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BIODIESEL PRODUCTION FROM SOIL FUNGI

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ABSTRACT:
Concern for the increase in energy demand and the depletion of fossil fuel reserves as resulted in a rapid rise in crude oil prices, and therefore, securing alternative source of energy is urgently required. Soil sample was collected from nearby garbage area and the fungi with fast growth rate were isolated and again cultivated as pure culture on PDA medium. Identification of isolated fungal strain was carried out by observing the macroscopic and microscopic character of the fungi. Soxhlet extraction by using hexane was carried out for lipid extraction from the dried fungi sample. Among the 6 strain of fungi which were purer cultured for and used for lipid extraction, Strain E had the high amount of lipid content and high growth rate as compared to other strains.

Keywords: Soxlet extraction, lipid extraction, Biodiesel.

INTRODUCTION:

Concern for the increase in energy demand and the depletion of fossil fuel reserves as resulted in a rapid rise in crude oil prices, and therefore, securing alternative source of energy is urgently required. The efficient use of organic cycle via waste to energy configuration can contribute to reducing the current energy crisis. Numbers of countries in the world, including India, are currently passing through the critical phase of population explosion and the growing population demands more energy input (Maddalena Rossi, Alberto Amaretti). Therefore, to ensure long-term sustainability, suitable alternative methods for oil production have to be developed which can be used as feedstock for several industrial applications. One of the most promising renewable energy resources is bio diesel(Gadallah Abu-Elreesh et, al), which is produced from renewable biomass by trens esterification of triacylglycerols, yielding mono alkyl ester of long-chain fatty acids with short-chain alcohols, for example fatty acid methyl ester and fatty acid ethyl. In different part of world, various renewable lipids have been chosen for production of biodiesel.

The production of biodiesel is based mostly on plant oils, even though animal fats and algal oils can also be used. In particular, soybean, rapeseed, and palm oils are adopted as the major feedstock for biodiesel production A. Akpinar-Bayizit–(2014). They are produced on agricultural land, opening the debate on the impact of the expansion of bioenergy crop cultures, which displace land from food production. Furthermore, their price restricts the large-scale development of biodiesel to some extent. Recently, the development of processes to produce single cell oils (SCOs) by using heterotrophic oleaginous microorganisms has triggered significant attention. These organisms accumulate lipids, mostly consisting of triacylglycerols (TAG) that form the storage fraction of the cell. The occurrence of TAG as reserve compounds is widespread among all eukaryotic organisms such as fungi, plants and animals, whereas it has been rarely described in bacteria

Several species of yeasts and filamentous fungi

are regarded as oleaginous, since they have the capability to synthesize and accumulate high amounts of TAG within their cells, up to 70% of their biomass weight. These lipids have similar composition and energy value to plant and animal oils, but their production do not compete for food resources, in particular, if it is based on

Inexpensive carbon sources, such as raw materials, by-products, and surplus. Furthermore,fungal SCOs have short life cycles, display rapid growth rates, unaffected by space, light or climatic variations, easier to scale up and have the ability to utilize a wide range of inexpensive renewable carbon sources such as lingo-cellulosic biomass and agro-industrial residues and wastewater.

Several studies reporting lipid accumulation by oleaginous yeasts and filamentous fungi on different renewable substrates such as glycerol, sewage water, whey and molasses have also been reviewed. (Petra Meeuwse Jhannes, et .al, 2011) The biodiesel quality depends upon the fatty acid composition of the oil feedstock.
For an oleaginous microbe to be considered as a suitable feedstock for biodiesel, the total lipid content (>20%) and the type of fatty acids (long chain saturated and/or monounsaturated fatty acids) are important criteria. (Mahesh Khot1 et.al, 2012)

Lipid content varies in every oleaginous fungi The aim of this study is to get fungal isolates from local sources which produce a large amounts of single cell oil and identifying the optimal SCO producing fungi and production and identification of lipids in the obtained soil fungi.

MATERIAL AND METHODS:

Sample collection and fungi isolation.

Soil sample was collected from nearby garbage area and was serial diluted. serial dilution was carried out by homogenizing 10 g of soil sample mixing in 100 ml of distil water and further dilution was carried out till 10^{-2},10^{-3},10^{-4},10^{-5},10^{-6} concentration. 1ml of each dilution was further poured in the petri plate filled with PDA (200:20:20) medium. The plates were kept in laminar air flow at room temperature for fungal growth for 76 hr. The fungi with fast growth rate were isolated and again cultivated as pure culture on PDA medium.

Fungi identification:

Identification of isolated fungal strain was carried out by observing the macroscopic and microscopic character of the fungi.

