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.

CHIN HOOI CHUAN

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ABSTRACT

SOLID STATE FERMENTATION OF PALM KERNEL CAKE
BY BACILLUS SUBTILIS

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 1.82×10^{10} 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 pepejal (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 β-mannananse, 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 40^0C, 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, manakala kandungan hemiselulosa telah berkurang sebanyak 2.6 kali berbanding dengan PKC sebelum penapian. Model kinetik punumbuhan logistik telah menggambarkan data 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 1.82×10^{10} 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
^{o}C	degree Celcius
%	percent
X	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

PKM	Palm Kernel Meal
SmF	Submerged Fermentation
SSF	Solid State Fermentation

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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 $$Ln\, X = \mu t + Ln\, X_o$$