Degradation of Metal Cyanide from Real Electroplating Wastewater by Mixed Culture of SUTS 1 and SUTS 2

Nootjalee Supromin[1], Siraporn Potivichayanon[2], and Rattana Toensakes[3]

***Abstract*—** The objective of this research was to investigate the capability of metal cyanide degradation by mixed culture of *Agrobacterium tumefaciens* SUTS 1 and *Pseudomonas monteilii* SUTS 2 in synthetic wastewater (copper and zinc cyanide) and electroplating wastewater (EPWW). The optimum conditions in the degradation of metal cyanide by the microbes were also investigated. It was found that the growth rate and metal cyanide degradation occurred optimally at 30:70 v/v of microbes per wastewater with 0.5 g/l of glucose as a carbon source. The rate of cyanide degradation was more than 99.99%, whereas the removal efficiency of metals (Cu and Zn) was 58.94%, 51.11%, and 44.38%, and the initial metal cyanide for each concentration of copper cyanide (CuCN) and zinc cyanide ($Zn(CN)_2$) was 5.0, 10.0, and 15.0 mg/l, respectively. Cyanide and copper could rapidly degrade in the first 24 hours, but zinc degradation slowly occurred over a period of seven days. When adding mixed cultures into EPWW to degrade the metal cyanide, it was found that the efficiency of the degradation of the cyanide and metal (Cu and Zn) reached 93.08% and 49.05% in seven days, respectively. Mixed cultures added into EPWW could promote high efficiency treatment and decrease the time for degradation, and it can effectively degrade metal cyanide into less toxic products, such as ammonia, nitrite, etc.

***Keywords*—**Mixed culture, metal cyanide degradation, optimal conditions, *Ex situ* bioremediation

I. Introduction

RAPID industrial development during recent decades has played an important role in the increase of environmental pollution. The surface water and soil contamination caused by the release of toxic chemicals can be dangerous to all classes of living organisms, if discharged without proper treatment. Cyanides are one of the most toxic and lethal chemicals. These substances are used on a large scale in the mining, electroplating, printed circuit board manufacturing, steel, and chemical industries. Consequently, these industries may discharge a large quantity of cyanide-containing liquid waste [1]. The cyanide anion can react with various metal cations to form metal-cyanide complexes [2], such as $Zn(CN)_4^{2-}$, $Cd(CN)_3^{-}$, $Ni(CN)_4^{2-}$, CuCN, and $Fe(CN)_6^{4-}$, etc. Metal complexes are usually very stable and toxic, so these must be removed from wastewater prior to their discharge into the environment [3]. The environmental impact of metal cyanides varies widely from one species to another depending on how readily natural pH. However, the contamination of the environment with cyanide primarily [4] affects human health and aquatic organisms [5], [6], [7] because cyanide and metal cyanide complexes are potent inhibitors of cellular metabolism [8]. However, cyanide can be removed and recovered or detoxified through several available methods, such as physical, chemical, and biological or biodegradation treatment methods. Although physical and chemical methods are effective removers of the compound, they have a few drawbacks over the biodegradation method. These are much more expensive, produce secondary pollution, and are suitable for low concentrations of cyanide; however, sometimes pretreatment is also required in these methods. The biological or biodegradation process is the process of breaking down and transforming hazardous materials into simple nontoxic substances through cyanide-oxidizing bacteria that break it down into harmless compounds. The bacterial detoxification would be quicker and cheaper, while providing high quality, environmentally acceptable effluents [9] compared to using chemical treatments to handle cyanide waste. To protect the environment and water bodies, effluents containing cyanide and its metals from various industries must be treated before discharging into the environment. In Thailand, the Ministry of Science, Technology and Environment proposed the industrial effluent standards for the discharge of wastewater containing cyanide and metals for the levels of cyanide (as HCN), copper (Cu), zinc (Zn), nickel (Ni), and chromium (Cr^{3+}) to be at 0.2, 2.0, 5.0, 1.0, and 0.75, respectively [10]. The contaminated metal-cyanide wastewater treatment has been studied but not thoroughly. For these reasons, the objective was to study the removal efficiency of the mixed culture to create an alternative method for treatment of metal-cyanide complex wastewater.

