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Efficacy of bacterial cellulose hydrogel in microfiber removal from contaminated waters: A sustainable approach to wastewater treatment

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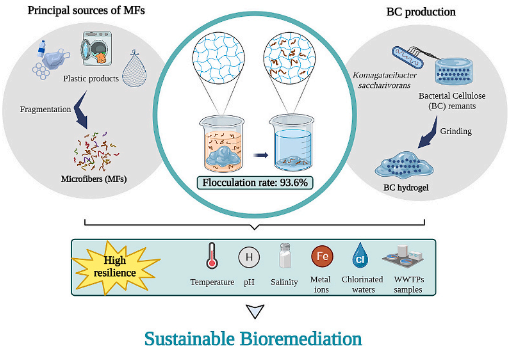
HIGHLIGHTS

- Hydrogel-form bacterial cellulose (BCH) shows up to 93.6 % MFs removal efficiency.
- Flocculation is especially influenced by BCH:MFs ratio and mixing intensity.
- BCH demonstrates resilience across diverse environmental conditions.
- The 3D porous structure of BCH is crucial in effective MFs retention.

GRAPHICAL ABSTRACT



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ABSTRACT

Microfibers (MFs), the dominant form of microplastics in ecosystems, pose a significant environmental risk due to the inadequacy of existing wastewater treatments to remove them. Recognising the need to develop sustainable solutions to tackle this environmental challenge, this research aimed to find an eco-friendly solution to the pervasive problem of MFs contaminating water bodies. Unused remnants of bacterial cellulose (BC) were ground to form a hydrogel-form of bacterial cellulose (BCH) and used as a potential bioflocculant for polyacrylonitrile MFs. The flocculation efficiency was evaluated across various operational and environmental factors, employing response surface methodology computational modelling to elucidate and model their impact on the process. The results revealed that the BCH:MFs ratio and mixing intensity were key factors in flocculation efficiency, with BCH resilient across a range of environmental conditions, achieving a 93.6 % average removal rate. The BCH's strong retention of MFs released only 8.3 % of the MFs, after a 24-hour wash, and the flocculation tests in contaminated wastewater and chlorinated water yielded 89.3 % and 86.1 % efficiency, respectively. Therefore, BCH presents a viable, sustainable, and effective approach for removing MFs from MFs-contaminated water, exhibiting exceptional flocculation performance and adaptability. This pioneer study using BCH as a

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bioflocculant for MFs removal sets a new standard in sustainable wastewater treatment, catalysing research on fibrous pollutant mitigation for environmental protection.

1. Introduction

The burst of the Industrial Revolution brought plastics into our daily lives but also generated persistent particulate waste that affects aquatic ecosystems and oceans, impacting wildlife and human health (Lebreton et al., 2017).

Microscopic plastic particles (MPs) can take various shapes and forms, such as spheres, fragments, and fibres (Mendonça et al., 2023). Among the different forms of MPs found in the environment, studies have found that the fibrous and filamentous forms (microfibres-MFs) are predominant in marine and freshwater ecosystems (Gago et al., 2018).

Recent investigations have detected the presence of MFs as the dominant MPs in the digestive system of fish, with an average of 1.67 particles per fish (Bessa et al., 2018). Moreover, Keisling et al. (2020) reported the detection of plastic particles in seafood, with an average of 0.72 microparticles per individual. It is estimated that seafood consumption in Europe alone could introduce up to 1.1×10^4 of these polymeric particles into consumers' bodies (Van Cauwenberghe and Janssen, 2014).

Although these fibrous MPs are the most dominant type of pollutant, research is still in its early stages. The bibliometric assessment (Fig. S1) shows that research primarily focuses on contamination, identification, transport, and impacts of MFs in the ecosystems. More recently, attention has turned to exploring the effect of MFs along the food chain and their potential implications for human health (Li et al., 2023). However, there is a notable absence of studies focused on treatment technologies for MFs removal, whose physicochemical and mechanical characteristics differ from those of MPs fragments.

MFs pollution mainly originates from the release of fibres while using and laundering textiles. These can include polyester, nylon, and cotton (Henry et al., 2019). De Falco et al. (2018) stated that 600,000 fibres can be released in a typical 5 kg wash load of polyester fabrics. Based on samples collected globally from shoreline sediments Browne et al. (2011) showed that polyester microfibres (56 %) were the most predominant, followed by acrylic microfibres (23 %).

The specific characteristics of MFs, such as their elongated and fibrous shape and a higher surface area to volume ratio, not only facilitate their widespread distribution in aquatic ecosystems but also make them difficult to eliminate through standard wastewater treatment processes (McIlwraith et al., 2019). Conventional approaches in wastewater treatment plants (WWTPs), such as membrane bioreactors, crossflow filtration, density separation, and centrifugation (Weis and De Falco, 2022), often fall short in removing MFs, are associated with substantial costs, and can generate toxic byproducts that exacerbate environmental pollution. It is estimated that WWTPs annually discharge about 227 million tonnes of MPs into the ocean (Mishra et al., 2020). Sutton et al. (2016) found a substantial presence of MFs in the effluents of nine WWTPs, where MFs accounted for up to 48 % of the particles in the effluents, regardless of whether the WWTPs used advanced or high-efficiency water treatment processes. In urban areas, the direct connection of domestic sewer channels to rivers leads to the entry of approximately 3 million tonnes of MFs into the ocean through adjacent rivers (Mishra et al., 2019).

Based on this, there is an urgent demand for innovative and eco-friendly methods to mitigate MFs from aquatic ecosystems sustainably. Products like Cora Balls, guppy filter bags (GuppyFriend Bag), and lint filters have already been developed and urbanised to deal with the pollutant release from household washing machine effluents. However, none of these techniques effectively filter these at a large-scale (Mishra et al., 2020). Another promising way entails using microorganisms to degrade or aggregate synthetic plastic particles. This approach has shown promise with several bacterial and fungal species, including *Pseudomonas* sp., *Rhodococcus* sp., *Aspergillus* sp., *Fusarium* sp., and their metabolic enzymes, demonstrating the capacity to break down synthetic polymers, as highlighted by Pathak and Navneet (2017). However, further research and practical implementation are essential to transform these findings into effective large-scale solutions for mitigating MPs pollution.

The use of microbial extracellular polymeric substances as natural flocculants has gained special attention in recent years for the substitution of synthetic flocculants in industrial (Aljuboori et al., 2015). Bacterial cellulose (BC), produced by specific strains of bacteria like *Komagataeibacter* and *Aerobacter*, have emerged as an effective material for efficient MPs removal (Faria et al., 2022). BC is synthesised through bacteria fermentation of carbon sources, forming a biopolymer composed of linear chains of glucose units linked by β-1,4-glycosidic bonds (Shoda and Sugano, 2005). Its unique physical and chemical properties, including high porosity, surface area, porosity, water-holding capacity, excellent biodegradability, and biological affinity (non-toxic), make it an attractive material for various applications (Islam et al., 2021). This natural biopolymer forms a complex 3D network of nanofibres with an inherent ability to trap and retain particles, including MPs (Mendonça et al., 2023).

Based on the urgent need for sustainable and highly efficient alternatives for removing MFs, this study assesses BCH derived from unused BC residues as a bioflocculant for remove MFs from MFs-contaminated waters. To this end, a systematic approach was adopted, using the Response Surface Methodology (RSM) to predict and optimize operational factors such as BCH:MFs ratio, duration of mechanical mixing, flocculation time, and mixing intensity. Chemical variations, including temperature, pH, salinity, and the presence and concentration of metal ions, were also investigated. To gain deeper insights into the retention strength and develop effective strategies for managing the formed BCH-MFs heteroaggregates, a 24-hour washing cycle was conducted. Laboratory-scale amplification experiments were carried out to ensure data reproducibility on a larger scale. Expanding the scope of the study, the same experimental protocol was applied to chlorinated waters and WWTP effluents, anticipating the potential application of BCH in the treatment of WWTP effluents and chlorinated recreational waters.

By developing technologies tailored to the specific challenges of MFs, this study has set a new standard for sustainable wastewater treatment, addressing one of the most pressing environmental challenges of the current era. Furthermore, this work aims to draw attention to the overlooked issue of fibrous pollutant mitigation, the need for specialization and also inspires new research into innovative solutions for MFs pollution.

2. Materials and methods

2.1. BCH production

BC biopolymers were produced following the procedure described in detail in Faria et al. (2022). Briefly, *Komagataeibacter saccharivorans* (Supplementary material ES1), a strain isolated from wine vinegar sourced from a local supermarket, was used to produce BC under static conditions, using Hestrin and Schramm (HS) liquid medium [2 % (w/v) glucose (G7021/Sigma-Aldrich), 0.5 % (w/v) peptone (84616.0500/VWR Chemicals), 0.5 % (w/v) yeast extract (84601.0500/VWR Chemicals), 0.27 % (w/v) disodium hydrogen phosphate (02494C/VWR Chemicals), 0.115 % (w/v) citric acid (0529-500G/VWR Chemicals), 1 % (w/v) ethanol, 0.4 % (w/v) acetic acid, pH 3.25] at 30 °C for 7 days, starting with an initial OD600 of 0.4 (UV-6300 PC Double Beam

Spectrophotometer, VWR) (Supplementary material ES1; Faria et al., 2022). The quantification of BC yield was conducted daily by measuring membrane weight (Supplementary material Fig. S2). The medium and materials were sterilised at 121 °C for 15 min. The biofilm was isolated and treated with 0.5 M NaOH to remove any adsorbed cells and retain culture medium components. Afterwards, the membranes were washed with distilled water a neutral pH was achieved.

