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Research Article

PRODUCTION OF ETHANOL USING HYDROLYSATE DERIVED FROM ACID PRETREATED DEFATTED BIOMASS OF *NANNOCHLOROPSIS LIMNETICA*

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ABSTRACT

Defatted microalgal biomass is one of the most promising third generation feedstock for biofuel production due to the contains considerable amounts of total reducing sugars than can be used as source for ethanol production by separate hydrolysis and fermentation. In the present study the defatted microalgal biomass of *Nannochloropsis limnetic* was acid pre treated and used as feedstock for ethanol production by *Saccharomyces cervevisiea*. The maximum yield of glucose 76.22±0.04 % obtained by acid pretreatment. The hydrolysate derived by acid pretreatment was used as substrate for ethanol production by *S. cervevisiea* and obtained the maximum ethanol 48.01±0.02 gL^{-1} with pH 4.0, at 30°C and 200 rpm. The results demonstrated that the defatted biomass of *N. limnetica* can be efficiently acid (H_2SO_4) pretreated to obtain high amount of fermentable sugars for enhanced yield of ethanol production at the optimum conditions.

INTRODUCTION

Fossil fuels have some of the environmental issues such as greenhouse gas emissions, air pollution, and acid rain (Scott *et al*., 2007). However, these can be reduced by using biofuels. Ethanol is one of the potential biofuel which can be used as a blend with gasoline to improve the octane number of the fuel, and reduce the greenhouse gas emissions (Wyman, 1996). Due to this positive impact the research has been directed towards economical ethanol generation. So bioethanol can be made using well known fermentation technology from a wide range of carbohydrate feedstock (Dinus, 2001; Bai *et al*., 2008)

The microalgae biomass is one of the most promising third generation feedstocks for biofuel production because their rapid growth rate, rich oil content, strong ability to sequester carbon dioxide and rapid conversion of carbon dioxide into a reusable gas such as methane or hydrogen. But the large-scale production of algal biofuels is not economically feasible yet. In biorefinery concept, every component of the biomass material would be used to produce commercially important products. The biorefining of microalgae biomass present opportunities to develop a sustainable and economical way of biofuel production. In a biorefinery, algae biomass is grown for the production of oil and other value added products. After all these molecules can be extracted and converted to final products, the bioethanol can be produced from the residual algae biomass (Nobre *et al*., 2013).

After extracted the lipids from the algal biomass which become as a defatted waste material. Many authors have been reported that the defatted microalgae as a valuable alternative protein ingredient for aquaculture and poultry (Ju *et al*., 2012; Leng *et al*., 2014) . Whole cell algal biomass or lipid extracted algal biomass requires proper pretreatment for lys is their cells to liberate fermentable sugars that can be readily metabolized and fermented for ethanol production by the fermenting microorganisms. There is no documentation till date about a viable and economic pretreatment method of algal biomass (Rodjaroen *et al*., 2007).

The microalgae biomass is degraded by mechanical or enzymatic methods and using as a raw material for ethanol production by fermentation using yeast. From the single biomass can be produced lipid based biofuels and ethanol biofules (Chaudhary *et al*., 2014). The yeast *Saccharomyces cerevisiae* YPS 128 a suitable organism for highest

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concentration of bioethanol production the by fermentation of the acid hydrolysate of *Chondrus crispus* (Kostas *et al*., 2016). Aim of the present study is to produce the bioethanol using hydrolysates derived from the acid pre-treatment of defatted biomass of *Nannochloropsis limnetica* by *Saccharomyces cerevisiae* and optimize their process conditions.

MATERIALS AND METHODS

Microalga

The fresh water green microalga *Nannochloropsis limnetica* was isolated from Kuppanatham Dam (12.40 ˚N and 78.75˚E) which is situated in Chengam Taluk's, Tiruvannamalai District, Tamil Nadu, India and cultivated in municipality waste water for biomass production.

Biomass harvesting and lipid extraction

Biomass was harvested by centrifugation at 14,000 rpm for 15minuts. The pellet was washed with distilled water and again centrifuged at the same rpm. Finally cell pellet (microalga biomass) was placed in a watch glass and dried in an oven at 60 °C for 12 hrs and cooled in a desiccator. The total lipids from biomass was extracted by method of Bligh and Dyer (1959). Then the defatted biomass was air-dried at room temperature.

