SOLID STATE FERMENTATION OF PALM KERNEL CAKE BY <u>BACILLUS SUBTILIS</u>



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SOLID STATE FERMENTATION OF PALM KERNEL CAKE BY <u>BACILLUS SUBTILIS</u>

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DECLARATION

The materials in this thesis are in original except for equations, excerpts, summaries and references, which have been duly acknowledged.

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ABSTRACT

SOLID STATE FERMENTATION OF PALM KERNEL CAKE BY <u>BACILLUS SUBTILIS</u>

It is known that the Palm Kernel Cake (PKC) is one of the potential ingredients of the poultry feed. Inclusion of PKC in poultry feed is very limited due to its high fiber content, low palatability, poor amino acid balance and low digestibility. It is expected that the microbial enzymes would be able to breakdown β -mannan (which is the most abundant fiber in PKC) into simple sugars during solid state fermentation (SSF). This may eliminate the anti-nutritional factors and increase the digestible sugars in PKC which can then be fully absorbed and metabolized by the chickens. In this research, effects of several important parameters during SSF of PKC on the growth of Bacillus subtilis ATCC 3366 were studied. These included the moisture content of PKC, temperature, and pH. In order to understand more about the substrate used for best operation, effect of particle size of PKC on the microbial growth was investigated. The literature review shows that there are no kinetic models to characterize the microbial conversion of PKC, which is essential information for bioreactor design. Hence, growth kinetics of B. subtilis during SSF of PKC was investigated. In current study, SSF operations were carried out in laboratory scale. Parameters monitored on the treated PKC include biomass, reducing sugar content, β-mannananse activity, acid detergent fiber (ADF) content, neutral detergent fiber (NDF) content, and hemicellulose content. From the present studies, it may be concluded that B. subtilis is found to be a potential strain in PKC enrichment. Optimum fermentation conditions observed were 150% moisture content, incubation temperature of 40°C, initial pH of 7, PKC particle size of 850 µm, incubation period of 36 hrs, while inoculum size was 20%. Soluble sugars in treated PKC were increased 5.4 folds; whereas Hemicellulose content was decreased 2.6 folds compared to the untreated PKC. Logistic growth kinetic model described the experimental data of biomass profile satisfactorily. The kinetics parameters μ and X_m were estimated from the model, which was 0.50 h^{-1} and 1.82 × 10¹⁰ CFU/gds respectively, at the optimum fermentation conditions. A kinetic equation that is capable of describing the effects of moisture content variations on growth was also developed.

Keywords: β-Mannanase, *Bacillus subtilis*, growth kinetics, palm kernel cake (PKC), solid state fermentation (SSF)

