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Direct Toxicity Assessment of Pollutants to Activated Sludge.

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ABSTRACT

In this contribution, modification of the conventional activated sludge respiration toxicity test into a fast and applicable noxiousness test has been performed. The real need to evaluate the harmful effects of industrial wastewater effluents before discharge into the sewerage system pushed forward to develop a modified, easy, cheap and short-term toxicity or noxiousness test. The modified noxiousness test is based on activated sludge respiration activity where the activated sludge is classified and employed directly without processing. Sludge with a dry matter content of more than 3 g/L gives better results. The results indicated that the modified tests were found compatible with the conventional respiration activity for synthetic and real wastewaters, an indication of possible application of the modified test in lieu of the conventional one. Noxiousness tests results carried out using activated sludge collected from two different municipal wastewater treatment plants in Egypt, were found to be comparable, thus enabling possible generalization of the results. A toxicity indicator (noxiousness number) has been developed based on IC50 for toxicity tests. This NOX can be used for comparison of toxicity levels between various wastewaters. The modified procedure was carried out without sludge conditioning in one-step.

Keywords: discharge, industrial wastewater, respiration activity test, noxiousness, noxiousness number

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INTRODUCTION

It is a common practice to design wastewater treatment plants for treating domestic wastewater, which should have no effect on the microorganisms involved in the biological treatment process [1]. Treatment plants have to handle the unexpected pollution added to its input stream. Municipal wastewater treatment plants are, generally, used for treating the majority of industrial wastewaters before being discharged to the receiving water environment. Regulations specify the minimum requirements of treated effluent; therefore, a biological treatment stage is required to ensure a high standard of clarification. This stage is highly sensitive to discharges of toxic industrial wastewater [2 and 3]. The harmful effect on the operation of the biological treatment stage reduces the quality of the treated wastewater discharged to the receiving surface or ground water. In the activated sludge (AS) process, the microorganisms involved are including prokaryotic and eukaryotic types. To assess the toxicity of industrial wastewater discharged to sewer, microbial testing systems are developed in order to protect the AS process. Factually, not all microorganisms respond to all toxic substances released to sewer in the same way [4 and 5]. Some widely used chemicals, are bio-degraded by AS microorganisms and some harmful intermediates are formed which affect other living organisms [6].

Monitoring the toxicity of complex industrial wastewater entering municipal wastewater treatment plants using bioassays could provide an early warning system to detect waters likely to reduce the efficiency of the plants [7]. There is a difference between the term “noxiousness” and the term “toxicity”, toxicity always refers to a specific test organism e.g. a special fish species, earthworm species [8 and 9], daphnia species [10 and 11], luminous bacteria [12], algae species [13], Lecane inermis rotifers [14] or special animal tissue [15]. Therefore, a toxicity test can only assess a negative effect on a very narrow section of the biosphere. Moreover, tests need a great effort expended on preparing and cultivating test organisms. This opposes the practical application of these tests in wastewater treatment. But noxiousness test has a broader meaning which refers to a multitude of mixture of different species which live together, it is a feature shared by all of the species within the tested habitat and which can be more or less influenced by different toxic substances. The result of noxiousness test is a summary parameter which is characteristic for the tested activated sludge heteropopulation. This is why municipal activated sludge which is a complex heteropopulation of aerobic bacteria is utilized in several activated sludge tests such as the activated sludge short-term respiration test [16], the activated sludge respiration inhibition test [17] or the activated sludge growth inhibitory test [18].

Activated sludge respiration is also used in continuously-operating “toximetres”, first developed and applied by the company BASF, Germany, in order to protect industrial sewage treatment plants from noxious influent shocks [19 and 20]. A toximetre must be installed within an influent bypass, to have enough time to deflect the influent toxic shock into a storage basin. These toximetres are fairly sophisticated mini-sewage treatment plants and they need good, round-the-clock maintenance. Moreover the research group of Strothmann represented a test system for monitoring the operation of wastewater treatment plants [21].The main objective of this study aims to transform the conventional activated sludge respiration toxicity test into a fast and applicable noxiousness test. The used activated sludge was collected from two different municipal wastewater treatment plants in Zeneen, Giza, Egypt (ZMWWTP) and Al Jabal al Asfar, Qalyubiyah, Egypt (JMWWTP).

