# TUBULAR PHOTOBIOREACTOR FOR MICROALGAE BIODIESEL PRODUCTION

Nkongolo Mulumba, Ihab H. Farag*
Chemical Engineering Department, University of New Hampshire (UNH) W301 Kingsbury Hall,
33 Academic Way, Durham, NH 03824-3591, phone 603-862-2313,
*Correspondence: ihab.farag@unh.edu

**ABSTRACT:** Biodiesel production from algae is a promising technique. Microalgae have the potential to produce 5,000-15,000 gallons of biodiesel/(acre-year). However, there are challenges; these include high yield of algae biomass with high lipid content and the effective technique to harvest the grown algae, extract the algal oil and transesterify the oil to biodiesel.

In this project Tubular PhotoBioReactor (TPBR) was designed and achieved a ten times increase in algae concentration. It produced 1g of dry algal biomass per liter of medium within 12 days, with a lipid content of 12% approximately. Healthy algal culture grew well in the TPBR reaching $56x10^6$ cells/mL of culture medium. The 10 fold increase is higher than those reported for open ponds and helical photobioreactor.

**Keywords:** microalgae biodiesel; microalgae harvesting, microalgae yield, microalgae lipid production, biofuels, cell production.

## 1 INTRODUCTION

All humankind has been relying on non-renewable fuel such as petroleum-based fuel since its discovery more than four thousand years ago. Increasing fuel demand worldwide, limited reserve and its effect on environment push scientists to look for a clean and renewable fuel in replacement of petroleum based fuel. Biodiesel has been loomed as a renewable fuel and potential replacement for petro-fuel.

**1.1 Background:** Biodiesel is a group of fatty methyl- esters produced by a transesterification reaction between fatty acids (triglyceride) and alcohol in presence of a catalyst [15], e.g.,

$$RCOO\text{-}CH_2CH(\text{-}OOCR')CH_2\text{-}OOCR + 3R'COH \rightarrow 3\ R\text{-}COR' + CH_2OH\text{-}CHOH\text{-}CH_2OH$$

where R represents fatty acids chains [2]. Fatty acids (FA) used in biodiesel production come from animal fat or vegetal oil. One of the biggest challenges in biodiesel production is finding sufficient feedstock capable of meeting the demand. Both oil and alcohol are extracted from food crops such as corn, canola, soybean, palm, etc. In effect this process is diverting a food crop to be an energy crop.

There is already an increase rejection of food for bio-fuel. The United Nations (UN), for instance, is expressing a strong disapproval of using food crops for bio-fuel, which they believe to be the key in foods price increase and food shortage, although none of these crops could produce enough oil to be converted in biodiesel able to meet worldwide demand

The United States alone consume about 70 billion gallons of diesel per year. This cannot be met with oil from corn, soybean nor canola, which yield nearly 50, 60 and 90 gallons of biodiesel per acre per year respectively. Nonetheless, microalgae could produce 5000 to 15000 gallons of biodiesel per acre per year in open pond [1, 7, 8]. Obviously, algal biodiesel could potentially replace petro-diesel.

Microalgae are unicellular microorganism, like simple plants with no root and leaves that grow through photosynthesis process. They capture carbon dioxide during photosynthesis and convert it into biomass which can be used as food, fertilizer, a source of medicine and biodiesel [6].

Growing algal cells in open ponds raise several concerns such as impossibility to control growth conditions and contamination risks. Algal cells in open ponds are exposed to the environment, subject to risk of contamination, light deficiency, and heterogeneous medium depending upon the mixing mechanism, the shape of the ponds and the depth of the culture. On the other hand, closed ponds (photobioreactor) mitigate fluid culture contamination, and enhance a full control of algal growth parameters such as light penetration, homogeneous culture, pH and carbon dioxide input. They would use less space with high algal biomass yield. However, they are expensive to build and maintain.