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II. Materials And Methods

A. Mixed Culture Inoculum

The bacteria used for cyanide degradation in this study is a mixed culture of *Agrobacterium tumefaciens* SUTS 1 and *Pseudomonas monteilii* SUTS 2, which were isolated from the wastewater treatment system of the cassava starch industry [11]. This site is contaminated with cyanide from the processing of cassava.

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B. Bacterial Growth Analysis

The mixed culture was cultivated in a buffer medium. A buffer medium was comprised per liter of KH_2PO_4 2.7 g, K_2HPO_4 3.5 g, 10 ml of a mineral salt solution (of the following composition: $FeSO_4{\bullet}7H_2O$ 300 mg, $MgCl_2{\bullet}6H_2O$ 180 mg, $Co(NO_3)_2{\bullet}6H_2O$ 130 mg, $CaCl_2$ 40 mg, $ZnSO_4$ 40 mg, and MoO_3 20 mg in 1 liter deionized water) and contained metal cyanide in the form of 0.5 mg/l of $Zn(CN)_2$ and 0.5 mg/l of CuCN. The final pH of the medium was adjusted to 7.2. The inoculum of 10 ml of mixed culture was added into an Erlenmeyer flask containing 90 ml of buffer medium and was incubated at room temperature, at 150 rpm on a rotary shaker. The growth of the mixed culture was studied by the colony counting technique on the buffer medium agar and incubated at 30°C for seven days.

C. Synthetic Wastewater and Electroplating Wastewater

Synthetic wastewater was prepared by adding metal cyanide in the form of $Zn(CN)_2$ and CuCN, which are commonly found in wastewater from electroplating plants. The $Zn(CN)_2$ and CuCN varied, and the concentrate was prepared separately from the buffer medium and autoclaved for 15 min at 15 psi at 121°C before use. The EPWW was collected from an electroplating factory in Nakhon Ratchasima, Thailand.

D. Experimental Design

The degradation of metal cyanide was set in a 500 ml Erlenmeyer flask and incubated at room temperature, at 150 rpm for seven days. In Experiment 1 and 2, 10 mg/l of $Zn(CN)_2$ and 10 mg/l of CuCN were added, respectively. The experiments were divided into four steps as follows:

1. Mixed culture and wastewater ratios

The ratios of microorganisms per synthetic wastewater varied at 10:90, 20:80, 30:70, 40:60, and 50:50 v/v (inoculum volume: synthetic wastewater volume). The exponential bacterial growth or logarithmic growth phase (from bacterial growth analysis) was used for these degradation processes.

2. Optimum concentrations of glucose

The appropriate ratio of mixed cultures per synthetic wastewater from the previous study was selected and added to the experiment with various glucose concentrations at 0.5, 0.7, and 1.0 g/l.

3. Degradation of cyanide in synthetic wastewater

Microbial degradation of the initial metal cyanide concentration in the form of zinc and copper cyanide was studied at 5 mg/l, 10 mg/l, and 15 mg/l, respectively, using an appropriate ratio of mixed cultures per synthetic wastewater and the optimum glucose concentration for microbes from the previous study.

4. Degradation of cyanide in electroplating wastewater

In this process, the removal efficiency of the mixed culture of SUTS 1 and SUTS 2 was investigated in real electroplating wastewater (EPWW) using the optimum conditions.

E. Analytical Method

In all experiments, the residual cyanide (residual CN^-), thiocyanate (SCN^-), copper (Cu), zinc (Zn), bicarbonate (HCO_3^-), ammonia nitrogen (NH_3-N), nitrite (NO_2^--N) and nitrate (NO_3^--N) were determined on Day 0, 1, 3, 5, and 7 of the incubation time according to standard methods for the examination of water and wastewater [12]. The reduction of sugar was analyzed by the dinitrosalicylic acid method (DNS) [13], measuring the dissolved oxygen (DO) and pH by multi-meter. The growth of the mixed culture was studied using the colony counting technique.

