Contents

			page
Abstract			1
Acknowledg	ments		iii v
Contents			
List of Figure			viii
			ix
List of Symb List of Abbre			x xi
LISCOLADDIE		5	XI
Chapter 1: I	ntrodu	ction	
1.1		se and Objectives of the Study	1
1.2	-	Results, Scope and Future Prospects of the Work	2
-		of Literature	_
2.1	Introd		5
2.2		sel: Definition, Global status, and classification	6
	2.2.1	First generation biodiesel	7
	2.2.2	Second generation biodiesel	8
	2.2.3	Third generation biodiesel	8
2.2	2.2.4 Ologoi	Fourth generation biodiesel inous microorganisms	8
2.3	•	Bacteria	9
	2.3.1 2.3.2	Filamentous fungi	9 10
	2.3.3	Microalgae	10
	2.3.4	Yeast	12
2.4		inous yeasts as oil producers	13
2.7	2.4.1	Heterotrophic nature and diverse physiological pathways	14
	2.4.2	Growth rate and cultivation	14
	2.4.3	Genetic manipulation	14
	2.4.4	Use of by-products	14
2.5		rehensive production of biodiesel using oleaginous yeasts	15
-	2.5.1	Characteristics of desirable yeast	15
	2.5.2	Extracellular enzymes produced by the oleaginous yeast	15
	2.5.3	Isolation and identification of oleaginous yeasts	17
	2.5.4	Lipogenesis in oleaginous yeast	18
	2.5.5	Factors affecting the growth of oleaginous yeasts	19
	2.5.6	Estimation and recovery of lipid	22
	2.5.7	Transesterification	23
	2.5.8	Biodiesel potential of the lipid extracts using analytical techniques	23
	2.5.9	Techno-economic analysis	24
2.6		tion of Industrial wastewater for biodiesel	25
	2.6.1	Fermentation (distillery) industry wastewater	28
	2.6.2	Biodiesel industry wastewater	28
	2.6.3	Food industry wastewater	29
	2.6.4	Municipal and sludge wastewater	29
	2.6.5	Paper & pulp industry wastewater	29
2.7	Object	lives	31
Chapter as I	colatia	n identification and characterization of Cyctobacidium aligenbagu	m
•		n, identification, and characterization of Cystobasidium oligophagu nd lipase producing oleaginous yeast	

3.1	Isolation and screening of oleaginous yeast strain		
3.2	Results and discussion		35
	3.2.1	Isolation and screening of the isolate	35
	3.2.2	Screening of oleaginous yeasts for cellulase and lipase activity	35
	3.2.3	Identification and characterization of the isolate	35

	3.2.4	Nile red fluorescence microscopy	37
	3.2.5	Batch cultivation of C. oligophagum JRC1 for total cellulase production	37
	3.2.6	Batch cultivation of C. oligophagum JRC1 for lipase production	38
	3.2.7	Batch cultivation of C. oligophagum JRC1 for lipid production and extraction	39
	3.2.8	Effect of carbon source on lipid production	42
	3.2.9	FT-IR and TLC analysis of the lipid extract	45
	3.2.10	Determination of FAME composition by GC-MS analysis	46
3.3	Conclu	sions	47

Chapter 4: Assessing oil accumulation in the oleaginous yeast Cystobasidium oligophagum JRC1 using dairy waste cheese whey as a substrate

0.1				
4.1	Cultiv	Cultivation of C. oligophagum JRC1 on the dairy waste cheese whey		
4.2	Result	Results and Discussion		
	4.2.1	Cheese whey waste characterization	51	
	4.2.2	Lipid production in deproteinized cheese whey in a shake flask experiments	51	
	4.2.3	Lipid production in untreated cheese whey in a shake flask experiments	53	
	4.2.4	Soluble COD removal and lipid generation	55	
	4.2.5	FT-IR, TLC, and ¹ H NMR analysis	57	
	4.2.6	Lipid profile and GC analysis of the samples	60	
	4.2.7	Biodiesel potential of FAME	62	
4.3	Concl	usions	63	

Chapter 5: Cystobasidium oligophagum JRC1 tolerance assessment on inhibitors released on Lignocellulosic biomass hydrolysis and growth on acid hydrolyzed agroindustrial wastes

5.1	Effect o	lignocellulosic inhibitors and acid hydrolysates	66
5.2	Results	and Discussion	67
	5.2.1	Effect of lignocellulosic inhibitors on the growth and lipid accumulation by C. Oligophagum JRC1	67
		5.2.1.1 Effect of 5-Hydroxymethyl Furfural (5-HMF)	67
	L	5.2.1.2 Effect of Furfural	69
	L	.2.1.3 Effect of Acetic acid	70
	L	5.2.1.4 Effect of mixture of inhibitors	71
	5.2.2	Cellular growth and lipid accumulation on lignocellulosic biomass	71
	5.2.3	Qualitative analysis of the lipid extracts	74
	L.	5.2.3.1 Thin layer chromatography	74
	L.	5.2.3.2 ¹ H NMR analysis	74
5.3	Conclus	ons	76

Chapter 6: Conclusions

Annexure A: Materials and methods

A.1	Materials	79
A.2	Methods	80
	A 2.1 Strain identification using 18s rRNA method	80
	A 2.2 Construction of phylogenetic tree using MEGA 6.0	80
	A 2.3 Scanning electron microscopy	80
	A 2.4 Nile-red fluorescence microscopy	80
	A 2.5 Cellulase assay	80
	A 2.6 Lipase assay	80
	A 2.7 Optimization for lipid accumulation	81
A.3	Analytical methods	81
	A 3.1 Estimation of total reducing sugar	81
	A 3.2 Estimation of total protein	81
	A 3.3 Estimation of total ammonical nitrogen	81
	A 3.4 Estimation of total Kjeldahl nitrogen (TKN)	81
	A 3.5 Estimation of Chemical oxygen demand	81

A 3.6 Determination of cell dry weight	82
A 3.7 Lipid extraction and gravimetric analysis	82
A 3.8 Transesterification of lipids	82
A 3.9 Thin layer chromatography	82
A 3.10 Fourier transform infrared (FT-IR) spectroscopy analysis	82
A 3.11 Nuclear magnetic resonance (NMR) analysis	82
A 3.12 Gas chromatography	83
A 3.13 Calculation of various lipid production parameters	83
A 3.14 Properties of Biodiesel	83
A 3.15 Statistical analysis	84
	85

References