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Removal of penicillin in aqueous solution using *Chlorella vulgaris* and *Spirulina platensis* from hospital wastewater

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ABSTRACT

Nowadays, antibiotics are considered as stable organic chemical compounds and their presence in hospital wastewater, pharmaceutical wastewaters and surface waters has led to some concerns. This study aimed to investigate the removal of penicillin as a pharmaceutical pollutant using *Spirulina platensis* and *Chlorella vulgaris* microalgae from aqueous solution. This study was carried out on synthetic samples at laboratory scale and wastewater samples of two hospitals in Birjand city, Iran. In this research, the effect of parameters such as retention time, the effect of pH variables and initial concentration on the removal efficiency of penicillin antibiotic by two microalgae were investigated. The results of this study showed that by increasing the number and biomass of microalgae, the removal efficiency of penicillin antibiotic increases. The optimal pH for removal of penicillin was obtained for both algae as 7 or neutral. Furthermore, the contact time at 8 and 9 d were selected as optimal time for *C. vulgaris* and *S. platensis*, respectively. The use of microalgae in the main hospitals of Birjand city wastewater has been efficient in the removal of penicillin antibiotics. Therefore, this method can be effective for the removal of antibiotics with similar structure.

Keywords: Aqueous solution; *Chlorella vulgaris*; Penicillin antibiotic; *Spirulina platensis*

1. Introduction

Drugs play a vital role in the modern life of human beings and are used to treat human and animal diseases. The presence of drugs is one of the most important and contemporary issues in the world that should seriously deal with. In the last decade, few scientists were interested in exploring the destination of the drugs that are found in nature with a large volume and thousands of diffusion channels, their effects on living organisms and the environment [1]. The drug must have a very high solubility in water to be absorbed by the target cells in the living body (human, animal and plant). Drugs have a very strong biological activity that affects living organisms. Moreover, they are highly resistant to biodegradation and are degraded under certain conditions by specific reactions not under normal conditions. Eventually, these drug compounds enter the water resources (drinking water, surface water and ground water) and affect the environment [2]. The rapid increase in the production of machinery used in the pharmaceutical industry during the Second World War was due to the armed forces' huge need for medicinal products and the production of new pharmaceuticals especially antibiotics during and after the Second World War that exacerbated the wastewater treatment problems caused by the pharmaceutical industry. In addition, the industrialization that has occurred in recent decades increased the discharge of liquid, solid and gaseous materials into the natural system, which, in turn, increased a variety of diseases and subsequently, the need for production of drugs in many countries. Therefore, a basic analysis was started in the United States to find the

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types of drugs produced and their amount of pollution in nature [3]. Accordingly, a number of studies focused on visiting and identifying of various types of drugs, hormones and other wastewater organic pollutants in an organized network of the rivers [4]. The results of these studies were used to treat wastewater generated in pharmaceutical industries. Today, organic materials as micropollutants, mainly originated from industry, agriculture, pharmaceutical and urban uses have been increasingly taken into account. The compounds used in personal health, pharmaceuticals and other usable materials like hormones may enter the environment by disposition of wastewater after purification. Nowadays, urban and industrial wastewater is recognized as the main source of the introduction of drugs into aquatic environments [5]. One of the early studies that address the presence of drug substances in the environment focuses on the presence of penicillin on the outlet of urban wastewater treatment plants in Germany [6]. New research has shown that antibiotics used in the treatment of human and animal diseases can be found in surface waters. These results show that antibiotics are not completely removed in the biological treatment processes and enter the receiving water; toxicity as their highest effect is harmful to the microorganisms in the environment and leads to the ecological imbalance. Besides, due to the presence of these materials in the environment, microorganisms get resistant against them [7]. More than 80 different types of active substances in the environment have been found in concentrations up to micrograms in the outlet of sewage treatment plants, surface water, groundwater and drinking water. For example, tetracycline and chlortetracycline at concentrations of 1.2 and 4 μg were found in urban wastewater [8]. Therefore, the purpose of this study was to investigate the effectiveness of the biological process for the removal of drug substances, including penicillin antibiotics from hospital wastewater. The effectiveness of this process is analysed by *Spirulina platensis* and *Chlorella vulgaris*, which are exposed to refined wastewater.

