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Rozprawa doktorska

Mikroplastik w systemach oczyszczania ścieków – efektywność usuwania i wpływ na przemiany biologiczne

Microplastic in wastewater treatment systems – removal efficiency and effect on biological processes

Praca wykonana w Katedrze Biotechnologii w Ochronie Środowiska

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[P2] Jachimowicz, P., Peng, R., Hüffer, T., Hofmann, T., Cydzik-Kwiatkowska, A. (2024). Tire materials disturb transformations of nitrogen compounds and affect the structure of biomass in aerobic granular sludge reactors. Journal of Hazardous Materials, 133223. **($MNiSW_{2024}$ = 200 pkt, IF = 12,2)**

[P3] Jachimowicz, P., Mądzielewska, W., Cydzik-Kwiatkowska, A. (2024). Microplastics in granular sequencing batch reactors: impacts on pollutant removal dynamics and microbial community. Journal of Hazardous Materials, 476, 135061. **($MNiSW_{2024}$ = 200 pkt, IF = 12,2)**

[P4] Jachimowicz, P., Nosek, D., Cydzik-Kwiatkowska, A. (2022). Chemical and microbiological changes on the surface of microplastic after long term exposition to different concentrations of ammonium in the environment. Science of the Total Environment, 830, 154784. **($MEiN_{2022}$ = 200 pkt, IF = 10,94)**

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Spis treści

Wykaz skrótów

2-A-BTH – 2-aminobenzotiazol

2-Me-S-BTH – 2-merkaptobenzotiazol

2-OH-BTH – 2-hydroksybenzotiazol

5-Me-BTH – 5-metylo-1H-benzotriazol

6PPD – N-(1,3-dimetylobutylo)-N'-fenylo-1,4-fenylenodiamina

6PPD-Q – N-(1,3-dimetylobutylo)-N'-fenylo-1,4-benzochinonodiimina

AGS – tlenowy osad granulowany

BPA – bisfenol A

BTH – benzotiazol

ChZT – chemiczne zapotrzebowanie na tlen

rChZT – chemiczne zapotrzebowanie na tlen po sączeniu próbki

DPG – 1,3-difenyloguanidyna

DTG – 1,3-di-o-toliloguanidyna

EPS – polimery zewnątrzkomórkowe

FCT – Flocculant F 290

FPM – Flopam EM 840

GSBR – reaktor sekwencyjny z tlenowym osadem granulowanym

HMMM – heksametoksymetylomelamina

LC/MS – chromatografia cieczowa połączona z tandemową spektrometrią mas

MP – mikroplastik

$N\text{-}NH_4$ – azot amonowy

$N\text{-}NO_2$ – azot azotanowy(III)

$N\text{-}NO_3$ – azot azotanowy(V)

OTU – operacyjna jednostka taksonomiczna

PA – poliamid

PAX – wodny roztwór chlorku poliglinu

PAO – organizmy gromadzące fosfor

PE – polietylen

PEL – Praestrol k 255 l

PET – politereftalan etylenu

PIX – wodny roztwór siarczanu żelaza(III)

PP – polypropylen

$P\text{-}PO_4$ – ortofosforany

PS – polystyren

PVC – poli(chlorek winylu)

RLM – równoważna liczba mieszkańców

s.m. – sucha masa

TM – mikroplastik z opon

TN – azot ogólny

UE – Unia Europejska

Streszczenie

Zanieczyszczenie mikroplastikiem (MP) stało się globalnym problemem środowiskowym, wywierającym negatywny wpływ na ekosystemy wodne i zdrowie publiczne. Celem rozprawy doktorskiej było określenie możliwości usuwania MP w systemach oczyszczania ścieków oraz jego wpływu na procesy biologiczne. Badania zostały podzielone na trzy etapy. W pierwszym etapie zbadano możliwości usuwania MP w osadnikach wstępnych. Badania wykazały, że stosowanie koagulantów i flokulantów może znacząco zwiększyć skuteczność usuwania MP. Zastosowanie FPM w dawce 25 µg/l umożliwiło usunięcie 96% MP o średnicy 80–212 µm. MP o wielkości 710–850 µm został całkowicie usunięty ze ścieków po użyciu PIX (38%) w dawce 95 µl/l, PEL w dawce 6,25 µg/l oraz PAX (36%) w dawce 90 µl/l i 180 µl/l. Zastosowanie koagulantów oraz flokulantów wpływało na wartości wskaźników zanieczyszczeń w ściekach oczyszczonych. W drugim etapie badań określono wpływ MP w ściekach dopływających na efektywność usuwania zanieczyszczeń oraz mikrobiom w GSBR. Rodzaj MP wpływał na akumulację MP w biomasie (TM>PET>PE) oraz przemiany związków organicznych, azotu i fosforu. Obecność TM wpływała na efektywność i kinetykę usunięcia $N\text{-}NH_4$ oraz szybkości usuwania/produkcji $N\text{-}NO_2$ i $N\text{-}NO_3$. Obecność PET w ściekach obniżała efektywność usuwania ChZT oraz $N\text{-}NH_4$, natomiast zwiększała efektywność usunięcia $P\text{-}PO_4$. Dozowanie PE wpływało na kinetykę przemian ChZT, $N\text{-}NH_4$, $N\text{-}NO_2$, $N\text{-}NO_3$ oraz $P\text{-}PO_4$ i obniżało stężenie $N\text{-}NO_2$ w ściekach oczyszczonych. Stwierdzono, że AGS degradował ponad 60% związków wymywanych z TM. Dawka TM w ściekach była skorelowana ze wzrostem udziału w biomasie mikroorganizmów z rodzajów *Xanthomonas*, *Ferruginibacter*, *Luteimonas*, *Hydrogenophaga*, *Devosia*, *Geobacter*, *Thiobacillus*, *Rubrivivax*, *Gemmatimonas* i *Acidovorax*, które są zdolne do degradacji mikrozanieczyszczeń. Dodatkowo adnotacja genomu przy wykorzystaniu bazy KEGG wskazała na zwiększenie potencjału metabolicznego zbiorowiska związanego z naprawą lub syntezą materiału genetycznego oraz biodegradacją wraz ze wzrostem dawki TM w ściekach. Dozowanie ze ściekami PET powodowało wzrost liczebności mikroorganizmów z rodzajów *Bacteroides*, *Singulisphaera*, *Gemmata*, *Ectothiorhodospira*, *Devosia, Dechloromonas*, *Woodsholea* i *Microcystis* w granulach tlenowych. W GSBR, do którego dozowano PE, obserwowano zwiększenie liczebności w biomasie mikroorganizmów z rodzajów *Mycobacterium*, *Oscillochloris*, *Fusobacterium*, *Lactobacillus*, *Stenotrophomonas*, *Bacteroides* i *Microcystis*. PET oraz PE w badanych dawkach nie wpływały na aktywność mikroorganizmów badaną w oparciu o liczebność transkryptów genów *16S r*DNA*, amoA, nirK* oraz *nirS* w biomasie,

natomiast obecność PET zwiększała liczebność transkryptów genów *nosZ* oraz *ppk1* w biomasie w cyklu GSBR. W trzecim etapie określono wpływ stężenia związków azotu w środowisku na charakterystykę powierzchni MP oraz zbiorowiska mikrobiologiczne w błonie zasiedlającej MP. Analiza morfologiczna wykazała zwiększenie chropowatości i powstawanie pęknięć na powierzchni MP w miarę trwania eksperymentu; spadek masy MP podczas eksperymentu wyniósł około 7%. Stężenie $N\text{-}NH_4$ w środowisku było pozytywnie skorelowane z sorpcją TN na powierzchni MP. W błonie biologicznej wykazano obecność mikroorganizmów o potencjale kolonizacji i degradacji tworzyw sztucznych (rodzaje *Acidovorax, Aneurinibacillus, Aquabacterium, Bacillus, Brevibacillus, Enterobacter, Gordonia, Hydrogenophaga, Ideonella, Pseudomonas, Rhodococcus, Rhodopseudomonas, Sphingomonas, Sphingopyxis, Streptomyces* oraz *Thermoactinomyce*). Choć zaobserwowano dużą liczbę rodzajów bakterii potencjalnie degradujących MP, ich względny udział procentowy w biomasie był niski. Na powierzchni MP rozwijały się mikroorganizmy potencjalnie patogenne z rodzajów *Enterobacter*, *Pseudomonas*, *Mycobacterium*, *Sphingopyxis*, *Aquabacterium* i *Brevundimonas*. Przeprowadzone badania poszerzają wiedzę dotyczącą wpływu MP na mikrobiom i efektywność oczyszczania ścieków i mogą służyć jako podstawa opracowania skutecznych strategii eliminacji MP ze środowiska wodnego.

Słowa kluczowe: mikroplastik, tlenowy osad granulowany, zbiorowiska mikrobiologiczne

Abstract

Microplastic (MP) pollution has become a global environmental issue, negatively impacting aquatic ecosystems and public health. The doctoral thesis aimed to determine the potential for MP removal in wastewater treatment systems and its effect on biological processes. The research was divided into three stages. The potential for MP removal in primary settling tanks was investigated in the first stage. The studies showed that using coagulants and flocculants can significantly increase the effectiveness of MP removal. The application of FPM at a dose of 25 µg/L enabled the removal of 96% of MPs with a diameter of 80–212 µm. MPs sized 710–850 µm were utterly removed from the wastewater after using PIX (38%) at a dose of 95 µl/L, PEL at a dose of 6.25 µg/L, and PAX (36%) at a dose of 90 and 189 µl/L. In the second stage of the research, the influence of MPs on pollutant removal efficiency and microbiome in GSBR was determined. The type of MP in wastewater influenced the accumulation of MPs in biomass (TM>PET>PE) and the transformation of organic compounds, nitrogen, and phosphorus. The presence of TM affected the efficiency and kinetics of $N\text{-}NH_4$ removal and the rates of $N\text{-}NO_2$ and $N\text{-}NO_3$ removal/production. The presence of PET in wastewater reduced the efficiency of COD and $N\text{-}NH_4$ removal while increasing the efficiency of $P\text{-}PO_4$ removal. The dosing of PE affected the kinetics of COD, $N\text{-}NH_4$, $N\text{-}NO_2$, $N\text{-}NO_3$, and $P\text{-}PO_4$ transformations and reduced the concentration of $N\text{-}NO_2$ in treated wastewater. It was found that AGS degraded over 60% of compounds leached from TM. The TM dose in wastewater was correlated with an increase in the proportion of microorganism biomass belonging to genera *Xanthomonas*, *Ferruginibacter*, *Luteimonas*, *Hydrogenophaga*, *Devosia*, *Geobacter*, *Thiobacillus*, *Rubrivivax*, *Gemmatimonas* and *Acidovorax* capable of degrading micropollutants. Additionally, genome annotation using the KEGG database indicated an increase in the metabolic potential of the community associated with genetic material repair or synthesis and biodegradation with increasing TM dose in wastewater. Dosing PET with wastewater increased the abundance of microorganisms from *Bacteroides*, *Singulisphaera*, *Gemmata*, *Ectothiorhodospira*, *Devosia*, *Dechloromonas*, *Woodsholea* and *Microcystis* genera in aerobic granules. In GSBR dosed with PE, the abundance of microorganisms from *Mycobacterium*, *Oscillochloris*, *Fusobacterium*, *Lactobacillus*, *Stenotrophomonas*, *Bacteroides* and *Microcystis* genera increased. PET and PE in the tested doses did not affect the activity of microorganisms based on the abundance of *16S r*DNA, *amoA*, *nirK*, and *nirS* genes in the biomass. However, the presence of PET increased the abundance of *nosZ* and *ppk1* genes in the biomass in the GSBR cycle. In the third

stage, the influence of nitrogen concentration in the environment on the surface characteristics of MPs and the microbial community in the biofilm colonizing MPs was determined. Morphological analysis showed increased roughness and the formation of cracks on the surface of MPs throughout the experiment; the decrease in MP mass during the experiment was approximately 7%. The $N\text{-}NH_4$ concentration in the environment was positively correlated with TN sorption on the MP surface. The presence of microorganisms with colonization and degradation potential for plastics (genera *Acidovorax*, *Aneurinibacillus*, *Aquabacterium*, *Bacillus*, *Brevibacillus*, *Enterobacter*, *Gordonia*, *Hydrogenophaga*, *Ideonella*, *Pseudomonas*, *Rhodococcus*, *Rhodopseudomonas*, *Sphingomonas*, *Sphingopyxis*, *Streptomyces*, and *Thermoactinomyce*) was demonstrated in the biofilm. Although many bacterial genera capable of degrading MPs were observed; their relative proportion in the biomass was low. Potentially pathogenic microorganisms such as *Enterobacter*, *Pseudomonas*, *Mycobacterium*, *Sphingopyxis*, *Aquabacterium*, and *Brevundimonas* were found on the MP surface. These studies expand knowledge regarding the impact of MPs on the microbiome and wastewater treatment efficiency. They may serve as a basis for developing effective strategies for MP elimination from the aquatic environment.

Keywords: microplastics, aerobic granular sludge, microbial communities

Wstęp

Światowa produkcja tworzyw sztucznych w 2022 roku osiągnęła poziom 400,3 miliona ton, w tym mniej niż 9% stanowiły tworzywa sztuczne pochodzące z recyklingu, a zaledwie 0,5% było tworzywami biopochodnymi. Średnia roczna stopa wzrostu produkcji w przedsiębiorstwach zajmujących się przetwórstwem gumy i tworzyw sztucznych w Polsce w latach 2011-2022 wyniosła 8,7%, co wskazuje, że tempo wzrostu w tym sektorze było wyższe niż obserwowane w innych branżach przetwórstwa przemysłowego (7,8%) (PlasticsEurope, 2023).

Wzrost produkcji niesie ze sobą wyzwania związane z ekoprojektowaniem wyrobów z tworzyw sztucznych oraz skuteczną zbiórką i zagospodarowaniem powstających odpadów (sortowanie, recykling, odzysk energii, składowanie). Niedostateczna infrastruktura do zbierania i przetwarzania odpadów, a także brak odpowiednich zachęt dla przedsiębiorców i innowacyjnych modeli biznesowych sprawiają, że potencjał odpadowych tworzyw sztucznych nie jest w pełni wykorzystany (Wagner, 2022). W rezultacie są one często niewłaściwie zagospodarowane lub składowane, co przyczynia się do zanieczyszczenia środowiska (Koelmans i in., 2022).

Terminem MP określa się tworzywa sztuczne o średnicy od 0,1 µm do 5,0 mm (Thompson i in., 2004). MP można podzielić ze względu na pochodzenie na pierwotny oraz wtórny. Pierwotny MP jest celowo używany w produktach takich jak kremy, szampony, żele do ciała i inne kosmetyki (Dąbrowska i in., 2022). Lei i in. (2017) stwierdzili, że około 7% środków do mycia twarzy zawierało MP; średnia masa MP w produkcie wyniosła 25,04 ± 10,69 mg MP/g, a średni rozmiar cząsteczek wyniósł 313 ± 130 µm. MP może występować w produktach nawet wtedy, gdy nie znajduje się na liście składników (Hernandez i in., 2017).

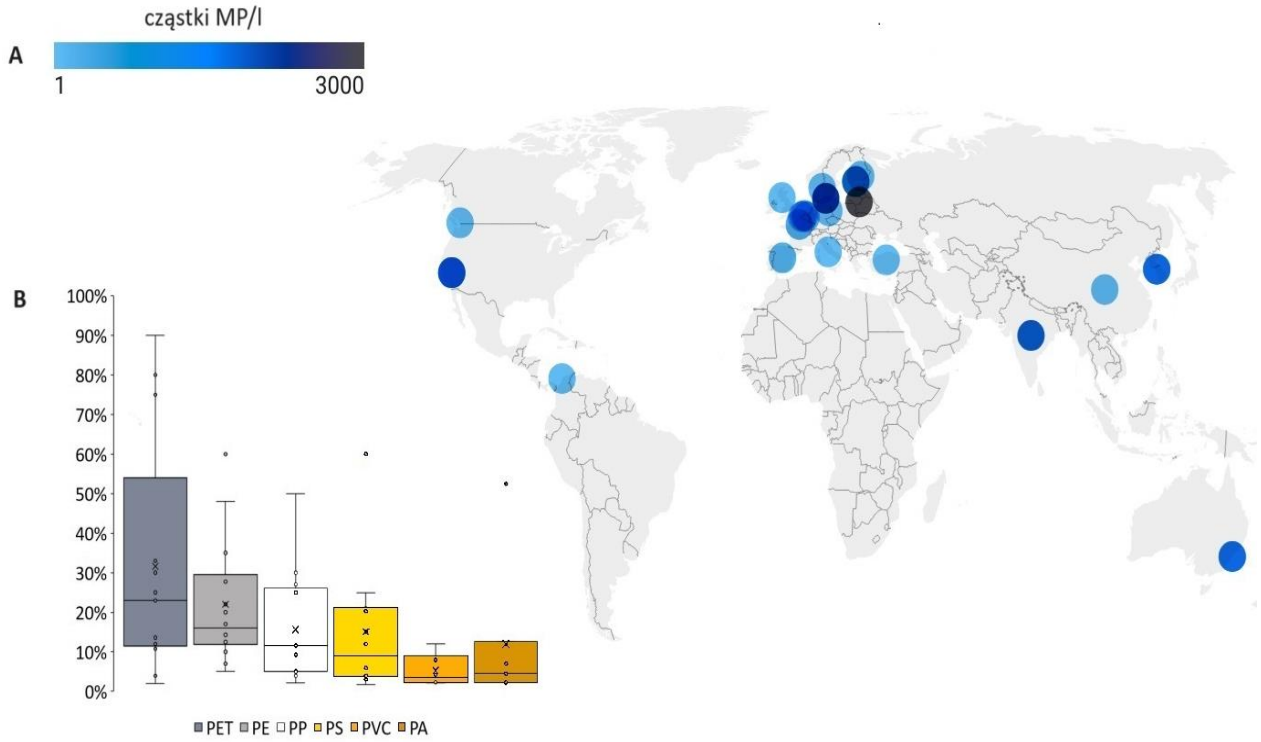
Wtórny MP to tworzywa sztuczne powstałe w wyniku biologicznej, chemicznej lub mechanicznej fragmentacji większych elementów plastikowych (Thompson, 2015). Badania przeprowadzone przez Nappera i Thompsona (2016) wykazały, że podczas prania 1 kg odzieży może być uwalnianych od 22 992 do 121 465 cząstek MP w formie włókien, które później mogą trafić do oczyszczalni ścieków. Badania Bao i in. (2022) pokazały, że w warunkach długotrwałego starzenia (90 dni), wywołanego światłem UV, zostało uwolnione 1473,27 ± 143,67 cząstek MP/g PVC. Proces degradacji tworzyw sztucznych nie kończy się na etapie MP – może prowadzić do powstawania jeszcze mniejszych cząsteczek nanoplastiku (Sorensen i Jovanović, 2021).

Obecność MP w środowisku budzi istotne obawy ze względu na potencjalnie negatywne skutki dla ekosystemów i zdrowia ludzi. MP obecne w wodzie mogą być łatwo wchłaniane przez organizmy wodne i przenoszone w łańcuchu pokarmowym (Vivekanand i in., 2021). Białowąs i in. (2022) stwierdzili występowanie tworzyw sztucznych w przewodzie pokarmowym odpowiednio 12,7% i 14,8% śledzi i dorszy z regionu południowego Bałtyku. Podczas produkcji tworzyw sztucznych stosuje się dodatki chemiczne, tj. plastyfikatory, stabilizatory termiczne, przeciwutleniacze, BPA czy barwniki, które mogą być uwalniane do środowiska wodnego (Yu i in., 2023). MP mogą działać jak nośnik zanieczyszczeń, sorbując substancje toksyczne z otoczenia (metale ciężkie, antybiotyki czy pestycydy), co zwiększa ryzyko ekspozycji organizmów żywych na te substancje (Brennecke i in., 2016; Hartmann i in., 2017). Dodatkowo powierzchnię MP mogą zasiedlać mikroorganizmy patogenne (Oberbeckmann i in., 2015). W związku z tym konieczne jest podejmowanie działań mających na celu ograniczenie emisji MP do środowiska, jak również monitorowanie jego obecności oraz wpływu na ekosystemy wodne i glebowe.

Ścieki komunalne, przemysłowe oraz wody ze spływu powierzchniowego trafiające do oczyszczalni ścieków mogą zawierać różnego rodzaju MP z produktów do pielęgnacji osobistej, chemicznych środków do prania, tekstyliów, czy ścierania opon samochodowych (Murphy i in., 2016). Wody opadowe mogą transportować od 0,3 do 179 mg TM/l (Wagner i in., 2018; Wik i Dave, 2009). Ten rodzaj MP jest niebezpieczny, gdyż stanowi złożoną mieszaninę chemiczną z dużą liczbą dodatków technologicznych. Oprócz gumy, do opon mogą być dodawane wypełniacze, środki wzmacniające, oleje, środki wulkanizacyjne, kleje oraz aktywatory – skład opon zależy od ich zastosowania. W wielu przypadkach stosuje się syntetyczne gumy na bazie ropy naftowej takie jak poliizopren, chloropren lub kauczuk styrenowo-butadienowy. Opony samochodów osobowych mogą zawierać w swojej strukturze do 85% gum syntetycznych (Wagner i in., 2018). TM może stanowić od 15 do 57% całkowitej ilości MP w środowisku (Wang i in., 2019).

Przegląd literatury wskazuje, że ścieki dopływające do oczyszczalni mogą zawierać MP w ilości 1,41–2962 MP/l (**rys. 1A**). W przypadku fińskich oczyszczalni ścieków o przepływie w zakresie 10 000–270 000 m^3/d, ze ściekami dopływało od $5{,}76 \cdot 10^8$ do $1{,}65 \cdot 10^{11}$ cząstek MP na dobę (Talvitie i in., 2017a; Talvitie i in., 2017b). Ilość MP w ściekach dopływających zależy od pory roku, typu kanalizacji, ilości ścieków, wielkości populacji, świadomości społecznej związanej z wykorzystaniem plastiku czy właściwości fizyko-chemicznych MP (Sol i in., 2020). Do najczęściej spotykanych rodzajów MP w ściekach dopływających zalicza się PET,

PE oraz PP (**rys. 1B**). Oczyszczalnie ścieków mogą być traktowane jako „hot spoty", z których MP ze ściekami oczyszczonymi lub osadami ściekowymi może się przedostawać do gleby czy rzek, a w rezultacie do mórz i oceanów (Edo i in., 2020). Badania w szkockiej oczyszczalni ścieków obsługującej populację 650 000 osób wykazały, że pomimo 98% skuteczności usuwania MP w układzie technologicznym, codziennie do środowiska uwalnianych było około 65 milionów cząstek MP (Murphy i in., 2016).



Rys. 1. Stężenie (A) oraz dominujące rodzaje (B) MP w ściekach dopływających do oczyszczalni ścieków (Akarsu i in., 2020; Bayo i in., 2020; Carr i in., 2016; Dris i in., 2015; Edo i in., 2020; Gies i in., 2018; Lee i Kim, 2018; Liu i in., 2019; Magni i in., 2019; Magnusson i Norén, 2014; Murphy i in., 2016; Patil i in., 2023; Pleskytė i in., 2023; Simon i in., 2018; Talvitie i in., 2017a; Talvitie i in., 2017b; Vercauteren i in., 2023; Ziajahromi i in., 2017)

Oczyszczalnie ścieków są głównie projektowane, by efektywnie usuwać związki organiczne, azotowe oraz fosforowe, natomiast nie są projektowane w celu usuwania takich mikrozanieczyszczeń jak MP. Osiągana na świecie efektywność usuwania MP w procesach oczyszczania ścieków waha się od około 60% do blisko 100%, w zależności od zastosowanej technologii (Ziajahromi i in., 2017; Talvitie i in., 2017a; Talvitie i in., 2017b; Pleskytė i in., 2023; Simon i in., 2018).