The hydrogel production involved transforming BC remnants into a usable hydrogel material (Fig. 1). These remnants, essentially residual or leftover fragments of BC, were subjected to a grinding procedure for 20 s (KHB3146, Kunft), resulting in a hydrogel. Following the grinding process, the hydrogel was sieved to isolate the fraction within 850 to 5000 μm. A moisture balance (Gibertini, Eurotherm) was employed to determine the BCH's dry weight (d.w.) by heating it to 106 °C for 120 min. It was found that BCH contains 95.9 ± 0.02 % water, and subsequent data will be expressed in terms of wet weight (w.w.). The characterisation of the produced BC is described in detail in Supplementary Material (ES2–4 and Fig. S3-S5) and Faria et al. (2024).

2.2. Microfibres

Commercial polyacrylonitrile wool (UV-Granulate, Magic Pyramid Bruecher & Partner KG) was ground (IKA Mills- MF 10.1 basic) into polyacrylonitrile MFs (hereinafter referred to as MFs). The MFs were sieved (Analysensieb–Retsch) to obtain the fraction 20–50 μm, the median fibre diameter fraction found in the ocean and the more difficult fraction to remove from WWTPs (Suaria et al., 2020). An aqueous solution of MFs was prepared using distilled water at a concentration of 1 g/L for use in the subsequent experiments. To make the study independent of the concentration of MFs used, it was conducted based on the BCH:MFs ratio, making it applicable to any concentration of MFs in polluted waters. The MFs were characterised by zeta potential, infrared spectroscopy, fluorescence microscopy, scanning electron microscopy and thermogravimetric analysis.

2.2.1. Infrared spectroscopy (FTIR)

ATR-FTIR (Attenuated total reflection-Fourier transform infrared spectroscopy) was employed to obtain the infrared spectra of the polyacrylonitrile MFs (previously dried). The ATR-FTIR spectra was recorded by a Perkin Elmer FTIR System Spectrum Two with a resolution of 4 cm^{-1} and an accumulation of 32 scans. The obtained spectrum was compared with the standard spectrum of polyacrylonitrile.

2.2.2. Zeta potential measurements

The zeta potential of MFs and BCH was measured in Milli-Q Water at 25 °C (pH 7) using a Zetasizer Nano ZSP (Malvern). The measurements were carried out in triplicate and averaged for three consecutive analyses of the same samples, with 100 runs each.

2.2.3. Thermogravimetric analyses (TGA)

A SETSYS Setaram TGA analyser with a platinum cell was used to assess the thermal behaviour of BCH, MPs, and the BCH-MFs hetero-aggregates. From room temperature to 800 °C, at a constant rate of 10 °C/min, the samples were heated and subjected to a 20 mL/min oxygen flow.

2.3. MFs removal efficiency with BCH

2.3.1. Removal process

The efficiency of the BCH as a bioflocculant was evaluated, under different operational scenarios and physicochemical variations, by treating MFs-contaminated waters (at a concentration of 1 g/L). Multiple tests were conducted, each in triplicate, to assess various operational parameters like BCH:MFs ratio (w.w./d.w.), mixing intensity (rpm - rotations per minute), mechanical mixing duration (min), and flocculation time (min); and physicochemical variations such as, temperature (°C), pH, salinity (‰), and the type and concentration of metal cations (4.5 mMol for type; 4.5, 9, and 18 mMol for concentration) were studied.

In 10 mL of MFs-contaminated water, a predetermined (see Section 2.3.2) quantity of BCH was added. The mixture was stirred (Agimatic-E JP Selecta) to increase the contact between MFs and BCH (named mechanical mixing) and allowed to settle (named flocculation time) to facilitate the formation of hetero-aggregates and examine their sedimentation/dispersibility. Following flocculation, hetero-aggregates were filtered using a sieve with an 850 μm pore size (Analysensieb–Retsch, Scansci). The free MFs, which failed to form aggregates, were quantified by solution turbidity at 750 nm (UV-6300PC Double Beam Spectrophotometer; VWR). The hetero-aggregates were sampled for further characterisation. This procedure was applied uniformly across all experiments. A control experiment involving only MFs-contaminated water was similarly processed, and it was observed that 100 % of the free MFs pass through the sieve, establishing a reliable baseline for comparison and demonstrating the method's effectiveness.

The flocculation rate was calculated according to Eq. (1), where OD_C is the optical density (OD) of the MFs-contaminated water (solution of 1 g/L of MFs without BCH), and OD_S, the OD of the filtered solution (Ndikubwimana et al., 2016):

$$\text{Flocculation rate }(\%) = \frac{OD_{C-}OD_S}{OD_C} \times 100 \tag{1}$$

The dispersion of the BCH-MFs hetero-aggregates were measured - Eq. (2), where *m* is the mass of BCH (g), and *v* is the volume of the BCH-MFs hetero-aggregate formed after the flocculation time:

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

2.3.2. Response surface methodology (RSM)

RSM modelling was carried out using the Design Expert software version 13 (Stat-Ease, Inc.) to produce a three-level factorial design with

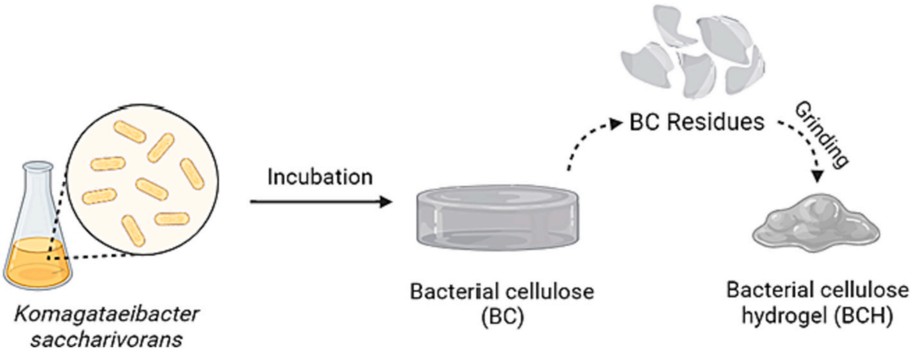

Fig. 1. Schematic representation of BCH obtention.

four variables. The Design Expert provided the equations that can be applied to determine the respective response(s) - flocculation rate and dispersion – in function of the variables - BCH:MFs ratio (5:1–100:1 w.w./d.w.); mixing intensity (60–300 rpm); mechanical mixing duration (5–120 min), and flocculation time (5–30 min). These generated models allowed to determine the conditions to achieve the highest flocculation rate (maximised flocculation). The residuals, lack-of-fit, and *p*–value were assessed using ANOVA analysis to evaluate the model's validity.

2.3.3. *Temperature, pH, salinity, and cations*

The influence of the pH (2, 4, 5.37, 6, and 8), salinity (0, 10,15, and 37 ‰), temperature (15, 20, 25 and 30 °C), as well as the effects of metal ions (4.5 mMol) such as Fe^{3+}, Ca^{2+}, Mg^{2+}, and K^{+}, was also considered. In addition, based on Faria et al. (2022), Fe^{3+} was chosen to evaluate the cation concentration effects (4.5, 9, and 18 mMol).

The different pH of the MFs-contaminated waters was adjusted (C5020, Consort) using HCl (0.1 M) and NaOH (0.5 M). The salinity concentrations were adjusted using NaCl (Handheld refractometer, OPTi Digital, VWR). All experiments were performed in triplicate, following these specific settings: a BCH:MFs ratio of 75:1 (w.w./d.w.), a mechanical mixing duration of 30 min, a mixing intensity of 60 rpm, and a flocculation time of 60 min. These conditions were previously determined as the maximised flocculation conditions in Section 2.3.2.

2.3.4. *Retention capacity*

The capacity of the BCH to retain MFs was evaluated using the BCH-MFs hetero-aggregates formed under the maximised flocculation conditions. In distilled water, the BCH-MFs hetero-aggregates were subjected to a 24 h washing cycle (in triplicate) with an agitation intensity of 300 rpm. The OD of the solutions was measured at 750 nm. The MFs retention rate was calculated according to Eq. (3), where the OD_S is the OD of the sample before the wash cycle, OD_{MF_S} is the OD of the MFs – contaminated water and OD_{SW} is the OD of the sample after the wash cycle, respectively:

$$\text{Retention rate } (\%) = \frac{\text{OD}_{SW}}{\text{OD}_{MF_s} - \text{OD}_S} \times 100 \qquad (3)$$

2.4. *Scale-up experiments*

The scale-up experiments were conducted with a volume of 500 mL, implementing a laboratory-scale upscaling by a factor of 50× to enhance the findings' reliability and consistency. This upscaling was executed while maintaining the maximised flocculation conditions previously established in Section 2.3.2: BCH:MFs ratio of 75:1 (w.w./d.w.), a mechanical mixing duration of 30 min, a mixing intensity of 60 rpm, and a flocculation time of 60 min. This approach ensured that the data collected at the larger scale aligned with our earlier findings and provided robust and reproducible results.

