Oil Shale, 2003, Vol. 20, No. 2
pp. 99-112
ISSN 0208-189X

STUDY OF BIODEGRADABILITY OF METHYL- AND HYDROXYPHENOLS BY ACTIVATED SLUDGE

R. LEPIK[*1], K. ORUPÕLD[*1],
S. VIGGOR[*2], T. TENNO[*1]

[*1] Institute of Physical Chemistry,
Chair of Colloid and Environmental Chemistry,
University of Tartu
2 Jakobi St., Tartu 51014, Estonia

[*2] Department of Environmental Technologies,
Center of Strategic Competence,
University of Tartu
23 Riia Rd., Tartu 51010, Estonia

The aim of this study was to investigate aerobic biodegradability of phenolic compounds characteristic of oil shale industry wastewaters by the activated sludge from the Kohtla-Järve wastewater treatment plant. For this purpose, the measurements of short-term oxygen uptake and biochemical oxygen demand were carried out for phenol, p-cresol, o-cresol, resorcinol, 5-methylresorcinol and 3,4-, 3,5-, 2,4- and 2,6-dimethylphenol as single substrates. The results obtained show that the microbial community of activated sludge was capable of oxidizing phenol, p-cresol, resorcinol and 5-methylresorcinol, the best-degrading dimethylphenol being 3,4-dimethylphenol. 3,5-, 2,4- and 2,6-dimethylphenol did not degrade during the short-term measurements; however, a long-term degradation of dimethylphenols was observed in the tests. The biodegradation of the studied compounds was also characterized by the kinetic parameters such as values of the maximum rate of oxygen uptake $V_{O_2,max}$, the maximum rate of substrate bio-oxidation V_{max} and half-saturation constants K_S. Variations in the measured parameters during the 2-year period of the study are discussed.

Introduction

Phenols are known components of wastewaters from pharmaceutical, plastic and paper industry. They are also found in the waste effluents of oil shale chemical industry and in the leachate of oil shale semicoke ash heaps. Oil shale chemical industry produces large amounts of phenol-containing wastewater and for this reason the risk of the contamination of natural water by phenolic compounds is quite high.

Phenols are toxic to several biochemical reactions and most living organisms and as such many of them are classified as U.S. EPA priority pollutants [1, 2]. However, with appropriate microorganisms and under the proper conditions, the biodegradation of phenolic compounds can occur.

In the present study oxygen uptake measurements were carried out in order to investigate the biodegradability of phenol and different methyl-, dimethyl- and hydroxyphenols by activated sludge adapted for the processing of phenolic wastewater from oil shale chemical industry. The purpose was to investigate the potential of the activated sludge from the Kohtla-Järve wastewater treatment plant to degrade the above-mentioned compounds and to predict the efficiency of their removal in the treatment plant.

Materials and Methods

Two methods based on the oxygen uptake measurement – the biochemical oxygen demand and the short-term oxygen uptake measurements were used.

The biochemical oxygen demand (BOD_5, BOD_7) is defined as the amount of oxygen (mg O_2/L) used by non-photosynthetic microorganisms at 20 °C during the incubation period of 5 or 7 days in the dark to metabolize biologically degradable organic compounds [3].

The method of short-term respirometric measurements [4–6] adapted from Čech *et al*. [7] was used for the determination of kinetic parameters and a short-term oxygen demand (BOD_{st}) of individual phenolic compounds. The short-term biochemical oxygen demand (BOD_{st}) is defined as the sum of oxygen demand for oxidation of all kinds of readily biodegradable organic compounds and ammonium, which are oxidized in the activated sludge suspension [8, 9]. Short-term measurement is considered more appropriate than estimation of biochemical oxygen demand for controlling purposes in the processing operation of the treatment plant.

An important distinction between the methods used is the different ratio of the initial substrate concentration to biomass. A low ratio of the initial substrate concentration to biomass was used in short-term measurements. Minimal changes occur in the degrading community under these conditions. The estimated kinetic parameters from those experiments are representative of the existing condition of the biomass in the wastewater treatment plant from which activated sludge is sampled. The biochemical oxygen demand measurements were carried out at a high ratio of the initial substrate concentration to biomass.

Activated sludge was sampled from the second-step aeration tank of the Kohtla-Järve wastewater treatment plant. The activated sludge used in the tests was stored in the laboratory and pre-aerated to achieve stable endogenous respiration. The concentrations of activated sludge samples were determined and quantified as dry matter content of suspended solids (MLSS) in grams per liter.

Phenol, *p*-cresol, *o*-cresol, resorcinol, 5-methylresorcinol and 2,4-, 2,6-, 3,4- and 3,5-dimethylphenol were used as single substrates in the experiments. All the chemicals used for the preparation of substrate solutions were of an analytical grade.

