Results & Discussion

4.0 RESULTS AND DISCUSSION

Azodyes are a group of chemicals that causes severe contamination in river and ground water. The textile industry is a major user of water, starting from washing raw wool or manmade fiber production upto garment manufacturing. Water resources are diminishing due to rapid population growth and industrial development, reuse of municipal and industrial wastewater after treatment and elimination of potential pollutants become more critical.

Water pollution is a major problem related to the economic/industrial growth of any country. The number of industries in India, during the last decade has grown more than ten times and accordingly the problems related to environmental degradation have increased many folds (Gowd and Govil, 2008).

Potential microbial processes for developing feasible remediation technology to combat environmental pollution due to dye bearing wastewater are routinely worked out by several research groups (Keharia and Madamwar, 2003).

The present study was focused on the isolation of efficient bacteria to decolourize the dye and to bioremediate the textile effluent with the isolate. The results and discussion pertaining to the present study " Decolourization of Azo dyes by newly isolated bacterium from the textile effluent" is dicussed as follows.

The study was carried out in five phases. In phase I of the study, the textile effluent and soil were collected from the industrial disposal site and the effluent was characterized for its physical features like colour, odour and turbidity. In phase II of the study, commercial dyes are collected and the microorganisms present in the soil was enriched in the medium containing dye mixture and isolated by serial diliution method. Finally they are tested for decolourization of some azo dyes. In phase III the isolated microorganisms were characterized by certain biochemical tests.

In phase IV, the decolourization was studied using various physicochemical parameters such as different culture conditions (static and strring), different values of pH (5-10), different initial dye concentrations (25-150 mg/l) and different temperatures (25-55^{o} c).

In phase V, other textile dyes were tested for decolurization with the isolated organism, textile effluent was treated with free microbial cell and immobilized cell and the BOD and COD of the treated effluent were determined.

4.1 COLLECTION AND CHARACTERIZATION OF TEXTILE EFFLUENT

The textile effluent was collected from the wastewater disposal site of the textile industry, in a sterile conical flask. The soil was collected from the disposal area in a sterile blue cap bottle.

The collected effluent was characterized for its physicochemical properties. Table 4 shows the physicochemical characteristics of the effluent.

TABLE 4
PHYSICOCHEMICAL CHARACTERISTICS OF THE TEXTILE EFFLUENT

PARAMETER	SAMPLE#	BIS LIMITS*
Colour	Blue	Absent
Odour	Offensive	Absent
Turbidity	Turbid	-
pH	10.2	6.0 – 9.0
Total suspended solids(mg/l)	2100	100
Total dissolved solids (mg/l)	11700	2100

* - Tolerance limits for textile effluent discharged into inland water source as per Bureau of Indian Standards (BIS) 2009.

- Mean value of duplicate samples.

According to BIS (2009) water should be odourless, colourless and clear. The presence of colour and odour in any water sample indicates the unpleasant nature of water. The textile industrial effluent studied was found to be blue coloured, turbid and also had an offensive odour.

According to BIS (2009) the normal pH range of water should be between 6.0 -9.0. The effluent sample analyzed had a pH value of 10.2 which is high when compared to standard value. Higher value of pH could be attributed to the presence of carbonates and bicarbonates (Saxene and Shrivastava, 2002).

The total suspended solids (2100 mg/l) and total dissolved solids (11,700 mg/l) in the sample was found to be high when compared with BIS standards. The presence of high level of total suspended solids and total dissolved solids might be due to the insoluble organic and inorganic matter present in the effluent (Nagarajn *et al.,* 2005).

4.2 DECOLURIZATION OF SELECTED AZODYES BY ISOLATED MICROORGANISM

The azodyes used in this study were collected from the local industries in and around coimbatore. In this study structurally different azodyes were used (Table 5)

TABLE 5

AZODYES USED FOR THE STUDY

Dyes	Chemical nature	Wave length nm
Malachite green	Triphenylmethane dye	617
Reactive red	Slufonated monoazo dye	511
Congo red	Direct dye	497
Reactive yellow	Monochloro-triazine dye	418
Reactive blue	Monoazo dye	590
Acid red	Diazo dye	505
Reactive navy blue	Vinyl sulfonate dye	560

The results of decolourization of azo dyes are represented in Table 7 Sand figure 2. This clearly indicated that, the isolated organism was very effective in decolourizing reactive red than reactive blue and reactive yellow. This isolate ADU-1 showed 97% of decolurization of reactive red in 48 hrs whereas for reactive blue and reactive yellow it showed only 89% and 80% of decolourization.

And also this result clearly showed the effectiveness of the isolate to remove the sulfonated azodyes. These dyes are normally considered to be more recalcitrant than carboxylated azodyes. These dyes were not decolourized easily because the permeation of the dyes through the cell membranre is the rate limiting step during bacterial reduction of azodyes. So such decolourization can be occurred by an oxygen insensitive azoreductase (Kodam *et al.*, 2005).

The reactive red was decolourized by *Enterobacter sp.*azo1 (91%), *Yersinia sp.*azo3 (87%) and *Serratia sp.* azo2 (46%) and *Erwinia sp.*azo4 (5%) after 7 days of incubation. Compared to these results our isolate showed better decolourization of reactive red (97%) within 48hrs of incubation (Jirasripongpun *et al.*, 2007).

