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PRODUCTION OF BIOFUEL FROM MICROORGANISMS

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Abstract:
The depletion of fossil-based fuels which lead to a need to find an alternative for fuel and the environmental impact produced by the conventional sources of energy over the planet has led to a new research work to seek the sustainable sources of clean and green energy. Biofuel is committed to becoming a worldwide leader in the development and deployment of renewable energy resources for fuels. Biofuel is an alternative fuel, made from renewable biological sources. Biofuel can also be produced from microbial sources like algae, bacteria, yeast, and fungi. Microbes exhibit the capacity to accumulate intracellular lipids within that in excess of 70 percent of their biomass during metabolic stress periods. The traditional feedstocks for Biofuel production in biomass are vegetable oils and animal fats resulting in competition with the food industry. Fungal lipids as a source for Biofuel was less studied. Hence the fungal lipids source as Biofuel was taken for this study.

Key Words: Fungal Lipids, Alternative Fuel and Biofuel & Microbial Fuel

Introduction:

The rapid growth of the world's population and increased industrialization leads to an increased need for bioenergy. A major challenge faced by mankind is the gradual and exhaustion of the earth fossil energy sources. The combustion of these energy materials used for heating of transportation fuels is one of the key factor responsible for global warming and environmental pollution. The world is facing declining liquid fuels will reserves at a time, when energy demand is exploding [1]. If consumption continues at the current rate, the fossil fuel supply could be gone before the end of the century. Biofuels are needed to move petroleum-derived transport fuels, which plays a major role in global warming and are of limited availability. Biofuel is potential renewable fuels that have attracted the most attention. Biofuel from microalgae seems to be the only renewable biofuel that has the potential to completely displace petroleum-derived transport fuels without adversely affecting the supply of food and other crop products. [2] Biomass energy, carbon neutral transport fuels are necessary for environmental and economic sustainability. [3]. Microbial lipid is a promising source of oil to produce biofuel if it can be generated from lignocellulosic materials [21]. The use of microbial systems for Biofuel production although not exploited industrially until now. The potential of the microorganism to grow on an almost numerous variety of food source which may play a significant role in building out the society from its current energy loss crisis. Increasing interest is generated to explore ways to reduce the high cost of biofuel especially the loss of the raw materials [4]. In addition, microbes can be tailored to utilize varies carbon sources as feedstock for the production of oils, such as waste or agriculture by-product. Many molds, yeast, and algae, fungi exhibit the capacity to accumulate intracellular lipids in excess of 70 percent of their biomass during metabolic stress periods [5]. However study in this area are not available in a larger number on the use of microbial sources for lipid production, Hence this present study was undertaken to exploits the microbes for biofuel production and to optimize the conditions for higher lipids production from oleaginous fungi. Lipids produced from microorganisms show great promise for biofuel production, but a major limiting factor is the high production cost attributed to feedstock [20]. The study is to investigate the feasibility of culturing the fungi with materials and to screen the best lipid producing from microorganisms.

Materials and Methods:

Isolation and Identification of Oleaginous Fungi [6]: Soil sample was collected from the different part of Tamil Nadu, to isolate the oleaginous fungi for lipid production disc of the isolate (PR) was inoculated in plain water agar. After one day the plain water agar plate is soaked with 4ml of Petri's salt solution and then the plate was incubated to 4 – 6 days [10]. Screening of oleaginous fungi was done by the method given by [6]. The culture was harvested and tested for lipids and biomass (gl-1).

Cell biomass Determination for Fungi [11]: Screened isolates of fungal biomass of mycelia were harvested from the incubated flasks by suction filtration through Whatman No 1 filter paper and thoroughly washed with distilled water. Then mycelia were filtered and dried at 60^{o}C in an oven for 15 hrs. The dried sample weight was taken and dry biomass was expressed in gl^{-1}

Extraction of Fungal Lipids: Fungal lipid from the dried mycelia extracted as per the method by Bligh and Tyer [12]. The influence of different nutritional such as carbon and nitrogen source and growth conditions such as pH and temperature were studied in PR. The lipids and biomass production by fungal isolate PR with different carbon sources were also studied with following Glucose 30.0 (0.16 M), Yeast extract 5.0g/l, Distilled

water 1000 ml, pH 5.4, Glucose in the broth were replaced separately with different carbon sources.[6] Different carbon sources like glucose, fructose, sucrose, and lactose were used in the concentration of 0.16 (M) in One gram of fungal isolate as mycelial suspension.

