Application of respirometry in the assessment of chromium contaminated waste waters treatment


Laboratoire de l’ingénierie des procédés d’environnement ‘LIPE’ Département de Chimie Industrielle,
Université Mentouri Constantine 25000 Algérie

Abstract

Restrictions concerning the disposal of waste waters are getting more severe, requiring a greater reliability for the waste water treatment plants as well as a better understanding and application of biological processes which depend upon the kinetics of the microorganisms’ growth. This latter is greatly influenced by the physico-chemical conditions of the hosting media.

The use of respirometry, the principle of which is based on measuring the consumed amount of oxygen by a sample of activated sludge for the metabolism of a given amount of substrate, seems to be able to contribute to the improvement of the plant management. In fact, it enables the estimation of certain characteristic variables for a good process or the detection of the influence of the physico-chemical conditions such as pH, salinity, metal toxicity, etc.

Obviously these conditions have an impact on the enzymatic reaction rates and hence on the metabolism and cell multiplication.

In the present study, inhibition by chromium which is a major contaminant present in tanning, metallic surface treatment and other industries waste waters, is likely to neutralise the biomass, was studied by means of the respirometry technique to detect the immediate influence of this type of stress on the activity of microorganisms (autotrophic and heterotrophic), as well as on its purifying power and its aggregation morphology.

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Keywords: Activated Sludge; Respirometry; Oxygen transfer; Microbial inhibition; Chromium

* Corresponding author. Tel.: +213-031-81-88-80; fax: +213-031-81-88-80.
E-mail address: docran@voila.fr
1. Introduction

The term respirometry is used to describe the measurement of oxygen consumption in wastewater samples or activated sludge. Under aerobic conditions the microorganisms consume oxygen in proportion to the organic matter and biomass in the sample. The first application of the respirometric technique for measuring wastewater oxidation was reported in 1924 by Otto Heinrich Warburg (1883–1970). It is based on manometry, which consisted in the analysis of gas pressure changes over an enclosed measuring system. Most of the respirometers available nowadays are based on Warburg’s respirometer (manometry) [01, 02]. After the invention of the Warburg respirometer several researchers have developed different kinds of respirometric measuring techniques based on manometry, electrolytic or direct measurement of oxygen consumption.

Using new technologically advanced respirometers, it is possible to utilize respirometry for biodegradation measurements in water media and soils, for kinetic analysis and biokinetic constants determination, for modeling biological wastewater treatment processes, for managing toxicity, nitrification capacity, activated sludge activity, biodegradability of poorly soluble substances such as oils and organic compounds [01, 03, 04, 05].

It is noted that standard domestic wastewater has generally a constant composition and rarely contains compounds or factors that would inhibit biodegradation. In contrast, industrial effluents are characterized as complex mixtures with varying concentrations of pollutants and inhibitory substances, such as heavy metals, volatile organic compounds, poly-aromatic and polychlorinated organic substances, which may affect system performance [06]. Respirometry is an advantageous technique in identifying compounds that might potentially have an adverse effect on a wastewater treatment system [07, 08, 09, 10, 11, 12].

A respiration inhibition test is important to managers of treatment facilities in order to check and to evaluate wastewaters toxicity. EC50, EC20 and EC10 values are used in respiration inhibition and nitrification inhibition tests to represent the concentration of wastewater, which produces 50%, 20% and 10% inhibition of the oxygen uptake rate in comparison to a control sample [05].

In the open respirometer technique, a larger sample volumes and longer essay times were required in comparison to the closed respirometer, this technique consumes small sample volumes and the essay requires less than 10 min [13].

In this paper chromium was chosen to represent the mineral toxic waste waters, the objectives of the present work were (i) to study the effect of the chromium addition on the oxygen uptake rate (ii) to study the effect of the chromium addition on morphology of the sludge biomass and its ability to deflocculation.

