Applied Water Science (2023) 13:166
https://doi.org/10.1007/s13201-023-01970-x

ORIGINAL ARTICLE

Use of *Saccharomyces cerevisiae* as new technique to remove polystyrene from aqueous medium: modeling, optimization, and performance

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Received: 23 April 2023 / Accepted: 12 July 2023 / Published online: 26 July 2023

Abstract

MPs are widely found in various environments. PS is the second most common microplastic in sediments, freshwater, soil, and coastal ecosystems. *S. cerevisiae* was studied as a biocoagulant due to its advantages such as ease of use, non-toxicity, large-scale cultivability and low cost. The aim of this study was to evaluate the efficiency of *S. cerevisiae* in removing PS from aqueous solutions. BBD was used to determine the optimal removal conditions. The MPs were washed, dried, crushed, sieved, and kept in a closed container to avoid exposure to light and moisture. PS removal was measured under various parameters such as the dose of *S. cerevisiae* (100–300 mg/L), the concentration of PS (200–900 mg/L), and the pH (4–10). The suspension of PS and *S. cerevisiae* was stirred and subjected to variable speeds to disperse yeast cells and contact with PS particles. The formed clots were settled under static conditions, and the suspended MPs in the aqueous solution were measured by filtering through Whatman filter paper and recording its weight after drying. The maximum PS removal efficiency was 98.81% under optimized conditions, i.e., the PS concentration of 550 mg/L, the yeast dose of 200 mg/L, and the pH of 7. With regard to the mentioned results, it can be said that *S. cerevisiae* can be used as a natural and environmentally friendly biocoagulant to remove PS.

Keywords *Saccharomyces cerevisiae* · Yeast · Microplastic · Polystyrene

Abbreviations

MPs	MPs
PS	Polystyrene
S. cerevisiae	*Saccharomyces cerevisiae*
BBD	Box–Behnken design
FT-IR	Fourier-transform infrared spectroscopy
EDX	Energy-dispersive X-ray
FESEM	Field emission scanning electron microscopy
ANOVA	Analysis of variance

