

Response to shock load of titanium dioxide nanoparticles on aerobic granular sludge and algal-bacterial granular sludge processes

Alfonz Kedves^{a,*}, Çağdaş Yavuz^a, Orsolya Kedves^b, Henrik Haspel^{a,c}, Zoltán Kónya^{a,c}

^a Department of Applied and Environmental Chemistry, University of Szeged, Szeged, Hungary

^b Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

^c HUN-REN-SZTE Reaction Kinetics and Surface Chemistry Research Group, Szeged, Hungary

ARTICLE INFO

Editor: Dr. Bernd Nowack

Keywords:

Aerobic granular sludge
Algal-bacterial granular sludge
Titanium dioxide nanoparticles
Shock load
Inhibition effect

ABSTRACT

Titanium dioxide nanoparticles (TiO₂ NPs) are extensively used in various fields and can consequently be detected in wastewater, making it necessary to study their potential impacts on biological wastewater treatment processes. In this study, the shock-load impacts of TiO₂ NPs were investigated at concentrations ranging between 1 and 200 mg L⁻¹ on nutrient removal, extracellular polymeric substances (EPSs), microbial activity in aerobic granular sludge (AGS), and algal-bacterial granular sludge (AB-AGS) bioreactors. The results indicated that low concentration (≤ 10 mg L⁻¹) TiO₂ NPs had no effect on microbial activity or the removal of chemical oxygen demand (COD), nitrogen, and phosphorus, due to the increased production of extracellular polymeric substances (EPSs) in the sludge. In contrast, the performance of both AGS and AB-AGS bioreactors gradually deteriorated as the concentration of TiO₂ NPs in the influent increased to 50, 100, and 200 mg L⁻¹. Specifically, the ammonia-nitrogen removal rate in AGS decreased from 99.9 % to 88.6 %, while in AB-AGS it dropped to 91.3 % at 200 mg L⁻¹ TiO₂ NPs. Furthermore, the nitrate-nitrogen levels remained stable in AB-AGS, while NO₃-N was detected in the effluent of AGS at 100 and 200 mg L⁻¹. Microbial activities change similarly as smaller decrease in the specific ammonia uptake rate (SAUR) and specific nitrate uptake rate (SNUR) was found in AB-AGS compared to those in AGS. Overall, the algal-bacterial sludge exhibited higher resilience against TiO₂ NPs, which was attributed to a) higher EPS volume, b) smaller decrease in LB-EPS, and c) the favorable protein to polysaccharide (PN/PS) ratio. This in turn, along with the symbiotic relationship between the algae and bacteria, mitigates the toxic effects of nanoparticles.

1. Introduction

Urbanization and the progress of civilization inevitably result in the production of large volumes of wastewater, which has led to a growing focus on and continuous research into effective nutrient removal in wastewater treatment (Di Trapani et al., 2011; Waqas et al., 2020). In comparison to activated sludge flocs, aerobic granules present a more attractive option due to their dense structure containing diverse microorganisms (Kedves et al., 2021; Kedves et al., 2020). The aerobic granular sludge (AGS) process offers several advantages, including adaptability to varying temperatures (Ab Halim et al., 2016), resilience to toxic substances (Liu et al., 2015; Zhao et al., 2015), and simultaneous nutrient removal capacities (Szabó et al., 2016; Li et al., 2019a). Consequently, aerobic granular sludge processes have garnered considerable attention in municipal wastewater treatment (Adav et al.,

2008). Recently, there has been growing interest in algal-bacterial granular sludge (AB-AGS) processes for wastewater treatment (Guo et al., 2021; Purba et al., 2020). This innovative approach takes advantage of the synergistic relationship between algae and bacteria to facilitate nutrient cycling and pollutant removal through biosynthesis and metabolic processes (Sanchez-Sanchez et al., 2023). Additionally, a symbiotic association can be established between algae and bacteria, wherein algae supply oxygen for bacterial organic oxidation, while bacteria provide CO₂ for microalgal photosynthesis (Zhang et al., 2018; Ji et al., 2020a). During the granulation processes, microorganisms produce extracellular polymeric substances (EPS), that form a unique gel-like matrix, helping microbial cells to create more complex polymers, which has two main components: polysaccharides (PS) and proteins (PN). This matrix shields microorganisms from fluctuating and harsh external conditions (Kedves et al., 2020). EPS can be classified

* Corresponding author at: Department of Applied and Environmental Chemistry, University of Szeged, H-6720 Szeged, Rerrich Béla tér 1, Hungary.
E-mail address: kedvesalfonz@chem.u-szeged.hu (A. Kedves).

into two types based on their location: loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS), which together form dual protective layers (Zheng et al., 2019). Indicators such as specific oxygen uptake rate (SOUR), specific ammonia uptake rate (SAUR), specific nitrite uptake rate (SNIUR), specific nitrate uptake rate (SNUR), and specific phosphorus uptake rate (SPUR) are critical for understanding the microbial activity and overall health of the sludge (Kedves et al., 2021; Wang et al., 2015a). These indicators reflect different aspects of the microbial metabolic processes, which are essential for evaluating the impact of hazardous materials on the performance of AGS and AB-AGS.

Nanomaterials are increasingly being used in the production of a wide range of industrial and consumer goods, thanks to their unique physical and chemical properties (Zheng et al., 2011). Titanium dioxide nanoparticles (TiO₂ NPs) find extensive use in various applications, including catalysts, paints, sunscreens, plastics, cosmetics, and industrial processes, owing to their chemical stability, high photocatalytic efficiency, and cost-effectiveness (Erdural et al., 2014; Larue et al., 2014; Dréno et al., 2019; Ismael, 2020). Previous studies have shown that the half-maximal inhibitory concentration (IC₅₀) of TiO₂ NPs is 37 mg L⁻¹ for denitrifying bacteria (Li et al., 2016), 35.9 mg L⁻¹ for algae (Aruoja et al., 2009), and only 11.9 mg L⁻¹ for human cells (Periasamy et al., 2015). TiO₂ NPs can exert their toxic effects by attaching to the cell surface, causing membrane damage, which leads to cell shrinkage and eventually cell death (Prokopiuk et al., 2023). If they attach to the cell and penetrate the cell wall, they can cause irreversible damage to its function (Li et al., 2015a). Furthermore, the negative impact of nanoparticles is influenced by the composition of the wastewater. Cervantes-Avilés et al. (Cervantes-Avilés et al., 2022) investigated the effects of TiO₂ NPs in synthetic, raw, and filtered wastewater, and based on the results, it was concluded that the nanoparticles negatively affected nutrient removal differently in each case, owing to the varying compositions of the wastewaters. Finally, their toxic effects can also occur through the release of metal ions from the nanoparticles, which alters the selective permeability of the cell membrane, affecting intracellular osmotic pressure (Xiao et al., 2022). The widespread use of these nanoparticles has led to the release of TiO₂ NPs into the environment, where they are detectable in soil, aquatic environments, and wastewater (Gottschalk et al., 2009). Wastewater treatment plants serve as one of the final barriers for removing contaminants from municipal and industrial wastewaters through microorganisms. One study reported that the amount of TiO₂ NPs reaches 10.7 µg L⁻¹ in influent wastewater at Southern California (Cervantes-Avilés and Keller, 2021), while another study found TiO₂ NPs present in sewage at concentrations of up to 5 mg L⁻¹ (Wang et al., 2017), and another investigation reported levels of these particles in sludge reaching up to 23 mg kg⁻¹ (Gottschalk et al., 2009). Because of the potential adverse effects of TiO₂ NPs on biological systems, it is crucial to evaluate their influence on the effectiveness of biological wastewater treatment systems.

The study by Zheng et al. (Zheng et al., 2011) found that the presence of TiO₂ NPs at a concentration of 50 mg L⁻¹ decreased the efficiency of ammonia removal in activated sludge by 55 %, attributed to a reduction in aerobic ammonia-oxidizing bacteria. In contrast, Li et al. (Li et al., 2017) observed a decline in the removal rates of organic matter and phosphorus, when the nanoparticle concentration reached 10 mg L⁻¹, with no significant impact on ammonia removal. Cervantes-Avilés et al. (Cervantes-Avilés et al., 2021) observed that increasing the concentration of TiO₂ NPs in the influent resulted in a decrease in the oxygen uptake rate and organic matter removal rate. Subsequent investigations by Zhou et al. and Li et al. (Zhou et al., 2019; Li et al., 2019b) noted a decrease in floc size, dewaterability, and microbial diversity in activated sludge by TiO₂ NPs addition. In aerobic granules, sludge settleability was improved at 50 mg L⁻¹ nanoparticle concentration due to the increased protein content along with rising nitrogen levels in effluent water (Jiang et al., 2022). However, TiO₂ NPs at concentrations of 30 and 50 mg L⁻¹ negatively affected denitrification processes in AB-AGS, while organic matter, ammonia, and phosphorus removal remained

stable (Li et al., 2015b). To date, there has been no comparative study on the impact of TiO₂ NPs on AGS and AB-AGS wastewater treatment technologies under identical operating conditions.

The present study investigated and compared the short-term effects of TiO₂ NPs at concentrations ranging from 1 to 200 mg L⁻¹ in two promising wastewater treatment systems, i.e. in AGS and AB-AGS. The removal rates of nitrogen compounds (ammonia, nitrite, and nitrate) and phosphorus were determined every 4 h during the experiment. Additionally, at the end of each experimental phase, the quantities of extracellular polymeric substances (EPSs) and microbial activity in the sludges were also assessed.

