RESEARCH ARTICLE

Biodiesel fuel production from algae oil using crude enzyme of newly isolated *Aneurinibacillus migulanus* strain

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ABSTRACT

Biodiesel is a green fuel and can be used as a blend with diesel or alone. It is a biodegradable and contains less CO_2 and NO_x. Biodiesel can be produced from many renewable resources like vegetable oil, fatty acids and algae oils. Oils can be used as feed stock for biodiesel production. Biodiesel can be produced through a transesterification reaction. Transesterification can be carried out using alkali based catalyst or enzymes based catalyst. Enzymes based catalyst has more advantages' than alkali based catalyst. In the present investigation was undertaken to transesterified crude algae oil with novel bacterial enzyme under optimized condition, The molar ratio of methanol to crude oil was 3:1 proportion and the reaction temperature was maintained at 40^0c and 150 rpm for 5hr reaction time.Product of reaction were analyzed using FTIR and GCMS, biodiesel were identified, and physicochemical properties of biodiesel were compared with standard, this study revealed that microalgae oil can be source of potential biofuel production.

Keywords: Algae oil, crude enzymes, biodiesel, transesterification.

INTRODUCTION

Microalgae are a diverse group of microorganisms; they are either prokaryotic or eukaryotic in nature, in recent year microalgae have gained attention as a possible solution to fossil fuel (Hossain, 2008). Current diesel are short supply and cause environment pollution and it is exhaustible fuels, to overcome this problem many alternative sources are being sought out, biodiesel from vegetable oil with alkali based transesterification currently being used and commercialized, vegetable oil is obtained from crop oil in India (Kumar *et al*., 2013). The oil contain of these crop is less than algae, algae can be grown on waste water and the production is harvested with 15 days as compare with oil crop, algae cultivation is chief and feasible.

Biomass is one of the better sources of bioenergy. Large scale production of biomass could contribute to sustainable development (Kulkarni et al., 2006) present fossil fuel will exhaust soon as the population of the world continuously increasing, biodiesel is one of the alternative fuel. This is

obtained by transesterification of triglyceride oil with methanol. It has been reported that sunflower oil, soybean oil palm oil as substitute of diesel fuel (Lang *et al*., 2002).

Transesterification can be alkali based or enzymes based catalytic reaction. Recent studies showed that microbial enzyme based transesterification is more attractive and economically chief as compare to alkali based catalyst. Byproduct glycerol can be easily recovered and biodiesel purification is economically fusible (Pandey and Benjamin, 200).

Most of the lipases for transesterification are obtained from fungi, yeast and bacteria, although a number lipases producing bacterial are available only few are commercially exploited. Of these the important genera Acromobacter Alcaligenes, bacillus, chromobacter, pseudomonas. etc, transesterification of palm oil with pseudomonas for production was reported (Liu and Salihon, 2011) In the present investigation algae oil as a feedstock in transesterification. Crude enzyme of novel strain *Aneurini bacillus migulanus* was isolated in our laboratory and used as catalyst in transesterification reaction, Reaction product biodiesel was analyzed with FTIR and GCMS and conformed. Physiochemical properties of biodiesel was studied and compared.

MATERIALS AND METHOD

Chemicals and equipments

Methanol, ethanols were purchased from SISCO research laboratory PVT.LTD. Crude Algae oil was collected form *Scenedesmus* sp YACCYB70 sp form our laboratory**.** The shaking incubator was supplied by SDFCL sd fine-Chem. limited. FTIR simzadu, and GCMS of Envirocare pvt. Laboratory Mumbai.

Enrichment of lipase producer

Soil sample was collected form oil meal industry at Gangakhed aseptically and stored in refrezer at 4^0C until use. 1gm of this soil was added in to 100 ml of basal media peptone $30gm^{-1}$yeast extract $10gm^{-1}$algae oil $10gm^{-1}$pH 7. inoculated media was incubated at 40^0C on rotory shaker for 144 hours.

Isolation, screening and identification of lipase producing bacteria

Loop full culture of enrichment medium was spot on tributrin agar plate and after incubation zone of clear around the colony was observed and culture was identified by 16SRNA molecular sequencing method.