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Introduction

Plastics used throughout life are made of organic and inorganic raw materials such as silicon, nitrogen, hydrogen, carbon, oxygen and chloride. Raw materials for making plastic are extracted from natural gas, coal and oil (Shah et al. 2008). According to the reports, the global production of plastic follows an increasing trend, so that their amount is expected to reach 445.25 million tons in 2025 from 1.5 million tons in 1950 (Rout et al. 2022). Plastics provide durable, lightweight, cheap and strong products, which is why around 300 million tons of plastic products are produced worldwide. Due to low recycling, 90% of plastics are discarded in aquatic environment (Du et al. 2022). Plastics in the environment can break down into smaller particles due to a process called photodegradation, which is caused by exposure to sunlight. This process can cause the plastic to become brittle and crack, leading to the formation of microplastics. Temperature can also play a role in the breakdown of plastics, as higher temperatures can accelerate the rate of degradation. Additionally, other factors such as wind, water, and mechanical stress can also contribute to the fragmentation of plastics into microplastic particles (Bonyadi et al. 2022a, b, c). Microplastic particles were first detected in the Sargasso Sea, an area in the North Atlantic Ocean. The term "microplastic" was first published in the scientific literature by Bayo et al. (2020) (Bayo et al. 2020). As an emerging pollutant, MPs may be more harmful than large plastic (Zhou et al. 2021). These emerging pollutants absorb other pollutants such as pharmaceuticals and personal care products (Esmaili et al. 2023), nanoparticles (Bonyadi et al. 2022a, b, c; Bonyadi et al. 2023), pesticides and herbicides (Pirsaheb et al. 2013), and heavy metals (Davodi et al. 2019). MPs enter the environment in two primary and secondary forms, which enter the environment directly from raw materials, and secondary, which are caused by the decomposition of large plastics by environmental factors such as sunlight, wind, and water (Napper et al. 2015). Due to their small size and sharp ends, MPs cause damage and inflammation in the organs of marine organisms. Also, due to their similarity to natural prey, MPs are ingested by organisms and cause malnutrition and reproductive disorders. In a study, it was demonstrated that MP can be transported through the gut into the circulatory system of aquatic species (Sun et al. 2019). These emerging pollutants accumulate in the body of marine organisms and enter the human body through the food chain (Okoye et al. 2022). MPs in various types, including polystyrene, polyethylene, polyvinyl chloride, polypropylene, and others, are released into seawater, fresh water, coastal waters, sediments, soil, foods, and even the human body [7, 8]. Polystyrene is the second most common microplastic in sediments, freshwater, soil, and coastal ecosystems, widely used in containers, protective packaging, lids, and bottles (Sun et al. 2019; Yilimulati et al. 2021). The mitigation of environmental pollution through the removal of organic pollutants from wastewater has emerged as a promising approach. Over the past few years, several studies have been conducted to explore the degradation of various pollutants through different techniques (Toolabi et al. 2017, 2018). So far, the removal of MPs has been done by different techniques such as membrane ultrafiltration (Gonzalez-Camejo et al. 2023), membrane bioreactor (Bayo et al. 2020), filtration (Bitter et al. 2022), rapid sand filtration (Bitter et al. 2022), sedimentation and disinfection. Among these, methods based on coagulation and flocculation mechanisms are more common due to the formation of large flocs of MPs that are removed from water by sedimentation. In a study, it has been proven that the coagulation process was effective in removing polyethylene MPs (Bayarkhuu et al. 2022). Using chemical coagulants to remove these pollutants causes environmental and health problems. The problems caused by the use of chemical coagulants in water treatment include the production of large amounts of sludge, the indestructibility of chemical compounds, having residues in water, biological accumulation in the body of organisms, and negative effects on human health such as the occurrence of Alzheimer's disease and dementia (Mazloomi et al. 2018). Therefore, these problems have caused the use of biological coagulants to be considered as a suitable alternative to chemical coagulants. Microorganisms can remove emerging pollutants from aqueous solutions through mechanisms such as biological decomposition, biosorption, and biocoagulation. Biological decomposition breaks down pollutants into simpler compounds, while biosorption involves adsorbing pollutants onto the surface of microorganisms (Nasoudari et al. 2021) The advantage of using biocoagulants includes positive features such as compatibility with the environment, cost-effectiveness, and biodegradability (Amran et al. 2021; Bonyadi et al. 2022a, b, c). Yeast, belonging to the family of unicellular fungi, is widely present in nature (Yang et al. 2014). *S. cerevisiae*, which is known as baker's yeast, in the processes of removing pollutants from the environment due to its advantages such as ease of use, non-toxicity, ability to be cultivated on a large scale and low cost (Hadiani et al. 2018). Skaf et al. (2020) investigated the removal of microplastic particles from simulated drinking water through alum (Skaf et al. 2020). Zhang et al. (2020) showed that enzymes extracted from *Aspergillus fungus* minimize polyethylene microplastic particles through biodegradation (Zhang et al. 2020). Sánchez (2020) investigated the potential of fungi to degrade petroleum polymers. According to its results, fungi can use MPs as a source of carbon and energy. Also, due to having an enzyme system with the ability to detoxify pollutants and the ability of their cells to penetrate three-dimensional layers, it can be said that fungal species have a high potential in reducing microplastic pollution (Sánchez 2020). Cunha et al. (2020) used microalgae based on biopolymers to remove nano and MPs (Cunha et al. 2020). *S. cerevisiae* is an effective tool for removing pollutants and microplastics from the environment due to its ability to adhere to microplastic particles and remove them through a process called biocoagulation. This yeast has been used in various biotechnological applications (Ramavandi et al. 2019; Sadeghi et al. 2019; Mazloomi et al. 2021), making it a promising and environmentally friendly solution for pollutant removal.

Therefore, the removal of polystyrene MPs by *S. cerevisiae* from aqueous solutions is important. Further, the characteristics of *S. cerevisiae* and polystyrene were investigated by FESEM, EDX and FTIR tests.

Materials and methods

Chemicals and reagents

Commercial grade plastic materials were prepared from Pishgaman Plastic Company. *S. cerevisiae* (ATCC 9763) was obtained from Iran Science and Technology Research Organization. The chemicals were obtained from Merck, Germany.

Preparation of PS MPs

First, the plastic granules were washed with concentrated hydrochloric acid. Then, they were dried in the oven at a temperature of 60 °C for 24 h. Then, the plastic granules were crushed into small particles and sieved in sizes less than 100 µm. The MPs were kept in a closed glass container in a dark environment to avoid exposure to light and moisture.

Design of experiments

In this study, the main parameters, such as pH (4–10), *S. cerevisiae* dose (100–300 mg/L), and initial PS concentration (200–900 mg/L), were tested (Table 1). The size of microplastic in all samples was less than 100 µm. All experiments were done in a 300-ml flux containing 200 ml of reaction mixture.

