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## Hydrolysis of surfactin over activated carbon

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## ABSTRACT

Surfactin is obtained through biocatalysis by microorganisms. In our biorefinery concept, it is purified on activated carbon (AC) during downstream processing. Besides cyclic surfactin, it is possible to obtain linear surfactin analogues, when AC with specific properties is used. In the present article, the hydrolysis of various cyclic surfactin analogues mediated by activated carbon is described. Hydrolysis products were identified using HPLC/UV/MS and (Q-TOF)MS/MS. Hydrolytic activity of six commercial and three modified activated carbons was evaluated. The porous texture of ACs was determined by sorption measurements and elemental composition of ACs surface – by SEM-EDS analysis. Their  $pH_{PZC}$  value and moisture, ash, and volatile matter content using proximate analysis were also determined. Properties of ACs were correlated with their hydrolytic activity, and the crucial role of alkaline  $pH_{PZC}$  was found. The beneficial effect of alkaline  $pH_{PZC}$  was further confirmed by acid modification of AC that had previously shown hydrolytic activity and lost this ability after the  $pH_{PZC}$  decrease.

## 1. Introduction

Biosurfactants are surface-active compounds that are produced by microorganisms or obtained by biotransformation [1,2]. Surfactin is a lipopeptide biosurfactant produced mainly by various strains of *Bacillus subtilis*. It is a mixture of homologues, which differ in length of the hydrophobic chain of  $\beta$ -hydroxy fatty acid (10–18 carbon atoms), isoforms which differ in the degree of chain branching, and analogues, which differ in the composition of amino acids in the hydrophilic heptapeptide ring [3–5]. Surfactin is one of the most effective biosurfactants, which can lower the surface tension of water from 72 to 27 mN/m at concentrations as low as  $1 \times 10^{-5}$  M [6–8]. Moreover, it possesses many bioactive properties, such as antimicrobial [9,10], antiviral [11], antifungal [12,13], and antitumor activities [14]. However, it also causes hemolysis of red blood cells, which is not beneficial from a medical point of view [4]. To overcome this problem and to enhance other properties of surfactin, new analogues are searched and synthesized [4,8,15]. One group of the studied compounds includes linear analogues of surfactin.

Cyclic surfactin (CS) may undergo chemical (alkaline or acid) or enzymatic hydrolysis. Its lactone or one of the peptide bonds are those

which are hydrolyzed. Consequently, the heptapeptide ring opens and linear surfactins (LS) are formed (Fig. 1).

Dufour et al. [4] studied the hemolytic activity of surfactin and its analogues, including LS. They found that all the tested linear analogues do not cause hemolysis of swine red blood cells, in contrast to cyclic surfactin. Moreover, LS inhibits the hemolytic effect of another surfactant (TX-100). Similar results were reported for another lipopeptide – battacin. Its linear analogue exhibits reduced hemolytic activity of mouse blood cells and also enhanced antimicrobial activity [16]. Also, other properties were modified due to surfactin linearization. The minimum surface tension of water increases from 27 mN/m for cyclic surfactin to 36 mN/m for linear surfactin and CMC value from  $1 \times 10^{-5}$  M to  $7-8 \times 10^{-5}$  M [17]. Despite the increase of both values, LS retains much of the significant surface activity of CS. The rise of these values is mainly due to the cleavage of the peptide ring and the increased hydrophilicity of LS [4,18]. Imura et al. [19] studied properties of concentrated solutions of LS and CS and structures of lyotropic liquid crystals that their form. They found that CS forms interdigitated lamella phases, while LS forms bicontinuous cubic phases.

The most common linearization is alkaline hydrolysis of surfactin which leads to saponification of lactone bond (Fig. 1a). Reagents that

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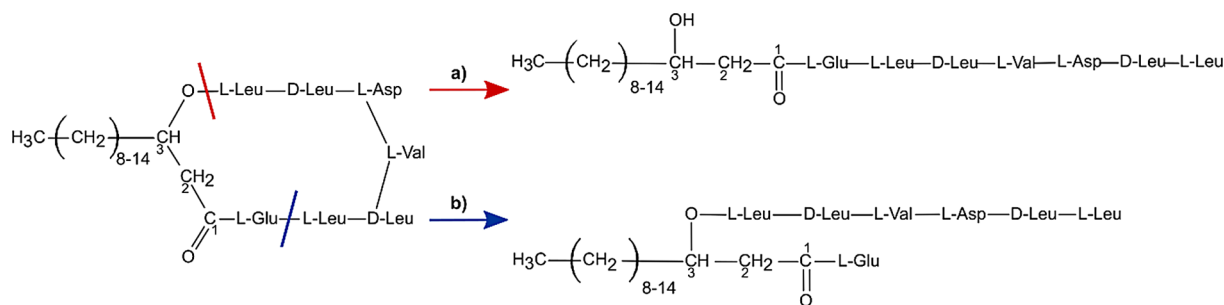


Fig. 1. Two possible ways of surfactin hydrolysis: a) hydrolysis of lactone bond, b) hydrolysis of the peptide bond between L-Glu1 and L-Leu2.

can serve as hydrolytic agents include sodium hydroxide solution in methanol [4,17,20], sodium methoxide solution in methanol [19,21] or an aqueous solution of sodium or ammonium hydroxide [18,22]. Acid hydrolysis using hydrochloric acid is also possible [22]. Enzymatic hydrolysis is induced by enzymes secreted by many microorganisms. *Streptomyces* sp. Mg1 strain produced enzyme – surfactin hydrolase – which hydrolyzes the lactone bond in surfactin. It makes this strain insensitive to the antimicrobial activity of surfactin [23]. In turn, V8 endoprotease obtained from *Staphylococcus aureus* hydrolyzed the peptide bond between L-Glu1 and L-Leu2 (Fig. 1b) [24]. Other lipopeptides also can be biodegraded by microorganisms' enzymes. Antibiotic daptomycin is hydrolyzed by 44% of 60 examined actinomycete, and another 29% caused its deacylation [25].

Basic hydrolysis allows obtaining LS with the highest purity (< 97%) [21]. However, the hydrolysis rate in strong alkaline solutions, such as 3 M NaOH, is lower than in weaker alkaline solutions, such as 1 M NaOH, and is 64% and 87% respectively. Acid hydrolysis with hydrochloric acid results in only 56% conversion to LS [22]. Enzymatic hydrolysis of the ester bond by surfactin hydrolase is also very efficient (95%) [23], but hydrolysis of the peptide bond by V8 endoprotease convert CS to LS in only 14%, even when the time of incubation was prolonged to four days [24].

There are also a few examples of direct biosynthesis of linear lipopeptides including surfactin by microorganisms [26–32]. Pathak et al. [30,31] obtained thirteen LS with a chain length of 13–16 carbon atoms also varying in amino acid composition, together with CS and other lipopeptides – fengycins and iturins. They used environment isolated strains of *Bacillus amyloliquefaciens* 6B and *B. tequilensis* P15. Dhali et al. [32] also obtained LS with a chain length of 13–16 carbon atoms together with CS from the genetic engineered strain of *B. subtilis*. Contamination of CS with LS is not surprising since cyclization of surfactin is the last step during its biosynthesis [8]. The *B. subtilis* KCTC12411BP marine strain produces three linear lipopeptides, named gageostatins A-C. They have identical amino acids composition in peptide as surfactin but the different chiral configuration of them (LLDLLL compared to LLDLLDL in surfactin) [26,27]. Another option is a genetic modification of microorganisms producing cyclic lipopeptides, in order to produce their linear analogues. Linear lipopeptides with a shorter sequence of amino acids than in surfactin were obtained this way [33,34]. Dufour et al. [4] and Pagadoy et al. [35] employed solid-phase peptide synthesis to obtain linear analogues of surfactin with different chain lengths. The same method was used by De Zoysa et al. [16] to obtain linear analogues of another cyclic lipopeptide - battacin - secreted by the soil bacterium *Paenibacillus tianmunensis*.

In our biorefinery concept, surfactin is obtained in biocatalytic reaction during fermentation of rapeseed meal by *Bacillus subtilis*. It is purified on activated carbon (AC) in downstream processing. We were searching for optimum conditions and sorbents for this process and observed that it is possible to obtain linear surfactin analogues when AC with specific properties is used.

Scarce literature data show that ACs can be a good adsorbent for biosurfactants [36–38], but there is no mention of hydrolysis that may

occur on this sorbent.

Activated carbons are highly porous materials with a broad range of applications in industry. They can serve as a versatile adsorbent for storage [39], separation [40,41], and purification [42] of gases and liquids [43,44], and also as capacitor components [45]. Moreover, they are cheap and non-toxic, can be prepared from a broad spectrum of organic precursors [46–48] and their surface can be functionalized to fit a specific application [49,50]. Fewer reports concern activated carbon's role as a catalyst of various reactions. It can serve as catalyst support [51], but also as a catalyst itself, which facilitates and mediates reactions, such as transesterification [52], hydrochlorination [53] or peroxide oxidation [54]. ACs have also been utilized in various hydrolysis reactions including dehydrohalogenation [55] and dehalogenation [56,57], but also ester hydrolysis [58].

We propose a new method for obtaining LS using a variety of AC as a catalyst. It is quite easy and inexpensive, which is essential in making LS production economically viable. Tuning properties of AC allow us to obtain linear or cyclic surfactin analogues in biorefinery depending on current demand.

## 2. Materials and methods

### 2.1. Materials

Surfactin sodium salt was purchased from Boruta-Zachem Biochemia (Bydgoszcz, Poland). Potassium hydroxide, sulfuric acid, nitric acid, and phosphoric acid (analytically pure) were purchased from Avantor (Avantor Performance Materials Poland S.A., Gliwice, Poland).

Granular activated carbons CWZ, BA11, and WACC were purchased from Carbon (Racibórz, Poland), TE50 – from Picatif (France), RB2 – from Norit (Denmark). BA11 and RB2 are coal-based, WACC and TE50 are coconut-shell-based, and CWZ is deciduous-tree-wood-based.

