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OPTIMAL OPERATING POLICY OF A FLUIDIZED BED BIOREACTOR USED FOR MERCURY UPTAKE FROM WASTEWATERS BY USING IMMOBILIZED *P. PUTIDA* CELLS

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Abstract

A model-based analysis of a three-phase continuously operated fluidized-bed bioreactor (TPFB) is developed in order to determine the multi-objective optimal and sustainable operating policy of a TPFB used for removing mercury ions from wastewater. More specifically, the analysis is focus on finding the optimal feeding policy of alginate porous beads of known particle size containing immobilized biomass (P. putida bacteria) that minimize the biomass consumption, while keeping a quasi-constant high mercury removal conversion, under quasi-stable reactor performances. The extended bioreactor model is accounting for the biomass growth, biodegradation, and its partial leakage and washout. Bioreactor dynamics prediction has been generated by using a simple Michaelis-Menten kinetic model adopted from literature. The resulted optimal feeding policy of the bioreactor points out the importance of the adoption of an extended and adequate process/reactor model able to solve difficult engineering operation problems by quickly adjusting the feeding conditions according to the time-varying characteristics of the biomass culture, and to the limited possibilities to control the process during the wastewater residence time in the bioreactor.

Key words: mercury uptake by immobilized *P. putida* culture; fluidized-bed biological reactor; multi-objective optimization; biomass growth, biodegradation, washout dynamic simulation

1. Introduction

The bioreactor optimal operation is one of the most difficult and challenging engineering problem, due to well-known bioprocess complexity and variability, besides the large number of variables influencing the bioprocess difficult to be accounted in a quite reduced kinetic model.

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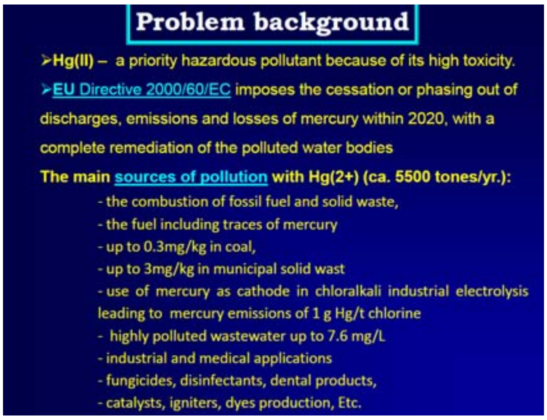


Problem background

>Hg(II) – a priority hazardous pollutant because of its high toxicity.

>EU Directive 2000/60/EC imposes the cessation or phasing out of discharges, emissions and losses of mercury within 2020, with a complete remediation of the polluted water bodies

The main sources of pollution with Hg(2+) (ca. 5500 tones/yr.):

- the combustion of fossil fuel and solid waste,
- the fuel including traces of mercury
- up to 0.3mg/kg in coal,
- up to 3mg/kg in municipal solid wast
- use of mercury as cathode in chloralkali industrial electrolysis leading to mercury emissions of 1 g Hg/t chlorine
- highly polluted wastewater up to 7.6 mg/L
- industrial and medical applications
- fungicides, disinfectants, dental products,
- catalysts, igniters, dyes production, Etc.

Fig. 1. The mercury removal problem from wastewaters [1, 2].

Classical procedures to remove mercury from wastewater are ineffective:

1. precipitation with H2S, with the disadvantage of handling large amounts of toxic H2S and resulting large volumes of toxic HgS sludge necessary to be deposited in special places, with no possibility of recycling
2. retention on the ion exchange columns, with the disadvantage of requiring costly adsorbents
3. retention on cheap sorbents, such as activated carbon, char from coal, volcanic tuff, agro-based waste materials, with the disadvantage of a limited adsorption capacity, and disposal problems

Biological procedure of reduction of Hg(2+) to volatile Hg(0) on bacteria cultures *(E. coli, Pseudomonas sp.)* and then removal of Hg(0) with sparging air from the bioreactor is highly effective: cheap, less pollutant, effective, sustainable (regenerable biomass), allows Hg(0) full recovering

Fig. 2. Classical removal procedures of mercury from wastewaters [1, 2].

