Mahesh Balwant Khot, Ph.D.

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EDUCATION

PhD (Microbiology) Shivaji University, Kolhapur, India	2012-2016
MSc (Microbiology) S.R.T.M. University, Nanded, India	2003-2005
PG Diploma (Patents Law), NALSAR University Hyderabad, India	2008-2009
BSc (Microbiology)	2000-2003

RESEARCH EXPERIENCE

Postdoctoral Researcher **2018- Present**

Laboratory of Renewable Resources, Biotechnology Centre, University of Concepcion, Concepcion, Chile
Role: Principal Investigator
Funded by: CONICYT- FONDECYT, Government of Chile
Project: Combinatorial approach of statistical optimization and mutagenesis for improved production of lipids as biodiesel feedstock from *Scheffersomyces coipomoensis* CB1

SERB National Postdoctoral Fellow **2016- 2018**

CSIR-Indian Institute of Petroleum, Dehradun, India
Role: Principal Investigator
Funded by: Science and Engineering Research Board (SERB) Government of India
Project: Yeast lipid based biorefinery for production of fatty acid methyl esters as biodiesel fuel and other value - added materials using low cost renewable feedstocks

Visiting Doctoral Researcher **2015**

Biotechnology Center, Universoty of Concepcion,Concepcion, Chile

Senior Research Fellow (Ph.D. Scholar) **2007- 14**

Shivaji University, Kolhapur, India; Savitribai Phule Pune University, Pune, India
Dissertation: Single cell oil of *Aspergillus terreus* IBBM1 as a potential feedstock for biodiesel

Research Project Assistant **2005- 07**

CSIR- National Chemical Laboratory, Pune, India

AREAS OF EXPERTISE

- ✓ Lignocellulosic biomass conversion to bio-based products in consolidated bioprocessing
- ✓ High cell density cultivation of bacterial, yeast and fungal strains in lab scale bioreactor systems
- ✓ BioProcess development including scale up from shake flask to 10 L fermenter
- ✓ Medium formulation, Growth optimization
- ✓ Downstream processing for intracellular microbial products

- ✓ Fungal lipid extraction, refining and analysis
- ✓ Microscopy: DIC, Fluorescence, Confocal, SEM,TEM
- ✓ GC-FID
- ✓ Fungal taxonomy

KEY ACHIEVEMENTS

- Authored and co-authored over 8 research articles in international peer-reviewed journals; 2 invited book chapters and 1 patent filed , Paper presentations at national and international scientific events *Annex 1

- Won funding up to 19,20,000/- INR (SERB, Govt. of India) and 81.624.000 CLP (FONDECYT, Chile) inclusive of research grant, salary

- Research work featured in national newspapers and international magazines.
  - ✓ **DNA** http://www.dnaindia.com/pune/report-fungi-may-solve-energy-problem-says-pune-scientist-1936887
  - ✓ **Sakal Times** http://epaper.sakaaltimes.com/SakaalTimes/27Sep2013/Normal/page2.htm http://goo.gl/reJAVU
  - ✓ **Biodiesel magazine –** http://www.biodieselmagazine.com/articles/8536/indian-researchers-study-lipid-productionpotential-of-fungi

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TEACHING EXPERIENCE

Contributory Faculty **2007-14**
Institute of Bioinformatics and Biotechnology, Savitribai Phule Pune University Pune, India

Course taught: 5-Year Integrated M.Sc. Biotechnology

Responsibilities: To conduct theory and practical sessions; Exam setting and evaluation; Dissertation supervision of postgraduate students

Subjects: Fermentation technology and downstream processing, Biochemical and Biophysical techniques, Biomolecules, Microbial Biotechnology