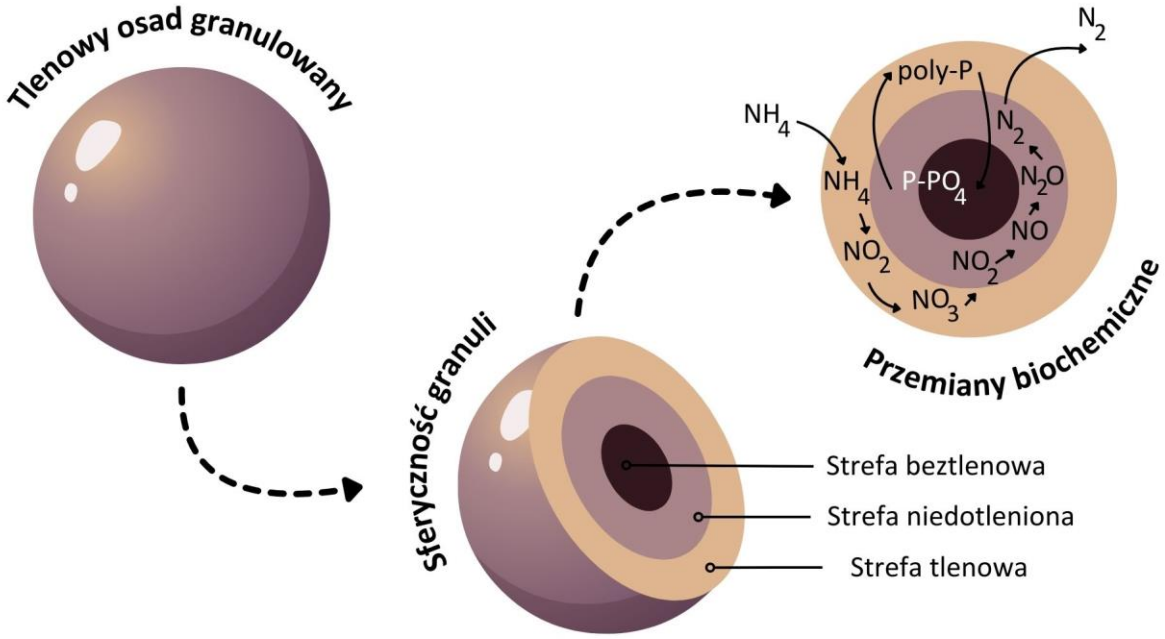
Większość oczyszczalni ścieków obejmuje oczyszczanie wstępne (mechaniczne), oczyszczanie biologiczne oraz doczyszczanie ścieków. W przypadku oczyszczania wstępnego zachodzi usuwanie zawiesin, a wraz z nimi MP (Liu i in., 2021). W osadnikach wstępnych MP jest usuwany w wyniku sedymentacji z zawiesinami łatwo opadającymi lub w wyniku flotacji z tłuszczami, które są odprowadzane wraz z osadem wstępnym (Ngo i in., 2019). Badania Lofty i in. (2022) wykazały, że w osadniku wstępnym wraz z zawiesinami jest usuwane 83% oraz około 17% MP wraz z osadem wyflotowanym. Podczas wstępnego oczyszczania ścieków było usuwane średnio od 4 do 98% MP (Liu i in., 2021).

Podczas oczyszczania biologicznego, MP jest głównie akumulowany w biomasie (Petroody i in., 2021). Badania Lee i Kim (2018) wskazują, że MP o średnicy od 106 do 300 μm lepiej sorbuje się na biomasie niż MP o średnicy >300 μm. W wyniku akumulacji w biomasie MP może być usunięty w komorze biologicznej z efektywnością od 32 do 99% (Kurt i Özdemir, 2022). MP może stanowić wektor zanieczyszczeń oraz substrat dla zbiorowisk mikrobiologicznych, które rozwijają się na jego powierzchni w formie błony biologicznej (Delacuvellerie i in., 2019). Istnieje luka badawcza dotycząca tego, w jaki sposób akumulowany w biomasie MP może oddziaływać na zbiorowiska mikrobiologiczne, a tym samym na efektywność usuwania zanieczyszczeń ze ścieków. Wykorzystanie na cele rolnicze osadów ściekowych zawierających zakumulowany MP (300–240 300 cząstek MP/kg s.m.), może powodować transmisję tego mikrozanieczyszczenia do środowiska glebowego (Cydzik-Kwiatkowska i in., 2022). Zastosowanie trzeciego stopnia oczyszczania ścieków może zmniejszyć stężenie MP o 5–20% w stosunku do poziomu usuwania osiągniętego na wcześniejszych etapach (Iyare i in., 2020).

Zidentyfikowano wiele bakterii zdolnych do degradacji tworzyw sztucznych (Ma i in., 2022; Taghavi i in., 2021; Usha i in., 2011). W badaniach Torena i in. (2021), z osadu czynnego wyizolowano mikroorganizmy degradujące PET. Badania prowadzono przez 168 dni, a do reaktora dozowano pożywkę, w której jedynym źródłem węgla był PET. Badania wykazały, że konsorcjum w którym dominował *Bacillus cereus* zdegradowało 17% masy PET. Yuan i in. (2020) wykazali, że degradacja MP zależała nie tylko od składu zbiorowiska mikroorganizmów, ale także od właściwości MP (rozmiar, kształt, występowanie grup funkcyjnych) i czynników środowiskowych (temperatura, pH, ilość związków organicznych). Poznanie ekologii mikroorganizmów zdolnych do degradacji tworzyw sztucznych jest kluczowe w ich późniejszym biotechnologicznym wykorzystaniu.

Dominującą metodą biologicznego oczyszczania ścieków jest osad czynny. Mimo swojego szerokiego zastosowania, systemy z osadem czynnym mają pewne wady, takie jak niskie stężenie biomasy w komorze napowietrzania, konieczność stosowania komór o odmiennych warunkach tlenowych w celu efektywnego usuwania biogenów, duże zapotrzebowanie na powierzchnię oraz wysokie zużycie energii (Gao i in., 2011). Obecnie jest rozwijana i wdrażana na skalę techniczną technologia AGS. Granula tlenowa to kuliste lub owalne zbiorowisko mikroorganizmów unieruchomionych w matrycy polimerów zewnątrzkomórkowych, które jest hodowane w GSBR (de Kreuk i in., 2007). W cyklu pracy GSBR występują przemiennie warunki żywienia i głodu. W okresie głodowania powierzchnia komórki bakteryjnej staje się bardziej hydrofobowa, co sprzyja adhezji i tworzeniu trójwymiarowej stabilnej struktury AGS o dużej gęstości (de Kreuk i van Loosdrecht, 2006). Technologię AGS, w porównaniu do konwencjonalnego osadu czynnego, wyróżniają niższe koszty eksploatacyjne, bardzo dobre właściwości sedymentacyjne biomasy, wysoka retencja biomasy, odporność biomasy na wysokie obciążenia ładunkiem zanieczyszczeń oraz wysoka tolerancja na obecność substancji toksycznych w ściekach (De Bruin i in., 2004; Adav i in., 2008).

Zwarta struktura AGS ogranicza dyfuzję tlenu do wnętrza granuli, co powoduje występowanie stref tlenowej, niedotlenionej oraz beztlenowej (**rys. 2**). Sprzyja to rozwojowi różnorodnych mikroorganizmów, które usuwają związki azotu, węgla i fosforu (Winkler i in., 2013). Nitryfikatory znajdują się w zewnętrznych, tlenowych warstwach AGS, podczas gdy denitryfikatory i PAO znajdują się w wewnętrznych warstwach granuli (Xia i in., 2018).



Rys. 2. Występowanie w strukturze AGS stref o zmiennych warunkach tlenowych wraz z zachodzącymi w nich wybranymi procesami

AGS zawiera większą ilość EPS niż osad czynny, co wpływa na właściwości sedymentacyjne i odwadnianie biomasy, jak również usuwanie związków toksycznych. Zwiększone wydzielanie przez bakterie zasiedlające granule tlenowe EPS bogatych w grupy funkcyjne (np. hydroksylowe, karboksylowe, sulfhydrylowe czy fosforanowe) powoduje wiązanie toksyn na powierzchnii EPS i chroni mikroorganizmy przed ich negatywnym działaniem (Melo i in., 2022).

Zdolność do zwiększonej produkcji EPS sprawia, że AGS jest bardziej odporny na substancje toksyczne i może być stosowany do oczyszczania ścieków zawierających mikrozanieczyszczenia. Badania przeprowadzone przez Cydzik-Kwiatkowską i in. (2017) wykazały, że efektywność usuwania BPA w GSBR-ach pracujących w 8-godzinnych cyklach przekroczyła 97%. Azot amonowy był efektywnie utleniany, gdy stężenie BPA w cyklu pracy spadło poniżej 2 mg/l. Zwiększenie stężenia BPA w ściekach powodowało zmniejszenie produkcji biomasy i zwiększone wydzielanie EPS, co skutkowało zwiększeniem średnicy granul. Zwiększenie średnic granul powodowało, że w AGS, obok mikroorganizmów tlenowych z rodzaju *Aquimonas* i *Pseudoxanthomonas*, pojawiły się mikroorganizmy fakultatywnie beztlenowe i beztlenowe, takie jak *Thauera* sp. i *Azoarcus* sp. Korelacje pomiędzy liczebnością *Methylobacillus* sp., *Nitrosospira* sp. i *Sphingomonas* sp. a stężeniem

BPA w ściekach wykazywały, że BPA był zarówno metabolizowany jak i bezpośrednio biodegradowany.

MP może wpływać na usuwanie zanieczyszczeń, strukturę biomasy oraz zbiorowiska mikrobiologiczne w AGS. Wyniki badań Dai i in. (2020) wykazały, że obecność MP PCV w ściekach sprzyja uwalnianiu i pobieraniu fosforu przez PAO oraz zmniejsza skuteczność usuwania azotu nieorganicznego. W obecności MP PCV zwiększyła się liczebność PAO i *Nitrospira* sp., ale zmniejszyła się liczebność bakterii denitryfikacyjnych. Badania Jachimowicz i in. (2022) wykazały, że obecność MP PE w ściekach dozowanych do GSBR stymulowała produkcję EPS i alginianu w granulach tlenowych, pogarszając właściwości sedymentacyjne biomasy i powodując jej wymywanie z reaktorów. Zrozumienie wpływu obecności MP w ściekach na mikrobiom i strukturę AGS jest kluczowe dla utrzymania efektywnego oczyszczania ścieków.

Badania przeprowadzone w ramach rozprawy doktorskiej mają istotne znaczenie nie tylko naukowe, ale także społeczne, związane z niepokojem naukowców i opinii publicznej dotyczącym obecności MP w środowisku oraz jej konsekwencjami. W dyrektywie Parlamentu Europejskiego i Rady UE z dnia 26 października 2022 r. w sprawie oczyszczania ścieków komunalnych, podkreśla się konieczność podejmowania działań w celu rozwiązania problemu mikrozanieczyszczeń, które obecnie są wykrywane w większości ścieków i wód UE. W ramach ustaleń planuje się standaryzację metodologii pomiaru MP w ściekach i osadach ściekowych oraz wprowadzenie obowiązkowego monitoringu stężenia MP w dopływie i odpływie z oczyszczalni o RLM powyżej 10 000. Wdrożenie dyrektywy będzie się wiązało z koniecznością zmian układów technologicznych oczyszczania ścieków.

W rozprawie doktorskiej skupiono się na możliwościach usuwania MP ze ścieków oraz na ocenie oddziaływania MP na procesy biologicznego oczyszczania ścieków. Badania prowadzone w dyscyplinie inżynierii środowiska, górnictwa i energetyki miały charakter interdyscyplinarny, łącząc wykorzystanie nowoczesnych narzędzi analitycznych do monitorowania mechanizmów usuwania zanieczyszczeń z badaniami składu gatunkowego oraz aktywności zbiorowisk mikroorganizmów. Takie podejście badawcze łączy elementy poznawcze i aplikacyjne w praktyce inżynierskiej.

Cel i zakres badań

Celem badań było określenie efektywności usuwania MP ze ścieków przy zastosowaniu flokulantów/koagulantów, oraz wpływu rodzaju i dawki MP w ściekach na efektywność usuwania MP i produktów jego degradacji, procesy biologicznego oczyszczania ścieków i mikrobiom w reaktorach z tlenowym osadem granulowanym. Dodatkowo badano wpływ stężenia związków azotowych w środowisku na rozwój mikroorganizmów zdolnych do kolonizacji powierzchni MP.

Zakres badań obejmował:

- określenie wpływu dawki dostępnych na rynku flokulantów (Flopam EM 840, Praestrol k 255 l, Flocculant F 290) i koagulantów (PIX, PAX) na usuwanie MP ze ścieków (Etap I, **P1**),

- określenie efektywności usuwania TM i produktów jego biodegradacji przez AGS (Etap II, **P2**),

- określenie wpływu stężenia i rodzaju MP (PET, PE oraz TM) w ściekach dopływających na efektywność oczyszczania ścieków oraz mikrobiom w reaktorach z AGS (Etap II, **P2, P3**),

- ocenę wpływu stężenia azotu amonowego w środowisku na rozwój mikroorganizmów zasiedlających powierzchnię MP oraz identyfikację zagrożeń związanych z MP jako wektorem zanieczyszczeń i mikroorganizmów patogennych (Etap III, **P4**).

Hipotezy badawcze

W ramach rozprawy doktorskiej weryfikowano następujące hipotezy badawcze:

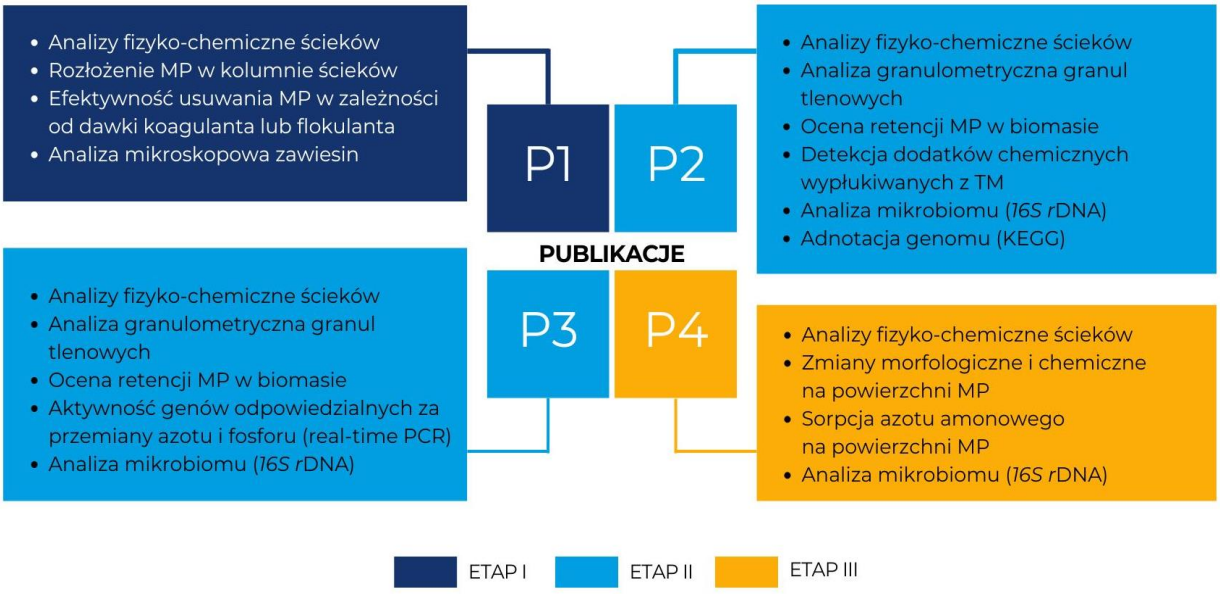
H1: zastosowanie koagulantów oraz flokulantów wpływa na efektywność usunięcia MP ze ścieków;

H2: dawka oraz rodzaj MP wpływają na efektywność oczyszczania ścieków oraz strukturę gatunkową i aktywność mikroorganizmów w AGS;

H3: stężenie azotu amonowego w środowisku wpływa na skład gatunkowy mikroorganizmów zasiedlających powierzchnię MP i potencjał metaboliczny mikrobiomu, w tym zdolność degradacji tworzyw sztucznych.

Materiały i metody badawcze

Badania zostały podzielone na trzy etapy (**rys. 3**). W etapie I badano skuteczność usuwania MP ze ścieków przy zastosowaniu koagulantów i flokulantów, w etapie II określono wpływ MP na procesy oczyszczania ścieków, natomiast w etapie III badano rozwój mikroorganizmów na powierzchni MP w zależności od stężenia azotu amonowego w środowisku.



Rys. 3 Etapy pracy doktorskiej

Etap I

Do badań wykorzystano ścieki dopływające do osadnika wstępnego w oczyszczalni ścieków „Łyna" w Olsztynie. Średnie stężenia zanieczyszczeń w ściekach wynosiły: 436,0 ± 32,0 mg rChZT/l, 41,4 ± 5,3 mg N-NH_4/l, 18,5 ± 2,5 mg P-PO_4/l, 2740 ± 350 mg s.m/l. Odczyn i przewodność elektryczna ścieków wynosiły odpowiednio 7,81 pH i 0,98 mS/cm.

Eksperymenty przeprowadzono w zlewkach o pojemności 1000 ml, które umieszczono w urządzeniu do koagulacji z mieszadłami łopatkowymi (Flock Tester Aqualytic). Do każdej zlewki dodano 1 l ścieków, które wymieszano z MP, a następnie dodano koagulanty (PIX i PAX) oraz flokulanty (FPM, PEL, FCT). Roztwory robocze koagulantów i flokulantów przygotowano 24 godziny przed użyciem przez rozcieńczenie 1 ml (postać płynna) lub 1 g (postać sucha) odpowiedniego związku w wodzie destylowanej. Roztwory robocze dodano do zlewek w ilości 1,0, 2,5 i 5,0 ml/l. W eksperymencie wykorzystano fluorescencyjny MP PE (Cospheric, Santa Barbara, CA, USA) o średnicach 710–850 µm i 180–212 µm. Do każdej zlewki dozowano po 25 cząsteczek MP obu badanych wielkości. Zawartość zlewek mieszano

przez 2 minuty (250 obr./min), następnie zastosowano powolne mieszanie (100 obr./min) przez 28 minut, a na końcu pozostawiono na 15 minut sedymentacji.

Eksperymenty przeprowadzono w trzech powtórzeniach dla każdej dawki koagulantu/flokulantu. Po zakończeniu eksperymentu z każdej zlewki pobrano trzy frakcje: frakcję osadu wyflotowanego (górne 100 ml ścieków wraz z zawiesinami), frakcję toni ścieków i frakcję osadu zsedymentowanego (100 ml ścieków i osadu z dna zlewki). Każda frakcja po eksperymencie została przefiltrowana przez sączki o średnicy porów 125 µm w leju Büchnera w celu określenia liczby cząstek MP. MP na sączkach zliczano za pomocą lupy optycznej z 10-krotnym powiększeniem (Delta Optical SZ-450) w ciemni w świetle UV w celu aktywacji barwnika fluorescencyjnego.

W ściekach oznaczono stężenie ChZT, N-NH_4 i P-PO_4 w trzech powtórzeniach. pH mierzono za pomocą TitroLine easy (Donserv). Obserwacje osadu prowadzono za pomocą mikroskopu optycznego wyposażonego w kamerę (Nikon Eclipse 50I). We frakcjach oznaczono stężenie zawiesin ogólnych.

Etap II

Badania dotyczące wpływu MP na biologiczne oczyszczanie ścieków prowadzono w GSBR. Objętość robocza GSBR wynosiła 3,0 l (wysokość 100 cm, średnica 10 cm), a stopień wymiany ścieków 60%/cykl. Prędkość dopływu powietrza wynosiła 0,85 cm/s. Cykl pracy GSBR trwał 480 minut i obejmował 5 minut napełniania, 60 minut fazy niedotlenionej, 400 minut fazy napowietrzania, 10 minut sedymentacji i 5 minut dekantacji. Praca GSBR była kontrolowana za pomocą automatycznego systemu sterowania.

Do zaszczepienia GSBR wykorzystano biomasę z Miejskiej Oczyszczalni Ścieków w Ornecie. Biomasa była adaptowana do warunków operacyjnych GSBR przez około 3 miesiące do momentu, gdy zakres zmian badanych wskaźników fizyko-chemicznych w ściekach nie przekraczał 5–10% w ciągu 14 dni.

W ramach eksperymentu przeprowadzono trzy serie różniące się rodzajem MP (PET, PE i TM) wprowadzanego ze ściekami. Każda seria eksperymentu była prowadzona w 4 różnych GSBR-ach. W seriach z PET i PE do reaktorów dodawano ścieki zawierające 1, 10 lub 50 mg MP/l, natomiast w przypadku TM stosowano dawki 10, 25 lub 50 mg MP/l. Dodatkowo w każdej serii eksploatowano reaktor kontrolny bez dodatku MP. Do GSBR dozowano ścieki syntetyczne o składzie: 700–800 mg ChZT/l, 40–60 mg N-NH_4/l, 8–12 mg P-PO_4/l oraz mikroelementy

(Coelho i in., 2000). Zastosowanie ścieków syntetycznych było konieczne, by zapobiec wprowadzaniu innych MP do GSBR. W każdej serii, co 6–9 cykli wykonywano analizy fizyko-chemiczne ścieków dopływających oraz oczyszczonych (ChZT, N-NH_4, N-NO_2, N-NO_3 i P-PO_4; APHA, 2012). Dodatkowo na koniec serii przeprowadzono badania kinetyczne zmian stężeń ChZT, N-NH_4, N-NO_2, N-NO_3 i P-PO_4 w cyklu pracy GSBR.

Stężenie biomasy w GSBR oraz stężenie zawiesin w ściekach oczyszczonych (APHA, 2012) oznaczano co 9–15 cykli. Na zakończenie każdej serii przeprowadzano analizę sitową biomasy z GSBR (Cydzik-Kwiatkowska i in., 2009). MP z biomasy izolowano za pomocą H_2O_2 (Li i in., 2018). Obecność MP potwierdzono również przy użyciu spektroskopii podczerwieni z transformacją Fouriera (FTIR) (Shimazu IRSpirit).

W seriach badawczych pobierano próbki biomasy, które przechowywano w temperaturze -20°C. Z próbek wyizolowano DNA przy pomocy FastDNA® SPIN Kit for Soil (MP Biomedical). Jakość i ilość wyizolowanego DNA badano spektrofotometrycznie (NanoDrop). Następnie DNA wysyłano do zewnętrznego laboratorium w celu przeprowadzenia analizy mikrobiomu poprzez sekwencjonowanie nowej generacji (platforma MiSeq Illumina) z zastosowaniem starterów 515 F/806 R (Caporaso i in., 2011), powielających fragmenty V3 i V4 genu *16S r*DNA. Uzyskane fragmenty sekwencji analizowano bioinformatycznie (Jachimowicz i in., 2024a).

W seriach z PE i PET, z próbek biomasy wyizolowano również RNA przy wykorzystaniu zestawu Total RNA kit (A&A Biotechnology). Ilość i jakość RNA kontrolowano spektofotometrycznie (NanoDrop). Usunięto DNA z wykorzystaniem DNAzy (Thermo Fisher Scientific). RNA przepisano na cDNA (RevertAid™ H Minus First Strand cDNA Synthesis Kit- Fermentas) podczas reakcji odwrotnej transkrypcji. Do analizy liczby kopii badanych genów wykorzystano technikę Real-Time PCR. Analizowano geny: *amoA* (monooksygenaza amonowa), *nirK* (reduktaza azotynowa), *nirS* (reduktaza azotynowa), *nosZ* (reduktaza dwutlenku azotu), *ppk1* (polifosfokinaza) oraz *16S r*DNA (ogólna aktywność bakterii) (Jachimowicz i in., 2024b). Krzywe standardowe skonstruowano przy użyciu plazmidów zawierających sklonowane inserty badanych genów. Do procedury klonowania użyto zestawu Clone Jet PCR cloning kit i kompetentnych komórek *Escherichia coli* 109 (Promega). Obecność insertów we wszystkich klonach potwierdzono metodą PCR z wykorzystaniem odpowiednich starterów. Skład mieszaniny reakcyjnej oraz profile termiczne Real-Time PCR przedstawiono w pracy Rusanowskiej (2015).

W serii z dodatkiem TM pobierano próbki ścieków oczyszczonych, by identyfikować substancje wymyte z TM oraz ich metabolity. Każda próbka była mieszaniną ścieków pobranych z GSBR w ciągu doby (3 cykle). Do próbek dodano standard wewnętrzny (100 µg 2-MeBTH), a następnie filtrowano je przez filtry nylonowe o wielkości porów 0,22 µm. W próbkach badano obecność aniliny, 2-A-BTH, DPG, 5-Me-BTH, 2-OH-BTH, BTH, 2-Me-S-BTH, HMMM, 6PPD, 6PPD-Q i DTG przy wykorzystaniu LC/MS (Agilent infinity 1290 II; Castan i in., 2022).

Etap III

Analizowano zmiany mikrobiomu na powierzchni MP oraz właściwości fizyko-chemiczne PET w zależności od stężenia azotu amonowego w środowisku. MP uzyskano w wyniku mielenia butelek PET pobranych z Zakładu Gospodarki Odpadami w Olsztynie. Butelki pochodziły od jednego krajowego producenta, co zapewniło homogeniczność próbki. Butelki wypłukano, usunięto z nich etykiety papierowe, a następnie pokrojono na kwadraty (5 cm × 5 cm). Uzyskany materiał został zmielony w automatycznym młynie SM 2000 (Retsch) na cząstki o średnicy < 5 mm.

Cylindryczny reaktor o wysokości 100 cm i szerokości 50 cm wypełniono do 20% objętości MP. MP był zraszany płynną pożywką nie zawierającą węgla (LCFBM) za pomocą dyszy umieszczonej w górnej części reaktora. Powietrze było wprowadzone przez perforowaną płytę na dnie reaktora w ilości 20 l/min. W reaktorze znajdowały się sondy do pomiaru wilgotności, temperatury i stężenia tlenu, a pracę reaktora kontrolował regulator elektroniczny. Jako inokulum wykorzystano osad czynny z Miejskiej Oczyszczalni Ścieków „Łyna" w Olsztynie. Inokulum przed wprowadzeniem do reaktora hodowano w płynnej pożywce LCFBM z dodatkiem plastików (1 × 1 cm) przez 30 dni w temperaturze 37˚C.

