Inhibition of Activated Sludge Respiration by Laundry Washing Agents

Silvie Hartmann, Hana Škrobánková, Ivo Havránek
Energy Units for Utilization of non Traditional Energy Sources (ENET)
VŠB-Technical University of Ostrava, Czech Republic

***Abstract*—Inhibition of the microbial activity caused by laundry washing agents was tested by two biotoxicity tests, respirometry and Vibrio fischeri. Eight samples of the different laundry washing agents (four powder and four liquid) were used. Respirometry tests showed higher inhibitory effects caused by powder laundry washing agents, whereas gel laundry washing agents were more toxic for *Vibrio fischeri*. The electrical conductivity of the laundry washing agents significantly affected the *Vibrio fischeri* inhibition.**

Keywords- laundry washing agents; toxicity; respirometry; Vibrio fischeri; waste water treatment plant .

I. INTRODUCTION

Laundry washing agents (washing powders/gels) referred in this paper as LWA are among the commonly used chemicals in households as well as in industry. A number of studies have investigated the toxic effect of individual constituents of common LWA although the toxic effect of complex LWA has yet to be adequately examined [1]. Specifically, surfactants, zeolites, bleaching agents, fabric brighteners, coloring agents and enzymes were found to be toxic to a wide range of aquatic organisms [2,3,4,5]. Over the years there has been a slow shift in the use of LWA from the traditional powder or granular form towards a gel form and recently the use of gel LWA is equivalent or even exceeds that of powder form particularly in the North America and Western Europe [6]. Gel and powder LWA differ not only in the composition but in the concentration of active substances as well. Gel LWA usually contain anionic surfactants such asalkyl sulfates, primary alkane sulfonates, alpha-olefin sulfonates [7] soap and fatty alcohol ethoxylates, whereas powder LWA consist of builders (zeolites), bleaching agents and other electrolytes in addition to surfactants [8].

In municipal waste water effluents LWA were reported in a concentration range from 0.008 to 6.2 mg L^{-1} [9]. Waste water treatment plants are compelled to manage the effluent polluted by a variety of household or industrial detergents that can only be very slowly degraded by the bacteria and inhibit the performance of the activated sludge bacteria [10].

Respiration is an essential activity of aerobic bacteria. For this reason the inhibition of respiration is a decisive factor for assessing the ecotoxicological risk of chemical substances in wastewater. Respirometry is used to assess the toxicity of waste water to heterotrophic and nitrifying bacteria in activated sludge. In contrast to *Vibrio Fischeri* luminescence toxicity test, activated sludge respirometry is a more direct method for measuring sludge activity and thus toxicity to sludge. Moreover the respirometry toxicity test involves a wide range of bacteria and protozoa [11]. The basis for respirometric tests is that the respiration rate of activated sludge or sludge microorganisms can be reduced in the presence of toxic substances. The most common way of measuring the bacterial respiration rate is the oxygen uptake rate [12]. The use of the *Vibrio fischeri* biotoxicity method is limited by interfering phenomena – loss of luminescence caused by absorption or diffusion of light in heavily-colored or turbid samples or the presence of organic, well biodegradable nutrients, which may cause a reduction in bioluminescence independently on the pollutants [10].

The purpose of this work was to determine the toxicity of powder and gel LWA on activated sludge microorganisms with the help of respiration inhibition tests using the Strathtox unit. In addition, the ecotoxicity test using *Vibrio Fischeri* was evaluated in order to compare the inhibitory effect.

