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Synthesis of a Bioflocculant Using Blood Plasma: A Promising Approach for Sustainable Water Treatment

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Abstract

The exponential growth of urban centers and the scarcity of treated water underscore the urgent need for environmental stewardship. The rising demand for alternatives to replace inorganic compounds in water treatment presents a significant opportunity to develop sustainable methods. This study evaluates the use of blood plasma-derived bioflocculants as a promising alternative. The methodology involves the separation and chemical treatment of porcine, bovine, and poultry blood plasma through acid hydrolysis with 0.1 M sulfuric acid to denature proteins, followed by drying and grinding to obtain a fine powder. The clarifying agents were characterized using Point Zero Charge (PZC), Fourier Transform Infrared Spectroscopy (FTIR), and Thermogravimetric Analysis (TGA), while turbidity removal efficiency was assessed using Response Surface Methodology with Central Composite Rotational Design (RSM–CCRD). The clarifying agents exhibited isoelectric points of 6.41, 6.47, and 6.64 for porcine, bovine, and poultry plasma, respectively. FTIR analysis confirmed amide groups and functional groups –CH, –CO, –OH, and H–S(=O)$_2$–OH, demonstrating potential for coagulation. TGA results indicated good thermal stability between 40 °C and 400 °C. The findings revealed turbidity removal efficiencies of 95%, 93%, and 90% for the agents derived from porcine, bovine, and poultry plasma, respectively. These results highlight the potential for innovation in developing water clarifiers from slaughterhouse by-products.

1. Introduction

Water bodies, in general, play an extremely vital role in the economic development of a country, besides being essential for human activities and survival [1]. Human practices in the environment slowly and gradually cause alterations that, over time, become more pronounced in water sources, posing risks to public health, impacting the environment, and hindering economic and social growth [2].

Water treatment plays a crucial role in this scenario as it aims to eliminate pathogens, impurities, and harmful substances, ensuring that the water meets the standards set by health authorities [3]. Among the indicators of water quality, turbidity is an important criterion, usually related to the efficiency of physical treatments for solid removal, where suspended particles and dissolved algae hinder light penetration. Compliance with this parameter, which is an aesthetic criterion that impacts the acceptance or rejection of the product, can be ensured through clarification treatments, which involve water purification processes such as coagulation/flocculation and sedimentation [4].

These processes are controlled by the charge neutralization mechanism [5]. This neutralization occurs in the presence of a flocculating agent with a charge opposite to that of the colloidal particles. The added product adsorbs and neutralizes the charges of the suspended particles, reducing the zeta potential (ζ) and the repulsive forces between the colloidal particles. Thus, it facilitates the aggregation of the suspended material to form flocs [6].

In water treatment systems, both in Brazil and other countries, the most commonly used coagulants are derived from inorganic compounds, mainly trivalent iron and aluminum salts, as well as synthetic

polymers, as indicated by Choy et al. [7] and Franco et al. [8]. However, there are growing concerns about the potential environmental impacts of these coagulants due to the presence of non-biodegradable and potentially ecotoxic residues in both the treated water and the sludge generated during coagulation and flocculation processes, as reported by Yegambaram et al. [9], Freitas et al. [10], and Michelan et al. [11]. In response to these challenges, interest has emerged using of natural coagulants, which are seen as a viable alternative due to their biodegradability and low toxicity [4].

According to Essandoh et al. [12] and Tannouri and Simmons [13], hemoglobin, the most abundant protein in blood, and serum albumins, which are the most abundant proteins in blood plasma, have been recognized for their potential to form agglutinations with other materials. Koul et al. [14] and Nath et al. [15] also identified that these protein molecules play a fundamental role in coagulation and flocculation processes. This is related to the fact that the net charge of a protein is affected by the pH of the medium in which it is found. Piazza and Garcia [16] describe that the protein acquires a negative charge when the pH exceeds the isoelectric point. However, when the pH is below this point, the protein's net charge becomes positive, and due to this, Quintapana [17] suggests its use as a possible clarifying agent.

The work carried out by Koul et al. [14] and Nath et al. [15] aligns with projections for the production of bovine, porcine, and poultry meat in Brazil. Such projections suggest an approximate production of 33 million tons of meat by the end of 2029 [18]. This increase in production will result in a greater generation of hard-to-treat waste, such as blood. This amount will produce approximately 11 million tons of blood, which is usually disposed of without proper treatment [19, 20]. As cited by Wang et al. [21], blood accounts for 7% to 11% of slaughterhouse waste. The direct discharge of untreated blood into sewage systems increases the organic pollution load in wastewater by 35% to 50%, posing risks to the environment [20].

Thus, it is understood that the low utilization of this waste, especially in less qualified facilities, is a major concern. It is imperative to implement measures to avoid this waste, which results in significant environmental pollution, economic losses, and the diversion of raw materials [22].

Considering the aforementioned context, there arises a need to explore the feasibility of a clarifying agent based on animal blood plasma. In this context, this project aims to synthesize and evaluate the efficacy of bioflocculants derived from plasma extracted from bovine, porcine, and poultry blood in reducing the turbidity of aqueous solutions. This study will investigate the performance of different bioflocculant concentrations across various pH ranges, comparing their effects with one another. This approach seeks to provide sustainable and effective alternatives for water treatment, contributing to reducing environmental impact and promoting the responsible use of natural resources.

2. Methodology

2.1. Reagents

The reagents used in the experimental procedures included: EDTA tetrasodium ($C_{10}H_{12}N_2Na_4O_8 \cdot 3H_2O$) of analytical grade, supplied by the brand Êxodo Científica; calcium carbonate ($CaCO_3$) of analytical grade, supplied by Dinâmica Química Contemporânea LTDA; sulfuric acid (H_2SO_4) and sodium chloride (NaCl), both of analytical grade, supplied by Vetec Química Fina; sodium hydroxide (NaOH) of analytical grade, supplied by Reatec; and hydrochloric acid (HCl) 37% of analytical grade, supplied by ACS Científica.

2.2. Plasma Collection and Treatment

Porcine, bovine, and poultry blood was collected from slaughterhouses located in the western region of Santa Catarina – Brazil, using EDTA 4Na as an anticoagulant at a concentration of 1.5 g/L of pure blood, following the methodology adapted from Kundu et al. [14].

As cited by Rakesh et al. [15], the most conventional process for plasma separation is centrifugation. The blood plasma was separated immediately after collection in a FANEM centrifuge, model 206 BL, at a rotation speed of 3600 rpm for 20 minutes. After phase separation, the supernatant plasma was removed, and the solid residue of blood cells was appropriately discarded for subsequent treatment.

The bioflocculant was obtained using the acid hydrolysis technique, following the methodology adapted from Araújo et al. [16]. The plasma was placed in a jacketed reactor, and the pH was adjusted to 3.5 using 0.2 M sulfuric acid. Agitation was applied using a FISATOM – 713D agitator at a speed of 300 rpm. The solution remained in this state for 4 hours at a constant temperature of 60°C. Subsequently, the solution was neutralized to a pH of 6.0 using 0.1 M sodium hydroxide. The pH measurements were carried out using a KASVI pH meter, model K39-1420A. The remaining solution was placed in Petri dishes and dried in a LOGEN oven, model LSDH-9140A-220, for 20 hours at 60°C to remove moisture. After drying, an IKA grinder, model A11BS032, was used to grind the material into powder for subsequent analysis. The powder was stored in a sealed container at room temperature, protected from light. To standardize the nomenclature and facilitate the interpretation of the experimental data, the clarifying agents derived from bovine, porcine, and poultry plasma will be referred to as BCA (Bovine Clarifying Agent), PCA (Porcine Clarifying Agent), and PLCA (Poultry Clarifying Agent), respectively, throughout this study.

2.3. Point Zero Charge (PZC)

The Point Zero Charge (PZC) parameter indicates the pH at which the net surface charge of a material is zero, providing crucial information about the electrostatic interactions between the material's surface and charged species [17]. The methodology used for pHpzc determination was adapted from Hirendrasinh et al. [18]. A 0.01 M NaCl solution was prepared, and 100 mL of this solution was added to different Erlenmeyer flasks, adjusting each one to pH values ranging from 2 to 12 using 0.1 M HCl and 0.1 M NaOH solutions. Subsequently, 0.4 g of the flocculant was added to each flask. The solutions were allowed to react for 48 hours in a SOLAB Shaker SL-222 incubator at 130 rpm and room temperature. The pHpzc value was estimated from the intersection of the final pH in relation to the initial pH.

