

***Degradation of Cyanide to Ammonia and Nitrate by Mixed Culture of
Agrobacterium Tumefaciens SUTS 1 and Pseudomonas Monteilii SUTS 2***

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Abstract

A mixed culture of bacteria capable of growth on cyanide was isolated from a wastewater stabilization pond of the cassava starch industry. The two species of bacteria found in this culture were identified as *Agrobacterium tumefaciens* SUTS 1 and *Pseudomonas monteilii* SUTS 2. The maximum growth rate of the mixed culture was 108 cells within 2 days. Cyanide degradation was studied using starting cyanide concentrations of 25, 50, and 150 mg/L. The residual cyanide, ammonia, nitrate, nitrite, pH, and cell counts were analyzed. At 25 and 50 mg/L cyanide, the mixed culture obtained a very high removal efficiency of more than 99.99%. Ammonia and nitrate were produced in the range of 0.14-0.28 mg/L and 1.71-2.69 mg/L, respectively. At 150 mg/L cyanide, the removal efficiency was lower whereas the concentrations of ammonia and nitrate increased to 1.40 mg/L and 5.21 mg/L, respectively. Nitrite was not detected in any experiment. Cell counts of the mixed culture increased when the cyanide concentration was lower but pH values increased when the cyanide concentration was higher.

Keywords: degradation, mixed culture of bacteria, removal efficiency, residual cyanide

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Introduction

The production processes of many industries such as those for petrochemicals, synthetic fuel processing, mining, coal, acrylic fibers and resin, plastic, and cassava starch use many cyanide compounds and generate high concentrations of cyanide (10-150 mg/L) and thiocyanate (50-650 mg/L) compounds (Knowles, 1976; Donberg *et al.*, 1992; Raybuck, 1992; Aronstein *et al.*, 1994; Liu *et al.*, 1996; ATSDR, 2006; Jeong, 2006). Furthermore, cyanide can also react with metals and heavy metals such as copper, zinc, nickel, cadmium, and iron to form metal-cyanide complexes (Young and Theis, 1991; Meeussen *et al.*, 1992). These complexes are usually very stable and toxic (Dzombak *et al.*, 2006). Thus, cyanide toxification depends on physical and chemical reactions and cyanide formation. Hydrogen cyanide is an extremely potent metabolic poison whereas metal-cyanide complexes vary in toxicity according to their concentration (Finnegan *et al.*, 1991). Cyanide can enter the human body by inhalation, ingestion, and adsorption. The fatal doses for human adults are 1-3 mg/kg body weight if ingested, 100-300 ppm if inhaled, and 100 mg/kg body weight if adsorbed (Huiatt, 1984). Moreover, cyanide is toxic to most species in freshwater or marine environments at a level of 0.1 mg/L at normal pH and temperature (Petrozzi and Dunn, 1994).

Treatment methods for cyanide in physicochemical and biological processes have been studied (Dumestre *et al.*, 1997; Dhillon and Shivaraman, 1999; Logsdon *et al.*, 1999; Adjei and Ohta, 2000; Botz and Mudder, 2002; Baxter and Cummings, 2006). Mostly, the use of pure cultures of microorganisms has been reported, for example, *Fusarium solani*, *Burkholderia cepacia* strain C-3 and *Pseudomonas* species. The biodegradation of cyanide and aromatic nitriles by *Agrobacterium tumefaciens* has been studied recently (Bauer *et al.*, 1998; Potivichayanon and Kitleartpornpairat, 2010). This bacterium, especially strain SUTS 1, when grown in the presence of cyanide, exhibited very high removal efficiency. It also utilized cyanide and produced ammonia and nitrate (Potivichayanon and Kitleartpornpairat, 2010) whereas strain d3 converted aromatic nitriles to amides (Bauer *et al.*, 1998). However, the addition of other bacterial strains in the experiment has to be considered because many species of bacteria are present in wastewater and may affect cyanide degradation (Kang and Park, 1997; Barclay *et al.*, 1998). Therefore, the objectives of this study were to investigate cyanide degradation by a mixed bacterial culture containing SUTS 1, to identify other cyanide-degrading bacteria and to study the production of ammonia and nitrate from cyanide degradation.

