

Membrane Fouling in Ultrafiltration of Spirulina (Arthrospira platensis) Protein Purification: Impacts and Mitigation Strategies to Improve Process Efficiency.

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ABSTRACT

This work investigates membrane fouling in the ultrafiltration of Spirulina (*Arthrospira platensis*), with the objective of studying its impact on protein extraction. Through a qualitative literature review, fouling was characterized as a dynamic and multiscale phenomenon that can be enhanced by physical and chemical interactions and by the system complexity. Results show that conventional models of single-protein systems do not represent multicomponent systems clearly, as the behavior is not linear. In addition, factors such as pH, operational conditions and membrane properties are highlighted as the main factors influencing fouling rate. The study indicates that fouling represents an important economic and technical challenge and suggests that integrated mitigation strategies with emerging technologies may improve the performance of protein extraction.

Index Terms - Membrane fouling, Spirulina, Protein purification, Ultrafiltration (UF)

INTRODUCTION

Various nutrients provide humans with the energy required to perform their functions and ensure the proper development of their systems. Moreover, the three main macromolecules responsible for human nutrition in food systems composition are proteins, lipids, and carbohydrates. Out of these macromolecules, proteins emerge as essential macronutrients for human life [1]. Playing an extremely important role in various physiological processes, proteins are a determining factor in balancing the diet of any organism [2]. The importance of proteins is closely related to their structural and functional diversity. Proteins are found in a wide range of sources and differ in amino acid composition and sequence, resulting in distinct chemical structures and biological properties [1, 2].

Furthermore, a study suggests that the world population will increase, estimating it to reach approximately 9.7 billion people by 2050 [3]. In addition, another study indicates that food consumption will increase by

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70% in 2080 [1], resulting in a proportional increase in protein demand, becoming essential to produce more food in the future.

Livestock farming, the traditional way of obtaining protein, generates a significant environmental impact. In this context, with the aim of increasing the supply of protein in food science, diversified protein offerings are emerging to reduce environmental impact [3]. A study [4] demonstrates that the production of plant-based alternative proteins emits up to 90% less greenhouse gases compared to beef, also using less water resources and pasture areas. Specifically in organisms such as microalgae, sustainable practices are fostered by cultivation in aquatic environments and carbon dioxide capture. In this way, the use of these practices could reduce pressure on agricultural systems by up to 50% and ensure sustainable production [3].

Spirulina as an Alternative

The scientific community explains the environmental impact caused by livestock farming, which is the traditional method of obtaining protein.

Microalgae are considered potential alternative protein sources capable of mitigating some of the environmental issues associated with conventional protein production. Spirulina (*Arthrospira platensis*) microalgae stand out due to their high protein content, which can reach 70% of dry biomass under optimal cultivation conditions [6]. Their cultivation and processing require less land and water than conventional animal protein production [7]. For these reasons, there is growing interest in Spirulina as a potential component of more sustainable protein systems. However, the recovery of this biomass via membrane separation processes involves the accumulation of retained material near the membrane surfaces. Concentration polarization, adsorption, pore blocking, and the formation of surface deposits are phenomena that collectively contribute to membrane fouling, highlighting the need for further research in this area.

Challenges of Protein Purification Process

To obtain proteins from microorganisms, a purification step is necessary. However, no single method can universally ensure efficient protein purification from complex systems. Protein physicochemical properties vary considerably, and many proteins are sensitive to changes in processing conditions. These characteristics can make filtration challenging [8].

In this context, one of the main challenges in protein purification is the presence of various impurities, many of which have characteristics similar to those of the target protein. Furthermore, the instability of proteins makes the process even more complex, as they can be easily modified due to changes in pH, temperature, or external agents, which can result in biological losses [8, 9].

The purification process involves multiple steps, and each step can affect the final protein recovery. Consequently, optimization of the overall process is important for maintaining protein yield. These multiple steps can also significantly increase the overall cost of the purification process. These challenges are even more frequent in complex biological matrices such as Spirulina, reinforcing the need to develop more efficient techniques [8, 9].

UF as a Solution

Low-pressure membrane filtration processes, specifically UF, have been employed for nearly 60 years to separate desired and undesired products from industrial feed streams. Alongside the achievements of membrane technology in numerous applications, these processes are also used in the food industry for concentration and purification purposes. UF is widely used in bioprocessing for the concentration and retention of proteins. Membrane separation is therefore an important step in several bioprocess applications, including protein purification, clarification, and concentration [5]. It is also necessary to highlight that the filtration method can directly affect the quality and cost of macromolecular protein production [8].

Membrane Fouling as a Limitation

During the ultrafiltration of multicomponent biological suspensions—such as streams derived from Spirulina—the accumulation of retained cells, proteins, polysaccharides, colloidal particles, and other organic compounds on the membrane surface increases mass transfer resistance. This reduces membrane permeability due to phenomena such as concentration polarization, adsorption, pore blocking, and the formation of cake or gel layers, ultimately leading to membrane fouling [5]. As fouling progresses, increasing the processing pressure or cleaning the membrane are options to prevent or mitigate extended processing times. The performance, efficiency, and economic viability of membrane ultrafiltration for protein recovery can be strongly affected membrane fouling.

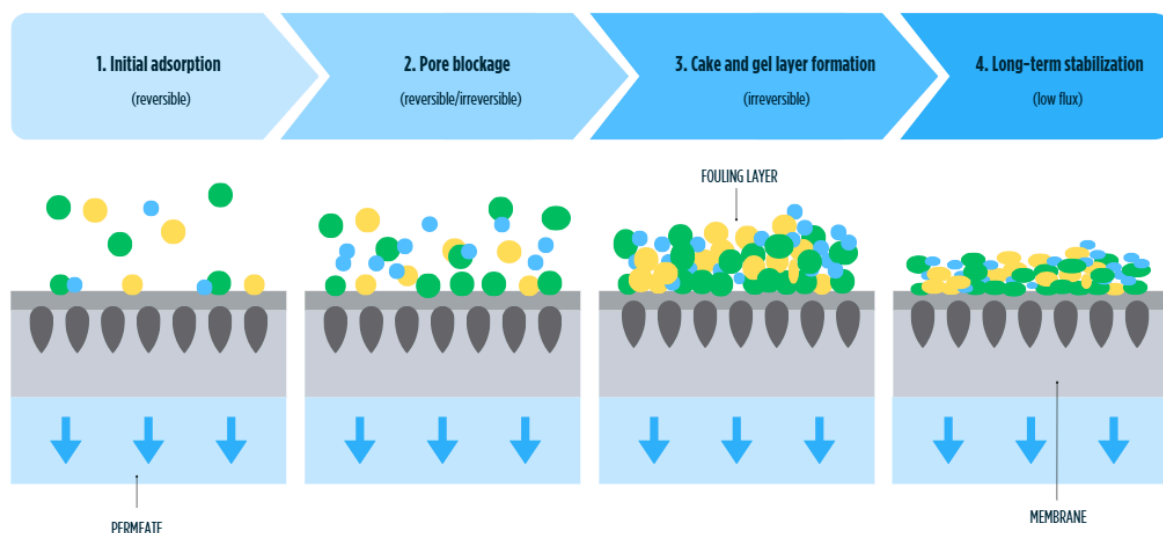


Figure 1: progression of membrane fouling during UF, from initial adsorption and pore blockage to cake and gel layer formation and long-term flux stabilization.

