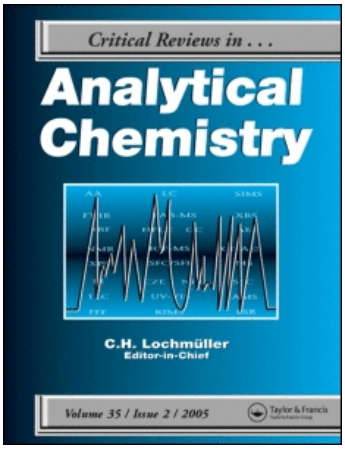

Critical Reviews in Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713400837

Biodegradation and Photooxidation Studies of Model Organic Pollutants

Katarina Senta Wissiak Grm; Margareta Vrtačnik; Boris Šket

Online publication date: 18 June 2010

To cite this Article Grm, Katarina Senta Wissiak , Vrtačnik, Margareta and Šket, Boris(2003) 'Biodegradation and Photooxidation Studies of Model Organic Pollutants', Critical Reviews in Analytical Chemistry, 33: 4, 333 — 338
To link to this Article: DOI: 10.1080/714037686
URL: http://dx.doi.org/10.1080/714037686

Critical Reviews in Analytical Chemistry, 33(4):333–338 (2003)

ISSN: 1040-8347
DOI: 10.1080/10408340390272647

Biodegradation and Photooxidation Studies of Model Organic Pollutants

Katarina Senta Wissiak Grm,[1] Margareta Vrtačnik,[1] and Boris Šket[2]

[1]University of Ljubljana, Faculty of Natural Sciences and Engineering, Vegova 4, POB 18/1, 1001 Ljubljana, Slovenia
[2]University of Ljubljana, Faculty of Chemistry and Chemical Technology, Vegova 4, POB 18/1, 1001 Ljubljana, Slovenia

ABSTRACT: In the paper we present the results of biodegradation and photooxidation studies of halosubstituted benzyl alcohols. The basic goal of our research was to examine the effect of the type and position of the halogen atom in the aromatic ring on the degradation rate and to use the experimental data for the development of HQSAR models for predicting degradation rates. Biodegradation results of 2-halosubstituted benzyl alcohols correlate with the size of the halogen atom, while for 4-halosubstituted benzyl alcohols, a good correlation with the energy of C—X bond was observed. In the photooxidation process, the impact of position of the halogen on the rate of reaction is smaller. The HQSAR model was derived only for photooxidation data sets, while for the biodegradation it was not possible to obtain a model with satisfactory statistical characteristics.

INTRODUCTION

The release of the chemicals into the environment, caused principally by industrial, agricultural and transport processes, results in air, soil and water pollution [1]. Consequently, the widespread utilization of chlorinated aromatic compounds, such as pesticides and herbicides, is attracting increased concern since halosubstituted aromatic chemicals have potentially adverse impacts on environmental systems. The degradation of these compounds is possible via chemical, photochemical and biological processes.

Biodegradation plays an extremely important role in minimising harmful effects on environmental systems, is broadly applicable to a range of organic chemicals, and represents a useful tool for better evaluation and exposure of hazards caused by chemicals present in the environment [2]. It represents a key process mediated by living organisms that results in the conversion of an organic chemical into organic and/or inorganic end products [3]. However, it requires long treatment periods, and therefore other physicochemical processes such as photocatalytic degradation are used to effect a change in the chemical species.

Halogen atoms, hydroxyl groups and aromatic rings are molecular fragments of many pesticides, therefore the biodegradation and photooxidation of 2-halosubstituted, 3-halosubstituted and 4-halosubstituted benzyl alcohols as model substances was studied. One of the purposes of our study was to examine how the type and position of the halogen atom on the aromatic ring affects the biodegradation and photooxidation rate in water. Since the biological and chemical degradation of halosubstituted aromatic chemicals is rather low, a photodegradation study was carried out using semiconductive oxides to accelerate their degradation [4].

