IMPORTANCE OF MICROBIAL SOURCES IN THE PRODUCTION OF BIODIESEL

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ROLE OF MICROBIAL SOURCES IN THE PRODUCTION OF BIODIESEL

- Feed Stock
- Esterification & Transesterification catalyst

CONVENTIONAL FEED STOCKS

- Vegetable oils
  - Cultivated
  - Tree-borne
- Animal Fats
- Used Oils
- Fatty Acids

OIL CONTENT OF SOME MICROALGAE SPECIES

Microalgae	Oil content (% dry wt)	Microalgae	Oil content (% dry wt)
Anabaena cylindrical	4-7	*Nannochloropsis sp.*	31-68
Botryococcus braunii	25-75	*Neochloris oleoabundans*	35-54
Chlamydomonas rheinhardii	21	*Nitzschia sp.*	45-47
Chlorella sp.	28-32	*Porphyridium cruentum*	9-14
Chlorella vulgaris	14-22	*Prymnesium parvum*	22-38
Chlorella pyrenoidosa	2	*Scenedesmus obliquus*	12-14
Crypthecodinium cohnii	20	*Scenedesmus quadricauda*	1-9
Cylindrotheca sp.	16-37	*Scenedesmus quadricauda*	16-40
Dunaliella bioculata	23	*Schizochytrium sp.*	50-77
Dunaliella primolecta	8	*Spirulina platensis*	4-9
Dunaliella salina	6	*Spirulina maxima*	6-7
Euglena gracilis	14-20	*Synechoccus sp.*	11
Isochrysis sp.	25-33	*Tetraselmis maculate*	3
Monallanthus salina	>20	*Tetraselmis sueica*	15-23
Nannochloris sp.	20-35		

SOURCES OF BIODIESEL A COMPARISON

FEED STOCK	OIL YIELD (L/ha)
Soybean	466
Canola	1,190
Jatropha	1,892
Oil Palm	5,950
Microalgae[a]	1,36,900
Microalgae[b]	58,700

[a]70% oil
[b]30% oil

Yosuf Chisti, *Biotechnology Advances*, **25** (2007) 294-306

ADVANTAGES OF MICROALGAE AS A SOURCE OF BIODIESEL

High Yield

- low cost of production

Algae can grow

- In places away from farm land
  (No destruction to food chain)
- Sewages
- Near to power plants
  (takes CO_2 from smokestacks and yields oil)

Oil Productivity

- Greater than best producing oil crops

Deoiled biomass

Higher grade protein → Animal Feed

Balanced N : P ratio → Organic Fertilizers

LIMITATION OF ALGAL-OIL FOR THE PRODUCTION OF BIODIESEL

High concentration of Polyunsaturated Fatty Acids

– Arachidonic acid ($C_{20:4}$)
– Docosahexanoic acid ($C_{22:6}$)
– Linolenic acid ($C_{18:3}$)
– Linoleic acid (18:2)

