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Effect of light on the biodegradation of selective azo dyes by *Escherichia coli* and *Pseudomonas sp.* and optimization of conditions for decolorization

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Abstract
The degradation of two azo dyes; congo red and acid red 27, commonly used in textile industries, was investigated using *Pseudomonas* sp. and *Escherichia coli* strains isolated from dye contaminated soil sludge. An aqueous medium containing 100 mg/L of dyes were used for the biodegradation experiments. The degradation results as color removal performance were obtained after 5 days incubation. Different factors like azoreductases specificity, pH and effects of a range of wavelengths of light on decolorization efficiencies were studied. *Pseudomonas* sp. showed highest degradation rate for congo red and *Escherichia coli* showed highest degradation rate for acid red 27. The color of congo red and acid red 27 dyes were removed up to 98% and 99%, respectively by *Pseudomonas* sp. and *E. coli* at the end of anaerobic incubation. Neutral pH was found to be the most appropriate and presence of light showed enhancement of biodegradation rate. Shorting of wavelength of light increased the rate of degradation. Irradiation of UV light before the introduction of microbial strains showed complete color removal only in 2 days period. UV/Visible spectrophotometer was used to monitor the decolorization process.
Keywords: Azo dyes; congo red; acid red 27; *Escherichia coli*; *Pseudomonas* sp.; Photo-biodegradation.

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Introduction

Synthetic dyes are used extensively in paper, textile, printing industries and other dying processes because they are easy to manufacture, have fastness to a variety of substrates and are available in huge shades in comparison to natural dyes [1]. Over 100,000 dyes are known to be used commercially and roughly one million tons annual production of these dyes is recorded worldwide. It is expected that more than 15% of the total dyestuff which is used in dyeing processes has unrestricted access to the environment [2]. The largest group of dyes used in textile industry is azo dyes. It constitutes approximately 60-70% of all the dyes produced [3].

One or more azo groups (R_1-N=N-R_2) having aromatic rings mostly substituted by sulfonate groups comprises the structure of these dyes. Conjugated system made by these complex aromatic substituted structures is responsible for high water solubility, intense color and resistance to degradation in natural conditions [4]. One of the most obvious indicators of water pollution is color in the effluent. It is aesthetically displeasing to discharge highly colored synthetic dye effluents to the environment and can harm the receiving aquatic bodies by restricting the light penetration. Moreover, cytotoxic nature of azo dyes breakdown products is also well known [5]. They are also carcinogenic and affect the skin causing skin cancer, ey e swelling and also effect digestive and respiratory track by inhalation. Dye effluents from the textile industries are toxic and must be controlled or treated to avoid their harmful effects to the environment and living organisms [6].

The meaning of treatment is to use practices, processes, materials, products, power or energy that eliminate or reduce the creation of hazards, wastes, pollutions or environmental disturbance. It also includes reduction of risk to health of humans and the ecology. Sufficient treatment of textile dyes wastewater requires a number of stages like color removal and degradation of aromatic chemicals [7]. Discharge of colored wastes in natural water bodies is banned according to the new environment regulations concerning textile products [8]. Therefore, it has become a necessity for clean production technology for textile industries that effective and economic treatment of effluents containing a variety of textile dyes should be obliged. The physical and chemical based treatment systems for removal of dyes from effluents are being widely used [9]. These systems have inherent disadvantages, however, as they generate a significant amount of sludge or cause secondary pollution due to formation of hazardous by-products. Interests have been generated by the new guidelines for effluent and sludge disposal in the wider use of biological treatment of dye wastewaters, as they are well-known to achieve complete mineralization without generating toxic sludge [10].

