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# Evaluating the Application of Cationic Acacia Tannins in Wastewater Treatment as Bio-Based Alternative to Synthetic Flocculants

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**How To Cite:** Arshadi, M.; Avander, J.; Antti, H.; et al. Evaluating the Application of Cationic Acacia Tannins in Wastewater Treatment as Bio-Based Alternative to Synthetic Flocculants. *Renewable Chemistry* **2026**, 2(1), 2. <https://doi.org/10.53941/rc.2026.100002>

Received: 20 April 2026

Revised: 6 July 2026

Accepted: 7 July 2026

Published: 22 July 2026

**Abstract:** Polyacrylamide (PAM), the most widely used flocculant in wastewater treatment, has raised sustainability concerns because it is derived from fossil-based raw materials. This study evaluated cationic tannins derived from *Acacia* as a bio-based alternative to PAM for the chemical treatment of dairy wastewater. Jar tests combined with design of experiments (DoE) and response surface optimization were used to investigate the effects of FeCl<sub>3</sub>, PAM, and tannin dosages on the removal of suspended solids (Susp), total phosphorus (TP), and chemical oxygen demand (COD). Samples were subjected to rapid mixing (150 rpm, 2 min), followed by slow mixing (80 rpm, 5 min) and a sedimentation time of 30 min. The combination of 360 µL L<sup>-1</sup> FeCl<sub>3</sub> and 100 mg L<sup>-1</sup> tannins achieved treatment efficiencies comparable to those obtained with 360 µL L<sup>-1</sup> FeCl<sub>3</sub> and 25 mg L<sup>-1</sup> PAM, indicating that approximately four times more tannin than PAM was required. At optimized FeCl<sub>3</sub>–tannin dosages, removal efficiencies approaching 100% for Susp, TP, and COD were obtained. Tannins used without FeCl<sub>3</sub> achieved approximately 98%, 70%, and 76% removal of Susp, TP, and COD, respectively. Treatment costs were estimated at 2.78 SEK m<sup>3</sup> for the FeCl<sub>3</sub>–tannin system compared with 1.83 SEK m<sup>3</sup> for the FeCl<sub>3</sub>–PAM system, corresponding to an additional cost of approximately 0.09 € /m<sup>3</sup>. However, costs could be reduced by optimizing the FeCl<sub>3</sub>-to-tannin ratio. Furthermore, sludge containing 100–200 mg L<sup>-1</sup> tannins showed enhanced biogas production. These results demonstrate that cationic Acacia tannins are a promising bio-based alternative to synthetic flocculants for dairy wastewater treatment, although further optimization and more full-scale validation are required.

**Keywords:** tannins; wastewater; flocculants; polyacrylamide

## 1. Introduction

Wastewater generated from domestic, industrial, and agricultural activities requires effective treatment to protect aquatic ecosystems and support sustainable water management [1]. In addition to enabling water reuse, wastewater treatment facilitates the recovery of valuable resources, such as biogas and fertilizers from sludge, thereby contributing to circular economy principles and several United Nations Sustainable Development Goals [2,3]. In Sweden, wastewater treatment generally combines mechanical, biological, and chemical processes to remove suspended solids, organic matter, and nutrients [4]. Chemical treatment, involving coagulation and



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flocculation followed by sedimentation or flotation, is particularly important for the removal of colloidal particles that cannot be separated by conventional physical methods.

Municipal and industrial wastewaters, especially those originating from the food-processing industry, contain negatively charged colloidal particles and phosphates that resist aggregation because of electrostatic repulsion. To destabilize these particles, metal salts such as ferric chloride are commonly used as coagulants, while high-molecular-weight polyelectrolytes are employed as flocculants to promote the formation of larger aggregates that can be removed by sedimentation or flotation [5,6]. In Sweden, cationic polyacrylamide (PAM) is the predominant flocculant used in wastewater treatment [7]. Although highly effective, PAM is derived from fossil resources and contains residual acrylamide monomers that raise concerns regarding toxicity and environmental sustainability [8,9]. Furthermore, cationic polymers have been reported to exhibit adverse effects on aquatic organisms [7,10], highlighting the need for renewable and environmentally benign alternatives.

Bio-based flocculants have attracted increasing attention as sustainable substitutes for conventional synthetic polymers. Among these, tannins are particularly promising because of their abundance, renewability, and commercial availability [11,12]. Condensed tannins account for more than 90% of global tannin production and are mainly obtained from sources such as quebracho wood, chestnut bark, and Acacia bark [13,14]. Through cationization, tannins can be modified to introduce positively charged groups, thereby enhancing their charge density and flocculation performance while preserving the desirable characteristics of the natural material [15,16]. Previous studies have demonstrated the effectiveness of cationic tannins in treating municipal wastewater and industrial effluents, with performances comparable to those of conventional coagulants and flocculants [17,18].

Therefore, the objective of this study was to evaluate the performance and economic feasibility of cationic Acacia tannins as a bio-based alternative to synthetic flocculants in dairy wastewater treatment. Particular emphasis was placed on assessing their applicability under full-scale operating conditions and comparing their performance with that of conventional treatment reagents.

## 2. Experimental

### 2.1. Reagents

Cationic tannins (Tanfloc SH), derived from *Acacia mearnsii* bark were provided by TANAC S.A. (Montenegro, Brazil) in powder form and a 10 w/v solution was prepared by dissolving 100 g tannin powder in 1 L distilled water. PAM was provided by a dairy with name Norrmejerier (NM) in powder form and a 0.5 w/v solution was prepared by dissolving 5 g PAM in 1 L distilled water. FeCl<sub>3</sub> was provided by NM in the form of a 30–40% solution.

### 2.2. Analytical Procedures

All analytical experiments were conducted at the laboratory of Swedish University of Agricultural Sciences:

Suspended solids (Susp) are the measurement of the amount of suspended solids that are present in a sample and was determined gravimetrically according to ISO 11923 as follows: 50 mL of sample was filtered through a separate pre-weighed 47 mm glass microfiber filter (Whatman) by vacuum filtration. The filters were placed in separate pre-weighed crucibles and placed in an oven at 105 °C for 2 h. After cooling, each crucible was weighed and the amount of Susp was determined and expressed as mg/L [19].

Total phosphorus (TP) is a measurement of all phosphorous compounds present in a sample and was determined spectrophotometrically according to ISO 6878 using the Phosphate (ortho/total) cuvette test with associated equipment (LT 200 dry thermostat and DR3900 spectrophotometer) provided by HACH, and expressed as mg/L [20].

Chemical oxygen demand (COD) measurements allow for analysis of how much organic matter that is present in a sample by measuring the amount of oxygen that is consumed for total chemical oxidation of all organic constituents. High levels of organic matter results in high chemical oxygen demand. COD was determined spectrophotometrically according to ISO 15705 using the COD cuvette test with associated equipment (LT 200 dry thermostat and DR3900 spectrophotometer) provided by HACH, and expressed as mg/L [21].



### 2.3. Experimental Design

Design of experiments (DoE) was used to investigate the effects and interactions of seven factors through simultaneous variation in a structured experimental design. Factor levels were defined at low and high settings, resulting in  $2^n$  experiments, with additional centre points included to estimate experimental variability and provide replicates. Response data were used to fit regression models, which were evaluated using  $R^2$  (goodness of fit),  $Q^2$  (predictive ability), model validity, and reproducibility. Model refinement was guided primarily by  $Q^2$ , the most sensitive indicator of model performance, and included the removal of outliers or statistically non-significant terms when appropriate [22].

