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Kinetics of the decolourization of a dyehouse effluent by *Providencia rettgeri* ODO

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The potential of an organism isolated from a mixture of azo dyes to decolourize textile wastewater was investigated. The organism identified by 16S rDNA as a strain of *Providencia rettgeri* was able to remove 94 ± 0.56% of the colour in a dyehouse effluent within 24 h in batch experiments. *P. rettgeri* strain ODO was able to use the effluent as sole carbon and nitrogen source. The optimum pH and temperature for colour removal ranged between 7–8 and 30–35 °C, respectively. Analysis of the data showed that the decolourization process exhibited first order kinetics with respect to the colour intensity and biomass concentration. The rate constant $k = 5.6 \times 10^{-3}$ L/gDCMh at 37 °C. The specific growth rate, μ, of the organism was 0.013 h^{-1} while the mean doubling time was 52.9 h. The results indicated that *P. rettgeri* strain ODO has the potential for colour removal from textile effluent.

Keywords: Dyehouse effluent; Decolourization; *Providencia rettgeri*; Decolourization kinetics

1. Introduction

There has been an enormous increase in the volume of wastewater generation due to industrialization, leading to a decline in the quality and availability of natural water in the environment. It is estimated that about 100 L of water is required to process 1 kg of textile material [1] while the textile industry consumes about two-third of the more than 7×10^5 tonnes of a wide variety of synthetic dyes produced annually [2–4]. Azo dyes are the largest and most versatile class of dyes. With more than 3000 different ones currently used for different dyeing purposes, they constitute 60–70% of dyes use in textile processing. Inefficiencies of the dyeing process lead to a direct loss of between 10 and 15% of the dyes in the effluents, giving rise to highly coloured wastewaters. Up to 280,000 tons of textile dyes are estimated to be lost in industrial effluents annually. The presence of azo compounds in textile wastewater raises serious environmental and health concerns [5–7].

In Nigeria, there is a preponderance of cottage textile dyeing industries which operate with a variety of dyes. More often than not, the effluents are released directly into sewage pipelines and open water drainages without any treatment because these industries do not have wastewater treatment plants. These dyes will eventually contaminate water bodies and ground water.

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Removal of colour in coloured effluents is important before discharge because the colour affects the aesthetic quality, the transparency, light penetration and gas solubility of water. These affect photosynthetic activity and hinder aquatic life. In addition, the self-purification ability of water is impaired [8,9].

Many of the physicochemical methods to remove dyes are either costly or complicated and time consuming. This effectively puts them beyond the reach of most cottage industries. In addition, many of these methods generate large amounts of solid waste and cannot remove completely the dyes/and or metabolic products from effluents [10–12]. As viable alternatives, microbial treatment processes are being investigated because they are cost effective, eco-friendly and applicable to a wide range of dyes. Bioremediation is fast becoming an alternative environmental restoration technology.

In this study, the ability of *Providencia rettgeri* strain ODO to decolourize a dyehouse effluent was investigated and the kinetics of the decolourization process were studied.

2. Experimental methods

2.1. Chemicals and reagents

The chemicals and reagents used are as stated in the methodology and were of analytical grade. The nutrient agar and nutrient broth used were products of Lab M, Lancashire, UK. Buffer tablets were from BDH chemicals, Poole, England. The azo dyes; Reactive blue 13 (RB13), Reactive red 58 (RR58) and Reactive yellow 42 (RY42) were obtained from a textile company in Lagos, Nigeria.

2.2. Dyehouse effluent characteristics and pre-treatment

The dyehouse effluent was collected in air-tight containers from a textile-dyeing house in Lagos, Nigeria. The dyehouse employs azo dyes in the dyeing process. The colour of the effluent sample was noted and the pH was measured using a pH meter (H18014 Haniva instruments, Romania). The concentration of dyes in the effluent sample was estimated as total dissolved solid by drying known volumes of the effluent samples at low temperature in pre-weighed vessels. An estimate of the dyes concentration was done by taking into consideration the information provided by the dyehouse on the proportion of auxiliary chemicals used in the processing.

Before the addition of media, the effluent sample was filtered using a Pyrex No. 1 glass filter to remove any insoluble solid particle. The pH was also adjusted to 7.2 using hydrochloric acid (HCl). The λ_{max} of the effluent was also determined using a UV–visible spectrophotometer (Shimadzu UV-1650PC). The effluent- media were always sterilized at 121 °C for 15–20 min before inoculation.