2.5. *WWTPs waters*

Real water samples were collected from a WWTP in Santana, Madeira (Portugal) for this investigation. The raw water's pH and conductivity were analyzed (C5020/Consort). The waters collected in the WWTP had a pH of 7.0 and a 399 mS/cm. To each litre of this water, 1 g of MFs was added, and the experiments were conducted in triplicate following the maximised flocculation conditions previously determined in 2.3.2.: a BCH:MFs ratio of 75:1 (w.w./d.w.), a mechanical mixing duration of 30 min, a mixing intensity of 60 rpm, and a flocculation time of 60 min.

2.6. *Chlorination*

To the water containing MFs, NaClO was added in a typical dose of chlorine for the treatment of recreational waters range of 10 mg/L (as Cl^{-}) (Li and Blatchley, 2008). The flocculation rate was evaluated at 0 and 24 h of contact with chlorinated water to investigate the possible effects of the Cl^{-} ions (Jin et al., 2023). The experiments were done in triplicate following the maximised flocculation conditions determined previously in 2.3.2.: a BCH:MFs ratio of 75:1 (w.w./d.w.), a mechanical mixing duration of 30 min, a mixing intensity of 60 rpm, and a flocculation time of 60 min.

2.7. *Fluorescence microscopy*

Fluorescence microscopy was utilised to investigate and document the retention of MFs in the BCH after flocculation. A Leica DM2700 device, attached to a Leica DFC450 C digital camera and a CoolLED pE-300 lite lighting system, was used.

2.8. *Scanning electron microscopy (SEM)*

SEM was employed to analyse the BCH and the hetero-aggregates. Before analysis, the 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 150×, 200× and 300×.

2.9. *Data and statistical analysis*

The results were presented as three replicates' mean values ± standard deviation (SD). Statistical analyses and data representation were performed using GraphPad Prim 9.0.0. The D'Agostino–Pearson omnibus and Kolmogorov–Smirnov normality tests were used to assess the Gaussian data distribution (statistical significance: p–value < 0.05). Parametric unpaired *t*-tests (or one–way ANOVA) were applied for normally distributed data. Statistical analysis was performed based on data from a minimum of independent experiments.

3. Results and discussion

MFs, the predominant form of MPs in marine and freshwater ecosystems, pose a significant environmental challenge. Their prevalence in aquatic organisms' digestive systems and subsequent entry into the human diet underscore the urgent need for mitigation strategies, especially considering the ineffectiveness of current wastewater treatments designed for MPs fragments in capturing these fine and fibrous pollutants.

In response to this challenge, this research evaluates the efficacy of BCH for removing MFs from MFs-contaminated waters, aiming to address the significant gap left by traditional treatment methods. By developing technologies specifically tailored to the unique characteristics of MFs, this study strives to establish a new benchmark for sustainable wastewater management, directly tackling one of the most pressing environmental issues of the present era. Furthermore, this study distinguishes itself by exploring the use of natural bioflocculants derived from renewable resources as a novel method for MF removal. In a field where such methodologies are scarce, this research takes a pioneering role, advancing the application of bio-based solutions to combat this urgent environmental problem.

3.1. *MFs characterisation*

Analysing the ATR-FTIR spectra of the MFs provided a straightforward method to confirm the structural composition of MFs as polyacrylonitrile fibres. This type of fibre has been applied across multiple industries, such as the textile, aerospace, and automotive sectors, in air and water systems and in construction materials to enhance mechanical properties (Kausar, 2019).

The ATR-FTIR spectrum (Fig. 2A) of the used MFs displayed characteristic absorption bands, providing valuable insights into their molecular structure. The prominent band at 2242 cm^{-1} corresponds to the C≡N stretching vibrations of the C—N group, evidencing the presence of nitrile groups in the polyacrylonitrile structure (Causin et al., 2005). This nitrile groups contribute to the overall hydrophobicity and chemical stability of the MFs. Additionally, the CH and CH_2 groups were observed by the C—H vibration bands at 2936 cm^{-1}, 1368 cm^{-1} and 1452 cm^{-1}. The band at 1736 cm^{-1} corresponds to the C═O stretching vibrations, along with the bands at 1233 cm^{-1} and 1068 cm^{-1} corresponding to C—O group stretching vibrations, indicating the presence of vinyl acetate monomeric units within the MFs (Aniagor et al., 2022). As mentioned by Karacan and Erdogan (2012), the polyacrylonitrile fibres are composed of approximately 90 % acrylonitrile and 10 % vinyl acetate monomeric units within its polymer structure, elucidating the observed characteristic bands of vinyl acetate groups in the employed MFs. These functional groups not only influence the overall chemical properties but also play a role in the interactions of the MFs with other substances. These characteristic groups collectively contribute to the observed negative surface charge of −13.9 ± 0.53 mV in the MFs.

Despite both the MFs and the bioflocculant BCH exhibiting negative surface charges (−30.3 ± 0.66 mV for BCH), their interaction in contaminated water environments can be facilitated through mechanisms beyond simple electrostatic repulsion. Moreover, the repulsive forces from the negatively charged surfaces might initially suggest limited interaction potential; however, the actual interaction dynamics in aqueous environments are influenced by several factors that can mitigate electrostatic repulsion.

In the context of bioflocculation, the presence of water and the specific physicochemical conditions can significantly alter interaction outcomes. For instance, the formation of H-bonds and hydrophobic interactions can play a crucial role in the aggregation process, despite the negative charges. Furthermore, in contaminated waters, the presence of ions and other charged species can act to shield the charges on the MFs and BCH, reducing the impact of electrostatic repulsion and enabling closer association between them.

Moreover, the unique surface characteristics of both the MFs and BCH can enhance their affinity for specific substances present in contaminated waters, potentially acting as bridging molecules to facilitate interaction. The adsorption of certain substances onto the surfaces of MFs and BCH can modify their effective surface charges, further promoting aggregation through mechanisms beyond electrostatic interactions.

Additionally, the partially preserved 3D network of BCH after grinding enhances its interaction with MFs. This network increases the area available for interactions and facilitates not only H-bonding and hydrophobic interactions but also may introduce structural entanglements with MFs, promoting aggregation. The structural integrity and unique properties of BCH's network can contribute significantly to the stability and efficiency of the bioflocculation process, demonstrating the importance of physical and structural attributes in facilitating these interactions despite inherent negative charges.

The effectiveness of these interactions in water treatment applications depends on the complex interplay of electrostatic and non-electrostatic forces, and can be influenced by the varied physicochemical compositions of contaminated waters. This underscores the

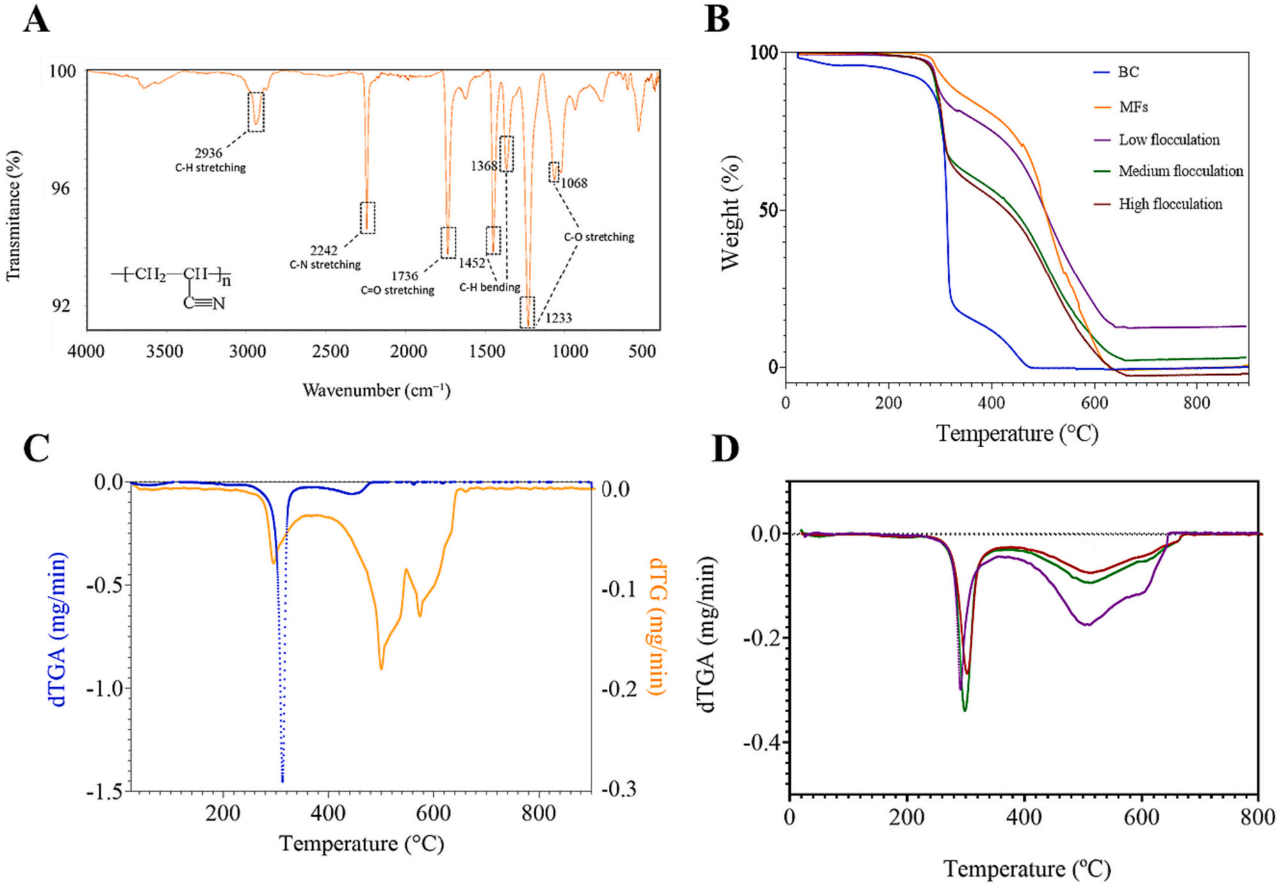

Fig. 2. Attenuated total reflectance – Fourier transform infrared spectroscopy (ATR-FTIR) spectra of polyacrylonitrile MFs (A). TGA (B) and dTGA (C and D) of BCH, MFs and the hetero-aggregates.

