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The removal of Acid Red 274 from wastewater: Combined biosorption and biocoagulation with *Spirogyra rhizopus*

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Abstract

In this study, the removal of Acid Red 274 dye by *Spirogyra rhizopus*, a green algae, was studied in a batch mode as a function of the initial pH, temperature, initial dye and algae concentrations. The optimum removal conditions were determined as initial pH 3.0, temperature 30 °C and algae concentration 0.5 g L^{-1}. The dye removal amounts increased linearly with increasing initial AR 274 concentrations and the removal equation at optimum temperature and initial pH value was obtained to be $q_{30} = 0.9724 \times C_0$ ($R^2 = 0.9996$). The linear isotherm model was applied to experimental data and was correlated with a linear equation $q_{eq} = 18.194 \times C_{eq}$ ($R^2 = 0.9928$) at 30 °C and initial pH 3.0. The removal amounts decreased while the removed concentrations of AR 274 dye increased with increasing *S. rhizopus* concentration. High AR 274 removal amounts were obtained as a result of the combined biosorption and biocoagulation.

Keywords: *Spirogyra rhizopus*; Acid Red 274; Biosorption; Biocoagulation; Linear isotherm

1. Introduction

Dye-synthesizing wastewater and textile wastewater are two types of poorly treated wastewater characterized by persistent colour, highly fluctuating pH, high COD and bio-toxicity [1]. The production of textile industry, as well as the volume of wastewater containing processed textile dyes, steadily increases [2]. The dye based effluents induce persistent colour coupled with organic load and affect the total ecological symbiotic balance of the receiving water stream [3–5]. The real hazard setting aside aesthetic considerations is caused when coloured agents interfere with the transmission of light through water and hinder photosynthesis, resulting in ecological imbalance [6]. Hence, removal of dyes from such wastewaters is a major environmental problem and complete dye removal is necessary because dyes will be visible even at low concentration [7,8]. There are more than 10,000 dyes available commercially, most of which are difficult to decolourize due to their complex aromatic molecular structure and synthetic origin [6–9]. They are specifically designed to resist fading upon exposure to sweat, light, water and oxidizing agents, and are as such very stable and difficult to degrade [10,11].

Dyestuffs are divided into classes according to their chemical constitutions and technological applications [4,12]. There are many structural varieties, such as acidic, basic, disperse, azo, diazo, anthraquinone based and metal complex dyes. Brightly coloured, water soluble and reactive acid dyes which are composed of ionisable groups such as sulphonates ($-SO_3^-$), carboxylates ($-CO_2^-$) or sulphates ($-SO_4^-$) are the most problematic with their high molecular weight, as they tend to pass through

