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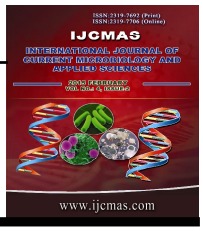

Original Research Article

Biosorption of Alizarin by *Burkholderia sp.*

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A B S T R A C T

The present study is an attempt to develop a suitable operational strategy for biosorption of alizarin from synthetic solution by optimizing the initial pH, inoculum concentration, and initial dye concentration. A bacterial strain capable of biosorption was isolated from rhizosphere of *Cassia occidentalis*. The isolate was characterized as *Burkholderia* sp. through ribotyping. Studies on dye removal were carried out in batch culture under both shaking and static conditions. The batch studies, using growing cells of the bacteria in sterile synthetic media containing dye, showed that the bacteria was able to tolerate dye concentration up to 1000 ppm. The growth of the bacterial cells was checked using spectrophotometric analysis. The organism's growth and its ability to remove alizarin by the organism were dependent on inoculum concentration (5–20 %), dye concentration (100 ppm–1000 ppm), and pH (5.0–8.0). The maximum decolorization (89%) was observed at pH 5.0 at inoculum concentration of 20%.

Keywords

Burkholderia sp., *Cassia occidentalis*, Rhizosphere, Dye removal, Biosorption, Alizarin

Introduction

Demand of water is increasing day-by-day and its limited supply has made wastewater treatment an attractive option. Some of the colour in the water bodies is of 'natural' origin (i.e. colour originating from microbial activities), but most of the colour, in the lower reaches of rivers, which is of synthetic origin, comes from industries, such as textile and foodstuff (Bayoumi *et al.*, 2010). Due to rapid industrialization and man's fascination with colour, the use of synthetic dyes has been greatly increasing. Synthetic dyes can be classified into six different classes based on their chemical structures, viz. azo, anthraquinone, sulphur, indigoid, triphenylmethane and phthalocyanine derivatives (Alhassani *et al.*, 2007).

Presence of dyes in water bodies impairs water quality and also lowers gas solubility as it significantly decreases the rate of photosynthesis by encumbering the light penetration into aqueous bodies (Bayoumi *et al.*, 2010). These dyes are recalcitrant owing to their stability against temperature, light, water and many chemical compounds (Zhang *et al.*, 2007). The wastewaters containing these dye products are becoming more and more complex. Hence, their

treatment is of top priority (Su *et al.*, 2009). To treat these complex industrial effluents, bacterial consortium has been used effectively (Khehra *et al.*, 2005; Asgher *et al.*, 2007; Moosvi *et al.*, 2007; Mohanaa *et al.*, 2008). Though the fraction of organic load of coloured organic compounds to wastewater is not major, their colour makes them aesthetically unacceptable. Not only water bodies, but also the nearby soil becomes contaminated because of the insufficient treatment of the wastes generated from these industries (Deng *et al.*, 2008).

Many physical and chemical methods have been in use since ages for the removal of these dyes from water bodies, including adsorption, coagulation–flocculation, oxidation and electrochemical methods. These methods, owing to their expensive nature, high sludge production, and release of by-products, have several disadvantages in application (Wang *et al.*, 2009a,b). Many low-cost materials because of their low adsorption capacity such as peat, bentonite, steel-plant slag, wood chops, carbon slurry, fly ash, bottom ash and de-oiled soya implying need for large amount of adsorbents (Xin *et al.*, 2012).

Microorganisms, because of their ease of application, environmental benignity, and low cost, are being used as an alternative for treatment of textile effluent (Zhang *et al.*, 2007). They have the property of producing less amount of sludge and are also economical in nature, therefore being more efficient (Cheunbarn *et al.*, 2008; Sartale *et al.*, 2009). Bio-treatment processes exploit the ability of microorganisms to degrade and metabolize various compounds. Microorganisms, including bacteria, fungi and algae, have the capability of efficiently removing a wide variety of dyes (Fu *et al.*, 2002; Forgacs *et al.*, 2004; Sartale *et al.*, 2009). Fungus works at lower pH, has longer hydraulic retention time, and may inhibit other beneficial microorganisms from growing along (Chen *et al.*, 2003). The term 'biosorption' involves the process of transport mechanisms for the removal of waste material with the help of microbial biomass including both active and passive methods (Özer *et al.*, 2005). Various chemical groups present on the cell surface are responsible for biosorption process to occur.

