Packed Bed Reactor Model for De-mercurization of Simulated Mercury-Laden Wastewater

Sanjukta Ghoshal • Pinaki Bhattacharya • Ranjana Chowdhury*

Chemical Engineering Department, Jadavpur University, Kolkata-700 032, India
Corresponding author: * ranjana.juchem@gmail.com

ABSTRACT

Microbial reduction of soluble bivalent mercury to its less toxic elemental metallic form was performed using mercury-resistant *Bacillus cereus* (JUBT1) isolated from the sludge of chloralkali industries. A lab-scale 1 m long and 0.05 m diameter packed bed biofilm reactor was designed to remove mercuric ions (Hg^{2+}) using isolated bacterial consortium. The bioreactor was continuously fed with sterile simulated wastewater containing $HgCl_2$ to reduce bivalent mercury to its elemental form by growing bacterial biofilm on porous packing material of the reactor. The performance efficiency of the reactor was studied varying different chemical and hydrodynamic parameters such as inlet concentration of mercury and inlet flow rate of simulated mercury-laden water, among others. The reactor was followed by an activated carbon-based adsorber to remove residual mercury in the reactor effluent. A maximum of 97-98% removal efficiency was obtained with respect to the concentration of Hg^{2+} in the inlet water. A deterministic mathematical model was developed to explain the performance of the packed bed reactor.

INTRODUCTION

Mercury (Hg), a highly hazardous heavy metal, is found to be present in appreciable quantity in the solid waste of caustic chlorine industries. Hg is also present in small or appreciable quantity in the industrial effluent of battery industries, goldsmith industries and industries for medical instruments. Due to its extensive applications, wide spread distribution, high toxicity and bio-magnification (Canstein *et al*. 1999; Okoronkwo *et al*. 2006), Hg poses a great threat towards environmental pollution. Hg is capable of binding with sulfhydryl, thioester and immidazole groups in its bivalent cationic form and thus inactivates the enzyme function (Horn *et al*. 1993; Okino 2000) causing absolute system disorder. Use of microorganisms for Hg removal is a very effective technique for the treatment of industrial wastewater (Essa *et al*. 2005; Ruiz 2005; Sorkhoh *et al*. 2010). The present techniques to abate Hg pollution are highly energy intensive and thus the process, when used for large scale Hg separation, becomes highly expensive. Application of bioremediation of contaminated soil is presently gaining a widespread application because of its low energy requirement, low cost (Oehmen *et al.* 2009; Mathivanan *et al.* 2010) as well as appreciably high purification efficiency. Bacteria containing Hg–resistance (mer) determinants are capable of reducing mercuric ions. Resistance to bivalent Hg ions in bacteria is conferred by the NADPH (Reduced Nicotinamide Adenine Dinucleotide Phosphate) dependent mercuric reductase, a cytoplasmic flavoenzyme (Horn *et al*. 1993; Deckwer *et al.* 1999; Brown *et al*. 2002; Schneider and Deckwer 2005; Kiyono and Pan-Hou 2006). Studies on biotransformation of Hg contaminated industrial effluent are now being carried out by different research groups with the objectives of transforming soluble bivalent Hg to its less toxic elemental metallic form which can be conveniently separated using conventional downstream processing. Kannan and Krishnamoorthy (2006) isolated Hg-resistant *Bacillus cereus* in one of their studies and found that the bacteria efficiently reduce Hg to its elemental form. It is now well established that Hg-resistant bacteria are able to perform this biotransformation with significantly high rate under controlled environmental conditions. Jayasankar *et al.* (2008) observed that several marine bacteria were highly resistant to Hg and were capable of detoxifying Hg along with some other heavy metals (De and Ramaiah 2007). Although much is known on the isolation, identification and culture medium formulation for Hg-resistant bacteria, only little attention has been paid on the understanding of growth kinetics of Hg-resistant cell as well as bioprocess engineering behaviour of the cells in different contacting devices in order to achieve favourable product distribution.

In the present investigation growth kinetics of the isolated strain (identified by 16S r DNA technology) of Hg-resistant bacteria namely, *B. cereus* (JUBT1) has been investigated. A biofilm reactor using immobilized form of the isolated strain has been operated to remove bivalent mercuric ions from simulated wastewater. A deterministic mathematical model has been developed for the biofilm reactor to predict its performance under the influence of different parameters like inlet concentration of mercuric ions and volumetric flow rate of the feed solution etc.

