

OPTIMIZATION OF MEDIA COMPOSITIONS AND
FERMENTATION CONDITIONS FOR ENHANCEMENT OF
SURFACTIN PRODUCTION BY RESPONSE SURFACE
METHODOLOGY (RSM)

UNIVERSITI SAINS ISLAM MALAYSIA
جامعة العلوم الإسلامية
ISLAMIC SCIENCE UNIVERSITY OF MALAYSIA

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UNIVERSITI SAINS ISLAM MALAYSIA

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METHODOLOGY (RSM)

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Thesis submitted in fulfilment for the degree of
MASTER OF SCIENCE

Faculty of Science and Technology
UNIVERSITI SAINS ISLAM MALAYSIA

Nilai

November 2015

APPROVAL

The thesis entitled “**Optimization of Media Compositions and Fermentation Conditions for Enhancement of Surfactin Production by Response Surface Methodology (RSM)**” submitted to the Faculty of Science and Technology, Universiti Sains Islam Malaysia and was accepted as fulfillment of the requirements for the degree of Master of Science.



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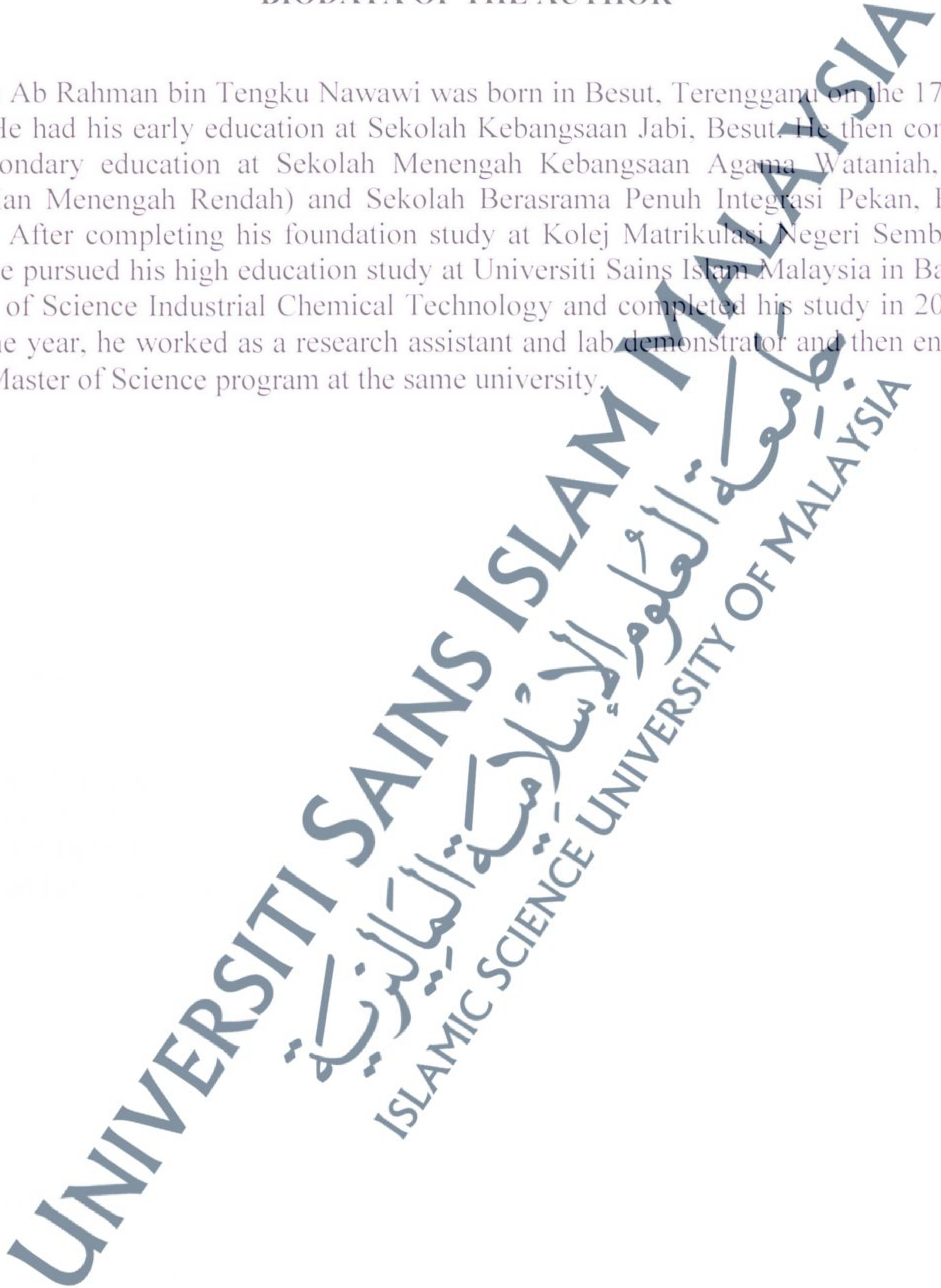
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ACKNOWLEDGEMENTS

