Faculty Resource Science and Technology

# UTILIZATION OF DIFFERENT NITROGEN SOURCES BY *SCENEDESMUS DIMORPHUS*

**NURUL AIN NAJIAH BINTI DAUD**

Bachelor of Science with Honours
(Aquatic Resource Science and Management)
2012

## DECLARATION

I hereby declare that the work in this project is my own except for qoutations and summaries which have been duly acknowledged. No portion of the work referred to in this dissertation has been summitted in support of an application for another degree qualification of this or any other university or institution of higher learning.

.........................................

Nurul Ain Najiah binti Daud

Aquatic Resources Science and Management Programme

Faculty of Resource Science and Technology

University Malaysia Sarawak

**Utilization of Different Nitrogen Sources by *Scenedesmus dimorphus***

**Nurul Ain Najiah binti Daud (24707)**

This project is submitted in partial fulfilment of the requirement
for degree of Bachelor of Science with Honours
(Aquatic Resource Science & Management)

**Supervisor: Associate Professor Dr. Norhadi Ismail**

Faculty of Resource Science and Technology
UNIVERSITI MALAYSIA SARAWAK
2012

## ACKNOWLEDGEMENT

The work included in this research has been carried out at the Department of Aquatic Science, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak (UNIMAS) from July 2010 to May 2012. I have really enjoyed working on this project from the very beginning!

I would above all like to express my deepest gratitude to my supervisor, Associate Professor Dr. Norhadi Ismail for his guidance, opinions, comments and encouragement as well as constant belief in me that I was the right person for this project. Also, great appreciation to all lecturers, including Associate Professor Dr. Ling Teck Yee and my examiner, Associate Professor Dr. Othman Bojo.

Besides, I would like to dedicate the precious acknowledgement to my collegues and lab mates that have been helping me throughout my project: Fizawati bt. Kamis, Nurul Nadia bt. Rajami, Nur Athirah bt. Saidi, as well as my senior, Samantha Siong Ling Chee and Angie anak Sapis for their help and tips as well as Chemistry Master student, Azlan. I am also grateful to the Aquatic Botany lab assistants, Mr. Zaidi Ibrahim and Mr. Mohd. Norazlan Bujang as well as other lab assistant, Mdm Lucy, Mr. Zulkifli Ahmad, Mr. Nazri and Mr. Mustapha Kamal. Thank you for valuable inputs and cooperation.

Thanks also goes to all my friends and people that have been contributed throughout my project. Last, but closest to my heart, I would like to thank my parents and siblings for all your love and encouragement. This would not have been possible without your support! You made all my days precious.

## TABLE OF CONTENTS


ACKNOWLEDGEMENT ........................................................................................ I

TABLE OF CONTENTS ........................................................................................ II

LIST OF ABBREVIATIONS ........................................................................................ V

LIST OF TABLES ........................................................................................ VI

LIST OF FIGURES ........................................................................................ VII

ABSTRACT ........................................................................................ 1

1.0 INTRODUCTION ........................................................................................ 2

2.0 LITERATURE REVIEW ........................................................................................ 4

2.1 General Information of Microalgae ........................................................................ 4

2.1.1 Macroalgae vs. Microalgae ........................................................ 5

2.1.2 Characteristics of Microalgae ........................................................ 5

2.1.3 Current Usage of Microalgae ........................................................ 6

2.1.4 *Scenedesmus dimorphus*........................................................ 6

2.2 Lipids from Algae ........................................................................................ 7

2.3 Biodiesel from Algae ........................................................................................ 10

2.4 Algal Growth Curve........................................................................................ 11

2.5 Fertilizer Nutrients in Animal Manure ........................................................................ 12

2.6 Harvesting of Microalgal Cultures........................................................................ 15

2.6.1 Flocculation ........................................................ 15

2.6.2 Centrifugation ........................................................ 15

2.6.3 Filtration ........................................................ 16

2.7 Lipid Extraction from Microalgae ........................................................ 16

3.0 MATERIALS AND METHODS ........................................................................................ 18

3.1 Microalgae (*Scenedesmus dimorphus*) Collection ………………………………... 18
3.2 Preparation of microalgae culture medium, Modified Bristol's Medium (MBM) 19
3.3 Preparation of Animal Manure Stock Medium................................................... 20
3.3.1 Chicken manure ………………………………………………………... 20
3.3.2 Cow manure……………………………………………………………. 20
3.4 Batch Culture studies of *S. dimorphus*............................................................ 21
3.4.1 Treatment 1 (Chicken Manure) ................................................................ 21
3.4.2 Treatment 2 (Cow Manure) ...................................................................... 21
3.5 Growth Measurement and Data Analysis ………………………………………. 24
3.6 Mass culture of *S. dimorphus*…………………………………………………… 24
3.6.1 Harvesting *Scenedesmus dimorphus* in mass culture …………………….. 25
3.7 Lipid Extraction by Soxhlet Method.................................................................... 25
3.8 Statistical Analysis............................................................................................... 26
Flow Chart ............................................................................................................... 27
4.0 RESULTS.............................................................................................................. 28
4.1 Batch Culture …………………………………………………………………….. 28
4.1.1 Chicken Manure ………………………………………………………… 28
4.1.2 Cow Manure …………………………………………………………….. 31
4.2 Growth Rate of *S. dimorphus*…………………………………………………….. 33
4.3.1 Growth Rate in Chicken Manure ………………………………………… 33
4.3.2 Growth Rate in Cow Manure ……………………………………………… 35
4.3 Cell Size and Structure Observed ……………………………………………... 37
4.4 Outdoor Mass Culture ………………………………………………………… 40
4.5 Lipid Yield ……………………………………………………………………….. 42