References Available Upon Request

ANNEXURE 1

List of Publications

1) **M Khot**, S Kamat, S Zinjarde, A Pant, B Chopade, A RaviKumar (2012) Single cell oil of oleaginous fungi from the tropical mangrove wetlands as a potential feedstock for biodiesel. Microbial Cell Factories 11 (1), 71. DOI: 10.1186/1475-2859-11-71
2) G Katre, C Joshi, **M Khot**, S Zinjarde, A RaviKumar (2012) Evaluation of single cell oil (SCO) from a tropical marine yeast *Yarrowia lipolytica* NCIM 3589 as a potential feedstock for biodiesel. AMB Express 2 (1), 36.DOI: 10.1186/2191-0855-2-36
3) S Kamat, **M Khot**, S Zinjarde, A RaviKumar, WN Gade (2013) 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. Bioresource Technology 135, 246-253.DOI: 10.1016/j.biortech.2012.11.059
4) H Kakkad, **M Khot**, S Zinjarde, A RaviKumar (2015) Biodiesel production by direct in situ transesterification of an oleaginous tropical mangrove fungus grown on untreated agro-residues and evaluation of its fuel properties. BioEnergy Research 8 (4), 1788-1799.DOI: 10.1007/s12155-015-9626-x
5) H Kakkad, **M Khot**, B Kulkarni, S Zinjarde, A RaviKumar, V. Ravi Kumar (2015) Conversion of dried *Aspergillus candidus* mycelia grown on waste whey to biodiesel by *in situ* acid transesterification. Bioresource Technology 197, 502-507.DOI: 10.1016/j.biortech.2015.07.118
6) **M Khot**, R Gupta, K Barve, S Zinjarde, S Govindwar, R Ameeta (2015) Fungal production of Single Cell Oil using untreated copra cake and evaluation of its fuel properties for biodiesel. Journal of Microbiology and Biotechnology 25 (4), 459-463.DOI: 10.4014/jmb.1407.07074
7) **M Khot**, D Ghosh (2017) Lipids of *Rhodotorula mucilaginosa* IIPL32 with biodiesel potential: Oil yield, fatty acid profile, fuel properties. Journal of Basic Microbiology 57, 345 –352. DOI: 10.1002/jobm.201600618
8) S. Bandhu, **MB Khot**, T. Sharma, OP Sharma, D Dasgupta, S Mohapatra, S Hazra, OP Khatri, D Ghosh D (2018) Single Cell Oil from Oleaginous Yeast Grown on Sugarcane Bagasse-Derived Xylose: An Approach toward Novel Biolubricant for Low Friction and Wear. ACS Sustainable Chemistry & Engineering 6 (1), 275 – 283. DOI: 10.1021/acssuschemeng.7b02425

Patent

Patent title	Applicant	Patent no.	Date of filing	Country	Status
Fungal bioconversion of lignocellulosics to single cell oil, fatty acid methyl esters and hydrolytic enzymes	Institute of Bioinformatics and Biotechnology, Savitribai Phule Pune University **Inventors**: Ameeta RaviKumar, **Mahesh Khot**, Kadambari Barve, Smita Zinjarde	583/MUM/2014	19/02/2014	India	Application Published

Book Chapters

1) N Bansal, M Khot, A Jana, A Nautiyal, T Sharma, D Dasgupta, S Mohapatra, S Yadav, S Hazra, D Ghosh (2018) Oleaginous Yeasts: Lignocellulosic Biomass Derived Single Cell Oil as Biofuel Feedstock, in: A Kuila and V. Sharma (Eds), Principles and Applications of Fermentation Technology. Wiley Scrivener Publishing, pp 263-306. doi:10.1002/9781119460381.ch14

2) M Khot, G Katre, S Zinjarde, A RaviKumar A (2018) Single Cell Oils (SCOs) of Oleaginous Filamentous Fungi as a Renewable Feedstock: A Biodiesel Biorefinery Approach, in: S Kumar, P Dheeran, M Taherzadeh, S Khanal S. (Eds), Fungal Biorefineries. Fungal Biology. Springer, Cham, pp 145-183. doi https://doi.org/10.1007/978-3-319-90379-8_8