As per the result of Patil *et al.*, (2008) the individual strain of *Bacillus odysseyi* showed the ability to decolourize reactive blue (50 mg/l) 82% at 24 hrs. The *Streptomyces coelicolor* decolourized reactive blue at 39% within 60min (Dube *et al.,* 2008). These results indicated that the isolate was not very effective in decolourizing reactive blue.

4.3 IDENTIFICATION OF THE ISOLATED MICROORGANISM

Pure culture was isolated after successive enrichment in dye broth containing all three dyes at 50mg/l. The isolate ADU – 1 grew to white colonies when plated on nutrient agar without dye. However, the colonies get colours like yellow, pink and blue on the dye agar plate and the agar medium became colourless especially where colonies are dense. The colourless colonies were observed after one day of incubation and this suggested the adsorbing of dye into cellmass. The colourless dye media after 7 days of incubation also demonstrated the decolourization activity of the tested microorganism, which could possibly be associated with either bioadsorption or biodegradation or both. The biochemical tests reaveled that the isolated bacteria belongs to *Aeromonas* species.

TABLE 6
BIOCHEMICAL ANALYSIS

S.No	Biochemical tests	Results
1	Gram stain	Negative
2	Glucose fermentation	Positive
3	Starch hydrolysis	Negative
4	Indole production	positives
5	MR reaction	positive
6	VP reaction	Positive
7	Catalse activity	Positive
8	Urease activity	Negative
9	Oxidase activity	Positive
10	Citrate activity	Positive

