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Journal of Hazardous Materials 153 (2008) 487–492
Journal of Hazardous Materials
www.elsevier.com/locate/jhazmat

Continuous treatment process of mercury removal from aqueous solution by growing recombinant *E. coli* cells and modeling study

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Received 27 February 2007; received in revised form 29 August 2007; accepted 29 August 2007
Available online 1 September 2007

Abstract

A continuous treatment process was developed to investigate the capability of genetically engineered *E. coli* to simultaneously accumulate mercuric ions and reproduce itself in a continuous stirred tank reactor (CSTR) system. The influence of dilution rate and initial Hg^{2+} concentration on continuous process was evaluated. Results indicated that the recombinant *E. coli* could effectively accumulate Hg^{2+} from aqueous solution with Hg^{2+} removal ratio up to about 90%, and propagate its cells at the same time in the continuous treatment system under suitable operational conditions. A kinetic model based on mass balance of Hg^{2+} was proposed to simulate the continuous process. The modeling results were in good agreement with the experimental data.

Keywords: Continuous treatment; Mercuric ion; Bioaccumulation; Genetically engineered *E. coli*

1. Introduction

By employing microorganisms as biosorbent to remove heavy metal contaminants, biosorption has received a great deal of attention [1,2]. Among those studies, harvested biomass (dead/pre-treated) was mostly used, where metal was adsorbed mainly at cell surface [3], rather than taken up into the cells. Thus, only a small fraction of bioaccumulation capacity was exploited. On the other hand, biosorption was sensitive to ambient conditions such as pH, ion strength, coexisting metals and organic substances [4], and also lacked specificity to desired metals. Moreover, regeneration of biomass was often involved with acid or base, possibly resulting in a second environmental pollution [5]. Further, biomass needed to be exchanged after a maximum of 5–10 sorption–desorption cycles. Therefore, biosorption alone might not suffice for effective metal remediation, and it seemed difficult to develop a continuous system based only on biosorptive removal of metals using microbial biomass [6].

Under such situation, application of active or growing cells might be a good alternative because of their ability of self-replenishment and the potential for optimization through development of resistant species and gene modification [7,8]. Employment of active microorganisms might not only allow development of a single-step process to remove most pollutants present in industrial effluents, but also avoid the need for a separate biomass production process (cultivating, harvesting, drying and storage, etc.) [9]. Furthermore, by utilizing organic wastes in wastewater as carbon substrates while bioaccumulating heavy metals, growing cells could offer a promising biotechnology for continuous treatment of heavy metal wastewater.

In our previous studies, genetically engineered *E. coli* JM109 expressing both mercury transport system and metallothionein (MT) was tested for its capability of simultaneous cell propagation and mercuric ion bioaccumulation in batch experiments [10]. In this paper, a continuous treatment process was developed to examine whether the recombinant strain could accumulate mercuric ion and reproduce its cells effectively at the same time in a continuous stirred tank reactor (CSTR). A mathematical model was further proposed to simulate the continuous process.

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doi:10.1016/j.jhazmat.2007.08.080

2. Materials and methods

2.1. Bacterial strain

E. coli JM109, which simultaneously expressed mercury transport system and MT, was used in this study [11,12]. Before use, cells were prepared as described previously [10].

2.2. Bioaccumulation procedure

For batch uptake, *E. coli* cells were harvested by centrifugation (10,000 × *g* for 10 min), washed with 10 mM phosphate buffer (pH 7.0), resuspended in different Hg^{2+} concentration solutions, and shaked 1 h at 150 r/min and 37 °C. Then the cells were removed by centrifugation and the supernatants of the solutions were analyzed for residual Hg^{2+} concentrations.

