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BACTERIAL OXIDATION OF FERROUS IONS IN SUSPENDED CELLS CULTURES AND BIOFILM OF *ACIDITHIOBACILLUS FERROOXIDANS* JCM 3863 IN PRESENCE OF COPPER IONS

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Abstract: Bacteria *Acidithiobacillus ferrooxidans* are usually implemented in many biotechnological processes as bioleaching of metals from low grade ores, purification of waste waters and gases, removal of metals from solid wastes etc. The bioprocess systems with biofilm of *Acidithiobacillus ferrooxidans* are capable to oxidize ferrous to ferric ions in higher concentrations. The aim of this work is to investigate bacterial oxidation in suspended cultures and biofilm of *Acidithiobacillus ferrooxidans JCM 3863* in presence of copper ions and different initial concentration of ferrous ions. Submerged cultures and biofilm have been cultivated in shake flasks at 200 rpm in two media – with 9 and 12 g/l initial concentration of ferrous ions. Biofilm and submerged cultures have been cultivated in five consecutive cycles in each nutrient medium in the presence of 5 g/l copper ions to complete oxidation of ferrous to ferric ions. In the end the biofilm thickness has been determined. Mean rates of oxidation were compared for each cycle of cultivation. In consecutive cultivation of suspended cells in media 9K after the second cycle the mean rates decrease significantly. In biofilm in media 9K is observed that the mean rates are constant and even increase in the last cycle. In consecutive cultivation of suspended cells in media 12K in the last two cycles almost the rate from the first cycle is reached after decrease in the medium cycles. In biofilm in media 12K there is a gradual increase of mean rates compared to the first cycle. In summary, the biofilm is more suitable for industrial biotechnological processes in the presence of copper ions and high concentrations of ferrous ions.

INTRODUCTION

Modern society is impossible without information technologies, but they are the source of a large amount of solid waste that are dangerous source of pollution. Bacteria *Acidithiobacillus ferrooxidans* are usually implemented in many biotechnological processes as bioleaching of metals from low grade ores, purification of waste waters and gases, removal of metals from solid wastes etc. At the same time they represent a valuable secondary raw material for the production of plastics and metals. Recycling of electronic waste is an important activity not only in terms of their treatment as waste, but also to extract valuable components and energy savings (Cui J. and Forssberg E., 2003; Khalid A. et al., 2014; Rivero Hudec M.A. et al. 2009). Biotechnological methods for extracting metals from secondary sources of raw materials does not require the incorporation of large amounts of energy to ensure the high temperatures and generate relatively small amounts of waste which are non-toxic (Ilyas S. et al., 2007). Genus *Acidithiobacillus* - species *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* (Free M.L., 2013; Singh U.Sh. and Kapoor K., 2010) are mainly involved in these processes. Research conducted by *Acidithiobacillus ferrooxidans* JSM 3863 (Gyurov P. et al., 2015; Ivanova E. et al., 2015; Mamatarkova V. et al., 2014) demonstrate their possible application for leaching of copper from printed circuit boards (Gyurov P. et al., 2015), copper and PMG (platinum and palladium) from automobile exhaust converters (Ivanova E. et al., 2015; Mamatarkova V. et al., 2014). Bioleaching rate depends on the concentration of ferric ions in the solution. Therefore, studies related to the behavior of *Acidithiobacillus ferrooxidans* at higher initial concentrations of iron and the possibility of their adaptation to them are of practical interest. It has been found that the biofilm of these bacteria are more resistant to high concentrations of ferric and ferrous ions (Nikolov L. et al., 1988; Karamanev D. and Nikolov L., 1991; Mamatarkova V., 2002) , as well as other environmental factors (Karamanev D. and Nikolov L., 1988). It is necessary to investigate bacterial oxidation in the presence of copper ions and elevated concentrations of iron.

The aim of this work is to investigate bacterial oxidation in submerged cultures and biofilm of *Acidithiobacillus ferrooxidans JCM 3863* in the presence of copper ions and different initial concentration of ferrous ions.

MATERIALS AND METHODS

Microorganisms

The experiments were carried out with the strain *Acidithiobacillus ferrooxidans* JCM 3863.

