Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

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Online publication date: 12 February 2010

To cite this Article Özer, Ayla and Turabik, Meral(2010) 'Competitive Biosorption of Acid Dyes from Binary Solutions onto *Enteromorpha prolifera*: Application of the First Order Derivative Spectrophotometric Analysis Method', Separation Science and Technology, 45: 3, 380 — 393
To link to this Article: DOI: 10.1080/01496390903418071
URL: http://dx.doi.org/10.1080/01496390903418071

Separation Science and Technology, 45: 380–393, 2010

ISSN: 0149-6395 print/1520-5754 online
DOI: 10.1080/01496390903418071

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Competitive Biosorption of Acid Dyes from Binary Solutions onto *Enteromorpha prolifera*: Application of the First Order Derivative Spectrophotometric Analysis Method

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The biosorption of Acid Red 274 and Acid Red 337 dyes from single and binary solutions on *Enteromorpha prolifera* was investigated in a single stage batch system. The first order derivative spectrophotometry was tested in order to analyse the studied binary solutions of the selected dyes. The single- and multi-component Langmuir and Freundlich isotherm models were applied to the experimental equilibrium data and the isotherm parameters were estimated. It was observed that the uptake amounts of the first component decreased with increasing concentration of the second component from binary solution. As a result, the binary biosorption of AR274 and AR337 dyes on *E.prolifera* have an antagonistic effect. The binary biosorption of AR274 and AR337 dyes in a single stage batch system was modelled and the equilibrium concentrations of the exit stream were found by using the experimental equilibrium curves and operating lines for the biosorption of single and binary dye solutions.

Keywords acid dyes; biosorption; derivative spectrophotometry; *Enteromorpha prolifera*

INTRODUCTION

Dyes are synthetic aromatic organic colorants, having potential application in textile, food, cosmetics, paper, and carpet industries (1,2). Wastewaters from these industries form visible pollution due to the presence of color in the final effluent and reduce photosynthetic activity by blocking the passage of light through the water (3,4). Disposal of this colored water into receiving waters can cause toxic effects on aquatic life. It may be mutagenic and carcinogenic and can cause severe damage to human beings (5,6). The main treatment techniques used to remove dyes from aqueous streams include coagulation and flocculation, reverse osmosis, electroflotation, membrane filtration, irradiation and ozonation, and adsorption (7). Adsorption is an effective and reliable physicochemical method for the removal of colors, odor, organic, and inorganic pollutants from the industrial wastewater (4). Numerous studies have been reported on decolorization of various dyes using activated carbon. Although activated carbon is the most widely used adsorbent in adsorption process, its high cost in production and regeneration make it uneconomical. A wide variety of microorganisms such as bacteria, fungi, algae either in their living or inactivated form are 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 (8). The major advantages of biosorption technology are its effectiveness in reducing the concentration of dyes to very low levels and the use of inexpensive biosorbent materials (9,10).

Although, the majority of the industrial effluents contain more than one pollutant species, few research papers have been published in literature about multi-component adsorption systems (3,11). The evaluation of competition between solutes in occupying the limited binding sites in multi-component systems and prediction of multi-component adsorption equilibrium are still complex problems in adsorption field (11). Another problem in multi-component adsorption system is the simultaneous determination of each dye concentration remaining in the solution. Generally, spectrophotometric methods are used for the analysis of components in multi-component systems, because these methods are more economic and simple, compared to methods such as chromatography and electrophoresis (12). But, the simultaneous analysis of components in multi-component solution by spectrophotometric methods can also be very complex due to the overlapping absorption bands of the components and spectral interferences (13,14). So, it is very important and necessary to develop reliable, fast, and sensitive methods for the determination of dyes in multi-component systems. Derivative spectrophotometry provides better selectivity and offers a solution in resolving the overlapping spectra

Received 27 March 2009; accepted 30 September 2009.
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in multi-component analysis without previous chemical separation (13–15). This method has been successfully applied in pharmaceutical and environmental analysis for the determination of drugs in multi-component systems (12,15–17), but has not yet been extensively applied as a method for the simultaneous analysis of dyes in biosorption from binary solutions.

Recent investigations have shown that selected species of seaweeds possess impressive biosorption capacities for the biosorption of heavy metal ions and dye anions but the simultaneous biosorption of dyes from binary dye solution by dried *Enteromorpha prolifera*, a green seaweed, has not been reported in the literature. In the present research, two acid dyes have been selected, namely, Acid Red 274 (AR274) and Acid Red 337 (AR337), due to their widespread use in textile industry and high stability in water. Acid dyes are organic sulphonic acids; their commercially available forms are usually sodium salts, which exhibit good water solubility. In sequence of their importance, acid dyes are mostly used with certain fiber types such as polyamide, wool, silk, modified acrylic, and polypropylene fibers, as well as blends of the aforementioned fibers with other fibers such as cotton, rayon, polyester, regular acrylic, etc. (18). In this study, the biosorption of AR274 and AR337 dyes from single and binary solutions on *Enteromorpha prolifera* was investigated in a single stage batch system. The zero order spectrophotometric method was used for the analysis of the AR274 and AR337 dyes from their single solutions while the first order derivative spectrophotometric method was applied for the biosorption from binary dyes solution. The single- and multi-component isotherm models were applied to the experimental data to determine the biosorbent capacity.

EXPERIMENTAL

Biosorbent

Enteromorpha prolifera, a kind of green algae, was obtained from the Mediterranean coast in Mersin, Turkey. The green algae genus *Enteromorpha* has great potential for commercial exploitation because of its abundant and varied chemical composition, as well as quality and concentration of basic nutrients for other living organisms (19). Aguilera-Morales et al. (2005) reported that the main constituents in *Enteromorpha spp* were minerals, protein, and ether extract and hemicellulose was dominant in the cellular wall of algae (19). *Enteromorpha spp* contains 9–14% protein, 2–3.6% ether extract, 32–36% ash, polyunsaturated fatty acids n-3 and n-4:10.4 and 10.9 g/100 g of total fatty acids, respectively (20). Christ et al. (1990) reported that the cell wall of *E.prolifera* was rich in sulphated polysaccarides which are strong ion-exchangers (21).

For the biosorption studies, the harvested fresh cells were rinsed with tap water, washed several times with distilled water, and then dried at 105°C for 24 h. After that, a given amount (10 g) of dried *E.prolifera* was suspended in 1 L double-distilled water by homogenizing in a commercial blender at room temperature.

Preparation of Acidic Dye Solutions

Acid Red 274 (AR274) and Acid Red 337 (AR337) dyestuffs were supplied by DyStar firm with commercial names Supranol Red 3BW and Telon Red FRL, respectively. The chemical structure of these dyes can not be presented here, since this data is protected by manufacturers. The stock solutions of AR274 and AR337 dyes were prepared in 1.0 g/L concentration. The experimental solutions were prepared by diluting the stock solution with distilled water. Before the biosorbent solution and dye solution are mixed, the pH of both solutions were adjusted to the desired initial pH with concentrated and diluted H_2SO_4 or NaOH solutions.

Batch Biosorption Studies

The 10 mL of biosorbent stock algae solution was mixed with 90 mL of solution containing a known concentration of AR274 and AR337 dyes in single species or in binary solution in an Erlenmeyer flask. The flasks were agitated on a shaker at constant temperature for 2 hours ample time for biosorption equilibrium. To determine the initial and unadsorbed dye concentrations, samples (4 mL) of biosorption medium were taken before mixing the biosorbent suspension and dye bearing solutions. They were centrifuged at 3500 rpm for 5 min. The dye remaining in the supernatant was analysed.

