Removing cyanide by mixed culture at liquid media with variation in pH and cyanide concentration

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Abstract. This preliminary study aims to investigate removal efficiency of cyanide by mixed culture *Thiobacillus* sp. and *Clostridium* sp. in a liquid media *stone mineral salt solution* (SMSs) with controlled condition. This research variation were pH 5, 7, and 9 and cyanide concentrate (ppm) 100, 300, and 500 within *erlenmeyer* 300 ml, for 48 hours at 30°C. In this research, cyanide artificial (KCN) was presented in liquid media. Analysis of cyanide concentration on liquid medium was done by *Gas Chromatography – Mass Spectrometer* (GC-MS) method. From the research, it was found that the cyanide removal was 50%, 55%, 53% for cyanide pH variation 5, 7, 9, respectively. In pH 7 was obtained a cyanide removal result of 55% and while for concentration (ppm) 100, 300, 500 each resulted in allowance of 45%, 57% 60%. The higher removal 60% is established at concentration of 500 ppm and pH 7. It could be concluded that mixed culture *Thiobacillus* sp. and *Clostridium* sp. could be an alternative to remove cyanide from contaminated water until concentration of 500 ppm with removal percentage of 60%.

1 Introduction

Environment defilement by cyanide compounds causing damage to our ecosystem. Source of cyanide compounds came from industrial activity, such as mining mineral, arsenic, lead, mercury, cadmium, chromium and sulfuric acid to extracting noble metal like gold ore and silver [1]. Gold ore as a result containing sulphides and cyanide in a large quantities, so the volume of cyanide waste water that generated by jewelry industry usually contain high metal concentration. Cyanide was totally toxic and dangerous for organism and environment [2]. Cyanide can have a negative impact to human's health and animal that causing death, also environment pollution. This toxic liquid residue must be minimalize to reduce the impact [3].

A cyanide is a chemical compound that contains the group C≡N. This group, consists of a carbon atom triple-bonded to a nitrogen atom. Cyanide is divided into three groups ; free cyanide associated with cyanide ion CN⁻ (produced by dissolving sodium or potassium cyanide in water) and hydrogen cyanide gas (HCN) ; cyanide ions and some metals such as Zn, Ni, Ag, Cd, Hg ; cyanide ions and Fe [4]. Cyanide removal can be done by adsorption process. The adsorption process takes place chemically using several adsorbents such as silica, manic alginates, activated carbon, polymeric materials. However, the adsorption process using *Granular Activated Carbon* (GAC) can damage the environment, highly cost and causing damage as a result from secondary pollution [5]. Bioremediation becomes one of the alternative methods to remove cyanide by utilizing

the ability of microorganism or plants to convert the toxicated cyanide compounds into simple compounds [2, 6, 7]. Cyanide is converted into a source of carbon and nitrogen by various enzymes which exist in microorganisms [8].

Bioremediation has several benefits that are able to eliminate cyanide compounds, have low operating costs, producing high quality materials, and eco-friendly processes [9]. Several types of bacteria that can exclude cyanides such as , i.e. *Klebsiella oxytoca* can remove 26%, *Bacillus* sp. can remove 65.5% [7], *Pseudomonas pseudoalcaligenes* can remove 99.9% [6], *Rhodococcus rhodochrous* can remove 47.78% [10], *Bacillus nealsonii* can remove 85.83% [11], *Thiobacillus thioparus* can remove 93% [12].

There are several factor of environmental conditions that affect the performance of microorganisms to remove cyanide, including cyanide concentrations, pH, temperature, nutrients, contact time between cyanides and microorganisms, and the presence of additional pollutants [13]. In some previous studies, researchers removed cyanide by using different bacteria with different conditions. Most of the bacteria that used was include in the class of mesophyll bacteria, whereas the bacteria can optimally grow in pH range 5,5-8,0. Cyanide concentration on cyanide removal depending on the type of bacteria that used, i.e. bacteria *Klebsiella oxytoca* at concentration of cyanide 157 ppm can remove 26%, *Rhodococcus rhodochrous* at a concentration of 150 ppm cyanide can remove 47.78% [10], *Bacillus nealsonii* at 100 ppm cyanide concentration can remove

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85.83% [11] *Thiobacillus thioautotrophicus* at concentration of 50 ppm cyanide remove 93% [12].

This research is a laboratory scale study with the objective of determining the optimum cyanide removal efficiency. The research uses mixed culture in *Thiobacillus* sp. and *Clostridium* sp. on liquid media SMSs with variation in pH and cyanide concentration.

2 Research methodology

2.1 Bacteria cultivation

Cyanide (KCN-) is used as an artificial contaminant which is subsequently put into bacteria (*Thiobacillus* sp. and *Clostridium* sp.). Medium of bacterial growth using *Stone Mineral Salt Solution* (SMSs). Batch cultures contained 80% (v/v) of SMSs, 10% (v/v) cyanide, and 10% (v/v) respectively of *Thiobacillus* sp. and *Clostridium* sp.