Mercury is considered a priority hazardous pollutant, due to its high toxicity. A maximum permissible concentration of 50 μg/L is imposed to discharged wastewaters [1]. Moreover, the European Union stated, under Directive 2000/60/EC, the cessation or phasing out of discharges, emissions and losses of mercury within 2020, with a complete remediation of the polluted water bodies [3]. ”Heavy metal pollution of the aquatic environment and particularly mercury pollution due to mining and industrial activities still represents a high worldwide concern. The main source of mercury is the combustion of fossil fuel and solid waste (Fig. 1), the fuel including traces of mercury up to 0.3 mg/kg in coal, and up to 3 mg/kg in municipal solid waste [3]. However, other significant sources of pollution can be also mentioned, such as the use of mercury as cathode in the chlor-alkali industrial electrolysis at large scale leading to significant mercury emissions (ca. 1 g Hg/t chlorine; [4]), and highly polluted wastewater (up to 7.6 mg/L [1]). Mercury is also used in numerous industrial and medical applications (fungicides, disinfectants, dental products, catalysts, igniters, dyes production, etc.)” [5-7].

The current treatment procedures (Fig. 2) to remove mercury from wastewater register in the following categories [1]: i) Precipitation with hydrogen sulfide, with the disadvantage of handling large amounts of toxic H_2S, and resulting large volumes of mercury contaminated sludge necessary to be deposited in special places, with no possibility of recycling the precipitated mercury; ii) Retention on the ion exchange columns, with the disadvantage of requiring costly adsorbents; it can be applied only for removing low loads of mercury from wastewater; besides, most of ion-exchange resins cannot be regenerated thus raising disposal problems, while the renewable resins are expensive; iii) Retention on various cheap sorbents, such as activated carbon, char from coal, volcanic tuff, modified cellulose or agro-based waste materials; iv) Renewable polymeric membranes also display promising sorption and filtration capabilities; v) Bioaccumulation into genetically modified bacteria cells by sequestering mercury via binding to metallothionein molecules or cytosolic chelating agents; this method is limited by the cell metabolic resources, e.g. by using cyanobacteria, *Bacillus sp.*, or *E. coli*.

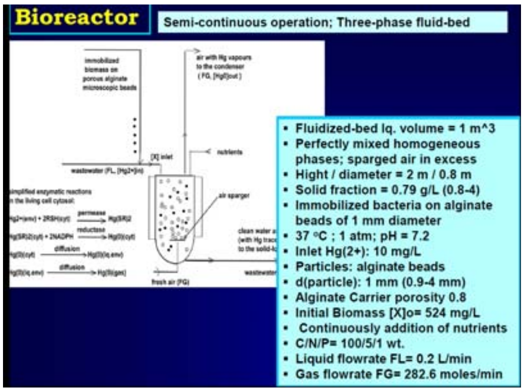
Microbial "detoxification" of wastewaters approached in this paper involves metabolic processes of certain cell cultures, that results in reducing the mercuric ions to elementary volatile mercury, which is less toxic for micro-organisms and more easily to be recovered from liquid / gas (air) phases. *Pseudomonas sp.*; *Aeromonas hydrophila*; *Escherichia coli* are to be mentioned among the resistant strains used [1]. The process takes place inside the living cells with a high efficiency; the *mer*-reductase / NADPH used being continuously synthetized and regenerated during the cell growth (see the simplified reactions in Fig. 3).

