Research

Surface Properties of Microalgal Biomass and Microplastics: Exploring Point of Zero-Charge and Contact Angle

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Abstract:
The surface properties of microalgal biomass and microplastics, focusing on the point of zero charge (PZC) and water contact angle (CA), were investigated. Microalgae, particularly *Chlorella vulgaris* and mixed microalgal consortia, were cultivated and analysed for their surface characteristics. Oven-dried and freeze-dried samples exhibited varying degrees of hydrophilicity, with freeze-dried microalgal consortia exhibiting more hydrophilic surface. The PZC values indicated a higher density of negative charges on the surface of *C. vulgaris* compared to the microalgal consortia biomass. Microplastics (MPs) from agricultural mulch films, including biodegradable and non-degradable types, were also examined. Results showed that naturally aged MPs exhibited more hydrophilic surfaces compared to their pristine counterparts. The PZC values of microplastics varied, with some showing neutral to slightly negative charges at environmental pH levels. The findings underscore the importance of surface characterization in understanding the sorption mechanisms of contaminants.

Keywords: Microplastics; Microalgal biomass; Surface properties; Point of zero-charge; Water contact angle; Sorption

1. Introduction

Sustainable development, as outlined by the United Nations (UN, 2015), can be enhanced with resource recovery and reuse in agricultural practices as well as reducing pollution in the environment due to insufficiently treated wastewater (WW). Nature-based solutions for wastewater treatment using microalgae are considered an efficient technology for the removal of contaminants of emerging concern (CECs) with simultaneous removal of nutrients from WW and incorporation into microalgal biomass (Maryjoseph & Ketheesan, 2020; Prosenc et al., 2021). Microalgae-based WW treatment process can take place in open ponds such as high-rate algal ponds (HRAP) or closed systems such as photobioreactors (PBRs). Either way, the end-products are treated WW and microalgal biomass. Treated WW can be used for irrigation, while microalgal biomass can be used to obtain high-value products: biostimulants to be used in agriculture, source of biopolymers, nutraceuticals (fatty acids, sterols, vitamins, minerals) and pigments (chlorophyll, phycocyanin, carotenoids) (Abdelfattah et al., 2023). Removed CECs can be either degraded or retained by microalgal biomass, including microplastics (MPs).

Increased yield in agricultural fields relies on the use of fertilizers (organic or mineral), CECs like pesticides and various materials, applied to the surface of the soil uncovered or covered with mulch (Alvarez et al., 2021; Bhuvaneswari et al., 2022; Hofmann et al., 2023). The most widely used material for mulch is non-biodegradable polyethylene (PE), which is lately being replaced with biodegradable and biobased polymers such as polybutylene co-adipate co-terephthalate (PBAT), thermoplastic starch (TPS), polylactic acid (PLA), polyhydroxy alkanoate (PHA) and their blends (Sintim et al., 2020). Mulch films, like all other plastics, can degrade in the environment when exposed to physical, chemical, and biological factors, forming MPs (i.e. particles in the size from 1 µm to 5 mm (ISO, 2023)). MPs are known contaminants in the soil, the source of which are overused agricultural materials (mulch, greenhouses), littering, wind deposition, irrigation with WW, composting, and fertilisation with organic fertilisers (Sa'adu & Farsang, 2023). MPs can alter the physical, chemical and microbial properties of soil and can be translocated between the environmental compartments (Dissanayake et al., 2022, Yadav et al., 2022).

Additionally, MPs were found to be an adsorbent and can act as a vector for other CECs such as antibiotics and pesticides, especially hydrophobic ones, and can enhance their persistence in soil (Šunta et al., 2020; Zhang et al., 2021; Wu et al., 2022). The sorption behaviour of CECs in the soil is dependent on the characteristics of the sorbate as well as on the characteristics of sorbents - in this case MPs and microalgal biomass (García-Delgado et al., 2020). There are many mechanisms involved in the sorption of contaminants on the surface of sorbents. Two of them are hydrophobic and electrostatic interactions that can be explained by the determination of the contact angle and point of zero charge of the sorbent, respectively.