2. Materials and methods

2.1. Preparation and characterization of TiO₂ NPs

All chemicals were used as received without any further purifications. Based on the previously published technique, TiO₂ NPs were synthesized through a modified nonaqueous solvothermal process (Li et al., 2015c). Titanium isopropoxide (TIP, 5 mL) was dissolved in 130 mL of anhydrous acetone, and after 20 min of stirring at room temperature, the solution was taken to a 250 mL Teflon-lined stainless-steel autoclave and kept at 180 °C for 10 h. The yellowish sediment was gathered by centrifugation, repeatedly washed with ethanol and dried at 60 °C. In the last step, the powder was calcined at 450 °C for 2 h to obtain pure white TiO₂ NPs. Structural and morphological characterization was done by using a Rigaku Miniflex-II X-ray diffractometer (Cu Kα radiation, λ = 1.5406 Å, 40 kV, 30 mA), a Bruker Vertex 70 FT-IR instrument (16 scans at 4 cm⁻¹ resolution), and a Hitachi S-4700 Type II scanning electron microscope (SEM) with 10 kV accelerating voltage equipped with a Röntec QX2 energy dispersive X-ray spectrometer (EDX) (Kedves et al., 2020).

2.2. Experimental procedure

The AGS sequencing batch reactors (SBRs) and the AB-AGS photo-sequencing batch reactors (PSBRs) were configured with an effective volume of 1.4 L and a hydraulic retention time of 8 h (detailed information can be found in *Supporting Information*). The average diameter of both types of granular sludge was 600 µm with mixed liquor suspended solids (MLSS) of 5.75 ± 0.15 g L⁻¹, and an initial sludge volume index (SVI₅) of 26.26 ± 0.13 mL g⁻¹, respectively. In each experiment, fresh granular sludge obtained from control bioreactors was utilized. TiO₂ NPs concentrations of 1, 5, 10, 50, 100, and 200 mg L⁻¹ were applied to investigate the effect of shock loads for 24 h. TiO₂ NPs were studied at environmentally relevant concentrations of 1 and 5 mg L⁻¹ (Yuan et al., 2021; Mu et al., 2012); additionally, higher concentrations were selected for investigation due to their extensive large-scale production (Mohammadparast and Mallard, 2023). Before use, the nanoparticles were placed in deionized water and sonicated for 10 min to prevent the use of aggregated particles during the experiments. Then, the desired amount of particles were introduced into the bioreactors along with synthetic wastewater (SWW). The SWW consisted of the following components per liter of deionized water: 1200 mg COD as glucose, 110 mg NH₄-N as NH₄Cl, 20 mg PO₄-P added as KH₂PO₄, 200 mg NaHCO₃, 25 mg CaCl₂, 45 mg MgSO₄, and 1 mL of trace element solution (Kedves et al., 2020).

2.3. Analytical methods

COD, nitrogen (including ammonia-nitrogen NH₃-N, nitrite-nitrogen (NO₂-N), and nitrate-nitrogen (NO₃-N)) and PO₄³⁻ contents of the effluent water were measured using test kits (Hanna Instruments) and a HANNA Instruments HI83306 Environmental Analysis Photometer. The concentration of titanium in the effluent was measured via Inductively-coupled plasma mass spectrometry (ICP-MS) using an Agilent 7900

instrument. The MLSS and SVI₅ of the sludge were measured as reported previously by Clesceri et al. (Clesceri et al., 1990). The effect of TiO₂ NPs on microbial activity was determined by measuring the specific oxygen uptake rate (SOUR), specific ammonia uptake rate (SAUR), specific nitrite uptake rate (SNIUR), specific nitrate uptake rate (SNUR), and specific phosphorus uptake rate (SPUR) at the end of each phase (see *Supporting Information*). Furthermore, the amounts of loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) in the sludge was determined using a modified heat extraction method (Li et al., 2023) (the sum of LB-EPS and TB-EPS constituted the total EPS amount). Briefly, a 10 mL sample was centrifuged at 5000 g for 30 min. The resulting supernatant was passed through a 0.22 µm membrane filter, and the filtrate collected represented the LB-EPS fraction. Once the supernatant was discarded after LB-EPS collection, the remaining sludge was resuspended in a 0.9 % (w/v) NaCl solution to its original volume. It was then heated to 80 °C for 30 min. The solution was centrifuged again at 10,000 g for 20 min, and the supernatant was filtered using a 0.22 µm membrane. The resulting filtrate was used to analyze the TB-EPS fraction. Both LB- and TB-EPS samples were stored at −30 °C until further analysis. The polysaccharide (PS) and protein (PN) contents in the EPS were

quantified using the Anthrone method (with glucose as the standard) and the modified Lowry method (with bovine serum albumin as the standard) as described by Frølund et al. (Frølund et al., 1996). EPS was calculated as the sum of PN and PS. All analyses were carried out in triplicate. The morphology of the sludge was investigated with a scanning electron microscope (SEM), the diameter of both types of granules were analyzed by using ImageJ 1.53a software (Kedves et al., 2021).

2.4. Statistical analysis

All measurements were conducted in triplicate, and the results were reported as the mean ± standard deviation (SD). To assess the significance of the findings, a one-way analysis of variance (ANOVA) was used. The significance level was set at 5 % for each analysis.

3. Results and discussion

3.1. Characterization of TiO₂ NPs

The X-ray diffraction patterns of the TiO₂ nanocrystals are displayed

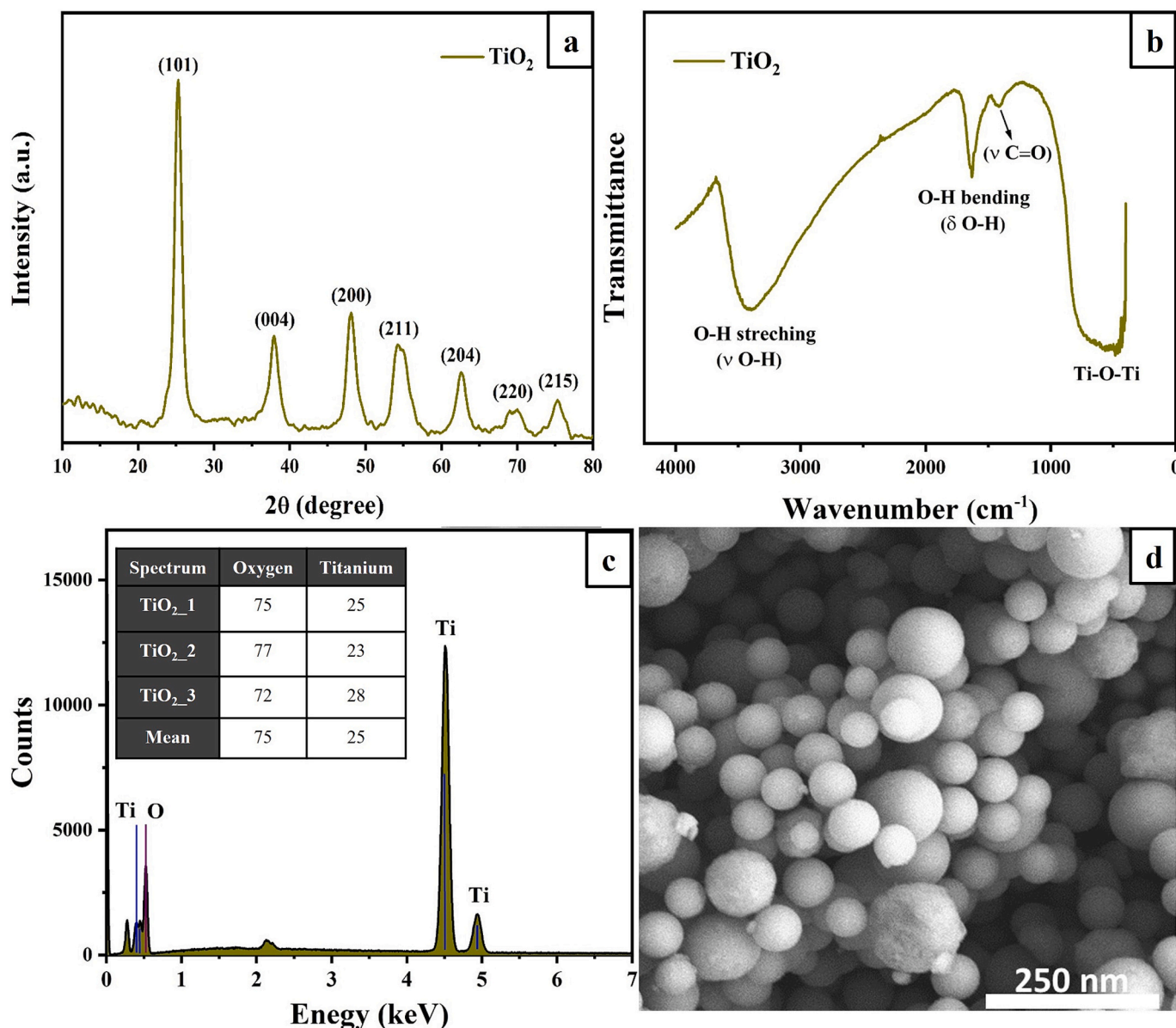


Fig. 1. Characterization of the TiO₂ NPs. a) X-ray diffraction pattern; The b) FT-IR spectrum, c) EDX spectrum, and d) SEM image of the nanoparticles.

in Fig. 1a. Reflections can be indexed as anatase TiO_2 NPs (JCPDS card file no. 21-1272), where the peaks appeared at 2θ of 25.2° , 37.8° , 48.1° , 54.6° , 62.6° , 69.7° , and 75.3° correspond to the (101), (004), (200), (211), (204), (220), and (215) crystal planes (John et al., 2020). To obtain more compositional details on TiO_2 NPs, FT-IR measurement was performed, and the resulting spectrum is shown in Fig. 1b. The vibrational modes that are commonly attributed to the stretching of Ti–O–Ti of the TiO_2 NPs are observed at 410 and 780 cm^{-1} . Furthermore, the vibrational modes at around 1632 and 3420 cm^{-1} are associated with the bending mode of the physisorbed water molecules on TiO_2 NPs, respectively (Eddy et al., 2021). Energy dispersive X-ray (EDX) analysis was carried out to confirm the chemical composition of the as-prepared nanoparticles, and a typical EDX spectrum is shown in Fig. 1c. A Ti:O atomic ratio of 1:3 was found from the average measured at three different points of the sample (inset Fig. 1c). Scanning electron microscopy image of the TiO_2 NPs sample is displayed in Fig. 1d. The TiO_2 crystals have spherical morphology of a diameter ranging between 30 and 130 nm .