1.2 **Biodiesel production from microalgae:** Production of biodiesel from algae has emerged as a possible unique way to compete petro-diesel. However, biodiesel yield depends upon lipid content of the algae strain used as a source of oil. Some algae strains could contain lipid up to 60% of dry biomass [2].
Medium composition and growth conditions are likely to be the most affecting parameters on oil content in algae.
Following the growth and harvesting of algae, the biodiesel production process consists of two steps: extracting oil using either solvent or mechanical extraction followed by biodiesel production through transesterification reaction using alcohol and catalyst. Certainly, two steps process increases production cost, and impact the environment negatively with the use of excessive solvent. In this project, the feasibility of producing biodiesel in one step process (in situ transesterification) will be explored.

## 2 OBJECTIVES

The objectives of this research project are to: 1-Select a microalgae-strain that, not only grows fast, but also contains high lipid contain. 2-Design a photobioreactor, which facilitates the control of algal growth conditions for high productivity. 3-Explore an alternative biodiesel production by combining oil extraction and transesterification process into one step (in situ algal biomass transesterification).

## 3 METHODS

**3.1 Algal strain selection:** Microalgae are unicellular photoautotrophic or photoheterotrophic microorganism, which grow like plant through photosynthesis process during which they capture carbon dioxide and photonic energy, and convert them into lipid. One of the biggest challenges in algae culture for biodiesel is to find a suitable strain with high lipid content and growth rate. Seven strains were selected based on their lipid content and growth rate information. First, they were cultured in 2L glass flask using fresh medium at room temperature with light supplied 24hr per day during the entire culture period. The medium contained soluble nutrients subdivided into two categories: 1- macronutrients rich in N, P, K and NaCl; 2- micronutrients containing metals traces especially Fe, Cu, Co, Mo and Zn. In addition, vitamins B1 and B7 were supplied to some strains as needed for a health growth.

The growth rate and lipid content for all seven batches were monitored over time. Lipid content was measured by Nile Red fluorescence [3] using a spectrofluorometer .The turbidity (absorbance) of the culture was measured using a Bausch and Lomb spectronic spectrophotometer.

**3.2 Photobioreactors.** The initial study of algae as feedstock for bio-fuel was done using open ponds. This exposes algal cultures to high risk of contamination and impact of weather. In addition, mixing mechanism has been very difficult for large ponds resulting in heterogeneous culture medium and limited exposure to light. As a result, algal cultures in open ponds have less productivity and enormous unhealthy batches. In recent years, researchers began studying closed system (photobioreactor) as a way to mitigate all treats encountered in open ponds. On the other hand, the construction as well as maintenance of a photobioreactor have shown high cost compared to an open pond.

Figure 1 shows the tubular photobioreactor (TPBR) designed in this project for algal cells growth. It has a main tank connected to two spiral tubes set in series. Both spiral parts were clear PVC tubes of 1" external diameter and 3/4" internal diameter. The volume of both spiral parts was 3.4 gal. The main tank served as a feeding point of medium to the PVC tubes with a maximum capacity of 5 gallons. Culture medium was pumped into the tubings at set at fixed flow rate. These tubes provided an area of 20 $ft^2$ exposed to the fluorescence light. Air compressor supplied air to the system for aeration and to serve as a source of carbon dioxide. The air flow rate was set in the ranges of 193 – 210 gal/hr.

