OPTIMIZATION OF MEDIUM AND CULTIVATION CONDITIONS FOR D-LACTIC ACID PRODUCTION USING CASSVA STARCH

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ABSTRACT

In this research, seven standard strains of lactic acid bacteria will be screened at first to evaluate their capability to produce D-lactic acid. Selected for investigation based on their D-lactic acid production capability with three media (MRS, RAM, BASAL).The results indicated that, all strains could produce lactic acid but at different yields. These isolates provided the concentration of lactic acid ranging from1.24-2.51, 0.44-3.24-0.49-2.10[g/l] and total acidity expressed ranging from 0.53-1.28, 0.61-1.97 and 0.69-1.58% in the three medium respectively. The maximum D-lactic acid 4.44[g/L] was obtained at 48hours fermentation with an initial cassava starch concentration. Therefore, 10 [g/L] of cassava starch concentration was chosen to be used as the inexpensive carbon source in RAM medium for D-lactic acid production by the isolate (*Lactobacillus delbruekii*).For nitrogen sources effect of different nitrogen sources on D-lactic acid production was investigated during this study (*Lactobacillus delbruekii)* was cultivated with 4-5 [g/L] yeast extract, Peptone. Yeast extract showed the higher D- lactic acid production followed by Peptone from 4.30 [g/L], 4.22 [g/L] respectively. For Dipotassium phosphate (K_2HPO_4) source. The maximum yield was 4.75[g/L] of D-lactic acid was produced. The process optimization will be started by optimization of medium composition using un-optimized medium and optimized medium, followed by complete growth kinetics studies in shake flask level. Result showed The D-lactic acid produced was in un-optimized medium and optimized medium 3.25[g/L], 5.47[g/L] respectively. Shake flask level scaled up in 16-L bioreactor for the production of D- lactic acid by using two strategy cultivations, with controlled pH and without controlled pH. The final results after 48 hours cultivation as follows, 9.12[g/L], 14.25[g/L] respectively, for the production of D- lactic acid.

ABSTRAK

Dalam kajian ini, tujuh jenis piawain bakteria asid laktik akan disaning pada mulanya untuk menilai keupayaan mereka untuk menghasilkan D-laktik asid. Dipilih untuk penyelidkan berdasarkan keupayaan pengeluaran asid D-laktik mereka dengan tiga medai (MRS, RAM, Basal) Keputusan menunjukkan bahawa, semua plawaian bakteria boleh menghasilkan asid laktik tetapi pada kadar hasil yang berbeza. pencilan menunj ukkan kepekatan asid laktik yang terdiri daripada from1.24-2.51,0.44-3.24-0.49-2.10[g/l] dan jumlah keasidan dikeluarkan antara 0.53-1.28, 0.61-1.97 and 0.69-1.58% dalam tiga media masing-masing.D-laktik asid maksimum 4.44 [g/L] telah diperolehi di penapaian 48h dengan kepekatan kanji ubi kayu awal. Oleh itu, 10 [g/L] kepekatan kanji ubi kayu telah dipilih untuk digunakan sebagai sumber karbon murah dalam media RAM untuk D-laktik pengeluaran asid oleh pencilan (*Lactobacillus delbruekii*). Untuk sumber nitrogen kesan sumber nitrogen yang berbeza pada D- pengeluaran asid laktik disiasat semasa kajian ini (*Lactobacillus delbruekii*) telah ditanam dengan 4-5 [g/l] ekstrak yis ,pepton. Ekstrak yis menunjukkan D- laktik asid pengeluaran yang lebih tinggi diikuti oleh pepton dari 4.30 [g/ L], 4.22 [g/L] masing-masing. Untuk dipotassium fosfat sumber (K_2HPO_4). Hasil maksimum adalah 4.75[g/L] D-laktik asid dihasilkan.Pengoptimuman proses akan bermula dengan mengoptimumkan komposisi media dangan media tidak-dioptimumkan dan media dioptimumkan, diikuti dengan pertumbuhan kajian Goncang tahap kelalang ditingkatkan. Keputusan menunjukkan Asid D-laktik dihasilkan adalah masing-masing di media un-dioptimumkan dan media dioptimumkan 3.25 [g/L], 5.47 [g/L]. Goncang tahap kelalang ditingkatkan dalam 16-L bioreaktor untuk pengeluaran D- asid laktik dengan menggunakan dua pengkulturan strategi, dengan pH dikawal dan tanpa pH dikawal. Keputusan akhir selepas penanaman 48 jam seperti berikut, 9.12 [g/L], 14.25 [g/L] masing-masing, untuk pengeluaran D- laktik asid.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AOAC	-	Association of Official Analytical Chemists
MC	-	MRS containing 0.5% CaCO3
MRS	-	De Man Rogosa and Shapes medium
RAM	-	Rogasa Agar Modified medium
et al.,	-	Ahmed (and others)
PLA	-	Poly lactic acid
LAB	-	lactic acid bacteria
RAM	-	Rogasa Agar Modified medium
rDNA	-	Ribosomal deoxyribonucleic acid
UV	-	Ultraviolet
CDW	-	Cell dry weight
DO	-	Dissolved Oxygen
OD	-	Optical Density
OD_{540}	-	Optical Density at 540 nm
OD_{600}	-	Optical Density at 600 nm
sp.	-	Species

