UNIVERSITI PUTRA MALAYSIA

# CITRIC ACID FERMENTATION BY LOCALLY ISOLATED ASPERGILLUS NIGER M2SG1-MS4

NOOROLLHAMEZON BT MOHD. NOOR.

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# CITRIC ACID FERMENTATION BY A LOCALLY ISOLATED *ASPERGILLUS NIGER* M2SG1-MS4

**By**

**NOOROLLHAMEZON BT MOHD.NOOR**

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

**February 2004**

# DEDICATION

**SPECIALLY DEDICATED TO:**

***My Beloved husband*** **AMINALLAH HJ JAAFAR,**
***'THANKS FOR YOUR CARING AND LOVING SUPPORT'***

***Ma &Abah*** **HALIMAH YUSOFF &MOHD.NOOR HJ MUHAMMAD**
***Umi & Ayah*** **HAMIDAH YUSOFF & ABDUL MANAN OMAR**
***Ma & Ayah*** **PATAHIAH YUSOFF & HJ JAAFAR**
***'THANKS FOR YOUR DOA AND ENCOURAGEMENT'***

**Along, Angah, Boboy & Baby NOMIE IRYANI, NOMIE SYAMIMI, MUHAMMAD MUFQI & NOMIE MARINI**
***'THANKS FOR YOUR UNDERSTANDING AND SACRIFICE'***

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master Science

**CITRIC ACID FERMENTATION BY A LOCALLY ISOLATED *ASPERGILLUS NIGER* M2SG1-MS4**

**By**

**NOOROLLHAMEZON BT MOHD.NOOR**

**February 2004**

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

**Institute : Bioscience**

The citric acid fermentation by locally isolated strain of *Aspergillus niger* was carried out using shake flask culture and 2 L stirred tank fermenter. The pure strain was obtained through monospores isolation step using spread plate technique. From morphological and biochemical properties, isolated monospore M2sg1-MS4 can be suggested belong to the strain *Aspergillus* species.

Optimization of fermentation medium for citric acid production by the locally isolated *A. niger* M2Sg1-MS4 was achieved using 200 g/L of glucose and 0.8 g/L of ammonium nitrate. Addition of 3% (v/v) methanol to the optimized medium improved citric acid production (62.5 g/L) by about two times higher compared to control. Trace elements used were similar to the trace elements used in a fermentation medium described by Osthman et al. (1999). Optimum fermentation condition for citric acid production by M2sg1-MS4 was obtained at initial pH range between 2.5-3.0, temperature of 30°C and agitation speed of 1000 rpm with 1.0 vvm airflow rate. Maximum citric acid concentration obtained under this condition

was approximately 62.5 g/L, which gave the yield and overall productivity of 0.41 g/g and 0.19 g/L/h, respectively.

Foaming is one of the problems normally encountered in submerged fermentation using bioreactor as a result of bubble aeration. In order to find approach for minimizing foam formation during citric acid fermentation in stirred tank fermenter, the effect of several fermenter configurations based on number of impeller and baffles used on foam formation tested. The fermenter with 4-baffles and a single Rushton turbine impeller was found suitable to avoid excessive foaming during the fermentation.

The development of dissolved oxygen tension (DOT) control strategies aimed at improving citric acid production in 2 L stirred tank fermenter was also carried out. Maximum citric acid production was obtained in batch fermentation in which DOT was controlled at 80% saturation through out the fermentation. Approximately, 69.06 g/L citric acid which gave yield and overall productivity of 0.47 g/g and 0.22 g/L/h respectively was obtained after 312 h of fermentation. This result indicates that the high DOT was needed for high glucose conversion to citric acid throughout the fermentation process.

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

**FERMENTASI ASID SITRIK OLEH STRAIN TEMPATAN *ASPERGILLUS NIGER* M2SG1-MS4**

Oleh

**NOOROLLHAMEZON BT MOHD.NOOR**

**Februari 2004**

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

**Institut : Biosains**

Fermentasi asid sitrik oleh strain pencilan tempatan telah dijalankan dalam kelalang bergoncang dan bioreaktor berpengaduk berisipadu 2 L. Strain tulin ini telah didapati melalui pemencilan spora tunggal dengan menggunakan teknik plat sebaran. Dari kajian morfologi dan ciri-ciri biokimia, spora tunggal M2sg1-MS4 telah dikenal pasti sebagai spesis kulat *Aspergillus niger*.

Media fermentasi yang optimum untuk penghasilan asid sitrik telah dicapai dengan menggunakan 200 g/L glukosa dan 0.8 g/L ammonia nitrat. Penambahan metanol sebanyak 3% (v/v) ke dalam media fermentasi telah meningkatkan penghasilan asid sitrik (62.5 g/L) sebanyak 2 kali ganda berbanding dengan ujikaji kawalan. Unsur surih yang digunakan adalah sama dengan unsur surih dalam media fermentasi yang dikemukakan oleh Osthman et al. (1999). Keadaan fermentasi yang optima untuk penghasilan asid sitrik telah dicapai dengan pH permulaan di antara 2.5-3.0, suhu 30°C and kadar pemutaran 1000 rpm dengan 1.0 vvm pengaliran udara. Anggaran asid sitrik yang didapati dari pengoptimaan ini, adalah sebanyak

62.5 g/L, yang memberikan 0.41 g/g kadar penghasilan dan 0.19 g/L/h produktiviti keseluruhan.

