are rich in pathogenic organisms, organic, inorganic and heavy metals that adversely affect human and aquatic life. Algae use these inorganic and heavy metals for its growth. In addition it also reduces pathogenic organism and release oxygen to be used by bacteria for decomposition of organic compounds in secondary treatment. In this review, we will discuss potential of microalgae in wastewater treatment, their benefits, strategies and challenges.

Keywords: Microalgae, Wastewater, Biomass production, Phycoremediation, Heavy Metals.

Introduction

Water pollution is one of the most critical environmental problems of today’s world (Abdel-Raouf, 2011).Industrialization and human activities adds huge waste materials into different water resources such as running water, lakes, rivers and sea. These pollutants are primarily originated by the discharge of in adequately treated industrial, agricultural and municipal wastewater (Lim et al; 2010). It contains high levels of inorganic and organic pollutants, heavy metals, pesticides and pathogens. These contaminates have detrimental effect on fauna and flora of aquatic life and also affects human health through food chain (Iqbal and Edyvean 2004; Akar and Tunali 2005; Carey and Migliaccio 2009).Waste water treatment is a foremost step in reducing pollutant, pathogenic organism to maintain water ecologyand to meet the demands of growing population for clean water with minimum environmental issues. Conventional methods have been used for many years toreduce the contaminant concentration and to improve the quality of wastewater effluent before it discharges to groundwater or re-enters water bodies (Rawat et al 2011). However, these methods are expensive, less effective in removing all type of contaminants, require high energy input and expertise help. In addition, conventional method produces large amount of sludge which require further handling and may generate additional pollution. These limitations of conventional treatment plant encourage researchers to explore alternative method for waste water treatment. Over the years, many modifications have been introduced to traditional treatment processes to improve the performance of treatment. However, the majority of these improvements come with some limitations such as high cost and complexity in the operation and maintenance.

Recently microalgae has been gaining interestdue to its dual role of bioremediation of waste water as well as generating algae biomass, which can be used in bioenergy generation, pharmaceuticals, organic fertilizer and animal feed (Batista et al 2015; Cai et al 2013). In addition algal wastewater treatment is more economic and eco-friendly method for effective removal of inorganic compound containing nitrogen and phosphorus, coliform bacteria, heavy metals and the reduction of chemical oxygen demand (COD) and biological oxygen demand (BOD) (Abdel-Raouf et al 2012; Cai et al 2013; Rawat et al 2011). Microalgae are unicellular photosynthetic organism that can assimilate large amount of carbon, nitrogen and phosphorus for their growth and oxygen production. The use of a wide range of microalgae such as *Chlorella, Scenedesmus, Phormidium, Botryococcus, Chlamydomonas and Spirulina* for treating domestic wastewater has been reported and efficacy of this method is promising (Olguın 2003; Chinnasamy 2010; Kong 2010). Microalgae are often preferred forbioremediation process due to its high photosynthetic efficiency, rapid uptake of nutrients,short life span coupled with simple nutritional requirement, so that these can be easily cultured and grown rapidly in bothindustrial and laboratory circumstances (Dwivedi 2012; Travieso 2006). The aim of this paper isto give an in-depth analysis and we will discuss potential of microalgae in waste water treatment, their benefits, strategies and challenges.

Conventional wastewater treatment

Untreated wastewater generally contains high levels of organic material, numerous pathogenic microorganisms, as well as inorganic nutrients and toxic compounds. Unreleased of these waste water in water bodies causes eutrophication and disposal of solid wastes in sanitary landfills is usually associated with soil, surface water and groundwater contamination, thus entails environmental and health hazards. Wastewater treatment is a process, where contaminants are removed from waste water to produce waste stream or solid waste suitable for discharge or reuse. Physical, chemical and biological methods are used to remove contaminants from wastewater. In order to achieve different levels of contaminant removal, individual wastewater treatment procedures are combined into a variety of systems, classified as primary, secondary, and tertiary wastewater treatment (Maier 2000). Conventional waste water treatmentis simple and commonly used method.It consist set of chambers in a series such that influentcan pass from one to other successively to upgrade the quality of water. It follows-Primary treatment, Secondary treatment and Tertiary treatment, shown in figure 1.

