TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABREVIATIONS AND SYMBOLS

μ	-	specific growth rate
λ	-	wavelength
$(NH_4)_2SO_4$	-	ammonium sulphate
Abs	-	absorbance
ADMI	-	American Dye Manufacturers Institute
Al	-	aluminum
APHA	-	American Public Health Association
AWW	-	autoclaved wastewater
BOD	-	biological oxygen demand
C	-	carbon
C.I	-	Colour Index
$CaCl_2$	-	calcium chloride
Cd	-	cadmium
CDM	-	chemically defined medium
CDW	-	cell dry weight
Cl	-	chloride
Co	-	cobalt
COD	-	chemical oxygen demand
Cr	-	chromium
$CrO4_2^-$	-	chromates
CrO_7^{2-}	-	dichromates
Cu	-	copper

d^{-1}	-	per day
DAF	-	dissolve air floatation
dATP	-	deoxyadenosine 5'-triphosphate
dCTP	-	deoxycytosine 5' triphosphate
DF	-	dilution factor
dGTP	-	deoxyguanosine 5' triphosphate
DNA	-	deoxyribonucleic acid
dNTP	-	deoxynucleotide triphosphate
DO	-	dissolve oxygen
DOE	-	Department of Environment
dTTP	-	deoxythymidine 5'-triphosphate
EDTA	-	ethylene diamine tetra acetic acid
FAD	-	flavin adenine dinucleotide(oxidized)
$FADH_2$	-	flavin adenine dinucleotide(reduced)
$FeCl_3$	-	ferric chloride
FMN	-	flavin adenine mononucleotide (oxidezed)
$FMNH_2$	-	flavin adenine mononucleotide (reduced)
FSTW	-	filter sterilized textile wastewater
gL^{-1}	-	gram per litre
gt	-	generation time
H^+	-	hydrogen ion
h^{-1}	-	per hour
H_2S	-	hydrogen sulphide
H_2SO_4	-	acid sulphuric
HCl	-	hydrochloric acid
HNO_3	-	acid nitric
HPLC	-	high performance liquid chromatography
HRT	-	hydraulic retention time
HSO_3^-	-	sulphite
IR	-	infrared
K	-	potassium

K_2CrO_4	-	potassium dichromate
K_2HPO_4	-	dipotassium hydrogen phosphate
kb	-	kilobase
KH_2PO_4	-	potassium dihydrogen phosphate
M	-	Molarity
mgL^{-1}	-	milligram per litre
$MgCl_2$	-	magnesium chloride
$MgSO_4.7H_2O$	-	magnesium sulphate heptahydrate
MIC	-	minimal inhibitory concentration
MW_r	-	molecular weight relative
N_2	-	nitrogen gas
Na	-	sodium
NaCl	-	sodium chloride
NAD	-	nicotinamide adenine dinucleotide
NAD^+	-	nicotinamide adenine dinucleotide(oxidized)
NADH	-	nicotinamide adenine dinucleotide(reduced)
NADP	-	nicotinamide adenine dinucleotide phosphate
NADPH	-	nicotinamide adenine dinucleotide phosphate(reduced)
NaOH	-	sodium Hydroxide
NB	-	nutrient broth
NH_4Cl	-	ammonium chloride
NH_4NO_3	-	ammonium nitrate
Ni	-	nikel
nm	-	nanometer
NMR	-	nuclear magnetic resonans
NO_3^-	-	nitrate
O_2	-	oxygen gas
OD_{600nm}	-	optical density at 600nm
Pb	-	plumbum
PCR	-	polymerase chain reaction
PHB	-	polyhydroxybutyrate

PO_4^{3-}	-	phosphate
ppm	-	part per million
Pt-Co	-	platinum cobalt
PVC	-	polyvinylchloride
RB15	-	reactive blue 15
RM	-	redox mediator
RNase	-	ribonuclease
rpm	-	rotation per minute
RR195	-	reactive red 195
rRNA	-	ribosomal RNA
S	-	sulphur
SBR	-	sequencing batch reactor
SDS	-	sodium dodecyl sulphate
SEM	-	scanning electron microscope
SFRed	-	sufimix supra red
SO_4^{2-}	-	sulphate
TAE	-	tris-acetate buffer
TCA	-	tricarboxylic acid cycle
td	-	doubling time
T_m	-	melting point
TOC	-	total organic carbon
TON	-	total organic nitrogen
Tris	-	2-hydroxymethyl-2-methyl-1,3-propanediol
TSS	-	total suspended solid
U	-	enzyme unit
UV	-	ultraviolet
UV-vis	-	ultraviolet-visible
v/v	-	volume per volume
w/v	-	weight per volume
Zn	-	zink