III. Results And Discussions

A. Growth of Mixed Culture Bacteria

The growth of the mixed culture of SUTS 1 and SUTS 2 in noncomplex cyanide has been reported [11]. In order to study the cyanide complex degradation, the growth of this mixed culture was investigated to obtain the exponential phase of bacterial growth in the metal cyanide condition. The maximum growth curve of *Agrobacterium tumefaciens* SUTS 1, *Pseudomonas monteilii* SUTS 2, and the mixed culture are depicted in Fig. 1. The mixed culture growth indicated that the maximum growth rate was higher than the pure culture of SUTS 1 or SUTS 2 and obtained approximately $2.52{\times}10^7$ cfu/ml within only two days. Therefore, the mixed culture at the maximum growth rate was chosen to study the cyanide and metal cyanide degradation in this research.

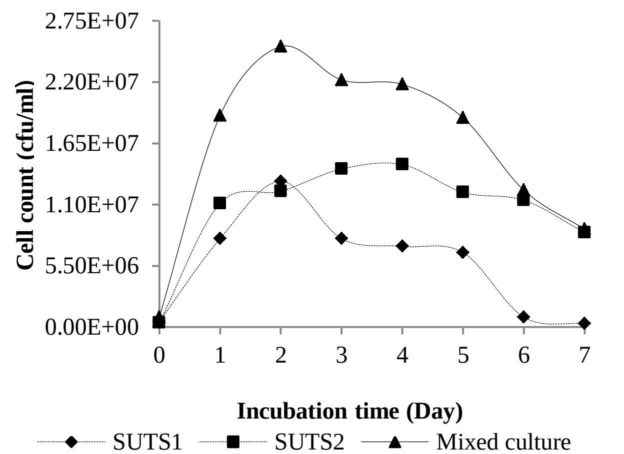

Fig. 1 Growth curve of SUTS 1, SUTS 2, and the mixed culture

B. Optimum Conditions for Metal Cyanide Degradation

The cell growth is an important factor because it is related to the ability of metal cyanide degradation. The initial cell count of the mixed culture was 7.60×10^6 cfu/ml at two days. The ratios studied were 10:90, 20:80, 30:70, 40:60, and 50:50 v/v. The DO, pH, and temperature were measured during the experiment and averaged 3.74 mg/l, 6.94, and 29.8°C, respectively. The results found that the appropriate ratio was 30:70 v/v when compared to other ratios. As shown in Fig. 2, the efficiency of the metal cyanide was degraded by the mixed culture at various ratios within seven days. Metal cyanides can be degraded suddenly by mixed culture after being added in the first time. Maximum metal cyanide removal rates were observed at three days. The mixed culture that has the highest growth was $1.16{\times}10^8$ cfu/ml obtained on Day 3 of the incubation. The cyanide was rapidly degraded by approximately 76.18% (initial concentration of cyanide was 15.28 mg/l) at Day 7, and it has capable cyanide degradation at 30 times compared to the control (no mixed culture cells). The maximum percentage of copper and zinc removal was 65.82% (initial copper and zinc concentrations were 8.05 mg/l and 5.24 mg/l, respectively). This study also found that copper was rapidly degraded by mixed culture as well as cyanide within the first 24 hours, whereas zinc degradation has occurred more slowly than cyanide and copper. Copper and zinc were degraded at 16 times when compared with the control. Therefore, the inoculum density exerted a significant effect on the biodegradation efficiency [14], whereas the first six hours of the lag phase *Rhodococcus* UKMP-5M has been reported for cyanide degradation [15]. The highest efficiency of cyanide removal occurred when microbes were in the exponential phases, whereas the removal efficiency was decreased when the microbes were in the stationary phase [8], [14]-[17]. The degradation rate of metal cyanide was associated with the cell growth phase. Therefore, the appropriate ratio between mixed cultures per synthetic wastewater was 30:70 v/v, which was used in the next step of the study.