MATERIALS AND METHODS

The toxicity bioassay used in this study is the conventional activated sludge respiration test [13] which is a relatively long procedure that requires nearly half a day for sludge conditioning using large centrifuge that may not be available in many laboratories at factories. In order to create a rapid and practicable noxiousness test, the laborious conventional test procedure was simplified and a modified test procedure without sludge conditioning was performed. Figure (1) illustrates equipments and the principles of sludge respiration activity measurement. The used activated sludge was collected from two different municipal wastewater treatment plants in Zeneen, Giza, Egypt (ZMWWTP) and Al Jabal al Asfar, Qalyubiyah, Egypt (JMWWTP). The sludge was aerated during transportation (within 1-2 h) to the laboratory using aeration pumps. Analysis of samples occurred within approximately 4 hours of collection. Similar to sludge collection real industrial wastewater collected from selective factories in Egypt, synthetic and real wastewater were employed in the conventional and modified test procedure. The synthetic wastewater including; sodium chloride, nitrilotriacetic acid, hexamethylenetetramine, chloramine T, tetrachloroethylene, di-Na-EDTA, phenol, thymol, 2, 4-dinitrotoluene, 2-nitrophenol, p-chlorophenol, 2, 4-dichlorophenol, aniline, 4-chloroaniline were prepared in the laboratory. Samples from industrial origin wastewater were collected from; groundwater from a contaminated site, paper mill, advanced oxidation process (AOP) treated effluent, slaughter, drainage water, pulp unit, leachate from a hazardous waste landfill, pesticide production, textile industry, rinsing water from plating industry, meat industry, pharmaceutical industry, crystal and glass industry, paint industry, and metal industry.

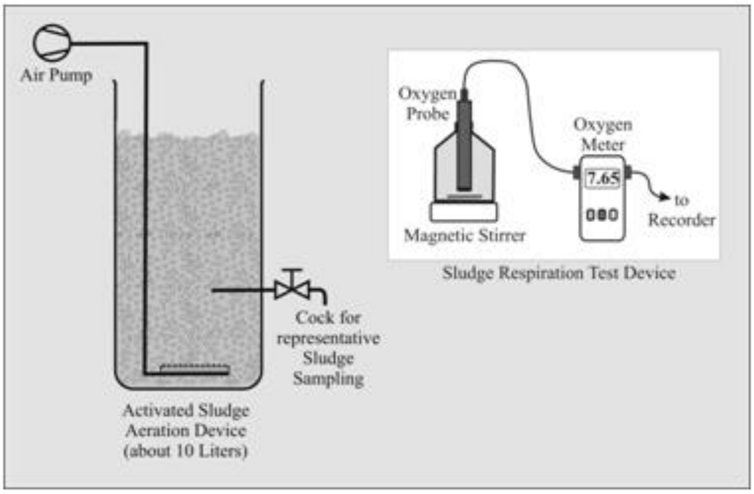

Figure 1: Basic graph of activated sludge respiration activity measurement set

The conventional sludge respiration activity

Sludge conditioning was performed as follows: 10 liters activated sludge sample was collected from a properly working municipal sewage treatment plant and coarse particles using an appropriate strainer were removed. Centrifugation (10 minutes at 10,000 m/s^2) was carried out and the supernatant discarded. Sludge concentrate was re-suspended using chlorine-free tap water and centrifugation and re-suspension steps were repeated, followed by aeration of sludge using an appropriate aeration device and the addition of antifoam (silicone oil emulsion) as necessary. Sludge is made ready for testing while being under continuous aeration and it can be used for about 24 hours. This time can be prolonged for another 24 hours if sludge is fed with about 50 ml of nutrient solution {distilled water (1 liter) + peptone (16 g) + meat extract (11 g) + urea (3 g) + sodium chloride (0.7 g) + calcium chloride dihydrate (0.4 g) + magnesium sulphate heptahydrate (0.2 g) + waterless di-potassium hydrogen phosphate (2.3 g), pH value adjusted to about 7} per liter of sludge. Subsequently, the conventional respiration activity method [13] measurement procedure was employed. Test wastewater was adjusted to a pH of 7 ± 0.5 and a temperature of 22 ± 2 °C. Eight batches with activated sludge, nutrient solution, distilled water and test wastewater, plus one blank (distilled water added instead of wastewater) according to the conventional procedure were prepared (Table 1). Aeration of each batch for 3 hours and the addition of anti-foam agent as necessary were carried out, followed by filling each batch into a 300 ml BOD bottle and agitation using a magnetic stirrer. Oxygen electrode was inserted into a bottle with spillover and without remaining air bubbles. Oxygen concentration was measured every minute and oxygen depletion curve was analyzed. The described measurement steps were repeated three times. The measurement time for the conventional method amounts to 10 minutes.

