OPTIMIZATION OF MEDIUM USING RESPONSE SURFACE METHODOLOGY FOR ERYTHROMYCIN PRODUCTION BY *Saccharoplyspora erythraea*

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To my beloved mother, father, brothers and sisters, love you all

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ABSTRACT

For the past decades, it was obvious and understood that there was a great industrial interest for optimization of medium components by statistical method for high erythromycin antibiotic production by *Saccharopolyspora erythraea* (formerly known as *Streptomyces erythraea*). In this research, medium components that were already screened in classical method by previous researcher were further optimized using statistical method known as Response Surface Methodology (RSM). Maximum erythromycin and cell dry weight observed in shake flasks studies after medium optimized in RSM were 412.5 mg/l and 4.9 g/l, respectively. It was found that by using optimized medium it increased the production of erythromycin and cell dry weight by 30.43% and 17.3% . Optimization of medium components in statistical method in which media components was first screened in full factorial using Plackett-Burman experimental design, showed that glucose and yeast extract had higher effect on erythromycin production. Further optimization was carried out in RSM using Box-Behnken experimental design in which optimized medium was selected. The optimized medium composed of g/L; glucose 45, yeast extract 8, sodium nitrate 4, dipotasium hydrogen phosphate 2.5, sodium chloride 1.0, and magnesium sulphate 0.5. This optimized medium was further studied in 16-L bioreactor using controlled and un-controlled pH conditions. For controlled pH bioreactor, the maximal erythromycin, and cell dry weight obtained were 567.5 mg/l and 5.65 g/l rspectively. It observed that the maximum erythromycin produced in controlled pH bioreactor was higher 12.9% than that cultivated in bioreactor under un-controlled pH condition and about 15.8% higher than that results obtained in shake flasks when used optimized medium. On the other hand, cell dry weight was about 17.7% higher when compared to cell produced in un-controlled bioreactor.

ABSTRAK

Sepanjang dekad yang lalu, ia adalah jelas dan difahami bahawa terdapat minat yang tinggi oleh industri untuk mengoptimumkan komposisi media dengan kaedah statistik bagi hasil pengeluaran antibiotik erythromycin yang tinggi oleh *Saccharopolyspora erythraea* (dahulunya dikenali sebagai *Streptomyces erythraea*). Dalam kajian ini, komposisi media yang telah ditapis menggunakan kaedah klasik oleh pengkaji terdahulu telah dioptimumkan menggunakan kaedah statistik yang dikenali sebagai Kaedah Permukaan Respon (RSM). Hasil erythromycin maksimum dan berat sel kering yang diperhatikan dalam kajian kelalang goncang selepas media dioptimumkan dalam RSM adalah 412.5 mg/l dan 4.9 g/l. Hasil kajian ini menunjukkan nilai perolehan yang tinggi iaitu 30.43% erythromycin dan 17.3% berat sel kering berbanding keputusan yang diperolehi dalam media yang belum dioptimumkan. Selain itu, pengoptimuman komposisi medium dengan kaedah statistik di mana komponen media telah ditapis dalam faktoran penuh menggunakan rekabentuk eksperimen Plackett-Burman, menunjukkan bahawa glukosa dan ekstrak yis mempunyai kesan yang tinggi terhadap pengeluaran erythromycin. Pengoptimuman selanjutnya telah dijalankan dalam RSM menggunakan rekabentuk Box-Behnken yang mana media yang telah di optimumkan dipilih. Media optimum terdiri daripada g/l; glukosa 45, ekstrak yis 8, natrium nitrat 4, dipotasium hidrogen fosfat 2.5, natrium klorida 1.0, dan magnesium sulfat 0.5.Media optimum ini seterusnya dikaji dalam bioreaktor 16-L menggunakan keadaan pH dikawal dan tidak dikawal. Untuk bioreaktor pH dikawal, erythromycin tertinggi serta beratsel kering yang diperolehi adalah 567.5 mg/l dan 5.65 g/l. Melalui pemerhatian di dapati bahawa erythromycin maksimum yang dihasilkan di dalam bioreaktor pH dikawal adalah lebih tinggi iaitu 12.9% berbanding yang tidak terkawal dan kira-kira 15.8% lebih tinggi berbanding keputusan yang diperolehi dalam termos goncang apabila menggunakan medium dioptimumkan. Berat sel kering, adalah kira-kira 17.7% lebih tinggi daripada bioreactor tidak dikawal.