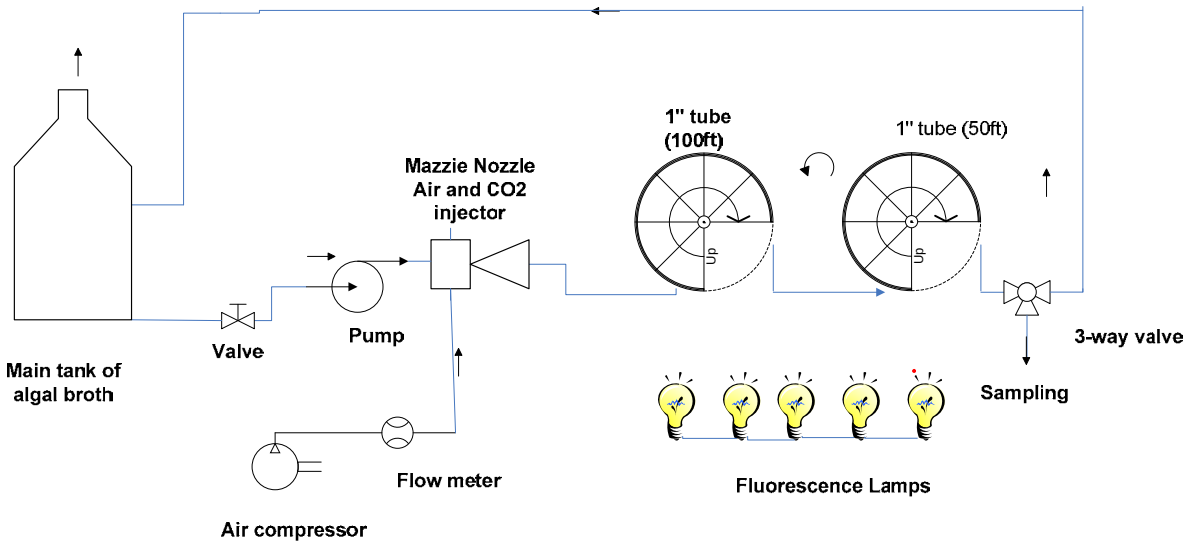


Figure 1: Tubular Photobioreactor

Culture flow rate was in turbulent regime with a Re >2000 to assure a good mixing and agitation. This TPBR could be used indoor with artificial source of photonic energy such as fluorescence lamps with low light intensity compared to sunlight. If weather permitted, the system could be set outdoor using sunlight as a source of photonic energy to minimize production cost.

**3.3 Algal culture and monitoring:** The selected algal strain was cultured in the TPBR to assess its functionality using fresh medium with no modification. Culture concentration and pH were measured over period of time varying between 12 to 14 days. A sample was taken every two day to measure the culture turbidity using a spectrophotometer (Bausch and Lomb spectronic) at 682 nm and cells count using a microscope. The pH of culture was measured as well using pH test strip. It is believed that strain released $OH^-$ during its growth, which followed photoautotrophic and mixotrophic mechanism [6] according to the following reactions:

Reaction 1: Photoautotrophic mechanism:

$$H_2O + HCO_3^- \rightarrow C\ (biomass) + \frac{1}{2} O_2 + 3\ OH^-$$

Reaction 2: Mixotrophic mechanism

$$a\ HCO_3^- + b\ CH_2O \rightarrow c\ C\ (biomass) + 3\ OH^- + d\ CO_2$$

The selected strain exhibited the typical growth curve of other micro-organisms, which included the following phases: lag, exponential, stationary and lysis. The length of each phase depended mixing mechanism, nutrients concentration, light penetration and the solubility of oxygen in the medium. Nutrients concentration and light penetration were the only parameters taken in consideration in this project. Nutrient composition was changed by varying the concentrations of $KNO_3$ and NaCl to assess their impact on algal biomass yield. After reaching a stationary or lysis phase, algal culture was harvested by centrifugation followed by lyophilization to produce dry algal biomass.

Crude lipid was extracted from dried algal biomass using either modified Folsch method [5] or Soxhlet extractor [9, 16]. In both methods, polar and non-polar solvent such as methanol and chloroform/hexane were used. The combination of polar and non-polar solvents enhances the extraction of both polar and non-polar lipid.

**3.3 In situ algal biomass transesterification:** Traditionally biodiesel is produced by transesterification of oil extracted from food crops such as soybean, canola and corn. This process included oil extraction then conversion into biodiesel. This two steps process is not only time and space consuming, but also increases the price of resulting product downstream.

Transesterifying algal lipid without oil extraction process could potentially reduce production cost. In 2006, Haas and Scott successfully conducted in situ transesterification [10]. A year later, Ferrentino and Farag initiated a study of in situ transesferification technique on dried algal biomass [4, 12, 13]. The current project explores an integrated transesterification of algal biomass into biodiesel.

