Biodegradation of Phenol in Petroleum Industry Produced Water by Estuary Mikroorganisms

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ABSTRACT

The study was undertaken to calculated the biodegradation kinetic parameters of phenol in water and sediment estuary. Biodegradation of phenol compound in brackish water have special characteristic due to the effect of high salinity and the activities of special groups of microorganisms. The biodegradation of this compound is provided from laboratory experiments in batch reactor system. The culture for the biodegradation assay is taken from the sediment in the estuary of Mahakam River. The growth medium is made of seawater, with phenol as the substrate. The biodegradation is observed for 40 days. The initial phenol concentration in Reactor was 450.65 to 469.57 mg/l. The kinetic study showed that specific growth rate microorganism (µ) in sea water was 0.092 to 0.1356 day^{-1}, the decay specific rate was 0.473 to 0.494 day^{-1}, with the degradation rate was 11.19 to 11.73 mg/l.day^{-1}. The dominant bacteria in the culture are Pseudomonas putida, Pseudomonas diminuta, Bacillus sphaericus, Enterobacter sp and pseudomonas sp.

Key words: phenol, biodegradation, seawater, sediment, estuary, microorganism

INTRODUCTION

One of pollutants which often generates problem is aromatic hydrocarbon. Hydrocarbon compounds which are often found, especially in territorial water are phenol and its derivates. Phenol and its derivates are found in various wastewater including those from the oil refining, petrochemical, coke and coal gasification (Semple and Cain, 1996).

Phenol which is toxic pollutant in last decades is often accumulated in territorial, surface water sediment and oil (Kuo and Genthner, 1996). Phenol consist of the base ring of benzene aromatic with one or more hydroxyl. The toxicities of phenol compounds are depend to atoms amount or coherent molecule on its ring. The chlorine phenol is more toxic on the amount of atom when the atom is bound to benzene ring. The chlorophenol more of toxic when the aquatic organism accumulated and persistence compares to other phenol.

Persistence For human phenol can irritate eye, ear and nose and also can cause systematical damage of nervous system, respiration and mouth (Priatna et al in Watson et al,1994). Simple phenol compounds such as phenol, cresol and tylenol are dissolve easily in water and degradable.

Phenol and its homolog can be degraded by various microorganism in the form of bacterium, fungi, mould, and algae (Semple and Cain, 1996). The microorganism abilities for degrading phenol compounds, are influenced by many factors such as environmental conditions and phenol concentrations.

MATERIAL AND METHOD

This experiment are in Microbiological Laboratory and Water Laboratory. The Aerobe Batch Reactors use artificial media with sea-water, sediment and biomass which are taken from the Estuary Area of Mahakam River in Kalimantan. This observation is divided to become 3 phases:

1. Activating the microorganism in the sediment by adding glucose and benzene gradually.
2. Determination the kinetic parameters of degradation phenol in sediment and seawater using the aerobe batch reactor. During the kinetic experiments, the environmental condition s such as pH, salinitas, temperature and Dissolved Oxygen (DO) are also measured.
3. Isolation and identification dominant bacteria in sediment.

Bacteria in sediment activated before packed into batch reactor, with addition of glucose and benzene. Activation this microorganism purpose to avoid death of its when packed into batch reactor. Addition glucose and benzene become 3 phases:

1. 2 % of glucose
2. 1% glucose + 1% Benzene Phase
3. 2 % Benzene.

Growth bacterium process after 24 hours with Total Plate Count (TPC).

The Aerobe Batch Reactor Experiment

This observation conducted in laboratory using aerobe batch reactor. This reactor in form of cylinder with diameter (D) of 14 cm and high (t) of 40 cm. The sediment for this experiment come from of Estuary Mahakam River in Kalimantan. While seawater away from other area. Salinity Sea-water save as to sea-water of Estuary Mahakam River Kalimantan. This Reactor provided with aerator. Air stream from top reactor so that look like waving sea-water.

