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Investigation of the removal of cyanide from aqueous solutions using biomass *Saccharomyces cerevisiae*

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ABSTRACT

The industrial revolution and the rise of factories and industrial towns caused the increasing production of wastewater that contains hazardous compounds in the aqueous ecosystems. The purpose of this study was to investigate the removal of cyanide from aqueous solutions using yeast of *Saccharomyces cerevisiae* biomass. In this experimental–interventional study, to measure the concentration of cyanide, the titrimetric method was used. After determining the concentration of cyanide in the samples exposed to the *S. cerevisiae* yeast, the removal rate was calculated. In the concentration of 5 mg/l of cyanide and at 15, 30, 60, 90 min of contact, yeast weight values of 0, 0.5, 1, 1.5 g/l, at the pH of 5, 7, 9 were studied. The data were statistically analyzed using SPSS 16 software. The study showed that with increasing the contact time of the yeast, cyanide removal efficiency increased. Concentration of 0.5 g/l of yeast at pH of 9 and contact time of 15 min had the lowest percentage of removal of cyanide, while concentration of 1.5 g/l of yeast at pH of 7 and contact time of 90 min had the highest level of removal of cyanide. About 0 g/l yeast concentration at all times showed a significant relationship ($p < 0.001$). According to the findings, this yeast is a suitable adsorbent for the removal of cyanide ions from wastewater. The yeast is easily produced in the very cheap fermentation process medium. Therefore, by replacing the expensive and noneconomical treatment methods with yeast biomass, contaminated wastewater can be treated.

Keywords: Cyanide; *Saccharomyces cerevisiae*; Biological absorption

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1. Introduction

Technological and industrial advancement followed by the formation of industrial revolution, rise of factories and industrial towns caused the increasing production of wastewaters containing hazardous compounds, such as hydrocarbons, oils and lipids, phenol and some other materials in aqueous ecosystems [1–3]. One of these materials is cyanide that is a toxic carbon–nitrogen radical and is very poisonous and dangerous. It is abundant in the wastewater of iron and steel, coal, automobile parts manufacturing, plastic works, plating industries as well as gold and silver mines. According to the statistics provided by different organizations and researchers, nearly 1.4 million tons of cyanide is annually entered into the environment by different industries worldwide, which are presently considered as one of the biggest threats for water resources, environment and even aqueous microorganisms [4–6]. The toxicity of the cyanide is because of its inclination to stick to iron in cytochrome oxidase, intervention in electron transfer and eventually causing anoxia in body tissues. Side effects resulted from short-term exposure to cyanide includes dizziness, headache, anesthesia, and convulsion, while long-term exposures lead to weight loss, effects on the thyroid, neural damages, and death [7–10]. The cyanide limits in freshwater determined by WHO is 0.07 mg/l, and by European Union is 0.05. The Indian Central Pollution Control Board (CPCB) and US environmental protection agency (USEPA) determined the cyanide limits as 0.02 mg/l in the wastewater [1,11–13]. Iranian environmental protection agency determined the cyanide limitation for discharging in surface waters as 0.2 mg/l, and in agricultural, irrigation, and absorbent wells as 0.1 mg/l [14,15].

However, the usual methods to standardize the industries' wastewater for aqueous ecosystems have been considered by researchers. These methods include surface absorption (active carbon), complex formation (metal addition), hydrolyze/distillation, electrowinning, oxidation (ozone, etc.), and biological methods [16,17]. Applying these methods often face limitations due to the higher costs of purification, additional purification requirement, sludge generation, increasing ions in solution, and low efficiency [18]. Biological methods, on the other hand, including biomass and absorption do not have the deficiencies of non-biological methods and are environmentally friendly. They produce no dangerous materials and are highly resistant against poisonous shocks [19–22]. The bioaccumulation may decrease the concentration of toxic ions from ppt to ppb in aqueous ecosystems by the aid of biological organisms including fungi creating complex [7,23,24]. Therefore, it is considered as an ideal option for purifying large amount of wastewater with low concentration of toxic ions. Certain studies have used *Saccharomyces cerevisiae* because of the high capacity of connection with toxic compositions and high ability of absorbing cell walls compared to other microorganisms [25–27]. This yeast is easily obtained from fermentation process in an unexpensive culture medium and it has considerable biomass. It can also be obtained from various food industries and fermentation processes as a waste material which its preparation is simple compared to other microbial wastes. In numerous studies, the effect of *S. cerevisiae* on chemical and toxic compositions has been investigated [28–31]. For example, Iwuoha et.al indicated that bioabsorption of *S. cerevisiae* can decrease 94.7% of acid hydrocyanic contents of warts on Cassava plant [32]. Prasad and Dhanya also detoxified cyanide contents of maize by *S. cerevisiae* [33]. Roy et.al found that bio-accumulation of *S. cerevisiae* was an effective factor for removing EU and Co [34]. The study performed by Ghaedi et al. entitled the absorption quality of biologic yeast of *S. cerevisiae* for removing Pb^{2+} from an aqueous solution showed that this yeast had the bioabsorption capacity of 89.9 mg/g [14]. Thus, due to the importance of the issue and the growth of industries in Iran, as a developing country, and the necessity of purifying wastewaters containing cyanide components and because of the water shortage and water resources protection, this study was performed to investigate the rate of cyanide removal from aqueous solutions simulated as using bioyeast of *S. cerevisiae*. Thereby, an effective step would be taken to restore the water required by human life.

