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Solving urban water microplastics with bacterial cellulose hydrogels: Leveraging predictive computational models

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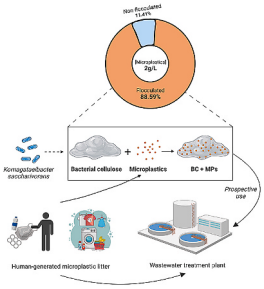
HIGHLIGHTS

- BC remnants can be scavenged to produce highly effective sustainable bioflocculants.
- Several operational parameters can be modulated to enhance the MPs flocculation rate.
- RSM is effective in predicting flocculation rates in a wide array of conditions.
- The BC hydrogel far outperforms commercial bioflocculants regarding MPs.

GRAPHICAL ABSTRACT



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ABSTRACT

The prevalence of microplastics (MPs) in both urban and aquatic ecosystems is concerning, with wastewater treatment plants being considered one of the major sources of the issue. As the focus on developing sustainable solutions increases, unused remnants from bacterial cellulose (BC) membranes were ground to form BC hydrogels as potential bioflocculants of MPs. The influence of operational parameters such as BC:MPs ratio, hydrogel grinding, immersion and mixing time, temperature, pH, ionic strength, and metal cations on MPs flocculation and dispersion were evaluated. A response surface methodology based on experimental data sets was computed to understand how these parameters influence the flocculation process. Further, both the BC hydrogel and the hetero-aggregation of MPs were characterised by UV–Vis, ATR-FTIR, IGC, water uptake assays, fluorescence, and scanning electron microscopy. These highlights that the BC hydrogel would be fully effective at hetero-aggregating MPs in naturally-occurring concentrations, thereby not constituting a limiting performance factor for MPs' optimal flocculation and aggregation. Even considering exceptionally high concentrations of MPs (2 g/L) that far exceed naturally-occurring concentrations, the BC hydrogel was shown to have elevated MPs flocculation activity (reaching 88.6%: 1.77 g/L). The computation of bioflocculation activity showed high reliability in predicting flocculation performance, unveiling that the BC:MPs ratio and grinding times were the most critical variables modulating flocculation rates. Also, short exposure times (5 min) were sufficient to drive robust particle aggregation. The microporous nature of the hydrogel revealed by electron microscopy is the likely driver of strong MPs bioflocculant activity, far outperforming dispersive commercial bioflocculants like xanthan gum and

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alginate. This pilot study provides convincing evidence that even BC remainings can be used to produce highly potent and circular bioflocculators of MPs, with prospective application in the wastewater treatment industry.

1. Introduction

A growing widespread concern about deeply entrenched environmental changes caused by small plastic particles has arisen (Andrady, 2011). These micropolymeric fragments, denominated microplastics (MPs), represent a global threat to several ecosystems: aquatic marine (Cole et al., 2011) and freshwater biospheres (Novotna et al., 2019); remote antarctic (Wang et al., 2014) and artic polar waters (Pang et al., 2020); and urban networks (Novotna et al., 2019); remote antarctic (Wang et al., 2014) and artic polar waters (Pang et al., 2020); and urban networks (Sun et al., 2019b).

Microplastics are defined as plastic particles with a diameter inferior to 5 mm (Arthur et al., 2009). Despite constituting the majority of marine debris, it is still not fully clear how MPs transversely affect aquatic biodiversity. Nonetheless, several studies have thoroughly reported extensive toxicity in marine vertebrates, microbiome, and benthic communities (Khalid et al., 2021a; Ugwu et al., 2021), as well as in freshwater ecosystems (Li et al., 2018). Moreover, MPs can also induce toxic effects on fundamental layers of the aquatic biome through the release of several adsorbed persistent organic pollutants (Cunha et al., 2019; Khalid et al., 2021b; Wang et al., 2020).

Less studied but not of lesser importance are urban water ecosystems. Given how critically important wastewater treatment plants (WWTPs) are to urban water distribution, these infrastructures have recently undergone extensive scrutiny. Currently, WWTPs do not possess the technological expertise to remove MPs, thus becoming a focal source of MPs' release into the environment. (Carr et al., 2016; Dey et al., 2021; Liu et al., 2021; Sarkar et al., 2021; Sun et al., 2019a). Even with the recent development of techniques to remove MPs from waters (Poerio et al., 2019a), these are still early-stage technologies with unsatisfactory environmental setbacks. Despite sustainability concerns, membrane systems such as membrane bioreactors (MBRs) show considerable promise. However, its membranes still represent a critical point of apprehension given their fossil-based/non-biodegradable nature and environmental unsustainable fouling removal mechanisms (Poerio et al., 2019b; Wang et al., 2014). Therefore, it is imperative to advance the R&D of biomembranes to tackle this issue.

Accordingly, bacterial cellulose (BC), first reported by Dr. Adrian Brown in the 19th century (Brown, 1886), is a natural extracellular polymer produced as an output of bacterial biosynthetic processes (Blanco Parte et al., 2020a; Rangaswamy et al., 2015). BC production is often restricted to bacterial species belonging to the *Komagataeibacter* genus, also known as *Acetobacter* and *Gluconacetobacter* (Lin et al., 2020a; Yamada et al., 2012). Aside from its biosustainable nature, structurally-stable BC can be produced using cost-effective raw and cheap alternative carbon sources, such as agricultural, industrial and food wastes (Islam et al., 2017; Vazquez et al., 2013).

The BC biofilm exhibits unique structural features, such as a hydrated ultrafine three-dimensional nanofibril structure and high porosity, resulting in the formation of a hydrogel. This hydrogel displays a strong water absorption and retention capacity, chemical stability, large specific surface area, excellent mechanical strength/degree of polymerisation, and exceptional biocompatibility (Andriani et al., 2020; Blanco Parte et al., 2020b; Choi and Shin, 2020; Klemm et al., 2005; Shah et al., 2013; Skvortsova et al., 2019). These distinctive properties are highlighted as BC has shown applicability and further promise in a wide range of fields, such as biomedical and tissue engineering (Liu et al., 2020; Moniri et al., 2017; Pang et al., 2020), food science (Azeredo et al., 2019; Lin et al., 2020b; Paximada et al., 2016), cosmetics/pharmaceutical industries (Swingler et al., 2021; Ullah et al., 2016), and paper/textile manufacturing (Reis et al., 2019). Despite some early environmental-related work focusing on BC and cellulose-based composites (Hussain et al., 2019; Ul-Islam et al., 2016), the most exciting advances regard the bioremediation potential of BC hydrogels in removing pollutants such as heavy metals, proteins, dyes and oil emulsions (Isik et al., 2018; Kurniawan and Yamamoto, 2013; B. v. Mohite and Patil, 2014; Wanichapichart et al., 2002). This highlights that BC might act as a highly multipurposed and sustainable alternative for complex bioremediation processes such as water treatment in WWTPs.

