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Inhibitory effects of polar and non-polar organic substances on activated sludge activity

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ABSTRACT

As is well known, several organic compounds, more often found in industrial wastewater, exhibit inhibitory effects on biomass activity, therefore, leading to poor wastewater treatment efficiency. This study presents the experimental results on the inhibition effects of 14 polar and non-polar organic compounds on the activity of activated sludge in the concentration range of 50–1,000 ppm. Two different activated sludge samples used, one of low activity and another of high activity. The organic compounds tested are: phenol, 2-chlorophenol, nitrophenol, resorcinol, dioxane, acetonitrile and benzotriazole (polar); and toluene, styrene, cyclohexane, tetrachloromethane, paraxylene, benzene and hexane (non-polar). The results demonstrate that polar compounds exhibit higher toxicity than non-polar compounds and hydrophobicity plays a crucial role. The effects of polarity, hydrophobicity, solubility and volatility are discussed and the conclusion is that inhibition is complex phenomenon requiring extensive theoretical and experimental studies.

Keywords: Recycle activated sludge; Inhibition; OUR; Organic compounds

1. Introduction

The biological treatment is one of the most widely used processes for the removal of organic pollutants from wastewater. The microorganisms of the activated sludge are usually bacteria, protozoa and rotifers [1,2]. The degradation extent of the organic compounds in an aerobic reactor is highly dependent on dissolved oxygen, temperature, organic matter, nutrients and micronutrients [3–5]. These parameters are also important for consideration of reasonable operation of wastewater treatment plants. Currently a large amount of organic contaminants present in wastewater are largely generated in industrial plants and a characteristic example is phenolic compounds. These compounds are considered as priority pollutants by US Environmental Protection Agency and due to the large use of phenol and its derivatives in many industries, such as petroleum processing plants and pharmaceutical industry, phenolic compounds are widespread in the environment [6]. A serious side effect of the existence of such compounds in wastewaters is that if not treated properly may lead to inhibition of biomass activity, therefore, leading to poor effluent quality [7].

The inhibitory effects of heavy metals and organic compounds found in effluents of many petrochemical and processing industries have attracted great interest. Studied organic compounds include aniline, benzotriazole, benzene, styrene, phenol, dichlorophenols, *p*-cresol, catechol, resorcinol, pyrogallol, hydroquinone, dinitrophenol and naphthenic acids [6,8]. One of the most complete studies is that of Cai et al. [9] who studied 24 aromatic chemicals typically existed in the industrial wastewater classified into three

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groups of benzenes, phenols and anilines. Many of these organic compounds are highly toxic, hardly degraded and could cause significant inhibition of activated sludge activity.

Different approaches have been explored to evaluate toxicity of wastewater constituents on activated sludge. These include biochemical oxygen demand (BOD), tests on pure cultures as well as mixed bacterial consortia, ecotoxicological tests, respiration method (Oxygen uptake rate, OUR), nitrogen and ammonia uptake rate by use of activated sludge [6,9–11]. The experimental reactors used are diverse, such us batch and continuous flow while the activated sludge used in several studies is different, such as primary activated sludge, acclimatized activated sludge and modified activated sludge [6,9,12]. Among all the approaches the respirometric method by use of activated sludge is considered the most effective, simple and low cost [9,13]. This method is based on the principle of the activated sludge respiration activity inhibition in the presence of toxicants where the decrease in the oxygen uptake is attributed to the compound's toxicity [13,14].

The purpose of this paper is to present new experimental results on the inhibitory effects of several organic compounds on municipal recycle activated sludge activity by use of OUR kinetics and discuss the possible influence of physical properties of the organic compounds on activated sludge activity by testing in total 14 organic compounds in the concentration range of 50–1,000 ppm. The compounds tested are: phenol, 2-chlorophenol, nitrophenol, resorcinol, dioxane, acetonitrile, benzotriazole, toluene, styrene, cyclohexane, paraxylene, benzene, hexane and tetrachloromethane.

