



Molecular clustering of microbial flora and bacterial degradation of textile dyes by isolates from contaminated soils

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ABSTRACT

Environmental contamination by textile dye effluents is of greater concern nowadays. The use of biological process for its elimination is a convenient way to get rid of it. This study sought to explore the potential use of bacterial isolates from textile dye contaminated soil in the degradation of the dyes. Different optimizing parameters such as pH, temperature and concentration have been worked out to find out the effective degradation. The work involved isolation of isolates (S1, S2 and S3) for the degradation of three different dyes (Black B1, BlueNE2RL, and Red BS11) at different parameters such as pH (pH6, pH7 and pH8), Temperature (Room temperature and Incubation temperature) and Concentration (5mg/l and 10mg/l). The results show varying degree of degradation under various optimizing conditions. The optimal conditions for the effective degradation for all three dyes were found to be at neutral pH and slightly acidic pH, at incubation temperature and at concentration of 5mg/l. Thus this study demonstrates means of developing a management strategy based on the biodegradation process for the effective removal of persistent textile dye.

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Introduction

Environmental pollution has been recognized as one of the major modern world problems. The increasing demand for water and the dwindling supply of it has made the treatment and reuse of industrial effluents an attractive option. Textile effluents are of concern because they colour the drains and ultimately the water bodies. They also diminish the water quality. The ability of micro-organisms to degrade and metabolize a wide variety of compounds has been recognized and exploited in various biotreatment processes. This study investigated the potential of bacteria isolated from textile industries contaminated soils.

Dyes are important class of chemicals that are widely used in many industrial processes like in textile mills, leather, food, paint, cosmetics and in pharmaceuticals. (Alhassani *et al.*, 2007, Moosvi *et al.*, 2007). Most of this dyes are synthetic in nature and are being classified based on their chemical structures into 6 different classes that is; azo, anthraquinone, sulfur, triphenylmethane, indigoid and phthalocyanine derivatives (Kodam and Gawai, 2006)

Technological advance has been on increase in diversity and complexity of synthesized textile dyes with the objective of product improvement through enhancement of dye properties such as resistance to fading, improved delivery of dyes to fabrics and the increased variety of shades. This increase in diversity and complexity of dyes is coupled with higher resistance to environmental degradation leading to the pollution problems by the textile effluents. (Moosvi *et al.*, 2007). In contrast, the appealing properties of these dyes are that they provide a wide range of brilliant shades and can be applied using a number of application methods that provide high wet fastness (Pearce *et al.*, 2003).

A larger proportion of the textile dye effluent are the azo dyes due to their capability to pass through normal water treatment procedures, which results into aesthetically unappealing water (Oranusi and Ogugbue, 2005). Moreover most of these dyes are toxic and potentially carcinogenic in nature and their removal from the industrial effluents is a major environmental problem (Alhassani *et al.*, 2007)

Textile dyes are of environmental interest because of their widespread use and their potential for forming toxic aromatic amines (Giovana *et al.*, 2003). Color is the first sign of contamination recognized in dye wastewaters and has to be removed before discharge into the environment (Padmavathy *et al.*, 2003). Residual color is a problem with reactive dyes because in current dyeing process as much as 50% of the dye is lost in the wastewater. These losses are due to the relatively low levels of dye fiber fixation and to the presence of unreactive hydrolyzed dye in the dye bath. The dye hydrolysis occurs when the dye molecule reacts with water rather than the hydroxyl groups of the cellulose. These problems are compounded by high water stability and characteristic brightness of the dyes (Pearce *et al.*, 2003). Azo dyes are the largest class of synthetic dyes used in the food industry, which are being characterized by the presence of one or more azo bonds (-N=N-) in association with one or more aromatic systems, which may also carry sulfonic acid groups. If these colorants come into contact with certain drugs within human body they can induce allergic and asthmatic reactions in sensitive individuals (Combes and Haveland, 1982). Several of the textile dyes are very stable to light, temperature and microbial attack making them recalcitrant compounds (Nyanhongo *et al.*, 2002). Most of the azo dyes-dye-degrading microorganism cleave the azo bond of the respective

azo dye and produce decolorized products (Wong and Yuen, 1996). The bacterial degradation of azo dyes is often mediated by azoreductases, which are more efficient under static and anoxic conditions (Baughman and Weber, 1994).

Over 10,000 dyes with a total of annual production with an excess of 7×10^5 metric tonnes worldwide are commercially available and typically 10-15% of this amount is discharged as industrial wastewaters due to the incomplete exhaustion of the dyes on to the fibers (Kodam and Gawai, 2006. Pearce *et al.*, 2003, Oranusi, 2005).

