



## INVESTIGATION OF nZVI AND TiO<sub>2</sub> NANOPARTICLES EFFECT ON THE MEMBRANE FOULING RATE IN ANAEROBIC MEMBRANE BIOREACTOR

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### Keywords

Nanoparticles,  
Membrane bioreactor,  
Landfill leachate,  
nZVI,  
TiO<sub>2</sub>.

### Abstract

In recent years, membrane bioreactor technology (MBRs) has been increasingly used for wastewater treatment. However, the MBR system faces membrane fouling problems, which lead to higher operating costs, membrane replacement expenses, and reduced competitiveness. Therefore, understanding membrane fouling is essential not only for solving these issues but also as a key factor in advancing membrane technology. In this study, the effects of nano zero-valent iron (nZVI) and titanium dioxide (TiO<sub>2</sub>) nanoparticles on the membrane fouling rate in a laboratory-scale submerged anaerobic membrane bioreactor (AnMBR) treating landfill leachate (LFL) were investigated under controlled conditions (50-300 mg/L nZVI and TiO<sub>2</sub>). Additionally, both nZVI and TiO<sub>2</sub> nanoparticles were compared regarding their impact on membrane fouling. The optimal membrane performance was observed at 100 mg/L TiO<sub>2</sub> and 100 mg/L nZVI, with membrane fouling rates of 36 mbar/day and 34 mbar/day, respectively. The addition of TiO<sub>2</sub> and nZVI significantly reduced membrane fouling (by 40% with TiO<sub>2</sub> and 70% with nZVI) in the AnMBR. When comparing nZVI and TiO<sub>2</sub> regarding membrane fouling rate, nZVI demonstrated the best performance. Based on the results, applying NPs in MBR systems significantly improves performance and reduces membrane fouling.

## ANAEROBİK MEMBRAN BİYOREAKTÖRÜNDE nZVI VE TiO<sub>2</sub> NANOPARTİKÜLLERİNİN MEMBRAN KİRLENME ORANI ÜZERİNDEKİ ETKİSİNİN ARAŞTIRILMASI

### Anahtar Kelimeler

Nanopartiküller,  
Membran biyoreaktör,  
Çöp sızıntı suları,  
nZVI,  
TiO<sub>2</sub>.

### Öz

Son yıllarda, membran biyoreaktör teknolojisi (MBR'ler) atıksu arıtımında giderek daha fazla kullanılmaktadır. Ancak MBR sistemi, daha yüksek işletme maliyetlerine, membran değiştirme masraflarına ve azalan rekabet gücüne yol açan membran kirlenme sorunlarıyla karşı karşıyadır. Bu nedenle, membran kirlenmesini anlamak yalnızca bu sorunları çözmek için değil, aynı zamanda membran teknolojisinin ilerlemesinde de önemli bir faktör olarak önemlidir. Bu çalışmada, nano sıfır değerlikli demir (nZVI) ve titanyum dioksit (TiO<sub>2</sub>) nanopartiküllerinin, LFL arıtan laboratuvar ölçekli bir batık anaerobik membran biyoreaktörde (AnMBR) membran kirlenme oranı üzerindeki etkileri kontrollü koşullar altında (50-300 mg/L nZVI ve TiO<sub>2</sub>) incelenmiştir. Ayrıca hem nZVI hem de TiO<sub>2</sub> nanopartikülleri membran kirlenmesi üzerindeki etkileri açısından karşılaştırılmıştır. Optimum membran performansı, sırasıyla 36 mbar/gün ve 34 mbar/gün membran kirlenme oranlarıyla 100 mg/L TiO<sub>2</sub> ve 100 mg/L nZVI'da gözlenmiştir. TiO<sub>2</sub> ve nZVI ilavesi, AnMBR'de membran kirlenmesini önemli ölçüde azaltmıştır (TiO<sub>2</sub> ile %40, nZVI ile %70). Membran kirlenme oranı açısından nZVI ve TiO<sub>2</sub> karşılaştırıldığında, nZVI en iyi performansı göstermiştir. Sonuçlara dayanarak, MBR sistemlerine NP uygulanması performansı önemli ölçüde artırmakta ve membran kirlenmesini azaltmaktadır.

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# INVESTIGATION OF nZVI AND TiO<sub>2</sub> NANOPARTICLES EFFECT ON THE MEMBRANE FOULING RATE IN ANAEROBIC MEMBRANE BIOREACTOR

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## Highlights

- The effects of TiO<sub>2</sub> and nZVI nanoparticles on membrane fouling were investigated in an AnMBR system.
- Both nanoparticles effectively reduced membrane fouling during landfill leachate treatment.
- Optimal antifouling performance was achieved at 100 mg/L of TiO<sub>2</sub> and nZVI.
- nZVI showed a higher fouling reduction efficiency (~70%) compared to TiO<sub>2</sub> (~40%).
- Nanoparticle addition is a simple and effective strategy to enhance MBR performance and stability.

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## Purpose and Scope

This study aims to investigate the effects of nano zero-valent iron (nZVI) and titanium dioxide (TiO<sub>2</sub>) nanoparticles on membrane fouling in an anaerobic membrane bioreactor (AnMBR) treating landfill leachate. The scope includes evaluating different nanoparticle doses to identify the optimal conditions for fouling mitigation and enhanced membrane performance.

