Cyanide kinetics and factors influencing its biodegradation

Lovasoa Christine RAZANAMAHANDRY[1], Harinaivo Anderson ANDRIANISA[1], Hela KAROUI[1]and Hamma YACOUBA[2]

[1]International Institute for Water and Environmental Engineering (2iE), Department of Water and Sanitary Engineering, Laboratory of Water, Decontamination, Ecosystem and Health (LEDES), 01 PO Box 594, 01, Ouagadougou, Burkina Faso

[2]International Institute for Water and Environmental Engineering (2iE), Department of Civil and Hydraulic Engineering, Laboratory of Water Resources and Hydrology (LEAH), 01 PO Box 594, 01, Ouagadougou, Burkina Faso

Corresponding author: Lovasoa Christine RAZANAMAHANDRY (c.razanamahandry@2ie-edu.org or tantely1989@gmail.com)

Abstract

Cyanide Degrading Bacteria (CDB) colonies were isolated from soil and water that collected into an Artisanal Small scale Gold Mining (ASGM) area. The biological degradation of the free cyanide (FCN) in laboratory scale was investigated using these CDB colonies. FCN test was conducted under various cultural conditions as pH, ranging from 5 to 10.5, carbon and nitrogen sources. After 24h, it was found that FCN removal efficiency was varied between 99.2 % for pH = 10.5 and 99.8 % for pH = 9.5. Glucose and ammonium sulphate were given a maximum FCN removal efficiency 100 % and 99.8 %, respectively, as carbon and nitrogen sources. All of nutrients sources have FCN removal efficiency above 94 %. The Michaelis–Menten kinetics enzymatic parameters were determined. The CDB colonies enzyme had a half-saturation constant, K_M of 14 mmol CN^- degraded L^{-1} for FCN and a specific activity of 28.4 mmol NH4+ formed mol^{-1} CN^- initial min^{-1}. It was conclude that the CDB colonies were highly promising for bioremediation environmental application. They could support a large pH range and a various nutrients available in natural environment.

Key Words:

- Mickaelis-Menten
- Bioremediation
- Cyanide degrading bacteria
- Kinetics
- Burkina Faso

1 Introduction

Cyanide is a chemical compounds that is widely used in mining and jewellery industries for gold extraction and recovery from crushed ores or electroplating residues (Huertas et al., 2010; Luque-Almagro et al., 2016). The mining industry releases large amounts of cyanide-containing liquid waste which often contains significant amounts of heavy metals as arsenic, lead, mercury, cadmium, chromium and sulphuric acid (Luque-Almagro et al., 2011, 2016) These hazardous effluents are a highly toxic (Huertas et al., 2010; White et al., 2000) to living organisms via at least three known mechanisms which involve the formation of (a) complexes with di- or tri-valent metals at the active site of metallo-enzymes such as cytochrome oxidase (Luque-Almagro et al., 2016; Solomonson & Spehar, 1981; Watanabe & Yano, 1993); (b) Schiff base intermediates leading to stable nitrile derivatives during enzyme catalysis; or (c) cyanohydrins with metabolic keto groups (Huertas et al., 2010; Solomonson & Spehar, 1981) . Knowing that the acute lethal dose of cyanide for mammals is as low as 0.5mg CN/kg of the body weight, the acute oral lethal dose of HCN for humans is reported to be 0.5– 3.5mg/kg of body weight (Chaouali et al., 2013). These toxic liquid residues must be treated by chemical and/or biological method to minimize the health and environmental risks (Luque-Almagro et al., 2016). Biological method that is less expensive and environmental friendly is often applied (Akcil, 2003; Pandey & Jain, 2002; Shete & Kapdnis, 2012). In order to apply the bioremediation in the natural environmental, studies of CDB colonies efficiency for removing cyanide have been paid more attentions (Kumar et al., 2016; Kumar et al., 2013; Safari et al., 2014; Van Zyl et al. 2011) Several parameters as pH, temperature and nutrients were more considered and varied for showing their ability to grow and survive under these conditions (Diaz & Caizaguano, 1999; Huertas et al., 2010; Nybom, 2013; Vieira & Nahas, 2005).

In this context, indigenous microorganisms could be able to degrade FCN in laboratory scale have been reported into the ASGM sites in Burkina Faso (Razanamahandry et al., 2016). Cyanide is used for extracting gold in these sites. The optimal conditions of CDB colonies needs to know deeply before their application in natural environment for remediating the entity polluted by cyanide. In which, factors that influence these CDB colonies activities and their enzymatic kinetics need to investigate. .

The aim of this study was to identify the factors influencing the cyanide biodegradation for the first time and to establish an enzymatic kinetics model of the cyanide biodegradation in second time.

2 Materials and Methods

2.1 Microorganisms source

Microorganisms were isolated from the water that collected in the artisanal small scale gold mining (ASGM) sites. The isolation procedure was shown in Razanamahandry et al. (2016)

2.2 Chemical reagents and analytical methods

The chemical reagents used, FCN and ammonium concentrations analytical methods and the living bacterial species measurement were described in Razanamahandry et al. (2016) .

Total cyanide (TCN) was determined by using Flow Injection Analysis (FIA) method.

