Inhibitory effects of non-ionic surfactants on the microbial activity of activated sludge system

Mª D. Coello Oviedo, D. Sales Márquez, R. Rodriguez-Barroso and J. Mª Quiroga Alonso

ABSTRACT

The purpose of the present work was to study the influence of a non-ionic surfactant, a nonylphenol with four ethoxylated units (NP4EO), on the activity of the microbial population present in a laboratory-scale activated sludge unit. Traditional control methods for this type of unit were used (measurement of suspended solids and chemical oxygen demand) as well as specific techniques for the measurement of bacterial activity (dehydrogenase activity and specific oxygen uptake rate) and the results were compared. It was shown that the Specific Oxygen Uptake Rate (SOUR) is the simplest and quickest way to carry out routine control of activated sludge activity, while measuring dehydrogenase activity provides more complete control. The results obtained indicated that there was no inhibition of microbial activity at a concentration of 5 ppm of NP4EO, which was not the case with a concentration of 10 ppm.

Key words | activated sludge, dehydrogenase activity, microbial activity, non-ionic surfactants, nonylphenol, specific oxygen uptake rate

INTRODUCTION

Non-ionic surfactants are one of the most commonly used groups of synthetic surfactants, comprising almost half of the totals amount of synthetic surfactants consumed in Europe. Alcohol ethoxylates (AE) and alkyl phenol ethoxylates (APE) have recently constituted around 74% of the total amount of non-ionics in use (Petrovic & Barceló 2004).

There has been a widespread trend in recent years to install wastewater treatment plants to put a stop to the progressive deterioration of natural sources of water supplies. As these plants mainly receive sewage with a high organic load, biologically based treatments are the most suitable, in particular, activated sludge treatment. The effluent quality of the plant is dependent on the proper running of the biological unit.

The correct functioning of this system requires not only control of the variables that can influence the development of microorganisms (pH, dissolved oxygen, nutrients, etc.), but also monitoring of activated sludge activity in order to avoid operational problems that may arise, such as bulking.

At present, when any of these problems occur, the solutions adopted usually involve shutting down the plant temporarily until a newly active biomass has been achieved, or continuing to operate with a deficient system. Whatever the solution, the plant loses operational capacity, which has a negative effect on the purification process.

The aforementioned operational problems can be caused by several factors, including the presence of inhibitory substances.

Surfactants are among the organic compounds found in wastewater which can inhibit the bacterial population in activated sludge systems (Uysal & Turkman 2007). The presence of these surfactants in wastewater is due to their use in many processes, such as the production of detergents, to modify viscosity and in emulsifiers. The different types of surfactant include alkylphenol.
polyethoxylates, non-ionic substances used in various industrial processes (treatment of heavy metals, textile processes, the paper industry, the agrochemical industry, etc.). The biodegradation of these surfactants creates metabolites of a toxic nature (AP1EO, AP2EO, etc.) (Ash & Ash 1987).

The aims of this study were to review the different ways of measuring the activity of the microorganisms inside an activated sludge unit in order to control it as well as to investigate the effect of a non-ionic surfactant on the microbial activity of the unit.

MATERIALS AND METHODS

Laboratory pilot scale plant reactor

The effect of the non-ionic surfactant on the microbiota present in the activated sludge unit was studied by means of a bioreactor designed especially for this purpose according to the specifications of the OECD screening method (BOE 1985) for determining the biodegradability of anionic and non-ionic surfactants (Figure 1).

Synthetic wastewater

The reactor was fed continuously with a synthetic nutrient. The following were dissolved in 1 L water: Peptone, 160 mg; Urea, 30 mg; Beef extract, 110 mg; NaCl, 7 mg; CaCl₂·2H₂O, 4 mg; Mg SO₄·7 H₂O, 2 mg; K₂HPO₄, 28 mg; and the non-ionic surfactant (NP4EO).

The synthetic sewage was freshly prepared each day using tap water and kept in a refrigerator. Prior to its use in the preparation of synthetic sewage, tap water was left for 24 h to reduce its free chlorine concentration (Coello et al. 2004).