Various methods have been used such as physical and chemical, which include adsorption method, coagulation process, ozone and hypochlorite treatment, for the treatment of dye waste effluents. All of these methods are either costly inefficient or result in the production of secondary waste products hence are not economically feasible. (Kodam and Gawai, 2007, Alhassan *et al.*, 2007). Unlike these methods the use of biological treatment methods has been found to be attractive due to their cost effectiveness, diverse metabolic pathways and versatility of microorganism (Togo *et al.*, 2008)

Many microorganisms belonging to different taxonomic groups like of bacteria fungi, actinomycetes and algae have been reported for their ability to degrade textile dyes (Moosvi *et al.*, 2007). Pure fungal cultures have been used to develop bioprocess for the mineralization of the dye (Chang *et al.*, 2004). In contrast, bacteria usage is normally faster to mineralize the dyes through combined metabolic mode of anaerobic-aerobic sequence. As the catabolic activities of microorganisms in mixed consortium complement each other, obviously syntrophic interaction present in the mixed communities can lead to complete mineralization of the dyes (Chang *et al.*, 2004). This attempt has been made to explore the potential of soil bacteria present in the contaminated site, itself to degrade the commercially available and commonly used textile dyes. Hence, the present work has an intense hope of identifying a biological method to degrade these potentially toxic dyes from the environmental compounds such as soil and water.

Objectives

The study was carried out with the following objectives.

1. To isolate the morphologically and biochemically different isolates from the textile effluent contaminated soil
2. To check the dye degradation potential of the select isolates at various parameters such as pH, Temperature and Concentration
3. To evaluate the optimal parameters for the effective dye degradation.

Materials And Methods

Sample collection

Soil samples were collected from contaminated sites of textile mills in and around Webuye Papermill in Western Kenya. The samples were collected using clean polythene bags and brought to the laboratory aseptically for the isolation of the bacterial organism capable of degrading the dyes

Dyes

The dyes used in this study were of commercial grade and procured from textile dye selling store and they were, Red BS 111, Blue NE2RL and Black B which were used frequently for dyeing the fabrics. These dyes were used for optimization experiments.

Isolation of bacteria degrading organism

About 15 mg of the collected soil sample was weighed and then dissolved in 85 ml of distilled water, kept in a shaker for 30 minutes. Aseptically 0.1 ml of the sample was pipetted to the

nutrient agar plates; spread plated and then was incubated at 37°C for 48 hours. The colony with distinct morphology and repeatedly was picked and streaked to get single isolated pure cultures.

The isolated colonies were subjected to Gram's staining, enzymatic reactions and biochemical tests to differentiate microorganisms with their morphological and biochemical characteristics. The different isolate were named as S1, S2, S3, S4 and S5. These isolate cultures were maintained in agar slant tubes.

Screening of the isolates capable of degrading the dyes (Prachi and Anushree, 2009)

Bushnell and Haas medium with yeast extract were used for screening of isolates for the ability to degrade the dyes.

Bushnell and Haas Medium composition

Ammonium nitrate	- 1 gm
Calcium chloride	- 0.2 gm
Ferric chloride	- 0.05gm
Magnesium sulfate	- 0.2 gm
di-Potassium hydrogen orthophosphate	- 1 gm
Distilled water	- 1000ml.

Preliminary batch experiments were carried out using 250 ml Erlenmeyer flasks containing 100 ml of the Bushnell and Haas medium in which 0.5mg and 1 mg of the dye were added. Then the solution was seeded with 10 ml inoculums of overnight culture isolates aseptically.

The samples were analyzed for the absorbance values using UV-Vis spectrophotometer-117. The samples from each conical flask were taken at different time intervals 0 hour, 24 hours, 48 hours and 72 hours respectively and they were quantified at the maxima absorbance of the dyes, Red BS 111 540 (nm), Blue NE2RL (530 nm) and Black B (525 nm) respectively with the obtained absorbance values, the percentage of decolorisation by the respective isolate (S1, S2 and S3) has been calculated with respect to different optimizing conditions such as pH, Temperature and Concentrations.

Different parameters were set to determine the appropriate on which the bacterial isolates would degrade the dye efficiently. The pH was altered by using either Hydrochloric acid or by Sodium Hydroxide which decreases and increases the pH respectively. Room temperature and Incubation temperatures were used for study.

Results

The Biodegradation potential of the three isolates from the contaminated sites namely S1, S2 and S3 were studied under different optimizing conditions such as PH temperature and concentration. Three dyes namely Black B1, Blue NE2RL, and Red BSIII has been used for the study. The results showed varying levels of degradation under different optimizing conditions. The results were expressed in percent degradation by observing the difference between the initial and final stage of observation.

Effect of pH

The degradation potential of the three isolates S1, S2 and S3 were checked against three different pH 6,7 and 8 respectively. Among the isolates S2 was efficiently degrading in almost with all the pH, ranging from 2% to 99%. The least being with S2 with an average degradation of 20.3%. But as a whole the degradation potential of all the three isolates has increased considerably at incubation temperature than at room temperature, similarly the condition at pH 7 was considered to be favorable at all conditions.

Table 1: Decolourization percentages of Black B dye

Dye	Black											
Temperature	Room Temperature						Incubation Temperature					
Concentration	5 mg/l			10 mg/l			5 mg/l			10 mg/l		
Time	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
Isolate	pH 6											
S1	2.2	12.5	6.9	7.9	13.8	16.8	25.5	34.9	53.3	32.1	44.6	66.7
S2	2.5	9.0	14.8	99.9	99.9	99.9	28.1	47.2	57.2	39.0	58.1	74.0
S3	3.1	3.8	10.3	8.0	14.1	24.2	28.9	42.2	53.8	34.3	47.1	71.3
Isolate	pH 7											
S1	10.5	21.2	24.2	14.6	16.4	16.7	19.3	29.3	40.3	12.9	21.0	31.4
S2	6.4	25.7	30.4	7.9	13.8	16.8	11.9	27.8	43.2	8.5	15.5	23.7
S3	9.9	12.0	37.2	13.5	20.9	22.6	13.8	21.7	42.3	9.9	18.3	22.6
Isolate	pH 8											
S1	8.8	41.2	59.8	8.3	13.6	23.4	38.5	46.5	63.6	4.5	8.5	18.5
S2	23.5	43.3	53.0	6.4	15.2	21.3	20.9	42.9	60.8	2.4	11.3	23.4
S3	17.7	22.7	36.6	12.1	21.0	22.8	21.3	56.3	73.3	9.5	12.5	17.3

Table 2: Decolourization percentages of Blue NE2RL dye.

