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ANTIMICROBIAL AGENTS USED IN THE MANUFACTURE OF CARE PRODUCTS – INHIBITORY EFFECT ON RESPIRATION RATE OF ACTIVATED SLUDGE

Madalina Mihalache, Irina Lucaciu, Catalina Stoica, Alina Banciu, Dragos Radulescu

National Research and Development Institute for Industrial Ecology-ECOIND, 57-73 Drumul Podu Dambovitei Street, district 6, 060652, Bucharest, irina.lucaciu@incdecoind.ro, Romania

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Introduction

2-(2,5-Dichlorophenoxy)-5-chlorophenol or Triclosan (TCS) shows a broad-spectrum antimicrobial agent used as antiseptic, disinfectant and preservative in many consumer products including anti-bacterial soaps, toothpastes, cosmetics, personal care products and materials such as medical devices, textiles, plastic ware, kitchen utensils, children's toys, and footwear. TCS is known to have an activity against a wide range of Gram-positive and Gram-negative bacteria and being classified as bacteriostatic at low concentrations (1 – 2 ppm) and bactericidal higher concentrations (≥ 8 ppm). The toxic effects of TCS on activated sludge micro-organisms are relatively little studied and known and there is a data gap on the degradation pathway of triclosan and its intermediary products. The limited data available indicate that triclosan exposure decreased respiration by heterotrophic and nitrifying bacteria from activated sewage sludge and effect levels of TCS on activated sludge micro-organisms vary depending on the level of acclimation. The minimum inhibitory concentrations (MIC) ranging between 0.1 to 21 mg /L of triclosan on 11 different bacterial strains was reported. It should be noted that the upper range minimum inhibitory concentrations (MICs) reported are well in excess of published solubility limit for TCS (10-12 mg/L at 25 °C). This study aims to present the inhibition effect of TCS on activated sludge microorganism's activity by measuring of the respiratory activity of microorganisms with the application of the Specific Oxygen Uptake Rate (SOUR) test, according to standard method ISO 8192:2007 (similar with OECD TG 209 Inhibition of Oxygen Consumption).

Materials and methods

Test substance: 2-(2, 5-Dichlorophenoxy)-5-chlorophenol or Triclosan (TCS) (Sigma Aldrich, USA) was tested. A stock solution of 10 mg in 1000 ml of ultrapure water was prepared. The synthetic nutrient medium was prepared according to OECD Guideline no.209.

Microbial inoculum

The activated sludge was taken from a municipal wastewater treatment plant which treats mostly domestic sewage (WWTP Bucharest, Romania). After collection the sludge was filtrated, washed with tap water and re-suspended in tap water.

The activated sludge suspension was aerated until usage in the respirometric test. Sludge characterization: pH=7.8; dry matter = 2.62 grams suspended solids / liter.

Respiration test method

Respiration inhibition test was performed according to standard method ISO 8192:2007 (similar with OECD guideline no.209). It gives information on inhibitory or stimulatory effects after a short exposure (usually 30 min up to 180 min or even more) of the test material on activated sludge microorganisms. The effect of TCS on the respiration rate of a sample of activated sludge was estimated under defined conditions in the presence of 3 different nominal concentrations: 1mg/L; 5 mg/L and 10 mg/L and in 2 replicates for each concentration. Each test mixture (1000 ml) contained: 50 mL synthetic medium, 300 ml activated sludge suspension, an appropriate amount of TCS stock solution to obtain the established test concentrations and deionized water up to the final volume of 1000 ml. Incubation of the test mixtures was performed at a constant temperature of 20°C±2°C and under continuous stirring for 3 hours. The sensitivity of each test concentration was examined with a control (activated sludge fed with a standard amount of synthetic nutrient medium) incubated under the same conditions as the test mixtures. The oxygen consumption (respiration rate) of each test concentration was measured with an oxygen-sensitive electrode system (WTW Model 3430), over the following incubation time intervals: every 3 hours during 24 hours, as well as after 48, 72 and 120 hours. The OUR was expressed as the decrease in dissolved oxygen content (mg O_2) per time unit (h):

$$OUR = \frac{initial\ oxygen\ concentration - final\ oxygen\ concentration}{\Delta t}$$

where: OUR (mg O_2/L*h); initial oxygen concentration (mg O_2/L); final oxygen concentration (mg O_2/L); Δt (time in h).

