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The effect of diazinon on the removal of carmoisine by *Saccharomyces cerevisiae*

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ABSTRACT

Carmoisine is a type of azo dye producing red color to foods. The use of carmoisine has been limited in many countries due to the presence of β-naphthylamine. The aim of this study was to investigate the effect of diazinon on the biotransformation of carmoisine using *Saccharomyces cerevisiae*. First, to obtain the optimized parameters of *S. cerevisiae* and carmoisine, the removal of carmoisine was examined using parameters, including retention time (0.5–24 h), yeast concentration (0.1%–1%), and carmoisine concentration (1–50 mg/L). Thereafter, a number of experiments were conducted in the presence of different factors, including the diazinon concentration of 0.001–1,000 mg/L, the retention time of 0.5–24 h, the optimized concentrations of carmoisine and *S. cerevisiae.* The results indicated that the removal efficiency of carmoisine for 1,000 mg/L diazinon and the control sample (0 mg/L diazinon) was 85.23% and 19.03%, respectively, within 0.5 h. Based on the results, there was a direct relationship between the biotransformation of carmoisine and the initial concentration of diazinon. We can conclude that *S. cerevisiae* has the ability to remove carmoisine with the lowest cost and high efficiency.

Keywords: Saccharomyces cerevisiae; Diazinon; Biotransformation; Carmoisine

1. Introduction

Dyes discharged into the environment usually originate from the textile, dyeing, printing, and cosmetic industries [1–3]. They have adverse effects on aquatic environments, which include decreased oxygen concentration due to the reaction with hydrosulfide in the structure of dyes and the prevention of the passage of light into water, leading to detrimental changes in the water ecosystem. Azo dyes are synthetic dyes characterized by the presence of one or more *chromophoric azo groups* [4]. Studies have shown that azo dyes have toxic, allergic, and irritating effects and cause dermatitis, cancer, and mutation in humans [1–3]. Carmoisine is a synthetic azo class of dyes that produce red color to foods. The presence of sulfonic groups in dye makes it polar and soluble in water. This dye is used in edibles such as jelly, jams, sweets, and preservatives. The use of carmoisine is banned in many developed countries due to the presence of b-naphthylamine, a known carcinogen in it that is created from reduction of azo groups [5,6]. This dye can cause drug sensitivity or asthma and allergy to many people. It enhances behavioral complications like hyperactivity, and sleeplessness in children and high doses of the dye also result in coma and even death. Thus, it is necessary to focus the research in removing the carmoisine from effluents using an applicable method without any by-product [7]. On the other hand, several applications of pesticides in agriculture can

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cause environmental issues such as contamination of water, soil, and the food. Pesticides include organophosphate, carbamate, and pyrethroid, and the organophosphorus compounds are the largest and most diverse pesticides. Because of their effect on a wide range of pests and also their low cost, organophosphate pesticides are used by farmers more than other pesticides [8]. Fig. 1 describes the chemical structure of carmoisine. Diazinon is an organophosphate compound originating from agricultural, municipal, and industrial effluents. It has adverse effects on humans, which include headache, nausea, respiratory tract constriction, the weakening of the central nervous system, and ultimately death [9]. Although physicochemical processes have wide applications in dye decolorization, microbial eco-friendly processes are truly in demand with regard to the effective degradation and mineralization of dye effluents. In this regard, *Saccharomyces cerevisiae* can metabolize and degrade dye compounds [10–12]. This microorganism can be produced in large quantities and used as a by-product in industry. Previous studies have corroborated the fact that diazinon is not toxic to *S. cerevisiae* at concentrations less than 50 g/L and does not deactivate the metabolic system [13]. In biodegradation processes, diazinon is degraded to the alkyl P–O and aryl P–O bonds and is used by microorganisms as a source of carbon and phosphorus. This can cause their growth and, therefore, increase their efficiency to remove chemicals and toxins [14,15]. Based on previous studies, azo dyes are biodegraded and broken into aromatic amines with side groups (–SO_3, –OH, –COOH, –Cl) [10,16]. McMullan et al. [16] reviewed the underlying mechanisms by which diverse categories of microorganisms from bacterial and fungal domains degrade dyestuffs. As mentioned earlier, carmoisine and diazinon are two major pollutants in the industrial and agricultural effluents. The simultaneous removal of these pollutants reduces the costs of initial investment and operation. For this reason, the removal of carmoisine in the presence of diazinon is carried out. The study was aimed at examining the effect of diazinon on the removal of carmoisine using *S. cerevisiae*.