Medium

Acidithiobacillus ferrooxidans JCM 3863 were cultivated in 9K liquid medium containing 9 g/l ferrous ions (Silverman M.P. and Lundgren D.G., 1959), and in 12K liquid medium – modified 9K media, supplemented with 12g/l ferrous ions. Biofilm was cultivated in the same media after his formation. Copper ions in the form of $CuSO_4.7H_2O$ in concentration 5 g/l were added in each media. As biological controls are used, the results obtained by cultivation of *Acidithiobacillus ferrooxidans* JCM 3863 in the described media without copper ions – for suspended cell cultures it is named "biological control", for biofilm – "cycle 0".

Analytical methods

Concentrations of ferrous, ferric ions and total iron were determined spectrophotometrically (Karamanev D. et al., 2002).

Determination of biofilm thickness

Thickness of biofilm was determined on the base of his dried weight, density and area of the carriers (Karamanev D. and Nikolov L., 1988; Nikolov L. et al., 2002; Mamatarkova V., 2002).

Experimental conditions

Acidithiobacillus ferrooxidans JCM 3863 were cultivated in shake flasks in batch at 190 rpm and 28±0.5°C. Suspended cells were cultivated in 100 ml liquid medium 9K or 12K (inoculum 1%). Biofilm was cultivated in 250 ml liquid media 9K or 12K, four carriers with formed biofilm in each flask, in fed-batch (Valkova M. et al., 1982; Nikolov L. et al., 2002; Mamatarkova V., 2002).

RESULTS AND DISCUSSION

Submerged cultures have been cultivated in five consecutive cycles in each nutrient medium in the presence of 5 g/l copper ions to complete oxidation of ferrous to ferric ions. The results are shown on figure 1. It is clear to see similarities and differences in the process dynamics of ferric ions production in medium 9K in submerged cultures. For the biological control and the first tree cycles, it can be accepted that they have similar dynamics and the full oxidation of ferrous ions is reached at 48 hours. A delay with 24 hours was observed in cycle 4, resulting in 72 h duration of the cycle, while at cycle 5 the oxidation is strongly delayed to 192 h.

Mean rates of ferrous ions oxidation and ferric ions production were calculated for each cycle of cultivation. The results are shown on figure 2.

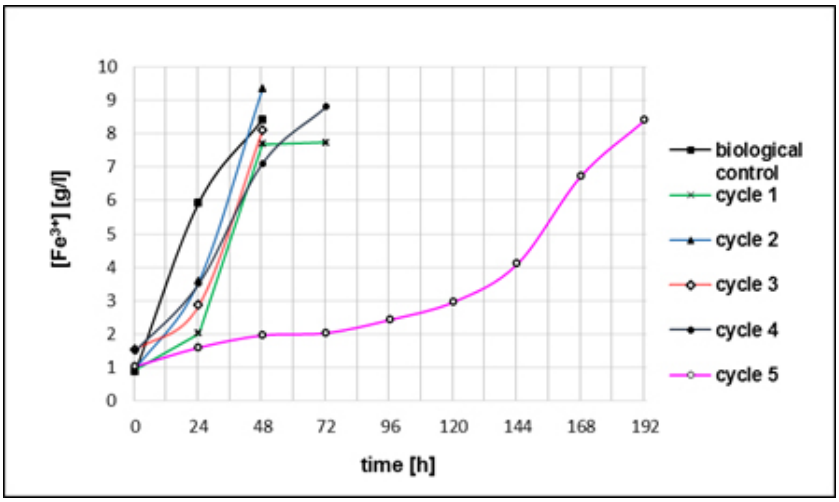

Fig. 1. Dynamics of ferric ions production in suspended cultures - biological control in 9K medium and five cycles in 9K medium with 5 g/l Cu^{2+}.

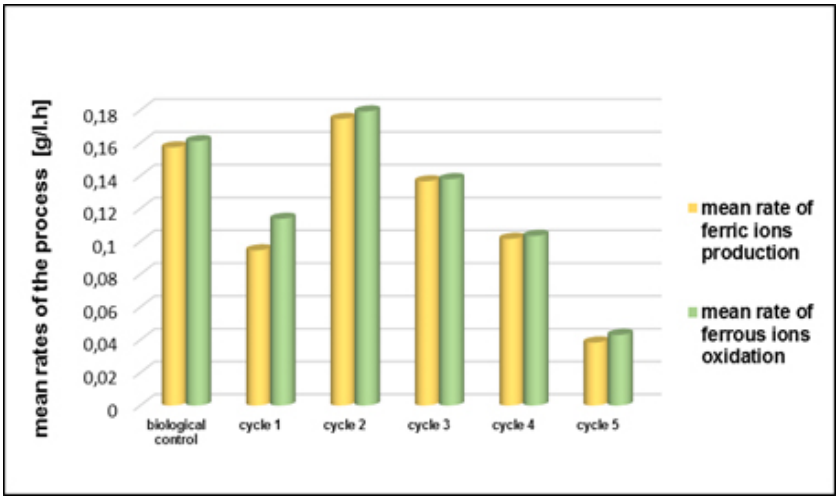

Fig. 2. Mean rates of ferric ions production in suspended cells - biological control in 9K medium and five cycles in 9K medium with 5 g/l Cu^{2+}.