3.2. Impact of TiO_2 NPs on extracellular polymeric substances production in AGS and AB-AGS

The granular sludge owes its excellent tolerance to pollutants to the high amount of extracellular polymeric substances (EPSs). The EPS are produced by microorganisms and consist of two main components: proteins (PN) and polysaccharides (PS). These substances are able to adsorb different compounds, improve the settling ability of sludge. Moreover, they protect microorganisms against harmful materials, such as heavy metals or nanoparticles (Zheng et al., 2019; Jiang et al., 2022). The PS and PN content of the initial AGS were 47.5 ± 4.1 and $56.5 \pm 5.2\text{ mg g}^{-1}\text{ MLVSS}$ (Fig. 2a), and 46.4 ± 3.9 and $56.5 \pm 4.7\text{ mg g}^{-1}\text{ MLVSS}$ in AB-AGS, respectively (Fig. 2b). Although nanoparticles at 1 mg L^{-1} did not cause any change in EPS, there was an increased polymer content in both granular sludges at 5 and 10 mg L^{-1} . This suggests, that TiO_2 NPs may have a toxic effect on microorganisms at these concentrations, as it was reported earlier in activated sludge (Li et al., 2017). Granular sludge, on the other hand, were able to secrete more EPS (mainly protein), thereby enabling the microorganisms to protect themselves against the nanomaterials (Xuan et al., 2010).

With further increases in the TiO_2 NPs content, the extent of EPS showed a declining trend. At 50 mg L^{-1} , the PN content in AGS and AB-AGS was 64.8 and $104.4\text{ mg g}^{-1}\text{ MLVSS}$, while the PS remained relatively stable. This, in turn, increases the PN/PS ratio to 1.34 and 2.32 , respectively. The results are in line with previous research, wherein 50

mg L^{-1} TiO_2 NPs had a positive effect on the EPS of AGS and AB-AGS (Jiang et al., 2022; Li et al., 2015b). The higher PN/PS ratio in the algal-bacterial sludge suggests more protected microorganisms in the center of the sludge against nanoparticles, as proteins are predominantly secreted inside the granules (Nanchaiah, 2018). In AGS, both PS and PN marginally decreased, when the contamination level reached 100 and 200 mg L^{-1} . This resulted in 10% and 20% declines in EPS volume, respectively. The PN/PS ratio was also lower than that in the initial sludge. This implies that microorganisms inside the granules were exposed to TiO_2 NPs, which then lead to a reduction in their metabolic activity. Conversely, in AB-AGS, the percentage of polymers decreased only by 3% and 12% , respectively, while the PN/PS ratio was comparable to that in the control sludge. This suggests that microorganisms in the core of the granular sludge were more protected against titanium-dioxide than in aerobic granular sludge. Consequently, the negative effect of nanoparticles on wastewater treatment efficiency may be lower. The observed higher EPS content in the AB-AGS system can be attributed not only to bacterial secretion but also to the contribution of algae, which are an integral part of the algal-bacterial consortia. Algae in AB-AGS are known to secrete EPS, including both PS and PN, at different rates compared to bacteria (Hakim et al., 2023; Li et al., 2022). Specifically, the algal contribution to EPS is significant in providing additional PS, which enhances the protective matrix of the granules (Liu et al., 2022). This additional source of PS and PN from algae leads to a more robust EPS matrix, which supports the structural integrity of the granular sludge and provides greater resilience against environmental stressors, such as TiO_2 NPs. Therefore, the higher protein content observed in the AB-AGS is not solely a result of bacterial activity but is also driven by the algal production of EPS. This combined contribution from both algae and bacteria helps explain the improved resistance of AB-AGS to TiO_2 NPs, as the algal-generated EPS complements the bacterial EPS, creating a more protective and resilient granular structure.

In granular sludge tightly bound proteins (TB-PN) and tightly bound polysaccharides (TB-PS) are integral to the structural integrity and stability of the granules, mainly form the internal structure of the granules, contributing to their dense and compact nature. Loosely bound proteins (LB-PN) and loosely bound polysaccharides (LB-PS), on the other hand, play a crucial role in the initial aggregation and surface interactions of the sludge particles, it is present in the outer part of the granules, influencing the overall microbial activity and biofilm formation within the granules (Zheng et al., 2019). As shown in the Fig. 3, the amount of TB-EPS in the initial AGS sludge is 2.1 times that of LB-EPS, while in AB-AGS it is only 1.7 times. This can be attributed to the fact that algae on the surface of AB-AGS are also capable of synthesizing EPS (Wu et al.,

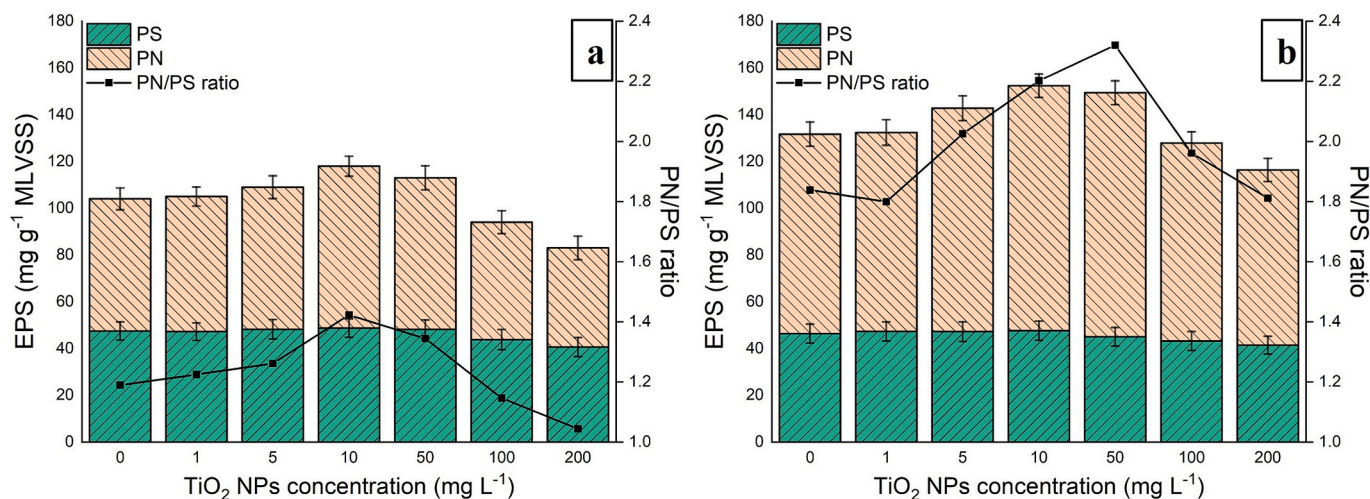


Fig. 2. Change in the extracellular polymeric substance (EPS) after introduction of titanium dioxide nanoparticles (TiO_2 NPs). EPS amount in a) aerobic granular sludge (AGS), and b) algal-bacterial aerobic granular sludge (AB-AGS) after addition of TiO_2 NPs.

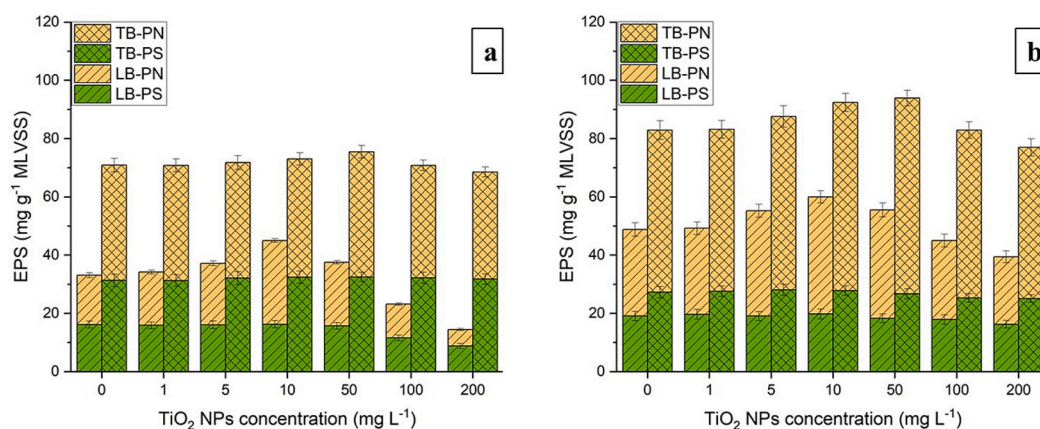


Fig. 3. Changes in loosely- and tightly bound polysaccharides and proteins (LB-PS, LB-PN, TB-PS, and TB-PN) after introduction of titanium dioxide nanoparticles (TiO₂ NPs). EPS amount in **a)** aerobic granular sludge (AGS), and **b)** algal-bacterial aerobic granular sludge (AB-AGS) after addition of TiO₂ NPs.

2023). Increasing the amount of TiO₂ NPs to 10 mg L⁻¹ resulted in an increase in LB-PS and LB-PN in both sludges, with a more pronounced increase in LB-PN. In AGS, the LB-PN increased from an initial 16.9 to 28.7 mg g⁻¹ MLVSS, while in AB-AGS it increased from 29.6 to 40.2 mg g⁻¹ MLVSS.