The chemical reagents that used for TCN analysis were formed by: NaOH , Chloramine T-trihydefficiency, Sulfamic Acid, Citric acid monohydefficiency, Succinic acid, Pyridine-4-carbonic acid, 1,3-Dimethylbarbituric acid, Tetraethylenepentamine, Disodium ethylenediamine tetraacetic acid, Sodium thiosulfate pentahydefficiency, Lead acetate trihydefficiency, Cyanide

standard solution 1000 mg L-1, potassium hexacyanoferefficiency II trihydefficiency, potassium hexacyanoferefficiency III, KCN. They are purchased at Sigma Aldrich, France.

2.3 Experimental procedure

2.3.1 Factor influencing the cyanide biodegradation

Eleven liquid mediums formed by 200 mL of FCN solutions with a concentration 60 mg CN^- L^{-1} were prepared in 250 mL Erlenmeyer flasks by using KCN from VWR (France). Note that 1 mg KCN equals 0.4 mg CN. 1 mL of CDB colonies was added into the Erlenmeyer flask.

FCN solutions pH was regulated at 5, 7, 9.5 and 10.5 values for testing the pH influence on the FCN biodegradation.

Nutrients influence was tested by adding 3 mL of nitrogen or carbon sources solutions 10 g L^{-1}, that were homogenized and autoclaved at 121 °C for 15 min, into the FCN solution. Nitrogen sources added can be divided into: (1) inorganic sources as ammonium sulphate and (2) organic source as meat extract, yeast extract and peptone. The carbon sources tested were: (1) monosaccharide as glucose, (2) disaccharides as sucrose and (3) monoglycerides as glycerol.

Then, the mixtures were shaken at 200 rpm during 24 hours by using a magnetic stirrer. 35 mL of the mixture were collected at the beginning of the experiment, and every 2 hours for monitoring FCN, living bacterial species and ammonium concentrations.

2.3.2 Enzymatic kinetics

Eight (08) Liquid mediums for each FCN and TCN solutions with a concentration varying 10, 20, 30, 40, 50, 60, 70 and 80 mg CN^- L^{-1} were freshly prepared into the Erlenmeyer flask. 1 mL of CDB colonies was added into the liquid medium and the mixture was shaken. 35 mL for each

liquid medium was taken for analysing the FCN, TCN, living bacteria and NH_4^+ product at the initial time (t = 0 mn) and after ten minutes (t = 10 mn).

2.4 Enzymatic kinetics model

Enzymatic reactions can be described by the Michaelis–Menten kinetics:

$$v = -\frac{dS}{dt} = \frac{dP}{dt} = \frac{v_{max}S}{K_M + S}$$

Where:

v : disappearance velocity of the substrate efficiency S

v_{max} : maximum disappearance velocity

K_M : half-saturation constant (the substefficiency concentration where the reaction velocity is half the maximum).

P: concentration of the product

v_{max} and K_M values could be evaluated by creating a Lineweaver– Burk plot in which the plot $1/v$ versus $1/S$ was shown, with:

$v_{max} = 1/b$ and $K_M = a/b$

where

a and b are the linear trend coefficients of Lineweaver– Burk plot: $y = ax + b$

2.5 Factors influencing the cyanide biodegradation

3 Results and discussion

3.1 pH influence

At the first time, the FCN biodegradation and the bacterial growth under various pH values during 24 hours were shown on the Figure 1. Two trend tendencies were observed. The first

group, in which the curves are in decreased, demonstrate the FCN removal efficiency and in the second group, where the curves are increased, relates the bacterial growth. The FCN was highly and quickly biodegraded during the first 10 h when the bacterial growth was relatively stable for all pH values. Then, the FCN decreased slowly until it's completely removal within 24 h. Yet, the bacterial growth became fast from 10 to 24 h. These curves trend have been reported on Razanamahandry et al. (2016) in which these results suggest that the FCN was removed by the living microorganisms and it is a biological mechanisms. The curve trends were the same for all pH values tested. This result suggests that the FCN was removed by the CDB colonies under various pH.

However, at the pH = 5 and 10.5, the bacterial growth is not very higher than under another pH value and the CDB colonies have a difficulty to remove completely the FCN from 10 to 24h. At the pH = 7, a little increase of CDB colonies density was observed and the FCN removal became more interesting, but FCN was completely removed at 24 h. At the pH = 9.5, FCN was completely removed by the CDB colonies from 15 to 24 h. The CDB activity was limited on the high acidity and alkalinity conditions. The pH 9.5 is the optimum pH obtained in which the bacterial growth is very high and the FCN removal efficiency is near the 100 %. Nevertheless, all of pH values were given FCN removal efficiency more than 95 % during 24h for 60 mg CN^{-} L^{-1}. Mekuto et al. (2013) have been reported at the same pH = 9.5 a maximum FCN removal efficiency 89.5 % over 8 days for degrading FCN concentration 200 mg CN^{-} L^{-1} by using *Bacillus sp.* consortium.

The CDB colonies that used have a large pH ranging (acidic, neutral and alkaline). The bioremediation application in environmental conditions of these CDB colonies is more

advantageous because whatever the pH range they could be remove with a high efficiency the FCN.