### 2.4. Screening

To assess the effectiveness of tannins and  $\text{FeCl}_3$ , a screening experiment using DoE was conducted. This approach required only a few experiments to gather crucial information on the factors studied. Two  $2^2$  full factorial design with 4 centre points was utilized, varying tannins, PAM and  $\text{FeCl}_3$  dosages. In total, 8 experiments per design were conducted. Table 1 shows the design matrix used in the two cases:

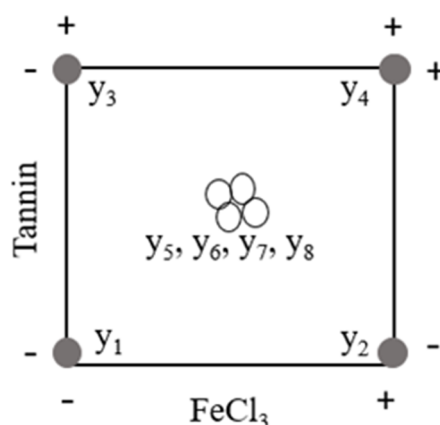
**Table 1.** Design matrix with  $\text{FeCl}_3$ -, Tannin- and PAM dosage as varying factors.

| Exp. No. | $\text{FeCl}_3$ Dosage ( $\mu\text{L/L}$ ) | Tannins Dosage ( $\text{mg/L}$ ) | PAM Dosage ( $\text{mg/L}$ ) | Response Value |
|----------|--------------------------------------------|----------------------------------|------------------------------|----------------|
| 1        | 120                                        | 25                               | 10                           | Y1             |
| 2        | 600                                        | 25                               | 10                           | Y2             |
| 3        | 120                                        | 175                              | 40                           | Y3             |
| 4        | 600                                        | 175                              | 40                           | Y4             |
| 5        | 360                                        | 100                              | 25                           | Y5             |
| 6        | 360                                        | 100                              | 25                           | Y6             |
| 7        | 360                                        | 100                              | 25                           | Y7             |
| 8        | 360                                        | 100                              | 25                           | Y8             |

The experimental design was determined using the Modde software version 12.1, with the run order randomized to minimize external condition effects. Data for response variables (Susp, TP, and COD) was inputted into Modde for analysis. A model was created, refined to maximize the  $Q^2$ -value.

### 2.5. Optimisation

After screening, DoE was used to optimize  $\text{FeCl}_3$  and tannin dosages with a central composite circumscribed design. Factors were investigated at high, low, intermediate, and axial levels. A graphical visualization of the experimental design region is shown in Figure 1.



**Figure 1.** Visualization of the CCC design region where points beyond the square represent axial points. In axial points, one factor is at an intermediate level and the other at one of its two axial levels. For instance, at  $y_6$ ,  $\text{FeCl}_3$  dosage is at its high axial point while tannin dosage is at intermediate level.

The experiments assigned as Table 2

**Table 2.** Design matrix with FeCl<sub>3</sub>—and tannin dosages as varying factors. Each experiment was executed in two replicates.

| Exp. No. | FeCl <sub>3</sub> Dosage (μL/L) | Tannins Dosage (mg/L) | Response Value |
|----------|---------------------------------|-----------------------|----------------|
| 1        | 120                             | 25                    | Y1             |
| 2        | 600                             | 25                    | Y2             |
| 3        | 120                             | 175                   | Y3             |
| 4        | 600                             | 175                   | Y4             |
| 5        | 20                              | 100                   | Y5             |
| 6        | 700                             | 100                   | Y6             |
| 7        | 360                             | 10                    | Y7             |
| 8        | 360                             | 190                   | Y8             |
| 9        | 360                             | 100                   | Y9             |
| 10       | 360                             | 100                   | Y10            |
| 11       | 360                             | 100                   | Y11            |
| 12       | 360                             | 100                   | Y12            |

In addition to Susp, TP, and COD, the cost of each dosage combination was included as a response variable. The cost for each experiment was calculated as

$$\text{Cost in Swedish Crowns (SEK)} = (A \times \mu\text{L FeCl}_3) + (B \times \text{mg tannins})$$

where A is the price per μL FeCl<sub>3</sub> and B is the price per mg tannin powder. The cost was indicated as SEK per 1 m<sup>3</sup> water. The data for the response variables was inputted into Modde, a model was created, and the results were analysed. The model was refined to achieve a high Q<sup>2</sup>-value.

## 2.6. Chemical Purification Using only Cationic Tannins

The study examined the effectiveness of tannins as a coagulant and flocculant. Six different dosages (100, 150, 200, 250, 300, and 350 mg/L) were tested in four replicates. The experiments followed a specific protocol without using FeCl<sub>3</sub>. Tannin was added in two stages to maintain the correct dosage. Mixing speeds were adjusted during the process for optimal results.

## 2.7. Effect of Cationic Tannins on Biogas Production

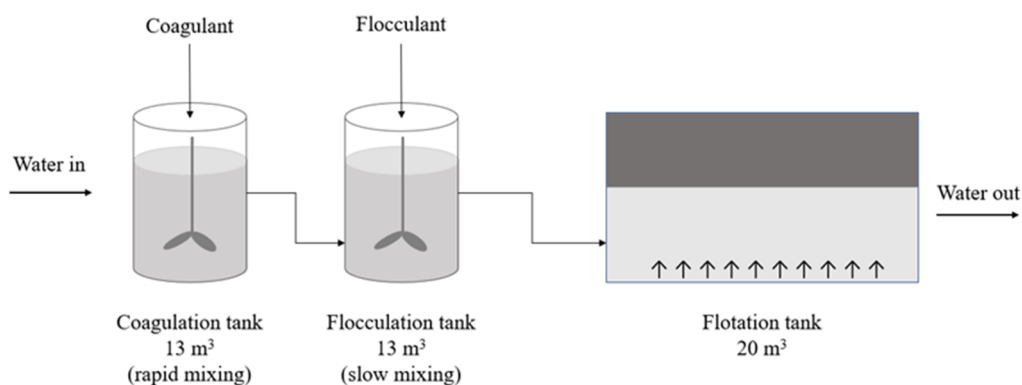
Sludge is commonly used in some industries for biogas production, so studying how cationic tannins affect gas production is crucial. To do this, 250 mL of “reactor sludge” was mixed with varying amounts of tannins (0, 25, 50, 100, and 200 mg) in glass bottles. Rubber stoppers were used to seal the bottles, which were then connected to water-filled gas washing bottles via rubber tubes submerged in E-flasks. The setup was placed in an oven at 37 °C. As gas formed in the sludge bottle, it would move to the gas washing bottle, creating pressure that would push water into the E-flask. More water in the E-flask indicated higher gas production in the sludge. Water levels in the E-flasks were measured using a graduated cylinder after 3, 20.5, and 48 h.

## 2.8. Vessel Tests

Jar tests were conducted using a flocculation tester (VELP Scientifica JLT6) with six speed-adjustable mixers. The experiments involved filling 1 L beakers with wastewater, setting the mixing speed at 150 rpm for 2 min, adding FeCl<sub>3</sub>, then reducing the speed to 80 rpm for 5 min while adding tannins. After 5 min, sedimentation occurred for 30 min, resulting in a clear and sludge phase. Samples were collected from the clear phase for analysis of Susp, TP, and COD. The different mixing periods for coagulation and flocculation were due to the need for rapid mixing to evenly distribute the coagulant during coagulation, and slower mixing to facilitate floc formation during flocculation. Various mixing speeds were based on our previous experiments. Susp, TP, and COD were also determined for a zero-sample to calculate % Removal. Jar tests were conducted on commercial tannins with FeCl<sub>3</sub> and PAM with FeCl<sub>3</sub>.

## 2.9. Full-Scale Trial

A trial was conducted at NM using FeCl<sub>3</sub> as a coagulant and tannin as a flocculant in a wastewater treatment system. The system included a coagulation tank with rapid mixing, a flocculation tank with slow mixing, and a flotation tank. See Figure 2.



