

Article

Potential of anaerobically digested poultry wastewater for metal biosorption by *Rhodobacter blasticus* and *Rhodobacter capsulatus*

M. H. Sayadi¹, M. Nourzadeh²

¹Department of Environmental Sciences, School of Natural Resources and Environment, University of Birjand, Birjand, Iran

²Department of Civil Engineering, School of Engineering, University of Birjand, Birjand, Iran

Email: Mh_sayadi@birjand.ac.ir

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Abstract

The aim of this research was to assess the elimination efficiency of heavy metals from poultry wastewater using the two bacterial species *Rhodobacter capsulatus* and *Rhodobacter blasticus*. In this research, the wastewater of breeder hens of Birjand city was used and in all the experiments the used bacterial amount was mixed with wastewater sample in a volumetric ratio of 1:10. The optimal bacterial mixture rate and pH were assessed. The results showed that the concentration of zinc and copper in the poultry wastewater constantly reduces with the lapse. *R. blasticus* had the highest elimination percent of heavy metals copper and zinc up to 72 hours, whereas eventually the two bacterial species mixture (1:1 ratio) together eliminated 84% and 90 % of zinc and copper respectively, but there was no significant difference between different bacterial treatments, period and residual concentration of zinc and copper ($p>0.5$). The elimination efficiency of zinc and copper was higher when pH level was between 7 and 9 (zinc was equivalent to 75% and 68% and copper was equivalent to 88% and 82% respectively) in comparison to the other pH's. Therefore, neutral pH is suitable for the wastewaters treated via bacteria. With the dilution of poultry wastewater, the elimination percent of zinc and copper increases and this indicates that the metals concentration present in poultry wastewater does not have a negative effect on the elimination. So, these bacterial types are able to grow in poultry wastewaters and treat them.

Keywords optimization; bacterial mixture; copper; zinc.

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1 Introduction

During the past decade, annually per capita, the global meat consumption has almost doubled (Schipanski and Bennett, 2012). At present, poultry production has the fastest growth of the meat industry in both urban and rural zones in most of the developing countries without consideration of the environmental issues (Atuanya and Aigbirior, 2001), wherein this growth is associated with a high volume of produced wastewaters that could

be used as a fertilizer for soil fertilization (Schipanski and Bennett, 2012). If these wastes are discharged into the environment without suitable processing, it is a serious pollution threat to the aquatic ecosystems, wherein this affair has attracted the attention of environmental supporters and scientists (Rodriguez et al., 2011; Su et al., 2014; Zhang and Liu, 2017). The wastewater produced in the poultry farms in addition to the fact that it is a resource enriched with phosphorus and nitrogen contains a high percent of heavy elements such as zinc and copper, which is used in the dietary regimen of this animal (Li et al., 2007). At present, varied physical and chemical techniques are adopted for the elimination of heavy metals viz. filtration, ion exchange, electrochemical filter, membrane processes, reverse osmosis, oxygenation/reduction (Rodriguez et al., 2011; Sayadi et al., 2016; Hayati et al., 2013), but these techniques in addition to high energy and cost expenditure are also associated with the production of secondary pollutants that create essential issues for the environment (Sayadi et al., 2012; Zojaji et al., 2015). Today, the utilization of biotechniques that are environment lovers and have a lesser environmental side-effects are acclaimed by the researchers, wherein in this field the photosynthetic bacteria (PB) process was considered and is used for the filtration of different types of wastewaters (Zhou et al., 2016; Huang et al., 2014). The research conducted on pig wastewater exhibited that bacteria have a high ability to treat the wastewater, especially the heavy elements (Zhou et al., 2016). The elimination of heavy metal cadmium from the aquatic solutions was also carried out via *Bacillus* sp. that had a notable efficiency (Huang et al., 2014). Even in other research, the elimination efficiency of copper metal via *Bacillus* sp. was 69.34% and for cadmium an elimination efficiency of 90.41% and 84.27% via *Pseudomonas* sp. and *Micrococcus* sp. respectively was reported (Rani et al., 2010). Even several reports in case of heavy metals absorption viz. cadmium, lead and nickel via *Rhodobacter sphaeroides* (Buccolieri et al., 2006) and *Rhodospirillum rubrum* (Smiejanet al., 2003) were reported. Hence, the aim of the present research was to assess the elimination efficiency of heavy metals from the poultry wastewater using the two species *Rhodobacter capsulatus* and *Rhodobacter blasticus*. Besides, in this research the effects of parameters such as optimal mixture ratio of two bacterial species, the preliminary concentrations of metals zinc and copper and pH level on the elimination efficiency were also tested.