2. Materials and methods

The main objective of the experiment was to determine the specific rate of oxygen uptake rate (sOUR), which is attributed to the biodegradation of organic carbon by heterotrophic bacteria. The simplified bioreaction, which takes place by heterotrophic bacteria is the following:

$$\text{Organic carbon} + O_2 + \text{nutrients} \rightarrow CO_2 + H_2O + \text{new biomass}$$

Following sampling, 1 L of activated sludge is placed under aeration overnight to reach endogenous conditions. Then, 160 mL of activated sludge is placed at a 1 L Erlenmeyer flask and mixed with 340 mL of water, 85 mL of sodium acetate solution (1 g/L) as a carbon source and a certain amount of inhibitor under continuous aeration and agitation so that the dissolved oxygen (DO) level remains high (minimum 4 mg/L). The aeration was achieved by using blowers to provide air at a rate of 20 L/min each. Agitation was provided by use of a magnetic stirrer. The temperature of activated sludge solution was maintained at 20°C ± 2°C and pH at 7.5 ± 0.4. If necessary, $NaHCO_3$ was added dropwise to adjust pH. DO of the liquid solution was measured using a dissolved oxygen probe (WTW), and the pH of solution was measured using a pH meter (Mettler Toledo). Oxygen consumption only due to organic compounds oxidation and not nitrification was studied in this research. For this, 5 mL of allylthiourea solution (1 g/L) was added to the slurry in order to inhibit nitrification process. The total suspended solids (TSS) of the reactors mixture was determined by filtrating 10 mL of sample on GF/C filters Whatman and subsequent drying in furnace at 105°C overnight. Under the experimental conditions used the average TSS was 1.54 ± 0.34 g/L. The properties of the compounds used in this research are summarized in Table 1.

For measuring the sOUR, after 10–60 min the biomass is transferred in a BOD flask under very mild agitation (magnetic stirrer) and without any aeration and DO is recorded with oxygen meter for 5–10 min. By plotting DO vs. time and calculating the slope the OUR_{max} can be calculated for each compound expressed in $mgO_2/gTSS\ h$ (sOUR). The results are corrected for temperature variations and the reference

Table 1
Organic compounds properties

	Dipole moment (debye)	Solubility in water at 25°C (mg/L)	Octanol–water partition coefficient ($\log K_{ow}$)	Vapor pressure at 25°C (mmHg)	Form
4-Nitrophenol	5.43	1.16×10^4	1.91	0.0005	Solid
Acetonitrile	3.44	10^6	–0.34	88.8	Liquid
Resorcinol (*m*-dihydroxybenzene)	2.09	7.17×10^5	0.8	0.000489	Solid
Benzotriazole	1.46	1.98×10^4	1.44	0.04	Solid
Phenol	1.24	8.28×10^4	1.46	0.35	Solid
2-Chlorophenol	1.13	1.13×10^4	2.15	2.53	Liquid
1,4-Dioxane	0.45	10^6	–0.27	38.1	Liquid
Toluene	0.31	526	2.73	24.8	Liquid
Styrene	0.13	310	2.95	6.4	Liquid
Hexane	0.08	9.5	3.9	153	Liquid
p-Xylene (1,4-dimethylbenzene)	0.07	162	3.15	8.84	Liquid
Cyclohexane	0	55	3.44	96.9	Liquid
Benzene	0	1,790	2.13	94.8	Liquid
CCl_4	0	793	2.83	115	Liquid

temperature of 20°C was used. For this purpose, the following temperature correction equation was used [15]:

$$sOUR_{20} = sOUR_T/1.09^{(T-20)}$$

OUR and TSS measurements are repeated at least twice for each reactor and the average value is used. Inhibition is calculated by subtracting the observed sOUR of the control reactor from that of the inhibitor reactor.

The recycle activated sludge used in this research was sampled from the Astana municipal wastewater treatment plant. Sampling was repeated in average of every 2 d and control experiment was done on each sample in order to account for activated sludge activity variability. Two experimental campaigns were implemented the first in summer 2015 and second in winter 2016.

3. Results and discussion

The activated sludge used in the two experimental campaigns exhibited very different activity. In winter 2016, sOUR was in the expected range with an average of 28.15 ± 3.24 mgO_2/gTSS h and low activity variability from day to day (11.5%). In summer 2015, wastewater treatment plant was in a start-up period following a replacement of the activated sludge and as a result sOUR was low with an average of 8.02 ± 2 mgO_2/gTSS h and high activity variability from day to day (24.87%). The results are shown in Figs. 1–14. As is evident, the two activated sludge samples are qualitatively similar and the observed differences do not alter the trends.

