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Utilization of Agro Waste as Carbon Sources for high Lipid Production by *Aspergillus Niger*

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Abstract: Effects of different agro waste as carbon source on biomass and lipid production of *Aspergillus niger* were studied. Different low cost feedstock substrate such as Orange peel, Banana peel, Potato peel, Sugarcane bagasse, Tomato waste, Rice bran,Rice straw and Rice husk was used as carbon source. There was a significant difference in the lipid accumulation process as related to the carbon sources was observed. It was found that when orange peel was used the biomass concentration and lipid content was 22.63g/l and 67.56% respectively and was high compared with other substrate used.Transesterification was carried out using acid catalyst and the yield was 69%. The FAME produced from the fungal lipid contains long chain fatty acids and higher number of saturated fatty acids and so it has the efficiency of acquiring high fuel and lubricating properties.
Keywords : fungi, *Aspergillus niger*, carbon source, lipid, biodiesel.

Introduction

In recent times the world has been confronted with most important issues global warming and energy crisis.it is clear that using of fossil fuels is the main cause for global warming[1], and the energy crisis is due to the depletion of fossil fuel resources[2]. Since 80% of global energy demand is produced from the fossil fuels, the fossil fuels are extensively utilized and this has led to climate change, environmental pollution and health problems[3]. In the other hand there is continues rise in the cost of crude oil due to the diminishing supply, thereby making production of fuels from alternative sources more feasible[4]. In order to displace fossil fuels, several biofuel candidates were proposed which can able to eliminate the vulnerability of energy sector[5]. The bio fuels have been mainly produced from waste oil and oil extracted from crops. There are several disadvantages 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. 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[6].

Single cell oil from oleaginous microorganisms has attracted the attention of the researchers because of its importance in the production of biodiesel and reducing pollution and waste. By using the single cell oil from the microorganism for high biodiesel production would cause low ecosystem impact in the world[7]. In this present study optimization of lipid production from *Aspergillus niger* using agricultural waste was investigated. Using agricultural waste for culturing fungi is considered as low cost feedstock. Different low cost feedstock substrate such as Orange peel, Banana peel, Potato peel, Sugarcane bagasse, Tomato waste, Rice bran,Rice straw and Rice husk was used as carbon source.Among all orange peel increased the lipid production in this oleaginous fungi.

Materials and Methods

Culturing condition and production media for fungi

The fungi *Aspergillus niger* was isolated from the soil contaminated by tannery effluents. It was maintained on Sabouraud Dextrose agar slant (SDB; pH 5.6; HI-media). 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 Orange peel, Banana peel, Potato peel, Sugarcane bagasse, Tomato waste, Rice bran, Rice straw and Rice husk. 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. The fungi were inoculated in the production medium and were kept in shaker at 150 rpm.

Lipid extraction from fungal mycelia

At the 5^{th} 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 60^0C. 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. The fatty acid profile was determined using GC-MS, (Agilent 6890) gas chromatograph equipped with a straight deactivated 2 mm direct injector liner and a 15m Alltech EC-5 column (250µ I.D., 0.25µ film thickness).

Tranesterification 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. The biodiesel was collected and analysed using GCMS.

