

4.5.1.1	Effect of biomass concentration on total sugar yield	110
4.5.1.2	Fermentation of reducing sugars to ethanol	111
4.5.1.3	SHF under non-agitated condition	113
4.5.1.4	SHF under agitated condition	115
4.5.2	Simultaneous saccharification and fermentation (SSF) of microalgal carbohydrate	117
4.5.2.1	SSF of microalgal carbohydrate under non-agitated condition	118
4.5.2.2	SSF of microalgal carbohydrate under agitated condition	120
4.5.3	Mathematical modeling of SHF under non-agitated and agitated conditions	122
4.5.4	Mathematical modeling of SSF of algal carbohydrate under non- agitated and agitated conditions	129
CHAPTER 5		
Summary and Conclusions		139-143
References		145-160
Appendix A-B		161-162
List of Publications		163

LIST OF FIGURES

S. No.	Figure	Page No.
1.1	Schematic representation of ethanol fermentation pathway	2
1.2	Starch synthesis in chloroplasts	6
1.3	Various methods of pre-treatment of micro-algal biomass	7
1.4	Production process of bioethanol	9
2.1	Blockage of lipid synthesis pathway by cerulenin in microalgae and accumulation of starch	23
2.2	Separate hydrolysis and fermentation of algae carbohydrate	34
2.3	Simultaneous saccharification and fermentation of algae carbohydrate	35
3.1	The arrangement of 18s, 5.8s and 28s rRNA genes	42
3.2	Reactions involved in starch determination	47
3.3	A setup of soxhlet apparatus (A), separating funnel containing chloroform with crude lipid mixture and methanol (B)	48
3.4	The bubble column photobioreactor used for microalgae growth	51
3.5	Set-up of stirred tank photobioreactor	52
3.6	Schematic diagram of internal loop photobioreactor (A) and external loop photobioreactor (B).	53
4.1	Pure colonies of isolated microalgae	72
4.2	Microscopic view of isolated microalgae	72
4.3	Agarose gel electrophoresis of PCR amplified 5.8s rRNA gene	73

4.4	Maximum likelihood phylogenetic tree of 18s r RNA sequence of isolated microalgae	74
4.5	Growth of <i>C. Sorokiniana</i> (A) and <i>T. obliquus</i> (B) with 0.5% (v/v) inoculum	75
4.6	Growth of <i>C. Sorokiniana</i> (A) and <i>T.obliquus</i> (B) with 2.0% (v/v) inoculum	76
4.7	Growth of <i>C. Sorokiniana</i> (A) and <i>T. obliquus</i> (B) with 5.0% (v/v) inoculum	76
4.8	Growth of <i>C. Sorokiniana</i> (A) and <i>T. obliquus</i> (B) at different pH	77
4.9	Specific growth rates of <i>C. sorokiniana</i> and <i>T. obliquus</i> at different temperatures	78
4.10	Growth of <i>C. Sorokiniana</i> (A) and <i>T. obliquus</i> (B) at different agitation conditions(rpm)	79
4.11	Microalgae grown in bubble column bioreactor	80
4.12	Growth of <i>C. Sorokiniana</i> (A) and <i>T. obliquus</i> (B) in bubble column bioreactor	81
4.13	Growth of microalgae at t=0 and t=12 days	82
4.14	Growth of <i>C. Sorokiniana</i> (A) and <i>T. obliquus</i> (B) in stirred tank bioreactor	82
4.15	Microalgae growth in the internal loop and external loop air-lift photobioreactor	83
4.16	Growth of <i>C. Sorokiniana</i> (A) and <i>T. obliquus</i> (B) in the internal loop air-lift photobioreactor with air and 2.0% CO ₂	84

4.17	Growth of <i>C. Sorokiniana</i> (A) and <i>T. obliquus</i> (B) in the external loop air-lift photobioreactor with air and 2.0% CO ₂	85
4.18	Specific growth rates of <i>C. sorokiniana</i> in different photobioreactors	86
4.19	Specific growth rates of <i>T. obliquus</i> in different photobioreactors	86
4.20	Comparision of biomass, carbohydrate and starch productivities of <i>C. sorokiniana</i> in bubble column, stirred tank, external loop airlift and internal loop airlift photobioreactors.	88
4.21	Comparision of biomass, carbohydrate and starch productivities of <i>T. obliquus</i> in bubble column, stirred tank, external loop airlift and internal loop airlift photobioreactors.	90
4.22	The carbohydrate, starch and lipid content of <i>C. sorokiniana</i> after cycloheximide treatment.	92
4.23	The carbohydrate, starch and lipid content of <i>T.obliquus</i> after cycloheximide treatment.	92
4.24	The carbohydrate, starch and lipid content of <i>C. sorokiniana</i> after cerulenin treatment.	93
4.25	The carbohydrate, starch and lipid content of <i>T. obliquus</i> after cerulenin treatment.	94
4.26	The carbohydrate content of <i>C. sorokiniana</i> after Nitrogen, Phosphorus and Suphur limitation	96
4.27	The carbohydrate content of <i>T. obliquus</i> after Nitrogen, Phosphorus and Suphur limitation	97

