Toxicity of Commercial Surfactants to Phosphate-Accumulating Bacterium

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Abstract

The toxicity of twelve commercial surfactants to phosphate-accumulating bacterium *Acinetobacter junii* was tested. The tested products were selected to cover a variety of surfactant types and classes (anionic, cationic, amphoteric and nonionic). One surfactant proved to be highly toxic ($EC_{50} < 1$ mg L^{-1}), six surfactants were toxic (EC_{50} values between 1 and 10 mg L^{-1}), two were harmful (EC_{50} values between 10 mg L^{-1} and 100 mg L^{-1}) and three surfactants with EC_{50} higher than 100 mg L^{-1} were considered as non-toxic to *A. junii*. The most toxic commercial surfactant was *Kutriacid* (linear alkylbenzene sulphonate) with EC_{50} value of 0.15 mg L^{-1}. When all of the tested surfactants were compared there could be no generalization in toxicity regarding the class or the length of the alkyl chain of surfactant. One observed feature is that all of the surfactants with branched alkyl chain had least toxic or no toxic effect to *A. junii*.

Keywords: *Acinetobacter junii*, bacteria, phosphate, surfactants, toxicity, wastewater.

1. Introduction

Surfactants generally consist of a hydrophilic head group (charged or non-charged) and a hydrophobic hydrocarbon tail. Depending on the charge of surfactant head group, they are generally classified as anionic, cationic, nonionic and amphoteric. The commercial surfactants are industrially manufactured solutions containing a single type of surfactant or mixtures of multiple structurally similar surfactants, water and impurities. These commercial products are used in industrial manufacture of detergents, personal care products, textiles, pharmaceuticals etc.[1]

The behaviour, fate and biological effects of surfactants in the environment need to be understood to assess their environmental risk. In a study by Warne *et al.*[2] surfactants were identified as the main contributors of detergent toxicity to aquatic organisms. After use in household or industry, residual surfactants and their degradation products are discharged to sewage system or directly to surface waters, and then dispersed into different environmental compartments (water, sediments, soil). Surfactants can also accumulate in the sludge sewage treatment flow[3] and exert an inhibitory effect on the sewage sludge microorganisms.

Such effect could have serious consequences, as the effectiveness with which the water treatment plant removes pollutants and breaks down sewage might be compromised.

In this study we wanted to investigate the possible toxicity of commercial surfactants to a specific type of microorganism inhabiting the activated sludge; a phosphate-accumulating bacterium *Acinetobacter junii*. The bacteria from the genus *Acinetobacter* were isolated from the phosphorus removing activated sludge plant[4] and although *Acinetobacter spp.* were present in extremely low number, their capacity to remove phosphate was the highest among all the phosphate-accumulating isolates.[5] These bacteria have become the model organism for enhanced biological phosphorus removal (EBPR) and elucidating the negative effects of surfactants on these bacteria could help to understand or prevent the possible failures of EBPR systems.

2. Materials and Methods

2. 1. Commercial Surfactants

The commercial surfactants were obtained from Croatian detergent producing companies. The 12 surfac-

tants (Table 1) tested in the study were selected based on their widespread usage in industrial production and to cover a variety of surfactant types and classes (anionic, cationic, amphoteric and nonionic). Commercial products were either used in the experiment immediately after their arrival in the laboratory, or stored at 4 °C. The valid material safety data sheet for each surfactant was provided by the manufacturer.

2. 2. Bacterium

The phosphate-accumulating bacterium *A. junii* (strain DSM 1532) was obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweg, Germany and maintained as described previously.[6]

2. 3. Simulative Wastewater

A chemically defined water solution that simulated the real wastewater was used as a growth medium for *A. junii*. The composition was as follows (in mg L^{-1} of distilled water): Na-propionate 300; peptone 100; $MgSO_4$ 10; $CaCl_2$ 6; KCl 30; yeast extract 20; KH_2PO_4 88. The pH was adjusted to 7.00 ± 0.04 with 1M HCl or 1M NaOH before autoclaving at 121 °C for 20 min.