2.3. Isolation and screening of organism for azo dye decolourization

The bacterium used in this study was isolated from textile dyes obtained from a textile company in Lagos, Nigeria. A mixture of three azo dyes: RB13, RR58 and RY42 was produced by adding 20 mg of each dye to 50 ml of sterile nutrient broth. This was incubated at 25 °C overnight and then cultured onto fresh nutrient agar. Organisms isolated were sub-cultured onto fresh nutrient agar plates.

The organisms were screened for the ability to decolourize azo dyes using 60 mg/L dyes mix in nutrient broth. Approximately, 0.05 g dry cell mass were added into 100 ml, 60 mg/L solution of three reactive azo dyes (RB13, RR58 and RY42; 20 mg/L each) in nutrient broth under static anoxic condition. Then, 6 ml samples were withdrawn at 6 h intervals for 24 h and centrifuged at 5000 rpm for 15 min to remove biomass. Percentage decolourization was recorded as $[(A_0 - A_t)/A_0] \times 100$, where A_0 and A_t are initial and final absorbance units, respectively, measured at the wavelength of maximum absorption of the dye mix, 546 nm.

2.4. Identification of bacterium and culture conditions

Presumptive identification of an isolate with significant azo dye decolourization activity was done using microbiological and biochemical methods while the identification was confirmed using 16S rDNA sequencing (Laragen Inc, USA). The 16S rDNA sequence was analysed at NCBI server (http://www.ncbi.nlm.nih.gov) using BLAST (blastn) tool and corresponding sequences were downloaded. A phylogenetic tree was constructed by the Neighbour-Joining method using the MEGA 4 package [13].

The bacterium was maintained on nutrient agar slant at 4 °C and proliferated in a 250-ml Erlenmeyer flask containing freshly prepared sterile broth (13 g/L nutrient broth) (LAB M, U.K) for 24 h. This formed the broth culture (BC) used in this study.

2.5. Dyehouse effluent decolourization studies

The pH of the effluent was adjusted to 7.2 using 0.1 M HCl and fresh nutrient broth was prepared with sterile effluent at a concentration of 13 g /L to produce effluent containing media (ECM). Volumes of BC were added to the ECM at a ratio 1:25 (V/V) in 250-ml Erlenmeyer flasks. The inoculated ECM were then incubated at 37 °C and 3 ml aliquots were taken at intervals up to 24 h after inoculation, centrifuged for 20 min at 5000 rpm to remove bacteria cells. The cell free supernatants were analysed for colour removal. All experiments were done in triplicate and an abiotic control was set up with each set of experiments. The abiotic control contains only the sterile ECM without the addition of BC.

Decolourization was followed by measuring the absorbance of the cell-free supernatant at the effluent pre-determined maximum absorbance wavelength ($\lambda_{max} = 548$ nm). The effluent colour removal rate (percentage decolourization) was calculated as follows:

$$P = [(A_o - A_t)/A_o] \times 100\% \quad (1)$$

where P = percentage decolourization, A_o = initial absorbance of effluent (pre inoculation), A_t = Absorbance of inoculated effluent at a specific time after inoculation.

The decolourization process was also studied by the analysis of the UV–visible spectra of the effluent pre- and post-incubation with *P. rettgeri* ODO using a Shimadzu 1650pc UV–visible spectrophotometer.

2.6. Effects of nutrient concentration on colour removal rate

The effect of nutrient concentration on the decolourization activity of *P. rettgeri* ODO was investigated by varying the concentration of the nutrient broth used in the ECM from 0 to 40 g/L.

2.7. Effects of initial pH and ambient temperature of effluent on colour removal rate

The effect of pH on decolourization at 37 °C was determined. Effluent pH was adjusted using either 0.1 M HCl or 0.1 M NaOH. ECM was prepared with effluent samples with pH between 4 and 9. The ECM was inoculated with BC and the decolourization rate at the various pH determined. To check temperature effects, decolourization experiments were set up as previously described in this paper but the incubation temperature was varied between 25 and 45 °C at pH 7.2.