Acid hydrolysis and preparation of defatted biomass hydrolysates

The defatted biomass was pre-treated by H_2SO_4. A 100g of defatted biomass was treated with 1 L of 5% (w/v) H_2SO_4 and autoclaved at 121^oC for 15min. After hydrolysis, the liquid fraction was separated by centrifugation at 14,000 rpm for 15min. Then the defatted biomass hydrolysate pH was adjusted to pH 7.0 using NaOH and stored at room temperature until further use.

Analytical procedures

Total carbohydrate content in defatted biomass was determined by the phenol sulphuric acid method (Dubois *et al*., 1956) using D-glucose as a standard. Simple sugars and sugar degradation products (propionic acid, acetic acid, formic acid and lactic acid) in hydrolysates were identified and quantified by high pressure liquid chromatography (HPLC) using a Waters system (Massachusetts, USA), equipped with an Aminex HPX-87-H column and a refraction index detector (Waters 2414). The eluent consisted of a 5 mM H_2SO_4 solution, previously filtered through a 0.20µm membrane filter and degasified at a flow rate of 0.6 mL/min. A volume of 20µL of sample was injected at 60^oC (Hernandez *et al*., 2015).

Microorganism Yeast

Compressed baker's yeast *Saccharomyces cerevisiae* was purchased from local market and used throughout this study for ethanol production. The yeast was maintained on agar plates supplement containing 0.3% yeast extract, 0.3% malt extract, 0.5% peptone, and 1.0% glucose at 5°C.

Inoculum preparation

Inoculum was prepared by transferring a loopful of *S. cerevisiae* stock culture to the medium supplement with 0.3% yeast extract, 0.3% malt extract, 0.5% peptone, and 1.0% glucose (pH 5.5) and cultivated at 35°C for 24 h in orbital shaker at 120 rpm. Cells of exponential phase were harvested by centrifugation (4,000rpm, 5min) and washed twice with sterilized distilled water and used for fermentation process.

Batch fermentation of hydrolysates derived from the acid pre-treatment of defatted biomass

Batch fermentation was carried out in 250 mL Erlenmeyer flasks containing 100mL sterilized hydrolysates of defatted biomass of *N. limnetica* as substrate. Then the flasks were inoculated with 1%(v/v) yeast cells and incubated in orbital shaker at 30°C at 150 rpm for 48hrs. Samples were taken after every 6 h once and estimated ethanol concentration from the culture broth by the method of Seo *et al*., (2009). An untreated defatted biomass was used as control.

Optimization of fermentation by one parameter at a time

The optimization experiments were carried out in 250 mL Erlenmeyer flask using 100mL sterilized hydrolysate of defatted biomass with 1% (v/v) inoculum at different concentration of hydrolysate (10 to 100% with 10% increment), different temperature (25 to 45^oC with 5^oC increment), pH (2, 3, 4, 5 and 6) and different agitation speed (100 to 300 rpm with 50rpm increment). Optimum condition of each parameter was used for further study. Sample of 5mL culture was withdrawn at 12h intervals and centrifuged at 14,000 rpm for 15 min. The cell free broth was used for ethanol estimation.

Statistical analysis

All the experiments were carried out in triplicates and results are presented as the mean ± standard deviation (SD) values of three independent replicates. The standard deviation did not exceed 5% of the average values. Data were further analyzed using one-way analysis of variance (ANOVA) using MINITAB 12 software. A significant difference was considered at the level of $p < 0.05$.

RESULTS AND DISCUSSION

The sugar composition of hydrolysate derived from the defatted biomass of *N. limnetica* using 5 % (w/v) H_2SO_4 pre-treatment in a autoclave at 121 °C for 15 min shown in Table 1. The sugar degradation products such as propionic acid, acetic acid, formic acid and lactic acid were not detected in hydrolysate by HPLC. The monosaccharide glucose 76.22 ± 0.04 % was obtained by acid pretreatment of defatted biomass of *N. limnetica*. Jeon *et al*., (2013) recovered only 32.4 % of dissolved carbohydrate from microalgal biomass by sonication pretreatment. Production of bioethanol from second-generation feedstock such as food crops (sugar cane and corn) has a lot of challenges due to their direct impact on food prices and deforestation. This could be overcome by using microalgal biomass. Because the carbohydrates are one of the major primary metabolites of microalgae. So the simple pre treatment is enough to recover the soluble carbohydrates for ethanol production. Whereas the lignocellulosic feedstock requires strong pretreatment prior to fermentation. Whole microalgae biomass as well as lipid extracted algal biomass has potential to be used as an economical and sustainable feedstock for the production of bioethanol.

