Single-step Ultrafiltration Technique for Recovery and Purification of Surfactin from Fermentation Broth

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ABSTRAK

Teknik Ultraturasan (UF) Tunggal untuk Pengekstrakan dan Pemurnian Surfaktin dari Cecair Penapaian

Beberapa penemuan dan prosedur telah ditambahbaik dalam kajian ini. Metode analisis surfaktin dan gula yang terdapat dalam cecair penapaian menggunakan Sistem Kromatografi Cecair Berprestasi Tinggi (HPLC) telah berjaya ditambahbaik untuk menjadikannya lebih peka dan cepat tanpa melakukan pengubahasuain yang ketara terhadap system yang sedia ada dengan hasil analisis pada minit ke 8 dan ke 5. Analisis yang cepat ini menjadikan ianya sangat praktikal untuk mengukur secara cepat semasa melakukan kerja optimasi. Seterusnya, kajian kinetik menyediakan maklumat yang lebih baik bagi memahami proses interaksi antara sel bakteria dan penggunaan glukosa ke atas hubungkait pengeluaran surfaktin oleh dua jenis bakteria iaitu, *Bacillus subtilis* MSH1 dan *Bacillus subtilis* ATCC 21332. Kepekatan maksimum surfactin (P_{max}) yang dicapai oleh *B. subtilis* MSH1 dan *B . subtilis* ATCC 21332 masing-masing adalah 226.17 mg/L dan 447.26 mg/L. Kajian kinetik menunjukkan pertumbuhan sel bakteria bagi *B. subtilis* MSH1 mempunyai nilai kadar pertumbuhan maksimum (μ_{max}), 0.224 h^{-1} dan mencapai kepekatan biomas maksimum (X_{max}) setinggi 2.90 g/L selepas 28 jam proses penapaian berlaku, manakala *B. subtilis* ATCC 21332, dengan nilai μ_{max}, 0.087 h^{-1} dan nilai X_{max}, 2.62 g/L selepas 45 jam proses penapaian. Dalam keadaan penapaian yang sama, *B. subtilis* MSH1 menunjukkan pertumbuhan kinetik yang lebih tinggi berbanding *B. subtilis* ATCC 21332 dan mempamerkan nilai-nilai μ_{max} dan X_{max} yang lebih baik. Kemudian, surfaktin yang ada dalam cecair penapaian telah diekstrak dengan menggunakan unit ultraturasan yang dilengkapi dengan membran hydrosart (HT) dan membran polietersulfon (PES) dengan saiz liang membrane iaitu 10 kDa dan 30 kDa (MWCO) menggunakan tekanan transmembran (TMP) di antara 0.5-2.0 bar. Fluks, pekali penolakan (*R*) surfaktin dan jumlah kandungan protein meresap dan tertinggal diukur semasa proses UF untuk menilai ciri-ciri semua membran ke arah pemulihan dan penulenan produk akhir surfactin. Surfaktin dapat dikekalkan dengan nilai *R* menghampiri dengan 1.00 bagi semua membran, dengan kadar penulenan diantara 82% hingga 88%. TMP yang dikenakan tidak mempunyai kesan yang signifikan ($P < 0.05$) pada R kerana liang membran bersaiz lebih kecil daripada surfaktin micelles. HT10 menunjukkan pemulihan dan ketulenan yang lebih baik untuk produk akhir berbanding dengan membran lain yang digunakan dalam penyelidikan ini. Kemudian, analisis ketegangan permukaan dan FTIR telah dilakukan untuk menilai fungsi produk akhir surfaktin. Keputusan menunjukkan nilai yang hampir sama dengan surfaktin standard walaupun dengan kehadiran bendaasing, namun ianya tidak menjejaskan fungsi asal surfaktin. Selain itu, FTIR spektrum mengesahkan kewujudan rantaian hydrocarbon dan juga ikatan peptide yang menunjukkan kewujudan struktur surfaktin.

ABSTRACT

Single-step Ultrafiltration (UF) Technique for Recovery and Purification of Surfactin from Fermentation Broth

Several findings and method were improvises as for the downstream processing of surfactin production. An efficient and sensitive High Perfomance Liquid Chomatography (HPLC) procedure for qualitative and quantitative analysis of surfactin and glucose present in complex fermentation has been successfully improvised without modification of current HPLC equipped with Variable Wavelength Detector (VWD) , with the total elution time of 5 minutes and 8 minutes respectively. This rapid analysis makes this technique ideal for fermentation broth optimization and assessment during fermentation process development. The method has been validated and has proven to quantify the glucose and surfactin respectively in standard solution as well as in fementation broth.The kinetic studies provides important kinetics information for better understanding of interactions of bacterial cell growth and glucose consumption towards surfactin production by *B. subtilis* MSH1 and *B. subtilis* ATCC 21332. The maximum concentration of surfactin (P_{max}) achieved by *B. subtilis* MSH1 and *B. subtilis* ATCC 21332 was 226.17 mg/L and 447.26 mg/L, respectively. The kinetic study of bacterial cell growth of both strains indicated that *B. subtilis* MSH1 had a specific growth rate (μ_{max}) of 0.224 h^{-1} and attained a maximum biomass concentration (X_{max}) as high as 2.90 g/L after 26 h of fermentation, while *B. subtilis* ATCC 21332, with μ_{max} of 0.087 h^{-1}, attained an X_{max} of 2.62 g/L after 45 h of incubation. Under identical fermentation conditions, *B. subtilis* MSH1 showed higher growth kinetics compared with *B. subtilis* ATCC 21332 and exhibited higher values of μ_{max} and X_{max}. Later, the surfactin present in fermentation broth were extracted by using benchtop cross-flow ultrafiltration unit equipped with hydrosart membrane(HT) and polyethersulfone membrane (PES) with a 10 kDa and 30 kDa molecular weight cut-off (MWCO) using transmembrane pressures (TMP) varying from 0.5-2.0 bar. Permeate flux, rejection coefficient (R) of surfactin and total protein contents in permeates and retentates were measured during the UF to evaluate the characteristic of all membranes towards the recovery and purity of the surfactin final fraction. Surfactin was retained almost completely with a rejection coefficient (R) close to 1.00 for all membranes, with permissible purity ranging from 82% to 88%. The TMPs applied had no significant effect ($P<0.05$) on R because the membranes pore size were smaller than surfactin micelles. HT10 was achieved better recovery and purity of the final product compared to other membrane used. Later, product characterization analysis was conducted to evaluate the functionality of surfactin final fraction by using surface tension and Fourier Transform Infra-red (FTIR). Result showed close proximity of surface activity in relation to surfactin standard which indirectly indicated the presence of impurities in the final fraction did not affect the original surfactin functionality. FTIR spectra confirmed that the UF retentate contains aliphatic hydrocarbons as well as a peptide-like moiety, which correspond to the structure of surfactin.