2. Materials and methods

2.1. Materials

All chemicals used in the experiments were of reagent grade. All solutions were prepared using distilled water. Diazinon and carmoisine were provided by Sigma-Aldrich (USA) and Merck (Germany) companies, respectively. *S. cerevisiae* cells harvested (PTCC (Persian Type Culture Collection): 5052) were locally obtained from the Iranian Research Organization for Science and Technology (IROST), Tehran, Iran.

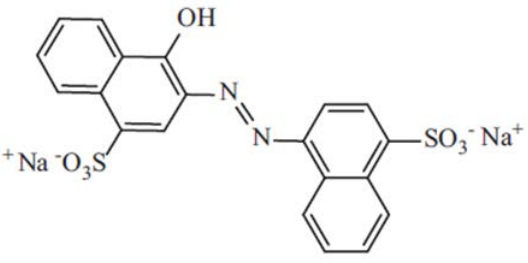

Fig. 1. Chemical structure of carmoisine.

2.2. Preparation of reaction mixtures

Initially, to obtain the optimized conditions of carmoisine removal by *S. cerevisiae*, 100 cc of the reaction mixture was prepared at the concentration of 1–50 mg/L of carmoisine, 0.1%–1% (m/v) of *S. cerevisiae*, and retention times of 0.5–24 h. Thereafter, 56 runs were carried out by considering different parameters, including a diazinon concentration of 0.001–1,000 mg/L, retention times of 0.5–24 h, and the optimized concentrations of carmoisine and *S. cerevisiae*. In similar conditions, a sample including 50 mg/L carmoisine and 1% *S. cerevisiae* without the presence of diazinon was studied as the control sample. All experiments were agitated at the fixed speed of 120 rpm and room temperature (28°C ± 2°C).

2.3. Analytical methods

A total of 10 mL of the sample was withdrawn from each Erlenmeyer flask at definite time intervals. The samples were centrifuged at 4,000 rpm for 12 min to remove the medium. Finally, the absorbance of each sample was read with a spectrophotometer at the wavelength of 515 nm. Dye concentrations were estimated from the standard curve of absorbance versus dye concentrations between 0 and 15 mg/L. When the concentrations were high, the sample solution was diluted until the absorbance fell into the absorbance range corresponding to 0–15 mg/L dye concentration. The removal efficiency (R, %) of carmoisine was calculated using the equation as follows:

$$R(\%) = \frac{C_o - C}{C} \times 100 \quad (1)$$

where C_0 is the initial dye concentration (mg/L), C is the dye concentration in solution after removal (mg/L). All experiments were run in duplicate and the average results are presented herein.

2.4. Statistical analysis

The significance of means within the groups of experimental data was assessed using one-way analysis of variance.

2.4. Toxicity test for diazinon

In this study, broth microdilution method was applied to determine the minimum inhibitory concentration (MIC) of diazinon against *S. cerevisiae*. In this method, 0.2 mL reaction mixture including a diazinon concentration of 0.048–25 g/L and a yeast suspension of 2.5 × 10^6 Cfu/mL was cultured in the RPMI medium. It was then incubated at 28°C for 24 h. Finally, the first concentration of diazinon that inhibited yeast growth was considered as MIC [17].

3. Results and discussion

3.1. The removal of carmoisine by Saccharomyces cerevisiae

In this study, initially, the effects of the concentration of diazinon, carmoisine, and *S. cerevisiae*, and the reaction