Eksperyment trwał 260 dni i obejmował trzy fazy: rozwój błony biologicznej (dzień 0–46; ~70 mg $N\text{-}NH_4$/l), adaptację do wysokiego stężenia $N\text{-}NH_4$ (dzień 46–86; ~430 mg $N\text{-}NH_4$/l) oraz adaptację do niskiego stężenia $N\text{-}NH_4$ (dzień 86–260; ~70 mg $N\text{-}NH_4$/l). W dopływie i odpływie z reaktora oznaczano stężenie ChZT, $N\text{-}NH_4$, $N\text{-}NO_2$ i $N\text{-}NO_3$ (APHA, 2012) oraz pH (TitroLine Easy, Donserv). Dodatkowo określano kinetykę zmian stężenia związków azotowych po wprowadzeniu świeżej porcji LCFBM do reaktora.

Aby określić ilości zanieczyszczeń zatrzymanych na powierzchni PET, pobrano próbkę MP z reaktora z głębokości około 10 cm. MP wysuszono strumieniem sprężonego powietrza i umieszczono na szczelnie zamkniętych szklanych płytkach Petriego w temperaturze

pokojowej. Stężenie ChZT oraz TN w MP określono umieszczając 1 g MP w kolbach reakcyjnych, a następnie wykonując oznaczenie zgodnie z APHA (2012). Wyniki uzyskane dla MP stanowiącego wsad do reaktora zostały odjęte od wyników uzyskanych dla MP pobranego w trakcie eksperymentu. Błona biologiczna została usunięta z MP ultradźwiękowo (10 min przy 25 kHz), a następnie odwirowana przez 30 minut przy 13 000 obr./min. Ilość biomasy określono zgodnie z APHA (2012). Zmiany masy MP w trakcie eksperymentu oceniano, ważąc 100 cząstek wsadowego MP oraz 100 cząstek MP pobranego podczas eksperymentu (po usunięciu biomasy). Przeprowadzono również obserwacje zmian chemicznych i morfologicznych powierzchni MP za pomocą FTIR (Nicoolet 6700, Thermo Scientific) oraz skaningowego mikroskopu elektronowego (SEM, Quanta FEG 250).

MP wraz z błoną biologiczną i cieczą pobrano w 0, 16, 30, 74 i 243 dniu eksperymentu. Każda próbka była odwirowywana przez 15 minut przy 8000 obr./min w wirówce MPW-380 (MPW). Z dna próbówki pobrano 500 µl biomasy, którą wykorzystano do analiz molekularnych opisanych w etapie II doktoratu.

Testy sorpcji $N\text{-}NH_4$ na MP przeprowadzono w szklanych zlewkach o pojemności 50 ml, stosując ścieki syntetyczne o początkowym stężeniu $N\text{-}NH_4$ wynoszącym 70 i 430 mg $N\text{-}NH_4$/l. Ilość dodanego MP wynosiła odpowiednio 10%, 2,5% i 0,5% (w/v). Próbki mieszano (150 obr./min, 24 godziny) w temperaturze pokojowej na mieszadle mechanicznym (TB51, Laboshake). Ilość sorbowanego $N\text{-}NH_4$ określono na podstawie różnicy stężenia początkowego i końcowego.

Podsumowanie wyników i weryfikacja hipotez badawczych

Etap I

Badania wykazały, że w osadniku wstępnym następuje skuteczne usuwanie MP ze ścieków, a efektywność procesu wzrasta wraz z wielkością MP. W próbce kontrolnej bez dodatku substancji chemicznych wspomagających sedymentację usuwane było > 70% MP, mniejsze cząstki MP były unieruchamiane w zawiesinach łatwoopadających. Zastosowanie FPM, PIX i PEL powodowało zatrzymanie mniejszych cząstek MP (80–212 μm) w osadzie z efektywnością od 62,0 do 86,4%. W przypadku FCT i PAX większość MP znajdowała się w osadzie wyflotowanym (36,0–64,0%). Odsetek cząstek MP w ściekach zmniejszał się ze zwiększaniem dawki wszystkich zastosowanych koagulantów/flokulantów z wyjątkiem PEL. Wykorzystanie FPM w dawce 5 ml/l wykazało szczególnie wysoką skuteczność (96%) w usuwaniu ze ścieków cząsteczek MP o średnicy 180–212 μm.

MP o wielkości 710–850 μm po zastosowaniu koagulantów/flokulantów znajdował się głównie w osadzie wyflotowanym (53,3–93,4%). Odsetek MP o średnicy 710–850 μm w ściekach był na poziomie 2–10% dla większości koagulantów/flokulantów, jedynie dla dawki FCT równej 2,5 ml/l udział wzrósł do 26,6%. MP został wyeliminowany ze ścieków po zastosowaniu 1,0 ml PIX/l, 2,5 ml PEL/l oraz dwóch najwyższych dawek PAX.

Sedymentacja ścieków bez dodatku koagulanta/flokulanta zmniejszyła stężenie ChZT (31%) i zawiesin ogólnych (16%), ale nie wpływała istotnie na usuwanie $N\text{-}NH_4$ i $P\text{-}PO_4$ (<2%). Największą efektywność usuwania ChZT oraz $P\text{-}PO_4$ obserwowano przy zastosowaniu koagulantów PIX i PAX (>40%).

Analiza mikroskopowa wykazała, że zwiększenie dawek koagulantów/flokulantów w zakresie od 1 do 5 ml/l stymulowało łączenie zawiesin i tworzenie większych agregatów. W próbce kontrolnej bez dodatku koagulantów/flokulantów dominowały zawiesiny o średnicy <100 μm. Największe zawiesiny o średnicy > 1 mm tworzyły się przy dawce 5 ml/l FCT i FPM.

Stwierdzono, że zastosowanie wybranych koagulantów oraz flokulantów zwiększa efektywność usuwania MP ze ścieków (H1).

Etap II

Wyniki eksperymentu wykazały wpływ rodzaju MP na jego deponowanie w AGS. PET oraz TM wykazywały większą tendencję do akumulacji w strukturze osadu, osiągając stężenia

odpowiednio 121–159 mg MP/g s.m. oraz 50–270 mg MP/g s.m. Ilość PET i TM deponowanego w osadach zwiększała się proporcjonalnie do dawki MP w ściekach. Wysoka efektywność usuwania MP ze ścieków, sięgająca 88,2%, wynikała z jego akumulacji w biomasie AGS. Podczas dozowania TM obserwowano, że dawka wpływała na zmianę struktury AGS, co powodowało wzrost udziału w biomasie granul o średnicy >350 μm oraz spadek udziału granul o średnicy 40–125 μm. W przypadku PE, ilość MP zatrzymanego w strukturze biomasy była zbliżona (około 30 mg MP/g s.m.) we wszystkich reaktorach.

Dawka PET wpłynęła istotnie na efektywność usuwania ChZT w GSBR, powodując spadek efektywności z 85,5% w reaktorze kontrolnym do 81,6% w GSBR z najwyższą dawką MP w ściekach. Dawka 10 oraz 50 mg PET/l obniżała szybkość usuwania ChZT z 5,43 mg/(g s.m.·h) w reaktorze kontrolnym do 5,05 oraz 4,50 mg/(g s.m.·h) odpowiednio przy dawce 10 oraz 50 mg PET/l. W przypadku MP PE, szybkość usuwania ChZT w cyklu GSBR wzrastała ze wzrostem dawki MP z 8,16 do 10,24 mg/(g s.m.·h), odpowiednio w reaktorze kontrolnym oraz przy dawce 50 mg PE/l.

Zwiększenie dawki MP z PET i TM istotnie zmniejszało efektywność oraz szybkość usuwania $N\text{-}NH_4$. Efektywność usuwania $N\text{-}NH_4$ w przypadku najwyższych dawek oby typów MP spadła w stosunku do reaktorów kontrolnych o odpowiednio 2,1% oraz 1,6% dla PET oraz TM. Szybkość usuwania $N\text{-}NH_4$ obniżyła się o 47% z 9,5 mg/l w reaktorze kontrolnym do 4,5 mg/l przy 50 mg TM/l. Obecność w ściekach MP PET obniżyła szybkość usuwania $N\text{-}NH_4$ z 0,38 mg/(g s.m.·h) w reaktorze kontrolnym do 0,32 mg/(g s.m.·h) przy dawce 50 mg PET/l. Dawka PE nie wpływała na efektywność usuwania $N\text{-}NH_4$, wpływała natomiast na szybkość usuwania, która wyniosła 0,58 mg $N\text{-}NH_4$/(g s.m.·h) w reaktorze kontrolnym i wzrosła do 0,73 mg $N\text{-}NH_4$/(g s.m.·h) w reaktorze, do którego dozowano 50 mg PE/l.

Dawka TM powodowała wzrost stężenia utlenionych form azotu w odpływie z GSBR z 23,2 ± 1,3 mg/l w reaktorze kontrolnym do 25,1 ± 1,3 mg/l w GSBR eksploatowanym przy stężeniu TM w ściekach na poziomie 50 mg/l. Wzrost dawki PE istotnie obniżał stężenie $N\text{-}NO_2$ w ściekach oczyszczonych. W przypadku PET dawka MP wpływała na zmniejszenie stężenia $N\text{-}NO_3$ w ściekach oczyszczonych z 3,74 ± 1,5 mg/l w reaktorze kontrolnym do 2,10 ± 1,0 mg/l w reaktorze, do którego dozowano 50 mg PET/l. Obecność MP PE oraz TM pozytywnie wpływała na szybkość usuwania lub produkcji $N\text{-}NO_2$ oraz $N\text{-}NO_3$. Wprowadzenie ze ściekami do GSBR TM w dawce 50 mg/l zwiększyło szybkość usuwania $N\text{-}NO_3$ z 0,18 ± 0,1 mg/(g s.m.·h) w reaktorze kontrolnym do 3,42 ± 0,4 mg/(g s.m.·h) w obecności TM.

W reaktorze, do którego dozowano 50 mg PET/l, efektywność usuwania P-PO_4 wzrosła o 7% w porównaniu z reaktorem kontrolnym. Szybkość uwalniania oraz usuwania P-PO_4 w cyklu pracy GSBR wzrastała wraz ze wzrostem dawki PE w ściekach. W GSBR, do którego dozowano ze ściekami 50 mg PET/l, szybkość uwalniania P-PO_4 wzrosła o 15%, a usuwania o 31% w porównaniu do reaktora kontrolnego. W fazie tlenowej, w GSBR, do których dozowano ze ściekami PET, szybkość usuwania P-PO_4 była znacząco wyższa (0,15–0,17 mg/(g s.m.·h)) niż w reaktorze kontrolnym (0,13 mg/(g s.m.·h)).

Badania ścieków odprowadzanych z GSBR, do których dozowano TM wykazały obecność aniliny, dodatku stosowanego do produkcji opon. Jej stężenie w odpływie korelowało dodatnio ze stężeniem TM w ściekach doprowadzanych do reaktorów biologicznych oraz ujemnie z efektywnością usuwania N-NH_4. Mikrobiologiczna degradacja amin aromatycznych, w tym aniliny, może prowadzić do uwolnienia N-NH_4 do środowiska. Porównując zawartość dodatków chemicznych w TM z ich stężeniami w odpływie z GSBR, wykazano, że AGS degradował i sorbował te związki z efektywnością > 60%. Degradacja związków wymytych z TM zachodziła z różną efektywnością – w ściekach oczyszczonych biologicznie występowały anilina, BTH, 2-A-BTH, DPG oraz 2-OH-BTH. Nie wykryto obecności 6PPD, którego stężenie przekraczało 4900 µg/g w odciekach z TM. Również spadek stężeń BTH w ściekach oczyszczonych w porównaniu do odcieków z TM wskazuje, że AGS skutecznie usuwał dodatki chemiczne wymyte z TM.

Dawka TM w ściekach korelowała pozytywnie z obecnością w AGS mikroorganizmów z rodzajów *Xanthomonas*, *Ferruginibacter*, *Luteimonas*, *Hydrogenophaga*, *Devosia*, *Geobacter*, *Thiobacillus*, *Rubrivivax*, *Gemmatimonas* i *Acidovorax*, które są zdolne do degradacji mikrozanieczyszczeń. Adnotacja genomu przy wykorzystaniu bazy KEGG wskazała na wzrost potencjału zbiorowiska mikroorganizmów do naprawy lub syntezy materiału genetycznego oraz biodegradacji zanieczyszczeń wraz ze wzrostem dawki TM w ściekach.

Analiza aktywności mikroorganizmów nie wykazała wpływu MP (PET oraz PE) na liczbę transkryptów genów *16S r*DNA*, amoA, nirK* oraz *nirS* w biomasie. Dozowanie ze ściekami PET zwiększyło liczebność transkryptów genów *nosZ* oraz *ppk1* w biomasie w cyklu GSBR. Podobnej korelacji nie odnotowano w GSBR, do którego był dozowany PE. Dozowanie ze ściekami PET powodowało wzrost udziału procentowego w AGS mikroorganizmów z rodzajów *Bacteroides*, *Singulisphaera*, *Gemmata*, *Ectothiorhodospira*, *Devosia, Dechloromonas*, *Woodsholea* i *Microcystis*. W przypadku GSBR do którego dozowano PE,

podobna zależność została zaobserwowana dla mikroorganizmów z rodzajów *Mycobacterium*, *Oscillochloris*, *Fusobacterium*, *Lactobacillus*, *Stenotrophomonas*, *Bacteroides* i *Microcystis*.

Stwierdzono, że stężenie i rodzaj MP w ściekach dopływających wpływają na mikrobiom AGS oraz przemiany związków organicznych, azotu i fosforu, które są kluczowe dla utrzymania wysokiej efektywności oczyszczania ścieków w GSBR (H2).

Etap III

Badania wykazały różnice w strukturze chemicznej pierwotnego MP oraz MP poddanego długotrwałemu oddziaływaniu zmiennych stężeń azotu amonowego w środowisku. Analiza FTIR próbek PET pobranych w trakcie eksperymentu wykazała zmniejszenie transmisji w zakresach długości fali odpowiadających deformacji pierścienia aromatycznego (1408 cm^{-1} oraz 792 cm^{-1}), co wskazuje na zmiany w grubości PET. Ponadto zaobserwowano zmiany w intensywności pasm odpowiadających różnym grupom funkcyjnym na powierzchni PET tj. asymetrycznego rozciągania estrowego (CO) jednostki glikolu etylenowego (1044 cm^{-1}), -CH_2 w konformacji trans jednostek glikolu etylenowego (1340 cm^{-1}), rozciąganiu karbonylowemu (C=O) (1712 cm^{-1}) oraz drganiach grup -OH i -CH (2800–3500 cm^{-1}). Zmiany te wskazują na degradację powierzchni MP w trakcie eksperymentu. Analiza SEM wykazała zmiany morfologiczne na powierzchni MP, takie jak zwiększenie chropowatości i powstawanie pęknięć, co jest zgodne z wynikami analizy z FTIR. Stwierdzono spadek masy MP podczas eksperymentu około 7%.

Efektywność usuwania N-NH_4 była znacznie wyższa w fazach I i III (18,1–23,7%) niż w fazie II (8,4%). Średnie stężenie ChZT w odpływie w fazie I eksperymentu wyniosło 212,3 ± 102,3 mg/l i było istotnie wyższe niż w fazie III eksperymentu (111,4 ± 39,8 mg ChZT/l). Różnice w efektywności usuwania ChZT, przy zbliżonym stężeniu azotu amonowego w ściekach dozowanych do reaktora, wynikały z wyższego stężenia biomasy błony biologicznej na powierzchni MP. Ilość błony biologicznej sukcesywnie wzrastała w miarę trwania eksperymentu; na koniec eksperymentu ilość biomasy na powierzchni MP była o 12% wyższa niż w I fazie badań. Stężenie N-NH_4 w środowisku było pozytywnie skorelowane z sorpcją TN na powierzchni MP. Przy stężeniu 70 mg N-NH_4/l w dopływie do reaktora, ilość azotu amonowego zatrzymanego na powierzchni MP wyniosła 26,6 mg N-NH_4/g MP, a szybkość jego usuwania wyniosła 1,11 mg N-NH_4/(g MP·h). Przy stężeniu azotu amonowego w środowisku na poziomie 430 mg/l najwyższa ilość sorbowanego N-NH_4 wynosiła 204,0 mg N-NH_4/g MP, a szybkość usuwania azotu amonowego wzrosła do 8,52 mg N-NH_4/(g MP·h).

Analiza molekularna błony biologicznej wykazała, że dominującymi bakteriami na poziomie typów były *Proteobacteria* (80,86%) i *Bacteroidetes* (14,17%). W fazie I udział procentowy *Proteobacteria* spadł z 80,86 do 47,96%, a *Bacteroidetes* z 14,7 do 7,51%; jednocześnie zaobserwowano znaczący wzrost udziału procentowego w biomasie *Actinobacteria* (z 0,60 do 28,55%). W fazie II najliczniejsze w błonie biologicznej były *Proteobacteria* (47,03%), *Nitrospirae* (18,33%) i *Bacteroidetes* (7,22%), podczas gdy w fazie III eksperymentu dominowały *Proteobacteria* (54,15%) i *Actinobacteria* (30,74%). Najwyższa liczba specyficznych OTU została zaobserwowana w fazie rozwoju błony biologicznej. Różnorodność mikrobiologiczna wyrażona indeksem Shannona-Wienera w I fazie eksperymentu wyniosła 2,96 i spadła do 1,78 w II fazie eksperymentu. W błonie biologicznej wykazano obecność mikroorganizmów zdolnych do kolonizacji i degradacji tworzyw sztucznych (*Acidovorax, Aneurinibacillus, Aquabacterium, Bacillus, Brevibacillus, Enterobacter, Gordonia, Hydrogenophaga, Ideonella, Pseudomonas, Rhodococcus, Rhodopseudomonas, Sphingomonas, Sphingopyxis, Streptomyces* oraz *Thermoactinomyce*). Choć zaobserwowano dużą liczbę rodzajów bakterii potencjalnie degradujących MP, należy podkreślić, że ich względny procentowy udział był niski, a ich obecność zmieniała się w czasie. Wykazano, że na powierzchni MP rozwijały się mikroorganizmy potencjalnie patogenne, tj. *Enterobacter* sp., *Pseudomonas* sp., *Mycobacterium* sp., *Sphingopyxis* sp., *Aquabacterium* sp. i *Brevundimonas* sp.

Badania wykazały zmiany morfologiczne oraz chemiczne powierzchni MP na skutek aktywności mikroorganizmów oraz czynników środowiskowych. Wykazano, że MP może być wektorem zanieczyszczeń i mikroorganizmów patogennych (H3).

Podsumowanie i wnioski

Przeprowadzone badania pozwoliły zweryfikować postawione w pracy hipotezy badawcze i wykazać, że:

- osadniki wstępne mogą skutecznie usuwać MP dopływający ze ściekami do oczyszczalni, przy czym efektywność procesu wzrasta z wielkością cząsteczek MP; zastosowanie koagulantów i flokulantów znacząco wpływało na rozkład MP w toni ścieków oraz skuteczność usuwania MP (**P1**),

- usuwanie MP ze ścieków w GSBR zachodziło głównie w wyniku akumulacji MP w biomasie; najlepiej akumulowany był TM (**P2**, **P3**),

- dawka i rodzaj MP wpływały na efektywność usuwania związków azotu i fosforu ze ścieków (**P2**, **P3**),

- obecność MP w ściekach wpływała na aktywność i strukturę mikrobiologiczną AGS (**P2**, **P3**),

- mikrobiom AGS efektywnie degradował zanieczyszczenia wypłukiwane z TM (**P2**),

- długa ekspozycja MP na wysokie stężenie azotu amonowego powodowała zmiany morfologiczne oraz chemiczne powierzchni MP, świadczące o jego degradacji (**P4**),

- stężenie azotu amonowego w środowisku wpływało na mikrobiom zasiedlający powierzchnię MP (**P4**),

- na powierzchni MP rozwijają się mikroorganizmy potencjalnie patogenne, więc MP może służyć jako wektor zanieczyszczeń (**P4**).

Spis literatury

1. Adav, S.S., Lee, D.J., Show, K.Y., & Tay, J.H. (2008). Aerobic granular sludge: recent advances. Biotechnology Advances, 26(5), 411–423.
2. Akarsu, C., Kumbur, H., Gökdag, K., Kıdey, S.A.E., & Sanchez-Vidal, A. (2020). Microplastics composition and load from three wastewater treatment plants discharging into Mersin Bay, northeastern Mediterranean Sea. Marine Pollution Bulletin, 150, 110776.
3. APHA (2012). Standard methods for the examination of water and wastewater. Washington, DC: American public health association.
4. Bao, R., Cheng, Z., Hou, Y., Xie, C., Pu, J., Peng, L., Gao, L., Chen, W., & Su, Y. (2022). Secondary microplastics formation and colonized microorganisms on the surface of conventional and degradable plastic granules during long-term UV aging in various environmental media. Journal of Hazardous Materials, 439, 129686.
5. Bayo, J., Olmos, S., & López-Castellanos, J. (2020). Microplastics in an urban wastewater treatment plant: The influence of physicochemical parameters and environmental factors. Chemosphere, 238, 124593.
6. Białowąs, M., Jonko-Sobuś, K., Pawlak, J., Polak-Juszczak, L., Dąbrowska, A., & Urban-Malinga, B. (2022). Plastic in digestive tracts and gills of cod and herring from the Baltic Sea. Science of the Total Environment, 822, 153333.
7. Brennecke, D., Duarte, B., Paiva, F., Caçador, I., & Canning-Clode, J. (2016). Microplastics as vector for heavy metal contamination from the marine environment. Estuarine, Coastal and Shelf Science, 178, 189–195.
8. Carr, S.A., Liu, J., & Tesoro, A.G. (2016). Transport and fate of microplastic particles in wastewater treatment plants. Water Research, 91, 174–182.
9. Caporaso, G., Lauber, L., Walters, W., Berg-Lyons, D., Lozupone, C., Turnbaugh, P., Fierer, N., & Knight, R., (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences, 108, 4516–4522.
10. Castan, S., Sherman, A., Peng, R., Zumstein, M.T., Wanek, W., Hüffer, T., & Hofmann, T., (2022). Uptake, metabolism, and accumulation of tire wear particle derived compounds in lettuce. Environmental Science and Technology 57 (1), 168–178.
11. Coelho, M.A.Z., Russo, C., & Araujo, O.Q.F. (2000). Optimization of a sequencing batch reactor for biological nitrogen removal. Water Research, 34(10), 2809–2817.

12. Cydzik-Kwiatkowska, A., Bernat, K., Zielińska, M., Bułkowska, K., & Wojnowska-Baryła, I. (2017). Aerobic granular sludge for bisphenol A (BPA) removal from wastewater. International Biodeterioration and Biodegradation, 122, 1–11.
13. Cydzik-Kwiatkowska, A., Białowiec, A., Wojnowska-Baryła, I., & Smoczyński, L. (2009). Characteristic of granulated activated sludge fed with glycerin fraction from biodiesel production. Archives of Environmental Protection, 41–52.
14. Cydzik-Kwiatkowska, A., Milojevic, N., & Jachimowicz, P. (2022). The fate of microplastic in sludge management systems. Science of the Total Environment, 848, 157466.
15. Dai, H.H., Gao, J.F., Wang, Z.Q., Zhao, Y.F., & Zhang, D. (2020). Behavior of nitrogen, phosphorus and antibiotic resistance genes under polyvinyl chloride microplastics pressures in an aerobic granular sludge system. Journal of Cleaner Production, 256, 120402.
16. Dąbrowska, A., Mielańczuk, M., & Syczewski, M. (2022). The Raman spectroscopy and SEM/EDS investigation of the primary sources of microplastics from cosmetics available in Poland. Chemosphere, 308, 136407.
17. De Bruin, L.M.M., de Kreuk, M.K., van der Roest, H.F.R., Uijterlinde, C., & van Loosdrecht, M.C.M. (2004). Aerobic granular sludge technology: an alternative to activated sludge? Water Science and Technology, 49(11–12), 1–7.
18. De Kreuk, M.K., Kishida, N., & Van Loosdrecht, M.C.M. (2007). Aerobic granular sludge – state of the art. Water Science and Technology, 55(8–9), 75–81.
19. De Kreuk, M.K., & van Loosdrecht, M.C. (2006). Formation of aerobic granules with domestic sewage. Journal of Environmental Engineering, 132(6), 694–697.
20. Delacuvellerie, A., Cyriaque, V., Gobert, S., Benali, S., & Wattiez, R. (2019). The plastisphere in marine ecosystem hosts potential specific microbial degraders including Alcanivorax borkumensis as a key player for the low-density polyethylene degradation. Journal of Hazardous Materials, 380, 120899.
21. Dris, R., Gasperi, J., Rocher, V., Saad, M., Renault, N., & Tassin, B. (2015). Microplastic contamination in an urban area: a case study in Greater Paris. Environmental Chemistry, 12(5), 592–599.
22. Edo, C., González-Pleiter, M., Leganés, F., Fernández-Piñas, F., & Rosal, R. (2020). Fate of microplastics in wastewater treatment plants and their environmental dispersion with effluent and sludge. Environmental Pollution, 259, 113837.
23. Gies, E.A., LeNoble, J.L., Noël, M., Etemadifar, A., Bishay, F., Hall, E.R., & Ross, P.S. (2018). Retention of microplastics in a major secondary wastewater treatment plant in Vancouver, Canada. Marine Pollution Bulletin, 133, 553–561.