Table 1 Analysis of sugar composition of hydrolysate derived from defatted biomass of *N. limnetica*

Sugar composition	Concentration (%)
Total carbohydrate content	22.42±0.02
Maltose	n.d
Glucose	76.22±0.04
Xylose	27.32±0.05
Ramnose	6.42±0.31
Fucose	5.02±0.04
Other sugars*	0.79±0.09
Acetic acid	n.d
Formic acid	n.d
Lactic acid	n.d
Propionic acid	n.d

n.d- Not detected
* Amount of sugars lower than 1% by mass.
Data are the mean ± SD of three measurements

Effect of different concentration of hydrolysate on ethanol production

The effect of different concentration of hydrolysate derived from defatted biomass of *N. limnetica* by acid pre-treatment. As shown in Figure 1, when increasing the concentration increased the ethanol production by *S. cerevisiae*. No any inhibitory effect was found in this experiment. Because the defatted microalgal biomass a rich source of carbohydrates, proteins, and with good mineral content. So they can be used as feed, fertilizer, and substrate for the production of bioethanol/bio-methane (Maurya *et al.*, 2016b). The maximum ethanol concentration 37.33 ± 0.09gL^{-1} was obtained at 100% hydrolysate of defatted biomass of *N. limnetica*. Hence, this concentration was used for further ethanol production.

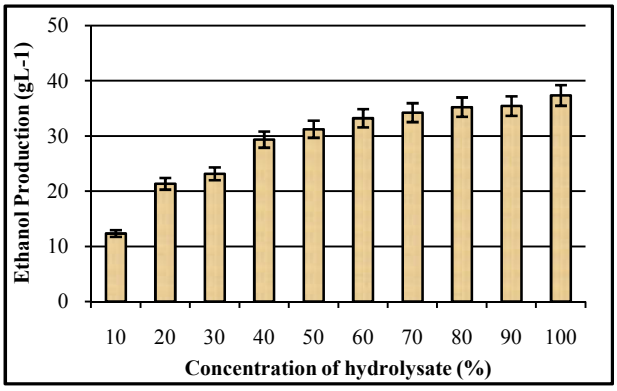

Figure 1 Effect of different concentration of hydrolysate derived from defatted biomass of *N. limnetica* on ethanol production by *S. cerevisiae*

Effect of temperature on ethanol production

The temperature optimization experiments were carried out at different temperature range from 25°C to 45°C with 5°C increment with 100% hydrolysate as ethanol production medium. As shown in the Figure 2, *S. cervevisiea* efficiently proceeded the fermentation process by converting sugars into maximum ethanol 39.41 ± 0.09 gL^{-1} at 30°C. The ethanol production and temperature are interdependent because the calculated 'r' values are significant at 0.05 levels at df_5. In this study, fermentation temperature 30°C was found to have maximum ethanol production. This study is accordance with Md. Fakruddin *et al*., (2012) study. The temperature 30°C was found as optimum temperature and used for further optimization of ethanol production. Generally *Saccharomyces* strains were ferment at the temperature 20-35°C (Aldiguer *et al*., 2004; Kadar *et al*., 2004).

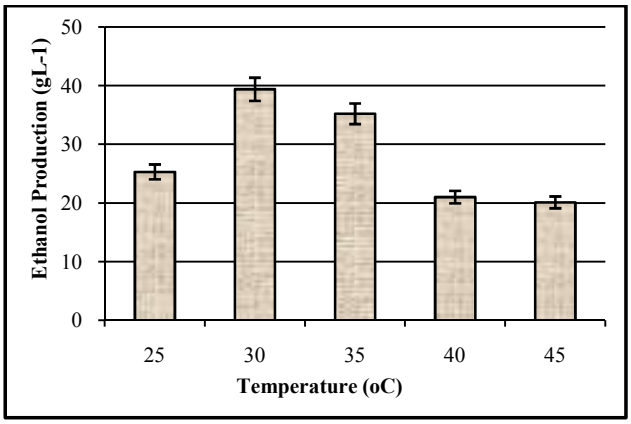

Figure 2 Effect of temperature on ethanol production by *S. cerevisiae* using hydrolysate derived from defatted biomass of *N. limnetica*

Effect of pH on ethanol production

The effect of initial pH on ethanol production from 100% hydrolysate of defatted biomass was studied. The initial pH of the fermentation was maintained from 2 to 6. Figure 3, shows that the *S. cerevisiae* fermented successfully the defatted biomass and produced maximum ethanol of 42.32±0.08gL^{-1} at pH 4.0, which is good agreement with the results of Lin *et al.*, (2012). The calculated 'r' values are significant at 0.05 levels at df_5. The 'r' value suggests that the fermentation capacity of *S. cerevisiae* is pH dependence. In the present study, initial pH 4.0 was found as a best pH for fermentation of hydrolysate of defatted biomass by *S. cerevisiae*. Hence, the optimum initial pH 4.0 was used for further optimization.

