

Microbial Degradation of Reactive Red 195 by Three Bacterial Isolates in Anaerobic-Aerobic Bioprocess

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Abstract Chemical Oxygen Demand (COD) and Decolorization removal efficiency of consortia of alkalophilic microorganisms was evaluated in a continuous anaerobic/aerobic reactor system. The biodegradation products of Reactive Red in the continuous anaerobic/aerobic reactor were studied by employing different analytical tools such as Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC). When the synthetic dye wastewater was subjected to a two stage reactor, a 96.0% color removal of Reactive Red was attained after the anaerobic treatment. However, after the aerobic treatment, overall decolorization efficiency was 100% as compared to the anaerobic stage. On the other hand, the removal efficiency of Chemical Oxygen Demand (COD) was greater after the aerobic treatment (66.6%) than the anaerobic treatment (94.26%). The band of the Thin Layer Chromatography shows fragments of dye decolorization products. On the other hand, the HPLC Chromatograms show that the dye decolorization products that are produced at the anaerobic stage were degraded. From HPLC chromatograms, three new peaks were obtained and one of it was degraded completely and the retention time and peak areas of the other two peaks was decreased. From the consortia, dye decolorizing microorganisms were isolated and tested for their decolorization efficiency in batch test. The isolates from the anaerobic/aerobic reactor were inoculated into a sterilized decolorized media with the addition of glucose as a carbon source to a final concentration of 0.1% and the isolates were incubated under aerobic condition. After 12 h the isolates growth (OD) was measured and all the isolates tested were able to grow using metabolites of the decolorized media as a sole nitrogen source.

Keywords: biodegradation, Reactive Red 195, dye biodegradation products, alkalophilic microorganisms

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1. Introduction

The world's ever increasing population and the progressive adoption of an industrial based life style has inevitably led to an increased anthropogenic impact on the biosphere. Textile industries are the most important industries in Gujarat and their numbers have increased. These industries have shown a significant increase in the use of synthetic complex organic dyes as the coloring material. Recently, pollution problems due to the discharge of effluents from a textile industry have increased tremendously. Approximately 75% of the dyes that are discharged by textile processing industries belong to the classes of reactive (36%), acid (25%) and direct (15%) dyes (Anjali, *et al.*, 2007). Among these classes of dyes, the azo dyes which are characterized by aromatic moieties linked together by azo bond (-N = N-) chromophores are the most important chemical classes of synthetic dyes and pigments, representing between 60% and 80% of the organic dyes used in industries such as textile, leather, plastic, cosmetic and food industries (Vandevivere, *et al.*, 1998). The removal of dyes from a

textile effluent is desired not only for aesthetic reasons, but also many azo dyes and their breakdown products are toxic to aquatic life (Chung and Stevens, 1993) and mutagenic to humans (Brown and Devito, 1993; Weisburger, 2002). Furthermore, their discharge into surface water obstructs light penetration and oxygen transfer into water bodies, hence affecting aquatic life (Asad, *et al.*, 2007). Since synthetic reactive azo dyes are made soluble in water and stable both chemically and photolytically by design, they are persistent and recalcitrant in the environment (Ramalho, *et al.*, 2005). Therefore, textile effluents containing dyes must be treated before their discharge into the environment. Although, it is difficult to treat textile industry effluents due to their high BOD, COD, heat, color, pH and the presence of metal ions (Dos Santos, 2005), now a days, different systems are developed and applied to treat textile waste water before they are being discharged into water bodies. Currently, physical, chemical and biological methods are employed to decolorize dyes (Hao, *et al.*, 2000). As compared to the physical and chemical methods, which are characterized by limited versatility, high operation cost and energy requirement, generation of a large amount of sludge and secondary pollution due to

