Journal of Hazardous Materials 172 (2009) 224–228

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Cyanide removal from cassava mill wastewater using *Azotobactor vinelandii* TISTR 1094 with mixed microorganisms in activated sludge treatment system

P. Kaewkannetra[a,b], T. Imai[c], F.J. Garcia-Garcia[d], T.Y. Chiu[e,f,*]

[a] *Department of Biotechnology, Faculty of Technology, Khon Kaen University, Khon Kaen 40002, Thailand*
[b] *Research Centre for Environmental Hazardous Substance Management, Department of Environmental Engineering, Faculty of Engineering, Khon Kaen University, Khon Kaen 40002, Thailand*
[c] *Department of Civil and Environmental Engineering, Faculty of Engineering, Yamaguchi University, Tokaiwadai Ube 755-8611, Japan*
[d] *Corrosion and Protection Centre, School of Materials, The University of Manchester, P.O. Box 88, Manchester M60 1QD, United Kingdom*
[e] *Earth Tech Engineering Ltd, Wentworth Business Park, Maple Road, Tankersley, Barnsley S75 3DL, United Kingdom*
[f] *Centre of Water Science, Cranfield University, Bedfordshire MK43 0AL, United Kingdom*

ARTICLE INFO

Article history:
Received 14 February 2009
Received in revised form 13 April 2009
Accepted 30 June 2009
Available online 5 July 2009

Keywords:
Cassava mill wastewater
A. vinelandii
Cyanide removal
Biological treatment
Activated sludge system

ABSTRACT

Cassava mill wastewater has a high organic and cyanide content and is an important economic product of traditional and rural low technology agro-industry in many parts of the world. However, the wastewater is toxic and can pose serious threat to the environment and aquatic life in the receiving waters. The ability of *Azotobactor vinelandii* TISTR 1094, a N_2-fixing bacterium, to grow and remove cyanide in cassava wastewater was evaluated. Results revealed that the cells in the exponential phase reduce the level of cyanide more rapidly than when the cells are at their stationary growth phase. The rate of cyanide removal by *A. vinelandii* depends on the initial cyanide concentration. As the initial cyanide concentration increased, removal rate increased and cyanide removal of up to 65.3% was achieved. In the subsequent pilot scale trial involving an activated sludge system, the introduction of *A. vinelandii* into the system resulted in cyanide removals of up to 90%. This represented an improvement of 20% when compared to the activated sludge system which did not incorporate the strain.

1. Introduction

Cassava starch is an important economic product of traditional and rural low technology agro-industry in Southeast Asia as well as in tropical Africa and Central America. Cassava composes approximately 57% of tropical root and tuber production. The cyanide content of cassava root varies with the plant variety and soil conditions and may range between 75 and 1000 mg (CN) kg^{-1}. Unfortunately, large amounts of natural cyanoglycosides found in cassava are released during the production of starch from the cassava tuber. These cyanogenic glycosides can enzymatically hydrolyze to cyanide which is often found within the wastewater discharge from these processing industries. Cyanide concentration in cassava mill wastewater has been reported to contain as high as 200 mg l^{-1} depending on the cyanoglycoside content of the cassava varieties [1]. The detected cyanoglucosides concentrations in effluents ranged between 10.4 and 274 mg l^{-1} [2].

Cyanide is toxic to humans and animals because it binds to key iron containing enzymes i.e. cytochrome oxidase required for cells to respire aerobically [3]. Ingesting cyanide can also result in either acute poisoning (including death) or chronic poisoning to humans and animals. Cyanide has also been associated with syndromes affecting the central nervous system in animals. Other effects of cyanide include the removal of trace elements from the environment. The resulting wastewater from cassava starch processing is therefore toxic and can pose a serious threat to the environment and aquatic life in the receiving waters. Hence, many countries have implemented a statutory limit of approximately 0.2 mg l^{-1} for cyanide discharge into natural water basins [4].