The fact that WWTPs still use inorganic flocculants presenting low flocculation efficiencies, no biodegradability, and several high health risk hazards/environmental sludge generation is daunting (Lee et al., 2014). Remarkably, several environmental-friendly alternatives such as microbial and plant-derived bioflocculants have gained recognition as truly unrealised potential sources of biomaterials with high flocculating power (Das et al., 2021; Li et al., 2020; Rebah et al., 2018). Still, and despite research having begun showing that microorganismal-derived biopolymers are highly effective in removing MPs from polluted water (Cunha et al., 2019, 2020a, 2020b; Faria et al., 2022), there is still a complex road ahead in aligning their environmental benefits with industrial and economic demands.

Following the urgent need to develop top-performing sustainable alternatives, this laboratory-scale work aims to evaluate the potential of BC hydrogels as bioflocculants and hetero-aggregators of MPs towards their prospective removal from contaminated WWTP waters. Besides, the production of BC membranes yields unused and leftover BC remnants. To fully embrace the circularity aspect of the process, only BC remnants were used to create the hydrogel. A response surface methodology was employed to predict and deepen the optimisation of parameters such as grinding times, BC:MPs ratio, temperature, and immersion time. The influence of different mixing times, pH, salinity, and presence of metal ions was investigated. To understand the BC/MPs hetero-aggregates durability, the retention capacity of the MPs in the BC hydrogel was also assessed.

2. Materials and methods

2.1. BC hydrogel production and characterisation

Komagataeibacter saccharivorans was used to produce the BC hydrogel (Supplementary ES1). The highest statistically significant BC production was achieved at 7 days (Fig. S1), after which the biofilm was isolated, treated with 0.5 M NaOH (80 °C for 45 min), and washed with distilled water until neutral pH, and kept at 4 °C pending usage (Faria, 2015; Faria et al., 2022). A moisture balance (Gibertini, Eurotherm, Novate Milanese, Italy) was used to determine the dry weight (106 °C during 120 min). The BC was characterised by infrared spectroscopy (ATR-FTIR; PerkinElmer, Llantrisant, United Kingdom), inverse gas chromatography (IGC; Surface Measurements Systems, London, United Kingdom), and water uptake capacity (Supplementary ES2-ES4). Visually, the hydrogel was characterised by fluorescence (Leica Microsistemas, Barcelona, Spain) and scanning electron microscopy (Hitachi, Montignyle-Bretonneux, France). To obtain the hydrogel, BC remnants (scraps) were ground from 1 to 20 min (Kunft, Worten, Portugal).

2.2. Microplastics

Commercial polystyrene (PS) (UV-Granulate, Magic Pyramid Bruecher & Partner KG, Frechen, Germany) was used in this study (Cunha et al., 2020a). This particular polymer was chosen given its vast occurrence in wastewater treatment plants (Sun et al., 2019b). A milling machine (Kunft, Worten, Portugal) was used to fragment the plastics,

which were then sieved (Analysensieb–Retsch, Scansci, Vila Nova de Gaia, Portugal) to obtain a fraction of MPs <100 μm (particle fraction difficult to remove from urban waters and known to be translocated into human tissues) (Cox et al., 2019; Sharma et al., 2021). A solution of polystyrene microplastics (*PS*-MPs) was prepared at a concentration of 2 g/L.

2.3. Flocculation activity

The flocculation activity of the BC hydrogel was measured using a standard clay-based methodology, as described by Kurane et al. (1986). Briefly, 1% of Ca^{2+} was added to the bentonite clay suspension (Fonte da Areia, Porto Santo (Cordeiro et al., 2010), (4 g/L). The final solution had a pH of 7, to which BC was mixed into in a ratio of 25:1 (BC hydrogel wet weight (w.w)/MPs or clay dry weight (d.w.)). At 20 ± 2 °C, the mixture was stirred at 300 rpm (Agimatic-E JP Selecta, V. Reis, Lisboa, Portugal) for 5 min and left resting for another 5 min. Subsequently, the solution was filtered (mesh size of < 112 μm; Krefeld, V. Reis, Lisboa, Portugal) and the optical densities (OD) of the unfiltered (without BC; OD_C) and filtered (OD_S) solutions were measured at 750 nm. The OD of the flocculant dispersion (OD_F) was subtracted from the OD_S, and the flocculating rate was calculated according to **Eq. (1)** (adaptation from Ndikubwimana et al. (2016)):

$$\text{Flocculation rate } (\%) = \frac{OD_C - (OD_S - OD_F)}{OD_C} \text{ x } 100 \quad (1)$$

The same experimental process was performed to assess the flocculation activity of the BC hydrogel relative to the particles being aggregated. All the experiments were conducted in triplicate.

2.4. MPs removal efficiency with BC hydrogel

2.4.1. Removal process

The MPs removal process was performed using a solution of PS-MPs with an exceedingly high concentration of 2 g/L. The BC hydrogel was added to the MPs-contaminated water, and different parameters were tested (in triplicate) to determine the hydrogel's behaviour in relation to several operational conditions (Supplementary Table S1). The mixture was adjusted relative to grinding times, BC:MPs ratio, temperature, pH, salinity and metal cations. The mixture was stirred at 300 rpm (Agimatic-E JP Selecta; V. Reis, Lisboa, Portugal) to potentiate hetero-aggregation formation (mixing times) and left resting for aggregate settling (immersion time) (Fig. S2) (based in (Ma et al., 2019)). The BC/MPs hetero-aggregate's height was measured to determine the dispersion (**Eq. (2)**), after which the solution was filtered (mesh size of <112 μm; Krefeld, V. Reis, Lisboa, Portugal). The absorbance of the MPs-contaminated water (solution of MPs or bioflocculant-free control) and the filtered solution were measured by the turbidity at 750 nm (UV–6300PC Spectrophotometer, V. Reis, Lisboa, Portugal). Moreover, the OD of the bioflocculant was subtracted to the OD obtained for each sample. The flocculation rate was calculated according to **Eq. (1)** and the dispersion was determined using **Eq. (2)**, where *m* is the mass of BC (g), and *v* is the volume of the BC/MPs hetero-aggregate formed after the immersion time:

$$\text{Dispersion } \left(cm^3 / g\right) = \frac{v}{m} \quad (2)$$

2.4.2. Flocculation: computational

A response surface methodology (RSM) was used in the present work, which is a combination of mathematical and statistical tools that may be applied in multi-variable processes and to optimise the parameters of an experimental design. Within this method, the design of the experiment (DOE) model is employed to establish the correlation between the variables (in this case, grinding time, BC:MPs ratio, temperature, and immersion time) that are influencing a specific variable (in this case, the flocculation rate, and the dispersion) and the output/response of that process. Despite the empirical nature of the resulting quantitative connection, there is a good chance that a viable model will be created that can be used to separate ineffective factors from effective ones and optimise operations (Breig and Luti, 2021). Several statistical analyses are taken into account to evaluate the model's validity (Domagalski et al., 2015), namely, the examination of the residuals (difference between the data point and the regression line), lack of fit (difference between the model prediction values and the experimental data), and analysis of the *p*-value and *F*-value (ANOVA analysis). The Minitab software was used to design the experiments considering the following parameters: grinding time (1–10 min), BC:MPs ratio (1.25:1–37.5:1 w. w./d.w.), temperature (15–30 ± 2 °C) and immersion time (0–120 min) (Supplementary Table S2).