In addition, the large pH rang could be explained by the presence of several CDB bacterial species in the colonies. Some authors have been found an optimal pH 6, 7, 5, 10 for FCN removal by using respectively the bacterial *Serretia marcescens RL2b* (Kumar et al., 2013) , *Rhodococcus sp. UKMP-5M* (Maniyam et al., 2011), *Pseudonomas Fluorescens* (Dursun et al., 1999), B*ulkhoderia cepacia* (Adjei and Ohta, 2000) . Yet, most of them were demonstrated at the presence of *Pseudomonas sp.* (Akcil et al., 2003; Huertas et al., 2010; Kao et al., 2003; Kiruthika, 2008) and *Bacillus sp.* (Mekuto et al., 2013; Wu et al., 2014) at the pH around 9.5.

FCN biodegradations were associated by an ammonium product during 24 h. The results are the same reported in Razanamahandry et al. (2016) that have been reported the FCN removed was converted into ammonium product that the living microorganisms degrade finally to form another product as nitrate as a final by-product (Chapatwala et al., 1998; Ingvorsen & Godtfredsen, 1991; Kao et al., 2003; Meyers et al., 1991; Potivichayanon & Kitleartpornpairoat, 2010; Watanabe & Yano, 1993)

Figure 2 shows that at the pH = 9.5 the cyanide removal efficiency is very high (99.8 %) after 24h. But, the maximum cyanide removal efficiencys are almost the same for each pH value. CDB colonies have not preferencies in term of pH. CDB colonies have a large range of pH (acidic – neutral- basic) for survival. This is an advantage for our CDB colonies because they could be easily adapted in the nature for either application of the bioremediation. Species that formed the consortium need to identify for knowing what are the species could be supported the acidic or neutral or basic conditions.

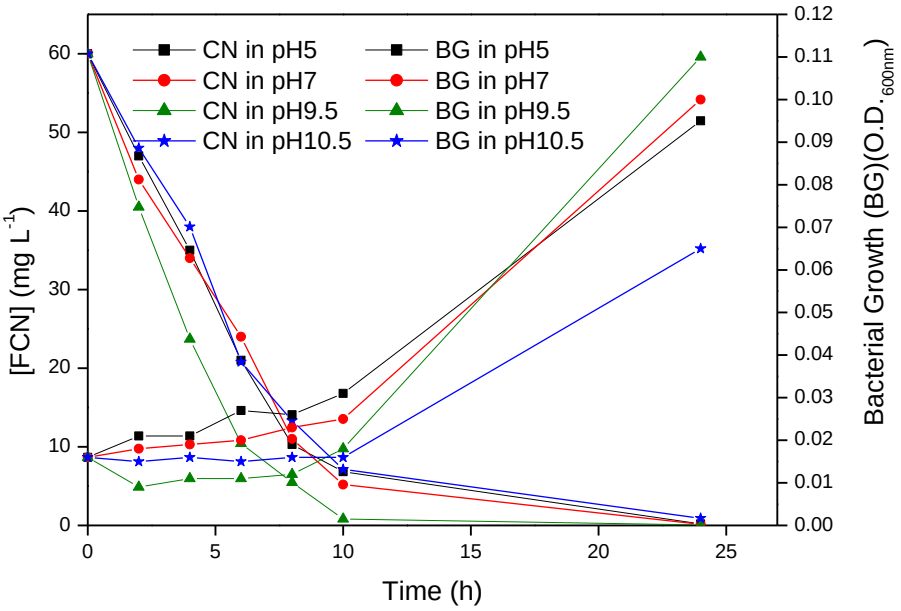


Figure 1 : FCN removal and CDB growth in liquid medium under various pH

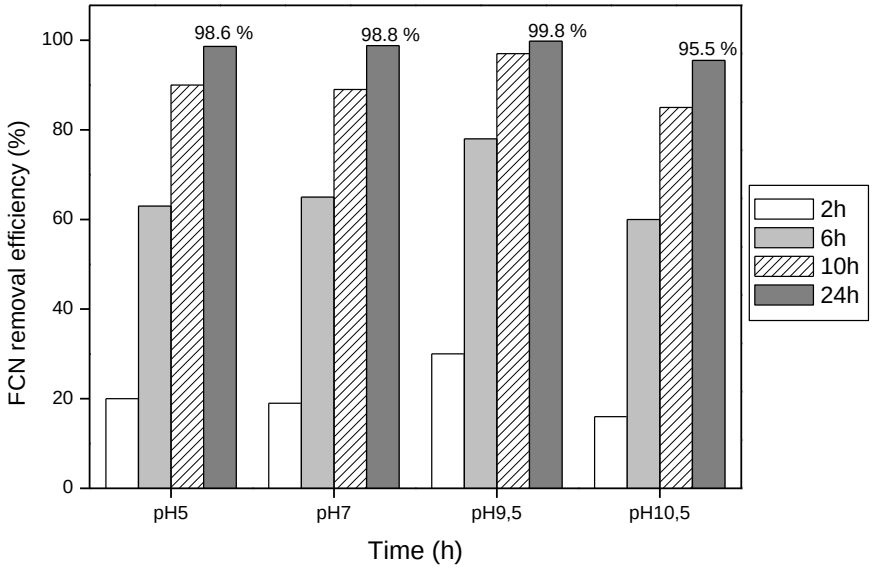


Figure 2: Effect of pH on the FCN biodegradation

3.2. Nutrients influence

Figure 3 below demonstrates the FCN removal and the CDB growth under various nutrients sources. Decreased curves illustrate the FCN removal and increased curves for the CDB growth. For the first 10 h where the initial FCN concentration is 60 mg CN^- L^{-1}, the FCN removal decreased quickly but the CDB increased slowly. Then, between 10 and 24h, the FCN was

completely removed and the CDB growth became very intense. The FCN was almost totally removed for the mediums where glucose and ammonium sulphate nutrients were added on 10h and 15 h respectively. Nevertheless under other nutrients the FCN removal was completely on exactly 24 h where the CDB growths are on the peak for all nutrients. But in the medium containing glucose and ammonium sulphate, the CDB growth is very high and have an optimal density respectively OD_{600nm} = 0.025 and OD_{600nm} = 0.011. Two hours after 24h, CDB growth declined. NH_4^+ production was detected during the entire test.

