# Pretreatment of textile effluent by ultrasound based hybrid methods for the enhancement of biodegradability

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This study presents the biodegradation of real textile effluent pretreated with different hybrid methods. The experiments were carried out with and without pretreatment of effluent to analyze the influence of different pretreatment methods. The advanced oxidation processes such as sonolysis, sono-sorption and sono-Fenton-sorption were employed for the pretreatment of the effluent. In sono-sorption and sono-Fenton-sorption, eco-friendly, cost effective and easily available tea waste was used as an adsorbent. The sorbent was activated using formaldehyde followed by sonolysis. The biodegradation was carried out using mixed culture and the optimal initial pH and incubation temperature were found to be 7 and 32 °C, respectively. The UV-vis and FTIR analyses of untreated and treated effluents confirmed the degradation of organic pollutants. The microbial growth data obtained were tested with Monod, Powell, Haldane, Luong and Edward equations and the obtained data were found to fit well with Haldane model.

Keywords: Textile effluent, Pretreatment, Advanced oxidation process, Sonolysis, Biological treatment

# Introduction

The synthetic dyes are often found in environment due to their wide use in various industries. The industries such as plastics, textile, food, paper and cosmetics are using these dyes in large quantities to colour their products. However, the textile industry ranks first in the consumption of dyes. It is estimated that approximately 10 - 15% of total amount of dyes produced were released into the environment mainly through wastewater<sup>1</sup>. Since the dyes are very toxic, treatment of such effluents is highly essential before disposing into the environment. But the biorefractory nature of these effluents restricts the applicability of biological treatment. Advanced oxidation processes (AOPs) can be successfully used to enhance the biodegradability of wastewater containing recalcitrant compounds. Though AOPs are highly efficient, the operating cost associated with that restricts their applicability as a sole treatment method. Therefore, methods involving AOP and biological method are reported as an economically attractive for treating such effluents<sup>2</sup>. Among the various AOPs, the

application of ultrasound (US) for the destruction of recalcitrant organics has gained greater attention in the recent past. Entezari and Al-Hoseini<sup>3</sup> employed sonosorption to remove methylene blue from aqueous solution and utilized waste newspaper as sorbent. The application of ultrasound increased the mass transfer and surface area of the sorbent, which in turn increased the methylene blue removal rate.

Shemer and Narkis<sup>4</sup> studied the removal of trihalomethanes from aqueous solution with ultrasound and Fenton's oxidation individually and also with the combination of both. Gopinath et al.<sup>5</sup> pretreated the Congored using sonolysis to enhance its biodegradability. In our previous study<sup>6</sup>, the treatment of real textile effluent using different AOPs such as sonolysis, sono-sorption and sono-Fenton-sorption (SFS) was reported and higher performance was reported for SFS. Onat et al.<sup>7</sup> studied the hybrid method involving ultrasound irradiation and biological treatment. Initially, decolorization of Reactive Red 2 (RR2), Reactive Blue 4 (RB4), and Basic Yellow 2 (BY2) was studied by continuous ultrasonic irradiation at 20 kHz and then was treated microbially using Rhodotorula mucilaginosa. The highest decolorization yield reported was 93%, which was observed for the

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Fable 1 -	Physicoche	emical chara	acteristics o	f real textile	effluent

Parameter	Values
Color	Greenish Blue
pH	5.0-5.2
COD, mg/L	29,333-30,667
BOD <sub>5</sub> , mg/L	9,260-9,780
TDS, mg/L	8,800
Density, kg/m <sup>3</sup>	973.8

sample with RR2 at 50 mg/L initial dye and 6 g/L cell concentrations. The studies reported in the literature deals with simulated textile wastewater prepared using one or more dyes. Therefore, the present study focuses on the biodegradation of real textile effluent with different pretreatment methods such as sonolysis, sono-sorption and sono-Fenton-sorption. The main purpose of combining different treatment processes was to achieve the destruction of recalcitrant compounds into biodegradable, which would facilitate the biodegradation of organic pollutants easily. The development of hybrid methods is much important for the treatment of textile effluent to overcome the operational problems associated with biological treatment alone employed.