It can be seen that after the second cycle of cultivation mean rates are permanently decreasing. The unessential differences between rates of ferrous ions oxidation and ferric ions production are caused by sedimentation of ferric ions as jarosite on the bottom and sides of the flasks. This type of sedimentation is always observed in cultivation of *Acidithiobacillus ferrooxidans* in media with ferrous ions (Nikolov L. and Mamatarkova V., 2005). Received results show that the *At. ferrooxidans* JCM 3863 culture obviously not adapted to presence of copper ions during five consecutive cycles of cultivation in medium 9K..

Analysis of cultivation dynamics of suspended cultures in media 12K show that in cycles 2 and 3 the process duration is 96 h, as for the other cycles (cycles 1, 4 and 5) and the biological control full oxidation is reached for 48 h (figure 3).

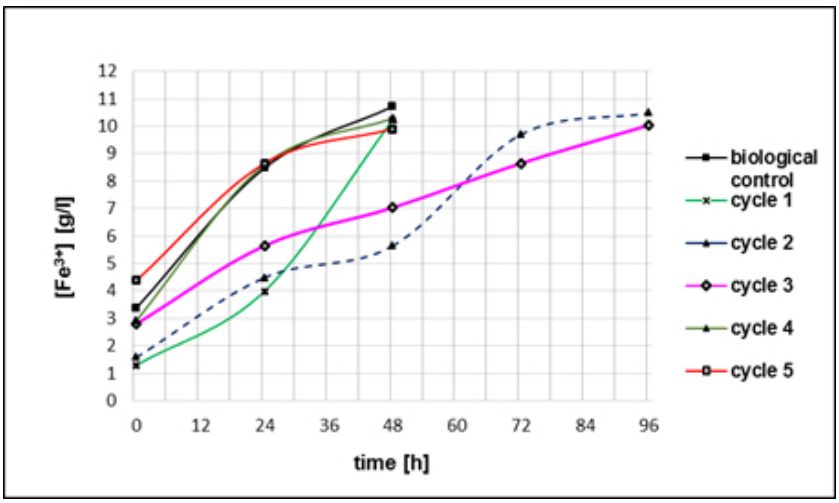

Fig. 3. Dynamics of ferric ions production in suspended cultures - biological control in 12K medium and five cycles in 12K medium with 5 g/l Cu^{2+}.

For each cycle of cultivation are calculated mean rates of ferrous ions oxidation and ferric ions production. The results are shown on figure 4.

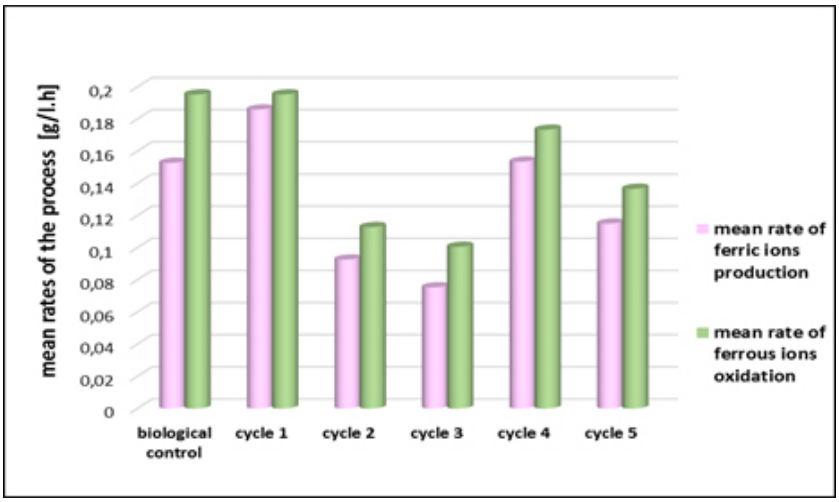

Fig. 4. Mean rates of ferric ions production in suspended cells cultures - biological control in 12K medium and five cycles in 12K medium with 5 g/l Cu^{2+}

