Mercury Removal From Petroleum Based Industries Wastewater By P. Putida

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ABSTRACT: Mercury pollution is one of a primary environmental issue and public health problem. The purpose of this research is to remove the mercury using pure culture Pseudomonas putida (ATCC 49128) at optimum growth parameters such as technique of culture, acclimatization time and speed of incubator speed. In this study, the optimum growth parameters ofP. putida were obtained to achieve the maximum of mercury removal. Thus, a field study were carried out at two different location based on petroleum industrial plants in Peninsular Malaysia. Processes involved in this research which is P. putida behavior in rehydrating free-dried growing method, growth parameters and optimum operating conditions. Analysis that carried out are turbidity, total dissolved solid and suspended solid where related to growth of P. putida. Efficiency of mercury removal from actual petroleum based industries plant 1 (P1) wastewater with the presence of 1000 ppb Hg increased from 84% after 4 hours to 90.5% after 96 hours. For plant 2, the sample with presence P. putida and nutrient broth had the highest of mercury removal which is 97.27%. This results show by using P. putida is efficient for mercury removal from actual petroleum.

Keywords: Pseudomonas putida, growth kinetic, mercury, petrochemical wastewater

1 INTRODUCTION

In recent years, there has been a growing need to eliminate hazardous pollutants from waters. Mercury pollution is one of a primary environmental issue and public health problem. From the start of the industrial period, the emissions of mercury have been increasing above levels in water and air [1] and also almost thousands of tons of mercury. The trace contaminant in hydrogen reservoirs made the mercury naturally occurred in all phases which are in oil, gas and water. The emissions of the mercury to the atmospheric environment were the main possibility from the use of fossil hydrocarbons as fuels. Besides that, it also possibility exist in production, transportation and in processing systems [2]. Wastewaters originate from production operations in the form of produced and in refining and gas processing as wastewater. Solid waste streams are generated in production, transportation and in refining. Air emissions originate from fugitive emissions (process equipment) and combustion. Combustion to be vastly dominant as a possible avenue by which mercury in oil and gas may be transferred from produced hydrocarbons to the environment as wastewater (US EPA, 1971).Oil and gas industry distinguishes between upstream and downstream operations. The upstream category refers to primary production and whatever processing is necessary to place the produced fluids in the transportation system. The term downstream operations refer to refining and gas processing to produce salableproducts [2]. In Minamata Bay, mercury-resistant Pseudomonas spp. was isolated from sediments near the drainage outlet to the Bay. Pseudomonas spp. dominated the bacteria with the highest resistance to mercury [3].

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The ability of P. putidato degrade hydrocarbon in wastewater such benzene, toluene, and o-xylene has a direct bearing on the development of strategies for dealing with environmental pollution [4]. Mechanism for mercury removal by P.putida were involved mechanism of redox transformation where the enzyme-catalysed reduction of the toxic mercury (Hg^{2+}) to non-toxic elemental mercury, Hg^0 [5,6]. Genetically, these genes involved in mercury reduction mechanism called mergenes, are arranged in an operon (mer operon) which consists of series of genes (MerR,MeO, MerP, MerT, MerA, MerD) and which are under control of the regulatory protein MerR which function as either a repressor (absence of Hg^{2+})or transcriptional activate (presence of Hg^{2+}) where MerO still unknown function, MerP as a periplasmic binding protein, MerT as transport gene, MerA is responsile for the production of mercury reductase enzyme and MerD as gen for regulation [7,8,9]. The function of Nicotinamide Adenine Dinucleotide Phosphate (NADPH) inside the cell is to reduce the Hg^{2+} to Hg^0 by mercury reductase (merA) which is related to glutathione reductase [10]. At the exponential or log phase, the growth rate of the cells increase in a short time and the cells grow at constant, maximum rate [11,12]. 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) [8].