5.0 DISCUSSION........................................................................................................ 43

5.1 Effect of animal manure as nitrogen sources and pH on cell growth …………. 43

5.2 Effect of animal manure at different pH level on cell growth…………………. 45

5.3 Effect of animal manure on mass culture on cell growth and lipid yield……… 46

5.4 Efficiency of micro algal harvest and lipid extraction method ……………….. 48

5.5 Limitations and Recommendations …………………………………………… 49

6.0 CONCLUSION..................................................................................................... 50

7.0 REFERENCES ………………………………………………………………. 51

8.0 APPENDICES……………………………………………………………….. 56

## LIST OF ABBREVIATIONS

| | |
|---|---|
| ACCase | Acetyl-CoA carboxylase |
| ANOVA | Analysis of variances |
| BOD | Biological oxygen demand |
| COD | Chemical oxygen demand |
| $CaCl_2 \cdot 2H_2O$ | Calcium chloride dihydrate |
| $CO_2$ | Carbon dioxide |
| $dH_2O$ | Distilled water |
| g | gram |
| h | hour |
| $H_2O$ | Water |
| $K_2HPO_4$ | Potassium hydrogen phosphate/ Dipotassium hydrogen phosphate |
| $KH_2PO_4$ | Potassium dihydrogen phosphate |
| L | Litre |
| m | meter |
| mg | milligram |
| ml | milliliter |
| $MgSO_4 \cdot 7H_2O$ | Magnesium sulphate |
| MBM | Modified Bristol's Medium |
| N | Nitrogen |
| NaCl | Sodium chloride |
| $NaNO_3$ | Sodium nitrate |
| $NO_3$ | Nitrate |
| P | Phosphorus |
| PGA | Polyglutamic acid |
| $PO_4$ | Phosphate |
| TAG | Triacylglycerols |
| UNIMAS | Universiti Malaysia Sarawak |
| UTEX | University of Texas |
| W | Watt |

## LIST OF TABLES


| | | |
|---|---|---|
| **Table 2.1:** | Microalgae strains currently being studied for biofuel | 4 |
| **Table 2.2:** | Chemical composition of algae expressed on a dry matter basis (%) | 8 |
| **Table 2.3:** | Vegetable Oil Yields | 9 |
| **Table 2.4:** | Production of oil from different crops | 11 |
| **Table 2.5:** | Average Nutrient Analysis of Major Types of Manure | 12 |
| **Table 2.6:** | Components in chicken manure stock solution | 13 |
| **Table 2.7:** | Nutrient characteristics of batches swine manure used in outdoor experiments, values in mg N or P per L | 13 |
| **Table 2.8:** | Physico-chemical properties of animal waste extract | 14 |
| **Table 2.9:** | Some common extraction methods explored in the last decade and their effectiveness at recovering lipids and lipid products | 17 |
| **Table 3.1:** | Chemical composition of Modified Bristol's Medium (MBM) | 19 |
| **Table 3.2:** | Chemical composition of Modified Bristol's Medium 1 (MBM1) | 20 |
| **Table 4.2.1:** | Result of Two-way Analysis of Variance (ANOVA) performed on the growth rate of *S. dimorphus*. | 34 |
| **Table 4.2.2:** | Result of Two-way Analysis of Variance (ANOVA) performed on the growth rate of *S. dimorphus*. | 36 |
| **Table 4.5.1:** | Influence of chicken and cow manure on dried biomass production and lipid content of *S. dimorphus*. | 42 |