## Design/methodology/approach

nZVI and TiO<sub>2</sub> nanoparticles were synthesized and characterized in our previous studies (Göçer et al., 2019; 2020; 2024) using XRD, SEM, EDX, FTIR, and BET surface area analyses. A laboratory-scale submerged anaerobic membrane bioreactor (AnMBR) with a working volume of 4 L (37×14×11 cm) was used to treat landfill leachate (LFL) collected from the Kahramanmaraş sanitary landfill. The reactor was operated at 30–36.5 °C with a hydraulic retention time (HRT) of 24 h and an MLSS concentration of 6000±500 mg/L. The filtration cycle consisted of 5 minutes of filtration followed by 0.5 minutes of relaxation and backwashing. Nanoparticle doses ranging from 50 to 300 mg/L were tested to evaluate their effects on membrane fouling.

## Findings

The addition of TiO<sub>2</sub> and nZVI nanoparticles effectively reduced membrane fouling in the AnMBR treating landfill leachate. The optimal performance was achieved at 100 mg/L for both nanoparticles, with fouling rates of 36 mbar/day for TiO<sub>2</sub> and 34 mbar/day for nZVI. Compared to the control, fouling was reduced by approximately 40% with TiO<sub>2</sub> and 70% with nZVI, demonstrating that nZVI provided superior antifouling performance. The results indicate that the addition of nanoparticles is a simple and efficient strategy to mitigate membrane fouling and enhance AnMBR performance. Implementing nZVI and TiO<sub>2</sub> nanoparticles in membrane bioreactors can extend the lifespan of membranes, reduce operational costs, and enhance the overall sustainability of wastewater treatment systems.

## Originality

This study compares the impacts of TiO<sub>2</sub> and nZVI nanoparticles on membrane fouling in anaerobic MBR systems treating landfill leachate. While both nanoparticles improved membrane performance, nZVI showed a greater ability to reduce fouling. The results aid in developing nanoparticle-assisted antifouling strategies and present a new approach to enhance the stability and efficiency of MBR operations.

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## 1. Introduction

Membrane bioreactors (MBRs) are widely used to treat and reuse wastewater because they offer many advantages over traditional activated sludge processes (Chang et al. 2002; Bagheri and Mirbagheri 2018; Ugarte et al., 2022; Rahman et al., 2023). Although many advantages of MBR, major drawback of membrane fouling (Gharibian and Hazrati, 2020; Tobino et al. 2016; Wu et al., 2020). In recent years, various studies have been carried out to reduce the membrane fouling rate (Amouamouha and Gholikandi 2018; Battistelli et al. 2019; Hawari et al. 2019; Li et al. 2020; Teng et al. 2020). These include modification of the membrane structure, improvement of operating conditions and improvement of biomass properties (Abdelrasoul et al. 2013; Banu et al. 2011; Gundogdu et al. 2019; Sichinga et al. 2016; Chang et al. 2002). Modification of biomass properties has been proven to be effective in effectively reducing membrane fouling. These modifications are carried out by the application of coagulants, adsorbents, nanoparticles (Alimoradi et al. 2018; Gkotsis et al. 2014). The addition of adsorbents and nanoparticles in the biological treatment system reduces the level of contamination, especially mitigation membrane fouling rate (Meng et al. 2009). Among NPs, nZVI and TiO<sub>2</sub> is investigated effect of the environmental remediation technology, wastewater treatment, and membrane fouling in MBRs Bagheri and Julkapli, 2016; Feng et al., 2016; Xiang et al., 2010; Yuniarto et al., 2013; Zhou et al., 2017; Zhu et al., 2012). nZVI is considered as one of the cost-effective, high removal efficiency and high adsorption capacity environmental remediation technologies. Additionally, nZVI has been widely evaluated to improve AnMBR performance by increasing biogas production, enhancing pollutant removal, and reducing membrane fouling (Zhou et al., 2017). TiO<sub>2</sub> nanoparticles have recently gained interest due to their photocatalytic properties, semiconductors, harsh conditions, commercial availability, and ease of preparation in degrading persistent pollutants in wastewater (Baolong et al., 2003; Xi et al., 2001). Thus, the nZVI and TiO<sub>2</sub> were used to alleviate membrane fouling and improve effluent quality in MBRs.

This research aimed to assess the influence of titanium dioxide (TiO<sub>2</sub>) and nano zero-valent iron (nZVI) nanoparticles on membrane fouling control in an anaerobic membrane bioreactor (AnMBR). The investigation emphasized three main aspects: (1) the comparative performance of TiO<sub>2</sub> and nZVI in reducing membrane fouling relative to a control setup, (2) the impact of different nanoparticle dosages on system behavior, and (3) the variation in fouling tendencies between TiO<sub>2</sub> and nZVI applications, (4) in addition, system performance was evaluated by assessing the effects of nZVI and TiO<sub>2</sub> concentrations on organic matter and nitrogen removal efficiencies, as well as on soluble microbial product (SMP) and extracellular polymeric substance (EPS) production.

## 2. Materials and Methods

### 2.1. nZVI and TiO<sub>2</sub> preparation

The preparation and analytical evaluation of TiO<sub>2</sub> and nZVI nanoparticles were comprehensively presented in our earlier publications (Göçer et al., 2019; 2020; 2024). Both nanoparticles were synthesized under controlled laboratory conditions, and their physicochemical features were identified through multiple instrumental analyses, including X-ray diffraction (XRD), scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), Fourier-transform infrared spectroscopy (FTIR), and specific surface area measurements using the BET method.

### 2.2. Characterization of LFL

A laboratory scale submerged AnMBR was fed from the sanitary landfill Kahramanmaraş/Turkey. The characteristics of landfill leachate are summarized in Table 1. It was stored at 4°C under laboratory conditions and characterized.

