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Mercury Removal from Actual Petroleum Based Industries Wastewater by *P. putida* in Membrane Bioreactor.

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Abstract

Mercury is an extremely toxic pollutant that currently being emitted and distributed globally. Several petroleum based industrial plants had showed high concentration of mercury compare to the Department of Environmental (DOE), Malaysia. Microbes have been used to solve environmental wastewater problems for many years. Objective of this study is to remove mercury from actual petrochemical wastewater using *Pseudomonas putida* (*P. putida*), in membrane bioreactor. To achieve the maximum of mercury removal, the optimum growth parameters of *P. putida* were obtained. Based on the optimum parameters of *P. putida* for specific growth rate, μ the removal of 4 mg/L was studied. Results showed mercury removal for sample with 4 mg/L mercury in bioreactor is 99.60% for the first 6 hours, 99.80% removal for 120 hours and 99.90 % after the microfiltration membrane system. The specific growth rate (μ) describes how fast the cells are reproducing. The higher the value of specific growth rate, then the faster the cells are growing. In this case, 6 hours was the optimum time for the mercury removal with the ratio of mercury mass over cell mass is 20.78 $\mu\text{gHg/g}$ cells for *P. putida*. Microfiltration membrane enhanced further the treatment of the wastewater by retaining the *P. putida* from escaping during the release of treated wastewater, reducing the turbidity by 94.2% (5.32 NTU) and concentration of suspended solids up to 60.4% (0.09 mg/L). The mechanism of mercury detoxification in the membrane bioreactor was based on reduction of Hg^{2+} to non-toxic Hg^0 by mercury reductase enzyme produced by *P. putida*. The findings from this study can be used as references for future application of petroleum based industries wastewater treatment as well as other industries related to mercury contamination in their wastewater treatment plant such as gold mining, chemical industries, agriculture etc.

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Keywords: *Mercury*, *Pseudomonas putida*, *growth kinetic*, *membrane bioreactor*, *petrochemical wastewater*

Introduction

Mercury is one of the most toxic elements found on earth. It binds to the sulfhydryl groups of enzymes and proteins, thereby inactivating vital cell functions. After being discharged into the environment, mercury enters the sediments where it remains for many decades. It is taken up by aquatic organisms in the form of highly toxic methylmercury and is subsequently biomagnified through the food chain, hence threatening the health of top predators, such as birds, fish, seals, and man, is thereby threatened (Braune et al., 1999 and Muir et al., 1999). Mercury (Hg) pollution of the environment by mining activities and

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petroleum based industries wastewater has resulted in worldwide contamination of large areas of soils and sediments (Miller et al.,1996; Degetto et al.,1997; Suchanek et al., 1998 and Horvat et al., 1999) and led to elevated atmospheric mercury levels (Ebinghaus and Slemr, 2000). Mercury contamination of hydrocarbon production and processing systems can be more than a mere nuisance (Li et al., 2009). Purification of areas polluted by heavy metals such as mercury is difficult, because the metals cannot be transformed into harmless elements (Al-Malack, 1996a). Over decades, communities have made concerted efforts to face this problem through treating and removing the heavy metals (Chandra et al., 2003). Various types of technology are available for removing mercury from water and wastewater, which include chemical precipitation, conventional coagulation, reverse osmosis, ultrafiltration, magnetic filtration, ion exchange and activated carbon adsorption and chemical reduction (Derek and Coates, 1997). Mercury remediation through common physico-chemical technologies are not only expensive but not environmental friendly and used only to treat high mercury concentration in wastewater treatment plant. Stringent legislation requires expensive and efficient method of treatment of wastewater in order to fulfill the discharge limit requirement (Demirbas, 2008). There are three principle advantages of biological technologies for the removal of pollutants: firstly, biological processes can be carried out *in-situ* at the contaminated site, secondly, bioprocess technologies are usually environmentally benign (no secondary pollution) and thirdly, they are cost effective (Vijayaraghavan and Yun, 2008). Biological systems have been thought to be adopted for the removal of toxic heavy metals such as mercury from petroleum based industries wastewater (Malakahmad et al., 2011). Bioremoval is a biological system for removing metal ions from polluted water has the potential to achieve greater performance at lower cost than non-biological wastewater treatment (Kondoh et al., 1998). Bacterial resistance to mercury is related to enzymatic reduction of Hg^{2+} to volatile Hg^0 (Devars et al., 2000). Mercury detoxification process originates from proteins of the microbial mercury resistance (*mer*) microbial mercury resistance, *mer* operon located in either plasmids or transposable elements in the mercury resistant microorganisms. Specific transport of bulk mercury across the cell membrane is achieved by two *mer* operon genes *merP* and *merT*, which express cystein-rich protein to deliver ambient mercuric toward intracellular mercuric reductase for subsequent reduction of mercuric ions to volatile Hg^0 (Weon and Ashok, 2001).

Objective of the Study

The current studies aimed at investigate reduction of Hg concentration using *P. putida* and to evaluate the performance of membrane bioreactor (MBR) for Hg removal in actual petroleum based industries wastewater application.

