DECOLOURIZATION OF TEXTILE DYES AND TEXTILE INDUSTRY EFFLUENT IN A FIXED BED BIOFILM REACTOR USING NATIVE MICROORGANISMS

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Degree of Doctor of Philosophy

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DECLARATION OF THE CANDIDATE AND SUPERVISOR

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Abstract

Textile and apparel industry produces huge quantities of wastewater with unfixed dyes, which generate colour and toxicity in discharged water, creating environmental pollution. Physical and chemical effluent decolourization techniques are widely used at present to remove colour in effluents in textile industries, however, they have several drawbacks and therefore not productive. Compared to physical and chemical methods, biological treatments have gained much attention globally as environmental-friendly and cost-effective techniques to decolourize textile industry effluent. Hence, in this work, decolourization potential of textile dyes by microbial strains, which were isolated from local-environment, and their applicability in industrial wastewater decolourization were investigated.

Five bacterial strains, with dye decolourizing potential were isolated from an effluent treatment facility of a local textile industry and identified using 16S rRNA gene sequencing analysis. Ability of these strains to decolourize selected textile dyes as individual strains and in a bacterial consortium was investigated using free bacterial cells cultured in 250 ml Erlenmeyer flasks containing 100 ml of decolourization media. Out of the isolated bacteria, *Proteus mirabilis* showed the highest capability to decolourize all dyes and was able to decolourize 50 ppm dye solutions of Yellow EXF, Red EXF, Blue EXF, Black WNN and Rhodamine under static conditions at 35 °C. Colour removal of 96, 94, 83, 95 and 30% respectively was observed after 72 h of treatment when decolourization media was inoculated with 2% (v/v) of bacterial culture. The developed bacterial consortium composed of *Proteus mirabilis*, *Morganella morganii* and *Enterobacter cloacae*, decolourized more than 90% of all four reactive dyes and 36% of Rhodamine dye after 72 h of incubation. Furthermore, the developed bacterial consortium was able to decolourize more than 83% of the synthetic dye mixture and 60% of the textile industry effluent, respectively after 46 h and 138 h of incubation at 35 ^{o}C temperature under static condition.

Effects of physico-chemical parameters (pH, temperature, concentration of dye, agitation and sources of carbon) for biological decolourization of dyes were studied in batch cultures with free cells. It was observed that dye decolourization was more effective under oxygen-limited, static conditions than shaking conditions and the maximum decolourization of dyes was observed at 40 ^{o}C and pH 7-8 in the media containing yeast extract as the carbon source.

Dye decolourization was further investigated in a fixed bed biofilm reactor where the biofilm was composed with the developed bacterial consortium. Decolourization of the synthetic dye mixture was done with three different concentrations of yeast extract in the feed and more than 90% decolourization of the synthetic dye mixture was observed when the concentration was 2 and 1 g/l in batch operation of the reactor. However, even when the concentration was reduced to 0.25 g/l, 75% decolourization of synthetic dye mixture was achieved in both batch and continuous operation of the reactor. Results showed that dye decolourization was more effective with attached cells (bacterial consortium) in the reactor than with free cells (used in flasks). Stability of the dense microbial communities in biofilms and their ability to survive and degrade dyes at extreme conditions could be the reason for observed high colour removals in the decolourization studies conducted in the reactor. Structural changes occurred in dyes due to biological treatments were studied using ultraviolet-visible spectral and high-performance liquid chromatography analyses. Metabolites formed due to biological degradation were analyzed using gas chromatography-mass spectrophotometry and found to be non-toxic and benign.

A maximum of 45% colour removal was observed when the diluted textile effluent was treated in the fixed bed biofilm reactor operated in continuous mode whereas 70% colour removal was achieved in 48 h with undiluted textile wastewater treated in batch mode. This shows the ability of the developed bacterial consortium to endure in highly complex and toxic environment in the fixed bed biofilm reactor and the potential application in textile industry wastewater treatment.

Key words: biological, decolourization, dyes, fixed bed biofilm reactor, textile effluent

DEDICATION

Dedicated to my parents and husband for their unconditional love, endless support and encouragement.

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LIST OF ABBREVIATIONS

AEBR	Anaerobic expanded bed reactor
AQDS	Anthraquinone-2, 6-disulfonate
AQS	Anthraquinone-2-sulfonate
BLAST	Basic local alignment search tool
BOD	Biochemical oxygen demand
CEA	Central environmental authority
COD	Chemical oxygen demand
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleoside triphosphate
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
EPL	Environmental protection license
EPS	Extracellular polymeric substances
EtBr	Ethidium bromide
FAD	Flavin adenide dinucleotide
FADH	Reduced form of flavin adenide dinucleotide
FBBR	Fixed bed biofilm reactor
FBR	Fluidized bed reactor
FMN	Flavin adenide mononucleotide
FTIR	Fourier-transform infrared spectroscopy
GCMS	Gas chromatography–mass spectroscopy
GOTS	Global organic textile standards
HDPS	High density polystyrene
HPLC	High performance liquid chromatography
HRT	Hydraulic retention time
IV	Intravenous tubing
LB	Luria–Bertani
MBBR	Moving bed biofilm reactor
MRSL	Manufacturing restricted substances list
MSDS	Material safety data sheets

MTBE	Methyl tertiary butyl ether
MTP	Microtiter plate
NADH	Reduced form of nicotinamide adenine dinucleotide
NADPH	Reduced form of nicotinamide adenine dinucleotide phosphate
NCBI	National center for biotechnology information
NIST	National institute of standards and technology
OD	Optical density
PCR	Polymerase chain reaction
PE	Polyethylene
PMMA	Poly (methyl methacrylate)
PP	Polypropylene
PTFE	Polytetrafluoroethylene
PU	Polyurethane
PVC	Polyvinyl chloride
RBC	Rotating biological contactors
rRNA	Ribosomal ribonucleic acid
SAMBR	Submerged anaerobic membrane bioreactor
SBR	Sequencing batch reactor
SEM	Scanning electron microscope
TAE	Tris-acetate-EDTA
UASB	Up-flow anaerobic sludge blanket
US EPA	United States environmental protection agency
UV	Ultraviolet
ZDHC	Zero discharge of hazardous chemicals