ملخص البحث

النتائج والأساليب العديدة كانت مرتجلة في هذا البحث. اسلوب تحليل surfactin والسكر موجود في سائل المتخمر باستخدام نظام High Perfomance Liquid Chomatography (HPLC) كانت ناجحة في إصلاحها لتكون أسرع و اكثر حساس بدون تغيّر على نظام الأصلي في نتيجة التحليل دقيقة 5 و 8. هذا تحليل سريع يجعلها أكثر عملية ليقيسها سريعة عند ممارسة الأعمال التحسينية. ويعد، زود البحث الحركي أحسن المعلومات لفهم عملية المعاملات تنمية خلية البكتريا و استعمال غلوكوز على العلاقة في تخريج surfactin من اثنان بكتريين أي من *Bacillus subtilis* MSH1 و *Bacillus subtilis* ATCC 21332 الحد الأقصى surfactin أي (P_{max}) الذي حققته *B. subtilis* MSH1 و *B. subtilis* ATCC 21332 على التوالي 226.17 ملغم / لتر و 447.26 ملغم / لتر. تظهر البحث الحركي نمو الخلايا من البكتيريا *B. subtilis* MSH1 لديه معدل النمو الأقصى(μmax) ، ($0.224\ H^{-1}$)، ويصل الحد الأقصى لتركيز الكتلة الحيوية (Xmax) حتى (2.90 جم / لتر) بعد 28 ساعة من حدوث التخمر، و ل *B. Subtilis* ATCC 21332, بقيمة μmax ، $0.08\ H^{-1}$و بقيمة X_{max}, هو 2.62 g/L بعد 48 ساعة من حدوث التخمر. في ظروف التخمر ذاتها، *B. subtilis* MSH1 أظهرت حركية النمو العالي مقارنة *B. subtilis* ATCC 21332 وعرض أفضل قيم μ_{max} و X_{max} ، استخرج surfactin موجود في السائل التخمير باستخدام وحدة الترشيح الفائق مجهزة (غشاء HT و polyethersulfone) غشاء PES الغشاء مع حجم المسام أي (10 كيلو دالتون) و (30 كيلو دالتون (MWCO)باستخدام الضغط عبر الغشاء (TMP) كانوا في مجموعة من 0،5-2،0 بار.يقيس التدفق و رفض معامل R surfactinومحتوى البروتين الكلي منشر واليسار أثناء عملية UF لتقييم خصائص الغشاء نحو الانتعاش وتنقية surfactin المنتج النهائي. يستطيع ان يبقى Surfactin بقيمة R قرباً ب 1.00 لجميع الأغشية، ومعدل تنقية من 82٪ إلى 88٪. كان TMP لا يأثره شديدا ($P < 0.05$) في R بسبب حجم المسام غشاء أصغر من surfaktin micelles. يظهر HT10 الانتعاش ونقاء المنتج النهائي أفضل من الأغشية الأخرى المستخدمة في هذا البحث. ثم، تم إجراء التوتر السطحي وتحليل FTIR لتقييم وظيفة المنتجات بالسطح. أظهرت النتائج أن تقريبا نفس السطحي الخاصية يحمي في وجود جسم غريب، ولكنه لا يؤثر على وظيفتها الأصلية السطحي. بالإضافة إلى ذلك، أطياف FTIR تؤكد وجود السلاسل الهيدروكربونية والسندات الببتيد، مما يدل على وجود هيكل السطحي.

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LIST OF ABBREVIATION

°C	Degrees Celsius
µ	Micro
ACN	Acetonitrile
ATCC	American Type Culture CollectionFTIR
B.	*Bacillus*
CMC	Criticial Micelle Concentration
CP	Concentration polarization
Da	Dalton
Eq	Equation
FTIR	Fourier Transform Infra-Red
g	gram
H	Hours
HPLC	High Performance Liquid Chromatography
HT	Hydrosart
J_f	pure water of fouled membrane
J_s	raw broth flux
J_w	the pure water flux of clean membrane
L	Liter
LC-MS	Liquid Chromatography Mass Spectrometry
LOD	Limit of Detection
LOQ	Limit of Quantification
M	Molarity
mg	Miligram

Min	Minutes
MSM	Mineral salts medium
NaOH	Sodium Hydroxide
PES	Polyethersufone
PES	Polyethersulfone
RSD	relative standard deviation
sp.	species
TFA	Trifluoroacetic Acid
TMP	Transmemmbrane Pressure
TPA	Total Peak Area
UF	Ultrafiltration
VWD	Variable Wavelength Detector