

Growth and Cyanide Degradation of *Azotobacter vinelandii* in Cyanide-Containing Wastewater System[§]

Koksunan, Sarawut¹, Sukanda Vichitphan¹, Lakkana Laopaiboon¹, Kanit Vichitphan^{1*}, and Jaehong Han^{2*}

¹Department of Biotechnology, Fermentation Research Center for Value Added Agricultural Products (FerVAAP), Khon Kaen University, Khon Kaen 40002, Thailand

²Metalloenzyme Research Group and Department of Biotechnology, Chung-Ang University, Anseong 456-756, Korea

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Azotobacter vinelandii, a strict aerobic nitrogen-fixing bacterium, has been extensively studied with regard to the ability of N₂-fixation due to its high expression of nitrogenase and fast growth. Because nitrogenase can also reduce cyanide to ammonia and methane, cyanide degradation by *A. vinelandii* has been studied for the application in the bioremediation of cyanide-contaminated wastewater. Cyanide degradation by *A. vinelandii* in NFS (nitrogen-free sucrose) medium was examined in terms of cell growth and cyanide reduction, and the results were applied for cyanide-contaminated cassava mill wastewater. From the NFS medium study in the 300 ml flask, it was found that *A. vinelandii* in the early stationary growth phase could reduce cyanide more rapidly than the cells in the exponential growth phase, and 84.4% of cyanide was degraded in 66 h incubation upon addition of 3.0 mM of NaCN. The resting cells of *A. vinelandii* could also reduce cyanide concentration by 90.4% with 3.0 mM of NaCN in the large-scale (3 L) fermentation with the same incubation time. Finally, the optimized conditions were applied to the cassava mill wastewater bioremediation, and *A. vinelandii* was able to reduce the cyanide concentration by 69.7% after 66 h in the cassava mill wastewater containing 4.0 mM of NaCN in the 3 L fermenter. Related to cyanide degradation in the cassava mill wastewater, nitrogenase was the responsible enzyme, which was confirmed by methane production. These findings would be helpful to

design a practical bioremediation system for the treatment of cyanide-contaminated wastewater.

Key words: *Azotobacter vinelandii*, bioremediation, cyanide, nitrogenase, wastewater

Cyanide is highly toxic to living organisms and particularly inactivates the respiration system by tight binding to cytochrome *c* oxidase. Cyanide is used in many industries such as chemical synthesis, pharmaceuticals, coal gasification, electroplating, plastics processing, gold and silver extraction, ore leaching, tanning, metallurgy, and agricultural chemistry [18]. Food processing industries handling crops like cassava and bitter almond also generate considerable quantities of cyanide waste from the cyanogenic glucosides in the plant material [1]. Because acute exposure to cyanide can cause death, and chronic exposure to even a low concentration can damage the thyroid and neuron system, the Environmental Protection Agency regulates its maximum tolerance level below 0.2 mg/l, and the Korean National Institute of Environmental Research requires a cyanide detection limit of 0.01 mg/l in drinking water.

Cyanide ion (CN⁻) and hydrogen cyanide (HCN) are often referred to as free cyanide. The relative amounts of both forms of free cyanide are mainly controlled by pH. The acid dissociation constant of HCN is 9.2 and most free cyanide in natural waters, which have a pH ranging between 6.0 and 8.5, is present as HCN. The percentage of HCN continues to increase as the pH drops, and at pH 7.0 about 99.5% of the cyanide exists as HCN, which is also highly soluble in water.

Currently, cyanide-containing wastewater is generally treated by chemical oxidation methods [17]. However, these methods are expensive, and hazardous chemicals of chlorine gas, permanganate, and ozone are used as the

*Corresponding authors

K.V.

Phone: +66 43 362 121; Fax: +66 43 362 121;

E-mail: kanvic@kku.ac.th

J.H.

Phone: +82 31 670 4830; Fax: +82 31 675 1381;

E-mail: jaehongh@cau.ac.kr

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reagents. Alternatively, bioremediation using microorganisms would be an environmentally preferred method for cyanide removal, compared with other techniques currently in use [3].