excessive chemical usage, biological treatment methods of textile industry waste water using biological processes are advantageous because they are relatively inexpensive, environmental friendly and generate less waste (Pandey, *et al.*, 2007; Ramya, *et al.*, 2007). The complete mineralization of dyes by a microorganism involves two major processes, reductive cleavage of the dyes' azo linkages under anaerobic conditions which results in the formation of generally a colorless but potentially hazardous aromatic amines and degradation of the aromatic amines aerobically. However; the most logical and economical process for the complete mineralization of dyes is achieved by combining the anaerobic and aerobic stages together (Van der Zee & Villaverde, 2005). Currently, many researches are carried out to decolorize the color of the dye in textile waste water with an anaerobic microorganism under anaerobic condition. But azo dye reduction under anaerobic condition leads to the accumulation of toxic aromatic amines which are resistant to anaerobic degradation (Franciscon, *et al.*, 2009). These dye metabolites are only degraded aerobically. Field *et al.*, (1995) showed that the aerobic stage of the combined anaerobic/aerobic treatment of dye wastes eliminated the additional COD, attributed to the removal of aromatic amines, which are anaerobically recalcitrant. As a consequence, anaerobic/aerobic sequential processes could prove for the reduction of both color and organic carbon. Therefore, sequential anaerobic/aerobic treatment can be used to decompose toxic and carcinogenic compounds efficiently (Sponza and Isik, 2005). In the case of Ankleshwar industrial zone, Gujarat, India, the wastes of textile industries are released into the nearby water body without proper treatment. This will have a great impact on the environment as well as human life. In some industries pretreatment were done aerobically before being discharged. But aerobic treatment of textile effluents containing azo dyes by an activated sludge is resistant to biodegradation. Hence, it is important to develop a system that can treat the dye containing wastewater of a textile industry. This can be achieved by using consortia of alkalophilic microorganisms in a sequential anaerobic/aerobic system.

2. Materials and Methods

2.1. Dyes and Chemicals

A reactive red azo dye (Figure 1) was kindly supplied by local textile industry. The dye was selected on the basis of structural diversity and frequent use in textile industries.

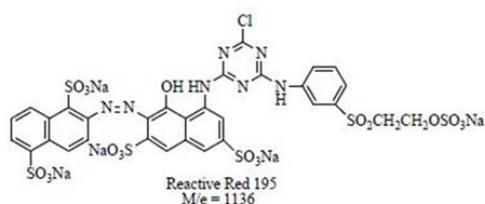


Figure 1. Chemical structure of Reactive Red 195

2.2. Composition of Synthetic Wastewater

The basic composition of a synthetic dye wastewater medium was (g L^{-1}): Na_2HPO_4 (3.6), KH_2PO_4 (1.0), $(\text{NH}_4)_2\text{SO}_4$ (1.0), MgSO_4 (1.0), CaCl_2 (0.1), $\text{FeC}_6\text{H}_5\text{O}_7$ (0.01), and 10 ml of trace element solution per liter was used for all the studies. The trace element solution used was of the following composition (mg L^{-1}): $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (10.0), $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (3.0), $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (1.0), $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ (2.0), $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ (3.0), H_3BO_3 (30.0) and $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ (1.0). The medium was supplemented with 10 mg/L. Soluble starch and yeast extract were added to the synthetic dye media to a final concentration of 0.5% (w/v) and 0.01% (w/v) respectively and sterilized along together with the media. Sodium carbonate, Na_2CO_3 , (25% w/v) was separately sterilized and added to the media in order to maintain an alkaline pH.

2.3. Configuration of the Continuous Reactor System

The basic configuration of the continuous reactor system consists of anaerobic and aerobic reactors respectively. In the continuous reactor system, the synthetic wastewater containing a reactive Red azo dye (0.01 g/L) were continuously pumped with a flow rate of 1 ml/min into an anaerobic reactor consisting of anaerobic consortia of alkalophilic mud samples (10% w/v) which are collected from common effluent treatment plant of Ankleshwar, Gujarat, India. The retention time of reactive Red azo dye in the anaerobic reactor was 2 h. The treated dye along together with some consortia of alkalophilic mud samples were then pumped into the aerobic reactor with a flow rate of 1 ml/min. The aerobic reactor was continuously supplied with air by using a pump. The continuous anaerobic/aerobic reactor also consists of three sampling ports (before the anaerobic reactor, after the anaerobic reactor and after the aerobic reactor) for the High performance Liquid Chromatography (HPLC), Thin Layer Chromatography (TLC) and Uv-Visible Spectrophotometry analysis.

