BIOMASS PRODUCTION OF LOCAL MICROALGAE ISOLATES IN NITRIFIED LANDFILL LEACHATE

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ABSTRACT

This study aims to examine phycoremediation and biomass production of local microalgae isolates cultivated in high nitrate concentration of nitrified landfill leachate (NLL). The microalgae samples were collected from landfill leachate treatment plant and were isolated as potential species that are able to grow in high nitrate concentration. The isolates were identified using APHA's Key for Identification of Freshwater Algae and 16S rRNA/18S rDNA molecular techniques. Maximum microalgae growth, nitrate removal performance, and biomass productivity were determined at NLL concentration of 10-30% v/v. Biomass produced was further characterized for carbohydrate, lipid, and protein composition. The findings indicated that three species of local microalgae were successfully isolated namely; *Chlorella* sp., *Scenedesmus* sp. and *Oscillatoria* sp. with highest specific growth rate (μ) of 0.62 day^{-1} (15% NLL), 0.71 day^{-1} (10% NLL), and 0.94 day^{-1} (10% NLL), respectively. Nitrate was removed by all microalgae isolates with nitrate removal rate of 26.5-27.5 $mgL^{-1}day^{-1}$ and maximum nitrate removal percentage of 84% (20% NLL) by *Oscillatoria* sp. Highest biomass productivity (106.87 $mgL^{-1}day^{-1}$), carbohydrate productivity (2921.83 $mgL^{-1}day^{-1}$), lipid productivity (1414.96 $mgL^{-1}day^{-1}$) were observed for *Oscillatoria* sp. cultured in 10% NLL. Highest protein productivity (5498.37 $mgL^{-1}day^{-1}$) was observed for *Oscillatoria* sp. cultured in 20% NLL. Highest carbohydrate, lipid, and protein content were observed for *Chlorella* sp. cultured in 30% NLL (37.50%), *Chlorella* sp. cultured in 10% NLL (18.23%), and *Oscillatoria* sp. cultured in 20% NLL (61.4%), respectively. The highest carbohydrate, lipid, and protein yield were achieved by *Oscillatoria* sp. cultured in 10% NLL (0.95 mg carbohydrate mg^{-1} NO_3^-), *Chlorella* sp. cultured in 10% NLL (0.5 mg lipid mg^{-1} NO_3^-), and *Oscillatoria* sp. cultured in 10% NLL (1.58 mg protein mg^{-1} NO_3^-), respectively. In conclusion, application of local microalgae isolates in phycoremediation of NLL and biomass production was feasible. As implication, local microalgae isolates performance in this research could serves as a basis for scaling up of microalgae biomass production.

