

Original Research Article

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Bioremediation and Detoxification of a Textile Azo Dye-Evans Blue by Bacterial Strain AKIP-2

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ABSTRACT

Keywords

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Increasing industrialization and urbanization result in the discharge of waste to the environment, which in turn creates more pollution. Environmental biotechnology is constantly expanding its efforts in the biological treatment of colored textile effluents, which is an environmental friendly and low cost alternative to physico-chemical decomposition processes. In the present study, effluent samples were collected from various textile and dyeing industries located in and around Kanchipuram, Tamilnadu, India and were exploited for the screening and isolation of bacterial strains that were capable of decolorizing the textile dye, Evans Blue. Optimization of cultural conditions (Temperature, pH, Agitation speeds and Dye concentrations) were carried out to maximize the bacterial growth of *E. coli* strain AKIP-2 and its decolorization efficiency towards Evans Blue. Both bacterial biomass and decolorization efficiency was found to be optimized at 35°C, neutral pH, after 24 h of incubation. Static conditions proved to be effective in maximizing decolorization. Increase in dye concentration decreased both bacterial growth and decolorization efficiency of *E. coli* strain AKIP-2.

Introduction

Textile industry has a major impact not only on the nation's economy but also on the environmental quality of life in many communities. Textile manufacturing consumes a considerable amount of water approximately 100 litres of H₂O kg⁻¹ of textile materials in its dyeing, finishing and manufacturing processes (Tang and Chen, 1996). Considering both the volume generated and the effluent composition, the textile industry wastewater is rated as the most polluting source among all industrial sectors (Koyuncu, 2002). Strong color of the

textile wastewater is the most serious problem of textile waste effluent. The disposal of these wastes into receiving water causes damage to the environment (Shyamala *et al.*, 2014). Dyes may significantly affect the photosynthetic activity in aquatic life because of reduced light penetration and may also be toxic to some aquatic life due to the presence of aromatics, metals, chlorides etc, (Husseiny, 2008; Hemapriya *et al.*, 2010). In addition to their visual effect and adverse impact in terms of chemical oxygen demand (COD), many synthetic dyes show their toxic, carcinogenic

and genotoxic effects (Pearce *et al.*, 2003). Traditional wastewater treatment technologies have proven to be markedly ineffective for handling wastewater of synthetic textile dyes because of the chemical stability of these pollutants. Color is one of the most obvious indicators of water pollution and the discharge of highly colored synthetic dyes in textile effluents can be damaging to the receiving water bodies (Nigam *et al.*, 1996; Shyamala *et al.*, 2014). Implementation of physical/chemical methods have inherent drawbacks of being economically unfeasible (more energy consumption and chemical uses), unable to remove the recalcitrant azo dyes and/or their organic metabolites completely, generating a significant amount of sludge that may cause secondary pollution problems (Zhang *et al.*, 2004; Hemapriya and Vijayanand, 2014). The microbial decolorization and degradation of azo dyes has been of considerable interest since it is inexpensive, eco-friendly and produces a less amount of sludge (Kalyani *et al.*, 2008; Saratale *et al.*, 2009). The effectiveness of microbial decolorization depends on the adaptability and the activity of selected microorganisms including bacteria, actinomycetes, fungi, yeasts, and algae capable of degrading azo dyes (Shyamala *et al.*, 2014).

Hence, the present investigation was intended to assess the potential of AKIP-2 strain to decolorize the synthetic textile azo dye, Evans Blue under aerobic conditions, and to optimize the culture conditions to maximize the biomass and decolorization efficiency of AKIP-2 Strain.

Materials and Methods

Sampling site and sample collection

The sampling area was the textile industries and dyeing units located in and around

Kanchipuram, Tamil Nadu, India. The effluent samples from both textile industries and dyeing units were characterized by its dark color and extreme turbidity.

Azo dye used

The commonly used textile azo dye, Evans Blue used in this study was procured from a local textile dyeing unit. Stock solution was prepared by dissolving 1 g of Evans Blue in 100 ml distilled water. The dye solution was sterilized by membrane filtration, since azo dyes may be unstable to moist-heat sterilization.

Isolation and screening of bacterial strains decolorizing evans blue

Effluent samples were serially diluted and spread over basal nutrient agar medium containing 50 ppm of Evans Blue. Colonies surrounded by halo (decolorized) zones were picked and streaked on nutrient agar plates containing Evans Blue. Different colonies of dye decolorizing bacteria were picked and re-streaked several times to obtain pure cultures.