MATERIALS AND METHODS

Bacterial strain

Hg-resistant bacteria have been isolated from the sludge of chloralkali industry initially as a mixed culture. A pure monoculture was purified from the mixed culture using conventional microbiological techniques. The morphology and genes of the isolated bacteria have been established using microbiological, biochemical and 16S-rDNA (ribosomal deoxyribonucleic acid) techniques (Genei, Bangalore).

Original Research Paper

Chemicals

Sucrose (SRL, India), yeast extract powder (LOBA Chemie, India), NaCl (Merck, India), $HgCl_2$ (Merck, India), NaH_2PO_4 (Merck, India) and Na_2HPO_4 (Merck, India) were used in the present experiment.

Bacterial enrichment and growth condition

Hg-resistant *B. cereus* (JUBT1) was isolated from the sludge of chloralkali industry (Hindusthan Heavy Chemicals, West Bengal) and was cultured in laboratory condition in Hg specific growth medium composed of (per liter) sucrose (10 g), yeast extract (10 g), NaCl (30 g) dissolved in sodium phosphate buffer (0.25 M). Temperature and pH of the culture broth were maintained at 30°C and 7, respectively. At first the selective sterile culture broth (0.2 Mpa, 121°C, 15 min) was inoculated with the sludge of the chloralkali industry. The growth medium was supplemented with $HgCl_2$ solution at a concentration of 0.002 g/m^3. The broth was incubated in a rotary shaker at 30°C for 7 days at 150 rpm. Subsequently, 10% of the suspension was transferred into another set of 50 ml Erlenmeyer flasks containing media of higher concentrations of Hg^{2+} in steps up to $0.03 g/m^3$ to help proliferation of the desired micro-organism. After 10 such transfers in 0.03 g/m^3 mercury-containing media, a loop-full of inoculum was streaked onto agar plates having similar composition and was incubated for 24 h at 37°C. Streaking of colony with same morphology was carried out for five times. Incubation was done for 24 h at 37°C. The pure cultures were refrigerated at 4°C for further uses.

Dry weight method for the determination of bacterial mass

The biomass concentration in the reaction broth was determined by dry weight method. In this method the broth was centrifuged at the rate of 10,000 rpm for 15 min at 4°C. The bacterial mass was then transferred to a pre-weighed aluminium cup and dried at 50°C overnight. The exact weight of the bacterial mass was determined by subtracting the weight of dry cup from that of the cup containing dry bacterial mass.

Mercury assay

The outlet samples from the packed bed reactor and the samples of the batch experiments were centrifuged at 10,000 rpm for 15 min and the supernatants were analyzed using cold vapour atomic absorption spectrophotometer (A-analyst, 200; Perkin-Elmer) to determine Hg concentration. The wavelength for determination of Hg was 253.7 nm.

Batch experiment

Growth kinetics of *B. cereus* (JUBT1) with respect to sucrose was determined by conducting batch experiments varying initial sucrose concentration from 1000 to 10,000 g/m^3 in Erlenmeyer flasks under shaking condition in absence of Hg. Separate batch experiments were performed varying initial concentration of Hg^{2+} from 0.005 to 0.03 g/m^3, the sucrose concentration being kept at 10,000 g/m^3. A constant temperature of 30°C and a neutral pH were maintained. Samples were withdrawn at an interval of 8 h over 72 h period.

Bioreactor design and set up

A laboratory scale packed bed reactor (**Fig. 1**) made up of stainless steel having 1 m length and 0.05 m diameter was used in order to treat the sterile simulated wastewater containing $HgCl_2$. The inlet concentration of Hg was varied from 0.005-0.03 g/m^3. The solution was pumped into the packed bed column in upward direction. The reactor was filled with rice husk. Isolated bacteria were grown as bio-film on the rice husk (**Figs. 4A, 4B**) inside the reactor. Performance of the bioreactor was studied using different parameters like inlet concentration of mercuric ions and volumetric flow rate of the feed solution etc. Samples collected from the reactor were analyzed for concentration of Hg^{2+} using atomic absorption spec-
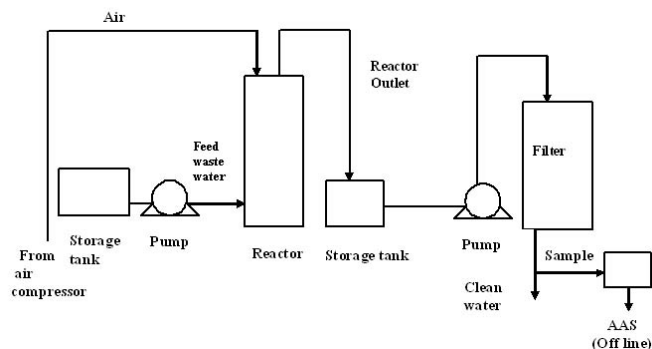

Fig. 1 Schematic diagram of bioreactor set up.

trophotometer. A 1 m long and 0.25 m diameter filter, packed with activated carbon, was placed after the reactor to adsorb the residual Hg from the reactor outlet (**Fig. 1**).