Dye	Blue NE2RL											
Temperature	Room Temperature						Incubation Temperature					
Concentration	5 mg/l			10 mg/l			5 mg/l			10 mg/l		
Time	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
Isolate	pH6											
S1	6.6	8.0	11.6	12.9	19.4	25.7	46.0	67.1	78.9	14.8	50.3	67.9
S2	9.5	19.0	19.6	8.9	13.2	21.2	20.0	69.5	62.0	27.0	42.8	55.8
S3	1.7	3.5	5.4	11.5	17.2	21.6	48.0	59.5	45.0	30.9	53.6	70.0
Isolate	pH 7											
S1	23.2	31.2	34.6	16.9	37.3	38.1	8.3	18.8	26.5	9.2	27.6	34.3
S2	19.2	24.6	41.3	12.9	26.7	29.2	12.0	22.6	30.2	13.5	19.5	26.2
S3	6.8	21.6	43.4	15.9	18.9	25.8	8.5	19.9	29.3	15.4	33.3	37.0
Isolate	pH 8											
S1	28.7	42.1	59.0	12.9	26.7	30.0	14.1	24.2	36.7	13.3	14.8	26.5
S2	28.2	64.6	73.8	6.0	13.5	19.1	14.8	27.5	44.6	10.3	11.2	26.6
S3	32.0	45.9	56.9	10.0	18.8	23.8	19.2	27.5	40.8	16.3	18.4	25.7

Table 3: Decolourization percentages of Red BS 111 dye.

Dye	Red BS 111											
Temperature	Room Temperature						Incubation Temperature					
Concentration	5 mg/l			10 mg/l			5 mg/l			10 mg/l		
Time	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
Isolate	pH6											
S1	13.0	14.0	17.5	4.4	17.0	22.5	45.6	55.4	72.8	17.0	26.3	56.5
S2	7.8	9.0	11.9	5.0	14.8	23.2	46.1	55.5	64.1	20.2	32.0	51.5
S3	12.8	16.6	14.5	7.2	14.2	22.4	48.5	59.6	71.5	14.3	28.7	51.1
Isolate	pH 7											
S1	10.2	9.0	28.5	13.9	23.1	41.0	9.5	28.3	52.8	12.2	14.8	27.2
S2	22.0	29.7	36.6	18.5	22.6	29.7	4.0	30.5	51.7	12.3	20.9	21.8
S3	15.4	23.8	31.3	14.0	22.4	26.8	1.5	26.0	34.0	14.2	27.9	32.0
Isolate	pH 8											
S1	19.1	25.9	37.2	9.9	23.1	31.9	15.7	19.9	32.3	7.3	15.3	23.1
S2	7.2	10.2	17.4	16.8	23.5	40.4	7.4	24.2	41.7	5.5	9.8	10.9
S3	39.1	47.8	55.9	14.3	20.4	37.1	7.4	23.6	21.4	7.4	13.4	19.3