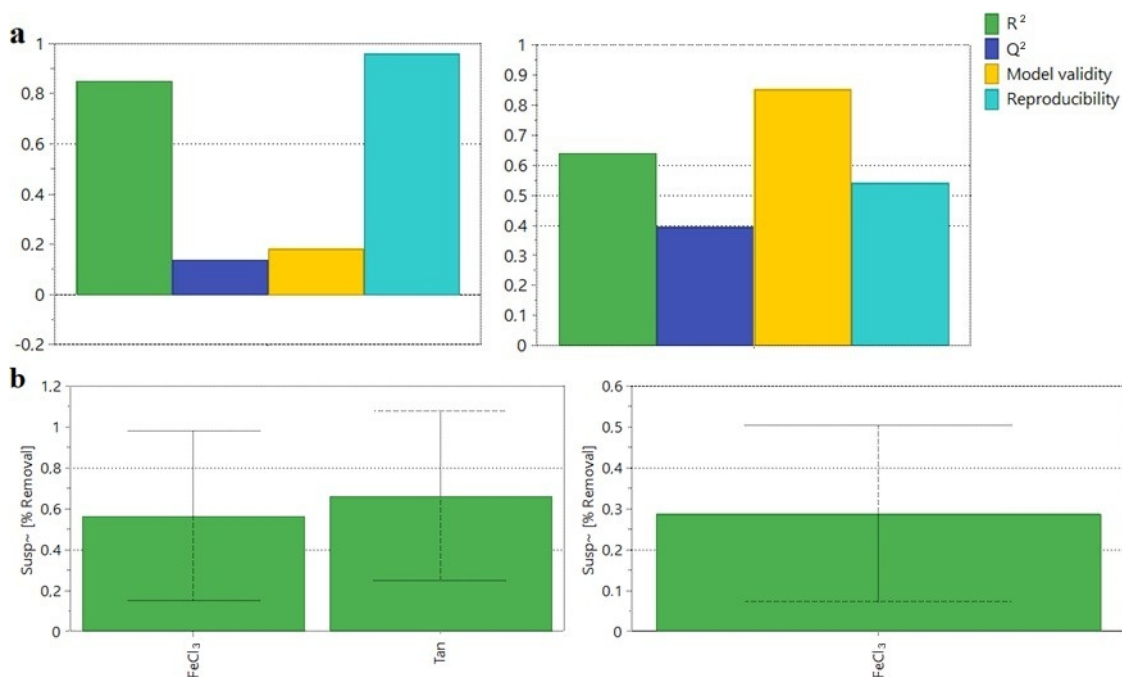
**Figure 2.** Schematic of a part of a wastewater treatment system. Arrows in flotation tank represents airflow.

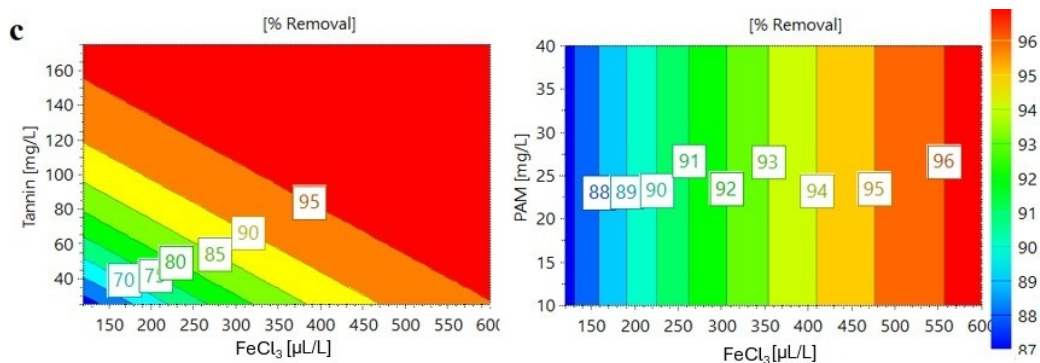
The experiment was executed as follows: A 5 w/v tannin solution was prepared by dissolving 50 kg tannin powder in 1 m<sup>3</sup> water in a tank equipped with a mixer. A dosing pump was installed with one rubber hose submerged into the tannin solution tank, and the other into the flocculation tank, pumping tannin solution into the flocculation tank. Before the dosing pump was started, the PAM pump was shut off and 50 L tannin solution (containing a total of 1.8 kg tannin powder) was poured directly into the flocculation tank to saturate the system and reach a desirable tannin concentration as quickly as possible. After this, the dosing pump was started. Initially, the water inflow was 60 m<sup>3</sup>/h and the dosing pump was set to achieve a tannin concentration of 150 mg/L in the system. After 15 min, the tannin concentration was doubled to boost the system with tannin solution for 15 min before returning to 150 mg/L. The following combinations of flow rate and tannin-concentration were also investigated: 60 m<sup>3</sup>/h and 75 mg/L, 30 m<sup>3</sup>/h and 100 mg/L, 30 m<sup>3</sup>/h and 200 mg/L, and finally, 60 m<sup>3</sup>/h and 250 mg/L. In addition, for a short period of time, the airflow in the flotation tank was shut off to see if this would have any effect on the flotation.

Samples were taken from untreated incoming water and purified outgoing water, all of which were analysed for Susp, TP, and COD levels.

### 3. Results

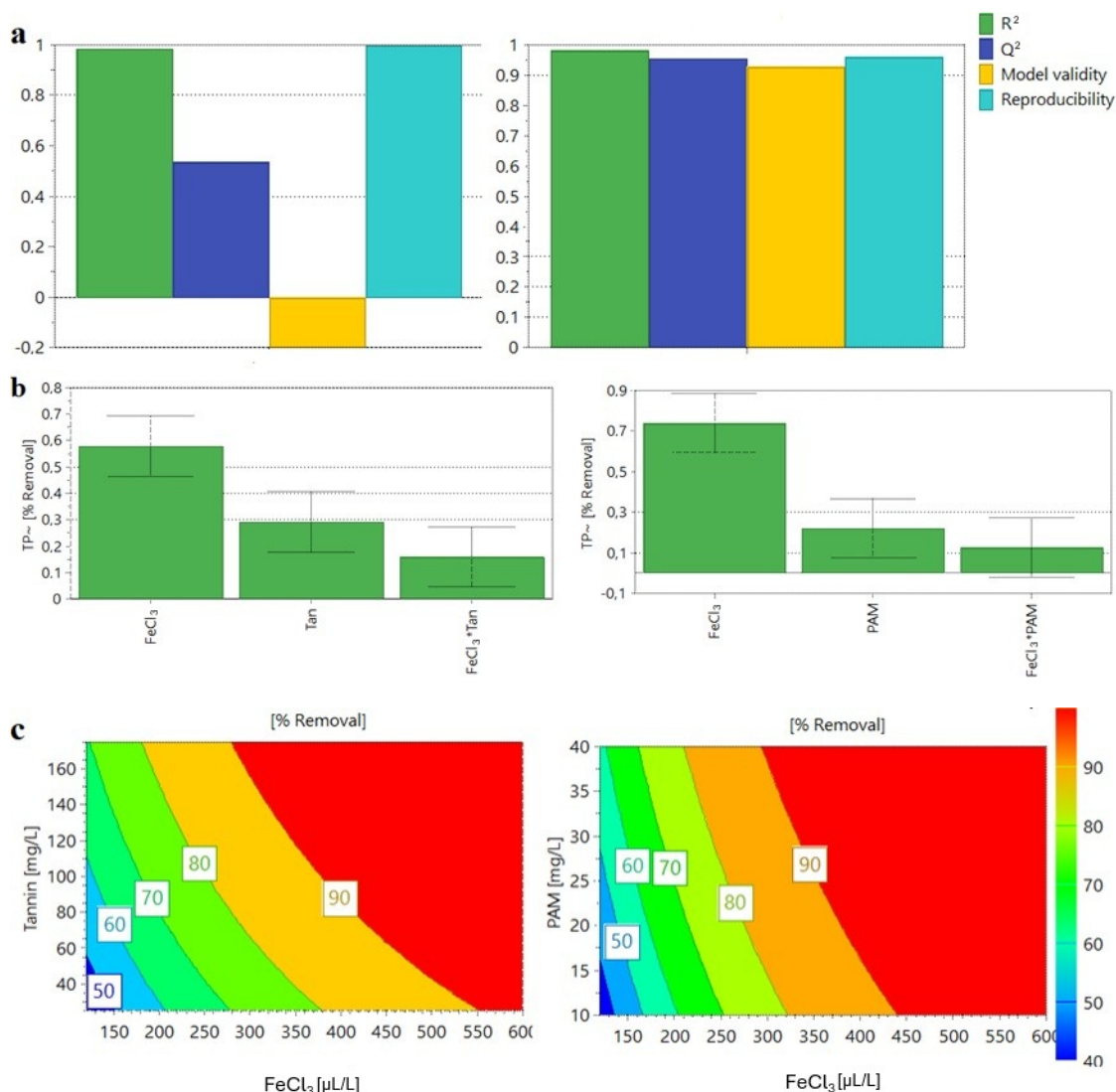
Results from Modde include select figures. Each response variable is presented separately, with FeCl<sub>3</sub>-tannins on the left and FeCl<sub>3</sub>-PAM on the right. Figure 3 shows model data for Susp, for TP, and for COD from both screening experiments.