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

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 [13,14]. 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 [15]. Using the natural logarithms on both sides of Equation 1, thus can be re-written as:

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

Specific growth rate, μ is defined as the increase in cell mass per unit time;

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

Number of generation, n

$$n = 3.3 \ln \frac{X_t}{X_0} = 3.3 \ln \frac{OD}{OD_0} \quad (4)$$

Generation time, g is defined the time required for formation of two cells from one doubling time where t is duration of exponential growth expressed in days, hours or minutes.

$$g = \frac{t}{n} \quad (5)$$

Growth rate constant, k is to measure of the number of generation that occurs per unit time in an exponential growing culture.

$$k = \frac{\ln 2}{g} \quad (6)$$

2 MATERIAL AND METHOD

2.1 Materials

In this research, the material used consisted of microorganism and chemicals for the preservation of the microbe in slant, culture medium and for analytical procedures.

2.1.1 Microorganism

Bacteria P.putidaused was obtained from Merck (Malaysia) Sdn. Bhd. as local agent dealing with bacteria. Contents of P.putidanutrient consist of 5% of pepton meat and 3% meat extract. Growth mediums for P. putida were prepared by suspending 8g nutrient powder in 1 L of de-ionized (DI) water. Growth media was sterilized in an autoclave at a temperature of 121^{o}C and a pressure of 15 psi for 25 minutes. Culture was kept below 5^{o}C and this culture stock was used for all subsequent works.

2.1.2 Chemicals

All chemicals and medium used in the experiments were analytical grade, including peptone, yeast extract, KH2PO4, $MgSO_4.7H_2O$, Urea, NaCl, HNO_3, HCl, NaOH, were purchased from Merck (Malaysia) Sdn. Bhd. Stannous Chloride solution ($SnCl_2$), $Hg(NO_3)^2$ as mercury standard solution (1000 000 ppb) and H_2SO_4 were obtained from Orbiting Scientific & Technology Sdn. Bhd.

2.1.3 Medium

The purpose of the medium was used for inoculum, growth and wastewater treatment purpose. For preparing the nutrient broth and nutrient agar, de-ionized water was added and heated using hot plate and stirred. Then, both of nutrients were sterilized in an autoclave at temperature of 121^{o}C at 15 psi for 25 minutes. At last, both of nutrients kept in a freezer at temperature below 5^{o}C.

2.1.4 Process of Culturing P.putidafrom Freeze-Dried

For the growth of culturing P.putida, the packing skin of the culture should be removed using sharp blade or soaking in water for a few minutes while the ampule briskly. Then, the gauze is covered around the ampule when the sample was sterilized with alcohol-dampened gauze to break the score area and the gauze also should not too wet because the alcohol can be sucked into the ampule when the scored area is broken. After the properly mixing culture in 0.50 ml nutrient broth, the suspension is transferred to an oven to 30^{o}C for 24 hours to let the culture to incubate.Lastly, the growth of the culture started when a few drops of suspension are transferred to slanting agar, nutrient broth and plate agar [16].

2.2 Equipments

2.2.1 Experimental Apparatus

Apparatus required for this research are auto-clave, H+P Varioklav Stream Sterilizer ESCO, Shaker (B. Braun, German) and microbiological incubator (Mermmert-Germany/BE 600)

2.2.2 Analysis Apparatus

Analysis apparatus that involved are mercury analyzer, (RA-300 Mercury, NIC), UV-Visible Spectrophotometer (U-1800, Hitachi), pH Meter (Mettler Toledo), vacuum pumo (HACH), Turbidity Meter HACH 2100P and analytical Balance (Mettler Toledo).

2.3 Method

Overall experiments for these experiments are involved sterilization, culture preservation, fermentation, procedure for analysis of samples and experimental data. Experiment are divided into 2 main stages and carried out in the shake flasks. The first stage is to investigate variables involved in fermentation during P.putidagrowth such as culturing technique, nutrient/substrate concentration, acclimatization time, pH, incubator shaker speed and temperature. For the second stage is to determine and study the effects of the variables on the capability of P.putida to reduce or remove mercury in wastewater.

2.4 Mercury Contamination Level in Petroleum Based Industries Wastewater

Study has been carried out at two different petroleum based industries which is located at East Coast and southwest of Peninsular Malaysia to determine contamination of mercury. Water discharge from plants in petroleum based industrial plant has been identified as sources of aquatic mercury contamination [2]. The first industry (P1) was located at the East Coast which is approximately 110 km south of Kuala Terengganu and the production of this industry involved in petroleum gas production. Meanwhile the second industry (P2) was located in the south west of Peninsular Malaysia and 90 km from Kuala Lumpur.