**Table 1.** Landfill Leachate Characterization

Parameters	Concentration (mg/L)	Parameters	Concentration (mg/L)
DOC	2446±400	NO <sub>2</sub> <sup>-</sup>	320±20
COD	8885±1500	NO <sub>3</sub> <sup>-</sup>	275±40
BOD	1500±300	Pt-Co (Color unit)	6380±300
NH <sub>4</sub> <sup>+</sup>	3101±200	Total Nitrogen (TN)	982±100

### 2.3. AnMBR design, operation, and experimental plan

In this research, a laboratory-scale anaerobic membrane bioreactor (AnMBR) equipped with a submerged hollow fiber membrane was employed. The reactor was rectangular, measuring 37 cm in length, 14 cm in width, and 11 cm in height, with an effective working volume of 4 L. The hydraulic retention time (HRT) was maintained at 24

hours, while the mixed liquor suspended solids (MLSS) concentration was controlled at  $6000 \pm 500$  mg/L. The system was operated under mesophilic conditions at  $30\text{--}36.5$  °C. Filtration was conducted in cycles consisting of 5 minutes of filtration, followed by 0.5 minutes of relaxation and backwashing. A schematic representation of the reactor setup is presented in Figure 1.

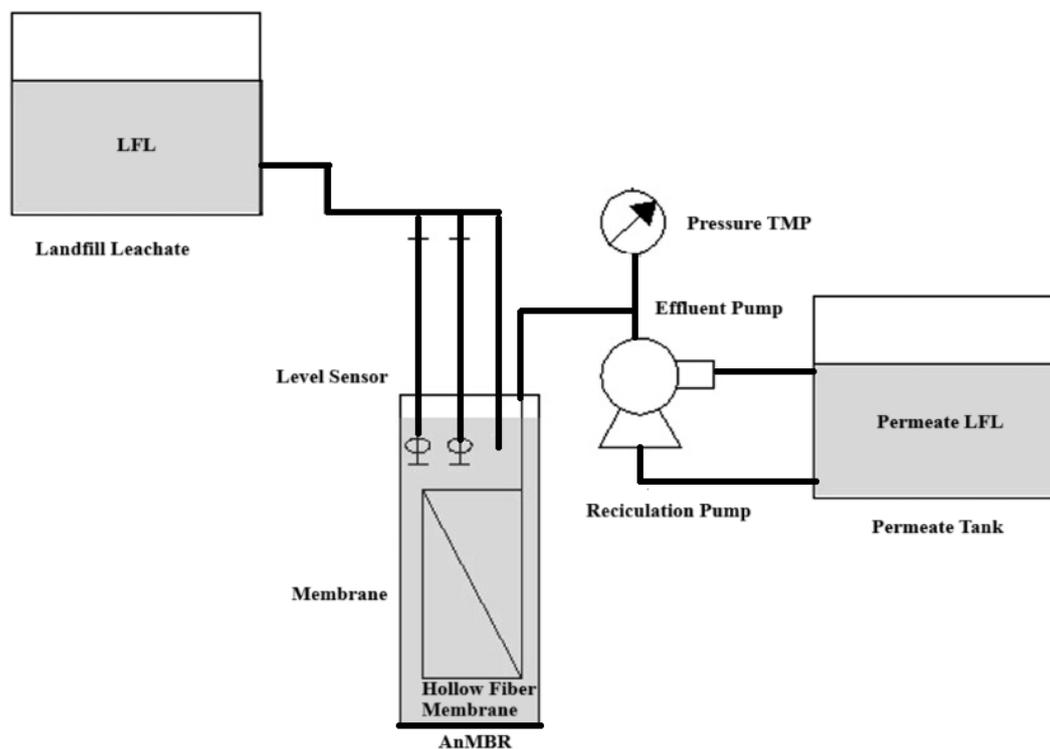


Figure 1. Schematic view of AnMBR

Membrane filtration performance and fouling behavior, expressed in terms of transmembrane pressure (TMP) and flux, were monitored throughout a 52-day operational period. The detailed operating parameters of the AnMBR system are summarized in Table 2. Five distinct operational phases (PI–PV) were defined based on variations in membrane fouling rate observed over the 52 days of operation.

Table 2. Operational conditions of AnMBR

Phases(P)	Days (d)	nZVI (mg/L)	TiO <sub>2</sub> (mg/L)	HRT (hours)	MLSS (mg/L)	Backwash and relaxation (time)
I	0-52	Control conditions (without nZVI addition)	Control conditions (without TiO <sub>2</sub> addition)	24	6000±500	5min-30s
II	0-52	50	50			
III	0-52	100	100			
IV	0-52	200	200			
V	0-52	300	300			

## 2.4. Analyses

The transmembrane pressure (TMP) was continuously recorded using a calibrated pressure sensor, while the permeate flux ( $J$ , LMH) of the AnMBR was determined according to Eq.(1):

$$J = \frac{Q_{\text{permeate}}}{A_{\text{membrane}}} \quad (1)$$

where  $J$  represents the permeate flux ( $\text{L}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ ),  $Q_{\text{permeate}}$  is the volumetric flow rate of the permeate ( $\text{L}\cdot\text{h}^{-1}$ ), and  $A_{\text{membrane}}$  denotes the effective surface area of the membrane ( $\text{m}^2$ ). The membrane fouling rate ( $\text{mbar}\cdot\text{day}^{-1}$ ) was calculated using Eq. (2):

$$\text{Membrane fouling rate (mbar/d)} = \frac{\text{TMP}_f - \text{TMP}_i (\Delta P)}{\Delta t} \quad (2)$$