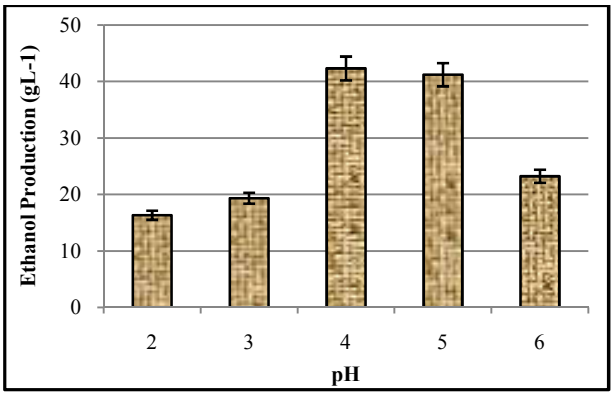

Figure 3 Effect of pH on ethanol production by *S. cerevisiae* using hydrolysate derived from defatted biomass of *N. limnetica*

Effect of different agitation speed on ethanol production

Effect of different agitation speed ranging from 100 to 300 rpm on ethanol production was studied. As shown in Figure 4, when agitation speeds increased from 100 to 300 rpm, ethanol production increased as the stirring rate increased from 100-200 rpm, and further increase in the agitation speed resulted in decreased ethanol production. The maximum ethanol production of 48.01±0.02gL^{-1} was obtained at 200rpm at 30°C and pH 4.0. Agitation speed 200rpm was found to be an ideal agitation speed because the calculated 'r' values are significant at 0.05 levels at df_5. The 'r' value suggests that the ethanol production and agitation speed are interdependent. The growth as well as performance of the microorganism cells can

improved by improve the mass transfer characteristics of culture broth by agitation (Md. Fakruddin *et al*., 2012).

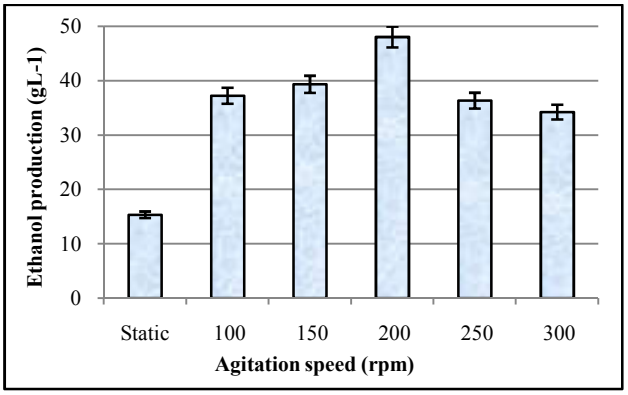

Figure 4 Effect of agitation speed on ethanol production by *S. cerevisiae* using hydrolysate derived from defatted biomass of *N. limnetica*

CONCLUSION

The acid (H_2SO_4) pretreatment in a autoclave at 121 °C for 15min enhanced the release of maximum glucose from defatted biomass of *N. limnetica*. The maximum ethanol production also was obtained from defatted biomass hydrolysis by acid hydrolysis of defatted biomass. This study results demonstrate that the acid hydrolysis is a simple method and enhances the conversion of defatted biomass to soluble sugar, and subsequent fermentation of the sugar residues significantly increases the production of ethanol. The hydrolysate of defatted biomass used as substrate for ethanol production and yeast *S. cervevisiea* efficiently proceeded the fermentation process by converting sugars into maximum ethanol 48.01±0.02gL^{-1} with pH 4.0, at 30^{o}C and 200 rpm.

