



UNIVERSITI PUTRA MALAYSIA

***PRODUCTION AND CHARACTERIZATION OF A BIOFLOCCULANT FROM
ASPERGILLUS FLAVUS AND ITS APPLICATION IN WATER TREATMENT***

AHMAD H. RAJAB ALJUBOOR

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TREATMENT**

By

AHMAD H. RAJAB ALJUBOORI

**Thesis Submitted to the School of Graduate Studies, University Putra
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Philosophy**

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Faculty : Engineering

Flocculants are useful agents in the aggregating of colloids, cells and suspended solids. Various flocculants are commonly used in industrial fields. These flocculants can be classified into inorganic, organic synthetic and naturally occurring flocculants. The use of inorganic and organic synthetic flocculants has caused some environmental and health problems. For example, the aluminum element in polyaluminium chloride it could induce Alzheimer's disease and polyacrylamide derivatives contain acrylamide monomers which are both neurotoxic and carcinogens.

In present study, the production and characterization of a bioflocculant named IH-7 by *Aspergillus flavus* was investigated to determine the optimal culture medium composition, environmental conditions, flocculation efficiency, flocculation mechanism and its application in water treatment.

The highest production was obtained when sucrose and peptone with C/N ratio of 5/1 were used in production media with initial pH 7 and the cultivation was incubated for 60 hours at 40°C. About 0.4 g of purified bioflocculant with average molecular weight of 2.574×10^4 Da, could be recovered from 1.0 L of fermentation broth. Chemical analysis showed that the purified IH-7 was mainly consisted of 28.5% protein and 69.7% polysaccharide. Fourier-transform infrared (FTIR) spectra indicated the presence of hydroxyl, amide, carboxyl and methoxyl groups in IH-7 molecules. The elemental analysis of purified IH-7 revealed that the weight fractions of the elements C, H, N, O and S were 29.9, 4.8, 3.3, 34.7 and 2.0%, respectively. The IH-7 bioflocculant showed good flocculation performance without cation addition. Thus, the study suggested charge neutralization is the flocculation mechanism of IH-7.

The study found bioflocculant IH-7 is a cation-independent bioflocculant able to flocculate activated carbon, kaolin clay, soil solids and yeast cells without cations addition. Overall, the IH-7 flocculation performance was better than PAC at a wide range of flocculant concentrations, 0.5 mg/L of bioflocculant IH-7 was able to achieve more than 90% of flocculating efficiency, within pH range of 4 to 8 and initial kaolin concentrations range of 0.5 to 10 g/L. In addition, IH-7 was found to be a thermo-stable bioflocculant which has great potential to replace common chemical flocculants in cold weather countries, as well as in treating turbid water with salinity up to 10% w/w. The treatment of river water by IH-7 bioflocculant in comparison with Polyaluminium chloride (PAC) showed that purified IH-7 performed better than PAC and crude IH-7 in treating river water. Water with final turbidity 5 NTU, total

solids 3.3 mg/L and Chemical Oxygen Demand 9.9 mg/L could be achieved when 4 mg/L of IH-7 was used to treat the river water. The use of IH-7 and PAC in discoloration of dye solutions for four types of dyes (Reactive Black 5, Methylene Blue, Fast Green and Crystal Violet), showed IH-7 bioflocculant and PAC were more effective to discolor the anionic dye solutions than cationic dye solutions.

This study concluded, extracellular polymeric substance named IH-7 produced by *Aspergillus flavus* has excellent flocculation properties for many types of suspended solids at wide range of conditions. In addition, IH-7 performed better than synthetic chemical flocculant in surface water treatment and discoloration applications.

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

**PENGHASILAN DAN PENCIRIAN BIOFLOKULAN DARIPADA
ASPERGILLUS FLAVUS DAN APLIKASINYA DALAM RAWATAN AIR**

Oleh

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Flokulan adalah agen yang berguna dalam mengagregat koloid, sel-sel dan pepejal terampai. Pelbagai flokulan biasanya digunakan dalam bidang perindustrian. Flokulan–flokulan ini boleh dikelaskan kepada flokulan bukan organik, sintetik organik dan semulajadi. Penggunaan flokulan bukan organik dan sintetik organik telah menyebabkan beberapa masalah alam sekitar dan kesihatan. Sebagai contoh, elemen aluminium dalam polialuminium klorida mampu menyebabkan penyakit Alzheimer dan terbitan daripada poliacrilamida mengandungi monomer acrilamida yang neurotoksik dan juga bersifat karsinogenik.

Dalam kajian semasa, penghasilan dan pencirian bioflokulan yang dinamakan sebagai IH-7 oleh *Aspergillus flavus* telah dikaji untuk menentukan komposisi medium kultur yang optimum, keadaan alam sekitar, kecekapan proses pemberbukuan (*flocculation*), mekanisma pemberbukuan dan aplikasinya dalam rawatan air.