The biosorbed dye amount at equilibrium, $q_{eq,i}$(mg dye/g biosorbent) can be calculated by using the Eq. (1):

$$q_{eq,i} = \frac{(C_{0,i} - C_{eq,i})V}{X_0} \quad (1)$$

where V is the solution volume containing dye (L) and X_0 is the mass of the biosorbent (g).

Dye Analysis

Shimadzu UV-160A spectrophotometer was used for analysis of AR274 and AR337 dyes. The zero order and first order derivative spectrophotometry (FODS) were used for the analysis of unadsorbed dye in biosorption from single and binary species, respectively. The first order derivative spectra of the studied dyes were recorded in 1 cm quartz cells at a scan speed of 1000 nm/min and a fixed slit width of 2 nm.

RESULTS AND DISCUSSION

The Simultaneous Analysis of AR274 and AR337 in Binary Solution

In this part of the study, the first order derivative spectrophotometry (FODS) was applied to the simultaneous

analysis of AR274 and AR337 dyes in the binary solution and the results were presented. The zero order and the first order derivative absorption spectra for single species and binary species of AR274 and AR337 dyes were separately recorded between 350 and 700 nm and presented in Figs. 1a–b, respectively. As can be seen in Fig. 1a, the zero order absorption spectrum of AR274 and AR337 dyes in binary solution overlapped while the maximum absorbances of AR274 and AR337 dyes in their single solutions were obtained at 527 nm and 492 nm, respectively. The overlapping of the AR274 and AR337 dyes spectra shows interference between the zero order spectra of AR274 and AR337 dyes, therefore their concentrations could not be determined by direct absorbance measurement in their mixture. Hence, it was not possible to estimate the amount of AR274 or AR337 dyes in binary solution. Therefore, derivative spectrophotometry (DS) was studied to assist in solving this problem and the first-order derivative spectrophotometry (FODS) was determined as suitable analysis methods for the analysis of the studied dyes in binary solution.

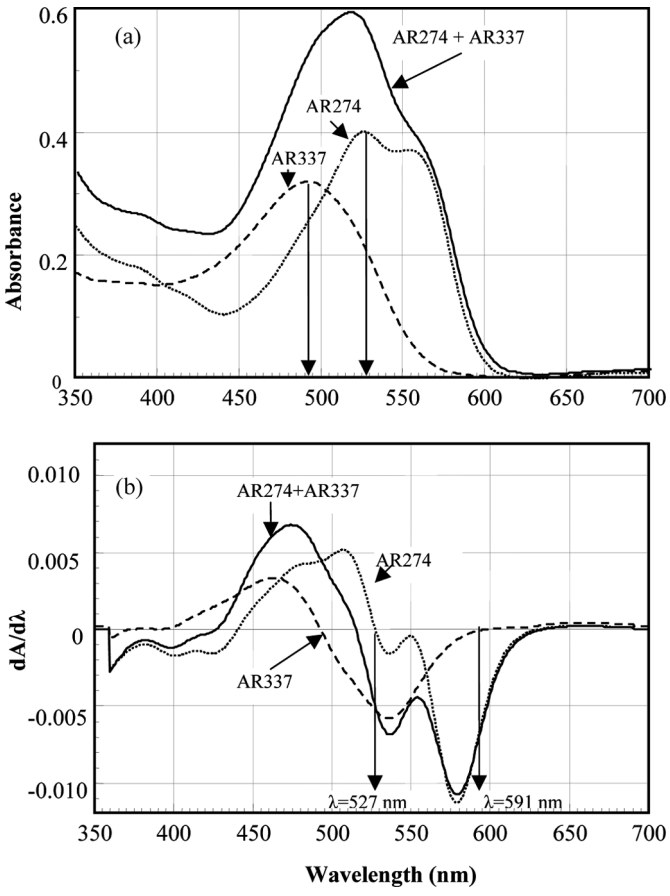

FIG. 1. Spectra of AR274 and AR337 dyes in single species and binary solution (a) Zero order spectra (b) First order derivative spectra (initial AR274 and AR337 concentration: 20 mg/L).

When the first-order derivative of zero order spectrum of dye is taken, the maxima and minima of the original function take zero values, and the inflections are converted into maxima or minima, respectively (14). The first-order derivative spectra of AR274 and AR337 dyes in single species and binary solution were given in Fig. 1b. Figure 1b showed that AR274 dye can be determined at 591 nm ($^1D_{591}$) in the presence of AR337, where the absorbance of AR337 is zero, and AR337 dye can be determined at 527 nm ($^1D_{527}$) in the presence of AR274, where the absorbance of AR274 dye is zero. Here, D is the absorbance value of the studied dye at the first order derivative wavelenght.

AR274 and AR337 dye concentrations in binary solution were determined by measuring the absorbance signal and using a calibration graph at the first order derivative wavelength. The calibration graphs for AR274 and AR337 dyes were prepared by reading absorbances of the solutions at different initial dye concentrations prepared in the volumetric flasks by using micro pipette at 591 nm ($^1D_{591}$) and 527 nm ($^1D_{527}$), respectively. The calibration equations and regression coefficients for AR274 and AR337 dyes in binary solution were obtained as the following;

$$^1D_{591}(\text{absorbance}) = 0.0004\ C_{AR274} + 0.00004 \quad \text{and} \quad R^2 = 0.9999$$

$$^1D_{527}(\text{absorbance}) = 0.0002\ C_{AR337} + 0.00090 \quad \text{and} \quad R^2 = 0.9943$$

respectively. It can be said that the unknown concentrations of AR274 and AR337 dyes in binary solution are exactly determined by the FODS according to regression coefficients of above equations.

In order to check the theoretical concentration of the studied dyes in binary solution, the recovery of each dye in aqueous solution was conducted and the recovered concentration was determined by the FODS. In the recovery studies, the AR274 and AR337 dyes in binary solution were prepared in three different ways. In the first way while the initial AR274 concentration was held at 20 mg/L, the initial AR337 concentrations were changed in the range of 10–50 mg/L; in the second way while the initial AR337 concentration was held at 20 mg/L, the initial AR274 concentration was changed in the range of 10–50 mg/L; and in the third way the initial AR274 and AR337 dye concentrations were changed in the range of 10–50 mg/L. The first order derivative spectra of three set binary solutions were recorded and the results of scanning obtained from the third set were presented in Fig. 2a–c. The AR274 and AR337 contents in binary solutions were determined from the first order derivative spectra by measuring the absorbance, which was set at the suitable wavelenght for the studied dye. The concentration of each dye was

