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Bioresource Technology 101 (2010) 2678–2684

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

The potential of free cells of *Pseudomonas aeruginosa* on textile dye degradation

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ARTICLE INFO

Article history:
Received 5 July 2009
Received in revised form 25 October 2009
Accepted 27 October 2009
Available online 24 November 2009

Keywords:
Biochemical oxidation
Pseudomonas aeruginosa
Procion Blue
Electro-oxidation

ABSTRACT

The objective of the present work was to reduce chemical oxygen demand (COD), color of textile effluent containing dye Procion Blue 2G and recycle the treated effluent. To achieve this objective, the degradation potential of bacterial strain *Pseudomonas aeruginosa* was tested. In degradation, the 'clean' electro-oxidation (EO) process was combined with bio-treatment such that an electro-oxidation step was included between two bio-degradation treatments. Bio-oxidation process was carried out under aerobic and anoxic conditions. Results showed more than 90% reduction in COD and complete removal of color at the end of two cycles of combined oxidation process with a post electro-oxidation. The treated effluent was then subjected to photo-oxidation to remove the microbes so that the water can be recycled after the removal of total dissolved solids (TDS).

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1. Introduction

Untreated dye effluent is highly colored and hence, reduces sunlight penetration, preventing photosynthesis. Many dyes are toxic to fish and mammalian life; they inhibit growth of microorganisms, and affect flora and fauna. They are also carcinogenic in nature and can cause intestinal cancer and cerebral abnormalities in the fetus (Doble and Kumar, 2005).

About 60% of reactive dyes applied onto cotton fabrics and yarn are hydrolyzed and discharged as effluent. The dye house releases two types of wastewater, viz., dye bath water and wash water/rinse water. The dye bath water mainly consists of complex dyestuff and various intermediate complexes. It was noticed that in a typical factory the effluent from the dye bath had COD 5000–6000, TDS 52,000, SS 2000 mg L^{-1} and pH 9. After dyeing, the fabrics are washed by rinsing in water to remove the excess dye present. The wastewater generated due to this operation is commonly called as 'wash water' having the value of COD 400–860, TS 4000, TDS 3200 mg L^{-1} and pH 8. The extent of environmental pollution due to dye bath water is very high.

The physical and chemical methods for the treatment of dye-containing effluent includes coagulation (Sanghi et al., 2006), flocculation (Mishra and Bajpai, 2005), membrane filtration (Ostar-Turk et al., 2005), electro-kinetic coagulation (Yang and McGarrahan, 2005), ion-exchange (Karcher et al., 2002), electro-chemical destruction (Bandara et al., 2007), photochemical precipitation (Lucas and Peres, 2006) chemical oxidation (El-Daly et al., 2005) , ozonation (Lackey et al., 2006) and adsorption with activated carbon (Orfao et al., 2006). Though some of these physicochemical processes have been shown to be effective, their application is limited due to the excess usage of chemicals, sludge generation with subsequent disposal problems, high installation as well as operating costs and sensitivity to a variable wastewater input (Robinson et al., 2001).

Many researchers have been tried to solve this problem using living or dead microorganisms (Yesilada et al., 2002; Bayramoglu et al., 2006; Arıca and Bayramoglu, 2007; Kumari and Abraham, 2007; Khalaf, 2008).

Another physical method has been one using ultraviolet light irradiation. Exposing effluent to ultraviolet light destroys pathogens. The beneficial factor is that, ultraviolet light treatment has no residual effect (Cheremisinoff, 2002).

Despite the existence of a variety of chemical and physical treatment processes, bioremediation of textile effluent still seems to be more cost-effective than other methods. The low cost of biotechnological treatment has been largely attributed to the small quantities or total absence of added reagents and microbial biomass to start up the bio-treatment process. Biological treatment for decolorization and degradation of textile effluents may be either aerobic, anaerobic, or a combination of both, depending on the type of microorganisms being employed (Bechtold et al., 2006). Due to the large variability of the composition of textile wastewaters, most of the traditional and individual physical, chemical and biological methods for treatment are inadequate.

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doi:10.1016/j.biortech.2009.10.077

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Hence, current research has been focused on a combination of physical or chemical treatment processes with biological methods for treating effluents in a cost-effective manner (Garcia-Montano et al., 2006; Ghoreishi and Haghighi, 2003; Ledakowicz et al., 2001; Lucas et al., 2007). However these combinations with biological methods have been either costly or merely transfer the pollutants from one phase to another phase.

Among these various physical or chemical combinations with biological methods, the electrochemical techniques have been receiving greater attention in recent years due to their distinctive advantages such as environmental compatibility, versatility, scalability and safety (Brillas et al., 2003). Previously, it has been reported that the non-bio degradable organics present in specialty chemicals industry wastewater have been treated successfully by coupling electro-oxidation and electro-coagulation with bio-oxidation (Basha et al., 2009).

In this present work, a single strain, *Pseudomonas aeruginosa* were used instead activated sludge because the use of a pure culture system ensures that the data are reproducible and that the interpretation of experimental observations is easier. The detailed mechanisms of bio-degradation can be determined using the tools of biochemistry and molecular biology, and these tools may also be used to up regulate the enzyme system to give modified strains with enhanced activities. Activated sludge contains mixed cultures only provide an average macroscopic view of what is happening in the system and results are not easily reproduced, making thorough, effective interpretation is difficult. The free cells of *P. aeruginosa*, was used for the bio-degradation of the dye effluent containing Procion Blue 2G with the combination of electro-oxidation. Biodegradation experiments were sequentially carried out in cycles for two different degradation environments (aerobic and anoxic) and for the two different cell concentrations.

