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***Venue:* Faculty of Life Sciences, Banasthali Vidyapith, Jaipur, Rajasthan,  
India.**



***Topic Presented:* Enzymatic Transesterification of Waste Cooking Oil for  
Biodiesel Production Using Lipolytic Fungal Isolate GH11**



**By**

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# Introduction

- **What is biodiesel?**

- ❖ Biodiesel is a domestic and renewable fuel for diesel engines majorly derived from vegetable oil or animal fat which meets the specification of ASTM D6751 or EN14214, and in which the final product is termed as fatty acid methyl esters (FAME) (chain length  $C_{14}$ - $C_{22}$ ) [1-3].

- **Merits of Biodiesel**

- ❖ Significant qualities of biodiesel include: better emission profile, biodegradability, renewability, portability, higher flash point, cetane number and combustion efficiency, lower sulphur and aromatic contents, safer handling and non-toxic [4-5].

- **Justification for the work**

- ❖ It is believed that large-scale production of biodiesel from edible oils may bring global imbalance to the food supply and demand market [1].
- ❖ The implementation of second generation biofuel where non-edible oil is used for biodiesel production has been earmarked as escape route out of the feedstock crisis as 85% of production cost accounts for feedstock [6].
- ❖ Moreover, enzymatic transesterification has been found to be a better alternative to the commonly used alkali catalyst as enzyme can catalyze feedstocks having free fatty acid  $>3.0\%$  w/w without production of soap. Biocatalyst also provide a better separation of final production as well [7].

# Biofuel Statistics

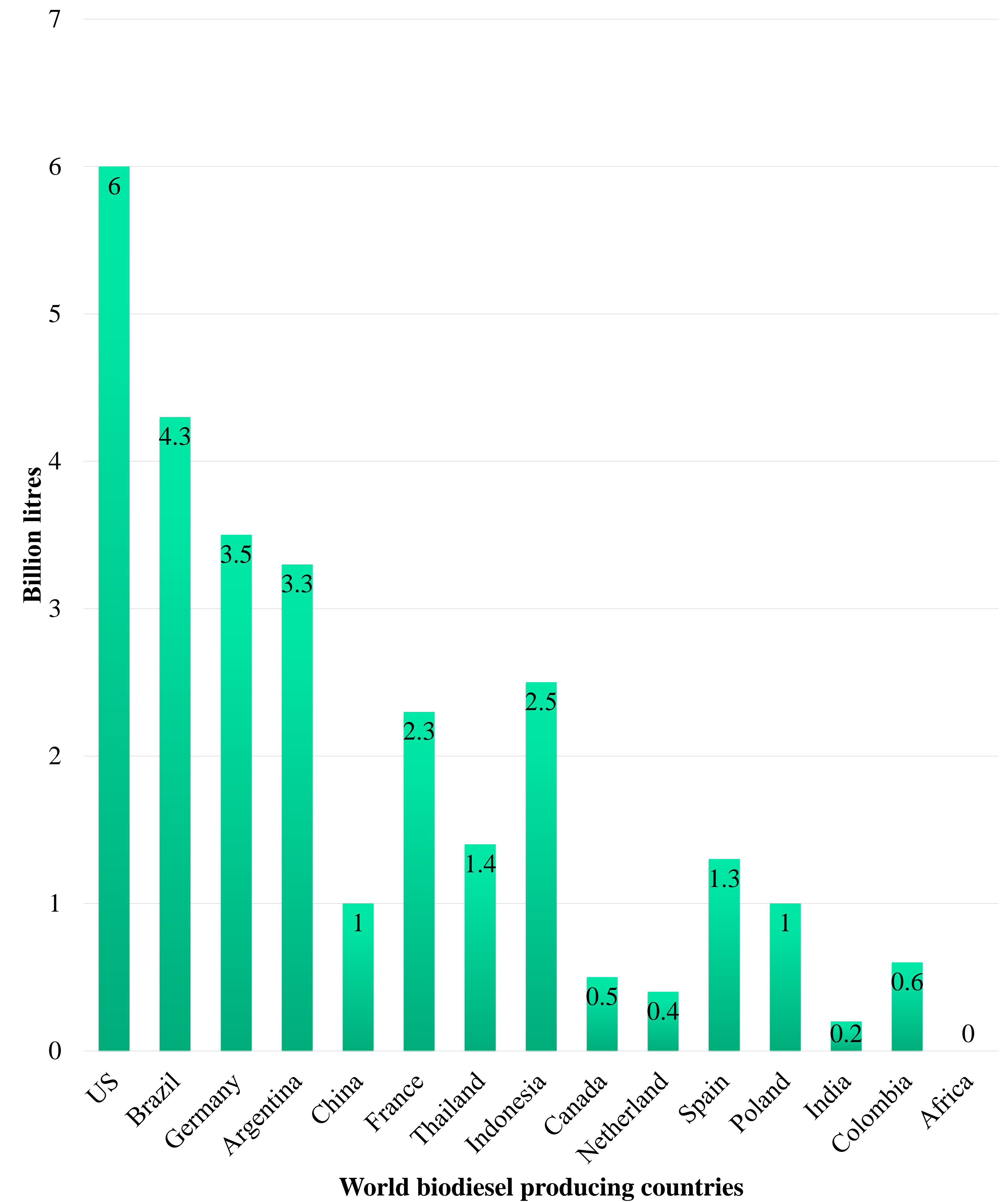
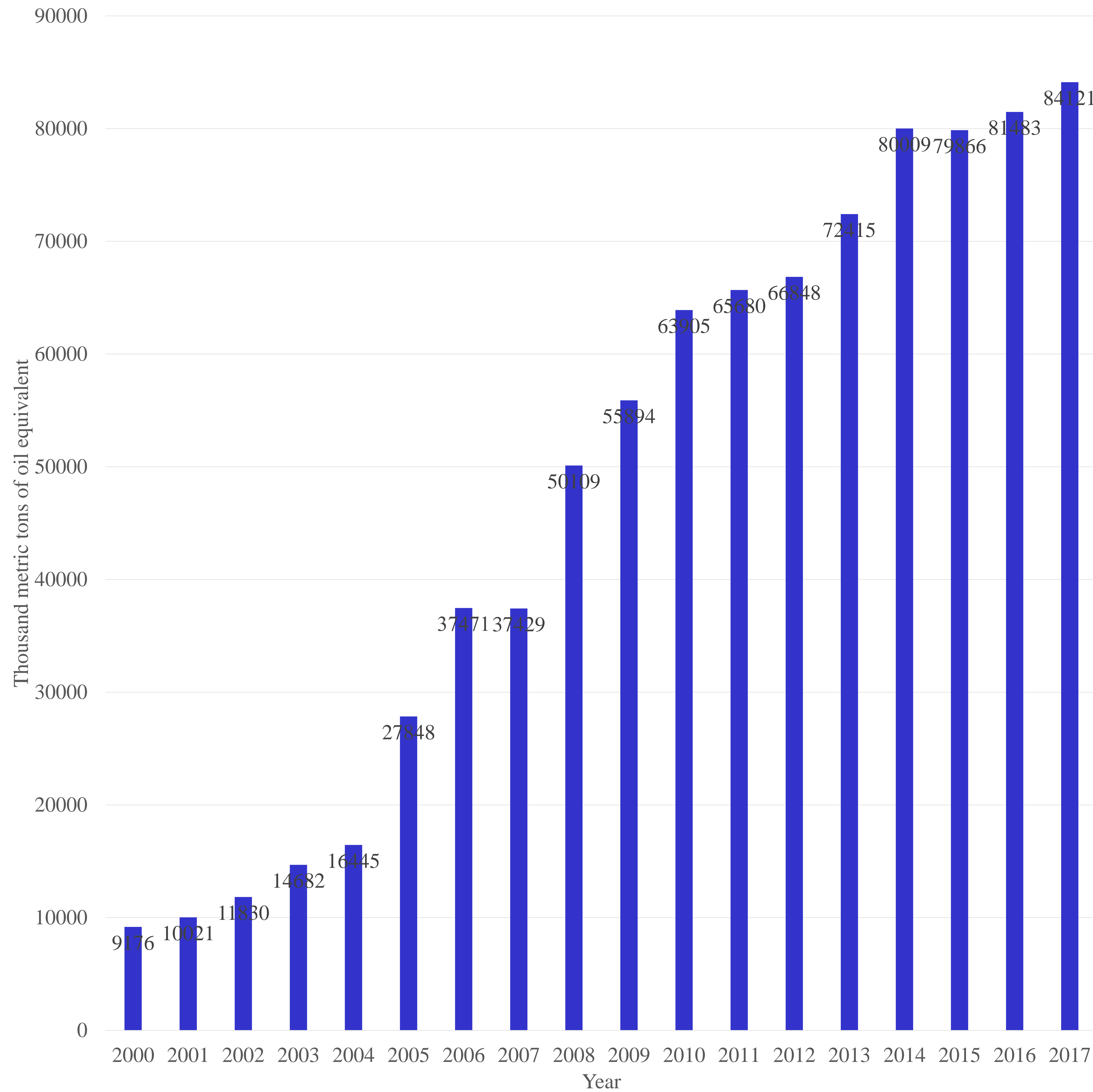