Azotobacter vinelandii is a widespread Gram-negative, strict-aerobic, and free-living bacterium that fixes nitrogen through the action of nitrogenase [14]. This organism has evolved a number of physiological mechanisms to allow it to fix nitrogen aerobically, despite the inherent oxygen sensitivity of nitrogenase enzyme. The biochemical study of nitrogen fixation by *A. vinelandii* is presented in many literatures [4, 9], because *A. vinelandii* grows very fast aerobically and produces a large quantity of nitrogenase under diazotrophic conditions. Nitrogenase is a robust enzyme that can also reduce many small molecules, including H^+ , acetylene, HCN, SCN^- , and N_3^- [4]. *Azotobacter* species have been used for biological treatments of olive-oil wastewater [6] and pulp-paper industry wastewater [8]. However, there are few studies of *A. vinelandii* for the degradation of cyanide from contaminated wastewater [10].

While we were working on the nitrogenase mechanism [12, 15], we noticed that no basic study on cyanide degradation by *A. vinelandii* has been done in the aspect of microbial biotechnology. Here we present cyanide degradation by *A. vinelandii* in NFS (nitrogen-free sucrose) medium in terms of cell growth and cyanide reduction. We also present a possible application in the bioremediation of cyanide-contaminated wastewater by means of the cassava-mill-wastewater model system.

MATERIALS AND METHODS

Microorganism

A. vinelandii was supplied by Microbiological Resources Centre (MIRCEN) of Thailand Institute of Scientific and Technological Research (TISTR) (Bangkok, Thailand). The microorganism from stock culture was streaked to activate on slants of NFS agar medium, and incubated aerobically at 30°C for 2 days before use.

Growth Medium and Inoculum Preparation

An NFS medium consisting of 2.0% sucrose, 0.2 g/l $MgSO_4 \cdot 7H_2O$, 0.073 g/l $CaCl_2 \cdot 2H_2O$, 10 mM $Na_2MoO_4 \cdot 2H_2O$, and 20 μM $FeCl_3 \cdot 6H_2O$ in pH 7.4 phosphate buffer (2.0 mM) was used in the experiments [16]. When it was necessary, 20 mM of urea was added as well. *A. vinelandii* grown on the slant was transferred in a cotton-wool-plugged 500 ml Erlenmeyer flask containing 300 ml of NFS medium. The culture was grown in a shaking incubator (New Brunswick Scientific, CT, USA) with 200 rpm at 30°C for 30 h, and the culture was used as a starter for cyanide biodegradation experiments.

Cell Growth Determination

Cell growth was determined by two different methods. The total cell count (direct microscopic cell counts) was determined using a hemacytometer (HBG Henneberg-Sander GmbH, Germany). Samples were centrifuged at 3,000 $\times g$ for 5 min and then washed twice with 2 mM phosphate buffer (pH 7.4). Appropriated dilutions were used

for the total cell count. Alternatively, the viable cell count was adopted by using the plate count technique. An appropriate dilution (0.1 ml of samples) was spread on NFS agar plates. The plates were incubated at 30°C for 48 h, and colony counts were enumerated for viable organisms. All the measurements were duplicated and the average values were shown in the data.

Cyanide Degradation by Different Growth Phases of *A. vinelandii*

After obtaining the growth curve, cell culture was harvested by centrifuging at 7,800 $\times g$ and 4°C for 5 min, when the cells were grown to the mid-exponential (24 h) or the early-stationary phase (30 h). The supernatants were discarded and cell pellets were resuspended with NFS medium by adjusting the absorbance to 1.0 at 600 nm. NaCN was then added to the cell suspension to give the desired final concentrations. The cell suspensions were then incubated on a shaking incubator with 200 rpm at 30°C. NFS medium with the same concentrations of NaCN without *A. vinelandii* was also measured for the cyanide reduction and used as a control.