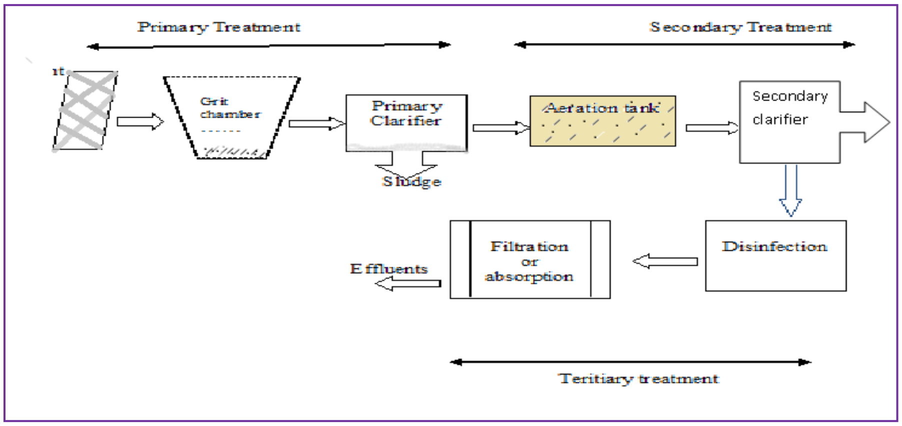

Figure 1- Conventional waste water treatment plant (Shelef&Sukenik 1984)

Primary treatment

The aim of primary treatment is to remove suspended and floatable material from wastewaterto produce homogenous suspension (Spellman 2000).Large, coarse, heavy inorganic and organic solids get removed passing through the screen and grit chamber (Kawamura, 2000).The screen chamber consists of bar screen of different shape and sizes that filter out different type of large objects from water. Effluent of screen chamber enter grit chamber to settle and remove sand, grit and stones to the bottom of the channel which is then allowedto pass slowly through alarge tank called primary sedimentation tanks or primary clarifiers (Qasim, 1998). In this large tank sludge settles in the bottom and floating material such as grease, oils rise to the surface and skims off. Primary treatment removes 50-65% of suspended solids and reduces 30–40 % of biochemical oxygen demand (BOD).

Secondary treatment

Primary effluents from primary sedimentation tank moved for secondary treatment. The aim of secondary treatment is to remove biodegradable organics matters and suspended solids through biological decomposition. Bacteria decompose the fine organic matter to produce clear effluents. They require supply of oxygen, which adds further cost to the treatment process. After biological treatment water is pumped to secondary sedimentation tank where secondary clarifiers operate in similar manner as in primary treatment.In secondary clarifier's leftover solids and the microorganism sink to the bottom and removed separately. The effluent from the secondary biological treatment usually contains a little BOD (5 to 10% of the original).

Tertiary treatment

Tertiary treatment is an advance treatment that involves the removal of inorganic nutrients (nitrogen, phosphorus), fine suspended particles, refractory organics, heavy metals and disease-causing pathogenic micro-organisms (Prabu, et al 2011). Tertiary treatment is generally carried out for collecting water from water bodies for re-use in industrial, agricultural and human personnel uses. In conventional method, tertiary treatment accomplished via chemical process involves chemical coagulation, flocculation, exchange, reverse osmosis and sedimentation followed by filtration and chlorination (Topare al 2011).

Microalgae wastewater treatment systems

The ultimate goal of wastewater management is the protection of the environment in a manner commensurate with public health and socio-economic concerns. For the effective treatment of wastewater, controlled growth of algae is in practice globally. Microalgae based treatment system is as effective as conventional treatment system with several advantages shown in table 1. It involves less sludge formation, requires low energy and reduces the release of green house gasses (CO_2) from treatment plant. In addition it is considered more economical as in remove wastes at low cost and produces valuable biomass which can be used as fertilizer, as animal feed and for bio energy production (Mata et al;2010., Rawat et al; 2011). The microalgae system can treat various types of wastewater like, domestic, sewage and industrial and reduce the excess nutrients (Nitrogen, phosphate), heavy metals and pathogenic organisms.

