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Using Oleaginous Yeast in Spent Tea for Determination of Lipid Accumulation Capacity

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Abstract*: Alternative energy becomes the most chief mover of economic growth and plays an important role in this warming world. The whole world comes across the depletion of fossil fuels and has the degradation of the environment. Due to the depletion of fossil fuels, demand is also considerable increasing in demand and rapid increase in the petroleum prices. So, there is a need in search for other alternatives to fossil fuels. Biodiesel is considering being new alternatives to explore our dependency on oil imports that are help in protecting the environment towards the sustainable development. In this study, oleanginous yeast such as Lipomyces starkeyi, Metschnikowia pulcherrima, and Yarrowia lipolytica was cultured where spent tea wastes were used as a substrate. Depolymerization techniques like acid and alkaline treatments were carried out on the spent tea waste substrate. The initial qualitative and quantitative analysis of glucose were carried out by UV spectroscopy gave a maximum result on alkaline treatment. Hence alkaline treatment was selected for culturing and the various oleaginous yeasts were inoculated in the alkali depolymerized samples. After growth of 7 days, biomass was separated from media and was found to be 10.5 g/l, 10 g/l, 8.75 g/l for Y. lipolytica, L. starkeyi and M. pulcherrima. Lipids were isolated from biomass by Bligh and dryer method. Lipid confirmation was sorted out using FTIR.

Keywords: Alternative energy, Biodiesel, Depolymerization techniques, UV Spectroscopy.

I. INTRODUCTION

The energy for the automotive vehicles is mainly obtained from the fossil fuels such as coal, petrol, diesel and etc. Due to the increasing in the population and consumption of the energies leads to depletion of energy resources. To overcome this issue is biodiesel for the replacement of fossil fuels. Biodiesel is basically derived from the process of trans-esterification of lipids and alcohols. Initially the lipids were extracted from the edible and non-edible oils but now a days the trends focusing on using oleaginous yeasts and algae for extracting lipids.

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Lipids are provided as an alternative valued feedstock for the biofuel production. Lipid accumulation occurs when nutrient in the medium becomes limited and carbon source becomes present in excess. Oleaginous yeast this amount of carbon change to lipid and accumulate in the form of tri-acyl glycerol (TAG)
in lipid bodies of the cells. Spent tea wastes are used for the production of Bio-oil, Bio-char in various industries and also producer gas since it contains cellulose, pectin, lignin, proteins, organic acids, tea fiber and caffeine, hemicellulose, carotenoids. For biodiesel production, other oil sources have been exposed. Currently, processes have been initiated to produce single cell oil (SCO) by some oleaginous microorganisms, such yeast, fungi, bacteria and microalgae. While the eukaryotic yeast, fungi and microalgae can able to synthetize triacylglycerol (TAG), which have similar composition of vegetable oils. Microorganisms are generally considered to be oleaginous only when they undergo lipid accumulation of more than 20% of their cell dry weight.

Oleaginous yeasts can able to accumulate 20–70 % of their cell mass as intercellular lipids are treated as alternative strategy for second generation fuel production which includes biodiesel. Comparing filamentous fungi and algae with yeasts (eukaryotic microorganisms) it has higher growth rate and grow to higher density lipids. Some oleaginous yeasts, such as *Lipomyces starkeyi*, *Yarrowia lipolytica*, *Metschnikowia pulcherrima*, *Rhodotorula glutinis*, *Rhodosporidium sp*., *Rhodotorula sp*. *Lipomyces sp*. has capable of accumulating the intracellular lipids as high as 70% of their biomass dry weight.

II. MATERIALS AND METHODS

A. Materials Collection

Spent Tea waste was collected from the tea stalls near Karpagam college of engineering, Coimbatore. For the growth of oleaginous yeast these spent tea wastes were used as a substrate. The Spent Tea was dried at sunlight for 2-3 days. After half of the moisture content was dried, it was taken and dried in hot air oven at 70^{o}C for 20mins. The dried spent tea waste was grinded using mortar and pestle. The coarse powder was achieved. Then the powder was sieved to get fine spent tea waste powder. Finally, the spent tea waste powder was ready to use as a substrate. The particle size of the final spent tea waste powder was analyzed by using particle size analyzer.

B. Depolymerization of Sample

The spent tea waste powder was depolymerized by alkali pretreatment. 3g of powdered spent tea waste sample was taken in 250ml conical flask.