2.4. Decolorization and Growth Measurement

In order to measure decolorization, sampling was done for every 24 h for 5 days from the three sampling sites of the continuous reactor (before the anaerobic reactor, after anaerobic treatment and after aerobic treatment). Then the samples were centrifuged at 8000 rpm for 10 min and were analyzed by UV-Vis spectrophotometry. Similarly, sampling was also done for every 24 h from the anaerobic batch decolorization test by isolates (E1, E2 and E3) from the continuous reactor, clarified and analyzed by UV-Vis spectrophotometry. For both tests, an uninoculated culture media with and without added dyes were used as a control and blank respectively. Decolorization efficiency of the sediment consortia in the anaerobic/aerobic reactor and the different isolates (E1, E2 and E3) in an anaerobic batch decolorization was expressed as

$$\text{Decolorization (\%)} = (A_0 - A)/A_0 \times 100,$$

Where, A_0 = the initial absorbance and

A = the absorbance after decolorization at the λ_{max} (nm) of the dye under study.

The average decolorization rate (μgh^{-1}) was calculated as

$C \times \% D \times 1000 / (100 \times t)$

Where C is the initial dye concentration and %D is the dye decolorization (%) after time t (h).

The bacterial growth or turbidity after decolorization of Reactive Red under batch test by three isolates isolated from the continuous reactor was calculated by determining the difference between the absorbance of the culture samples before and after centrifugation at 600 nm.

$$\text{Turbidity} = \text{OD}_{(\text{before centrifugation})} - \text{OD}_{(\text{after centrifugation})}$$

2.5. Decolorization under Different Culture Conditions

Decolorization under different culture conditions was studied by changing carbon source, nitrogen source and dye concentration. In effect, different decolorization efficiency was obtained at different conditions. The effect of aeration was examined under three culture conditions, namely, static (no shaking), agitated (aerobic) and anaerobic. In the case of aerobic decolorization batch tests using the three isolates (E1, E2 and E3), the culture flasks were shaken on a rotary shaker running at 110 rpm/min. In the anaerobic batch test, flasks containing decolorizing medium were sealed with rubber septa and incubated under anaerobic condition and in the static condition flasks were placed in the incubator directly.

2.6. Decolorization by Active and Inactive Cells

After the dye was decolorized in an anaerobic batch test by the isolate E1, the decolorized medium was autoclaved, centrifuged at 8000 rpm for 10 min, incubated with the dye and their decolorization activity was monitored by UV-Vis spectrophotometry.

2.7. Isolation of Bacterial Strains from the Continuous Reactor

Isolation was made from the continuous reactor system that was acclimatized for a month with alkalophilic consortia. Serial dilutions (10^{-1} to 10^{-6}) of the samples collected from both anaerobic and aerobic reactors were inoculated into a mineral salt agar medium (MSM) by the spread plate technique. Isolates were inoculated into a synthetic dye media containing 10 mg/L and incubated at 30°C for 5 days. The isolates were tested for their decolorization efficiency under anaerobic batch conditions and the strain that achieved the best decolorization efficiency was selected for further study.

2.8. Analysis

2.8.1. Color Measurement

Color in the influent and effluent samples of the anaerobic/aerobic reactors and color in the anaerobic batch treatment with the selected isolate, E1, was measured with Shimadzu-1800 UV-Vis spectrophotometer at the maximum visible absorbance wavelength of the dye. In both cases samples were centrifuged at 8000 rpm for 10 min and absorbance values of supernatants were recorded for color measurements. In order to measure the real color removal, the absorbance

values of the treated media at λ max was calibrated with the control samples containing no dye (Awoke, 2008).