Decolorization assay

A loopful of bacterial culture AKIP-2 was inoculated in 100 ml of nutrient broth and incubated at 150 rpm at 37°C for 24 h. Then, 1 ml of 24 h old culture was inoculated in 100 ml of nutrient broth containing 50 ppm of Evans Blue and re-incubated at 37°C till complete decolorization occurs. Suitable control without any inoculum was also run along with experimental flasks. 1.0 ml of sample was withdrawn every 12 h and centrifuged at 10,000 rpm for 15 min. Decolorization extent was determined by measuring the absorbance of the culture supernatant at 590 nm using UV-visible spectrophotometer (Hitachi U 2800), according to Hemapriya *et al.*, (2013).

Decolorization efficiency (%) = $\frac{\text{Dye (i)} - \text{Dye (r)}}{\text{Dye (i)}} \times 100$

Where, Dye (i) refers to the initial dye concentration and Dye (r) refers to the residual dye concentration. Decolorization experiments were performed in triplicates.

Bacterial strain and culture conditions

Bacterial strain that showed maximum decolorization percentage on Evans Blue was aerobically cultured in nutrient broth containing 50 ppm of Evans Blue. The pH was adjusted to 7.0. For frequent use, the culture was maintained by transfer to a fresh medium at 24 h intervals. When required for prolonged periods, it was maintained by sub-culturing once every 7 days on slants, prepared by solidifying the above mentioned medium with 2.0 (w/v) agar.

Optimization of various culture conditions for bacterial biomass and evans blue decolorization by AKIP-2

Effect of temperature, pH, agitation rates and dye concentrations

The effect of temperature, pH, agitation rates and dye concentration on both bacterial biomass and dye decolorizing ability of AKIP-2 strain was studied. This was carried out by incubating the bacterial strain at different temperatures (20-60°C), different pH values of the medium (pH 4.0-10.0), different agitation speeds (0-200 rpm) and various dye concentrations (200-1000 ppm). Bacterial biomass and decolorization percentage was measured at optimum growth (24 h).

Results and Discussion

Effect of incubation time

Incubation time played a significant role in maximizing both bacterial growth and dye

decolorizing ability of *E.coli* strain AKI-2. Results of the present study revealed that the dye decolorizing ability of the isolate was dependent on the bacterial growth. The bacterial cells started multiplying within 4 h and reached their maximum growth within 24 h and thereafter started to decline, due to the depletion of nutrients and accumulation of toxic metabolites (Fig 1 and 2). In contrast, decolorization of Methyl orange by *Bacillus* sp. strain TVU-M4 was achieved after 32 h of incubation (Shyamala *et al.*, 2014).

Effect of temperature

The results shown in Fig 3 and 4 revealed that *E.coli* strain AKI-2 showed strong decolorizing activity and highest bacterial growth from 30-40°C, with optimum being 35°C after 24 h of incubation. The incubation at 30, 50 and 60°C was found to decrease both bacterial biomass and dye decolorizing ability of the bacterial strain; however the decolorization percentage of the isolate was found to be greatly inhibited at temperature below 30°C. Decolorization of Congo Red by *Bacillus* sp. VT-II was maximized at 40°C (Sawhney and Kumar, 2011).

Effect of pH

E. coli strain AKI-2 grew well in a broad range of pH (5.0-10.0) and its decolorizing ability does not have strict pH requirement. Bacterial biomass and dye decolorizing ability was found to be optimized at neutral pH (7.0) (Fig.5 and 6). Similarly, neutral pH was found to be effective in maximizing both bacterial growth and dye decolorization efficiency of many bacterial strains.

The pH tolerance of the decolorizing bacteria is quite important because the reactive azo dyes bind to cotton fibers by the addition or substitution mechanisms under alkaline conditions (Aksu *et al.*, 2007).