UF processes can be optimized by trial and error. This method is the most accessible; however, the labor requirements and the multiplicity of factors that influence the performance of UF result in delays and uncertain costs. Factors such as membrane material, pore size, protein type and concentration, crossflux rate, transmembrane pressure, and temperature make UF optimization difficult. These factors can increase processing time and labor requirements, require large quantities of expensive protein materials, and involve materials that may have limited availability. With investment in equipment, it is possible to overcome these limitations, implementing high-throughput screening systems or ultracompact membrane devices as more effective options [8]. In recent years, machine learning has also demonstrated increasing potential in materials science and process engineering due to its strong ability to process and learn from large, high-dimensional datasets to establish predictive models. It has been used to design new membrane materials and to deepen the interpretation of the membrane structure-performance relationship, assessing the importance of its characteristics [8].

Protein fouling can entail significant operating costs for membrane filtration units, but economic studies addressing protein fouling are limited. An economic analysis of biocatalyst separation reported that the membrane (and its replacement), transmembrane pressure, and fluid flux rate are the most significant cost components. As a quantitative indication, the cost of fouling in reverse osmosis of surface water was reported to be 24% of operating expenses; an analogous study for protein fouling would be useful [5].

Research Gap and Objective

The interest in alternative sources of proteins such as Spirulina has increased. However, there is still a lack of studies integrating the impact of membrane fouling in UF methods specifically to protein purification in microalgae. In addition, the complexity of Spirulina's composition still remains highly misunderstood,

particularly in UF, despite its widespread use in protein processing. Moreover, the existing studies generally focus on the general concept of fouling, without emphasis on mitigation strategies. Therefore, the main goal of this study is to analyse the impact of membrane fouling on UF in the extraction of proteins from Spirulina and evaluate different fouling mitigation methods so that the efficiency of protein recovery increases. The research question of this study is: “What is the impact of membrane fouling on the performance of UF in the purification of proteins from Spirulina, and which mitigation strategies can be employed to optimize this process?”

METHODOLOGY

This study was characterized as a qualitative literature review. The literature used was collected from various scientific sources, including SciELO, CAPES Journals Portal, Google Scholar, Elsevier, Academia.Edu, Science.gov, E-Journals, ResearchGate, Editora Científica, and ScienceDirect. Furthermore, it is important to note that the works used were published between 1983 and 2025, with most being published after the year 2000, and the keywords "membrane fouling," "ultrafiltration," "protein purification," "microalgae," and "spirulina" were used to find them. Initially, approximately 218 articles were identified. Filtering was performed based on title analysis, selecting 45 studies directly related to the proposed theme. Subsequently, the selected articles were analyzed in full.

The sources were selected based on their relevance, publication year, and the amount of information related to the topics of this research. The analysis was performed by comparing different studies to identify patterns in the methods and evaluate mitigation strategies. Therefore, the findings were organized according to the main factor influencing membrane fouling and the possible approaches to mitigate its effect during Spirulina protein purification.

LITERATURE REVIEW

This section will present the scientific literature necessary for the discussion and substantiation of the project. Concrete data and relevant information will be presented.

The Principles of UF

Efficiency depends on the physicochemical composition of the solution, the operating parameters, and certain membrane characteristics. Imbalances in these parameters often result in inefficient separation, caused by an increase in protein concentration on the membrane surface. Depending on the macromolecules, a high osmotic pressure can develop at the membrane-solution interface, which would reduce the effective driving force for flux, promoting fouling. For high molecular weight proteins, the effect of osmotic pressure is less important [11, 14, 16].

Within this context, membrane separation processes can be characterized based on two parameters: the permeate flux, which represents the flux rate (volumetric, mass, or molar) of permeate per unit area of the membrane, and the selective capacity of the membrane, which, depending on the type of process in question, can be defined in different ways. In processes where the driving force is the pressure gradient, the rejection coefficient (defined by the ratio between the concentration of the species in the feed and in its permeate) is used to define the selective capacity of the membrane in comparison to a given species. A rejection coefficient of zero indicates that the species is not selectively retained by the membrane. In contrast, a coefficient approaching one indicates substantial or complete retention of the species under the evaluated conditions [12, 14].

Furthermore, the retention ability of UF membranes is defined based on their molecular weight cutoff (MWCO). Although this is an arbitrary choice when other conditions are considered, such as feed concentration, transmembrane pressure, pH, among others, the MWCO has been adopted by most of the UF community, as it provides a good starting point. Since the development of UF membranes in the 1960s, thousands of different membranes have been commercialized. Most membranes are composed of two or more layers: a surface layer of approximately 10 μm , conferring selectivity to the membrane, and the others of a microporous nature, conferring mechanical support, with the pore size progressively increasing [16].

UF membranes are also classified according to the material used in their fabrication: organic, ceramic, and metallic. Among them, organic membranes have a lower cost and a well-established process of fabrication. However, they are characterized by having low mechanical resistance, short life period, and are more susceptible to membrane fouling. Moreover, organic membranes include polyethylene, polysulfone, and polyvinylidene fluoride, of which the last one has the highest antifouling capacity. Although they are expensive and difficult to produce, ceramic and metallic membranes present better mechanical and resistance properties in comparison to organic membranes [16].

Metallic membranes are easier to recover from fouling than organic ones. The membrane used in UF is of paramount importance to the process, considering that fouling has become a major obstacle limiting the development of membrane bioreactor applications (MBR) [15].

Despite advances in membrane technology, protein purification by UF remains challenging in complex systems. Further research is needed to improve membrane selectivity and to optimize operating conditions that may affect protein stability and separation performance [16].

