Inter*net* Electronic Journal of Molecular Design

June 2003, Volume 2, Number 6, Pages 383–391

Editor: Ovidiu Ivanciuc

Special issue dedicated to Professor Haruo Hosoya on the occasion of the 65th birthday
Part 10

Guest Editor: Jun–ichi Aihara

Structure–Biodegradation Relationship Study of Commercial Linear Alkylbenzene Sulfonates

Li Li,[1] Xiao Yi Zhang,[2] Zheng Wu Wang,[3] and Gan Zuo Li[3]

[1] Department of Chemistry, Binzhou Teachers College, Binzhou, Shandong, 256600, China
[2] Department of Chemistry, Guizhou Normal University, Guiyang, 550001, China
[3] Key Laboratory of Colloid and Interface Chemistry for State Education Ministry, Shandong University, Jinan, Shandong, 250100, China

Received: June 30, 2002; Revised: November 16, 2002; Accepted: January 17, 2003; Published: June 30, 2003

Citation of the article:
L. Li, X. Y. Zhang, Z. W. Wang, and G. Z. Li, Structure–Biodegradation Relationship Study of Commercial Linear Alkylbenzene Sulfonates, *Internet Electron. J. Mol. Des.* **2003**, *2*, 383–391, http://www.biochempress.com.

Inter*net* Electronic Journal of Molecular Design

BioChem Press
http://www.biochempress.com

Structure–Biodegradation Relationship Study of Commercial Linear Alkylbenzene Sulfonates[#]

Li Li,[1,*] Xiao Yi Zhang,[2] Zheng Wu Wang,[3] and Gan Zuo Li[3]

[1] Department of Chemistry, Binzhou Teachers College, Binzhou, Shandong, 256600, China
[2] Department of Chemistry, Guizhou Normal University, Guiyang, 550001, China
[3] Key Laboratory of Colloid and Interface Chemistry for State Education Ministry, Shandong University, Jinan, Shandong, 250100, China

Received: June 30, 2002; Revised: November 16, 2002; Accepted: January 17, 2003; Published: June 30, 2003

Internet Electron. J. Mol. Des. **2003**, *2* (6), 383–391

Abstract

Motivation. Biodegradation (metabolism by microorganisms) is one of the most important processes determining the fate of organic chemicals in the environment. Structure–biodegradation relationships for linear alkylbenzene sulfonates (LAS) can be used to interpret the mechanism of LAS biodegradation and predict biodegradation rates by using theoretical descriptors computed from the chemical structure.

Method. Relationships between the electronic structure and biodegradability of commercial LAS are investigated by using semiempirical quantum chemistry method with the PM3 Hamiltonian.

Results. Electronic and stereo effects are the main factors affecting the course of LAS biodegradation, and oxygen atoms may be the real donors.

Conclusions. The different biodegradation rates of these compounds can be modeled by using some structural and electronic parameters such as the energy of frontier molecular orbitals, the dipole moment, the bonding orbital parameters, the charge density and the net charges at the active site.

Keywords. Structure–biodegradation relationships; linear alkylbenzene sulfonate; quantum descriptors; PM3.

1 INTRODUCTION

Biodegradation (metabolism by microorganisms) is one of the most important processes determining the fate of organic chemicals in the environment [1]. There have recently been increasing interests in determining the relationship between the molecular structure and the biodegradation. Knowing the relationship between the structure and the biodegradation of chemicals could assist the prediction of their biodegradation rates and may be used in mechanistic studies of the processes, which determine biodegradation rates.

[#] Dedicated to Professor Haruo Hosoya on the occasion of the 65th birthday.
* Correspondence author; E–mail: lli_yu@163.com.

Commercial linear alkylbenzene sulfonate (LAS) is a widely used synthetic surfactant in detergents and household cleaning products. For example, the consumption of LAS in Europe, North America and Japan was approximately 950,000 metric tons in 1994 [2]. Numerous studies on the environmental fate and effects of LAS have been published [3]. These studies confirm that LAS is completely biodegradable; environmental concentrations of LAS are low [4].