Lipase production

Novel bacterial strain grown in basal medium at 40^0C in shaking incubator at 144 rpm for 5 days, Crude lipase was obtained by centrifugation at 100000rpm for 10 minutes. the cell free supernant was considered as crude enzyme**.**

Enzyme assay

Titrometric method was used for the determination of enzyme activity. 10% olive oil emulsion in 2% gum acacia was used as a substrate. The reaction mixture composed of 0.5ml substrate emulsion, 0.4ml 0.1M tris-HCL buffer (PH 7.2) 0.1ml crude lipase solution, blank was prepared without enzyme. Instead of enzyme distilled water was used. Reaction was carried out at 30^0C for 30 min..The reaction was terminated by the addition of 2ml acetone and titrated against 0.05N NaOH using phenopthelin as an indicator. Amount of NaOH required to achieve end point (colorless-pink) was recorded. Enzyme unit calculated as 1 unit of lipase activity was defined as the amount of enzyme released from fatty acid in 1 minute under standard assay condition**.**

Transesterification reaction optimization and production of biodiesel

The enzymatic transesterification reaction was carried out in a 50 ml conical flask and optimized with respect to temperature and the molar ratio of methanol to algae oil in orbital shaker. Standard methods were used and studies were carried out in triplicate. The initial conditions were sat at 6ml algae oil, 3 ml methanol, and 10% volume of crude lipase 40^0C 150 rpm and 5 hour reaction time.

Analytical method

The analysis of reaction product carried out using FTIR and GCMS

RESULT AND DISCUSSION

Identification of bacterial isolate

Based on morphology and biochemical test the bacterial isolate was identified as *Aneurinibacillus migulanus,* 16sRNA methodology was carried out. The isolate under study was 99% *Aneurinibacillus migulanus*. Nucleotide sequence was sending to

deposit at NCBI. This novel strain produce lipase enzyme at is maximum amount on fifth day and the enzyme unit was detected 56.66U/ml.it was reported that the extracellular lipases production normally appear in the fermentation medium when the bacterial cell growth reach to the end of logarithmic phase. (Heshm *et al*., 2005) similar study also studied by Selvakumar 2008; Tembhukar, 2012. In our study crude enzyme was used in transesterification reaction which was obtained from novel strain, this enzyme found to be potential as catalytic in biodiesel production, the FTIR report and the peak at1753.29 peak of IR conform the formation of methyl ester shown in photo plate 2.GCMS peak named 19:1 w7c10-methyl conforms the formation of methyl ester shown in fig 2. This finding revealed that the novel bacterial enzyme transforms algae oil in to methyl ester (biodiesel). Similar result with other oil shown by Ya-Nang et al., 2011; Shah et al., 2003, Wai-Du and De-hun, 2004; Meng and Salihon, 2011). Many lipase are used in transesterification form microbes such as lipases obtained from: *Rhizomucor miehei, Rhizopus oryzae, Candida antarctica, Candida rugosa, Pseudomonas cepacia* and *Thermomyces lanuginosa*, It was reported that the trans-esterificatin of several vegetable oils by bacterial lipases including *Pseudomonas* lipase showed a stronger activity compared to fungal lipases such as Lipozyme TL IM (Grepen, 2005), but the commercial immobilized lipase B from *C. antarctica* (Novozym 435) is the most commonly used enzyme Lipozyme TLI [Du *et al*., 2004).

Table 1: Physicochemical properties of *Scenedesmus* sp YACCYB70 algae oil biodiesel

S.No.	Parameters	Units	Test Method Astm
1	Density, at 15°C	gm/cc	0.85
2	Viscosity, at 40°C	mm2/s	4.2
3	Moisture	%	1.8
4	Flash point	°C	110
5	Cetane Number	--	54
6	Acid value	mg of KOH/gm	0.52
7	Calorific value	KCal/kg	9110

