Journal of Hazardous Materials 162 (2009) 74–79

Contents lists available at ScienceDirect

Journal of Hazardous Materials

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

Biodegradation of cyanide containing effluents by *Scenedesmus obliquus*

Fatma Gurbuz[a], Hasan Ciftci[b], Ata Akcil[b,*]

[a] *Suleyman Demirel University, Department of Biological Sciences, TR32260 Isparta, Turkey*
[b] *Suleyman Demirel University, Department of Mining Engineering, Mineral Processing Division, BIOMIN Group, TR32260 Isparta, Turkey*

ARTICLE INFO

Article history:
Received 17 December 2007
Received in revised form 4 May 2008
Accepted 6 May 2008
Available online 9 May 2008

Keywords:
Cyanide
Degradation
Gold mining
Scenedesmus obliquus
Environment

ABSTRACT

Biological degradation of cyanide has been shown a viable and robust process for degrading cyanide in mining process wastewaters. Several algal cultures can effectively degrade cyanide as carbon and/or nitrogen source for their growth. In this study, cyanide effluent degradation by *Scenedesmus obliquus* was examined. Gold mill effluents containing WAD cyanide concentration of 77.9 mg/L was fed to batch unit to examine the ability of *S. obliquus* for degrading cyanide. Cyanide was reduced down to 6 mg/L in 77 h. Microbial growth and metal uptake of Zn, Fe and Cu was examined during cyanide degradation. The cells well adapted to high pH and the effluent contained cyanide and the metals. It is important that Zn level reduced down 50%, of the starting concentration. pH was kept at 10.3 to prevent loss of cyanide as HCN, due its volatile nature. The bio treatment process was considered to be successful in degrading cyanide in the mine process water.

1. Introduction

Cyanide is highly toxic and its toxicity is related to its physicochemical specification. It has played a key role in extracting gold and other metals such as silver, copper and zinc from ores in many countries of the world. The free cyanide form HCN, CN^-, is classified as the most toxic due to its high metabolic inhibition potential [1,2]. There have been many incidents throughout the world due to improper handling or failure during the transportation. As an example, in 1990, a leak in Colorado (USA) was reported to have destroyed aquatic life along a 17-mile stretch of one river, and in the same year, 10 million gallons of cyanide solution spilled into the South Carolina River (USA), killing thousands of fish [3]. Exposure to cyanide in solution through consumption of surface water is the main exposure route for most animals affected by cyanide poisoning, but concurrent exposure through inhalation and skin absorption may also occur. Cyanide contamination of rivers, lakes, and seas can permanently damage some species. Toxicological studies have indicated that short-term exposure to high levels of cyanide can harm the nervous, respiratory and cardiovascular system of animals. In the environment, cyanide may degrade forming products of generally lower toxicity, but which may also be problematic in the environment.

Many industrial activities such as coal processing, organic synthesis, metal plating, and ore leaching, generate significant quantities of cyanide which is well-known metabolic inhibitor. Cyanidation of oxidized ore is a widespread technology used on an industrial scale for silver and gold recovery from oxidized ores and sulphidic concentrates. Currently, there are about 875 gold and silver operations through the world, of which about 460 utilize cyanide. According to some estimates up to 90% of gold is produced using cyanide [4,5]. Chemical and physical processes have been applied in most cases for cyanide degradation from tailings slurries and wastewaters. However, there are still problems facing the mining industry because of stringent environmental regulations and the cost of compliance with those regulations [5,6]. Volatilization and sorption are the two physical processes that contribute to the loss of cyanide from surface water. At pH <9.2, most of the free cyanide in solution exists as hydrogen cyanide, the volatile form. Volatilization rates are dependent upon pH (lower pH, faster rates) and aeration [7]. The most common alkali metal cyanides (sodium and potassium cyanide) may be lost from surface waters primarily through volatilization, whereas the sparingly soluble metal cyanides (copper, nickel and zinc) are removed from water predominantly by sedimentation and biodegradation [8].

Microbiological treatment potentially offers the cheapest means of cyanide degradation [9–11]. Biodegradation of cyanide in natural surface waters is dependent on such factors as cyanide concentrations, pH, temperature, availability of nutrients, and acclimation of microbes [12]. Microbial degradation of cyanide from tailings and wastewater is proven and viable alternative to chemical and physical treatment processes. Biological treatment can be applied in many situations, under many conditions, and in many configurations including in situ, aerobic and anaerobic, active and passive,

* Corresponding author. Tel.: +90 2462111775; fax: +90 2462370434.
E-mail address: ata@mmf.sdu.edu.tr (A. Akcil).

0304-3894/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2008.05.008

and suspended and attached growth. It has been employed in full-scale facilities worldwide both for conventional cyanidation and heap leach applications [12,13]. Microorganisms are known to possess various enzymes able to convert cyanide into naturally occurring compounds, including mineralization products [14–16]. However, utilization of bacteria to degrade cyanide and thiocyanate on a scale larger than laboratory bench scale has not been previously accomplished even though the available literature indicated that certain strains of bacteria possessed the capacity to degrade cyanides [13,17,18]. Under aerobic conditions the biodegradation of cyanides and thiocyanate in wastewaters, initially produces ammonia, which is converted to nitrite and nitrate in the presence of nitrifying bacteria, whereas anaerobic biodegradation under denitrification conditions may produce nitrogen. Complete biodegradation of simple and metal complexed cyanides and thiocyanate from mining wastewaters by various species of *Pseudomonas*, *Vibrionacas*, and *Enterobacterias* has been reported [19]. Sulphate ions were produced from thiocyanate degradation. Whilst bacteria and fungi have often been identified as cyanide detoxifying microorganisms, cyanide detoxification by algae has been shown in only a few studies [20–22].

