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Article

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Algal biochar as a sorbent for the treatment of non-production dairy wastewater

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Abstract

The aim of this study was to determine the potential of using biochar derived from *Chlorella sp*. algal biomass as an effective and environmentally friendly sorbent for the preliminary removal of contaminants present in acidic spent washing baths. The research included characterising the obtained biochars using SEM/EDS, an evaluation of sorption efficiency using solid - phase extraction, and an analysis of sorption kinetics in solutions. The SEM/EDS analysis revealed an increase in the porosity of the materials with rising process temperature, and the highest C+O+N content was observed for biochar produced at 600°C (92.45%). In the solid - phase extraction method, the best contaminant removal results were also obtained for the biochar produced at 600°C: OWO was reduced from 13.61 to 9.57 mg/dm³ (29.7%), sulfates from 108 to 35.3 mg/dm³ (67.3%), and chlorides from 35.1 to 5.3 mg/dm³ (84.9%). Kinetic analysis indicated that the sorption maximum was rapidly reached after 15-30 minutes due to the removal of all contaminants. The results confirm that biochar derived from *Chlorella sp.* algal biomass can be used as a material to aid in the treatment of acidic spent washing baths, but only as a preliminary or supplementary method. A novel aspect of this study is the use of biochar derived from *Chlorella sp.* algal biomass, which aligns with the principles of circular economy, as it enables the conversion of algal biomass into a useful sorbent to support wastewater treatment processes.

Keywords: biomass, sorption, biochar, algae, dairy wastewater

1. Introduction

In recent years, environmental protection has become one of the key priorities of international policy, especially in highly developed countries. The ongoing degradation of the environment is largely a consequence of the generation of significant amounts of both municipal and industrial waste, the management of which can be technologically complex and economically unfeasible [1,2,3] Of particular importance is the issue of water and wastewater management in industrial enterprises, where increasing legal requirements and economic pressure are forcing the implementation of solutions that reduce water consumption and the volume of wastewater discharged [1,2,3] The reuse of water in closed-loop systems is now not only a matter of compliance with environmental regulations but also a factor that enhances companies' competitiveness on domestic and international markets. Industrial wastewater is generated at various stages of technological processes, and its qualitative and quantitative composition is closely dependent on the specific nature of a given industry [2] Assessing its harmfulness requires knowledge not only of the overall composition of the effluent from the entire industrial plant but also of the composition of the effluent streams from individual production departments. The selection of treatment technologies should ensure maximum efficiency in removing pollutants while minimising energy and financial costs [3,4]. One of the industrial sectors in Poland and Europe that generates large amounts of waste and consumes significant amounts of water is the agri-food industry [3,4,5]. Due to legal regulations imposed on businesses by the European Union, the sector's activities have been focused on the reuse of resources at all stages of the production process in recent years [3,4] Within this sector, the dairy industry is characterised by a high water consumption, used for technological, energy, and sanitary-hygienic purposes. Dairy wastewater contains residues of milk and dairy products, fats, lactose, proteins, as well as cleaning and disinfecting agents. It is characterised by elevated levels of total organic

carbon, sulfates, and chlorides, as well as a significant content of suspended solids and biogenic compounds, particularly nitrogen and phosphorus. The literature [1,2,3,4] on dairy wastewater indicates that assessing the content of organic compounds and inorganic ions is essential when designing treatment systems, as well as when considering the possibility of recycling water back into the process cycle. Total organic carbon (TOC) is an indicator of the content of organic compounds present in water and wastewater. Its removal helps reduce the load of organic substances that may contribute to the deterioration of oxygen conditions. The removal of sulfates is important because they may originate, among other sources, from cleaning agents used to wash process equipment. Their presence increases the mineralisation of wastewater, and under anaerobic conditions, sulfates can be reduced to sulfides, which promotes the formation of odours and corrosion. Chlorides, on the other hand, are not effectively removed by conventional biodegradation processes [6,7]. They can affect the conductivity, salinity, and corrosiveness of wastewater, and at higher concentrations, they can also affect the stability of activated sludge. Excessive discharge of these elements into the aquatic environment leads to eutrophication; therefore, their effective removal is a crucial element of the treatment process [5,6,7] Traditional wastewater treatment systems include mechanical, biological (aerobic and anaerobic), and physicochemical stages; however, in the case of dissolved, poorly biodegradable organic pollutants and parts of the biogenic fraction, biological processes prove insufficient. In this context, pretreatment, using sorption as a method to complement conventional technologies, is an important element of the treatment process [5,6,7]. Sorption is a physicochemical process involving the binding of molecules or ions from the liquid phase to the surface or within the porous structure of a solid phase (sorbent). In an aqueous solution, the sorption mechanism may involve physical adsorption (van der Waals interactions, electrostatic forces), chemisorption (formation of covalent or coordination bonds), ion exchange, and surface complexation [8,9]. This process occurs at the solid-solution interface and is determined by the properties of both systems: the adsorbate, pH, ionic strength, and the temperature of the solution, as well as the specific surface area, porosity, and the presence of functional groups on the sorbent's surface [10].

