

Inhibition of activated sludge respiration by heavy metals

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Abstract—Inhibition of the respiration microbial activity in the activated sludge caused by heavy metal concentrations (Cr, Cd, Cu and Ni) was studied by means of respirometric method using Strathtox respirometer (Strathkelvin Glasgow). The studied sludge samples were obtained from the two waste water treatment plants with different types of pollution (municipal waste water and domestic waste water).

Keywords—heavy metals; toxicity; respirometry; sludge; waste water treatment plant

I. INTRODUCTION

At the beginning of the twentieth century, the method of biological treatment was established, and now it is the basis of waste water treatment worldwide. It uses naturally occurring bacteria at very much higher concentrations in tanks. These bacteria, collectively with some microbes and protozoa contained in sludge, are together referred to as activated sludge. The bacteria remove small molecules of organic carbon and consequently, there occurs bacteria growth and the waste water is purified. The effluent (treated waste water) can then be released to water streams or the sea. The control of the treatment process is very complicated due to the large number of parameters that can affect it. Waste water treatment plants have to deal with recalcitrant chemicals that can only be degraded by the bacteria very slowly, and with toxic chemicals that inhibit the performance of the activated sludge bacteria. Excessive concentrations of toxic chemicals can generate a toxic shock that kills the bacteria [1; 2]. The purpose of this work was to determine the toxicity of heavy metals (Cd, Cr, Cu, Ni) on activated sludge microorganisms with the help of the respiration inhibition tests using the Strathtox unit.

II. RESPIRATION INHIBITION

A. Heavy metals in waste waters

The potential influence of heavy metals on the water environment has been a major interest over the last decades. Heavy metals such as copper, zinc, nickel and cadmium are commonly present in untreated waste waters coming from households and from the industry, particularly mining, smelting, metallurgy, electroplating, coking and chemical production, and metal-finishing industry. The toxicity of the metal is directly related to its solubility in the presence of the sludge [3]. The concentrations of heavy metals in waste waters

vary significantly depending on the industrial activities and on the chemical form of occurrence. Levels of heavy metal concentrations in municipal wastewaters are expected to be considerably lower because industrial effluents will be diluted by domestic waste waters [4].

Several studies mention that heavy metals can change the microbial structure of activated sludge by modifying both cell density and species richness, even at moderate concentrations, thus having a noxious effect on the growth and survival of microorganisms [5; 6; 7; 8]. Heavy metals incline to affect the metabolic functions of microorganisms in activated sludge and lower the effectiveness of the biological processes in waste water treatment plants [9].

Trace amounts ($\mu\text{g/l}$) of some metal ions such as Cu, Zn, Pb, Ni, Co are required by some organisms as cofactors for the enzymatic activities. Though, for most of the organisms, heavy metal ion concentrations at the level of mg/l are known to be toxic because of irreversible inhibition of some enzymes by the heavy metal ions. Toxicity of heavy metal ions on activated sludge bacteria varies depending on the type and concentrations of heavy metal ions and the microorganisms as well as the environmental conditions such as pH, temperature, dissolved oxygen (DO), presence of other metal ions, ionic strength and also the operating parameters such as, sludge age (Sludge Residence Time - SRT) and hydraulic residence time [10].

Former studies have shown that adapted sludge maintains a high removal efficiency of dissolved organic matter although exposed to constant input of heavy metals [11]. This suggests that adaptation can reduce the negative effects of toxic substances on biological reactions, and then some microbial groups can become predominant. Another study found that shock loads of toxicants produce remarkable effects on activated sludge whether it is adapted or not [12]. Obviously, as the characteristics of microorganisms in complex activated sludge system for nutrient removal have not been known yet, the study of the effects of toxic metals on activated sludge becomes important, especially the issue which kinds of adapted microorganisms can resist the toxicity of heavy metals and consequently what levels they can tolerate [13].