2. Methods

2.1. Materials and instruments

For this experimental-laboratory study, penicillin (Jaber Ebne Hayyan Pharmaceutical Co., Iran) Pen L.A. 1200000 type was used. To conduct experiments and determine the concentration of residual penicillin at completion of the experiment, high-performance liquid chromatography (Agilent Technologies, USA) was used [9]. The used instrument was calibrated prior to the experiment according to the manufacturer's instructions.

The microalgae were grown until they reached high concentrations and then the experiment was carried out. For this purpose, *S. platensis* is cultivated in Zarrok's medium and *C. vulgaris* is cultivated in Bold's Basal Medium. Aeration were accomplished using air from a compressor (RESUN AC-9603-0.12MPa) and two fluorescent lamps light from the intensity of 3,500 lux in periods of 12 h of darkness/brightness Erlenmeyer flask exposure levels was used to determine [10,11].

In this study, at the first for determining the optimal conditions for the removal of penicillin by *C. vulgaris* and *S. platensis* in synthetic medium was studied. Penicillin concentrations of 0.4, 0.8, 1.2, 1.6 and 2 mg/L at pH values of 5, 6, 7 and 8 and a number of microalgae (first day 3×10^7 cells/L) were investigated.

2.2. Cell counting and dry weight measurement

The interactions of microalgae cultivation on removal of penicillin were studied. The direct microscopic cell count by Thoma haemocytometer was performed with the optical microscope (Labomed, USA). Dry weight of *C. vulgaris* and *S. platensis* were measured by centrifuging at 4,500 rpm for 30 min and then washed by deionized water, finally dried at 105°C for 40 min.

2.3. Sampling and analysing the hospital wastewater

The hospitals wastewater samples were taken from two main hospitals of Birjand city capital city of South Khorasan Province, Iran. The samples were collected at three different times between 8 am and 6 pm and kept in dark glass bottles at 4°C, finally mixed. The hospital wastewater containing a vast variety of microorganisms such as bacteria which can remove penicillin, so the samples were sterilized via an autoclave at 121°C for 30 min. The concentration of penicillin in the hospital wastewater is determined, and then the removal of the penicillin at the optimum conditions such as pH, retention time and cells and biomass of microalgae was studied.

2.4. Statistical analysis

In this study, the raw data were stored in Ms Excel and then the relationships among the parameters under studied were interpreted by Kruskal Wallis analysis (the *P*-value of the Kolmogorov–Smirnov test was less than 0.05) using SPSS (version 17) software.

3. Results and discussion

3.1. Dry weight and number of microalgae

As Table 1 shows, the highest dry weight was observed for *C. vulgaris* and *S. platensis*, 4.2 and 4.4 g/L, respectively, after 6 d. The highest cell numbers were also observed on the 6th day, 0.67×10^7 and 0.53×10^7 for *C. vulgaris* and *S. platensis*, respectively. Based on the statistical analysis of the data obtained from dry weight, it was found that the dry biomass of algae has a significant difference (P-value < 0.05) in different days.

Table 1 shows the growth of microalgae (number of algae) and changes in this parameter during the 8-d period of cultivation. Based on the statistical analysis of the data obtained from the number of microalgae, it was found that the number of microalgae significantly increased during incubation (P-value < 0.05). Also, there was no significant difference between the number of algae in *C. vulgaris* and *S. platensis*.

Mousavi et al. [12] studied the growth of *C. vulgaris* in different cell densities and acknowledged that less phytoplankton density would reduce the removal efficiency, and this is consistent with the results of this study; they also pointed out that very high concentrations will reduce the amount of light penetration among treatments and increase the self-shadow

Table 1
Biomass (dry weight) and number of microalgae of *Chlorella vulgaris* and *Spirulina platensis*

Microalgae	Time	Day			
		1	4	6	8
Chlorella vulgaris	Biomass of algae (g/L)	3.570 ± 0.74	3.600 ± 0.68	4.240 ± 0.73	4.010 ± 0.79
	Number of algae (cells/L)	0.410×10^7	0.490×10^7	0.670×10^7	0.530×10^7
Spirulina platensis	Biomass of algae (g/L)	3.690 ± 0.59	4.110 ± 0.94	4.380 ± 0.87	4.200 ± 1.23
	Number of algae (cells/L)	0.450×10^7	0.490×10^7	0.530×10^7	0.490×10^7

effects, thereby limiting the growth and metabolic activity in phytoplankton cells.