Fig a. World Biofuel Production (2000-2017) – Source OECD, 2017

Fig b. Major World Biodiesel Productions per countries for year 2017– Source OECD, 2017



# Methodology

- **Samples Collection, Isolation and Screening**
  - Different species of higher fungi were collected from Botanical garden, University of Ibadan, (latitude 7°26"N and longitude 3°54" E), while lower fungi were isolated from Palm-oil mill effluent from palm oil refining factory in Fiditi Village, Oyo State, Nigeria. The samples were screened for lipase production using solid-state and submerged fermentations.
- **Lipase Production & immobilization**
  - The best lipase producing fungi was immobilized on silica gel matrix (60–150 mesh) crossed linked with 4% glutaraldehyde solution [8].
- **Biodiesel production**
  - The enzymatic transesterification of spent soybean oil was investigated using both free and immobilized lipases [9]. The produced biodiesel (FAME) was analyzed using Fourier Transformed Infra-red spectroscopy [8].
- **Optimization of transesterification conditions**
  - The effect of production parameters such as acyl acceptor, substrate to molar ratio of methanol, solvent, reaction temperature, reaction time, and repeated use of immobilized enzymes were optimized [10].



# Sampling Location



University of Ibadan

Latitude 7°26"N & Longitude 3°54" E

km

map





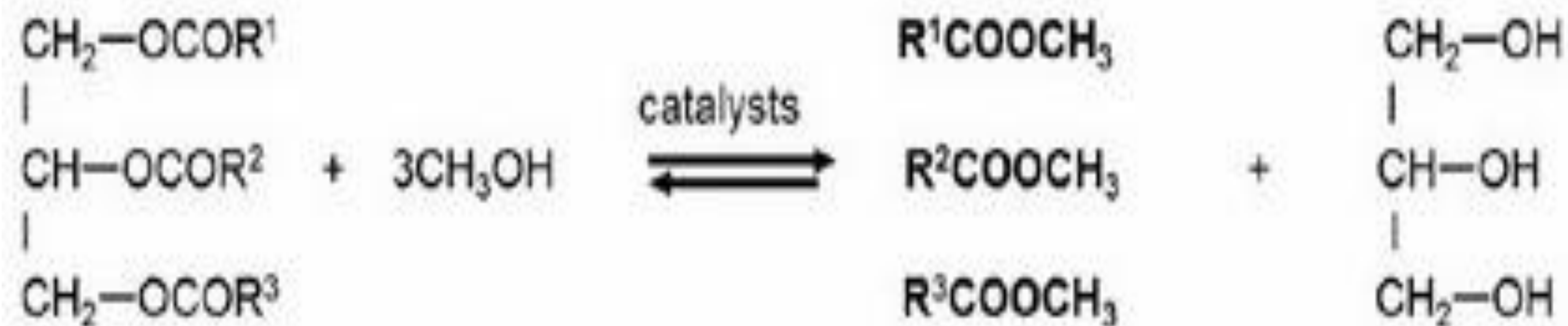
University of Ibadan Main Entrance gate



# Sampling Location







Plant Oil  
(Triglyceride)  
(Rapeseed,  
Soybean, etc)

Methanol

**Biodiesel Fuel (BDF)**  
(Methyl ester compounds)

Glycerin

$\text{R}^1, \text{R}^2, \text{R}^3$ : Fatty Acid Chain (C11~C17)