The Contact angle (CA) is a measure of the wettability of a solid surface by a liquid, the most commonly used is water. CA is defined as the interface angle, formed at the three-phase boundary, that is generated after the application of a drop of liquid on the solid surface, between the gas-solid surface, gas -liquid surface and solid-liquid surface. The CA is a result of the balanced forces due to the interfacial tensions of solid surface, liquid and air. According to the Young-Laplace's equation, the CA (θ) can be expressed as in Eq. (1):

$$cos\theta_{\gamma} = \frac{\gamma_{SV} - \gamma_{SL}}{\gamma_{LV}} \quad (1)$$

where θ_{γ} represents the contact angle, γ_{SV} represents the surface energy of the solid-air interface, γ_{SL} represents the surface energy of the solid-liquid interface, and γ_{LV} represents the surface energy at the liquid-air interface. The water CA values of less than 90° indicate the surface is hydrophilic, while the values of more than 90°C indicate that the surface is hydrophobic (Guo & Zhao, 2024; Kholodov et al., 2015).

The point of zero charge (PZC) is a pH value at which the surface density of positive charges is equal to that of negative charges under given analytical parameters (temperature conditions, composition of aqueous solutions) (Rey et al., 2011). PZC is determined using the salt addition method. The method is based on measuring the differences between the initial and final pH values in the salt solutions (from pH 2-11) over a certain period. Based on the measured differences, the surface can exhibit a negative charge in case of lower final pH values and a positive charge in case of higher final pH values as can be seen in **Figure 1** (Bakatula et al., 2018; Zia-Ur-Rehman et al., 2020).

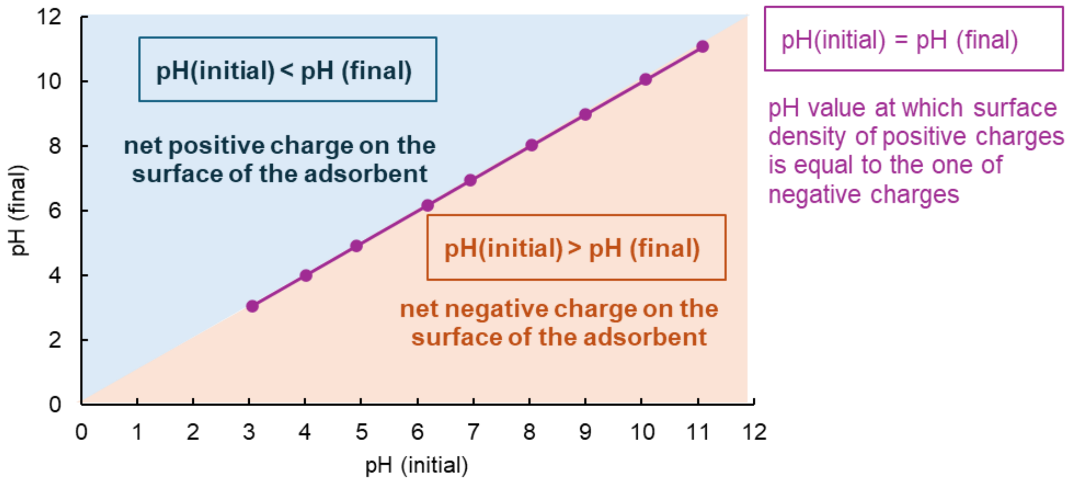

Figure 1. The surface charge of the adsorbent concerning the differences in pH values, determined in salt solution with the salt solution method for determination of the point of zero charge (adopted from Zia-Ur-Rehman et al., 2020).