First and foremost, I am grateful to Allah S.W.T., for the good health and the strength that was necessary to complete this study and made this dissertation possible.

Although only my name appears on the cover of this thesis, a huge deal of people has contributed to its completion. Thusly, I offer my sincerest gratitude to my supervisor, Dr Mohd Hafez bin Mohd Isa, who has supported me throughout my thesis with his perseverance and expertise since I began working as research assistant under his supervision. I attributed the level of my Master degree to his sincere encouragement.

I am also indebted to the lectures at Chemistry Department in USIM whom I have interacted during the course of my graduate studies particularly Dr Zaiton, AP Dr Salina, AP Dr Siti Salhah and Dr Shukri for the many constructive discussions that helped me understand my research area better.

In my daily work, I have been blessed with a friendly and comforting group of fellow students and staff of USIM. A great appreciation toward my lab mates (Qadri, Awatif, Hajar and Shima) who exhibited excellent support and help during my Master study. The same goes to En. Freddy, Kak Sarina, Kak Normah, Puan Siti Aminah that provided well-equipped facility for me to finish my lab work. I also want to take this opportunity to express the profound gratitude for my deep heart to all members of Faculty of Science and Technology for their willingness guidance and assistance for me to complete this thesis.

Most importantly, none of this would have been possible without the love and patience of my family. As the matter of the fact, I dedicated this thesis to my father Tengku Nawawi, my mother Rukiah and my siblings who have been a constant source of love, concern, support and strength all these years.

Abstrak

Surfaktin adalah biosurfaktan lipopeptida yang dihasilkan oleh pelbagai baka *Bacillus subtilis*. Surfaktin menunjukkan sifat permukaan-aktif yang sangat baik dan sifat-sifat biologi bermanfaat yang mungkin penting dalam pelbagai bidang saintifik dan industri. Dalam eksperimen ini, pencilan tempatan *B. subtilis* dinamakan sebagai *B. subtilis* 3M telah digunakan dan dikulturkan ke dalam media Cooper. Dengan menggunakan teknik fermentasi kelalang goncang, pelbagai faktor komposisi media dan keadaan fermentasi memberi kesan terhadap pengeluaran surfactin dan biojisim telah dioptimumkan menggunakan kaedah tindak balas permukaan (RSM). Glukosa (20-60 g/L), ammonium nitrat (0.03-0.07 M), ferus sulfat (40-200 μ M) dan mangan sulfat (0.5-2.5 mM) mewakili faktor untuk komposisi media. Sementara itu, masa fermentasi (48-144 jam), jumlah inokulum (1-20 %) dan suhu (20-50 °C) telah dipilih sebagai faktor untuk keadaan fermentasi. Julat bagi setiap faktor telah ditentukan dengan menggunakan kaedah pengoptimuman satu-faktor-pada-satu-masa (OFAT). Seterusnya, reka bentuk komposit pusat (CCD) yang digabungkan dengan RSM telah digunakan untuk mengoptimumkan komposisi media dan kaedah fermentasi bagi pengeluaran surfaktin dan biomas dalam kultur media. Kemudian, data eksperimen telah dimasukkan ke dalam persamaan polinomial tertib kedua dengan menggunakan analisis regresi berganda dan diikuti pula dengan analisis varians (ANOVA). Plot tindak balas permukaan 3D daripada model matematik telah digunakan untuk menentukan keadaan optima. Untuk komposisi media, hasil pengeluaran optima surfaktin diperhatikan ketika media Cooper ditambah dengan