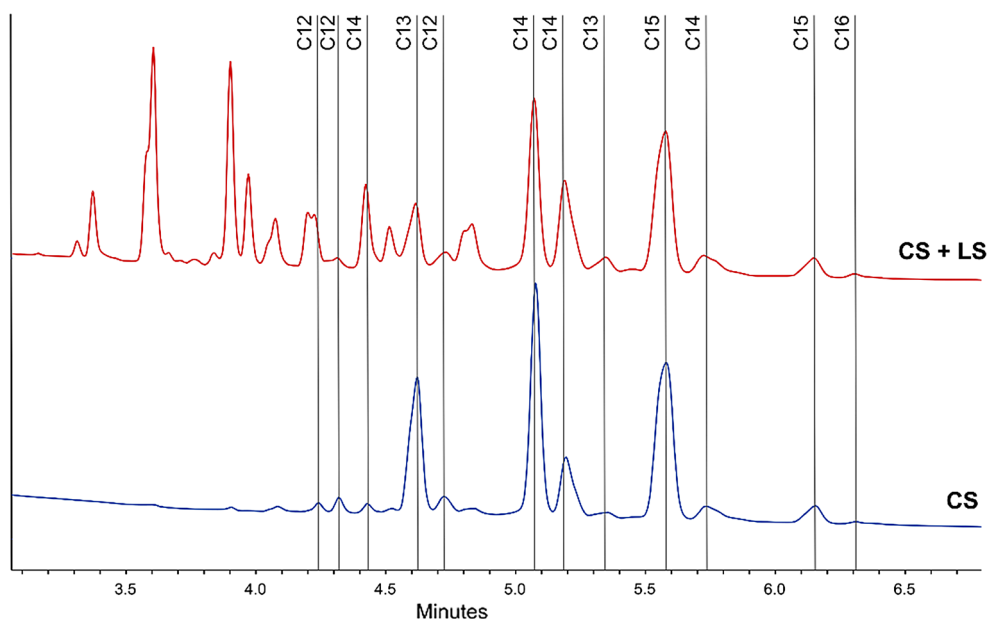
Char Novicarbon was purchased from Ekomotor (Wrocław, Poland). It was obtained from beech wood in the process of carbonization in a revolving tube kiln (final temperature: 600 °C, heating speed: 10 °C/min) in a flow of inert gas (N<sub>2</sub>, flow: 10 dm<sup>3</sup>/h). After carbonization, the obtained product was cooled to room temperature in the kiln in a flow of nitrogen.

### 2.2. Activated carbon characteristic

Activated carbons (AC) were characterized by proximate analysis (moisture, ash, and volatile fraction) in compliance with Polish Standard (PN-80/G-04511, 04512, 04516). Surface area and porosity were determined by the method described elsewhere [46].

AC pH<sub>PZC</sub> was determined by a protocol based on ISO 787-9:1981 standard for determination of pH value of an aqueous suspension of pigments and extenders. Freshly distilled water (20 ml) was poured into a flask containing 0.5 g of AC. The flask was sealed and shaken for 72 h. After that time, the pH was measured in triplicate.

Elemental composition of AC surface was determined using



**Fig. 2.** HPLC-UV chromatograms of cyclic surfactin (CS) and linear and cyclic surfactin (LS + CS) solutions (fragment from 3 to 7 min). Cyclic surfactin peaks are indicated by the vertical lines, and the chain length is depicted.

**Table 1**

Mass-to-charge ( $m/z$ ) values of molecular ion peaks corresponding to UV-chromatogram peaks of cyclic and linear surfactin (positive ionization was applied). Also, the retention time ( $t$ ) is indicated. For cyclic surfactin, only peaks with the highest intensity are present in the table.

The chain length of surfactin analogues	Linear surfactin		Cyclic surfactin	
	$t$ [s]	$m/z$	$t$ [s]	$m/z$
C12	3.308	1012.79	4.309	994.82
C12	3.368	1012.81		
C12	3.452	1012.73		
C13	3.578	1026.80	4.612	1008.79
C13	3.601	1026.79		
C13	3.660	1026.80		
C13	3.705	1026.81		
C14	3.898	1040.81	5.068	1022.81
C14	3.967	1040.84	5.186	1022.83
C15	4.197	1054.85	5.575	1036.88
C15	4.221	1054.84		
C15	4.276	1054.81		

scanning electron microscopy – energy-dispersive X-ray spectroscopy (SEM-EDS). The samples of AC were observed using Quanta 250 system (FEI, Hillsboro, OR, USA) operated at 15 kV, equipped with Tracor-Northern EDS. The EDS detector has an ultra-thin light-element window, which allow to detect elements with atomic number  $> 5$ . The results are mean from six randomly chosen spots or areas on the surface of each AC.

### 2.3. LC solvents

Acetonitrile (ACN) (HPLC gradient grade) was purchased from VWR (VWR International, Radnor, PA, USA). Water (LC-MS grade), methanol (LC-MS grade), and acetic acid (HPLC gradient grade) were purchased from Merck (Merck KGaA, Darmstadt, Germany). 2-propanol (HPLC grade) and trifluoroacetic acid (TFA) (HPLC grade) were purchased from Sigma-Aldrich (Saint Louis, MO, USA).

### 2.4. Surfactin hydrolysis

We ground each of the AC to obtain uniform batches with a

granulation of 0.2–1 mm, rinsed them with distilled water, and dried them in an oven (105 °C) overnight. In a typical experiment, we poured 1.5 ml of surfactin solution (0.8 mg/ml) into a 2 ml microtube containing 0.02 g of AC. Samples were shaken in a microshaker for a maximum time of 144 h. For each AC, we prepared a series of 12 samples, which were collected and analyzed after different hydrolysis time.

Repeatability measurement was performed for two activated carbons, which showed the best hydrolytic performance: Picactif and WACC. For this purpose, a short version of the aforementioned experiment was employed. We poured 1.5 ml of surfactin solution (0.863 mg/ml) into a 2 ml microtube containing 0.02 g of AC. For each AC, we prepared two series of 4 samples, which were shaken in a microshaker and collected after 0.5, 1, 2 and 24 h.

### 2.5. Analysis

We measured the surfactin concentration using HPLC/UV analysis. Before analysis, each sample was purified using a syringe filter (Nylon, 0.22  $\mu\text{m}$ ). Analysis was performed on a Series 200 HPLC system (Perkin Elmer, Waltham, MA, USA) equipped with Kinetex XB-C18 column (100  $\times$  4.6 mm, 2.6  $\mu\text{m}$ ) (Phenomenex, Torrance, CA, USA). The surfactin was eluted for 13 min by ACN/H<sub>2</sub>O/TFA (3.8 mM v/v) at 1.2 ml/min under isocratic conditions. The spectrum was recorded at 205 nm. The results are mean of two measurements presented with error bar ( $\pm$  standard deviation).

To identify the hydrolysis products, UPLC/UV/MS analysis of obtained solutions was performed on an Acquity Arc UPLC system (Waters, Millford, MA, USA) equipped with QDa detector and Cortec C18 column (50  $\times$  4.6 mm, 2.7  $\mu\text{m}$ ). Surfactin was eluted by solvent A: acetonitrile and 0.1% (v/v) formic acid and solvent B: water and 0.1% (v/v) formic acid at 1 ml/min under 8.25 min linear gradient conditions (%A: 0 min – 50%, 0.15 min – 50%, 2.25 min – 80%, 5.25 min – 90%, 6.75 min – 100%, 7.50 min – 50%, 8.25 min – 50%). The spectrum was recorded at 210 nm. MS detection was performed in positive and negative polarization. Spectra were recorded in the mass range of 500–1250 Da. Capillary voltage was +0.8 kV, cone voltage 25 V.

We confirmed the structure of hydrolyzed surfactin by tandem mass spectrometry. All LC/MS and LC/MS/MS experiments were performed on a Xevo G2 Q-ToF connected to an Acquity UPLC chromatography

