Evaluation of Pre-Treatment Techniques for Butanol Production from Microalgae-Based Biodiesel Residue

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Abstract- **In the present study an attempt has been made to evaluate the potential of cyanobacterialbiomass for lipid extraction subsequent utilization of residual biomass for sugar analysis. Cyanobacterial biomass was collected from the pond located in Banaras Hindu University, Varanasi campus and identified as cyanobacterial biomass of Lyngbyalimnetica. Appropriate amount of dry cyanobacterial biomass was subjected to lipid extraction by Bligh & Dyer and modified Bligh & Dyer method. Maximum 0.153 g/g of lipid was extracted with 2:1 ratio of chloroform:methanol. Lipid extracted cyanobacterial biomass was subsequently utilized for the pre-treatment to release sugar for butanol fermentation. Maximum 0.283 g/g of sugar was obtained when the biomass was treated with 1.63M H2SO4 for 60 min at 100°C.**

Keywords: Cyanobacteria, Lipid, Carbohydrate, Biodiesel, Bioalcohol, Extraction.

I. INTRODUCTION

Biofuel is a renewable fuel which can be produced from biological oils derived from plants, microbes. Biofuel is non-toxic and biodegradable, alternative fuel that is obtained from renewable resources (Hossain et al., 2008). Demand of global energy is increasing with increase the population of world and industrialization. Now a day's consumption rate of fossil fuels such petroleum, coal, natural gases are run out by 2040 (Demirbas, 2010). At the current both energy crisis and climate change are the key issues all over the world. According to some energy expert there will be severe energy crisis in coming 50 years. The demand of clean and renewable biofuel has rapidly increased now a day due to several global issues such as growing of human population, fossil fuel depletion and global climate change.The traditional feedstocks are based on annual crops, problems in storage and potential shortages of raw materials in bad agricultural years pose additional threats to current biodiesel production systems (Trent, 2012).

Photoautotrophic algal are strains as unicellular or multicellular microorganisms with greater energy conversion efficiency than plants. Amidst these, cyanobacterial biomass (blue-green algae) retains huge carbon dioxide sequestration characteristics, thereby reducing the greenhouse effect with resulting huge biomass production that can be used for different purposes (Darochet al., 2013).Cyanobacterial growth and storage occur within the different parts of the cells and its depend on various factors such as temperature (incubation temperature), the availability of different types of nutrient, intensity of light, pH of medium, processes of shaking (Carvalho et al., 2009). The light intensity is an important parameter for cyanobacterial growth and its optimal temperature is also important and it controls the concentration of different types of chemical constituents of biomass (Zhang et al., 2015). Many species of cyanobacteria grow in low light intensity (< 65 μmol m^{-2} s^{-1}) while some other species can grow in large intensity (> 90 μmol m^{-2} s^{-1}). Many species can grow either autotrophic or heterotrophic but some of the species grow in both conditions however, autotrophic growth rate is much faster than heterotrophic growth rate (Csavina et al., 2011). Recently the environmentally friendly approach has received renewed attention as a pretreatment method for enhancing enzymatic biomass in lipid and carbohydrate production processes and there are different types of pretreatment processes for algal extraction.Optimization of essential operating parameters such as treatment temperature, time, and concentration of pretreatment agent is the first and important step toward maximizing the sugar release from biomass (Wang, Liu, and Wang, 2011).Large carbohydrate storage within the structure of cyanobacteria with negligible lignin content and large fermentable sugar makes it more suitable for such purpose An additional advantage of utilizing cyanobacterial biomass for bioalcohol fermentation is no or little release of toxins during pre-processing (pretreatment/hydrolysis) that decrease the efficiency of fermentative bacteria and reduce the end-product titter marginally (Ho et al., 2012). Use of petroleum based fuels is now accepted as unsustainable due to the continuous depletion in its level and large accumulation of CO_2 in the environment.

II. MATERIALS AND METHODS

All experiments were done in duplicates and the average value has been represented. Sample was collected from the different pond in Banaras Hindu University campus.

2.1 Collection and Cultivation

Cyanobacterial biomass has been proved as a potential source of lipid as well as carbohydrate that can be used for biodiesel and bioalcohols production, respectively. Efficiency of identified cyanobacterial biomass (obtained

from previous study (Kushwaha et al. 2018)) of Lyngbyalimnetica and Oscillatoriaobscurawas evaluated for lipid extraction and subsequent utilization of residual biomass for sugar analysis.

2.2 Lipid extraction

The potential of cyanobacterial biomass of L. limnetica and O. obscura for lipid extraction has been evaluated. Modified Bligh and Dyer method was used to extract lipid by changing the ratio of solvent chloroform: methanol (1:1–2:1) to get the maximum lipid yield. For 2 mL of sample volume approx 8.0 mL of chloroform: methanol was mixed in an appropriate proportion and lipid was extracted by cell rupturing using mortar-pestle. Further 4.0 mL of chloroform was added in the mixture to complete the extraction process. A known volume of distilled water (4.0 mL) was added in the mixture to separate the chloroform and methanol layers and mixed well. This mixture was then incubated at 40ºC for 30 min for clear separation of layers. After the incubation tubes containing sample were centrifuged at 5000 rpm for 10 min (Eltek TC 4100F, Mumbai, India), bottom chloroform layer was separated in a pre-weighted container and placed in an oven for 60 min at 60ºC until a constant volume was achieved. Figure1 show that over all process for lipid extraction and sugar analysis and sugar recovery for biobutanol production.

