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THE USE OF RESPIROMETRY FOR THE EVALUATION OF AN INDUSTRIAL WASTEWATER TOXICITY OVER ACTIVATED SLUDGE

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ABSTRACT

The discharge of industrial wastewaters with toxic substances in wastewater treatment plants may cause relevant inhibition of microbiological metabolism. A number of toxicity tests available mostly focus on ecological impacts on water recipients by using macro invertebrates as bio-indicators. However, toxic effects occurring in wastewater treatment plants cannot be properly addressed with this approach. A promising method that has been used to investigate microbiological inhibition caused by industrial wastewaters is the respirometric test. As part of a treatability study, the present investigation aimed to assess the toxic effects of an industrial wastewater generated in a wood-floor industry, located in Nybro, Sweden over activated sludge microorganisms. The wastewater results from washing/cleaning of machineries that continuously apply urea-formaldehyde resins on wood particle boards. The respirometric method was able in a short time to assess both the inhibition and the stimulation of microbial metabolism caused by this particular tested effluent. Metabolic inhibition was positively correlated with dilution factors and formaldehyde concentrations within the aqueous phase. Whereas 1,989.4 mg L^{-1} of formaldehyde caused 50% of metabolic inhibition, concentrations below 156 mg L^{-1} did stimulate it. High EC_{50} values suggest that in the presence of other compounds, antagonistic processes are taking place, reducing toxic effects of formaldehyde. Finally it was concluded that toxicity tests carried out with single substances in synthetic waters have limited value if the ultimate goal is to develop real wastewater treatment systems. It is important to highlight that formaldehyde was here used as an indicator and the correlation between inhibition of respiration and formaldehyde concentration is actually reflecting the effect of not only this particular substance but the complex mixture of substances presented in the glue wastewater.

KEYWORDS

Toxicity, Respirometry, Formaldehyde, Activated Sludge, Industrial Wastewater.

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1. INTRODUCTION

Adequate knowledge of the negative effects of toxins and inhibitors in any biological wastewater treatment system is essential for an optimal application of the processes [1]. However, although toxic and microbial inhibitory compounds are usually found in industrial wastewater [2], most efforts carried out to date have concentrated on the toxic impacts of direct discharges into the natural water environment [3]. Moreover, toxic effects over microbial metabolism in wastewater treatment plants have traditionally been addressed for individual-isolated substances [3,4]. In reality, effluents, particularly industrial ones, are complex mixtures of organic and inorganic compounds with specific interactions among them. Assessing the properties and effects of isolated compounds has limited merits, considering the possibility of either synergistic or antagonistic effects. Whereas antagonism is the condition in which the toxic effects caused by one compound are reduced by the presence of a second one, synergism, is the condition where the toxic effects caused by the original compound are increased by the presence of a second compound in the system [1].

The present investigation aimed to evaluate the toxic effects of a real industrial wastewater over microbial populations found in the activated sludge of a wastewater treatment plant. Although, the correlation between formaldehyde concentrations and toxicity was the main focus, the "whole effluent approach" suggested by [4] was followed. By testing a real effluent generated by a wood floor industry and observing the result of the interactive processes occurring among different compounds, the toxicity could be assessed as close as possible to the real scenario. Toxicity data related to formaldehyde as a single compound - widely reported in the literature - was here used in order to compare the isolate toxicity effects with the effects on the presence of different compounds.

2. MATERIAL AND METHODS

Industrial effluent (glue wastewater): The wastewater studied is generated during procedures of cleaning/ washing of machineries that continuously apply urea-formaldehyde resins on wood particle boards, at the wood floor industry AB Gustaf Kähr located in Nybro, Sweden. Samples of the studied effluent were taken just after the cleaning procedure and sent immediately to the laboratory. Formaldehyde was analyzed with the VDI Vorschrift method.

Activated sludge: The activated sludge used for these experiments was taken from Kalmar Municipal Wastewater Treatment Plant, Kalmar, Sweden. The mixed liquor volatile suspended solids - MLVSS ranged between 3.98-4.2 g L^{-1}.

