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INFLUENCE OF NEEM LEAF POWDER AS A NATURAL COAGULANT IN MBR EMPLOYED TO TREAT DAIRY WASTEWATER

VPLIV PRAHU IZ LISTOV NEEMA KOT NARAVNEGA KOAGULANTA V MBR, UPORABLJENEM ZA ČIŠČENJE ODPADNE VODE IZ MLEKARN

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Abstract

This study pretreats dairy wastewater prior to MBR processing using neem leaf powder as a natural coagulant. Scanning Electron Microscopy, X-ray Diffraction, Fourier Transform Infrared Spectroscopy, and Energy-Dispersive X-ray Analysis revealed that neem powder has a high surface area, an amorphous-crystalline structure, and coagulation-promoting functional groups and elements. Jar tests optimized coagulant dosage, pH, mixing time, and settling duration. Under ideal conditions, turbidity and COD decreased significantly. Both MBR systems, with and without neem pretreatment, were tested for COD reduction efficiency, transmembrane pressure, permeate flux, specific oxygen uptake rate, and sludge settleability. In the experimental MBR reactors, neem-based pretreatment substantially enhanced COD removal efficiency, peaking at ~99% at a 6-hour hydraulic retention time (HRT), compared to ~90% without pretreatment. The neem-integrated system showed reduced TMP accumulation (17.9 kPa vs. 27.6 kPa), increased flux retention (22.7 vs. 12.9 liters per square meter per hour), and enhanced microbial activity, as measured by Mixed Liquid Volatile Suspended Solids (MLVSS) (23.5 mg O_2/g MLVSS/h). These results indicate that neem powder may enhance biodegradability and reduce membrane fouling, making dairy wastewater management more sustainable.

Keywords: MBR, Coagulant, Neem leaf powder, COD reduction, Transmembrane Pressure, Specific Oxygen Uptake Rate, Sludge Settleability, Fouling.

Izvleček

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V tej študiji se je odpadna voda iz mlekarne pred čiščenjem z membranskim bioreaktorjem (MBR) predhodno obdelala z uporabo prahu iz listov neema kot naravnega koagulanta. Analize z vrstično elektronsko mikroskopijo (SEM), rentgensko difrakcijo (XRD), Fourierjevo transformacijsko infrardečo spektroskopijo (FTIR) in energijsko disperzijsko rentgensko analizo (EDAX) so pokazale, da ima prah neema veliko površino, amorfno-kristalno strukturo ter funkcionalne skupine in elemente, ki spodbujajo koagulacijo. Testi v kozarcih so optimizirali odmerek koagulanta, pH, čas mešanja in trajanje usedanja. V idealnih pogojih sta se motnost in KPK znatno zmanjšala. Oba sistema MBR, s predhodno obdelavo z neemom in brez nje, sta bila testirana glede učinkovitosti zmanjšanja KPK, transmembranskega tlaka (TMP), permeatnega toka, specifične hitrosti privzema kisika (SOUR) in usedanja blata (SVI). V eksperimentalnih reaktorjih MBR je predhodna obdelava na osnovi neema bistveno povečala učinkovitost odstranjevanja KPK, ki je dosegla vrh pri ~99 % pri 6-urnem hidravličnem zadrževalnem času (HRT) v primerjavi s ~90 % brez predhodne obdelave. Sistem, integriran z neemom, je pokazal zmanjšano kopičenje TMP (17,9 kPa v primerjavi z 27,6 kPa), povečano zadrževanje toka (22,7 litra na kvadratni meter na uro (LMH) v primerjavi z 12,9 LMH) in okrepljeno mikrobno aktivnost, merjeno s hlapnimi suspendiranimi snovmi v mešanici aktivnega blata in vode (MLVSS) (23,5 mg O_2/g MLVSS/h). Ti rezultati kažejo, da lahko prah neema poveča biorazgradljivost in zmanjša mašenje membran, s čimer postane upravljanje odpadne vode iz mlekarn bolj trajnostno.

Ključne besede: MBR, koagulant, prah listov neema, zmanjšanje KPK, transmembranski tlak, specifična hitrost privzema kisika, usedanje blata, mašenje.

1. Introduction

Pretreatment of dairy effluent is crucial for membrane bioreactor (MBR) systems, as it reduces high concentrations of fats, oils, and organic matter that can inhibit biological activity and cause membrane fouling. Efficient pretreatment processes, such as the use of coagulants and flocculants or the growth of biosurfactant-producing yeasts, including *Candida bombicola*, could considerably increase the removal of these contaminants, achieving more than 90% chemical oxygen demand (COD) removal and reducing substrate inhibition in biological treatment (Aragaw TA & Bogale FM, 2023). Also, proper pretreatment, such as fine screening, is essential for ensuring the stability and sustainability of MBRs, as the accumulation of solids may affect membrane performance (Cote et al., 2006). A combination of physical, chemical, and biological pretreatment methods may maximize the efficiency of the entire dairy wastewater treatment process, improve permeate quality, and minimize operational costs (Ramsuroop, 2024).

Coagulation is an effective pretreatment method for membrane bioprocesses in treating dairy wastewater, as it offers several advantages over direct membrane filtration. Coagulation also improves COD uptake, achieving 96-99% when coupled with ultrafiltration (UF) or nanofiltration (NF), compared to only 61% with direct NF (Formentini-Schmitt et al., 2023). It also contributes to a considerable reduction in membrane fouling, with studies showing reductions in fouling rates by 10-37% when coagulation is used as a pretreatment before membrane filtration (Esteki et al., 2024). This pretreatment not only increases membrane lifetime but also enhances permeate flux, as reported, with an 80% increase in a combined microfiltration-UF system (Zielińska & Galik, 2017). Moreover, natural coagulants, including *Moringa oleifera*, have demonstrated positive effects on treatment efficiency and the sustainability of dairy wastewater purification (Formentini-Schmitt et al., 2023). Overall, coagulation as a pretreatment is highly effective at enhancing the efficiency of membrane bioprocesses; hence, it is an excellent approach to dairy wastewater management.

Coagulation is a superior pretreatment method for membrane bioprocesses in dairy wastewater treatment compared to direct membrane filtration. On the one hand, coagulation can significantly improve COD removal, achieving 95% in hybrid systems (Alimoradi et al., 2018). On the other hand, residual aluminum from the coagulation process may cause operational issues in subsequent MBR stages, including increased transmembrane pressure and irreversible membrane fouling (Alimoradi et al., 2018). Moreover, the efficiency of the coagulation process can be increased by combining it with advanced oxidation processes (AOPs); however, residual chemicals may complicate downstream processing (El-Sonbati et al., 2020). However, natural coagulants, including *Moringa oleifera*, have been shown to reduce fouling and improve membrane performance, suggesting that alternative pretreatment strategies can alleviate the drawbacks of chemical coagulation (Formentini-Schmitt et al., 2023). Although coagulation can enhance initial treatment results, its long-term effects require adequate control to avoid undermining membrane integrity and functional efficiency.

Moringa oleifera and other plant-based natural coagulants offer significant benefits over traditional chemical coagulants in membrane bioprocesses for treating dairy wastewater. These natural alternatives are cost-effective and environmentally friendly because they produce less sludge and do not generate hazardous byproducts when chemical coagulants are not used (Bahrodin et al., 2021). Research shows that natural coagulants can achieve removal efficiencies comparable to or better than those for major water pollutants, including biological oxygen demand (BOD), COD, and turbidity, with removals frequently exceeding 60% (Koul, B et al., 2022). Also, these coagulants are biologically based, and thus the production of non-treatable wastes is minimal; hence, they are applicable to sustainable wastewater management (Badawi et al., 2023). Moreover, natural coagulants do not significantly affect the pH of treated water, thereby preserving water quality (Alazaiza et al., 2022). Overall, the shift towards natural coagulants is a promising approach to improving the efficiency and sustainability of dairy wastewater treatment processes.

Using neem powder as a coagulant in a membrane bioreactor for treating dairy wastewater has several disadvantages. Neem powder is considered environmentally friendly and more economical because it is derived from abundantly available, low-cost biomass, requires simple processing, works effectively at lower dosages, and produces less sludge, thereby reducing chemical, energy, and disposal costs in wastewater treatment compared to chemical coagulants. On the other hand, its effectiveness can vary across various pH conditions, and it may require the addition of other coagulants, such as powdered activated carbon to improve efficiency (Kani et al., 2016). Moreover, the optimal neem powder concentration may vary significantly, and it is critical to adjust it precisely to achieve desirable pollutant removal rates (COD and BOD) (Mohan et al., 2019). Also, the organic matter in dairy wastewater can slow the coagulation process and worsen turbidity reduction (Al-Tayawi et al., 2023). Finally, the presence of residual neem-derived organic compounds may complicate downstream processing in the membrane system, potentially influencing membrane fouling and overall treatment efficiency (Maeda, 2024). Therefore, although neem powder can be seen as an attractive, sustainable alternative, its practical application in dairy wastewater treatment still needs improvement, and its limitations should be understood.

