ORIGINAL ARTICLE

Optimization of the Biodecolorization Activity of the Ayapers dye Removal by *Halmonas* sp. Isolated from Urmia Lake

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Abstract

Background: Among chemical dyes, Azo dyes, as environmental synthetic pollutants, are most commonly used in a bunch of different colors in different industries, especially in textile industry to a large extent.

Methods: Due to some limitation and difficulties to remove these pollutants from the environment, the biological filtering method, as the economically and optimal methods, are preferred. Using the Taguchi method and evaluation of such factors in the environment as temperature pH, color density and concentration of salt, we studied the optimal condition of Halomunas PTCC1132 bacteria decolorization in order to compare it with the Aryapress dye removal from aquatic environment. Therefore, 16 experiments were designed according to Array Table in 4 factors and 4 levels. The results were then analyzed using a computer the program named Qualitek-4.

Results: The results showed that this salt, loving bacterium Halomonas strain PTCC1714, has the ability of bleaching in a wide range of salts till 20%, pH (5-9) and dye tolerance up to 5 gr/lit (500 ppm), and has the highest rate of decolorization in 100 ppm.

Conclusions: According to the results with an optimal growth condition- the temperature of 40^{c}, pH of 7.5, and the salt concentration of 10% up to 93% - the strain was capable of removing the Azo dye Aryapress color with the concentration of 100 ppm which is a considerable amount and can be used in biological treatment of industrial textile sewage.

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Introduction

Due to increases in the activity of industries such as paper, textiles, leather industries, and because of non-compliance with environmental requirements and large amounts of pollutants, including various synthetic dyes makes them the most polluting industrial effluents.[1] Different combinations of synthetic colors are commonly used in textile industries which results in different sewages in terms of composition.[2] Among the industries, textile industry typically produces colored wastewater which is considered as an escalating problem since the colors, which are mostly synthetic and toxic in nature, including poly-aromatic molecules makes them resistant to biodegradation.[3] These colors are said to block the oxygen and penetration of sunlight and pose a threat to aquatic life and biological processes.[4, 5] Worldwide, approximately 700,000 tons of colors are released into the environment annually. Immobilization of microorganisms is an advantageous technique for the textile dyes treatment; it has been shown that enhancement of the microbial performance helps to overcome the negative effects of recalcitrant substrates.[6, 7]

Among synthetic dyes, Azo dyes account for the majority of all dyestuff used in various industries and are known as important environmental contaminants because they are dissolvable in solution and cannot be removed through conventional biological treatments.[8-10] Since these color's synthetic origin is electron deficient xenobiotic aromatic compounds, containing Azo bonds (-N=N-), with high resistance against biological degradation, their removal is a crucial issue.[11-13] Such contaminants contribute to the major fraction of BOD, COD, TOC values and high toxicity.[14] Further, numerous reports have mentioned that these colors are degradable in anaerobic conditions, producing aromatic type of products which are toxic and have carcinogenic, mutagenic and allergenic effects just after being released into the environment.[15-17]

Many different effluent treatment approaches have been proposed to remove Azo dyes using physical and chemical methods such as absorption, coagulation, sedimentation, filtration, etc. Some limitations of these methods include non-environmentally friendly, high costs, and large amounts of sludge production which can lead to secondary pollution.[18] Biological methods, bacterial decolorization and degradation have proved to be efficient, simpler, more cost-effective; they lead to less sludge production and non-hazardous products if they are incorporated and applied with physico-chemical methods.[19-23] In biological treatment, various microorganisms catalyze the natural treatment processes in the ecosystem.[24]

Many of the textile companies are placed on high salinity land and deserts of Iran which leads to discharge of their wastewater to the highly saline environments. In some cases, the local salt, such as sodium and potassium chloride are used to improve the dye performance. In such environments, the salt tolerant or halophilic microbes are necessary for biological azo dye degradation.[25, 26] Next to being tolerant to high salt concentrations, halophilic bacteria have been shown to tolerate the oxyanions and heavy metals.[27, 28] The current study describes decolorization of azo-dyes from Arya press by the strain *Halomonas sp.* PTCC1714 isolated from Uremia Lake**.** Effects of initial dye concentration, pH and temperature are quantified.

