

Algal biochar in dairy wastewater treatment

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Abstract

The article presents a novel solution based on dairy wastewater sorption on a biochar substrate obtained through thermal decomposition of *Chlorella sp.* algae biomass. The algal biomass obtained in the culture medium containing wastewater from dairy production was separated from the culture medium through sedimentation and centrifugation and then freeze-dried. After freeze-drying, the dry biomass was pyrolysed at 600°C in a CO₂ atmosphere. The EDS analysis showed that the oxygen-to-carbon (O/C) and nitrogen-to-carbon (N/C) ratios in the obtained material averaged 0.24 and 0.54 respectively. The arrangement and structure of the obtained biochar was evaluated using Raman spectroscopy. The observed spectra revealed the presence of D bands located at 1346–1354 cm⁻¹ and corresponding to disordered carbon structures, as well as G bands located at 1585–1594 cm⁻¹ and corresponding to tensile vibrations. The D/G intensity ratio was determined at 0.28. The next phase of the research involved sorption of dairy wastewater from cleaning processes containing 1 g of the obtained biochar using solid phase extraction. The study results confirmed high sorption efficiency of the obtained algal biochar. Turbidity was reduced by 93%, suspension by 88%, sulphates by 61%, chlorides by 80%, and organic carbon by 17%. The research confirmed the possibility of using wastewater from dairy production as a natural culture medium for *Chlorella sp.* algae cultivation to manufacture valuable biochar, which could be used as a sorption bed in the treatment of dairy wastewater from cleaning processes.

Keywords

algae, biomass, biochar, dairy wastewater, *Chlorella sp.*, sorption

1. INTRODUCTION

Water is fundamental in the majority of manufacturing processes. As wastewater from industrial sources may vary in terms of composition and contain pollutants in concentrations that could affect the quality of receiving water, it may not be discharged to any surface water bodies or sewage systems (Zander and Dajnowiec, 2009). The changing Polish laws on environmental protection increasingly emphasise the importance of wastewater treatment (Szwarc et al., 2017). The industry that generates a large amount of wastewater is the agriculture and food production sector, mainly dairy production. In this industry, the amount of the wastewater discharged depends on the size of the plant and the nature of production. A typical Polish dairy plant discharges between 450 and 600 m³/d of wastewater on average (Dziosa and Makowska, 2017). Dairy plants generate two types of wastewater, i.e. wastewater from dairy production processes (dairy production wastewater) and wastewater from cleaning processes (used baths). Dairy production wastewater contains milk and processed milk residues, nitrogen and phosphorus and it has alkaline pH. Wastewater from cleaning processes is diluted and apart from organic substances it also contains cleansing agents (acids, alkalis and detergents) (Dziosa and Makowska, 2017; Zander and Dajnowiec, 2009). To treat dairy wastewater, membrane technologies, biological treatment methods, and activated sludge can be used (Zander and Dajnowiec, 2009). However, new and more effective wastewater treatment methods are

sought to better manage all wastewater streams and meet the requirements of the circular economy. An innovative approach to dairy wastewater treatment assumes the use of sorption and a biochar substrate (Al-Dhabi and Arasu, 2022; Hou et al., 2021; Ślęzak et al., 2018). Biochar is produced as a result of thermal decomposition of biomass at an elevated temperature (300–900°C) in the absence of oxygen, i.e. through pyrolysis (Jeguirim and Limousy, 2017; Meng and Wang, 2020). During pyrolysis, complex chemical compounds are broken down into compounds with lower molecular weight. As a result, oil, gas or biochar are produced. Factors that affect the share of each product are the temperature cascade, the process end temperature, the type and decomposition time of the substrate used, the pressure, and the inert gas (Pan et al., 2010). The end composition of the post-pyrolysis product is determined by initiating and secondary reactions between product particles (Law et al., 2022; Yu et al., 2023). In an inert gas atmosphere, the least stable chemical bonds present in the biomass break. As a result of progressing polymerisation and polycondensation, the following are created: carbon oxides (II) and (IV), non-condensable gases (H₂, CO, CH₄), mixtures of unsaturated hydrocarbons (C₂H₄, C₂H₆, C₃H₈), and water (Sun et al., 2022). The biochar mass produced via pyrolysis is uniform and contains large amounts of elemental carbon (Chen et al., 2017). Major parameters characterising biochar properties include: chemical composition, stability, porosity, specific surface area, bulk density, pH and ion-exchange capacity (Chen et al., 2017; Vieira Costa et al.,



