Appl Microbiol Biotechnol (1997) 48: 636–641

ORIGINAL PAPER

M. (Lüle) Albek · Ü. Yetiş · C. F. Gökçay

Effects of Ni(II) on respirometric oxygen uptake

Received: 4 January 1997 / Received revision: 10 June 1997 / Accepted: 29 June 1997

Abstract The effects of Ni(II), substrate and initial biomass concentrations on biochemical oxygen demand (BOD) were studied by using an electrolytic respirometer. The effects of Ni(II) (2.5, 5.0, 10.0, 25.0 mg/l) and substrate (325, 650, 1300 mg/l as chemical oxygen demand) in a synthetic wastewater with differing initial biomass concentrations (1, 10, 100 mg/l) were investigated. The biomass-to-metal ratio was found to be the most important parameter affecting the measured BOD values. The maximum specific growth rates were calculated and the results of batch respirometric experiments were analysed both by graphical and statistical methods. In statistical analyses, a factorial experimental design approach was followed and results were treated by multiple regression techniques. A mathematical model was developed to express the maximum oxygen uptake in terms of nickel, substrate and initial biomass concentrations and their magnitudes of their effects were compared. The biomass-to-metal ratio was found to be very significant so that another model that expresses oxygen uptake in relation to the biomass-to-metal ratio and also to substrate concentration was developed. Finally, the effect of Ni(II) was demonstrated to depend on both substrate and initial biomass concentrations. This effect was stimulatory at low concentrations of Ni(II), and complete inhibition was never observed even at the highest concentration of Ni(II) studied, which was 25.0 mg/l.

M. (Lüle) Albek (✉)
Environmental Engineering Department, Anadolu University, Yunusemre Campus, 26470 Eskişehir, Turkey
Tel.: 90 222 3350580/3631
Fax: 90 222 3353616

Ü. Yetiş · C. F. Gökçay
Environmental Engineering Department, Middle East Technical University, 06531 Ankara, Turkey

Introduction

The heavy metals in wastewaters originate from their wide usage in metal-finishing industries. Heavy-metal toxicity studies on the biological wastewater treatment process are important, as most municipal wastewater treatment facilities also accept industrial effluents. Considering the occasional failure of industrial pretreatment facilities in treating metals, there is a continuous need to investigate the effects of heavy metals on such biological processes.

The toxicity of heavy metals in biological treatment processes depends mainly upon two factors, namely, metal species and concentration. Other factors such as pH, microorganism concentration and influent strength and type are also reported to affect the toxicity of metals, though to a lesser degree. Moreover, previous studies on the toxicity of Ni(II) to the activated-sludge process have shown that biomass and substrate concentrations have considerable effects on its performance (Sujaritannonta and Sherrard 1981). Yetiş et al. (1992), emphasized the importance of the biomass-to-metal ratio in biological oxygen demand (BOD) experiments. However, a gap still exists in the literature on the combined effects of initial seed biomass and substrate concentrations in the context of metal toxicity to oxygen uptake. Among the methods to determine oxygen uptake, a relatively new instrument, the electrolytic respirometer, greatly simplifies respiration studies by allowing precise measurements of oxygen consumption over a long period of time, as reviewed by Berkün and Tebbutt (1976), Young and Baumann (1976), Tribe and Maynard (1988), Cailas and Gehr (1989), Larson and Perry (1981).

Accordingly the objective of this study was to investigate the effects of substrate and biomass concentrations on the respirometric oxygen demand in the presence of heavy metals.

Materials and methods

Respirometer

An electrolytic respirometer (Bioscience, model BI-1000), which is designed to measure the rate of respiration by microorganisms in solutions, soils and sludges, was used in the experiments.

The seeded microorganisms in the reactor vessels normally metabolize the organic matter in the sample, which is kept at a constant temperature in a water bath, and oxygen is utilized while CO_2 is produced. The carbon dioxide in the head space is scrubbed by a caustic solution kept in a designated well. The result is a slight vacuum inside the reactor relative to the outer atmosphere, which causes a decrease in the electrolyte solution level in the outer chamber of the electrolysis cell. When an approximately 1-mm change in electrolyte level has occurred, the contact between the sensor electrode and electrolyte is broken causing an electric current to flow through the electrolyte between two submerged electrodes. The passing current, causes hydrolysis of the water, splitting it into oxygen and hydrogen. The oxygen generated restores the original pressure inside the reactor vessel. When the pressure inside the cell is re-established, the electrolyte solution rises in the outer chamber of the cell, and the hydrolysis stops. The amount of oxygen generated is calculated from the current passing and the value stored in a computer. The hydrogen gas is vented to the atmosphere.