ABSTRAK

Palm Kernel Cake (PKC) merupakan salah satu daripada bahan yang berpotensi untuk digunakan sebagai ayam ternakan. Pengunaan PKC dalam makanan ayam ternakan adalah terhad kerana ia mempunyai kandungan fiber yang tinggi, tidak lazat, kandungan asid amino yang kurang seimbangan, dan sukar untuk dicernakan. Dijangka bahawa enzim mikrob dapat memecahkan β-mannan (terdapat dalam kuantiti yang banyak sekali dalam PKC) menjadi gula ringkas ketika penapaian keadaan pepeial (SSF). Ini mungkin dapat mengurangkan factor anti-khasiat, dan seterusnya meningkatkan kandungan gula ringkas dalam PKC yang dapat digunakan terus untuk metobolisme ayam ternakan. Dalam kajian ini, kesan beberapa parameter yang penting ketika SSF menggunakan PKC dan Bacillus subtilis ATCC 3366 telah disiasat. Ini termasuklah kelembapan substrat, suhu, dan pH. Kesan saiz partikel PKC juga telah dikaji supaya dapat memahami berkenaan substrat yang paling sesuai untuk operasi SSF. Hasil dari kajian literatur menunjukkan bahawa tiada model yang didapati untuk menggambarkan penukaran PKC ketika SSF, yang merupakan informasi yang diperlukan untuk rekabentuk bioreaktor. Oleh sebab itu, kinetik penumbuhan B. subtilis semasa SSF menggunakan PKC telah dikaji. Operasi SSF dalam kajian ini dijalankan dalam skala makmal. Parameter yang dikaji untuk PKC selepas penapaian termasuklah biojisim, kandungan gula ringkas, aktiviti ßmannananse, kandungan acid detergent fiber (ADF), neutral detergent fiber (NDF), dan hemiselulosa. Berdasarkan kajian ini, boleh disimpulkan bahawa B. subtilis adalah strain bakteria yang berpotensi untuk memperkayakan khasiat PKC. Keadaan penapaian optimum termasuklah 150% kelembapan; suhu pada 40°C, pH asal pada 7, partikel saiz pada 850 µm, tempoh penapaian pada 36 jam, manakala saiz inoculum pada 20%. Gula terlarut dalam PKC selepas penapaian telah meningkat sebanyak 5.4 kali, <mark>manaka</mark>la kandungan hemiselulosa telah berkurang sebanyak 2.6 kali berbanding dengan PKC sebelum penapian. Model kinetik punumbuhan logistik telah menggamb<mark>arkan data</mark> eksperimen untuk profil biojisim dengan memuaskan. Parameter-parameter kinetik μ and X_m telah ditaksirkan dari model tersebut, dengan nilai 0.50 jam⁻¹ dan 1.82 × 10¹⁰ CFU/gds masing-masing, pada keadaan penapaian optimum. Persamaan kinetik yang berupaya menggambarkan kesan variasi kelembapan terhadap penumbuhan sel juga telah didapatkan.

ABBREVIATIONS

μ	Specific Growth Rate Constant (hr-1)
g	gram
mg	milligram
gds	gram dry substrate
L	liter
mL	milliliter
t	time
h	hours
min	minutes
S	seconds
μm	micron
nm	nanometer
M	molar (mol / L)
mM	mili-molar (mol / L)
U	unit enzyme
°C	degree Celcius
%	percent
Х	Cell concentration (CFU / gds or CFU / mL)
X _o	Initial cell concentration (CFU / gds or CFU / mL)
X _m	Maximum cell concentration (CFU / gds or CFU / mL)
ADF	Acid Detergent Fiber
NDF	Neutral Detergent Fiber
CFU	Colony Forming Unit
DF	Dilution Factor
MC	Moisture Content
PKC	Palm Kernel Cake

РКМ	Palm Kernel Meal
SmF	Submerged Fermentation
SSF	Solid State Fermentation



	Page
	number
TITLE	ii
DECLARATION	iii
ACKNOWLEDGMENT	iv
ABSTRACT	v
ABSTRAK	vi
ABBREVIATIONS	vii
THESIS CONTENT	ix
LIST OF FIGURE	xiv
LIST OF TABLE	xx
CHAPTER 1: INTRODUCTION	1
1.1 CONVERSTION OF PALM KERNEL CAKE INTO POULTRY	
	1
1.2 PROBLEM STATEMENT	2
1.3 HYPOTHESIS	3
1.4 RESEARCH OBJECTIVES	3
CHAPTER 2: PALM KERNEL CAKE AND SOLID STATE	5
FERMENTATION	
2.1 PALM KERNEL CAKE	5
2.1.1 Palm Kernel Cake and Palm Kernel Meal	6
2.1.2 Market of Palm Kernel Cake	8
2.2 APPLICATIONS OF PALM KERNEL CAKE	10
2.2.1 Raw Palm Kernel Cake as Feedstuff	10