Commercial LAS, however, is a blend of LAS molecules that vary in terms of alkyl chain length, position of the benzene ring along the alkyl chain, and concentration of co–products called dialkyltetralin sulfonates (DATS) and single methyl–branched isomers of linear alkylbenzene sulfonate (*iso*–LAS). Because of the widespread and high volume use of commercial LAS, significant quantity of LAS co–products reaches the environment. Consequently, several workers [5,6] studied biodegradation and environmental fate of DATS and LAS isomers. Nielsen *et al*. [7] measured LAS and LAS co–products biodegradation using ^{14}C labeled method with porous pot activated sludge test. They concluded that the mineralization of iso–LAS (and standard LAS for comparison) was immediate and rapid, whereas DATS mineralization proceeded at a slower rate.

In the present work, we try to interpret the differences in biodegradation of LAS and its co–products from the electronic structure characteristics and to explore the mechanism of LAS biodegradation. The electronic descriptors of LAS and its co–products, including the orbital energy, the dipole moment, the charge distributions (Mulliken population analyses) and the local electronic characteristics of surfactant molecule and so on, are calculated by using semiempirical quantum chemical method at the PM3 level.

2 MATERIALS AND METHODS

2.1 Chemical Data

The biodegradation data of DATS and iso–LAS were taken from the Nielsen's paper, and are presented in Table 1 and Table 2 [7]. The molecular structure of LAS and its co–prodcuts are illustrated in Figure 1.

2.2 Computational Methods

All 3D model molecular structures were constructed by using Hyper Chem. 5.1 for Windows. The MM+ force field was used to optimize the three–dimensional molecular geometry for model compounds. All calculations in this study were performed on a GHPCC workstation with MOPAC 7.0 software under UNIX operating system, using the PM3 method.

Table 1. Fate of LAS Coproducts in Porous Pot Activated Sludge Treatment

		Parent Removal (%)	Mineralization (%$^{14}CO_2$)	Percent in Biomass	Ultimate Biodegradation (%)	Residual in Liquids (%)
LAS		98.4	57.5	28.6	86.1	13.9
DATS		98.6	0.8	1.8	2.6	97.4
iso–LAS	IA	99.7	53.0	26.2	79.2	20.8
	IB	99.5	51.6	27.8	79.4	20.6
	IIA	99.3	58.2	31.5	89.7	10.3
	IIB	98.5	7.6	4.2	11.8	88.2

