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# FTIR Analysis of Lipid Produced by Oleaginous Yeast *Lipomyces starkeyi* Using Agricultural Waste

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***Abstract***

**Biodiesel is obtained from a chemical reaction called transesterification (ester exchange). The reaction converts esters from long chain fatty acids into mono alkyl esters. Chemically, biodiesel commonly is a fatty acid methyl ester. Lipid is a class of organic compounds that are fatty acids or their derivatives and are insoluble in water but in organic solvents. Lipids include fatty acids, oils, waxes, sterols and triglycerides. Biodiesel is typically made by chemically reaction on lipids with an alcohol producing fatty acid esters. Lipids from agricultural waste is used as a resource for the preparation of biodiesel. The waste has a hard husk protecting the kernel inside. It contains 25% Cellulose, 30% lignin, 15% hemicellulose and 21% ash. Agricultural Waste was dried and ground in a mixer and further ground for 1hr in ball mill. Then they were dried and subjected to hydrolysis techniques such as acid and alkaline hydrolysis. After hydrolysis pH was adjusted as required for the species. After pH adjustment, media was autoclaved and inoculated with 6% of microorganism. In this study, hydrolyzed agricultural wastes were used for culturing oleaginous yeast *Lipomyces starkeyi. UV visible spectroscopy is used to find the growth curve of microbes. Lipid extraction is done from the biomass obtained on acid hydrolysis and characterized by FTIR. It confirms the presence of lipid.***

***Keywords—Agriculture waste, Hydrolysis, oleaginous yeast, biomass, Lipid.***

## I. Introduction

Energy security is the constant availability and supply of affordable energy consumer and industry. Much of the world extraordinary economic progress over the century has been facilitated by reliable source of energy. However, at beginning of the new millennium, one of the biggest challenges is continuing to meet rising energy demand in a sustainable way and energy security has become a constant and universal issue. Threats to energy security risk due to fossil fuel scarcity or disruptions to fossil fuel supplies from international markets, risk due to a lack of investment in domestic national energy infrastructure, risk from technology and infrastructure failures and industry activism or terrorism. Due to increased energy demand, India must import energy to meet current demand. There is potential for bio-fuel to leverage indigenous source of input, potentially increasing income and opportunities in rural areas. Currently, bio fuel production is minimal, accounting for only one percent of global production. Supporting a future bio energy sector will likely require policy support and local interest, technological breakthroughs, and cost effective feedstock production. Bio fuels are potentially important to India because of the significant number of lives they could impact and the economic changes. India is currently the second most populous nation in the world with a growing population of over 1.147 billion people. However, there are possibilities to improve poverty through economic growth bio-fuels could help resolve some of India s economic problems. As the fifth largest energy consumer in the world, India additionally provides a good market for bio fuels. 70% of its crude oil is imported from around the world and experts anticipate that over 94% of its crude oil will be purchased from abroad by 2030, if energy trends continue on their current trajectory. Bio-fuels offer potential opportunities to decrease the nation's dependence on foreign energy imports. In India, bio fuels are an alternative energy option due to the availability of feedstock crops. Yard waste is a important feedstock for microbial lipid biofuel production. Large quantity of Agricultural waste is generated and hence it can be pretreated and cultured with oleaginous yeast for biodiesel production.

Oleaginous microorganisms have the excess oil content of 20 % of the biomass weight. Oleaginous microorganisms such as yeasts, fungi, algae and bacteria are used for the production of microbial oils or single cell oils. Lipids are produced from the oleaginous microorganisms in the quantity of 40% of their biomass. Biodiesel is produced from the oleaginous yeasts such as *Yarrowialipolytica, Metschnikowia pulcherima and Lipomycesstarkeyi.*

## II. MATERIALS AND METHODS

*A. Sample Collection*

Agricultural waste collected from fields around in Coimbatore. Agricultural waste consist of corn leaves and sugar cane leaves are inedible, they are used in various non-food applications as low-valuable waste materials. However, it was demonstrated that these waste can be considered as a valuable source of bioactive components.