43.4 g/L glukosa, 0.052 M ammonia nitrat, 140 μ M ferus sulfat dan 1.73 mM mangan sulfat. Dalam keadaan ini, hasil maksima surfaktin telah diramalkan pada 190.8 mg/L. Bagi keadaan fermentasi, hasil optima pengeluaran surfaktin diperhatikan ketika media Cooper dalam keadaan masa penapaian ke 93 jam, 8.2% daripada jumlah inokulum dan suhu pada 25 °C. Surfaktin diperhatikan telah terhasil sebanyak 249.7 mg/L melalui syarat-syarat tersebut. Secara keseluruhannya, kedua-dua kaedah optimasi komposisi media dan keadaan fermentasi dengan menggunakan RSM terbukti sangat berkesan meningkatkan hasil surfaktin berbanding dengan media Cooper konvensional

Abstract

Surfactin is a lipopeptide biosurfactant produced by various strains of *Bacillus subtilis*. Surfactin showed excellent surface-active properties and prominent biological properties that may be important in various scientific and industrial fields. In this experiment, local isolate of *B. subtilis* denoted as *B. subtilis* 3M was used and cultured into the Cooper's media. By using shake flask fermentation technique, various parameters of media compositions and fermentation conditions (in Cooper's media) that affected surfactin and biomass production were optimized using response surface methodology (RSM). Glucose (20-60 g/L), ammonium nitrate (0.03-0.07 M), ferrous sulfate (40-200 μ M) and manganese sulfate (0.5-2.5 mM) represented factors for media compositions. On the other hand, fermentation time (48-144 h), inoculum volume (1-20 %) and temperature (20-50 $^{\circ}$ C) were selected as factors for fermentation conditions. The ranges for each factor were determined by applying one-factor-at-a-time (OFAT) optimization. Henceforth, central composite design (CCD) was employed combining with RSM to optimize both media compositions and fermentation conditions for the production of surfactin and biomass in the media culture. Thusly, the experimental data was fitted to a second-order polynomial equation using multiple regression analysis and analyzed using analysis of variance (ANOVA). The 3D response surface plots from the mathematical models were utilized to determine optimum conditions. For media compositions, optimal surfactin production was observed of Cooper's media supplemented with 43.4 g/L glucose, 0.052 M ammonium nitrate, 140 μ M ferrous sulfate and 1.73 mM manganese sulfate. Under these

conditions, the maximum predicted yield of surfactin was 190.8 mg/L. As for fermentation conditions, optimal surfactin production was observed of Cooper's media in the conditions of 93 hours of fermentation time, 8.2 % of inoculum volume and 25 °C of temperature. Surfactin yield under these conditions was observed at 249.7 mg/L. Thus, optimization of both media compositions and fermentation conditions by using RSM managed to enhance surfactin yield to some extent significantly than conventional Cooper's media.