24. Gao, D., Liu, L., Liang, H., & Wu, W.M. (2011). Aerobic granular sludge: characterization, mechanism of granulation and application to wastewater treatment. Critical Reviews in Biotechnology, 31(2), 137–152.
25. Hartmann, N.B., Rist, S., Bodin, J., Jensen, L.H., Schmidt, S.N., Mayer, P., Meibom, A., & Baun, A. (2017). Microplastics as vectors for environmental contaminants: Exploring sorption, desorption, and transfer to biota. Integrated Environmental Assessment and Management, 13(3), 488–493.
26. Hernandez, L.M., Yousefi, N., & Tufenkji, N. (2017). Are there nanoplastics in your personal care products? Environmental Science & Technology Letters, 4(7), 280–285.
27. Hreiz, R., Latifi, M.A., & Roche, N. (2015). Optimal design and operation of activated sludge processes: State-of-the-art. Chemical Engineering Journal, 281, 900–920.
28. Iyare, P.U., Ouki, S.K., & Bond, T. (2020). Microplastics removal in wastewater treatment plants: a critical review. Environmental Science: Water Research and Technology, 6(10), 2664–2675.
29. Jachimowicz, P., Jo, Y.J., & Cydzik-Kwiatkowska, A. (2022). Polyethylene microplastics increase extracellular polymeric substances production in aerobic granular sludge. Science of the Total Environment, 851, 158208.
30. Jachimowicz, P., Peng, R., Hüffer, T., Hofmann, T., & Cydzik-Kwiatkowska, A. (2024a). Tire materials disturb transformations of nitrogen compounds and affect the structure of biomass in aerobic granular sludge reactors. Journal of Hazardous Materials, 465, 133223.
31. Jachimowicz, P., Mądzielewska, W., & Cydzik-Kwiatkowska, A. (2024b) Microplastics in granular sequencing batch reactors: effects on pollutant removal dynamics and the microbial community. Available at SSRN 4744814.
32. Koelmans, A.A., Redondo-Hasselerharm, P.E., Nor, N.H.M., de Ruijter, V.N., Mintenig, S.M., & Kooi, M. (2022). Risk assessment of microplastic particles. Nature Reviews Materials, 7(2), 138–152.
33. Kurt, Z., & Özdemir, I. (2022). Effectiveness of microplastics removal in wastewater treatment plants: A critical analysis of wastewater treatment processes. Journal of Environmental Chemical Engineering, 10(3), 107831.
34. Lee, H., & Kim, Y. (2018). Treatment characteristics of microplastics at biological sewage treatment facilities in Korea. Marine Pollution Bulletin, 137, 1–8.
35. Lei, K., Qiao, F., Liu, Q., Wei, Z., Qi, H., Cui, S., Xue Y., Deng Y., & An, L. (2017). Microplastics releasing from personal care and cosmetic products in China. Marine Pollution Bulletin, 123(1–2), 122–126.

36. Li, X., Chen, L., Mei, Q., Dong, B., Dai, X., Ding, G., & Zeng, E.Y., (2018). Microplastics in sewage sludge from the wastewater treatment plants in China. Water Research, 142, 75–85.
37. Liu, X., Yuan, W., Di, M., Li, Z., & Wang, J. (2019). Transfer and fate of microplastics during the conventional activated sludge process in one wastewater treatment plant of China. Chemical Engineering Journal, 362, 176–182.
38. Liu, W., Zhang, J., Liu, H., Guo, X., Zhang, X., Yao, X., Cao, Z., & Zhang, T. (2021). A review of the removal of microplastics in global wastewater treatment plants: Characteristics and mechanisms. Environment International, 146, 106277.
39. Lofty, J., Muhawenimana, V., Wilson, C.A.M.E., & Ouro, P. (2022). Microplastics removal from a primary settler tank in a wastewater treatment plant and estimations of contamination onto European agricultural land via sewage sludge recycling. Environmental Pollution, 304, 119198.
40. Ma, J., Gong, Z., Wang, Z., Liu, H., Chen, G., & Guo, G. (2022). Elucidating degradation properties, microbial community, and mechanism of microplastics in sewage sludge under different terminal electron acceptors conditions. Bioresource Technology, 346, 126624.
41. Magni, S., Binelli, A., Pittura, L., Avio, C.G., Della Torre, C., Parenti, C.C., & Regoli, F. (2019). The fate of microplastics in an Italian Wastewater Treatment Plant. Science of the Total Environment, 652, 602–610.
42. Magnusson, K., & Norén, F. (2014). Screening of microplastic particles in and down-stream a wastewater treatment plant. Digitala Vetenskapliga Arkivet. diva-portal.org
43. Melo, A., Quintelas, C., Ferreira, E.C., & Mesquita, D.P. (2022). The role of extracellular polymeric substances in micropollutant removal. Frontiers in Chemical Engineering, 4, 778469.
44. Murphy, F., Ewins, C., Carbonnier, F., & Quinn, B. (2016). Wastewater treatment works (WwTW) as a source of microplastics in the aquatic environment. Environmental Science and Technology, 50(11), 5800–5808.
45. Napper, I.E., & Thompson, R.C. (2016). Release of synthetic microplastic plastic fibres from domestic washing machines: Effects of fabric type and washing conditions. Marine Pollution Bulletin, 112(1–2), 39–45.
46. Ngo, P.L., Pramanik, B.K., Shah, K., & Roychand, R. (2019). Pathway, classification and removal efficiency of microplastics in wastewater treatment plants. Environmental Pollution, 255, 113326.

47. Oberbeckmann, M.G.J., Löder, M., & Labrenz M., (2015). Marine microplastic-associated biofilms – a review. Environmental Chemistry, 12(5), 551–562.
48. Patil, S., Kamdi, P., Chakraborty, S., Das, S., Bafana, A., Krishnamurthi, K., & Sivanesan, S. (2023). Characterization and removal of microplastics in a sewage treatment plant from urban Nagpur, India. Environmental Monitoring and Assessment, 195(1), 47.
49. Petroody, S.S.A., Hashemi, S.H., & van Gestel, C.A. (2021). Transport and accumulation of microplastics through wastewater treatment sludge processes. Chemosphere, 278, 130471.
50. Plastics Europe, (2023). Plastics — The Facts 2022. An Analysis of European Plastics Production, Demand and Waste Data. https://plasticseurope.org/ (dostęp: 15.04.2024)
51. Pleskytė, S., Uogintė, I., Burbulytė, A., & Byčenkienė, S. (2023). Characteristics and removal efficiency of microplastics at secondary wastewater treatment plant in Lithuania. Water Environment Research, 95(12), 10958.
52. Propozycja Dyrektywy Parlamentu Europejskiego i Rady z dnia 26.10.2022 (2022/0345) dotycząca oczyszczania ścieków komunalnych. (dostęp: 15.04.2024)
53. Rusanowska, P., (2015). Różnorodność i liczebność bakterii denitryfikacyjnych w tlenowym osadzie granulowanym. Rozprawa doktorska, Uniwersytet Warmińsko-Mazurski w Olsztynie.
54. Simon, M., van Alst, N., & Vollertsen, J. (2018). Quantification of microplastic mass and removal rates at wastewater treatmentplants applying Focal Plane Array (FPA)-based Fourier Trans-form Infrared (FT-IR) imaging. Water Research, 142, 1–9.
55. Sol, D., Laca, A., Laca, A., & Díaz, M. (2020). Approaching the environmental problem of microplastics: Importance of WWTP treatments. Science of the Total Environment, 740, 140016.
56. Sorensen, R.M., & Jovanović, B. (2021). From nanoplastic to microplastic: A bibliometric analysis on the presence of plastic particles in the environment. Marine Pollution Bulletin, 163, 111926.
57. Taghavi, N., Singhal, N., Zhuang, W.Q., & Baroutian, S. (2021). Degradation of plastic waste using stimulated and naturally occurring microbial strains. Chemosphere, 263, 127975.
58. Talvitie, J., Mikola, A., Koistinen, A., & Setälä, O. (2017a) Solutions to microplastic pollution – Removal of microplastics from wastewater effluent with advanced wastewater treatment technologies. Water Research, 123, 401–407.

59. Talvitie, J., Mikola, A., Setälä, O., Heinonen, M., & Koistinen, A. (2017b). How well is microlitter purified from wastewater? – A detailed study on the stepwise removal of microlitter in a tertiary level wastewater treatment plant. Water Research, 109, 164–172.
60. Thompson, R.C. (2015). Microplastics in the marine environment: sources, consequences and solutions. W: Marine Anthropogenic Litter. Springer International Publishing, 185–200, Edytorzy: Melanie Bergmann, Lars Gutow, Michael Klages.
61. Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W., & Russell, A.E. (2004). Lost at sea: where is all the plastic? Science, 304(5672), 838–838.
62. Torena, P., Alvarez-Cuenca, M., & Reza, M. (2021). Biodegradation of polyethylene terephthalate microplastics by bacterial communities from activated sludge. The Canadian Journal of Chemical Engineering, 99, S69–S82.
63. Usha, R., Sangeetha, T., & Palaniswamy, M. (2011). Screening of polyethylene degrading microorganisms from garbage soil. Libyan Agriculture Research Center Journal International, 2(4), 200–204.
64. Vercauteren, M., Semmouri, I., Van Acker, E., Pequeur, E., Janssen, C.R., & Asselman, J. (2023). Toward a better understanding of the contribution of wastewater treatment plants to microplastic pollution in receiving waterways. Environmental Toxicology and Chemistry, 42(3), 642–654.
65. Vivekanand, A.C., Mohapatra, S., & Tyagi, V.K. (2021). Microplastics in aquatic environment: Challenges and perspectives. Chemosphere, 282, 131151.
66. Wang T., Li B., Zou X., Wang Y., Li Y., Xu Y., Mao L., Zhang C., & Yu W. (2019). Emission of primary microplastics in mainland China: invisible but not negligible. Water Research, 162, 214–224.
67. Wagner, M. (2022). Solutions to plastic pollution: A conceptual framework to tackle a wicked problem. Microplastic in the Environment: Pattern & Process, 333–352.
68. Wagner, S., Hüfer, T., Klöckner, P., Wehrhahn, M., Hofmann, T., & Reemtsma, T. (2018). Tire wear particles in the aquatic environment – a review on generation, analysis, occurrence, fate and effects. Water Research 139, 83–100.
69. Wik, A., & Dave, G. (2009). Occurrence and effects of tire wear particles in the environment – A critical review and an initial risk assessment. Environmental Pollution, 157(1), 1–11.
70. Winkler, M.H., Kleerebezem, R., De Bruin, L.M.M., Verheijen, P.J.T., Abbas, B., Habermacher, J., & Van Loosdrecht, M.C.M. (2013). Microbial diversity differences within aerobic granular sludge and activated sludge flocs. Applied Microbiology and Biotechnology, 97, 7447–7458.

71. Xia, J., Ye, L., Ren, H., & Zhang, X.X. (2018). Microbial community structure and function in aerobic granular sludge. Applied Microbiology and Biotechnology, 102, 3967–3979.
72. Yu, Y., Kumar, M., Bolan, S., Padhye, L.P., Bolan, N., Li, S., & Li, Y. (2023). Various additive release from microplastics and their toxicity in aquatic environments. Environmental Pollution, 123219.
73. Yuan, J., Ma, J., Sun, Y., Zhou, T., Zhao, Y., & Yu, F. (2020). Microbial degradation and other environmental aspects of microplastics/plastics. Science of the Total Environment, 715, 136968.
74. Ziajahromi, S., Neale, P.A., Rintoul, L., & Leusch, F.D. (2017). Wastewater treatment plants as a pathway for microplastics: development of a new approach to sample wastewater-based microplastics. Water Research, 112, 93–99.

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Coagulation and Flocculation before Primary Clarification as Efficient Solutions for Low-Density Microplastic Removal from Wastewater

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Abstract: Microplastic (MP) removal from wastewater was investigated using various types and doses of commercial coagulants (PIX, PAX) and flocculants (FPM, PEL, FCT) before primary clarification in a wastewater treatment plant (WWTP). Dosing with FPM, PIX, and PEL caused small MPs (180–212 µm) to be transferred mainly to the settled sludge (up to 86.4% of MP at a dose of 5 mL FMP/m^3), while dosing of FCT and PAX caused these MPs to be transferred to the floated sludge (up to 64% MP at a dose of 5 mL PAX/m^3). The efficiency of MP removal from wastewater was the highest (90%) with 2.5 mL PAX/m^3; the generated primary sludge had a low MP content and could be safely managed in subsequent stages of sludge treatment. At the highest doses, PIX significantly increased the removal of P-PO_4 (up to 94%) and COD (up to 73%). FPM and FCT resulted in over 40% efficiency of ammonium removal—such disturbance in wastewater composition may negatively affect further biological treatment. Effective removal of MP in the mechanical part of WWTP resulting from coagulation and flocculation enables the safe use of the excess sludge for agricultural purposes.

Keywords: microplastic; wastewater; wastewater treatment plant; coagulants; flocculants; micropollutants

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1. Introduction

Microplastic (MP) is a micropollutant commonly present in wastewater. Primary microplastics include industrial 'scrubbers' used to blast clean surfaces, plastic powders used in molding, and plastic nanoparticles used in various industrial processes. Kalčíková et al. [1] showed that concentrations of microbeads varied from 0.42 to 11.12 mg per 100 mL of the tested personal care products (facial and body scrubs). As primary MPs utilized in consumer products are washed down the drain, there is a growing concern regarding the direct emission of MP into aquatic environments [2]. Hidayaturrahman and Lee [3] have shown that microbeads comprise the highest percentage of microplastic particles (with regards to the shape) present in effluents the different wastewater treatment plants (WWTPs). Due to the smooth surface of microbeads, lower adsorption capacity in the mechanical part of WWTPs is expected compared to that of irregular shaped particles of MP [4].

The presence of MP in wastewater entering the biological part of WWTPs, with MP accumulation in the biomass, negatively affects the metabolic potential of the biomass. It was observed that polyether sulfone (PES) at a concentration of 0.5 g MP/L slightly inhibited ammonium removal and significantly decreased the specific rate of nitrate reduction. The presence of PES inhibited the activity of nitrite oxidase in biomass and increased the metabolism of amino acids. After increasing the level of nitrate reductase 1 in bacterial cells as a result of PES MP addition, the quantities of cytochrome c-containing subunit II and cytochrome b-containing subunit I decreased, leading to the accumulation of nitrite nitrogen and affecting nitrogen metabolism. High-throughput sequencing showed that the presence of PES in wastewater reduced the abundance of denitrifying *Bacillales* in aerobic

granules and stimulated the growth of *Anaeroline*, which can anaerobically metabolize hydrocarbons that are present in MP [5].

The highest MP removal occurs in the mechanical part of wastewater treatment plants (WWTPs) (50–98%), and is related to the high efficiency of removing suspended solids with adsorbed MPs [6]. Most technological solutions for MP removal that currently in testing postulate the addition of a third treatment stage in the form of membranes, sand traps, coagulation, or ozonation chambers [7]. However, the addition of a third treatment stage increases the WWTP's operating costs and does not prevent the accumulation of MPs in the sludge [3]. Research by Park et al. [8] at 50 South Korean wastewater treatment plants (WWTPs) showed that the concentration of MP in the influent varied from 14 to 470 MP/L. High MP removal efficiency in wastewater treatment systems, reaching > 90% [9,10], results from the fact that large amounts of MP (22–89%) are transferred to sludge. Koutnik et al. [11] reported that the concentration of MP in sewage sludge might reach 39020 MP/kg of dry mass (DM).

In wastewater treatment, coagulation lowers the electrokinetic potential of colloidal particles and suspensions and allows their aggregation. During coagulation, chemicals react with wastewater, and some soluble pollutants are precipitated and then removed by settling [12]. In the studies of El-Gohara et al. [13], the addition of aluminum coagulant (600 mg/L) to wastewater from the personal care-products industry resulted in efficiencies of COD and total suspended solids (TSS) removal of 76% and 94%, respectively. Coagulation efficiency depends on the appropriate selection of coagulant dose, and for dose optimization, the total costs of the process must be considered [14]. To remove TSS from wastewater, flocculants can be used that cross-link suspensions to form large, quickly settling agglomerates [15]. There is no data in the literature on using coagulants or flocculants (C/Fs) to intensify MP removal from wastewater in the primary clarifier.

The study investigated the effect of the dose and type of C/Fs on the efficiency of polyethylene (PE) MP removal from wastewater before the primary clarifier. It also aimed to determine how the amount and type of C/Fs affect the removal of other pollutants from wastewater in the context of disturbance of biological wastewater treatment. Removal of MPs in the mechanical part of WWTPs will eliminate the negative impact of MP on the biological part of the WWTPs, and will enable agricultural use of the excess sludge. In this study, MP in the form of PE was tested because this type of plastic predominates in wastewater that contains personal care products [16].

2. Materials and Methods

2.1. Substrate

Wastewater for the experiment was collected before the primary clarifier at the WWTP "Łyna" in Olsztyn (Poland). The concentration of pollutants in the wastewater averaged 436.0 ± 32.0 mg COD/L, 41.4 ± 5.3 mg N-NH_4/L, 18.5 ± 2.5 mg P-PO_4/L, 2740 ± 350 mg DM/L. The pH and salinity (mS/cm) of the wastewater were 7.81 and 0.98, respectively.

Known concentrations of fluorescent polyethylene microbeads (Figure S1; Cospheric, Santa Barbara, CA, USA) with diameters of 710–850 µm (green) and 180–212 µm (blue) were added to the wastewater.

2.2. Experiment Set-Up

The experiment was carried out in 1000 mL beakers in a coagulation apparatus with paddle stirrers (Flock Tester AL 46-6, Aqualytic, Dortmund, Germany). Wastewater and the appropriate C/Fs were added to the beakers. The contents of the beakers were intensively mixed for 2 min (250 RPM), then slowly mixed for 28 min (100 RPM), followed by 15 min of settling.

In the experiment, flocculants Flopam EM 840 MEB (FPM), Praestrol k 255 l (PEL), Flocculant F-290 (FCT), and coagulants PIX 113 (PIX), PAX 18 (PAX) (Table 1) were used. Working solutions of C/Fs were prepared 24 h before use by diluting 1 mL (liquid form) or

1 g (dry form) of the respective C/F in distilled water. The working solutions were dosed into the beakers at doses of 1.0, 2.5, and 5.0 mL/L. Experiments and analyses were carried out in triplicate for each type and dose of C/F.

Table 1. Properties of the C/Fs.

Name of C/Fs (Company)	Chemical Composition	The Concentration of C/Fs Used (mL of Solution/L of Wastewater)
FPM (Korona JV)	Hydrocarbons: C_{12}–C_{15}, n-alkanes, isoalkanes, cyclic, <2% aromatic; ethoxylatedisotridecanol	1.10 mg/L
PEL (Stockhausen GMbH & Co.)	The copolymer of acrylamide and a cationic acrylic acid derivative in isoparaffin hydrocarbons	1.03 mg/L
FCT (Korona JV)	Adipic and sulfamide acid	0.90 mg/L
PIX (kemipol)	40–42% aqueous solution of iron(III) sulfate, consisting of 11.8 ± 0.4% of the total iron and up to 1% of free sulphuric acid	0.53 mg/L
PAX (kemipol)	Aqueous solution of polyaluminium chloride containing 17.0 ± 0.6% Al_2O_3 and 20.0 ± 2.0% of chloride ions	1.4 mg/L

Three fractions were collected from the experimental beakers: surface phase (upper 100 mL of wastewater and floated suspensions), liquid phase, and sludge phase (100 mL of sludge and wastewater from the bottom of the beaker); it was assumed that MP was present in the surface and sludge phase. Each collected fraction was filtered on filters with a pore diameter of 125 µm in a Büchner funnel at a constant vacuum of 500 g/cm^2 (Figure 1). Each sample was filtered several times to avoid overloading a filter with suspended solids which may obscure the MP count. The readings from all filters were summed to describe the content of MP in a particular sample. Filtration was carried out in a laminar flow box to avoid loss of particles. The MP particles on the filters were counted using an optical binocular (Delta Optical SZ-450) with a 10× zoom in the darkroom, using UV to activate the fluorescent dye.

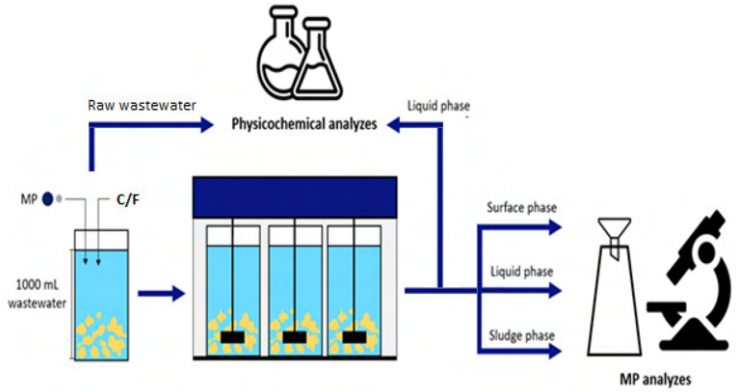

Figure 1. The scheme of the experiment.

Chemical analyses of raw and liquid phases (in triplicate) included COD, TSS, N-NH_4, and P-PO_4 [17]. The pH was measured with a TitroLine easy (Donserv). Observations of the settled sludge were carried out using an optical microscope equipped with a camera (Nikon Eclipse 50I).

3. Results and Discussion

3.1. Removal of MP

The research assumed that the transition of MP from wastewater to floated or settled sludge would effectively eliminate MP in the primary clarifier, minimizing its negative effect on biomass in aeration tanks and on the quality of excess sludge. The studies by Petroody et al. [18] showed that the primary clarifier can remove 75.2% of the incoming MP and that the particle removal efficiency increased proportionally to the MP size. Plastic particles of 37–300 µm were removed with an efficiency of 70%, while particles larger than 500 µm were removed with an efficiency of 85%. Low-density MPs float on the surface of the wastewater with grease or oil and are mainly removed by surface skimming [19].

In the present study, coagulants influenced the distribution of MP in the wastewater column (Figure 2A). Application of FPM, PIX, and PEL caused mainly smaller MP particles to be retained in the sludge phase (62.0–86.4%). In the cases of FCT and PAX, most of the MP was present in the surface phase (36.0–64.0%). For all C/Fs, except PEL, the percentage of MP particles in the liquid phase decreased with an increasing dose of C/Fs. Although different coagulants demonstrated different removal effects on MPs, their specific removal mechanism can still be explained by classical coagulation removal mechanisms, such as charge neutralization, adsorption, and sweep flocculation. The hydrolysates of metal coagulant can be adsorbed on the surfaces of negatively charged MPs, neutralizing the original charge on the MP surface and reducing the electrostatic repulsion, accordingly making the MPs unstable. The positively charged coagulant hydrolyzed monomers can adsorb the surrounding negatively charged MPs to form flocs [20]

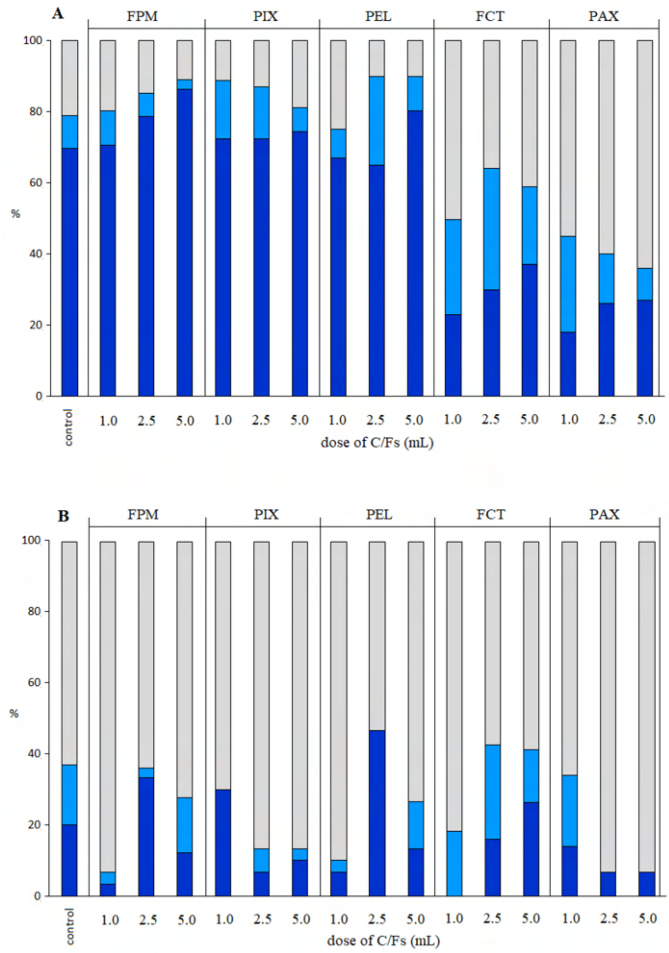

Figure 2. Distribution of MP with a diameter of 180–212 µm (**A**) and 710–850 µm (**B**) in the surface phase (grey), the liquid phase (light blue), and the sludge phase (dark blue).

In the controls, even without using C/Fs, the removal of MP was observed as a result of the sedimentation process. Researchers [1,21] reported that MP was captured in biomass of sludge settling at the bottom of the reactor. This was caused by the adhesion of dissolved organic matter particles to the MP structure, creating an eco-crown and decreasing hydrophobicity [22].