Despite these challenges, UF stands out as an efficient and easily scalable separation technique for applications involving proteins. Its performance, however, depends strongly on membrane characteristics, feed composition, and operating conditions.

Although protein concentration is a routine and successful operation in bioprocesses, protein fractionation or purification remains a technological challenge and requires further research effort. Currently, it is known that separation based solely on molecular size is not effective in cases where the proteins to be separated have very similar molar masses. However, many studies show that manipulating solute-solute and solute-membrane interactions allows the separation of proteins with similar molar masses using high-performance processes, still based on membrane separation.

The main reasons for using membranes in different processes include their classic advantages, such as the possibility of separating molecules in a customized and inexpensive way, consistent product quality requiring minimal intervention, minimization of temperature damage, recycling possibilities and low effluent generation, moderate energy consumption, low pressure drop, minimization of shear stress, absence of solvents and phase changes [16]. However, despite these advantages, UF performance can be limited by membrane fouling, which reduces permeate flux and increases resistance to filtration.

Membrane Fouling Mechanisms

During filtration, permeation resistance is increased by the inherent accumulation of retained particles on the membrane surface (CP) and by the deposition of particles on the membrane surface or within the membrane pores (fouling) [13]. The solute concentration on the wall can reach its solubility limit and solute precipitation on the membrane surface can occur, forming a gel layer [11].

CP is a reversible phenomenon caused by an increase in the concentration of solutes rejected by the membrane in the region near its surface. After a certain time, steady-state conditions will be established and the accumulation of solute or particles on the membrane surface can affect permeate flux in different ways. First, the decrease in permeate flux can occur mainly due to the creation of an osmotic flux that counteracts the pressure gradient caused by the accumulated solute. Although some proteins can increase this osmotic flux through high concentrations achieved on the membrane surface, this effect is more common in low molecular weight solutes. In addition, it is possible that a gel layer may form due to the high solute concentration, again creating resistance. Another important factor is the accumulation of solute, which, when carried to the surface by mass convection, can lead to the formation of a concentration gradient in the membrane, opposite to the pressure gradient. The concentration gradient can increase the transport resistance in the direction of the permeate, since it causes the diffusive movement of solutes from the membrane surface to the interior. Thus, in the UF process, this phenomenon is more pronounced due to the high solvent flux [16].

The decline in flux can be caused by several factors, such as the phenomenon of osmotic pressure, CP, solute adsorption, gel layer formation and pore clogging. All these factors induce additional resistances to transport across the membrane. The extent of this phenomenon is strongly dependent on the type of membrane process and the feed solution used [11, 16].

Fouling is a phenomenon in which the membrane adsorbs or interacts in some way with the solute in the feed stream, resulting in a decrease in flux, an increase in solute rejection by the membrane, or both. Unlike the polarized gel layer, fouling is generally neither reversible nor time-dependent. As a result of the interaction between the membrane, the adsorbed solutes and the solutes present in the feed stream, fouling can occur. The three factors that trigger this process are the properties of the membrane's constituent material, the properties of the solute and the operating parameters [14, 16].

How Proteins Interact With Each Other and With the Membrane

The phenomenon of membrane fouling is directly related to the interactions between proteins and between proteins and the membrane. A recent study [17] explains that these non-covalent interactions can influence membrane permeability during the process. Molecular and computational approaches have been used to investigate protein–membrane interactions and to improve the understanding of the forces governing adsorption. In addition, comprehending these interactions depends directly on the characterization of the surface charges of the involved systems. Protein charges, for example, are determined by their origin and arrangement of the exposed amino acids, as well as the condition of the environment, such as the solvent and pH. In that regard, it is important to characterize the surface in contact with the protein, as this is an important factor effectively evaluating these charges and subsequent long-range interactions [17, 18].

From a physicochemical perspective, interactions between proteins and membrane surfaces can be described by several contributions, including steric repulsion, electrostatic interactions, and van der Waals forces. The repulsion term is associated with the interaction between electronic clouds, while the electrostatic term refers to the Coulomb interactions among the charges in the proteins and the membrane, being directly influenced by the dielectric properties of the medium. Meanwhile, hydrophobic interactions are represented by van der Waals forces, generally affected by the potential of Lennard-Jones. Together, these contributions define the overall interaction potential responsible for protein adsorption [17].

Moreover, several factors can alter these interactions, and among them, the pH of the solution has an important role as it can affect the surface charges of proteins and their interaction with the surface of the membranes. In this context, the effect of pH on fouling varies with the pH level. When the pH is equal to the isoelectric point of the protein, membrane fouling is very high [18]. Thus, flux reaches a minimum at this pH and can increase at both lower and higher pH values, for which several explanations have been proposed [17, 18].

Changes in pH can alter the protonation state of protein functional groups and consequently modify protein charge. These changes can affect electrostatic interactions at the molecular level and influence protein–protein and protein–membrane interactions [17, 18].

All these modifications in the protonation state can influence the charge properties of the protein being filtered, which alters the interactions between proteins and membranes. Therefore, the consequence of

these variations is that the behavior of proteins is pH-dependent, especially near the isoelectric point. In this condition, decreasing electrostatic repulsion should favor aggregation and further increase the hydrophobicity of proteins, which will ultimately result in a decrease in flux. Thus, the simultaneous action of fouling mechanisms and protein properties (which vary according to system conditions) modify the final flux behavior and, mainly, retention in UF [18].

Although research on fouling shows more single protein systems, extracting proteins like those from Spirulina in real-world processes involves much more complex biological mixtures [19], as species generally mix with each other. In the present study, protein transmission may differ from that pre-established in single proteins, and for this reason it is necessary to understand protein-protein interactions and develop techniques that are more efficient.

Given this scenario, a study [19] shows that these interactions can alter separation performance. One example is the presence of proteins with completely opposite charges, which can lead to electrostatic attraction and the formation of complexes, causing lower protein transmission.

Ionic strength can modify electrostatic interactions by screening the charges surrounding proteins. Increased ionic strength may therefore reduce electrostatic repulsion and influence protein aggregation and deposition on membrane surfaces [20].

Therefore, membrane fouling during microalgae protein purification in multicomponent systems cannot be characterized simply as an extension of single-protein behaviour. Instead, it is governed by complex interactions between different species, and interspecific interactions may dominate the overall behaviour, resulting in fouling patterns that differ from those observed in simple systems [21].