One of the materials used as a sorbent is biochar, produced through pyrolysis [11,12]. Biochar is characterised by a well-developed structure of micropores and mesopores, a large specific surface area, and the presence of functional groups (including carboxyl, hydroxyl, and carbonyl groups), which enable the effective adsorption of organic and inorganic pollutants [13,14]. The physicochemical properties of biochar, such as pH, degree of structural order, and pore size distribution, depend on the type of feedstock and the parameters of the pyrolysis process (temperature, time, heating rate) [15,16]. An appropriate modification of these parameters allows for the design of materials with targeted sorption properties, tailored to the specific characteristics of the wastewater being treated [16,17,18]. Algae, particularly those of the genus *Chlorella sp.*, are a particularly promising feedstock for biochar production. They are characterised by a high biomass productivity and a favourable chemical composition (nitrogen and phosphorus content). The use of algae as a substrate for biochar production aligns with the concept of circular economy, enabling the simultaneous management of excess biomass from algal blooms and the production of an effective, low-cost sorbent for industrial wastewater treatment [19,20,21].

The aim of this study was to determine the potential of biochar derived from *Chlorella sp*. algal biomass as an effective and environmentally friendly sorbent for removing pollutants present in acidic, non-production-related spent washing baths, such as sulfates (SO_4^{2-}), total organic carbon (TOC), and chlorides (Cl^-).

2. Materials and Methods

2.1.Algal Biomass

Chlorella sp. algal biomass was obtained from in - house cultures. The strains used as starting material for the cultures were sourced from the CCBA collection at the Institute of Oceanography, University of Gdańsk, and were designated as BA 103. After the cultivation process was completed, the biomass was separated from the culture medium through a series of sedimentation and centrifugation steps. Centrifugation was performed using a Centrifuge 5430 (Eppendorf) equipped with a 6 - place FA-45-30-11 rotor at 8000 rpm for 10 minutes. In the next step, the biomass was frozen using liquid nitrogen. To completely remove water, a freeze-drying process was performed at - 80°C under reduced pressure below 10 Pa using a FreeZone 2.5 Plus (Labconco) freeze-dryer. The use of freeze-drying as the final stage of sample preparation made it possible to obtain a high-quality product by reducing the risk of undesirable chemical reactions, such as oxidation, and by inhibiting microbial activity. Additionally, the resulting dry preparation exhibited greater stability. The detailed methodology for culturing and harvesting algal biomass was described in the author's previous articles [22].

2.2. Method for Producing Biochar

The pyrolysis of algal biomass was carried out using an *FCF-V12RM* furnace equipped with an *MRT-4 PID* controller (*Czylok)*. The process was conducted under a 3-stage cascade heating regimen at temperatures of 400ºC, 500ºC, 600ºC, 700˚C, 800˚C, and 900˚C. The total duration of the pyrolysis process at each set temperature was 105 min, and the residence time of the sample at the maximum temperature was 15 min. The biochar was left in the furnace to cool spontaneously to a temperature of 25 ± 2º C. The aging time ranged from 18 to 24 h and depended on the final temperature of the process. Carbon dioxide was used as the inert gas at a flow rate of 5.0 dm^3/min. The biochar was ground in a *Testchem* laboratory vibratory mill. The characteristics of the obtained biochars were described in the author's previous articles [23].