Lyophilized algal biomass was pulverized using a mortar and pistol to release as much lipid as possible. Then a sample was mixed with methanol in the presence of an alkaline catalyst such as KOH or NaOH to produce biodiesel. A sonicator was used not only to break algal cell wall for lipid release, but also to mix released lipid with alcohol/catalyst.

## 4 RESULTS AND DISCUSSION

**4.1 Algal strain selection.** Each of the seven algal strains was cultured in 2L-glass flask operating in batch mode. Algae concentration was monitored by measuring the turbidity, and the algae oil content was monitored by the Red Nile method. Figure 2 is a plot of algal concentration and lipid content in the broth. It shows that $x_7$ is the best. Therefore this strain was retained for the rest of the project experiments.

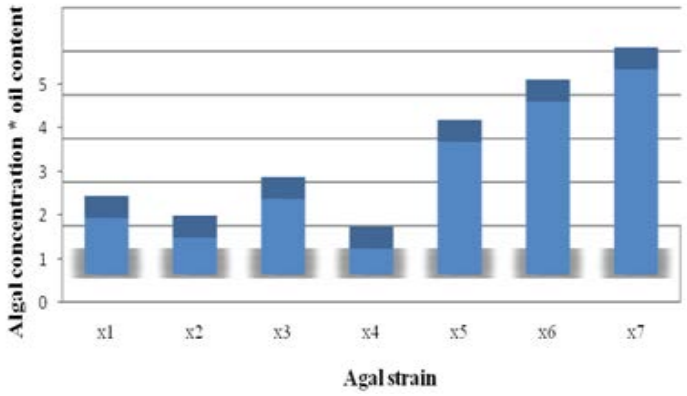


Figure 2 Lipid Content of different Algae broth solution

**4.2 Algae growth and culture monitoring:** Figure 3 is the growth curve of the selected algae strains. It plots the number of cells per ml of broth solution versus time. It shows that the cell count increases by factor of 10, since it started at $4x10^6$ Cells/mL (inoculums concentration of 0.1g/L) and reached about $56x10^6$ Cell/mL after 12 days. Based on these measurements and assuming that one nutrient is limiting the growth, it was possible to determine the parameters of the Monod Equation:

$$\mu = \mu_{\max} * \frac{S}{K+S} \quad ,$$

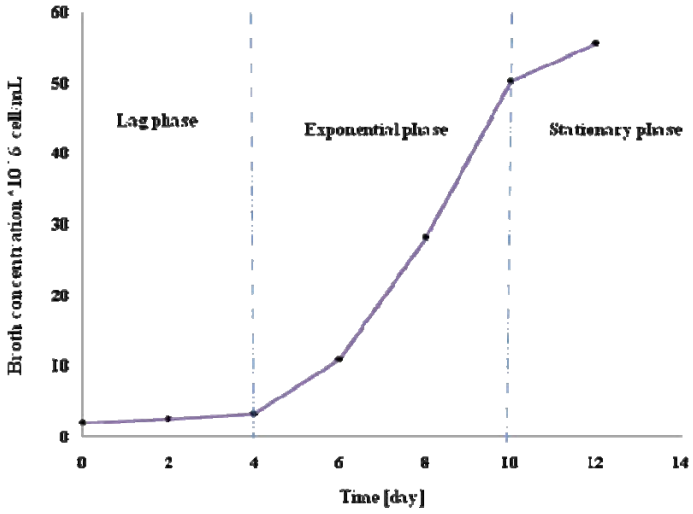


Figure 3 Growth Curve of Selected Algae Strain

where μ is the specific growth rate of the cells, $\mu_{max}$ is the maximum specific growth rate for the algae species, S is the concentration of the limiting nutrient, and K is the half saturation coefficient. The maximum specific growth rate of algae was estimated to be 0.6 per day.