*B. Pretreatment*

Collected waste was dried and ground in a mixer. Sample passing through 75µ sieve was collected and further ground for 1hr, 2hr, 4hr in ball mill. Obtained particle was analyzed for its size in particle size analyser. Since the size plays an important role in pretreatment more focus given for size reduction

*1) Acid Pretreatment:*1g of sample, 3% of acid remaining 97 ml of water. Acid hydrolysis was carried out with $H_2SO_4$on the substrate. Varying concentrations of $H_2SO_4$solution was employed to optimize the required quantity. Heated at 120°C at 15psi pressure in autoclave. Kept aside to cool for 24 hours and filtered.

2)Alkali Pretreatment:1g of sample, 3% of 0.1N of alkali on Sodium Hydroxide (NaOH) remaining 97% of water. Alkali hydrolysis was carried out with NaOH on the substrate. Varying concentrations of NaOH solution was employed to optimise the required quantity. Heated at 120°C at 15psi pressure in autoclave. Kept aside to cool for 24 hours and filtered.

*C.Microorganisms used for lipid production*

The various oleaginous yeasts can be used for lipid production from the depolymerized substrate samples. The oleaginous yeasts used in this study is *Lipomyces starkeyi*. *L.starkeyi* were grown in the optimum pH of 8 based on literatures.

*D.Preparation of medium for culture:*

After hydrolysis the sample is cooled and kept at stand by for 24 hours. Then it is filtered with a whatman filter paper. After filteration pH was adjusted as required for the species. After pH adjustment, media was autoclaved and innoculated with 6% Microoraganism. Culture was maintained at a temperature of 25˚C and 120 rpm in orbital shaker. In this study, hydrolyzed agricultural wastes were used for culturing oleaginous yeast *Lipomycesstarkeyi*, The yeasts strain inoculated in the laminar air flow chamber to prevent the entry of other microorganisms and it was grown under aerobic condition at 25°C in a rotary shaker at 150 rpm.

*E. Biomass extraction:*

The grown biomass was separated from the liquid medium by centrifuging in a large volume centrifuge at 8100 rpm for 5 minutes and temperature 25˚c. The supernatant was discarded and the pellet was recovered. After centrifuge the biomass was dried in 50˚c.

*F.Lipid extraction for alkaline hydrolyzed samples:*

The biomass from alkaline hydrolyzed sample was taken and the lipid was extracted by chloroform methanol method. Then, the lipid was analysed by FTIR.

*1)chloroform methanol method*:The tissue is homogenized with chloroform/methanol (2/1) to a final volume 20 times the volume of the tissue sample (1g in 20 ml of solvent mixture). After dispersion, the whole mixture is agitated during 15 – 20 minutes in an orbital shaker at room temperature. The homogenate is either filtered (funnel with a folded filter paper) or centrifuged to recover the liquid phase. The solvent is washed with 0.2 volume (4 ml for 20ml) of water or better 0.9 % NaCl solution. After vortexing some seconds, the mixture is centrifuged at low speed (2000 rpm) to separate the two phases. Remove the upper phase by siphoning and kept it to analyze gangliosides or small organic polar molecules. If necessary (need of removing labelled molecules), rinse the interface one or two times with methanol/water (1/1) without mixing the whole preparation. After centrifuged and siphoning of the upper phase, the lower chloroform phase containing lipids is evaporated under vacuum in a rotary evaporator or nitrogen stream if the volume is under 2 – 3 ml.

*2)Lipid verification by FTIR:* The lower layer in the centrifuge tube containing chloroform and the upper layer was lipid. The lipid was extracted and it was kept in the trough plate for running the analysis in the FTIR of PerkinElmer make. The samples were run at 4000 – 450 $cm^{-1}$ wavenumber and verified by Fourier transform Infrared spectroscopy (FTIR).

## III. RESULTS AND DISCUSSIONS

*A.Particle size analyser*

*1)Particle size result 1 hr*: The powdered vegetable waste was sieved through 75 µ mesh and the passed powder was put into the planetary mill for grinding. Finally, the powder was collected and its particle size was analysed by particle size analyser of M/S Malvan Make. The size of the powder was reduced to 173.7 nm.