**Figure 3.** (a) summary of the model statistics (left:  $R^2 = 0.85$ ,  $Q^2 = 0.14$ , Model validity = 0.18, Reproducibility = 0.96, right:  $R^2 = 0.64$ ,  $Q^2 = 0.40$ , Model validity = 0.85, Reproducibility = 0.54, (b) coefficient plot showing significance of factors, (c) contour plot showing optimum settings. FeCl<sub>3</sub>- and tannin dosages as varying factors (left), FeCl<sub>3</sub>- and PAM dosages as varying factors (right). Response: Susp.

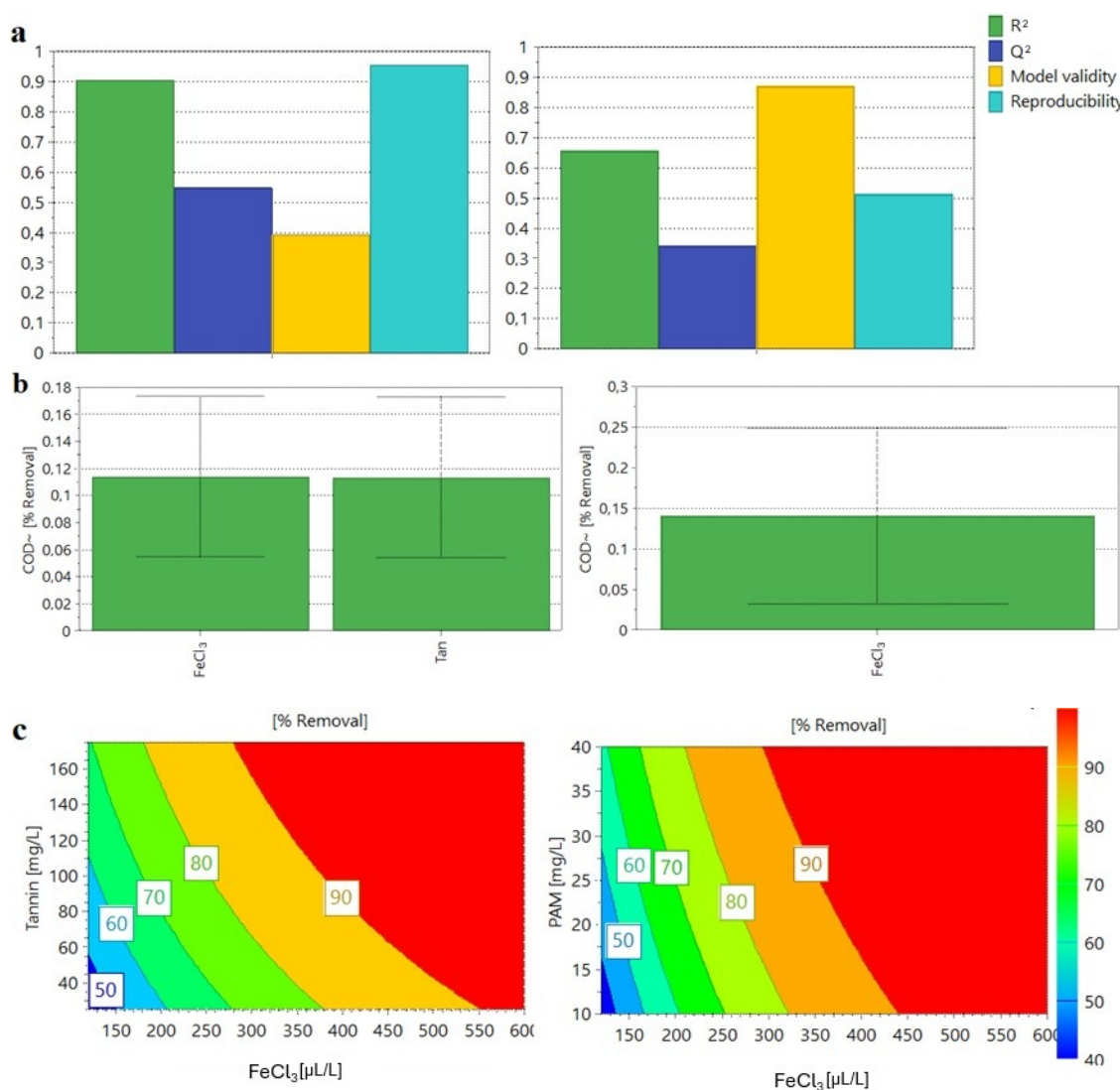
The model fitted for FeCl<sub>3</sub>-PAM is significant in all parameters, while the model for FeCl<sub>3</sub>-tannins has a low validity value (0.18). FeCl<sub>3</sub> and tannin dosages are both significant in explaining the response. For FeCl<sub>3</sub>-PAM, only FeCl<sub>3</sub> is significant for Susp removal. Increasing tannin dosage at a specific FeCl<sub>3</sub> dosage improves Susp removal but increasing PAM dosage does not. In both cases, Susp removal above 95% was achieved. Refer to Figure 4 for detailed illustrations.



**Figure 4.** (a) summary of the model statistics (left:  $R^2 = 0.99$ ,  $Q^2 = 0.54$ , Model validity =  $-0.2$ , Reproducibility =  $1.0$ , right:  $R^2 = 0.98$ ,  $Q^2 = 0.96$ , Model validity =  $0.93$ , Reproducibility =  $0.96$ ), (b) coefficient plot showing significance of factors, (c) contour plot showing optimum settings.  $\text{FeCl}_3$ - and tannin dosages as varying factors (**left**),  $\text{FeCl}_3$ - and PAM dosages as varying factors (**right**). Response: TP.

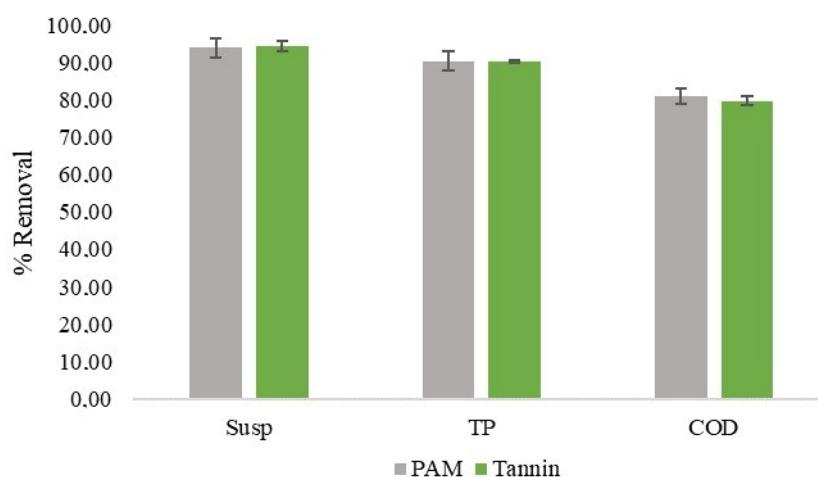
Regarding the TP models obtained from the screening, the  $\text{FeCl}_3$ -PAM model is significant in all parameters, while the  $\text{FeCl}_3$ -tannins model has a negative validity value of  $-0.2$  as shown in Figure 4a. The combination of  $\text{FeCl}_3$  and tannin dosages is also significant in explaining the response.  $\text{FeCl}_3$  and PAM dosages are both significant in explaining the response, but the combination of these two factors is on the verge of significance (confidence interval extends over 0), reaching an optimum at high  $\text{FeCl}_3$  and tannin/PAM dosages, resulting in TP removal above 90% in both cases.