ix

	2.2.2	Fermented / Treated Palm Kernel Cake as Feedstuff	11
	2.2.3	Palm Kernel Cake as Substrate in Enzyme Production	13
2.3	PALM KERNEL CAKE AS A FEEDSTUFF FOR POULTRY		
2.4	SOLI	D STATE FERMENTATION	15
	2.4.1	Enrichment of Palm Kernel Cake through Solid State	16
		Fermentation	
	2.4.2	Engineering Aspects in Solid State Fermentation of Palm	16
		Kernel Cake	
2.5	STRA	IN SELECTION	19
	2.5.1	Introduction of Bacillus subtilis	19
	2.5.2	Application of Bacillus subtilis in Solid State Fermentation	20
	2.5.3	β-Mannanase Production by Bacillus subtilis	20
2.6	KINET	TIC MODELS USED IN SOLID STATE FERMENTATION	20
CHAP	TER 3:	MATERIALS AND METHODS	23
3.1	INTRO	DUCTION	23
3.2	MICRO	OORGANISM AND CULTURE MAINTENANCE	24
	3.2.1	Inoculum Preparation	25
3.3	SUBM	IERGED FERMENTATION OF BACILLUS SUBTILIS	25
	3.3.1	Submerged Fermentation with Locust Bean Gum (LBG)	26
	3.3.2	Submerged Fermentation with Palm Kernel Cake (PKC)	27
	3.3.3	Optimum Enzyme Activity Conditions	27
3.4	SOLIE	STATE FERMENTATION OF BACILLUS SUBTILIS	27
	3.4.1	Solid State Fermentation with Palm Kernel Cake	30
	3.4.2	Kinetic Analysis of Bacillus subtilis in Solid State	32
		Fermentation of Palm Kernel Cake	
		3.4.2.a Logistic Model	32

Х

		3.4.2.b Exponential Model	35
3.5	ANAL	YSIS	38
	3.5.1	Measurement of Biomass	38
	3.5.2	Measurement of Reducing Sugars	40
	3.5.3	β-Mannanase Assay	46
	3.5.4	Hemicellulose Content	51
	3.5.5	Soluble Protein Analysis	53
CHAF	PTER 4	RESULTS AND DISCUSSION	56
4.1	SUBMERGED FERMENTATION OF BACILLUS SUBTILIS		56
	4.1.1	Growth Profile	56
	4.1.2	β-Mannanase Activity	58
	4.1.3	Optimum β-Mannanase Activity Conditions	58
		4.1.3.a Enzyme Activities At Different Temperature	58
		4.1.3.b Enzyme Activities At Different pH	59
4.2	SOLIE	STATE FERMENTATION OF PALM KERNEL CAKE BY	60
	4.2.1	Consumption and Production of Reducing Sugar	60
		4.2.1.a Effect of Moisture Content	62
		4.2.1.b Effect of Temperature	63
		4.2.1.c Effect of pH	63
		4.2.1.d Effect Particle Size of PKC	65
	4.2.2	Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF)	66
		and Hemicellulose Content	
		4.2.2.a Effect of Moisture Content	66
		4.2.2.b Effect of Temperature	67
		4.2.2.c Effect of pH	68

	4.2.2.d	Effect of PKC Particle Size	69
4.2.3	β-Mannar	nase Activity	69
	4.2.3.a	Effect of Moisture Content	69
	4.2.3.b	Effect of Temperature	70
	4.2.3.c	Effect of pH	71
	4.2.3.d	Effect of PKC Particle Size	72
4.2.4	Biomass		73
	4.2.4.a	Effect of Moisture Content	73
	4.2.4.b	Effect of Temperature	75
	4.2.4.c	Effect of pH	77
	4.2.4.d	Effect of PKC Particle Size	78
4.2.5	Soluble P	rotein Content	80
4.2.6	Discussio	n	81
	4.2.6.a	Initial Moisture Content of PKC	81
	4.2.6.b	Incubation Temperature	83
	4.2.6.c	Initial pH of PKC	83
	4.2.6.d	Particle size of PKC	85
	4.2.6.e	Incubation Period	85
	4.2.6.f	Enzyme Activity of <i>B. subtilis</i> in SSF	92
	4.2.6.g	pH Value Profiles	93
GROV		TICS DURING SOLID STATE FERMENTATION	95
OF PALM KERNEL CAKE BY BACILLUS SUBTILIS			
4.3.1	Growth Ki	netics of Bacillus subtilis	95
	4.3.1.a	Logistic Model	95
	4.3.1.b	Exponential Model	102
	4.3.1.c	Comparison of µ	114
4.3.2	Inclusion	of Moisture Content Effect into Growth Model	115