where  $\text{TMP}_f$  and  $\text{TMP}_i$  are the final and initial transmembrane pressures (mbar), respectively, and  $\Delta t$  represents the operation time (days). All collected samples were first centrifuged at 4,000 rpm for 5 min using an Eppendorf 5415R centrifuge (Hamburg, Germany), followed by filtration through sterile 0.45  $\mu\text{m}$  syringe filters (Sartorius AG, Göttingen, Germany). Total organic carbon (TOC) and total nitrogen (TN) concentrations were determined using a total organic carbon analyzer equipped with TN detection (Shimadzu TOC-VCPN, Kyoto, Japan). Concentrations of inorganic nitrogen species, including ammonium ( $\text{NH}_4^+$ ) in influent and effluent samples were quantified by ion chromatography (Dionex ICS-3000, Sunnyvale, CA, USA). Chemical oxygen demand (COD) was analyzed using the dichromate closed-reflux colorimetric method in accordance with Standard Methods (Method 5220D). According to Göçer et al. (2025), SMP concentrations were measured by analyzing protein and carbohydrate contents in the filtrate. For extracellular polymeric substances (EPS) analysis, reactor samples were centrifuged, and EPS was then extracted from the biomass. Carbohydrate and protein contents in both SMP and EPS fractions were determined using the phenol-sulfuric acid and Lowry methods, respectively (DuBois et al. 1956, Lowry et al 1951).

### 3. Results and Discussion

#### 3.1. Effect of nZVI and $\text{TiO}_2$ Concentration on Organic and Nitrogen Removal Performance

The results indicate that nZVI concentration significantly and proportionally affected the removal of COD, TOC, and nitrogen compounds in the AnMBR system (Table 3). Control conditions showed moderate COD removal (49.7%) and low TOC removal (22.6%), which is typical for anaerobic treatment of landfill leachate containing refractory organic compounds (Kjeldsen et al., 2002; Renou et al., 2008). At low to moderate nZVI levels (50–100 mg/L), COD removal slightly increased (50–56%), while TOC removal remained limited (Table 3). This suggests that at these concentrations, nZVI mainly enhanced the breakdown of particulate and easily biodegradable organics, with a weaker impact on soluble and recalcitrant carbon fractions (Liu et al., 2022; Nabi et al., 2023). Nitrogen removal efficiencies (TN and  $\text{NH}_4^+$ ) stayed negligible, which is expected in anaerobic conditions where nitrification is suppressed (Anjum et al., 2017). The best performance was observed at 200 mg/L nZVI, with COD and TOC removal efficiencies rising significantly to 68.9% and 46.8%, respectively (Table 3). This improvement likely results from increased microbial activity, along with the catalytic and adsorptive properties of nZVI that help facilitate electron transfer and organic matter conversion (Maaz et al., 2019; De Souza Vandenberghe et al., 2022). However, raising nZVI concentration further to 300 mg/L caused a substantial decrease in COD removal efficiency (34.1%-Table 3). This decline is probably linked to nanoparticle aggregation, excessive iron release, and microbial inhibition, which reduce the reactive surface area and hinder mass transfer processes (Hu et al., 2020b; Zhang et al., 2020b). Across all operating conditions, TN and  $\text{NH}_4^+$  removal efficiencies (Table 3) remained low (<14%), confirming that nZVI mainly promotes carbon removal rather than nitrogen transformation in the anaerobic AnMBR system (Anjum et al., 2017; Nabi et al., 2023). An optimal nZVI dosage (200 mg/L) notably enhanced COD and TOC removal through microbial stimulation and catalytic effects, while excessive nZVI led to performance decline due to aggregation and microbial stress. Results show that  $\text{TiO}_2$  addition affected organic matter removal in the AnMBR system in a concentration-dependent but less stable way compared to nZVI (Table 4). Control conditions showed moderate COD (49.9%) and TOC (28.5%) removal efficiencies (Table 4). At low  $\text{TiO}_2$  levels (50–100 mg/L), COD removal improved to 52.9–61.8%, while TOC removal stayed around 27–29% (Table 4). This indicates  $\text{TiO}_2$  effectively degrades biodegradable organics but has limited impact on refractory carbon fractions (Zhang et al., 2018; Nabi et al., 2023). The highest COD removal (61.8%) was achieved at 100 mg/L  $\text{TiO}_2$ , suggesting an optimal concentration for organic degradation. Increasing  $\text{TiO}_2$  to 200 mg/L caused a decline in both COD and TOC removal efficiencies (44.4% and 14.4%, respectively) (Table 4). This decline might result from nanoparticle aggregation and microbial stress, which decrease effective surface area and inhibit microbial activity (Hu et al., 2020; Zhang et al., 2020b). At 300 mg/L  $\text{TiO}_2$ , COD and TOC removal slightly recovered but remained below the best performance, indicating unstable system performance at high nanoparticle concentrations. Conversely, TN removal gradually increased with higher  $\text{TiO}_2$  levels, reaching 23.5% at 300 mg/L. This could be connected to enhanced microbial uptake or adsorption of nitrogen compounds onto  $\text{TiO}_2$  surfaces; however,  $\text{NH}_4^+$  removal stayed negligible under all tested conditions, consistent with the lack of nitrification in anaerobic systems (Anjum et al., 2017). Overall,  $\text{TiO}_2$  improved organic matter removal only within a narrow concentration range, with higher doses causing performance issues. This highlights the need to optimize dosage for stable operation of the AnMBR system.  $\text{TiO}_2$  boosted COD removal at moderate levels (~100 mg/L), but excessive doses led to poor performance due to particle aggregation and microbial inhibition.

