Biological Removal of Zinc (II) by Fusarium solani under Different Modes of Operational Strategies: A Comparative Study

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Abstract

The goal of the current batch biosorption experiment was to determine how well Fusarium solani (isolated from soil) resting cells could remove zinc (II) from aqueous solution. With an increase in pH (up to pH 5.0) and an increase in initial zinc (II) concentration of up to 500 mg/L, the specific zinc (II) removal increases. By increasing biomass content from 2.5 to 5.5 g/L, the specific zinc (II) removal remained nearly constant. The investigations also utilised resting cells from various growth stages and at an initial zinc (II) concentration of 500 mg/L, the maximum specific zinc (II) uptake (51.7 mg/g) was accomplished (36 h old). The comparison of the results revealed that cells in a growing state (63.9 mg/g) can achieve the highest selective uptake. Hence, the uptake of zinc(II) under various operational strategies was only done with growing cells. The biological uptake of zinc (II) was then done in a fed batch mode of operation using Fusarium solani growing cells. Increased volume pulse feeding (IVPF) and constant volume pulse feeding (CVPF) were used to study the fed batch process and the impacts of these operational methods on biological performance were contrasted with those of a traditional batch process.

According to batch experiments, at pH 5.0 and an initial zinc (II) ion concentration of 500 mg/l, the maximum specific zinc (II) removal was 63.9 mg/g. The maximal specific zinc (II) removal in the IVPF process was determined to be 45.47 mg/g and 33.58 mg/g, but the values in the CVPF process were 33.12 mg/g and 22.59 mg/g respectively for the first and second pulse feedings in both cases. The zinc (II) uptake found in prior investigations carried out by the current authors employing the continuous mode of operation was then compared with these results. The process could be run for a longer period of time with a maximum specific zinc (II) removal of 52.8 mg/g when operating in a continuous mode which was shown to be the optimal operational strategy.

Keywords: Zinc (II), Fusarium solani, Resting cells, Growing cells, Batch, Pulse feeding, Fed batch.

Introduction

Water is such a vital resource in today's society that life without water is simply not conceivable. Water scarcity is gradually becoming a significant issue for us in this age of fast industry, urbanisation and overpopulation. Additionally, all types of living things are at risk from water contamination, notably as a result of heavy metals produced in industrial effluent. When zinc ions bioaccumulate in the food supply chain, they can cause anaemia because they have a great affinity for replacing iron in plasma proteins and red blood cells. This poses a threat to human life. The World Health Organization[5] states that the maximum zinc concentration in drinking water is 5.0 mg/L. To avoid negative impacts on the ecosystem and public health, zinc (II) ions must be removed or reduced to the authorised levels before discharge[10].

Chrome plating, wood preservation, metal cleaning and processing, alloy preparation and other industrial activities produce wastewaters that contain Zn,[6,7]. Precipitation, electrochemical treatment, coagulation, reverse osmosis, membrane filtration and electrolysis are some of the traditional techniques for extracting zinc (II) from wastewaters[2]. Cost and waste creation are important considerations in the majority of these processes, especially for low zinc concentrations (II) like 1-100 mg/L, making these techniques ineffective or expensive[14]. This is a serious environmental problem since zinc (II) will always be present in the environment, making cleanup challenging[1]. Due to its persistence, it builds up throughout the food chain until it eventually reaches extremely high levels in living things, posing major health hazards[9,16].

In order to clean the without producing hazardous waste byproducts, ecologically friendly techniques must be created. The term "bioremediation" refers to the environmentally friendly removal (and even further recovery) of these pollutants from the metal-contaminated wastewaters by the active or passive capture of both organic and inorganic species from aqueous solutions by microbial biomass[10]. Microorganisms are said to extract zinc (II) from solutions based on water using their growing, resting and non-living cells[6].

However, the majority of research on the removal of zinc (II) has utilised dead fungal cells[11] and there is very little data on the usage of active and resting cells. Because they do not need growth media or nutrients or worry about toxicity, non-living cells offer benefits over resting and growing cells.

Moreover, adsorbed metal may be easily desorbed and biomass that has been rejuvenated can be used once more. Yet, the biggest disadvantage is that as non-living biomass dries, biochemical cell energy responses cease whereas both growing and resting cells can remain biochemically active[12,15,16]. The benefit of resting cells is that they need very little energy for upkeep in order to maintain metabolic activity. A mixture of surface reactions, intracellular and extracellular precipitation and extracellular complexation processes can also be used to sequester metal[11].