2.8.2. Biodegradation Assay Via TLC

After complete decolorization by the efficient decolorizer isolate (E1) which was selected from the isolates, the decolorized medium was centrifuged at 8000 g for 10 min. And the supernatant extracted with chloroform after alkalization to pH 8 to extract the biotransformed products. Then the extracted product was evaporated in a rotary evaporator. The concentrated extract was dissolved in 1 ml chloroform and used for thin layer chromatography (TLC). The mobile phase for the organic and the aqueous extracts was petroleum ether: chloroform: methanol (4:1:1). The bands of decolorization metabolite were observed under UV light.

2.8.3. HPLC Analysis of Decolorization Metabolites

To determine the dye fragments produced upon decolorization in a continuous reactor system using microbial consortia the treated samples were used directly for HPLC analysis. HPLC analysis was carried out on a Cecil model Adept CE 4900 chromatograph equipped with a Cecil model CE 4200 UV detector, a column model CE 4601, and a Lichrosorb C-18 column with a 4.6 mm inside diameter and 25 cm height. In the continuous reactor, sampling was done before the anaerobic treatment, after anaerobic treatment and aerobic treatments and centrifuged at 8000 g for 10 min, clarified by 0.45 filters, and analyzed with HPLC. In the batch test, the decolorized media under anaerobic condition with the efficient isolate were centrifuged, pH adjusted to 7.5, autoclaved, supplemented with glucose to a final concentration of 0.1% and inoculated with three isolate aerobically that are capable of decolorizing under aerobic, incapable of decolorizing under aerobic and the efficient isolate that is capable of decolorizing under anaerobic condition. Samples before autoclaving (after centrifugation) and incubation with the three isolates were centrifuged at 6000 rpm for 10 min, clarified by 0.45 filters, and analyzed with HPLC. In the analysis of dye fragments, a mobile phase composed of a phosphate buffer solution (0.7 g/L Na_2HPO_4 , 0.58g/L $\text{NH}_4\text{H}_2\text{PO}_4$) and methanol with a flow rate of 1 ml min^{-1} were used. The elutes were monitored by the UV absorption at 220 nm and 217 nm. To determine the dye fragments produced upon decolorization, the treated samples were used directly for HPLC analysis.

2.8.4. COD, Total Nitrogen and NH_4^+ Measurements

In the continuous reactor, samples before the anaerobic treatment, after anaerobic treatment and after aerobic treatment were taken and analyzed for chemical oxygen demand (COD), Total nitrogen, ammonia and nitrate. Chemical Oxygen Demand, Total Nitrogen and NH_4^+ measurement were done according to the standard methods of HACH instruction manual and instruments.

The percent removal efficiency of Chemical Oxygen Demand (COD) and Total Nitrogen (TN) was calculated as:

$$\% \text{ Removal efficiency} = \frac{C_i - C_f}{C_i} \times 100,$$

Where, C_i = the initial concentration of the feed C_f = final concentration after treatment.

3. Results

3.1. Color Measurement from the Continuous Reactor

From Figure 2a, the maximum absorbance of the dye ($\lambda_{\max} = 540 \text{ nm}$) was 0.631. After the anaerobic stage (Figure 2b), the maximum absorbance of the dye was found to be 0.025. The color removal efficiency of the reactor was approximately 96.0%. After the dye was treated in an anaerobic condition, it was pumped into a second stage known as the aerobic reactor. From Figure 4c, the absorbance of the dye after aerobic treatment or the effluent was found to be 0.0. And the color removal efficiency of reactive Red 184 at λ_{\max} was 100%. Thus the major color removal was attained at the anaerobic stage than the aerobic stage. After biodegradation of the dye, the absorbance peaks in the visible region disappeared while the absorption peak in the UV range did not diminish, indicating complete decolorization.