time on the removal efficiency of carmoisine were studied. Fig. 2 demonstrates the effect of different concentrations of *S. cerevisiae* (0.5%–1%) on the removal efficiency of carmoisine within 5 h. The maximum removal efficiency of diazinon was 92.44% at the concentration of 1% *S. cerevisiae*. With regard to the results of Fig. 2, the increase in the concentration of *S. cerevisiae* has led to the increased removal efficiency of carmoisine. As can be observed, with the augmentation of *S. cerevisiae* from 0.5% to 1% m/v, the removal efficiency of diazinon grew from 17.68% to 92.44% ($P < 0.05$). Fig. 3 indicates the effect of the initial concentrations of carmoisine (1–50 mg/L) on the removal efficiency of carmoisine. Based on Fig. 3, there is a direct relationship between the removal efficiency of carmoisine and its initial concentration. The results indicated that the curve slope initially increases and then gradually decreases along with the elevation of the carmoisine concentration. In this regard, the highest removal rate was obtained at the initial diazinon concentration of 50 mg/L, as the collision between *S. cerevisiae* and carmoisine increases. This indicates that the increase in concentration of the dye decreases the resistance toward dye uptake and increases the mass driving force among adsorbent and adsorbate, which increases the percentage adsorption of the dye. These results are in disagreement with Shanmugam and Mahadevan [18] when adjusting the initial concentration of azo dye from 20 to 40 mg/L resulted in reducing their removal efficiencies of reactive dye from 57% to 31%. With regard to previous studies, biotransformation has been known as the main mechanism of biodegradation of azo dyes [19,20]. Jadhav et al. [10] completely removed 100 mg/L of methyl red using *S. cerevisiae* and the reaction time range was considered 0.5–24 h. The biological reactions have typically been dependent on the reaction time and the yeast mass. The results presented in Fig. 3 indicate that carmoisine is removed rapidly from 0.5 to 5 h, while from 5 to 24 h, the curve slope is stable and the removal efficiency levels off ($P < 0.05$). Accordingly, the highest removal rate (92.44%) was obtained at the carmoisine concentration of 50 mg/L and the reaction time of 5 h. This may be related to the saturation of adsorbent site on the outer interface of the biomass by dye species. The time needed to reach equilibrium is defined as "equilibrium time." The rapid uptake of the dye indicates that the sorption process could be ionic in nature where the anionic dye molecules bind to the various positively charged organic functional groups present on the surface of the biomass [21]. Mahmoud [20] demonstrated that the dye of ramazole blue was removed completely by bacteria at the concentration of 100 mg/L [21]. According to the results in Figs. 2 and 3, the optimized values of concentrations of *S. cerevisiae* and carmoisine were found to be 1% and 50 mg/L, respectively.

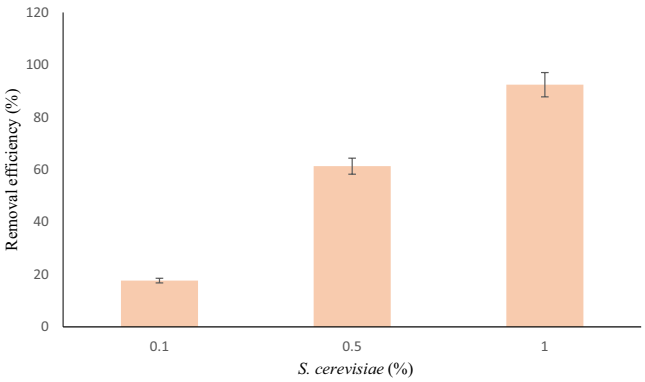

Fig. 2. The effect of the initial *S. cerevisiae* concentration on the removal efficiency of carmoisine in 5 h.

3.2. The effect of diazinon on the removal of carmoisine

Fig. 4 displays the effect of the initial diazinon concentration (0–1,000 mg/L) and reaction time (0.5–24 h) on the removal efficiency of carmoisine. The maximum removal