It can be seen that the mean rates of ferrous ions oxidation in the biological control and cycle 1 are commensurable. In cycle 2 and 3 mean rate visible decrease with over 50%. In the last two cycles 4 and 5 mean rates increase again and are similar to this in biological control and cycle 1. The enhancement of mean rates in cycles 4 and 5 shows adaptation of the culture. The unessential differences between rates of ferrous ions oxidation and ferric ions production are caused by sedimentation of ferric ions as jarosite on the bottom and sides of flasks, like in media 9K.

Biofilm has been cultivated in five consecutive cycles in each nutrient medium in the presence of 5 g/l copper ions to complete oxidation of ferrous to ferric ions, like in suspended cells cultures. Cultivation of biofilm in medium 9K and 5 g/l copper ions shows that with the exception of cycle 4 and 5 the full oxidation is reached at 48 hours. Results show that dynamics of ferric ions production by biofilm in the absence of copper ions (cycle 0) and the cycles 3 and 5 in the presence of copper ions are similar in the first 24 hours – above 5 g/l ferric ions are produced (figure 5). In cycles 1 and 2 (the first two cycles of cultivation of biofilm in the presence of copper ions) the production of ferric ions is smaller. This difference in dynamics may be due to the adaptation of biofilm in cycles 1 and 2 to the presence of copper ions. The difference in the final concentrations of ferric ions is due to the growth of the biofilm and formation of jarosite (Grishin S.I. et al., 1988; Nikolov L. and Mamatarkova V., 2005).

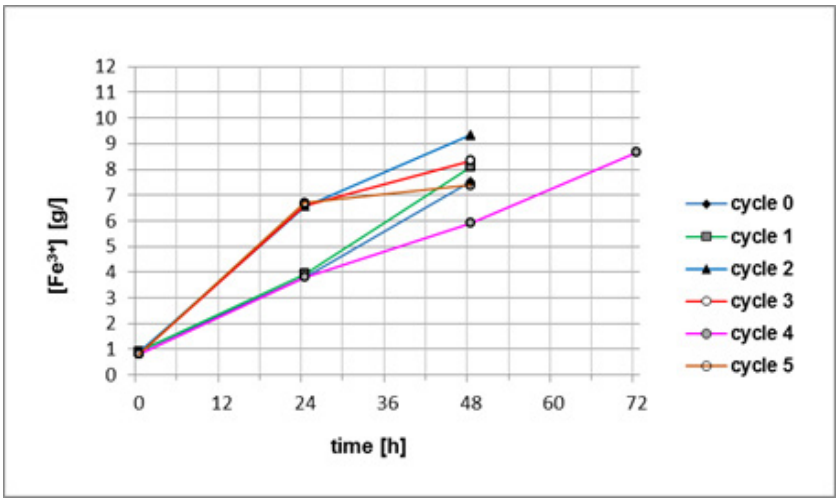

Fig. 5. Dynamics of ferric ions production in biofilm - biological control in 9K medium (cycle 0) and five cycles in 9K medium with 5 g/l Cu^{2+}.

Mean rates of ferrous ions oxidation and ferric ions production are calculated for each cycle of cultivation. The results are shown on figure 6.

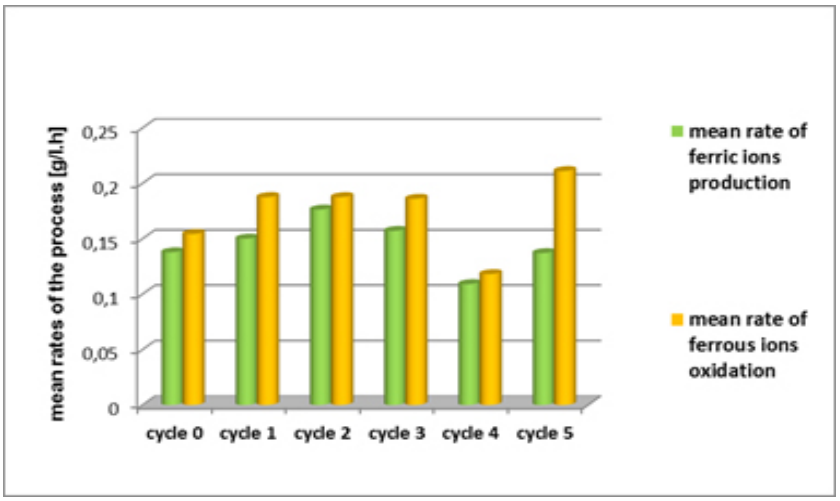

Fig. 6. Mean rates of cultivation of biofilm - biological control (cycle 0) in 9K medium and five cycles in 9K medium with 5 g/l Cu^{2+}.