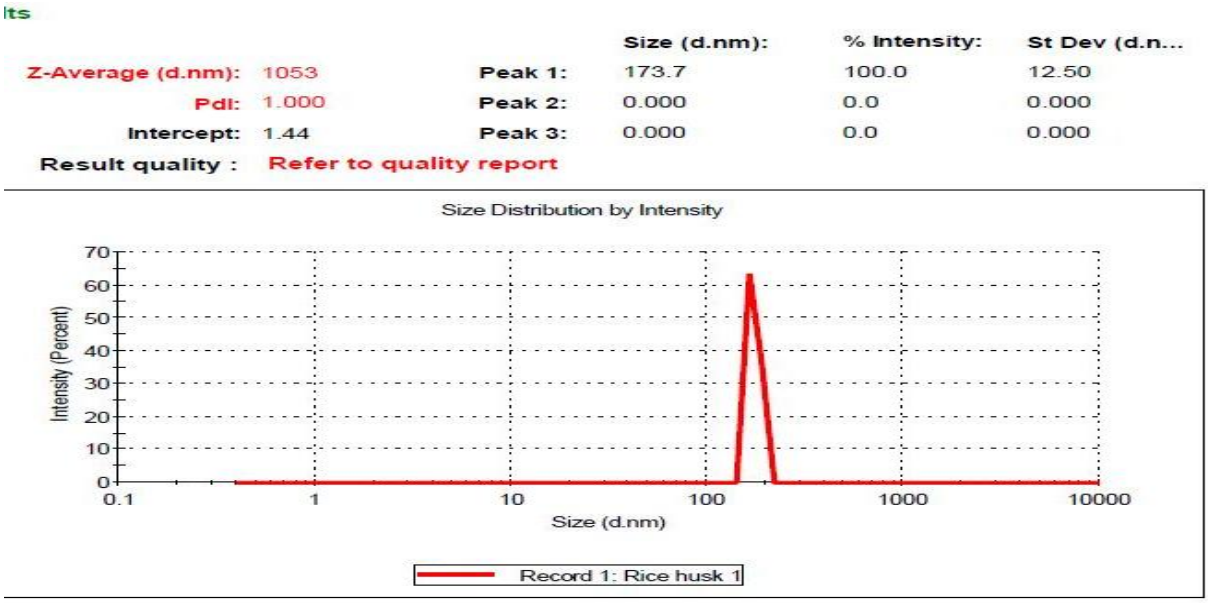


Fig.3.1. particle size result 1hr

*2)Particle size result 2 hr:* The particle size after 2hr ball milling was found out as 264.5 nm. It is clearly seen that the particle size is reducing further when compared to 1 hr ball milling.