The presence of differnet chemical groups such as phosphate, carboxylate, hydroxyl and amino groups on the microbial cell surface helps in sequestration of waste materials (Özer *et al.*, 2005). Factors affecting the decolorization process include pH, initial dye concentration, class of dye, temperature and ionic strength (Kaushik *et al.*, 2010). In the removal process, the application of biomass under growing condition has the advantage of avoiding separate processes of biomass production, harvesting, drying and storage (Michaels *et al.*, 1986).

Though much work has been done on bacterial biodegradation of dyes, degradation of anthraquinone dyes is still in its initial stages. The colour of anthraquinone dye is because of its anthraquinone nucleus. Modification of this colour is due to the number, types and position of substituents (Lee *et al.*, 2004). Anthraquinone dyes are carcinogenic and mutagenic to both animals and microorganisms. Therefore, removal of these dyes by microbes is of great interest in environmental chemistry. Further research is needed to isolate new microorganisms, which have the capability of removing a wide range of structurally different dyes, and to study their physiological characteristics. This will not only enhance our understanding of the mechanisms involved in dye biodegradation, but also

better our insight into its biotechnological application (Wang *et al.,* 2009a,b). *Burkholderia* sp. is able to utilize a wide variety of chlorophenols and aromatic compounds as carbon and energy sources. Members of *Burkholderia* genus, apart from inhabiting the soil, also find their place as plant or human pathogens, and are classified into the β-subgroup of the *Proteobacteria* (Lü *et al.,* 2003).

In the present study, the biosorption of alizarin, in both shaking and static conditions, has been attempted using growing cultures of *Burkholderia* sp., isolated from rhizosphere of *Cassia occidentalis*, with consideration of three important factors: pH, inoculum concentration, and dye concentration.

Materials and Methods

Preparation of dye solution

Alizarin (properties in Table 1), with maximum absorbance wavelength (λ_{max}) at 528 nm, was purchased from Fisher Scientific (India). Synthetic dye solutions were prepared by diluting 1.0 g/l of stock solution of dyes, obtained by dissolving weighed amount of Alizarin in 1 l of distilled water.

Media and bacterial culture

Isolation of anthraquinone degraders from rhizosphere soil was performed through enrichment technique on Bushnell and Haas medium amended with dye, alizarin (100 mg/l), along with glucose and yeast extract (Moosvi *et al.,* 2007). Dye containing media were inoculated with 10 ml of rhizosphere soil suspension (10% w/v) and incubated at 37° C, with repeated rounds of fresh media amended with dye, till stable cell growth and dye decolourisation were attained. This was then plated on the same media amended with alizarin to isolate strains capable of decolorizing the dye by showing distinct clear zones. Such a strain was selected for further decolourisation assay. The isolate was characterized as *Burkholderia* sp. by ribotyping (Ocimum Biosolutions, New Delhi, India). Comparison of sequence data to known sequences in the GenBank™ Database was done using the Basic Local Alignment Search Tool (BLAST). The bacterial strain was grown in 250 ml Erlenmeyer flasks containing 100 ml of Bushnell and Haas medium which contained: glucose, 0.1 g; $MgSO_4$, 0.02 g; K_2HPO_4, 0.1 g; $CaCl_2$, 0.002 g; $FeCl_3$, 0.005 g; NH_4NO_3, 0.1 g; Yeast extract, 0.05 g. The pH of the media was adjusted to the required values by using 1 N H_2SO_4 and 1 N NaOH before autoclaving. The flasks were kept in shaking (220 rpm) and static conditions at 37° C. A 10 % (v/v) of secondary culture, except for the studies of inoculum concentration effect, was used as an inoculum in all batch biosorption experiments.

Batch biosorption using dye solution

The studies in batch cultures were carried out using synthetic dye solution in 250 ml Erlenmeyer flasks with working volume of 100 ml, which contained desired initial concentration of the dye. The media was inoculated with 10 % (v/v) inoculum. Each flask was incubated at 37° C with static and shaking (220 rpm) condition. The samples were drawn at regular intervals of time and analyzed for residual concentration of dyes. The cell growth was checked using spectral analysis. All the experiments were carried out in triplicates. The effect of different parameters *viz.* inoculum concentration (5–20 %), pH (5.0–8.0), and dye concentration (100–1000 ppm) were studied for optimization of the batch studies.