As the substrates differed in the theoretical oxygen demand (thOD), the average amount of oxygen consumed per unit of thOD was also calculated. The thOD is the calculated oxygen concentration (expressed as mole of O_2 per mole of the substrate) required for the mineralization of the given organic compound into carbon dioxide and water.

The uncertainties of the measurements were calculated as standard deviations of the mean and expressed at the confidence level of 95 % (coverage factor equal to 2).

Biochemical Oxygen Demand Measurement

The biochemical oxygen demand measurement was carried out according to the international standard method for the BOD_5 measurement [3].

The test solutions containing an individual phenolic compound at 0.015 mM were prepared with air-saturated distilled water containing phosphate buffer (pH = 7.2) and mineral nutrients [3]. 1 mL of activated sludge suspension was added as seeding per 1 L of the medium. A nitrification inhibitor, allylthiourea, at the final concentration of 0.5 mg/L was added to the samples. The BOD bottles were incubated at 20 °C in the dark to prevent any photochemical reaction. The dissolved oxygen concentration was measured by an electrochemical oxygen sensor (BODetector, Estonia) every day during the 7-day incubation period. There was one BOD bottle for each day. Blank controls were run with each test to measure the endogenous respiration of microorganisms.

The unit of BOD_7 used in this study was mole of O_2 per mole of the substrate. Biodegradation was followed by the depletion of oxygen concentration in the test bottles, corrected for the blank value and divided by the expected theoretical oxygen demand (thOD) to calculate the percentage of biodegradation. Oxygen uptake rates (OUR) expressed in mg $O_2/L \cdot h$ were also calculated for each substrate.

Short-Term Oxygen Uptake Measurement

In the case of the short-term respirometric measurement after the recording of the endogenous oxygen uptake of activated sludge, a substrate was added and the substrate-dependent exogenous oxygen uptake was measured. An oxygenmeter (OXI 325, WTW, Germany) was used to measure the oxygen concentration. Data were recorded after every 1-2 second by a computer. The tests were performed at 20 ± 2 °C and completed in 5–20 min depending

on the phenolic compound. A series of tests were carried out for each substrate with the same activated sludge.

From the measured data the oxygen uptake rate (OUR) as a function of time and the oxygen demand caused by substrate addition (ΔC_{O_2}) were calculated (Fig. 1). The ΔC_{O_2} gives an estimate of the short-term oxygen demand (BOD_{st}) caused by the addition of a substrate during the test. It was possible to calculate the ΔC_{O_2} values only if the oxygen uptake rate, approximately equal to the endogenous oxygen uptake rate at the end of the test, was achieved.

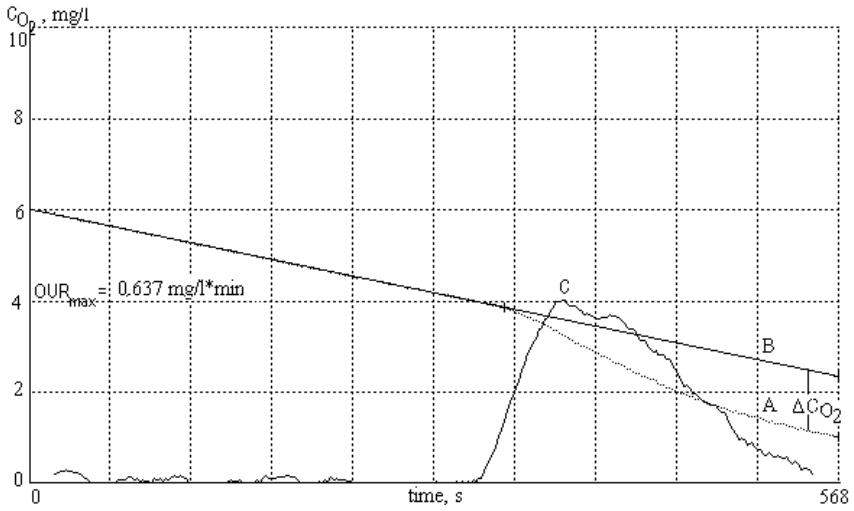

Fig. 1. Measured oxygen concentrations (curve *A*), oxygen concentration profile due to the endogenous respiration of activated sludge (curve *B*) and calculated OURs (curve *C*) in the case of the addition of phenol (0.015 mM). The maximum OUR, OUR_{max}, expresses the reaction rate for a certain amount of the added substrate

More specific respirometric analyses were carried out with five substrates, i.e. phenol, resorcinol, 5-methylresorcinol, *o*- and *p*-cresol at concentrations from 0.005 mM to 0.05 mM.