Before discussing the results is useful to mention that comparison of sOUR values and inhibition from different studies is difficult and data are scattered, partly due to the different methods used. For instance, in the related literature there are extensive data on the inhibitory effects of organic compounds on isolated cultures, as for example, in the study of Katritzky et al. [10] who present LC_{50} data for 104 compounds. However, the structure of activated sludge is complex and is comprised of microorganisms, extracellular polymeric substances (EPS) and particles from wastewater that get entrapped in the flocs. EPS provide an essential polymeric matrix in which microorganisms are embedded and remain aggregated, accounting for the flocculent nature of activated sludge [16]. Furthermore, while proteins are usually the predominant component of activated sludge EPS, polysaccharides are usually found in pure cultures [17]. Thus, inhibitory effects are better represented by experiments on actual wastewater treatment plant activated sludge and thus only these studies are discussed here.

Literature review reveals that many factors influence the inhibition process, as polarity and hydrophobicity, expressed by the octanol–water partition coefficient (K_{ow}). In general, polar compounds are soluble in water and thus can give high

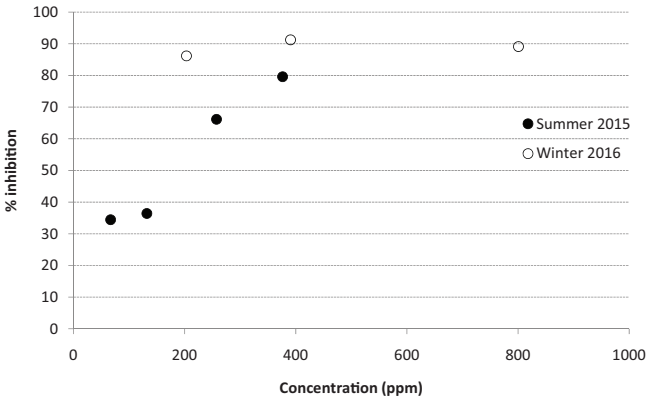

Fig. 2. Nitrophenol inhibition.

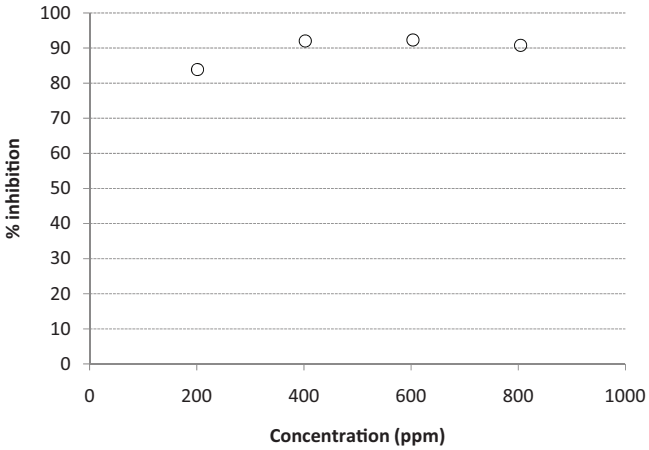

Fig. 3. Chlorophenol inhibition (winter 2016).

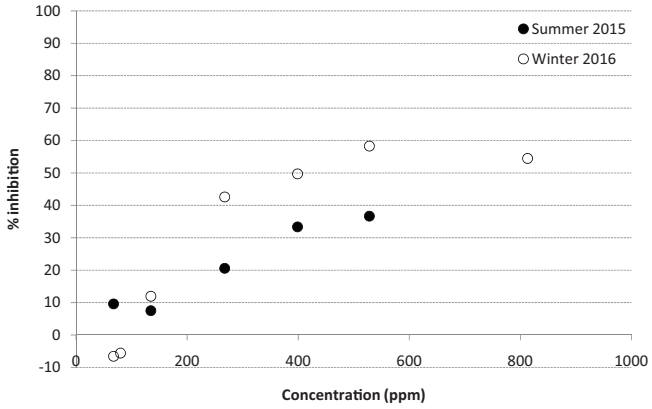

Fig. 1. Phenol concentration.

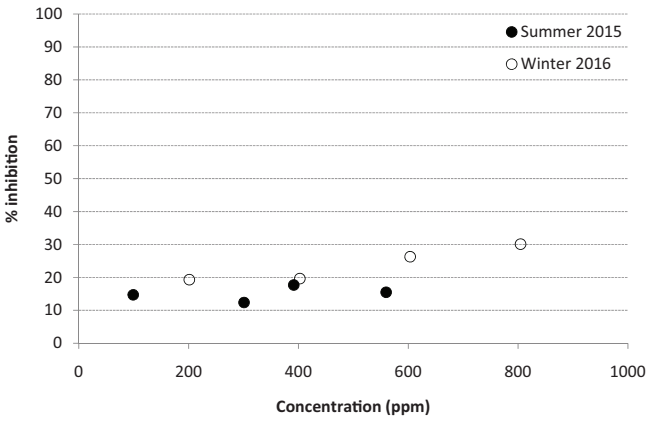

Fig. 4. Resorcinol inhibition.