Equation for biodiesel production



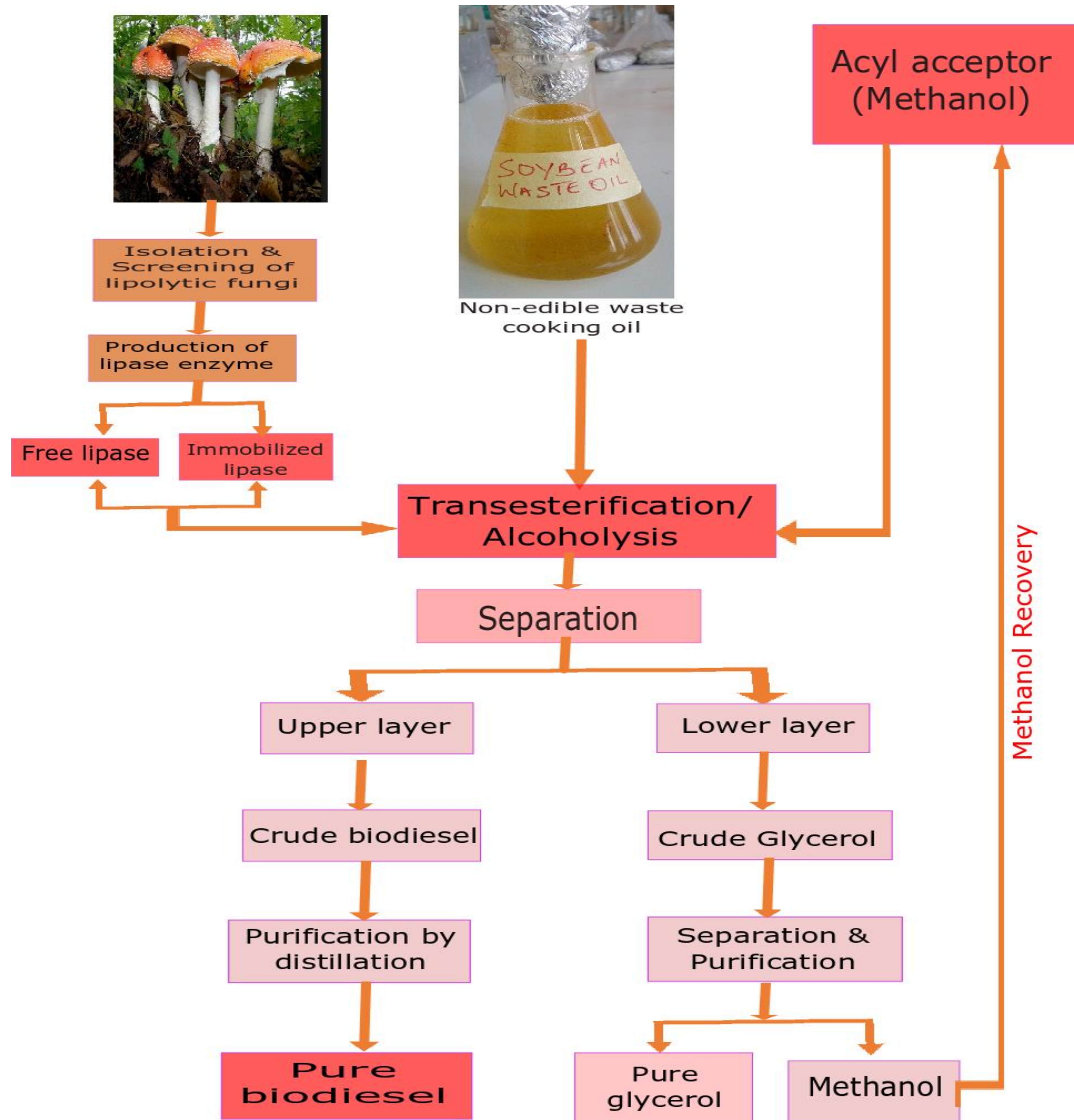


Fig 1: Step-wise procedure for Enzymatic production of biodiesel using waste cooking oil as feedstock



## Results

- Forty eight (48) fungi were isolated and screened. Isolate GH11 showed the best lipase activities both in solid-state and submerged fermentations, as such, the isolate was immobilized and used for biodiesel production
- The effects of production parameters were characterized and optimized.
- Acyl acceptor, Oil to methanol molar ratio, solvent addition, amount of enzyme, reaction temperature and time had significant difference ( $p > 0.05$ ) on biodiesel yield.