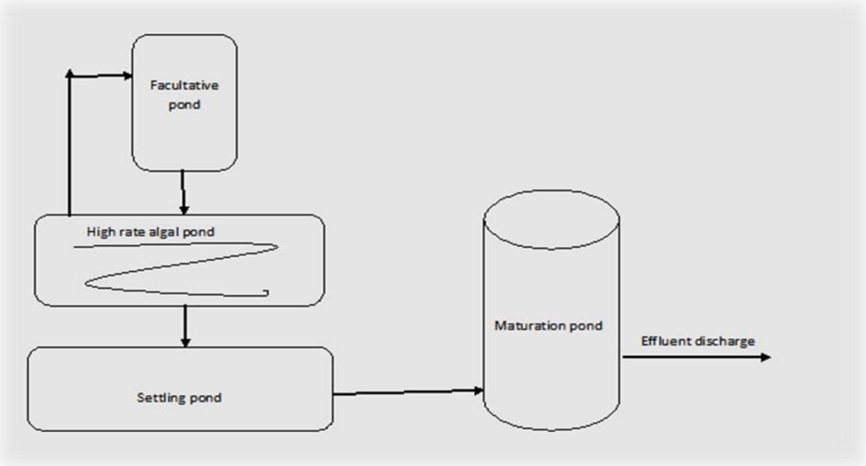

Figure 2: Advance integrated algal waste water treatment (www.thepoultrysite.com)

Table 1: Advantages of algae based waste water treatment over conventional method

Algal based wastewater treatment	Conventional Method
Low operating cost	Expensive method
Very low biological and chemical sludge formed	Large amount of toxic sludge is formed that may cause other types of pollution
Possible to recover valuable metals	Not possible to recover metals
Microbial biomasses have various industrial applications.	Could not be used for any other purposes
More efficient method especially to remove heavy metals.	Less efficient to remove heavy metals
Reduces the release of green house gases	Releases green house gases like CO_2

Oswald (1990) proposed Advanced Integrated Waste Water System to achieve comprehensive waste water treatment and to minimize the drawback of conventional treatment (Figure 2). In comparison to conventional ponds it is more economical, ecofriendly, less sludge formation coupled with resource recovering and recycling (Graham et al 2009; Bayramoglu 2006). Depending on the objective to be achieved it may serve in primary treatment, secondary treatment, in tertiary treatment. It may be used solely or in combination of conventional treatment. These ponds consist of at least four basic ponds including a facultative pond, a high rate algal pond, an algae settling pond, and finally a maturation pond.

Facultative stabilization Pond

It is a pond of relative depth nearly1-3m, in which different kinds of organisms work together to uptake pollutants. Raw sewage enter to this pond in low velocities facilitate settling of solids at the bottom, where anaerobic bacteria act on solid sewage and release biogas like CO_2. Aerobic bacteria function near the surface, breakdown the pollutants into simple compounds then assimilate them and use them as nutrients source for their cell growth. Main characteristic of this pond arelong detention time and no mixing required. Stabilization ponds are widely used because they are simple in design, operation and economic. However, stabilization ponds have the disadvantage of having high levels of total suspended solids in the effluent (Bich 1999; Craggs et al, 2014).A properly designed facultative pond could remove almost 100 % suspended solids and 60–70 % of the biochemical oxygen demand (BOD) from the influent wastewater (Davies-Colley, 2005). Effluent from this pond requires further treatments and microalgae.

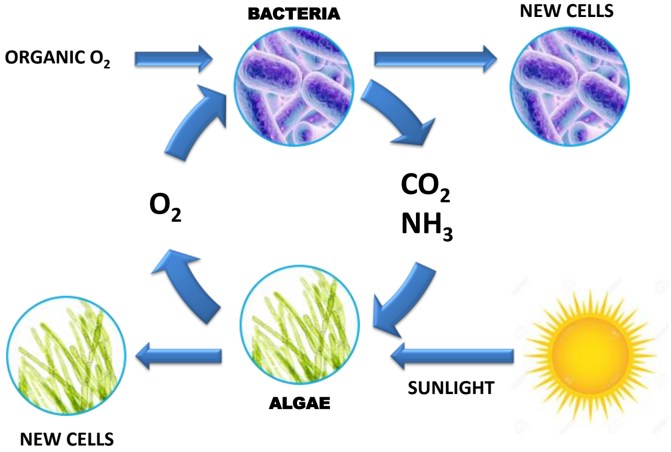

Figure 3: Symbiotic relationship of microalgae and bacteria in wastewater treatment (www.thepoultrysite.com)