Fig.1 Effect of incubation time on bacterial biomass of *E. coli* strain AKI-2

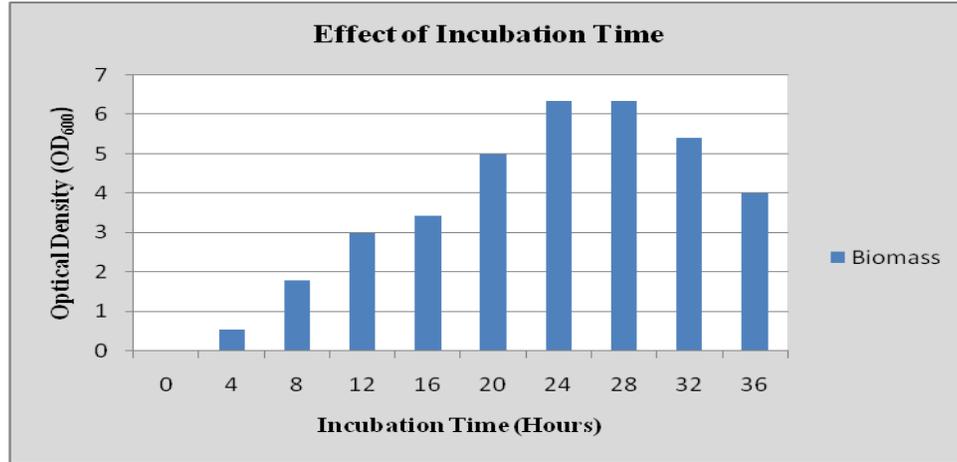


Fig.2 Effect of incubation time on decolorization of Evans blue by *E. coli* strain AKI-2

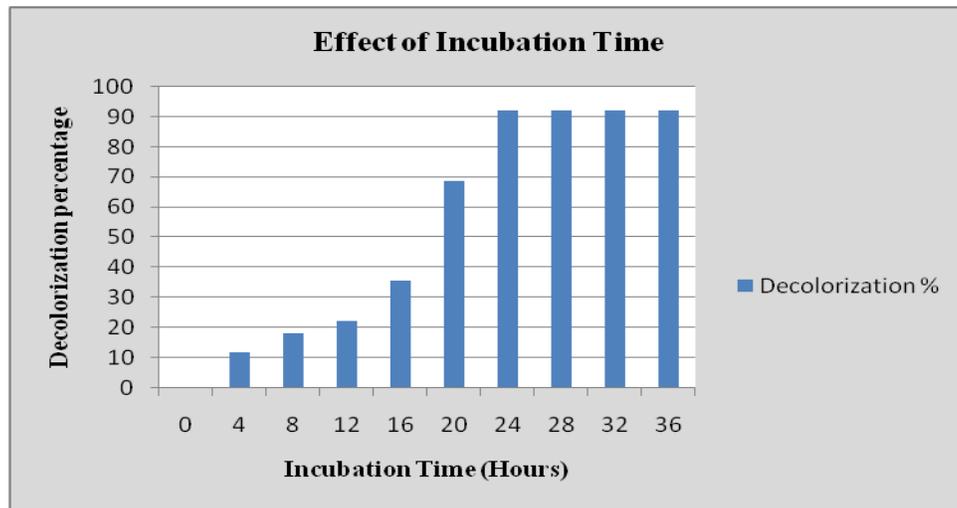


Fig.3 Effect of temperature on bacterial biomass of *E. coli* strain AKI-2

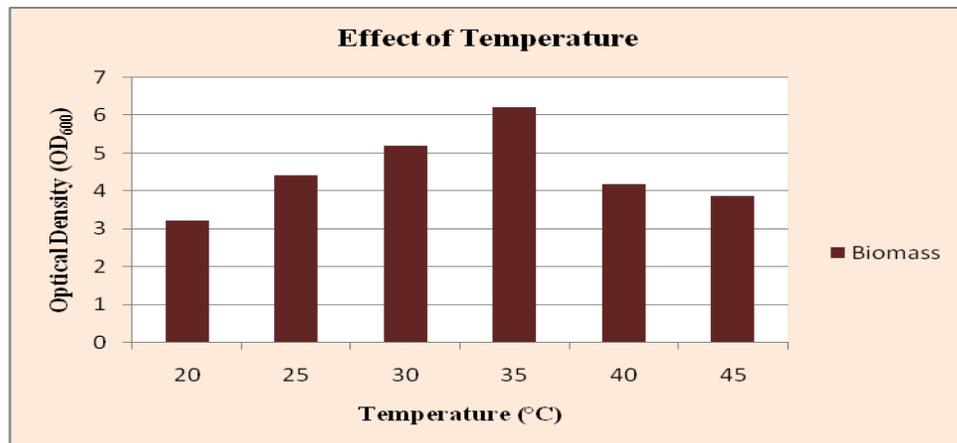


Fig.4 Effect of incubation time on decolorization of evans blue by *E. coli* strain AKI-2