Materials and Methods

Chemicals and equipment

Sodium hydrogen phosphate (NaHPO_4), sodium sulfate (Na_2SO_4), magnesium chloride ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$), calcium chloride (CaCl_2), sodium chloride (NaCl), zinc sulfate (ZnSO_4), and molybdenum trioxide (MoO_3) were purchased from Ajax Finechem. Dipotassium hydrogen phosphate (K_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), ferrous sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), and cobalt nitrate ($\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) were purchased from Fisher Scientific. Ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) and potassium cyanide (KCN) were purchased from Merck. Bacto agar

was purchased from Difco. All chemicals used in this study were analytical reagent grade.

A rotary shaker (Excelsa E5; New Brunswick Scientific), an autoclave (TKA steroclave; Teknolabo), a light microscope (Olympus BX51; Olympus), an incubator (Mettler BE 500; Mettler), and an automated DNA sequencer (3100-Avant Genetic Analyzer; ABI) were used in the experiment.

Mixed culture of microorganisms and cultivation

Microorganisms were obtained by repeated grab sampling from a stabilization pond of a cassava starch industry in Nakhon Ratchasima, Thailand. In order to enrich the mixed culture of microorganisms, 10 mL of sample were inoculated into a 500 mL Erlenmeyer flask containing 100 mL of enrichment medium and incubated for 7 days at 30°C on a rotary shaker (180 rpm) (Potivichayanon and Kitleartpornpairat, 2010). The experiment was done in duplicate. An enrichment medium composed of 4.0 g NaHPO₄, 2.13 g Na₂SO₄, 3.10 g K₂HPO₄, 200 mg MgCl₂•6H₂O, 2.0 mg FeCl₃•6H₂O, and 1.0 mg CaCl₂ in 1 L of de-ionized water at pH 7.2 was used. The medium was autoclaved for 15 min at 15 psi and 121°C before use.

Isolation and identification of cyanide-degrading bacteria

In order to screen cyanide-degrading bacteria from the mixed culture, 10 mL of the mixed culture in an enrichment medium flask was used for each Erlenmeyer flask containing 100 mL of buffer medium (BM) and 25 mg/L potassium cyanide was added and the mixture was incubated at 30°C, 180 rpm for 7 days. After that, cyanide-degrading bacterial isolation was performed using the spreading plate technique on a buffer medium containing potassium cyanide (BMK) agar (BMK with 18 g/L of Bacto agar) which was incubated at 30°C for 7 days. Following this, the morphology and number of colonies were observed under a light microscope. The morphology of the bacterial colony was analyzed using Gram staining (Bergey and John, 1994). In addition, bacterial cells were identified by DNA sequencing using an automated DNA sequencer (3100-Avant Genetic Analyzer, ABI).

Mixed culture growth analysis

The growth of the mixed culture was studied using the colony counting technique. The number of viable colonies was determined daily by the spreading plate technique on BMK agar containing 25 mg/L KCN. From the flask containing the mixed culture in BMK, 0.1 mL was obtained and diluted ten-fold with sterile 0.85% NaCl solution. Then, 0.1 mL of this diluted mixture was thoroughly spread onto BMK agar plates. The plates were incubated at 30°C for 7 days. The viable colonies on plates were then counted and those containing 30-300 colonies were used to calculate the viable cell concentration as colony forming units/milliliter (CFU/mL) (APHA, AWWA, WEF, 1995). In addition to the mixed culture growth analysis, the pure culture growth of the isolated bacteria was studied in the same method of mixed culture growth analysis (Potivichayanon and Kitleartpornpairat, 2010).

Media condition

Buffer medium (BM) was used as the medium (Potivichayanon and Kitleartpornpaioat, 2010): 1 L of BM contained 2.7 g KH_2PO_4 , 3.5 g K_2HPO_4 and 10 mL of trace salts solution (300 mg $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 180 mg $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 130 mg $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, 40 mg CaCl_2 , 40 mg ZnSO_4 and 20 mg MoO_3 in 1 L de-ionized water). The final pH was adjusted to pH 7.2. The medium was autoclaved for 15 min at 15 psi and 121°C before use. Different concentrations of potassium cyanide (KCN) were added to the BM for the cyanide degrading experiment.