2 Materials and Methodology

2.1 Microorganism

The microorganism species and culture media of two species *Rhodobacter capsulatus* (*R. capsulatus*) and *Rhodobacter blasticus* (*R. blasticus*) were procured from Birjand University and even for the entire experiments these two bacterial species were used. The species were cultured at 30°C and 3000 Lx light intensity and in a time period of 5 days. For the conduct of experiments, 200 ml of these two bacterial species was used from RCVBN media for inoculation in 500 ml experimental Erlenmeyer (Hulsen et al., 2014).

2.2 Poultry wastewater

The poultry wastewater was procured from breeder hens' farm located in the west of Birjand city, South Khorasan province. Prior to conduction of experiments on the collected sample, the wastewater was centrifuged at 6000 rpm for a period of 15 minutes; this task was carried out to eliminate the suspended solid particles. The prepared sample was maintained at 4°C during the experimental course.

2.3 Bioreactors

The bioreactors used for the entire experiments were 500 ml flasks that prior to the experiment were sterilized via an autoclave at 121°C for 30 minutes. The entire experiments were carried out at environmental temperature of 25±2°C and 3000 Lx light intensity (Hulsen et al., 2014). In the course of experiments the used bacterial amount was mixed with the wastewater sample at a 1:10 volumetric ratio.

2.4 Experimental design

To achieve an optimal bacterial mixture, the mixture ratios of two species *R. capsulatus* and *R. blasticus* were considered 1:0, 0:1, 4:1, 2:1, 1:1, 1:2 and 1:4 respectively. To realize an optimal pH at five levels 5, 6, 7, 8, 9 and 10 the experiments were conducted, NaOH and HCl were used to adjust the preliminary pH. Considering that the obtained preliminary concentration of heavy metals zinc and copper were 60 and 48 mg/l respectively in the poultry wastewater, the treatments of heavy metals concentration were tested at 5 levels for zinc (60, 40, 30, 20, 15 and 10) and copper (3, 6, 18, 12, 24, 48) mg/l respectively. Besides, to measure the concentration of residual zinc and copper in the solutions, daily for 7 days the atomic absorption system (Contra AA700) was used. All the experiments were repeated three times and all the results were reported as an average.

2.5 Analysis data

The data from the input and output values of heavy metals were analyzed via SPSS software version 16. Initially using Kolmogorov-Smirnov (K-S) test the data normality was assessed and later using the one-way variance analysis test (ANOVA) the difference between the average of the test groups were assessed and besides the required figures were traced with Excel software.

3 Results and Discussion

3.1 Removal of Cu and Zn by *R. capsulatus* and *R. blasticus*

In the present study, initially the separate effects of two bacterial species viz. *R. capsulatus* and *R. blasticus* were assessed in the elimination of heavy metals Cu and Zn from the poultry wastewater that were equivalent to 60 and 48 mg/l respectively. Later, the two bacterial species were mixed at 1:1 ratio, so that the reciprocal effects of these two species are assessed on the elimination efficiency; its results are tabulated in Table 1.

Table 1 shows that with lapse the Cu and Zn concentrations in poultry wastewater are constantly reducing. *R. blasticus* up to 72 hours in relation to other treatments had the highest rate of heavy metals Cu and Zn elimination and *R. capsulatus* had the lowest elimination rate at different time periods. The significant point was that at 168 hours the elimination rate of zinc via *R. capsulatus* (residual concentration 14.04 mg/l) was higher than *R. blasticus* (residual concentration 15.09 mg/l) and at 144 hours the elimination rate of copper via *R. capsulatus* (residual concentration 9.17 mg/l) was higher than *R. blasticus* (residual concentration 9.81 mg/l).

