STUDY THE *MIX CULTURE* AND *WILD STRAIN SP*. OF MICROALGAE IN HIGHLY CONCENTRATED MUNICIPAL WASTE WATER

NADZIRAH BINTI SHA'ARI

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Faculty of Chemical & Natural Resources Engineering
Universiti Malaysia Pahang

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ABSTRACT

Municipal wastewater is one of the major concerns of the environmental problems. The wastewater is contaminated, so the treatment is essential to minimize its effect to nature before it discharge. Wastewater could used as media in biodiesel production has a potential source of low-cost lipids production. The objectives of this study is to identify the nutrient removal efficiency and lipid production by mixed algae culture and wild strain sp. in concentrated municipal wastewater (centrate). All these experiment will be conducted in batch operation to monitor the biomass cultivation for biodiesel production. From this study, the *mixed culture* of freshwater algae could remove chemical oxygen demand (COD), ammoniacal nitrogen and total phosphorus by 74.5%, 90.9% and 94.2 % respectively from raw centrate and produced 14.8% of dry biomass lipid content much higher value from other sample which is *wild strain sp.* of freshwater microalgae.

KAJIAN MENGENAI KULTUR CAMPURAN DAN SPESIS LIAR MIKROALGA DI DALAM AIR SISA KUMBAHAN BERKEPEKATAN TINGGI

ABSTRAK

Sisa kumbahan yang tidak diselia dengan baik merupakan salah satu punca masalah pencemaran alam sekitar. Rawatan sisa kumbahan perlu dijalankan sebelum ia dikeluarkan untuk mengurangkan kesannya terhadap alam sekitar. Sisa-sisa tersebut juga boleh digunakan sebagai media di dalam penghasilan biodiesel dan mempunyai kos penghasilan lipid yang rendah. Objektif kajian ini adalah untuk mengenal pasti kecekapan penyingkiran nutrien dan pengeluaran lipid oleh kultur campuran alga dan spesis alga liar daripada air di dalam air sisa kumbahan berkepekatan tinggi. Eksperimen ini akan dijalankan secara berasingan untuk memantau perkembangan biomas sebagai pengeluar biodiesel. Kajian ini mendapati bahawa kultur campuran alga daripada air tawar boleh menyingkirkan ammonia, jumlah fosforus dan keperluan oksigen kimia (COD) sebanyak 74.5%, 90.9% dan 94.2% daripada "centrate" mentah dan kajian ini juga boleh mengeluarkan 14.8% peratusan biomas lipid yang dikeringkan lebih tinggi daripada sampel lain.

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LIST OF ABBREVIATIONS

FAME	-	Methyl ester of fatty acids
CO_2	-	Carbon dioxide
O_2	-	Oxygen
%	-	Percentage
L	-	Liter
ha	-	Hectare
m^2	-	Meter square
NH_4^+	-	Ammonium ion
NO_3^-	-	Nitrate ion
PO_4^{3-}	-	Phosphate ion
SS	-	Suspended solids
Mm	-	Millimeter
COD	-	Chemical oxygen demand
BOD	-	Biological oxygen demand
mg	-	milligram
N	-	Nitrogen
P	-	Phosphorus
ml	-	Milliliter
NH_3	-	Ammonia
mg/L	-	Milligram per liter
g/L	-	Gram per liter
nm	-	Nanometer
OD680	-	Optical density at 680nm
POME	-	Palm oil mill effluent
˚C	-	Degree Celsius

CHAPTER 1

INTRODUCTION

1.1 Background of Study

The increasing amount of waste originating from human activities gives the negative impact on the environment and in particular the water quality. Some of the wastewater generated will be collected and wastewater will be treated before being discharged into rivers. The process of treatment is necessary to ensure there is minimum impact on environment. There are many methods that can be used to perform the treatment. Combining wastewater remediation with algal biomass production is likely one of the most economically and environmentally sustainable ways to produce bio-energy and bio-products since wastewaters provide not only water source but also most of the necessary nutrients for algae growth, and thus significant reductions in production costs associated with credits for wastewater

treatment, as well as mitigation of the greenhouse gas emissions can be achieved (Pittmann et al., 2010)

The research of growing algae in municipal wastewaters has been under investigation for more than half a century (Oswald et al., 1978 ; Tam and Wong, 1989). Municipal wastewater collected from various stages during treatment process has been tested for their ability of supporting algae growth in previous studies. These different types of wastewater include primary clarifier effluent [Tam and Wong, 1989], and [Lau et al., 1995]), activated sewage filtrates [Tam and Wong, 1989], and effluent from secondary treatment tank (Oswald et al., 1978). The centrate, a highly concentrated municipal wastewater stream generated through dewatering of sludge from the primary settling, has the characteristics of rich in nutrients including phosphorus, ammonia, and organic nitrogen. At this moment, rare studies have been conducted to test the suitability of growing algae in raw centrate, thus our current research effort in this area is necessary and important.

