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Research Article Open Access

Integrated Approach to the Problems of Dye Wastewater by Sonolysis and Biological Treatment

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Abstract

The degradation of a reactive dye by combined sonolysis (40 kHz) and biodegradation was studied using Acid red (AR 66) as a model dye. The combined action of ultrasound and biodegradation using *Bacillus subtilis* was characterized as a function of dye concentration, pH and temperature. Under optimum conditions, AR 66 could be completely degraded within 10 h. The COD analysis was performed and was found that about 90-95% COD reduction was achieved by this hybrid technique. The biodegradation data for AR 66 were fitted to Bridge- Haldane equation with good correlation.

Keywords: Sonolysis; Sonicator; Ultrasound; Biodegradation; *Bacillus* sp; Acid Red; Bioremdiation; Azo dye

Introduction

Today's highly industrialized environment is charged with a multitude of potentially toxic chemicals. The presence of harmful pollutants in the discharge wastewaters often contaminates the surface water and soil. Pollution of aquatic and soil is a worldwide problem that can result in uptake and accumulation of toxic chemicals in food chains and also harm to the flora and fauna of affected habitats [5]. Though a substantial progress has been made in reducing industrial releases in the recent years, major releases still occur [9]. Particularly, effluents of textile dyeing/finishing mills are often complex with intense color, chemical oxygen demand (COD), suspended solids, and a variety of refractory matter such as heavy metals and nonionic surfactants. Moreover, the industry suffers from excessive water consumption, due to manifold washing of dyed fabrics to remove dye residual from their surfaces. Accordingly, the management of dye house effluents requires an integrated approach to the problems of "effluent treatment" and "water consumption" by developing suitable treatments that produce harmless effluents and recyclable water. The primary route by which dye enters the environment from dye manufacturers and tanneries is through the production of wastewater, and also through the disposal of sludge containing dyes precipitated from the effluent by flocculation [15]. Colorants that enter the wastewater streams normally pass through a waste water treatment plant where they are eliminated to a large degree by adsorption on the sludge [13]. It is therefore necessary to treat textile effluents prior to their discharge into the receiving water.

The physical and chemical techniques include physico-chemical flocculation combined with flotation, electro flotation, and flocculation with Fe (II)/$Ca(OH)_2$, membrane-filtration, electro kinetics coagulation, electrochemical destruction, ion-exchange, irradiation, precipitation, ozonation, adsorption and the katox treatment method involving the use of activated carbon and air mixtures [11]. Some of these techniques have been shown to be effective, although they have shortcomings. Major disadvantages include excess amount of chemical usage or sludge generation with obvious disposal problems; costly plant requirements or operating expenses; lack of effective color reduction, particularly for sulfonated azo dyes; and sensitivity to a variable wastewater input.

Textile industries consume substantial volumes of water and chemicals for wet processing of textiles. These chemicals are used for desizing, scouring, bleaching, dyeing, printing and finishing. They range from inorganic compounds and elements to polymers and organic products [2]. There are more than 8000 chemical products associated with the dyeing process listed in the Color Index (Society of Dyers and Colorists, 1976) while over 100000 commercially available dyes exist with over 7 x 105 metric tons of dyestuff produced annually [16]. The presence of very small amounts of dyes in water (less than 1 ppm for some dyes) is highly visible and affects the aesthetic merit, water transparency and gas solubility in lakes, rivers and other water bodies [10].

Dyes usually have a very low rate of removal ratio for BOD to COD (BOD/COD less than 0.1). Biological methods, being cheap and simple to use, have been the main focus of recent studies on dye degradation and decolorization. Traditional methods like thermal degradation, photo catalytic degradation, and electrochemical degradation are used for treatment of these pollutants [8]. During the past two decades, several physico-chemical decolorization techniques have been reported, few, however, have been accepted by the textile industries. Their lack of implementation has been largely due to high cost, low efficiency and inapplicability to a wide variety of dyes [5].