In this study, degradation of dye effluent was a sequential treatment process consisting of pre electro-oxidation, aerobic-anoxic-aerobic bio-oxidation as one cycle and a post electro-oxidation. The treated effluent again was subjected to irradiation to remove the microbial populations present in the effluent.

2. Methods

The chemicals used for the experiments and the analysis techniques were of AR grade.

2.1. Dye solution preparation

Procion Blue hydrolyzed dye-bath waste has been collected from a dyeing industry located in Tiruppur, Tamilnadu, India, and was dried by keeping it in a hot air oven. To prepare the study sample, the dried dye sample was dissolved in double distilled water (2000 mg L^{-1}). The color of the effluent was purple blue with a high alkaline pH range of 8–10. The COD of the effluent was found to be 2579 mg L^{-1}. When the dye bath was taking longer time to dry, the study sample of required concentration has also been prepared with the known density of dye bath effluent.

2.2. Culture preparation

A culture of *P. aeruginosa* was obtained from Microbiology Laboratory, Bharathidasan University, Trichy. The bacterial cultures have been grown in 250 mL Erlenmeyer flasks with 100 mL of nutrient broth medium, containing (g L^{-1}) of peptone 5, NaCl 5, beef extract 3 and incubated for 24 h at 303 K at static condition. The acclimatization has been done by gradually exposing *P. aeruginosa* to the increasing concentrations of dye as per earlier report (Kalme et al., 2006).

2.3. Electrochemical oxidation system

A batch electrolytic cell has been used for the electrochemical degradation process. The schematic diagram of experimental setup has been depicted elsewhere (Basha et al., 2009). The setup consists of an undivided electrolytic cell of 200 mL working capacity, closed with a PVC lid having provisions to fix the cathode and anode keeping 1.5 cm inter-electrode distance. The titanium substrate insoluble anode (TSIA with $Ti/RuO_x–TiO_x$ coating) was employed as anode in the form of expanded mesh. A stainless steel plate (of dimension $8 \times 5 \times 0.2$ cm) was used as the cathode. A multi-output 2 A and 35 V, DC power source (with ammeter and voltmeter) was connected to the cell. Stirring was done with a magnetic stirrer. All the electro-oxidation studies have been carried out at 5.0 A dm^{-2} at room temperature.

2.4. Biochemical oxidation system

All the bio-degradation experiments have been carried out at room temperature in batch mode in 250 mL Erlenmeyer flasks kept in shaking condition (140 rpm) with the help of rotary shaker for aerobic oxidation and kept in static condition for anoxic degradation. Anoxic conditions will occur if the rate of oxidation of organic matter by bacteria is greater than the supply of dissolved oxygen. When the sampling is done the flasks were agitated. Only to restrict the oxygen supply agitation was not done during the anoxic degradation stage (for 5 d) under anoxic condition.

2.5. Irradiation system

In irradiation process, a 120 W medium pressure mercury lamp has been used for illumination. A 250 mL beaker was placed inside a photo reactor to carryout irradiation studies for the treated effluent. Continuous stirring was done by magnetic stirrer.

2.6. Experimental procedure

The mode of operation that has been adopted for treating high strength refractory organic contaminants in wastewater has been as shown in the flowchart Fig. 1. A known quantity (200 mL) of dye effluent containing the Procion Blue 2G (with the initial COD of 2579 mg L^{-1}) has been taken in electro-oxidation reactor and subjected to input of certain quantity of electricity, Q (4 or 8 Ah L^{-1}, charge per liter), in the first step of operation to improve biodegradability.

In electrolysis, NaCl has been added to the effluent prior to electrolysis as a supporting electrolyte with the concentration of 2.5 g L^{-1}. After electrolysis, to subside all the chemical reactions, the content of the reactor was kept idle for 12 h.

In the second step, biochemical oxidation experiment has been started with known inoculum concentrations. A known volume, m (50 mL L^{-1} or 100 mL L^{-1}), of acclimatized bacterial broth culture was taken into 250 mL Erlenmeyer flask containing a 200 mL dye effluent (electrochemically pre-treated). Further, the flasks were kept in orbiterary shaker for 5 days in aerobic condition. In the third step of operation, the flask is (added with 25 mL L^{-1} of acclimatized bacterial broth culture if required) subjected to anoxic degradation for 5 days in static condition.

The sample of 1 mL has been collected at the initial stage and at the end of electro-oxidation experiments and subjected to COD analysis. In bio-oxidation studies, 1 mL of sample has been taken for every 24 h (and also during the culture addition in effluent) and subjected to COD analysis. In biochemical operation 0.1 mL of sample has been collected from the effluent every day and spread on agar plates to ensure the presence of microbes. This constitute one cycle.