# LIST OF FIGURES


| | | |
|---|---|---|
| **Figure 2.1:** | *Scenedesmus dimorphus* colony cells | 7 |
| **Figure 2.2:** | Schematic representation of algae growth rate in batch culture (solid line) and nutrients concentration (dashed line) | 12 |
| **Figure 2.3:** | Schematic relation of poultry manure/cow manure, paper waste and algae sludge for biomethane, biofertilizer and biodiesel via microalga *Chlorella vulgaris* cells | 13 |
| **Figure 3.1:** | Stock culture of *Scenedesmus dimorphus* | 18 |
| **Figure 3.2(a):** | Illustration of Treatment 1 that using different concentrations of chicken manure and at different pH. | 22 |
| **Figure 3.2(b):** | Illustration of Treatment 1 that using different concentrations of cow manure and at different pH. | 23 |
| **Figure 3.3:** | Soxhlet Lipid Extraction Method | 25 |
| **Figure 3.4:** | Flow chart of the overview experimental designed | 27 |
| **Figure 4.1.1:** | Growth curve of *Scenedemus dimorphus* in batch culture of chicken manure | 30 |
| **Figure 4.1.2:** | Growth curve of *Scenedemus dimorphus* in batch culture of cow manure | 32 |
| **Figure 4.2.1:** | Growth rate of *Scenedesmus dimorphus* in different concentrations medium of chicken manure (%) at pH 6, 7 and 8 in batch culture. | 33 |
| **Figure 4.2.2:** | Growth rate of *Scenedesmus dimorphus* in different concentrations medium of cow manure (%) at pH 6, 7 and 8 in batch culture. | 34 |
| **Figure 4.3.1:** | *Scenedesmus dimorphus* fat cells structure. (a) Modified Bristol Medium (MBM), (b) Chicken manure, and (c) Cow manure. (Scale bar = 10µm). | 37 |
| **Figure 4.3.2:** | Single form of *Scenedesmus dimorphus* throughout the exponential growth phase (Scale bar = 10µm). | 38 |
| **Figure 4.3.3:** | *Scenedesmus dimorphus* colony of two and four during the early stage of experiment and also declining phase (Scale bar = 10µm). | 38 |
| **Figure 4.3.4:** | Dividing cells of *Scenedesmus dimorphus* were also found throughout the counting process at Day 12 (Scale bar = 10µm). | 38 |
| **Figure 4.3.5:** | Four and, seven and eight-celled in colony of *Scenedesmus dimorphus* were found in linear growth phase as well as in late exponential phase (Scale bar = 10µm). | 39 |
| **Figure 4.4.1:** | Growth curves of *Scenedesmus dimorphus* in 3.5% of chicken manure and 22.5% of cow manure at pH 8 during outdoor mass cultivation. | 40 |
| **Figure 4.4.2:** | Growth rate of *Scenedesmus dimorphus* in different concentrations medium of animal manure (%) at pH 8 during outdoor mass cultivation. | 41 |

## Utilization of Different Nitrogen Sources by *Scenedesmus dimorphus*

**Nurul Ain Najiah binti Daud**

Aquatic Resource Science and Management
Faculty of Resource Science and Technology
Universiti Malaysia Sarawak

## ABSTRACT

Microalgae cultivation has received much attention in biofuel researches as it possessed several advantages, including high photosynthetic efficiency as well as high growth rates and high biomass production compared to other energy crops. The present study aims to evaluate the possibility of using chicken and cow manure as organic sources of nitrogen. In this study, the freshwater green microalgae *Scenedesmus dimorphus* was cultivated in growth medium at pH 6, 7 and 8, which was enriched with different concentrations of chicken and cow manure as nitrogen sources. The concentration of chicken manure and cow manure which gave maximum algal growth rates were 3.5% and 22.5% respectively. These two N concentrations were used for the outdoor algal mass culture in 10 L medium for 20 days. The algal biomass in the mass culture was harvested at the end of the experiments and their lipid was extracted in n-hexane by using a Soxhlet extraction method. Results showed that the lipid yield produced by the alga when cultured in chicken manure was about 11.23% and 11.14% when grew in cow manure. This experiment showed that *S. dimorphus* can be grown in animal manure as their source of N.

**Keywords:** *Scenedesmus dimorphus*, chicken manure, cow manure, lipid content.

## ABSTRAK

*Penternakan mikroalga telah mendapat perhatian disebabkan dalam kajian biofuel yang mempunyai beberapa kelebihan termasuklah efisiennya fotosintetik dan mempunyai kadar pertumbuhan yang tinggi dan menghasilkan biojisim yang tinggi berbanding dengan tumbuhan biasa. Kajian ini dijalankan bertujuan untuk mentaksir kemungkinan menggunakan baja-najis ayam dan lembu sebagai sumber organic nitrogen. Dalam kajian ini, mikroalga hijau air tawar* Scenedesmus dimorphus *diternak di dalam medium pada pH 6, 7 dan 8, yang diperkaya dengan kepekatan baja-najis ayam dan baja-najis lembu yang berlainan sebagai sumber nitrogen. Kepekatan baja-najis ayam dan baja-naijs lembu pada kadar pertumbuhan yang maksima adalah di 3.5% dan 22.5%. Kedua-dua kepekatan N tersebut telah digunakan untuk pengkulturan luar secara besar-besaran di dalam 10 L medium selama 20 hari. Biojisim alga dari pengkulturan luar besar-besaran dituai di akhir eksperimen dan kandungan lipid diekstrak dalam n-hexane dengan menggunakan kaedah pengekstrak Soxhlet. Keputusan menunjukkan hasil lipid apabila dikultur dalam baja-najis ayam adalah dianggarkan sebanyak 11.23% dan 11.14% apabila dikultur dalam baja-najis lembu dianggarkan sebanyak 11.14%. Eksperimen ini menujukkan bahawa* S. dimorphus *boleh tumbuh di dalam baja-najis haiwan sebagai sumber N.*

***Kata kunci:*** Scenedesmus dimorphus, *baja-najis ayam, baja-najis lembu, lipid*

## 1.0 INTRODUCTION

The world has put increasingly more attention into the development and utilization of alternative energy resources as a result of continued fossil fuel depletion, increasing air pollution and global warming (Farrel *et al*., 2006). The biofuel production from photosynthetic microorganisms is considered as an effective strategy to produce renewable energy (Vicente *et al*., 2004). One promising candidate of biomass for alternative fuel production is microalgae, which have high growth rates (Rittmann, 2008).