Surfactants

The surfactant used in these experiments was a nonylphenol polyethoxylate with an average of four ethoxylated units (NP4EO) and a wide range of oligomers of 2 and 6 ethoxylated units (Figure 2).

Analytical procedures

The analytical procedures employed were designed to determine microbial activity and to control the functioning of the pilot plant. The functioning of the pilot plant was monitored by periodic measurements of total suspended solids (TSS), volatile suspended solids (VSS) and by determining the COD of the effluent, according to standard methods APHA-AWWA-WPCF (1995).

Microbial activity was calculated by measuring dehydrogenase activity (DHA) and the specific oxygen uptake rate (SOUR).

Dehydrogenase activity was recorded using the method of Koopman et al. (1984), while the determination of the Specific Oxygen Uptake Rate (SOUR) was carried out following the method recommended by APHA, AWWA and WPCF (1995).

Analytical method used for the analysis of NP4EO

The separation, identification and quantification of the oligomers contained in the commercial formula under study was carried out by means of normal phase HPLC with

![Figure 1](image1.png)

![Figure 2](image2.png)
fluorescence detector, operating at 225 nm (excitation) and 304 nm (emission).

A 5-μm particle size Hypersil AP-2, NH2 column was used for the stationary phase (internal diameter: 4.6 mm, length: 250 mm). The analytical column was used in conjunction with a μBondapak NH2 precolumn.

The samples of effluent were pretreated before being injected into the chromatograph. They were dried at a temperature of 45–55°C, evaporation being aided by means of a nitrogen current. Once the solvent had evaporated, the dry residue was resuspended in 1 mL of hexane/isopropanol (80/20). The yield of this process of concentration and purification was 94%.

Identification was accomplished by injecting internal standard solution of known composition. In order to quantify the peaks registered and calculate the amount of NPEO in a sample taken from the assay, the concept of equality of the molar absorption coefficients of all oligomers was applied (Wang & Fingas 1993).

RESULTS AND DISCUSSION

Operation of the reactor

The plant was started up by filling the aeration vessel with 3.5 L of activated sludge; the concentration of suspended solids being 2.8–3 mg/L. Synthetic sewage (without any surfactant) was then applied.

The flow rate to the activated sludge units were progressively increased from 100 mL/h to a maximum of 400 mL/h, which, given the volume of the reactors, represents a HRT (hydraulic rate time) of 7.5 hours. The microorganisms were adapted in this way to the synthetic medium.

Only synthetic wastewater was fed continuously until stable COD removal was obtained.

Influence of the concentration of NP4EO

Results of the experiments conducted for non-ionic surfactant runs are presented in this section. In all figures, the mean values of measurements are presented. The effect of a non-ionic surfactant (NP4EO) on the microbiota present in the activated sludge unit was studied by the methods of control and measurement of microbial activity.

5 ppm of NP4EO

Figure 3a shows the evolution of suspended solids during the assays. Volatile suspended solids (VSS) expressed the changes in the amount of biomass in the system. It can be observed that after a slight initial descent as a result of contact with the surfactant, the trend of this variable remains practically constant throughout this assay (as in the control) (Coello et al. 2004).

The COD removal efficiency of the reactor during the experiments is presented in Figure 3b. This decreased from

![Figure 3](https://iwaponline.com/wst/article-pdf/60/4/1033/448972/1033.pdf)
day 5 onwards, COD removals being observed to vary from 85% to 70%. This minimum could be caused by the inhibition and evacuation of superior microorganisms in the reactor due to the presence of the surfactant. This compound product provokes the appearance of dispersed flocs in the clarifier and therefore a decrease in the efficiency of the process. The system recovers from the 8th day of experimentation onwards, turning to some performance depuratives similar to those of the control (COD removal greater than 80%) (Liwarska & Bizukojc 2007).