Statistical and data analyses

Non-linear regression analysis of the experimental data derived from batch experiments was performed using the method of least squares to determine the growth kinetics of the isolated bacterial strain, i.e. *B. cereus* (JUBT1). The regression coefficients lie in the range of 0.96 to 0.98.

RESULTS AND DISCUSSION

In the present investigation, a set of batch experiments has been performed to determine the kinetic parameters of growth of the Hg-resistant bacteria, *B. cereus* (JUBT1), isolated from the sludge of chloralkali industry. Kannan and Krishnamoorthy (2006) isolated seven strains of *B. cereus* from Pulicat lake sediment, India and identified them on the basis of fatty acid profile. All the isolates exhibited high tolerance to mercuric compounds. All the strains exhibited longer lag phase at 200 µg/ml $HgCl_2$ concentration. They have also added that growth of bacterial strains in liquid culture medium also depends on the bioavailability and toxicity of mercuric compounds. Several organic matters like yeast extract and also some negatively charged ions like chlorides which bind with the metal and alter the bioavailability, toxicity and rate of diffusion of metals in the medium. Therefore, they concluded that the growth of bacterial cells shows some small differences according to the increasing concentrations of toxic mercuric compounds in liquid medium. Initial concentrations of Hg and that of limiting substrate, sucrose, have been varied in order to compare their effect on Hg reduction capacity of the bacterial culture. It was found that growth of the isolated bacteria under study increases with the increase in Hg concentration which was varied from 0.005-0.03 g/m^3. So considering Hg as the second substrate the multiple substrate growth kinetic model was used to describe the dual substrates limited growth of the bacteria. De and Ramaiah (2007) found that the toxic effect of Hg enhanced the lag phase of the bacteria but as the bacteria adapted to the toxic Hg by means of detoxification growth was normal. In another study, De et al. (2007), isolated six Hg-resistant bacteria and reported the similar growth pattern, in some cases a steep increase follows a long gap whereas in another cases they observed exponential growth without any lag.

Determination of growth kinetics of bacterial strain

Systematic batch studies have been conducted for the determination of growth kinetics of the isolated bacterial strain varying the initial concentration of bivalent Hg in the range of 0.005-0.03 g/m^3 and that of sucrose, the only carbon source, in the range of 1000-10,000 g/m^3. For each set of batch experiment, biomass and residual Hg concentration of
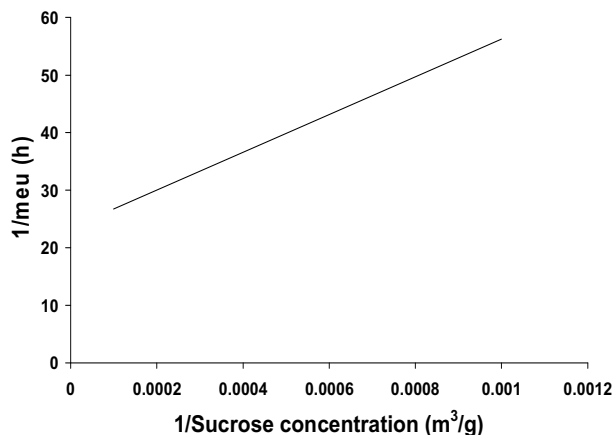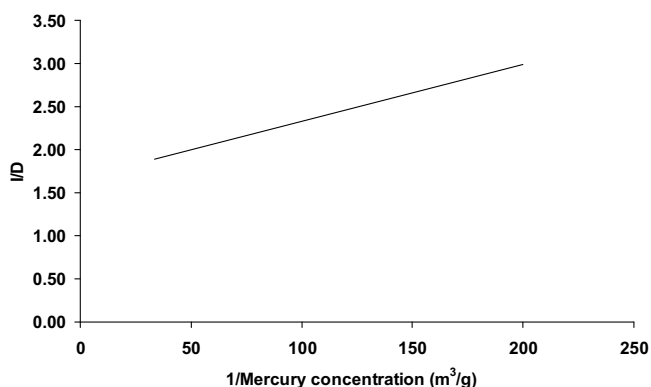
Fig. 2 Graph for μ_{max} and K_{S1}.