2. Materials and methods

This experimental–interventional study was performed in the chemistry laboratory center of Health Faculty of Kashan University of Medical Sciences in 2014. The commercial strain of dried yeast and inactivated *S. cerevisiae* was purchased from Dezfool factory registered under no. 87977 and manufacturing license of 23/11557. *S. cerevisiae* was used as biomass in this study to remove cyanide. The yeast was filtered through a screen with the mesh size of 1 mm, and then, the slag yeast was added to the samples with specified weights. Determined weights of the yeast was added into 500 cc samples of the cyanide aqueous solution and was mixed in a special Meyer flask by an electric mixer with a speed of 300 rpm for the determined times. Then, samples were passed through Whatman filter paper rated 0.45 using vacuum pump

and Buchner funnel to separate weight values of yeast from the solution. After that, in order to prepare the artificial and handmade contaminated water, the potassium cyanide (produced by Merck in Germany) was added in to the distilled water. Titrimetric method standard no. 4500 was used to measure the residual cyanide concentration and silver nitrate ($Ag^{+}(NO_3^{-})$) was used as the indicator made by Merck. In this step, titration was allowed as long as the solution changed its color from canary yellow to pinkish. The amount of consumed titrant was recorded and the concentration of residual cyanide was calculated by the following formulation. After specifying the concentration of the cyanide samples exposed to *S. cerevisiae* in given weights and considering the cyanide initial concentrations, the removal rate was calculated. Also, factors effective on the process including exposure time of 15, 30, 60, and 90 min, weight values of applied yeast including 0. 0.5, 1, 1.54 g/l, cyanide concentration of (5 mg/l) and different pH levels including 5, 7, and 9 through separated steps were investigated under laboratory conditions. The cyanide levels were calculated through the following equation:

$$\text{mg CN/L} = \frac{((A-B)\times 1000)}{\text{ml orginal sample 1}} \times \frac{250}{\text{ml portion used}}$$

where A is the consumed titrant (ml) for the sample and B is the consumed titrant for control (ml). In order to be ensured of the obtained results, all experiments were performed three times and the average of measured values was used. All the measures taken in the laboratory were based on standard methods [35]. Finally, the obtained data were statistically analyzed by SPSS 16 software.

2.1. Results and discussion

In this study, the effects of variables of yeast concentration, exposure time, and pH on the cyanide removal efficiency were investigated. The highest level of cyanide removal efficiency was obtained in 30, 60, and 90 min with pH of 5 and 1 g/l yeast concentration so that after 30 min the cyanide concentration reduced to 2.1 mg/l from 3.8 mg/l. Also, after 60 and 90 min of exposure, this value was 1.4 and 0.5 mg/l, respectively. In this level of pH, the yeast concentrations of 0.5 and 1.5 mg/l were in line and had a small difference in the removal process (Fig. 1). The cyanide concentration was identically removing by passing of time in all pH values so that its concentration did not reach lower than 3.5 mg/l in average. In pH of 7 and concentration of 1.5 g/l of the yeast, the highest level

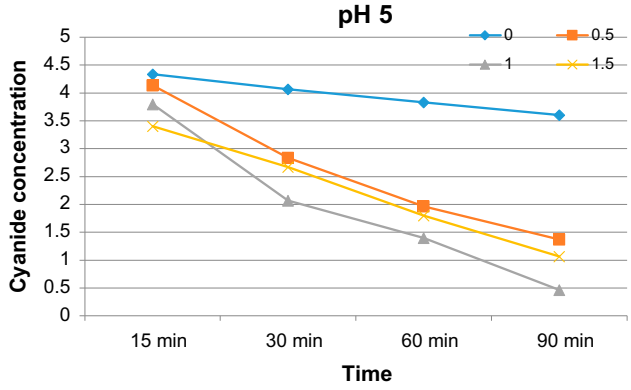

Fig. 1. Cyanide averaged absorbed concentration mg/l by the yeast g/l over different times at pH 5.