Both COD models fitted during the screening are significant in all parameters, as shown in Figure 5a.  $\text{FeCl}_3$  and tannin dosages are both important in explaining the response. For the  $\text{FeCl}_3$ -PAM combination, only  $\text{FeCl}_3$  is significant for COD removal, shown in Figure 5b. Increasing tannin dosage at a certain  $\text{FeCl}_3$  level improves COD removal, while increasing PAM dosage does not, as seen in Figure 5c. Both cases achieved COD removal above 84%.



**Figure 5.** (a) summary of the model statistics (left:  $R^2 = 0.91$ ,  $Q^2 = 0.55$ , Model validity =  $0.39$ , Reproducibility =  $0.96$ , right:  $R^2 = 0.66$ ,  $Q^2 = 0.34$ , Model validity =  $0.87$ , Reproducibility =  $0.51$ ), (b) coefficient plot showing significance of factors, (c) contour plot showing optimum settings.  $\text{FeCl}_3$ - and tannin dosages as varying factors (**left**),  $\text{FeCl}_3$ - and PAM dosages as varying factors (**right**). Response: COD.

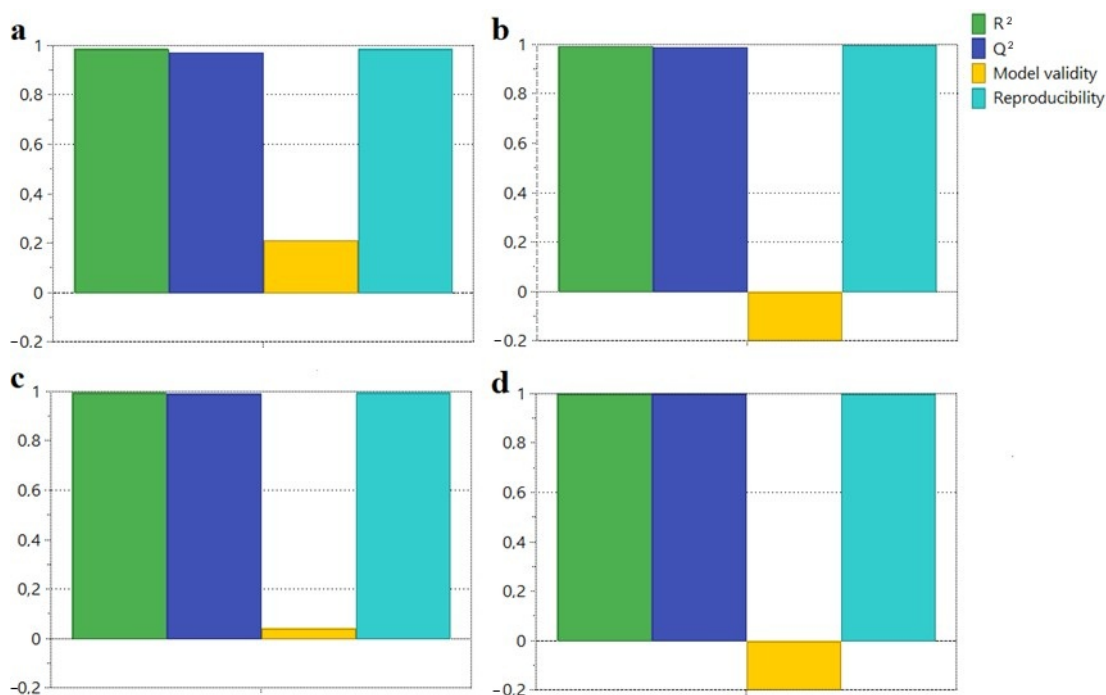
Results from the four replicates in each screening are shown in Figure 6, with  $\text{FeCl}_3$  at  $360 \mu\text{L/L}$ , tannin at  $100 \text{ mg/L}$ , and PAM at  $25 \text{ mg/L}$ . The  $t$ -test did not find a significant difference between the  $\text{FeCl}_3$ -tannin and  $\text{FeCl}_3$ -PAM combinations ( $p$ -value  $> 0.05$ ).



**Figure 6.** Results of four replicates with PAM dosage = 25 mg/L, tannin dosage = 100 mg/L and  $\text{FeCl}_3$  dosage = 360  $\mu\text{L/L}$  (in combination with either PAM or tannins). Error bars represent standard deviation. Obtained  $p$ -value from  $t$ -test > 0.05.

### 3.1. Optimization

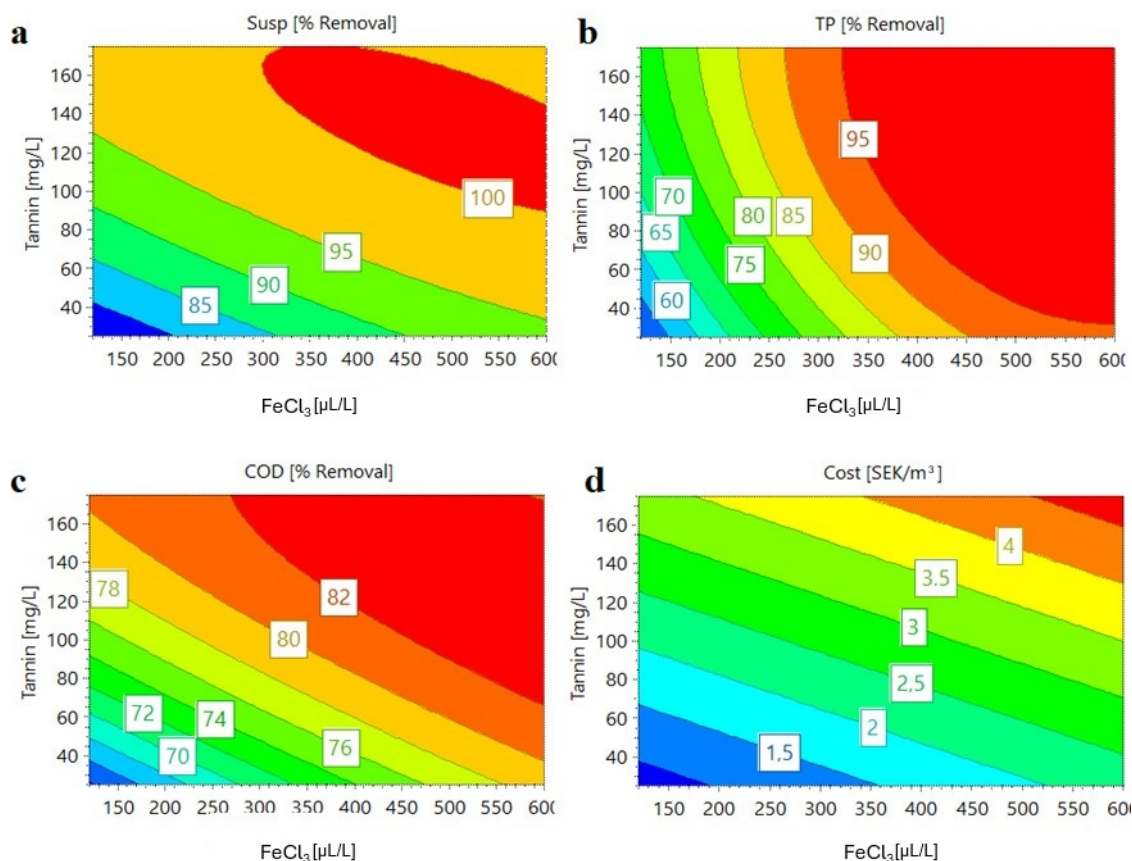
The results include figures from Modde. All models from optimization are significant in parameters, except model validity, which is low for Susp and COD, and negative for TP and cost. Figure 7 illustrates the results.



**Figure 7.** Summary of the model statistics. (a) Susp ( $R^2 = 0.99$ ,  $Q^2 = 0.97$ , Model validity = 0.21, Reproducibility = 0.99), (b) TP ( $R^2 = 0.99$ ,  $Q^2 = 0.99$ , Model validity = -0.2, Reproducibility = 1.0), (c) COD ( $R^2 = 1.0$ ,  $Q^2 = 0.99$ , Model validity = 0.04, Reproducibility = 0.99), (d) Cost ( $R^2 = 1.0$ ,  $Q^2 = 1.0$ , Model validity = -0.2, Reproducibility = 1.0).