Table 1 Mean±SD concentrations of remaining copper and zinc (mg/L) at different times.

Time (h)	<i>R. blasticus</i>		<i>R. capsulatus</i>		<i>R. blasticus and R. capsulatus</i>	
	Zn	Cu	Zn	Cu	Zn	Cu
0	60	48	60	48	60	48
24	49.23±17.22	36.96±9.48	56.22±19.07	41.72±8.34	52.91±17.60	40.32±14.69
48	37.18±9.84	26.98±9.25	53.64±10.47	39.45±6.64	42.75±10.57	31.45±8.56
72	27.44±6.38	18.50±4.97	47.73±9.68	32.68±4.97	31.10±6.23	21.07±6.06
96	23.69±7.40	15.62±4.00	36.11±8.04	23.20±3.52	22.52±7.51	13.70±3.29
120	20.07±5.74	11.46±3.94	25.92±4.95	15.55±3.36	15.68±5.45	8.63±3.02
144	18.56±4.31	9.81±5.18	19.67±3.89	9.17±2.39	12.70±3.54	6.64±1.96
168	15.09±4.05	5.83±1.49	14.04±2.76	7.56±1.42	9.85±1.97	4.95±1.57

Whereas from 96 hours onwards the two bacterial species mixture showed the highest elimination rate (Table 1). The results of the Kolmogorov-Smirnov test showed that P-value for residual concentration of Cu and Zn were (0.701 and 0.578 respectively) which is higher than 0.5. Hence, these data are normal and results

of one-way variance analysis test showed that there is a nil significant difference between different bacterial treatments, time and residual concentration of Zn and Cu ($p > 0.5$).

The persistence of heavy metals in aquatic solutions is dependent on several activities of microorganisms present in the aquatic solutions, especially bacteria, although its absorption mechanism is not properly specified (Willison and Magnin, 2013). The elimination of heavy metals from aquatic solutions via microorganisms, especially bacteria have a direct relationship with time and copper and zinc metals absorption was influenced by time (Özdemiret al., 2009).

The Figs. 1 and 2 are indicators of an elimination percent of zinc and copper. As depicted by Figs. 1 and 2, *R. blasticus* to 72 hours had the highest elimination percent of heavy metals, zinc and copper and *R. capsulatus* had the lowest elimination percent (except 168 hours for zinc and 144 hours for copper) at different time periods of the experiment.

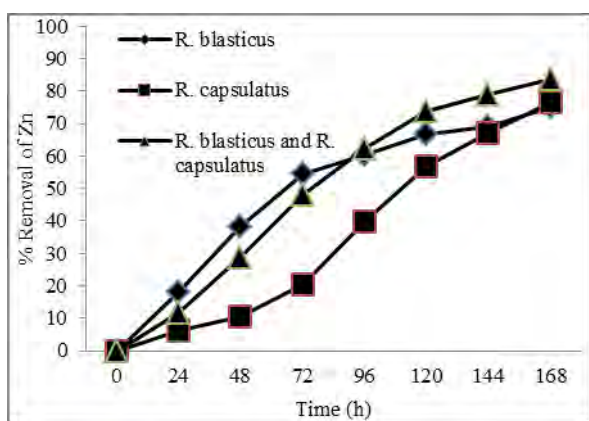


Fig. 1 The percentage removal of the zinc by bacteria at different times.

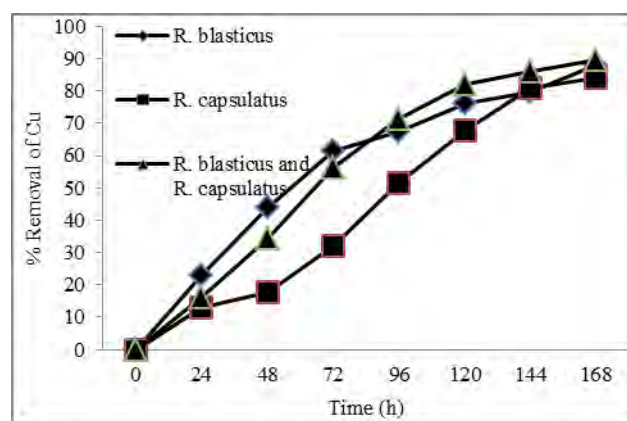


Fig. 2 The percentage removal of the copper by bacteria at different times.