On the other hand, growing systems have a clear advantage over non-growing systems and dead cells have been removed concurrently with the cells' growth. Separate industrial procedures for biomass cultivation, harvesting, drying, processing and storing can all be avoided. However, nutritional media is necessary for organism growth and cell proliferation is impeded when metal concentrations are high. This is one of the main disadvantages of employing growing systems for metal biosorption[3]. Using creatures that can withstand metals can help to overcome this issue. Tolerance and removal capabilities are important properties of growing biomass employed in a metal removal process.

The goal of the current study is to assess the capability of Fusarium solani resting cells to remove zinc (II) from aqueous solution. Using synthetic zinc(II) solution in batch bioreactors, the effects of pH, initial zinc(II) concentration, biomass concentration and culture age on zinc(II) removal from aqueous solutions were investigated. To remove the most zinc (II) from the medium while Fusarium solani was growing, studies were conducted utilising several operational tactics.

The fed batch operational techniques (pulse feeding operation) were designed to minimise the inhibitory effects of the dissolved components in the media while retaining the availability of new nutrient medium. Both constant volume pulse feeding (CVPF), which is intermittent feeding while simultaneously withdrawing the medium and increasing volume pulse feeding (IVPF), which is intermittent feeding without withdrawing the medium, were used for pulse feeding.

Material and Methods

Preparation of the organism and inoculum: In the current investigation, Fusarium solani, which was isolated from soil, was cultivated in a 250 ml Erlenmeyer flask at 30°C and 180 rpm using media with the following content (g/L)*:* 10.0, glucose, 0.5, K_2HP0_4, 1.0, NaCl, 0.1, $MgS0_4$, 0.5, NH_4N0_3 and 5.0, yeast extract. The media had a pH of 6.0. For the zinc (II) removal experiments, an inoculum of 10% (v/v) of a 36-hour-old culture was used.

Preparation of biomass: After 36 h of growth (when sugar was completely utilized), the fungus cells were centrifuged for five minutes at 5000 rpm at 30^0C followed by three washes with distilled water. In each experiment, a weighed quantity of washed resting cells (4.5 g biomass/L, on a dry weight basis) served as the biosorbent. By separating out the quantity of washed cells utilised in the experiment and drying it at 80^0C for 24 hours, dry cell weight was calculated gravimetrically.

Preparation of zinc (II) solutions: Zinc (II) solutions of various concentrations [100- 500 mg/L] were made by diluting the stock solution which were prepared by adding the necessary amounts of $ZnCl_2$ to a stock solution [2.092 g $ZnCl_2$] and letting it dissolve in one litre of distilled water.

Batch studies: The Erlenmeyer flask (250 ml) was filled with a weighted portion of the resting cells (4.5 g/L on a dry weight basis) along with 100 mL of a solution of zinc (II) with a defined concentration. The pH of the solution containing zinc(II) was adjusted to the necessary value before mixing the biomass and glucose (0.05 g/L) solely needed for cell maintenance. The flask was then infected and incubated in a shaker at 150 rpm for 24 hours at 30°C. The samples were periodically removed, centrifuged for five minutes at 5000 rpm and the supernatant liquid was separated and its residual zinc (II) concentration was determined.

To investigate the effects of pH (2–6), starting zinc(II) concentration [100–500 mg/L], biomass concentration [2.5–5.5 g/L] and culture age (12–48 h), batch experiments were conducted in a consistent manner. There were three duplicates of each experiment run. The biomass that was obtained through centrifugation was cleaned, dried and its dry weight was calculated gravimetrically.

Fed batch studies

Increase Volume Pulse Feeding (IVPF): Using the IVPF mode of operation, a fed batch bioreactor was used (working volume-3 l), A 500 mg/l starting zinc (II) concentration at pH 5.0 medium was given a 10% (v/v) inoculum concentration. The medium volume was initially kept to 1 l and the process was monitored till the substrate was fully consumed. It took five days. 1 l of new medium was introduced to the reactor on the fifth day and a similar addition was made on the twelfth day. The procedure was examined till day 20.