In recent years, microalgae cultivation has received much attention on account of their utility (Ono and Cuello, 2006) as it possessed several advantages, including high photosynthetic efficiency as well as high growth rates and high biomass production compared to other energy crops (Goswami, 2011). Several microalgae strains have been reported to have the ability to accumulate large quantities of lipids. Recent studies have found that the lipid content of algae could be increased through changing cultivation conditions, such as $CO_2$ (Chiu *et al*., 2009), nitrogen depletion (Li *et al.*, 2008), phosphate limitation (Reitan *et al.*, 1994), temperature, high salinity (Rao *et al.*, 2007), high iron concentration (Liu *et al.*, 2008) and nutrient concentration (Converti *et al*., 2009). Although ammonia and urea were often used in mass cultivation owing to the relatively low-cost (Matsudo *et al.*, 2009), selecting proper nitrogen source for each algal species is important in improving biomass and oil productivity (Li *et al*, 2008).

Biodiesel fuel is becoming more promising as it is from non toxic, biodegradable and renewable resources as well as its use leads to a decrease in the emission of harmful air pollutants (Gouveia and Oliveira, 2009). Biodiesel is usually produced from oleaginous crops such as rapeseed, soybean, sunflower and palm through a chemical process of their oils with short chain alcohols (Lang *et al.*, 2001). Microalgae are able to produce 15-300 times more oil for biodiesel production compared to traditional crops on an area basis (Dragone *et al.*, 2008). Meanwhile when compared with conventional crop plants which are usually harvested once or twice a year, microalgae have a very short harvesting cycle which allowing multiple or continuous harvests with significantly increased yields (Schenk *et al.,* 2008).

Since fatty acid methyl esters originating from vegetable oils and animal fats are known as biodiesel, from the energetic point of view, lipids are the most desirable components of microalgae cells (Sostaric *et al*., 2009). In addition, the organic matter produced by photosynthetic microalgae can be transformed into a wide range of valuable products, such as biodiesel, food additives and health care products (Pulz and Gross, 2004). Most research works have focused on the growth and lipid accumulation through cultivation in photo-bioreactors and the lipid content of algae could be increased dramatically under certain stress factors (Qiang and Junda, 2011). However, little research has been conducted on cultivation of microalgae by using chicken and cow manure as organic nitrogen source to determine the lipid content.

The objectives of this study were, (i) to evaluate the possibility of using chicken and cow manure as organic sources of nitrogen for *Scenedesmus dimorphus* culture, (ii) to compare the cell growth of *S. dimorphus* cultured in different concentrations of animal manure and pH levels, (iii) to gain the information about the lipid yield of *S. dimorphus* cultured in animal manure.

## 2.0 LITERATURE REVIEW

### 2.1 General Information of Microalgae

Algae are a diverse group of eukaryotic organisms that belong to the Phylum Protista. These organisms use light energy to convert $CO_2$ and $H_2O$ into carbohydrates and other cellular products. During this process oxygen is released. Algae contain chlorophyll *a*, which is required for photosynthesis. Many algae contain other pigments that extend the range of light that can be used by these organisms for photosynthesis. Organisms that are classified as algae are quite diverse (Luz *et al.,* 1997).

All algae are primarily made up of proteins, carbohydrates, fats, and nucleic acids in varying proportions. While the percentages can vary with the type of algae, some types of algae are made up of up to 40% fatty acids based on their overall mass. It is this fatty acid that can be extracted and converted into biofuel (Xiaodong *et al*., 2009).

There are a number of species of algae that are being studied for their suitability as feed stock for biofuel production. Table 2.1 gives a list of these species. (Encarnación *et al.*, 2010).

**Table 2.1:** Microalgae strains currently being studied for biofuel (Encarnación *et al.,* 2010).

| Species/Taxa | Class | Explanation |
|---|---|---|
| *Neochloris oleoabundans* | Chlorophyceae | |
| *Scenedesmus dimorphus* | Chlorophyceae | Preferred species for oil production for biodiesel. Problem – produces thick sediment if not constantly agitated |
| *Phaeodactylum tricornutum* | Bacillariophyceae | |
| *Pleurochrysis carterae* | Prymnesiophyceae | Unicellular coccolithophorid alga. Able to calcify subcellularly |
| *Prymnesium parvum* | Prymnesiophyceae | Toxic algae |
| *Tetraselmis chuii* | Chlorophyceae | Marine unicellular alga |
| *Isochrysis galbana* | Prymnesiophyceae | Microalga |
| *Botryococcus braunii* | Trebouxiophyceae | Can produce long chain hydrocarbons representing 86% of its dry weight |
| *Dunaliella tertiolecta* | Chlorophyceae | Oil yield of about 37%. Fast growing. |