2.4.3. Flocculation: experimental

All the flocculation tests (Supplementary Table S1) were conducted using the above mentioned method. Different ratios of BC (wet weight) and MPs solution (2 g/L) were explored to determine its influence on the flocculating activity (1.25:1–37.5:1, w. w./d.w.). The impact of the grinding times of the hydrogel (1–20 min), the effects of different immersion times (0–360 min), mixing times (5–30 min), and temperatures (4-30 °C) were tested. The influence of the pH (3–8) was also considered: MPs solutions were adjusted (HI96100, Hanna Instruments Póvoa de Varzim, Portugal) using HCl (0.1 M) and NaOH (0.5 M). In addition, various salinity concentrations (Hand Held Refractometer, Labbox, Spain) of 0, 10, 15 and 37‰, as well as the effects of metal ions (4.5 mM) such as Fe^{3+}, Ca^{2+}, Mg^{2+}, and K^{+} were measured. Supplementing this parameter, Fe^{3+} was chosen to evaluate the effects of several cation concentrations (4.5, 9 and 18 mM). All the experiments were conducted in triplicate.

2.4.4. Retention capacity

The capacity of the BC hydrogel to retain MPs was evaluated. After collecting the BC/MPs hetero-aggregates formed with the ratios BC:MPs of 25:1 and 50:1, the hetero-aggregates were kept under agitation (300 rpm) for 24 h in distilled water to clear the hydrogel of MPs. This will ensure the release of all removable microparticles. The BC/MPs hetero-aggregates were formed with the following conditions: 2 min of grinding time, 5 min of mixing time, and 60 min of immersion time at 20 °C. One wash cycle for each of the ratios was performed in triplicate. The OD of the solutions was measured at 750 nm and the MPs retention rate was calculated according to **Eq. (3)**, where the OD_S is the sample before the wash cycle, OD_{MPs} is the MPs-contaminated water, and OD_{SW} is the sample after the wash cycle, respectively:

$$\text{Retention rate } (\%) = \frac{OD_{SW}}{OD_{MPs} - OD_S} \times 100 \quad (3)$$

2.5. Fluorescence microscopy

Fluorescence microscopy was used to document the retention of MPs in the BC hydrogel, using a Leica DM2700 device attached to a Leica DFC450C digital camera and a CoolLED pE-300 lite lighting system (Leica Microsistemas, Barcelona, Spain). The MPs' fluorescence was observed with a 450–490 nm/515-565 nm excitation/emission filter I3.

2.6. Scanning electron microscopy

The BC hydrogel and BC/MPs hetero-aggregates were analysed using scanning electron microscopy (SEM; Montigny-le-Bretonneux, France). Samples were lyophilised, coated with a thin layer of carbon (EMITECH K950X Turbo Evaporator) and deposited on a steel plate. An HR-FESEM SU-70 Hitachi Scanning Electron Microscopy equipment (5 kV beam; 15.6 mm working distance; field emission mode) was used to acquire the SEM micrographs. Images were collected at magnifications of 100× and 400x.

2.7. MPs removal with commercial bioflocculants

The BC hydrogel flocculation capacity was compared to commercial bioflocculants, xanthan gum (11472781/MP Biomedicals) and alginate (A3249/PanReac). The flocculating tests were conducted using the method described previously: after an immersion time of 1 h, 1 mL was removed (*ca.* 3.5 cm from the top) and its OD absorbance was measured at 750 nm.

2.8. Data and statistical analysis

The results were presented as the mean values ± standard deviation (SD) of three replicates. Data representation and statistics were performed using GraphPad Prism 8. The D'Agostino-Pearson omnibus and Kolmogorov-Smirnov normality tests were used to assess the Gaussian distribution of data. Parametric unpaired *t*-tests (or one-way ANOVA) were applied for normally distributed data, while non-parametric unpaired Mann-Whitney (or Kruskal-Wallis) tests were applied for non-Gaussian distributed data (statistical significance: p-value < 0.05). Statistical analysis was performed in at least three independent experiments.

3. Results and discussion

3.1. BC physicochemical characterisation

The ATR-FTIR spectra (Fig. S3) of the bacterial cellulose (BC) exhibited the characteristic absorption bands of cellulose at around: 3347 cm^{-1} (O–H stretching vibrations); 2899, 1434, 1373 and 1312 cm^{-1} (C–H and *C*–H_2 stretching vibrations); 1632 (C–H or O–H bending vibration), and 1064 cm^{-1} (C–O stretching vibration) (Faria et al., 2019; Pecoraro et al., 2007; Pretsch et al., 2009).

Further, in terms of industrial applicability and economic scalability, BC drying greatly increases logistics and implementation practicality. Thus, the BC remnants were ground and oven-dried. Firstly, the water content of the hydrogel was determined to be 98.70 ± 0.08%. Also, considering that the BC is fully immersed in water during the MPs removal process, it is crucial to understand how the water uptake capacity (swelling behaviour) of the BC evolves with time. The rehydration potential, which plays a significant role in allowing for particle aggregation (Hamidi et al., 2008), is central in allowing the BC hydrogel to recover its full structural integrity. Accordingly, the oven-dried (40 ± 2 °C) BC hydrogel exhibited a solid swelling behaviour of 300% after 2 h and saturated at 580% its dried weight after 8 h (Fig. S4). This confirms that the BC hydrogel can be easily dried and re-hydrated to regain its original properties.

3.2. Flocculation activity

Clay is typically used as a standard in flocculation assays (Cunha et al., 2020c). A flocculation activity assay using clay showed that the BC hydrogel (500 mg d. w./L) flocculated 27.12 ± 0.63% of particles present in the water. Despite the BC hydrogel not flocculating clay particles as efficiently, this biopolymer still showed a solid theoretical basis for fulfilling its objective: the flocculation and hetero-aggregation of MPs. Thus, the flocculation of MPs was studied under the same conditions used for the clay flocculation, and a MPs flocculation rate of 74.54 ± 3.00% was obtained (Fig. S5). The differences in the flocculation rate of BC with clay and PS-MPs are due to their acid/base properties. Inverse Gas Chromatography (Supplementary ES3) reveals that BC has an approximately amphoteric character ($K_b/K_a = 0.80$) and that clay has a basic character ($K_b/K_a = 5.50$) much more basic than PS-MPs ($K_b/K_a = 1.94$). Thus, interactions between the acidic and basic groups of BC hydrogel are more significant with the PS-MPs.