Result and Discusision

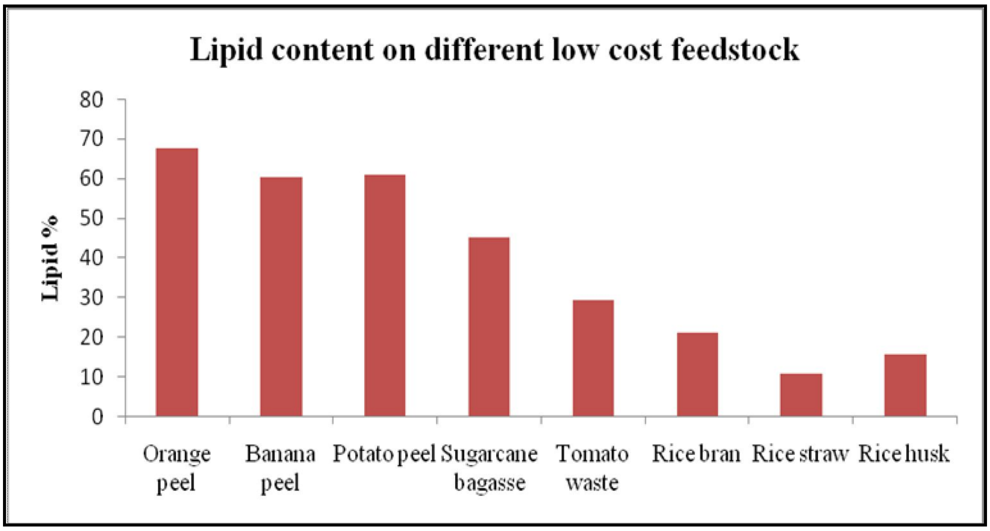

Figure 1 Lipid concentration of fungi grown in different low cost substrate

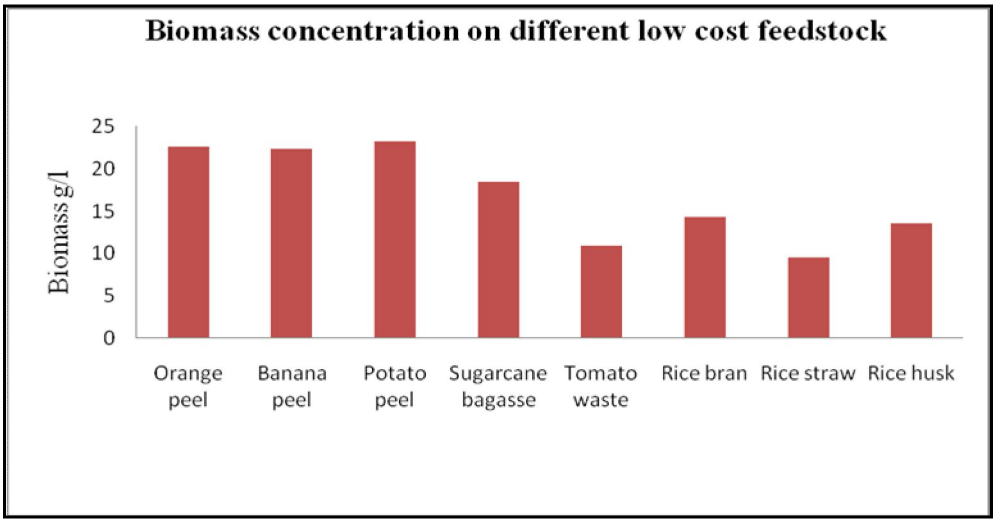

Figure 2 Biomass concentration of fungi grown in different low cost substrate

The growth and lipid production of oleaginous fungi *Aspergillus niger* in various agricultural waste was studied. By accumulating lipids within a short period of time and growing well on a variety of wastes and inexpensive materials, such as nutritional residues from agriculture and industry, the oleaginous microorganisms plays a vital role in lowering the cost of fuels[8].It was found that all the carbon sources used supported the growth and production of lipid to a considerable extent. Various low cost substrate were used which includes Orange peel, Banana peel, Potato peel, Sugarcane bagasse, Tomato waste, Rice bran, Rice straw and Rice husk. Figure 1 and figure 2 show the biomass concentration and lipid concentration of fungi grown in different low cost substrate respectively.The capability of *Aspergillus niger* to grow on the various agricultural waste carbon substrates indicates their utilization by the fungus and also found that growth of fungus was high in these raw carbon sources when compared to commercially available carbon sources. However, due to the proportion of amylase to amylopectin which tend to vary from one starch/polysaccharide sources to another there is a difference in utilization between the raw and commercial carbon by microorganisms[9]. If the media contain high sugar content the fungi metabolise it for synthesis proteins[10].

It was observed that there was an increase in biomass concentration and lipid concentration when the fungi were cultured in media containing orange peel as carbon source. The lipid content was 67.56% in orange peel containing medium. In the view of utilizing waste material and cost effectiveness, using orange peel can prove highly economical at pilot scale.It was revealed from the metabolic performance by fungal strains that the orange peel could support microbial growth for production of high lipid concentration. Gema,*et al.,*[11] observed that the lipid content was 15–20 mg of oil per gram of dry mass when the fungi *Cunning hamella echinulata* was cultivated in orange peel.