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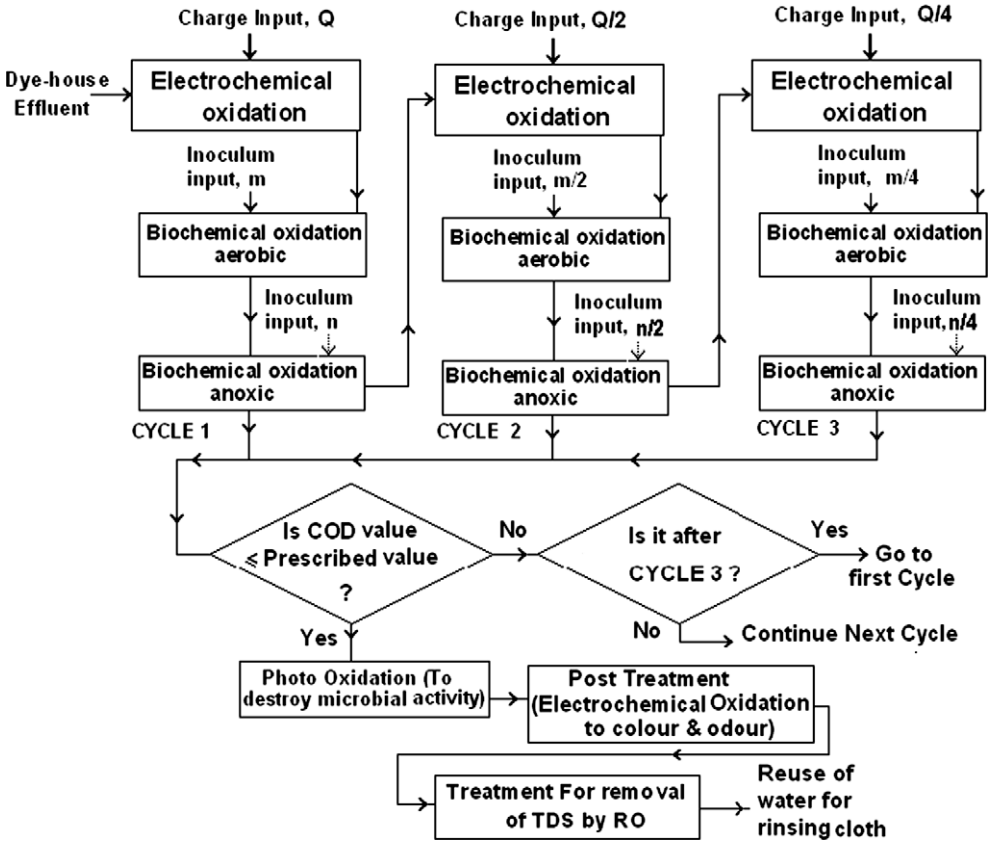

Fig. 1. A generalized flow chart for treating high strength refractory organic contaminants in wastewater.

In the second cycle, the filtered effluent has been treated electrochemically by passing 2 or 4 Ah L^{-1} electric charge at the first step of operation. After 12 h of electrochemical treatment, 25 or 50 mL L^{-1} of *P. aeruginosa* broth culture has been added to effluents, again subjected to aerobic oxidation for 5 days and to anoxic degradation (after adding 25 mL L^{-1} acclimatized bacterial broth culture if required) for 5 days in static condition. All COD analysis was carried out as before. This constitutes the second cycle of the experiment.

In the third cycle, the effluent was pre-treated electrochemically with an electrical charge input of 2 Ah L^{-1}. For biochemical operation 25 mL L^{-1} of bacterial broth culture was added.

If the COD value was found to be less than the prescribed value in any of the each sequential cycles of bio-degradation, the treated effluent was separately subjected to post electro-oxidation for maximum of an hour. The effluent was sequentially subjected to irradiation separately for one hour. The sample of 0.1 mL was collected from both the effluents and spread on agar plates. The absence of microbes in treated effluent is ensured when the microbial growth was not observed in spread agar plate for 3 days and the effluent could be sent for TDS removal. The treated effluent could be reused after TDS removal.

2.7. Analytical measurement

The sample of 1 mL has been collected at each step of the cycle and subjected to COD analysis. Samples of 10 mL have been taken from the raw, pre-treated, biologically treated (containing initial broth culture) and post treated (containing initial broth culture) effluent and were subjected to UV–visible spectroscopy (UV) and Fourier Transform Infrared Spectroscopy (FT-IR) analysis.

2.7.1. Determination of COD, BOD and Biodegradablity index (BI)

COD of all samples were determined by the dichromate closed reflux method using thermo reactor TR620-Merck and BOD by Winkler's method, strictly following the APHA (Clesceri et al., 1998). Experiments were repeated until the error was less than 3%. The biodegradability index is defined as the ratio of BOD to COD. The value ranges from 0 to 1. Morais and Zamora (2005) reported that samples with biodegradability index smaller than 0.3 are not appropriate for biological degradation. According to Chamarro et al. (2001) for complete bio-degradation, the effluent must present a bio degradability index of at least 0.4.

2.7.2. Extraction and FT-IR analysis

Each 5 ml of untreated and treated effluent was taken, centrifuged (5000 rpm) and filtered through a 0.45 μm membrane filter (Millipore). The filtrate was then extracted three times using diethyl ether. The extract was dried over anhydrous Na_2SO_4 and further evaporated to obtain solid mass in a rotary evaporator. To prepare the pellets, the dried solid masses were mixed with spectroscopically pure KBr. The pellets were fixed in sample holder and the analyses were carried out in the mid IR region of 400–4000 cm^{-1} using Thermo Nicolet Nexus 670 FT-IR Spectrophotometer.