Literature Review

High concentrations of mercury are found in several regions of the world. Mercury in crude oil above certain limits can be problematic to refinery operations as mercury would deactivate catalysts and reduces the quality of refined products. The Environmental Protection Agency (EPA) of the United State has specified that waste with mercury below 260 mg/L concentrations must be stabilized before disposal. The biosorption process possesses metal-sequestering properties and can be used to decrease the concentration of mercury ions in solution. It can effectively sequester dissolved mercury ions out of dilute complex solutions efficiently and quickly, thus making it an ideal candidate for treating high

volume and low concentration complex wastewaters (Wang and Chen, 2006). Strain grows well with benzene, toluene, ethylbenzene, and p-cymene. Mutants of strain that are capable of growth with n-propylbenzene, n-butylbenzene, isopropylbenzene and biphenyl are easily obtained (Ouyang et al., 2006). In addition to aromatic hydrocarbons, the broad substrate toluene dioxygenase in strain can oxidize trichloroethylene (TCE), indole, nitrotoluenes, chlorobenzenes, chlorophenols and many other aromatic substrates. Although *P. putida* cannot use TCE as a source of carbon and energy, it is capable of degrading and detoxifying TCE in the presence of an additional carbon source (Walia et al., 2003).

Microorganisms are known to mediate four typed of enzymatic transformations of mercury (Barkay and Wagner-Dobler, 2005) which are reduction of Hg^{2+} to Hg^0 , breakdown of organ mercury compounds (including MeHg^+), resulting in formation of Hg^0 , and methylation of Hg^{2+} oxidation of Hg^0 to Hg^{2+} . The detoxification (resistance) mechanism for mercury is based on the unique peculiarities of this metal: the electrochemical potential of $\text{Hg}^{2+}/\text{Hg}^0$ at pH 7 is +430mV, which means living cells are able reduce Hg^{2+} to elemental form Hg^0 , which is non-toxic to human and microorganism (Singh et al., 2008). Secondly, the melting point of mercury is extraordinary low (melting point -39°C , boiling point 35°C), so that elemental mercury does not remain inside the cell but leave it by passive diffusion and is then either evaporated into the air or precipitated due to its low solubility in water. In either case, the bacterial cell is effectively freed of toxic Hg^{2+} or organ mercury compounds. The biochemical basis of resistance to inorganic mercury compounds such as $\text{Hg}(\text{NO}_3)_2$ appears to be quite similar in several different species. This enzyme has been characterized in plasmid-carrying strains of *P. putida*. This reductase is flavoprotein, which catalyzes the NADPH dependent on reduction of Hg^{2+} to Hg^0 then this allows for more $\text{CH}_3\text{Hg}^{2+}$ to be converted to Hg^{2+} for compound of organic mercury.

The rate increase cell (or biomass) is depending on the concentration of cells present in the reactor. The number of cells increased exponentially and the exponential growth varies with the type of microorganism and growth conditions. The growth follows a geometric progression ($2^0, 2^1, 2^2, 2^n$) (Bitton, 2005).

$$X_t = X_0 e^{\mu t} \quad (1)$$

Where X_t is the concentration of biomass or the number of cells in the bioreactor after time, t . X_0 is the initial number or biomass of cells and μ is the specific growth rate. This model of microbial growth is referred to as the exponential growth model (Park et al., 2002, Tsai and Juang, 2006). Biomass concentrations are typically expressed in g.L^{-1} of dry weight or density/turbidity of cell under measurement optical density at 600 nm wavelength is used (Shuler and Kargi, 2002).

Using the natural logarithms on both sides of Equation 1, thus can be re-written as:

$$\ln X_t = \ln X_0 + \mu t \quad (2)$$

Where μ is given by

$$\mu = \frac{\ln X_t - \ln X_0}{t} \quad (3)$$

The specific growth rate (μ) describes how fast the cells are reproducing. The higher the value of specific growth rate, then the faster the cells are growing. When cells are not growing, then their specific growth rate is zero. During exponential phase, the specific growth rate is relatively constant, (Bitton, 2005, Brock et al., 2006).

A mathematical relationship during the exponential growth involved the following parameters (Shuler and Kargi, 2002, Ee, 2004, Brock et al., 2006):

- Specific growth rate, μ is defined as the increase in cell mass per unit time, e.g., grams cells (g) per gram cells (g) per hours: ($\text{g} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$). The specific growth rate is commonly units are in reciprocal hours (hr^{-1}), however, it can also be expressed in reciprocal seconds (s^{-1}) or minutes (min^{-1}) or any other units of time.
- Number of generation, $n = 3.3 \ln X_t / X_0 = 3.3 \ln OD / OD_0$
- Generation time, g is defined the time required for formation of two cells from one, doubling time, $g = t/n$, where t is duration of exponential growth expressed in days, hours, or minutes depending on the organism and the growth condition.
- Growth rate constant to measure of the number of generation that occur per unit time in an exponential growing culture), $k = \ln 2/g = 0.693/g$

Armed with knowledge of n and t , g and k can be calculated. These parameters will show the different microorganisms growing under the different culture conditions. This is often useful for optimizing for testing the positive or negative effect of some treatment on the bacteria culture (Shuler and Kargi, 2002, Brock et. al 2006).

In this research membrane filtration was use to retain *P. putida* from escaping during the release of treated wastewater. Livingston (1994) reported that the function of the membrane is to separate wastewater from cell medium (*P. putida*), where biodegradation takes place under controlled conditions and to clarify by dead-end filtration. Thus, an attempt is made to enhance *P. putida* growth activities process using microporous hollow-fiber membrane. Cells immobilized on microporous hollow fiber have been employed for biotechnology application such as cell culture and biodegradation (Chung et al., 2005). Another research be achieved showed *P. putida* V1 as a promising candidate in the bioremediation of ecosystems contaminated with methylmercury, presenting high salinity (up to 10%) and other heavy metals, such as nickel and lead (Lucélia et. al. 2014). In all cases, the optimal mercury uptake by biomass was at pH 7.40. It is expected that the adsorption of metals decrease at low pH value because of competition for binding sites between cations and protons (Sahoo and Das, 1992). When the pH value is higher than 7, hydroxo species of the metals can be formed and do not bind to the adsorption sites on the surface of the adsorbent

(Kacar et al., 2002). Application of low air flux is creating a highly concentrated sink for Hg in the process (Barna et. al 2014). Other method had been used as remediation techniques to control Hg contamination in soils and to avoid adverse health effects. Amongst, thermal treatment can be applied to soils with very high concentrations of Hg (up to 34,000 mg/kg) and its remedial efficiency is fairly high (up to 99%). As each Hg contaminated site is unique, a specific and deep evaluation of the site must be carried out prior to the application of the selected technique. This will lead for improvement of the above-mentioned remediation techniques (Jingying et al 2015).

