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# Microalgae Biomass Production in High Nutrient Wastewater: A Review

*Pengeluaran Biojisim Mikroalga dalam Air Kumbahan Bernutrien Tinggi: Satu Tinjauan*

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## Abstract

The ability of microalgae in phycoremediation of wastewater and biomass production for animal food, fertilizer, and source of renewable energy has sparked the interest to investigate the microalgae nitrate assimilation and biomass production in high nutrient wastewater, especially in nitrified landfill leachate (NLL). This paper reviews the dual role of autotrophic microalgae in phycoremediation of high nutrient wastewater and as a source of feedstock for energy generation. The use of microalgae for nutrient removal, factors affecting the growth and analysis of the biomass for potential bioenergy substrate are discussed.

**Keywords** microalgae, wastewater, biomass production, bioenergy, renewable energy, waste management, landfill leachate

## Abstrak

Keupayaan mikroalga dalam fikoremediasi air kumbahan dan pengeluaran biojisim untuk makanan haiwan, baja, dan sumber tenaga boleh diperbaharui telah mencetuskan minat untuk menyiasat asimilasi nitrat oleh mikroalga dan pengeluaran biojisim dalam air kumbahan bernutrien tinggi, terutamanya dalam *leachate* tapak pelupusan bernitrat (NLL). Kertas ini mengkaji dwi peranan mikroalga autotrofik dalam fikoremediasi air kumbahan bernutrien tinggi dan sebagai sumber bahan mentah untuk penjanaan tenaga. Penggunaan mikroalga dalam penyingkiran nutrien, faktor-faktor yang mempengaruhi pertumbuhan dan analisis biojisim yang berpotensi untuk substrat biotenaga dibincangkan.

**Kata kunci** mikroalga, air kumbahan, pengeluaran biojisim, biotenaga, tenaga boleh diperbaharui, pengurusan sisa, cecair larut resap

## Background

Landfilling is the most applicable method in waste management due to its availability, low maintenance, and offer dumpling high quantities of waste at economical costs in comparison to other disposal methods such as incineration. However, the production of leachate and odors are the main disadvantages of this disposal method (Foul, Isa & Hung, 2009). Leachate represents the water which passes through the waste from precipitation and water generated from the waste within the landfill site, thus forming liquid that contains suspended solids, soluble components of the waste and products from the degradation of the waste by various microorganisms (Williams, 2005). Freshly produced landfill leachates are characterized by low pH, high Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) (Idris, Saed & Hung, 2004). The most common toxic components in leachate are ammonia and heavy metals, which can be hazardous even at low levels, if they accumulate in the food chain. Treatment of landfill leachates presents unique problems from engineering point of view mainly because of high COD (600-15 000 mg/L) and ammonium ion (500-3000 mg/L) contents.

In Malaysia currently, the main waste disposal method is landfilling. The latest assessment on landfill sites carried on January, 2011 revealed that there are 296 landfills throughout Malaysia; 166 are still in operation and 130 are closed landfill. However, only eight of the existing landfills are Level 4 sanitary landfills (Ministry of Housing and Local Government [MHLG], 2011). The sanitary landfill is constructed to minimize the pollution caused by the waste. Sanitary landfill is a fully engineered disposal option in that the selected wasteland is carefully engineered in advance before it is pressed into service (Ramachandra, 2006). Operators of sanitary landfills can minimize the effects of leachate through proper selection of site and leachate treatment method.

Various leachate treatment methods exist nowadays are with different efficiency, availability, and cost. Leachate treatment can be classed into three categories including biological, chemical, and physicochemical. Composition of landfill leachate present variations depending on the type of material deposited into the landfill, landfill condition such as pH, temperature, moisture, age, climate and the characteristic of the precipitation entering the landfill. The composition of leachate should be characterized before a suitable treatment strategy is developed (Kargi & Pamukoglu, 2002). Therefore, ongoing research to search for the best and low cost and maintenance method is required before ones can choose any treatment technology suitable for the different composition of landfill leachate.

