Model-based sensitivity analysis of a fluidised-bed bioreactor for mercury uptake by immobilised *Pseudomonas putida* cells

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A model-based sensitivity analysis was performed in order to evaluate the importance of the individual operating parameters of a three-phase fluidised-bed biological reactor used for removing mercury ions from wastewater. The parameters analysed involve the immobilised biomass load (bacteria *P. putida* on alginate beads, particle size, inlet flow-rate, mercury ion loads in the fed wastewater, and the solid fraction in the reactor. Predictions were generated by using pseudo-first-order, Michaelis–Menten, or pseudo-Haldane kinetic models. The results highlight the major influence of the biomass/solid load and of the liquid residence time on the reactor efficiency. Also, the resultant significant differences in the model predictions underline the importance of using a more accurate kinetic model for process design and control purposes.

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**Introduction**

Heavy metal pollution of the aquatic environment, particularly mercury pollution due to mining and industrial activities, continues to represent a major concern worldwide. The main source of mercury is the combustion of fossil fuels and solid waste, with traces of mercury up to 0.3 mg kg\(^{-1}\) in coal, and up to 3 mg kg\(^{-1}\) in municipal solid wastes (Di Natale et al., 2006). However, other significant sources of pollution need to be recognised, such as the use of mercury as cathode in chloralkali electrolysis on a large scale leading to significant mercury emissions (ca 1 g of Hg per t of chlorine, Leonh"auser et al. (2006)), and highly polluted wastewater (up to 7.6 mg L\(^{-1}\), Green-Ruiz (2006)). Mercury is also used in numerous industrial and medical applications (fungicides, disinfectants, dental products, catalysts, igniters, dye production, etc., Deckwer et al. (2004)).

Mercury is considered to be a priority hazardous pollutant due to its high toxicity, and a maximum permissible concentration of 50 µg L\(^{-1}\) is imposed on discharged wastewaters (Deckwer et al., 2004). Moreover, the European Union required, under Directive 2000/60/CE (European Commission, 2000), the cessation or phasing out of discharges, emissions, and losses of mercury by 2020, with complete remediation of polluted water bodies (Di Natale et al., 2006). Among possible treatment procedures for removing mercury from wastewaters are the following: i) precipitation with toxic H\(_2\)S, resulting in the need for safe disposal of large volumes of mercury-contaminated sludge (mercury recycling from HgS is not possible, Wagner-D"obler et al. (2000)); ii) mercuric ion retention by ion exchange columns (albeit renewable adsorbents are very expensive, Hosseini-Bandegharaei et al. (2011)); iii) retention on cheap sorbents, such as activated carbon, char from coal, volcanic tuff (Di Natale et al., 2006), immobilised enzymes on alginate (Bhattacharyya et al., 2010), modified agro-waste materials.
(O’Connell et al., 2008; Demirbas, 2008), or renewable polymeric membranes; iv) biosorption on cheap waste-biomass resulting from industrial fermenters (Alluri et al., 2007) or on activated sludge from wastewater treatment plants (Dąbrowska, 2012); v) sequestration of low loads of mercury into modified bacteria (Bacillus sp. or E. coli, Chen et al. (1998) and Cain et al. (2008)).

A relatively recent technology known as “microbial detoxification” uses cellular reduction of mercuric ions inside bacteria, the volatile mercury being eliminated and recovered from liquid/gas phases. Monocultures of Pseudomonas sp. (Deckwer et al., 2004; von Canstein et al., 1999; Leonhäuser et al., 2006), Aeromonas hydrophila (Leonhäuser et al., 2006), Escherichia coli (Philippidis et al., 1991), or mixed cultures of resistant Gram-positive and negative bacteria using various sources of carbon (Oehmen et al., 2009; Carvalho et al., 2011) have been tested. The current study focuses on this remediation technology using resistant bacteria strains of P. putida. Instead of “neutralising” the toxic mercuric ions by building complex chelates, thus consuming lots of cell energy and metabolites to maintain chelate homeostasis, the bacteria developed an efficient defence system by simply reducing the ions to volatile, less toxic, and readily eliminable metal from the cell by membrane diffusion. The cellular mer-reductase synthesis is controlled by a complex regulatory circuit related to the mer-operon expression (Maria, 2009).