At a constant biomass concentration and under the conditions where oxygen would not be a limiting factor, the maximum oxygen uptake rate, OUR_{max}, is related to the added substrate concentration (S) and can be calculated by the following equation:

$$\mathrm{OUR}_{\max} = \nu V_{\max} \frac{S}{K_S + S} \tag{1}$$

where V_{max} is the maximum rate of substrate bio-oxidation;

K_S is the half-saturation constant of the substrate;

coefficient ν expresses the quantity of oxygen consumed per unit of substrate in the degradation process.

If the concentration of the biodegradable substrate is high, the OUR_{max} value will approximate its maximum value, i.e., the maximum rate of oxygen uptake for the biodegradation process of the given compound, $V_{O_2,max}$, can be calculated as follows:

$$V_{O_2,max} = \nu V_{max} \tag{2}$$

The dependence of OUR_{max} values on substrate concentration was investigated (Fig. 2), and on the basis of the Michaelis-Menten kinetics the kinetic parameters $V_{O_2,max}$ and K_S were determined from the above dependence by the non-linear regression and by using the method of the least squares. The values of the maximum rates of oxygen uptake $V_{O_2,max}$ in mg O_2/L · min were expressed per gram of dry weight of activated sludge.

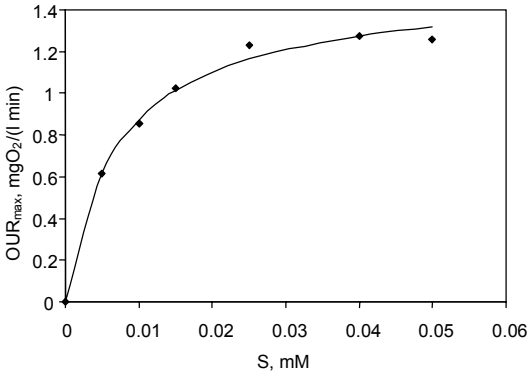

Fig. 2. Relationship between OUR_{max} values and added phenol concentrations. Activated sludge sample dates from 18 February 2002. The symbols correspond to the actual data, whereas the solid line is the Michaelis-Menten model curve

Results and Discussion

To investigate the biodegradability of the phenols studied, the short-term as well as biochemical oxygen demands and also the kinetic parameters estimated from the respirometric analysis were used. Results from the experiment series run within a period of two years are summarized in Tables 1–3.

Results from the conventional BOD measurements are given in Table 1. BOD_7 values formed 71–90 % of the thOD of the studied phenolic compounds, except in the case of 2,6-dimethylphenol where the BOD_7/thOD ratio did not exceed 25 %. The values of the ratio BOD_5/thOD were relatively similar to the respective values of the BOD_7/thOD.

The obtained BOD_5/thOD values for phenol, *o*- and *p*-cresol and 3,4-dimethylphenol were in the range of those published in literature, but in the case of resorcinol, the value obtained in the present study was somewhat higher [1]. High values of the standard deviation (S.D.) of the mean in Table 1 indicate a relatively high variation in these parameters between the test series. It can be explained by the differences of the microbial community of activated sludge in time. Standard deviations of short-term BOD measurements were lower than those of the conventional BOD measurements.

Table 1. **Mean Values of the Ratios of Biochemical Oxygen Demand (BOD_5, BOD_7) to Theoretical Oxygen Demand (thOD) with Standard Deviations of the Mean (S.D.) in the Case of Studied Phenolic Compounds (mean ± S.D.)**

Substrate	thOD, mol O_2/mol	BOD_5/thOD	BOD_7/thOD
Phenol	7.0	0.76 ± 0.11 (n = 11)	0.76 ± 0.12 (n = 11)
o-Cresol	8.5	0.68 ± 0.10 (n = 11)	0.74 ± 0.10 (n = 11)
p-Cresol	8.5	0.77 ± 0.08 (n = 11)	0.81 ± 0.08 (n = 11)
Resorcinol	6.5	0.78 ± 0.10 (n = 10)	0.84 ± 0.09 (n = 10)
5-Methylresorcinol	8.0	0.75 ± 0.09 (n = 11)	0.79 ± 0.09 (n = 11)
3,4-Dimethylphenol	10.0	0.67 ± 0.17 (n = 10)	0.71 ± 0.13 (n = 10)
3,5-Dimethylphenol	10.0	0.57 ± 0.15 (n = 2)	0.82 ± 0.65 (n = 2)
2,4-Dimethylphenol	10.0	0.74 ± 0.09 (n = 3)	0.90 ± 0.25 (n = 3)
2,6-Dimethylphenol	10.0	0.22 ± 0.22 (n = 3)	0.25 ± 0.18 (n = 3)

n is the number of experimental series.