2.7.3. UV–visible Spectrophotometer analysis

For UV-analysis, 5 mL of treated and untreated samples were taken and centrifuged at 12,000 rpm for 10 min. The supernatant of untreated and treated samples were analyzed by monitoring the changes in its absorption spectrum using UV–visible spectrophotometer (V-530, Japan) with a cell having 1 cm optical path length.

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3. Results and discussions

3.1. Electrochemical oxidation

The mechanism of electrochemical oxidation of wastewater is a complex phenomenon involving coupling of electron transfer reaction with a dissociate chemisorptions step. The electrochemical oxidation of organic contaminants on noble oxide coated catalytic anode (RuO_x–TiO_x) is as follows:

In the first step, H_2O is discharged at the anode to produce adsorbed hydroxyl radicals according to the reaction

$$(RuO_x{-}TiO_x) + H_2O \rightarrow (RuO_x{-}TiO_x)(\cdot OH) + H^+ + e^- \quad (1)$$

In the second step, generally the adsorbed hydroxyl radicals may interact with the oxygen already present in the oxide anode with possible transition of oxygen from the adsorbed hydroxyl radical to the oxide forming the higher oxide (RuO_x–TiO_{x+1})

$$(RuO_x{-}TiO_x)(\cdot OH) \rightarrow (RuO_x{-}TiO_{x+1}) + H^+ + e^- \quad (2)$$

At the anode surface the active oxygen can be present in two states, either as physisorbed adsorbed (RuO_x–TiO_x)($\cdot$OH) or/and as chemisorbed (RuO_x–TiO_{x+1}). When NaCl is used as supporting electrolyte, chloride ion may anodically react with (RuO_x–TiO_x)($\cdot$OH) to form adsorbed OCl^- radicals according to the following:

$$(RuO_x{-}TiO_x)(\cdot OH) + Cl^- \rightarrow (RuO_x{-}TiO_x)(\cdot OCl) + H^+ + 2e^- \quad (3)$$

In presence of chloride ion, the adsorbed hypochlorite radicals may interact with the oxygen already present in the oxide anode with possible transition of oxygen from the adsorbed hypochlorite radical to the oxide forming the higher oxide (RuO_x–TiO_{x+1}) according to the following reaction and also (RuO_x–TiO_x)($\cdot$OCl) simultaneously react with chloride ion to generate active oxygen (dioxygen) and chlorine according to the following reactions:

$$(RuO_x{-}TiO_x)(\cdot OCl) + Cl^- \rightarrow (RuO_x{-}TiO_{x+1}) + Cl_2 + e^- \quad (4)$$

$$(RuO_x{-}TiO_x)(\cdot OCl) + Cl^- \rightarrow (RuO_x{-}TiO_x) + \frac{1}{2}O_2 + Cl_2 + e^- \quad (5)$$

The reactions of anodic oxidation of chloride ions to form chlorine in bulk of solution as given by Eqs. (4) and (5) are as follows:

$$(Cl_2{-}H_2O) \xrightarrow{k_2} H^+ + Cl^- HOCl \quad (6)$$

$$HOCl \underset{k_{3'}}{\overset{k_3}{\leftrightarrow}} H^+ + OCl^- \quad (7)$$

$$Dye + OCl^- \xrightarrow{k_3} CO_2 + H_2O + Cl^- \quad (8)$$

Since organic compounds of the effluent are electrochemically inactive, the primary reaction that occurs at the anodes is chloride ion oxidation (Eqs. (4) and (5)) with the liberation of Cl_2, which is a robust oxidizing agent. As regards to the reactions in the bulk, gaseous Cl_2 dissolves in the aqueous solutions due to ionization as indicated in Eqs. (6) and (7). The hypochlorite ions can oxidize the long chain hydrocarbons to lower ones thereby improving the biodegradability to an extent, especially during the early stage of the treatment. The pseudo steady state theory can be applied to each of the intermediates products (HOCl and OCl^-) taking part in the bulk solution. Taking all other reactions as irreversible processes, the rates of reactions r_i for the sequence are

$$-r_{Cl_2} = k_2[Cl_2] \quad (9)$$

$$r_{HOCl} = k_2[Cl_2] - k_3[HOCL] + k'_3[H^+][OCl^-] = 0 \quad (10)$$

$$-r_{OCl^-} = k_3[HOCl] - k'_3[H^+][OCl^-] - k_4[Dye][OCl^-] = 0 \quad (11)$$

$$-r_{Dye} = k_4[Dye][OCl^-] \quad (12)$$

From the above equations we can easily deduce the following expression:

$$-r_{Cl_2} = -r_{Dye} = k_4[Dye][OCl^-] \quad (13)$$

Finally as regards to the bulk solution it is also to be noted that $-r_{Cl_2} = r_{Cl^-}$ from material balance of Eq. (6), that is

$$-r_{Cl_2} = r_{Cl^-} = k_2[Cl_2] = -r_{Dye} = k_4[Dye][OCl^-] \quad (14)$$

Where the rate of reaction r_i and the rate constants k_i (i = 2, 3 and 4) are defined with respect to bulk and the rate expression for main electrode reaction can be written as