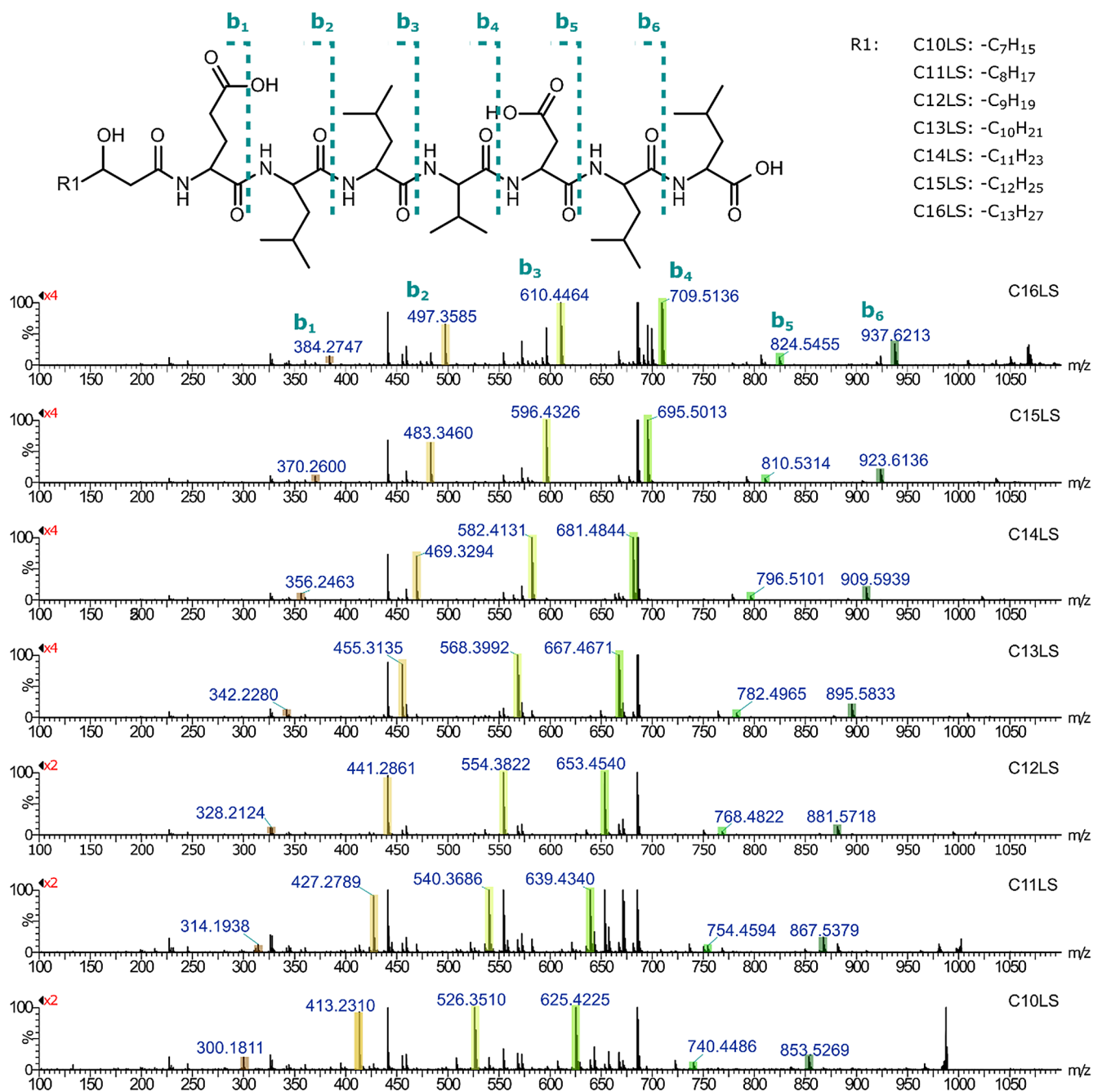


Fig. 3. MS/MS(Q-TOF) spectra of hydrolyzed surfactin. B ions of seven linear surfactin analogues differ in chain length (C10LS – C16LS). Ions are consistent with the predicted structure depicted above.

system (Waters, Millford, MA, USA) equipped with Acquity CSS C18 column (100 × 1.0 mm). Surfactin was eluted by solvent A: 40% water and 0.1% acetic acid in methanol and solvent B: 40% 2-propanol and 0.1% acetic acid in methanol under linear gradient conditions (%A: 0 min – 100%, 1 min – 100%, 1.1 min – 50%, 14 min – 30%, 15 min – 0%, 19.9 min – 0%, 20 min – 100%, 25 min – 100%) at 0.05 ml/min. Detection was performed in positive polarization in sensitivity mode. Capillary voltage +3.5 kV, sampling cone 40, extraction cone 4.0, source temperature 120 °C, gas temperature 350 °C, gas flow rate 800 l/h (13.31/min), cone gas 50 l/h (0.831/min). No additional make-up solution was required. Mass correction via lock-spray fluidics (5 µl/min) was carried out using enkephalin (556.2771 Da) as a calibrator. In LC/MS experiments 500–1100 Da spectra were collected. In LC/MS/MS experiments spectra were collected in the range of 100–1100 Da,

collision energy was 30 V, 7 precursor ions were chosen for fragmentation (initial and end time in brackets): 984.6268 (4–8 min), 998.6411 (5–9 min), 1012.6565 (5–9 min), 1026.6775 (6–9 min), 1040.6958 (7–11 min), 1054.7095 (7–12 min), and 1068.7228 (9–12 min). Also, MS<sup>3</sup> fragmentation was performed using the 685.4501 Da in-source-fragment as a precursor.

## 2.6. Acid modification of activated carbon

The Norit activated carbon was modified with three inorganic acids: sulfuric acid, nitric acid, and phosphoric acid. We ground the AC to obtain uniform batches with granulation of 0.2–1 mm, rinsed them with distilled water, and dried them in an oven (105 °C) overnight. We poured 20 g of 5% (wt.) acid into 50 ml Erlenmeyer flask containing 5 g

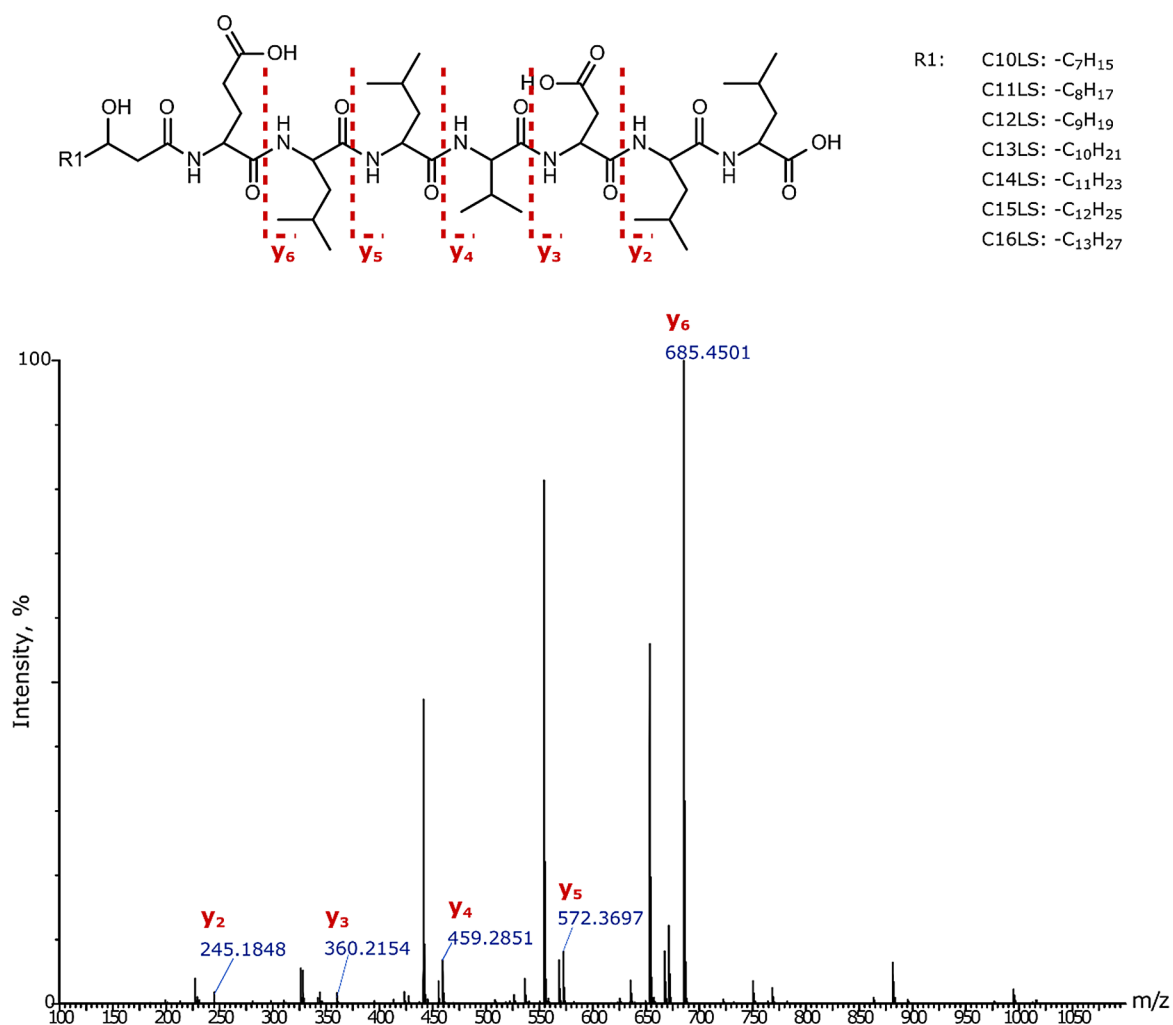


Fig. 4. MS/MS(Q-TOF) spectra of hydrolyzed surfactin. Y ions are the same for all of seven linear surfactin analogues differ in chain length (C10LS – C16LS). Ions are consistent with the predicted structure depicted above.

of AC. Samples were shaken in a shaker for 15 h (150 rpm). The modified AC samples obtained were washed with hot distilled water for 0.5 h, then soaked for 1 h in a 0.01 M solution of potassium hydroxide, and then were being washed again with hot distilled water until pH was close to neutral. Then, samples were dried in an oven (105 °C) overnight. Obtained ACs were designated as N-H<sub>2</sub>SO<sub>4</sub>, N-HNO<sub>3</sub>, and N-H<sub>3</sub>PO<sub>4</sub>, respectively.

### 3. Results and discussion

#### 3.1. Surfactin hydrolysis

We observed additional peaks on HPLC/UV chromatograms of surfactin solution after its hydrolysis on AC (Fig. 2). Mass spectra revealed that the molecular ion peaks corresponding to these peaks have *m/z* values 18 Da higher than cyclic surfactin analogues (Table 1). Five, new HPLC/UV peaks with the highest intensity have similar order and ratios of peaks retention time. Therefore, we assumed that the new peaks come from the linear surfactin products obtained by hydrolysis. Literature data suggest that usually, the lactone bond undergoes hydrolysis [4,17–23], leading to peptide ring opening.

#### 3.2. Confirmation of linear surfactin structure

To confirm our assumptions we performed tandem mass spectrometry of the new surfactin analogues. Careful examination of single

mass spectra revealed that C10–C16 chain-length analogues of surfactin are present in solution both in cyclic and hydrolyzed forms. Therefore, we performed fragmentation of ions with seven different *m/z* values corresponding to surfactin analogues with different chain length.