All tests were done at room temperature. At first, based on the parameters defined in Table 1, 200 ml of reaction mixture containing the different amounts of PS and *S. cerevisiae* was prepared. Then, the prepared suspension was subjected to variable speed of 400 rpm for 1 min, to disperse yeast cells and contact with PS particles. Subsequently, in order to promote the formation of sizable clots, the suspension was agitated at 100 rpm for a duration of 15 min. Upon completion of this stage, the mixture was transferred to an Imhof funnel and allowed to settle undisturbed for a period of 30 min, facilitating the formation of clots. The supernatant was filtered using Whatman paper to measure the suspended MPs in the aqueous solution, and then filter paper. It was dried in an oven at 60 °C for 24 h and the weight of the paper was recorded (Zhou et al. 2021). PS removal efficiency was calculated by the following formula:

Table 1 Range and levels of main factors applied for the PS removal

Factor	Variable level			
	Code	− 1	0	+ 1
PS Conc. (mg/L)	*A*	200	550	900
S. cerevisiae (mg/L)	*B*	100	200	300
pH	*C*	4	7	10

$$R\,(\%) = \frac{M_1 - M_2}{M_1} \times 100\% \tag{1}$$

where M_1 and M_2 are PS weight before and after the removal process, respectively.

Modeling PS removal

This study was designed applying the response level method, BBD, to optimize the removal efficiency of microplastic by *S. cerevisiae*. The quadratic model, suggested by BBD, was expressed as the following formula:

$$Y = \beta_0 + \sum\nolimits_{i=1}^{k} \beta_i x_i + \sum\nolimits_{i=1}^{k} \beta_{ii} x_i^2 + \sum\nolimits_{1\le i\le j}^{k} \beta_{ij} x_i x_j \tag{2}$$

In this formula, Y, β_i, β_{ij}, β_{ii}, β_0, and x_j or x_i, respectively, express predicted response, regression coefficients for linear impacts, interaction coefficients, quadratic coefficients, the constant coefficients, and coded values of factors (Anbarani et al. 2023).

Results and discussion

Characterization

FT-IR

Figure 1a illustrates the FT-IR spectra for PS before removal process. The peak at 807.87 cm^{-1} was associated with the presence of a benzene ring in PS, which disappeared after coagulation (Zhou et al. 2021). The peak at 1656.29 cm^{-1} indicated the presence of C=O in PS (Yao et al. 2023). The band appeared at 2926.04 cm^{-1} is related to C–H asymmetric stretching (Lin et al. 2020). The peak at 3377.56 cm^{-1} was attributed to the O–H stretching vibration (Du et al. 2022). The peak at 1402.20 cm^{-1} introduces the PS carbon chain. The peak appeared at 1241.07 cm^{-1}, which corresponds to the ester groups in PS (Sun et al. 2021).

Figure 1b represents the FT-IR spectra after the removal process. The C=O peak for the flocs shifted from 1656.29 to 1657.08 cm^{-1} (Lu et al. 2021). After the elimination process, the O–H peak changed to 3404.22 cm^{-1} (Chen et al. 2016). The peak at 1152.62 cm^{-1} indicates ether and ester groups. In fact, O–H and ester provide an active site for the attachment of coagulant particles (Wang et al. 2023; Onukwuli et al. 2021). A new peak appeared at 1601.48 cm^{-1}, which confirms the presence of (C=O–) group. Based on previous studies, (O–H) and (C=O–) groups offer evidence of microplastic removal by yeasts (Zhang et al. 2020). The change

