Preliminary Evaluation of Inhibitory Effects of Some Substances on Aerobic and Anaerobic Treatment Plant Biomasses

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This study proposes two experimental procedures carried out to assay preliminary toxicity of seven compounds ($CrCl_3$, $FeCl_3$, $NaBO_3$, NaCl, $NaNO_2$, $NaNO_3$, C_2HCl_3) on two biomasses coming from an aerobic activated sludge system and an anaerobic upflow sludge bed reactor, both operating in an industrial wastewater treatment plant. The aim was to obtain information about procedures to enhance the plant efficiency in the treatment of industrial wastewaters containing inhibitory compounds.

To evaluate the aerobic biomass a respirometric test was used, as the anaerobic biomass was examined by methanogenic activity batch tests. Inhibition measures show similar results in experiments carried out with increased concentrations of the considered compounds on both biomasses. To control the reality of the toxicity assay procedures, high concentrations of NaOCl were tested among the other compounds during the study.

Key words:
Aerobic biomass, anaerobic biomass, toxicity assay, respirometry, methanogenic activity.

Introduction

The typical presence in industrial wastewaters of many toxic, xenobiotic and persistent compounds has relevance on the conservation of water quality, public health and, in generally, on the protection of the environment. Furthermore, when traditional biological treatment technologies are used to abate wastewater pollution, toxics can compromise efficiencies of this natural remediation inhibiting performances of, both, aerobic[1,2] and anaerobic biomasses utilized in treatments.[3,4]

To assay, as a preventive measure, the value of inhibition of a compound on a biomass is a useful method to know, if a particular biological process could be negatively influenced by the toxicity of a liquid waste.[5,6] This could be the situation, for instance, of a liquid waste coming from a new industrial activity which must be treated or pre-treated by an already working biological plant. A preliminary distinction must be made between inhibitions related to aerobic and anaerobic biomasses characterizing the biological treatment plant, in fact the biomass metabolism and the toxicity resistance in an aerobic or anaerobic environment are rather different.[7]

Generally, while toxicity of aerobic biomass reduces the possibility to utilize oxygen during metabolism performance, the anaerobic process is influenced on its limiting methanogenic phase.[7] For these reasons a preliminary assay of toxicity can be deduced from the influence of toxic concentrations on overall biomasses metabolism, controlling respirometric and methanogenic behavior.[8,9]

Amongs all toxicity tests on aerobic biomasses reported by bibliography, the respirometric assays are undoubtedly the most common applications,[10,11,12] and so this experimental evaluation is widely used for its simplicity, rapidity, cost-effectiveness and the possibility to test the health quality of the aerobic biomass during operations without altering other conditions. The procedure used in this work reflects the respirometric toxicity test of OECD (Organization for Economic Cooperation and Development) method n. 209 called "Activated sludge, Respiration Inhibition Test". The original method verified by several authors[13,14] was modified in some parts to attain the particular biomass of concern.

Anaerobic process involves a complex interaction of several groups of bacteria, often linked by their individual substrate and product specificities. Four main steps determine anaerobic process: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The methanogens responsible for methanogenesis are generally considered to be more sensitive to environmental conditions, such as toxicant concentrations, rather than other anaerobic microor-

Table 1 – *Compounds and mass concentrations used for tests*

$CrCl_3$ γ/mg L^{-1} Cr	$FeCl_3$ γ/mg L^{-1} Fe	$NaBO_3$ γ/mg L^{-1} B	NaCl γ/mg L^{-1} Cl	$NaNO_2$ γ/mg L^{-1} N	$NaNO_3$ γ/mg L^{-1} N	C_2HCl_3 γ/mg L^{-1}
2.6	10.0	4.0	2890.0	1.0	10.0	1.2
5.2	20.0	8.0	4760.0	2.0	20.0	12.0
56.0		12.0	9400.0			
84.0		24.0	17400.0			
			37500.0			

ganisms, and the methane formation from acetate is also known to be the rate-limiting step in the methanogenic phase.[7] Measures of methanogenic activity may be quite useful in many situations,[15] but a particularly interesting application of this kind of test is to determine the toxic or inhibition effect that some selected compounds or the overall wastewater exert on an anaerobic sludge.[9,16]

