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ACTIVATED SLUDGE INHIBITION TESTS FOR ACRYLIC/ METHACRYLIC ACIDS AND DERIVATES

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Abstract. Acrylic acid and derivates are commercially important and widely used compounds. They have been the subject of numerous animal toxicity studies covering a variety of exposure routes, but there is little or contradictory literature data on the activated sludge inhibition. This paper reports the results obtained from EC_{50} activated sludge inhibition tests for α-, β-unsaturated acids (acrylic and methacrylic acid) and their derivatives: amides (acrylamide, methacrylamide) and esters (methyl acrylate, ethyl acrylate, butyl acrylate, and respectively methyl methacrylate, ethyl methacrylate and butyl methacrylate). The tests were carried out in similar conditions according to the international standard SR EN ISO 8192 – Water quality – Tests for inhibition of oxygen consumption by activated sludge. The experimental results show that toxicity increases in the series of amides–acids–esters, the acrylic compounds are more toxic than the methacrylic ones and toxicity increases in the esters series along with an increase in the number of carbon atoms in the alkyl group.

Keywords: sludge inhibition, acrylic/methacrylic acid and derivatives, EC_{50}, respirometric tests.

AIMS AND BACKGROUND

The main objective of this researches was to assess the toxicity on activated sludge of α-, β-unsaturated acids (acrylic and methacrylic acid) and their derivatives: amides (acrylamide, methacrylamide) and esters (methyl acrylate, ethyl acrylate, butyl acrylate, and respectively methyl methacrylate, ethyl methacrylate and butyl methacrylate) and to evaluate structure–toxicity relationship.

The world-wide production of acrylic acid in 1994 was estimated to be approximately 2 million tones. The market trend is quite dynamic during the last decade with apparent annual growth rates about 5%, according to Merchant Research & Consulting Ltd. (UK) consulting agency, global acrylic acid production capacity will expand to 4 million tones/year by 2009.

Acrylic monomers are important building blocks for polymers used in widely diverse applications. They are used to prepare thermoplastic coatings, manufacture

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acrylic and methacrylic fibers, synthesise higher acrylic esters, prepare adhesives and sealants, and used in paper treatment as coating binders for pigments, as ingredients of latex paints, floor polishes, in treatments of leather goods, as viscosity index improvers for lubricating oil, and as chemical intermediates in the preparation of amphoteric surfactants, glass substitutes, gel electrophoresis (SDS-PAGE), and materials that must meet flame-retardant standards[1,2].

Releases of acrylic monomers into the environment are to be expected during production, processing, use and disposal mainly via waste water.

Over past 40 years, the fate of chemical pollutions in the environment has become an important issue[3]. Most efforts have concentrated on the fate of chemicals in the environment, especially in wastewater treatment processes, methods for investigating toxicity and biodegradation processes being developed as international standardised methods. These compounds have been the subject of numerous animal toxicity studies[4,5] covering a variety of exposure routes, but there is little or contradictory literature data on activated sludge inhibition[6,7]. In order to have comparable results, all the tests for EC_{50} activated sludge inhibition were carried out in similar conditions according to the International Standard SR EN ISO 8192 – Water quality – Tests for inhibition of oxygen consumption by activated sludge[8].

EXPERIMENTAL

The chemicals tested were carried out on acrylic and methacrylic acid, acrylamide, methacrylamide, methyl acrylate, ethyl acrylate, butyl acrylate and respectively methyl methacrylate, ethyl methacrylate, and butyl methacrylate. The chemical formulas, molecular weights, and chemical abstract registry numbers are listed in Table 1. The substances were provided by the manufacturer (Merk); in all cases, chemical purity being >98%.

Table 1. Tested substances basic characteristics

Substance	Mol. wt. (g/mol)	Formula	Structure	CAS RN
Acrylic acid	72.06	$C_3H_4O_2$	CH_2 = CH–COOH	79-10-7
Acrylamide	71.08	C_3H_5NO	CH_2 = CH–$CONH_2$	79-06-1
Methyl acrylate	86.09	$C_4H_6O_2$	CH_2=CHCOOCH_3	96-33-3
Ethyl acrylate	100.11	$C_5H_8O_2$	CH_2=CHCOOCH_2CH_3	140-88-5
Butyl acrylate	128.17	$C_7H_{12}O_2$	CH_2=CHCOO$(CH_2)_3CH_3$	141-32-2
Methacrylic acid	86.09	$C_4H_6O_2$	CH_2=C(CH_3)COOH	79-41-4
Methacrylamide	85.11	C_4H_7NO	CH_2=C$(CH_3)CONH_2$	79-39-0
Methyl methacrylate	100.11	$C_5H_8O_2$	CH_2=C(CH_3) –$COOCH_3$	80-62-6
Ethyl methacrylate	114.14	$C_6H_{10}O_2$	CH_2=C(CH_3)COO(C_2H_5)	97-62-3
Butyl methacrylate	142.20	$C_8H_{14}O_2$	CH_2=C(CH_3)COO(C_4H_9)	97-88-1