Ultrasound is a sound wave with a frequency above the human audible range of 16 Hz to 16 kHz. In recent years, numerous unit operations involving physical as well as chemical processes are reported to have been enhanced by ultrasonic irradiation. These could be a better way of augmentation for the processes as an advanced technique. The important point here is that ultrasonic irradiation is physical method activation rather than using chemical entities. Detailed studies have been made in the unit operations related to leather such as diffusion rate enhancement through porous leather matrix, cleaning, degreasing, tanning, dyeing, oil–water emulsification process and

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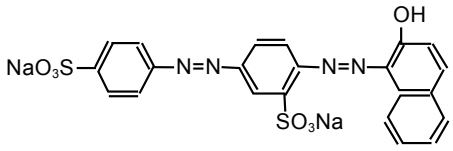
solid–liquid tannin extraction from vegetable tanning materials as well as in precipitation reaction in wastewater treatment. The fundamental mechanism involved in these processes is ultrasonic cavitation in liquid media. In addition to this there also exist some process specific mechanisms for the enhancement of the processes.

The ability of Microorganisms to carry out dye decolorization has received much attention recently. Microbial decolorization and degradation of dyes is seen as a cost-effective method for removing these pollutants from the environment. Low intensity ultrasonic irradiation can improve activity of microorganisms effectively, and thus it can be used to enhance the biological treatment of wastewater and increase the treatment efficiency through improving microbial activity in the biological reactor. The research achievements from home and abroad on the application of ultrasound (US) in bioengineering and biology were comprehensively summarized. The biological effect of low intensity US and the main mechanisms of biological activity enhancement were discussed, and the future application of low intensity US in biological wastewater treatment was analyzed accordingly. In this present study, there have been benefits such as improvement in process efficiency, process time reduction, performing the processes under milder conditions and avoiding the use of some toxic chemicals to achieve cleaner processing.

Materials and Methods

ACID RED 66

Acid Red 66 (C.I.26905) was received from Aldrich Chem. The structure of Acid red was shown in structure 1.



Structure 1: Acid red

Microorganism isolation and culture conditions

The Microorganism used in this study was isolated from the soil sample of tannery effluent contaminated sites at Pallavaram, Chennai, India. The soil sample (10% w/v) was inoculated in nutrient broth containing Acid red (50 mg/l). The flask was incubated at 37^{0}C under static conditions. After 2 days of incubation, 1.0 ml of the culture was serially diluted and samples (0.1 ml) were drawn from 10^{-5} dilution. The samples were inoculated (spread plate method) on the nutrient agar plates containing 200 mg/l Acid red. After 2 days of incubation at 37^{0}C, the colonies were screened for their ability to form a clear zone around them [3]. These organisms were isolated and sub cultured in nutrient broth containing Acid red (200 mg/l). Further morphological and biochemical tests were carried out to identify the organism. The Microorganism responsible for the degradation of dye was found to be *Bacillus sp*. and was used for further studies.

Morphology

Bacteria come in a range of different shapes. Rod-shaped cells are called bacilli (as in *Bacillus anthracis*), spherical cells are called cocci (as in *Staphylococcus aureus*), and helical bacteria come in three forms, vibrio - curved or comma-shaped rods (such as *Vibrio cholerae*), spirilla - thick, rigid helices (such as *Rhodospirillum rubrum*).Bacterial slides are monitored under the microscope using the 40X objective (total magnification = 400X) and they have been found to be rod shaped.

Anaerobic growth

Microorganisms that grow in the presence of Oxygen are called aerobic microbes and that grow in the absence of oxygen are called anaerobic microbes. The isolated microorganism is grown under both aerobic and anaerobic conditions [6]. The biomass in each case is determined separately by dry method. Under aerobic conditions the microorganism show maximal growth and under aerobic conditions negligible growth is seen. Hence the organism is tested negative for anaerobic growth

Indole test

Indole is a component of the amino acid tryptophan. Some bacteria have the ability to break down tryptophan for nutritional needs using the enzyme tryptophanase. When tryptophan is broken down, the presence of Indole can be detected through the use of Kovacs' reagent. Kovac's reagent, which is yellow, reacts with Indole and produces a red color on the surface of the test tube. The isolated organism is tested negative for Indole test [7].

Acclimatization

The acclimatization was performed by gradually exposing *Bacillus sp*. to the increasing concentrations of Acid red (Figure 1). *Bacillus sp*. was consecutively transferred into the nutrient medium with increasing concentrations of Acid red dye. During the investigation, nutrient broth concentration was decreased from 90% (w/v) to 0% (w/v). Finally the organism was provided with Acid red solution as sole source of nutrient. Acclimatization experiments were carried out at the temperature of 37^{0}C.