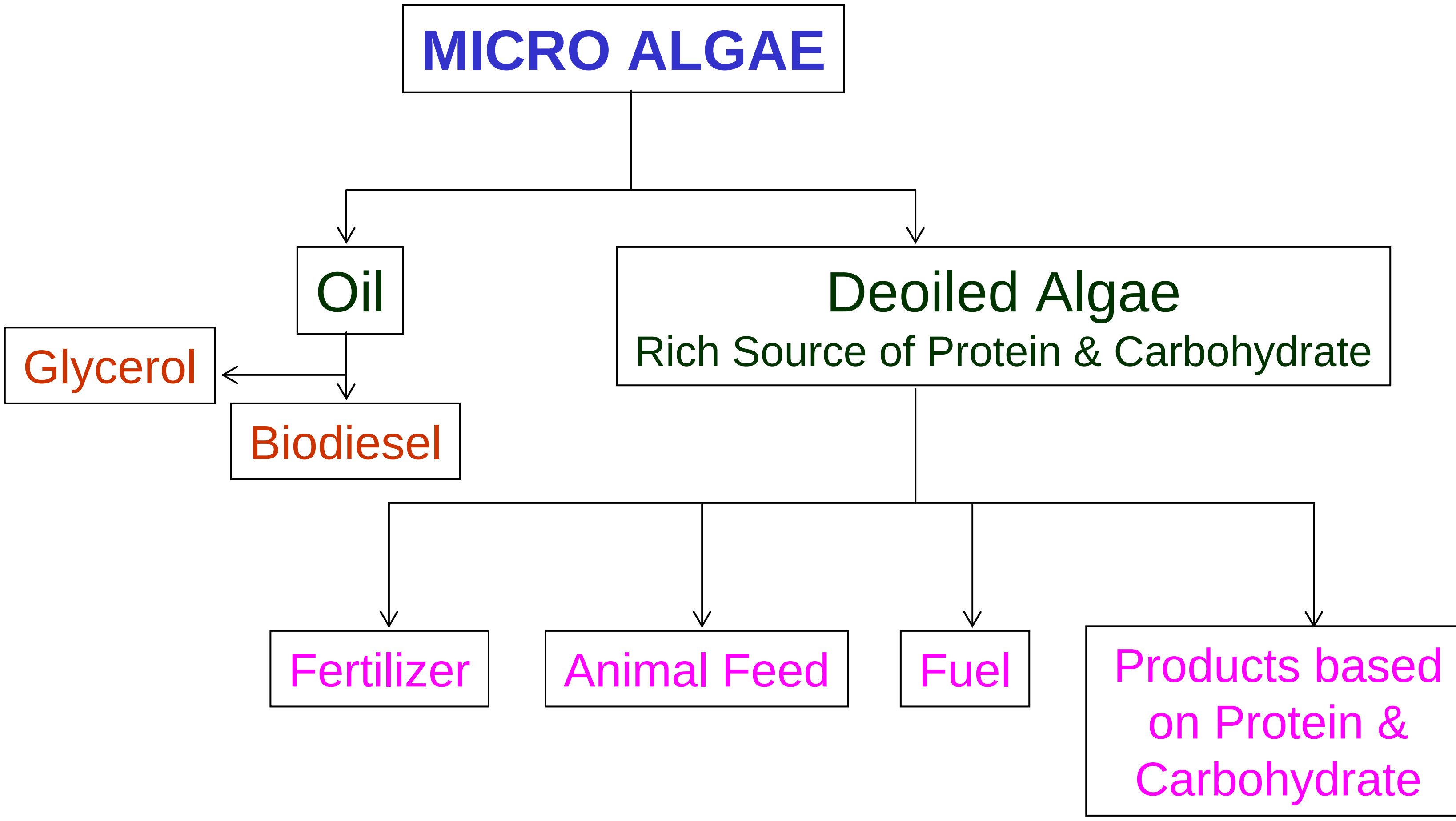
- Storage stability → (?)

Yosuf Chisti, *Biotechnology Advances*, **25** (2007) 294-306

POSSIBLE SOLUTION

- Identification of Right Microbe which Produces Oil with optimum FA Composition
- Partial Hydrogenation
  - To reduce unsaturation to desired levels