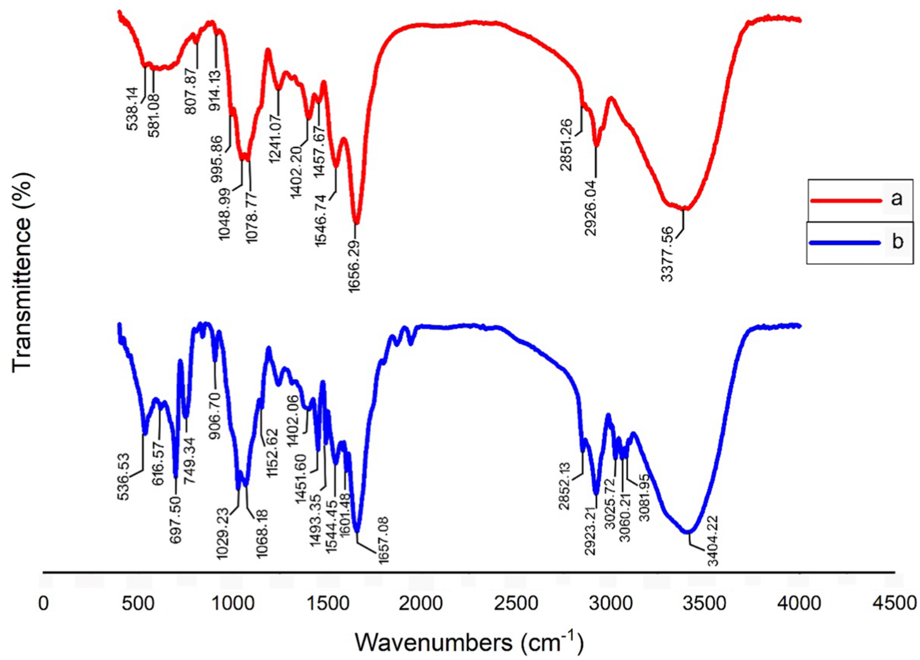

Fig. 1 FTIR spectra of **a** before and **b** after PS removal

in the peak from 1402.20 to 1402.06 cm^{-1} was caused by a decrease in carbon content in the sludge. The peak at 2923.21 cm^{-1} represents the C-H group, which did not change significantly compared to before removal.

FESEM

The microscopic morphology of *S. cerevisiae* and PS after elimination is represented in Fig. 2. According to Fig. 2, PS particles are accumulated on the surface of *S. cerevisiae* fact, the pores of S. cerevisiae are a suitable space for keeping PS particles (Machado et al. 2008). Hydrophobins are proteins produced by yeasts. Negatively charged MPs can bind to the hydrophobin surface. As a result, large spherical clots are produced. Therefore, it can be said that the surface charge neutralization process has occurred. Usually, due to being hydrophobic, yeasts have unique properties such as high adhesion, firm structure, and suitable surface activity that enable attachment to plastic surfaces. The effect of yeast on plastic includes changes in molecular weight, elasticity, crystallinity and fragmentation of functional groups on the plastic surface (Sánchez 2020). This evidence proves that *S. cerevisiae* acts as a coagulant.

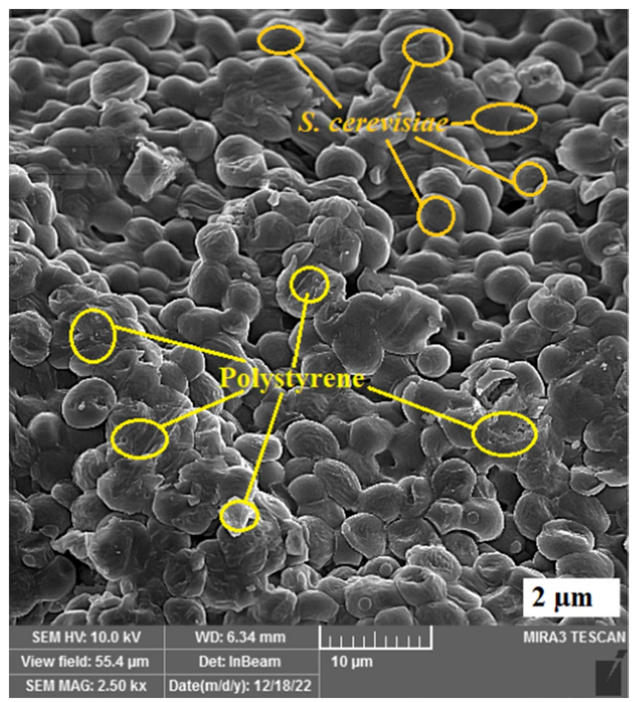

Fig. 2 FESEM images of the floc. PS and *S. cerevisiae*

EDX

The technique of energy-dispersive X-ray spectroscopic patterns (EDX) was used to analyze the elements of PS particles. Figure 3a–b shows the EDX of PS particles before and after removal process. In this analysis, the percentages of carbon, nitrogen, oxygen and phosphorus elements are mentioned. As shown in Fig. 3a, the percentage of carbon in PS particles before the coagulation process was 88.09%, and other elements accounted for small percentages. It can also be seen carefully in Fig. 3b that the percentage of carbon in the mixture of PS and *S. cerevisiae* has decreased and other elements