Proper treatment of dye-contaminated water released from fabric industry and other sectors of dye-stuff is compulsory to avoid contaminants from ground area and ground water hazards. At present a

number of physicochemical and scientific techniques for the elimination of colors from effluents are practiced [11]. Among these, biotechnological techniques are getting improved interest globally as eco-friendly procedures that are progressively becoming effective and cost-reductive for the remedies of contaminated wastewater from dye effluents [12]. Many bio-treatment techniques are dependent on the utilization of debris to initiate the dye deterioration procedures [13]. Usually, it is however, important to guarantee complete mineralization and cleansing of contaminations for an efficient remedy. Azo dye breakdown products differ in their revolt to biodegradation because of their complicated components and xeno-biotic characteristics [4]. Furthermore, biodegradation is also retarded because dye degrading microbes are delicate to elevated levels of sodium which is added in the dye treatment [14]. This can restrict the growth of the microbes and treatment time becomes much long. By the development and solitude of very useful, azo-dye degrading microbes which can tolerate salts, process of biodegradation has now become an effective way to enhance wastewater treatment techniques with particular microbe variations and enhanced bioremediation of azo colors has been obtained [15].

Biodegradation is a procedure in which various active microbes such as natural, augmented or genetically intended microbes are presented to the bioreactor or the dye contaminated places and scientific procedures of degrading pollutants are accelerated. It accomplishes more constant outcomes [16]. As used here, photo-biodegradation is the term for the use of chosen variations of microbes in contrast to the imprecise microbe societies such triggered debris produced microbes. In the procedure of healing the pollutants although activated debris is used. The microbial varieties included in this content are unspecified and the process is a vague [17]. This may cause to unreliable outcomes, such that in some situations, up to 90% of the colors in an effluent can stay without treatment after a triggered debris procedure. However, activated sludge can offer a useful beginning technique from which personal variations or consortia-microbes can be separated and seems to be classy for use in the form of inoculants to the waste material [18]. The microbe varieties can then be analyzed to figure out the ecological aspects for the development and propagation of enhanced amount of amount of deterioration.

For complete decolorization process, the influence of individual isolates should be improved by using other extremely useful dye-degrading parameters [3]. In this way it is thought that the mixed hybrid techniques of the microbe combined with other parameters are more productive than the treatments using the single isolates. All of these may have variable kinetics of degradation showing performance at variable degradation levels. Light may also be proved such a useful parameter in degradation process. Collaboration of light with microbe activity can also result handy elimination of harmful metabolites and otherwise may result in the development of cofactors. Although a number of microbe verities can lower impacts of azo colors [19]. Relatively few microbe varieties in collaboration with light have appeared much efficient for use in photo-biodegradation of plastics. Before light along with individual isolates can be suggested, extensive analysis is needed to comprehend the part of effective biodegradation parameter [20]. So a study was planned to isolate new strains capable of degrading two azo dyes; congo red and acid red 27 and to optimize their degradation capability considering pH and wavelengths of light.

Materials and Methods

Dyes

Congo red and acid red 27, typical azo dyes used in dyeing textiles and cotton yarn, were of commercial quality (Fig. 1). These dyes were purchased from a textile factory in Faisalabad, Pakistan.

Identification of microbes

Microbial strains were isolated from the dye contaminated soil sludge. Samples were cultured primarily in nutrient broth at 37^{o}C for 24 hr. These microbes were identified by using staining techniques and biochemical analysis based on the methods of Bergey's Manual of Systematic Bacteriology using standard microbiological procedures [21]. All the characteristics confirmed that the isolates strains are *Pseudomonas* sp. and *E. coli*. These microbes were sub-cultured on to the MacConkey agar medium to get more specificity and selectivity [22]. Cultures were placed in the incubator at 37^{o}C for 5 days and well formed red colonies were observed on the agar gel. These microbial cultures were stored at 4^{o}C for further use.