Susp removal in Figure 8a shows a negative effect with high  $\text{FeCl}_3$  and tannin dosages. Optimum TP and COD removal, seen in Figures 8b,c, occurs at high  $\text{FeCl}_3$  and tannin levels, resulting in over 95% and 82% removal, respectively. Susp removal reaches nearly 100%, indicating effective treatment with these settings.

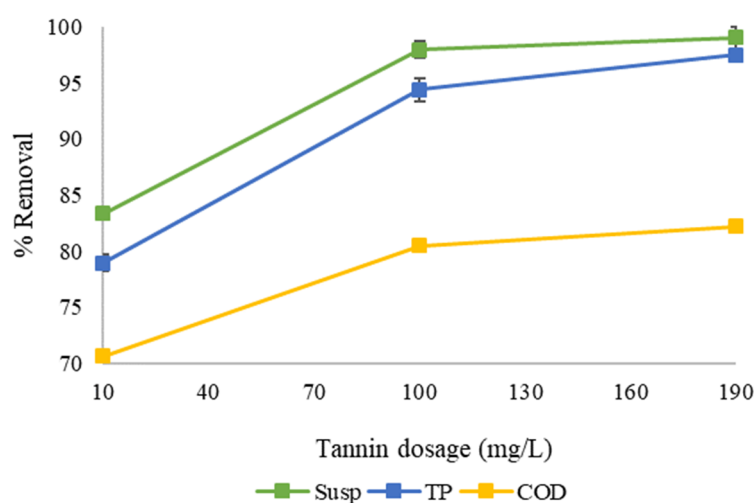




**Figure 8.** Contour plots showing optimum settings. (a) Susp, (b) TP, (c) COD, (d) Cost.

The most cost-effective option is at low tannin and high FeCl<sub>3</sub> dosages, with removal rates of around 70% for Susp, TP, and COD. A balanced approach is to use low tannin and high FeCl<sub>3</sub> dosages, resulting in a cost of approximately 2.5 SEK/m<sup>3</sup> and removal rates of around 80% for COD and 95% for Susp and TP, as shown in Figure 8.

Figure 9 depicts the impact of increasing tannin dosage while keeping FeCl<sub>3</sub> constant at 360 μL/L. Enhanced removal of Susp-, TP, and COD is most notable when tannin dosage rises from 10 to 100 mg/L. Beyond 100 mg/L, the removal efficiency diminishes.



**Figure 9.** Results of different tannin dosages with constant FeCl<sub>3</sub> dosage at 360 μL/L. (10 mg/L: 2 replicates, 100 mg/L: 8 replicates, 190 mg/L: 2 replicates). Error bars represent standard deviation. Specific values (in mg/L) from these experiments are presented in Table 3.



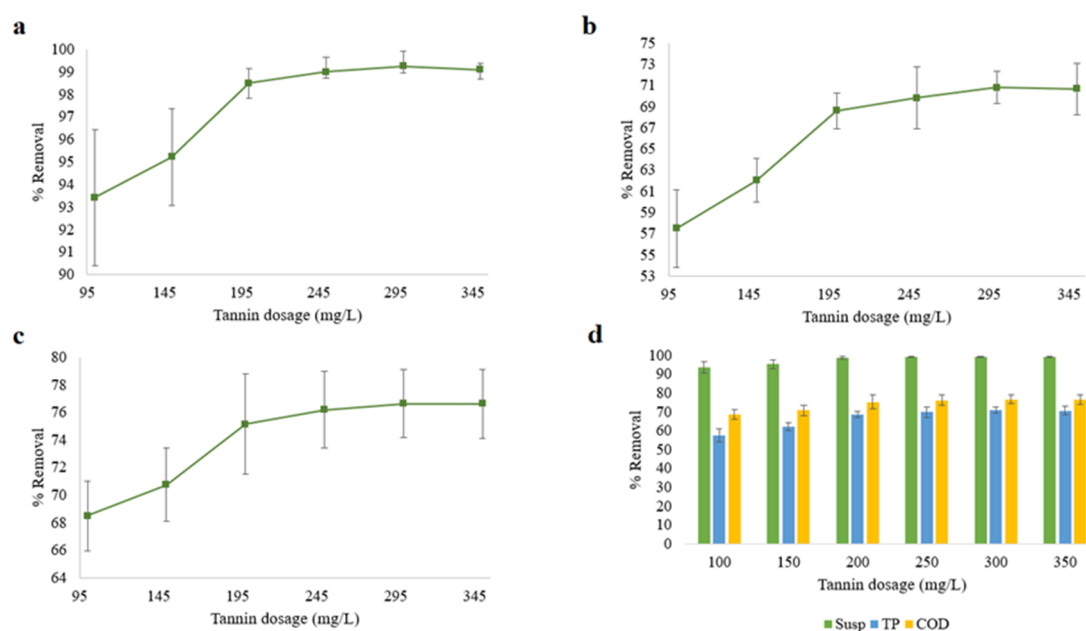
Increasing tannin dosage from 10 to 190 mg/L (with constant  $\text{FeCl}_3$  dosage of 360  $\mu\text{L/L}$ ) reduced Susp from 202 to 12 mg/L and decreased TP from 6.41 to under 1 mg/L, and COD from 1112 to 644 mg/L. Cost increased by approximately 3 SEK/ $\text{m}^3$  according to Table 3.

**Table 3.** Specific values for response variables from selected experiments. % Removal of Susp, TP and COD are presented graphically in Figure 11.

| $\text{FeCl}_3$ Dosage ( $\mu\text{L/L}$ ) | Tannin Dosage (mg/L) | Susp (mg/L)           | TP (mg/L)           | COD (mg/L)        | Cost (SEK/ $\text{m}^3$ ) |
|--------------------------------------------|----------------------|-----------------------|---------------------|-------------------|---------------------------|
| 360                                        | 10                   | 202.0 ( $\pm 5.7$ )   | 6.41 ( $\pm 0.23$ ) | 1112 ( $\pm 38$ ) | 1.25                      |
| 360                                        | 100                  | 27.50 ( $\pm 9.84$ )  | 1.77 ( $\pm 0.33$ ) | 695 ( $\pm 39$ )  | 2.78                      |
| 360                                        | 190                  | 12.00 ( $\pm 11.31$ ) | 0.79 ( $\pm 0.01$ ) | 644 ( $\pm 54$ )  | 4.31                      |

### 3.2. Chemical Purification Using only Cationic Tannins

The most significant enhancement in Susp, TP, and COD elimination was seen with a tannin dosage of approximately 200 mg/L. Beyond this point, the effectiveness of increasing tannin dosage decreased, as shown in Figure 10a–c. Susp showed the highest removal rate at 98%, followed by COD and TP at around 76% and 70% respectively, as depicted in Figure 10. Specific removal values for Susp, TP, and COD (in mg/L), along with costs (in SEK/ $\text{m}^3$ ), can be found in Table 4.



**Figure 10.** Results from 4 replicates using only tannins. (a) Susp, (b) TP, (c) COD, (d) compilation of all previous mentioned. Error bars represent standard deviation. Specific values (in mg/L) from these experiments are presented in Table 4.

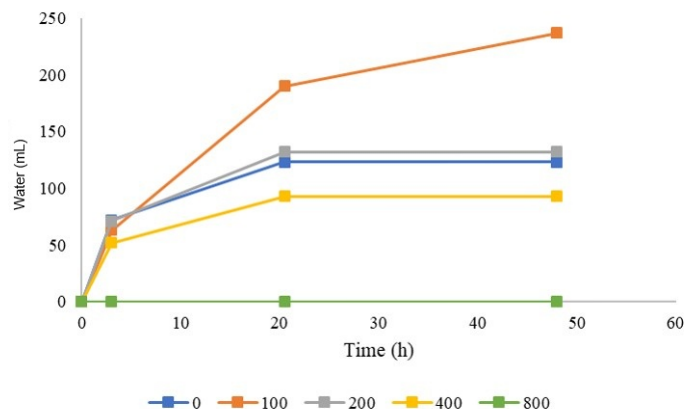
A tannin dosage of 300 mg/L resulted in improved outcomes: Susp decreased from 119 to 13.50 mg/L, TP decreased from 26.25 to 18.28 mg/L, and COD decreased from 1611 to 1202 mg/L compared to 100 mg/L dosage.