For continuous bioaccumulation, bacterial cells were inoculated into 350 ml sterile Luria-Bertani medium (LB, peptone 10 g/L, yeast extract 5 g/L and NaCl 10 g/L) containing ampicillin (50 mg/L) and kanamycin (30 mg/L), and incubated in a water-jacketed stirred tank reactor (12 cm in height and 15 cm in diameter) at 150 r/min, 37 °C. As cell growth reached stationary phase, 1:1 diluted LB medium (peptone 5 g/L, yeast extract 2.5 g/L and NaCl 5 g/L) containing desired Hg^{2+} concentration was continuously pumped into the reactor at different flow rates F (or dilution rates D, $D = F/V$) by a peristaltic pump. Effluent samples were collected manually at the different time intervals to measure OD_{600}, and then the cells in effluent were centrifugalized, producing supernatant for Hg^{2+} analysis. For effluent flowing out of the system, a 0.45 μm-pore-diameter nitrocellulose filter was used to prevent the cells from releasing to the environment. During the experiments, the reaction volume (V) in the reactor kept constant at 350 mL, and the stirred speed and temperature were kept 150 r/min and 37 °C, respectively. The experiments were carried out by triplicate at the same conditions and the results expressed as mean values. Hg^{2+} removal ratio of the continuous process (E, %) was obtained via $(C_{in} - C_{out})/C_{in}$, where C_{in} and C_{out} were Hg^{2+} concentrations of influent and effluent, respectively (mg/L); Hg^{2+} removal capability of the continuous process (R, mgHg^{2+}/Lh) was acquired from F_{in} $(C_{in} - C_{out})/V$, where F_{in} was the flow rate of influent (L/h).

2.3. Analytical methods

Hg^{2+} was determined by cold vapor atomic absorption spectrophotometry. The dry weight of cells was determined after the cell pellet had been dried at 80 °C for 24 h. The value of 0.35 g dry weights per liter of OD_{600} 1.0 was obtained in the experiment. Hg^{2+} uptake capacities of *E. coli* cells were expressed as mg Hg^{2+} accumulated by per gram dry weight of cells.

3. Results and discussions

3.1. Determination of bioaccumulation isotherm

Bioaccumulation equilibrium of recombinant *E. coli* JM109 was investigated by contacting the cells with solutions containing different concentrations of Hg^{2+} for 1 h. Two isotherm equations were applied for the equilibrium modeling of bioaccumulation system, Langmuir and Freundlich isotherms:

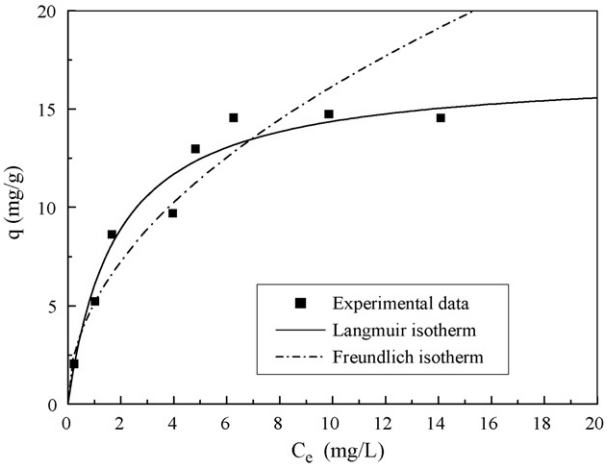

Fig. 1. Comparison of Langmuir and Freundlich isotherm.

- Langmuir equation:

$$q = \frac{q_m C_e}{K + C_e} \quad (1)$$

where C_e is the residual or equilibrium concentration of Hg^{2+} (mg/L), q the bioaccumulation of Hg^{2+} (mg/g), q_m the maximum bioaccumulation capacity (mg/g), and K is the dissociation constant (mg/L).

- Freundlich equation:

$$q = kC_e^{1/n} \quad (2)$$

where k and n were modeling parameters.

Fig. 1 shows the Langmuir and Freundlich bioaccumulation isotherms of Hg^{2+} by recombinant *E. coli* cells. As shown from the figure, Langmuir isotherm model was more suitable to describe the bioaccumulation equilibrium of Hg^{2+} than Freundlich equation, which led to a larger deviation as Hg^{2+} residual concentration increased.

From Langmuir equation, q_m and K values could be drawn as 16.98 mg/g and 1.813 mg/L, respectively.

3.2. Continuous treatment process by growing E. coli cells in CSTR

In our previous study, genetically engineered *E. coli* JM109 was proved to be able to simultaneously accumulate Hg^{2+} and propagate its cells in Hg^{2+} solution containing organic matters [10]. However, those experiments were carried out in shaking flasks. To evaluate the ability of recombinant *E. coli* cells to remove Hg^{2+} from aqueous solution and grow at the same time in a continuous treatment system, a continuous stirred tank reactor was employed in this study. For a continuous process, dilution rate (D) and initial metal ion concentration (C_{in}) being two important operational factors were thus focused.