Feed solution

A synthetic wastewater with a known chemical oxygen demand (COD) and nickel concentration was used in the experiments. The composition of the synthetic wastewater is listed in Table 1.

A stock nickel chloride solution was prepared and added to the feed solution in appropriate volumes to obtain 2.5, 5.0 and 10.0 mg/l Ni(II) overall concentrations. A phosphate salt was introduced as a source of phosphorus for the microorganisms and to maintain a stable pH. All other macro- and micronutrients were added in sufficient quantities to make carbon the growth-limiting nutrient. Proteose-peptone (Oxoid), which is frequently used as the carbon source for such systems in order to obtain a stable mixed culture growing on a synthetic wastewater simulating a municipal wastewater (Yetiş et al. 1992; Gökçay and Dilek 1991; Yetiş and Gökçay 1989), was utilized in the preparation of 325, 650 and 1300 mg/l COD concentrations.

Seed biomass

The activated sludge that was used as a source of seed to the reactors was grown in a semi-batch culture in the laboratory in a similar synthetic wastewater that did not contain any Ni(II). The batch activated-sludge culture was grown in a volume of 6 l and provided fresh activated sludge for each seeding. Active biomass taken from the reactor was centrifuged at 3000 rpm for 15 min and the pellet was washed with phosphate buffer solution. The washed and homogenized pellets were then used as the initial inocula to the reactors.

Table 1 Composition of the synthetic wastewater

Constituents	Concentration (mg/l)
Proteose-peptone	Variable
NaCl	407.4
Na_2SO_4	44.6
K_2HPO_4	44.6
$MgCl_2 \cdot 6H_2O$	3.7
$FeCl_2 \cdot 2H_2O$	3.7
$CaCl_2 \cdot 2H_2O$	3.7
$MnSO_4$	0.057
NaOH	0.008
$ZnSO_4$	0.046
$CuSO_4$	0.076

Analytical techniques

Seed/biomass concentrations were measured in a spectrophotometer at 550 nm wavelength against distilled water serving as reagent blank, and were converted to milligrams per litre volatile suspended solids via a calibration curve developed in this study.

Experimental design and analysis

A multifactorial approach to the experimental design was employed, so that the effects of combinations of parameters as well as significant solo effects could be determined. For this purpose a two-level (maximum and minimum) approach was used and the final interactions were analysed by the same technique. Another statistical method, namely, multiple regression, was also used to model the oxygen uptake.

Results

In this study, 48 experiments, along with their duplicates, were performed at different combinations of Ni(II) (0, 2.5, 5, 10, 25 mg/l), initial biomass (1, 10, 100 mg/l) and substrate (325, 650, 1300 mg/l as COD) concentrations. For all experiments, plots of the oxygen accumulated against time were obtained. The final cumulative oxygen-uptake values obtained were those recorded at approximately 160–200 h; since the change observed in oxygen uptake from this time on was considered insignificant, these values was taken to be approximately equal to the ultimate value.

Effects of Ni(II) on oxygen uptake

The results of the experiments at different substrate, Ni(II) and biomass concentrations are summarized in Fig. 1. From this figure, it can be seen that, at 1300 mg/l substrate concentration, the total oxygen uptakes were approximately the same for all Ni(II) concentrations. However, flasks not containing Ni(II) exhibited a slightly higher oxygen uptake. This was particularly noticeable with increasing substrate concentration. The total oxygen uptake for 1300 mg/l substrate was approximately two times higher than that obtained with 650 mg/l substrate, as expected.