Synthetic medium: In each experimental batch, 1 L of activated sludge was spiked with a synthetic medium according to Table 1.

Table 1: Synthetic medium used for respirometric test in each 100 mL of activated sludge.

	Component	mg 100 mL^{-1}
Nutrients	NH_4Cl	160
	$CaCl_2*2H_20$	22.5
	$MgCl_2*6H_20$	30
	$FeCl_3*4H_2O$	6
	$C_6H_{12}O_6$	2000
Buffer solution	KH_2PO_4	81
	K_2HPO_4	113
Trace elements	$MnC_{12}*4H_2O$	0.15
	H_3BO_3	0.015
	$ZnCl_2$	0.015
	$CuCl_2$	0.009
	$Na_2MoO_4*2H_2O$	0.003
	$CoCl*6H_2O$	0.15
	$NiCl_2*6H_2O$	0.015
	Na_2SeO_3	0.015

Respirometry: A toxicity test based on respirometric method was set-up on the basis of the established OECD Method 209 [5], which provides a screening procedure to indicate suitable non-inhibitory concentrations to be used in biodegradability tests. The original procedure established in the method was slightly modified, since the method is not appropriate for testing VOCs due losses that occurs as a consequence of continuous aeration on the test beacker.

Experimental Procedures: Each batch of respirometry was conducted in three different 600 ml-beacker as described below:

- **Control 1 and Control 2:** (synthetic medium + 100 mL activated sludge + 150 mL distilled water);
- **Test:** (synthetic medium + 100 mL activated sludge + 150 mL wastewater with distilled water in the following dilution factors: 1:100; 1:50; 1:25; 1:10; 1:5; 1:2.5 and no dilution).

After filling the beackers, aeration with pressurized air for 40 min was carried out. To reduce the formaldehyde volatilization at the test beackers, the addition of the effluent in different dilutions took place immediately after the aeration. Finished the aeration and the effluent addition, the liquids were carefully and rapidly transferred into BOD flasks kept in 30°C in a water bath in order to measure the respiration rate. The dissolved oxygen-DO was measured and computed with a Digital Oxymeter WTW Multi 340i in each minute during 15-25 min, depending on the time that either the DO was close to its complete depletion or that the oxygen uptake rate-OUR has reached steady conditions. The respirometric test using BOD bottles (narrow bottle neck) allowed considering negligible the oxygen transfer between the external ambient and the liquid phase, being possible to estimate the respiration rate uniquely by using the dissolved oxygen variation within the flask.

The inhibitory effect of the tested effluent (percentage of inhibition) at a particular concentration is expressed as a percentage of the mean respiration rates of the two controls (OECD, 1984). It is calculated as:

$$\% \text{ Inhibition} = 1 - \left[\left(\frac{2Rs}{(Rc1 + Rc2)} \right) * 100 \right] \qquad \text{Eq (1)}$$

Where:
Rs = Oxygen consumption rate of tested effluent in the different concentrations (mg L^{-1} h^{-1});
Rc_1 = Oxygen – consumption rate of Control 1 (mg O_2 L^{-1} h^{-1});
Rc_2 = Oxygen- Consumption Rate of Control 2 (mg O_2 L^{-1} h^{-1});

Concentrations in which the percentage inhibition showed 20%, 50% and 90% (EC_{20}, EC_{50} and EC_{90}) were derived by plotting % inhibition against Logarithm of Concentration.

3. RESULTS

Curves of DO concentration and OUR in time for glue - Plots of DO against time in seven different dilution factors (1:100, 1:50, 1:25, 1:10, 1:5, 1:2.5 and with no dilution) are presented in Figure 1 and Figure 2. As expected, the DO decreases down to a level considered stable. The lower the dilution is, longer is the time required to reach this condition. Whereas only 9 min were needed to reach a well established plateau when diluted 100 times (Figure 1a and Figure 2b), in 25 min of experiment no stable condition was reached in the pure effluent – no dilution (Figure 2a and Figure 2b). This behavior is well correlated with the toxic effects over the microbial metabolism, which is reasonably higher in more concentrated wastewater.