In this study two simple experimental procedures were carried out to assay preliminary toxicity of seven compounds (Fe^{3+}, NO_2^-, NO_3^-, Cr^{3+}, Cl^-, BO_3^-, C_2HCl_3) (Table 1) contained in an industrial liquid waste, which are intended to be potentially toxic for the considered wastewater treatment plant biomasses (aerobic and anaerobic), with the aim to check possible inhibition effects on two treatment plant reactors (aerobic activated sludge and upflow anaerobic systems), which have to be chosen for wastewater depuration.

The procedures used in this work to assay biomasses inhibition allow to determine to some extent instantaneous toxicity effects of the selected compounds and they don't consider the potential adaptability of the biomasses to a toxic substrate after an adequate acclimatization time, however, the results obtained can give preliminary information about the effect of not controlled occurring inhibition on liquid wastewater treatment.

Materials and methods

Aerobic toxicity assay

This procedure is an adaption of the OECD nr. 209 method, experiments were carried out as a batch test in three 900 mL volume closed respirometers at 20 ± 2 °C, pH 6.5–8.5 (fig. 1 A). One respirometer was chosen as a control and in the others

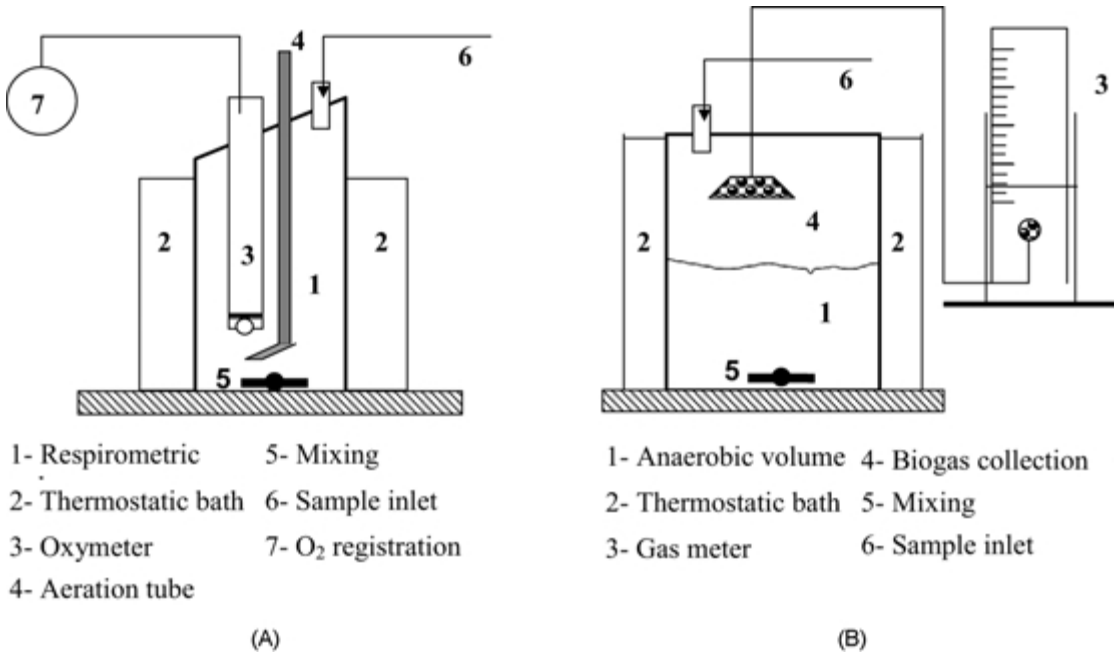


Fig. 1 – *Aerobic (A) and anaerobic (B) laboratory batch reactors used in inhibition tests*

a selected concentration of toxic compounds was added. The measurement of dissolved oxygen and OUR (Oxygen Uptake Rate) was achieved by three oxymeters (with temperature control), if required pH value was corrected by addition of HCl and NaOH solutions. The inoculated biomass was taken from the activated sludge reactor of the industrial wastewater treatment plant, object of the study.