References

1. Aldiguer, A.S., Alfenore, S., Cameleyer, X and Goma, G. 2004. Synergistic temperature and ethanol effect on *Saccharomyces cerevisiae* dynamic behaviour in ethanol bio-fuel production. *Bioproc Biosyst Eng.,* 26:217-22.
2. Bligh, E.G. and Dyer, W.J. 1959. A rapid method for total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37:911-917.
3. Dubois, M, Gilles, K.A., Hamilton, J.K., Rebers, P.A and Smith, F. 1956. Colorimetric method for determination of sugars and related substances, *Anal. Chem.,* 28: 350-356.
4. Hernandez, D.B., Riano, R., Coca, M and Garcia-Gonzalez, M.C. 2015. Saccharification of carbohydrates in microalgal biomass by physical, chemical and enzymatic pre-treatments as a previous step for bioethanol production, *Chem. Engg. J.,* 262:939-945.
5. Jeon, B-H, Choi J.A., Kim, H.C., Hwang, J-H., Abou-Shanab, R.A.I., Dempsey, B.A., Regan, J.M and Kim, J.R. 2013. Ultrasonic disintegration of microalgal biomass and consequent improvement of bioaccessibility/bioavailability in microbial fermentation. *Biotechnol Biofuels.,* 6(37):1-9.
6. Ju, Z.Y., Deng, D-F and Dominy, W.2012. A defatted microalgae (*Haematococcus pluvialis*) meal as a protein ingredient to partially replace fishmeal in diets of Pacific white shrimp (Litopenaeus vannamei, Boone, 1931), *Aquaculture*, 354-355: 50-55
7. Kadar, Z., Szengyel, Z and Reczey, K., 2004. Simultaneous saccharification and fermentation (SSF) of industrial wastes for the production of ethanol. *Ind. Crop Prod.,* 20: 103-110.
8. Leng, X., Hsu, K-N., Austic, R.E and Lei, X.2014. Effect of dietary defatted diatom biomass on egg production and quality of laying hens, *J. Anim Sci Biotechnol.,* 5(1):3.
9. Lin Y., Zhang, W., Li, C., Sakakibara, K., Tanaka, S and Kong, H. 2012. Factors affecting ethanol fermentation using *Saccharomyces cerevisiae* BY4742, *Biomass Bioenergy,* 47:395-401.
10. Md. Fakruddin, Md. Abdul Quayum, Monzur Morshed Ahmed and Naiyyum Choudhury, 2012. Analysis of Key Factors Affecting Ethanol Production by *Saccharomyces cerevisiae* IFST-072011. *Biotechnology,* 11: 248-252.
11. Nobre, B.P., Villalobos, F., Barragan, B.E., Oliveira, A.C., Batista, A.P., Marques, P.A.S.S., Mendes, R.L., Sovova, L., Palavra, A.F. and Gouveia, L. 2013. A biorefinery from *Nannochloropsis* sp microalga - Extraction of oils and pigments. Production of biohydrogen from the leftover biomass. *Bioresource Technol.,* 135: 128-36.
12. Rodjaroen, S., Juntawong, N., Mahakhant, A and Miyamoto, K. 2007. High biomass production and starch accumulation in native green algal strains and cyanobacterial strains of Thailand Kasetsart. *J Nat Sci.,* 41:570-575.
13. Seo, H.B., Kim, H.J., Lee, O.K., Ha, J.H., Lee, H and Jung, K.H. 2009. Measurement of ethanol concentration using solvent extraction and dichromate oxidation and its application to bioethanol production process. *J Ind Microbiol Biot.,* 36:285-292.
14. Maurya, R., Paliwal, C., Chokshi, K., Pancha, I., Ghosh, T., Satpati, G.G., Pal, R., Ghosh, A and Mishra, S. 2016b. Hydrolysate of lipid extracted microalgal biomass residue: an algal growth promoter and enhancer. *Bioresource Technol.,* 207: 197-204.
15. Chaudhary, L., Priya, P., Soni, N., Singh, P and Tiwari, A. 2014. Algae as a Feedstock for Bioethanol Production: New Entrance in Biofuel World, *Inter. J. ChemTech Res.,* 6(2): 1381-1389.
16. Kostas, E.T., White, D.A., Du, C and Cook, D.J. 2016. Selection of yeast strains for bioethanol production from UK seaweeds, *J. Appl. Phycol.,* 28:1427-1441.
17. Dinus, R.J. 2001. Genetic improvement of poplar feedstock quality for ethanol production, *Appl. Biochem. Biotechnol.,* 91-93: 23-34.
18. Bai, F.W., Anderson, W.A and Moo-Young, M.2008. Ethanol fermentation technologies from sugar and starch feedstock's, *Biotechnol. Adv.,* 26: 89-105.
19. Wyman, C. 1996. Handbook on bioethanol: production and utilization. Washington, DC: Taylor & Francis.
20. Scott, E., Peter, F and Sanders, J. 2007. Biomass in the manufacture of industrial products - the use of proteins and amino acids. *Appl. Microbio. Biotechnol.,* 75: 751-762.