ELSEVIER

Journal of Hazardous Materials B139 (2007) 332–339

Journal of Hazardous Materials

www.elsevier.com/locate/jhazmat

Critical appraisal of respirometric methods for metal inhibition on activated sludge

E. Ubay Cokgor*, S. Ozdemir, O. Karahan, G. Insel, D. Orhon

Istanbul Technical University, Faculty of Civil Engineering, Department of Environmental Engineering, 34469 Maslak, Istanbul, Turkey

Received 20 January 2006; received in revised form 8 June 2006; accepted 13 June 2006
Available online 16 June 2006

Abstract

This paper evaluates the merit of oxygen uptake rate measurements for the assessment of metal inhibition on activated sludge. For this purpose, experiments are conducted to calculate EC_{50} levels of nickel and hexavalent chromium using the ISO 8192 procedure, yielding results that are highly variable and difficult to correlate, depending on the type of substrate and the initial food to microorganism ratio. Similar experiments based on continuous respirometric measurements to give the entire oxygen uptake rate profile provide a much better insight on the impact of inhibition on different biochemical processes taking place in the reactor. The results indicate that percent reduction of the amount of dissolved oxygen utilized after an appropriate reaction time is a much better index for the assessment of the inhibitory effects.

Keywords: Activated sludge; Heavy metals; Inhibition; Oxygen uptake rate; Respirometry

1. Introduction

Today, wastewater management is greatly complicated by the presence of biologically resistant substances, such as heavy metals, persistent organic compounds, etc. These substances, commonly called inhibitors, often interfere and impair the performance of biological treatment systems. Inhibitory effects are mostly reported for the operation of activated sludge systems as reduction in the removal of organic carbon, impairment of solid separation and modification of sludge compacting properties [1]. The increasing trend towards combining industrial wastewater with domestic sewage for joint treatment increases the possibility of contamination of the influent by inhibitors and especially by heavy metals such as nickel, chromium, etc. Although the mechanisms by which heavy metals affect biological processes are not fully defined, it is well documented that relatively low concentrations of various heavy metals may stimulate activated sludge while increased doses lead to partial or total impairment of system performance [2–5].

Extensive research effort has been devoted to test and establish an experimental procedure, simple yet sensitive and relevant to activated sludge population, yielding a numerical index that would identify, with an acceptable accuracy, the inhibitory effect and this way, help to define a threshold value for the protection of activated sludge systems. This effort resulted in an abundance of different assays to screen presence and effects of toxicants and inhibitors in wastewater, such as chemical and microscopic analyses; measurement of the inhibition of growth and viability of bacterial cells; respirometric procedures; bioluminescent analyses [6,7]. The common feature of all these procedures is the fact that each one yields a markedly different index making it very difficult to define an appropriate threshold value for the tested inhibitor, therefore resulting in stringent and overprotective limits.

Respirometric approaches have recently gained increasing attention for the interpretation of wastewater characteristics and activated sludge behavior. Oxygen uptake rate profiles have been interpreted to assess new processes such as biological storage [8], to identify different COD fractions and to determine major rate coefficients [9–12]. This can be attributed to the continuing improvement of respirometric techniques [13]. Respirometry has also been used in the assessment of toxicity and inhibition, based on the observations that the oxygen consumption rate of activated sludge decreases when the wastewater contains toxicants or inhibitors [14,15]. One of these methods is the ISO 8192 Test for inhibition of oxygen consumption by activated

* Corresponding author. Tel.: +90 212 285 65 76; fax: +90 212 286 65 45.
E-mail address: ubay@itu.edu.tr (E.U. Cokgor).

0304-3894/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2006.06.038

sludge [16]. An important parameter in this method is the EC_{50} value defining the effective concentration of the inhibitor inducing 50% reduction in the oxygen uptake rate. This test has been widely applied to evaluate heavy metal inhibition: Vankova et al. [17] reported 1-h EC_{50} for Cr(VI) as 40–90 mg/L and 0.5-h EC_{50} for Cr(III) as 49 mg/L. The 0.5-h EC_{50} for Cu(II) was found as 123 mg/L, using this procedure [1]. In a similar study conducted by Gutierrez et al. [18], 3-h EC_{50} levels for Cr(VI), Zn(II), Cd(II) and Cu(II) have been calculated as 19, 56, 34 and 32 mg/L, respectively.

In this context, the objective of this study was to provide a critical appraisal of respirometric methods for metal inhibition on activated sludge. Nickel (Ni-II) and hexavalent chromium (Cr-VI) were selected for this purpose and tested using the procedure defined in ISO 8192 [16]. Parallel studies were also conducted to observe the entire OUR profile, measured by means of respirometry in a batch reactor initially fed with the desired substrate and seeded with an acclimated biomass to the selected substrate.