The doubling time of the algae strain in TPBR was calculated as follows and showed that the broth concentration was doubling every 26 hr with a maximum growth rate of 0.6 per day.

$$T_d = \frac{Ln2}{\mu_{max}} = 1.16 \text{ day or } 26hr$$

Figure 4 is the plot of pH versus time. It shows that the pH of the selected algae strain culture started around 6.5 at the beginning of the culture and attained a plateau of pH 9 when the culture reached a stationary phase. The two batches (batch I and II) cultured at different time using same medium composition and same inoculums concentration, showed that the pH varied in the same way and reached a plateau at pH 9. The increase of pH was due to the release of $OH^-$ as shown in reactions 1 and 2.

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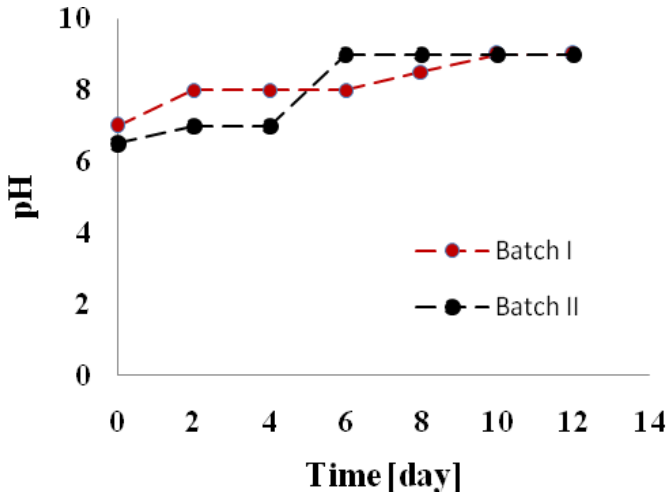


Figure 4 pH of Selected Algae Strain vs. time

**4.3 Algae growth rate**: Four batches of same algae were grown in the TPBR and harvested after 12 days. Algal Broth for each batch was centrifuged and lyophilized after discarding excess water. The weight of each dry algal biomass was measured. The concentration of harveste**d** broth plotted versus batch in Figure 5 shows that an average yield of algal biomass of 1.1g of algal biomass per L of broth with a dilution rate of 0.083 $day^{-1}$. It is important to note that the starting concentration for the broth was 0.1g of algal biomass per mL of broth. It is also important to note that algal biomass concentration reached in TPBR is twice the yield in raceway ponds [8].

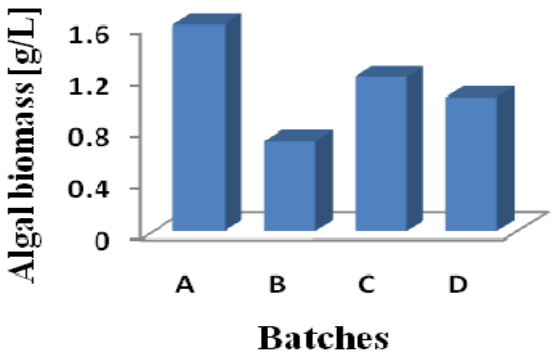


Figure 5 Algae concentration for harvested broth versus batch

Thus the ratio of initial concentration to the final concentration was 11, which was higher than the ratio 4 reported for open ponds [2] or helical photobioreactors [11].

The volumetric productivity [11] for this TPBR was determined as P=DX, where D was dilution rate in [$hr^{-1}$] and X was the biomass concentration in [g/L].