Graphs showing the degradation pattern of the dyes

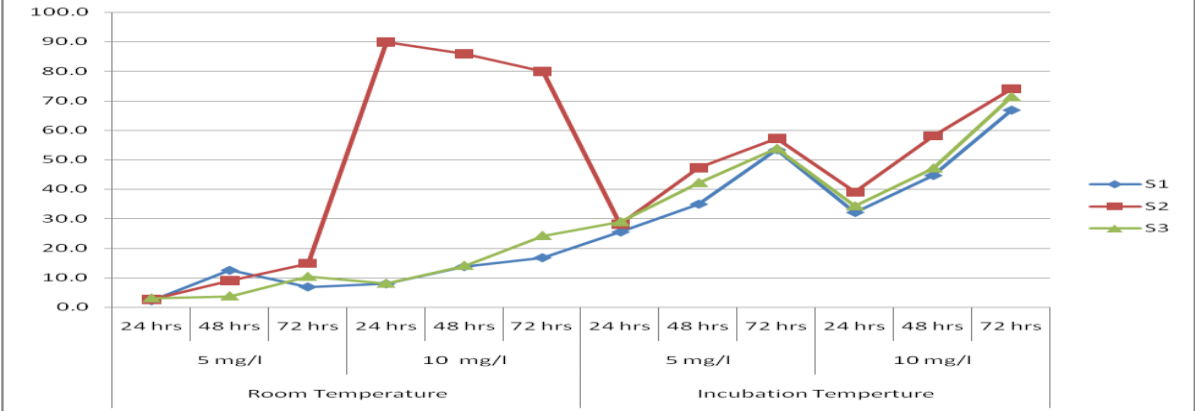


Fig 1 - Black B - pH 6

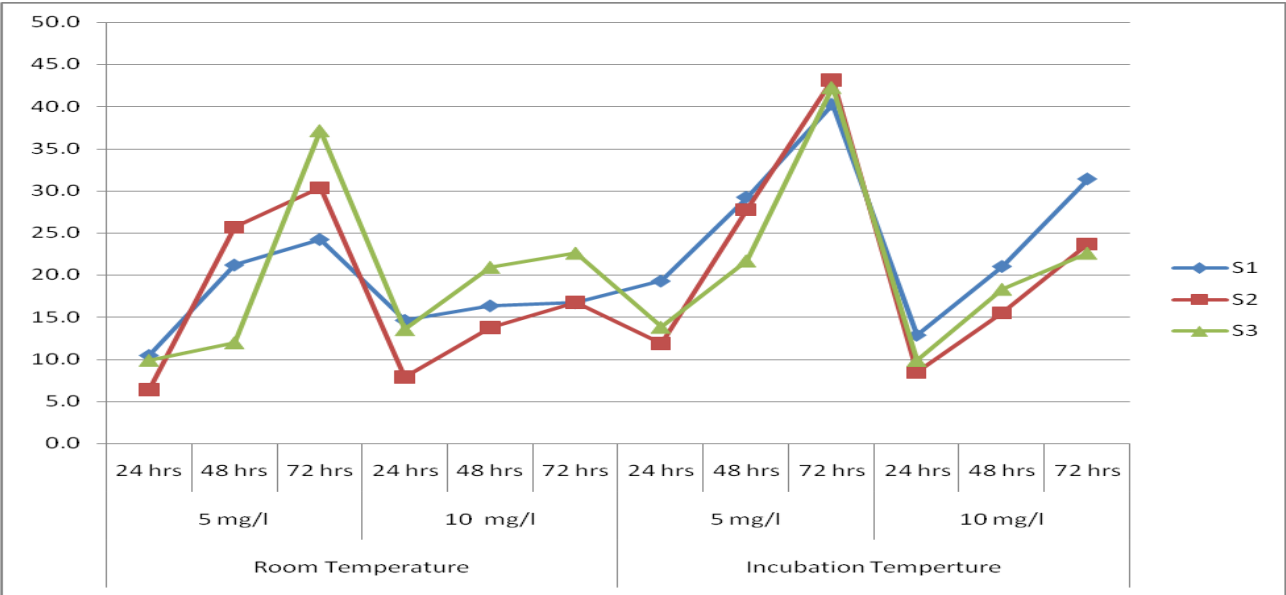


Fig 2 - Black B – pH 7

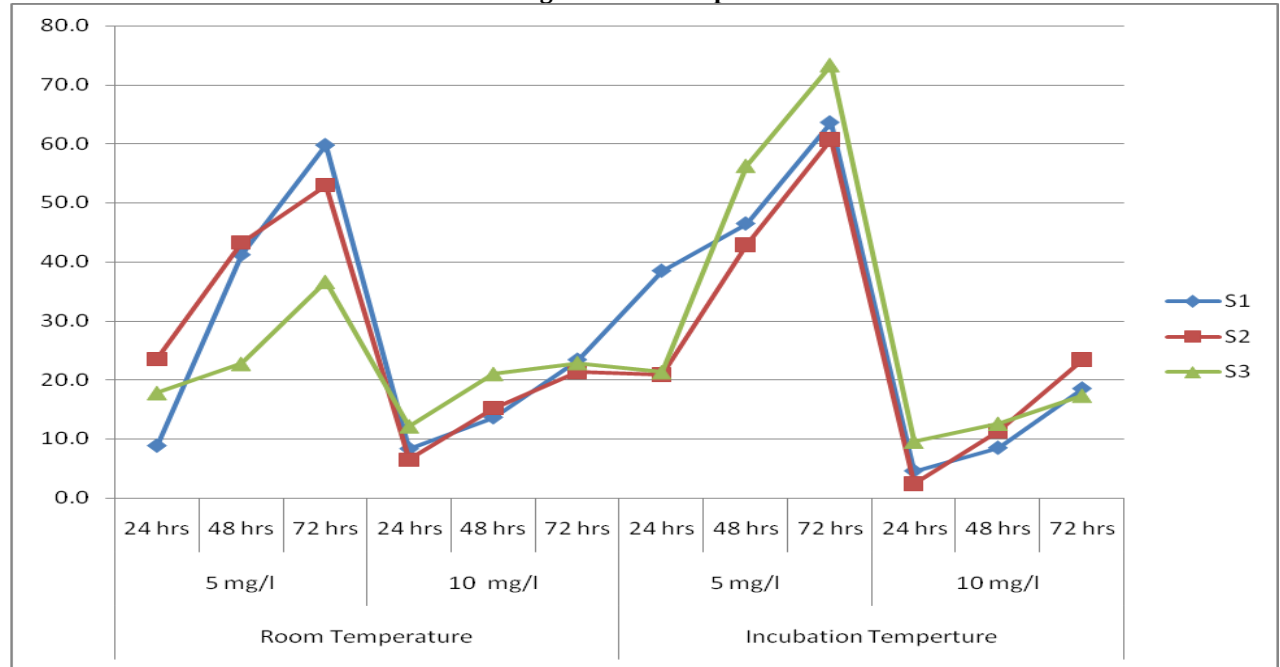


Fig. 3 - Black B – pH 8

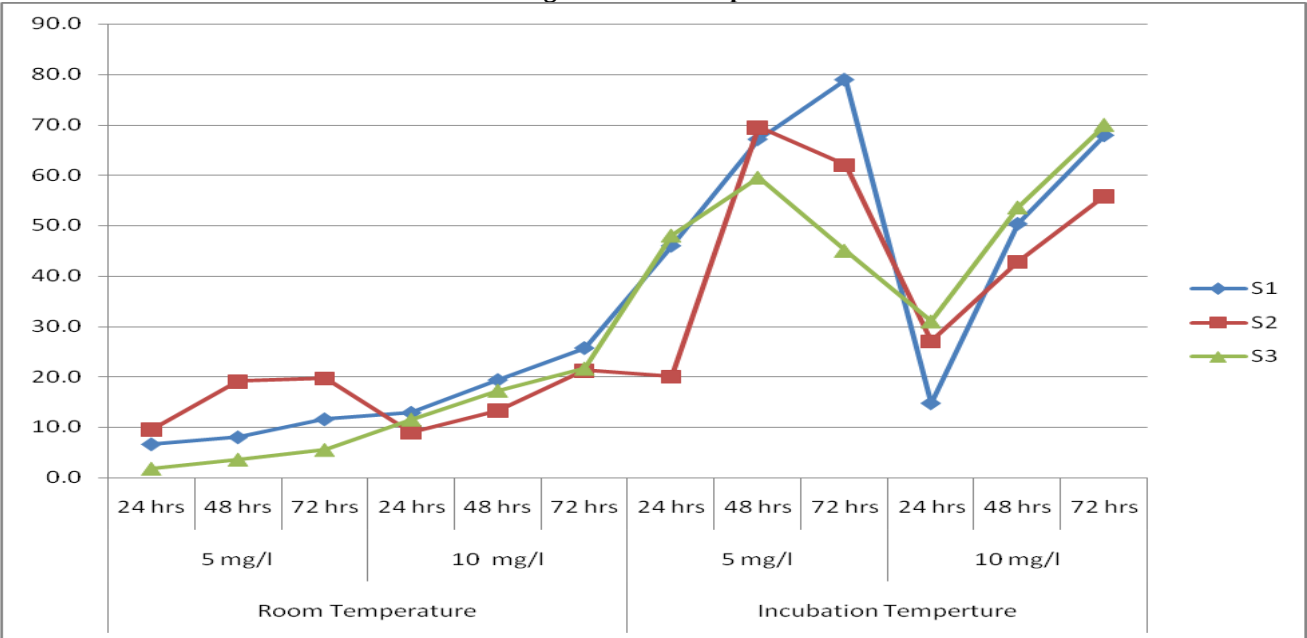