2.3. Scanning electron microscopy with X-ray microanalysis (SEM/EDS)

The microstructure of the biomass and algal biochar was examined using a high-resolution scanning electron microscope (SEM) SU-70 (*Hitachi)*, equipped with a *Schottky* field-emission electron gun, a secondary electron detector, and an X-ray microanalyser (EDS) NSS 312 (*Thermo Scientific)*. The materials were tested under the following conditions: 500x magnification, 15 kV accelerating voltage, 50 A emission current, 100 s analysis time, and a radiation detection angle of approximately 30º (corresponding to a working distance of approx. 15 mm). EDS analyses were performed using a standardless method; for concentrations of identified elements up to 100%, the final measurement results are reported with an accuracy of 0.1%.

2.4. Sorption Process

The sorption process was carried out using two methods: sorption on biochar in solution and solid-phase extraction. Acidic spent washing baths obtained from one of the local dairies were used as the liquid to be treated. Their composition is presented in Table 1 at .

Table 1. Characteristics of non-production dairy wastewater subjected to the sorption process.

Parameter	*Value*
pH	2.43
TOC [mg/dm^3]	13.61
Sulfates [mg/dm^3]	108
Chlorides [mg/dm^3]	35.1

2.4.1. Extraction to the solid phase

The study utilised solid-phase extraction (*SPE*) on columns with a volume of 3 cm^3, each containing 1 g of biochar produced under various thermal conditions. The test involved wetting the bed with 10 cm^3 of distilled water and then passing 50 cm^3 of dairy wastewater through the biochar bed.

2.4.2 Sorption process on biochar in solution

In 100 cm^3 conical flasks, 1 ± 0.001 g of the biochar sample was weighed into each flask, followed by the addition of 50 cm^3 of wastewater and sealing the flasks against evaporation with a glass stopper. The flasks were placed on a *Unimax 2010* shaker (*Heidolph)* and shaken for 5, 10, 15, 30, 60, and 90 min at a speed of 160 rpm and a temperature of 20ºC. After the specified time, the contents of the flasks were transferred to 50 cm^3 test tubes and centrifuged using an *Eppendorf Centrifuge 5430* at 8,000 rpm for 15 min. Samples were taken from the solution above the biochar for physicochemical analysis.

Changes occurring in the treated wastewater during the sorption process were evaluated based on the changes in the following physicochemical parameters: total organic carbon (TOC), sulfates (SO_4^{2-}) and chlorides (Cl^-) using cuvette tests (*Hach Lange)* (vials containing ready-to-use chemical reagent kits designed to measure specific parameters) and a *UV-VIS DR 6000* spectrophotometer (*Hach Lange*). The parameters are listed in Table 2.

Table 2. List of parameters determined using cuvette tests in the sorption process.

No.	*Parameter Determined*	*Measurement range [mg/dm³]*	*Cuvette*	*Method/standard*	*Wavelength [nm]*
1.	TOC [mg/dm^3]	2–65	LCK380	DIN 38409-H3	435
2.	Sulfates [mg/dm^3]	40–150	LCK 153	Barium sulfate	430
3.	Chlorides [mg/dm^3]	1–1,000	LCK 311	Iron(III) thiocyanate	468

The sorption capacity (q) of biochar (Equation 1) was determined using the following formula:

$$q = \frac{c_o - c_t}{M} \times V \quad (1)$$

where:

C_o – initial concentration of pollutants [mg/dm^3]

C_t – concentration pollutants after time [mg/dm^3]

V – solution volume [dm^3]

M – mass of biochar [g]

The experiments were conducted in triplicate, and the results are presented as the average of these replicates.

3. Results and Discussion

To investigate the morphological characteristics and microstructure of *Chlorella sp*. algal biomass and the biochar produced from it, images were obtained using Scanning Electron Microscopy (SEM) (Fig. 1).

a) b) c)

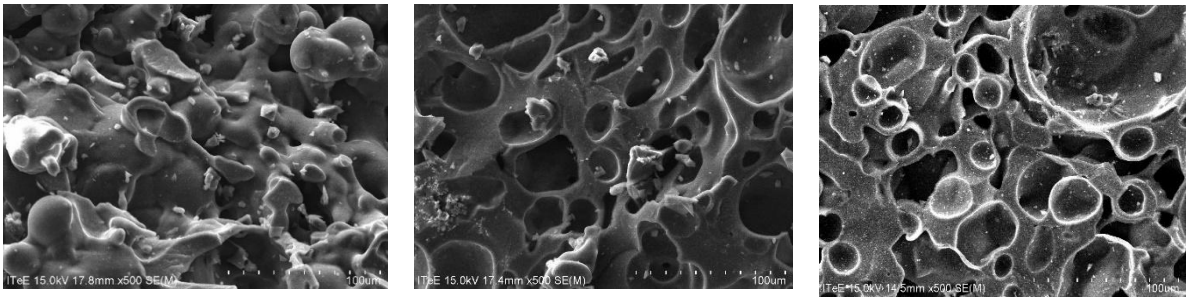


Fig. 1. SEM images, magnification x500: a) algal biomass, b) biochar produced at 400°C, c) biochar produced at 900°C.