Cyanide Analysis

Cyanide was assayed by a modified ninhydrin method described by Drochioiu [5]. The sample was centrifuged at 3,000 $\times g$ for 5 min, and 2.0 ml of diluted supernatant was mixed with 2.0 ml of ninhydrin solution. After 10 min incubation at room temperature, the absorbance was measured at 485 nm, using NaCN in the range of 0–150 ng/ml as a standard solution. All the measurements were duplicated and the average values were shown in the data.

Fermenter Experiments

The experimental set-up basically consisted of a 5 L fermenter (B. Braun Biotech International, Germany) with a 3 L working volume of NFS medium. The fermenter was sterilized by autoclaving at 15 psi for 15 min. Fermentation was maintained at an agitation rate of 200 rpm at 30°C. Aeration was performed with a sparger at the flow rate of 2 L/min. The gas scrubber was filled with 0.1 N NaOH placed at the exit of the air stream to trap the evaporation of HCN. When the cells were grown to the stationary phase (30 h), the aeration was stopped and NaCN was added to give final concentrations of 3.0, 3.5, and 4.0 mM, respectively, in the separated experiments. The cell growth and cyanide degradation were measured with the time intervals.

Cassava Mill Wastewater Preparation

The cassava mill wastewater was collected directly from a cassava mill processing site near Khon Kaen, Thailand. Samples were centrifuged at 5,000 $\times g$, for 10 min at 4°C. The supernatant from the centrifuged cassava mill wastewater was analyzed and used in this experiment. The major components in the wastewater such as cyanide concentration, ammonia, COD, BOD, total nitrogen, and total carbohydrate were analyzed using the standard method [2]. For the controlled experiments, cyanide in the cassava mill wastewater was eliminated by autoclaving at 15 psi for 15 min. Fermentation was maintained at an agitation rate of 200 rpm at 30°C. Aeration was performed with a sparger at the flow rate of 2 L/min. The gas scrubber was filled with 0.1 N NaOH placed at the exit of the air stream to trap the evaporation of HCN. When the cells were grown to the stationary phase (30 h), the aeration was stopped and NaCN was added to give a final concentration of 4.0 mM. The cell growth and cyanide degradation were measured with the time intervals.

Whenever the supplements were provided to the cultures, 0.2 g/l $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.073 g/l $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 10 μM $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, and 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ were added to the cassava mill wastewater.

Nitrogenase Activity Measurement

To determine the nitrogenase activity in the cells, cyanide was used as the substrate of nitrogenase and the production of methane was detected [13]. The cell suspension (40 ml) was transferred to a serum bottle (60 ml) and the bottle was sealed with a rubber stopper and an aluminum cap. For the cyanide reduction, the reaction was initiated by injection of 4.0 mM NaCN. The serum bottles were incubated at 30°C with 200 rpm in a gyrotory shaker (New Brunswick Scientific, Enfield, CT, USA) for 24 h. After incubation, the gas phase (50 μl) was taken from the serum bottle and methane production was analyzed on a Shimadzu GC-17A gas chromatography system (Kyoto, Japan) equipped with a flame ionization detector and a Rt U-plot 80/100 capillary column (Restek, Bellefonte, PA, USA). The operating conditions were set as follows: column temperature 50°C; injector temperature 200°C; detector temperature 200°C; flow rate of carrier gas (He) 40 ml/min.

RESULTS

Growth of *A. vinelandii* in NFS Medium

A. vinelandii starter was inoculated into an NFS medium with and without urea because nitrogenase is only expressed under diazotrophic conditions. Cell growth was determined over a time period and the growth patterns are shown in Fig. 1. The growth curve obtained from batch culture comprised a characteristic lag phase, exponential phase, and stationary phase. The lag period of both conditions was observed for the initial 6 h incubation. The exponential phase was observed after the lag phase between 6 and 30 h. The exponential phase presents a period of logarithmic cell doublings and the specific growth rate (μ) of *A. vinelandii* cultured in NFS medium with and without urea was 0.136 and 0.123 h^{-1} , respectively.