Lipid content was calculated using the following equation.

$$\text{Lipid Yield} = \frac{W_2 - W_1}{\text{sample weight}} \ (\text{g/g})$$

Where, W_1=initial weight of container (g), W_2=final weight of container (g)

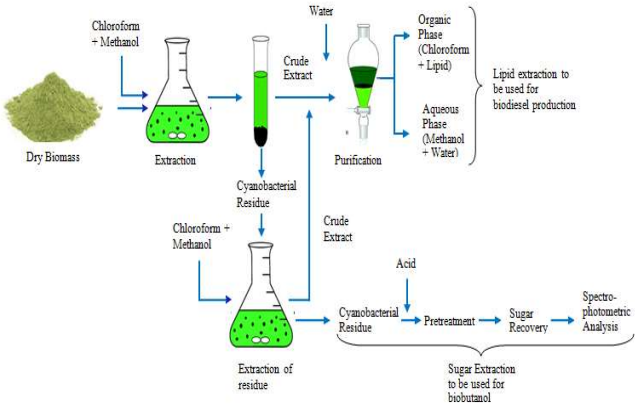

Figure 1: Simultaneous recovery of lipid and fermentable sugar from cyanobacterial biomass.

2.3 Batch pretreatment and sugar estimation

The effects of pretreatment agents like acidic and alkali pretreatments were tested at various parameters such as pretreatmentagent concentration, temperature and time to obtain maximum sugar concentration has been analyzed in the previous study (Kushwaha et al., 2018). Lipid extracted biomass was subsequently utilized for the acid pretreatment at optimized conditions (100°C for 60 min with 1.63 M H_2SO_4) to release sugars. The clear supernatant obtained from biomass digestion was adjusted to pH 7.0±0.2 and analyzed for the amount of total sugar spectrophotometrically using phenol- sulfuric acid method (Masuko et al, 2005).

III. RESULT AND DISCUSSION.

3.1 Lipid extraction and utilization of cyanobacterial residue for sugar release

Lipid content in cyanobacteria was varies from species to species and varies from different concentration of solvent. According to the culture conditions used in this study the cyanobacteria samples were determined to have total lipid content of biomass. The biomass oil content of the used cyanobacterial strain a highly dependent on the specific growth condition not only influences by the cyanobacteria species.Potential of cyanobacterial biomass of L. limnetica and O. obscura was evaluated for lipid extraction and subsequent utilization of the residual biomass for sugar release to be used for butanol fermentation. Simple grinding method was employed to rupture the cell wall of the biomass and maximum 0.123 and 0.139 g/g band pre-treatment processes were done after that lipid was extracted with a mixture of chloroform and methanol (2:1 v/v) and then the lipid extracted biomass was subsequently utilized for the acid pre-treatment at optimized conditions to release sugar in Figure (2) and Figure (3) for different cyanobacteria species.

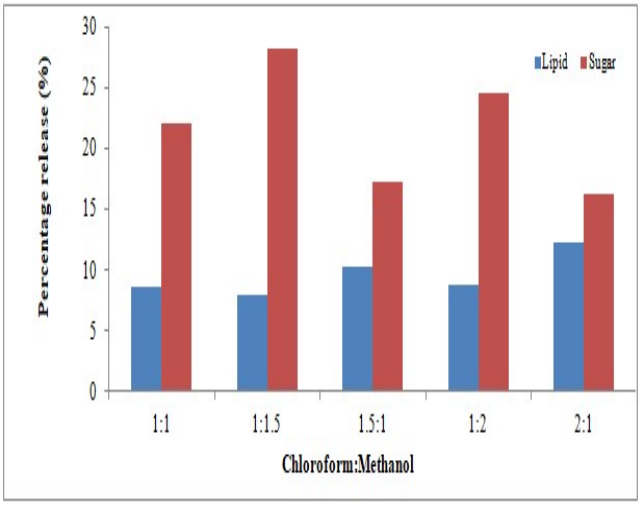

Figure 2: Sequential recovery of lipid and carbohydrate of species L. limnetica

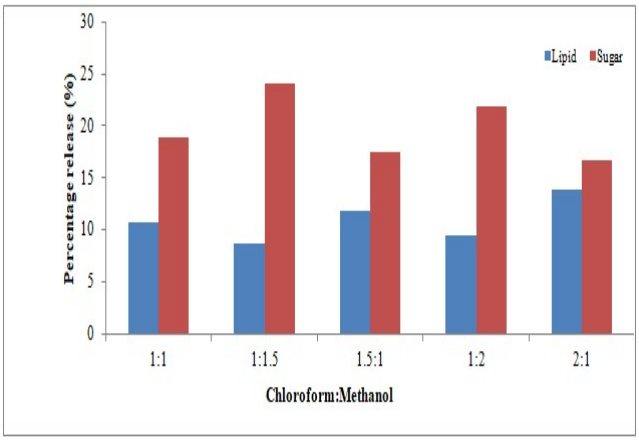

Figure 3: Sequential recovery of lipid and carbohydrate of species *O. obscura.*

Nearly 0.162 and 0.167 g/g of sugar was found from the residue obtained from the first step while maximum 0.283 and 0.241 g/g of sugar release was found from 1:1.5 (chloroform: methanol) lipid extracted biomass residue. Smoothening of the residual biomass surface was observed when it was treated with high chloroform concentration to extract lipid. This could be the possible reason of lower sugar yield from both the residual biomass.

IV. CONCLUSIONS

The lipid extraction and carbohydrate accumulation potential of two cyan bacterial species such as O. obscura and L. limnetica isolated from the water body. These two species have more potential to produce bio fuel. These cyan bacteria were easily grown in laboratory condition. The biomass of cyanobacteria was very cheap and has more production rate of bio fuel comparing to other biomass sources.

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