Studies on Effect of different CO_2 Concentrations on the Biomass and Lipid yields of Scenedesmus Quadricauda for Bio-Diesel Production

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Abstract :- Energy is essential and vital for development. The use of fossil fuels as energy is now widely accepted as unsustainable due to depleting resources and also due to the accumulation of greenhouse gasses in the Environment. Renewable and carbon neutral biodiesel is necessary for environmental and sustainability. The micro algae which are one of the most abundant organisms in the world got final attention because of its highest capacity to produce bio fuels in per acre as compared to other power crops and more yielding capacity (58,700L/h). These tiny organisms don't require big lands or farmhouses. They can be grown in open ponds, plastic bags, glass vessels and photo bioreactors. Algae can accumulate the CO_2 and reduce the CO_2 levels in environment to prevent the global warming. The micro algae have emerged as high lipid content to produce the bio diesel. Scenedesmus quadricaudaare a green pyramid shape planktonic micro algae. It is of potentially great importance in the field of biotechnology. It has been shown to grow best at a temperature of 28^0 C, Light period of 12 hrs in CHU 13 medium. It has high lipid content (25 % - 86 %).In this study an attempt has been made to evaluate the CO_2 sequestration efficiency of Scenedesmus quadricauda for its maximum biomass production and lipid content. The algae were grown in culture rack and open race way ponds. Flask cultures were maintained at 32^0 C temperature and 8000 Lux light intensity with different light and dark periods. Different concentrations of CO_2 inputs were studied to know optimum CO_2 concentration to get Maximum yield of Biomass, Lipid and Fatty Acid Methyl Esters (FAME) .For open raceway pondstudies the algal cultures were grown out side in the open environment. physico-chemical parameters like pH and D.O along with light and temperatures were monitored for both flask and open race way pond cultures. Best yields were noted in open raceway ponds at 28-40 ^{0} C Temperatures and 80-120Klux light intensities, at 12% CO_2 for S. quadricauda with a biomass yield of 1.485 g/L, lipid yield of 0.452 g/L and FAME yield of 0.21g/L.

I. INTRODUCTION

The scarcity of conventional fossil fuels, growing emissions of combustion-generated pollutants, and their increasing costs will make biomass sources more attractive. Petroleum-based fuels are limited reserves concentrated in certain regions of the world. These sources are on the average of reaching their peak production. The fossil fuel resources are shortening day by day. The scarcity of known petroleum reserves will make renewable energy sources more attractive. Ayhan Demirbas*et al.,* (2009).

Petroleum and diesel come in the category of non-renewable fuel and will last for a limited period of time. Mankind's continuous use of fossil fuels is unsustainable as they are limited resources of energy and their combustion will lead to generation of the energy-related emissions of Green House Gases (GHG) like carbon dioxide, sulfur dioxide, nitrogen dioxide and volatile organic compounds. During the past few decades, global atmospheric concentrations of GHG have frequently risen with a growth rate of CO_2 emissions. Thus, increasing CO_2 concentrations is considered to be one of the main causes of global warming.

The production of biodiesel has recently received much attention worldwide. Because of the world energy crisis, many countries have started to take a series of measures to resolve this problem. Finding alternative energy resources is a pressing mission for many countries, especially for those countries lacking conventional fuel resources. In the 1930s and 1940s, vegetable oils have been used as diesel fuels in the emergency situation. With the rapid development of the modern industry, the demand for energy has been greatly increased in recent years, and therefore alternative energy sources are being explored. Thus, the term "biodiesel" has appeared very frequently in many recent reports. (clayton*et al.,* 2008).

The world total biodiesel production was estimated to be around 1.8 billion liters in 2003. (Guanhua*et al.,* 2010). Although there was no increase in biodiesel production between 1996 and 1998, a sharp increase in biodiesel production was observed in the past several years. It is speculated that the production of biodiesel will be further tremendously increased because of increasing demand for fuels and "cleaner" energy globally.