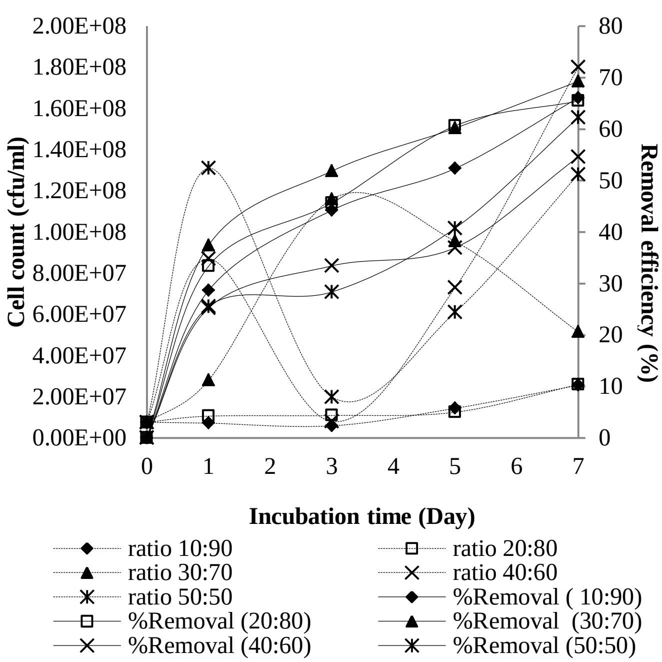

Fig. 2 Relationship between cell growth and removal efficiency of metal cyanide by mixed culture

Glucose is a carbon and energy source for many microorganisms [8], [15], [18]-19] and can be utilized easily by the mixed culture to lead to the growth and biodegradation of metal cyanide. The previous studies showed that no growth was observed in the media containing metal cyanide as the sole source of carbon and nitrogen [15], [20]. An interesting note in the previous studies was that it was observed that the experiment in which there were no added organic nutrients resulted in the decrease of metal cyanide biodegradation. However, the system efficiency could be increased by supplementation with glucose, and its efficiency was increased by increasing the glucose concentration [19]. Therefore, this study investigated the relationship between the amounts of available glucose per growth rate of mixed culture using the ratio of mixed culture per synthetic wastewater at 30:70 v/v. The medium broth was prepared by mixing copper and zinc cyanide followed by glucose in various concentrations (0.5, 0.7, and 1.0 g/l). The initial concentrations of cyanide, copper, and zinc were 15.14 mg/l, 8.25 mg/l, and 5.38 mg/l, respectively. The result showed that, at the first 24 hours, the mixed culture could be rapidly grown using glucose as a carbon and energy source with the degradation efficiency of about 69.54%, 77.22%, and 68.58%, respectively (data not shown). In addition, the maximum microbial growth rate obtained within three days was 2.50×10^{9}, 6.65×10^{9}, and 2.90×10^{9} cfu/ml from the initial cell count of 1.03×10^{7} cfu/ml. At Day 7 of incubation, the mixed culture can degrade the glucose by about 91.39%, 93.70%, and 90.71%, respectively. According to these conditions, the cyanide degradation efficiency occurred rapidly during the first three days. Complete degradation (>99.99%) of cyanide occurred within seven days, at initial glucose concentrations 0.5 and 0.7 g/l, whereas the addition of 1.0 g/l of glucose resulted in the efficiency of cyanide degradation at 93.26%. However, it has residual cyanide at 1.02 mg/l, and the result of metal removal in the form of copper and zinc was 62.49%, 57.02%, and 60.72%, respectively. It was observed that with the addition of initial glucose levels of 0.5 and 0.7 g/l, cyanide removal could be higher than that of the initial glucose at 1.0 g/l. It may be that the excessive glucose has directly affected the microorganisms regarding the metal cyanide degradation. Other studies have reported that ferrous cyanide complex removal yields decrease when the glucose concentration was more than 0.45 g/l [8]. Therefore, the initial glucose concentration of 0.5 g/l was efficient to remove metal cyanide. Furthermore, the rate of cyanide degradation was enhanced almost three times in the presence of glucose when compared to the control [21].