Degradation experiments

The Acid red stock solution of 1000 mg/l was prepared and from that required concentrations of dye solution was made. The degradation experiments were carried out with and without pretreatment. The pretreatment technique employed in the present investigation was sonolysis. The degradation experiment was carried out using various initial concentrations of dye. The pure dye solution was used as a sole source of energy. 250 ml Erlenmeyer flasks were used and 100 ml of solution was taken. The various concentrations of 20, 50, 80, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 mg/l solutions were prepared and inoculated with 1 ml of 24 h old *Bacillus sp.* inoculum. The flasks were incubated at 37^{0}C. Small aliquots (3 ml) of the solution was taken at regular intervals (3 h intervals) and centrifuged at 6000 rpm for 10 min. the supernatant was analyzed for the maximum absorbance using UV-Vis spectrophotometer at 496nm. The cell pellet was washed twice with distilled water and used for biomass estimation. The Decolorization was calculated as follows.

$$\%Decolorization = \frac{(Initial\,absorbance - Final\,absorbance)\,X100}{Initial\,absorbance} \quad (1)$$

For studying the effect of temperature, about 100 ml of dye solution (300 mg/l) was inoculated with 1 ml of 24 h old *Bacillus sp.* inoculums and incubated at various temperatures of 31, 33, 35, 37, 39 and 41^{0}C. Small volumes of sample were taken and decolorization was determined. For studying the effect of pH on decolorization, about 100 ml of dye solution (300 mg/l) was adjusted with various pH levels of 3, 4, 5, 6, 7, and 8. The solutions were then inoculated with 1 ml of 24 h old *Bacillus sp.* inoculum and incubated at 37^{0}C. Small volumes

of samples were taken at regular time intervals (3 h interval) and the decolorization was determined [1].

Pretreatment

Pretreatment was done by sonolysis. The sonochemical reactor (3 L, 30 KHz, Saisonics) was used to pre-treat the dye solution. About 1 liter of the dye solution of 300-mg/l concentrations was loaded in the sono-chemical reactor, and the pretreatment was carried out for various time limits of 0.5, 1, 1.5, 2, 2.5 and 3 hours. The solution was cooled to room temperature. 100 ml of pretreated sample was taken in Erlenmeyer flasks and inoculated with acclimatized strains of *Bacillus sp* [4]. The flasks were incubated at 37^{0}C. Smaller amount of samples were taken at regular time intervals (3 h interval) and the decolorization was determined.

COD analysis

About 500 ml of dye solution of concentration 300 mg/l was inoculated with 24 h old *Bacillus* sp. of inoculums (1% v/v) and the flask was incubated at 37^{0}C. About 15 ml of aliquots were taken at regular intervals (3 h intervals) and analyzed for COD. The COD removal was calculated by using following formula.

$$\%COD\,removal = \frac{(Initial\,COD - Final\,COD)\,X100}{Initial\,COD} \quad (2)$$

Results and Discussion

Identification and acclimatization of microorganism

The isolated organism was analyzed morphologically and biochemically to identify the species and genus. Table 1 shows the results obtained through various biochemical characteristic testing experiments. The isolated organism was identified as *Bacillus sp.* from these results and was used for further studies. Dye degrading bacteria were acclimatized by increasing concentrations of dye and decreasing concentrations of nutrient broth. During this phase the initial stage solutions were containing nutrient broth, which provided the organisms with suitable energy source. The growth at these stages was observed to be good. Though the higher dye concentrations inhibit the bacterial growth initially, the bacteria were induced to produce certain enzymes for taking part in the metabolism reaction. The culture was undisturbed for 48 h and the growth was observed after 2 days of incubation. The growth was determined based on the turbidity of the solution. Also the samples were drawn after 48 h and streaked on the plates containing Acid red agar (Acid red and agar) and incubated at 37°C and good growth was observed. Finally the organism was inoculated in pure dye solution (without any nutrients) and was used for further studies.

The isolated organism was analyzed morphologically and biochemically to identify the species and genus. Table 1 shows the results obtained at various experiments. From the results obtained by the sequence analysis of 16S rRNA (Table 2) and the phylogenetic tree (Figure 1) the organism was identified as *Bacillus subtilis* and it is used for further studies.