At 650 mg/l substrate, it could be concluded that 2.5 mg/l and 5.0 mg/l Ni(II) with 100 mg/l initial seed biomass did not stimulate the system any more than the contents of the flask that was devoid of nickel. However, the accumulated oxygen uptake progressively increased with the nickel concentration in flasks that contained 100 mg/l initial seed biomass. For example, among this set, the lowest oxygen uptake was recorded in the flask containing 2.5 mg/l Ni(II) and the highest was at

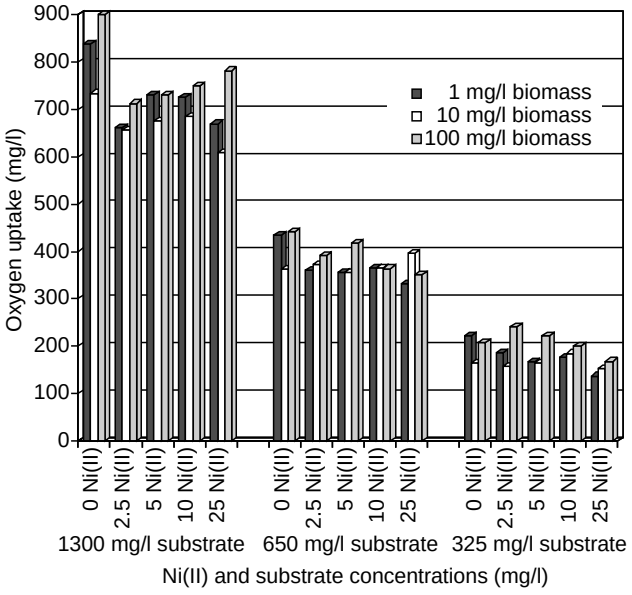

Fig. 1 Oxygen uptake values at different substrate, Ni(II) and biomass concentrations

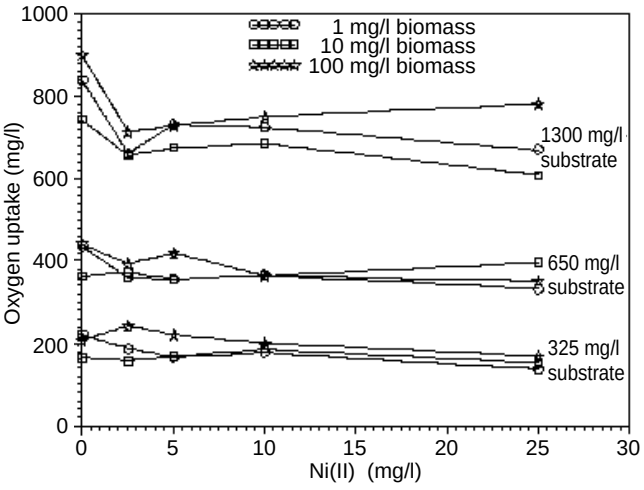

Fig. 2 Oxygen uptake versus Ni(II) graph at different biomass and substrate concentrations

25 mg/l Ni(II). At 325 mg/l substrate, a slight stimulatory effect on O_2 uptake could also be observed with Ni(II), especially at the highest (100 mg/l) biomass concentration (Fig. 1).

Figure 2, shows cumulative oxygen uptake values in flasks containing 2.5, 5.0, 10.0 and 25.0 mg/l Ni(II) and various initial seed concentrations.

Statistical approach

In order to analyse the magnitudes of the effects of independent variables on oxygen uptake, a factorial design approach was adopted. Since BOD is directly proportional to the substrate concentration, a normalization of the dependent parameter (BOD) with respect to the initial substrate concentration was necessary. Accordingly the independent variables were assigned as Ni(II), biomass and substrate concentrations, while the dependent variable became the normalized maximum oxygen uptake, or normalized BOD_L, i.e. "BOD_L/substrate". The linear model shown in Eq. 1 was adopted to study the solo and combination effects.

Two levels were adopted for each factor, these being the upper level (denoted + in Table 2), and the lower level (denoted − in Table 2). The design matrix and the lower and upper levels assigned for each factor are given in Table 2. The algebraic solution of the experimental matrix is depicted in Fig. 3. From this figure it can be calculated that the effect of Ni(II) on oxygen uptake is inhibitory, while biomass and substrate are stimulatory. The inhibitory effect of Ni(II) could be minimized by maintaining high initial biomass or substrate concentrations in the test medium. Again from this figure it can be deduced that biomass concentration and, to a lesser extent, substrate concentration both lift the inhibitory effect of Ni(II) on oxygen uptake. However, in the case of Ni(II), substrate and biomass in combination, the resultant effect was still inhibitory, though to a lesser extent than when nickel was considered alone.