Operation Of Aerobe Batch Reactor

Analysis Compound of Phenol

Attempt phenol degradation in reactor batch aerobe I and II. Its both made no difference where into each reactor added sediment counted 85 gram, 5 litter sea-water and concentration compound phenol 161.5 mg/l that is adapted with compound concentration of phenol in produce water. When processing DO not less 6 mg/l so that the condition of aerobe remain to awake. pH during process of degraded take place 6.5 – 8.5 where optimal pH for the growth of bacterium 6.5-8.5 (Michael, 1986).
Rang temperature during process from 22-24.5 ^{0}C according to laboratory room temperature. Salt rate early process that is 33 and mount until 41 because the have decreasing of volume. Early phenol for the reactor I was 469.57 mg/l, reactor II was 450.65 mg/l, its has high concentration because sediment sample contain high phenol concentration. Sample take from sample point and used centrifuge to separate water and sediment. The sampling every 2 days for 40 days

Measurement Of Sample Water

Use method of aminoantipirin by spectrophotometer specthronic 20 brand of Milton Roy Company. Put 50 ml sample into funnel apart 100 ml. Then Add 0.5 ml buffer pH 10, 0.3 ml amino antipirin and 0.3 ml $K_3Fe(Cn)_6$, let 5 minute Add 2 x 5 ml CHCL$_3$ (chloroform), shake 2-3 minute, let until both separate condensation. take underside (chloroform) and pack into covet use funnel which have cotton. Afterwards measure color formed at wavelength 465 nm at spectrophotometer. Concentration of Phenol calculated with calibrate standard curve (SMEWW 5530 C 20 edition).

Measurement Of Sample Sediment

In principle is equal to inspection water, but the sediment dried by screening. After dry just packed into gourd of distil and added aquades until volume 50 ml. Packing into funnel and next step equal to inspection phenol in water.

RESULT AND DISCUSSION

Inspection Total Plate Count (TPC)
To activate bacterium in sediment before packed into reactor addition of glucose and benzene step by step. First phase that is addition of glucose counted 2%. Second Phase addition 1% glucose + 1%

benzene. While third phase addition 2% benzene. Growth of bacterium seen from bacteria amount with method Total Plate Count. The Result after 24 hour can be seen at figure 1.
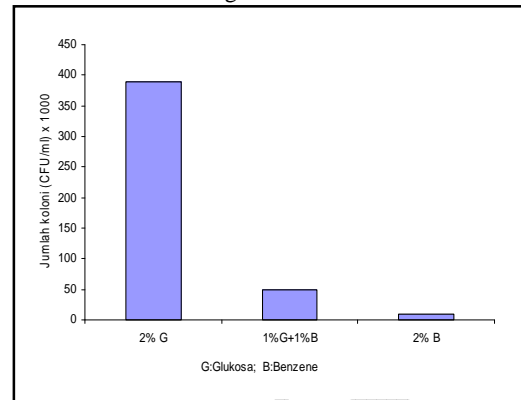

Figure 1 Colony Count after 24 Hours Observation

From result test TPC can be seen that bacterium colonies amount 3.9 x 105 (CFU / ml) when addition of glucose 2%. Addition of glucose 1% and benzene 1% bacterium colonies amount go decrease to 4.9 x 104 (CFU / ml). While addition of benzene 2% bacterium colonies amount decrease to 9.9 x 103 (CFU / ml). Degradation of this bacterium colonies amount happened because existing bacterium in sediment are mix culture, so that not all bacteria there can use benzene as his food. Election of glucose early stage activation because glucose are especial food for bacterium.. Addition of benzene next phase only in the place of glucose, because benzene is simple aromatic compound, stable and easy degradation by hallophilic bacterium(Nichelson, 2004). The purpose added glucose and benzene to prevent the bacteria vacuum because the sediment have save during 1 weeks.

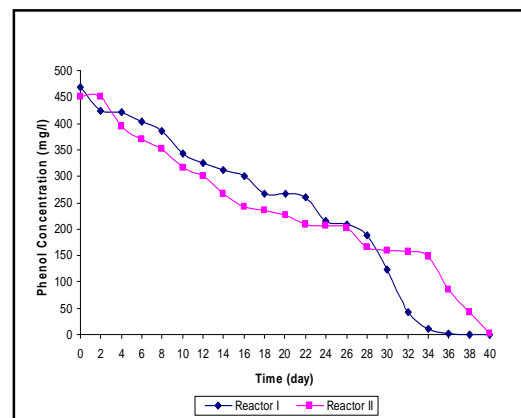**Phenol Degradation In Water**

Figure 2 Phenol Concentration at Reactor I and II

Figure 2 showing degradation pattern phenol concentration at reactor I and II. Degradation rate reactor I is 11.73 mg/L per day and reactor II is

11.19 mg/L per day. Phenol concentration increased to time. Pattern off degradation reactor I and II is same until days 28, Degradation rate relative lower. Degradation at reactor I is fast than reactor II after days 28 because doing thinning 1/7 time for add to volume.