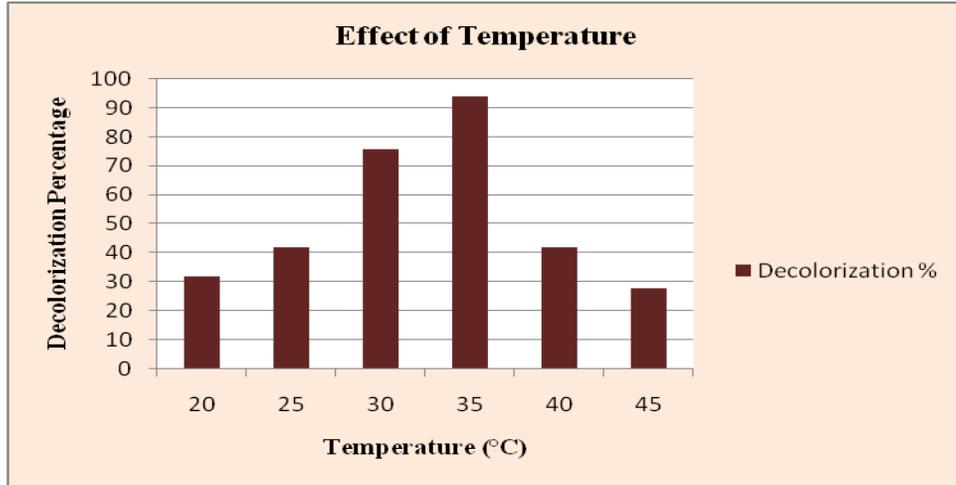


Fig.5 Effect of pH on bacterial biomass of *E. coli* strain AKI-2

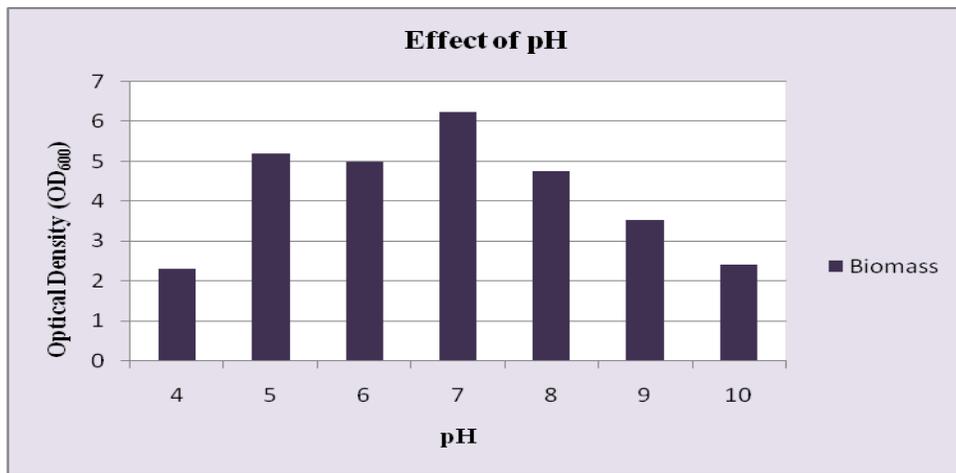


Fig.6 Effect of pH on decolorization of evans blue by *E. coli* strain AKI-2