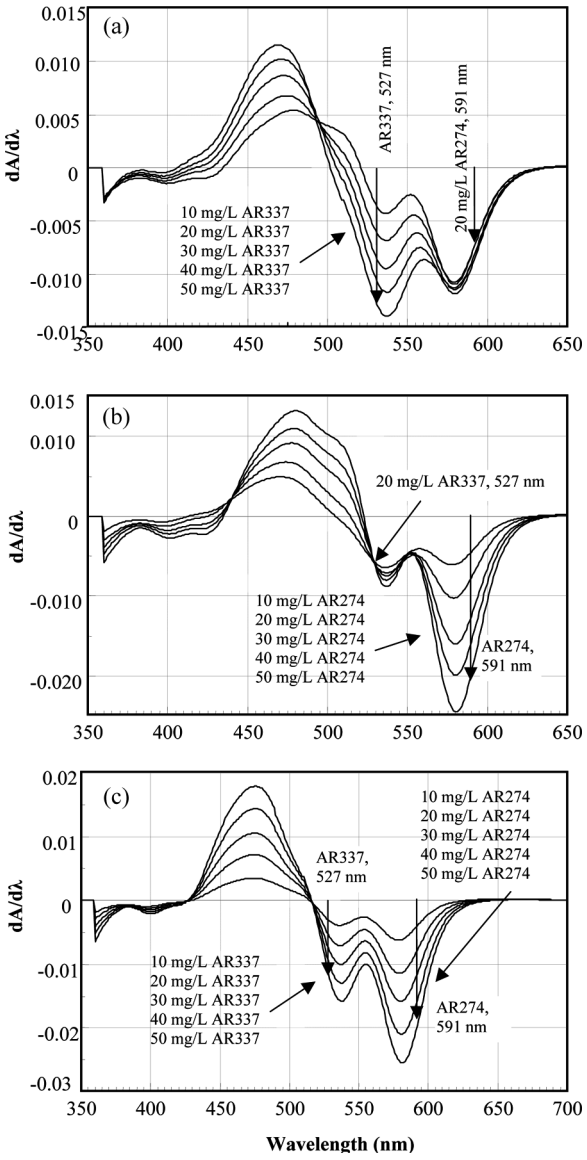

FIG. 2. First order derivative spectra of AR274 and AR337 in binary solutions (a) AR274 concentration: 20 mg/L, constant; AR337 concentration: in the range of 10–50 mg/L (b) AR337 concentration: 20 mg/L, constant; AR274 concentration: in the range of 10–50 mg/L (c) Both initial AR337 and AR274 concentrations were changed in the range of 10–50 mg/L.

calculated from the calibration graphs and it was named as "*found*". The results obtained from the resolution of synthetic binary solutions were summarized in Table 1. The recoveries were calculated by dividing the found dye concentration to theoretical dye concentration. As can be seen from Table 1, the recoveries for the determination of the AR274 and AR337 concentrations in binary solutions by the first order derivative spectrophotometric method were in the range of 95–108%. The statistical *t*-tests were also applied to theoretical and "*found*" concentrations and the differences between theoretical and found concentrations were determined to be insignificant ($P < 0.630$). According to recovery studies and *t*-test results, it can be said that AR274 and AR337 dyes in binary solution can be determined accurately by using the first order derivative spectrophotometry (FODS). As a result, derivative spectrophotometry can also be proposed for the analysis of binary dye solutions in which their spectra overlapped.

The Effect of the Initial pH on the Biosorption of Acid Dyes

Initial pH is one of the most important environmental factors influencing not only site dissociation, but also the solution chemistry of 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. The effect of initial pH on sorption capacity of dried algae for each dye was studied in the pH range of 2.0–6.0 at 50 mg/L initial dye concentration and the results were presented in Fig. 3. The maximum uptake value (mg/g) was obtained at initial pH value of 2.0 due to the existence of a significantly high electrostatic attraction between the positively charged surface of the algae cells and anionic dyes, AR274 and AR337. Acidic dyes are called anionic dyes because of the negative electrical structure of the chromophore groups and composed of ionizable groups such as sulphonates, carboxylates, or sulphates to favor their solubilization in water (22,23). The dyes can interact with active groups such as polysaccharides, proteins, and lipids on the cell surface in a different manner (24).

The interaction between the sorbate and the sorbent is affected by the pH of an aqueous medium in two ways: first, since dyes are complex aromatic organic compounds having different functional groups and unsaturated bonds, they have different ionization potentials at different pH, resulting in the pH-dependent net charge on dye molecules. Second, the surface of the sorbent include many functional groups, so the net charge on the sorbent, which could be measured in the form of zeta potential or isoelectric point, is also pH-dependent (25). Çetinkaya et al. (1999) reported that the isoelectric point of the algal biomass would be at a pH of 3.0 (26). However, at pH values below the isoelectric point, the biomass will have a net positive charge due to protonation of functional groups. It is expected that positively charged functional groups on the sorbent surface will favor the adsorption of negatively charged dye anions due to electrostatic attraction which could be the primary mechanism (25,27,28). As the initial pH increases, the number of negatively charged sites on the biosorbent surface increases and the number of positively charged sites decreases. A negative surface charge does not favor the adsorption of dye anions due to the electrostatic repulsion (29). It was concluded that the effect of initial pH on the

TABLE 1
Determination of the recoveries for the AR274 and AR337 dyes from a binary solution by the first order derivative spectra

Theoretical C_{AR274} (mg/L)	Theoretical C_{AR337} (mg/L)	Found C_{AR274} (mg/L)	Found C_{AR337} (mg/L)	Recovery AR274%	Recovery AR337%
20	10	19.45	9.55	97.25	95.50
20	15	20.20	14.57	101.00	97.13
20	20	20.95	20.60	104.75	103.00
20	25	20.45	25.13	102.25	100.52
20	30	20.20	30.65	101.00	102.17
20	35	20.95	35.18	104.75	100.51
20	40	21.70	40.70	108.50	101.75
20	45	21.15	45.73	105.75	101.62
20	50	21.05	50.75	105.25	101.50
10	20	10.68	21.10	106.80	105.50
15	20	15.19	20.60	101.27	103.00
20	20	19.45	21.10	97.25	105.50
25	20	24.46	19.60	97.84	98.00
30	20	29.97	19.10	99.90	95.50
35	20	34.74	19.05	99.25	95.25
40	20	39.75	19.10	99.37	95.50
45	20	44.26	19.09	98.35	95.45
50	20	49.02	19.10	98.04	95.50
10	10	10.42	10.05	104.20	100.50
15	15	15.19	15.07	101.27	100.47
20	20	20.70	20.60	103.50	103.00
25	25	25.71	24.62	102.84	98.48
30	30	29.97	30.15	99.90	100.50
35	35	35.98	34.67	102.80	99.06
40	40	41.25	39.69	103.13	99.26
45	45	45.51	44.22	101.13	98.27
50	50	51.27	50.25	102.54	100.50

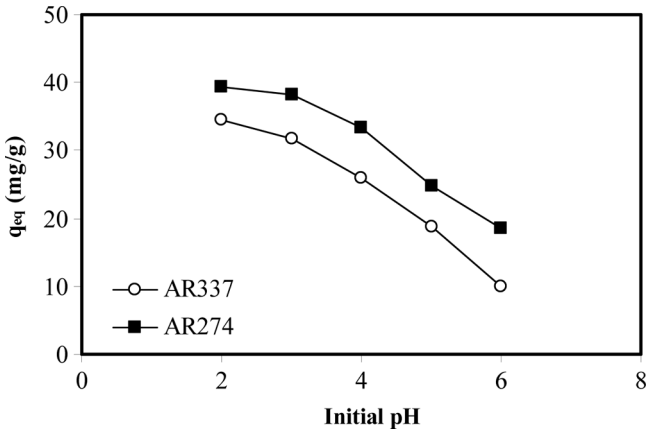

FIG. 3. The effect of initial pH for AR274 and AR337 biosorption on *E.prolifera* in binary mixture (initial temperature 30°C, 50 mg/L initial dye concentration).

biosorption of acidic dyes from the binary solution was due to the significantly high electrostatic attraction between the positively charged surface of biosorbent and anionic dyes.