Lipid estimation method:

The fungal biomasses from each pure culture were collected and dried at 60 C. The samples of dry masses were then crushed with mortar and pestle and added to 10 ml of distilled water. 2 ml 1:1 sulphuric acid was added and then 25 ml n-hexane was added to the mixture to dissolve the lipids. The solutions were left on a rotary shaker overnight. The layer of hexane was extracted out and filtered through a 40 Whatman filter paper with 6 gm of sodium sulphate. The filtrate was then poured into a dry crucible (whose dry weight was measured) and was left at room temperature for the hexane to vaporize leaving only the lipid. The total weight was measured.
The oil extraction procedure was repeated for all the fungal biomasses. The weight differences for the respective fungal samples were recorded (Anshuman Bhanja and Gauri Minde, 2014).

Lipid extraction method:

Fungal biomass with higher growth rate and higher lipid content was selected for the further large culture, lipid extraction and biodiesel production. The fungal biomass was further cultivate in suspension medium of PD (potato: dextrose -20:2) and was incubated at room temperature for 7-10 days. The suspension culture was then filtered by using muslin cloth and was dried at 60°C. the dried bio mass was then crushed using mortar and pester and turned into powder. Soxhlet extraction by using hexane was carried out.

Soil sample were serial diluted and 1 ml of each concentration were poured in the petriplate and kept in laminar air flow. After 48 hr the fungal growth was obtained. Fungi with high growth rate were further isolated and pure culture was carried out of about 6 fungal strains. Fungal strains were identified by observing morphological character under microscope. The pure culture was further cultivated in submerged culture. The cultured was collected and dried in oven. The dried sample was further used for lipid extraction .Dry weight of Unidentified 1 was 260 mg and the total amount of lipid estimated was 59 mg ,unidentified 2 with 254 mg dry weight and total amount of lipid 61 mg, *Aspergilius 1* with 372 dry weight and total amount lipid extracted 79 mg, *Aspergillus 2* with dry weight 252 mg and total amount of lipid extracted 55 mg, *Aspergillus 3* with dry weight 372 and total amount of lipid 62 mg, unidentified 3 with dry weight 554 and total amount of lipid 151 mg was obtained. Unidentified 3 had the high amount of lipid content and high growth rate as compared to other strains.

CONCLUSION:

Unidentified 3 was found to be high growth rate and high lipid yield as compared to other strains isolated from soil sample. Soil fungi with high growth rate and with high lipid content can cultured in large culture and can be used for lipid extraction which further by using transesterification method can be used as biodiesel.

REFERENCES:

Anshuman Bhanja, Gauri Minde, Sandip Magdum, and V. Kalyanraman, Comparative Studies of Oleaginous Fungal Strains (*Mucor circinelloides* and *Trichoderma reesei*) for Effective Wastewater Treatment and Bio-Oil Production Hindawi Publishing Corporation Biotechnology Research International Volume 2014, Article ID 479370,

Gadallah Abu-Elreesh* and Desouky Abd-El-Haleem, An effective lipid-producing fungal sp. strain DGB1 and its use for biodiesel production African Journal of Biotechnology Vol. 12(34), pp. 5347-5353, 21 August, 2013

Maddalena Rossi, Alberto Amaretti, Stef ano Raimondi and Alan Leonardi Getting Lipids for Biodiesel Production from Oleaginous Fungi

Petra Meeuwse • Johannes Tramper • Arjen Rinzema Modeling lipid accumulation in oleaginous fungi in chemostat cultures: I. Development and validation of a chemostat model for Umbelopsis is a bellina, Bioprocess Biosyst Eng (2011)

Mahesh Khot1 , Srijay Kamat1,2, Smita Zinjarde1 , Aditi Pant1 , Balu Chopade1 and Ameeta Ravi Kumar1 Single cell oil of oleaginous fungi from the tropical mangrove wetlands as a potential feedstock for biodiesel , Khot et al. Microbial Cell Factories 2012

RESULT AND DISCUSSION:

Sr.No	Sample	Name	Dry weight (mg)	Amount of lipid extracted (mg)	Percent lipid
1.	Strain A	Unidentified 1	260	59 ± 4	22.69%
2.	Strain B	Unidentified 2	254	61 ± 4	24.01%
3.	Strain C	*Aspergillus 1*	372	794	21.23%
4.	Strain D	*Aspergillus 2*	252	554	21.82%
5.	Strain F	*Aspergillus3*	372	624	16.66%
6.	Strain E	Unidentified 3	554	151 ± 4	27.25%