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EFFECT OF WASTE CARBON ON BIOMASS AND LIPID CONCENTRATION USING *ASPERGILLUS NIGER*

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ABSTRACT
Effect of different waste containing carbon source on biomass and lipid production of *Aspergillus niger* were sudied. Different low cost and easily available carbon based feedstock substrate such as Potato peel and Rice husk was characterized and used as carbon source. These feedstocks were used for the culturing of *Aspergillus niger*. There was a significant difference in the lipid accumulation process as related to these carbon sources. It was found that, increase in biomass concentration increased the lipid production when the oleaginous fungal species *Aspergillus niger* were cultivated in media containing potato peel as waste carbon source. The lipid extracted (%) was found to be 66.2% in Potato peel, 58.3% in Rice husk and 61.6% control. This show that the oleaginous fungal species *Aspergillus niger* has an potential to cultivate in waste carbon sources and reduces the production cost. This process leads to the integrated process for production of alternative fuel with integration of waste management.

KEYWORDS: *Aspergillus niger*, Waste carbon, Biodiesel, Potato peel and Rice husk.

INTRODUCTION
In recent times, the domain has been challenged with most important issues in global warming and energy crisis.[1] It is vibrant that use of fossil fuels is the main cause for global warming, whereas energy crisis is due to the depletion of fossil fuel resources.[2-4] Since 85% of total energy demand is produced from the fossil fuels which are extensively utilized and this has led to climate change, environmental pollution and health problems.[5] In order to produce other fuel than fossil fuels, several biofuel contenders were proposed which can able to eliminate the vulnerability of energy sector. The bio fuels have been mainly produced from waste oil and oil extracted from crops.[6] There are several difficulties while food crops are used for the biofuel production such as escalation of food prices, food shortages and large quantities of water are required for proper irrigation of biofuel crops.[7-8] The economically and technically viable biofuel resources should possess quality such as cost effectiveness (cost less than petroleum fuels), minimal use of water and land and also should enable air quality improvement.[9]

MATERIAL AND METHODOLOGY
The oleaginous fungal species *Aspergillus niger* were isolated from soil samples by the use of the serial dilution method and plating method. 10 grams of each soil sample was dissolved in 90 mL of sterile distilled water. This was further added in 250 mL of conical flasks; the flasks were shaken vigorously for five minutes. The suspensions were serially diluted up to one thousand folds.[4,10] One mL of the one thousand dilution aliquot was transferred aseptically onto sterile Petri dishes and then 10 - 15 mL of molten PDA medium was poured into the Petri dishes. The plates were incubated at 30°C for 5 days. Single fungal colonies were isolated and transferred repeatedly to new plates until pure cultures were obtained, which were maintained in PDA slants and stored at 4°C.

Media Preparation
The composition of media used for producing high lipid content with low cost substrate contained (g/l) Carbon source, 100; Peptone, 10; NaCl, 1.5; Lysine, 0.5; $ZnSO_4$. $7H_2O$, 0.5; pH 6, and maintained at 30^0C. To the above media composition 25g/l of glucose was also added. In the above mentioned media the carbon source was replaced by various low cost substrates such as Potato peel and Rice husk.[11] These low cost carbon substrates were sun dried until they were crispy to touch and were grounded into fine powder. The fine powdered carbon source raw materials were then sieved with 0.2 mm in

diameter sieve and kept in desiccators until required for use.[11-12] The fungi were inoculated in the production medium and were kept in shaker at 150 rpm.

Same procedure was followed for comparison with control in which carbon source was taken as pour glucose.

Biomass analysis

Fungal biomass was analyzed by filtering the contents in conical flasks using 0.09 mm stainless steel sieve (Starsdet, Germany). Filtered biomass was washed twice with distilled water and then dried at 100°C until a constant weight.[13]

Lipid extraction from fungal mycelia

At the 5th day of incubation the mycelia was harvested by simple filtration method using using Whatman No.1 filter paper and washed with distilled water followed by drying at 60^{0}C. Dry biomass weight was determined gravimetrically and expressed in g/l. The lipid was extracted from the dried biomass by soxhlet extraction method using n-hexane as solvent.