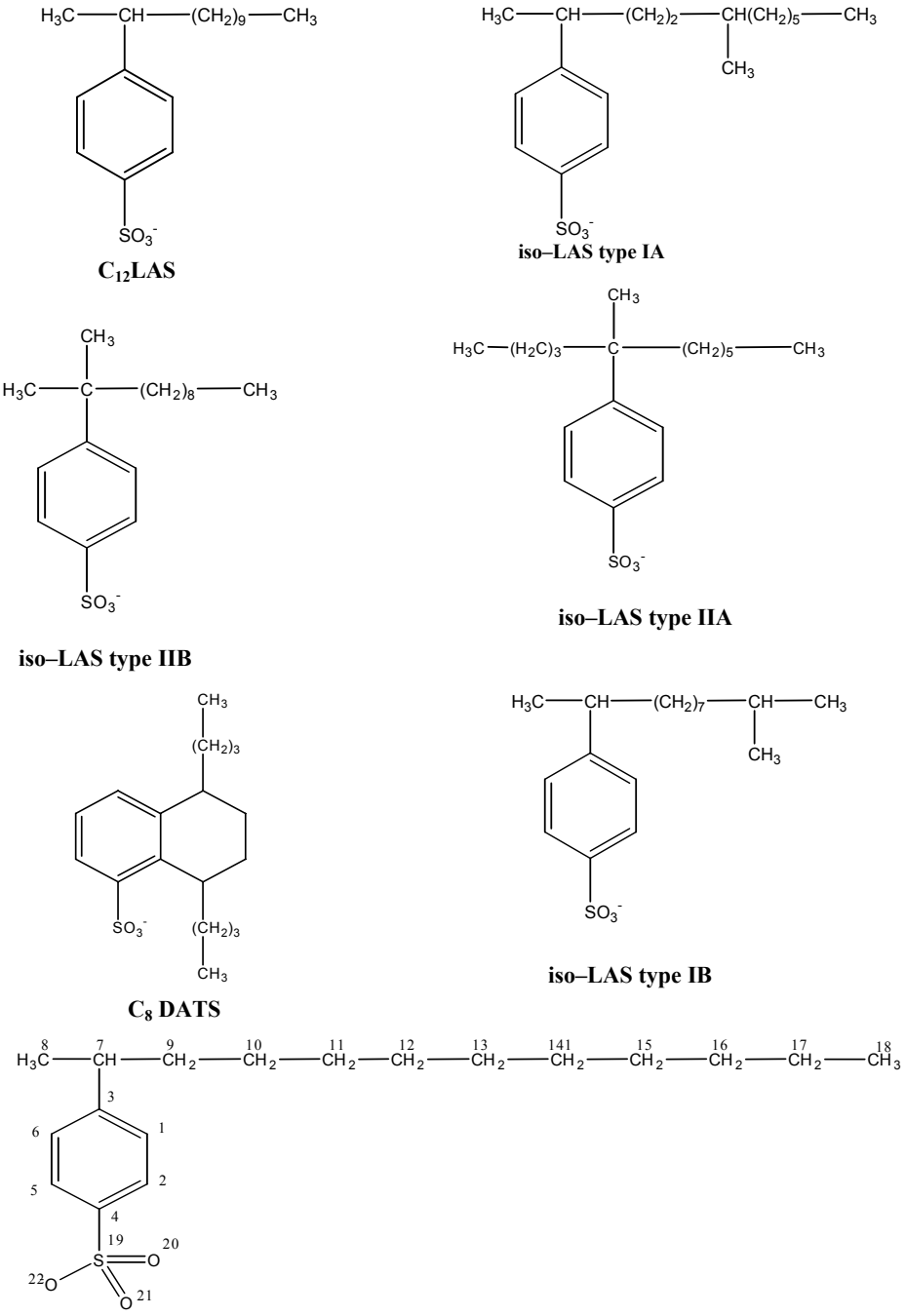

Figure 1. Chemical Structures of C_{12} LAS and its Co–products.

Table 2. Mineralization of Radiolabled Compounds in Aquatic Environments

		Rate ($t_{1/2}$ day)	Extent [%T $^{14}CO_2$ (30day)]
LAS		3.4; 4.6	60; 76
DATS		14.2; 8.2	19; 19
iso–LAS	IA	3.1	71
	IB	3.2	70
	IIA	4.0	63
	IIB	8.6	47

3 RESULTS AND DISCUSSION

3.1 Analysis of Experimental Results

From the biodegradation data (Table 1), it can be seen that for the entire iso–LAS compounds more than 98% parent marital are removed, and that most of them have values for mineralization being greater than 50% and for ultimate biodegradation being in the range 79–90%. However, DATS and one of the iso–LAS isomers, LASIIB, only have values 0.8 and 7.6 percent for mineralization, and 2.6 and 11.9 percent for ultimate biodegradation, respectively. Table 2 shows that DATS and iso–LAS IIB proceed for mineralization at much slower biodegradation rates than the other iso–LAS and LAS. The mineralization rates of the other iso–LAS are as rapid as that of LAS.

3.2 The Electronic Effect in Mechanistic of LAS Biodegradation

Intensive studies on biodegradation of LAS and its coproducts have shown that the their biodegradation pathway include the following steps: the first ones are ω– and β– oxidations of the alkyl chain, which result in some sulfophenylcarboxylic acids with a chain of 4–5 carbon atoms. The next ones are the split of the aromatic ring and the subsequent desulphonation [8]. The initial step in the biodegradation of LAS as well as its coproducts has been shown to be an oxidative reaction, which proceeds on the alkyl chain at one of the terminal methyl groups.