- **PAPER PRESENTATIONS/ WORKSHOPS ATTENDED**

1) **Mahesh Khot.** *Solid state fermentation of chemically untreated sugarcane bagasse for fungal production of single cell oil as biodiesel feedstock*. Paper presented at 3rd Iberoamerican Congress, 4th Latin American Congress and 2nd International symposium on Lignocellulosic materials ‘Biorefineries’, Concepcion-Chile, 2015.
2) **Mahesh Khot.** *Single cell oil from lignin rich waste by oleaginous fungus for biodiesel production*. Paper presented at 1st National Conference on Energy and Environment, Pune-India, 2014.
3) **Mahesh Khot,** Kadambari Barve, Rohini Gupta and Ameeta Ravikumar**.** *Biotechnological conversion of solid agro-wastes to single cell oils*. Paper

presented at 1st International Brainstorming Workshop on Waste to Energy in India, Mumbai-India, 2012.

4) Attended the National Workshop on ‘Applications of Transmission Electron Microscopy in Life Sciences’ organized by CSIR-Central Drug Research institute, Lucknow, India on 23-25 January 2017.
5) Attended the ‘Short Course on Microwave Assisted Organic Synthesis’ at IIT-Bombay, Mumbai-India, 2012.

Chapter 8
Single Cell Oils (SCOs) of Oleaginous Filamentous Fungi as a Renewable Feedstock: A Biodiesel Biorefinery Approach

Mahesh Khot, Gouri Katre, Smita Zinjarde, and Ameeta RaviKumar

Abstract Single cell oils (SCOs) from oleaginous fungi are fast occupying centre stage as biodiesel feedstock and offer many advantages over plant- and algal-based oils. The biorefinery concept involves the production of SCOs along with other coproducts by these fungi when grown on waste agro-residue biomass. Filamentous fungi, in general, are able to effectively utilize this waste biomass as they have the capacity to produce lignocellulosic enzymes, namely, cellulase, xylanase, etc. The utilization of these wastes as growth substrates would not only solve the problem of waste disposal but also help in reducing the production cost of biodiesel. This chapter deals with production of SCOs from various filamentous fungi as feedstock for biodiesel when grown on lignocellulosic wastes. Two important parameters to be considered for biodiesel production are feedstock selection and fuel properties of biodiesel which are strain and growth substrate specific. Approaches to improve the process efficiency like optimization of fermentation conditions, one-step transesterification and metabolic engineering as well as the physico-chemical properties of biodiesel are also discussed.

8.1 Introduction

Extensive utilization of coal and petroleum has forced reserves of these non-renewable fuels to near depletion resulting in these fuel sources becoming increasingly limited and expensive to acquire. Therefore, there exists an acute need for alternative fuels along with sustainable methodologies for their production. These methodologies should be cost-effective and environment-friendly and yet produce high yields of quality biodiesel. Furthermore co-synthesis of other valuable

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S. Kumar et al. (eds.), *Fungal Biorefineries*, Fungal Biology,
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ACS Sustainable Chemistry & Engineering

Research Article

pubs.acs.org/journal/ascecg

Single Cell Oil from Oleaginous Yeast Grown on Sugarcane Bagasse-Derived Xylose: An Approach toward Novel Biolubricant for Low Friction and Wear

Sheetal Bandhu,[†,‡,⊥] Mahesh B. Khot,[†,⊥] Tripti Sharma,[†,‡] Om P. Sharma,[§] Diptarka Dasgupta,[†,‡] Swati Mohapatra,[∥] Saugata Hazra,[∥] Om P. Khatri,[*,‡,§] and Debashish Ghosh[*,†,‡]

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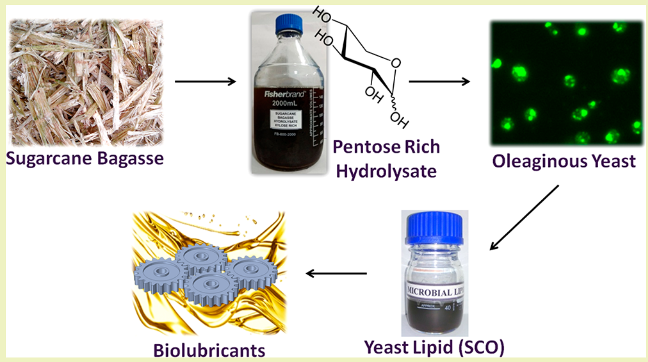
Supporting Information