Transesterification of extracted lipid

Transesterification of lipid was carried out to produce FAME (Fatty acid methyl esters) using either acid or alkali or enzyme catalyst. To the fungal lipid 20 ml of methanol and 2ml of concentrated sulphuric acid was added and mixed vigorously for 2 hours at 70ºC. After the completion of reaction the mixture was allowed to cool at room temperature and then transferred to the separating funnel for obtaining two layers containing upper biodiesel layer and lower glycerol layer.[14] The biodiesel was collected and analyzed using CHNSO.

RESULTS AND DISCUSSION

Flasks were removed from the incubator at designed time intervals and subjected to analysis. Samples taken from the flasks were centrifuged at 5000 rpm for 10 min. Biomass samples were dried at 80°C until a constant weight and were used for lipids extraction.

The biomass concentration of fungi *Aspergillus niger* grown in different low cost substrate is shown in Figure 1.

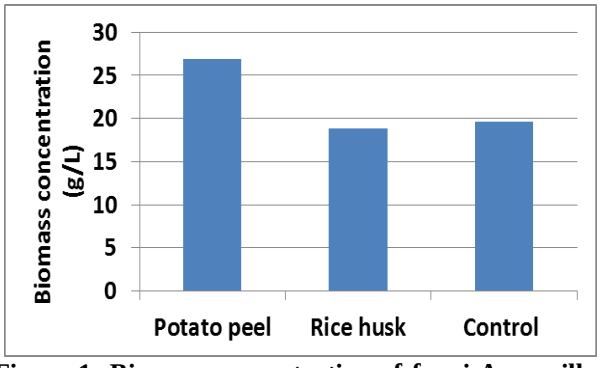

Figure 1: Biomass concentration of fungi Aspergillus niger.

The growth and development of oleaginous fungal species *Aspergillus niger* was studied in various types of waste carbon sources from agricultural or domestic waste. By storing lipids in a short duration of time and moreover culturing well in various types of carbon wastes and low-cost materials, like nutritional filtrates from agricultural and industrial residues.[15] The oleaginous microbial species has an potential to plays in depressing the cost of oils. It was observed that all types of carbon sources can be supported for the growth and reproduction of oleaginous microbial species to a considerable extent. Various types of low cost carbon substrates were utilized like Potato peel, Rice husk etc. Figure 1 represents the biomass concentration of oleaginous fungal species *Aspergillus niger* in different types of low cost carbon substrate. The proficiency of *Aspergillus niger* to cultivate well on the various types of waste carbon substrates shows their utilization by oleaginous fungal species *Aspergillus niger* and also observed that the growth of oleaginous fungal species *Aspergillus niger* was well in Potato peel as raw carbon sources as compared to the commercial nutrient medium.

Lipid Extration

It was found that, increase in biomass concentration increased the lipid production when the oleaginous fungal species *Aspergillus niger* were cultivated in media containing potato peel as waste carbon source. The lipid production was found to be 60.56% in potato peel containing waste carbon medium. For utilizing waste source material and to efficient the cost analysis, using potato peel can also demonstrate the highly economical at pilot scale. It can also revealed from this study that metabolic rate and performance of oleaginous fungal species *Aspergillus niger* could support the growth for the production of huge lipid concentration. The lipid content was extracted from dried biomass of oleaginous fungal species *Aspergillus niger* by using soxhlet extraction method with n-hexane as a extraction solvent.[16] Figure shows the lipid % of oleaginous fungal species *Aspergillus niger* cultivated in different low cost substrate.

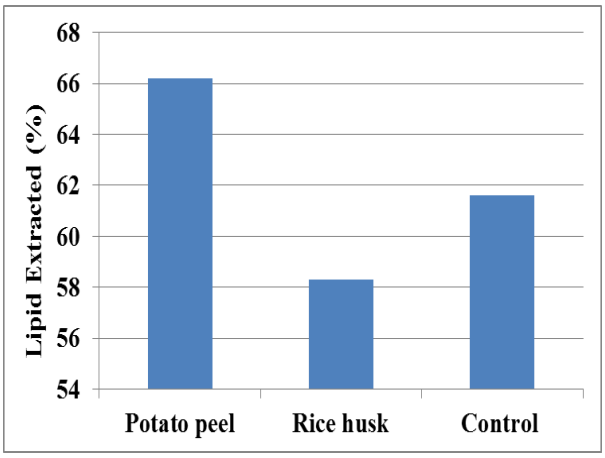

Figure 2: Lipid % of oleaginous fungal species Aspergillus niger.