The SEM images indicate changes in the morphology of the materials occurring during the pyrolysis. The algal biomass is characterised by a compact structure and a smooth surface. In the biochar produced at 400°C, the first irregular pores are visible, though the material's structure remains largely compact [18]. As the pyrolysis temperature increases to 900°C, a more porous structure develops, and the number and size of the pores increase. Pores with dimensions corresponding to macro- and mesopores are observed. At the same time, the pore walls become thinner, indicating the progressive removal of volatile components and the restructuring of the material's carbon structure. The SEM results were confirmed in a publication by Vieira Costa et al. [24] where the authors used scanning electron microscopy to evaluate the morphology of hydrochar and biochar obtained from microalgal biomass. SEM observations showed that thermal treatment destroyed the original structure of the biomass and resulted in an irregular surface containing pores, depressions, and crevices. These features could serve as active sites for pollutant binding, which confirmed the potential for using the resulting materials as adsorbents in wastewater treatment. In the next stage of the research, a surface analysis was conducted to identify the main chemical elements (carbon, oxygen, and nitrogen) present in the algal biomass and the resulting biochar (Table 3)

Table 3. Results of EDS analysis (% w/w) for carbon, oxygen, and nitrogen content in the biomass sample and 6 samples of the analysed biochar.

	Biomass	***Biochar 400***	***Biochar 500***	***Biochar 600***	***Biochar 700***	***Biochar 800***	***Biochar 900***
C	29.59	47.85	48.33	49.86	46.85	47.29	45.50

O	8.65	14.25	15.59	16.54	12.99	13.44	8.89
N	30.58	24.68	23.56	26.05	22.11	20.56	19.05
C+O+N (% of total weight)	68.82	86.78	87.48	92.45	81.95	81.29	73.44

Based on this data, it was found that the total content of the analysed elements (C+O+N) was the highest for biochar produced at 600°C, at 92.45%, while the lowest was for biomass (68.82%) and biochar produced at 900°C (73.44%). During the pyrolysis, the carbon content increased relative to that of the biomass. The carbon content rose from 47.85% for the 400°C biochar to 49.86% for the 600°C biochar. At higher temperatures, a gradual decrease in the carbon content was observed, down to 45.50% in 900°C biochar. At the same time, the nitrogen content decreased as the process temperature increased, from 24.68% for 400°C biochar to 19.05% for 900°C biochar. A similar trend was observed for oxygen, whose content, after reaching a maximum for 600°C biochar (16.54%), decreased to 8.89% for 900°C biochar [25] The observed changes in elemental composition resulted from the progressive thermal decomposition of the organic components of the algal biomass, primarily proteins, lipids, and polysaccharides. The initial increase in the oxygen content, observed up to 600°C, may have resulted from changes in the proportions of individual elements and the behavior of oxygen-containing surface groups in the solid pyrolysis product. At higher temperatures, dehydration and decarboxylation reactions intensified. The high nitrogen content in the biomass and the resulting biochar was primarily due to the presence of proteins and other nitrogen-containing compounds in the algal cells. The increase in temperature caused the breakdown of peptide bonds [25]. To assess changes in surface composition, the O/C and N/C ratios were calculated, and their values are presented in Table 4.

Table 4. Proportions of the analysed elements from EDS analysis in biomass and biochar samples.