Fig. 3 Graph for K and K_{S2}.

Table 1 Values of the kinetic parameters.

Kinetic parameter	Value
μ_{max} (h^{-1})	0.043
K_{S1} (g/m^3)	1410.787
K	0.6
K_{S2} (g/m^3)	0.00396

the culture broth have been determined at a particular time interval. Biomass concentration has been determined using dry weight method. Residual Hg concentration has been measured using atomic absorption spectrophotometer. The kinetic parameters namely μ_{max} – maximum specific growth rate and K_s – saturation constant related to sucrose have been calculated by performing batch type experiments varying the initial concentrations of carbon source, sucrose (1000-10,000 g/m^3) without addition of HgCl$_2$ in the liquid medium. Similar experiments have been conducted varying Hg concentration from 0.005-0.03 g/m^3 at a constant value of sucrose concentration of 10,000 g/m^3. In one of their studies, Wagner-Dobler *et al.* (2003) observed that the enzymatic Hg biotransformation mechanism followed by common bacterial culture is reaction controlled. This reaction is a bireactant mechanism having substrate inhibition where Hg (II) acts as an uncompetitive inhibitor and the reducing cofactor NADPH$_2$ acts as a competitive inhibitor. According to them the complete enzyme kinetics within the cell can be described appropriately by the King-Altman method. They have calculated the reaction rate of Hg biotransformation considering NADPH$_2$ concentration to be constant. From the experiment they observed that both the natural isolates having mer-resistance genes as well as the genetically engineered microorganism used in the experiment exhibited the general kinetic behaviour. In another study, Wagner Dobler (2003) also stated that enzymatic detoxification of mercuric ions follows typical inhibition kinetics with respect to optimum Hg concentration in the culture broth. This absolute
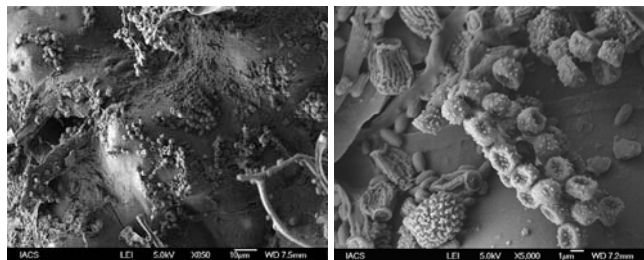

Fig. 4 Scanning electron micrographs of *Bacillus cereus* (JUBT1) grown on packing material. Left: magnification = X850; Right: magnification = X5000.

value for optimum Hg concentration depends on the buffer and medium used, cell density and growth stage of the microbial culture used and also on characteristics of the bacterial strains. They noticed that in case of unbuffered Hg solution microbial growth occurs up to 1-10 mg/l and in case of buffered solution growth occurs almost up to the concentration of 70 mg/l. In the present study, it has been observed that the growth of microorganism under study increases with the increase in Hg concentration in the present range under study. Therefore, the growth of the organism has been considered to be limited by dual substrates and a multiple substrate growth kinetic model has been attempted. The model is as follows:

$$\mu = \frac{\mu_{max} C_{S1}}{K_{S1} + CS_1} * \frac{KC_{S2}}{K_{S2} + CS_2} \tag{1}$$

In **Fig. 2**, the reciprocal of specific growth rate obtained in absence of mercuric ions, has been plotted against that of sucrose concentration. The values of μ_{max} and K_{S1} have been determined both graphically and through non-linear regression analysis. To determine the rate constants, K and K_{S2} with respect to Hg, in **Fig. 3** the reciprocal of a parameter δ, defined as $\delta = \mu_{\text{in presence of Hg}}/\mu_{\text{in absence of Hg}}$ has been plotted against the inverse of Hg concentration. Non-linear regression analysis has also been done to determine the same parameters. All kinetic parameters, namely, μ_{max}, K_{S1}, K_{S2} and K have been reported in **Table 1**. Kinetic parameters of microbial detoxification of Hg-laden wastewater have never been reported.

Biofilm

The characteristics of the scanning electron micrograph of the biofilm developed by growth of *Bacillus cereus* (JUBT1) on the reactor packing for 120 days have been shown in **Figs. 4A** and **4B**. From the micrographs it is clear that a distinct biofilm has been developed on the packing.