Methodology

Materials

The materials used in this research consisted of deionized water, growth media positive *P. putida*, Hg, model wastewater and actual petrochemical wastewater. Various chemicals and materials are also used for sample analysis of wastewater and also as cleaning chemical.

Bacteria *P. putida* in bioreactor membrane system

Mercury can be successfully removed by *P. putida*. However, high growth of *P. putida* could affect the amount of suspended solid in the wastewater. Hence, a separation method to remove the suspended solid is proposed and in this study, a membrane bioreactor is employed. Figure 1 show the experimental setup of the membrane bioreactor system. The system consists of a bioreactor, reservoir, pump and hollow fiber cartridge (microfiltration), and the various parts are connected using tubes. The growth of *P. putida* is measured using a sterilisable pH electrode. The built-in temperature control system used will maintain the temperature of the culture by controlling the inflow and outflow of cooling water through the water jacket available on the outer layer of the reservoir. The 2.00 L bioreactor was used for growing *P. putida* and removing of mercury process from wastewater is maintained at 37°C. The reservoir is used to store the samples before introducing to the membrane system, the hollow fiber cartridge. *P. putida* was grown in an incubator at 30°C before transferring into the reservoir of the bioreactor. The quantity of cell culture normally used is in the range of 3.00% – 10.00% (v/v) of medium volume (Ee, 2004). In this case, 0.20 L of cell culture is added to the total system.

For membrane operation, firstly the cell sampling or drain valve is closed and ensured the cartridges of hollow fiber in the upper and lower manifolds are ensured to be secured. Then the tubes of the pump should be correctly positioned and tensioned within the pump head. Flexible tubing is connected from the retentate outlet on the upper manifold to one of the tubing barbs on the reservoir caps and another flexible tubing is directed from the upper permeate line to a collection reservoir to receive the product. The reservoir cap on the silicone gasket is repositioned and sanitary clamps were put in place. The backpressure tubing valve is opened for several times and the pump is operated at slow speed; 0.5 minutes is needed for the pressure to build up as the mechanically dampened pressure gauges responded slowly. As a result the speed of the pump increased slowly, the inlet pressure might build up and the data can be collected (Chung et al., 2005).

Meanwhile, the bioreactor was set up in “back-flush” configuration, where the feed stream flows from the outside of the hollow fibers and permeate is collected from the inside of the hollow fibers (Wagner-Dobler, 2003). This bioreactor is operated in a batch fluid recycle mode, where the retentate is circulated to the bioreactor for continuous operation and permeate as product is collected in another container. The circulation was achieved with the use of a pump and the flow rate of permeate is controlled by varying the pressure of the feed stream.

To reuse the hollow fiber cartridge, its membrane is cleaned with 20.00 mg/L NaOCl, thoroughly rinsed with de-ionized water, and stored accordingly (Reardon et al., 2000). Membrane bioreactor systems, module Hollow Fiber (HF), operating condition and model wastewater containing Hg at different concentration as a samples. Water quality parameter are measured including total dissolved solid, suspended solid, temperature, turbidity and Hg concentration.