Figure 3c shows the evolution of the specific oxygen uptake rate and dehydrogenase activity against time; a similar trend being observed in both measurements. At the beginning of the assay, the values of both measures are seen to increase up to a maximum value on the 5th day of the experimentation. This behaviour of the microbial activity variables is probably provoked by the presence of the surfactants. Different authors have verified (Figuers et al. 1997; Mezzanotte et al. 2003) that the presence of non-ions in the influent did not deteriorate dehydrogenase activity of activated sludge biomass. The degradation of surfactants by means of β-oxidation increases the pool of acetyl-CoA in the cells to a greater degree than the catabolism of acetate and amino acids. Microbial activity decreased after the 6th day of the assay. This drop in activity rates reached a minimum on day 9. During the period in which superior microorganisms are eliminated; the activity microbial parameters registered lower values. The microorganisms adapt after this short period of inhibition; microbial activity values subsequently being similar to those determined in the assay control (Table 1).

It can thus be concluded that at a concentration of 5 ppm, the nonylphenol caused inhibitory effects on the activated sludge system, acting mainly on superior microorganisms. This inhibition did not reach important levels and had very little effect on the purification process. However, it should be noted that the decantation process deteriorated, which had direct effects on the effluent quality as regards the amount of suspended solids in the effluent.

10 ppm of NP4EO

The results obtained in this assay were different from the previous one.

The evolution of Suspended Solids is shown in Figure 4a. The values for this parameter fell over time, with a minimum level of suspended solids between days 15 and 20 (1.5–2 g/L). Such a trend could be produced by the
possible toxic effect of NP4EO at a greater concentration. After the 15th day, the TSS and VSS variables show a more or less constant trend until the end of the assay. This could be due to the saponifying properties of surfactant.

As far as the efficiency of purification is concerned, i.e. the percentage of COD removal, it can be seen from Figure 4b that there is a significant decrease, probably due to the removal of part of the microbiota (in keeping with the decrease in VSS). The plant was not operating suitably, as good clarification did not take place due to the loss of biomass in the effluent resulting from the absence of superior microorganisms.

The efficiency of depuration descended along the assay, concretely the degree of COD removal on day 15th was minor to 50%. The COD removal rate was lower than in the control run (Table 2). This result shows that a higher concentration of surfactant has a negative effect on the efficiency of the system. In the system, the microorganism is inhibited by the presence of non-ionic surfactant. The effect on the microbiota is greater, especially on the superior microorganism, which disappear as was observed using optical microscopy. This disappearance cause a fall in flocculation levels, which in turn produces a loss of biomass through the effluent and the rate removal COD decrease.

Finally, Figure 4c shows the specific oxygen uptake rate and dehydrogenase activity, giving an idea of the real activity which exists in an activated sludge system with a concentration of 10 ppm of the non-ionic surfactant, NP4EO. Dehydrogenase activity and the specific oxygen uptake rate behave analogously. Microbial activity increases considerably during the first three days of exposure of the microbiota to the surfactant; this period was shorter than in the assay with 5 ppm due to the higher concentration.

A similar effect has already been described by other authors (Edwards & Sherrard 1982; Coello et al. 2004), who reported that the initial increase in activity caused by contact with the toxin is followed by a decrease in activity due to the inhibition resulting from the exposure of the microbiota to the toxic compound.

When the concentration of surfactant was 10 ppm, the microbial activity decreased to minimum values (SOUR = 138 mgO₂/g VSS d and DHA = 31 mgO₂/g VSS d). These values represent more than 60% inhibition of activity microbial.

After reaching these minimum values, microbial activity remained practically constant until the end of the assay. However, those results are much lower than those obtained in the control assay (Table 1), thus reflecting the inhibition caused by the addition of the surfactant.

The results obtained in the experiment allow us to state that the applied dose of surfactant produces negative effects on the activated sludge system.

Relation between residual concentration of NP4EO and microbial activity

Table 2 shows an example of surfactant concentration measurements in the effluent and the separation percentage for the continuous assay with 5 ppm of NP4EO.