**Table 3.** Effect of nZVI Concentration on Organic and Nitrogen Removal

		nZVI			
Phases (P)		COD(mg/L)	TOC(mg/L)	TN(mg/L)	NH <sub>4</sub> <sup>+</sup> (mg/L)
PI	Influent	10382±3340	3853±1231	1619±269	5481±994
	Permeate	5220±2696	2984±1004	1456±323	5350±1400
	Efficiency (%)	49.72	22.55	10.07	2.39
PII	Influent	16176±862	5636±619	2476±452	6584±344
	Permeate	7135±2899	4366±553	2592±424	6518±451
	Efficiency (%)	55.89	22.53	0	1.00
PIII	Influent	17402±1132	7444±408	2744±163	5850±281
	Permeate	8668±2107	5207±258	2378±177	5837±167
	Efficiency (%)	50.19	30.05	13.34	0.22
PIV	Influent	27217±1659	10738±729	2730±124	5623±930
	Permeate	8454±3950	5716±1327	2513±200	5701±849
	Efficiency (%)	68.94	46.77	7.95	0
PV	Influent	27628±1108	10941±314	2585±93	6587±162
	Permeate	18199±2981	7643±1332	2582±132	6460±326
	Efficiency (%)	34.13	30.14	0.12	1.93

**Table 4.** Effect of TiO<sub>2</sub> Concentration on Organic and Nitrogen Removal

		TiO <sub>2</sub>			
Phases (P)		COD(mg/L)	TOC(mg/L)	TN(mg/L)	NH <sub>4</sub> <sup>+</sup> (mg/L)
PI	Influent	10817±3365	4175±1270	1651±292	5481±994
	Permeate	5418±2705	2984±1066	1510±335	5350±1400
	Efficiency (%)	49.91	28.52	8.54	2.39
PII	Influent	9785±183	5117±253	1775±78	5860±616
	Permeate	4605±572	3654±415	1574±57	5926±729
	Efficiency (%)	52.93	28.59	11.32	0
PIII	Influent	12521±355	4503±409	1715±44	5850±281
	Permeate	4786±678	3264±134	1541±55	5837±167
	Efficiency (%)	61.77	27.51	10.14	0.22
PIV	Influent	9975±1878	5047±615	1592±110	5623±930
	Permeate	5551±1843	4318±371	1372±99	5701±849
	Efficiency (%)	44.35	14.44	13.81	0
PV	Influent	10163±1990	5471±343	1761±159	6587±162
	Permeate	5356±1040	4253±176	1348±137	6460±326
	Efficiency (%)	47.29	22.26	23.45	1.93

### 3.2. Effect of nZVI and TiO<sub>2</sub> on the membrane fouling rate

Metal oxide nanoparticles such as nZVI, TiO<sub>2</sub>, ZnO and CeO<sub>2</sub> are used for the degradation of organic pollutants. nZVI and TiO<sub>2</sub> nanoparticles are among the newest technologies for environmental remediation and wastewater treatment due to easily available, low cost and very effective adsorbents (Lefevre et al., 2016; Sacca et al., 2013; Patil et al., 2016; Sevcu et al., 2012). Recently, TiO<sub>2</sub> and nZVI have been known as nanoparticles used to mitigate membrane fouling rate. The reduction in membrane fouling observed with nZVI and TiO<sub>2</sub> addition can be explained by multiple synergistic mechanisms. First, nanoparticles alter sludge physicochemical properties by promoting microbial aggregation and strengthening the sludge flocs' structure, which reduces the release of SMP and EPS, both key contributors to membrane fouling. Second, stimulates microbial metabolic activity and selectively

suppresses fouling-related bacterial groups, leading to a less adhesive and more permeable cake layer. Third, the presence of nanoparticles enhances cake layer porosity and disrupts the compact fouling structure through its particulate and magnetic characteristics, thereby lowering hydraulic resistance. These combined biological and physical effects ultimately result in a reduced fouling rate in the nanoparticles-assisted AnMBR. The effect of nZVI and  $\text{TiO}_2$  on the membrane fouling rate are shown in Figure 2-3.