This paper is aiming at using a multi-objective criterion and an elaborated bioreactor model to derive sustainable optimal operating policies of a TPFB used for mercury uptake from wastewaters by immobilized cultures of *P. putida*. The target optimization criteria concern the sustainability of the TPFB operation, leading to the concomitant optimality of: I) an economic criterion (maximum of mercury removal conversion); ii) a safety-stability criterion (quasi-stationary removal conversion over a defined time-horizon); iii) an environmental criterion (minimum wasted biomass with mercury traces leaving the TPFB).

2. Bioreactor model and optimization problem formulation

To simulate the TPFB reactor performances and to derive the optimization problem solution, an extended dynamic ideal model of the bioreactor was adopted (Fig. 5 [1]), including a simple Michaelis-Menten kinetics of the bioprocess derived from a structured extended model (Fig. 4, [7-9]), thus allowing its use for bioprocess optimization. The model includes the main characteristics of the bioprocess on the solid support, and the solid-liquid-gas interfacial transport of the mercury, even if additional calculations are necessary to derive the particle effectiveness and to solve the mass flux equality at solid-liquid-gas interface during the transient regime. The extended bioreactor model is accounting for the biomass growth, biodegradation, and its partial leakage and washout (Fig. 4, [7-9]). The nominal operating conditions of the TPFB are presented in Fig. 3.

The TPFB model main hypotheses are presented by [1]. In short, due to the well-mixing conditions, the mercury ions diffuse from bulk phase through the external diffusional film surrounding the particles and then through the internal pores until reaching the immobilized biomass where the mercury is crossing the cell membrane, and then it is reduced to metallic mercury (see the simplified reactions in Fig. 3). The resulted metallic mercury from the cell cytosol diffuses through the cell membrane and then it is transported as micro-drops (of ca. 5 μm diameter [3]), or dissolved (solubility of 26 μg/L at 26°C) to the gas-liquid interface from where it passes as vapors into the homogeneous gas phase (air). Low amounts of mercury bio-accumulated into bacteria are neglected in the model. Mercury mass balance in the liquid and gas phases includes the inlet-outlet and transport terms (Fig. 5).

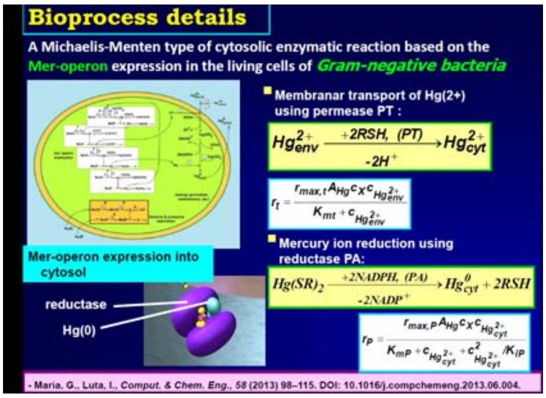


Bioreactor

Semi-continuous operation; Three-phase fluid-bed

- Fluidized-bed lq. volume = 1 m^3
- Perfectly mixed homogeneous phases; sparged air in excess
- Hight / diameter = 2 m / 0.8 m
- Solid fraction = 0.79 g/L (0.8-4)
- Immobilized bacteria on alginate beads of 1 mm diameter
- 37 °C ; 1 atm; pH = 7.2
- Inlet Hg(2+): 10 mg/L
- Particles: alginate beads
- d(particle): 1 mm (0.9-4 mm)
- Alginate Carrier porosity 0.8
- Initial Biomass [X]o= 524 mg/L
- Continuously addition of nutrients
- C/N/P= 100/5/1 wt.
- Liquid flowrate FL= 0.2 L/min
- Gas flowrate FG= 282.6 moles/min

Fig. 3. The main characteristics and nominal operating conditions of the semi-continuous TPFB bioreactor used for mercury removal from wastewaters [1, 2].