Factors Affecting Fouling

As filtration proceeds, membrane performance can change during protein extraction, with a common flux-versus-time behavior characterized by a decrease in flux. This phenomenon occurs precisely due to membrane fouling, characterized by the deposition of residues on the membrane surface. Furthermore, the concentration of solute molecules accumulated at the membrane can become very high, exceeding the solubility limit of the medium and forming a gel layer on the surface. This phenomenon occurs mainly when the solution contains proteins in its composition; it is also possible that some residues penetrate the membrane directly and block the pores [22, 23, 24].

Furthermore, the adsorption process can occur in the outermost layers of the membrane, as well as within the membrane pores. The occurrence of these phenomena varies according to the membrane process and feed solution used. That is, regardless of the solution, fouling will depend on many physical and chemical factors, such as concentration, temperature, pH, ionic strength and specific interactions such as hydrogen bonding and dipole-dipole interactions [25, 26].

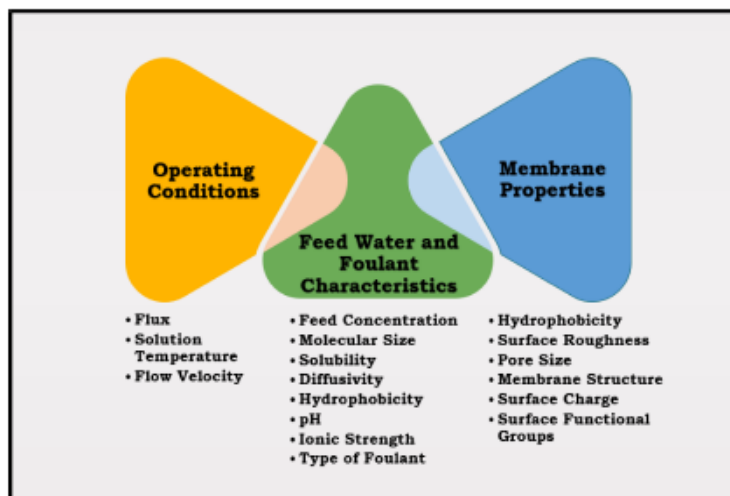


Figure 2: factors influencing membrane fouling formation during UF, including operating conditions, membrane characteristics, and feed-solution properties [44].

In addition, there are several factors that can modify the results of the protein extraction process during UF. First, resistance to deposition depends on the pore size of the membrane; the larger the pore, the lower the resistance to deposition [27].

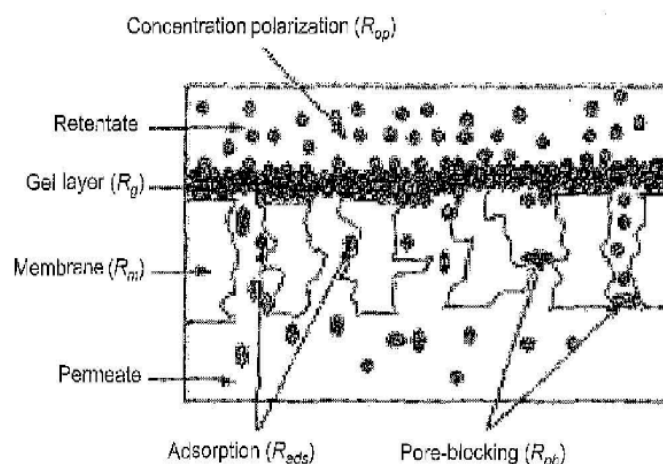


Figure 3: overview of the main resistances to mass transport in UF, illustrating how membrane and fouling-related resistances can affect permeate flux[11].

Furthermore, the hydrophobicity of the particles must also be considered as a relevant factor since it can influence their deposition and interactions within the fouling layer. Hydrophobic particles have a greater tendency to aggregate, which may result in the formation of a less dense gel layer than that formed by hydrophilic particles. For intense fouling to occur, the presence of both proteins and hydrophilic particles

may favor the formation of a denser fouling layer compared to a mixture of hydrophobic particles and proteins [28, 29, 30].

Finally, protein adsorption within UF pores can significantly reduce the effective pore size of the membrane. In this way, the adsorption phenomenon will always depend on the extension and the force of the interaction, which is controlled by physical and chemical factors of the protein, the adsorption surface and the solvent. Moreover, electrostatic interactions directly affect the protein adsorption rate, with the highest adsorption occurring near the isoelectric point (where it is electrically neutral). On the other hand, it is understood that the dependence of adsorption on pH is determined by the relative contribution of intramolecular hydrophobic interactions to the stabilization of the protein structure when dissolved, demonstrating its high variability. Thus, adsorption increases with increasing protein concentration and substantially reduces the membrane's water permeability [31, 32].

The hydrophilicity and hydrophobicity of a membrane are generally characterized by the contact angle θ . The higher the value of θ , the greater the hydrophobicity of the membrane surface. The value of θ is related to the morphology of the membrane surface and the size of the membrane pores. Furthermore, the hydrophilicity of the membrane material can alter its antifouling performance, and for this reason hydrophilic membranes are less affected by adsorption [15, 31, 32].

However, researchers have concluded that more hydrophilic polyethersulfone (PES) membranes suffer from more severe fouling, which may be related to the maximum pore opening of the PES membrane. It is worth noting that the hydrophilicity and hydrophobicity of the membrane generally only have a significant effect on membrane fouling in the initial stage of filtration. After initial fouling, the chemical properties of the fouling agents will replace the chemical properties of the membrane itself as the main influencing factor [15].

When the surface charge of the membrane is equal to the charge of the product to be filtered, this can increase the membrane flux. Therefore, if a product with a charge similar to that of the membrane is to pass through the UF process, this can reduce membrane fouling due to the repelling effect of similar charges [15].

The degree of membrane fouling worsens with prolonged operating time, resulting in an increase in transmembrane pressure difference and a decrease in water flux. In addition, the smaller the membrane pore size, the slower the increase in transmembrane pressure difference and the longer the membrane cleaning cycle time; the larger the membrane pore size, the more severe the fouling and the shorter the cleaning cycle time. However, due to the high porosity of the membrane surface and the interwoven fiber network-like pore structure, the membrane can be cleaned effectively, the membrane performance can be effectively restored after repeated cleanings, and the membrane water flux undergoes minimal changes [33, 34, 35].