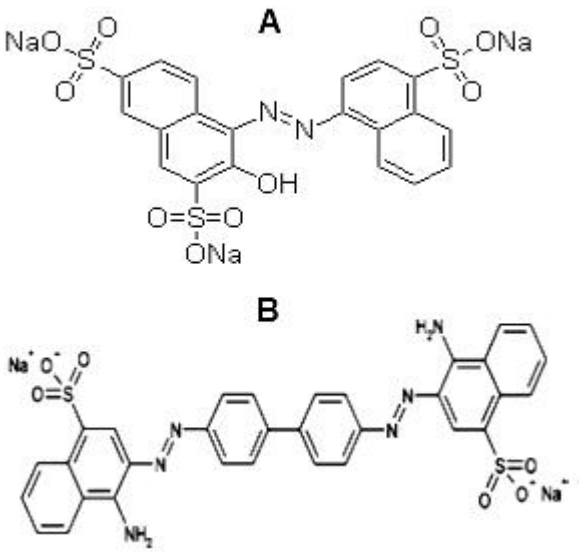

Fig. 1: Acid red 27 (A) and congo red (B) chemical structures

Synthetic media

The support medium used was 1% peptone, 0.5% yeast extract and 1% NaCl for the microbes. Glucose (1g/L) was used as co-substrate [23]. It provides the electrons for the reductive cleavage of the azo dyes, namely CR and AR 27. The biodegradation experiment was conducted for 5 days using 100 ppm dye solutions throughout the study.

Experimental procedure

Two facultative anaerobes strains, *Pseudomonas* sp., and *E. coli* were identified and separated from dyes contaminated soil sludge. They were cultured and maintained separately on MacConkey agar medium [22]. Microbes from the culture plates were inoculated in the dye solution in 100 ml flasks. Other nutrients like glucose, yeast extract, peptone and NaCl were also introduced to the dye solution to support the process of degradation. To determine the effect of pH on biodegradation, different pH values (2, 7, 8.5, 12) were achieved by using the solution of 0.1M of sodium hydroxide and 0.01M solution of hydrochloric acid. To determine the effect of different wavelength lights, special wooden box was crafted and assembled with a lamp holder inside it, fixed on the ceiling. Lamps emitting light in different colors (wavelength ranging from 300 to 800) were applied to analyze the light effects on the process of biodegradation. The microbes were not exposed to the UV light, instead, the dye solutions were irradiated for half an hour and then microbes were inoculated. Process of photo-biodegradation was monitored and samples were taken after continuous intervals manually with the help of pipette. Degradation rates were determined by measuring absorbance at 497nm for congo red and at 540nm for acid red 27 using UV/Visible spectrophotometer.

Table 1: Wavelengths emitted by lamps used for experiments

Color of light	Wavelength range	Maximum emission
Red	650-750 nm	700 nm
Orange	600-650 nm	620 nm
Yellow	550-600 nm	580 nm
Green	500-550 nm	530 nm
Blue	450-500 nm	470 nm
Ultra Violet	300-350 nm	315 nm

Results and Discussions

The strain *Pseudomonas* sp. was identified by the following characteristics: Gram-negative, rod shaped aerobic, polar flagellate, non-spore-forming, catalase-positive, oxidase-positive, nitrate reducing and acid forming from glucose [24]. *Escherichia coli* strain was identified by the following characteristics: Gram-negative, rod shaped, endospore forming, facultative anaerobic, catalase-positive, lactose positive, and could reduce nitrate [25].

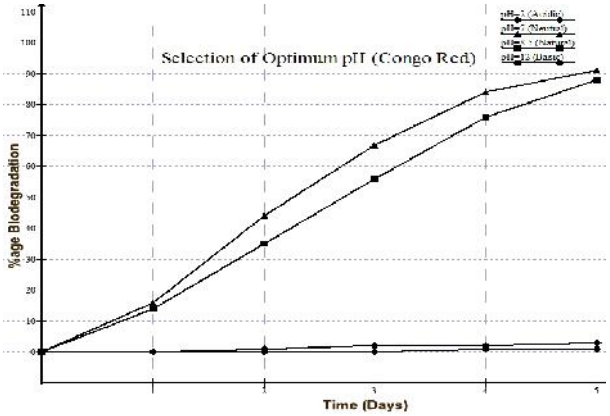

Fig. 2: Biodegradation of congo red at different pH values

Experiments were conducted using 100 ppm concentration of dyes in 100 ml solution having variable pH at room temperature. Biodegradation process is highly dependent on the pH of the solution. It has been observed that change in pH have significant effects on the biodegradation of both dyes. At acidic and basic pH, degradation process was approximately ceased, while at pH 7 the process of degradation was optimum than that of pH 8.5 (Fig 2). Therefore, pH of 7 was the optimum pH for the biodegradation of both

dyes i.e. congo red and acid red 27 (Fig 3). The reason might be related with the growth of microbial strains used. Optimum pH for the maximum growth of *Pseudomonas* spp. is 7.0–7.5 [26] while the growth of *Escherichia coli* is optimum at the pH of 6.0-7.0 [27]. More growth of bacterial at neutral pH exhibited more degradation of both dyes.