1.2 Problem statement

Municipal wastewater are a complex mixture of human waste, suspended solids, debris and a variety of chemicals that come from residential, commercial and industrial activities. It become one of the major concerns of the environment problems. As the wastewater is found to be highly contaminated, it could not be discharged directly into the environment. Therefore, wastewater treatment is essential to minimize the effect of the contaminants to nature. The major problem with most wastewater treatment is the very high concentration of nutrients,

particularly total Nitrogen and Phosphorus concentration as well as toxic metals which require high cost of chemical based treatment to remove high concentration of nutrients during wastewater treatment. Therefore, we use algae-based wastewater treatment to provide cost-effective and sustainable means of algal growth for biofuels production.

1.3 Objectives of study

The objectives of this study is to identify the nutrient removal efficiency and lipid production by mixed algae culture and wild strain sp. in concentrated municipal wastewater (centrate).

1.4 Scope of study

Scopes of the study were identified in order to achieve the research objectives. The scope research includes:

- To investigate the growth rate, biomass yield, nutrients removal efficiency and lipid productivity for algae grown in highly concentration of municipal waste (centrate).
- Growth of microalgae will be determined by dry biomass measurement.
- To investigate amount of nutrient removal during algae-based wastewater treatment.

1.5 Significance of Study

The main benefit to be gain from this experiment is the production of algae with high lipid content in concentrated municipal wastewater (centrate). Additional research into the removal of pollutants, such inorganic salts of nitrogen and phosphorus and organic materials from wastewater using algae can reduce the environmental pollution and lighten the operations of wastewater treatment plant. The significant advantage of algal processes in wastewater treatment over the conventional chemical-based treatment methods is the potential cost saving and the lower level technology that is utilised, therefore making this approach more attractive to developing countries.

1.6 Summary

This introduction comprises of five subchapters. The first subchapter is about the background of research itself. For the second part comprises of the problem statement for this process. Then, it will state the main objectives of the research, following by scope of the research and significance of research itself.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Literature review is a research report that already done by other researcher or intelligent people about their project. This report includes journal, book, article and many. All this report will help in the project that will be carried out.

More knowledge required because it will help to well understanding about the project. It will help to do the experiment properly and accurately and fast. Other than that, it can help during presentation session so that the panel can understand what the purpose or target of the research conduct.

Growth of algae using highly concentrated municipal waste water as carbon source is the project topic. The material relate about this topic were taken to give information and understanding to the reader.

2.2 Pollution

Pollution is the action of environmental contamination with man-made waste. This includes mainly land, water and air. Pollution can come in various forms including the lesser-known noise, light and thermal pollution.

2.2.1 Water Pollution

Water pollution is the action of environmental contamination with man-made waste into water. The source of this waste could be raw sewage, chemicals, trash or fertilizer. Water pollution has severe human consequences, since less than 3% of the Earth contains water that is potable or safe for drinking (Dubay et al., 1999).

Water pollution comes from industrial, agricultural and domestic sources. The industrial sector made major water pollution. As the factories become larger, they need more water to remove both chemicals wastes and heat. According to Kapuchella (1986), water pollution can be defined as any change in water caused by Homo sapiens and considered by Homo sapiens to be unfavourable to Homo sapiens or to other forms of life. Basically, water pollution is any human action that impairs the use of water as resource.

Water pollution can also be defined as the addition to water of an access material that is harmful to the living organisms or which impairs the beneficial uses of water. Pollution makes water physically impure or filthy. It changes the natural qualities of water, so that it becomes unsuitable for the uses to which it is normally

put to. Pollutants may be present in water either as suspended particles or as dissolved compound or both (Sodhi, 2005).

Water pollutants are classified into four board categories which are chemical, physical, physiological and biological as shown in Table 2.1.

Table 2.1 Types of Water Pollutants

Chemical	Physical	Physiological	Biological
Organic Pollutants	Colour	Taste	Weeds
Inorganic Pollutants	Turbidity	Odour	Algae
	Suspended matter		Viruses
	Radioactivity		Bacteria
	Thermal		Protozoa
			Parasitic worms

(Source : Sodhi, 2005)

a) Municipal Waste water

Municipal wastewater effluent consists of sanitary waste, collected in sewers and from households. It also includes waste from industry, commercial establishments, and institutions. Depending on the level of treatment, municipal wastewater typically contains human and other organic waste, nutrients, pathogens, microorganisms, suspended solids, and household and industrial chemicals not removed by the treatment process. In some cases, urban runoff or stormwater is collected in the same collection system as sanitary waste, adding different ingredients to municipal wastewater entering wastewater facilities.