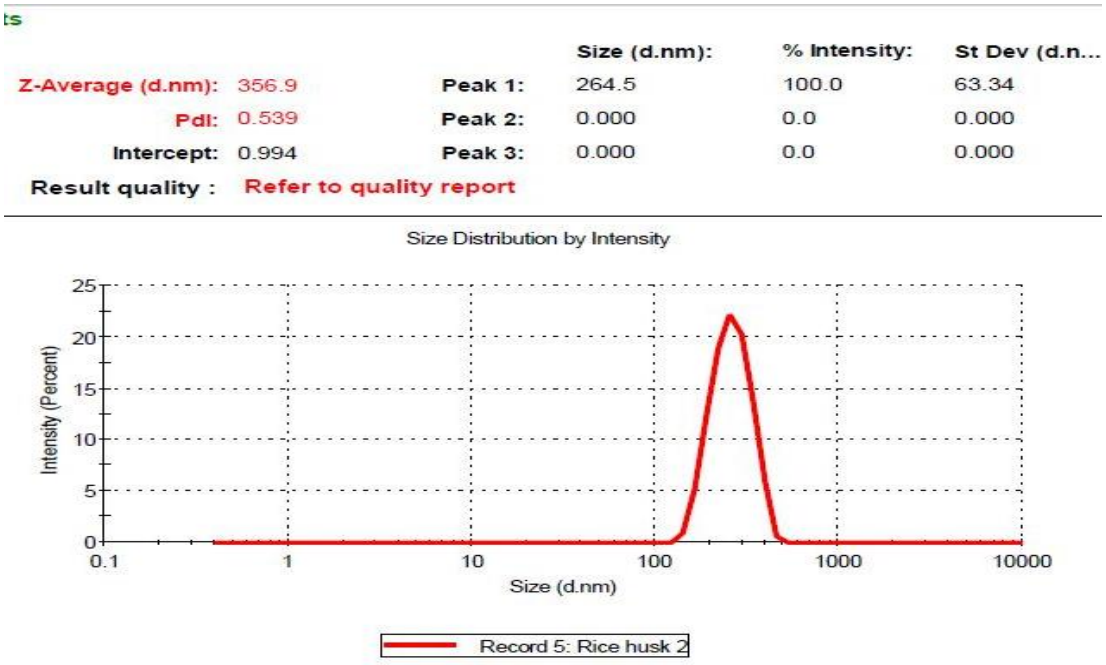


Fig 3.2.particle size result 2 hr

*3)Particle size result 4 hr:* Further the size is reduced by increasing the duration of ball milling upto 4 hrs. After 4 hrs powdered sample was anlysed in particle analyser and it was found to be 410.2 nm

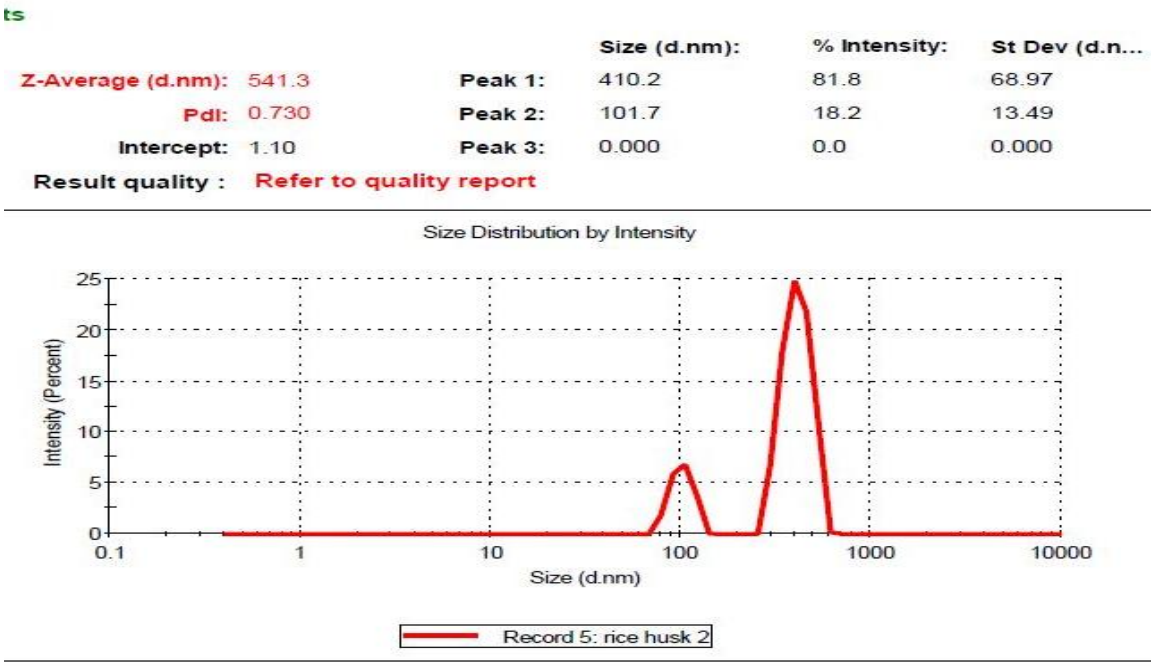


Fig 3.3.particle size result 4hr

*B.Growth curve analysis by UV spectrophotometer:*

*1)Growth curve of Lipomyces starkeyi on alkali pretreatment: After size reduction the sample was given alkali pretreated and the pH was adjusted to 8. After autoclave the sample is inoculated culture of Lipomyces starkeyi. And it was kept in rotary shaker as explained in the methods.the growth of the organism is monitored by finding the optical density using Spectrophotometer.* The optical density is measured at 640 nm