The positive effect of biomass on oxygen uptake is also understandable, as the initial seed biomass put into the test system is also a form of substrate to the microorganisms.

Table 2 The design matrix and the maximum and minimum concentrations of factors in the design matrix

Design matrix			Maximum and minimum concentrations of factors			
Ni(II)	Biomass	Substrate	Ni(II) (mg/l)	Biomass (mg/l)	Substrate (mg/l COD)	Normalized BOD_L (mg/l BOD_L/rB/l substrate)
−	−	−	10	1	325	0.68
+	−	−	25	1	325	0.42
−	+	−	0	100	325	0.64
+	+	−	25	100	325	0.51
−	−	+	0	1	1300	0.64
+	−	+	25	1	1300	0.51
−	+	+	0	100	1300	0.69
+	+	+	25	100	1300	0.60

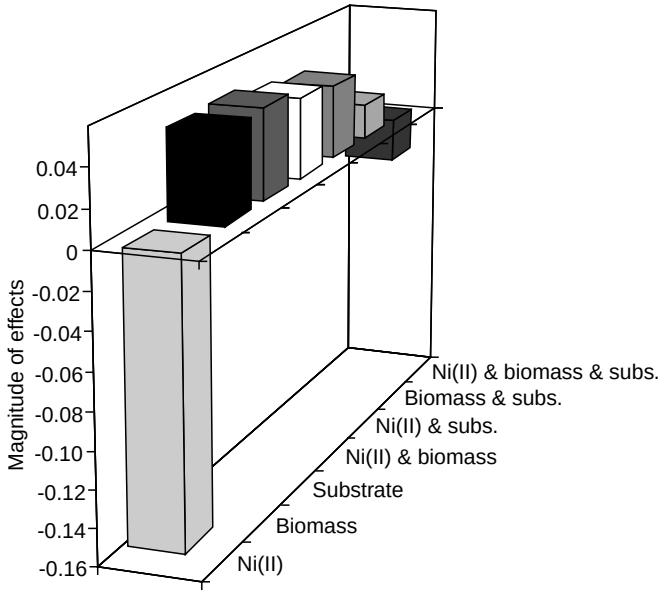

Fig. 3 Estimates of factor effects on normalized biological oxygen demand

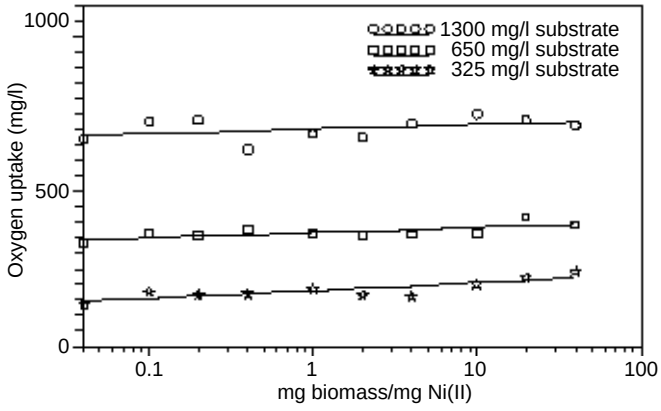

Fig. 4 Oxygen uptake versus biomass-to-metal ratio

Multiple regression for a linear model of BOD_L

From the above factorial analysis, it was found that all the parameters tested are important to some extent and hence must be considered in a linear multiple regression model of the type:

$$y = k_1X_1 + k_2X_2 + k_3X_3 + k_{12}X_{12} + k_{13}X_{13} + k_{23}X_{23} + k_{123}X_{123} + C \quad (1)$$

where y is the maximum oxygen uptake or BOD_L (mg/l); X_1 is the Ni(II) concentration (mg/l), X_2 the biomass concentration (mg/l) and X_3 the substrate concentration (mg/l).