**Table 4.** Specific values for Susp, TP, COD and Cost (tannins only). % Removal of Susp, TP and COD are presented graphically in Figure 10.

| Tannin Dosage (mg/L) | Susp (mg/L)           | TP (mg/L)            | COD (mg/L)         | Cost (SEK/ $\text{m}^3$ ) |
|----------------------|-----------------------|----------------------|--------------------|---------------------------|
| 100                  | 119.0 ( $\pm 55.0$ )  | 26.25 ( $\pm 3.92$ ) | 1611 ( $\pm 327$ ) | 1.70                      |
| 150                  | 86.50 ( $\pm 39.03$ ) | 62.02 ( $\pm 2.05$ ) | 1501 ( $\pm 348$ ) | 2.55                      |
| 200                  | 27.50 ( $\pm 11.82$ ) | 19.68 ( $\pm 4.62$ ) | 1284 ( $\pm 365$ ) | 3.40                      |
| 250                  | 18.00 ( $\pm 4.90$ )  | 18.80 ( $\pm 3.97$ ) | 1226 ( $\pm 317$ ) | 4.25                      |
| 300                  | 13.50 ( $\pm 5.51$ )  | 18.28 ( $\pm 4.16$ ) | 1202 ( $\pm 296$ ) | 5.10                      |
| 350                  | 16.67 ( $\pm 7.02$ )  | 18.38 ( $\pm 4.40$ ) | 1203 ( $\pm 297$ ) | 5.95                      |

### 3.3. Effect of Cationic Tannins on Biogas Production

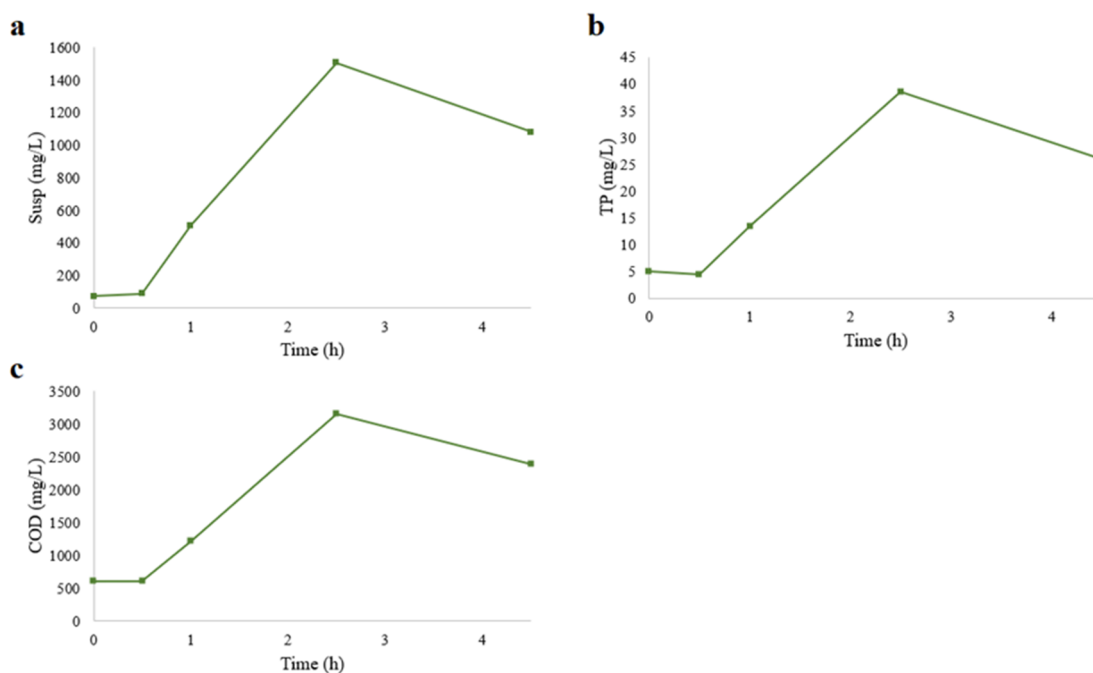
Gas production was highest in sludge with tannin concentrations of 100 and 200 mg/L compared to zero tannin. Sludge with 400 mg/L had slightly lower gas production, while at 800 mg/L gas production was non-existent. Results shown in Figure 11.



**Figure 11.** Gas production in tannin sludge. The amount of tannin has been recalculated as 0, 100, 200, 400 and 800 mg/L. More water indicates more gas production.

### 3.4. Full-Scale Trial

During the full-scale trial, Susp, TP, and COD did not reach values seen under normal conditions. Susp increased from ~70 to 1500 mg/L, TP from ~5 to 40 mg/L, and COD from ~500 to 3000 mg/L from 30 min to 2.5 h. PAM dosage was stopped, 1.8 kg of tannins was added to the tank, and tannin dosage varied from 75–300 mg/L under a flow rate of 60 m<sup>3</sup>/h. This is shown in Figure 12.



**Figure 12.** Results from full scale trial, (a) Susp, (b) TP, (c) COD. The two first points are collected under normal conditions (FeCl<sub>3</sub> and PAM) just before 1.8 kg tannins were poured directly into the flocculation tank. The tannin pump was started after 1 h and the FeCl<sub>3</sub> dosage was constant (360 µL/L) throughout the experiment.

The sample collected after 4.5 h showed the lowest values for Susp, TP, and COD (around 1100, 25, and 2250 mg/L respectively) following initiation of the tannin pump. Tannin dosage of 200 mg/L and flow rate of 30 m<sup>3</sup>/h were maintained for about 1 h (with airflow in flotation tank). The increase in the outflow as shown in

Figure 12 is due to the breakdown of the flocks by the pump in the flotation tank. It is clear that tannins build weaker flocks compared to PAM.

### 3.5. Cost Analysis

A cost comparison was performed between the conventional  $\text{FeCl}_3$ -PAM system and the alternative  $\text{FeCl}_3$ -Acacia tannin system based on the optimum dosages determined in this study. The treatment cost for the  $\text{FeCl}_3$ -PAM system was estimated at 1.83 SEK  $\text{m}^3$ , whereas the corresponding cost for the  $\text{FeCl}_3$ -Acacia tannin system was 2.78 SEK  $\text{m}^3$ , corresponding to an increase of approximately 52% (0.09 € /  $\text{m}^3$  treated wastewater). The higher cost of the tannin-based treatment was primarily attributed to the requirement for a tannin dosage approximately four times greater than that of PAM to achieve comparable treatment performance.

Despite the higher cost, the combination of Acacia tannins and  $\text{FeCl}_3$  achieved removal efficiencies for suspended solids, total phosphorus, and chemical oxygen demand comparable to those obtained with the conventional  $\text{FeCl}_3$ -PAM system. Furthermore, the results indicate that treatment costs could potentially be reduced by optimizing the  $\text{FeCl}_3$ -to-tannin ratio. Considering the renewable origin of tannins and the increasing interest in replacing fossil-based synthetic polymers, Acacia tannins represent a promising bio-based alternative for dairy wastewater treatment, although further optimization and economic evaluation are required.

## 4. Discussions

The study aimed to assess if cationic tannins could be a cost-effective and efficient alternative to PAM and other synthetic flocculants in treating dairy wastewater. Results from the screening with  $\text{FeCl}_3$ -tannin and  $\text{FeCl}_3$ -PAM showed that both  $\text{FeCl}_3$  and tannins were significant in explaining all responses, but only  $\text{FeCl}_3$  was significant for Susp and COD removal when used with PAM. This was unexpected as PAM typically leads to larger floc formation for easier settling and decreased suspended particles and organic material. The insignificant role of PAM could be due to model inaccuracy or external factors.  $\text{FeCl}_3$  was found significant for all responses, with phosphate ion precipitation and sweep coagulation playing roles in TP, Susp, and COD removal. The screening confirmed that tannins and PAM had similar performance when combined with  $\text{FeCl}_3$  at specified dosages, with tannins needing four times the dosage of PAM to achieve comparable results. Overall, the study demonstrated the potential of cationic tannins as a viable alternative to traditional flocculants in wastewater treatment processes.