Most of the smaller MP particles (34.0% of all MPs) remained in the liquid phase and potentially entered the biological part of the WWTP when FCT was used (2.5 mL/L). The most efficient removal of small MPs from the liquid phase was observed at a dose of 5 mL FPM/L—only 4% of those MPs remained in the treated wastewater. Ma et al. [23] observed that dosing with C/F resulted in high efficiency of removal of MPs with small particle diameter. Addition to wastewater of 0.2 mmol/L of $FeCl_3 \cdot 6H_2O$ and 15 mg/L of cationic polyacrylamide flocculant led to 1–5% average efficiency of removal of MPs with diameters of 0.5–5 mm. In contrast, MP with diameters 140 µm and 15 µm were removed with 10% higher efficiency [24]. In the present study, MP in the form of granules was used. Granular MP is more complicated to remove than, e.g., MP in the form of fibers, due to the smaller reactive surface of granules, which limits the interaction with chemical compounds. Shahi et al. [25], in experiments on MP removal from water, reported that even at very high doses of coagulant, e.g., 50 mg/L (economically unjustified), the removal efficiency of granular MP did not exceed 32%. Our research shows that this efficiency was much higher (even up to 96%) when the coagulation process was applied to wastewater before the primary clarifier.

After the application of C/Fs, the larger MP particles (710–850 µm) were mainly found in the surface phase (53.3–93.4%) (Figure 2B). The percentage of larger MP particles in the liquid phase remained at the level of 2–10% for most of the C/Fs, only for 2.5 mL FCT/L did this percentage increase to 26.6%. Lofty et al. [26] reported that after a seven-day sampling period, a higher concentration of particles with 1000–5000 µm diameters was present in the scum than in the sludge (1271 vs. 771 MP/L) in the primary settling tank. The higher concentration of MPs in the scum resulted from a lower density than water, or from their flocculation with floating fats, oils, and grease. The tested MP was eliminated after applying 1.0 mL PIX/L, 2.5 mL PEL/L, and PAX at the two highest doses. The weight of MP particles with diameters of 710–850 µm was significantly greater than that of MP particles with diameters of 180–212 µm (0.321 mg vs. 0.007 mg). Therefore, the total weight of removed MP was determined by removing the larger MP particles. Murphy et al. [27] found that microbeads were effectively removed by skimming. This was consistent with other studies conducted by Michielssen et al. [28] and Sutton et al. [29]; both studies found that microbeads were absent in the effluent of WWTPs. In contrast, a survey conducted on WWTPs in New York showed that 4 out of 10 WWTPs still released microbeads [30]. This difference might be due to the different amounts of fat, grease, and oil in the wastewater, as these compounds are important for microplastics skimming.

3.2. Effectiveness of Pollutant Removal from Wastewater

Based on the quality of wastewater after C/Fs treatment, a dendrogram was created, grouping samples with similar effectiveness of removal of individual pollutant indicators (Figure 3). Settling of wastewater for 15 min without the addition of C/Fs reduced the concentration of COD (31%) and total suspended solids (16%), but did not significantly affect the reduction of N-NH_4 and P-PO_4 (<2%). High efficiency of N-NH_4 removal was observed for wastewater treated with FPM—36% and 41% of ammonium nitrogen was removed at doses of 2.5 and 5.0 mL/L, respectively. Such high efficiency of N-NH_4 removal may result from the fact that FPM contains polyacrylamide, which can absorb N-NH_4 on its surface [31].

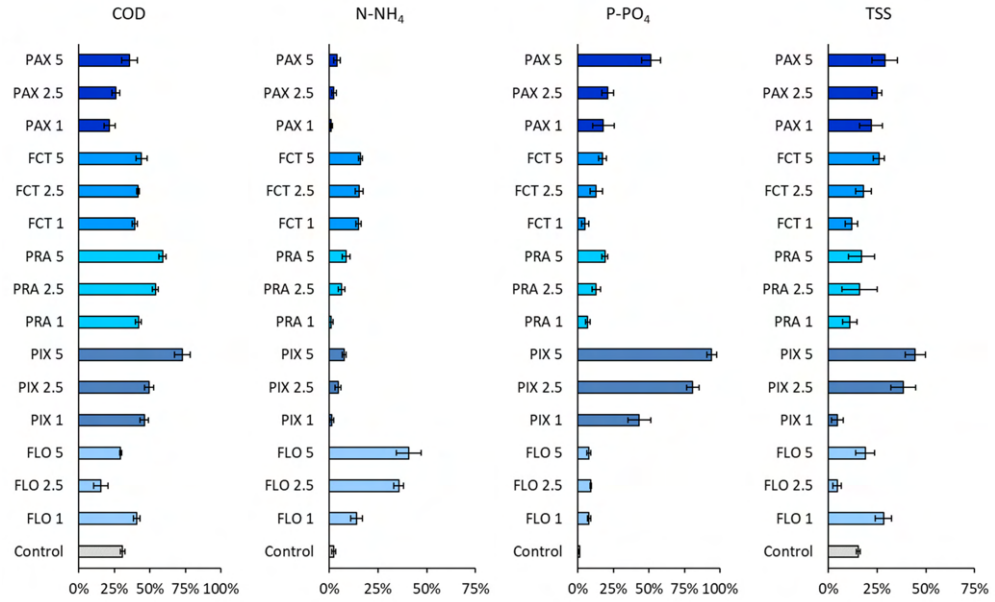

Figure 3. The effectiveness of pollutant removal from raw wastewater depending on the type and dose of C/Fs.

Application of PIX at a dose ≥ 2.5 mL/L resulted in high efficiency of TSS removal (up to 45%). High efficiency of P-PO_4 was observed for PIX and PAX. The efficiency of P-PO_4 increased from 43% at the dose of 1 mL PIX/L, to 94% at the dose of 5 mL PIX/L. Fe(III) salts in PIX were dissociated, and the Fe(III) ions reacted with P-PO_4—the obtained salts were precipitated. The efficiency of COD removal increased with increasing doses of C/Fs. The highest efficiency of COD removal was observed for PEL and PIX at 5 mL/L—the use of 5 mL PIX/L reduced the COD concentration in wastewater by over 70%. Efficient COD removal resulted from the combination of charge neutralization, entrapment, adsorption, and complexation with coagulant metal ions present in PIX into insoluble particulate aggregates [32]. Too much removal of one of the nutrients in the mechanical part of WWTP due to C/F application may affect the operation of the biological part of WWTP. COD is necessary for the effective biological removal of P and N. For example, lowering the COD/N ratio from 4.5 to 2.3 in the influent decreased denitrification efficiency and, consequently, the removal of nitrogen from wastewater [33]. Optimization of the dose of coagulants in the mechanical part of the WWTP is necessary to avoid the transfer of coagulants to the biological part. Philips et al. [34] showed that the continuous dosing of iron(III) salt to the activated sludge gradually inhibited total and nitrifying activity. This toxicity can be attributed to decreased pH due to forming iron hydroxides. The observed loss of nitrification was also partly a consequence of the negative effect of ferric iron on the structure of activated flocs.

Microscopic analysis showed that the addition of C/Fs affected the structure of the settled agglomerates (Figure 4). In the control wastewater, suspensions with diameter < 100 µm predominated. The largest suspensions with diameter > 1 mm were formed at the dose of 5 mL/L FCT and FPM. The presence of aromatic groups in FPM facilitates hydrophobic interactions between the flocculant polymer and the surface of the particle, which enhances agglomeration. Furthermore, the structure of this polymer, which is more branched than the other polymers, enables it to adsorb more particles and form denser aggregates [35]. In this study, microscopic analysis showed that increasing the C/F doses stimulated cross-linking and increased the density of aggregates. The high specific surface area of those aggregates favored the removal of undissolved pollutants from the liquid phase of the wastewater. Coagulants destabilize the surface charge of colloids and stabilize the surface charge of the suspended MPs, which allows the particles to come close enough to enable van der Waals interactions and particle agglomeration [36]. The maximum particle size that can be incorporated into an existing

floc is proportional to the size of the floc [24]. The studies of Kalčíková et al. [1] showed that PE microgranules had a very high affinity for activated sludge and accumulated in sludge and fragmented organic matter at the bottom of the reactor.

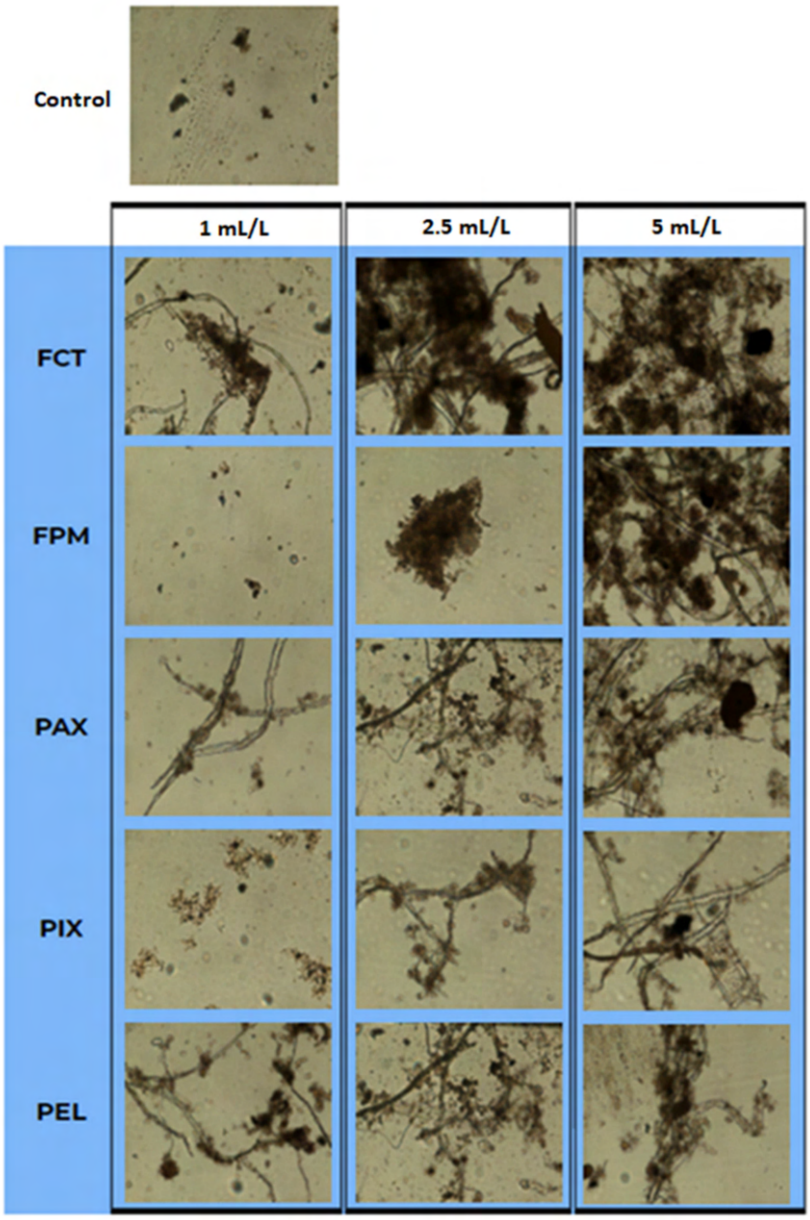

Figure 4. Microscopic analysis of solids from the sludge phase depending on the type and dose of C/Fs.

In all cases, the addition of C/Fs to wastewater changed the pH value (Figure 5). The change in pH value towards alkaline was noted for FCT and PEL. FPM and PIX decreased responses by maximum 0.50 and 0.54 pH units, respectively. The dose of 1.0 mL PAX/L increased the pH value; the other doses resulted in its decrease.

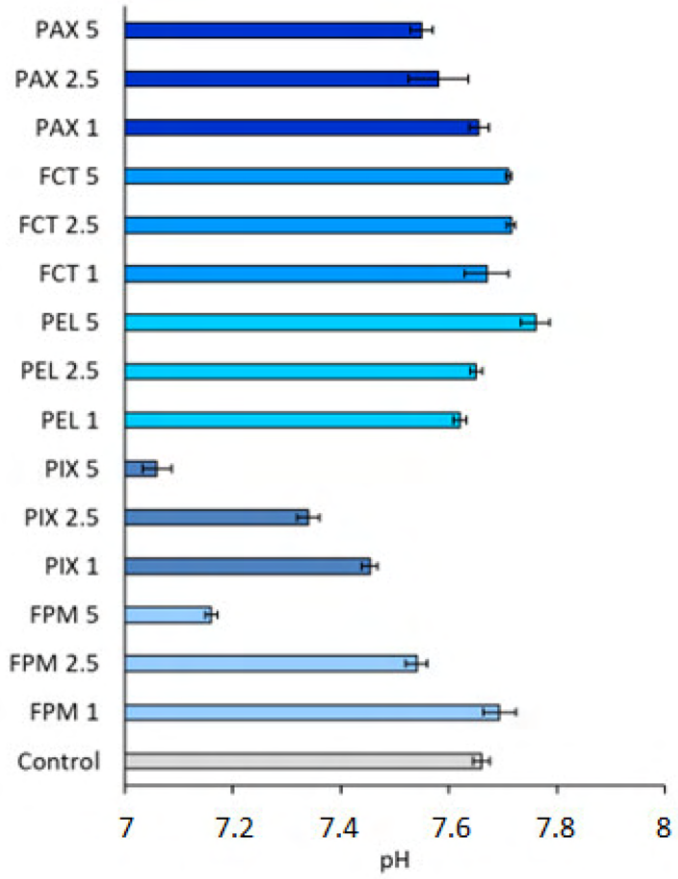

Figure 5. The pH of wastewater after dosing with C/Fs.

4. Conclusions

The addition of C/F to raw wastewater caused from 66.6 to 97.3% of MP to be transferred from wastewater to the floated or settled sludge, enabling effective MP removal in the primary clarifier. MPs with larger diameters (710–850 µm) were mainly found in the floated sludge. The highest efficiency of MP removal from wastewater (90%) was observed for 2.5 mL PAX/L—the low content of MP in the generated primary sludge enabled its safe use in subsequent stages of sludge management. Increasing the dose of C/F resulted in cross-linking of the sludge structure and increasing sludge density, which favored the incorporation of MP. The highest amount of PIX (5 mL/L), although most efficient for MP removal, resulted in 94 and 73% efficiencies of P-PO_4 and COD removal, respectively, which may adversely affect the functioning of the biological part of the wastewater treatment plant.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijerph192013013/s1, Figure S1: Photography of MP under UV light in the filter after filtration of wastewater

Author Contributions: Conceptualization, P.J. and A.C.-K.; methodology, P.J.; software, P.J.; validation, P.J.; formal analysis, P.J.; investigation, P.J. and A.C.-K.; resources, P.J.; data curation, P.J.; writing—original draft preparation, P.J.; writing—review and editing, P.J and A.C.-K.; visualization, P.J.; supervision, P.J., A.C.-K.; project administration, P.J.; funding acquisition, A.C.-K. All authors have read and agreed to the published version of the manuscript.

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References

1. Kalčíková, G.; Alič, B.; Skalar, T.; Bundschuh, M.; Gotvajn, A.Ž. Wastewater treatment plant effluents as source of cosmetic polyethylene microbeads to freshwater. *Chemosphere* **2017**, *188*, 25–31. [CrossRef] [PubMed]
2. Fendall, L.S.; Sewell, M.A. Contributing to marine pollution by washing your face: Microplastics in facial cleansers. *Mar. Pollut. Bull.* **2009**, *58*, 1225–1228. [CrossRef] [PubMed]
3. Hidayaturrahman, H.; Lee, T.-G. A study on characteristics of microplastic in wastewater of South Korea: Identification, quantification, and fate of microplastics during treatment process. *Mar. Pollut. Bull.* **2019**, *146*, 696–702. [CrossRef] [PubMed]
4. Ding, L.; Luo, Y.; Yu, X.; Ouyang, Z.; Liu, P.; Guo, X. Insight into interactions of polystyrene microplastics with different types and compositions of dissolved organic matter. *Sci. Total Environ.* **2022**, *824*, 153883. [CrossRef] [PubMed]
5. Qin, R.; Su, C.; Liu, W.; Tang, L.; Li, X.; Deng, X.; Chen, Z. Effects of exposure to polyether sulfone microplastic on the nitrifying process and microbial community structure in aerobic granular sludge. *Bioresour. Technol.* **2020**, *302*, 122827. [CrossRef]
6. Sun, J.; Dai, X.; Wang, Q.; van Loosdrecht, M.C.M.; Ni, B.-J. Microplastics in wastewater treatment plants: Detection, occurrence and removal. *Water Res.* **2019**, *152*, 21–37. [CrossRef]
7. Hu, Y.; Gong, M.; Wang, J.; Bassi, A. Current research trends on microplastic pollution from wastewater systems: A critical review. *Rev. Environ. Sci. Biotechnol.* **2019**, *18*, 207–230. [CrossRef]
8. Park, H.J.; Oh, M.J.; Kim, P.G.; Kim, G.; Jeong, D.H.; Ju, B.K.; Kwon, J.H. National reconnaissance survey of microplastics in municipal wastewater treatment plants in Korea. *Environ. Sci. Technol.* **2020**, *54*, 1503–1512. [CrossRef]
9. Gies, E.A.; LeNoble, J.L.; Noël, M.; Etemadifar, A.; Bishay, F.; Hall, E.R.; Ross, P.S. Retention of microplastics in a major secondary wastewater treatment plant in Vancouver, Canada. *Mar. Pollut. Bull.* **2018**, *133*, 553–561. [CrossRef] [PubMed]
10. Lares, M.; Ncibi, M.C.; Sillanpää, M.; Sillanpää, M. Occurrence, identification and removal of microplastic particles and fibers in conventional activated sludge process and advanced MBR technology. *Water Res.* **2018**, *133*, 236–246. [CrossRef]
11. Koutnik, V.S.; Alkidim, S.; Leonard, J.; DePrima, F.; Cao, S.; Hoek, E.M.; Mohanty, S.K. Unaccounted Microplastics in Wastewater Sludge: Where Do They Go? *ACS ES&T Water* **2021**, *1*, 1086–1097. [CrossRef]
12. Kwak, D.H.; Lee, K.C. Effect of floated sludge recycling on phosphorus removal in dissolved air flotation. In Proceedings of the Institution of Civil Engineers—Water Management; Thomas Telford Ltd.: London, UK, 2015; Volume 168, pp. 270–279. [CrossRef]
13. El-Gohary, F.; Tawfik, A.; Mahmoud, U. Comparative study between chemical coagulation/precipitation (C/P) versus coagulation/dissolved air flotation (C/DAF) for pre-treatment of personal care products (PCPs) wastewater. *Desalination* **2010**, *252*, 106–112. [CrossRef]
14. Kumar, V.; Al-Gheethi, A.; Asharuddin, S.M.; Othman, N. Potential of cassava peels as a sustainable coagulant aid for institutional wastewater treatment: Characterisation, optimisation and techno-economic analysis. *Chem. Eng. J.* **2021**, *420*, 127642. [CrossRef]
15. Somasundaran, P.; Runkana, V.; Kapur, P. Flocculation and dispersion of colloidal suspensions by polymers and surfactants: Experimental and modeling studies, Coagulation and Flocculation. *Coagul. Flocculation* **2005**, *126*, 767–804. [CrossRef]
16. Chua, E.M.; Shimeta, J.; Nugegoda, D.; Morrison, P.D.; Clarke, B.O. Assimilation of polybrominated diphenyl ethers from microplastics by the marine amphipod, *Allorchestes compressa*. *Environ. Sci. Technol.* **2014**, *48*, 8127–8134. [CrossRef]
17. APHA (Ed.) *Standard Methods for the Examination of Water and Wastewater*, 18th ed.; APHA: Washington, DC, USA, 1992.
18. Petroody, S.S.A.; Hashemi, S.H.; van Gestel, C.A. Factors affecting microplastic retention and emission by a wastewater treatment plant on the southern coast of Caspian Sea. *Chemosphere* **2020**, *261*, 128179. [CrossRef]
19. Ngo, P.L.; Pramanik, B.K.; Shah, K.; Roychand, R. Pathway, classification and removal efficiency of microplastics in wastewater treatment plants. *Environ. Pollut.* **2019**, *255*, 113326. [CrossRef]
20. Xu, Q.; Huang, Q.S.; Luo, T.Y.; Wu, R.L.; Wei, W.; Ni, B.J. Coagulation removal and photocatalytic degradation of microplastics in urban waters. *J. Chem. Eng.* **2021**, *416*, 129123. [CrossRef]
21. Jachimowicz, P.; Jo, Y.J.; Cydzik-Kwiatkowska, A. Polyethylene microplastics increase extracellular polymeric substances production in aerobic granular sludge. *Sci. Total Environ.* **2022**, *851*, 158208. [CrossRef]
22. Wang, X.; Liang, D.; Wang, Y.; Peijnenburg, W.J.; Monikh, F.A.; Zhao, X.; Fan, W. A critical review on the biological impact of natural organic matter on nanomaterials in the aquatic environment. *Carbon Res.* **2022**, *1*, 13. [CrossRef]
23. Ma, B.; Xue, W.; Ding, Y.; Hu, C.; Liu, H.; Qu, J. Removal characteristics of microplastics by Fe-based coagulants during drinking water treatment. *Res. J. Environ. Sci.* **2019**, *78*, 267–275. [CrossRef] [PubMed]
24. Lapointe, M.; Farner, J.M.; Hernandez, L.M.; Tufenkji, N. Understanding and improving microplastic removal during water treatment: Impact of coagulation and flocculation. *Environ. Sci. Technol.* **2020**, *54*, 8719–8727. [CrossRef] [PubMed]
25. Shahi, N.K.; Maeng, M.; Kim, D.; Dockko, S. Removal behavior of microplastics using alum coagulant and its enhancement using polyamine-coated sand. *Process Saf. Environ. Prot.* **2020**, *141*, 9–17. [CrossRef]
26. Lofty, J.; Muhawenimana, V.; Wilson, C.A.M.E.; Ouro, P. Microplastics removal from a primary settler tank in a wastewater treatment plant and estimations of contamination onto European agricultural land via sewage sludge recycling. *Environ. Pollut.* **2022**, *304*, 119198. [CrossRef] [PubMed]

27. Murphy, F.; Ewins, C.; Carbonnier, F.; Quinn, B. Wastewater treatment works (WwTW) as a source of microplastics in the aquatic environment. *Environ. Sci. Technol.* **2016**, *50*, 5800–5808. [CrossRef] [PubMed]
28. Michielssen, M.R.; Michielssen, E.R.; Ni, J.; Duhaime, M. Fate of microplastics and other small anthropogenic litter (SAL) in wastewater treatment plants depends on unit processes employed. *Environ. Sci. Water Res. Technol.* **2016**, *2*, 1064–1073. [CrossRef]
29. Sutton, R.; Mason, S.A.; Stanek, S.K.; Willis-Norton, E.; Wren, I.F.; Box, C. Microplastic contamination in the San Francisco bay, California, USA. *Mar. Pollut. Bull.* **2016**, *109*, 230. [CrossRef]
30. Schneiderman, E.T. *Discharging Microbeads to Our Waters: An Examination of Wastewater Treatment Plants in New York*; New York State Office of the Attorney General: New York, NY, USA, 2015; pp. 1–11. Available online: http://www.ag.ny.gov/pdfs/2015_Microbeads_Report_FINAL.pdf (accessed on 1 September 2022).
31. Shafey, E.L.; Abdel-Latif, A.M.; Abd El-Salam, H.M. The facile synthesis of poly (acrylate/acrylamide) titanium dioxide nanocomposite for groundwater ammonia removal. *Desalination Water Treat.* **2021**, *212*, 61–70. [CrossRef]
32. Dayarathne, H.N.P.; Angove, M.J.; Aryal, R.; Abuel-Naga, H.; Mainali, B. Removal of natural organic matter from source water: Review on coagulants, dual coagulation, alternative coagulants, and mechanisms. *J. Water Process Eng.* **2021**, *40*, 101820. [CrossRef]
33. Cydzik-Kwiatkowska, A.; Rusanowska, P.; Zielińska, M.; Bernat, K.; Wojnowska-Baryła, I. Structure of nitrogen-converting communities induced by hydraulic retention time and COD/N ratio in constantly aerated granular sludge reactors treating digester supernatant. *Bioresour. Technol.* **2014**, *154*, 162–170. [CrossRef]
34. Philips, S.; Rabaey, K.; Verstraete, W. Impact of iron salts on activated sludge and interaction with nitrite or nitrate. *Bioresour. Technol.* **2003**, *88*, 229–239. [CrossRef]
35. Mierczynska-Vasilev, A.; Kor, M.; Addai-Mensah, J.; Beattie, D.A. The influence of polymer chemistry on adsorption and flocculation of talc suspensions. *Chem. Eng. J.* **2013**, *220*, 375–382. [CrossRef]
36. Akbal, F.; Camcı, S. Copper, Chromium and nickel removal from metal plating wastewater by electrocoagulation. *Desalination* **2011**, *269*, 214–222. [CrossRef]