Fouling in Complex Biological Systems

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Membrane fouling by solutions containing dissolved proteins is a problem caused by UF treatment in the food processing industry. One of the main problems in pressure-driven membrane processes is the reduction of permeate flux to values well below the theoretical capacity of the membrane. The typical variation in flux over time consists of a rapid initial drop, followed by a long and gradual decline. One of the main causes of this decline is membrane fouling, which directly affects UF performance due to the deposition of a layer on the membrane surface or the total or partial blockage of pores [36, 37].

The major challenge today is to prevent or reduce membrane fouling. Since fouling has many different characteristics, it is important to adapt measures to a specific process. In this sense, it is important to consider several points, such as altering the membrane flux regime, modifying the external membrane surface, and cleaning it. For these reasons, it is normal to characterize the filtration behavior and conduct more experiments with transmembrane pressure to study the variation of this flux over time and under many different conditions. However, the interpretation of data from experiments with constant pressure often causes problems, since the variation in flux produces variable conditions (of concentration, rheology, solubilities, etc.) in the boundary layer throughout any experiment [37]

The interactions between macromolecular and particulate components in complex fluids can result in unexpected and rapid changes in fouling. The kinetics and number of adsorbed macromolecules will determine the initial phase of fouling and the progressive closure of the pores or membrane surface [38].

A common practice to experimentally determine the critical flux in complex fluid systems is to establish a percentage increase in flux over a fixed time period. At low fluxes, the transmembrane pressure remains relatively stable, with little fouling occurring, and at an ever-increasing rate of increase in the transmembrane pressure (TMP) at fluxes exceeding the critical values. Since the zero rate of TMP increase is generally not reached in the filtration of complex fluids, it is not possible to define a critical flux according to its strictest definition [38].

In fluids with macromolecules and particles, membrane fouling occurs even at low flux rates and becomes significantly accentuated when the so-called critical flux (in its weak form) is reached. Although seemingly simple in principle, identifying the critical flux value from flux variation experiments strongly depends on the conditions used. For complex fluids, fouling interactions of the colloidal component are affected by the potential for small macromolecules to penetrate and adsorb onto the membrane structure and the fouling layer structure. On the other hand, colloids or particles can affect the initial position of macromolecules, adsorbing them onto their surfaces or providing a secondary layer that traps these aggregated macromolecules [38].

In fluids where several classes of solutes and particles are present simultaneously, the dispersive mechanisms can be represented by several equations. However, the mechanisms do not have the same magnitude for all classes of solutions/particles, especially in relation to their size and surface charge. In addition to transport aspects that can influence and determine fouling, as they are variable in the UF

system, favoring or preventing fouling, an increase in the concentration of macromolecules, colloids, or particles due to accumulation in the boundary layer leads to drastic changes in the collective properties of these particles: their diffusion coefficient, osmotic pressure, and, moreover, their stability are altered. Note that, even for fluxes below the defined steady state, the fluid near the membrane can reach a concentration such that its stability is compromised, which locally initiates the formation of aggregates that, in turn, soon deposit on the membrane [39].

The Challenges of Spirulina

The analytical characterization of Spirulina harvested using a submerged UF membrane system showed that the chemical composition of Spirulina cultivated in mineral medium was crude protein, 72.5%; carbohydrates, 8.3%; total lipids, 6.3%; chlorophyll, 4.8%; and ash, 5.2%. The biomass typically consists of carbohydrates, proteins, and nucleic acids, as previously obtained through Fourier Transform Infrared Spectroscopy (FTIR) analysis on encrusted membranes [40].

Small particles and macromolecules classified as soluble microbial products or extracellular polymeric substances play an important role in membrane fouling, as they tend to deposit on the membrane surface due to low backtransport velocity and strong static interaction with membrane surfaces. The accumulation of an algal cake layer and the adsorption of algal organic matter cause membrane fouling [40].

Spirulina can grow in a variety of culture media and reach high population densities in tropical and subtropical ecosystems, being considered an emerging source of organic matter [41]. Furthermore, its morphology presents unique characteristics, which can influence the growth of this microalgae in different ecosystems.



Figure 4: morphology of Spirulina (*Arthrospira platensis*) observed by optical microscopy at 400× magnification, illustrating its filamentous structure relevant to its behavior during membrane filtration.
Photo by G. Caretta [7].

In microalgal processing, membrane technologies can support biomass concentration and the recovery of valuable compounds. However, membrane fouling can limit these applications by reducing permeate flux and increasing cleaning requirements [42].

Membrane technologies have been tested for microalgae processing, as they enable biomass concentration and the recovery of value-added compounds [42]. For solutions derived from *Spirulina* microalgae, the efficiency of multicomponent separation using membranes is affected by fouling. This phenomenon is triggered by an increase in the concentration of retained multicomponents near the membrane surface—a phenomenon known as concentration polarization.

Continuous accumulation can lead to adsorption, pore blocking, and the formation of cake or gel layers. Such deposits increase mass transfer resistance and can progressively reduce permeate flux [40, 41, 42]. Furthermore, the complex composition of *Spirulina*-derived streams implies that different classes of compounds can interact with one another and with the membrane surface, potentially altering the structure and properties of the fouling layer.

The literature on algal membrane processes indicates that fouling can increase cleaning requirements, energy consumption, and processing time [42]. The severity and dominant mechanism, however, depend on the species, feed composition, membrane characteristics, and operating conditions. Consequently, fouling during *Spirulina* processing should be considered as a system-dependent phenomenon rather than as a single mechanism. This consideration provides a basis for evaluating the mechanisms and mitigation strategies discussed in this review.

Therefore, during the harvesting and concentration of *Spirulina* by ultrafiltration, fouling results from the progressive accumulation of retained cells, proteins, polysaccharides, extracellular polymeric substances, and other organic materials near and on the membrane surface. This accumulation initially promotes concentration polarization and may subsequently lead to adsorption, pore blockage, and the formation of cake or gel layers. As these phenomena develop, membrane resistance increases and permeate flux decreases, making the process progressively less efficient and increasing the need for cleaning and operational control [40, 41, 42]. This behavior is particularly relevant for *Spirulina* because its multicomponent composition can promote simultaneous interactions between different classes of foulants and the membrane surface.

Fouling Mitigation Strategies

Membrane fouling is widely known as a major challenge in membrane separation processes, as it directly affects process efficiency [43]. This phenomenon mainly limits current applications such as *Spirulina* extraction, where there is still a significant lack of in-depth studies on possible mitigation techniques [43]. Membrane fouling is the accumulation of residues on the membrane surface during UF, and this phenomenon can be affected by many different factors such as operating conditions and characteristics of the feed solution [43, 44]. Given this scenario, the search for mitigation strategies has been extensively studied, and they can be classified into membrane modification and optimization of operating conditions [44]. Regarding materials, hydrophilic membranes have been chosen because they have proven to be more effective, since they can form a layer of water on the membrane surface that prevents particle adsorption

and can decrease the fouling rate [3]. In addition, strategies based on membrane modification consist, for example, of incorporating hydrophilic nanoparticles, as these can improve performance and increase permeate flux, which reduces contaminant deposition [43,44].

