ISTANBUL UNIVERSITY PRESS

RESEARCH ARTICLE

Eur J Biol 2019; 78(1): 5-10

Cyanide Removal in Electroplating, Metal Plating and Gold Mining Industries' Wastewaters by Using *Klebsiella pneumoniae* and *Klebsiella oxytoca* Species

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Please cite this article as: Avcioglu NH, Seyis Bilkay I. Cyanide Removal in Electroplating, Metal Plating and Gold Mining Industries' Wastewaters by Using *Klebsiella pneumoniae* and *Klebsiella oxytoca* Species. Eur J Biol 2019; 78(1): 5-10.

ABSTRACT

Objective: In this study, cyanide containing wastewaters of electroplating, metal plating and gold mining industries were selected in order to investigate the usability of *Klebsiella pneumoniae* and *Klebsiella oxytoca*.

Materials and Methods: In order to investigate their cyanide removal capability *K. pneumoniae* and *K. oxytoca* cultures both, separately and with sterile crude extracts were used.

Results: It is observed that cyanide in electroplating wastewater was effectively removed by the cultures and sterile crude extracts of *K. pneumoniae* (both 100%) and *K. oxytoca* (100% and 70%, respectively). Additionally, culture of *K. pneumoniae*, mixture of *K. pneumoniae* and *K. oxytoca* cultures, sterile crude extract of *K. oxytoca* and mixture of *K. pneumoniae* and *K. oxytoca* sterile crude extracts degraded cyanide efficiently (94%, 96%, 90% and 93%, respectively) in metal plating wastewater.

Conclusion: It is determined that *K. pneumoniae* and *K. oxytoca* cultures and crude extracts can be a promising alternative for cyanide removal in wastewaters of different industries.

Keywords: *Klebsiella pneumoniae, Klebsiella oxytoca*, cyanide, wastewater, biodegradation

INTRODUCTION

Cyanide consumption and cyanogen waste production are approximately 1.5 million tones and 14 million kg per year respectively in worldwide (1). Furthermore, cyanide is an important inhibitor of cytochrome oxidase c and some other metalloproteins and it binds with methemoglobin irreversibly in blood. Therefore, removal of cyanide especially in industrial wastewaters by using effective methods is an important issue in order to protect the health of living organisms (2-5). In this respect, researchers focused on the treatment of this toxic compound by biological treatment methods. Accordingly, biological systems are being used in the removal of cyanogen compounds. Based on the investigations obtained from the literature, various plants such as *Sorghum bicolor* and *Linum usitassimum var. omega-gold* (6), *Zea mays* (7), various fungi such as *Aspergillus niger* (8), *Cryptococcus humicolus* (9), *Fusarium lateritium* (10, 11), *Fusarium oxysporum* (12, 13), *Fusarium solani* (14, 15), *Rhizopus arrhizus* (16), *Scenedesmus obliquus* (17), *Trichoderma sp.* (18) and *Trichoderma harzianum* (19) were found as cyanide degrading species. Additionally, *Agrobacterium tumefaciens* (20), *Azotobacter vinelandii* (21), *Bacillus nealsonii* (22), *Klebsiella oxytoca* (2, 4, 5), *Paracoccus sp.* (23), *Pseudomonas fluorescens* (24-26), *Rhodococcus sp.* (27, 28), *Serratia marcescens* (29, 30), *Serratia odorifera* (31), *Halomonas sp.* (32) are some of the cyanide degrading bacteria. Accordingly, cyanide removal is directly carried out with some metabolic pathways (hydrolytic, oxidative, reductive and substitutional/transfer reactions) which include cyanide degrading enzymes. By means of these enzymatic pathways, bacteria/fungi detoxify cyanide to nontoxic chemicals as ammonia (33). In this respect, in this study cyanogen waste forming industrial processes' wastewaters

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Submitted: 24.07.2018 • **Revision Requested:** 19.10.2018 • **Last Revision Received:** 17.01.2019 • **Accepted:** 06.02.2019

(electroplating, metal plating and gold mining) were prepared and cyanide removal efficiencies of *Klebsiella pneumoniae* and *Klebsiella oxytoca* cultures, sterile crude extracts and mix of the cultures and sterile crude extracts of them were investigated.