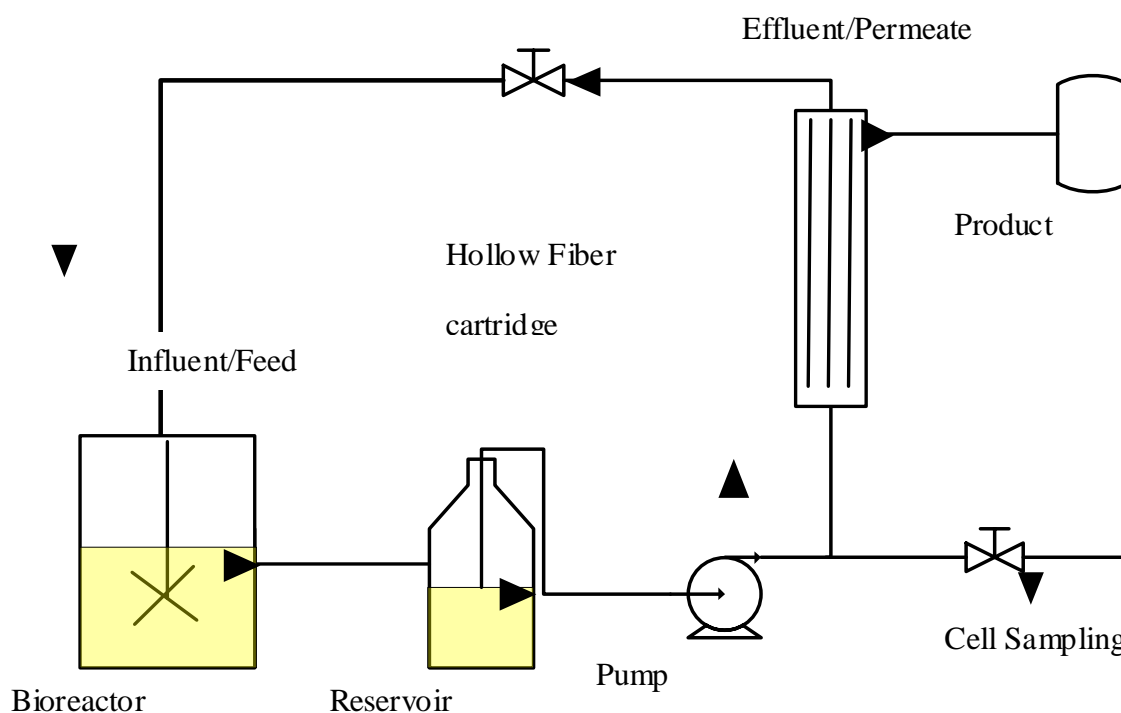


Figure 1: Diagram of Membrane Bioreactor (MBR)

Mercury removal from actual petroleum based industries wastewater

Petroleum based industries wastewater sample from petrochemical plant was added with 4 mg/L mercury solution. A study on mercury removal at 4 mg/L was performed at pH 7 with temperature at 37°C for 120 hours in a membrane reactor. Flux of 120 l/m²/hr (LMH) is observed with inlet pressure (P_{inlet}) of 11 psi, (P_{outlet}) is 1 psi and TMP is 5 psi. The feed flowrate is 0.22 L/min for the hollow fiber membrane polysulfone with membrane area of 0.011m² and 0.20 um of pore size.

Data Analysis And Discussion

Determination of Standard Calibration Curve Using Cell Dried Weight

Experiment on the standard growth curve by using dried cell weight is conducted in order to confirm the growth pattern by directly measuring cell density (OD). From the observation of results presented in Table 4.1 and Figure 4.1, *P. putida* standard curve using cell dry weight method to be used as reference for dried weight. In this case the cell dried weight (g/L) is 0.39 times optical density. It shows that an increase in concentration of biomass will increase the optical density.

Table 4.1:
P. putida standard growth curve using cell dried weight

Sample Number	Weight (g)	Weight Cell (g)	Cell Weight (g)	Total Volume (L)	Concentration Biomass (g/L)	Optical Density (OD)
1	0	0	0	0	0.10	0
2	.95	.95	.001	.013	0.10	.21
3	0	0	0	0	0.11	0
4	.94	.94	.001	.013	0.15	.23
5	0	0	0	0	0.20	0
	.94	.94	.001	.010		.26
	0	0	0	0		0
	.95	.95	.002	.010		.40
	0	0	0	0		0
	.95	.96	.002	.010		.53

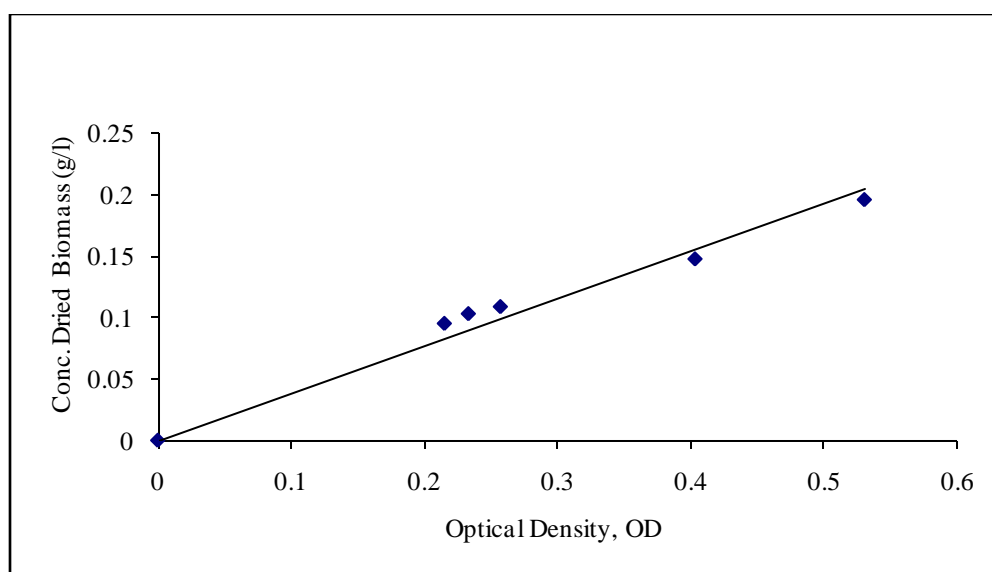


Figure 4.1: *P. putida* standard curve using cell dry weight method

Mercury removal and *P. putida* growth behavior

Results showed in Table 4.2 for *P. putida* growth related parameter in the membrane bioreactor system. It is found that the cell exhibits a typical batch growth curve of microbial culture, which is divided into the lag, exponential, stationary and death phases. In the lag phase, cells immediately show an exponential growth phase with a sharp increase in cell density in the first 6 hours with a fast decline in mercury concentration as a result of mercury removal by *P. putida* activities (Figure 4.2). After that, the cells consistently grow in the range of optical density (OD) 1.99 - 2.02, at minimum mercury concentration of 16.40 – 6.00 $\mu\text{g/L}$. The highest cell density obtained is 2.155 with exponential growth of 1.11 and specific growth rate, μ of 0.03 hr^{-1} . The best degradation efficiency is found at pH 7 with temperature at 37°C .