When the oxygen demand of the substrate in conventional BOD measurements was studied as a function of time, it was found that oxygen uptake proceeds rapidly within the first two or three days and then slows down (Fig. 3). The maximum OUR (expressed in mg O_2/L · h) was achieved within 50 hours varying in different tests and showing higher values for phenol and *p*-cresol. In the case of *o*-cresol and dimethylphenols the maximum values were achieved by the 74th hour, and a lag period of approximately one day was observed before oxidation occurred. For the biodegradation of *o*-cresol a 1-day lag period has also been reported in literature [1].

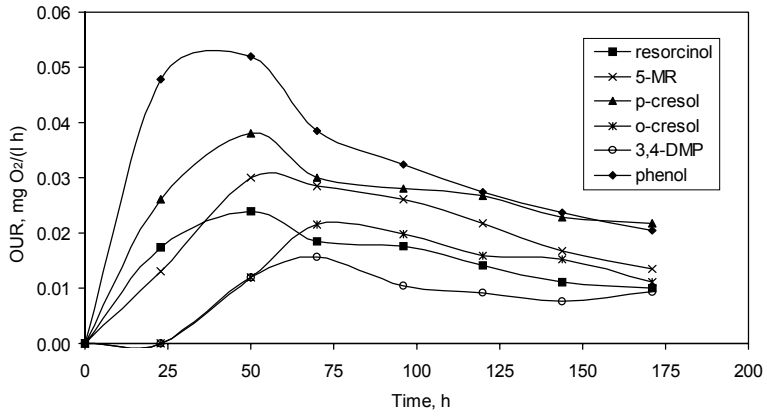

Fig. 3. Calculated OUR values as a function of time for different phenolic compounds: MR – methylresorcinol and DMP – dimethylphenol

The dependences of the measured ΔC_{O_2} values on the added substrate concentrations in short-term tests for different compounds are presented in Fig. 4. The slopes of the lines in Fig. 4 characterize the average amount of oxygen in mg utilized per mmole of the substrate.

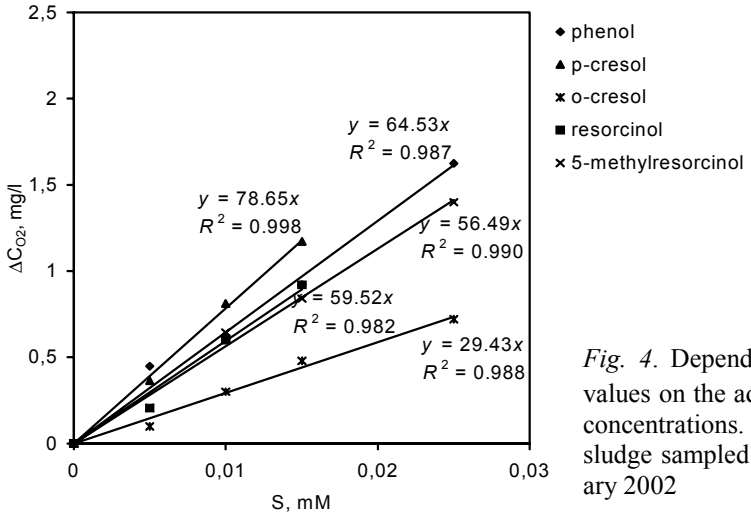

Fig. 4. Dependence of ΔC_{O_2} values on the added substrate concentrations. The activated sludge sampled on 11 February 2002

The results of the short-term oxygen demand measurement show that microorganisms in the Kohtla-Järve activated sludge quickly consume resorcinol, phenol, *p*-cresol, and 5-methylresorcinol. Short-term oxygen demand of these compounds was up to 30–40 % of the theoretical oxygen demand, showing the highest value for resorcinol (Table 2). The amounts of oxygen consumed in the tests suggest that these phenolic compounds were oxidized into common cellular metabolites.

Table 2. **Mean Values of the Ratio of Short-Term (BOD_{st}) Oxygen Demand to Theoretical Oxygen Demand (thOD) and Maximum Rate of Oxygen Consumption (OUR_{max}) with Standard Deviation of the Mean (S.D.) in the Case of Studied Phenolic Compounds (mean ± S.D.)**