2.2 Bacteria growth

Population growth of *Thiobacillus* sp. and *Clostridium* sp. in mixed culture is done by using *Total Plate Count* (TPC) method on petri dish containing *Nutrient Agar* (NA).

Number of colonies per ml or per gram
= {number of colonies per cup x (1 / dilution factor)} (1)

2.3 Analysis of cyanide removal

The cyanide removal will be analyzed using *Gas Chromatography - Mass Spectrometer* (GC - MS) method. The environmental conditions to remove cyanide in a batch culture are adjusted to a temperature of 30 °C, and contact time in 48 hours. The variations of pH 5, 7, 9 and variations of cyanide concentration (ppm) 100, 300, 500 is shown in Fig. 1.

Fig. 1. Cyanide discharge test on liquid media (SMSs).

Variation of pH used in this research are 5, 7, and 9. Cyanide level will be counted using Formula 1 for each pH variation.

Formula 1 :

$$R = \frac{Au}{Ab} \times \frac{Vb}{Vu} \times \frac{Ve}{Wu} \quad (2)$$

*R: Cyanide Level (ppm), Au: Chromatogram area of the sample, Ab: Standard Chromatogram area, Cb: Standard Concentration (ng/μl), Vb: Volume of injected standard solution (μl), Vu: Volume of injected sample solution (μl), Ve: Final volume of the sample extract (μl), Wu: Sample weight (g).

3 Result and discussion

3.1 Bacterial growth on cyanide

Thiobacillus sp. and *Clostridium* sp. can growth on a medium containing cyanide with a concentration of 100 ppm, 300 ppm, 500 ppm can be seen in Figure 2.

Fig. 2. Test of bacterial growth on a medium containing 100 ppm, 300 ppm, 500 ppm cyanide within 48 hours.

In Fig. 2, it is shown that there is inhibition zone within 48 hours. This can be happened due to the condition of bacteria that can grow on toxic compounds. Thus the bacteria are suspected to be able to remove the cyanide pollutant compound. As described [14] that the diameters formed in the inhibition zone may be an indication of bacterial susceptibility to antibacterial agents. The inhibitory power of bacterial growth can be seen in the area around the paper discs that overgrown by bacteria. If inhibition zone formed around the paper discs that spilled before with various concentrations of cyanide, it indicates that the bacteria are resistant to cyanide. Conversely, if inhibition zone is seen around the paper discs, so the bacteria are sensitive to cyanide.

3.2 Cyanide removal based on pH variation

Data of cyanide removal can be seen from the result of GC-MS temporary test that shown in Table 1.

Table 1. Percentage of Cyanide Levels Removal at Variation of pH.

pH	Cyanide Removal (%)			
	X ₁	X ₂	X ₃	X _{Average}
5	55	45	50	50
7	40	65	60	55
9	52	63	44	53

In table 1, the highest removal (55%) is occurs when temperature 30°C, with pH conditions of 7 for 48 hours. The optimum pH range for the growth of several bacteria such as *Clostridium* lies between 6.5-7.5; *Klebsiella oxytoca* is 7 obtained a cyanide removal 70% and [10]

Rhodococcus rhodochrous bacteria is optimum at pH 7 obtained a cyanide removal 47,78%. Table 1 also showed that the bacteria *Thiobacillus* sp. and *Clostridium* sp. could live in large pH range nevertheless optimum bacterial cultivation conditions in the environment, will result in greater of removal.

3.3 Cyanide removal based on cyanide concentration variation

With the optimum pH condition (pH 7), then proceed the cyanide concentration variations.

Table 2. Percentage of cyanide levels removal at variation of cyanide concentration.

Concentration (ppm)	Cyanide Removal (%)			
	X ₁	X ₂	X ₃	X _{Average}
100	40	45	50	45
300	61	48	62	57
500	65	45	70	60

In Table 2, the percentage remove of cyanide removal of 60% was achieved at a concentration of 500 ppm. This is in accordance with the assertion [15] in previous studies which stated that a mixed culture capable of degrading cyanide by about 57% - 76%. [7] also said that the mixed cultures of *Bacillus safensis*, *Bacillus lichenformis*, *Bacillus tequilensis*, and *Agrowaste* were able to degrade cyanide around 59.75% -89.5% whit concentration 100 ppm, and *Pseudomonas pseudoalcaligenes* bacteria capable of degrading cyanide by 99.9% [6]. Yet, to reach greater removal the optimum bacterial cultivation conditions in environment should be maintained.

4 Conclusions

Mixed of bacteria *Thiobacillus* sp. and *Clostridium* sp. can remove cyanide level, it is proved by Gas Chromatography – Mass Spectrometer (GC – MS) in pH conditions of 7 and cyanide concentrations of 500 ppm, resulting 60% removal percentage.

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