The CDB colonies could be able to degrade FCN under all of nutrients tested. They have been converted the FCN to another compounds less toxic. FCN removal was quickly by using glucose and ammonium sulphate nutrients as carbon and nitrogen source respectively. The CDB colonies have more ability to growth in the medium with nitrogen sources than the medium with carbon sources. CDB could not survive without FCN and nutrients.

Mirizadeh et al. (2014) have been reported that the CDB colonies could be able to grow in the FCN medium that containing carbon and/ or nitrogen source as nutrients. They suggest the CDB colonies were formed by two groups of bacteria such as heterotroph and lithotroph bacteria. The CDB colonies that could be oxidizing respectively an organic and inorganic form were called heterotroph and lithotroph bacteria. The optimum FCN removal condition was obtained by using the nutrients: glucose and ammonium sulphate. Yet, the bacterial growth was $OD_{600\ nm}$ = 0.011 at the end of FCN substrate.

Glucose was found by many authors as a nutrient that give an optimum FCN removal (Kiruthika, 2008; Kumar et al., 2013; Mirizadeh et al., 2014). In addition, the same authors also have been found that when the nutrients are unavailable the CDB growth decreased.

The CDB colonies used during the test were formed by two groups of bacteria such as heterotroph and lithotroph bacteria. They need nutrients as glucose and ammonium sulphate for more removing FCN in short time. Their enzymes activities are not inhibited by the carbon and nitrogen sources used (Brandt et al., 2014; Couto & Toca-Herrera, 2007; Li et al., 2012)

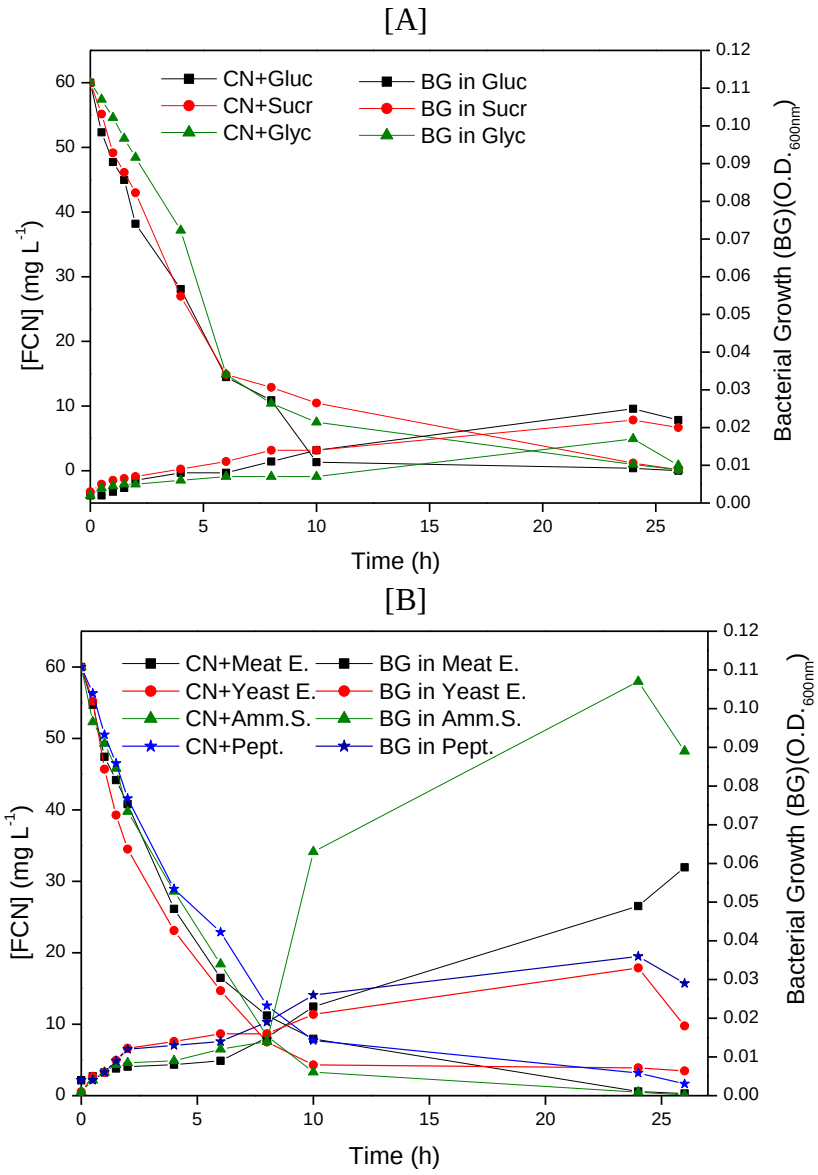


Figure 3: FCN removal and CDB growth in liquid medium enriched by nutrients: carbon sources [A] and nitrogen sources [B]

Figure 4 illustrate the FCN removal efficiency for all nutrients tested. Optimum FCN removal efficiency was observed with the presence of glucose and ammonium sulphate with a FCN removal efficiency respectively 100 % and 99.8 % after 24 h. Yet, the entire nutrients used have shown FCN removal efficiency more than 94 %. Three nutrients have the minimal and the same value, around the 94 %, of the FCN efficiency as peptone, meat extract and yeast extract.