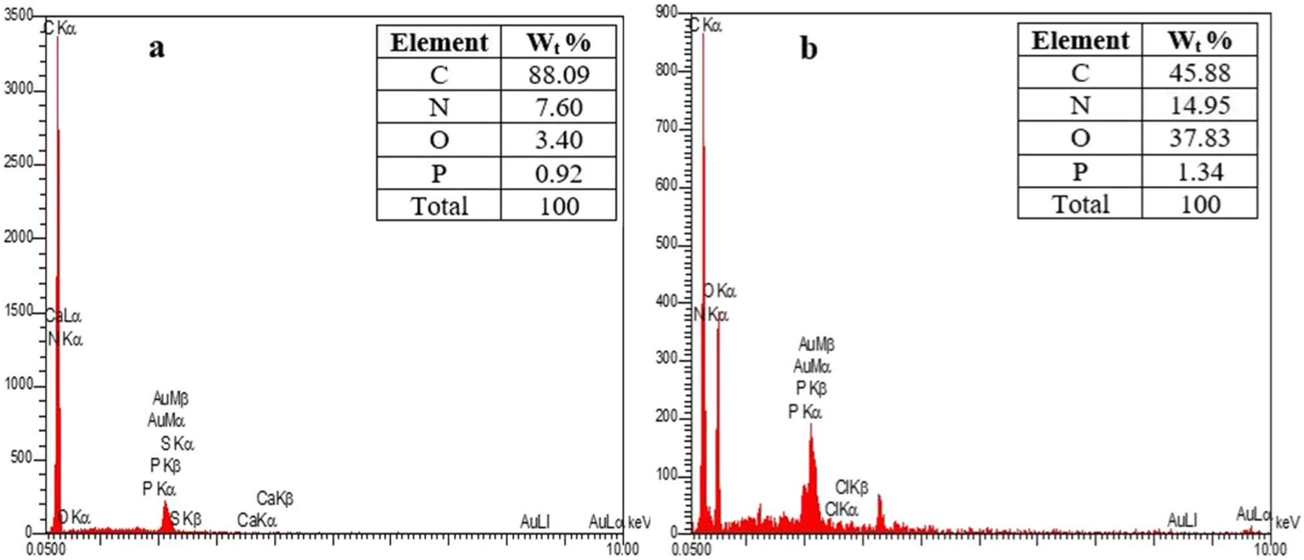

Fig. 3 EDX spectrum of PS **a** before and **b** after PS removal

Table 2 BBD matrix for PS removal by *oak* seed

Run no	Coded variable			Removal (%)	Run no	Coded variable			Removal (%)
	A	*B*	*C*			*A*	*B*	*C*	
1	200	200	4	73.5	10	550	200	7	98.81
2	200	200	10	88.25	11	550	200	7	87.81
3	200	100	7	59.75	12	550	300	4	65.81
4	200	300	7	51.25	13	550	100	4	50.45
5	550	100	10	51.6	14	900	200	4	75.38
6	550	300	10	84.4	15	900	300	7	63.66
7	550	200	7	88.45	16	900	200	10	71.5
8	550	200	7	90.63	17	900	100	7	27.77
9	550	200	7	92.9					

Table 3 Statistical adequacy evaluation of models

Source	Sequential *p* value	Lack of Fit *p* value	Adjusted R^2	Predicted R^2
Linear	0.4919	0.0030	−0.0293	−0.4204
2FI	0.6889	0.0019	−0.1627	−1.3611
Quadratic	< 0.0001	0.4385	0.9444	0.8014
Cubic	0.4385		0.9473	

have increased. So that the amounts of carbon, oxygen, nitrogen and phosphorus in the studied samples were 45.88%, 37.83%, 14.95%, and 1.34%, respectively. The high content of carbon and oxygen facilitates the formation of hydrogen bonds with the coagulant and improves the ability of PS to bridge between clots or absorb them (Yao et al. 2023).

Response model

In this test, the effect of *S. cerevisiae* on PS removal from aqueous media was investigated. Table 2 indicates PS removal by *S. cerevisiae*.