Constant Volume Pulse Feeding (CVPF): Intermittent feeding and synchronous medium withdrawal make up this style of operation. An inoculum concentration of 10% (v/v) was added to a medium containing 500 mg/l of starting zinc (II) concentration at pH 5.0 in a fed batch bioreactor (working volume: 3 l). When the substrate had been fully consumed, the volume of the medium was originally retained at 3 l and the removal of zinc (II) was observed for 5 days. On the fifth day, the first pulse feeding was carried out. From the reactor, one litre of homogeneous medium was removed and replaced with new medium (1 l) containing the necessary amount of zinc (II) to maintain a concentration of 500 mg/l of zinc (II) (3 l).

The pulse feeding was carried out on the 12th day and the procedure was observed until the 20th day. The residual zinc (II) concentration and residual substrate concentration of the liquid samples were measured in both IVPF and CVPF procedures. Gravimetric analysis was used to estimate the collected biomass. There were three duplicates of each experiment run.

Assay Techniques: The residual zinc (II) concentration was estimated by Atomic Absorption Spectrophotometer at 214.7 nm[8] and Di-nitro salicylic acid (DNS) technique was used to measure the amount of sugar in the liquid at 540 nm[3].

Results and Discussion

Batch studies: Fusarium solani was grown in a variety of initial zinc (II) concentrations (0-500 mg/l) and at initial medium pH 6.0. Figures 1a and 1b show the changes in remaining glucose concentration (g/l) and biomass concentration (gm of dry biomass weight per litre of liquid medium) respectively with time (h) in the medium. Figure 1a shows that in the absence of zinc (II) ions, glucose was fully used after 36 hours of incubation. The lag period was discovered to be approximately the same in the presence of zinc (II) up to 500 mg/l initial zinc (II) ion concentration. The lag period in the absence of zinc (II) was 3 hours, followed by the exponential phase of growth, as can be seen in the figure 1b (up to 30 h). Within 36 hours, Fusarium solani's growth reached its stationary phase.

Both the growth rates of *Fusarium solani* (Fig. 1b) and the rate at which glucose was utilised (Fig. 1a) were observed to be reduced when the initial zinc (II) concentration increased from 0-500 mg/l, despite the fact that glucose was utilised entirely in each case. Table 1 displays the pH shift over time at various initial zinc (II) concentrations (0-500 mg/l) during *Fusarium solani* development. pH fell from 6.0 to 4.8 in 15 hours in the absence of zinc (II) ion i.e. the early stages of the exponential development (Figure 1b) gradually climbed to 5.6 in 36 hours, when glucose was fully utilised (Figure 1a). The generation of pyruvate and hydrogen ions during the growth of the *Fusarium solani* may be the cause of the pH dropping over time at various initial zinc (II) concentrations indicated in table 1.

An elevation in pH may result from the coenzymes' conservation of the available hydrogen ions when they make the reduced coenzymes required for the tricarboxylic acid cycle's oxidation of Acetyl-CoA, which is derived from the breakdown of pyruvate and converts it to CO_2 and H_2O. The pH declined from an initial value of 6.0 to 5.1–5.3 in the early period of the exponential growth and then progressively increased to 6.5 after glucose was fully consumed in all concentrations of zinc (II) ions, showing a comparable trend.

Table 2 shows the effect of pH and initial zinc (II) concentration on specific zinc (II) removal by the resting cells of the *Fusarium solani*. pH was studied in the range 2.0-6.0, using a biomass content of 4.5 g/L at an initial zinc (II) concentration of 50 mg/L (dry wt. basis). At pH=5.0 and at initial zinc (II) concentrations ranging from 50 to 500 mg/L, the specific zinc (II) removal per g of dry biomass was determined.

Table 3 displays the uptake of zinc (II) in relation to the biomass concentrations of the resting cells (2.5–5.5 g biomass/L) at an initial 500 mg zinc (II)/L concentration and at pH=5.0. *Fusarium solani* cells were taken at different growth stages (12, 24, 36 and 48 hours) and kept in a resting state in the absence of zinc (II) (by the addition of low maintenance energy in the form of glucose) and were utilised to examine the impact of culture age (physiological state of growth) on zinc (II) removal by the same organisms' resting cells at an initial 500 mg/L concentration and at pH=5.0. The results are reported in table 3.

Figure 2 compares the specific zinc (II) removal results from the current investigation utilising resting cells at various pH levels to the results from past studies by the same authors employing the same fungus (*Fusarium solani*) under growing conditions[11] and as an adsorbent using non- living cells[12].