$$-r'_{Cl} = r'_{Cl_2} = k_1[Cl^-] \quad (15)$$

where k_1 is heterogeneous electrochemical rate constant. Hence, in the following section an attempt has been made to establish a relation between the reacting species in bulk and at the electrode surfaces. The basic relationship applicable to all electrochemical reactions is Faraday's law that relates to the amount of substance reacted at the surface to the charge (I_At) passed is $M_A I_A t/nF$ (assuming 100% current efficiency) and the characteristic measurable parameter is current density, i_A, which is I_A/A_e. Thus the electrochemical reaction rate (for the disappearance of reactant A) can be expressed as

$$-(V_R/A_e)d[A]/dt = i_A/nF \quad (16)$$

where I_A is the current passed in time t, M_A is the molecular weight, n is the number of electrons transferred per mole of reaction, A_e electrode area, V_R reactor volume and F is the Faraday (96,500 Coulomb or As/mol). It has to be noted $-r_A = -d[A]/dt = i_A a/nF$, where a is specific electrode area (A_e/V_R). Assuming the main electrode reaction is governed by a simple Tafel type expression, then

$$-(V_R/A_e)d[A]/dt = i_A/zF = k'[A]\exp(bE) \quad (17)$$

$$\text{or} \quad -r'_{Cl^-} = r'_{Cl_2^-} = k_1[Cl^-] = k_1 a[Cl^-]_s \exp(bE) \quad (18)$$

The electrochemical oxidation of Cl^- ion to Cl_2 at the anode causes depletion of Cl^- ion near the anode. The reactant Cl^- is transported from the bulk of electrolyte to electrode surface due to concentration gradient, for further reaction. This process may be assumed to be under diffusion control. Then,

$$i_A/zF = k_L([Cl^-] - [Cl^-]_s) \quad (19)$$

Elimination of $[Cl^-]_s$ using Eqs. (18) and (19) results as

$$i_A/zF = k_1[Cl^-] \quad (20)$$

$$\text{Where} \quad 1/k_1 = 1/K_L + 1/k'a\exp(bE) \quad (21)$$

From a material balance of species Cl^- by taking note of Eqs. (13) and (14) we can write

$$i_A/zF = k'[Cl_2] \quad (22)$$

$$i_A/zF = k''[Dye][OCl^-] \quad (23)$$

During electrolysis, since the constant current is applied, the rate of generation of $[OCl^-]$ will remain constant under a given set of experimental condition, but it varies as the applied current is altered. Then

$$i_A/zF = k_L[COD] \quad (24)$$

Adopting the same classification for the reactors as for conventional reactors, thus the electrochemical reaction rate (for removal of COD) can be expressed as

$$-(V_R/A_e)d[COD]/dt = k_L[COD] \quad (25)$$

$$\text{Or} \quad [COD] = [COD]_o \exp(-k_L at) \quad (26)$$

where a is the specific electrode surface (cm^{-1}), defined as the ratio of active electrode surface to the electrolyte volume and k_L, the mass transfer coefficient ($cm\ s^{-1}$).

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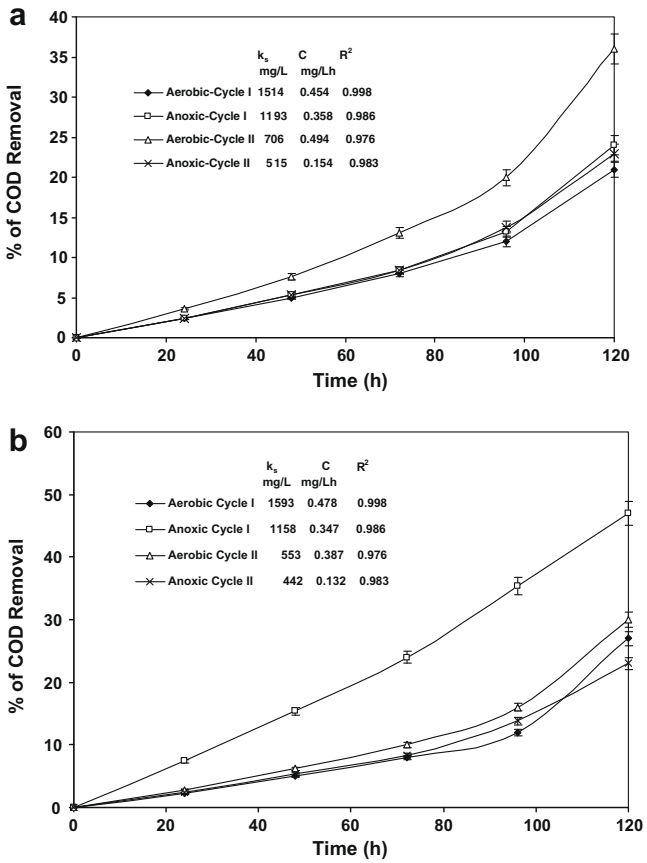

Fig. 2. Variation of %COD with time in two cycles for two degradation environments (aerobic and anoxic) using two volumes of *P. aeruginosa* inoculum (a) 50 mL L^{-1} and (b) 100 mL L^{-1}.

3.2. Effect of pre treatment, bio-degradation and post treatment on COD reduction

In dye degradation, electro-oxidation process with initial charge input of 8 Ah L^{-1} was coupled with bio-degradation process.

In 30 min of pretreatment of electro-oxidation, it was observed that the COD decreased from 2579 to 1832 mg L^{-1} (29% reduction) for the applied charge of 1.6 Ah. The average value of mass coefficient (k_L) was computed by the Eq. (26) which is 0.001188 cm/s. This was found almost identical for all electro-oxidation steps in different cycles, as well for post and all other electrolysis steps.