3.2. Effect of pH on removal of penicillin by microalgae

In fact, pH plays an important role in the overall process and sorption capacity resulting from its effect on the adsorbent surface charge, the degree of ionization of the materials in the solution, the separation of the agent groups in the active sites and the chemistry of solution [13]. Therefore, pH can dramatically affect removal of the organic and inorganic materials from solutions. This study examined the concentration of penicillin as 0.4 g/L, initial dry biomass of *C. vulgaris* as 0.28 g/L, the initial number of *C. vulgaris* as 3.1×10^7, the initial dry biomass of *S. platensis* as 0.28 g/L and the initial number of *S. platensis* as 3.2×10^7 at pH 5, 6, 7 and 8 for 6 d.

As shown in Table 2, at pH = 7, the removal of penicillin is higher in *C. vulgaris* and *S. platensis*. It is also noteworthy that both the biomass and the number of algae at pH of 7 are the highest for both types of microalgae. It can therefore be concluded that the levels of removal of penicillin are a function of the amount of biomass and the number of algae. The effects of *Rhizopus arrhizus* as a biological absorbent in the removal of penicillin G were investigated and showed that the pH of 7 was the most effective. In this study, the highest removal of penicillin occurred at pH of 7 [14]. At higher pH values, the degree of penicillin G ionization and the value of OH^- ion increase, thereby inhibiting the penicillin G release, and increasing electrostatic repulsion between sites with negative charge of the ion in absorbent and penicillin G [15].

3.3. The effect of time on removal of penicillin by algae

The amount of removed penicillin is a function of contact time. To obtain optimal time to remove penicillin by these types of microalgae, experiments were carried out at optimal pH of 7, penicillin concentration of 0.4 mg/L, initial dry biomass of *C. vulgaris* of 0.32 g/L, the initial number of *C. vulgaris* of 3.4×10^7 cells/L, the initial dry biomass of *S. platensis* of 0.34 g/L and the initial number of *S. platensis* of 3.7×10^7 for 16 d. The results of the effect of contact time on penicillin sorption have been shown in Figs. 1 and 2. It is obvious that with increasing contact time the removal of penicillin increased. After about 6 d all penicillin were removed. Therefore, contact times of 6th day were selected as optimal time span for penicillin by *C. vulgaris* and *S. platensis*. According to the results of this study, the cells and biomass of both algae are a function of detention time, which means that over time, the cells and biomass of both types of algae has increased due to the increased probability of contact among the penicillin molecules and adsorbent [16]. Penicillin was absorbed in the early days at a high rate and over time, the amount of removal decreased probably due to decreased concentrations of soluble penicillin and reduction of active sites in the surface of microalgae. The results showed that penicillin sorption in the thin layer, which is the first sorption step, occurs faster, but penetration in the pores as a subsequent step of the sorption process, which leads to increased sorption at the adsorbent internal surfaces, is delayed [16].

Sadeghi et al. [17] conducted a study on the ability of yeast *Saccharomyces cerevisiae* in biological removal of antibiotic ciprofloxacin from aqueous solutions and indicated that the optimal amount of 10 mg/L of ciprofloxacin was removed as 23.53% 4 d after the onset of the reaction and the highest removal of the antibiotic or 100% was achieved during the first 4 d of the reaction [17]. Therefore, it can be concluded that during the reaction, removal of the antibiotics occur in optimal conditions in the first 6 d and after the time of equilibrium, no significant removal is observed. Hosseini et al. concluded that the removal percentage of tetracycline antibiotic increased by increasing the reaction time from 15 to 90 h, and then remained almost constant until 120 h of reaction. The highest removal of penicillin antibiotic was achieved in 5 d [18].