A. Source of Biodiesel

The bio-fuel production from photosynthetic microorganisms is considered as a process to produce renewable energy for global warming mitigation. Recently, microalgae have received much attention as a renewable energy resource because the photoautotrophic mechanism can convert atmospheric carbon dioxide into biomass, fatty acids, and lipids (spolaore*et al.*, 2006; chist, *et al.*, 2007).

Many research works have been conducted to produce microalgal lipids as source of bio-fuel production (Takagi *et al.*, 2000; chist, 2007; Hu *et al.*, 2008). For mass production of bio-fuel, the economic feasibility of microalgal culture greatly depends on the productivity of biomass and lipids.

An alternative fuel to petro diesel must be technically feasible, economically competitive, environmentally acceptable, and easily available. The current alternative diesel fuel can be termed biodiesel. Biodiesel can offer other benefits, including reduction of green house gas emissions, regional development and social structure, especially to developing countries. However, for quantifying the effect of biodiesel it is important to take into account several other factors such as raw material, driving cycle, and vehicle technology. Use of biodiesel will allow a balance to be sought between agriculture, economic development, and the environment. Biodiesel methyl esters improve the lubrication properties of the diesel fuel blend. Biodiesel reduced long term engine wear in diesel engines. Biodiesel is a good lubricant (about 66% better than petro diesel).

Common name	Biodiesel (bio-diesel)
Common chemical name	Fatty acid (m)ethyl ester
Chemical formula range	C_{14}–C_{24} methyl esters or$C_{15_25}H_{28_48}O_2$
Kinematic viscosity range (mm2/s, at313 K	3.3–5.2
Density range (kg/m3, at 288 K)	860–894
Boiling point range (K)	>475
Flash point range (K)	420–450
Distillation range (K)	470–600
Vapor pressure (mm Hg, at 295 K)	<5
Solubility in water	Insoluble in water
Physical appearance	Light to dark yellow, clear liquid
Odor	Light musty/soapy odor

Table 1:- Technical Properties of Biodiesel

Flash point	D 93 325 K min 403 K
Water and sediment	D 2709 0.05 max %vol 0.05 max %vol
Kinematic viscosity	D 445 1.3–4.1 mm2/s 1.9–6.0 mm2/s
Sulfated ash	D 874 – 0.02 max %wt
Ash	D 482 0.01 max %wt –
Sulfur	D 2622/129 – 0.05 max %wt
corrosiosin	D 130 No. 3 max No. 3 max
Cetane number	D 613 40 min 47 min
Aromaticity	D 1319 35 max %vol –
Carbon residue	D 4530 – 0.05 max %mass
Carbon residue	D 524 0.35 max %mass
Distillation temp	D1160 555 k min-611k max

Table 2 :- ASTM Standards of Bio diesel and Petro Diesel Fuels

B. Algae as Feed Stock for Renewable Resources

Algae are simple organisms that are mainly aquatic and microscopic. Microalgae are sunlight-driven cell factories that convert carbon dioxide to potential bio fuels, foods, feeds and high-value bioactive. In addition, these photosynthetic micro-organisms are useful in bioremediation applications and as nitrogen fixing bio fertilizers. Microalgae can provide several different types of renewable bio fuels. These include methane produced by anaerobic digestion of the algal biomass; biodiesel derived from microalgal oil; and photo biologically produced bio hydrogen. The idea of using microalgae as a source of fuel is not new, but it is now being taken seriously because of the escalating price of petroleum and, more significantly, the emerging concern about global warming that is associated with burning fossil fuels.