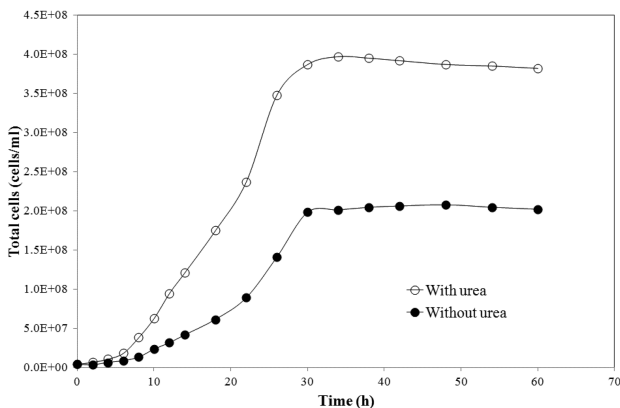


Fig. 1. Growth curve of *Azotobacter vinelandii* in NFS medium with (open circle) and without urea (filled circle).

Whereas the stationary phase for both conditions was obtained after 30 h of incubation, the cell numbers on the stationary phases were different. Total cell counts of *A. vinelandii* in NFS medium with urea showed twice as many cells.

Cyanide Biodegradation by *A. vinelandii* from Mid-Exponential and Stationary Phases

The cyanide reductions by *A. vinelandii* harvested from two different growth phases are shown in Fig. 2 as a function of time. Cells in both mid-exponential and stationary phases showed the ability to reduce 1.0 mM of cyanide. However, cells in the stationary phase had an ability to reduce cyanide better than cells in the mid-exponential phase. Cell suspensions in the stationary and mid-exponential phases could reduce cyanide at about 75.7% and 55.7%, respectively, after 60 h of incubation time.

Cell Growth and Cyanide Degradation Under Different Concentrations of NaCN

To the cells from the stationary phase (30 h after incubation), NaCN was added to produce the final concentration of 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 mM, respectively, and the cell growths were measured. The NFS media containing the same concentrations of NaCN without *A. vinelandii* were used as controls of cyanide degradation determination. The growth patterns of *A. vinelandii* were similar with the concentrations between 0.5 and 3.0 mM of NaCN, but total cells numbers decreased during the stationary phase at the concentrations of higher than 4.0 mM (Fig. 3). With these cultures, cyanide degradation by *A. vinelandii* was also measured and the results are shown in Table 1. Relevant to the growth curves in Fig. 3, cyanide degradation by *A. vinelandii* was only observed at the concentrations

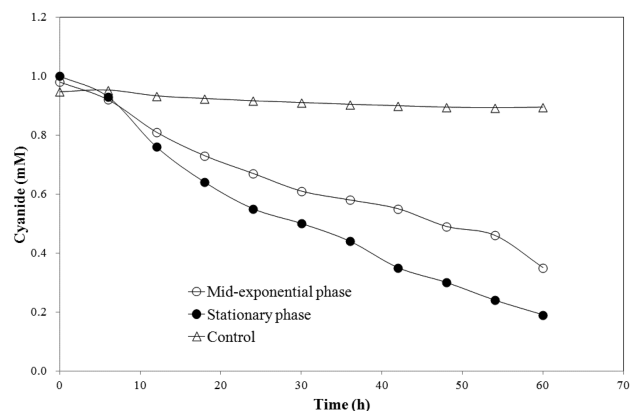


Fig. 2. Cyanide degradation by *Azotobacter vinelandii* in NFS medium under diazotrophic conditions.

NaCN (1.0 mM) was added to the cell suspensions obtained from the mid-exponential (open circle) and stationary phases (filled circle).