The Effect of the Temperature on the Biosorption of Acid Dyes

Investigation of temperature effect on the biosorption of acidic dyes from the binary solution is very important in the real application of biosorption since various textile and other dye effluents are produced at relatively high temperatures (22). As shown in Fig. 4, the biosorption of AR274 and AR337 dyes in binary mixture increased with increasing temperature up to 30°C due to higher affinity of sites for these dyes and an increase of binding sites on the biosorbent, and decreased with further increase in temperature. The further increase of temperature may alter the surface activity of *E.prolifera* resulting the decrease of the equilibrium uptake capacity (q_{eq}) in the temperature interval of 30–50°C means that the biosorption processes of these dyes

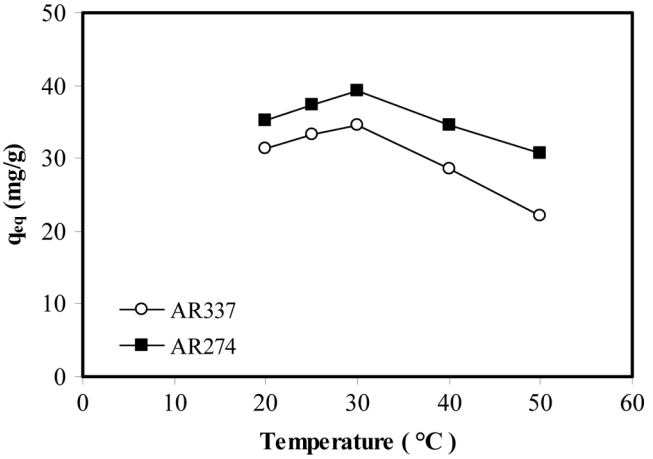

FIG. 4. The effect of temperature for AR274 and AR337 biosorption on *E.prolifera* in binary mixture (initial pH 3.0, 50 mg/L initial dye concentration).

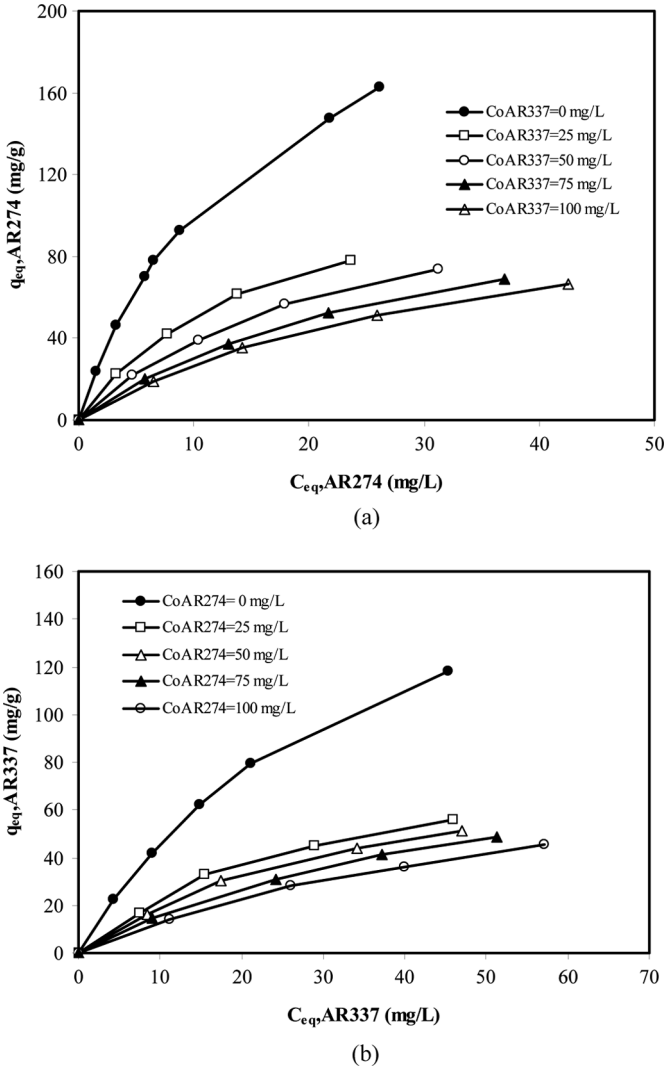

FIG. 5. (a) Non-linearized adsorption isotherms of AR274 in the absence and presence of increasing AR337 dye concentrations (b) Non-linearized adsorption isotherms of AR337 in the absence and presence of increasing AR274 dye concentrations (Temperature 30°C, initial pH 2.0).

by *E.prolifera* are exothermic in nature and may be concluded to be a physisorption. If adsorption is governed only by physical phenomena, an increase in temperature will be followed by a decrease in adsorption equilibrium (30).

The Effect of the Initial Dye Concentration for the Biosorption of Acid Dyes

The combined effects of two or more components on microorganism biosorption depend on the number of pollutant competition for binding sites, pollutant combination as well as levels of pollutant concentration, order of pollutant addition, and residence time (31). To investigate the effect of the initial dye concentration on the biosorption of AR274 dye from binary solution on *E.prolifera*, the initial AR274 dye concentration was changed from 25 to 100 mg/L while the initial AR337 dye concentration was held constant at 25, 50, 75, and 100 mg/L for each run at initial pH 2.0 and 30°C temperature. Then, while the initial AR337 concentration in the binary solution was changed from 25 to 100 mg/L, the initial AR274 concentration was held constant at 25, 50, 75, and 100 mg/L for each experiment set. The non-linearized adsorption isotherms of AR274 in the absence and presence of increasing AR337 and, those of AR274 in the absence and presence of increasing AR337 dye concentrations were given in Fig. 5a and Fig. 5b, respectively. The individual and total biosorbed dye amounts at equilibrium [q_{eq}; mg/g; $q_{eq,total} = (q_{eq,AR274} + q_{eq,AR337})$ mg/g)], the individual biosorption yields [Biosorption $\text{yield}_i\ \% = 100(C_{o,i} - C_{eq,i})/C_{o,i}$] and total biosorption yields [Biosorption $\text{yield}_{total}\ \% = 100\Sigma(C_{o,i} - C_{eq,i})/\Sigma C_{o,i}$] were also given in Table 2 for the biosorption of AR274 and AR337 dyes from the single and binary species.

As can be seen from Table 2, for the single species biosorption of AR274 dye, the biosorption capacity of biosorbent increased from 23.7 to 92.4 mg/g while the individual biosorption yield decreased from 94.05 to 91.30%, respectively, with increasing the initial AR274 concentration from 25 to 100 mg/L. For the biosorption of AR274 in the presence of 100 mg/L initial AR337 concentration, the biosorption capacity of biosorbent increased from 18.9 to 66.5 mg/g as the biosorption yield of *E.prolifera* decreased from 74.15 to 61.00%, respectively, with increasing initial AR274 concentration from 25 to 100 mg/L. When the initial dye concentration of solutions was low, all dye molecules present in the solution could interact with the binding sites and thus the biosorption yields (%) were higher than those at high initial dye concentration in solution. Because of the saturation of biosorption sites, the lower biosorption yields were observed at high initial dye concentration in solution.

The similar trends were also observed for the biosorption of other AR274 and AR337 combinations on *E.prolifera* (Table 2). Table 2 also presents the effect of AR274 on the equilibrium uptakes and biosorption yields for AR337 dye from the binary solution on *E.prolifera*. From Table 2, the biosorbed AR337 dye amounts in the absence and presence of AR274(100 mg/L of initial AR274 dye concentration) at equilibrium were obtained as 79.4 mg/g and 45.6 mg/g, respectively. The total biosorption yield for AR337 biosorption from binary solution was obtained to be 52.93% while the individual biosorption yields for AR337 biosorption in the absence and in the presence of AR274 dye were found as 79.00% and 44.36%, respectively. It was observed that the AR274 and AR337 dyes uptake capacities of *E.prolifera* reduced in the presence of the other dye. When the initial AR337 dye concentration was increased step by step and also AR274 dye concentration held constant in binary solution, the reducing effect of AR337 dye on AR274 dye uptake capacity of *E.prolifera* increased. This reducing effect was dominant at higher initial AR337 concentration because of a fixed quantity of biosorbent could only offer a finite number of surface binding sites where all dye molecules compete for these biosorption sites on the surface. In multi-component systems, components may have different adsorption type from single component systems, adsorption of components i and j may increase (synergism), decrease (antagonism), or may remain unchanged (32). As a result, the reduced values of the uptake capacity of *E. prolifera* for AR274 and AR337 in multi-component solutions clearly indicated the competition between constituent dyes for the available surface area, and the combined biosorption is generally found as antagonistic behaviour resulting in a lower biosorption yield.