Substrate	BOD_{st}/thOD		OUR_{max}, mg O_2/(mmol · min · MLSS)	
	2000	2001–2002	2000	2001–2002
Phenol	0.38 ± 0.03 (*n* = 9)	0.31 ± 0.02 (*n* = 12)	9.93 ± 2.23 (*n* = 9)	10.43 ± 1.22 (*n* = 11)
o-Cresol	0.16 ± 0.03 (*n* = 9)	0.13 ± 0.03 (*n* = 5)	3.11 ± 1.02 (*n* = 9)	2.30 ± 0.20 (*n* = 5)
p-Cresol	0.38 ± 0.03 (*n* = 9)	0.30 ± 0.05 (*n* = 5)	8.77 ± 2.21 (*n* = 9)	8.91 ± 1.83 (*n* = 5)
Resorcinol	0.43 ± 0.02 (*n* = 11)	0.34 ± 0.06 (*n* = 6)	7.40 ± 1.19 (*n* = 11)	4.83 ± 1.46 (*n* = 6)
5-Methylresorcinol	0.36 ± 0.03 (*n* = 11)	0.25 ± 0.02 (*n* = 5)	9.43 ± 1.91 (*n* = 11)	5.49 ± 0.88 (*n* = 5)
3,4-Dimethylphenol	0.16 ± 0.04 (*n* = 10)		2.99 ± 0.63 (*n* = 10)	
3,5-Dimethylphenol	0.03 ± 0.03 (*n* = 2)		1.60 ± 1.31 (*n* = 2)	

n is the number of experimental series.

The BOD_{st}/thOD values received in the present study were compared with those obtained by Orupõld *et al*. [5] with the activated sludge of the Kohtla-Järve wastewater treatment plant at a different time. The obtained values for phenol and 5-methylresorcinol correlate well with the results reported by Orupõld *et al*. [5], but the ratio of BOD_{st}/thOD for resorcinol in the present work was about 1.6 times higher. The differences in the BOD_{st} values could be caused by variations in the influents of the treatment plant and operation conditions, which can influence the microbial community of activated sludge thereby affecting the rate and extent of biodegradation.

Short-term oxygen demands formed 20–40 % of the BOD_7 of different phenolic compounds, except in the case of 3,5-dimethylphenol where the BOD_{st}/BOD_7 value was only up to 4 %.

The best-degrading dimethylphenol was 3,4-dimethylphenol, in which case the measured short-term oxygen demand was 16 % from the thOD. 3,5-, 2,4- and 2,6-dimethylphenols did not degrade in the time frame of the short-term BOD measurement. However, the results of the BOD_5 and BOD_7 measurement show a long-term degradation of dimethylphenols. Activation of enzymes required for degradation of these phenols perhaps takes a longer time than is the duration of the short-term oxygen demand measurement. While resistant to biodegradation as single substrates, dimethylphenols may degrade in mixture with other phenols or aromatic compounds, as enzymes can be induced by the more readily degradable substrates.

The results of short- and long-term measurements showed that 2,6-dimethylphenol is the worst-degrading phenolic compound investigated in the present work. The ring cleavage retardation of that compound may be explained by the blocked hydroxylation step because the both of two methyl groups are in the *ortho* position relative to the hydroxyl group and another hydroxyl group cannot be inserted next to the initial hydroxyl group. It is known that in the case of *ortho*-methylsubstituted phenols, hydroxylation is directed at the 6-position, both 2- and 6-hydroxylation seem to be possible in *meta*- and *para*-substituted phenols like 3,4-dimethylphenol [10].

To assess the biodegradability of phenols, the maximum OUR corresponding to the initial rate of degradation reaction for the given amount of a substrate was also used. OUR_{max} depends on the number of microorganisms in the sludge suspension and for this reason it was calculated per dry matter content (MLSS) of activated sludge. In this study, the OUR_{max} values for various substrates were compared at the concentration of 0.015 mM with the data shown in Table 2. Among the studied substrates, phenol had the highest value of OUR_{max}. OUR_{max} values for 5-methylresorcinol, *p*-cresol and resorcinol by the activated sludge of the Kohtla-Järve biotreatment plant exceeded those for *o*-cresol and dimethyphenols.

It is interesting that the OUR_{max} values for resorcinol and 5-methylresorcinol differed considerably if we compare the two parts of the experiment (Fig. 5). However, significant differences also existed between activated sludge samples taken at different times.