2.8. Estimation of biomass

Biomass concentration was determined as dry cell mass (DCM). Biomass was determined by measuring optical density at 600 nm (OD_{600}) essentially as described by Dong et al. [14]. Harvested cells were diluted until the optical density at 600 nm (OD_{600}) was 1.0 with sterile water. Aliquots of the cell suspension (1, 2, 3, 4 and 5 ml) were dehydrated in pre-weighed tubes at 40 °C until constant weight. A relation was made using an analogue of Beer–Lambert's law:

$$OD_{600} = \varepsilon Cl$$

$$\text{Biomass } (g\text{DCM}/L) = OD_{600}/\varepsilon l$$

where OD_{600} = optical density (absorbance) of sample at 600 nm, C = concentration of light absorbing entity in the sample that produces the OD_{600}, ε = extinction coefficient, l = the light path length = 1 cm and $C = OD_{600}/\varepsilon l$.

2.9. Kinetic modelling

For the purpose of data fitting and estimation of the specific colour removal rate, the relative colour intensity (RCI) was calculated from the decolourization experiments as follows:

$$\text{RCI} = [A_t/A_0] \times 100$$

where A_t = absorbance of inoculated effluent at a specified time interval, A_0 = absorbance of untreated effluent and the uninoculated effluent was assigned a RCI of 100.

2.10. Estimation of kinetic parameters

For isothermal kinetics of substrate conversion by enzymes and/living cells, a Michaelis–Menten type rate equation (equation (1)) is widely used [15].

$$v = d[S]/dt = V_m[S]/(K_m + [S]) = k_2[E_0][S]/(k_{-1} + (k_2/k_1) + [S]) \quad (1)$$

where v = velocity (rate) of the reaction, $[S]$ = substrate concentration, t = time, V_m = maximal velocity, K_m = Michaelis constant, k_1, k_{-1}, k_2 are rate constants and $[E_0]$ = enzyme activity.

According to Yu et al. [15], because of the non-linearity of the above equation, a convenient general kinetic model is employed

$$dC/dt = -kM^m C^n \quad (2)$$

where t = time, M = cell mass concentration (gDCM/L), m = partial reaction order, C = dye concentration, n = partial reaction order, k = specific decolourization rate.

Depending on the partial reaction order of dye concentration, the relationship between effluent dye concentration and time is given by:

$$(C/C_0)^{(1-n)} = 1 - [(1-n)kM^m]/C_0^{(1-n)}]t \quad (n \neq 1) \quad (3)$$

$$(C/C_0) = \exp(-kM^m t) \quad (n = 1) \quad (4)$$

The order of colour removal with respect to colour intensity and specific colour removal rate were subsequently obtained from a plot of ln RCI vs. time (h).

The order of decolourization with respect to biomass was evaluated from the log of the ratio of initial dye concentration to the concentration at a given time. This was determined from a plot of ln (RCI_0/RCI) vs. biomass concentration where:

RCI_0 = relative colour intensity of uninoculated effluent and
RCI = relative colour intensity of inoculated effluent.

Other process parameters; specific growth rate (μ, h^{-1}) and mean doubling time (t_d, h) for *P. rettgeri* ODO were calculated following standard methods [16].

2.11. Statistical analysis

All experiments were performed in triplicate and results expressed as mean ± standard deviation except in the evaluation of the media concentration on colour removal, which was done in duplicate.

3. Results

3.1. Characterization of dyehouse effluent

The colour of the effluent was observed to be deep purple and the pH was 7.4. The UV–visible spectral scan revealed absorbance peaks at 205, 244, 260, 290, 294, 584 and 610 nm indicative of the presence of various chromophores in the effluent. The concentration of azo dyes in the effluent sample was estimated to be approximately 150 mg/L taking into consideration the proportion of the auxiliary chemicals present in the effluent, predicated on the information available from the dyehouse source.

3.2. Identification of the bacterium strain

The isolate used in this study was found to be a Gram-negative, rod shaped, aerobic bacterium which was identified by 16S rDNA sequencing as *P. rettgeri*. It has been designated as *P. rettgeri* strain ODO and the 16S rDNA sequence has been deposited at NCBI with accession no GU395555 (figure 1).