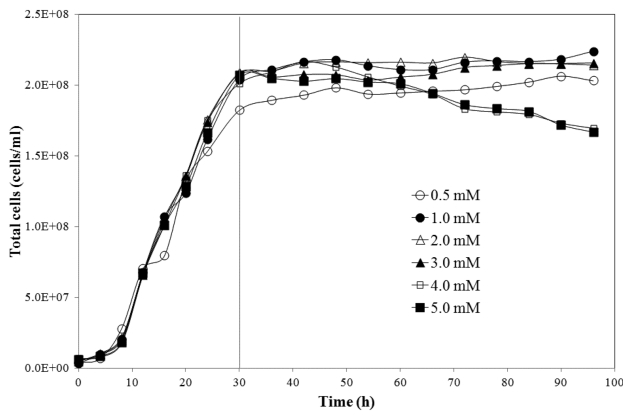


Fig. 3. Growth curves of *Azotobacter vinelandii* from the early stationary phase with various concentrations of NaCN in NFS media. The vertical line represents the time (30 h) of cyanide addition.

between 0.5 and 3.0 mM of NaCN. No cyanide reduction by *A. vinelandii* was observed at the concentrations of higher than 4.0 mM. Cyanide reduction in the controls mainly represents evaporation of cyanide by the aeration of media.

Cell Growth and Cyanide Degradation in the Presence of Nitrogen Source

To study the effect of nitrogen source, *A. vinelandii* was grown in NFS medium with 20 mM of urea until it reached to the stationary phase. NaCN was then added to the cells in the final concentrations of 1.0, 2.0, and 3.0 mM, respectively. In the presence of urea, the growth curve of *A. vinelandii* was affected by all the concentrations of NaCN (Fig. S5) and no cyanide degradation was observed (data not shown).

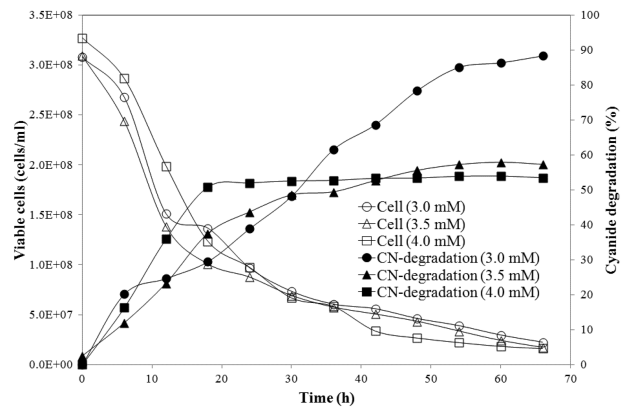


Fig. 4. Viable cells and cyanide degradation of *Azotobacter vinelandii* when 3.0, 3.5, and 4.0 mM of NaCN were added at the stationary phase (30 h). Filled labels show the cyanide degradation percentage after correction with controls, and open labels show the viable cell numbers at each cyanide concentration.

Cell Growth and Cyanide Degradation in Large-Scale Fermenter

In flask-scale experiments, the growth curve and cyanide tolerance under diazotrophic conditions of *A. vinelandii*, as well as cyanide degradation by the cells from the stationary phase were studied. Next, the cell growth and cyanide degradation were determined in a 3 L culture to scale up the 300 ml flask experiments. When the concentrations near the maximum cyanide tolerance, 3.0, 3.5, and 4.0 mM of NaCN, were added into the NFS medium after 30 h, the cell growth at all concentrations was stopped during the first 18 h after NaCN addition. The cell numbers showed slight increases at all sodium cyanide concentrations in 18 h after cyanide additions (Fig. S6). However, when cyanide degradation and cell viability were measured, the

Table 1. Cyanide degradation by *A. vinelandii* from the early-stationary phase with various concentrations of NaCN in NFS media.