Reactor modeling

The following assumptions have been made during the development of the mathematical model,
1. Inlet feed stream is sterile;
2. Biofilm thickness remains unchanged throughout the operation;
3. The process is under steady state;
4. Reactor packing is random;
5. Biomass growth is according to Monod type of kinetic model;
6. Bed porosity(ε) remains same during the operation;
7. Both external and internal mass transfer resistances are negligible.
Applying the above assumptions the differential system equations based on Monod kinetics have been developed.
Making material balance for biomass over a differential volume of axial length Δz spanning over length "z" and "z+ Δz":
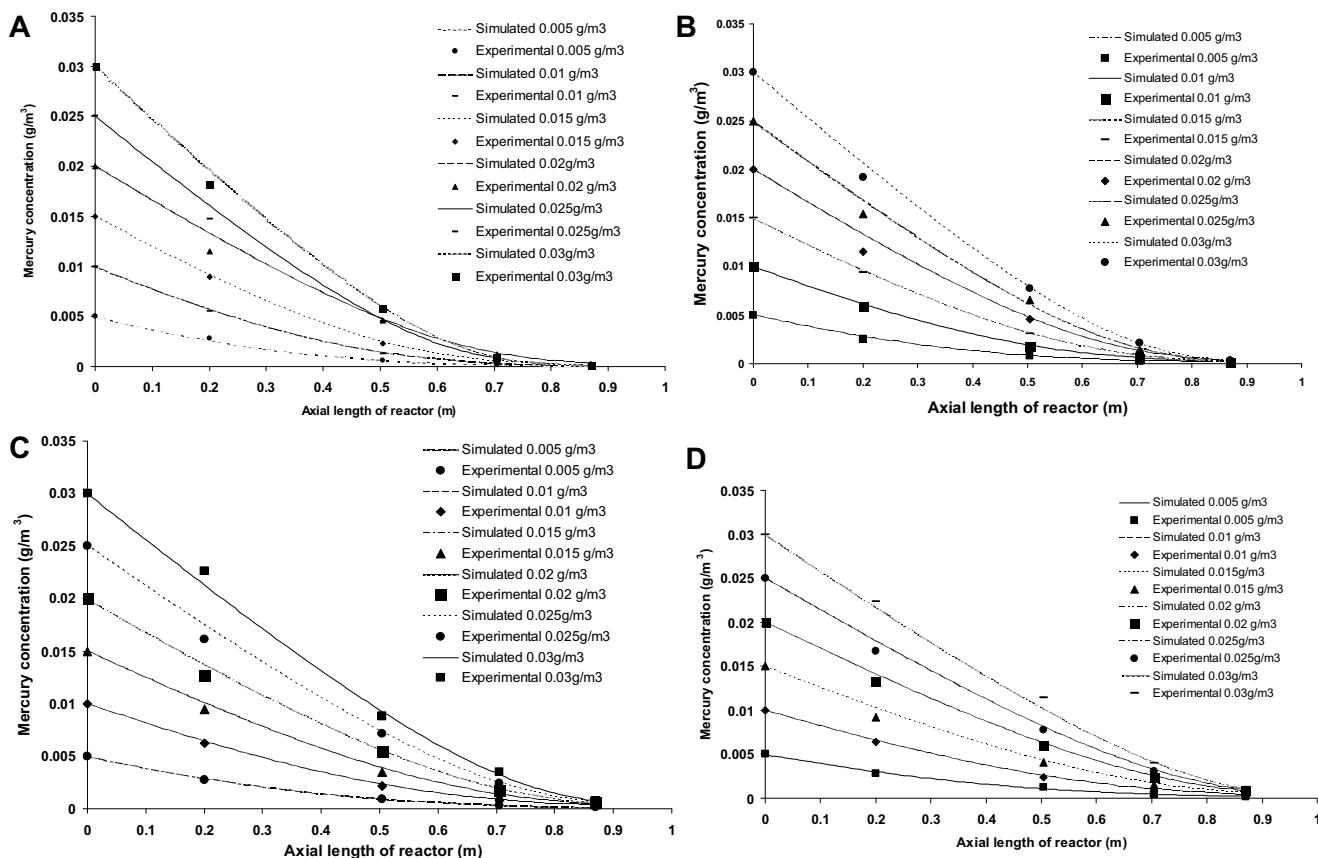
Fig. 5 Simulated and experimental profiles of mercury concentration along the axial length of the reactor (taking inlet mercury concentration as a parameter). (**A**) Superficial velocity of inlet $HgCl_2$ solution 0.0197 m/h. (**B**) Superficial velocity of inlet $HgCl_2$ solution 0.0217 m/h. (**C**) Superficial velocity of inlet $HgCl_2$ solution 0.0235 m/h. (**D**) Superficial velocity of inlet $HgCl_2$ solution 0.0248 m/h.