Although much research has been conducted on sustainable and bio-based pretreatment technologies, quantitative data to facilitate an assessment of neem powder's performance as a pretreatment technique prior to MBR are scarce. The lack of standardization of operational parameters and lab-scale studies reduces their reproducibility and industrial application for treating dairy wastewater. Thus, an attempt was made to assess the performance of neem powder as a pretreatment to the MBR system in terms of COD removal efficiency, microbial growth rate, and fouling analysis under optimal operational conditions. The work aims to achieve the novel integration of locally available, bio-based materials as a green strategy for MBR treatment of dairy wastewater, marking an innovation in sustainable and cost-effective wastewater treatment.

2. Materials and Methods

The methodology used to conduct the study is shown in Figure 1. The study was carried out in three phases: phase I involved the collection and characterization of coagulant, wastewater, and membrane. Phase II was carried out as pretreatment to the MBR process, and the treatment of pretreated dairy wastewater in the MBR was carried out as Phase III.

Figure 1: *Methodology adopted for the study.*

Slika 1: *Uporabljena metodologija.*

Figure 2 (a): *Sun-dried neem leaf.*

Slika 2 (a): *Na soncu posušeni listi neema.*

2.1 Collection and Characterization of Wastewater

Wastewater samples used in the current investigation were collected from a dairy processing plant at Aavin in Karaikudi, Sivaganga district, Tamil Nadu, India. Characteristic analysis was performed in the laboratory in accordance with APHA guidelines. (Table 1).

Characterization of dairy wastewater reveals elevated BOD and COD levels compared with the industrial effluent standards prescribed by the Central Pollution Control Board (CPCB), along with substantial organic and inorganic content.

Table 1: Characteristics of dairy wastewater.

Preglednica 1: Značilnosti odpadne vode iz mlekarne.

Parameter	**Unit**	**Value**
BOD	mg/L	350
COD	mg/L	1019.36
Organic matter	mg/L	1920
Turbidity	NTU	52.3
Color	-	Light grey
Odor	-	Unpleasant
Hardness	mg/L as $CaCO_3$	1250
Alkalinity	mg/L as $CaCO_3$	110
SVI	mg/L	18.87
pH	-	6.9

2.2 Collection and Preparation of Neem Powder

Neem leaves *(Azadirachta indica)* were collected from the Alagappa Chettiar Government College of Engineering and Technology premises in Karaikudi, Sivaganga district, Tamil Nadu, India. The collected neem leaves were sun-dried for 3-4 days and then ground into a fine powder. Neem powder that passes through a 75 µm sieve was stored and used as a coagulant dosage, as shown in Figure 2 (a) and (b).

Figure 2 (b): *Neem leaf powder.*

Slika 2 (b): *Prah listov neema.*

To minimize property changes associated with sun-drying, neem leaves were dried under standardized conditions by spreading them in a single layer on raised mesh trays at a fixed campus location, ensuring uniform exposure and air circulation. Ambient temperature and relative humidity were monitored during drying, and the process was terminated at a predefined moisture endpoint (≤5% w/w, oven method) rather than by drying time to account for variability in solar intensity. Sun-dried neem leaves were ground using the same equipment and sieved through a 75 µm mesh to obtain a uniform particle-size fraction. Batch consistency was ascertained by measuring moisture content, total protein, and bulk density, as shown in Table 2 (Lwasa et al., 2024).

2.3 Coagulation Studies

Coagulation studies using neem powder were conducted to optimize the dosage, pH, rotational speed, and settling time for the batch coagulation process. Each experiment was repeated three times, and the average value was taken into consideration for further computation. Studies were conducted in the jar test apparatus, where the dosage was added as a solution by mixing 1 g of neem leaf powder with 100 ml of distilled water. The initial dosage was taken as the optimized dosage for the remaining experiments. The mixture was then allowed to disperse uniformly for an hour using a magnetic stirrer. The efficacy and optimal response in all studies were assessed based on turbidity removal efficiency. The turbidity removal efficiency is determined by:

$$Reduction\ in\ Turbidity = \frac{Initial\ turbidity - Final\ Turbidity}{Initial\ Turbidity} \times 100 \quad (1)$$

Table 2: Characterization of neem leaf powder.

Preglednica 2: Značilnosti prahu listov neema.

Parameter	**Batch 1**	**Batch 2**	**Batch 3**	**Mean ± SD**	**%RSD**	**Acceptance criterion**
Moisture content (% w/w)	4.8	4.89	4.83	4.83 ± 0.07	1.45	≤ 5%
Particle size (<75 µm, %)	96.4	95.8	96.9	96.4 ± 0.55	0.57	≥ 95%
Bulk density (g/cm^3)	0.41	0.43	0.42	0.42 ± 0.01	2.4	±10%
Total protein (mg/g)	22.6	23.1	22.9	22.9 ± 0.25	1.1	±10%

2.4 Performance analysis of MBR with and without treatment

2.4.1 COD reduction analysis under different HRT

Two sets of experimental studies were designed; the first set of experimental work was performed with a coagulation process as a pretreatment, and the other without pretreatment. The coagulation treatment process was performed based on the outcomes of preliminary studies. COD reduction analysis was conducted in both reactors under varying HRT. HRT was varied from 2 to 7 hours at 1-hour intervals. The COD reduction was assessed based on the following equation:

$$COD\ reduction = \frac{Initial\ COD - Final\ COD}{Initial\ COD} \times 100 \quad (2)$$

2.4.2 Microbial Activity Analysis

In microbial activity in MBR systems, Specific Oxygen Uptake Rate (SOUR) was determined at HRTs of 2, 3, 4, 5, and 7 hrs as described in equation 3. At every HRT, mixed-liquid samples were taken at steady state from the neem-pretreated and non-pretreated MBR reactors. The experiments were performed in a reactor (1 L) with a sealed sample under measured aeration with a probe of dissolved oxygen (DO) (Model: YSI ProODO) at 25 ± 2 °C. Over 10 min., DO levels were measured while the sample was gently stirred.

$$SOUR = \frac{-\Delta DO}{(\Delta t \times MLVSS)} \quad (3)$$

Here, ΔDO denotes the change in dissolved oxygen concentration (mg/L) during test duration Δt (h), and MLVSS denotes the concentration of mixed liquor volatile suspended solids (g). The negative sign indicates that DO has decreased over time due to microbial respiration.

2.4.3 Membrane Fouling Analysis

To evaluate membrane fouling patterns and operational performance, two parameters, transmembrane pressure (TMP) and permeate flux, were measured daily for 7 days in two identical membrane bioreactor (MBR) systems: one with and one without neem leaf powder pretreatment as explained in equation 4. Both reactors were run at identical hydraulic retention time (HRT), organic loading rate (OLR), and aeration. A calibrated digital pressure transducer was placed across the membrane module that continuously monitored TMP.

$$J = \frac{V}{(A \times t)} \quad (4)$$

Here, J=Permeate flux (L/m²·h), V=Volume of collected permeate (L), A=Membrane surface area (m²), t=Filtration time (h).

2.3 Reactor Setup

The MBR process, with and without pretreatment (Figures 3), was conducted to assess the impact of pretreatment on COD reduction in dairy wastewater.

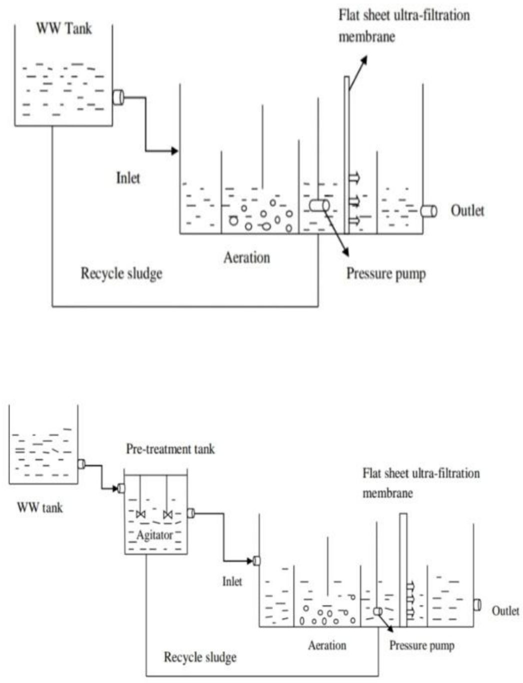

Figure 3: *Reactor Without pretreatment and With pretreatment.*

Slika 3: *Reaktor brez predobdelave in s predobdelavo.*

The reactor was designed for continuous flow with a working volume of 6 L, and a submerged flat-sheet Polyether Sulfone (PES) membrane was employed. The following operational parameters were used during the experimental study: MLSS concentration: 3125 mg/L; Aeration rate: 0.5 m3/m2.hr; Temperature: room temperature varying around 21–25 °C; Flow rate: to maintain the HRT in the range of 2–7 hours, the flow rate was varied from 14–50 ml/min using a peristaltic pump. Properties of the membrane employed in the current investigation are presented in Table 3.

