REMOVAL OF KYANIDES FROM MODEL WATERS

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Abstract
This thesis is introducing a theme of biodegradation cyanide in a model sample of wastewater by microorganism *Escherichia coli* and *Pseudomonas aeruginosa*. Apart from monitoring an effect of choosen microorganism on a speed of degradation there was also an effect of an initial concentration of cyanides and temperature monitored. Another tested factor was an efect of used organic substrate on degradation of cyanides. It was prooved that tested microorganisms are able to successfully eliminate those cyanides from sample.

Key words:
Biodegradation, wastewater treatment, cyanide, *Escherichia coli*, *Pseudomonas aeruginosa*

Introduction
With the industrial development environmental pollution is increasing, including surface water. If the wastewater contains toxic substances, there is a danger to all classes of living organisms. Cyanide is among the highly toxic substances. Although cyanide is poisonous, it is one of the most indispensable industrial chemicals. For example, cyanide is used to extract gold and silver from minerals in the mining industry in technologies associated with coal heat treatment, coating and heat treatment of metals or for the purpose of chemical synthesis. The resulting waste water may also contain heavy metals such as nickel, copper, zinc, silver, and iron, with which it forms complexes cyanides. Cyanides can be present in the environment and waste water as free cyanide (HCN, CN^-, NaCN), metal complexes, cyanates or nitriles. For removing cyanides from waste water there are many ways. Recently, as the most environmentally friendly seem to be the biological degradation, to which is this work dedicated.

Methodics:
For testing biodegradation CN^- was used a referential strain of *Escherichia coli* CCM 3954 of the Czech Collection of Microorganisms at Masaryk University in Brno. *Pseudomonas aeruginosa* was cultured from samples of waste water from urban biological wastewater treatment plant. This strain was identified on the basis of growth and biochemical properties.

In experiments using a modified M9 medium by authors Kao et al. (2003), which was prepared as follows. Into the reservoir bottle of 500 ml volume were successively weighed 8.95 g of Na_2HPO_4 * $12H_2O$; 1.5 g KH_2PO4 and 0.25 g of NaCl. Chemicals were dissolved in 498 ml of distilled water. Subsequently, the bottle sterilized in an autoclave at 121 ° C for 15 minutes. After cooling to 50 ° C were added 2 ml MgSO 4 and 0.1 ml of 1M CaCl2. To thus prepared stock solution of the modified M9 medium was added a solution of KCN, so as to achieve the desired concentration of CN^- (10 and 50 mg/l). Then was added the organic carbon source (glucose, ethanol or methanol). Finally, it was inoculated with 2.5 ml of the test organism of scale 1 McFarland turbidity. Testing the degradation of cyanide ions was carried out at 25 ° C and 37 ° C with stirring. The individual samples were in regular intervals collected 1.5 ml of medium to determine the concentration of cyanide ions. To check whether there is any contamination of the sample and whether the sample tested microorganism is still viable, after each determination was plated onto MH agar. The concentration of cyanide was determined according to ISO 75 7415.

Results and discussion:
Firstly, all three organic substrates were tested on their effect on cyanides without the presence of microorganisms. While in methanol and ethanol, there was no effect observed, in glucose a sharp drop of cyanide was observed, as shown in Figure 1. Within 12 days decreased the concentrations of cyanide ions in the sample with 0.2% glucose to 66.56%, in the sample containing 0.4% glucose to 55.66% and in the sample with 1.6% glucose to 69.81%. The presence of undesirably microorganisms was verified by inoculating to the broth. This significant decrease in cyanide solution was probably caused by the reaction of the aqueous cyanide solution with glucose according Kiliani-Fischer synthesis (CAREY, 2006). Consequently glucose wasn´t used for testing degradation CN- microorganisms. However, glucose as a component of the M9 medium was used by authors KAO et al. (2003) in experiments with bacteria Klebsiella oxytoca and Figueira CIMINELLI et al. (1996) with bacteria E. coli.