Bio-degradation experiments were carried out in *cycles* for two different *P. aeruginosa* inoculum volumes (50 and 100 mL L^{-1}) and for two degradation environments (aerobic and anoxic) as per the protocol shown in Fig. 1. The results are presented in Fig. 2a, b and Table 1.

Bio-degradation process was a sequential one and it was carried out in both aerobic and anoxic conditions in two cycles of operation indicated in Fig. 2a for 10 mL of inoculum containing *P. aeruginosa* and in Fig. 2b for 20 mL of inoculum. The figures clearly indicate that there are remarkable changes in the course of the degradation process by *P. aeruginosa* by biochemical reaction. The performance is more or less same in both the cycles. From Fig. 2a the COD reduction obtained for two degradation environments (aerobic and anoxic) were 21% and 24% in first cycle and 36% and 23% in second cycles respectively for the effluent containing10 mL of inoculum. Similarly from Fig. 2b the COD reduction obtained for two degradation environments (aerobic and anoxic) as 27% and 47% in first cycle and 30% and 23% in second cycles respectively for the effluent containing 20 mL of inoculum.

As presented in Table 1, in the second cycle of degradation scheme, the overall %COD reduction obtained in the combined process treatment of dye-house effluent together with post electro-oxidation for the effluent containing 10 and 20 mL of inoculum are 90.2 and 91.6, respectively.

To ensure that the microorganisms will grow, they must be allowed to remain in the system long enough to reproduce. This period depends on their growth rate, which is related directly to the rate at which they metabolize or utilize the waste. If the environmental conditions are controlled properly, the growth rate of the microorganisms can ensure effective waste stabilization. In the system the rate of growth of bacterial cells can be defined by the following relationship:

$$r_g = \frac{dX}{dt} = \mu X \tag{27}$$

where r_g, rate of bacterial growth; μ, specific growth rate; X, concentration of microorganisms. Experimentally it has been found that the effect of a limiting substrate or nutrient can often be defined adequately using the following expression proposed by Monod.

Table 1
Performance of combined process treatment of dye-house effluent.

Parameters and operating conditions	Units	Environment I	Environment II
Pollutant	Procion Blue 2G – Hydrolyzed dye		
Batch reactor holdup, V_R	mL	200	200
Initial COD	mg L^{-1}	2579	2579
Electrochemical mediated oxidation		Cycle I	
Charge input, Q	Ah L^{-1}	8	8
COD (after electro-oxidation)	mg L^{-1}	1832	1832
Biochemical oxidation	*Pseudomonas aeruginosa*		
Inoculums input, m	mL L^{-1}	50	100
COD (after inoculum addition)	mg L^{-1}	1920	2020
COD (after 5 days of aerobic oxidation)	mg L^{-1}	1520	1475
COD (after 5 days of anoxic process)	mg L^{-1}	1152	782
Electrochemical mediated oxidation		Cycle II	
Charge input, $Q/2$	Ah L^{-1}	4	4
COD (after electro-oxidation)	mg L^{-1}	971	660
Biochemical oxidation	*Pseudomonas aeruginosa*		
Inoculum input, $m/2$	mg L^{-1}	25	50
COD (after inoculum addition)	mg L^{-1}	1020	800
COD (after 5 days of aerobic oxidation)	mg L^{-1}	653	560
COD (after 5 days of anoxic process)	mg L^{-1}	502	431
Post electro-oxidation	mg L^{-1}	253	217
Overall % of COD Removal		90.2	91.6

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$$\mu = \mu_m \frac{S}{k_s + S} \tag{28}$$

where μ_m, maximum specific growth rate; S, concentration of growth-limiting substrate in solution; k_s, half-velocity constant, substrate concentration at one-half the maximum growth rate. Because the quantity of the new cell produced has been observed to be reproducible for given substrate, the following relationship has been developed the rate of substrate utilization and the rate of growth

$$\frac{dX}{dt} = -Y\frac{dS}{dt} \tag{29}$$

Y, maximum yield coefficient (defined as the ratio of the mass of cell formed to the mass of substrate consumed). $r_{su} = dS/dt$ = substrate utilization rate. Combining the Eqs. (25)–(27) the expression for the rate of substrate utilization can be written as follow:

$$\frac{dS}{dt} = -\mu_m \frac{XS}{Y(k_s + S)} \tag{30}$$

In Eq. (28), the term $\mu_m X/Y$ is often replaced by the term C, defined as the maximum rate of substrate utilization, the resulting expression is

$$\frac{dS}{dt} = -\frac{CS}{k_s + S} \tag{31}$$

In bacteria systems used for wastewater treatment, the distribution of cell age is such that not all the cells in the system are in the log-growth phase. Consequently, the expression for the rate of growth must be corrected to account for the energy required for cell maintenance. However, if most of the cells in the bacterial system are in log-growth phase, the solution of Eq. (29) is

$$k_s \ln\left(\frac{S}{S_o}\right) + (S - S_o) = -Ct \tag{32}$$

Where S_o is the initial substrate concentration. We can rewrite the Eq. (30) as

$$\frac{\ln\left(\frac{S}{S_o}\right)}{(S - S_o)} = -\frac{C}{k_s}\frac{t}{(S - S_o)} - \frac{1}{k_s} \tag{33}$$

The plot of $\ln(S/S_o)/(S - S_o)$ vs. $t/(S - S_o)$ will be linear if the above kinetics (Monod) follows. From the intercept, half-velocity constant can be computed and the maximum utilization rate of substrate is obtained by dividing slope by intercept.