Table 3: Basic properties of the membrane.

Preglednica 3: Osnovne lastnosti membrane.

Parameters	Description
Type of MBR	Submerged
Module design	Flat-sheet ultrafiltration
Membrane material	Polyethersulfone
Pore size	0.01-0.1 µm

3. Results and discussion

3.1 Surface Analysis of Neem Powder

Neem powder has been widely characterized, especially in terms of its use in the coagulation process using a Scanning Electron Microscope (SEM), Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), and Energy-Dispersive X-ray Analysis (EDAX).

The surface morphology of neem powder was investigated using a SEM. It was found that the particle size of neem powder ranged between 0.09 and 0.24 mm, with an average particle size of 0.165 mm (Figure 4). Circularity ranged from 0.107 to 0.786, with an average value of 0.368, indicating that most particles were relatively irregular in shape, as average circularity was below the commonly used threshold of 0.4 for highly irregular particles."Therefore, the surface area of neem powder is larger, it can bind colloidal particles, and its roughness leads to better interlocking, resulting in better floc formation (Wylie et al., 2022). XRD was used to evaluate the crystal structure of neem powder and determine the product's crystallinity and phase composition. XRD results showed that neem powder has an amorphous structure at large, with few crystalline phases (Figure 5).

It was linked to the presence of crystalline peaks, which were attributed to organic compounds such as tannins and flavonoids, known to facilitate neem's coagulative properties (Afzhal et al., 2023). The presence of functional groups in neem powder between 3950 and 620 cm-1 (Figure 6) was identified using FTIR.

The FTIR spectrum exhibited strong absorption bands corresponding to hydroxyl (-OH), carbonyl (C=O), and amine (N-H) groups. The major band observed was at about 1054.7 cm^1. Terpenoids and flavonoids are among the several substances known to be present in neem leaves. The 1054.7–1060 cm^{-1} peak may be caused by C-O bonding in some of these substances (Table 4).

EDAX was used to assess the elemental composition of neem powder (Figure 7).

The EDAX spectrum indicated the presence of elements such as carbon, oxygen, nitrogen, and sulphur. The two main elements of the sample are carbon (C) and oxygen (O). The sample's carbon content is roughly 65.29 weight percent (wt.%) or 73.35 atomic percent (at.%). The sample's oxygen content is approximately 29.92% by weight or 25.24% by atomic percent. There are 3.27 weight percent (or 1.13 at.%) of potassium, 0.47 weight percent (or 0.16 at.%) of calcium, and 1.05 weight percent (or 0.12 at. %) of indium. This sample contains a considerable amount of oxygen and is rich in carbon. The spectrum of neemleaf powder exhibited dominant peaks of carbon and oxygen, confirming the organic biopolymeric nature of the material, primarily composed of cellulose, hemicellulose, lignin, proteins, and polyphenols. Prominent calcium and potassium peaks were also observed, which is consistent with literature reports indicating Ca (1.8–3.2%) and K (0.9–2.2%) as major inorganic constituents of neem leaves (Andersa et al., 2024). The presence of Ca^{2+} plays a critical role in coagulation through charge neutralization, electrical double-layer compression, and cation-mediated cross-linking of functional groups ($–COO^–$, –OH, $–NH_2$) present in neem biopolymers, thereby enhancing adsorption-bridging and floc formation (Choy et al., 2014). Potassium, although monovalent, increases ionic strength, thereby facilitating particle collisions and aggregation. Elements such as Mg, Fe, Zn, Mn, and C, which have been reported in the literature at low concentrations (<0.6% for Mg and <0.1% for most trace metals), were not distinctly visible in the spectrum, which is attributed to the inherent detection limits of EDS for light and trace elements in organic matrices (Goldstein et al., 2018). A minor indium peak was detected, indicating trace-level environmental uptake, which is environmentally plausible and consistent with reports of indium occurrence in plant tissues near anthropogenic sources; however, its low intensity suggests no significant influence on coagulation performance (Jensen et al., 2018). Overall, the EDS results corroborate the literature-based elemental composition and support the conclusion that the coagulation efficiency of neem leaf powder arises from the synergistic action of multivalent cations (especially Ca^{2+}) and organic biopolymers, enabling effective charge neutralization and adsorption-bridging mechanisms comparable to conventional coagulants. The obtained EDAX spectrum was compared with the literature presented in Table 5.

Elevated indium in neem-derived coagulants can impair coagulation performance and produce metal-enriched sludge. Indium competes with native multivalent cations (Ca^{2+}, Mg^{2+}, Fe^{3+}) for binding sites on neem biopolymers, altering adsorption-bridging and reducing floc strength; in addition, indium exposure changes plant cell-wall composition (increased lignification), lowering the release of soluble active polymers (Qian et al., 2024). The resulting sludge concentrates and may exhibit altered dewatering and leachability characteristics, potentially necessitating stabilization or recovery operations (Yandem & Jabłońska-Czapla, 2024). Therefore, routine measurement of both feedstock and sludge, coupled with sequential extraction and consideration of recovery options, is recommended.