MATERIALS AND METHODS

Bacterial Strains

In this study, *K. oxytoca* and *K. pneumoniae*, which were determined as similar to *K. oxytoca* ATCC 13182 and *K. pneumoniae* ATCC 700721 by 16S rRNA analysis in our previous study (34), were used. *Klebsiella* strains were inoculated in to the Brain Heart Infusion Broth media containing 10% glycerol and stored at -20°C for later analysis.

Synthetic Wastewater

Electroplating, metal-plating and gold mining industries' wastewaters were selected in order to investigate the cyanide biodegradation capability of cultures, sterile crude extracts, mix cultures and mix sterile crude extracts of *K. pneumoniae* and *K. oxytoca* species. Accordingly, synthetic wastewaters were prepared as follows; (as mgL^{-1}) 1.2 Ag, 1.2 Cd, 7.0 Cr, 4.5 Cu, 4.1 Ni, 0.6 Pb, 4.2 Zn and 5.0 CN for electroplating wastewater, (as mgL^{-1}) 0.43 Ag, 0.69 Cd, 2.77 Cr, 3.38 Cu, 3.98 Ni, 0.69 Pb, 2.61 Zn and 1.2 CN for metal-plating wastewater and (as mgL^{-1}) 10.0 As, 0.02 Cd, 0.1 Cr, 400.0 Cu, 40.0 Fe, 0.05 Hg, 20.0 Mn, 50.0 NH_4, 10.0 Ni, 0.1 Pb, 6.0 Sel, 2.0 Ag, 100.0 Zn, 1000.0 CN and 2000.0 SCN- (thiocyanate) for gold mining industry wastewater (35).

Biodegradation Studies

Firstly, *K. pneumoniae and K. oxytoca* were incubated into the enrichment media (Luria Bertani Broth) at 37°C, 150 rpm for log phase and after the incubation period cultures of *Klebsiella* species were adjusted to OD_{600}= 1.0 and mix cultures of *K. pneumoniae and K. oxytoca* were formed by combining these species' cultures with the proportion of 1:1 (v/v) in order to use in the biodegradation studies of cyanide containing wastewaters.

Sterile crude extracts of *K. oxytoca* and *K. pneumoniae* species were prepared as follows; enriched *Klebsiella* cultures were inoculated into cyanide biodegradation media containing g/L: 1 glucose, 0.5 K_2HPO_4, 0.5 K_2HPO_4 and 0.05 $MgSO_4$ (24) and at the end of the incubation period, cultures were centrifuged at 4000 rpm for 5 minutes and were sterilized by 0.45 µM cellulose acetate filter (Millipore) to form sterile crude extracts of *Klebsiella* species. Mix of the sterile crude extracts of *K. pneumoniae* and *K. oxytoca* species were formed by combining these species' sterile crude extracts with the proportion of 1:1 (v/v).

Cultures, sterile crude extracts and mixtures of them were inoculated into the wastewaters with the concentration of 1:10 (v/v) and incubation was performed in optimal conditions at 25°C, 150 rpm for 3 days for *K. pneumoniae* and at 30°C, 100 rpm for 5 days for *K. oxytoca*. The experiment was performed in triplicate. Additionally, cyanide containing wastewater which doesn't contain bacteria was used as control in all experiments.

Analysis of Biodegradation Products

Residual cyanide concentration and ammonia formation in synthetic wastewaters were assessed by using picric acid method (36) and Nesslerization method (37) respectively. Additionally, growth of *K. pneumoniae*, *K. oxytoca* cultures separately and mix cultures of *K. pneumoniae* - *K. oxytoca* species in these wastewaters were measured by using spectrophotometer (Shimatzu-UV 1700) at 600 nm.