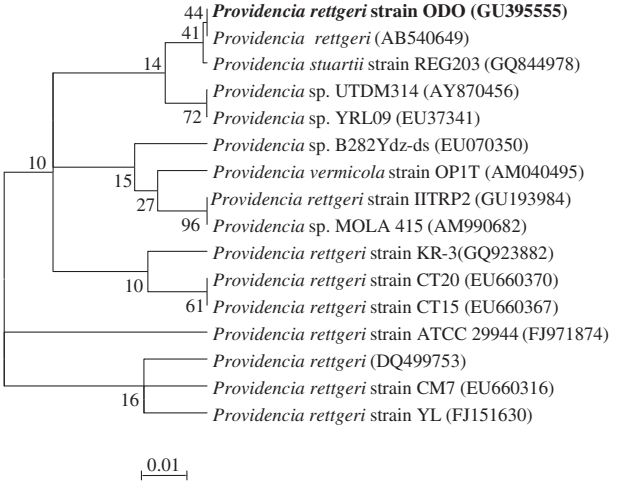

Figure 1. Phylogenetic analysis of 16S rDNA sequence of bacterial isolate *P. rettgeri* strain ODO. Distance tree constructed by neighbour joining method using MEGA 4. The sequences have been retrieved from NCBI database, showing the phylogenetic relationships of *P. rettgeri* strain ODO and other species of genus *Providencia*. Numbers at nodes shows the level of bootstrap support based on data for 1000 replication. Bar, 0.01 substitutions per nucleotide position and numbers in parentheses represent Gen Bank accession numbers.

3.3. *Colour removal by* P. rettgeri *strain ODO in BC*

P. rettgeri strain ODO demonstrated a great potential to decolourize textile effluents. It achieved 46 ± 3.0 and 95 ± 1.7% decolourization of a simulated effluent (60 mg/L reactive azo dyes mix) after 12 h and 24 h, respectively. Figure 2 shows that 94 ± 0.56% of the initial colour of the dyehouse effluent was also removed in 24 h by this organism. The changes in the UV–visible spectra of the effluent as a result of the colour removal activity of *P. rettgeri* ODO are shown in figure 3.

Biomass concentration in the ECM was estimated as decolourization progressed at 6, 12, 24, 48 and 72 h. Results show that $\varepsilon = 0.097$ L/gDCM and $OD_{600} = 1.0 = 10.34$ *g*DCM/L. Thus, biomass concentration was estimated from $C = OD_{600}/\varepsilon l$

$$\text{Biomass concentration} = OD_{600} \text{ of sample} \times 10.34g \text{ DCM/L}.$$

The results (figure 2) indicate that colour removal is growth associated.

3.4. *Effect of nutrient concentration on effluent decolourization*

The pattern of decolourization observed for the various concentrations of nutrient broth in the ECM was similar, the colour removal peaked around 24 h. The decolourization increased with increasing nutrient (broth) concentration (figure 4). But, *P. rettgeri* strain ODO was able to achieve a significant reduction in effluent colour without the addition of any nutrient or co-substrate.

3.5. *Effect of pH and temperature on effluent decolourization*

The optimal initial pH for the decolourization of the dyehouse effluent by *P. rettgeri* strain ODO was in the range 7–8. Although the results show higher colour removal rates at pH

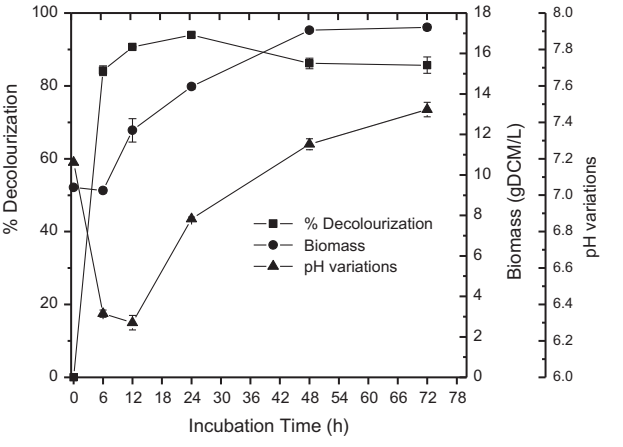

Figure 2. Changes in biomass and pH during decolourization of dyehouse effluent by *P. rettgeri* ODO.