Nanoparticles at a concentration of 200 mg L⁻¹ did not cause significant changes in the amount of TB-EPS, which may suggest that due to the structure of the granules, the toxic substance was unable to penetrate

the interior of the granules (as they were able to produce sufficient TB-EPS), thus not affecting the predominantly anoxic and anaerobic microorganisms present inside. In contrast, there was a significant decrease in the volume of LB-EPS. For aerobic granules, the amount of LB-PS decreased by 45.6 % compared to the initial amount, while the amount of LB-PN decreased by 66.6 %. This large decrease suggests that aerobic microorganisms on the surface of the granules were unable to protect against the high concentration of nanoparticles, potentially

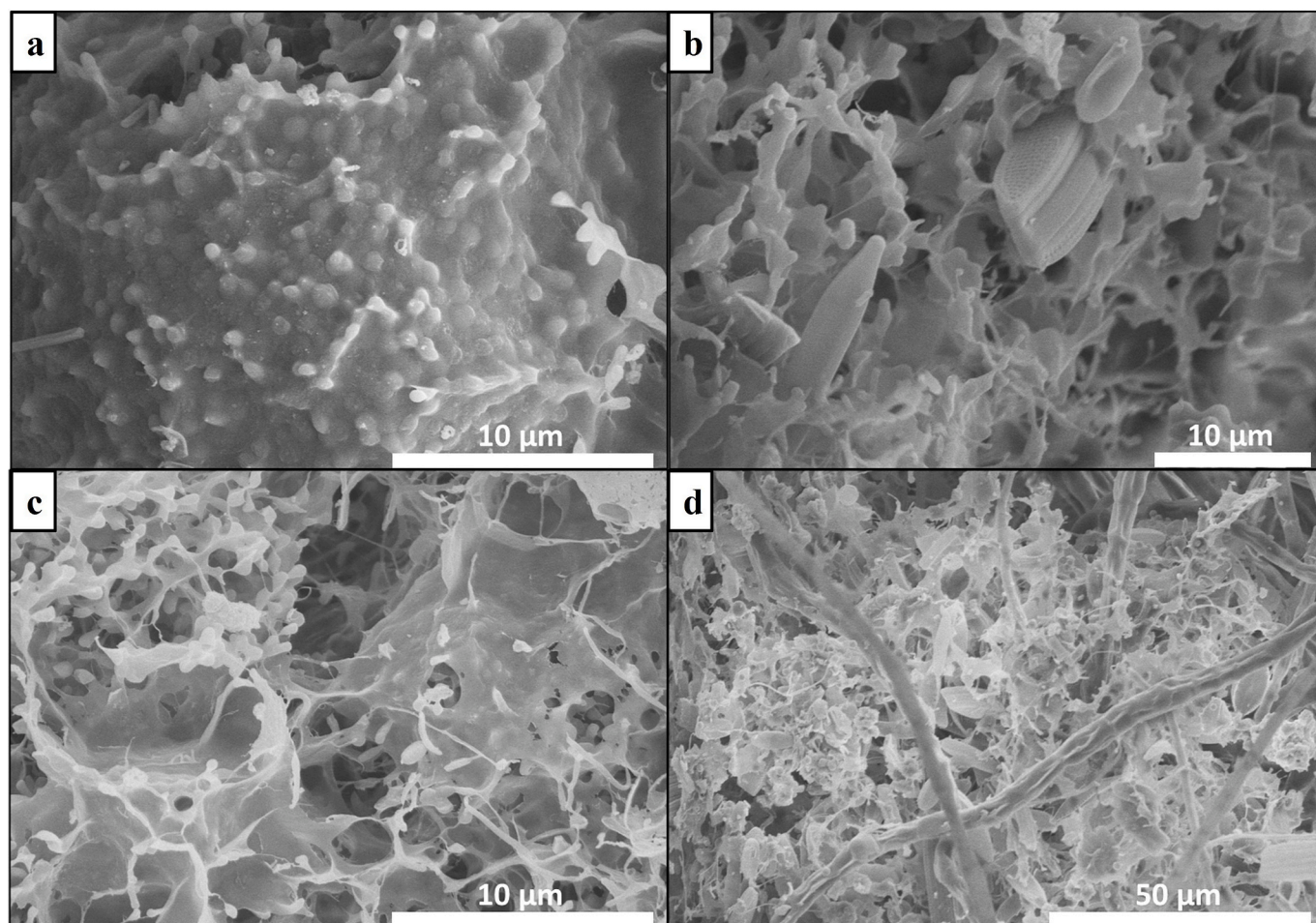


Fig. 4. Scanning electron microscope (SEM) images of granular sludge (GS). The exterior of the **a)** aerobic and **b)** algal-bacterial GS. The surface of the **c)** aerobic and **d)** algal-bacterial GS after the addition of 200 mg L⁻¹ TiO₂ NPs.

reducing their nutrient removal capacity. Although the LB-EPS amount in AB-AGS also decreased, the amounts of LB-PS and LB-PN only decreased by 14.6 % and 22.1 %, respectively. These observations indicate that algae on the surface of the granules play an important role in EPS production and defense against toxic substances (Fig. 4b and d).

3.3. Impact the shock load of TiO₂ NPs on bioreactor performance

The surface of AGS (400–800 μm in diameter) exhibited various embedded microorganisms within the EPS matrix (Fig. 4a), while algae were also visible on the exterior of AG-AGS (500–1000 μm in diameter) (Fig. 4b).

Without the presence of nanoparticles, the effluent COD, ammonia-, nitrite-, nitrate-nitrogen, and PO_4^{3-} contents in AGS were 82 ± 5 , 0.08 ± 0.02 , 0.05 ± 0.01 , 0.49 ± 0.03 , and $0.54 \pm 0.06 \text{ mg L}^{-1}$, respectively. In contrast, AB-AGS demonstrated more efficient nutrient removal with the corresponding concentrations of 74 ± 6.3 , 0.05 ± 0.01 , 0.03 ± 0.01 , 0.45 ± 0.04 , and $0.36 \pm 0.05 \text{ mg L}^{-1}$, respectively. Upon addition of 50, 100, and 200 mg L^{-1} TiO₂ NPs into the bioreactors, the nutrient removal declined (Fig. 5). The performance of AGS and AB-AGS, however, remained stable at lower TiO₂ NP concentrations.

The addition of 50 mg L^{-1} TiO₂ NPs to AB-AGS did not change the COD removal significantly, while in the AGS bioreactor, COD concentration in the effluent increased from 82 ± 2.3 to $117 \pm 4.4 \text{ mg L}^{-1}$ after 24 h (Fig. 5a). Further increase in TiO₂ NPs concentration to 100 and 200 mg L^{-1} , on the other hand, resulted in reduced COD removal in both SBRs. The COD values in the effluent were 142 ± 3.8 and $183 \pm 5.2 \text{ mg L}^{-1}$ in AGS, and 129 ± 3.7 and $161 \pm 4.1 \text{ mg L}^{-1}$ in AB-AGS, respectively. Furthermore, at 200 mg L^{-1} nanoparticle concentration, no drop in the removal rate in the AB-AGS was observed after 4 h of operation. This suggests higher tolerance for the latter compared to that of the

aerobic granular sludge within a very narrow time interval. Li et al. (Li et al., 2017) investigated the effect of TiO₂ NPs on activated sludge and found, that the nanoparticles at concentrations as low as 10 mg L^{-1} negatively influenced COD removal with a 10 % drop after 1 day of operation. In another study, exposure of activated sludge to 50 mg L^{-1} TiO₂ NPs reduced the organic matter removal by 64 % (Li et al., 2019b). While Zhou et al. (Zhou et al., 2019) and Cervantes-Avilés et al. (Cervantes-Avilés et al., 2021) also reported a declined chemical oxygen demand reduction efficiency when the concentration of nanoparticles were lower than 50 mg L^{-1} . The significant differences between activated and granular sludges may originate from the differences in their structure. In activated sludge, microorganisms are more exposed to pollutants, as flocs have very loose structure. This allows the nanomaterials to attach to the surface of heterotrophic microorganisms and pass through the cell membrane (Wang et al., 2017). In contrast to previous literature results, our findings suggest that heterotrophic microorganisms within the granules displayed increased resilience against the adverse effects of NPs. Compared to activated sludge, the elevated secretion of EPS within the granules could mitigate the toxicity of NPs on the sludge, which results in a lower decline in organic removal efficiency.

The removal rate of ammonia also decreased by the continuous increase in nanoparticle concentration. At 50, 100, and 200 mg L^{-1} TiO₂ NPs the $\text{NH}_3\text{-N}$ removal efficiency dropped from 99.9 % to 97 %, 94.3 %, and 88.6 % in SBR, and from 99.9 % only to 98.8 %, 96.9 %, and 91.3 % in PSBR, respectively (Fig. 5b). The latter implies that the algal-bacterial consortia are more tolerant against contaminations, which is even more pronounced in the case of nitrite. Whilst addition of 200 mg L^{-1} nanoparticle to AB-AGS increased the nitrite concentration from 0.03 to 2.6 mg L^{-1} , the $\text{NO}_2\text{-N}$ concentration grew from 0.05 to only 3.74 and 8.04 mg L^{-1} at 100 and 200 mg L^{-1} TiO₂ NPs, respectively (Fig. 5c).