B. Toxicity

Even though there are several thousands of chemical reactions engaged in the metabolism of bacteria, we can identify three major processes that are applicable to the biological treatment of sewage. These are Ingestion; Respiration; Growth and division. These three processes are highly integrated.

Ingested organic carbon is processed in two ways. Some of it goes along the pathway of catabolism or respiration and ends up as carbon dioxide. This carbon is lost to the system. The remaining organic carbon follows the anabolism or growth pathway and ends up in newly formed biomass. This carbon is therefore kept in the system. The purpose of respiration is to provide the energy that is necessary for the growth and for the maintenance of the bacteria [14].

Toxic chemicals in the waste water can enter the bacteria and inhibit one or more enzymes of the pathways involved in either anabolism or catabolism. If the catabolic reactions of respiration are affected, the rate of respiration and energy production is reduced and the rate of growth is therefore reduced. On the other hand, if the anabolic pathways of biosynthesis are inhibited, the rate of growth is reduced, and this is accompanied by a decrease in the rate of respiration, as the requirement for energy is reduced. It shows that wherever the toxicity takes effect, there is inhibition of both respiration rate and rate of biodegradation [15].

III. MATERIALS AND METHODS

A. Respirometry method

Respiration is an essential activity of aerobic bacteria. For this reason respiration inhibition is a significant decisive factor for assessing the ecotoxicological risk of chemical substances in wastewater. Respirometry is used to assess waste water toxicity to heterotrophic and nitrifying bacteria in activated sludge. In contrast to bioluminescence, activated sludge respirometry is a more direct method for measuring sludge activity and thus toxicity to sludge [16]. The basis for respirometric tests is that the respiration rate of activated sludge or sludge microorganisms can be reduced in the presence of toxic substances. The most common way of measuring the bacterial respiration rate is the oxygen uptake rate [17].

B. Experimental apparatus

The respirometer Strathtox (Strathkelvin Instruments Ltd. Glasgow) is a laboratory instrument which is used to conduct a range of temperature controlled tests on activated sludge samples, so it can simulate the conditions of the treatment process. This equipment is based on the respirometry applications in the biomedical field and uses 6 oxygen electrodes simultaneously. Sludge sample volumes have been reduced to 20 ml, the rates measured on these 20 ml samples are more or less equal to those measured in a 1 liter sample. The use of 6 oxygen electrodes allows the respiration rate of a control sample of sludge to be measured at the same time as that of samples of the same sludge mixed with 5 different concentrations of waste water. Respiration inhibition tests, involve the measurement of the concentration of waste water

causing a 50% (or other selected percentage) inhibition of the respiration rate [18].

C. Sludge source

The Waste Water Treatment Plant (WWTP) Ostrava – Privoz was designed for a population equivalent of 638 000. This facility treats an average flow of 110 994 m³/day with 211 mg/l BOD, 426 mg/l COD and 325 mg/l of Total Suspended Solids (average annual concentrations in waste water input to WWTP). The input contains maximum year concentrations of 4.72 µg/l Cd; 375 µg/l Cr; 65 µg/l Cu and 115 µg/l Ni. The WWTP Hermanice II was designed for a population equivalent of 3600 with no industrial inflow.

The activated sludge was collected from the secondary treatment tanks of a WWTP Ostrava – Privoz and WWTP Hermanice II. It was kept aerated during transit to the laboratory, using a portable 12V aeration device. In the laboratory, 600 ml of the activated sludge was placed into the stock flask. In the stock flask the sludge was maintained at a constant temperature selected for the test and kept fully aerated. The synthetic sewage feed for the unit calibration was prepared by ISO 8192.

D. Heavy metals

The studied heavy metals are listed in Table I. All solutions were made up in distilled water of different concentrations. Cr, Cu, Cd and Ni were chosen as representative metals, commonly found in municipal waste water of Ostrava. Store solutions of metals were prepared prior to testing and each was diluted to 20 concentrations as being relevant for pollutant concentrations causing respiratory inhibition of activated sludge.