It was found that the lipid concentration and biomass concentration was high and comparatively same when potato peel and banana peel was used as carbon source. The lipid concentration for carbon sources potato peel and banana peel was 60.96% and 60.26% respectively. It was reported that the increased fungal biomass was due to the increased of reducing sugar content in the culture media[12]. For growth and metabolic activities fungi requires various organic carbon sources[13]. It was documented in the study of Venkata Subash, *et al.,*[14], that the *Aspergillus sp* showed high lipid productivity and fungal biomass growth potential utilizing CWL (corncob waste liquor) as substrate.

When sugarcane bagasse was used the lipid content was 45.04%. Sugarcane bagasse can be used as low cost carbon energy for culturing fungi. Unlike sugar, Sugarcane bagasse contains complex lignocellulosic material which is not charred after autoclaving[15].Sevgi Ertugrul Karatay, *et al.,*[16] reported that the yeast cells C. lypolitica, C. tropicalis and R. mucilaginosa accumulated 59.9%, 46.8% and 69.5% lipid in their cells, respectively when molasses was used for culturing. It was studied that *Aspergillus alternata* and *Cladosporium cladosporioides* showed high lipid productivity of 40.7% lipids per dry mass and38.5% lipids per dry mass respectively when sugarcane molasses was used as substrate[7]. Zhu,*et al.,*[17] reported that due to the high sugar content in sugarcane molasses, the growth of oleaginous microbes will be high but the high nitrogen content of this sugar may prevents its lipid accumulation and also he studied the cell growth and lipid accumulation of *Trichosporon fermentans* in molasses with different total sugar concentrations ranging from 5% to 30% and obtained maximum of 29.9 g/l biomass and 31.8% lipid content at 15% total sugar concentration.

The lipid concentration of Aspergillus niger was 10.87%, 15.68% and 21.08-% respectively when rice straw, rice husk and rice bran was used as substrate. *Trametes hirsute* was cultured in a medium containing wheat bran or rice straw as a typical lignocellulosic material and it converted the biomass into ethanol with a theoretical yield of 78.8% which shows that it can use rice straw as carbon sources[18].*Trichosporon fermentans* produced 28.6g/l of biomass and 40.1% lipid when rice straw hydrolysate was used as carbon sources[19]. It was found about 29.51% of lipid content when tomato waste was used as carbon sources. Fakas,*et al.,*[20] found that when fungi *Cunninghamella echinulata* was cultured on tomato waste hydrolysate (TWH) media having glucose as carbon source, the lipid production was 0.48 g of lipid per gram of dry biomass, corresponding to 8.7 g of lipid per litre of growth medium.

Acid catalyst was used for biodiesel (Fatty acid methyl ester) production by transesterification process and produced FAME was characterised by GC-MS. The biodiesel yield was 69%. Table 1 and table 2 shows the GCMS result of fatty acids present in the lipid of *Aspergillus niger* and FAME. The FAME produced from the fungal lipid contains long chain fatty acids and higher number of saturated fatty acids and so it has the efficiency of acquiring high fuel and lubricating properties[21].

Table 1: Fatty acids composition of lipid extracted from *Aspergillus niger* grown in media containing orange peel as carbon sources

Fatty acid		Concentration (%)
Myristic acid	14:0	5.45
Pentadecanoic acid	15:0	11.24
Palmitic acid	16:0	15.98
Stearic acid	18:0	8.94
Palmitoleic acid	16:1	19.25
Oleic acid	18:1	19.78
Linolenic acid	18:3	16.86

Table 2: GC profile of FAME (Fatty acid methyl esters)

Sl.No.	FAME
1	Pentadecanoic acid methyl ester
2	Stearic acid methyl ester
3	Mysteric acid methyl ester
4	Oleic acid methyl ester
5	Linolenic acid methyl ester

Conclusions

Higher biomass and lipid content was obtained when the filamentous fungi was cultured in the media containing orange peel as carbon source. The lipid has good fatty acid and it was converted into methyl esters with the yield of 69% by transesterification process using acid catalyst. Since this *Aspergillus niger* can be grown on agricultural wastes it can be used for producing cheap microbial oil and can control environmental pollution.

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