Equilibrium Modelling

Modelling of biosorption data is important for predicting and comparing biosorption performance. Equilibrium studies give the capacity of the adsorbent and describe the adsorption isotherm by constants whose values express the surface properties and affinity of the adsorbent (33).

TABLE 2
The individual uptake amounts, total uptake amounts and biosorption yields for the biosorption of AR274 and AR337 dyes from single species and binary solutions

$C_{0\ AR337}$ (mg/L)	$C_{0\ AR274}$ (mg/L)	$q_{eq\ AR337}$ (mg/g)	$q_{eqAR274}$ (mg/g)	$q_{eqTotal}$ (mg/g)	Biosorption yield, % AR337	Biosorption yield, % AR274	Biosorption yield, % Total
0.0	25.2	0.0	23.7	23.7	0.00	94.05	94.05
0.0	49.3	0.0	46.1	46.1	0.00	93.51	93.51
0.0	76.1	0.0	70.3	70.3	0.00	92.38	92.38
0.0	101.2	0.0	92.4	92.4	0.00	91.30	91.30
26.9	0.0	22.6	0.0	22.6	84.00	0.0	84.00
24.3	26.0	16.8	22.8	39.6	69.14	87.69	78.73
24.8	49.5	16.3	41.8	58.1	65.72	84.44	78.20
23.8	75.6	14.7	61.7	76.4	61.76	81.61	76.86
25.5	101.9	14.3	78.3	92.6	56.08	76.84	72.68
51.0	0.0	41.9	0.0	41.9	82.16	0.0	82.16
48.6	26.3	33.1	21.7	54.8	68.11	82.44	73.14
47.9	49.6	30.4	39.2	69.6	63.47	78.97	71.36
55.0	74.5	30.8	56.6	87.4	56.00	75.93	67.47
54.0	105.2	28.0	74.0	102.0	51.85	70.37	64.10
77.0	0.0	62.1	0.0	62.1	80.65	0.0	80.65
74.0	26.1	45.0	20.3	65.3	60.81	77.87	65.25
77.9	50.4	43.8	37.4	81.2	56.22	74.19	63.28
78.4	74.2	41.2	52.5	93.7	52.55	70.79	61.42
76.3	105.8	36.3	68.8	105.1	47.58	65.03	57.72
100.5	0.0	79.4	0.0	79.4	79.00	0.0	79.00
101.7	25.5	55.7	18.9	74.6	54.77	74.15	58.65
98.5	49.4	51.5	35.2	86.7	52.28	71.19	58.60
99.7	77.0	48.4	51.1	99.5	48.55	66.33	56.30
102.8	109.0	45.6	66.5	112.1	44.36	61.00	52.93

In order to model the biosorption equilibrium, the biosorption studies of AR274 and AR337 dyes in single and binary solution on *E.prolifera* were done in a single stage batch reactor by varying the initial concentrations of the studied dyes at initial pH 2.0 and temperature 30°C. The experimental isotherms for the biosorption of AR274 and AR337 dyes from single and binary solution were given in Fig. 5a–b, respectively. Figure 5a–b show that the equilibrium uptake amounts decreased with increasing the other dye concentration in the biosorption medium.

Application of Single-Component Isotherm Models to Equilibrium Data

The single-component biosorption isotherms were described using the Langmuir (34) and Freundlich (35) isotherm models as follows:

Langmuir isotherm model:

$$q_{eq} = \frac{q_{\max} K_a C_{eq}}{1 + K_a C_{eq}} \tag{2}$$

Freundlich isotherm model:

$$q_{eq} = K_F C_{eq}^{1/n} \tag{3}$$

where q_{eq} (mg/g) and C_{eq} (mg/L) are the biosorbed dye amount and the residual dye concentration in solution at equilibrium, respectively. q_{max} is the monolayer coverage dye capacity of biosorbent and K_a (L/mg) is a constant related to the energy of adsorption; K_F ((mg/g)(L/mg)$^{-1/n}$) and $1/n$ are the Freundlich constants indicating adsorption capacity and intensity, respectively.

The single-component Langmuir and Freundlich isotherm models were applied to the biosorption data of AR274 and AR337 dyes on *E.prolifera* and the isotherm constants were presented in Table 3. As shown from Table 3, *E.prolifera* have a higher maximum monolayer coverage capacity (q_{max}) for AR274 dye than that of AR337 dye, for the biosorption from both in single and binary solution. This type of behavior can be explained by structural differences of these two dyes. The q_{max} values in biosorption of the studied dyes from the binary solution decreased with increasing the other dye concentration. For example, the maximum monolayer coverage capacity of *E.prolifera* for AR274 dye decreased from 246.9 to 120.5 mg/g with the presence of 100 mg/L initial AR337 concentration, while the maximum monolayer coverage capacity of *E.prolifera* for AR337 dye decreased from 218.3 to 95.2 mg/g in the presence of initial AR274 concentration of 100 mg/L. K_a, calculated from the Langmuir isotherm model indicates the affinity for binding of dye anions (36). It was determined that the affinity of *E.prolifera* toward AR274 was higher than AR337 as can be noticed by the high K_a values of AR274. However, the calculated K_a values for AR274 and AR337 dye tended to decrease in the presence and increasing concentration of the other dye (Table 3). These decreasing K_a values for both dye showed that a competition occured between these two dyes for biosorption sites, thus the affinity of *E.prolifera* toward AR274 and AR337 dye decreased.

The single-component Freundlich isotherm model was also applied to the biosorption data of AR274 and AR337 dyes in single and binary solutions on *E.prolifera* and the Freundlich constants, K_F and n, were evaluated (Table 3). For the biosorption from binary solution, the n and K_F values decreased with the presence and increasing concentration of other dye indicating that the biosorption intensity was contrarily affected and the biosorption capacity of biosorbent decreased with increasing concentration of the other dye, respectively.

TABLE 3
The single-component Langmuir and Freundlich isotherm constants for the biosorption of AR274 and AR337 dyes from single species and binary solutions (initial pH 2.0, temperature 30°C)

		Langmuir Isotherm			Freundlich Isotherm		
Component	$C_{0\ AR337}$	q_{max}	K_a	R^2	K_F	n	R^2
AR274	0	246.9	0.0698	0.999	27.80	1.894	0.976
	25	128.2	0.0652	0.999	11.00	1.570	0.991
	50	125.0	0.0452	0.999	8.27	1.537	0.992
	75	121.9	0.0344	0.999	6.55	1.506	0.992
	100	120.5	0.0288	0.999	5.65	1.494	0.992
AR337	0	218.3	0.0264	0.999	18.81	2.219	0.960
	25	114.9	0.0232	0.995	4.88	1.531	0.968
	50	104.2	0.0221	0.997	4.11	1.497	0.984
	75	96.2	0.0198	0.999	3.19	1.427	0.995
	100	95.2	0.0158	0.999	2.62	1.404	0.995

Isotherm models for describing biosorption behavior of dyes in single and binary solution was analysed by comparing the experimental and calculated equilibrium data. The comparison of the experimental and calculated equilibrium data ($q_{eq,exp}$; $q_{eq,calc}$) from single-component isotherm models for the biosorption of AR274 and AR337 dyes from single species and binary solution were presented in Fig. 6a–d along with their error values. An error function is defined to enable the optimization process and to evaluate the fit of the isotherm equation to the experimental data. In this study, the average relative error (ARE) (37) function was examined. ARE function minimizes the fractional error distribution across the entire concentration range:

$$\mathrm{ARE} = \frac{100}{p}\sum_{i=1}^{P}\left[\frac{(q_{eq,calc} - q_{eq,\exp})}{q_{eq,\exp}}\right] \qquad (4)$$

where P indicates the number of measurements.