The curves of oxygen uptake rate-OUR against time are shown in Figure 3 and Figure 4. Reasonably, dilution and OUR are positively correlated. Comparing the controls and the tested effluent in all dilution factors, it is noticed that the peak of OUR of the second shifts in steps. Interestingly, the peaks of OUR in the dilution factors of 1:100 and 1:50 are higher when compared with the respective controls (Figure 3a and Figure 3b). This result suggests the presence of compounds that in this particular condition stimulate microbial metabolism, as long as the dilution factor is kept between 50 and 100 times. The peak of OUR is considerably lower when testing the pure effluent (Figure 4c). According to Figure 4d, OUR peaks vary both in x-axis (time) and y-axis (maximum oxygen up-take rate), being clear that the dilution factor plays an important role and some peaks come in a shorter period and in lower values than others. In the batch respirometry with 100, 50, 25 and 10 times of dilution, the respiration rate achieved peaks of 38.4, 40.2 and 29.4 and 23.4 mg L^{-1} $hour^{-1}$ in 4, 5, 6 and 7 minutes respectively. An interesting fact is that the shortest period to achieve the oxygen consumption peak is observed when testing the pure effluent; but on the other hand, this peak presents a rapid decrease once it is reached (Figure 4d). This might be correlated with the toxicity posed by the pure effluent.