concentration solutions of easily migrating molecules in an aquatic environment. Partition coefficient values represent the tendency of the chemical to partition itself between an organic phase and an aqueous phase and the higher the partition coefficient the more hydrophobic the compound. Hydrophobic chemicals will tend to avoid the aqueous phase and concentrate in more lipophilic phases of biota in activated sludge and attack and destroy their cells [9]. Cai et al. [9] presented both experimental data and models, which demonstrated the strong influence of polarity and hydrophobicity. Henriques [17] found that EPS matrix functions as a protective barrier for the bacteria inside activated sludge flocs to chemicals that it has the potential to interact with, such as hydrophobic (octanol) and positively charged (cadmium) compounds, but that the toxicity response for soluble, hydrophilic toxins (*N*-ethylmaleimide and cyanide) is not significantly influenced by the presence of the polymer matrix. Hydrophilic compounds diffuse more easily though EPS, reaching thus

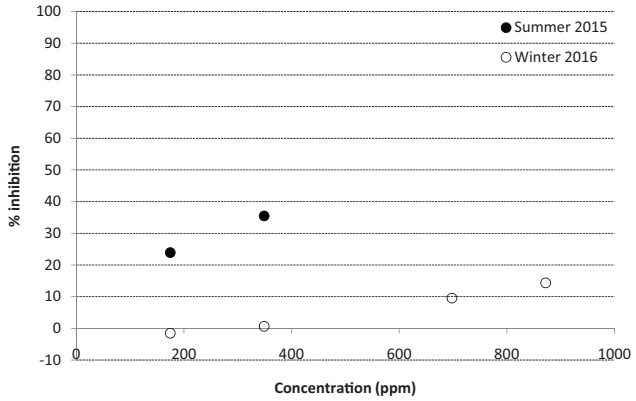

Fig. 5. 1,4-Dioxane inhibition.

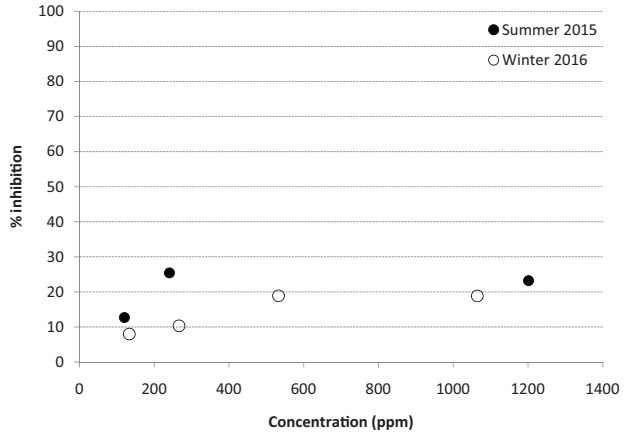

Fig. 6. Acetonitrile inhibition.

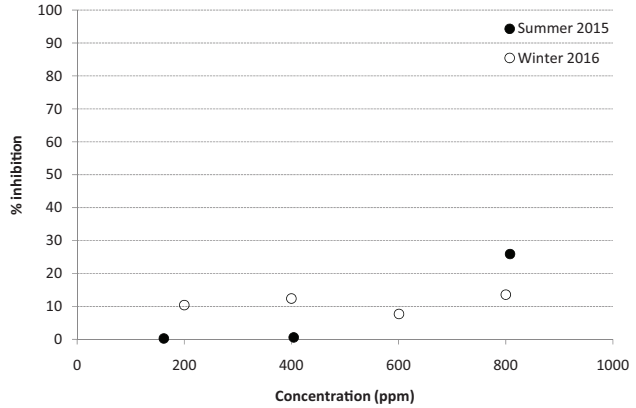

Fig. 7. Tetrachloromethane inhibition.