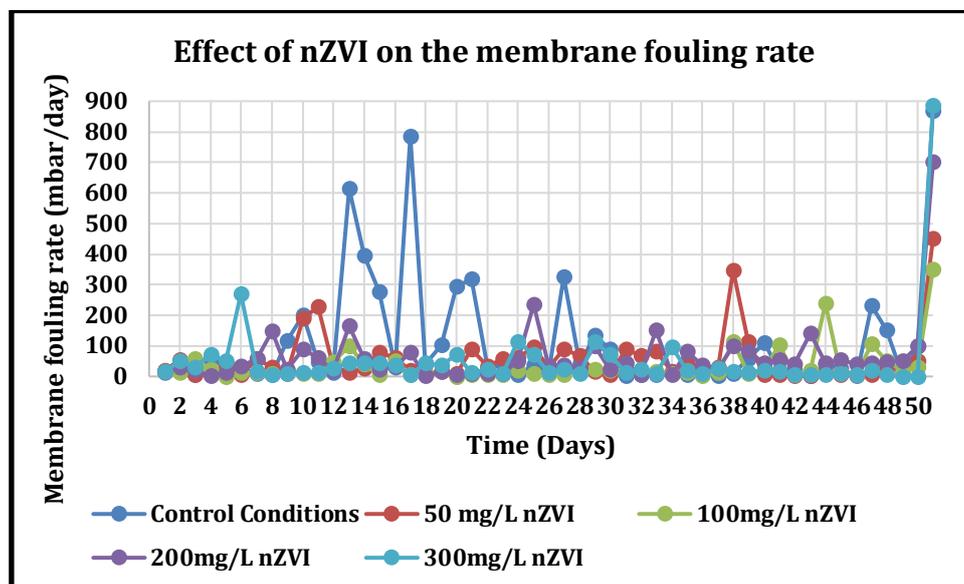


Figure 2. Effect of nZVI on the membrane fouling rate

The membrane fouling behavior was assessed under control conditions and at nZVI concentrations of 50, 100, 200, and 300 mg/L. The optimal nanoparticle dosage was identified as 100 mg/L, corresponding to TMP and flux values of -234 mbar and 0.65 LMH, respectively. Increasing the nZVI concentration beyond this level (from 100 to 300 mg/L) resulted in a negative impact on both the fouling rate and membrane performance (Fig. 2). Thus, while moderate nZVI addition enhanced membrane performance and delayed fouling, excessive concentrations caused a sharp decline in TMP and overall operational stability. According to literature since it is quite difficult to treating LFL, there are no studies based on the TMP-flux relationship. Jiang et al. (2003) investigated the treatment of petrochemical wastewater in submerged MBR. Zhou et al. (2017) reported that the TMP decreased with the increase in nZVI concentration and delayed membrane fouling rate. Their study was similar to our study. Mei et al. (2014) reported that using a laboratory-scale continuous system (An/Ae/Anox) and adding ZnO nanoparticulate matter played an important role in reducing membrane fouling. In another study they reported that using ZnO nanoparticulate matter accelerated the fouling of membrane pores in a laboratory-scale continuous system (Anox/Ae) but reduced the fouling caused by the cake layer (Wang et al., 2014). Previous studies have reported the successful application of various nanoparticles, such as  $\text{TiO}_2$  and Ag, to alleviate membrane fouling in bioreactor systems (Chang et al., 2002; Hong and He, 2014; Kim and Van der Bruggen, 2010). Consistent with these findings, the present results demonstrated that the incorporation of nZVI nanoparticles effectively reduced the rate of membrane fouling. The membrane fouling rate was evaluated under control conditions, 50 mg/L, 100 mg/L, 200 mg/L and 300 mg/L  $\text{TiO}_2$  operating conditions (Fig. 3). The addition of nanoparticles and the optimum  $\text{TiO}_2$  concentration was determined as 100 mg/L. In 100 mg/L  $\text{TiO}_2$  operating conditions; it was observed that there were very serious decreases in TMP value by reducing membrane fouling rate (Fig. 3). In another study, Bae et al. (2005) reported that membrane fouling was significantly reduced when  $\text{TiO}_2$  was immobilized onto ultrafiltration membranes. They attributed this improvement to the increased presence of nanoparticles on the membrane surface, which enhanced the antifouling effect.

As a result, the rate of membrane fouling decreased with increasing  $\text{TiO}_2$  concentration, as the nanoparticles enhanced the fouling mitigation effect through their presence on the membrane surface and within the cake layer. Simultaneously, the overall fouling resistance declined, likely due to the reduced adhesion of organic pollutants to the membrane surface and pore walls. The addition of  $\text{TiO}_2$  nanoparticles was also found to thin the cake layer and delay the onset of fouling. Similar findings regarding improved membrane performance and reduced fouling have been reported in previous studies by Guo et al. (2012), Meng et al. (2009), and Wang and Wu (2009). The comparison of both  $\text{TiO}_2$  and nZVI membrane fouling rates is shown in Figure 4. The membrane fouling rate was evaluated under control conditions, 50 mg/L, 100 mg/L, 200 mg/L and 300 mg/L nZVI and  $\text{TiO}_2$  operating conditions. According to nZVI, membrane fouling rate were determined as 114, 56, 34, 65 and 50 mbar/day, respectively (Fig. 4). Also, according to  $\text{TiO}_2$  membrane fouling rate were determined as 59, 80, 36, 40 and 104

mbar/day, respectively (Fig. 4). Membrane fouling rate was observed nZVI and TiO<sub>2</sub> concentration increased membrane performance improved and fouling rate decreased (from control conditions to 100 mg/L). It was observed that membrane performance deteriorated and fouling rate increased when nZVI and TiO<sub>2</sub> concentrations was increased from 100 mg/L to 200 mg/L and 300 mg/L (Fig. 4). In contrast, the control reactor operated without TiO<sub>2</sub> exhibited a considerably higher fouling rate, primarily due to the formation of a denser cake layer. Overall, the incorporation of 100 mg/L TiO<sub>2</sub> or nZVI nanoparticles was found to effectively mitigate membrane clogging and extend filtration cycles by delaying the onset of fouling.

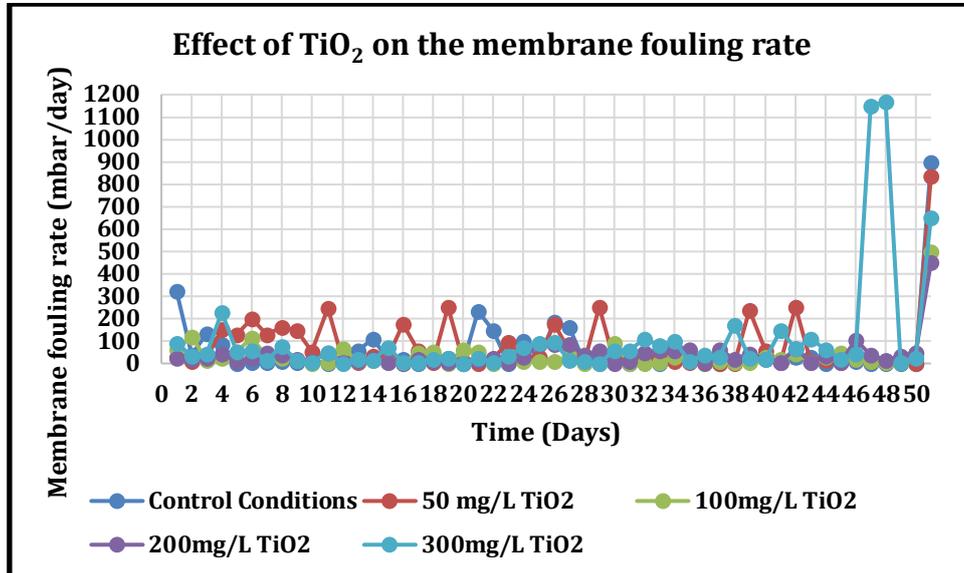


Figure 3. Effect of TiO<sub>2</sub> on the membrane fouling rate

At higher nZVI concentrations (200–300 mg/L), the deterioration in AnMBR performance can be explained by nanoparticle aggregation and excessive iron release. Aggregated nZVI particles can accumulate on the membrane surface, clogging membrane pores and leading to the formation of a denser and less permeable cake layer. Furthermore, high Fe(II) concentrations can interact with organic matter to form iron-organic complexes, intensifying membrane fouling. In addition, excessive nZVI can partially inhibit microbial activity, reducing the efficiency of organic matter removal. These findings suggest the existence of an optimal nZVI dosage range for effective AnMBR operation.

