Solid state fermentation of chemically untreated sugarcane bagasse for fungal production of single cell oil as biodiesel feedstock

Mahesh Khot [1,2], Sanjay Govindwar[2], V. RaviKumar [3], B.D.Kulkarni [3], Ameeta RaviKumar[1]

[1] Institute of Bioinformatics and Biotechnology (IBB), Savitribai Phule Pune University, Ganeshkhind, Pune-411007, India
[2] Department of Microbiology, Shivaji University, Kolhapur-416004, India
[3] Chemical Engineering and Process Development Division, CSIR-National Chemical Laboratory, Pune 411 008, India

BIODIESEL – LIQUID BIOFUEL OF CHOICE

Biodiesel = FAMEs (Fatty Acid Methyl Esters)

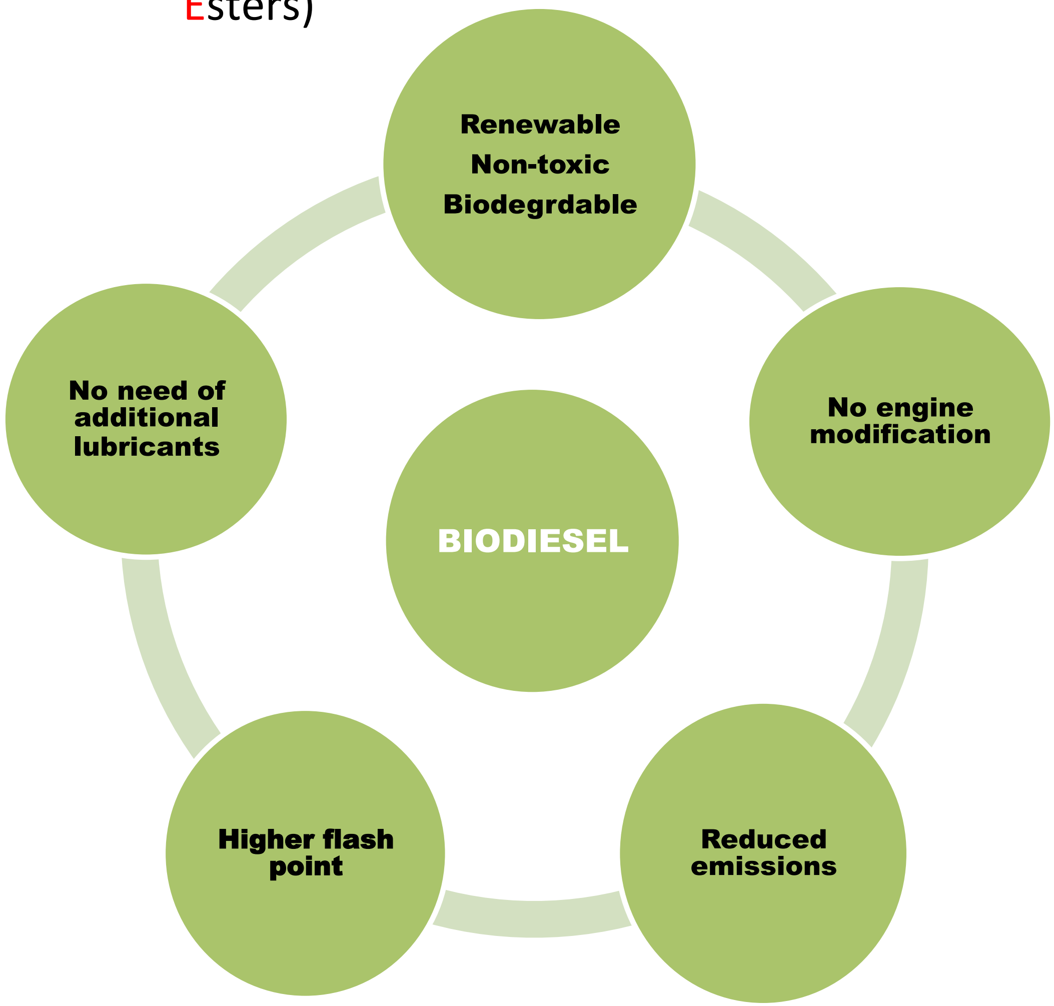


Figure 1 Advantages of biodiesel over petro- diesel

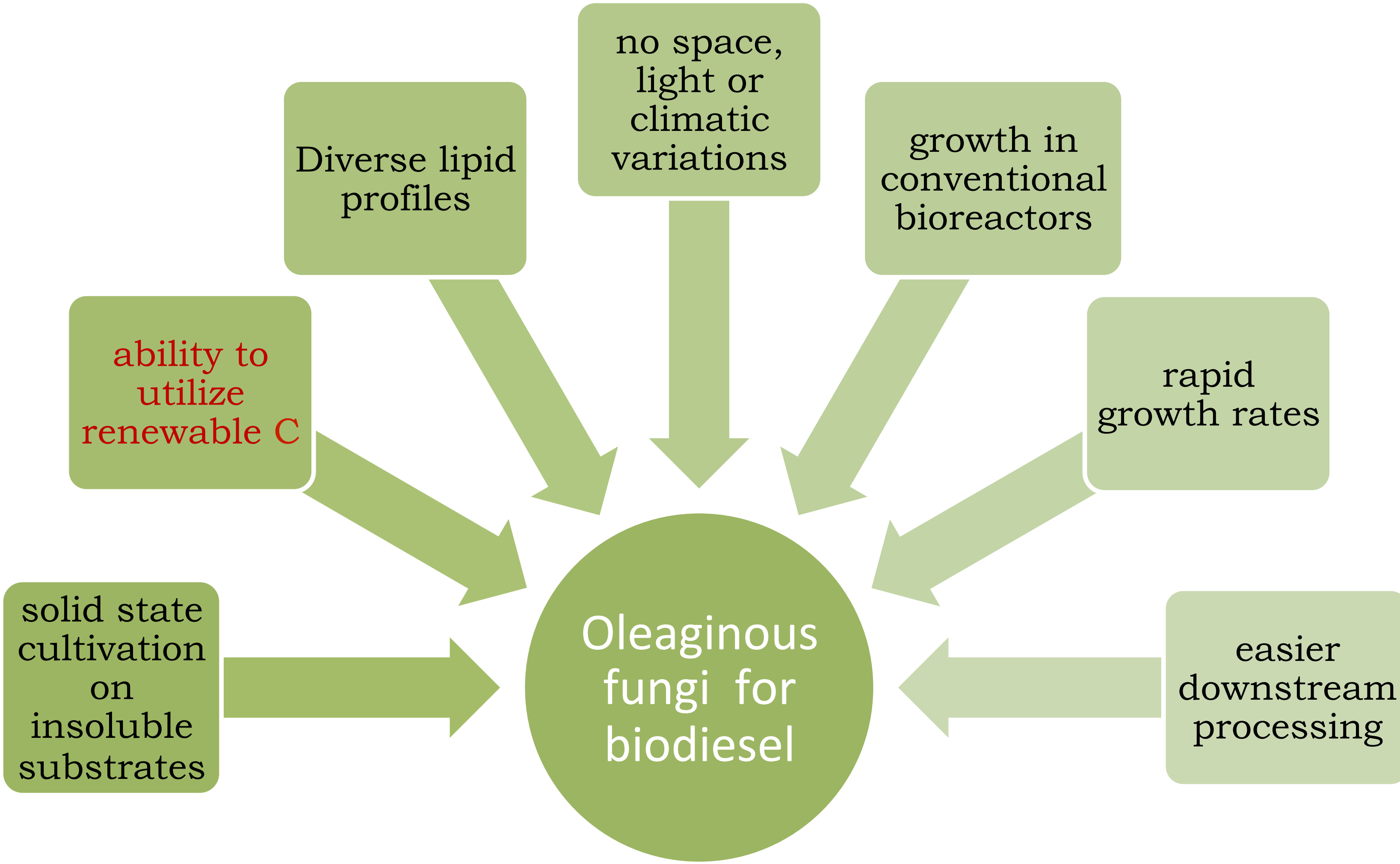
Microbial lipids → Single Cell Oil → Biodiesel