A high separation of the surfactant in the effluent was observed throughout the assay. This separation may be due to the biodegradation process of the ethoxylated chain as a result of the hydrolytic enzymes of the microorganisms, which break down the ethoxylated groups of the chain into ethylenglycol and then into CO₂ and H₂O. Another reason could be the ease with which these compounds adsorb onto the suspended solids present in the reactor, which is greater due to the decrease in the number of ethoxylated units resulting from the biodegradation process. A slight decrease in the percentage of biodegradation was also observed after Day 7.

These values for NP4EO removal are in keeping with the COD values described in the previous section (initial decrease in COD until Day 7–8 and an increase in COD values over the following days). Thus, as other authors have stated (Jonkers et al. 2001), it can be seen that the principal microorganisms of the biodegradation process are bacteria,
which develop in the absence of superior microorganisms (such as protozoans). These superior microorganisms decrease in number during the first part of the experiment (the first 7 days) as a result of the toxic effect of the surfactant. This facilitates the growth of bacteria, increases the percentage of biodegradation and decreases the effluent COD values. With the reappearance of the protozoans as a result of their adaptation, the percentage of biodegradation of the surfactant decreases, thus causing an increase in effluent COD values.

The relation between activity measurements and percentages of surfactant biodegradation also confirms the previous results. After a slight increase in microbial activity, brought about by the influx of the toxin as already established by several authors, a decrease in bacterivorous ciliates is produced, thus giving rise to the maximum rate of biodegradation. Activity returns to normal values from day 9 onwards due to the presence of bacterivorous ciliates, which leads to a decrease in the percentage of surfactant biodegradation.

Figure 5 shows an example of the chromatograms corresponding to the evolution of the different oligomers of NP4EO during the assay. An analysis of these chromatograms reveals a similar distribution to that found by other authors (Brown 1986; Brunner et al. 1988) in treatment plants, although they differ from those obtained by the same authors in discontinuous experiments, in which the products of biodegradation are almost exclusively NP1EO, NP1EC and NP2EC (Uysal & Turkman 2007). Besides the different system used (continuous experiments in this study and discontinuous assays in others), this difference could be due to the presence of a high concentration of suspended solids in the system. Consequently, the shorter chain oligomers which form during the process (NP1EO, NP2EO) and whose concentration should increase considerably due to their low solubility tend to adsorb onto the suspended solids. The percentage in the medium of longer chain oligomers, which are more soluble and more easily biodegradable, remains practically stable, as those lost through biodegradation are replaced by the continuous process (Petrovic & Barceló 2004) (Table 2).

However, a more detailed analysis of the percentages of areas of the respective homologues reveals a slight increase in shorter chain and more toxic homologues (NP1EO, NP2EO and NP3EO), although this increase is not sufficient to cause significant variation in the microbial activity of the system.
CONCLUSIONS

Synthetic wastewater containing different concentrations of non-ionic surfactant was subjected to biological treatment in a laboratory scale activated sludge unit. The general conclusions which can be drawn regarding the effects of the non-ionic surfactant NP4EO on the system are the following:

A concentration of 5 ppm of the non-ionic surfactant nonylphenol does not have lethal effects on the biomass of the activated sludge. It does, however, inhibit superior microorganisms, mainly rotifers. This brings about a growth in bacteria populations, thus causing an improvement in effluent quality. Although there are fluctuations in the microbial activity of the microorganisms in the activated sludge due to contact with the surfactant, the system soon recovers and returns to normal activity values. A clear inhibition of the microbiota in general is not produced at a concentration of 5 ppm, and although the activity of the more sensitive microorganisms, i.e. the superior microorganisms, is inhibited by the presence of the surfactant, the rest of the microbiota develops normally.

With a concentration of 10 ppm of NP4EO, the results are significantly different from those obtained at the lower concentration. There is a general decrease in values of all the variables, thus showing that the surfactant takes effect just as its degradation begins and its degradation products, which are much more toxic than NP4EO, start to appear in the medium. An important inhibition occurred in activated sludge system. All the variables were affected by the presence of NP4EO to this concentration.

REFERENCES