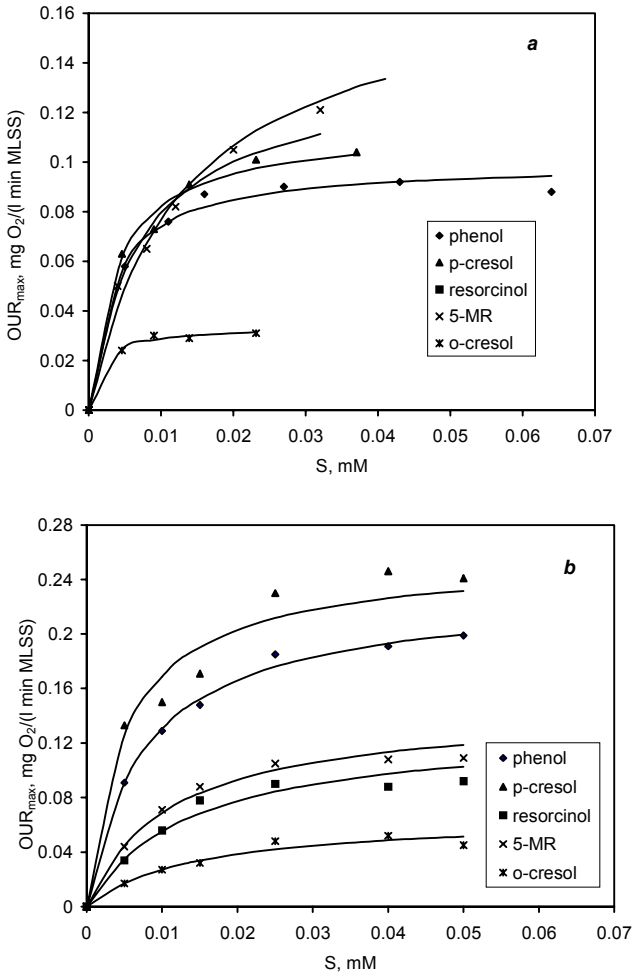

Fig. 5. Relationships between OUR_{max} values and concentrations of different substrates with specific activated sludge samples: (*a*) from 5 November 2001 having dry matter content equal to 3.90 g/L and (*b*) from 29 January 2002 with dry matter content equal to 5.65 g/L. The symbols correspond to the actual data, whereas the solid line is the Michaelis-Menten model curve. MR – methylresorcinol

The results for various phenolic compounds gained with the same activated sludge (shown in Fig. 5) suggest that considerable differences exist in their metabolism. Fig. 6 illustrates the time-dependent variations of oxygen uptake for phenol and resorcinol biodegradation.

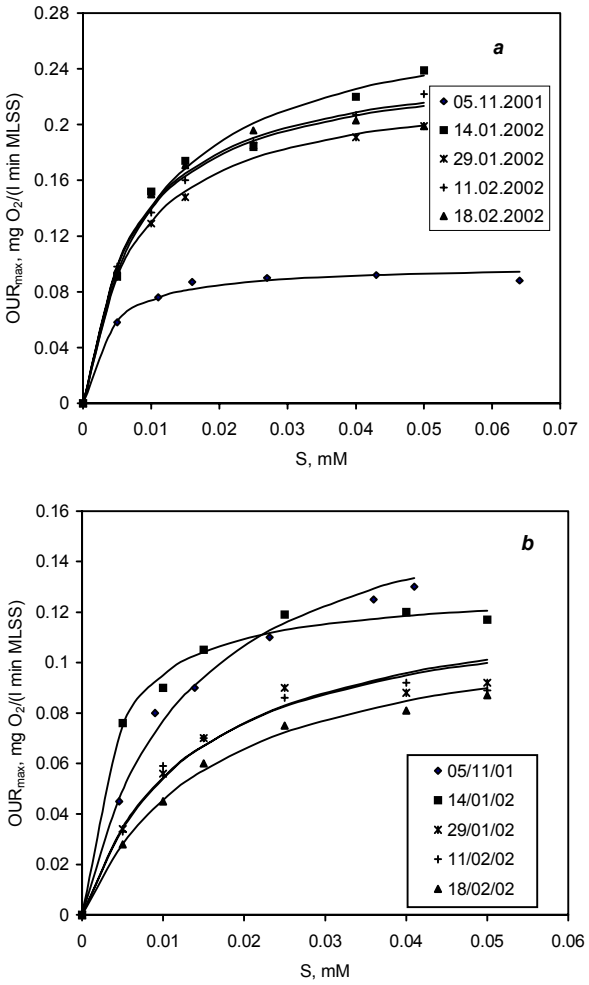

Fig. 6. Time-dependent variations of oxygen uptake for *(a)* phenol and *(b)* resorcinol biodegradation. The symbols correspond to the actual data, whereas the solid line is the Michaelis-Menten model curve

It should be noted that the average OUR_{max} value was 3.3 times higher for *p*-cresol compared with that for *o*-cresol. The curves of OUR resulting from the addition of the same amount of *p*-cresol and *o*-cresol are illustrated in Fig. 7*a* and 7*b*, respectively. The shapes of OUR curves show the differences in the substrate degradation processes by activated sludge. The BOD_{st}/thOD ratio of *o*-cresol was ca 2.3 times lower compared with the value for *p*-cresol (see Table 2).