At the final experimental period (168 hours) *R. blasticus* with a value of 75% and 88% and *R. capsulatus* with a value of 77 and 84%, respectively eliminated zinc and copper from the poultry wastewater, whereas the two bacterial species mixture could together eliminate 84% and 90% of zinc and copper respectively.

The notable point was that during the entire experimental period, the elimination efficiency of copper for *R. blasticus* and *R. capsulatus* and the two bacterial mixture species was higher in relation to elimination percent of zinc. In the study of Pokrovsky et al. (2014) it was reported that copper absorption value via *Rhodospseudomonas palustris* was higher than zinc and this is because of the difference in the size and thickness of zinc and copper ions. The smaller hydrated radius of copper led to better absorption of this metal in the bacteria. Besides, the structural differences and varied chemical mixtures of the cell walls of the bacteria caused the difference in their resistible rate against the metallic ions which is also true in the absorption via other microorganisms (Ginn and Fein, 2009).

Rhodobacteria are positive gram bacteria that have a teichoic acid wall which causes the creation of negative load in the bacterial cell wall and is the factor of superficial absorption of metals with positive load. However, the gram negative bacterium with thin lipopolysaccharide cellular wall located on the peptide and glycan layer is the factor for the superficial absorption of metals with positive load. In general, the negative load rate of gram positive bacteria is higher than the gram negative bacteria and as a result has the higher ability to eliminate heavy metals (Pokrovsky et al., 2013; Matyar et al., 2008).

The figure slope of *R. blasticuse* lamination percent was higher at the beginning of the experimental period, but for *R. capsulatus* bacteria was higher at the end of the experimental period wherein has a direct

relationship with the bacterial growth rate (Wen et al., 2016). The reason for this kind of *R. blasticus* behavior can be expressed as follows whereby the growth rate of this species in relation to *R. capsulatus* in the initial 72 hours was faster, but after lapse the growth rate in *R. blasticus* population reduces and it can be safely stated that this bacteria has entered the stationary phase. In relation to *R. blasticus*, the comparative phase of *R. capsulatus* was longer, in the first 72 hours *R. capsulatus* grows slowly and later the growth rate increases significantly (Huisman et al., 1996; Wen et al., 2016).

3.2 The influence of mixture ratio of two bacterial species on the elimination efficiency of Cu and Zn

The results of Table 1 as well as Figs 1 and 2, depicts that the highest elimination value of heavy metals at the end of the experimental period is related to a combination of these two bacterial species. Therefore, to obtain an optimal ratio the two bacterial mixtures viz. *R. blasticus* and *R. capsulatus* were mixed at 7 ratio and the elimination experiments were carried out where in the results of zinc and copper elimination percent is tabulated in the Table 2.

Table 2 The percentage removal of Zn and Cu in various ratios of *R. blasticus* and *R. capsulatus*.

Time (h)	1:1		1:0		0:1		1:2		2:1		4:1		1:4	
	Zn	Cu	Zn	Cu	Zn	Cu	Zn	Cu	Zn	Cu	Zn	Cu	Zn	Cu
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	12	16	18	23	6	13	11	14	14	21	17	22	7	14
48	29	34	38	44	11	18	25	31	34	42	36	41	13	19
72	48	56	54	61	20	32	46	55	50	60	51	59	20	30
96	62	71	61	67	40	52	59	70	62	65	58	64	42	49
120	74	82	67	76	57	68	70	75	69	73	63	75	56	65
144	79	86	69	80	67	81	75	83	75	77	65	81	65	80
168	84	90	75	88	77	84	80	85	79	86	74	89	78	81