E. Respiration inhibition test

The respiration inhibition is one of the tests preferred in the case of heterogeneous cultures of microorganisms in an aqueous medium. The standardized method for testing inhibitory effects of substances on the respiratory activity of microorganisms in activated sludge is described in the documents ISO 8192:1986 revised by ISO 8192:2007 and OECD 209 – Regulation EC 440/2008. The respiration inhibition test measures the respiration inhibition caused by 5 different concentrations of waste water compared to the respiration of a control sample of activated sludge.

Tests were carried out in six 20 ml glass tubes. Synthetic sewage (2 ml) and test mixture (depending on concentration – diluted with distilled water) were added to the tubes. The tubes were kept stirred with a magnetic stir-bar in a waterbath of Strathtox unit. After reaching of the constant temperature of 20°C, 8 ml of activated sludge were quickly added to the tubes and oxygen electrodes were inserted into the tubes for recording the respiration rate values. As soon as the oxygen

TABLE I. METAL TOXICANTS USED FOR RESPIRATORY INHIBITION TESTING

Metals	Chemical form	Producer	Purity (Minimum Assay)
Cadmium	CdCl ₂ · 2.5H ₂ O	Lachema	Analar grade, 99.0%
Chromium	CrO ₃	Lachema	Analar grade, 98.0%
Copper	CuCl ₂ · 2H ₂ O	Lachema	Analar grade, 99.0%
Nickel	NiCl ₂ · 6H ₂ O	Lachema	Analar grade, 98.0%

content of the tube with the fastest respiration rate had fallen to near zero, the test was stopped. Percentage inhibition was calculated as Equation (1):

$$(1 - R_s/R_c) * 100 \quad (1)$$

where R_s = sample oxygen uptake rate
 R_c = control oxygen uptake rate

IV. RESULTS AND DISCUSSION

The four metals were tested in different concentration intervals, taking into account their solubility limit and their average concentration values detected in industrial waste water. Figures show the characteristic inhibition profile observed in the respirometric tests for the four tested metals. There can be observed evident inhibitory effect of the metals on the biomass activity. As shown in Figs. 1; 2; 3; 4 there are differences in the behavior of activated sludge from two different types of WWTPs. As described above, WWTP Ostrava Privoz is a large plant treating also industrial waste water specific for the region of Ostrava with heavy industry (steel industry, coking plants etc.) unlike the Hermanice II WWTP, a small plant treating no industrial inflow.

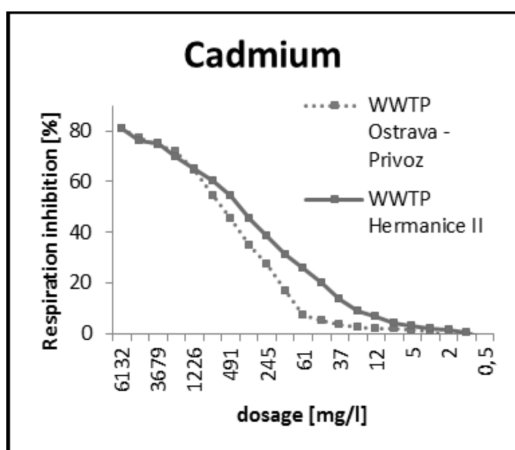


Fig. 1. Characteristic inhibition profile of cadmium.

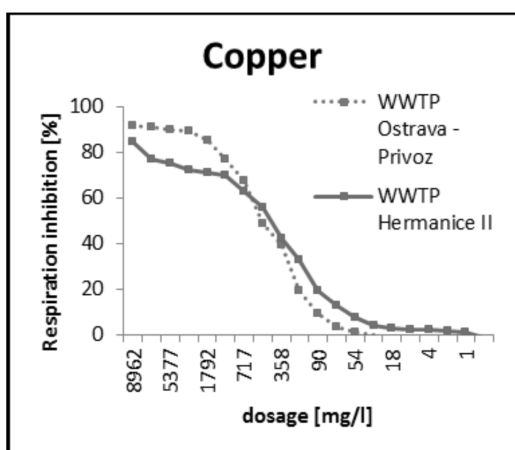


Fig. 2. Characteristic inhibition profile of cooper.