2.4. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis is used to study the secondary structures and functional groups of proteins, allowing detailed analysis of molecular interactions by correlating them with the vibrations emitted by chemical bonds [19]. The FTIR spectra of the flocculants were obtained using an IRSpirit (Shimadzu) spectrophotometer at a wavelength range of 4000 to 400 cm^{-1}. The dry material samples were analyzed using the ATR (Attenuated Total Reflectance) method, with a prior background evaluation (blank).

2.5. Thermogravimetric Analysis (TGA)

TGA is a technique used to determine the composition of materials, including the quantities of highly-volatile matter, medium-volatility materials, combustible material, and ash content (ASTM E1131-20). TGA was conducted on a mass of approximately 30 mg of pure plasma and bioflocculant from each type of blood, placed in an open alumina sample holder. The analysis conditions were carried out with a heating rate of 10°C/min, from 40°C to 600°C, under a nitrogen atmosphere at a flow rate of 50 mL/min.

2.6. Clarification Studies

The analyses to determine the ideal bioflocculant and its optimal performance parameters were conducted through experimental design using Response Surface Methodology (RSM) with Central Composite Rotational Design (CCRD), which determined the variation of concentrations and pH of the plasma samples selected for the study. The methodology used for the clarification study was adapted from Wang et al. [20] and Pacheco et al. [21], in which a standardized solution of calcium carbonate ($CaCO_3$) in water with a concentration of 7 g/L was prepared under agitation for 5 minutes, until complete homogenization.

Four hundred mL of the standardized calcium carbonate solution were transferred to 1000 mL beakers allocated in equipment specifically designed for the Jar Test method. The pH of the calcium carbonate solution was adjusted using 0.1 M sulfuric acid (H_2SO_4) and sodium hydroxide (NaOH) solutions. After the pH adjustment, a 15 mL aliquot of the standardized solution was collected to determine the initial turbidity using an AKSO Turbidity Max device. Next, the bioflocculant was added to the solution, and agitation was initiated at 160 rpm for 2 minutes to disperse the material in the solution. Subsequently, the system was maintained at 60 rpm for 5 minutes for floc formation. After this period, the agitation was turned off, and the samples were left undisturbed for 40 minutes to allow for the decantation of the particulate matter. After this decantation time, 80 mL aliquots were withdrawn for the final turbidity measurement. The process was performed using calcium carbonate samples adjusted to pH values of 4.2, 5.0, 7.0, 9.0, and 9.8, with varying concentrations of 0.3, 0.5, 1.0, 1.5, and 1.7 g/L, according to the experimental design. A sample of the calcium carbonate solution was maintained without the addition of bioflocculant to compare the effect of the obtained material with the natural decantation process of calcium carbonate.

3. Results and Discussion

3.1. Material Characterization

3.1.1. Point Zero Charge (PZC)

The pHpzc values of porcine (PCA), bovine (BCA), and poultry (PLCA) clarifying agents were approximately 6.41, 6.47, and 6.64, respectively, as shown in Fig. 1.

Figure 1 reveals that the pHpzc values are within a neutral range, with values close to 6.5. This result demonstrates that the PCA, BCA, and PLCA flocculants can be more efficient in removing anionic contaminants when the pH of the solution is above the pHpzc. This is because the surface of the flocculants under these conditions is positively charged, maximizing the attraction effects described by Coulomb's Law, as observed in Deng et al. [22], thus favoring the removal of anionic species. However, in acidic pH, flocculation efficiency is compromised due to the increase in electrostatic repulsion.

3.1.2. Fourier Transform Infrared Spectroscopy (FTIR)

Figure 2 illustrates the FTIR spectra for porcine, bovine, and poultry blood plasmas, along with their respective clarifying agents.

The general analysis of the infrared spectra reveals that the shape and position of the peaks remain consistent between different samples, indicating the structural stability of the protein components after acid hydrolysis treatment. This finding suggests that the process does not promote a complete breakdown of protein structures nor the significant formation of new functional groups, as noted by Wang et al. [19], with only a change in the intensity of the existing bands.

Deeper analysis of the FTIR spectra, as illustrated in Figs. 2a, 2b, and 2c, reveal that wavelengths near 3300 cm^{-1} indicate the presence of characteristic amide A bands, which are attributed to the stretching vibrations of –NH groups, as described by Singh et al. [23] and Kong et al. [17]. This evidence suggests the presence of partially preserved protein structures after treatment. Furthermore, additional studies by Garcia and Wilson [24] and Agbovi and Wilson [25] support these findings by reporting distinct oscillations around 2900 cm^{-1} associated with the stretching of $–NH_3$, –CH, $–CH_2$, and $–CH_3$ groups. These results imply the formation of the amide B band, indicating spatial changes in the structure of the treated proteins.

Studies by Wang et al. [19], Singh et al. [23], and Garcia and Wilson [24] also highlight the presence of multiple adsorption peaks in the infrared region, particularly within the amide I and amide II bands, which reveal significant details about the protein structures in question. Specifically, the amide I bands, located nearly 1650 cm^{-1}, are primarily attributed to the stretching vibrations of the C = O, –CO, and –COO– groups. On the other hand, the amide II bands, around 1500 cm^{-1}, are associated with the vibrations and shortening of the –CN groups. Both are observed with greater intensity in porcine and bovine plasmas, as well as in and clarifying agents, as illustrated in Figs. 2a and 2b. These findings are consistent with

previous studies conducted by Wei et al. [26] and Kong et al. [17], further reinforcing the reliability and consistency of the obtained results.

A significant increase in stretching around the 1100 cm^{-1} wavelength was also observed in Fig. 2a, indicating specific alterations in the FTIR spectrum for CS. Recent studies by Mishra et al. [27], Safdar et al. [28], and Singh et al. [23] emphasize that this behavior is closely related to the vibrations of the –CO, –C–OH, –COC, and H–S(= $O)_2$–OH groups. These findings suggest subtle structural modifications in plasma proteins. According to Saguer, Álvarez, and Ismail [29], the hydrolysis process at acidic pH, combined with exposure to high temperatures, promotes the partial denaturation of molecules. Meutter and Goormaghtigh [30] discuss how this process disrupts the secondary and tertiary conformations of the protein, altering its spatial arrangement.

The presence of carboxyl, hydroxyl, and amine groups in bioflocculant molecules is critical for determining their flocculation effect, as they promote suitable binding sites that attract dispersed particles in solution, thereby enhancing the flocculants effective in binding mechanisms between the particles and the flocculants [31–34]. Another significant factor that may improve flocculant activity is the introduction of sulfonic acid functional groups on plasma surfaces after acid hydrolysis, as indicated by peaks observed around 1110 cm^{-1} [35, 36]. According to Yu et al. [37], Feng et al. [26] and Artifon et al. [38], the presence of these groups in bioflocculants enhances their capacity to remove colloidal particles and suspended metals.

3.1.3. Thermogravimetric Analysis (TGA)

Thermogravimetric analysis (TGA) was conducted on flocculants and pure plasmas to elucidate their thermal responses at different temperature ranges. Figure 3 illustrates the TGA curves, emphasizing the mass losses associated with thermal decomposition, oxidation, and volatilization [25].

Examining Figs. 3a and 3b, it is observed that the initial mass loss occurs between 40°C and 270°C, primarily due to water desorption and the volatilization of low-molecular-weight compounds [24]. Between 270°C and 400°C, significant mass loss is attributed to the removal of moisture associated with hydroxyl and carboxyl functional groups [39]. The final stage of mass loss, observed above 400°C, results from the thermal degradation of the polymer matrix and the decomposition of organic material [24]. The average mass loss percentages for the three thermal zones in the flocculants were 16.27%, 45.21%, and 26.38%, respectively. In contrast, for pure plasma, the mass losses in the same zones were 19.05%, 49.41%, and 29.48%, respectively.