of cyanide removal was observed in all times so that within 15 min of exposure, the cyanide concentration was lowered to half and by passing of time it quickly reached 0.2 up to the end of experiment. The concentration of cyanide decreased to 1 and 0.6 mg/l, respectively in the concentrations of 0.5 and 1 g/l of the yeast over 90 min of exposure (Fig. 2). For pH of 9, the yeast concentration of 1 and 1.5 g/l had both the highest removal efficiency among the others so that at the end of 90 min the cyanide concentration was 0.5 mg/l (Fig. 3). As seen in Fig. 3, the cyanide removal efficiency in terms of the yeast concentration shows that by increasing the yeast concentration over 1 g/l (1.5 g/l) over 30 min of exposure, desorption occurs and fully overlaps to 1 g/l concentration of the yeast.

Table 1 shows the average and standard deviations of cyanide removal rate during different times in terms of pH and yeast concentration. As seen, the concentration of 0.5 g/l of the yeast at pH of 9 and

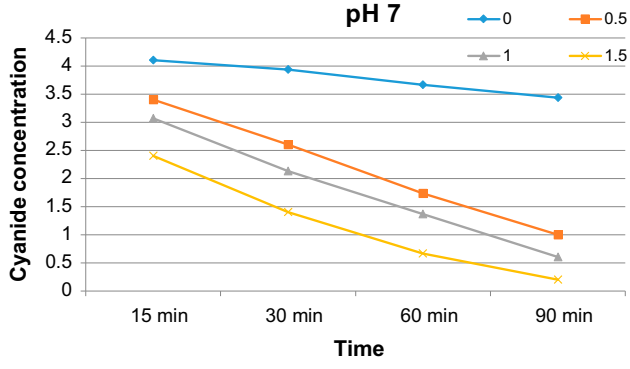

Fig. 2. Cyanide averaged absorbed concentration mg/l by the yeast g/l over different times at pH 7.

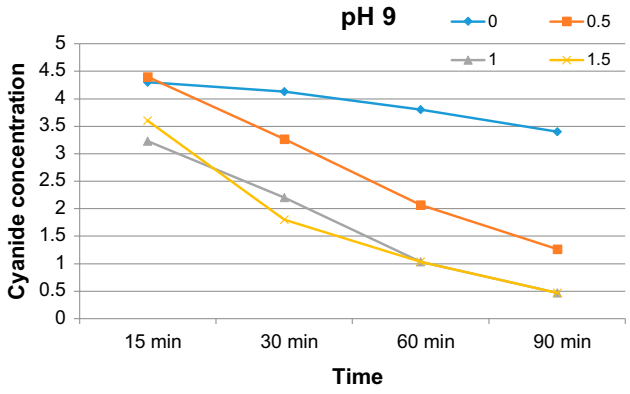

Fig. 3. Cyanide averaged absorbed concentration mg/l by the yeast g/l over different times at pH 9.

exposure time of 15 min has the lowest rate of cyanide removal. Although it was expected that the removal rate would increase with the increase in pH but it did not happen and even reduction was somehow observed. As Table 1 shows, on the average, the highest rate of cyanide removal is realized when pH is 7 and yeast concentration is 1.5 g/l with exposure time of 90 min.

Based on performed statistical analyses (one-way variance analysis), the cyanide removal rate at different pH levels and times was not significant statistically compared to other concentrations (PV = 0.5). However, the relationship between time and yeast concentration with other concentrations was investigated separately. As Table 2 shows, there is a significant relationship. As seen in the table, due to the absence of the yeast (0 g/l concentration), there is a significant relationship in all times except for 15 min of exposure time at the yeast concentration of 0 g/l. In all times, there is a significant relationship when the yeast concentration is 0.5, 1, and 1.5 g/l except the concentration of 0.5 at the exposure time of 15 min which is because of low yeast concentration. There is not any significant relationship at any time for the concentration of 1 g/l of the yeast compared to 1.5 g/l.