Culture Conditions: 50 ml of broth was dispensed in 250 ml sterilized conical flasks and incubated at 30^{o}C and kept in an incubator shaker at 200 rpm for 7 days. The mycelium was filtered through Whatman No.1 filter paper. Liquids and biomass content of filtered fungal mycelium were analyzed. From the literature, survey glucose was found to be the best source for lipid production. Hence different concentration of glucose was taken to find out the optimum level for maximum lipids production.

Lipids Production and Glucose Utilization by Fungal Isolate (PR): Lipids production and glucose utilization by fungal isolate (PR) The rate of depletion of glucose source and the formation of lipids in a fungal isolate (PR) was studied. The screening broth with 0.6 M of glucose concentration was dispensed into the 250 ml Erlenmeyer flask and sterilized. 1g of the fungal isolate (PR) was inoculated as suspension of mycelial and incubated in a shaker with an incubator for seven days at 220 rpm. After incubation, flasks were withdrawn and filtered using Whattman No 1 filter paper. The filtrates were analyzed for glucose content and mycelia were analyzed for lipids and biomass content. Glucose in the culture filter was determined by the method of Miller [13]. The culture filtrate was centrifuged at 6000 rpm at 30^{o}C from that 0.2 ml supernatant was pipetted out into a test tube.1 ml alkaline copper tartrate reagent was added to the above solution and placed over boiling water bath for 10 min. After cooling, 1 ml of arsenic molybdate reagent (given below) was added. The orange-red color developed was observed and measured in colorimeter at 620 nm after 10 min. the standard curve was prepared with the absorbance value of the standard and value of samples was plotted accordingly to calculate the amount of glucose present.

The fungal isolate (PR) was tested for its ability with nitrogen sources of different variety like yeast extract, ammonium chloride, and ammonium sulphate. The broth of Yeast extract was replaced with different nitrogen source individually like Yeast extract, Ammonium chloride, and Ammonium sulphate. For large scale production of lipid by fungal isolate (PR) in bioreactor nutritional and growth factors were adopted for the Nutrient adopted Glucose 0.6 M, Yeast extracts 10.0 g, pH 6.5 and Temperature 30 o C. [14]. Optimal fermentation medium was utilized for fermentation process in a 5-L bioreactor (Lark innovative technologies, India), equipped with disc impeller, oxygen, and pH electrodes. The temperature, agitation speed, gas purging flow rate, pumping rates, antifoam addition, and the vessel level was also monitored by the equipment. The pH value and temperature were kept constant throughout the experiment time [15]. The fermentation broth was inoculated *Rhodotorula glutinis* (24 hrs) at a 10 percent level containing (28x10 CFU/ml) in the broth. Fermentation was carried out for a period of five days. The biomass was harvested by centrifugation and lipids produced by yeast strains were estimated by harvesting the biomass. GC-MS Analysis was done for identification of lipids and fatty acids by Gas Chromatography- Mass Spectrometer (GC- MS) [16]. The physicochemical properties of the fungal isolate were done in order to test its efficiency to use as Biofuel. According to American standards for testing of material (ASTM) (2003), the following properties were analyzed. The characters to be as a biofuel was analyzed by their physical and fuel properties including density, iodine value, acid value, viscosity, cloud point, pure point, gross heat of combustion and volatility.

Results and Discussion:

Lipids production by fungal isolates among the five isolates studied PR sample shows high lipid production and biomass (28 % and 10g/l) (Fig.1). So the PR sample was selected for further studies.

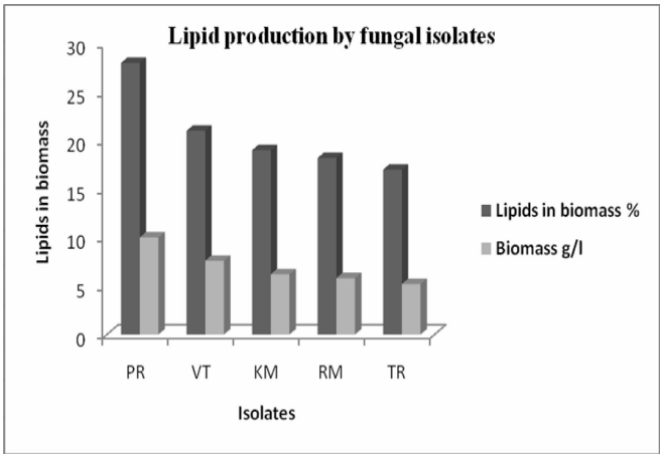

Figure 1: Lipid production by fungal isolates

In order to optimize the temperature for maximum lipids production by fungal isolate (PR), the fungus was grown at different temperature ranges of 20, 25, 30, 35 and 40^{o}C and the Effect of temperature on lipids production by fungal isolate was identified. Fungus accumulated significantly more lipids (43.2%) at 30^{o}C and least amount of lipids (14.3%) was recorded at 40^{o}C (Fig.2). The study indicated that the fungal isolate (PR) optimum temperature was 30^{o}C on biomass as well as lipid production reported by [17].