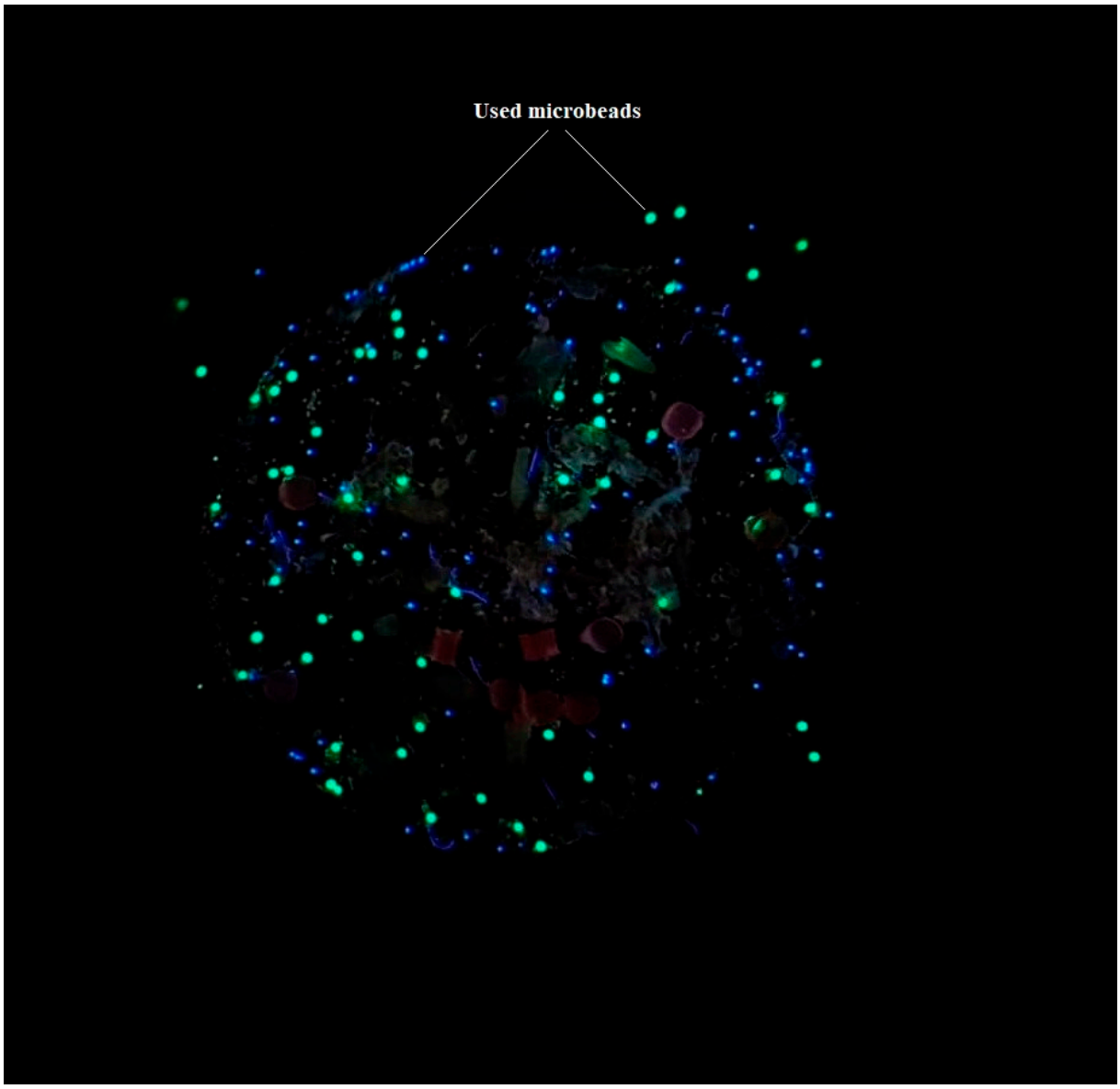

Figure S1: Photography of MP under UV light in the filter after filtration of wastewater

Olsztyn, 4.04.2024

(miejscowość, data)

Piotr Jachimowicz

(imię i nazwisko kandydata)

Inżynieria środowiska, górnictwo i energetyka

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polegał na: udziale w zaplanowaniu koncepcji badań, opracowaniu metodyki, wykonaniu części eksperymentalnej, opracowaniu i interpretacji wyników, graficznym opracowaniu wyników oraz przygotowaniu pierwszej wersji manuskryptu.

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Olsztyn, 4.04.2024

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polegał na: udziale w zaplanowaniu koncepcji badań, interpretacji wyników badań, pozyskaniu funduszy na prowadzenie badań, korekcie artykułu przed złożeniem do wydawnictwa.

Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez Pana Piotra Jachimowicza jako części rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów naukowych opublikowanych w czasopismach naukowych. Oświadczam, iż samodzielna i możliwa do wyodrębnienia część w/w pracy wykazuje indywidualny wkład kandydata Pana Piotra Jachimowicza polegający na:

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A. Cydzik-Kwiatkowska

(podpis)

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Research Paper

Tire materials disturb transformations of nitrogen compounds and affect the structure of biomass in aerobic granular sludge reactors

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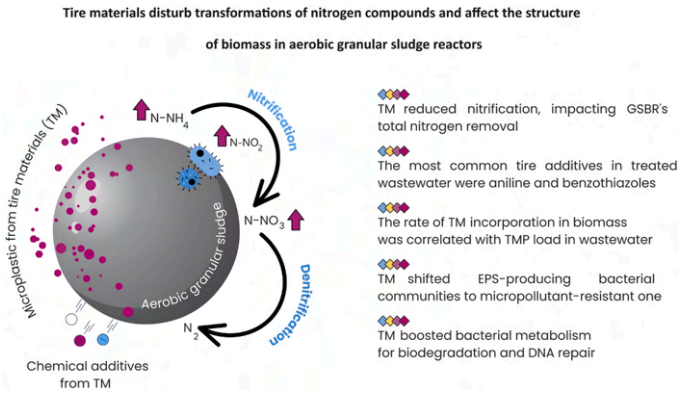
HIGHLIGHTS

- Tire materials (TMs) reduced nitrification, impacting nitrogen removal by aerobic granules.
- The most common tire additives in treated wastewater were aniline and benzothiazoles.
- The rate of TMs incorporation in biomass was correlated with TM load in wastewater.
- TMs shifted EPS-producing bacteria communities to micropollutant-resistant one.
- TMs boosted bacterial metabolism for biodegradation and DNA repair.

GRAPHICAL ABSTRACT



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ABSTRACT

Tire materials (TMs) present a notable hazard due to their potential to release harmful chemicals and microplastics into the environment. They can infiltrate wastewater treatment plants, where their effects remain inadequately understood, raising concerns regarding their influence on treatment procedures. Thus, this study investigated the impact of TMs in wastewater (10, 25, 50 mg/L) on wastewater treatment efficiency, biomass morphology, and microbial composition in aerobic granular sludge (AGS) reactors. TM dosage negatively correlated with nitrification and denitrification efficiencies, reducing overall nitrogen removal, but did not affect the efficiency of chemical-oxygen-demand removal. The presence of TMs increased the diameter of the granules due to TM incorporation into the biomass. The most frequently leached additives from TMs were N-(1,3-dimethylbutyl)-N′-phenyl-1,4-phenylenediamine, benzothiazole (BTH), and 2-hydroxybenzothiazole. In the treated wastewater, only BTH and aniline were detected in higher concentrations, which indicates that tire additives were biodegraded by AGS. The microbial community within the AGS adapted to TMs and their chemicals, highlighting the potential for efficient degradation of tire additives by bacteria belonging to the genera *Rubrivivax*, *Ferruginibacter*, and *Xanthomonas*. Additionally, our research underscores AGS's ability to incorporate TMs

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into biomass and effectively biodegrade tire additives, offering a promising solution for addressing environmental concerns related to TMs.

1. Introduction

The number of passenger cars per capita in the European Union has increased by 13.5% over the last decade and is constantly growing. There are also countries where this number has increased much more intensively over the past decade, i.e., Romania (66.8%), Estonia (43.8%), Poland (41.7%), and Slovakia (41.6%) [19]. This increased demand for automobiles has led to a corresponding increase in tire consumption.

Tire materials (TMs) are complex mixtures, containing various substances. Besides the rubber/elastomer itself, reinforcement agents (carbon black, silica, silanes), processing oils, vulcanisation agents (ZnO, S, Se, Te, thiazoles, organic peroxides, nitro compounds), and additives (e.g., halogenated cyanoalkanes, phenols, aromatic and aliphatic esters) may be added [50]. The composition of raw tire materials varies depending on the tire type. For example, in comparison with truck tires, tires of passenger cars have a higher weight percentage of additives (10% vs 14%), fillers (24% vs 26%), and textiles (0% vs 4%) [2]. Synthetic rubber (i.e., styrene butadiene rubber) is used in 75% of passenger car tires and 10% of truck tires [7]. Most additives are not covalently bound to the polymer, which allows them to be released during and after the use of the plastic product. Several tire-specific chemicals have been found in environmental compartments; benzothiazoles (BTH), phthalates, and phenols are significant ingredients of vulcanisation accelerators and have been used as tire contamination tracers [31]. Tread wear has also been shown to be a significant source of Zn and polyaromatic hydrocarbons in the environment [53].

During vehicle driving, tire wear produces small diameter (<5 mm) tire particles. These particles can be deposited on roads, emitted into the air, reach surrounding soils, or flow into sewers and end up in wastewater treatment plants (WWTPs) [4]. The estimation of the mass flow of TMs from highways to wastewater treatment systems is in the range of 3200–10800 t/year [50]. Accumulation of TMs in WWTP sludge has been estimated to reach up to 42.7 g/kg d.w. Wik and Dave [54]. The presence of these particles in sludges may result in the release of potentially toxic compounds into the aquatic environment and in TMs being transported to agricultural soils [9].

WWTPs are designed to optimize the activity of microbial communities for the oxidation of carbon and ammonia, the reduction of nitrate, and the accumulation of phosphate. Bacterial metabolism determines the effectiveness of biological wastewater treatment and micropollutant removal via enzymatic degradation [12,56]. The structure of microbial consortia in WWTPs results from the operational parameters of the purification process. Bacteria usually aggregate to form suspended flocs. Activated sludge flocs settle relatively slowly, requiring large primary and secondary settling tanks before clear effluent can be released. Alternatively, aerobic granular sludge (AGS) aggregates have been formed in sequencing batch reactors (SBRs) with short fill periods and various substrates [16]. Compared to conventional activated sludge, AGS systems have better tolerance to micropollutant toxicity, more compact microbial structure, and excellent settling properties, leading to high biomass retention [39]. The high tolerance of AGS to microplastics (MP) results from the microorganisms intensively producing extracellular polymeric substances (EPS) as a defense mechanism in stressful conditions [25]. Micropollutants can affect granules' morphology and the efficiency of pollutant removal by AGS.

Micropollutants such as e.g. Bisphenol A can induce changes in granule morphology and kinetics of nitrogen and COD conversions, however, the AGS system maintains very highefficiency of BPA removal [15].

Because TMs are a mixture of various compounds, they are degraded by multiple groups of microorganisms. Ding et al. [18] showed positive correlations between the presence of TMs and an increased abundance of hydrocarbon-degradation-related bacteria (e.g., *Alcanivorax* sp., *Neptuniibacter* sp., *Ketobacter* sp., and *Marinobacter* sp.) and bacteria related to sulfate reduction (e.g., *Desulfobacter* sp. and *Desulfofrigus* sp.). Based on functional gene prediction, Liu et al. [36] found that TMs affected the most abundant genes present in bacteria communities in marine sediments related to aromatic compound degradation, hydrocarbon degradation, aerobic chemoheterotrophy, thiosulfate respiration, and anaerobic nitrogen metabolism. While most of the current scientific reports focus on TM's influence on microbial consortia in the aquatic environment, the existing literature about their effect on biomass on WWTPs is very limited.

The aim of the research was to determine the effect of TMs in wastewater on the treatment efficiency and the structure of biomass in AGS reactors and to verify the amount of TM-leached chemical additives in wastewater effluent. The current state of research on the impact of TMs on the microbiological communities in wastewater treatment plants responsible for transforming essential compounds, i.e., carbon, nitrogen and phosphorus compounds, is still being determined. The above studies complement the research gap concerning the impact of TMs in biological wastewater treatment plants, i.e., the creation of specific ecological niches caused by micropollutants and changes in the kinetics and efficiency of pollutant removal.

2. Material and methods

2.1. Experimental setup

4 granular sludge batch reactors (GSBRs) were operated at different TM loading. The working volume of the reactor was 3.0 L (height/diameter ratio of 3.0), the wastewater exchange ratio was 60%, the superficial air velocity was 0.85 cm/s, the temperature was 20 °C, and reaction was 7.5–8.5 pH; the reactors were aerated up to saturation. The cycle length was 480 min (5 min of filling, 60 min of anoxic phase, 400 min of aeration phase, 10 min of settling, and 5 min of decantation). Cycles were established using automatic timers to start and stop filling, aeration, and effluent withdrawal.

The GSBRs inoculum was collected from a municipal WWTP in Orneta (Poland). The WWTP operates with AGS technology. The AGS was acclimated to the substrate for 90 days in batch mode before running the experiment. The operation of the GSBRs was considered stable if the range of changes in investigated characteristics of the effluent did not exceed 5–10% within 14 days. To illustrate the impact, the TM experiment was conducted for a period of 90 cycles (30 days). To the control GSBR (R1), no TMs were added; reactors R2, R3, and R4 were fed with wastewater containing 10, 25, and 50 mg TM/L, respectively. Currently, there is a significant research gap concerning TM concentrations in influents to wastewater treatment plants. Therefore, in our experiment, to set the TM doses we used information about TM amounts (ranging from 10 to 130 mg TM/L) entering roadside gully pots with stormwater, which may subsequently reach wastewater treatment plants [26]. TM having a size distribution of 400–700 μm, was provided by an Austrian tire recycling company that produces tire granulate from used trucks and car tires. The synthetic wastewater composition was adapted from those described by Coelho et al. [11], and sodium acetate was used as an organic carbon source. The pollutant concentrations in the influent were 700–800 mg COD/L, 40–60 mg N-NH_4/L, and 8–12 mg P-PO_4/L.

The chemical oxygen demand (COD), nitrogen ammonia (N-NH_4), nitrite (N-NO_2), nitrate (N-NO_3), and orthophosphates (P-PO_4) were

measured according to APHA [1] in the influent and effluent of the GSBRs. The analyses were done three times a week during stable reactors' performance. In the GSBRs, pollutant concentrations were also measured during the reactor cycle to examine the kinetics of the removal of organics, nitrogen, and phosphorus by AGS.

2.2. Biomass analysis and TM detection

The concentrations of mixed liquor suspended solids (MLSS), sludge volumetric index (SVI) in the reactors, and total suspended solids (TSS) in effluent were determined according to APHA [1]. The particle size analysis of AGS was performed with a wet sieving technique using RetschAS 200 sieving device. The following sieve size classes were applied 40, 90, 125, 250, 355, 500, and 710 μm. For sieving, 1000 mL of AGS were taken from each GSBR at the end of the experiment. The sieving process was supported by tap water (temperature 12 °C) from the spray nozzle, which was located above the uppermost sieve. The water left the sieve stack together with the last fraction through the outlet in the collector. Rinsing was carried out until the liquid leaving the sieve stack outlet was no longer turbid with solid particles. In this experiment, sieving lasted 15 min, and the vibration amplitude was 1.5 mm [14].

The mass of TM in the sludge was determined in the entire volume of the reactor. Biomass samples were collected after the run of the experiment, consequently frozen, and stored at − 20 °C. After defrosting, sludge samples were homogenised (wet weight) for extraction. In brief, 100 mL of sludge was added to an Erlenmeyer flask with 900 mL of deionised water and 1.2 g of sodium chloride (up to saturation). After stirring for 15 min, the mixture was settled for 2 h. Then, the top water layer was filtered in a vacuum filtration unit using a sieve with a pore size of 40 μm. The extraction was conducted in triplicate; thus, all the extracts were collected in the sieve. The sieve in the vacuum filtration unit was then washed with more than 600 mL of distilled water to remove salt residues. The extract in the sieve and bottom layer was sequentially treated with 300 mL hydrogen peroxide solution (H_2O_2, 30%) overnight to remove and disintegrate organic materials. The mixture was poured into 900 mL of distilled water, vacuum-filtrated through glass fiber filters, rinsed again several times with deionised water, and dried in a desiccator for 3 days [33].

Fourier Transform Infrared Spectroscopy (FTIR, Shimazu IRSpirit) was used to observe changes in functional groups of pristine TM and TM from each GSBR. A diamond crystal attachment was equipped to establish the reference and TM surface spectra. Measurements were taken with a resolution of 4 cm^{-1} in wavenumber ranges from 4000 to 400 cm^{-1} (32 scans per sample). Three different points, i.e., the central point and two different edge points of each sample, were examined. Selected spectra from the sample were compared with their reference spectra from the library. Background spectra were regularly measured against air with the same setting as described above. Between measurements, the FTIR crystal was cleaned with ethanol to avoid cross-contamination.

2.3. Chemical analysis of TM additives and their transformation products in the effluents

During the experiment, 200 mL wastewater samples were collected (every 3 days) from reactors for chemical analysis of TM additives and their transformation products. Each representative sample was the sum of wastewater effluent collected over 3 cycles on the same day and frozen at − 20 °C for future analysis. After defrosting samples, internal standard (100 μg of 2-methylbenzothiazole (2-MeBTH)) was spiked. The sample were filtered (0.22 μm nylon syringe filters), and 1 mL of the sample was transferred into the clean glass vial and adjusted the pH to 4. The presence of 11 compounds was analysed in effluents, i.e., aniline, 2-aminobenzothiazole (2-A-BTH), 1,3-diphenylguanidine (DPG), 5-methyl-1 H-benzotriazole (5-Me-BTH), 2-hydroxybenzothiazole (2-OH-BTH), BTH, 2-mercaptobenzothiazole (2-Me-S-BTH), hexamethoxymethyl-melamine (HMMM), N-(1,3-dimethylbutyl)-N′-phenyl-1,4-phenylenediamine (6PPD), 2-anilino-5-[(4-methylpentan-2-yl)amino]- cyclohexa-2,5-diene-1,4-dione (6PPD-Q), and 1,3-di-o-tolylguanidine (DTG).

TM-derived compounds were quantified using an ultra-high performance liquid chromatography (UHPLC) (Agilent infinity 1290 II, Agilent Technologies, Santa Clara, US), which was connected to a 6470 LC/TQ triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, US) running in multiple reaction monitoring (MRM) mode with a C18 column (Acquity HSS T3, 1.8 μm, 100 mm Waters) [10]. The flow rate was set to 0.3 mL/min, and the column temperature was 40 °C. The mobile phase consisted of (A) 0.1% formic acid water and (B) acetonitrile with 0.1% formic acid with the gradient as follows: 0 min, 5% B; 100% B 25 min; 100% B 30 min; 5% B 33 min. For quantification, a calibration curve with 8 standards (0.1 μg/L – 1000 μg/L in methanol) for each compound of interest and 1 μg of 2-methyl benzothiazole was used as an internal standard to calculate the additive content in each sample. The transitions from the precursor ion to each product are given in Table S1. The results of the LC-MS/MS were evaluated using the MassHunter software.

The content of additives in TM used was also determined (Table 1) using solvent extraction. 0.2 g of TM was placed in 15 mL test tubes with Teflon-lined screw caps. 20% acetone in dichloromethane (10 mL) was added to the tubes, and the test tubes were placed in an ultra-sonication bath (Branson 8210, Danbury CT, USA) for 1 h. 1 μg of 2-methyl benzothiazole, was added to the extract as an internal standard. The extract was then vortexed, filtered by 0.2 μm Nylon filters (SUN-SRi, Rockwood, TN, USA), transferred into new test tubes, and the extraction was repeated with fresh solvent. 0.2 mL of purified water was added to the pooled extracts to avoid volatilization of the additives during solvent evaporation. The extracts were evaporated to 0.2 mL under a gentle stream of nitrogen and mixed with 0.8 mL methanol and analysed with the LC-MS/MS.

2.4. Changes in the microbial community in the GSBRs

To assess the impact of TM on the microbial community's structure in the GSBRs, biomass and wastewater samples (10 mL) were collected from the reactors at the 7th (21st cycle), 14th (42nd cycle), and 28th day (84th cycle). The samples were stored at − 20 °C until DNA was extracted. DNA was isolated with a FastDNA® SPIN Kit for Soil (MP Biomedical); DNAs isolated from the biomass collected during the three replications in a particular reactor were pooled together.

The quantity and quality of DNA were assessed with the use of a NanoDrop spectrophotometer (Thermo Scientific) and agarose electrophoresis. The samples were then amplified using primers 515 F/806 R (GTGCCAGCMGCCGCGGTAA/GGACTACHVGGGTWTCTAAT) targeting the V4 region of the bacterial 16 S rDNA gene [8] and sequenced using MiSeq Illumina Platform in Research and Testing Laboratory

Table 1
Content of additives in TM (n = 3).

Compound name	Content of additive in TM (μg/g)	LOD	LOQ
2-A-BTH	8 ± 1	0.06	0.19
2-OH-BTH	300 ± 8	0.21	0.65
2-Me-S-BTH	110 ± 8	47.65	144.39
2-Me-BTH	n.d.	0.06	0.20
BTH	500 ± 30	13.15	39.83
5-Me-BTH	n.d.	0.06	0.18
6PPD	4900 ± 200	0.18	0.20
6PPD-Q	120 ± 6	0.03	0.10
Aniline	40 ± 6	0.04	0.11
DPG	30 ± 0.5	0.10	0.29
DTG	n.d.	0.03	0.11
HMMM	240 ± 20	0.01	0.03

n.d. – not detected

(USA). Chimeras were removed from denoised, clustered raw reads using UCHIME in a de novo mode [21]. Reads were converted to a FASTA format; sequences with low-quality tags or less than half the expected length were eliminated. The UPARSE algorithm was employed to cluster the remaining sequences into operational taxonomic units (OTUs, [20]). For taxonomic identification, each cluster's centroid sequence was compared to the NCBI database using the USEARCH global alignment algorithm. The obtained sequences were analysed bioinformatically using Microbiome Analyst tool for meta-analysis of microbiome data [17]. The sequences have been deposited in the NCBI Sequence Read Archive (SRA, Accession: PRJNA1009698).

The metagenomes were predicted from 16 S rDNA data using the Marker Data Profling tool in MicrobiomeAnalyst. This method enables the mapping of gene abundance profiles, which were predicted from Greengenes. The bacterial OTUs were imported into PiCrust, and the functional genes were identified from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. KO data resulted from the PiCrust prediction were then imported to Shotgun Data Profling tool in MicrobiomeAnalyst, filtered by a modified setting (low count filter: min count=4, prevalence=20%, low variance filter: 10% inter-quantile range), and normalized using Total Sum Scaling method.

2.5. Statistical analysis

The samples were subjected to measurements in triplicates to ensure robust and reliable data. All calculations and statistical analyses were carried out utilizing Statistica 13 software. In order to assess statistically significant differences in concentrations, ANOVA testing was employed. Subsequently, for comprehensive multivariate comparisons, Tukey's Honestly Significant Difference (HSD) post hoc testing was performed.

In our investigation, we sought to explore the relationships between different species of bacteria, reactor performance, the concentration of tire additives present in wastewater and the dose of TM, and to achieve this, Pearson correlation analysis was applied. This analytical approach allowed us to discern potential correlations between bacterial species and TM dosage.

Throughout the study, we set the criterion for statistical significance at $p < 0.05$, ensuring a stringent evaluation of the obtained results. This stringent criterion enhances the reliability and validity of our findings, reinforcing the robustness of the statistical analyses employed in this research.

3. Results and discussion

3.1. The accumulation of TM in biomass had a substantial effect on GSBR performance

During a stable GSBR performance, the average COD concentrations in the effluents were 68.1 ± 11.7, 67.7 ± 12.3, 68.2 ± 12.4, and 69.3 ± 11.1 mg/L in R1, R2, R3, and R4, respectively, indicating similar effectiveness of COD removal in all reactors. The COD removal rate was in the range of 55.50 – 55.88 mg/(L•h) in the reactors (Table 2); about 75% of the COD was removed within the first 2 h of the cycle (Fig. S1). There was no significant effect of TM dose and time of the experiment on the effectiveness of COD removal ($p > 0.05$).

In all GSBRs, ammonium removal efficiency, resulting from nitrification and biomass synthesis, exceeded 98.0%. Notably, the efficiency of N-NH_4 removal exhibited a significant negative correlation ($r = -0.79$, $p < 0.05$) with the TM dosage. A similar association was also present with the N-NH_4 removal rate, which declined as the TM dosage increased (Table 2, Fig. S2). In R1, N-NH_4 removal was 9.5 (mg/(L•h)), and after 3 h, the effluent concentration was below 0.5 ± 0.1 mg/L. N-NH_4 removal in R4 was 4.5 (mg/(L•h)), and at the end of the cycle, N-NH_4 concentration was 2.0 ± 0.3 mg/L (Fig. S1). The slowdown in the reaction rate may be due to slow leaching of such substances as aniline from the TM, which may result in an additional load of N-NH_4 [43].

Table 2
Removal efficiency and kinetic changes in GSBRs.