$$u A \varepsilon C_x \Big|_{z+\Delta z} - u A \varepsilon C_x \Big|_z = \mu C_x (1-\varepsilon) A a_p l \Delta z \qquad (2)$$

Dividing both sides by $A\varepsilon u\Delta z$ and limiting $\Delta z \to 0$,
Biomass:

$$\frac{dC_x}{dz} = \mu . C_x \frac{(1-\varepsilon).a_p.1}{u.\varepsilon} \qquad (3)$$

The yield coefficient with respect to any substrate, S, is defined as follows:

$$Y_{X/CS} = -\left(\frac{dC_x}{dC_s}\right) \qquad (4)$$

Therefore, the differential mass balance equations for sucrose and Hg may be written in the following forms,
Sucrose:

$$\frac{dC_{S1}}{dz} = -\frac{1}{Y_{X/CS1}}.\mu.C_x \frac{(1-\varepsilon)a_p.1}{u.\varepsilon} \qquad (5)$$

Hg:

$$\frac{dC_{S2}}{dz} = -\frac{1}{Y_{X/CS2}}.\mu.C_x.\frac{(1-\varepsilon).a_p.1}{u.\varepsilon} \qquad (6)$$

Equations (3), (5) and (6) have been solved using 4th-order Runge-Kutta method with the aid of a suitable Visual FORTRAN program.

In **Figs. 5A-D**, simulated values of Hg concentrations in the effluent drawn at different axial position have been plotted against the corresponding axial length using inlet concentration of Hg as a parameter. Experimental results have been plotted in the same figure. From the close analysis of the figure, it is evident that simulated results are in good agreement with the experimental results.

Similarly, in **Figs. 6A-F**, both the experimental and simulated values of Hg concentrations in the effluent drawn

at different axial position have been plotted against the axial length of the reactor using superficial velocity of reactor inlet as a parameter. This plot represents that the removal efficiency of Hg increases with the decrease in superficial velocity of the feed stream. This may be justified by the fact that with the decrease of superficial velocity, the residence time of Hg-laden stream in the reactor increases resulting in more transport and assimilation of Hg in the cellular system. It is also evident from the plot that simulated results are in good agreement with the experimental data for all the initial Hg concentrations studied.

In the present investigation a maximum of 90-95% Hg removal efficiency has been observed for the bio-film reactor. The efficiency increases to 97-98% when an activated carbon filter has been added after the reactor. Previous workers also observed the efficacy of removal of mercuric ions to lie in the range of 98-99% using the combination of bioreactor based on Hg-resistant microbial cultures and a carbon filter (Wagner-Dobler *et al.* 1999; Canstein *et al.* 2002; Wagner-Dobler *et al.* 2003). However, the efficiency of Hg detoxification obtained solely by the bio-film reactor has not been reported. In most of the reported works month-wise performance the bioreactor, coupled with carbon filter, has been reported. The comparison of experimental data with any theoretical prediction has not been made in the literature.