The ability of the organism to degrade the dye and consume it as a nutrient source was not achieved immediately. After regular variations under numerous generations had been successfully cultured under

S.No	Analysis	Result
1)	Gram staining	+
2)	Motility test	+
3)	Morphology	Rod
4)	Anaerobic growth	-
5)	Catalase test	+
6)	Indole test	-
7)	Glucose fermentation test	-
8)	Lactose fermentation test	-
9)	Amylase test	+
10)	Nitrate broth test	+
11)	Voges-Proskauer test	+
12)	Citrate	+
13)	MR/VP test	-/+

Table 1: Biochemical characters.

FOREWARD PRIMER
CCGTGGGTAACCTGCCTGTACACTGGGATAACTCCGGGAAACCGGAGCTAATACCGGATAGTTCCTTGAACCG CATGGTTCAAGGATGAAAGACGGTTTCGGCTGTCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGA GGTAACGGCTCACCAAGGCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACAC GGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCG CGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTGCAAGAGTAACTGCTTGC ACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAA GCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTC AACCGGGGAAGGTCV
REVERSE PRIMER
TGCGAGCCCTTTCAGCCCAATAATCCGGCAAAGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTT AGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTGCAAGCAGTTACTCTTGCACTTGTTCTTCCCTAACAACAGA GCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCC CTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTA CGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCCGCGGGTCCATCTGTAAGTGACAGC CGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTAT CCCAGTCTTACAGGCAGGTTACCCACGTGTTACTCACCCGTCCGCCGCTAACATCCGGGAGCAAGCT

Table 2: Sequence analysis of 16S rRNA gene of Bacillus subtilis.

varying environmental conditions (nutrient broth concentrations), the organism gained the ability to degrade and consume the dye as a sole source of nutrition. The acclimatization resulted in the variation of species for its fittest survival under odd nutritional conditions.

Pretreatment of acid red by sonolysis

The sonolysis pretreatment has showed considerable decolorization. The sonolysis involved directing ultrasound waves into the dye solution. During this process, an adiabatic conversion of liquid phase molecules into gas phase occurs by oscillating pressures created by the high frequency (30 kHz) ultrasound irradiation. These are called gaseous pockets or Micro bubbles [13]. The dissolved compounds of the solution diffuse through the Micro bubbles and the bubbles expand in size until they reach a critical size.

This collapse creates very high temperature of about 2000 to 5000 K temperature and very high pressure of about 1000 atm pressure. Due to extreme conditions, the compounds thus undergo pyrolitic fragmentation. The pyrolysis reactions lead to the dissociation of complex substances into hydroxyl, hydrogen and organic radicals. The structural destruction of dyes disable them to absorb the visible light because their absorbance is dependent on aromatic and azo groups present which is degraded by ultrasound.

Figure 2 shows clearly that the sonochemical degradation of Acid red increases with increase in time. Almost 84% of degradation was achieved in 180 min (i.e., 3 hrs). The prolonged exposure of dye solution to Ultrasound may enhance the generation of oxidative species [8]. This is initiated by the hemolytic cleavage of water molecules by pyrolytic reactions, which may be represented as follows:

$$H_2O \rightarrow OH^{\bullet} + H^{\bullet} \quad (3)$$

$$2OH + 2H \rightarrow H_2O_2^{\bullet} + H_2^{\bullet} \quad (4)$$

After the first step of reaction when H_2O was splitting in to radicals, further reactions depend on other oxidative radicals present in the micro bubbles. Anyhow the OH and H_2O_2 are strong oxidants. The production rate of oxidants depends on the temperature and pressure prevails at the time of bubble collapse. The lifetime of the micro bubbles is another important phenomenon that controls the production of oxidative species.

Biodegradation of acid red

The biodegradation assays carried out using isolated strain of *Bacillus sp.* showed that the Acid red was easily degraded by the organism. The organism when it was acclimatized developed the enzyme system so that the azo reductase activity became a major phenomenon of it. Being reduced by the azo reductase, the azo bonds might be degraded and the total system was now having enough carbon and nitrogen compounds for the organism to consume as nutrient. In other cases, when some necessary nutrients were provided initially and the dye was degraded by the organism, the intermediate compounds and sometimes the end products might be toxic than the dye itself. In such cases the decolorization could not be a single parameter to decide the degradation of toxicity of the dye.

Without pretreatment

The dye decolorization was observed for non pretreated dye samples. The decolorization was almost 100% within 24-27 h for 100-300 mg/l of dye concentrations. The complete decolorization time was increasing with increase in the dye concentration. For 1000 mg/l of dye concentration the decolorization was almost 95% after 48 hours.