الملخص

سورفكتين هي سلالات مختلفة تنتجها من العصومة الرقيقة المسمى بسيلوس سوبتليس (Bacillus subtilis). وقد أظهر سورفكتين سطحها نشطة ممتازة وفيها الخصائص البيولوجية الجيدة البارزة التي قد تكون مهمة في مجالات العلمية والصناعية. وبدأ هذه التجريبية باختار البنسيلين المحلي المسمى ب سوبتليس (B. Subtilis 3M) ووضع في موضع مثقف في مغذ كوبر (Cooper's media)، وبه هذا الترتيب المذكور عاها وجعل على الماكينة هزة قارورة التخمير، ثم استوعبها بمعايير مختلفة من وسائل الاعلام في تركيب التجريبية وظروف التخمر المتنوع (في مغذ كوبر) في تحصيل الانتاج المناسب الجيد ومع ذلك باستخدام منهج الاستجابي السطحي (RSM)، ومن العوامل للإعلام التراكيبي في الجلوكوز (20 – 60 جرام/لتر)، ونترات الأمنيوم (0.03 – 0.07 م)، وكبيرتات الحديدوز (40 – 200 ميكرون)، وكبيرتات المنغنيز (5.0 – 5.2 مم). وأما الوقت التخمير للتجريبية فاخياره ما بين 48 و 144 ساعات، وحجم اللقاح هو 1-20%، ودراسة الحرارة هي ما بين 20 – 50 درجة مئوية، وكلها المذكورة هي العوامل للظروف التخمير، ولكل عامل له مقدار المتوسط أو المدى باستخدام المنهج (OFAT) – المنهج تطبيق عامل واحد على واحد في وقت. وبعد ذلك، لزيادة منهج التصميم على المنهج الاستجابي السطحي (RSM) وهو المنهج تصميم مركب المركزي (CCD) ليتناول كل وسائل الإعلام التراكيبي وكل عوامل للظروف التخمير في ثور الانتاج لسورفكتين (Surfactin). وبعد ذلك التركيب جعلت معلومات الانتاج إلى جهاز لتركيز البيانات التجريبية فحسبها في معادلة متعددة للحدود من الدرجة الثانية باستخدام المنهج تحليل الانحدار المتعدد ثم حلها بطريقة تحليل التباين (ANOVA)؛ واستخدمت المؤامرات للسطحي 3D استجابة من النماذج الرياضية لتحديد الظروف المثلى. وبالنسبة لوسائل الإعلام الت، فرأى الاستنتاج ما كان في مغذ كوبر حينما أضافه بزيادة 43.4 غرام/ لتر الجلوكوز، و 0.052 م نترات الأمنيوم، و 140 ميكرومتر كبيرتات الحديدوز، و 1.73 ملي كبيرتات المنغنيز. وفي ظل هذه المرحلة، توقع أقصى الوزن من سورفكتين 190.8 ملي غرام/لتر. وبالنسبة لظروف التخمر، فرأى استنتاج ما كان في مغذ كوبر في الساعة 93 من التجريبية، وحينما وصل 8.2% حجم اللقاح، وحينما وقع في الدرجة الحرارة 25 درجة مئوية. وقد أدرك أن وزن لسورفكتين بعد التجريبية هو 249.7 ملي غرام/ لتر بشروط الخاصة.

والنتيجة بين وسائل الإعلام التراكيب وظروف التخدير باستخدام المنهج الاستجابي السطحي (RSM) هي أن (RSM) تمكن أن تزداد العائد لسورفكتين إلى حد كبير من وسائل الاعلام كوبر التقليدية، وهي طريقة قديمة.

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ABBREVIATIONS

3D	Three dimensional
°C	Degree Celsius
ACN	Acetonitrile
ANOVA	Analysis of variance
BHI	Brain heart infusion
CaCl ₂	Calcium chloride
CCD	Central composite design
CMC	Critical micelle concentration
CV	Coefficient of variation
DHB	2,5-dihydroxybenzoic acid
EDTA	Ethylenediamine tetraacetic acid
HCl	Hydrochloric acid
HPLC	High pressure liquid chromatography
Na ₂ HPO ₄	Disodium hydrogen phosphate
FDA	Food and Drug Administration
FeSO ₄	Ferrous sulfate
g	Relative centrifugal force
h	hour
HSV	Herpes simplex virus
g/L	gram per liter
KH ₂ PO ₄	Potassium dihydrogen phosphate
MALDI-ToF	Matrix-assisted laser desorption/ionization – time of flight
MgSO ₄	Magnesium sulfate
MnSO ₄	Manganese sulfate

MRSA	Methicilin-resistant <i>Staphylococcus aureus</i>
NaNO ₃	Sodium nitrate
NH ₄ NO ₃	Ammonium nitrate
OFAT	One-factor-at-a-time
Rpm	round per minute
RSM	Response surface methodology
TFA	Trifluoroacetic acid
v/v	volume/volume
VW	Visible wavelength
vvm	volume per volume per minute