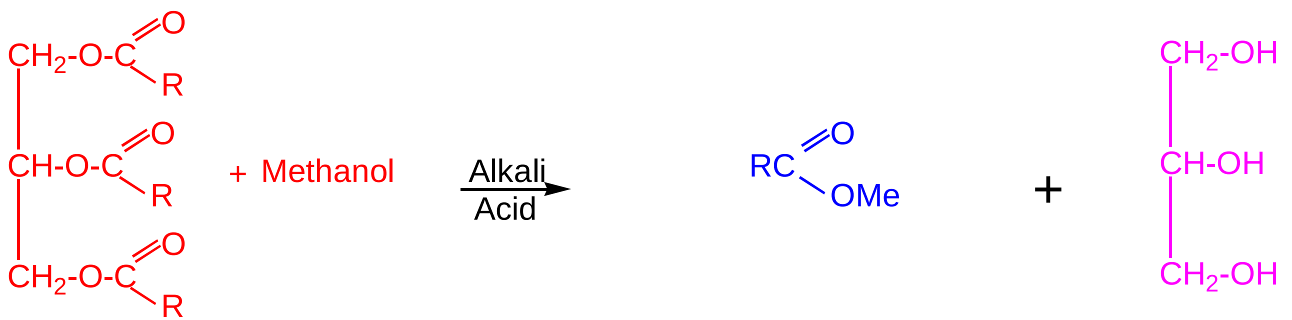
INTEGRATED APPROACH



CHEMISTRY OF BIODIESEL

VERY SIMPLE CHEMISTRY…

VERY LOW FFA – only Transesterification

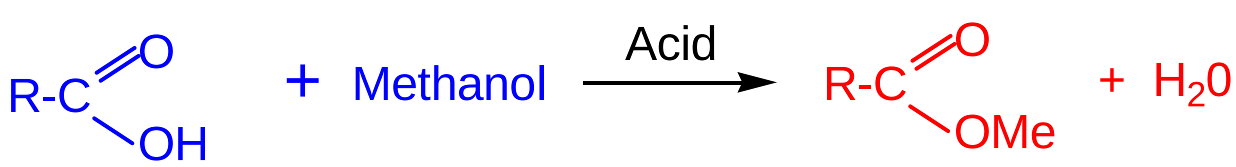


OIL / FAT (TRIGLYCERIDE)

Fatty Acid Methyl Ester (Biodiesel)

Glycerol

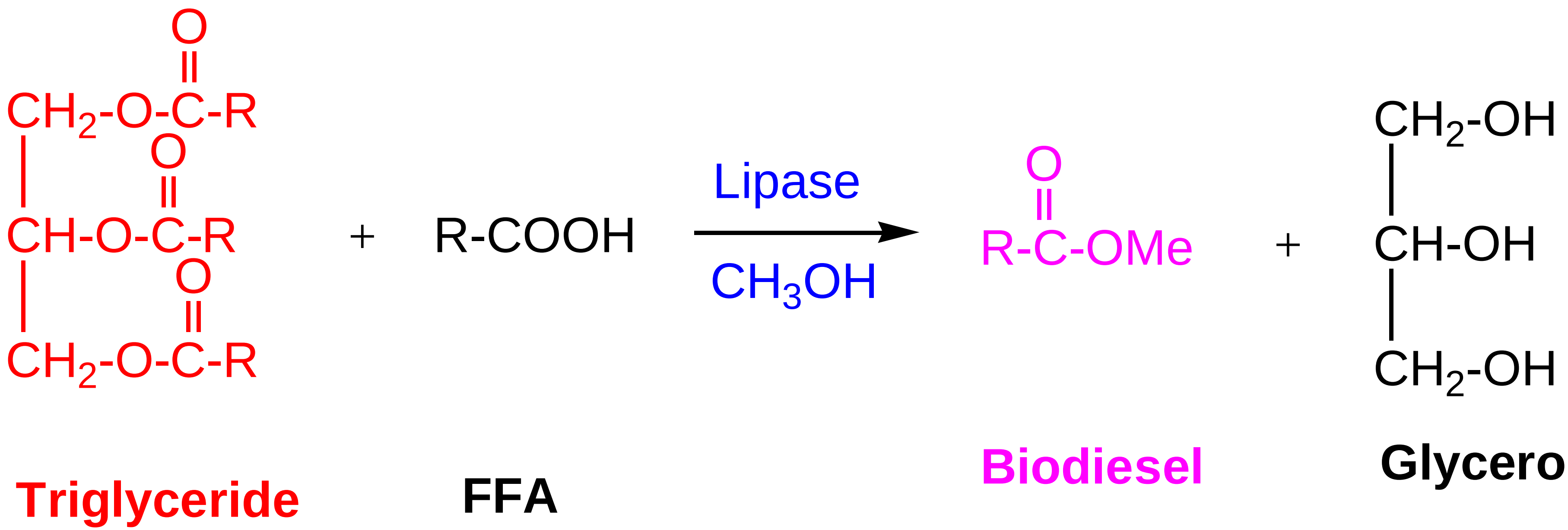
HIGH FFA – ESTERIFICATION followed by TRANSESTERIFICATION