### 2.1.1 Macroalgae vs. Microalgae

There are two groups of algae: macroalgae and microalgae. Macroalgae are large in term of size and multicellular often seen growing in ponds (Carlsson *et al.*, 2007). The largest multicellular algae are called seaweed, as an example of this is the giant kelp which can grow over 25 m in length (Johnson, 2009). Microalgae, on the other hand are tiny organisms of micrometers in size. These unicellular algae normally grow in suspension within a water body (Chang, 2007). Besides, microalgae are often responsible for the appearance of cloudiness within a pond or aquarium. Both types of algae grow extremely fast. The largest seaweed, giant kelp is known to grow as fast as 50 cm per day and can reach a length up to 80 m (Thomas, 2002). Likewise, microalgae are known to contain large amounts of lipids within their cell structure and they are increasingly becoming an interest as a biofuel feedstock.

### 2.1.2 Characteristics of Microalgae

Microalgae recognized as one of the oldest living organisms, are thallophytes which means plants lacking roots, stems and leaves. They have chlorophyll *a* as their primary photosynthetic pigment and lack of sterile covering of cells around the reproductive cells (Brennan and Owende, 2010). While the mechanism of photosynthesis in these microorganisms is similar to that of higher plants, they are generally efficient converters of solar energy because of their similar cellular structure. Moreover, they have more efficient access to water, carbon dioxide and other nutrients because the cells grow in aqueous suspension (Chisti, 2007).

Microalgae can be either autotrophic or heterotrophic. If they are autotrophic, they use inorganic compounds as a source of carbon. Autotrophs can be photoautotrophic as it using light as a source of energy or chemoautotrophic as it oxidizing inorganic compounds for energy. If they are heterotrophic, microalgae use organic compounds for growth. Heterotrophs can be photoheterotrophs as it using light as a source of energy or chemoheterotrophs as it oxidizing organic compounds for energy. Some photosynthetic microalgae are mixotrophic, combining heterotrophy and autotrophy by photosynthesis (Lee, 2001). For autotrophic algae, photosynthesis is a key component of their survival, whereby they convert solar radiation and $CO_2$ absorbed by chloroplasts (Brennan and Owende, 2010).

### 2.1.3 Current Usage of Microalgae

Microalgae have several useful characteristics which enable them to be used in a variety of ways (Johnson, 2009). The high lipid, carbohydrate and protein contents of many algal species have driven research in a wide spectrum of uses. These vary from food products to biofuels, to use for phycoremediation. The exponential growth of algae under ideal nutrient loads has lead to the idea of algae as a phycoremediation tool (Olguin, 2003), considering that the nutrients that algae needs are often a waste products, such as nitrogen and phosphorus (Johnson, 2009). Algal biomass can also be used as a biosorpent to clean contaminated waste streams (El Sikaily and Khaled, 2006).

### 2.1.4 *Scenedesmus dimorphus*

*Scenedesmus dimorphus* shown in Figure 2.1 is a green microalgae, bean shaped of approximately 10μm in size. It is categorized as a heavy bacterium; *S. dimorphuss* has a lipid content of 16-40%, being one of the preferred species for oil yield in the production of biodiesel. One of the problems with this microalga is that it is heavy and forms thick sediments if not kept in constant agitation. The optimal growth temperature for this strain falls between 30-35°C. *S. dimorphus* will use any and all light it is given and should be further researched for use in mass production (John *et al*., 2005). According to Goswami (2011), *S. dimorphus* contains a large amount of intracellular lipids which starts to accumulate when there is a stress condition.


**Figure 2.1:** *Scenedesmus dimorphus* colony cells. (Source NBRP-Algae, 2009).

## 2.2 Lipids from Algae

Microalgae contain lipids and fatty acids as its membrane components, metabolites, storage products, and sources of energy. Diatoms and cyanobacteria have been found to contain high levels of lipids - over 30%. Due to the high lipid content, these microalgal strains are of great interest in the search for sustainable sources for the production of biodiesel (Damiani, *et al.,* 2010). Table 2.2 shows the chemical composition of algae. It has been found that algae can contain between 2% and 40% lipids by weight.

**Table 2.2:** Chemical composition of algae expressed on a dry matter basis (%) (Mata *et al.*, 2010).

| Strain | Protein | Carbohydrates | Lipids | Nucleic acid |
|---|---|---|---|---|
| *Scenedesmus obliquus* | 50-56 | 10-17 | 12-14 | 3-6 |
| *Scenedesmus quadricauda* | 47 | - | 1.9 | - |
| *Scenedesmus dimorphus* | 8-18 | 21-52 | 16-40 | - |
| *Chlamydomonas reinhardtii* | 48 | 17 | 21 | - |
| *Chlorella vulgaris* | 51-58 | 12-17 | 14-22 | 4-5 |
| *Chlorella pyrenoidosa* | 57 | 26 | 2 | - |
| *Spirogyra* sp. | 6-20 | 33-64 | 11-21 | - |
| *Dunaliella bioculata* | 49 | 4 | 8 | - |
| *Dunaliella salina* | 57 | 32 | 6 | - |
| *Euglena gracilis* | 39-61 | 14-18 | 14-20 | - |
| *Prymnesium parvum* | 28-45 | 25-33 | 22-38 | 1-2 |
| *Tetraselmis maculata* | 52 | 15 | 3 | - |
| *Porphyridium cruentum* | 28-39 | 40-57 | 9-14 | - |
| *Spirulina platensis* | 46-63 | 8-14 | 4--9 | 2-5 |
| *Spirulina maxima* | 60-71 | 13-16 | 6-7 | 3-4.5 |
| *Synechoccus* sp. | 63 | 15 | 11 | 5 |
| *Anabaena cylindrica* | 43-56 | 25-30 | 4-7 | - |