Table 1: Preparation of batches according to the conventional method

Aliquot in ml	Batches								
	Blank	1	2	3	4	5	6	7	8
Activated sludge	500	500	500	500	500	500	500	500	500
Nutrient solution	32	32	32	32	32	32	32	32	32
Test wastewater	0	0.5	5	10	50	100	200	300	468
Distilled water	468	467.5	463	458	418	368	268	168	0
Total	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000

The modified sludge respiration activity procedure

The modified procedure was carried out without sludge conditioning in one-step; activated sludge was collected from a properly-working municipal sewage treatment plant (10 liters), aerated and employed directly for the measurement procedure at pH of 7 ± 0.5 and temperature 22 ± 2 °C. Seven batches with activated sludge, nutrient solution {chlorine free tap-water (1 liter) + peptone (13 g) + ammonium chloride (11.5 g) + sodium dihydrogen phosphate (2,67 g)}, tap-water and test wastewater, plus a blank (chlorine-free tap water added instead of wastewater) were prepared (Table 2). Each batch was filled into a 300 ml BOD bottle and agitated using a magnetic stirrer. Oxygen electrode was inserted into a bottle with spill over and without remaining air bubbles. Oxygen concentration was measured every minute and oxygen depletion curve was analyzed three times and the test time for the modified method is 5 minutes. The modified test has several advantages over the conventional one as follows: omission of laborious sludge conditioning, centrifugation process is unnecessary, no need to use anti-foaming reagent during preparation of sludge or measuring procedure, utilization of sludge with higher dry matter content, shortening of sludge respiration activity measurement to 5 minutes, measuring of wastewater noxiousness in higher concentrations and tap water is used instead of the distilled water.

Table 2: Preparation of batches according to modified method

Aliquot in ml	Batches							
	Blank	1	2	3	4	5	6	7
Activated sludge	100	100	100	100	100	100	100	100
Nutrient solution	10	10	10	10	10	10	10	10
Test wastewater	0	0.1	0.5	5	10	50	100	190
Tap water	190	189.9	189.5	185	180	130	90	0
Total	300	300	300	300	300	300	300	300

RESULTS AND DISSCUSSION

Comparison between conventional and modified noxiousness (toxicity) test procedure

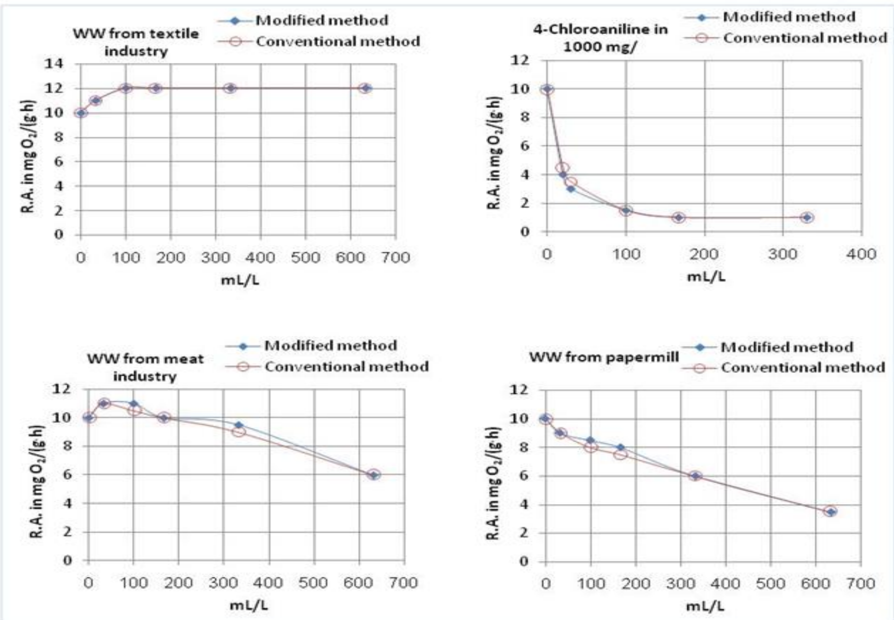

Figure 2: Comparison between conventional respiration activity methods and modified noxiousness test using different wastewaters