4.3

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS FOR 119 FUTURE WORK

REFERENCES	122

APPENDIX

APPENDIX A: ADF, NDF, and Hemicellulose Content of PKC 129 During Various SSF Operations

APPENDIX B: Relationship Between Hemicellulose Content and 131 Enzyme Activity During Various SSF Operations

APPENDIX C: Relationship Between Hemicellulose and Reducing 133 Sugar Contents During Various SSF Operations

APPENDIX D: Paper Presented During ICCBPE 2005 135 (International Conference on Chemical and Bioprocess Engineering)

APPENDIX E: Paper Presented During INRET 2006 (International 140 Conference on Natural Resources Engineering and Technology)

LIST OF FIGURES

- Figure 2.1 Derivative of palm kernel cake from oil palm.
- Figure 2.2 Extraction of palm kernel oil.

particle size.

- Figure 2.3 The various empirical kinetic profiles that have been described in SSF systems: (A) exponential; (B) logistic; (C) linear; (D) fast-acceleration / slow-deceleration.
- Figure 3.1 Structure of locust bean gum, which consists of a long chain β -(1-4)mannose polymer with α -(1-6)-galactose units appearing as single unit side chains. The ratio of galactose to mannose in LBG is roughly 1:4.
- Figure 3.2 Data of Mannose Calibration Curve for Hach Spectrophotometer
- Figure 3.3 Mannose Standard Curve using Hach Spectrophotometer
- Figure 3.4 Data of Mannose Calibration Curve for Novaspec II Spectrophotometer
- Figure 3.5 Mannose Standard Curve using Novaspect II Spectrophotometer
- Figure 3.6 Soluble Protein Standard Curve using Novaspec II Spectrophotometer
- Figure 3.7 Graph $Ln\left[\left(\frac{X_o}{X_m - X_o}\right)\left(\frac{X_m - X}{X}\right)\right]$ versus t. Fermentation conditions: Moisture content 110%, 30 °C, pH 7, 20 % inoculum, and 850 µm

Figure 3.8 Graph Ln X versus t. Fermentation conditions: Moisture content 110%,

- 30°C, pH 7, 20 % inoculum, and 850 μm particle size.
- Figure 4.1 Growth Profiles of *B. subtilis* during SmF, using both LBG and PKC as carbon sources. Fermentation conditions: 30°C, 150 rpm.

- Figure 4.2 Effect of Temperature on Activity of β -Mannanase produced by *B.* subtilis. Assay conditions: Locust bean gum as substrate, temperatures: 30 – 60°C, and sodium acetate buffer (pH 5.0).
- Figure 4.3 Effect of pH on Activity of β -Mannanase produced by <u>B. subtilis</u>. Assay conditions: Locust bean gum as substrate, sodium acetate buffer (pH 5 8), and temperature at 55 °C.
- Figure 4.4 Reducing sugar content of PKC during SSF of PKC *B. subtilis*, at different moisture contents.
- Figure 4.5 Reducing sugar content of PKC during SSF by *B. subtilis* at different temperature.
- Figure 4.6 Reducing sugar content of PKC during SSF by *B. subtilis*, using different pH of PKC.
- Figure 4.7 Reducing sugar content of PKC during SSF by *B. subtilis*, using different particle size of PKC
- Figure 4.8 β-Mannanase profiles during SSF of PKC by *B. subtilis* at different moisture contents.
- Figure 4.9 β-Mannanase profiles during SSF of PKC by *B. subtilis* at different temperature.
- Figure 4.10 β-Mannanase profiles during SSF of PKC by *B. subtilis* using different pH of PKC.
- Figure 4.11 β-Mannanase profiles during SSF of PKC by *B. subtilis* using different particle size of PKC.
- Figure 4.12 Viable cell count of *Bacillus subtilis* in log scale during SSF of PKC, at different moisture contents: (a) 90 %; (b) 110 %; (c) 130 %; (d) 150 %; (e) 170 %; (f) 200%.