Materials and Methods

Media and Growth Condition

To achieve an effective color removal of textile wastewater by bacteria, it is essential to have an optimum medium composition and culture conditions for bacterial growth as the removing color by bacteria which is strictly regulated by the cell metabolism. The bacterial strains used in the present study, Halomonas bacterium sp. PTCC1714 was isolated from Urmia Lake (north of Iran) and obtained from National Iranian Center of Genetic. The best salty growth condition (medium) was prepared including (g) NaCl 100, glucose 10, NH4Cl 2.3, K2HPO4 0.6, MgSO4. H2O 1.4, FeSO4.7H2O 0.01 in a total volume of one liter. For each experiment (totally 16 experiments), 20 cc of the salt medium was poured into the Erlenmeyer (100 ml) and then according to the conditions specified by means of software, the pH and color concentration were adjusted, and 5% pollution was injected to each Erlenmeyer. The Erlens were placed into the incubator (37◦C) containing a shaker to be heated with 120 rpm within 5 days. Finishing the experiment, the amount of color removed from the environment was assessed using a spectrophotometer.

Decolorization in Different Environmental Conditions

Among the factors contributing to the growth and decolorization of the bacteria, four were considered as the main factors to examine their effects on decolorization by *Halomonas sp.* PTCC1714: temperature, pH, medium salt and color concentration. With four different operating modes adjusting four levels (Table 1), 16 experiments were designed, and different combination of those is presented in Table 2.

The average results of 16 experiments and the effect of each factor on the range of Halomonas (PTCC 1714) decolorization are seen from the above Table. The results were analyzed by the software - Qualitek-4- and the effect of each level was calculated.

Color and Turbidity Presence in the Environment

Generally, dye degradation is introduced as the amount of absorbance decreases at the maximum wavelength absorbtion. The spectrum absorption of Aryapress Azo dye was detected in the range of 200 to 800 nanometer using spectrophotometer in order to identify the maximum absorbed wavelength of the color. In this regard, the maximum absorbed wavelength was found to be 490 nanometer for Aryapress dye.

2 ml sample was selected from each heated sample (Erlens) in order to measure the spectrum absorption related to each color. The samples were then subjected to a centrifugation with 120 rpm for 5 minutes. Therefore, the supernatant liquid was separated and light absorption evaluated at 490 and 499 wavelengths; also, the decolorization (%) was calculated according to the following formula:

$$D(\%) = \frac{(A - A^{\circ})}{A} \times 100$$

Table 1: The studied factors with four levels applied in experiments designs

Factors/levels	Level 1	Level 2	Level 3	Level 4
Cultivation temperature (◦C)	31	33	35	40
Salt value (w/v) %	5	10	15	20
Dye concentration (ppm)	100	500	1000	5000
Cultivation pH	5	6.5	7.5	9

Table 2: The selected parameters for designing sixteen orthogonal arrays according to Taguchi's method

Experiments	Temperature (°C)	pH	Dye Concentration (ppm)	Salt Concentration (%)	decolorization (%)
1	31	5	100	5	24.6
2	31	6.5	500	10	42.1
3	31	7.5	1000	15	15.45
4	40	9	5000	20	13.4
5	33	5	500	15	13.8
6	33	6.5	100	20	16.6
7	33	7.5	5000	5	18.5
8	33	9	1000	10	9.54
9	35	5	1000	20	44.5
10	35	6.5	5000	15	26.8
11	35	7.5	100	10	78
12	35	9	500	5	51.5
13	40	5	5000	10	85.5
14	40	6.5	1000	5	55.8
15	40	7.5	500	20	64.7
16	40	9	100	15	83.7

A=absorption to the environment after decolorization

A°=absorption to the indicator (first environment)