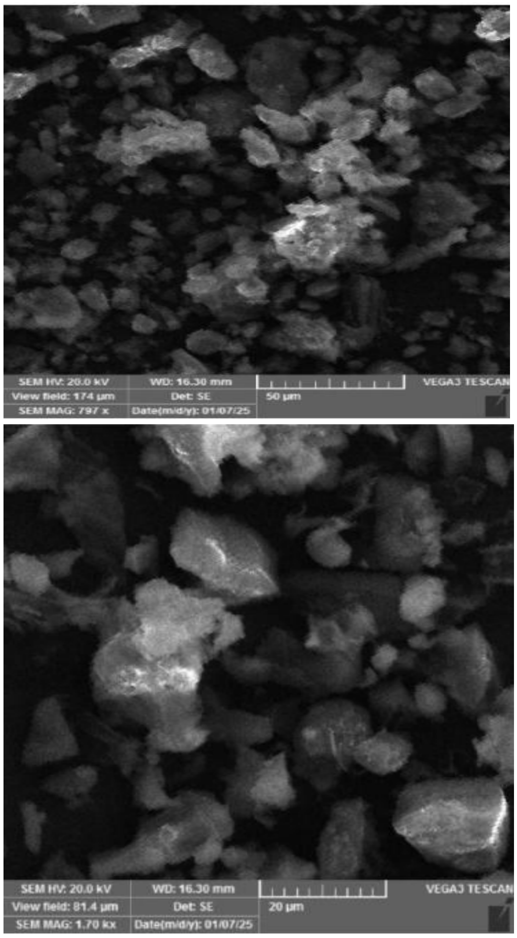

Figure 4: *SEM images of neem leaf powder.*

Slika 4: *SEM-slike prahu listov neema.*

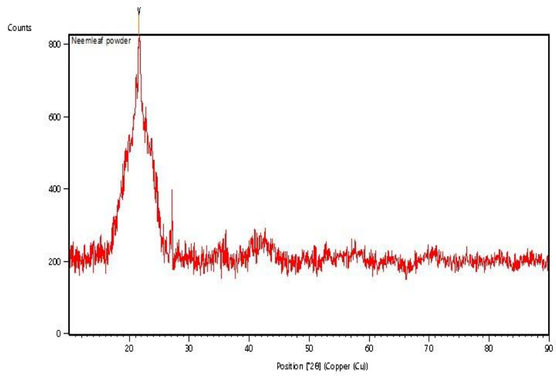

Figure 5: *XRD analysis of neem leaf powder.*

Slika 5: *XRD-analiza prahu listov neema.*

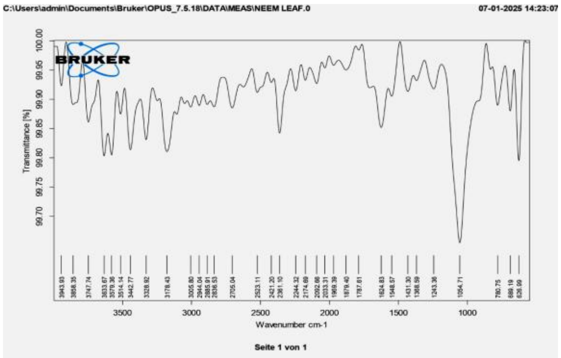

Figure 6: *FTIR image of the neem leaf Powder.*

Slika 6: *FTIR-slika prahu listov neema.*

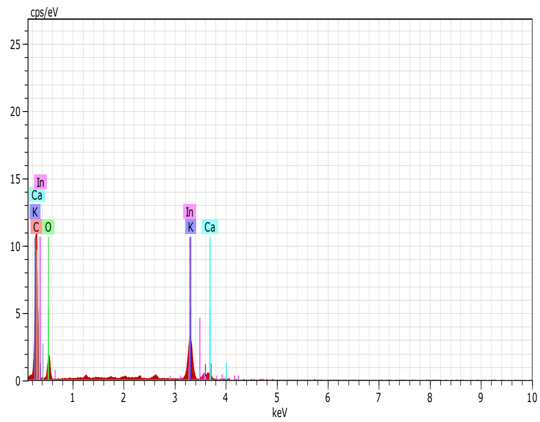

Figure 7: *EDAX spectrum of neem leaf powder.*

Slika 7: *EDAX-spekter prahu listov neema.*

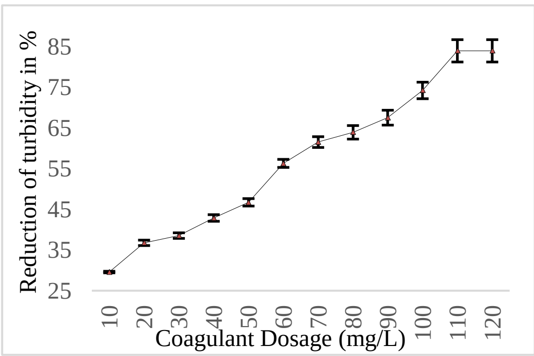

Figure 8.a: *Variation of turbidity reduction with coagulant dosage.*

Slika 8.a: *Sprememba zmanjšanja motnosti z odmerkom koagulanta.*

Table 4: *FTIR Spectrum.*

Preglednica 4: *Spekter FTIR.*

Wave number	Possible Functional group	Interpretation
3394-3200	O-H/N-H stretch	The broad peak indicates a hydroxyl group (phenols, alcohols, or amines)
2295,2854	C-H stretch (alkane)	Medium peaks are saturated hydrocarbons
1739	C=O Stretch (carbonyl)	Strong, sharp peaks are esters, aldehydes, or carboxylic acids
1626,1604	C=C stretch (aromatic)	aromatic rings
1511-1442	N-H bend/ aromatic	Proteins or secondary amines
1240-1020	C-O/C-N Stretch	Esthers, esters, or amine-related structures
890-690	Aromatic C-H bending (out of plane)	Substituted benzene ring

Table 5: *Comparative analysis of EDAX Spectrum.*

Preglednica 5: *Primerjalna analiza EDAX-spektra.*

Element	Presence in %	Observation	Agreement	Reference
C	Major organic fraction	Strong peak	Fully consistent	Choy et al. (2014)
O	Major organic fraction	Strong peak	Fully consistent	Andersa et al. (2024)
Ca	1.8–3.2 %	Strong peak	Fully consistent	Andersa et al. (2024)
K	0.9–2.2 %	Clear peak	Fully consistent	Patel et al. (2021)
Mg	0.22–0.58 %	Not visible	Expected (below detection limit)	Patel et al. (2021)
Fe	0.015–0.072 %	Not visible	Expected	Goldstein et al. (2018)
Zn, Mn, Cu	<0.02 %	Not visible	Expected	Singh et al. (2020);
Indium (In)	Trace / environmental	Minor peak	Environmentally plausible	Yandem & Jabłońska-Czapla (2024)

3.2 Coagulation Studies

The optimal coagulant dosage was determined in a study assessing the effect of varying it. The optimal dosage was used in experiments to optimize rapid mixing, slow mixing time, pH, and settling time. All experiments were conducted in triplicate (n = 3), and results are presented as mean ± SD. The results from the optimization study are shown in Figure 8.

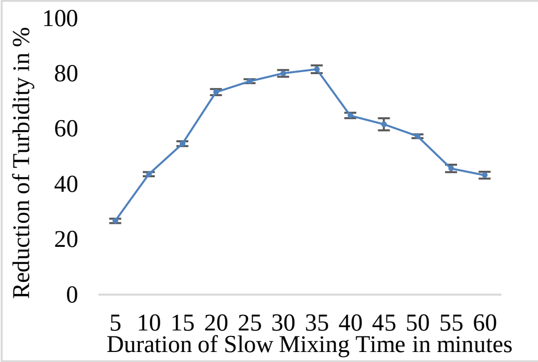

Figure 8.b: *Variation of turbidity reduction with slow mixing time.*

Slika 8.b: *Sprememba zmanjšanja motnosti s počasnim mešanjem.*

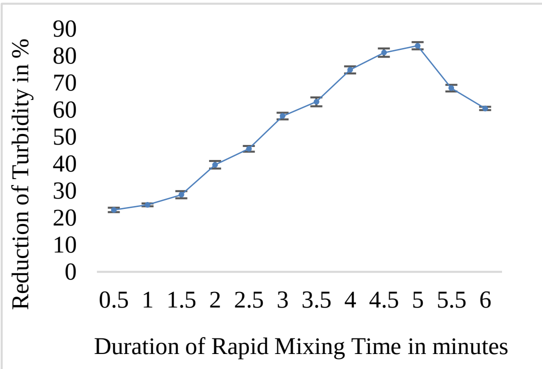

Figure 8.c: *Variation of turbidity reduction with rapid mixing time.*

Slika 8.c: *Sprememba zmanjšanja motnosti s hitrim mešanjem.*

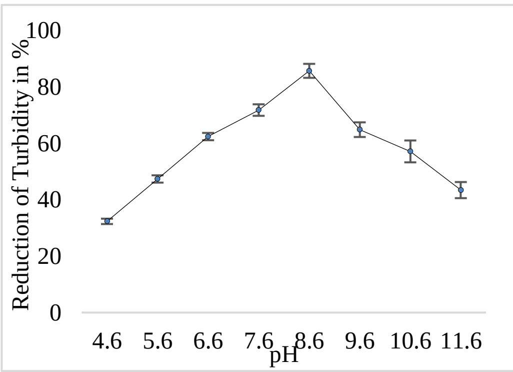

Figure 8.d: *Variation of turbidity reduction with pH.*

Slika 8.d: *Sprememba zmanjšanja motnosti s pH.*

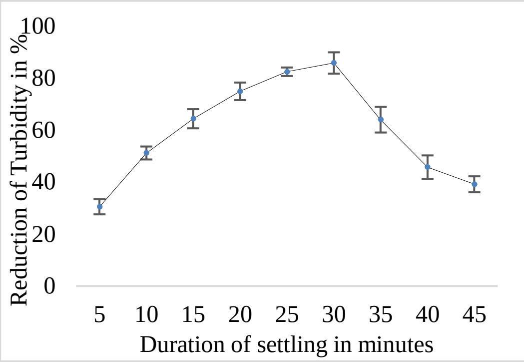

Figure 8.e: *Variation of turbidity reduction with duration of settling.*

Slika 8.e: *Sprememba zmanjšanja motnosti s trajanjem usedanja.*

At low dosages of neem leaf powder, there are not enough active sites to effectively bind with the particles, causing turbidity in the wastewater. The initial increase in dosage leads to a proportional increase in turbidity removal, as more particles are neutralized and aggregated for sedimentation (Khan et al., 2023). The optimal dosage is reached when the maximum number of particles is neutralized and aggregated, leading to the highest turbidity removal efficiency. Once the optimal dosage is surpassed, the turbidity removal efficiency remains constant because all available particles have been effectively neutralized. Additional coagulants do not contribute to further turbidity reduction and may even destabilize the formed flocs, potentially increasing turbidity if overdosed (Khan et al., 2023). This plateau effect is a common characteristic in coagulation processes, where the balance between coagulant dosage and particle availability is crucial for optimal performance. Mixing time ensures sufficient contact between coagulant and particles, promoting effective floc formation and subsequent sedimentation (El-tawel et al., 2023). Slow mixing time allows collision and clumping of the particles, resulting in bigger and more stable flocs (Tahraoui et al., 2024). In this way, the optimal mixing time allows for the maximum interaction between neem leaf powder and the suspended particles, thereby maximizing flocculation and sedimentation (Tsoutsa et al., 2024). Continuous mixing after the optimum period resulted in floc breakdown due to inappropriate shear forces, thereby reducing the efficiency of turbidity removal. Overmixing will also result in the resuspension of settled particles, thereby negating the effects of coagulation, and this too will increase turbidity levels. Neem leaf powder is found to be effective at various pH levels, but the interaction between the coagulant and particles is optimized at 8.6, resulting in more turbidity removal (Namdeti et al., 2023).