LIST OF APPENDICES

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABREVIATIONS AND SYMBOLS

μ	-	specific growth rate
λ	-	wavelength
$(NH_4)_2SO_4$	-	ammonium sulphate
Abs	-	absorbance
ADMI	-	American Dye Manufacturers Institute
Al	-	aluminum
APHA	-	American Public Health Association
AWW	-	autoclaved wastewater
BOD	-	biological oxygen demand
C	-	carbon
C.I	-	Colour Index
$CaCl_2$	-	calcium chloride
Cd	-	cadmium
CDM	-	chemically defined medium
CDW	-	cell dry weight
Cl	-	chloride
Co	-	cobalt
COD	-	chemical oxygen demand
Cr	-	chromium
$CrO4_2^-$	-	chromates
CrO_7^{2-}	-	dichromates
Cu	-	copper

d^{-1}	-	per day
DAF	-	dissolve air floatation
dATP	-	deoxyadenosine 5'-triphosphate
dCTP	-	deoxycytosine 5' triphosphate
DF	-	dilution factor
dGTP	-	deoxyguanosine 5' triphosphate
DNA	-	deoxyribonucleic acid
dNTP	-	deoxynucleotide triphosphate
DO	-	dissolve oxygen
DOE	-	Department of Environment
dTTP	-	deoxythymidine 5'-triphosphate
EDTA	-	ethylene diamine tetra acetic acid
FAD	-	flavin adenine dinucleotide(oxidized)
$FADH_2$	-	flavin adenine dinucleotide(reduced)
$FeCl_3$	-	ferric chloride
FMN	-	flavin adenine mononucleotide (oxidezed)
$FMNH_2$	-	flavin adenine mononucleotide (reduced)
FSTW	-	filter sterilized textile wastewater
gL^{-1}	-	gram per litre
gt	-	generation time
H^+	-	hydrogen ion
h^{-1}	-	per hour
H_2S	-	hydrogen sulphide
H_2SO_4	-	acid sulphuric
HCl	-	hydrochloric acid
HNO_3	-	acid nitric
HPLC	-	high performance liquid chromatography
HRT	-	hydraulic retention time
HSO_3^-	-	sulphite
IR	-	infrared
K	-	potassium

K_2CrO_4	-	potassium dichromate
K_2HPO_4	-	dipotassium hydrogen phosphate
kb	-	kilobase
KH_2PO_4	-	potassium dihydrogen phosphate
M	-	Molarity
mgL^{-1}	-	milligram per litre
$MgCl_2$	-	magnesium chloride
$MgSO_4.7H_2O$	-	magnesium sulphate heptahydrate
MIC	-	minimal inhibitory concentration
MW_r	-	molecular weight relative
N_2	-	nitrogen gas
Na	-	sodium
NaCl	-	sodium chloride
NAD	-	nicotinamide adenine dinucleotide
NAD^+	-	nicotinamide adenine dinucleotide(oxidized)
NADH	-	nicotinamide adenine dinucleotide(reduced)
NADP	-	nicotinamide adenine dinucleotide phosphate
NADPH	-	nicotinamide adenine dinucleotide phosphate(reduced)
NaOH	-	sodium Hydroxide
NB	-	nutrient broth
NH_4Cl	-	ammonium chloride
NH_4NO_3	-	ammonium nitrate
Ni	-	nikel
nm	-	nanometer
NMR	-	nuclear magnetic resonans
NO_3^-	-	nitrate
O_2	-	oxygen gas
OD_{600nm}	-	optical density at 600nm
Pb	-	plumbum
PCR	-	polymerase chain reaction
PHB	-	polyhydroxybutyrate

PO_4^{3-}	-	phosphate
ppm	-	part per million
Pt-Co	-	platinum cobalt
PVC	-	polyvinylchloride
RB15	-	reactive blue 15
RM	-	redox mediator
RNase	-	ribonuclease
rpm	-	rotation per minute
RR195	-	reactive red 195
rRNA	-	ribosomal RNA
S	-	sulphur
SBR	-	sequencing batch reactor
SDS	-	sodium dodecyl sulphate
SEM	-	scanning electron microscope
SFRed	-	sufimix supra red
SO_4^{2-}	-	sulphate
TAE	-	tris-acetate buffer
TCA	-	tricarboxylic acid cycle
td	-	doubling time
T_m	-	melting point
TOC	-	total organic carbon
TON	-	total organic nitrogen
Tris	-	2-hydroxymethyl-2-methyl-1,3-propanediol
TSS	-	total suspended solid
U	-	enzyme unit
UV	-	ultraviolet
UV-vis	-	ultraviolet-visible
v/v	-	volume per volume
w/v	-	weight per volume
Zn	-	zink

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background studies

Dyes are classified according to their application and chemical structure. They are composed of a group of atoms responsible for the dye colour, called chromophores, as well as an electron withdrawing or donating substituents that cause or intensify the colour of the chromophores, called auxochromes (Christie, 2001). It is estimated that almost 10^9 kg of dyes are produced annually in the world, of which azo dyes represent about 70% by weight (Zollinger, 1987). Therefore, azo dyes can be considered as the most important group of synthetic colourants. They are generally considered as xenobiotic compounds that are very recalcitrant against biodegradative processes. Many dyes are visible in water at concentrations as low as 1 mgL^{-1}. Textile-processing wastewaters, typically with dye content in the range 10-200 mgL^{-1} (O'Neill *et al.,* 1999), are usually highly coloured and when discharged in open waters presents an aesthetic problem. As dyes are designed to be chemically and photolytically stable, they are highly persistent in natural environments. The release of dyes may therefore present an ecotoxic hazard and introduces the potential danger of bioaccumulation that may eventually affect man by transport through the food chain.