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conventional treatment systems unaffected [13]. Various treatment methods based on physical or chemical processes viz., physico-chemical flocculation combined with flotation, electroflotation, flocculation with Fe(II)/$Ca(OH)_2$, membrane filtration, electrokinetic coagulation, irradiation, ion-exchange, precipitation, ozonation and adsorption have been investigated for treating dye bearing effluents [14]. The coagulation/flocculation process is in extensive use for pre-, main or post-treatment. The limitations of coagulation/flocculation are that it is not always effective and there are problems associated with sludge disposal. Thus, interest in coagulant recovery stems not only from the potential savings in chemical costs but also from substantial reductions in sludge volume [13]. The adsorption of pollutants from aqueous solution plays an important role in wastewater treatment since it eliminates the need for huge sludge-handling processes. The most widely used industrial sorbent is activated carbon. However, it is an expensive material unless regeneration becomes relatively easy. An alternative inexpensive sorbent able to reduce the cost of a sorption system has always been searched. A low cost sorbent is defined as one which is abundant in nature, or is a by-product or waste material from another industry [15,16]. Various materials such as coal, fly ash, wood, silica gel, rice husk, cotton waste, bark, sugar industry mud, palm-fruit brunch, etc. have been investigated to remove dyes from aqueous solutions for colour removal [17–21]. A wide variety of microorganisms such as bacteria, fungi, algae either in their living or inactivated form were also used as alternative adsorbents to activated carbon. The term 'biosorption' refers to different modes involving a combination of active and passive transport mechanisms to remove unwanted materials by microbial biomass. Biosorption has distinct advantages over the conventional methods: the process does not produce chemical sludges, it could be highly selective, more efficient, easy to operate and hence cost effective for the treatment of large volumes of wastewaters containing low pollutant concentrations [22]. The use of inactivated biomass is also advantageous as the process is free from nutrient supply and moreover there are no toxicity constraints in the organism employed [23]. Algae have been found to be potentially suitable biosorbents because of their cheap availability both in fresh and saltwater, relatively high surface area and high binding affinity [23,24]. Biosorption in algae has mainly been attributed to the cell wall properties where both electrostatic attraction and complexation can play a role [25]. Algal cell surface is naturally formed by various chemical groups such as hydroxyl, carboxylate, amino and phosphate which are believed to be responsible for the sequestration of unwanted materials from effluents. The dye removal, especially by using algae, may be attributed to the accumulation of dye ions on the surface of biopolymers and further to the diffusion of the dye molecules from aqueous phase onto the solid phase of the biopolymer. Extracellular polymers consist of surface functional groups, which enhance sorption of the dye molecules onto the surface of the polymer (floc) during dye removal process. The secreted biopolymer will adsorb dye molecules remaining on the floc and settle [3,26]. Also, the released metabolic intermediates (long chain biopolymers) having excellent coagulation capacity and the dye remaining in the aqueous phase tend to adsorb onto the surface of the polymers and tend to settle (biocoagulation) [3]. The mechanism of colour removal by biological flocculation in the case of algal biomass may be reasoned from the interaction of high molecular weight extracellular polymer and these polymers bond electrostatically or physically and subsequently bridge the dispersion into a three dimensional matrix of sufficient magnitude to subside under quiescent conditions [3].

The purpose of this study is to investigate the removal of Acid Red 274 dye by *Spirogyra rhizopus*, a naturally cheap and abundant biomass.

2. Material and methods

2.1. Algal biomass

The alga was obtained from a natural water channel in Mersin, Turkey and identified as *S. rhizopus*. *Spirogyra* species are isogamous filamentous green algae and appear as an elongated thread or filament composed of cylindrical cells. *Spirogyra* belongs to chlorophyta class having plastid pigment with a starch grain characteristic. Flagella are absent in *Spirogyra* [3]. *S. rhizopus* after collecting from the channel was washed twice with tap water in order to remove adhering insect larvae, soil, etc. It was dried in sunlight and then in an oven at 105 °C for 24 h until all the moisture evaporated, put in distilled water and blended to obtain larger surface area. A stock solution of 10 g/L of adsorbent was prepared.

2.2. Dye solutions

The test solutions containing Acid Red 274 dye were prepared by diluting 1.0 g/L of stock solution of dye which was obtained by dissolving weighed amount of AR 274 in 1 L of distilled water. The range of concentrations of prepared dye solutions changed between 25 and 1000 mg/L. Higher dye concentration in solution was obtained by dissolving weighed amount of AR 274. The pH of each solution was adjusted to the required value with concentrated and diluted H_2SO_4 and NaOH solutions before mixing the biomass suspension.

2.3. Batch studies

Experiments were conducted in 250 mL Erlenmayer flasks containing 100 mL of dye synthetic solutions. The flasks were agitated on a shaker at 150 rpm. Samples (5 mL) were taken before mixing the algae solution and dye bearing solution and at pre-determined time intervals for the residual dye concentration in the solution. Samples were centrifuged at 3500 rpm for 5 min and the supernatant liquid was analysed.

2.4. Dye concentration analysis

The concentration of AR 274 dye remaining in the solution was measured colourimetrically using a spectrophotometer (JASCOV-530 UV/VIZ). The absorbance values were read at 527 nm.

3. Results and discussion

The removal of AR 274 dye by dried *S. rhizopus* was investigated as a function of initial pH, the initial dye concentration, temperature and algae concentration. Results were given in q (mg/g) and q_{eq} (mg/g), removed dye amount per unit mass of alga at any time t and equilibrium; C_{eq} (mg/L), remaining dye concentrations in solution at equilibrium.