Degradation rate phenol compound in water influenced by many factors. Either by nature of itself phenol, concentration of phenol, attendance of other compound and ability of microorganism to degradation of phenol and environment(Baker and of Mayfield, 1980). Environmental factor which influence decomposition of compound phenol that are temperature (Baker and of Mayfield, 1980), sunlight (Et al Hwang., 1986), salinity and nutrient to supporter of growth bacterium (Rubin and of alexander, 1983) and attendance of other compound (Et al Southworrth., 1985)

Phenol degradable easy because its compound soluble in water. (Et al Watson 1995:54). This theory explain there will be decrease of phenol concentration although not be added microorganism into reactor.

In water occur photochemical reaction with phenol use sunlight yielding radical hydroxyl and peroxy. Half life time reported 100 to 19,2 hour (Howar, 1989). Phenol react with nitrate ion, dissolve in water form dihydroxybenzenes, nitrophenols, and nitrosophenol of nitroquinone, possibility by radical altered to become radical hidroxy and phenoxyl (al et niessen., 1977). Phenol is also found will react with acid of nitrite in discard water and form cyanide. (Et al Adachi., 1987), as well as forming chlorophenol in drinking water chlorinese (Et al Jarvis., 1985) and p-benzoquinone in dioxide chlorine.
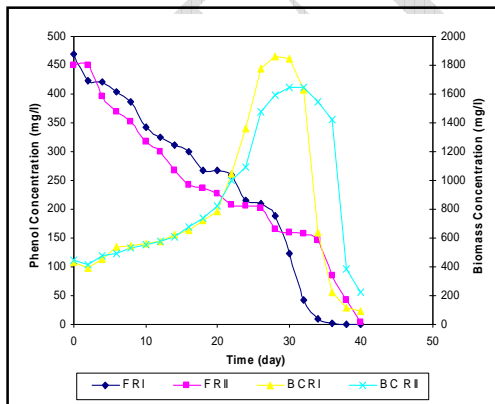

Figure 3 Time course plot of phenol concentration and biomass concentration during Biodegradation in Reactor I and II

Growth of biomass interpreted as total mass cell. In this observation growth mass cell measured with method VSS. Figure 3 showing growth of biomass with decrease of phenol concentration in water. In reactor I early phenol concentration is 469.57 mg/l biomass concentration 432 mg/l. While reactor II 450.65 mg/l and biomass 445 mg/l.

Growth Phases of Biomass in Reactor I and II:

1. Lag phase
In this phase microorganism need time for adaptation with new environment. At the moment microorganism to synthetic enzyme to metabolism new of nutrient , in this case it is phenol compound. Lag phase in reactor I and II take place enough during length time that is 0 – 20 days. This matter is caused by high phenol concentration early more than 400 mg /l, its of toxic for microorganism. Where phenol concentration at range 460 - 1000 mg/l. Its can cause inhibit of growth bacterium and fungi (Kwasniewska and Caesar, 1983). Concentration having the character of toxic for protozoa that is 33- 144 mg/l (Bringmann and Kuhn, 1980) and for lower alga again that is 6-8 mg/l.(Brigman and Kunh, 1980).

2. Exponential Phase
In reactor I exponential phase happen when phenol concentration 266.58-209.64 mg/l with biomass concentration 786-1780 mg/l. Its phase take place during 6 day that's 20-26 day. In reactor II exponential phase happened when phenol concentration 226.24-202.8 mg/l with biomass concentration 826-1480 mg/l.Its phase take place during 6 day that's 20-26 day. In reactor II exponential phase happened when phenol concentration 226.24-202.8 mg/l with biomass concentration 826-1480 mg/l. Exponential phase take place during 6 day that 20-26 day. It phase occur maximally conversion of phenol by biomass this phase take place as long as good condition environment and biomass concentration constant.

3. Stationer Phase
In Reactor I stationer phase occur 6 days that 26-32. Biomass concentration range 1780-1630 mg/l with phenol concentration 209.04-42.28 mg/l. In reactor II stationer phase occur during 10 days that's 26-36 mg/l. In reactor II stationer phase occur during 10 day that's 26-36, biomass concentration1480-1420 mg/l with phenol concentration 202.8-84.56 mg/l. In this phase happened balance grown and death of biomass cell.

4.Death Phase
In reactor I death phase take place at 32-36 day, when phenol concentration 42.28 – 2.86 mg/l and biomass concentration 1630-226 mg/l. In reactor II death phase take place on 36-40 when phenol concentration 84.56-2.94 mg/l and biomass concentration 1420-224 mg/l.