LIST OF SYMBOLS

%	-	Percent
>	-	Greater than
F	-	Feed rate (g $L^{-1}h^{-1}$)
KS	-	Substrate utilization constant
S	-	Substrate concentration (g L^{-1})
So	-	Feed substrate concentration (g substrate L^{-1})
t	-	Time
t0	-	Initial time (h)
V	-	Volume
v/v	-	Volume per volume
vvm	-	Volume per volume per minute
X	-	Biomass concentration (g L^{-1})
X0	-	Original biomass concentration (g L^{-1})
%	-	Percent
>	-	Greater than
F	-	Feed rate (g $L^{-1}h^{-1}$)
KS	-	Substrate utilization constant
So	-	Feed substrate concentration (g substrate L^{-1})
t	-	Time
t0	-	Initial time (h)
V	-	Volume
v/v	-	Volume per volume

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Research Background

The Cassava (*Manihotesculenta crantz*) is imperishable bush with a massive storage root (El-Sharkawy, 2003). The dry substance of roughly 35-40% is for the most part comprised of starch and the protein substance being low. Both roots and leaves are used in food preparation. All parts of the plant contain cyanogenic glycosides. Upon processing, these are hydrolyzed to release cyanohydrins, which in turn release the toxic HCN (Brimer and Preedy, 2014).

Billions huge amounts of cassava are delivered yearly around the world. Notwithstanding this exclusive half of generation is prepared into consumable one leaving a huge amount of starch cassava that can be harmful to environment because release of HCN (Petrov *et el*., 2008). The financial obligation to the modern cassava processor depends just on this amount and this let us consider how we can get advantage however much as could reasonably be expected from this colossal measure of cassava.

Starch can be a suitable substrate for producing biochemical materials due to its rich degradability, providing a beneficial approach of cassava (Sommart *et al*., 2000). Microbial biomass protein and natural acid can move out as profitable final items by the biotechnological treatment of starch. Changing starch into esteemed reflection and fitting activity of starch stream are among the by and large examined viewpoints in cassava research (Adnan and Tan, 2007).

Lactic acid is a standout amongst the most imperative natural acids which could be created from cassava. This natural acid is generally utilized as a part of numerous applications. These days, 80% of lactic acid is utilized as a part of food,and sustenance related commercial enterprises. The business creation of lactic acid is extremely regular and it is conceivable either by chemical synthetic or by fermentation process (Xiaodong *et al*., 1997).

Acetaldehyde and hydrogen cyanide derived lacto nitrile hydrolysis is a frequently used technique for its synthesis. However bacterial fermentation of simple sugars is mostly used for biotechnological production of the acid, accountable for almost 50% of the total lactic acid capacity (Huang *et al*., 2003).

Lactic acid, mixture of two isomers dependably comes about while delivering synthetically. Though, a solitary or a blend of two isomers in different extents is yielded, contingent on substrate microorganism and growth conditions utilized as a part of fermentation process. Use of cheap crude materials, for example, starch, sugarcane beet-sugar, molasses and other carb rich materials make natural generation favorable over chemical synthesis (Tsao *et al*., 1999).