Although cyanide is poisonous, it is one of the most indispensable industrial chemicals and is produced on large scale for use in mining. However, excessive use of cyanide for the dissolution of Au is associated with environmental risk. In order to protect the environment and people, reasonable levels of protection should be provided through promulgation of standards that not only protect designated beneficial uses, but also can be achieved through treatment and analyzed accurately using approved methods.

The preferred method of extracting gold present in ore is as water-soluble complexes in aqueous solution of sodium cyanide (NaCN) and then extracting it from the solutions. The alkalinity ensures that free cyanide ions, which are essential for the reaction, are not lost as free cyanide gas.

The reaction (Eq. (1)) is electrochemical and describes how gold is dissolved through the combined presence of oxygen and cyanide. During the reaction the gold forms a gold cyanide complex in alkaline solution. The resultant gold-bearing solution (as described by Eq. (1)) to give $Au(CN)_2^-$ is named as 'pregnant liquor' solution. After the first reaction, the procedure involves passing through activated carbon (Eq. (2)) or by cementation with zinc Eq. (3) however, there many variations of each procedure, leaving a solution which loaded with cyanide, metalloid cyanide complexes, thiocyanate (SCN^-) and thiocyanate complexes along with other chemicals [5,23].

$$2Au + 4NaCN + H_2O + (1/2)O_2 \rightarrow 2NaAu(CN)_2 + 2NaOH \quad (1)$$

$$NaAu(CN)_2 \xrightarrow{\text{Activated carbon}} Au(CN)_2^- + Na^+ \quad (2)$$

$$2NaAu(CN)_2 + Zn \rightarrow 2Au + Na_2Zn(CN)_4 \quad \text{(Cementation)} \quad (3)$$

The amount of NaCN generated annually, the land area needed to dump the waste and the quantity of water used for processing 250.000 tons of ore (3 ppm) per year are, land area (needed) 1.56 ha, used NaCN 125 tons, process water 365.000 m^3 [24].

The aim of this work was to investigate cyanide degradation of gold mining wastewaters by *Scenedesmus obliquus*. Uptake of Cu, Fe and Zn was examined in short-term experience to assess the uptake capacities of the algae. The most common forms of cyanide in the environment are free cyanide, metallocyanide complexes, and synthetic nitriles. Free cyanide, specifically HCN at pH 9.2 and lower, is the primary toxic agent in the aquatic environment. The toxicity of complex cyanides is related to their ability to release cyanide ions in solution; relatively small fluctuations in pH significantly affect their biocidal properties [25]. Hence, the study was conducted at pH 10.3. Gold ores was provided from Ovacik Gold Mine (Izmir, Turkey). In the history of Turkey the first use of cyanide for gold recovery has been at the Ovacik Gold Mine.

2. Materials and methods

2.1. Reagents and conditions

Stock solutions of NaCN were prepared according to process for extracting gold from gold ores. A 1-L stock solution of cyanide was prepared by dissolving 1.885 g NaCN with NaOH (solid). All glassware was washed by soaking 10% HNO_3 and rinsed with de-ionized water. All reagents were analytical grade. Factors increasing the toxicity of free cyanide in aquatic ecosystems include low dissolved oxygen, low pH, but not hardness other than the effect hardness or alkalinity has on pH. The acute toxicity of cyanide on aquacultures in solutions containing only free cyanide decreases with increasing pH, indicating the molecular form (HCN) is more toxic (2.3 times) than the anionic form (CN^-) [26]. It has been earlier showed that when the pH greater than 10.0 there is a little hydrogen ion present and nearly all of the free cyanide is present a CN^- [27]. Gurbuz et al. [21] were obtained the optimum cyanide removal at pH 10.3 due to cyanide at this pH was less toxic than lower pH values. Hence, the pH of the solutions was maintained 10.3 and was adjusted by using 1N NaOH, before and after stirring to prevent the cyanide loss as HCN. Temperature was maintained at room temperature throughout the process.

2.2. Organism and culture conditions

S. obliquus was isolated from Lake Egirdir (Isparta, Turkey) by single cell culturing technique and inoculated in modified SAG medium. The medium was essentially composed of KNO_3 0.1 g; $K_2HPO_4{\cdot}7H_2O$ 0.02 g; $MgSO_4{\cdot}7H_2O$ 0.001 g; $FeCl_3{\cdot}6H_2O$ 0.001 g and 50 ml of soil extract for a liter. Further isolation was made on plate containing 2% agar in the same culture (20 ml) media and were poured into sterile Petri dishes. A dumb-bell tipped bent glass rod was dipped into the algae suspension and streaked on the surface of the agar at two or three places. By this method, single cells were obtained [28]. The isolate were identified by manuals by Prescott [29] and John et al. [30]. The culture was aerated with filtered air and illuminated at 4000 lx light intensity with a light/dark cycle of 12/12 [31].