The second purpose of our study was to derive QSAR models for predicting the degradation rates of model substances. Since experiments for degradation studies of pollutants are time consuming and costly, in the recent decades QSAR techniques have been used as valuable tools in predicting the environmental fate and toxicity of pollutants. However, the complexity of QSAR

approaches makes the use of QSAR non-trivial [5]. Therefore a novel, highly predictive QSAR technique, HQSAR (Hologram Quantitative Structure Activity Relationship) was developed. HQSAR attempts to correlate molecular structure with biological and/or chemical activity for a series of compounds using molecular holograms constructed from counts of sub-structural molecular fragments. The HQSAR approach eliminates the need for determination of 3D structure, binding conformations, and molecular alignment, on which the majority of QSAR techniques are based. The technique is compositional and topological in nature, since only elemental and bond-type data are included in molecular holograms. The values of regression coefficient (r^2) and cross-validated r^2 (q^2) are accepted as statistical measures of merit for a QSAR model [6].

MATERIALS AND METHODS

Biodegradation

Monosubstituted benzyl alcohols were obtained from Aldrich Chemical Company Inc., methylene chloride from Riedel-de Häen, naphthalene and sodium hydroxide from Kemika, Zagreb.

Samples of liquor sludge were collected from the ŠTUDA waste water treatment plant, Domžale, and were decanted, filtered and transferred to a container in which they were aerated in the laboratory for approximately 24 hours. The sludge was grown on pepton/glucose until 2.68 mg/mL per dry weight or 2.19 mg/mL per dry weight was obtained. The biodegradation of benzyl alcohols was examined in a series of reaction mixtures containing 0.1 mg benzyl alcohol/mL media. (100 mg benzyl alcohols/L media).

The experiment was set up using a modified biodegradability MITI test, which is both standardised and suitable for the determination of aerobic biodegradability in an aqueous medium [7]. The test material was dissolved in a 300 mL medium with neutral pH, to which was added 5.3 mL (2.68 mg/per mL dry weight) or 4 mL (2.19 mg/per mL dry weight) of inoculum of aerobic micro-organisms.

In order to quantitatively determine the amount of undegraded benzyl alcohol in the reaction mixture, samples of 10 mL-aliquots were extracted prior to analysis, and analysed with the Hewlett Packard HP 6890 Series Gas Chromatograph System.

Methylene chloride, acting as a solvent, and naphthalene, as an internal standard, were used. The amounts of benzyl alcohols were determined using a 30 m long HP-5 column with an inner diameter of 0.32 mm.

Photooxidation

2-Methyl imidazol was obtained from Fluka Chemie AG, and H_2O_2 from Belinka, Ljubljana. All other chemicals used in the experiment were obtained from Aldrich Chemical Company Inc.

A typical experiment for the photooxidation of halosubstituted benzyl alcohols was performed as follows: a suspension containing 20 mg of halosubstituted benzyl alcohol and 100 mg of TiO_2 (anatas) in 50 ml double distilled water was exposed to an ultrasonic bath for 5–10 minutes in order to obtain a fine suspension. The suspension was then irradiated in a quartz photoreactor equipped with a cooling system using Hg HPQ 125 W.

During irradiation the mixture was bubbled with oxygen, and samples were removed every 10 minutes. The samples were further analysed by

TABLE 1
Training and Predicting Data Sets

Biodegradation		Photooxidation	
Training set	Predicting set	Training set	Predicting set
2-F benzyl alcohol	2,4-difluoro benzyl alcohol	2-F benzyl alcohol	3-F benzyl alcohol
2-Cl benzyl alcohol	2,4-dichloro benzyl alcohol	2-Cl benzyl alcohol	3-Cl benzyl alcohol
2-Br benzyl alcohol		2-Br benzyl alcohol	3-Br benzyl alcohol
4-F benzyl alcohol		4-F benzyl alcohol	
4-Cl benzyl alcohol		4-Cl benzyl alcohol	
4-Br benzyl alcohol		4-Br benzyl alcohol	
Benzyl alcohol			

GC and GC/MS using 2-methyl imidazol as an internal standard.

Analytical gas chromatography (GC) was carried out on a Hewlett Packard 6890 Series Gas Chromatograph System using a 30 m Innowax column.