2.4.1 Case Study

For first case study, samples are collected at two different points which are at the exit point and inlet point of the treatment plant from wastewater treatment plant of a petroleum gas processing industry (P1) situated in the East Coast of Peninsular Malaysia.Meanwhile for second case

study, samples are collected from a wastewater treatment plant of a petroleum refinery (P2) situated in the South West of Peninsular Malaysia at six different location at zone 1 and 34 samples were collected at various points within zone 2.

Table 1: *Sampling Point*

Location	Period of Sampling (Days)
East Coast of Peninsular Malaysia Industry (P1)	
Exit point of the treatment plant (P1L1)	14 (one sample per day)
Inlet point of the treatment plant (P2L2)	12 (one sample per day)
South West of Peninsular Malaysia Industry (P2)	
Zone 1 (P2L1)	5 (one sample per day at six different location)
Zone 2 (P2L2)	5 (34 samples)

2.4.2 Wastewater Sample Collection

Before the samples were collected, the containers were rinsed several times with water and wastewater samples were collected around the center of wastewater pond. Normally to prevent turbulence and air bubble, the samples were slowly filled into the containers.

2.4.3 Samples Storage and Preservation

This proper storage is to ensure any undesired interferences present in contaminated are removed. After that, the glassware are rinsed with a tap water and 1:1 hydrochloric acid (HCl) solution followed by with de-ionized water. Lastly, samples were put in the oven to dry. Preservation process is to slow the chemical and biological changes that continue to happen after collection which is by adding acid. Normally, the samples are always analyzed after the collection. The pH control (acid addition), refrigeration and freezing at below 5^{o}C are the preservation methods which are to protect the samples.

2.5 P. putida in Shake Flask

Process that involved mercury removal in shake flask by P.putidaare sterilization of glassware, preparation of bacteria inoculum, determination of P.putida growth, determination cell dry weight and determination of mercury.

2.5.1 Sterilization of glassware

Before sterilization, cotton plug and an aluminum foil were used to cover the test tube and Erlenmeyer flasks. Then, all glassware were autoclaved at a temperature of 121^{o}C and pressure at 15 psi for 25 minutes. After that, the autoclave was allowed to cool for 15 minutes before opening it and then add all the glassware into another sterile container. The medium that has been fermented in shake flasks were also sterilized with the standard procedure of autoclave sterilization [17].

2.5.2 Preparation of Bacteria Inoculum

A loop-full of P.putida colony, ATCC 49128 that have been cultivated on nutrient agar (NA) is picked for the preparation of inoculum and then transferred into 10 ml of nutrient broth (NB) which is 10% of the medium volume. After that, the culture is incubated at 30^{o}C for 24 hours. The quantity of inoculum supposedly to use about 3% to 10% of the medium volume [18], but in this study 10% of the medium was used. The colony is transferred to 25 ml inoculum flask containing 90 ml nutrient broth after 24 hours. Suitable temperature for cell to grow at 37^{o}C while being vigorously shaken at 180 rpm and then samples are analyzed by UV spectrophotometer at optical density (OD) of 600nm to monitor growth of P.putida [15]. Table 2 showed summary of the operating conditions for P.putida growth in shake flask.

Table 2: *Effect of operating condition for P.putida growth studied in shake flask*

Experiment	Operating Conditions Study	Parameter Value
1	Acclimatization Time (hr)	24 48 72
2	Orbital Shaker Speed (rpm)	140 180 200
3	Temperature (^{o}C)	25 30 33 37 42
4	Substrate Concentration (g/L)	4 8 12 16
5	pH	4.0 7.0 10.0 6.7 (Control)
6	Mercury at low and high concentration (ppb)	1000 (Low) 4000 (High)

2.5.4 Determination of P.putida Growth

UV spectrophotometer was used to identify the growth of P.putidaand the method is based on the absorption of light by suspended cells in media of the sample culture. For every hour, 5 ml of liquid samples was taken until decay phase is occurred at all concentration and the shake flasks are detached from the orbital shaker during the sampling. The growth of P.putida is examined at 600nm absorbance using a UV spectrophotometer.