2. Materials and methods

The experimental setup has been prepared with the acclimation of activated sludge obtained from a domestic WWTP with the synthetic sewage described by ISO 8192 [16], with a synthetic wastewater mixture suggested by Henze [19], with glucose, and starch–acetic acid mixture as well-defined model substrates, in four fill and draw reactors. The acclimated biomass was used in the inhibition tests defined by ISO 8192 [16] and in the tests conducted with an on-line respirometer. The ISO 8192 tests have been conducted for the four different substrate mixtures listed above for the assessment of the effect of substrate on the test results and the standard test has been run for different initial food to microorganism ratios (S_0/X_0) to investigate the effect of the initial conditions imposed on the test results.

2.1. Reactor operation for biomass acclimation

Activated sludge was cultivated in four laboratory-scale fill-and-draw reactors, which had a working volume of 3 and/or 4 L. The reactors were operated at a sludge age of 10 days and a hydraulic retention time (HRT) of 1 day. Synthetic wastewater with 400 mg COD/L consisting of an appropriate mixture of acetic acid, propionic acid, ethanol, glutamic acid and glucose prepared in accordance with Henze [19] to stimulate the readily biodegradable COD fraction in domestic sewage was fed to the first reactor 1 (R1). Glucose with 1000 mg COD/L as sole carbon source was added into the second reactor (R2). The third reactor (R3), was fed with starch–acetic acid mixture with 350 mg COD/L as 43% starch and 57% acetic acid of the total COD concentration, respectively. Last reactor having 400 mg COD/L (R4) was introduced with nutrient solution consisting peptone, meat extract, urea, NaCl, $CaCl_2{\cdot}2H_2O$, $MgSO_4{\cdot}7H_2O$, K_2HPO_4 as proposed by ISO 8192 method [16]. Dissolved oxygen (DO) concentration and temperature in the biological treatability tests were kept at minimum of 3 mg DO/L and constant at 20 °C, respectively.

2.2. Standard inhibition test

The ISO 8192 method describes the way to assess the inhibitory effects of a test substance on oxygen consumption of activated sludge by measuring the respiration rate under defined conditions in the presence of a defined biodegradable substrate and different concentrations of the test substance. These inhibitory effects may include effects on respiration and nitrification. Percent inhibition of oxygen consumption (I), used to calculate the inhibitory effect of a test substance at a particular concentration, is given in Eq. (1). The concentration of inhibiting oxygen consumption by 50% (EC_{50}) is calculated or deduced by interpolation [16]. It is basically the interpolation of effective inhibitor concentration (EC_{50}), which induces a 50% reduction in the oxygen uptake rate, (OUR), compared with a blank control. The blank control is prepared using the same amounts of activated sludge seed and biodegradable substrate feed as the test dilutions. The physicochemical control is carried out to monitor the possible physicochemical oxygen consumption. This dilution includes the test substrate and substrate medium, without the addition of activated sludge. The assessment is based on a single, individual OUR measurement at 30 min after the start of the experiment. A second OUR measurement is also suggested, after 3 h if required, according to the ISO 8192 guidelines [16]:

$$\frac{R_B - (R_T - R_{PC})}{R_B} \times 100 = I \quad (1)$$

where R_T is the oxygen-consumption rate by test mixture, R_B the oxygen-consumption rate by the blank control and R_{PC} is the oxygen-consumption rate by the physicochemical control.

For the determination of effective concentration, EC_{50} value, a synthetic feed solution with a known COD concentration (400 mg COD/L peptone–meat extract (ISO substrate), starch–acetic acid, readily biodegradable synthetic substrate mixtures and 1000 mg COD/L glucose was added to test vessels and the pH of the solutions were neutralized prior to the assay. A nitrification inhibitor (Formula 2533™, Hach Company) was added to the OUR reactors to prevent any possible interference induced by nitrification, at a concentration of 133 mg for 250 mL volume. Then, a microbial inoculum was added to the vessels at a desired S_0/X_0 ratio and the solution was filled up to a final volume of 250 ml with demineralized water. The test material was added to each vessel at the desired heavy metal concentrations with 5 min lags. The toxic compounds added were nickel(II) nitrate, hexahydrate and potassium dichromate. Nickel(II) nitrate hexahydrate ionizes in aqueous solution and Ni(II) ions are dissipated. Potassium dichromate is an anhydride of the potassium salt of dichromic acid and dissolving potassium dichromate in water produces chromate ions with Cr(VI).

Dissolved oxygen measurements were performed by a WTW Inolab Oxi Level 2 oxygen meter in 50 ml air-tight vessels. The initial dissolved oxygen concentration in the test vessels was 7–8 mg/L.

Additionally, in order to investigate the effects of initial food to microorganism ratio (S_0/X_0) on heavy metal inhibition, several tests were performed both changing COD and microorganism concentrations. In these experiments, peptone–meat extract

mixture was used as a carbon source. Initial COD concentrations were arranged between 255 and 850 mg COD/L. Initial concentrations of biomass were between 400 and 2670 mg VSS/L.

Respirometric tests were also conducted with acclimated biomass seeding alone to obtain endogenous oxygen uptake rate (OUR) level of biomass. Samples with desired S_0/X_0 ratios are added to the reactor and the OUR data was monitored. Control analysis without heavy metal addition was conducted before inhibition analysis for each study. OUR measurements were performed with an RA-1000 (Applitek Co., Nazareth, Belgium) continuous respirometer.

