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MICROALGAE BIOMASS PRODUCTION AND NITRATE REMOVAL FROM LANDFILL LEACHATE

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Abstract

Microalgae biomass production during high nitrate landfill leachate phycoremediation is a viable process with numerous of commercial potential including production of valuable resources for bio-chemicals, bio-fertilizers, animal feeds and bio-fuels. Excessive nitrate in the landfill leachate could be assimilated into algal biomass, instead of being discharged and possibly cause hazardous effect to environmental and health. In this study, the biomass production, nitrate removal and the composition of protein, carbohydrate, and lipid content of locally isolated microalgae strain of Oscillatoria sp., Chorella sp., and Scenedesmus sp., from phycoremediation of landfill leachate were analyzed to evaluate its potential application. The strains were cultivated in a diluted nitrified leachate with 500 mg/L of NO3- concentration. The results obtained showed that nitrate removal was feasible with up to 74% of NO3- removal by Oscillatoria sp. About 59% of protein (w/w), 26% of carbohydrate (w/w) and 20% of lipids were observed in Oscillatoria sp., Scenedesmus sp., and Chorella sp., respectively.

Keywords: Microlagae, biomass production, landfill leachate, nutrient removal.

INTRODUCTION

Bioremediation performed by microalgae was termed as phycoremediation, which was firstly introduced by John (2000). The use of algae to treat wastewater has been in vogue for more than 50 years, with one of the first descriptions of this application being reported by Oswald in 1957 (Rawat et al, 2011). The usage of microalgae to treat wastewater is an environmental friendly method with no secondary pollution as long as the biomass produced is reused and efficient nutrient recycling is allowed. The microalgae consume the minerals in the waste as part of their growth process. In addition to treating the water, the created biomass has a variety of applications including production of bio-diesel, animal feed, products for pharmaceutical and cosmetic purposes (Christi, 2007; Milledge, 2011; Spolaore, Joannis-Casan, Duran, Isambert, 2006), or it can even be used as a source of heating or electricity (Thornton, et al., 2010). In addition they form an important food source for shellfish or other aquatic species (Woertz, Feffer, & Nelson, 2009). This wide variety application of microalgae explains the interest in controlling their growth.

Microalgal biomass production offers more advantages compared to conventional biomass production. They are aquatic species that do not require arable land for cultivation, thus they do not 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, microalgae cultivation can be performed on any sources of water. Some species can thrive in brackish water or seawater and wastewater from food and agroindustrial sectors (Markou, Angelidaki, Georgakakis, 2012). 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 wastewater can be considered as resource. 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).

In recent years, many researchers have studied the potential of dual application of microalgae for wastewater treatment and biomass production (Rawat, et al., 2011; Kong et al., 2010; Griffiths, 2009). The high nitrogen level in wastewater had become a growing concern, which has increased the necessity to develop simple, efficient, and cost effective nitrogen removal techniques. High nitrate wastes (>1000 ppm) are usually generated by fertilizer, metal finishing, cooking and organic chemical industry, nuclear industry (Nair, Dhamole, & DSouza, 2010) and nitrified landfill leachate (Yusof, et al., 2010).

The presence of nitrate should be seen as nutrient resources that should be manipulated, recycled and used to produce useful product. One of the interesting means seen is to use microalgae to assimilate nitrate in high nitrate wastewater. Microalgae strains under consideration for this dual application must be highly productive in managed culture and resistant to variable and extreme environmental conditions (Gacheva & Pilarski, 2008).