Effect of pH

The decolorization was observed at various pH levels of the dye solution. Figure 3 shows the %decolorization at various pH levels. As

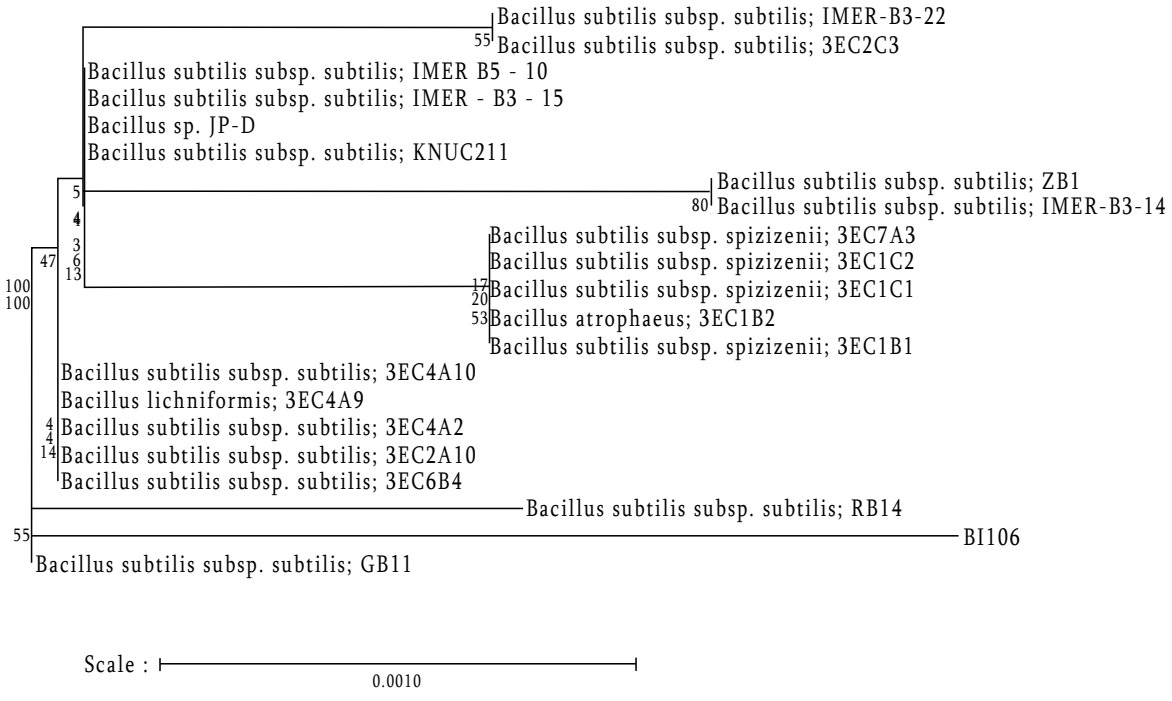


Figure 1: The phylogenetic tree of *Bacillus subtilis*..

the graph suggests, the pH variation to weakly acidic and basic didn't show any considerable variation in the decolorization rather the time of the decolorization increased few hours. The very low pH, which is strongly acidic at pH 3, showed almost inhibition to the decolorization. The decolorization was anyhow good at neutral (pH 7.0) and mild alkaline (pH 8.0) pH levels. This can be directly related to the optimum pH level for the growth of the organism. The organism, which was almost degrading the dye solutions under variable pH levels of pH 4.0-8.0, is a good sign for the treatment of textile effluent, which varies in the pH levels in a wide range.

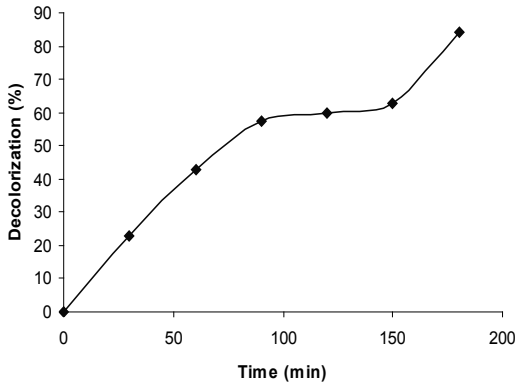


Figure 2: Decolorization of Acid by Sonication.