2. Experimental materials and methods

2.1. Open respirometer experiments

The open respirometer (flowing gas/static liquid) consisted in a 500 ml working volume reactor in which the sample was placed, stirred with a magnetic stir-bar and continuously aerated at the bottom of the respirometer using an air pump, the DO concentration (C) measurements as a function of time (t) was recorded using the LINSEIS recorder. Oxygen uptake rate is dependent on temperature as well as
microbial activity which increase with it [14]. Therefore, it is important to keep a constant temperature during the entire experiment, which is often performed at 20 ± 1 °C using a thermal enclosure.

![Schematic picture of Open respirometer built in (lipe) laboratory](image)

**Fig. 1.** Schematic picture of Open respirometer built in (lipe) laboratory

2.2. Activated sludge biomass source

The activated sludge was obtained from the aeration tank of the local wastewater treatment plant «IBN ZIAD», Constantine, Algeria. It is of an extended aeration type activated sludge process with an anoxic zone ahead (AO). The four aeration compartments are equipped with four surface turbines (p: 4 × 93 kW), aeration control is based on an alternation system (air-on/air-off) which corresponds to 15 cycles per day with a cumulative aeration time of 13h/day.

The samples were taken from the final compartment (A4), in order to minimize residual levels of pollution. The collected sludge was used immediately and no adjustment procedure was undertaken.

2.3. Sludge preparation

The sludge can be settled and the supernatant was then replaced with tap water in order to reduce the initial concentration of substrate in the sludge, this washing procedure was repeated until the theoretical concentration of soluble matter was reduced by more than 95% [15], in this work the procedure consisted to aerating the biomass in order to degrade all exogenous substrate initially present in the sample.

2.4. Carbon and nitrogen source

The substrate kind is a decisive factor for the type and rate of biochemical reactions taking place in an activated sludge system [16].

In this study, the choice was directed towards a binary substrate consisting of sodium acetate and ammonium chloride (a very easily degradable organic matter for heterotrophic and autotrophic bacteria). In this experiment, the injections of carbon and nitrogen to the reactor were such that the resulting chemical oxygen demand (COD) and (NH₄⁺ -N) in the reactor are equal to 15,2 mg/l et 1,6 mg/l respectively, the concentration of NH₄⁺-N is less than the concentration that inhibits nitrifying bacteria [17]. The concentration of biomass in the respirometer is in the range of 5,28 mg/l, hence the initial S₀/X₀ ratio is about 2,87mg COD/mg TSS for the carbon substrate and 0,30 mg NH₄⁺-N /mg TSS for the nitrogen substrate.

2.5. Nitrification inhibitor

When sludge from nitrifying treatment plant is used some of the oxygen consumption is used for nitrification during measurements of organic degradation, a nitrifying inhibitor ‘allyl thiourée’ C₄H₈N₂S (ATU) [18] is often, used for this purpose, which inhibits the conversion of ammonia to nitrite. For OUR tests levels of 12 mg/l of inhibitor is typically used. However, investigations have shown that the additions of 10 mg/l of ATU impacts the endogenous respiration of the sludge which results in a lowered
Therefore, one should be aware of how the results are used depending on the application of the method.

For the same experimental procedure, Baudouin, 2004 recommends the addition of 20 mg/l of ATU in the respirometer but Delgado, 2009 advocates a concentration about 10 mg/l of ATU, in this study the concentration of the inhibitor is 5 mg/l in the respirometer (experimental constraints).

2.6. The toxic inhibitor

To use the respirometer for assessing the effect of environmental physico-chemical conditions on microbial biodegradation of carbon and nitrogen compounds, chromium minerals was chosen since it is likely to inhibit wastewater treatment.

Although the discharge standards allowed in the most liquid discharges industries do not exceed 0.3 mg/l [23], the maximum amount of chromium introduced, under the form of chromium chloride hexahydrate (CrCl₃·6H₂O), in the respirometer is 30 mg/l simulating an accidental spill.

2.7. Experimental procedure

The experimental procedure includes the following steps:

- The first step is setting the endogenous sludge, where the respirometer is filled with activated sludge aerated and not supplied with a volume of 500 ml during the night preceding the tests (agitation 100 round/min, T: 20 °C) in this way, the substrate that could be initially in the sludge is completely consumed by bacteria and this results in a plateau reached in terms of dissolved oxygen concentration.