The overall aim of this research was to determine the surface properties (PZC and CA) of MPs and dry microalgal biomass as sorbents of CECs, and to investigate how the drying process affects the surface characteristics of microalgal biomass. This will help predict the behavior of different combinations of MPs, CECs, and microalgal biomass when added to soil.

2. Material and Methods

2.1. Microplastics

Surface properties of microplastic particles (MPs) from agricultural mulch were assessed, namely of two biodegradable and one non-degradable film. Polymer types of plastic mulch used were co-polymer of polybutylene adipate terephthalate and polylactic acid (PBAT/PLA), TPS, and high-density PE (PE_black). Obtained mulch of each polymer type was manually cut into smaller particles to obtain large MPs in the size between 1 mm and 5 mm.

2.2. Microalgal biomass

Microalgal biomass analysed consisted of a monoculture *Chlorella vulgaris* (*C. vulgaris*), cultivated in the laboratory, and a mixed microalgal consortia, cultivated during the WW treatment process in HRAP.

Monoculture of *C. vulgaris*, acquired from AlgEn (Algal Technology Centre, Slovenia), was cultivated in a sterile Bold's Basal Medium (BBM; PhytoTech Labs, USA). *C. vulgaris* was cultivated in sterile 250 ml Erlenmeyer flasks, which were sealed with foam caps to enhance gas exchange. The inoculated flasks were continuously agitated at 150 rpm using an orbital shaker (RS-OS-20, Phoenix Instrument, Germany) within a custom-built growth chamber. Cultivation conditions were as follows: 25 °C, artificial illumination provided by FLUORA lamps (OSRAM, Germany) with an average illumination intensity of 1380 lx. The obtained biomass of *C. vulgaris* was harvested using a UNIVERSAL 320 centrifuge (Hettich Zentrifugen, Germany) at 8000 rpm for 5 min.

Mixed microalgal consortia was harvested from the HRAP pilot plant (45° 52.5' N, 13° 54.3' E), used for treating effluent from primary treatment of WW at the Central Wastewater Treatment Plant Ajdovščina (CWWTP Ajdovščina). 1 L of WW containing microalgal biomass from HRAP was concentrated with centrifugation at 8000 rpm for 5 min (UNIVERSAL 320, Hettich Zentrifugen, Germany).

2.3. Measurements of contact angle

CA of MPs and microalgal biomass were measured with tensiometer Theta Attention (Biolin Scientific, Sweden) using the sessile drop method (Kholodov et al., 2015; Cramer et al., 2022). MPs and microalgal biomass were mounted onto the microscopic object glass slides using double-sided adhesive tape (4965, Tesa, Germany). A droplet of water (5 μL) was extruded from the syringe onto the sample, while simultaneously the magnifying camera on the tensiometer recorded (OneAttension software) the droplet formation on the sample (repeatability n=9). The geometry of each droplet was evaluated by the Young-Laplace's equation and CA was measured at the three-phase contact point between the surface of the sample, the water droplet and the air.

2.4. Measurements of point of zero charge

The salt addition method was used to determine the point of zero charge (PZC) of MPs and dry microalgal biomass according to the modified method by Santaeufemia et al. (2021). Briefly, charges in the pH were measured with a Multi 3620 IDS meter and Sentinx 940 probe (WTW, Germany) in nine pH solutions (3-11) containing 40 mL of 0.01 M $CaCl_2$. In each solution, the pH value was adjusted to the initial pH of the solution ($pH_{initial}$) using 0.1 M NaOH and/or 0.1 M HCl. To each of the solutions with adjusted pH, 40 mg of MPs or microalgal biomass was added and left to stir on magnetic stirrers (Velp Scientifica, Italy) at approx. 300 rpm. After 1 h, the pH was measured and denoted as pH_{final}. Difference in the pH (ΔpH) for each of the solutions was calculated according to the Eq. (2) and the PZC was obtained from the intersection of ΔpH with the x-axis on the graph ΔpH vs. $pH_{initial}$.