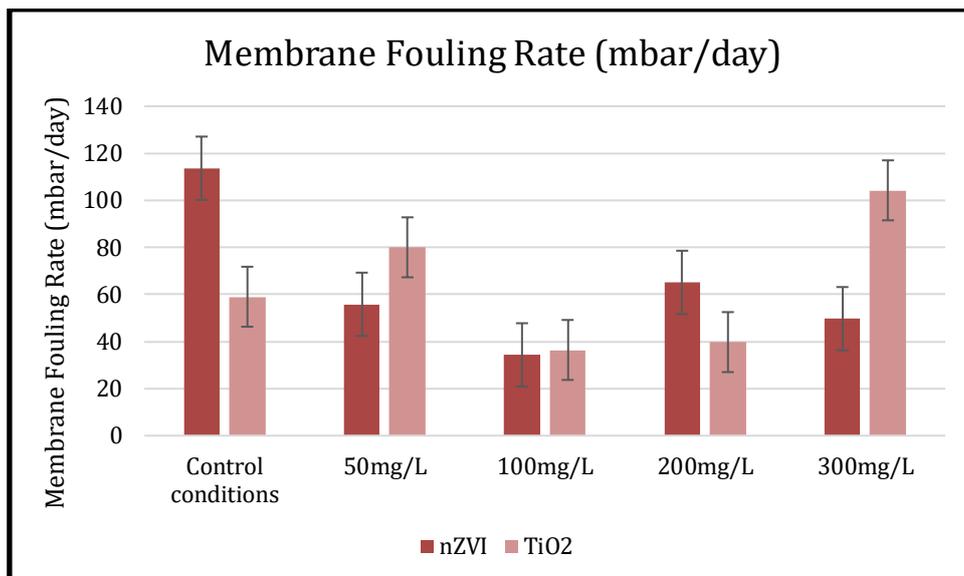


Figure 4. Comparison of the membrane fouling rate of nZVI and TiO<sub>2</sub>

### 3.3. Effect of nZVI and TiO<sub>2</sub> Concentration on SMP, EPS Production and Membrane Fouling

SMP and EPS are the primary contributors to membrane fouling in AnMBR systems, particularly through pore blocking and cake layer formation (Le-Clech et al., 2006; Meng et al., 2009). The results indicate that nZVI concentration significantly affected SMP and EPS production, thereby affecting membrane fouling (Table 5). Control (PI) and low nZVI dosage conditions (50 mg/L-PII) resulted in relatively high SMP and EPS concentrations, indicating limited regulation of microbial metabolism and insufficient fouling mitigation (Guo et al., 2012). In contrast, moderate nZVI addition (100–200 mg/L, PIII and IV) substantially reduced SMP carbohydrate concentrations in the permeate and EPS levels in the supernatant. Since carbohydrate rich biopolymers are highly hydrophilic and strongly associated with membrane fouling, their reduction results in a more porous and less compact cake layer, along with the lowest fouling rates (Meng et al., 2009; Tian et al., 2013). However, further increasing nZVI concentration to 300 mg/L led to elevated EPS carbohydrate production, despite reduced SMP levels (Table 5). This effect is likely attributed to nanoparticle agglomeration and excessive iron release, which induced microbial stress and stimulated EPS secretion as a protective response (Hu et al., 2020b; Zhang et al., 2020b). Consequently, the antifouling benefits were partially offset at high nZVI concentrations. Overall, these findings demonstrate that optimum nZVI dosages (100–200 mg/L) effectively mitigate membrane fouling by suppressing SMP and EPS production, whereas excessive nZVI addition may deteriorate membrane performance (Table 5).

**Table 5.** Effect of nZVI on SMP, EPS and Membrane Fouling

Phases (P)		nZVI			
		SMP		EPS	
		Carbohydrate	Protein	Carbohydrate	Protein
PI	Influent	946±1.73	31±0.57		
	Supernatant	848±1.22	44±0.57	13±0.57	4±0.46
	Permeate	985±1.52	39±1.52		
PII	Influent	820±1.21	39±1.52		
	Supernatant	930±1.75	41±0.57	13±0.57	6±0.46
	Permeate	764±1.28	33±1.52		
PIII	Influent	849±1.22	37±1.52		
	Supernatant	832±1.3	41±0.57	10±0.57	14±0.57
	Permeate	424±1.15	36±0.57		
PIV	Influent	816±1.73	40±0.55		
	Supernatant	932±1.52	29±0.55	6±0.57	9±0.57
	Permeate	508±0.57	34±0.57		
PV	Influent	613±0.57	25±1.57		
	Supernatant	744±0.57	38±1.57	20±0.57	3±0.57
	Permeate	499±0.58	22±1.57		