- The second and the third step is the determination of the coefficient of oxygen transfer (kLa) and the endogenous respiration. Air injection is stopped, oxygen concentration is brought close to 1 mg/l, then air supply is restarted and the variation of dissolved oxygen concentration is recorded. Deaeration curve obeys to equation (1):

\[
\frac{dS_O}{dt} = k_L a (S_{O, sat} - S_{end}) - OUR_{end}
\]  

(1)

Where \( S_O \), \( S_{O, sat} \) and \( S_{end} \) are the instantaneous, the saturation and the endogenous DO concentrations, respectively.

- The fourth step is the injection of a certain amount of the synthetic binary substrate which gives rise to an increase in oxygen consumption by microorganisms (autotrophic and heterotrophic) and therefore a respirometric peak is obtained (equation (3) and (4)), once the substrate oxidized the dissolved oxygen concentration tends to return to the endogenous level as a result of the aeration.

\[
OUR = OUR_{exo} + OUR_{end}
\]  

(2)

\[
\frac{dS_O}{dt} = k_L a (S_{O, sat} - S_O) - OUR_t
\]  

(3)

\[
OUR_{exo} = k_L a (S_{O, end} - S_O) - \frac{dS_O}{dt}
\]  

(4)

- The fifth step is to observe the effect of adding the toxic product on the parameters of respirometric peak, this will be clear by simultaneous injection of quantity of toxic and the same amount of substrate.
• After returning to the endogenous level, an adequate amount of nitrification inhibitor and the same amount of substrate are added, in order to differentiate between the toxic effect on the two components of the exogenous respiration of heterotrophs and autotrophs.

3. Experimental results and discussion

3.1. $k_{La}$ and OUR$_{end}$ assessment

The oxygen mass transfer coefficient $k_{La}$ and the endogenous respiration rates (OUR$_{end}$) were obtained using a non-steady state procedure; they were calculated in a previous experiment as the slope of DO decrease in the reactor without external aeration and without external substrate. The experimental profiles obtained are plotted in Fig. 2, all the experiments were conducted under the same operational conditions.

The reproducibility of $k_{La}$ measurements is not very good, the results varied considerably between two experiments. It is recommended, to determine this parameter for each experiment [19].

In fact, in this study $k_{La}$ was determined at the beginning of each experiment since the endogenous respiration varies widely with microbial activity.

3.2. Identifiability (substrate /biomass (ratio))

Many authors have highlighted the importance of initial conditions in biodegradation tests. In particular the initial substrate to biomass concentration ratio denoted $S/X$, which determines the type of respirometric response [24]. Indeed, this ratio influences both the metabolic processes involved during the degradation, the identification of kinetic parameters and the separation between easily and slowly biodegradable fractions.

The respirometric tests were conducted in this study by setting an initial ratio $S_0/X_0$ to 2.87 mg COD/mg TSS (experimental constraints), while setting the same experimental conditions ($T = 20\pm1$, Agitation: 100 r/min, $V= 500$ ml).

The area formed under the curve for each peak, represents the quantity of oxygen consumed (necessary) for the assimilation of exogenous substrate added.
Respirometric method can be used, as well, to assess the initial substrate to biomass ratio $S_0/X_0$. Table 1 shows the effect of variation of this ratio on oxygen consumption. As the initial ratio is doubled oxygen consumption increases by 17%. Furthermore, when it is augmented 10 times, oxygen consumption increased by only 28%. These results indicate that substrate is much more abundant in solution than biomass. Therefore the ratio is high, which is confirmed by the literature (> 2 mg DCO/ mg MVS).