It is well known that in ordinary circumstances, the highest occupied molecular orbit (HOMO) and its adjacent occupied molecular orbits (namely HOMO (–1), HOMO (–2), etc.) have the donating property electron. The data from the Table 3 indicate that the principal ingredients of HOMO and the next highest occupied molecular orbits (NHOMO) mainly include these atoms, C_1, C_4, O_{20}, O_{21}, O_{22}, i.e. the *C* atoms in the benzene and the O atoms in the sulfo group. This shows that the O atoms (or benzene group) are probably the active site of oxidative attack in the model compounds. The data of the local molecular orbital calculation show that the three highest local occupied molecular orbits mainly consist of S–O bonds, which is primarily contributed by the O atoms. Furthermore, from the data in the Table 4, it can be seen that negative charges mainly focus on the O atoms, and their net negative charges ($Q_O > -0.95$) are higher than those of the terminal

atom C_{18} ($Q_{C_{18}}$>–0.15). This phenomenon suggests that C_{18} is not the real center of supplying electron in the enzymatic reaction, but the O atoms may be, since they have much more capacity to combine with the positive charge regions of enzyme. In conclusion, though reactivation is possible through short chain or long chain aliphatic alcohols or carboxylic, the O atoms are probably the active centers in the reaction and the 'real' electron donor. Also we should focus on the charge distributions of the O atoms and other atoms concerned to them.

Table 3. Frontier energies E_{HOMO} and E_{LUMO}, ionization potential I, and principal components of HOMO and NHOMO

		E_{HOMO} (eV)	E_{LUMO} (eV)	ΔE (eV)	I (eV)	Principal components of HOMO and NHOMO				
						C_1	C_4	O_{20}	O_{21}	O_{22}
LAS		–5.915	3.393	9.308	5.915	4.7%	35.36%	16.87%	22.61%	15.74%
DATS		–5.996	3.417	9.413	5.996	4.7%	18.33%	36.73%	12.12%	15.13%
iso–LAS	IA	–5.917	3.390	9.307	5.917	5.0%	37.11%	17.14%	17.03%	23.76%
	IB	–5.914	3.394	9.308	5.914	5.0%	37.11%	16.90%	17.24%	23.79%
	IIA	–5.917	3.383	9.300	5.917	5.0%	37.26%	17.17%	17.10%	23.77%
	IIB	–5.940	3.346	9.286	5.940	5.1%	37.10%	17.10%	17.03%	23.70%

Table 4. Bonding contribution (BC), dipole moment (μ_{TOT}, μ_X) and net charges (Q_N)

		the BC Value (ev)		μ(D)		Q_N (e.s.u.)			
		HOMO	terminal C–H	μ_{TOT}	μ_X	C_4	C_{18}	S	O
LAS		0.8203	1.9216	29.139	–28.313	–0.479	–0.1085	2.2996	–0.9410
DTAS		0.9226	2.0343	13.166	–10.586	–0.480	–0.1072	2.2918	–0.9294
iso–LAS	IA	0.8203	2.0303	29.139	–28.313	–0.480	–0.1083	2.2990	–0.9406
	IB	0.8203	2.0089	32.082	–29.671	–0.480	–0.1205	2.2994	–0.9408
	IIA	0.8217	2.0671	29.917	–27.572	–0.479	–0.1086	2.2988	–0.9405
	IIB	0.8214	2.0556	22.853	–21.224	–0.478	–0.1106	2.2994	–0.9405

Table 5. Energies of local molecular orbits (LMO), indices of frontier electronic density (F_r^E) and the bond order (BO)

		LAS	DATS	LASIA	LASIB	LASIIA	LASIIB
Energies of LMO (eV)	O_{22}–S	–6.8743	–7.2067	–6.8756	–6.8713	–6.8791	–6.9014
	O_{21}–S	–6.9009	–7.2185	–6.9068	–6.9040	–6.9097	–6.9275
	O_{20}–S	–6.9062	–7.3844	–6.9078	–6.9050	–6.9143	–6.9362
	C_4–S	–14.914	–15.025	–14.914	–14.912	–14.909	–14.926
	C_{18}–H	–16.904	–15.307	–16.834	–16.851	–16.829	–16.397
F_r^E	C_{18}	0	2.41×10^{-5}	0	0	0	3.0×10^{-8}
	C_4	0.3587	0.1626	0.3590	0.3588	0.3594	0.3597
	O	0.2702	6.64×10^{-2}	0.2717	0.2717	0.2700	0.2691
BO	S–O	1.0972	1.1019	1.0969	1.0972	1.0970	1.0974
	C–H	0.9886	0.9870	0.9868	0.9858	0.9869	0.9853