Parameters		R_1	R_2	R_3	R_4
COD removal efficiency (%)		91.5 ± 1.5	91.5 ± 1.5	91.5 ± 1.6	91.3 ± 1.4
TN removal efficiency (%)		86.7 ± 3.3	84.4 ± 2.9	79.6 ± 4.6	76.0 ± 4.8
N-NH_4 removal efficiency (%)		99.7 ± 0.2	99.6 ± 0.3	98.8 ± 0.5	98.1 ± 0.8
Nitrification efficiency (%)		50.7 ± 1.4	50.3 ± 1.4	49.2 ± 1.5	48.2 ± 2.0
Denitrification efficiency (%)		61.7 ± 7.6	56.4 ± 6.2	48.1 ± 8.8	42. ± 9.1
P-PO_4 removal efficiency (%)		61.9 ± 7.3	64.6 ± 7.6	64.2 ± 5.7	65.0 ± 7.8
Nsyn (mg N/L)		18.25 ± 0.5	18.31 ± 0.5	18.33 ± 0.5	18.31 ± 0.5
NOx (mg N/L)		23.2 ± 1.3	23.7 ± 1.4	24.6 ± 1.5	25.1 ± 1.3
r_{COD} (mg/(L•h))		55.50	57.00	55.50	55.88
$r_{N\text{-}NH4}$ (mg/(L•h))		9.50	6.33	4.69	4.50
$r_{N\text{-}NO2}$ (mg/(L•h))	anoxic phase	2.16	2.33	2.23	3.70
	aerobic phase	1.02	0.82	1.24	1.64
$r_{N\text{-}NO3}$ (mg/(L•h))	anoxic phase	0.18	0.87	1.35	3.42
	aerobic phase	0.11	0.36	0.40	0.81
$r_{P\text{-}PO4}$ (mg/(L•h))	anoxic phase	2.05	2.07	2.04	2.78
	aerobic phase	0.60	0.88	0.83	0.83

Nsyn – nitrogen used for biomass synthesis; NOx – oxidized form of nitrogen; * - in the GSBR, two different kinetics removal were observed.

Sathicq et al. [46] reported that organic nitrogen leaching from TM could account for about 10% of the N-NH_4 concentration in the water system. In addition, TM accumulated in biomass can block oxygen diffusion in the granule structure, which can also affect the N-NH_4 removal rate [35]. The percentage of N-NH_4 removal in the cycle was 100% for R1 and R2. The efficiency in R3 and R4 exhibited a decrease, with the N-NH_4 removal percentages reaching 98.6% and 94.7%, respectively. From 18.25 m/L to 18.33 mg/L of nitrogen was used from biomass synthesis; there was no significant difference between the GSBRs (Table 2).

The N-NO_2 concentration increased during the first hour of the GSBR cycle (Fig. S1), especially in the reactors with higher concentrations of TM in the influent. TM dosage correlated significantly with the quantity of N-NO_2 in the treated wastewater ($r = 0.74$, $p < 0.05$). Investigations of a long-term exposure to TMs, had similar findings to those in our study and indicated that TM stress may disrupt both N-NH_4 oxidation during nitrification and N-NO_3 reduction during denitrification. This disruption can decrease efficiency of total nitrogen removal [59]. Notably, the rate of N-NO_2 production significantly increased after the first hour of the GSBR cycle in R4 (from 0.82 to 1.64 mg/(L•h)). The rate of N-NO_2 removal increased in the GSBRs operated at the TM doses of 25 and 50 mg/L. The rate of N-NO_3 removal in the anoxic phase increased as the TM dose in the GSBRs was increased, as did the N-NO_3 production rates during the aerobic phase.

The NO_x concentrations in the GSBR effluents, exhibited a TM dose-dependent trend. In R1, NO_x concentration averaged 23.2 ± 1.3 mg N/L. As the TM dose increased, NO_x concentrations in R2 and R3 increased and averaged 23.7 ± 1.4 mg N/L and 24.6 ± 1.5 mg N/L, respectively. The highest dose of TM in R4 resulted in a concentration of NO_x at a level of 25.1 ± 1.3 mg N/L.

The nitrification efficiency correlated negatively with the TM dose ($r = -0.54$, $p < 0.05$), with the efficiency being 50.7 ± 1.4, 50.3 ± 1.4, 49.2 ± 1.5, and 48.2 ± 2.0 for R1, R2, R3, and R4, respectively. The nitrification efficiency decreased as the experiment progressed (r =

−0.60, $p < 0.05$), indicating that TM accumulation in biomass had a negative impact on the process. According to Liu et al. [35], TM did not immediately affect nitrogen removal rates; however, prolonged exposure resulted in a decrease in bacterial activity. Our findings revealed that there was also a substantial negative correlation between the denitrification efficiency ($r = -0.67$, $p < 0.05$) and the TM dose, which might be due to the impaired nitrification process.

The amount of TM in the reactors also correlated negatively with the overall nitrogen removal efficiency ($r = -0.72$, $p < 0.05$). This could be attributed to the potential inhibitory effects of TM on microbial activity, including enzyme and electron transport activity. Previous studies have reported that prolonged operation of WWTPs can lead to a decline in the relative abundance of nitrifiers (e.g., *Nitrospira*) and denitrifiers (e.g., *Zoogloea*), which could contribute to the reduction of nitrogen removal efficiency [58].

In all TM-fed reactors, the effectiveness of P-PO_4 removal was over 64% (Table 2, Fig. S2). The average P-PO_4 effluent concentrations were 3.05 ± 0.6, 2.83 ± 0.6, 2.86 ± 0.4 and 2.80 ± 0.6 mg/L in R1, R2, R3, and R4, respectively. The release rate of P-PO_4 in anoxic conditions was significantly higher in the reactor with the highest dose of TM than in the control reactor (2.78 vs. 2.05 mg/L, Table 2). This difference is due to the leaching of the carbon compounds from TM [46], which polyphosphate-accumulating organisms can use as carbon sources [47]. The efficiency of P-PO_4 removal was negatively correlated with the efficiency of COD removal ($r = -0.59$, $p < 0.05$). The reason for this is the incorporation of an anaerobic feeding phase in the GSBR cycle, during which organic carbon is taken up by phosphorus accumulating organisms and stored as intracellular polymers [37]. There was also a significant negative correlation between the experiment time and the efficiency of P-PO_4 removal ($r = -0.78$, $p < 0.05$). The reason for this is the aging of the sludge, which leads to a decrease in the abundance of phosphorus-accumulating bacteria. Phosphate removal processes are strongly dependent on the sludge retention time. The periodic removal of excess sludge from the reactor stimulates biomass turnover and the growth of new cells, which can accelerate the consumption of both organics and nutrients. Some studies have shown that the biological removal of phosphorus is favored at low sludge retention time [55].

The TM dosage affected the granulometric structure of the biomass in the reactors (Fig. 1). In the control reactor, granules with a diameter of 90–125 μm dominated, accounting for 29.0% of the total biomass. The TM dose strongly correlated with the granulometric structure of the biomass in the reactors ($p < 0.05$, $r = 0.97$), as reflected by a substantial increase in the percentage of > 355 μm granules in R2 (37%), R3 (45%), and R4 (48%). As the TM dosage in the GSBRs was increased, the percentage of small granules with a diameter of 40–90 μm decreased ($r = -0.95$, $p < 0.05$), while the share of granules with a diameter of 500–710 μm increased ($r = 0.96$, $p < 0.05$). The share of 355–500 μm granules correlated negatively and significantly with the shares of 40–90 μm granules ($r = -0.97$, $p < 0.05$) and 90–125 μm granules ($r = -0.98$, $p < 0.05$). The increase in granule diameters resulted from the attachment of TM particles (400–700 μm) to the granule structure. Similar findings have been reported in the previous studies, where prolonged exposure of MPs to biomass resulted in the formation of large agglomerates [25]. The high roughness and porosity of TMs allow them to bind to biomass, leading to increased adhesion and protection against shear forces [6].

MPs that are removed from treated wastewater are accumulated mostly in sludge [22]. Similarly, there was a linear relationship between the amount of TM dosed to reactors and the amount of TM per mg of biomass at the end of the experiment (Fig. 2, $r = 0.99$). The amount of TM in the sludge at the end of the experiment was 0.05, 0.12 and 0.27 mg TM/mg MLSS in R2, R3 and R4, respectively. The high affinity of MPs for sludge has been attributed to the negative charge of the AGS and the positive surface charge of MPs [29].

Changes in the presence of the bands in FTIR spectra indicated that the TM trapped in the biomass during the experiment differed from the TM initially used (Fig. S3). In the TM from biomass, strong peaks within the 2800–3000 cm^{-1} region indicated typical C-H stretching vibrations in alkane chains. Peaks in the 1540–1695 cm^{-1} range were ascribed to C═C stretching vibrations in aromatic compounds. The absorption peak at 1612 cm^{-1} was probably caused by olefin C═C stretching vibration [52]. These changes suggest an alteration in TM structure, which may result in the release of the substances into wastewater.

3.2. AGS has the capability to degrade of tire additives present in wastewater

Aniline, a product of the degradation of aromatic amine-based tire additives, was present in all reactors, with the highest concentration in R4 (up to 16 μg/L) (Fig. 3). Throughout the entire experiment, aniline concentrations were consistently highest in R4. The decrease in aniline concentration over time in reactors R2, R3, and R4 indicated

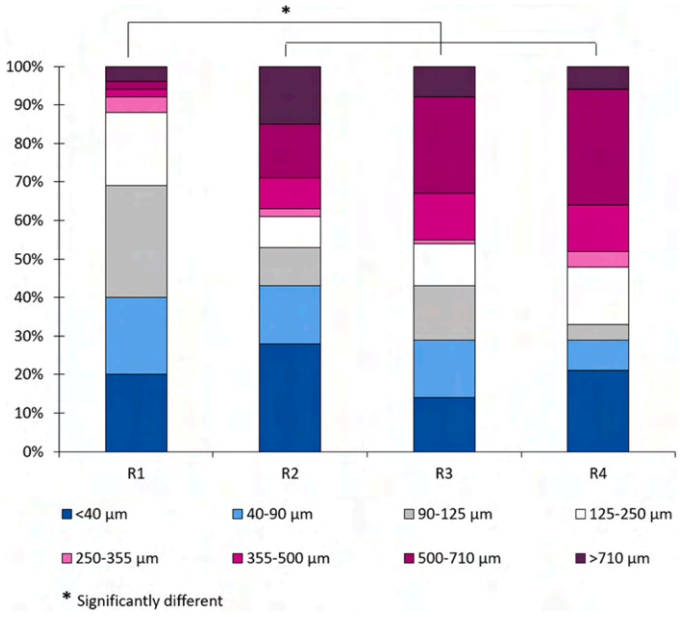

Fig. 1. Distribution of granule particle sizes in the reactors at the end of the experiment.

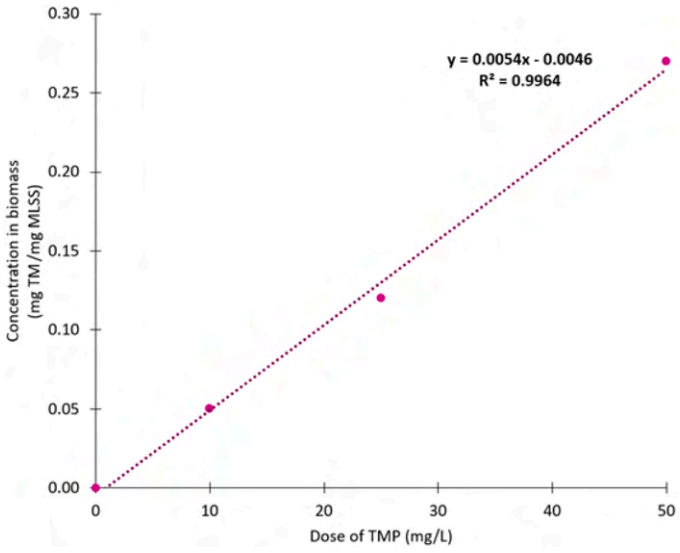

Fig. 2. Correlation between TM dose in influent wastewater and TM concentration trapped on biomass in the reactors.

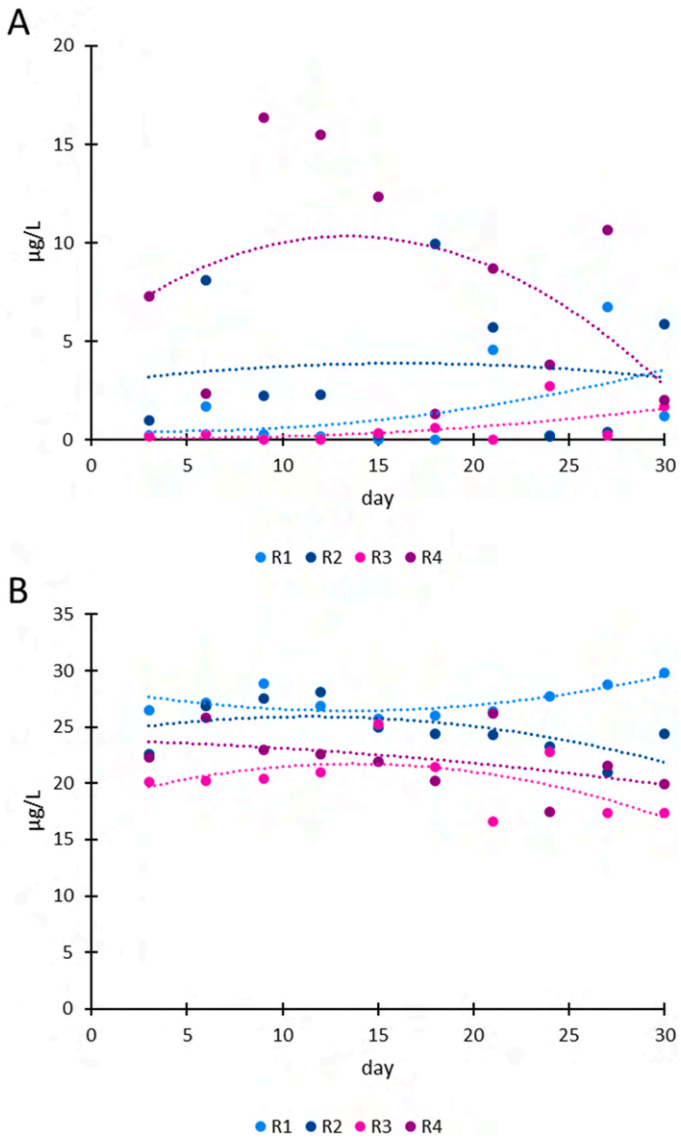

Fig. 3. Quantification of (A) aniline and (B) BTH in treated wastewater from the reactors.

adaptations in microbial community within the biomass. R1 exhibited a completely different trend, with an increase in aniline concentration over time. This may have been caused by leaching or flushing from inoculum previously collected from the wastewater treatment plant.

The concentration of aniline in the treated wastewater negatively correlated with ammonium removal efficiency ($r = -0.76$, $p < 0.05$) and positively correlated with the TM dosage ($r = 0.65$, $p < 0.05$). This suggests the leaching of aniline from TM and its breakdown and subsequent utilization in the nitrogen cycle. Bacterial degradation of monocyclic aromatic amines, such as aniline, typically results in the release of ammonia. Ammonium ions may be released after a ring cleavage. The initial conversion of aniline to catechol is a multi-step reaction catalyzed by three enzymes: a glutamine synthetase-like enzyme, a glutamine amidotransferase-like enzyme, and an aniline dioxygenase [3].

BTH was detected in all reactors, including the control reactor, with concentrations ranging from 15 to 30 µg/L. Significant differences observed in the concentration of BTH in the GSBR effluents indicated that bacterial community adapted to the presence of TM. Kowalska et al., [30] observed that the major mechanism for the elimination of BTH in membrane bioreactors treating wastewater was biohydroxylation. The concentrations of 2-A-BTH, DPG, and 2-OH-BTH were below 5 µg/L (Fig. S4). Several studies have shown that microbial degradation of BTH yields 2-OH-BTH, which is further hydroxylated to 2,6-dihydroxy-BTH. In addition, 2,6-dihydroxy BTH is converted by monooxygenase into catechol (dihydroxy benzene) and dicarboxylic acid by catechol 1,2-dioxygenase [34].

Comparing the chemical additive contents in TM (Table 1) with their concentrations in the effluent (Fig. 3), it can be concluded that AGS exhibits significant capabilities for the biodegradation of these pollutants. The observed decrease in concentrations suggests that the microbial community within the AGS effectively degrades the chemical additives from TM. Zhang et al. [61] showed that using activated sludge technology, tire additives and their transformation products can be removed at levels of 16.7–100% during wastewater treatment. Our results highlight the potential of aerobic granular sludge as a promising treatment method for efficiently reducing the levels of these contaminants in wastewater, contributing to improved water quality and environmental protection.

3.3. The microbial community structure changes to increase the potential for degrading TM compounds

The microbiological structure of the AGS changed with increasing TM concentration in the influent, indicating an adaptation of the bacteria to the micropollutants present in wastewater. It demonstrated the succession ability of microorganisms capable of degrading individual toxic substances in the TM mixture.

The microbial community composition within AGS was characterised using 16 S rRNA gene amplicon sequencing, resulting in 557114 high-quality reads with an average of 42854 ± 22658 reads per biomass sample (Table S2). In the 16 S rRNA gene libraries obtained from biomass samples at an order level, *Rodocyclales* (23.34–59.47%), *Sphingobacteriales* (13.16–27.32), *Xanthomonodales* (6.89–17.21%) and *Cytophagales* (2.63–5.82%) predominated. There was an evident difference in microbial communities between the control reactor (R1) and the reactor with the highest dose of TM (R4). There was an increasing abundance of *Sphingobacteriales, Xanthomonodales,* and *Burkholderiales* but a decrease of *Rodocyclales* in the R4 microbial community compared to R1 (Fig. 4).

The main microorganisms present in the community forming the core of the microbial structure (Fig. 5), which occurred regardless of the TM dose, include genera *Thauera, Pseudofulvimonas, Azocarus, Woodsholea, Zoogloea, Pseudoxanthomonas, Nannocystis, Mesorhizobium, Rhodobacter, Hydrogenophaga*, *Xanthomonas, Nordella, Legionella,* and *Geobacter*. The results show that the core of the microbial structure consisted of EPS-forming microorganisms, i.e., *Thauera, Zoogloea, Pseudoxanthomonas, Rhodobacter,* and *Xanthomonas* [24,42,63]. EPS production plays an important role in forming and maintaining granules' stability and integrity, protecting bacteria from severe external conditions like toxic compound presence. This supports the importance of EPS producers in the long-term stability of the AGS treatment process treating industrial wastewater [42]. There was a significant difference between the microbial community composition of the inoculum and the samples collected during the experiment.

No significant difference was obtained between OTU-based α-diversity of the analysed samples at the genus level in terms of Chao, ACE, Shannon, and Simpson indexes (Table S2). Non-metric multidimensional scaling (NMDS) at the genus level showed that the β-diversity (using the Bray-Curtis dissimilarity measure) of microbial community composition of the biomass significantly changed over time in the experiment, but a dose of TM didn't have a significant effect (Fig. S5). In the case of α- and β-diversity, a significant difference was observed between the inoculum and the rest of the biomass samples. Muñoz-Palazon et al. [41] suggested that the microbial composition of the granular biomass depends more on technological configurations and environmental factors than on inoculum community structure.

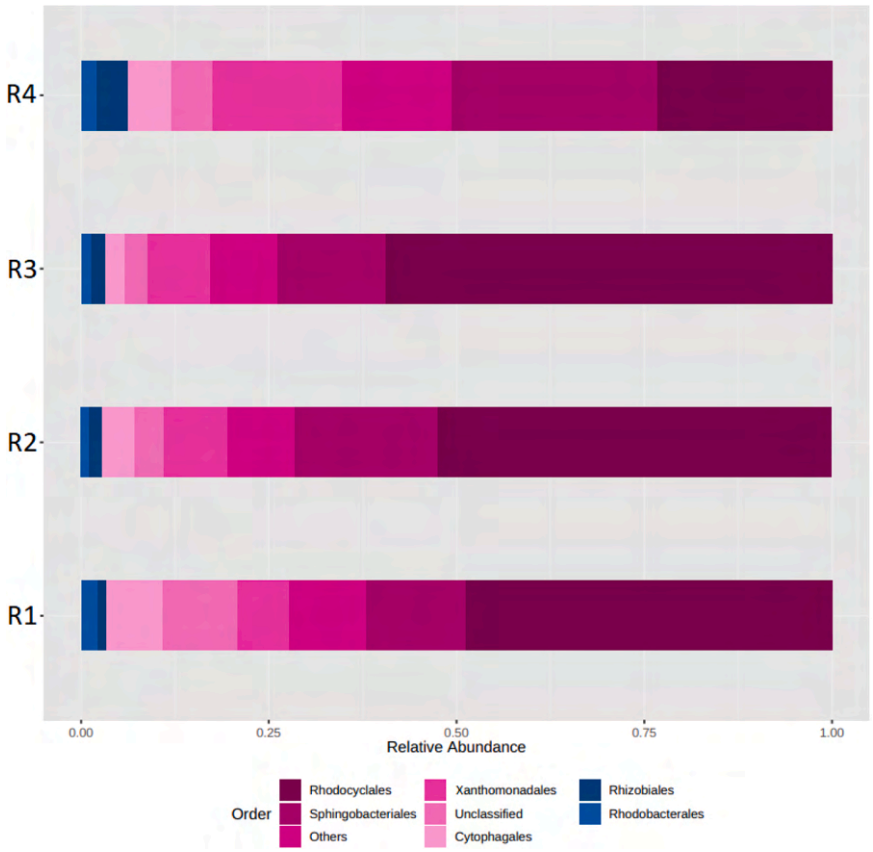

Fig. 4. Merging relative abundance profiling microbial community in reactors with different TM dose (order level).

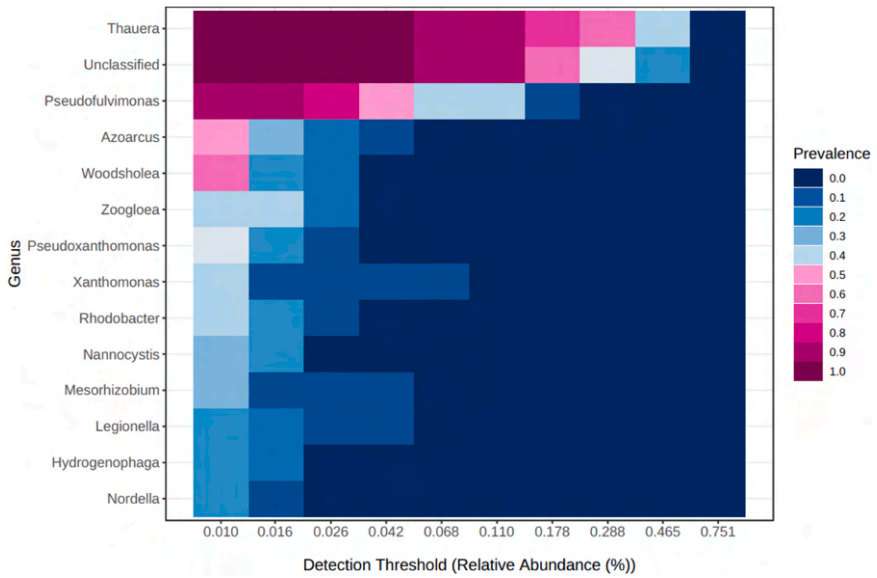

Fig. 5. The main microorganisms present in the community forming the core of the microbial structure.

Pearson correlations were calculated between individual microorganisms and the dose of TM to illustrate changes in the community caused by the presence of TMs (Fig. 6). The performance of specific microbial communities in the degradation of xenobiotics is dictated by specific functional microbes that are prevalent in the community. Bacteria from genera *Luteimonas* ($r = 0.77$, $p < 0.05$), *Hydrogenophaga* ($r = 0.70$), *Devosia* ($r = 0.69$), *Geobacter* ($r = 0.64$), Gemmatimonas ($r = 0.62$) and *Acidovorax* ($r = 0.56$) that were identified in this study are known to participate in the degradation of various aromatic hydrocarbons such as benzene, toluene, ethylbenzene, and xylenes [23, 44]. The concentration of BTH in the effluent was significantly correlated with the abundances of microorganisms belonging to such genera as *Hydrogenophaga* ($r = -0.77$, $p < 0.05$), *Geobacter* ($r = -0.68$, $p < 0.05$), *Aminobacter* ($r = -0.79$, $p < 0.05$), Prosthecobacter ($r = -0.76$, $p < 0.05$), and *Rubrivivax* ($r = -0.65$, $p < 0.05$). There was no correlation between the concentration of aniline in the effluent and

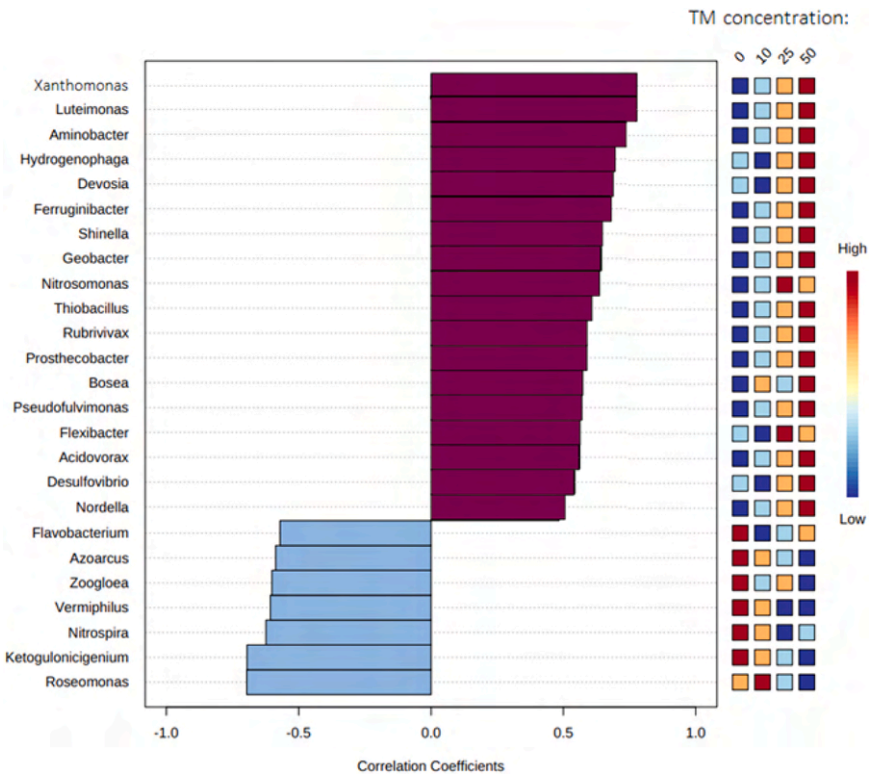

Fig. 6. Top 25 genus correlated with dose of TM in the wastewater.

abundances of individual microorganisms. This implies that this compound must engage pre-existing metabolic pathways through co-metabolism [43].