These data indicate that both the flocculants and pure plasmas exhibit three distinct stages of thermal degradation. The substantial mass loss observed between 270°C and 400°C suggests significant volatilization of components and structural dehydration. In contrast, the degradation occurring above 400°C reflects the disintegration of the polymer structure and the decomposition of organic constituents. The higher mass loss in pure plasma across all zones, compared to the flocculants,

indicates a composition with a higher volatile content and a more thermally susceptible organic structure.

3.2. Clarification Studies

3.2.1. Mathematical modeling

Given that the quality of effluent treatment is intrinsically linked to effluent parameters, such as pH, mathematical modeling emerges as a key resource for optimizing procedures and predicting the efficiency of these processes [40]. Based on the results obtained from the CCRD experimental design, an analysis was conducted using Minitab software for statistical evaluation and the development of a mathematical model that describes the relationship between bioflocculant dosage, its source, the characteristics of the effluent to be treated, and flocculation efficiency. The interactions between parameters, as determined through statistical analysis, are illustrated in Fig. 4 and Table 1.

Table 1
– *p-value* for analysis of variance (ANOVA).

	Linear			Square		Two-factor interaction		
Model	**C**	**pH**	**δ**	**C²**	**pH²**	**C x pH**	**C x δ**	**pH x δ**
0.019	0.777	0.026	0.174	0.989	0.003	0.990	0.956	0.022

C – Concentration; pH – Hydrogen potential; δ – Blood type.

Source: Elaborated By the authors.

Using ANOVA statistical analysis with a significance level of 5%, it was determined that the parameters that significantly influence the model are pH in its linear form, pH in its squared form (pH²), and the combination of pH and blood type (δ) through a two-factor interaction. Consequently, the mathematical models 1, 2, and 3, which define the efficiency (η) of the bioflocculant, are presented in Equations 1 to 3 for porcine, bovine, and poultry, respectively.

$$\eta_{porcine} = -0.659 + (0.358 \times pH) - (0.02306 \times pH^2) \quad (1)$$

$$\eta_{bovine} = -1.194 + (0.443 \times pH) - (0.02306 \times pH^2) \quad (2)$$

$$\eta_{poultry} = -0.472 + (0.360 \times pH) - (0.02306 \times pH^2) \quad (3)$$

3.2.2. Evaluation of Efficiencies Using the Response Surface Methodology (RSM)

The removal of suspended solids from the solution was evaluated through coagulation and flocculation processes using the Jar Test. Two primary effects were investigated to assess process efficiency: the variation in flocculant concentration and the pH of the medium. The results obtained through the RSM method are presented in Figs. 5, 6, and 7.

Based on the results presented, distinct performance behaviors for each of the evaluated clarifying agents are evident. Current legislation in various countries, including Brazil's Portaria GM/MS Nº. 888, the European Parliament Directive (EU) 2020/2184 [50], and the U.S. Environmental Protection Agency (EPA) Primary Drinking Water Regulations [55], indicates that fresh surface water typically has pH values ranging from 6.5 to 9.0. This range aligns with the experimental pH levels that demonstrated good turbidity reduction efficiency.

Analyzing Fig. 5, which illustrates the behavior of the PLCA, reveals a clear correlation between pH and product concentration. The optimal pH range for achieving efficiencies above 94% is between 7.5 and 8.2, with bioflocculant concentrations exceeding 1.3 g/L. Additionally, broader pH ranges, varying from 6.7 to 9.0, are noteworthy as they demonstrate efficiencies above 90% regardless of bioflocculant concentration. This behavior indicates that within this broader pH range, variations in clarifying agent concentration do not significantly affect treatment efficiency. Such robustness suggests valuable operational flexibility, allowing for adjustments in clarifying agent dosage without compromising process effectiveness. However, at pH levels below 6.5 or above 9.2, even changes in clarifying agent concentration fail to maintain the desired efficiency, which drops below 80%.

In case of BCA, as illustrated in Fig. 6, the most promising results are observed within more alkaline pH ranges, specifically between 8.0 and approximately 11.0, with efficiencies exceeding 88% across various concentrations. The optimal range is identified as being between pH 9.0 and 10.0, where efficiencies surpass 93%. These findings suggest that more alkaline environments enhance the optimized performance of BCA. Additionally, similar to the observations made for PLCA, the concentration of the flocculant within the optimal pH range does not significantly affect efficiency, thereby ensuring the robustness of the process. Conversely, when the bioflocculant is applied at pH levels below 7.5, there is a notable decline in efficiency, with values dropping below 70%.

In contrast to the previously presented results, the PCA emerges as an even more promising alternative, demonstrating higher efficiencies under similar pH conditions compared to other clarifying agents, while requiring lower concentration. As illustrated in Fig. 7(b), within the pH range of 7.2 to 9.5, variations in concentration do not significantly affect clarification efficiency, which consistently remains above 95%. However, it is noteworthy that within this pH range, concentrations exceeding 2.5 g/L achieve efficiency levels close to 100%. These findings are further supported by the analysis in Fig. 6(a), which highlights the relationship between pH and concentration in the clarification process, underscoring the importance of considering multiple factors when designing wastewater treatment strategies. Additionally, FTIR analyses validate these results, revealing more intense peaks for hydroxyl, carbonyl, and sulfonic acid functional groups in the bioflocculant derived from porcine plasma.

From Table 2, one can compare the optimal results achieved for each clarifying agent with the standardized calcium carbonate solution, in relation to the sample that did not have a flocculant agent added.

Table 2
– Efficiency of clarification as a function of medium pH, concentration and flocculant origin.

Clarifying agent	pH	Concentration – $g.L^{-1}$	Initial Turbidity – NTU	Final Turbidity – NTU	Efficiency (η) – %
Sample 0	8.2	–	453.0	412.0	9.10
Poultry	8.0	0.09	534.0	28.6	94.64
	8.0	1.50	534.0	29.0	94.57
	8.0	2.91	534.0	27.9	94.78
Bovine	8.0	0.09	452.0	23.5	94.80
	8.0	1.50	452.0	22.3	95.07
	8.0	2.91	452.0	27.9	93.83
Porcine	8.0	1.50	494.0	26.2	94.70
	10.0	2.50	456.0.	22.2	95.13
	8.0	2.91	439.0	26.8	94.03

The results obtained, as demonstrated above, reveal a promising performance of the proposed method in comparison to previous studies. For instance, the results from Medeiros [4], who investigated the efficacy of bioflocculants derived from *Moringa oleifera*, showed turbidity removal efficiencies of around 87%. Similarly, studies conducted by Amran et al. [52] reported turbidity removal rates of approximately 88% using products derived from *Carica papaya* seeds. Lee et al. [53] examined the clarification efficiency of kaolin/hematite using modified hemoglobin at different dosages and pH levels, discovering that the optimal dosage of hematite achieved a turbidity reduction of 99%. In another study, Lee et al. [54] analyzed native bovine blood and three types of chemically modified blood in comparison to kaolin and hematite suspensions, with polymerized bovine blood demonstrating an 81% precipitation rate with hematite suspensions.

It has been also observed that the combination of adding a clarifying agent to the solution, along with pH adjustment, significantly enhances the sedimentation process of colloidal particles present in the initial solution compared to the same solution without the clarifying agent. The bioflocculant prepared from porcine plasma (PCA) demonstrated high removal efficiency across a broad pH spectrum, achieving removal rates exceeding 95%. These results underscore the synergy between the clarifying agent's action and environmental conditions, highlighting the effectiveness of this combined approach for efficiently removing suspended impurities from surface water.

4. Conclusion

This study focused on the utilization of porcine, bovine, and poultry blood plasma for the synthesis of a bioflocculant applicable in water treatment. The characterization of the resulting clarifying agents was conducted using PZC, resulting in isoelectric points of 6.41, 6.47, and 6.64 for PCA, BCA, and PLCA, respectively. FTIR analyses indicate alterations in the spatial structure of the protein complexes, emphasizing the presence of amide groups such as and functional groups –CH, –CO, –OH, and H–$S(=O)_2$–OH on the peripheral regions of the molecules, which enhance the capacity to remove colloidal particles. The thermal stability of the materials was confirmed by TGA, demonstrating the thermal robustness of the synthesized compounds within a temperature range of 40 °C to 400 °C. According to ANOVA analysis, it was determined that only the origin of the blood and pH significantly affect the process efficiency, with optimal performance observed at alkaline pH levels, where the electrostatic affinity of the molecules facilitates the formation of flocs and the sedimentation of particulate matter.