As demonstrated in Table2, up to 72 hours, *R. blasticus* (1:0) alone had the highest elimination percent (copper 75% and zinc 88% respectively). But , with span of time the highest elimination percent pertained to 1:1 where at the experimental end (168 hours), had the highest elimination percent (copper 90% and zinc 84% respectively). The ratios 0:1 and 1:2 had the lowest elimination percent amount of zinc up to 144 hours and copper up to 120 hours respectively. It is important to mention that at different experimental periods and in the entire ratios the copper elimination percent value was higher than zinc (Table 2). In the study of Rani (2013) reported that the copper elimination rate via *Bacillus* sp. was 69.34%, whereas in the present study with two bacterial mixtures of *R. blasticus* and *R. capsulatus* in an optimal ratio 1:1 the copper elimination efficiency in the poultry wastewater reached 90%.

3.3 Effect of pH on removal percent Cu and Zn

The most suitable pH range wherein the possibility of bacterial growth and activity exists is 5.5 to 9.4 and if the pH level is over limitedly high or low it causes reduction of enzyme catalysis which in turn affects and eventually eliminates the bacterial growth (Koku et al., 2002). Accordingly, the effects of different pH's i.e. 5, 6, 7, 8, 9 and 10 were assessed on Zn and Cu percent elimination from the poultry wastewater. The Figs. 3 and 4 show elimination percent of zinc and copper in different pH's and periods. The highest elimination efficiency pertained to different periods at pH 7 wherein at pH 7 and 168 hours, the elimination rate of zinc and copper were 75% and 88 % respectively. As depicted in Figs. 3 and 4, the elimination efficiency of zinc and copper when pH level was between 7 and 9 (zinc was equivalent to 75% and 68% and copper equivalent to 88% and 82% respectively) which was higher than the pH's 5, 6 and 10. Therefore, it can be confirmed that neutral pH is the most suitable level for wastewaters that are treated via the bacteria. pH is one of the most important parameters that have much effect in biosorption of heavy metals and at low pH's the heavy metals

absorption reduces. In several cases, with pH increase the metals biosorption increases up to an extent and further continues to pH 9, but after that quickly the biosorption reduces.

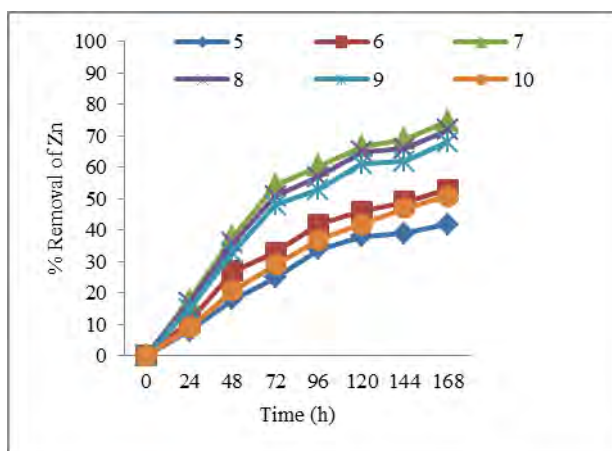


Fig. 3 The percentage removal of zinc by the optimum ratio of bacteria at different times and pH.

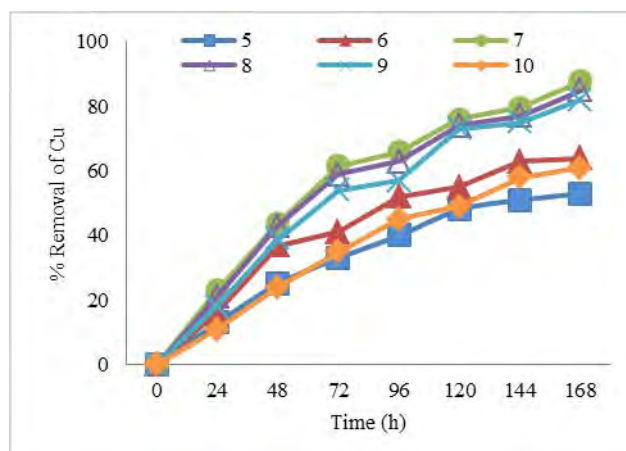


Fig. 4 The percentage removal of copper by the optimum ratio of bacteria at different times and pH.