2.5.5 Mercury Determination

Firstly, the samples were pretreated with strong acid and an oxidizing agent to change the compound into divalent mercury ions (Hg^{2+}) and used a mercury analyzer system to measure the content of mercury with maximum concentration at 15 ppb. Besides that, the samples must be diluted before analyzing because the mercury content in the sample is high concentration. Dilution of samples containing hydrogen sulphate (97%) and ultrapure with 1:1

ratio is prepared which is 40 ml of hydrogen sulphate (97%) with 40 ml ultrapure water is mixed using measurement cylinder and then poured into a clean glass bottle. Then, a mixture of hydrogen sulphate and stanum chloride is prepared where 2 g of stanum chloride, 1 ml of hydrogen chloride and 19 ml of ultrapure water are used. The mixture is stirred until the stanum chloride is completely dissolved and then 10 ml of sample is poured into a test tube. Lastly, the test tube is plugged into the socket of Mercury Analyzer for 3 minutes.

2.6 Petroleum Based Industries Wastewater Analysis

2.6.1 Temperature

Temperature of the samples was measured using dwi-function pH meter model Mettler Toledo Delta 320 with accuracy +/- 1^{o}C. The electrode of the meter is immersed 10.00 cm below the surface of the sample for 2 – 5 minutes or until a stable reading is obtained.

2.6.2 Turbidity

Turbidity of the sample was determined using turbidity meter model WTW Turb 350 IR. 10 ml of sample is filled in the sample tubes, clear colorless glass that has been thoroughly cleaned of both the inside and outside before being used for testing. Then, the sample tube is placed in the equipment and the read button was pressed. The reading is recorded when a static is recorded when static value appeared on the screen.

2.6.3 pH

The pH meter model Mettler Toledo Delta 320 is used to measure the pH of the sample. While the electrode of the pH meter is immersed 5 cm below the surface of the sample for 2 – 5 minutes, the sample is stirred to ensure homogeneity. Calibration of the pH meter is carried out once a week using buffer solution at pH 4, 7 and 10.

2.6.4 Total Suspended Solid (TSS)

Filter disk is dried in oven at 103^{o}C to 105^{o}C for 1 hour and cooled in desiccators and weighed. Then, filter is wetted with a small volume of distilled water and 10 ml of sample were pipetted onto the center of filter disk. Filter paper is washed with three times successively with 10 ml of distilled water and continues suction for about 3 min after filtration. After that, filters are removed from the filtration apparatus and transferred to an aluminum weighing dish. Lastly, filter disk is dried in oven at 103^{o}C to 105^{o}C for 1 hour and cooled in desiccators and weight.

2.6.5 Total Dissolved Solid (TDS)

Total suspended solid is to measure of the combined content of all inorganic substances in a liquid in molecular, ionized or micro-granular suspended form by using TDS meter. The solids in the sample must be small enough to survive filtration through a sieve of 2 micrometer.

3 RESULT AND DISCUSSION

These studies on the treatment of petroleum based industries wastewater at two different plants were carried out by using mercury resistant microorganism such as P.putida in an effort to develop an environmental friendly, cost effective, integrated, end-of-pipe remediation technology.

3.1 Plant 1

Plant 1 is located in the East Coast of Peninsular Malaysia and the first production division with the commencement of gas production. The samples are carried out at two different point which is inlet (P1L1) and outlet (P1L2) point of the treatment and a very low mercury concentration of 0.01 ppb was found in the wastewater at location P1L1. Therefore, mercury concentration of 1000 ppb was added into the sample to study on mercury removal at 1000 ppb at pH 7 with temperature at 37^{o}C. The experiment was conducted in orbital shaker at 180 rpm for 4 hours. The samples were prepared into three types samples which are:

i. Sample A: 20 ml (Nutrient broth, NB + P.putida) added into 180 ml NB
ii. Sample B: 20 ml (Nutrient broth, NB + P.putida) added into 180 ml wastewater
iii. Sample C: 20 ml (Nutrient broth, NB + P.putida) added into 180 ml wastewater with 1000ppb mercury

Table 3: *Growth behavior of P. putida in different media condition for sample A, sample B and sample C*

From the result, the growth of P.putida in sample B and sample C took quite sometimes to grow after inoculation. Exponential growth obtained was 3.01 for sample A, 2.66 for sample C and 1.73 for sample B. It show that the presence of mercury in the sample wastewater affect the growth of bacteria.