Time (h)	NaCN (mM)					
	0.5	1.0	2.0	3.0	4.0	5.0
0	0.48	1.01	2.02	2.95	3.95	5.10
6	0.29	0.85	1.55	2.38	3.88	4.95
12	0.11	0.71	1.28	1.78	3.82	4.89
18	0.07	0.58	1.13	1.29	3.79	4.81
24	0.04	0.49	1.03	1.07	3.78	4.79
30	0.03	0.40	0.83	0.89	3.74	4.73
36	0.03	0.34	0.72	0.84	3.68	4.66
42	0.02	0.25	0.62	0.75	3.63	4.57
48	0.01	0.26	0.55	0.64	3.57	4.49
54	0.00	0.20	0.47	0.57	3.51	4.42
60	0.00	0.16	0.32	0.51	3.44	4.35
66	0.00	0.13	0.19	0.46	3.36	4.24
Cyanide reduction (%) in 66 h	100.0	87.1	90.6	84.4	14.9	16.8
Control (%) in 66 h	9.0	14.6	18.9	15.1	14.9	16.8

results showed that cyanide degradation at the concentration over 3.5 mM was not continuous after the first 18 h and the cell viability decreased rapidly for the period of time (Fig. 4). The final cyanide reductions after corrections by controls were 88.3%, 57.2%, and 53.4% after 66 h of incubation with 3.0, 3.5, and 4.0 mM of NaCN, respectively.

Growth and Cyanide Degradation by *A. vinelandii* in Cassava Mill Wastewater

The major components of cassava mill wastewater are shown in Table S1. The result shows that the cassava mill wastewater contained both a carbon source and nitrogen source required for *A. vinelandii* growth. The acidity of cassava mill wastewater, pH 5.60, also favored cyanide reduction by *A. vinelandii*, because most free cyanide exists in the HCN form and inhibition of nitrogenase by CN^- is minimized. The concentration of cyanide (3.18 mM) was found close to the range that *A. vinelandii* could biodegrade cyanide compared with the flask and fermenter experiments. This cassava mill wastewater was used to study cyanide degradation by *A. vinelandii* with the controlled cyanide concentration and supplements addition.

Growth of *A. vinelandii* with or without supplements in the sterilized cassava mill wastewater was measured and the specific growth rates (μ) in cassava mill wastewater were 0.091 and 0.105 h^{-1} , respectively (Fig. S7). The exponential phases were observed after 6 h inoculation and the stationary phases were exhibited in 30 h for both conditions, and that was the same as the results obtained from the NFS media experiments. Although *A. vinelandii* can grow well without mineral supplements, supplementation increased cell counts more than 50%. It appears the cassava mill wastewater system does not contain enough Mo and Fe ions, which are required for the biosynthesis of nitrogenase.

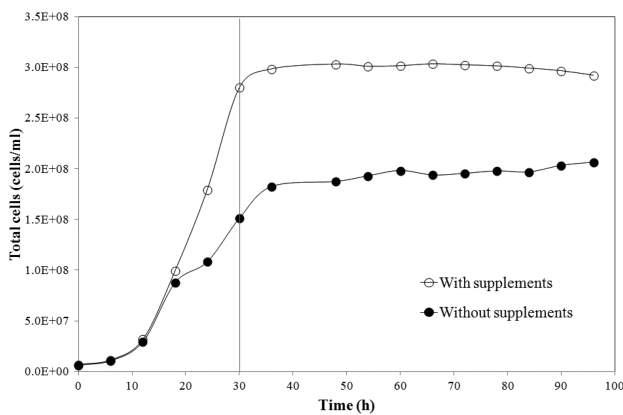


Fig. 5. Growth curves of *Azotobacter vinelandii* in cassava mill wastewater with (open circle) and without supplements (filled circle), when 4.0 mM NaCN was added in the early-stationary phase (30 h).

The vertical line represents the time of cyanide addition.