Fatty Acid

Biodiesel

LIPASE CATALYZED PREPARATION OF BIODIESEL



Lipases simultaneously converts both FFA and TG into Biodiesel

LIPASES USED FOR BIODIESEL PRODUCTION

***Candida antarctica* (Novozym 435)**	*Mucor miehei* (Lipozyme)
***Candida rugosa* (Lipase A4)**	*Rhizopus delemar*
***Thermomyces lanuginosus*(LipozymeTL IM)**	*Geotricum candidum*
Pseudomonas fluorescens	*Porcine pancreas*
***Pseudomonas cepacia* (Lipase PS-30)**	*Fusarium heterosporum*
Rhizopus oryzae	*Aspergillus niger*
***Rhizomucar miehei* (Lipozyme IM 60)**	*Chromobacterium viscosum*
***Pseudomonas fluorescens* (Amano AK)**	*Candida antartica* (SP 435)
Rhizomucor miehei (Lipozyme IM-77)	Immobilized *R. oryzae* cells within BSPs
Immobilized *R. oryzae* whole cells	*Rhizopus niveus* (Newlase F)
Burkholderias cepacia (IM BS–30)	*Mucor javanicus* (Lipase M)
Candida Sp. (SP 382)	*Cryptococus* ssp. S-2 (Strain CS2)

COMPARISION BETWEEN ALKALI AND LIPASE-CATALYSIS METHODS

Condition	Alkali-catalysis Process	Lipase-catalysis Process
Reaction temperature	60 -70°C	30 -40°C
Free Fatty acids in raw materials	Saponified product	Methyl esters
Water in raw materials	Interference with the reaction	No influence
Yield of methyl esters	Normal	Higher
Recovery of glycerol	Difficult	Easy
Purification of methyl esters	Repeated Washings	None
Production cost	Cheap	Relatively expensive

INHIBITION OF LIPASE ACTIVITY

Factors effecting the lipase activity

- Short chain alcohols – MeoH and EtOH
- Glycerol
- Gums

HOW TO OVERCOME?

MeOH

- Step wise addition
- Use of solvents

Glycerol

- Treatment with solvents like IPA, 2-butanol, n-butanol etc.
- Dialysis method using flat sheet membrane

Gums

- Degumming

Other Pretreatments

- Treating the used enzyme intermittently with methyl esters and/ or oils

EFFECT OF PRESENCE AND ABSENCE OF SOLVENT ON ENZYME - BASED BIODIESEL PRODUCTION

Alcohol	Oil Source	Solvent	Lipase	Yield (%)
Methanol	Tallow	Hexane	*Mucor miehei*	94.8
	Tallow	None	*M. miehei*	19.4
Ehtanol	Tallow	Hexane	*M. miehei*	98.0
	Tallow	None	*M. miehei*	65.5
Isopropanol	Tallow	None	*M. miehei*	90.3
2-Butanol	Tallow	None	*M. miehei*	96.4