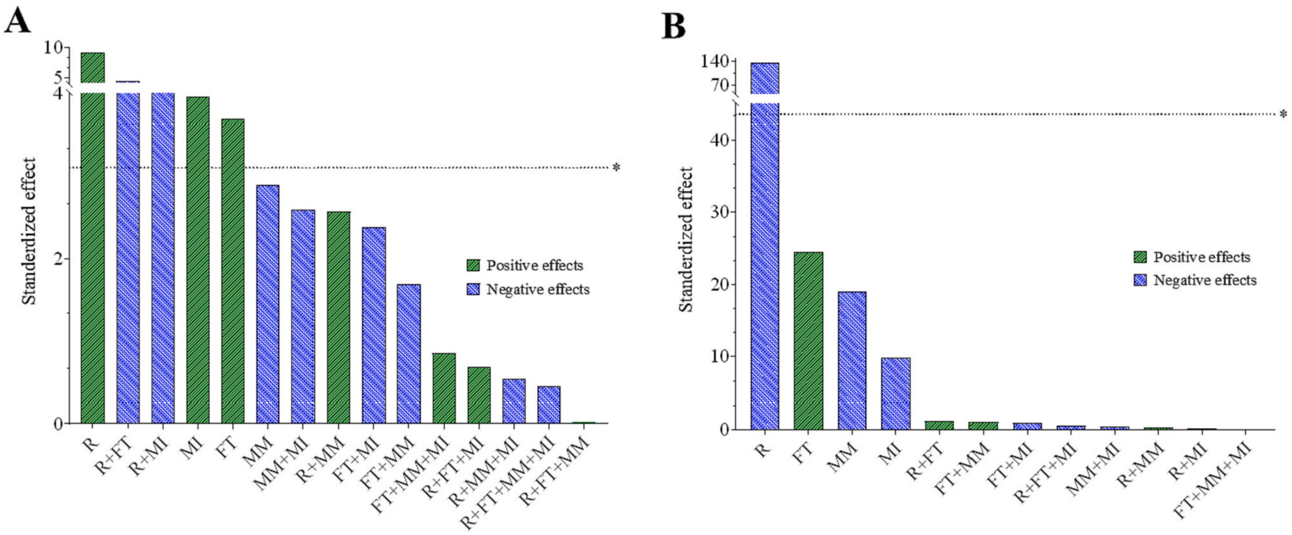

Fig. 3. Pareto chart on the degree of influence of the operational factors in determining the (A) flocculation rate and (B) dispersion of MFs. *Horizontal line represents the threshold of statistical significance. *R*: BCH:MFs ratio; *MI*: mixing intensity; *FT*: flocculation time; *MM*: mechanical mixing duration.

necessity for a comprehensive examination of the environmental conditions and the specific properties of the substances present.

3.2. *Response surface methodology (RSM)*

RSM was employed to modulate the parameters of the experimental design and was applied to predict how several potential operational conditions found in WWTPs would affect the MFs flocculation rate and the dispersion of the herero-aggregates towards the removal of MFs from MFs-contaminated waters. The parameters BCH:MFs ratio (w.w./d.w.) (5:1, 52.5:1, 100:1), mixing intensity (60, 180, 300 rpm), mechanical mixing duration (5, 17.5, 30 min), and flocculation time (5, 62.5, 120 min) were tested to determine the conditions for the maximised flocculation of MFs using the BCH.

Based on experimental values and RSM computational modulation, the operational conditions with the most statistically significant impact on the flocculation of MFs were determined (Fig. 3). Accordingly, the BCH:MFs ratio (*R*) emerged as the most influential, followed by the mixing intensity (*MI*) and flocculation time (*FT*), all of which exceeded the threshold of statistical significance (Fig. 3A). These three parameters positively affected the flocculation rate, meaning that an increase in these variables should lead to an improvement in the flocculation rate. An increased flocculant ratio to MFs might promote more effective binding and aggregation, improving flocculation. Higher *MI* could enhance the contact and collision frequency between MFs and BCH, facilitating their aggregation. And extending the *FT* could allow for a more complete interaction between BCH and MFs, enabling the formation of hetero-aggregates.

As far as dispersion is concerned, only the BCH:MFs ratio displays statistical significance, as depicted in Fig. 3B. It had a negative effect, indicating that an increase in the BCH:MFs ratio should result in a reduction in the dispersion of hetero-aggregates formed during the flocculation process. An increase in the ratio BCH:MFs, could lead to the formation of denser aggregates, causing them to settle faster, directly influencing dispersion.

The contour plots presented in Fig. 4 are a vital component of the study as they serve the purpose of optimizing the operational conditions. These aim to determine the conditions appropriate for the study's desired response(s), specifically targeting maximum flocculation. This strategic approach is essential to harness the full potential of BCH, identifying the specific conditions under which it performs at its best and ensure its practical suitability within industrial applications, such as WWTPs.

The results of these contour plots provide valuable insights into the relationship between various parameters, including the BCH:MFs ratio and mixing intensity, and their impact on flocculation and dispersion rates. The findings reveal that, when specific conditions are met (e.g., with the model assuming a flocculation time of 62.5 min and a mechanical mixing duration of 17.5 min), a higher flocculation rate can be achieved by increasing the BCH:MFs ratio and mixing intensity (Fig. $4A_1$ and $4A_2$).

When it comes to the dispersion (with the same hold values, assumed by the model) (Fig. $4B_1$ and $4B_2$), the contour plots show that an increase in the BCH:MFs ratio leads to a decrease in dispersion, independently of the mixing intensity. This observation is consistent with the Pareto chart (Fig. 3B), which highlights the statistically significant negative effect of the BCH:MFs ratio on dispersion.

The regression equations that define the experimental correlations with the flocculation rate and the dispersion are displayed in Eq. 4 and Eq. 5, respectively. When the values of individual variables are modified, these equations can be employed to forecast the expected behaviour of flocculation or dispersion. For instance, with a *R* of 5:1 (BCH:MFs), an *FT* of 90 min, an *MM* of 10 min and an *MI* of 100, flocculation rate would be 64 %, and dispersion would be 205 cm^2/g.

$$\begin{gathered}\boldsymbol{Flocculation\ rate}\ (\%) = 64.7 + 0.05R + 0.09FT – 0.18*MM + 0.09*MI – \\ 0.00008*R*FT + 0.00017*R*MM – 0.00004*R*MI – 0.00024*FT*MM – \\ 0.00018*FT*MI – 0.00082*MM*MI – 0.00003*R^2 + \\ 0.00016*FT^2 + 0.00479*MM^2 – 0.00008*MI^2\end{gathered} \tag{4}$$

$$\boldsymbol{Dispersion}\ \left(\boldsymbol{cm^2/g}\right) = 252.56 - 0.658{*}R\text{–}0.026{*}FT\text{–}0.779{*}MM\text{–}0.049{*}MI + 0.000044{*}R{*}FT + 0.001198{*}R{*}MM + \left(1.717\ x\ 10^{-6}\right){*}R{*}MI + 0.000694{*}FT{*}MM\text{–}0.000062{*}FT{*}MI\text{–}0.000127{*}MM{*}MI + 0.0004{*}R^2\text{–}0.0001{*}FT^2\text{–}0.0132{*}MM^2 + 0.0001{*}MI^2 \tag{5}$$

The applicability and validity of the models were measured using ANOVA analysis and by the comparison (R^2) between the experimental and predicted values. No significant lack-of-fit ($p \geq 0.05$) was found, and the predicted R^2 values and the R^2 values obtained experimentally do not differ significantly for both responses (Fig. 5). An *F*-value of 51.52 and a *p*–value of <0.05 for the flocculation rate confirm the appropriateness of the model. Furthermore, for the dispersion, the *p*–value is <0.05, and the *F*-value is 6750.91, proving that this model is also applicable. Considering this, the model equations' validity and the computed models' reliability are confirmed.