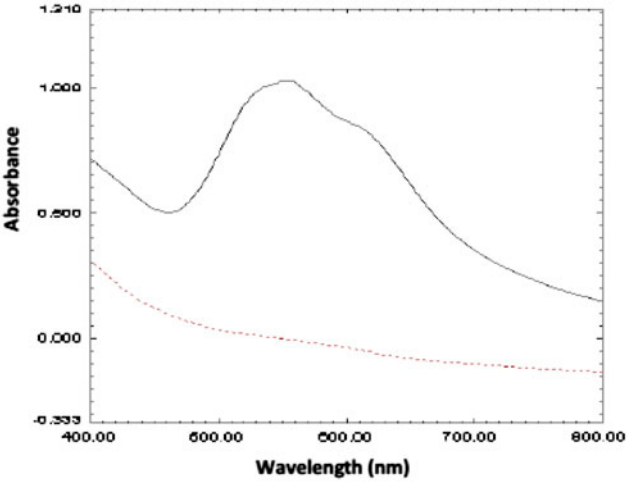

Figure 3. Spectral changes in effluent after inoculation with *P. rettgeri* ODO. The continuous line is the effluent spectra while the discontinuous line shows the spectra after inoculation with *P. rettgeri*.

4 and 5, this was attributable to dye precipitation. In addition, the results were not significantly different at pH 4 and 5 for the abiotic control, showing that the results obtained were not due to the activity of the bacterium. The optimum temperature for the decolourization of the dyehouse effluent by *P. rettgeri* strain ODO was found to be in the range 30–35 °C (figure 5).

3.6. Kinetic parameters of decolourization by P. rettgeri

Figure 6 shows the time course of colour removal from the dyehouse effluent by the *P. rettgeri* strain. The value of the specific colour removal rate, k, was obtained from the slope of the plot of ln (RCI) against time (h). $k = 0.0056$ L/gDCMh at 37 °C.

The order of colour removal was determined with respect to two controlling factors; effluent colour intensity and the biomass. The Spearman's coefficient of correlation in the

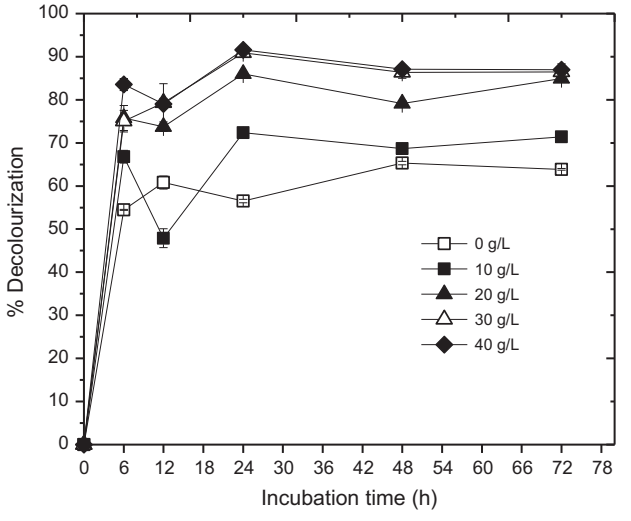

Figure 4. Effect of media concentration on the colour removal activity of *P. rettgeri* ODO.

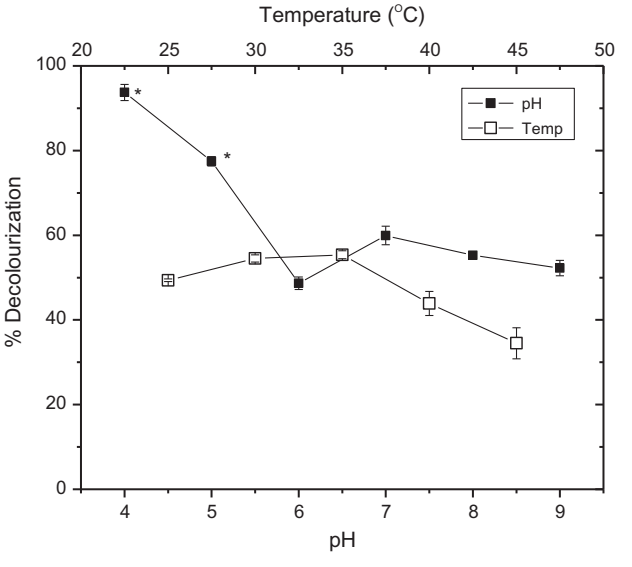

Figure 5. Effect of initial pH and temperature on the decolourization of dyehouse effluent by *P. rettgeri* (*dyes were precipitated at pH 4 and 5). The temperature for the pH study was 37 °C while the pH for the temperature study was 7.2.

plot of ln RCI vs. t (figure 6) was $R^2 = 0.8834$, implying a high degree of linearity between the incubation time and effluent colour intensity. Thus bio-decolourization was first order with respect to colour intensity.