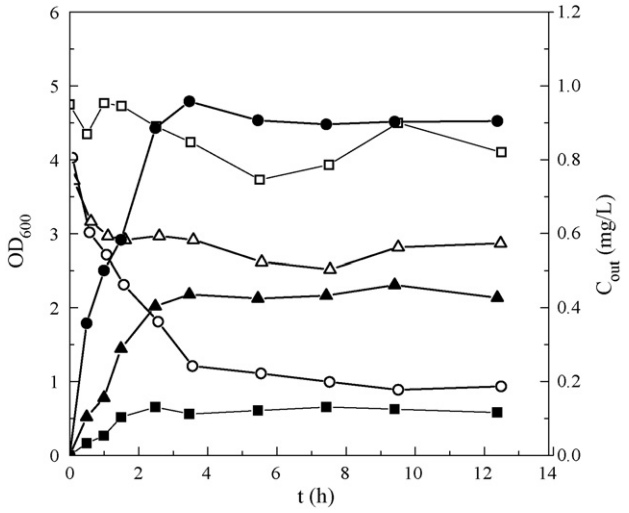

Fig. 2. Continuous treatment under constant influent mercurial concentration (C_{in} = 2 mg/L). White symbols refer to OD_{600} and black symbols refer to C_{out}. (□, ■) $D = 0.20\,h^{-1}$; (△, ▲) $D = 0.57\,h^{-1}$; (◯, ●) $D = 0.72\,h^{-1}$.

3.2.1. Effect of dilution rate

Fig. 2 shows the effect of dilution rate on the continuous process, where the influent was 1:1 diluted LB + 2 mg/L Hg^{2+}. It could be seen that both Hg^{2+} removal and cell growth proceeded successfully in the continuous system. Hg^{2+} concentration of effluent reached a plateau within 4 h, whose values were 0.12 mg/L, 0.42 mg/L and 0.90 mg/L with dilution rates being $0.21\,h^{-1}$, $0.57\,h^{-1}$ and $0.72\,h^{-1}$, respectively, indicating the increase of dilution rate decreased Hg^{2+} removal ratio of the process. Fig. 3 displays the Hg^{2+} removal ratio under different dilution rates. Ninety-two percentage of Hg^{2+} removal ratio could be achieved as dilution rate was $0.2\,h^{-1}$, while this value dropped to 54% with dilution rate coming up to $0.72\,h^{-1}$. Considering the rapid accumulation of metal ions by recombinant cells [11], the decrease of Hg^{2+} removal ratio could be because the cell growth was affected by the varying dilution rate, which might also affect Hg^{2+} removal ratio. From Fig. 2, OD_{600} of *E. coli* cells basically remained constant from the beginning as dilution rates were relatively low, suggesting bacterial cells could get used to the varying situation of the reactor rapidly and grew very well under low dilution rates. However, as dilution rate came up to a high value ($0.72\,h^{-1}$), cell growth was influenced seriously. OD_{600} dropped from the starting 4.0 to 0.94 and then kept stable. Fewer cells in the reactor would certainly result in a relatively lower Hg^{2+} removal ratio. Indeed CSTR in our experiment could be considered as a chemostat, where there is a critical dilution rate D_{cri}, whose value equals to the maximum specific cell growth rate μ_{max} at certain operational condition. When dilution rate of the system is higher than D_{cri}, all the cells in the reactor will be washed out, making the operation completely fail.

For a continuous process, removal capability (how many mg Hg^{2+} can be removed per reaction volume per hour by the system, R, mg/L h) was supposed to be paid more attention. Fig. 3 also exhibits Hg^{2+} removal capability of the process under different dilution rates. Among four studied dilution rates, $0.57\,h^{-1}$ was the best with the highest removal capability of 0.91 g/L h. At low dilution rates, though Hg^{2+} removal ratios were high, Hg^{2+} removal capabilities were too low, making the treatment system economically ineffective. On the other hand, higher dilution rate, even if no washout was observed, might not necessarily produce higher treatment capability. Therefore, a suitable dilution rate of a continuous treatment system should be determined by comprehensively taking both heavy metal removal ratio and removal capability into account.