All the respirometers were filled with 900 mg (as VSS: Volatile Suspended Solids) of the aerobic biomass diluted to 880 mL with tap water to obtain a concentration of about 1 g L^{-1} as VSS in the respirometric volume. Control was fed with 20 mL of a base substrate composed by only sodium acetate (20 g L^{-1} CH_3COONa solution), test respirometers were fed with an amount of base substrate and with little volume (1–3 mL) of concentrated toxic solution to reach the same 20 mL added volume and the designed starting toxic concentration in the respirometer. After the first 30 minutes of aeration, to guarantee the attainment of stable conditions in the respirometer, the aeration was stopped and the substrate-test solution was added. The comparison of the control and the test OUR measurement, during the following 15 min, gives the instantaneous inhibition effect of the toxic on the biomass.

Given the respiration rate of the biomass as the slope of the dissolved oxygen measurement on time, it is possible to establish, for all the tested substances, inhibition effect (in %) of the respiration rate occurring in the control, so:

$$I = \left(1 - \frac{R_t}{R_c}\right) \cdot 100 \qquad (1)$$

Anaerobic toxicity assay

Experiments were carried out as a batch test at 37 °C. Working volume of bottles was 1 L 50 % filled. For the measuring of biogas production, during the designed one day reaction, a calibrated cylinder was used (fig. 1B). The biogas was bubbled through a solution of NaOH for entrapment of CO_2 and H_2S. The substrate concentration of COD 2 g L^{-1} was used. The substrate contains sodium acetate, d-glucose and nutrients (nitrogen and phosphorus). 1.0 g L^{-1} of $NaHCO_3$ was added to each test for keeping pH about 7. One mL solution of trace element per liter for each test was also added, this solution contained γ/mg L^{-1}: $FeCl_3 \cdot 4H_2O$ (2000), $CoCl_2 \cdot 6H_2O$ (2000), $MnCl_2 \cdot 4H_2O$ (500), $CuCl_2 \cdot 2H_2O$ (30), $ZnCl_2$ (50), $NiCl_2 \cdot 6H_2O$ (30), H_3BO_3 (50), $(NH_4)\ Mo_2O_7 \cdot 2H_2O$ (90), $Na_2SeO_3 \cdot H_2O$ (100), EDTA (1000), and HCl 12 mmol L^{-1}. The sludge from an upflow anaerobic reactor treating industrial wastewater was used as inoculum. This reactor runs with an anaerobic biomass which in origin was the activated sludge biomass of the industrial treatment plant mentioned above, subsequently acclimated in the anaerobic upflow conditions for about one year. VSS values were in all tests 24 – 30 g L^{-1}. The environmental conditions for each test were the same (temperature, reaction time, base substrate fed, VSS in biomass, pH, amount of nutrients).

The anaerobic activity can be calculated from the methane production rate (dV_{CH4}/dt) or from the substrate degradation rate ($d\gamma/dt$), and be expressed also as grams od COD for each gram of VSS per day or grams of COD_{CH4} for each gram of VSS per day. The degree of inhibition was calculated comparing methane production in the control batch reactor fed with only substrate with the one in the batch test fed with substrate and toxic solution.

A simple method for the determination of methanogenic and nonmethanogenic activity was followed,[9] while calculations and activity expression are presented as reported by *Soto* et al.[16] If the activity of methane in anaerobic process is expressed by the following expression,

$$a_{CH_4} = (dV_{CH_4}/dt)/(\gamma_{X_0} \cdot V_R X_1) \qquad (2)$$

the degree of inhibition can be calculated from the subsequent definition:

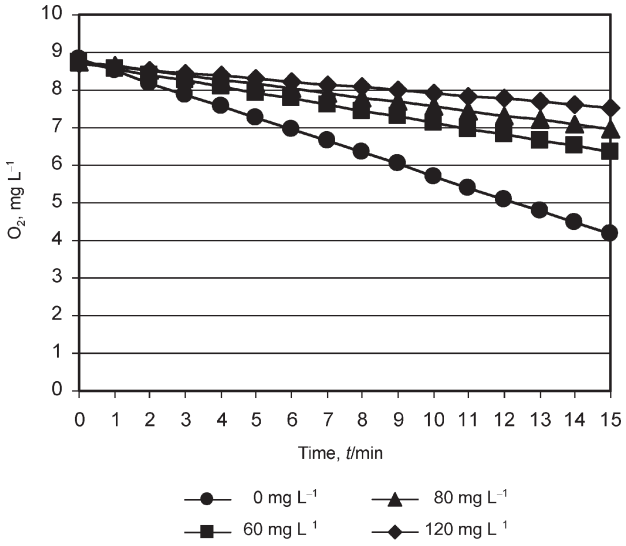
$$I = \left(1 - \frac{a_1}{a_0}\right) \cdot 100 \qquad (3)$$

The determination of all parameters used in the experimental session was carried out as proposed by *Standard methods.*[17]

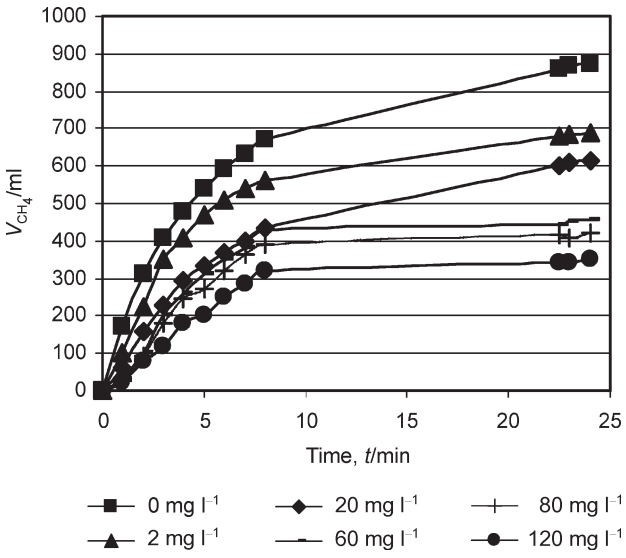
Results and discussion

As a preliminary experiment, the aerobic and anaerobic biomasses inhibition tests were conducted utilizing increasing concentrations (from 2 to 120 mg L^{-1}) of sodium ipochlorite (NaOCl), with this experimental assay, the whole response on inhibition stimulation was verified. Fig. 2 illustrates the respirogram obtained in the aerobic test and the fig. 3 shows the course of methanogenic test during anaerobic prove.

In respirometric tests the rate of oxygen uptake in endogenous condition of biomass reveals how the biomass works and if some compound at a definite concentration interferes with its equilibrated metabolism. In the same way, since methane is major final product of anaerobic digestion process, biogas production is a useful indicator in monitoring an anaerobic process suffering from any toxicant.



F i g . 2 – *Endogenous respirometric behavior during NaOCl tests*



F i g . 3 – *Cumulative methane production during NaOCl tests*

In table 2 results inhibition tests performed on aerobic and anaerobic biomasses are depicted. In the first column the chemical formula of the inhibitor is shown, in the second the concentration used in the tests as mg per liter of useful volume of the batch reactors, and in the third and fourth column the percent inhibition degree of the corresponding aerobic and anaerobic tests are indicated, respectively.

Note that predominantly all the substances at the concentrations used don't give particularly heavy inhibition on, either, the aerobic and anaerobic biomasses tested, further values under 50 % inhibition are considered mild and likely recovered by a suitable biomass acclimation.

