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, manioc alginates, activated carbon, polymeric materials. However, the adsorption process using Granular Activated Carbon (GAC) can damage the environment, highly cost and causing demage 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].

Bioremedial 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 pseudalcaligenes can remove 99.9% [6], Rhodococcus rhodochrous can remove 47.78% [10], Bacillus nealsomni 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 nealsomni at 100 ppm cyanide concentration can remove
85.83% [11] *Thiobacillus thioparus* 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
\[ N = \frac{\text{number of colonies per cup} \times (1 / \text{dilution factor})}{(1)} \]

#### 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).](image)

*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:
\[ R = \frac{A_x}{B_x} \times \frac{V_b}{V_e} \times V_u \]

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

| pH  | X1   | X2   | X3   | XAverage |
|-----|------|------|------|----------|
| 5   | 55   | 45   | 50   | 50       |
| 7   | 40   | 65   | 60   | **55**   |
| 9   | 52   | 63   | 44   | 53       |

In Table 1, the highest removal (55%) 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) | X1 | X2 | X3 | XAverage |
|---------------------|----|----|----|----------|
| 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 Basillus safensis, Basillus licheniformis, 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 concentations of 500 ppm, resulting 60% removal percentage.

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