Lipids stored by oleaginous microorganisms, with ≥ 20% lipids (Algae, Fungi, Bacteria)

Emerging Biodiesel Feedstock

- ✓ Short life cycle
- ✓ Less labor
- ✓ Easy scale up
- ✓ No conflict with land
- ✓ Net energy gain

A Bulk Product with low value V/s Lipids as sources of ω-3 or 6 PUFAs


no space, light or climatic variations
Diverse lipid profiles
growth in conventional bioreactors
ability to utilize renewable C
rapid growth rates
solid state cultivation on insoluble substrates
Oleaginous fungi for biodiesel
easier downstream processing

Solid State Fermentation (SSF)

- ✓ Bioprocess in absence or near absence of free water on insoluble solid matrix with moisture to suuport microbial growth
- ✓ the solid matrix is the source of C & nutrients,

Or

an inert growth support with impregnated nutrients

- ✓ the lower energy requirement and cultivation costs
- ✓ higher product yields,
- ✓ less wastewater generation,
- ✓ reduced transport costs,
- ✓ potential environmental benefits by utilizing solid agro-industrial wastes

Consolidated Bioprocessing

Consolidated Bioprocessing

- lignocellulose into desired biofuel in one step without added enzymes or chemicals

Advantages

- It reduces capital and processing costs by simplification of the process scheme and integration of as many unit operations as possible
- Use of a single microbial strain capable of both degrading insoluble components of lignocellulosic biomass and produce desired products at high yield and titer