The measured standard deviation (SD) values for coagulation process variables indicate the intrinsic experimental variability and sensitivity of each operational factor during wastewater treatment using a neem leaf-derived natural coagulant. Reduced SD values in coagulant dosage indicate that dosage effects on treatment efficacy were relatively uniform across replicates, whereas an increase in SD at higher dosages signals nonlinear responses and operational sensitivity at elevated levels, aligning with the need for precise dosage calibration to achieve optimal floc formation in natural coagulants (Ali et al., 2025). The elevated pH SD highlights its crucial role in charge neutralization and colloid destabilization, making it a sensitive parameter in coagulation optimization, as evidenced by jar-test studies in which pH adjustments markedly affected removal efficiency (Mahapatra, 2025). Similarly, the settling time SD was significant, indicating variability in floc settling dynamics attributed to heterogeneous floc size and density, a typical characteristic of natural coagulants, where sedimentation performance can fluctuate considerably (Ali et al., 2025). Conversely, mixing times (both rapid and slow) generally exhibited lower SD values, indicating more stable hydrodynamic conditions during floc formation; however, some variability indicates that mixing regimes can affect floc breakage and reaggregation under specific conditions (Corral Bobadilla et al., 2019). Collectively, these SD patterns indicate that pH and settling time are more significant sources of uncertainty in process optimization than mixing times, and effective optimization algorithms should account for this variability rather than relying exclusively on mean values.

3.3 Performance Analysis of MBR with and without pretreatment

The influence of pretreatment on MBR performance was assessed based on COD reduction, microbial activity, membrane fouling analysis, and sludge characterization.

3.3.1 COD reduction analysis under different HRT

Two reactors were fabricated, one with a coagulation process as pretreatment and without pretreatment. The coagulation treatment process was performed based on outcomes obtained from the preliminary studies. COD reduction analysis was conducted in both reactors under varying HRT. HRT was varied from 2 to 7 hrs with a 1-hour time interval. HRT determines the duration wastewater remains in the treatment system, impacting the biological processes and overall system performance. Studies have shown that optimizing HRT can significantly enhance the treatment efficiency of MBRs used for dairy wastewater, which is characterized by high organic loads.

COD reduction is high for 2 hours of HRT, then decreases, reaching a maximum at the 5th hour. After 5 hours of treatment, the COD reduction decreases over time for both pretreated and untreated samples. COD removal is consistently higher with pretreatment than without across all HRTs. Maximum COD reduction with pretreatment occurs at 6 hours (~98%). Without pretreatment, the peak reduction in COD is around 90% at 5 hours. Post-peak (after 6 hours), a decline in COD removal is observed in both cases. Increased HRT allows more time for microbial degradation and settling of organic matter. As shown, COD removal improves from 2 hr to 5 hr/6 hr due to extended contact time (Li-Yih Lin et al., 2022).

At 7 hours of HRT, COD removal efficiency declines. This decline may be due to the following:

- End-product inhibition: Accumulated metabolites may inhibit microbial activity.
- Microbial decay phase: Extended HRT beyond optimal leads to cell lysis or reduced metabolic activity.
- Oversettling of biomass may reduce the active contact between organics and microbes (Shi, Z et al., 2021).

Coagulation as a pretreatment improves COD removal efficiency by selectively removing particulate, colloidal, and high-molecular-weight organic matter that is weakly biodegradable and difficult to eliminate by biological processes alone. Coagulation/flocculation destabilizes negatively charged colloids and uses adsorption-bridging mechanisms to aggregate suspended and colloidal organic matter into settleable flocs, thereby directly reducing influent COD and transforming residual COD into a more soluble and biodegradable fraction (increased BOD/COD ratio). The decrease in non-biodegradable and slowly biodegradable COD reduces the organic load and toxicity entering downstream biological or membrane processes, enhances microbial activity, and alleviates membrane fouling by decreasing extracellular polymeric substances (EPS) and fine particles. Thus, the bioreactor can operate at elevated biomass concentrations and extended solids retention times, facilitating more thorough biodegradation of residual soluble COD, while the membrane mostly serves as a polishing barrier. Extensive research on industrial and dairy effluents has shown that coagulation pretreatment can reduce influent COD by 20–60%, thereby substantially increasing overall COD removal to 95–99% in integrated systems, underscoring its essential role in achieving high treatment efficacy (Rahman et al., 2023).

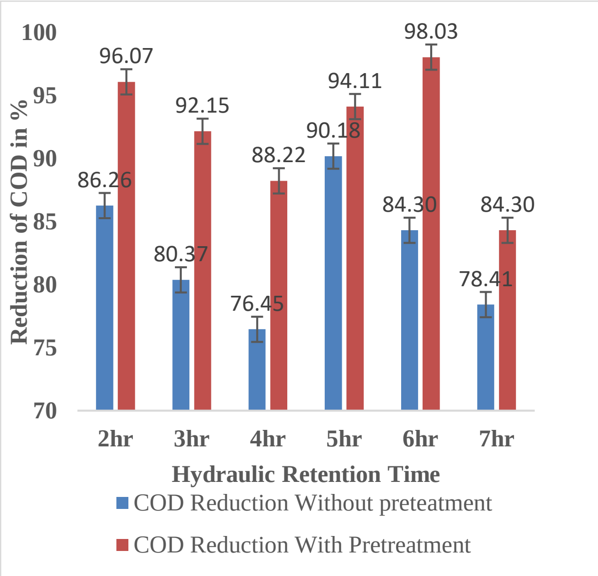

Figure 9: *Reduction of COD in MBR with pretreatment and without pretreatment under varying HRT.*

Slika 9: *Zmanjšanje KPK v MBR s predhodno obdelavo in brez predhodne obdelave pri različnih HRT.*

The reduction in COD across varying HRTs indicates that pretreatment significantly improves wastewater treatment efficiency. As shown through comparative analysis, COD removal improved substantially with pretreatment, reaching a maximum efficiency of approximately 99% at a 6-hour HRT, compared to a peak of around 90% without pretreatment at a 5-hour HRT. This trend aligns with findings from Khan et al. (2023), who reported that natural pretreatment agents such as neem leaf powder enhance floc formation and organic matter breakdown, thereby improving downstream biodegradability. The first trend of COD removal increasing as the HRT increases is explained by the fact that the longer the contact time, the more effective the microbial degradation and settling of particulate matter should be, as confirmed by Gardena et al. (2025). Nevertheless, in addition to the optimal HRT, COD removal was reduced at 7 hours in both cases. Such a decrease can be explained by microbial inhibition from metabolic byproducts or a lack of substrates, as observed by Yin, Q. (2024) in more complex biological systems. These results indicate the importance of optimizing the strategies used before treating and the retention time to get maximum efficiency of COD reduction during the treatment of wastewater.

3.3.2. Microbial Activity Analysis

The variation in SOUR with hydraulic retention time (HRT) is presented in Table 6. In both the neem-pretreated and control systems, SOUR increased with HRT beyond 2 hours, peaked at 5 hours, and then gradually decreased at longer HRTs. SOUR also reached a maximum of 23.5+/-1.1 mg O2/g MLVSS/h at 5 hrs HRT, which was significantly higher ($p<0.05$) than the control system, which reached a maximum of 15.2+/-0.8 mg O2/g MLVSS/h at the same HRT. An increase indicates that the neem powder pretreatment enhanced the biodegradability of the influent, thereby increasing microbial respiration. The recorded rise in SOUR to 5 hours indicates the best compromise between organic loading and microbial metabolic activity, providing sufficient time for enzymes to degrade organics and for oxygen transfer (Koul et al., 2022; Khan et al., 2023). In addition to this maximum HRT, SOUR started to decrease, but at all HRT values, it was 25–40 % higher in the neem-pretreated system than in the untreated one, which confirms the role of neem in stimulating microbial activity (Shi et al., 2021; Yin et al., 2024). Higher SOUR might be explained by the elimination of inhibitory organics and colloidal particles at the coagulation step, which most probably triggered enhanced oxygen transfer and decreased membrane fouling, as shown in the past by Esteki et al. (2024) and Bahrodin et al. 2021.

3.3.3 Membrane Fouling Analysis

Figure 10 presents a combined analysis of TMP and permeate flux over a 7-day operational period for two MBR systems: one treating dairy wastewater without pretreatment, and the other with neem leaf powder pretreatment. The dual-axis graph visually compares the dynamic fouling behavior and membrane performance of the two configurations under identical operating conditions.

***Table 6**: Variation of SOUR in MBR with and without pretreatment.*

***Preglednica 6:** Sprememba SOUR v MBR s predobdelavo in brez nje.*

HRT (hours)	SOUR without pretreatment (mg O_2/g MLVSS/h)	SOUR with neem pretreatment (mg O_2/g MLVSS/h)
2	10.8 ± 0.6	16.3 ± 0.9
3	12.5 ± 0.7	18.9 ± 1.0
4	14.3 ± 0.8	21.1 ± 1.1
5	15.2 ± 0.8	23.5 ± 1.1
6	14.9 ± 0.9	22.8 ± 1.2
7	13.7 ± 0.7	20.6 ± 1.0

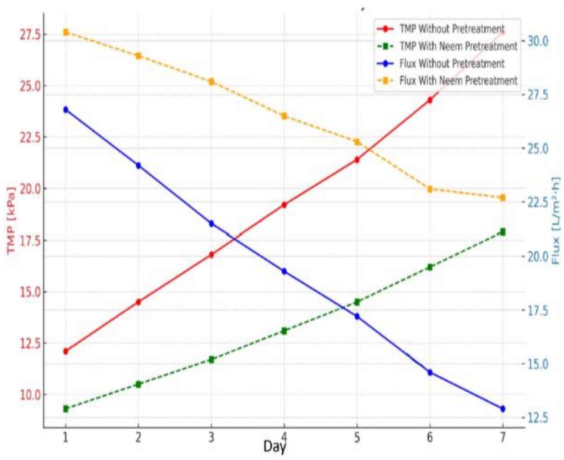

Figure 10: Variation of permeate flux and TMP of MBR with and without pretreatment.