C. Removal Efficiency of Metal Cyanide in Wastewater

Various species of microorganisms are capable of degrading cyanide by oxidative, reductive, hydrolytic, and substitution/transfer processes [22]. Microorganisms can degrade and transform cyanide complex to less toxic products to the environment using cyanide as the sole carbon and/or nitrogen source [8]. In response to metals in the environment, microbes have evolved mechanisms of metal resistance and detoxification. Resistance mechanisms may be general or specific depending on the specific metal. However, some heavy metals are necessary for enzymatic functions and bacterial growth, and uptake mechanisms exist that allow for the entrance of metal ions into the cell, such as zinc, which is another essential trace element. It is not biologically redox reactive and is thus not used in respiration. It is, however, important in forming complexes (such as zinc fingers in DNA) and as a component in cellular enzymes [23]. Bacteria cells accumulate zinc by a fast, unspecific uptake mechanism, and it is normally found in higher concentrations (but is less toxic) than other heavy metals [23]-[24]. In addition, *Pseudomonas* sp. has toxic resistance by the accumulation of copper ions in the periplasm and the outer and inner cell walls [25]. Such mechanisms can be achieved under the appropriate conditions, such as the pH, temperature, nutrients, cell count of microorganisms, initial cyanide concentration, etc. [14], [26]. The rate increase of the initial metal cyanide concentration may result in toxicity to the bacteria cell. Therefore, the capability of the mixed culture for degradation at the various initial concentrations of copper cyanide and zinc cyanide (5, 10, and 15 mg/l) was determined for further experiments on the removal of metal cyanide in real EPWW (see Table I). The results illustrated that the mixed culture of SUTS 1 and SUTS 2 resulted in an increased cell count, with the maximum growth on Day 3, which was 1.15×10^9 cfu/ml, 2.25×10^9 cfu/ml, and 2.45×10^8 cfu/ml, respectively. Moreover, cyanide for all three concentrations was not found (removal efficiency >99.99%). In addition, it was found that cyanide is capable of rapidly degrading within the first 24 hours. The removal of metal cyanide in the form of copper and zinc cyanide was 58.94%, 51.11%, and 44.38%, respectively. Copper was more rapidly removed than zinc, which was enhanced almost 3.5, 2.4, and 2.0 times for the initial copper and zinc cyanide at 5, 10, and 15 mg/l. Therefore, at higher metal cyanide concentrations, the mixed culture growth and substrate degradation rates were decreased. This may be because higher metal cyanide concentrations have a potentially inhibitory effect on cell growth [8], [14], [27]. Each type of microorganism has a different capability for degrading metal cyanide. Sirianuntapiboon and Chuamkaew reported that cyanide removal efficiency at higher than 15 mg/l led to negative effects on the growth of bio-film and the consequent reduction in the removal efficiency of the system [28]. Instead, *E. coli* BC6 used copper cyanide as a nitrogen source limited at least 5 mg/l, as some species can be grown in conditions that have concentrations of zinc cyanide up to 100 mg/l, but it decreases the growth rate [29]. The previous study has reported that the complete degradation of metal cyanides (copper and zinc cyanide) could be obtained in 24 hours at an initial cyanide concentration of 50 mg/l [14]. Thus, this study found that the mixed culture (*Agrobacterium tumefaciens* SUTS 1 and *Pseudomonas monteilii* SUTS 2) was able to degrade metal cyanides (copper and zinc cyanide) rapidly within 24 hours (bioavailability). The addition of the mixed culture (*Ex situ* bioremediation) in EPWW enhances the effectiveness of the degradation of metal cyanide, and the duration of treatment was reduced. EPWW is contaminated with cyanide, copper, and zinc at initial concentrations of 4.48 mg/l, 3.34 mg/l, and 4.03 mg/l, respectively. There were indigenous microbes in the EPWW and the mixed culture that added an initial cell count of indigenous microbes and mixed culture of 4.80×10^4 cfu/ml and 1.23×10^7 cfu/ml, respectively. The result found that the mixed culture can be grown, and it has an increased cell count. The removal efficiency of cyanide and its metals (copper and zinc) were 93.08% and 49.05%, respectively, whereas that of the control (only indigenous microbes) were 47.32% and 33.55%, respectively (Fig. 3). It could be concluded that the efficiency of the cyanide and metal degradation was enhanced almost two times and 1.5 times in the presence of the mixed culture in EPWW, but it still has residual cyanide, copper, and zinc at 0.31 mg/l, 1.43 mg/l, and 2.38 mg/l, respectively, on Day 7 of the incubation period. An interesting result of this study was that the mixed culture was able to remove the copper better than the control experiment.