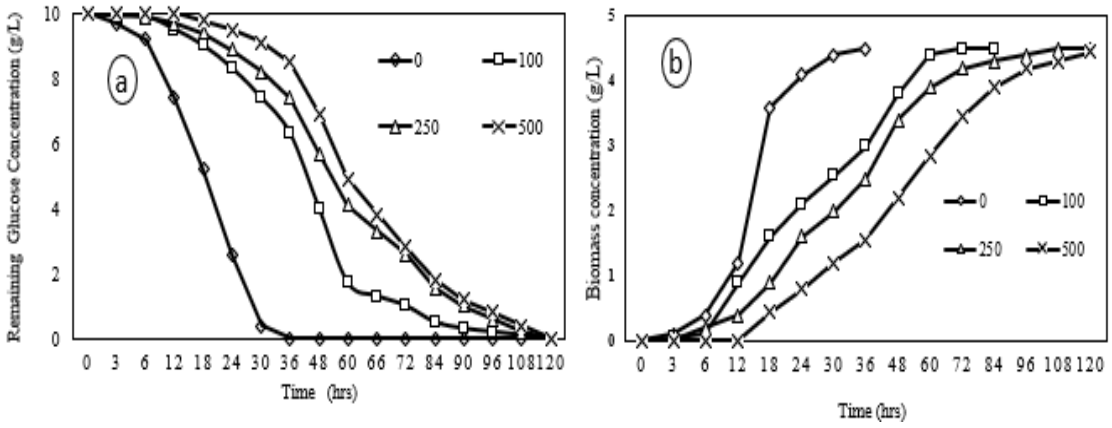

Figure 1: (a) Change in remaining glucose concentration (b) Biomass concentration of Fusarium solani with time at different initial zinc (II) concentrations

Table 1
Showing the pH change when Fusarium solani grows at various durations and initial zinc (II) concentrations.

Initial zinc (II) concentration (mg/L)	pH values										
	Time(h)	0	6	12	15	18	24	36	48	72	96	120
0		6.0	5.2	4.9	4.8	5.0	5.1	5.6	--	--	--	--
50		6.0	5.8	5.5	5.4	5.3	5.1	5.3	5.5	6.5	--	--
100		6.0	5.8	5.6	5.5	5.8	5.3	5.5	5.9	6.0	6.2	6.5
250		6.0	5.8	5.6	5.5	5.8	5.3	5.5	5.9	6.0	6.2	6.5
500		6.0	5.8	5.6	5.5	5.4	5.3	5.2	5.1	6.3	6.4	6.5

Table 2
Effect of pH and initial zinc (II) concentration on zinc adsorption

	Initial Zinc (II) conc [mg/L]	Resting cells of Fusarium solani	pH				
			2	3	4	5	6
Effect of pH	50	Adsorption [%]	10	20	34	100	98
		Specific Zinc removal [mg/g]	3.13	5.33	7.72	11.02	10.89
Effect of initial Zinc (II) concentration (mg/l)	50	Adsorption [%]	-	-	-	100	-
		Specific Zinc removal [mg/g]	-	-	-	11.02	-
	100	Adsorption [%]	-	-	-	81.9	-
		Specific Zinc removal [mg/g]	-	-	-	15.23	-
	200	Adsorption [%]	-	-	-	73	-
		Specific Zinc removal [mg/g]	-	-	-	24.65	-
	300	Adsorption [%]	-	-	-	68	-
		Specific Zinc removal [mg/g]	-	-	-	35.43	-
	400	Adsorption [%]	-	-	-	62	-
		Specific Zinc removal [mg/g]	-	-	-	47.89	-
	500	Adsorption [%]	-	-	-	57.5	-
		Specific Zinc removal [mg/g]	-	-	-	51.7	-

Table 3
Effect of biomass concentration and culture age on specific zinc (II) removal

	Initial zinc(II) concentration (mg/l)	Resting cells of Fusarium solani	Zinc (II) removed (mg/l)	Specific zinc removal (mg/g)
Effect of biomass concentration	500	Biomass concentration (g/l)		
		2.5	123	49.2
		3.5	174.6	49.8
		4.5	232.6	51.7
		5.5	284.3	51.7
Effect of culture age	500	Culture age (h)		
		12	48.72	40.6
		24	173.43	42.3
		36	232.65	51.7
		48	232.65	51.7