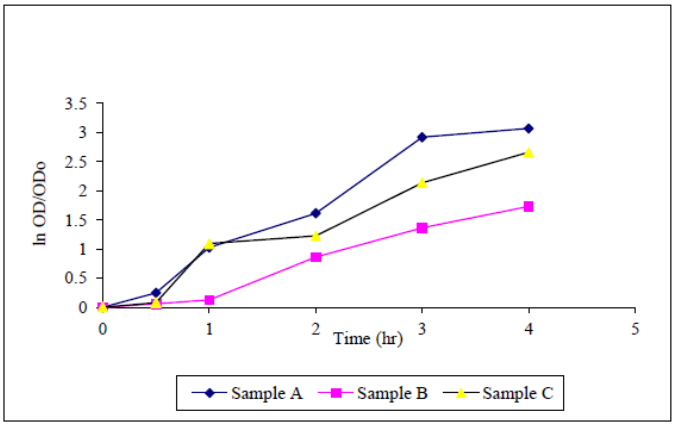

Figure 1: *The growth of P. putida in petroleum based industries wastewater*

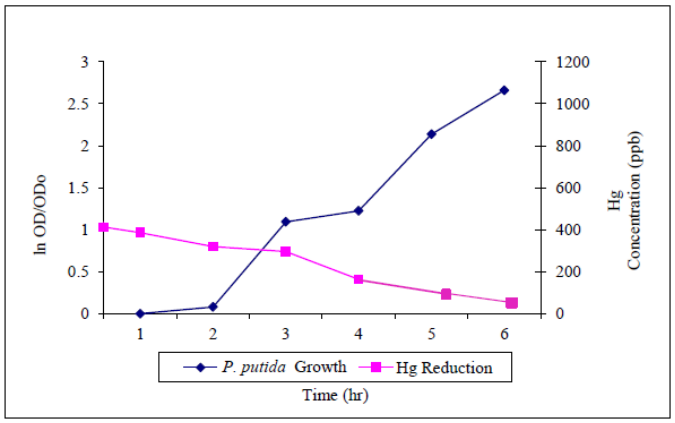

Figure 2: *Mercury (1000 ppb) removal from petroleum based industries wastewater by P. putida at optimum operating condition in the shake flask*

From the Figure 2, it showed the mercury removal from petroleum based industries wastewater byP.putida. The efficiency of mercury removal after 4 hours and 96 hours increased from 84% to 90.5% which is the mercury content was reduced from 840 ppb to 905 ppb.

3.2 Plant 2

Plant 2 is located at Southern of Peninsular Malaysia and the industry based on petroleum where the main processes carried out at the plant are crude distillation unit, naphtha hydro-treating unit, catalytic, reforming unit with continuous catalytic regenerator, saturated gas concentration unit, sour water stripping unit, kerosene and heavy naphtha treating unit and mercury removal unit. For the industrial Plant 2, the wastewater parameters are first determined such as pH, temperature, total dissolved solid (TDS), suspended solid (SS) and turbidity. Then, the mercury retention efficiency and wastewater parameters of the microbial detoxification system are determined using the actual sample of petroleum based industries wastewater. From the analysis, the wastewater is contained 22.00 ppb mercury at pH 6.09, temperature at 25^{o}C, with total dissolved solid (TDS) was 260.00 mg/L, total suspended solid (TSS) at 27.33 mg/L and turbidity of 68.30 NTU. Concentration of mercury is lower than the requirement of Department of Health (DOE) under Standard B which is 50 ppb but higher than Standard A at 5 ppb. This experiment was conducted in a shake flask at optimum operating condition for 52 hours to investigate the mercury (1000ppb) removal from petroleum based industries wastewater by P. putida. The wastewater samples from plant 2 prepared is as follow:

i. P .putida + wastewater
ii. P. putida + Nutrient Broth (NB)
iii. Wastewater only
iv. Wastewater + Nutrient Broth (NB)
v. Wastewater + Nutrient Broth (NB) + P. putida