An overall Michaelis–Menten type kinetic model was proposed by Deckwer et al. (2004) for mercury uptake by the P. putida sp. cells, applied in a simplified quasi-first-order form for \( [\text{Hg}^2+] < 1 \text{ mg L}^{-1} \), or in an extended Haldane form. By using individual experiments with cultures of intact cells or cells “permeabilised” to ionic species, Philippidis et al. (1991) demonstrated that mercury membrane permeation was the slowest process step. Eventually, a reduced Michaelis–Menten model for mercury uptake by E. coli cells was proposed, with the adjustable rate constants being a function of the copy-numbers of mer-plasmids existing in the cloned cells. It was concluded that modified E. coli cells reported an increased efficiency in removing mercury ions up to a certain limit (controlled by the permease proteins) to not exhaust the internal cell resources. A complex dynamic structured model for cellular mercury uptake, including 26 individual or lumped cellular species and 33 metabolic reactions, was proposed by Maria (2009, 2010) based on data from the literature. The structured model can simulate the cell adaptation to environmental changes by means of the mer-operon genetic circuit controlling the mer-gene expression levels.

Although the process kinetics was well established, few systematic engineering studies on process development have been reported on choosing the best construction and operating alternative. Amongst them, valuable experiments on the lab- or pilot-scale were carried out in a fixed-bed reactor (using P. putida sp. immobilised on pumice granules, Wagner-Döbler et al. (2000)), in a three-phase fluidised-bed (TPFB) reactor (using P. putida immobilised on alginate beads, Deckwer et al. (2004)), or using a CSTR reactor with suspended P. putida cells (Leonhäuser et al., 2006). Despite the fixed-bed reactor being simple, robust, and achieving high space-time conversions, it suffers from bed-clogging problems. An appropriate alternative is the aerated TPFB reactor with the possibility of purging the (immobilised) biomass to prevent mercury bioaccumulation.

This paper seeks to extend the engineering studies of the TPFB biological reactor by Deckwer et al. (2004) by developing a model-based sensitivity analysis to establish the degree of influence of various operating parameters on mercury-removal efficiency. The parameters analysed entail the biomass (immobilised Pseudomonas putida) load on alginate beads, mercury ion loads in the fed wastewater, average size of suspended particles, inlet flow-rate, and the solids fraction in the reactor. Using a pseudo-first-order, or a simple or extended Michaelis–Menten kinetic model, predictions of the process efficiency were generated. The results afford a direct comparison of individual control parameter importance, thus aiding the subsequent process optimisation step. The differences of up to 7–8 % in mercury conversion predicted by different models also indicate the importance of using an accurate non-linear kinetic model for process design and control (ultimately replacing the “default” adaptive linear controller).

**Fluidised bed reactor and apparent kinetic model**

The continuously operated TPFB bioreactor is that used by Deckwer et al. (2004) to remove mercury from wastewater on a bench-scale using P. putida sp. immobilised on porous alginate granules of 0.9 mm average diameter. The reactor and carrier characteristics and the nominal (reference) operating conditions \( \phi_{\text{ref}} \) are presented in Table 1. A good diffusion of the substrate (mercuric ions) into bacteria is assumed to take place in the support large pores, while an equilibrated biomass metabolism is ensured by the added nutrients in the fed wastewater. The liquid flow-rate (or the liquid residence time), the aeration rate, pH, and temperature are closely monitored to ensure a sustainable bioprocess. The biomass content of the support varies (up to 0.5–0.6 g of biomass per g of support, Deckwer et al. (2004)), but a quasi-constant level is assumed to be maintained by employing a purge/renewal system of the “biocatalyst” particles. The outlet gas containing the volatile metallic mercury is passed through an adsorption device for recovering the metal.