Degradation of cyanide

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

Analytical methods

In all experiments, ammonia (NH_3), nitrate (NO_3^-), nitrite (NO_2^-), and residual cyanide were determined according to standard methods (APHA, AWWA, WEF, 1995). The concentration of ammonia was analyzed by distillation and the titrimetric method (APHA, AWWA, WEF, 1995), nitrate was analyzed by the brucine method (APHA, AWWA, WEF, 1998), nitrite was analyzed by the colorimetric method (APHA, AWWA, WEF, 1995), and residual cyanide was analyzed by the titrimetric method (APHA, AWWA, WEF, 1995).

Cyanide removal efficiency calculation

All experiments were performed in duplicate. The removal efficiency (RE) of the mixed culture was calculated as shown in the following formula:

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

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

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

Results and Discussion

Isolation and identification of single strains from a mixed culture

In order to isolate and identify single strains of bacteria from a mixed culture, the external morphology was examined using a compound microscope. The distinguishable colonies showed that there were two different dominant types of microorganisms. The colony of the first type was circular in shape, convex, smooth and non-pigmented to light beige. The second colony type was circular in shape, convex, smooth, white and opaque. Both types represented Gram negative stains with

a rod shape. The colony sizes of the first and second types were 5 to 7.5 mm and 1 to 2 mm, respectively. Identification of the bacterial strains was performed on the basis of morphology using Bergey's manual (Bergey and John, 1994). Following this, DNA sequences were analyzed at the Mahidol University-Osaka University Collaborative Research Center for Bioscience and Biotechnology (MU-OU: CRC). The nucleotide base sequence of the gene which codes for 16S ribosomal RNA was compared with GenBank's databases (Subject or Reference gene) of various gene sequences (Benson *et al.*, 2011). The results of the first and second types showed a 100% identity of *Agrobacterium tumefaciens* SUTS 1 (Potivichayanon and Kitleartpornpaioat, 2010) and *Pseudomonas monteilii* SUTS 2 (Figure 1 and Figure 2), respectively. Therefore, the mixed culture contained two species of bacteria capable of growing in the presence of cyanide. Several species of the *Pseudomonas* genus have previously been studied for cyanide degradation (Harris and Knowles, 1983; Dorr and Knowles, 1989; Watanabe *et al.*, 1998; Dhillon and Shivaraman, 1999; Cipollone *et al.*, 2004); however, there have been no reports on *P. monteilii*. Furthermore, the mixed culture of *P. monteilii* SUTS 2 with the high cyanide removal potential of *Agrobacterium tumefaciens* SUTS 1 may increase the overall removal efficiency.

Score = 1181 bits (639), Expect = 0.0
 Identities = 639/639 (100%), Gaps = 0/639 (0%)
 Strand=Plus/Plus

Query	1	GGCAGACGGGTGAGTAACGCGTGGGAACATACCCTTTCCTGCGGAATAGCTCCGGGAAAC	60
Sbjct	39	GGCAGACGGGTGAGTAACGCGTGGGAACATACCCTTTCCTGCGGAATAGCTCCGGGAAAC	98
Query	61	TGGAATTAATACCGCATACGCCCTACGGGGAAAGATTTATCGGGGAAGGATTGGCCCGC	120
Sbjct	99	TGGAATTAATACCGCATACGCCCTACGGGGAAAGATTTATCGGGGAAGGATTGGCCCGC	158
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Sbjct	159	GTTGGATTAGCTAGTTGGTGGGGTAAAGGCCCTACCAAGGCGACGATCCATAGCTGGTCTG	218
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Sbjct	339	CCTTAGGGTTGTAAAGCTCTTTCACCGATGAAGATAATGACGGTAGTCGGAGAAGAAGCC	398
Query	361	CCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTTCGGAATT	420
Sbjct	399	CCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTTCGGAATT	458
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Sbjct	519	CTGCGGAAGTGCCTTTGATACTGGGTATCTTGAGTATGGAAGAGGTAAGTGGAAATCCGA	578
Query	541	GTGTAGAGGTGAAATTCGTAGATATTCGGAGGAACACCAAGTGGCGAAGGCGGCTTACTGG	600
Sbjct	579	GTGTAGAGGTGAAATTCGTAGATATTCGGAGGAACACCAAGTGGCGAAGGCGGCTTACTGG	638
Query	601	TCCATTACTGACGCTGAGGTGCGAAAGCGTGGGGAGCAA	639
Sbjct	639	TCCATTACTGACGCTGAGGTGCGAAAGCGTGGGGAGCAA	677