The two test methods were investigated using synthetic wastewater of 4-chlorophenol in 1000 mg/L, and industrial paper mill wastewater from deinking unit, from the thermomechanical pulper unit and wastewater from grinding mill as three different wastewater types. The results indicated in

Figure (2) represent the impact of the synthetic and real wastewater towards activated sludge collected from ZMWWTP applying the modified and conventional method. There is no relevant difference between the use of freshly-sampled activated sludge as per the modified method and conditioned sludge according to the conventional method. Therefore, the modified method simplifies the testing procedure and shortens test time considerably, that proves reliability of this modification. The modified test presents clear, graphically viable results for the noxiousness of a wastewater regardless of its type. The following investigations and experiments were performed for the modified test method:

Understanding the meaning of the slopes of modified sludge respiration activity curves

The modified sludge respiration activity curves and their meaning were investigated using different synthetic wastewater. Curves shown in Figure (3) have different slopes: Negative slope curves indicate noxious wastewater ingredients. Zero or positive slope curves indicate non-noxious wastewater ingredients. In some investigations; it should be noted that in case of increasing wastewater concentration the curve may invert to a negative slope due to the excess of dients.

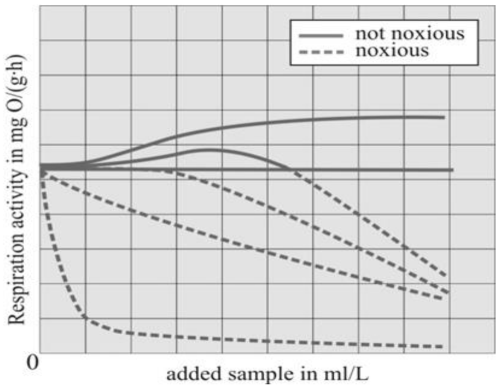

Figure 3: Rapid noxiousness test slopes of sludge respiration activity curves and their meaning

Effect of activated sludge dry matter content on the modified respiration activity test (noxiousness test)

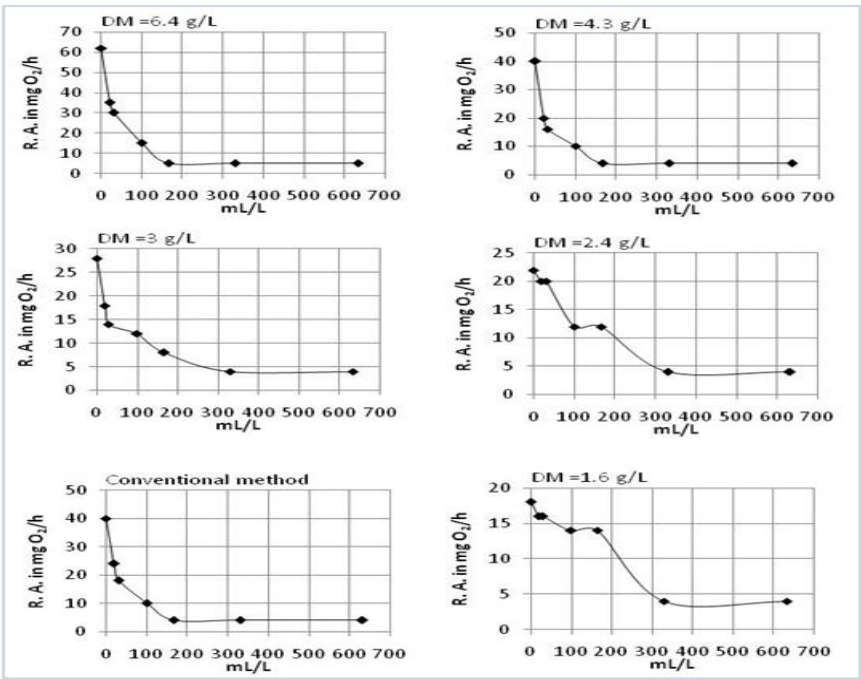

Figure 4: Influence of sludge dry matter content (from 1.6 g/L to 6.4 g/L) on sludge respiration activity, tested model wastewater is 2,4-dichlorophenol (1000 mg/L)

Figure (4) shows the results of six sludge respiration tests, using synthetic wastewater of 2, 4-dichlorophenol (1,000 mg/L). The graphs shows that sludge with dry matter content below 3 g/L do not result in clearly-shaped curves. Sludge with a dry matter content of more than 3 g/L gives better results that conclude that, the dry matter content of the sludge should be more than 3 g/L.