It is visible that after the addition of copper ions, the mean rates of oxidation are the same in three consecutive cycles. The mean rates of ferric ions production are different in each cycle. These differences are caused by significant sedimentation of ferric ions as jarosite on the bottom and sides of flasks and mostly on the carriers, which leads to increasing of biofilm, as mentioned above. This sedimentation explains the differences between the rates of ferrous ions oxidation and ferric ions production. The different dynamic of sedimentation in the different cycles and flasks can explain the differences in the mean rates of ferric ions production.

In biofilm in medium 12K after the first cycle of cultivation in the presence of copper ions biofilm oxidizes ferrous ions by similar dynamics in the next four cycles. The duration of each cycle is longer in comparison to the cultivation of biofilm in 9K medium with 5 g/l Cu^{2+} (see figure 5). The removal of copper ions from the medium in cycle 6 leads to similar dynamics as in cycle 0 before the addition of copper ions. This shows that the influence of copper ions is reversible. Similar results were established for cultivation of *Acidithiobacillus ferrooxidans* JCM 3863 in the presence of other metals as silver and mixture of nickel, cobalt and copper ions (Mamatarkova V. et al., 2013; Tzenov M. et al., 2015).

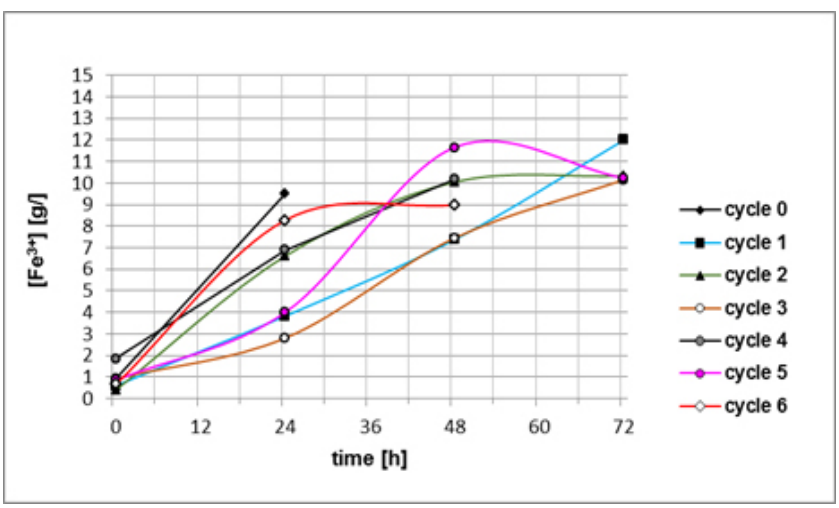

Fig. 7. Dynamics of ferric ions production in biofilm - biological control in 12K medium (cycle 0) and five cycles in 12K medium with 5 g/l Cu^{2+}.

Mean rates of ferrous ions oxidation and ferric ions production were calculated for each cycle of cultivation. The results are shown on figure 8.

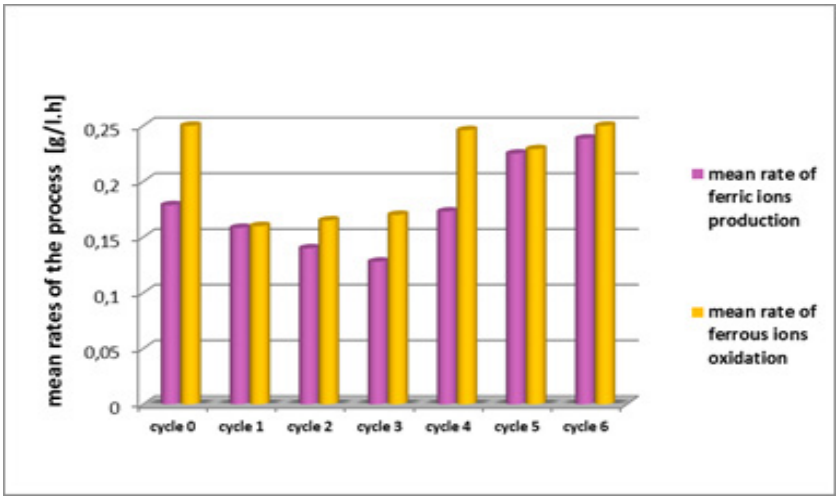

Fig. 8. Mean rates of cultivation of biofilm - biological control (cycle 0) in 12K medium and five cycles in 12K medium with 5 g/l Cu^{2+}.