It has been shown that many species of microalgae are suited to the processing of nutrient rich wastewater (Sing & Dhar, 2007; Shi, Podola & Melkonian, 2007; Martinez, et al., 2000). However, the treatment of landfill leachate using microalgae has received very little attention. Landfill leachate produced in Malaysia contains high level of ammonia (Yusof, et al., 2010). Biological leachate treatment method by means of Nitrification Activated Sludge System (NASS) has successfully remove 99% ammonia from the leachate by converted it to nitrate, to comply with the stringent discharge limit imposed (10 mg/L ammonia for Standard A). The presence of high nitrate in the nitrified landfill leachate proposed the possibility to use it as the growth media for microalgae. By cultivating microalgae, which consume polluting nutrients in nitrified landfill leachate and processing this resource, the goals of wastewater treatment and biomass production can be combined.

Lot of microalgae strains has been collected, preserved and stored. However, not all strains are suitable for this purpose since the presence of high concentration of nitrate in the wastewater and others compounds may cause toxicity. Local strains collected from wastewater would be the best candidates since there are more likely adapted to the local temperature, weather, light regimes, and more importantly the environment of high nitrate and other pollutions. This paper examines the feasibility of nitrate removal from landfill leachate using microalgae. The biochemical composition of the microalgae biomass was also evaluated.

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RESEARCH METHOD

Materials and Methods

Experimental set up

Three sets of experiments were run in parallel to determine microalgae growth, nitrate removal, and biochemical properties of biomass harvested. The microalgae were cultured in Erlenmeyer flask containing three different medium; BG-11 plus nitrate medium (control), nitrified landfill leachate (NL), and nitrified landfill leachate with nutrient added (NLA). The cultures placed on culture rack at temperature range of 25oC-30oC. Aeration were provided by commercial aquarium pump, and light by cool white fluorescent lamp adjusted to 2000-3000 lux intensity and 12:12 light dark cycles.

Microalgae

Microalgae strains used were collected from Jeram Sanitary Landfill, Selangor. The species were screened, isolated, purified, and identified using 18S rRNA molecular technique. The selected strains were identified as Oscillatoria sp., Chlorella sp., Scenedesmus sp. The species were selected on the basis of survivability and the ability to grow in high nitrate concentration up to 3000 mg/L.

Characteristics of Wastewater

Landfill leachate was collected from Landfill Treatment Pond of Jeram Sanitary Landfill Leachate. The leachate had mean values of pH 8.78, COD 2050 mg/L and BOD5 320 mg/L. The mean available ammonium and nitrite is 744.38 mg/L and 1226 mg/L respectively. Sulfate concentration was 78.3 mg/L and phosphate was below detection range. Table 1 summarized the characteristic of the landfill leachate. The raw landfill leachate was nitrified using ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) following the process of Nitrification Activated Sludge System (NASS) (Yusof et al., 2010) This process produced nitrified landfill leachate (NL) with mean available nitrate 2430 mg/L. NL was diluted to 20% with distilled water before used as culture media. NLA is NL with the addition of 0.23 mM K2HPO4 and 0.03 mM MgSO4.7H2O.

Table 1 : Characteristic of Landfill Leachate

Parameter	Concentration (mg/L)
pH	8.78
COD	2050
BOD_5	320
Lithium	0.24
Sodium	2540.39
Ammonium	744.38
Pottasium	1760.69
Magnesium	102.85
Calcium	122.806
Fluoride	n.a.
Chloride	2820.00
Nitrite	1226.00
Nitrate	n.a.
Phosphate	n.a.
Sulfate	78.3

Micoalgae Harvesting

Microalgae cell was harvested during stationary phase (day 14). The dry weight was determined after oven drying at 60oC. Biomass for biochemical composition analysis was freeze dried using Scanvac Cool Save Freeze Dryer. The culture was centrifuged at 5000 rpm for 5 minutes. The supernatant was removed and the cell was washed twice with deionized water. The microalgae cell was kept overnight in -20oC freezer before freeze dried. The lyophilized microalgae cell was stored at room temperature for analysis.

Nitrate Analysis

Known volume of samples will be harvested by centrifugation at 5000 rpm for 5 min. The supernatant collected will be analyzed for nitrate using Dionex ICS-1100 Ion Chromatography.