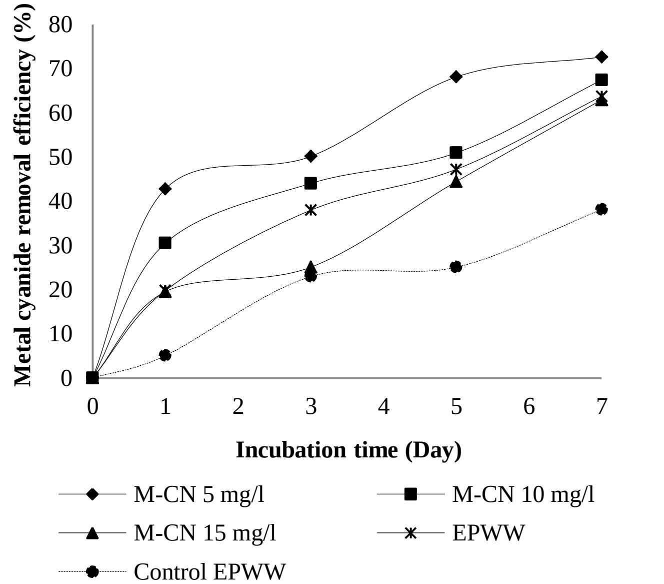

Fig. 3 Removal efficiency of metal cyanides in synthetic wastewater and EPWW

D. Relationship between Metal Cyanide Degradation with By-product after Degradation

Metal cyanide was degraded by microbes and transformed into less toxic forms, such as ammonia (NH_3), nitrite (NO_2^-), nitrate (NO_3^-), sulfate (SO_4^{2-}), bicarbonate (HCO_3^-), and thiocyanate (SCN^-) [26], [28], [30]-[33]. By-products could be easily utilized by microbes. The results found that by-product concentrations were increased when increasing the metal cyanide concentration (see Table II). Ammonia was not detected from the metal cyanide degradation at the initial 5 mg/l by the mixed culture. Ammonia was used as nitrogen source [22], [34], and it was rapidly used by the microbes [35]-[36]. In addition, with the initial concentrations of metal cyanide at 10 mg/l and 15 mg/l, the ammonia on Day 7 was 0.93 mg/l and 2.38 mg/l, respectively. Ammonia has occurred after the cyanide was degraded approximately 60%, and the ammonia has increased throughout the period of seven days. This is in accordance with other studies that reported that ammonia has increased with increases in cyanide concentration [28]. However, it is different with ammonia that occurred in the EPWW. The result indicated that the maximum ammonia that occurred on the first day of the study was 2.10 mg/l, and throughout the period of seven days, the ammonia has a tendency to decrease. When compared with the control set, it was found that ammonia was unchanged and slowly decreased after five days. The accumulation of ammonia was observed in previous studies [22], [31], [34] and the increasing of ammonia concentrations in the system has affected the decreasing cyanide degradation because the ammonia may inhibit the enzymes of the microbes in cyanide degradation [34], [37]. Therefore, the result of the studies found that the mixed culture was able to use ammonia as a nitrogen source better than the indigenous microbes, and residual ammonia in this experiment was not found because *Pseudomonas* sp. could degrade cyanide and convert it to ammonia in both aerobic and/or anaerobic conditions [38] similar to *Agrobacterium tumefaciens* SUTS 1, which was able to degrade the cyanide to ammonia and nitrate as a final by-product [11]. In addition, Holt et al. reported that *Agrobacterium* sp. could utilize ammonia salt and nitrate as the sole nitrogen sources or ammonia can be oxidized by microbes and converted to nitrite and nitrate [39]. The study found that nitrite and nitrate have increasing concentrations from the first day that were not detected. Nitrite and nitrate concentrations were increased when the metal cyanide concentration increased, whereas the ammonia obtained maximum concentrations by Day 3. After that, nitrite and nitrate have a decreasing trend until Day 7. In addition to the nitrogen form, thiocyanate could occur from the reaction between cyanide and sulfur (S^-). It is less toxic than cyanide. This study also found that thiocyanate has occurred in both synthetic wastewater and EPWW and was also used as source of carbon, nitrogen, and sulfur or it was converted to ammonia and carbon dioxide by microorganisms [2].