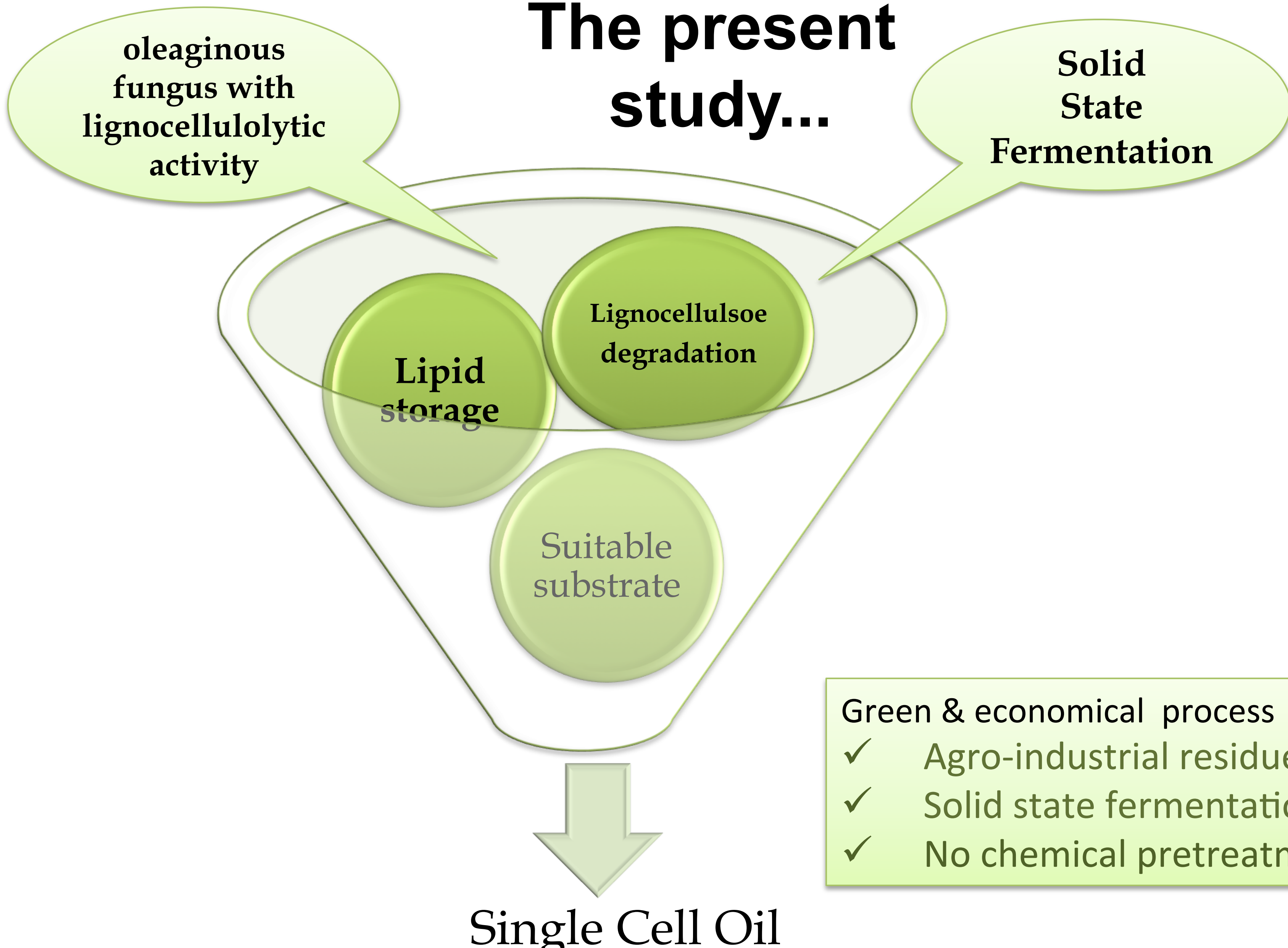
Applications in biodiesel

- oleaginous fungi with their battery of lignocellulolytic enzymes would be used for the direct conversion of an agro-residue without chemical treatment into SCO as biodiesel feedstock

Mangrove Oleaginous fungi

- contribute to the intense carbon processing of this ecosystem,
- are ideal candidates for direct conversion of untreated agro-residue into lipids

The present study...



Green & economical process

- ✓ Agro-industrial residues
- ✓ Solid state fermentation
- ✓ No chemical pretreatment

Objective

Evaluation of consolídated bioprocessing

for

direct conversion of

Sugarcane bagasse

into single cell oil (SCO)

as a biodiesel feedstock

by a mangrove oleaginous fungal strain

***Aspergillus terreus* IBB M1**

Why *Aspergillus terreus* IBB M1?

Khot *et al. Microbial Cell Factories* 2012, 11:71
http://www.microbialcellfactories.com/content/11/1/71

MICROBIAL CELL FACTORIES

RESEARCH Open Access

Single cell oil of oleaginous fungi from the tropical mangrove wetlands as a potential feedstock for biodiesel

Mahesh Khot[1], Srijay Kamat[1,2], Smita Zinjarde[1], Aditi Pant[1], Balu Chopade[1] and Ameeta RaviKumar[1*]

ELSEVIER

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Coupled production of single cell oil as biodiesel feedstock, xylitol and xylanase from sugarcane bagasse in a biorefinery concept using fungi from the tropical mangrove wetlands

Srijay Kamat[a,b], Mahesh Khot[b], Smita Zinjarde[b], Ameeta RaviKumar[b], Wasudeo Namdeo Gade[a,*]

[a] Department of Biotechnology, University of Pune, Pune 411 007, India
[b] Institute of Bioinformatics and Biotechnology, University of Pune, Pune 411 007, India

Experimental

Substrate

- ✓ *bagasse* procured as dried material from local sugar mill
- ✓ Water washes
- ✓ dried to constant weight
- ✓ milling to < 1mm
- ✓ Used as substrate for SSF without any enzymatic/ chemical treatment

culture conditions

- ✓ *Spore inoculum: A.terreus* IBB M1 grown on Malt extract agar (7 days, 28°C)
- ✓ 250 mL wide mouth Erlenmeyer flasks each containing 5 g substrate and 20 mL of moistening agent composed of (in g/L)