2.3. Measurement of algal growth

Algal growth in the flasks was monitored by measuring the chlorophyll *a* concentration [32] and cell counting by Improved Neaubouer, along with the cyanide levels. Acetone (90%) was the extraction solution to extract the pigments from the separated algal cells. 10 mL algal suspension was centrifuged at 3000 rpm for 2 min and supernatant was discarded. The pelleted cells were suspended in 3 mL of solvent. The volume was made up to 5 mL. After further centrifuging, the chlorophyll *a* concentration in the extract was calculated by reading the absorption (*A*) of the pigment extract in a spectrophotometer at the given wavelength against a solvent blank using the following equation:

$$\text{Chlorophyll } a \ (12.7 \times A_{663}) - (2.69 \times A_{645}) \quad (4)$$

2.4. Cyanidation and degradation processes

The gold ores were supplied from the Ovacik Gold Mine [33], and treated with NaCN for 24 h as described in Fig. 1. Cyanidation process was carried out in a 2 L glass container which had gold ore

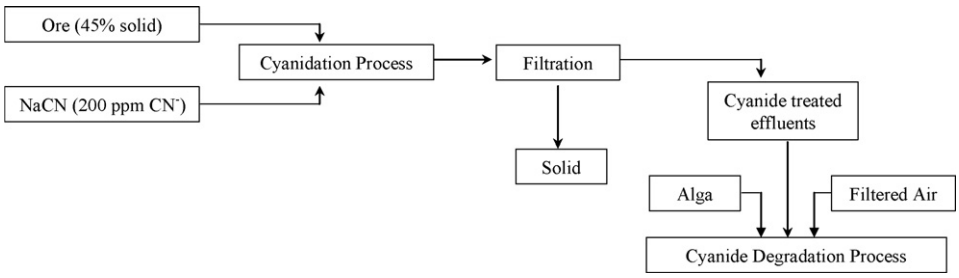

Fig. 1. Cyanidation process of gold deposits and algal treatment of the filtrated cyanide wastes.

(45%, w/v) and $NaCN^-$ (200 mg/L). The pulp was mixed by overhead stirrer for 24 h and filtered through a filter (Whatman No. 40) at end of the mixing duration. The flasks, containing filtered effluents of 250 mL, were sealed with silicone rubber bungs. After cyanidation, the processed effluents were measured for cyanide concentration. Analytically, the "toxicologically significant" or ecologically important" forms of cyanide are differentiated most accurately from the others by the WAD cyanide procedure. This procedure is widely used by industry and regulatory agencies for compliance and monitoring purposes. The WAD analytical procedure measures free (CN^-, HCN) and the weakly metal complexed (zinc, cadmium, silver, copper, and nickel) forms of cyanide [5,34]. Subtraction of the WAD cyanide value from the total cyanide value obtained from a split sample provides a measure of, the essentially non-toxic and stable, iron cyanide levels present.

Free cyanide and weak acid cyanide reacts with the picric acid reagent to produce an orange color that can be measured colorimetrically at a wavelength of 520 nm using known standards for quantification. The dissolved alkali metal picrate was converted by cyanide to the colored salt of *iso*-purpuric acid and its concentration was measured.

Calibration curves were prepared for each test ($R^2 = 0.999$ for all tests). Control groups, one of which contained growth medium and CN^- solution but no algal cells, and the other group contained algal cells and the growth media and none cyanide solution to test the effect of cyanide on algal growth, were carried out under identical conditions. All experiments and colorimetric readings were performed in duplicates according to colorimetric picric acid method [35]. Cyanide concentration was measured by Shimadzu UV/vis 1601, calibration curve was performed for every test ($R^2 = 0.999$). The most critical environmental factors associated with aerobic biological treatment include pH, temperature, oxygen levels, and nutrient availability. Temperature should be above 20 °C to support adequate biological growth and treatment. Limiting nutrient in the solution is found to be phosphate, need for the nutrient is ranging from about 1 to 5 mg/L [36]. The concentration of the metals in the waste solution was measured by AAS (PerkinElmer A Analyst800). The concentration of the nutrients was calculated according to processes; NO_3^- [37], PO_4^{3-} and NH_4^+ [38].

Biodegradation assays were carried out according to the method of Stauber et al. [39]. Exponentially growing cells (2 weeks old) were harvested by centrifuging. Cells were inoculated to the flasks containing 200 mL 72 mg/L of cyanide waste solution and to the flasks of control groups. These flasks were incubated at room temperature, aerated with filtered air and illuminated (cool white fluorescent tubes) with a light/dark cycle of 12/12.

Table 1
Composition of the wastewater after cyanidation process

Parameters	Composition
pH	9.85
Cyanide (WAD) (mg/L)	77.91
Nitrate (mg/L)	25.0
PO_4 (mg/L)	9.8
NH_4 (mg/L)	10
Zn (mg/L)	2.9
Cu (mg/L)	0.69
Fe (mg/L)	3.854
Pb (mg/L)	<0.45
Cd (mg/L)	<0.03
Cr (mg/L)	<0.08

3. Results and discussion

3.1. Analysis of the constituent in cyanide waste

Depending on the constituents in the remaining solution and their respective concentration after cyanidation, the processed wastewater is hazardous to the environment and needs special treatment and remediation. Gold ores were processed according the treatments in Ovacik Gold Mine. Treated process solutions are contained free contaminants such as copper, iron, mercury nitrate and zinc as well as metal cyanide complexes of them. The processed effluents were measured for the components and the results were presented in Table 1. Phosphate and nitrate were as nutrient vital components for the growth of *S. obliquus* in the effluents. Phosphate concentration was found 9.8 mg/L and nitrate was 25.0 mg/L. Although the effect of phosphate concentration on the degradation of cyanide has not been examined, it has been presented earlier by Blumenroth et al. [40] that with lower phosphate concentration, degradation rate of cyanide was decreased down considerably.

3.2. Cyanide degradation assay

A large number of processes exist for the removal of cyanide. Chemical and physical processes have been applied in most cases for cyanide degradation from tailings slurries and wastewaters. However, there are still problems facing the mining industry because of stringent environmental regulations and the cost of compliance with those regulations and many require or produce chemicals that have an ecotoxicological impact. Microbial cyanide oxidation is proven, economical technology for destroying free and complex cyanide in process solution, wastewaters and spent heaps [41,42].