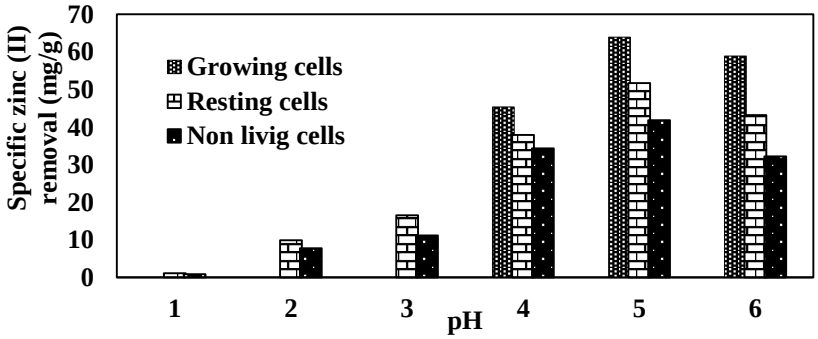

Figure 2: Comparison of the highest specific uptake of zinc (II) during growth, resting conditions and as non-living cells at an initial concentration of 500 mg zinc (II)/L

In figure 2, growing cells up to pH 3.0 are used without any zinc (II) removal. This is because, at pH levels below 3.5, the organism's ability to develop was impeded, yet, at the same pH, selective zinc (II) removal was possible utilising both resting and non-living cells. Likewise, increased zinc (II) removal in Fusarium solani resting cells compared to non-living cells may be caused by extracellular and intracellular zinc buildup that is not dependent on metabolism. Induced cell activity via the membrane redox enzymes was obtained during growth of the Fusarium solani (separately for resting cell production) in the absence of zinc (II) in a growth media and maintaining the cells under resting condition using only a small amount of glucose as a maintenance energy source.

The extracellular buildup is the only reason why non-living cells remove less zinc (II) than live cells do. The specific zinc (II) uptake by Fusarium solani throughout its growth was found to be at its highest (63.9 mg/g) when glucose was fully utilised at a higher pH value (5.0).

At the same pH, the zinc (II) removal by resting cells was much lower (51.7 mg/g) whereas the removal by non-living cells was significantly lower (32.2 mg/g). Higher zinc (II) removal by growing cells of Fusarium solani might have been significantly influenced by growth-associated enzymatic activity at higher pH values. Although while pronated cell functional groups are less accessible at higher pH values, resting cells nevertheless significantly remove zinc (II) due to enzymatic activity.

Enzymatic activity was no longer observed once the cells were dried for zinc (II) adsorption. However, when the cells were expanding and taking zinc (II) from the broth, Fusarium solani was shown to have the maximum zinc (II) removal at pH 5.0. Throughout its growth, the Fusarium solani is likely to employ a complicated strategy of intracellular/extracellular accumulation or intracellular/ extracellular decrease. The results presented above clearly demonstrate that multiple processes contribute to zinc (II) uptake by Fusarium solani under various growth circumstances (non living, resting and growing cells). The pH environment appears to have a big impact on how zinc (II) is removed.

While in the case of non-living cells, a straightforward physical adsorption mechanism is involved and using the cells in resting conditions, it is claimed that extracellular and intracellular accumulation may occur simultaneously. The Fusarium solani is believed to have a complicated growth process that involves intracellular/extracellular enzymatic conversion as well as intracellular/extracellular accumulation. However, thorough research is required to determine the precise mechanism of zinc (II) removal.

Using growing cells of Fusarium solani at an initial zinc (II) concentration of 500 mg/l, batch tests on zinc (II) removal showed that the maximum specific zinc (II) removal occurred at pH 5.0. Almost 100% removal was achieved at lower concentrations up to 100 mg/l whereas only 57.51% removal was seen at the same concentration. When operating in continuous mode, a similar pattern was seen. Just 52.8 mg/g, or 47.52%, of the original 500 mg/l concentration of zinc (II) was removed at the 500 mg/l concentration. Other methods namely, IVPF and CVPF (two separate modes of pulse feeding operations) were tried to run the process in a fed batch reactor since it was unable to achieve complete removal at higher zinc (II) concentrations.

Due to the availability of new medium containing nutrients and the dilution of the medium limiting the inhibitory effect of the dissolved components in the medium, it was anticipated that fed batch operations would remove more zinc (II) than batch operations.

Figure 3 depicts the variations in residual zinc (II) concentration over time in the constant volume pulse feeding (CVPF) mode of operation at 500 mg/l initial zinc (II) concentration and at pH 5.0 in the presence of Fusarium solani. In CVPF, the medium volume was initially maintained at 3 l and the process was observed for 5 days. The medium included 500 mg/l zinc (II) content.