II. MATERIALS AND METHODS

A. Laundry washing agents

Eight samples of commercially available LWA were used; four powder (Persil Universal; Ariel Automat White Flowers Pro-Zim 7; Denkmit Aktiv-Shutz; Korrekt Universal detergent) and four gel (Persil Expert Duo-Caps; Ariel Colour & Style gel; Denkmit Reisetube; Korrekt Washing gel). The powder and gel form of the same brand of LWA were examined. The ingredients of each LWA were evaluated based on the composition information available on the packing or website. The differences between the LWA ingredients are presented in Table 1. All solutions of the LWA were prepared with tap water at a temperature of 35°C. The amount of the LWA was calculated according to the typical dosage of the LWA for one washing load (with the 40 l water consumption). The dosage of gel LWA (in grams) was calculated according to the total amount of solids in each LWA (US EPA METHOD 1684 Total, Fixed, and Volatile Solids in Water, Solids, and Biosolids).

B. Activated sludge

The Waste Water Treatment Plant (WWTP) Biocleaner BC-35 (Envi – Pur) was designed for a population equivalent under 500 with no industrial inflow. The activated sludge was collected from the aeration tank of the Biocleaner BC-35 WWTP and was maintained aerated

during transit to the laboratory using a portable 12V aeration device. In the laboratory, 600 ml of the activated sludge was placed into the stock flask. In the stock flask the sludge was maintained at a constant temperature selected for the test (25°C) and fully aerated. The synthetic sewage feed for the unit calibration was prepared by ISO 8192.

TABLE I. COMPOSITION OF TESTED LWA

composition	POWDER LWA				GEL LWA			
	1	2	3	4	1	2	3	4
zeolites	YES	YES	>30%	YES	NO	NO	NO	NO
oxygen based whitening/bleaching agent	YES	15-30%	15-30%	YES	NO	NO	NO	NO
anionic surfactants	5-15%	5-15%	5-15%	<5%	15-30%	5-15%	5-15%	<5%
nonionic surfactants	<5%	<5%	5-15%	YES	15-30%	<5%	<5%	<5%
amfoteric surfactants	NO	NO	NO	NO	NO	NO	<5%	NO
polycarboxylathes	YES	YES	<5%	NO	NO	NO	NO	NO
phosphonates	YES	YES	<5%	NO	<5%	YES	NO	NO
soaps	YES	NO	NO	YES	NO	YES	NO	NO
enzymes	YES	YES	YES	YES	YES	YES	NO	NO
(optical) brighteners	YES	YES	YES	YES	YES	NO	NO	NO
parfumes/fragrances	YES	YES	YES	YES	YES	YES	YES	YES
conservants	NO	NO	YES	NO	NO	YES	YES	YES

1= Persil; 2 = Ariel; 3 = DenkMit; 4 = Korrekt
YES ingredient present in the LWA
NO ingredient not present in the LWA according to the producer

C. *Testing methods*

Two kinds of biotoxicity tests were used for the evaluation of toxicity – respirometry test and *Vibrio Fischeri* luminescent test.

Respirometry. The respirometer Strathtox (Strathkelvin Intruments Ltd. Glasgow) was used for measuring the respiration inhibtion of the LWA samples. The EN ISO 8192:2007 standard test method and OECD 209 – Regulation EC 440/2008 for testing inhibitory effects of substances on the respiratory activity of microorganisms in activated sludge were applied. The respiration inhibition test measures the respiration inhibition caused by 5 different concentrations of waste water compared to the respiration of a control sample of activated sludge. Tests were carried out in six 20 ml glass tubes. A synthetic sewage feed (2 ml) and test mixtures of LWA (depending on concentration – diluted with distilled water) were added to the tubes. The synthetic sewage feed for the respiration inhibition measurements was prepared according to the ISO 8192. The tubes were stirred with a magnetic stir-bar in a waterbath of Strathtox unit. After reaching of a constant temperature of 25 °C, 8 ml of activated sludge were quickly added to the tubes and oxygen electrodes were inserted into the tubes to record the respiration rate values. The tests were considered completed with the oxygen content of the tube reaching the value near zero. The percentage inhibition was calculated as follows:

$$(1-R_s/R_c) \quad 100 \qquad (1)$$

where R_s = sample oxygen uptake rate
R_c = control oxygen uptake rate

Biotoxicity test. The biotoxicity luminiscent tests of the LWA samples (water solution samples made up with the higher 0.5 g/L and lower 0.05 g/L dosage of LWA samples) were performed according to EN ISO 11348 – 2 (Water quality – Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test) – Part 2: Method using liquid – dried bacteria) standard test method. The marine luminescent bacteria (*Vibrio fischeri)* were employed to evaluate the biotoxicity of the LWA samples. The bacteria were exposed to the LWA leachates for 30 minutes at 15 °C as determined by a luminometer. Biotoxicity was expressed as the light inhibition ratio H_{30}. The analyses were carried out in the IGI laboratories VŠB – Technical University (TU) of Ostrava, CZ.

III. RESULTS

The eight possible toxicants were tested for the respiration inhibition and biotoxicity in different concentration intervals, taking into account the average dosage for one washing cycle. The chemical parameters such as electrical conductivity, pH, and Chemical Oxygen Demand (COD) were measured before the toxicity measurements for each of the LWA solution and are presented in Table 2.

TABLE II. CHEMICAL PARAMETERS OF THE LWA

LWA	el. conductivity * [µs/cm]	pH*	COD** [mg/l]
Persil (P)	2420	10.24	158
Ariel (P)	2590	10.28	139
DenkMit (P)	1264	9.63	267
Korrekt (P)	3800	10.09	31.4
Persil (G)	222	7.47	3804
Ariel gel (G)	794	7.69	1030
DenkMit gel (G)	1258	7.51	747
Korrekt gel (G)	1796	7.78	667

P = powder G = liquid
* Concentration of the LWA solution = 2 g/L
** Concentration of the LWA solution = 0.5 g/L

Electrical conductivity measures the concentration of dissolved salts, both positively and negatively charged ions. Sodium, calcium, magnesium, chloride and sulphate ions

are the main contributing ions in greywater. The most common sources of these ions in greywater are sodium-based soaps, nitrates, and phosphates found in LWA and powdered soaps [13]. Considerably higher values of electrical conductivity and pH were observed in all four samples of powder LWA. Whereas, higher COD values were found for the gel LWA samples. The COD and electrical conductivity results were consistent with the results obtained by Singh et al. [13], who reported that higher COD of gel LWA solutions may be expected compared to the LWA formulation.

A. *Respirometry tests*

The inhibition profiles of the LWA with different dosage are shown in Figure 1. The higher rate of the inhibitory effect of the LWA on the biomass activity was observed for the powder samples. All the tests were performed in two replicates and the average value was used for data analysis.

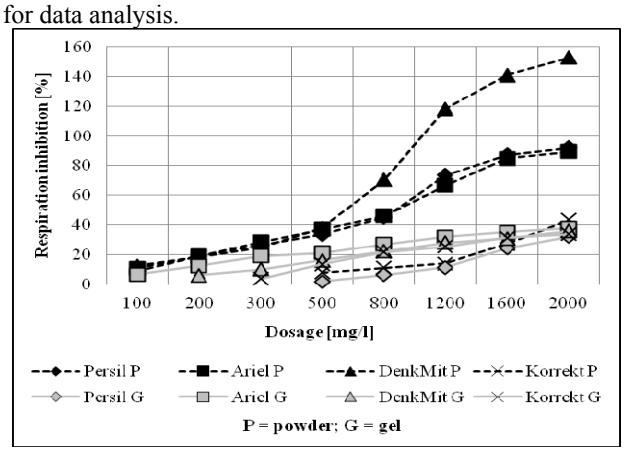

Figure 1. Respiration inhibition profiles of solid (black) and liquid (grey) LWA.

B. *Biotoxicity tests*

An inhibition in the range of 80 – 99.85% was obtained for the LWA solutions of 0.5 g/L after 30 minutes. Therefore, a further dilution was applied and H_{30} inhibition values were established on the solution samples with 0.05 g/l of LWA. The values of the biotoxicity inhibition for all LWA are listed in Table 3.