Fig 4 - Blue NE2RL - pH 6

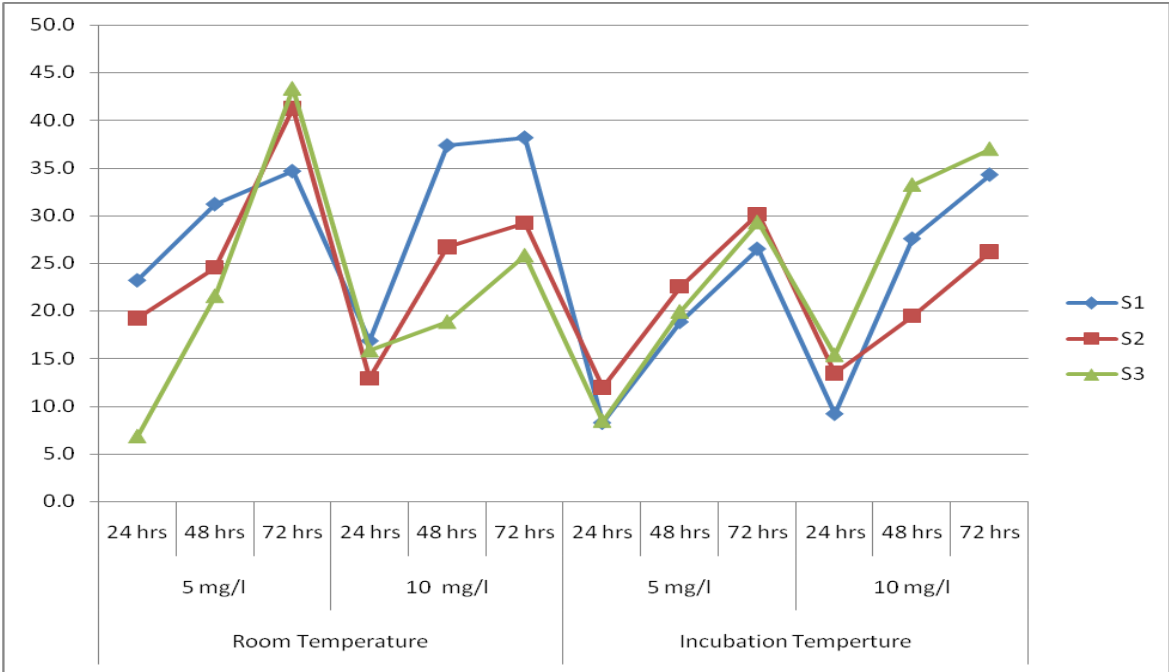


Fig 5 - Blue NE2RL - pH 7

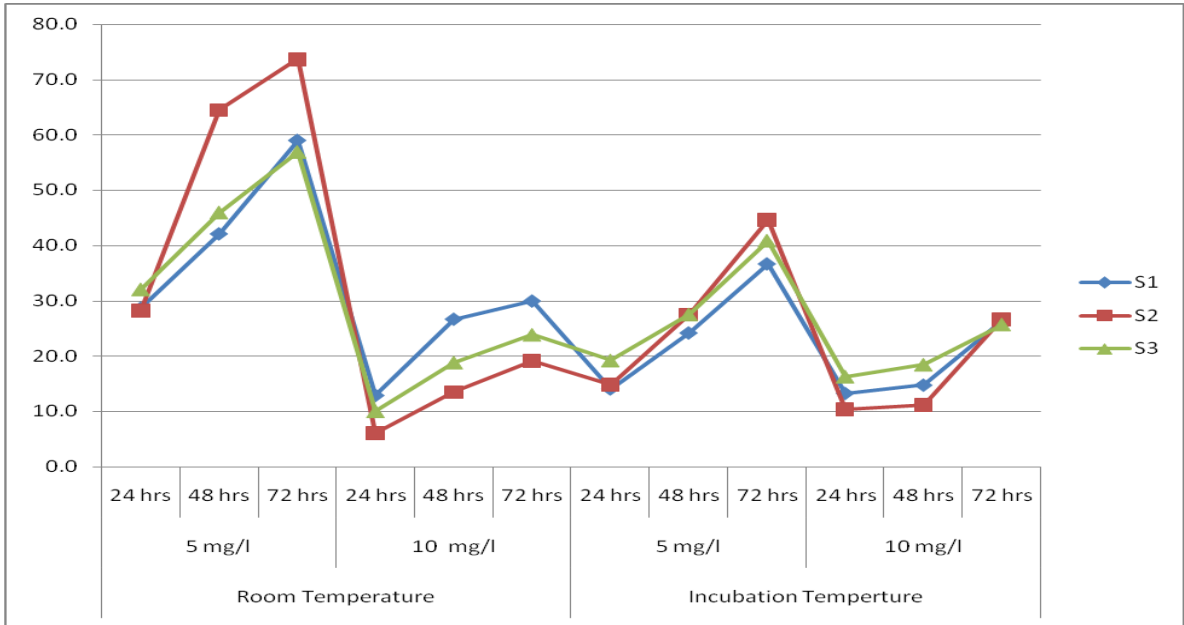


Fig 6 - Blue NE2RL - pH 8

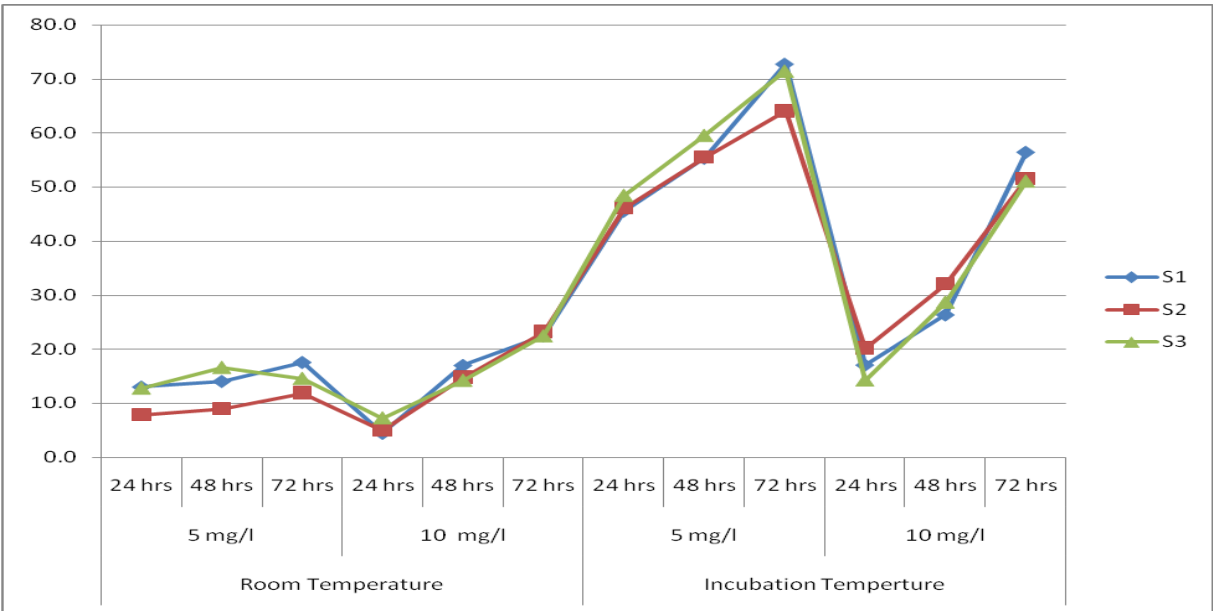


Fig 7 - Red BS111 - pH 6

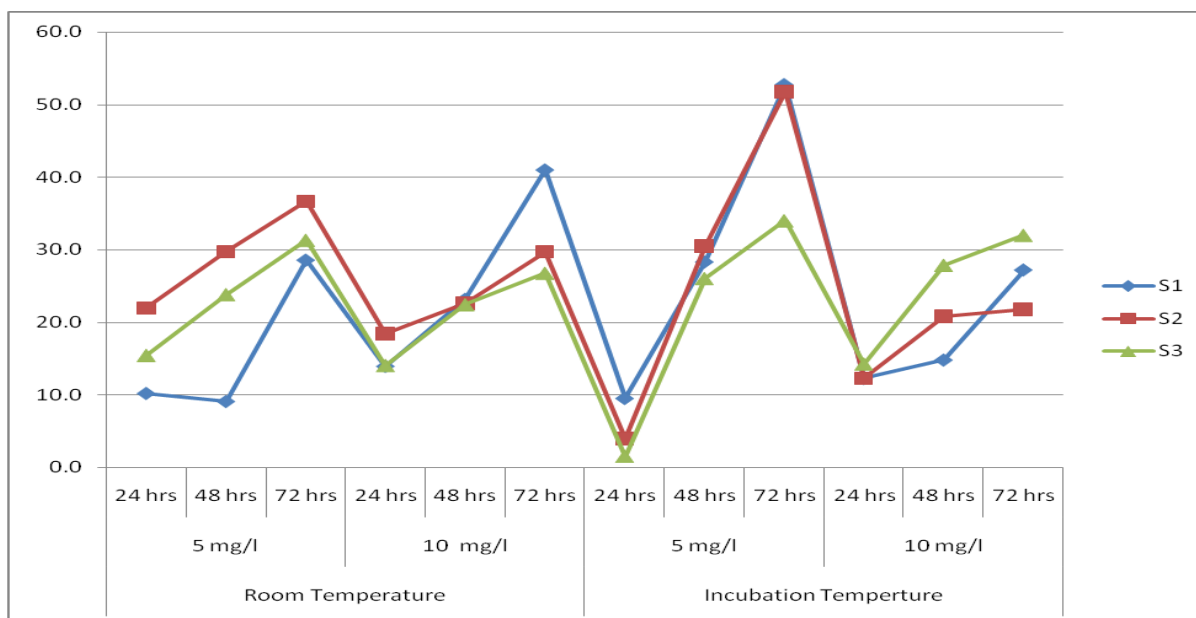


Fig 8 - Red BS111 -pH 7