The different studies demonstrated that an optimal pH level is around 7 for zinc and copper (Özdemir et al., 2009; Magnin et al., 2014; Esposito et al., 2001) which in concurrence with the present study. pHs 5, 6 and 10 have the lowest elimination efficiency and among these pH=5 with final elimination of 42%, had the weakest function, this affair is due to the reason that the bacteria in this condition did not have growth ability, as a result the microbial population after some time reduces and little by little are eliminated (Wen et al., 2016).

3.4 Effects of preliminary Zn and Cu concentration on the elimination rate

In the present study, the poultry wastewater for zinc and copper was diluted at 6 levels viz. 60, 40, 30, 20, 15, 10 and 18, 24, 48, 12, 6 and 3 mg/l, respectively and the preliminary concentration effects of zinc and copper on the elimination percent of these metals experimented with an optimal bacterial ratio of 1:1 at an optimal pH 7 is tabulated in Table 3.

The results demonstrate that with poultry wastewater dilution, the elimination percent of zinc and copper increases in a manner that in the lowest concentrations the highest elimination percent rate exists and this indicates that the concentration of existing metals in the poultry wastewater are generally lesser than it could have a negative effect on the bacterial growth and followed by the elimination of these metals. Therefore, these bacterial types are able to have a rapid growth in the poultry wastewater and treat them.

The elements of zinc and copper such as iron, nickel-cobalt since been essential micronutrients for bacterial growth; at high concentrations have negative effects on the bacterial growth (Ji and Silver, 1995). In the study of Magnin et al. in 2014, it was reported that zinc at 65 mg/l concentration had nil effects on the growth of *R. capsulatus*. The bioaccumulation is a complex process wherein the metallic ions could be transported in the specific organelles and are influenced by the enzymatic processes and again are transported outside with energy utilization (Silver and Phung, 2005). According to the results of Hunang et al. (2014) initially the metals are absorbed to the bacterial cell level and later some of the ions are transported into the cell and accumulate in the cytoplasm. If the metals concentration is high in the environment and bioaccumulation in the cell increases the growing bacteria with energy utilization, transport the metals outside the cell and protect the cell from the high toxicity level of metals (Hunang et al., 2014).

Table 3 The percentage removal of zinc and copper in different concentration (mg/L).

Time (h)	Concentration of Zn						Concentration of Cu					
	60	40	30	20	15	10	48	24	18	12	6	3
0	0	0	0	0	0	0	0	0	0	0	0	0
24	18	36	47	74	76	86	23	48	73	87	96	99
48	38	52	63	82	82	97	44	71	84	89	100	100
72	54	64	75	84	86	100	61	79	86	92	100	100
96	61	70	87	88	92	100	67	84	91	96	100	100
120	67	81	90	95	98	100	76	96	100	100	100	100
144	69	85	92	99	100	100	80	99	100	100	100	100
168	75	88	95	100	100	100	88	100	100	100	100	100

Even Wang and associates reported reduction of bioaccumulation with increase of metals concentration and showed that with increase of metals concentration the bioaccumulation rate reduces via different bacteria (Wang et al., 2012). Resultantly, it can be said that in low metals concentration, the absorption takes place at a higher rate. Even the earlier studies emphasized that zinc and copper elimination takes place more in the lower concentrations (Salehizadesh and Shojaosadati, 2003).

4 Conclusions

R. blasticus in comparison to the other treatments had the highest elimination rate of heavy metals viz. zinc and copper up to 72 hours and *R. capsulatus* had the lowest elimination rate at different time periods. The significant point was that at 168 hours the elimination rate of metals viz. zinc and copper was higher via *R. capsulatus* than *R. blasticus*. The elimination efficiency of zinc and copper was higher when pH level was between 7 and 9 in relation to other pH's, therefore, it can be safely stated that neutral pH is suitable for the wastewaters that are treated via bacteria. With poultry wastewater dilution, the percent elimination of metals viz. zinc and copper increases as in the lowest concentrations, the highest elimination percent rate exists, thus these bacterial species are able to rapidly grow in the poultry wastewaters and treat them.

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