Algal oils have been found to be very high in unsaturated fatty acids. Some of these unsaturated fatty acids that are found in different algal species include: arachidonic acid, eicospentaenoic acid, docasahexaenoic acid, gamma-linolenic acid, and linoleic acid (Damiani, *et al.,* 2010). When comparing the lipid yield of algae to vegetable sources, algae can produce between 20,000 and 100,000 liters per hectare. Table 2.3 shows the oil yields for selected terrestrial plants (Mata *et al.*, 2010).

**Table 2.3:** Vegetable Oil Yields (Mata *et al.*, 2010).

| Crop | liters oil/ha | US gal/acre | Crop | liters oil/ha | US gal/acre |
|---|---|---|---|---|---|
| corn (maize) | 172 | 18 | camelina | 583 | 62 |
| cashew nut | 176 | 19 | sesame | 696 | 74 |
| oats | 217 | 23 | safflower | 779 | 83 |
| lupine | 232 | 25 | rice | 828 | 88 |
| kenaf | 273 | 29 | tung oil | 940 | 100 |
| calendula | 305 | 33 | sunflower | 952 | 102 |
| cotton | 325 | 35 | cocoa (cacao) | 1026 | 110 |
| hemp | 363 | 39 | peanut | 1059 | 113 |
| soybean | 446 | 48 | opium poppy | 1163 | 124 |
| coffee | 459 | 49 | rapeseed | 1190 | 127 |
| linseed (flax) | 478 | 51 | olive | 1212 | 129 |
| hazelnut | 482 | 51 | castor bean | 1413 | 151 |
| euphorbia | 524 | 56 | pecan nut | 1791 | 191 |
| pumpkin seed | 534 | 57 | jojoba | 1818 | 194 |
| coriander | 536 | 57 | jatropha | 1892 | 202 |
| mustard seed | 572 | 61 | macadamia nut | 2246 | 240 |

The lipid and fatty acid contents of microalgae differ according to the culture conditions. It has been found that in some cases the lipid content can be enhanced by imposing nitrogen starvation. Biochemical studies have indicated that acetyl-CoA carboxylase (ACCase), a biotin containing enzyme that catalyzes an early step in fatty acid biosynthesis, might be involved in the control of the lipid accumulation process. In light of this fact, it might be possible to increase lipid production rates by increasing the activity of this enzyme by genetically engineering the microalgae (Brune, *et al.*, 1999).

Microalgae are classified as the most primitive form of plants. The mechanism of photosynthesis in microalgae is similar to that in higher plants, but they are usually more efficient converters of solar energy because of their simple cellular structure. Due to the fact that the cells grow in aqueous suspension, microalgae have more efficient access to water, $CO_2$, and other nutrients. These factors account for the ability of microalgae to produce larger quantities of oil per unit area of land as compared to terrestrial oilseed crops (Charity *et al.*, 2009).

### 2.3 Biodiesel from Algae

Biodiesel fuel has received considerable attention in recent years as it is made from non-toxic, biodegradable and renewable resources and provides environmental benefits. In addition, biodiesel is usually produced from oleaginous crop (Antolin *et al.*, 2002). The use of microalgae as feed stock can be a suitable alternative because algae are the most efficient biological producer of oil on the planet and a versatile biomass source and may soon be one of the Earth's most important renewable fuel crops (Gouveia and Oliveira, 2009). This is due to its higher photosynthetic efficiency and biomass productivities, fast grower than higher plants and growing in liquid medium which can be handled easily to grow in variable climates and non-arable land included (Chisti, 2007). Algae also use far less water than traditional crops. Furthermore, they do not displace food crop cultures as their production is not seasonal and can be harvested daily (Chisti, 2008).

Biodiesel from algae in itself is not significantly different from biodiesel produced from plant oils. All biodiesel essentially are produced using triglycerides which commonly called fats from the plant or algal oils. Algae produce a lot of polyunsaturated which may present a stability problem since higher levels of polyunsaturated fatty acids tend to decrease the stability of biodiesel (Lang *et al.*, 2001). However, polyunsaturated also have much lower melting points than monounsaturated or saturates, thus algal biodiesel should have much better cold weather properties than many other bio-feedstock. The most significant difference with oil-seed crops is the yield of algal oil as shown on Table 2.4, and hence biodiesel. Microalgae are by a factor of 8 to 25 for palm oil and a factor of 40 to

120 for rapeseed, the highest potential energy yield temperate vegetable oil crop (Encarnacion *et al*., 2010).