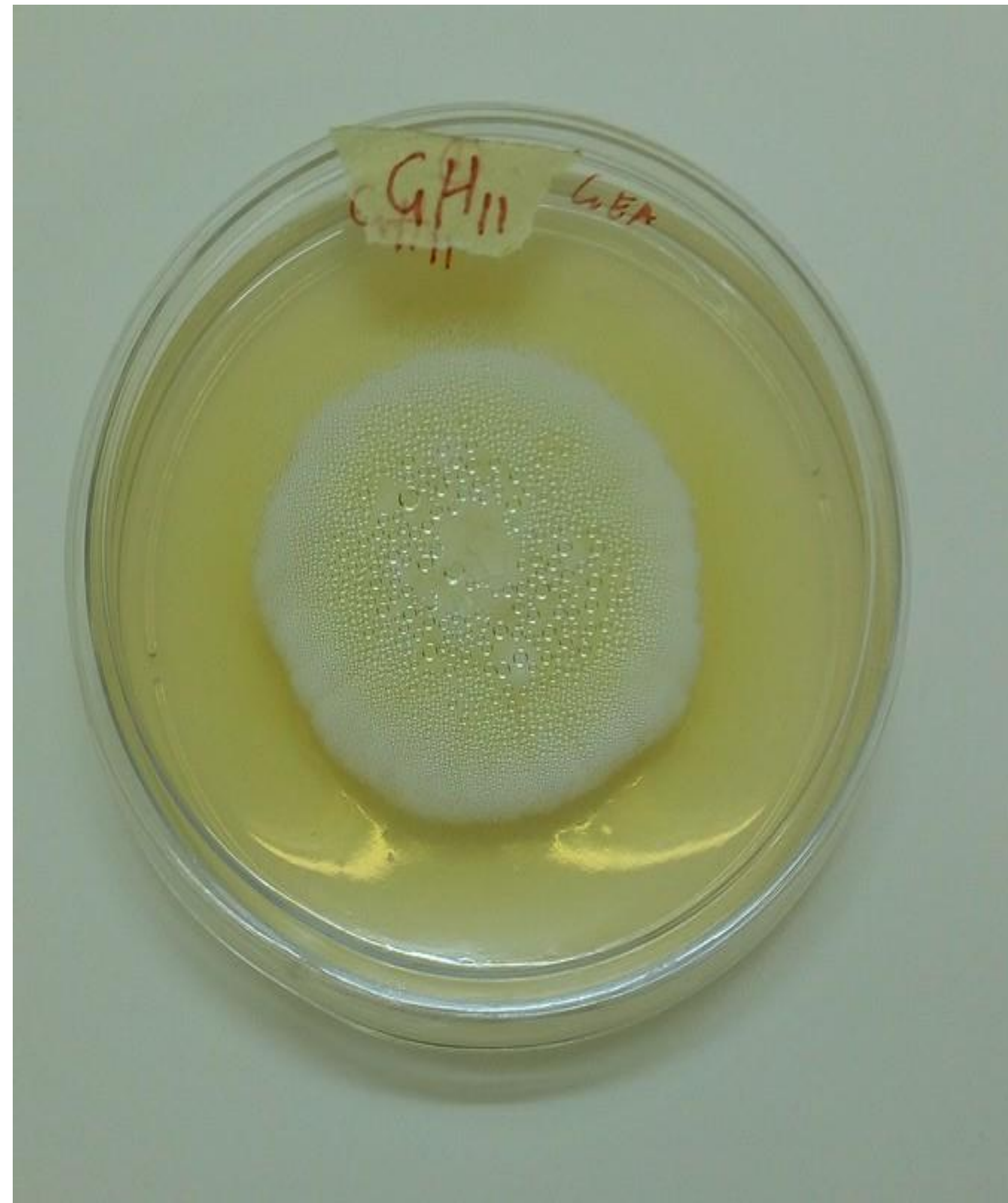


Fig 2. Front (A) & back (B) view of isolate GH11 on PDA medium



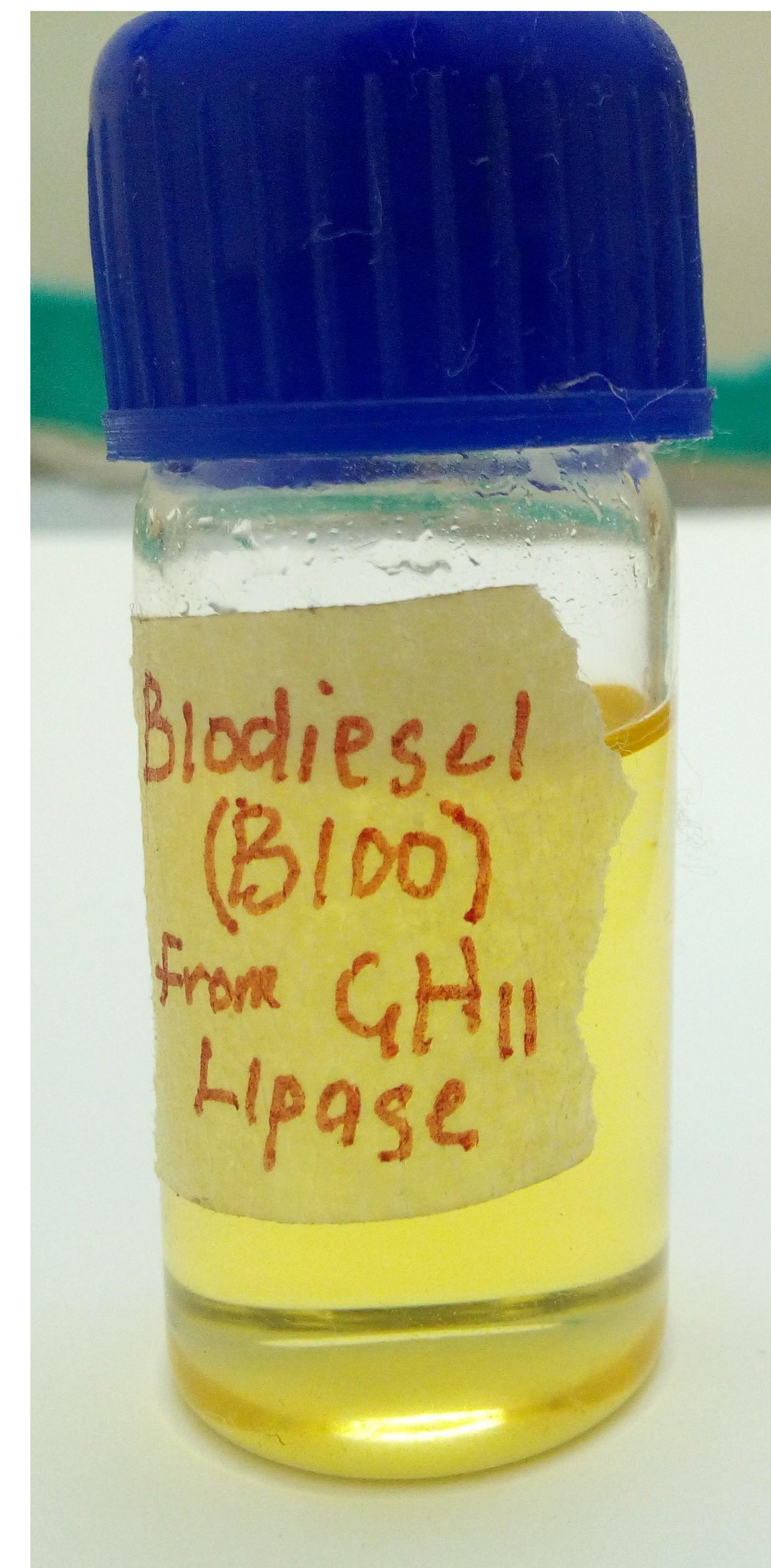
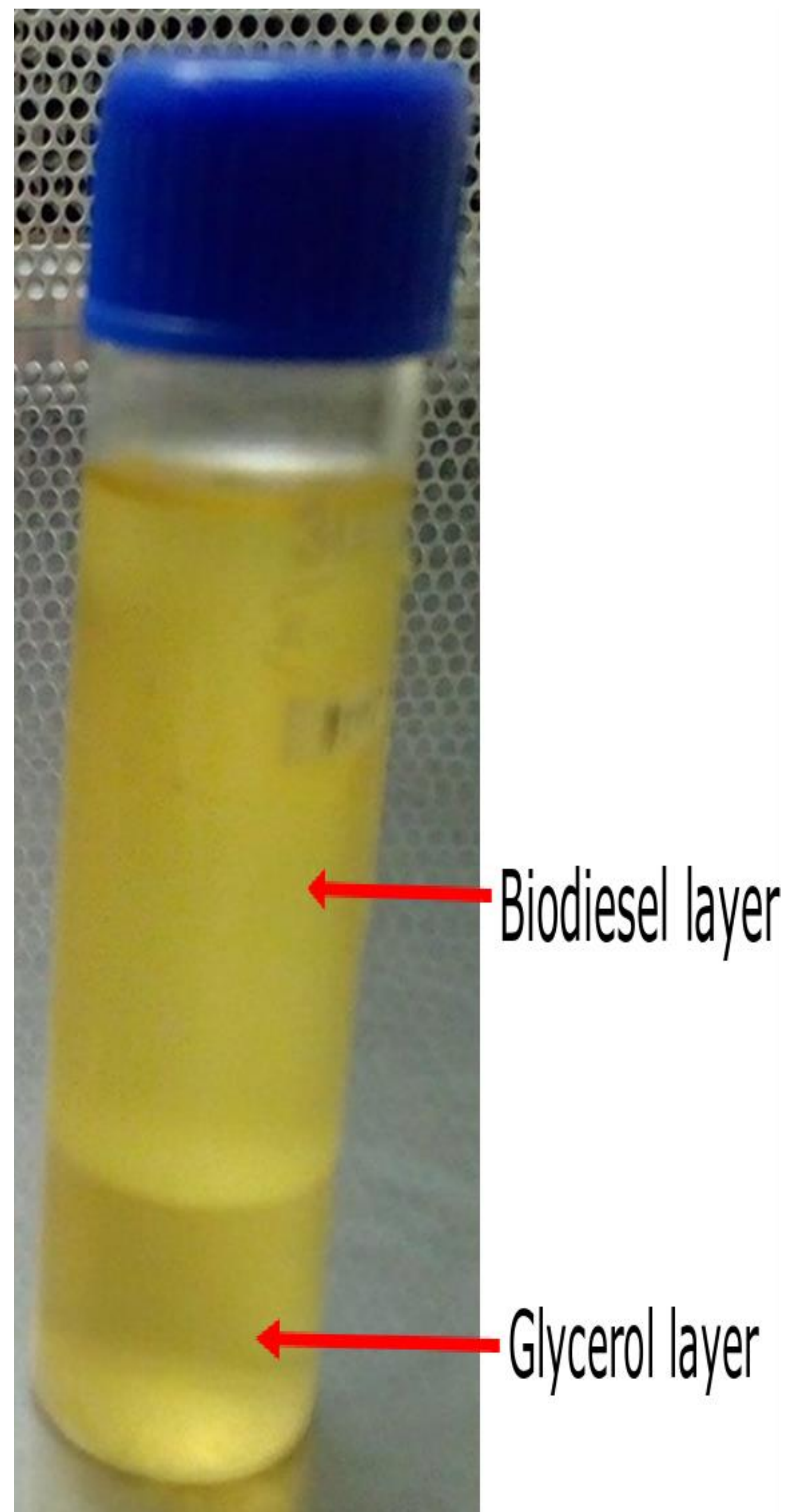


Fig 3. (A) Overview of biphasic layers' formation in biodiesel production  
(B) Purified Biodiesel (B100) from isolate GH11