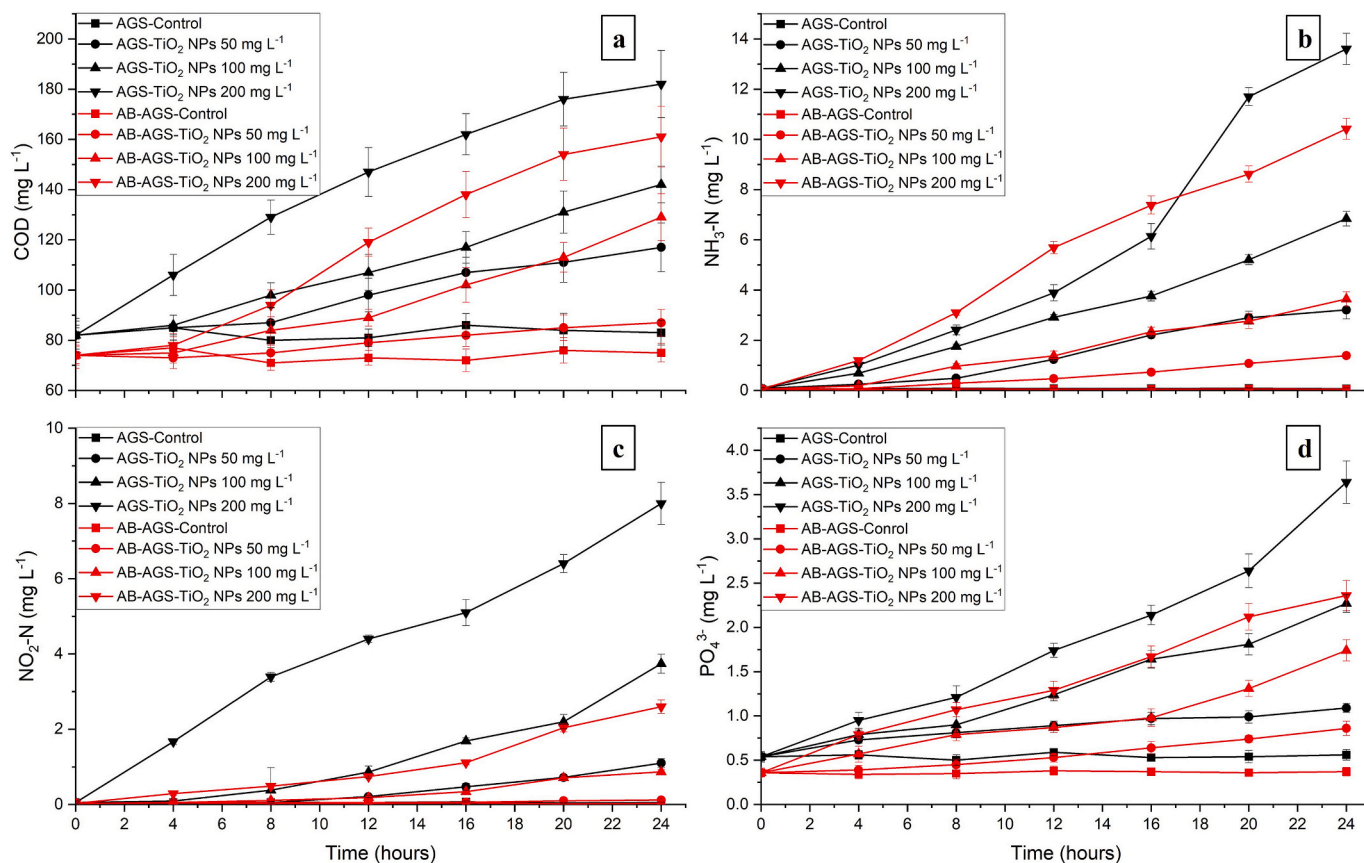


Fig. 5. Impacts of titanium dioxide nanoparticles (TiO₂ NPs) on the aerobic granular sludge (AGS) and algal-bacterial aerobic granular sludge (AB-AGS) performance. a) Chemical oxygen demand (COD), b) ammonia-nitrogen ($\text{NH}_3\text{-N}$), c) nitrite-nitrogen ($\text{NO}_2\text{-N}$), and d) phosphorus (PO_4^{3-}) contents.

During the experiments, the effluent nitrate concentration remained stable in the algal-bacterial sludge, whereas it slightly increased to 5.38 and 8.26 mg L⁻¹ in AGS upon introducing 100 and 200 mg L⁻¹ TiO₂ NPs, respectively. The differences observed in nitrite and nitrate removal efficiency between AGS and AB-AGS may be attributed to the amount of LB-EPS present, as nitrite- and nitrate-removing microorganisms are primarily located in the inner layers of the granules (Xia et al., 2018). Therefore, microorganisms may be better protected against the negative effects of nanoparticles in an algal-bacterial sludge with higher LB-PS and LB-PN contents, due to the adhesion of toxic nanoparticles on the surface of the granules, they did not penetrate the anoxic and anaerobic zones where nitrite and nitrate removal also occurs. Additionally, significant changes were observed in the polymer matrix on the surface of AGS (Fig. 5c). This suggests that TiO₂ NPs were able to penetrate into the interior of the granules, inhibiting denitrifying microorganisms. Zheng et al. (Zheng et al., 2011) investigated the impact of TiO₂ NPs on activated sludge, and found that the removal of ammonia declined to 24.4 % at 50 mg L⁻¹ nanoparticle contamination with stable nitrite and nitrate concentrations stable in the effluent wastewater. Studies on the effect of ≥ 50 mg L⁻¹ ZnO NPs on AGS reported decreased nitrite and nitrate removal, similar to our results (He et al., 2017a; He et al., 2017b). This discrepancy may arise from the low ammonia-to-nitrite conversion in the activated sludge, which in turn can be degraded first to nitrate and then to nitrogen by the microorganisms.

Cervantes-Avilés et al. (Cervantes-Avilés et al., 2021) established that TiO₂ NPs at 1.5 and 2 mg L⁻¹ caused a shift in bacterial diversity, resulting in a decline in organic matter and nitrogen removal efficiency in activated sludge. Studies by Li et al. (Li et al., 2017) and Zheng et al. (Zheng et al., 2011) have also shown that the addition of TiO₂ NPs can lead to significant shifts in the microbial community structure, particularly affecting the diversity of nitrifying and denitrifying bacteria. These changes can impact the overall efficiency of nitrogen removal, as well as the removal of organic matter. In our study, although we did not perform a detailed microbial diversity analysis, the observed decline in nitrification rate at higher concentrations of TiO₂ NPs (≥ 100 mg L⁻¹) suggests that the microbial community in both AGS and AB-AGS may have shifted. Specifically, the reduction in nitrifying bacteria could explain the decrease in ammonia and nitrite removal. These findings are consistent with previous reports, which indicate that TiO₂ NPs can exert selective pressure on microbial populations, reducing the abundance of key functional groups involved in nitrogen and organic matter cycling.

A significant difference was found in phosphate removal between the two bioreactors (Fig. 5d). At 100 and 200 mg L⁻¹ titanium dioxide concentrations, the phosphate removal efficiency decreased from 97.3 % to 88.6 % and 81.8 %, and from 98.2 % to 91.3 % and 88.2 % in AGS and PSBR, respectively. The results show higher nanoparticle tolerance in AB-AGS, as the PO₄³⁻ removal at 200 mg L⁻¹ TiO₂ NPs was nearly identical to that in AGS at 100 mg L⁻¹. This could be originated from the higher EPS amount in algal sludge. The P-accumulating organisms (PAOs) are embedded in the polymer (Wang et al., 2015b; Li et al., 2015d), which prevent nanoparticles from attaching to cell surface and permeating into the cells. A further reason could be the high sensitivity of PAOs to various environmental factors, such as increased nitrite and nitrate concentrations (Kedves et al., 2020; Li et al., 2015b). Thus, higher nitrogen levels in AGS SBRs may further inhibit phosphorus removal. Moreover, algae uptake and store phosphorus from water (Schaedig et al., 2023; Abeysiriwardana-Arachchige et al., 2020), which also highlights the importance of algae in PO₄³⁻ removal in PSBRs. The greater resistance of AB-AGS to TiO₂ NPs compared to AGS can be attributed to several factors. Firstly, AB-AGS has a higher amount of EPS, which provides better protection to the microorganisms by adsorbing and sequestering nanoparticles, thereby reducing their toxic effects. Additionally, the symbiotic relationship between algae and bacteria in AB-AGS contributes to its resilience. The algae produce oxygen through photosynthesis, which supports bacterial respiration and metabolic activity (Hakim et al., 2023), enhancing overall stability and resistance to

pollutants. Moreover, the higher protein-to-polysaccharide ratio in AB-AGS suggests a stronger and more protective EPS matrix, further mitigating the impact of TiO₂ NPs. Finally, it is possible that due to filamentous growth, the specific surface area of the granules increased, which caused the nanoparticles to be more evenly distributed across the granules, rather than concentrated in one place. Thus, TiO₂ NPs did not inhibit the metabolism of the algae, as the negative effects of nanoparticles on algae (see Fig. 4b and d) are primarily exerted by adhering to their surface, preventing them from receiving sufficient light (Aruoja et al., 2009).

3.4. Impact of TiO₂ NPs on the microbial activity of AGS and AB-AGS

To assess the impact of TiO₂ NPs on the microbial activity, the SAUR, SNIUR, SNUR, and SPUR of AGS and AB-AGS were examined at various nanoparticle concentrations. As shown in Fig. 6a, the SAUR of aerobic granular sludge remained stable after the introduction of 1 and 5 mg L⁻¹ nanoparticles, while it did not change in the algal-bacterial sludge even at 10 mg L⁻¹ TiO₂ NPs. In AGS, the increase in TiO₂ NP concentration from 0 to 100 and 200 mg L⁻¹ decreased the ammonia uptake from 4.71 to 3.51 and 2.97, and from 5.61 to 4.87 and 4.29 mg N (g MLVSS•h)⁻¹ in AGS and AB-AGS, respectively. The initial SNIUR in AGS and AB-AGS was 3.74 and 4.41 mg N (g MLVSS•h)⁻¹. This decreased first to 3.26 and 3.03, and then further to 4.29 and 3.94 mg N (g MLVSS•h)⁻¹ at 100 and 200 mg L⁻¹ TiO₂ NP concentrations, respectively (Fig. 6b). The changes in SAUR and SNIUR indicate that elevated TiO₂ NPs concentration could influence the microbial activities of both ammonia- and nitrite-oxidizing microorganisms. Furthermore, the variations in SAUR and SNIUR were consistent with the NH₃-N and NO₂-N removal at all nanoparticle concentrations. Similar phenomena were observed in previous studies, where increasing amounts of ZnO and TiO₂ NPs led to a decrease in SAUR and SNIUR, consequently affecting the removal of NH₃-N and NO₂-N in activated sludge (Li et al., 2017; Wang et al., 2016). The SNUR in the control aerobic and algal-bacterial sludge was 15.4 and 16.7 mg N (g MLVSS•h)⁻¹, respectively. With TiO₂ NP concentrations of 50, 100, and 200 mg L⁻¹, the SNUR decreased by 4 %, 12 %, and 20 % in AGS, respectively. In contrast, in AB-AGS, the nitrate uptake remained unchanged up to 50 mg L⁻¹ TiO₂ NPs and only dropped by 3 % and 6 % at higher concentrations (Fig. 6c). This observation suggests that the nitrate removal in algal-bacterial sludge remained unaffected, which indicates that the increased titanium content did not inhibit the microbial activity of denitrifying bacteria. As shown in Fig. 6d, at 50, 100, and 200 mg L⁻¹ NPs, the SPUR dropped by 7 %, 11 %, and 23 %, and only by 3 %, 7 %, and 15 % in AGS and AB-AGS, respectively. These differences explain the higher PO₄³⁻ concentrations in the effluent from AGS. The increase in titanium concentration in the influent resulted in greater inhibition of aerobic PAOs in the SBR compared to that in PSBR. The mild change in SPUR can also be attributed to several factors: i) heavy metal tolerance (Xiao et al., 2023), ii) the algae's capability to accumulate and store a large amount of cellular polyphosphate (Ji et al., 2020b), and iii) the enhanced removal of nitrogen by algae (Zhou et al., 2022). Therefore ammonia, nitrite, and nitrate did not hinder the activity of PAOs. For AB-AGS, the presence of algae not only aids in oxygen production but also contributes to the overall structural integrity and resilience of the granules. The algae secrete additional EPS, which enhances the overall protective ability of the sludge. Furthermore, the metabolic activities in AB-AGS are less affected due to the synergistic interactions between algae and bacteria, which maintain higher levels of microbial activity even under nanoparticle stress.