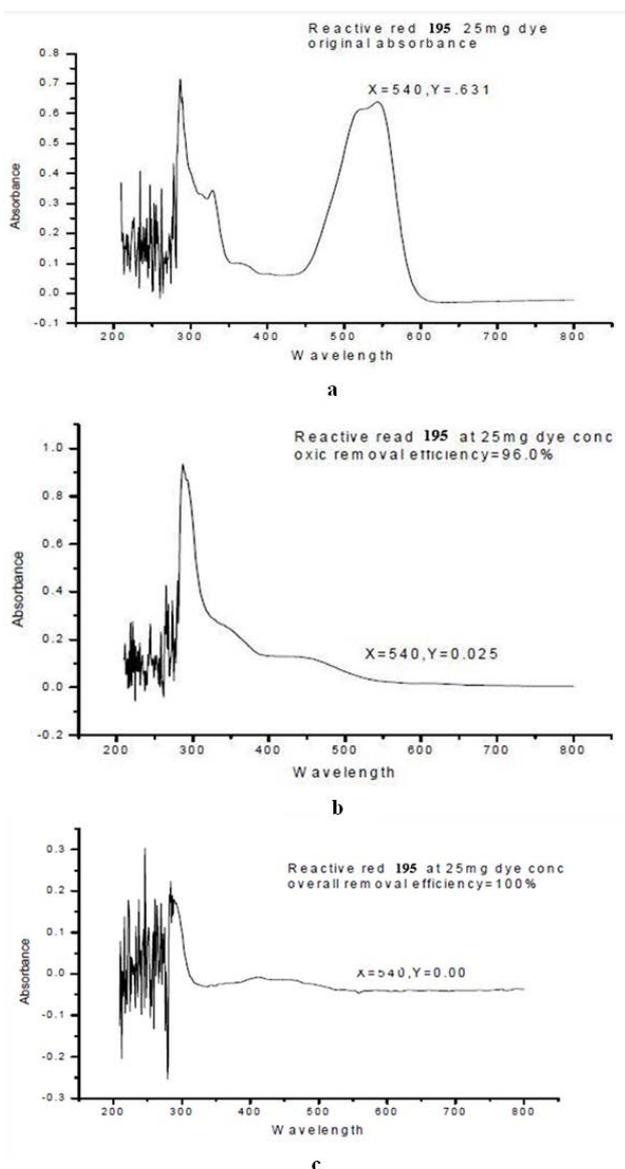


Figure 2. Color removal efficiency of a) dye b) anaerobic effluent c) aerobic effluent

3.2. HPLC Analysis of Biodegradation Products

At 220 nm, HPLC analysis of the parent compound, the dye (Figure 3a) shows the presence of two peaks with retention times 0.62 and 1.35 minute. The peak area of the peak with retention time 0.62 was 47.6 mv. For the second peak with retention time 1.35, the area of the peak was 23.3 mv. At the same wavelength, the dye after anaerobic treatment was analyzed by HPLC and it gives three new peaks with different retention times than the chromatograms of the parent molecule (Figure 3b). The retention times of the peaks were, 0.46, 0.58 and 1.23 minute. The peak areas of the three peaks were 121, 245 and 46 mv respectively. The appearance of these three new peaks with different retention times after anaerobic treatment can be attributed by the cleavage of the azo bond by the consortia into aromatic compounds. It should be noted that due to the unavailability of authentic standards, the chromatographic peaks appearing in samples taken after the anaerobic system could not be identified or quantified. Therefore, it is reasonable to consider that the three peaks contain aromatic amines originating from total reduction of Reactive Red azo dye. Further HPLC analysis of the effluent after aerobic treatment at 220 nm, gives three peaks with retention times, 0.46, 0.58 and 1.25 minute (Figure 3c). The areas of the three peaks were 12, 61, 52 mv respectively. At the end of aerobic phase, the HPLC analysis seems to indicate that the decolorization metabolites produced during the anaerobic phase were removed in the subsequent aerobic phase. Figure 5c shows that when the products produced during the anaerobic stage were metabolized aerobically, a less aromatic compound was formed. This is because the product peak area of the peaks decreased tremendously.