PENGHASILAN BIOJISIM MIKROALGA PENCILAN TEMPATAN DI DALAM CECAIR LARUT RESAP TERNITRIFIKASI

ABSTRAK

Penyelidikan ini bertujuan mengkaji fikoremediasi dan penghasilan biojisim daripada mikroalga pencilan tempatan di dalam cecair larut resap ternitirifikasi (CLRT) yang berkepekatan nitrat tinggi. Sampel mikroalga diperolehi dari loji rawatan cecair larut resap dan dipencilkan untuk spesies yang berpotensi hidup dalam kepekatan nitrat yang tinggi. Pencilan dikenalpasti menggunakan *APHA's Key for Identification of Freshwater Algae* dan teknik molekul 16S rRNA/18S rDNA. Pertumbuhan mikroalga maksimum, prestasi penyingkiran nitrat dan produktiviti biojisim dinilai pada kepekatan CLRT 10-30% v/v. Biojisim yang dihasilkan seterusnya dicirikan untuk komposisi karbohidrat, lipid dan protein. Dapatan kajian ini menunjukkan bahawa tiga spesies mikroalga telah berjaya dipencilkan iaitu; *Chlorella* sp., *Scenedesmus* sp. dan *Oscillatoria* sp. dengan kadar pertumbuhan spesifik (μ) tertinggi 0.62 hari^{-1} (15% CLRT), 0.71 hari^{-1} (10% CLRT) dan 0.94 hari^{-1} (10% CLRT), masing-masing. Nitrat disingkirkan oleh semua pencilan mikroalga dengan kadar penyingkiran nitrat 26.5-27.5 mgL^{-1} hari^{-1} dan peratusan penyingkiran nitrat maksimum 84% (20% CLRT) oleh *Oscillatoria* sp. Produktiviti biojisim (106.87 mgL^{-1}hari^{-1}), produktiviti karbohidrat (2921.83 mgL^{-1}hari^{-1}) dan produktiviti lipid (1414.89 mgL^{-1}hari^{-1}) tertinggi telah diperhatikan pada *Oscillatoria* sp. yang dikultur di dalam 10% CLRT. Produktiviti protein tertinggi (5498.37 mgL^{-1}hari^{-1}) telah diperhatikan pada *Oscillatoria* sp. yang dikultur di dalam 20% CLRT. Kandungan karbohidrat, lipid dan protein tertinggi diperhatikan pada *Chlorella* sp. yang dikultur di dalam 30% CLRT (37.50%), *Chlorella* sp. yang dikultur di dalam 10% CLRT (18.23%) dan *Oscillatoria* sp. yang dikultur di dalam 20% CLRT (61.4%), masing-masing. Hasil karbohidrat, lipid, and protein tertinggi telah dicapai oleh *Oscillatoria* sp. yang dikultur di dalam 10% CLRT (0.95 mg karbohidrat mg^{-1} NO_3^-), *Chlorella* sp. yang dikultur di dalam 10% CLRT (0.5 mg lipid mg^{-1} NO_3^-) dan *Oscillatoria* sp. yang dikultur di dalam 10% CLRT (1.58 mg protein mg^{-1} NO_3^-), masing-masing. Kesimpulannya, aplikasi mikroalga pencilan tempatan dalam fikoremediasi CLRT dan penghasilan biojisim boleh dilakukan. Implikasinya, prestasi mikroalga pencilan tempatan dalam kajian ini boleh menjadi asas untuk penghasilan biojisim mikroalga skala tinggi.

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LIST OF ABBREVIATIONS

AOB	Ammonia-Oxidizing Bacteria
BLAST	Basic Local Alignment Search Tool
BOD_5	5-Day Biochemical Oxygen Demand
C	Carbon
CLRT	*Cecair Larut Resap Ternitrifikasi*
COD	Chemical Oxygen Demand
DGGE	Denaturing Gradient Gel Electrophoresis
DO	Dissolved Oxygen
MSW	Municipal Solid Waste
N	Nitrogen
NASS	Nitrifying Activated Sludge System
NCBI	National Centre for Biotechnology Information
NH_3	Ammonia
NH_3^+	Ammonium
NLL	Nitrified Landfill Leachate
NO_2^-	Nitrite
NO_3^-	Nitrate
NOB	Nitrite-Oxidizing Bacteria
P	Phosphorus
SBR	Sequencing Batch Reactor
TKN	Total Kjeldahl Nitrogen
TSS	Total Suspended Solid

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CHAPTER 1

INTRODUCTION

1.1 Background

Landfilling is the most applicable method in waste management due to its availability and low maintenance. Compared to other disposal method such as incineration, landfilling allows for waste to be dumped in high quantity at economical costs, though this disposal method leads to the production of leachate and odours (Foul, Aziz, Isa, & Hung, 2009). Leachate is the water that passes through the waste from precipitation and within the landfill site. The water then form a 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 high chemical oxygen demand (COD) (Idris, Saed, & Hung, 2004). The most common toxic components in leachate are ammonia (NH_3) and heavy metals, which can be hazardous if they accumulate in the food chain even at low level. Treating landfill leachates presents a unique problem from engineering point of view mainly because the substance contains high COD (600-15000 mgL^{-1}) and ammonium ion (NH_4^+) (500-3000 mgL^{-1}) (Kargi & Pamukoglu, 2003).

Landfilling is the current main waste disposal method in Malaysia. The latest assessment of landfill sites in July 2015 revealed that there are 296 landfills throughout the country; 165 are still operating and 131 are closed landfills. However, only 10 of the existing landfills are sanitary landfills (National Solid Waste Management Department [NSWMD], 2015). Sanitary landfill is constructed to minimize the pollution caused by solid waste. It is a fully engineered disposal option in which the selected wasteland is carefully engineered in advance before being pressed into service (Ramachandra, 2006). Operators of sanitary landfills can minimize the effects of leachate by selecting an appropriate site and a leachate treatment method. However, constructing a sanitary landfill is costly and requires advanced technology and maintenance.