2023). The physical and chemical properties of biochar decide on its fitness for a particular purpose and are connected with the type of the biomass used and the conditions of production (Pan et al., 2010; Xu et al., 2023). What makes the pyrolysis process more beneficial compared to other processing methods is the ease of storing and transporting biomass, which significantly reduces the costs incurred (Sun et al., 2022; Wu et al., 2023). Additionally, emission of pollutants to the atmosphere is significantly reduced compared to traditional combustion. Given its chemical composition, algal biomass is perfect for pyrolysis, especially when containing the following species of algae: *Chlorella vulgaris* and *Chlorella sp.* (Huo et al., 2018; Makowska and Dziosa, 2018; Milledge and Heaven, 2014). To grow, algae need access to biogenic components, particularly nitrogen and phosphorus, which can be found in dairy wastewater and are difficult to remove using traditional wastewater treatment methods (Kusmayadi et al., 2022; Szwarc et al., 2017; Urbanowska et al., 2019).

The article describes a study centred around the cultivation of *Chlorella sp.* algae in dairy wastewater and processing of the obtained biomass into a biochar sorbent to treat used baths.

2. MATERIALS AND METHODS

2.1. Dairy wastewater

In the study, the dairy production wastewater and used baths from a local dairy plant were applied. Their characteristics are presented in Tables 1 and 2 below.

Table 1. Characteristics of wastewater from dairy production.

Parameter	Wastewater from dairy production
Total nitrogen	54.0 mg/dm ³
Total phosphorus	17.5 mg/dm ³
pH	7.38

Table 2. Characteristics of wastewater from cleaning processes (used baths).

Parameter	Wastewater from cleaning processes
Chlorides	35.1 mg/dm ³
Turbidity	45.7 NTU
Suspension	73 mg/dm ³
TOC	13.61 mg/dm ³
Sulphur	108 mg/dm ³
pH	2.43

2.2. Algae cultivation

For the purpose of the cultivation, *Chlorella sp.* algae were used. Algal strains came from our own cultivation isolated from the BA 103 culture obtained from the Culture Collection of Baltic Algae (CCBA) at the Institute of Oceanography at the University of Gdańsk. As a culture medium, dairy production wastewater was used. Cultivation was prepared in 3 dm³ glass laboratory reactors. Each reactor was equipped with a heating jacket and a thermostat for temperature adjustment. In the lid, two circulation outlets and an anchor stirrer were mounted. The outlets enabled gas exchange and the stirrer prevented cell sedimentation and ensured even exposition to light and access to nutrients. Detailed parameters of the cultivation are presented in Table 3.

Table 3. *Chlorella* algae cultivation parameters.

Parameter	Value
Temperature	26°C
Mixing	260 rpm
Lighting	White light intensity 700 ÷ 900 Lux
Photoperiod	16 h/8 h (day/night)
Time	44 days

Cultivation was monitored based on the optical density (OD) measurement of *Chlorella sp.* biomass growth. Every four (4) days, cuvette tests were carried out for 3 cm³ of the material to measure the absorbance of radiation at a wavelength specific for *Chlorella sp.* algae (686 nm). Distilled water was used as a reference sample. The cultivation continued for 44 days until depletion of nutrients from the culture medium, as measured with a Hach DR 6000 UV-VIS spectrophotometer and Hach Lange LCK cuvette tests (Table 4).

Table 4. Parameters determined using cuvette tests in algae cultivation.

