Novel cyanide electro-biodegradation using *Bacillus pumilus* ATCC 7061 in aqueous solution

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Abstract

Background Electro-biodegradation is a novel technique for cyanide degradation in aqueous solutions. Many physical, chemical, and biological methods have been developed and used to treat cyanide degradation. The biological methods are more environmentally-friendly and economically cost-effective when compared to other techniques, however, the process reaction time period is much longer and the efficiency is lower.

Methods In this research, the bacterial strain, *Bacillus pumilus* ATCC 7061, was tested for the first time to introduce the Cyanide Electro-biodegradation technique. By using a direct current power supply, electrons were generated in an electro-biodegradation cell containing culture media at free cyanide concentrations of 100 to 500 mg/l, under alkaline conditions.

Results Experimental tests showed that when electrons were added and bacteria were inoculated into the aqueous media containing 100, 200, 300, 400 and 500 mg/l of free cyanide, the cyanide degradation efficiency increased from 16.2, 21.6, 29.5, 38.7 and 44.5% to 98.6, 99.3, 99.7, 99.8 and 99.7%, in 36, 72, 137, 233 and 301 h, respectively. The results show that by adding electrons, the process reaction time decreases and cyanide degradation efficiency increases significantly.

Conclusions The results presented here demonstrate for the first time the importance and the significance of the electro-biodegradation technique in the efficient degradation and removal of cyanide present in aqueous solutions.

Keywords Cyanide removal · Wastewater treatment · Biodegradation · *Bacillus pumilus* · Alkaline conditions

Background

The highly toxic compound, cyanide, is often used in several industrial processes comprising photo finishing, metal plating, coal washing, synthetic fiber production and the processing of

gold and silver, all of which contribute to serious environmental problems [1–5].

Cyanide compounds, particularly HCN, are harmful to almost all living organisms, and their existence in natural water bodies and even soils can be dangerous to aquatic ecosystems and human health [2].

Numerous chemical, physical and innovative techniques have been presented in the past to remove cyanide from contaminated waters and soils such as Adsorption, oxidation, electrolysis, Electro Chemical Oxidation, Electro Coagulation (EC), Photo electro chemical degradation, Simultaneous Adsorption and Biodegradation (SAB) and Sequencing Batch Reactor (SBR) [6–11]. However, their applications were often restricted, due to variations in environmental conditions, operational costs and the formation of hazardous by-products [9, 11, 12]. In contrast, biological processes have been recognized to be cost-effective, environmentally friendly [9] and well-adapted for the treatment of gold mining effluents [12]. According to Kumar et al. [13], the best alternative for the degradation of cyanide constituents into less toxic ones is the use of microorganisms [2].

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Although cyanide is toxic to life, it is naturally produced by many organisms encompassing microbes and plants [14]. Conversely, many microbial species have been shown to degrade cyanide and its complexes [15]. Many of these species are capable of using cyanide as a sole source of carbon or nitrogen. Up to now, several bacterial, fungal and algal species such as, *Pseudomonas*, *Rhodococcus*, *Klebsiella*, *Bacillus*, *Citrobacter*, *Stemphylium loti*, *Aspergillus niger*, *Trichoderma* spp., *Chlorella* sp., *Arthrospira maxima*, *Chroococcus*, *Bacillus pumilus* and etc., have been effectively used to biologically degrade cyanide [1, 16–23]. In all previous researches, not only the capability of microbes for cyanide degradation was evaluated, but also the rate at which cyanide can be degraded was also considered as an important and critical aspect of such biological processes. Due to the environmental significance of cyanide biodegradation methods, many researchers have attempted to accelerate the rate of the biological reaction and degradation of cyanide by using a combination of various methods, or by altering the relevant environmental conditions of the biological reaction [1, 7, 24–27]. The electrochemical and biological degradation of cyanide represents an attractive and effective approach due to the degradation achieved and eco-environmental compatibility, but without the generation of secondary pollutants [28].

The effects of various parameters such as pH, free cyanide concentration, type of growth medium, etc., have been previously studied. However, the importance of the bacterial electron transfer chain, which is important for bacterial respiration [29], has not been emphasized even though exposure to cyanide is considered by many bacteria as a stressful condition.