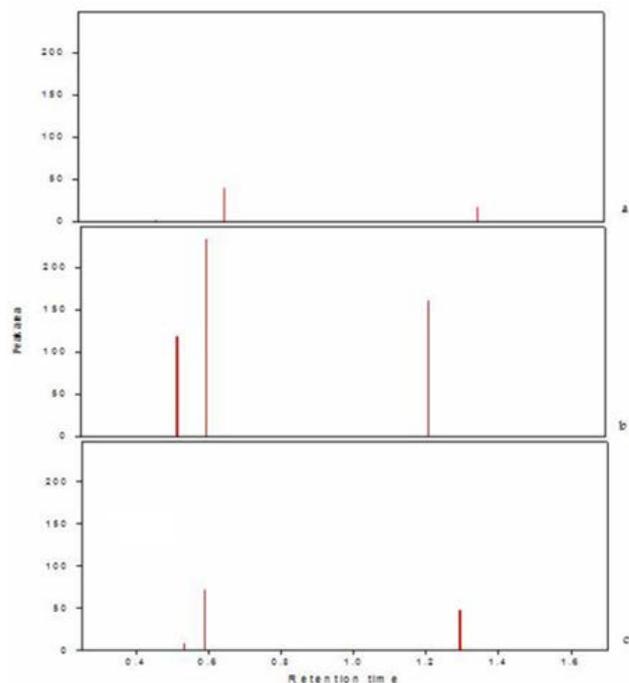


Figure 3. HPLC chromatograms of the a) dye, b) anaerobic effluent c) aerobic effluent at 220nm

3.3. Thin Layer Chromatography/TLC

The TLC chromatograms under UV light showed that the decolorized sample had two bands indicating that Reactive Red was cleaved into two fragments (data not shown).

3.4. Decolorization of Reactive Red by Active and Inactive Cells

In the study of active versus inactivated cells, half of the decolorized media by the isolate E1 in an anaerobic batch experiment were autoclaved and half of the remaining decolorized media were left without being autoclaved. In the study it is only active (non autoclaved) cells were able to decolorize the dye whereas inactivated cells are unable to do so. This shows that decolorization was primarily taking place by degradation than adsorption.

3.5. COD, Total Nitrogen, and NH_4^+ Measurements

Table 1. The influent and effluent parameters of anaerobic/aerobic system

Parameter	Sample		
	feed	anaerobic effluent	aerobic effluent
COD (mg/L)	1290	430	74
Total Nitrogen (mg/L)	104.8	52.2	23.2
Absorbance at λ_{max} (nm)	540	220	217
$\text{NH}_3\text{-N}$ (mg/L)	0	25.6	54
pH	9.97	8.92	9.42

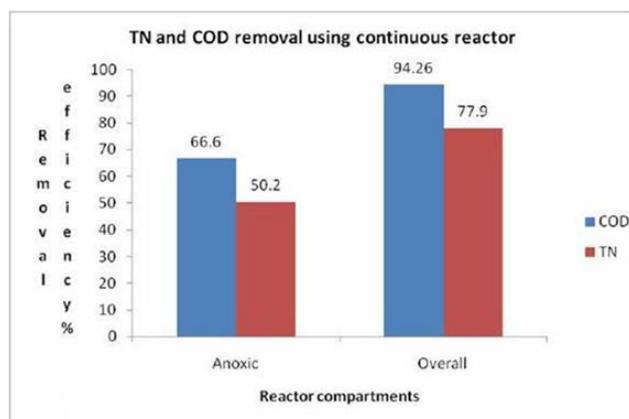


Figure 4. Comparison of COD and Total Nitrogen Removal in an anaerobic and Overall

3.6. Isolation of Isolates from the Continuous Reactor

isolate E1, which is isolated from the anaerobic reactor were tested for its decolorization efficiency, as a function of time, with various concentration of dyes ranging from 1 mg/L, 2.5 mg/L and 5 mg/L and it shows a pronounced efficiency in decolorizing reactive Red 184 in 48 h. Similarly, the decolorization efficiency of E2 and E3, which were isolated from the aerobic reactor, were tested under aerobic and anaerobic conditions, respectively. Isolate E2 decolorized the dye within four days of incubation in a shaker (data not shown). When the isolate E3 was incubated with the dye under aerobic condition, its decolorization efficiency under this condition was almost negligible. Unlike isolate E2, it was capable of decolorizing the dye in 48 hours of incubation. In order to test whether the three isolates are able to grow on the decolorized media using it as a sole nitrogen source or not the decolorized medium by isolate E1 under anaerobic condition were centrifuged at 600 g for 10 min and the pH of the supernatant was adjusted to 7.5. And then the supernatant was autoclaved and placed in to a 50 ml flask.