Bioprocess details

A Michaelis-Menten type of cytosolic enzymatic reaction based on the Mer-operon expression in the living cells of *Gram-negative bacteria*

- Membranar transport of Hg(2+) using permease PT :

$$Hg^{2+}_{env} \xrightarrow[-2H^{+}]{+2RSH,\ (PT)} Hg^{2+}_{cyt}$$

$$r_t = \frac{r_{max,t} A_{Hg} c_X c_{Hg^{2+}_{env}}}{K_{mt} + c_{Hg^{2+}_{env}}}$$

- Mercury ion reduction using reductase PA:

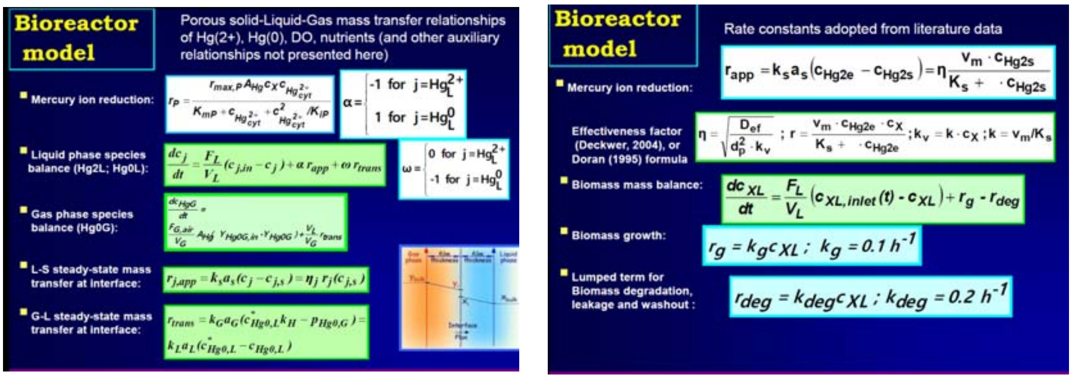
$$Hg(SR)_2 \xrightarrow[-2NADP^{+}]{+2NADPH,\ (PA)} Hg^{0}_{cyt} + 2RSH$$

$$r_p = \frac{r_{max,p} A_{Hg} c_X c_{Hg^{2+}_{cyt}}}{K_{mp} + c_{Hg^{2+}_{cyt}} + c^{2}_{Hg^{2+}_{cyt}} / K_{ip}}$$

Mer-operon expression into cytosol

- Maria, G., Luta, I., *Comput. & Chem. Eng.*, *58* (2013) 98–115. DOI: 10.1016/j.compchemeng.2013.06.004.

Fig. 4. The main mercury uptake kinetic model in on the immobilized cultures of *P. putida* in the approached TPFB [1, 2].



Bioreactor model

Porous solid-Liquid-Gas mass transfer relationships of Hg(2+), Hg(0), DO, nutrients (and other auxiliary relationships not presented here)

- Mercury ion reduction:

$$r_p = \frac{r_{max,p} A_{Hg} c_X c_{Hg^{2+}_{cyt}}}{K_{mp} + c_{Hg^{2+}_{cyt}} + c^{2}_{Hg^{2+}_{cyt}} / K_{ip}} \qquad \alpha = \begin{cases} -1 & \text{for } j = Hg^{2+}_{L} \\ 1 & \text{for } j = Hg^{0}_{L} \end{cases}$$

- Liquid phase species balance (Hg2L; Hg0L):

$$\frac{dc_j}{dt} = \frac{F_L}{V_L}(c_{j,in} - c_j) + \alpha\, r_{app} + \omega\, r_{trans} \qquad \omega = \begin{cases} 0 & \text{for } j = Hg^{2+}_{L} \\ -1 & \text{for } j = Hg^{0}_{L} \end{cases}$$

- Gas phase species balance (Hg0G):

$$\frac{dc_{HgG}}{dt} = \frac{F_{G,air}}{V_G} A_{Hg} (y_{Hg0G,in} - y_{Hg0G}) + \frac{V_L}{V_G} r_{trans}$$