Analytical methods

The bacterial growth was determined by spectral analysis. The biomass was centrifuged at 5000 rpm at room temperature for 2 min; supernatant was taken to measure percent dye decolorization. The pellet was washed twice by medium and O.D. was taken for determination of cell growth at 600 nm. The concentration of dye was determined by measuring absorbance at 528 nm. This absorbance was compared with the standard curve plotted using different concentrations of the dye. The measurement of dye absorbance was done using UV / VIS spectrophotometer (Eppendorf, Germany).

The following formula was used to calculate the percentage decolorization:

$$\% \text{ Decolorization} = \{(Conc_i - Conc_f)/Conc_i\} \text{ X } 100$$

Where, $Conc_i$ is the initial concentration (mg/l) of dye and $Conc_f$ is the residual concentration (mg/l) of dye at different time intervals.

Results and Discussion

The strain was isolated from rhizosphere of *Cassia occidentalis*, through enrichment technique on Bushnell and Haas medium amended with dye, alizarin [(100 mg/l) dye selected on the basis of structural resemblance with Emodin (a secondary metabolite abundantly found in rhizodeposits of *Cassia occidentalis*)] along with glucose and yeast extract (Moosvi *et al.,* 2007). Various parameters such as pH, inoculum concentration and dye concentration were analysed to get the maximum decolorisation. The isolate was characterized as *Burkholderia* sp. by ribotyping. Comparison of sequence data to known sequences in the GenBank™ Database was done (the sequence similarity was found to be 98%) using the Basic Local Alignment Search Tool (BLAST).

Figure 1 shows the cell growth of *Burkholderia* isolate with respect to time. The maximum increase in the cell growth in shake flasks was observed till 6 h, which correlated well with the growth curve of *Burkholderia* sp. The study was carried out both in shaking (220 rpm) and static condition at 37° C.

Optimum biosorption conditions

Effect of inoculum concentration

As the inoculum concentration increased from 5 % to 20 %, the percent decolourization and cell growth, both in shaking and static condition also increased (Figure 2). The minimum biosorption concentration for the maximum decolourization of the dye was found to be 20 %.

Effect of pH

From figure 3 it is clear that the minimum pH for maximum cell growth and maximum biosorption was 5.0 and it decreased significantly above this pH. Unlike in studies conducted by Alalewi *et al.* (2012) and Bayoumi *et al.* (2010) where the optimum pH for growth of *Burkholderia* sp. is found to be 8.0, and Lü *et al.* (2003) where optimum pH is 6.8, in our study, its optimum pH for growth was 5.0 (Lü *et al.,* 2003; Bayoumi *et al.,* 2010; Alalewi *et al.,* 2012).

Effect of initial dye concentration

As the dye concentration increased from 100 ppm to 1000 ppm, the cell growth decreased, indicating higher dye

concentration to be toxic for the strain (Figure 4). The percent decolorization decreased with an increase in initial concentration of dye. This can be attributed to the fact that, at lower dye concentration, the ratio of biosorbent sites to the dye concentration was higher, whereas, at higher dye concentration, the biosorbent sites were completely saturated (Özer *et al.,* 2005; Kaushik *et al.,* 2010) Similar studies have been reported earlier (Alhassani *et al.,* 2007; Wang *et al.,* 2009a,b).

Effect of inoculum concentration

The increase in cell growth can be attributed to the fact that at higher initial inoculum concentration, higher will be the amount of cells present, and vice-versa. The linear increase in percent decolorization in accordance with the inoculum concentration is because of availability of more binding sites for dye molecules and increased surface area (Özer *et al.,* 2005). Further constant biosorption trend, after 12 h, may be explained by the equilibrium established between the dye molecules present in the solution with those adsorbed on the cell surface and also because of the saturation of the dye binding sites on the biosorbent surface (Özer *et al.,* 2005).

Other studies on *Burkholderia* sp. however, show no correlation between inocula size and percent decolorization (Bayoumi *et al.,* 2010; Alalewi *et al.,* 2012). The possible reason for this could be the mechanism of biodegradation in their studies, rather than biosorption as in our study. It can be hypothesized that the enzymes responsible for the degradation have their respective maximum turnover numbers, whereas, biosorption is completely a surface phenomenon, and hence depends upon the inoculum size.