In order to comply with government legislations, wastewater containing cyanide must be treated before discharging into the environment. Current wastewater treatments for cyanide removal employ chemical and physical methods which are often expensive and involve the use of additional hazardous reagents (chlorine and sodium hypochlorite) [5]. In many instances, complete degradation of some cyanide complexes is not achieved [6,7]. Biological treatment on the other hand is a cost-effective and environmentally acceptable method for cyanide removal. Several researchers report that biological processes could be used to remove cyanide compounds from wastewater [8–10]. Some organisms resistant to cyanide have been reported to remain active even at

* Corresponding author at: Earth Tech Engineering Ltd, Wentworth Business Park, Maple Road, Tankersley, Barnsley S75 3DL, United Kingdom. Tel.: +44 122 622 4176; fax: +44 122 622 4488.
E-mail address: TzeYen.Chiu@eu.earthtech.com (T.Y. Chiu).

0304-3894/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2009.06.162

concentrations higher than 1 mM of cyanide [3]. *Bacillus*, *Pseudomonas*, and *Klebsiella oxytoca* have been reported to biodegrade cyanide to non-toxic end-products and using cyanide as the sole nitrogen source under aerobic and/or anaerobic environment [10–12].

One of the problems in biological treatment of industrial wastewaters, such as cassava wastewater, is their low nitrogen (N) and high chemical oxygen demand (COD) content leading to a nutritionally unbalanced wastewater. COD removal performance of the biological treatment falls due nitrogen (N) deficiency when biologically utilizable nitrogen concentration is below N/COD < 0.05 [13]. Nitrogen balancing by external addition of nitrogen compounds to industrial wastewater is often necessary. A cheaper alternative to the conventional approach of external nitrogen addition to the nitrogen deficient wastewater is to ultilize nitrogen fixing bacterial in activated sludge. *Azotobactor vinelandii*, an aerobic nitrogen-fixing Gram-negative bacterium found in soils has the capacity to fix nitrogen gas (N_2) to compound of ammonium (NH_4^+) through the action of nitrogenase [14–16] which is inhibited by free oxygen. However, this enzyme is protected from oxygen inhibition by iron and molybdenum salts [17]. Additionally, the enzymes within these strains have been reported such as rhodaneses and nitrogenases to be involved in cyanide detoxification [18–20].

To the authors' knowledge, there has been no work undertaken on the cyanide removal efficiency of *A. vinelandii* from cassava wastewater. Most of the work undertaken for this bacterial strain in wastewater treatment is concerned with nitrogen fixation in olive-oil and pulp and paper industries [21–23] and the role of the enzymes rhodanese and nitrogenase in cyanide detoxification although this information may be useful in understanding certain aspects of the cyanide potential of *A. vinelandii*.

The major objective of this study was to investigate the performance of *A. vinelandii* supplemented activated sludge for biological treatment of cyanide in cassava wastewater. The ability of *A. vinelandii*'s to grow in cassava wastewater is assessed and its cyanide removal potential is investigated under various cyanide concentrations. Subsequently, the cyanide removal efficiency of an existing activated sludge wastewater treatment system is studied in the presence and absence of this strain.

2. Materials and methods

2.1. Microorganism

Pure *A. vinelandii* culture (TISTR 1094) was obtained from Thailand Institute of Scientific and Technological Research (TISTR), Thailand. *Azotobacter* was activated in a nutrient broth on a shaker at 30 °C and 200 rpm for 24 h. Subsequent cultivation and maintenance were carried out in a nitrogen-free sucrose (NFS) medium containing 2% sucrose, 0.2 g l^{-1} $MgSO_4{\cdot}7H_2O$, 0.073 g l^{-1} $CaCl_2{\cdot}2H_2O$, 10 μM $Na_2MoO_4{\cdot}2H_2O$, 20 μM $FeCl_3{\cdot}6H_2O$ and 2 μM phosphate buffer pH 7.4 [24]. The cell growth was counted every 6 h for 80 h and the cell was used as the starter for shake flask and the activated sludge studies. All chemicals used were of analytical grade (SC Scientific and Merck Chemical, Bangkok, Thailand).

2.2. Growth monitoring and cyanide removal in flask scale

Inoculums of the starter were transferred to flasks containing 100 ml NFS and 300 ml cassava wastewater at the final pH of 7–8.5 for acclimatization. The cyanide content was varied using different amounts of NaCN ranging from 0 to 150 mg l^{-1}. The cell growth in terms of total cell was counted and cyanide concentration was monitored during incubation until end of incubation time. The microorganism cell growth was analyzed using haemacytometer after the sample was taken from the shake flask, centrifuged at a speed of 6200 rpm for 5 min and washed twice with phosphate buffer.