In Table 1, treatment of synthetic dye wastewater with a continuous anaerobic/aerobic process showed that evolution of NH_4^+ in the subsequent aerobic process increases from the anaerobic reactor (25.6 mg/L) to the aerobic reactor (54 mg/L). The pH after the anaerobic treatment decreases to 8.92 and it increases to 9.42 after aerobic treatment (Table 1). As Figure 4 shows, the Chemical Oxygen Demand (COD) and Total Nitrogen (TN) removal efficiency after anaerobic treatment were 66.6% and % 50.2 % respectively. And the overall COD and Total nitrogen removal efficiency after aerobic treatment were 94.26 % and 77.9 % respectively.

Since the cells already utilized the carbon source during decolorization, glucose was separately autoclaved and added to each flask to a final concentration 0.1%. The three flasks were inoculated with the three isolates and incubated under aerobic condition. The sole nitrogen source for the isolates was the dye decolorization metabolite, which mainly consist of aromatic amines. Samples were taken every 12 hours and the cell growth (turbidity) was measured at 600 nm (Figure 5). All the three isolates are able to grow.

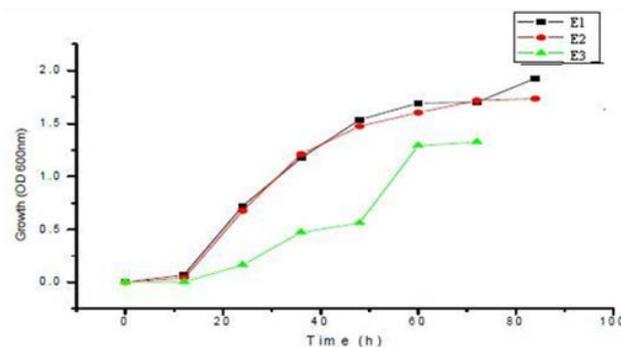


Figure 5. Growth profile of three isolates, E1, E2 and E3 on decolorization product of isolate E1 under aerobic condition

4. Discussion

In the present study, consortia of alkalophilic microorganisms from common effluent treatment plant are used to decolorize Reactive Red 195 in a continuous anaerobic/aerobic reactor system. In the system 100 percent color removal was obtained at the end of the treatment (Table 1). As the dye was decolorized, the azo bond of Reactive Red serves as a final electron acceptor and cleaved into aromatic amines (Chung and Stevens, 1993). Although color removal is greater at the anaerobic stage, the presence of the anaerobic stage alone is not effective for complete decolorization. If dye reduction is not taking place at the anaerobic stage, it will leave the aerobic stage intact (Van der Zee and Villaverde, 2005).