Effect of pH

pH of wastewater effluents has got a wide range because of different waste products being released (Alhassani *et al.,* 2007); hence the pH range of the strain for biosorption was to be established. The study of effect of initial pH is crucial, as it not only affects the biosorption capacity, but also the structure of the dye, colour stability, and solubility properties of some of the dyes (Özer *et al.,* 2005; Deng *et al.,* 2008). It has been reported that influence of the initial pH is more as compared to the final pH (Waranusantigul *et al.,* 2003). The possible reasons could be, firstly the cell growth was inversely affected at pH 8.0. Secondly, the neutralization of the charge might be responsible for the dye adsorption on cell surface. Normally, at lower pH, relatively positive charges are there on the cell surface as compared to the dyes tested. Thus, the cells may have relatively higher affinity for the dyes (Chen *et al.,* 2003).

Effect of initial dye concentration

The rate of mass transfer of the molecules present in the liquid onto the solid phase will be dependent on the initial dye concentration (Özer *et al.,* 2005). Dye degradation has been reported by *Burkholderia* sp. in earlier studies as well (Bayoumi *et al.,* 2010; Alalewi *et al.,* 2012).

Lü *et al.* (2003) have shown than the species is also capable of degrading many anthraquinones and aromatic compounds, hence our isolate holds biotechnological potential. Besides, our study being a biosorption study unlike the earlier ones, where the dye removal was due to the biodegradation process, has got more industrial application due to the less retention time for the treatment of wastewater.

The results of this study indicate that the isolated bacteria, *Burkholderia* sp., proved to be efficient in decolorizing alizarin with high efficiency (89 %), at dye concentration of 100 ppm at pH 5 with an inoculum concentration of 20 %. The strain can also tolerate and decolorize the dye at concentration level of 1000 ppm. It is able to grow and efficiently decolorize alizarin in a broad pH range of 5.0–8.0. The decolorization capability in a broad pH range will be an advantage in the case of practical applications to dye effluents, because the pH of dye-containing wastewater varies greatly because of the different kinds of wastes being released. The biosorption pattern of dyes was depending on various parameters like pH, inoculum concentration and dye concentration dependent. The isolate holds potential in its application in environment-friendly manner in treatment of dye effluent under field conditions.

Table.1 Characteristics of Alizarin

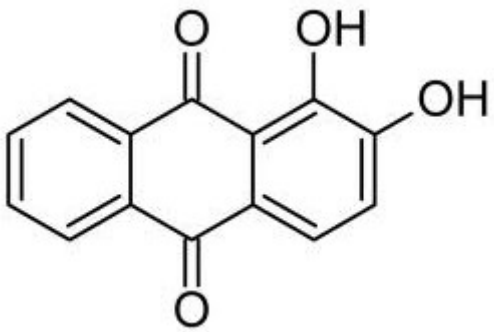
Dye	Color index name (C.I. No.)	Molecular structure	Wavelength (nm)
Alizarin	58000 C		528 nm

Figure.1 Cell growth (OD at 600 nm) with respect to time for *Burkholderia* sp. culture. Error bars in the figures represent the standard deviation (*n*=3)