Therefore, It was observed that lipid concentration and the biomass concentration of oleaginous fungal species *Aspergillus niger* was found to be high and comparatively with same trend when potato peel was used as the source of carbon. The lipid concentration from different carbon sources is shwon in Figure.

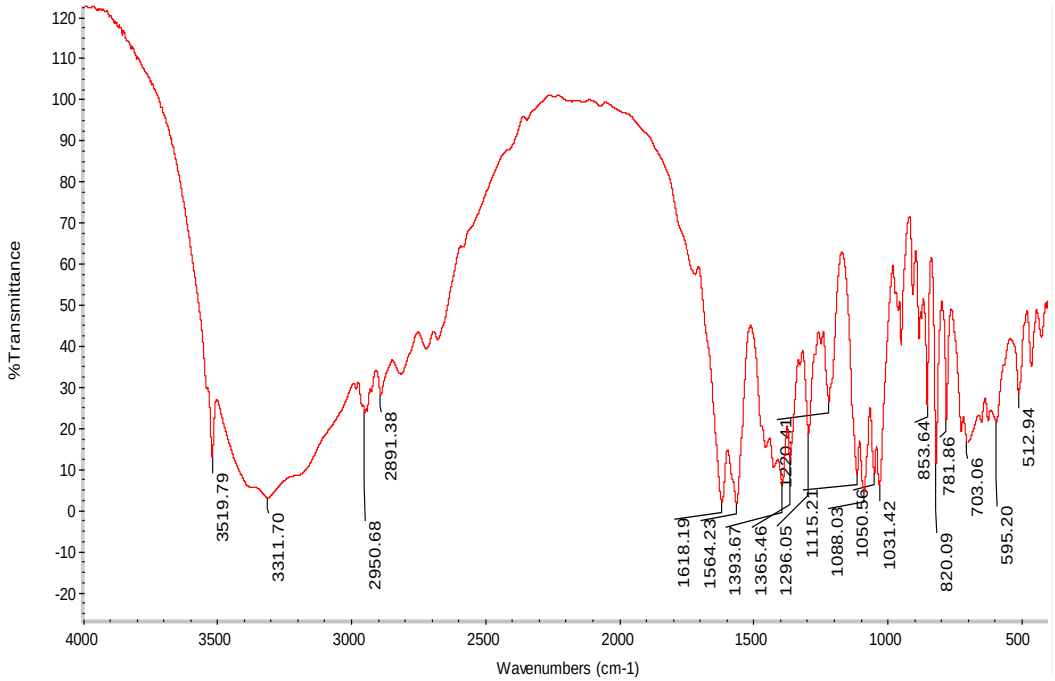

Figure 3: IR spectrum of transesterified oil.

From Figure 3, the region 678.55 cm^{-1} – 960 cm^{-1} designate the occurrence of =C-H functional groups. They represent the bending types of vibrations at a low energy and a frequency region in the spectrum and are all double bounded. They are attributed to olefinic (alkenes) functional groups in the biodiesel and they are unsaturated.[17] They are part of fatty acid methyl esters with unsaturated bond in the biodiesel, such as methyl oleate and methyl linoleate.[18]

CONCLUSION

It was found that, increase in biomass concentration increased the lipid production when the oleaginous fungal species Aspergillus niger were cultivated in media containing potato peel as waste carbon source. The lipid extracted (%) was found to be 66.2% in Potato peel, 58.3% in Rice husk and 61.6% control. This show that the oleaginous fungal species *Aspergillus niger* has an potential to culvate in waste carbon sources and reduces the production cost. High concentration of biomass and lipid content was produced when the oleaginous fungal species *Aspergillus niger* was cultivated in media containing waste carbon potato peel as carbon source.

The lipid has optimum fatty acid and it was further trans-esterified into methyl esters by using transesterification process with methanol. The FTIR analysis and Elemental analysis proves the characteristics of extracted oil.