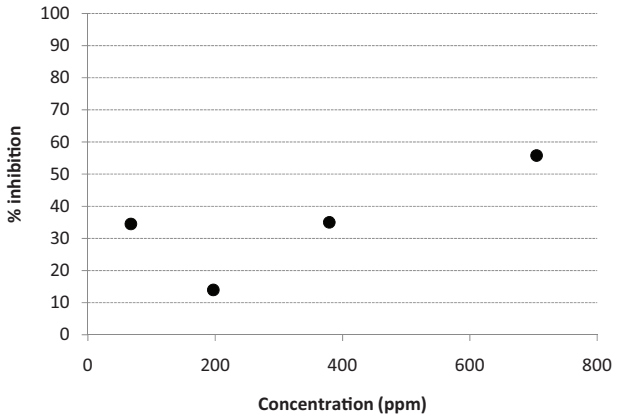

Fig. 8. Benzotriazole inhibition (summer 2015).

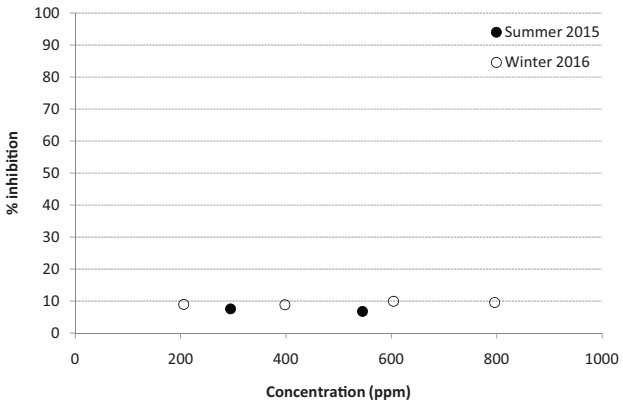

Fig. 9. Toluene inhibition.

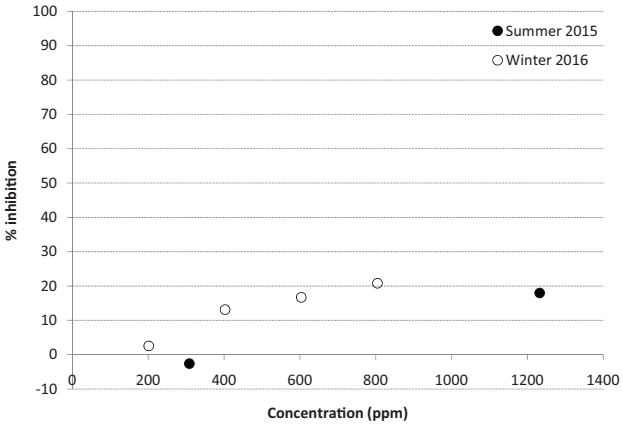

Fig. 10. Styrene inhibition.

the cells and interacting with them. Henriques [17] found that hydrophobic octanol ($\log K_{ow} = 3$) is much less toxic than hydrophilic *N*-ethylmaleimide ($\log K_{ow} = 0.58$) although solubility is relatively low and similar for both compounds, 540 and 1,000 mg/L, respectively. Thus, an ideal inhibitor balances between polarity, solubility and hydrophobicity, which are not easy as generally, polar compounds are hydrophilic. Furthermore, solubility, which is basically governed by polarity and volatility are expected to influence inhibition as well [9]. Finally, chemistry plays a significant role, for instance compounds with aromatic ring appear to be hardly biodegradable to microbial community, especially when are chlorinated.

Having these in mind, the behavior of the studied compounds can be sufficiently explained. As is seen in Figs. 1–14, all compounds showed some inhibition but is clear that polar compounds are more toxic than non-polar, especially chlorophenol, nitrophenol and in lesser extend phenol and benzotriazole. The very high toxicity of chlorophenols and nitrophenols is well documented [18,19]. The rest of polar compounds, acetonitrile, dioxane and in lesser extend resorcinol are very hydrophilic, which probably explains the relatively low inhibition levels. Also, acetonitrile and dioxane have very high volatility, which seems to contribute to their low inhibition levels. It should be mentioned that dioxane although its relatively low dipole moment is considered polar due to its very high solubility in water (Table 1). Among the non-polar compounds styrene showed relatively high inhibition, probably because of the low volatility compared with the rest of non-polar compounds. The rest of non-polar compounds are hydrophobic but they have very low polarity and high volatility and thus showed low inhibition. Also, with the exception of benzene and CCl_4 the non-polar compounds become insoluble within the concentration range investigated, thus hindering their inhibitory potential.