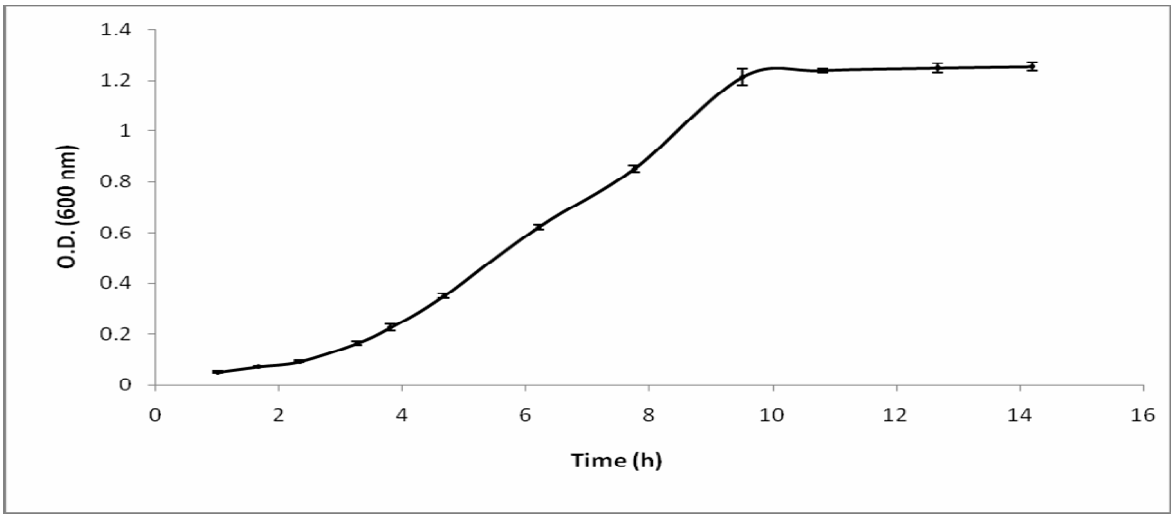


Figure.2 Effect of different inoculum concentration with time: percent dye removal under shaking condition (a); percent dye removal under static condition (b); cell growth under shaking condition (c); cell growth under static condition (d). Error bars in the figures represent standard deviation (*n*=3)