RESULTS

Cyanide Removal by Using Bacterial Cultures and Crude Extracts

Cyanide removal capabilities of cultures and sterile crude extracts of *K. pneumoniae* and *K. oxytoca* species in electroplating, metal plating and gold mining wastewasters were investigated in this study. As a result, it is found that *K. pneumoniae* culture degraded the cyanide content in electroplating and metal plating industries' wastewaters with efficiencies of 100% and 94%, respectively (Figures 1a and 1b). However, this strain was not effective in the biodegradation process of cyanide in gold mining industry wastewater (Figure 1c). Additionally, when biodegradation results are evaluated for *K. oxytoca*, it is found that results are in parallel with the biodegradation ability of *K. pneumoniae* culture and accordingly this strain also fully degraded the cyanide content in electroplating wastewater,

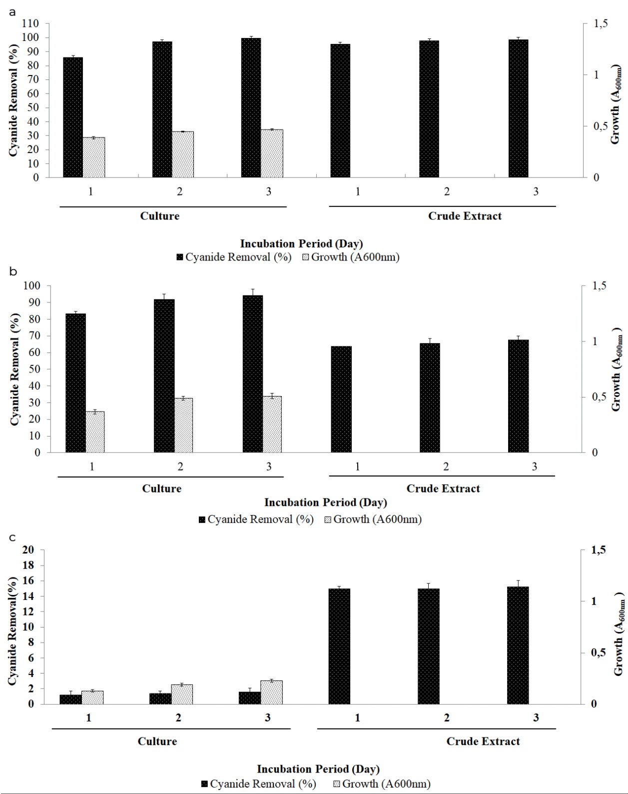

Figure 1. Cyanide removal in electroplating (a), metal plating (b) and gold mining (c) wastewaters with culture and crude extract of *K. pneumoniae*.

