



Microalgae Cultivation in Wastewater for Nutrient Removal

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Abstract

Wastewater is the spent or used water containing dissolved or suspended solids such as pollutants and microbes, including nutrients. The wastewater discharged into the water bodies are hazardous to environment and cause various health problems in human beings. Eutrophication is one such major environmental problem caused due to the discharge of nutrient rich wastewater into the nearby water bodies. Several methods, commonly chemical treatment and conventional biological methods are used to remove nutrients (phosphorus and nitrogen) from wastewater. However, high cost and more sludge production, are the major disadvantages limiting its use and hence researchers are now focusing on microalgae for nutrient removal from wastewater as it is less expensive and results in less sludge production. This paper provides a short review on the current scenario in the cultivation of microalgae in wastewater for nutrient removal. The resulting biomass from the treatment process can be used for the biodiesel production or for bioactive compound extraction.

Key words: Wastewater, microalgae, nitrogen, phosphorous, immobilization.

Introduction

Wastewater is a general term used to represent the water with poor quality that contains more amounts of pollutants and microbes. If wastewater is discharged into the nearby water bodies, it can cause serious environmental and health problems to human beings. Wastewater treatment is an important measure to reduce the pollutant and other contaminants present in wastewater. The first step in wastewater treatment method is primary treatment which removes the solids, oil, and grease from wastewater. Secondary treatment or biological treatment is the second step, which exploits microorganisms to eliminate the chemicals present in wastewater. Final step is the tertiary treatment; which eliminates the microbes from wastewater before discharging into the river (Rawat *et al.*, 2010).

Effluent produced from the secondary treatment plant contains more amounts of nutrients (nitrogen and phosphorus) and if these effluents are discharged into water bodies; it causes eutrophication and affects the ecosystem. To remove these nutrients, several processes are used, but the disadvantages of this type of treatment are high cost and increased sludge production (Yuan *et al.*, 2011). As an alternative to the conventional treatment methods, microalgae are suggested to remove the nutrients from wastewater (Mallick, 2002). The use of microalgae or macroalgae (seaweeds) to remove pollutants and nutrients from the wastewater is called phycoremediation. Microalgae wastewater treatment is eco friendly and offers the advantage of a cost effective way of nutrient removal and biomass production (Mulbry *et al.*, 2008). The microalgae grown in wastewater can be used as energy source, fertilizer, fine chemicals production and as feed to animals (Vilchez *et al.*, 1997; Mulbry *et al.*, 2006). The aim of this paper is to summarize the different types of strategies

currently examined to cultivate the microalgae in wastewater for nutrient removal.

Processing of wastewater

Wastewater contains different types of materials like soluble organic, inorganic, insoluble inorganic materials, macro solids, toxins, etc. By processing, it is ensured that only the soluble fraction of the waste like carbon, nitrogen and phosphate are used for the microalgae cultivation. Besides pollutants, bacteria and protozoa are also present in wastewater. If this wastewater is used as a culture medium, microbes present in it will compete for microalgae growth and nutrition. Hence ultra violet light source, filtration, autoclaving and centrifugation are the different types of pre-treatment method used to remove suspended solids and algae feeding organism like protozoa and bacteria. Filtration of secondary effluent using 0.20 μm pore filters gave the highest biomass growth (*Chlorella* sp.) with the total nitrogen removal of 92% and total phosphorus removal of 86%. In case of UV radiation (pre-treatment method), UV- B at the dose of 1620 mJ cm^{-2} gave the maximum biomass growth (*Chlorella* sp.) when compared to 270 mJ cm^{-2} of UV- B dose (Cho *et al.*, 2011). The *Chlorella* sp. cultivated in the autoclaved centrate showed higher growth rate than the raw centrate (Li *et al.*, 2011). After sterilizing the wastewater, phosphorus level in it gets reduced and it might have effect on the algal growth. This nutrient level is increased by adding the chemicals likes KH_2PO_4 , K_2HPO_4 and Na_2HPO_4 . In some pre-treatment process, centrifugation alone is used to process the wastewater e.g. piggery wastewater, but in case of wastewater from olive oil mill, both filtration and centrifugation are used to condition wastewater (Hodaifa *et al.*, 2010). The debris present in the wastewater is removed by

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filtration method using cotton gauze and diatomaceous earth filters (Chinnasamy *et al.*, 2010).

