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Improvement in lipids extraction processes for biodiesel production from wet microalgal pellets grown on diammonium phosphate and sodium bicarbonate combinations

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HIGHLIGHTS

- Microalgae grown on Sodium Bicarbonate ($NaHCO_3$) and Diammonium Phosphate (DAP) combination.
- Effect of thermolysis and sonolysis was highlighted as effective cell disruption methods.
- Biodiesel synthesis was accomplished after lipids extraction from wet microalgae biomass.
- Transesterification of extracted lipids with methanol has resulted fatty acid methyl esters (FAMEs).

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ABSTRACT

Biomass productivity and growth kinetics for microalgae grown on sodium bicarbonate and diammonium phosphate were investigated. Different carbon and nitrogen ratios have shown different growth rates and biomass productivity and C:N ratio 50:10 as mg L^{-1} has shown the best production than all. For effective lipids extraction from biomass thermolysis and sonolysis were carried out from wet biomass. Sonolysis at 2.3 W intensity for 5 min has released 8.58 mg at neutral pH. More quantity of lipids was extracted when extraction was made at pH 4 and 10 which resulted 9 mg and 9.28 mg lipids respectively. Thermal treatment at 100 °C for 10 min has released 12.82 mg lipid at neutral pH. In the same thermolysis at pH 4 and 10 more quantity of lipids was extracted which were 15.16 mg and 14.81 mg respectively. Finally transesterified lipids were analyzed through GC–MS for FAME composition analysis.

1. Introduction

Microalgae are unicellular autotrophic, mixotrophic and heterotrophic microscopic organisms having a great potential to synthesis a wide range of valuable bio-based products (Liang et al., 2009). Microalgae can synthesis a considerable content of its biomass in the form of lipids, carbohydrates and proteins. Proteins and carbohydrates are valuable food ingredients. Lipids on the other hand could have its dual applications, i.e. in the field of food and bioenergy. For its bioenergy implications scientist are still working on its life cycles, growth kinetics, maximum biomass production and making it available for energy harvesting (Liu et al., 2013). From microalgal lipids biodiesel could be synthesized through transesterification. Biodiesel is a promising biofuel because it is a sustainable alternative of conventional petroleum fuel. In recent years, most of bioenergy research groups have focused on the use of intercellular microalgal-lipids (Triglycerides or TAGs) for the synthesis of biodiesel (Chisti, 2007; Laura Azócar et al., 2014). The lipids extraction from wet microalgal biomass is low because of decreased cells disruption, dissolution of neutral lipids and interference of water molecules with the organic solvents and therefore it needs efficient processes to increase the lipids extraction yield (Keris-Sen et al., 2014). Despite of these certain constraints in the commercial application of this technology, still researchers are hopeful for the development of sustainable technologies for microalgal biodiesel (Chisti, 2013).

Biodiesel production form microalgae follow downstream processing which is a multistep and difficult process. In downstream processes the first hurdle becomes at constant and maximum biomass production with a significant portion with lipids. Secondly lipids extraction from dry biomass is very expensive and energy intensive process. Therefore lipids extraction from wet biomass is gaining more attention (Wawrik and Harriman, 2010). Key factors

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Table 1
Sequence-I: Constant N:P ratio by means of DAP (mg L^{-1}) and variable Carbon ratio by means of $NaHCO_3$ (mg L^{-1}).

S. No.	Treatments	C – ratio	$NaHCO_3$ (mg L^{-1})	N:P – Ratio	DAP (mg L^{-1})	K – ratio	KCl (mg L^{-1})	C:N:P:K (mg L^{-1})
01	T-1	50	337	9:5	50	10	19.1	50:9:5:10
02	T-2	100	674	9:5	50	10	19.1	100:9:5:10
03	T-3	150	842	9:5	50	10	19.1	150:9:5:10
04	T-4	200	1011	9:5	50	10	19.1	200:9:5:10
05	C	Control *	BG – 11 Medium					

* Control was made by using BG-11 growth medium.

responsible for microalgal growth and cultivation are nutrients availability, carbon–nitrogen ratio (C:N), light penetration, carbon source, pH, salinity and temperature. Carbon–nitrogen ratio (C:N) is a key factor responsible for the shift of intracellular metabolism towards lipids synthesis and accumulation in cell sap. Greater lipids accumulation can be achieved by nitrogen deprivation. In this process nitrogen concentration is kept less than carbon in nutrients medium. More amount of carbon is utilized in the form of bicarbonates (Demirbas, 2011). It is evident from a number of investigations that inorganic carbon in the form of bicarbonate (HCO_3^{-1}) solubilization in the culture medium could have effects on the lipids synthesis rather than subjecting gaseous carbon injections in the form of CO_2. For a wide range of microalgae species for their growth in synthetic culture mediums, sodium bicarbonate is promisingly used to provide water soluble input of HCO_3^{-1} (Pruvost et al., 2011).