TABLE I
METAL CYANIDE DEGRADATION BY MIXED CULTURE IN SYNTHETIC WASTEWATER AND EPWW ON DAY 7 OF THE STUDY

Category	Metal cyanide concentration (mg/l)		Cyanide (CN^-) (mg/l)		Copper (Cu) (mg/l)		Zinc (Zn) (mg/l)		Cell count (cfu/ml)	
	CuCN	$Zn(CN)_2$	Before	After	Before	After	Before	After	Before	After
Synthetic wastewater	5	5	7.48±0.30	N.D. *	3.37±0.03	0.28	2.70±0.01	1.99±0.01	1.18E+07	7.65E+08
	10	10	14.08±0.04	N.D. *	7.54±0.17	2.10±0.02	5.22±0.02	3.65±0.01	1.18E+07	7.90E+07
	15	15	17.86±0.42	N.D. *	11.08±0.17	4.54±0.02	8.67±0.03	6.10±0.04	1.68E+07	9.50E+06
EPWW	EPWW		4.48±0.16	0.31±0.03	3.34±0.01	1.43	4.03±0.01	2.38±0.01	1.23E+07	6.95E+05
	Control		4.48±0.16	2.36±0.16	3.34±0.01	2.10±0.01	4.03±0.01	2.82±0.01	4.80E+04	1.00E+05

Remarks: N.D. = Not Detected
* Efficiency more than 99.99%

TABLE II
BY-PRODUCTS AFTER THE DEGRADATION PROCESSES BY MIXED CULTURE ON DAY 7 OF THE STUDY

Category	Metal cyanide concentration (mg/l)		Ammonia (NH_3) (mg/l)		Nitrite (NO_2^-) (mg/l)		Nitrate (NO_3^-) (mg/l)		Thiocyanate (SCN^-) (mg/l)	
	CuCN	$Zn(CN)_2$	Before	After	Before	After	Before	After	Before	After
Synthetic wastewater	5	5	N.D.	N.D.	N.D.	0.04	N.D.	0.92	N.D.	1.53
	10	10	N.D.	0.93±0.08	N.D.	0.02	N.D.	3.45	N.D.	0.84
	15	15	N.D.	2.38	N.D.	0.03	N.D.	3.08±0.01	N.D.	1.30
EPWW	EPWW		2.10±0.14	N.D.	0.03	0.02	13.37±0.04	8.54±0.04	0.10	0.05
	Control		2.10±0.14	1.45±0.08	0.03	0.04	13.37±0.04	18.44±0.04	0.10	0.06

Remarks: N.D. = Not Detected

IV. CONCLUSION

Metal cyanide contaminated in both the synthetic wastewater and EPWW was degraded by *Agrobacterium tumefaciens* SUTS 1 and *Pseudomonas monteilii* SUTS 2 (mixed culture) in optimum conditions. The ratio of microbes per wastewater was 30:70 v/v with 0.5 g/l of glucose. It enhanced the higher removal efficiency. In addition, the mixed culture can effectively degrade metal cyanide into less toxic products. However, wastewater discharge criteria for the factory situated in the industrial estate of Thailand for cyanide, copper, and zinc were not more than 0.2 mg/l, 2.0 mg/l, and 5.0 mg/l, respectively [10]. It was found that only cyanide could not pass according to the standard criterion. Therefore, the next study will investigate the optimal conditions between aerobic and anoxic conditions for metal cyanide degradation by mixed culture. These conditions are factors that may encourage a higher efficiency of degradation.

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