Noxiousness test of various synthetic and real wastewaters collected from ZMWWTP

Noxiousness test is done for some synthetic and real wastewaters, using activated sludge collected from ZMWWTP are shown in Figures (5-7). Figure (5) graphically represents noxiousness of different wastewaters. Each graph illustrates the concentration at which the wastewater becomes toxic, di-Na-EDTA (2000 mg/L) the start point to be toxic is reached in a concentration of 200 ml di-Na-EDTA/liter of total mixture, 2-nitrophenol and thymol have a high inhibitory toxic effect at their lowest concentrations but nitrilotriacetic acid has no inhibitory toxic effect at any concentration to the activated sludge. In addition, Figure (6) represents the impact of the table salt [1000 mg/L] towards the collected activated sludge. It is clear that it has no inhibitory toxic effect at any concentration to the activated sludge. Moreover, Figure (7) shows the high toxicity of wastewater collected from drainage water which has no inhibitory toxic effect at any concentration to the activated sludge, slaughter wastewater the start point to be toxic is reached in a concentration of 130 ml/L, wastewater collected from glass industry had a high inhibitory toxic effect at their lowest concentration at low concentration is not toxic but when reach 10 ml/L start to have a high inhibitory toxic effect.

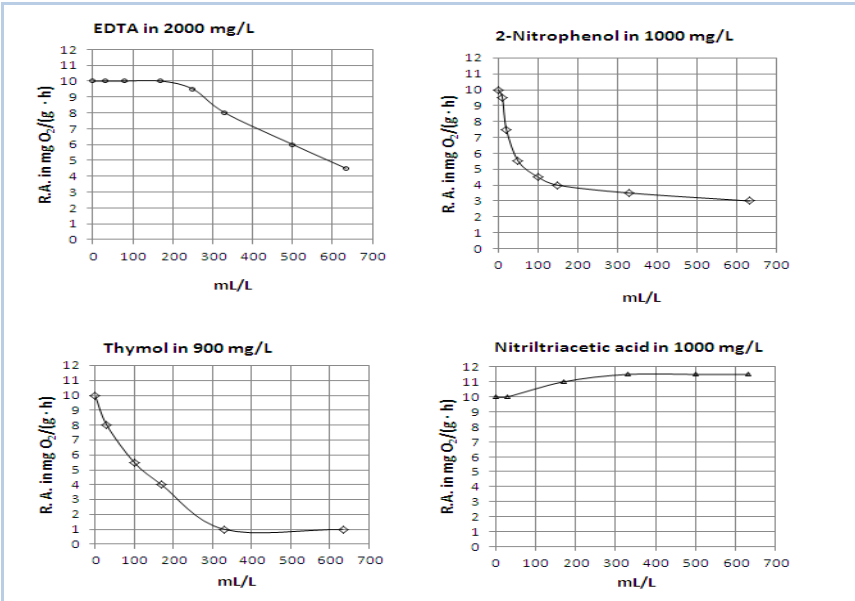

Figure 5: Sludge respiration activity curves of different model wastewaters, showing their noxious properties using activated sludge collected from (ZMWWTP)

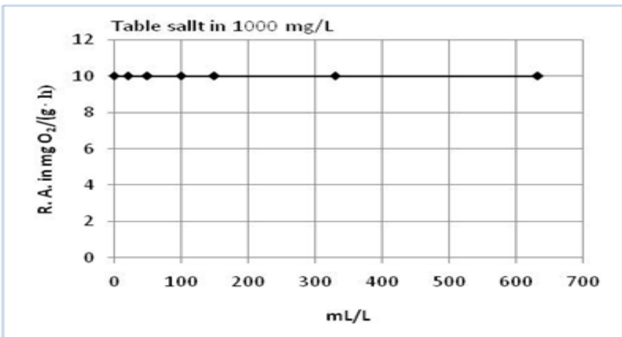

Figure 6: Sludge respiration activity curve of sodium chloride in 1,000 mg/L, showing its noxious property using activated sludge collected from (ZMWWTP)and (JMWWTP)