Parameter	Measurement range [mg/dm ³]	Method/standard	Wavelength [nm]
Total nitrogen	20 ÷ 100	EN-ISO 11905-1	345
Total phosphorus	0.05 ÷ 1.5 2 ÷ 20	EN ISO 6878-1 DIN 38405	880

The authors assumed that in the event of a decline in the phosphorus content below 1 mg/dm³, the cultivation would end.

2.3. Separation of biomass from the culture medium

After cultivation, the *Chlorella sp.* biomass was isolated from the culture medium through sedimentation, centrifugation, and freeze-drying. In the centrifugation process, an Eppendorf

Centrifuge 5430 with an FA-45-30-11 rotor was used; the biomass was centrifuged at 8,000 rpm for the total of 10 min (Figure 1).



Figure 1. *Chlorella sp.* algae biomass centrifuged from the culture medium.

Centrifuged biomass was then dried using liquid nitrogen. To remove any water residues, the biomass was freeze-dried at a temperature of -80°C and a pressure below 10 Pa using a Lobconco FreeZone 2.5 plus freeze dry system (Fig. 2).



Figure 2. Freeze-dried *Chlorella sp.* algae biomass.

Freeze-drying helped to obtain a high quality product by mitigating the risk of adverse reactions (e.g. oxidation) and microorganism activity.

2.4. Obtaining biochar from the algal biomass

The obtained freeze-dried biomass was pyrolysed using a Czylok FCF-V12RM furnace with a PID MRT-4 controller. Pyrolysis was carried out under cascade heating conditions (3-stage) at 600°C (Fig. 3). The biomass was pyrolysed for the total of 105 min, and the sample was kept at the maximum temperature for the total of 15 min.

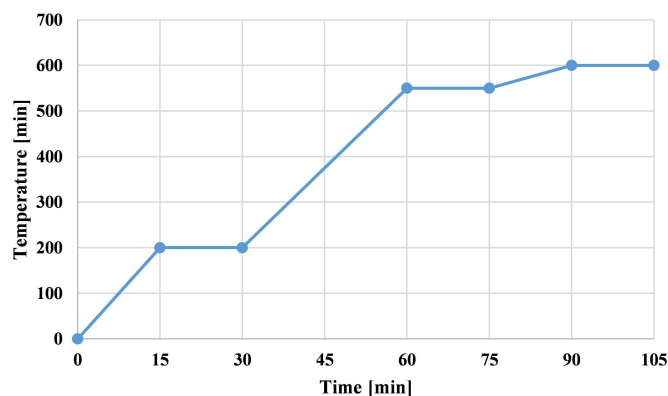


Figure 3. Changes in the heating temperatures of the algal biomass sample during the pyrolysis process.

Biochar was left in the furnace to cool down to room temperature ($25 \pm 2^{\circ}\text{C}$) and seasoned for 24 h. Carbon dioxide with a flow of $5.0 \text{ dm}^3/\text{min}$ was used as inert gas. Biochar was ground using a Testchem vibratory grinder. Granulation of the obtained biochar was analysed using 0.125 mm, 0.100 mm, 0.075 mm, and 0.040 mm sieves.

2.5. Method for testing the properties of biomass and biochar from *Chlorella* algae

The algal biomass and biochar microstructure were analysed using a Hitachi SU-70 Schottky Field Emission scanning electron microscope (SEM) coupled with an X-Ray Microanalysis EDS NSS 312 by Thermo Scientific. The analyses were carried out under the following conditions: magnification 500x, acceleration voltage of 15 kV, emission voltage of 50 A, analysis time of 100 s, and inclination angle of approx. 30° (corresponding to working distance of approx. 15 nm). The EDS analysis was standardless, producing results within 0.1% relative error of the known composition of the identified elements.

