Synthesis of Lipid By Oleaginous Yeast Yarrowia Lipolytica 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 Yarrowia lipolytica. UV visible spectroscopy used to find the growth curve of microbes. Lipid extraction is done from the biomass obtained on acid hydrolysis and characterized by FTIR.*

Keywords- Agriculture waste, Hydrolysis, oleaginous yeast, biomass,Lipid.

I. INTRODUCTION

Biodiesel is technically defined as alkyl (usually methyl and ethyl) esters of long chain fatty acids derived from vegetable oils or animal fats. When used as fuel in diesel engines and heating systems, biodiesel has many merits, such as high energy density, more favorable combustion emission profile, improved lubricating properties, and others. It is also an environmentally benign fuel compared to petroleum-based diesel, as biodiesel is renewable, biodegradable, non-toxic, and essentially carbon dioxide neutral. In brief, these merits make biodiesel a good sustainable energy carrier. The common way to produce biodiesel is by transesterification of pre-extracted triacylglycerides (TAG) with an alcohol. The majority of current research has concentrated on the base-catalyzed technology, since it is much faster than the acid catalyzed process and the reaction conditions are moderated. However, the most profitable raw materials (e.g. waste cooking oils, low-value fats and brown greases) usually have a high content of free fatty acids (FFAs) and water, where alkaline catalysis produces soaps by neutralizing the FFAs and Saponifying TAG in the presence of residual water. Both soap formations are side-reactions, leading to partial consumption of the catalyst, reducing biodiesel yield, and significantly complicating subsequent purification processes. Thus, sensitivity to FFAs and moisture represents a severe issue for large scale production of biodiesel with those inexpensive feed stocks. Today, there is a major need for energy sources throughout the world. Domestic, industrial activities and automobiles requires energy resources. The use of vehicles is increasing nowadays with number of vehicles increasing year after year. Fossil fuels such as coal and oil are majorly utilised for energy resources are getting depleted due to domestic and other industrial activities. The usage of fossil fuels relating to global warming and other greenhouse gas emissions. During the fossil fuel usage, the carbon dioxide is emitted into the atmosphere and cause global warming. The better solution for this issues is replacing the fossil fuel with biodiesel. Biodiesel have various advantages over fossil fuels. Vegetable oil based biodiesel was introduced and investigated in the 1890s, when Rudolph Diesel invented diesel engines to be used for machines in the agricultural sector (Orchad et al., 2007). Some of these includes reduced greenhouse gas emission and energy security. It is produced by the process of bio-diesel reaction of lipids and alcohol. Initially the lipids were extracted from edible and non-edible oils. But nowadays lipids were extracted from the oleaginous yeasts and algae. Use of food grains for biodiesel production has increased more concern and limited the edible oil usage. The non-edible oil needs lot of investment and resources. Hence researches were turned towards single cell oil for the production of biodiesel. Growth of single cell organisms required organic energy resources for their growth. Organic wastes are plently available throughout the world. Hence, this study focuses on the preparation of organic waste specifically vegetable waste. Depolymerisation of vegetable waste and the growth of oleaginous yeasts namely *Yarrowia lipolytica, Metschnikowia pulcherrima and Lipomyces*

starkeyi on depolymerized vegetable waste for single cell oil. So, the biodiesel is produced from these wastes as a substrate.

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. METHODS AND MATERIALS

2.1. 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.

2.2. 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.

2.2.1. Acid pretreatment

1g of sample, 3% of acid remaining 97 ml of water. Acid hydrolysis was carried out with H_2SO_4on the substrate. Varying concentrations of H_2SO_4solution 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.

2.2.2. 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.

2.3. 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 *Yarrowia lipolytica* *Y.lipolytica* is grown in the optimum pH of 6 based on literatures.

2.4. Preparation of medium for culture

After hydrolysis Cooled and kept at stand by for 24 hours 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 *Yarrowia lipolytica***.** 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.

2.5. 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.

2.6. 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.

2.6.1. 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.

2.6.2. 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

3.1. Particle size analyser

3.1.1.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 ultra sonication and particle size analyser. The size of the powder was reduced to 750 nm.

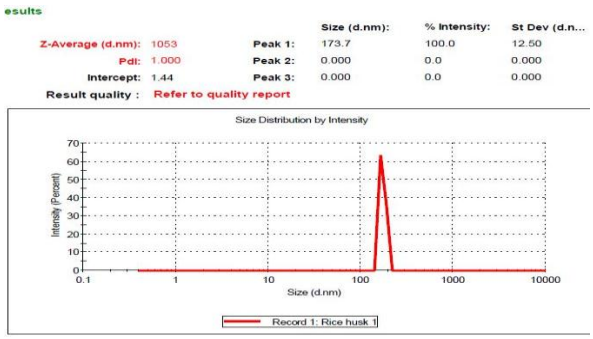


Fig.3.1. particle size result 1hr

3.1.2. Particle size result 2 hr

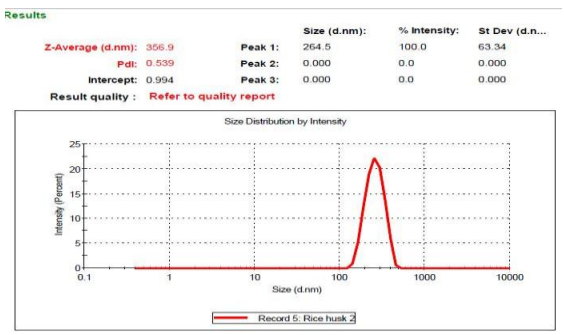


Fig 3.2.particle size result 2 hr

From the graph the particle size was analysed on 264.5 nm after 2hr grounded in ball mill. Particle size was analysed on particle size analyser. And its effective on pretreatment.