Table 2
The effect of pH on removal of penicillin in aqueous solutions after 6 d

Parameters	*Chlorella vulgaris*				*Spirulina platensis*			
pH	5	6	7	8	5	6	7	8
Penicillin concentration (mg/L)	0.201	0.157	0.061	0.068	0.259	0.188	0.094	0.112
Number of algae (cells/L)	3.570×10^7	3.60×10^7	4.240×10^7	4.010×10^7	3.690×10^7	4.110×10^7	4.380×10^7	4.200×10^7
Biomass of algae (g/L)	0.410	0.490	0.670	0.530	0.450	0.490	0.530	0.490

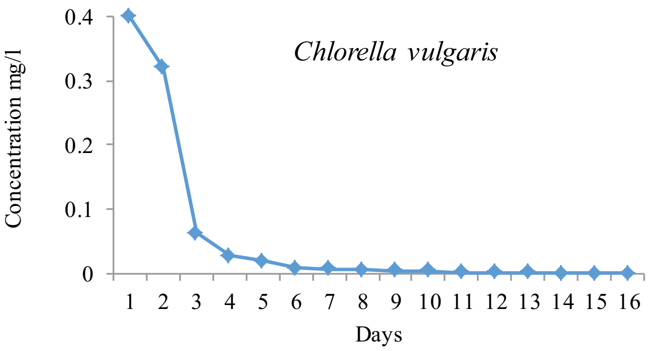

Fig. 1. The trend of time in removing penicillin by *Chlorella vulgaris*.

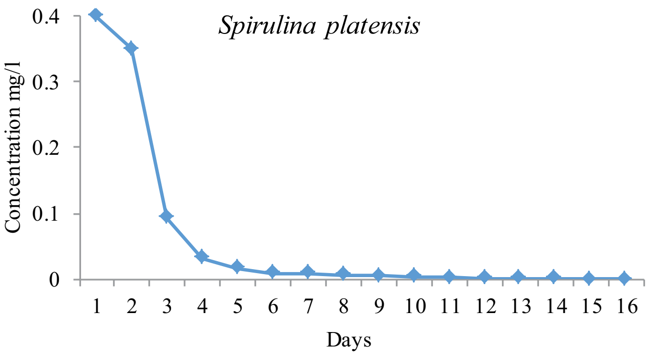

Fig. 2. The trend of time in removing penicillin by *Spirulina platensis*.

3.4. The effect of the initial concentration of penicillin on the removal efficiency by microalgae

The effect of the initial concentration of penicillin on the removal efficiency of pollutant by microalgae in the under-study system was observed with changes in the initial concentrations of the pollutant in mg/L at concentrations of 0.4, 0.8, 1.2, 1.6 and 2. The experiments were carried out at optimal pH of 7 and the number of algae was 3.2×10^7 and 3.3×10^7 for *C. vulgaris* and *S. platensis* and biomass of 0.3 and 0.29 g/L, respectively, for *C. vulgaris* and *S. platensis*, with 6 d detention times. The effect of the initial dose of the adsorbent on the removal efficiency is shown in Table 3, which is also increased by increasing the amount of pollution of the removal, while after the concentration of 1.6 mg/L, the amount of penicillin is less absorbed, indicating a negative effect of the pollutant on the microalgae function in removal of penicillin. Therefore, the sorption of penicillin G decreases with increasing concentrations due to the reduction in the cells and biomass of algae, but the sorption capacity increases, which is due to an increased probability of contact between adsorbent and absorbent material. The reason for reducing the sorption efficiency by increasing the initial concentration is the fact that the number of sorption sites is constant but the number of absorbing molecules increases [19,20]. Compared with the behaviour of two types of algae, the ability to remove penicillin has been different, so that at low concentrations (less than 1.6 mg/L), *C. vulgaris* has a little higher ability to remove penicillin, but at higher concentrations, *S. platensis* has a higher ability to remove penicillin. There is no significant difference among the number of algae and biomass also between the algae at removal efficiency of penicillin (P-value > 0.05).

The passive biosorption (adsorption) was the principal mechanism in culture with low penicillin G concentrations (below 20 mg/L) [21]. The results showed that the removal efficiency of penicillin increases with increasing adsorbent content. Increasing the removal efficiency of the process by increasing the amount of adsorbent is due to the increase in the specific surface area and also the internal porosity of the adsorbent, which will increase the efficiency of removal of penicillin, although increasing the dose of adsorbent increases efficiency, but the amount of penicillin per gram of absorbent decreases and this is due to the lack of saturation of the active sites in the sorption of pollutants, so that as the absorbent dose increases, the total capacity of the active sites present in the adsorbent surface is not fully utilized and this reduces the amount of sorption in the adsorbent mass unit [22]. Aghdam and Hejazi [23] investigated the removal of ampicillin, kanamycin and rifampicin by algae. The use of binary combination of ampicillin and kanamycin with concentrations of 0.1–0.5 mg/L resulted in complete removal of pollution.