Pembentukan buih yang berlebihan merupakan satu masalah yang sering dihadapi kesan daripada gelembung udara dalam fermentasi menggunakan bioreaktor. Beberapa konfigurasi bioreaktor yang menggunakan pengaduk dan 'baffle' telah dikaji untuk mendapatkan kaedah yang terbaik bagi mengurangkan pembentukkan buihsemasa proses penapaian asid sitrik menggunakan bioreaktor berpengaduk. Konfigurasi bioreaktor dengan 4 'baffle' dan satu pengaduk turbin Rushton berupaya mencegah pembentukan buih yang berlebihan semasa proses penapaian asid sitrik.

Strategi pengawalan oksigen terlarut untuk tujuan meningkatkan penghasilan asid sitrik telah dijalankan dengan menggunakan fermenter 2L berpengaduk. Penghasilan asid sitrik yang maksima telah diperolehi dengan fermentasi pengawalan oksigen terlarut pada ketepuan 80% sepanjang tempoh proses fermentasi. Sebanyak 69.06 g/L asid sitrik dengan 0.47 g/g kadar penghasilan dan 0.22 g/L/h produktiviti telah dicapai selepas 312 jam fermentasi. Keputusan ujikaji tersebut menunjukkan pengawalan oksigen terlarut yang tinggi sepanjang proses fermentasi diperlukan untuk metabolisma penukaran glukosa kepada penghasilan asid sitrik yang tinggi.

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I certify that an Examination Committee met on 18th February 2004 to conduct the final examination of Noorollhamezon Binti Awang on her Master of Science thesis entitled "Citric Acid Fermentation by a Locally Isolated *Aspergillus niger* M2SG1-MS4" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

**Ling Tau Chuan, Ph.D.**
Faculty of Engineering
Universiti Putra Malaysia
(Chairman)

**Raha Abdul Rahim, Ph.D.**
Associate Professor
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Member)

**Rosfarizan Mohamad, Ph.D.**
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Member)

**Wan Mohtar Wan Yusoff, Ph.D.**
Professor
Faculty of Technology and Information Science
Universiti Kebangsaan Malaysia
(Independent Examiner)

**GULAM RUSUL RAHMAT ALI, Ph.D.**
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 27 MAY 2004

This thesis 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 are as follows:

**ARBAKARIYA ARIFF, Ph.D.**
Associate Professor
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Member)

**ROSFARIZAN MOHAMAD, Ph.D.**
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Member)

**HIRZUN MOHD YUSOF, Ph.D.**
Sime Darby Technology Centre
(Member)

**AINI IDERIS, Ph.D.**
Professor / Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 28 JUN 2004

## DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other at UPM or other institutions.

**NOOROLLHAMEZON BINTI MOHD. NOOR**

Date: 25/5/04

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## LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| h | : | hour |
| P | : | Product Concentration (g/L) |
| $P_{max}$ | : | Maximum citric acid concentration (g/L) |
| X | : | Cell Concentration (g/L) |
| $X_{max}$ | : | Maximum cell concentration (g/L) |
| t | : | Time (hour) |
| CA | : | Citric Acid |
| ME | : | Malt Extract Agar |
| FF | : | Filamentous Fungi |
| %T | : | Percent transmittance |
| IF | : | Inoculation fluid |
| $Y_{p/s}$ | : | Yield of citric acid based on glucose consumed (g/g) |

# CHAPTER 1

# INTRODUCTION

Citric acid (2-hydroxy-1,2,3-propanetricarboxylic acid ) was first extracted and crystallized from lemon juice by Swedish chemist, Scheele in 1784. Natural occurring citric acid are mainly found in citrus fruits, tomatoes, other fruits and an intermediate of the tricarboxylic acid (TCA) cycle system in all living cells. Those that were found in fruits and vegetables are natural citric acid whilst from the living cell was obtained through fermentation process.

Citric acid is widely used in food and beverage industries, pharmaceutical, cosmetics and environmental bioremediation. In the food industry citric acid is used as preservatives, flavour-enhancer, and inhibitors to the development of metal-catalysed off-flavours and colour deterioration. Pharmaceutically citrate is used in blood transfusion and the free acid as effervescent products. Uses of citric acid in cosmetics include those in astringent lotions to adjust pH and act as an antioxidant, sequesterant and synergists. The uses of citric acid in environmental bioremediation include the replacement of sodium tripolyphosphate in detergent wherein the latter caused algae bloom discharged in water bodies, act as heavy metal chelator in soil and is used to remove clog in pipes.

The world demand for citric acid is about 0.75 million tones per year, exclusively produced by fermentation process. The increasing use of citric acid in a variety of industries has demanded a steady increase in citric acid production. In Malaysia, citric acid is fully imported with a total consumption of about 5,000 tonnes per annum. This will give a total current domestic market demand of approximately RM30 million per annum (at citric acid price of RM4700 per tonne) and this is bound to increase in the coming years with increasing number of industries related to the use of citric acid. Thus, locally produced citric acid will reduce the cost of the product and savings in foreign exchange. This prompted investigation of the feasibility of producing citric acid locally using cheap and easily available raw materials such as tapioca waste, sago starch and pineapple skin waste as carbohydrate source.