Table 2
One-way variance analysis between yeast concentration and exposure time

Time				Concentration (g)	
90	60	30	15		
<0.001	<0.001	<0.001	0.3	0	0.5
<0.001	0.012	0.003	0.023	1	
<0.001	0.001	<0.001	0.002	1.5	
<0.001	<0.001	<0.001	0.002	0	1
0.7	0.24	0.46	0.37	1.5	
<0.001	<0.001	<0.001	<0.001	0	1.5

3. Results and discussion

This study indicates that as the exposure time increases, the cyanide removal efficiency also increases. By the increase in the reaction time, the possibility of the contact of yeast and absorbed material (cyanide) increases. As a result, the absorption capacity of yeast cell walls is increased and a higher rate of removal is reported. The increasing absorption level as a result of increasing exposure time may be due to the presence of the micropores structure on the

Table 1
The average and standard deviation of cyanide removal rate during various time lapses based on pH and yeast concentration

Concentration					pH
1.5	1	0.5	0	Time	
32 ± 6.92	24 ± 10.58	17.33 ± 4.61	13.33 ± 2.30	15	5
46.66 ± 8.32	58.66 ± 12.22	43.33 ± 7.02	18.66 ± 2.30	30	
64 ± 10.58	72 ± 11.13	60.67 ± 3.05	23.33 ± 1.15	60	
78.66 ± 10.06	90.66 ± 4.16	72.66 ± 5.03	28 ± 0	90	
52 ± 8	38.66 ± 16.16	32 ± 6.92	18 ± 3.46	15	7
72 ± 10.58	57.33 ± 7.02	48 ± 10.58	21.33 ± 6.42	30	
86.67 ± 6.11	72.67 ± 10.26	65.33 ± 4.61	26.67 ± 4.16	60	
96 ± 3.46	88 ± 6	80 ± 4	31.33 ± 8.08	90	
28 ± 8	35.33 ± 32.66	12 ± 13.85	14 ± 5.29	15	9
64 ± 14.42	56 ± 4	34.66 ± 14.04	17.33 ± 2.30	30	
79.33 ± 7.02	71.33 ± 4.16	58.67 ± 12.22	24 ± 3.46	60	
90.66 ± 6.11	89.33 ± 5.03	74.66 ± 7.02	32 ± 4	90	

absorbent surface of the yeast. The results indicate that as the initial concentration of the yeast increases from 0 to 1.5 g/l, the cyanide removal efficiency increases. When the yeast concentration is lower than 1 g/l in the solution, in addition to surface absorption, ions may infiltrate into the yeast cell and as a result promote the absorption level per 1 g of the yeast, while this process is blocked when the yeast concentration is increased above 1 g/l at exposure time of 90 min. Another reason is that extra accumulation and aggregation of cell components occurs in the high yeast concentrations which results in reduction of active locations for suitable ion absorption. The highest removal efficiency occurs at 90 min contact time and concentration of 1 g/l. In spite of increasing initial concentration of the yeast lead to increasing number of absorption places. Results indicate that the highest cyanide removal efficiency is observed at pH of 7 or the neutral condition. When alkaline pH is 9, the removal efficiency is higher compared to acidic level of 5. As seen in the charts, the effects of 3 different pH levels (5, 7, and 9) are studied on cyanide removal rate by the yeast. Generally, the level of yeast adaption to alkali conditions grows when exposure time increases. Generally, charts indicate that regardless of different absorption rates, the absorption happens anyway at all pH levels and the cyanide ion is removed from the solution that suggests the application of biological methods for removing toxic ions of cyanide from industrial wastewaters in different pH levels. Aksu found the considerable effects of color bioaccumulation of textile industry wastewater by *S. cerevisiae* (more than 85 mg/l) [36]. Brady and Duncan succeeded to considerably decrease the metal cations from industries wastewater by *S. cerevisiae* bioaccumulation during a certain time and temperature [37]. Parvathi et.al in their investigation on bioabsorption of lead from battery-manufacturing factories wastewater by *S. cerevisiae* observed that increasing pH of metal solution leads to higher rate of ion absorption and the highest level was when pH was 5 and gradually decreased when pH exceeded this level [38]. This result is not consistent with present study maybe due to different ions used. Also, using dead cells of *S. cerevisiae* in this study has certain advantages over alive cells; nonalive cells can be stored longer in room temperature, they are not affected by toxic metal ions, and need no nutrient to grow up. Moreover, initial treatment and killing the cells through chemical and physical methods leads to more absorption compared to live cells. It has been indicated that the protein on cell walls creates a complex reacting with ions and since nutrients are removed from cell walls, the absorption level increases.

4. Conclusions

Based on the obtained results, we can express that *S. cerevisiae* yeast is a suitable absorbent for removing cyanide ions from wastewater. This yeast is prepared with low cost through fermentation process by culture medium. Thus, by replacing costly treatment methods with biomasses, wastewater can be treated.

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