$$\Delta \boldsymbol{pH} = \boldsymbol{pH_{initial}} - \boldsymbol{pH_{final}} \quad (2)$$

3. Results and Discussion

3.1. Surface properties of dry microalgal biomass

Microalgae are unicellular organisms mainly present in aquatic environments. Based on the previous research by Ozkan & Berberoglu (2013), it was expected for the surface of the tested microalgal biomass to exhibit a hydrophilic nature, regardless of different sample preparations. CA of laboratory culture of *C. vulgaris* was determined to be similar in case of direct (oven) drying of the biomass (86.1±9.0°) and freeze-dried biomass (82.8±6.3°). Contrary, the surface of microalgal consortia was more hydrophilic when biomass was freeze-dried (65.0±6.6°) compared to when it was oven (direct) dried (93.1±4.4°). The drying method can affect the hydrophobicity/hydrophilicity of microalgal biomass due to the structural changes in the cell wall. Ultimately, the breakage of the cell wall can expose more hydrophobic intracellular components therefore, making the surface more hydrophobic (Machado et al., 2022).

The surface charge of microalgal biomass was found to be similar for both tested microalgal biomasses, regardless of the used drying method. PZC of the culture of *C. vulgaris* was slightly acidic, determined at pH of 5.7 and 5.6, for direct and freeze drying, respectively (**Figure 2**). Microalgal consortia biomass exhibited slightly higher PZC at pH values of 6.2 and 6.7, for direct and freeze drying, respectively. Obtained PZC values are in the range of previously reported PZC values for dry microalgal biomass, tested in different salt solutions (Mohammed et al., 2019; Bakatula et al., 2018). Therefore, at environmental pH values (7-8), the surface of *C. vulgaris* had a higher density of negative charge compared to the microalgal consortia. Negative surface charge in microalgal biomass is attributed to the presence of carboxylic and amino functional groups on the membrane of the microalgal cells (Li et al., 2022).