A number of leachate treatment methods exist nowadays with different degree of efficiency, availability, and cost. Leachate treatment methods can be biological, chemical, or physical. Ongoing studies to search for the best and the least costly maintenance method are required before one can choose the most suitable treatment technology to treat landfill leachate.

Leachate produced in Malaysia contains high NH_4^+ concentration (Yusof et al., 2010). This is due to the fact that solid waste collected is not segregated at source, causing most of the putrescible fraction to end up in the landfill. Under an anaerobic condition, the organic nitrogen (N) in the landfill waste, such as protein, is hydrolyzed into amino acids before fermenting further to other compounds including NH_3. The anaerobic hydrolysis of municipal solid waste (MSW) containing proteins is slower than the one containing carbohydrates, resulting in a slow release of soluble N, and this breeds a high concentration of NH_4^+ in leachate.

Removing NH_4^+ is principally important in the tertiary treatment of landfill leachate in order to comply with the imposed discharge limit (10 mgL^{-1} for Standard A). This amount restriction serves to mitigate the toxic effect of NH_3 to the aquatic life (Yusof et al., 2010). The biological leachate treatment method by means of Nitrifying Activated Sludge System (NASS) has been efficient in treating leachate with high NH_4^+ concentration, and there are growing interests to involve microalgae in the treatment system with the aim to produce useful biomass. So far NASS has successfully treated landfill leachate of 1450 mgL^{-1} NH_4^+ concentration (Yusof et al., 2010). In Yusof et al.'s experiment, complete nitrification was achieved with a very high NH_4^+ removal percentage (~100%), and that the ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) had completely nitrified NH_4^+ to nitrate (NO_3^-) (Figure 1.1), producing nitrified landfill leachate (NLL) with high NO_3^- concentration.

The high NO_3^- level in wastewater has become a growing concern which necessitates the development of a simple, efficient, and cost effective N removal technique. High NO_3^- wastes (>1000 mgL^{-1}) are usually generated from fertilizers, metal finishing, cooking, organic chemical industry, and nuclear industry (Nair, Dhamole, & DSouza, 2010), and their treatments have become a challenge for these industries. NO_3^- should be seen as a nutrient resource that should be manipulated and used to produce useful products, and one of the means is to use microalgae to assimilate NO_3^- in high NO_3^- wastewater especially NLL. Not only has this approach treated wastewater, but the utilization of NO_3^- as a nutrient source will produce biomass that has various applications.

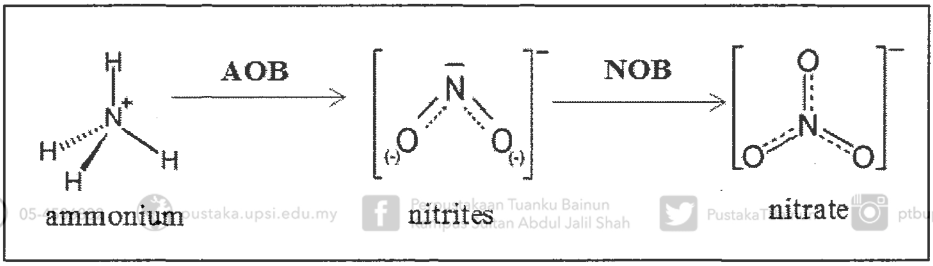

Figure 1.1. Nitrification in NASS.

The use of microalgae to remove pollutant from the environment is termed as phycoremediation, which was first introduced by John (John, 2000). The use of microalgae 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, Kumar, Mutanda, & Bux, 2011). Phycoremediation is defined as the use of macroalgae or microalgae for removing biotransformation of pollutants, including nutrients and xenobiotics from wastewater and CO_2 from waste air (Olguin, Galicia, Mercado, & Perez, 2003). This field has evolved from the early work by Oswald (1963). Wastewater phycoremediation is an eco-friendly process with no secondary pollution (sludge) as long as the biomass produced is reused and allows efficient nutrient recycling (Oswald, 1963; Olguin et al., 2003).