Table 4.2: Parameter related

P. putida growth behavior and activity during mercury removal in membrane bioreactor

Growth Parameter	Min	Max
Specific Growth Rate, μ (hr^{-1})	0.0277	
OD	0.713	2.155
Ln OD/OD ₀	0.912	1.106
Number Of Generation, n	1.307	1.585
Generation Time, g (hr)	4.069	80.328

Growth Rate Constant, k (hr^{-1})	0.009	0.170
Hg Removal (%)	99.60 for 6 hr	99.80 for 120.00 hr and 99.90 after membrane

Based on the analysis of the data obtained from the experiments, it is found from the graph that the maximum number of generation, n , obtained is 1.59 as shown in Figure 4.3, the generation time g of *P. putida* cell, is 80.33 hours as shown in Figure 4.5 while the growth rate constant k is 0.17 hr^{-1} as shown Figure 4.5 which is a way of measuring how fast the cells are dividing in a culture and defined on the basis of generation time or doubling time.

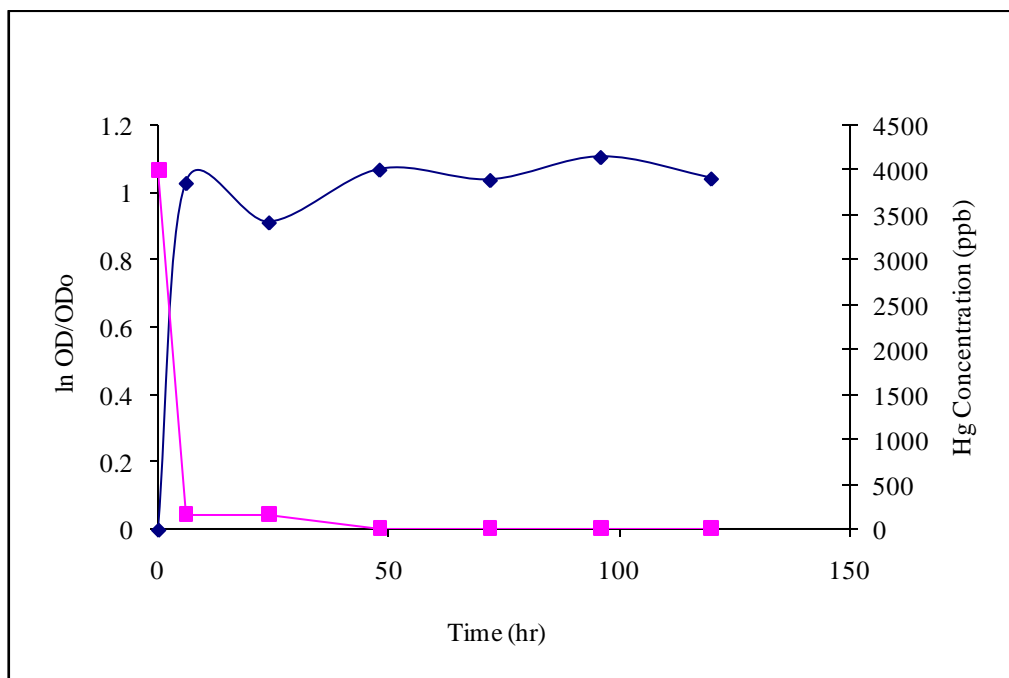


Figure 4.2: Mercury (4 000.00 ppb) removal by *P. putida* in Membrane Bioreactor

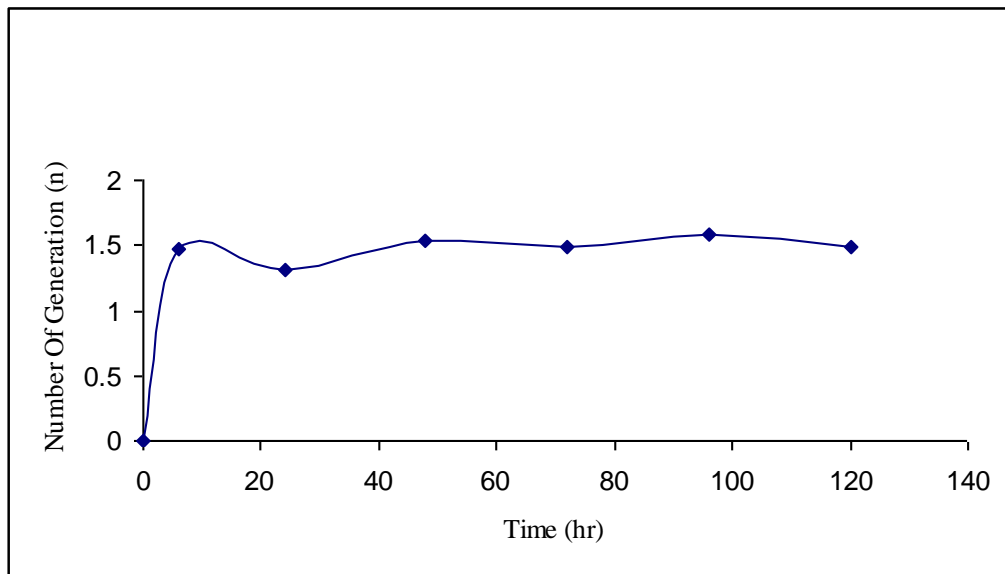


Figure 4.3 : Number of generation, n of *p. putida* in membrane bioreactor during mercury removal