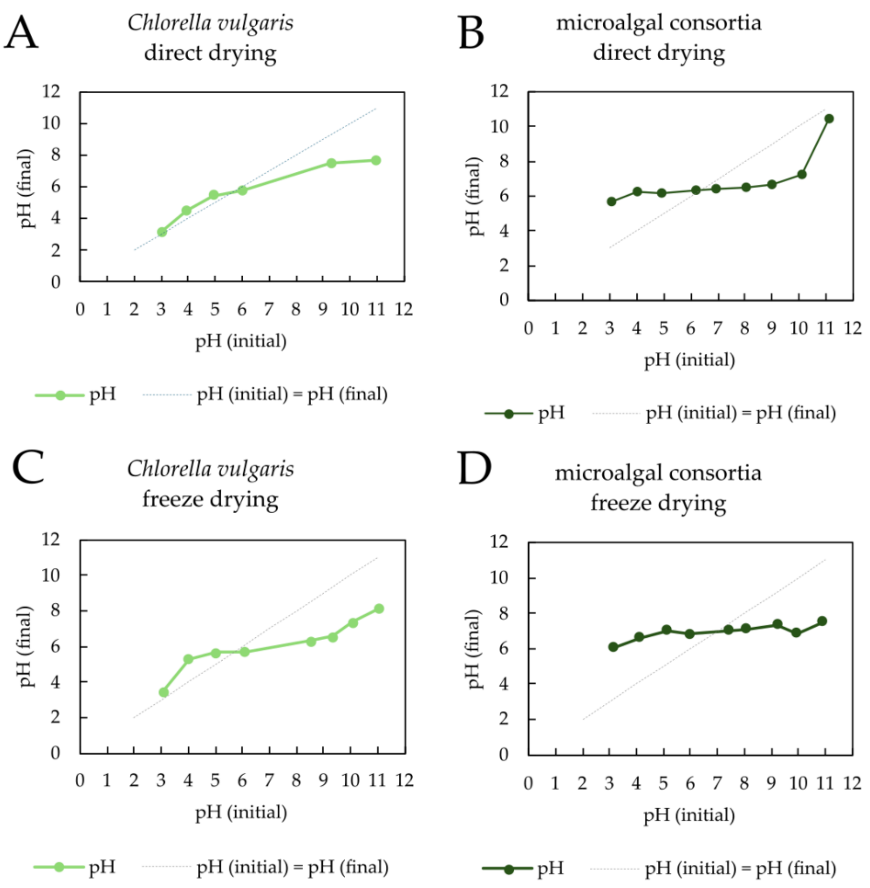

Figure 2. Initial (pH (initial)) vs. final (pH (final)) pH measurements of salt solution containing microalgal biomass, dried in two different ways: direct oven drying (*Chlorella vulgaris* (A) and microalgal consortia (B)) and freeze-drying (*Chlorella vulgaris* (C) and microalgal consortia (D)). The point of zero charge (PZC) of each microalgal biomass is characterised by the crossing of the pH (final) curve at different pH (initial) values with the linear curve of equal pH (initial) and pH (final) in salt solution (pH (initial) = pH (final)).

3.2. Surface properties of microplastics

MPs, in general, are reported to primarily have a hydrophobic nature, since most of the structural polymers are hydrophobic. The wettability of the tested mulch MPs in this study based on the polymer types was as follows: PBAT/PLA (84.7±5.9°) > TPS (76.5±4.5°) > PE_black (73.0±7.8°). For pristine MPs, the obtained water CA for PLA and PBAT are in accordance with the literature, 75-85° and 82-92°, respectively (Tümer et al., 2022; Pan et al., 2024). Contrary, TPS film exhibited more hydrophobic surface compared to the reported CA values in the range from 53° to 62° (Jantanasakulwong et al., 2016; Zhong et al., 2022), and PE mulch exhibited more hydrophilic surface compared to the reported values for PE from 89° to 99° (Aktas et al., 2023; Accu Dyne Test, 2025). The discrepancy with the literature could be due to the possible presence of additives in mulch films that alter the hydrophobicity/hydrophilicity of the material to achieve the desirable characteristics of mulch (moisture control and funnelling the excess rainfall away from the roots, heating properties of plastic to regulate soil temperature) (Kasirajan & Ngouajio, 2012).

Exposure to environmental factors (physical, chemical, and biological) can cause ageing and degradation of MPs. Thus, the surface of MPs can become hydrophilic (Harraq & Bharti, 2021). This was observed also in our case, where all tested polymer types exhibited more hydrophilic surface of the naturally aged particles compared to their pristine counterparts. The CA of naturally aged MPs followed

PE black (67.6±7.3°) ≈ PBAT/PLA (65.5±5.0°) > TPS (57.3±3.5°).

The surface of tested MPs exhibited neutral to slightly negative charge (**Figure 3**). Neutral charge at environmental pH values was observed for pristine PBAT/PLA and TPS MPs, and naturally aged PE_black (all PZC 7.3) and PBAT/PLA (PZC 6.8). A slightly acidic and therefore more negatively charged surface was determined in the case of pristine PE_black (PZC 6.3) and naturally aged TPS (PZC 6.1).