The figure suggests that on the fifth day, when glucose was fully used, the total residual zinc (II) content in the presence of Fusarium solani declined to 220 mg/l. This shows that the medium's 280 mg/l of zinc (II) was physiologically removed. It was discovered that the specific zinc (II) removal value which was 62.23 mg/g, was almost identical to the value (63.9 mg/g) achieved in batch operation. On the fifth day, 1L of homogeneous medium was removed from the reactor and fresh media (1 l) were added to restore the volume to its original 3 l. The nutrients and calculated amount of zinc (II) needed to keep the zinc (II) content at 500 mg/l were present in this fresh 1 l medium. This procedure was watched over until the 12th day in order to make sure that Fusarium solani had access to fresh nutrients and that the medium had been diluted.

The biological removal of zinc (II) was determined to be 129.2 mg/l on the 10th day and it remained constant until the 12th day, when 12% of the available glucose was still unutilized and 33.12 mg/g of specific zinc (II) removal was determined. Similar to the first, a second pulse feeding was performed on day 12 and the process was observed through day 20. On the 17th day, it was discovered that the total removal was 72.3 mg/l; this value remained constant until the 20th day and 55% of the glucose was left unutilized. The specific removal of zinc (II) was 22.59 mg/g.

Due to the pulse feeding during CVPF operation, it is possible that Fusarium solani was unable to adapt to the variable growth environment. Another option is the elimination of viable cells during medium withdrawal. Because of the inefficient use of glucose and the decreased growth, less zinc (II) was removed.

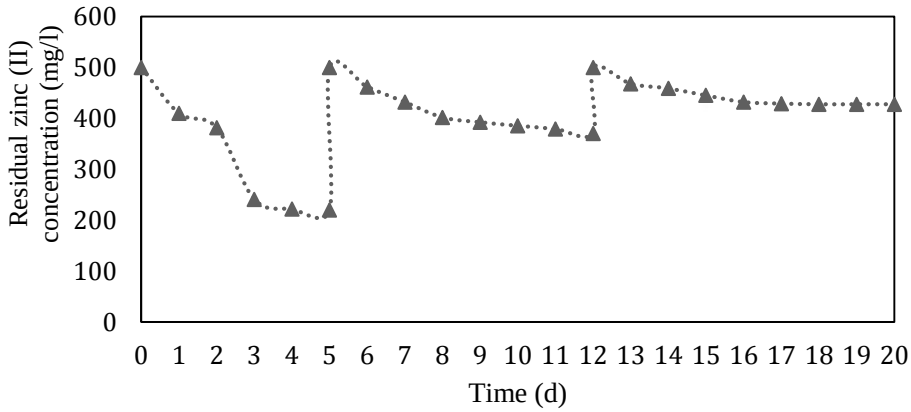

Figure 3: Shows how the residual zinc (II) concentration changes over time when utilising a medium in constant volume pulse feeding (CVPF) mode of operation.

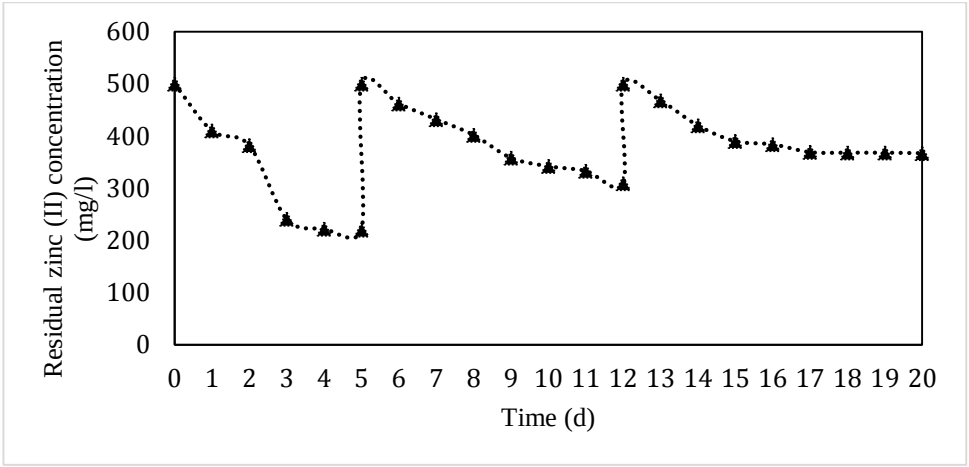

Figure 4: Shows how the residual zinc (II) concentration changes over time in the increasing volume pulse feeding (IVPF) mode of operation utilising sterile growth media at 500 mg/l initial zinc (II) concentration and pH 5.0

As no additional improvement in zinc (II) removal was seen after the first and second pulse feeding on the fifth and twelfth days, after 20 days the CVPF method was stopped.