Cyanide removal by viable microbial cells achieved from solutions up to 350 mg/L. However, concentration above 300 mg/L was found to be toxic to alive microorganism [43]. On the other hand, *S. obliquus* tolerated 400 mg/L CN^- without adaptation to the medium [21]. The degradation of cyanide effluents were tested by using *Burkholderia cepacia*. It was shown that cyanide concentration of about 220 mg/L was degraded down to 50 mg/L [39]. Another report was done by Akcil et al. [13] and they reported that *Pseudomonas* sp. reduced the cyanide concentration of 200 mg/L down to 1 mg/L in 70 h. Dumustre et al. [44] demonstrated that fungus *Fusarium solani*

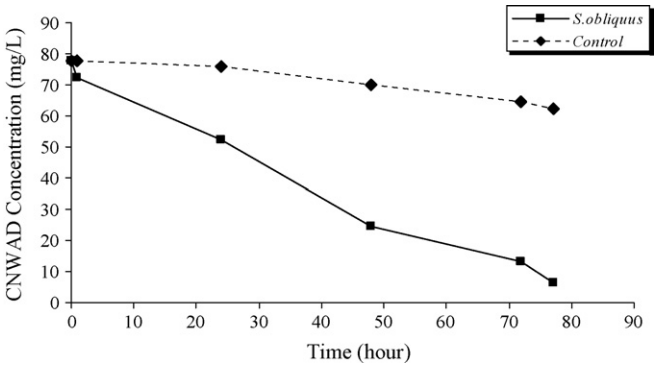

Fig. 2. The degradation rate of cyanide by *Scenedesmus obliquus*.

IHEM 8026 degraded cyanide under alkaline conditions. Although *F. solani* was unable to grow on cyanide, it degraded 1.176 mmol of CN/h. In another study *Fusarium oxysporum* CCMI 876 and *Methylobacterium* sp. RXM CCMI 908 were studied for biodegradation. Cyanide was degraded by *F. oxysporum* CCMI 876 at a rate of 0.059 mM/h leading to 96% cyanide conversion and leaving a residual 0.21 mM [45]. Ezzi and Lynch [46] evaluated degradation of cyanide by certain strains of the *Trichoderma* spp. and showed that cyanide (2000 mg/L) concentrations reached low levels within 32 days of incubation by the presence of glucose.

In this work, unlike the other studies reported, we used the process effluents to find out the adaptation of the alga and sustainability of the application for industrial use. *S. obliquus* was exposed to the processed effluent which concentration of cyanide was found 77.91 mg/L. Cyanide level was gradually decreased down to approximately 6 mg/L. Control group revealed that escape of free cyanide to the gas phase took place although initial pH was kept 10.3. The results showed that *S. obliquus* degraded cyanide 91% in 77 h experiment. The decrease of cyanide concentration in the control group was found 19.8% during the period of the experiment. Fig. 2 shows the daily measured total cyanide concentration over 4 days.

3.3. Growth rate of S. obliquus

The effect of cyanide on the growth of *S. obliquus* was determined via spectrophotometric analysis of chlorophyll-(*a*) and cell counting during cyanide degradation assay. The cell growth was affected by the cyanide when it was compared to control group which only contains SAG growth media and none cyanide. Chlorophyll *a* was suggested as the most important photosynthetic pigment in algal cells and any inhibition of its activity would cause a reduction of photosynthesis, leading to a decrease in cell [47]. Tam et al. [48] showed 85% of Ni removal by viable cells of *Chlorella miniata*. The removal rate was maintained for five cycles, but the efficiency of removal Ni thereafter progressively declined, reaching 70% at the end of 10th cycle. Cells were still viable after successive Ni biosorption, but division rate and chlorophyll *a* activity were adversely affected.

Chlorophyll *a* was measured as 4.4 μg/L at the start of the test. During the first 24 h of exposure, slight decrease in growth of *S. obliquus* was observed (Fig. 3). It was detected as 3.60 μg/L, whereas the control group showed an increase in the biomass Chlorophyll *a* was measured as 3.60 μg/L at the beginning of the test then increased to 4.0 μg/L. HCN, a low molecular-weight, non-ionic, hydrophilic chemical specie, is easily able to penetrate into the cell membrane. Once it is inside the cell HCN is converted to CN^- form which may inhibit enzymes within the cells [49]. This might be the cause of decrease on the growth of algae in the study although pH was 10.3, even at this stage there was found a small amount of HCN form present in the solution. Increase in the growth of *S. obliquus* was detected at 48 h due adaptation of the cells. Chlorophyll *a* was measured as 3.90 μg/L and continued to increase. The difference in growth rate between test groups and controls were found similar. Fig. 3 presents the effect of cyanide on growth and algal adaptation.

3.4. Metal uptake

This experiment was carried out in order to determine how much quantity of metals could be removed by adsorption which were biologically degraded and became free in the effluents from metal cyanides. Uptake of the metals in the effluents showed that sorption of the metals influenced and affected due to competitiveness. The uptake capacities of the metals (Zn, Cu, Cd, Al) decreased in uptake capacities as the complexity of the solution increased. The remarkable ability of biomass to uptake and concentrate the heavy metals, is becoming a useful tool for treating industrial solutions contaminated with heavy metals [50,51]. *Scenedesmus* is a microalgae genus commonly used in heavy metal removal experiments. It has proven removal capacity for U^{6+} [52], Cu^{2+}, Cd^{2+} [53] and Zn^{2+} [54].