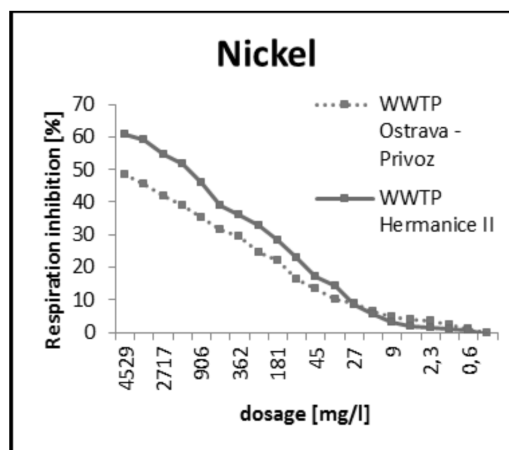


Fig. 3. Characteristic inhibition profile of nickel.

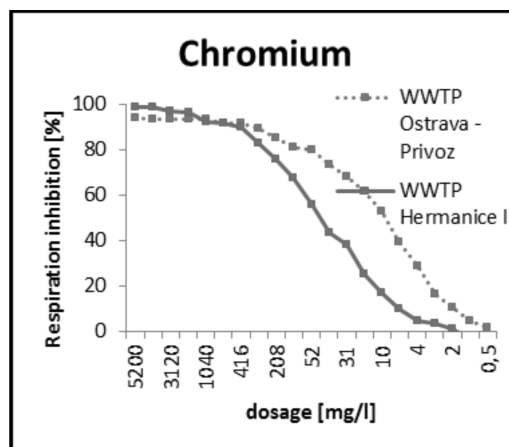


Fig. 4. Characteristic inhibition profile of chromium.

Table II shows the comparison of respiration inhibition in % of four selected metals for various concentrations; from very high of 4000 mg/l to low of 10 mg/l. Large differences between the two types of WWTPs can be observed, which shows the variance in microbiology of activated sludge and behavior of microorganisms.

Generally, heavy metals incline to have a bacteriostatic effect. The increasing metal concentrations lead to mortality. The results suggest that some types of microorganisms in different activated sludge have developed mechanisms to deal with elevated concentrations of heavy metals in their environment [19].

The relative degree of maximum respiration inhibition for WWTP Ostrava has been found to be:

Very high concentrations:

Ostrava $Cr^{6+} > Cu^{2+} > Cd^{2+} > Ni^{2+}$
 Hermanice II $Cr^{6+} > Cd^{2+} > Cu^{2+} > Ni^{2+}$

Low concentrations:

Ostrava $Cr^{6+} > Ni^{2+} > Cd^{2+} > Cu^{2+}$
 Hermanice II $Cr^{6+} > Cd^{2+} > Ni^{2+} > Cu^{2+}$

TABLE II. COMPARISON OF THE RESPIRATION INHIBITION EFFECTS OF DIFFERENT METALS ON ACTIVATED SLUDGE BIOMASS

Toxicant	Dosage of metals causing inhibition in [%]							
	4000 [mg/l]		500 [mg/l]		50 [mg/l]		10 [mg/l]	
	Ostrava	Hermanice II	Ostrava	Hermanice II	Ostrava	Hermanice II	Ostrava	Hermanice II
Cu	89	73	48	55	1	7	0	2
Cd	76	75	45	54	5	19	2	6
Ni	47	60	31	39	13	17	5	3
Cr	93	97	91	91	79	55	52	17

TABLE III. AVERAGE AMOUNT OF METALS PER YEAR IN WASTE WATER FLOWING INTO WWTP OSTRAVA AND IN WASTE WATER FROM EXTERNAL IMPORTERS.