ABSTRACT: Yeast lipid as single cell oil (SCO) is evaluated as an alternative renewable source of vegetable oils for a biolubricant formulation. The *Rhodotorula mucilaginosa* IIPL32 yeast strain is cultivated on lignocellulosic pentosans derived from sugarcane bagasse to produce the SCO. The chemical composition and distribution of variable fatty acids in the yeast SCO are characterized by NMR, FTIR, and GC × GC analyses. The high viscosity index and a low pour point of yeast SCO owing to the favorable composition of saturated and unsaturated fatty acids promise its potential as a renewable and environmentally friendly lube base oil. The yeast SCO as lube base oil significantly reduced the coefficient of friction (72%) and wear (24%) compared to those of conventional mineral lube base oil (SN 150). The fatty acids in the yeast SCO formed a good quality tribo-chemical thin film on the engineering surfaces, which not only reduced the friction but also protected the contact interfaces against wear. This study demonstrates that yeast SCO being renewable, biodegradable, and nontoxic, provides favorable physicochemical and tribophysical properties for good quality lubricant formulation and it can be a good alternative to the conventional mineral lube oil-based lubricants.

KEYWORDS: *Single cell oil, Yeast, Fatty acids, Biolubricant, Friction*

INTRODUCTION

Climate change, diminishing petroleum reserves, increasing oil demand, and rising CO_2 levels in the environment are widespread challenges and cause adverse impacts on the ecosystem and health hazards to living beings. In this context, bioeconomy-based energy and associated products from renewable sources are gaining immense attention. Single cell oil (SCO) referring to lipids of oleaginous yeast has been studied as a source of unsaturated fatty acids for a diversified range of applications. Recently, triacylglycerol accumulating yeasts have emerged as a promising feedstock for fuels and oleochemicals.[1−4] The suitability of oleaginous yeasts as an SCO source lies in their capability to produce biomass in conventional bioreactors; no competition with food production; rapid growth rates with high biomass and lipid productivity; growth independent of space and facile approach to adopt light or climatic variations; ability to utilize lignocellulosic sugars; and ease of scale-up and amenability to genetic manipulations, etc.[5] As a result, oleaginous yeast shows immense potential for producing SCO as an intermediate "'building block"' of fuels (biodiesel and biojet-fuel), soaps, plastics, paints, detergents, textiles, rubber, surfactants, lubricants, additives for the food and cosmetic industries, and other oleochemicals. SCO can be used in its native form or converted to desired commodities and high-value specialty oleo-chemicals through a variety of chemical, physical, and biochemical methods.[6] SCO has been successfully commercialized for polyunsaturated fatty acid (PUFA) enriched specialty oils for use in the food and supplement industries.[7] The production of yeast-derived SCO for renewable oleo-chemicals is still in its preliminary stage and attracts immense interest for a diversified range of applications as an alternate to edible and nonedible oleo-chemical commodities.

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SHORT COMMUNICATION

Journal of Basic Microbiology

Lipids of *Rhodotorula mucilaginosa* IIPL32 with biodiesel potential: Oil yield, fatty acid profile, fuel properties