Penghasilan tertinggi telah diperoleh apabila sukrosa dan pepton dengan nisbah C/N sebanyak 5/1 telah digunakan dalam medium penghasilan dengan pH permulaan 7 dan kulat telah dieram selama 60 jam pada 40°C. Kira-kira 0.4 g bioflokulan yang telah ditulenkan dengan purata berat molekul 2.574×10^4 Da, mungkin boleh diperoleh daripada 1.0 L air rebusan penapaian. Analisis kimia menunjukkan bahawa IH-7 yang ditulenkan sebahagian besarnya terdiri daripada protein 28.5% dan polisakarida 69.7%. Spektrum Fourier-transform inframerah (FTIR) menunjukkan kehadiran kumpulan – kumpulan hidroksil, amida, karboksil dan metoksil dalam molekul IH-7. Analisis unsur IH-7 tulen mendedahkan bahawa pecahan berat unsur-unsur C, H, N, O dan S adalah masing - masing sebanyak 29.9, 4.8, 3.3, 34.7 dan 2.0%. Bioflokulan IH-7 menunjukkan prestasi pemberbukuan yang baik tanpa tambahan kation. Oleh itu, kajian ini mencadangkan bahawa peneutralan caj adalah mekanisme pemberbukuan untuk IH-7.

Kajian mendapati bioflokulan IH-7 adalah bioflokulan bebas kation yang dapat menggumpal karbon yang diaktifkan, tanah liat kaolin, pepejal tanah dan sel-sel yis tanpa tambahan kation. Secara keseluruhan, prestasi pemberbukuan IH-7 adalah lebih baik daripada PAC pada julat kepekatan flokulan yang besar, 0.5 mg/L bioflokulan IH-7 mampu mencapai lebih daripada 90% kecekapan pemberbukuan, dalam julat pH 4 hingga 8 dan julat kepekatan awal kaolin antara 0.5 hingga 10 g/L. Di samping itu, IH-7 telah didapati sebagai bioflokulan stabil suhu yang mempunyai potensi besar untuk menggantikan flokulan kimia yang biasa digunakan di negara-negara bercuaca sejuk, serta dalam merawat air yang keruh dengan

kemasinan sehingga 10% w/w. Rawatan air sungai oleh bioflokulan IH-7 jika dibandingkan dengan Polialuminium klorida (PAC) menunjukkan bahawa IH-7 yang dituliskan bertindak lebih baik daripada PAC dan IH-7 mentah dalam merawat air sungai. Air dengan kekeruhan akhir 5 NTU, jumlah pepejal 3.3 mg/L dan Permintaan Oksigen Kimia 9.9 mg/L boleh dicapai apabila 4 mg/L IH-7 digunakan untuk merawat air sungai. Penggunaan IH-7 dan PAC dalam penyahwarna larutan pewarna untuk empat jenis pewarna (Reaktif Hitam 5, Metilena Biru, Fast Green dan Violet Kristal), menunjukkan bioflokulan IH-7 dan PAC lebih berkesan untuk menyahwarna larutan pewarna anionik berbanding larutan pewarna kationik.

Kajian ini merumuskan bahawa bahan polimer luar sel yang dinamakan IH-7 yang dihasilkan oleh *Aspergillus flavus* mempunyai ciri-ciri pemberbukuan yang unggul untuk pelbagai jenis pepejal terampai dalam pelbagai keadaan. Di samping itu, IH-7 menunjukkan prestasi yang lebih baik daripada flokulan kimia sintetik dalam rawatan air permukaan dan aplikasi penyahwarna.

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I certify that a Thesis Examination Committee has met on 08 / 05 / 2012 to conduct the final examination of Ahmad H. Rajab Aljuboori on his thesis entitled "Production and Characterization of a Bioflocculant from *Aspergillus flavus* and Its Application in Water Treatment" 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.

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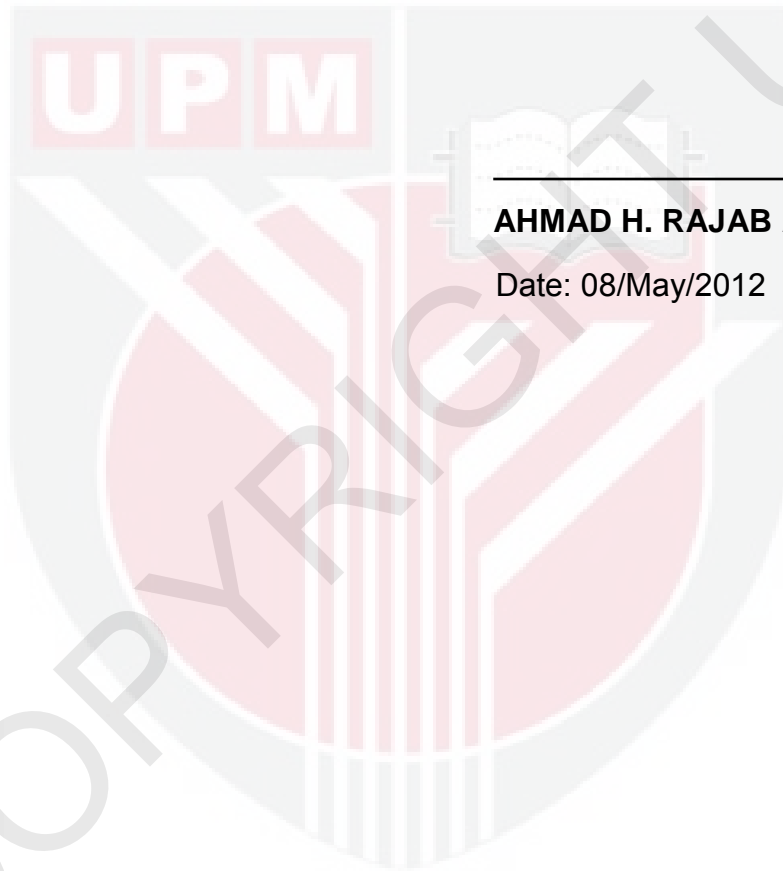
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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.



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Date: 08/May/2012

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