In the past 2-3years the production of biodiesel from algae has been an area of considerable interest. This is because

1. Algae have higher productivities than land plants with some species having doubling times of a few hours.
2. Some species can accumulate very large amounts of triacylglycerides (TAGs), the major feedstock for biodiesel production.
3. High quality agricultural land is not required to grow the biomass.
4. On the other hand, one of the major disadvantages of microalgae for biofuel production is the low biomass concentration in the micro algal culture due to the limit of light penetration, which in combination with the small size of algal cells makes the harvest of algal biomasses relatively costly. The large water content of harvested algal biomass also means its drying would be an energy-consuming process. The higher capital costs of and the rather intensive care required by a microalgae farming facility compared to a conventional agricultural farm is another factor that impedes the commercial implementation of the biofuels from microalgae strategy.

C. Advantages of Microalgae

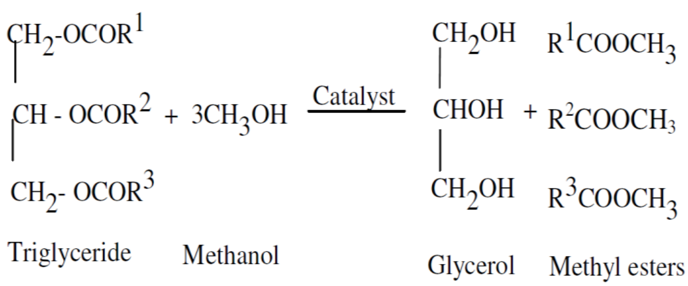
The advantages of microalgae over higher plants as a source of transportation biofuels are numerous

1. Oil yield per area of microalgae cultures could greatly exceed the yield of the best oilseed crops.
2. Microalgae grow in an aquatic medium, but need less water than terrestrial crops;
3. Microalgae can be cultivated in seawater or brackish water on non-arable land, and do not compete for resources with conventional agriculture.
4. Microalgae biomass production may be combined with direct bio-fixation of waste CO_2 (1 kg of dry algal biomass requiring about 1.8 kg of CO_2).
5. Fertilizers for microalgae cultivation (especially nitrogen and phosphorus) can be obtained from wastewaters.
6. Algae cultivation does not need herbicides or pesticides.

7. The residual algal biomass after oil extraction may be used as feed or fertilizer, or fermented to produce ethanol methane.
8. The biochemical composition of algal biomass can be modulated by varying growth conditions and oil content can be highly enhanced.

D. Biodiesel Production

Parent oil used in making biodiesel consists of triglycerides in which three fatty acid molecules are esterifies with a molecule of glycerol. In making biodiesel, triglycerides are reacted with methanol in a reaction known as transesterification or alcoholysis. Transestrification produces methyl esters of fatty acids, that are biodiesel, and glycerol The reaction occurs stepwise: triglycerides are first converted to diglycerides, then to monoglycerides and finally to glycerol.



Transesterification requires 3mol of alcohol for each mole of triglyceride to produce 1 mol of glycerol and3 mol of methyl esters. The reaction is equilibrium. Industrial processes use 6mol of methanol for each mole of triglyceride (Fukuda *et al.,* 2001). This large excess of methanol ensures that the reaction is driven in the direction of methyl esters, i.e. towards biodiesel. Yield of methyl esters exceeds 98% on a weight basis (Fukuda et al., 2001). Transesterification is catalyzed by acids, alkalis (Fukuda *et al.,* 2001; Meher*et al.,* 2006) and lipase enzymes (Sharma *et al.,* 2001). Alkali-catalyzedtransesterification is about 4000 times faster than the acid catalyzed reaction (Fukuda *et al.,* 2001).

Consequently, alkalis such as sodium and potassium hydroxide are commonly used as commercial catalysts at a concentration of about 1% by weight of oil. Alkoxides such as sodium meth oxide are even better catalysts than sodium hydroxide and are being increasingly used. Use of lipases offers important advantages, but is not currently feasible because of the relatively high cost of the catalyst (Fukuda *et al.,* 2001). Alkalicatalyzed transesterification is carried out at approximately 60°Cunder atmospheric pressure, as methanol boils off at 65 °C at atmospheric pressure. Under these conditions, reaction takes about 90min to complete. A higher temperature can be used in combination with higher pressure, but this is expensive. Methanol and oil do not mix, hence the reaction mixture contains two liquid phases. Other alcohols can be used, but methanol is the least expensive. To prevent yield loss due to saponification reactions (i.e. soap formation), the oil and alcohol must be dry and the oil should have a minimum of free fatty acids. Biodiesel is recovered by repeated washing with water to remove glycerol and methanol.