So, the first aim of applying the BC hydrogel was to predict how the flocculation rate of the BC hydrogel would vary under several potential operational conditions found in wastewater treatment plants. Thus, response surface methodology (RSM) was employed (Supplementary Table S2) to determine the degree to which parameters such as grinding time, ratio, temperature, and immersion time might influence the flocculation rate of the BC hydrogel (Fig. 1). Based on registered experimental values and computational modulation, the parameter exhibiting the most statistically significant impact on the flocculation of MPs was the ratio (BC:MPs). The grinding time, immersion time and temperature ranked as the following most impactful parameters to flocculation activity. Therefore, these parameters were sequentially explored to determine the optimal conditions for flocculation of MPs using the BC hydrogel.

Under favourable conditions, the best biosorption performance is usually reached at the optimal bioflocculant dosage (Salehizadeh and Yan, 2014; Zeng et al., 2019). Despite a greater porous surface area available theoretically leading to an increase in particle flocculation, there is a saturation point at which the bioflocculant concentration no longer improves flocculation performance (Abdel Maksoud et al., 2020a). Based on the model in Fig. 1, the effect of the BC:MPs ratio was analysed to determine the most cost-effective dosage for the MPs flocculation process. As shown in Fig. 2A, the percentage of MPs removal reached a maximum of 80.42 ± 2.48% with a BC:MPs ratio of 25:1. At this ratio, the BC hydrogel is no longer the limiting factor of optimal flocculation and aggregation of MPs. Further, it is shown that the flocculation activity is at its highest when the dispersion is at its lowest. It is noteworthy that the dispersion is calculated after flocculation.

To understand how different grinding times affect the efficiency of the hydrogel as a bioflocculator in the optimal ratio (25:1 BC:MPs), the BC biopolymer was ground from 1 to 20 min (Fig. 2B). Unsurprisingly, it becomes apparent that the hydrogel's structural integrity is critical in modulating particle aggregation. The highest flocculation rates (83.67 ± 0.79% and 80.42 ± 2.48%) were obtained for grinding times of 1 and 2 min, with no significance found between. A consistent decrease in flocculation rate is observed with increased grinding times, reaching a plateau at 10 min. Dispersion does not show to move inversely to flocculation rates.

According to the RSM model in Fig. 1, the third parameter affecting flocculation the most is the hydrogel's immersion time. Using the optimal parameters of 25:1 BC:MPs ratio and 2 min of grinding time, the effect of progressively increased immersion times is shown in Fig. 2C. Over time, the flocculation rate increased in contrast to decreased dispersion. It is documented that a maximum flocculation rate is reached at an immersion time of 60 min. Longer immersion times are expected to yield better flocculation results, given that time is a crucial variable in

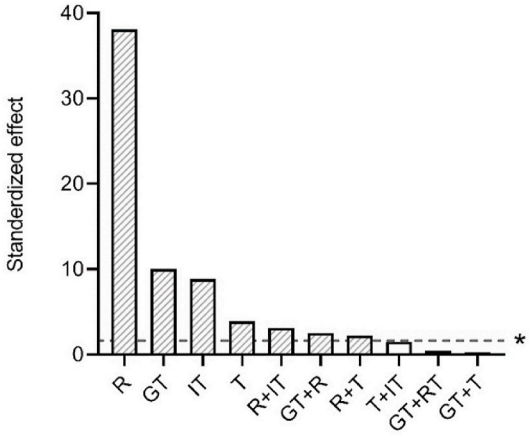

Fig. 1. Pareto chart on the degree of influence of four parameters (grinding times, ratio, temperature, and immersion times) in determining flocculation activity. * The horizontal line represents the threshold of statistical significance. R: Ratio; GT: Grinding time; IT: Immersion time; T: Temperature.

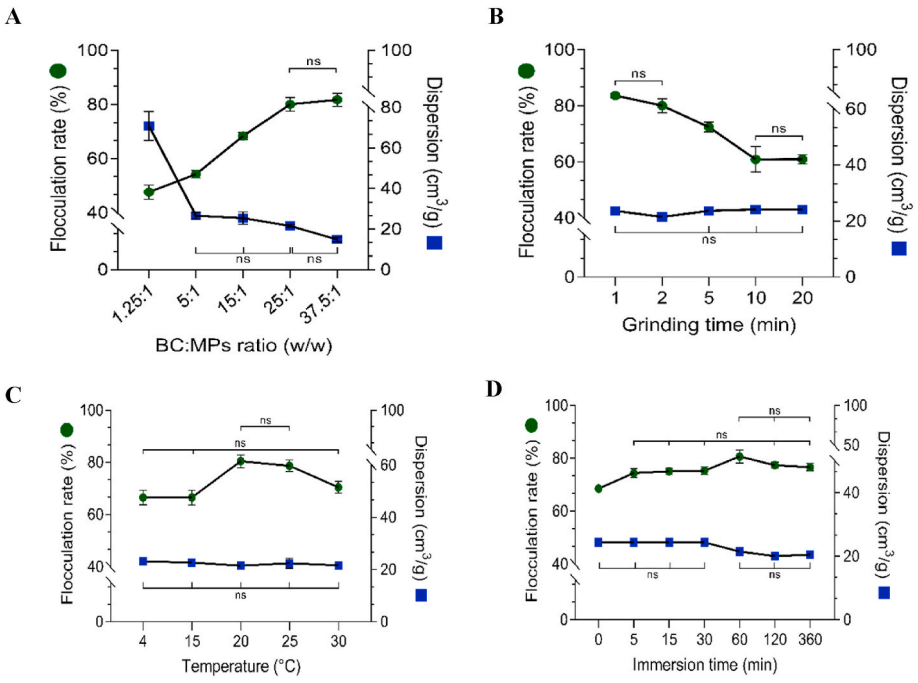

Fig. 2. Effect of different conditions on the flocculation and dispersion activities of the BC hydrogel regarding MPs. (**A**) BC hydrogel:MPs ratio; (**B**) grinding time; (**C**) immersion time; (**D**) temperature. *ns*: non significantly different ($p \geq 0.05$).

potentiating particle adsorption, providing MPs a longer frame to access the biopolymer's active sites (Abdel Maksoud et al., 2020b) and reach an equilibrium (Sargin et al., 2019) (Sargin et al., 2019).