2.3. The activated sludge (AS) experiments

Two 20 l bench scale reactors of conventional activated sludge (AS) systems were used in this study. The system consisted of a 20 l plexiglas unit with two compartments, an aeration tank and a sedimentation tank. Aeration was supplied by stone air diffusers to maintain the dissolved oxygen level of 2 mg l^{-1}. The operation of the bench scale systems started by the addition of mixed microbial sludge from a full-scale activated sludge wastewater treatment plant of cassava mill factory (Kalasin province, north-eastern of Thailand), followed by a sludge acclimatization period where cassava mill wastewater, also obtained from the same factory, was introduced to the system. Cassava mill wastewater was gradually replaced by synthetic high strength influent, followed by the addition of cyanides. A mixed liquor suspended solid (MLSS) of around 2500–3000 mg l^{-1} within the aeration tank was maintained. Synthetic wastewater was prepared using the following analytical grade substances: 9.01 mg $CaCl_2$, 5.02 mg $FeCl_2$, 63.6 mg KH_2PO_4, 38.8 mg $MgSO_4{\cdot}7H_2O$, 684 mg $NaHCO_3$, 940 mg NH_4HCO_3, 1802 mg sugar, 120 mg urea and diluted with 2 l of wastewater. In addition with the desired cyanide concentrations was fed into the aeration tank at a flow rate of 20 l d^{-1}. Samples of the influent and effluent were collected daily and analyzed for COD and cyanide concentrations. Two AS systems ran in parallel with one acting as a control set (without *A. vinelandii* addition) and the other with *A. vinelandii* added to the activated sludge culture.

2.4. Analytical methods

To determine total cell count the samples were serially diluted with sterile saline solution (0.85% w/v NaCl). The appropriately diluted samples (0.1 ml) were plated and incubated at 30 °C for 24 h to form fully developed colonies. The cell count was estimated by counting colonies grown on nutrient agar medium using a haemacytometer [25]. For all counts, the average of at least three replicate plates was used for each tested dilution. In order to establish the reliability and reproducibility of the plate count technique, 10 independent samples were drawn (at the same time) from the shake flask experiment and were serially diluted and plated. Each dilution was plated in three different plates and the colonies were counted and the standard deviation was calculated using Microsoft Excel's built-in STDEV function ("non-biased" or "n^{-1}" method). The standard deviation was 6%.

The wastewater characteristics such as pH, suspended solids (SS), MLSS, COD ammonia were analyzed following standard methods [26] and the cyanide content was analyzed using a modified ninhydrin method as follows [27]: wastewater sample was centrifuged at 6200 rpm, for 5 min. Then, 2 ml of supernatant was mixed with 2 ml ninhydrin solution. After 10 min incubation at room temperature, the absorbance was measured at 485 nm using spectrophotometer (UV-1201 Shimadzu). Samples were analyzed in triplicates. Standard deviations were less than 5% of the average.

3. Results and discussion

3.1. Characteristics of cassava mill wastewater

Table 1 shows the composition of cassava wastewater. The high organic content of 16,000 mg l^{-1} in the wastewater found in this study is in agreement with those found typically in large scale starch processing industries [28,29]. These organic pollutants originate from the washing and extracting processes in the tapioca

Table 1
Composition of cassava mill wastewater.

Parameter	Concentration (mg l^{-1})	
	Current study	[28,29,32,33,37]
pH	5.5	3.8–5.7
SS	2200–4000	330–7600
Cyanide	86	19–61
Ammonia	37	37.8–102
Chemical oxygen demand (COD)	16,000	7000–41,406
Total kjeldahl nitrogen (TKN)	350	–
Total carbohydrate	16	–

industry [30,31]. In order to produce 1 ton of starch, a tapioca processing factory discharges about 12 m^3 of wastewater containing 6125–13,500 mg l^{-1} (COD), 1466–7600 mg l^{-1} (SS) and pH 4.5–5.0 [32,33]. These values indicate that the wastewater is highly biodegradable and therefore, there is no need for the supply of external organic carbon source for cyanide-degrading microoganisms. The wastewater pH was low (pH 5.5) and probably arises from the acidification process of starch due to organic acid production [34]. The low carbohydrate content (16 mg l^{-1}) of the wastewater was in agreement with this observation. A high cyanide concentration of 86 mg l^{-1} was observed in this study comparing the other cassava mill wastewater studies (Table 1) and this could arise from the different variety of cassava that is being treated. Elsewhere, cyanide concentration in cassava mill wastewater was reported contain up to 200 mg l^{-1} depending on the cyanoglycoside content of the cassava varieties [1].