The optimized model statistics show high  $Q^2$  values for all response variables, indicating the reliability of the obtained models. Optimum conditions for TP and COD removal are achieved with high tannin and  $\text{FeCl}_3$  dosages, while Susp shows a transitional phase where excessive  $\text{FeCl}_3$  and tannins have a negative impact. This can be attributed to overdosage leading to charge neutralization issues and inhibited particle aggregation due to charge reversal. In addition, overdosage impairs polymer bridging, where excessive flocculant inhibits the formation of bridges between particles. Figure 11 demonstrates that increasing tannin dosage from 10 to 100 mg/L, at a constant  $\text{FeCl}_3$  dosage of 360  $\mu\text{L/L}$ , significantly improves Susp, TP, and COD removal. However, further tannin addition beyond this point does not yield as noticeable improvements, likely due to polymer bridging inhibition. After a certain tannin threshold, most colloidal particle surfaces become occupied, making it harder for tannins to form bridges. Nonetheless, a slight improvement in removal efficiency may still occur as particle-complexes increase in size, aiding settling despite not reaching the same level as uninhibited polymer bridging.

Susp, TP, and COD were selected as response variables at Norrmejerier. Limits were set for these parameters: 250 mg/L Susp, 10 mg/L TP, and 500 mg/L COD. Tannin dosage of 10 mg/L (with 360  $\mu\text{L/L}$   $\text{FeCl}_3$ ) met the limits for Susp and TP but not for COD. Experiments with PAM also failed to meet the restrictions. Comparative cost analysis showed that using PAM cost 1.83 SEK/ $\text{m}^3$  while tannins cost 2.78 SEK/ $\text{m}^3$ , making tannins approximately 1 SEK/ $\text{m}^3$  more expensive. However, factors like reducing carbon footprint, corporate social responsibility (CSR), and goodwill should also be considered. Carbon footprint denotes greenhouse gas emissions; replacing fossil-based with bio-based materials can help. CSR refers to business responsibility towards society, including the environment. Goodwill represents a company's value beyond assets, like reputation. Shifting from fossil-based to bio-based reagents can positively impact these aspects, important in today's business landscape. It's crucial to consider these factors, not just costs, when making decisions.

The tannins used in the experiments were purchased from Brazil for commercial production, but it could be possible to produce tannins at Swedish pulp mills using bark as a side stream. Extracting valuable tannins before incinerating the bark could reduce costs and be more sustainable. More research is needed for effective extraction methods for Scandinavian tree species.

The experiments showed low model validity values, which could be due to low replicate error from accurate determination of the response. Using only tannins without  $\text{FeCl}_3$  added showed that the TP and COD restrictions were not met, even at a tannin dosage of 350 mg/L. Increasing the tannin dosage from 100 to 200 mg/L improved removal but further increasing the dosage did not have much effect. Cationic tannins must be used with  $\text{FeCl}_3$  or other metal salts to achieve desirable results for TP and COD restrictions.

Recycling phosphates collected during wastewater treatment, preferably as fertilizer, is important due to phosphorus being a finite resource. When  $\text{FeCl}_3$  is used as a coagulant, most phosphates are present as iron phosphate, which studies have shown can still be effective fertilizers for plants. Cationic tannins must be used in combination with  $\text{FeCl}_3$  or other metal salts for nutrient recycling, which should not be an issue.

Gas production was not affected until a tannin dosage of 400 mg/L, which is not economically feasible. A more practical dosage of 100 mg/L showed improved Susp, TP, and COD removal, with even better gas production than the control sample. Successful jar tests with  $\text{FeCl}_3$  and tannins were not replicated in full-scale trials due to floc formation that couldn't be separated in the flotation tank. Optimal Susp, TP, and COD values were achieved after 4.5 h with a tannin dosage of 200 mg/L and a flow rate of 30 m<sup>3</sup>/h, indicating that higher tannin dosage and lower flow rate are preferable. Tannin flocs appeared fluffy and lightweight, requiring more time to settle compared to PAM flocs. In the flotation tank, flocs remained small and didn't separate effectively from the water. Changes in water volume, flotation method, and oxygen saturation may have impacted the results. Further research is needed to understand the effects of oxygen levels and pH on tannin flocculation. Large-scale settings should be simulated to optimize the use of cationic tannins as a flocculant.

Cationic tannins' impact on gas production was investigated, but further research is needed to ensure they don't harm bacterial cultures in biogas production systems. Dewatering properties of tannin-sludge were not evaluated, despite its importance in reducing volume and weight of sludge for transportation and sustainability. Additional studies are required to fully assess the efficacy and implications of using cationic tannins in wastewater treatment processes.

It should be noted that the reagents used in the cationization process are less environmentally friendly than tannins themselves. Similarly, the reagents used in synthetic flocculants are not eco-friendly either. Thus, using tannin-based flocculants in wastewater treatment is preferred for both people and the environment. Further research may lead to more sustainable cationization processes in the future. The industry supports the shift from synthetic to bio-based alternatives in wastewater treatment, including management at sewage treatment plants and relevant organizations. Swedish sewage treatment plants meeting certain sustainability criteria can be Revaq certified. Currently, Revaq does not require the use of PAM, but a bio-based alternative could potentially lead to restrictions in the future.

## 5. Conclusions

Cationic tannins were evaluated as flocculant in the chemical purification step during wastewater treatment from dairy. In lab scale experiments, cationic tannins from Acacia can be successfully applied as flocculant in the chemical purification step of wastewater from the dairy industry. It is a viable alternative to PAM, and possibly other synthetic flocculants, in terms of performance but slightly more expensive as a greater amount is required. However, the cost should be considered alongside values such as reducing carbon footprint, corporate social responsibility (CSR) and goodwill. Factors that are becoming increasingly important for many businesses today. The key findings from each individual experiment can be summarized as:

- The effect of cationic tannins in combination with  $\text{FeCl}_3$  was comparable to the combination of PAM and  $\text{FeCl}_3$ .
- The tannin dosage should be four times the PAM dosage for comparable results which is approximately 0.09 € /m<sup>3</sup> more expensive. However, the cost may be reduced by altering the ratio of  $\text{FeCl}_3$ -/tannin-dosage.
- At optimal tannin and  $\text{FeCl}_3$  dosages, a Susp-, TP and COD removal close to 100% was reached.
- Tannins alone were able to achieve a Susp, TP and COD removal around 98%, 70% and 76% respectively.
- The biogas production was increased in sludge containing a tannin concentration of 100- and 200 mg/L.
- The system that was utilized in this full-scale trial was not suitable for tannins, however, higher tannin dosage combined with lower flow rate was favourable.

## Author Contributions

M.A. (Corresponding author): Conceptualization, methodology, project administration, formal analysis, writing—original draft preparation, writing—review and editing. J.A.: Investigation, experimental work, data

curation, validation, formal analysis, visualization. T.M.: Methodology, coordination of pilot-scale activities, formal analysis, writing—review and editing. H.A.: Supervision, statistical analysis, software support, validation, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

### Funding

This research was funded by Vinnova (Sweden's Innovation Agency) through the OptiBark project, Grant No. 2019-04585, "Bio-based materials and chemicals from industrial bark and willow (*Salix*) through optimized processes".

### Institutional Review Board Statement

Not applicable.

### Informed Consent Statement

Not applicable.

### Data Availability Statement

The data presented in this study are available from the corresponding author upon reasonable request.

### Acknowledgments

The authors acknowledge that this manuscript is based on the Master's thesis of Julia Avander [23], completed at the Swedish University of Agricultural Sciences (SLU). The thesis has been substantially revised and expanded to produce the present journal article. The authors sincerely thank Ulla-Britt Östman and Olof Ögren for their supervision of the experimental work and for their valuable comments and constructive feedback.

### Conflicts of Interest

Given the role as a member of Editorial Board, Mehrdad Arshadi had no involvement in the peer review of this paper and had no access to information regarding its peer-review process. Full responsibility for the editorial process of this paper was delegated to another editor of the journal.

### Use of AI and AI-Assisted Technologies

No AI tools were utilized for this paper.

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