The purpose of the present research was to study the applicability of the electro-biodegradation technique for cyanide destruction in a batch cell, and compare its effectiveness with the common biological treatment systems for the efficient degradation of cyanide. Hence, this research investigates the effects of the simultaneous addition of electrons and bacterial inoculation on the cyanide destruction efficiency and the process reaction time period in an aqueous solution.

Materials and Methods

Bacterial strain and growth conditions

The bacterial strain, *Bacillus pumilus* ATCC 7061 was obtained as a lyophilized sample from the Persian Type Culture Collection (PTCC) at the Iranian Research Organization for Science and Technology (IROST), Tehran, Iran. The *B. pumilus* was chosen because it is capable of good growth under alkaline conditions, and can survive under stressful environmental conditions, such as the presence of cyanide. Furthermore, some of the microbes that are capable of cyanide degradation have also been found to be pathogenic and dangerous to humans. Accordingly, *B. pumilus* was used because it has been generally recognized as safe for humans and their environment.

Live cultures of the bacterial strain were prepared according to American Type culture Collection (ATCC) protocol and were subsequently used to inoculate solid and liquid media (Fig. 1).

After an initial growth in nutrient broth (NB), a bacterial sample was then used to inoculate NB media which were incubated at 30 °C in an orbital incubator, with shaking at 180 rpm based on ATCC protocol. During the growth period, bacterial samples were taken at regular time intervals and used for subsequent growth analysis.

Preparation of culture media

Nutrient broth powder (Merck, Germany) was used for bacterial growth throughout the experiments. It was prepared according to the company instructions. The sodium chloride (NaCl) powder (Merck, Germany) was dissolved as an electrolyte in distilled water, at a final concentration of 0.1 M. In order to prevent formation of the highly toxic and volatile hydrogen cyanide (HCN), all growth and biodegradation experiments were carried out at alkaline pH values (9) [23]. The pH of the medium was adjusted by using reagent grade sodium hydroxide (NaOH) solution (0.1 M) (Merck, Germany), and then sterilised by being

Fig. 1 **a** Streaking inoculation on sterilized petri dish containing Nutrient agar (NA) medium, to isolate a single pure colony from the *B. pumilus* - ATCC 7061 strain, **b** single colony of *B. pumilus* under 10X

magnification, **c** rod-shaped cells obtained from the single colony under 100X magnification

autoclaved at 121 °C and a pressure of 15 lb./in² for 15 min.

In all bench-scale experiments, standard cyanide stock solution (1000 mg/l CN[−]) was prepared by adding 1.88 g of dry reagent-grade sodium cyanide (NaCN) to a 1 l volumetric flask and diluting to the mark with distilled water. The solution was mixed well, and then sterilised by using a 0.2 µm membrane filter, which was removed from the sterile package by flamed forceps and placed on an autoclaved jar under the biological hood. The sterilized syringe was used for withdrawing solution from the container and injecting slowly into the membrane filter, thereby allowing the liquid to pass completely through the filter. The resulting sterilized solution was then diluted in culture media (NB) or distilled water to the desired final cyanide concentration that was to be used for the particular Electro-Biodegradation experiment or calibration of the analytical instruments.

Growth measurements

In this study, bacterial growth was analysed by measuring optical density (OD₆₀₀) of the culture samples at a wavelength of 600 nm using a UV/VIS spectrophotometer (Beckman, USA).

Cyanide concentration and pH measurements

Cyanide concentration and pH values were measured using a Thermo Scientific Orion ISE meter (ThermoFisher Scientific,

USA). A Thermo Scientific Orion Cyanide electrode #9606BNWP (ThermoFisher Scientific, USA) was employed to determine cyanide concentration according to the company instructions and the Orion™ 9207BN pH/ATC Triode™ probe (ThermoFisher Scientific, USA) was used for pH measurements.

Electro-biodegradation experiments

The experiments were performed in a rectangular-shaped electro-biodegradation cell with an inner length of 26 cm, a width of 11 cm, and a height of 10 cm [30]. The applied electrical potential was kept constant using a programmable DC Power supply. Two aluminum electrodes were installed inside the cell, and the electrode control box was capable of reversing the polarity and switching the electrodes every 1–5 s. A constant voltage gradient of 2.0 V with a 6 mA/cm² current density was used for all experiments [31]. The schematic diagram of experimental set up of electro-biodegradation cell was shown in Fig. 2.