Metals	Input channel A of WWTP Ostrava [µg/l]			Input channel D of WWTP Ostrava [µg/l]			Importers of WW to WWTP Ostrava [mg/l]		
	Avg.	Min.	Max.	Avg.	Min.	Max.	Avg.	Min.	Max.
Cr	19,1	10,7	29,3	46,2	5,9	375,6	7,2	0,0015	320
Cd	0,8	0,2	1,9	1,0	0,2	4,7	0,0226	0,0000145	0,374
Cu	42,7	13,0	65,0	40,4	16,0	58,0	10,9	0,004	483
Ni	28,4	5,0	86,0	34,0	5,0	115,0	5,44	0,0025	109

The toxicity at very high concentrations shows the differences between Cd and Cu for different types of activated sludge and between Ni and Cd at low concentrations. It can be also concluded that Ni is much more toxic at low concentrations [20]. For all cases Cr6+ has been found to be the most toxic, causing highest respiration inhibition. In comparison to relative toxicity of metals common to the UK, EU and the USA of the following order Hg > Cd > Ni/Pb > Cu > Zn and for anaerobic inhibition in a municipal sludge Ni > Cu > Cd > Cr > Pb [3] it can be assumed that aerobic or anaerobic process plays a significant role for respiration inhibition. In most municipal waste water treatment plants, the occurrence of heavy metals can significantly affect the efficiency of the plant, reducing the chemical oxygen demand adsorption capacity and the settling characteristic of the sludge. In literature [21] it was reported that the order of inhibitory effect as follows in this nitrification system: Ag > Hg > Cd > Cr3+ = Cr6+. The nitrifying micro-organisms are more susceptible to heavy metal inhibition than the micro-organisms responsible for the oxidation of carbonaceous material [11]. Our study shows that Cr6+ is the most toxic heavy metal for aerobic activated sludge with a 52 % respiration inhibition above the dose of 10 mg/l. Vaipolou and Gikas [22] summarize that clear conclusions about the critical chromium concentrations that affect activated sludge growth cannot be derived. Literature data on Cr6+ effects on activated sludge are controversial. Some of them mention that activated sludge growth is stimulated at Cr6+ concentrations up to 5 mg/l, above which it is inhibited, while others report growth stimulation at concentrations up to 25 mg/l. However, all reports agree that Cr6+ is definitely an activated sludge growth inhibitor at higher concentrations. A number of factors have been identified to influence chromium toxicity on activated sludge, such as pH, biomass concentration, presence of organic substances or other heavy metals, adaptation process, exposure time, etc. [23; 24].

The obtained respiration inhibition data were compared with the average year amounts of metals flowing into WWTP Ostrava and also with waste water from external importers treated in WWTP Ostrava, these data are reported in Table III. Average concentration amounts of metals from the Ostrava WWTP input are in µg/l units. Compared to results of respiration inhibition caused by metals in mg/l, it can be concluded that regular inflow cannot influence the activated sludge respiration. On the other hand, high exceptional concentrations in waste water from external importers treated in WWTP Ostrava can influence the activity of the biomass.

V. CONCLUSIONS

Respiration inhibition tests of four metals – Cr, Cd, Cu and Ni, representative heavy metals occurring in many industrial waste waters, have been carried out by a respirometric method based on the ISO 8192:2007 and OECD 209 – Regulation EC 440 and the Strathtox device.

All investigated heavy metals inhibit activated sludge growth at relatively low concentrations. However, the critical concentrations are only achievable by shock loads, above which they significantly affect activated sludge respiration. As with many heavy metals, adaptation can significantly increase microbial tolerance to heavy metals.

The need for monitors of influent waste water toxicity at municipal WWTPs has been demonstrated. It can be concluded that for the needs of process control early detection of metals allows the quick intervention of appropriate control strategies to reduce the biomass-toxicant contact time together with respiration inhibition.

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