**Table 2.4:** Production of oil from different crops (Encarnacion *et al*., 2010).

| Oil | Gallons of Oil per Year per Acre |
|---|---|
| Corn | 18 |
| Soybeans | 48 |
| Safflower | 83 |
| Sunflower | 102 |
| Rapessed | 127 |
| Oil Palm | 635 |
| Microalgae | 5000-15000 |

## 2.4 Algal Growth Curve

Under suitable cultivation conditions and sufficient nutrients, microalgae can grow profusely (Gouveia and Oliveira, 2009). Commonly they double their biomass within 24 h or within 3.5 h during exponential growth phase (Chisti, 2007).

Figure 2.2 represents the algae growth curve in a batch culture (solid line) and nutrients concentration (dashed line), where five reasonably well defined growth phases can be recognized: (1) lag phase; (2) exponential growth phase, representing the maximum growth rate under the specific conditions; (3) linear growth phase; (4) stationary growth phase; (5) decline or death phase. Meanwhile the opposite pattern of dashed curve indicates the nutrients depletion during the stationary phase and onwards.

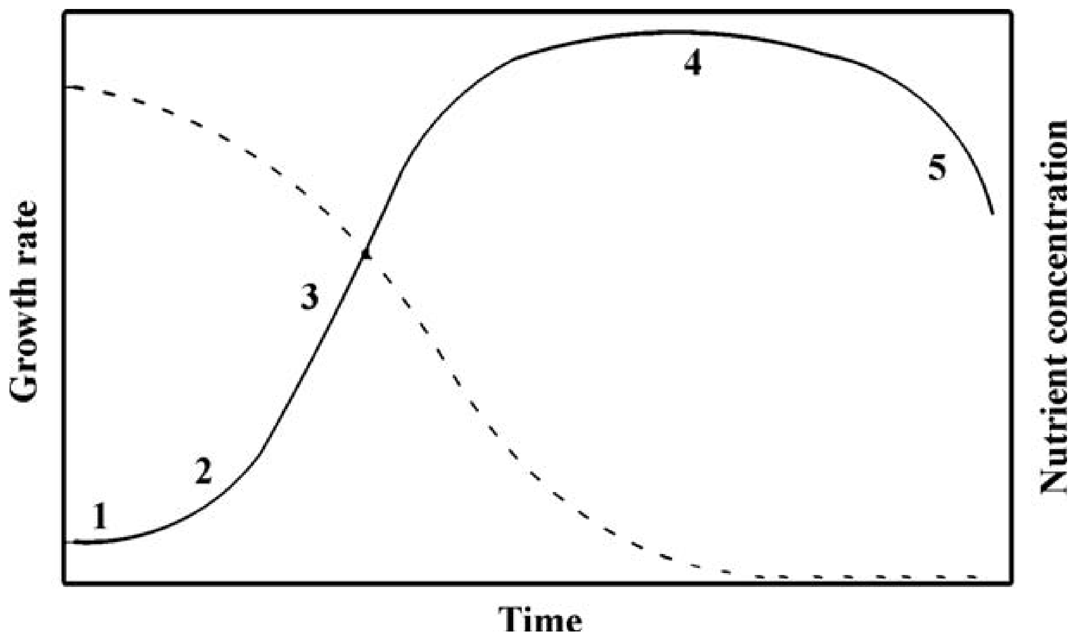


**Figure 2.2:** Schematic representation of algae growth rate in batch culture (solid line) and nutrients concentration (dashed line) (Mata *et al.*, 2010).

## 2.5 Fertilizer Nutrients in Animal Manure

Manure is a by-product containing many plant nutrients and organic matter. Animal manure can be an asset rather than a liability for producers when effectively managed and properly used (Hailin, 2010). The approximate fertilizer nutrient content for various manure is shown in Table 2.5. Besides, cultivation of microalgae in chicken manure (Table 2.6) documented by Li (2011), in order to test a simple and cheap method to produce biomass from microscopic algae in specifically modified bioreactors, which can be used for manual algae biomass production.

**Table 2.5:** Average nutrient analysis of major types of manure (Hailin, 2010).

| Manure type | Dry Matter (%) | Nutrient Content (lbs./ton) | | |
|---|---|---|---|---|
| | | Total N | $P_2O_5$ | $K_2O$ |
| Feedlot Manure | 62 | 24 | 21 | 25 |
| Broiler Litter | 77 | 63 | 61 | 50 |
| | | Nutrient Content (lbs./1000 gal) | | |
| Lagoon Effluent | 0.5 | 4.2 | 1.0 | 5.0 |
| Lagoon Sludge | 7 | 15 | 16 | 11 |
| Dairy Slurry | 3 | 13 | 11 | 11 |