NOVEL IMMOBILIZATION TECHNIQUES

Immobilization

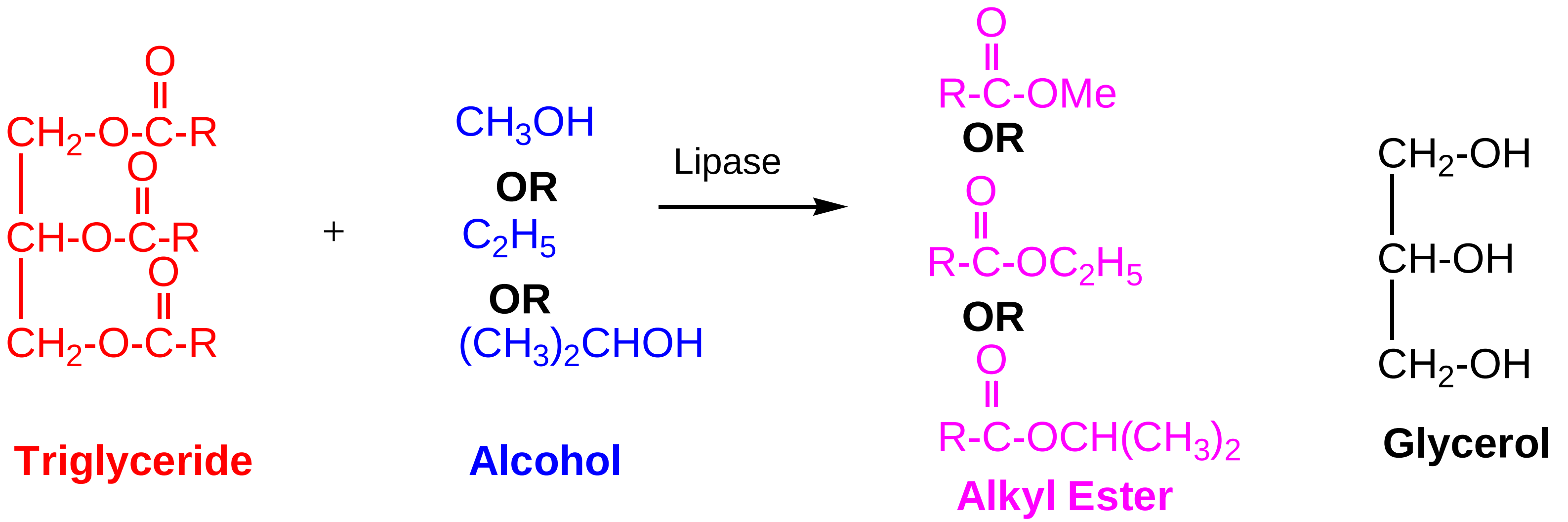
- Enhances the stability
- Can be recovered and reused

Special Methods

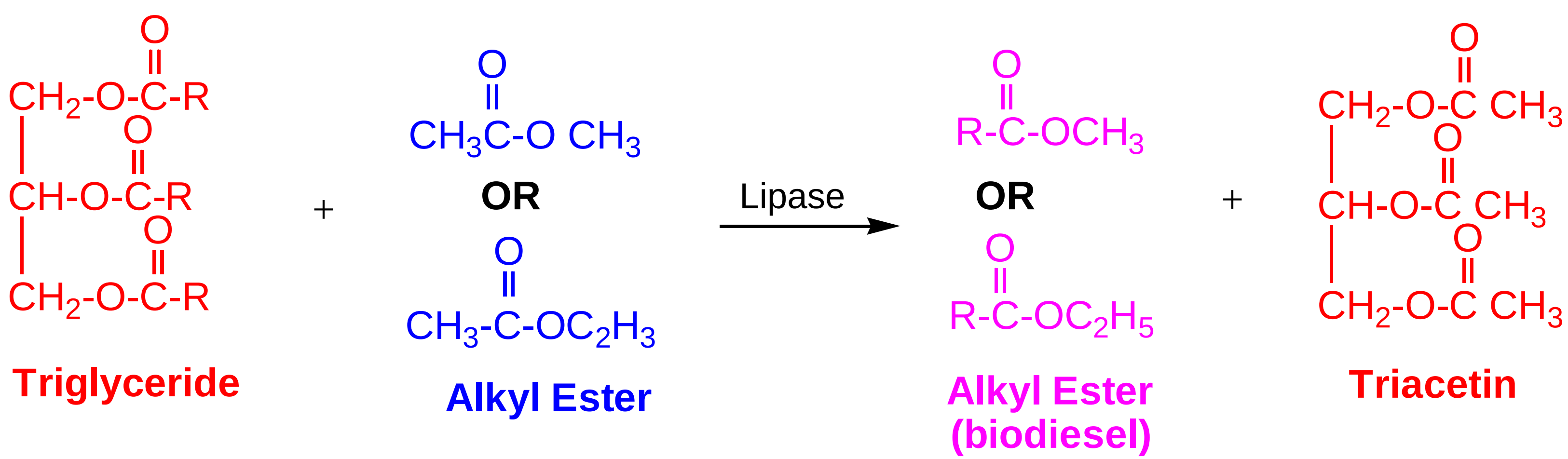
- o Phyllosilicate sol gel matrix-based
- o Entrapment in sol gel polymer matrix
- o On macroporous acrylic resin
- o With biomas support particles (BSPs)
- o Cross linking treatment to BSPs