3.1.3. Particle sizeresult 4 hr

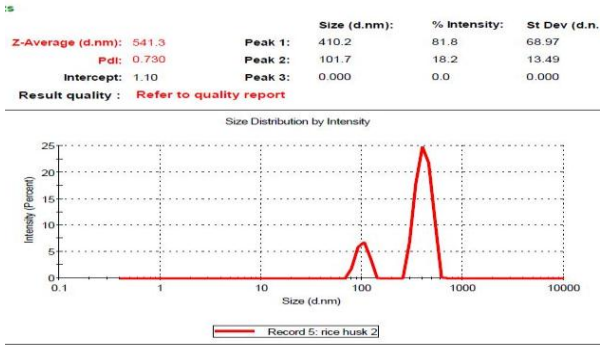
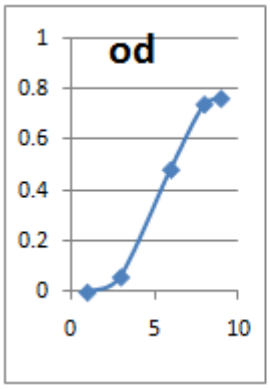


Fig 3.3.particle size result 4hr

From the graph the particle size is 101.7nm obtained on after 4hr grounded in ball mill. The particle size analyser is used for particle size analysis.

3.2. Growth curve analysis for uv spectrophotometer

3.2.1. Growth curve of *Yarrowialipolytica* on alkali pretreatment

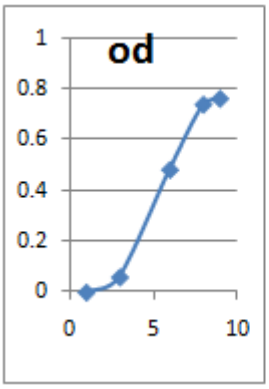


days	OD reading
1	0
3	0.01
6	0.546
8	0.743
9	0.766

Fig 3.6. Growth curve of *Yarrowialipolytica*on alkali pretreatment

From the graph the maximum biomass growth on 9 days with alkali pretreatment using *Yarrowia lipolytica*. The max optical density observed was 0.766 at 640 nm.

3.2.4. Growth curve of *Yarrowialipolytica* on acid pretreatment



days	OD reading
1	0
3	0.01
6	0.546
8	0.743
9	0.766

Fig 3.7. Growth curve of *Yarrowia lipolytica*on on acid pretreatment

From the graph the maximum biomass growth on 9 days of acid pretreatment on *Yarrowialipolytica*. The optical density 0.567 was measured on 640 nm. Compare to the maximum biomass growth on alkali and acid, the alkali maximum growth of biomass.

3.3. FTIR result

3.3.3.*Yarrowialipolytica* FTIR analysis

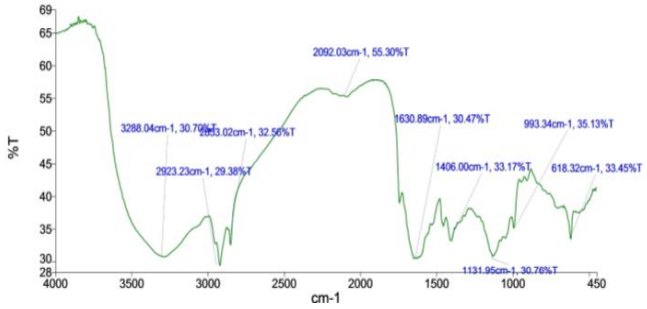


fig 3.12. *Yarrowialipolytica*FTIR analysis

description	library name	score
DIETHYLENE GLYCOL MONO(CA RBOXYMETHYL)	FLUKA	0.490778

From the graph it is confirmed the presence of the lipid and it Diethylene glycol mono about 49 %

3.4. Lipid extraction

Table 3.1. lipids obtained from biomass

Species	Glucose (mg/l)	Biomass (mg/l)	Lipids (mg/l)
Yarrowia lipolytica	200	112	44.8

From the table the lipid produced by *Yarrowialipolytica* is 44.8 mg/l

IV. SUMMARY AND CONCLUSION

Rice husk was collected from rice mills and around in Coimbatore. Rice husk can be pre-treated and cultured used for lipid production with oleaginous yeasts.Literature was collected and studied about distillery spent wash and oleaginous yeast, lipid accumulation. Rice husk was collected and dried was ground in a mixer and further ground for 1hr, 2hr, 4hr in ball mill. From the particle size analysis result 1hr, 2hr, 4hr are 173.7nm, 264.5nm, 101.7nm. Then they were dried and subjected to various hydrolysis techniques such as acid hydrolysis & alkaline hydrolysis. After hydrolysis pH was adjusted as required for the species. After pH adjustment, media was autoclaved and innoculated with Microorganism. The organisms were cultured in standard media and its growth was analysed. In this study, hydrolyzed agricultural wastes were used for culturing oleaginous yeast *Yarrowia lipolytica***.** UV visible spectrophotometer is used for biomass growth analysis, from the result the biomass growth of *yarrowia lipolytica* was found as 112 mg/l corresponding to the max optical density of 0.766 at 640 nm. From this biomass, high amount of lipids can be extracted. Folch method is used for lipid extraction and it gives lipid as 44.8 mg/ l. The FTIR spectrum confirms the presence of lipid and *Yarrowia lipolytica*a produced 49% lipid. This lipid can be used for the production of Biodiesel. This illustrate that the Agriculture waste can be a alternative source for producing biodiesel.

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