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LIST OF ABBREVIATION

°C	Degree Celsius
DMS0	Dimethyl sulfoxide
DMF	Dimethylformamide
DO	Dissolved Oxygen
DOT	Dissolved Oxygen Tension
ER	Enoylreductase
FDA	Food and Drug Adminstration
g	Gram
g/L	Gram per liter
GI	Gastrointestinal
HPLC	High Performance Liquid Chromatography
KS	Ketosynthase
KR	Ketoreductase
L	Liter
MAI	*Mycobacterium aviumintracellulare*
MAC	Mycobacterium ovium complex
NCIMB	National Collection of Industrial and Marine Bacteria
PKS	Polyketide synthases
PMV	Packed mycelium volume
RSM	Response surface methodology
rpm	Rotation per minute
TE	Thioesterase
U	Unit for enzyme activity
vvm	Volume/volume/minute
%	Percentage

CHAPTER I

INTRODUCTION

1.1 Background

An antibiotic is defined as an organic compound produced by a microorganism which is inhibitory or repressor to the growth and metabolism of other microorganisms in small concentrations (Satoshi and Yoshitake, 1986; Wolfgang, 2005; Samaneh and Razieh, 2011). Since the time Sir Alexander Fleming discovered Penicillin, first antibiotic in 1928, several antibiotics were isolated but few have been approved in pharmaceutical application because of their mode of action against foreign microorganisms that cause a disease to human being (Oreste, 2003).

Recently success of erythromycin in antibiotic market over the other antibiotics was due to that erythromycin has high quality and it is cheap in price (Ying-ping *et al.,* 2010). It is produced by the strain *Saccharopolyspora erythraea,* originally isolated from soil collected in Philippines (Christopher *et al.,* 2001). It belongs to the chemical class of antibiotics known as macrolides. Structurally it contains a large lactone ring linked with amino sugars through glycosidic bonds (Xiang *et al.,* 2008; Samaneh and Razieh, 2011). Clinically, it is mainly used to cure

respiratory infection related diseases (Wolfgang, 2005; Ying-ping *et al*., 2010). With regard to antimicrobial spectrum and clinical utility, it matches to penicillin, but it is also active against organisms that become resistant to penicillin and streptomycin. For this reason, it is often recommended or suggested to those patients with allergies when penicillin is prescribed.

As stated by Ju *et al.* (2008) recently, erythromycin received much attention because of the increasing applications of its semi-synthetic modified derivatives to infection diseases, such as azithromycin, roxithromycin and clarithromycin. Research done by El-Enshasy *et al*. (2007) described that nowadays erythromycin was also used for treatment of feeding intolerance in preterm infants, and as an antimalarial in combination with other drugs to reduce the pathogen resistance.

Several researchers were previously studied improvement and optimization of the medium components for high erythromycin production using classical medium optimization. However, according to existed literature review pertains to the optimization and productions of this antibiotic, few studies have previously conducted optimization of medium components such as carbon, nitrogen and phosphate sources using response surface methodology (RSM).

The aim of this study was to optimize medium components for high erythromycin antibiotic production by the strain *S. erythraea* via submerged fermentation using statistical technique known as response surface methodology (RSM). Moreover, the growth curve of media before and after optimized was also experimented, in addition effects of pH controlled and uncontrolled mode towards the antibiotic production was performed in 16-L Bioreactor

1.2 Problem Statement

Medium components for high erythromycin production by *Saccharopolysora erythraea* have been researching for several years and it was observed that the cultivation of erythromycin was affected by the composition of cultivation media. According to previous researchers, very few authors were reported the optimization of media compositions for high erythromycin production by statistical method. Therefore, there is a great demand for optimization of medium composition by the application of response surface methodology to maximize erythromycin production, decrease time and cost.

1.3 Objective of the Study

The main objective of the present work is to maximize erythromycin production through optimization of media composition by *Saccharopolyspora erythraea* WICC-B12 using Response Surface Methodology (RSM) , and to study kinetics of erythromycin production in 16-L bioreactor under controlled and un-controlled-pH mode.