The structure and arrangement of the biochar obtained was analysed based on the D/G intensity ratio determined using a Jasco NRS 5100 Raman spectroscope with an excitation laser with a wavelength of 532.12 nm and an CCD detector. The measurement parameters were as follows: diffraction grating: 600 lines/mm; laser power: 4.8 mW; numerical aperture: $d4000 \mu\text{m}$; resolution: 1.37 cm^{-1} (aperture $10 \times 1000 \mu\text{m}$); magnification: 50x, exposure time: 60 s with light accumulation: 20. The tested samples were placed on glass plates

and for each of them at least three (3) spectra were recorded ($100\text{--}3,700\text{ cm}^{-1}$). The spectra were processed using Spectra Manager Analysis software by the means-movement method enabling noise elimination and smoothing.

2.6. Sorption

Sorption was carried out using solid phase extraction (SPE). A 3 cm^3 measuring column containing 1 g of the algal biochar obtained was used. The test consisted in adding 10 cm^3 of distilled water to the biochar substrate and filtrating 50 cm^3 of the wastewater from the cleaning processes through it. Changes in the treated wastewater taking place during sorption were evaluated based on changes in physical and chemical parameters: total organic carbon (TOC), sulphates (SO_4^{2-}), and chlorides (Cl^-), turbidity, and total suspended solids.

Carbon, sulphate and chloride content in the wastewater cleaned through sorption was determined using Hach Lange cuvette tests and a DR 6000 UV-VIS spectrophotometer. The determined parameters are presented in Table 5.

The content of total suspended solids in the tested samples was determined using a DR 6000 UV-VIS spectrophotometer and a 25 cm^3 glass cuvette with an optical path length of 1 inch. Turbidity of the wastewater prior to and after treatment was determined in accordance with the EN ISO 7027.1:2016-09 standard using a Hach Lange 2100Q IS turbidimeter. The measurement involved defining the turbidity coefficient using sensors that determine the amount of the light scattered or absorbed and the light transmitted. Tested samples had the volume of 15 mL.

3. RESULTS AND DISCUSSION

Chlorella sp. culture was prepared in laboratory reactors on a culture medium made of dairy wastewater. During cultivation, the authors evaluated biomass growth – based on optical density measurements (Fig. 4) – and changes in the initial and final total nitrogen and phosphorus content in the culture medium.

The tests were carried out for the total of 44 days, until depletion of nutrients in the culture medium and with the negative physiological changes, which occur in microalgae cells during the die-off phase, in which aerobic conditions of the culture deteriorate and cells die, prevented. Therefore, along with the biomass growth, the consumption of nutrients from the

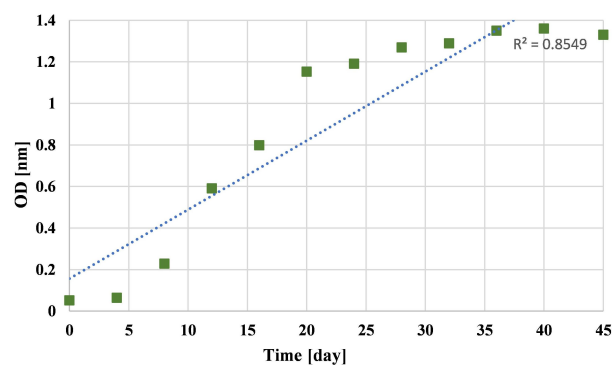


Figure 4. *Chlorella* algae biomass growth in the culture using dairy production wastewater.

culture medium was monitored. During the experiment, total nitrogen content was reduced by 40%, from 64.1 mg/dm^3 to 36.46 mg/dm^3 . Phosphorus content was reduced by 96%, from 19.7 mg/dm^3 to 0.70 mg/dm^3 . Once phosphorus content in the culture medium dropped below 1 mg/dm^3 , the cultivation ended. The obtained biomass was separated from the culture medium, centrifuged and freeze-dried.

The next stage of the test involved thermal decomposition of the algal biomass obtained. To this end, biomass was pyrolysed at 600°C .

To get a true image of the surface and morphology of the biochar obtained from the biomass of *Chlorella sp.* microalgae, scanning electron microscopy was employed (Fig. 5a). For the purposes of comparison, images of the biomass structure before pyrolysis were also taken (Fig. 5b).