3.2. Growth monitoring and cyanide degradation in flask scale

The growth pattern of *A. vinelandii* in cassava mill wastewater is shown in Fig. 1. The initial lag period was observed after 6 h of incubation and stationary phase was exhibited within 30 h. The specific growth rate (μ) and cell number were about 0.1 and 2.2×10^8 cell ml^{-1} respectively.

Fig. 2 shows the growth pattern of *A. vinelandii* under various sodium cyanide concentrations (50, 100 and 150 mg l^{-1}) and the cell reached to stationary phase after 30 h respectively. The results suggest that *A. vinelandii* are able to survive under the maximum range of cyanide concentration typically found in the cassava mill industry. Preliminary studies carried out at higher cyanide concentrations (>250 mg l^{-1}) in nitrogen-free sucrose medium show growth inhibition of *A. vinelandii*. The mechanism of cyanide reduction could be associated with the N_2-fixing enzymes of *A. vinelandii*.

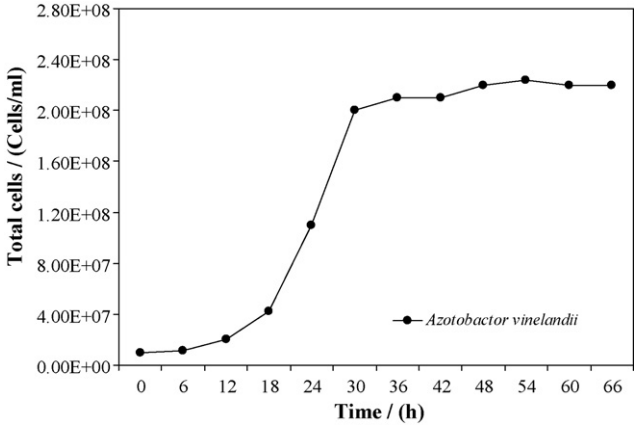


Fig. 1. Growth pattern of *A. vinelandii* in cassava wastewater as a function of time.

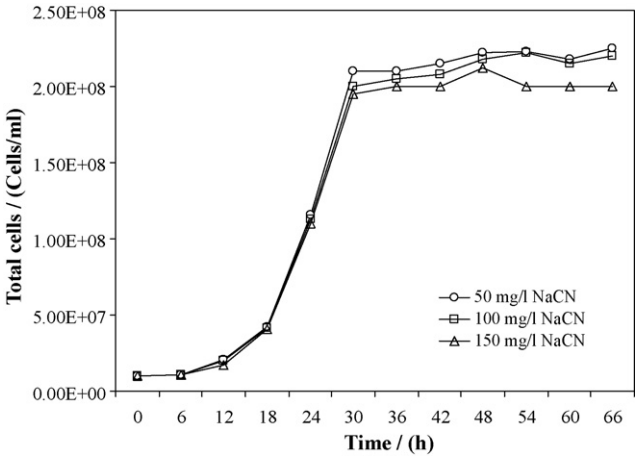
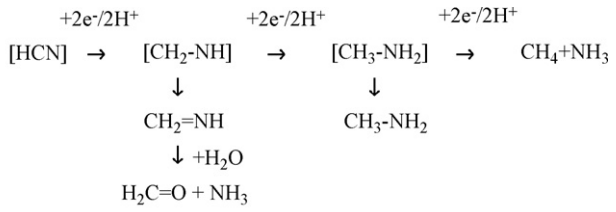


Fig. 2. Growth pattern of *A. vinelandii* in cassava wastewater under various NaCN concentrations as a function of time.



[] indicates substrate bound to the enzyme.

Fig. 3. Schematic of possible reactions involved in cyanide reduction (adapted from [20]).