Figure 1. The comparison between nucleotide base sequences of bacterial gene of *Agrobacterium tumefaciens* SUTS 1 (Query or Unknown gene) and gene sequences of GenBank's databases (Sbjct or Reference gene).

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Score = 1168 bits (632), Expect = 0.0
Identities = 632/632 (100%), Gaps = 0/632 (0%)
Strand=Plus/Plus

Query 1 CTTGCTCCTTGATTTCAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGG 60
      |||
Sbjct 40 CTTGCTCCTTGATTTCAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGG 99

Query 61 GGGACAACGTTTCGAAAGGAACGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGA 120
      |||
Sbjct 100 GGGACAACGTTTCGAAAGGAACGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGA 159

Query 121 CCTTCGGGCCTTGCGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGAGGTAATG 180
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Sbjct 160 CCTTCGGGCCTTGCGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGAGGTAATG 219

Query 181 GCTCACCAAGGCGACGATCCGTAAGTGGTCTGAGAGGATGATCAGTCACACTGGAACCTGA 240
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Sbjct 220 GCTCACCAAGGCGACGATCCGTAAGTGGTCTGAGAGGATGATCAGTCACACTGGAACCTGA 279

Query 241 GACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGC 300
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Query 301 CTGATCCAGCCATGCCCGGTGTGTGAAGAAGTCTTCGGATTGTAAAGCACTTTAAGTTG 360
      |||
Sbjct 340 CTGATCCAGCCATGCCCGGTGTGTGAAGAAGTCTTCGGATTGTAAAGCACTTTAAGTTG 399

Query 361 GGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTGACGTTACCGACAGAATAAGCACCG 420
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Sbjct 400 GGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTGACGTTACCGACAGAATAAGCACCG 459

Query 421 GCTAACTCTGTGCCAGCAGCCGCGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACT 480
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Sbjct 580 GGGAAGTGCATCCAAAAGTGGCGAGCTAGAGTACGGTAGAGGGTGGTGGAAATTCCTGTG 639

Query 601 TAGCGGTGAAATGCGTAGATATAGGAAGGAAC 632
      |||
Sbjct 640 TAGCGGTGAAATGCGTAGATATAGGAAGGAAC 671

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Figure 2. The comparison between nucleotide base sequences of bacterial gene of *Pseudomonas monteilii* SUTS 2 (Query or unknown gene) and gene sequences of GenBank's databases (Sbjct or reference gene).

Mixed culture bacterial growth

The growth of the mixed culture of SUTS 1 and SUTS 2 was studied (Figure 3). The highest growth rate was obtained on day 2 of the incubation period. The colony forming units per mL during this time was approximately 5.30×10^8 CFU/mL. In the previous study, the growth of SUTS 1 was reported (Potivichayanon and Kitleartpornpairat, 2010). It showed the highest growth on day 4 of the incubation period that was 4.70×10^8 CFU/mL. In further experiment, SUTS 2 was studied and showed highest on day 3 of the incubation period obtained 2.00×10^8 CFU/mL (Figure 3). Therefore, the mixed culture of SUTS 1 and SUTS 2 was higher cell growth than the pure culture of SUTS 1 and also the pure culture of SUTS 2. For these reason, the mixed culture was chosen to study the cyanide degradation in this research.