Leachate produced in Malaysia contains high $N\text{-}NH_4^+$ (Yusof *et al.,* 2010). Biological leachate treatment method by means of nitrification activated sludge system (NASS) showed an excellent way in treating leachate. However, there are growing interest to involve microalgae in the treatment system with the aim of producing useful biomass. NASS had successfully treated high ammonium concentration leachate (1450 mg/L) (Yusof *et al.,* 2010). Through nitrification, the ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) convert ammonium to nitrate. Removal of ammonia is principally important in the tertiary treatment of landfill leachate to comply with the stringent discharge limit imposed (10 mg/L for Standard A) to mitigate the toxic effect of ammonia to the aquatic life (Yusof *et al.,* 2010) and there is no discharge limit imposed for $NO_3^-$ (Environmental Quality (Control Of Pollution From Solid Waste Transfer Station and Landfill) Regulations 2009, 2009). However, excessive nitrate in nitrified leachate should be eliminated prior discharge, since $NO_3^-$ if released into environment can create serious problems, such as eutrophication of rivers and deterioration of water quality. It also causes potential hazard to human health, because nitrate in the gastrointestinal tract can be reduced to nitrite ions. In addition, nitrate and nitrite do have the potential to form N-nitrous compounds, which are potent carcinogens (Foglar *et al.,* 2004). Typical toxic responses to nitrate exposure are methaemoglobinaemia, abortion and still-born babies.

The high nitrogen level in wastewater has become a growing concern, which has increased the necessity to develop simple, efficient, and cost effective nitrogen removal techniques. High nitrate wastes (> 1000 ppm $NO_3^-$) are usually generated by fertilizer, metal finishing, cooking, organic chemical industry and nuclear industry (Nair, Dhamole

& DSouza, 2010) where treatment has become a challenge for these industries. The presence of $NO_3^-$ should be seen as nutrient resources that should be manipulated and used to produce useful product. One of the interesting means seen is to use microalgae to assimilate nitrate in high nitrate wastewater especially nitrified landfill leachate. This approach not only treated the wastewater, but the utilization of $NO_3^-$ as nutrient sources would produce biomass which can be used in various applications. Microalgae have been used for century for pharmaceutical, oil, biogas, and fertilizers. However, not all strains of microalgae can grow well in high nitrate concentration and producing notable biomass.

The presence of nitrate in the treated landfill leachate by means of NASS has sparked the interest to use the nitrified landfill leachate as the growth media for microalgae. The microalgae not only further treated the leachate by reducing the nitrate concentration, but at the same time producing biomass that has various applications. To date, many microalgae strains has been collected, preserved and stored. However not all strains are suitable for this purpose since the presence of high concentration of $NO_3^-$ in the wastewater and others compounds that may cause toxicity. Local strains would probably be the best candidates since they are more likely adapted to the local temperature, weather, light regime, and more importantly the environment of high nitrate and other pollutions. This paper reviews the dual role of autotrophic microalgae in phycoremediation of high nutrient wastewater and as a source of feedstock for energy generation. The use microalgae for nutrient removal, factors affecting the growth and analysis of the biomass of for potential bioenergy substrate are discussed.

## Potential of Microalgae System

The rapid development and industrialization coupled with an increased awareness on the need for a clean environment have forced industrialists, environmentalists and governments to look for cheap, efficient and long lasting solutions to waste water treatment and recycling of nitrogen and phosphorus. In recent years, the importance of biological waste treatment systems has attracted the attention of workers all over the world and has helped in developing low cost waste treatment systems. There are reports in which intensive algal culture have been used for the removal of nutrients from wastewaters (Singh & Dhar, 2007). The use of microalgae in wastewater treatment could prove beneficial in different ways since they also bring about oxygenation and mineralization (Thornton *et al.,* 2010) in addition to being a food source of shellfish or other aquatic species (Woertz, Feffer & Nelson, 2009).

The usage of microalgae to clean wastewater is an environmental friendly method. The microalgae consume the minerals in the waste as part of their growth process. In addition to cleaning the water, the created biomass has a variety of applications including production of bio-diesel, animal feed, products for pharmaceutical and cosmetic purposes, or it can even be used as a source of heating or electricity (Thornton *et al.,* 2010). This wide variety application of microalgae explains the interest in controlling their growth.

Microalgae system has a number of unique benefits. They are aquatic species that do not require arable land for cultivation. Therefore, for cultivation, it does not need to compete with agricultural commodities for growing space. In fact, microalgae cultivation facilities can be built on marginal land that has few other uses (Campbell, 2008). In addition, the water used in algae cultivation can be fresh water or saline, and salt concentrations up to twice that of seawater can be used effectively. This means that algae need not to compete with other users for fresh water. Innovations to microalgae production allow it to become more productive while consuming resources that would otherwise be considered as waste (Campbell, 2008). In some circumstances waste water can be considered as resource. The increasing scarcity of fresh water resources in many countries also makes this recycling of wastewater attractive (Schenk *et al.,* 2008). Microalgae also have a greater capacity to absorb $CO_2$ than land plants, and are also not prone to photosynthetic inhibition under conditions of intense sunlight (Campbell, 2008). Microalgae biomass can be produced at extremely high volumes and this biomass can yield a much higher percentage of oil than other sources (Campbell, 2008).