## Materials and methods

#### Materials

The effluent used in the present study was collected from a textile dyeing unit located at southern part of Tamil Nadu, India and its characteristics are presented in Table 1. It is learnt that the dyeing unit uses different azo dyes for coloring the textiles. The tea waste was collected from the canteen and its physical and chemical properties are given in Table 2. The chemicals and reagents used in the present study were of analytical reagent.

#### Microorganism

In the present study, microbial consortium isolated from textile effluent was used. This was added into the nutrient broth containing peptone 5 g/L, beef extract 1 g/L, yeast extract 2 g/L, sodium chloride 5 g/L and effluent (3 g COD/L) and the contents were incubated at 37°C. It was then acclimatized in nutrient broth medium containing effluent (6 g COD/L) and then the sludge was acclimatized to withstand even higher concentrations of dye. The morphological and biochemical tests performed with indigenous consortium Table 2-Chemical and physical properties of tea waste used in the experiment

Chemical characteristics	
Parameter	Value (%)
Water soluble components	5.2
Insoluble components	83.8
Moisture	8.0
Ash	3.0
Physical Characteristics	
Parameter	Value
Bulk density	0.279 g/cm <sup>3</sup>
Particle size	80 - 354 mm

#### Table 3 - Morphological and Biochemical analyses of microorganisms

Parameter Analysis	Bacillus sp.	Pseudomonas sp.		
Morphology	Rod	Rod		
Gram staining	+	-		
Oxidase	-	+		
Indole	-	-		
Methyl Red	+	-		
VP test	+	-		
Citrate	+	+		
HS Production	+	-		
Motility	+	-		
Gelatin hydrolysis	-	+		
D-Glucose	+	+		
Gas production	-	-		
L-Arabinose	+	+		
Lactose	+	-		
Maltose	+	-		
Mannitol	-	+		
Mannose	+	+		
Catalase	+	+		
Nitrate reduction	+	+		

(Table 3) revealed that the consortium majorly contains *Pseudomonas* sp. and *Bacillus* sp.

#### Biodegradation

About 200 ml of effluent was placed in an Erlenmeyer flask and inoculated with 2 ml of microbial consortium. The samples withdrawn from the flask at regular time intervals were centrifuged at 6000 rpm for 20 min and the supernatant was subjected to further analysis. The degradation of effluent was assessed based on % COD reduction and it was determined using Eq. 1.

% COD reduction = 
$$\frac{C_0 - C}{C_0} \times 100$$
 ...(1)

where,  $C_0$  and C are the initial COD in mg/L and COD at any time t in min, respectively. The cell pellets, thus collected were washed twice with distilled water and the biomass was estimated using wet weight method. The effect of initial pH (4-9), temperature (28 to 44 °C) and initial concentration (20-100% v/v) on the biodegradation of textile effluent was studied. The initial concentration was varied by diluting the effluent to the desired concentration.

# Pretreatment

## Sonolysis

Sonolysis was performed in a bath type sonicator operating at 30 kHz (Oscar Ultrasonics, OU-Mini type) and the ultrasonic waves were emitted from the bottom of the reactor. The temperature of the sonicator (volume of 3.5 L) was controlled using cooling coil. About 500 ml of effluent was charged into the reactor and was subjected to ultrasound irradiation at different desired time limits. The sonicated effluent (200 ml) was then added into Erlenmeyer flask and inoculated with microbial consortium. The samples were withdrawn at regular time intervals and subjected to COD analysis.

#### Sono-sorption

Sono-sorption was performed using activated tea waste as sorbent under the influence of ultrasonic irradiation. Tea waste (discarded tea dust after use) is an eco-friendly, cost effective and easily available waste material and India is the largest producer and consumer of tea. For better performance, the activation of tea waste is essential and in the present study, tea waste was activated by formaldehyde followed by ultrasound<sup>6</sup>. About 500 mL of effluent and 6 g/L of activated tea waste (optimized size of  $80 - 120 \mu m$ ) were charged into the sonochemical reactor and the mixture was irradiated for the desired time. The samples were withdrawn at regular time intervals and subjected to COD analysis.