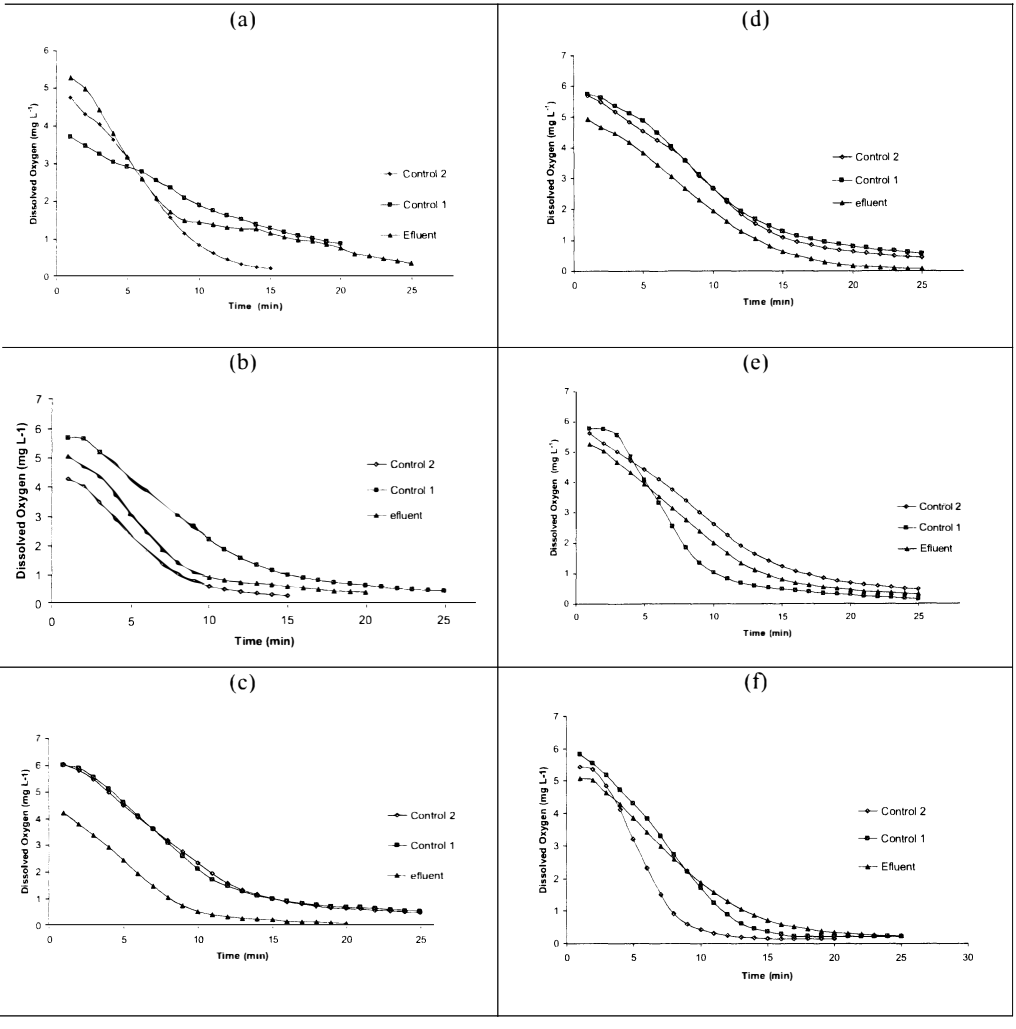

Figure 1: DO concentration along the toxicity experiment: (a) dilution 1:100; (b) dilution 1:50; (c) dilution 1:25; (d) dilution 1:10; (e) dilution 1:5; (f) dilution 1:2.5.

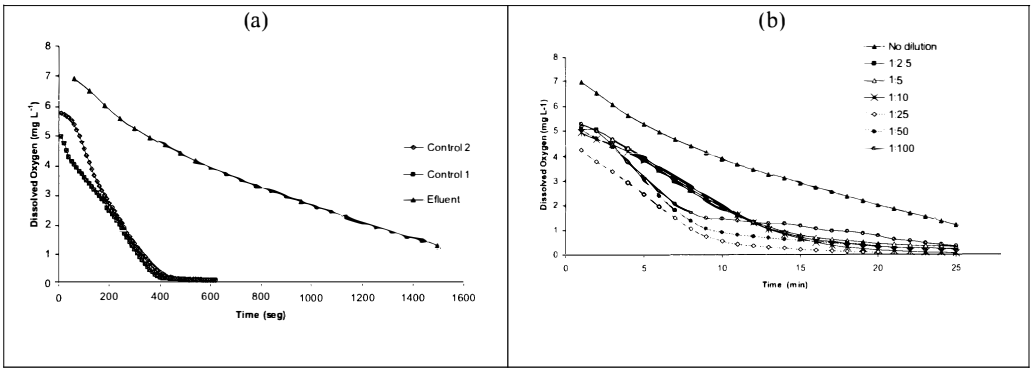

Figure 2: Dissolved Oxygen concentration along the toxicity experiment. (a) Dilution 1:1; (b) Test effluent in all dilution factors