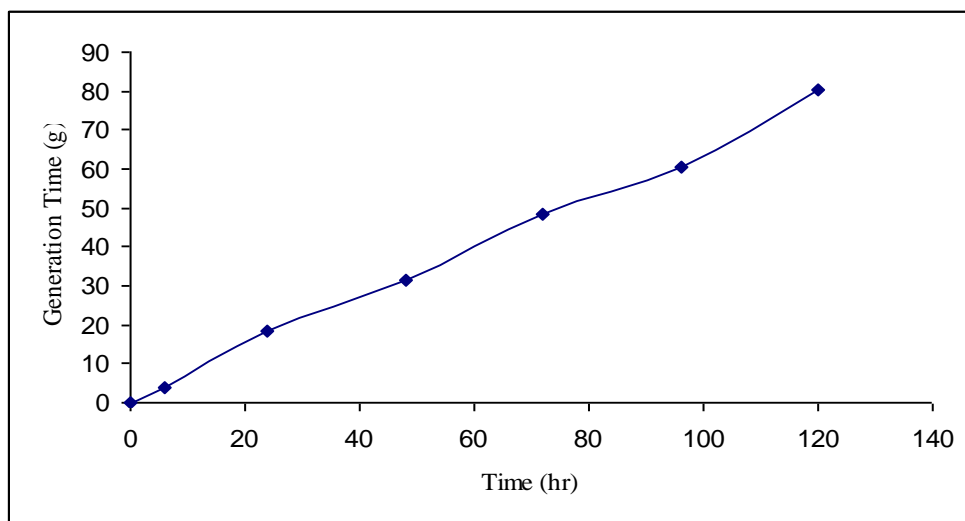


Figure 4.4: Generation time, g of *p. putida* in membrane bioreactor during mercury removal

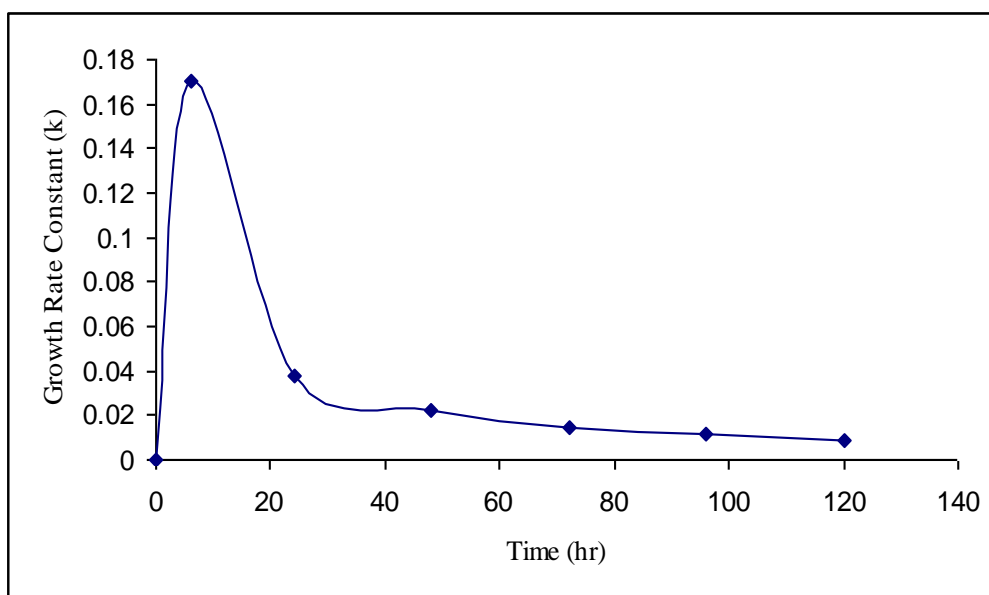


Figure 4.5: Growth rate constant, *k* of *p. putida* in membrane bioreactor during mercury removal

From Table 4.3, the overall percentage of mercury removal in membrane bioreactor is very high, which is more than 99.00 % and the highest removal efficiency is 99.90 % for petroleum based industries wastewater with 4mg/L mercury concentration that was treated for 120.00 hours. In this case, results indicated that mercury level at 3.60 µg/L in treated petroleum based industries wastewater is below than the discharge limit required by DOE for Standard B (50.00 µg/L) and even better than the requirement for Standard A of 5.00 µg/L (DOE, 2011). The study also has shown that the percentage of mercury removal efficiency in bioreactor is 99.60 % for first 6.00 hours and 99.80% for 120 hours before the sample is introduced to a membrane system. In this case, 6.00 hours was the optimum time for the mercury removal with the ratio of mercury mass over cell mass is 20.78 ugHg/ gcell for *P. putida*.

Table 4.3: Results of parameter related *P. putida* growth behavior and activity during mercury removal in membrane bioreactor

Time (hr)	Optical Density (OD)	Biomass concentration (g/L)	Hg concentration (ug/L)	µgHg/gcell	% Hg removal
0.00	0.00	0.00	4000.00	-	-
6.00	1.995	0.77	16.00	20.78	99.6
120.00	1.775	0.68	16.00	23.82	99.6

0	48.0	2.076	0.80	12.00	3.30	0	
0	72.0	2.013	0.78	8.00	5.00	1	99.7
0	96.0	2.155	0.83	6.00	0.25	0	99.8
00	120.	0.00	0.00	3.60	23	7.	99.8
r	Afte				64	0	99.8
mbrane	Me					-	99.9
						0	

From the investigation, it is confirmed that the function of microfiltration membrane system is introduced to retain *P. putida* in bioreactor. As a result, the cross-flow membrane filtration system would allow maximum microbe-metal interaction and reduced the diffusional barrier for the metal and produced high cell densities can be produced. In addition, cell harvesting and metal clean up could be processed in one membrane system. Chen et al. (1998) and Chung et al. (2003) reported that the evidence from the SEM image that *P. putida* cells are indeed entrapped and grow in microvoids of the hollow fibers.