Figure 4 depicts the variations in residual zinc (II) concentration over time in the increasing volume pulse feeding (IVPF) mode of operation at a pH of 5.0 and an initial initial zinc (II) ion concentration of 500 mg/l. In IVPF, the media volume was initially kept at 1l and the process was observed for 5 days until all the glucose had been used. The media included 500 mg/l zinc (II) concentration. In 5 days, a removal of 62.23 mg/g of specific zinc (II) was seen. On the fifth day, 1l of fresh media was added for the initial pulse feeding, which was then watched over until the twelfth day. The biological removal of zinc (II) in the presence of Fusarium solani was 191 mg/l on the 10th day, remained constant on the 11th day and was found to be 45.47 mg/g on the 12th day.

In a similar manner, a second pulse feeding was performed on the 12th day and on the 17th day, the removal was discovered to have fallen to 131 mg/l. It stayed consistent up until the 20th day when glucose was discovered to be 15% underutilised. 33.58 mg/g was the specific uptake of zinc (II). Following the first and second pulse feedings, considerable removal of zinc (II) was achieved in the IVPF mode as compared to the CVPF mode. It was noticed that the values were lower than those produced in batch mode. As a result, the process was unable to continue under fed batch conditions.

Table 4 compares zinc (II) removal in various modes of operations[11-13].

The findings from past studies utilising the same Fusarium solani under batch and continuous mode of operations are compared to the zinc (II) removal results obtained in the current study employing CVPF and IVPF mode of operations in table 4. The removal of zinc (II) in batch mode was 57.51%. The process was terminated after 20 days in CVPF mode due to a sharp decline in the percentage of zinc (II) removal after each feeding. The IVPF operation likewise showed a similar tendency.

Zinc (II) removal in continuous mode of operation was determined to be 81% using a two-stage reactor. The process could be successfully operated for a long period in continuous mode with higher zinc (II) removal as compared to other operational strategies, making it the best operational strategy for continuous removal of zinc (II) out of the aforementioned techniques..

Table 4
Comparison of the removal of zinc (II) during batch, IVPF, CVPF and continuous operation

Mode of operation		Conc. of zinc (II) removal (mg/l)	zinc (II) removed (%)	Rate of zinc (II) removal (mg/l/d)	Specific zinc (II) removal (mg/g)
Batch		287.56	57.51	57.51	63.9
IVPF	(0-5 d)	280	56	56	62.23
	1st pulse feed(6-12d)	191	38.2	27.28	45.47
	2nd pulse feeding (13-20 d)	131	26.2	18.71	33.58
CVPF	(0-5 d)	280	56	56	62.23
	1st pulse feed (6-12d)	129.2	25.84	18.45	33.12
	2nd pulse feeding (13-20 d)	72.3	14.46	10.32	22.59
Continuous	Single stage	237.6	47.52	57.95	52.8
Multi stage	First stage	237.6	81 (over all)	57.95	52.8
	Second stage	167.4		33.85	37.2

The overall removal rate of zinc (II) with batch operation was 3.03 times higher than the rate attained in CVPF operation after the first pulse feeding and 5.4 times higher after the second pulse feeding.

Additionally, it was discovered that the removal rate in batch operation is 2 times higher and 3.0 times higher than the rate attained after the first and second pulses of feeding in IVPF operation. On the other hand, in a single stage bioreactor, continuous mode operation was shown to have a greater overall removal rate than batch operation whereas second stage operation was found to have a lower rate of zinc (II) removal.

Conclusion

The removal of zinc (II) in a single stage bioreactor can be carried out for an extended period of time at a greater removal rate. The ability to produce increased zinc (II) removal and to extend the process duration seems to be benefits of the multistage operation. In lower concentrations, a single stage reactor would be efficient but at larger concentrations, a multi stage reactor system would be required to receive enhanced removal of zinc (II) in a continuous manner.

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