2. 4. Toxicity Test Design

The *A. junii* was pregrown on nutrient agar plates for 16 h at 30 ± 0.1 °C. The biomass was then resuspended in 9 mL of sterile 0.3% NaCl solution. A 5 mL of such suspension was inoculated into the flasks containing 500 mL of simulative wastewater, after which the starting number of bacterial cells was determined. Appropriate amounts of surfactant commercial product were then aseptically added in each flask. The sterility of surfactant solutions was previously confirmed. A 250 mL was taken from each flask to measure the concentrations of active matter of surfactant in the simulative wastewater. The bottles with remaining 250 mL of wastewater were then aerobically incubated for 24 h at 30 ± 0.5 °C in a water-bath with shaker (70 rpm) and rate of 1 L per minute of filtered air was provided. Bottles which served as negative control contained no surfactant. For each surfactant a preliminary range-finding test covering several orders of magnitude in surfactant concentrations was carried out in order to determine the concentrations range for each surfactant used in final test. Tested concentrations ranged from 0 to (in g L^{-1}):

Table 1: Specifications of the tested commercial surfactants. * The data was retrieved from the valid material safety data sheet. N/A; the information was not listed in the data sheet.

Class of surfactant	Commercial product	CAS Number *	MW (g mol^{-1})	Active matter * (W %)	General chemical description *	Common name	Acronym
Anionic	Hostapur SAS 60	85711-69-9	328	60	Sulfonic acid-sec-alkane, sodium salt; C14-17	Secondary alkane sulphonate	SAS
	Kutriacid 95A	85536-14-7	322	97	Benzenesulfonic acid-sec-alkyl derivates; C10-13-iso, C12 rich	Linear alkyl benzene sulphonate	LAS
	Lutensit TC-EHS	126-92-1	232	30–60	Sodium-2-ethyl hexyl sulphate	Alkyl sulphate	AS
	Na-Cumolsulfonat	28348-53-0	222	> 40	1-methyl-ethyl-benzenesulfonic acid	Alkyl benzene sulphonate	ABS
	Solfodac AC-3-1	85536-14-7	322	98	Benzenesulfonic acid-sec-alkyl derivates; C10-13-iso, C12 rich	Linear alkyl benzene sulphonate	LAS
	Texapon LS 35	85586-07-8	302	30–50	Aqueous solution of sulfuric acid, mono alkyl esters, sodium salt; C12-16	Alkyl sulphate	AS
Cationic	Servamine KOO 330	3528-63-0	350	N/A	1H-imidazole-1-ethanamine	Quaternary ammonium compound	QAC
Amphoteric	Oxidet DM-4	3332-27-2	257	30	Dimethyl myristyl amine oxide; C14	Amine oxide	AO
Nonionic	Arylpon VPC	90803-88-6	444	50-70 fatty amine, ethoxylated (CAS 26635-93-8) 10-20 fatty alcohol, ethoxylated (CAS 68920-66-1)	Fatty oleylamine, ethoxylated; C18. Fatty alcohol, ethoxylated; C16-18.	Alkyl ethoxylate + ethoxylated amine	AE+EA
	Empilan KI 8	78330-21-9	505	60–100	Alcohols ethoxylated C11-14-iso, C13 rich	Alcohol ethoxylate	AE
	Ethomeen T/15	61791-26-2	350	100	Tallow alkylamine ethoxylate	Ethoxylated amine	EA
	Genapol PF 20	9003-11-6	12700	N/A	Polymerization product of ethylene oxide and propylene oxide	Ethylene oxide/ propylene oxide polymer	EOP

10, *Hostapur*; 0.1, *Kutriacid*; 15, *Lutensit*; 15, *Cumolsulfonat*; 1, *Solfodac*; 1, *Texapon*; 1, *Servamine*; 0.1, *Oxidet*; 1, *Arylpon*; 5, *Empilan*; 0.1, *Ethomeen*; 15, *Genapol*. The concentration that caused 50% of growth inhibition of *A. junii* when compared to negative control was determined and expressed as EC_{50}. The EC_{50} values were expressed as concentrations of commercial product (mg L^{-1}), concentrations of active matter of surfactant in commercial product (mg L^{-1}) and as molar concentrations of active matter of surfactant in commercial product (mol L^{-1}), in the wastewater.

2. 5. Analytical Methods

The viable cell counts were performed by aseptically placing a 1 mL of sample in a tube containing 9 mL of sterile 0.3% NaCl solution. Sample was then serially diluted (10^{-1} to 10^{-7}) and 0.1 mL was inoculated onto nutrient agar plates (spread plate method). After the incubation (24h/30 ± 0.1 °C) the grown colonies were counted and reported as colony forming units (CFU) per one mL of simulated wastewater.