Microalgae can also effectively reduce NO_3^- concentration in wastewater and it can grow in leachate (Che Sa, Surif, Mohamed Ibrahim, & Wan Omar, 2011; Mustafa, Phang, & Chu, 2012). This presents a possibility of collecting microalgae biomass from leachate, which is one of the better sources of energy. Compared to terrestrial plants, microalgae are promising biomass species owing to their fast growth rate and high CO_2 fixation ability (Campbell, 2008). They have long been recognized as a potential source for biofuel production because of their high oil content and rapid biomass production. After oil extraction, the remaining biomass fraction can be used as a high protein feed for livestock (Roeselers, Loosdrecht, & Muyzer, 2007). This reduces waste and gives further value to the process.

The presence of NO_3^- in treated landfill leachate by means of NASS proposes the possibility of using NLL as the growth medium for microalgae. Microalgae have the capability of fixing CO_2 and assimilating NO_3^- from wastewater, and converting them into useful biomass. Therefore, the production of biomass from microalgae should be coupled with wastewater treatment (Figure 1.2). The growth of microalgae does not only purify leachate by reducing NO_3^- concentration but it also creates microalgae biomass at the same time. Microalgae biomass has various applications, including in the production of bio-diesel (Chisti, 2007), as animal feed (Milledge, 2011), products for pharmaceutical and cosmetic purposes (Spolaore, Joannis, Duran, & Isambert, 2006), or it can even be used as a source of heating or electricity (Thornton et al., 2010). In fact, microalgae biomass forms an important food source for shellfish or other aquatic species (Woertz, Feffer, Lundquist, & Nelson, 2009). These various applications of microalgae explain the interest in controlling their growth.