$P=0.004\ g.L^{-1}hr^{-1}$

A low algal biomass concentration shows a high impact on volumetric productivity in TPBR being 10 times lower than the volumetric productivity in helical photobioreactor [11]. However, if a culture starts with an inoculum at higher concentration, for instance 1.0 g/L, the volumetric productivity in TPBR is expected to be much higher compared to those of other photobioreactors

**4.4 Algae Oil Content**: Oil content (g oil per g of dry algal biomass) of harvested algae was determined by measuring weights of extracted oil using soxhlet extractor and hexane. Figure 6 presents algal oil content (in %) for each batch. The oil content varies from 7 to 13 % with an average of 11% or 0.11 g of oil per g of dry algal biomass.,

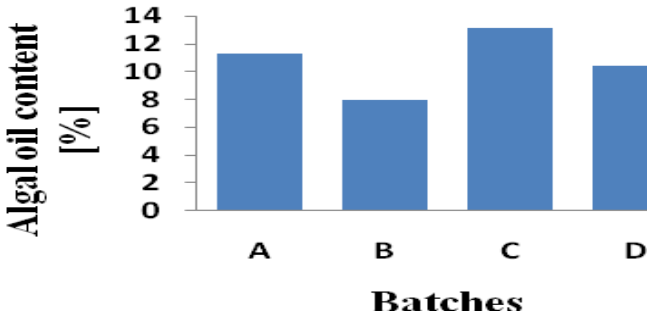


Figure 6 Algae oil content broth versus batch

**4.5 Effect of $KNO_3$ and NaCl on algal growth:** Five difference batches (flask A, B, C, D and E) of the same algae strain were cultured using different composition of $KNO_3$ and NaCl in the medium as follow: flask A as a control, flask B with no NaCl, Flask C with double concentration of NaCl, flask D with no $KNO_3$ and flask E with double concentration of $KNO_3$. Figure 7 shows the variation of turbidities (Optical Density) of each of five algal cultures with respect to time. It shows that $KNO_3$ and NaCl concentration are directly proportional to the algal concentration; doubling the concentration of $KNO_3$ or NaCl increases the concentration of algal culture. In fact, no growth was observed in flask D, which did not contain any $KNO_3$,

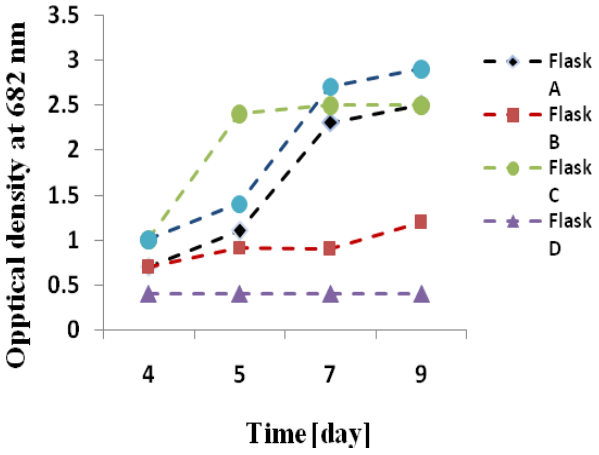


Figure 7 Effect of $KNO_3$ and NaCl on algal absorbance versus time

**4.6 Light effect on algal growth in TPBR:** The light penetration distance was evaluated using the following empirical formula [14, 16].

$$d_p = \frac{6000}{C}$$

Where $d_p$ and C are respectively light penetration distance (in cm) and algal broth concentration (in mg/L). The light penetration for the TPBR with an algal biomass concentration of 1.1g/L or 1100 mg/L is 5.5 cm. The outside diameter of the PVC tubes used in TPBR was 2.5 cm or 1"; therefore, light was not a limited factor.

## 5 CONCLUSION

Seven strains were tested and the best was selected for its highest oil content and per liter of broth. The selected strain was cultured in the TPBR, which was designed to achieve high productivity. Four different batches cultured in the TPBR showed that a high yield of algal biomass could be attained by comparing the ratio of initial broth concentration to the final concentration being high (11) to those of existing close or open system.

In addition to the yield in algal biomass being 1.1g/L with a volumetric productivity of 0.04 $gL^{-1}hr^{-1}$ or 0.096 $kg.m^{-3}.day^{-1}$, the dry algal biomass contained an average lipid concentration of 11% in mass. Also, the modification of medium composition showed that doubling the concentration of $KNO_3$ and NaCl significantly increases algal biomass yield. It is important to note that light was not a limited factor in algal grown in TPBR.

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