UNIVERSITI PUTRA MALAYSIA

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By

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CHARACTERIZATION AND OPTIMIZATION OF BIOFLOCCULANT DERIVED THROUGH IMPLEMENTATION OF SUBMERGED AND SOLID-STATE FERMENTATION OF *Bacillus subtilis* UPMB13

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October 2014

Chair : Mohd Kamil bin Yusoff, PhD

Faculty : Environmental Studies

Breakthroughs in bioproduction field have opened up vast opportunities in the exploration of bio-based products as substitutes to chemical derivatives for water treatment technologies. In that framework the interest of finding an ecologically benign solution specifically focusing on treating suspended solids pollution was explored. As production cost becomes the limiting factor which restricts wider applications of bioproducts for alternative water treatment, fermentation technology was applied in this study; basic substrates were utilized through non-elaborative techniques for bioproduction of a biopolymeric flocculant. The main goal of this study is to produce a biopolymer with flocculating capabilities which can substitute the commonly used commercial flocculants through two fermentation strategies; namely, the submerged (SmF) and solid-state (SSF) fermentations. The flocculating performances were measured through kaolin assays based on the clarity of the suspension and the visible flocs formed after treatment with the bioflocculant. The characterizations of the bioflocculants produced were scrutinized for further understanding of their nature and properties which contributed to their flocculating abilities. The bioflocculant produced through the better fermentation strategy was further studied for its flocculating performances and in comparison to other commercial flocculants. A novel low molecular weight (10-50 x 10^3 Da), high flocculating biopolymer denoted as UPMBF13 was successfully produced through *de novo* pathway from the SmF and the SSF of *Bacillus subtilis* UPMB13. It was found to consist of poly-γ-glutamic acid and polysaccharide derivatives, with hydroxyl, carboxyl, methoxyl and carbonyl functional groups and was either fibrous (SmF) or granular (SSF) in natures, which are the major known characteristics of a bioflocculant. The best production strategy for UPMBF13 was found to be through the SmF by manipulating the optimum conditions (media: no additional supplement; duration: 24-72 hrs; temperature: 25-30^{o}C; pH: 7.0-8.0; shaking

speed: 100-200 rpm) for growth. This led to a maximum performance of 95% in flocculating activity with large visible floc formations, comparable to those from the commercial flocculant polyacrylamide (maximum activity: 98%), and superior to that of polyaluminium chloride (maximum activity: 47%). The production of UPMBF13 through SSF was also verified to be possible, but yielded an inferior product (maximum activity: 71%) with barely any flocs formed upon treatment. Furthermore, the SmF strategy yielded at an average two-fold the amount of UPMBF13 at 2.70 g/L while the SSF produced about 1.25 g/kg in 72 hrs. Overcoming the inferior performance of the SSF by scaling-up the process to a pilot-scale level (near-to-adiabatic, non-sterilized condition with continuous oxygen flow) led to a competitive environment where the autochthonous microbes proliferated over UPMB13 and produced their own bioflocculants which obscured the performance of UPMBF13. In general, the results from this study confirmed that the production of UPMBF13 is feasible through *de novo* pathway with no additional input of L-glutamic acid supplement. High flocculating performance was achieved solely with basic substrates without further manipulations and modifications. Furthermore, UPMBF13 is cation-independent once extracted and purified, requiring no additional cation source for its application in suspended solid treatments.

Keywords: *Bacillus subtilis* UPMB13, bioflocculant, extracellular polymeric substance, biopolymer, submerged fermentation, solid-state fermentation

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENCIRIAN DAN PENGOPTIMUMAN BIOFLOKULAN YANG DIHASILKAN MELALUI IMPLEMENTASI KAEDAH FERMENTASI TERENDAM DAN FERMENTASI KEADAAN PEPEJAL KE ATAS *Bacillus subtilis* UPMB13