NOVEL ACYL DONORS

TRANSESTERIFICATION



INTERESTERIFICATION



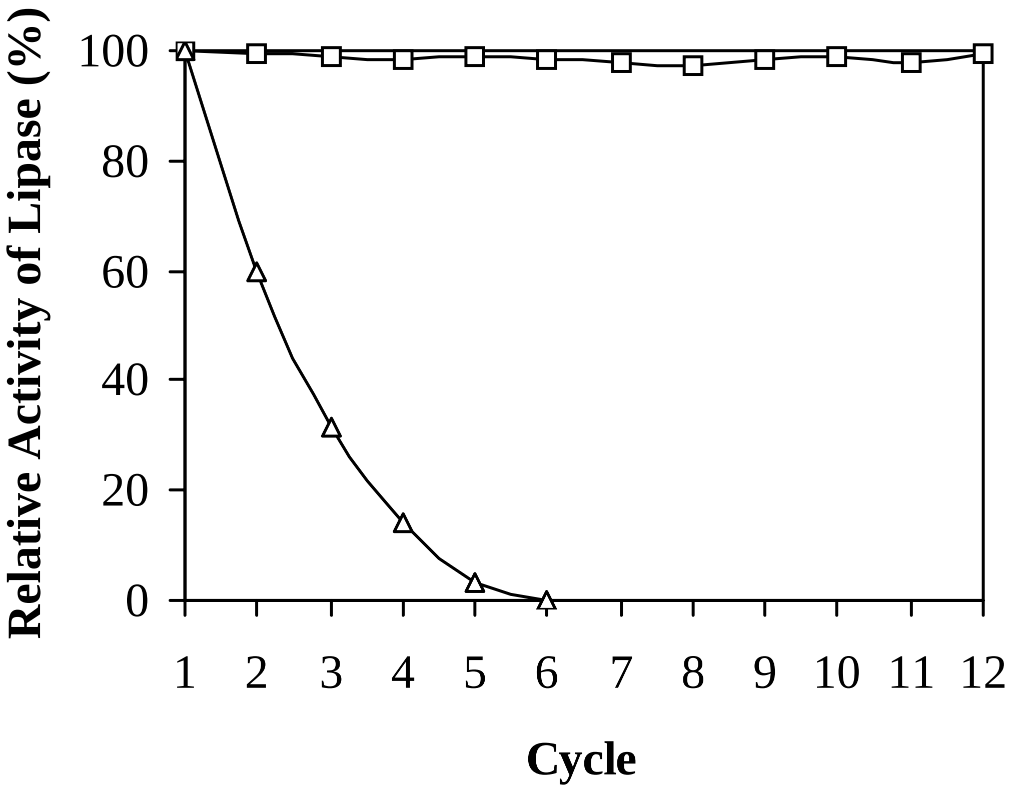
LIPASE-MEDIATED CONVERSION OF VEGETABLE OILS INTO BIODIESEL USING ETHYL ACETATE AS ACYL ACCEPTOR

$$\text{Oil + Ethyl acetate} \xrightarrow[\text{50°C, 12 hr, 1:11}]{\text{Novozyme 435 (10\%)}} \text{Ethyl ester + Triacetin}$$

Oil	Yield (%)
Crude Sunflower oil	92.7
Crude jatropha oil	91.3
Crude Karanja oil	90.0

MK Modi, JRC Reddy, BVSK Rao and RBN Prasad, *Bioresource Technologies*, **98** (2007) 1260-1264.