Nitrogenase commonly found in *A. vinelandii* has been demonstrated to be involved in cyanide reduction and is illustrated in Fig. 3 [20,35].

HCN is initially converted into CH_2=NH (methyleneimine) by two electrons and two protons and when this intermediate escapes from the active site, it is hydrolyzed to H_2C=O and NH_3 [20,35]. Under these circumstances, NH_3 is produced without concomitant CH_4 production. In the next step, an additional two electrons and two protons produce CH_3NH_2, which can also escape. Finally, CH_4 and NH_3 are formed in equal quantities by the next pair of electrons and protons.

The final cyanide reduction increased when initial cyanide concentration was increased (Table 2). Cyanide reductions of 56%, 58%, and 65.3% were obtained corresponding to initial NaCN concentrations of 50, 100 and 150 mg l^{-1} respectively. This is in agreement with Fisher et al. [20] who observe increased NH_3 and CH_4 at NaCN

Table 2
Sodium cyanide reduction in cassava wastewater by *A. vinelandii*.

Time (h)	NaCN concentration (mg l^{-1})		
0	50	100	150
6	48	92.5	137.5
12	44	77.4	121
18	41.2	66.2	109
24	35	58.8	88.5
30	34	51.5	79.4
36	32	49	76
42	29	47.5	71
48	26.5	46.1	68.5
54	24	45.1	65.5
60	22.5	43.1	59
66	22.1	42	52
% cyanide reduction	56	58	65.3

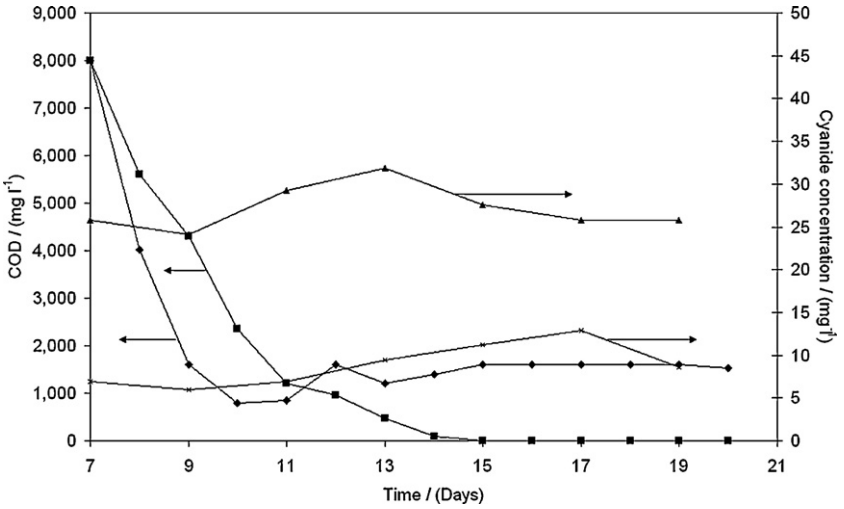


Fig. 4. COD and cyanide concentrations observed in the effluent of AS treatment systems as a function of time (♦: C-AS and ■: E-AS for COD; ▲: C-AS and x: E-AS for cyanide).

concentrations of up to 5 mM indicative of increased cyanide reduction. The rate of removal seems to be dependent on the initial NaCN concentration. When the concentrations increased from 50 to 150 mg l^{-1}, the rate of cyanide removal was found to increase from 0.4 to 1.5 mg l^{-1} h^{-1}. In addition, rate of cyanide removal was observed to be faster when the cells are at their exponential growth phase (6–30 h) as compared to when the cells are at their stationary phase (over 36 h). This suggests that rate of cyanide removal is linked to the metabolic activity of *A. vinelandii*.

3.3. The activated sludge (AS) experiments

Figs. 4–6 show the wastewater quality such as COD, cyanide concentrations, TS and pH in two both sets of AS: control set (C-AS) and experimental set (E-AS). The effluents of both AS systems achieved similar COD and TS removals of about 74.5% and 26.1% respectively. The pH of wastewater in both AS ranged from pH 7 to pH 9 which lies within the discharging wastewater standard. This implies that both systems have similar efficiencies for treating the wastewater.