Positive polarization provided better sensitivity for the majority of peaks, and only these results are presented hereafter. For all ions, we observed fragments typical for peptide fragmentation, i.e. *b*/*y* ion pairs (Figs. 3 and 4). Cleavage of a peptide usually occurs at the peptide amide bond, which produces *b* ions, if the *N*-terminal fragment retains the charge, and *y* ions, if the *C*-terminal fragment retains the charge [59]. We found *b*<sub>1</sub> – *b*<sub>6</sub> and *y*<sub>1</sub> – *y*<sub>6</sub> fragments on spectra of all analogues. They are consistent with predicted structures, in which the lactone bond undergoes hydrolysis and linear surfactin analogues are formed (*y*<sub>1</sub> ion has low intensity, and it is not visible on the full-scale spectra).

One can observe that the presence of these ions does not exclude the possibility of the hydrolysis of other bonds, such as the amide bond between L-Glu1 and L-Leu2 or between D-Leu6 and L-Leu7, because neither *b*<sub>0</sub> nor *y*<sub>7</sub> ions are present on the spectra. However, in the first case another *y* ions series ought to be present; in the second – another *b* ions series, but fragmentation ions from both series are missing on the recorded spectra.

Moreover, we performed a MS<sup>3</sup> fragmentation of the in-source generated fragment. The fragment of *m/z* value of 685.4501 Da was identified as the *y*<sub>6</sub> ion. It is the fragment with the highest intensity in the spectra of all analogues. *Y* and *b* ions of this in-source generated



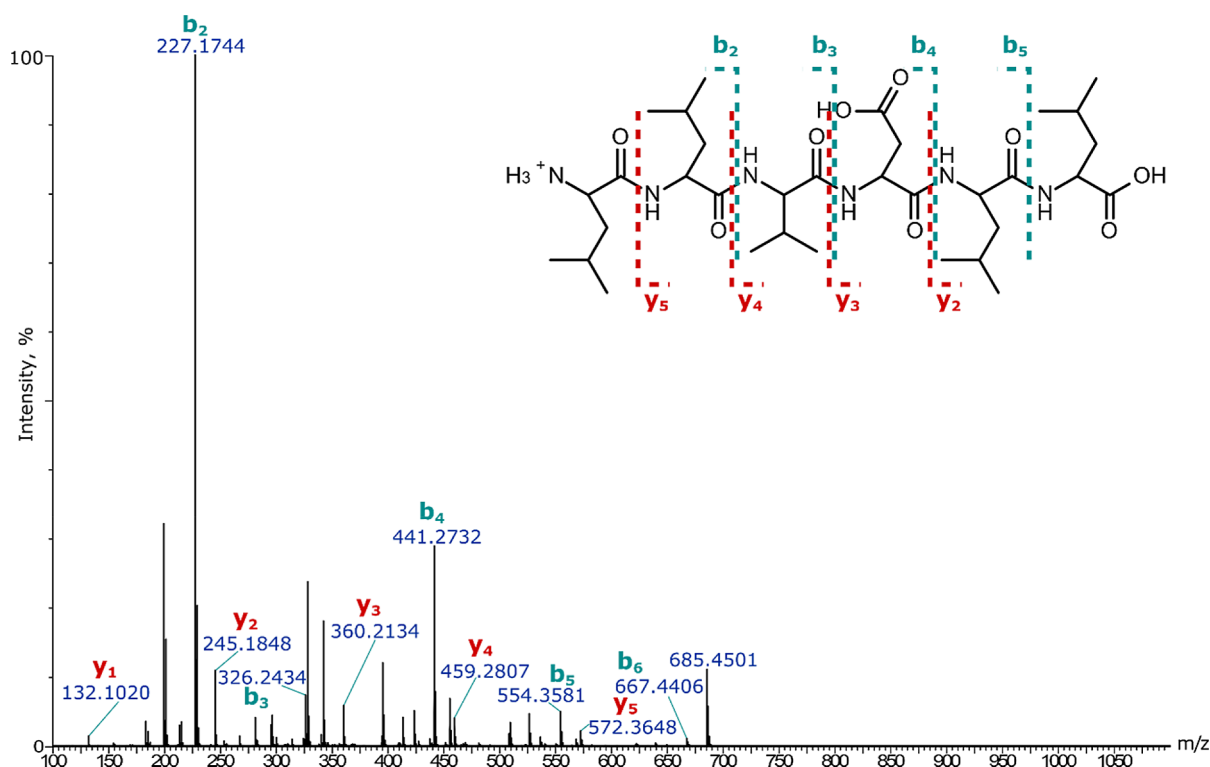


Fig. 5. MS/MS(Q-TOF) spectra of the 685.4501 Da in-source-fragment created from all seven linear surfactin analogues differ in chain length (C10 – C16). B and y ions are consistent with the predicted structure.

Table 2

Activated carbons' parameters:  $\text{pH}_{\text{PZC}}$ ,  $W_{\text{A}}$  – moisture,  $A_{\text{d}}$  – ash (dry state),  $V_{\text{daf}}$  – volatile fraction (dry, ash-free state).

Sorbent	$\text{pH}_{\text{PZC}}$	$W_{\text{A}}$ [%]	$A_{\text{d}}$ [%]	$V_{\text{daf}}$ [%]
BA11	$8.96 \pm 0.41$	$2.13 \pm 0.21$	$9.26 \pm 0.10$	$1.17 \pm 0.49$
CWZ	$8.42 \pm 0.05$	$17.49 \pm 0.02$	$7.49 \pm 0.67$	$9.29 \pm 1.97$
Norit	$9.71 \pm 0.37$	$0.90 \pm 0.07$	$4.91 \pm 0.02$	$2.14 \pm 0.09$
Novicarbon	$7.99 \pm 0.02$	$4.20 \pm 0.00$	$4.40 \pm 0.00$	$20.00 \pm 0.00$
Picactif	$9.75 \pm 0.10$	$3.10 \pm 0.00$	$2.20 \pm 0.00$	$3.20 \pm 0.00$
WACC	$9.68 \pm 0.16$	$8.55 \pm 1.22$	$3.29 \pm 0.55$	$2.98 \pm 0.32$

fragment was found, and the order of amino acids in the peptide fragment was confirmed (Fig. 5).

### 3.3. Correlation between activated carbon parameters and surfactin hydrolysis

All the tested activated carbons caused surfactin hydrolysis, but the only tested char – Novicarbon did not. Among the sorbents, hydrolysis rate differs depending on the AC used. In order to explain these results, we performed sorbents characterization and tried to correlate their parameters with the hydrolysis rate. Proximate analysis and  $\text{pH}_{\text{PZC}}$  values of AC are collected in Table 2, porous texture parameters – in

Table 3

Activated carbon parameters: distribution of pores volume ( $V_{\Sigma\text{mic}}$  – the sum of micropores volume,  $V_{\Sigma\text{mes}}$  – sum of mesopores volume,  $V_{\text{tot}}$  – total pores volume).

Sorbent	$V_{0-0.4 \text{ nm}}$ [ $\text{cm}^3/\text{g}$ ]	$V_{0.4-2 \text{ nm}}$ [ $\text{cm}^3/\text{g}$ ]	$V_{\Sigma\text{mic}}$ [ $\text{cm}^3/\text{g}$ ]	$V_{2-3 \text{ nm}}$ [ $\text{cm}^3/\text{g}$ ]	$V_{3-5 \text{ nm}}$ [ $\text{cm}^3/\text{g}$ ]	$V_{5-10 \text{ nm}}$ [ $\text{cm}^3/\text{g}$ ]	$V_{10-50 \text{ nm}}$ [ $\text{cm}^3/\text{g}$ ]	$V_{\Sigma\text{mes}}$ [ $\text{cm}^3/\text{g}$ ]	$V_{\text{tot}}$ [ $\text{cm}^3/\text{g}$ ]
BA11	0.000	0.384	0.384	0.029	0.013	0.014	0.017	0.073	0.457
CWZ	0.000	0.303	0.303	0.028	0.016	0.022	0.018	0.084	0.387
Norit	0.000	0.360	0.360	0.028	0.012	0.008	0.008	0.056	0.416
Novicarbon	0.068	0.056	0.124	0.008	0.006	0.005	0.004	0.023	0.147
Picactif	0.000	0.410	0.410	0.020	0.011	0.008	0.010	0.049	0.459
WACC	0.000	0.373	0.373	0.016	0.005	0.006	0.010	0.037	0.410

Tables 3 and 4, and elemental composition of AC surface in Table 5. All AC have alkaline  $\text{pH}_{\text{PZC}}$  and a predominantly microporous structure, in which micropores accounted for 78–91% of the total adsorption pores volume. Only Novicarbon has pores of width below 0.4 nm, which are characteristic for chars [46]. The highest content of oxygen on the sorbent surface has Novicarbon – over 10%. The remaining AC have a little lower content of 6.80–9.91%, except for a Norit which surface contains less than 1% of oxygen. The highest content of alkaline metals (Na, K) has two AC – Picactif and WACC.

We cannot determine the hydrolysis rate, because we failed in our attempts to separate pure linear analogues. However, in order to compare hydrolysis rates on different ACs, we can compare the area of linear analogues peaks on HPLC/UV chromatograms, since they were recorded on the same apparatus at the same time. The fastest hydrolysis took place on Picactif and WACC activated carbons (Fig. 6). In the first case, a decrease of peak area after 96 h of hydrolysis can be associated with adsorption of the hydrolysis products on the activated carbon. No hydrolysis occurred when Novicarbon activated carbon was used. However, we can observe trace amounts of linear surfactin in cyclic surfactin used as a substrate for hydrolysis.