- Figure 4.13 Viable cell count of *Bacillus subtilis* in log scale during SSF of PKC, at different temperature: (a) 30 °C; (b) 40 °C; (c) 50 °C.
- Figure 4.14 Viable cell count of *Bacillus subtilis* in log scale during SSF of PKC, at different initial pH of PKC: (a) pH 6; (b) pH 7; (c) pH 7.5.
- Figure 4.15 Viable cell count of *Bacillus subtilis* in log scale during SSF of PKC, using different particle size of PKC: (a) 325 μm; (b) 655 μm; (c) 850 μm.
- Figure 4.16 Soluble protein content of treated PKC during SSF by *B. subtilis.* (Fermentation conditions: 40°C, 150% moisture content, pH 7, 850 μm particle size).
- Figure 4.17 Growth of *B. subtilis* during SSF of PKC without adjusting the initial pH of PKC with NaOH solution (Fermentation conditions: 100 % moisture content; 30 °C; initial pH 5.4; 850 µm particle size; 20 % inoculum size).
- Figure 4.18 Relationship between the end products during SSF of PKC by *B. subtilis* (Fermentation conditions: 150% moisture content; 40°C, initial pH 7, particle size 850 µm, 20% inoculum size).
- Figure 4.19 ADF and NDF contents of PKC during SSF of PKC by *B. subtilis* (Fermentation conditions: 150 % moisture content; 40°C; initial pH 7; 20% inoculum; 850 µm particle size).
- Figure 4.20 Relationship between Hemicellulose content and β-Mannanase activity during SSF of PKC by *B. subtilis* (Fermentation conditions: 150 % moisture content; 40°C; initial pH 7; 20% inoculum; 850 µm particle size).

- Figure 4.21 Relationship between Hemicellulose and reducing sugar content during SSF of PKC by *B. subtilis* (Fermentation conditions: 150 % moisture content; 40°C; initial pH 7; 20% inoculum; 850 μm particle size).
- Figure 4.22 pH value profile of PKC during SSF of PKC by *B. subtilis* (Fermentation conditions: 150 % moisture content; 40°C; initial pH 7; 20% inoculum; 850 µm particle size).
- Figure 4.23 pH value profile of PKC during SSF of PKC by *B. subtilis* at different moisture content. (Fermentation conditions: 40°C; initial pH 7; 20% inoculum; 850 µm particle size)
- Figure 4.24 Growth profiles of *B. subtilis* during SSF of PKC at different moisture contents, in log scale. Fermentation conditions: 30°C, pH 7, 20 % inoculum, and 850 µm particle size. Moisture content ranged between (a) 90 %; (b) 110 %; (c) 130 %; (d) 150 %, (e) 170 %; and (f) 200 %. The solid line represents the logistic curve that fitted to the biomass data.
- Figure 4.25 Growth profiles of *B. subtilis* during SSF of PKC at different incubation temperature, in log scale. Fermentation conditions: 150 % moisture content, pH 7, 20 % inoculum, and 850 µm particle size. Temperature ranged between (a) 30 °C; (b) 40 °C; (c) 50 °C. The solid line represents the logistic curve that fitted to the biomass data.
- Figure 4.26 Growth profiles of *B. subtilis* during SSF of PKC at different initial pH of substrate, in log scale. Fermentation conditions: 150 % moisture content, 30 °C, 20 % inoculum, and 850 μm particle size. pH ranged between (a) pH 6; (b) pH 7; (c) pH 7.5. The solid line represents the logistic curve that fitted to the biomass data.
- Figure 4.27 Growth profiles of *B. subtilis* during SSF of PKC at different particle size of substrate, in log scale. Fermentation conditions: 150 % moisture content, 30 °C, pH 7, 20 % inoculum. PKC particle size ranged between (a) 325 μm; (b) 655 μm; (c) 855 μm. The solid line

represents the logistic curve that fitted to the biomass data.