Add 9ml of NaOH and 91ml of distilled water. Then neutralize the sample at pH of 4, 6 and 8 to add oleaginous yeast. After all samples are neutralized, it was filtered by whattman filter paper and kept in auto clave for 120°C for 20 minutes at 15 Psi

C. Inoculation of Oleagious Yeast

The various oleaginous yeasts can be used for lipid production from the depolymerized substrate samples. The oleaginous yeasts used are *M.pulcherrima*, *Y.lipolytica* and *L.starkeyi* were grown in the pH of 4, 6 and 8 respectively. The acid depolymerized samples were kept laminar flow chamber to add the inoculum. The three oleaginous yeasts were added into the acid depolymerized spent tea waste samples. They were grown under an aerobic condition at 25°C in an orbital shaker at 150 rpm.

D. Culturing in Orbital Shaker

The inoculum added samples are kept in orbital shaker for 7 days at 120 rpm, 28°C and it has been rotated till it attains the death phase. The inoculum was allowed to grow in the samples. The samples were tested for optical density at each day in double beam UV spectrophotometer. After it attains the maximum OD value the samples were taken out and centrifuged to obtain the biomass to extract the lipids.

E. Growth Curve Determination

Three oleaginous yeasts were cultured were cultured with pretreated samples and optical density (OD) had been measured for every 24 hours in a Double beam UV visible spectrophotometer at a wavelength of 590 nm. The standard solution had been prepared and it has been used as a blank solution for the initial measurement of OD. For obtaining accurate optical density OD values between 0.1 to 1. The dilution had been done several times for the cells to be suspended in the medium solution

F. Biomass Extraction

After the growth of 7 days, inoculum added samples were centrifuged for 3.30 minutes at 8000 rpm for 28°C in cooling centrifuge. The biomass was settled at the bottom of the centrifuge tube. Finally, the biomass was separated by micro pipette. Now, the biomass was ready for lipid extraction.

G. Lipid Extraction from Biomass Bligh and Dryer Method

1 mL of biomass sample was added to 3.75 ml of chloroform: methanol (1:2) ratio. Then it is kept in the cyclo mixer for about 10-15 minutes. Again 1.25 ml of chloroform was added and kept in cyclo mixer for about 1 minute. Then 1.25 ml of water is added and again kept in cyclo mixer for 1 minute. Mixture homogenized with magnetic stirrer @ 300 rpm for 1 Hr. Finally, it is kept in large volume centrifuge to centrifuge the sample to extract the lipids. Here the two layers are in which lower is collected and kept separately in another tube. Then 1.88 ml of chloroform was added to the remaining sample in tube and it is kept in thermo mixer for 1 min and centrifuged for 10 minutes at 9000 rpm. Now the lower layer obtained from the second mixer is mixed with first lower layer collected separately and evaporated for the small volume of the sample.

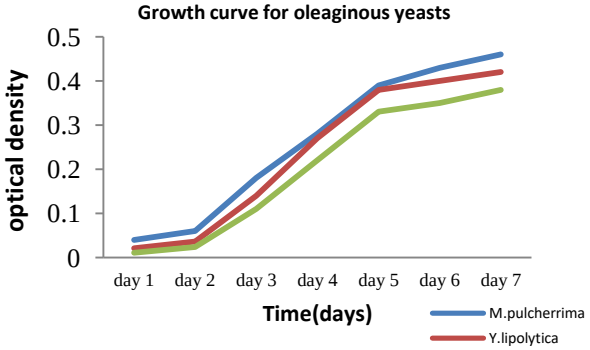
III. RESULTS AND DISCUSSION

A. Growth Curve Analysis

The inoculum added samples were allowed to grow in the orbital shaker for 7 days. The growth of the organisms was measured daily by double beam UV-visible spectrophotometer. The growth of the organisms was given by the optical density value at 590nm i.e. absorbance given by the UV. From the optical density value, *Yarrowia lipolytica* had the maximum growth at 7 days. *Metschnikowia pulcherrima* had the minimum growth after 7 days.