## Sono-Fenton-sorption

Sono-Fenton-sorption experiments were conducted similar to sono-sorption and in addition, Fenton's reagent (50 mg/L of Fe (II) and 400 mg/L of  $H_2O_2$ ) was added into the sonochemical reactor along with 500 mL of effluent and 6 g/L of activated tea waste<sup>6</sup>. The pH of the solution was maintained at 3 to prevent the precipitation of iron as iron hydroxide<sup>8</sup>.

## **Analytical methods**

Chemical oxygen demand (COD) was determined by open reflux method<sup>9</sup>. A digital calibrated pH meter (Elico, Li 127 type) was used to determine the pH. The untreated and the effluent after different treatments were analyzed using Fourier transform infrared spectroscopy (Perkin Elmer Spectrum 1 FT-IR spectrometer) and the absorbance spectra were recorded from 4000 – 400 cm<sup>-1</sup>.

## Ecotoxicity test

The ecotoxicity of the textile effluent was performed before and after biodegradation using disc diffusion method. The test was performed as outlined by NCCLS<sup>10</sup>. The test was carried out with *Escherichia coli* (MTCC 443) and the diameter of inhibition zone was measured in mm using a ruler. The values reported in present study include the disc diameter of 6 mm.

## Kinetics

The knowledge on microbial growth kinetics is essential to understand the degradation capability of the microorganism and biokinetic constants are essential to scale-up the process. Since the growth of microbes is a result of anabolic and catabolic enzymatic activities, substrate utilization or growth associated product formation can be quantitatively described on the basis of growth models. In this study, the data obtained were tested with Monod, Powell, Haldane and Luong equations.

The specific growth rate was determined using Eq. 2.

$$\mathbf{\mu} = \frac{1}{x} \left( \frac{dx}{dt} \right) \qquad \dots (2)$$

where,  $\mu$  is the specific growth rate (1/h), x is the biomass concentration (g/L) at time t (h) and dx is change in biomass concentration with respect to change in time, dt. The growth of microbes without any inhibition can be modeled using Monod equation<sup>11</sup> and is given as

$$\mu = \frac{\mu_{max} \cdot S}{K_S + S} \qquad \dots (3)$$

where  $\mu_{max}$  is the maximum specific growth rate (1/h), S is the substrate concentration and  $K_s$  is the half saturation constant (g/L). This model is simple and describes the dependence of specific growth rate on the initial substrate concentration. The specific growth rate was studied for different substrate concentrations by changing the initial concentration of the effluent by diluting with distilled water. The biomass concentration was determined



Fig. 1 - Effect of operating parameters on % COD reduction of effluent during biodegradation. Fig. 1(a) Conditions: Effluent volume: 200 ml, Incubation time: 24 h, initial concentration of effluent: 100% (undiluted), incubation temperature: 32 °c;



through wet weight method. The original Monod model was modified by Powell<sup>12</sup> and is given as:

$$\mu = \frac{(\mu_{max} + m).S}{K_S + S} - m \qquad ...(4)$$

where m is the maintenance rate (1/h).

Monod and Powell models are particularly fit the non-inhibitory growth data well whereas in the present study, as the biodegradation was performed for real textile effluent of very high COD content, it is required to test the data obtained with different growth kinetic models accounting inhibition too. In the present study, growth data obtained were tested with Haldane, Edward and Luong model.

A modified version of Monod model is Haldane model<sup>13</sup> which incorporates inhibitory effects of toxic substrates and is given as:

$$\mu = \frac{\mu_{max} \cdot S}{K_s + S + \left(\frac{S^2}{K_i}\right)} \qquad \dots (5)$$

where  $\mathbf{K}_{i}$  is the substrate inhibition constant (g COD/L).

Edward<sup>14</sup> proposed a mathematical model similar to Haldane model and is given as:

$$\mu = \frac{\mu_{max} \cdot S}{K_s + S + \left(\frac{S^2}{K_i}\right) \left(1 + \frac{S}{K}\right)} \qquad \dots (6)$$

This model has an additional term [1+(S/K)] in the denominator, which includes a positive constant K and the higher value of K indicates the more suitability of Edward model compared to Haldane model.