The cyanide removals of about 70% and 90% are observed in C-AS and E-AS sets respectively. The ability of the control system to remove cyanides at high influent concentration of 86 mg l^{-1} is attributed to the presence of acclimatized microorganisms that are able to remove cyanide in the wastewater. Additionally, other abiotic removal processes might be involved. Previous researchers have reported almost 60% cyanide removal in activated sludge process by stripping i.e. the volatilization of HCN from aqueous phase

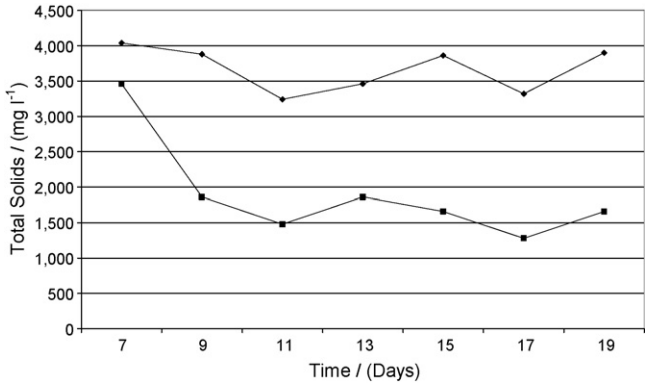


Fig. 5. Total solids concentration obtained from the effluent of AS treatment systems as a function of time (♦: control-AS, ■: experiment-AS).

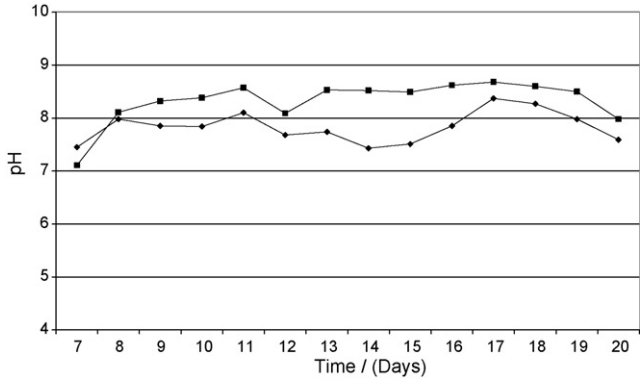


Fig. 6. pH values of the effluent of AS treatment systems as a function of time (♦: control-AS, ■: experiment-AS).

to air phase. This is highly likely to occur in the current study since the system pH was pH 7–8.5 (Fig. 6) and cyanide in this pH range is normally in the form of HCN which is highly volatile. Adding *A. vinelandii* resulted in increased COD and cyanide removals. It seems that *A. vinelandii* is able to co-cultivate with the mixed microbial microorganisms within the AS wastewater treatment and aid in an enhanced removal of cyanide. This is the first study in which *A. vinelandii* is illustrated to possess the ability to remove cyanide under wastewater conditions. The improved COD removal under *A. vinelandii* supplemented conditions is in agreement with Kargi and Ozmuci [17] although their comparisons were made for the biological treatment of nitrogen deficient synthetic wastewater.

An interesting observation in the present study is the apparent link between the decrease in cyanide concentrations and the reduction in the levels of COD in the wastewater. This is illustrated when comparing the cyanide and COD reductions with and without *A. vinelandii*. Analogously, tetracyanonickelate degradation by *A. vinelandii* is found to be dependent on the availability of a carbon source [36].

4. Conclusions

The ability of *A. vinelandii*, a N_2-fixing bacterium, to grow and remove cyanide in cassava wastewater was evaluated. This study demonstrates that *A. vinelandii* are able to survive and thrive within the typical range of CN concentrations found in the cassava mill industry. The *A. vinelandii* supplemented activated sludge cultures

showed that *A. vinelandii* possess the ability to co-cultivate with other MMS and aid in an enhanced removal of cyanide. Cyanide removal increased from 70% to 90% in the presence of *A. vinelandii*. This is the first study in which *A. vinelandii* was shown to be able to remove cyanide under wastewater conditions.

Acknowledgments

The authors would like to gratefully acknowledge the Research Centre for Environmental and Hazardous Substance Management, Faculty of Engineering, Khon Kaen University, Thailand and the JSPS-NRCT Asia Core Program for their financial contributions, the Research Centre of Fermentation of Value Added Agricultural Products (FerVAAP) for their facilities. Finally, special thanks to P. Seubchuir and A. Srisaen who conducted some of the experiments.

