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WHOLE EFFLUENT RISK ESTIMATION FOR A SMALL RECIPIENT WATERCOURSE

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Whole effluent toxicity is most often considered as a static parameter. However, toxicity might change as degradation processes, especially biodegradation goes by and intermediate products appear. These intermediates can even be more toxic than the original effluent was, posing higher risk to the ecosystem of the recipient water body. In our test series it was assessed how toxicity of a municipal wastewater sample changes during biodegradation taking into consideration different temperature regimes (10, 20 and 30 °C). Results proved our null hypothesis: after the high initial toxicity of the fresh effluent sample toxicity did show a further increase. Biodegradation resulted in toxicity reduction only after an approx. 2 week-period.

Keywords: Whole effluent toxicity – environmental risk – biodegradation – bioluminescence – ToxAlert

INTRODUCTION

In many countries, both chemical and biological analysis/testing of effluents are required by regulations either before discharging to sewage treatment plants or before discharging to the recipient. However, most legislation is directed towards regulation of discharges to the receiving environment. In Europe, the most comprehensive legal mean is the EU Water Framework Directive (2000), which aims at maintaining and improving the aquatic environment in the Community, and is concerned primarily with protecting receiving waters from pollution.

Numerous authors have used ecotoxicological tests to assess the effectiveness of wastewater purification/treatment processes [3, 7]. Toxicity, however, is not a static

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parameter: toxic potential of an effluent will change due to degradation processes such as photolysis, hydrolysis, oxidization and biodegradation. The risk of toxic effects in the recipient depends primarily on the time-related variation of toxicity of the effluent.

Ready biodegradability of an effluent is a key parameter to assess hazards an effluent poses to its environment either it is treated in a municipal treatment plant or discharged to a recipient surface water [17].

The latest version of OECD tests for ready biodegradability [13] aims at predicting whether a chemical has the potential to be easily biodegraded in the environment. In these protocols usually oxygen uptake is measured, involving long-term (14- to 28-day) respirometer testing. However, these tests which use chemical end-points such as COD do not give an indication on how toxicity of the chemical will change due to the formation of intermediate products. These tests were not designed to predict the behaviour of effluents in the aquatic ecosystem. Therefore although some methods have been described to evaluate the biodegradability of chemicals in environmental water [10], no relevant OECD Test Guidelines have been proposed so far. Instead, simulation tests exist such as the stream model of Shimp et al. [15] or the die-away test of Anderson et al. [1].

Biodegradation in the environment is affected by many factors such as concentration of the effluent, exposure and the composition of microbial communities. In a recipient water presence of competent bacteria can be expected [20]. For testing biodegradability, it has been recognised that a mixed community of natural origin is more capable of degrading a wide range of compounds than pure cultures of selected strains [13].

During degradation processes not only concentration of the chemicals (and therefore exposure) will change but also, photo-degradable, hydrolytically unstable, oxidizable and biodegradable substances in addition may form such breakdown products which can be even more toxic than the parent substance was. Evaluation of the biodegradability is a key element of hazard identification of whole effluents, and it should comprise toxicity testing as well [22–23].

The fact that during degradation such intermediate products might appear which are more toxic is not only a hypothetical suggestion [4] but has been demonstrated experimentally for selected types of industrial wastewaters of high organic matter content [8]. It seems very likely that toxic wastewater effluents can pose even more serious risk to the environment long after they were discharged.

Our basic aim was to test how degradation processes, especially biodegradation affect the toxicity of communal wastewater and to predict its behaviour at different temperature regimes of 10, 20 and 30 °C. Also, tests were designed to reveal if inoculum taken from the recipient water body can influence the biodegradation process, in other words, what potential different microbial communities have to degrade communal wastewater.

MATERIALS AND METHODS

Raw wastewater sample was collected from the municipal treatment plant of Veszprém. Capacity of the plant is 12,000 m^3/day.

For toxicity assessment, a commercial assay marketed as ToxAlert® (Merck) was used using the luminescent seawater bacterium *Vibrio fischeri* NRRL B-1117. Bacterial bioluminescence is attributed to the activation of the enzyme luciferase with luciferin [11]. The attenuation of light emitted by bacteria in presence of a toxicant is related to the inhibition of this reaction. Reduction in light output may be measured after exposure to a toxic sample for 5 to 30 minutes.

This test was selected as (i) being similar to measuring respiration inhibition, since luminescence is a measure of the rate at which the bacteria produce ATP in the course of their respiratory metabolism and (ii) having a very short exposure, therefore being able to give an indication of actual (instantaneous) toxicity.