For two ACs with the highest hydrolytic activity (Picactif and WACC), we checked the repeatability of hydrolysis (Fig. 7). Irrespective of hydrolysis time, the standard deviation of linear surfactin peaks total area is a little below 10%. Only after 24 h of hydrolysis, it is higher for

**Table 4**

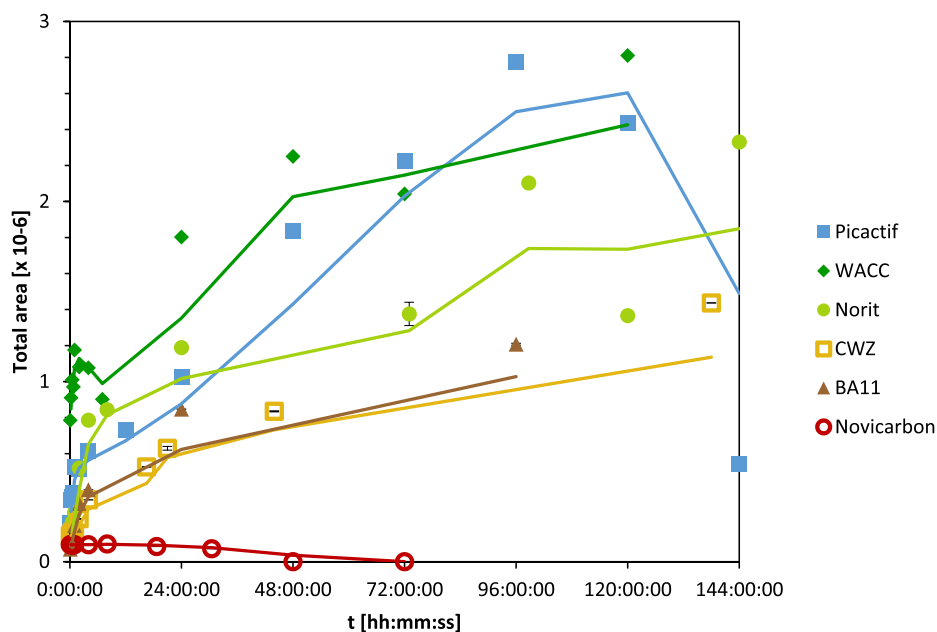
Activated carbon parameters: distribution of pores surface area ( $S_{\Sigma\text{mic}}$  – the sum of micropores surface area,  $S_{\Sigma\text{mes}}$  – the sum of mesopores surface area,  $S_{\text{tot}}$  – total pores surface area,  $S_{\text{BET}}$  – pores BET surface area).

Sorbent	$S_{0-0.4 \text{ nm}}$ [m <sup>2</sup> /g]	$S_{0.4-2 \text{ nm}}$ [m <sup>2</sup> /g]	$S_{\Sigma\text{mic}}$ [m <sup>2</sup> /g]	$S_{2-3 \text{ nm}}$ [m <sup>2</sup> /g]	$S_{3-5 \text{ nm}}$ [m <sup>2</sup> /g]	$S_{5-10 \text{ nm}}$ [m <sup>2</sup> /g]	$S_{10-50 \text{ nm}}$ [m <sup>2</sup> /g]	$S_{\Sigma\text{mes}}$ [m <sup>2</sup> /g]	$S_{\text{tot}}$ [m <sup>2</sup> /g]	$S_{\text{BET}}$ [m <sup>2</sup> /g]
BA11	0	1061	1061	24.9	7.0	4.0	1.6	37.5	1099	993
CWZ	0	837	837	23.3	8.7	6.3	1.8	40.1	877.1	749
Norit	0	992	992	22.8	6.4	2.4	0.7	32.3	1024	896
Novicarbon	179	153	332	6.5	3.3	1.6	0.4	11.8	343.8	72
Picactif	0	1132	1132	17.2	5.8	2.7	0.9	26.6	1159	1028
WACC	0	1030	1030	13.9	2.8	1.7	0.9	19.3	1049	903

**Table 5**

Sorbent surface elemental composition determined by SEM-EDS (the results are presented as an element weight percentage on AC surface).

Element	Weight [%]					
	BA11	CWZ	Norit	Novicarbon	Picactif	WACC
C	87.75 ± 2.68	89.13 ± 1.41	96.02 ± 2.00	88.35 ± 4.18	90.76 ± 1.46	88.64 ± 2.06
O	8.96 ± 1.62	9.91 ± 1.89	0.96 ± 1.92	10.57 ± 4.08	6.80 ± 2.14	8.80 ± 2.49
Mg	0.04 ± 0.05	0.03 ± 0.03	0.50 ± 0.33	0.07 ± 0.07	0.03 ± 0.07	0.04 ± 0.03
Si	0.71 ± 0.17	0.11 ± 0.03	0.27 ± 0.28	0.05 ± 0.06	0.09 ± 0.07	0.14 ± 0.08
S	0.45 ± 0.12	0.07 ± 0.06	0.52 ± 0.22	0.02 ± 0.05	–	0.14 ± 0.10
Ca	0.51 ± 0.27	0.29 ± 0.68	0.87 ± 0.61	0.54 ± 0.55	–	–
Al	0.59 ± 0.09	0.09 ± 0.03	0.04 ± 0.08	0.01 ± 0.02	–	0.05 ± 0.02
K	0.06 ± 0.09	0.24 ± 0.23	0.11 ± 0.21	0.33 ± 0.22	1.30 ± 0.48	1.78 ± 1.16
Cu	–	–	0.72 ± 1.18	–	0.83 ± 0.74	–
Na	0.14 ± 0.15	–	–	–	0.12 ± 0.16	0.27 ± 0.22
P	0.03 ± 0.05	–	–	0.04 ± 0.06	0.04 ± 0.06	0.09 ± 0.08
Cl	0.05 ± 0.07	0.11 ± 0.04	–	–	–	0.07 ± 0.11
Fe	0.84 ± 0.34	–	–	–	–	–



**Fig. 6.** The total area of linear surfactin peaks on HPLC/UV chromatograms in solution after hydrolysis on various activated carbons.

Picactif (17%) and lower for WACC ( $< 0.05\%$ ) (see Fig. 8 and Table 8).

We found a strong positive correlation between  $\text{pH}_{\text{PZC}}$  of AC and the total area of linear surfactin peaks on HPLC-UV chromatograms ( $R^2 = 0.8607$ ) (Fig. 8a). Novicarbon, with the lowest  $\text{pH}_{\text{PZC}}$  value (7.99), was the only AC which did not hydrolyze surfactin. The other ACs, with  $\text{pH}_{\text{PZC}}$  higher than 8.4, caused hydrolysis. It is in agreement with literature data, where alkaline hydrolysis of surfactin is the most effective one [4,17–23]. Also, hydrolysis of another compound which is catalyzed on AC is alkaline [55,57,60]. Moreover, we found a weak positive correlation ( $R^2 = 0.4472$ ) between the content of alkaline

metals (Na and K) on AC surface and the total area of linear surfactin peaks on HPLC-UV chromatograms (Fig. 8b), which supports an aforementioned conclusion. Based on SEM-EDS results we calculated mean oxygen content on the AC surface and found its weak negative impact on surfactin hydrolysis by AC (Fig. 8c). The most abundant AC oxygen surface group are acidic in nature (carboxylic, phenol, anhydride and lactone groups) [61]. Therefore, the higher oxygen content on the AC surface the weaker activity of the sorbent in the reaction of surfactin hydrolysis. A similar relationship was found by Chen et al. [55] who discovered that hydrolysis of 1,1,2,2-tetrachloroethane

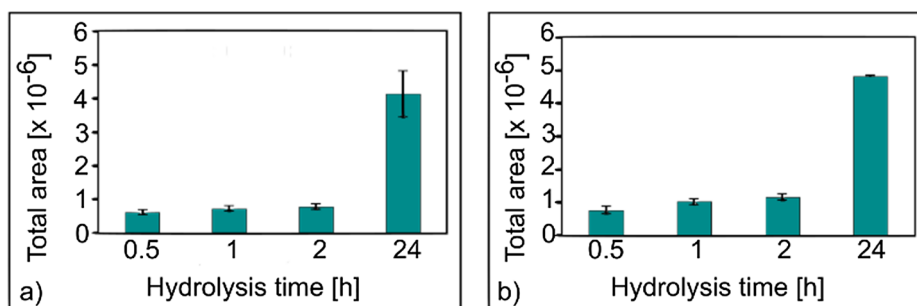


Fig. 7. Repeatability of surfactin hydrolysis by two ACs: (a) Picactif and (b) WACC. Hydrolysis is expressed as the total area of linear surfactin peaks on HPLC/UV chromatograms in solution.

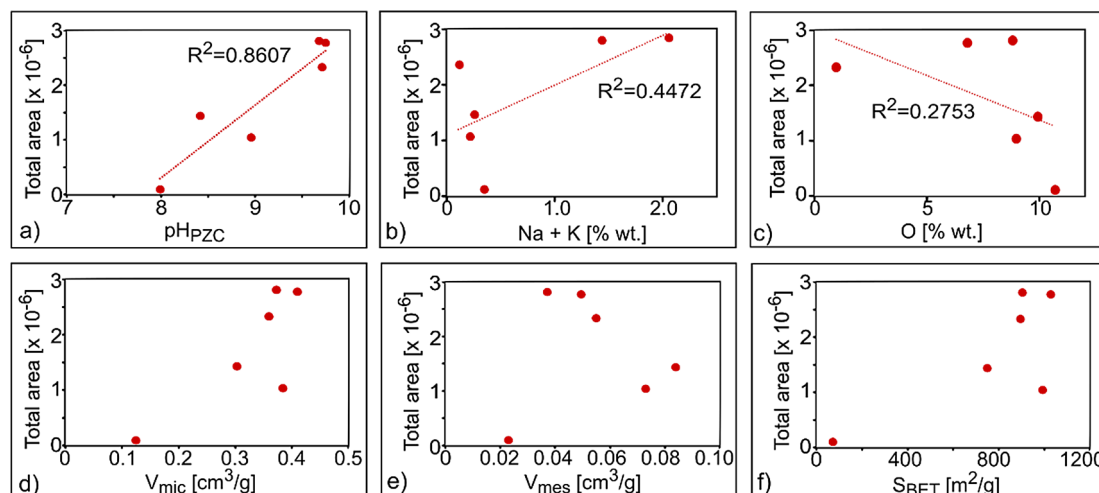


Fig. 8. Effect of various activated carbon parameters on surfactin hydrolysis rate expressed as maximum total peak area: (a)  $\text{pH}_{\text{PZC}}$ , (b) content of Na and K on AC surface, (c) content of oxygen on AC surface, (d) micropores volume ( $V_{\text{mic}}$ ), (e) mesopores volume ( $V_{\text{mes}}$ ), (f) BET surface area ( $S_{\text{BET}}$ ).