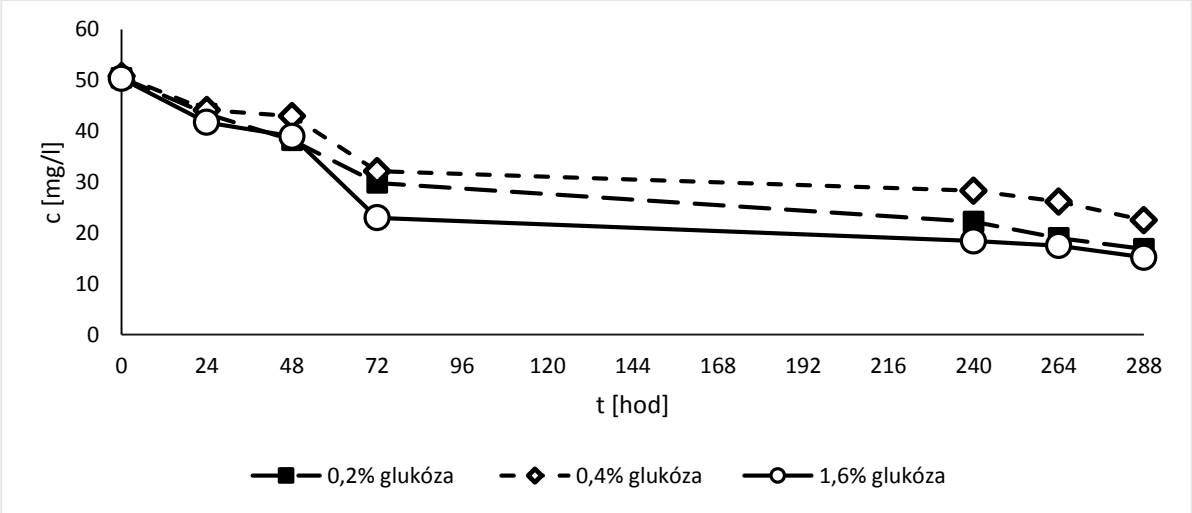

Fig. 1 Comparison of stability of cyanide ions, glucose was used as a substrate

Degradation of cyanides for Escherichia coli
To test the ability of degrading cyanide bacteria E. coli were used two organic substrates, ethanol and methanol. After the previous testing of these substrates without bacterial suspension were selected concentration of labeled methanol 2, ethanol 2 (Which correspond to the value of COD = 2150 mg / l) and Methanol 4 Ethanol 4 (which correspond to the value of COD = 8600 mg / l). Both of these substrates were tested in two concentrations of cyanide ions, 50 and 10 mg / l. At the concentration of 50 mg / l, the samples were incubated at 25 °C and 37 °C. Samples with a concentration of 10 mg / l were only incubated at 25 °C.
During 6 days of the experiment the bacteria degraded the cyanide more extensively in samples containing ethanol as the organic substrate (Figure 2). For the sample identified as *ethanol 2* it was only 34.42%, the sample labeled as *ethanol 4* it was 29.89%. In contrast, the decrease in the concentration of cyanide ions for the sample labeled as *methanol 4* it was only 8.64%. Figure 3 shows the results of experiments at 37 ° C. Also here, the highest loss of cyanide was when used as an organic substrate of a lower concentration of ethanol (*ethanol 2*). Leaving aside the increase of degradation of cyanide in a sample labeled as *methanol 4* to more than double (here the question is whether the low degradation at temperatures of 25 ° C is not affected by any mistake in conducting the experiment), we can conclude that *E. coli* bacteria degraded cyanide better at temperature of 25 ° C.
The ability of E. coli bacteria to degrade CN- was further verified in samples of cyanide ions with concentration of 10 mg / l. Experiments were conducted only at 25 ° C.