PLATE 1

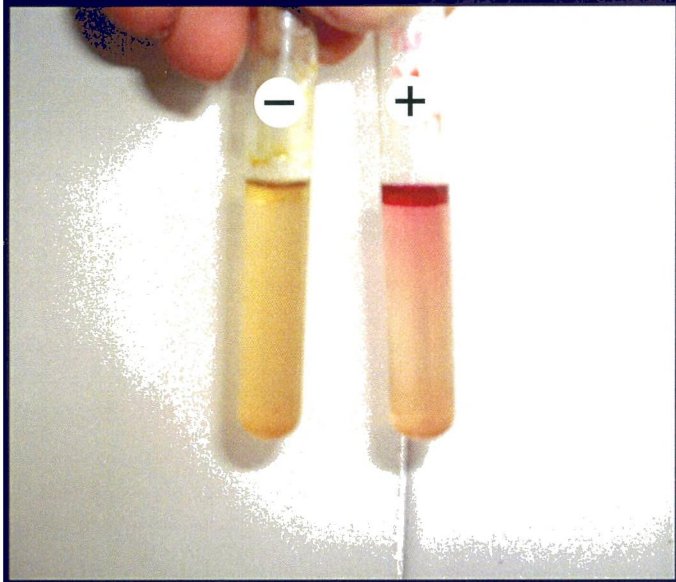
ISOLATED COLONIES ON

Reactive Red Dye

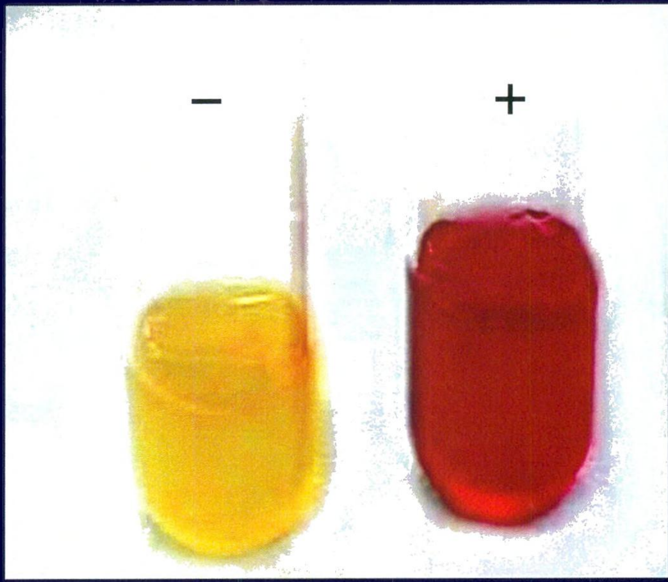
Reactive Blue Dye

PLATE 2

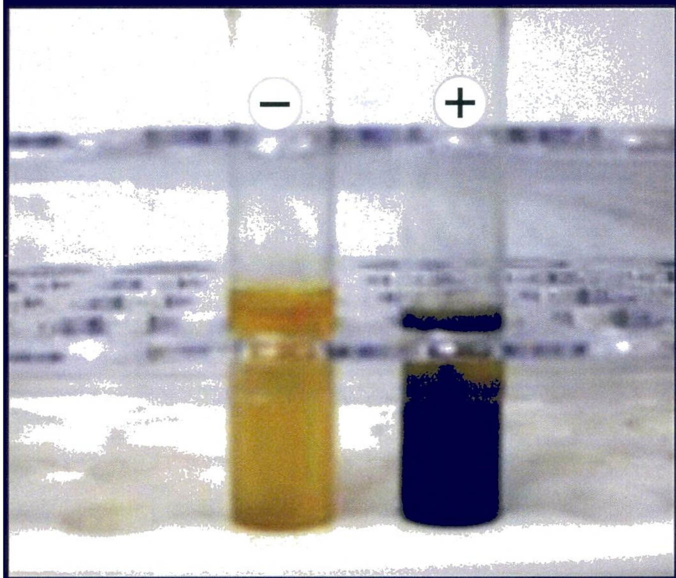
BIOCHEMICAL TESTS



Indole Production

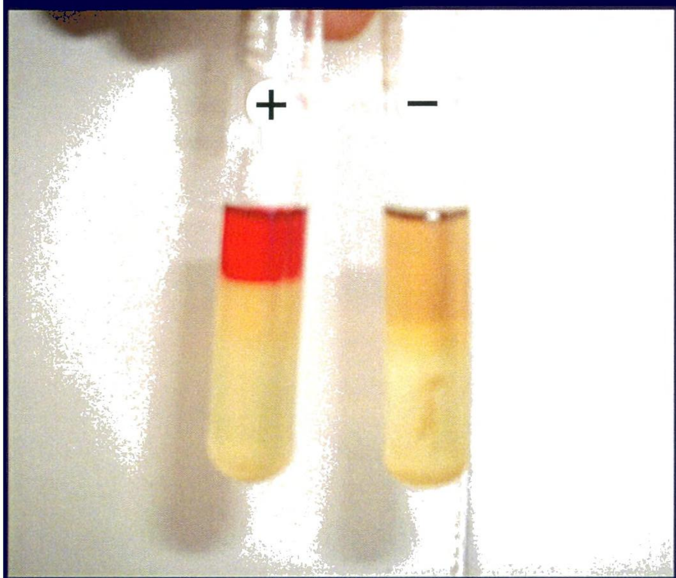


Glucose Fermentation



H_2S Production

Methyl Red

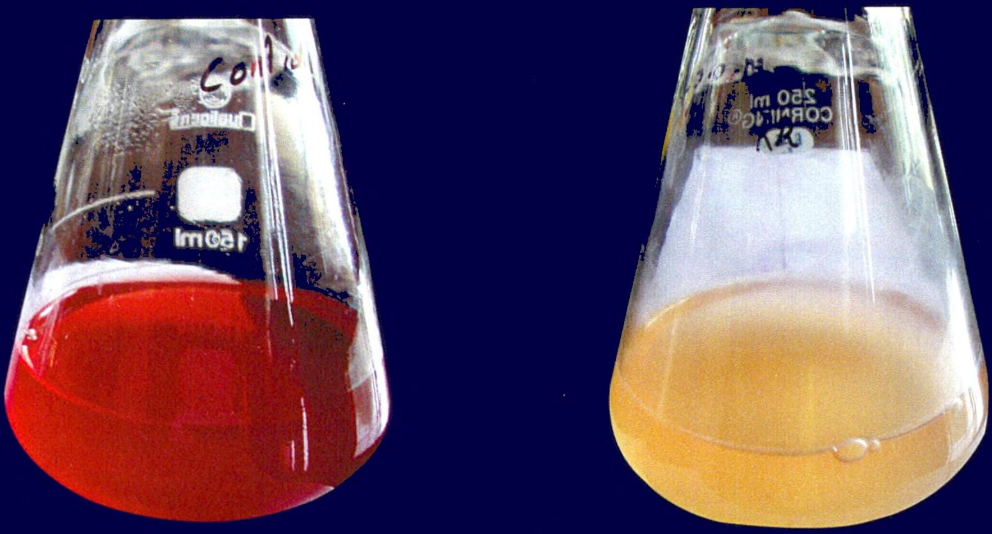


VP Reaction

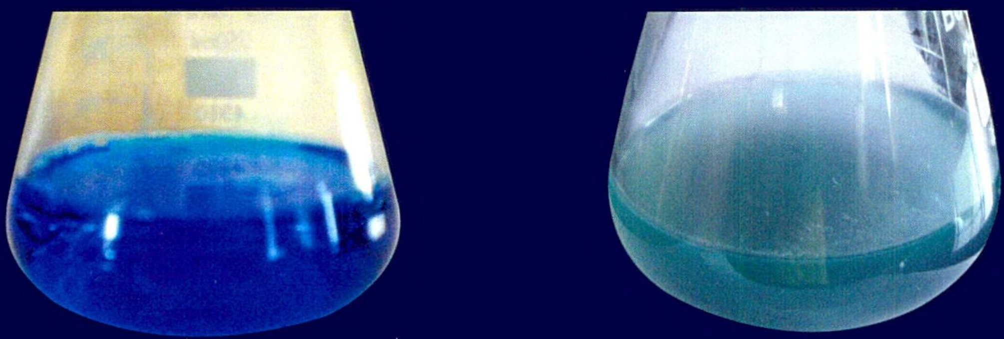
Catalase Test

PLATE 3

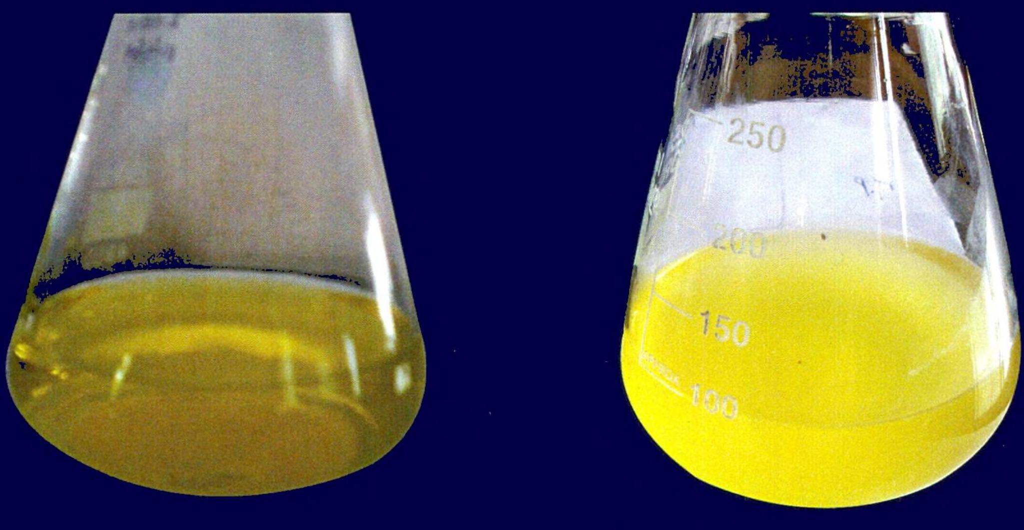
DECOLOURIZATION



Reactive Red



Reactive Blue



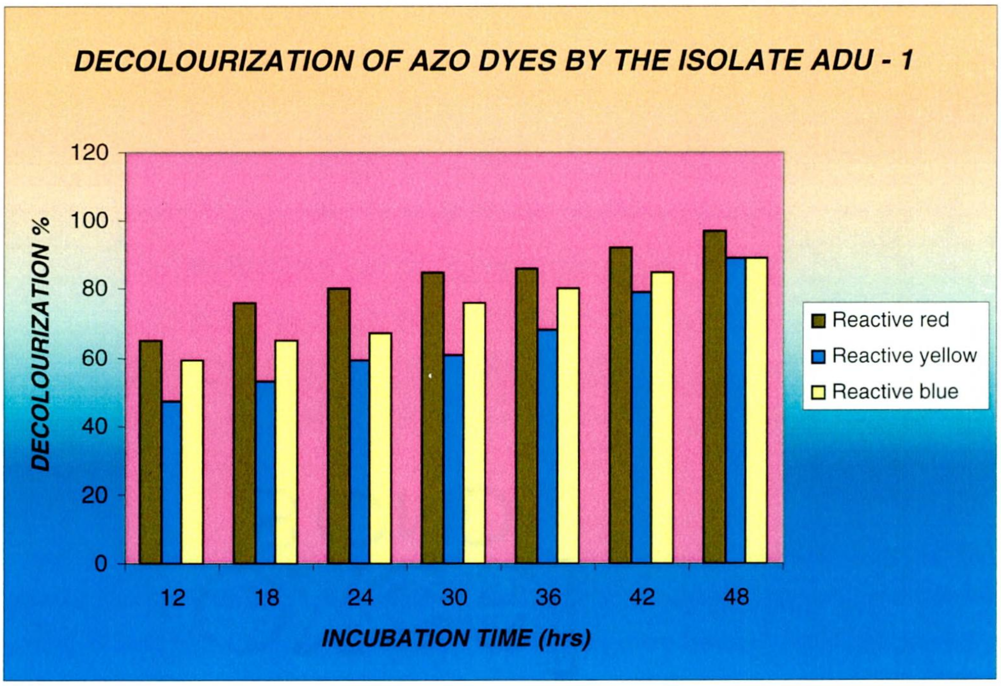
Reactive Yellow

TABLE 7

DECOLOURIZATION OF AZODYES BY THE ISOLATE ADU - 1

S.No	Time (hrs)	Decolourization %		
		Reactive red	Reactive blue	Reactive yellow
1	12	65	59	47
2	18	76	65	53
3	24	80	67	59
4	30	85	76	61
5	36	86	80	68
6	42	92	85	79
7	48	97	89	80

FIGURE - 2



4.4 EFFECT OF PROCESS PARAMETERS ON DECOLOURIZATION OF REACTIVE RED USING THE ISOLATE

The effect of pH, temperature, initial concentration of dye and culture conditions were studied with an aim to determine the optimal conditions required for maximum decolorization using reactive red which is decolourized more rapidly than other dyes.

4.4.1 EFFECT OF pH

Bacterial culture generally exhibited maximum decolourization rate at pH values near 7. Decolourization of reactive red at various pH values by the isolated strain was shown in figure 3 and table 8. It shows that decolorization was increased from pH 5-9 and maximum decolourization 97% was achived at 48 hrs of incubation at pH7.