3.2.2. Effect of initial Hg^{2+} concentration

With dilution rate keeping $0.36\,h^{-1}$, operation situations under different Hg^{2+} concentrations in influent (1:1 diluted LB) of 2 mg/L, 4 mg/L and 8 mg/L were displayed in Fig. 4. At certain dilution rate, the growth of the cells was dependent on Hg^{2+} concentration of the solution. Low Hg^{2+} concentration in influent affected cell growth slightly, so during the process OD_{600} easily kept stable at 2 mg/L of initial Hg^{2+} concentration, or just declined a little at 4 mg/L Hg^{2+} concentration. However, when initial Hg^{2+} concentration increased to 8 mg/L, an obvious inhibition effect on cell growth was observed, OD_{600} dropping from 3.6 to 0.96. As discussed previously, for a chemostat there is a washout dilution rate D_{cri} which equals to μ_{max} at certain con-

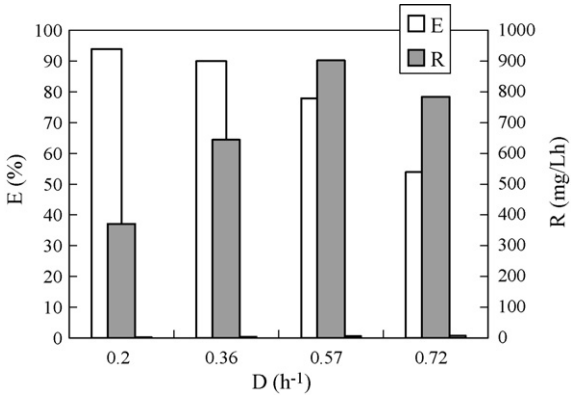

Fig. 3. Hg^{2+} removal ratio and Hg^{2+} removal capability of the continuous process under different dilution rates.

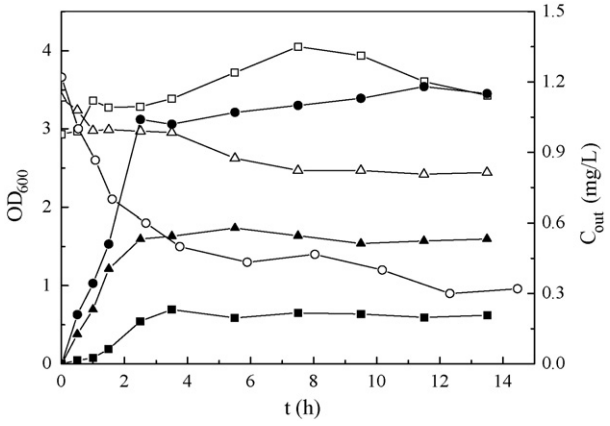

Fig. 4. Continuous treatment under constant dilution rate ($D = 0.36\,h^{-1}$). White symbols refer to OD_{600} and black symbols refer to C_{out}. (□, ■) C_{in} = 2 mg/L; (△, ▲) C_{in} = 4 mg/L; (◯, ●) C_{in} = 8 mg/L.

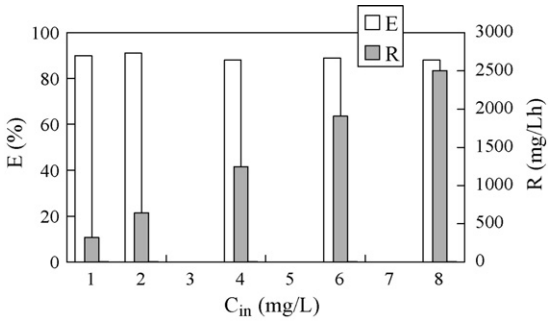

Fig. 5. Hg^{2+} removal ratio and Hg^{2+} removal capability of the continuous process under different initial Hg^{2+} concentrations.

dition. Apparently μ_{max} may be decreased with the increase of toxic metal ion concentration in the solution. Accordingly, it is predictable that even if dilution rate of the continuous system keeps constant, there must be an initial toxic metal ion concentration, as long as it is high enough, to make μ_{max} less than operational dilution rate, leading to washout.

From Fig. 4, it can be concluded that Hg^{2+} was effectively accumulated by the growing cells under three conditions, with Hg^{2+} concentration in the solution declining from 2 mg/L, 4 mg/L and 8 mg/L to 0.2 mg/L, 0.51 mg/L and 1.13 mg/L, respectively. Fig. 5 displays the Hg^{2+} removal ratio and Hg^{2+} removal capability under different initial Hg^{2+} concentrations in influent as dilution rate kept at 0.36 h^{-1}. All the removal ratios were about 90%, indicating high efficiency of recombinant *E. coli* cells to accumulate Hg^{2+} from the solution. In the meantime, Hg^{2+} removal capability of the system was enhanced with the increase of initial Hg^{2+} concentration. As initial Hg^{2+} concentration was 8 mg/L, Hg^{2+} removal capability reached 2.5 g/L h. This should be attributable to the high bioaccumulation capacity of the recombinant cells, which did not show up at low Hg^{2+} concentration. As initial Hg^{2+} concentration went up, the cells could accumulate more mercuric ions in the solution, thus producing high removal capability. However, just as mentioned above, it should be noted that higher initial Hg^{2+} concentration, if seriously inhibiting cell growth, might result in lower removal capability, or even complete failure of the continuous treatment, though this situation was not observed in our study.