For experimental confirmation, untested values of the variables were investigated to confirm the computation of both response factors. As shown in Fig. 4, when mixed at 180 rpm, a BCH:MFs ratio of 10:1 (a*) achieved 75.0 ± 0.03 % of flocculation. The experimental value falls within the predicted flocculation rate (65.8 %). When put to the test, mixing at 120 rpm, a BCH:MFs ratio of 43:1 (b*) produced an 85.1 ± 0.01 % of flocculation rate, lying within the anticipated flocculation rate of 78.4 %. Finally, similar conclusions were drawn when testing an 81:1 BCH:MFs ratio and an agitation intensity of 240 rpm (c*). The flocculation yielded 87.3 ± 0.03 %, compared to the predicted 93.0 %. Therefore, the model can reliably predict and distinguish the conditions where the flocculation of MFs by BCH performs exceptionally well and those where it doesn't, which is crucial for its practical implementation in industrial applications.

The fine-tuning of an intended outcome represents one of the pivotal stages in the framework of the RSM. Therefore, in order to fully harness BCH's potential and ensure its suitability for industrial context, the maximised flocculation values of the variables were determined. With a focus on cost-efficiency in water treatment industries, each factor was carefully adjusted to maximize flocculation, as this facilitates the

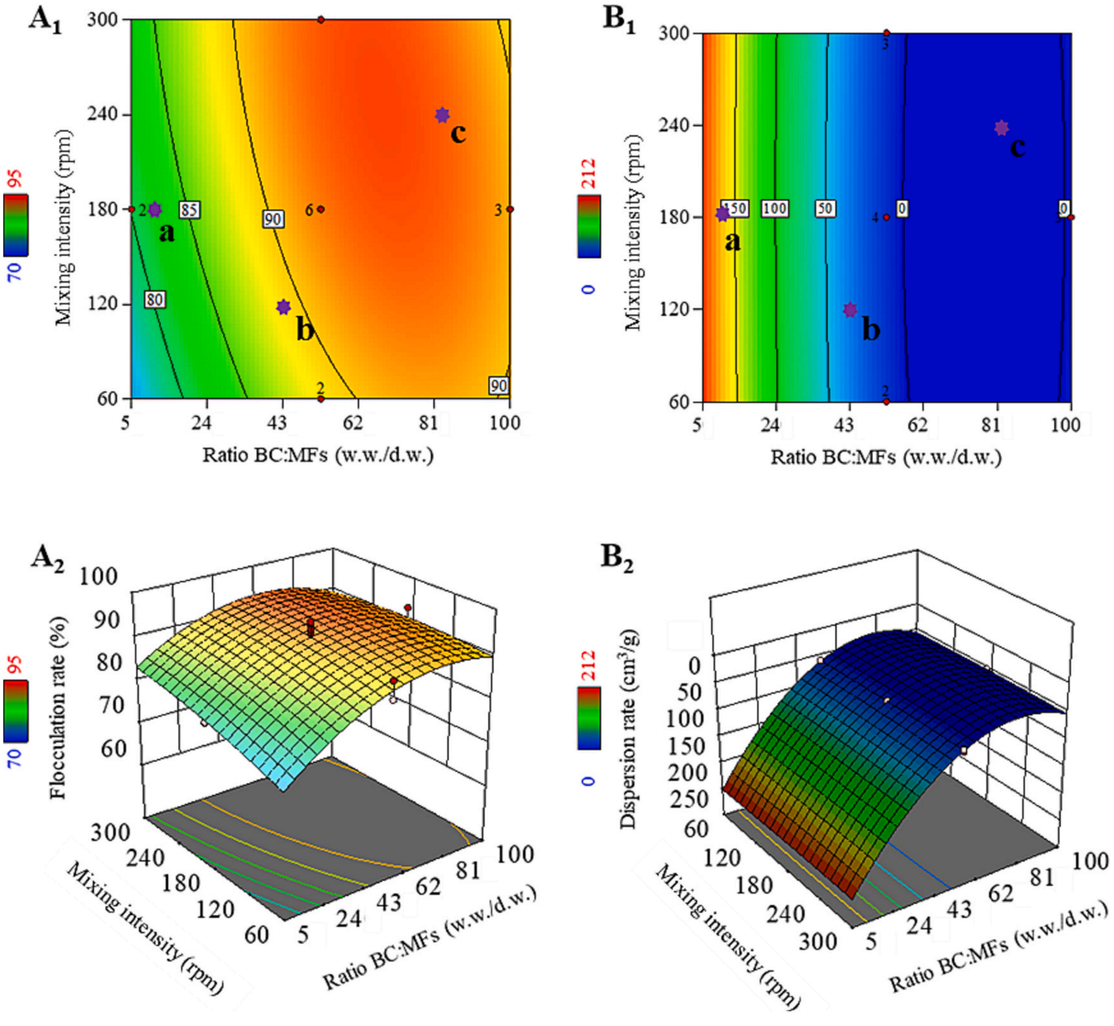

Fig. 4. Plots of the (A) flocculation and (B) dispersion rates of the BCH in relation to MFs removal from MFs-contaminated waters. Contour plots: 1 in subscript (A_1, B_1); 3D surface plots: 2 in subscript (A_2, B_2). *experimentally-confirmed values.

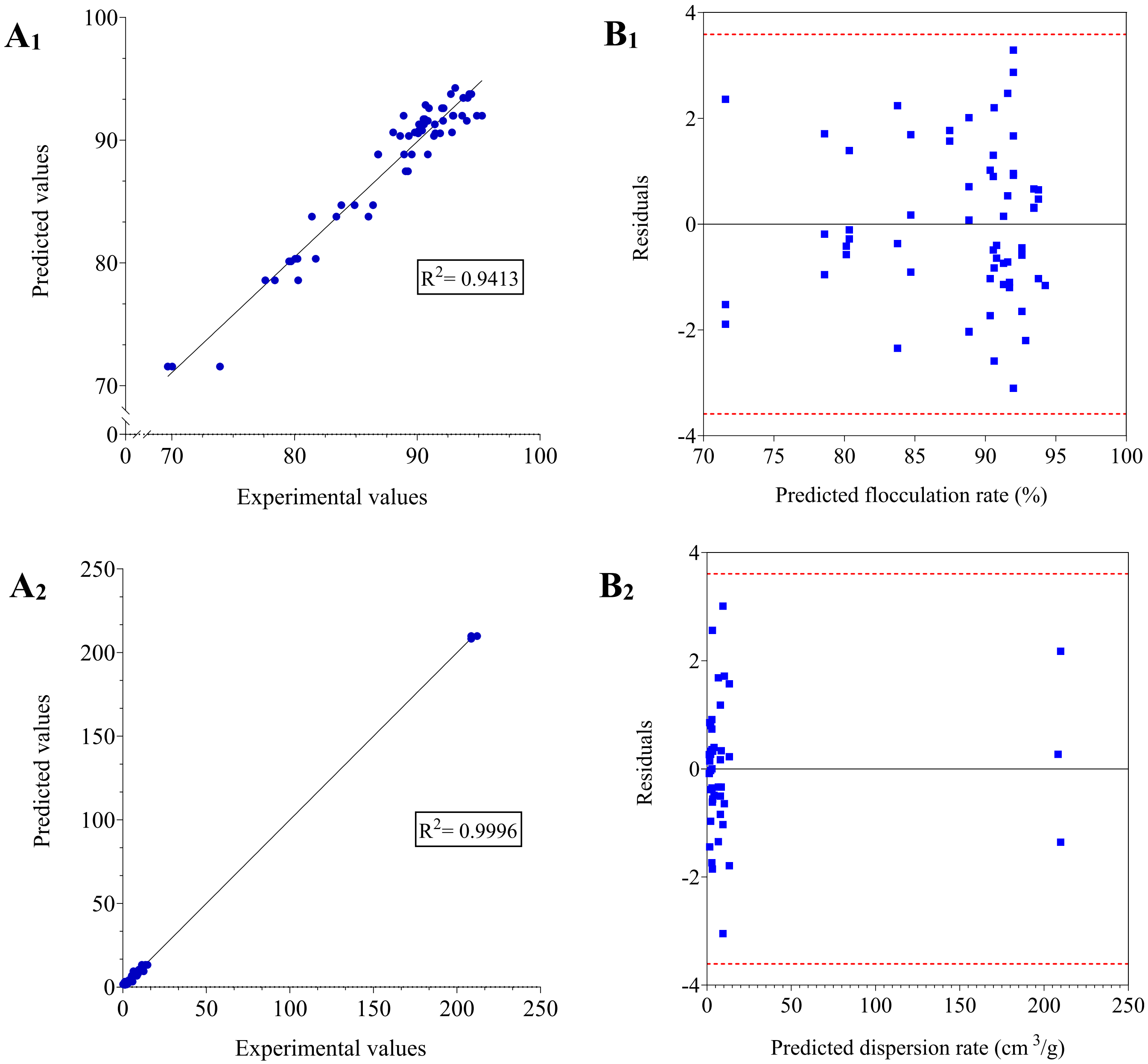

Fig. 5. Relation between the actual and predicted values (A), and residual plot of the predicted values (B) of the flocculation (1 in subscript: A_1, B_1) and dispersion (2 in subscript: A_2, B_2) rates of the BCH in relation to MFs removal from MFs-contaminated waters.

subsequent removal of hetero-aggregates during the water treatment process. The results revealed a maximum flocculation rate of 93.6 ± 0.01 % when using a BCH:MFs ratio of 75:1, mixing at 60 rpm, conducting a mechanical mixing duration of 30 min, and allowing a flocculation time of 60 min. Li et al. (2022) achieved a flocculation rate between 86 and 89 % after 2 h of settling using the inorganic flocculant polyaluminum chloride. Another study, by Duan et al. (2022), achieved a flocculation rate of 93.2 % using the inorganic coagulant magnesium hydroxide. Compared with these studies, the BCH surpassed expectations, achieving an exceptional flocculation rate. Furthermore, BCH is derived from a waste product that is naturally produced by bacteria as part of their lifecycle.