Results are summarized in Figs. 15 and 16 where the maximum inhibition is plotted vs. K_{ow} and dipole moment, respectively. It is interesting to note that there is K_{ow}-inhibition correlation up to $\log(K_{ow})$ of about 2 and the more hydrophobic the compound the higher the inhibition is. It is also interesting that, with only few exceptions, this value coincides with the $\log(K_{ow})$ upper limit of the polar compounds used in this study and in the study of Cai et al., as shown in Fig. 17 [9]. Above this limit, inhibition abruptly becomes very low for all non-polar compounds, which are clustered closely to each other and there is no particular trend. Concerning polarity is evident that all non-polar compounds are clustered in low inhibition values and although polar compounds show higher inhibition values there is no correlation between dipole moment and inhibition.

To facilitate the discussion, the data presented by Cai et al. [9] are processed and showed in Figs. 18 and 19 where the inhibition, expressed as LC_{50} (g/L) is plotted vs. K_{ow} and dipole moment, respectively. The present study and that of Cai et al. [9]

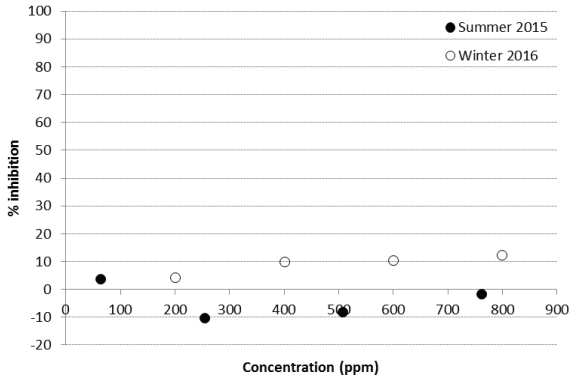

Fig. 11. Cyclohexane inhibition.

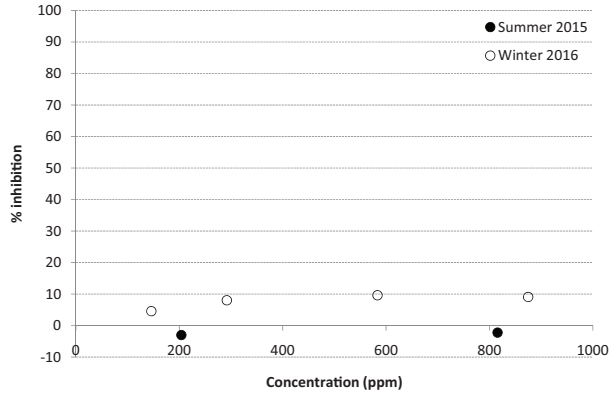

Fig. 12. Paraxylene inhibition.

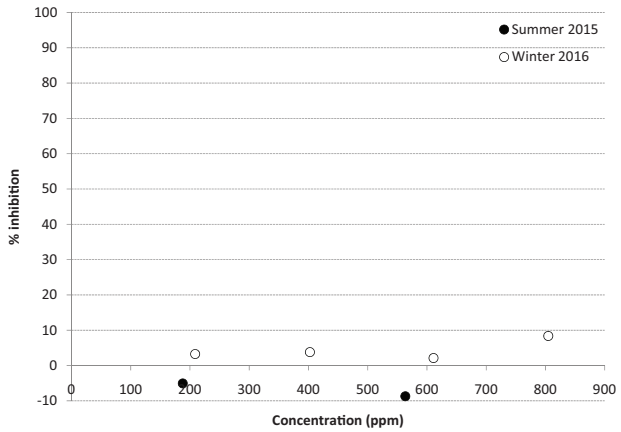

Fig. 13. Benzene inhibition.

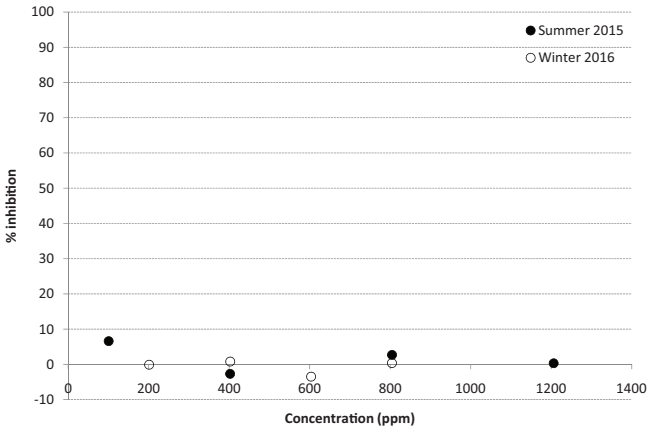

Fig. 14. Hexane inhibition.