The efficiency values obtained during the research exceeded 95%, 93%, and 90% for PCA, BCA, and PLCA, respectively. These results indicate the successful development of efficient bioflocculants derived from waste, produced through a simple and cost-effective method. Furthermore, the findings highlight the significant potential for research and innovation in the development of water clarifiers from plasma proteins, presenting a promising area for further research and process optimization.

Declarations

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References

1. Okumura, A. T. R., Silva, A. G., Silva, N. R. S., Lopes, E. R. N., Bifano, R. B. A., & Quilenato, R. V. (2020). Determinação da qualidade da água de um rio tropical sob a perspectiva do uso do solo e cobertura vegetal. *Revista Brasileira de Geografia Física, 13*(4), 1835–1850.
2. Coelho, F. R., De Rubin, J. C. R., & Silva, A. M. T. C. (2021). Análise de qualidade da água no alto curso do rio Meia Ponte entre 2013 e 2018. Revista EVS - Revista de Ciências Ambientais e Saúde,

47(1). https://doi.org/10.18224/evs.v47i1.8616

3. Lemos, K. S., Filho, S. Q. A., & Cavallini, G. S. (2020). Avaliação comparativa entre os coagulantes sulfato de alumínio ferroso e policloreto de alumínio para tratamento de água: estudo de viabilidade econômica. DESAFIOS - Revista Interdisciplinar da Universidade Federal do Tocantins, 7(1), 109–119. https://doi.org/10.20873/uftv7-7824
4. Medeiros, J. B. (2023). Uso da Moringa oleifera Lam como coagulante natural no tratamento de águas superficiais (Trabalho de Conclusão de Curso, Universidade Federal da Paraíba).
5. Wei, Y., Ji, Q., Chen, L., Hao, J., Yao, C., & Dong, X. (2017). Preparation of an inorganic coagulant-polysilicate-magnesium for dyeing wastewater treatment: Effect of acid medium on the characterization and coagulation performance. Journal of the Taiwan Institute of Chemical Engineers, 72, 142–148. https://doi.org/10.1016/j.jtice.2017.01.020
6. Macczak, P., Kaczmarek, H., & Ziegler-Borowska, M. (2020). Recent achievements in polymer bio-based flocculants for water treatment. Materials, 13(18), 3951. https://doi.org/10.3390/ma13183951
7. Choy, S. Y., Prasad, K. M. N., Wu, T. Y., Raghunandan, M. E., & Ramanan, R. N. (2014). Utilization of plant-based natural coagulants as future alternatives towards sustainable water clarification. Journal of Environmental Sciences, 26, 2178–2189. https://doi.org/10.1016/j.jes.2014.09.024
8. Franco, C. S., Batista, M. D. A., Oliveira, L. F. C., Kohn, G. P., & Fia, R. (2017). Coagulation with Moringa oleifera seed prepared by different methods in water turbidity of 20 to 100 UNT. Engenharia Sanitária e Ambiental, 22(4), 781–788. https://doi.org/10.1590/s1413-41522017145729
9. Yegambaram, M., Manivannan, B., Beach, T. G., & Halden, R. U. (2015). Role of environmental contaminants in the etiology of Alzheimer's disease: A review. Current Alzheimer Research, 12(2), 116–146. https://doi.org/10.2174/1567205012666150204121719
10. Freitas, J. H. E. S., Santana, K. V., Nascimento, A. C. C., Paiva, S. C., Moura, M. C., Coelho, L. C. B. B., Oliveira, M. B. M., Paiva, P. M. G., Nascimento, A. E., & Napoleão, T. H. (2016). Evaluation of using aluminum sulfate and water-soluble Moringa oleifera seed lectin to reduce turbidity and toxicity of polluted stream water. *Chemosphere, 163*, 133–141. https://doi.org/10.1016/j.chemosphere.2016.08.019
11. Michelan, D. C. G. S., Santos, W. N. A., Rosa, T. S., Santos, D. G., & Jesus, R. C. S. (2021). Uso do coagulante/floculante emergente à base de moringa no tratamento de água com verificação da composição e toxicidade do lodo produzido. Engenharia Sanitária e Ambiental, 26(5). https://doi.org/10.1590/S1413-415220200314
12. Essandoh, M., Garcia, R. A., & Nieman, C. M. (2018). Chemical and enzymatic protein cross-linking to improve flocculant properties. ACS Sustainable Chemistry & Engineering, 6(10), 13214–13222. https://doi.org/10.1021/acssuschemeng.8b02395
13. Tannouri, N., & Simmons, D. B. D. (2023). Characterizing the origin of blood plasma proteins from organ tissues in rainbow trout (Oncorhynchus mykiss) using a comparative non-targeted proteomics

approach. Comparative Biochemistry and Physiology Part D: Genomics and Proteomics, 46, 101070. https://doi.org/10.1016/j.cbd.2023.101070

14. Koul, B., Bhat, N., Abubakar, M., Mishra, M., Arukha, A. P., & Yadav, D. (2022). Application of natural coagulants in water treatment: A sustainable alternative to chemicals. Water, 14(22), 3751. https://doi.org/10.3390/w14223751
15. Nath, A., Mishra, A., & Pande, P. P. (2021). A review on natural polymeric coagulants in wastewater treatment. Materials Today: Proceedings, 46(14), 6113–6117. https://doi.org/10.1016/j.matpr.2020.03.551
16. Piazza, G. J., & Garcia, R. A. (2016). Biobased flocculants derived from animal processing protein by-products. In Z. Berk (Ed.), *Protein Byproducts* (pp. 135–146). Academic Press. https://doi.org/10.1016/B978-0-12-802391-4.00008-2
17. Quinapanta, E. G. V. (2021). *Obtención de un clarificante orgánico a partir de plasma sanguíneo bovino, ovino y porcino...* (Trabalho de Conclusão de Curso, Universidad Central del Ecuador).
18. MAPA. (2019). *Projeções do agronegócio: Brasil 2018/19 a 2028/29: Projeções de longo* prazo (126 p.). Ministério da Agricultura, Pecuária e Abastecimento. ISBN 978-85-7991-127-9
19. Langone, M., Ferrentino, R., Freddi, F., & Andreottola, G. (2019). Anaerobic digestion of blood serum water integrated in a valorization process of the bovine blood treatment. Biomass and Bioenergy, 120, 1–8. https://doi.org/10.1016/j.biombioe.2018.10.015
20. Nazifa, T. H., Saady, N. M. C., Bazan, C., Zendehboudi, S., Aftab, A., & Albayati, T. M. (2021). Anaerobic digestion of blood from slaughtered livestock: A review. Energies, 14(18), 5666. https://doi.org/10.3390/en14185666
21. Wang, S., Hawkins, G. L., Kiepper, B. H., & Das, K. C. (2018). Treatment of slaughterhouse blood waste using pilot scale two-stage anaerobic digesters for biogas production. Renewable Energy, 126, 552–562. https://doi.org/10.1016/j.renene.2018.03.076
22. Velarde, A. et al. (2018). *Guia de boas práticas: Insensibilização por gás em suínos*. Diálogos - União Europeia - Brasil, [s. l.], p. 22.
23. Rakesh, P. E., Maria, M. S., Chandra, T. S., & Sen, A. K. (2017). Capillary flow-driven microfluidic device with wettability gradient and sedimentation effects for blood plasma separation. Scientific Reports, 7, 43457. https://doi.org/10.1038/srep43457
24. Araújo, L. F., Dias, M. V. C., Brito, E. A., & Júnior, S. (2009). Microbial production of protein from reservoir of acerola (Malpighia emarginata D.C) for animal feeding. Tecnologia & Ciência Agropecuária, 3(3), 47–53. https://doi.org/10.5935/1981-2965.20210019
25. Kong, D., & Wilson, L. D. (2020). Uptake of methylene blue from aqueous solution by pectin–chitosan binary composites. Journal of Composites Science, 4(3), 95. https://doi.org/10.3390/jcs4030095
26. Hirendrasinh, P., Thanki, A., Singh, N. K., Pandey, S., Yadav, M., & Yadav, T. C. (2020). Parametric and kinetic investigations on segregated and mixed textile effluent streams using Moringa oleifera seed powders of different sizes. Journal of Water Process Engineering, 34, 101159. https://doi.org/10.1016/j.jwpe.2020.101159