Microalgae Biochemical Composition

Lipid analysis was performed using modified Bligh & Dyer (1959) method. Known amount of dry sample of microalgae was mixed with 100 ml distilled water. 50 ml of blended sample was taken and microwaved for 5 minutes at high temperature. 1:1 v/v of chloroform-methanol was added to sample, to the ratio of 1:1 sample: solvent. The mixture was poured in a separatory funnel and shaken for 15 minutes. 2 layers of methanol-water upper layer and lipid-chloroform lower layer were obtained. The lipid-chloroform layer was taken and the solvent was evaporated using rotary evaporator for 1 hour at 40oC under vacuum.

Carbohydrate was determined using phenol-sulphuric acid method. Glucose Standards in the range of 0.02 – 0.1 mg/ml was prepared. 1ml of each sample/ standard was pipetted into test tube. 1ml of 5% phenol was added to each tube. 5ml of 96% sulphuric acid was added to each tube and shaken well. After 10 minutes, the contents in the tubes were vortexed and place in a water bath at 25-30 oC for 20 minutes. The absorbance was measured at 490 nm. The amount of total carbohydrate present was calculated using the standard graph prepared.

Protein analysis was performed using Pierce BCA Protein Assay Kit. The working reagent (WR) was prepared by mixing 50 parts of BCA Reagent A (sodium carbonate, sodium bicarbonate, bicinchonicic acid and sodium tartrate in 0.1 M sodium hydroxide) with 1 part BCA Reagent B (containing 4% cupric sulfate). The diluted albumin (BSA) standards were prepared in the range of 5 – 250 ug/ml final BSA concentration. 0.1 ml of each sample was pipetted into test tube. 2.0 ml of WR was added to each tube and mixed well. The test tubes were covered and incubated at 60oC for 30 minutes. After 30 minutes, the tubes were cooled at room temperature and the absorbance was recorded at 562nm. Protein concentration was determined based on standard curve prepared.

RESULT AND DISCUSSION

The present investigation was focused on analyzing the efficiency of the selected microalgal genera to grow in nitrified landfill leachate and their capacity to assimilate nitrate. There was a significant difference amongst different genera examined for nitrate removal and biochemical composition.

Table 2 shows the percentage of nitrate removal. Oscillatoria sp. in NLA show the highest percentage of nitrate removal, 74.6% compared to the other species. Nitrate removal of Oscillatoria sp. in NL (72.9%) and NLA (74.6%) also higher than in BG-11 (59.8%). Moreover Scenedesmus sp. also showed higher nitrate removal in NL (48.7%) and NLA (48.5%) compared to in BG-11 (37.3%). However, for Chorella sp. nitrate removal percentage in BG-11 was the highest (46.70%), followed by in NLA (31.7%) and NL (26.4%). Nutrient limitation was observed from Chorella sp. in BG-11. On day 7, the growth rate started to decrease causing the reduction of nitrate assimilation rate. This might due to phosphate and sulfate deficiency. In this research, in term of leachate nitrate removal efficiency, Oscillatoria sp. was the most efficient followed by Scenedesmus sp. and Chlorella sp. A research conducted by Singh and Dhar (2007) revealed that Oscillatoria was most efficient in scavenging nitrate while ammonia scavenging ability was highest in Chorella vulgaris. In addition, a study conducted by Shi, Podola and Melkonian (2007) found that two green microalgae Chlorella vulgaris and Scenedesmus rubescens had removed nitrate efficiently from municipal wastewater, to less than 10% of their initial concentration within 9 days.

In this study, species selected were able to survive up to 20% leachate concentration (~500 mg/L Nitrate). This suggested that the selected strains are high nitrate and leachate tolerant. In the study of Che Sa, Surif, Mohamed Ibrahim, Wan Omar (2011), they found that there was inhibition effect on Chlorella sp. growth at higher leachate concentration (10% leachate, nitrate) due to higher toxic materials. However, the research suggest the feasibility of using leachate (5%) as a low cost medium to culture microalgae for production microalgae biomass for biodiesel production.