3.1. The effect of initial pH

pH is one of the most important environmental factors influencing not only site dissociation, but also the solution chemistry of the dyes: hydrolysis, complexation by organic and/or inorganic ligands, redox reactions, precipitation are strongly influenced by pH and, on the other side, strongly influence the speciation and the adsorption availability of the dyes. Fig. 1 shows the effect of solution pH on the removal of AR 274 with *S. rhizopus* at 25 °C, 100 mg L^{-1} of initial metal ion concentration. The removal amounts (mg/g) decreased with an increase in solution pH from 3.0 to 6.0. At low pH values, the surface of algae would also be surrounded by hydronium ions which increase the acid dye interaction with positively charged binding sites of the *S. rhizopus* by greater attractive forces. Direct, acid and reactive dyes have anionic characters. Higher removal values obtained at lower pH values may be due to the electrostatic attractions between negatively charged dye anion and positively charged cell surface. Acid Red 274 has its vital substituents, sodium sulphonate groups which ionizes into two sodium cations and two coloured sulfonate anions in an aqueous solution. Therefore, the functional groups which are charged positively on the biosorbent surface

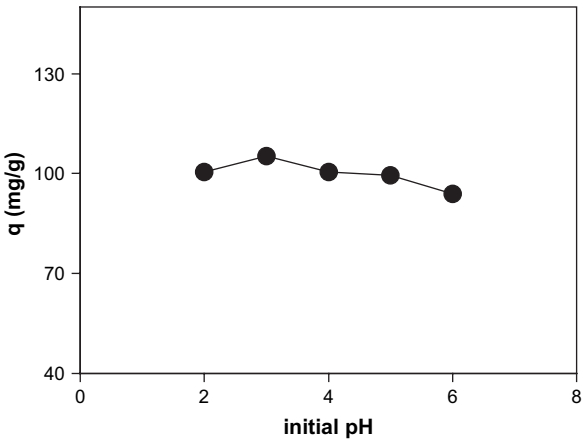

Fig. 1. The effect of solution pH on the removal of AR 274 with *S. rhizopus* (temperature 25 °C, 1 g/L algae concentration, 100 mg/L initial dye concentration, 100 rpm).

can attract dye $(acid)^{2-}$ and remove it from aqueous solution.

3.2. The effect of initial dye concentration

The initial concentration provides an important driving force to overcome all mass transfer resistance of all molecules between the aqueous and solid phases [26–29]. In this study, the effect of initial dye concentration on the AR 274 dye removal by *S. rhizopus* was investigated in the range of 25–3600 mg/L of the initial dye concentrations and the variation in the removed amounts with contact time is given in Fig. 2. An important part of AR 274 dye was removed by *S. rhizopus* in the range of contact time 3–5 min for all of the studied initial dye concentrations and then the removed dye amount was reached to equilibrium. At low initial dye concentrations, the removal process occurred very fast owing to the large difference in concentration between the biosorbent surface and solution. The removed dye amounts by *S. rhizopus* and the equilibrium dye concentrations remaining in solution for different initial dye concentrations are given in Fig. 3 at initial pH 3.0 and temperature 30 °C. As seen from Fig. 3, the dye removal amounts increase linearly with increasing initial AR 274 concentration while the equilibrium AR 274 dye concentrations were very low in the studied range of 25–3600 mg/L initial dye concentration. The equation of q versus C_0 was found as q (mg/g) = 0.9724 × C_0 (mg/L) (R^2 = 0.9996) at 30 °C and initial pH 3.0 for the range above initial dye concentrations. According to the equation, the higher removal amounts can be obtained at higher initial dye concentrations. Currently, since industrial wastewaters would not contain high values of dye concentration, experiments at initial dye concentrations higher than 3600 mg/L were not conducted. The same trend was