Table 4: *Mercury removal by P. putida from petroleum based industrial Plant P2 wastewater*

Growth Parameter / **Sample**	**Min**					**Max**				
	i	ii	iii	iv	v	i	ii	iii	iv	v
Specific Growth Rate, μ(hr^{-1})	0.02	0.05	0.02	0.11	0.11	-	-	-	-	-
OD	0.14	0.18	0.01	0.01	0.01	0.55	2.34	0.03	2.28	2.68
Exponential cell growth, (ln OD/OD_0)	0.45	0.11	0.00	0.26	0.88	1.40	2.55	0.90	5.43	5.59
Number of Generation, n	0.64	0.16	0.00	0.38	1.25	2.00	3.65	1.29	7.78	8.01
Generation Time, g (hr)	0.08	0.05	0.00	0.10	0.04	26.63	18.44	4.46	7.98	6.60
Growth Rate Constant, k (hr^{-1})	1.56	2.26	0.00	0.52	6.30	8.86	1.35	0.15	6.88	17.88
Hg Removal (%)	94.09	-	12.73	56.82	97.27	-	-	-	-	-

From the Table 4, the specific growth rate, μ obtained are 0.02 hr^{-1} for sample (i), 0.05 hr^{-1} for sample (ii), 0.02 hr^{-1} for sample (iii), 0.12 hr^{-1} for sample (iv) and 0.11 hr^{-1} for sample (v). The growth behavior of P.putidabetween different conditions is shown in Figure 3.

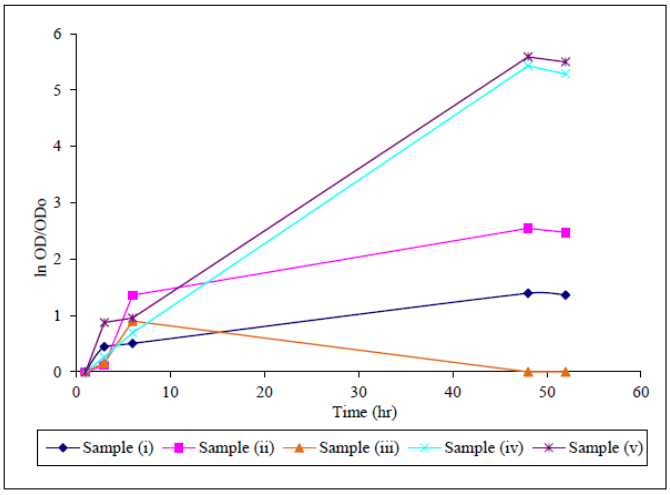

***Figure 3: P.** putida and other natural microbe growth in petroleum based industries wastewater*

The results indicate that in the presence of nutrient broth with 8 g/L concentration samples (ii), (iv) and (v) showed high cell activity with increasing optical density and the exponential growth especially for samples (iv) and (v) where the nutrient was added in wastewater sample. Moreover, it is noted that the pattern of results obtained from sample (iv) and (v) are quite similar and are consistent with the cell growth behavior as has been indicated. However based on data analysis, mercury removal for sample (iv) is 56.82% compared 97.27% for sample (v).