At the same time, strategies for optimizing operating conditions are being developed and widely used to mitigate fouling [43, 44, 45]. Techniques such as increasing shear on the membrane surface, inducing turbulence, and using instabilities in the flux can greatly reduce CP and thus minimize residue accumulation [45]. Other approaches may include operating below the critical flux and using reverse flux, as these help to recover membrane performance and reduce flux loss [45]. Finally, there are also some physical methods that are used, such as backwashing and backpulsing, which can promote the removal of waste particles that impair the protein extraction process and wet permeability over time [43, 44, 45].

In addition, pulsating flux techniques and the use of turbulence promoters (which are advanced hydrodynamic techniques) have also shown significant progress in permeate flux, as these destabilize the fouling layer and increase mass transfer [45]. The air sparging technique, which consists of using air or gas in the system, has also been reported as an effective approach, like backwashing and backpulsing, it promotes the removal of deposits and manages to increase permeate flux [45].

Therefore, to mitigate the membrane fouling phenomenon, an integrated approach may be beneficial, combining the appropriate selection of materials, membrane modification, and control of processing conditions. The possible application of these strategies can improve the final performance of the process and also facilitate the cleaning of Spirulina membranes, which will make the processing more efficient and economically viable [45].

RESULTS

General Impact of Membrane Fouling on UF Performance

The literature review showed that membrane fouling can impact UF performance by decreasing permeate flux over time [5, 36, 37]. In principle, the three highlighted stages were rapid initial adsorption through protein-membrane interactions; intermediate pore blockage due to gel formation in the upper membrane layer; and, finally, long-term stabilization at low flux [5, 41]. This problem results in reduced protein recovery efficiency, increased operating costs, and higher energy demand [5].

CP as a Precursor to Fouling

The formation of a boundary layer of accumulated solutes due to CP leads to an increase in osmotic pressure, a reduction in the driving force for filtration, and a tendency towards the formation of a gel layer

[11, 13, 16]. The studies selected for this review address CP as a reversible step, but also as a facilitator for irreversible fouling [13, 16].

Role of Protein–Membrane and Protein–Protein Interactions

The selected studies indicate that protein–membrane and protein–protein interactions are important contributors to fouling development. These interactions were associated with adsorption, aggregate formation, and changes in separation performance, particularly in systems containing multiple components [17, 19, 20, 21].

Differences Between Single-Protein Systems vs Complex Mixtures (Spirulina)

The studies selected for this review indicate that the behavior observed in single–protein models cannot be directly extrapolated to Spirulina systems. In Spirulina, the simultaneous presence of proteins, polysaccharides, and extracellular polymeric substances can promote complex interactions and aggregate formation, making fouling behavior more difficult to predict [19, 21, 40, 41].

Main Factors Affecting Fouling

The scientific studies selected for this review point to three main factors for fouling: operational parameters, membrane properties, and feed characteristics [14, 16, 25, 26].

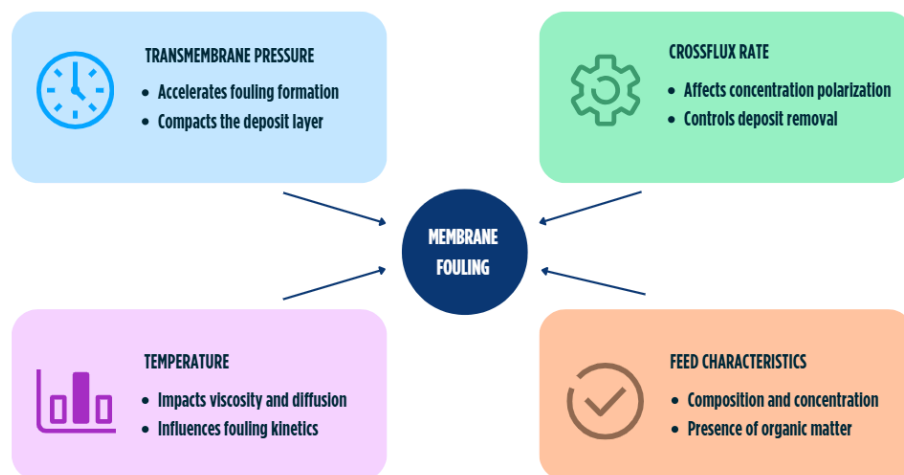


Figure 5: main operational and feed-related factors influencing membrane fouling during UF.

Among these factors, feed characteristics, particularly concentration, pH, and ionic strength, were consistently associated with changes in fouling behavior [17, 18, 20, 25, 26, 31, 32]. Operational parameters, including transmembrane pressure, crossflux rate, and temperature, also influenced fouling

development, while membrane properties such as pore size, hydrophobicity, hydrophilicity, and membrane material affected process performance [8, 15, 16, 27]. The selected studies also indicated that critical flux is more difficult to define in complex fluids such as microalgae systems [36, 37, 38].

Economic and Operational Impacts

The selected studies indicate that membrane fouling also has important economic and operational consequences for UF. Increased cleaning frequency, membrane replacement, and energy consumption can contribute significantly to operating costs, particularly in complex systems such as Spirulina [5, 42].

Identified Mitigation Strategies

Membrane fouling mitigation strategies were mainly classified into four types: membrane modification, operational adjustments, hydrodynamic techniques, and cleaning methods [43, 44]. Membrane modification approaches involve using hydrophilic materials and incorporating nanoparticles, as these approaches can reduce fouling intensity and improve permeate flux [3, 43, 44]. Regarding operational strategies, controlling the transmembrane pressure, adjusting crossflux conditions, and operating below the critical flux stood out as approaches aimed at limiting the fouling growth rate [45]. At the same time, some hydrodynamic techniques such as turbulence promoters, air sparging, and pulsatile flux were highlighted for their ability to increase mass transfer and also reduce residue accumulation during UF [45]. Furthermore, physical methods such as backwashing and backpulsing have been frequently reported in the literature as effective approaches for restoring membrane performance over time [43, 44, 45].