High rate algae ponds (HRAPs)

It is a second pond of this system characterized by shallower depth of 30-50cm, shorter retention time. In this pond, heterotrophic bacteria and photosynthetic algae work together in the pond to purify wastewater. Shallow ponds provide better light for photosynthesis of algae and thereby maximize their growth (Davies-Colley, 2005). During photosynthesis algae release oxygen which is utilized by aerobic bacteria for oxidation of organic compounds. Oxygen released by algal photosynthesis is utilized by aerobic bacteria to remove theremaining BOD in the wastewater (Figure 3). In addition, algal photosynthesis increases pH above 9–10, helps in killing most of pathogenic bacteria. Advantage of HRAPs over facultative pond is that it helps to remove odor and pathogenic organism efficiently. In addition high productivity of algae is achieved in these ponds with sufficient removal of nutrients at a shorter retention time. The release of free oxygen is of major significance in phycoremediation of wastewater as using microalgae in the treatment process decreases the need for the mechanical aeration in secondary treatment (Munoz and Guieysse 2006; Guieysse et al 2011). Mechanical aeration is one of the costly processes in wastewater treatment. It can account for 50% of the total treatment cost (Metcalf et al 2003). Thus, using microalgae in the treatment process can provide an environment friendly aeration method. Also using microalgae in wastewater treatment can significantly contribute in reducing CO_2 emissions (green house gas) from wastewater treatment plants.

Algae Settling Pond

The effluent from HRAP entered to settling ponds. In this pond, algae are allowed to settle in the bottom of pond, thereby clarified effluent is achieved. Settled algae are rich in nutrients and can be utilized for further applications (Ramadan & Ponce, 2003). The effluents from algal settling ponds are low in BOD and nutrients. Some time advanced harvesting processes might be necessary to achieve complete algae removal from the effluent. If AIWPS's are designed properly to enhance disinfection, chlorination should not be necessary (Ramadan and Ponce, 2003).

Maturation Pond

It is the last pond of this system where effluents from the settling pond are stored for additional 10–15 days in maturation pond (Green et al 1995). The aim of this pond is to achieve enhanced levels of disinfection. In addition, it could also be utilized as storage pond for irrigation applications and a habitat for aquatic life.

Phycoremediation

The term phycoremediation was introduced by John (2000) to refer the use of macro-algae or micro-algae for the removal or biotransformation of pollutants, including nutrients and xenobiotics from wastewater and CO_2 from waste air (Moreno-Garrido 2008; Mulbry et al 2008; Olguın 2003). Extensive work has been conducted to explore the feasibility of using microalgae for wastewater treatment, especially for the removal of nitrogen and phosphorus, several heavy metals from effluents (Mallick 2002; Aslan 2006; Hameed 2007; Hernandez et al 2007; Lebeau and Robert 2003). Number of researchers investigated microalgae utilizes low quality water effluents such as municipal/industrial/ agricultural as feed for growth of their biomass (Grönlund & Fröling 2014; Carlsson et al 2007).

Thereby cultivation of microalgae in wastewater meets both the objective of nutrient removal and production of algal biomass which has many industrial applications (Han et al 2007; Rawat et al, 2011).

Phycoremediation of Inorganic Nutrients (N,P)

The capability of microalgae to degrade hazardous organic pollutants is well known. Table 2 illustrated many microalgae species have been already successfully used for the removal of inorganic nutrients from different source of waste water such asdomestic, municipal and industrial wastewaters.

Table 2: Microalgae in removing nutrients from different waste water sources

Waste water	Microalgae strain	P	N	References
Domestic wastewater	Mixed algae growth	99%	90%	Metcalf et al 2003
Domestic wastewater	*Botryococcusbraunii*	100%	79%	Nurdogan & Oswald 1996
Wastewater from textile industry	*Chlorella vulgaris*	33%	45%	Sydney et al 2011
Synthetic wastewater	*Chlorella vulgaris*	96%	97%	Peng et al 2010
Primary wastewater	*Haematococcus pluvialis*	100%	100%	Kang et al 2006
Soybean processing wastewater	*Chlorella pyrenoidosa*	70%	89%	Kang et al 2006
Primary and secondary wastewater	*Desmodesmus communis*	99%	99%	Hongyang et al2011
Urban waste water	*Chlorella*sp.and*Chlamydomonas* sp.	100%	84%	Samorì et al2014