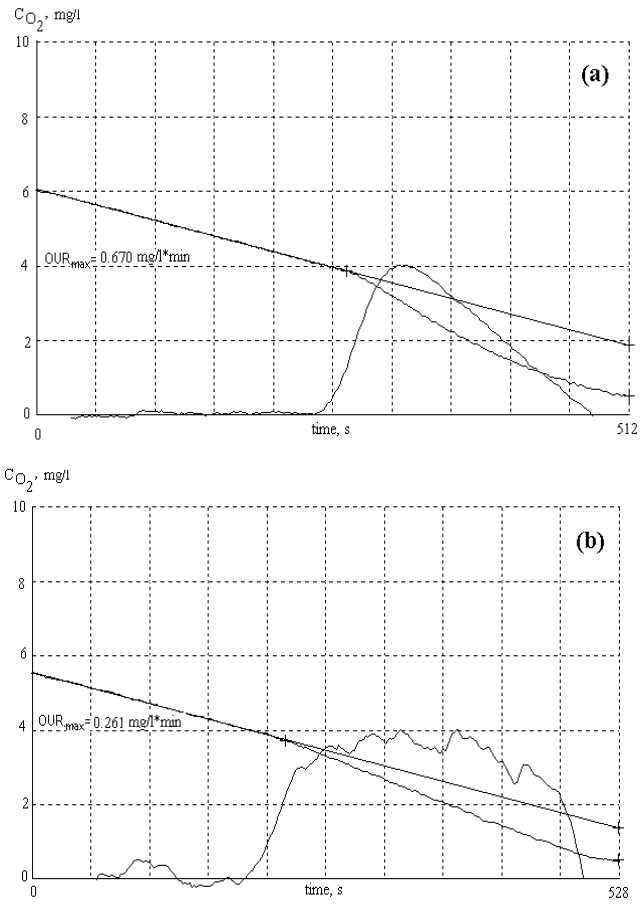

Fig. 7. Measured oxygen concentrations and calculated oxygen uptake rates upon the addition of 0.015 mM of (*a*) *p*-cresol and (*b*) *o*-cresol gained with the same sample of activated sludge

One possibility of explaining this phenomenon could be that in activated sludge containing a mixture of different microorganisms, which has a wider spectrum of metabolic properties, the degradation of *p*-cresol occurs through two distinct catabolic pathways at the same time.

Among the studied substrates, phenol had the highest value of the maximum rate of the oxygen uptake, $V_{O_2,max}$, followed by *p*-cresol, 5-methylresorcinol and resorcinol.

It was also possible to estimate the maximum rate of substrate oxidation V_{max} by dividing the $V_{O_2,max}$ values by stoichiometric coefficient ν. The average value of the maximum rate of substrate oxidation was also the highest for phenol. The fact that this value was higher than the values for *p*-cresol, 5-methylresorcinol, resorcinol and *o*-cresol indicates that phenol was more readily oxidized by the studied activated sludge than cresols or resorcinols.

Although the rates of the degradation of 5-methylresorcinol and resorcinol were lower compared with those of phenol, the obtained results showed that the studied biomass was able to oxidize the resorcinolic compound. Considering that K_S is related to the affinity of the microbial community for the substrate, the results showed the highest affinity of activated sludge to *p*-cresol. These results are summarized in Table 3.

Table 3. **The Values of Estimated Kinetic Parameters $V_{O_2,max}$, V_{max}, and K_S Describing the Biodegradation for Different Substrates**

Substrate	$V_{O_2,max}$, mg O_2/(L · min · MLSS)		V_{max}, μmol/(L · min · MLSS) (mean ± S.D.)	K_S, μM (range)
	Mean ± S.D.	Range		
Phenol	0.238 ± 0.031	1.5–5.0	3.5 ± 0.5	3.0–13.0
o-Cresol	0.058 ± 0.017	0.7–2.2	1.7 ± 1.0	2.0–16.0
p-Cresol	0.171 ± 0.034	1.2–3.0	2.1 ± 1.0	2.0–6.0
Resorcinol	0.123 ± 0.017	1.3–2.5	1.8 ± 0.4	4.0–13.0
5-Methylresorcinol	0.135 ± 0.037	1.6–3.3	2.1 ± 1.0	5.0–19.0

Examination of the values shows that the uncertainty of the measurement associated with K_S was larger than the uncertainty of the measurement associated with $V_{O_2,max}$. This is because the respiration rate associated with a high substrate concentration is close to the maximum, making $V_{O_2,max}$ well defined. K_S, on the other hand, is determined in the region of a low substrate concentration.