Slika 10: Sprememba permeatnega toka in temperature pretoka MBR s predobdelavo in brez nje.

Without Neem Pretreatment:

- TMP steadily increased from 12.1 to 27.6 kPa, indicating progressive fouling due to the accumulation of suspended solids, colloidal matter, and microbial biofilm on the membrane surface.
- Flux declined sharply from 26.8 to 12.9 LMH, with over 50% loss in permeability due to severe fouling.

With Neem Pretreatment:

- TMP rose more gradually, reaching only 17.9 kPa by Day 7. Neem pretreatment results in a 35% reduction in fouling pressure compared to the untreated system.
- The flux remained relatively stable, decreasing moderately from 30.4 to 22.7 LMH, indicating improved membrane permeability and hydraulic performance.

The contrasting TMP and flux profiles between the two systems suggest that neem pretreatment significantly mitigates membrane fouling, primarily by removing colloidal and organic matter before the wastewater reaches the membrane module. The results align with findings by Esteki et al. (2024), who demonstrated that natural coagulant-based pretreatment reduces TMP buildup and extends membrane life. The higher and more stable flux observed in the neem-pretreated system can be attributed to effective particle destabilization and floc formation, reducing fine particle penetration into membrane pores, reduced organic and microbial load, lowering the potential for biofilm growth and pore clogging and formation of larger, less compressible cake layers, which shear forces can more easily remove (Hassanzadeh et al., 2016; Bahrodin et al., 2021). Furthermore, the lower TMP suggests a lower series resistance, meaning fewer energy losses during filtration and a less frequent need for backwashing or chemical cleaning (Zielińska & Galik, 2017).

Permeance was calculated by normalizing the permeate flux to the applied transmembrane pressure ($J/\Delta P$). The MBR without pretreatment shows a rapid decline in permeance (Figure 11), indicating accelerated membrane fouling and increased filtration resistance. In contrast, the neem-pretreated system maintains significantly higher and more stable permeance throughout the operational period, demonstrating effective fouling mitigation by removing particulate and colloidal organic matter during coagulation pretreatment.

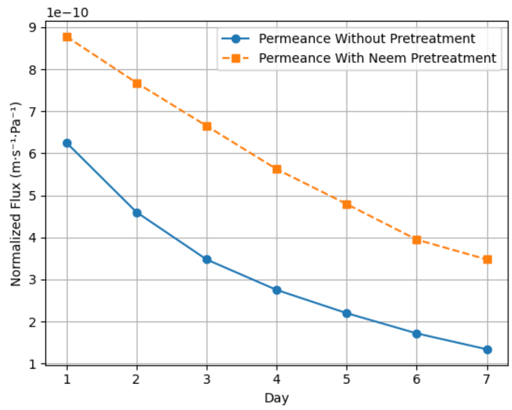

Figure 11: *Pressure-normalized flux (permeance) variation over time for the MBR operated with and without pretreatment.*

Slika 11: *Sprememba tlakovno normaliziranega pretoka s časom za MBR, s predobdelavo in brez nje.*

3.3.4 Sludge Characterization Analysis

To assess the settleability and compaction properties of activated sludge in MBR systems, the Sludge Volume Index (SVI) was measured daily over a 7-day operational period for both neem-pretreated and non-pretreated reactors. The calculation of SVI followed the general procedure presented in APHA (2017) as follows:

$$SVI = \frac{Settled\ Sludge\ Volume\ after\ 30\ min\ (\frac{mL}{L})}{MLSS\ (\frac{mg}{L})} \times 1000 \quad (4)$$

Samples of mixed liquor were collected from each reactor and poured into Imhoff cones. After 30 minutes of undisturbed settling, the volume of settled sludge was recorded. Concurrently, MLSS concentration was measured gravimetrically as per Standard Methods 2540 D. SVI values were expressed in mL/g. Each test was conducted in triplicate, and the results were averaged. The test assesses floc formation and sludge compatibility, which directly influence membrane fouling, effluent clarity, and sludge handling efficiency (Sisay et al., 2023).

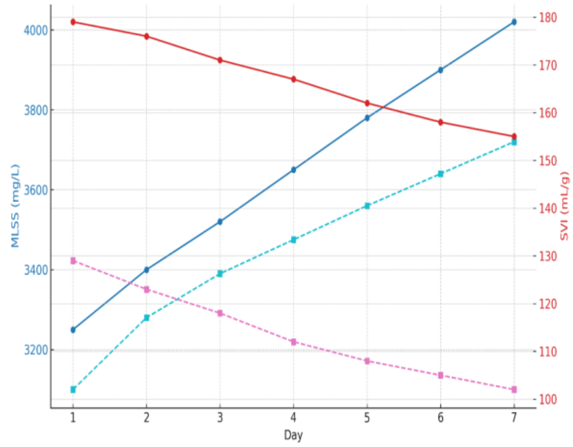

Figure 12: *Variation of MLSS and SVI in the MBR system with and without treatment.*

Slika 12: *Sprememba MLSS in SVI v sistemu MBR z obdelavo in brez nje.*

SVI values in the neem-pretreated reactor consistently ranged from 102 to 129 mL/g, indicating excellent settleability and well-formed flocs (Figure 11). By contrast, the non-pretreated system exhibited higher SVI values (155–179 mL/g), indicating bulkier, poorly settling sludge that is prone to membrane fouling and poor dewatering. As per Zhou et al. (2023) and Chen et al. (2022), SVI values <120 mL/g are suggestive of sludge that settles well, while values above 150 mL/g suggest filamentous or dispersed growth, a typical feature in reactors without pretreatment or with high colloidal organic loading. The lower SVI in the neem-pretreated system corresponds to denser, more compact sludge flocs that settle faster and occupy less volume. This results in reduced suspended solids in the permeate, improving effluent clarity and lowering fouling potential, as compact sludge minimizes cake-layer formation. These benefits have been corroborated in recent studies, where natural coagulants, such as *Moringa* and neem, have improved sludge dewaterability, compressibility, and membrane life (Nasr et al., 2021; Afzal et al., 2023). Neem contains polyphenolic compounds and bioflavonoids that stimulate the formation of compact biofloc structures, as it is a natural flocculant and microbial modulator. The result presented above is consistent with Li et al. (2020), who found that SVI and floc stability increased after adding tannin-rich extracts to the MBR.

After neem-powder coagulation pretreatment of dairy wastewater, the generated sludge should be thickened and subjected to mechanical dewatering (e.g., centrifugation or belt filter pressing), with optional conditioning using polymers or short-duration thermal treatment to reduce moisture content and stabilize sludge characteristics before further processing in the MBR or downstream biological units (Manea & Bumbac, 2024). Sludge produced from neem-based coagulation is typically rich in biodegradable organic matter and comparatively low in toxic metals, making it suitable for biological stabilization routes such as anaerobic digestion for biogas recovery or co-composting with bulking agents, provided that C/N ratio adjustment and co-digestion strategies are adopted to enhance methane yield and pathogen reduction (Ali et al., 2025). In integrated neem-pretreatment MBR systems, routine sludge characterization (moisture content, volatile solids, C/N ratio, pathogen indicators, and residual plant-derived compounds) is essential, as the nature of the natural coagulant influences sludge biodegradability, dewaterability, and digestion performance (Manea & Bumbac, 2024). Where stabilized biosolids meet regulatory standards, co-composted neem-sludge can be valorized as a nutrient-rich soil amendment, supporting circular resource recovery, while in energy-focused treatment trains, dewatered sludge may be integrated into anaerobic digesters or AnMBR systems with careful monitoring of process stability and foaming risks to ensure sustainable and compliant sludge management (Ali et al., 2025).

4. Conclusions

This study comprehensively evaluated the performance enhancement of the MBR system through the integration of neem leaf powder pretreatment in treating high-strength dairy wastewater. The investigation covered critical performance indicators, including microbial activity (SOUR), membrane fouling (TMP and flux trends), sludge settleability (SVI and MLSS evolution),

The neem-pretreated system consistently outperformed the non-pretreated configuration across all operational metrics:

SOUR values were significantly higher in the neem system, indicating enhanced microbial activity and oxygen uptake capacity.

