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Biodegradation of Cyanide by a Novel Cyanide-degrading Bacterium

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***Abstract*—**The objectives were to identify cyanide-degrading bacteria and study cyanide removal efficiency. *Agrobacterium tumefaciens* SUTS 1 was isolated. This is a new strain of microorganisms for cyanide degradation. The maximum growth rate of SUTS 1 obtained 4.7×10^8 CFU/ml within 4 days. The cyanide removal efficiency was studied at 25, 50, and 150 mg/L cyanide. The residual cyanide, ammonia, nitrate, nitrite, pH, and cell counts were analyzed. At 25 and 50 mg/L cyanide, SUTS 1 obtained similar removal efficiency approximately 87.50%. At 150 mg/L cyanide, SUTS 1 enhanced the cyanide removal efficiency up to 97.90%. Cell counts of SUTS 1 increased when the cyanide concentration was set at lower. The ammonia increased when the removal efficiency increased. The nitrate increased when the ammonia decreased but the nitrite did not detect in all experiments. pH values also increased when the cyanide concentrations were set at higher.

***Keywords*—**Biodegradation, Cyanide-degrading bacteria, Removal efficiency, Residual cyanide

I. Introduction

CYANIDE compounds are widely used in many industries such as mining industries, coal industries, acrylic fibers and resins production industries, plastic production industries, and cassava starch industries [1]-[5]. As a result of these industrial activities, cyanide-containing compounds and complexes are discharged into the environment as industrial wastes, for example hydrogen cyanide (HCN), thiocyanate (SCN^-), and cyanate (CNO^-). Furthermore, cyanide can be also reacted with metals and heavy metals such as copper, zinc, nickel, cadmium, or iron and formed metal-cyanide complexes [6], [7]. These complexes are usually very stable and toxic [8]. Thus, cyanide toxification depends on physical- chemical reaction and formation. Hydrogen cyanide is an extremely potent metabolic poison whereas metal-cyanide complexes vary in toxicity according to their concentration [9]. Cyanide can be entered to human body by inhalation, ingestion, and adsorption. The fatal doses for human adults are 1-3 mg/kg body weight if ingested, 100-300 mg/L if inhaled, and 100 mg/kg body weight if adsorbed [10].

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The treatment methods for cyanide in common use today are physicochemical processes, for example, copper-catalyzed chemical process or hydrogen peroxide process [11], [12]. However, these processes have high operating costs and also produce chemical waste that must be disposed of before discharge. Recently, biological processes have been studied because they are inexpensive and no effect to the environment [13]-[16]. For these reasons, the objectives in this study were to isolate and identify cyanide-degrading bacteria and to study the removal efficiency of this microorganism.

II. Materials and Methods

A. Bacterial isolation and identification

Cyanide-degrading bacterium was isolated from wastewater treatment system of cassava starch industry and purified by repeatedly transferring the cells to enrichment medium. An enrichment medium composed of $NaHPO_4$ 4.0 g, Na_2SO_4 2.13 g, K_2HPO_4 3.10 g, $MgCl_2 \cdot 6H_2O$ 200 mg, $FeCl_3 \cdot 6H_2O$ 2.0 mg, and $CaCl_2$ 1.0 mg in 1 liter of deionized water and pH was adjusted to 7.2. The medium was autoclaved for 15 min at 15 psi and 121°C before use. About 10 ml of microorganisms were inoculated into 100 ml of this medium and incubated for 7 days at 30°C on a rotary shaker (180 rpm).

In order to screen cyanide-degrading bacterium, 10 ml of microorganisms were transferred into 500 ml Erlenmeyer flask containing 100 ml of buffer medium (BM) and added 25 mg/L potassium cyanide and incubated at 30°C, 180 rpm. After 7 days, cyanide-degrading bacterial isolation was done by spreading plate technique on buffer medium containing potassium cyanide (BMK) agar (BMK and 18 g/L of Bacto agar) and incubated at 30°C for 7 days. After that the morphology and number of colonies were observed under a light microscope. Colony of bacterial morphology was analyzed using Gram stain [17] and identified by DNA sequencing using Automate DNA sequencer (3100-Avant genetic analyzer, ABI).