In this case, the reduction of ionic mercury to water insoluble metallic mercury, which is catalyzed by mercury resistant bacteria, is the mechanism by which mercury is removed from a solution. While volatilization of the reduced Hg^0 from batch microbial cultures were captured as metallic mercury within the bioreactor (Wagner-Dobler et al., 2000b).

The solubility of elemental mercury in water is 60.00 ug/L (Barkay, 2003), which is the minimum concentration of mercury that can be obtained in the effluent of this type of mercury retention bioreactor. To reach the wastewater discharge limit reliably, a polishing step, such as carbon filtration is necessary (Wagner-Dobler, 2003). Observation from this study also showed that when the bioreactor operated in the proximity or in the stationary phase of the curve, the amount of excess sludge is considerably reduced. In this condition, the substrate removal efficiency is very high and thus confirming that one of the advantages of using membrane bioreactor (MBR) rather than conventional activated sludge.

Petroleum Based Industries Wastewater Quality Analysis

Typical quality of feed (petroleum based industries wastewater) and membrane bioreactor permeate (water quality) as a function of time throughout the study is shown in Table 4.4. Figure 4.6 and Figure 4.7 indicate petroleum based industries wastewater turbidity

and suspended solid at the initial stage with *P. putida*. Figure 4.8 shows the results of comparison between turbidity and suspended solids after mercury removal by *P. putida* in the membrane bioreactor. Suspended solid in petroleum based industries wastewater is consistently less than 0.30 mg/L although cell density showed further enhancement of the growth with an increase in optical density and turbidity fluctuated from 82.20 to 91.90 mg/L. In this situation, it is noted that mercury concentration decreased at a very fast rate in the first 6.00 hours of the operation when the membrane bioreactor is operated at optimum operating condition.

Table 4.4:

Effect of mercury removal from petroleum based industries wastewater to water quality by p. putida in membrane bioreactor

Time (hr)	Turbidity (NTU)	Suspended Solid (mg/L)	Optical Density	Biomass concentration (g/L)	Hg Concentration (ug/L)
0.00	88.20	0.13	0.00	0.00	4000.00
6.00	94.50	0.25	0.00	0.77	16.00
24.00	90.40	0.23	0.78	0.80	12.00
48.00	84.60	0.27	0.08	0.78	8.00
72.00	82.10	0.22	0.01	0.83	6.00
96.00	78.50	0.09	0.16	0.78	6.00
120.00	91.90	0.02	0.02	0.00	4.00
After Membrane	5.32		0.00		

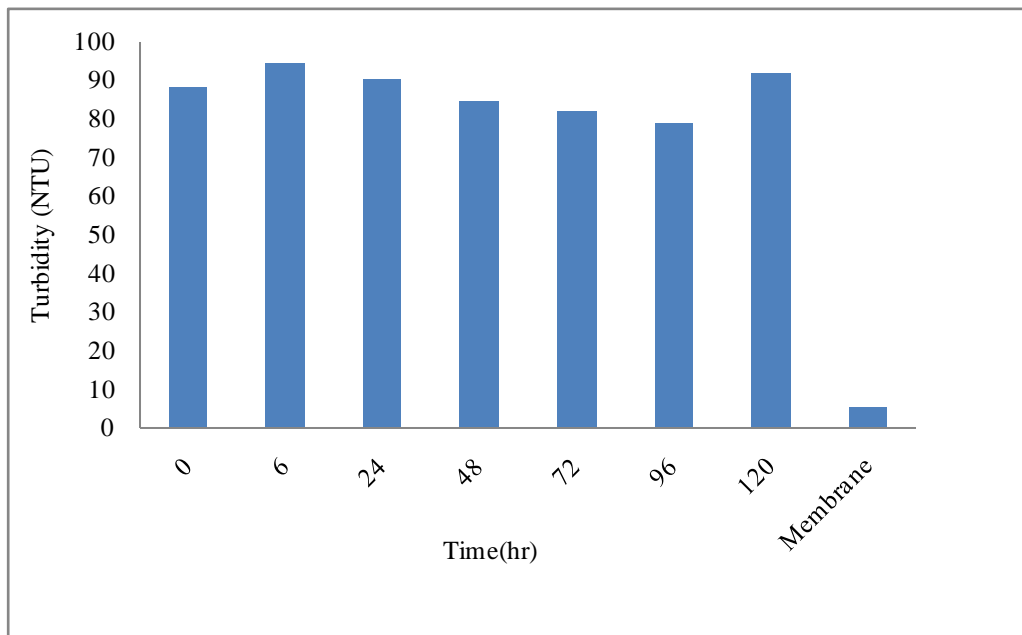


Figure 4.6: Turbidity in membrane bioreactor after mercury removal by *p. putida*