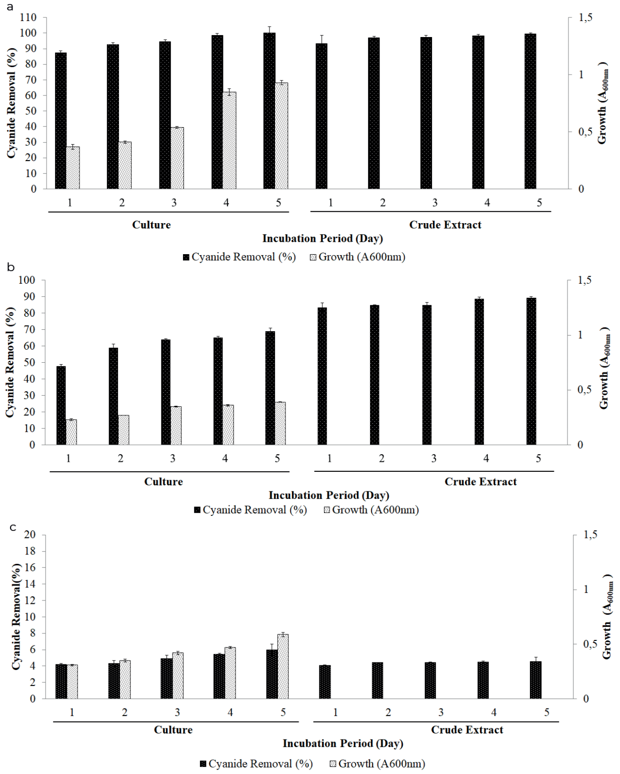

Figure 2. Cyanide removal in electroplating (a), metal plating (b) and gold mining (c) wastewaters with culture and crude extract of *K. oxytoca*.

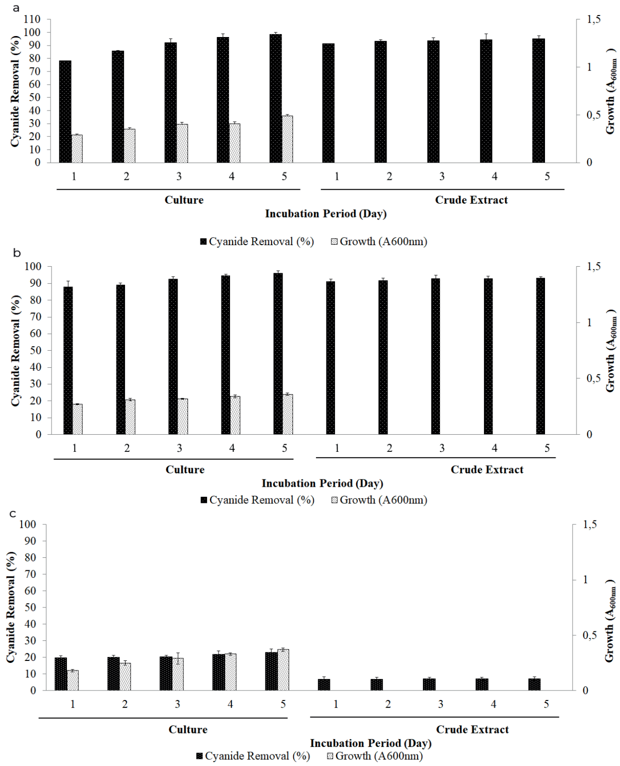

Figure 3. Cyanide removal in with culture and crude extract of *K. pneumoniae* and *K. oxytoca* in electroplating (a), metal plating (b) and gold mining (c) wastewaters.

70% of the cyanide content in metal plating wastewater and it was not effective in the biodegradation of cyanide content in the gold mining wastewater (Figure 2). Furthermore, when crude extracts are used for the removal of cyanide, it is found that sterile crude extract of *K. pneumoniae* fully degraded cyanide in electroplating wastewater and degraded 65% of the cyanide content in metal plating industry wastewater (Figures 1a and 1b). Besides, sterile crude extract of *K. oxytoca* degraded both of these wastewaters with the efficiencies of 100% and 90%, respectively (Figures 2a and 2b).

Cyanide Removal by Using Mix Cultures and Mix Crude Extracts

Mix cultures and mix crude extracts of *Klebsiella* species were formed in order to evaluate the interactions between these *Klebsiella* species in different cyanide removal processes. Accordingly, it is observed that mix of cultures and mix of crude extracts of *K. pneumoniae* and *K. oxytoca* species are also effective in the biodegradation of cyanide in electroplating and metal plating wastewaters (Figures 3a and 3b). Besides, when these species are combined with each other, synergetic interaction between these *Klebsiella* species was observed and biodegradation ability increased to 23% in gold mining wastewater (Figure 3c).