B. Bacterial growth analysis

The growth of isolated bacterium was studied by colony count technique. The number of viable colonies was determined daily by spreading plate technique on BMK agar containing 25 mg/L KCN. 0.1 ml of isolated bacterium in BMK was obtained from the flask and a ten-fold dilution was performed with sterile 0.85% NaCl solution. After that 0.1 ml

of each dilution was spreaded thoroughly on BMK agar. The plates were incubated at 30°C for 7 days. The plates containing 30-300 colonies were counted and used for calculation of viable cell concentration as colony forming units/ml (CFU/ml) [18].

C. Media condition

Buffer medium (BM) was used as media in this study. 1 liter of BM contained KH_2PO_4 2.7 g, K_2HPO_4 3.5 g, and 10 ml of trace salts solution ($FeSO_4$•$7H_2O$ 300 mg, $MgCl_2$•$6H_2O$ 180 mg, $Co(NO_3)_2$•$6H_2O$ 130 mg, $CaCl_2$ 40 mg, $ZnSO_4$ 40 mg, and MoO_3 20 mg in 1 liter deionized water). Final pH was adjusted 7.2. The medium was autoclaved for 15 min at 15 psi and 121°C before use. Potassium cyanide (KCN) in different concentration will be added to BM for cyanide degrading experimental study.

D. Cyanide degrading experiment

The isolated cyanide-degrading bacterium was inoculated in BM containing KCN at 25, 50, or 150 mg/L. The biodegradation of cyanide was set at 10:100 (inoculum's volume: BM's volume) in 500 ml Erlenmeyer flask and incubated at 30°C on a rotary shaker (180 rpm) for 7 and 15 days. After incubation times, bacterial growth, ammonia, nitrate, nitrite, and residual cyanide were analyzed. In addition, the abiotic experiment was performed at 50 mg/L KCN in similar BM for 15 days.

E. Analytical methods

Ammonia (NH_3), Nitrate (NO_3^-), Nitrite (NO_2^-), and residual cyanide were determined according to Standard Method [18]. The concentration of ammonia was analyzed by titrimetric method, nitrate was analyzed by brucine method, nitrite was analyzed by colorimetric method, and residual cyanide was analyzed by titrimetric method.

F. Cyanide removal efficiency calculation

The duplicate treatments were done for all. The removal efficiency (RE) of cyanide-degrading bacterium was calculated as shown in following formula.

$$RE\ (\%) = \frac{\text{Initial concentration- Residual concentration}}{\text{Initial concentration}} \times 100$$

Initial concentration = Initial concentration of cyanide (mg/L)
Residual concentration = Residual concentration of cyanide (mg/L)

III. Results

A. Isolation and identification of cyanide-degrading bacteria

Cyanide-degrading bacterium was isolated from wastewater treatment system of cassava starch industry. The external morphology was examined by using a compound microscope, and the characteristics of colonies were described in Table 1. The identification of bacterial strain was performed on the basis of its morphology by Bergey's manual [17]. After that the DNA sequencing was performed. The result showed 100% of identity was *Agrobacterium tumefaciens* SUTS 1. *Agrobacterium tumefaciens* SUTS 1 has never been reported as microorganisms capable for the removal of cyanide.

TABLE I
EXTERNAL MORPHOLOGY OF BACTERIAL COLONY

Type	Size of colony (mm)	External morphology	Gram stain	Shape
Agrobacterium tumefaciens SUTS 1	5-7.5	Circular shape, convex, smooth, nonpigmented to light beige	Negative	Rod

B. Cyanide-degrading bacterial growth

The growth of this bacterium was studied after the isolation. The growth of *Agrobacterium tumefaciens* SUTS 1 is shown in Fig. 1. The highest growth rate was obtained on day 4 of the incubation time. The colony forming units per ml during this time were approximately 10^8 cells.