3.5. Removal of penicillin by the microalgae from the hospitals wastewater

Among the various elements of hospital wastewater, antibiotics are interested due to the biological activity that

Table 3
Effect of different concentrations of penicillin on removal efficiency after 6 d

	Chlorella vulgaris					*Spirulina platensis*				
Initial concentration (mg/L)	0.4	0.8	1.2	1.6	2	0.4	0.8	1.2	1.6	2
Number of algae (cells/L)	4.38×10^7	4.41×10^7	4.35×10^7	4.34×10^7	4.37×10^7	4.42×10^7	4.40×10^7	4.39×10^7	4.38×10^7	4.39×10^7
Biomass of algae (g/L)	0.69	0.70	0.69	0.69	0.70	0.55	0.54	0.54	0.53	0.53
Final concentration (mg/L)	0.003	0.007	0.012	0.018	0.145	0.003	0.009	0.016	0.021	0.111
% Removal	99.25	99.13	99.00	98.88	92.75	99.25	98.88	98.67	98.69	94.45

Table 4
Removal of penicillin from hospital wastewater at optimum conditions by *Chlorella vulgaris* and *Spirulina platensis*

Parameters	*Chlorella vulgaris*		*Spirulina platensis*		Control	
	Hospital 1	Hospital 2	Hospital 1	Hospital 2	Hospital 1	Hospital 2
Initial concentration of penicillin (mg/L)	0.042	0.047	0.042	0.047	0.042	0.047
Dry biomass (g/L)	0.86	0.88	0.72	0.73	–	–
Number of cells (cells/L)	9.64×10^7	9.85×10^7	7.89×10^7	7.94×10^7	–	–
Final concentration of penicillin (mg/L)	0	0	0	0	0.04	0.04

has been shown to enhance the resistance of the pathogenic microorganisms [1]. In this study, the removal of penicillin antibiotic from the actual hospitals wastewater was investigated by the microalgae. As shown in this research, the effective parameters on removal of penicillin from wastewater, that is, pH, time, biomass and cells of algae were optimized. The optimal pH values were 7 and detention time was 6 d for *C. vulgaris* and *S. platensis*. The initial biomass of the microalgae of *C. vulgaris* was at 0.34 g/L and the initial biomass of the microalgae of *S. platensis* was 0.27 g/L. Moreover, the primary number of algae was 3.3×10^7 cells/L for *C. vulgaris* and the initial number of algae was 3.2×10^7 cells/L for *S. platensis*. As shown in Table 4, the initial concentrations of penicillin antibiotics in the wastewaters of the first and second hospitals were 0.042 and 0.047 mg/L, respectively. It was found that the dry biomass (P-value > 0.05) and number of cells (P-value > 0.05) of microalgae has no significant difference in different hospitals.

Table 4 shows the effects of two microalgae on removal of penicillin in the wastewater of the two hospitals in Birjand city. In the optimum conditions, the final concentration of penicillin was achieved to nil by both microalgae while in the control samples there is no removal of penicillin; it means the total penicillin has been removed by these algae. de Godos et al. demonstrated that algae reactors were able to remove tetracycline at 99% ± 1% in the absence of light. Removal of tetracycline in the presence of algae biomass in dark conditions was rapidly conducted at the initial stage (0–4 h) of the removal of tetracycline and then the removal was reduced after 43 h [24].

4. Conclusion

Today, most researchers seek to provide effective and economically efficient methods for the treatment of wastewater. Therefore, according to the results obtained, it can be noted that in connection with sewage containing medicinal substances, the use of microalgae can be very beneficial and can be justified both in terms of efficiency and economically. This study showed that as the cells and biomass of microalgae increase, the removal efficiency of penicillin increases too. The optimal pH was obtained to remove penicillin for both algae as 7 or neutral and the contact time of 6 d was selected as optimal time. This research shows that the microalgae in sewage of two hospitals containing pharmaceutical materials grew and was able to remove the penicillin antibiotic completely under optimal conditions, while in the control samples there is no removal of penicillin. Therefore, this method can be effective for the removal of antibiotics with similar structure. It is obvious that such high efficiency without the high cost and adverse environmental effects are highly desirable.

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