- L-S steady-state mass transfer at interface:

$$r_{j,app} = k_s a_s (c_j - c_{j,s}) = \eta_j\, r_j(c_{j,s})$$

- G-L steady-state mass transfer at interface:

$$r_{trans} = k_G a_G (c^{*}_{Hg0,L} k_H - p_{Hg0,G}) = k_L a_L (c^{*}_{Hg0,L} - c_{Hg0,L})$$

Bioreactor model

Rate constants adopted from literature data

- Mercury ion reduction:

$$r_{app} = k_s a_s (c_{Hg2e} - c_{Hg2s}) = \eta \frac{v_m \cdot c_{Hg2s}}{K_s + \cdot c_{Hg2s}}$$

Effectiveness factor (Deckwer, 2004), or Doran (1995) formula

$$\eta = \sqrt{\frac{D_{ef}}{d_p^2 \cdot k_v}}\ ;\ r = \frac{v_m \cdot c_{Hg2e} \cdot c_X}{K_s + \cdot c_{Hg2e}}\ ; k_v = k \cdot c_X\ ; k = v_m / K_s$$

- Biomass mass balance:

$$\frac{dc_{XL}}{dt} = \frac{F_L}{V_L}(c_{XL,inlet}(t) - c_{XL}) + r_g - r_{deg}$$

- Biomass growth:

$$r_g = k_g c_{XL}\ ;\ k_g = 0.1\ h^{-1}$$

- Lumped term for Biomass degradation, leakage and washout :

$$r_{deg} = k_{deg} c_{XL}\ ;\ k_{deg} = 0.2\ h^{-1}$$

Fig. 5. TPFB bioreactor math model (left), and auxiliary relationships (right). See [1] for details.

3. Solving the optimization problem, and results

To solve this complex multi-objective optimization problem, the "weighting function method" has been used (joint function Φ in Fig. 6-right), by associating to each criterion (Φ_j, j=1-3) weighting factors (giving less weight to the biomass consumption: $w_{x_{Hg}}$, w_σ, and w_X in this case), chosen depending on the relative importance given to each objective. The formulated normalized three objective functions for the optimization problem, accounts for (Fig. 6):

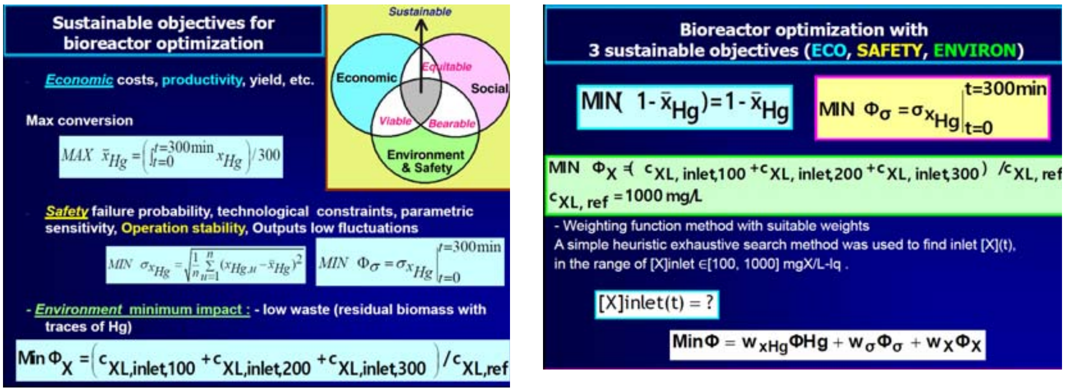
i) an economic criterion, that is maximum of the average mercury ion reduction conversion (x_{Hg}), over the considered running-time interval t∈[0, 300] min.;

ii) a safety criterion, that is a minimum standard deviation (denoted by σ) of x_{Hg} from its average value over the considered running-time interval;

iii) an environmental minimum impact criterion, that is minimum biomass consumption over the considered running-time interval.