Table 1. Effect of the initial $S_0/X_0$ ratio on the amount of oxygen consumed

<table>
<thead>
<tr>
<th>K_La (h⁻¹)</th>
<th>OUR end (mg/l.min)</th>
<th>the amount of oxygen consumed (mg/l)</th>
<th>(S₀/X₀ : 2.87 mg COD/mg TSS)</th>
<th>the amount of oxygen consumed (mg/l)</th>
<th>(S₀/X₀ : 5.74 mg COD/mg TSS)</th>
<th>the amount of oxygen consumed (mg/l)</th>
<th>(S₀/X₀ : 28.7 mg COD/mg TSS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.92</td>
<td>0.054</td>
<td>108.84</td>
<td></td>
<td>127.59</td>
<td></td>
<td>139.09</td>
<td></td>
</tr>
</tbody>
</table>

3.3. Respirometric response

The evolution of dissolved oxygen concentration in experimental stages is represented in the following figures:

Fig. 4. an example for the Respirogramme obtained at 20°C (1 mg/l Cr (III) addition)

Fig. 5. Respirogram obtained at 20°C (0.1 mg/l Cr (III) addition)

Fig. 6. Respirogram obtained at 20°C (0.5 mg/l Cr (III) addition)

Fig. 7. Respirogram obtained at 20°C (4 mg/l Cr (III) addition)
3.4. Effect of inhibition on OUR profiles

OUR profiles were obtained by means of respirometric measurements under the same experimental conditions and the same initial S₀/X₀ ratio. An example of the OUR profiles for the control reactor with and without toxic inhibitor addition are plotted in Fig.10.

The assessment and interpretation of the oxygen uptake rate (OUR) is now recognized as the most important tool to quantify major parameters and processes from significant experimental and modelling studies.

At first the OUR measurement gives a very fast response, when substrates are added, however in the second phase of the experiment, when substrate and chromium are added the respiration rate decreased as can be seen through the slope of the ascending part of the curve (central part of the curve of figure 10). Moreover, the position of the inflexion point on the two parts of the curve is different, indicating that the quantity of oxygen consumed is different as well.

The amount of oxygen consumed during the tests evaluating the effect of different pulses of chromium on microbial activity. The amount of oxygen consumed without Cr addition differs from one experience to another due probably to microbial activity (Table 2).

The reduction percentages in the amount of oxygen consumed with Cr (III) concentration added in comparison to the reference test is calculated according to the equation (5):

\[
I\% = \frac{\int [OUR(t)]_0 dt - \int [OUR(t)]_I dt}{\int [OUR(t)]_0 dt} \times 100
\]

Where %I is the reduction of respiratory activity, expressed as a percentage, \(\int [OUR(t)]_0 dt\), the amount of oxygen consumed in the absence of inhibitor (mgO₂/L) and \(\int [OUR(t)]_I dt\), the amount of oxygen consumed in the presence of inhibitor (mgO₂/L).
Table 2. The amount of oxygen consumed and the percentages of reductions in the amount during the tests evaluating the effect of different pulses of toxic on microbial activity.

<table>
<thead>
<tr>
<th>Concentration of Cr (III) added (mg/l)</th>
<th>0.1</th>
<th>0.5</th>
<th>1</th>
<th>4</th>
<th>10</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>OURend (mg/l.min)</td>
<td>0.05736</td>
<td>n.d</td>
<td>0.0652</td>
<td>n.d</td>
<td>0.01008</td>
<td>0.10961</td>
</tr>
<tr>
<td>KLa (h⁻¹)</td>
<td>34.42</td>
<td>n.d</td>
<td>9.78</td>
<td>n.d</td>
<td>60.24</td>
<td>16.4</td>
</tr>
<tr>
<td>the amount of oxygen consumed (mg/l) (without Cr addition)</td>
<td>579.595</td>
<td>n.d</td>
<td>171.825</td>
<td>n.d</td>
<td>2415.1</td>
<td>187.925</td>
</tr>
<tr>
<td>the amount of oxygen consumed (mg/l) (with Cr addition)</td>
<td>688.284</td>
<td>n.d</td>
<td>78.296</td>
<td>n.d</td>
<td>1326.43</td>
<td>114.43</td>
</tr>
<tr>
<td>I (%)</td>
<td>+18.75</td>
<td>nd</td>
<td>-54.43</td>
<td>nd</td>
<td>-45.077</td>
<td>-39.10</td>
</tr>
</tbody>
</table>

n.d: not determined

The increase of the amount of oxygen consumed with 0.1 mg/l Cr addition (Table 2) can be explained that microorganisms adapted well to this level of toxicity, there were no inhibition moreover it could be possible that growth of the biomass contributed as well and it is much faster. Thereafter, the reduction of the amount of oxygen consumed became clearer as the concentration of chromium added is greater than 0.5 mg/l (Fig. 4, 5, 6, 7, 8, 9).