The decolourization percentage was decreased as pH was increased further from pH 8-10. This clearly showed that the decolourization rate decreased under extremely alkaline (>pH9) and acidic condition (<pH6).

This pH tolerance range was similar to that observed in halophilic and halotolerant bacteria, which also decolourize azodyes (Asad *et al.,* 2007). Alkaline tolerance is an important consideration for industrial applications because processes using reactive azodyes are usually performed under alkaline condition (Aksu, 2003).

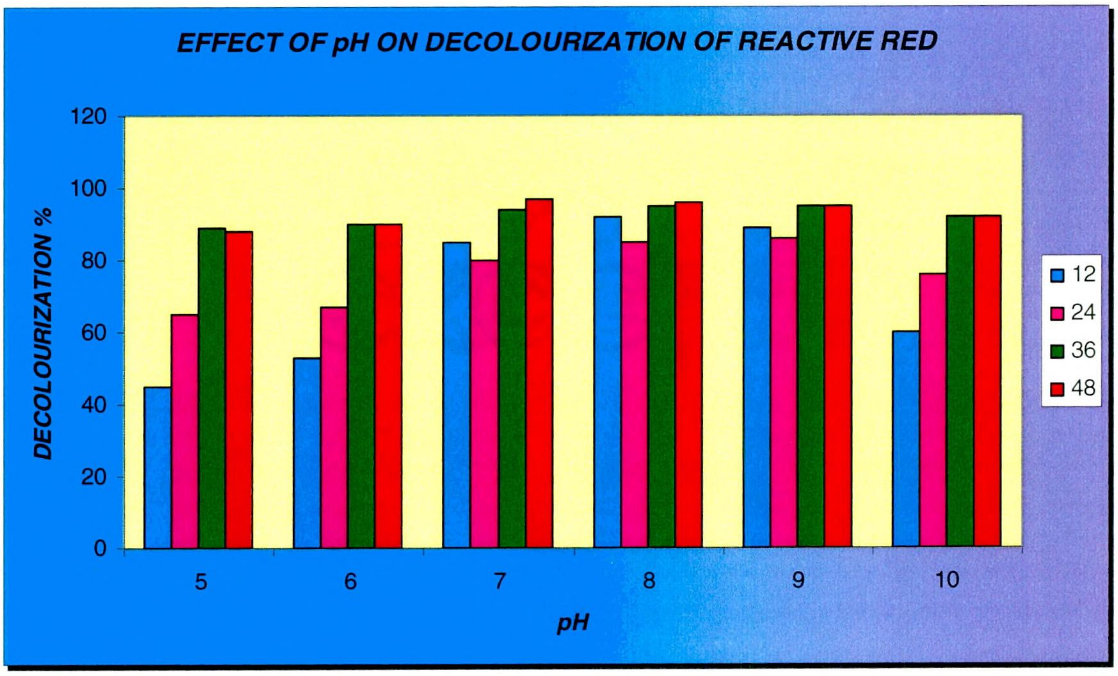
The decolourization rate of reactive red was peaked at pH10 after 12 hrs incubation by *Clostridium biferrmentans* (Joe *et al.,* 2008).

TABLE 8

EFFECT OF pH ON DECOLOURIZATION OF REACTIVE RED BY THE ISOLATE ADU - 1

S.No	pH	Incubation time (hrs)			
		12	**24**	**36**	**48**
1	5	45	65	89	88
2	6	53	67	90	90
3	7	85	80	94	97
4	8	92	85	95	96
5	9	89	86	95	95
6	10	60	76	92	92

FIGURE – 3



4.4.2 EFFECT OF TEMPERATURE

In this experiment effect of temperature was determined by varying the temperature range from 25-55^{0}c at different incubation time from 12-48 hrs in which the initial dye concentration was 5omg/l and pH was 7.

Table 9 and figure 4 shows that dye decolourization of the isolated ADU – 1 was found to increase with increase in incubation temperature from 25-45^{0}c. The maximum decolurization (79%) was observed at 40^{0}c after 48hrs of incubation. Further increase in temperature resulted in gradual reduction in decolourization acitivity of the isolate ADU -1.

Decline in decolourization activity at higher temperature can be attributed to the loss of cell viability or to the denaturation of the azoreductase enzyme (Pearce *et al.*, 2003).

4.4.3 EFFECT OF INITIAL DYE CONCENTRATION

Figure 5 and table 10 shows the effect of initial concentration of dye ranging from 50-150 mg/l of dye at pH 7 and at 35^{0}c with varing incubation time from 12-48 hrs. It is clear from the figure that percentage removal of dye increased with an increase in time. Further percentage removal of dye decreased with an increase in dye concentration. Percentage removal of dye was found to be 98,94,86,68,60,39 at 25,50,75,100,125 and 150 mg/l initial concentrations of the dye after 48 hrs of incubation. An increased concentration of the dye takes more time for the decolourization (Kalme *et al*., 2007).