But we have to explain why biodegradation of model compounds at first takes place in the terminal CH_3 group. In organism, the full enzyme is made up of apoenzyme and coenzyme NAD^+ in the biodegradation of model compounds. The coenzyme NAD^+ has the effect of transferring electrons and hydrogen. In the process of LAS biodegradation, the coenzyme NAD^+ transfers H atom and assists chemical reaction between enzyme and substrates. The reasonable explanation of that biodegradation of model compounds at first takes place in the terminal CH_3 group is that on one hand, there is no transferred H atom in the S–O bond, and on the other hand, the degrees of

S–O bonding are higher than those of the terminal C–H bonding in the model compounds (Table 5). It shows that it is more difficult to rupture the S–O bond. Therefore the first step of oxidation takes place by rupturing terminal C–H bond in terminal CH_3 group combining with coenzyme NAD^+.

In addition, Linden and Ravenswaay [9] found experimentally that stereo effect causes hydrocarbon hydroxylation by a *Pseudomonds aeruginosa* strain to be substrate–specific. They pointed out that the active center of the responsible hydrocarbon hydroxylase is in the interior of the molecule, i.e., in the enzyme molecule, and that substrate hydrocarbon molecules must have a very particular stereo size so that it can be bound to the active center of the enzyme. Swisher [10] and Wickhold [11] have independently found that the LAS components which can be biodegraded most rapidly are those with one–methyl group being the farthest from the sulphophenyl configuration, they called this phenomenon "distance principle". They considered that the obvious explanation of the distance principle is based on stereo hindrance of enzymatic conversion of LAS by the aromatic molecular fraction. Koshland put forward the induced–fit theory in the inductive interaction between enzyme and substrate. He thought that active center is flexible. It is regarded that the partly internal energy, which is caused without accurate fit in the interaction between enzyme and substrate, is used to make substrate molecule deformed in the process of induced–fit of enzyme and substrate. An enzyme engages with substrate and makes the reaction take place (Figure 2). Therefore, the stereo effect has great influence on biodegradability.

Fig 2**. The Induced–Fit Process of Enzyme and Substrate**

The dipole moment of the molecule is often used to quantify the polarity effect, which is the measure of molecular deformation and contains information about potentially inductive interactions in the molecule [12,13]. From the data in Table 4, it can be seen that the dipole moment of the model structures in the X–axis is the largest, and this phenomenon indicates that the model compounds are easier to be deformed in this direction. The optimized molecular structures shows that the alkyl chain is situated in the X negative coordinate and the included angle with X coordinate is very smaller, which means that the model molecules are easily deformed and the carbon chain forms special stereo size in the inductive interaction. Consequently the deformed molecule inserts in the enzyme, the O atom combines with the active position of the enzyme, and coenzyme NAD^+ removes a H atom from crispate alkyl chain. This process is called ω–oxidation, from the above analysis it is clear that the electronic and stereo effects have the greatest influence on the biodegradation of LAS and its co–products.

3.3 The Influence of Charge Distributions on Biodegradation of Model Compounds

The total dipole moment of molecules (μ_{tot}) reflects the global charge distribution and the polarity of a molecule. In Table 4, the μ_x of DATS molecule (–10.586 D) is the lowest in the model compounds. So the carbon chain of DATS molecule is not easily deformed, which causes the lowest biodegradation of DATS. Table 4 also shows that the μ_{tot} of DATS molecule (13.166 D) is the lowest and the next is type IIB of iso–LAS (22.853 D) whereas μ_{tot} of other LAS isomer molecules are nearly the same as that of LAS (29.139 D). Then in the inductive interaction between enzyme and model compounds, LAS and its isomers except for type IIB are easier to be induced and polarized than DATS and the type IIB molecules, and they are easily flexible. So their biodegradability is very high. Due to the most difficult deformation of DATS molecule, stereo hindrance is much larger in the inductive interaction, which causes the lowest biodegradability.