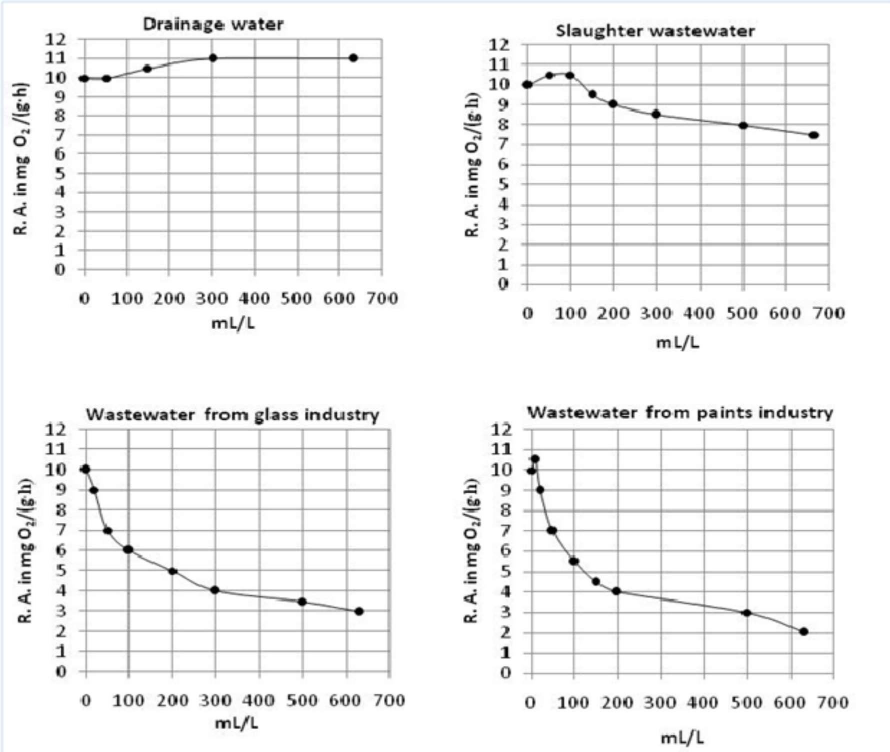

Figure 7: Sludge respiration activity curves of different real wastewaters, showing their noxious properties using activated sludge collected from (ZMWWTP)

Application of the modified toxicity test using activated sludge collected from JMWWTP

Noxiousness test is carried out for the same synthetic wastewater used with activated sludge collected from ZMWWTP was carried out for the activated sludge collected from JMWWTP. Nearly the same results have been obtained as shown in Figures (6, 8 and 9), that could generalize the test.

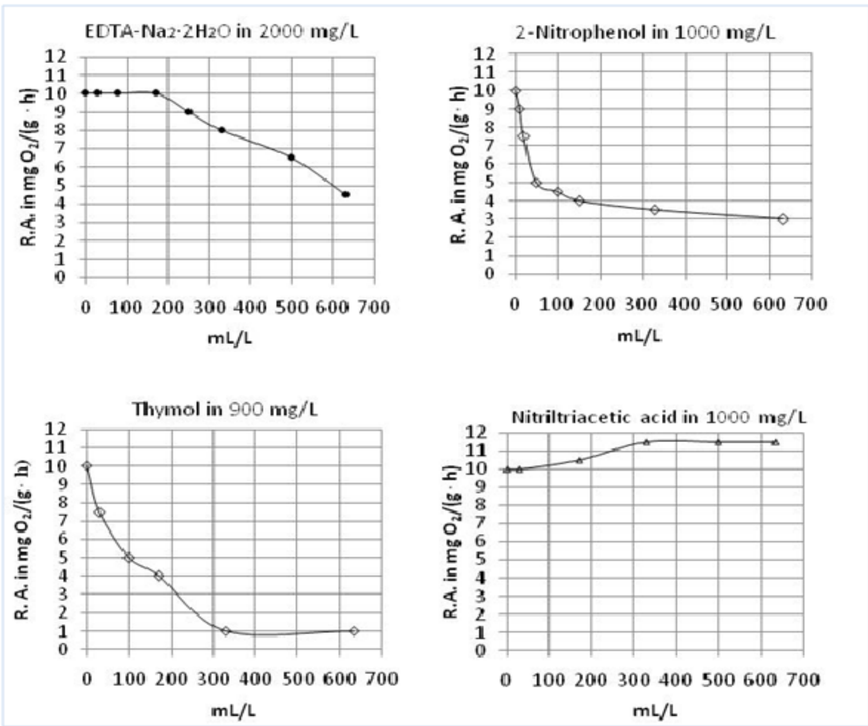

Figure 8: Sludge respiration activity curves of different model wastewaters, showing their noxious properties using activated sludge collected from (JMWWTP)