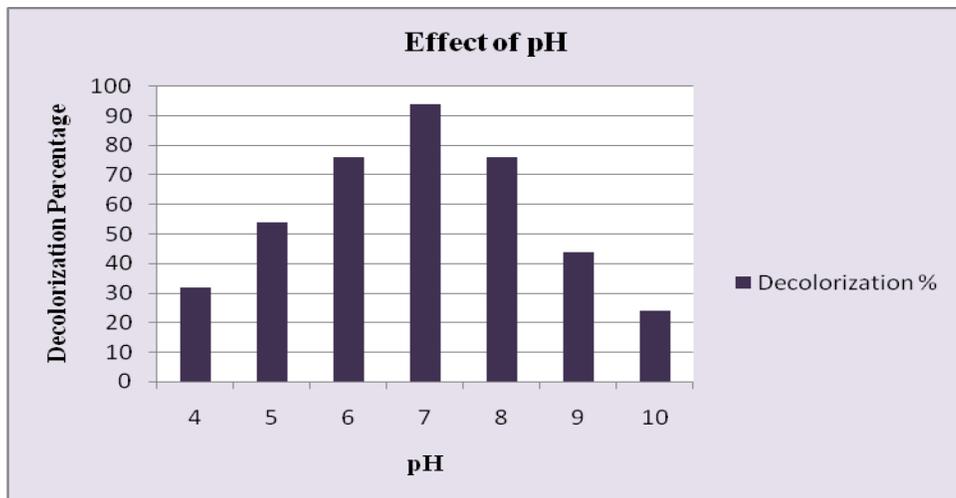


Fig.7 Effect of agitation speed on bacterial biomass of *E. coli* strain AKI-2

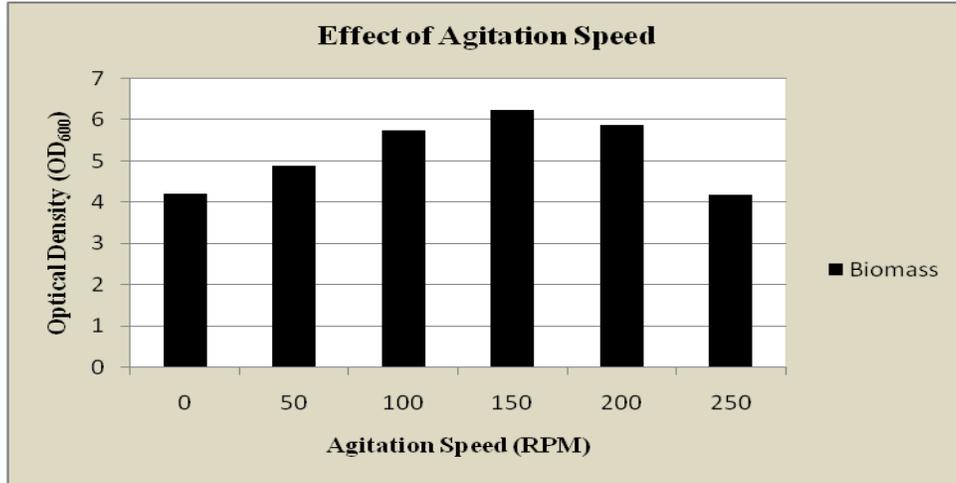


Fig.8 Effect of agitation speed on decolorization of evans blue by *E. coli* strain AKI-2