2.3. Analytical methods

COD was determined using the procedure defined by ISO 6060 [20]. For soluble COD determination, samples were subjected to vacuum filtration by means of Millipore membrane filters with a pore size of 0.45 μm. The Millipore AP40 glass fiber filters were used for total suspended solids (TSS) and volatile suspended solids (VSS) measurements. TSS, VSS analyses were performed as defined in Standard Methods [21].

3. Experimental results

3.1. EC_{50} experiments

The respirometric assessment of the inhibitory impact of two heavy metals, nickel (Ni-II), and hexavalent chromium (Cr-VI), on substrate utilization by activated sludge has been carried out with the complex peptone–meat extract substrate as described in the standard procedure [16]. The ISO procedure requires running a set of aerated batch reactors with a range of inhibitor concentrations, using the selected substrate and biomass previously acclimated to the same substrate adjusted to initial concentrations of 400 mg COD/L and 1700 mg VSS/L, respectively, and therefore, corresponding to an initial food to microorganism (S_0/X_0) ratio of around 0.24 mg COD/mg VSS. The EC_{50} level as defined by ISO 8192 after 30 min of reaction time for peptone–meat extract substrate was calculated which corresponds to 33 mg/L for Ni(II) and 60 mg/L for Cr(VI) at a constant temperature of 20 °C, as illustrated in Figs. 1 and 2.

The experimental data on both figures indicate a good logarithmic correlation between the inhibitor concentration and

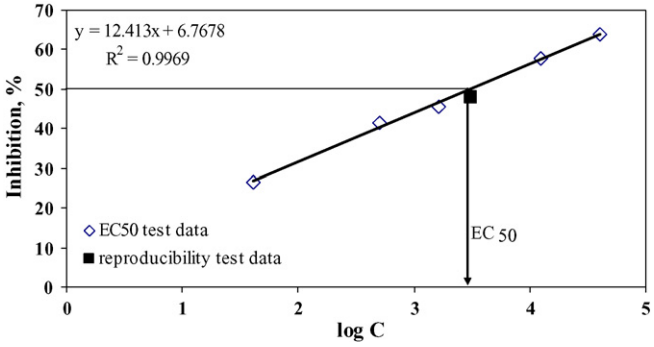

Fig. 1. Results of the EC_{50} test for nickel using peptone–meat extract substrate.

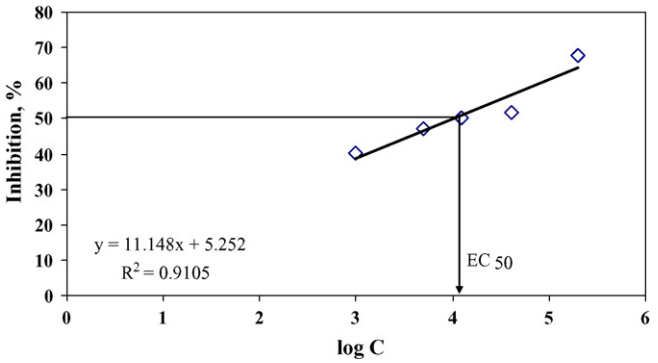

Fig. 2. Results of the EC_{50} test for hexavalent chromium using peptone–meat extract substrate.

percent decrease in the corresponding OUR level for the range of heavy metal concentrations tested in the study. In both cases, the EC_{50} was calculated by the interpolation of the linearized plots. The reproducibility of the results was tested by running an additional experiment using the calculated EC_{50} value of 33 mg/L for Ni(II), which yielded, as shown in Fig. 1, an OUR decrease of 48%, only slightly lower than the expected 50% decrease, a result which supports the reliability of the experimental procedure.

3.2. The effect of substrate on EC_{50} values

The type of substrate is a decisive factor for the type and rate of biochemical reactions taking place in an activated sludge system. Therefore, the impact of inhibition is likely to be quite substrate-specific. To test the effect of substrate, experiments on Ni inhibition were also conducted with *glucose*, an easily biodegradable substrate very commonly used in similar experiments [8,22], a *five-component mixture* defined and suggested by Henze [19] as a synthetic easily biodegradable substrate for respirometric testings and finally, an *acetic acid–starch mixture* approximating a typical easily biodegradable–slowly biodegradable substrate combination commonly observed in different wastewaters [23,24]. The results jointly displayed in Table 1, show that EC_{50} values vary in a wide range of 33–182 mg/L underlining the importance of substrate selection in such tests.

3.3. The effect of reaction time and S_0/X_0 ratio on EC_{50} values

Two sets of additional experiments were conducted with 33 mg/L nickel(II) addition, to visualize the effect of the reaction time and the initial S_0/X_0 ratio on the assessment of EC_{50} values.