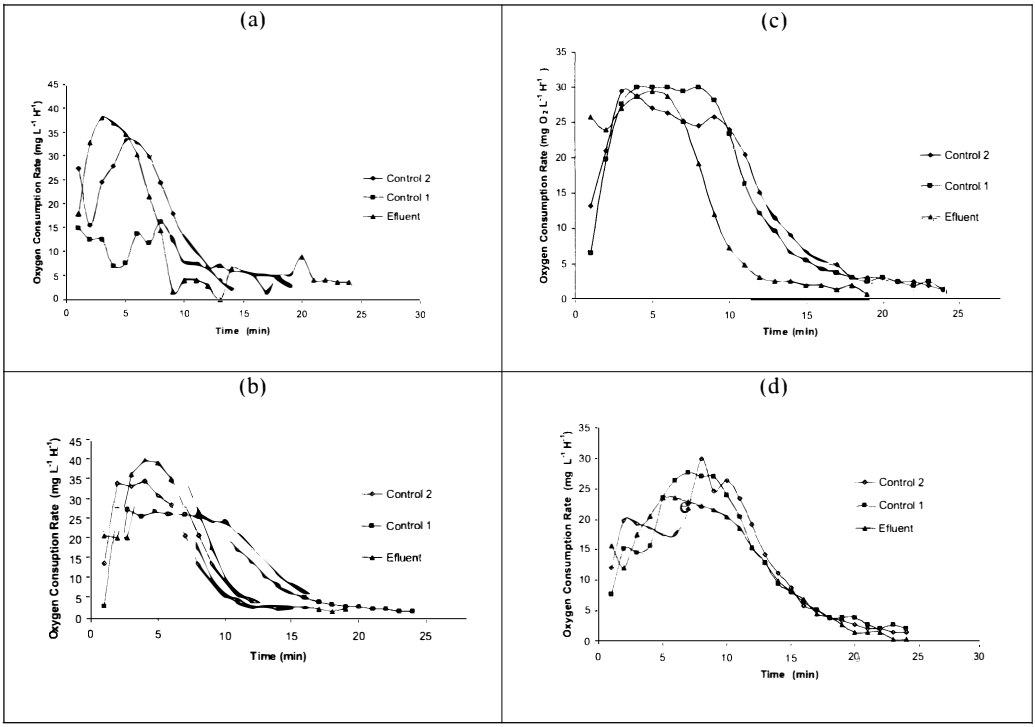

Figure 3: Oxygen Up-Take Rate along the toxicity experiment. (a) Dilution 1:100; (b) dilution 1:50; (c) dilution 1:25; (d) dilution 1:10

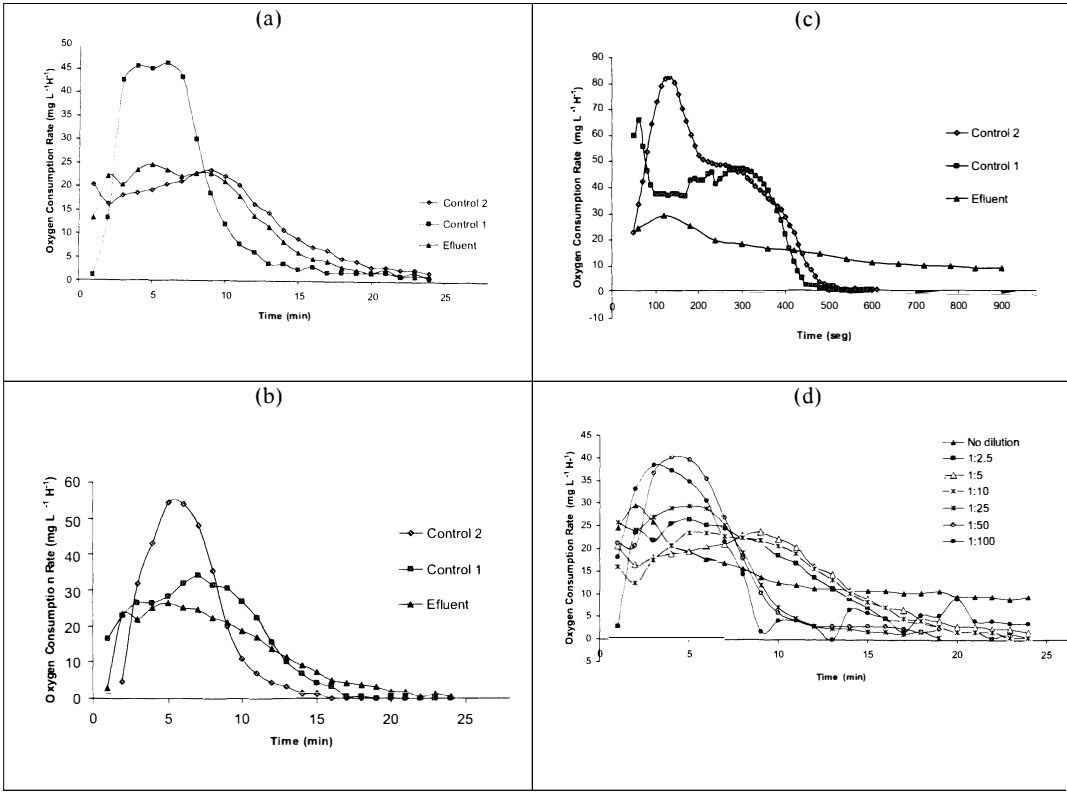

Figure 4: Oxygen Up-Take Rate along the toxicity experiment: (a) dilution 1:5; (b) dilution 1:2.5; (c) Dilution 1: 1; (d) Test effluent in all dilution factors