D=decolorization percentage

Design of Experiments (Taguchi Orthogonal Array)

In recent years, the Taguchi method, as a valuable optimization method, has been widely used to optimize and design experiments and provide the final optimum condition of an independent evaluation of the factors with a minimum number of experiments.[29] The main principle of the Taguchi method, an easy and robust type of fractional factorial method, is to study the sensitivity and effects of various variables on its other sets by modifying them simultaneously and can reduce research costs and variations error in experimental processes.[30-33] The Taguchi approach applies the systematic orthogonal arrays (OA) for designing the experiment and optimizes the control parameters to provide the best results.

The first step in Taguchi method in the current study is to determine the affecting factors as well as their levels, considering the fact that the decolorization of Aryapress Azo dye by bacteria needs to be proportional to the bacterial growth conditions; also, the textile plants effluent treatment conditions should be met.

In general, the design of the experiments is carried out in three ways:

1. One factor at a time (single agent method): One of the factors is changed, while other factors are constant; then, the best value (which shows the optimal response) is determined for each factor. This is done for all factors. In this case, if there are interactions between the factors, it is difficult to determine the optimal conditions and it will also take a lot of time.

2. Full Factor Method: In this method, all possible states between the variables and their levels are tested, which requires a lot of time, effort and energy. This method is usually not recommended.

3. Fractional method of a complete factorial. In this method, a number of possible combinations of variables are selected; the choice of this number is based on the experimental design so that the variance of the error is similar to the state that all possible combinations are executed. Following the tests and after evaluating the answers, the best answer is selected. In this method, the number of tests is greatly reduced. The Taguchi method is a designing test of a third-type method.[34]

Results

Statistical Analysis

Sixteen experiments were designed for decolorization of Aryapress dye by means of Halomonas bacteria according to Taguchi method. The outcome of Taguchi method is analyzed by ANOVA- a statistical technique- to study the performance characteristics of any given input parameters and can define the experimental data.

By Analysis of variance (ANOVA), the contribution of each factor and the optimum level is determined as a control key on the performance. The main purpose of the ANOVA is to divide the whole variation into its proper ingredients as well as assess the effect of any factor on the variance, considering the whole variance of all the factors. Determination of effective parameters in dye removal using analysis of variance is shown in Table 3. The last column determines the contribution of each factor (%) in dye removal.[35]

Taguchi experimental design technique uses a computer program, Qualitek-4, for automatic designing of the experiments, selecting the orthogonal arrays, allocating the parameters to the appropriate column, and portion of any factors in Taguchi approach.[36, 37] Subsequently, the effect of each level was calculated (Table 4)

As seen in Tables 1 and 4, factors including temperature in Level 4 (means 40 ° C), pH in level 3 (pH 7.5), color concentration at the first level (100 ppm) and salt percentage at level 2 (10% NaCl) are shown to be most effective in decolorization of Aryapress dye by means of *Halomonas* strains PTCC 1714.

The outcome was also studied by the interactions between the factors at different levels on the range of *Halomonas* (PTCC 1714); decolorization resulted in different percentages of Azo dye removal (Table 5). These interactions are measured based on the index between the two selective factors which could be different between 44.47- 15.11%. The lowest impact relates to temperature versus pH factor (15.11%) and the highest percentage concerns the interaction between factor pH and salt concentration (44.47%).

Comparing the intensity of interaction between the elements in this table revealed that the effect of a factor in optimizing the decolorization was affected by other factors.