Specific Growth Rate
Specific growth rate observation during six day, showing exponential phase. In Figure 4 slope linear line is specific growth rate. Specific growth rate (μ) for reactor I is 0.135/day and reactor II 0.092/day. Specific growth rate is influenced by phenol concentration.
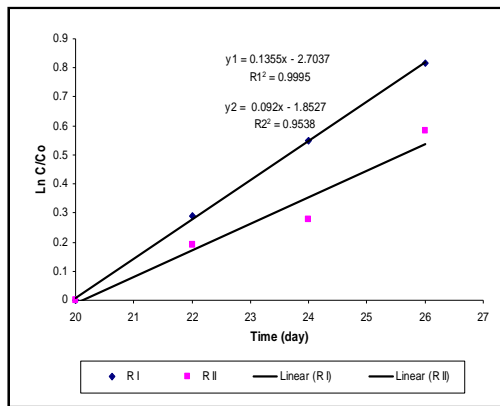
Figure 4 Determine specific growth rate (μ)
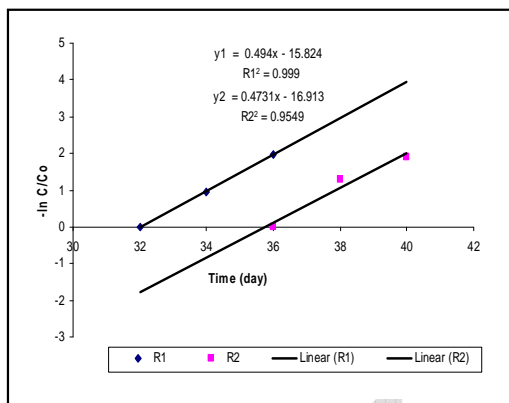
Decay Specific Rate (K_d)

Figure 5 Determine specific decay rate (k_d)

In Figure 5 slope linear line is decay specific rate. Decay specific rate observation during death phase. Decay specific rate for Reactor I is 0.494 day^{-1} and Reactor II is 0.473 day^{-1}.
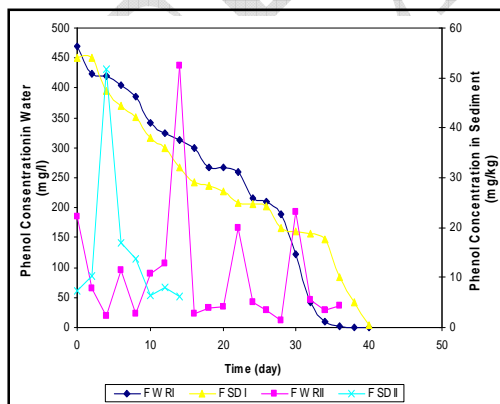
Phenol Degradation in Sediment

Figure 6 Phenol Concentration in Sediment

In Figure 6 showing phenol in water transport to sediment and adsorption by sediment. Degradation rate in Reactor I is 5.017 mg/kg. Day and Reactor II 7.557 mg/kg.day^{-1} . Factor influencing degradation phenol in sediment are precipitation in sediment, ability microorganism and environment. In Reactor

II phenol fast degradation than Reactor II because in Reactor II high precipitation. Absorption coefficient phenol by sediment and soil increase if increase organism organic matter. (Moontizaan, 1994). Partici coefficient for phenol by sediment and soil increase if increase organic matter (Moontizaan, 1994). Partici coefficient (Koc) in clay and sediment 39 and 91 dm^3/kg (Moontizaan, 1994). Phenol have night mobility in sediment because photochemical reaction and oxygen phenol also degrade become methane and CO_2. Phenol not persistent in sediment because quickly biodegradation and transport to groundwater.

Isolation and Identification Bacteria
Use scrape method Bargey's Manual
Isolation and identification to purpose bacteria dominant in sediment. Its capable to degradation of phenol. The results identification of dominant culture bacteria were *Pseudomonas putida, Pseudomonas diminuta, Bacillus sphaericus, Enterobacter sp,* and *pseudomonas sp.*

CONCLUSION

The result present in this paper demonstrate the biodegradation kinetic parameter of phenol compound in seawater and sediment.. The biomass are taken from the sea sediment in the estuary of Mahakam River. The biodegradation is observes for 40 days. The kinetic study showed that specific growth rate microorganism (μ) in sea water in the Reactor was 0.1356 to 0.092 day^{-1}, the decay specific rate in the Reactor was 0.473 to 0.494 day^{-1} and the degradation rate in the reactor was 11.19 to 11.73 mg/l.day^{-1}. The phenol degradation in sediment was 0.912 to 5.017 mg/kg.day^{-1}. The dominant bacteria in the culture are *Pseudomonas putida, Pseudomonas diminuta, Bacillus sphaericus, Enterobacter sp,* and *pseudomonas sp* is evidence use to phenol as source carbon.

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