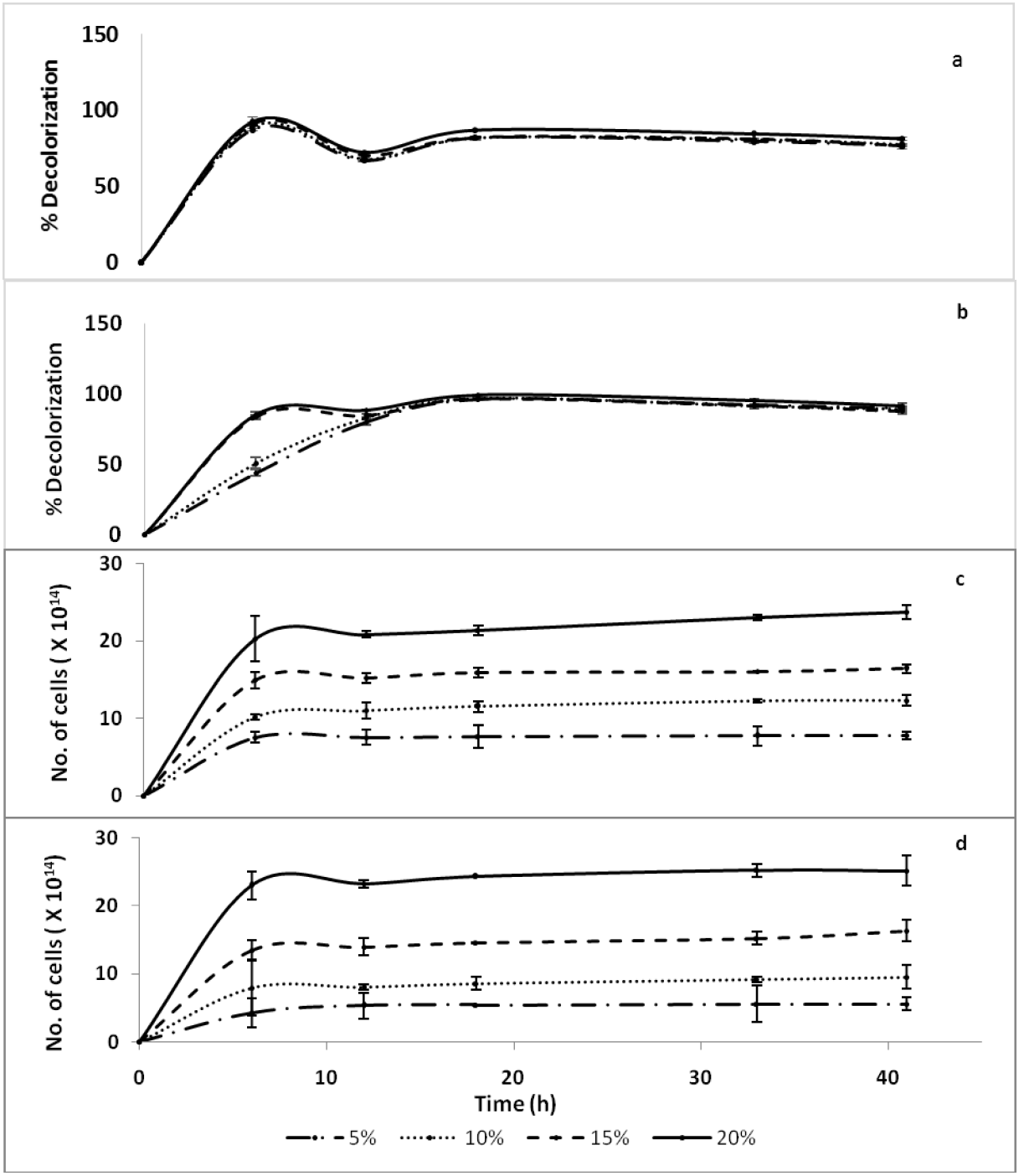


Figure.3 Effect of varying pH with time: percent dye removal under shaking condition (a); percent dye removal under static condition (b); cell growth under shaking condition (c); cell growth under static condition (d). Error bars in the figures represent standard deviation (*n*=3)