	Biomass	***Biochar 400***	***Biochar 500***	***Biochar 600***	***Biochar 700***	***Biochar 800***	***Biochar 900***
O/C	0.29	0.30	0.32	0.31	0.28	0.28	0.19
N/C	1.03	0.51	0.49	0.52	0.47	0.43	0.42

The O/C ratios determined for the biochar samples produced at 400, 500, and 600°C were similar to one another and amounted to 0.30, 0.32, and 0.31, respectively. This indicates that, within this temperature range, a comparable amount of oxygen relative to carbon was retained on the surface of the materials. This may indicate the presence of oxygen-containing functional groups, important for the sorption properties of biochar [25]. For biochar samples produced at the higher temperatures of 700 and 800°C, the O/C ratio decreased to 0.28, while for the 900°C sample, it reached its lowest value of 0.19. This trend indicates a progressive decrease in the oxygen-to-carbon ratio as the pyrolysis temperature increases. The decrease in the O/C ratio in biochar produced at higher temperatures can be attributed to

the thermal transformation of biomass, including the removal of volatile compounds and the gradual loss of oxygen-containing functional groups [25]. The N/C ratios for all biochar samples were lower than those of the feedstock biomass, indicating a decrease in the relative nitrogen content of the material after pyrolysis. Among the biochar samples studied, the highest N/C ratio of 0.52 was obtained for biochar produced at 600°C, while the lowest value of 0.42 was obtained for biochar produced at 900°C. The observed trend confirms that an increase in process temperature promotes a gradual decrease in the nitrogen-to-carbon ratio. This may result from the decomposition of nitrogenous components of algal biomass and their partial removal in the form of volatile products during pyrolysis [25]. The SEM/EDS results indicate that the pyrolysis of algal biomass leads to changes in the surface properties of the materials. On the one hand, there is an increase in the carbon content and the development of a porous structure, which increases the potential availability of sorption surface area. On the other hand, as the temperature increases, the proportion of surface functional groups containing oxygen and nitrogen - which can act as active sites in the pollutant binding process - may decrease. The most favorable properties among the analysed materials were obtained for biochar produced at 600°C. This means that at this temperature, the pyrolysis process may have resulted in a material characterised by a well-developed porous structure while simultaneously preserving functional groups capable of interacting with substances present in wastewater. The results obtained are consistent with the study by Yang et al. [26], in which biochar derived from Chlorella sp. and Spirulina sp. residues, produced at 600°C, exhibited the highest Pb(II) adsorption capacity, amounting to 131.4 and 154.5 mg/g, respectively. The authors emphasized, however, that the high sorption was not due solely to surface O and N groups, but also to the presence of mineral components, ion exchange, and precipitation. Similarly, Myung et al. [27] demonstrated that biochar from Spirulina platensis produced at 600°C was an effective material for immobilizing Pb and Zn, and that the sorption mechanism involved surface complexation, cation exchange, electrostatic interactions, and precipitation. González-Hourcade et al. [28] demonstrated for biochar derived from microalgae at 600°C that the presence of micropores and nitrogen- and oxygen-containing groups contributed to the material's adsorption properties, with pore filling being the dominant mechanism for acetaminophen sorption. Figs. 2-4 present the results obtained for the sorption process conducted using the solid-phase extraction method with biochar produced at various pyrolysis temperatures.

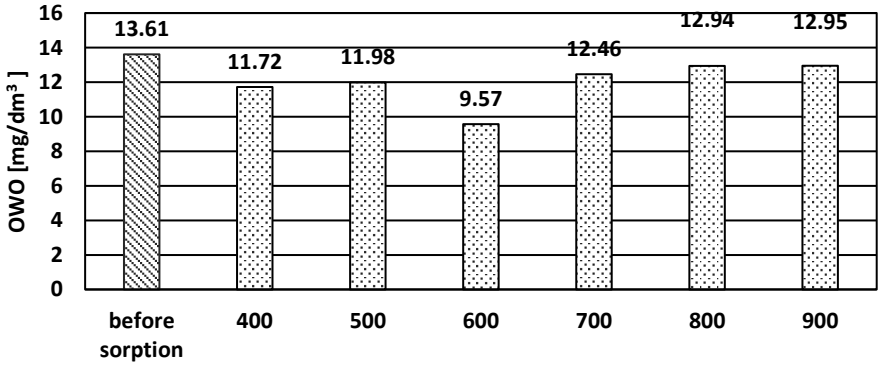


Fig. 2. Changes in the concentration of total organic carbon during sorption by the solid-phase extraction method, presented in a comparative analysis for biochar samples 400, 500, 600, 700, 800, and 900.