In order to have comparable results, all tests for EC_{50} activated sludge inhibition were carried out in similar conditions according to SR EN ISO 8192 – Water quality – Tests for inhibition of oxygen consumption by activated sludge (A method) using activated sludge from a municipal wastewater treatment plant. This standardised inhibition test assesses the inhibitory effect of tested substances on oxygen consumption efficiency of activated sludge microflora by measuring the respiration speed in the presence of different concentrations of test substances.

The tests were carried out according to standard, in two stages: preliminary tests (using at least three concentrations of the test material, one biological control vessel and one abiotic control) in order to estimate the range of concentrations needed for the definitive test; and the definitive tests which were conducted using at least five concentrations (deduced from the preliminary test) of the test material, one biological control and one abiotic control.

The test vessel contains 10 ml of synthetic medium (peptone, meat extract, urea, sodium chloride, calcium chloride, magnesium sulphate, potassium monohydrogen phosphate), 10 ml of activated sludge in concentration of 2-4 g d.w./l, the test material and dilution water (aerated distilled water). The biological blank control has the same composition with the test vessel, but without the test material while the abiotic control has the test material, but does not have activated sludge. The pH of the mixture should be 7.5±0.5 and the initial concentration of dissolved oxygen in the vessels must be close to the saturation limit. The tests were performed at room temperature ($22^oC \pm 2^oC$) in stirring conditions (magnetic stirrer) so that the determinations of dissolved oxygen in the incubation vessels to be reproducible.

The measurements of dissolved oxygen in each incubation vessel were made with a WTW oxygen electrode, every 30 min for 180 min. The oxygen consumption rate of the test mixtures can be determined based on the measured values, from the linear part of the graphs of oxygen concentration versus time. The oxygen consumption rate R, in milligrams per liter per hour, can be calculated according to equation (1):

$$R=[(Q_1-Q_2)/\Delta_t]\times 60 \quad (1)$$

where Q_1 is the oxygen concentration at the beginning of the selected section of the linear phase, mg/l; Q_2 – the oxygen concentration at the end of the selected section of the linear phase, mg/l; Δ_t – the time interval between these two measurements, min.

The percentage inhibition of oxygen consumption at each concentration of test substance is given by equation (2):

$$I = R_b - (R_t - R_a)/R_b \times 100$$

where R_t is the rate of oxygen consumption obtained for the test material; R_b – the rate of oxygen consumption obtained for the blank control (biological); R_a – the rate of oxygen consumption obtained for the abiotic control.

The concentration which inhibits the oxygen consumption by 50% (EC_{50}) can be calculated or interpolated by plotting the percentage inhibition of oxygen consumption against the logarithm of the test material concentration.

RESULTS AND DISCUSSION

All ten compounds were tested in order to evaluate activated sludge EC_{50} inhibition. The concentrations used in the definitive experiments were established in preliminary tests (according to standard).

In the presence of easy biodegradable substances, activated sludge consumes oxygen at a higher rate than in their absence; addition of a toxic concentration of a test compound results in a decrease of the oxygen consumption rate. The rate was calculated (using equation (1)) based on the dissolved oxygen concentration measured every 30 min, from the linear part of the graphs of oxygen concentration versus time. The inhibitory concentration corresponding to each tested concentration was determined using equation (2), by comparison of the rate in mixtures of test material with that of a biological control. Activated sludge EC_{50} was determined by plotting the percentage inhibition of oxygen consumption against the logarithm of the test material concentration (inhibition curve as in examples for acrylic acid and methyl acrylate – Figs 1 and 2).

Acrylic acid (neutralised with NaOH 1N, pH=7.5±0.5) was tested in seven concentrations deduced from the preliminary test, respectively 1, 3, 5, 7, 9, 12, and 15 g/l. Measured values of oxygen consumption during the test as well as the calculated consumption rates and inhibition percentages are presented in Table 2.