In this study mixed microalgae culture (Sathish and Sims, 2012) (*Scenedesmus dimorphus* sp., *Chlorococcus* sp. and *Chlorella* sp.) were investigated for the effect of Diammonium phosphate (DAP) analog with sodium bicarbonate ($NaHCO_3$) ratios on biomass production and lipids accumulation (Pruvost et al., 2011). After harvesting thermolysis and sonolysis were employed for enhanced cell disruptions (Cheng et al., 2014; Keris-Sen et al., 2014). After lipids extraction acid catalyzed trans-esterification of the extracted lipids was performed in the presence of methanol to synthesis biodiesel (Rasoul-Amini et al., 2011)

2. Methods

2.1. Culture mediums for microalgal growth

The fresh mixed cultures of microalgae were identified by using methods described by Zarina et al. (2005), Zarina and Masud-ul-Hasan (2005). These cultures were kept under ambient conditions and provided BG-11 medium for microalgae survival. The mixed cultures of microalgae were grown indoors in well mixed photobioreactors according to the methods explained by Ahmad et al. (2012). A slightly modified version of the microalgae growth media was used for culturing as shown in Tables 1 and 2. For the investigation of the effect of nitrogen (N), phosphorous (P) and carbon (C), Diammonium phosphate (DAP) and sodium bicarbonate ($NaHCO_3$) were used in different ratios along with BG-11 medium with slight modifications for N:P and carbon combinations (White et al., 2013). These are shown in the Tables 1 and 2 which were selected as eight treatments for cultivation and their controls were also made by keeping the composition of BG-11 medium. For effective growth the potassium (K) was maintained at constant concentration.

The synthetic culture mediums of different treatments were made by altering the C:N:P ratio by using different molar mass ratios of DAP and $NaHCO_3$ as major nitrogen, phosphorus and carbon suppliers. The remaining constituents of the mediums were kept constant as in BG-11 medium i.e. by eliminating the usage of $NaNO_3$ and K_2HPO_4. Control in the cultivation experiments was made by keeping the throughout media composition as BG-11. BG-11 medium was made by preparing nine stock solutions. First stock solution was made by taking 15.0 g of $NaNO_3$ in a liter of deionized water. Other seven stock solutions were made by taking 2.0 g, 3.75 g, 1.80 g, 0.30 g, 0.30 g, 0.05 g and 1.00 g of K_2HPO_4, $MgSO_4{\cdot}7H_2O$, $CaCl_2{\cdot}2H_2O$, citric acid, ammonium ferric citrate green, $EDTANa_2$ and Na_2CO_3 respectively in 500 mL deionized water for each solution. The final stock solution for trace elements was made by taking 2.86 g, 1.81 g, 0.22 g, 0.39 g, 0.08 g and 0.05 g of H_3BO_3, $MnCl_2{\cdot}4H_2O$, $ZnSO_4{\cdot}7H_2O$, $Na_2MoO_4{\cdot}2H_2O$, $CuSO_4{\cdot}5H_2O$, and $Co(NO_3)_2{\cdot}6H_2O$ respectively in a liter of deionized water. The culture medium was made by taking 100 mL of $NaNO_3$ solution, then 10.0 mL each of K_2HPO_4, $MgSO_4{\cdot}7H_2O$, $CaCl_2{\cdot}2H_2O$, citric acid, ammonium ferric citrate green, $EDTANa_2$ and Na_2CO_3 solutions and 1.0 mL of trace elements solution and making the final volume up to 1000 mL with distilled water (Keris-Sen et al., 2014) .

2.2. Microalgae cultivation and growth kinetics

Microalgae were grown in lab scale photobioreactor with 2 L aqua culture medium capacity. Photobioreactor consist of 2.5 L transparent plastic bottles, air pump, delivery tubing, LED light, illumination sheets and electricity supply as illustrated in Fig. 1a. Culture medium was prepared by using distilled water and dissolving calculated concentrations of nutrients according to the set conditions. A fresh culture of microalgae inoculum was used to initiate cultivation at a maintained pH 7 (Pruvost et al., 2011). For the provision of light LED lamps were used (Chen et al., 2011). These had the specifications of; wattage of 8 W, Beam angle 40°, 560 lumen (Lm), power of current 69 ± 5 mA, color of light 6500 K, frequency 50 Hz. The light intensity was calculated by using Digital Lux meter (model No. LX1010BS). The light power was maintained at 800 LUX (933.96 lumens "lm") at 90° angle in the area of 30 cm^2, at which it provides 15.56 watts (W) energy approximately at the distance of 15 cm over the reactor bottles (Choochote et al., 2010). For the determination of microalgae growth (growth kinetics), 10 mL of the samples were taken on daily basis from the culture bottles and their optical density (OD) was determined at 600 nm by using spectrophotometer (OD-600). OD is the indicator for the microalgae density. From the same samples cell count for the microalgae cells was made to correspond the OD-600 and number of cells per mL and 1000 mL. Following mathematical equation was used for the calculation of growth rate (GR) per day to fit for OD (Zhu et al., 2013):

$$\mathbf{GR} = \frac{(\ln \mathbf{OD_t} - \ln \mathbf{OD_o})}{\boldsymbol{t}} \quad (1)$$

where $\mathbf{OD_0}$ is for optical density at time taken at zero at initial day, and $\mathbf{OD_t}$ is for the optical density at time taken at the respective day and $\boldsymbol{t}$ refers to the time taken for growth. OD of the culture was compared on daily basis with OD of the blank samples. At the exponential phase the specific growth rate (μ, per day) of the microalgae