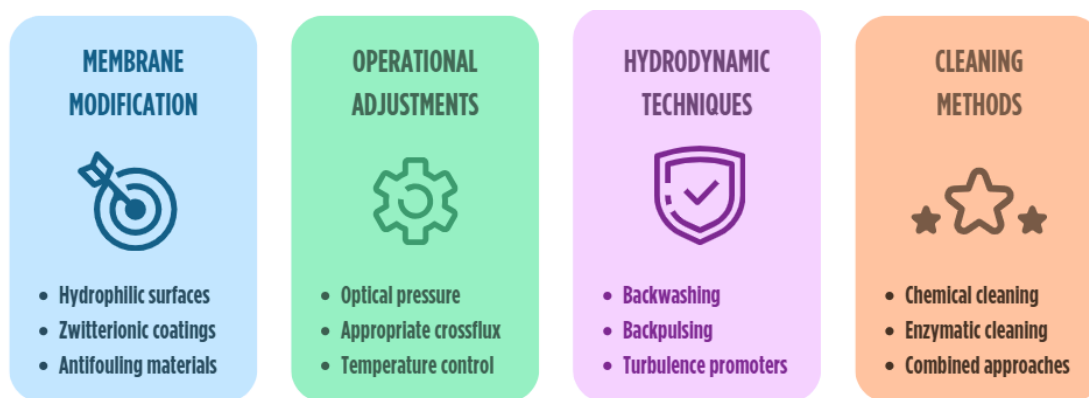


Figure 6: main membrane fouling mitigation strategies identified in the literature, including membrane modification, operational adjustments, hydrodynamic techniques, and cleaning methods.

However, it is important to highlight that the literature does not present these strategies in isolation, but always in combination, as combined techniques make it easier to achieve more promising results [45].

Emerging Approaches

The selected studies showed a strong association between membrane fouling mitigation and the integration of advanced methodologies and data-driven systems. Machine learning provides the basis for models that can analyze a series of high-dimensional datasets to detect fouling behavior even before severe fouling occurs. Furthermore, these models can also support the optimization of membrane materials and their operating conditions, the two main factors discussed during the literature review of this project. At the same time, the possibility of more quickly evaluating the various factors of this process through high-throughput screening systems and specific devices such as ultra-compact membranes was highlighted, as these can reduce the conventional trial-and-error approaches currently used in the industry. These methods have been described in the literature as recent developments aimed at improving the understanding of the relationships between membrane structure and performance and increasing process efficiency under complex UF conditions. Thus, these methods are recognized as recent technological approaches, which can improve understanding of the relationship between membrane structure and performance, as well as enhance the final efficiency of the process [8].

ANALYSIS

The results indicate that irreversible membrane fouling should not be interpreted as a unique phenomenon, but rather as a sequence of interdependent mechanisms driven by CP. Subsequently, mechanisms such as adsorption, pore blockage, and cake and gel layer formation can occur. These mechanisms, in turn, develop at a rate directly related to the three main fouling factors: operational parameters, membrane properties, and feed characteristics. In this context, it is necessary to evaluate how these mechanisms behave in different systems.

The membrane UF systems researched by the scientific community in this review mostly indicate simple, single-component systems. Simple systems favor experiments aimed at evaluating the interference of these variables in membrane fouling. However, in industrial applications, feed systems that require UF are commonly multicomponent systems, such as the Spirulina system considered in this review. The literature review indicates that protein-based models are insufficient to describe fouling behavior in Spirulina systems because of the complex interactions among the different components of the feed system and the consequent unpredictability of the process. Further studies are needed to vary the factors that influence membrane fouling in Spirulina UF and identify the most influential factors.

The pH stands out as an important and highly sensitive variable among those mentioned in the literature. At the isoelectric point, electrostatic repulsion decreases and protein interactions are intensified. Thus, fouling may become more pronounced under these conditions. Intermolecular interactions can also influence residue deposition and membrane pore blockage. These interactions include protein–protein and protein–membrane interactions, which have been highlighted in the literature. Finally, it is also necessary to highlight the operational parameters. The first parameter highlighted was high transmembrane pressure,

as it can accelerate fouling formation and compact the deposit layer. Furthermore, hydrophobicity mainly influences the initial stages, without direct long-term interference. These factors directly influence permeate flux behavior.

As reported in the literature, there is an identified pattern that permeate flux behavior generally follows during UF. First, there is a rapid initial decline caused mainly by protein adsorption on the membrane surface. After this, due to the formation of a fouling layer and the blockage of pores, a slower and more gradual decrease occurs, eventually leading to flux stabilization at lower values. Unlike simple systems, where critical flux is used to define a threshold below which fouling is almost irrelevant, in complex systems like Spirulina (which contains multiple components including proteins), fouling mechanisms occur simultaneously and in a non-linear fashion. For this reason, the transition between systems with high and low fouling rate becomes imprecise, and fouling can occur even under low flux conditions, limiting the true application of the critical flux concept. This behavior directly influences the operational and economic performance of the process.

Analysed Aspect:	Main Finding:	Reference:
Reduction in permeate flux	Reduced by pore blockage.	[5,36,37,41]
CP	Formation of a boundary layer due to CP that can increase osmotic pressure and reduce driving force.	[11,13,16]
Purification efficiency	Reduction in protein recovery and alteration in selectivity.	[5,14]
Operational costs	-Energy increase -Washing necessity -Membrane substitution	[5, 42]
Spirulina	Intensifies fouling.	[40,41,21,38,39]

Table 1: impact of membrane fouling on UF performance.

To mitigate membrane fouling and maintain process continuity, frequent cleaning cycles are necessary, leading to increased process costs and consequent economic problems. This also results in operational downtime and reduced productivity, as the process becomes slower and more expensive. Furthermore, the literature states that the need to maintain higher TMP to sustain the flux significantly increases the final energy consumption of the process, while irreversible fouling means that the membrane must be replaced due to its accelerated degradation. All these effects together increase the final cost of the protein extraction process from complex materials such as Spirulina. Therefore, fouling can compromise process efficiency and economic viability, highlighting the potential importance of control strategies.

The effectiveness of fouling mitigation strategies depends on their integration rather than isolated application. Mitigating membrane fouling in UF of multicomponent systems is not efficient when a

single, specific strategy is applied in isolation. This review suggests, based on the literature analyzed, that UF performance in multicomponent systems may be improved by integrating fouling mitigation strategies. In the specific case of Spirulina systems, the complexity of the system may contribute to more complex fouling behaviour. Thus, combined strategies may be beneficial for mitigating the effects of this phenomenon and improving flux stability. In this context, several technologies have gained prominence as promising tools, since they can improve fouling control.