As seen in Figs. 6a–d, the error values (ARE) obtained from the Langmuir isotherm model are lower than those of the Freundlich isotherm model. In addition, the correlation coefficients (R^2) obtained from the Langmuir model are better than those of Freundlich isotherm model for the biosorption from single- as well as in binary systems (Table 3). As a result, the single-component Langmuir isotherm model fitted well to the biosorption data of AR274 and AR337 dyes on *E.prolifera*.

Application of Multi-Component Isotherm Models to Equilibrium Data

Equilibria and capacity relationship for single-component systems are well established and quantitatively expressed by various types of adsorption isotherms (38). For multi-component systems, interference and competition phenomena for adsorption sites occur and lead to a more complex mathematical formulation of the

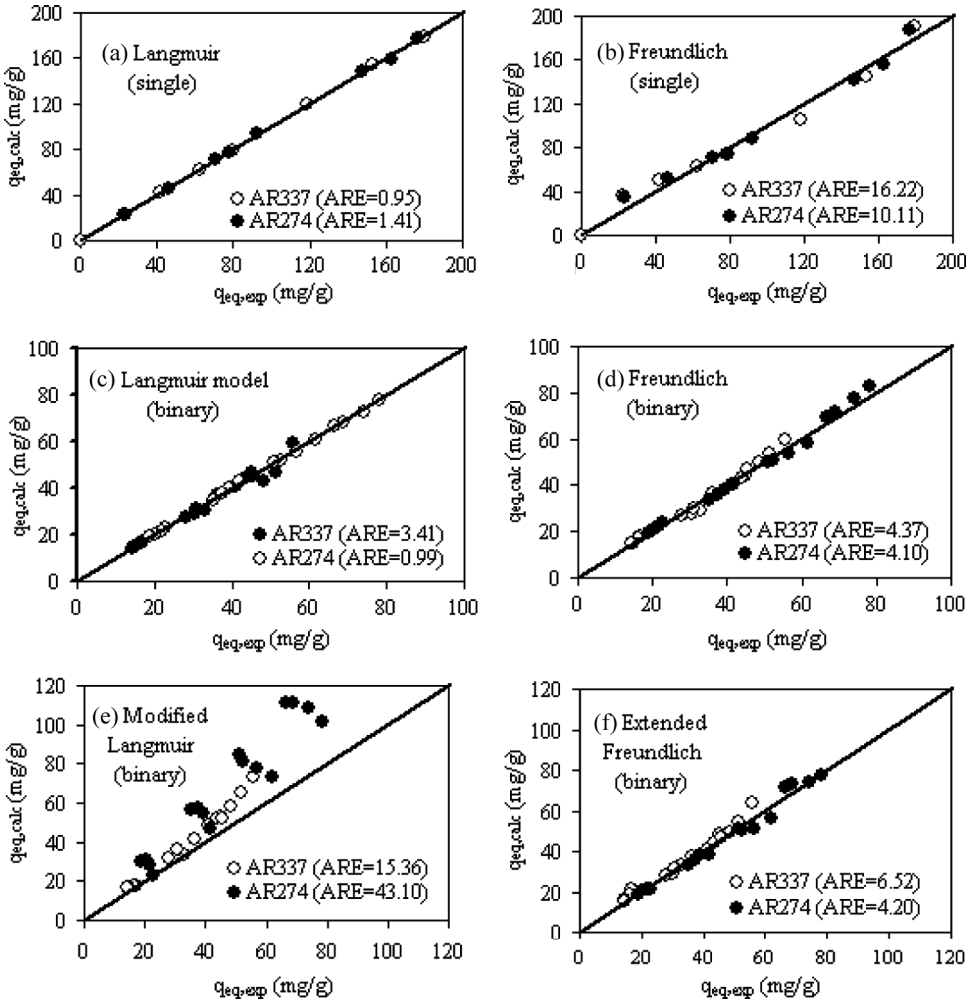

FIG. 6. The comparison of the experimental and calculated equilibrium data ($q_{eq,exp}$; $q_{eq,calc}$) obtained from single-and multi-component isotherm models with their error values for the biosorption of AR274 and AR337 dyes in single species [(a) Langmuir (b) Freundlich] and binary solutions; [(c) Langmuir (d) Freundlich (e) Modified Langmuir (f) Extended Freundlich isotherm models].

equilibrium. Several isotherm models have been proposed to describe equilibrium and competitive adsorption for such a system (38,39).

The modified Langmuir equation (39) describes the multi-component adsorption isotherm with the following equation:

$$q_{eq,i} = \frac{q_{\max,i} K_{a,i}(C_{eq,i}/\eta_i)}{1 + \sum_{j=1}^{N} K_{a,j}(C_{eq,j}/\eta_j)} \quad (5)$$

where $C_{eq,i}$ and $q_{eq,i}$ are the residual dye concentration and biosorbed dye amount of i component at equilibrium, respectively. $K_{a,i}$ and $q_{\max,i}$ are derived from the corresponding individual Langmuir isotherm equation and η_i is the Langmuir correction coefficient of the ith component that is estimated from competitive biosorption data. η_i is characteristic of each species and depends on the concentration of the other components.

Also, the extended Freundlich isotherm model Eq. (38) is given as follows:

$$q_{eq1} = \frac{K_{F1} C_{eq1}^{1/(n_1+x_1)}}{C_{eq1}^{x_1+y_1} C_{eq2}^{z_1}} \quad (6)$$

$$q_{eq2} = \frac{K_{F2} C_{eq2}^{1/(n_2+x_2)}}{C_{eq2}^{x_2+y_2} C_{eq1}^{z_2}} \quad (7)$$

where K_{F1}, K_{F2}, n_1 and n_2 can be estimated from the corresponding individual Freundlich isotherm equations and the other six parameters (x_1; y_1; z_1 and x_2; y_2; z_2) are the extended Freundlich adsorption constants of the first and second components in binary dye solution.

In this part of the study, the multi-component modified Langmuir and extended Freundlich isotherm models were applied to the biosorption data of AR274 and AR337 dyes from binary solution on *E.prolifera* and isotherm constants were given in Table 4. The single- and multi-component Langmuir and Freundlich isotherm constants were used to calculate q_{eq} values. The comparison of the experimental and calculated q_{eq} values for the biosorption from the binary solution was presented around the 45° line in Fig. 6e-f with their ARE values. In comparison with the modified Langmuir model, the extended Freundlich model fitted to the biosorption data with the lower ARE values of 4.20 for AR274; and 6.52 for AR337 dye, respectively. Since, all of the data points are distributed above the 45° line for the modified Langmuir model for both dyes with high ARE values, this indicates that the modified Langmuir model could not represent the biosorption data of AR274 and AR337 dyes for the binary systems. However, while the correlation slightly deviated at very high solution concentrations for the extended Freundlich model, it is significantly high at the modified Langmuir model. This is characteristic for the Langmuir model which is not valid for high concentrations assuming a limited number of identical sites for biosorption. The reason of these deviations could be due to the lower uptake (q_{exp}) of recessive component (AR337) in the biosorption process than the calculated uptake (q_{calc}) from multi-component isotherm models.