In our previous study, a continuous treatment process was developed to remove Hg^{2+} from electrolytic wastewater by genetically engineered *E. coli* [11]. A hollow-fiber bioreactor was used in the system to retain the cells and more than 99% of mercuric ions in the wastewater were effectively accumulated. Chen and Kim realized a continuous treatment on Hg^{2+} removal by recombinant *E. coli* in a hollow-fiber bioreactor [13]. Other researchers also set up some continuous processes to accumulate heavy metal ions with biomass [5,14,15]. However, in those systems microorganisms did not grow and reproduce as they accumulated heavy metal ions from aqueous solution. As a result, if the accumulation capacity of all the cells in the system was saturated, i.e., the breakthrough point reached, the treatment process had to be ceased and fresh cells had to be reloaded to the system to restart the treatment, making the process technically and economically ineffective. Seriously, those could not be considered 'continuous' processes. Our technology, on the contrary, could overcome this disadvantage and therefore realize a 'real' continuous treatment process via combining heavy metal wastewater with organic wastewater in the system, where bacterial cells utilize organic matters as nutrients to propagate themselves as they accumulate metal ions and accordingly the reloading process of fresh cells can be eliminated.

3.3. Kinetic modeling of continuous treatment process

Analysis of experimental data is important for developing an equation that can be used to optimize an operation procedure. To evaluate the characteristics of continuous process, we proposed a mathematical model based on the mass balance of Hg^{2+} in the reactor. To simplify the model, three postulations were put forward:

1. The reactor could be considered as an ideal continuous stirred tank reactor, so the mixing of all substances in the reactor could reach even rapidly.
2. Considering the Hg^{2+} uptake was very fast [11], the bioaccumulation equilibrium of Hg^{2+} by recombinant cells could be achieved in the reactor and described by Langmuir model [13].
3. Our kinetic model was proposed to simulate the changing curve of Hg^{2+} in effluent and thus predict the final value of Hg^{2+} concentration as the continuous process reached stable operation. However, from the beginning of the treatment, the cells kept growing and propagating as they accumulated Hg^{2+}, so the concentration of the cells was varying all the time before the process came to the stable phase. The varying concentration of the cells made the model complicated. Considering the difficulty to simultaneously simulate the changing situation of cell propagation and Hg^{2+} removal, we assumed that the average concentration of the cells before its stable phase could be employed for the development of the model.

The mass balance of Hg^{2+} of the reactor was demonstrated in Fig. 6. Apparently from time t to $t + dt$, Hg^{2+} amount pumped into the reactor (Hg_{in}) should be the sum of Hg^{2+} amount accumulating in the reactor (Hg_{ac}) and Hg^{2+} amount flowing out of the reactor (Hg_{out}):

$$Hg_{in} = Hg_{ac} + Hg_{out} \quad (3)$$

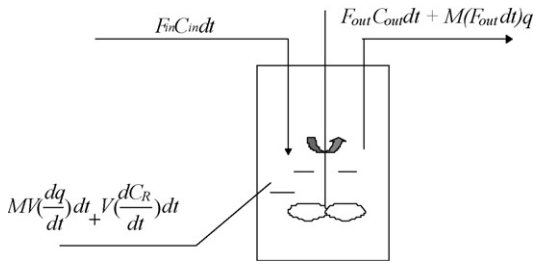

Fig. 6. Mass balance of Hg^{2+} of the reactor.

From Fig. 6:

$$Hg_{in} = F_{in} C_{in}\, dt \quad (4)$$

where F_{in} is the flow rate of influent (L/h) and C_{in} is the Hg^{2+} concentration of influent (mg/L).

Hg_{ac} included two parts, Hg^{2+} in the solution in the reactor and Hg^{2+} accumulated by the cells that still stayed in the reactor:

$$Hg_{ac} = MV\left(\frac{dq}{dt}\right) dt + V\left(\frac{dC_R}{dt}\right) dt \quad (5)$$

where M is the average concentration of the cells in the solution (g/L), V the solution volume in the reactor (L), q the Hg^{2+} uptake by the cells (mg/g) is calculated by Eq. (1), and C_R is the Hg^{2+} concentration in the reactor (mg/L).