Ammonia Formation

In this study, ammonia was observed as a final product of cyanide biodegradation process. Accordingly, it is examined that ammonia was formed by both of *Klebsiella* species' cultures in electroplating wastewater and were formed by *K. pneumoniae* and *K. oxytoca* cultures in metal plating wastewater during cyanide biodegradation processes. Although the least cyanide biodegradation capability of all *Klebsiella* species was observed in gold mining wastewater, by means of including 1000 mg/l CN and 2000 mg/L SCN- in its content, the most amount of ammonia (6.9 mg/L and 4.68 mg/L) were formed by *K. pneumoniae* and *K. oxytoca* cultures, respectively.

Ammonia formation in wastewaters during cyanide biodegradation processes by using mix cultures and mix crude extracts of *Klebsiella* species were also investigated. As a result, it is observed that, 0.51 mg/L, 0.12 mg/L and 7.9 mg/L ammonia were formed in cyanide removal processes of electroplating, metal plating and gold mining industries respectively by using mix cultures of *Klebsiella* species and 0.49 mg/L, 0.12 mg/L and 7.9 mg/L ammonia were formed in cyanide removal processes by using their mix of crude extracts.

DISCUSSION

Cyanide removal by using biological methods seems more advantageous and environmentally friendly than chemical ones as they form nontoxic end products (2, 5, 38). Accordingly, microorganisms, which are being investigated for cyanide removal efficiencies, must be able to live in wastewaters containing heavy metals and some other toxic chemicals and also synthesize enzymes for cyanide removal processes.

In all over the world, up to 20% of cyanogen wastes originate from electroplating industries (39). Additionally, electroplating wastewater is an important cyanogen waste former by means of using and forming sodium and potassium salts of cyanide in its production processes (40). In this study, *K. pneumoniae* and *K. oxytoca* species' cultures fully degraded the cyanide content in electroplating wastewater and 94% and 70% of the cyanide content in metal plating wastewater respectively. Some of other researches, *B. safensis, B. licheniformis* and *B. tequilensis* strains which were isolated from electroplating wastewater, degraded 200 and 400 CN^{-}/L cyanide with the efficiencies of 65.5% and 44.3% respectively (41) and *P. montelii* which was also isolated from wastewater, degraded 93% of the cyanide content in wastewater (40). Therefore, in the present study, *Klebsiella* species are found to be effective agents for the removal of cyanogen wastes for further treatment processes.

In this study, as presented above, biodegradation of cyanide by using *Klebsiella* species in gold mining wastewater was also investigated and it is observed that both of the *Klebsiella* species were not effective in this wastewater (Figures 1c and 2c). This is probably due to the fact that, the content of this wastewater contains not only heavy toxic ions (As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Sel, Mg and Zn) and high concentrations of cyanide compounds (SCN-, CN^{-}), and it also contains NH_4^+ which is known as a competitive agent in cyanide biodegradation. Accordingly, cyanide removal by *K. pneumoniae and K. oxytoca species* was not effective in the removal of cyanide in this wastewater because of the presence of these chemicals. In parallel with our research, it is found that the presence of ammonia in the biodegradation media directly affects the biodegradation ability of microorganism as it utilizes ammonia instead of cyanide inside the medium (41).

Additionally, ion effect on cyanide biodegradation capability of microorganisms was also investigated in some other researches and it is observed that growth amount and cyanide biodegradation ability of *Burkholderia cepacia* inhibited in the presence of 1mM Cu and Fe ions and biodegradation is also directly related with the presence of Ni, Co, Mn and Mo ions (42) and it is determined that Pb and Cd and Fe ions inhibited this process approximately 20% and 30-35% respectively (43). In a similar study concentrated on ion effect on cyanide removal, it was determined that although Cu and Ca ions directly affected the bacterial growth and cyanide biodegradation, Mg and Mn ions affected only growth (44). Additionally, Ibrahim *et al.* (30) found that Hg ion shows toxic effect on the growth of *Serratia marcescens* and cyanide biodegradation with this bacterial strain dropped sharply from 92% to 24.7% in the presence of this ion. As a result, all wastewaters investigated in this study contain different concentrations of various ions and *K. pneumoniae* and *K. oxytoca* species achieved cyanide removal. Therefore, these results directly demonstrate the usability of these *Klebsiella* species in the treatment processes of ion containing cyanogen wastes.