Table 4 Coefficients of estimation for the QM of PS removal by *saccharomyces cerevisiae*

Factor	Coefficient estimate	*df*	Standard error	95% CI low	95% CI high	VIF
Intercept	91.72	1	2.04	86.9	96.54	
A-Conc	−4.31	1	1.61	−8.11	−0.4954	1
B-Dose	9.44	1	1.61	5.63	13.25	1
C-pH	3.83	1	1.61	0.0167	7.64	1
AB	11.1	1	2.28	5.71	16.49	1
AC	−4.66	1	2.28	−10.05	0.73	1
BC	4.36	1	2.28	−1.03	9.75	1
A^2	−13.51	1	2.22	−18.76	−8.26	1.01
B^2	−27.6	1	2.22	−32.85	−22.35	1.01
C^2	−1.05	1	2.22	−6.3	4.2	1.01

Table 5 ANOVA for the QM of PS elimination by *S.cerevisiae*

Model	Sum of squares	*df*	Mean square	*F*-value	*p* value
	5834.72	9	648.30	31.22	< 0.0001
A-Conc	148.26	1	148.26	7.14	0.0319
B-Dose	713.48	1	713.48	34.36	0.0006
C-pH	117.12	1	117.12	5.64	0.0492
AB	492.62	1	492.62	23.72	0.0018
AC	86.77	1	86.77	4.18	0.0802
BC	76.04	1	76.04	3.66	0.0972
A^2	768.51	1	768.51	37.01	0.0005
B^2	3207.99	1	3207.99	154.50	< 0.0001
C^2	4.66	1	4.66	0.2246	0.6500
Residual	145.35	7	20.76		
Lack of Fit	66.52	3	22.17	1.13	0.4385
Pure Error	78.83	4	19.71		
Cor Total	5980.07	16			
R^2	0.97		Predicted R^2	0.80	
Adjusted R^2	0.94		Adeq Precision	18.87	

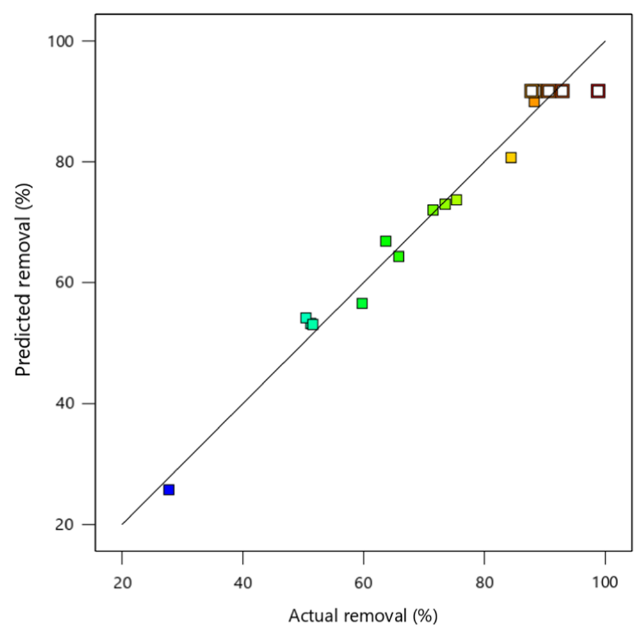

Fig. 4 Distribution of experimental versus predicted removal for PS by *S. cerevisiae*

Based on Table 2, the lowest and highest PS removal efficiency was 27.77% and 98.81%, respectively. The experimental findings for 2FI, linear, cubic and quadratic models were statistically estimated to determine the best model. Table 3 exhibits the decision of the statistical adequacy of the models.

Table 4 demonstrates the quadratic model coefficients of PS elimination by *S. cerevisiae* particles. Table 4 shows the fit of the quadratic model (QM) to the experimental data.

From Table 4, the QM of PS removal pursuant to the coded factors is presented in Eq. (3):

$$\text{Removal }\% = 91.72 - 4.32\text{A} + 9.44\text{B} + 3.83\text{C} + 11.10\text{AB} - 4.66\text{AC}\ 4.36\text{BC} - 13.51\text{A}^2 - 27.60\text{B}^2 - 1.05\text{C}^2 \quad (3)$$

Accordingly, each model consists of two variable and fixed components. With regard to the various laboratory parameters, the elimination rate was predicted to be 91.72%. The coded factors of A, B, and C had the coefficients of −4.31, +9.44, and +3.83, respectively. The dose of *S. cerevisiae* (code B) had the greatest effect on the removal efficiency with a coefficient of 9.44. AB had the highest interaction effect with a coefficient of 11.1 and B^2 had the highest square effect with a coefficient of 27.6. Table 5 demonstrates the analysis of variance (ANOVA) for the QM of PS elimination by *S. cerevisiae* particles.