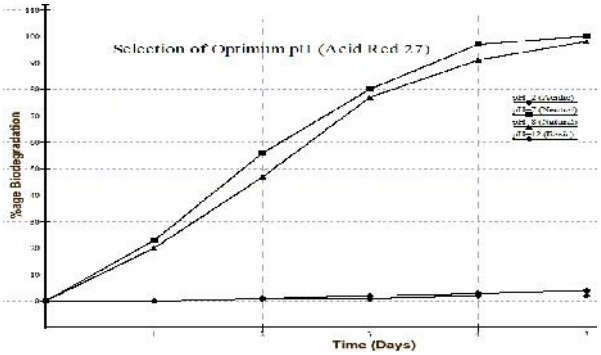

Fig. 3: Biodegradation of acid red 27 at different pH values

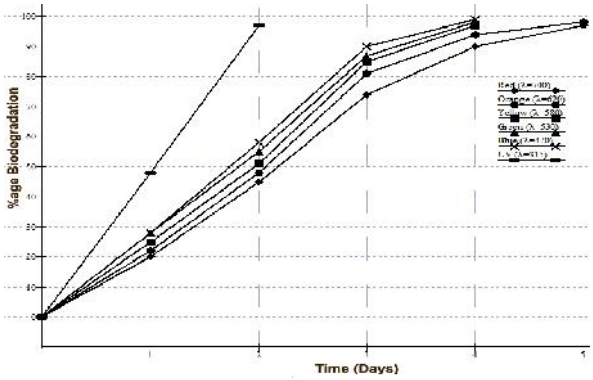

Fig. 4: Photo-biodegradation of congo red in the presence of different wavelengths of light

The effect of different wavelengths on photo-biodegradation was studied by using light from five different sources (wavelength ranging from 300-800). The maximum degradation of both dyes was observed at 350 nm wavelength of UV light. The results showed that decrease in the wavelength of light increased the rate of degradation (Fig 4 and 5). Enhancement of photo-biodegradation process by decreasing the wavelength was might be due to the increase of energy of radiation. As the energy of radiations increased they become complementary to the red color of the dye. In this way excitation of dye bonds was enhanced and photo-biodegradation was also enhanced.

In addition, energy from the photons of light can break the chemical bonds by photo-oxidation [28, 29]. Light, especially UV radiation, increases the availability of dissolved organic matter to bacteria [30] which is the primary substrate for bacterial growth in ecosystems [31]. Non-ionizing radiation, such as ultraviolet (UV) light, exerts its mutagenic effect by exciting electrons in molecules [32].

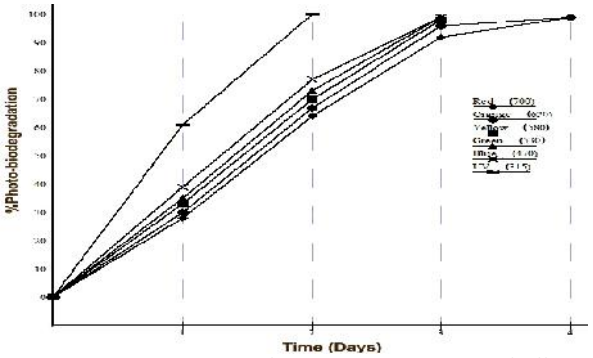

Fig. 5: Photo-biodegradation of acid red 27 in the presence of different wavelengths of light

Conclusions

Color accumulation is one of the burning issues due to large no. of pollution hazards. Biodegradation is one of the cheapest and most effective process but thought to be slower. However, rate of biodegradation can be enhanced by using hybrid techniques. Light is one of the best source of energy which can increase the degradation rates in environment friendly way. Shorter wavelengths of light i.e. ultra violet light increased the biodegradation process more effectively. This research work recommended the use of specific azoreductases containing microbes at the neutral pH and in the presence of low intensity shorter wavelengths of light to degrade azo dyes like congo red and acid red 27. Further studies are required to find out more efficient biodegrading microbial strains and to optimize the degradation process.

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