Conclusions

- The results of our work indicated that the activated sludge from the Kohtla-Järve wastewater treatment plant was able to oxidize the studied phenolic compounds without adaptation before the performing of the tests. This is possible due to a specific microbial population, which is formed in the treatment plant processing wastewater containing the given compounds. Differences existed in the maximum rate of oxygen uptake ($V_{O_2,max}$), as well as in the amounts of oxygen consumed per mole of substrate. $V_{O_2,max}$ is a direct measure of the metabolic activity of a microbial community when the reaction rate is independent of substrate concentration.
- It can be concluded that the method of the measurement of short-term oxygen demand (BOD_{st}) for the purpose of this study has some advantages over the conventional BOD method, because BOD_{st} is determined under conditions more closely approximating those in a wastewater treatment plant considering the ratio of the initial substrate to biomass concentration. Another advantage of the short-term method concerns the

relatively fast-obtained results. The results of short-term oxygen uptake measurements can also be used for optimization of the operation of a wastewater treatment plant, because BOD_{st} and $V_{O_2,max}$ can give information about activated sludge processes in the treatment plant.

However, it should be considered that in the wastewater treatment plant, a mixed substrate system exists and the other carbon sources are available for the microorganisms. Many different compounds that could also influence the biodegradation of the tested substrate may occur in the influents.

- The values of biodegradation parameters obtained with various activated sludge samples varied considerably. The rate of the biodegradation of phenolic compounds largely depends on the properties of activated sludge (floc composition and size, dry matter content, microbial population), which in turn are conditioned by the concentration, and the properties of the substrate in the treatment plant.
- The results also showed that the biodegradability of phenols is related to their structures. It depends on the properties and the number of substituents of aromatic nucleus. Biodegradation was effected on the positions of methyl groups of methylphenol. The *p*-substituted phenols are more readily degraded than the *m*- or the *o*-substituted ones. The obtained results showed that phenol, hydroxyphenols and cresols were degraded by activated sludge faster than dimethylphenols. This is in agreement with the previously obtained data where phenol and cresols showed to be easily biodegradable compared to dimethylphenols [11, 12].

REFERENCES

1. *Verschueren, K.* Handbook of Environmental Data on Organic Chemicals. 2nd Ed. – New York: Van Nostrand Reinhold, 1983.
2. *Patnaik, P.* Handbook of Environmental Analysis: Chemical Pollutants in Air, Water, Soil, and Solid Wastes. – Lewis Publishers, 1997.
3. International Organization for Standardization, *International Standard ISO 5815: Water Quality – Determination of biochemical oxygen demand after 5 days (BOD_5), Dilution and seeding method*, Geneva, 1986.
4. *Painter, H. A., King, E. F.* Biodegradation of water-soluble compounds // The Handbook of Environmental Chemistry Vol. 2, part C. / O. Hutzinger (ed.). Berlin: Springer-Verlag, 1985. P. 87–120.
5. *Orupõld, K., Maširin, A., Tenno, T.* Estimation of biodegradation parameters on phenolic compounds on activated sludge by respirometry // Chemosphere. 2001. Vol. 44, No. 5. P. 1273–1280.
6. *Hellat, K., Maširin, A., Nigu, P., Orupõld, K., Tenno, T.* Biodegradability studies of Kohtla-Järve wastewaters by activated sludge measurements // Oil Shale. 1997. Vol. 14, No. 4. P. 509–518.

7. *Čech, J. S., Chudoba, J., Grau, P.* Determination of kinetic constants of activated sludge microorganisms // Water Science Technology. 1985. Vol. 17. P. 259–272.
8. *Spanjers, H., Olsson, G., Klapwijk, A.* Determining short-term biochemical oxygen demand and respiration rate in an aeration tank by using respirometry and estimation // Water Research. 1994. Vol. 28, No. 7. P. 1571–1583.
9. *Lukasse, L. J. S., Keesman, K. J., van Straten, G.* Estimation of BOD_{st}, respiration rate and kinetics of activated sludge // Water Research. 1997. Vol. 31, No. 9. P. 2278–2286.
10. *Pieper, D. H., Stadler-Fritzsche, K., Knackmuss, H. J., Timmis, K. N.* Formation of dimethylmuconolactones from dimethylphenols by *Alcaligenes eutroghus* JMP 134 // Applied and Environmental Microbiology. 1995. Vol. 61, No. 6. P. 2159–2165.
11. *Brenner, A., Chozick, R., Irvine, R. L.* Treatment of a high-strength mixed phenolic waste in a SRB // Water Environmental Research. 1992. Vol. 64. P. 128–131.
12. *Kahru, A., Põllumaa, L, Reiman, R., Liiders, M., Maloverjan, A.* The toxicity and biodegradability of eight main phenolic compounds characteristic to the oil-shale industry wastewaters: a test battery approach // Environmental Toxicology. 2000. Vol. 15. P. 431–442.

Presented by J. Kann
Received April 26, 2002