The respirometry results are differ from the results obtained by Azizullah et al. [1], and Singh et al. [13], who reported higher inhibitory effects for the gel LWA for the fresh water algal organisms (*E. gracilis*) and for Swiss chard plants. The biotoxicity tests using *Vibrio fischeri* bacteria confirmed the higher toxicity of the powder detergents. All four powder LWA contained relatively high amounts of zeolite, which, according to Hrenović et al. [3] is toxic for the specific type of bacteria (*Acinetobacter junii*), commonly found in the activated sludge and can possibly affect the respirometry inhibition.

TABLE III. COMPARISON OF THE BIOTOXICITY INHIBITION THE RESPIRATION INHIBITION

LWA	H30 * [%]	H30 ** [%]	Respiration inhib. * [%]
Persil (P)	99.85	39.37	33.45
Ariel (P)	99.85	43.7	36.95
DenkMit (P)	98.64	69.18	37.65
Korrekt (P)	79.94	39.68	7.8
Persil (G)	99.03	94.4	1.75
Ariel (G)	95.92	79.19	21.1
DenkMit (G)	83.57	83.83	16.15
Korrekt (G)	87.45	70.52	13.35

P = powder G = liquid
* Dosage of detergent solution = 0.5 g/L
** Dosage of detergent solution = 0.05 g/L

The inhibition of *Vibrio fischeri* is affected by the concentration of soluble ions (Na^+, Ca^{2+}, K^+) [14]. The highest *Vibrio fischeri* inhibition was found for the sample with the lowest concentration of soluble substances (Figure 2). Data were analyzed by the IBM SPSS Statistics software, Version 21.0.0. to calculate the linear correlation, using the Pearson´s two tailed correlation test. A statistically significant correlation ($N = 8$; $R = 0.926$; $p > 0.01$) was obtained. In addition, *Vibrio fischeri* inhibition is also affected by the quantity of the nanoparticles in the solution, as well as its color and turbidity [15].

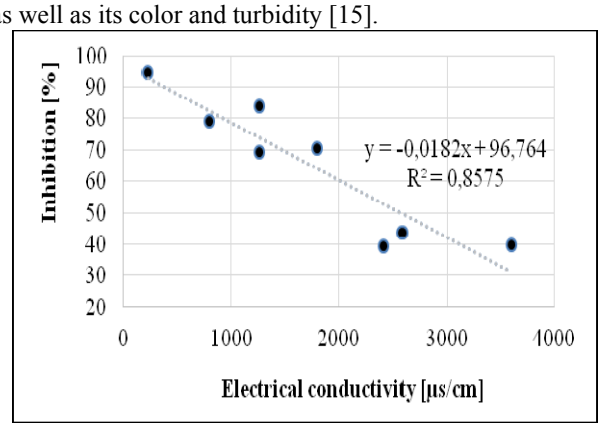

Figure 2. Dependence of the *Vibrio fischeri* inhibition on the electrical conductivity of LWA.

IV. CONCLUSIONS

All LWA samples adversely affected the bacteria in both toxicity tests examined in this paper – respirometry test and *Vibrio fischeri* test. The responses of bacteria on the type of LWA (powder/gel) were different and the following conclusions can be drawn:

- The powder LWA cause higher inhibition in the respirometry test than in the *Vibrio fisheri* test.
- The gel LWA showed a very high inhibition (H_{30} = 70 – 94%), even for small dosage (0.05 g/L) of the LWA in the *Vibrio fisheri* tests.

The results showed that bacteria used for the tests in this paper reacted differently on the same tested conditions. The activated sludge bacteria (mainly *Pseudomonas*) showed higher inhibition for the powder laundry washing agents,

whereas *Vibrio fischeri* bacteria reacted to the contrary, due to the higher sensitivity on the dissolved matter concentration. Both toxicity tests should be examined together to get an overall information about the complex laundry washing agent inhibition. However *Vibrio fischeri* inhibition test should be primarily used for the waste water effluent quality monitoring.

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