27. Wang, L., Ma, Y., Shen, R., Zhang, L., He, L., Qu, Y., Ma, X., Guo, Z., Chen, C., Li, H., & Kong, X. (2024). Effect of ultrasonic treatment on the physicochemical properties of bovine plasma protein–carboxymethyl cellulose composite gel. *Foods, 13*(5), 732. https://doi.org/10.3390/foods13050732
28. Wang, J., Zhang, Z., Liu, D., Li, J., Wang, X., Song, B., Yue, B., Zhao, K., & Song, Y. (2017). Hydrolysis of polyaluminum chloride prior to coagulation: Effects on coagulation behavior and implications for improving coagulation performance. *Journal of Environmental Sciences, 57*, 162–169. https://doi.org/10.1016/j.jes.2016.10.014
29. Pacheco, I. S., Alves, A. G. T., Santana, L. C., Ribeiro, E. A. M., Canobre, S. C., & Amaral, F. A. (2022). Performance of cationic hemicelluloses arising from peanut shell residue from agroindustry in application as primary coagulant in physical-chemical treatment of dairy wastewater. *Journal of Water Process Engineering, 47*, 102661. https://doi.org/10.1016/j.jwpe.2022.102661
30. Deng, Z., Huang, D., He, Q., & Chassagne, C. (2022). Review of the action of organic matter on mineral sediment flocculation. *Frontiers in Earth Science, 10*. https://doi.org/10.3389/feart.2022.965919
31. Singh, A., Benjakul, S., Prodpran, T., & Nuthong, P. (2021). Effect of psyllium (*Plantago ovata* forks) husk on characteristics, rheological and textural properties of threadfin bream surimi gel. *Foods, 10*(6), 1181. https://doi.org/10.3390/foods10061181
32. García, D. J. V., & Wilson, L. D. (2022). Utilization of bioflocculants from flaxseed gum and fenugreek gum for the removal of arsenicals from water. *Materials, 15*(23), 8691. https://doi.org/10.3390/ma15238691
33. Agbovi, H. K., & Wilson, L. D. (2018). Design of amphoteric chitosan flocculants for phosphate and turbidity removal in wastewater. *Carbohydrate Polymers, 189*, 360–370. https://doi.org/10.1016/j.carbpol.2018.02.024
34. Wei, W., Hu, W., Zang, X. Y., Zhang, F. P., Sun, S. Q., Liu, Y., & Xu, C. H. (2018). Analysis of protein structure changes and quality regulation of surimi during gelation based on infrared spectroscopy and microscopic imaging. *Scientific Reports, 8*, 23645. https://doi.org/10.1038/s41598-018-23645-3
35. Mishra, S., & Kundu, K. (2019). Synthesis, characterization and applications of polyacrylamide grafted fenugreek gum (FG-g-PAM) as flocculant: Microwave vs thermal synthesis approach. *International Journal of Biological Macromolecules, 141*, 792–808. https://doi.org/10.1016/j.ijbiomac.2019.09.033
36. Safdar, B., Pang, Z., Liu, X., Jatoi, M. A., Mehmood, A., Rashid, M. T., Ali, N., & Naveed, M. (2019). Flaxseed gum: Extraction, bioactive composition, structural characterization, and its potential antioxidant activity. *Journal of Food Biochemistry, 43*(11), e13014. https://doi.org/10.1111/jfbc.13014
37. Saguer, E., Álvarez, P., & Ismail, A. A. (2012). Heat-induced denaturation/aggregation of porcine plasma and its fractions studied by FTIR spectroscopy. *Food Hydrocolloids, 12*(1), 1–6. https://doi.org/10.1016/j.foodhyd.2011.06.012

38. Meutter, J. D., & Goormaghtigh, E. (2021). Protein structural denaturation evaluated by MCR-ALS of protein microarray FTIR spectra. *Analytical Chemistry, 93*(40), 13456–13462. https://doi.org/10.1021/acs.analchem.1c01416
39. Ayangbenro, A. S., Babalola, O. O., & Aremu, O. S. (2019). Bioflocculant production and heavy metal sorption by metal resistant bacterial isolates from gold mining soil. *Chemosphere, 231*, 113–120. https://doi.org/10.1016/j.chemosphere.2019.05.092
40. Vimala, R. T. V., Escaline, J. L., & Sivaramakrishnan, S. (2020). Characterization of self-assembled bioflocculant from the microbial consortium and its applications. *Journal of Environmental Management, 258*, 110000. https://doi.org/10.1016/j.jenvman.2019.110000
41. Yang, Z., Wang, W., & Liu, S. (2017). Flocculation of coal waste slurry using bioflocculant produced by *Azotobacter chroococcum*. *Energy & Fuels, 31*(2), 1460–1467. https://doi.org/10.1021/acs.energyfuels.6b03052
42. Tawila, Z. M. A., Ismail, S., Amr, S. S. A., & Elkhair, E. K. A. A. (2019). A novel efficient bioflocculant QZ-7 for the removal of heavy metals from industrial wastewater. *RSC Advances, 9*, 27825–27834. https://doi.org/10.1039/C9RA04683F
43. Lau, K. S., Azmi, N. A. S., Chin, S. X., Zakaria, S., & Chia, C. H. (2023). Chitosan-bead-encapsulated polystyrene sulfonate for adsorption of methylene blue and regeneration studies: Batch and continuous approaches. *Polymers, 15*(5), 1269. https://doi.org/10.3390/polym15051269
44. Pishnamazi, M., Ghasemi, S., Khosravi, A., Sahebi, A. Z., Zadeh, A. H., & Borghei, S. M. (2021). Removal of Cu(II) from industrial wastewater using poly(acrylamide-co-2-acrylamide-2-methyl propane sulfonic acid)/graphene oxide/sodium alginate hydrogel: Isotherm, kinetics, and optimization study. *Journal of Water Process Engineering, 42*, 102144. https://doi.org/10.1016/j.jwpe.2021.102144
45. Yu, Y., Sol, Y., Zhou, J., Chen, A., & Shah, K. J. (2021). Preparation and characterization of high-efficiency magnetic heavy metal capture flocculants. *Water, 13*(13), 1732. https://doi.org/10.3390/w13131732
46. Feng, X. X., Yuan, Y., Yongjun, S., Xing, Z., & Aowen, C. (2021). Heavy metal removal from aqueous solutions by chitosan-based magnetic composite flocculants. *Journal of Environmental Sciences, 108*, 22–32. https://doi.org/10.1016/j.jes.2021.02.014
47. Artifon, W., Immich, A. P. S., Da Silva, A., De Souza, A. A. U., & De Oliveira, D. (2023). A novel bioflocculant extracted from excess-activated sludge for dye-containing wastewater treatment. *Journal of Water Process Engineering, 56*, 104568. https://doi.org/10.1016/j.jwpe.2023.104568
48. Maliehe, T. S., Basson, K. A., & Dlamini, N. G. (2019). Removal of pollutants in mine wastewater by a non-cytotoxic polymeric bioflocculant from *Alcaligenes faecalis* HCB2. *International Journal of Environmental Research and Public Health, 16*(20), 4001. https://doi.org/10.3390/ijerph16204001
49. Graça, J. K., & Botari, A. (2023). Modelagem matemática do processo de floculação de águas com turbidez elevada: estudos e análise comparativa entre métodos e modelos. *Revista Ambiente & Água, 18*. https://doi.org/10.4136/ambi-agua.2888

50. European Union. (2020). *Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption (recast)*. http://data.europa.eu/eli/dir/2020/2184/oj
51. ASTM International. (2020). *Standard Test Method for Compositional Analysis by Thermogravimetry (E1131–20)*.
52. Amran, A. H., Zaidi, N. S., Syafiuddin, A., Zhan, L. Z., Bahrodin, M. B., Mehmood, M. A., & Boopathy, R. (2021). Potential of *Carica papaya* seed-derived bio-coagulant to remove turbidity from polluted water assessed through experimental and modeling-based study. *Applied Sciences, 11*, 5715. https://doi.org/10.3390/app11125715
53. Lee, C., Garcia, R. A., Bumanlag, L. P., & Liang, C. (2023). Eficiência de floculação da hemoglobina fosforilada em sistemas caulim e hematita. *Journal of Applied Polymer Science, 140*. https://doi.org/10.1002/app.54409
54. Lee, C., Garcia, R. A., Bumanlag, L. P., & Liang, C. (2022). Influence of methylation and polymerization on flocculant properties of bovine blood. *ACS Omega, 7*(3), 3037–3043. https://doi.org/10.1021/acsomega.1c06126
55. U.S. Environmental Protection Agency. (2024). *pH*. Washington, DC.