Various vast organizations are worried in the advancement of procedure and creation of lactic acid on account of the colossal potential interest of lactic acid as a segment and feedstock in different commercial industrial, for example, nourishment handling drinks and pharmaceutical. For delivering lactic acid biologically starch and sugars are to a great extent utilized as substrates as a part of the exchange forms (Huang *et al*., 2005).

The production of lactic acid using microbial resources has been broadly researched and several lactic acid producing bacteria *Streptococcus, Tetragenococcus, Vagococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Carnobacterium, Enterococcus Clostridium* and *Weissella* have been reported (Stiles and Holzapfel 1997). Producing lactic acid biologically is mostly completed by the bacterial fermentation of simple sugars. Due to their large production yield and growth rate bacterial strains like *Lactobacillus* and *Lactococcus* have received worldwide profit (Richter and Berthold, 1998).

However, the (1) high costs for substrate pretreatment, hydrolysis (2) particular nutrients supplementation (3) controlling pH through fermentation (4) lactic acid recovery and purification are the major cases with the processes of bacterial fermentation. For example, producing lactic acid by traditional means form starch demands pretreatment by gelatinization and liquefaction performed for 10-15 minutes between 100-130 °C temperature after that enzyme scarification to glucose and following its processing to lactic acid by fermentation. The two-step process includes of successive enzymatic hydrolysis and microbial fermentation makes unappealing from economic perspectives. Generally the composition of cassava is 800 g kg^{-1} water, 20 g kg^{-1} protein, 1 g kg^{-1} fat, 170 g kg^{-1} carbohydrate and 9g kg^{-1} other components like inorganic minerals ,vitamins and metals (Alves *et al*., 2007).

The modulation of all the desired nutrients for various fermentation is the advantage of such a feedstock as compare to other agro industrial wastes (sugar beet, or extract of wheat bran, peel of citrus fruits). All others require collection with carbon or nitrogen sources and other trivial nutrients. Therefore least preprocessing and supplementation are the advantages of using cassava as a feedstock. The production cost of lactic acid would be decreased by producing using starch.

1.2 Problem Statement

This Research includes two problem statements, economical and environmental
Several years of intensive research has been done for the production of lactic acid based on the importance of this acid in food, and chemical industries. However, most of previous studies were focused on using standard medium for lactic acid bacteria cultivations such as MRS broth contain expensive carbon and nitrogen sources.

Billions huge amounts of cassava are delivered yearly around the world. Notwithstanding this exclusive half of generation is prepared into consumable one leaving a huge amount of starch cassava that can be harmful to environment because release of HCN. The financial obligation to the modern cassava processor depends just on this amount and this let us consider how we can get advantage however much as could reasonably be expected from this colossal measure of cassava.

Therefore, there was a need to develop cheap culture medium to improve the process economy of lactic acid production. Thus, in the present work the potential use of cassava as cheap and available carbon source for the development of more efficient and cheap process for lactic acid production will be investigated.

This work included investigation of lactic acid production beginning, from screening of possibility of Lactic acid bacteria (LAB) for lactic acid production. At least 7 standard strains of lactic acid bacteria obtained from Wellness Industries Culture Collection (WICC) at the Institute of Bio product Development (IBD), University Technology Malaysia, of will be screened and selected for D-lactic acid production. Several optimum production parameters **(**particularly nutrient sources using cassava starch as a carbon source, nitrogen sources, phosphate source, optimum temperature, pH, cassava concentration and inoculums size**)** for D-lactic acid production will be determined.

1.3 Research objectives

The main objective of this study the production of D-lactic acid through culture media optimization and cultivation strategy in semi-industrial scale16-L bioreactor by using cassava starch as a main carbon source.

1.4 Scope and limitation of the study

The scope of the research will be as follows:

1.4.1 Screening and selection of the possibility strain of (LAB) for D-lactic acid production using starch of cassava as feedstock.

1.4.2 Optimization of medium composition by using one factor at time (OFAT) using cassava based medium.

1.4.3 Growth kinetics in shake flask cultures using the un-optimized medium.

1.4.4 Growth kinetics in shake flask cultures using the optimized medium

1.4.5 Study the effect of bioprocessing conditions on D-lactic acid production in semi industrial scale 16-L bioreactor.

1.4.6 Batch cultivation of *Lactobacillus* sp. in semi-industrial scale 16-L bioreactor.

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