Lastly, the temperature has been documented as a modulating factor during the flocculation process (Abdel Maksoud et al., 2020b; Vajihinejad et al., 2019). Fig. 2D shows the relationship between temperature and flocculation rate at optimal conditions (25:1 BC:MPs ratio, 2 min of grinding and 60 min of immersion time). The BC hydrogel showed a lower flocculation rate at lower temperatures (4 and 15 °C). Room temperatures (20–25 °C) facilitate the flocculation process,

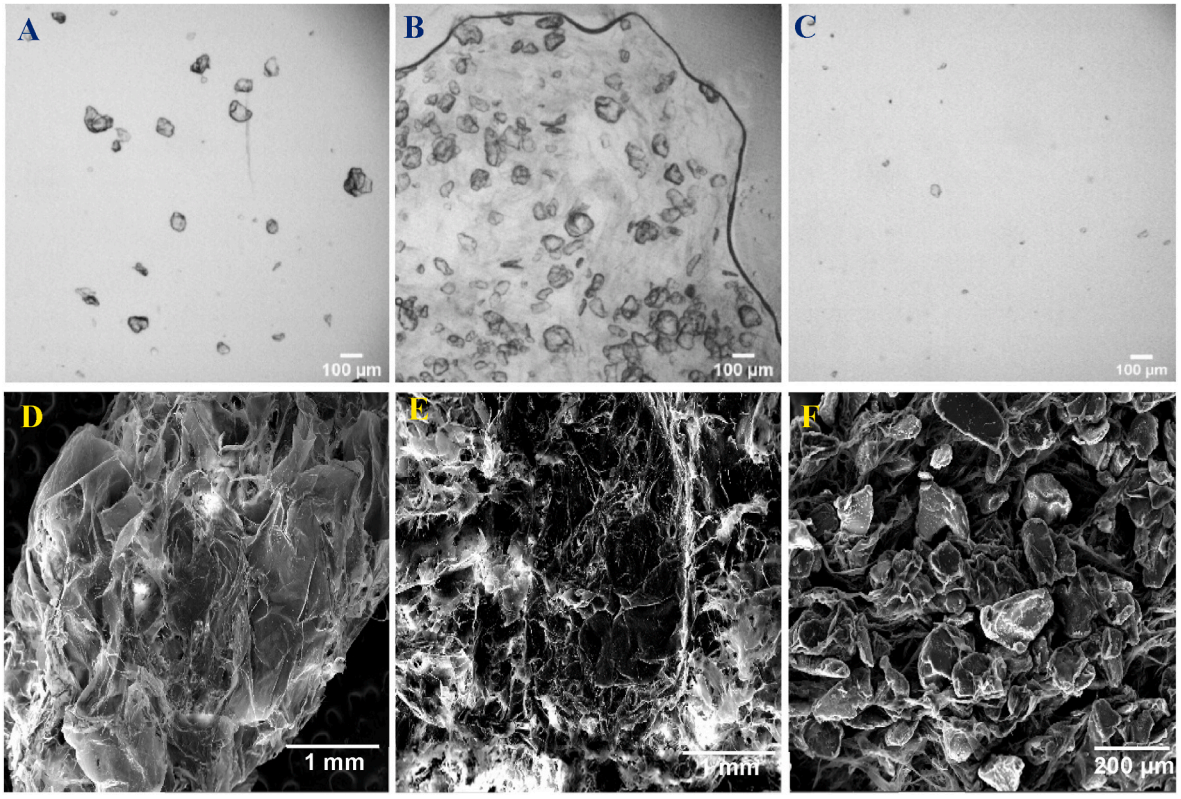

Fig. 3. Fluorescence micrographs: (**A**) MPs solution before flocculation; (**B**) BC/MPs hetero-aggregate; (**C**) MPs remaining after flocculation. Scanning electron micrographs: (**D**) BC hydrogel after 1 min of grinding; (**E**) BC hydrogel after 20 min of grinding; (**F**) MPs adsorbed and embedded in the BC hydrogel.

highlighting the lack of necessity for specific operating conditions using BC hydrogels. The relation between lower flocculation activity and lower temperature has been hypothesised to be driven by the attenuation in the binding force between the bioflocculant and the particles (Abdel Maksoud et al., 2020b). Considering that the dispersion is not inversely affected here, temperature might play a role in particle binding but not particle release.

3.3. Microscopy: BC hydrogel and MPs hetero-aggregation

Fluorescence and scanning electron microscopy were employed to visualise the retention of MPs in the BC hydrogel network. The micrographs unveiled that the MP particles are retained in the cellulosic network of the bioflocculant (Fig. 3B), with a substantial reduction in the number of particles outside the hydrogel (Fig. 3A, C).

Scanning electron microscopy confirmed the expected three-dimensional (3D) fibrillar network and porous gel-like microstructure of the BC hydrogel. Considerable structural differences between the BC hydrogel ground for 1 min (Figs. 3D) and 20 min (Fig. 3E) are observed. Moreover, both the adsorption and incorporation of MPs in the hydrogel are displayed in Fig. 3F. The porous nature of the hydrogel is likely the primary factor driving the flocculation and aggregation of MPs. As a consequence of excessive grinding, the breakage in the 3D structural integrity and consequent pore collapse likely causes the decreased flocculation rates observed in Fig. 2B.

3.4. Experimental meets computational flocculation

In order to understand how flocculation would vary in a vast array of experimental conditions, a contour plot was computed. Based on the experimental design parameters expressed in Supplementary Table S2, contour plots of the variables under study were generated to display the region of optimal factor setting for both flocculation (Fig. 4A) and dispersion (Fig. 4B). With the model assuming a hold temperature of 22.5 °C and an immersion time of 60 min, it is clear that decreasing grinding times while increasing the ratio of BC:MPs are expected to yield higher flocculation rates (Fig. 4A). On the other hand, assuming a grinding time of 5.5 min and a temperature of 22.5 °C is expected to yield higher dispersion at lower ratios of BC:MPs independently of the immersion time.

To experimentally confirm the reliability and predictability of the computed model, several previously untested values were trialled. As shown in Fig. 4A, a 30:1 BC:MPs ratio with a grinding time of 3 min (a*) yielded the expected >80% flocculation rate (87.2%). Further, a 20:1 BC:MPs ratio with a grinding time of 5 min (b*) yielded the expected 64–72% flocculation rate (71.48%). A 1.25:1 BC:MPs ratio with a grinding time of 9 min (c*) yielded a flocculation rate of 52.57%. Despite this latter not matching the exact predicted region of values, the experimental value still falls in the lower flocculation rate region (blue). This highlights that the model can reliably predict and distinguish high from low-performance MPs bioflocculant conditions for the BC hydrogel, widely facilitating potential industrial optimisations. The same line of thought was applied for the computation of dispersion (Fig. 4B). A 30:1 BC:MPs ratio with an immersion time of 20 min (a*) yielded a dispersion of 8.06 cm^3/g. The same was observed for the 20:1 BC:MPs ratio with an immersion time of 60 min (b*), yielding the expected 15–20 cm^3/g dispersion (17.62 cm^3/g). Predicted dispersive values of 3.75:1 BC:MPs ratio and an immersion time of 40 min (c*) yielded a dispersion of 158.12 cm^3/g, highlighting not how exactly predictive the model is but how it can separate optimal from poor bioflocculant performance. Both predicted and confirmed experimental values corroborate the data presented in Fig. 1, showing that the ratio and grinding times are the most important variables modulating the flocculation rate.

Fig. 5 shows a simplified factorial plot exhibiting the flocculation and dispersion in response to each variable. Individual plots exhibit the value in which the flocculation/dispersion is highest and lowest. The optimised paraments are presented as a grinding time of 1 min, a BC:MPs ratio of 31.65:1 (BC hydrogel:MPs ratio), temperature of 22.9 °C and an immersion time of 76 min. The optimal values were used experimentally and confirmed to yield the highest flocculation rate (88.59%) and the lowest dispersion (17.62 cm^3/g). This confirms that lower grinding times, higher BC:MPs ratios, room temperatures, and increased immersion times are associated with increased flocculation rates and decreased dispersion.