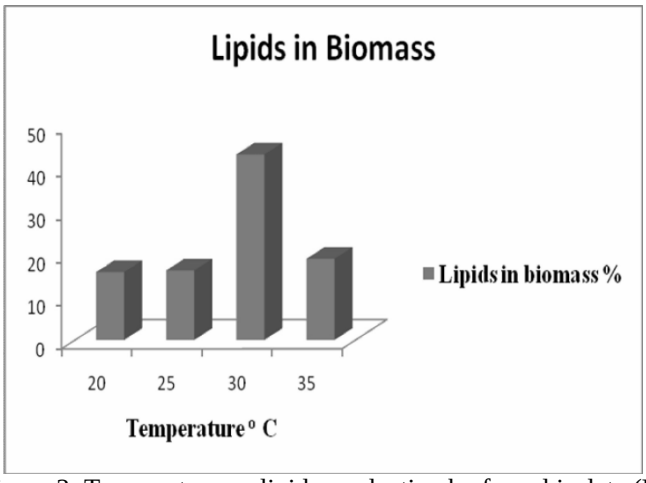

Figure 2: Temperature on lipids production by fungal isolate (PR)

Effect of pH on lipids production by fungal isolate (PR) The influence of pH by fungal isolate (PR) was investigated at different pH ranged from 5.5, 6.5, 7.5 and 8.5 on lipids production. Maximum lipids (43.5 %) and biomass (10.7gl-1) content were obtained significantly when the fungus was grown at a pH of 6.5. It is observed that the least amount of lipids (10.2%) and biomass (5.6%) content was recorded at pH 7.5 and no growth and lipids accumulation was observed at a pH of 8. [18] [19] reported that best pH for biomass and lipid production together with a high proportion of PUFA's (polyunsaturated fatty acids) was around 6.0.

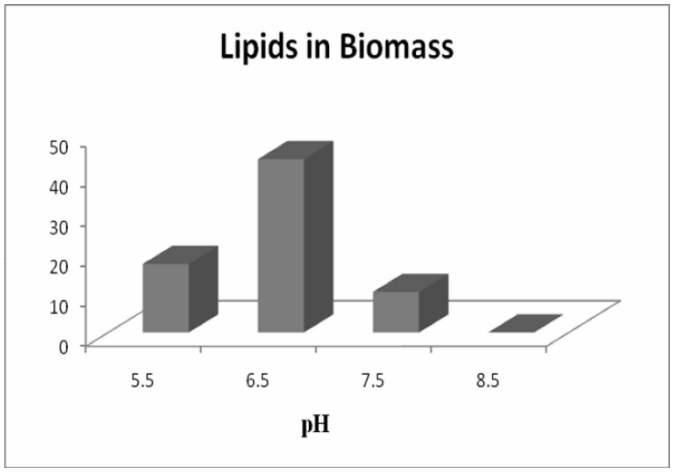

Figure 3: pH on lipids production by fungal isolate (PR)

Physico-Chemical Properties of Fungal Lipids:

Lipids produced through fermenter was analyzed for its physicochemical properties in order to use as Biofuel, values were listed. The obtained values were compared with ASTM standards. The physical properties such as, specific gravity of 0.95 g/cc, viscosity at 40^{o}C of 50.81cSt, calorific value of 32.09 Kcal/kg, Flash 218^{o}C, Fire point 230^{o}C cloud 4^{o}C, pour point 7^{o}C and the chemical properties of lipids like Free Fatty acids (FFA) of 14.54% , acid value 27.2 were obtained. The viscosity of fungal lipids (54.81 cSt) and acid value (28.2) were higher when compared to the standard (1.9- 6.08cSt and < 0.8) respectively. Whereas other properties like flashpoint, carbon residue were within the limit of the standard. As the value of flash and fire point of fungal lipids satisfies the standard limits to be as biofuel. It can be used as such, like vegetable oil. Since the viscosity, acid value and FFA of the fungal isolate is higher than the standard level, which may cause the engine blockage and it has to be reduced through trans esterification process. Fatty acids profiles such as palmitic acid (36.4 min), stearic (33.34) and oleic acid (32.60) were identified based on their retention times by GC-MS.