Growth curve for oleaginous yeasts

Days	M.pulcherrima	Y.lipolytica	L.starkeyi
1	0.09	0.021	0.011
2	0.15	0.036	0.024
3	0.23	0.14	0.11
4	0.31	0.27	0.22
5	0.35	0.38	0.33
6	0.4	0.4	0.35
7	0.43	0.48	0.38



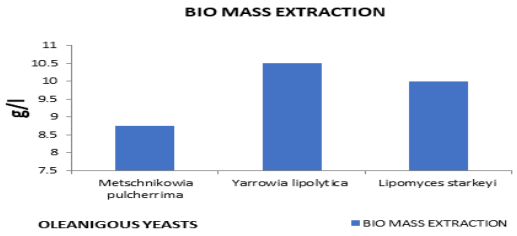
OD reading at 590 nm tracing growth curve of oleaginous yeasts for a period of 7 days

B. Biomass Extraction

The biomass was extracted from the three oleaginous yeast to determine the yeast which can produce a higher amount of lipid. The biomass weight of the alkali hydrolyzed samples was 8.75 g/l, 10.5 g/l and 10 g/l for *Metschnikowia pulcherrima, Yarrowia lipolytica and Lipomyces starkeyi* respectively.

Biomass Extraction

Oleaginous yeast	g/L
M.pulcherrima	8.75
Y.lipolytica	10.5
L.starkeyi	10

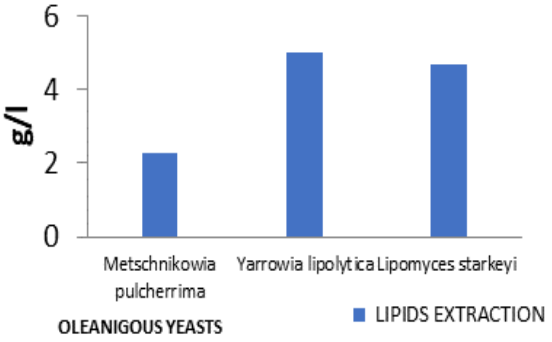


C. Extraction of Lipids

After the biomass was extracted from the alkali hydrolyzed samples, the lipids had been extracted from the bligh and dryer method. The weight of the lipids of the alkali hydrolyzed samples were 2.3 g/l, 5 g/l and 4.7 g/l for *Metschnikowia pulcherrima, Yarrowia lipolytica and Lipomyces starkeyi* respectively.

Extraction of Lipids

Oleaginous yeast	g/L
M.pulcherrima	2.3
Y.lipolytica	5.0
L.starkeyi	4.7

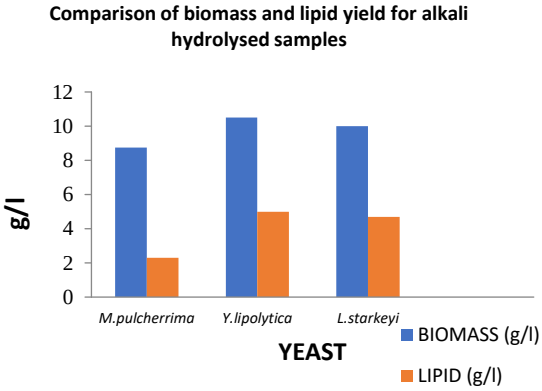


D. Comparison of Biomass and Lipid Yield for Alkali Hydrolyzed Samples

The biomass weight of the alkali hydrolyzed samples was 8.75 g/l, 10.5 g/l and 10 g/l for *Metschnikowia pulcherrima, Yarrowia lipolytica and Lipomyces starkeyi* respectively. From the biomass obtained, the lipid was extracted accordingly. The lipid yield of *Yarrowia lipolytica* was higher of 5 g/l and *Metschnikowia pulcherrima* was lower of 2.3 g/l.

Comparison of biomass and lipid yield for acid hydrolysed samples

Species	Biomass (G/L)	Lipid (G/L)
M.pulcherrima	8.75	2.3
Y.lipolytica	10.5	5
L.starkeyi	10	4.7



IV. CONCLUSION

From the study, Alkali hydrolysis samples, after 7 days of growth, the biomass was separated and found to be 10.5, 10, 8.75 g/l for *Y. lipolytica, L. starkeyi and M. pulcherrima*. From the biomass, the lipid was extracted and found to be higher of 5 g/l in *Yarrowia lipolytica* and lower of 2.3 g/l in *Metschnikowia pulcherrima* and the lipid yield of *Lipomyces starkeyi* was 4.7 g/l. Verification of lipids by FTIR gave the presence of unsaturated fatty acids like methyl linoleate, butyl stearate, ethyl palmitate, ethyl myristate and ethyl linoleate with a spectral match of 92%. From the above study, it has been concluded that the yeast *Yarrowia lipolytica* was found to have the highest lipid yield among the three oleaginous yeast used for the study. The extraction of lipids can be verified using FTIR (Fourier Transform InfraRed spectroscopy) and with the help of the higher lipid accumulation it can be further used for the biodiesel production through various transesterification methods.

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