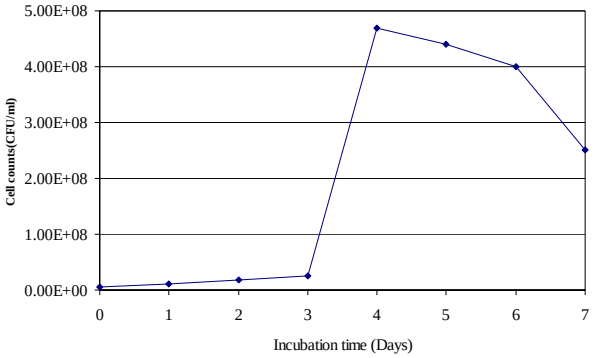

Fig. 1 Growth curve of *Agrobacterium tumefaciens* SUTS 1

C. Abiotic experiment

In the abiotic experiment, the cyanide removal efficiency was only 12.5% with 43.75 mg/L of residual cyanide after 15 days of incubation. The ammonia and nitrate concentrations were 0.14 mg/L and 1.48 mg/L, respectively whereas nitrite was not detected. pH was 7.21 throughout the experiment.

D. Biodegradation of cyanide

The cyanide removal efficiency of *Agrobacterium tumefaciens* SUTS 1 was studied at 25, 50, and 150 mg/L cyanide (Table II to IV). At 25 mg/L cyanide, the results showed that SUTS 1 obtained 75% removal efficiency with 6.25 mg/L residual cyanide within 7 days of study and the growth increased from 3.1×10^8 CFU/ml to 2.2×10^9 CFU/ml. After 15 days, the removal efficiency increased more than 87% and the cell of SUTS 1 increased to 4.1×10^9 CFU/ml. In addition, the ammonia and nitrate concentration

increased to 0.14 mg/L and to 1.58 mg/L, respectively whereas the nitrite did not detect.

TABLE II
CYANIDE DEGRADATION AT 25 mg/L OF CYANIDE CONCENTRATION

Time (Days)	RE (%)	Residual cyanide (mg/L)	NH_3 (mg/L)	NO_3^- (mg/L)	NO_2^- (mg/L)	pH	Cell counts (CFU/ml)
0	0.00	25.00	0.00	0.00	0.00	7.1	3.10E+08
7	75.00	6.25	0.14	1.49	0.00	7.2	2.20E+09
15	87.50	3.13	0.14	1.58	0.00	7.2	4.10E+09

When cyanide concentration was increased to 50 mg/L, the removal efficiency was similar to previous experiment that was 75% and 87.5% after 7 and 15 days of incubation. The growth of SUTS 1 increased from 8×10^7 CFU/ml to 2.3×10^8 CFU/ml on day 7 and to 2.1×10^9 CFU/ml on day 15. The ammonia increased from 0.14 mg/L to 0.28 mg/L whereas the nitrate decreased from 2.38 mg/L to 2.15 mg/L after 15 days. When cyanide concentration was increased to 150 mg/L, SUTS 1 exhibited more than 89% removal efficiency in only 7 days of incubation time and increased to 97.90% removal efficiency on day 15 with 3.15 mg/L. In addition, the cells of SUTS 1 decreased from 9.0×10^6 CFU/ml on day 7 to 4.5×10^6 CFU/ml on day 15 of incubation. The relationship between cyanide removal efficiency and growth of SUTS 1 in all experiment was shown in Fig. 2. The ammonia concentration increased from 0.56 mg/L to 0.70 mg/L on day 7 whereas it was not detected on day 15. On the other hand, the nitrate concentration decreased from 3.31 mg/L to 1.45 mg/L on day 7 but it increased to 7.21 mg/L on day 15. However, the nitrite was not detected from all experiment. pH was in the range of 7.10-7.21 in lower cyanide concentration and increased to 7.44 in higher cyanide concentration.

TABLE III
CYANIDE DEGRADATION AT 50 mg/L OF CYANIDE CONCENTRATION

Time (Days)	RE (%)	Residual cyanide (mg/L)	NH_3 (mg/L)	NO_3^- (mg/L)	NO_2^- (mg/L)	pH	Cell counts (CFU/ml)
0	0.00	50.00	0.00	2.42	0.00	7.19	8.00E+07
7	75.00	12.50	0.14	2.38	0.00	7.19	2.30E+08
15	87.50	6.25	0.28	2.15	0.00	7.21	2.10E+09