II. SCOPE AND OBJECTIVES

A. Scope

The present study an attempt has been made to evaluate the biodiesel production from Scenedesmusquadricauda by using different CO_2 concentrations, in different conditions.

B. Objectives

1. To carry out Isolation and enrichment of micro algae Scenedesmusquadricauda species from a local water body.
2. To perform Optimization of process parameters includes Light intensity, Temperature, pH and DO for the maximum production of algae using different CO_2 concentrations for maximum biomass production.
3. To Extract lipids from Scenedesmus Quadricauda using distillation method with benzyl alcohol as solvent.

III. MATERIALS AND METHODS

A. Isolation and Cultivation

Mixed algal cultures were collected from different fresh water bodies of Hyderabad. Microscopic observations were carried out and found presence of Chlorella vulgaris, Scenedesmusquadricauda, Oscillatoria (Cynophyceae), Lyngbya, Cyclotellaand Closterium. Scenedesmusquadricaudawas isolated by plating in its respective enrichment media BG 11. The strain was subjected to purification by serial dilution followed by plating. The individual colonies were isolated and inoculated into selective liquid medium and incubated at 25 ± 1^{O}C under 8000Lux light intensity with 8:16 hrs light/dark regime. The purity of the culture was ensured by repeated plating and by regular observation under microscope Fig. 2.

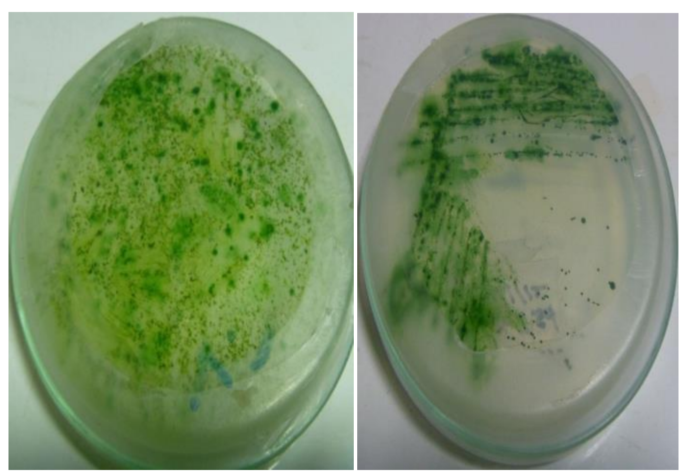


Figure 1 :- Spread plate and quadrant streak plate techniques used for S. quadricauda isolations.

B. Indoor Experimental Setup for CO_2 Experiments

The lab scale setup was made in a five stair culture rack with each shelf containing a series of seven flasks inter connected through a PV pipe which passes CO_2 gas and air mixture into each flask. 500ml Erlenmeyer flasks with working volume of 200ml each were setup Fig.1.

Figure 2 :- Culture Rack containing S. quadricauda connected to PV pipes

C. Outdoor Experimental Setup with Open Raceway Ponds

Studies with open raceway ponds of 2x1m with working volumes 200L medium constructed sideways driven by a motor paddle which gave 15cm/s flow rate were taken up. A vacuolated (Stainless steel) SS pipe has been placed beneath the medium into each pond running along the raceway design delivering the CO_2 gas. A motor driven 25L capacity movable SS harvester containing nylon filters was attached to the pond for harvesting after every batch.