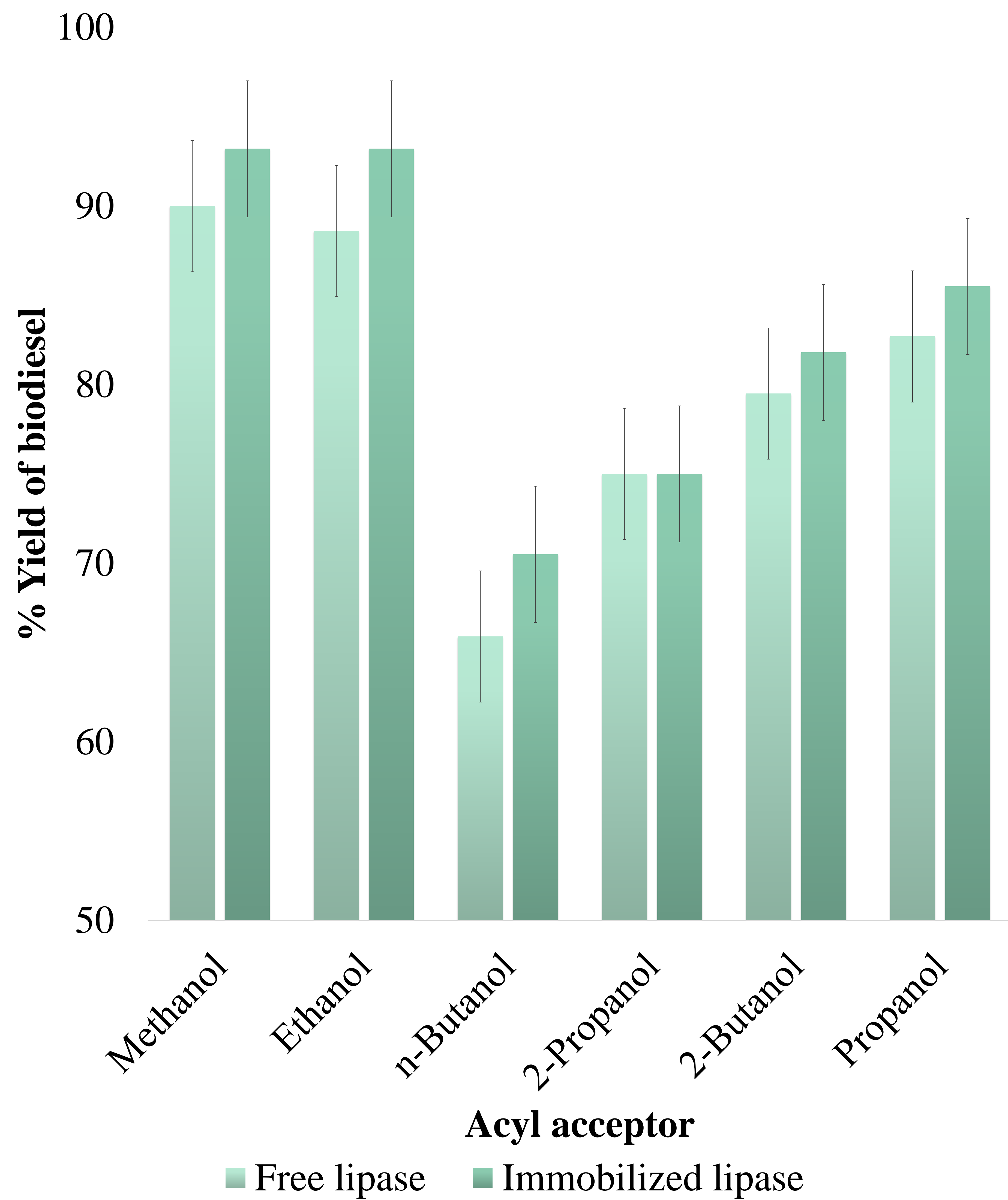


Fig 4. Effect of Acyl acceptor on biodiesel production

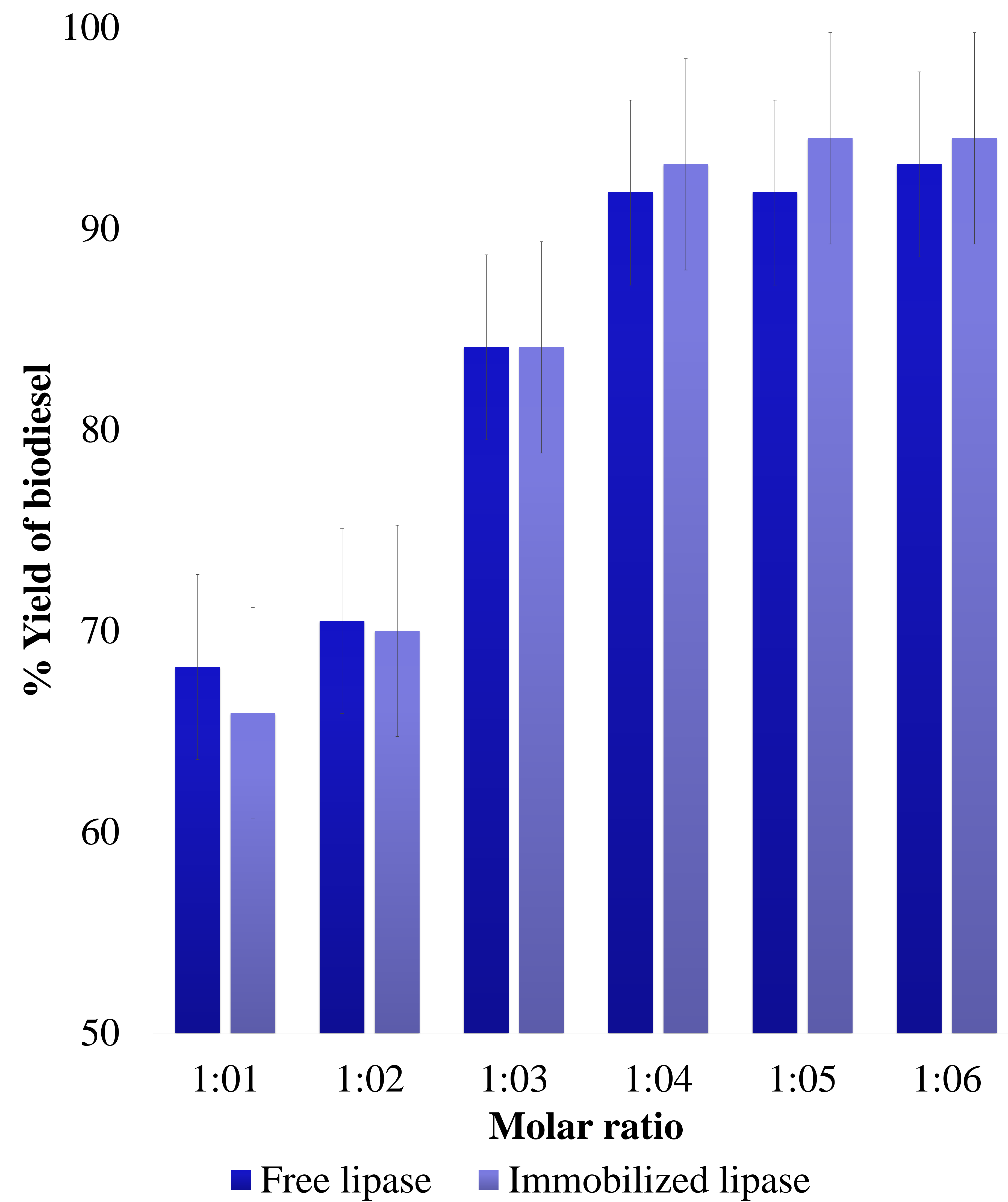


Fig 5. Effect of Oil to methanol molar ration on biodiesel production



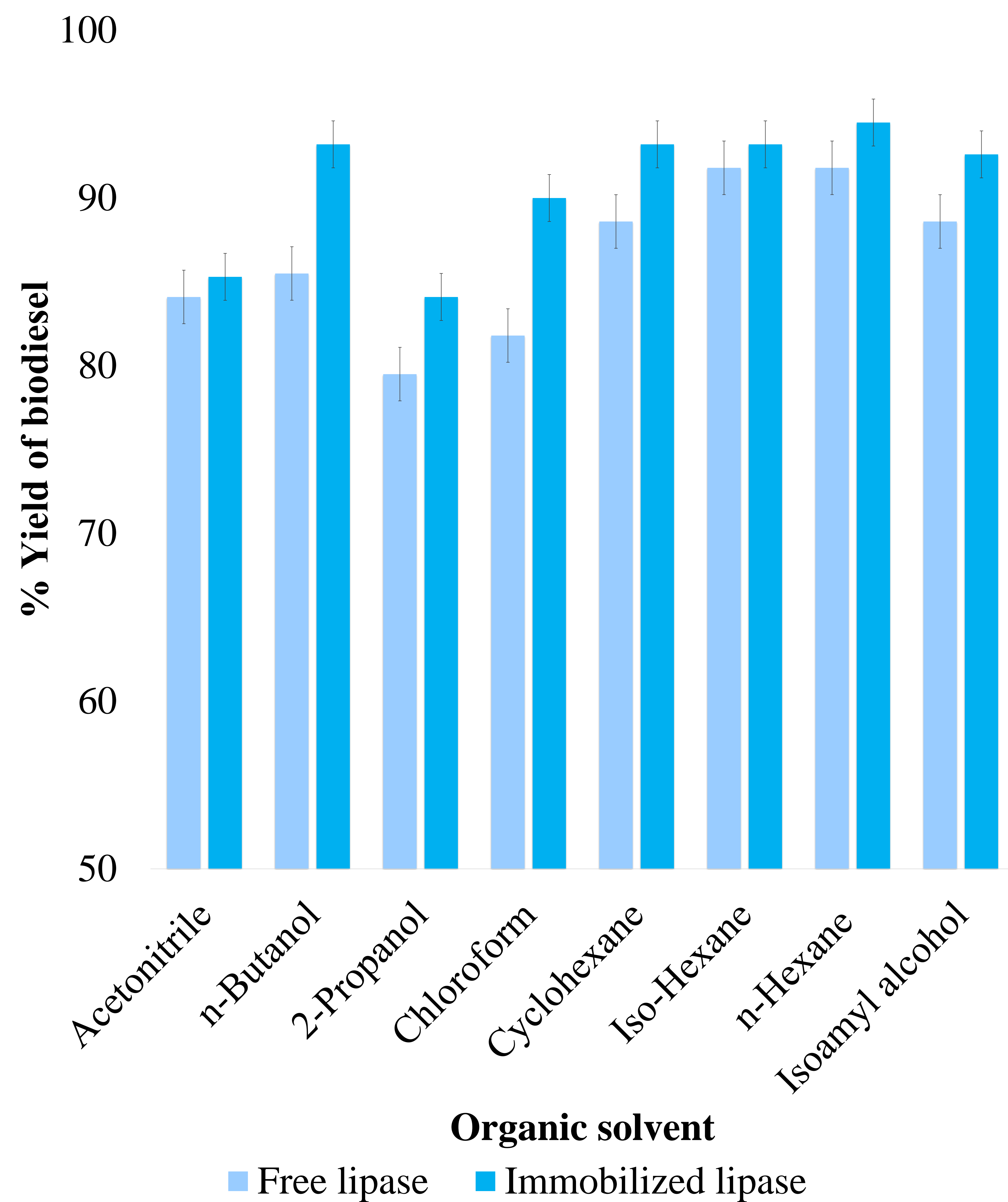


Fig 5. Effect of Oil to methanol molar ration on biodiesel production

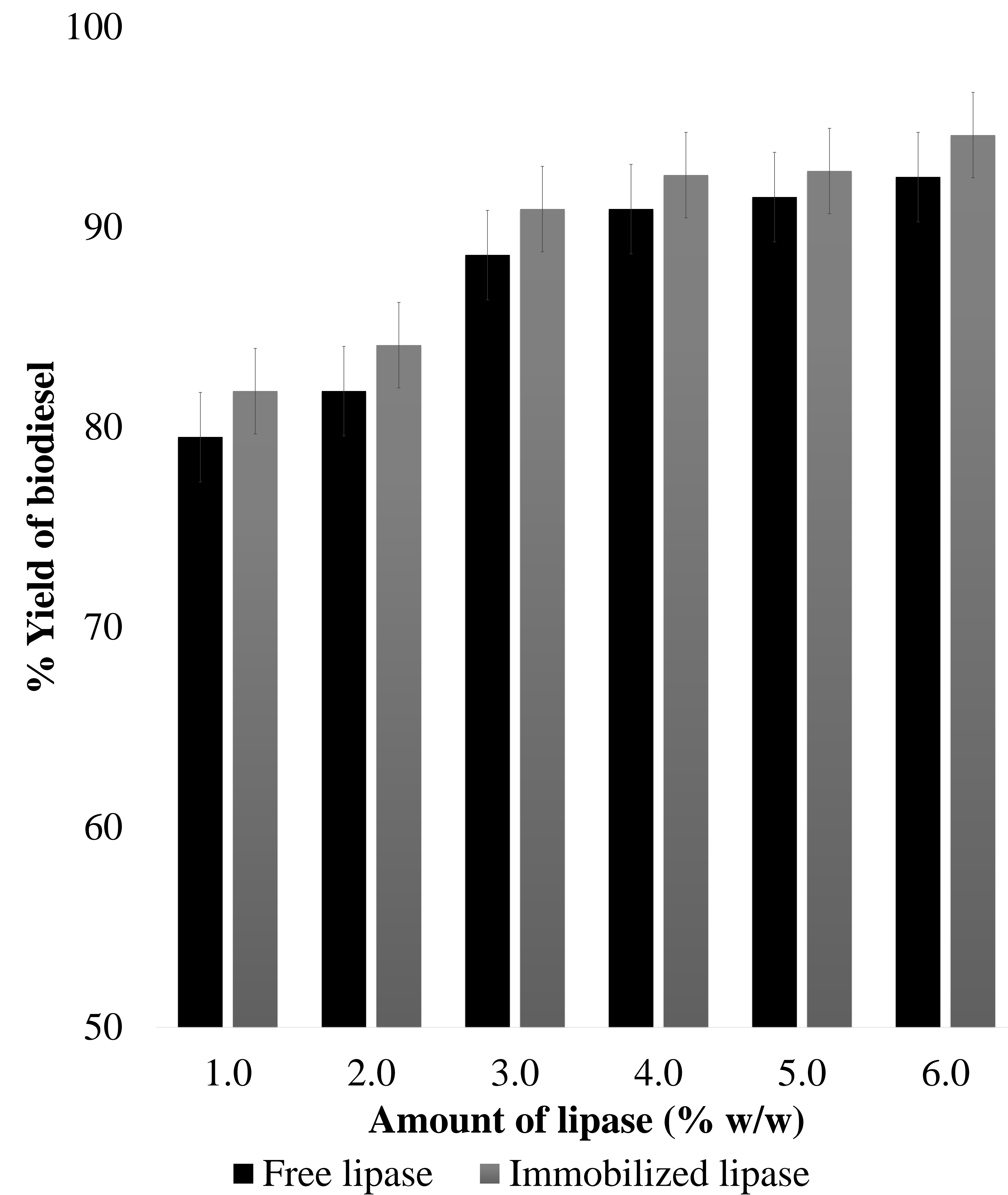


Fig 7. Effect of amount of lipase on biodiesel production



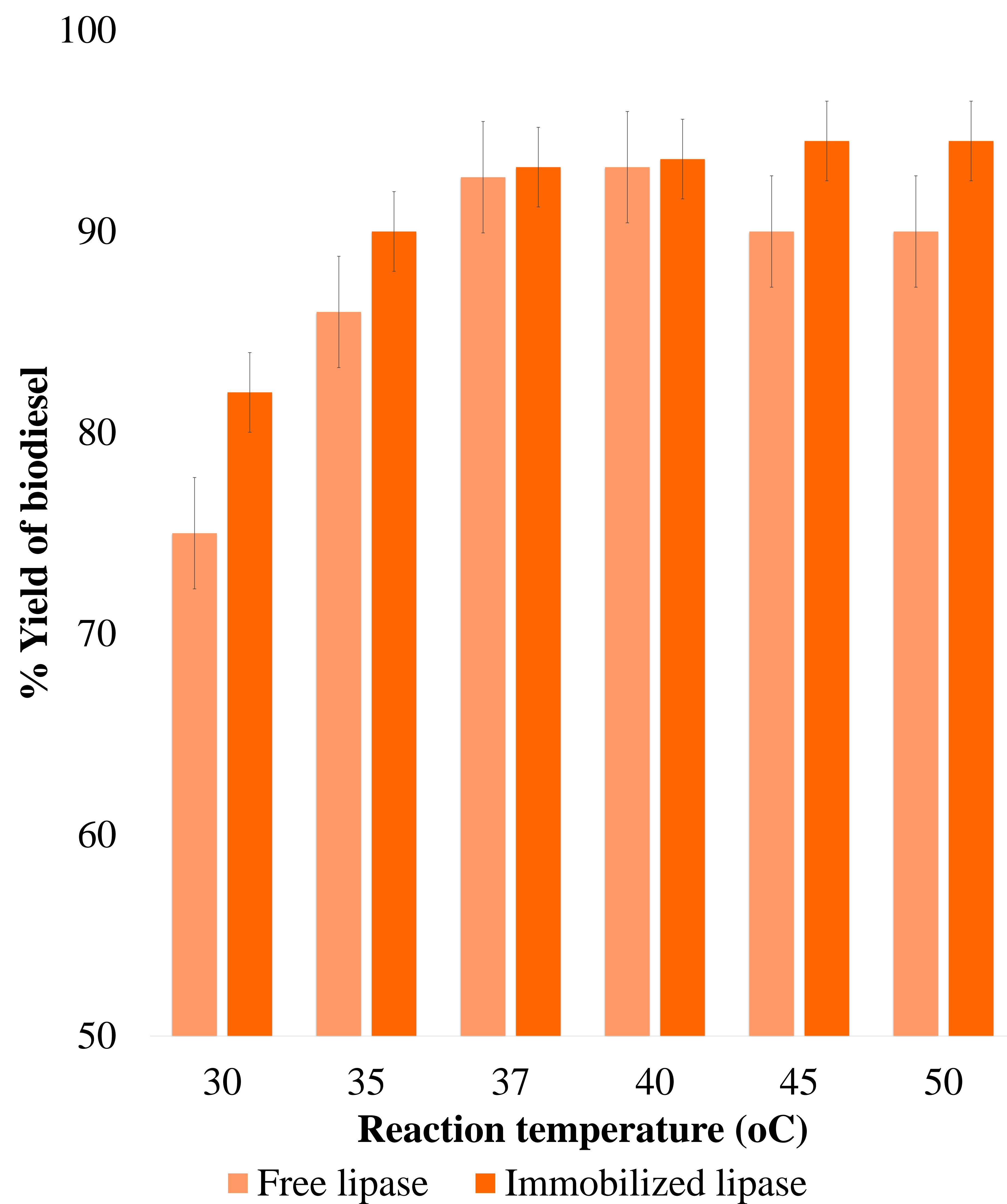