TABLE IV
CYANIDE DEGRADATION AT 150 mg/L OF CYANIDE CONCENTRATION

Time (Days)	RE (%)	Residual cyanide (mg/L)	NH_3 (mg/L)	NO_3^- (mg/L)	NO_2^- (mg/L)	pH	Cell counts (CFU/ml)
0	0.00	150.00	0.56	3.31	0.00	7.39	1.00E+08
7	89.97	15.05	0.70	1.45	0.00	7.38	9.00E+06
15	97.90	3.15	0.00	7.21	0.00	7.44	4.55E+06

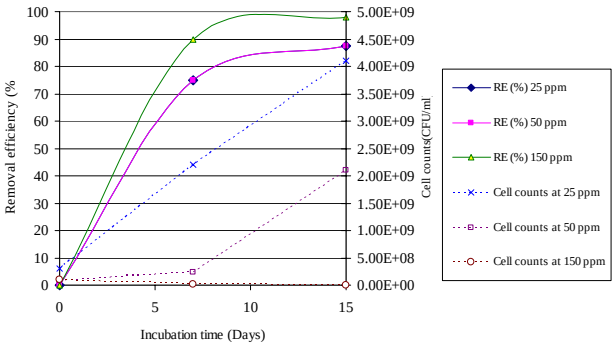

Fig. 2 The relationship between cyanide removal efficiency and growth of SUTS 1 at 25, 50 and 150 mg/L of cyanide concentration

IV. DISCUSSION

A cyanide-degrading bacterium, isolated from wastewater treatment system contaminated with cyanide was identified as *Agrobacterium tumefaciens* SUTS 1. Mostly, the genus Agrobacterium has been studied about plant biotechnology and transformation [19]-[21]. However, the cyanide degradation by this bacterium has never been reported. In addition, the studies on the biodegradation of cyanide have been reported including studies on the isolation and identification of cyanide-degrading microorganisms. The several species of genus of Pseudomonas have been studied for cyanide degradation [15], [22]-[25]. Furthermore, *Klebsiella pneumoniae*, *Moraxella*, *Serratia*, and *Alcaligenes* species were isolated and identified as cyanide-degrading bacteria [26], [27] which utilized cyanide as a source of carbon and nitrogen. Chapatwala *et al.* [28] reported that the immobilized cell can degrade the higher concentration of cyanide more than the non-immobilized cell of *Pseudomonas putida*. In contrast, the suspended cell of *Agrobacterium tumefaciens* SUTS 1 can grow in cyanide condition and also exhibited increasing removal efficiency when the cyanide concentration increased.

In this study, it is evident that *Agrobacterium tumefaciens* SUTS 1 was able to degrade cyanide to ammonia and nitrate. The ammonia revealed the increasing concentration when the cyanide removal efficiency increased. But the nitrate exhibited the increasing concentration when the ammonia concentration decreased or did not detect. It may be due to cyanide can be degraded to ammonia and converted finally to nitrate as a final by-product [23], [26], [28]-[32]. However, the others by-product such as methane, carbon dioxide, or nitrite can be occurred [33]. In addition, the nitrite was determined in this experiment, but it was not detected similar to the study of Sirianuntapiboon and Chuamkaew [34]. It may be due to the increasing rate of nitrification reaction that ammonia is oxidized rapidly to nitrate and may be due to the concentration of oxygen that oxygen is utilized in the cyanide degradation process by microorganism [28], [35].

V. Conclusion

Agrobacterium tumefaciens SUTS 1 was isolated and used for cyanide degradation. SUTS 1 exhibited more than 97% cyanide removal efficiency although the cyanide concentration was increased to 150 mg/L. The growth studied in term of colony forming units per ml (CFU/ml) of SUTS 1 was in the range of increasing that was from approximately 10^8 to 10^9 cells and 10^7 to 10^9 cells when the cyanide concentration was set at 25 mg/L and 50 mg/L, respectively. However, the cells of SUTS 1 decreased from 10^8 to 10^6 cells when the experiment was at 150 mg/L cyanide. The increasing removal efficiency yields higher ammonia and pH values. As a consequence, the nitrate increased whereas ammonia decreased or did not detect at the highest cyanide concentration due to the conversion of ammonia to nitrate.

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