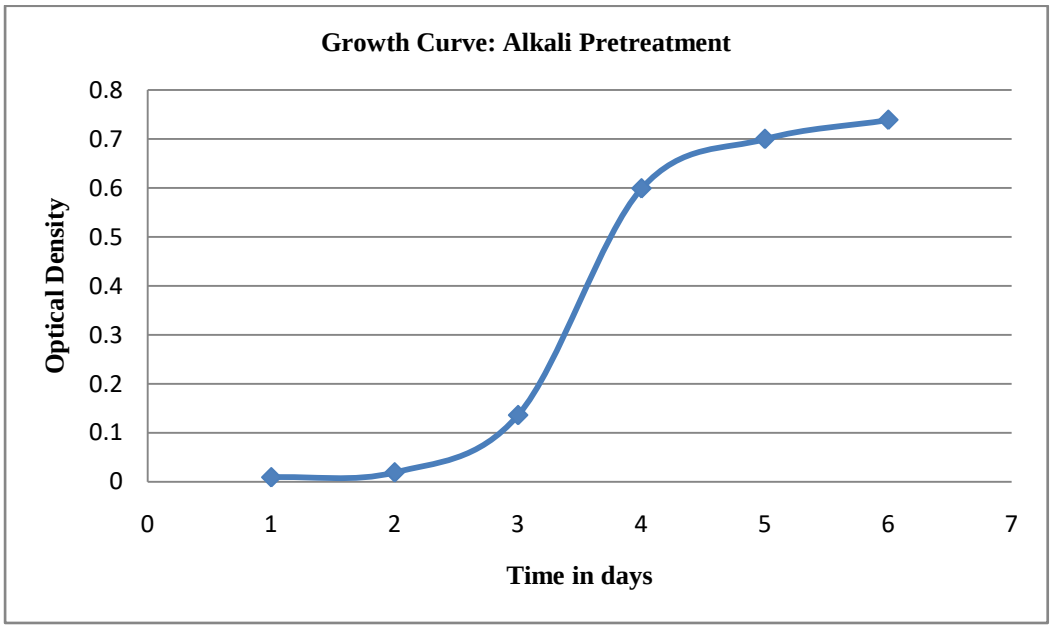


Fig 3.4.Growth curve of *Lipomyces starkeyi* on alkali pretreatment

From the study the maximum biomass growth was reached on 6 days on alkali pretreatment for *Lipomyces starkeyi*.

2) Growth curve of *Lipomyces starkeyi* on acid pretreatment: As explained above the sample was given Acid pretreatment and the growth study was conducted.

