

BIODEGRADATION OF REMAZOL BLACK B BY BACTERIAL
CONSORTIUM NAR-2

SEYEDEH NAZANIN KARDI

A dissertation submitted in partial fulfillment of the
requirements for the award of the degree of
Master of Science (Biotechnology)

Faculty of Biosciences and Medical Engineering
Universiti Teknologi Malaysia

JULY 2013

BIODEGRADATION OF REMAZOL BLACK B BY BACTERIAL
CONSORTIUM NAR-2

SEYEDEH NAZANIN KARDI

UNIVERSITI TEKNOLOGI MALAYSIA

*Specially dedicated to my beloved Dad and Mom, Reza
Kardí and María Hadíghí.*

*To my adorable husband
Níma
And
My granny*

ACKNOWLEDGMENTS

My gratitude to God Almighty, because with His blessings and grace, this thesis has finally seen its end.

I wish to express my sincerest appreciation to Prof. Dr. Noor Aini Abdul Rashid for her comments, encouragement, constructive advice and guidance throughout the process of completing this project. Thank you for the opportunity given to me.

I would like to thank our lab assistants and all research assistants. Million thanks to post graduate Azura Ahmad for her invaluable assistance and useful discussions. My appreciation also goes to all lecturers in the Department of Biosciences for their advice and the knowledge shared in the past one and half years.

Last but not least, my utmost appreciation to my loving parents, Reza and Maria for their eternal support, undying love, sacrifices and encouragement I am nothing without you both. Special thanks go to my adorable husband, Nima for the support and sacrifices.

ABSTRACT

The ability of the bacterial consortium NAR-2 consisting of A1, C1 and L17 to degrade the model azo dye Remazol Black B (RBB) was studied in batch and in continuous systems. Continuous decolourisation was performed in a borosilicate glass column (12 mm x 20 mm) packed with Surfactant Modified Clinoptilolite immobilised with bacterial consortium NAR-2. In batch studies, 90.79% decolourisation of RBB was achieved under microaerophilic condition within 80 minutes by inoculating 10% (v/v) of bacterial consortium NAR-2 at a 1:1:1 ratio. This was achieved in modified P5 medium pH 7 and incubated at 45°C under microaerophilic condition. In column bioreactor studies, decolourisation was observed at 45°C and carried out by varying the flow rates and dye concentrations. Flow rate at 0.2, 0.4, 0.6, 0.8, and 1.0 ml/min were tested and dye concentration of 0.1, 0.3, 0.5, 0.7, and 1.0 g/L were used. Almost 95.87% decolourisation of 0.1 g/L RBB was achieved at the flow rate 0.2 ml/min. By fixing 0.2 ml/min as default flow rate, varying concentrations of RBB were examined. Above 90% decolourisation was achieved with 0.1, 0.3 and 0.5 g/L RBB but at 0.7 and 1.0 g/L the percentage drop to 36 and 28%, respectively. Decolourisation percentage began to drop at higher dye concentration. Biomass leached out from the column was determined using viable cell count. From both flow rate and dye concentration experiments, it can be seen that C1 cell wash out was the highest as compared to A1 and L17. Analyses of decolourized and biodegradation products of RBB using total aromatic amines (TAA) showed that reduction of RBB resulted in the formation of aromatic amines. Further aerobic degradation for 15 days showed the amines concentration reduced from an initial of 18 mg/L to 2 mg/L following aerobic treatment in batch whereas in column experiment, the amines concentration dropped significantly from 34 mg/L to 11 mg/L.

ABSTRAK

Keupayaan konsortium bakteria NAR-2 terdiri daripada A1, C1 dan L17 untuk menyahwarnakan model azo pewarna Remazol Black B (RBB) telah dikaji dalam kelompok dan dalam sistem lengkap berterusan. Penyahwarnaan lengkap berterusan dilakukan dengan menggunakan kolum kaca borosilika (12 mm x 20 mm) dimampatkan dengan konsortium bakteria NAR-2 yang disekat gerak di atas clinoptilolite dengan permukaan yang telah diubah suai dengan surfaktan. Dalam eksperimen kelompok, 90.79% penyahwarnaan RBB telah dicapai di bawah keadaan mikroaerofilik dalam tempoh 80 minit dengan menginokulasi 10% (v/v) konsortium bakteria NAR-2 pada nisbah 1:1:1. Ini telah dicapai dalam medium P5 terubah suai pada pH 7 dan dieram pada 45°C di bawah keadaan mikroaerofilik. Dalam eksperimen kolum bioreaktor, penyahwarnaan telah diperhatikan pada 45°C dan dijalankan dengan mengubah kadar alir dan kepekatan pewarna. Kadar alir 0.2, 0.4, 0.6, 0.8, dan 1.0 ml/min, dan kepekatan pewarna 0.1, 0.3, 0.5, 0.7, dan 1.0 g / L telah dikaji. Hampir 95.87% penyahwarnaan 0.1 g/L RBB telah dicapai pada kadar alir 0.2 ml/min. Dengan menetapkan 0.2 ml/min sebagai kadar alir tentu awal, RBB pada kepekatan berbeza diperiksa. Lebih daripada 90% penyahwarnaan dicapai dengan 0.1, 0.3 dan 0.5 g/L RBB tetapi pada kepekatan 0.7 dan 1.0 g/L, peratusan menurun kepada 36 dan 28%, masing-masing. Peratusan penyahwarnaan mula berkurangan pada kepekatan pewarna yang lebih tinggi. Biomas yang terlarut lesap dari kolum ditentukan dengan menggunakan kiraan sel berdaya hidup. Berdasarkan kedua-dua eksperimen kadar alir dan kepekatan pewarna, dapat dilihat bahawa sel C1 yang terlarut resap adalah yang tertinggi berbanding A1 dan L17. Analisis produk ternyahwarna dan biodegradasi RBB menggunakan jumlah amina aromatik (TAA) mengesahkan bahawa penyahwarnaan RBB menghasilkan amina aromatik. Lanjutan degradasi aerobik selama 15 hari menunjukkan kepekatan amina menurun daripada 18 mg/L kepada 2 mg/L dalam eksperimen kelompok manakala dalam eksperimen kolum, kepekatan amina menurun dengan ketara daripada 34 mg/L hingga 11 mg/L.