Microalgae is an unicellular photosynthetic organism, utilizes inorganic nutrients such as N, P and CO_2 from the environment to synthesize organic compound for the biomass growth and produce O_2 as a byproduct according to the following overall stoichiometric formula for photosynthesis:

$6\ H_2O + 6\ CO_2 + light \longrightarrow C_6\ H_{12}O_6 + 6\ O_2$

Secondary effluents are rich inorganic nutrients (nitrogen, phosphorus) that can result to eutrophication and formation of dead zones in water bodies. Microalgae assimilate different forms of inorganic nitrogen (such as nitrites, nitrates, ammonia) from waste water and convert them into organic nitrogen sources required for protein, DNA,RNA and cell synthesis (Conley 2009). Microalgae prefer ammonia and after depletion of ammonia it assimilates nitrate than nitrite, depending on their availability in wastewater. All forms of inorganic nitrogen species are reduced to ammonium inside the cell by their enzymatic systemas ammonium easily gets converted into glutamine without any redox reaction and thus utilizes less cellular energy (Flynn et al 1997). Phosphorus (P) is the second essential nutrient for microalgae, which isutilizedfor the synthesis of various compounds such as ADP, ATP, nucleic acids, phospholipids and protein (Conley et al 2009). Different forms of phosphorus mainly orthophosphate are accumulated in wastewater due to different human activities particularly through phosphorus containing fertilizers. Microalgae have a ability to absorb excess phosphorus and stores it as granules of polyphosphate in the cell for future utilization.

Phycoremediation of pathogenic organisms

Wastewater contains a wide range of pathogens such as viruses, bacteria, parasite and protozoa which can cause various diseases such as cholera, gastroenteritis, typhoid fever, dysentery, and hepatitis (Rasoul-Amini 2014). Several studies have showed that microalgae have high efficiency in removing pathogens and increase the mortality of coliform bacteria through direct and indirect means (García and Bécares 1997).Several mechanisms have been proposed to explain the disinfection ability of microalgae which includes: increase in pH of waste water,making unfavorable condition for pathogen growth, secretion of antibacterial substances,production of toxic extracellular compounds by certain species of algae, depletion of nutrients and organic matter in wastewater, essential for the growth of pathogens.

Phycoremediation of Heavy Metal

Heavy metals are toxic in nature and causes serious threat to the human being and flora and fauna of receiving water bodies (Nageswara and Prabhakara, 2011).Once the metal releases into the water bodies, they persistthere and get accumulated in biological system over the time (Zahira, 2005). They are carcinogenic in nature and have detrimental effect on aquatic organisms and ultimately human being, as they get concentrated through food chain. Most of the heavy metal salts are soluble in water, form aqueous solutions (Zahira et al 2005). Therefore difficult to separate by ordinary physical means of separation. Conventional methods used for the removal of heavy metal ions include chemical precipitation, floatation, adsorption, ion exchange, membrane filtration, chemical oxidation/reduction and electrochemical processes. However, these techniques have some limitations, such as expensive, ineffective removal of metal ions, high energy demands, and the generation of toxic sludge which requires additional elimination stages (Ahalya et al. 2003, Manikandan et al 2011).Now there is need for economical, effective and safe methods for removing heavy metals from waste waters. In this endeavor,

Microalgae emerged as an alternative technique over traditional methods (Aksu 2002). It is one of the most prominent eco-friendly method for removal of heavy metals from industrial effluents like mining, metals plating, paint industry. Metal removal capacities of microalgae have been recognized in previous studies as highlighted in Table 3. Both live and dead cells can be successfully used for the removal of heavy metals from waste water. Metals removal capacity of microalgae varies from species to species depending on its cell wall composition. The presence of various negatively charged functional groups such as carboxyl, carbonyl, amino, amido, hydroxyl, sulfhydryl, sulphonate and phosphorus, etc. on algal cell wall exhibits chemical affinity to positively charged metal ions and facilitate surface binding via chelation, adsorption, coordination, ion exchange and microprecipitation etc(Kajan 1992).