When cells reached to the stationary phase (30 h), NaCN solution was added to the fermenter containing cassava mill wastewater in the final concentration of 4.0 mM NaCN. The cell numbers in cassava mill wastewater with and without supplement were 3.0×10^8 cells/ml and 2.0×10^8 cells/ml, respectively, after 66 h of incubation in the presence of cyanide (Fig. 5). The viable cell and cyanide reduction were also determined under the conditions with and without supplements as a function of time after the addition of NaCN (Fig. 6). The number of the initial viable cells cultured in cassava mill wastewater with supplements was twice higher than that without supplement. The viable cells in cassava mill wastewater with supplements slowly decreased from 3.0×10^8 cells/ml to 6×10^7 cells/ml within 48 h of incubation. However, the viable cells without supplements rapidly decreased from 1.5×10^8 cells/ml to 2×10^7 cells/ml within 12 h of incubation in the presence of 4.0 mM NaCN. The cyanide reduction was also determined during incubation time (Fig. 6). The cyanide reductions were determined as 64.0% and 45.9%, respectively, with and without supplements in the cassava mill wastewater after 66 h of incubation in the presence of 4.0 mM NaCN.

Nitrogenase Activity During the Cyanide Degradation

Under the cassava mill wastewater system, the role of nitrogenase in the conversion of cyanide to methane was studied. Production of methane was observed from the cassava mill wastewater containing 4.0 mM NaCN (Fig. S10), and nitrogenase from *A. vinelandii* was found to be involved in the cyanide degradation process. However, methane production was not observed after 60 h of incubation, which might due to the toxic effect of sodium cyanide to viable cells. The results also concluded that

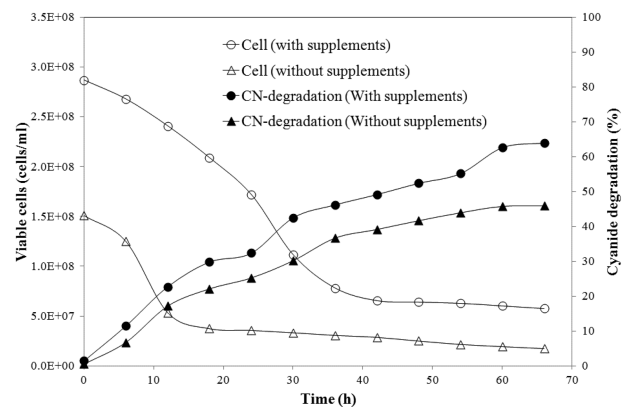


Fig. 6. Viable cells and cyanide degradation of *Azotobacter vinelandii* in cassava mill wastewater when 4.0 mM of NaCN was added at the stationary phase (30 h).

Filled labels show cyanide degradation percentage after correction with controls, and open labels show viable cell numbers.

nitrogenase enzyme is produced by *A. vinelandii*, when ammonium in cassava mill wastewater was limited during *A. vinelandii* growth. The ammonium ion concentration in cassava mill wastewater was 2.04 mM.

DISCUSSION

Strict aerobic *A. vinelandii* is a good producer of nitrogenase, and the Mo, Fe-containing enzyme reduces dinitrogen into ammonia. Nitrogenase is also known to degrade cyanide. Therefore, *A. vinelandii* was used for the bioremediation of aqueous cyanide in this study. As shown in Fig. 1, total cells of *A. vinelandii* at the stationary phase in NFS medium with and without urea were different, even though the growth curves and specific growth rates of *A. vinelandii* under both conditions were similar. Because nitrogenase in *A. vinelandii* is the responsible biochemical unit for the cyanide degradation, it is important to find the optimized conditions that allow efficient expression of nitrogenase. It is known that nitrogenase is only expressed under diazotrophic conditions and any nitrogen sources, including urea, completely suppresses nitrogenase biosynthesis [14].

Monitoring cyanide degradation by *A. vinelandii* from mid-exponential and stationary phases (Fig. 2) suggested that the cells in the stationary phase could produce and accumulate nitrogenase better than the cells in mid-exponential phase. This finding is contrary to the report by Kaewkannetra *et al.* [10], but consistent with the previous study by Kao *et al.* [11] that reported the resting cells of *Klebsiella oytoca* were able to biodegrade cyanide through the action of nitrogenase. Therefore, the cells in the stationary phase were selected to study cyanide degradation in this study. *A. vinelandii* from the stationary phase was able to grow and degrade cyanide even with 3.0 mM of NaCN (Table 1 and Fig. S4).