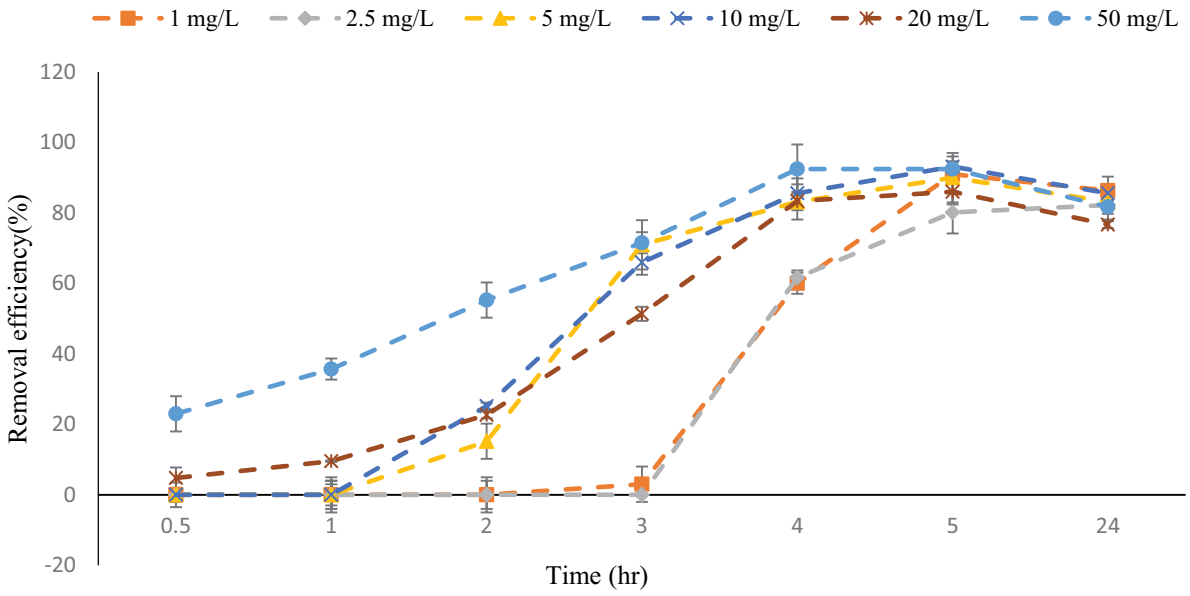

Fig. 3. The effect of the initial concentration of carmoisine (1–50 mg/L) on the removal efficiency at different reaction times (0.5–24 h).

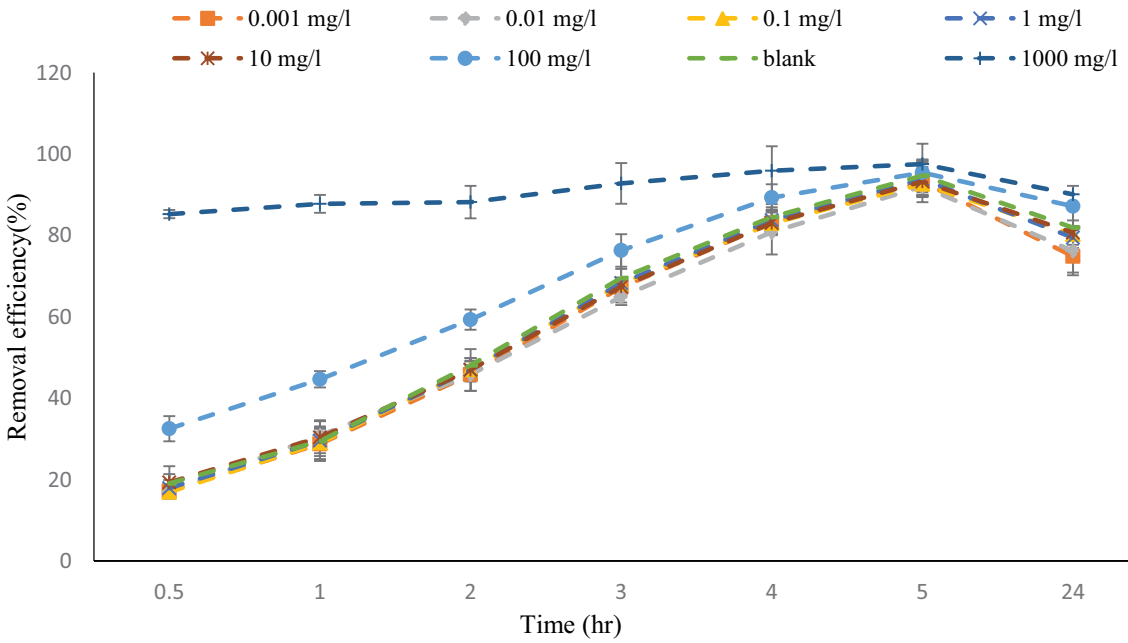

Fig. 4. The removal efficiency of carmoisine by *S. cerevisiae* at the different concentration of diazinon and reaction time.