TABLE 9

EFFECT OF TEMPERATURE ON DECOLOURIZATION OF REACTIVE RED

S.No	Temperature ^{0}C	Incubation time (hrs)			
		12	**24**	**36**	**48**
1	25	13	16	29	40
2	30	19	25	35	42
3	35	30	50	59	63
4	40	47	60	71	79
5	45	39	53	65	72
6	50	29	45	50	58
7	55	28	20	42	51

FIGURE – 4

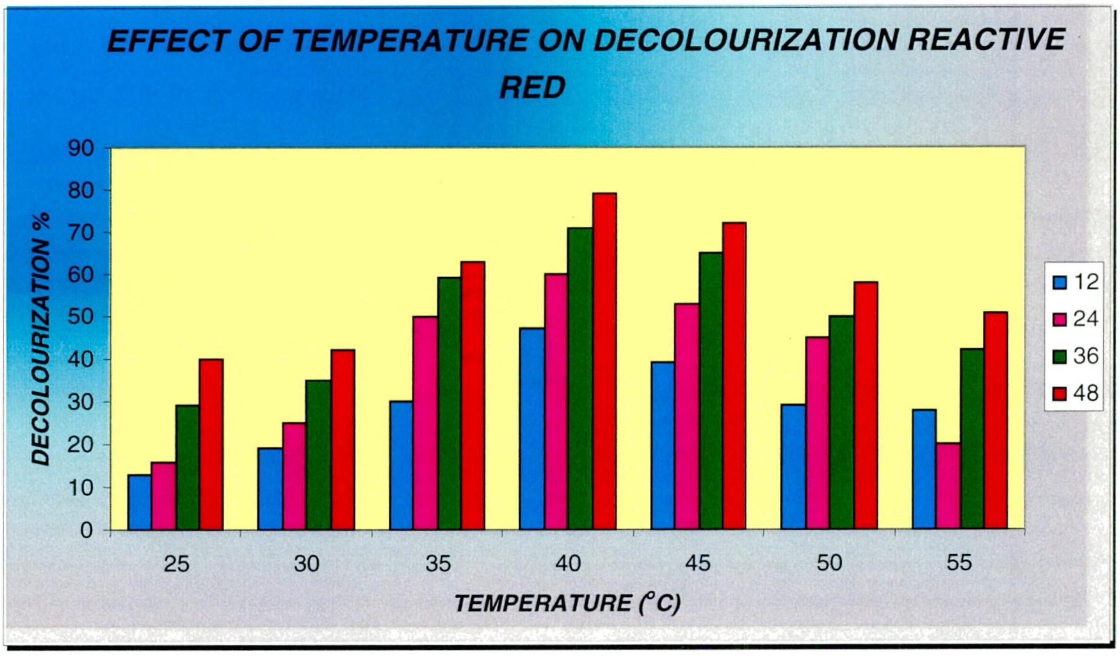
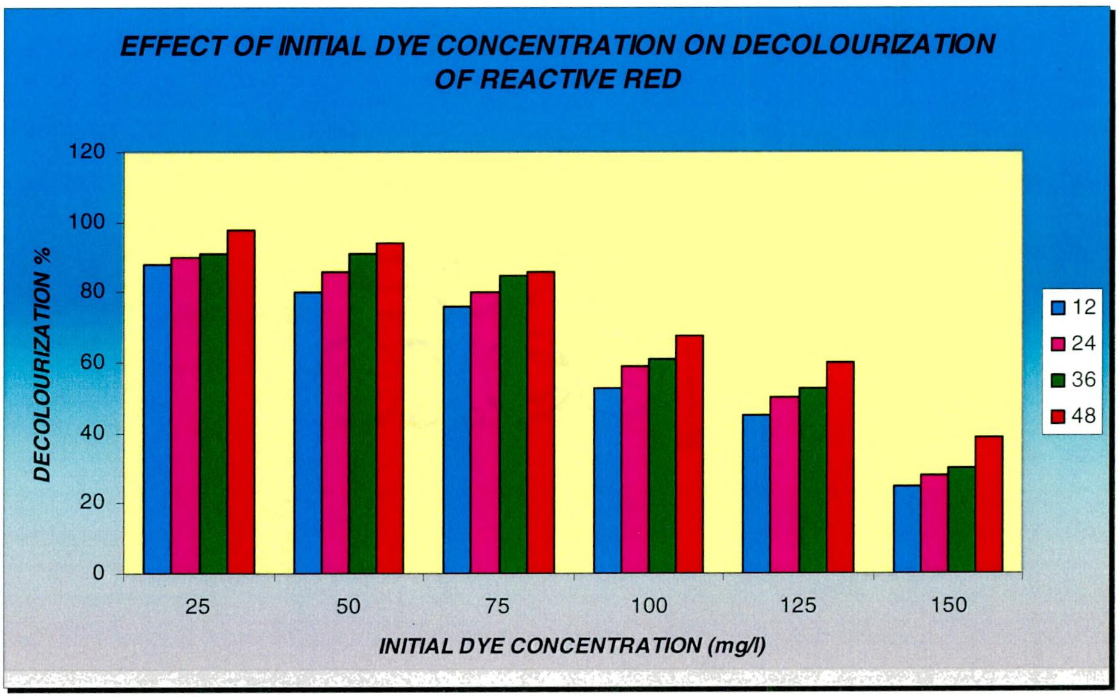


TABLE 10

EFFECT OF INITIAL DYE CONCENTRATION ON DECOLOURIZATION OF REACTIVE RED

S.No	Initial dye concentration mg/l	Incubation time (hrs)			
		12	24	36	48
1	25	88	90	91	98
2	50	80	86	91	94
3	75	76	80	85	86
4	100	53	59	61	68
5	125	45	50	53	60
6	150	25	28	30	39

FIGURE-5



4.4.4 EFFECT OF DIFFERENT CULTURE CONDITIONS

Figure 6 and table 11 clearly shows that compared to the agitation culture, the colour removal were best in static culture with 54-90% of decolourization at 48hrs of incubation.