The concentrations of active matter of surfactants in the wastewater were measured using standard methods for surfactant measurements. Anionic surfactants were measured as methylene blue active substances[7] (range; 0.001–2.0 mg L^{-1}) and nonionic surfactants were measured by indirect tensammetric method[8] (range; 0.05–15.0 mg L^{-1}). The cationic surfactant was measured spectrophotometrically using the bromphenolblue method[9] (range; 0.05–2.5 mg L^{-1}) and amphoteric surfactant using the alkaline methylene blue method[10] (range; 10–100 mg L^{-1}). To measure concentrations that were above sensitivity range appropriate dilutions were made. For amphoteric amine oxide, the concentrations below the sensitivity range were taken as nominal values.

The pH of simulated wastewater was measured with WTW 330 pH-meter. All the measurements were done in triplicate.

3. Results and Discussion

The toxicities of the 12 surfactants (commercial products) to phosphate-accumulating bacterium *A. junii* are presented in Table 2. Based on Croatian regulations on chemical classification[11] seven products proved to be toxic (EC_{50} values between 1 and 10 mg L^{-1}) and five surfactants with EC_{50} higher than 100 mg L^{-1} were considered as non-toxic. The most toxic commercial product was *Kutriacid* with EC_{50} value of 1.4 mg L^{-1}.

The pH values in the reactors with surfactants were measured after 24 h of incubation and compared to the control reactors. The investigated surfactants by used concentrations did not influence the final pH values of the medium (7.40 ± 0.53), when compared to the control reactors without surfactant addition (7.75 ± 0.19). The possible negative effect of pH toward the *A. junii* can thus be safely excluded and the toxicity ascribed only to the presence of surfactant in the simulative wastewater.

The commercial surfactant products are used for manufacture of detergents, shampoos, cosmetics etc. After usage these products are discharged into sewage and further diluted in the wastewater. So to reliably represent the toxic effect of particular commercial surfactant in the environment, in further text the EC_{50} values are expressed as measured concentrations of active matter in the commercial product. Toxicity values of active matter of surfactant also allow comparison of different surfactants regarding their physicochemical properties. The EC_{50} values are expressed in mg L^{-1} for comparison with other studies on surfactant toxicities, since most of these are also expressed in mg L^{-1}.

3. 1. Anionic Surfactants

Toxicity of six commercially produced anionic surfactants was tested in this study. *Kutriacid* and *Solfodac* (linear alkyl benzene sulphonates; LAS) proved to be the most toxic type of anionic surfactants (Table 2) with EC_{50}

Table 2. Toxicity values expressed as EC_{50} (mg L^{-1}) of commercial product and EC_{50} and IC_{95} (mg L^{-1} or mol L^{-1}) of active surfactant matter with coefficient of variation (CV) for tested surfactants. [c_0 CFU (10^6 CFU mL^{-1})] = 35. 0 ± 9. 21.

Commercial surfactant	EC_{50} (mg L^{-1})	EC_{50} active matter (mg L^{-1})	EC_{50} active matter (mol L^{-1})	IC_{95} active matter (mg L^{-1})	IC_{95} active matter (mol L^{-1})	CV (%)
Hostapur	110.0	20.4	6.22×10^{-5}	148.4	4.52×10^{-4}	27.27
Kutriacid	1.4	0.15	4.66×10^{-7}	9.6	2.98×10^{-5}	35.71
Solfodac	3.1	1.8	5.60×10^{-6}	4.3	1.33×10^{-5}	9.68
Lutensit	15000	> 3471	$>1.50 \times 10^{-2}$	> 3471	$> 1.5 \times 10^{-2}$	–
Texapon	8.2	1.8	5.96×10^{-6}	175.6	5.81×10^{-4}	24.39
Cumolsulfonat	15000	> 72.6	$>3.27 \times 10^{-4}$	> 72.6	$> 3.27 \times 10^{-4}$	–
Servamine	4.3	2.8	8.00×10^{-6}	6.18	1.76×10^{-5}	6.98
Oxidet	3.1	1.0	3.89×10^{-6}	2.8	1.09×10^{-5}	9.68
Arylpon	4.1	1.9	4.28×10^{-6}	4.4	9.91×10^{-6}	12.20
Empilan	630.0	570	1.13×10^{-3}	> 4524	$> 8.96 \times 10^{-3}$	9.52
Ethomeen	1.8	1.1	3.14×10^{-6}	6.1	1.74×10^{-5}	33.33
Genapol	15000	> 14340	1.13×10^{-3}	> 14340	$> 1.13 \times 10^{-3}$	–

values of 0.15 mg L⁻¹ and 1.8 mg L⁻¹, respectively. Straight chain alkyl sulphate (AS) *Texapon* was the second most toxic anionic surfactant, while *Lutensit* (AS with branched chain) showed no toxic effect. Branched chain secondary alkane sulphonate (SAS) *Hostapur* and alkyl benzene sulphonate (ABS) *Cumolsulfonat*, also with branched alkyl chain, proved to be harmfull to *A. junii.*