The optimization problem variables are the concentration of the feeding biomass [$c_{XL,\text{ inlet},100}; c_{XL,\text{ inlet},200}; c_{XL,\text{ inlet},300}$] over three considered running-time intervals [0-100], [100-200], and [200-300] min. during the batch time t∈[0, 300] min. (Fig.7-8) [1].

The best TPFB reactor operating policy B (Fig.8) was obtained for the relative weights: $w_{x_{Hg}} = w_\sigma = 1;\ w_X = 0.1$. by giving less weight to the biomass consumption, thus ensuring a maximum conversion, but also quite-uniform over the running time, with the expense of a higher consumption of biomass. This operating policy is preferred because the biomass is cheap and renewable, while the removal of mercury from wastewater is a very important ecological issue deserving lots of investments, and operational costs.



Sustainable objectives for bioreactor optimization

Economic costs, productivity, yield, etc.

Max conversion

$MAX\ \bar{x}_{Hg} = \left(\int_{t=0}^{t=300\text{min}} x_{Hg}\right)/300$

Safety failure probability, technological constraints, parametric sensitivity, Operation stability, Outputs low fluctuations

$MIN\ \sigma_{x_{Hg}} = \sqrt{\frac{1}{n}\sum_{u=1}^{n}(x_{Hg,u}-\bar{x}_{Hg})^2}$ $MIN\ \Phi_\sigma = \sigma_{x_{Hg}}\Big|_{t=0}^{t=300\text{min}}$

- *Environment minimum impact* : - low waste (residual biomass with traces of Hg)

$\text{Min}\ \Phi_X = \left(c_{XL,\text{inlet}100} + c_{XL,\text{inlet}200} + c_{XL,\text{inlet}300}\right)/c_{XL,\text{ref}}$

Bioreactor optimization with 3 sustainable objectives (ECO, SAFETY, ENVIRON)

$MIN(1-\bar{x}_{Hg}) = 1-\bar{x}_{Hg}$ $MIN\ \Phi_\sigma = \sigma_{x_{Hg}}\Big|_{t=0}^{t=300\text{min}}$

$MIN\ \Phi_X = (c_{XL,\text{ inlet}100} + c_{XL,\text{ inlet}200} + c_{XL,\text{ inlet}300})/c_{XL,\text{ ref}}$

$c_{XL,\text{ ref}} = 1000$ mg/L

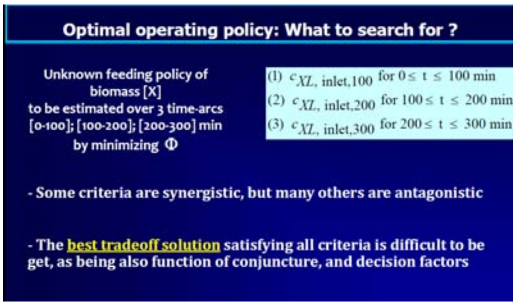
- Weighting function method with suitable weights

A simple heuristic exhaustive search method was used to find inlet [X](t). in the range of [X]inlet ∈[100, 1000] mgX/L-lq .

[X]inlet(t) = ?

$\text{Min}\Phi = w_{xHg}\Phi Hg + w_\sigma\Phi_\sigma + w_X\Phi_X$

Fig. 6. The considered three optimization objectives of the semi-continuous TPFB bioreactor used for mercury removal from wastewaters (left), and the joint optimization criterion Φ (right)[1,2].



Optimal operating policy: What to search for ?