Table 1
Results of the EC_{50} test for nickel for various substrates

Substrate type	EC_{50} (mg/L)
Peptone–meat extract mixture (ISO substrate)	33
Glucose	180
Readily biodegradable synthetic substrate mixture	182
Starch–acetic acid	145

Table 2
Effect of reaction time and the initial S_0/X_0 ratio on nickel percent inhibition (I, %) at a constant biomass level of 1700 mg/L

Time	S_0/X_0 ratio (mg COD/mg VSS)			
	0.15	0.24	0.30	0.50
30 min	66	47	48	50
1 h	69	60	59	58
2 h	74	64	63	71
3 h	80	59	54	71
5 h	66	64	Not available	75
6 h	63	54	60	68

Table 3
Effect of reaction time and F/M ratio on nickel percent inhibition (I, %) at a constant substrate COD level of 400 mg/L

Time	S_0/X_0 ratio (mg COD/mg VSS)			
	0.15	0.24	0.5	1.0
30 min	49	47	59	68
1 h	90	60	64	59
2 h	89	64	77	74
3 h	68	59	68	80
5 h	–	64	Not available	–
6 h	45	54	72	75

In the first set, the biomass was held constant at 1700 mg VSS/L and the COD of the peptone–meat extract substrate was changed from 255 to 850 mg/L to obtain four different initial S_0/X_0 ratios in the range of 0.15–0.50 mg COD/mg VSS. The value of 0.24 mg COD/mg VSS tested in the experiment corresponds to the S_0/X_0 ratio defined in the ISO procedure. In each experiment, OUR measurements were carried out six times along the reactor operation between 30 min (standard EC_{50} testing) to 6 h. The results obtained are outlined in Table 2.

A significant variation of the percent inhibition is depicted as a function of the initial S_0/X_0 ratio. For the standard reaction time of 30 min, the results were quite reproducible except for the lowest S_0/X_0 ratio of 0.15 mg COD/mg VSS where the level of inhibition increased 66%. For other observations at different times a wider spread inhibition was observed. The most pronounced effect of the initial S_0/X_0 ratio was obtained after a reaction time of 3 h, where percent inhibition varied between 54% to 80% for different S_0/X_0 ratios. The results given in Table 2 do not seem to indicate a regular trend for the effect of reaction time on the magnitude of inhibition. These results are in agreement with previous observations, which have shown that the uptake of heavy metal by activated sludge could be characterized by a short-term (3–10 min), rapid metal uptake phase followed by a long-term (over many hours) and slow phase uptake [25]. In another study Vankova et al. [26] showed that depending on exposure time, EC_{50} value for Cr(VI) initially decreased (toxicity of Cr(VI) increased) and this decrease was a consequence of the gradually inactivated proteins of active cells.

The second set of experiments were conducted for a constant initial COD of 400 mg/L and a range of biomass concentrations between 400 and 2670 mg VSS/L to yield four different S_0/X_0 ratios from 0.15 to 1.0 mg COD/mg VSS. The results, as given in Table 3, illustrate a similar significant effect of both the S_0/X_0 ratio and the reaction time on inhibition. The important interpretation of the results of both sets of experiments in Tables 2 and 3, should be the observation of the significant difference in the level of inhibition for the same S_0/X_0 ratio – i.e. S_0/X_0 ratios of 0.15 and 0.5 mg COD/mg VSS – obtained with different amounts of initial substrate and biomass.

3.4. Effect of inhibition on OUR profiles

Understanding of substrate utilization by activated sludge systems operated under aerobic conditions and its interpretation by mechanistic modeling may be considered as one of the most significant achievements in environmental sciences. This approach essentially provided: (i) identification of substrate components with different biodegradation rates and COD fractionation; (ii) discovery and model description of new biochemical processes, such as hydrolysis, storage and utilization of biopolymers, etc, aside from biomass growth, taking part in substrate utilization. As a result, activated sludge models today exhibit a multi-component structure differentiating a number of substrate and biomass fractions as model components. Accordingly, they incorporate and define a sequence of biochemical processes involving different model components. Assessment and interpretation of the oxygen uptake rate (OUR) is now recognized as a the most important tool to quantify major parameters and processes all significant experimental and modeling studies. A single OUR value, as in the ISO 8192 test may be used at best as a rough index of the rate of biochemical reactions at the time of measurement. However, the OUR profile in an initially fed batch reactor under predefined starting up conditions with substrate and acclimated biomass, accurately reflects the sequence of biochemical reactions under aerobic conditions. Therefore, comparison of two OUR profiles with and without inhibitor addition can give the necessary information about an inhibitory impact on the entire biochemical reactions spectrum.

