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APPENDIX A

CONFERENCE AND PUBLICATION

1.0 Conference

During the study, the author had attended two international conferences. The details as below:

a. International Conference on Chemical, Environment and Biological Sciences (ICEBS-2014) Sept. 17-18, 2014 Kuala Lumpur (Malaysia); Oral presentation
b. The 6th International Conference on Postgraduate Education (ICPE-6), Dec. 17-18, 2014 Melaka (Malaysia); poster presentation

2.0 Publication

2.1 Published article

During the study, the author had publish three article. The details as below:

1) A Simple and Effective Isocratic HPLC Method for Fast Identification and Quantification of Surfactin. (Muhammad Qadri Effendy Mubarak, Abdul Rahman Hassan, Aidil Abdul Hamid, Sahaid Khalil & Mohd Hafez Mohd Isa) (Had published in Sains Malaysiana 44(1)(2015): 115-120).

2) Single-step Crossflow Ultrafiltration for Recovery and Purification of Surfactin Produced by Bacillus subtilis ATCC 21332. (Muhammad Qadri Effendy Mubarak & Mohd Hafez Mohd Isa, and Abdul Rahman Hassan); ISBN 978-81-929653-5-2 http://dx.doi.org/10.15242/IICBE.C914

3) A Simple and Effective Isocratic HPLC Method for Identification and Quantification of Glucose in Complex Fermentation (Muhammad Qadri Effendy Mubarak & Mohd Hafez Mohd Isa) ; (ISBN 978-967-0764-10-8)

2.2 Article submitted for possible publication

The author sent the article for possible publication. The article in review process. The details as below:

1) Kinetic of surfactin production by *Bacillus subtilis* in a 5 L stirred-tank bioreactor (Review Process)

APPENDIX B

B.1 Certificate from International Conference on Chemical, Enviroment and Biological Sciences (CEBS-2014)

C914034

IICBE INT'L CONFERENCE

www.iicbe.org

Certificate of Participation

This Certificate is awarded to

Muhammad Qadri Effendy Muharak

Master Student, Faculty of Science and Technology, Universiti Sains Islam Malaysia, Negeri Sembilan, Malaysia

for Paper Titled

Single-step Cross-flow Ultrafiltration for Recovery and Purification of Surfactin Produced by Bacillus subtilis ATCC 21332

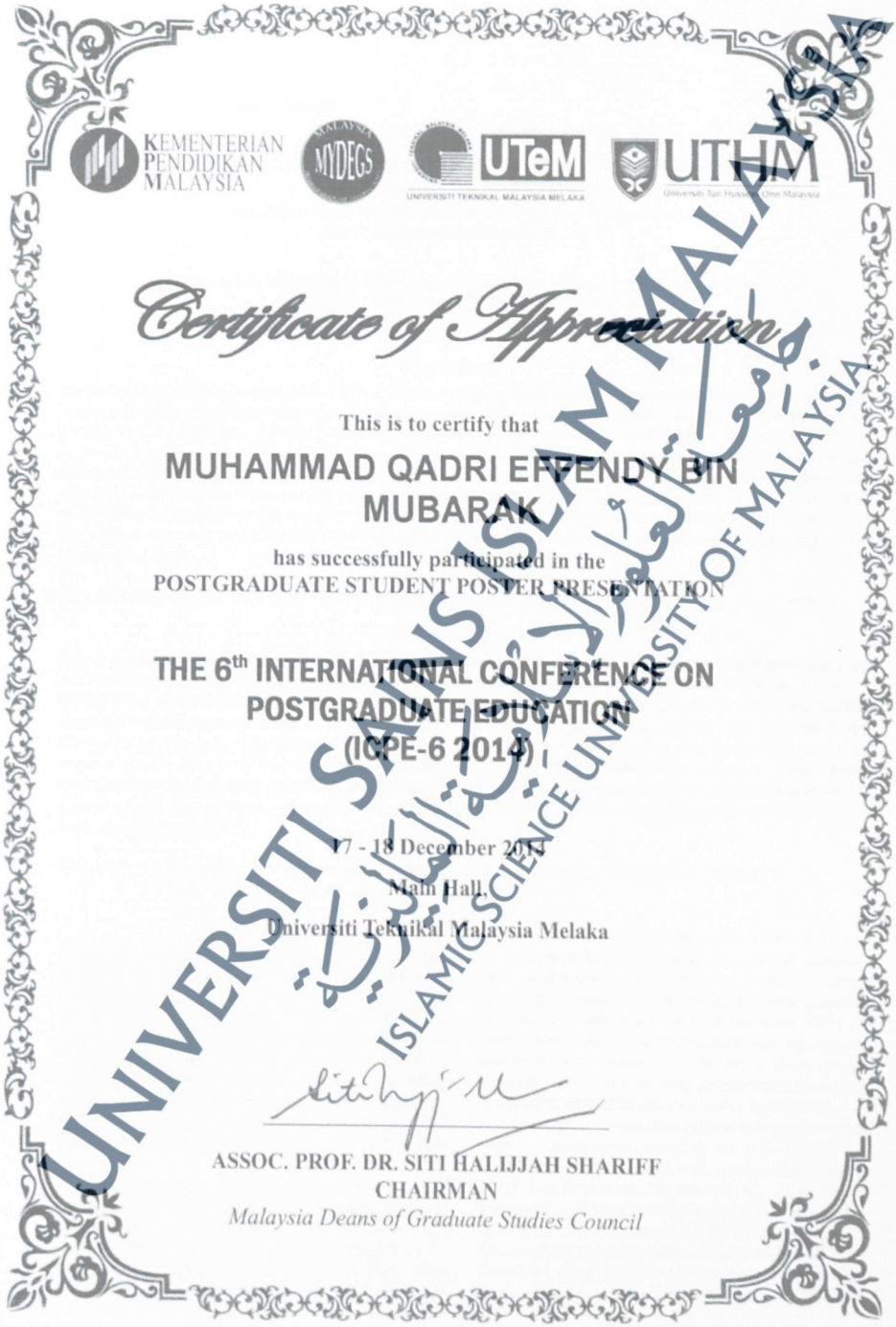
in technical presentation, recognition and appreciation of research contributions to