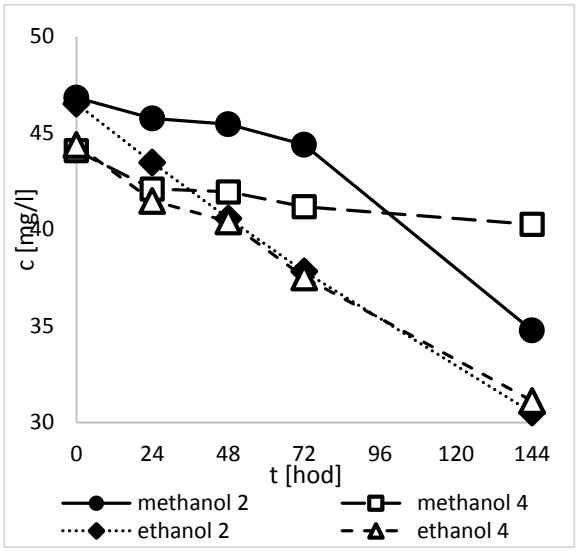

Fig. 2: The dependence of degradation CN^- (50 mg /l) versus time for E. coli at 25 °C

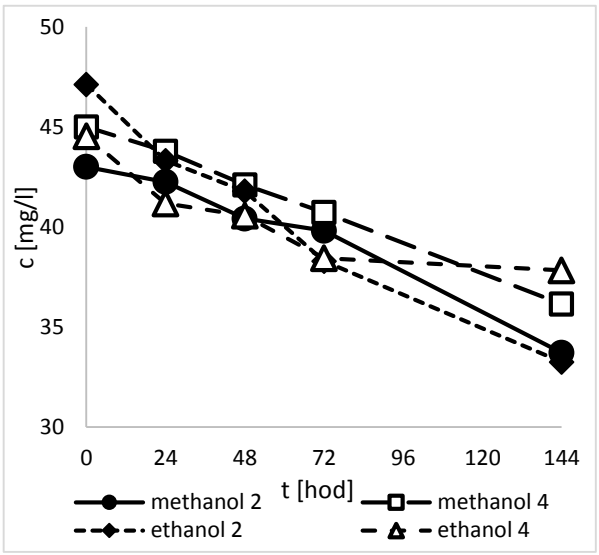

Fig. 3: The dependence of degradation CN^- (50 mg/l) versus time for E. coli při 37 °C

Even at this concentration there was a significant decrease in the concentration of CN^- in samples containing ethanol substrate (Fig. 4). After 6 days, there was a decrease of 38.64% of cyanide in a sample labeled *ethanol 4* and 36.05% loss for sample *ethanol 2*. The samples with methanol showed a lower percentage decrease (17.99% for sample *methanol 2* and 20.39% for *methanol 4*).