This part of the experimental study investigated the assessment of the impact of Ni(II) and Cr(VI) inhibition by using OUR profiles. For this purpose, OUR profiles were obtained by means of respirometric measurements using aerated batch reactors operated at the same initial S_0/X_0 ratio of 0.24 mg COD/mg VSS, as described in detail in the previous section. The OUR profile for the control reactor (no inhibitor addition) Cr(VI) experiment is plotted in Fig. 3. Available techniques today enable to interpret this OUR curve to yield: (i) major COD fractions of the substrate, (ii) significant biochemical mechanisms for substrate utilization, and (iii) the magnitude of applicable model coefficients associated with biochemical processes. A detailed account of such an evaluation is beyond the scope of this paper and it is presented elsewhere by Insel et al. [27] who reported that the peptone–meat extract substrate defined by ISO 8192 and used in this study had at least three COD components with readily biodegradable, rapidly hydrolysable and slowly hydrolysable fractions and substrate utilization involved primary growth with simultaneous glycogen storage, subsequent utilization of the stored glycogen for secondary growth and endogenous respi-

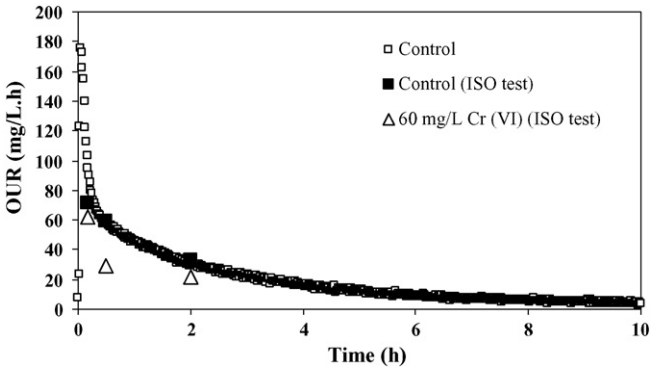

Fig. 3. OUR profile of the peptone–meat extract control test for Cr(VI) inhibition and results of the ISO measurements.

ration. The plot of the corresponding OUR profile in Fig. 3 also shows control and inhibition OUR results of the ISO test run at three different times of 10, 30 and 120 min after the start of the experiment. The individual OUR measurements of the control fit well at 30 and 120 min but significantly diverge from the OUR profile at 10 min. It should be noted that the individual OUR measurements correspond to different phases of the reaction sequence; the first one at 10 min reflects the joint effect of primary growth and substrate storage; the second one at 30 min, the utilization of the stored substrate and the third one at 120 min utilization of the slowly biodegradable substrate fraction together with endogenous respiration according to the model. Therefore, the expected impact of inhibition is likely to take place on different biochemical mechanisms.

The respirometric assessment of the OUR profiles obtained using peptone–meat extract as substrate and both with and without inhibitor addition for Ni(II) and Cr(VI) are plotted in Figs. 4 and 5. Similarly Figs. 6 and 7 show the OUR profiles with and without nickel addition using glucose and starch–acetate mixture. EC_{50} values of previously obtained for Ni(II) and Cr(VI) associated with different type of substrates were used in the experiments.

Two major observations derived from OUR profile experiments may be summarized as follows: (i) OUR profiles for controls using different substrates exhibit totally different characters so that a given time interval from the start of the experiment – say 30 min as defined in the ISO 8192 test – may have a totally different implication as far as effect on biochemical reactions are concerned; (ii) the cumulative OUR reductions observed after 30 min, for inhibitor additions at EC_{50} levels do not support results of the ISO tests; they are far more different than the expected 50% decrease as outlined in Table 4 and highly dependent on the type of substrate, as 30 min reaction time corresponds to a different phase of the specific biochemical reaction sequence associated with each substrate.

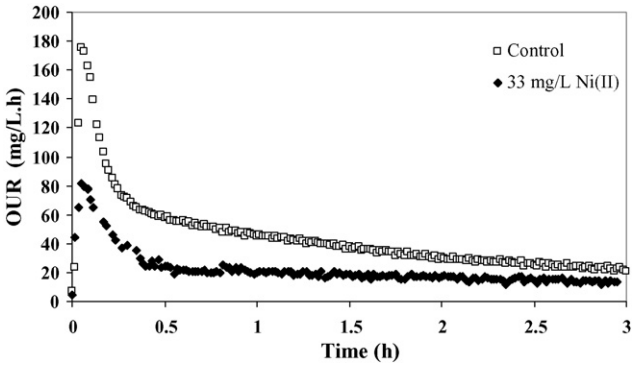

Fig. 4. OUR profiles obtained for peptone–meat extract for control and 33 mg/L Ni(II) addition.

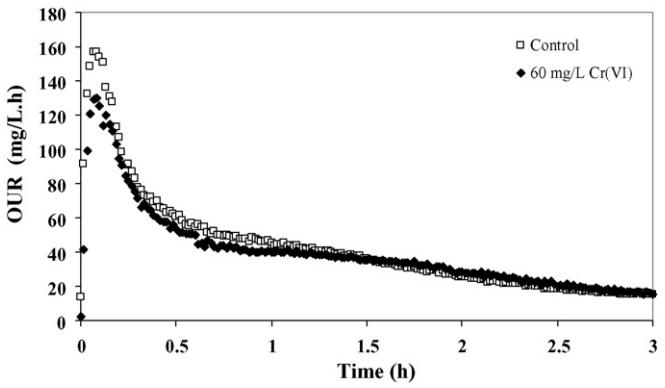

Fig. 5. OUR profiles obtained for peptone–meat extract for control and 60 mg/L Cr(VI) addition.