Some toxicity data obtained from the literature are listed in Table 3. For some surfactants literature data could not be found so the presented toxicity data were obtained from the valid material safety data sheet provided by the manufacturer.

Table 3: Toxicity data for various types of surfactants.

Surfactant	Species	EC 50 (mg L⁻¹)	References
SAS	*Pseudomonas putida*	> 1.0	Data sheet*
LAS	*Daphnia magna*	8.1	17
	Dunaliella sp.	3.5	37
AS	*Gloeocapsa sp.*	50	38
	Vibrio fischeri	2.6	39
	Acinetobacter junii	2.25	40
ABS	*Daphnia magna*	> 100	Data sheet*
EOP	Bacteria	> 1.0	Data sheet*
QAC	*Pseudomonas putida*	6.0	23
	Vibrio fischeri	0.5	
	Acinetobacter junii	0.12	41
AO	*Photobacterium phosphoreum*	2. 4	42
	Daphnia magna	6. 8	
AE	*Ceriodaphnia dubia*	0. 39	2
		5. 36	
EA	*Daphnia magna*	0. 17	
	Nitrifying bacteria	64. 0	Data sheet*
AE+EA	Bacteria	> 1. 0	Data sheet*

*The data was retrieved from the valid material safety data sheet provided by the manufacturer.

Since the anionic surfactants are the most popularly used type of surfactant, their toxicity towards aquatic organisms was widely researched. The chronic toxicity of anionic surfactants occurs at concentrations usually greater than 0.1 mg L⁻¹.[12] Hennes-Morgan and Oude[13] found the ranges of toxicity values for the LAS, alkyl ethoxy sulphates and AS on *Daphnia magna* to be broad and largely overlapping. The EC₅₀ values were ranging roughly between 1–15 mg L⁻¹ of these anionic surfactants. However, the LAS tended to be the most toxic followed by the alkyl ethoxy sulphates that were either more toxic than or as toxic as the AS surfactants.

Some studies indicated that molecular lipophilicity exerts a considerable influence on the toxicity of anionic surfactants,[14] and the effect depends on both the character of the polar head group and the length of apolar alkyl chain.[15] It seems that in our study the toxicity of anionic surfactants did not depend on the type of active group; the

surfactants that share the same sulphonate active group were ranging from highly toxic to non-toxic to *A. junii*. To compare the toxicity of different surfactants regarding the chain length, the EC₅₀ value should be expressed in mol L⁻¹ (Table 2) to demonstrate the toxicity as a function of number of surfactant molecules. The order of toxicities of anionic surfactants were as follows: *Kutriacid* C₁₀₋₁₃ ₍₁₂ rich₎ > *Texapon* C₁₂ > *Solfodac* C₁₀₋₁₃ ₍₁₂ rich₎ > *Hostapur* C₁₃₋₁₇. Based on these results it seems that the toxicity of anionic surfactants decreases with increase in alkyl chain length. However, these conclusions cannot be assuredly verified from current experimental data. The exact distribution of alkyl chain length homologues of the tested surfactants could not be established so their contribution to the observed toxicity could not be evaluated.

Although Kotani *et al.*[16] stated that the the C₁₈ AS compounds caused human skin cell injury whereas C₁₀ and C₁₆ compounds caused more severe membrane destruction and protein denaturation, most literature data report that the toxicity of anionic surfactants increases with increasing lipophilicity of the molecule.[17] Holman and Macek[18] found the toxicity of LAS homologues to aquatic organisms was increasing with increase in chain length and/or molecular weight. In a quantitative structure-activity relationship study Versteeg *et al.*[19] found that toxicity of anionic surfactants to aquatic organisms increases with increasing length of the alkyl chain. However, this trend was not observed when the commercial products that contained LAS of different alkyl chain lengths were tested.[2] Hall *et al.*[20] evaluated the acute toxicities of 17 industrial surfactants having a wide range of physicochemical characteristics and reported that the base structure of the surfactants (aromatic or aliphatic, branched or linear) were not a factors controlling toxicity.

