UNIVERSITI PUTRA MALAYSIA

IMPROVED CULTIVATION OF Pediococcus acidilactici BY In Situ REMOVAL OF LACTIC ACID USING POLYMERIC RESIN

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IMPROVED CULTIVATION OF *Pediococcus acidilactici* BY *In Situ* REMOVAL OF LACTIC ACID USING POLYMERIC RESIN

By

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IMPROVED CULTIVATION OF *Pediococcus acidilactici* BY *In Situ* REMOVAL OF LACTIC ACID USING POLYMERIC RESIN

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Lactic acid bacteria (LAB) are industrially important microorganisms recognized for fermentative ability mostly in their probiotic benefits as well as lactic acid production for various applications. Nevertheless, fermentation employing LAB often suffers end-product inhibition which reduces the cell growth rate and the production of metabolite. The inhibition of lactic acid is due to the solubility of the undissociated lactic acid within the cytoplasmic membrane and insolubility of dissociated lactate, which causes acidification of cytoplasm and failure of proton motive forces. This phenomenon influences the transmembrane pH gradient and decreases the amount of energy available for cell growth. The utility of adsorbent resins for *in-situ* lactic acid removal to enhance the cultivation performance of *Pediococcus acidilactici* was studied in shake flask culture and 2 L stirred tank bioreactor. Five different types of anion-exchange resin (namely Amberlite IRA 67, IRA 410, IRA 400, Duolite A7 and Bowex MSA) were screened for the highest uptake capacity of lactic acid based on Langmuir adsorption isotherm. Weak base anion-exchange resin, Amberlite IRA 67 gave the highest maximum uptake capacity of lactic acid (0.996 g lactic acid/g wet resin) compared to the other anion-exchange resins. The effect of different loading concentrations (5 - 40 g/L) of anion-exchange resin on the performance of batch cultivation of *P. acidilactici* was also evaluated. High loading concentrations of anion-exchange resin showed an inhibitory effect on the growth of *P. acidilactici*. The application of IRA 67 anion-exchange resin in batch and constant fed-batch fermentation improved the growth of *P. acidilactici* about 67 times and 56 times, respectively compared to the control batch fermentation without resin addition. Nevertheless, the *in situ* addition of dispersed resin in the culture created shear stress by resins collision and caused direct shear force to the cells. The growth of *P. acidilactici* in the integrated bioreactor-internal column system containing anion-exchange resin was further improved by 1.4 times over that

obtained in the bioreactor containing dispersed resin. The improvement of the *P. acidilactici* growth indicated that extractive fermentation using solid phase is an effective approach for reducing by-product inhibition and increasing product titer.

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

PENINGKATAN PENGKULTURAN *Pediococcus acidilactici* MELALUI PENYINGKIRAN ASID LAKTIK SECARA *In Situ* MENGGUNAKAN RESIN POLIMER

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Bakteria asid laktik (LAB) merupakan mikroorganisma industri yang penting dan dikenali kerana keupayaan fermentasinya terutama dalam faedah probiotik dan juga penghasilan asid laktik untuk pelbagai aplikasi. Walaubagaimanapun, fermentasi oleh LAB sering mengalami perencatan akibat daripada produk yang dihasilkan dan keadaan ini mengakibatkan penurunan dalam kadar pertumbuhan sel dan penghasilan metabolit. Perencatan akibat asid laktik adalah disebabkan oleh kelarutan asid laktik yang tidak berpisah di dalam membran sitoplasma dan ketidaklarutan asid laktik yang berpisah, di mana keadaan ini menyebabkan pengasidan sitoplasma dan kegagalan kuasa proton motif. Fenomena ini mempengaruhi kecerunan pH transmembran dan menurunkan jumlah tenaga untuk pertumbuhan sel. Penggunaan resin penjerap untuk penyingkiran asid laktik secara *in situ* bagi meningkatkan prestasi pengkulturan *Pediococcus acidilactici* telah dikaji di dalam kelalang kon dan bioreaktor berpengaduk 2 L. Lima jenis resin penukaran anion (iaitu Amberlite IRA 67, IRA 410, IRA 400, Duolite A7 dan Bowex MSA) telah diperiksa untuk mendapatkan resin penjerap yang mempunyai kapasiti pengambilan asid laktik yang tertinggi melalui isoterma penjerapan Langmuir. Resin penukaran anion bes lemah, Amberlite IRA 67 telah menunjukkan pengambilan maksimum asid laktik yang tertinggi (0.996 g asid laktik/g resin basah) berbanding resin penukaran anion yang lain. Kesan kepekatan muatan (5 – 40 g/L) resin penukaran anion terhadap prestasi fermentasi sesekelompok *P. acidilactici* juga turut dikaji. Kepekatan muatan resin yang tinggi menunjukkan kesan perencatan terhadap pertumbuhan *P. acidilactici*. Pengaplikasian resin penukaran anion di dalam fermentasi sesekelompok dan fermentasi suapan sesekelompok secara konstan masing-masing menunjukkan peningkatan dalam pertumbuhan *P. acidilactici* sebanyak 67 kali dan 56 kali berbanding fermentasi sesekelompok tanpa pengunaan

resin. Walaubagaimanapun, penambahan resin secara *in situ* dan tersebar dalam kultur telah menghasilkan tegasan ricih yang disebabkan oleh pelanggaran antara resin dan menyebabkan daya ricih langsung ke atas sel. Pertumbuhan *P. acidilactici* di dalam sistem bioreaktor bersepadu kolum dalaman yang mengandungi resin penukaran anion menunjukkan peningkatan sebanyak 1.4 kali melebihi pertumbuhan yang diperolehi dalam bioreaktor dengan penambahan resin secara tersebar. Peningkatan dalam pertumbuhan *P. acidilactici* menunjukkan bahawa fermentasi ekstraktif menggunakan fasa pepejal merupakan pendekatan yang efektif dalam mengurangkan perencatan akibat daripada produk yang dihasilkan dan meningkatkan jumlah penghasilan produk.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