The values of R^2, predicted R^2, adjusted R^2, and adequacy precision were determined 0.97, 0.80, 0.94 and 18.87, respectively. Based on Table 5, the *p* value for all the coded factors, including *S. cerevisiae* dose, PS concentration, and pH, was obtained to be less than 0.05. Therefore, all three variables are significant. In this model, the difference between R^2 and predicted R^2 was less than 0.2. Therefore, this model is correct. Figure 4 illustrates actual versus predicted removal, showing the adequacy of the model to provide a good prediction for PS removal.

The effect of main parameters on removal efficiency

Figure 5 indicates the impact of important parameters, containing *S. cerevisiae* dose, PS concentration, and pH and on the removal rate. It is necessary to note that to express the effect of one parameter on a response; other factors are fixed at the zero level. For example, when the pH variable increases from level 1 to + 1, other variables such as *S. cerevisiae* dose *and PS* concentration are constant at zero level.

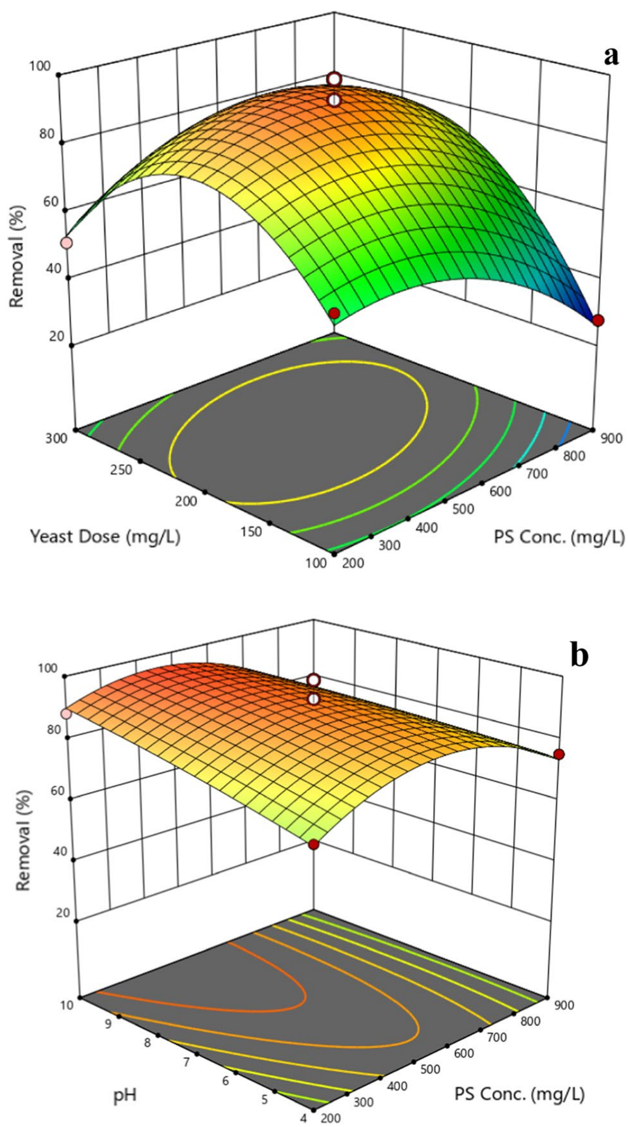

Fig. 5 Response surface plot about the effects of dose vs. concentration (**a**) and pH versus concentration (**b**)