The FCN degradation quickly by glucose and ammonium sulphate on ten 10 h could be explaining by these best FCN removal efficiency. Alavi (2011) have been reported that glucose is a monosaccharide sugar that could be degraded easily by the CDB colonies than another sugars as sucrose and glycerol. In addition, the ammonium sulphate was constituted by a higher nitrogen percentage than another nitrogen sources. Therefore, nitrogen that more available in ammonium sulphate could be explain the quickly and highest of CDB growth.

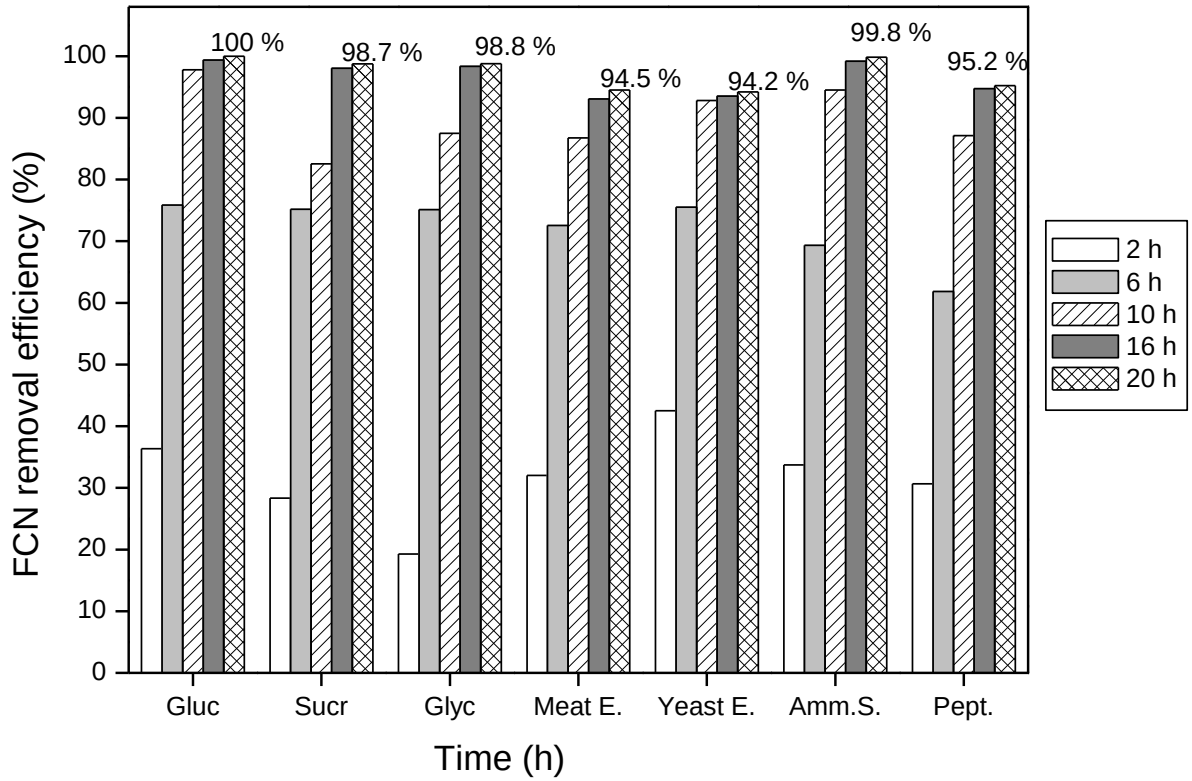


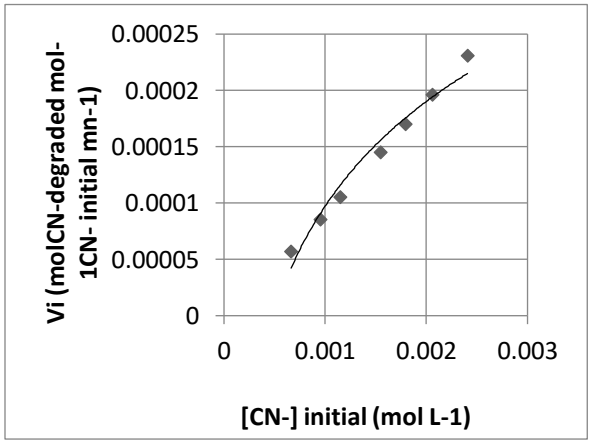
Figure 4: Effects of nutrients sources on the FCN biodegradation, where: Gluc = glucose; Sucr = sucrose; Glyc = glycerol; Meat E. = Meat Extract; Yeast E. = Yeast Extract; Amm.S. = Ammonium Sulphate; Pept. = Peptone