Therefore; for the removal of color as well as for removal of the products that are produced as a result of dye decolorization at the anaerobic stage, a continuous anaerobic/aerobic system is crucial. In the study, greater color removal efficiency was achieved at the anaerobic stage than the aerobic stage. The color removal efficiency after the anaerobic stage was found to be 96.0%. On the other hand, the color removal efficiency of the overall was found to be 100%. The color removal efficiency of the aerobic reactor was only 4%. Thus, for complete color removal, a continuous anaerobic/aerobic system was found to be essential. Qualitatively, dye decolorization products were identified by High Performance Chromatography (HPLC). The chromatograms of HPLC of the anaerobic/aerobic treatment process showed the formation of new products at the anaerobic stage and oxidation of the product at the aerobic stage. At the anaerobic treatment process a third new peak and another two peaks with a lower retention time and greater peak area were identified. This is due to the presence of one azo bond in Reactive Red dye. According to (Pandey, 2007), azo dye reduction is taking place primarily by anaerobic reduction of the azo bond of the dye followed by aerobic oxidation of biodegradation products. The peak areas of the three metabolites decrease to a greater extent. This suggests that continuous anaerobic/aerobic treatment of dye containing effluents is a promising technology for dye decolorization as well as oxidation of decolorization products. Based on the structure of most of the reactive azo dyes, the prediction is that under anaerobic condition, the products of dye reduction would result in the formation of aromatic compounds (Carvalho, 2007). And the chromatogram of the Thin Layer, showed the presence of two fragmented dyes. This is because the Reactive Red under study was a monoazo dye and cleavage of the azo linkage would result in two structurally distinct aromatic amines. According to the Literature (Sani and Banerjee, 1999), biodegradation of dyes by bacteria could be due to adsorption or biodegradation. After biodegradation, half of the decolorized media was autoclaved and inoculated into another media containing Reactive Red. The remaining non-autoclaved decolorized media was inoculated into another media containing Reactive Red. Then the biodegradation was monitored daily both visually and in a UV-Visible Spectrophotometer. In effect it is the non-autoclaved decolorized media showed biodegradation. This is because biodegradation of dyes proceeds primarily by degradation than physical adsorption. The continuous anaerobic/aerobic reactor system was evaluated for Chemical Oxygen Demand (COD), total nitrogen and ammonia evolution. In the system, COD removal efficiency was 66.6% at the anaerobic stage and the overall removal efficiency was 94.26%. This shows that most of the chemicals and the dye are used by the consortia. Similarly, the removal efficiency of total nitrogen increases from 50.2 at the anaerobic stage to 77.9 in the overall. This is because the nitrogen sources in the original dye media were used as a nitrogen source for growth by the consortia. On the other hand, ammonia evolution increases after aerobic treatment than after anaerobic treatment in the continuous reactor system. During decolorization of Reactive Red by consortia in a continuous anaerobic/aerobic system, the pH of the synthetic dye containing wastewater, when it was treated

anaerobically and increases slightly (Table 1). According to (Sponza, 2005) the lowering of pH at the anaerobic stage is due to the formation of acids. As Figure 5 shows, the three isolates (E1, E2, and E3) were able to grow using the decolorized media as a sole nitrogen source and with the supplement of glucose as a carbon source. This shows that decolorization products can be further degraded into simpler molecules.

5. Conclusions

Treatment of synthetic wastewater containing azo dye, RR 195 by consortia of alkalophilic microorganisms was conducted under a continuous anaerobic/aerobic system. Biodegradation efficiency of the system and analysis of the degradation products was studied. During the treatment, most of the color was removed at the anaerobic stage (96%) than at the aerobic stage (4%). Ultimately, a 100% color removal efficiency was attained in the over anaerobic/aerobic system. In addition to color removal efficiency, Chemical Oxygen Demand (COD) and Total Nitrogen (TN) removal efficiency was studied. In the study a 66.6% and 50.2% COD and TN removal efficiency were obtained after anaerobic treatment respectively. In the overall treatment system, 94.26% and 77.9% COD and TN removal efficiency were obtained respectively. After biodegradation of RR 195 in a continuous anaerobic/aerobic system, the intermediate products were studied by High Performance Liquid Chromatography (HPLC) and Thin Layer Chromatography (TLC). From HPLC analysis, it has been found that, the intermediate products which were appeared in the anaerobic treatment were treated at the aerobic reactor. Hence, a continuous anaerobic/aerobic system was found to be efficient in removing most of the biodegradation products. TLC analysis has shown that the consortia were able to degrade the dye under study in to two fragments. The efficiency of a single isolate for color removal isolated from a continuous anaerobic/aerobic system containing consortia of alkalophilic microorganisms was evaluated in a batch test. In the study, isolate E1 has shown a 100% color removal after 48 h of incubation. Similarly, the efficiency of three isolates from the continuous reactor were evaluated whether they can utilize the decolorized media as a sole nitrogen source or not. During the study, all the three isolates have shown growth.

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