**Table 2.6:** Components in chicken manure stock solution (Li, 2011).

| Component | % on total product | Concentrations (g/2L) |
|---|---|---|
| Nitrogen (N) total | 4.0 | 0.56 |
| Phosphorus ($P_2O_5$) total | 3.0 | 0.42 |
| Potassium ($K_2O$) | 2.5 | 0.35 |
| Magnesium (MgO) total | 1.0 | 0.14 |
| Sulphur S) total | 1.0 | 0.14 |
| Calcium (CaO) | 9.0 | 1.26 |
| Carbon (C/N=9) | 36.0 | 5.04 |

**Table 2.7:** Nutrient characteristics of batches swine manure used in outdoor experiments, values in mg N or P per L (De Pauw *et al.*, 1980).

| | $NH_4$-N | $NO_2$-N | $NO_3$-N | Total inorganic N | Ortho-P | N:P | pH |
|---|---|---|---|---|---|---|---|
| Maximum | 174.0 | 449.0 | 79.0 | 1789.0 | 330.7 | 420.0 | 9.2 |
| Minimum | 77.3 | 0.0 | 0.0 | 80.7 | 2.0 | 1.6 | 8.6 |
| Average | 614.7 | 19.9 | 13.9 | 674.2 | 32.7 | 72.2 | 8.9 |

The feasibility of swine manure tested (Table 2.7) as the sole nutrient source for the *Chlorella saccharophila* (Krüger), and algae developing naturally in enriched seawater and which could be suited as food organisms.

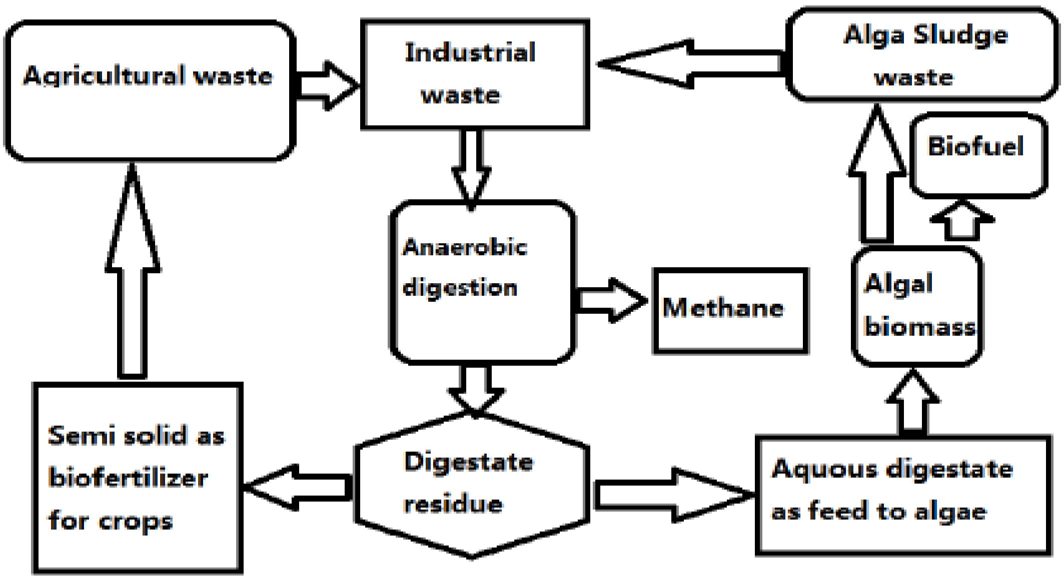


**Figure 2.3:** Schematic relation of poultry manure/cow manure, paper waste and algae sludge for biomethane, biofertilizer and biodiesel via microalga *Chlorella vulgaris* cells (Iyovo *et al.,* 2010).

According to Schefferle (1965), poultry manure is rich in nutrients especially the much needed nitrogen and phosphorus as well as cells growth promoter like glycine is released from poultry manure on decomposition. Magid *et al.* (1995) studied that some nutrients in poultry manure by composition included (g/kg) potassium 37.5, phosphate 25.5 and nitrogen 55.7. Nitrogen is normally in the form of uric acid and about 66% can be available on decomposition (Iyovo *et al.*, 2010). In addition, studies indicated that other trace elements, such as magnesium, calcium, iron,copper, zinc, nickel, lead and chromium existed in digested poultry manure (Bao *et al.*, 2008). Hence, poultry manure has been a traditional organic fertilizer and an attractive source of nutrients which can be retrieved and utilized (Iyovo *et al.*, 2010). There are varieties of animal waste that have been studied used to supplement nutrients for microalgae (Table 2.8).

**Table 2.8:** Physico-chemical properties of animal waste extract (Agwa *et al*., 2012).

| Parameters | Goat waste | Pig waste | Cow waste | Grass cutter waste | Poultry waste |
|---|---|---|---|---|---|
| pH | 7.22 | 6.32 | 6.28 | 6.0 | 6.66 |
| $NO_3$ | 5.06 | 5.06 | 4.60 | 2.61 | 3.83 |
| $PO_4$ | 7.04 | 11.26 | 16.5 | 8.24 | 8.80 |
| BOD | 240 | 320 | 270 | 320 | 160 |
| COD | 844 | 1620 | 1390 | 1540 | 1796 |
| Conductivity | 272 | 293 | 370 | 915 | 827 |