Table 2. Oxygen consumption determination (mg O_2/l), calculated respiration rates and inhibition percentages for each tested concentration (acrylic acid)

Time	Concentration (g/l)							BCS	ACS
	1	3	5	7	9	12	15		
0	9.27	9.27	9.24	9.2	9.26	9.25	9.23	**9.33**	**9.42**
30	**8.16**	**8.35**	**8.44**	**8.47**	**8.54**	**8.6**	**8.6**	7.84	9.36
60	7.29	7.61	7.77	7.88	7.97	8.11	8.09	6.46	9.32
90	6.5	7	7.26	7.43	7.54	7.7	7.77	5.24	9.34
120	5.76	6.4	6.69	6.93	7.09	7.3	7.38	4.04	9.33
150	5.02	5.79	6.19	6.49	6.66	6.91	7.01	2.76	9.3
180	**4.29**	**5.2**	**5.67**	**6.03**	**6.24**	**6.57**	**6.67**	**1.48**	**9.28**
Rate	1.66	1.36	1.19	1.06	1.01	0.89	0.85	2.62	0.05
Inhibition	38.34	49.93	56.30	61.40	63.31	67.64	69.17		
Lg concentration	3	3.47	3.69	3.84	3.95	4.08	4.17		

BCS – biologic control sample; ACS – abiotic control sample.

The rates of oxygen consumption ranged between 2.62 mg/l/h for the biological control and 0.85 mg/l/h for the most concentrated test vessel. It is observed a gradual diminution of oxygen consumption rate along with the increase of toxic concentration in the test vessel, the inhibition percentage increases along with the concentration of the tested compound from 38 to 69%.

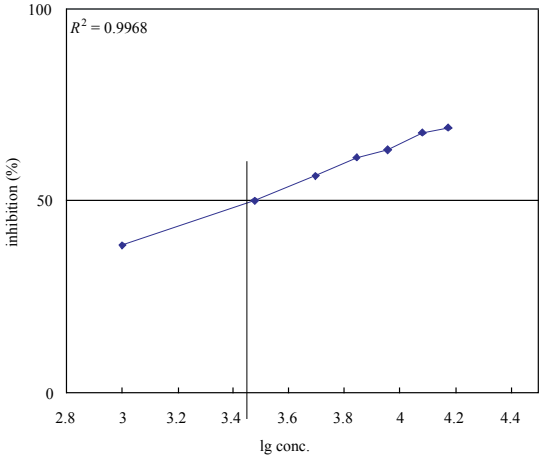


Fig. 1. Activated sludge inhibition curve for acrylic acid

Activated sludge EC_{50} inhibition for neutralised acrylic acid is 2818 mg/l.

Methyl acrylate was tested at five concentrations, i. e. 500, 700, 900, 1200 and 1500 mg/l. Measured values of oxygen consumption during the test as well as the calculated consumption rates and inhibition percentages are presented in Table 3.

Table 3. Oxygen consumption determination (mg O_2/l), calculated respiration rates and inhibition percentages for each tested concentration (methyl acrylate)

Time	Concentration (mg/l)					BCS	ACS
	500	700	900	1200	1500		
0	7.92	**7.89**	**7.8**	**7.85**	**7.85**	**7.8**	**8.08**
30	**6.89**	6.79	6.79	6.82	6.81	6.24	8.06
60	5.5	5.54	5.71	5.83	5.89	4.64	8.06
90	4.24	4.44	**4.78**	**4.96**	5.04	3.1	7.96
120	2.99	**3.41**	4.14	4.18	**4.23**	**1.53**	**7.92**
150	**1.9**	2.47	3.12	3.51	3.72	0.39	7.73
180	1.15	1.78	2.66	3.06	3.27	0.22	7.72
Rate	2.6	2.24	2.01	1.92	1.72	3.135	0.08
Inhibition	19.61	31.10	38.33	41.09	47.68		
Lg concentration	2.69	2.84	2.95	3.07	3.17		

BCS – biologic control sample; ACS – abiotic control sample.

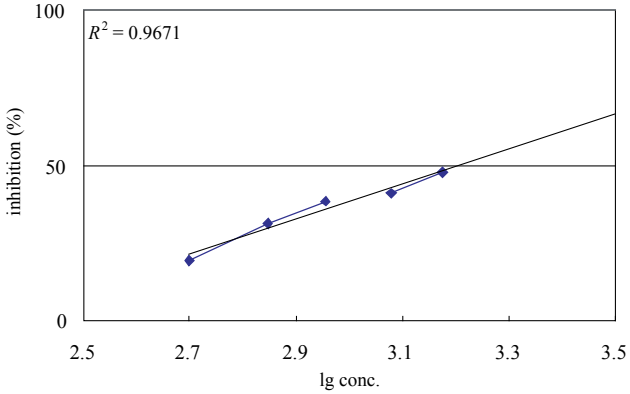


Fig. 2. Activated sludge inhibition curve for methyl acrylate

The rates of oxygen consumption ranged between 3.13 mg/l/h for the biological control and 1.72 mg/l/h for the most concentrated test vessel. It is observed a gradual diminution of oxygen consumption rate along with the increase of toxic concentration in the test vessels, the inhibition percentage increases along with the concentration of the tested compound from 19.61 to 47.68%. Activated sludge EC_{50} inhibition for methyl acrylate is 1585 mg/l.