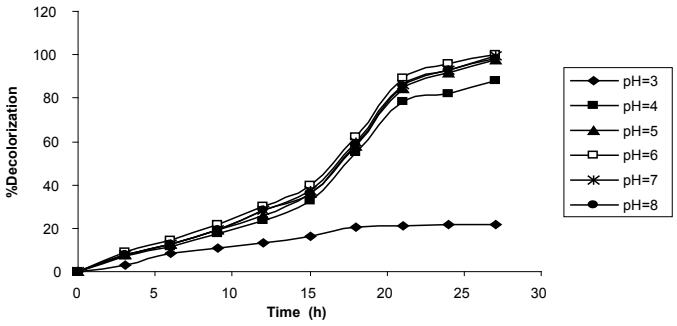


Figure 3: Effect of pH for decolorization of Acid red (300 mg/l; at 37°C).

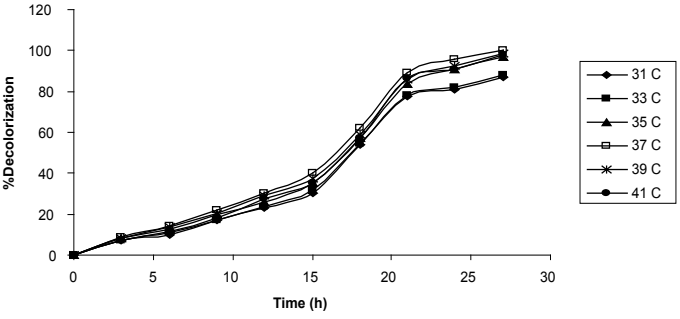


Figure 4: Effect of temperature for decolorization of Acid red (300 mg/l; at pH 7.0).

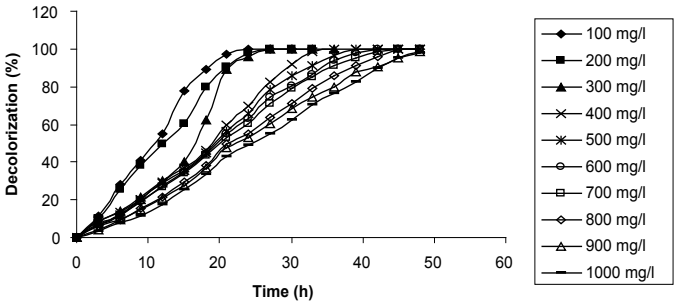


Figure 5: Effect of initial concentration for decolorization of Acid red.

Effect of temperature

The decolorization was observed at various temperatures. The effect of temperature on percentage decolorization is shown in Figure 4. The *Bacillus sp.* was showing very low inactivation under the temperature levels of 31°C and 41°C. The complete degradation was attained almost at the same time limit for 35°C, 37°C and 39°C. Based on the experimental data obtained through experiments optimum temperature was 37°C. In general the increase in the temperature of the reaction increases the reaction rate. But here the reactions are strictly biological which depends on the living organisms. On the other hand, the enzymatic reactions are temperature sensitive. The proteins are not stable with high temperatures (more than 45°C) and denaturation of protein may result. The temperature conditions here studied depend on the atmospheric temperature in which the effluent may be discharged. Depending on the average high and low temperatures of this zone, the temperature limits were selected. The organism itself can survive at a wide range of temperatures and hence there was no significant change in the decolorization time.

Effect of Initial concentration

Decolorization was studied at all studied initial dye concentrations (100-1000 mg/l). The percentage decolorizations of various initial concentrations of dye (100-1000 mg/l) were plotted against time in Figure 5.

For 100 mg/l of dye concentration, the complete decolorization was achieved after 24 hours of incubation. However the decolorization was not inhibited by the increase in the concentrations up to 1000 mg/l, despite the increased process timings.

Though the decolorization was observed at higher concentrations of 1000-1500 mg/l, it was effective with consensus of time only at lower concentrations (100-300 mg/l). The effluent concentrations normally vary widely time to time. So the ability of the organism to degrade the dye at wide range of concentrations is an important factor for effective degradation [17]. By various experiments performed with different initial concentrations, we came to know that the isolated species of *Bacillus sp.* had the ability to degrade different concentrations of Acid red.

With pretreatment

Figure 6 shows the decolorization of various pretreated samples (300 mg/l). The rate of decolorization varies for different pretreated samples. The non pretreated sample took 27-29 hours for complete degradation, whereas the 3 h treated sample took only 6 to 8 hours for complete degradation.