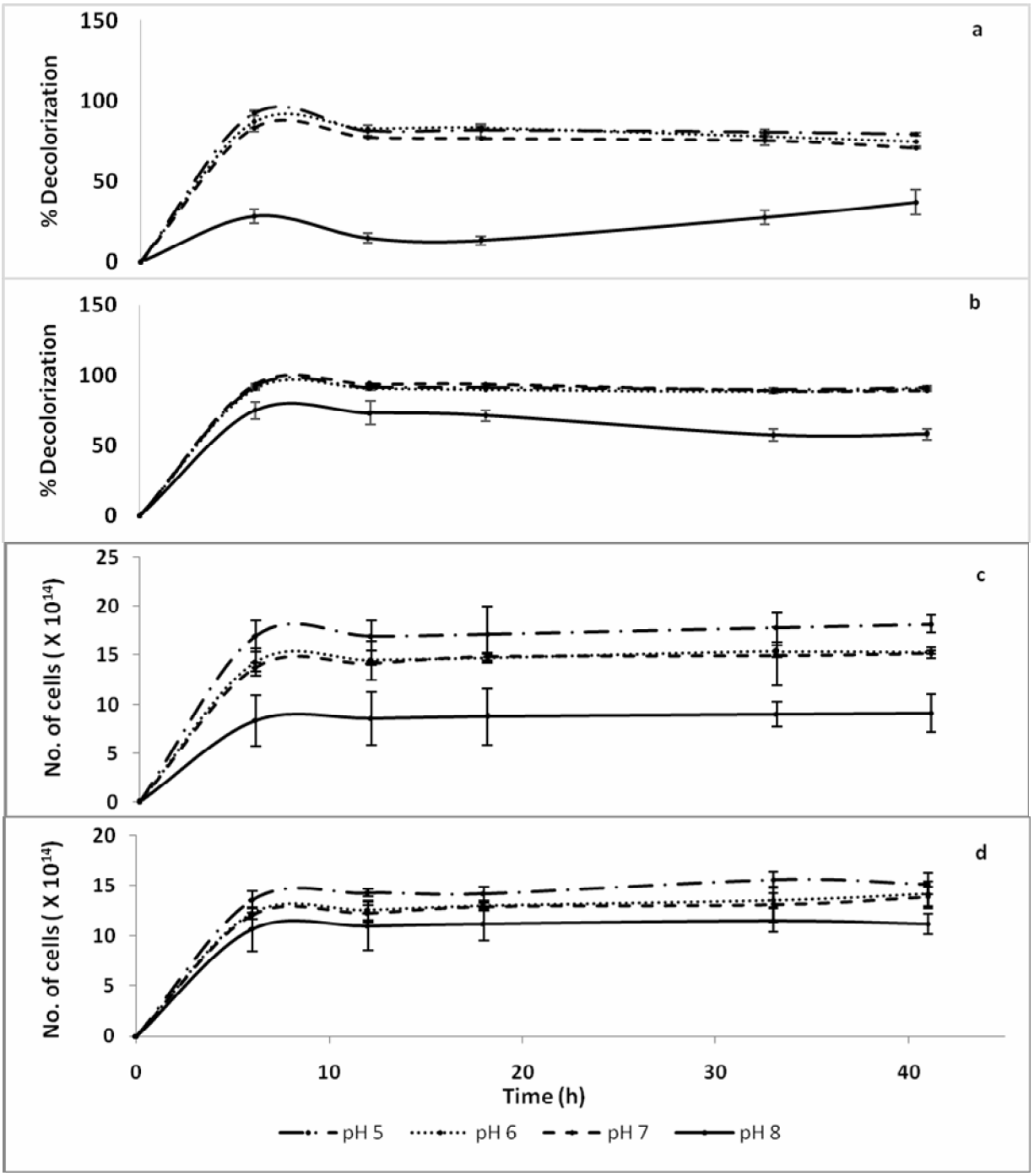
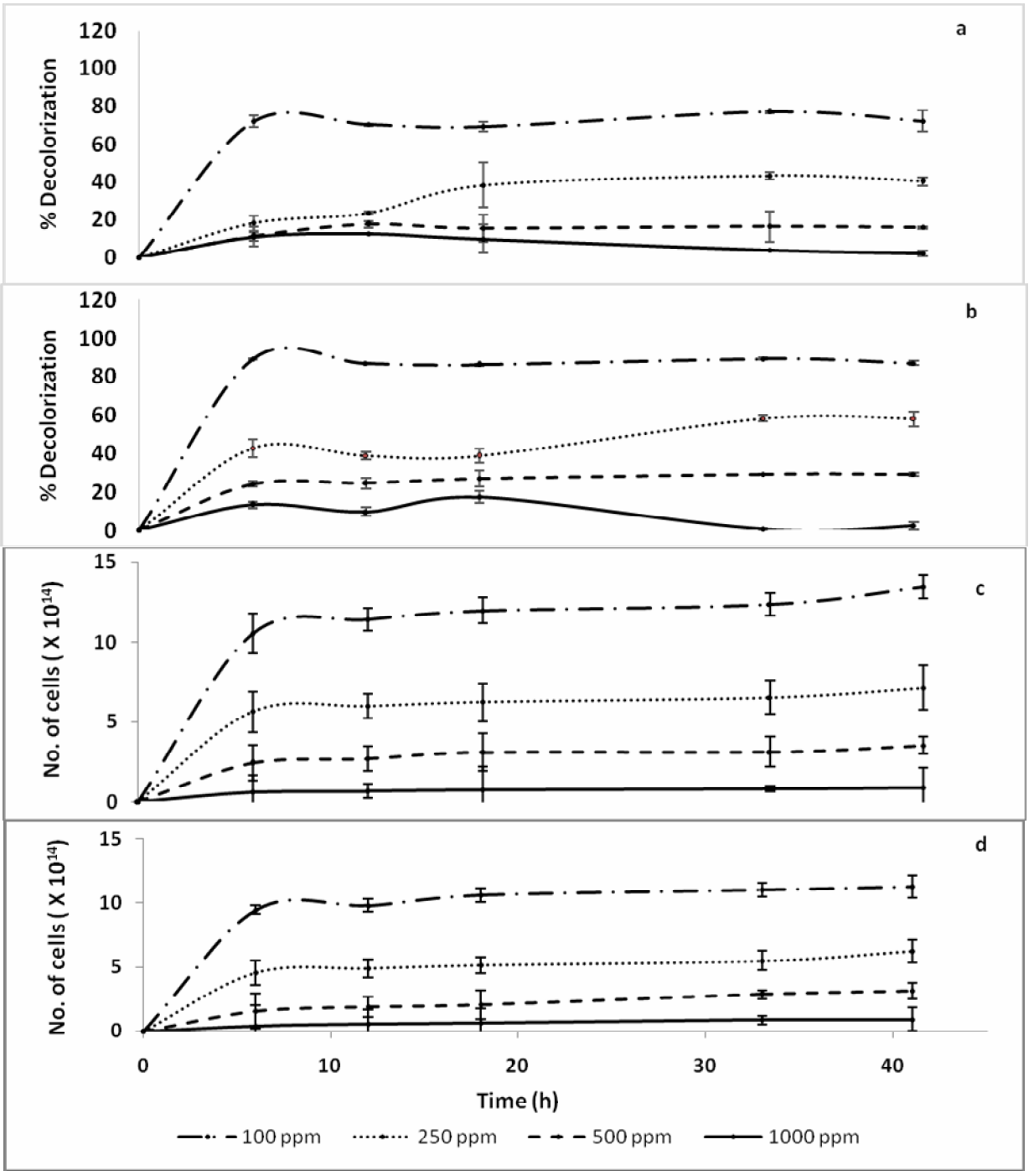


Figure.4 Effect of different dye concentration with time: percent dye removal under shaking condition (a); percent dye removal under static condition (b); cell growth under shaking condition (c); cell growth under static condition (d). Error bars in the figures represent standard deviation (*n*=3)



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