2.3 Algae

Algae, particularly green unicellular microalgae have been proposed for a long time as a potential renewable fuel source (Benemann et al., 1977; Oswald and Golueke, 1960). Algae have been recognized for their potential to provide an efficient and high quality feedstock without competing with food, feed or arable land surface. The great advantage of using algae as feedstock for current biofuels is the low-cost biofuels processing technology and the established downstream production chains and even the effective integration to fuel markets, as supply chains may not exist for all advanced biofuels (Slade et al., 2009), hindering their market penetration. Algae have the potential to reduce Green House Gaseous emissions through the replacement of fossil fuels.

Algae can be divided into two groups which are macroalgae and microalgae. Macroalgae is the plant that has no root stems and leaves. Sometimes they are look-alike leaves or stems. Macroalgae can be divided into three groups which are green macroalgae, red macroalgae and brown macroalgae. There are about 200 species of macroalgae are used worldwide and about 10 species of them in intensive culture (Bowles, 2007)

2.4 Microalgae

Microalgae are a group of autotrophic unicellular microorganisms categorized in the eukaryotic and prokaryotic microalgae photosynthetic microorganisms. Microalgae can grow almost anywhere, requiring sunlight and some simple nutrients,

and the growth can be accelerated by the addition of specific nutrients. They are can be found either in marine or freshwater. It is a microscopic photosynthetic mechanism similar with land based plant. But it is a simpler cellular structure and submerged in an aqueous environment where it is easy to reproduce with water, Carbon dioxide (CO_2), sunlight and other nutrients (Bowles, 2007).

Microalgae commonly used are the *Cynophyceae* (blue-green algae), *Chlorophyceae* (green algae), *Bacillarlophyceae* (including the diatoms) and *Chrysophyceae* (including golden algae). The most important factors for optimizing the microalgae culture are light intensity, temperature, nutrients, pH and CO_2 concentration (Travieso et al., 2006).

Currently biofuel produced from algae is expensive but cost can be reduced by growing it on cheap medium as algae require few nutrients for its growth which can be derived from wastewater and even it can be grown on land unsuitable for other crops thus eliminating the competition with food crops. Monoculture of algae is difficult to maintain therefore mixed algae cultures can be used for nutrient removal from wastewater and biodiesel production. The oil yield of algae was estimated to be 5000 to 20,000 gallons per acre per year (Chisti, 2008).

2.5 Requirement for Microalgae Growth

2.5.1 Light Intensity

Light is the important factor for microalgae culture. There is effect on the microalgae photosynthesis kinetic. Algae are phototropic and that light energy is the

growth limiting substrate. This is nice because the energy source which is sunlight can be gotten for free. But it is very difficult to expose microalgae culture to a sufficient amount of light energy and to utilize this energy efficiently for biomass production (Jannsen, 2002). According to the research that done by Jeon (2005), the volumetric photosynthetic activity increased with increasing the incident light intensity, and eventually reached the maximum value. Therefore, when microalgae cultured in more depth or cell concentration of water, the light intensity must be increased (Siti Norasyikin, 2010).

2.5.2 Temperature

Temperature is the important environmental parameters effect on growth of algae and hetetrophic bacteria in wastewater. Temperature is known to influence the biomass composition, nutrient requirement, nature of metabolism and the metabolic reaction rate. Microorganisms do not have the ability to regulate their internal temperature. This may in turn effect the microorganism growth and substrate utilization rate (Jeon, 2005).

The optimum temperature for bacterial growth is between 15^{o}C and 19^{o}C (Jou, 2003). For microalgae, the most commonly cultured temperature between 16^{o}C and 27 ^{o}C (Siti Norasyikin, 2010).

2.5.3 Nutrients

Microalgae are easy to cultivate. Nutrient need to be provided is simple such as ammonium or nitrates, phosphates, trace amounts of certain metals and most importantly the greenhouse gaseous carbon dioxide (Janssen, 2002). In general, the concentration of phytoplankton cells in culture was higher than that found in nature (Siti Norasyikin, 2010). Thus, microalgae should be cultivated with adequate nutrition content to ensure the optimum cultivation of microalgae.

2.5.4 pH

In the other hand, pH values also the important factor for optimizing the microalgae culture. The pH values of microalgae cultures were changed by the time from initial phase up to stationary phase depending on the algal species and the growth media. Variation in pH can affect metabolism and growth of algae in a number of ways, including altering the equilibrium of inorganic carbon (C) species, changing availability of nutrients and at extremes directly affecting cell physiology. Most algae have pH optima for growth and photosynthesis in the neutral to alkaline pH range, but species may be found growing in acid conditions as low as pH 1±0 (Albertano et al., 1971)

The pH value needs to be controlled within certain limits. The additional CO_2 that needs to be added for the alga culture to grow rapidly lowers pH, while the respiration and usage of CO_2 increases pH. Nutrients also have to be added without affecting the pH value too much