It is visible that after the addition of copper ions, the mean rates of oxidation decrease in the first three cycles of cultivation of biofilm. In cycle 4 and 5 mean rates increase. Oxidation rates of ferrous ions is commensurable with this on cycle 0, before the addition of copper ions. The results show that the biofilm is adaptive to various concentration of ferrous and copper ions in media.

After the end of biofilm cultivation, its thickness was determined. Results are presented on figure 9. It can be seen that in medium 9K in the presence of copper ions the biofilm grew slower (thickness 243 μ for 600 h) that in medium 12K in the presence of copper ions (thickness 300 μ for the same period). In our previous studies (Mamatarkova V. et al., 2013; Tzenov M. et al., 2015) we established that for 600 h cultivation in 9K medium in the absence of any metal ions, biofilm thickness reached 375 μ. This shows that presence of copper ions in medium influence the growth of biofilm.

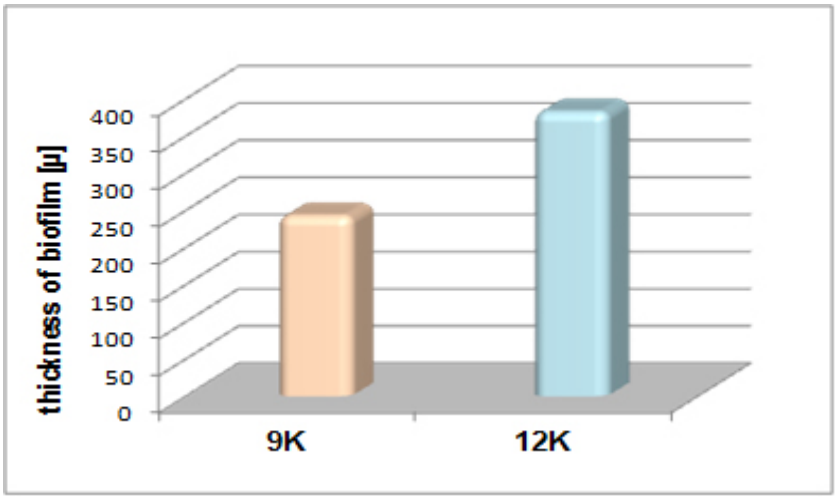

Fig. 9. Biofilm thickness after cultivation in 9K and 12K media with 5 g/l Cu^{2+}.

The mean rates of ferrous ions oxidation in suspended cultures and in biofilm of *Acidithiobacillus ferrooxidans* JCM 3863 were compared. Results are shown on figure 10 and 11.

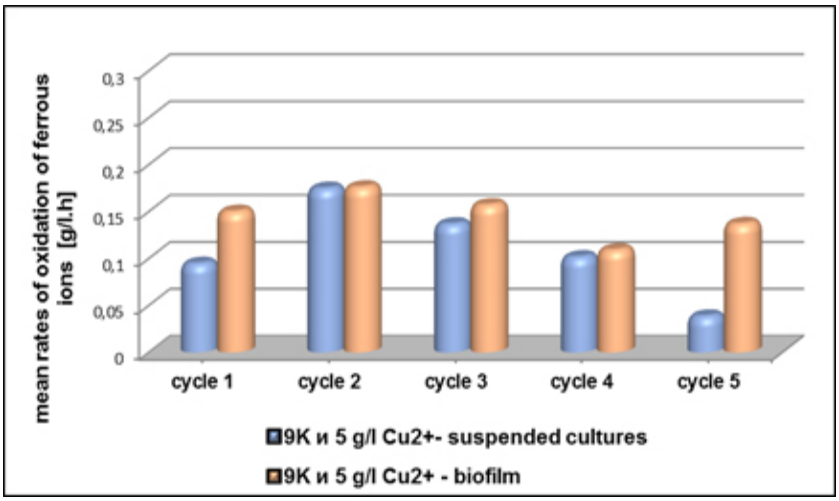

Fig. 10. Mean rates of ferrous ions oxidation - in medium 9K with 5 g/l Cu^{2+}.