To confirm the practical application of the RSM methodology, production was scaled up to predict the performance of the hydrogel in MFs remediation for potential use in WWTPs. The outcomes met the expectations at 89.1 ± 0.25 %, compared to the flocculation rate obtained in the laboratory scale (93.6 ± 0.01 %). The experimental values closely matched the predicted values, affirming the success of the chosen conditions in achieving the aforementioned target. In summary, RSM demonstrates exceptional predictive accuracy and precision.

3.3. Characterisation and analysis of the hetero-aggregates

The interaction between BCH and MFs has been meticulously analyzed using fluorescence and SEM, illuminating the complex mechanisms behind MF sorption. These techniques provide a thorough visualization of the MFs' interaction with BCH, highlighting the sorption process's efficiency.

The BC polymer is renowned for its distinct and characteristic three-dimensional nanofibrillar network and laminar microstructure, featuring a dense and fibrillar microporous arrangement. This complex structure, stabilized by robust H-bonds due to abundant hydroxyl groups (Takawa et al., 2022) undergoes significant transformation upon conversion to BCH, disrupting the original microporous architecture

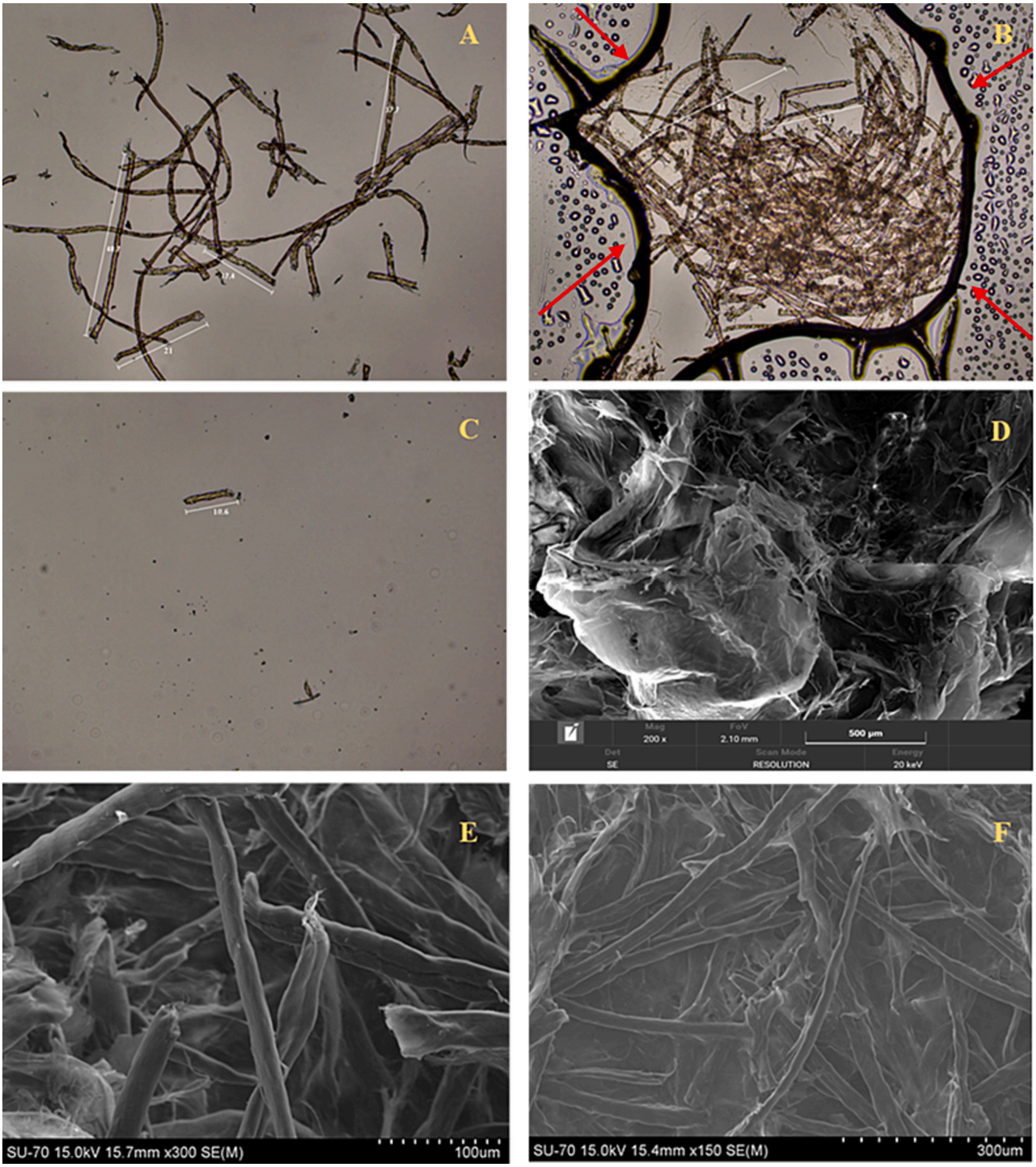

Fig. 6. Fluorescence microscopy: (A) MFs solution before flocculation; (B) BCH-MFs hetero-aggregate; (C) MFs remaining after flocculation. Scanning electron microscopy: BCH (200×) (D), and MFs adsorbed in the BCH ((E) 300× and (F) 150×). *The red arrows represent the BCH-MFs hetero-aggregate.

(Fig. 6). This alteration could introduce new active sites, optimizing MFs' interaction and increasing sorption efficacy, consequently leading to an increased flocculation rate.

As seen in Fig. 6B, the MFs are ensnared within the BCH's porous framework, significantly reducing the prevalence of particles external to the hydrogel (Fig. 6A and C). SEM micrographs of the hetero-aggregates reveal the encapsulation of both diminutive and slender, elongated MFs within the BCH network. This complex leads to the formation of aggregates between BCH and MFs.

This comprehensive microscopy analysis not only confirms the high capacity of BCH to retain MFs but also the morphological alteration of BCH upon interaction with MFs.

This alteration was visually evident upon flocculation onset, transitioning BCH from a viscous paste to a structurally modified hydrogel, signalling a reorganization of the hydrogel's structural composition. Such visible reorganization enhances sedimentation capabilities, facilitating the removal of larger aggregates. Examining SEM micrographs, the entrapment of MFs within the BCH fibres highlights the structural reorganization and the pivotal role of BC's fibrillar structure in these interactions. This entanglement, which results in flocculation and the creation of hetero-aggregates, plays a crucial role in the process's effectiveness of removing MFs fromMFs-contaminated waters.

Through the calculation of the flocculation rates and analysis/modelling using RSM, it was demonstrated that a higher flocculation rate is achieved with a higher BCH:MFs ratio. However, what does this imply regarding the composition of the hetero-aggregates? Do high flocculation rates lead to MFs-saturated hetero-aggregates, or do these aggregates predominantly contain BCH? What causes low flocculation rates? Thermogravimetric Analysis (TGA) was conducted to answer these questions, starting with the thermal behaviour study of BCH and MFs individually and then of the hetero-aggregates obtained under different conditions.

As depicted in Fig. 2B to D, the TGA of the BCH displays a thermal profile characterised by a single-step process. This process shows initial and peak decomposition temperatures at 266 and 347 °C, respectively, aligning closely with prior publications' data (Hao et al., 2018). On the contrary, the TGA profile for the MFs revealed a weight loss pattern characterised by three distinct stages, with no noticeable weight loss occurring before reaching 260 °C, typical of polyacrylonitrile compounds. As the temperature continues to rise, the polymer degradation initiates with cyclisation, followed by oxidation and dehydrogenation processes (Hao et al., 2018). The TGA profile of BCH-MFs hetero-aggregates (examples given in Fig. 2C and D) exhibited the characteristic decomposition peaks of BCH and MFs, where the first step at 298 °C was associated with the degradation of the cellulose skeleton; the following steps can be attributed to the degradation of MFs backbone.