The model relates the variation of substrate concentration with time. It is characterized by certain constants, explicitly by maximum rate of substrate utilization, C, substrate affinity constant, k_s, which expresses COD removal with time. Monod kinetics constants are evaluated from the % of COD removal with time for *P. aeruginosa* and their regression coefficients are also presented in Fig. 2a and b itself. High regression coefficients (mostly greater than 0.98) were found for the two sets studies. This suggests that two parameters Monod kinetics model is very suitable for describing the kinetic model for the removal of COD by biochemical oxidation using *P. aeruginosa* in that studied concentration.

3.3. FT-IR spectra analysis

In degradation experiments, the treated effluent (containing 20 mL inoculum) samples were taken from the end of each treatment (pre-treated, bio-degraded and post treated) and subjected to FT-IR analysis. The spectrum results were compared with the control. The selected dye molecule for this study possess 1,4-diamino anthraquinone and trichloro triazine moiety with two sulfonic acid groups. From the spectra of untreated sample, C–H stretching absorption was found at 2936, 2853 cm^{-1}. Characteristic absorption peak at 1641 cm^{-1} is due to the carbonyl group in diamino anthroquinone. The absorption peak at 1558 cm^{-1} is due to –N=C–. The peaks at 1485, 1413 cm^{-1} are due to the aromatic carbon stretching; 1142 cm^{-1} absorption peak is due to SO_3H group; 622 cm^{-1} absorption peak is due to C–Cl. The above absorption peaks are characteristic peaks for the Procion Blue 2G. From the spectra for pre-treated sample, the –N=C– characteristic peak disappeared at 1558 cm^{-1}. From the results, it is evident that the trichloro triazine moiety may be degraded in electro-oxidation process. The spectra of bio-degraded sample, the characteristic peaks of SO_3H group completely disappeared at 1142 cm^{-1} and other functional groups were degraded in the bio-degradation process. The spectra of post treated sample, shows the absorption peaks at 1638, 1373, 1146, 1034 and 609 cm^{-1}. These peaks were due to the presence of simple aliphatic acid during the mineralization process of the dye molecule. The experimental results are found to be identical with these results (up to 90% removal of COD).

3.4. Effect of combined degradation on UV–visible spectra

The UV–visible spectra for untreated, pre-treated, bio-degraded and post treated samples are taken. The spectrum of untreated sample exhibits the absorption band at 322 and 282 nm. The peak at 322 nm is due to the n–π^* transition of diamino anthraquinone group and 282 absorption peak is due to π–π^* transition of aromatic ring. The decrease in absorption peaks at 322 and 282 nm indicates a rapid degradation of Procion Blue 2G. The decrease in the intensity of n–π^* transition is due to the degradation of triazine moiety in electro-oxidation process. Spectra of bio-degraded sample shows the decrease in the intensity of π–π^* transition which confirms the degradation of Procion Blue 2G. Spectra of post treated sample shows peaks at 253, 285 and 325 nm, it may be due to the simple aliphatic acids such as acetic acid, oxalic acid etc., present in the final stage of degradation processes of Procion Blue 2G.

From COD, FT-IR and UV–visible spectrophotometer analysis results, the integrated technology of electro-oxidation with biological oxidation was found to be effective in the degradation and decolorization of Procion Blue 2G.

3.5. Effect of irradiation on microbial population

From spread plate analysis, the absence of microbes in treated effluent is ensured when the microbial growth was not observed in spread agar plate for 3 days of microbial growth for the sample taken after 1 h of irradiation and the irradiated effluent could be sent for TDS removal. The treated effluent could be reused after TDS removal.

4. Conclusion

The integrated electro and biochemical degradation process for the dye solution containing Procion Blue 2G removed the complete color and more than 90% of overall COD at the end of the treatment when initial charge of 8 Ah L^{-1} applied for the experiments to enhance bio-degradation.

Hence, it was concluded that the free bacterial cells of *P. aeruginosa* have enough potential to treat the bio recalcitrant dye by the way of electro and bio-degradation by integration.

Further, the possibility to recycle the treated effluent, less cost compared to chemical treatment and negligible sludge generation also adds to economy of treatment.

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References

Arıca, M.Y., Bayramoglu, G., 2007. Biosorption of Reactive Red-120 dye from aqueous solution by native and modified fungus biomass preparations of *Lentinus sajor-caju*. J. Hazard. Mater. 149, 499–507.

Bandara, J., Wansapura, P.T., Jayathilaka, S.P.B., 2007. Indium tin oxide coated conducting glass electrode for electrochemical destruction of textile colorants. Electrochim. Acta 52, 4161–4166.

Basha, C.A., Chithra, E., Sripriyalakshmi, N.K., 2009. Electro-degradation and biological oxidation of non-biodegradable organic contaminants. Chem. Eng. J. 149, 25–34.

Bayramoglu, G., Celik, G., Yakup Arica, M., 2006. Biosorption of Reactive Blue 4 dye by native and treated fungus *Phanerocheate chrysosporium*: batch and continuous flow system studies. J. Hazard. Mater. B137, 1689–1697.