TMP buildup was slower, and flux retention was higher, suggesting effective fouling mitigation due to reduced colloidal and organic loading.

SVI values in the neem system ranged from 102 to 129 mL/g, representing excellent sludge settleability, compared to 155 to 179 mL/g in the control.

MLSS concentration increased steadily, accompanied by improved biomass retention and floc stability in the neem reactor.

Statistical analysis (paired t-tests) confirmed the significance of improvements in TMP and flux trends ($p < 0.001$), reinforcing the operational and environmental viability of neem-based pretreatment strategies. Overall, this work demonstrates that neem leaf powder, a natural, locally available, and sustainable coagulant, has strong potential to enhance the performance, stability, and environmental sustainability of MBR systems. Its application is particularly promising for decentralized wastewater treatment systems in agro-industrial and rural settings, where resource optimization is critical.

The present study has certain limitations that should be considered when interpreting the results. The chemical composition of neem leaves can vary with season, plant age, and geographical origin, potentially influencing coagulation efficiency and reproducibility unless standardized preparation protocols are adopted. In addition, the experimental duration was relatively short, and long-term operation may reveal different trends in membrane fouling behavior and sludge characteristics. The experiments were conducted at the laboratory scale; therefore, hydraulic conditions, sludge handling, and operational stability may differ during scale-up to pilot or full-scale MBR systems. Addressing these challenges through extended operation, standardized neem processing, and pilot-scale validation will be essential to confirm the robustness and practical applicability of the proposed neem-based pretreatment strategy.

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CRediT authorship contribution statement

Prakash: Supervisor, Monitoring of Experimental Setup, Review & Manuscript correction.

Pauline: Conceptualization, Methodology, result analysis.

Mahalakshmi: Conduct of Experiments, Preparation of graphs & Manuscript preparation.

Data Availability

The authors confirm that the data supporting the findings of this study are available within the article.

Declaration of Interest

The authors declare that they have no known competing financial interests.

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References

Afzal, Muhammad & Khan, Shahzad & Zeshan, Basit & Riaz, Muhammad & Ejaz, Umer & Saleem, Ayesha & Zaineb, Rida & Sindhu, Haseeb & Yean, Chan & Ahmed, Naveed. (2023). Characterization of Bioactive Compounds and Novel Proteins Derived from Promising Source Citrullus colocynthis along with In-Vitro and In-Vivo Activities. *Molecules* 28, 1743. https://doi.org/10.3390/molecules28041743.

Alazaiza, M. Y. D., Albahnasawi, A., Ali, G. A. M., Bashir, M. J. K., Nassani, D. E., Al Maskari, T., Amr, S. S. A., & Abujazar, M. S. S. (2022). Application of Natural Coagulants for Pharmaceutical Removal from Water and Wastewater: A Review. *Water 14*(2), 140. https://doi.org/10.3390/w14020140.

Albahnasawi, A., Ali, G. A. M., Bashir, M. J. K., Nassani, D. E., Al Maskari, T., Amr, S. S. A., & Abujazar, M. S. S. (2022). Application of Natural Coagulants for Pharmaceutical Removal from Water and Wastewater: A Review. *Water*, *14*(2), 140. https://doi.org/10.3390/w14020140.

Alguacil, F. J. (2024). Recent advances in indium recovery. *Metals*, *14*(11), 1282. https://doi.org/10.3390/met14111282.

Ali, S. K., Abdou, M. M., Emara, M. M., Farag, R. S., & Mubarak, M. F. (2025). *Eco-friendly solutions: A comprehensive review of natural coagulants for sustainable water treatment*. *Environmental Geochemistry and Health*, *47*, 535. https://doi.org/10.1007/s10653-025-02803-3.

Al-Tayawi, Aws & Sisay, Elias & Beszédes, Sándor & Kertész, Sz. (2023). Wastewater Treatment in the Dairy Industry from Classical Treatment to Promising Technologies: An Overview. *Processes* 11, 2133. https://doi.org/10.3390/pr11072133.

andem, G., & Jabłońska-Czapla, M. (2024). Indium as an emerging contaminant: Occurrence and environmental impact. *Archives of Environmental Protection*, *50*(3). https://doi.org/10.24425/aep.2024.151688.

Andersa, K. N., Tamiru, M., Teka, T. A., Ali, I. M., Chane, K. T., Regasa, T. K., & Ahmed, E. H. (2024). Proximate composition, phytochemicals, and safety of neem leaf flour: A review. *Food Science & Nutrition*, *12*, 6929–6937. https://doi.org/10.1002/fsn3.4336.

Aragaw TA and Bogale FM (2023), Role of coagulation/flocculation as a pretreatment option to reduce colloidal/ bio-colloidal fouling in tertiary filtration of textile wastewater: A review and future outlooks. *Front. Environ. Sci*. 11,1142227. https://doi.org/10.3389/fenvs.2023.1142227.

Badawi, A. K., Salama, R. S., & Mostafa, M. M. M. (2023). Natural-based coagulants/flocculants as sustainable market-valued products for industrial wastewater treatment: a review of recent developments. *RSC advances 13*(28), 19335–19355. https://doi.org/10.1039/d3ra01999c.

Bahrodin, Muhammad & Zaidi, Nur Syamimi & Hussein, Norelyza & Sillanpää, Mika & Prasetyo, Dedy & Syafiuddin, Achmad. (2021). Recent Advances on Coagulation-Based Treatment of Wastewater: Transition from Chemical to Natural Coagulant. *Current Pollution Reports* 7. https://doi.org/10.1007/s40726-021-00191-7.

Chen, Y., Jiang, X., Yang, M., & Wang, Z. (2025). Biotechnology revival: *in situ* sludge minimization in wastewater. *Frontiers in microbiology*, *16*, 1603215. https://doi.org/10.3389/fmicb.2025.1603215.

Choy, S. Y., Prasad, K. M. N., Wu, T. Y., & Ramanan, R. N. (2014). A review on common vegetables and legumes as promising plant-based natural coagulants. *International Journal of Environmental Science and Technology, 11*, 1733–1748. https://doi.org/10.1007/s13762-013-0386-0.

Corral Bobadilla, M., Lostado Lorza, R., Escribano García, R., Somovilla Gómez, F., & Vergara González, E. P. (2019). *Coagulation: Determination of key operating parameters by multi-response surface methodology using desirability functions*. **Water, 11**(2), 398. https://doi.org/10.3390/w11020398.

Cote, Pierre & Brink, David & Adnan, Ali. (2006). Pretreatment Requirements for Membrane Bioreactors. *Proceedings of the Water Environment Federation* 1846-1855. https://doi.org/10.2175/193864706783750169.

Daverey, Achlesh & Pakshirajan, Kannan. (2011). Pretreatment of Synthetic Dairy Wastewater Using the Sophorolipid-Producing Yeast Candida bombicola. *Applied biochemistry and biotechnology* 163, 720-8. https://doi.org/10.1007/s12010-010-9077-y.

El-Sonbati, Adel & Diab, M. & Morgan, Sh.M. & Abou-Dobara, Mohamed & El-Ghettany, A.A.. (2019). Synthesis, characterization, theoretical and molecular docking studies of mixed-ligand complexes of Cu(II), Ni(II), Co(II), Mn(II), Cr(III), UO2(II) and Cd(II). *Journal of Molecular Structure* 1200, 127065. https://doi.org/10.1016/j.molstruc.2019.127065.

Formentini-Schmitt, Dalila & Alves, Álvaro & Veit, Marcia & Bergamasco, Rosângela & Vieira, Angelica & Fagundes-Klen, Márcia. (2023). Ultrafiltration Combined with Coagulation/Flocculation/Sedimentation Using Moringa oleifera as Coagulant to Treat Dairy Industry Wastewater. *Water* 224. https://doi.org/10.1007/s11270-013-1682-2.

Gardana, Rivelino & Mikhael, Magenta & Maulidiany, Nopa & Wandi, Asep. (2025). Effect of Hydraulic Retention Time on Food Chain Reactor (FCR) Performance in Industrial Wastewater Treatment. Syntax Literate; *Journal Ilmiah Indonesia*. https://doi.org/10. 3165-3180. https://doi.org/10.36418/syntax-literate.v10i3.57832.

Goldstein, J. I., Newbury, D. E., Michael, J. R., Ritchie, N. W. M., Scott, J. H. J., & Joy, D. C. (2018). *Scanning electron microscopy and X-ray microanalysis* (4th ed.). Springer. https://doi.org/10.1007/978-1-4939-6676-9.

Hassanzadeh, A., Tanisali, E., Hassas, B. V., Karakaş, F., & Celik, M. S., (2016). Effect of particle morphology on coagulation of silica. In *IMPC 2016 - 28th International Mineral Processing Congress* (IMPC 2016 - 28th International Mineral Processing Congress; Vol. 2016-September.