- Figure 4.28 Growth profiles of *B. subtilis* during SSF of PKC at different moisture contents, in log scale. Fermentation conditions: 30°C, pH 7, 20 % inoculum, and 850 µm particle size. Moisture content ranged between (a) 90 %; (b) 110 %; (c) 130 %; (d) 150 %, (e) 170 %; and (f) 200 %. The solid line represents the exponential curve that fitted to the biomass data.
- Figure 4.29 Growth profiles of *B. subtilis* during SSF of PKC at different incubation temperature, in log scale. Fermentation conditions: 150 % moisture content, pH 7, 20 % inoculum, and 850 μm particle size. Temperature ranged between (a) 30 °C; (b) 40 °C; (c) 50 °C. The solid line represents the exponential curve that fitted to the biomass data.
- Figure 4.30 Growth profiles of *B. subtilis* during SSF of PKC at different initial pH of substrate, in log scale. Fermentation conditions: 150 % moisture content, 30 °C, 20 % inoculum, and 850 µm particle size. pH ranged between (a) pH 6; (b) pH 7; (c) pH 7.5. The solid line represents the exponential curve that fitted to the biomass data.
- Figure 4.31 Growth profiles of *B. subtilis* during SSF of PKC at different particle size of substrate, in log scale. Fermentation conditions: 150 % moisture content, 30 °C, pH 7, 20 % inoculum. PKC particle size ranged between (a) 325 μm; (b) 655 μm; (c) 855 μm. The solid line represents the exponential curve that fitted to the biomass data.
- Figure 4.32 Growth profiles of *B. subtilis* during SSF of PKC at different moisture contents, in log scale. Fermentation conditions: 30°C, pH 7, 20 % inoculum, and 850 µm particle size. Moisture content ranged between (a) 90 %; (b) 110 %; (c) 130 %; (d) 150 %, (e) 170 %; and (f) 200 %. The solid line represents the exponential curve with an additional equation (dX / dt = 0), that fitted to the biomass data.

- Figure 4.33 Growth profiles of *B. subtilis* during SSF of PKC at different incubation temperature, in log scale. Fermentation conditions: 150 % moisture content, pH 7, 20 % inoculum, and 850 µm particle size. Temperature ranged between (a) 30 °C; (b) 40 °C; (c) 50 °C. The solid line represents the exponential curve with an additional equation (dX / dt = 0), that fitted to the biomass data.
- Figure 4.34 Growth profiles of *B. subtilis* during SSF of PKC at different initial pH of substrate, in log scale. Fermentation conditions: 150 % moisture content, 30 °C, 20 % inoculum, and 850 µm particle size. pH ranged between (a) pH 6; (b) pH 7; (c) pH 7.5. The solid line represents the exponential curve with an additional equation (dX / dt = 0), that fitted to the biomass data.
- Figure 4.35 Growth profiles of *B. subtilis* during SSF of PKC at different particle size of substrate, in log scale. Fermentation conditions: 150 % moisture content, 30 °C, pH 7, 20 % inoculum. PKC particle size ranged between (a) 325 μm; (b) 655 μm; (c) 855 μm. The solid line represents the exponential curve with an additional equation (dX / dt = 0), that fitted to the biomass data.
- Figure 4.36 Specific growth rate constant, µ at different moisture content during SSF of PKC by *B. subtilis*.
- Figure 4.37 Maximum biomass concentration, X_m at different moisture content during SSF of PKC by *B. subtilis*.

LIST OF TABLES

- Tabe 2.1 Typical Chemical Composition and Energy Content of raw PKC
- Table 2.2
 Quality Characteristics of PKC Surveyed in 1989
- Table 2.3Published data on enzyme production using PKC as substrate in solidstate fermentation
- Table 2.4 Various Types of Bioreactors in SSF of PKC
- Table 2.5 Specific classification of *B. subtilis*
- Table 2.6:
 Application of B. subtilis in solid state fermentation
- Table 2.7Differential and integrated form of the various empirical growth
equations that have been applied to SSF systems
- Table 3.1
 Moisture contents adjustment for 5 grams of PKC
- Table 3.2Data for graph $Ln\left[\left(\frac{X_o}{X_m X_o}\right)\left(\frac{X_m X}{X}\right)\right]$ versus t. Fermentationconditions: Moisture content 110%, 30 °C, pH 7, 20% inoculum, and

conditions: Moisture content 110%, 30 °C, pH 7, 20% inoculum, and 850 µm particle size.