With the results obtained from the flask-scale experiments, cell growth and cyanide degradation in a 3 L fermenter scale were measured (Figs. S6 and 4). It was concluded that 3.0 mM of NaCN is the maximum concentration of cyanide degradation with viable cells, and the cyanide degrading ability was reduced as the viable cells were decreased. The scale-up experiments also confirmed that the results from the flask experiments can be applied to large-scale experiments.

Finally, cyanide degradation by *A. vinelandii* was applied to the wastewater system and cassava mill wastewater was tested with the controlled concentrations of cyanide. As shown in Table S1, the cassava mill wastewater contained both a carbon source and nitrogen source required for *A. vinelandii* growth. Although it is not a strict diazotrophic condition, low concentration (2.04 mM) of ammonia was found not to inhibit the expression of nitrogenase (Table S2). However, it did not have enough Mo and Fe ions required

for the expression of nitrogenase, as shown by Fig. S7. Supplementation of these minerals enhanced cell growth and cyanide degradation in the cassava mill wastewater experiment (Fig. 6). Gas chromatography analyses of methane, one of the end-products of cyanide degradation, production from the cassava mill wastewater also confirmed that nitrogenase was responsible for cyanide degradation.

Hydrogen cyanide (HCN) is a substrate for nitrogenase, but cyanide ion (CN⁻) is a strong inhibitor ($K_i = 27 \mu\text{M}$) for nitrogenase [13]. However, free cyanide is mostly present in the form of HCN under the experimental conditions of pH 7.4, and no significant inhibition of nitrogenase was observed based on the cell growth. The acidity of the cassava mill wastewater was found to be pH 5.6 (Table S1), which resulted in the HCN form of free cyanide. Therefore, it is important to control the pH of wastewater for the bioremediation of cyanide. Low-molecular-weight HCN is a non-ionic and hydrophilic chemical species, and it can easily penetrate the cell membrane to inhibit metalloenzymes within the cells [7]. Although nitrogenase metabolizes HCN, we cannot exclude the possibility of cyanide inhibition of other biochemical pathways in the cell. The protective function of nitrogenase in the cell against cyanide inhibition was evident by the cyanide inhibition on the cell growth in the urea-containing NFS media. Without nitrogenase, the growth of *A. vinelandii* was inhibited even with 0.01 mM of NaCN (Fig. S3). Kao *et al.* [11] also found that *K. oytoca* could not degrade cyanide when 20 mM ammonia was added in the medium as a nitrogen source.

The results from this study provided basic information on the ability of *A. vinelandii* for cyanide degradation. The cyanide biodegradation ability of *A. vinelandii* in NFS medium in a shake flask and in a fermenter revealed that the *A. vinelandii* in the early-stationary phase had a better efficiency to reduce cyanide than the cells in mid-exponential phase. Moreover, *A. vinelandii* could degrade NaCN up to 3.0 mM in a shake flask and up to 4.0 mM in a fermenter. On the other hand, the ability of *A. vinelandii* to degrade of cyanide was dramatically inhibited when 20 mM of urea was added to NFS medium, due to the absence of nitrogenase, which is responsible for the cyanide-degrading ability. When the optimized conditions for the cyanide degradation by *A. vinelandii* were applied to the 3 L cassava mill wastewater containing 4.0 mM of NaCN, 64.0% of cyanide was degraded in 66 h with supplementation of the Mo and Fe ions required for the biosynthesis of active nitrogenase. Nitrogenase activity in *A. vinelandii* was confirmed in the cassava mill wastewater system, which concluded that *A. vinelandii* induced nitrogenase to reduce NaCN as an alternative nitrogen source. Results from this study provide us the characteristics and mechanisms of cyanide conversion by *A. vinelandii*. These findings would be helpful in designing a practical

system inoculated with *A. vinelandii* for the treatment of wastewater containing cyanide.

Acknowledgments

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