Analyses of all the experimentally obtained hetero-aggregates for different flocculation rates demonstrated the presence of MFs within the bioflocculant BCH matrix. Additionally, a trend of increasing intensity of steps associated with the MFs' backbone degradation was observed for

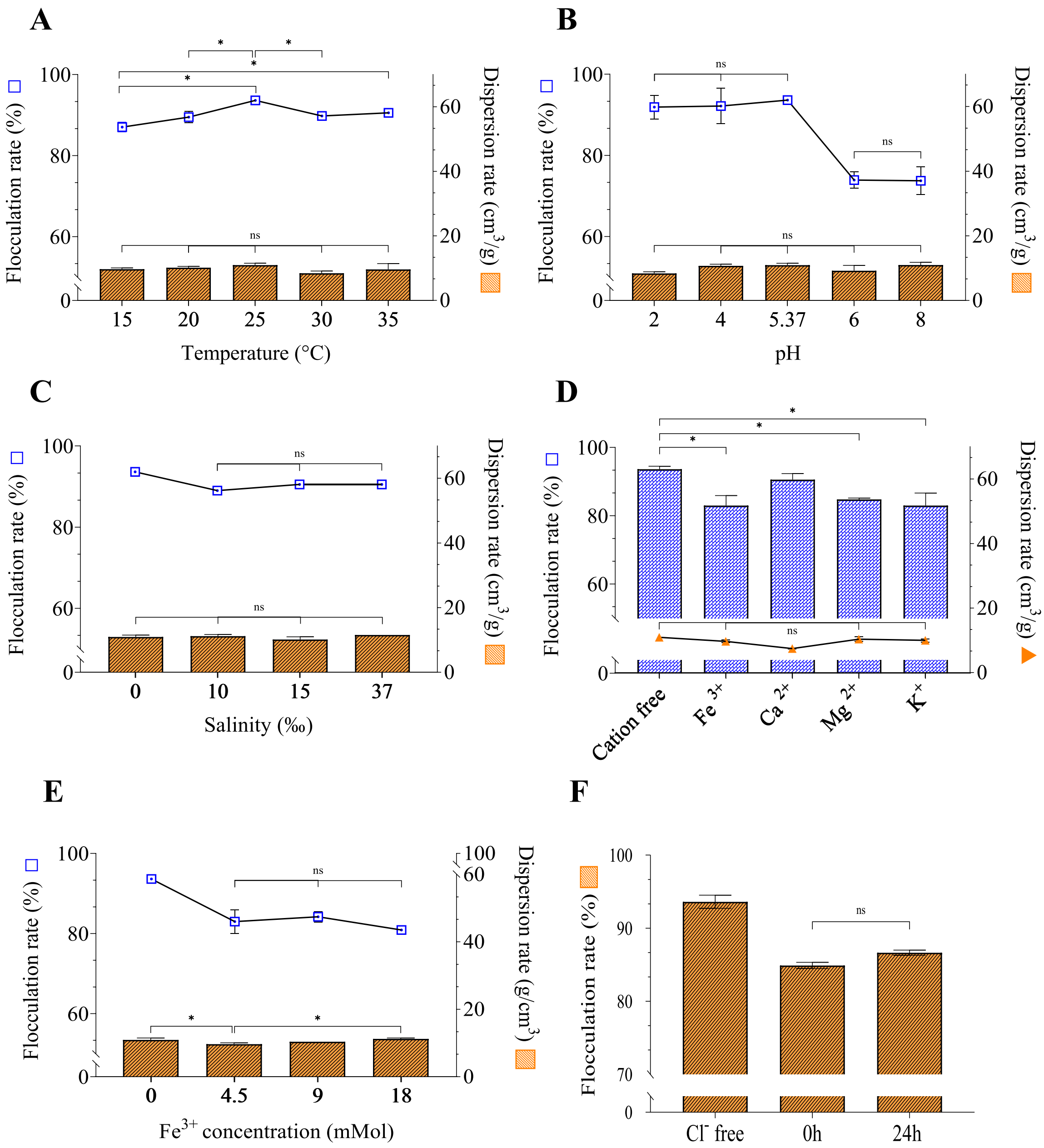

Fig. 7. Flocculation and dispersion rates of BCH in relation to MFs removal from MFs-contaminated waters, under different physicochemical variations: (A) temperature; (B) pH; (C) salinity; (D) metal ions presence (4.5 mMol); (E) different concentrations of Fe^{3+}; (F) after water chlorination (0 h) and after 24 h of chlorination. *significantly different ($p < 0.05$). *ns*: non-significantly different ($p \geq 0.05$).

lower flocculation rates, as exemplified by the three provided hetero-aggregate examples in Fig. 2B and C - one representing conditions leading to high flocculation rates, one medium, and one low. The thermal steps associated with the MFs' backbone correspond to larger percentages of the initial mass of BCH-MFs hetero-aggregates when the flocculation rate is lower. This reveals that *(i)* at high flocculation rates, the hetero-aggregates' composition is predominantly BCH, and *(ii)* at low flocculation rates, hetero-aggregates contain higher quantities of MFs. Hence, it is concluded that the amount of BCH is the key factor influencing the obtained flocculation rates, reaching a saturation point those results in diminished flocculation efficiency.

3.4. Resilience of the BCH/MFs flocculation process

In addition to the operational factors associated with WWTPs efficiency, physicochemical variations in water quality, including factors such as pH, temperature, metal ions, and salinity, can impact the sorption mechanism and flocculation kinetic, disturbing or improving the flocculation performance (Aljuboori et al., 2015). To explore these effects, various conditions were investigated while adhering to the maximised flocculation parameters identified previously using RSM. These parameters include a BCH:MFs ratio of 75:1, mixing at 60 rpm, a mechanical mixing duration of 30 min, and a flocculation time of 60 min.

The flocculation process appears to be sensitive to variations in **temperature** as observed with other flocculants (Fitzpatrick et al., 2004). As depicted in Fig. 7A, the flocculation rate is maximised at room temperature (25 °C), achieving 93.6 ± 0.01 %. A decrease in temperature to 15 °C correlates with a slight decline of 6.6 % in the flocculation rate. The observed reduction can be attributed to the impact of chemical kinetics. Flocculation is essentially a dynamic process where particles aggregate to form larger flocs. This aggregation rate is influenced by the frequency and effectiveness of collisions between the flocculating agent - BCH - and the particles to be removed - MFs. At lower temperatures, the diffusion of the biopolymer, in this case, BCH, is slowed. This reduction in diffusion rate means that the biopolymer particles may take longer to reach and interact with the suspended MFs in the water. Additionally, the collision frequency of suspended particles is reduced, resulting in fewer collisions between the biopolymer particles and the MFs (Pan et al., 2009). The slower diffusion and reduced collision frequency inhibit the efficient interaction between BCH and MFs, decreasing the flocculation activity. Conversely, for the system studied in this work, an increase in temperature to 35 °C does not significantly impact flocculation activity compared to room temperature. Regarding dispersion, as shown in Fig. 7A, it is evident that temperature changes did not markedly affect this aspect.

These findings suggest that BCH holds promise as a natural flocculant in regions with tropical, subtropical, and temperate climates. The robust performance across a range of temperatures highlights the versatility and adaptability of BCH, further supporting its potential for widespread use in addressing MFs pollution in various environmental settings.

The influence of water **pH** on the flocculation process was systematically investigated to understand its impact. The pH, linked to hydrogen ion (H^+) concentration, significantly impacts the flocculation process by influencing the surface charge distribution on both MFs and BCH. It also affects H-bonding and crosslinking within BCH, thereby altering its flocculating activity (Lai et al., 2018). pH ranging from 4 to 8 was tested (Fig. 7B), revealing significant insights into the pH-dependent behaviour of the flocculation process. The results show that the highest flocculation (93.6 ± 0.01 %) rate was achieved at a pH of 5.37. However, as the pH deviated towards higher/alkaline values, the flocculation rate significantly decreased (*c.a.* 20 %), reaching a minimum of 73.7 ± 3.42 % in pH 8. These variations can be explained by changes in the H^+ concentration, potentially altering the ionization of functional groups on the surfaces of the MFs and BCH: *(i)* at lower pH levels, with higher H^+ concentration, functional groups on the MFs and BCH surfaces are more likely to become protonated; and *(ii)* at higher pH levels the deprotonation may occur, with decreased H^+ concentration, leading to the loss of H^+ from functional groups. These changes in surface charges directly affect the sorption of MFs to BCH. Additionally, water pH may induce conformational changes and structural alterations in the formed hetero-aggregates, affecting their stability (Surendhiran and Vijay, 2013). Regarding hetero-aggregate dispersion, no significant modifications were observed with pH changes. Thus, these results show that the functional groups on both MFs and BCH surfaces play a pivotal role in the efficiency of the flocculation process. This underscores the importance of pH optimization for maximizing BCH's flocculation activity in removing MFs from MFs-contaminated water sources, where pH variations may occur due to natural conditions or specific treatment processes.

Understanding the impact of varying ionic strengths, especially those influenced by **salinity** variations, is crucial for optimizing the flocculation capabilities of biopolymers like BCH in WWTPs. Fig. 7C illustrates how different salinities affect BCH flocculation rates, providing insights into its adaptability. The highest flocculation activity is observed in freshwater. However, increasing salinity to typical seawater levels (37 ‰) reduces the flocculation rate, dropping to 88.0 ± 4.50 %. This reduction is mainly due to the effect of sodium ions (Na^+), which interact with negatively charged functional groups of both BCH and MFs, leading to partial charge neutralization. The negatively charged surfaces of MFs may change in the presence of Na^+, altering their interaction with BCH. Furthermore, Na^+ ions raise the ionic strength of the solution, which can impact the colloidal stability and influence the aggregation behaviour of BCH and MFs. Despite these influences, hetero-aggregate dispersion remains stable, indicating the flocculation process's resilience to ionic strength changes.