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This study analyzes the single cell oil (SCO), fatty acid profile, and biodiesel fuel properties of the yeast *Rhodotorula mucilaginosa* IIPL32 grown on the pentose fraction of acid pre-treated sugarcane bagasse as a carbon source. The yeast biomass from nitrogen limiting culture conditions (15.3 g L^{-1}) was able to give the SCO yield of 0.17 g g^{-1} of xylose consumed. Acid digestion, cryo-pulverization, direct *in situ* transesterification, and microwave assisted techniques were evaluated in comparison to the Soxhlet extraction for the total intracellular yeast lipid recovery. The significant differences were observed among the SCO yield of different methods and the *in situ* transesterification stood out most for effective yeast lipid recovery generating 97.23 mg lipid as FAME per gram dry biomass. The method was fast and consumed lesser solvent with greater FAME yield while accessing most cellular fatty acids present. The yeast lipids showed the major presence of monounsaturated fatty esters (35–55%; 18:1, 16:1) suitable for better ignition quality, oxidative stability, and cold-flow properties of the biodiesel. Analyzed fuel properties (density, kinematic viscosity, cetane number) of the yeast oil were in good agreement with international biodiesel standards. The sugarcane bagasse-derived xylose and the consolidated comparative assessment of lab scale SCO recovery methods highlight the necessity for careful substrate choice and validation of analytical method in yeast oil research. The use of less toxic co-solvents together with solvent recovery and recycling would help improve process economics for sustainable production of biodiesel from the hemicellulosic fraction of cheap renewable sources.

KEYWORDS
in situ transesterification, pentose utilization, *Rhodotorula mucilaginous*, yeast biodiesel, yeast lipid extraction

1 | INTRODUCTION

The oleaginous yeast lipids, collectively termed as single cell oil (SCO) has emerged as an intermediate "building block" for renewable fuels, platform chemicals, food, and feed ingredients [1]. However, the biofuel production (e.g., Biodiesel) from oleaginous yeasts is still in its infancy and would be competitive with other commodity type oils by the use of bio-based feedstock. Literature shows that the agricultural wastes and agro-industrial residues as the substrate for oleaginous yeasts can cut SCO production costs [2,3]. The abundant availability, low-cost, and renewable nature of the lignocellulosic biomass make it an attractive source of sugars for SCO production from oleaginous yeasts.

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Short Communication

Conversion of dried *Aspergillus candidus* mycelia grown on waste whey to biodiesel by *in situ* acid transesterification

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[a] Institute of Bioinformatics and Biotechnology, Savitribai Phule Pune University, Pune 411007, India

[b] Chemical Engineering and Process Development Division, CSIR-National Chemical Laboratory (CSIR-NCL), Pune 411008, India

HIGHLIGHTS

- A tropical oleaginous mangrove isolate *A. candidus* grown on waste whey.
- Optimization of acid catalyzed *in situ* transesterification step.
- Biomass is the predominant factor in conversion to FAME or biodiesel.
- Higher conversion or process efficiency with lower biomass loading.
- Biodiesel properties meet national and international norms.

ARTICLE INFO

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Biodiesel

ABSTRACT

This study reports optimization of the transesterification reaction step on dried biomass of an oleaginous fungus *Aspergillus candidus* grown on agro-dairy waste, whey. Acid catalyzed transesterification was performed and variables affecting esterification, *viz.*, catalyst methanol and chloroform concentrations, temperature, time, and biomass were investigated. Statistical optimization of the transesterification reaction using Plackett–Burman Design showed biomass to be the predominant factor with a 12.5-fold increase in total FAME from 25.6 to 320 mg. Studies indicate that the transesterification efficiency in terms of conversion is favored by employing lower biomass loadings. *A. candidus* exhibited FAME profiles containing desirable saturated (30.2%), monounsaturated (31.5%) and polyunsaturated methyl esters (38.3%). The predicted and experimentally determined biodiesel properties (density, kinematic viscosity, iodine value, cetane number, TAN, water content, total and free glycerol) were in accordance with international (ASTM D6751, EN 14214) and national (IS 15607) standards.

1. Introduction

Renewable fuel alternatives such as biodiesel are produced by transesterification of plant, algal oils or animal fats and consist of mono-alkyl esters of long-chain fatty acids (fatty acid methyl esters, FAMEs). One of the challenges for commercialization of biodiesel production is its high raw material cost especially with respect to vegetable oils. Lipids of oleaginous filamentous fungi (single cell oil, SCO) are emerging as a promising sustainable feedstock for biodiesel production. Filamentous fungi possess several advantages for SCO production, *viz.*, produce suitable FAME profiles composed of desirable fatty acids, ability to use a range of renewable carbon sources and also pelleted growth for easy downstream processing and scale-up (Meeuwse et al., 2013).