Inhibition of respiration as a function of formaldehyde concentration: Table 2 and Figure 5 show the respiration inhibition as a function of formaldehyde concentration in the glue wastewater. The equation obtained ($R^2 = 0.95$) was used to estimate the values of EC_{20} (20 % of inhibition), EC_{50} (50 % of inhibition) and EC_{90} (90 % of inhibition), respectively 587.5 mg L^{-1}, 1,989 mg L^{-1} and 10,194 mg L^{-1}. The high value of EC_{50} found in this study, when compared with available data in the literature, suggests antagonistic interactions with favorable outcomes for the microbial metabolism in this particular case. [2] observed 50 % of activated sludge respiration inhibition at 62.7 mg L^{-1} similar to [4] which observed EC_{50} of 57 mg L^{-1} of formaldehyde in its pure form in both cases In another investigation [4] reported formaldehyde toxic effects at the same concentrations as previously tested were much more significant in the presence of other compounds, such as phenols, etc. This result might be related with either synergistic interactions or toxicity due the other compounds, instead of formaldehyde.

Whereas [6] have reported an EC_{50} of 150 mg L^{-1} of formaldehyde that was present in wastewater generated during the wood-glue manufacturing, in the current investigation the inhibition started to take place only when the formaldehyde was over 156 mg L^{-1} (Table 2). In formaldehyde concentrations below these values, a stimulation of microorganism's respiration is observed (negative values of inhibition in Figure 5). This stimulation might be correlated with existing compounds in the tested effluent functioning as additional sources of carbon (hydrocarbons), as well as nutrients (urea originated from the glue resin), that up to a certain concentration stimulate enzymatic activities instead of causing toxicity (Figure 3a and 3b). According to Table 2, an alternative interpretation is that apart from the formaldehyde concentrations and its effects, other compounds may be responsible for respiration inhibition, starting when the dilutions are reduced, in this particular case down to ≤ 25 times.

Table 2. Inhibition of respiration as a function of formaldehyde concentration.

Dilution Factor	Formaldehyde (mg L^{-1})	Inhibition (%)
100	39	- 60
50	78	- 30
25	156	1
10	390	17
5	780	32
2.5	1560	40
no dilution	3900	59

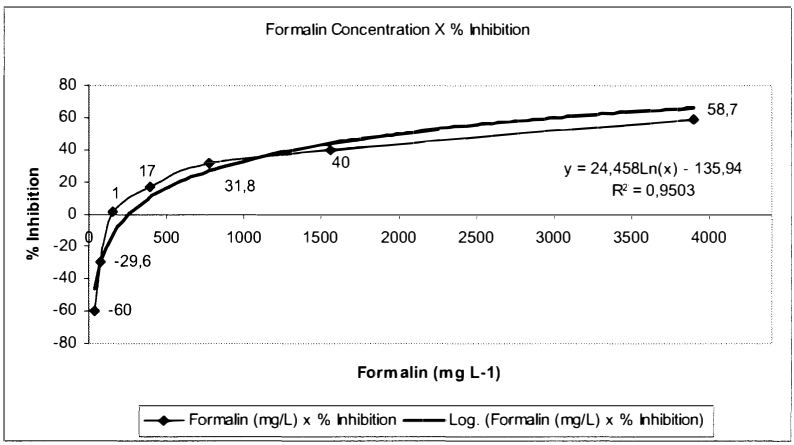

Figure 5: Inhibition of respiration as a function of formaldehyde in glue wastewater.

4. CONCLUSIONS

Toxicity tests carried out with single substances have limited value, if the ultimate goal is to develop real wastewater treatment systems. The interaction of different compounds in real complex effluents can either increase or reduce the toxic effect of a specific substance,

condition suggested by the results in the present study. It also explains different values of formaldehyde toxicity in the literature. Since formaldehyde was here taken as an indicator, the correlation between inhibition of respiration and formaldehyde concentration is actually reflecting the effect of not only this particular substance but the complex mixture of substances presented in the glue wastewater. The comparison with formaldehyde toxicity data available in literature tested in its pure form raise some questions regarding different effects that may be observed when its pure form is compared to its effects in the presence of other compounds. The new tests are expected to sort out aspects regarding the effects of formaldehyde in both conditions (pure and real effluent), and to contribute to better understanding of the nature of interactive processes on toxicity effects.

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