Thus, Na^+ ions play a multifaceted role in the BCH-MFs flocculation process. They affect the electrostatic repulsion and potentially alter the charge characteristics of both components, impacting the flocculation dynamics. Given the relatively modest decrease in flocculation rate (5.7 %) and the consistent dispersion, BCH shows potential for removing MFs across environments with different ionic strengths, such as ocean, estuarine, and river waters.

The presence of **metal ions** in wastewater treatment is a common occurrence due to various industrial processes, domestic discharges, and environmental factors (Gholipour et al., 2020). Depending on the specific wastewater sources, these metal ions can include cations such as Fe^{3+}, Ca^{2+}, Mg^{2+}, K^+, among others. Further, in certain instances, intentionally adding specific metal ions to wastewater can improve the flocculation of the natural flocculant (Yim et al., 2007). Therefore, an investigation was conducted to explore the impact of cations like Fe^{3+}, Ca^{2+}, $Mg^{2+,}$ and K^+ on the flocculation rate of BCH, as illustrated in Fig. 7D. The results showed that the presence of cations Fe^{3+}, Mg^{2+}, and K^+ led to a decreased flocculation rate (up to 11 %). This decline can be attributed to the potential masking of the sorption sites of the biopolymer. The competition between positively charged ions and the negatively charged sites of the BCH can lead to reduced availability of sorption sites, thus diminishing flocculation efficiency. Given the naturally high flocculation rate exhibited by the hydrogel, it becomes apparent that satisfactory removal yields can be attained without the need to introduce cations for capturing MFs. This approach not only aids in averting environmental pollution but also contributes to cost savings in WWTPs (Salehizadeh and Yan, 2014).

Despite typically being present in trace amounts, Fe^{3+} is one of the most toxic and widely distributed ions in wastewater effluents (Chai et al., 2021). Therefore, different concentrations of Fe^{3+} were tested (4.5, 9, and 18 mMol) to evaluate the flocculation capacity of the BCH in the presence of this cation. As observed previously, the presence of Fe^{3+} at a concentration of 4.5 mMol resulted in a roughly 11 % reduction in the flocculation rate. Notably, flocculation neither increased nor decreased significantly with increased cation concentration, as depicted in Fig. 7E. Moreover, the presence of elevated Fe^{3+} concentrations had minimal influence on the dispersion of the BCH (between 0.3 and 1.3 %). This highlights the resilience of the biopolymer's performance, even when faced with demanding chemical variations.

To gain a deeper understanding of the retention force and develop effective strategies for managing the hetero-aggregates formed during flocculation under maximised flocculation conditions, a 24-h washing cycle was carried out. The outcomes demonstrated that out of the initially flocculated 93.6 % of MFs, merely 8.3 ± 0.01 % of the MFs were liberated from the BCH.

The structural resilience of the BCH is showcased by its ability to effectively lock in MFs, offering a powerful combination of robust particle aggregation and exceptional particle entrapment. This can be attributed to the sorption within BCH's porous structure, along with the potential influence of strong electrostatic interactions that enhance particle aggregation. Overall, these findings emphasise BCH's efficacy as a solution for removing MFs and its potential utility in industrial contexts like WWTPs.

3.5. Impact of the chlorination process

Chlorination, a widely employed water treatment method, plays a critical role in safeguarding public health by ensuring the safety and purity of water. This process involves the controlled introduction of chlorine into water supplies, typically in the form of chlorine compounds (Zhai et al., 2017). Chlorine, known for its powerful disinfectant properties, effectively eliminates harmful microorganisms, such as bacteria and viruses, that may be present in untreated water (Kim et al., 2003). Given that flocculation processes rely on chemical interactions, it becomes imperative to investigate the potential impact of Cl^{-} ions in treated water undergoing MFs removal by BCH.

Experimentally, after the chlorination of the MFs-contaminated water, the flocculation rates decreased by up to 9.7 % in the maximised flocculation conditions (Fig. 7F). It's well-established that during chlorination processes, the generation of byproducts can occur due to the presence of free chlorine ions (Kim et al., 2003). These byproducts might, in some way, influence the flocculation rate. A 24 h chlorination did not significantly change the flocculation rate from the initial measurement time.

Thus, the hydrogels made from BC can be used in recreational waters (e.g., swimming pools) and water treatment stations that use chlorine or sodium hypochlorite to mitigate MFs contamination.

3.6. BCH performance on WWTPs

WWTPs are vital pillars of urban infrastructure, tasked with treating and purifying wastewater originating from diverse sources, including households, industries, and businesses. Their overarching mission is to safeguard public health and the environment by efficiently eliminating contaminants and pollutants from wastewater before it is reused or released into natural water bodies. In the framework of the present study, WWTPs represent a focal point where innovative solutions, such as BCH, can be applied to address contemporary environmental challenges, notably the presence of MFs. As the results of the investigation into MFs removal with BCH are examined, it becomes evident that the real-world applications extend beyond the laboratory setting. The goal is to bridge the gap between controlled laboratory experiments and their real-world application within WWTPs.

Considering these factors, experiments were conducted to assess the effectiveness of BCH in extracting MFs from the effluents of WWTPs. The results show that the flocculation rate decreased by only 4.5 % compared to the highest yield (93.6 %) obtained in distilled water. The hydrogel had a high capacity for adsorbing the MFs present in the wastewater despite a slight decrease in flocculation rate. The presence of organic matter (e.g., fulvic and humic acid), salts, ions, and metal cations can influence the flocculation and sedimentation processes of emerging pollutants, potentially leading to reduced efficacy (Matilainen et al., 2010). Based on these findings, BCH is revealed to be a good potential solution for removing MFs that are not eliminated in WWTPs through traditional treatments.

3.7. Future perspectives in real-world scenarios

The increasing challenge of MFs pollution, primarily from synthetic textiles, presents a critical environmental and waste management issue. Unlike MPs fragments, MFs (fibrous MPs) possess unique physicochemical properties that require specialized mitigation approaches. BCH, derived from BC remnants, has emerged as a promising material for environmental applications, particularly in addressing MFs pollution. Its effectiveness, demonstrated by an average removal rate of 93.6 %, makes it a viable candidate for integration into water treatment facilities and WWTPs.

The adaptability of BCH to varying conditions such as temperature, pH, salinity, and metal ions enhances its potential for real-world applications. Its robust retention capabilities, indicated by only an 8.3 % release of absorbed MFs after a 24-h wash cycle, further validate its effectiveness in continuous water treatment processes.

While studying individual effects of temperature, pH, and salinity provide important insights into the resilience of the BCH-MFs flocculation process, the interplay of these factors shapes the flocculation outcomes in real-world scenarios. It was found that the optimal flocculation occurs at 25 °C and pH 5.37, with effectiveness diminishing in higher salinity conditions. However, the practical impact on natural or industrial water bodies can be complex. In estuaries, where salinity and pH levels may significantly shift due to the confluence of freshwater and seawater, such fluctuations can impact the charge distribution on MFs and BCH, influencing flocculation efficiency. Seasonal temperature variations further complicate the flocculation kinetics, affecting the rate of interaction between BCH and MFs.

Furthermore, future research should be directed towards optimizing BCH application in larger-scale water treatment processes. This includes determining optimal dosage requirements, contact times, and compatibility with existing treatment technologies. The goal is to bridge the gap between laboratory findings and real-world implementation, contributing to sustainable and practical solutions for MFs removal in water treatment settings.

In summary, while this study lays the groundwork for BCH's application in water treatment, additional research is necessary to upscale and effectively implement these solutions in real-world scenarios.

4. Conclusion

Given that microfibers (MFs) are the dominant type of microplastic in ecosystems and considering the current wastewater treatment's inability to effectively capture MFs, the present research evaluates bacterial cellulose hydrogel (BCH) to address this critical gap and serve as a catalyst for future research, highlighting the issue of fibrous pollutant mitigation. The results highlighted the excellent performance of BCH in removing MFs, with a flocculation rate of 93.6 % achieved under optimal conditions: a BCH:MFs ratio of 75:1 (w.w./d.w.), 30 min of mechanical mixing, 60 rpm mixing intensity, and 60 min of flocculation time.

Computational modelling demonstrates the key role of the BCH:MFs ratio and mixing intensity in influencing the flocculation rate. BCH displayed a remarkable ability to capture and retain MFs under various physicochemical variations, such as temperature, pH, salinity, and the presence of metal ions. The flocculation rate remained nearly unchanged when utilizing water from WWTPs and chlorinated recreational waters. This robust performance is due to both electrostatic interactions and the sorption of MFs within the 3D hydrogel's structure, contributing to the observed strong and irreversible hetero-aggregation during the flocculation process. Consequently, employing BCH, a natural and biodegradable bioflocculant, signifies a transformative approach to remediation methods. Its exceptional efficacy in removing MFs, even in demanding environments, highlights BCH's promise as a groundbreaking, eco-friendly solution for addressing MFs pollution.

CRediT authorship contribution statement

Filipa Rodrigues: Writing – original draft, Investigation, Formal analysis, Conceptualization. **Marisa Faria:** Writing – original draft, Formal analysis, Data curation. **Ivana Mendonça:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Edward Sousa:** Formal analysis. **Artur Ferreira:** Resources, Project administration. **Nereida Cordeiro:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT to improve text fluency and readability. After using this tool, the authors reviewed and edited the content as needed and took full responsibility for the content of the publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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.scitotenv.2024.170846.

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