Table 6

Modified activated carbons' parameters:  $\text{pH}_{\text{PZC}}$ ,  $W_{\text{A}}$  – moisture,  $A_{\text{d}}$  – ash (dry state).

AC	$\text{pH}_{\text{PZC}}$	$W_{\text{A}}$ [%]	$A_{\text{d}}$ [%]
N- $\text{H}_2\text{SO}_4$	$7.28 \pm 0.02$	$4.96 \pm 0.00$	$2.34 \pm 0.03$
N- $\text{HNO}_3$	$7.02 \pm 0.13$	$4.15 \pm 0.00$	$2.10 \pm 0.10$
N- $\text{H}_3\text{PO}_4$	$7.84 \pm 0.11$	$3.81 \pm 0.00$	$2.71 \pm 0.19$
Norit	$9.71 \pm 0.37$	$0.90 \pm 0.07$	$4.91 \pm 0.02$

catalyzed by AC was less effective with increasing content of oxygen on the AC surface. The effect of oxygen content on hydrolysis yield is not so pronounced as the effect of activated carbons  $\text{pH}_{\text{PZC}}$  probably because some amount of surface oxygen may come from alkaline and alkaline earth metal oxides present on AC surface. These oxides may provide basic sites on the AC surface. Also,  $\pi$  electrons from graphene layers forming the AC structure may give basic character to AC pores [61]. Probably these two types of active sites are responsible for surfactin hydrolysis. The hydrolysis rate seems not to be influenced by AC

Table 7

Modified activated carbon parameters: distribution of pores volume ( $V_{\Sigma\text{mic}}$  – the sum of micropores volume,  $V_{\Sigma\text{mes}}$  – sum of mesopores volume,  $V_{\text{tot}}$  – total pores volume).

AC	$V_{0-0.4 \text{ nm}}$ [ $\text{cm}^3/\text{g}$ ]	$V_{0.4-2 \text{ nm}}$ [ $\text{cm}^3/\text{g}$ ]	$V_{\Sigma\text{mic}}$ [ $\text{cm}^3/\text{g}$ ]	$V_{2-3 \text{ nm}}$ [ $\text{cm}^3/\text{g}$ ]	$V_{3-5 \text{ nm}}$ [ $\text{cm}^3/\text{g}$ ]	$V_{5-10 \text{ nm}}$ [ $\text{cm}^3/\text{g}$ ]	$V_{10-50 \text{ nm}}$ [ $\text{cm}^3/\text{g}$ ]	$V_{\Sigma\text{mes}}$ [ $\text{cm}^3/\text{g}$ ]	$V_{\text{tot}}$ [ $\text{cm}^3/\text{g}$ ]
N- $\text{H}_2\text{SO}_4$	0.000	0.369	0.369	0.026	0.015	0.006	0.006	0.053	0.422
N- $\text{HNO}_3$	0.000	0.364	0.364	0.023	0.011	0.007	0.006	0.047	0.411
N- $\text{H}_3\text{PO}_4$	0.000	0.369	0.369	0.028	0.011	0.007	0.008	0.054	0.423
Norit	0.000	0.360	0.360	0.028	0.012	0.008	0.008	0.056	0.416

porous texture characteristic. We did not find any correlation between micro- or mesopores volume, or BET surface area, and surfactin hydrolysis rate (Fig. 8d, e and f).

### 3.4. Confirmation of a key role of $\text{pH}_{\text{PZC}}$ on surfactin hydrolysis rate

In order to confirm that alkaline  $\text{pH}_{\text{PZC}}$  is the key to surfactin hydrolysis over AC, we prepared a series of acid-modified ACs. Norit AC, which efficiently hydrolyzes surfactin, was treated with three inorganic acids: sulfuric acid, nitric acid, and phosphoric acid. Parameters of the prepared ACs are collected in Tables 6–8. We can notice that porous structure parameters did not change significantly as a result of the modification. The only difference is in the AC chemistry, which reflects in lower, close to neutral,  $\text{pH}_{\text{PZC}}$  values and more than twice lower ash content. Mild modification conditions (room temperature and/or diluted acids) usually results with slight porous texture increase and significant ash content reduction [62,63]. Then, the modified AC was used for surfactin hydrolysis. In all cases, no hydrolysis occurred. At this moment, we confirmed that  $\text{pH}_{\text{PZC}}$  is the main AC parameter deciding on



**Table 8**

Modified activated carbon parameters: distribution of pores surface area ( $S_{\Sigma mic}$  – the sum of micropores surface area,  $S_{\Sigma mes}$  – the sum of mesopores surface area,  $S_{tot}$  – total pores surface area,  $S_{BET}$  – pores BET surface area).

AC	$S_{0-0.4 \text{ nm}}$ [m <sup>2</sup> /g]	$S_{0.4-2 \text{ nm}}$ [m <sup>2</sup> /g]	$S_{\Sigma mic}$ [m <sup>2</sup> /g]	$S_{2-3 \text{ nm}}$ [m <sup>2</sup> /g]	$S_{3-5 \text{ nm}}$ [m <sup>2</sup> /g]	$S_{5-10 \text{ nm}}$ [m <sup>2</sup> /g]	$S_{10-50 \text{ nm}}$ [m <sup>2</sup> /g]	$S_{\Sigma mes}$ [m <sup>2</sup> /g]	$S_{tot}$ [m <sup>2</sup> /g]	$S_{BET}$ [m <sup>2</sup> /g]
N-H <sub>2</sub> SO <sub>4</sub>	0	1020	1020	22.3	5.2	1.8	0.6	29.9	1050	932
N-HNO <sub>3</sub>	0	1006	1006	19.6	6.0	2.2	0.5	28.3	1034	895
N-H <sub>3</sub> PO <sub>4</sub>	0	1020	1020	23.0	6.2	2.0	0.8	32.0	1052	915
Norit	0	992	992	22.8	6.4	2.4	0.7	32.3	1024	896

surfactin hydrolysis effectiveness. Moreover, we showed that it is not important what kind of acid serves as a donor of acid groups since all three tested acids prevented hydrolysis.

#### 4. Conclusions

Fermentation of rapeseed meal by *Bacillus subtilis* results with some value-added products such as biosurfactants, biopolymers, and feed additives. In our biorefinery, we utilize activated carbon for purifying surfactin after its biosynthesis. Modification of AC properties allows us to produce linear surfactin analogues through hydrolysis catalyzed on activated carbon. Structures of obtained compounds were predicted based on HPLC/UV/MS analysis. It revealed that the molecular ion peaks corresponding to new HPLC-UV peaks have  $m/z = 1012.8$ , 1026.8, 1040.8, and 1054.8. These correspond to 18 Da higher six major surfactin standard peaks of four homologs that differ in their  $\beta$ -hydroxy fatty acid chain length: C12, C13, C14, and C15. To identify the surfactin bond that undergoes hydrolysis, tandem mass spectrometry was performed. Fragmentation ions from the b and y series were found in the spectra of ions of all seven analogues – C10-C16, and in the spectra of the in-source generated linear surfactin peptide fragment with  $m/z = 685.4501$  Da. Fragmentation paths of all ions enable us to state that hydrolytic cleavage occurs at the lactone bond. The peptide ring opens, that results in linear surfactin analogues.

All five commercially available AC exhibited hydrolytic activity. The only sorbent which did not hydrolyzed surfactin was commercial char – Novicarbon. The hydrolysis rate differs depending on the AC's properties and contact time. The longer the contact time, the higher is the concentration of hydrolyzed surfactin in solution. The only exception is Picactif activated carbon, on which partial adsorption of the hydrolysis products occurred, reflecting in a decrease of product concentration after 96 h of hydrolysis. We measured repeatability of surfactin hydrolysis for two AC with the highest catalytic activity. The standard deviation of the total area of linear surfactin peaks on HPLC/UV chromatograms is around 10%. A strong positive correlation was found between  $pH_{PZC}$  of AC and a total area of linear surfactin peaks on HPLC/UV chromatograms ( $R^2 = 0.8607$ ). Also, weak positive effect of alkaline metal (Na and K) content on the AC surface on hydrolysis rate was found. It is in agreement with the literature data, where alkaline hydrolysis of surfactin is the most effective one. In turn, the higher oxygen content on AC surface the weaker hydrolytic activity of AC. Similar behaviour was noticed when AC was used as a catalyst of 1,1,2,2-tetrachloroethane hydrolysis. The decisive role of basic sites on the catalytic activity of AC during surfactin hydrolysis was confirmed by acid modification of one of AC. As a result of acids action,  $pH_{PZC}$  of AC was reduced to almost neutral, and ash content was reduced over twice. Modified AC did not show any hydrolytic activity which confirms the key role of alkaline  $pH_{PZC}$  of AC in surfactin hydrolysis. The report demonstrates a new method of obtaining linear surfactin analogues, which exploits the catalytic properties of activated carbon.

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#### Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bioorg.2019.03.070>.