CONCLUSION

It is evident from the study that the biodetoxification of bivalent Hg using isolated Hg-resistant bacterial strain from chloralkali effluent is not only feasible but may also offer a promising route for industrial application as well. The results of the present investigation clearly indicate that the microbial Hg detoxification procedure is a highly effective system to purify the environment from Hg pollution. It is found that the treatment procedure is dependent on optimi-
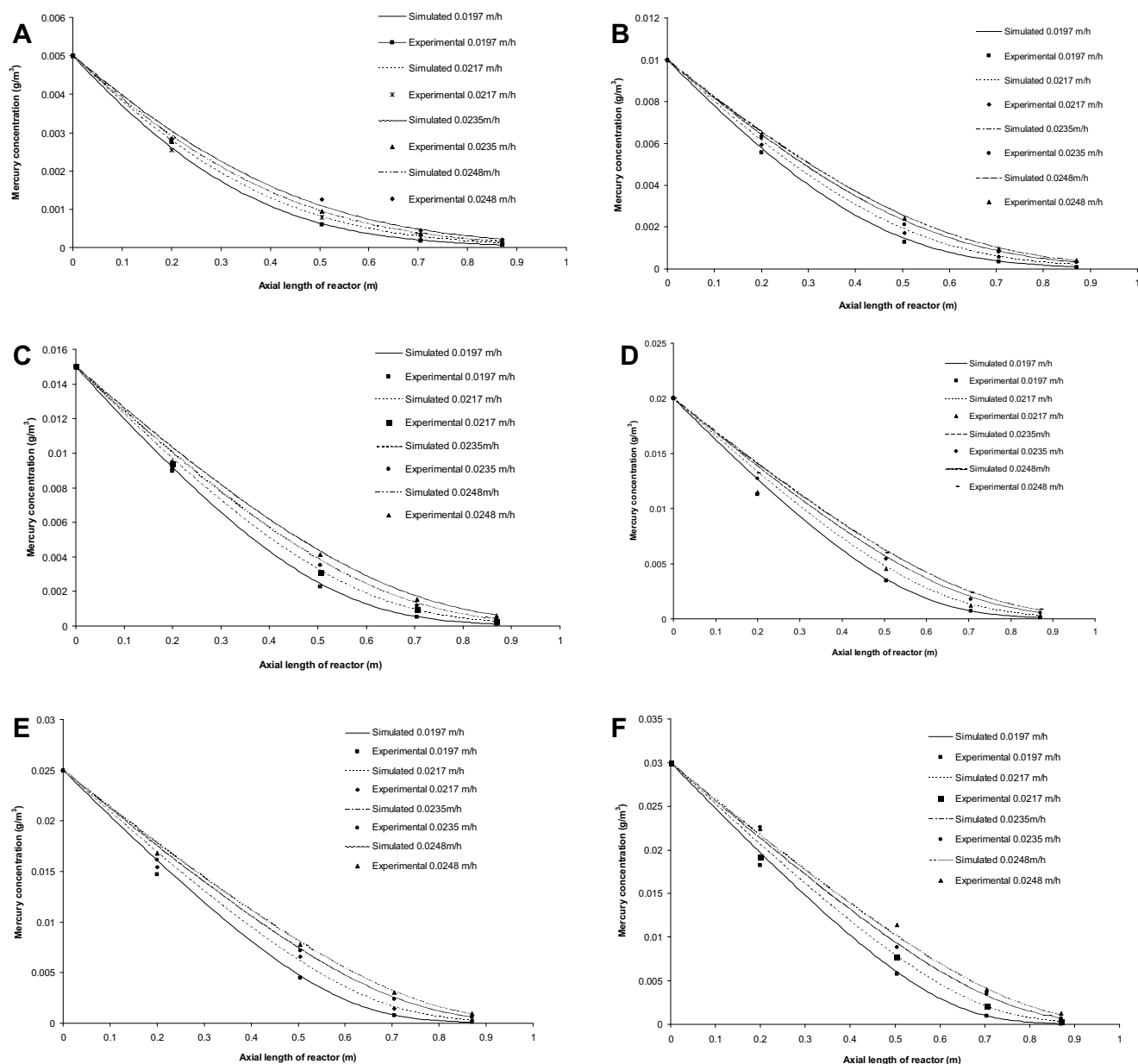
Fig. 6 Simulated and experimental profiles of mercury concentration along the axial length of the reactor (taking superficial velocity as a parameter). (**A**) Initial mercury concentration 0.005 g/m³. (**B**) Initial mercury concentration 0.01 g/m³. (**C**) Initial mercury concentration 0.015 g/m³. (**D**) Initial mercury concentration 0.02 g/m³. (**E**) Initial mercury concentration 0.025 g/m³. (**F**) Initial mercury concentration 0.03 g/m³.

zation of a large number of controlling factors and it is achieved only through modeling of those rate determining factors. It is expected that the model will be helpful to predict the performance behaviour of similar systems meant for the treatment of heavy metals through biochemical route.