Figures

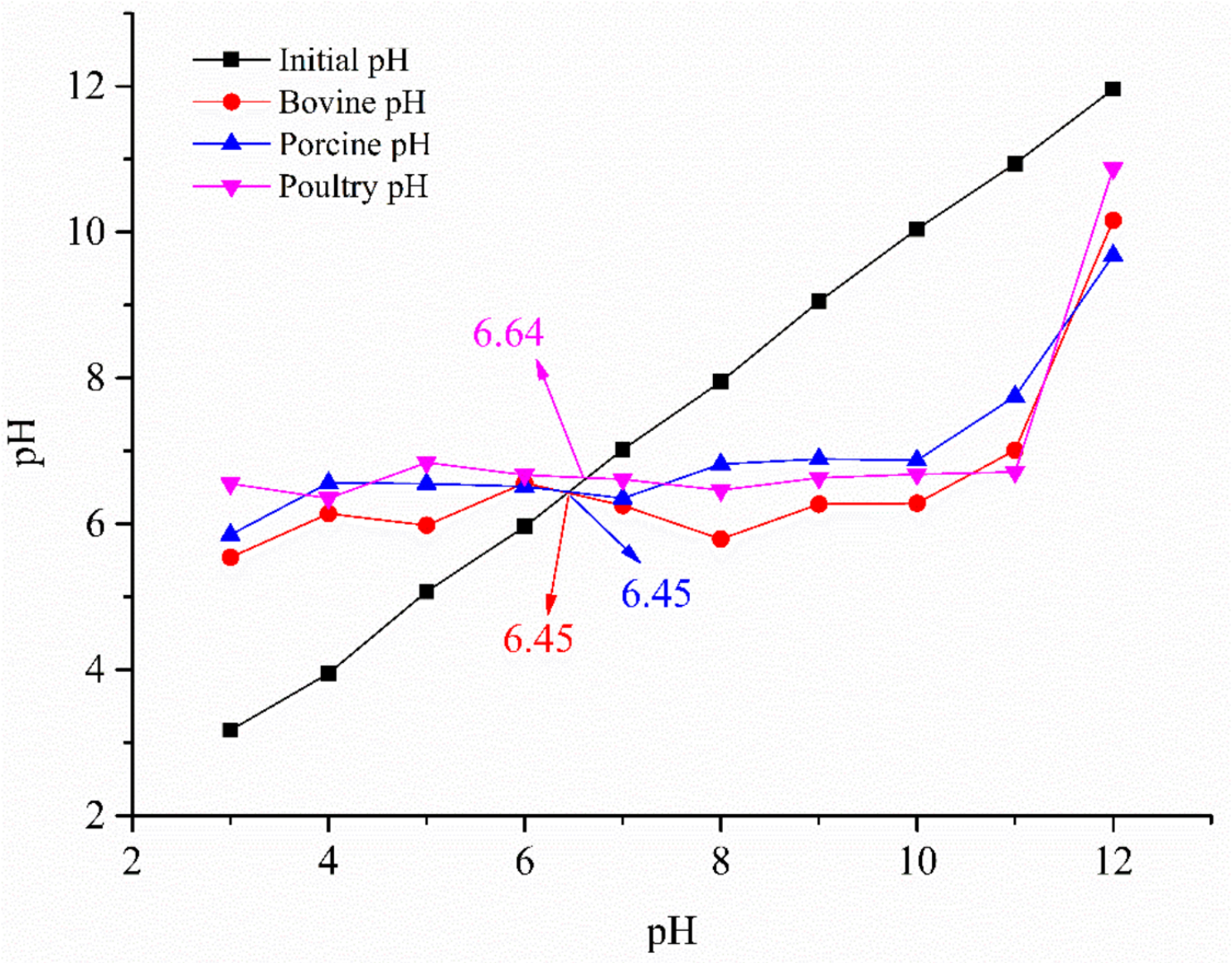


Figure 1

Point Zero Charge (PZC) as a function of varying initial pH for PCA, BCA, and PLCA.

Source: Elaborated by the authors, 2024.

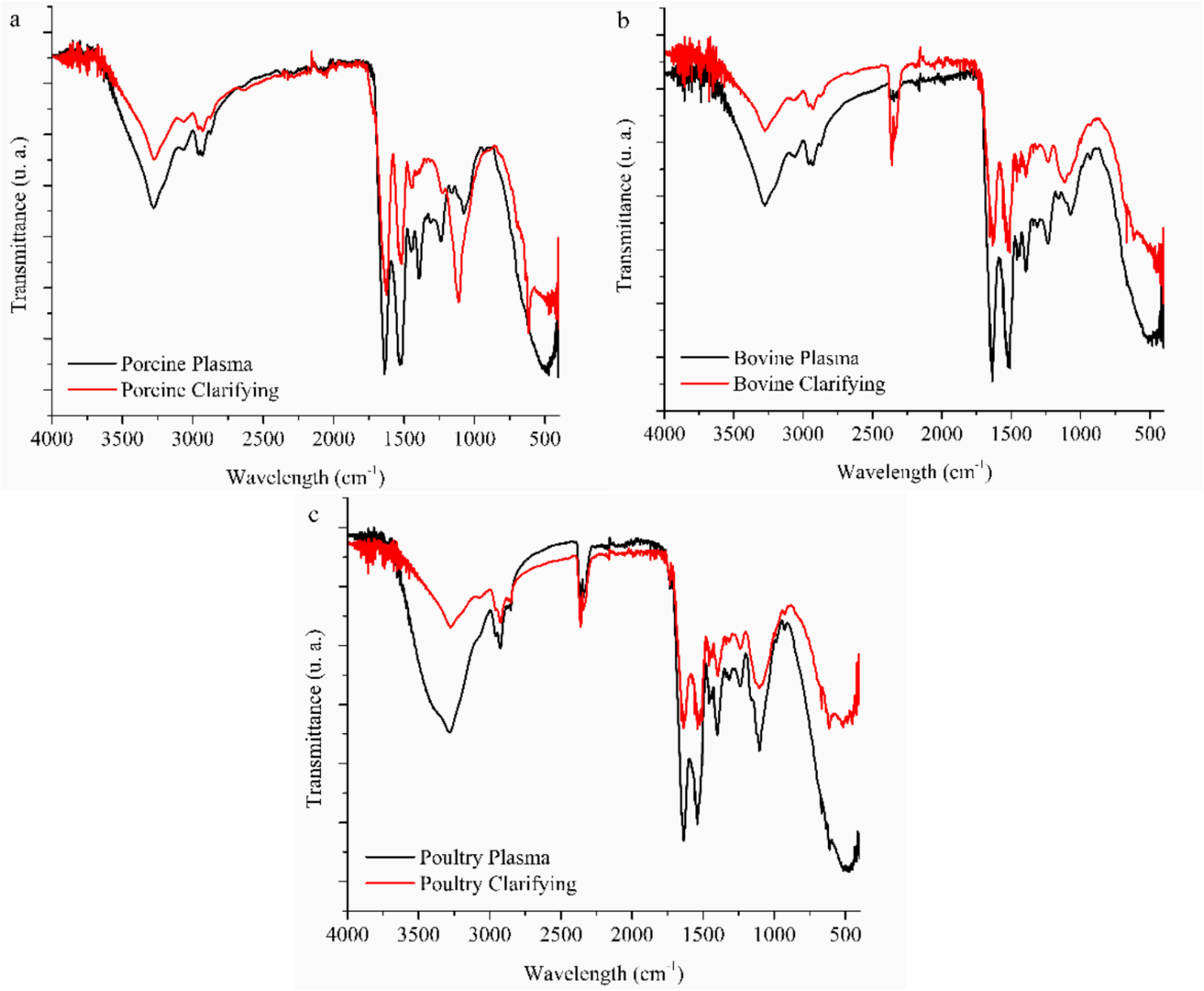


Figure 2

Comparison of FTIR spectra of porcine (a), bovine (b), and poultry (c) blood plasmas with their respective clarifying agents.