The inhibitory effect of substrate on microbial growth under batch conditions can also be modeled using Luong equation<sup>15</sup>. The assumptions of this model include no lag phase, organism death, endogenous respiration, substrate used for maintenance energy or inhibition by the products<sup>16</sup>. Luong model is given as:

$$\boldsymbol{\mu} = \frac{\boldsymbol{\mu}_{max} \cdot \boldsymbol{S}}{K_{S} + \boldsymbol{S}} \left( \mathbf{1} - \frac{\boldsymbol{S}}{S_{m}} \right)^{n} \qquad \dots (7)$$

where  $S_m$  is the critical inhibitor concentration above which the biodegradation stops and n is the positive integer. All equations were fitted with the data by nonlinear regression method using MATLAB<sup>®</sup>.

## **Results and discussion**

#### Biodegradation

## Effect of initial pH and initial concentration

In order to find the optimum pH for the effective degradation by microbial consortium, experiments were performed at six different initial pH and the results obtained are shown in Fig.1a. After 24 h of incubation, the % COD reduction at pH 4 was 12.4 and at pH 5 and 6, the reduction was 20.45 %. At neutral conditions, the maximum COD reduction (24.7%) was observed. The degradation was less at basic conditions and the COD reduction was about 15.91 and 13.5% for pH of 8 and 9 respectively. From the results, the optimum pH arrived was 7 and further experiments were carried out at the optimum pH. The results indicated that the microbial consortium isolated from the real effluent was found to be efficient on degrading the organic pollutants under wide pH ranging from 4 to 9. Therefore, the microbial consortium can be effectively used to treat the textile effluents, which are generally prone to have varying pH levels. The influence of initial concentration on biodegradation was studied and the results (Fig. 1b) showed the decrease in COD reduction with an increase in initial concentration. This may due to the increase in organics load and inhibition of microbial growth at higher concentrations.

#### Effect of temperature

The influence of incubation temperature on the time required for the complete degradation by microbial consortium was studied by varying the temperature from 28 to 44°C and the results obtained are shown in Fig.1c. No growth was observed at 44°C and the growth was significant over the temperature range from 28-40°C. The maximum % COD reduction was observed at 32°C and hence, further experiments were carried out at 32°C. Cetin and Donmez<sup>17</sup> investigated the decolourization of reactive dyes by microbial consortium isolated from textile effluent and reported no growth of microbial consortium at 45°C and reported maximum color removal at 35°C.

#### **Pretreatment of effluent**

The % COD reduction of effluent using microbial consortium at optimum conditions was found to be 24.7% after the incubation period of about 24 h. To enhance the degradation rate, three pretreatment methods such as sonolysis, sono-sorption and sono-Fenton-sorption were employed.

#### Sonolysis

The effluent was irradiated with ultrasound at different time intervals and then was subjected to biodegradation. The COD reduction after 24 h of incubation was observed to be 24.7, 38.2, 48.3 and 55.1% when the effluent was pretreated with sonolysis for 0, 30, 60 and 90 min, respectively. Here, 0 min indicates no pretreatment and the effluent was degraded by biological treatment. The application of ultrasound into the aqueous effluent generates tiny bubbles and when these bubbles reach high pressure region it collapses. This violent collapse generates very high temperature of several thousand Kelvin and pressure of several hundred atmospheres<sup>18, 19</sup>. The higher temperatures generated pyrolytically cleave the pollutants and also produce hydroxyl radicals, which are strong oxidizing agents. Thus, during ultrasound irradiation, the complex organic molecules were converted into simpler molecules through oxidation and pyrolytic decomposition<sup>20</sup>. These simpler molecules can easily be biodegraded and this leads to better reduction in COD.

#### Sono-sorption

Sonolysis can be improved by the addition of solid particles into the reaction medium. It is well established that the presence of solid particles will accelerate the sonolytic degradation as these added particles enhance the nucleation sites in the liquid bath and hence, cavitation was significantly improved. Keck et al.<sup>21</sup> reported that presence of inert particles doubled the reaction rate compared to the rate observed in the absence of particles in the sonicator. This may be attributed to the induced turbulence and additional convective mass transfer inside the pores caused by high velocity micro jets produced by the ultrasound irradiation<sup>22</sup>. Therefore, effluent was pretreated with sono-sorption at different time intervals and then was subjected to biodegradation. The COD reduction observed after 24 h of incubation was 57.3, 73 and 82% when the effluent was pretreated with sonosorption for 30, 60 and 90 min respectively. Thus, the effluent after sono-sorption pretreatment was easily biodegraded and hence, significant reduction in COD was observed with biological treatment. This is attributed to the fact that the complex organic pollutants were initially adsorbed on the surface of the sorbent. The organic molecules are present on the surface and hence, well exposed to the oxidizing agents. This phenomenon indicates that the sorbent acts as a catalyst for the better pyrolytic decomposition of textile effluent by ultrasound.