ATP	Adenosine triphosphate
ATPS	Aqueous two-phase system
BET	Brunauer-Emmett-Teller
BHI	Brain heart infusion
BOD	Biochemical oxygen demand
CFU	Colony forming units
CI	Chloride
CLA	Conjugated linoleic acid
CSTF	Continuous stirred tank fermentor
DNA	Deoxyribonucleic acid
DOT	Dissolved oxygen tension
EMP	Embden-Meyerhof-Parnas
Fe^{3+}	Ferric ion
GRAS	Generally regarded as safe
H_2O_2	Hydrogen peroxide
HCI	Hydrochloric acid
HEC	Hydroxyethylcellulose
IBS	Irritable bowel syndrome
ISPR	*In-situ* product removal
LAB	Lactic acid bacteria
LDH	Lactate dehydrogenase
$mOsm.kg^{-1}$	Milliosmole per kilogram
MRS	De Man Rogosa and Sharpe
NaCI	Sodium chloride
NAD^+	Nicotiamide adenine dinucleotide
NADH	Nicotiamide adenine dinucleotide
NaOH	Sodium hydroxide
PEI	Poly(ethyleneimine)
PLA	Polylactic acid
PPM	Parts per million
psi	Pounds per square inch
RNA	Ribonucleic acid
RP-HPLC	Reverse-phase high performance liquid chromatography
rpm	Rotation per minute
rRNA	Ribosomal ribonucleic acid
SEM	Scanning electron microscope
TSBYE	Trypticase soy broth yeast
v/v	Volume/volume
vvm	Volumetric air flow rate
w/v	Weight/volume

CHAPTER 1

INTRODUCTION

Lactic acid bacteria (LAB) have recently attracted the captivated attention of scientific and medical researchers due to their contribution in the part of gut microflora formation, which in turn, beneficial to the host as probiotic microorganisms (Sreekumar et al., 2010). The fermentation of LAB through carbohydrate metabolization produces lactic acid as the major metabolic end-product. Lactic acid has been found to have many potential applications in chemical, food and pharmaceutical industry. Nevertheless, the major problem in the application of LAB culture either as probiotics or for lactic acid production is the reduced growth and biomass concentration owing to end product inhibition. Lactic acid accumulation inhibits LAB growth due to pH alteration into acidic condition which in turn affects LAB growth and reduces its viability. It is also known that the main challenge in engineering of biomass production from LAB fermentation is to overcome the problem of product inhibition (Aguirre-Ezkauriatza et al., 2010).

The acidification of cytoplasm and failure of proton motive forces are the reasons for the end product inhibition in LAB fermentation (Wee et al., 2006). As the concentration of lactate increases or the pH of the medium decreases, the concentration of undissociated lactic acid in the medium also increases (Broadbent et al., 2010). The undissociated lactic acid is cytoplasmic membrane soluble and thus can pass through the bacterial membrane via simple diffusion and dissociates inside the cell, whilst the dissociated lactate is insoluble (Wee et al., 2006). Eventually, this will affect the transmembrane pH gradient where the transmembrane pH gradient can no longer be maintained and disabled the cellular functions. Besides, the amount of energy that may be used for cell growth also reduces as it is being used for maintaining the transmembrane pH gradient. In addition, the reduction of intracellular pH and acidification of cytoplasm can reduce the activity of metabolic enzyme and also lead to the metabolic enzyme denaturation (Piard and Desmazeaud, 1991).

Among batch, fed-batch and continuous fermentation which are commonly used for biomass production in microbial fermentation, batch fermentation is identified as the most frequently used mode due to the simplicity of the process (Abdel-Rahman et al., 2013). Nevertheless, batch fermentation of LAB is intensely inhibited by the presence of organic acids and low pH values (Cui et al., 2016). Meanwhile, there are numerous reports on fed-batch fermentation that were conducted to overcome the end product inhibition in LAB fermentation which in turn enhanced biomass production (Boon et al., 2007; Aguirre-Ezkauriatza et al., 2010; Ming et al., 2016). However, the use of fed-batch and pH controlled fermentations for overcoming end product inhibition in LAB fermentations are often inefficient due to high osmotic pressure and the presence of acid anions (Cui et al., 2016). Therefore, there are various strategies have been developed to remove and recover lactic acid from

fermentation broth to overcome end product inhibition in LAB fermentation such as solvent extraction (Chen at al., 2012), electrodialysis (Habova et al., 2004) and aqueous two-phase systems (Aydogan et al., 2011). Besides, the application of recombinant microorganism in overcoming end product inhibition by improving acid tolerance of LAB has also been explored (Patnaik et al., 2002). In addition, an extractive fermentation using anion exchange resin for the adsorption of lactic acid to reduce inhibition in the fermentation of LAB has also been reported (Garret et al., 2015; Cui et al., 2016). However, little literature is currently available on the mechanism of *in situ* lactic acid removal using anion exchange resin and its effect on the growth of LAB.

The present study was aimed to provide alternatives in overcoming end product inhibition and enhancing biomass production of LAB fermentation. The specific objectives of this study were:

1. To investigate the effects of fermentation conditions on growth of *P. acidilactici* in batch fermentation.
2. To investigate the feasibility of using constant fed-batch fermentation with anion exchange resin for improvement of *P. acidilactici* cultivation.
3. To evaluate the possibility of using anion exchange resin with integrated bioreactor-internal column system for *in situ* lactic acid removal and enhancement of *P. acidilactici* cultivation performance.

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