1.4 Scope of the Study

Three scopes were focused to achieve the research objective

1. Optimization of media compositions, using response surface methodology (RSM) in a shake flask.
2. Study growth curve kinetics of erythromycin before and after medium optimization in shake flask.
3. Study the effect of controlled and uncontrolled pH condition towards erythromycin production in 16-L Bioreactor.

REFERENCES

Bohumil, S. (1983). *Methods in Industrial Microbiology*. England: Ellis Horwood Limited.

Carrington, R. (1986). A Review of Antibiotics Isolation Techniques. *In* Stowel, J.D., Bailey, P.P. and Winstanley, D.J. (Ed.).*Bioactive Microbial Products 3: Down Stream processing* (pp. 45-58). London: Academic press.

Chen, H.C. and Wilde, F. (1990).The effect of dissolved oxygen and aeration rate on antibiotic production of *Streptomyces fradiae. Biotechnology and Bioengineering*, 37, 591-595.

Christopher, C., Scott, F., Sally. O., Lawrence, C., Stefan, Z., Elaine, W., Timothy, L., John, C., Mark, B., Salej, P., Gary, A. and Peter, L. (2001). *Saccharoplyspora erythraea*-catalyzed bioconversion of 6-deoxyerythronolide B analogs for production of novel erythromycins. *Biotechnology*, 92, 217-228.

Dongzhi, W., Xiangzhao, M., Yaling, Sh., Liang, Y., Shi, C., Yuping, Y., Jingya, Y., Hu, Z. and Zixing, D. (2007). Optimizing medium composition for

accumulation of the FR-008/Candicidin derivatives CS101 by a mutant of *Streptomyce sp*. Using statistical experimental methods. *Process Chemistry*, 42, 878-883.

El-Diwany, A., Mohamed, A., Hesham, A. and El-Sayed, A. (2000).Optimization of the cultivation medium for natamaycin production by *Streptomyces natalensis. Basic Microbiology*, 3, 157-166.

El-Enshasy, H.A., Mohamed N.A., Farid, M.A, and El-Diwany, A.L. (2007). Improvement of erythromycin production by *Saccharopolyspora erythraea* in molasses based medium through cultivation medium optimization. *Bioresource Technology*, 99, 4263-4268.

Elmahdi, I., Bangz, F., Dixon, K., Harrop, T., Sugden, D. and Lye, G.J. (2003).pH control in microwell fermentations of *S. erythraea* CA340: influence on biomass growth kinetics and erythromycin biosynthesis. *Biomedical Engineering*, 16, 299-310.

Feng, C. and Xiandi, G. (1997). Influence of medium components on astaxanthin and production of *Haematococus pluvialis. Process Biochemistry*, 4, 385-391.

Gavin, J.C., David, L. and Michael, E.B. (1996). Oxygen limitation can induce microbial secondary metabolite formation: investigations with miniature electrodes in shaker and bioreactor culture. *Microbiology*, 141, 663-669.

Gary, W.O. (2000*). A First Course in Design and Analysis of Experiments*. New York: W.H. Freeman Press.

Guillaume, E.V. and Michelle, S. (2005). Experimental Design in Microbiology.*In* Jose, L.B.(Ed.). *Microbial Processes and Products* (pp. 25-63). Totowa, New Jersey: HUMANA Press.

Hassan, A., Narmeen, A. and Geban M. (2010). Response Surface Methodology as a tool for optimizing the production of antimicrobial agent from *Bacillus licheniformis* SN2. *Current Research in Biotechnology*, 3, 1-14

Hamedi, J., Rostamza, M. and Noohi, A. (2007). Enhancement in production of erythromycin by *Saccharopolyspora erythraea* by the use of suitable industrial seeding-media. *DARU*, 16, 1-12.

Herbert, A. (2002). Introduction to the macrolide antibiotics. *In* Schonfeld, W. and Krist, H.A. (Ed.).*Macrolide Antibiotics* (pp. 1-11). Berlin: Burkhauser Press.