The colour removal process was also found to be a first-order kinetic reaction with respect to biomass concentration as revealed by $R^2 = 0.9989$ in the plot of $\ln(RCI_0/RCI_t)$ vs. [Biomass] (figure 7). Where, RCI_0 = initial relative colour intensity = 100, and RCI_t = relative colour intensity at specific incubation time after inoculation. The data points

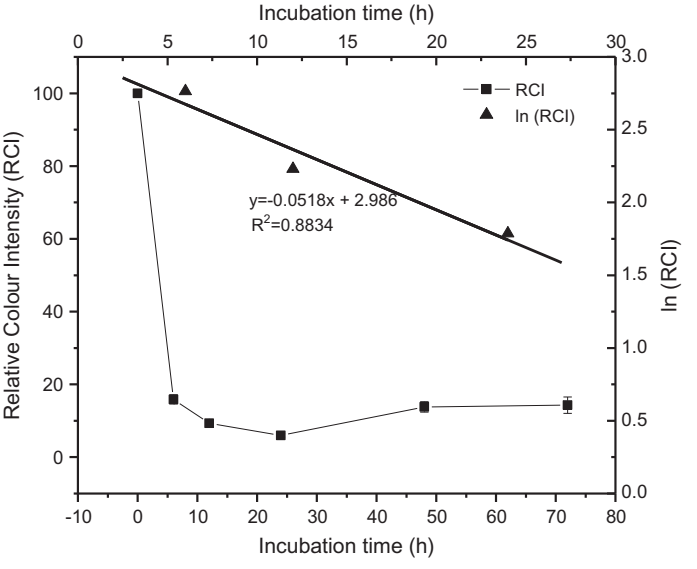

Figure 6. Time course of colour removal from dyehouse effluent, data fitting and estimation of biokinetic parameters for colour removal by the *P. rettgeri* ODO with an initial biomass of 9.38 *g*DCM/L. Secondary axes are for a plot of lnRCI vs. incubation time.

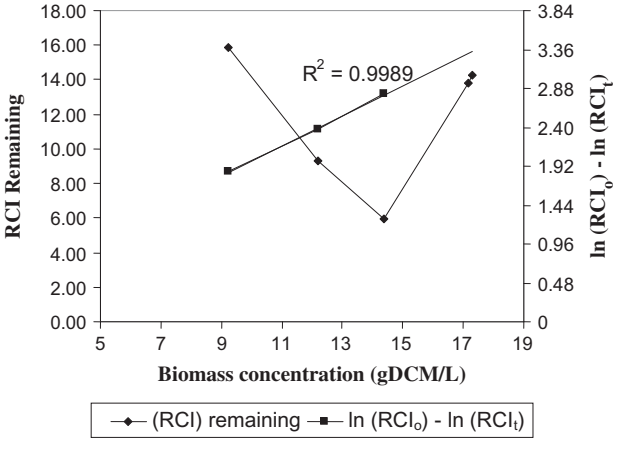

Figure 7. Effect of *P. rettgeri* biomass concentration on colour removal.

selected for this analysis were for RCI remaining after 6, 12 and 24 h, respectively, during which the organism was in the early to middle exponential growth phase and most active (see figure 2).

3.7. Exponential growth kinetics of P. rettgeri

The biomass concentration was observed to increase in the batch decolourization culture as colour removal proceeded. The specific growth rate, μ, calculated from the slope of ln [biomass] vs. time was $0.013\,h^{-1}$ (figure 8). The mean doubling time was subsequently determined as 52.9 h.

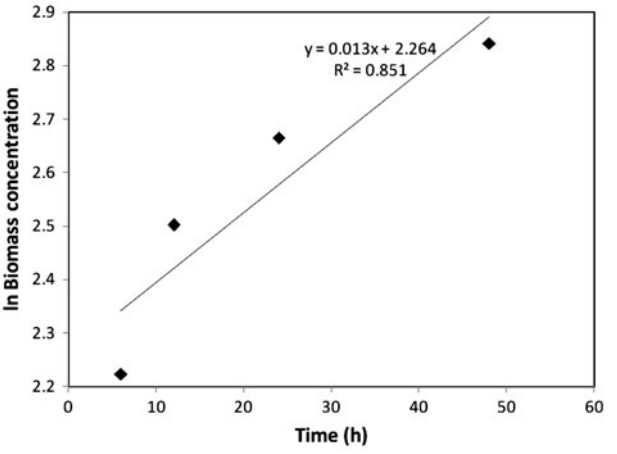

Figure 8. Data fitting and estimation of kinetic parameters for cell growth.

