UNIVERSITI PUTRA MALAYSIA

# EFFECT OF FERMENTER CONFIGURATION, ANTIFOAM AND AGITATION SPEED ON THE PERFORMANCE OF KOJIC ACID FERMENTATION BY ASPERGILLUSFLAVUS LINK STRAIN 44-1

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**By**

**SURYANI KAMARUDIN**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of Requirement for the Degree of Master of Science**

**March 2002**

**SPECIALLY DEDICATED TO:**

*'ALHAMDULILLAH'*

***My Beloved Husband*** FAIZALAMIRI ABU BAKAR,
*'THANKS FOR YOUR CARING AND LOVING SUPPORT'*

*Emak & Ayah* KHALIJAH YAHAYA & KAMARUDIN ABDUL SAMAT
*'THANKS FOR YOUR DOA AND ENCOURAGEMENT'*

*To all my family at* PARIT, PERAK & KUALA LIPIS, PAHANG
*'THANKS FOR YOUR MORAL SUPPORT'*

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**Chairman:** **Associate Professor Arbakariya Ariff, Ph.D.**

**Faculty :** **Food Science and Biotechnology**

The effect of agitation speed on growth morphology and cell breakage of *Aspergillus flavus* Link strain 44-1 and its relationship to kojic acid production was carried out using 2 L stirred tank fermenter. From the shake flask experiments, it was found that the addition of silicone based antifoam, even at very low concentration (0.001% v/v), reduced growth and kojic acid production significantly. In order to find the suitable approach for minimizing foam formation during kojic acid fermentation in stirred tank fermenter, the effect of several fermenter configuration based on number of impeller and baffles used on foam formation have been tested. The degree of foaming was greatly influenced by the fermenter configuration. The fermenter with 4-baffles and a single Rushton turbine was found suitable to avoid excessive foaming during the fermentation.

Two mechanisms of cell damage, (i) breaking up, and (ii) shaving off were noticed at agitation speed of 600 and 800 rpm. The breaking up results in the formation of new pellet and fragments while the shaving off results in the formation of shaved pellet and free filamentous mycelia.

Agitation speed also greatly influenced the growth morphology of *Aspergillus flavus*, in which, at low speed the pellets were weak and fluffy while at high speed the more stable pellets were formed. The agitation speed 600 rpm was found optimal for growth (13.11 g/L) of *Aspergillus flavus* and kojic acid production (32.95 g/L), which was associated to growth in the form of pellet with an average size of 1.32 mm. This gave the yield and overall productivity of 0.331 g/g and 2.059 g/L.day, respectively.

The models based on logistic and Luedeking-Piret equations were found suitable to describe kojic acid fermentation by *Aspergillus flavus* Link strain 44-1 at certain fermentation conditions. Kojic acid production was found non-growth associated process and the kinetic parameters values obtained from modelling can be used to verify the fermentation data.

The *Aspergillus flavus* culture during kojic acid fermentation was found to be a non-Newtonian Pseudoplastic type with flow behavior index less than one. The flow was in transitional region with Reynolds number between 0 and

$10^4$. The power consumption in this experiment was proportional to the agitation speed applied by the impeller and ranged between 0.3 to 7.0 J/s.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains.

**KESAN KONFIGURASI FERMENTER, ANTIBUIH DAN HALAJU PENGADUK TERHADAP FERMENTASI ASID KOJIK OLEH *ASPERGILLUS FLAVUS* LINK STRAIN 44-1**

Oleh

**SURYANI KAMARUDIN**

**Mac 2002**

**Pengerusi:** **Profesor Madya Arbakariya Ariff, Ph.D.**

**Fakulti:** **Sains Makanan dan Bioteknologi**

Satu eksperimen telah dijalankan dengan menggunakan bioreaktor berisipadu 2L untuk mengkaji kesan halaju pengaduk ke atas morfologi pertumbuhan dan pemecahan sel *Aspergillus flavus* Link 44-1 serta hubungannya terhadap penghasilan asid kojik. Penambahan silikon anti-buih walaupun pada kepekatan rendah (0.001%) telah mengurangkan pertumbuhan sel dan penghasilan asid kojik dengan berkesan. Kesan beberapa konfigurasi fermenter yang melibatkan penggunaan pengaduk dan 'baffle' telah dikaji untuk mendapatkan kaedah terbaik meminimakan penghasilan buih semasa proses penapaian asid kojik di dalam fermenter tangki berpengaduk.. Konfigurasi fermenter memberi kesan yang besar terhadap darjah pembuihan. Fermenter dengan 4 'baffle' dan pengaduk jenis Rushton turbin tunggal berupaya mencegah pembuihan yang serius semasa proses penapaian asid kojik.