T a b l e 2 – *Results of the inhibition tests*

Compound tested	Cconcentration γ/mg L^{-1}	Inhibition degree aerobic test I/%	Inhibition degree anaerobic test I/%
Cr^{3+}	2.6	2.7	6.9
	5.2	9.3	9.5
	56.0	11.7	16.4
	84.0	21.4	20.2
$Fe^{3}+$	10.0	3.1	7.8
	20.0	11.6	11.1
BO_3^- (as B)	4.0	12.5	1.1
	8.0	26.2	13.2
	12.0	28.4	18.1
	24.0	44.4	28.6
Cl^-	2890.0	9.8	11.3
	4760.0	25.3	23.4
	9400.0	54.7	33.9
	17400.0	76.8	55.6
	37500.0	89.5	75.7
NO_2^- (as N)	1.0	4.4	4.8
	2.0	5.2	10.1
NO_3^- (as N)	10.0	0	2.7
	20.0	0	6.4
C_2HCl_3	1.2	0	14.5
	12.0	2.6	32.2

Fe^{3+} and NO_2^- concentrations used in tests induce similar little inhibition on, both, the aerobic and anaerobic biomass, NO_3^- seems to produce no effect on aerobic biomass at the concentrations assayed, and only a minor degree of inhibition is measured on anaerobic biomass. The inhibition effect of Cr^{3+} is somewhat evident on both biomasses.

The most significant inhibition effects are given by the concentrations of Cl^- and C_2HCl_3 used in the tests, in particular the inhibition results to be induced by the highest concentrations of Cl^- added in, both, aerobic and anaerobic tests. Cl^- appear to compromise biomasses performance. The presence of high concentrations of Cl^- represent an important inhibition problem during wastewater treatment of industrial waste, this is related to the fact that high salt concentrations can cause plasmolysis and resulting activity inhibition of cells.

Another consideration must be reserved to evidence regarding the tests with C_2HCl_3. A more evident inhibition degree on anaerobic biomass with

respect to the aerobic one appears from the results obtained, but in respirometric tests a lot of substance is expected to strip out with an important decreasing of the trichloroetylene concentration in the batch reactor.

Conclusion

Two simple laboratory tests were carried out to assay if some considered concentrations of potentially toxic substances, found in an industrial liquid waste could give inhibitory effects on aerobic and anaerobic biomasses which are employed in reactors of a wastewater treatment plant.

Results show similar inhibition in aerobic and anaerobic biomasses tested:

– A minor inhibition under 10 % appears in the aerobic and anaerobic tests with all considered concentrations of NO_2^- and NO_3^-, further Fe^{3+} and Cr^{3+} at lower concentrations also present similar behaviour.

– High Cr^{3+} concentrations cause most measurable effects on both aerobic and anaerobic biomasses, while BO_3^- seems to have generally higher inhibition potentials and influences with high intensity aerobic treatment. As well, the highest concentration of C_2HCl_3 tested showed an evident inhibition on anaerobic biomass. These performances must be considered in the evaluation of the most suitable treatment of wastewater containing such concentrations of inhibitory compounds.

– The highest concentrations tested of the Cl^- causes a 70 % inhibition on aerobic biomass and reaches 85 % on anaerobic biomass. For this reason, particular attention must be taken in the presence of high concentration of chlorides coming from salinity of liquid waste of concern.

– Further experiments must be carried out, regarding the combination of two or more inhibitory substances, before taking a final decision about what kind of the existing biological processes adapt more to the treatment of industrial liquid wastes.

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List of symbols

COD_S – soluble chemical oxygen demand, mg L^{-1}

COD_{CH4} – chemical oxygen demand value of methane, mg L^{-1}

OUR – oxygen uptake rate, mg L^{-1} min^{-1}

VSS – volatile suspended solids, mg L^{-1}

I – degree of inhibition, %

R_t – respiration rate for the test respirometer, mg L^{-1} min^{-1}

R_c – respiration rate for the control respirometer, mg L^{-1} min^{-1}

dV_{CH4}/dt – methane production rate, L d^{-1}

$d\gamma_S/dt$ – substrate degradation rate, g L^{-1} d^{-1}

t – time, d

V_{CH4} – cumulative methane production, L

V_R – useful volume of the reactor, L

X_1 – conversion factor which represents the COD value of the unit of methane volume (this factor depends on the temperature and moisture of gas), dimensionless

γ_{X0} – initial biomass concentration, mg L^{-1}

a_0 – activity for control test without inhibitor agent, L g^{-1} d^{-1}

a_1 – activity for test with inhibitor and base substrate, L g^{-1} d^{-1}

γ – mass concentration

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