Therefore, this oleaginous fungal species *Aspergillus niger* can be grown on different types of agricultural wastes and moreover can be used for the production of cheap microbial oil and finally can control environmental pollution.

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REFERENCE

1. Angerbauer C, Siebenhofer M, Mittelbach M and Guebitz G.M., Conversion of sewage sludge into lipids by Lipomyces starkeyi for biodiesel production, Bioresour Technol., 2008; 99: 3051-6.
2. Magdy Mohamed Khalil Bagy, Mohamed Hemida Abd-Alla, Fatthy Mohamed Morsy and Elhagag Ahmed Hassan., Two stage biodiesel and hydrogen production from molasses by oleaginous fungi and Clostridium acetobutylicum ATCC 824, International journal of hydrogen energy, 2014; 39: 3185-3197.
3. Sevgi Ertugrul Karatay and Gönül Dönmez., Improving the lipid accumulation properties of the yeast cells for biodiesel production using molasses, Bioresource Technology, 2010; 101: 7988–7990.
4. Venkata Subhash G and Venkata Mohan S., Lipid accumulation for biodiesel production by oleaginous fungus Aspergillus awamori: Influence of critical factors, Fuel, 2014; 116: 509–515.
5. Rawat I, Ranjith Kumar R, Mutanda T, and Bux F., Review on biofuel oil and gas production processes

from microalgae, Applied Energy, 2013; 103: 444–467.
6. Anoop Singh, Poonam Singh Nigam and Jerry D. Murphy., Mechanism and challenges in commercialisation of algal biofuels, Bioresource Technology, 2011; 102: 26–34.
7. Liam Brennan and Philip Owende., Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products, Renewable and Sustainable Energy Reviews, 2010; 14: 557–577.
8. Caporgno, M., R. Trobajo, N. Caiola, C. Ibanez, A. Fabregat, and C. Bengoa. Biogas production from sewage sludge and microalgae co-digestion under mesophilic and thermophilic conditions. *Renewable Energy*, 2015; 75: 374-380.
9. Ehimen, E., Z. Sun, C. Carrington, E. Birch, and J. Eaton-Rye. Anaerobic digestion of microalgae residues resulting from the biodiesel production process. *Applied Energy*, 2011; 88(10): 3454-3463.
10. Mussgnug, J. H., V. Klassen, A. Schl□uter, and O. Kruse. Microalgae as substrates for fermentative biogas production in a combined bio refinery concept. *Journal of Biotechnology*, 2010; 150: 51-56.
11. Alcantara, C., and Garcia-Encina P., and Munoz R. Evaluation of mass and energy balances in the integrated microalgae growth-anaerobic digestion process. *Chemical Engineering Journal*, 2013; 221: 238-246.
12. Olguin, E. Phycoremediation: Key issues for cost-effective nutrient removal processes. Biotechnology Advances, 2003; 22: 81-91.
13. Richmond, A. Open systems for the mass production of photoautotrophic microalgae outdoors: physiological principles. Journal of Applied Phycology, 1992; 4: 281-286.
14. Ichsana, H. Hadiyanto, and R. Hendrokoc. Integrated biogas-microalgae from waste waters as the potential biorefinery sources in indonesia. *Energy Procedia*, 2014; 47: 143-148.
15. Prajapati, S. K., A. Malik, and V. K. Vijay. Comparative evaluation of biomass production and bioenergy generation potential of Chlorella spp. through anaerobic digestion. Applied Energy, 2014; 114: 790-797.
16. Hongyang, S., Z. Yalei, Z. Chunmin, Z. Xuefei, and L. Jinpeng. Cultivation of Chlorella pyrenoidosa in soybean processing wastewater. *Bioresource Technology*, 2011; 102: 9884-9890.
17. Tam, N. F. Y. and Y. S. Wong. Comparison of growth and nutrient removal efficiency of Chlorella pyrenoidosa in settled and activated sewages. *Environmental Pollution*, 1990; 65: 93-108.
18. Wang, H., H. Xiong, Z. Hui, and X. Zeng. Mixotrophic cultivation of Chlorella pyrenoidosa with diluted primary piggery wastewater to produce lipids. *Bioresource Technology*, 2012; 104: 215-220.