Microalgae cultivation in artificial wastewater for nutrient removal

Artificial wastewater is defined as a synthetic medium, which contains an inorganic nutrient composition similar to real wastewater, however it lacks toxic components, microbes, etc., which are originally present in real wastewater. Artificial wastewater is used to analyse the nutrient removal ability of the microalgae, acclimatization of the cells prior to cultivating in real secondary effluent and to identify the growth inhibiting concentration of certain types of nutrients. One major drawback of using the artificial wastewater is that the result obtained in it could not be reproduced in real secondary effluent (Pittman *et al.*, 2011).

Some species of microalgae are used in the biological treatment of the wastewater e.g., *Chlorella* sp. *Chlorella sorokiniana* (*C. sorokiniana*) and *Chlorella vulgaris* (*C. vulgaris*) efficiently remove nitrogen than phosphorous from effluent. Hernandez *et al.*, 2006 carried out extensive research to analyze the effect of starvation and co-immobilization of *C. sorokiniana*, *C. vulgaris* with microalgae growth-promoting bacterium (MGPB) *Azospirillum brasilense* for nutrient removal capability. The synthetic wastewater used mimicked the chemical composition similar to real domestic wastewater collected from the city of Mexico. Both the *C. sorokiniana* and *C. vulgaris* microalgae cultures were co-immobilized with bacteria and starved for 3 and 5 days by cultivating in sterile saline with continuous light. This study concluded that the starvation for 3 days does not affect the growth of *C. vulgaris* and *C. sorokiniana*, whereas starvation for 5 days negatively affects the growth of *C. vulgaris*. In case of nutrient removal efficiency, both the cultures showed more phosphorus removal capability when co-immobilized with *Azospirillum brasilense* and starvation of these cultures further increased the phosphorus removal efficiency of *C. sorokiniana*.

Several studies examined the effect of semi-continuous culture method on the nutrient removal ability of the *Scenedesmus* sp. The artificial wastewater used in this study to culture *Scenedesmus* sp. had the nutrient content composition similar to that of secondary wastewater treatment effluent of Ensenada, Mexico. The *Scenedesmus* sp. was cultured in artificial wastewater with a daily replacement of 30% and 50% of the culture with fresh medium (semicontinuous culture). The result of this study showed that the *Scenedesmus* sp. had

higher biomass concentration in 30% dilution and removed more than 50% of the phosphorus in both the dilutions. In case of nitrogen removal, 50% dilutions showed 66% of removal, whereas 30% diluted culture showed 50% of removal (Votolina *et al.*, 1998).

In another study, the same artificial medium was used to analyze the amount of nitrogen incorporated into *Scenedesmus obliquus* (*S. obliquus*) biomass and also the loss of nitrogen in the form of ammonium. *S. obliquus* was cultivated in an artificial medium with the daily replacement of 50% and 70% of the cell suspension with the fresh medium. The result of this study concluded that only lesser amount of nitrogen in the form of nitrate were utilized from the medium by *S. obliquus* and the remaining form of nitrogen is lost by ammonia stripping (Nunez *et al.*, 2001).

Microalgae cultivation in real wastewater for nutrient removal

Wastewater contains large amount of materials like toxins, microbes, etc. Processing is the main step to remove these unwanted materials from wastewater. The type of processing in turn depends on the characteristics of wastewater. For nutrient removal from the wastewater, single microalgae strain or consortium of microalgae along with microalgae growth-promoting bacteria are used.

Several studies were performed to analyze the capability of microalgae consortium along with symbiotic bacteria for nutrient removal capacity from wastewater. One such study was the analysis of euglenophyt, cyanobacterium, green microalgae and two types of indigenous microalgae consortium along with symbiotic bacteria for the nutrient removal capability from diluted (1:4 & 1:8) piggery wastewater. The result of this study showed that the unialgal cultures like *Euglena viridis* (*E. viridis*), *Chlorella sorokiniana* (*C. sorokiniana*) were able to grow in both types of diluted wastewater, while *Scenedesmus obliquus* (*S. obliquus*) and consortium 2 were able to grow in eight times (1:8) diluted wastewater, whereas consortium1 and *Spirulina platensis* showed no growth. In case of phosphorus and nitrogen removal capacity, *E. viridis* and *C. sorokiniana* showed more nitrogen removal in both the dilutions; on the other hand, *C. sorokiniana*, *S. obliquus* and *E. viridis* showed phosphorus (20 - 65%) removal in eight times diluted wastewater (de-Godos *et al.*, 2010).