#### Sono-Fenton-sorption

The sono-sorption process may be further improved by incorporating Fenton process. This is because of the production of highly reactive hydroxyl radicals and other oxidants by both sonolysis and Fenton processes. Thus, the produced powerful oxidants and pyrolytic action of ultrasound irradiation degraded the organic pollutants present in the effluent effectively and in addition, the mass transfer rate was enhanced significantly in the presence of ultrasound. The pretreatment of effluent by sono-Fenton-sorption for 30 and 60 min showed 71.9 and 84.3% COD reduction respectively, after 24h of incubation. Complete degradation (100%) was achieved when the effluent was pretreated with SFS for a period of 90 min followed by the biodegradation.

#### **Comparison of pretreatment methods**

The presence of non-biodegradable compounds in the textile effluent prevents the treatment by biological methods. Therefore, the effluent was subjected to pretreatment to convert complex organics into simpler molecules, which can be treated biologically. In this study, the real textile effluent was subjected to different pretreatment methods and the results presented in Fig. 2 showed that better performance was observed with SFS. The pretreatment of effluent for 30 min with sonolysis, sono-sorption and sono-Fenton-sorption enhanced the



Fig. 2 - Effect of different pretreatment methods such as (a) sonolysis, (b) sono-sorption, (c) sono-Fenton-sorption, for different time duration, followed by biodegradation for 24 h.
Conditions: Frequency: 30 kHz, effluent volume: 500 mL, initial pH: 5.2, sorbent dosage: 6 g/L.

COD reduction in biological treatment from 24.7 to 38.2, 57.3 and 71.9%, respectively. In order to confirm the influence of pretreatment, biodegradation of pretreated effluent (by sono-Fenton-sorption; initial COD of 16 g/ L) and the diluted effluent (initial COD of 16 g/L) was carried out. Even though both the effluents had similar COD levels, the COD reduction observed for the diluted effluent was 89.9% after 24 h incubation. On the other hand, complete degradation was achieved when the effluent was pretreated with SFS. This confirmed that, enhancement in COD reduction was attributed by synergistic effect and not due to the effect of less substrate concentration. The pretreatment of effluent oxidized the highly complex organic pollutants into simpler molecules, which made biodegradation simple.

#### **Degradation analysis**

The FT-IR spectral comparison between untreated textile effluent and effluent after biological degradation with pretreatment of sonolysis, sono-sorption, sono-Fenton-sorption (treatment of 90 min each) clearly confirmed the degradation of organic compounds present in the effluent (Fig. 3). It can be observed that, majority of the peaks present in the FT-IR spectrum of the untreated effluent were either shifted or disappeared during the treatment. The peaks in the range of 3800 - 3200 cm<sup>-1</sup> correspond to –OH bridging groups in all the systems and might be attributed to O-H stretching of either phenolic or alcoholic group. The peaks at 1396 and 763 cm<sup>-1</sup> in the untreated effluent spectrum (Fig. 3a) confirmed the presence of phenolic group. The peak at 1396 cm<sup>-1</sup> represents the presence of aromatic nitro



Fig. 3 - FT-IR spectra of effluent (a) untreated; (b-d) treated with different pretreatment methods followed by biodegradation with (b) sonolysis, (c) sono-sorption, (d) sono-Fenton-sorption

compounds and the peak at 763 cm<sup>-1</sup> indicate the presence of mono-substituted aromatic compounds in the untreated effluent. The -N=N- stretching vibrations formed peaks around 1623 - 1653 cm<sup>-1</sup>, while the more complex amide band was located at 1279 cm<sup>-1</sup>. The peak at 2127 cm<sup>-1</sup>may be due to stretching vibrations of C=N. After biodegradation with pretreatment of sonolysis and sono-sorption (Figs. 3b and 3c), the absence of peaks at 1396 and 1279 cm<sup>-1</sup> confirmed the mineralization of aromatic nitro and complex amide compounds. On the