HQSAR Modelling of Data Sets

In our study, the HQSAR technique, introduced by Tripos, Inc. was used. The internal self-consistency of the HQSAR model is defined by the criterion $r^2 \geq 0.9$, while the criterion $q^2 \geq 0.5$ could be considered as an acceptable measure of the ability of the model to interpolate within the training set population.

Training and predicting data sets used for deriving the models are presented in Table 1.

Logarithms of biodegradation conversion efficiency and logarithms of the invert photodegradation rate constants were applied for defining the activity of halosubstituted benzyl alcohols included in the data sets.

RESULTS

Biodegradation

The results of the biodegradation of 4-halosubstituted benzyl alcohols compared with the results of the biodegradation of benzyl alcohol are presented in Table 2.

Based on the results of the biodegradation of 4-halosubstituted benzyl alcohols presented in Table 2 it is evident that we can compare the biodegradation of benzyl alcohol and 4-Br benzyl alcohol. The highest biodegradation level was obtained for both compounds and was also completed after 16 days of biodegradation.

TABLE 2
Amounts of Conversion for 4-Halosubstituted Benzyl Alcohols (Inoculum Added: 2.19 mg/mL Per Dry Weight)

	Conversion (%)	
Sample	After 0 days	After 16 days
Benzyl alcohol	0.0	100.0
4-fluoro benzyl alcohol	0.0	42.2
4-chloro benzyl alcohol	0.0	56.2
4-bromo benzyl alcohol	0.0	100.0

TABLE 3
Amounts of Conversion for 2-Halosubstituted Benzyl Alcohols (Inoculum Added: 2.68 g/mL Per Dry Weight)

	Conversion (%)	
Sample	After 0 days	After 16 days
Benzyl alcohol	0.0	100.0
2-fluoro benzyl alcohol	0.0	100.0
2-chloro benzyl alcohol	0.0	9.3
2-bromo benzyl alcohol	0.0	4.2

The amount of conversion is lowest in the case of the biodegradation of the 4-F derivate (42.2%) and 4-Cl derivate (56.2%) after 16 days of biodegradation. It can be assumed that the energy of the C—X bond may be one of the prevailing factors affecting the biodegradation rate of 4-halosubstituted benzyl alcohols.

The results of the biodegradation of 2-halosubstituted benzyl alcohols compared with the results of the biodegradation of benzyl alcohol are presented in Table 3.

The biodegradation figures of 2-halosubstituted benzyl alcohols indicate exactly the reverse effect of the halogen atom to that in the 4-halosubstituted molecules. Conversion is the lowest in the case of 2-Br benzyl alcohol (4.2%) and highest in the case of benzyl alcohol and 2-F benzyl alcohol, for which biodegradation is complete. Noting the very similar atomic radius of hydrogen and fluorine it can be deduced that the oxidase specificity in the case of benzyl alcohol and 2-F benzyl alcohol is similar. In the case of 2-Br benzyl alcohol, the interaction is inhibited due to the large size of the bromine

TABLE 4
Rate Constants of Photodegradation [$\times 10^{-4}$ s^{-1}] of Benzyl Alcohol and Selected Halosubstituted Benzyl Alcohol in the Presence of TiO_2 Powder

Halogen atom	Position 2	Position 3	Position 4
F	4.8	12.6	4.2
Cl	5.5	11.8	5.0
Br	8.3	3.1	12.0
Benzyl alcohol		4.3	

TABLE 5
Actual Versus Predicted Values of Log (1/k)

Substance	$\log (1/k)_{actual}$	$\log (1/k)_{predicted}$
4-bromo benzyl alcohol	2.9206	2.9844
2-bromo benzyl alcohol	3.0806	3.0117
2-chloro benzyl alcohol	3.2595	3.2587
4-chloro benzyl alcohol	3.3010	3.2979
2-fluoro benzyl alcohol	3.3186	3.3473
4-fluoro benzyl alcohol	3.3765	3.3568

TABLE 6
Actual Versus Predicted Values of Log (1/k) for Predicting Data Sets

Substance	$\log (1/k)_{actual}$	$\log (1/k)_{predicted}$
3-chloro benzyl alcohol	2.9278	2.9272
3-fluoro benzyl alcohol	2.8992	2.8986
3-bromo benzyl alcohol	3.5085	3.5078