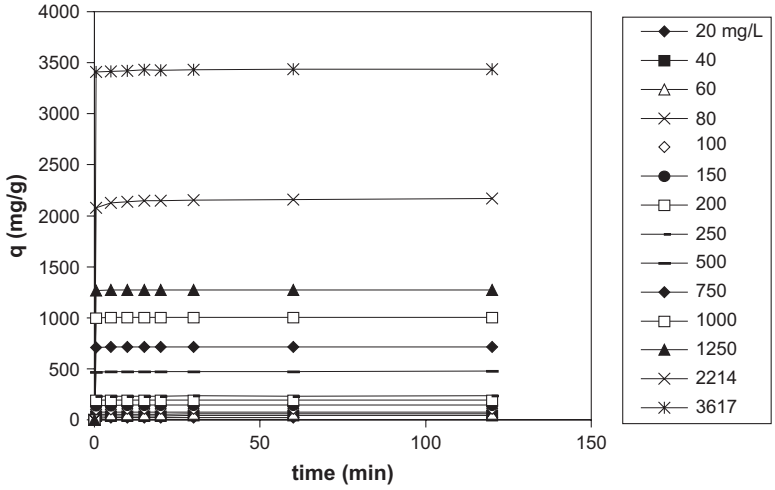

Fig. 2. The variation in the removed AR 274 amount with contact time for different initial dye concentrations (temperature 30 °C, 1 g/L algae concentration, initial pH 3.0, 100 rpm).

obtained for the adsorption of Acid Orange 7 by spent brewery grains [27]. Kannan and Sundaram [7] also reported that the amount adsorbed on various carbons increased exponentially with increasing initial concentration of methylene blue. Mohan et al. studied the removal of reactive yellow 22 dye by active *Spirogyra* sp. and they reported that the reactive yellow 22 dye–active *Spirogyra* sp. treatment mechanism can be explained by biosorption, bioconversion and biocoagulation [3]. High removal amounts obtained for AR 274–inactivated *S. rhizopus* system can be attributed to biosorption and biocoagulation in our present study.

3.3. Modelling of the AR 274 removal equilibrium

The most widely used isotherm equation for modelling equilibrium is the Langmuir equation, based on the assumption that there is a finite number of binding sites which are homogeneously distributed over the adsorbent surface, these binding sites have the same affinity for adsorption of a single molecular layer and there is no interaction between adsorbed molecules. The Langmuir isotherm is given by $q_{eq} = Q^{\circ}KC_{eq}/(1 + KC_{eq})$, where Q° is the maximum adsorbate loading capacity (mg/g), q_{eq} is adsorbate loading capacity at equilibrium (mg/g), C_{eq} is the concentration in fluid at equilibrium (mg/L), K is adsorption constant (L/mg). This isotherm is of the favorable type. When K is large and $KC_{eq} \gg 1$, the isotherm is strongly favorable, and when $KC_{eq} < 1$, the isotherm is nearly linear [30]. The Langmuir equation obeys Henry's Law at low equilibrium concentration; it implies $q_{eq} = a_L C_{eq}$, $a_L = Q^{\circ}K$, hence, it is analogous to Henry's Law [31]. For the AR 274–*S. rhizopus* treatment process, very low equilibrium concentrations were obtained resulting in higher removal amounts as seen from Fig. 3, in the studied range of 25–3600 mg/L initial dye concentrations. Therefore, the linear isotherm model was applied to experimental data as shown in Fig. 4 and the variation in the q_{eq} with C_{eq} was correlated with a linear equation of $q_{eq} = 18.194 \times C_{eq}$ ($R^2 = 0.9928$) at

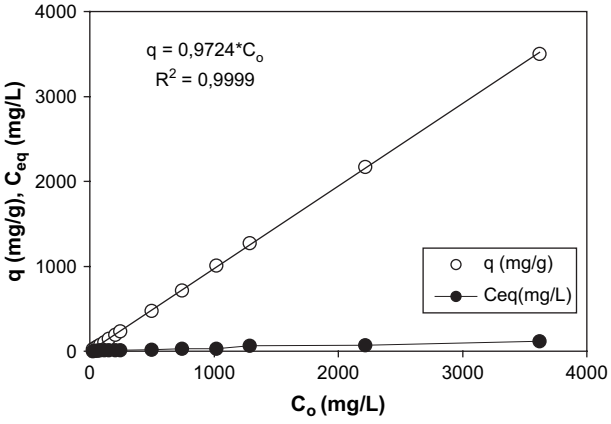

Fig. 3. The variation in the removed AR 274 amounts by *S. rhizopus* with initial dye concentrations (temperature 30 °C, 1 g/L algae concentration, initial pH = 3.0, 100 rpm).