The protocol described by BS EN ISO 11348.3, Part 3 – Method using freeze-dried bacteria [2] was used. The ToxAlert®100 luminometer calculates all values automatically.

For providing a competent bacterial community, inoculum was taken from the recipient stream, Channel Séd [21]. Inoculum and the sample were mixed in a 1:1 ratio. For each test, the dilution series of 6.25%, 12.5%, 25%, 50% and 100% sample was used as suggested by the WET method manuals [18, 19]. Samples were aerated prior to toxicity testing.

Three temperature regimes were set: one series of samples was kept at 10 °C, the second at room temperature (approx. 22 °C) and the third at 30 °C.

Samples were marked during the assay as follows:

R10: raw sample at 10 °C

R22: raw sample at room temperature (22 °C)

R30: raw sample at 30 °C

RS10: raw sample + inoculum from the recipient water body (Séd) at 10 °C

RS22: raw sample + inoculum from the recipient water body (Séd) at room temperature (22 °C)

RS30: raw sample + inoculum from the recipient water body (Séd) at 30 °C

Toxicity tests were conducted regularly. First measurement was made at the beginning of the study (Day 0), followed by the second one on Day 5, assuming a lag period of 5 days [12]. From Day 5 to Day 26, toxicity measurements were completed approx. at weekly intervals, as our previous study [18] suggested that most significant changes can be expected during the first month. Also, most biodegradability test protocols cover 28 days [13]. Bioassays were completed from Day 26 to Day 96 approx. at two-week intervals, till Day 96. In order to represent real-world conditions, the minimum incubation time is considered 8 weeks [14, 16]. However, Strevett et al. [16] report that for some chemicals this period may be insufficient, requiring an incubation time of 100 days. A final assay was completed on Day 153.

COD measurements were also made, following the protocol described by MSZ ISO 6060: 1991 standard.

RESULTS

Figures 1–2 show toxicity changes from Day 0 to Day 153. The raw communal wastewater exerted a high toxicity (expressed as 80.9% bioluminescence inhibition) and risk to the environment. With no inoculum added, this toxicity even increased during the first 12 days, showing somewhat different patterns in the three exposure regimes: at room temperature (sample R22) inhibition was already as high as 91.8%, still increasing to Day 12, reaching its maximum, 97.8% inhibition. Under the other two temperature regimes, 10 °C (sample R10) and 30 °C (sample R30) first a slight decrease was shown, than by Day 12 the maximum toxicity appeared, reaching 94.9 and 97.3% inhibition, respectively. Afterwards, between Day 12 and Day 19 first a rapid decrease could be observed, than from Day 19 on, a steady, slower decrease, finally reaching a "tolerable" level of approx. 30% of inhibition by Day 26 in the case of R22 (34.55%), by Day 40 in the case of sample R10 (34.15% inhibition) and finally by Day 54 in the case of sample R30 (32.45% inhibition). It should be noted, however, that this sample showed an anomalous low inhibition of 26.1% on Day 19, which can be most likely attributed to test error.

In the presence of the inoculum, the maximum toxicity was lower for samples RS10 and RS22 on Day 12 than it was the case with samples without inoculum. Afterwards, a much more rapid decrease was initiated, resulting in inhibition below 30% even by Day 19. (There was a slightly higher inhibition value for RS22 on Day 54.) Sample RS30, however, did show a somewhat different pattern: toxicity increased to 94.3% of inhibition by Day 12, than a rapid and steady decrease could be observed, but finally toxicity started to increase again, showing a 40.7 of inhibition by Day 153.

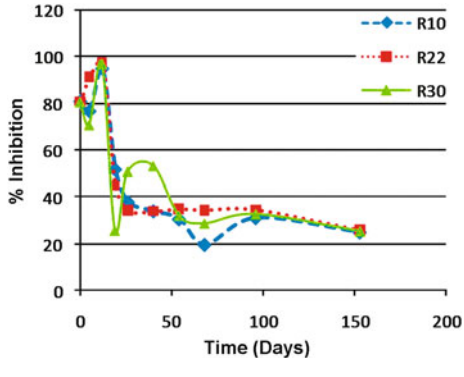

Fig. 1. Toxicity changes of the raw wastewater sample without inoculum, at different temperature regimes