The percentage inhibition of the respiratory activity was expressed as the percentage reduction OUR values relative to the control and determined by the formula:

$$\%INH = \frac{OUR_0 - OUR_{TCS}}{OUR_0} * 100$$

where: OUR_0= dissolved oxygen content for blank control sample (mg O_2/L*h) and OUR_{TCS}= dissolved oxygen content for TCS mixture (mg O_2/L*h).

Results and conclusions

The inhibitory effect of 1mg/L; 5 mg/L and 10 mg/L Triclosan on a mixed culture of activated sludge microorganisms with the application of Specific Oxygen Uptake Rate (SOUR) test was investigated.

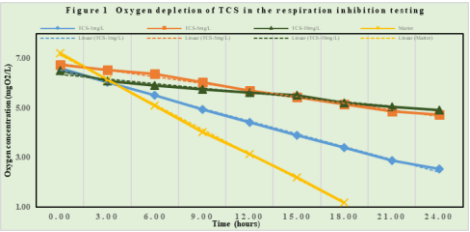

Fig. 1. Oxygen depletion of TCS in the respiration inhibition testing

After 3 hours' incubation, the oxygen concentration was recorded every period of time for each tested mixture and also, control sample. The OUR of each TCS concentration was calculated by linear regression of oxygen concentration readings during the linear part of the curves (O_2-depletion) (Figure 1).

The specific oxygen consumption rates (SOURs) – uptake of oxygen by activated sludge per unit mass of dry sludge (suspended solids), in unit time, were also calculated. The values of respiration rates of activated sludge for TCS tested concentrations and blank control calculated (after 3 hours' incubation) and the percentage inhibition of respiratory activity of sludge (% $_{INH}$) for each test solutions are summarized in Table 1.

Table 1. Respiration Rate and Inhibition of TCS

Test sample	Nominal Concentration (mg/L)	Respiration rates		Inhibition (%)
		Oxygen Uptake Rate mg O_2/(Lxh)	Specific Oxygen Uptake Rate mg O_2/(gxh)	
Control	-	0.33	0.42	-
TCS	1	0.18*	0.23	46.4
TCS	5	0.10*	0.13	69.3
TCS	10	0.05*	0.06	85.9

** average of 2 replicates*

Although the inhibition test continued for another 96 hours, no acclimatization of the activated sludge to TCS was observed. The inhibition levels calculated after 120 hours were 38%, 65% and 76% for the tested concentrations (1 mg/L, 5 mg/L and 10 mg L respectively).
A median inhibition concentration (IC50) related to the respiration rate of activated sludge was calculated according with concentration-effect curve of TCS (Figure 2).

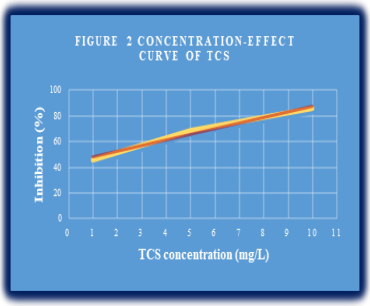

Fig. 2. Concentration effect curve of TCS

The experimental results obtained in the first 24 hours of testing were used to estimate the IC50 value of the triclosan of approximately 1.5 mg/L. Published studies on inhibitory effects TCS on heterotrophic oxidation and also on the rate of nitrification showed that low concentrations (1 – 2 ppm) may cause inhibition of respiration of activated sludge microorganisms.
Consequently, it can be appreciated that the result obtained by us is comparable to previous published data.

The results of this study would have been much more accurate if the respiration inhibition test was performed on a higher TCS concentrations and at different level of acclimation of sludge microorganisms. To confirm the results of this study, our research will continue on various TCS concentrations and using both activated sludge and reference bacterial strains (eg. *Escherichia coli* and *Pseudomonas aeruginosa*).