Category:	Strategy:	Mechanisms of action:	References:
Membrane modification:	Hydrophilic methods	Formation of a water layer which can reduce adsorption.	[3,43,44]
	Nanoparticles incorporation	It improves the flux and enhances antifouling properties.	[43,44]
Operational optimization:	Operate below the critical flux	It reduces the fouling formation.	[45]
	Control the transmembrane pressure	Avoids the compaction of the fouling layer.	[45]
Hydrodynamic techniques:	Turbulence	Reduces CP.	[45]
	Air sparging	Remove deposits from the surface layer.	[45]
	Pulsatile flux	Destabilizes the fouling layer.	[45]
Physical cleaning methods:	Backwashing	Remove deposited residues.	[43,44,45]
	Backpulsing	Permeability restoration.	[43,44,45]

Table 2: Mitigation strategies for membrane fouling.

The technologies mentioned represent a transition from traditional trial-and-error approaches to predictive methods of control of UF systems. According to the literature, machine learning allows the analysis of a wide range of complex, high-dimensional datasets to simulate fouling behavior and, consequently, optimize operating conditions in the most efficient way. In this context, methods such as advanced screening systems and ultracompact membrane devices are gaining importance as they contribute to accelerating experimental evaluation, reducing study time, material consumption, and ultimately optimizing the process. Based on these studies, we understand that with the rise and application of technology in predictive control of UF, UF performance can potentially be optimized more efficiently and in a shorter timeframe.

DISCUSSION AND LIMITATIONS

The findings of this review suggest that membrane fouling is a major factor affecting the efficiency of Spirulina protein purification by UF. Unlike simplified protein systems, Spirulina contains multiple components that interact with the membrane and with each other, making fouling more complex and difficult to predict. In this system, fouling therefore needs to be considered in relation to feed composition, membrane properties, and operating conditions rather than as an isolated phenomenon.

In Spirulina UF, CP plays an important role because the accumulation of solutes near the membrane can increase local concentration and osmotic pressure, reducing the effective driving force for filtration. This condition can favor subsequent fouling mechanisms, including protein adsorption, pore blockage, and cake or gel layer formation. Thus, fouling should be understood as a dynamic process in which reversible and irreversible phenomena can interact and progressively reduce membrane performance [11, 13, 16].

Spirulina is not a simple feed for membrane filtration. Along with proteins, it contains polysaccharides, peptides, pigments, and extracellular substances. During UF, these different components may interact with the membrane surface and with one another, and some may also form aggregates. Because of this composition, results obtained with single-protein solutions may not describe what occurs in a Spirulina feed. This difference highlights the need to investigate fouling under multicomponent conditions [19, 21, 40, 41].

Membrane properties such as pore size, surface charge, and hydrophilicity can affect fouling, especially at the beginning of filtration. Proteins may attach to the membrane surface or enter its pores. Hydrophilic membranes can reduce protein attachment because water remains on the membrane surface. However, as material accumulates, a fouling layer forms and can change how the membrane behaves. This means that membrane properties alone may not be enough to control fouling. Combining membrane selection with appropriate operating conditions may be a more effective approach [17, 18].

Membrane characteristics such as pore size, surface charge and hydrophilicity mainly influence the initial stages of fouling, such as adsorption and pore blockage. An example is the use of hydrophilic membranes, which tend to reduce protein adhesion initially due to the formation of a surface hydration layer. However, once the fouling layer is established, the properties of the fouling layer alter the system and its behavior, causing the original characteristics of the membrane to become secondary. Therefore, this suggests that improvements in membrane design, although important, may not be sufficient in isolation. Instead, integrated approaches combining material selection with operational optimization may provide more effective fouling management.

Fouling also has direct implications for the feasibility of Spirulina protein purification. The reduction in permeate flux can increase energy consumption, cleaning frequency, and operational downtime, thereby increasing process costs [5, 42]. Therefore, controlling fouling is not only necessary to maintain UF

performance but may also be important for the economic viability of Spirulina protein production at larger scales.

The literature also indicates that fouling mitigation may be more effectively approached through complementary strategies rather than isolated interventions. Because fouling in Spirulina results from interacting mechanisms and variables, combining membrane modification, operational optimization, hydrodynamic control, and cleaning methods may provide more effective control of flux decline and deposit formation [43–45].

Emerging approaches such as machine learning and high-throughput screening may further support Spirulina UF by enabling the analysis of complex datasets and the prediction of fouling behavior. These approaches are particularly relevant to multicomponent systems, in which conventional trial-and-error optimization may be insufficient [8].

Even though the contributions of this project are significant, this study faced some limitations that need to be considered. Among them, it is important to highlight that as the project is a literature review, it depends on the scientific articles already published, and the majority of them study simple systems, which generally do not include Spirulina. Another limitation to consider is the lack of standardization in experimental conditions reported in the studies, which made it more difficult to compare different strategies and techniques. The lack of scientific articles focusing on microalgae UF was also a barrier, and when specifically related to Spirulina, the number of published studies is very limited. Finally, it is also necessary to highlight the fact that this study does not include experimental validation, and it will be included as a future step of the project.

CONCLUSION

The literature review indicates that membrane fouling presents a major challenge in the UF of Spirulina (*Arthrospira platensis*) proteins. Spirulina represents a complex multicomponent system where protein-protein and protein-membrane interactions lead to non-linear and often unpredictable fouling behavior. CP is the precursor to membrane fouling, followed by various interdependent mechanisms: protein adsorption, pore blockage, and gel or cake layer formation on the membrane surface. Factors such as pH, especially near the isoelectric point, ionic strength, protein concentration, operating conditions, and membrane properties directly influence these interactions in this complex system. Permeate flux and process efficiency can decrease as fouling progresses toward a more irreversible stage. The results of this review suggest that isolated mitigation strategies may be insufficient and that integrated approaches combining membrane modification, operational optimization, hydrodynamic techniques, and cleaning methods may provide more effective fouling control, while emerging technologies show potential for predictive optimization. Furthermore, the literature indicates that membrane fouling can increase energy consumption, cleaning frequency, and the need for membrane replacement, representing an important challenge during UF. Therefore, future perspectives of this research will be to experimentally analyze

different combined mitigation strategies to determine which combination is the most efficient for Spirulina, while also analyzing the technical and economic parameters to make this technology industrially viable.

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