pH of the study was kept at 10.3 due to cyanide degradation. Hence uptake of the metals was low. The order of uptake for competitive conditions was Zn(II) > Fe(III) > Cu(II). The uptake of Zn was 50% and 46% for Fe at the end of test period (Fig. 4). Copper uptake behaved differently from the other two metals in the effluents. It was found that there was no difference in removal of Cu concentration in the solution which implies that the removal of Cu at lower pH could be more complete than at a high pH. Karahan et al. [55] studied biosorption of Cu by *S. obliquus* and found the optimum pH for removal was 5.5. Zhou et al. [56] also found that increasing pH from 3.0 to 5.0 increased the sorption of Cu by *S. platensis*. However, at pH 8.6 they found decrease in the sorption and suggested that Cu started to precipitate at this pH. The presence of divalent ions may alter the biosorption of heavy metals by algal biomass, particularly if these ions are alkaline or alkaline earth elements. Cobalt biosorption by *Ascophyllum nodosum* was improved by the presence of potassium ions [57]. Costa et al. [58] was found the presence of sodium and magnesium contributed to the increase in the uptake capacity for Zn by *Sargassum* sp. Uptake of Zn ions by living *S. obliquus* in the effluents could occur due to an independent metabolism binding to cell walls and metabolism-dependent uptake where metals ions are transported across the cell membrane. This phenomenon was reported by living organisms elsewhere [59,60].

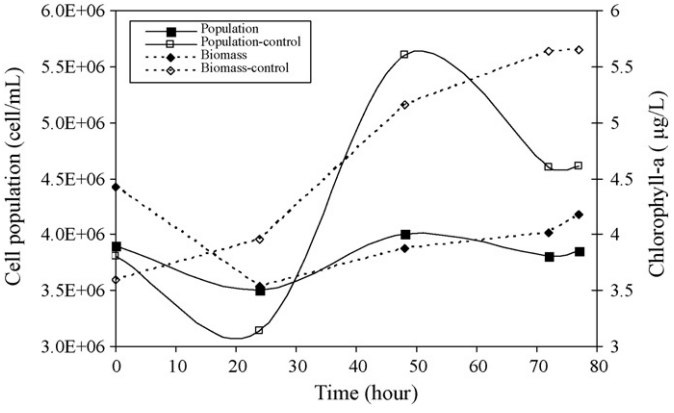

Fig. 3. The effect of cyanide on the growth of *S. obliquus*. (Biomass; chlorophyll *a*) (population; cell/mL).

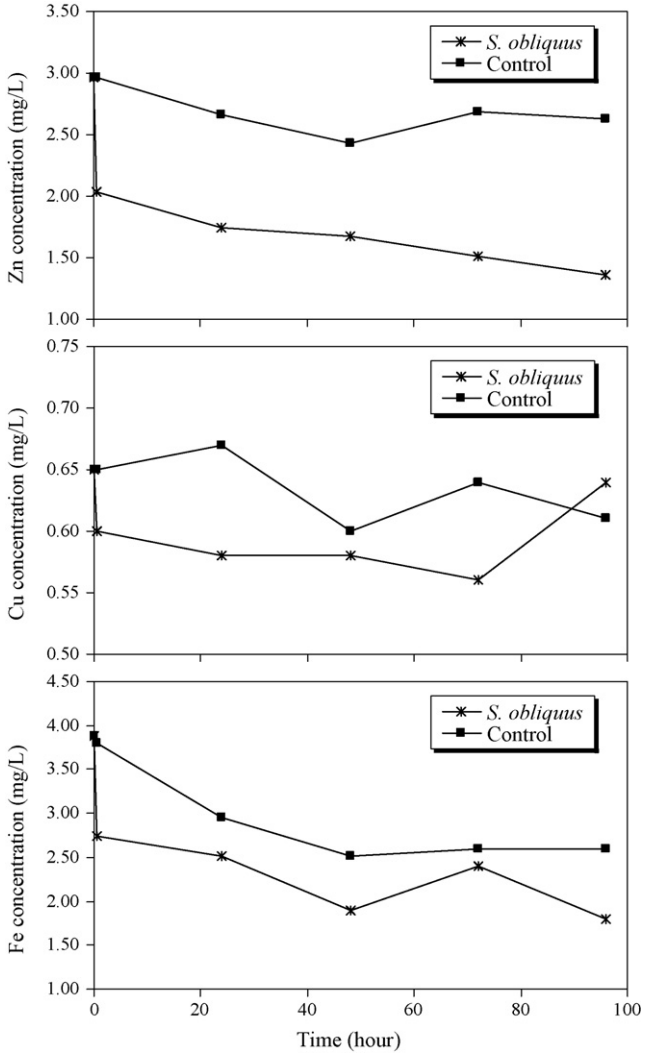

Fig. 4. The effect of time on the uptake of Zn, Cu and Fe in the waste effluent by *S. obliquus*.

In our study Fe uptake in the solution, reduced down to 1.50 mg/L up to 48 h. Then the following 24 h, release of the metal was observed due to dissociation of iron cyanide complexes. This phenomena followed by decrease of Fe concentration due to either adsorption or precipitation of the metal on the cell surface (Fig. 4).

Perales-Vela et al. [61] were investigated the effects of sublethal concentrations of Cu^{2+} in the growth and metabolism of *Scenedesmus incrassatulus* and found that sensitivity occurred at: growth > photosynthetic pigments content = photosynthetic O_2 evolution > photosynthetic electron transport > respiration. As a redox-active metal, Cu is known to directly participate in the formation of toxic reactive oxygen species, thereby causing oxidative stress [62]. On the other hand, Zn does not directly accelerate the formation of reactive oxygen species due to its redox inertness, and it therefore, exerted comparatively less stress on the test organism. However, algae have some protective mechanisms under stress conditions. Rijstenbil and Gerringa [63] have proved that sexual reproduction is promoted under Cu^{2+} stress and can serve as protective mechanism. Sexual reproduction as a response to severe heavy metal shock has also been observed in *Scenedesmus* spp. In the case of Cd^{2+} and Cu^{2+}, it has been found that *S. incrassatulus*, can respond to metal stress by expressing phenotypic plasticity that may allow these cells to survive in a hostile environment [64].