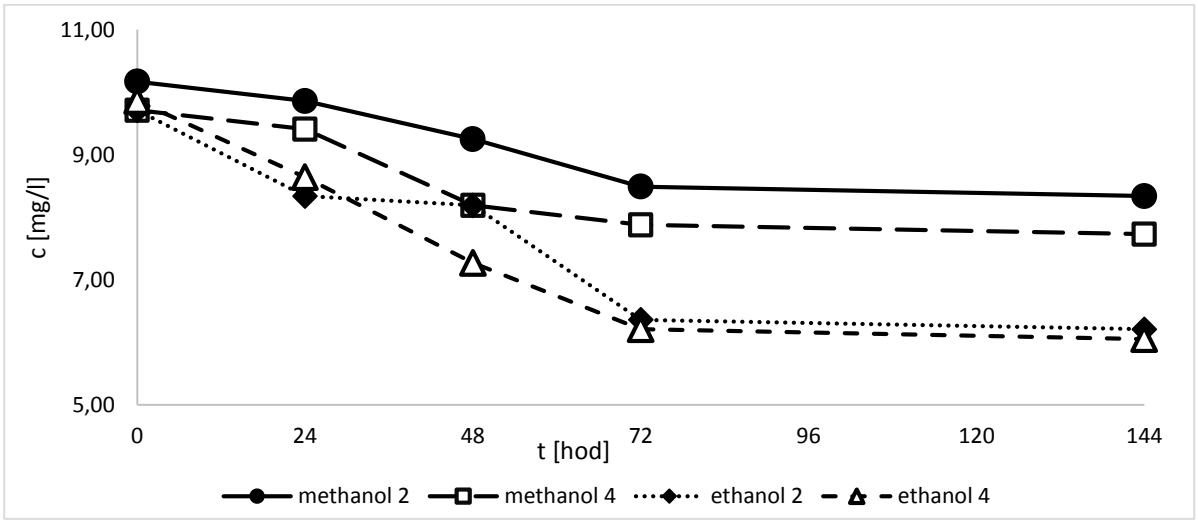

Fig. 4: The dependence of degradation of cyanide ions (10 mg / l) versus time for E. coli at 25 °C

Degradation of cyanides for Pseudomonas aeruginosa

To test the ability of degradation of cyanides *P. aeruginosa*, were again used two organic substrates, methanol and ethanol. Their concentration and the designation was chosen for better comparison with the case of elected walkable testing of bacteria *E. coli*. Cyanide concentration and the temperature was again chosen as in the previous case testing, so that for the cyanide concentration of 50 mg / l 25 ° C and 37 ° C, and for a concentration of 10 mg / l, the tests were conducted only at 25 ° C.

The Fig. 5 shows that after 6 days the bacteria degraded most cyanides in a sample with *ethanol 2* and it was 23.75%. For samples *methanol 4* and *ethanol 4* was observed almost the same percentage of loss (14.05% and 14.78%). At least degraded *P. aeruginosa* cyanides in samples with *methanol 2* and it was only 8.06%. The Fig. 6 then shows the degradation of cyanide at 37 ° C. It can be seen that the loss of cyanide ions is almost comparable for the experiments conducted at 25 ° C. The largest loss of

24.05% was observed in sample with *ethanol 2*. The samples with *ethanol 4* and *methanol 4* provide again similar to the percentage loss, 16.04% and 14.64%. The lowest degree of degradation CN^- was also achieved at this temperature for a sample labeled *methanol 2*,it was 9.57% of the original value. With these experiments it was found, that for bacteria *P. aeruginosa* temperature had no effect on the rate and extent of degradation CN^-.

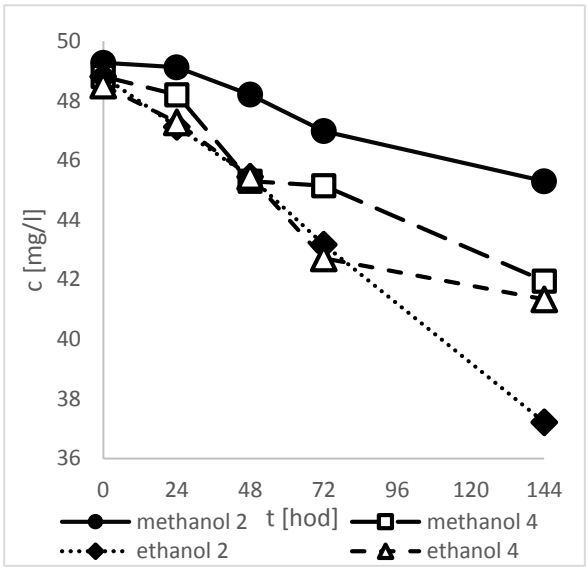

Fig. 5: The dependence of degradation CN^- (50 mg /l) versus time for *P.aeruginosa* at 25 °C