TABLE 4
The Modified Langmuir and Extended Freundlich isotherm model parameters for the biosorption of AR274 and AR337 dyes from single species and binary solutions (initial pH 2.0, temperature 30°C)

Component	Modified Langmuir isotherm model	Extended Freundlich isotherm model		
	η_i	x_i	y_i	z_i
AR274	2.047	0.506	1.768	0.502
AR337	1.974	0.338	0.692	0.553

The Modelling of the Biosorption From Binary Solutions in a Single Stage Bacth System

The biosorption in a batch system can be considered as a single stage equilibrium. The same quantity of solution (V_0) is treated at the single stage by a given amount of biosorbent (X_0) to reduce the dye concentration of solution from C_0 to C_{eq} (Fig. 7). From Fig. 7, the mass balance for the dye in the single stage is given by;

$$V_0 C_{0,i} + X_0 q_{0,i} = V_0 C_{eq,i} + X_0 q_{eq,i} \quad (8)$$

$$-\frac{V_0}{X_0} = \frac{(q_{eq,i} - q_{0,i})}{(C_{eq,i} - C_{0,i})} \quad (9)$$

The biosorbed dye amount at the beginning in a single stage batch reactor (q_0) is equal to 0.0. Equation 9 will provide the operating line passing through (C_0, q_0) and (C_{eq}, q_{eq}) for the single stage.

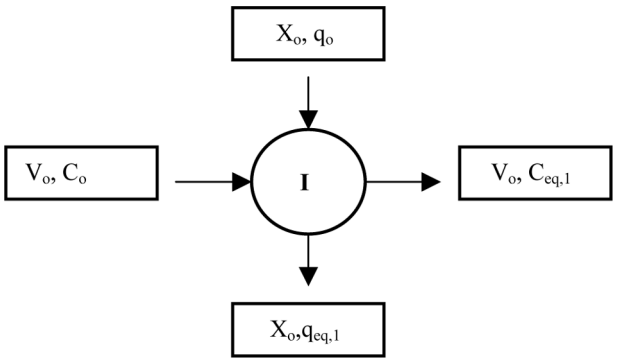

FIG. 7. A single stage batch system.

Substituting the single-component Langmuir and Freundlich isotherm model equations (Eqs. 1 and 2) for q_{eq} in Eq. 9 gives Eqs. (10) and (11), respectively.

$$-\frac{V_0}{X_0}=\frac{q_{\max,i}K_{a,i}C_{eq,i}}{(C_{eq,i}-C_{0,i})(1+K_{a,i}C_{eq,i})} \tag{10}$$

$$-\frac{V_0}{X_0}=\frac{K_{F,i}C_{eq,i}^{1/n}}{(C_{eq,i}-C_{0,i})} \tag{11}$$

Equation 9 can be rewritten for the biosorption from the binary solution. The biosorbed amounts ($q_{0,1}$ and $q_{0,2}$) of the first and second component at the beginning of the biosorption are equal to 0.0. When the modified Langmuir and extended Freundlich model equations (Eqs. 3–5) were substituted for $q_{eq,1}$, the Eqs. (12) and (13) can be obtained for biosorption of the first component from the binary solution.

$$-\frac{V_0}{X_0}=\frac{q_{\max}K_aC_{eq,1}/\eta_1}{(C_{eq,1}-C_{0,1})(1+K_aC_{eq,1}/\eta_1+K_aC_{eq,2}/\eta_2)} \tag{12}$$

$$-\frac{V_0}{X_0}=\frac{K_{F1}C_{eq1}^{1/(n_1+x_1)}}{(C_{eq,1}-C_{0,1})(C_{eq1}^{x_1+y_1}C_{eq2}^{z_1})} \tag{13}$$

These equations can also be rewritten for biosorption of the second component from the binary solution in the same manner.

The residual AR274 and AR337 concentrations for the biosorption from the single species and binary solution at equilibrium can be obtained by using their experimental equilibrium curves and operating lines. The experimental equilibrium curves and operating lines for the studied biosorption processes obtained by plotting C_{eq} and q_{eq} values at 30°C and initial pH 2.0 were given in Fig. 8. For example, in order to determine the equilibrium AR274 and AR337 dye concentrations in the exit stream leaving the single stage batch system in single species and binary solution, the initial dye concentrations were located at the proper point

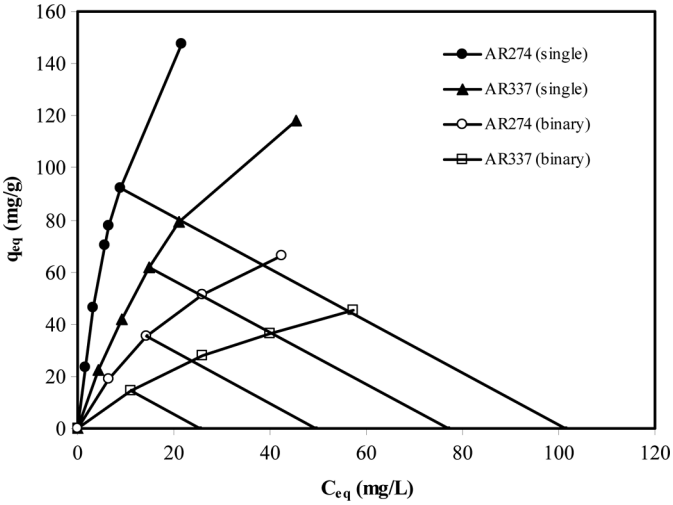

FIG. 8. The equilibrium curves and operating lines obtained from the biosorption of AR274 and AR337 dyes from single species and binary solution on *E.prolifera* in the single stage batch system (pH 2.0, Temperature 30°C, $V_0/X_0=-1.0$).

on the absisca ($C_{0,AR274}=101.2\,mg/L$ (single), $C_{0,AR337}=77.0\,mg/L$ (single), $C_{0,AR274}=49.4\,mg/L$ (binary), $C_{0,AR337}=25.5\,mg/L$ (binary); $q_0=0.0$); then the operating lines passing through (C_0, q_0) with a slope of $V_0/X_0=-1.0$ are drawn for the studied biosorption processes. According to Eq. 9, the intersection coordinates of the operating lines and the equilibrium curves give C_{eq} and q_{eq} values. The C_{eq} values from Fig. 8 were found as $C_{eq,AR274}=8.5\,mg/L$ (single), $C_{eq,AR337}=15.6\,mg/L$ (single), $C_{eq,AR274}=15.0\,mg/L$ (binary) and $C_{eq,AR337}=12.5\,mg/L$ (binary) while the experimental C_{eq} values were obtained as $C_{eq,AR274}=8.8\,mg/L$ (single), $C_{eq,AR337}=14.9\,mg/L$ (single), $C_{eq,AR274}=14.2\,mg/L$ (binary) and $C_{eq,AR337}=11.2\,mg/L$ (binary).