Fig 8. Effect of Reaction temperature on biodiesel production

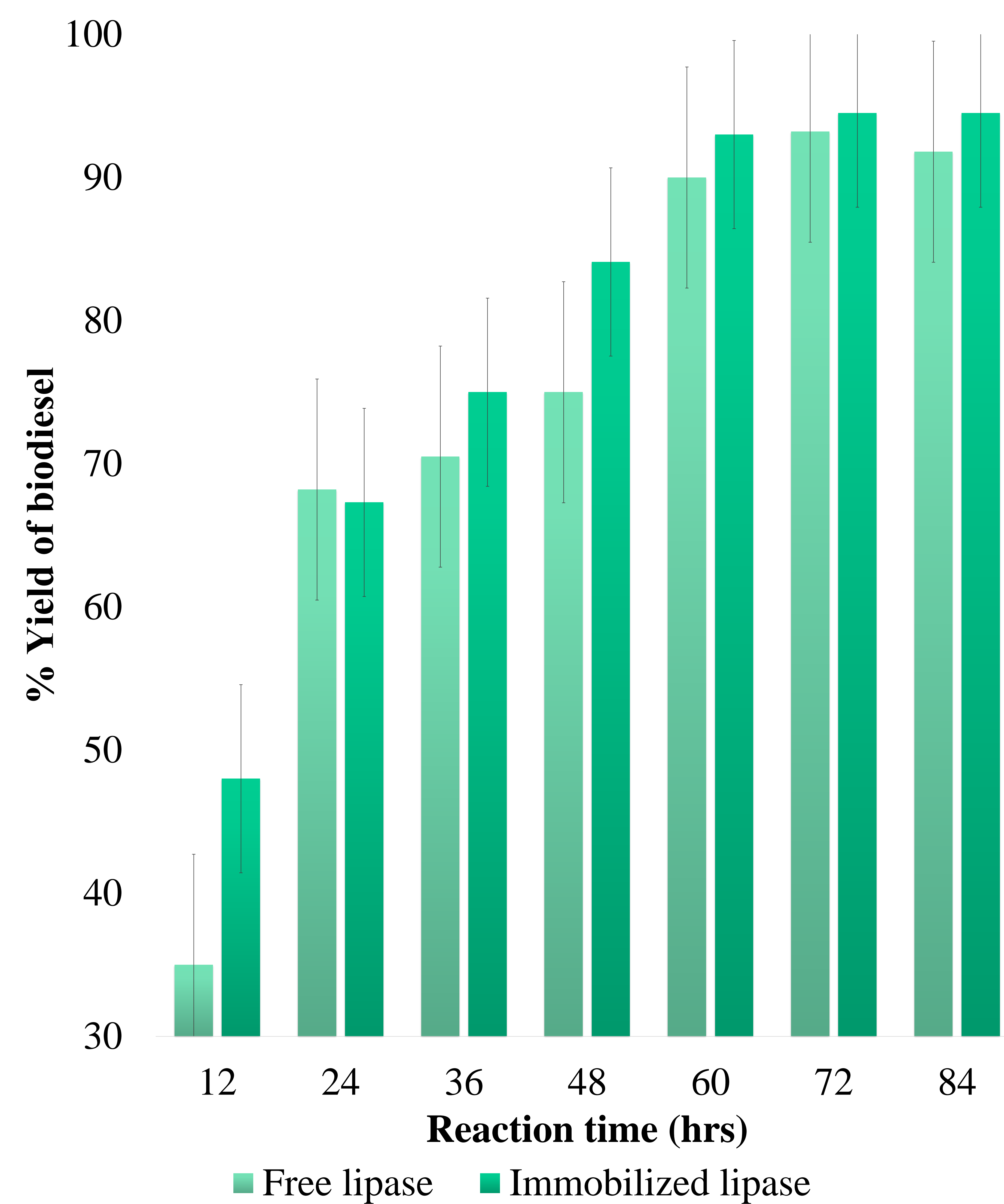


Fig 9. Effect of Reaction time on biodiesel production



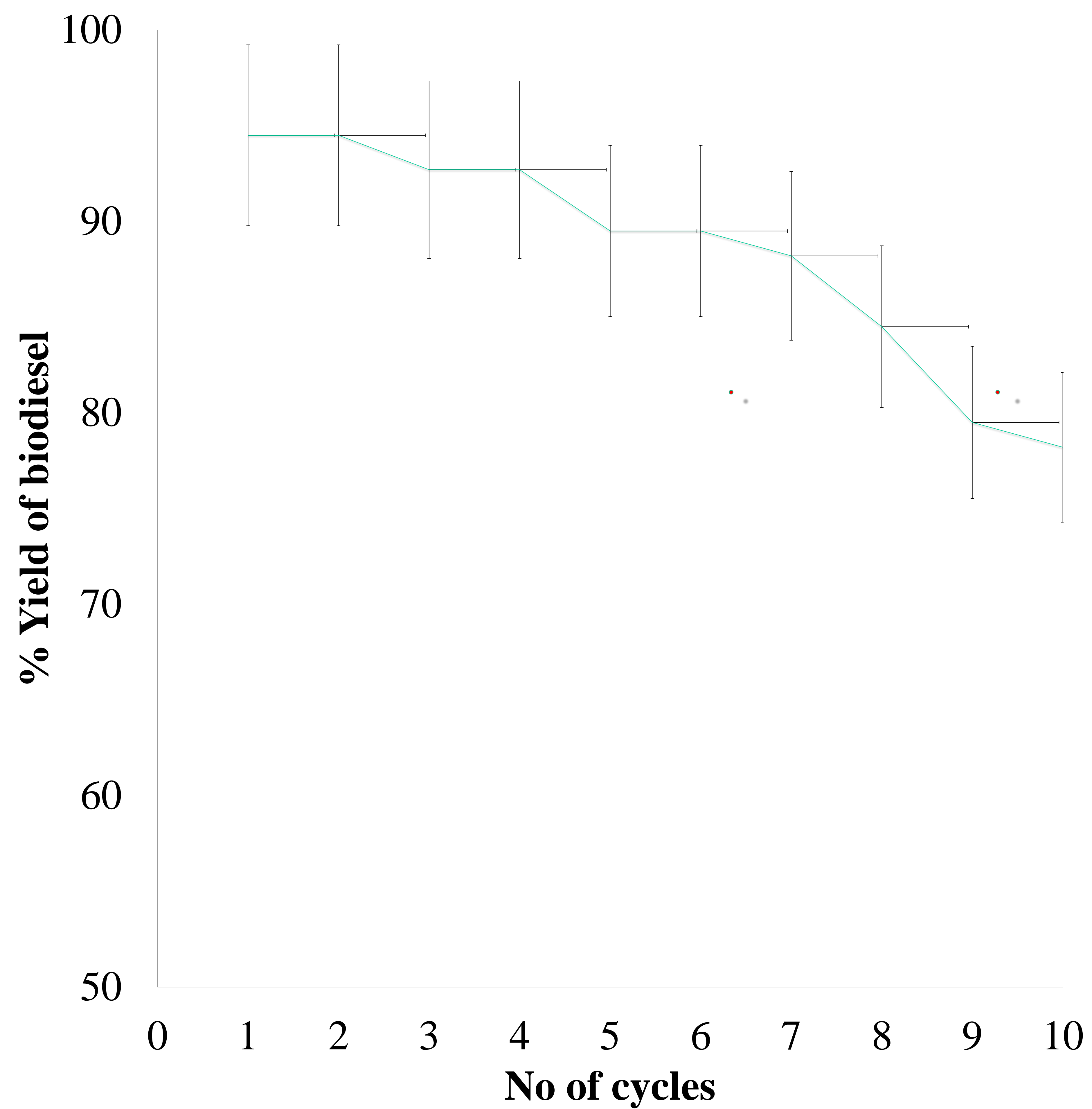


Fig 10. Effect of repeated used of Immobilized lipase on biodiesel production

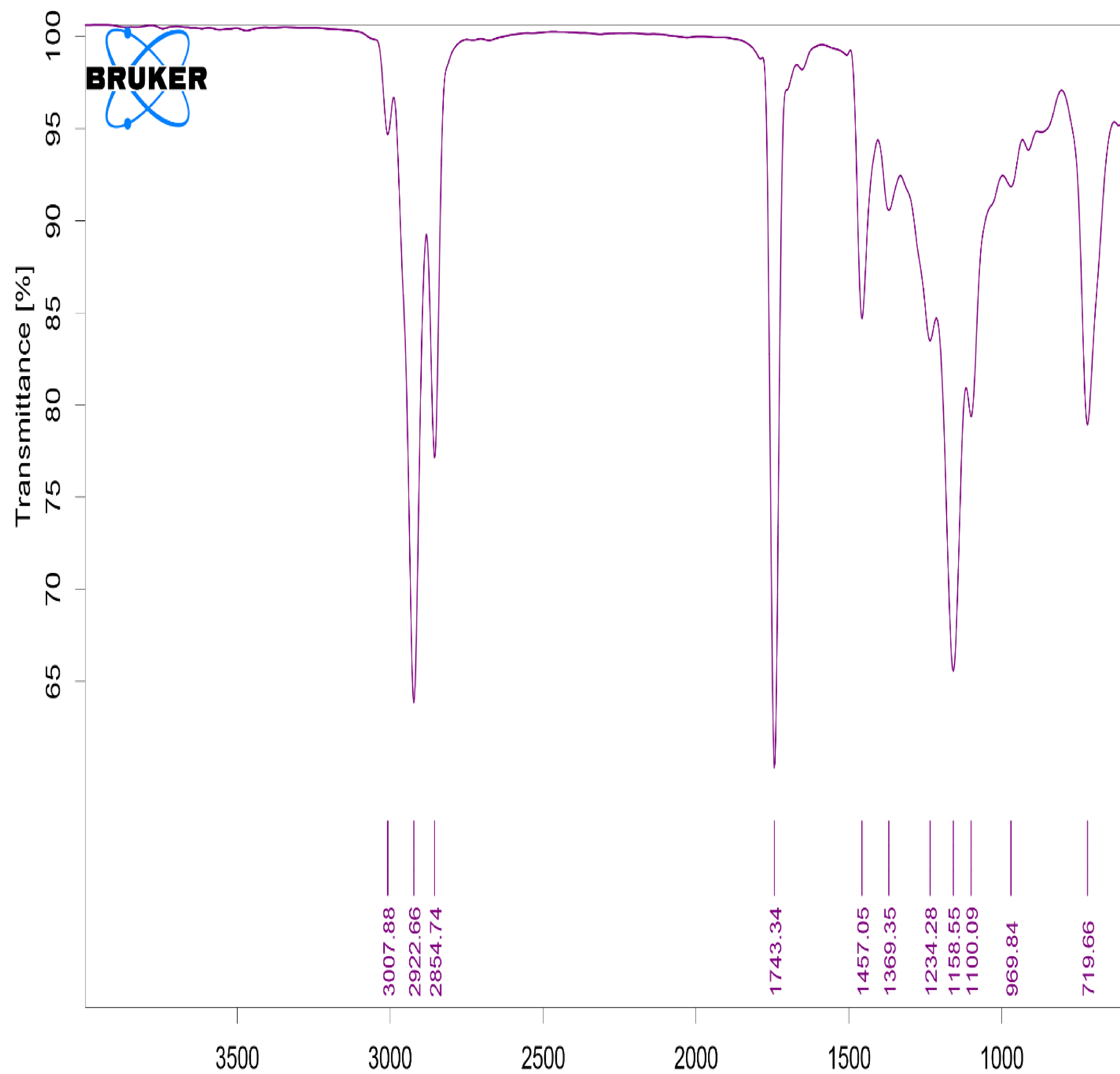


Fig 11. Fourier Transform Infrared (FT-IR) spectroscopy analysis of fatty ester



## Conclusion

- The lipolytic fungi isolate GH11 has demonstrated a great potential to catalyze transesterification of relative inexpensive waste soybean cooking oil (WSCO) for biodiesel production.
- The production of eco-friendly and biodegradable diesel fuel from WSCO also offers four-facet of solutions: economic, environmental, sustainability and waste management.
- Optimization of production parameters such as Acyl acceptor, Oil to methanol molar ratio, solvent addition, amount of enzyme, reaction temperature and time has been confirmed to have significant difference ( $p > 0.05$ ) on biodiesel yield.



## On-going study

- Characterization of fatty esters using HPLC, GC-MC & NMR
- Determination of biodiesel properties such as flash point, cetane number, viscosity, carbon and sulphur residues etc.
- Details analysis of immobilized enzymes using FESEM & XRD



## Selected Citations

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