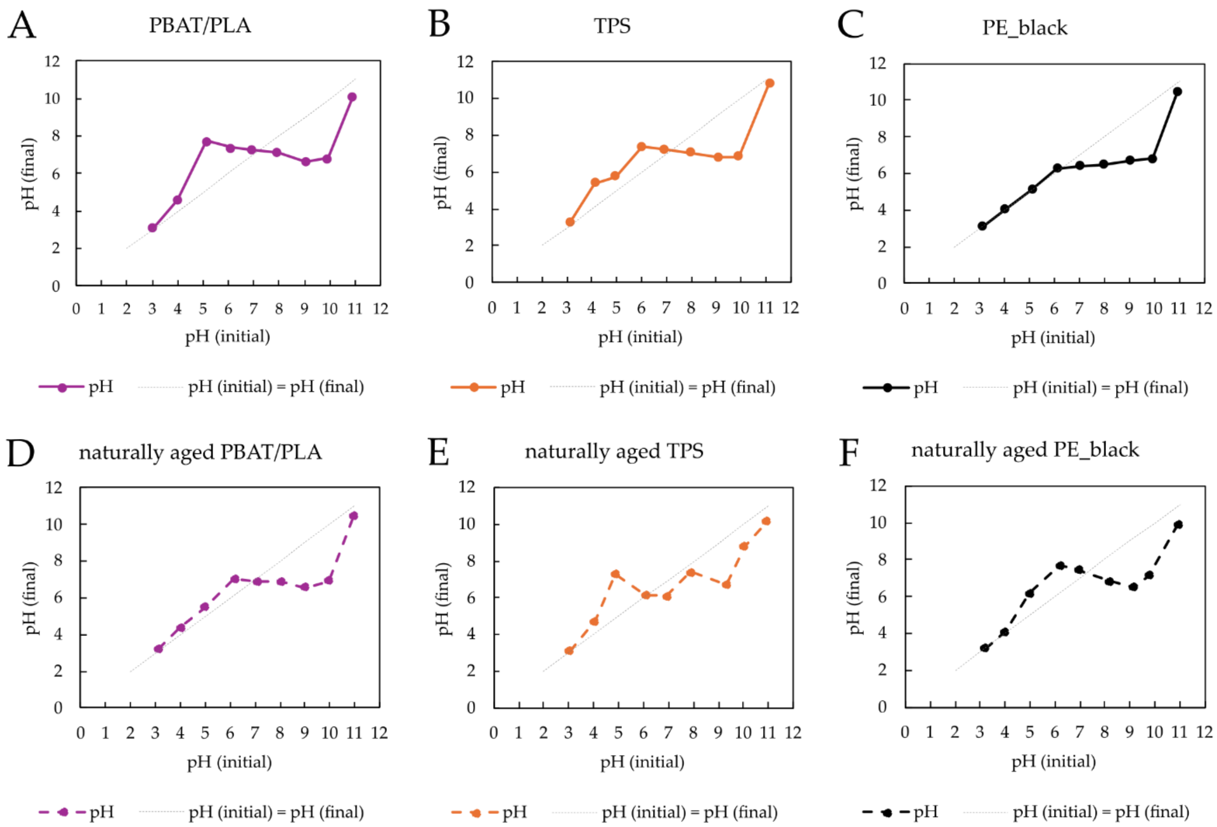

Figure 3. Point of zero charge of pristine microplastics (polybutylene adipate terephthalate (PBAT)/polylactic acid (PLA) – A, thermoplastic starch (TPS) – B, and polyethylene (PE_black) – C) and naturally aged microplastics (PBAT/PLA – D, TPS – E, and PE_black – F). The point of zero charge (PZC) of each type of microplastics is characterised at the crossing of pH_f curve at different pH_i values with the linear curve of equal pH_i and pH_f in salt solution (pH (initial) = pH (final)).

5. Conclusions

Surface characterization of sorbents is important in order to explain the sorption mechanisms that drive the sorption process of certain organic contaminants. Two methods for characterization of surfaces – point of zero charge (PZC) and water contact angle (CA) – were used to characterize the surface of microalgal biomass and microplastic particles (MPs) as sorbents. The drying method of microalgal biomass can modify the surface characteristic, especially the polarity of the surface due modifications of the cell wall and elimination of intracellular hydrophobic substances. The effects of used drying method can vary, based on the composition of the used microalgal biomass. On the other hand, ageing of MPs can change the surface charge and wettability (CA), therefore affecting the sorption process of organic contaminants in the aquatic and terrestrial environment.

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Conflicts of Interest: The authors declare no conflict of interest.

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