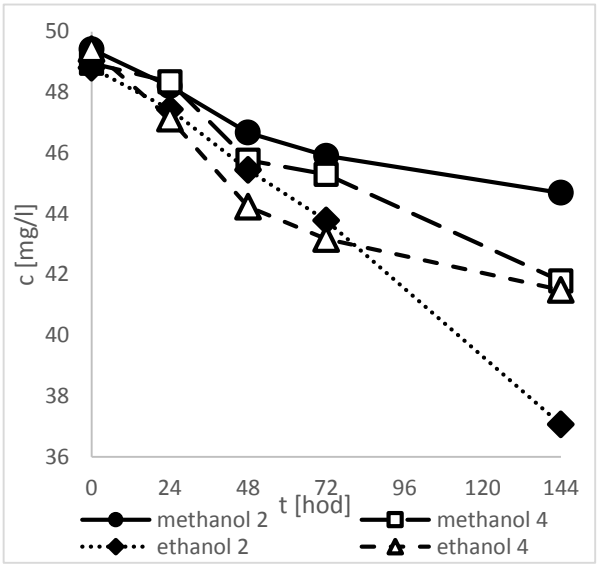

Fig. 6: The dependence of degradation CN^- (50 *m*g/l) versus time for *P.aeruginosa* při 37 °C

The ability of *P. aeruginosa* to degrade CN^- was further verified in samples with cyanide ion concentration of 10 mg / l (Fig. 7). Experiments were conducted only at 25 ° C. Just as was the case with *E. coli*, *P. aeruginosa* occurred even at this concentration of cyanide ions to a considerable decrease in samples that contained substrate ethanol (in the sample *ethanol 4* was removed by 35.32% and 27.47% of cyanide in sample with *ethanol 2*).

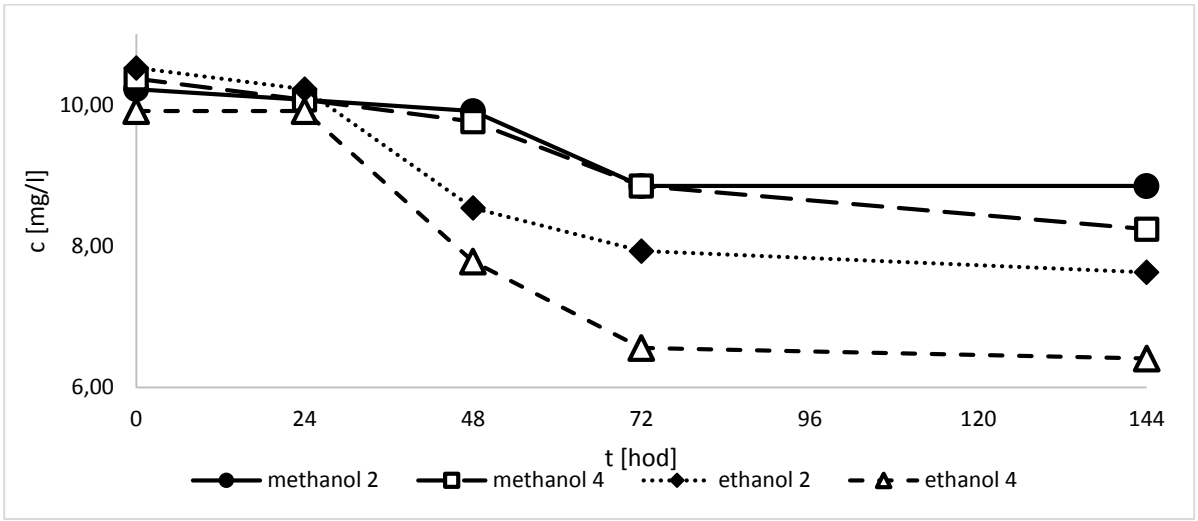

Fig. 7*:* The dependence of degradation of cyanide ions (10 mg / l) against time for P. aeruginosa at 25 °C

Conclusion

Experiments have demonstrated the ability of both tested species of bacteria, *E. coli* and *P. aeruginosa*, to degrade cyanides, although the effectiveness of this degradation under the testing conditions is not so great, in a practical point of view. For both tested bacterial species, ethanol showed as appropriate organic substrate, which in most cases provided higher degradation of cyanides. For *E. coli* was also shown the influence of the temperature on the degradation of cyanide ions, with *P. aeruginosa*, this effect has not been demonstrated.

Acknowledgments

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