0.5 NH_4Cl, 1.5 yeast extract, 15 NaCl, 5 Na_2HPO_4, 7 KH_2PO_4, 1.5 $MgSO_4.7H_2O$, 0.1 $CaCl_2.2H_2O$, and trace metals

- ✓ steam sterilized, cooled and inoculated with 1mL of spore suspension per g of substrate.
- ✓ 30°C , 8 days, static

Methods

- ✓ Fermented Biomass heat dried to constant weight
- ✓ Scanning Electron Microscopy (SEM) analysis
- ✓ Total lipids extracted after cryopulverization of biomass, purified and determined gravimetrically as SCO
- ✓ FAME preparation by alkali catalyzed transesterification
- ✓ Fatty acid analysis by GC-FID
- ✓ Prediction of biodiesel fuel properties based on linear mixing rules and/or empirical, linear regression equations using FAME profile

Prediction of biodiesel fuel properties

Fuel property	Prediction model based on fatty acid profile
Density	
Kinematic viscosity	$\mathbf{v_{mix}} = \sum(\mathbf{A_c v_c})$
Cetane number	

c_i is the concentration (mass fraction) and q_i is the density of component individual FAME) present in FAMEs as detected by GC-FID.

v_{mix} – the kinematic viscosity of sample, Ac – the relative amount (%/100) of individual FAME component; v_c – viscosity of individual FAME component obtained from the database.

CN_i represent reported CN of pure FAME available in database and W_i is the mass fraction of individual FAME component detected and quantified by GC-FID.