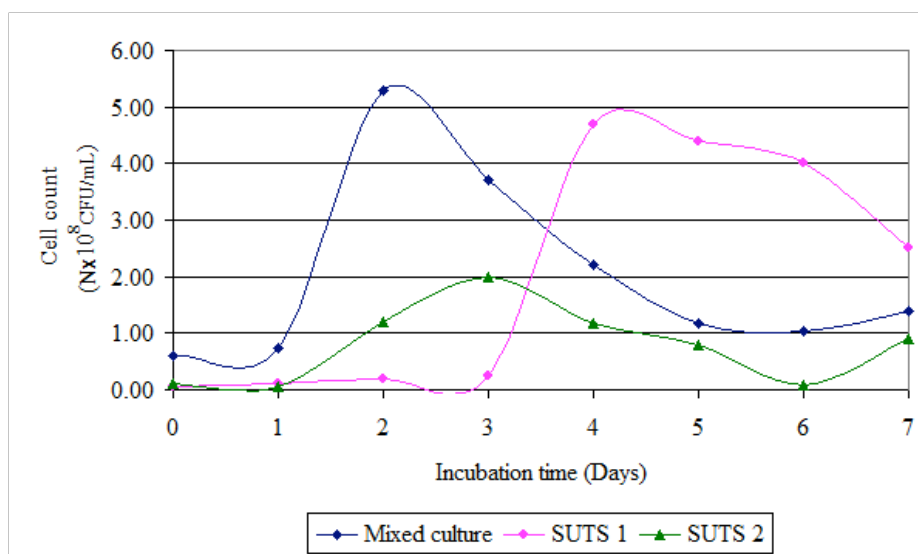


Figure 3. Growth curve of the mixed and pure culture of *Agrobacterium tumefaciens* SUTS 1 and *Pseudomonas monteilii* SUTS 2.

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. The pH was 7.21 throughout the experiment.

Degradation of cyanide to ammonia and nitrate

The degradation of cyanide to ammonia and nitrate by the mixed culture of *Agrobacterium tumefaciens* SUTS 1 and *Pseudomonas monteilii* SUTS 2 was studied using starting concentrations of 25, 50, and 150 mg/L cyanide (Tables 1). The relationship between cyanide removal efficiency and the growth of mixed culture in all experiments is shown in Figure 4. At 25 mg/L cyanide, the results showed that the mixed culture of SUTS 1 and SUTS 2 obtained a 75% removal efficiency with 6.25 mg/L residual cyanide left within 7 days of study and the growth rate increased from 4.70×10^8 CFU/mL to 4.10×10^9 CFU/mL. After 15 days, residual cyanide was not detected; therefore, a high removal efficiency of 99.99% had been obtained. From these results, the mixed culture of SUTS 1 and SUTS 2 showed the higher efficiency than the pure culture SUTS 1 that obtained only 87.50% removal efficiency (Potivichayanon and Kitleartpornpairat, 2010), similar to mixed culture of two *Pseudomonas* species that presented the complete degradation of cyanide and phenol within 40 h in the batch culture (Kang and Park, 1997). However, cells in the mixed culture decreased to 3.80×10^9 CFU/mL. In addition, the ammonia and nitrate concentrations increased to 0.16 mg/L and 1.98 mg/L, respectively, whereas nitrite was not detected. The pH was approximately pH 7.21-7.22.

Table 1. Cyanide degradation at a concentration of 25, 50 and 150 mg/L cyanide.

Time (Days)	Residual cyanide (mg/L)			NH ₃ (mg/L)			NO ₃ ⁻ (mg/L)			pH		
	25 mg/ L	50 mg/ L	150 mg/L	25 mg/ L	50 mg/ L	150 mg/ L	25 mg/ L	50 mg/ L	150 mg/ L	25 mg/ L	50 mg/ L	150 mg/ L
0	25.0 0	50.0 0	150.0 0	0.00	0.28	0.70	0.00	1.98	3.00	7.22	7.16	7.38
7	6.25	12.5 0	36.05	0.14	0.00	1.12	1.71	2.69	3.31	7.22	7.24	7.38
15	0.00	0.00	34.00	0.16	0.28	1.40	1.98	2.36	5.21	7.21	7.18	7.43