Table 3: Biosorption of heavy metals by microalgae

Algal species	Targeted Metals	References
Chlorella vulgaris	Copper, nickel	Fourest&Volesky 1997
Chlorella vulgaris; Scenedesmusacutus	Cadmium, chromium, zinc	Mehta et al 2001
Stichococcusbacillaris	Lead	Travieso et al 1999
Spirogyra hyaline	Cadmium, Mercury, Lead, Arsenic and Cobalt	Mahan et al 1992

Cladophorafracta	97-99% removal of copper, cadmium, mercury	Kumar &Oammen 2012
Chlamydomonusreinhartii; Selenestrumcapricornutum	Chromium, copper, silver	Deng et al2007
Spirogyra hyline	Cadmium, lead, zinc, copper, nickel	Elmahadi& Greenway, 1991
Spirogyra condensates and Rchizocloniumhieroglyphicum	Chromium	Gupta &Rastogi, 1992
Chlorella emersonii	Mercury	Onyancha et al2008
Chlorella sorokiniana	Cadmium , Chromium, lead, nickel	Wilkinson et al 1990
Chlamydomonasreinhardtii	Cadmium, lead, mercury	Akhtar et al 2003

Challenges and strategies

Previous extensive work has been strongly proven that microalgae are economic, efficient and sustainable way to treat wastewater. It has a considerable potential to remove significant amount of inorganic nutrients, heavy metal, pathogenic organism, chemical toxins and sequestrate atmospheric Co_2. However, there is need to improve the technology for large scalewastewater treatment such as nitrogen uptake could be increased if the microalgaewere preconditioned by starvation , concentrated algal cultures were used to decrease the land and space requirements. Primary strategies of microalgal waste water treatment is the selection of ideal algal strain and improvement algal strain improvement by various methodologies such as lipidomics, genomics, proteomics and metabolomics to maximize the use of waste water for higher biomass productivity. Furthermore, it is recommended that a biorefinery based production strategy is the best solution to combine and integrate various processes to maximize economic and environmental benefits, while minimizing waste and pollution (Briens et al 2008; Singh 2010). In biorefinery approach all the components of the biomass raw material are used to produce useful products. Despite, the salient drawbacks in utilization microbial biomass is its harvesting. Harvesting is one of the most challenging processes in integrated microalgae wastewater treatment because of its small size, high dilution rate, and electronegative cell surface charge.Harvesting is a process of separating the algae from water, accounting for 30% of total microalgae biomass production cost (Wiley et al, 2009).Many processes practiced for harvesting microalgae includes sand filtration, high-speed centrifugation, electrocoagulation and flocculation (Akhtar et al 2004; Mallick 2002). Although they have limitation of high cost, long processing time, high energy need and low recovery. A biological method bioflocculation which is an innovative dewatering method has been proposed. These processes haveseveral advantages over their free-cell counterparts including high biosorption capacity, occupy less space, are easier to handle, and can be used repeatedly for product generation(Akhtar et al 2004; Mallick 2002).In addition, immobilized cells are resistant to harsh environments such as pH, salinity and metal toxicity; recoveringthe cells in a less-destructive way (Hall-Stoodley et al2004; Liu et al 2009).However, any single method has not been proven best for harvesting microalgae..

Conclusion

Algal based wastewater treatment is a viable alternate technology for treating wastewater in an economical and sustainable way over conventional treatment processes. Using microalgae in wastewater treatment can greatly improve the treatment process through reducing the pollutant concentrations, pathogenic organism,biological oxygen demand(BOD), CO_2, emission, and reducing aeration requirement. Furthermore, microalgae have the potential touse wastewaterfor the growth of its biomass having numerous benefits such as animal feed, fertilizer, production of biofuels and nutraceuticals. Integration of wastewater treatment with generation of microalgal biomass reduces the production cost. Therefore, further investigationsare needed forlarge scale cultivation of microalgae in wastewater, assessment ofenvironmental and safety risk and to explore the use of these microalgae in valuable products.Harvesting is a major hurdle to integrated microalgal wastewater treatment since it is the most costly process in microalgae treatment. Many harvesting methods have been practiced; however, further research is required to develop more economical harvesting methods.With the advantages of low cost raw material and no secondary pollution, algae could be promising for purification of waste water.

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