Results

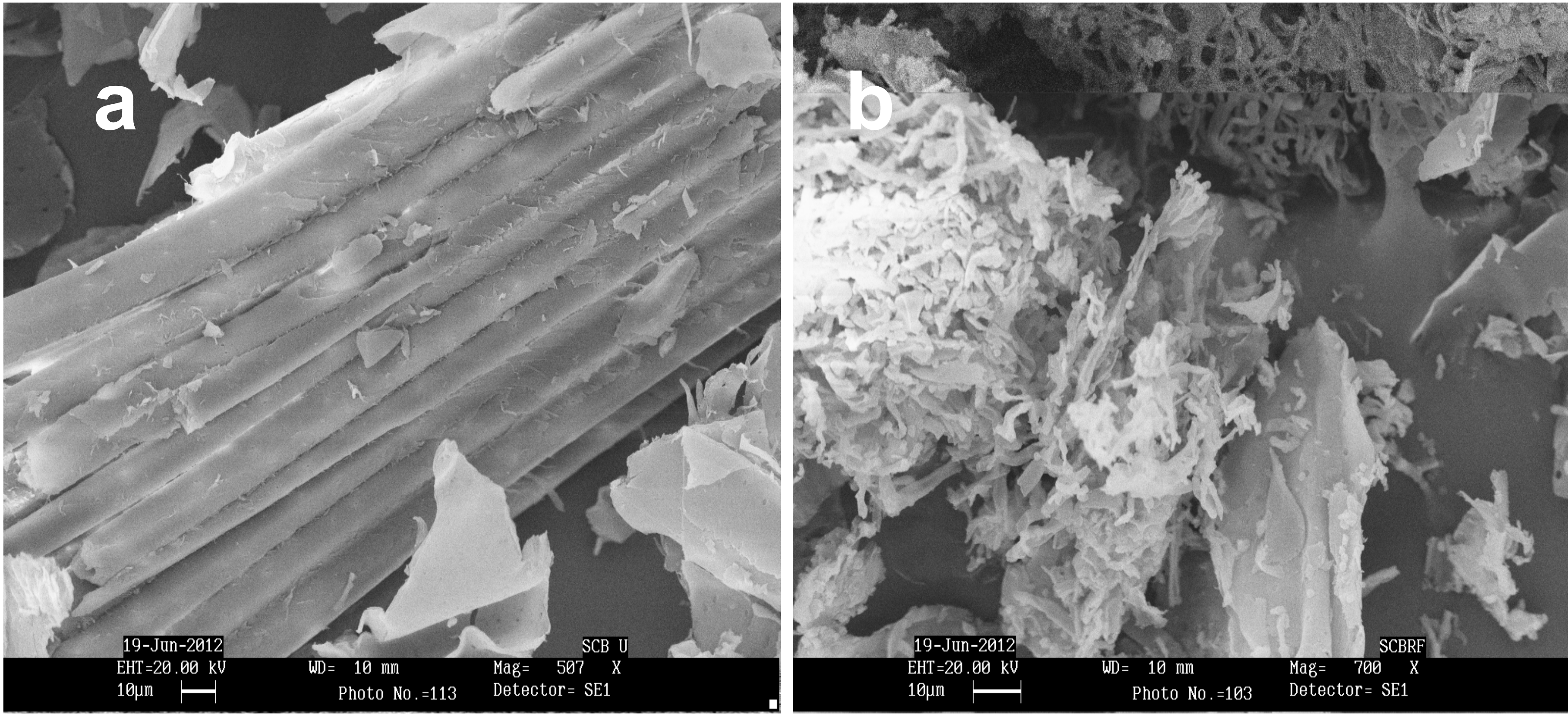


Fig.1 SEM analysis of substrate sugarcane bagasse in native state (a) and after fungal growth (b)

SCO Yield : **27.8 mg/g of substrate**

↓

A. terreus **IBB M1 isolated from mangrove wetlands of the Indian west coast with 54% lipid content and suitable fatty acid profile**

↓

Given the mangrove habitat and extensive water washes of bagasse,

A. terreus **IBB M1 has the capability to grow on insoluble sugars present in bagasse**

↓

one-step
Saccharification - oil accumulation

↓

Consolidated bioprocessing

Fatty acid profile of SCO

Fatty acid methyl ester (FAME)	% wt
Saturated (SFA)	**Total SFA = 71.94**
Caprylic acid (C8: 0)	**2.497**
Capric acid (C10: 0)	**9.754**
Lauric acid (C12: 0)	**15.967**
Myristic acid (C14: 0)	**15.431**
Palmitic acid (C16: 0)	**12.223**
Stearic acid (C18:0)	**8.579**
Arachidic acid (C20:0)	**5.668**
Monounsaturated (MUFA)	**Total MUFA= 11.81**
Oleic acid (C18:1)	**11.515**
Polyunsaturated (PUFA)	**Total PUFA= 16.25**
Linoleic acid (C18:2)	**8.809**
γ- Linolenic acid (C18:3)	**7.45**

Fuel properties of FAMEs

Fatty acid profile of oil ffedstock determines the quality of biodiesel

Good biodiesel= MUFA with regulated amounts of SFAs and PUFAs

High SFA → High cetane number, better oxidative stability

MUFA [oleate, C18:1] → Better ignition quality, nitrogen oxide emissions, fuel stability and flow properties

PUFAs with ≥ 4 double bonds → poor oxidative stability and increases viscosity

Our studies → desirable MUFAs along with the low levels of undesirable PUFAs suggesting the potential suitability of the produced SCO as biodiesel feedstock

Fuel properties of FAMEs

Fuel property/quality parameter	Value	Biodiesel fuel standard specifications		
		ASTM D6751	EN 14214	IS 15607
Density (15°C;g/cm^3)	0.86	ns	0.86 - 0.90	0.86 - 0.90
Kinematic viscosity (40°C; mm^2/s)	3.2	1.9-6.0	3.5 - 5.0	3.5 - 5.0
Cetane number	53	47 min	51 min	51 min
Linolenic acid content (C18:3) (%)	7.45	ns	12 max	ns
FAMEs with ≥4 double bonds (%)	-	ns	1 max	ns

Conclusions

- ✓ Direct conversion of chemically untreated sugarcane bagasse into SCO was demonstrated via SSF using an oleaginous mangrove fungal strain *A. terreus* IBB M1
- ✓ SCO is suitable as biodiesel fuel feedstock based on fatty acid profile & fuel property analysis
- ✓ The solid state cultivation process is cost-effective and environment-friendly
- ✓ It is being studied further by design of experiments methodology

धन्यवाद

Thank You

Questions?