The use of renewable carbon sources especially locally available agro-industrial waste is essential to improve the process economics of microbial biodiesel production (Huang et al., 2013). One such substrate is whey, a major waste obtained after the precipitation of milk casein during dairy processes. Currently, the total world production of whey is approximately 85 million tons of which India contributes approximately 8 million tons. Most of the total global production of whey is disposed by land application and direct discharge into water bodies. Such large discharge without treatment causes serious environmental pollution problems and hence the management of whey effluent is an important challenge. In this connection, controlled fermentation processes employing whey based media have been reported to yield valuable bio-products including ethanol, hydrogen, lactic acid and a succinate based animal feed additive while simultaneously reducing

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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*]

Abstract

Background: Single cell oils (SCOs) accumulated by oleaginous fungi have emerged as a potential alternative feedstock for biodiesel production. Though fungi from mangrove ecosystem have been reported for production of several lignocellulolytic enzymes, they remain unexplored for their SCO producing ability. Thus, these oleaginous fungi from the mangrove ecosystem could be suitable candidates for production of SCOs from lignocellulosic biomass. The accumulation of lipids being species specific, strain selection is critical and therefore, it is of importance to evaluate the fungal diversity of mangrove wetlands. The whole cells of these fungi were investigated with respect to oleaginicity, cell mass, lipid content, fatty acid methyl ester profiles and physicochemical properties of transesterified SCOs in order to explore their potential for biodiesel production.

Results: In the present study, 14 yeasts and filamentous fungi were isolated from the detritus based mangrove wetlands along the Indian west coast. Nile red staining revealed that lipid bodies were present in 5 of the 14 fungal isolates. Lipid extraction showed that these fungi were able to accumulate > 20% (w/w) of their dry cell mass (4.14 - 6.44 g L^{-1}) as lipids with neutral lipid as the major fraction. The profile of transesterified SCOs revealed a high content of saturated and monounsaturated fatty acids i.e., palmitic (C16:0), stearic (C18:0) and oleic (C18:1) acids similar to conventional vegetable oils used for biodiesel production. The experimentally determined and predicted biodiesel properties for 3 fungal isolates correlated well with the specified standards. Isolate IBB M1, with the highest SCO yield and containing high amounts of saturated and monounsaturated fatty acid was identified as *Aspergillus terreus* using morphotaxonomic study and 18 S rRNA gene sequencing. Batch flask cultures with varying initial glucose concentration revealed that maximal cell biomass and lipid content were obtained at $30gL^{-1}$. The strain was able to utilize cheap renewable substrates *viz*., sugarcane bagasse, grape stalk, groundnut shells and cheese whey for SCO production.

Conclusion: Our study suggests that SCOs of oleaginous fungi from the mangrove wetlands of the Indian west coast could be used as a potential feedstock for biodiesel production with *Aspergillus terreus* IBB M1 as a promising candidate.

Keywords: Mangrove wetlands, Oleaginous fungi, Single cell oil, Fatty acid methyl ester, *Aspergillus terreus*

Background

Biodiesel as an alternative fuel has been in the forefront of the liquid biofuel sector for the last two decades. The use of edible vegetable oils such as soybean, rapeseed and non-edible oils such as *Jatropha* in the United States, Europe and India, respectively, as oil feedstock for biodiesel production needs to be augmented with newer technologies. To meet the demand of the biodiesel industry, alternative oil sources are being explored and developed. Recently, microbial lipids (single cell oils, SCOs) accumulated by oleaginous microorganisms e.g., microalgae and fungi, with 20% or more of their cell mass being composed of lipids, have emerged as a potential feedstock for biodiesel production [1,2].

The applications of oleaginous fungi for biodiesel are very few although they have several advantages over conventional plant and algal resources as they can be

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