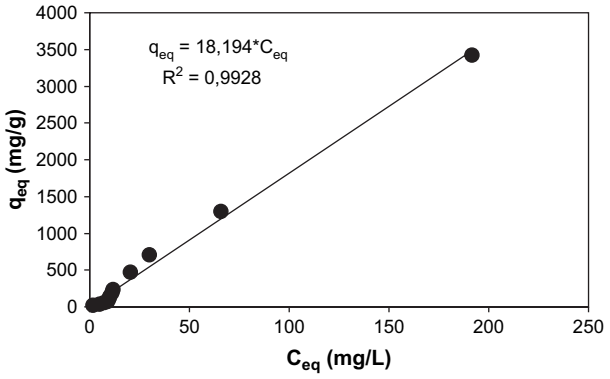

Fig. 4. Applied linear isotherm model for AR 274 removal using *S. rhizopus* (temperature 30 °C, 1 g/L algae concentration, initial pH = 3.0, 100 rpm).

30 °C and initial pH 3.0. Hence, it was concluded that the removal of AR 274 dye by using *S. rhizopus* fitted very well to linear isotherm model with $a_L = Q^\circ K$ value of 18.194 at 30 °C and initial pH 3.0.

3.4. The effect of temperature

The effect of temperature on the AR 274 removal by *S. rhizopus* was investigated as a function of the contact times for the studied temperature range (20–50 °C). The data showed that a great amount of AR 274 was removed in the first 3–5 min and this amount did not change with further increase in contact time (not shown data). The removed amounts for AR 274 by *S. rhizopus* are given in Fig. 5 as a function of the initial dye concentration at 20, 30 and 40 °C. As seen from Fig. 5, the dye removal amounts increase linearly with initial AR 274 concentration for different temperatures. The experimental removal equations at 20, 30 and 40 °C for the range of 25–3600 mg/L initial dye concentrations were found as:

$$q_{20} = 0.9337 \times C_0 \left(R^2 = 0.9988\right) \quad (1)$$

$$q_{30} = 0.9724 \times C_0 \left(R^2 = 0.9996\right) \quad (2)$$

$$q_{40} = 0.9664 \times C_0 \left(R^2 = 0.9995\right) \quad (3)$$

High correlation coefficients indicated that the variation in the removed amounts of AR 274 with initial dye concentrations can be well defined by the linear equation form. It is very clear that the removed amount at 30 °C is higher than those of other temperature of the expected removal amount to be obtained at same initial dye concentration. Fig. 6 depicted the effect of temperature on the removal amounts. As seen from Fig. 6, the optimum removal temperature was determined as 30 °C and the removal amount decreased with further increase and decrease in temperature. An increase in the removal amounts in the interval of 20–30 °C deals with an increase in the uptake capacity of *S. rhizopus*. Increasing the temperature may alter the surface activity of *S. rhizopus* resulting in a decrease in the removal amounts, indicating that the removal process is exothermic in nature.

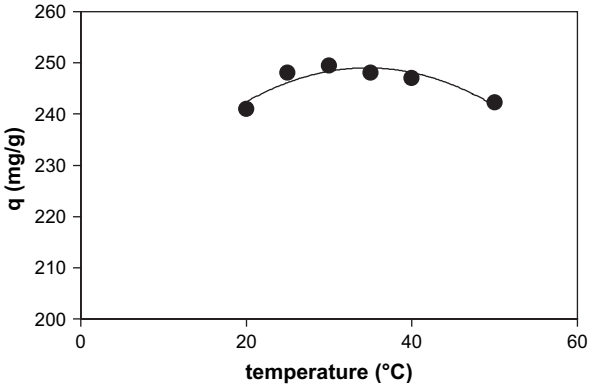

Fig. 6. The effect of temperature on the removed AR 274 amounts (1 g/L algae concentration, 250 mg/L initial dye concentration, initial pH = 3.0, 100 rpm).