4. Conclusions

Main objective of the present study was to evaluate the efficiency degradation rate of cyanide in the effluents of gold mill as alternative chemical processes. WAD cyanide is generally considered to be the best current measure for assessing human and animal toxicity. In the study, WAD cyanide determined due to "Free" cyanide has been shown to be analytically inexact at desired regulatory levels and WAD cyanide levels are more easily determined below one part per million (ppm) and more relevant from an environmental standpoint [65].

Cyanide degradation was affected by the other metals being present in the solutions. In our previous study cyanide was degraded down to respectively 1 mg/L with synthetic solution of cyanide at almost every concentration tested (100–400 mg/L) by *Scenedesmus* [21]. In the current study, viable cell of *S. obliquus* was degraded and detoxified cyanide in the cyanide effluent down to 6.42 mg/L at over 60 h period. *Scenedesmus* biomass and cell count showed us after a period of adaptation increase of biomass took place. Cells well adapted high pH and *S. obliquus* degraded over 90% of cyanide.

It is important that the uptake rate of Zn(II) ions by *S. obliquus* were 50% and 46% for Fe(III) ions at the end of test period. The use of biological cyanide degradation eliminates the need for toxic or corrosive chemical oxidizers. Algae have many advantages for handling waste effluents due to easy handling and culturing. They offer economical approach as a cheap material for degradation cyanide and waste material. Bearing in mind the advantages of algae, there is a strong potential that such system could be exploited commercially for the treatment of waste effluents or radioactive wastewaters. Therefore, algae can be used for the treatment of cyanide wastes as an alternative to fungi and bacteria.

References

[1] L.P. Solomonson, Cyanide as a metabolic inhibitor, in: B. Vennesland, E.E. Conn, C.J. Knowles, J. Westley, F. Wissing (Eds.), Cyanide in Biology, Academic Press, London, UK, 1981, pp. 11–28.
[2] C.J. Knowles, A.W. Bunch, Microbial cyanide metabolism, Adv. Microbiol. Physiol. 27 (1986) 73–111.
[3] E. Young, Mining the Earth, Worldwatch paper 109 (1992) 24.
[4] T. Mudder, M. Botz, The Cyanide Monograph, 2nd ed., Mining Journal Books, London, UK, 2001.
[5] T. Mudder, M. Botz, A. Smith, The Chemistry and Treatment of Cyanidation Wastes, 2nd ed., Mining Journal Books, London, UK, 2001.
[6] J. Whitlock, T. Mudder, The Homestake wastewater treatment process. Part I. Design and startup of a full-scale facility, in: T.I. Mudder, M. Botz (Eds.), The Cyanide Monograph, 2nd ed., Mining Journal Books Limited, London, England, UK, 1998.
[7] K. Hagelstein, The ecotoxicological properties of cyanide, in short course notes on management of cyanide in mining, in: Australian Centre for Minesite Rehabilitation Research, Perth, Western Australia, April 14–16, 1997.
[8] USEPA, RMZ Briefing on Cyanide, Office of Pollution Prevention and Toxic's Cyanidation Mining Initiative and Mining Inquiry, November, 1992.
[9] J.L. Whitlock, T.I. Mudder, The Homestake wastewater treatment process: biological removal of toxic parameters from cyanidation wastewaters and bioassay effluent evaluation, fundamental and applied biohydrometallurgy, in: Proceedings of the Sixth International Symposium on Biohydrometallurgy, Vancouver, BC, Canada, August 21–24, 1985, pp. 327–339.
[10] R.H. Lien, P.B. Altringer, Case study: bacterial cyanide detoxification during closure of the green springs gold heap leach operation, in: US Bureau of Mines, Prepublication of Paper to be Presented at the International Biohydrometallurgy Symposium, Jackson Hole, Wyoming, August 22–25, 1993 (also presented at Mine Operations and Closure Short Course, Helena, Montana, April 27–29).
[11] J. Whitlock, Recent advances in technologies for biological treatment of thiocyanate, cyanide, heavy metals and nitrates, in: C. Young (Ed.), Cyanide: Social, Industrial and Economic Aspects, The Metals Society, Warrendale, PA, 2001, pp. 195–203.
[12] A. Akcil, T. Mudder, Microbial destruction of cyanide wastes in gold mining: process review, Biotechnol. Lett. 25 (2003) 445–450.
[13] A. Akcil, A.G. Karahan, H. Ciftci, O. Sagdic, Biological treatment of cyanide by natural isolated bacteria (*Pseudomonas* species), Miner. Eng. 16 (2003) 560–567.