The agitated culture showed good growth but less decolourization activity. These results indicated that decolourization dose not depend on biomass concentration but were significantly correlated with dissolved oxygen levels. A similar observation was reported by Moosvi *et al.,* (2007) where within 38hrs, maximum decolourization of 93% was observed under static conditions compared to only 24% in shaking conditions.

In static incubation, transfer of oxygen is limited to the broth surface and the cell culture would most likely sedimented at the bottom of the flask and become rapidly oxygen-depleted (Stolz, 2001; Chen, 2002). In the absence of oxygen, the azodye acts as sole oxidant or electron acceptor, and its reduction rate is then governed exclusively by the rate of formation of the electron donor (reduced azodyes).

Although the culture was able to grow aerobically, anoxic decolourization of the various dyes by the aerobic and facultative microbial consortium was best achieved under anaerobic or static condition. Similar observations have been reported by Kapdan *et al.,* (2000) and Khehra *et al.,* (2005).

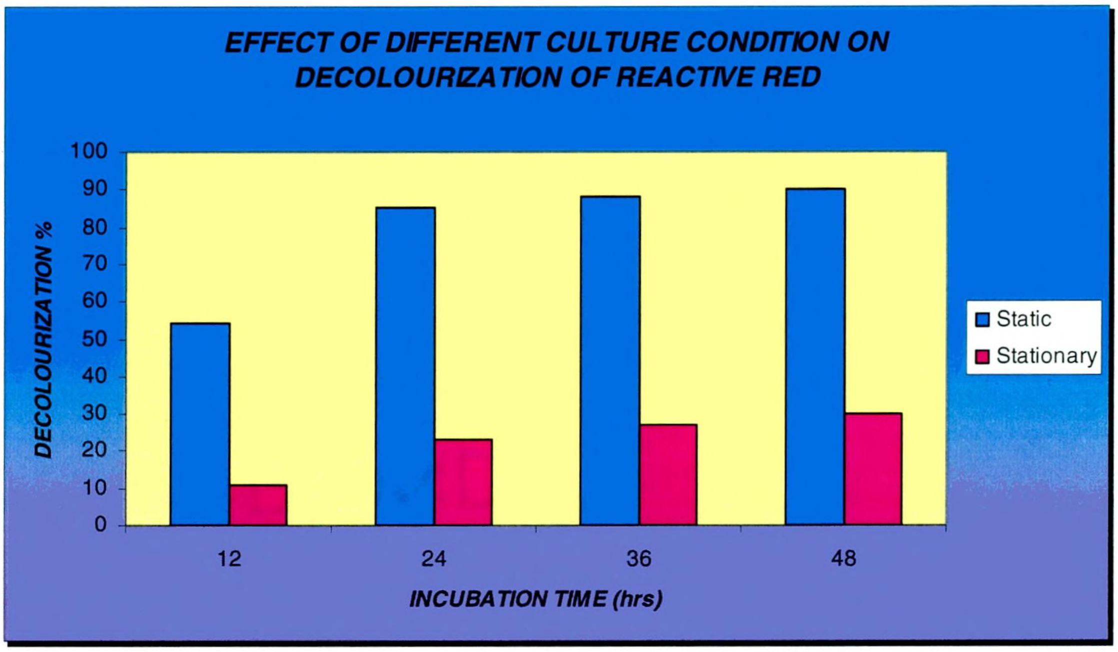
Decolouriztion probably would not take place in extremely anaerobic conditions (O2 – free nitrogen sparging) as pointed out by Chen (2002), which indicates that a minimum amount of oxygen present in the facultative anaerobic condition (static condition) was still needed for the consortium to maintain their basic cellular activity for effective decolourization.

TABLE 11

EFFECT OF DIFFERENT CULTURE CONDITIONS ON DECOLOURIZATION OF REACTIVE RED

S.No	Incubation time (hrs)	Decolourization % at	
		static	stationary
1	12	54	11
2	24	85	23
3	36	88	27
4	48	90	30

FIGURE – 6



4.5 TREATMENT OF TEXTILE EFFLUENT AND OTHER TEXTILE DYES.

Table 12 and figure 7 shows that among the 4 dyes tested navy blue was the easiest dye to degrade when compared with other dyes. Decolourization percentage was 83, 75, 63 and 47 for navy blue, acid red, malachite green and congo red respectively after 48hrs of incubation at 50mg/l dye concentration.

These results indicated that some azodyes are more resistant to removal by bacterial cells and this may be attributed to their structural differences.