The regression equations defining the experimental correlations are displayed in **Eq. (5)** and **Eq. (6)**, where *GT* is the grinding time, *R* is the BC:MPs ratio, *T* is the temperature, and *IT* is the immersion time. These equations can be applied to predict the response of flocculation or dispersion when adjusting the levels of each variable.

$$\text{Flocculation rate}\ (\%) = -86.2 - 3.12GT + 0.118R + 11.30T + 0.275IT + 0.0566GT^2 - 0.000093R^2 - 0.2478T^2 - 0.001814IT^2 \quad (5)$$

$$\text{Dispersion}\ \left(cm^3/g\right) = 81.0 + 0.21GT - 0.1224R - 1.67T - 0.127IT + 0.027GT^2 + 0.000094R^2 + 0.0376T^2 + 0.00063IT^2 \quad (6)$$

The applicability of the models was evaluated using residuals, lack of fit, and ANOVA analysis. The residual *vs.* predicted values shows a uniform distribution, and the lack of outliers suggests clear indicators of normality (Figs. S6A and S6C). None of the models had a significant lack-of-fit (*p*-value >0.05), and each model had a coefficient of determination over 0.96 (Figs. S6B and S6D), indicating the suitability of the models. The values of the adjusted R^2 and the predicted R^2 did not differ significantly (<0.2). Additionally, since the coefficient of variations was low, the values are considered to be good indicators of the reproducibility of the responses studied. The *p*-value less than 0.05 and the *F*-value of 152.5 for the flocculation rate and 112.04 for the dispersion,

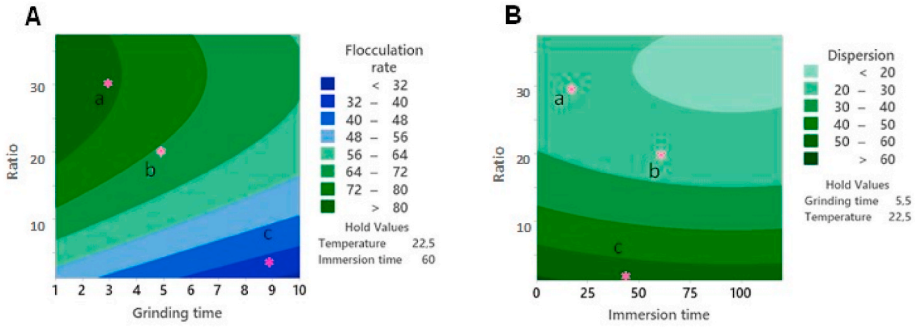

Fig. 4. Contour plots: (**A**) flocculation rate as a function of the ratio and grinding times, with constant temperature and immersion times; (**B**) dispersion as a function of the BC hydrogel:MPs ratio (w.w/d.w.) and immersion times. *experimentally-confirmed values.

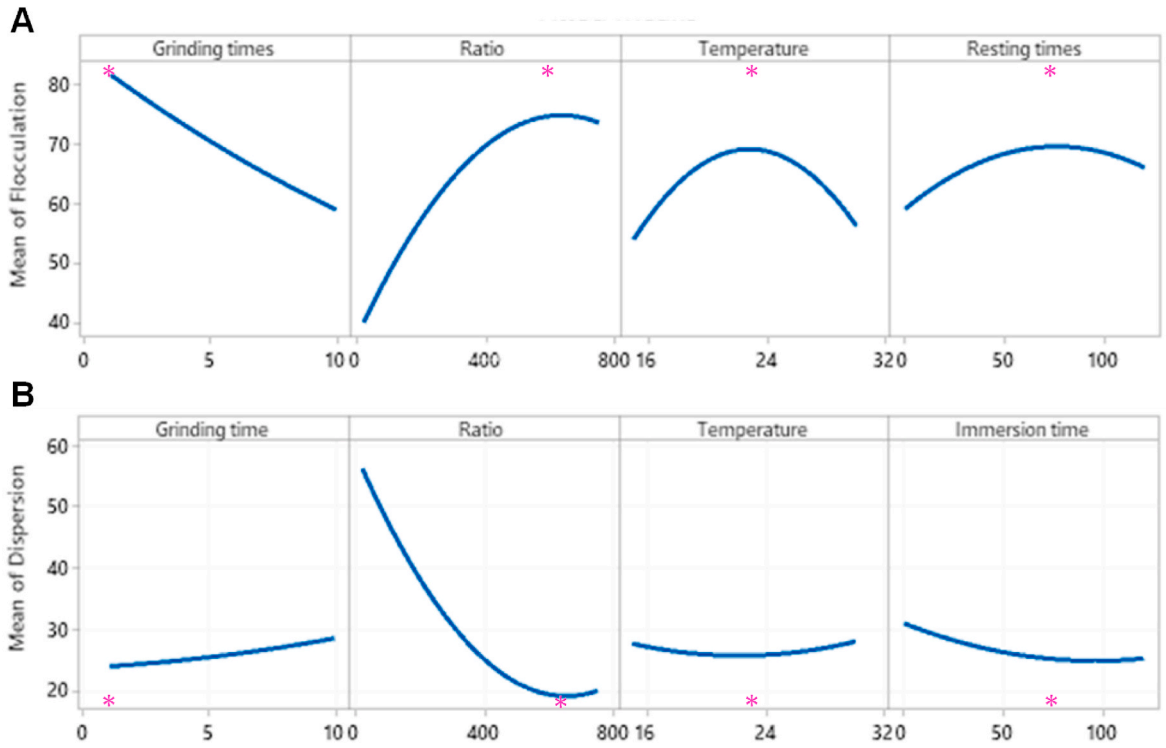

Fig. 5. Factorial plot of (**A**) flocculation rate and (**B**) dispersion, in function of grinding times, BC:MPs ratio, temperature, and immersion times. *experimentally-confirmed values.

also led to the conclusion that the model is applicable.