It can be seen from this Table that the most effective parameter contributing to the decolorization is temperature, followed by salt concentration, color concentration and the pH, respectively. The ANOVA results illustrated that the contribution of temperature

Table 3: ANOVA results for decolorization rate of Aryapres biodegradation through *Halomonas* strain PTCC1714

Factor/ Column	D.F	MS	(V)	SS	PC (%)
Temperature	3	7574.315	2524.771	6834.12	65.04
pH	3	191.064	63.688	0	0
Dye Concentration	3	804.512	268.17	64.317	0.612
Salt Concentration	3	1197.467	399.155	457.272	4.351

SS: Sum of squares; D.F: Degrees of freedom; MS: Mean squares; PC: Percentage contribution; V: Variance

Table 4: The effect of different levels of the factors on the decolorization rate of Halomonas (PTCC 1714)

Factors/levels (%)	First level (%)	Second level (%)	Third level (%)	Forth level (%)
Temperature	19.712	11.605	49.25	65.299
pH	39.349	33.325	40.439	33.752
Color concentration	44.849	41.625	27.189	32.202
Salt concentration	31.752	51.427	31.987	30.699

Table 5: The estimation of the interaction between different measures in diverse parameters

The effect of pair factors (SI)	The intensity of interaction (%)	The optimum situation (the optimum levels)
Salt * pH	44.47	(1, 2)
Salt * Dye	41.71	(2, 4)
Salt * Temperature	22.97	(2, 4)
Dye * Temperature	18.55	(4, 4)
Dye * pH	17.44	(1, 4)
PH * Temperature	15.11	(1, 4)

Table 6: The optimum conditions for biodegradation of Aryapress color through *Halomonas* strain PTCC1714

Column/Factor	Factor	Level	Portion
Temperature	Temperature	4	28.833
pH	pH	3	3.973
Dye concentration	Dye concentration	1	8.383
Salt concentration	Salt concentration	2	14.96
Sum of all factors portion			56.149
The main mean of work			36.466
Predictable results in optimum condition			92.615

(65.04%) was by far more significant than the salt concentration which was 4.351%. It is obvious that the effect of other factors such as pH (0 %) and dye concentration (0.612) wass very low.

The contribution of each factor during the specified period is shown in Table 6. According to Tables 3 and 6, the temperature has the key role in dye removal from aqueous environment and the optimum conditions for strain Halomonas PTCC1714 is expected to be almost 93%.

The Effect of Temperature on Decolorization

Studies have shown that the Azo dye removal increased in the optimum temperature to some extent. In addition, temperature is effective on the growth rate, biomass operation and the reaction mechanism.[38, 39] Chung-HsinWu revealed that the decolorization rate of C.I. Reactive Red 2 (RR2) in ultrasound (US), US/ TiO_2, ultraviolet (UV)/TiO_2 and UV/US/TiO_2 systems increased as temperature enhanced.[40, 41] Kuo CY applied photo-Fenton and photo-Fenton-like systems for decolorizing C.I. Reactive Red 2 (RR2) and revealed that the fixed rate of dye removal enhanced as temperature and oxidant concentration rose.[42, 43] Similar studies have been reported by other researchers to find the effectiveness of temperature on decolorization.[44, 45]

Another study showed that the maximum rate of bleaching with the optimal cell cultivation was achieved between 35 to 40c, and decolorization decreased in the higher temperatures which can be due to the cell viability reduction or denaturation of the azorodoctaz enzyme.[46] Further studies have reported that decolorization rate by the strain of Citobacter sp. Ck3 increase by rising temperature from 27 to 37c, while it is stopped at 42c. These results show that temperature has a crucial effect on decolorization; the highest the temperature the more the decolorization. And this strain had the maximum dye removal effect and growth at the temperature of 42 0C.

The Effect of pH in Decolorization

One of the important factors for dyes removal process is pH. The proper pH for decolorization is mostly in the normal pH or a little alkaline pH, while decolorization rate is reduced quickly in the high alkaline or high acidic environment. In present study, the effect of pH was studied at pH values 5.0, 6.5, 7.5 and 9. According to many researches, it is revealed that bacteria growth will decrease in the low primary pH which leads to a low decolorization. However, the *Halomunas* growth will increase at pH 7 to 8 which results in high decolorization. Several papers have reported studies on the high effect of pH on the dye removal efficiency. Decolorization performance of three types of organic dyes was studied by Photo Assisted Chemical Oxidation process (PACO), and the effect of pH and some more factors were investigated. According to the results, the most decolorization percentage occurred at high initial pH of 9, while dye removal decreased by increase in solution pH from 9 to 12.[36]