[14] K. Ingvorsen, B. Hojer-Pedersen, S.E. Godtfredsen, Novel cyanide-hydrolyzing enzyme from *Alcaligenes xylosoxidans* subsp. denitrificans, Appl. Environ. Microbiol. 57 (1991) 1783–1789.
[15] J. Stratford, A.E. Dias, C.J. Knowles, The utilization of thiocyanate as a nitrogen source by a heterotrophic bacterium: the degradative pathway involves formation of ammonia and tetrathionate, Microbiology 140 (1994) 2657–2662.
[16] Y. Kita, H. Nishikawa, T. Takemoto, Effects of cyanide and dissolved oxygen concentration on biological Au recovery, J. Biotechnol. 124 (2006) 545–551.
[17] G.R.V. Babu, O.K. Vijaya, V.L. Ross, J.H. Wolfram, K.D. Chapatwala, Cell-free extract(s) of *Pseudomonas putida* catalyzes the conversion of cyanides, cyanates, thiocyanates, formamide, and cyanide-containing mine waters into ammonia, Appl. Microbiol. Biotechnol. 45 (1996) 273–277.
[18] Y.B. Patil, K.M. Paknikar, Development of process for biodetoxification of metal cyanides from wastewaters, Process Biochem. 35 (2000) 1139–1151.
[19] C. Boucabeille, A. Bories, P. Ollivier, Degradation of thiocyanate by a bacterial coculture, Biotechnol. Lett. 16 (1994) 425–430.
[20] F. Gurbuz, A. Karahan, A. Akcil, H. Ciftci, Degradation of cyanide by natural algae species, in: V. Yanko-Hombach, A. Arnold, P. Hallock, S. Ishman, M. McGann, W.C. Parker (Eds.), Extended Abstracts of the Third International Congress Environmental, Micropaleontology, Microbiology and Meiobenthology, EMMM'2002, Vienna, Austria, 2002, p. 93.
[21] F. Gurbuz, H. Ciftci, A. Karahan, A. Akcil, A biotechnological approach: degradation of cyanide effluents by *Scenedesmus obliquus*, in: Biotechnology Congress, Çanakkale, Turkey, August 25–31, 2003 (in Turkish).
[22] F. Gurbuz, H. Ciftci, A. Karahan, A. Akcil, Microbial detoxification of cyanide solutions: a new biotechnological approach using algae, Hydrometallurgy 72 (2004) 167–176.
[23] K. Bosecker, B Blumenroth, Microbial treatment of cyanide and heavy metals containing wastewater from gold mining, in: E. Unal, B. Unver, E. Tercan (Eds.), Proceedings of the 17th International Mining Congress and Exhibition of Turkey, The Chamber of Mining Engineers of Turkey, 2001, pp. 203–207 (ISBN 975-395-417-4).
[24] F. Korte, Ökologisch-chemiche Betrachtungen zum vorgeschlagenen Goldgewinnonugsprojekt unter Anwendung der Cyanidlaugung in der Türkei (in German) durch die, Tüprag Metal Mining Ltd., 1993.
[25] M.M. Botz, T. Mudder, A.U. Akcil, Cyanide treatment: physical, chemical and biological processes, in: M. Adams (Ed.), Development in Mineral Processing, 2005, pp. 672–700, Chapter 15.
[26] M.J. Logsdon, K. Hagelstein, T. Mudder, The Management of Cyanide in Gold Extraction, International Council on Metals and the Environment, Mining Journal Books Ltd., Ottowa Ontaria, Canada, 1999, p. 40.
[27] J.S. Scott, J.C. Ingles, Removal of cyanide from gold mill efluents, in: Canadian Mineral Processors 13th Annual Meeting Ottowa, Ont., Canada, January 20–22, 1981, pp. 380–418.
[28] A. Richmond, Handbook of Microalgal Mass Culture, CRC, Baco Raton, 1986, p. 528.
[29] G.W. Prescott, Algae of the Western Great Lakes Area Cranbrook Institute of Science Bulletin Fifth Edition Dubuque Iowa, USA, 1973.
[30] D.M. John, B.A. Whitton, A.J. Brook, The Freshwater Algal Flora Of The British Isles, An Identification Guide to Freshwater and Terrestrial Algae, Cambridge University Press, Cambridge, UK, 2002.
[31] J.P.K. Wong, Y.S. Wong, N.F.Y. Tom, Nickel biosorption by two chlorella species (*C. vulgaris*, *C. miniata*) (local isolate), Biosour. Technol. 73 (2000) 133–137.
[32] E.W. Becker, Microalgae, Biotechnology and Microbiology, Cambridge University Press, UK, 1994, p. 293.
[33] A. Akcil, First application of cyanidation process in Turkish gold mining and its environmental impacts, Miner. Eng. 15 (2002) 695–699.
[34] Colorado S, Guidelines for Cyanide Leaching Projects Colorado Department of Natural Resources, Mined Land Reclamation Division, USA, March, 1992.
[35] D.M. White, W. Schonobel, Treatment of cyanide in sequencing batch biofilm reactor, Water Res. 32 (1998) 254.
[36] A. Akcil, Destruction of cyanide in gold mill effluents: biological versus chemical treatments, Biotechnol. Adv. 21 (2003) 501–511.
[37] ISO, Water Quality—Determination of Nitrate. Part 3. Spectrometric Method Using Sulfosalicylic Acid, ISO 7890-3, International Organization for Standardization, Geneva, 1988.
[38] APHA, in: L. Clesceri, A.E. Greenberg, A.D. Eaton (Eds.), Standard Methods for the Examination of Water and Wastewater, APHA, Washington, DC, 1998.
[39] J.L. Stauber, J. Tsai, G.T. Vaughan, S.M. Peterson, C.I. Brockbank, Algae as indicators of toxicity of the effluent from bleached eucalypt kraft pulp mills, National Pulp Mills Research Program Technical Report No.3, CSIRO, Canada, 1994.