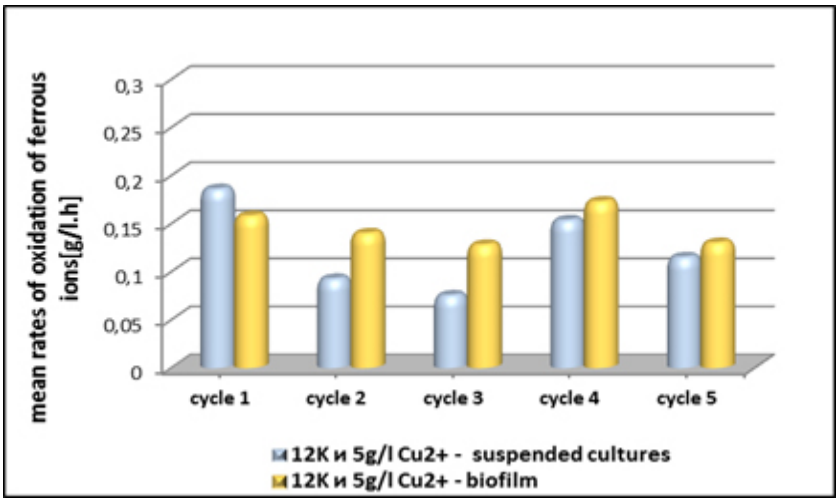

Fig. 11. Mean rates of ferrous ions oxidation - in medium 12K with 5 g/l Cu^{2+}.

Mean rates of ferrous ions oxidation were compared for each cycle of cultivation in the presence of copper ions. In consecutive cultivation of suspended cells and biofilm in medium 9K after the first cycle the mean rates increased in cycles 2 and 3. Cultivation of biofilm in medium 9K shows that the mean rates are higher compared to that in suspended cells cultures. This difference is most pronounced in cycle 5. These results show that the cells of *Acidithiobacillus ferrooxidans* JCM 3863 in biofilm are more resistant to copper ions and may be considered that biofilm adapt to the presence of copper ions. Similar results about resistance of biofilm to various conditions of cultivation were obtained with other strains *Acidithiobacillus ferrooxidans* (Karamanev D., Nikolov L., 1988; Karamanev D., Nikolov L., 1991).

Variability in mean rate of ferrous ions oxidation can be observed in consecutive cultivation of suspended cells and biofilm in media 12K. In biofilm, the mean rates are nearly constant but the changes are similar to these in suspended cells cultures. Mean rate of ferrous ions oxidation is higher in biofilm again.

CONCLUSIONS

The present results of this research can show that the biofilm is suitable for application in industrial biotechnological processes in the presence of copper ions and higher concentrations of ferrous ions. The further investigations should be directed to the development of biofilm process system for bioleaching of copper and other metals from real electronic and other solid wastes.