efficiency was 97.51% at 1,000 mg/L diazinon concentration for 5 h. The results (Fig. 4) represent that the removal efficiency of carmoisine grows along with the increase in the reaction time from 0.5 to 5 h and then decreases gradually up to 24 h. In this study, a significant difference was detected between the removal efficiency of carmoisine at diazinon concentrations of 1,000 mg/L and 0 mg/L for the first 3 h ($P < 0.05$). Fig. 5 illustrates the effect of the initial diazinon concentration (0–1,000 mg/L) on the removal efficiency within 0.5 h. Based on the findings (Fig. 5), the removal efficiency of carmoisine for 1,000 mg/L diazinon and the control sample (0 mg/L diazinon) was found to be 85.23% and 19.03%, respectively, within 0.5 h. Accordingly, the removal efficiency of diazinon at the concentration of 1,000 mg/L was approximately 4.5 times as large as its removal efficiency in the control sample. Carmoisine is a type of azo dye with two groups of sulfonate which can produce a negative charge in aqueous solutions. The cell surface of *S. cerevisiae* has three

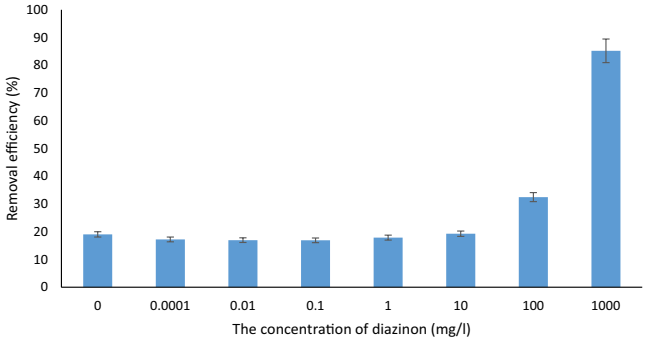

Fig. 5. The effect of the initial concentration of diazinon (0–1,000 mg/L) on the removal efficiency in 0.5 h.

carboxylic, phosphonate, and amine functional groups. These groups have an important role in the biological activity of *S. cerevisiae*. Compared with other groups, amine groups are more active in absorbing contaminants and creating a positive charge on yeast. However, carboxyl and phosphonate groups produce a negative charge on yeast [22]. Due to electrostatic repulsion, carboxyl and phosphonate groups are not suitable for the adsorption of anionic dyes. Meanwhile, amine groups are found to form NH_4^+ mainly in protein molecules. It can, therefore, be concluded that *S. cerevisiae* removes carmoisine because of the electrostatic gravity between the positive charge of the amino groups in yeast and the SO_3 groups in the dye [23,24]. *S. cerevisiae* can degrade the alkyl P–O and aryl P–O bonds of diazinon and use it as a source of carbon and phosphorus [14,15]. Hence, it can be concluded that the removal efficiency of carmoisine grows in the presence of diazinon due to the increased metabolic activities of *S. cerevisiae* [8]. Kristina et al. [14] found that the bacterial strains of *Arthrobacter* and *Streptomyces* could have a synergistic effect on the biodegradation of diazinon [14]. Fig. 6 exhibits the GC-MS spectrum of diazinon as an intermediate during the biotransformation process. The results of Fig. 6 suggest that diazinon degrades to 16 compounds with the detection quality of over 50% (based on the molecular weight). Furthermore, by-products with a molecular weight ranging from 84.094 to 652.117 atomic mass unit were detected within different retention times of 2.185 until 32.072 min. The clearest peaks were related to acetaldehyde, propylhydrazone, cyclohexasiloxane-dodecamethyl, and heptane compounds, whose detection quality was over 80%. The major peak at the time of 17.367 min was related to pentasiloxane-dodecamethyl compound, whose detection quality was 47%. Finally, the results of this study showed that the MIC value of diazinon against *S. cerevisiae* was 6.25 g/L.