The Effect of Dye Concentration on Decolorization

The efficiency of dye removal will be reduced by increasing the color of sewage. Mou and colleagues (1991) have reported the reverse effect of high color concentration on dye removal process as well as on its rate.[37] Wuhrmann and colleagues (1980) have observed that the speed of decolorization was so fast in the beginning, while it reduced suddenly by the rise of concentration. This effect is said to be the result of metabolites made during the color reduction time. The dye removal activity of the strain was studied from 100 to 500 ppm. Thus, it was revealed that decolorization increased by color concentration improvement, and the most activity was at 100 ppm. Azo dyes generally include one or more sulfonic acid groups on the aromatic rings of their own structure whose performance is as a detergent in limiting the microorganism's growth in high color concentration. On the other hand, there are some reports revealing that colors inhibit the synthesis of nucleic acids and cell growth. Since dye removal through cells is usually dependent on the growth of the cells, the decolorization is reduced, which is due to the decrease in cell growth in high concentrations of color.[38]

Effect of Salt Concentration on Dyeing

The bacterium is a salt-loving one from the Urmiah Lake, and has certain mechanisms, enzymes and carriers in its membrane to produce osmotic balance in its living environment. Therefore, it can transfer the color to the inner parts of its own body in order to do surface adsorption as well as by transferring the electrons and degradable materials through the member. As a result, this strain is more capable in decolorization compared with the non-salt-loving specious. Since bacteria PTCC 1714 have the optimum growth conditions at 10% salt concentration, the highest level of dye removal is in this concentration. There are some studies showing the effectiveness of high salt in decolorization.[39]

Discussion

Isolating and developing the new strain will be beneficial in textile waste-water treatment. Currently, biological methods are often used to remove dyes in waste-water because of their excellent decolonization ability and are cheaper and environment friendly.[40] Microbial de-colorization is an ecofriendly and cost competitive alternative to chemical decomposition process.[41] The chemical and physical methods are less effective, costly, of limited applicability and produce wastes, which are difficult to dispose of(8).[42] To efficiently apply and decolorize the dyes in wastewater, we must consider many factors To demonstrate this, we experimentally studied the effectiveness of different parameters for the removal of synthetic dyes in the current research. The analysis was carried out with an initial dye concentration of 100-500 ppm, temperature of 31, 33, 35 and 40 c, pH of 5, 6.5, 7.5 and 9, and salt concentration of 5, 10, 15 and 20 ppm. The results were then optimized using the Taguchi method by performing 16 experimental runs. Taguchi method is widely used for optimization of variable reactions by significantly reducing the time to the upper limit and the number of experiments as well as determining the most suitable conditions.[45] By using the Taguchi method and ANOVA (analysis and variance) for designing the test, specifying the optimal conditions for the growth of the *Halomonas* strain PTCC 1714 and obtaining the optimum level of output factors, it was determined that the bacterium should have the optimum ability (93%) in decolorization of the Ariapress which is considerable in comparison with other methods.[46]

According to the results, it was revealed that the Arya press dye decolorization rate increased as the temperature increased as this strain had the highest removal rate at 40 °C, dye concentration of 100 ppm, salt concentration of 20% and pH of 6.5. Considering the characteristics of this strain (including growth in difficult conditions, tolerance of the swage complex conditions, and low cost of cultivation), it can be said that the mentioned strain is considered as a proper solution for the textile wastewater treatment, as the main industry in Iran, which face high volumes of color pollutants every year. Thus, the goal of this study was to optimize the parameters which increase the efficiency of decolorization of Aryapress Azo dyes by using the bacterium Halomonas strain PTCC 1714. The software Qualitek-4 was also used for automatic designing of the experiments in the Taguchi method.

Conflict of Interest: None declared.

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