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REFERENCES

1. Cui J., Forssberg E. 2003. Mechanical recycling of waste electric and electronic equipment: A review. *Journal of Hazardous Materials*, 99 (3): 243–263.
2. Free M. L. 2013. Hydrometallurgy: Fundamentals and Applications, Chapter 5: Metal Extraction. John Wiley & Sons, pp.159-165.
3. Grishin S.I., Bigham J.M., Tuovinen O.H. 1988. Characterization of Jarosite Formed upon Bacterial Oxidation of Ferrous Sulfate in a Packed-Bed Reactor. *Applied and Environmental Microbiology*, 54 (12): 3101-3106.
4. Gyurov P., Mamatarkova V., Karadjova I. 2015. Potentialities of Metals Bioleaching From Printed Circuit Boards by *Acidithiobacillus ferrooxidans* JCM 3863. Youth Science Conference Kliment's days, november 2015, Sofia, Bulgaria, Abstract Book p. 40.
5. Ilyas S., Anwar M.A., Niazi S.B., Ghauri M.A. 2007. Bioleaching of metals from electronic scrap by moderately thermophilic acidophilic bacteria. *Hydrometallurgy*, 88: 180-188.
6. Ivanova E., Mamatarkova V., Karadjova I. 2015 Ecological application of *Acidithiobacillus ferrooxidans* JCM 3863 for biohydrometallurgical recovery of rare and precious metals from spent catalytic converters. *Ecological Engineering and Environment Protection* (EEEP'2015), Science Conference, Burgas, Bulgaria.
7. Karamanev D., Nikolov L. 1988. Influence of Some Physicochemical Parameters on Bacterial Activity of Biofilm: Ferrous Iron Oxidation by *Thiobacillus ferrooxidans*. *Biotechnology and Bioengineering*, 31: 295-299.
8. Karamanev D., Nikolov L. 1991. A comparison between the reaction-rates in biofilm reactors and free suspended cells bioreactors. *Bioprocess Engineering*, 6 (3): 127-130.
9. Karamanev D., Nikolov L., Mamatarkova V. 2002. Rapid simultaneous quantitative determination of ferric and ferrous ions in drainage waters and similar solutions. *Minerals Engineering*, 15 (5): 341-346.
10. Khalid A., Rhamdhani M.A., Brooks G., Masood S. 2014. Metal Extraction Processes for Electronic Waste and Existing Industrial Routes: A Review and Australian Perspective. *Resources,* (3): 152-179.
11. Mamatarkova V. 2002. Dynamics of Bacterial Oxidation of High Concentrations of Iron Ions. PhD Thesis. Sofia University "St Kl. Ohridski", Sofia, Bulgaria.
12. Mamatarkova V., Karadjova I., Mitov G., Gerginova N., Geneva S. 2013. Influence of silver ions on oxidation activity of *Acidithiobacillus ferrooxidans* JCM 3863 in biofilm. International Conference "Bioscience – Development and New Opportunities", Sofia, Bulgaria, Abstract Book, p. 98.

13. Mamatarkova V., Ivanova E., Karadjova I. 2014. Investigation of the possibility of using *Acidithiobacillus ferrooxidans* JCM 3863 for biohydrometallurgical recovery of rare and precious metals from spent catalytic converters, *Annuaire de l'Université de Sofia "St. Kliment Ohridski"*, Faculte De Biologie, 100 (4): 63-69, First National Conference of Biotechnology, Sofia 2014.
14. Nikolov L., Mamatarkova V. 2005. Dynamics of Sedimentation of Ferric Ions in Biodisk Reactor. *Ecological Engineering and Environment Protection, No* 1: 42-51.
15. Nikolov L., Karamanev D., Mamatarkova V., Mehochev D., Dimitrov D. 2002. Properties of the biofilm of *Thiobacillus ferrooxidans* formed in rotating biological contactor. *Biochemical Engineering Journal*, 12 (1): 43-48.
16. Nikolov L., Valkova-Valchanova M., Mehochev D. 1988. Oxidation of High Ferrous Iron Concentrations By Chemolithotrophic *Thiobacillus Ferrooxidans* In Packed-Bed Bioreactors. *Journal of Biotechnology*, 7 (2): 87-94.
17. Rivero Hudec M.A., Sodhi M., Goglia-Arora D. 2009. Biorecovery of Metals from Electronic Waste. 7th Latin American and Caribbean Conference for Engineering and Technology WE1-1, San Cristóbal, Venezuela.
18. Silverman M.P., Lundgren D.G. 1959. A study on the chemoautotrophic iron bacterium *Ferrobacillus ferrooxidans*. I. An improved medium and a harvesting procedure for securing high cell yields. *Journal of Bacteriology*, 77: 642-647.
19. Singh, U.Sh., Kapoor K. 2010. Microbial Biotechnology. Oxford Book Company, Jaipur, India, ISBN: 978-93-80179-24-7.
20. Tzenov M., Mamatarkova V., Karadjova I. 2015. Bacterial oxidation of ferrous ions by biofilm of *Acidithiobacillus ferrooxidans* JCM 3863 in the presence of a mixture of Ni^{2+}, Co^{2+} and Cu^{2+}. Youth Science Conference Kliment's days, Sofia, Bulgaria, Abstract Book, p. 30.
21. Valkova M., Nikolov L., Todorov Ts. 1982. Microbial oxidation оф ferrous sulfate in biodisk reactor. Вълкова М., Николов Л., Тодоров Ц., 1982, "Микробиологично окисление на феросулфат в биодисков реактор", *Annuaire de l'Université de Sofia "St. Kliment Ohridski"*, Faculte De Biologie, 3 (75): 58-65.