Dua mekanisma kerosakan sel telah dikenalpasti semasa penapaian asid kojik dengan halaju pengaduk antara 600 dan 800 rpm. Mekanisma kerosakan sel tersebut ialah (i) pemecahan sel dan (ii) pencukuran sel. Pemecahan sel akan menghasilkan 'pellet' baru beserta sel yang pecah. Pencukuran sel pula akan menghasilkan 'pellet' yang licin (tanpa rambut) dan miselia berfilamen bebas.

Halaju pengaduk memberi kesan yang besar terhadap morfologi pertumbuhan *Aspergillus flavus*. Pada halaju ynag rendah, 'pellet' menjadi lemah dan bercabang sementara pada halaju yang tinggi, 'pellet' akan menjadi lebih stabil. Halaju pengaduk optima untuk pertumbuhan *Aspergillus flavus* ialah 600 rpm dengan penghasilan sel sebanyak 13.11 g/L dan asid kojik sebanyak 32.95 g/L. Halaju pengaduk 600 rpm juga telah menyumbang pembentukan sel berbentuk 'pellet' dengan saiz purata 1.32 mm. Penghasilan keseluruhan ialah 0.331 g/g dan produktiviti keseluruhan ialah 2.059 g/L. hari.

Persamaan model Logistik, Luedeking-Piret dan Luedeking-Piret terubah, dapat menerangkan perlakuan penapaian asid kojik oleh *Aspergillus flavus* Link 44-1 pada keadaan tertentu. Penghasilan asid kojik merupakan proses pertumbuhan bukan-berkait dan data eksperimen dapat dibuktikan melalui parameter kinetik yang diperolehi daripada proses permodelan yang dilakukan.

Kultur *Aspergillus flavus* semasa penapaian asid kojik didapati bersifat Pseudoplastik bukan-Newtonian, dengan indeks perlakuan aliran kurang daripada satu. Aliran bendalir pula dikategorikan dalam bahagian peralihan dengan nombor Reynolds di antara 0 dan $10^4$. Julat kuasa yang digunakan di dalam eksperimen ini adalah di antara 0.3 – 7.0 J/s dan nilainya berkadaran dengan halaju pengaduk yang digunakan.

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## TABLE OF CONTENT

## LIST OF FIGURE

## LIST OF TABLE

## LIST OF EQUATION

## LIST OF SYMBOL

| Symbol | Description |
|---|---|
| S | strain |
| P | stress |
| F/A | force per unit area |
| C5 | carbon five |
| $\mu$ | viscosity |
| $\mu_a$ | average apparent viscosity |
| $\iota$ | shear stress |
| K | consistency index |
| $n$ | flow behavior index |
| $N$ | rotational speed |
| P | power |
| $D_p$ | particle diameter |
| $D_a$ | agitator diameter |
| $D_t$ | tank diameter |
| $N_{Re}$ | Reynolds number |
| $N_P$ | Power number |
| dV/dy | shear rate |
| $(dV/dy)_{av}$ | average shear rate |
| W | disk width |
| J | baffles width |

## LIST OF ABBREVIATION

| | |
|---|---|
| PPO | polyphenol oxidase |
| DOT | dissolved oxygen tension |
| $NaNO_3$ | natrium nitrate |
| $(NH_4)_2SO_4$ | ammonium sulfate |
| $NH_4NO_3$ | ammonium nitrate |
| $(NH_4)_2HPO_4$ | ammonium hydrogen phosphate |
| $KH_2PO_4$ | kalium hydrogen phosphate |
| $MgSO_4$ | magnesium sulfate |
| $MgSO_4.7H_2O$ | magnesium sulfate anhydrous |
| CPA | cyclopiazonic acid |
| SEM | scanning electron microscope |
| CZ | Czapek agar |
| NaOH | natrium hydroxide |
| HCl | hydrogen cloride |
| $FeCl_3$ | ferric chloride |

# CHAPTER 1

# INTRODUCTION

Kojic acid has many potential applications. It is widely use in medical, food industry, cosmetic and industrial chemistry. In food industry kojic acid is used as an antispeck and antimelanosis (blackening) agents for agriculture products. It is also used in the post harvest process to improve the quality and the productivity of the products. Kojic acid is also used as a chelating agents and activator in insecticide production. In medical field, kojic acid is used as an antifungal and antibacterial reagents, painkiller, antibiotic and anti-inflammatory drugs. In cosmetic industry, kojic acid is widely used as a whitening agent and ultra violet filter in skin care product in replace of hydroquinone, which is carcinogenic. The application of kojic acid is increased with the development of the industries related to its application.

Filamentous fungi and streptomycetes are the most common microorganisms used for organic acid and antibiotic production. Due to their complex morphologies in submerged cultures during industrial process, control of fungal fermentation requires great attention. Industrially, kojic acid is produced by aerobic fermentation of *Aspergillus* species such as *A. albus, A. flavus, A. nidulans, A. parasiticus, A. tamarii* and *Penicillium daleae* using various types of carbon source. Although kojic acid has been produced and applied industrially for some time, details of industrial techniques of