OPERATIONAL STABILITY OF ENZYME



Operational stability of lipase in interesterification and ethanolysis of crude jatropha oil at 50°C and 150 rpm using 10% Novozym 435 (% wt/wt of oil). For interesterification: ethyl acetate to oil molar ratio of 11:1 (single step addition of ethyl acetate), 12 h. For ethanolysis: ethanol to oil molar ratio of 4:1 (4 step addition of ethanol), 8 h. □ Interesterification. △ Ethanolysis.

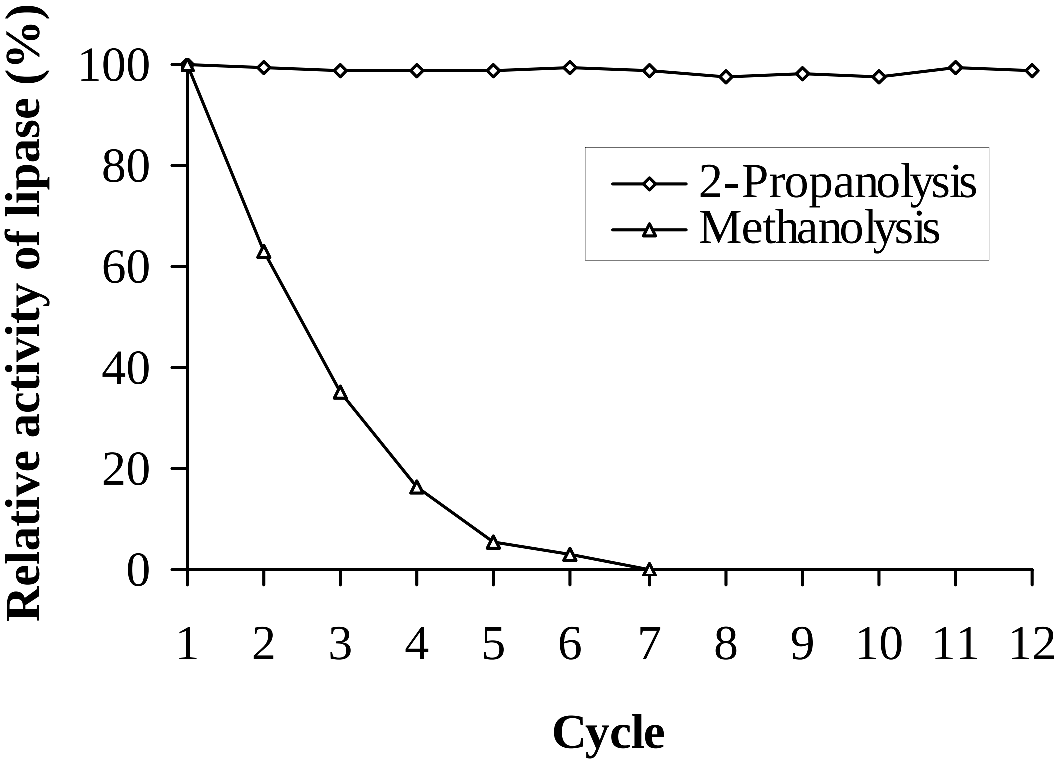
LIPASE-MEDIATED TRANSFORMATION OF VEGETABLE OILS INTO BIODIESEL USING PROPAN-2-OL AS ACYL ACCEPTOR

$$\text{Oil + IPA} \xrightarrow[\text{50}^{\circ}\text{C, 8 hr, 1:4}]{\text{Novozym 435 (10\%)}} \text{IPA esters + Glycerol}$$

Oil	Yield (%)
Crude Sunflower oil	93.4
Crude jatropha oil	92.8
Crude Karanja oil	91.7

MK Modi, JRC Reddy, BVSK Rao and RBN Prasad, Biotechnlogy Letters, 28: 637-640 (2006)