The comparison of the surface of the samples prior to and after pyrolysis shows an important change in morphology. The microimage of the *Chlorella sp.* microalgae biomass shows few pores; the surface of the biomass is smooth and residues of the culture medium are visible. Images of the structure of the biochar obtained confirm the presence of pores of various sizes and shapes, particularly macropores and mesopores. The EDS microanalysis enabled identification of main chemical elements in the biochar obtained. Carbon content amounted to 41.26%/w/w, oxygen – 9.78%/w/w and nitrogen – 22.15%/w/w. The EDS analysis showed that the oxygen-to-carbon (O/C) and nitrogen-to-carbon (N/C) ratios in the obtained material averaged 0.24 and 0.54, respectively. The arrangement and structure of the obtained biochar was evaluated using Raman spectroscopy (Fig. 6). The observed spectra revealed the presence of D bands located at 1346--

Table 5. Parameters determined during sorption using cuvette tests.

Parameter	Measurement range [mg/dm^3]	Method/standard	Wavelength [nm]
TOC	2 ÷ 65	DIN 38409-H3	435
Sulphur	40 ÷ 150	Barium sulphate	430
Chlorides	1 ÷ 1,000	Iron thiocyanate (III)	468

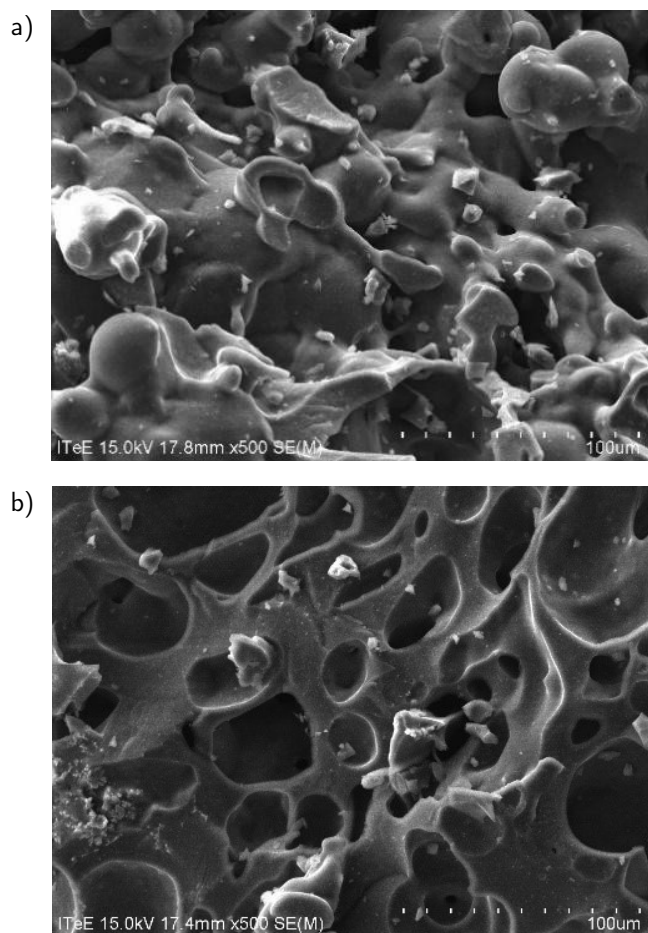


Figure 5. SEM images of the sample a) biochar from algal biomass and b) algal biomass at magnification $\times 500$.

1354 cm^{-1} and corresponding to disordered carbon structures, as well as G bands located at $1585\text{--}1594\text{ cm}^{-1}$ and corresponding to tensile vibrations. The D/G intensity ratio, which is one of the key parameters characterising the structure of carbon materials, was measured at 1353 cm^{-1} and 1591 cm^{-1} . In the case of algal biochar, it averaged 0.28 (Parab et al., 2012). In the case of the commercial active carbon tested under the same conditions, the D/G intensity ratio was 1. The low value of the tested parameters confirms high sorption capacity of the biochar produced.