Previous researches have shown that the electrolysis of aqueous media generates hydroxide ions (OH[−]) at the cathode and hydrogen ions (H⁺) at the anode. The acidic condition in the medium cause the generation of cyanide hydrogen that not only toxic and deathlike but also has a direct effect on cyanide degradation process reaction. Therefore, in this research polarity was reversed by switching the electrodes to avoid acidic conditions in the culture media [32].

Fig. 2 Schematic diagram of electro-biodegradation cell

Data availability The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Investigation of cyanide electro-biodegradation

Electro-biodegradation and biodegradation of cyanide were investigated in culture media at free cyanide concentrations of 100, 200, 300, 400 and 500 mg/l [23, 33, 34].

In both electro-biodegradation and biodegradation experiments, bacterial cultures were harvested near the end of the logarithmic phase of growth, and were then used at a concentration of 4% (v/v) [16, 23, 35] to inoculate culture media containing different concentrations of free cyanide (100 to 500 mg/l). The cultures were subsequently incubated in the electro-biodegradation cell, in the presence or absence of electrical potential. In order to prevent the formation of the toxic gas, hydrogen cyanide, during cyanide degradation, alkaline conditions must be provided and based on the PTCC protocol the best temperature for cultivation is 30 °C. Therefore cultivation was carried out at a pH of 9 and a temperature of 30 °C. Bacterial growth and cyanide degradation were eventually measured and compared during the cultivation period.

All experiments were replicated three times, and the average of all three experiments was expressed as the final result.

Results and discussion

The experimental results of electro-biodegradation as well as biodegradation of cyanide, at three free cyanide concentrations of 100, 200 and 300 mg/l, are presented in Table 1, Figs. 3a, b and 4a, b.

Figure 3a, b shows the results of bacterial growth, biodegradation and electro-biodegradation of cyanide versus reaction time in the presence of 100 and 200 mg/l of cyanide. As observed from the graphs, free cyanide levels decreased gradually in the biodegradation experiments, but declined considerably during the electro-biodegradation process. On the other

hand, the bacterial population density which remained constant at the lag phase, elevated significantly thereafter, reaching a peak following the logarithmic phase of growth, which was then proceeded by a sudden drop in cell density during the death phase. Moreover, it appeared that the trend in bacterial growth and cyanide reduction are directly related.

Analysis of cyanide biodegradation by *B. pumilus* indicated that free cyanide concentrations decreased from 99.8 and 194.9 to 1.42 and 2.33 mg/l after 230 and 396 h of cultivation, showing cyanide removal efficiencies of 98.5 and 98.8%, respectively. When electro-biodegradation technique using a batch cell, CN^- levels were reduced from 98.6 and 199.7 to 1.37 and 1.44 mg/l, demonstrating cyanide removal efficiencies of 98.6 and 99.3% within 36 and 72 h, respectively, which were significantly shorter than the biodegradation process reaction time period. In addition, as it is clearly shown in Fig. 3a, b, the lag phases of growth in electro-biodegradation were 9 and 18 h compared with 135 and 218 h during the biodegradation process, at free cyanide concentrations of 100 and 200 mg/l, respectively, which clearly demonstrate the influence of the added electrons in decreasing the biodegradation reaction time period from 230 to 36 and 396 to 72 h. A glance at the bacterial growth curves shows that, although growth periods are different, the maximum bacterial population size and density is approximately similar in both methods at various cyanide concentrations. The results obtained imply that bacterial metabolism is activated by the electrokinetic process that consequently reduces the biodegradation reaction time period significantly.

Figure 4a illustrates the results of cyanide biodegradation and bacterial growth with respect to time in the presence of 300 mg/l of free cyanide. The bacterial growth curve clearly indicates that the *B. pumilus* strain cannot grow in media containing more than 200 mg/l of CN^- , even after 600 h of incubation. Furthermore, cyanide degradation decreased gradually to 60% at 250 h and remained steady thereafter, which is a common observation in natural cyanide degradation, suggesting the possible absence of the biodegradation process.