Hg_{out} also included two parts, Hg^{2+} in effluent and Hg^{2+} accumulated by the cells flowing out of the reactor:

$$Hg_{out} = F_{out} C_{out}\, dt + MqF_{out}\, dt \quad (6)$$

where F_{out} and C_{out} were the flow rate (L/h) and Hg^{2+} concentration (mg/L) of effluent, respectively.

From Eqs. (3)–(6) we have

$$F_{in} C_{in}\, dt = V\, dC_R + MV\, dq + F_{out} C_{out}\, dt + MqF_{out}\, dt \quad (7)$$

As V kept constant during the process, so

$$F_{in} = F_{out} \quad (8)$$

For CSTR, with postulation 2, it could be deduced:

$$C_R = C_e = C_{out} \quad (9)$$

where C_e is the equilibrium concentration of Hg^{2+} (mg/L).

Combining Eq. (7) with Eqs. (8) and (9), finally we have

$$\frac{dC_{out}}{dt} = \frac{D(C_{in} - C_{out})(K + C_{out})^2 - (K + C_{out})MDq_m C_{out}}{(K + C_{out})^2 + MKq_m} \quad (10)$$

where D is the dilution rate ($D = F/V$, h^{-1}) and K and q_m were parameters demonstrated in Eq. (1).

From Eq. (10) the curve of theoretical C_{out} versus t could be obtained. Part of the modeling results was shown in Fig. 7. It could be concluded that despite some deviation observed, the calculated results were in fairly good agreement with the experimental data, indicating the kinetic model proposed in this paper could be used to simulate the continuous treatment process and predict the changing of Hg^{2+} concentration in effluent.

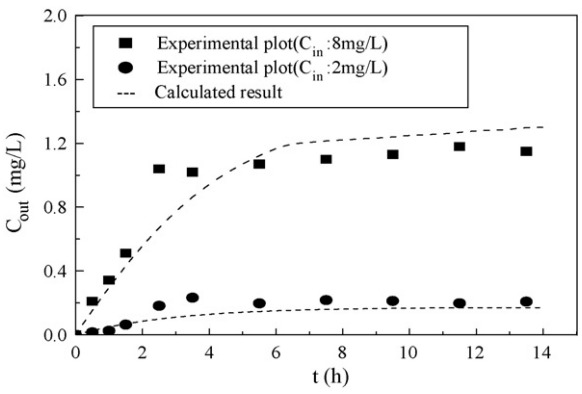

Fig. 7. Comparison of experimental data with calculated results ($D = 0.36\ h^{-1}$).

Deviation between modeling results and experimental data might mainly come from two aspects. Deduced from Eq. (10), curve C_{out} versus t was influenced by not only D and C_{in}, but also M, q_m and K. As mentioned in postulation 3, cell concentration was varying prior to stable phase, so using average cell concentration might result in deviation. On the other hand, though Langmuir model (q_m and K) has been widely employed to describe biosorption and bioaccumulation processes [4,5,16] and worked well in our study (Fig. 1), theoretically it was based on the assumption of monolayer surface adsorption, which might also produce some error.

4. Conclusion

Genetically engineered *E. coli* JM109, which expressed both Hg^{2+} transport system and metallothionein, was tested for its ability to simultaneously grow and bioaccumulate Hg^{2+} in aqueous solution containing mercuric ions and organic matters in a continuous stirred tank reactor system. The effect of two operational factors, dilution rate and initial Hg^{2+} concentration, was focused. It could be concluded that by operating under suitable dilution rate and initial metal ion concentration, the recombinant *E. coli* cells could propagate themselves during the whole process, reaching a stable phase after several hours of growing. At the same time, the growing cells could effectively accumulate Hg^{2+} from the solution in the continuous system, possibly suggesting the potential of our technology to develop a 'real' continuous treatment process different from those by employing inactivated biomass. Furthermore, a mathematical model was put forward based on the mass balance of Hg^{2+}. The good agreement between calculated results and experimental data indicated that the kinetic model could be used to simulate the continuous process.

Acknowledgement

The financial support of Shenzhen research project QK200601 and 863 program of China (2005AA601010-05) are gratefully acknowledged.

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