Compared to the initial OWO value of 13.61 mg/dm^3, the highest removal efficiency for this component was achieved in biochar 600, for which the OWO value after the sorption process was 9.57 mg/dm^3. The results for the other biochars were as follows: biochar 900-12.95 mg/dm^3, biochar 800-12.94 mg/dm^3, biochar 700-12.46 mg/dm^3, biochar 500-11.98 mg/dm^3, and biochar 400-11.72 mg/dm.

The biochar produced at 600ºC exhibited the highest capacity for removing organic compounds, while the sorption efficiency decreased for biochars produced at the higher pyrolysis temperatures of 700-900 ºC. A high effectiveness of the biochar samples used was also observed in the removal of sulfate anions (Fig. 3).

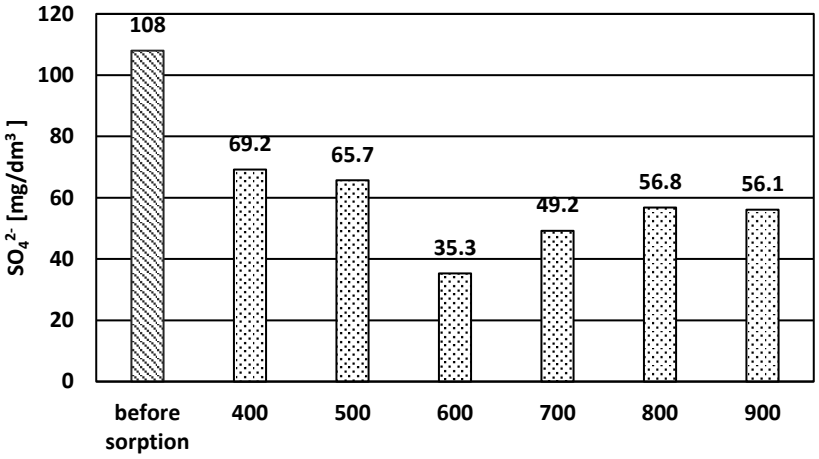


Fig. 3. Changes in the concentration of sulfate anions SO_4^{-2} during sorption by solid-phase extraction, presented in a comparative analysis for biochar produced at 400, 500, 600, 700, 800, and 900.

The highest efficiency in removing sulfate anions was achieved for biochar produced at 600ºC. The concentration of SO_4^{-2} after the sorption process decreased from an initial value of 108 mg/dm^3 to 35.3 mg/dm^3. For the remaining biochar samples, the sulfate anion concentration was reduced to the following values: 400 biochar-69.2 mg/dm^3, 500 biochar-65.7 mg/dm^3, 800 biochar-56.8 mg/dm^3, biochar 900-56.1 mg/dm^3, and biochar 700-49.2 mg/dm^3. In the next stage of the study, the effectiveness of biochars in the removal of chloride anions was evaluated (Fig. 4).

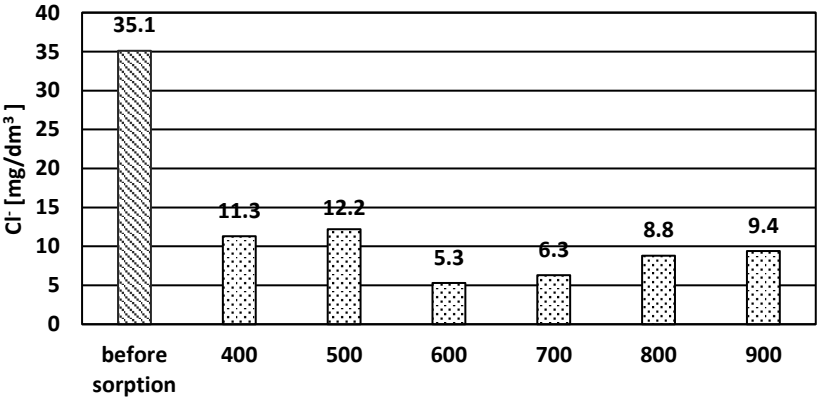


Fig. 4. Changes in the concentration of chloride anions Cl^- during sorption by solid-phase extraction, presented in a comparative analysis for biochar types 400, 500, 600, 700, 800, and 900.