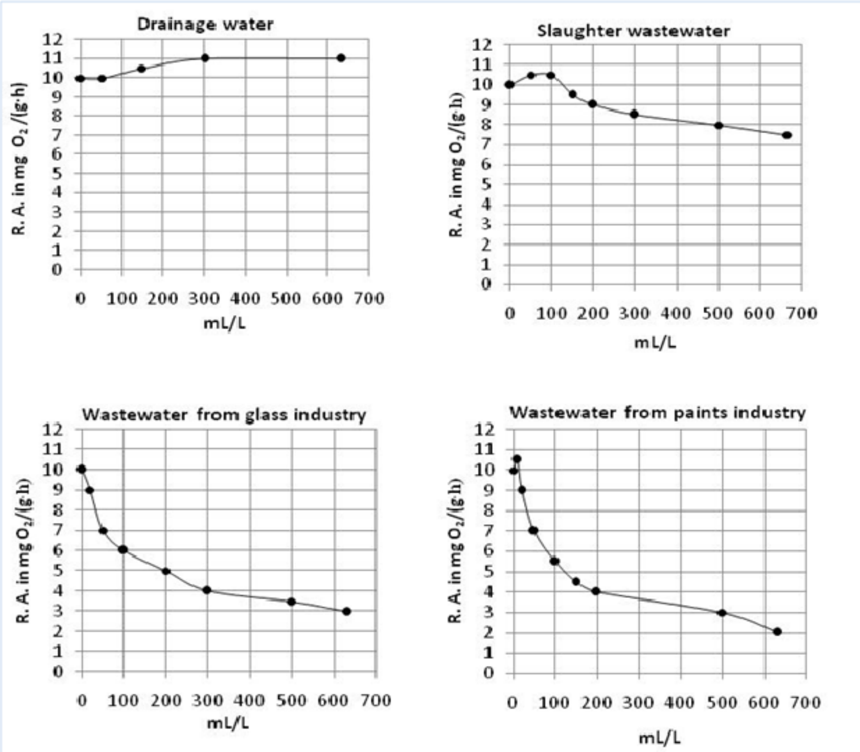

Figure 9: Sludge respiration activity curves of different real industrial wastewaters, showing their noxious properties using activated sludge collected from (JMWWTP)

Noxiousness Numbers (NOX)

The previous noxiousness Figures (5-9) present reliable and quick noxious information about the tested wastewater. However, the results are not demonstrative enough for practical use, to provide a comparison to the lethal dose values LC_{50} from toxicity tests (Substance concentration which is lethal for 50 % of the tested organisms). Therefore a similar procedure is recommended in order to obtain an IC_{50}-value (Substance concentration which causes a 50 % respiration inhibition of municipal activated sludge) and to create a noxiousness number for any wastewater.

Procedure:

i. Set an arbitrary substance test concentration S_{Test} – here 1000 mg/L. (If this standard test concentration is more or less than 1000 mg/L, then choose a test concentration, C_{Test} in mg/L).
ii. Determine the graphical IC_{50} value of the respiration activity curve. It is the added sample volume in ml/L which inhibits respiration activity by 50 %.
iii. This IC_{50} value is then used for the further calculation of the noxiousness number (NOX) as follows (with units):

$$NOX = \frac{1}{IC_{50}\ \ ml/L} \cdot \ 10{,}000\ \frac{ml}{L} \ \cdot \ \frac{S_{test}\ mg/L}{C_{test}\ mg/L}$$

Where:

- 10,000 ml/L is an auxiliary factor used to obtain convenient noxiousness numbers between zero and some 1,000.
- Substance concentrations S_{Test} and C_{Test} make sense only for model wastewaters with defined ingredients.
- The noxiousness number (NOX) has no unit.