The efficiency of the process was thus improved up to 77% by pretreatment with sonolysis. It is noted that the power consumption for the low frequency Ultrasound is also low. Instead of going for a complete sonolysis process, the pretreated process may be cost effective as the pretreatment only needs small power input. As the obnoxious dyes were deposited, all but the strongest chromophores were killed off.

COD analysis

The COD analysis of various samples withdrawn during the degradation process through biodegradation and sonolysis were done and the results are shown in Figure 7 and Figure 8. The percentage Chemical Oxygen Demand removal increased with respect to time and the maximum COD removal was achieved at the end of the process. The variation of the COD during sonolysis was measured and is shown in Figure 8. The decrease in COD was observed during the process. The process showed a quick decrease in the COD to a certain noticeable levels (70-80%) with in the short span of time compared to the biodegradation.

This suggests that the degradation by sonolysis may be a better pretreatment method. Though the COD levels did not decrease to a certain extent in many previous literatures, the COD removal here was achieved due to the settling of dye intermediates, which was observed during the sonolysis process.

Growth kinetics

Generally two approaches were followed to fit the data obtained for the biodegradation experiments. First approach considered dye as a non-inhibitory substrate and the corresponding model equation is given below.

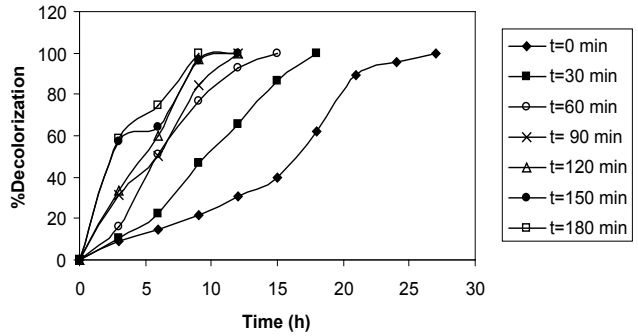


Figure 6: Effect of pretreatment time for the decolorization of Acid red.

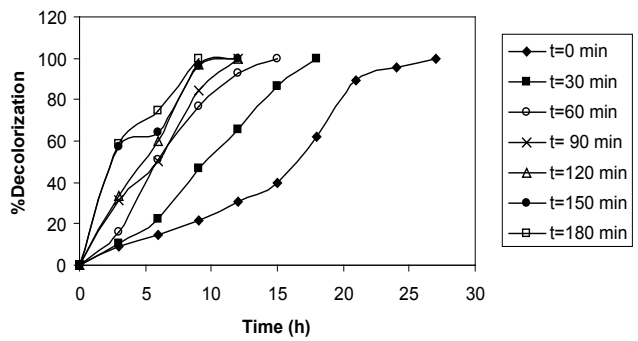


Figure 7: Effect of incubation time for %COD removal by biodegradation (without sonication).

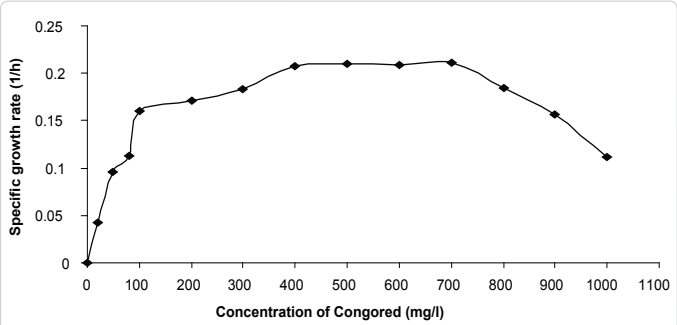


Figure 8: Effect of treatment time for % COD removal by sonolysis.

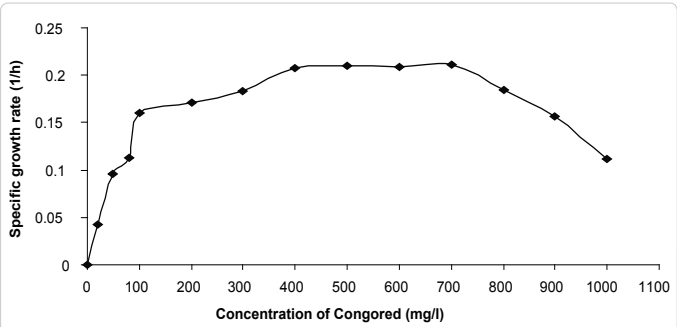


Figure 9: Growth model for the *Bacillus sp.* under various substrate concentrations.

$$\mu_g = \frac{\mu_{max} S}{K_s + S} \quad (5)$$

Where μ_g is growth rate in h^{-1}, μ_{max} is maximum specific growth rate in h^{-1}, S is the substrate concentration in mg/l, K_s is the half saturation constant in mg/l.