Ison, A.P., Lilly, M.D. and Heydarian, S.M. (1996). The Effect of culture conditions on the production of erythromycin by *S. erythrae* in batch culture. *Biotechnology Letters*, 18, 1181-1186.

Ju, C., Xiang, Z., Hai-feng, H., Ying-ping, Z. and Si-liang, Z. (2008). Oxygen uptake rate optimization with nitrogen regulation for erythromycin production and scale-up from 50 L to 372 m^3 scale. *Bioresource Technology*, 100, 1406-1412.

Katz, L. (1997). Manipulation of modular polyketide synthases. Chemistry Reviews, 97, 2557-2576.

Katz, L. and Donaldo, S. (1993).Polyketide synthesis: Prospects of hybrid antibiotics. *Annual Review Microbiology*, 47, 875-912.

Kheirolomon, A., Kazemi, A.V., Arjumad, M. and Doosty, H. (2001).Optimization of penicillin G Acylase production. *Sceintia Iranica*, 8, 159-165.

Nereida, C., Edie, M., Milagros, C. and Piere, C. (2002).Optimization of a culture medium containing fish silage for L-lysine production by *Corynebacterium glutamicum*. *Bioresource Technology*, 85, 207-2011.

Oreste, A. (2003). *Bacteria versus Antimicrobial Agent: an Integrated Approach* (pp. 240-270). Washington: ASM Press.

Peter, G. (2002). *The Optimal Design of Blocked and Split-Plot Experiments*. New York: Springer Press.

Peter, F., Patrick, C., Debra, J. and James, S. (1992). Identification of DEBS 1, DEBS 2 and DEBS 3, the multi enzyme polypeptide of the erythromycin-producing polyketide synthase from *Saccharopolyspora erythraea. Federation of European Biochemical Societies (FEBS)*, 23, 235-228.

Pualine, M. (1995).*Bioprocess Engineering Principles*. London: Academic Press Limited.

Samaneh, Y. and Razieh, R. (2011).Effect of medium composition fermentation conditions on erythromycin production by *S. erythraea*. *Clinical Biochemistry*, 8, 992-998.

Satoshi, O. and Yoshitake, T. (1986).Macrolide Antibiotics.*Biotechnology.* 4 (5), 360-387.

Stanbury, P.F. and Whitaker, A.(1984*). Principles of Fermentation Technology*. Oxford: Pergoman Press.

Staunton, J. and Wilkinson, B.(1997) Biosynthesis of erythromycin and rapamycin. *Chemistry Reviews*, 97, 2611-2630.

Thaer, (2011) Production of antibacterial metabolites by strain no.10/2 (*S. albovinaceus*) and media optimization studies for the maximum metabolite production. *IJPI's Biotechnology and Biotherapeutics*, 5, 222-228.

Tripathi, C. and Vineeta, S. (2008). Production and statistical optimization for a novel olivanic acid by *Streptomyces olivaceus* MTCC 6820. *Process Chemistry*, 43, 1313-1317.

Thomas, P. R. (2007). *Modern Experimental Design.* New Jersey: John Wiley & Sons, Inc.

Wolfgang, M. (2005).Production of Erythromycin with *Saccharopolyspora erythraea*. *In* Jose, L.B.(Ed.). *Microbial Processes and Products* (pp. 65-89). Totowa, New Jersey: HUMANA Press.

Xing, Z., Yonghong, W., Xiangling, F., Fengqiu, A. and Guhong Wang.(2011) improvement of antibiotic activity of *Xenorhabdus bovienii* by medium optimization using response surface methodology. *Microbial Cell Factories,* 10, 98-107.

Xiang, Z., Hai-feng, H., Changa-fa, C., Ju, C., Ying-ping, Z. and Si-liang, Z. (2008).Application of oxygen uptake and response surface methodology for erythromycin production by *Saccharopolyspora erythraea. Industrial Microbial Biotechnology*, 35,1637-1642.

Ying-ping, Z., Xiang, Z., Hai-feng, H., Changa-fa, C., Ju, C. and Si-liang, Z. (2010).Response surface methodology for optimization of the erythromycin production by Fed-batch fermentation using an inexpensive biological nitrogen source. *Biochemical Engineering*, 24, 95-100.