Bechtold, T., Burtscher, E., Hung, Y.-T., 2006. Treatment of textile wastes. In: Wang, L.K., Hung, Y.-T., Lo, H.H., Yapijakis, C. (Eds.), Waste Treatment in the Process Industries. Taylor & Francis Group, Boca Raton, pp. 363–398.

Brillas, E., Cabot, PL., Casado, J., 2003. Electrochemical methods for degradation of organic pollutants in aqueous media. In: Tarr, M.A. (Ed.), Chemical Degradation Methods for Wastes and Pollutants Environmental and Industrial Applications. Marcel Dekker, Inc., New York.

Chamarro, E., Marco, A., Esplugas, S., 2001. Use of fenton reagent to improve organic chemical biodegradability. Water Res. 35 (4), 1047–1051.

Cheremisinoff, N.P., 2002. Handbook of Water and Wastewater Treatment Technologies. Butterworth–Heinemann, Woburn.

Clesceri, L.S., Greenberg, A.E., Eaton, A.D., 1998. Standard Methods for the Examination of Water and Wastewater, 20th ed. APHA, Washington, DC.

Doble, M., Kumar, A., 2005. Biotreatment of Industrial Effluents. Elsevier Butterworth–Heinemann, Burlington.

El-Daly, H.A., Habib, A.-F.M., El-Din, M.A.B., 2005. Kinetic investigation of the oxidative decolorization of Direct Green 28 and Direct Blue 78 by hydrogen peroxide. Dyes Pigment. 66, 161–170.

Garcia-Montano, J., Torrades, F., Garcia-Hortal, J.A., Domenech, X., Peral, J., 2006. Combining photo-Fenton process with aerobic sequencing batch reactor for commercial hetero-bireactive dye removal. Appl. Catal. B 67, 86–92.

Ghoreishi, S.M., Haghighi, R., 2003. Chemical catalytic reaction and biological oxidation for treatment of non-biodegradable textile effluent. Chem. Eng. J 95, 163–169.

Kalme, S.D., Parshetti, G.K., Jadhav, S.U., Govindwar, S.P., 2006. Biodegradation of benzidine based dye Direct Blue-6 by *Pseudomonas desmolyticum* NCIM 2112. Bioresour. Technol. 98, 1405–1410.

Karcher, S., Kornmuller, A., Jekel, M., 2002. Anion exchange resins for removal of reactive dyes from textile wastewaters. Water Res. 36, 4717–4724.

Khalaf, M.A., 2008. Biosorption of reactive dye from textile wastewater by non-viable biomass of *Aspergillus niger* and *Spirogyra* sp. Bioresour. Technol. 99, 6631–6634.

Kumari, K., Abraham, T.E., 2007. Biosorption of anionic textile dyes by nonviable biomass of fungi and yeast. Bioresour. Technol. 98, 1704–1710.

Lackey, L.W., Mines Jr., R.O., McCreanor, P.T., 2006. Ozonation of acid yellow 17 dye in a semi-batch bubble column. J. Hazard. Mater. B138, 357–362.

Ledakowicz, S., Solecka, M., Zylla, R., 2001. Biodegradation, decolourisation and detoxification of textile wastewater enhanced by advanced oxidation processes. J. Biotechnol. 89, 175–184.

Lucas, M.S., Dias, A.A., Sampaio, A., Amaral, C., Peres, J.A., 2007. Degradation of a textile reactive Azo dye by a combined chemical-biological process: Fenton's reagent-yeast. Water Res. 41, 1103–1109.

Lucas, M.S., Peres, J.A., 2006. Decolorization of the azo dye reactive black 5 by Fenton and photo-Fenton oxidation. Dyes and Pigments 71, 236–244.

Mishra, A., Bajpai, M., 2005. Flocculation behaviour of model textile wastewater treated with a food grade polysaccharide. J. Hazard. Mater. B118, 213–217.

Morais, J.L., Zamora, P.P., 2005. Use of advanced oxidation processes to improve the biodegradability of mature landfill leachates. J. Hazard. Mater. 123, 181–186.

Orfao, J.J.M., Silva, A.I.M., Pereira, J.C.V., Barata, S.A., Fonseca, I.M., Faria, P.C.C., Pereira, M.F.R., 2006. Adsorption of a reactive dye on chemically modified activated carbons—influence of pH. J. Colloid Interf. Sci. 296, 480–489.

Ostar-Turk, S.S., Simonic, M., Petrinic, I., 2005. Wastewater treatment after reactive printing. Dyes Pigment. 64, 147–152.

Robinson, T., McMullan, G., Marchant, R., Nigam, P., 2001. Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative. Bioresour. Technol. 77, 247–255.

Sanghi, R., Bhattacharya, B., Singh, V., 2006. Use of *Cassia javahikai* seed gum and gum-g-polyacrylamide as coagulant aid for the decolorization of textile dye solutions. Bioresour. Technol. 97, 1259–1264.

Yang, C.-L., McGarrahan, J., 2005. Electrochemical coagulation for textile effluent decolorization. J. Hazard. Mater. B127, 40–47.

Yesilada, O., Cing, S., Asma, D., 2002. Decolourisation of the textile dye Astrazon Red FBL by *Funalia trogii* pellets. Bioresour. Technol. 81, 155–157.