Source: Elaborated by the authors, 2024.

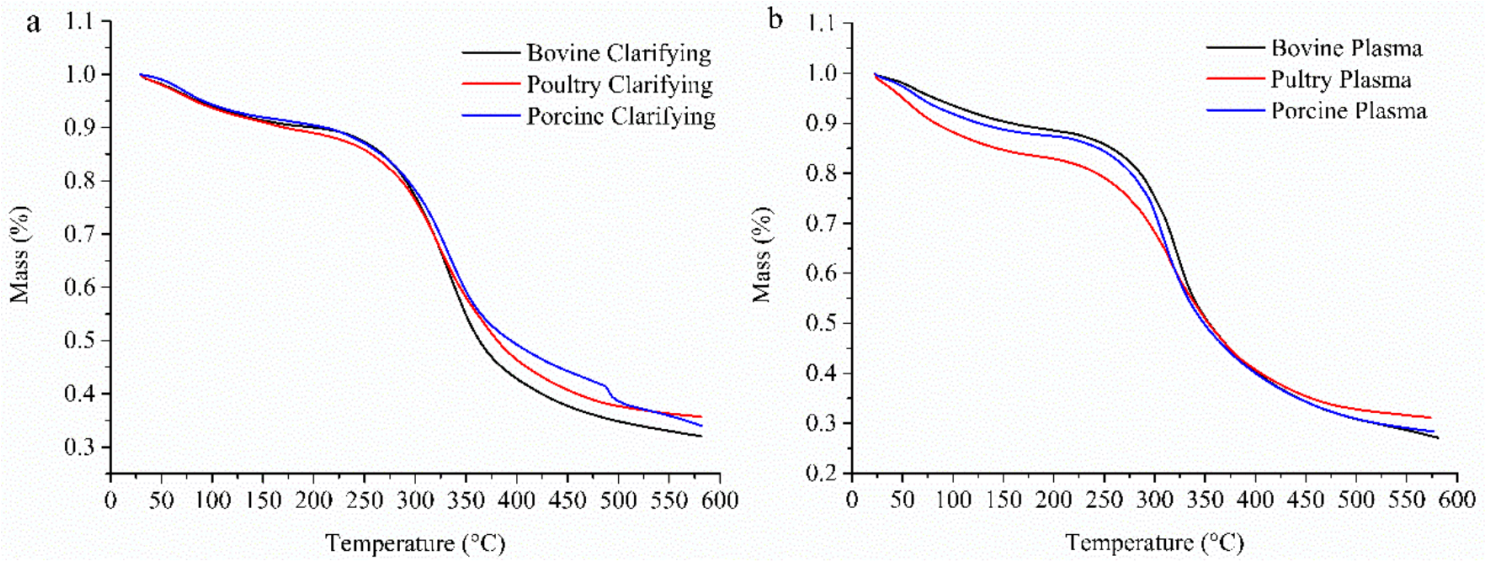


Figure 3

TGA curves for the clarifying agents (a) and pure plasmas (b).

Source: Elaborated by the authors.

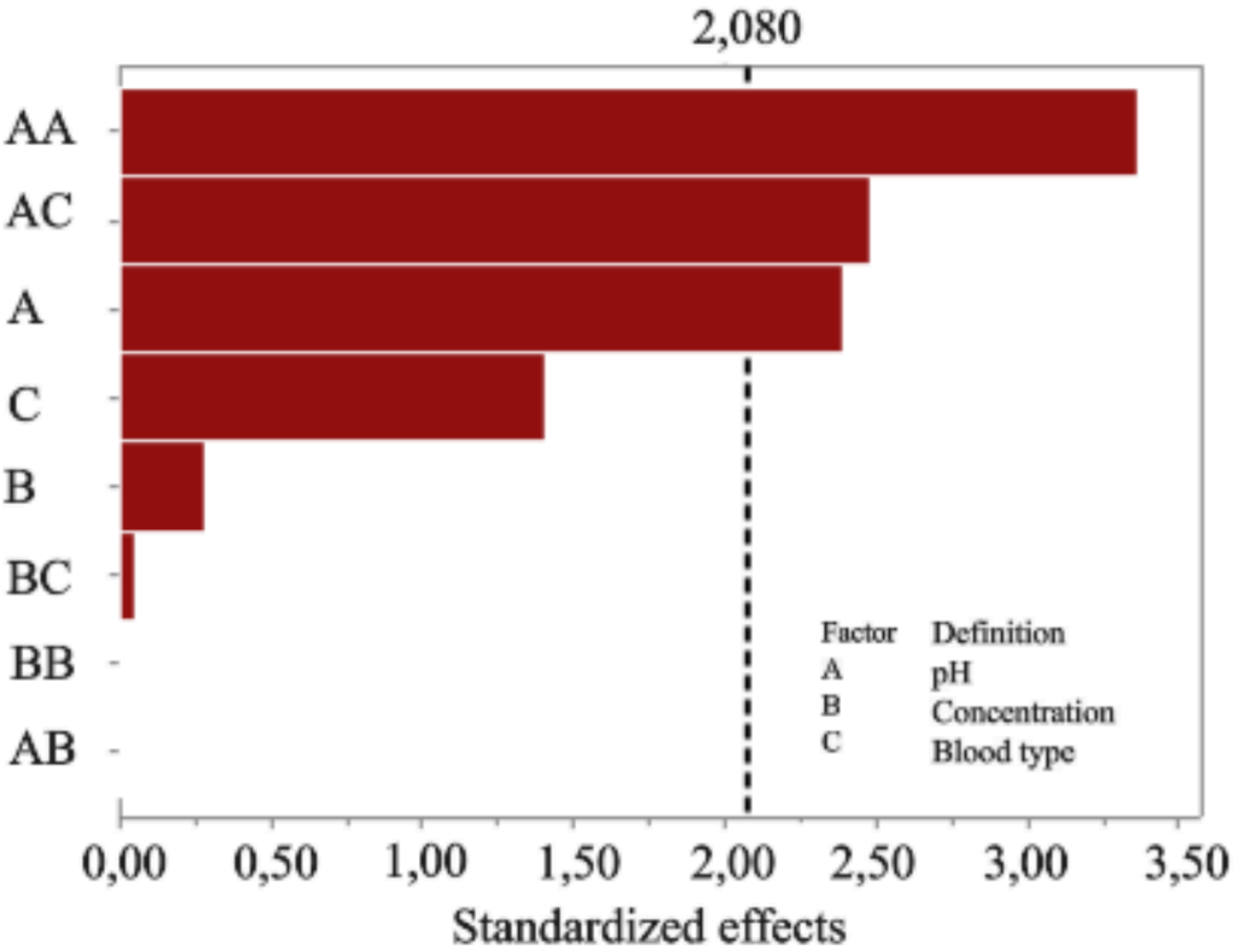


Figure 4

Pareto chart of standardized effects.

Source: Elaborated by the authors, 2024.