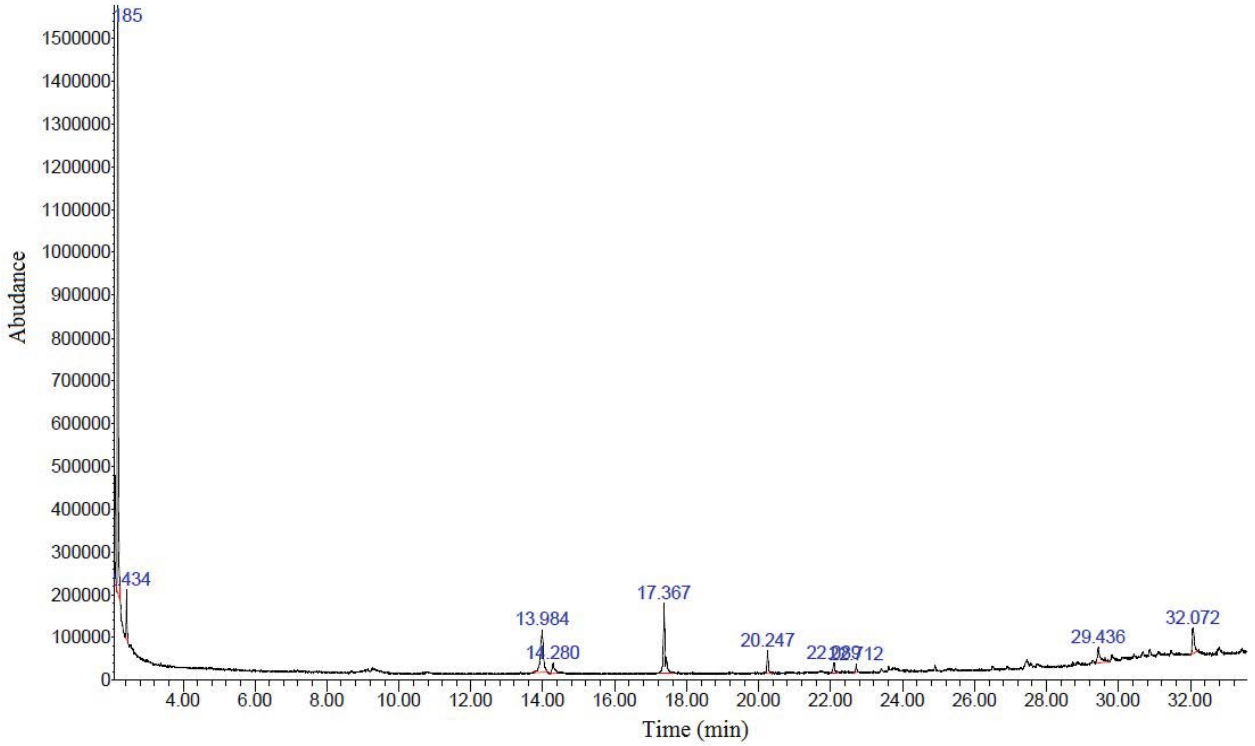

Fig. 6. GC-MS spectrum of degraded product of diazinon as an intermediate during the biotransformation process.

4. Conclusion

Based on the results obtained in this study, the removal efficiency of carmoisine has a direct relationship with the concentration of *S. cerevisiae*, reaction time, and the initial concentrations of diazinon and carmoisine. It can be concluded that diazinon does not have toxic effects on *S. cerevisiae* and increases the removal efficiency of carmoisine.