Alternatively, purified enzymes from bacterial crude extracts can be used in biodegradation studies instead of their cultures. When purified enzymes are used, crude extracts directly degraded toxic compounds effectively in a short time. In this respect, sterile crude extracts of *Klebsiella* species were used in order to investigate the ability of these unpurified extracellular substances in biodegradation processes of different wastewaters. No purification method was used for purification of these sterile crude extracts to obtain cyanide degrading enzymes. In this respect, these extracellular substances may contain not only enzymes but also biodegradation products and some other unusable chemicals. Therefore, this study pointed out the usability of unpurified extracellular products of *Klebsiella* species in cyanide biodegradation and it is obvious that if it is purified, the efficiency of biodegradation will be much higher. Additionally, it can be noted that, *Klebsiella* enzymes can be used solely for cyanide biodegradation and alternative immobilized systems can be developed in future researches.

Antagonistic and synergetic interactions between bacteria are being used in environmental studies in order to treat various toxic chemical compounds in literature. Therefore, mix cultures of *Klebsiella* species' and mix of their crude extracts were formed and biodegradation abilities of these mixes were also investigated in this study. As a result, it is observed that mix cultures and mix crude extracts of *Klebsiella* species are also effective in cyanide biodegradation processes in electroplating and metal plating industries' wastewaters. Additionally, synergetic interaction between these species was also examined in the cyanide biodegradation of gold mining industry' wastewater. In the literature, it is observed that when *Citrobacter sp.* MCM B-181, *Pseudomonas sp.* MCM B-182, *Pseudomonas sp.* MCM B-183 and *Pseudomonas sp*. MCM B-184 strains are used solely, cyanide removal efficiencies of these strains are found as 92.5%, 88.4%, 73.2% and 68.8%, respectively but with a consortium and by using a reactor, cyanide removal increased to 99% , and when *Bacillus sp*., *Klebsiella sp*., *Pseudomonas sp*. and another *Pseudomonas sp*. are used, cyanide biodegradation amount increased to approximately 98% by means of synergetic interactions between these strains (44). In another study, it is observed that when a consortium included *B. subtilis* and *P. stutzeri* strains are used cyanide biodegradation increased from 66.9 % to 88.5 % (45). According to Razanamahandry et al. (46) enzymes which are synthesized by consortia are durable for a long time and the usage of these consortia in degradation studies can increase biodegradation efficiencies. Therefore, this research demonstrated the importance of synergetic interactions between microorganisms in environmental researches.

CONCLUSION

To sum up, the results of this research demonstrated the usability of cultures, unpurified sterile crude extracts and mixes of cultures and crude extracts of *K. oxytoca* and *K. pneumoniae* species in cyanide removal processes of electroplating and metal plating industries' wastewaters efficiently. Additionally, different than other researches, this study also determined the importance of sterile crude extracts of *K. oxytoca* and *K. pneumoniae* species in the biodegradation of cyanide. The results are promising for the future researches on cyanide removal by using bacterial strains and their crude extracts.

Peer-review: Externally peer-reviewed.

Author Contributions: Conception/Design of study: N.H.A, I.S.B.; Data Acquisition: N.H.A.; Data Analysis/Interpretation: N.H.A.; Final Approval and Accountability: N.H.A, I.S.B.; Drafting Manuscript: N.H.A.; Critical Revision of Manuscript: N.H.A.; Technical or Material Support: N.H.A., I.S.B.; Supervision: N.H.A., I.S.B.

Conflict of Interest: The author has no conflict of interest to declare.

Financial Disclosure: Hacettepe University Scientific Research Projects Coordination Unit 014 D03 601 004.

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