Table 2. Percentage of Microalgae Nitrate Removal

Species	Media	Percentage (%)
Oscillatoria sp.	BG-11	59.8
	NL	72.9
	NLA	74.6
Chlorella sp.	BG-11	46.7
	NL	26.4
	NLA	31.7
Scenedesmus sp.	BG-11	37.3
	NL	48.7
	NLA	48.5

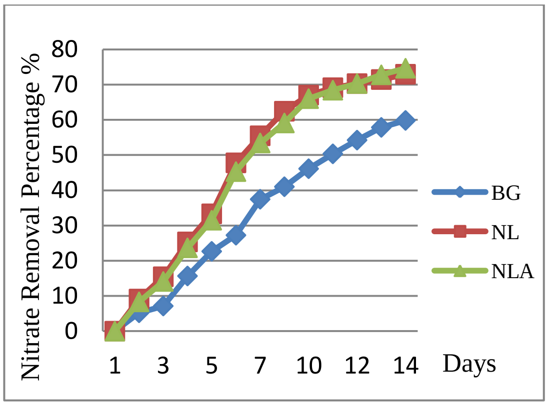

Figure 1: Nitrate removal percentage of *Oscillatoria sp.*

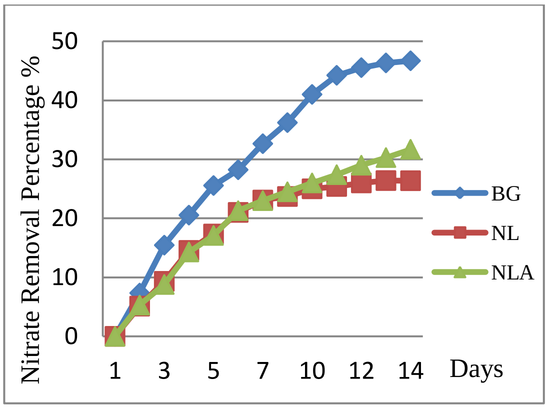

Figure 2 Nitrate removal percentage of *Chlorella sp.*

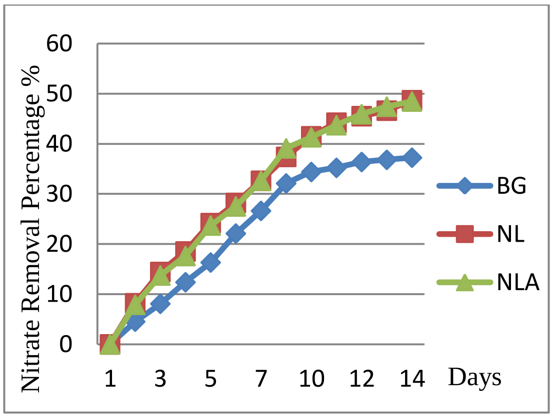

Figure 3. Nitrate removal percentage of *Scenedesmus sp.*

Table 3 showed the biochemical composition of microalgae expressed on a dry matter basis. The lipid, carbohydrate, and protein content of microalgae were calculated by weight percentage of dry matter basis (% w/w). Highest lipid percentage, 19.7% was observed from Chorella sp. in NL. Highest carbohydrate percentage, 26.2% was observed from Scenedesmus sp. in BG-11. Most of the species produced high protein biomass because they assimilate high concentration of nitrate. High percentage of protein was observed from Chorella sp. in NLA, Oscillatoria sp. in NLA, and Oscillatoria sp. in NL which were in 58.8 %, 58.8 % and 57.7% respectively.