Adsorbent dose effect

Determining the appropriate dose for *S. cerevisiae* is important. According to Fig. 5a, the highest PS removal efficiency (98.81%) was discovered at the dose of 200 mg/L *S. cerevisiae* (p value < 0.05). Generally, with the increase in S. cerevisiae dosage, the removal rate of PS enhances. The reason for the low removal efficiency can be explained as small, fragile and unstable flocs are formed in low dosages of yeast. Therefore, the removal of microplates is reduced due to the suspension of flocs and their weak sedimentation. By increasing the dose of *S. cerevisiae*, yeast cells linked to MPs stick together and form larger flocs, which leads to their rapid sedimentation and high efficiency (Ma et al. 2019). Due to its hydrophobin properties, *S. cerevisiae* can adhere to hydrophobin substrates through cell penetration into the layers of MPs (Sánchez 2020). Frantz et al. (2020) performed coagulation and flocculation using shrimp waste, and the results showed that the percentage of turbidity removal at a dose of 200 mg/L of coagulant was approximately 100%, but at coagulant doses higher than 200 mg/L, the removal decreased (Frantz et al. 2020). Machado et al. (2008) investigated the removal of heavy metals by *S. cerevisiae*. The results showed that at the dose of 0.25 g/L, sedimentation was weak, but at the dose of 0.5 g/L, complete sedimentation occurred. This evidence is consistent with the concept of "critical cell density" proposed by Miki et al. 1982 (Miki et al. 1982). "Critical cell density" means that the presence of a low dose of *S.cerevisiae* cells limits the possibility of attachment and the rate of coagulation is practically zero (Machado et al. 2008). In a study, yeast enzymes could shorten the length of microplastic polyethylene chains and eliminate or reduce MPs (Restrepo-Flórez et al. 2014). In another study, fungi can remove polyurethane and polyethylene better than bacteria (Muhonja et al. 2018; Sánchez 2020).

Effect of initial PS concentration

PS concentration was one of the important factors in PS removal. Figure 5a indicated that the PS removal efficiency was increased by 8.12% from 200 to 500 mg PS concentration and then decreased by 16.73% from 500 to 900 mg/L concentration (p value < 0.05). Considering that PS particles are mostly negatively charged. Therefore, at high concentrations of PS, repulsive forces prevail between particles, which lead to their floating in aqueous solutions. At low concentrations of PS, van der Waals forces between PS particles are dominant due to the bonding with a balanced ratio of yeasts, which results in the production of large and strong flocs. Yao et al. (2023) investigated the microplastic characteristics of PS in the coagulation process. The results showed that when the PS concentration increased from 0.25 to 1 g/L, the removal efficiency was high. While the concentration of PS increased from 1 to 5 g/L, the percentage of PS removal decreased (Yao et al. 2023).

pH effect

The solution pH can affect the process of removing MPs by affecting the balance of negative and positive charges in PS particles and yeast cells, respectively (Zhang et al. 2021). Figure 5b shows the removal efficiency of PS MPs at different pH. From Fig. 5b, PS removal enhances with increasing pH (p value < 0.05). According to the findings, maximum and minimum removal of PS occurred at pH 10 and 4, respectively. The reason can be interpreted as that in alkaline pH, there is more negative charge on the surface of PS. As a result, it increases the probability of yeast cells with positive surface charge to bind to PS particles and also increases coagulation (Sillanpää et al. 2018). On the other hand, the size of the flocs in alkaline pH is larger than in acidic pH, provides optimal conditions for flocs deposition and thus increases the PS removal efficiency (Zhou et al. 2021).

Optimum operational conditions

In this study, we utilized BBD to analyze the results and determine the optimal conditions for dye removal. Based on the quadratic model, we found that the highest removal rate (97.4%) was achieved at a pH of 10, a yeast dose of 219.96 mg/L, and PS level of 462.97 mg/L.

Conclusion

In this study, the use of *S. cerevisiae* yeast as a natural coagulant to remove polystyrene MPs was evaluated. To achieve the optimal conditions of PS removal using Box–Behnken model, the effect of different parameters such as yeast dose, PS concentration, and pH was investigated. In this study, FT-IR, FESEM and EDX analyses were performed. FT-IR was performed to determine the functional groups active in the process. FESEM also investigated the effect of surface morphology, which showed the accumulation of *S. cerevisiae* yeast on the surface of PS particles. EDX analysis was also performed to determine the percentage and name of the elements in polystyrene before and after the coagulation process. According to the findings, the relationship between the removal efficiency with yeast dose and pH was direct, while it was inverse with PS concentration. Moreover, alkaline conditions were favorable for PS removal. The maximum PS removal efficiency was 98.81% under optimized conditions, i.e., the PS concentration of 550 mg/L, the yeast dose of 200 mg/L, and the pH of 7. With regard to the mentioned results, it can be said that *S. cerevisiae* can be used as a natural and environmentally friendly coagulant to remove PS.

Acknowledgements Not applicable.

Author contributions MZ performed the experiments and wrote the paper; AE designed the experiments and analyzed data; ZB wrote and edited the paper and conceived and designed the experiments.

Funding The authors would like to thank the financial support provided by the Student Research Committee of Mashhad University of Medical Science (Iran) through the Grant Number of 4010688.

Data availability All necessary data are included in the document.

Declarations

Conflict of interest The authors declare that they have no conflict of interests.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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