The second approach considered dye as an inhibitory substrate and represented by substrate inhibitory model known as Haldane's model. The corresponding equation is given below.

$$\mu_g = \frac{\mu_{max} S}{K_s + S + (S^2 / K_i)} \quad (6)$$

where K_i is the substrate inhibition constant in mg/l.

The respective models were fitted (Figure 9, 10 and 11) with the experimental data obtained and are given below.

$$\mu_g = \frac{0.272S}{106.68 + S} \quad (R^2 = 0.98) \quad (7)$$

The Haldane's model is mentioned as,

$$\mu_g = \frac{0.46S}{175.88 + S + (S^2 / 587.54)} \quad (R^2 = 0.98) \quad (8)$$

The maximum specific growth rate is an important parameter in the modeling of growth kinetics. Its value is the slope of the line when the organisms grow exponentially

From the Figure 9, it can be predicted that the growth rate started decreasing at the concentration of 600 mg/l, which exactly fits with the model prediction. The growth rate decreased at a high rate as the concentration started increasing more than 700 mg/l. The growth of the organism was inhibited at concentrations about 1500 mg/l. The linearized growth model was shown in Figure 10.

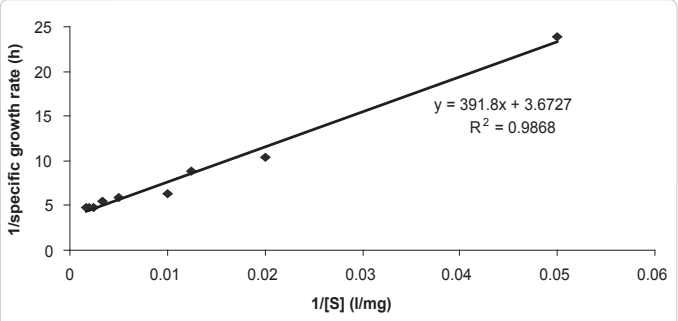


Figure 10: Linearized growth model.

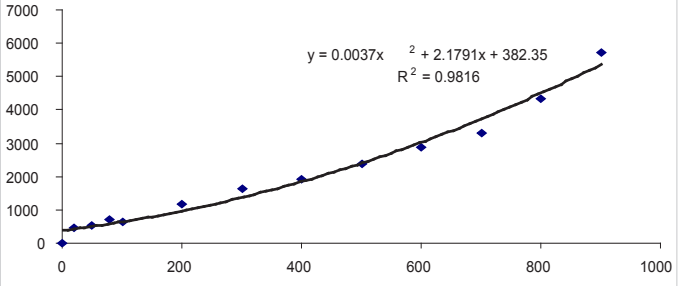


Figure 11: Haldane's model.

In this study the single substrate was used for the growth and hence the growth rate mainly depends on the substrate concentration. The inhibition constant obtained in the Haldane's model shows that the cell growth started ceasing at the concentration of 587.54 mg/l of Acid red and shown in Figure 11.

The cell growth rate constant suggests that at relatively low concentrations the efficiency of the process is high. However the large scale treatment of the wastewater containing Acid red can be a difficult task as the concentrations of substrate may vary eventually.

Conclusion

These results indicate that the ultrasonic irradiation enhanced the degradation. The addition of chloride ion (50 ppm) into the dye solution in the cases of sonolysis, the decomposition efficiency did not change significantly by the addition of chloride ion. These results indicate that the decomposition of dye using the irradiation of ultrasound is not influenced by chloride ion. The isolated Microorganism, *Bacillus sp*., was acclimatized in such a way that could utilize AR 66 dye as a sole source of nutrition. The efficiency of AR 66 degradation by this isolate was characterized as a function of dye concentration, pH, as well as temperature. Under optimum conditions, AR 66 could be degraded within 10 h. The COD analysis was performed and was found that about 90-95% COD reduction was achieved by this hybrid technique. The biodegradation data for AR 66 were fitted to Bridge- Haldane equation with good correlation.

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