The biochar obtained was used as a sorbent in the treatment of used baths from a local dairy plant. Figure 7 presents the effectiveness of the treatment of used baths with biochar from algae cultivated on dairy wastewater used as a sorbent.

Sorption confirmed high efficiency of treatment of used baths. Turbidity was reduced by 93% (from 45.7 to 3.15 NTU), total suspended solids by 88% (from 73 to 9 mg/dm^3), sulphate content by 61% (from 108 to 42.5 mg/dm^3), and chloride content by 80% (from 35.1 to 6.9 mg/dm^3). The worst results were observed in relation to the total organic carbon content, which was reduced only by 17% (from 13.61 to 11.35 mg/dm^3). The obtained results are similar to those obtained for biochar from a synthetic culture medium.

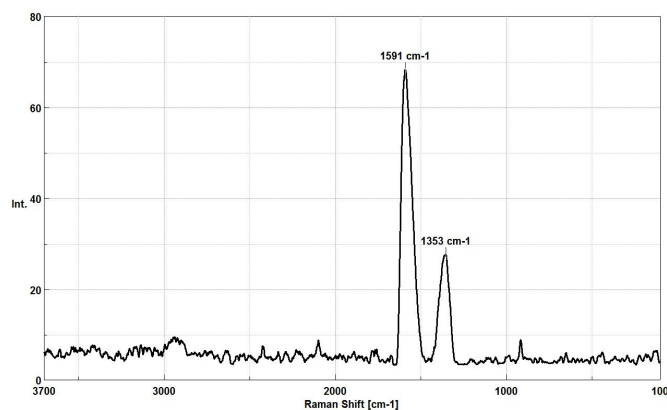


Figure 6. Raman spectrum of biochar from *Chlorella* algae.

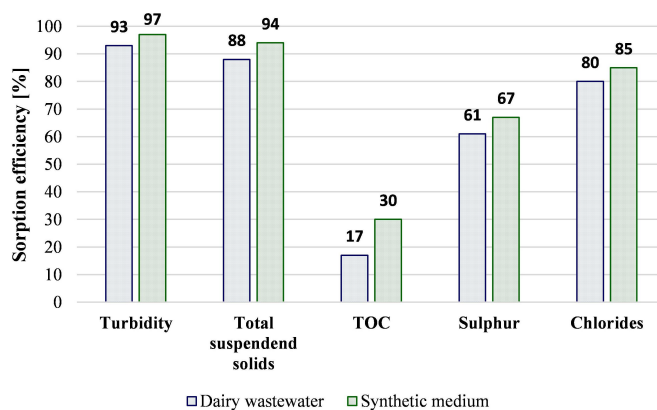


Figure 7. Summary of sorption efficiency results in the solid phase extraction method for biochar from *Chlorella sp.* algae biomass.

It should be stressed that the study showed dependencies between practical application of algal biomass and several factors that can affect its properties as a new sorption biomaterial. The biochar obtained from the algal biomass is highly effective during sorption, which increases its application possibilities.

4. CONCLUSIONS

The verification tests confirmed the possibility of using the dairy post-industrial waste as a culture medium for *Chlorella sp.* algae, converting the post-cultivation biomass in the pyrolysis process, and using the biochar obtained as a sorbent in the treatment of wastewater from cleaning processes. During the experiments, total nitrogen and phosphorus content in the culture medium was reduced by 40% and 96% respectively. The biochar obtained as a result of pyrolysis had an advanced specific surface area. The sorption efficiency varied between 17 and 93%. The obtained sorption properties justify the continuation of the research to further improve the process of sorbent production on the basis of algae cultivated on a culture medium containing post-industrial wastewater, which is in line with the circular economy assumptions concerning effective water and wastewater management. In the course

of their further work, the authors plan to functionalise the structure of biochar produced from algal biomass and increase the removal efficiency of various pollutants.

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