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References

[1] P. Monda, C.B. Majumder, B. Mohanty, Effects of adsorbent dose, its particle size and initial arsenic concentration on the removal of arsenic, iron and manganese from simulated ground water by Fe^{3+} impregnated activated carbon, J. Hazard. Mater., 150 (2008) 695–702.
[2] A.A. Duker, EJ.M. Carranza, M. Hale, Arsenic geochemistry and health, Environ Int., 31 (2005) 631–641.
[3] S. Amna, M. Qaisar, B. Muhammad, Investigation on *Melia azedarach* biomass for arsenic remediation from contaminated water, Desal. Wat. Treat., 53 (2015) 1632–1640.
[4] A.A. Najafpoor, H. Alidadi, H. Esmaeili, Optimization of anionic dye adsorption onto *Melia azedarach* sawdust in aqueous solutions: effect of calcium cations, Asia-Pacific J. Chem. Eng., 11 (2016) 258–270.
[5] V. Gupta, Application of low-cost adsorbents for dye removal – a review, J. Environ. Manage., 90 (2009) 2313–2342.
[6] M.M. Biswas, K.E. Taylor, J.K. Bewtra, N. Biswas, Enzymatic treatment of sulfonated aromatic amines generated from reductive degradation of reactive azo dyes, Water Environ. Res., 79 (2007) 351–356.
[7] M.R. Sohrabi, A. Khavaran, S. Shariati, S. Shariati, Removal of Carmoisine edible dye by Fenton and photo Fenton processes using Taguchi orthogonal array design, Arabian J. Chem., 10 (2017) S3523–S3531.
[8] M.H. Ehrampoush, A. Sadeghi, Z. Bonyadi, Optimization of diazinon biodegradation from aqueous solutions by *Saccharomyces cerevisiae* using response surface methodology, AMB Express, 7 (2017) 68.
[9] R. Wauchope, The pesticide content of surface water draining from agricultural fields – a review, J. Environ. Qual., 7 (1978) 459–472.
[10] J.P. Jadhav, G.K. Parshetti, S.D. Kalme, S.P. Govindwar, Decolourization of azo dye methyl red by *Saccharomyces cerevisiae* MTCC 463, Chemosphere, 68 (2007) 394–400.
[11] N. George, C.P. Singh, S. Sondhi, Biodegradation and analytical methods for detection of organophosphorous pesticide: chlorpyrifos, Int. J. Pure Appl. Sci. Technol., 20 (2014) 79–94.
[12] R.A. Peinado, J.J. Morenoa, Yeast biocapsules: a new immobilization method and their applications, Enzyme Microb. Technol., 40 (2006) 6.
[13] W. Dieter, Mutagenicity studies on organophosphorus insecticides, Mutat. Res., 32 (1975) 133–150.
[14] T.D. Kristina, T. Polonca, S. David, Microorganisms trigger chemical degradation of diazinon, Int. Biodeterior. Biodegrad., 62 (2008) 293–296.
[15] B. Abasalt, N.F. Ghazal, J. Pouran, Cleaning from the inside: biodegradation of organophosphate pesticides by *Pseudomonas plecoglossicida*, Biotechnol. Health Sci. 1 (2014) e19193.
[16] G. McMullan, C. Meehan, A. Conneely, Microbial decolorization and degradation of textiles dyes, Appl. Microbiol. Biotechnol., 51 (2001) 81–87.
[17] D.W. Sparling, G. Fellers, Comparative toxicity of chlorpyrifos, diazinon, malathion and their oxon derivatives to larval Rana boylii, Environ. Pollut. 147 (2007) 535–539.
[18] B.K. Shanmugam, S. Mahadevan, Metabolism and biotransformation of azo dye by bacterial consortium studied in a bioreaction calorimeter, Bioresour. Technol., 196 (2015) 500–508.

[19] Ö. Özşen, İ. Kıran, İ. Dağ, F. Demirci, Biotransformation of abietic acid by fungi and biological evaluation of its metabolites, Process Biochem., 52 (2017) 130–140.
[20] M.S. Mahmoud, Decolorization of certain reactive dye from aqueous solution using Baker's Yeast (*Saccharomyces cerevisiae*) strain, HBRC J., 12 (2016) 88–98.
[21] A. Sadeghi, M. Dolatabadi, S.N. Asadzadeh, Ability of the yeast *Saccharomyces cerevisiae* for biological removal of ciprofloxacin antibiotic in aqueous solution, J. North Khorasan Univ., 7 (2015) 71–79.
[22] Y.F. Joseph, S.E.G. Nour, Performance and kinetic studies on biosorption of Astrazon Blue dye by dried biomass of Baker's yeast as a low cost biosorbent, Biosci. Biotechnol. Res. Asia, 4 (2017) 359–370.
[23] S.M. Ahmed, I. Fawzia, N.S. El-Gendy, A kinetic study for the removal of anionic sulphonated dye from aqueous solution using nano-polyaniline and Baker's yeast, Arabian J. Chem., 9 (2016) S1721-S1728.
[24] I. Muhammad, E.C. David, K. Azeem, Microbial biotechnology for decolorization of textile wastewaters, Rev. Environ. Sci. Biotechnol., 14 (2015) 73–92.