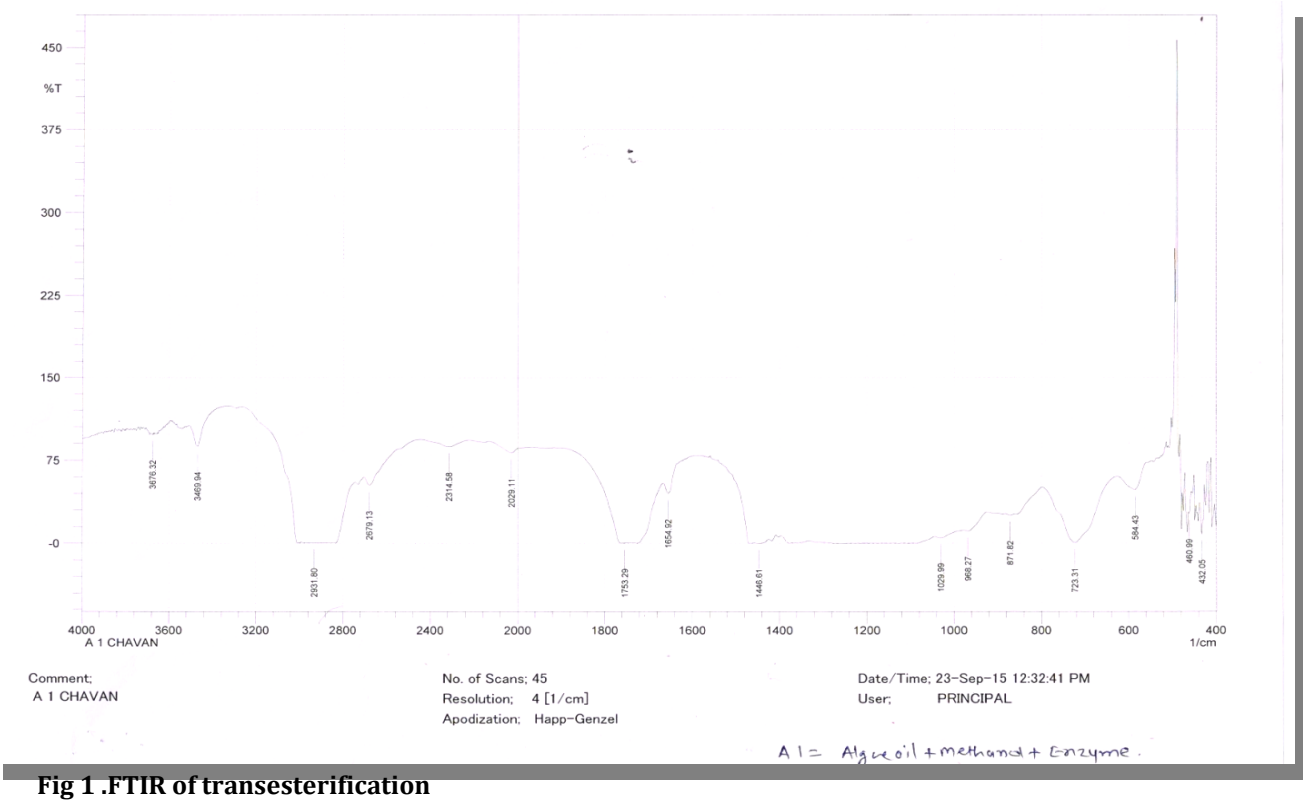
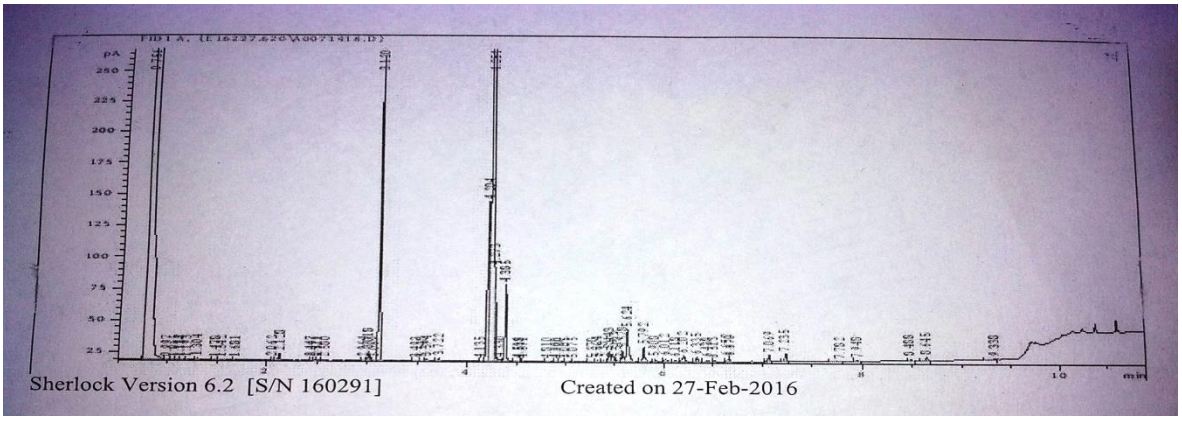


Fig 1 .FTIR of transesterification



GCMS Chromatogram of Biodiesel

In this study since the final results of methyl ester conversation rate Crude enzymes from *Aneurinibacillus migulanus* were considered as the most suitable lipase for transesterification reaction of algae oil and methanol to methyl ester. Physiochemical properties of biodiesel showed in table 1. This properties showed that the flash point 120 and Cetane number 54 indicate this biodiesel could be used to run the public transport after proper purification ,further investigation need to done in the area of biofuel.

CONCLUSION

Form the result and discussion we conclude that newly isolated Aneurinibacillus ***migulanus*** produced novel lipase which can carry out transesterification, using algae oil as substrate. Lipase enzyme need to purified and further enzymatic investigation should carry out for better result. Physicochemical properties of biodiesel are in close proximity with the known biodiesel.

Conflicts of interest: The authors stated that no conflicts of interest.

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