Table 1. Microalgal cultures examined for piggery wastewater treatment

S. No	Types of Cultures	Microalgae
1	Consortium 1	<i>Scenedesmus</i> <i>Chlamydomonas</i> <i>Microspora</i> <i>Oocystis</i> <i>Chlorella</i> <i>Nitzschia</i>
2	Consortium 2	<i>Chlorella</i> strains
3	Green microalgae	<i>Chlorella sorokiniana</i> <i>Scenedesmus obliquus</i>
4	Cyanobacterium	<i>Spirulina platensis</i>
5	Euglenophyt	<i>Euglena viridis</i>

In a similar study, native microalgae consortium (lacking symbiotic bacteria); freshwater and marine microalgae were analyzed for the biomass production and nutrient removal capability from the treated carpet mill effluent. Totally, fifteen microalgae were isolated from the wastewater using BG11 medium. Pure cultures of the isolate were mixed in equal proportion to form the native microalgae consortia. In addition to the native algal consortia, two fresh water microalgae (*Botryococcus braunii*, *Chlorella saccharophila* var. *saccharophila*) and marine algae (*Dunaliella tertiolecta* and *Pleurochrysis carterae*) were selected for this study. The result of this study, indicated that the native algal consortia showed better biomass production in both treated and untreated wastewater than the unialgal cultures and the nutrient removal capability of native microalgae consortia showed more phosphate (98.8–99.1%) and nitrate removal(99.7– 99.8%) in 72 hours (Chinnasamy *et al.*, 2010).

Immobilized microalgae for nutrient removal from wastewater

In phycoremediation, the main problem identified is the harvesting of microalgae after treating the wastewater (James, 1998; Mallick, 2002; Aslan and Kapdan, 2006). Immobilization of microalgae is one of the solutions for harvesting problem (Vilchez *et al.*, 2001; Jimnez-Perez *et al.*, 2004). Carrageenan, chitosan and alginate are commonly used polymers for the microalgae immobilization. Immobilized microalgae beads are easy to harvest and reuse. There are several factors affecting the nutrient removal efficiency of immobilized microalgae

1. Number of microalgae immobilized beads inoculated to

treat wastewater

2. Gel permeability for the nutrients present in the wastewater.
3. Cell density present in each bead.
4. Environmental factors.
5. Chemical components present in wastewater.
6. Type of gel matrix used for immobilization.

Some studies were done to identify the key factor affecting the immobilized microbe's nutrient removal efficiency. In one study, the effect of two key factors such as gel thickness and cell concentration in the gel were analyzed for the nutrient removal efficiency. *Scenedesmus* sp. used in this study was isolated from the municipal wastewater and immobilized in calcium alginate screen with two different gel thicknesses (2mm & 3mm). This study concluded that the cell density within the gel was an important factor affecting the orthophosphate and ammonia removal than gel thickness. In the same study, effect of starvation on nutrient removal efficiency of the immobilized *Scenedesmus* sp. was also evaluated. *Scenedesmus* sp. was starved for 48 hours in a photo bioreactor at 100% relative humidity and used to treat both the artificial and real secondary effluent. The result of this study concluded that the cell concentrations in the gel and microalgae starvation were considered as important factors to enhance the ammonia and orthophosphate removal from the effluent. *Scenedesmus* sp. showed more nutrient removal in real secondary effluent than in artificial wastewater (Zhang *et al.*, 2008).

Effect of immobilization on the growth and nutrient removal of *Scenedesmus obliquus* (*S. obliquus*) and *Chlorella vulgaris* (*C. vulgaris*) in artificial and urban wastewater was analyzed.

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Both the cultures were immobilized in alginate beads. Immobilized cultures showed immediate growth when inoculated into the medium than the free cell, which showed lag period. *C. vulgaris* had higher cell densities within the beads than *S. obliquus*. In case of nitrogen uptake, immobilized microalgae had higher nitrogen uptake than the free cell in both the types of wastewater but in phosphorus removal efficiency, immobilized cultures removed more phosphorus in artificial wastewater than in urban wastewater (Ruiz-Marin *et al.*, 2010).

Conclusions

Based on this review, it was concluded that the microalgae alone cannot efficiently remove the nutrients from the wastewater. The microalgae growth - promoting bacteria (MGPB), starvation and dilution of the wastewater are the different ways used to enhance microalgae nutrient removal rate. Microalgae cultivated in the wastewater can be used for the biodiesel production and as feed for animals. This dual process (microalgae cultivation in effluent coupled with biodiesel production) has several advantages such as less cost and less energy input for biodiesel production, and less greenhouse gas emission during biodiesel production.

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