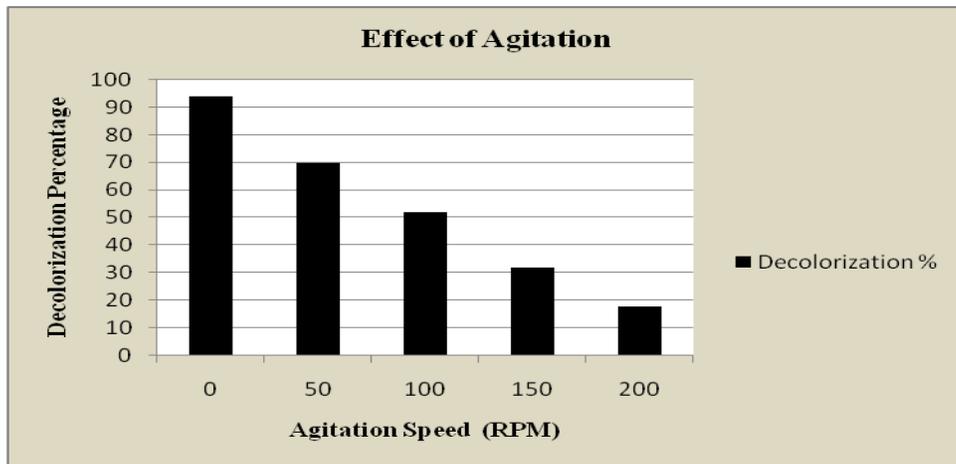


Fig.9 Effect of dye concentration on bacterial biomass of *E. coli* strain AKI-2

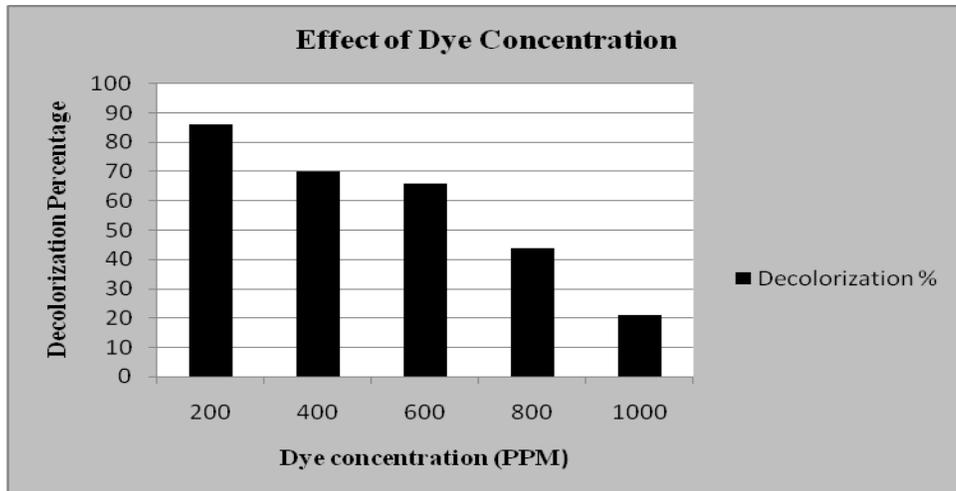
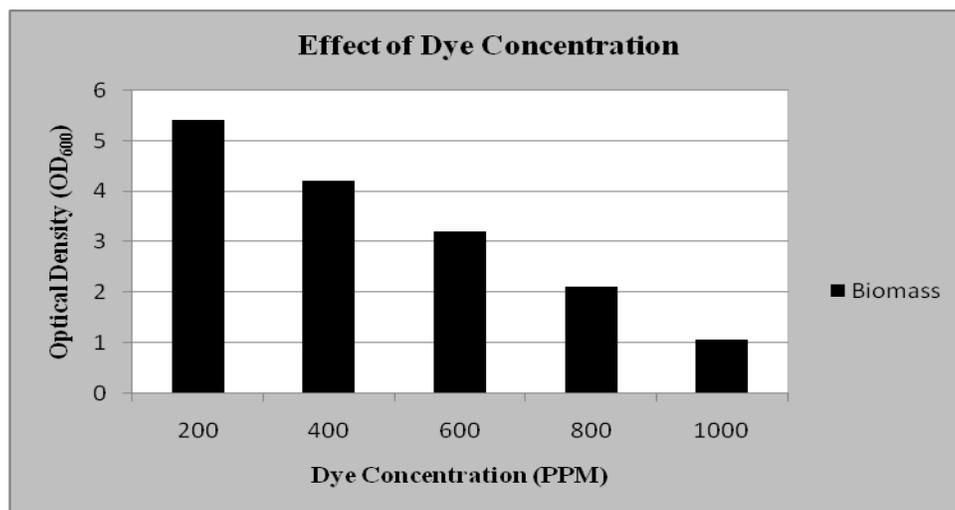


Fig.10 Effect of dye concentration on decolorization of evans blue by *E. coli* strain AKI-2



Effect of dye concentrations

The influence of different dye concentrations (0-1000 ppm) were analyzed on bacterial biomass and decolorization ability of *E.coli* strain AKI-2. The results shown in Fig 7 and 8 revealed that the decolorization rate increased linearly with increase in initial dye concentration upto 100 ppm. As the dye concentration increased in the culture medium, a decline in color removal was attained. At high concentration (1000 ppm), Evans blue greatly suppressed both bacterial biomass and decolorization ability.

Effect of agitation speeds

Microorganisms vary in their oxygen requirement. The effect of various agitation speeds (0-250 rpm) on the bacterial growth and color removal capacity of *E.coli* strain AKI-2 was studied at 35°C after 24 h of incubation. The decolorization ability of the isolate was found to be maximized at static conditions. Shaking conditions highly repressed the decolorizing ability of *E.coli*. In contrast, the bacterial biomass was found to be maximized when incubated at 200 rpm (Fig 9 and 10).

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