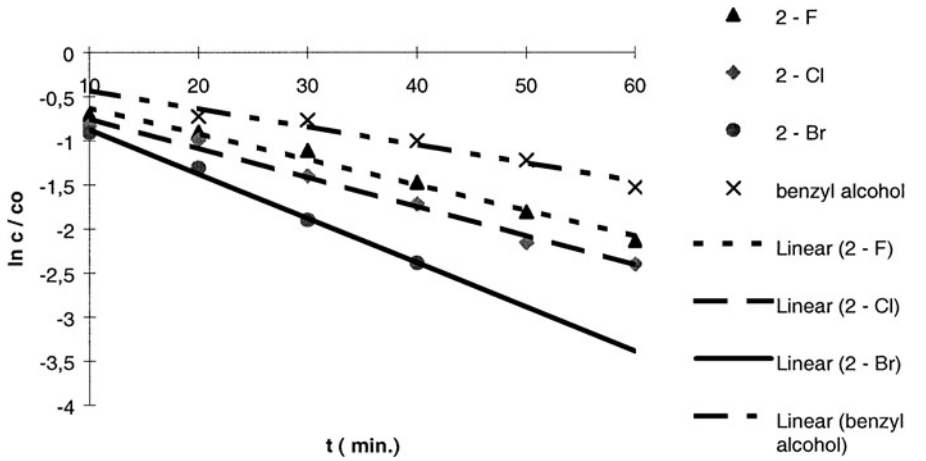

FIGURE 1. Kinetics of the photocatalytic degradation of 2-halobenzyl alcohols in the presence of TiO_2 powder.

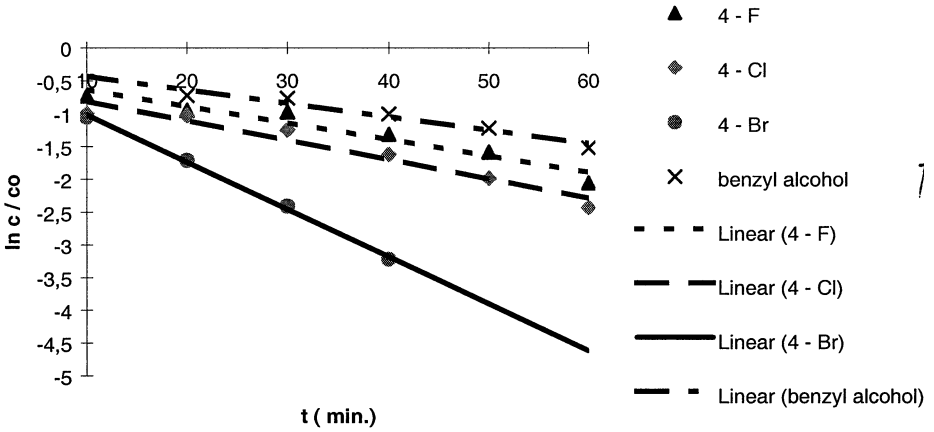

FIGURE 2. Kinetics of the photocatalytic degradation of 4-halobenzyl alcohols in the presence of TiO_2 powder.

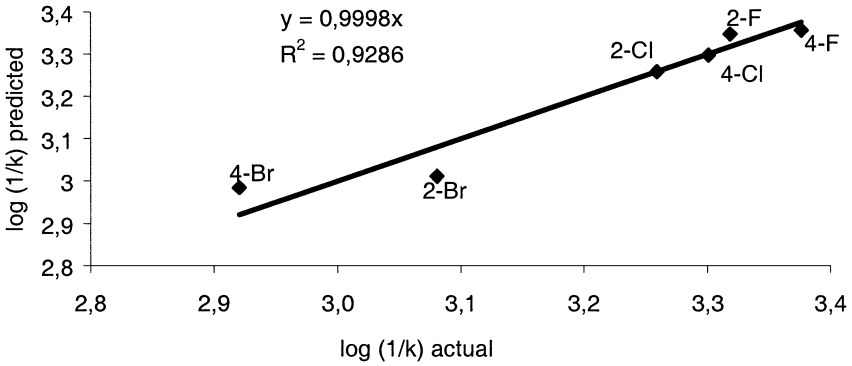

FIGURE 3. Data trendline.

atom (comparable to the methyl group), resulting in a lower level of biodegradation.