4. Discussion

Light absorbance at the visible spectrum is a characteristic property of dyes [17] and azo dyes are able to absorb in the visible range due to the presence of one or more azo bonds [18]. Thus, the peaks at 548 and 610 nm in the spectral analysis of the effluent are of significance since they showed the presence of dyes, which typically absorb light in the visible region. The peaks are characteristic of purple and greenish blue, respectively [19]. This is in agreement with the information obtained from the dyehouse that the effluent contains purple and navy blue dyes.

The UV–visible spectra scan of the inoculated effluent showed the loss of the peaks at 548 and 610 nm after 24 h indicating that the chromophores in the visible range were degraded. The shifts in λ_{max} value of a dye or disappearance of the characteristic peaks in the visible spectrum have been reported to indicate the degradation of such dyes [20–22]. Many reported bio-decolourization studies used conditions such as single dyes or dyes mixes under conditions which do not approximate an industrial environment. It is difficult to extrapolate the results to real textile effluents which are known to contain usually a complex mixture of dyes and auxiliary chemicals that are applied to impart to the finished product specific properties or to facilitate certain production stages [23]. The effluent from a dyehouse was employed in this study and a very high percentage of the colour was removed from the effluent by *P. rettgeri* strain ODO under static anoxic condition; this suggests that the strain may be useful for textile wastewater treatment.

The colour removal process was found to be growth linked. But, the decrease in colour removal observed after 24 h may be due to the organism entering the late growth phase or may be caused by the reaction of degradation products with degraded and undegraded dyes [24].

Previous studies showed that the composition and concentration of nutrients affect microbial decolourization [6,21,22]. The process is also affected by the initial pH of the effluent and the ambient temperature [25,26]. In this study, the decolourization increased with increasing nutrient (broth) concentration. But, *P. rettgeri* strain ODO was able to achieve a significant reduction in effluent colour without the addition of any nutrient or co-substrate, indicating that the organism was able to use the dyehouse effluent as the sole

source of carbon and nitrogen. The optimal initial pH for the decolourization process at 37 °C was 7–8, since there was no significant difference in the values obtained at pH 7 and 8. The optimal temperature for the process was found to be 30–35 °C.

The dyes were observed to be precipitated out at pH 4–5, indicating precipitation and filtration as a potential physicochemical treatment protocol. There are various associated challenges such as the cost of equipment required and the generation of an acidic wastewater. All these make biotreatment a more viable and eco-friendlier alternative.

The bio-decolourization process was growth-related as biomass concentration was observed to increase with incubation time, necessitating an evaluation of the kinetics of cell growth in the batch culture which is applicable mainly to growth-related fermentation processes [27]. The organism appeared to be in its early to middle exponential (or logarithmic) growth phase between 6 and 24 h, in which decolourization was progressive until it reached its peak at 24 h. The organism was probably in its late growth phase between 24 h and 48 h, during which increase in biomass is not necessarily a function of increasing number of cells but mainly attributable to the accumulation of lipids and carbohydrates. This may be responsible for the increase observed in the RCI remaining when biomass concentration exceeded 15 *g*DCM/L after 24 h. Between 48 and 72 h, the organism may have entered into its stationary phase where its activities are expected to be mainly for maintenance or survival purposes [27]. These may also affect the organism's ability to produce the enzyme(s) involved in the decolourization process. Microbial decolourization and degradation have often been linked to the capacity of the organism to produce the relevant enzyme(s) [28,29].

The pattern of effluent decolourization by the *P. rettgeri* strain was found to be first order with respect to biomass concentration and effluent colour intensity.

5. Conclusion

The use of a microbial species as a tool in bioremediation depends on the ability to metabolize the pollutant(s) in question and identification of the optimal process conditions. From the results obtained in this study, it can be concluded that *P. rettgeri* strain ODO has the potential for decolourization of dyehouse effluents, since observations indicate that it decolourized a dyehouse effluent without nutrient supplementation or addition of co-metabolites.

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