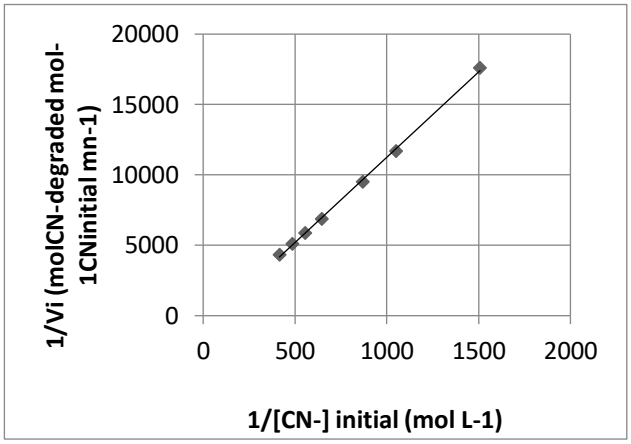
3.3 FCN biodegradation kinetics

The disappearance velocity v of the substrate efficiency S was determined by two ways, from the substrate concentrations degraded (CN- degraded) and from the product formed (NH4+ formed) as shown in Figure 5. Figure 5a shows the typical Michaelis– Menten-type kinetics for enzyme activity increasing with FCN concentrations tested. The Lineweaver–Burk plot (Figure 5b) obtained for the conversion of FCN shows that the Km for FCN is 14 mM. CDB colonies tested have a specific activity of 28.4 mmol NH4+ formed mol^{-1} CN^- initial min^{-1}.

[a] [b]

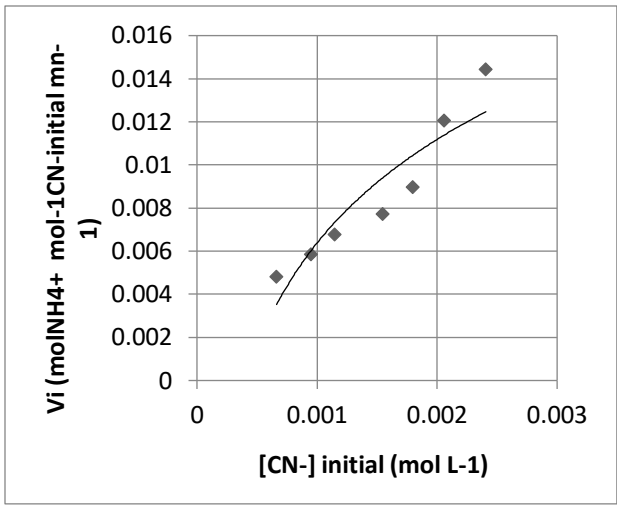
From substrate

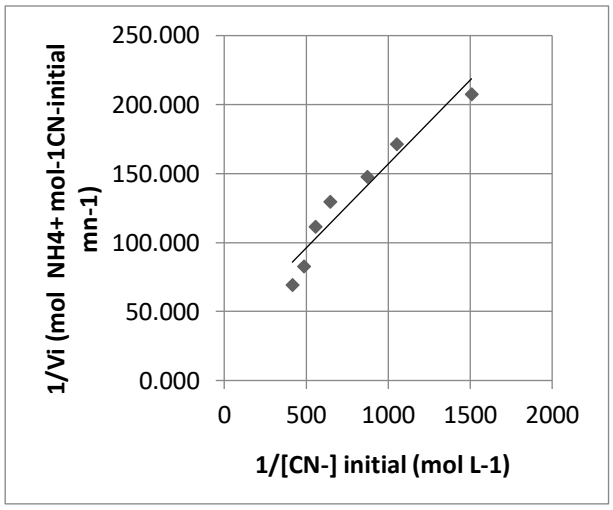




$y = 12.114x + 861.6$
$R^2 = 0.9989$
Vmax = 1.16 mmol CN - degraded mol^{-1} CN initial min^{-1}
Km = 14 mmol CN - degraded L^{-1}

From product





$y = 0.1217x + 35.239$
$R^2 = 0.9329$
Vmax = 28,4 mmol NH4+ formed mol^{-1} CN- initial min^{-1}
Km = 3 mmol NH4+ formed L^{-1}

Figure 5: Determination of Michaelis–Menten parameters of FCN removal by CDB colonies in liquid medium. [a] Non-linear regression, [b] Lineweaver– Burk plot

4 Conclusions

After 24 h, the bacteria consortium seems to prefer the nutrients glucose and ammonium sulfate as a carbon and nitrogen source respectively with FCN removal efficiency 100 % and 99.82 %. Nevertheless, the FCN removal efficiency is more than 94 % for all of nutrients. The CDB colonies tested could be survive under large pH ranging (acidic – neutral – alkaline) and use different carbon and nitrogen sources. All of carbon and nitrogen sources have not been caused the oxidative stress in the cells. Their enzymatic activity was not inhibited. They could be easily adapted in several type of soil for further future application of the bioremediation. Yet, the organic matter in the site soil needs to study before applying this bioremediation on the first time. In the second time, the enzymatic and proteins type for each species will be to determine after its isolation and identification.

Acknowledgments

This present study was funded by the Swiss Agency for Development and Cooperation under the 3E Programme, Grant Number 81016359/1. Authors would like to particularly thank Dr Seyram SOUSOU who gave additional advices for the data analysis, to the laboratory technicians and Miss Chérifatou IBRAHIMOU master students at 2iE who contributed in data collection and FCN analysis.