Oleh

ZUFARZAANA ZULKEFLEE

Oktober 2014

Pengerusi : Mohd Kamil bin Yusoff, PhD

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Kejayaan cemerlang dalam bidang biopembuatan telah membuka banyak peluang dalam penerokaan produk berasaskan biologi sebagai pengganti kepada derivatif-derivatif kimia dalam teknologi rawatan air. Selaras dengan itu, kepentingan mencari penyelesaian yang lebih mesra alam, khususnya dalam merawat pencemaran pepejal terampai telah diterokai. Oleh kerana kos pengeluaran telah dikenal pasti sebagai faktor pengehad yang menyekat penggunaan bioproduk sebagai rawatan air alternatif secara meluas, teknologi fermentasi telah digunakan dalam kajian ini; substrat asas telah digunakan melalui teknik mudah untuk biopembuatan flokulan berasaskan biopolimer. Matlamat utama kajian ini adalah untuk menghasilkan sejenis biopolimer dengan keupayaan flokulasi yang boleh menggantikan flokulan-flokulan komersial yang biasa digunakan melalui dua strategi fermentasi iaitu kaedah fermentasi terendam (SmF) dan fermentasi keadaan pepejal (SSF). Kadar prestasi flokulasi diukur melalui ujian menggunakan kaolin berdasarkan kejernihan ampaian dan penghasilan flok selepas rawatan menggunakan bioflokulan tersebut. Pencirian bioflokulan yang dihasilkan diteliti untuk pemahaman lebih lanjut tentang sifat-sifat asas yang menyumbang kepada kebolehannya sebagai flokulan. Bioflokulan yang dihasilkan melalui kaedah fermentasi yang lebih baik telah dikaji lebih lanjut bagi mengenalpasti faktor bagi meningkatkan kadar prestasi flokulan dan mengkaji perbandingan prestasi bioflokulan tersebut dengan beberapa flokulan komersial. Sebuah biopolimer novel yang berprestasi tinggi dengan jisim molekular rendah (10-50 x 10^3 Da), dinamakan sebagai UPMBF13 telah berjaya dihasilkan melalui laluan *de novo* dari SmF dan SSF *Bacillus subtilis* UPMB13. Ia didapati terdiri daripada asid poli-γ-glutamik dan derivatif polisakarida dengan kumpulan berfungsi hidroksil, karboksil, metoksil and karbonil serta bersifat samada bergentian (SmF) atau berbutir (SSF), di mana kesemuanya dikenali sebagai ciri-ciri

major bioflokulan. Kaedah fermentasi terendam telah dibuktikan sebagai kaedah terbaik bagi pengeluaran UPMBF13 melalui manipulasi pertumbuhan dan penyediaan keadaan optimum (media: tiada supplemen tambahan; tempoh: 24-72 jam; suhu: 25-30^{o}C; pH: 7.0-8.0; kelajuan goncangan: 100-200 rpm). Ini telah membawa kepada kadar prestasi flokulasi maksimum sebanyak 95% dengan pembentukkan flok besar dapat diperhatikan, setanding dengan flokulan komersial poliakrilamida (flokulasi maksimum: 98%) dan lebih tinggi daripada polialuminium klorida (flokulasi maksimum: 47%). Kaedah fermentasi keadaan pepejal juga telah dibuktikan mampu dilaksanakan bagi pengeluaran UPMBF13, namun menghasilkan produk yang lebih rendah prestasi (flokulasi maksimum: 71%) dengan hampir tiada pengahasilan flok. Tambahan pula, kaedah SmF berjaya menghasilkan UPMBF13 pada kuantiti dua kali ganda; iaitu 2.7 g/L, manakala kaedah SSF hanya menghasilkan 1.25 g/kg UPMBF13 dalam masa 72 jam. Bagi mengatasi prestasi rendah kaedah SSF, percubaan menaikkan skala proses SSF di tahap skala perintis (keadaan hampir adiabatik, tidak steril dengan aliran oksigen berterusan) telah meningkatkan persekitaran yang lebih berdaya saing di mana mikroorganisma sedia ada yang hadir dalam substrat telah mengatasi pertumbuhan UPMB13 dan menghasilkan bioflokulan mereka sendiri yang mengatasi prestasi UPMBF13. Secara amnya, hasil kajian mengesahkan pengeluaran UPMBF13 boleh dilaksanakan melalui laluan *de novo* tanpa memerlukan penambahan asid L-glutamik. Kadar prestasi flokulasi yang tinggi telah dicapai dengan hanya menggunakan substrat asas tanpa manipulasi dan pengubahsuaian lanjut. Tambahan lagi, UPMBF13 bebas kebergantungan terhadap kation setelah diekstrak, oleh itu tiada sumber kation tambahan yang diperlukan bagi merawat pencemaran pepejal terampai.

Kata kunci: *Bacillus subtilis* UPMB13, bioflokulan, bahan polimer luar sel, biopolimer, fermentasi terendam, fermentasi keadaan pepejal

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I certify that a Thesis Examination Committee has met on 27 October 2014 to conduct the final examination of Zufarzaana binti Zulkeflee on her thesis entitled "Characterization and Optimization of Bioflocculant derived through Implementation of Submerged and Solid-state Fermentation of *Bacillus subtilis* UPMB13" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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