#### References

- [1] P. Biniarz, M. Lukaszewicz, T. Janek, Screening concepts, characterization and structural analysis of microbial-derived bioactive lipopeptides: a review, *Crit. Rev. Biotechnol.* 37 (2017) 393–410, <https://doi.org/10.3109/07388551.2016.1163324>.
- [2] M. Henkel, M. Geissler, F. Weggenmann, R. Hausmann, Production of microbial biosurfactants: Status quo of rhamnolipid and surfactin towards large-scale production, *Biotechnol. J.* 12 (2017) 1–10, <https://doi.org/10.1002/biot.201600561>.
- [3] P. Jajor, D. Piłakowska-Pietras, A. Krasowska, M. Lukaszewicz, Surfactin analogues produced by *Bacillus subtilis* strains grown on rapeseed cake, *J. Mol. Struct.* 1126 (2016) 141–146, <https://doi.org/10.1016/j.molstruc.2016.02.014>.
- [4] S. Dufour, M. Deleu, K. Nott, B. Wathelet, P. Thonart, M. Paquet, Hemolytic activity of new linear surfactin analogs in relation to their physico-chemical properties, *Biochim. Biophys. Acta – Gen. Subj.* 1726 (2005) 87–95, <https://doi.org/10.1016/j.bbagen.2005.06.015>.
- [5] P. Biniarz, M. Lukaszewicz, Direct quantification of lipopeptide biosurfactants in biological samples via HPLC and UPLC-MS requires sample modification with an organic solvent, *Appl. Microbiol. Biotechnol.* 101 (2017) 4747–4759, <https://doi.org/10.1007/s00253-017-8272-y>.
- [6] H. Heerklotz, J. Seelig, Detergent-like action of the antibiotic peptide surfactin on lipid membranes, *Biophys. J.* 81 (2001) 1547–1554, [https://doi.org/10.1016/S0006-3495\(01\)75808-0](https://doi.org/10.1016/S0006-3495(01)75808-0).
- [7] A. Zou, J. Liu, V.M. Garamus, Y. Yang, R. Willumeit, B. Mu, Micellization activity of the natural lipopeptide [Glu 1, Asp 5 ] surfactin-C15 in aqueous solution, *J. Phys. Chem. B* 114 (2010) 2712–2718, <https://doi.org/10.1021/jp908675s>.
- [8] F. Peypoux, J.M. Bonmatin, J. Wallach, Recent trends in the biochemistry of surfactin, *Appl. Microbiol. Biotechnol.* 51 (1999) 553–563, <https://doi.org/10.1007/s002530051432>.
- [9] J. Falardeau, C. Wise, L. Novitsky, T.J. Avis, Ecological and mechanistic insights into the direct and indirect antimicrobial properties of *Bacillus subtilis* Lipopeptides on plant pathogens, *J. Chem. Ecol.* 39 (2013) 869–878, <https://doi.org/10.1007/s10886-013-0319-7>.
- [10] K.M. Kim, J.Y. Lee, C.K. Kim, J.S. Kang, Isolation and characterization of surfactin produced by *Bacillus polyfermenticus* KJS-2, *Arch. Pharm. Res.* 32 (2009) 711–715, <https://doi.org/10.1007/s12272-009-1509-2>.
- [11] X. Huang, Z. Lu, H. Zhao, X. Bie, F.X. Lü, S. Yang, Antiviral activity of antimicrobial lipopeptide from *Bacillus subtilis* fmbj against Pseudorabies virus, porcine parvovirus, Newcastle disease virus and infectious bursal disease virus in vitro, *Int. J. Pept. Res. Ther.* 12 (2006) 373–377, <https://doi.org/10.1007/s10989-006-9041-4>.
- [12] A. Sarwar, M.N. Hassan, M. Imran, M. Iqbal, S. Majeed, G. Brader, A. Sessitsch, F.Y. Hafeez, Biocontrol activity of surfactin A purified from *Bacillus* NH-100 and NH-217 against rice bakanae disease, *Microbiol. Res.* 209 (2018) 1–13, <https://doi.org/10.1016/j.micres.2018.01.006>.
- [13] K. Paraszkiwicz, P. Bernat, P. Siewiera, M. Moryl, L.S. Paszt, P. Trzcziński, Ł. Jałowicki, G. Plaza, Agricultural potential of rhizospheric *Bacillus subtilis* strains exhibiting varied efficiency of surfactin production, *Sci. Hortic. (Amsterdam)* 225 (2017) 802–809, <https://doi.org/10.1016/j.scienta.2017.07.034>.
- [14] S. Kim, J.Y. Kim, S.-H. Kim, H.J. Bae, H. Yi, S.H. Yoon, B.S. Koo, M. Kwon, J.Y. Cho, C.-E. Lee, S. Hong, Surfactin from *Bacillus subtilis* displays anti-proliferative effect via apoptosis induction, cell cycle arrest and survival signaling suppression, *FEBS Lett.* 581 (2007) 865–871, <https://doi.org/10.1016/j.febslet.2007.01.059>.
- [15] S.M. Mandal, A.E.A.D. Barbosa, O.L. Franco, Lipopeptides in microbial infection control: Scope and reality for industry, *Biotechnol. Adv.* 31 (2013) 338–345, <https://doi.org/10.1016/j.biotechadv.2013.01.004>.
- [16] G.H. De Zoysa, A.J. Cameron, V.V. Hegde, S. Raghothama, V. Sarojini, Antimicrobial peptides with potential for biofilm eradication: synthesis and structure activity relationship studies of baccatin peptides, *J. Med. Chem.* 58 (2015) 625–639, <https://doi.org/10.1021/jm501084q>.