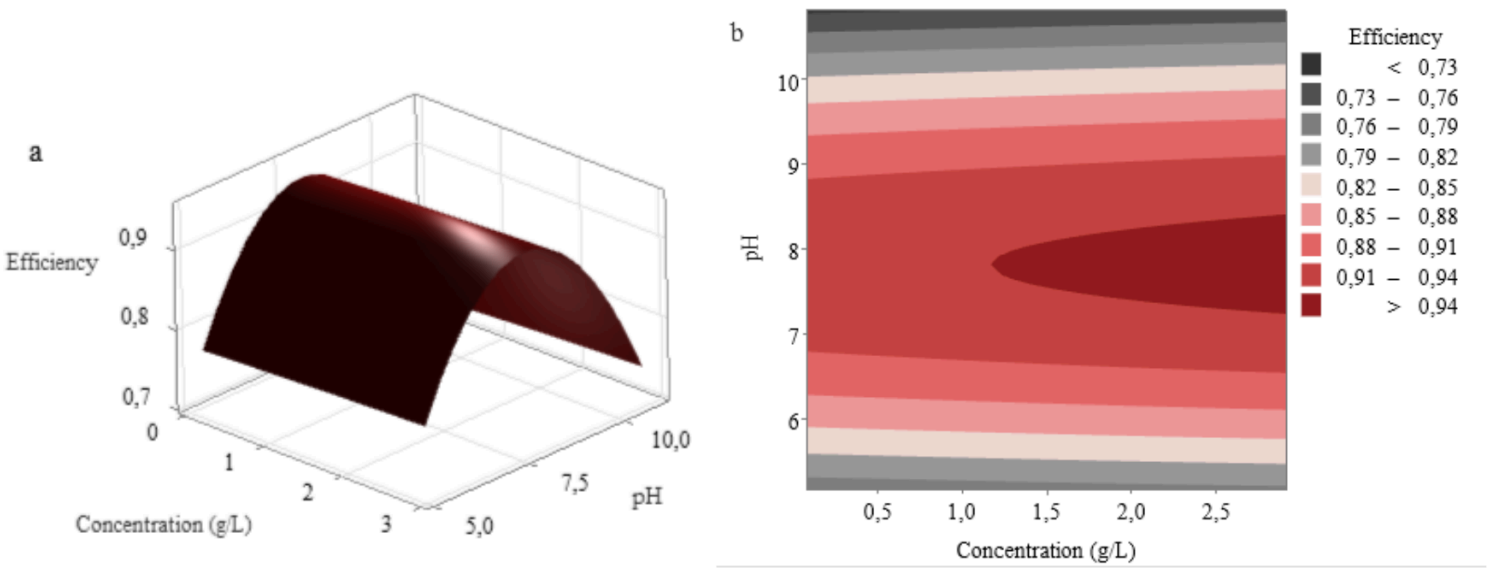


Figure 5

Response surface for the variation of flocculant dosage and pH on the surface (a) and contour (b) for poultry clarifying agent (PLCA).

Source: Elaborated by the authors.

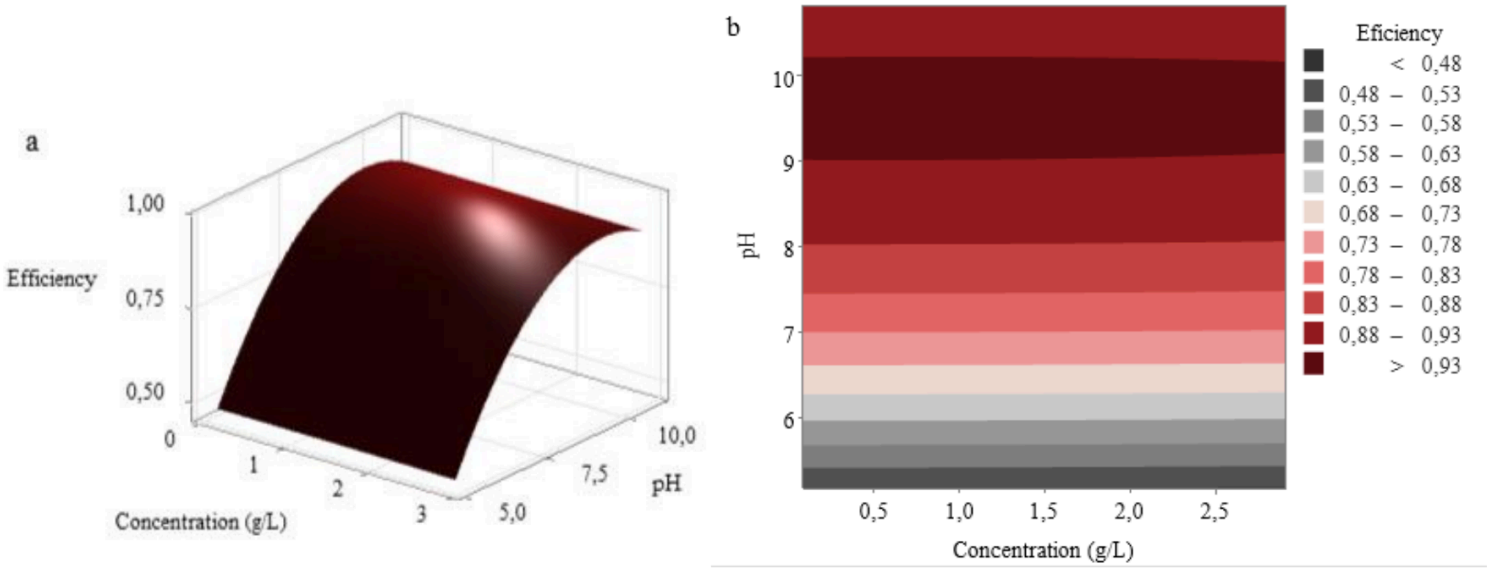


Figure 6

Response surface for the variation of flocculant dosage and pH on the surface (a) and contour (b) for bovine clarifying agent (BCA).

Source: Elaborated by the authors.

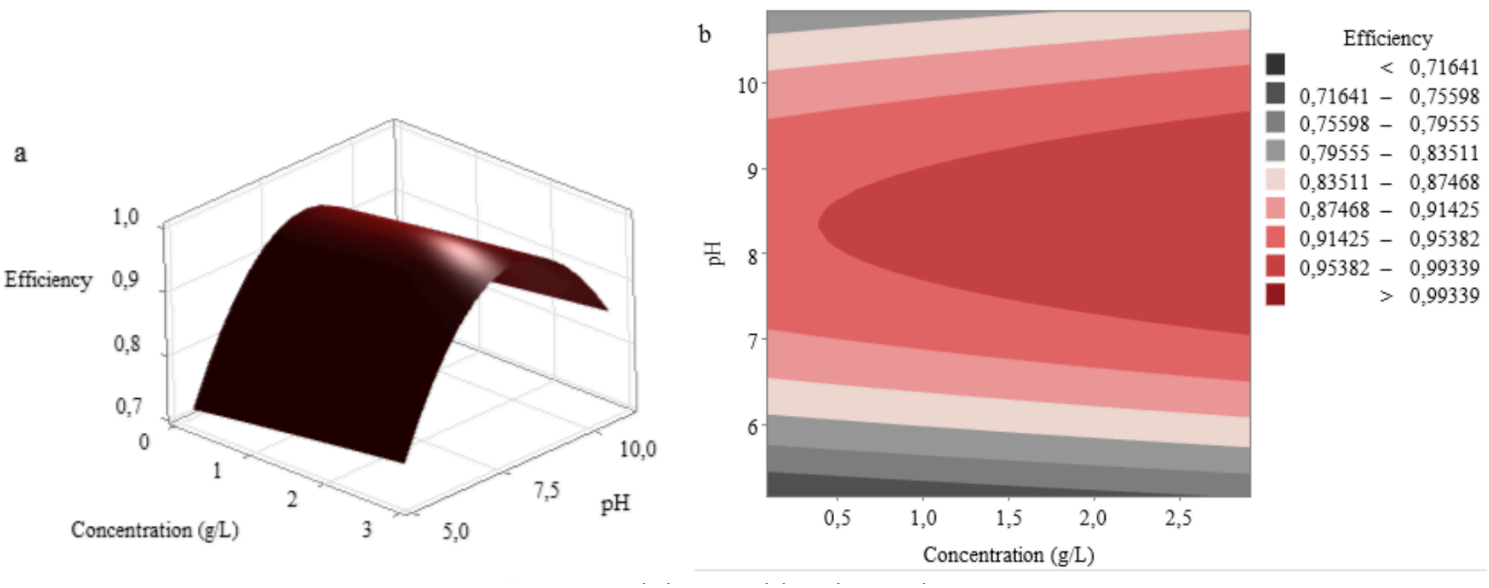


Figure 7

Response surface for the variation of flocculant dosage and pH on the surface (a) and contour (b) for porcine clarifying agent (PCA).

Source: Elaborated by the authors.

Supplementary Files

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- GraphicalAbstract.png