Fig. 4 - Monod, Powell and Haldane models fitted to experimental data

other hand, the presence of peaks at 1651 and 1639 cm<sup>-1</sup> after sonolysis followed by biodegradation and at 1644 cm<sup>-1</sup> after sono-sorption followed by biodegradation indicates the presence of azo bond in the dyes. In addition, the mono-substituted aromatic compounds constituted the peaks at 757 and 733 cm<sup>-1</sup> after sonolyis and sono-sorption followed by biodegradation respectively. This confirmed the incomplete degradation after sonolysis followed by biodegradation (COD reduction of 55.1%) and sonosorption (COD reduction of 82%). Figure 3d shows the FT-IR spectrum of the effluent treated with SFS followed by biological treatment. The peak at 3418 cm<sup>-1</sup> may be due to the hydroxyl stretching of alcoholic group. The C=O stretching vibration produced the peak at 1707 cm<sup>-</sup> <sup>1</sup> and C=N stretching vibration produced the peak at 2145 cm<sup>-1</sup>. The results clearly indicate the absence of complex aromatic compounds and this confirmed the complete degradation of organic pollutants present in the effluent.

#### Ecotoxicity test

The degradation of effluent may produce organic intermediates or final products, which are sometimes more toxic than the untreated effluent. In this study, disc diffusion susceptibility test was carried out with *Escherichia coli*, to analyze the toxicity of effluent, before and after treatment. Under similar conditions, the diameter of inhibition zone was found to be 19 mm for untreated effluent whereas it was about 16 and 11mm for effluent after biodegradation with pretreatment using sonolysis and sono-sorption, respectively. No such inhibition zone was observed for the effluent treated by

Table 4-Growth kinetic parameters obtained from different models							
S. No.	Model	$\mu_{max}(1/h)$	K <sub>s</sub> (g COD/L)	K <sub>i</sub> (g COD/L)	K	m	$\mathbb{R}^2$
1	Monod	0.07678	0.8894	-	-	-	0.9262
2	Powell	0.07677	0.8902	-	-	2.498x10 <sup>-5</sup>	0.9262
3	Haldane	0.3036	20.12	10.36	-	-	0.9671

SFS followed by biodegradation. It clearly indicates the hybrid method of SFS followed by biodegradation completely removed the toxicity of the effluent.

## **Growth kinetics**

The growth data obtained were fitted with Monod, Powell and Haldane models and model parameters values obtained are presented in Table 4. Fig. 4 shows the fits obtained for different models and it can be seen from the figure that the trend observed for Monod and Powell models is almost similar. This may be due to the fact that the value of maintenance coefficient obtained was very less and hence, the model results in almost similar to that of Monod model. The substrate affinity constant  $(K_s)$ was found to be 0.8918 (Monod model) and 0.8941 g COD/L (Powell model) and these values are much lesser than initial substrate concentration (29.67 g COD/L). The kinetic models accounting inhibition such as Haldane, Edward and Luong models were also tested. Haldane model was found to fit the data well with high correlation coefficient.

The critical substrate concentration  $(S_{crt})$  was estimated using Eq. 8, above which the substrate removal rate falls due to the inhibition effect.

$$S_{ort} = \sqrt{K_s K_l} \qquad \dots (8)$$

where,  $K_s$  and  $K_I$  are substrate affinity constant (g COD/L) and substrate inhibition constant (g COD/L), respectively. The critical substrate concentration obtained in the present study was 14.426 g COD/L. The other models such as Edward and Luong models did not fit the data well.

## Conclusions

The more stringent legislations with respect to the discharge of textile effluents motivate to develop novel treatment techniques for the benefit of industries across the world. The obtained results proved that the treatment of real textile effluent using novel hybrid pretreatment, sono-Fenton-sorption followed by biodegradation, is very effective on COD reduction. The FT-IR analyses confirmed that the degradation of organic pollutants and

the treated effluent was proved as non-toxic through ecotoxicity test. The microbial growth data obtained were fitted with different models and Haldane model was found to fit the data well.

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