The value of lipid percentage obtained from all species is considered low for biodiesel generation. Lipid content for pure cultures of algae have been reported to range from 1%-85%. More than 20% extractable algal oil is useful for biodiesel generation (Brune, Lundquist & Benemann, 2009). The low percentage of lipid might be due to high concentration of nitrate in the culture. The effect of nitrogen on the lipid content of Chlorella has been studied in the research by Nigam, Prakash Rai & Sharma (2011). They found that as the nitrate concentration in the medium decreases, biomass production also decreased, but the lipid content increased. In addition, according to their findings, they found that at the similar concentration of nitrate source, lipid tends to accumulate more in stationary phase in comparison to exponential phase. As nitrate source is increased in the medium, enhancement in biomass concentration is recorded.

According to Yeesang and Cheirsilp (2011), under nitrogen deficient conditions, algal cells accumulate carbon metabolites as lipids. Nitrogen limitation also triggers the accumulation of carbohydrates. It has earlier been reported that under nitrogen starvation conditions, nitrogen containing macromolecules and carbon reserve compounds like carbohydrates and fats are accumulated (Dayananda et al., 2006). In this condition, many microalgal strains could transform proteins or peptides to lipids or carbohydrates as energy reserve components. Research by Chaichalerm et al., (2012) also supported that lowest nitrogen concentration was considered as the optimum medium for microalgae lipid production. However, in this research high nitrate wastewater was used, thus producing biomass with significant protein content. High protein content of the microalgae (>50%) is suitable for providing animal feed replacement (Brune, Lundquist & Benemann, 2009). After the available lipid and carbohydrate content was extracted, the residual biomass can be used as fertilizer, soil amendment, or feed for fish or livestock (Roeselers et al., 2007).

Table 3. Biochemical Composition of Microalgae Expressed on a Dry Matter Basis (%)

Microalgae	Media	Lipid, %	Carbohydrate, %	Protein, %
Oscillatoria sp.	BG	10.2	16.6	52.1

	NL	15.2	13.6	57.7
	NLA	15.1	13.6	58.8
Chlorella sp.	BG	13.4	19.3	52.4
	NL	19.7	21.9	45.5
	NLA	16.6	17.9	58.8
Scenedesmus sp.	BG	12.4	26.2	33.0
	NL	16.2	23.4	34.1
	NLA	14.4	24.3	49.4

The main interest in research nowadays is the cultivation of microalgae for lipids production to generate biodiesel. Average biodiesel production yield from microalgae can be 10 to 20 times higher than the yield obtained from vegetable oils (Chisti, 2007). Under optimum condition, most common microalgae (Scenedesmus and Chlorella included) have oil levels between 20 to 75% by weight of dried biomass (Elumalai, Baskaran, Prakasam & Senthil Kumar, 2011).

There are several other thermochemical and biological conversion technologies, in which biomass from microalgae could be used as a substrate. However, the possible uses of these technologies were limited by the high protein content or the low carbohydrate content of the majority of the microalgal species (Markou, Angelidaki, Georgakakis, 2012). Moreover, in the majority of biomass conversion technologies, carbohydrates are the main substrate for production of biofuels (Lardon et al., 2009). Nevertheless, microalgae biomass composition could be manipulated by several cultivation techniques, such as nutrient starvation or other stressed environmental conditions, which cause the microalgae to accumulate carbohydrates. Furthermore, lipid content and biomass productivity depend on environmental conditions, culturing methods, and growth phase. In particular, nitrogen limitation increases lipid content in some species. However, nitrogen limitation decreases growth rate, which can lead to decreased overall lipid productivity.

Conclusion

Present investigation revealed the significant ability of microalgae to grow in nitrified landfill leachate. The three selected microalgae species are able to survive in high nitrate environment. Even though the nitrate level may slower the growth rate, however their ability to tolerate high nitrate environment and produce biomass might useful in dual application of high nitrate wastewater treatment and biomass production. Such microalgae biomass can be used as bio-fertilizer for paddy crop or as animal feed after ascertaining toxicological aspects. It will be attempted to manipulate the composition of the growth media if it is required for optimal biomass production for biodiesel.

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