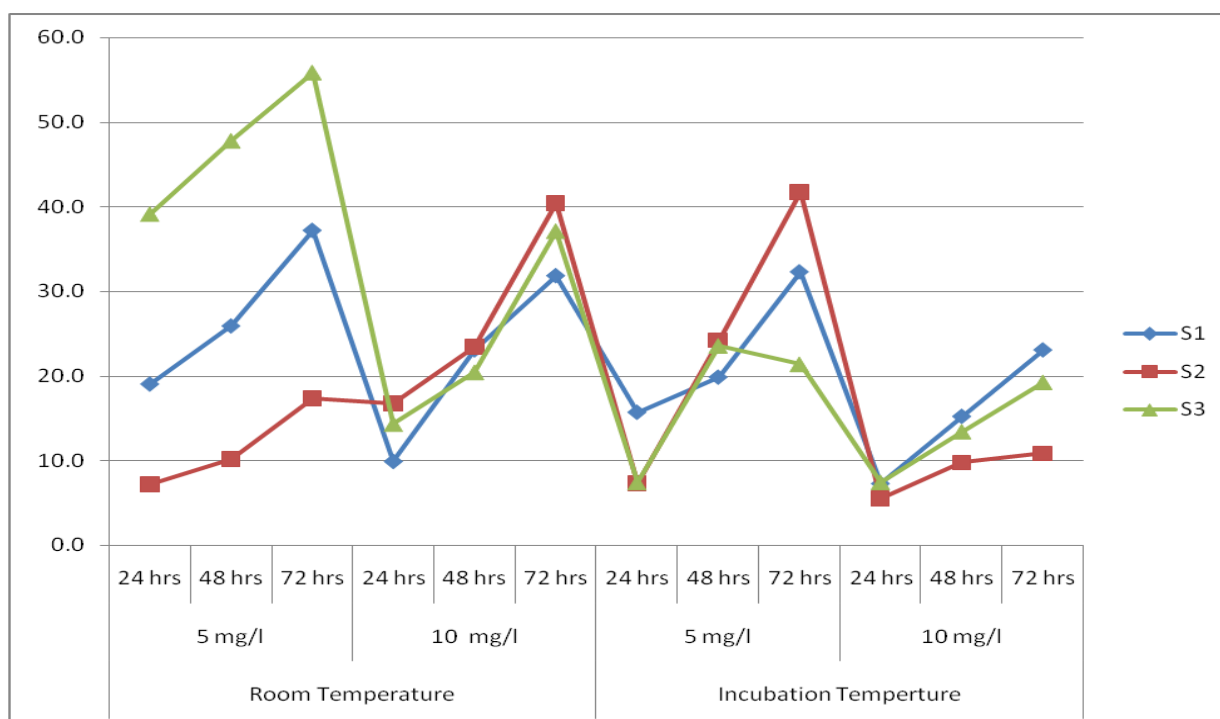


Fig 9 - Red BS111 - pH 8

The degradation pattern of the isolates for the Blue dye showed a different trend when compared with Black dye. The isolate S2 showed a better role at pH 8 ranging from 28.2% to 73%. The isolate S1 and S3 showed the least values at pH6 whereas it has increased considerably at pH7 and pH8. But the pH factor was not found to be a determining factor to have a reasonable choice of breakdown of the dye for the isolates S1 and S3. The isolate S2 considerably differ in its action. Thus a varying trend was observed for the blue dye. (Table 1 – 3)