4. Conclusions

The shock-load impact of titanium dioxide nanoparticles (TiO₂ NPs) on biological wastewater treatment processes was studied at concentrations ranging between 1 and 200 mg L⁻¹. TiO₂ NPs exerted various effects on nutrient removal, the level of extracellular polymeric

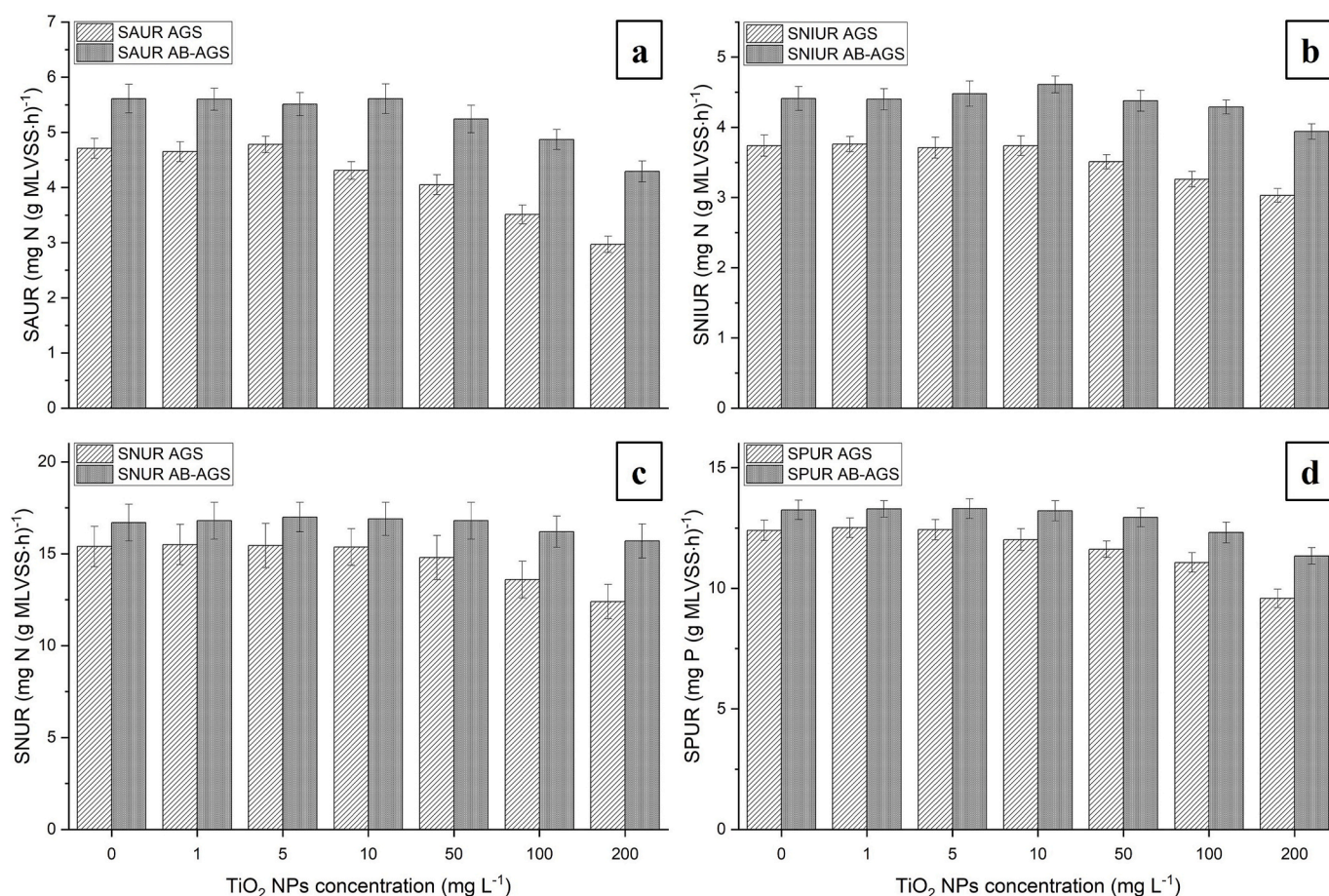


Fig. 6. Impact of titanium dioxide nanoparticles (TiO₂ NPs) on the microbial activities of aerobic granular sludge (AGS) and algal-bacterial aerobic granular sludge (AB-AGS). **a)** specific ammonia uptake rate (SAUR); **b)** specific nitrite uptake rate (SNIUR); **c)** specific nitrate uptake rate (SNUR); **d)** specific phosphorus uptake rate (SPUR).

substances (EPSs), and the microbial activity in aerobic granular sludge (AGS) and algal-bacterial granular sludge (AB-AGS) bioreactors. The fluctuations in nutrient removal mirrored those in microbial activities across varying TiO₂ NP concentrations. Low nanoparticle concentrations (≤ 10 mg L⁻¹) had no discernible impact on the bioreactors, whereas COD, nitrogen, and PO₄³⁻ removal decreased at concentrations ≥ 50 mg L⁻¹. At the highest titanium content, PO₄³⁻ removal efficiency declined from 97.3 % to 81.8 %, and from 98.2 % to 88.2 % in AGS and AB-AGS, respectively. The reduction in specific phosphorus uptake rate (SPUR) was less pronounced in algal-bacterial sludge, resulting in lower phosphorus levels in the effluent. The enhanced tolerance of AB-AGS to TiO₂ NPs may be attributed to a higher supply of LB-EPS and TB-EPS (especially lower drop in LB-EPS) and a higher protein-to-polysaccharide (PN/PS) ratio. These findings provide valuable insights into understanding the shock-load impact of TiO₂ NPs on the performance of both aerobic granular and algal-bacterial granular sludges.

CRediT authorship contribution statement

Alfonz Kedves: Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Çağdaş Yavuz:** Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation. **Orsolya Kedves:** Visualization, Software, Methodology, Investigation, Formal analysis. **Henrik Haspel:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Formal analysis. **Zoltán Kónya:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition,

Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgment

O.K. acknowledges financial support of the New National Excellence Program of the Ministry for Culture and Innovation from the source of the National Research, Development and Innovation Fund (ÚNKP-23-4-SZTE-556).

H.H. acknowledges financial support of the János Bolyai Research Grant of the Hungarian Academy of Sciences (BO/682/22) and the New National Excellence Program of the Ministry for Culture and Innovation from NKFI Fund (ÚNKP-22-5-SZTE-574). Project no. RRF-2.3.1-21-2022-00009 (National Laboratory for Renewable Energy) has been implemented with the support provided by the Recovery and Resilience Facility of the European Union within the framework of Programme Széchenyi Plan Plus. This project has received funding from the HUNREN Hungarian Research Network.

Z.K. is grateful for K 21 138714 and SNN_135918 project from the

source of the National Research, Development and Innovation Fund. TKP2021-NVA-19 under the TKP2021-NVA funding scheme of the Ministry for Innovation and Technology are acknowledged.