From the results of multiple regression analysis it can be deduced that, in spite of a good fit to the model obtained, with a multiple regression coefficient (r^2) of 0.9671, the results were significant at the 95% confidence level except for X_3. By using ANOVA the model was improved. The improved model then became

$$y = k_1X_1 + k_2X_2 + k_3X_3 + C \quad (2)$$

and produced a multiple regression coefficient (r^2) of 0.972338. The improved model can be re-written as

$$y = -2.03X_1 + 0.39X_2 + 0.55X_3 + 12.11 \quad (3)$$

Effects of biomass-to-metal ratio on oxygen uptake

Another normalization approach was made with respect to biomass and metal. Accordingly the oxygen-uptake data were plotted against the biomass/Ni(II) ratio as depicted in Fig. 4. A linear relationship between the biomass-to-metal ratio and oxygen uptake was obtained for all initial substrate concentrations. The oxygen uptake increased linearly at a constant rate with the biomass-to-metal ratio at all the substrate concentrations tested. This observation suggests that the effect of biomass-to-metal ratio on oxygen uptake is independent of the substrate concentration.

Effects of Ni(II)/biomass ratio on maximum specific growth rate

At high substrate concentrations microorganisms will grow at their maximum rate, and this can be defined by the following equation, which is a simplification of the Monod expression at relatively high substrate levels:

$$\frac{dX}{dt} = \mu_m X \quad (4)$$

Integration of Eq. 4 yields

$$X = X_0\, e^{\mu_m t} \quad (5)$$

where X_0 is the initial biomass concentration at time zero, mass/volume.

The amount of microorganisms produced is a function of the substrate removed, which will require a proportional amount of oxygen consumption. Thus, the change in cell mass can be related to the measured oxygen consumption in the respirometer:

$$\frac{dX}{dt} = Y\frac{dS}{dt} = \frac{Y}{a}\frac{d[O]}{dt} \quad (6)$$

where a is the oxygen consumption coefficient, the mass of O_2 used/mass of substrate oxidized, and $d[O]/dt$ is the rate of oxygen consumption (mass volume^{-1} time^{-1}).

The derivative of Eq. 5 is taken to obtain the rate of change of cell mass under maximum growth conditions and this is substituted into Eq. 6 to yield:

$$\frac{d[O]}{dt} = \left[\frac{\mu_m X_0 a}{Y}\right] e^{\mu_m t} \quad (7)$$

taking the natural logarithm of both sides results in:

$$\ln \frac{d[O]}{dt} = \ln \left[\frac{\mu_m X_0 a}{Y} \right] + \mu_m t \quad (8)$$

Thus, the respirometric data can be used to determine μ_m, from the slope of the semi-logarithmic plot of $d[O]/dt$ against time, for each test run with varying Ni(II), biomass and substrate concentrations. The variance of μ_m values calculated through this approach was never above 0.02 1/h, the correlation coefficient for each set of $d[O]/dt$ versus time values was never below 0.80.

The Ni(II)/biomass ratio was plotted against these calculated μ_m values as shown in Fig. 5. From this figure it is readily seen that μ_m is independent of substrate concentration. However, it seems to be an important function of the metal-to-biomass ratio. A Ni(II)/biomass ratio of 2.5 seemed important in the sense that a gradual increase in growth rate was noticeable up to this value, although there was a quite big fluctuation in μ_m, at Ni(II)/biomass ratios of 1 and 5, which remained unexplained. After the ratio of 2.5 was passed, inhibition was evident as the Ni(II)-to-biomass concentration ratio increased. Therefore, it can be concluded that 2.5 mg Ni(II)/mg biomass stimulates the system while 25 mg/l Ni(II)/mg biomass does not entirely upset the performance of the system.

A linear model analysing the effect of biomass-to-metal ratio on BOD_L by multiple regression

In order to formulate the effects of the biomass-to-metal ratio on oxygen uptake, single and multiple effects of substrate concentration and biomass-to-metal ratio were analysed by multiple regression. Although a model with a correlation coefficient (r^2) of 0.9897 was obtained when quadratic terms were included, the significance levels of the quadratic terms {[substrate]^2, [substrate] × (biomass-to-metal ratio) and (biomass-to-metal ratio)^2}were negligible, hence these were omitted from the regression model. The improved model, not including the quadratic terms, becomes