The initial chloride concentration was 35.1 mg/dm³. The highest sorption efficiency was achieved for biochar 600, for which the chloride concentration decreased to 5.3 mg/dm^3. For biochar 400, a value of 11.3 mg/dm^3 was obtained, and for biochar 500, a value of 12.2 mg/dm^3. For biochars produced at higher temperatures, the final chloride concentration was 6.3 mg/dm^3 for biochar 700, 8.8 mg/dm^3 for biochar 800, and 9.4 mg/dm^3 for biochar 900. The best properties of biochar produced at 600°C can be

attributed to a favorable combination of porosity, surface groups containing oxygen and nitrogen, and mineral components derived from algal biomass.

In the next stage of the research, based on the results obtained from the solid-phase extraction method and a detailed analysis of sorption on biochar in solution, the biochar produced at 600°C was selected (Figs. 5 - 6).

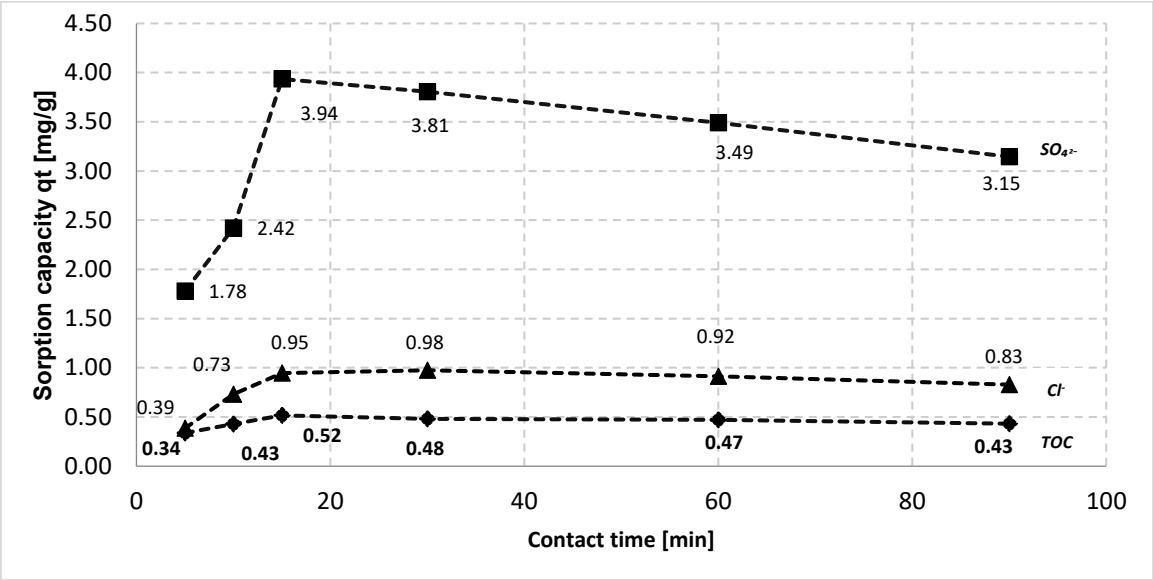


Fig. 5. Sorption capacity of biochar 600 for the removal of OWO, SO_4^{2-}, and Cl^- using the biochar sorption method.

The highest sorption capacity in the study was obtained for sulfate ions, which amounted to 3.94 mg/g after 15 minutes.This was due to the large number of free active sites on the biochar surface and the large concentration gradient between the solution and the sorbent surface. For chlorides, the highest value was 0.98 mg/g after 30 minutes, while for OWO, the sorption capacity was 0.52 mg/g after 15 minutes. In all cases, a slight decrease in sorption capacity was observed after the maximum was reached, which may indicate the partially reversible nature of sorption or the leaching of weakly bound components from the biochar surface.