3.5. The effect of algae concentration

The effect of the algae concentrations on the removal of AR 274 was investigated at five different adsorbent concentrations in the range of 0.5–3.0 g/L. The variation in the removal amounts with algae concentrations is given in Fig. 7. By increasing the algae concentration, the removed dye concentrations increased while the removal dye amounts per unit mass decreased. It is readily understood that the number of available adsorption sites increases with an increase in

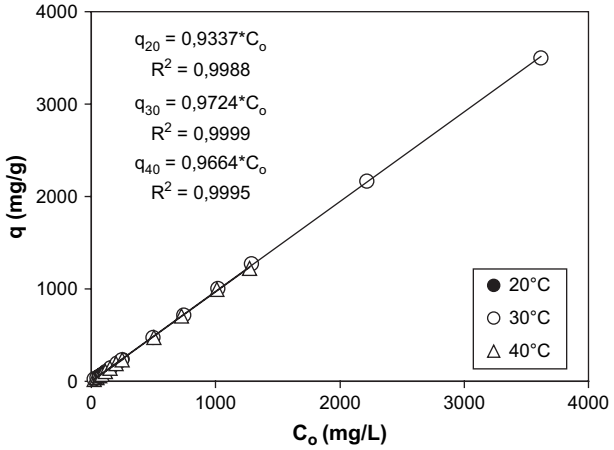

Fig. 5. The removal amounts of AR 274 as a function of initial dye concentrations at different temperatures (1 g/L algae concentration, initial pH = 3.0, 100 rpm).

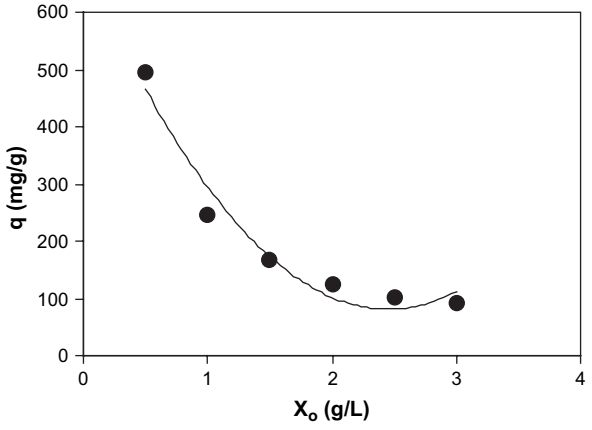

Fig. 7. The variation in the removed AR 274 amount with algae concentrations (temperature 30 °C, 250 mg/L initial dye concentration, initial pH = 3.0, 100 rpm).

adsorbent concentration and it, therefore results in increase in the removed dye concentration. Furthermore, the increase in the removed concentration may be attributed to the fact that biocoagulation is also effective on the removal mechanism of AR 274 dye by *S. rhizopus*. The decrease in the removal capacity with increasing algae concentration is mainly due to the unsaturation of adsorption sites through the adsorption reaction. Another reason may be due to the particle interaction, such as aggregation, resulted from high algae concentration. Such aggregation would lead to a decrease in total surface area of the sorbent and an increase in diffusional path length [19].

4. Conclusion

The present study revealed the potential ability of the algal *S. rhizopus* to decolourize wastewaters. Almost complete removal of AR 274 dye from synthetic wastewater with concentration lower than 25 mg/L of initial AR 274 dye concentration can be achieved by using *S. rhizopus* resulting from biocoagulation and biosorption. The removal equilibrium was modelled by using the linear isotherm model and the equation was obtained as $q_{eq} = 18.194 \times C_{eq}$ ($R^2 = 0.9928$) since the dye equilibrium concentration remaining in solution was very low even for high initial dye concentrations. The R^2 value indicated that the linear type of isotherm fitted well the equilibrium experimental data. According to equation $q_{30} = 0.9724 \times C_0$ ($R^2 = 0.9996$), the removed AR 274 amounts by *S. rhizopus* relating to initial dye concentrations were relatively high when compared with other biomass reported in literature. The monolayer coverage of *Tremella fuciformis* used for Sulfur Black 1 removal was 934 mg/g [14] while 728.2 and 693.2 mg/g were obtained for the monolayer coverage of chitosan used for the removal of AR 73 and AR 18, respectively [6]. The monolayer coverage of chemically cross-linked chitosan beads used for the removal of AR 14 was reported as 1940 mg/g [8]. As a result, *S. rhizopus* is shown to be highly effective as a coagulant as well as an adsorbent in the removal of AR 274 dye. The use of a green algal species growing in natural freshwater, sometimes taken as a biological pollution indicator, for the purification of wastewaters involving acid dyes, thought as a kind of chemical pollution is very important for process economy.

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