International Conference on Chemical, Environment & Biological Sciences (CEBS-2014)

IICBE Chair

Sept. 17-18, 2014
Kuala Lumpur (Malaysia)

B.3 Ceritificate from The 6th International Conference on Postgraduate Education (ICPE-6 2014) for poster presentation.



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MUHAMMAD QADRI EFFENDY BIN MUBARAK

has successfully participated in the
POSTGRADUATE STUDENT POSTER PRESENTATION

THE 6th INTERNATIONAL CONFERENCE ON POSTGRADUATE EDUCATION (ICPE-6 2014)

17 - 18 December 2014
Main Hall
Universiti Teknikal Malaysia Melaka

ASSOC. PROF. DR. SITI HALIJJAH SHARIFF
CHAIRMAN
Malaysia Deans of Graduate Studies Council

APPENDIX C

C.1. Paper publish for Sains Malaysiana 44(1)(2015): 115-120 entitled " A Simple and Effective Isocratic HPLC Method for Fast Identification and Quantification of Surfactin".

Sains Malaysiana 44(1)(2015): 115–120

A Simple and Effective Isocratic HPLC Method for Fast Identification and Quantification of Surfactin

(Kaedah Isokratik HPLC Ringkas dan Berkesan bagi Pengenalpastian dan Kuantifikasi Surfaktin dengan Cepat)

MUHAMMAD QADRI EFFENDY MUBARAK, ABDUL RAHMAN HASSAN, AIDIL ABDUL HAMID, SAHAID KHALIL & MOHD HAFEZ MOHD ISA*

ABSTRACT

The aim of this study was to establish a simple, accurate and reproducible method for the identification and quantification of surfactin using high-performance liquid chromatography (HPLC). Previously reported method of identification and quantification of surfactin were time consuming and requires a large quantity of mobile phase. This new method was achieved by application of Chromolith® high performance RP-18 (100 × 4.6 mm, 5 µm) as the stationary phase and optimization of mobile phase ratio and flow rate. Mobile phase consisted of acetonitrile (ACN) and 3.8 mM trifluoroacetic acid (TFA) solution of 80:20 ratio at flow rate of 2.2 mL/min was obtained as the optimal conditions. Totalization time of the obtained surfactin peaks was four times quicker than various methods previously reported in the literature. The method described here allowed for fine separation of surfactin (standard sample >98% purity) and surfactin in fermentation broth.

Keywords: High-performance liquid chromatography (HPLC); isocratic; mobile phase; stationary phase; surfactin

ABSTRAK

Tujuan kajian ini adalah untuk menyediakan satu kaedah yang mudah, tepat dan boleh ulang untuk mengenal pasti dan mengkuantifikasi surfaktin menggunakan kromatografi cecair berprestasi tinggi (HPLC). Sebelum ini dilaporkan kaedah kenal pasti dan kuantifikasi surfaktin memerlukan masa yang panjang dan jumlah fasa bergerak yang banyak. Kaedah baru telah diperoleh dengan menggunakan kolum Chromolith® high performance RP-18 (100 × 4.6 mm, 5 µm) sebagai fasa pegun dan pengoptimuman nisbah dan kadar aliran fasa bergerak. Fasa bergerak terdiri daripada cecair asetonitril (ACN) dan 3.8 mM asid trifluoroacetic (TFA) dengan nisbah 80:20 pada kadar aliran 2.2 mL/min sebagai kadar optimum. Masa analisis yang diperoleh adalah empat kali lebih pantas daripada kaedah yang dilaporkan sebelum ini. Kaedah yang diguna pakai di sini dapat memisahkan piawan surfaktin (>98% ketulenan) dan surfaktin dalam sampel fermentasi dengan baik.