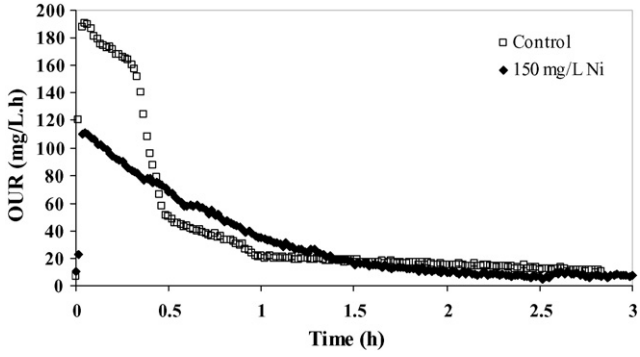

Fig. 6. OUR profiles obtained for glucose and 150 mg/L Ni(II) addition.

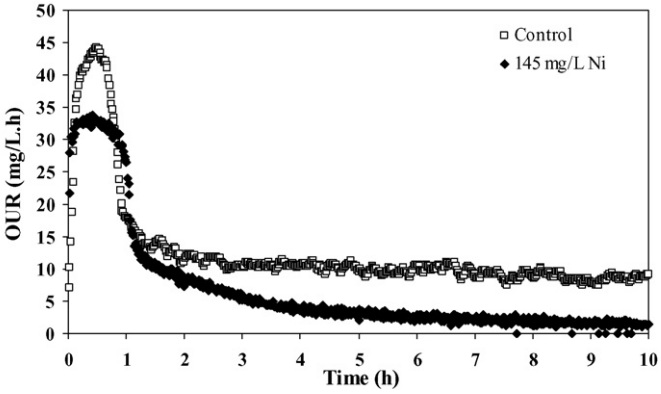

Fig. 7. OUR profiles obtained for starch–acetate mixture and 145 mg/L Ni(II) addition.

Table 4
Cumulative oxygen utilization and percent inhibition for different mixtures

Mixture	Cumulative oxygen utilization (mg/L)			% Inhibition on cumulative oxygen utilization		
	30 min	2 h	3 h	30 min	2 h	3 h
Control (peptone only)	48	108	127	–	–	–
Cr(VI)	41	99	120	15	8	6
Ni(II)	29	59	73	40	45	43

4. Discussion

Evaluation of the OUR profile for inhibitory impacts requires its full understanding and interpretation in terms of the kinetics and stoichiometry of the relevant biochemical processes. Each OUR measurement along the profile may be conveniently converted to the numerical value of the reaction rate taking place at that stage, i.e. growth, endogenous respiration, etc., so that measuring OUR values at different times actually means measuring rates of different processes. The area under OUR profile, however, gives the equivalent of the biodegradable substrate utilized in the experiment in terms of the amount of dissolved oxygen consumed [11,12,28]. Obviously, an inhibitory action cannot affect the amount of biodegradable substrate but only slows down its rate of utilization, resulting in (i) a lower oxygen consumption at a given time after the start of the experiment, and (ii) a wider spread of the OUR profile over a longer time. The reduction in the amount of dissolved oxygen consumed is a much better index as it reflects the overall effect of inhibition on all microbial processes involved.

In this context, the simulated and experimental cumulative dissolved oxygen consumptions ascertained using the corresponding OUR profiles obtained in the study are plotted in Fig. 8. The plots illustrate distinctly different inhibition effects of Ni(II) and Cr(VI) on activated sludge: while the effect of Cr(VI) is relatively small and tampers down as the reaction proceeds, that of Ni(II) is much more pronounced and equally persists throughout the observation period.

A more detailed evaluation of the cumulative dissolved oxygen curves presented in Table 4, shows the values of cumulative dissolved oxygen consumption and percent inhibition of its utilization at three different time intervals of 30 min, 2 and 3 h. Data outlined in this table clearly indicates that the relatively small impact of Cr(VI) is only observed at the initial phase

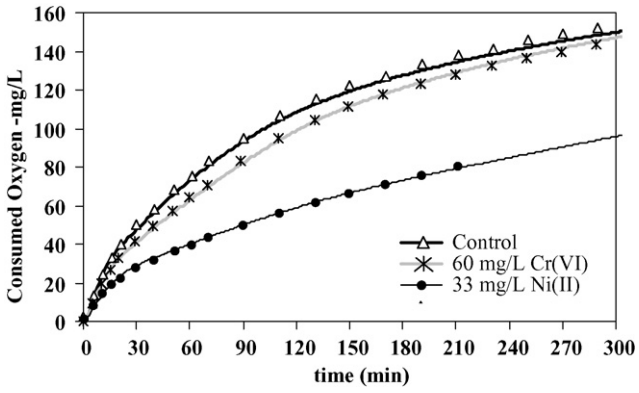

Fig. 8. Cumulative oxygen uptake profiles for peptone–meat extract and heavy metal mixtures.

whereas the inhibition due to Ni(II) remains stable during the whole period. Insel et al. [27] brought a mechanistic explanation to this observation by model calibration where the effect of Cr(VI) appears to be limited to microbial growth, while Ni(II) affects more hydrolysis of slowly biodegradable substrate, aside from growth process.