A concentration of 1 mg/l reduces more than 50 % of the amount of oxygen consumed. The experimental data indicate that beyond this concentration there is no more inhibition effect.

The experimental data indicate a no good concordance between the inhibitor concentration and the percent decrease in the amount of oxygen consumed for the range of chromium metal concentration tested in the study, Because under low S₀/X₀ 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, increasing the S₀/X₀ ratio generally reduce the effect of inhibition [16], because cell multiplication becomes important and the degradation rate increases exponentially, experiment with low S₀/X₀ ratios are therefore more sentive in determining toxicity.

3.5. Effect of inhibition in heterotrophic activity

Active biomass represents 70% of the total biomass and heterotrophic bacteria represent 90 % of the active biomass; the analytical means provide access to these ratios [25].

At low Cr concentration (0.1 mg/l), heterotrophic bacteria are already partially inhibited compared to the reference test, for higher concentration (30 mg/l) the inhibition is almost the same (Table 3).

Table 3. Effect of chromium toxic addition on the amount of oxygen consumed by heterotrophic bacteria

<table>
<thead>
<tr>
<th>Concentration of Cr (III) added (mg/l)</th>
<th>0.0</th>
<th>0.1</th>
<th>0.5</th>
<th>1</th>
<th>4</th>
<th>10</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of the amount of oxygen consumed by heterotrophic bacteria / the total amount of oxygen consumed.</td>
<td>65.57</td>
<td>41.71</td>
<td>n.d</td>
<td>n.d</td>
<td>n.d</td>
<td>87.098</td>
<td>41.49</td>
</tr>
</tbody>
</table>
3.6. Effect of chromium toxic addition on the floc forming

The observed changes of microbial flora could be associated with the respirometric technique to indicate a decay or inhibition of the microorganisms in the sludge.

![Micrographs of sludge samples obtained (a) without toxic inhibitor, (b) with addition of 30 mg/l Cr (III) (× 10)](image1)

![Micrographs of sludge samples obtained (a) without toxic inhibitor, (b) with addition of 30 mg/l Cr (III) (× 20)](image2)

Aggregation of various microbial species, formed as bioflocs, is important in maintaining a desirable performance in many aerobic biological waste water treatment plants; several parameters influence the formation or not of bioflocs, like overloading of organic substrates, DO limitation, temperature variations and toxicant transients, such as heavy metal [26], phenol [27] and electrophilic compounds [28,29]. Changes in microbial composition and floc agglomeration in the respirometer was determined by microscope observation without and with 30 mg/l Cr (III) toxic inhibitor addition (Fig. 11 and Fig. 12), chromium addition may induce a lethal shock on biomass, rather than physiological impact (no deflocculation was visualized by microscopic observation).

4. Conclusions

In this paper respirometric technique is used to study the effect of chromium contaminated waste waters on microbial activity.

OUR measurement was used to evaluate the effect of chromium addition on oxygen, consumption. It was found that beyond 1mg/l of chromium the inhibition did not increased. However at low concentration of the inhibitor a catalytic effect seem to take place, which is probably due to the biomass increase. Moreover, a microscopic examination of microorganisms does not show a morphological changes or any deflocculation. From the results obtained, OUR test is more sensitive to toxic elements introduction than microscopic examination.

To increase the sensitivity of this method, the inhibition OUR experiments can be repeated by setting low S0/X0 ratios and other type of substrates.

Acknowledgement

The wastewater treatment plants at « IBN ZIAD», Constantine in Algeria, are all acknowledged for providing sludge for the experiments.
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