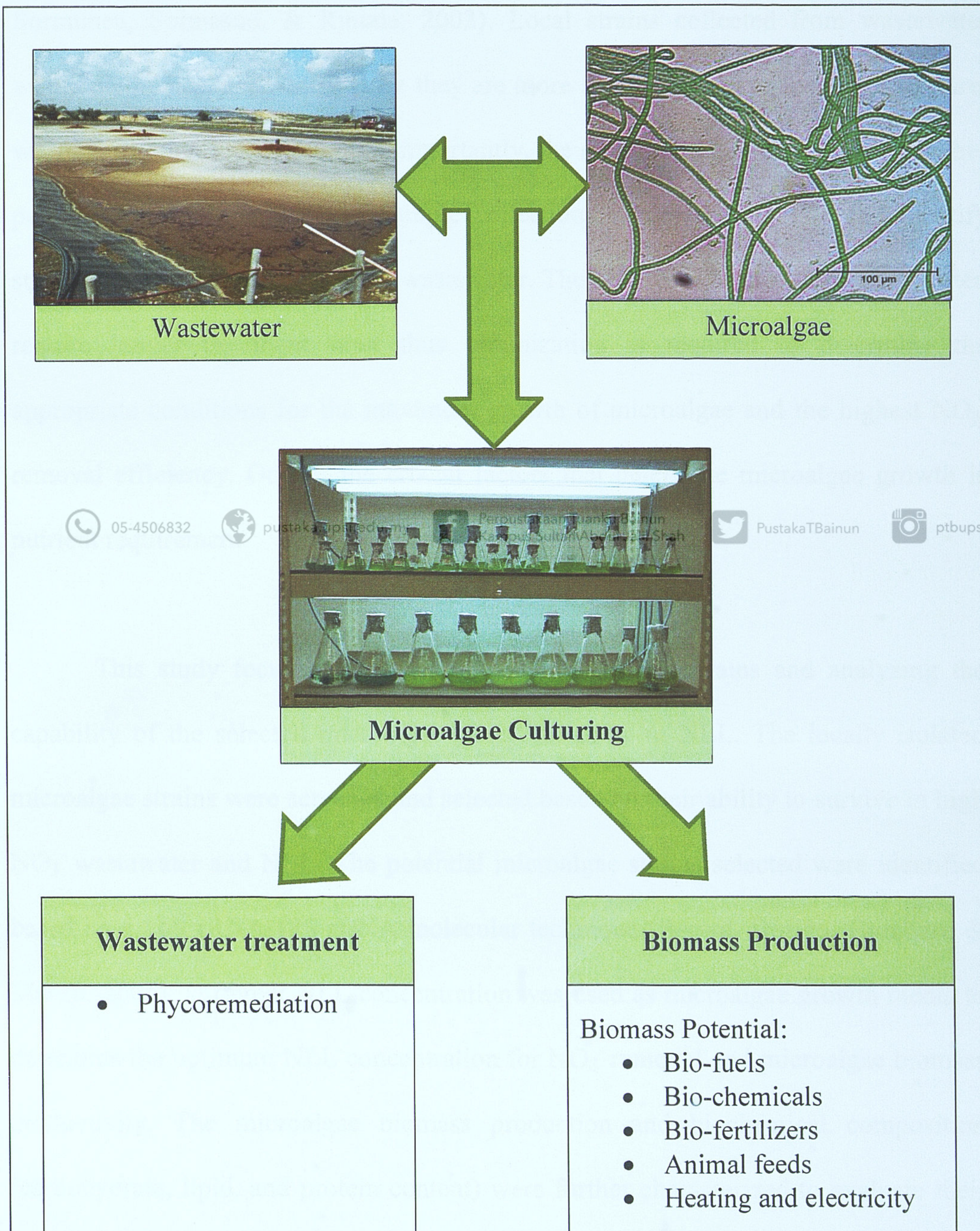

Figure 1.2. Coupled microalgae system: wastewater treatment and biomass production.

A number of microalgae strains have been collected, preserved, and stored (Gachon et al., 2007). However not all strains are suitable for current research purpose. This is due to the presence of high concentration of NO_3^- in the wastewater and other compounds that may cause toxicity to the microalgae (Marttinen, Kettunen, Sormunen, Soimasuo, & Rintala, 2002). Local strains collected from wastewater would be the best candidates since they are more likely to adapt to local temperature, weather, light regimes, and more importantly, the environment of high NO_3^- and other pollutions. However, much of sample collection is needed to isolate and identify strains that can grow in high NO_3^- wastewater. The biological treatment methods often require longer treatment time thus optimization is required to determine the appropriate conditions for the maximum growth of microalgae and the highest NO_3^- removal efficiency. One of the crucial factors that determine microalgae growth is nutrient requirement.

This study focuses on isolating local microalgae strains and analyzing the capability of the selected microalgae genera to grow in NLL. The locally isolated microalgae strains were screened and selected based on their ability to survive in high NO_3^- wastewater and NLL. The potential microalgae strains selected were identified based on a 16S rRNA/18S rDNA molecular technique (Berard, Dorigo, Humbert, & Martin, 2005). Different NLL concentration was used as microalgae growth media to determine the optimum NLL concentration for NO_3^- removal and microalgae biomass productivity. The microalgae biomass production and biochemical composition (carbohydrate, lipid, and protein content) were further characterized to evaluate their potential and commercial application.

1.2 Problem Statement

In NASS nitrification does not constitute an effective removal of N although the process effectively neutralizes the oxygen demand exerted by NH_3, reduces BOD, and prevents the toxic effects of NH_3. This is because in this process, NH_3 is actually converted to NO_3^-. The NASS treatment is acceptable for landfill leachate treatment since there is no discharge limit imposed for NO_3^- in Environmental Quality (Control of Pollution from Solid Waste Transfer Station and Landfill) Regulations 2009 (Department of Environment, 2009a). However, excessive NO_3^- in NLL should be eliminated prior to discharge in order to prevent serious environmental and health problems. Among the established international regulations include the European Community and the USA Environmental Protection Agency, which set 5.6 mgL^{-1} and 10 mgL^{-1} as the amount of NO_3^- discharged to the environment respectively (Aslan & Cakici, 2007).

If released into the environment, NO_3^- can create serious problems, such as the eutrophication of rivers and the deterioration of water quality (Zimmo, van der Steen, Gijzen, 2004). The release may also be hazardous to human health because NO_3^- in the gastrointestinal tract can be reduced to nitrite ions. In addition, NO_3^- and nitrite (NO_2^-) can be transformed to potent carcinogens N-nitrous compounds (Foglar, Briski, Sipos, & Vukovic, 2005). Common toxic responses to NO_3^- poisoning are methaemoglobinaemia (Ergas & Reuss, 2001), abortion, and still-born babies (Bruning & Kaneene, 1993). Therefore, NLL should be further treated to completely remove NO_3^- from wastewater before being discharged to the environment.