Microorganisms such as bacteria, yeast and fungi have been shown to be able to produce citric acid by fermentation process. Industrial productions of citric acid cover two main areas; 1) isolation, strain improvement and maintenance, and 2) fermentation, product recovery and purification. Currently, citric acid is produced through aerobic fermentation predominantly by fungus, *A. niger* which was first used by Currie in 1917. The isolation and screening of microorganisms capable of producing citric acid will not be sufficient to be used for industrial production. Thus, strain improvement will be required in order to obtain a higher production yield of citric acid industrially. The strain can be improved either by random mutagenesis using ultra-violet irradiation or chemical mutagenesis. Due to lack of sexual cycle and refractory to classical genetic approaches, mutagenesis and screening has been the method of choice for strain improvement in industrial production of citric acid (Bigelis, 1989).

Furthermore, documented know-how for the production of citric acid by fungal fermentation are heavily protected by patents. Thus, attempts to produce the citric acid at large scale is restricted and being monopolised by few organisations. Aim so, research have to be step-up for the development of a local citric acid production capabilities. This would entail own process development that cores optimisation of medium and culture condition, mode of fermenter operation, designs of fermenter and cell immobilisation.

The objectives of this study will focus on process optimization aimed at enhancing citric acid fermentation by a local isolate, *A. niger* strain M2sgl which include the following:

1. To identify and characterised the locally isolated citric acid producing fungus.
2. To obtain a pure culture of high citric acid producer through monospore isolation technique.
3. To study culture conditions and medium composition for improvement of citric acid production by the local isolate.
4. To establish important fermenter configuration and operating variables that minimizes foaming and enhanced the performance of citric acid fermentation in stirred tank fermenter.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Properties and Application of Citric Acid

Citric acid (2-hydroxy-1, 2, 3-propanetricarboxylic acid) is a primary metabolite product and is formed in the tricarboxylic acid cycle. The chemical structure of citric acid, which is classified as an acidifying agent and buffer component is shown in Figure 1. Citric acid was first isolated from lemon juice by the Swedish chemist Scheele (1784). The first commercial production of citric acid was started in 1823 by John and Edmund Sturge in England. Mihir and Fujio (1997) described the synthesis of citric acid from glycerol and other raw materials using different routes since early 1880.

Citric acid crystallises in the form of colourless or white, crystalline powder, and odourless (Sodeck, 1981). The melting points for anhydrous citric acid crystal and monohydrate form are 153°C and 100°C respectively (Tariq et al., 1995). Citric acid is soluble in water and ethanol, and sparingly soluble in ether and practically insoluble in chloroform. Citric acid has a molecular formula of $C_6H_8O_7$ with a molecular weight of 192.1 for the anhydrous and 210.1 for the monohydrate form (Sodeck, 1981; Akihiko et al., 1996). Clark (1964) reported that citric acid has maximum peak ultraviolet absorption spectra at 214 nm.

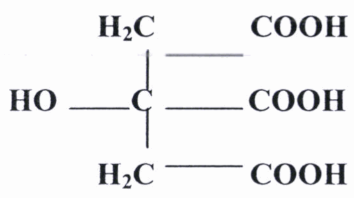


**Figure 1:** The chemical structure of Citric acid (Elizabeth, 1972).

Citric acid has gained universal acceptance as a safe food ingredient. The Food and Drug Administration in the USA lists citric acid and its sodium, potassium and calcium salts as multiple purpose generally recognized as safe (GRAS) food additives. Citric acid is principal food acid used in the preparation of various beverages and confectionery. It also functions as an antioxidant for inhibiting rancidity in fats and oils. In pharmaceutical, citrates are used in blood transfusion and the free acid is used in effervescent products. It is rapidly metabolized in the human body and can serve as a source of energy. Cosmetics uses of citric acid include those in astringent lotions to adjust pH and act as an antioxidant, sequesterant and synergists. In industrial uses, citric acid were used as biodegradable ingredient to replace Sodium Tripoly Phosphate in detergent which makes it an environmental friendly.

**Table 1:** Summary of uses of citric acid in various sector.

| Sector | Uses |
|---|---|
| Pharmaceutical | Included in the formulation for many types of effervescent tablets. |
| | As the anion in a range of pharmaceutical preparations which employ a basic substance as the active agent. |
| | Blood anticoagulant |
| Food and Beverages | Flavour enhancers |
| | pH control agent for gelation control, buffering and preservative enhancement. |
| | Chelating agent |
| Cosmetic | As an antioxidant and synergist |
| | Included in various cream, ointments and shampoo |
| | As plaque inhibitor in a range of toothpaste. |
| Industrial | Have cleansing and sequestering properties and can therefore be used in detergents, industrial and domestic cleaners. |
| | For treatment of boiler water and in metal plating |
| | In tanning and textiles. |