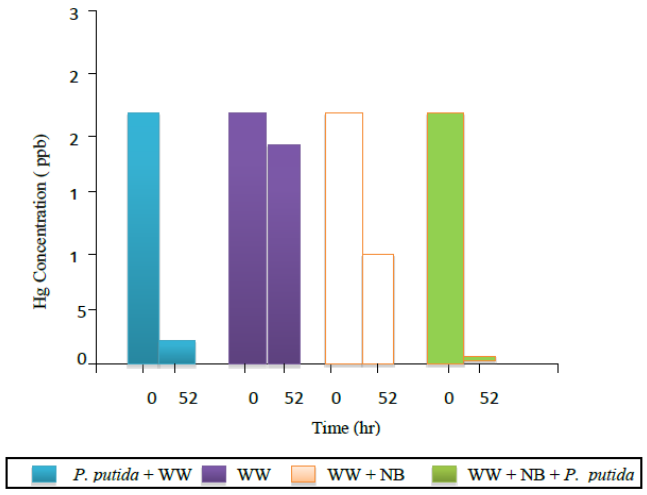

***Figure 4:** Mercury removal by P. putida in petroleum based industries wastewater*

3.3 Petroleum Based Industries Wastewater Quality Analysis

3.3.1 Total Dissolved Solid

The total dissolved solid is affected P.putida growth in the wastewater of petroleum based industries and the degree of concentration is shown in Table 5. It described the combined content of all inorganic substances contained in the liquid in molecular, ionized or micro-granular (colloidal sol) suspended form.

***Table 5:** Petroleum based industries wastewater (WW) total dissolved solid with respect to the treatment*

Sample Petroleum Based Industries Wastewater (WW)	Before Treatment (mg/L)	After Treatment (mg/L)	Degree of Concentration (Metcalf and Eddy, 2003)
(i) P. putida + WW	260.00	430.00	Low
(ii) P. putida + NB	260.00	1800.00	High
(iii) WW	260.00	250.00	Very low
(iv) WW + NB	260.00	1860.00	High
(v) WW + NB + P. putida	260.00	2150.00	Very high

From the result, it shown that the total dissolved solid increased when the supplementation of nutrient broth (NB) to the system as illustrated in Figure 5. However, the total dissolved solid for sample (iii) in the media almost reach a stable value of 250.00 mg/L

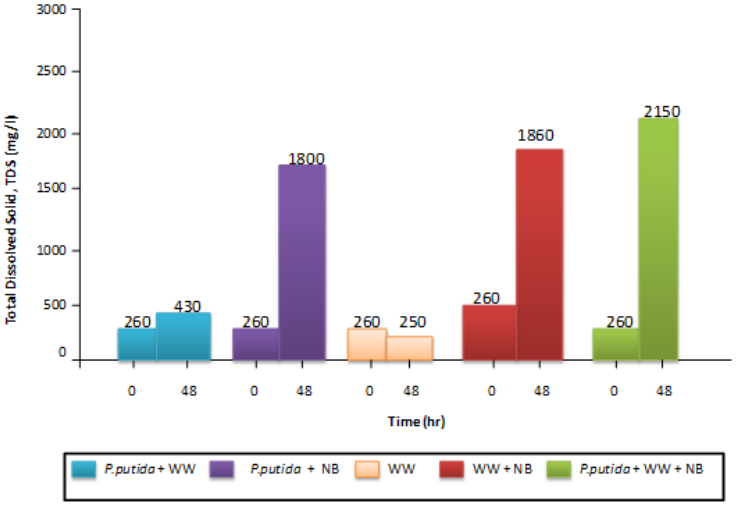

***Figure 5:** Effect of P. putida growth to total dissolved solid of petroleum based industries wastewater*

3.3.2 Total Suspended Solid (TSS)

Solids suspended in water may consist of inorganic or organic particles or immiscible liquids. However, if the concentrations are enhanced through, this can lead to alterations to physical, chemical and biological properties of the water body [19].

Table 6: *Petroleum based industries wastewater (WW) suspended solid with respect to the treatment*

Sample of Petroleum Based Industries Wastewater (WW)	Before Treatment (mg/L)	After Treatment (mg/L)	Degree of Concentration (Metcalf & Eddy, 2003)
(i) P. putida + WW	27.33	0.54	Very low
(iv) WW + NB	27.33	1.62	Very low
(v) WW + NB + P. putida	27.33	1.12	Very low

From Table 6, it show the suspended solid decreased from 27.33 mg/L to almost below 2.00 mg/L. The value of suspended solids obtained are categorized as very low [20] and also lower than the requirement by Environmental Quality Act (EQA) 2011 2011, Environmental Quality Sewage and Industrial Effluents for Standard B which is 100.00 mg/L. Therefore, the Figure6 illustrated the change of suspended solid for sample (i), (iv) and (v).