agree that both polarity and hydrophobicity are crucial. The two studies also agree on the existence of a K_{ow}-inhibition correlation and the absence of such correlation between dipole moment and inhibition. Finally, in both studies the toxicity order of the phenolic compounds is the same, dichlorophenol > phenol > resorcinol. However, there are major differences between the two studies, of the most important being the very high toxicity of non-polar and hydrophobic compounds observed by Cai et al. [9] in contrast to the results of the present study. The differences in the experimental protocols, the use of different toxicity index (LC_{50} vs. maximum % inhibition) and the different compounds used may explain some but not all these differences. One issue identified is some confusion on the models and values presented by Cai et al. [9]. In particular, Cai et al. [9] recognize dipole moment as an important factor related with the toxicity of chemicals and they developed a multiple linear regression equation based on K_{ow} and dipole moment. We have used this model and the toxicity order is phenol > styrene > xylene > toluene, very different than the one reported by Cai et al. [9] which is xylene > toluene > styrene > phenol. Also, their experimental results showed that LC_{50} for toluene is almost four times lower than 2,4-dichlorophenol, which is a questionable result as the toxicity of chlorinated aromatics is well documented. For example, in respirometry experiments, toluene showed almost 10 times higher LC_{50} than 3,5-dichlorophenol [20].

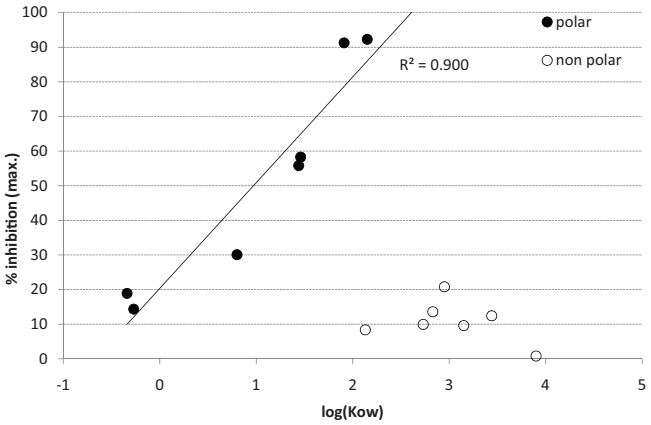

Fig. 15. Maximum observed inhibition vs. hydrophobicity (winter 2016 data).

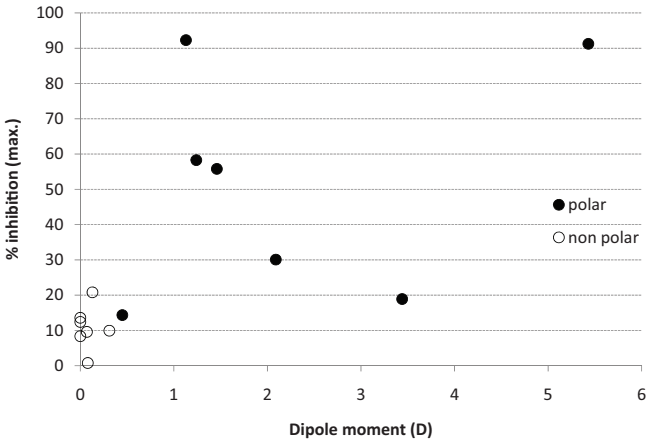

Fig. 16. Maximum observed inhibition vs. polarity (winter 2016 data).

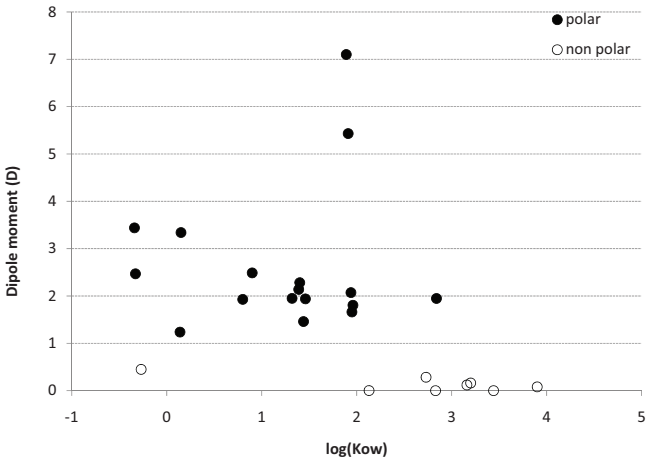

Fig. 17. Polarity vs. hydrophobicity for the investigated compounds in the present study and that of Cai et al. [9].