References

Adjei, M., & Ohta, Y. (2000). Factors affecting the biodegradation of cyanide by Burkholderia cepacia strain C-3. *Journal of Bioscience and Bioengineering*, *89*, 274–277.

Akcil, A. (2003). Destruction of cyanide in gold mill effluents: biological versus chemical treatments. *Biotechnology Advances*, *21*(6), 501–511. http://doi.org/10.1016/S0734-9750(03)00099-5

Akcil, A., Karahan, A. G., Ciftci, H., & Sagdic, O. (2003). Biological treatment of cyanide by natural isolated bacteria (Pseudomonas sp.). *Minerals Engineering*, *16*, 643–649.

Alavi, A. (2011). *Remediation of a heavy metal and PAH-contaminated sediment by a rhamnolipid foam*. The Department of Building , Civil and Environmental Engineering Presented in Partial Fulfillment of the Requirements for the Degree of Master of Applied Science at Concordia University Montreal, Quebec, Canada.

Brandt, U., Waletzko, C., Voigt, B., Hecker, M., & Steinbüchel, A. (2014). Mercaptosuccinate metabolism in Variovorax paradoxus strain B4--a proteomic approach. *Applied microbiology and biotechnology*, *98*(13), 6039–50. http://doi.org/10.1007/s00253-014-

5811-7

Chaouali, N., Gana, I., Dorra, A., Khelifi, F., Nouioui, A., Masri, W., … Hedhili, A. (2013). Potential Toxic Levels of Cyanide in Almonds (Prunus amygdalus), Apricot Kernels (Prunus armeniaca), and Almond Syrup. *ISRN toxicology*, *2013*, 610648. http://doi.org/10.1155/2013/610648

Chapatwala, K. D., Rabu, G. R. V., Vijaya, O. K., Kumar, K. P., & Wolfram, J. H. (1998). Biodegradation of cyanides, cyanates and thiocyanates to ammonia and carbon dioxide by immobilized cells of Pseudomonas putida. *J Ind Microbiol and Biot.*, *20*, 28–33.

Couto, S., & Toca-Herrera, J. (2007). Laccase production at reactor scale by filamentous fungi. *Biotechnol. Adv.*, *25*, 558–569.

Diaz, X; Caizaguano, R. (1999). Bioremem diation of cyanide leaching residues. *Escuela Polit6cnica Nacional, Instituto de Investigaci6n Tecnol6gica, P.O.Box 17-01-2759, Quito - Ecuador*, 595–605.

Dursun, A.Y. ; Alık, A. C.; Aksu, Z. (1999). « Degradation of ferrous(II) cyanide complex ions by Pseudomonas fluorescens ». *Process Biochemistry*, *34*, 901–908.

Gurbuz, F., Ciftci, H., & Akcil, A. (2009). Biodegradation of cyanide containing effluents by Scenedesmus obliquus. *Journal of hazardous materials*, *162*(1), 74–9. http://doi.org/10.1016/j.jhazmat.2008.05.008

Huertas, M. J., Saez, L. P., Rolda, M., Luque-Almagro, V. M., & Martinez-Luque, M. et al. (2010). A Alkaline cyanide degradation by Pseudomonas pseudoalcaligenes CECT5344 in a

batch reactor.Influence of pH. *Journal of Hazardous Materials*, *179*, 72–78.

Ingvorsen, K., & Godtfredsen, S. E. (1991). Novel Cyanide-Hydrolyzing Enzyme from Alcaligenes xylosoxidans subsp . denitrificans. *Applied and Environmental Microbiology*, *57*(6), 1783–1789.

Kao, C., Liu, J., Lou, H., Lin, C., & Chen, S. (2003). Biotransformation of cyanide to methane and ammonia by Klebsiella oxytoca. *Chemosphere*, *50*, 1055–1061.

Kao, C. M., Liu, J. K., Lou, H. R., Lin, C. S., & Chen, S. C. (2003). Biotransformation of cyanide to methane and ammonia by Klebsiella oxytoca. *Chemosphere*, *50*(8), 1055–1061. http://doi.org/10.1016/S0045-6535(02)00624-0

Kiruthika, A. J. S. (2008). Cyanide Detoxification and Recovery of Gold from Gold Effluent. *Advanced biotechnoogy*, 20–26.

Kumar, R., Saha, S., Dhaka, S., Kurade, M. B., Kang, C. U., Baek, S. H., & Jeon, B.-H. (2016). Remediation of cyanide-contaminated environments through microbes and plants: a review of current knowledge and future perspectives. *Geosystem Engineering*, *0*(0), 0. http://doi.org/doi=10.1080%2F12269328.2016.1218303

Kumar, V., Kumar, V., & Bhalla, T. C. (2013). In vitro cyanide degradation by Serretia marcescens RL2b. *International Journal of Environment Sciences*, *3*(6), 1969–1979. http://doi.org/10.6088/ijes.2013030600018

Li, Z., Bi, J., Yang, J., Pan, J., Sun, Z., & Zhu, X. (2012). Requirement of a Tsp2-type tetraspanin for laccase repression and stress resistance in the basidiomycete Cryptococcus

neoformans. *Applied and environmental microbiology*, *78*(1), 21–7. http://doi.org/10.1128/AEM.06072-11