The deterioration of red dye by the isolates showed a similar pattern as that of Black dye. As a whole isolate S3 was found effective at all the pH (6, 7 and 8) whereas S2 has not shown any effective degradation. The values of S3 ranged from 4% to 55%. The least degradation was effected for S1 isolate for all the three pH and for all the three dyes respectively. Thus pH

has varying effect which influences the degradation potential of the isolates. (Fig 1-9)

Effect of Temperature:

Temperature plays a vital role in determining the stability of the dye where it is present in the environment component. Room temperature (27°C), incubation temperature (37°C) were optimized for the study. (Table 1 – 3)

Normally any microorganism can show considerable improvement in growth or activity when kept at favorable temperature. This is the same with degradation activity also. The variation between the room temperature and incubation temperature were highly significant against all the other parameters such as concentration, dye and time. For all the isolates only at 24 hours incubation time, the temperature doesn't play any significant role, whereas it was in the order of 10% to 60% increase at longer incubation times such as 48 to 72

hours. The maximum degradation was effected by S3 isolate which showed 73% at incubation temperature. Thus, temperature factor have higher significant variations in the degradation parameters against other parameters. (Fig 1-9)

Effect of Concentration:

The concentration factor has a major effect over the biodegradation property. Since the strength of the dye has a major impact over the organism, utmost concern has to given to this. The study has been optimized with two concentrations such as 5mg/l and 10mg/l. But still concentration factor does not play any major significant role when compared to pH and Temperature. (Table 1 – 3)

However, again varying degradation pattern was observed among the isolates. The percentage degradation ranged from 2% in 5mg/l to 99% in 10mg/l for S2 isolate and from 1% in 5mg/l to 70% in 10mg/l for S3 respectively. Though S2 showed considerable variation with other parameters, it was not there with concentration. (Fig 1-9)

As a whole, all the parameters (pH, Temperature and Concentration) has its own role to play with the isolates S1, S2 and S3 and thereby enhance the degradation of the highly resistant textile azo dyes

Discussion

The degradation potential of the contaminated soil isolates S, S2 and S3 against the three dyes namely Black B1, Blue NE2R1 and Red BS111 is discussed with relevant earlier studies in this section. All these dyes belong to Azo group and its degradation potential at different optimizing conditions such as pH, Temperature and Concentration were discussed here.

Effect of pH:

It has been reported that the optimum pH for colour removal is often at neutral pH value or a slightly alkaline pH value (Pearce *et al.*, 2003). This factor was similar to the present study. Moreover the rate of colour removal tends to decrease rapidly at strongly acid or strongly pH values. This can be confirmed with the extrapolation of the present results at extreme pH values. Thus in the present study the suffered dye concentration showed better degradation. Biological reduction of an azo bond can result in the increase of pH due to the formation of aromatic amine metabolites which are more basic than the original azo compound (Wilmott NJ, 1997). Chang *et al* found that the dye reduction rate increased nearly 2.5 folds as the pH was raised from 5.0 to 7.0, while the rate became insensitive to pH in the range of 7.0 to 9.5. Thus, extreme pH values determine to bacterial degradation. However the present study has not focused at these extreme pH values. Thus the degradation potential does not show any remarkable ineffectiveness.

Effect of Temperature:

It has been reported that in many systems, the rate of colour removal increases with increasing temperature within a defined range that depends on the system. The temperature required to produce the maximum rate of colour removal tends to correspond with the optimum cell growth temperature of 35-45°C (Pearce *et al* 2003). The same trend was observed in the present study. The decline in colour removal activity at higher temperatures can be attributed to the loss of cell viability or to the denaturation of azo reductase enzyme. However, it has been shown that with certain whole bacterial cell preparations the azo reductase enzyme is relatively thermo stable up to temperatures of 60°C over short period of time (Pearce *et al* unpublished). Thus the present study has showed better degradation at

incubation temperatures which was similar to the above referred study.

Effect of Concentration:

The concentration of the dye substrate can influence the efficiency of dye removal through a combination of factors including the toxicity of the dye at higher concentrations. Wuhmann *et al* (1980) reported that the degradation follows first order reaction. The higher the concentration the longer the time required to remove the dye colour. Thus, in the present study the degradation was low at 5mg/l and was found to increase from 24 hours to 72 hours. Sani *et al* (1999), Dubling and Wright (1995) also reported the same trend with reference to concentration and also that the non enzymatic reduction mechanism that is controlled by process that are independent of the dye concentration.

Thus, all the parameters such as pH, Temperature and concentration have equal importance and effectiveness in the process of textile dye degradation.

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