- [17] M. Morikawa, Y. Hirata, T. Imanaka, A study on the structure & function relationship of lipopeptide biosurfactants, *Biochim. Biophys. Acta (BBA)-Mol. Cell Biol. Lipids* 1488 (2000) 211–218.
- [18] M. Osman, Y. Ishigami, K. Ishikawa, Y. Ishizuka, H. Holmsen, Dynamic transition of  $\alpha$ -helix to  $\beta$ -sheet structure in linear surfactin correlating to critical micelle concentration, *Biotechnol. Lett.* 16 (1994) 913–918, <https://doi.org/10.1007/BF00128624>.
- [19] T. Imura, S. Ikeda, K. Aburai, T. Taira, D. Kitamoto, Interdigitated lamella and bi-continuous cubic phases formation from natural cyclic surfactin and its linear derivative, *J. Oleo Sci.* 62 (2013) 499–503.
- [20] M. Deleu, R. Brasseur, M. Paquot, H. Legros, S. Dufour, P. Jacques, J. Destain, P. Thonart, A. Brans, B. Joris, J.-M. Frere, Novel use of lipopeptide preparations, US2006166869A1, 2006.
- [21] T. Taira, S. Ikeda, D. Kawamura, H. Sakai, M. Abe, D. Kitamoto, T. Imura, Monolayer behavior of cyclic and linear forms of surfactins: thermodynamic analysis of Langmuir monolayers and AFM study of Langmuir-Blodgett monolayers, *J. Oleo Sci.* 63 (2014) 407–412, <https://doi.org/10.5650/jos.ess13138>.
- [22] M. Osuman, I. Yutaka, I. Yasuko, Straight-chain surfactin, JPH0892279(A), 1996.
- [23] B.C. Hoefler, K.V. Gorzelnik, J.Y. Yang, N. Hendricks, P.C. Dorrestein, P.D. Straight, Enzymatic resistance to the lipopeptide surfactin as identified through imaging mass spectrometry of bacterial competition, *Proc. Natl. Acad. Sci.* 109 (2012) 13082–13087, <https://doi.org/10.1073/pnas.1205586109>.
- [24] I. Grangemard, J. Wallach, F. Peypoux, Evidence of surfactin hydrolysis by a bacterial endoprotease, *Biotechnol. Lett.* 21 (1999) 241–244, <https://doi.org/10.1023/A:1005444717166>.
- [25] V.M. D'Costa, T.A. Mukhtar, T. Patel, K. Koteva, N. Waglchner, D.W. Hughes, G.D. Wright, G. De Pascale, Inactivation of the lipopeptide antibiotic daptomycin by hydrolytic mechanisms, *Antimicrob. Agents Chemother.* 56 (2012) 757–764, <https://doi.org/10.1128/AAC.05441-11>.
- [26] F.S. Tareq, H.J. Shin, H.-S. Lee, J.-S. Lee, Y.-J. Lee, M.A. Lee, Gageostatins lipopeptides produced from a Marine-derived *Bacillus subtilis* having antimicrobial activity, KR20180003520A, 2018.
- [27] F.S. Tareq, M.A. Lee, H.-S. Lee, J.-S. Lee, Y.-J. Lee, H.J. Shin, Gageostatins A-C, antimicrobial linear lipopeptides from a marine *Bacillus subtilis*, *Mar. Drugs* 12 (2014) 871–885, <https://doi.org/10.3390/md12020871>.
- [28] M.J. Balunas, R.G. Linington, K. Tidgewell, A.M. Fenner, L.D. Ureña, G. Della Togna, D.E. Kyle, W.H. Gerwick, Dragonamide E, a modified linear lipopeptide from *Lymbia majuscula* with antileishmanial activity, *J. Nat. Prod.* 73 (2010) 60–66, <https://doi.org/10.1021/np900622m>.
- [29] K. Desjardine, A. Pereira, H. Wright, T. Matainaho, M. Kelly, R.J. Andersen, Tauramamide, A Lipopeptide Antibiotic Produced in Culture by *Bre W* *ibacillus laterosporus* Isolated from a Marine Habitat: Structure Elucidation and Synthesis, 2007, pp. 1850–1853.
- [30] K.V. Pathak, A. Bose, H. Keharia, Identification and characterization of novel surfactins produced by fungal antagonist *Bacillus amyloliquefaciens* 6B, *Biotechnol. Appl. Biochem.* 61 (2014) 349–356, <https://doi.org/10.1002/bab.1174>.
- [31] K.V. Pathak, A. Bose, H. Keharia, Characterization of novel lipopeptides produced by *Bacillus tequilensis* P15 using liquid chromatography coupled electron spray ionization tandem mass spectrometry (LC-ESI-MS/MS), *Int. J. Pept. Res. Ther.* 20 (2014) 133–143, <https://doi.org/10.1007/s10989-013-9375-7>.
- [32] D. Dhali, F. Coutte, A.A. Arias, S. Auger, V. Bidnenko, G. Chataigné, M. Lalk, J. Niehren, J. de Sousa, C. Versari, P. Jacques, Genetic engineering of the branched fatty acid metabolic pathway of *Bacillus subtilis* for the overproduction of surfactin C14 isoform, *Biotechnol. J.* 12 (2017) 1–10, <https://doi.org/10.1002/biot.201600574>.
- [33] F. De Ferra, F. Rodriguez, O. Tortora, C. Tosi, G. Grandi, Engineering of peptide synthetases. Key role of the thioesterase-like domain for efficient production of recombinant peptides, *J. Biol. Chem.* 272 (1997) 25304–25309, <https://doi.org/10.1074/jbc.272.40.25304>.
- [34] T. Stachelhaus, A. Schneider, M. Marahiel, Rational design of peptide antibiotics by targeted replacement of bacterial and fungal domains, *Science* (80-) 269 (1995) 69–72, <https://doi.org/10.1126/science.7604280>.
- [35] M. Pagadoy, F. Peypoux, J. Wallach, Solid-phase synthesis of surfactin, a powerful biosurfactant produced by *Bacillus subtilis*, and of four analogues, *Int. J. Pept. Res. Ther.* 11 (2005) 195–202, <https://doi.org/10.1007/s10989-005-6790-4>.
- [36] L. Montastruc, T. Liu, F. Gancel, L. Zhao, I. Nikov, Integrated process for production of surfactin. Part 2. Equilibrium and kinetic study of surfactin adsorption onto activated carbon, *Biochem. Eng. J.* 38 (2008) 349–354, <https://doi.org/10.1016/j.bej.2007.07.023>.
- [37] K.V. Dubey, A.A. Juwarkar, S.K. Singh, Adsorption-desorption process using wood-based activated carbon for recovery of biosurfactant from fermented distillery wastewater, *Biotechnol. Prog.* 21 (2008) 860–867, <https://doi.org/10.1021/bp040012e>.
- [38] A. Carmen dos Santos Mendes de Oliveira, M. da Silva Bezerra, C.E. de Araujo Padilha, A.M. Melchuna, G.R. de Macedo, E.S. dos Santos, Recovery of rhamnolipids produced by *Pseudomonas aeruginosa* using acidic precipitation, extraction, and adsorption on activated carbon, *Sep. Sci. Technol.* 48 (2013) 2852–2859, <https://doi.org/10.1080/01496395.2013.809107>.
- [39] Z.J. Sroka, M. Kułczyński, J. Kaczmarczyk, R. Michałowski, Application of carbon adsorbent to methane storage in fuel tanks of vehicles, *Polish J. Environ. Study* 18 (2009) 211–215.
- [40] A.A. Abdurashed, A.A. Jilil, S. Triwahyono, M.A.A. Zaini, Y. Gambo, M. Ibrahim, Surface modification of activated carbon for adsorption of SO<sub>2</sub> and NO<sub>x</sub>: a review of existing and emerging technologies, *Renew. Sustain. Energy Rev.* 94 (2018) 1067–1085, <https://doi.org/10.1016/j.rser.2018.07.011>.
- [41] N. Kostoglou, C. Koczwar, C. Prehal, V. Terziyska, B. Babic, B. Matovic, G. Constantinides, C. Tampaxis, G. Charalambopoulou, T. Steriotis, S. Hinder, M. Baker, K. Polychronopoulou, C. Doumanidis, O. Paris, C. Mitterer, C. Reholz, Nanoporous activated carbon cloth as a versatile material for hydrogen adsorption, selective gas separation and electrochemical energy storage, *Nano Energy* 40 (2017) 49–64, <https://doi.org/10.1016/j.nanoen.2017.07.056>.
- [42] H. Nam, S. Wang, H.R. Jeong, TMA and H<sub>2</sub>S gas removals using metal loaded on rice husk activated carbon for indoor air purification, *Fuel* 213 (2018) 186–194, <https://doi.org/10.1016/j.fuel.2017.10.089>.
- [43] M. Dabioch, R. Skorek, A. Kita, P. Janoska, K. Pytlakowska, P. Zerzucha, R. Sitko, A study on adsorption of metals by activated carbon in a large-scale (municipal) process of surface water purification, *Open Chem.* 11 (2013) 742–753, <https://doi.org/10.2478/s11532-013-0209-4>.
- [44] K. Bratek, W. Bratek, M. Kułczyński, Activated carbons prepared by corn cobs activation in water purification, *Polish J. Environ. Study* 14 (2005) 115–118.
- [45] Z. Lin, E. Goikolea, A. Balducci, K. Naoi, P.L. Taberna, M. Salanne, G. Yushin, P. Simon, Materials for supercapacitors: when Li-ion battery power is not enough, *Mater. Today* 21 (2018) 419–436, <https://doi.org/10.1016/j.mattod.2018.01.035>.
- [46] H. Fałtynowicz, J. Kaczmarczyk, M. Kułczyński, Preparation and characterization of activated carbons from biomass material – giant knotweed (*Reynoutria sachalinensis*), *Open Chem.* 13 (2015), <https://doi.org/10.1515/chem-2015-0128>.
- [47] A. Perrin, A. Celzard, A. Albiniak, J. Kaczmarczyk, J.F. Maréché, G. Furdin, NaOH activation of anthracites: effect of temperature on pore textures and methane storage ability, *Carbon N. Y.* 42 (2004) 2855–2866, <https://doi.org/10.1016/j.carbon.2004.06.030>.
- [48] O. Ioannidou, A. Zabaniotou, Agricultural residues as precursors for activated carbon production – a review, *Renew. Sustain. Energy Rev.* 11 (2007) 1966–2005, <https://doi.org/10.1016/j.rser.2006.03.013>.
- [49] T.J. Bandosz, C. Petit, On the reactive adsorption of ammonia on activated carbons modified by impregnation with inorganic compounds, *J. Colloid Interface Sci.* 338 (2009) 329–345, <https://doi.org/10.1016/j.jcis.2009.06.039>.
- [50] L. Tang, L. Li, R. Chen, C. Wang, W. Ma, X. Ma, Adsorption of acetone and isopropanol on organic acid modified activated carbons, *J. Environ. Chem. Eng.* 4 (2016) 2045–2051, <https://doi.org/10.1016/j.jece.2016.03.031>.
- [51] M. Kułczyński, Selective catalytic reduction no by ammonia over ceramic and active carbon based catalysts, in: A. Ahsan (Ed.), *Heat Anal. Thermodyn. Eff., Intech*, 2011. <http://www.intechopen.com/books/heat-analysis-and-thermodynamic-effects/selective-catalytic-reduction-no-by-ammonia-over-ceramic-and-active-carbon-based-catalysts>.
- [52] B.E. Narowska, M. Kułczyński, M. Łukaszewicz, E. Burchacka, Use of activated carbons as catalyst supports for biodiesel production, *Renew. Energy* 135 (2019) 176–185.
- [53] W. Zhao, M. Zhu, B. Dai, Cobalt-nitrogen-activated carbon as catalyst in acetylene hydrochlorination, *Catal. Commun.* 98 (2017) 22–25, <https://doi.org/10.1016/j.catcom.2017.04.049>.
- [54] A.L. Garcia-Costa, J.A. Zazo, J.J. Rodriguez, J.A. Casas, Intensification of catalytic wet peroxide oxidation with microwave radiation: activity and stability of carbon materials, *Sep. Purif. Technol.* 209 (2019) 301–306, <https://doi.org/10.1016/j.seppur.2018.07.054>.
- [55] W. Chen, Y. Li, D. Zhu, S. Zheng, W. Chen, Dehydrochlorination of activated carbon-bound 1,1,2,2-tetrachloroethane: implications for carbonaceous material-based soil/sediment remediation, *Carbon N.Y.* 78 (2014) 578–588, <https://doi.org/10.1016/j.carbon.2014.07.041>.
- [56] H.S. Hsieh, J.J. Pignatello, Activated carbon-mediated base hydrolysis of alkyl bromides, *Appl. Catal. B Environ.* 211 (2017) 68–78, <https://doi.org/10.1016/j.apcatb.2017.04.010>.
- [57] K. Mackenzie, J. Battke, F.D. Kopinke, Catalytic effects of activated carbon on hydrolysis reactions of chlorinated organic compounds: Part 1.  $\gamma$ -Hexachlorocyclohexane, *Catal. Today* 102–103 (2005) 148–153, <https://doi.org/10.1016/j.cattod.2005.02.038>.
- [58] T. Budinova, M. Razvigorova, N. Petrov, V. Minkova, R. Taranjiska, Catalytic hydrolysis of soya oil with carbon adsorbents, *Carbon N.Y.* 36 (1998) 899–901, [https://doi.org/10.1016/S0008-6223\(97\)00201-7](https://doi.org/10.1016/S0008-6223(97)00201-7).
- [59] V.H. Wysocki, K.A. Resing, Q. Zhang, G. Cheng, Mass spectrometry of peptides and proteins, *Methods* 35 (2005) 211–222, <https://doi.org/10.1016/j.ymeth.2004.08.013>.
- [60] K. Mackenzie, J. Battke, R. Koehler, F.D. Kopinke, Catalytic effects of activated carbon on hydrolysis reactions of chlorinated organic compounds: Part 2. 1,1,2,2-Tetrachloroethane, *Appl. Catal. B Environ.* 59 (2005) 171–179, <https://doi.org/10.1108/13683040810881216>.
- [61] J.L. Figueiredo, Functionalization of porous carbons for catalytic applications, *J. Mater. Chem. A* 1 (2013) 9351–9364, <https://doi.org/10.1039/c3ta10876g>.
- [62] J. Choma, M. Jaroniec, W. Burakiewicz-Mortka, J. Klinik, A. Tybel, Zmiany powierzchniowych i strukturalnych właściwości węgla aktywnych wywołane ich utlenianiem (Oxidation-induced changes in the surface and structural properties of active carbons), *Ochr. Środowiska* 70 (1998) 13–20.
- [63] A.M. Carvajal-Bernal, F. Gómez, L. Giraldo, J.C. Moreno-Piraján, Chemical modification of activated carbons and its effect on the adsorption of phenolic compounds Modificación química de carbones activados y su efecto en la adsorción de compuestos fenólicos 17 (2015) 109–119.