At 50 mg/L cyanide, the removal efficiency was similar to the previous experiment with 25 mg/L and 75% removal efficiency, and both showed a removal efficiency of more than 99.99% after 15 days of incubation. As similar result from previous experiment, the mixed culture showed the higher efficiency than the pure culture of SUTS 1 that obtained only 87.50% (Potivichayanon and Kitleartpornpairat, 2010). The growth of the mixed culture increased from 1.00×10^8 CFU/mL to 2.50×10^9 CFU/mL on day 7 but it decreased to 4.10×10^8 CFU/mL on day 15. At 50 mg/L cyanide, no ammonia was detected on day 7 whereas the nitrate had increased from 1.98 mg/L on day 0 to 2.69 mg/L on day 7. After 15 days, the ammonia had increased to 0.28 mg/L whereas nitrate levels showed a slight decrease to 2.36 mg/L. The levels of ammonia increased with the increasing concentration of cyanide as cyanide removal efficiency increased. In addition, nitrate concentration was detected at increasing value where the ammonia concentration decreased or was not detected. This may have been because the cyanide was degraded to ammonia and then converted to nitrate as the final by-product (Dorr and Knowles, 1989; Ingvorsen *et al.*, 1991; Meyers *et al.*, 1991; Petrozzi and Dunn, 1994; Chapatwala *et al.*, 1998; Watanabe *et al.*, 1998; Kao *et al.*, 2003). Other by-products such as methane, carbon dioxide and nitrite can also occur (Chapatwala *et al.*, 1998; Kao *et al.*, 2003; Ebbs, 2004), although no nitrite was detected and methane and carbon dioxide were not investigated in the present study.

Most organisms capable of biodegrading cyanide may be sensitive to the concentration of cyanide, with biodegradation and/or the growth rate decreasing above specific thresholds for each organism (Raybuck, 1992). From this reason, the cyanide concentration was set high and increased to 150 mg/L. The mixed culture of SUTS 1 and SUTS 2 exhibited more than 75% removal efficiency after only 7 days incubation, which increased to 77.33% removal efficiency after day 15 with 34 mg/L of residual cyanide. In addition, the cells of SUTS 1 and SUTS 2 decreased from 3.00×10^8 CFU/mL to 1.40×10^7 CFU/mL on day 7 but they slightly increased to 2.00×10^7 CFU/mL on day 15 of incubation. This may be due to the microorganisms trying to adapt to a higher cyanide concentration in the same way as a commercially marketed strain of *Fusarium* can treat and tolerate cyanide concentrations of up to 100 mg/L (Ebbs, 2004; Sirianuntapiboon and Chuamkaew, 2007). The ammonia concentration increased from 0.70 mg/L to 1.12 mg/L on day 7 and to 1.40 mg/L on

day 15. In addition, nitrate was also in the range of increased values from 3.00 to 5.21 mg/L by the end of the experiment. From the results, when the cyanide concentration was increased, the concentration of ammonia and nitrate increased but the removal efficiency decreased. Furthermore, the formation of degradation products, such as ammonia or nitrate, may also limit the efficacy of microorganisms (Ebbs, 2004), as shown in the present experiment. When higher values of ammonia and nitrate were detected, the removal efficiency of the mixed culture was limited to only 77.33%. This might have been due to the high concentration of ammonia and nitrate, which may have affected the ability of the mixed culture to further degrade cyanide. However, *Agrobacterium tumefaciens* SUTS 1 and *Pseudomonas monteilii* SUTS 2 may be capable of respiration in the presence of nitrate and use nitrate as an alternate electron acceptor for their growth (Bergey and John, 1994). Therefore, the cell numbers of SUTS 1 and SUTS 2 still increased in this study. In addition, nitrite was not detected in any of the experiments, similar to the results of Sirianuntapiboon and Chuamkaew (2007). This may have been due to the increasing rate of nitrification reaction whereby ammonia is rapidly oxidized to nitrate, and it may also have been due to the concentration of oxygen, since oxygen is utilized in the cyanide degradation process by microorganisms (Chapatwala *et al.*, 1998; Tchobanoglous *et al.*, 2004). The pH values may have also limited the removal efficiency and cell growth (Ebbs, 2004). When pH was detected around 7.16-7.24, the removal efficiency and the cells of the mixed culture exhibited very high values. On the other hand, when the pH increased to 7.43, cell growth and removal efficiency exhibited were at lower levels. Previous studies have shown that the pH should be in the range of 6 to 9 (Baxter and Cummings, 2006).