References

- Ab Halim, M.H., Nor Anuar, A., Abdul Jamal, N.S., Azmi, S.I., Ujang, Z., Bob, M.M., 2016. Influence of high temperature on the performance of aerobic granular sludge in biological treatment of wastewater. *J. Environ. Manag.* 184, 271–280. <https://doi.org/10.1016/j.jenvman.2016.09.079>.
- Abeyesiriwardana-Arachchige, I.S.A., Munasinghe-Arachchige, S.P., Delanka-Pedige, H. M.K., Nirmalakhandan, N., 2020. Removal and recovery of nutrients from municipal sewage: algal vs. conventional approaches. *Water Res.* 175, 115709. <https://doi.org/10.1016/j.watres.2020.115709>.
- Adav, S.S., Lee, D.J., Show, K.Y., Tay, J.H., 2008. Aerobic granular sludge: Recent advances. *Biotechnol. Adv.* 26, 411–423. <https://doi.org/10.1016/j.biotechadv.2008.05.002>.
- Aruoja, V., Dubourgier, H.C., Kasemets, K., Kahru, A., 2009. Toxicity of nanoparticles of CuO, ZnO and TiO₂ to microalgae *Pseudokirchneriella subcapitata*. *Sci. Total Environ.* 407, 1461–1468. <https://doi.org/10.1016/j.scitotenv.2008.10.053>.
- Cervantes-Avilés, P., Keller, A.A., 2021. Incidence of metal-based nanoparticles in the conventional wastewater treatment process. *Water Res.* 189, 116603. <https://doi.org/10.1016/j.watres.2020.116603>.
- Cervantes-Avilés, P., Caretta, C.A., Brito, E.M.S., Bertin, P., Cuevas-Rodríguez, G., Duran, R., 2021. Changes in bacterial diversity of activated sludge exposed to titanium dioxide nanoparticles. *Biodegradation* 32, 313–326. <https://doi.org/10.1007/s10532-021-09939-w>.
- Cervantes-Avilés, P., Saber, A.N., Mora, A., Mahlknecht, J., Cuevas-Rodríguez, G., 2022. Influence of wastewater type in the effects caused by titanium dioxide nanoparticles in the removal of macronutrients by activated sludge. *Environ. Sci. Pollut. Res.* 29, 8746–8757. <https://doi.org/10.1007/s11356-021-16221-2>.
- Clesceri, L.S., Greenberg, A.E., Trussell, R.R., 1990. *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, Washington DC.
- Di Trapani, D., Capodici, M., Cosenza, A., Di Bella, G., Mannina, G., Torregrossa, M., Viviani, G., 2011. Evaluation of biomass activity and wastewater characterization in a UCT-MBR pilot plant by means of respirometric techniques. *Desalination* 269, 190–197. <https://doi.org/10.1016/j.desal.2010.10.061>.
- Dréno, B., Alexis, A., Chuberre, B., Marinovich, M., 2019. Safety of titanium dioxide nanoparticles in cosmetics. *J. Eur. Acad. Dermatol. Venereol.* 33, 34–46. <https://doi.org/10.1111/jdv.15943>.
- Eddy, D.R., Ishmah, S.N., Permana, M.D., Firdaus, M.L., Rahayu, I., El-Badry, Y.A., Hussein, E.E., El-Bahy, Z.M., 2021. Photocatalytic phenol degradation by silica-modified titanium dioxide. *Appl. Sci.* 11. <https://doi.org/10.3390/app11199033>.
- Erdural, B., Bolukbasi, U., Karakas, G., 2014. Photocatalytic antibacterial activity of TiO₂-SiO₂ thin films: the effect of composition on cell adhesion and antibacterial activity. *J. Photochem. Photobiol. A Chem.* 283, 29–37. <https://doi.org/10.1016/j.jphotochem.2014.03.016>.
- Frølund, B., Palmgren, R., Keiding, K., Nielsen, P.H., 1996. Extraction of extracellular polymers from activated sludge using a cation exchange resin. *Water Res.* 30, 1749–1758. [https://doi.org/10.1016/0043-1354\(95\)00323-1](https://doi.org/10.1016/0043-1354(95)00323-1).
- Gottschalk, F., Sonderer, T., Scholz, R.W., Nowack, B., 2009. Modeled environmental concentrations of engineered nanomaterials (TiO₂, ZnO, ag, CNT, fullerenes) for different regions. *Environ. Sci. Technol.* 43, 9216–9222. <https://doi.org/10.1021/es901553>.
- Guo, D., Zhang, X., Shi, Y., Cui, B., Fan, J., Ji, B., Yuan, J., 2021. Microalgal-bacterial granular sludge process outperformed aerobic granular sludge process in municipal wastewater treatment with less carbon dioxide emissions. *Environ. Sci. Pollut. Res.* 28, 13616–13623. <https://doi.org/10.1007/s11356-020-11565-7>.
- Hakim, M.A., Sevillano, K., Ewerts, H., van de Vossenberg, J., van der Steen, N.P., 2023. Development of microalgal-bacterial aerobic granules for ammonium removal from wastewater in a photo sequencing batch reactor. *Mater. Today Proc.* 77, 209–216. <https://doi.org/10.1016/j.matpr.2022.11.263>.
- He, Q., Yuan, Z., Zhang, J., Zhang, S., Zhang, W., Zou, Z., Wang, H., 2017a. Insight into the impact of ZnO nanoparticles on aerobic granular sludge under shock loading. *Chemosphere* 173, 411–416. <https://doi.org/10.1016/j.chemosphere.2017.01.085>.
- He, Q., Gao, S., Zhang, S., Zhang, W., Wang, H., 2017b. Chronic responses of aerobic granules to zinc oxide nanoparticles in a sequencing batch reactor performing simultaneous nitrification, denitrification and phosphorus removal. *Bioresour. Technol.* 238, 95–101. <https://doi.org/10.1016/j.biortech.2017.04.010>.
- Ismael, M., 2020. A review and recent advances in solar-to-hydrogen energy conversion based on photocatalytic water splitting over doped-TiO₂ nanoparticles. *Sol. Energy* 211, 522–546. <https://doi.org/10.1016/j.solener.2020.09.073>.
- Ji, B., Zhang, M., Gu, J., Ma, Y., Liu, Y., 2020a. A self-sustaining synergetic microalgal-bacterial granular sludge process towards energy-efficient and environmentally sustainable municipal wastewater treatment. *Water Res.* 179, 115884. <https://doi.org/10.1016/j.watres.2020.115884>.
- Ji, B., Zhang, M., Wang, L., Wang, S., Liu, Y., 2020b. Removal mechanisms of phosphorus in non-aerated microalgal-bacterial granular sludge process. *Bioresour. Technol.* 312, 123531. <https://doi.org/10.1016/j.biortech.2020.123531>.
- Jiang, Y., Shang, Y., Zhang, W., Zhang, X., Li, J., Shao, S., 2022. Assessing the effect of SiO₂ and TiO₂ nanoparticles on granule stability and microbial community shift in aerobic granular sludge process. *Chemosphere* 307, 135677. <https://doi.org/10.1016/j.chemosphere.2022.135677>.
- John, A.K., Palaty, S., Sharma, S.S., 2020. Greener approach towards the synthesis of titanium dioxide nanostructures with exposed {001} facets for enhanced visible light photodegradation of organic pollutants. *J. Mater. Sci. Mater. Electron.* 31, 20868–20882. <https://doi.org/10.1007/s10854-020-04602-1>.
- Kedves, A., Sánta, L., Balázs, M., Kesserű, P., Kiss, I., Rónavári, A., Kónya, Z., 2020. Chronic responses of aerobic granules to the presence of graphene oxide in sequencing batch reactors. *J. Hazard. Mater.* 389. <https://doi.org/10.1016/j.jhazmat.2019.121905>.
- Kedves, A., Rónavári, A., Kónya, Z., 2021. Long-term effect of graphene oxide on the aerobic granular sludge wastewater treatment process. *J. Environ. Chem. Eng.* 9, 1–7. <https://doi.org/10.1016/j.jece.2020.104853>.
- Larue, C., Castillo-Michel, H., Sobanska, S., Trcera, N., Sorieul, S., Cécillon, L., Ouerdane, L., Legros, S., Sarret, G., 2014. Fate of pristine TiO₂ nanoparticles and aged paint-containing TiO₂ nanoparticles in lettuce crop after foliar exposure. *J. Hazard. Mater.* 273, 17–26. <https://doi.org/10.1016/j.jhazmat.2014.03.014>.
- Li, F., Liang, Z., Zheng, X., Zhao, W., Wu, M., Wang, Z., 2015a. Toxicity of nano-TiO₂ on algae and the site of reactive oxygen species production. *Aquat. Toxicol.* 158, 1–13. <https://doi.org/10.1016/j.aquatox.2014.10.014>.
- Li, B., Huang, W., Zhang, C., Feng, S., Zhang, Z., Lei, Z., Sugiyama, N., 2015b. Effect of TiO₂ nanoparticles on aerobic granulation of algal-bacterial symbiosis system and nutrients removal from synthetic wastewater. *Bioresour. Technol.* 187, 214–220. <https://doi.org/10.1016/j.biortech.2015.03.118>.
- Li, Z.Q., Que, Y.P., Mo, L.E., Chen, W.C., Ding, Y., Ma, Y.M., Jiang, L., Hu, L.H., Dai, S.Y., 2015c. One-pot synthesis of mesoporous TiO₂ micropheres and its application for high-efficiency dye-sensitized solar cells. *ACS Appl. Mater. Interfaces* 7, 10928–10934. <https://doi.org/10.1021/acsami.5b02195>.
- Li, W.-W., Zhang, H.-L., Sheng, G.-P., Yu, H.-Q., 2015d. Roles of extracellular polymeric substances in enhanced biological phosphorus removal process. *Water Res.* 86, 85–95. <https://doi.org/10.1016/j.watres.2015.06.034>.
- Li, D., Li, B., Wang, Q., Hou, N., Li, C., Cheng, X., 2016. Toxicity of TiO₂ nanoparticle to denitrifying strain CFY1 and the impact on microbial community structures in activated sludge. *Chemosphere* 144, 1334–1341. <https://doi.org/10.1016/j.chemosphere.2015.10.002>.
- Li, Z., Wang, X., Ma, B., Wang, S., Zheng, D., She, Z., Guo, L., Zhao, Y., Xu, Q., Jin, C., Li, S., Gao, M., 2017. Long-term impacts of titanium dioxide nanoparticles (TiO₂ NPs) on performance and microbial community of activated sludge. *Bioresour. Technol.* 238, 361–368. <https://doi.org/10.1016/j.biortech.2017.04.069>.
- Li, C., Liu, S., Ma, T., Zheng, M., Ni, J., 2019a. Simultaneous nitrification, denitrification and phosphorus removal in a sequencing batch reactor (SBR) under low temperature. *Chemosphere* 229, 132–141. <https://doi.org/10.1016/j.chemosphere.2019.04.185>.
- Li, K., Qian, J., Wang, P., Wang, C., Fan, X., Lu, B., Tian, X., Jin, W., He, X., Guo, W., 2019b. Toxicity of three crystalline TiO₂ nanoparticles in activated sludge: bacterial cell death modes differentially weaken sludge dewaterability. *Environ. Sci. Technol.* 53, 4542–4555. <https://doi.org/10.1021/acs.est.8b04991>.
- Li, Z., Wang, J., Chen, X., Lei, Z., Yuan, T., Shimizu, K., Zhang, Z., Lee, D.-J., 2022. Insight into aerobic phosphorus removal from wastewater in algal-bacterial aerobic granular sludge system. *Bioresour. Technol.* 352, 127104. <https://doi.org/10.1016/j.biortech.2022.127104>.
- Li, Y.Q., Zhao, B.H., Chen, X.T., Zhang, Y.Q., Yang, H.S., 2023. Co-existence effect of copper oxide nanoparticles and ciprofloxacin on simultaneous nitrification, endogenous denitrification, and phosphorus removal by aerobic granular sludge. *Chemosphere* 312. <https://doi.org/10.1016/j.chemosphere.2022.137254>.
- Liu, W., Zhang, J., Jin, Y., Zhao, X., Cai, Z., 2015. Adsorption of Pb(II), Cd(II) and Zn(II) by extracellular polymeric substances extracted from aerobic granular sludge: efficiency of protein. *J. Environ. Chem. Eng.* 3, 1223–1232. <https://doi.org/10.1016/j.jece.2015.04.009>.
- Liu, W., Huang, W., Cao, Z., Ji, Y., Liu, D., Huang, W., Zhu, Y., Lei, Z., 2022. Microalgae simultaneously promote antibiotic removal and antibiotic resistance genes/bacteria attenuation in algal-bacterial granular sludge system. *J. Hazard. Mater.* 438, 129286. <https://doi.org/10.1016/j.jhazmat.2022.129286>.
- Mohammadparast, V., Mallard, B.L., 2023. The effect and underlying mechanisms of titanium dioxide nanoparticles on glucose homeostasis: a literature review. *J. Appl. Toxicol.* 43, 22–31. <https://doi.org/10.1002/jat.4318>.
- Mu, H., Zheng, X., Chen, Y., Chen, H., Liu, K., 2012. Response of anaerobic granular sludge to a shock load of zinc oxide nanoparticles during biological wastewater treatment. *Environ. Sci. Technol.* 46, 5997–6003. <https://doi.org/10.1021/es300616a>.
- Nancharaiyah, Y.V., Reddy, G. Kiran Kumar, 2018. Aerobic granular sludge technology: mechanisms of granulation and biotechnological applications. *Bioresour. Technol.* 247, 1128–1143. <https://doi.org/10.1016/j.biortech.2017.09.131>.
- Periasamy, V.S., Athinayanan, J., Al-Hadi, A.M., Al Juhaimi, F., Alshatwi, A.A., 2015. Effects of titanium dioxide nanoparticles isolated from confectionery products on the metabolic stress pathway in human lung fibroblast cells. *Arch. Environ. Contam. Toxicol.* 68, 521–533. <https://doi.org/10.1007/s00244-014-0109-4>.
- Prokopiuk, V., Yefimova, S., Onishchenko, A., Kapustinik, V., Myasoedov, V., Maksimchuk, P., Butov, D., Bespalova, I., Tkachenko, A., 2023. Assessing the cytotoxicity of TiO₂-x nanoparticles with a different Ti3+/Ti2+/Ti4+ ratio. *Biol. Trace Elem. Res.* 201, 3117–3130. <https://doi.org/10.1007/s12011-022-03403-3>.
- Purba, L.D.A., Ibiyeye, H.T., Yuzir, A., Mohamad, S.E., Iwamoto, K., Zamyadi, A., Abdullah, N., 2020. Various applications of aerobic granular sludge: a review. *Environ. Technol. Innov.* 20, 101045. <https://doi.org/10.1016/j.eti.2020.101045>.
- Sanchez-Sanchez, C., Baquerizo, G., Moreno-Rodríguez, E., 2023. Analysing the influence of operating conditions on the performance of algal-bacterial granular sludge processes for wastewater treatment: a review. *Water Environ. J.* 37, 657–670. <https://doi.org/10.1111/wej.12873>.