## Microalgae in Wastewater Treatment

Treatment of wastewater using microalgae is gaining momentum nowadays due to several advantages in comparison to other biological technologies (Sousa, 2011). The ability of microalgae for nutrient removal, biomass production, and lipid production for biofuel production is actively investigated. Various types of wastewaters have been applied as nutrient medium for microalgae growth including agro-industrial wastewater, industrial wastewater, municipal sewage wastewater, and landfill leachate.

Many researchers have conducted investigation to prove the capability of microalgae for nutrient removal especially nitrogen. The ability of two microalgae species *Chorella vulgaris* and *Scenedesmus* sp. isolated from a wastewater stabilized pond for ammonia and phosphorus removal from agro-industrial wastewater of dairy industry and pig farming was studied by Gonzalez, Canizarez & Baena (1997). This study shows the potential of using these microalgae to reduce pollution of heavily contaminated wastewater.

Hu & Sommerfeld (2004) has conducted a study to screen and isolate high performance microalgae for bioremediation of nitrate contaminated wastewater. Microalgae from various water bodies throughout the metropolitan Phoenix area were sampled to obtain the species that have high growth rate and nitrate uptake. Three unicellular green microalgae, *Chlorella* sp., *Chlorococcum* sp., and *Scenedesmus* sp., one filamentous green alga, *Ulothrix* sp., and one filamentous cyanobacterium, *Pseudanabaena* sp., have been isolated. The research found that the unicellular algal species exhibit higher specific growth rates than the filamentous ones, paralleling the higher nitrate uptake rates. Among the three isolated unicellular green algae, *Scenedesmus* sp. appears to be the more desirable candidate for high performance nitrate removal.

Singh & Dhar (2007) has studied nitrogen (N) and phosphorous (P) scavenging potential in microalgae. Microalgae strains including *Nostoc muscorum, Anabaena variabilis, Oscillatoria princeps, Plectonema* sp. and *Chlorella* sp. grown under secondary treated sewage effluent and standard BG-11 medium have been examined in this study. According to the research findings, the mean dry weight and the pigments were highest in microalgae under BG-11 medium in comparison to those grown in sewage effluent. This investigation revealed the significant ability of microalgae to grow in secondary treated sewage effluents and to scavenge N and P from wastewater. *Chlorella vulgaris* was most efficient in scavenging ammoniacal nitrogen while nitrate-scavenging ability was highest in *Oscillatoria*. All the microalgal genera also removed available phosphorous efficiently.

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Kim *et al.* (2007) added fermented swine urine (3%) (v/v) to a control medium (CT), named KEP I, and an aquatic microalgal culture (10% Bold's Basal Medium) for growing mixed *Scenedesmus* species. After 31 days of culturing, the growth rate, dry weight, amino acid levels and secondary metabolites including chlorophyll *a*, astaxanthin, lutein, $\alpha$ and $\beta$-carotene increased at a greater degree in *Scenedesmus* grown in KEP I medium than in CT medium. Total lipids were much less in cells grown in KEP I than those grown in CT. The KEP I medium could improve the cost efficiency of industrial mass batch cultures for $CO_2$ sequestration, bioremediation, phytonutrients, agricultural fertilizers, and microalgal stock for the species preservation of aquaculture strains for use in young fish feed. It may also serve to attenuate negative environmental impact via the recycling of animal wastewater.

Wang *et al.* (2010) conducted a study to investigate the effectiveness of using digested dairy manure as a nutrient supplement for cultivation of oil-rich green microalgae *Chlorella* sp. Different dilution multiples of 10, 15, 20, and 25 were applied to the digested manure and algal growth was compared with regards to growth rate, nutrient removal efficiency, and final algal fatty acids content and composition. Slower growth rates were observed with less diluted manure samples. The algae removed ammonia, total nitrogen, total phosphorus, and COD by 100%, 75.7–82.5%, 62.5–74.7%, and 27.4–38.4%, respectively, in differently diluted dairy manure. The total fatty acid content of the dry weight increased from 9.00% to 13.7% along with the increasing dilution multiples. COD in digested dairy manure, beside $CO_2$, proved to be another carbon source for mixotrophic *Chlorella* (Wang *et al.,* 2010). Based on the results from this study, a process combining anaerobic digestion and algae cultivation can be proposed as an effective way to convert wastewater into profitable by products as well as to reduce contaminations to environment.