- Table 3.3 Data for graphLn X versus t. Fermentation conditions: Moisture content 110%, 30 °C, pH 7, 20% inoculum, and 850 µm particle size.
- Table 4.1
 Percentage of increase in reducing sugar content for treated PKC at different moisture content.
- Table 4.2 Percentage of increase in reducing sugar content for treated PKC at different temperature.
- Table 4.3Percentage of increase in reducing sugar content for treated PKC using
different initial pH of PKC.

- Table 4.4
 Percentage of increase in reducing sugar content for treated PKC using different particle size of PKC.
- Table 4.5Percentage of reduction in Hemicellulose content of PKC after 60 hrs of
SSF, at different moisture content.
- Table 4.6Percentage of reduction in Hemicellulose content of PKC after 60 hrs of
SSF, at different temperature.
- Table 4.7Percentage of reduction in Hemicellulose content of PKC after 60 hrs of
SSF, at different initial pH of PKC.
- Table 4.8Percentage of reduction in Hemicellulose content of PKC after 60 hrs of
SSF, using different particle size of PKC.
- Table 4.9
 Lag phase period of *B. subtilis* during SSF of PKC at different moisture content.
- Table 4.10
 Lag phase period of *B. subtilis* during SSF of PKC at different temperature.
- Table 4.11 Lag phase period of *B. subtilis* during SSF of PKC at different initial pH.
- Table 4.12 Lag phase period of *B. subtilis* during SSF of PKC using different particle size of PKC.
- Table 4.13 Reducing sugar, hemicellulose, and protein contents of raw PKC and fermented PKC by *B. subtilis.* (Fermentation conditions: 150% moisture content; 40°C, initial pH 7, particle size 850 µm, 20% inoculum size, 24 hrs)
- Table 4.14
 Reduction in Hemicellulose content of PKC uses different strains of microbes during solid state fermentation.
- Table 4.15
 Increasing in protein content of substrates using different strains of microbes during solid state fermentation.

- Table 4.16 β-Mannanase enzyme activity uses different strains of microbes during submerged and solid state fermentation.
- Table 4.17 Estimated values of µ and X_m at different initial moisture of substrate during SSF of PKC by *B. subtilis* using logistic model.
- Table 4.18 Estimated values of µ and X_m at different incubation temperature during SSF of PKC by *B. subtilis* using logistic model.
- Table 4.19 Estimated values of µ and X_m at different initial substrate pH during SSF of PKC by *B. subtilis* using logistic model.
- Table 4.20 Estimated values of µ and X_m at different substrate particle size during SSF of PKC by *B. subtilis* using logistic model.
- Table 4.21 Estimated values of μ and X_o at different initial moisture of substrate during SSF of PKC by *B. subtilis* using exponential model.
- Table 4.22 Estimated values of µ and X_o at different incubation temperature during SSF of PKC by *B. subtilis* using exponential model.
- Table 4.23 Estimated values of μ and X_o at different initial substrate pH during SSF of PKC by *B. subtilis* using exponential model.
- Table 4.24 Estimated values of μ and X_o at different substrate particle size during SSF of PKC by *B. subtilis* using exponential model.
- Table 4.25Estimated parameters using both logistic and exponential equations at
various SSF conditions.
- Table 4.26 Values of parameters for equation $\mu = A_0 + A_1 MC + A_2 MC^2 + A_3 MC^3 + A_4 MC^4 ; \text{ and}$ $X_m = B_0 + B_1 MC + B_2 MC^2 + B_3 MC^3 + B_4 MC^4$

LIST OF EQUATIONS

Equation 3.1

$$X = \frac{X_m}{1 + \left(\frac{X_m}{X_o} - 1\right)e^{-\mu t}}$$

Equation 3.2
$$Ln\left[\left(\frac{X_{o}}{X_{m}-X_{o}}\right)\left(\frac{X_{m}-X}{X}\right)\right] = -\mu t$$

Equation 3.3

 $X = X_o e^{\mu t}$

Equation 3.4

 $Ln X = \mu t + Ln X_o$