Kata kunci: Fasa bergerak; fasa pegun; isokratik; kromatografi cecair prestasi tinggi (HPLC); surfaktin

INTRODUCTION

Surfactants, which can be either chemically or biologically produced, are defined as surface active agents that have wide ranging and attractive properties (Akbarian et al. 2007). Surfactants produce by biological synthesis are known as biosurfactants and can be generated by a variety of bacteria, yeasts and fungi through utilization of various carbon feedstocks such as sugars and oil (Chen et al. 2007). Biosurfactants are usually produced during the stationary phase and excreted as secondary metabolites during growth in microbial culture broth (Georgiou et al. 1992; Wei et al. 2007).

Biosurfactants offer various advantages over chemical surfactants, including being less toxic, but more biodegradable and environmentally friendly, as well as able to maintain their physico-chemical properties at different temperatures and pH (Mulligan 2005). Due to these attractive properties, there has been increased interest in biosurfactants for applications in various fields, such as food, medical, pharmaceutical, cosmetics and agriculture industries (Banat et al. 2000). In addition, biosurfactants possess several unique therapeutic properties of biomedical importance (Singh & Cameotra 2004) and can exert anti-adhesive activity against several pathogenic microorganisms (Heinemann et al. 2000). These features make biosurfactants favourable alternatives to chemically synthesised surfactants for a variety of applications.

Surfactin also exhibit many pharmacological activities, such as antimicrobial, antiviral, anti-inflammatory and antimycoplasma activity, as well as the ability to inhibit fibrin clot formation and haemolysis. Additionally, they have antitumor activity against Ehrlich's ascites carcinoma cells and can inhibit the cyclic adenosine 3, 5-monophosphate phosphodiesterase (Davies et al. 2001; Fernandes et al. 2007). Research on the formulation, characterization and pharmacokinetics of surfactin

C.2. Paper publish for International Conference on Chemical, Environment & Biological Sciences (ICEBS-2014) entitled "Single-step Crossflow Ultrafiltration for Recovery and Purification of Surfactin Produced by *Bacillus subtilis* ATCC 21332".

International Conference on Chemical, Environment & Biological Sciences (CEBS-2014) Sept. 17-18, 2014 Kuala Lumpur (Malaysia)

Single-step Cross-flow Ultrafiltration for Recovery and Purification of Surfactin Produced by *Bacillus subtilis* ATCC 21332

Muhammad Qadri Effendy Mubarak, Mohd Hafez Mohd Isa, and Abdul Rahman Hassan

***Abstract*—**Surfactin is a powerful biosurfactant that has attractive behavior produced by fermentation of various strains of *Bacillus*. However, the downstream processing is a major obstacle due to the impurities present in the fermentation broth. Downstream technique was applied by a single-step cross-flow ultrafiltration (UF) technique using a benchtop cross-flow filtration unit equipped with a hydrosart membrane with a 10 kDa molecular weight cut-off (MWCO) (HT10) and a polyethersulfone membrane with 10kDa MWCO (PES10) membranes to recover and purify surfactin from the fermentation broth. Four different transmembrane pressures (TMP) varying from 0.5 bar to 2.0 bar were applied for each filtration process. Permeate flux, rejection coefficient (R) of surfactin and protein contents both in permeates and retentates were measured during the UF to evaluate the characteristic of both membranes towards the recovery and purity of the final product. Surfactin was retained almost completely with a rejection coefficient (R) close to 1 for both membranes, with permissible purity ranging from 82% to 88%. Four different TMP applied on the membrane had no significant effect (P>0.05) because the pore size of the membranes were smaller than surfactin micelles. In this study, HT10 achieved better recovery and purity of the final product compared to PES10. Later, FTIR and surface tension measurements were conducted to assess the purity and functionality of the recovered and purified surfactin from both UF membranes used in this study.