OPERATIONAL STABILITY OF ENZYME



Operational stability of lipase over repeated cycles in alcoholysis of crude jatropha oil using alcohol to oil molar ratio of 4:1, catalyzed by Novozym 435 (200 mg) at 50 °C and 150 rpm for 8 h. For 2-propanolysis 0.55 g of propan-2-ol added to 2 g of oil in single step. For methanolysis 0.29 g methanol added to 2 g oil in four steps (72.5 mg in each step). 100% Relative activity of lipase in absolute terms corresponds to 93.4 and 92.8% alkyl esters conversion in methanolysis and 2-propanolysis, respectively.

MICROBIAL LIPASE-MEDIATED PREPARATION OF BIODIESEL USING VARIOUS TYPES OF OILS AND ALCOHOLS

Oil	Alcohol	Lipase(s)	Conditions	Conver-sion (%)
Soybean	Methanol	*C. Antarctica* (Novozym 435)	Oil: methanol, 1:8; lipase: 4 wt% of oil; 30°C, 3.5 hr	97
Sunflower	Methanol	*C. antarctica* (Novozyme 435)	Oil: methanol, 1:4; lipase: 7 wt% of oil; water: 400 ppm; 50°C; 16 hr.	97
Jatropha	Methanol, ethanol	*Chromobacterium viscosum, C. rugosa,*	Oil: alcohol, 1:4; lipase: 10 wt% of oil; 40°C; 8 hr.	62-92
Castor	Ethanol	*C. antarctica* (Novozym 435), *T.lanuginosus* (Lipozyme IM)	Oil: ethanol, 1:10; lipase: 20% of oil; 65°C Oil: ethanol, 1:3; lipase: 20 wt% of oil: 65°C.	81.4 98
Cottonseed	Methanol	*C. antarctica* (Novozym 435)	Oil: methanol, 1:4; lipase: 30 wt % of oil; 50°C; 7 hr.	72-94
Restaurant grease	Methanol, ethanol, propanol, isopropanol, butanol	*C. antarctica* (SP 435), *T. lanuginosus, P. cepacia* (IMPS-30)	Oil: alcohol, 1:4; lipase: 10 wt% of grease; 40°C; 8-48 hr.	87-95
Canola	Methanol	*C. antarctica* (Novozym 435)	Oil: methanol, 1:3.5; lipase: 42.3% of oil; water: 7.2%; 38°C; 12.4 hr.	97.9
Rice bran oil	Methanol	*C. antarctica* (Novozym 435); *Rhizomucor miehei* (1M-60)	Oil: methanol, 1:3.6; lipase: 5 wt% of oil; 50°C; 4 to 6 hr.	98
Waste bleaching earth containing palm oil	Methanol; ethanol; 1-propanol; 1-butanol; isobutanol	*C. cylindrace, C. rugosa, R. oryzae, A. niger, Rhizopus japonicus,*	Oil: alcohol, 1:4; lipase: 11 to 475 IU/g of waste activated bleaching earth; 30-37°C; 4-8 hr.	78-96
Waste edible oil	Methanol	*C. antarctica* (Novozym 435)	Oil: methanol, 1:3; lipase: 4 wt% of oil; 30°C.	98
Jatropha, karanj, sunflower	Ethyl acetate	*C. Antarctica* (Novozyme-435)	Oil: ethyl acetate, 1:11; 50°C; 12 hr.	90 – 92.7

CONCLUSIONS

Microbial Sources as Feed Stocks

- Microbial Biodiesel … Technically feasible
- Critical evaluation of Algal Biology through Genetic Engineering
- To adopt bio-refinary concept for cost reduction
- Economics need to be improved to make it competitive with petrodiesel

Microbial Sources as Catalysts

- Microbial Enzymes are Effective Catalysts for the production of Biodiesel
- Biotechnological Potential of Microbial Lipases is Steadily Increaing
- Enzyme cost hampers the cost of Biodiesel

THANK YOU