Results for the electro-biodegradation of cyanide and growth of *B. pumilus* versus time in the presence of 300 mg/l of cyanide are presented in Fig. 4b. As shown in the graph,

Table 1 Results of electro – biodegradation and biodegradation of cyanide at 100–300 mg/l of CN^-

Cyanide treatment method	CN^- concentration (mg/l) before degradation	Time (h)	% Removal of CN^-	CN^- concentration (mg/l) after degradation
Biodegradation	99.8	230	98.5	1.42
	194.9	396	98.8	2.33
	303.6	622	65.3	105.5
Electro-biodegradation	98.58	36	98.60	1.37
	199.7	72	99.27	1.44
	298.6	137	99.7	0.99

Fig. 3 Effect of electro biodegradation and biodegradation process on cyanide removal, and bacterial growth trend at 100 (a) and 200 (b) mg/l CN^- versus time by *Bacillus pumilus* ATCC 7061; each experiment was replicated three times

B. pumilus growth was exceptionally strong and cyanide was degraded significantly by the electro-biodegradation process. CN^- levels decreased from 298.6 to 0.99 mg/l and cyanide removal efficiency was found to be 99.7% within 137 h of cultivation. In addition, the bacterial population increased after 19 h and reached an optimal value after 107 h of cultivation, during which cyanide degraded by almost 95%. These data indicate that the addition of electrons is the main factor in accelerating the rate of bacterial growth and cyanide biodegradation.

Based on these experimental results it appears that electro-biodegradation is significantly effective in cyanide degradation. Although reversing polarity by switching the electrodes was used during the process, it was decided to run comparative cyanide removal experiments by using aluminum electrodes, which achieved optimal results as in previous research [31], at different cyanide concentrations, ranging from 100 to 500 mg/l, in the presence and absence of bacterial species, all under the same conditions. These set of experiments verified that the cyanide degradation process was not because of an electro-oxidation process. The details of the results are presented in Table 2.

Figure 5a–e illustrates the results of the electro-biodegradation of cyanide, bacterial growth, and cyanide degradation in a cell without bacterial inoculation versus time, in the presence of various cyanide concentrations. As is presented in the graphs, cyanide was degraded rapidly by electro-biodegradation when compared to that of electro-oxidation, which decreased slowly and remained steady thereafter, at non-permissible cyanide levels. Thus, it seems the addition of electrons plays an important and critical role in reducing the reaction time, and is thus more practical and applicable for higher levels of cyanide than those by existing biological treatment systems.

As is clearly demonstrated in Fig. 5a–e, durations of the lag phase in the presence of 100, 200, 300, 400 and 500 mg/l CN^- were 3, 7, 19, 46 and 93 h, respectively, which were then followed by rapid bacterial growth (logarithmic growth). Moreover, the maximal microbial growth yield was approximately the same in all different media, which shows that maximum bacterial activation occurred in the presence of cyanide as a source of nitrogen and other nutrients in the culture medium. It is evident that while the bacterial population reached a peak and then suddenly decreased, the cyanide content in the

Fig. 4 Cyanide biodegradation (a), cyanide electro-biodegradation (b) and bacterial growth trends in media containing 300 mg/l CN^- vs time, by *Bacillus pumilus* ATCC 7061; each experiment was replicated three times

medium was found to be minimal. This suggests that the microorganism needs a source of nutrients for metabolism and growth, even under stressful conditions, and that source in this case is cyanide.

As shown in the graphs, CN^- levels were reduced from 98.6, 199.7, 298.3, 399.3 and 498.8 to 1.37, 1.44, 0.99, 1.1 and 1.53 mg/l, showing cyanide removal efficiencies of 98.6, 99.3, 99.7, 99.72 and 99.69%, respectively. In addition, increasing CN^- concentrations from 100 to 500 mg/l in the media increased the cyanide removal period from 36 to 301 h, which means the electro-biodegradation period is a function of the cyanide concentration. Furthermore, according

to the data, while the bacterial population density elevated rapidly soon after the lag phase, cyanide degraded significantly. It seems that cyanide was used as a nitrogen source for bacterial growth [33]. Therefore, the results obtained demonstrate that the electro-biodegradation process is substantially practical and significantly effective in accelerating cyanide degradation.