Selected data presented in Tables 2 and 3 are plotted in Fig. 9 to better illustrate the change of percent inhibition with exposure time at two S_0/X_0 ratios of 0.5 and 0.15 mg COD/mg VSS, set for constant initial substrate and biomass. This figure provides a clear indication that under low S_0/X_0 ratios, the level of inhibition is much more pronounced at the beginning of the experiment due to fast consumption of substrate and the resulting steeper OUR profile, since biomass growth is the dominant biochemical process at this phase. Increasing the S_0/X_0 ratio generally reduces the effect of inhibition. Experiments with low S_0/X_0 ratios are therefore more sensitive in determining toxicity, reaching a maximum level within a shorter exposure time.

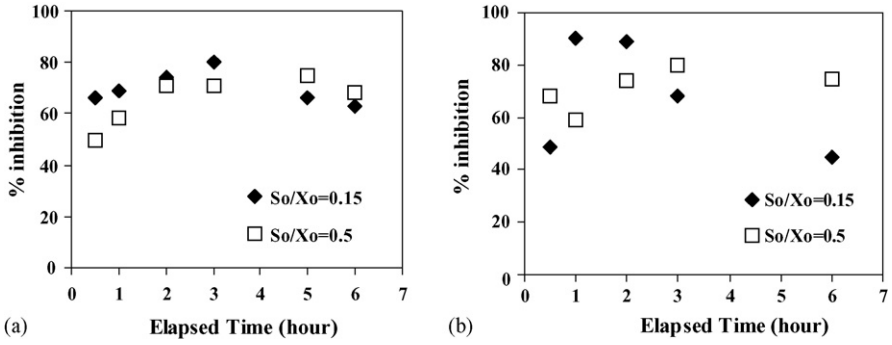

Fig. 9. Effect of initial S_0/X_0 ratio on percent inhibition under (a) constant biomass (b) constant substrate concentration, for different exposure times.

Furthermore, the analysis of OUR profiles in Figs. 4 and 5 shows that the growth phase lasts only 0.5 h and hydrolysis represented by the second plateau takes place within the next 0.5–3.0 h. In the following endogenous decay phase, the impact on inhibition stays at minimum level, an equally important observation also showing that the inhibition effect is predominantly dependent upon the selection of the initial S_0/X_0 ratio. Consequently, evaluation of complete OUR profile is required for the assessment of the inhibition level of a toxic compound. In the context, mechanistic biodegradation models can and should be effectively utilized as convenient tools to better quantify the inhibition effect of a certain toxic compound at different stages of the biodegradation process.

5. Conclusions

In the light of experimental results obtained and their evaluation, the concluding remarks of the study concerning the merit of using respirometric measurements for inhibition assessment may be summarized as follows.

Inhibition assessment by means of a single OUR measurement as in the ISO 8192 procedure only provides an index value, as in many other procedures using different methods and techniques. Relevancy of the OUR index and its superiority to other similar inhibition indexes proposed in the literature is questionable.

The EC_{50} value defining the inhibitor concentration that causes a 50% reduction in the OUR after a certain reaction time, although reproducible for the experimental conditions defined in the ISO procedure, appears to be highly variable depending on the type of substrate and the initial S_0/X_0 ratio used in the experiment. Experimental results have shown that the EC_{50} value is also affected by the relative amounts of substrate and biomass for the same S_0/X_0 ratio. Therefore, EC_{50} values are bound to be erratic in nature and difficult to interpret.

OUR measurements in standard ISO test are performed indirectly, using an oxygen meter. Technologies available today enable direct assessment of the entire OUR profile, which reflects the full sequence of biochemical reactions taking place in the reactor. As illustrated in this paper, inhibition assessment can be made on the entire profile, evaluating reductions on the amount of dissolved oxygen consumption or by model calibration, which quantifies the impact of inhibition on the rates of different processes. Based on results and evaluations in this study, determination of percent inhibition with the aid of cumulative oxygen consumption after an appropriate reaction time i.e. 2 h, appears to be much more meaningful index for the numerical assessment of inhibition.

Acknowledgments

This study was supported by the Scientific and Technical Research Council of Turkey (TUBITAK) under Project no. CAYDAG 102Y071. This study was also conducted as part of the sponsored research activities of Environmental Biotechnology Centre of The Scientific and Technical Research Council of Turkey.