***Keywords*—**Hydrosart membrane, polyethersulfone membrane, transmembrane pressure, rejection coefficient.

I. Introduction

Bacillus subtilis is a sporulating gram bacterium that thrives in the soil and is nonpathogenic to human beings [1], enabling its application in various fields and making it one of the most studied Gram-positive bacteria [2]. The ability of *B. subtilis* strains to produce a series of lipopeptides (such as iturin and fengycin) has been documented over 60 years [3] and has created great potential for its application in various fields as an alternative surfactant to replace chemical surfactants. Surfactin is a heptapeptide linked to a β-hydroxy fatty acid chain of 13–16 carbon chains and includes a series of isoforms. It is a high-value biopolymer that offers advantageous properties for mankind. However, surfactin is an expensive lipopeptide, which makes it unable to compete effectively with chemical surfactants because the downstream process contributes up to 60% of the production cost [4] due to the complexity of fermentation broth, which contains impurities such as protein, sugar, lipid, compounds and different types of amino acids [5].

In recent years, a lot of effort has been expended in cutting down the downstream processing cost including using foam fractionation [6], acid precipitation [7], [8] extraction using organic solvent, adsorption chromatography or a combination of these techniques. Unfortunately, these techniques give low surfactin purity (<15%) which is insufficient and improvement in downstream processing techniques and performance is hugely important. In addition, some of the approaches involving multi-step treatment of fermentation broth make it impractical and less attractive for industrial application [9], [10], [11]. In addition, most of the conventional methods dealing with toxic organic solvents such as chloroform and dichloromethane make the final product suffer from the loss of biosurfactant activity. Hence, there is a demand to develop more economic and environmentally friendly method to improve current downstream processing method.

Surfactin separation efficiency from fermentation broth is the essential issue in developing commercial-scale processes. One of the alternative techniques for downstream processing is membrane filtration. There is a lot of interest in applying a membrane system for the purpose of recovery and purification of biosurfactants [9], [10], [11]. Membrane filtration using pressure-driven force applied to a membrane to dissolve and suspend species based on the size and molecular scale [4] is widely used in various chemical and biochemical processes. More importantly, the membrane approach process involves no phase change [12], which enables the molecules structure to be preserved. In much of the literature, membrane filtration meets downstream separation needs because the concentration and purification of the final product surpasses the limitations of traditional methods [13], [14].

Membrane filtration has been considered in this work

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C.3. Paper publish for The 6th International Conference on on Postgraduate Education (ICPE-6 2014) entitled "A Simple and Effective Isocratic HPLC Method for Identification and Quantification of Glucose in Complex Fermentation Broth".

A Simple and Effective Isocratic HPLC Method for Identification and Quantification of Glucose in Complex Fermentation Broth

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Keywords: HPLC, Glucose, Stationary phase, Optimal condition

Abstract

The aim of this study was to establish a simple, accurate and reproducible method for the identification and quantification of glucose in fermentation broth using high-performance liquid chromatography (HPLC) without modification in current HPLC system that equipped with variable wavelength detector (VWD). The method was achieved by the application of Chromolith® high performance NH2 (100 mm x 4.6 mm, 5 µm) column. Mobile phase consisted of 3.8 mM trifluroacetic acid (TFA) solution with flow rate of 0.5 ml/min were pumped at 100% was obtained as the optimal conditions. Under the optimized conditions, the method showed good linearity in the range of 0.1–80.0 g/L with determination coefficients (R^2) (0.998). Limit of detection (LOD) and limit of quantification (LOQ) were 1.0 g/L and 2.0 g/L respectively with good repeatability (RSD<5%, n=5). The proposed method has been successfully applied to the analysis of the glucose in fermentation broth allowed for fine separation of glucose both in glucose solution and fermentation broth.

Introduction

Bioprocess industry especially related to fermentation activities has identified the advantages of doing specific parameters measurements as closely to the fermentation process as possible [1]. In order to make use of increasing knowledge about the process control, new analytical methods have to be invented and applied to meet the intend tendency. Fermentation monitoring covers a vast area, encompassing numerous analytical fields [1] because the effective control of fermentation processes demands the measurement of as many significant parameters as possible as frequently as possible [2]. Therefore, it is important to have an accurate and consistent set of approaches for measurement for each parameter considered in fermentation process to meet with measurement process. Carbohydrates (glucose, fructose, lactose, sucrose, etc) are essential sources for cell growth and product synthesis. It was consumed in biomass growth for biosurfactant production and in biomass maintenance [3]. Glucose is assimilated during bacterial growth until another essential source depleted [4]. Therefore, the measurement of glucose which is often the mainly carbon sources in fermentation process especially for biosurfactant is particular interest [5]. A reliable technique for glucose analysis would undoubtedly improve greatly the efficiency of fermentation monitoring and allow the information for development of new feedback control strategies.

Materials and Methods

Glucose standard and chemicals

All chemical used are analytical grade unless stated. Glucose Anhydrous (J. Kollins) and Trifluroacetic acid (TFA) solution (Merck) of HPLC grade was used. Deionised water was prepared using a system equipped with 0.2µm filter (Elga).