References

[1] H. Siller, J. Winter, Degradation of cyanide in agroindustrial or industrial wastewater in an acidification reactor or in a single-step methane reactor by bacteria enriched from soil and peels of cassava, Applied Microbiology and Biotechnology 50 (1998) 384–389.
[2] C. Balagopalan, L. Rajalekshmy, Cyanogen accumulation in environment during processing of cassava (*Manihot esculenta* Crantz) for starch and sago, Water Air and Soil Pollution 102 (1998) 407–413.
[3] S.C. Chena, J.K. Liu, The response to cyanide of cyanide-resistant *Klebsiella oxytoca* bacterial strain, FEMS Microbiology Ecology 175 (1999) 37–43.
[4] Y.B. Patil, K.M. Paknikar, Development of a process for biodetoxification of metal cyanide from wastewater, Process Biochemistry 35 (2000) 1139–1151.
[5] A. Watanabe, K. Yano, K. Ikebukuro, I. Karube, Cyanide hydrolysis in cyanide-degrading bacterium, *Pseudomonas stutzeri* AK61, by cyanidase, Microbiology 144 (1998) 1677–1682.
[6] M.M. Figueria, V.S.T. Ciminelli, M.C. De Andrade, V.R. Linardi, Cyanide degradation by an *Escherichia coil* strain, Canadian Journal of Microbiology 42 (1996) 519–523.
[7] H. Yanase, A. Sakamoto, K. Okamoto, K. Kita, Y. Sato, Degradation of the metal–cyano complex tetracyanonickelate (II) by *Fusarium oxysporium* N-10, Applied Microbiology and Biotechnology 53 (2000) 328–334.
[8] H.J. Gijzen, E. Bernal, H. Ferrer, Cyanide toxicity and cyanide degradation in anaerobic wastewater treatment, Water Research 34 (2000) 2447–2454.
[9] A. Akcil, A.G. Karahan, H. Ciftci, O. Agdic, Biological treatment cyanide by natural isolated bacteria (*Pseudomonas* sp.), Materials Engineering 16 (2003) 643–649.
[10] S. Sirianuntapiboon, C. Chuamkaew, Packed cage rotating biological contactor system for treatment of cyanide wastewater, Bioresource Technology 98 (2007) 266–272.
[11] C.M. Kao, J.K. Liu, H.R. Lou, C.S. Lin, S.C. Chen, Biotransformation of cyanide to methane and ammonia *Klebsiella oxytoca*, Chemosphere 50 (2003) 1055–1061.
[12] S. Ebbs, Biological degradation of cyanide compounds, Current Opinion in Biotechnology 15 (2004) 231–236.
[13] Metcalf and Eddy, Inc., Wastewater Engineering: Treatment, Disposal and Reuse, 3rd ed., McGraw Hill, USA, 1991.
[14] A. Marín, L.J. Liermann, S.L. Brantley, V. Lebron, The release of Fe and Mo from silicate by *Azotobacter vinelandii*, in: Proceeding of Eleventh Annual V.M. Goldschmidt Conference, University Park, PA, USA, January 15–17, 2001, p. 3789.
[15] M.G. Yates, The enzymology of molybdenum-dependent nitrogen fixation, in: G. Stacey, R.H. Burris, H.J. Evans (Eds.), Biological Nitrogen Fixation, Chapman & Hall, New York, 1992, pp. 685–735.
[16] Y.B. Yan, Q. Wu, R.Q. Zhang, Dynamic accumulation and degradation of poly 3-hydroxyalkanoate in living cell of *Azotobacter vinelandii* UWD characterized by C13 NMR, FEMS Microbiology Letters 193 (2000) 269–273.
[17] F. Kargi, S. Ozmuci, Performance of *Azotobacter* supplemented activated sludge in biological treatment of nitrogen deficient wastewater, Process Biochemistry 38 (2002) 57–64.
[18] S.A. Raybuck, Microbes and microbial enzymes for cyanide degradation, Biodegradation 3 (1992) 3–18.
[19] R. Colnaghi, S. Pagani, C. Kennedy, M. Drummond, Cloning, sequence analysis and overexpression of the rhodanese gene of *Azotobacter vinelandii*, European Journal of Biochemistry 236 (1996) 240–248.