Taking into account the knowledge of the optimal ratio (31.65:1) and considering a MPs-contaminated water with 2 g/L, the BC hydrogel was able to hetero-aggregate roughly 1.77 g/L of polystyrene MPs, which is far beyond any urban concentration reported to date. Also, the optimal amount of BC to use for different levels of MPs contamination can be determined. For example, for a MPs-contaminated water with 50 mg/L, a mass of 2.06 g of BC hydrogel or 18.2 mg of dry BC remnants per litre

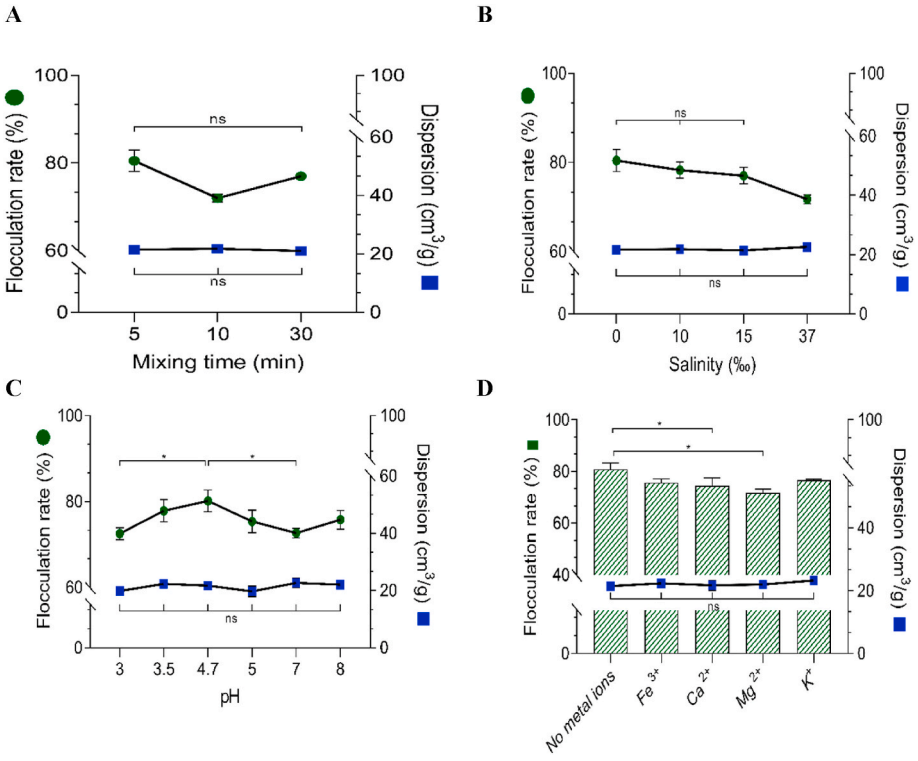

Fig. 6. Effect of different conditions on the flocculation rate of the BC hydrogel towards MPs-contaminated water and dispersion after flocculation. (**A**) Mixing time; (**B**) pH; (**C**) salinity; (**D**) metal ions presence (4.5 mMol). * significantly different ($p < 0.05$). *ns*: non significantly different ($p \geq 0.05$).

should be used (Fig. S7).

3.5. Mixing time, pH, salinity, and cations

Beyond the two parameters linked to the bacterial cellulose (BC) hydrogel: BC:MPs ratio and grinding time and the two parameters associated with operational conditions: temperature and immersion time, variables such as mixing time, pH, salinity, and the presence of metal cations would be anticipated to considerably influence the flocculating activity of the biopolymer.

Mixing during the flocculation process is expected to facilitate particle adsorption to the biopolymer, leading to the formation of hetero-aggregates formed by BC and microplastics (BC:MPs) (Aljuboori et al., 2015; Al-Shamrani et al., 2002). Fig. 6A demonstrates that a shorter mixing time of 5 min displays a higher flocculation rate (80.42 ± 2.48%) compared to longer mixing times of 10 or 30 min. These findings denounce that short exposure times are enough to drive strong particle aggregation, highlighting that MPs absorb and hetero-aggregate in the BC hydrogel fairly quickly. Importantly, increased mixing times can lead to the breakage of BC/MPs hetero-aggregates and consequent decrease in flocculation rates (Gregory, 1991). However, dispersion does not change as mixing times increase, dismissing the possibility of meaningful polymer destruction and strengthening the hypothesis that particle aggregation happens rapidly. The fact that a mixing time of 5 and 30 min yielded statistical similar flocculation rates suggest that over a long enough period of time, BC/MPs hetero-aggregates are able to re-arrange and re-form.

The pH is another important factor that is known to affect the activity of bioflocculants (Nwodo and Okoh, 2013) by influencing the stability of the microparticles and the hetero-aggregate formation (Ugbenyen and Okoh, 2014). Thus, a wide variation of pH ranging from 3 to 8 was tested to understand its influence on flocculation and dispersion (Fig. 6B).

Results show that the highest flocculating activity was achieved at a pH of 4.7. Despite both lower and higher pH ranges showing slightly decreased flocculation but not dispersion, these findings show that the bioflocculant would be suitable for prospective application in neutral, acid, and alkaline wastewaters. The differences observed here are likely due to a pH-dependent shift in zeta potentials (Qiu et al., 2020) and bridging/charge neutralisation mechanisms likely to influence particle aggregation (Yu and Somasundaran, 1996).

Ionic strength, or salinity, is known to influence biopolymers' ability to flocculate target particles (Abdel Maksoud et al., 2020b). Results in Fig. 6C show that only in high salinity is the flocculation rate of the BC hydrogel affected. Despite constituting a slight decrease, this effect may be due to electrolytic interference with the active sites of the BC, as they may obstruct the adsorption of the MPs (Abdel Maksoud et al., 2020b). The dispersion was not affected by increased salinity conditions, highlighting that the BC hydrogel flocculates MPs stably under severely distinct ionic strength environments.

Lastly, metal ions are frequently added to enhance flocculation activity (Elkady et al., 2011; Gong et al., 2008; Ugbenyen et al., 2012) and are likely to be ubiquitous during wastewater treatment. In general terms, cations are known to promote flocculation by neutralising/-stabilising negative charges of suspended particles and bridging them together, thus increasing particle adsorption onto the bioflocculant (Wu and Ye, 2007; Yim et al., 2007). Therefore, metal cations (Fe^{3+}, Ca^{2+}, Mg^{2+}, and K^{+}) were added to understand their influence on flocculation activity. The flocculation rate of the BC hydrogel remained statistically unchanged, except for when exposed to Mg^{2+} (Fig. 6D). Despite a small decrease observed there and the fact that the dispersions were unchanged, highlights that the bioflocculant capacity of the BC hydrogel is vastly resistant to chemical changes in operational parameters. Moreover, given that Fe^{3+} is one of the most widespread toxic components in wastewater effluents (Chai et al., 2021) and that different cation concentrations can also influence flocculation rates (Lee et al., 2012), different concentrations were tested (Fig. S8). Despite high Fe^{3+} concentrations, the flocculation rate of the BC hydrogel was barely affected. The dispersion was also not affected, further strengthening the potential for this biopolymer to perform even under harsh operational conditions.

3.6. Retention capacity

Importantly for understanding the BC/MPs hetero-aggregates durability, the ability of the BC hydrogel to irreversibly retain the MPs was evaluated. After flocculation, the BC/MPs hetero-aggregates were subjected to a wash cycle of 24 h to evaluate if the hydrogel releases previously retained MPs. With a 25:1 (w.w./d.w.) BC:MPs ratio, only 13.01 ± 0.94% of the MPs were removed after a wash cycle of 24 h (Fig. 7). The BC hydrogel's capacity to retain aggregated MPs highlights its structural fortitude that pairs strong particle aggregation with high particle retention. Aside from its microporous nature, electrostatic interactions might help to explain the strength of particle aggregation observed here (Cunha et al., 2020b; Salehizadeh and Shojaosadati, 2001).