ACKNOWLEDGEMENTS

REFERENCES

Brown NL, Shih YC, Leangt C, Glendinning KJ, Hobman JL, Wilson JR (2002) Mercury transport and resistance. *Biochemical Society Transactions* **30**, 199-204

Brunke M, Deckwer WD, Frischruth A, Horn JM, Frischruth H, Rhode M, Rohricht M, Timmis KN, Weppen P (1993) Microbial retention of mercury from waste streams in a laboratory column containing mer A gene bacteria. *FEMS Microbiology* **11**, 145-152

Canstein HV, Li Y, Leonhauser J, Haase E, Felske A, Deckwer WD, Wagner-Dobler I (2002) Spatially oscillating activity and microbial succession of mercury-reducing biofilms in a technical-scale bioremediation system. *Applied and Environmental Microbiology* **68**, 1938-1946

Carlos Green-Ruiz (2005) Mercury (II) removal from aqueous solutions by nonviable *Bacillus* Sp. from a tropical estuary. *Bioresource Technology* **97**, 1907-1911

De J, Ramaiah N (2007) Characterization of marine bacteria highly resistant to mercury exhibiting multiple resistances to toxic chemicals. *Ecological Indicators* **7**, 511-520

De J, Ramaiah N, Vardanyan L (2008) Detoxification of toxic heavy metals by marine bacteria highly resistant to mercury. *Marine Biotechnology* **10**, 471-477

De J, Ramaiah N, Bhosle NB, Garg A, Vardanyan L, Nagle VL, Fukami K (2007) Potential of mercury-resistant marine bacteria for detoxification of chemicals of environmental concern. *Microbes and Environments* **22**, 336-345

Essa AMM, Macaskie LE, Brown NL (2005) A new method for mercury removal. *Biotechnology Letters* **27**, 1649-1655

Kannan SK, Krishnamoorthy R (2006) Isolation of mercury resistant bacteria and influence of abiotic factors on bioavailability of mercury – A case study in Pulicat Lake North of Chennai, South East India. *Science of the Total Environment* **367**, 341-353

Kiyono M, Pan-Hou H (2006) Genetic engineering of bacteria for environmental remediation of mercury. *Journal of Health Science* **52**, 199-204

Mathivanan K, Balasubramanian V, Rajaram R (2010) Bacterial resistance to mercury pollution through genetic transformation. *World Applied Sciences Journal* **8**, 400-403

Oehmen A, Fradinho J, Serra S, Carvalho G, Capelo JL, Velizarov S,

Crespo JG, Reis MAM (2009) The effect of carbon source on the biological reduction of ionic mercury. *Journal of Hazardous Materials* **165**, 1040-1048

Okino S, Iwasaki K, Yagi O, Tanaka H (2000) Development of a biological mercury removal-recovery system. *Biotechnology Letters* **22**, 783-788

Okoronkwo NE, Igwe JC, Okoronkwo IJ (2007) Environmental impacts of mercury and its detoxification from aqueous solutions. *African Journal of Biotechnology* **6**, 335-340

Schneider M, Deckwer WD (2005) Kinetics of mercury reduction by *Serratia marcescens* mercuric reductase expressed by *Pseudomonas putida* strains. *Engineering Life Science* **5**, 415-424

Sorkhoh NA, Ali N, Dashti N, Al Mailem DM, Al Awadhi H, Eliyas M, Radwan SS (2010) Soil bacteria with the combined potential for oil utilization, nitrogen fixation, and mercury resistance. *International Biodeterioration and Biodegradation* **64**, 226-231

Wagner-Dobler I (2003) Pilot plant for bioremediation of mercury-containing industrial wastewater. *Applied Microbiology and Biotechnology* **62**, 124-133

Wagner-Dobler I, Canstein HV, Li Y, Leonhauser J, Deckwer WD (2003) Process-integrated microbial mercury removal from wastewater of chlor-alkali electrolysis plants. *Engineering in Life Sciences* **3**, 177-181

Wagner-Dobler I, Canstein HV, Li Y, Timmis KN, Deckwer WD (1999) Removal of mercury from chloralkali electrolysis wastewater by a mercury-resistant *Pseudomonas putida* strain. *Applied and Environmental Microbiology* **65**, 5279-5284

Appendix: Abbreviations list

μ	= Specific growth rate of bacterial cell (h^{-1})
μ_{max}	= Maximum specific growth rate of bacterial cell (h^{-1})
K_{S1}	= Saturation constant (g/m^3)
K_{S2}	= Saturation constant for mercury (g/m^3)
C_{S1}	= Sucrose concentration (g/m^3)
C_{S2}	= Mercury concentration (g/m^3)
$Y_{X/CS1}$	= Yield coefficient of sucrose
$Y_{X/CS2}$	= Yield coefficient of mercury
C_X	= Biomass concentration (g/m^3)
K	= Constant
Z	= Axial length of the reactor (m)
ε	= Bed porosity
a_p	= Specific surface area of packing (m^2/m^3)
u	= Superficial velocity (m/h)
l	= Biofilm thickness (m)