[20] K. Fisher, M.J. Dilworth, C.H. Kim, W.E. Newton, *Azotobacter vinelandii* nitrogenases with substitutions in the FeMo-cofactor environment of the MoFe protein: effects of acetylene or ethylene on interactions with H^+, HCN, and CN^-, Biochemistry 39 (2000) 10855–10865.
[21] C. Balis, J. Chatzipavlidis, F. Flouri, Olive mill waste as substrate for nitrogen fixation, International Biodeterioration & Biodegradation 38 (1996) 169–174.
[22] C. Ehaliotis, K. Papadopoulou, M. Kotsou, I. Mari, C. Balis, Adaptation and population dynamics of *Azotobactor vinelandii* during aerobic biological treatment of olive-mill wastewater, FEMS Microbiology Ecology 30 (1999) 301–311.
[23] D.J. Gapes, N.M. Frost, T.A. Colark, P. Dare, R.G. Hunter, A.H. Slade, Nitrogen fixation in treatment of pulp and paper wastewater, Water Science and Technology 40 (1999) 85–92.
[24] G.W. Strandberg, P.W. Wilson, Formation of the nitrogen-fixing enzyme system in *Azotobacter vinelandii*, Canadian Journal of Microbiology 14 (1968) 25–31.
[25] W.F. Harrigan, M.E. McCance, Laboratory Methods in Microbiology, Academic Press, London, 1996.
[26] APHA, AWWA, Standard Methods for the Examination of Water and Wastewater, 15th ed., APHA, AWWA, Washington, DC, USA, 1995.
[27] G. Drochioiu, Fast and highly selective determination of cyanide with 2,2-dihydroxy-1,3-indanedione, Talanta 56 (2002) 1163–1165.
[28] H.N.P. Mai, L.N. Thai, N.T. Viet, G. Lettinga, Effect of organic loading rate on treatment efficiency for tapioca processing wastewater using UASB, in: Proceedings of International Conference on Industry and Environment in Viet Nam, HCMC, Viet Nam, April 20–21, 2001, pp. 224–233.
[29] L.T.K. Oanh, K. de Jong, H.N.P. Mai, N.T. Viet, Removing suspended solids from tapioca processing wastewater in upflow anaerobic filter (UAF), in: International Conference: Industry and Environment in Vietnam, Ho Chi Minh City, Vietnam, 2001.
[30] T. Nandy, S.N. Kaul, V.S.S. Sekhar, Waste management in the tapioca based starch industry, Journal of Environmental Studies 48 (1995) 81–96.
[31] L.V. Khoa, Tapioca starch industry in Vietnam and environmental pollution, in: Proceedings of the Workshop on Pollution Reduction in Tapioca Processing Industry in Viet Nam, HCMC, Viet Nam, January 16, 1998.
[32] D. Peters, D.D. Ngai, D.T. An, Agro-processing wastewater assessment in peri-urban Hanoi, CIP Program Report, 2000, pp. 451–457.
[33] P.G. Hien, L.T.K. Oanh, N.T. Viet, G. Lettinga, Closed wastewater system in the tapioca industry in Vietnam, Water Science and Technology 39 (1999) 89–96.
[34] X. Colin, J.-L. Farinet, O. Rojas, D. Alazard, Anaerobic treatment of cassava starch extraction wastewater using a horizontal flow filter with bamboo as support, Bioresource Technology 98 (2007) 1602–1607.
[35] J.G. Li, B.K. Burgess, J.L. Corbin, Nitrogenase reactivity: cyanide as substrate and inhibitor, Biochemistry 21 (1982) 4393–4402.
[36] C.M. Kao, S.H. Li, Y.L. Chen, S.C. Chen, Utilization of the metal-cyano complex tetracyanonickelate (II) by *Azotobacter vinelandii*, Letters in Applied Microbiology 41 (2005) 216–220.
[37] N.P.M. Huynh, Integrated treatment of tapioca processing industrial wastewater based on environmental bio-technology, Ph.D. Thesis, Wageningen University, Netherlands, 2006.