It is understandable that the O atom can be act as an active center. The net charges in the O atom of LAS and its iso–LAS isomers are higher than those of DATS (Table 4). Then the O atom of these compounds can supply electrons better than those of DATS. Those results can also gave another explanation of that biodegradation of DATS is lowest in the compounds.

Although the electronic characteristics (the bond order, ΔE, net charge of terminal carbon atom, and so on) of type IIB are almost similar to those of LAS as well as its isomers, its μ_{tot} is obviously lower, which consequently reduces the molecular deformation and polarity and increases the stereo hindrance in the interaction between the enzyme and substrates and makes it has lower biodegradability.

3.4 The Influence of Frontier Orbital Energies on Mineralization of Model Compounds

According to the frontier molecular orbital theory (FMO) of chemical reactivity, a large HOMO–LUMO gap (ΔE) implies high stability of the molecule in chemical reaction [12]. From the data in table 3, it is obvious that the energy gap of DATS molecule is higher than those of iso–LAS and LAS molecules. So it is very stabile and has the lowest biodegradability. The energy E_{HOMO} of type IIB (E_{HOMO} = –5.940 eV) is very lower than that of other iso–LAS molecules. So then it is poor to supply an electron and its mineralization is lower than those of other iso–LAS (E_{HOMO} = –5.92 eV) molecules. From the data in Table 5, it can be seen that the HOMO and the two next occupied orbits (O_{20}–S, O_{21}–S, O_{22}–S) calculated from the local molecular orbits in the model compounds mainly consist of S–O bonds, which is mainly contributed by the O atom.

3.4 The Influence of Electronic Characteristic Bonding Orbital Parameters of Bond Orbit on Biodegradation of the Model Compounds

The frontier orbital electron densities of atoms provide a useful means for the detailed characterization of donor–acceptor interaction [14]. According to the frontier electron reactivity theory, the majority of chemical reactions take place in the orientations where overlap of the HOMO and LUMO of the respective reactants can reach the maximum [15]. In the case of a donor molecule, the HOMO density is critical factor for the charge transfer.

The HOMO densities F_r^E is defined by the following equation [16]:

$$F_r^E = \sum \left(C_{HOMO,n}\right)^2 / \varepsilon_{HOMO}$$

Where $C_{HOMO,\,n}$ is the coefficients of the atomic orbits X_n in the HOMO, and ε_{HOMO} is the HOMO energy. From Table 5, the value of the HOMO density in the O atoms of DATS molecule is the lowest and that of the type IIB is next. So DTAS and the type IIB mineralization proceeded at a slower biodegrading rate, which is in accord with the experimental results.

The bond order (BO) provides a measure of bonding formed by the two adjacent atoms. The higher value of BO, the steadier the bond will be. Table 4 illustrates that the value of BO of S–O bonds in the LAS, LASIA, LASIIA and LASIB are lower than those of DATS and the type IIB. The O atoms in the LAS and its isomers are easier to provide an electron for enzyme than those of DATS and LASIIB. So the biodegradation rates of DATS and LASIIB are lower than those of LAS and its isomers. This phenomenon is due to the higher bound of valence electrons of the O atoms in DATS and the type IIB molecules.

In addition, the bonding contribution (BC) value of the HOMO for DATS (0.9226) is very much larger than that of other LAS isomers (the BC value about 0.82). Because the BC values of terminal C–H bond for LAS and its isomers are less than those of DATS and the type IIB, the terminal C–H bonds in DATS and the type IIB molecules are harder to be ruptured than those of LAS and its isomers. This phenomenon also indicates that the biodegradation of LAS and its isomers are immediate and rapid, whereas the biodegradation of DATS and the type IIB proceed at much slower rates.