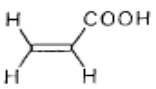
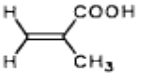
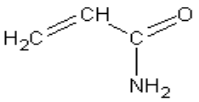
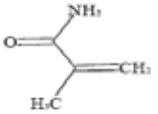
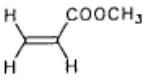
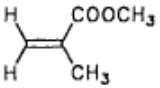
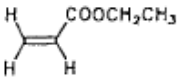
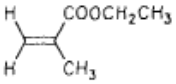
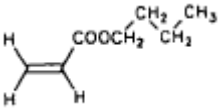
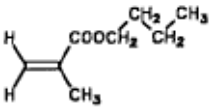
The same experimental procedure was applied to all compounds, and EC_{50} calculation was made similar to the examples above. Determined EC_{50} values for each tested compound are summarised in Table 4.

The obtained results on inhibition of activated sludge show consistency within the structurally related groups:

- The EC_{50} inhibition concentration decreases in the series of amides–acids–esters from the order of tens of grams in the case of amides to grams-tens of grams in the case of neutralised acids and grams-hundred milligrams in the case of esters (Fig. 3) which means that toxicity increases in the series of amides–acids–esters.
- Activated sludge EC_{50} values for the acrylic compounds are smaller than for the methacrylic ones, the difference being higher in the case of acids (EC_{50} for acrylic acid is ≅ 5 times smaller than for the methacrylic acid) and decreases in the case of esters along with the increase of the number of carbon atoms in the alkyl group (Fig. 3).
- Toxicity increases in the esters series along with the increase of the number of carbon atoms in the alkyl group (Fig. 4).

The information generated by these experiments may be helpful in estimating the effect of acrylic/methacrylic acid and derivatives on mixed bacterial communities in the aquatic environment, especially from biological treatment systems.

Table 4. EC_{50} activated sludge inhibition for acrylic/ methacrylic acids and derivatives

Substance	Structure	Tested concentration range	Calculated inhibition range (%)	EC_{50} (mg/l)
Acrylic acid		1-15 g/l	38-69	2818
Methacrylic acid		5-25 g/l	35-58	13500
Acrylamide		10-30 g/l	21-66	20200
Methacrylamide		10-30 g/l	22-58	24800
Methyl acrylate		500-1500 mg/l	19-47	1585
Methyl methacrylate		3-7 g/l	46-88	3162
Ethyl acrylate		500-2000 mg/l	38-73	793
Ethyl methacrylate		1700-2500 mg/l	52-78	1640
Butyl acrylate		100-500 mg/l	39-77	191
Butyl methacrylate		200-550 mg/l	43-98	204

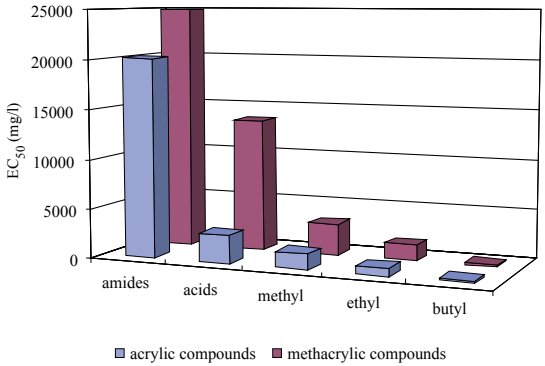


Fig. 3. Structure activated sludge EC_{50} inhibition relationship in the series of acrylic/methacrylic acid and derivates

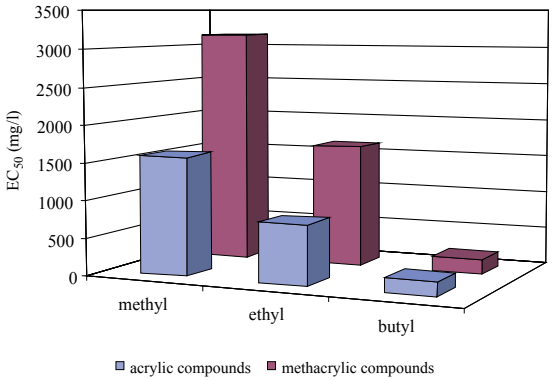


Fig. 4. Structure activated sludge EC_{50} inhibition relationship in the series of acrylic/methacrylic acid alkyl esters

CONCLUSIONS

Acrylic/methacrylic acid and derivatives (amides, esters) were tested for activated sludge inhibition (EC_{50}) in similar conditions according to international standard SR EN ISO 8192/2001. The experimental results show that:

- toxicity increases in the series of amides–acids–esters;
- the acrylic compounds are more toxic than the methacrylic ones;
- toxicity increases in the esters series along with the increase of the number of carbon atoms in the alkyl group.

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