Surfactants containing branched alkyl chain were non-toxic (*Lutensit*) or considerably less toxic (*Cumolsulfonat*, *Hostapur*) than straight chain anionics. Common perception is that anionic surfactants with branched alkyl chain are poorly degradable and therefore environmentally unacceptable. However, recent studies showed that alkyl sulphates with either a small number of methyl branches along the hydrophobic chain[21] or 2-alkyl branches[22] were as rapidly and completely biodegraded as their linear counterparts. In our study branched chain anionics proved to be more environmental friendly than their linear chain counterparts.

3. 2. Cationic Surfactants

Servamine belongs to the quaternary ammonium compound (QAC) type of cationic surfactant. The *Servamine* proved to be toxic to *A. junii* with EC₅₀ value of 2.8 mg L⁻¹ (Table 2), which is similar to EC₅₀ values for the most toxic representatives of other classes of tested surfactants.

The QACs are known to be toxic in low concentrations to Gram-positive bacteria.[23] Augustin *et al.*[24] hypot-

hesized that QACs can disarrange the biological wastewater treatment process. The prevalence of QAC-resistant bacteria was elevated in effluents of sewage treatment plants receiving QACs.[25] In previous study from our laboratory[26] the QAC with 12 carbon atoms in alkyl chain acted less toxic against phosphate-accumulating bacterium *A. junii* than the QAC with 16 carbon atoms in alkyl chain. Garcia *et al.*[27] carried out the acute toxicity tests on *D. magna* and *Photobacterium phosphoreum* for six cationic surfactants: the EC_{50} on *D. magna* ranged from 0.13 to 0.38 mg L^{-1} whereas EC_{50} on *P. phosphoreum* ranged from 0.5 to 0.63 mg L^{-1}. They did not observe the incremental difference in toxicity between homologues of different chain length.

Since *Servamine* was the only representative of QAC in this study, and it contains imidazole ring instead of long alkyl chain, no relations between the chain length and toxicity of cationic surfactants could be concluded.

3. 3. Amphoteric Surfactants

In this study one amphoteric surfactant, *Oxidet*, belonging to amine oxide (AO) group was tested. The pure C_{14} AO component of *Oxidet* proved to be toxic to *A. junii* with EC_{50} of 1.0 mg L^{-1} (Table 2). The AO has been shown to have anti-microbial activity (Table 3) that increases with increasing chain length, exhibiting a cut-off effect at chain lengths of approximately C_{14}.[28] Garcia *et al.*[29] showed that the toxicity to *Daphnia* sp. increased when increasing the alkyl chain length. The relations between the chain length and toxicity of AO against *A. junii* could not be established in this study since *Oxidet* was the only representative of this particular type of surfactant.

3. 4. Nonionic Surfactants

The *Ethomeen*, an ethoxylated amine (EA) was the most toxic nonionic surfactant with EC_{50} of 1.1 mg L^{-1} (Table 2). *Empilan*, a member of alcohol ethoxylate (AE) group and *Genapol*, ethylene oxide/propylene oxide polymer, showed no toxic effect whilst *Arylpon*, a mixture of 60% EA and 15% (AE), proved to be toxic with EC_{50} of 1.9 mg L^{-1}. Since AE type of surfactant proved to be nontoxic to *A. junii*, it seems that the toxicity of *Arlypon* mixture comes from 60% of present EA. Lewis[30] found that surfactants in mixture had antagonistic, additive and synergistic interactions on toxicity to aquatic organisms. In our case it seems that the toxicity of surfactant mixture is defined by the most toxic surfactant present in the mixture.

The literature indicates that nonionic surfactants are in general more toxic to aquatic organisms than anionic surfactants.[13] Such generalization could not be considered in this study since the toxicity of nonionic and anionic surfactants were wide ranged and overlapping.