4 CONCLUSIONS

Computer simulations allow the investigation of atomistic behavior revealing unique insights into the contributions of individual atoms and bond to complex molecules. We have successfully interpreted the difference of biodegradation of commercial LAS based on electronic characteristic of the model compounds and obtained the following conclusion:

(*a*) According to LMO theory and the net charges in the atoms, the O atoms in the model

compounds may be the real electron donor, and the deformed alkyl chain forms effective spatial size, which make LAS and its co–products molecules can insert in the enzyme and coenzyme NAD^+ to react with H atoms in the terminal CH_3, and break the C–H bonds.

(*b*) The lowest energy of HOMO and a larger HOMO–LUMO gap of DATS molecule reduce its ability to electron donation; therefore, the mineralization of DATS proceeds at a slower rate.

(*c*) In addition to electronic effect, the stereo effect is an important factor in the biodegradation of commercial LAS.

Acknowledgment

The authors wish to express the deepest gratitude to the State Natural Science Foundation of China (grant No. 29973023) and the Guizhou Province Scientific and Technological Foundation of China (grant No. 20003079) for their financial support.

5 REFERENCES

[1] M. Alexander, Biodegradation of Organic Chemicals of Environmental Concern, *Science* **1981**, *211*, 132–138.
[2] SRI International, Chemical Economics Handbook, 610.5000E, **1995**.
[3] Environmental and Human Safety of Major Surfactants. Volume 1. Anionic Surfactants. Part 1. Linear Alkylbenzene Sulfonates; Final report from A. D. Little, Inc. to the Soap and Detergent Association: New York, 1991; pp III–1—V–58.
[4] J. Q. Guan, J. Sh. Li, Biodegradation of Surfactants in the Environment, *Environment Science of China* **1994**, *15*, 81–86.
[5] A. Di Corcia, R. Samperi, A.Marcomini, Investigation of the Fate of Linear Alkyl Benzenesulfonates and Coproducts in a Laboratory Biodegradation Test by Using Liquid Chromatography/Mass Spectrometry, *Environ*. *Sci*. *Technol*. **1999**, *33*, 4112–4118.
[6] A. Di Corcia, F. Cassasa, A. Marcomini, et al, Investigation of the Fate of Linear Alkyl Benzenesulfonates and Coproducts in a Laboratory Biodegradation Test by Using Linquid Chromatography/Mass Spectrometry, *Environ*. *Sci*. *Technol*. **1999**, *33*, 4112–4118.
[7] M. N. Allen, N. B. Larry, et al, Biodegradation of Coproducts of Commercial Linear Alkylbenzene Sulfonate, *Environ*. *Sci*. *Technol*. **1997**, *31*, 3397–3404.
[8] P. M. Schöberl, Basic Principles of LAS Biodegradation, *Tenside*, *Surfactants*, *Deterg*. **1989**, *26* (2), 86–94.
[9] J. C. Ravenswaay, van der Linden, Biodegradation of Linear Alkylbenzene Sulfonate, *A*. *C*. *A*. *van Leeuvenhoek* **1971**, 37, 339–345.
[10] R. D. Swisher, W. E. Gledhill, et al, Proc. Of the VIIth International Congress on Surface Active Substances **1978**, *4*, 218–224.
[11] R. Wickbold, Biodegradation of the Surfactant Linear Alkylbenzenesulfonate, *Tenside Detergents* **1975**, *12*, 25–31.
[12] M. Karelson, V. S. Lobanov, Quantum–Chemical Descriptors in QSAR/QSPR Studies, *Chem*. *Rev*. **1996**, *96*,1027–1043.
[13] M. V. Diudea (Ed.), *QSPR/QSAR Studies by Molecular Descriptors*, Research Studies Press, Letchworth, 1986, 20–25.
[14] A. R. Katritzky, V. S. Lobanov, M. Karelson, QSPR: The Correlation and Quantitative Prediction of Chemical and Physical Properties from Structure, *Chem*. *Soc*. *Rev*. **1995**, 279–287.
[15] K. Fukui, *Theory of Orientation and Stereoselection*; SpringerVerlag: New York, 1975, pp34–39.
[16] R. Franker, *Theroetical Drug Design Method*; Elsevier: Amsterdam, 1984; pp 115–123.