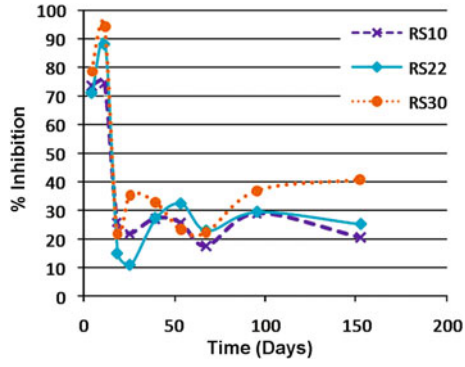

Fig. 2. Toxicity changes of the raw wastewater sample with inoculum, at different temperature regimes

Figure 3 shows COD changes from Day 0 to Day 153. COD changes, on the contrary, did show a more uniform patter, first a rapid than a steady decrease for all sam-

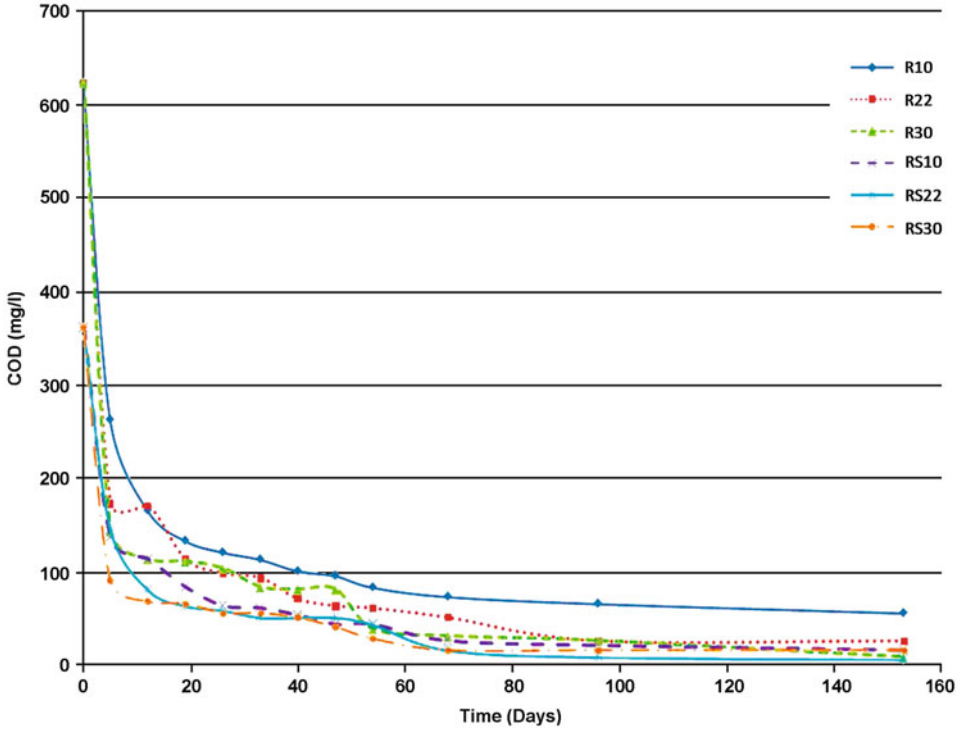

Fig. 3. COD changes of the raw wastewater sample with and without inoculum, at different temperature regimes

ples, with no differentiation between inoculum-free and inoculated samples. Also, different temperature regimes seemed to have no effect on the pattern of COD reduction.

DISCUSSION

Although pattern of toxicity changes varied amongst samples as described above, there were common features, proving our null hypothesis. During the first period, till Day 12 toxicity of all effluent samples increased, both for raw and inoculated ones and for all temperature regimes, posing high environmental risk during approx. two weeks. A decline in toxicity began only after this peak. The two-week period before effective biodegradation begins can be regarded as an average: household wastewaters are very complex mixtures, for example Eriksson et al. [5] have estimated that grey wastewater from Danish households could potentially contain more than 900 different xenobiotic organic compounds (XOCs). Only from wastewater originated from bathrooms, almost 200 different organic chemicals were identified [6]. The very diverse nature of household wastewater makes it very difficult to follow the processes by which biodegradable substances break down and to analyse the toxicity of each intermediate product separately.

Whole effluent toxicity, as the definition implies, gives a good estimation on the aggregate environmental hazard of the effluent. Our results underline a serious environmental risk: raw municipal wastewater if untreated might undergo such degradation which results in toxic intermediates, prolonging the period during which ecosystem of the recipient freshwater might be impacted. However, it seems also reasonable that in the presence of competent, pre-adapted microbial community biodegradation can be enhanced and risk can be sooner mitigated.

Naturally, it is not feasible technologically to regularly monitor a 3-month biodegradation process. The *Vibrio fischeri* bioluminescence inhibition test, however, can give some indication for the predicted behaviour of selected effluent types [9].

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