4.28	The starch content of <i>C. sorokiniana</i> after Nitrogen, Phosphorus and Sulphur limitation	97
4.29	The starch content of <i>T. obliquus</i> after Nitrogen, Phosphorus and Sulphur limitation	98
4.30	Microscopic view of microalgae before and after the pre-treatments	101
4.31	The sugar released after acidic pre-treatment of <i>C. sorokiniana</i> and <i>T. obliquus</i> biomass	103
4.32	The sugar released after NaOH pre-treatment of <i>C. sorokiniana</i> and <i>T. obliquus</i> biomass	104
4.33	The sugar released after hydroxyl radical-aided thermal pre-treatment of <i>C. sorokiniana</i> and <i>T. obliquus</i> biomass	105
4.34	The sugar released after ultrasonication pre-treatment of <i>C. sorokiniana</i> and <i>T. obliquus</i> biomass	106
4.35	The sugar released after enzymatic pre-treatment and ultrasonication followed by enzymatic pre-treatment of <i>C. sorokiniana</i> and <i>T. obliquus</i> biomass	108
4.36	The sugar released after pre-treatment of <i>C. sorokiniana</i> and <i>T. obliquus</i> biomass by different pre-treatment methods	108
4.37	The SEM images of <i>C. sorokiniana</i> (A-Before pre-treatment and B-After pre-treatment) and <i>T. obliquus</i> (C-Before pre-treatment and D-After pre-treatment)	109
4.38	The effect of biomass concentrations on total reducing sugars by acidic pre-treatment	112

4.39	The effect of biomass concentrations on total reducing sugars by ultrasonication followed by enzymatic pre-treatment	113
4.40	The sugar depletion and ethanol production profiles of SHF in <i>C. sorokiniana</i> biomass after acidic and enzymatic hydrolysis under non-agitated condition	114
4.41	The sugar depletion and ethanol production profiles of SHF in <i>T. obliquus</i> biomass after acidic and enzymatic hydrolysis under non-agitated condition	115
4.42	The sugar depletion and ethanol production profiles of SHF using <i>C. sorokiniana</i> biomass after acidic and enzymatic hydrolysis under agitated condition	116
4.43	The sugar depletion and ethanol production profiles of SHF in <i>T. obliquus</i> biomass after acidic and enzymatic hydrolysis under agitated condition	117
4.44	Concentration profiles of carbohydrate (S), glucose (G), and Bioethanol (E) of SSF using <i>C. sorokiniana</i> biomass under non-agitated condition.	119
4.45	Concentration profiles of carbohydrate (S), glucose (G), and bioethanol (E) of SSF using <i>T. obliquus</i> biomass under non-agitated condition.	119
4.46	Experimental concentration profiles of carbohydrate (S), glucose(G), and bioethanol (E) vs. time(t) under agitated conditions for <i>C. sorokiniana</i>	121

4.47	Experimental concentration profiles of carbohydrate (S), glucose(G), and bioethanol (E) vs. time(t) under agitated conditions for <i>T. obliquus</i>	121
4.48	The concentration profiles of reducing sugar (G), yeast cell mass (X) and ethanol (E) during SHF under non-agitated conditions	123
4.49	The experimental and predicted profiles of reducing sugar consumption during SHF under non-agitated condition. Line (---) shows the model data while cross(x) shows the experimental data	125
4.50	The experimental and predicted profiles of yeast cell mass during SHF under non-agitated condition. Line (---) shows the model data while cross(x) shows the experimental data	126
4.51	The experimental and predicted profiles of ethanol production during SHF under non-agitated condition. Line (---) shows the model data while cross(x) shows the experimental data	126
4.52	The concentration profiles of reducing sugar (G), yeast cell mass (X) and ethanol (E) during SHF under agitated condition	127
4.53	The experimental and predicted profiles of reducing sugar consumption during SHF under shaking condition. Line (---) shows the model data while cross (x) shows the experimental data	128
4.54	The experimental and predicted profiles of yeast cell mass during SHF under shaking condition. Line (---) shows the model data while cross (x) shows the experimental data	128
4.55	The experimental and predicted profiles of ethanol production during	129

	SHF under shaking condition. Line (---) shows the model data while cross(x) shows the experimental data	
4.56	The concentration profiles of reducing sugar (G), carbohydrate (S), yeast cell mass (X) and ethanol (E) during SSF	130
4.57	Profile of predicted (lines) and experimental (x) carbohydrate depletion during the simultaneous saccharification and fermentation (SSF) process under non-agitated conditions	132
4.58	Profile of predicted (lines) and experimental (x) glucose (G) during the simultaneous saccharification and fermentation (SSF) process under non-agitated conditions	133
4.59	Profile of predicted (lines) and experimental (symbols) cell mass concentration during the simultaneous saccharification and fermentation (SSF) process under non-agitated conditions	134
4.60	Profile of predicted (lines) and experimental (symbols) bioethanol during the simultaneous saccharification and fermentation (SSF) process under non-agitated conditions	134
4.61	The concentration profiles of reducing sugar (G), starch (S), yeast cell mass (X) and ethanol (E) during SSF under agitated conditions	136
4.62	Profile of predicted (lines) and experimental (x) starch depletion during the simultaneous saccharification and fermentation (SSF) process under agitated conditions	136

4. 63 Profile of predicted (lines) and experimental (x) glucose (G) during the simultaneous saccharification and fermentation (SSF) process under agitated conditions 137
- 4.64 Profile of predicted (lines) and experimental (x) cell mass (X) concentration during the simultaneous saccharification and fermentation (SSF) process under agitated conditions 137
- 4.65 Profile of predicted (lines) and experimental (x) bioethanol (E) during the simultaneous saccharification and fermentation (SSF) process under agitated conditions 138