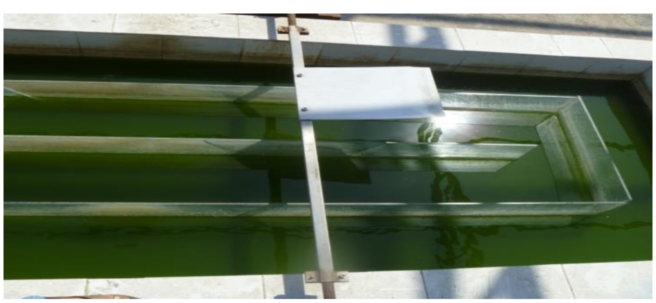


Figure 3 :- Open Raceway Ponds

D. Optimization Studies for Operational Parameters

- *CO_2 Inputs*

Different percentage concentrations of CO_2 (CO_2/air mixture) were studied for both indoor and outdoor experiments they are 0.038% (atmospheric), 6%, 8%, 10%, 12%, 14%, 16%, 18%, 29%, 22% and 24% with flow rate 2.5ml/200ml/2mins/hour in indoor conditions and 2.5L/200L/2mins/hour in outdoor conditions.

- *Light and Temperature*

The experiments were conducted over a period of 180 days starting from March to August. In indoor flask cultures the light and temperatures were maintained at 8KLux and 32^{O}C respectively equivalent to that of open atmospheric conditions. The light intensities ranged between 80-120KLux and temperatures ranged between 28-40^{O}C.

- *Biomass Harvesting and Drying*

The fully grown microalgae were bulk harvested with harvester in outdoor ponds and through filtration with nylon double layered cloth in indoor lab experiments after reaching to late stationary phase. After separating, the rigid biomass was sun dried till the total moisture was evaporated. The dried biomass was ground in a mortar and pestle into a fine powder, weighed and stored in cool dry place.

Figure 4:- Harvester

E. Extraction of Oil

The powdered biomass was subjected to solvent extraction using benzyl alcohol in a flask poured into 500ml round bottom flask connected to a Buchner funnel through which the extracted solvent was filtered twice. The final extract was then subjected to distillation process in an oil bath maintained at 210ºC as the boiling point of benzyl alcohol was 205ºC. As benzyl boils off at 205C collected into another flask the remaining extract was noted as algae-oil and collected separately into a pre-weighed container to measure the weight and then subjected for GC MS analysis.

F. Transesterification

The *S. quadricauda* oil was subjected to methanol KOH transesterification in a conical flask at 65ºC temperature for 12 - 24h and later FAME and glycerol layers were separated in a separating funnel. The estimated FAMEs were weighed to measure the quantity.

IV. RESULTS AND DISCUSSION

Effect of CO_2 concentration of the S. quadricaudain indoor was shown Figure 6. As increase in CO_2 concentrations from 6%-24% led to significant decline in Biomass, Lipid and FAME yield because the required amount of light intensity was not sufficient to speed up the photosynthesis reaction in the algae.

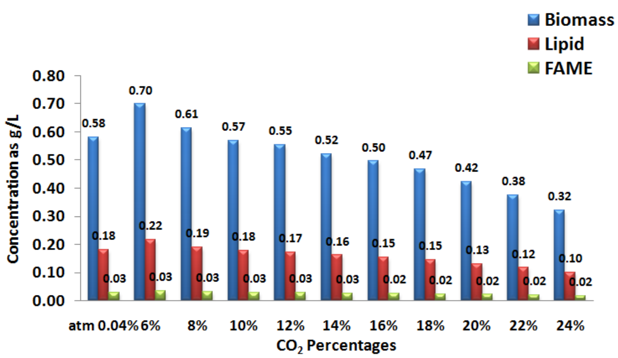


Figure 5:- Biomass, lipid and FAME yields of S.Quadricaudaat different percentage CO_2 concentrations in indoor culture rack

In outdoor open raceway pond experiment the growth was significantly enhanced compared to indoor culture. An increase in the CO_2 concentration from 0.04%-12% led to increase in the Biomass production from 0.7g/L to 1.47g/L (Fig.7). Further any increment in the CO_2 led to significant decline in the Biomass, Lipid & FAME yields. The possible reason was that as CO_2 increases unutilized CO_2 will be converted to HCO_3 thereby reducing the pH of the medium resulting in the bleaching of chlorophylls leading to cell decline. When the CO_2 levels are low, algal growth will be inhibited by low carbon source.