[40] P. Blumenroth, K. Bosecker, A. Michnea, N. Sasaran, Development of a bioremediation process for mining wastewaters, in: R. Amils, A. Ballester (Eds.), Biohydrometallurgy and the Environment Towards the Mining of the 21st Century, Elsevier, Amsterdam, The Netherlands, 1999, pp. 547–558.
[41] I. Finnegan, Microbial bio-remediation of one carbon wastes excluding photosynthesis, in: Wastecon'92 11th Congress, Institute of Waste Management, Rand Afrikaans University, Johannesburg, South Africa, 1992.
[42] S. Trapp, M. Larsen, A. Pirandello, J. Danquah-Boakye, Feasibility of cyanide elimination using plants, Eur. J. Miner. Process. Environ. Protect. 3 (2003) 128–137.
[43] D.J. Adams, J.V. Komen, T.M. Pickett, Biological cyanide degradation, in: C. Young (Ed.), Cyanide: Social, Industrial and Economic Aspects, The Metals Society, Warrendale, PA, 2001, pp. 203–213.
[44] A. Dumustre, T. Chone, J.M. Portal, M. Gerard, J. Berthelin, Cyanide degradation under alkaline conditions by strains of *Fusarium solani* isolated from contaminated soils, Appl. Environ. Microbiol. 63 (1997) 2729–2734.
[45] M. Graça Campos, P. Pereira, J.C. Roseiro, Packed-bed reactor for the integrated biodegradation of cyanide and formamide by immobilised *Fusarium oxysporum* CCMI 876 and *Methylobacterium* sp. RXM CCMI 908, Enzyme Microb. Technol. 38 (2006) 848–854.
[46] M.I. Ezzi, J.M. Lynch, Biodegradation of cyanide by *Trichoderma* spp. and *Fusarium* spp., Enzyme Microb. Technol. 36 (2005) 849–854.
[47] J.P.K. Wong, L. Chang, Effects of copper, chromium and nickel on growth, photosynthesis and chlorophyll *a* synthesis of Chlorella pyrenodosa 251, Environ. Pollut. 72 (1991) 127–139.
[48] N.F.Y. Tam, J.P.K. Wong, Y.S. Wong, Repeated use of two *Chlorella* species, *C. vulgaris* and WW1 for cyclic nickel biosorption, Environ. Pollut. 114 (2001) 85–92.
[49] F. Pablo, J.L. Satuber, R.T. Buckney, Toxicity of cyanide and cyanide complexes to the marine diatom *Nitzchia closterium*, Water Res. 31 (1997) 2435–2442.
[50] N. Kuyucak, B. Volesky, Biosorbents for recovery of metals from industrial solutions, Biotechnol. Lett. 10 (1998) 137–142.
[51] F. Veglio, F. Beolchini, Removal of metals by biosorption: a review, Hydrometallurgy 44 (1997) 301–316.
[52] X. Zhang, S. Luo, Q. Yang, H. Zhang, J. Li, Accumulation of uranium at low concentration by the green alga *Scenedesmus obliquus*, J. Appl. Phycol. 9 (1997) 65–71.
[53] P.A. Terry, W. Stone, Biosorption of cadmium and copper contaminated water by *Scenedesmus abundans*, Chemosphere 47 (2002) 249–255.
[54] R.O. Cañizares-Villanueva, S. González-Moreno, A.R. Domínguez-Bocanegra, Growth, nutrient assimilation and cadmium removal by suspended and immobilized *Scenedesmus Acutus* cultures: influence of immobilization matrix, in: F. Chen, Y. Jiang (Eds.), Algae and Their Biotechnological Potential, Kluwer Publishers, Dordrecht, The Netherlands, 2001, pp. 147–161.
[55] A.G. Karahan, F. Gurbuz, H. Tuncmen, Biosorption and desorption of Cu and Cd by microalgae, J. Fish. Aquat. Sci. 18 (2001) 173–182 (in Turkish).
[56] J.L. Zhou, P.L. Huang, R.G. Lin, Sorption and desorption of Cu and Cd by macroalgae and microalgae, Environ. Pollut. 101 (1998) 67–75.
[57] N. Kuyucak, B. Volesky, Accumulation of cobalt by marine algae, Biotechnol. Bioeng. 33 (1989) 809–814.
[58] A.C.A. Costa, L.M.S. Mesquita, J. Tornovsky, Batch and continuous heavy metals biosorption by a brown seaweed from a zinc-producing plant, Miner. Eng. 9 (1996) 811–824.
[59] G.M. Gadd, C. White, L. De Rome, Heavy metal and radionuclide uptake by fungi and yeast, in: R. Norris, D.P. Kelly (Eds.), Biohydrometallurgy, A. Rowe Chippenham, Wilts., UK, 1988.
[60] A.C.A. Costa, S.G.F. Leite, Metals biosorption by sodium alginate immobilized *Chlorella homosphara* cells, Biotechnol. Lett. 13 (1991) 559–562.
[61] H.V. Perales-Vela, S. Gonzaïlez-Moreno, C. Montes-Horcasitas, R.O. Cãnizares-Villanueva, Growth, photosynthetic and respiratory responses to sub-lethal copper concentrations in *Scenedesmus incrassatulus* (Chlorophyceae), Chemosphere 67 (2007) 2274–2281.
[62] E. Pinto, T.C.S. Sigaud-Kutner, M.A.S. Leitao, O.K. Okamoto, D. Morse, P. Colepicolo, Heavy metal-induced oxidative stress in algae, J. Phycol. 39 (2003) 1008–1018.
[63] J.W. Rijstenbil, L.J.A. Gerringa, Interactions of algal ligands, metal complexation and availability, and cell responses of the diatom *Ditylum brightwellii* with a gradual increase in copper, Aquat. Toxicol. 56 (2002) 115–131.
[64] M. Penã-Castro, F. Martínez-Jerónimo, F. Esparza-García, R.O. Cãnizares- Villanueva, Phenotypic plasticity in *Scenedesmus incrassatulus* (Chlorophyceae) in response to heavy metal stress, Chemosphere 57 (2004) 1629–1636.
[65] T. Mudder, A. Smith, Solution management during decommissioning of heap leach operations, Paper presented at society for mining, Metallurgy, and Exploration's Annual Meeting, SMME Preprint, Phoenix, Arizona, February 24–27, 1992.