It is important to mention that such discrepancies in inhibition experimental results are frequent in the related literature. Ochoa-Herrera et al. [21] note that the concentrations of Cu(II) reported to inhibit microbial activity vary widely, even

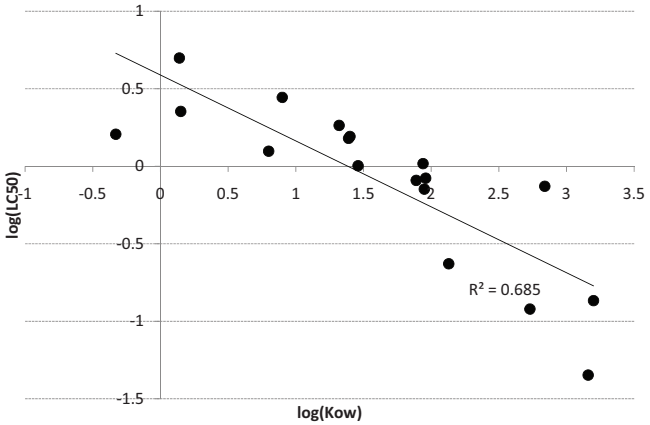

Fig. 18. LC_{50} vs. hydrophobicity [9].

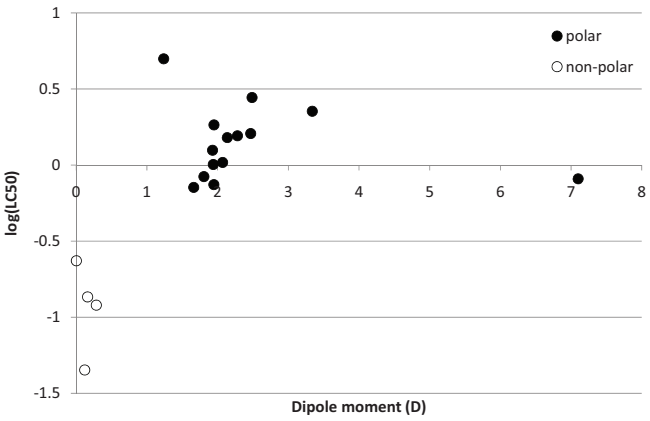

Fig. 19. LC_{50} versus polarity [9].

for microorganisms in the same trophic group. They concluded that the significant variation observed in the inhibitory levels are most likely due to differences in the experimental conditions utilized in the various assays as composition of the medium, pH values, temperature, etc. Stasinakis et al. [22] studied the effect of Cr(VI) on activated sludge activity and mention that the results were controversial, in most cases and the discrepancy in the experimental results could be explained by the dependency of metal toxicity on various parameters, such as the operational conditions of activated sludge process, the acclimatization of the biomass to the toxic compound and the chemical and microbial speciation. Ferro Orozco et al. [23] reported that the xenobiotic degradation could be modified by the presence of readily biodegradable substrates. In another study, Stasinakis et al. [24] reported that organotin compounds inhibition is diminished at high TSS concentrations. More relevant to our discussion is the effect of substrate; Stasinakis et al. [22] observed that the use of synthetic substrate leads to a gradual reduction of the microorganisms variability and that the activated sludge systems receiving synthetic substrate, may not be as resilient to changes in conditions as those developed on a more diverse substrate such as domestic wastewater. Cai et al. [9] are using wastewater from WWTP while in the present study acetic acid was used as carbon source. Thus, based on the above analysis, it is plausible that the differences in carbon source contribute to the observed discrepancies. Thus, the interactions between the inhibitors and carbon sources need to be further investigated.

4. Conclusions

The results demonstrate that while all compounds exhibit some inhibition polar compounds are more toxic than non-polar, especially chlorophenol and nitrophenol. However, highly hydrophilic and volatile polar compounds exhibit relatively low inhibition. Among the non-polar compounds styrene showed relatively high inhibition, probably because of the low volatility compared with the rest of non-polar compounds. The rest of non-polar compounds are sparingly soluble and volatile and thus showed very low inhibition. Hydrophobicity is enhancing inhibition but up to a certain limit of a $\log(K_{ow})$ of about 2 while above this limit, inhibition abruptly becomes very low for all non-polar compounds. Inhibition is a complex phenomenon governed by hydrophobicity, polarity, solubility, volatility and reactivity of the organic compounds.

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