Coloured wastewater is a consequence of batch processes both in the dye manufacturing and dye-consuming industries such as printing, cosmetics, plastics, food, drugs and biological stain. The annual market for dyes is more than 7 X 10^5 tonnes per year (Robinson, 2001). Two per cent of dyes that are produced are discharged directly in aqueous effluent, and 10% are subsequently lost during the textile colouration process (Easton, 1995). The main reason for dye loss is the incomplete exhaustion of dyes onto the fibre. Coloured wastewater is particularly associated with those reactive azo dyes that are used for dyeing cellulose fibres. These dyes make up approximately 30% of the total dye market (Kamilaki, 2000).

Azo dyes are generally persistent under aerobic conditions. However, under anaerobic conditions, they undergo relatively easy reductive fission, yielding aromatic amines. The latter compounds, in turn, generally require aerobic conditions for their degradation. Therefore, without adequate treatment, these dyes are stable and can remain in the environment for an extended period of time. For instance, the half-life of hydrolysed Reactive Blue 19 (RB19) is about 46 years at pH 7 and 25ºC (Hao *et al*., 2000). In addition to the environmental problem, the textile industry consumes large amounts of potable water. In many countries where potable water is scarce, this large water consumption has become intolerable and wastewater recycling has been recommended in order to decrease the water requirements. Therefore, because of their commercial importance and usage in many industries, the impact (Guaratini and Zanoni, 2000) and toxicity (Walthall and Stark, 1999; Tsuda *et al*., 2001) of dyes that are released in the environment have been extensively studied.

In Malaysia, the textile industry is a major source of wastewater since there are more than 200 textile factories in this country (Rakmi, 1993). Textile wastewater accounts for 22% of the total volume of industrial wastewater produced in the country. It has a strong colour in the form of persistent organics and also variety of the other pollutants including chloride, ammonia, organic nitrogen, nitrate, phosphate and heavy metals such as Fe, Zn, Cu, Cr and Pb. Depletion of dissolved oxygen content in water bodies can have a serious effect on aquatic life. Hence, various wastewater treatment methods such as physico-chemical and biological methods, usually in a combination are applied to treat the effluent to the discharge limits **(**Zee, 2002)

Removal of dyes is a major concern when treating textile-processing wastewater. The vast majority (60-70%) of the more than 10,000 dyes applied in textile-processing industries are azo compounds, i.e. molecules with one or several azo (N=N) bridges linking substituted aromatic structures (Carliell *et al.*, 1995). Their discharge is undesirable, not only for aesthetic reasons, but also because many azo dyes and their breakdown products such as aromatic amines have been proven toxic to aquatic life and mutagenic to humans (Chung and Cerniglia, 1992).

Thus, a wide range of methods has been developed for the removal of synthetic dyes from waters and wastewaters to decrease their impact on the environment. Various physicochemical techniques include membrane filtration, coagulation/flocculation, precipitation, flotation, adsorption on inorganic or organic matrices, ion exchange, ion pair extraction, ultrasonic mineralisation, electrolysis, advanced oxidation (chlorination, bleaching, ozonation, Fenton oxidation and photocatalytic oxidation) and chemical reduction. In addition, biological pre-treatment, main treatment and post treatment techniques include bacterial and fungal (microbiological or enzymatic decomposition) biosorption and biodegradation in aerobic, anaerobic, anoxic or combined anaerobic/aerobic treatment processes can be employed to remove colour from dye containing wastewaters (Hao *et al.*, 2000). Enzymatic biodegradation involved an enzyme produced by the microorganisms called azoreductases, which can reductively cleavage the azo bond, -N=N- (Stolz, 2001).

In general, each technique has its limitations. The efficacy of the various methods of dye removal, such as chemical precipitation, chemical oxidation, and adsorption along with their effects on subsequent biological treatment was compared in an earlier paper (Tunay *et al.,* 1996). Currently, microbial biodegradation has become a promising approach for dye treatment because it is cheaper, effective and more environmentally friendly. The ability of microorganisms to carry out dye decolourization has received much attention. Microbial decolourization and degradation of dyes is seen as a cost-effective method for removing these pollutants from the environment. Therefore, in this thesis, pure bacterial culture was successfully isolated and used for the transformation of azo dyes to non-coloured intermediates and/or even to partially mineralize them, which are safe and less toxic to the environment. The summary of the experimental design carried out in this study is shown in Appendix P.

1.2 Objectives of the study

The present study was aimed at investigating the ability of potential bacteria to degrade dyes with the specific objectives: -

1. To isolate, screen and characterize potential dye degrading bacteria from raw textile wastewater.
2. To optimize physical and chemical condition for azo dye decolorization by selected bacteria in synthetic medium.
3. To determine enzyme localization and azoreductase activity for Reactive Red 195 colour removal using selected bacteria.
4. To analyze biodegradation products of Reactive Red 195 and its intermediate in synthetic medium using selected bacteria.