The values of $C_{eq,AR274}$ and $C_{eq,AR337}$ in the exit stream leaving a single stage batch system at the biosorption of AR274 and AR337 dyes from binary solution for $V_0/X_0=-1.0$ were also calculated using the single- and multi-component Langmuir and Freundlich isotherm

TABLE 5
Comparison of the experimental and calculated C_{eq} values found from the Langmuir and Freundlich isotherms for the biosorption of AR274 and AR337 dyes from single species on *E.prolifera* ($V_0/X_0=-1$)

Single	AR274			Single	AR337		
$C_{o,AR274}$ (mg/L)	$C_{eq,exp}$ (mg/L)	$C_{eq,L}$ (mg/L)	$C_{eq,F}$ (mg/L)	$C_{o,AR337}$ (mg/L)	$C_{eq,exp}$ (mg/L)	$C_{eq,L}$ (mg/L)	$C_{eq,F}$ (mg/L)
25.2	1.5	1.5	0.8	26.9	4.3	4.4	1.9
49.3	3.2	3.3	2.7	51.0	9.1	9.1	6.8
76.1	5.8	5.7	5.8	77.0	14.9	15.1	14.6
101.2	8.8	8.6	9.6	100.5	21.2	21.7	23.2

TABLE 6
Comparison of the experimental and calculated C_{eq} values found from the modified Langmuir and Extended Freundlich isotherms for the biosorption of AR274 and AR337 dyes from binary solution on *E.prolifera* ($V_0/X_0 = -1$)

Binary		AR337			AR274		
$C_{o,AR337}$ (mg/L)	$C_{o,AR274}$ (mg/L)	$C_{eq,exp}$ (mg/L)	$C_{eq,multiL}$ (mg/L)	$C_{eq,multiF}$ (mg/L)	$C_{eq,exp}$ (mg/L)	$C_{eq,multiL}$ (mg/L)	$C_{eq,multiF}$ (mg/L)
24.3	26.0	7.5	7.1	6.3	3.3	3.3	3.9
48.6	26.3	15.5	15.5	15.5	4.6	4.6	4.6
74.0	26.1	29.0	26.1	28.1	5.8	7.1	6.5
101.7	25.5	46.0	39.8	43.7	6.5	10.1	8.7
24.8	49.5	8.5	7.9	7.7	7.7	7.8	7.9
47.9	49.6	17.5	16.9	18.0	10.4	10.6	10.2
77.9	50.4	34.1	30.7	33.8	13.0	14.3	13.1
98.5	49.4	47.0	41.5	45.7	14.2	16.8	14.9
23.8	75.6	9.1	8.4	8.5	13.8	13.9	13.9
55.0	74.5	24.2	21.9	23.8	18.0	18.6	18.0
78.4	74.2	37.2	34.0	37.2	21.7	22.7	21.7
99.7	77.0	51.3	46.7	50.7	25.9	27.7	26.1
25.5	101.9	11.2	10.2	10.5	23.7	23.8	23.7
54.0	105.2	26.0	24.4	26.0	31.2	31.5	31.2
76.3	105.8	40.0	37.2	39.6	37.0	37.7	37.1
102.8	109.0	57.2	53.8	56.6	42.5	43.5	42.6

constants, from Eqs.10–13 by using Microsoft Excel. The values C_{eq} calculated from the isotherm models for the biosorption of AR274 and AR337 dyes from the single species and binary solution were given in Tables 5 and 6, respectively. The C_{eq} calculated from the single-component Langmuir isotherm model for the single species dye biosorption were approximately equal to the experimental values (Table 5). As a result, the Langmuir isotherm model demonstrated good correlation with the single biosorption data of AR274 and AR337 dyes on *E.prolifera.* As seen in Table 6, the C_{eq} values calculated from the extended Freundlich isotherm model for the binary biosorption data fitted better to the experimental data than those of the modified Langmuir model in the presence of the second dye. This could be explained by higher deviations from the modified Langmuir isotherm model than those of the extended Freundlich isotherm model at very high dye concentrations in binary solution.

On the other hand, the isotherm constants or the operating lines obtained from the experimental equilibrium data can be used to find the stage number and/or the final concentration of the exit stream for the desired purification without any experiments (40). As a result, the removal of a given amount of solute can be accomplished with greater economy of adsorbent if the solution is treated with separate small batches of the adsorbent rather than in single batch, with filtration between each stage.

CONCLUSION

In this study, the biosorption from a binary acidic dye solution, Acid Red 274 and Acid Red 337, on *Enteromorpha prolifera*, a green seaweed, was investigated in a single stage batch system. Derivative Spectrophotometry (DS) was applied for the analysis of the studied acidic dyes in binary solution. The first order derivative spectrophotometry (FODS) was also determined as a suitable analysis method for the analysis of the studied dyes in binary solution. The wavelenghts to determine unknown concentrations of AR274 and AR337 dyes in binary solution using FODS were found as 591 and 527 nm, respectively, while the analysis of AR274 and AR337 dyes in single solution were conducted at 527 and 492 nm, respectively. According to recovery studies and t-test results, it can be said that AR274 and AR337 concentrations in binary solution can be correctly determined using FODS. The optimum biosorption conditions for the biosorption of AR274 and AR337 dyes from binary solution were determined to be initial pH 2.0 and temperature 30°C. The single-component adsorption isotherm models such as the Langmuir and Freundlich isotherm models and the multi-component adsorption isotherm models such as the modified Langmuir and extended Freundlich isotherm models were applied to the experimental data obtained from the biosorption of AR274 and AR337 dyes from single species and binary solution on *E.prolifera*. It was found that the

monolayer coverage capacity of *E. prolifera,* according to the Langmuir isotherm model was obtained as 247 mg/g for AR274 dye and 218 mg/g for AR337 dye. These values decreased to 121 mg/g for AR274 and 100 mg/g for AR337 when both dye concentrations were kept at 100 mg/L and the combined biosorption of AR274 and AR337 dyes on *E. prolifera* is generally found to be antagonistic. The single-component Langmuir and Freundlich constants obtained from the biosorption of AR274 and AR337 dyes in single solution were used to describe the biosorption equilibrium of AR274 and AR337 dyes in binary solution.

For the biosorption from binary solution, the residual dye concentrations at equilibrium at a single stage for "a volume of solution containing dye (V_o)/a given quantity of dried algae (X_o)" ratio was calculated by using the tested isotherm constants. It was observed that the experimental biosorption equilibrium data for binary dye solution are in good agreement with those calculated using the applied isotherm constants. Also, the equilibrium AR274 and AR337 concentrations in the exit stream of a single stage batch system were obtained by using the experimental equilibrium curves and operating lines.

NOMENCLATURE

ARE	average relative error
C_{ad}	the biosorbed dye concentration (mg/L)
$C_{ad,eq}$	the biosorbed dye concentration at equilibrium (mg/L)
C_{eq}	the residual dye concentration in solution at equilibrium(mg/L)
$C_{eq,exp}$	the experimental dye concentration in solution at equilibrium(mg/L)
$C_{eq,i}$	the residual concentration of i th component at equilibrium (mg/L)
$C_{eq,F}$	the equilibrium concentration calculated from Freundlich isotherm model (mg/L)
$C_{eq,L}$	the equilibrium concentration calculated from the Langmuir isotherm model (mg/L)
$C_{eq,multiF}$	the equilibrium concentration calculated from the extended Freundlich isotherm model (mg/L)
$C_{eq,multiL}$	the equilibrium concentration calculated from the multi component Langmuir isotherm model (mg/L)
C_0	initial dye concentration (mg/L)
$C_{0,i}$	initial concentration of i th component (mg/L)
D	the absorbance value of each dye at the first order derivative wavelength.
$K_{a,i}$	the single-Langmuir isotherm constant of ith component (L/mg)
$K_{F,i}$	the single-Freundlich isotherm constant of ith component ((mg/g)(L/mg)$^{-1/n}$)
n_i	the single-Freundlich isotherm constant of i th component
p	number of experimental data points
$q_{eq,i}$	the biosorbed amount of i th component at equilibrium (mg/g)
$q_{eq,cal}$	the calculated value of the biosorbed dye at equilibrium (mg/g)
q_{max}	maximum monolayer coverage capacity of biosorbent (mg/g)
R^2	correlation coefficient
T	temperature (°C)
V	the solution volume containing dye (L)
X	the mass of biosorbent (g).
x_i, y_i, z_i	extended Freundlich isotherm constants of i th component

Greek Symbols

η_i	modified Langmuir isotherm constant for i th component
λ	wavelenght (nm)

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