For experiments in indoor culture flasks at 32^{O}C temperatures and 8KLux light intensity, best result was seen at 6% CO_2 for S. quadricauda. For experiments in open raceway ponds at 28-40^{O}C temperatures and 80-120KLux light intensities, best result was seen at 12% CO_2 for S. quadricauda.

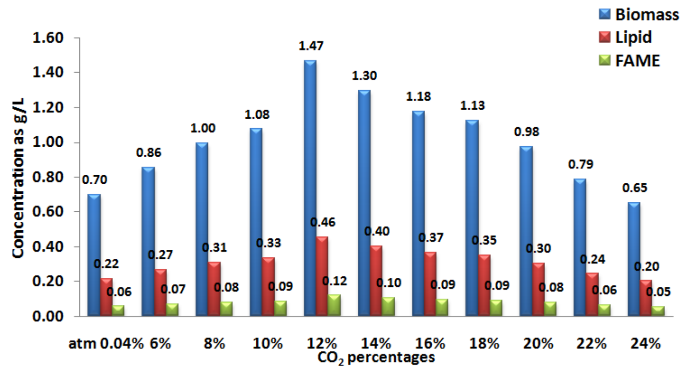


Figure: 6 Biomass, lipid and FAME yields of S. quadricaudaat different percentages of CO_2in outdoor open raceway pond

ei was able to produce more biomass (1.485 g/L) in open ponds compared to that of culture flasks (1.193 g/L). S. quadricauda was able to tolerate CO_2 up to 24% but best yields were noted at 6% CO_2 in indoor flasks and 12% CO_2 in open raceway ponds (Table.6). Lipid yields were also high in open ponds (0.452 g/L) compared to shake flasks (0.27 g/L). The difference in biomass yields between indoor and outdoor cultures were 19.66%. Lipid yields were 30.4% of dry biomass and FAME yields were 57% of derived lipids in open ponds.

S. quadricauda	Indoor experiments in culture rack 6% CO_2	Outdoor open raceway pond experiments with 12% CO_2
Biomass Yield	1.193 g/L	1.485 g/L
Lipid Yield	0.27 g/L	0.452 g/L
FAME yield	0.119g/L	0.21g/L

Table 3:- Best yields of S. quadricauda in two different culture modes

A. PH and DO variations

The pH values ranged between 6.0-9.0 in indoor and 6.5-9.5 in outdoor experiments. pH value shifted towards slight acidic side during lag phase with CO_2 inputs. pH shifted to alkaline side after lag phase due to high photosynthetic rate.

B. Dissolved Oxygen

Oxygen levels in the medium were monitored as dissolved oxygen. The oxygen concentrations were below 4ppm during lag phase. It reached to 11ppm during the growth phase due to high photosynthetic rate in open ponds. In indoor cultures the DO values ranged between 2-7ppm.

C. GC MS analysis result

The GC MS analysis showed a profile of fatty acids like Palmitic acid, Stearic acid, Oleic acid, Linolenic acid commonly in *S. quadricauda* in the two culture modes.

D. Transesterification results

FAME amounts were higher in open raceway ponds compared to indoor flasks with maximum of 0.21g/L.

V. CONCLUSION

Scenedesmusquadricauda was found to be tolerant to wide environmental conditions like light intensities, temperatures, predators in open conditions. Benzyl alcohol is the best solvent for the algal oil,*S. Quadricauda* was able to produce more biomass in open ponds compared to that of culture flasks. *S. quadricauda* was able to tolerate CO_2 up to 24% but best yields were noted at 6% CO_2 in flasks and 12% CO_2 in open raceway ponds.

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