References

[1] K. Wong, M. Zhang, X. Li, W. Lo, A luminescence-based scanning respirometer for heavy metal toxicity monitoring, Biosens. Bioelectron. 12 (1997) 125–133.
[2] G.N. McDermott, M.A. Post, B.N. Jackson, M.B. Ettinger, Nickel in relation to activated sludge and anaerobic digestion process, J. Water Pollut. Control Fed. 37 (1965) 163–177.
[3] M.M. Bagby, J.H. Sherrard, Combined effects of cadmium and nickel on the activated sludge process, J. Water Pollut. Control Fed. 53 (1981) 1609–1619.
[4] S. Sujarittanota, J.H. Sherrard, Activated sludge nickel toxicity studies, J. Water Pollut. Control Fed. 53 (1981) 1314–1322.
[5] U. Yetis, G.N. Demirer, C.F. Gokcay, Effect of Cr(VI) on the biomass yield of activated sludge, Enzyme Microb. Technol. 25 (1999) 48–54.
[6] C.J. Kelly, N. Tumsaroj, C.A. Lajoie, Assessing wastewater metal toxicity with bacterial bioluminescence in a bench-scale wastewater treatment system, Water Res. 38 (2004) 423–431.
[7] G. Ricco, M.C. Tomei, R. Ramadori, G. Laera, Toxicity assessment of common xenobiotic compounds on municipal activated sludge: comparison between respirometry and microtox, Water Res. 38 (2004) 2103–2110.
[8] O. Karahan-Gul, N. Artan, D. Orhon, M. Henze, M.C.M. van Loosdrecht, Experimental assessment of bacterial storage yield, ASCE J. Environ. Eng. 128 (11) (2002) 1030–1035.
[9] G.A. Ekama, P.L. Dold, G.V.R. Marais, Procedures for determining influent COD fractions and the maximum specific growth rate of heterotrophs in activated sludge systems, Water Sci. Technol. 18 (1986) 91–114.
[10] D. Orhon, E. Ates, S. Sozen, E.U. Cokgor, Characterization, COD fractionation of domestic wastewaters, Environ. Pollut. 95 (2) (1997) 191–204.
[11] D. Orhon, D. Okutman, G. Insel, Characterisation and biodegradation of settleable organic matter for domestic wastewater, Water SA 28 (3) (2002) 299–305.
[12] G. Insel, D. Orhon, P.A. Vanrolleghem, Identification and modelling of aerobic hydrolysis mechanism—application of optimal experimental design, J. Chem. Technol. Biotechnol. 78 (4) (2003) 437–445.
[13] H. Spanjers, P.A. Vanrolleghem, G. Olsson, P.L. Dold, Respirometry in control of activated sludge process: principles, IWA Technical Report, London, UK, 1998.
[14] M. Albek, U. Yetis, C.F. Gokcay, Effects of Ni(II) on respirometric oxygen uptake, Appl. Microbiol. Biotechnol. 48 (1997) 636–641.
[15] C.J. Kelly, N. Tumsaroj, C.A. Lajoie, Assessing wastewater metal toxicity with bacterial bioluminescence in a bench-scale wastewater treatment system, Water Res. (38) (1997) 423–431.
[16] ISO 8192, Water quality—test for inhibition of oxygen consumption by activated sludge, 1995.
[17] S. Vankova, J. Kupec, J. Hoffmann, Toxicity of chromium to activated sludge, Ecotoxicol. Environ. Safety 42 (1999) 16–21.
[18] M. Gutierrez, J. Etxebarria, L. Fuentes, Evaluation of wastewater toxicity: comparative study between microtox and activated sludge oxygen uptake inhibition, Water Res. 36 (2002) 919–992.
[19] M. Henze, Characterization of wastewater for modelling of activated sludge processes, Water Sci. Technol. 25 (6) (1992) 1–15.
[20] ISO, Water quality —determination of the chemical oxygen demand, Ref. no. ISO 6060-1986.
[21] Standard Methods for the Examination of Water and Wastewater, 19th ed., American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC, USA, 1995.
[22] O. Karahan, A. Martins, D. Orhon, M.C.M. van Loosdrecht, Experimental evaluation of starch utilization mechanism by activated sludge, Biotechnol. Bioeng. 93 (5) (2006) 964–970.
[23] J.J. Beun, F. Paletta, M.C.M. van Loosdrecht, J.J. Heijnen, Stoichiometry and kinetics of poly-β-hydroxybutyrate metabolism in aerobic, slow growing, activated sludge cultures, Biotechnol. Bioeng. 67 (4) (2000) 379–389.

[24] T. Mino, D.C. San Pedro, T. Matsuo, Estimation of the rate of slowly biodegradable COD (SBCOD) hydrolysis under anaerobic, anoxic and aerobic conditions by experiments using starch as model substrate, Water Sci. Technol. 31 (2) (1995) 95–103.

[25] C.T. Tien, C.P. Huang, Kinetics of Heavy Metal Adsorption on Sludge Particulate. Heavy Metals in the Environment, Elsevier, Amsterdam, 1991, pp. 313–327.

[26] S. Vankova, J. Kupec, J. Hoffmann, Toxicity of Chromium to Activated Sludge, Ecotoxicol. Environ. Safety (42) (1999) 16–21.

[27] G. Insel, O. Karahan, S. Ozdemir, I. Pala, T. Katipoglu, E.U. Cokgor, D. Orhon, Unified basis for the respirometric evaluation of inhibition for activated sludge, J. Env. Sci. Health A 41 (9) (2006) (in press).

[28] D. Okutman, S. Ovez, D. Orhon, Hydrolysis of settleable substrate in domestic sewage, Biotechnol. Lett. 23 (23) (2001) 1907–1914.