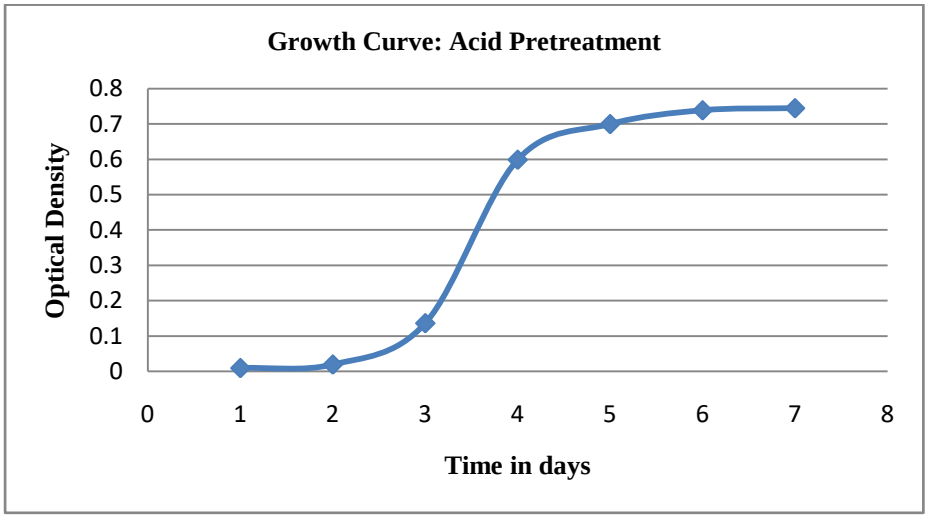


Fig.3.5.Growth curve of *Lipomycesstarkeyi* on acid pretreatment

From the graph the maximum biomass growth was reached on 6days for *Lipomyces starkeyi*. The OD was measured on 640 nm.

*C. Lipid Extraction:* The lipid was extracted based on the methodology given above. The lipid was obtained and it is found to be 51.2mg/l

Table 3.1.Llipids obtained from biomass

| | Glucose (mg/l) | Biomass (mg/l) | Lipids (mg/l) |
|---|---|---|---|
| *Alkali treatment* | 195 | 105 | 51.2 |
| *Acid treatment* | 204 | 110 | 52.5 |

D. FTIR analysis:

The lipid obtained was further analysed by FTIR of make PerkinElmer for finding the types lipids present in it. It was found that around 59 % of lipid consist of L(-)-GLYCERALDEH YDH and it confirmed that the lipid is existing in the extract and it can be used for the specific purposes

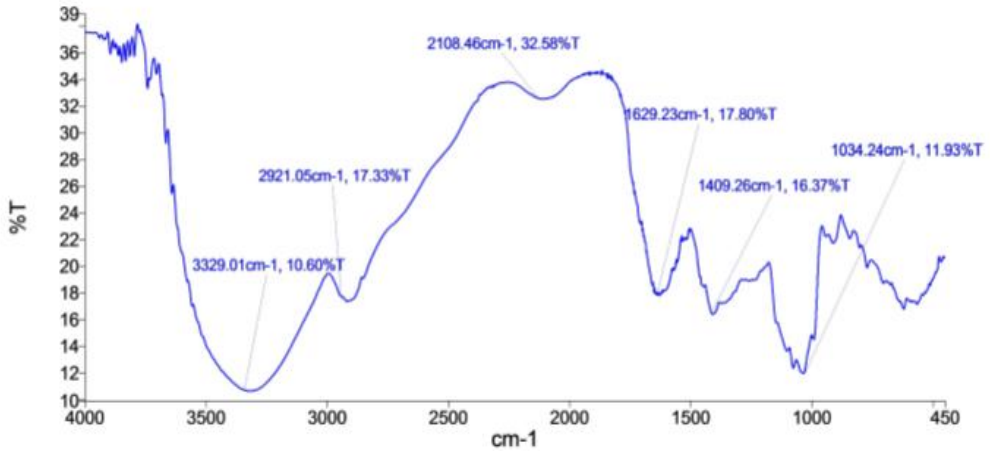


fig.3.11. *Lipomycesstarkeyi* FTIR analysis

| Description | Library name | Score |
|---|---|---|
| L(-)-GLYCERALDEH YDH UNNATURAL FORM | FLUKA | 0.594675 |

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## IV. CONCLUSION

Ball milling was found to be an effective method for size reduction when compared to normal grinding. The Acid pre treatment method was foud to be an effective pretreatment method. Acid treatment gives higher glucose 204 mg/l and the same for alkali pretreatment is 195 mg/l. After pH adjustment, media was autoclaved and inoculated with *Lipomyces starkeyi*. Maximum biomass was obtained by the acid treated medium and it was found to be 204 mg/l and the same for alkali treated medium is 105 mg/l. The lipid obtained from biomass using acid treated medium was found to be 110 mg/l and it is 105mg/l for biomass grown in alkali treated medium. The FTIR used for the analysis of lipid and it is confirmed that 59 % lipid consist of L(-)-GLYCERALDEH YDH .

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