During the past ten years, fossil fuel depletion and global warming issues have strongly motivated research on the fuel production from biomass (Lardon *et al.,* 2009). Microalgae can grow rapidly and be among the promising photosynthetic biomass sources (Tsukahara & Sawayama, 2005). These have prompted some researchers to couple wastewater treatment and biomass production for production of biofuel. Woertz *et al.* (2009) studied the nutrient removal and lipid production of microalgae for biofuel feedstock during treatment of dairy and municipal wastewaters supplemented with $CO_2$. In their study, the microalgae inoculum containing a wide-ranging mixture of green algae and diatoms collected from local ponds treating municipal wastewater was used. The results from both types of wastewater suggest that $CO_2$-supplemented algae cultures can simultaneously remove dissolved nitrogen and phosphorus to lower level while generating a feedstock potentially useful for liquid biofuel production.

Chinnasamy *et al.* (2010) conducted a study using a wastewater containing 85–90% carpet industry effluents with 10–15% municipal sewage, to evaluate the feasibility of algal biomass and biodiesel production using native algal strains, isolated from carpet wastewater. Preliminary growth studies indicated both fresh water and marine algae showed good growth in wastewaters. A consortium of 15 native algal isolates showed >96% nutrient removal in treated wastewater. Biomass production potential and lipid content of this consortium cultivated in treated wastewater were 9.2–17.8 tonnes $ha^{-1}$ $year^{-1}$ and 6.82%, respectively. About 63.9% of algal oil obtained from the consortium could be converted into biodiesel.

Several improvement methods have been made to increase nutrient removal abilities, biomass quantity and quality of microalgae. One of them is by immobilization technique. In a research conducted by Alejandro, Leopoldo & Tom (2010), two species of microalgae

*Scenedesmus obliquus* and *Chlorella vulgaris* growing as immobilized and free cells were compared to test its ability to remove N and P in batch cultures of urban wastewater. The *Scenedesmus obliquus* showed a higher N and P uptake rate in urban wastewater than *Chlorella vulgaris.* From a practical point of view, the protein and lipids content analysis suggest that immobilized systems could facilitate the separation of the biomass from the treated wastewater. However, in terms of nutritional value of the biomass, immobilized systems do not represent an advantage over free-cell systems.

## Factors Affecting Microalgae Growth

Study on optimization of several factors affecting the microalgae growth contaminants removal is crucial. The optimization of strain-specific cultivation conditions is of confronting complexity, with many interrelated factors that can each be limiting. The most important parameters regulating algal growth are nutrient quantity and quality, temperature, light cycle and intensity, levels of $CO_2$ and $O_2$, pH and salinity, and mixing (Barsanti & Gualtieri, 2006). Knowledge about the influence and ranges of these parameters could help to promote microalgae growth.

Optimal media formulation is also critical to ensure sufficient and stable supply of nutrients to attain maximal growth acceleration and cell density, and ultimately to produce biomass at higher efficiencies (Schenk *et al.,* 2008). Optimization of mineral nutrients can increase culture productivity. Nitrogen and phosphorous are generally early targets in mineral optimization of media formulations, but other minerals are also vitally important for support of the structural and metabolic biochemistry of the cell. Mineral ions also have significant impact upon areas such as osmoregulation and osmoadaptive capacity, and molecular configuration of photosynthetic complexes (Schenk *et al.,* 2008). Ranges for nutrients are described by Graham, Graham & Wilcox (2009). Algal production can also be a multi-phased process with each step having independent optimal conditions such as nitrogen limitation in oil production or sulphur limitation in $H_2$ production (Schenk *et al.,* 2008).

The temperature of cultures medium should be controlled on the level that allows the algae to grow. The optimal temperature reported for phytoplankton cultures is generally between 20$^o$C and 30$^o$C (Thornton *et al.,* 2010). Photosynthesis depends on the light intensity and frequency. Since algae are photosynthetic organisms, there is a need to set the cultures in areas of varying temperatures and with sufficient light to promote photosynthesis. The photosynthetic rate is proportional to irradiance. The higher the irradiance, the longer the dark period that can be afforded by the system without loss of growth. Optimal light intensity for algae is 2,500-5,000 lux. The growth of algae becomes saturated at a range of 150−200 μmol photon $m^{-2}s^{-1}$ (Thornton *et al.,* 2010). The light source may be supplied by artificial illumination from 4ft white fluorescent lamps emitting about 15 u E $m^2$/s (Adoki, 2008).