We observed that the toxicity of nonionic surfactants increases with increase in chain length; C_{16-18} *Arylpon*

was considerably more toxic than $C_{11-14 \,(13 \, rich)}$ *Empilan*. The most toxic nonionic *Ethomeen* is a tallow alkyl amine, meaning it is a mixture of alkyl amines with many different chain lengths ranging from C_{14} to C_{19}, with C_{18} being abundant. We also observed that the *Empilan* with 8 ethoxylated groups (EO) was considerably less toxic than *Ethomeen* with 5 EO. Still, no conclusions could be drawn from these observations since the different nature of hydrophilic groups in tested surfactants does not allow us to establish the effect of alkyl chain length or the number of ethoxylated groups on toxicity. Feijtal and Van de Plassche[31] stated that there was no relationship between the carbon chain length or the number of ethoxylated groups (EO) and the toxicity for AE surfactants. Warne *et al.*[2] found that the AE containing eight EO was notably more toxic than the AE containing three EO but could not state with any degree of confidence that there was a trend of increased toxicity with increased ethoxylation. In contrary, Wong *et al.*[32] stated that toxicity of AEs tended to increase with increasing alkyl chain length and decreasing number of EO groups.

The branched alkyl chain of *Empilan* might be responsible for its lack of toxicity which was confirmed by Madsen *et al.*[33] observation of neglible aquatic toxicity for branched nonionic surfactants.

The biodegradation of surfactants was not expected with pure culture of *A. junii* since usually a consortium of bacteria is required for successful biodegradation.[34] The *Genapol* seemed to be biodegraded by *A. junii* since the number of bacteria in the reactors with *Genapol* after 24 h incubation was up to four times greater when compared to control reactor with no *Genapol* added. However, since the surfactant was not the sole carbon source in the growth media, the increase in cell number could simply be due to the lack of antibacterial activity of the surfactant, or for some miscellaneous reason. No literature data about the biodegradation of this substance could be found. The material safety data sheet information states the biodegradation of > 47% which was the lowest among all the surfactants used in the study. The increase in number of bacterial cells after the 24 h of incubation when compared to control was not observed for other two non toxic surfactants *Lutensit* and *Cumolsulfonat*. The number of cells was actually slightly decreasing; however, the EC_{50} was not obtained at 15 g L^{-1} of commercial product so we classified them as non-toxic, but not possibly biodegradable by *A. junii*.

It should be noted that toxicity values of commercial surfactants against *A. junii* reported in this study are probably the upper limits. The adsorption of surfactants to insensitive organisms, dead cells in flocs and inorganic matter in the wastewater treatment plant should protect viable susceptible bacteria from the toxic effect of surfactants. Traina *et al.*[35] found that dissolved humic substances reduced the aqueous bioavailability of LAS and Cano *et al.*[36] found that organic carbon in sediments reduced the toxicity of ABS to *Hyalella azteca*.

4. Conclusions

The results showed that the toxicities of nonionic and anionic surfactants to phosphate-accumulating bacterium *A. junii* were wide ranged and overlapping. When all of the tested surfactants were compared there could be no generalization in toxicity regarding the class or the alkyl chain length of the surfactant. One observed feature is that surfactants with branched alkyl chain had least toxic or no toxic effect to *A. junii*.

Our experiments proved that the most abundantly used types of surfactants (LAS, AS and QAC) are also the most toxic ones to *A. junii*. Ever expanding detergent and cosmetics industry will assuredly give rise to concentrations of these commercially manufactured surfactants in sewage effluents. Thus, it is important to investigate the influence of these abundantly present chemicals on phosphate-accumulating bacteria inhabiting the activated sludge in order to prevent or understand the possible failures of EBPR systems.

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Povzetek

Testirali smo vpliv strupenosti dvanajstih vrst različnih (anionskih, kationskih, amfoternih in neionskih) komercialnih surfaktantov na kulturo *Acinetobacter junii.* Sedem je bilo strupenih (EC_{50} vrednosti med 1 in 10 mg L^{-1}), medtem ko smo jih pet opredelili kot nestrupene (EC_{50} višja od 100 mg L^{-1}). Najbolj strupen surfaktant je bil *Kutriacid* ($EC_{50} = 1.4$ mg L^{-1}). Strupenost anionskih surfaktantov je naraščala s krajšanjem alkilne verige, vendar ni bila odvisna od vrste aktivne skupine, medtem ko je strupenost neionskih surfaktantov naraščala z dolžino verige. Iz primerjave vseh testiranih surfaktantov ni bilo možni dobiti splošnih zaključkov o vplivu vrste ali dolžine verige na strupenost, ugotovili smo le, da bolj razvejana veriga povzroča manjšo strupenost ali pa ni strupena za *A. junii.*