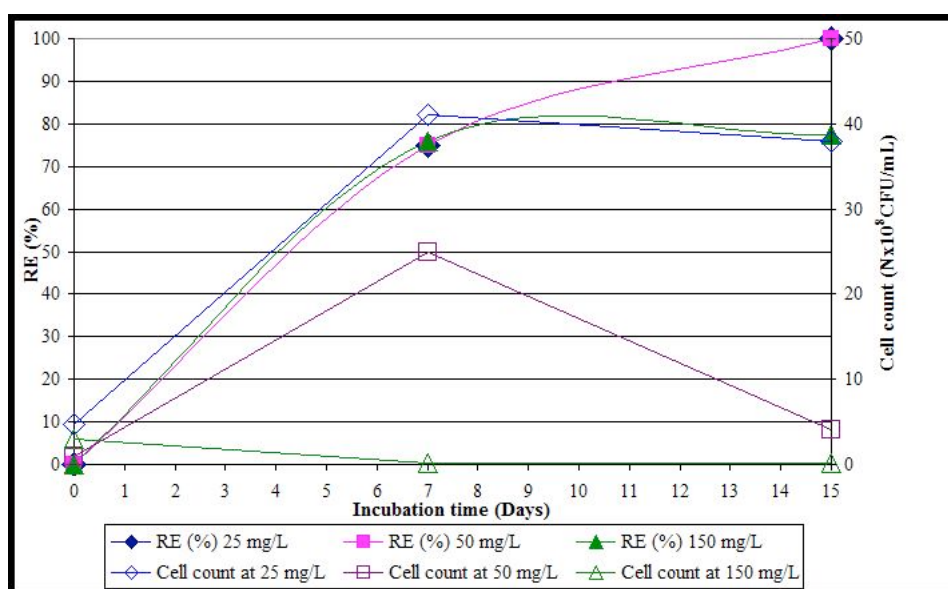


Figure 4. The relationship between cyanide removal efficiency and growth of the mixed culture of SUTS 1 and SUTS 2 at 25, 50 and 150 mg/L cyanide.

Therefore, *Agrobacterium tumefaciens* SUTS 1 and *Pseudomonas monteilii* SUTS 2 were able to degrade cyanide to ammonia and nitrate. Similar to other species, such as mixed culture of *Klebsiella pneumoniae*, *Moraxella*, *Serratia*, and *Pseudomonas*

species and pure culture of *Alcaligenes* species, cyanide is used as a source of carbon and nitrogen (Ingvorsen *et al.*, 1991; Kang and Kim, 1993). Moreover, the rate of cyanide degradation also increased due to mixing of bacterial culture (Kang and Park, 1997). Chapatwala *et al.* (1998) reported that immobilized cells of *Pseudomonas putida* could degrade higher concentrations of cyanide than non-immobilized cells. In contrast, the suspended cells in the mixed culture of *Agrobacterium tumefaciens* SUTS 1 and *Pseudomonas monteilii* SUTS 2 grew in cyanide conditions and also exhibited very high cyanide removal efficiency.

Conclusions

A mixed culture isolated from a wastewater treatment system contaminated with cyanide was identified as *Agrobacterium tumefaciens* SUTS 1 and *Pseudomonas monteilii* SUTS 2. The cyanide degradation by *Agrobacterium tumefaciens* SUTS 1 was recently reported. The pure culture of SUTS 1 exhibited more than 97% cyanide removal efficiency when the cyanide concentration was increased to 150 mg/L. The mixed culture of SUTS 1 and SUTS 2 exhibited the highest removal efficiency of more than 99.99% with no detection of residual cyanide. In addition, the cells of two bacteria grew with the ability to utilize cyanide and produced ammonia and also nitrate. Future work of this study will focus on the capability of the mixed culture of SUTS 1 and SUTS 2 in more technologically advanced wastewater treatment systems.

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