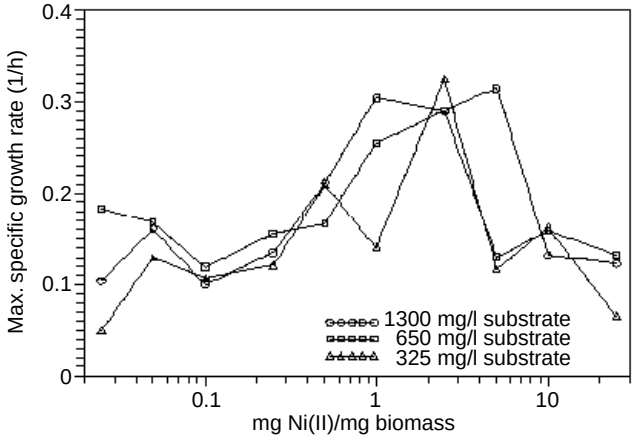

Fig. 5 Maximum specific growth rate versus Ni(II)/biomass ratio

$$y = 0.531505 X_3 + 1.390403 X_4 + 4.316008 \quad (9)$$

where, X_3 is the substrate concentration (mg/l) and X_4 the biomass-to-metal ratio.

Discussion

The oxygen-uptake rate and oxygen-transfer capacity are of great importance in biotechnology and biological wastewater treatment. Respirometric measurement of the oxygen consumption of the activated sludge has been recognized as one of the most common and practical ways of determining the extent of microbial activity. However, for a waste containing toxic materials, like heavy metals, this method of oxygen-uptake measurement has limited use. Organic matter and biomass concentrations in the reactor cell appear to influence the oxygen consumption and, in turn, cause the generation of data that are not meaningful in the evaluation of treatment plant performance. In this study, the effects of Ni(II), initial substrate and initial biomass concentrations on oxygen uptake were evaluated to assess the influence of heavy metals on oxygen uptake in relation to biomass and substrate concentrations.

It was found that a direct relationship between Ni(II) concentration and total oxygen uptake within the concentration range tested does not exist. Moreover, it was generally observed that, at low concentrations (2.5 and 5.0 mg/l), the effect of Ni(II) is stimulatory whereas 25 mg/l Ni(II) was inhibitory but did not upset the system entirely. Furthermore, the effect of Ni(II) becomes significant with decreasing substrate concentration. In addition, especially at high substrate concentrations, the effect of the initial biomass on oxygen uptake is not very significant.

There is a linear relationship between the biomass-to-metal ratio and the oxygen-uptake values. The linearity shows an approximately parallel trend at different concentrations of substrate, as shown Fig. 4. Therefore the effect of the biomass-to-metal ratio on oxygen uptake remains unchanged whatever the substrate concentration. The ratio of Ni(II) to biomass is also important for the maximum specific growth rate. A ratio of 2.5 is found to be stimulatory for such systems.

References

Berkün M, Tebbutt THY (1976) Respirometric determination of BOD. Water Res 10: 613–617

Cailas MD, Gehr R (1989) The oxygen uptake rate approach for analyzing respirometric biochemical oxygen demand data. II. Application and evaluation. Water Res 23: 993–1001

Gökçay CF, Dilek FB (1991) Effects of nickel, chromium and initial feed concentration on the batch growth of a microbial consortium developed from sewage. Environ Technol 12: 1–11

Larson RJ, Perry RL (1981) Use of the electrolytic respirometer to measure biodegradation in natural waters. Water Res 15: 697–702

Sujarittanonta S, Sherrard JH (1981) Activated sludge nickel toxicity studies. Water Pollut Control Fed 53: 1314–1322

Tribe HT, Maynard P (1988) A new electrolytic respirometer. Proc R Soc Edinb Sect B (Biol Sci) 94: 178–181

Yetiş Ü, Gökçay CF (1989) Effects of nickel(II) on activated sludge. Water Res 23: 1003–1007

Yetiş Ü, Dilek FB, Gökçay CF (1992) Effects of heavy metals on biological activity in BOD bottle-seed biomass concentration. In: Second International Symposium on Waste Management Problems in Agro-Industries, September 23–25, Istanbul, Turkey

Young JC, Baumann ER (1976) The electrolytic respirometer. I. Factors affecting oxygen uptake measurements. Water Res 10: 1031–1040