Jensen, H., Gaw, S., Lehto, N. J., Hassall, L., & Robinson, B. H. (2018). The mobility and plant uptake of gallium and indium. *Chemosphere, 209*, 675–684. https://doi.org/10.1016/j.chemosphere.2018.06.111.

Kani, Mophin Kani & Anju,. (2016). Exploring The Use of Orange Peel And Neem Leaf Powder As Alternative Coagulant in Treatment of Dairy Wastewater.., *International Journal of Scientific & Engineering Research* 7 (4), 238-244.

Khan, Q., Imran, U., Ullman, J. L., & Khokhar, W. A. (2023). Turbidity removal through the application of powdered azadirachta indica (neem) seeds. *Journal of Engineering and Technology 42*(1), 1. https://doi.org/10.22581/muet1982.2301.01.

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.

Li Z, Wang X, Alberdi A, Deng J, Zhong Z, Si H, Zheng C, Zhou H, Wang J, Yang Y, Wright A-DG, Mao S, Zhang Z, Guan L and Li G (2020). Comparative Microbiome Analysis Reveals the Ecological Relationships Between Rumen Methanogens, Acetogens, and Their Hosts. Front. Microbiol. 11:1311. https://doi.org/10.3389/fmicb.2020.01311.

Li-Yih Lin, Jiun-Lin Horng, Chieh-An Cheng, Chun-Yung Chang, Bor-Wei Cherng, Sian-Tai Liu, Ming-Yi Chou. (2022), Sublethal ammonia induces alterations of emotions, cognition, and social behaviors in zebrafish (Danio rerio), *Ecotoxicology and Environmental Safety* 244,114058, https://doi.org/10.1016/j.ecoenv.2022.114058.

Lwasa, A., Mdee, O. J., Ntalikwa, J. W., & Sadiki, N. (2024). *Performance analysis of plant-based coagulants in water purification: A review. Discover Water, 4*(108). https://doi.org/10.1007/s43832-024-00171-0.

Maeda, Y. (2024). Fouling of Reverse Osmosis (RO) and Nanofiltration (NF) Membranes by Low Molecular Weight Organic Compounds (LMWOCs), Part 1: Fundamentals and Mechanism. *Membranes 14*(10), 221. https://doi.org/10.3390/membranes14100221.

Mahapatra, S. S. (2025). *Improving the efficiency and optimization of wastewater treatment using response surface methodology (RSM).* Water Environment

Journal, 39(1), Article e70020. https://doi.org/10.1111/wej.70020.

Manea, E. E., & Bumbac, C. (2024). Sludge composting — Is this a viable solution for wastewater sludge management *Water, 16*(16), 2241. https://doi.org/10.3390/w16162241.

Mohan, Siva & K, Vidhya & C.T., Sivakumar & M, Sugnathi & V, Shanmugavadivu & Devi, M.. (2019). Textile Waste Water Treatment by Using Natural Coagulant (Neem-Azadirachta India). International Research Journal of Multidisciplinary Technovation. 1. 636-642. https://doi.org/10.34256/irjmtcon90.

Namdeti, R., Joaquin, A. A., Meka, U. R., Ali Azam Al Amri, M. A., & Kashoub, A. S. A. M. (2023). Biocoagulants as Ecofriendly Alternatives in the Dairy Wastewater Treatment. *Advances in Research*, 16–23. https://doi.org/10.9734/air/2023/v24i1929.

Nasr, M., Islam, M., Shehata, S. (2021) Smart Healthcare in the Age of AI: Recent Advances, Challenges, and Future Prospects. IEEE Access, 9, 145248–145270. https://doi.org/10.1109/ACCESS.2021.3118960.

Patel, S., Thakur, A. S., & Shah, D. L. (2021). Nutritional and mineral profiling of *Azadirachta indica* leaves collected from different agro-climatic regions of India. *Journal of Food Composition and Analysis, 96*, 103703. https://doi.org/10.1016/j.jfca.2020.103703.

Qian, R., Li, Y., Liu, Y., Sun, N., Liu, L., Lin, X., & Sun, C. (2024). Integrated transcriptomic and metabolomic analysis reveals the potential mechanisms underlying indium-induced inhibition of root elongation in wheat plants. *Science of the Total Environment, 908*, 168477. https://doi.org/10.1016/j.scitotenv.2023.168477.

Rahman, T. U., Roy, H., Islam, M. R., Naddeo, V., & Islam, M. S. (2023). Advancement of membrane bioreactor technology toward sustainable industrial wastewater treatment. *Membranes, 13*(2), 181. https://doi.org/10.3390/membranes13020181.

Ramsuroop, J., Gutu, L., Ayinde, W. B., Basitere, M., & Manono, M. S. (2024). A Review of Biological Processes for Dairy Wastewater Treatment and the Effect of Physical Parameters Which Affect Their Efficiency. *Water*, *16*(4), 537. https://doi.org/10.3390/w16040537.

Reem M. El-taweel, Nora Mohamed, Khlood A. Alrefaey, Sh Husien, A.B. Abdel-Aziz, Alyaa I. Salim, Nagwan G. Mostafa, Lobna A. Said, Irene Samy Fahim, Ahmed G. Radwan,(2023), A review of coagulation explaining its definition, mechanism, coagulant types, and optimization models; RSM, and ANN, *Current Research in Green and Sustainable Chemistry 6, 2023, 100358. https://doi.org/10.1016/j.crgsc.2023.100358.*

S, M., K, V., C.T, S., M, S., V, S., & M, D. (2019). Textile Waste Water Treatment by Using Natural Coagulant (Neem-Azadirachta India). *International Research Journal of Multidisciplinary Technovation*, *1*(6), 636–642. https://doi.org/10.34256/irjmtcon9.

Sara Esteki, Milad Karsaz, Burhan Ghofrani, Reza Yegani, Sima Majidi,2024,Combination of membrane bioreactor with chemical coagulation for the treatment of real pharmaceutical wastewater: Comparison of simultaneous and consecutive pretreatment of coagulation on MBR performance, *Journal of Water Process Engineering*, 60,105108. https://doi.org/10.1016/j.jwpe.2024.105108.

Shi, Z., Usman, M., He, J., Chen, H., Zhang, S., & Luo, G. (2021). Combined microbial transcript and metabolic analysis reveals the different roles of hydrochar and biochar in promoting anaerobic digestion of waste activated sludge. *Water research*, 205, 117679. https://doi.org/10.1016/j.watres.2021.117679.

Singh, S., Kumar, M., & Chauhan, J. S. (2020). Assessment of heavy metals in medicinal plant leaves grown in urban and peri-urban areas of India. *Environmental Science and Pollution Research, 27*, 14721–14733. https://doi.org/10.1007/s11356-020-07976-8.

Sirwan Alimoradi , Rasha Faraj , Ali Torabian. (2018). Effects of residual aluminum on hybrid membrane bioreactor (Coagulation-MBR) performance, treating dairy wastewater, *Chemical Engineering and Processing - Process Intensification*. 133, 320-324. https://doi.org/10.1016/j.cep.2018.09.023.

Sisay, E. J., Al-Tayawi, A. N., László, Z., & Kertész, S. (2023). Recent Advances in Organic Fouling Control and Mitigation Strategies in Membrane Separation Processes: A Review. *Sustainability* *15*(18), 13389. https://doi.org/10.3390/su151813389.

Tahraoui, H., Toumi, S., Boudoukhani, M., Touzout, N., Sid, A. N. E. H., Amrane, A., Belhadj, A.-E., Hadjadj, M., Laichi, Y., Aboumustapha, M., Kebir, M., Bouguettoucha, A., Chebli, D., Assadi, A. A., & Zhang, J. (2024). Evaluating the Effectiveness of Coagulation–Flocculation Treatment Using Aluminum Sulfate on a Polluted Surface Water Source: A Year-Long Study. *Water* *16*(3), 400. https://doi.org/10.3390/w16030400.

Tsoutsa, E. K., Tolkou, A. K., Kyzas, G. Z., & Katsoyiannis, I. A. (2024). New Trends in Composite Coagulants for Water and Wastewater Treatment. *Macromol* *4*(3), 509-532. https://doi.org/10.3390/macromol4030030.

Wylie, M. R., & Merrell, D. S. (2022). The Antimicrobial Potential of the Neem Tree *Azadirachta indica*. *Frontiers in pharmacology 13*, 891535. https://doi.org/10.3389/fphar.2022.891535.

Yin, Q., He, K., Collins, G. *et al.* Microbial strategies driving low concentration substrate degradation for sustainable remediation solutions. *npj Clean Water* 7, 52 (2024). https://doi.org/10.1038/s41545-024-00348-z.

Zielińska, M., Galik, M. Use of Ceramic Membranes in a Membrane Filtration Supported by Coagulation for the Treatment of Dairy Wastewater. *Water Air Soil Pollut* 228, 173 (2017). https://doi.org/10.1007/s11270-017-3365-x.