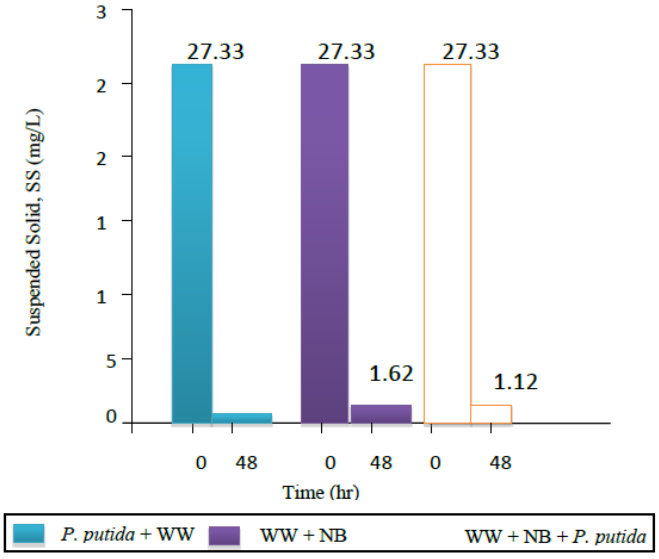

Figure 6: *Effect of P. putida growth to suspended solid of petroleum based industries wastewater*

3.3.3 Turbidity

Turbidity is the measure of light scattering properties of water and also influenced by particle size and shape of suspended solid. From Table 7 shown the effect of P.putida growth and the activity about the turbidity of petroleum based industries wastewater and the experiments were carried out for 48 hours in shake flask.

Table 7: *Petroleum based industries wastewater (WW) turbidity with respect to the treatment*

Sample Petroleum Based Industries Wastewater (WW)	Before Treatment (NTU)	After Treatment (NTU)
(i) P. putida + WW	68.30	213.00
(ii) P. putida + NB	68.30	395.00
(iii) WW	68.30	56.40
(iv) WW + NB	68.30	879.00
(v) WW + NB + P. putida	68.30	2150.00

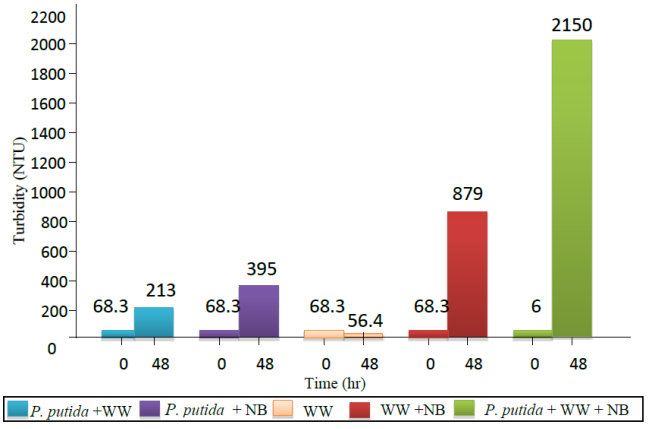

Figure 7: *Effect of P. putida growth to turbidity of petroleum based industries wastewater*

The result show all the samples had turbidity of 68.30 NTU and the samples (v) which is has the highest increment of turbidity value by more than 30 times when the presence of P.putida and nutrient broth (NB) followed by sample (iv) that increased by 13 times increased when only nutrient broth in the sample. Then, turbidity value sample (ii) is 6 times increased and sample (i) was slightly increased by 3 times while sample (iii) remains unchanged. This demonstrated that turbidity of the sample is largely caused by total dissolved solids and total suspended solids [21]. However, the amount of solids in suspensions will depend on the degree of agitation of the wastewater sample.

4 CONCLUSION

The objective of this research is to investigate reduction of Hg concentration in actual petroleum based industries wastewater using P.putidaat optimum condition and has successfully proven that it could reduce the mercury concentration. The efficiency of mercury removal from actual petroleum based industries wastewater with the presence of 1000 ppb Hg increased from 84% after 4 hours

to 90.5% after 96 hours for Plant 1(P1). For plant 2, the sample with presence P.putida and nutrient broth had the highest of mercury removal which is 97.27%. This results show by using P.putida is efficiency of mercury removal from actual petroleum.

5 ACKNOWLEDGEMENT

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