The addition of TiO<sub>2</sub> nanoparticles into the AnMBR system exerted a dosage-dependent effect on the production of EPS and SMP, directly modulating membrane fouling (Table 6). Control conditions (PI), the effect of SMP carbohydrates suggested a high fouling inclination, reinforcing the established role of these soluble organics as primary foulants (Le-Clech et al., 2006; Meng et al., 2009). PII conditions (50 mg/L) significantly reduced both SMP and EPS carbohydrate levels within the supernatant. This improvement is likely attributable to the adsorption of soluble organic matter onto the nanoparticles and the partial inhibition of biopolymer synthesis, which hinders the development of a carbohydrate-rich gel layer (Guo et al., 2012; Tian et al., 2013). Conversely, increasing the dosage to 100–200 mg/L resulted in a significant increase in supernatant SMP and EPS (both proteins and carbohydrates-Table 6). Consequently, suggests that TiO<sub>2</sub> concentrations (100 and 200mg/L) may induce nanoparticle biopolymer interactions, prompting a protective hyper-secretion of EPS that results in a resistant cake layer (Zhang et al., 2018; Hu et al., 2020). PV conditions (300 mg/L TiO<sub>2</sub>) led to a decrease in SMP and EPS concentrations, potentially due to nanoparticle agglomeration. The resulting biological instability suggests that high dosages are ineffective for consistent, long-term fouling mitigation. Ultimately, while low-dose TiO<sub>2</sub> shows promise for fouling control, higher concentrations introduce complex nanoparticle microbe interactions that destabilize the filtration process.

**Table 6.** Effect of SMP and EPS on the membrane fouling rate

		TiO <sub>2</sub>			
Phases(P)		SMP		EPS	
		Carbohydrate	Protein	Carbohydrate	Protein
PI	Influent	946±1.73	31±0.57		
	Supernatant	848±1.22	44±0.57	13±0.57	4±0.46
	Permeate	985±1.52	39±1.52		
PII	Influent	665±4.16	37±0.48		
	Supernatant	691±0.57	37±0.48	7±4.30	2±0.14
	Permeate	512±1.51	34±1.09		
PIII	Influent	673±1.22	34±0.45		
	Supernatant	1009±1.3	37±0.92	12±1.31	9±0.35
	Permeate	486±1.15	31±0.69		
PIV	Influent	680±1.73	36±1.15		
	Supernatant	858±1.52	37±0.81	15±1.15	12±0.57
	Permeate	457±0.57	27±1.29		
PV	Influent	529±0.57	34±1.09		
	Supernatant	707±0.57	38±1.57	6±1.46	2±0.12
	Permeate	499±0.58	34±0.45		

A comparison between nZVI and TiO<sub>2</sub> indicates that nZVI facilitates a more consistent and robust mitigation of membrane fouling within the AnMBR. 100 and 200 mg/L dosages of nZVI gradually decreased the production of SMP and EPS, specifically targeting carbohydrate-rich fractions, the primary drivers of membrane fouling (Meng et al., 2009; Guo et al., 2012). Due to promoting the development of a highly porous and less compressible cake layer, ultimately yielding the lowest recorded membrane fouling rates. In contrast, the effect of TiO<sub>2</sub> remained highly sensitive to concentration and exhibited less predictability. While a 50 mg/L dosage aided in decreasing biopolymer levels, the concentration of 100–200 mg/L triggered defensive EPS secretion. As a result of a microbial stress response to nanoparticle exposure, the formation of dense cake layers formed that undermined long-term filtration efficiency. The technical superiority of nZVI is rooted in its multifunctional nature, encompassing both physicochemical and biological pathways. Also, nZVI catalyzes microbial activity and modulates community dynamics through redox-mediated pathways and magnetic aggregation. Conversely, the efficacy of TiO<sub>2</sub> is largely limited by adsorption and surface-level interactions and lacks the biologically stimulating redox activity specific to nZVI. Consequently, while these results highlight nZVI as a more sustainable and reliable agent for fouling control, the higher concentrations of TiO<sub>2</sub> reduction efficacy stem from adverse nanoparticle-microbe interactions.

#### 4. Conclusions

TiO<sub>2</sub> and nZVI nanoparticles were added to the anaerobic membrane bioreactor (AnMBR) system, and the main results of this study are summarized as follows:

- The AnMBR systems containing TiO<sub>2</sub> and nZVI showed a slower decline in flux and transmembrane pressure (TMP) compared to the control reactor. The addition of nanoparticles effectively reduced the rate of membrane fouling.
- The decrease in membrane clogging was mainly due to the presence of TiO<sub>2</sub> and nZVI nanoparticles, which improved membrane surface properties and lowered cake layer formation.
- The best operational performance was achieved at 100 mg/L of TiO<sub>2</sub> and 100 mg/L of nZVI, corresponding to membrane fouling rates of 36 mbar/day and 34 mbar/day, respectively.
- Adding TiO<sub>2</sub> and nZVI resulted in a significant reduction in fouling, with decreases of about 40% and 70%, respectively.
- Overall, incorporating TiO<sub>2</sub> and nZVI nanoparticles into AnMBR systems offers a simple yet highly effective method for reducing membrane fouling and improving long-term reactor performance.
- 100 and 200 mg/L nZVI concentrations decreased SMP and EPS production, resulting in a more porous cake layer and lower membrane fouling rates, whereas excessive 300 mg/L nZVI promoted EPS accumulation due to microbial stress and nanoparticle aggregation.

- Low TiO<sub>2</sub> dosages decreased SMP and EPS, whereas higher concentrations result in biopolymer production, limiting their effectiveness for long-term membrane fouling control.
- nZVI outperformed TiO<sub>2</sub> in fouling mitigation and biopolymer production (both SMP and EPS), whereas TiO<sub>2</sub> showed unstable effects at higher concentrations due to stress-induced EPS secretion.

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## Conflict of Interest

No conflict of interest was declared by the authors.

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