Increasing the used ratio of BC:MPs to 50:1 (similar to less polluted wastewater) significantly decreased the release of MPs to 1.54 ± 0.03%. Notably, the concentration used here (2 g/L) far exceeds any naturally occurring abundance of MPs, highlighting that the BC hydrogel would never act as the limiting factor to proper bioremediation in real wastewater treatment scenarios.

3.7. MPs removal with commercial bioflocculants

To benchmark the performance of the BC hydrogel, its flocculation capacity was compared to two most common commercially available flocculants: *(i)* xanthan gum, a natural exopolysaccharide produced by *Xanthomonas campestris* with several industrial applications (García-Ochoa et al., 2000; Lee and Song, 2015); *(ii)* alginate, a natural organic polymer obtained from brown algae (*Phaeophyceae*) that is also widely used industrially (Augst et al., 2006; Lee and Mooney, 2012). Both xanthan gum and alginate are unsatisfactory flocculants (−95.18 ± 0.10% and −87.75 ± 4.55%, respectively; Fig. S9), compared to the BC hydrogel. Not only do these commercial bioflocculants not flocculate the microplastic particles but end up effectively dispersing them. Given that xanthan gum is a negatively charged polyelectrolyte (Mrokowska and Krztoń-Maziopa, 2019), the presence of negatively charged polystyrene MPs (Cunha et al., 2020b) might result in electrostatic repulsion. The same is expected with alginate (Klemm et al., 2005), which again promotes MPs' dispersion, not flocculation. In the case of BC, it is a positively charged biopolymer (Cunha et al., 2020a), which explains the higher flocculation rate.

3.8. Call for research

At this point, it is expected that exponentially available data will

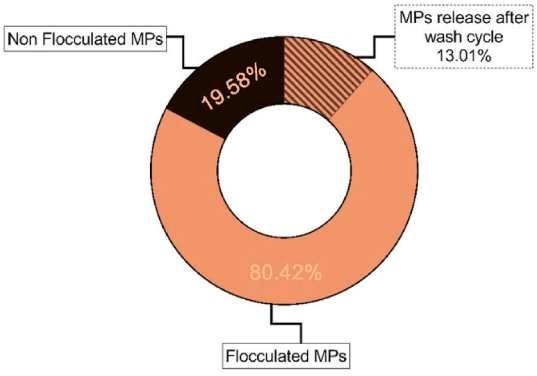

Fig. 7. Retention capacity MPs by the BC hydrogel after 24 h wash cycle.

reinforce the apprehension and clear away any remaining skepticism and indifference regarding MPs pollution. Environmental contamination by MPs is arguably the most substantial ramification of the whole plastic pollution issue. The urban outgrowth and by-products are extremely worrisome. As the demand for biosustainable alternatives grows across every economic sector, a bacterial-based alternative for urban water remediation is presented here. This follows reports from our laboratory of similarly effective microalgal-based extracellular polymeric substances in aggregating and removing MPs (Cunha et al., 2020a, 2020b). Also, a recent work from our laboratory (Faria et al., 2022) showed the potential of BC membranes as biosustainable and ecologically inert alternatives to conventional hazardous fossil-based polymeric membranes used in wastewater treatment plants (WWTPs) for the removal of MPs. Given how particle concentration might affect flocculation capacity, this study follows our previous data demonstrating that these biopolymers perform just as well in the presence of low concentrations of MPs (Cunha et al., 2020a, 2020b; Faria et al., 2022). Thus, our focus was to understand how these biopolymers and hydrogels perform longitudinally even in the upmost extreme pollutive conditions. It is now critical to understand how to optimise culture conditions at an industrial scale to produce and create BC membranes at cost-efficiency. Also, it is still technically unfeasible to quantitatively analyse the removal of emerging micropollutants such as MPs from real wastewater samples. Accordingly, future studies that focus on modulating and re-creating quantitative (-able) complex matrixes that closely models real WWTPs water is currently in development. From a financial point of view, it is important to calculate the CAPEX (capital expenditure) required to achieve economic breakeven and potential margin expansions, compared to the current input costs of inorganic and synthetic polymeric membranes. Still, it is critical to realise that the economic outlook is a vital constant of the equation but cannot outweigh the main concern: to develop a biodegradable, biosustainable and truly circular alternative to current applications. The fact that BC membranes and its scraps/remnants/remainings can be easily manipulated further strengthens its application narrative. Taking everything into consideration, the pollution caused by MPs is not just another issue. It is one of the greatest generational issues of our time and should be addressed as such.

4. Conclusion

This pilot research aimed to assess how several operational parameters would affect the flocculation performance of microplastics (MPs) using a bacterial cellulose (BC) hydrogel assembled with BC remnants. Also, the main priority remains to develop sustainable, circular, and ecologically inert solutions to bioremediation processes in wastewater treatment plants. Results show that the BC hydrogel can achieve a MPs flocculation rate of 88.59% under optimal conditions of 31.65:1 BC hydrogel:MPs ratio (or 3.2:10 BC dry weight:MPs ratio), grinding time of 1 min, immersion time of 76 min and temperature of 22.9 °C. It was confirmed that the BC:MPs ratio and the BC grinding time equate the most crucial variables potentiating the flocculation process. Further, the microporous cellulosic network of the BC hydrogel, revealed by scanning electron microscopy, and its acid character and positively charged nature are the likely main contributors to the strong hetero-aggregation of MPs observed using fluorescence microscopy.

The computational and experimental confirmation of the flocculation model provides largely facilitating conditions for potential industrial optimisations. Interestingly, the BC hydrogel was shown to be vastly resistant to a wide range of chemical variations in operational parameters, such as ionic strength and metal cations concentration. Short exposure times were shown to be sufficient in driving a strong aggregation of MPs, highlighting the speed at which the hetero-aggregation process occurs. Lastly, the BC hydrogel was shown to be substantially more effective in flocculating MPs, unlike commercial alternatives such as xanthan gum and alginate, which dispersed the micropolymeric particles instead. The data presented here is compelling in showing that a BC hydrogel forged with unused scraps from BC membranes carries immense promise as a truly sustainable and circular solution to bioremediation processes.

Author contributions statement

IM, MF and NC contributed to the conception and design of the study. IM, JS and CC executed the experiment. IM, CC and NC analysed, interpreted the data and wrote the manuscript. NC and AF made possible the execution of the experiment through providing administrative and financial support, supervising the experiment, and making the critical revisions regarding important intellectual content of the manuscript. All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests. Nereida Cordeiro reports financial support was provided by Foundation for Science and Technology. Nereida Cordeiro reports financial support was provided by European Territorial Cooperation Programme PCT-MAC 2014–2020. Marisa Faria reports financial support was provided by Foundation for Science and Technology. Ivana Mendonca reports financial support was provided by Fundação Amadeu Dias.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2022.137719.

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