Example: In the diagram, Figure (10), IC_{50} can be determined as 200 ml/L. If one assumes that substance concentration C_{Test} must be limited for solubility reasons to only 100 mg/L, then the NOX number is (units omitted):

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NOX = (1/200) · 10,000 · (1,000/100) = 500

Table (3) represents noxiousness numbers (NOX) of various synthetic and real wastewaters in collected in Egypt. Noxiousness numbers (NOX) are practice-oriented, unit-less, formal numbers which roughly characterise the noxiousness of a wastewater and enable a comparison of the noxiousness of different wastewater. For example, in Table (3) wastewater N° 30 is more noxious than N° 17 by 1000 times.

Table 3: Examples of noxiousness numbers of various wastewaters

N°	Type of wastewater (model wastewater or real wastewater)	Substance concentration S_{Test} = 1,000 mg/L $C_{Test} < S_{Test}$	IC_{50} (ml/L) (from diagram)	Noxiousness number (NOX) (calculated)
1	Model wastewater, sodium chloride	1,000 mg/L	not noxious	0
2	Model wastewater, nitrilotriacetic acid	1,000 mg/L	not noxious	0
3	Groundwater from a contaminated site	-	not noxious	0
4	Model wastewater, hexamethylenetetramine	1,000 mg/L	not noxious	0
5	Model wastewater, chloramine T	100 mg/L	not noxious	0
6	Model wastewater, tetrachloroethylene	100 mg/L	not noxious	0
7	Paper mill wastewater, grinding unit	-	not noxious	0
8	Paper mill wastewater, de-inking unit	-	not noxious	0
9	AOP treated effluent from N° 18	-	not noxious	0
10	AOP treated effluent from N° 20	-	not noxious	0
11	Slaughter wastewater	-	not noxious	0
12	AOP treated effluent from N° 27	-	not noxious	0
13	Model wastewater, di-Na-EDTA	2,000 mg/L	633	8
15	Drainage water	-	1300	8
15	Paper mill wastewater, pulper unit	-	750	13
16	Model wastewater, phenol	1,000 mg/L	633	16
17	Rinsing water from plating industry	-	600	17
18	Wastewater from a pharmaceutical industry	-	250	40
19	Crystal and glass industry (Lab. Unit)	-	200	50
20	Wastewater from paints industry	-	120	83
21	Wastewater from a metal industry	-	110	91
22	Model wastewater, thymol	9,00 mg/L	100	111
23	Model wastewater, 2,4-dinitrotoluene	100 mg/L	633	160
24	Model wastewater, 2-nitrophenol	1,000 mg/L	60	167
25	Model wastewater, p-chlorophenol	1,000 mg/L	30	333
26	Wastewater containing different phenols	-	25	400
27	Model wastewater, 2,4-dichlorophenol	1,000 mg/L	17	590
28	Model wastewater, aniline	1,000 mg/L	16	625
29	Wastewater from a pesticide production	-	4	2,500
30	Leachate from a hazardous waste landfill	-	0.5	20,000

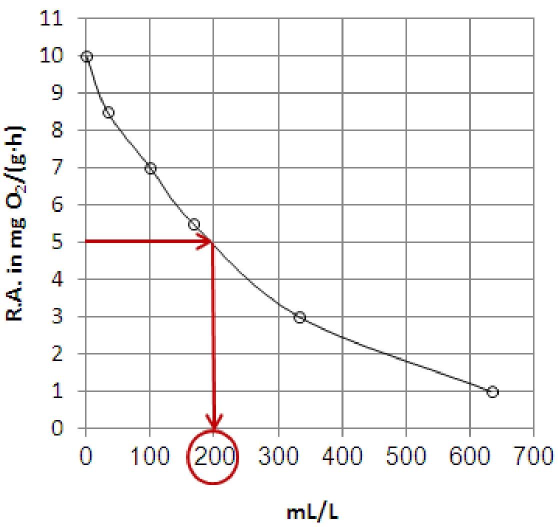

Figure 10: Chart clarifies the IC50 determination of a noxious wastewater showed the sample volume that inhibits 50% R.A.

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CONCLUSIONS

The short term modified test procedure is found to be compatible with the lengthy conventional test procedure, hence it can be applied for economic point of view, saving time and cost. Noxiousness test could be considered an alarm test before discharging wastewater into the sewerage system. Noxiousness test results for activated sludge collected from two different municipal wastewater treatment plants in (ZMWWTP) and (JMWWTP) are compatible, an indication of possible generalization of the results. The created noxiousness number is considered adequate for comparison between various wastewaters toxicity.

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