The dye degradability was also found to be dependent upon the number and position of hydroxyl and sulpho groups in proximity relative to the azobond. A hydroxyl group in position 2 of the napthol ring – induced the reaction, whereas sulpho groups in the ortho and para position hindered the reaction (Khadijah *et al.*, 2009).

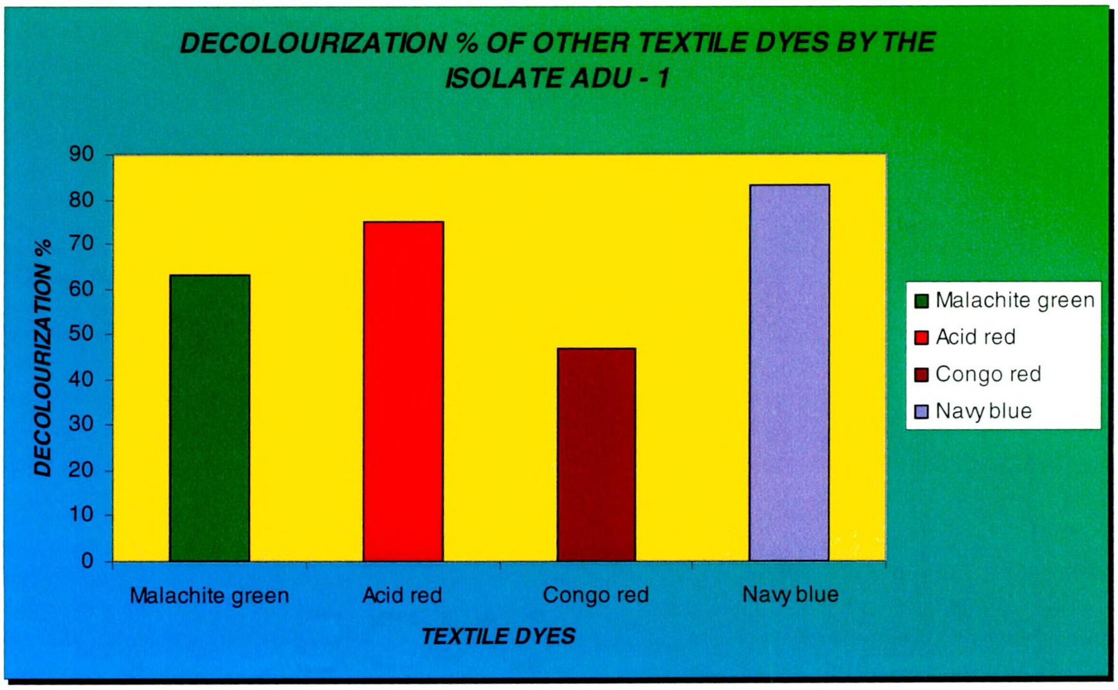
In addition dyes with simple structures and low molecular weight show higher rates of decolourization when compared to highly substituted, high molecular weight dyes. Similar observation was obtained on the investigation of the degradability on different structural azodyes (Chen *et al.*, 2003).

TABLE 12

DECOLOURIZATION OF VARIOUS TEXTILE DYES BY THE ISOLATE ADU – 1

S.No	Textile Dye	Decolourization % after 48 hrs of incubation
1	Malachite green	63
2	Acid red	75
3	Congo red	47
4	Navy blue	83

FIGURE – 7



As per the result of Khalid *et al.,* (2008) rapid decolourization of (99 and 92%) acid red by the bacterial strain *Shewanell putrefaciens* and *Aeromonas punctata* was observed at 4 hrs. But the isolate ADU – 1 show 75% decolourization of acidblue only after 48 hrs. So this isolate was not efficient in decolourization of acid blue compared to these strain.

As per the result of Patil *et al.*,(2008) the strain *Bacillus odysseji* decolourize Navy blue (86%) at 60 hrs of incubation compared to this the isolate ADU – 1 was efficient in decolourization (83%) of Navyblue within 48% hrs.

The textile effluent was treated with free bacterial cell and the cells were immobilized in sodium alginate. BOD and COD of the treated effluent was determined. Table 13 indicates that the isolate ADU – 1 significantly reduced the BOD (200 mg/l) and COD (1,000 mg/l) of the textile effluent into BOD (19 mg/l) and COD (213 mg/l) after treatment with free bacterial cells for about 48 hrs.

And also free cells were very effective in reducing the BOD and COD when compared to the immobilized cells.

This is because the rate of diffusion of substrate across the beads appears to limit its utilization. Slight agitation (120 rpm) of the beads favoured rapid degradation caused by increased O_2 supply to the beads and translocation of substrates and products.

TABLE 13

COD AND BOD OF TREATED EFFLUENT

Environmental parameters	**Untreated textile effluent**	**Effluent treated with free cell**	**Effluent treated with immobilized cell**
COD mg/l	1,000	213	500
BOD mg/l	200	19	110

The immobilized cells of *Pseudomonas putida* P8 degrade catechol more efficiently than the free cells. *P.putida* was capable of utilizing acetonitrile as a sole source of carbon and nitrogens when immobilized in calcium alginate (Puvaneswari *et al.*, 2006).