- Schaedig, E., Cantrell, M., Urban, C., Zhao, X., Greene, D., Dancer, J., Gross, M., Sebesta, J., Chou, K.J., Grabowy, J., Gross, M., Kumar, K., Yu, J., 2023. Isolation of phosphorus-hyperaccumulating microalgae from revolving algal biofilm (RAB) wastewater treatment systems. *Front. Microbiol.* 14. <https://doi.org/10.3389/fmicb.2023.1219318>.
- Szabó, E., Hermansson, M., Modin, O., Persson, F., Wilén, B.M., 2016. Effects of wash-out dynamics on nitrifying bacteria in aerobic granular sludge during start-up at gradually decreased settling time. *Water (Switzerland)*. 8. <https://doi.org/10.3390/w8050172>.
- Wang, S., Gao, M., Wang, Z., She, Z., Jin, C., Zhao, Y., Guo, L., Chang, Q., 2015a. Effect of oxytetracycline on performance and microbial community of an anoxic-aerobic sequencing batch reactor treating mariculture wastewater. *RSC Adv.* 5, 53893–53904. <https://doi.org/10.1039/C5RA06302G>.
- Wang, Y., Jiang, X., Wang, H., Guo, G., Guo, J., Qin, J., Zhou, S., 2015b. Comparison of performance, microorganism populations, and bio-physiochemical properties of granular and flocculent sludge from denitrifying phosphorus removal reactors. *Chem. Eng. J.* 262, 49–58. <https://doi.org/10.1016/j.cej.2014.09.065>.
- Wang, S., Gao, M., She, Z., Zheng, D., Jin, C., Guo, L., Zhao, Y., Li, Z., Wang, X., 2016. Long-term effects of ZnO nanoparticles on nitrogen and phosphorus removal, microbial activity and microbial community of a sequencing batch reactor. *Bioresour. Technol.* 216, 428–436. <https://doi.org/10.1016/j.biortech.2016.05.099>.
- Wang, S., Liu, Z., Wang, W., You, H., 2017. Fate and transformation of nanoparticles (NPs) in municipal wastewater treatment systems and effects of NPs on the biological treatment of wastewater: a review. *RSC Adv.* 7, 37065–37075. <https://doi.org/10.1039/c7ra05690g>.
- Waqas, S., Bilal, M.R., Man, Z., Wibisono, Y., Jaafar, J., Indra Mahlia, T.M., Khan, A.L., Aslam, M., 2020. Recent progress in integrated fixed-film activated sludge process for wastewater treatment: a review. *J. Environ. Manag.* 268, 110718. <https://doi.org/10.1016/j.jenvman.2020.110718>.
- Wu, Q., Li, S., Wang, H., Wang, W., Gao, X., Guan, X., Zhang, Z., Teng, Y., Zhu, L., 2023. Construction of an efficient microalgal-fungal co-cultivation system for swine wastewater treatment: nutrients removal and extracellular polymeric substances (EPS)-mediated aggregated structure formation. *Chem. Eng. J.* 476, 146690. <https://doi.org/10.1016/j.cej.2023.146690>.
- Xia, J., Ye, L., Ren, H., Zhang, X.-X., 2018. Microbial community structure and function in aerobic granular sludge. *Appl. Microbiol. Biotechnol.* 102, 3967–3979. <https://doi.org/10.1007/s00253-018-8905-9>.
- Xiao, M., Xin, J., Fan, J., Ji, B., 2022. Response mechanisms of microalgal-bacterial granular sludge to zinc oxide nanoparticles. *Bioresour. Technol.* 361, 127713. <https://doi.org/10.1016/j.biortech.2022.127713>.
- Xiao, X., Li, W., Jin, M., Zhang, L., Qin, L., Geng, W., 2023. Responses and tolerance mechanisms of microalgae to heavy metal stress: a review. *Mar. Environ. Res.* 183, 105805. <https://doi.org/10.1016/j.marenvres.2022.105805>.
- Xuan, W., Bin, Z., Zhiqiang, S., Zhigang, Q., Zhaoli, C., Min, J., Junwen, L., Jingfeng, W., 2010. The EPS characteristics of sludge in an aerobic granule membrane bioreactor. *Bioresour. Technol.* 101, 8046–8050. <https://doi.org/10.1016/j.biortech.2010.05.074>.
- Yuan, L., Li, Y., Zeng, T., Wang, D., Liu, X., Xu, Q., Yang, Q., Yang, F., Chen, H., 2021. Revealing how the entering nano-titanium dioxide in wastewater worsened sludge dewaterability. *Chem. Eng. J.* 411, 128465. <https://doi.org/10.1016/j.cej.2021.128465>.
- Zhang, B., Lens, P.N.L., Shi, W., Zhang, R., Zhang, Z., Guo, Y., Bao, X., Cui, F., 2018. Enhancement of aerobic granulation and nutrient removal by an algal-bacterial consortium in a lab-scale photobioreactor. *Chem. Eng. J.* 334, 2373–2382. <https://doi.org/10.1016/j.cej.2017.11.151>.
- Zhao, X., Chen, Z., Wang, X., Li, J., Shen, J., Xu, H., 2015. Remediation of pharmaceuticals and personal care products using an aerobic granular sludge sequencing bioreactor and microbial community profiling using Solexa sequencing technology analysis. *Bioresour. Technol.* 179, 104–112. <https://doi.org/10.1016/j.biortech.2014.12.002>.
- Zheng, X., Chen, Y., Wu, R., 2011. Long-term effects of titanium dioxide nanoparticles on nitrogen and phosphorus removal from wastewater and bacterial community shift in activated sludge. *Environ. Sci. Technol.* 45, 7284–7290. <https://doi.org/10.1021/es2008598>.
- Zheng, X., Zhang, Y., Chen, W., Wang, W., Xu, H., Shao, X., Yang, M., Xu, Z., Zhu, L., 2019. Effect of increased influent COD on relieving the toxicity of CeO₂ NPs on aerobic granular sludge. *Int. J. Environ. Res. Public Health* 16. <https://doi.org/10.3390/ijerph16193609>.
- Zhou, L., Zhuang, W.Q., De Costa, Y., Xia, S., 2019. Potential effects of suspended TiO₂ nanoparticles on activated sludge floc properties in membrane bioreactors. *Chemosphere* 223, 148–156. <https://doi.org/10.1016/j.chemosphere.2019.02.042>.
- Zhou, Y.Y.Y., Zhou, Y.Y.Y., Chen, S., Guo, N., Xiang, P., Lin, S., Bai, Y., Hu, X., Zhang, Z., 2022. Evaluating the role of algae in algal-bacterial granular sludge: nutrient removal, microbial community and granular characteristics. *Bioresour. Technol.* 365, 128165. <https://doi.org/10.1016/j.biortech.2022.128165>.