Figure 6 represents the cyanide content in the medium, cyanide electro-biodegradation efficiency and bacterial growth data in three graphs, all sharing the element of time (ranging from 0 to 350 h) along their horizontal axis. The colors used to show the trends are also the same in all graphs,

Table 2 Results of electro-biodegradation and electro-degradation in the presence of 100 to 500 mg/l of free cyanide in the culture media

Cyanide treatment method	CN ⁻ concentration (mg/l) before degradation	Time (h)	% Removal of CN ⁻	CN ⁻ concentration (mg/l) after degradation
Electro-biodegradation	98.6	36	98.60	1.37
	199.7	72	99.27	1.44
	298.3	117	99.70	0.99
	399.3	209	99.72	1.10
	498.8	301	99.69	1.53
Electro -degradation	98.74	36	19.75	79.24
	194.2	72	20.75	153.9
	301.1	117	52.57	142.8
	407.4	233	57.45	173.4
	502.9	301	60.04	200.9

Fig. 5 Effect of the electro-biodegradation process on cyanide removal and bacterial growth vs time in the presence of different cyanide concentrations (a 100, b 200, c 300, d 400, e 500 mg l⁻¹). A comparison is made

with the results of cyanide degradation in a cell without inoculation. Each experiment was replicated three times

Fig. 6 Cyanide electro-biodegradation (a), process efficiency (b) and bacterial growth (c) curves vs time in the presence of different CN⁻ concentrations (100–500 mg/l)

and represent different cyanide concentrations, yet they illustrate different categories on the vertical axis of each graph.

According to Fig. 6a, as time passes, free cyanide undergoes a downward trend in all its scales in terms of density. All figures move from their point of climax to plateau at zero. This is exactly the opposite when Fig. 6b is taken into consideration. Here, time is a factor working for the process efficiency to peak. All trends begin from zero and tend to reach the upper limit of cyanide removal efficiency during the reaction time period. In Fig. 6a, b, the smaller the value of each trend at the beginning, the shorter is the duration of the reaction.

As illustrated in Fig. 6c, bacterial growth takes a three-phase trend through which it initially experiences lag phase thereby remaining steady, and then moving up with some

negligible fluctuations in the growth phase, and along with the axis of time it moves down as soon as it peaks, which is the final dead phase in each trend.

By considering the results which have been obtained and clearly illustrated, the trend of cyanide degradation, bacterial growth and process efficiency versus time are strongly dependent on the CN⁻ concentration in the medium.

The purpose of presenting the above experimental results is to provide an initiative about the electro-biodegradation technique for cyanide removal from wastewaters, and evaluate the effect of this method on the biodegradation process over time. The results which have been obtained from this study showed that by using the electro-biodegradation technique, the time required for the reaction process can be significantly decreased and also higher cyanide concentrations can be degraded when compared to the common biodegradation methods. In addition, the study illustrated that the application of electrokinetics to the biodegradation process has considerable effects on bacterial growth and accelerates cyanide biodegradation simultaneously.

Conclusion

In conclusion, by using the electro-biodegradation technique, time of the reaction process at concentrations of 100, 200, 300, 400 and 500 mg/l of CN⁻ was 36, 72, 117, 209, and 301 h with approximately 99% cyanide removal efficiency, respectively. Thus the process was significantly shorter and much more practical when compared to the experiments that involved biodegradation in the absence of an electric current, which had much longer reaction periods and were incapable of degrading cyanide at concentrations over 200 mg/l. Furthermore, not only the process period was shorter, but also the bacterial population grew faster when an electric current was applied. It is thus quite clear that cyanide degradation using the electro-biodegradation method is a function of retention time, bacterial population density and cyanide concentration.

Cyanide is present as a toxic and hazardous combination in industrial and mining wastewaters, leading to potential severe environmental pollution. Thus, by using the electro-biodegradation of cyanide, one can effectively reduce the overall cost, duration of the degradation process and related environmental problems. Therefore, the process of electro-biodegradation of cyanide may be a more attractive future option for efficient treatment of industrial and mining effluents.

Consequently, variations in electrodes type, culture media and using diverse microbial species are recommended to gain the maximum benefits of the cyanide electro-biodegradation process.

Finally, the present paper was the first report of its kind regarding the cyanide electro-biodegradation capacity of *Bacillus pumilus* ATCC 7061.

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Authors' contributions AO carried out this study as a part of his PhD thesis, conducted the experiments, collected and interpreted the data, and wrote the manuscript. SZST, PS and FDA supervised the study in all steps and edited the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare that they have no competing interests.

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