Photooxidation

The results illustrating photodegradation of benzyl alcohol, 2-halosubstituted benzyl and 4-halosubstituted benzyl alcohols in the presence of TiO_2 powder are presented in Figures 1 and 2.

From the photooxidation data for 2-halosubstituted and 4-halosubstituted benzyl alcohols it is evident that the photooxidation rate is linked to the energy of the C—X bond, since it increases from fluoro to bromo derivative, whereas in the case of 3-halosubstituted benzyl alcohols exactly the reverse trend was observed. (Table 4).

HQSAR

Several HQSAR runs were performed over different hologram lengths by varying hologram construction parameter settings: fragment lengths, atom counts, connections, bond types, number of hydrogen atoms and donor-acceptor moieties. The trials were successful only for the catalytic photooxidation process, while for the biodegradation it was not possible to obtain a model with satisfactory statistics. The best model statistics for the photooxidation model ($r^2 = 0.933$; $q^2 = 0.570$) were obtained if molecular fragment lengths were 4–7.

The comparison of the actual and predicted values of log (1/k) calculated by the model, is presented in Table 5.

Linear regression trendline for actual and predicted values of log (1/k) and its equation is presented in Figure 3.

The regression equation was used for predicting photooxidation rates of the 3-halo substituted benzyl alcohols, which were excluded from the training sets. The predictions are in good agreement with the experimentally determined values, Table 6.

CONCLUSIONS

Biodegradation

The biodegradation results for 2- and 4-halosubstituted benzyl alcohols indicate that the crucial step connected with their degradation is different. In the case of 2-halosubstituted benzyl alcohols the results indicate that the crucial step is linked to the size of the halogen atom, whereas for 4-halosubstituted benzyl alcohols it is linked to the energy of the C—X bond.

Photooxidation

The impact of the position of the halogen atom on the degradation is smaller in comparison with the results of the biodegradation of the same substances, with the exception of 4-bromo derivate, where the photooxidation rate is higher than in the case of the 2-bromo derivate. However, in the case of the 3-halosubstituted benzyl alcohols the rate of photooxidation is exactly the reverse: the highest value was obtained in the case of the fluoro derivate, and the lowest in the case of the bromo derivate, which indicates that the electronic effects of the halogen atom in this case predominate over the effect of the energy of the C—X bond.

HQSAR Modelling

A HQSAR model with satisfactory statistics and predicting power was derived only

for the photooxidation data set, while for the biodegradation the trials resulted in models with $q^2 < 0.05$ and $r^2 < 0.9$. We concluded that the data set for biodegradation trials did not contain sufficient representative molecules for designing an appropriate hologram. It might be possible to improve the model statistics by the application of more sophisticated QSAR techniques.

ACKNOWLEDGEMENT

The authors would like to thank the Ministry of Education, Research and Sports of the Republic of Slovenia for financial support of the study, and Tripos, Inc., who granted us permission to use their HQSAR techniques.

REFERENCES

1. Vrtačnik, M.; Radinja, J. 1996. Substances with high toxicity for bio-systems, in Toxic waste management, UNESCO, Ljubljana, 19–88.
2. West, R.J.; Gonsior, S.J. 1996. *Environ. Toxicol. Chem.* **15**: 472.
3. Larson, R.J.; Cowan, C.E. 1995. *Environ. Toxicol. Chem*. **14**: 1433.
4. Al-Ekabi, H.; Serpone, N. 1988. *J. Phys. Chem*. **92**: 5726–5731.
5. Lowis, D.R. 1997. *Tripos, Technical Notes* **1**: 5.
6. Tong, W.; Lowis, D.R.; Perkins, R.; Chen, Y.; Welsh, W.J.; Goddette, D.W.; Heritage, T.V.; Sheehan, D.M. 1998. *J. Chem. Info. Comp. Sci*. **38**(4): 669–667.
7. Commission of the European Communities. 1987. Council Directive 67/548/EEC.