Luque-Almagro, V. M., Blasco, R., Martínez-Luque, M., Moreno-Vivián, C., Castillo, F., & Roldán, M. D. (2011). Bacterial cyanide degradation is under review: Pseudomonas pseudoalcaligenes CECT5344, a case of an alkaliphilic cyanotroph. *Biochemical Society transactions*, *39*(1), 269–74. http://doi.org/10.1042/BST0390269

Luque-Almagro, V. M., Moreno-Vivián, C., & Roldán, M. D. (2016). Biodegradation of cyanide wastes from mining and jewellery industries. *Current Opinion in Biotechnology*, *38*, 9–13. http://doi.org/10.1016/j.copbio.2015.12.004

Maniyam, M. N., Sjahrir, F., & Ibrahim, A. L. (2011). Biodegradation of Cyanide by Rhodococcus Strains Isolated in Malaysia. *International Conference on Food Engineering and Biotechnology*, *9*, 21–25.

Mekuto, L., Jackson, V. A., Ntwampe, K. S., & Obed; (2013). Biodegradation of Free Cyanide Using Bacillus Sp . Consortium Dominated by Bacillus Safensis , Lichenformis and Tequilensis Strains : A Bioprocess Supported Solely with Whey. *Bioremediation & Biodegradation*, (18), 1 –7. http://doi.org/10.4172/2155-6199.S18-004

Meyers, P. R., Gokool, P., Rawlings, D. E., & Woods, D. R. (1991). An efficient cyanide-degrading Bacillus pumilus strain. *Journal of General Microbiology*, *137*(6), 1397–1400. http://doi.org/10.1099/00221287-137-6-1397

Mirizadeh, S., Yaghmaei, S., & Ghobadi Nejad, Z. (2014). Biodegradation of cyanide by a new isolated strain under alkaline conditions and optimization by response surface methodology

(RSM). *Journal of environmental health science & engineering*, *12*(1), 85. http://doi.org/10.1186/2052-336X-12-85

Nybom, S. (2013). Biodegradation of Cyanobacterial Toxins.

Pandey, G., & Jain, R. K. (2002). Bacterial Chemotaxis toward Environmental Pollutants : Role in Bioremediation MINIREVIEWS Bacterial Chemotaxis toward Environmental Pollutants : Role in Bioremediation †, *68*(12). http://doi.org/10.1128/AEM.68.12.5789

Potivichayanon, S., & Kitleartpornpairoat, R. (2010). Biodegradation of Cyanide by a Novel Cyanide- degrading Bacterium. *World Academy of Science, Engineering and Technology*, *4*(6), 1093–1096.

Razanamahandry, L. C., Andrianisa, H. A., Karoui, H., Kouakou, K. M., & Yacouba, H. (2016). Biodegradation of free cyanide by bacterial species isolated from cyanide-contaminated artisanal gold mining catchment area in Burkina Faso. *Chemosphere*, *157*, 71–78. http://doi.org/10.1016/j.chemosphere.2016.05.020

Safari, M., Amache, R., Esmaeilishirazifard, E., & Keshavarz, T. (2014). Microbial metabolism of quorum-sensing molecules acyl-homoserine lactones, γ-heptalactone and other lactones. *Applied Microbiology and Biotechnology*, *98*(8), 3401–3412. http://doi.org/10.1007/s00253-014-5518-9

Shete, H. G., & Kapdnis, B. P. (2012). Production and Characterization of Cyanide Hydratase from Micromonospora braunna Abstract : *Universal Journal of Environmental Research and Technology*, *2*(6), 609–615.

Solomonson, L. P., & Spehar, A. M. (1981). Cyanide as a metabolic inhibitor. In B. Vennesland, E. E. Conn, C. J. Knowles, & J. Westley & F. Wissing (Reds), *Cyanide in Biology* (bll 11–28). London : Academic Press.

Toyama, T., Momotani, N., Ogata, Y., Miyamori, Y., Inoue, D., Sei, K., … Ike, M. (2010). Isolation and characterization of 4-tert-butylphenol-utilizing Sphingobium fuliginis strains from Phragmites australis rhizosphere sediment. *Applied and environmental microbiology*, *76*(20), 6733–40. http://doi.org/10.1128/AEM.00258-10

Van Zyl, A. W., Harrison, S. T. L., & Hille, R. P. V. (2011). Biodegradation of thiocyanate by a mixed microbial population. In *Mine Water - Managing the Challenges. IMWA* (bll 119–124). Aachen, Germany.

Vieira, F. C. S., & Nahas, E. (2005). Comparison of microbial numbers in soils by using various culture media and temperatures. *Microbiological research*, *160*(2), 197–202. http://doi.org/10.1016/j.micres.2005.01.004

Watanabe, A., & Yano, K. (1993). Cyanide hydrolysis in a cyanide-degrading bacterium , Pseudornonas stutzeri AK61 , by cyanidase, (1 998), 1677–1682.

White, D. M., Pilon, T. A., & Woolard, C. (2000). BIOLOGICAL TREATMENT OF CYANIDE CONTAINING WASTEWATER, *34*(7), 2105–2109.

Wu, C.-F., Xu, X.-M., Zhu, Q., Deng, M.-C., Feng, L., Peng, J., … Wang, J.-H. (2014). An effective method for the detoxification of cyanide-rich wastewater by Bacillus sp. CN-22. *Applied microbiology and biotechnology*, *98*(8), 3801–7. http://doi.org/10.1007/s00253-013-5433-5