Carbon dioxide ($CO_2$) enrichment of photoautotrophic algal cultures can significantly increase biomass production (Schenk *et al.,* 2008). For a high photosynthesis rate, the balance between $CO_2$ and $O_2$ has to be taken into consideration (Thornton *et al.,* 2010). The typical ratios of C, N, and P in high growth rate microalgae are C=6:N=1; and C=48:P=1 (Barsanti & Gualtieri, 2006). The proper range of $CO_2$ concentration during algal growth is 0.8%-1.0% (Cheng, Zhang, Chen & Gao, 2006).

Maintenance of an acceptable pH range throughout culturing is of utmost importance as it impacts all aspects of media biochemistry (Schenk *et al.,* 2008). Deviations from the optimum pH and salinity will cause productivity problems. The pH values for optimum growth of microalgae are in ranges of 7-12 (Thornton *et al.,* 2010). Both, ionic absorption from the media and the metabolic biochemistry of the cell, exert significant pressure upon pH, and in high performance cultures, their effect is powerful enough to overcome the neutralizing capacity of exogenous buffering agents. Currently microinjection of strong acids and alkalis, metabolic balancing in heterotrophic cultures and regulated $CO_2$ dissolution in both photoautotrophic and heterotrophic cultures, are the most practical and economical strategies for pH control (Schenk *et al.,* 2008).

While shaking cultures under controlled conditions may present valuable preliminary data, valid scale-up models require properly engineered lab-scale bioreactors in order to match mixing and mass transfer conditions (Schenk *et al.,* 2008). Mixing is necessary as it prevents sedimentation of the cells and provides even distribution of $O_2$, $CO_2$, and nutrients (Anderson, 2005). In laboratory condition, aeration and agitation are provided by means of aquarium aerators/air pumps (Adoki, 2008).

## Analysis of Microalgae Biomass

The potential of biomass produced is identified by analyzing its chemical and physical properties. Most of the researchers focus on analyzing the total lipids production with the aim of producing biofuel. After extraction of lipids, the remaining biomass can still be used as bio-fertilizers for paddy crop or as animal feed after ascertaining toxicological aspects (Singh & Dhar, 2007).

The algal growth and cell density was monitored by measuring the absorbance at 660 nm using spectrophotometer (Farahani *et al.,* 2006). The concentration of the dried algal biomass was analyzed based on weight mass (Gacheva & Pilarski, 2008). Procedure for biomass estimation is described by Dayananda, Kumudha, Sarada & Ravishankar (2010). The known volume of culture was harvested at 5000 rpm for 5 minutes and the pellet was washed twice with distilled water and freeze dried. The calorific value of the dried biomass was determined using an automatic adiabatic bomb calorimeter, and net calorific value was calculated. The C, H, and N analysis was carried out in CHN analyzer and the oxygen content was calculated by differences. The elemental analysis data was used to determine the empirical formula of the biomass.

The biochemical analysis of total protein, carbohydrate and lipid was done to determine nutrient composition. The protein content was measured following the method of Lowry (1951) (Gacheva & Pilarski, 2008) and also micro Kjeldahl method (APHA, 2005). The total protein concentration was determined by measuring the absorbance at 660 and 730 nm using spectrophotometer (Farahani *et al.,* 2006). The carbohydrate content was determined following the antrone method of Jaaska (1964) (Gacheva & Pilarski, 2008). The total carbohydrate from the lyophilized algal biomass was estimated spectroscopically using phenol-sulphuric method and glucose as the standard (Dayananda *et al.,* 2010). Total lipid content was measured as described by Petkov (1990); 20-30 mg of biomass was extracted 2 times with a mixture of chloroform and methanol (2:1) (Gacheva & Pilarski, 2008).

## Conclusion

The increasing deterioration of the environment and the need for energy and food forced the exploration of the feasibility of wastewater recycling and resource recovery. Within this context, bioremediation with microalgae has been particularly attractive because of their photosynthetic capabilities converting solar energy into useful biomass and incorporating nutrients such as nitrogen and phosphorus which cause pollution and eutrophication. They play a major role as producers in waste stabilization ponds and other polluted aquatic environments. Thus, microalgae should be involved in wastewater treatment especially landfill leachate with more emphasis might be placed on wastewater treatment and microalgae biomass production. The work represents major milestone in the effort to demonstrate that microalgae have potential for high nitrate wastewater treatment and biomass production.

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