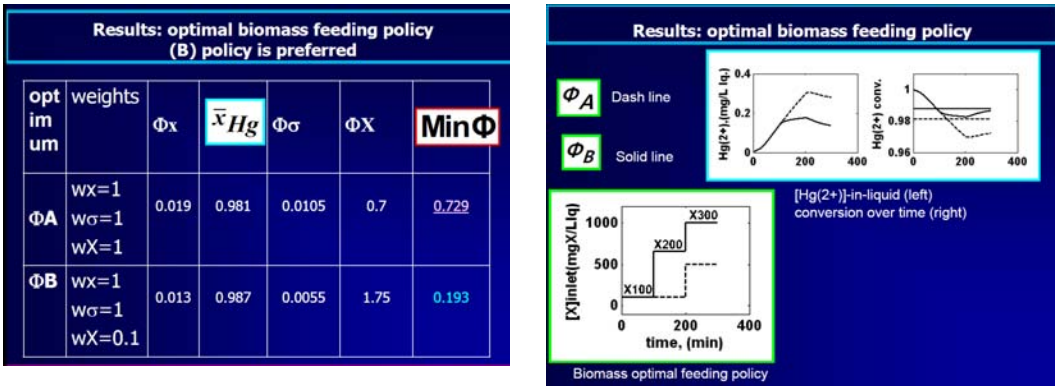
Unknown feeding policy of
biomass [X]
to be estimated over 3 time-arcs
[0-100]; [100-200]; [200-300] min
by minimizing Φ

(1) $c_{XL,\ \mathrm{inlet},100}$ for $0 \le t \le 100$ min
(2) $c_{XL,\ \mathrm{inlet},200}$ for $100 \le t \le 200$ min
(3) $c_{XL,\ \mathrm{inlet},300}$ for $200 \le t \le 300$ min

- Some criteria are synergistic, but many others are antagonistic

- The best tradeoff solution satisfying all criteria is difficult to be get, as being also function of conjuncture, and decision factors

Fig. 7. The optimization problem variables $[X]_L$ over the considered three running-time intervals during the batch time t∈[0, 300] min [1].



Results: optimal biomass feeding policy
(B) policy is preferred

optimum	weights	Φx	x_{Hg}	Φσ	ΦX	MinΦ
ΦA	wx=1 wσ=1 wX=1	0.019	0.981	0.0105	0.7	0.729
ΦB	wx=1 wσ=1 wX=0.1	0.013	0.987	0.0055	1.75	0.193

Results: optimal biomass feeding policy

[Hg(2+)]-in-liquid (left)
conversion over time (right)

Biomass optimal feeding policy

Fig. 8. The TPFB bioreactor performances for two optimal operating policies (A, and B). (left) realized optimization indices; (right) species dynamics [1].

4. Conclusions

Derivation of alternative optimal operation policies of a TPFB bioreactor used for testing mercury removal efficiency by using immobilized bacteria on a suitable porous support (alginate beads) is an essential engineering analysis to be used in further process scale-up and control.

In the simulated case study, the very effective bacteria metabolism allows an efficient reduction of the cytosolic mercury (92-99% conversion), excretion and transport of the volatile metal to the gas phase for a high pollutant load in the wastewater. By keeping a reasonable small size of alginate particles (1-2 mm), the most important control parameters appear to be the biomass load in the bioreactor and the wastewater/biomass feed flow rate.

The use of an accurate Michaelis-Menten kinetic model of the reduction bioprocess, and of a reasonable extended bioreactor model are essential steps for deriving satisfactory and interpretable results, that is prediction of various optimal operating alternatives. The study points- out the high importance of considering a detailed biomass mass balance in the bioreactor model for ensuring a satisfactory precision of the results.

Finally, the use of immobilized (modified) resistant bacteria in fluidized-bed aerators is proved as being a viable, effective, and less costly alternative for mercury removal from wastewaters at a large-scale. The optimal choice of the operating parameters, and especially the optimal biomass continuous addition policy can be ranged to ensure a high and quasi-stationary process conversion (around 99%), eventually leading to mercury loads in the bioreactor effluent lower than the regulations' threshold, with the expense of a moderate biomass consumption. This disadvantage is compensated by the use of a cheap and renewable biomass, and by the tremendous ecological importance of removing the very toxic mercury from wastewaters, or from surface waters.

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