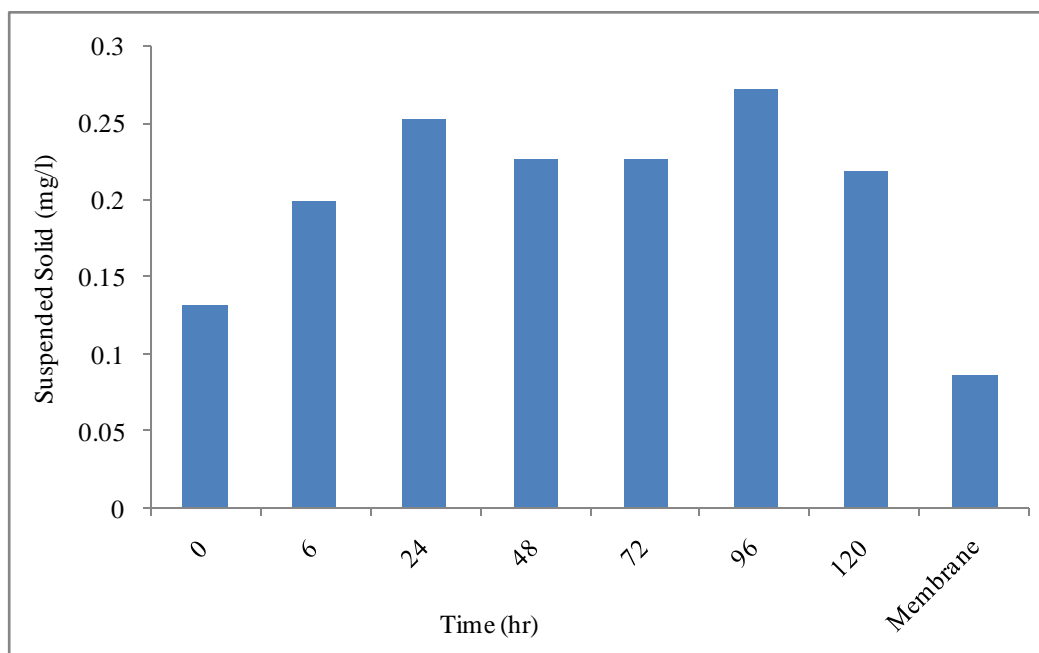


Figure 4.7: Suspended solids in membrane bioreactor after mercury removal by *p. putida*

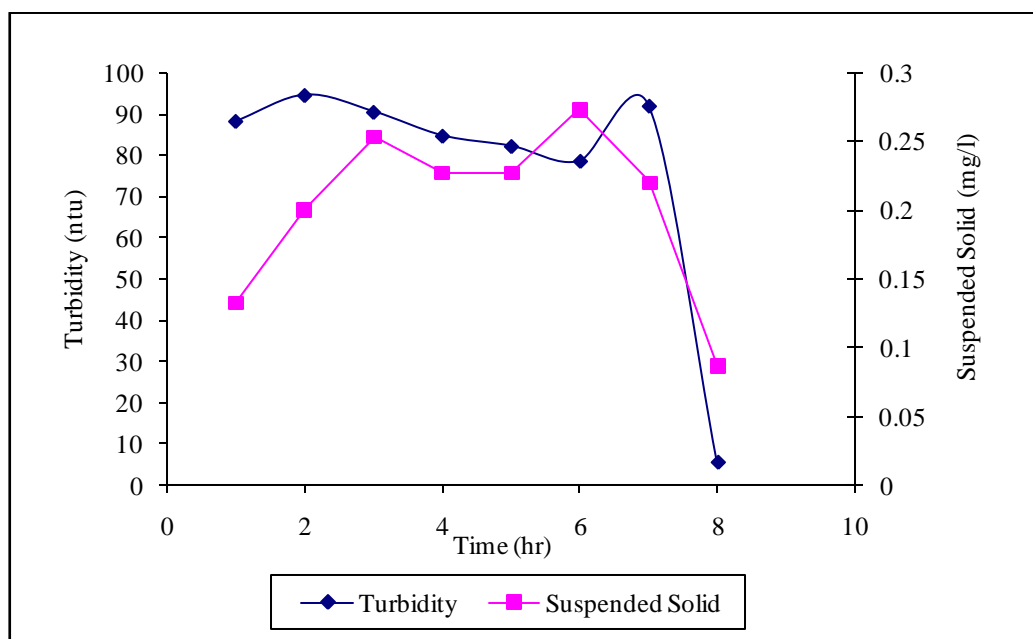


Figure 4.8: Comparison of turbidity and suspended solids in membrane bioreactor after mercury removal by *p. putida*

Based on the results analysis on effect of mercury removal to turbidity and suspended solids of petroleum based industries wastewater in membrane bioreactor, it is found that the percentage of turbidity removed in the membrane microfiltration phase is 94.20 %, while the percentage of suspended solids removed is 60.40%. Consequently, it can also be seen that there is no cell content in the permeate as the measured optical density (OD) is 0.00.

Conclusion

The investigation clearly demonstrates that *P. putida* growth and activity in bioreactor can be enhanced by optimizing the operating parameters and the use of microfiltration membrane. The biological method and MBR design are emerging as a potential alternative that have been demonstrated to be robust, highly selective, efficient and environmental friendly (green technology) in minimizing mercury in wastewater with low mercury concentration. Bioremediation is often considered a cost effective and environmental friendly method and is gradually making inroads in environmental clean-up application. In this study *P. putida* naturally possess the ability to degrade and transform toxic mercury (Hg^{2+}) to less toxic elemental mercury (Hg^0) through enzymatic transformation. By retaining *P. putida* in

the bioreactor, this would result in maximum microbe-mercury interaction and could reduce diffusional barrier for mercury hence producing high cell densities. At the same time, the elemental mercury formed could be retained in the system. Using membrane bioreactor will result in an improvement in the overall performance of the microfiltration process and the wastewater quality. In addition, the turbidity is improved by 94.2% (5.32 NTU) and suspended solid removed by 60.4% (0.09). With this minimum concentration of mercury in petroleum based industries wastewater after improving the wastewater quality, the effluent can be discharged from wastewater treatment plant, hence meeting the limit of DOE requirement of Malaysia.

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