Conclusion:

The present study reveals that the Microbial Biofuel produced from fungi is very economical and eco-friendly because of the less amount of carbon content. The values of the fungal isolate are higher than the

standard level, which may cause the engine blockage and it has to be reduced through transesterification process. The study summarized that the use of fungal lipids from (PR) as Biofuel should undergo some pretreatment and transesterification to meet out the standard levels of ASTM (American Society for Testing and Materials) D6751 specification.

References:

1. Matthew N Campbell, Biofuel: Algae as a Renewable Source for Liquid Fuel, Biofuel: Algae as a Renewable Source for Liquid Fuel. Guelph Engineering Journal, (1), 2 - 7. ISSN: 1916-1107. ©2008
2. Reda A. I. Abou-Shanab, Byong-Hun Jeon, Hocheol Song, Yongje Kim, Jae-Hoon Hwang, Yonsei University, Department of Environmental Engineering, The Online Journal on Power and Energy Engineering (OJPEE) Vol. (1) – No. (1) Reference Number: W09-0002 4 Algae-Biofuel: Potential Use as Sustainable Alternative Green Energy 305-350, S. Korea
3. Yusuf Chisti, 2007. Biofuel from microalgae, Biotechnological Advances. 25(3): 294-306.
4. Miao, X.L, Wu, Q.U. Biofuel production from heterotrophic microalgal oil. Bioresource Technology 2006, 97, 841-846.
5. Ratledge, C. and J. P. Wynn. 2002. The biochemistry and molecular biology of lipid accumulation in the oleaginous microorganism. Adv. Appl. Microbiol. 51: 1-51.
6. Ahmed. S.U., K.S. Sudheer, P. Ashok, K. Sanjit, and B. N. Prasad. 2006. Effects of various process parameters on the production of linolenic acid in submerged fermentation. Food Technol. Biotechnol., 44(2): 283-287
7. Frances Duncan, 2005. Applied Microbiology Laboratory Manual By Frances Duncan With contributions from Valerie Walker and Neil Clark Fourth Edition.
8. Alexopolous, C. J. 1996. Characterization of fungi. In. fourth (Ed.), Introductory mycology
9. Watanabe, T. and M. Yoshida. 1983. Quantitative assay of sporangia and zoospore production by *Pythium aphanidermatum* with a soaking plain water agar culture method. *Ann. Phytopath. Soc*. Japan 49: 137-142.
10. Tucker, C. M. 1931. Taxonomy of the genus *Phytophthora de Bary*. Res. Bull. Missouri Agri Exp.stn. 153: 1- 208
11. Cerik, M., P. Andrani and J. Sajbidor. 1996. Effect of extraction methods on lipid yield and fatty acid composition of lipid classes containing λ-linolenic acid extracted from fungi. JAOCS, 73(3): 357-365
12. Bligh E.G. and N.J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can J. Biochem. Physiol., 37: 911-917.
13. Miller, J. (1972). Notes on sugar determination J. Biol. Chem., 200: 245-247
14. Lindberg, A. M., G. Molin. 1993. Effect of temperature and glucose supply on the production of polyunsaturated fatty acids by the fungus Mortierella alpine CBS 343.66 in fermentor cultures. Appl. Microbiol. Biotechnol., 39: 450-455.
15. Chuan-Chao Dai, Jie Tao, Feng Xie, Yi-jun Dai and Mo Zhao Biofuel generation from oleaginous yeast *Rhodotorula glutinis* with xylose assimilating capacity, 2007
16. Saxena S, Brody AL, Schwartz JM, Baxter LR (1998) Neuroimaging and frontal-subcortical circuitry in obsessive-compulsive disorder. Br J Psychiatry 35(Suppl):26–37
17. Sumner, J. L., E.D. Morgan, and H. C. Evans. 1969. The effect of growth temperature on the fatty acid composition of fungi of the order Mucorales. Can. J. Microbiol., 15: 515-520
18. Bajpai, P.K., P. Bajpai and O.P. ward. 1991. Arachidonic acid production by fungi. Appl. Environ. Microbiol. 57: 1255-1258.
19. Yamada, H., S. Shimizu, and Y. Shinmen. 1987. Production of arachidonic acid by Mortierella elongate IS-5. Agric. Biol. Chem. 51: 785-790.
20. Yubin Zheng, Xiaochen Yu, Jijiao Zeng, and Shulin Chen, 2012, Feasibility of filamentous fungi for biofuel production using hydrolysate from dilute sulfuric acid pretreatment of wheat straw. Biotechnol Biofuels. 5: 50.
21. Zhang J, Hu B, Microbial lipid production from corn stover via Mortierella isabellina. Appl Biochem Biotechnol. 2014 Sep; 174(2):574-86