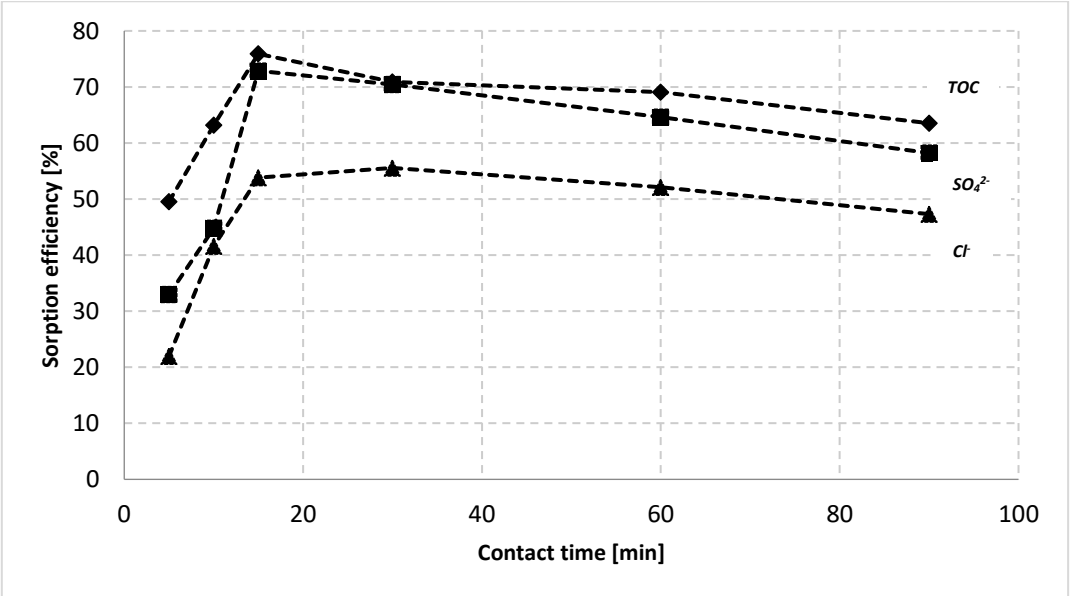


Fig. 6. Sorption efficiency of biochar 600 in the solution sorption method.

In terms of percentage efficiency, the highest degree of contaminant removal in the kinetic study was obtained for OWO (75.90%; 15 min), followed by SO (72.87%; 15 min) and Cl^- (55.56%; 30 min). After exceeding the 15-30-minute time window, further prolonging the contact time did not improve the purification effect [29,30].

The analysis showed that biochar obtained from *Chlorella sp.* algal biomass may be a promising sorbent for assisting in the treatment of acidic spent washing baths from the dairy industry. The most favorable properties were obtained for biochar produced at 600°C, which was confirmed in this study by the simultaneous development of a porous structure and the retention on the material's surface of elements such as oxygen and nitrogen, which potentially participate in interactions with selected wastewater components. The results obtained both in the solid-phase extraction method and in kinetic sorption studies in solution indicate the possibility of reducing the concentrations of OWO, sulfates, and chlorides, with the process proceeding most intensively during the initial contact time between the sorbent and the wastewater. The results are based on laboratory studies and do not yet allow for a full assessment of the sorbent's durability, its regeneration potential, or the process's effectiveness under technical conditions. For this reason, sorption on biochar derived from algal biomass should be considered a preliminary or supplementary method that can support conventional dairy wastewater treatment systems, particularly in terms of the secondary treatment of selected effluents and the protection of subsequent process stages.

4. Conclusion

- The study demonstrated the potential for using biochar derived from *Chlorella sp.* algal biomass as a sorbent material to aid in the treatment of acidic spent washing baths from the dairy industry.
- The SEM/EDS analysis found that as the pyrolysis temperature increased, the porous structure of the resulting materials developed; however, the biochar produced at 600°C exhibited the most favorable surface properties.
- This material was characterised by the highest proportion of C+O+N elements, amounting to 92.45%, which may indicate the presence of carbon structures and surface groups containing oxygen and nitrogen, potentially participating in interactions with selected wastewater components. In studies conducted using the solid-phase extraction method, the best treatment results were also obtained for the 600°C biochar.
- The use of this sorbent reduced the concentration of OWO by 29.7%, sulfates by 67.3%, and chlorides by 84.9%. In sorption studies conducted in solution, the process efficiency was evaluated based on sorption capacity. The analysis of the process kinetics showed that sorption occurred most intensively during the initial stage of contact between the sorbent and the wastewater. Maximum sorption capacities were achieved after 15 minutes for OWO and sulfates, and after 30 minutes for chlorides. Further extending the contact time to 60-90 minutes did not improve the results, which may indicate that a state close to sorption equilibrium had been reached, a limited number of available active sites, or the partially reversible nature of the binding of certain components.
- The results confirm that biochar derived from *Chlorella sp.* biomass can be used as a material suitable for the preliminary or supplementary treatment of acidic spent washing baths. However, sorption on biochar should not be treated as a standalone technology for the complete treatment of dairy wastewater, but rather as a stage that supports integrated systems. Its potential application may include partial reduction of the pollutant load or secondary treatment.

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The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.