Sulfur-oxidizing bacteria that were observed in the AGS, specifically genera *Thiobacillus* ($r = 0.61$, $p < 0.05$) and *Bosea* ($r = 0.57$, $p < 0.05$), can oxidize disulfide linkages and therefore devulcanise rubber material [49,57]. Our study showed a significant increase in bacteria of the *Xanthomonas* genus ($r = 0.77$, $p < 0.05$) with an increased dose of TM. These bacteria can grow adhesively on rubber and require direct contact with the material to degrade the polyisoprene chains in its structure. *Xanthomonas* produces the RoxA enzyme, which can break down natural and synthetic polyisoprene material by oxidatively cleaving the double bonds of poly(cis-1,4-isoprene). The RoxA enzyme is known to cleave poly(cis-1,4-isoprene) through a dioxygenase mechanism, which initiates polyisoprene degradation [49].

The positive correlation of genera *Rubrivivax* ($r = 0.59$, $p < 0.05$) with the dose of TM can be connected with the abillity of this bacteria to grow in the presence of high concentrations of aniline and other aromatic compounds that are used as chemical additives of TM [40]. The genera *Ferruginibacter* ($r = 0.68$, $p < 0.05$) and *Shinella* ($r = 0.64$, $p < 0.05$) can hydrolyse several kinds of organic pollutants, and may utilise aniline as the sole source of carbon, nitrogen and energy [58,62]. *Ferruginibacter* sp. were found to be tolerant to the inhibition effect of aniline (100–200 mg/L) and could play important roles in pollutant reduction in the WWTP system [58]. The microbial community of AGS can remove aniline and nitrogen synchronously [60].

After the addition of TM, the growth of genera *Roseomonas* ($r = -0.69$, $p < 0.05$), *Ketogulonicigenium* ($r = -0.69$, $p < 0.05$), *Nitrospira* ($r = -0.62$, $p < 0.05$), *Vermiphilus* ($r = -0.61$, $p < 0.05$), *Zoogloea* ($r = -0.60$, $p < 0.05$), *Azoarcus* ($r = -0.58$, $p < 0.05$) *Flavobacterium* ($r = -0.57$, $p < 0.05$) was to inhibited, suggesting a sensitivity of these bacteria to TM additives and their transformation products. The decrease in the abundance of genera *Zooglea* and *Flavobacterium* may be caused by the development of other EPS-forming microorganisms capable of growing under unfavourable conditions, such as *Xanthomonas, Thauera,* and *Pseudoxanthomonas*. Banjerdkij et al. [5] suggest that cross-synthesis can be the major system *Xanthomonas* uses to protect itself from Cd and Zn toxicity. *Thauera* sp. can efficiently degrade aromatic compounds, antibiotic and the increased number of these bacteria in AGS indicates that they tolerate elevated of concentrations toxic substances [48].

The bacteria from genera *Nitrosomonas* ($r = 0.64$, $p < 0.05$) and *Prosthecobacter* ($r = 0{,}59$, $p < 0.05$) were identified as ammonia-oxidizing bacteria (AOB) in the AGS and prevailed at the higher dose of TM. At the same time, *Nitrospira* ($r = -0.62$, $p < 0.05$) were most abundant in the R1. This agrees with the results from reactor performance, which showed a fast recovery of ammonium but not nitrite oxidation, corroborating that nitrite-oxidizing (NOB) bacteria were nitrifiers mostly affected by TM. Several studies reported that NOB were more sensitive to other operational factors and environmental conditions than AOB [45].

Microbial activities in WWTPs play a critical role in the efficiency of pollutant removal processes. Our study predicted bacterial functionality based on Clusters of Orthologous Groups (COG)-level functional categories (Fig. S6). The most abundant categories were general function ($14.30 \pm 0.08\%$), translation ribosomal structure and biogenesis ($13.43\% \pm 0.38$), amino acid transport and metabolism ($8.36\% \pm 0.16$), coenzyme transport and metabolism ($8.05\% \pm 0.19$), energy production and conversion ($8.00\% \pm 0.14$), inorganic ion transport and metabolism (7.29%), and nucleotide transport and metabolism ($5.91\% \pm 0.17$). The TM dose did not significantly affect the overall microbial community's metabolism ($p < 0.05$).

Changes in some metabolic pathways in individual reactors during the experiment were demonstrated. Similar conclusions were obtained by Wang et al. [51], who observed that functional genes in microbial communities on the tire MPs changed with time. The increased abundance of microorganisms and genes associated with metabolism contributed to the degradation of xenobiotics. In our study, a statistically significant correlation was observed in the R1 reactor between the experiment cycle and energy production and conversion ($r = 0.99$,

$p < 0.05$). This probably was due to adaptation to the degradation of energy-rich and easily degradable compounds present in wastewater [64]. In R2, a correlation ($r = -0.99$, $p < 0.05$) between time and secondary metabolites, biosynthesis, transport and catabolism was observed. This may be related to the toxicity of TM additives at the beginning of the reactor operation, which did not occur in later cycles, which is to associated with the adaptation of the microbial community. In R4, a correlation was noted between the time of reactor operation (cycles) and functions related to nucleotide transport and metabolism ($r = 0.99$, $p < 0.05$) and RNA processing and modification ($r = 0.99$, $p < 0.05$). Increased activity in the above metabolic pathways may be associated with physiological needs for DNA repair or RNA synthesis in the microbial community. Similarly, Jiang and Zhang [27] observed that in razor clam *Sinonovacula constricta* the exposure to high concentrations of polystyrene nanoplastics changed purine metabolism to increase purine nucleotides and consume the reserves for synthesising purine nucleotides. Other research reported that inducing excessive reactive oxygen species production due to exposure to TM causes oxidative stress reactions, cell membrane damage, DNA/RNA oxidiation, and trigger functional enzyme inactivation [32].

The top 25 functional gene predictions (Fig. 7) indicated that several predicted pathways were significantly enriched ($p < 0.05$). Correlated gene prediction is connected to the metabolism, repair or synthesis of genetic material and biodegradation. Correlations between the dose and the activity of acetyl-CoA/propionyl-CoA carboxylase (K01964) have been shown, which may be related to the use of inorganic carbon released as a result of hydrolysis of TM forms and its use by the microbial community [28]. It is similar to catechol 2,3-dioxygenase (K07104), which can degrade aromatic hydrocarbons [38]. Much of the gene prediction is uncharacterised, which proves AGS's high biodiversity and adaptation to micropollutants present in wastewater [13]. There was no significant correlation observed between specific enzymes of the nitrogen cycle and the TM dosage. This suggests a co-metabolism between bacteria that oxidize N-NH_4 and aniline. However, a notable correlation ($r = 0.61$, $p < 0.05$) was observed between the TM dosage and the abundance of gene responsible for of aromatic compounds degradation (K00446).

4. Conclusion

The study shows that the increased TM dose in influent wastewater affects the cycle's removal/production rate of nitrogen compounds and reduces the nitrification and denitrification efficiency in the GSBRs. A linear relationship was observed between the amount of TM dosed to the reactors and the amount of TM trapped in the AGS biomass, which resulted in a change in the granulometric structure in GSBRs. The most commonly found chemical additives from TM in treated wastewater are aniline and benzothiazoles. The adaptation causes the difference in the microbial structure of the AGS community to the occurring micropollution and the increase in the population of microorganisms capable of degrading aromatic hydrocarbons, rubber, and heavy metals metabolism. The higher concentration of TM increased the activity of metabolic pathways associated with physiological needs for DNA repair, RNA synthesis, and biodegradation of xenobiotics in the microbial community. Understanding TM influence not only emphasizes the importance of the presence of micropollutants in waste management systems but also underscores the urgent need for comprehensive strategies to mitigate the potential environmental and operational disruptions they can

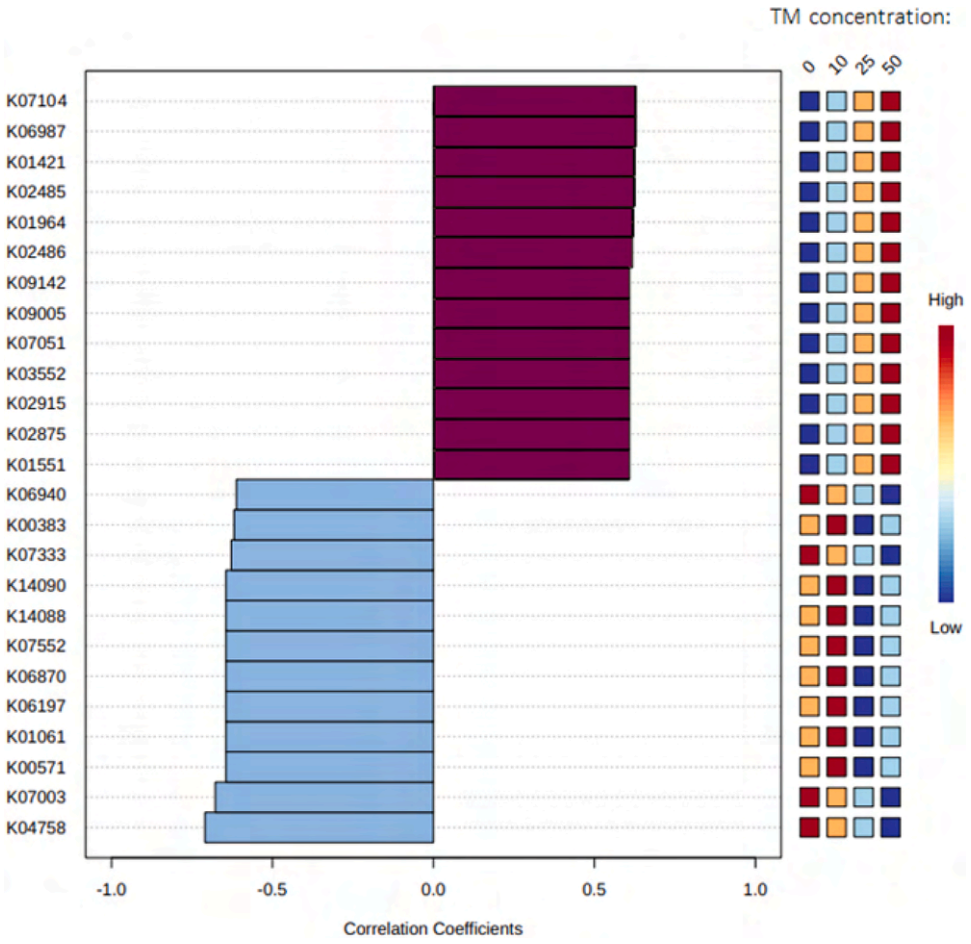

Fig. 7. Top 25 gene predicitons correlated with dose of TM in reactors.

cause across WWTP treatment systems.

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CRediT authorship contribution statement

Piotr Jachimowicz: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Visualization Project administration, Funding acquisition. **Ruoting Peng:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft. **Thorsten Hüffer:** Conceptualization, Methodology, Writing – review & editing, Supervision. **Thilo Hofmann:** Writing – review & editing, Supervision. **Agnieszka Cydzik-Kwiatkowska:** Conceptualization, Methodology, Resources, Project administration, Writing – review & editing, Supervision.

Environmental Implication

The presence of chemical additives from TM and their transformation products in WWTP has significant environmental implications. Compounds such as aniline, BTH, and DPG, if released into the environment through wastewater discharge, can have adverse effects on aquatic ecosystems and organisms due to their persistence and potential toxicity. The research highlights the need for improved wastewater treatment processes to remove these contaminants effectively. Implementing advanced methods like aerobic granular sludge technology offers a promising approach to mitigate the environmental impact of tire additive pollutants, contributing to enhanced water quality and environmental protection efforts.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Piotr Jachimowicz reports financial support was provided by National Science Centre Poland.

Data Availability

The data that has been used is confidential.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2023.133223.

References

[1] APHA, A., 2012. Standard Methods for Examination of Water And Wastewater. AmericanPublic Health Association, Standard Methods,, Washington. ISBN 978-087553-013-0.
[2] Araujo-Morera, J., Verdejo, R., López-Manchado, M.A., Santana, M.H., 2021. Sustainable mobility: the route of tires through the circular economy model. Waste Manag 126, 309–322. https://doi.org/10.1016/j.wasman.2021.03.025.
[3] Arora, P., 2015. Bacterial degradation of monocyclic aromatic amines. Front Microbiol 6, 820. https://doi.org/10.3389/fmicb.2015.00820.
[4] Baensch-Baltruschat, B., Kocher, B., Kochleus, C., Stock, F., Reifferscheid, G., 2021. Tyre and road wear particles-a calculation of generation, transport and release to water and soil with special regard to German roads. Sci Total Environ 752, 141939. https://doi.org/10.1016/j.scitotenv.2020.141939.
[5] Banjerdkij, P., Vattanaviboon, P., Mongkolsuk, S., 2003. Cadmium-induced adaptive resistance and cross-resistance to zinc in Xanthomonas campestris. Curr Microbiol 47, 0260–0262. https://doi.org/10.1007/s00284-003-4025-5.
[6] Barros, A.R., Adorno, M.A.T., Sakamoto, I.K., Maintinguer, S.I., Varesche, M.B.A., Silva, E.L., 2011. Performance evaluation and phylogenetic characterization of anaerobic fluidized bed reactors using ground tire and pet as support materials for biohydrogen production. Bioresour Technol 102 (4), 3840–3847. https://doi.org/10.1016/j.biortech.2010.12.014.
[7] Camatini, M., Crosta, G.F., Dolukhanyan, T., Sung, C., Giuliani, G., Corbetta, G.M., Cencetti, S., Regazzoni, C., 2001. Microcharacterization and identification of tire debris in heterogeneous laboratory and environmental specimens. Mater Charact 46 (4), 271–283. https://doi.org/10.1016/S1044-5803(00)00098-X.
[8] Caporaso, G., Lauber, L., Walters, W., Berg-Lyons, D., Lozupone, C., Turnbaugh, P., Fierer, N., Knight, R., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci USA 108 (Suppl), 4516–4522. https://doi.org/10.1073/pnas.1000080107.
[9] Castan, S., Henkel, C., Hüffer, T., Hofmann, T., 2021. Microplastics and nanoplastics barely enhance contaminant mobility in agricultural soils. Commun Earth Environ 2 (1), 1–9. https://doi.org/10.1038/s43247-021-00267-8.
[10] Castan, S., Sherman, A., Peng, R., Zumstein, M.T., Wanek, W., Hüffer, T., Hofmann, T., 2022. Uptake, metabolism, and accumulation of tire wear particle-derived compounds in lettuce. Environ Sci Technol 57 (1), 168–178. https://doi.org/10.1021/acs.est.2c05660.
[11] Coelho, M.A.Z., Russo, C., Araujo, O.Q.F., 2000. Optimization of a sequencing batch reactorfor biological nitrogen removal. Water Res 34 (10), 2809–2817. https://doi.org/10.1016/S0043-1354(00)00010-5.
[12] Cydzik-Kwiatkowska, A., Zielińska, M., 2016. Bacterial communities in full-scale wastewater treatment systems. World J Microbiol Biotechnol 32 (4), 1–8. DOI: 10.1007/s11274-016-2012-9.
[13] Cydzik-Kwiatkowska, A., Grzyb, M., Jachimowicz, P., 2021. Metatranscriptome analysis of bisphenol A-exposed aerobic granular sludge. Energies 14, 3263. https://doi.org/10.3390/en14113263.
[14] Cydzik-Kwiatkowska, A., Białowiec, A., Wojnowska-Baryła, I., Smoczyński, L., 2009. Characteristic of granulated activated sludge fed with glycerin fraction from biodiesel production. Arch Environ Prot 41–52.
[15] Cydzik-Kwiatkowska, A., Bernat, K., Zielińska, M., Bułkowska, K., Wojnowska-Baryła, I., 2017. Aerobic granular sludge for bisphenol A (BPA) removal from wastewater. Int Biodeterior Biodegrad 122, 1–11. https://doi.org/10.1016/j.ibiod.2017.04.008.
[16] De Kreuk, M.K., Kishida, N., Van Loosdrecht, M.C.M., 2007. Aerobic granular sludge–state of the art. Water Sci Technol 55 (8-9), 75–81. https://doi.org/10.2166/wst.2007.244.
[17] Dhariwal, A., Chong, J., Habib, S., King, I.L., Agellon, L.B., Xia, J., 2017. MicrobiomeAnalyst: a web-based tool for comprehensive statistical, visual and meta-analysis of microbiome data. Nucleic Acids Res 45 (W1), W180–W188. https://doi.org/10.1093/nar/gkx295.
[18] Ding, J., Meng, F., Chen, H., Chen, Q., Hu, A., Yu, C.P., Chen, L., Lv, M., 2022. Leachable additives of tire particles explain the shift in microbial community composition and function in coastal sediments. Environ Sci Technol 56 (17), 12257–12266. https://doi.org/10.1021/acs.est.2c02757.
[19] Eurostat, 2022. Passenger cars in the EU. Available online: ⟨https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Passenger_cars_in_the_EU⟩ (accessed on 30 Aprill 2023).
[20] Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods 10 (10), 996–998.
[21] Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics. https://doi.org/10.1093/bioinformatics/btr381.
[22] Gies, E.A., LeNoble, J.L., Noel, M., Etemadifar, A., Bishay, F., Hall, E.R., Ross, P.S., 2018. Retention of microplastic in a major secondary wastewater treatment plant in Vancouver, Canada. Mar Pollut Bull 133, 553–561. https://doi.org/10.1016/j.marpolbul.2018.06.006.
[23] Huang, W.C., Jia, X., Li, J., Li, M., 2019. Dynamics of microbial community in the bioreactor for bisphenol S removal. Sci Total Environ 662, 15–21. https://doi.org/10.1016/j.scitotenv.2019.01.1.
[24] Jachimowicz, P., Cydzik-Kwiatkowska, A., Szklarz, P., 2020. Effect of aeration mode on microbial structure and efficiency of treatment of TSS-rich wastewater from meat processing. Appl Sci 10 (21), 7414. https://doi.org/10.3390/app10217414.
[25] Jachimowicz, P., Jo, Y.J., Cydzik-Kwiatkowska, A., 2022. Polyethylene microplastics increase extracellular polymeric substances production in aerobic granular sludge. Sci Total Environ 851, 158208. https://doi.org/10.1016/j.scitotenv.2022.158208.
[26] Järlskog, I., Jaramillo-Vogel, D., Rausch, J., Gustafsson, M., Strömvall, A.M., Andersson-Sköld, Y., 2022. Concentrations of tire wear microplastics and other traffic-derived non-exhaust particles in the road environment. Environ Int 170, 107618. https://doi.org/10.1016/j.envint.2022.107618.
[27] Jiang, Q., Zhang, W., 2021. Gradual effects of gradient concentrations of polystyrene nanoplastics on metabolic processes of the razor clams. Environ Pollut 287, 117631. https://doi.org/10.1016/j.envpol.2021.117631.
[28] Kajla, S., Kumari, R., Nagi, G.K., 2022. Microbial CO_2 fixation and biotechnology in reducing industrial CO_2 emissions. Arch Microbiol 204 (2), 149. https://doi.org/10.1007/s00203-021-02677-w.

[29] Kalčíková, G., Alič, B., Skalar, T., Bundschuh, M., Gotvajn, A.Ž., 2017. Wastewater treatment plant effluents as source of cosmetic polyethylene microbeads to freshwater. Chemosphere 188, 25–31. https://doi.org/10.1016/j.chemosphere.2017.08.131.
[30] Kowalska, K., Felis, E., Sochacki, A., Bajkacz, S., 2019. Removal and transformation pathways of benzothiazole and benzotriazole in membrane bioreactors treating synthetic municipal wastewater. Chemosphere 227, 162–171. https://doi.org/10.1016/j.chemosphere.2019.04.037.
[31] Kumata, H., Sanada, Y., Takada, H., Ueno, T., 2000. Historical trends of N-cyclohexyl-2-benzothiazolamine, 2-(4-morpholinyl) benzothiazole, and other anthropogenic contaminants in the urban reservoir sediment core. Environ Sci Technol 34 (2), 246–253. https://doi.org/10.1021/es990738k.
[32] Lennicke, C., Cochemé, H.M., 2021. Redox metabolism: ROS as specific molecular regulators of cell signaling and function. Mol Cell 81 (18), 3691–3707. https://doi.org/10.1016/j.molcel.2021.08.018.
[33] Li, X., Chen, L., Mei, Q., Dong, B., Dai, X., Ding, G., Zeng, E.Y., 2018. Microplastics in sewage sludge from the wastewater treatment plants in China. Water Res 142, 75–85. https://doi.org/10.1016/j.watres.2018.05.034.
[34] Liao, C., Kim, U.-J., Kannan, K., 2018. A review of environmental occurrence, fate, exposure, and toxicity of benzothiazoles. Environ Sci Technol 52 (9), 5007–5026. https://doi.org/10.1021/acs.est.7b05493.
[35] Liu, Q., Li, L., Zhao, X., Song, K., 2021. An evaluation of the effects of nanoplastics on the removal of activated-sludge nutrients and production of short chain fatty acid. Process Saf Environ Prot 148, 1070–1076. https://doi.org/10.1016/j.psep.2021.02.029.
[36] Liu, Y., Zhou, H., Yan, M., Liu, Y., Ni, X., Song, J., Yi, X., 2022. Toxicity of tire wear particles and the leachates to microorganisms in marine sediments. Environ Pollut 309, 119744. https://doi.org/10.1016/j.envpol.2022.119744.
[37] Lopez-Vazquez, C.M., Oehmen, A., Hooijmans, C.M., Brdjanovic, D., Gijzen, H.J., Yuan, Z., van Loosdrecht, M.C.M., 2009. Modeling the PAO-GAO competition: effects of carbon source, pH and temperature. Water Res 43, 450–462. https://doi.org/10.1016/j.watres.2008.10.032.
[38] Lünsmann, V., Kappelmeyer, U., Benndorf, R., Martinez-Lavanchy, P.M., Taubert, A., Adrian, L., Duarte, M., Dietmar, P., Bergen, M., Muller, J., Heipieper, H., Jehmlich, N., 2016. In situ p rotein-SIP highlights Burkholderiaceae as key players degrading toluene by para ring hydroxylation in a constructed wetland model. Environ Microbiol 18 (4), 1176–1186. https://doi.org/10.1111/1462-2920.13133.
[39] McSwain, B.S., Irvine, R.L., Hausner, M., Wilderer, P., 2005. Composition and distribution of extracellular polymeric substances in aerobic flocs and granular sludge. Appl Environ Microbiol 71 (2), 1051–1057. https://doi.org/10.1128/AEM.71.2.1051-1057.2005.
[40] Mujahid, M., Prasuna, M.L., Sasikala, C., Ramana, C.V., 2014. Integrated metabolomic and proteomic analysis reveals systemic responses of rubrivivax benzoatilyticus JA2 to aniline stress. J Proteome Res 14 (2), 711–727. https://doi.org/10.1021/pr500725b.
[41] Muñoz-Palazon, B., Pesciaroli, C., Rodriguez-Sanchez, A., Gonzalez-Lopez, J., Gonzalez-Martinez, A., 2018. Pollutants degradation performance and microbial community structure of aerobic granular sludge systems using inoculums adapted at mild and low temperature. Chemosphere 204, 431–441. https://doi.org/10.1016/j.chemosphere.2018.04.062.
[42] Paulo, A.M., Amorim, C.L., Costa, J., Mesquita, D.P., Ferreira, E.C., Castro, P.M., 2021. Long-term stability of a non-adapted aerobic granular sludge process treating fish canning wastewater associated to EPS producers in the core microbiome. Sci Total Environ 756, 144007. https://doi.org/10.1016/j.scitotenv.2020.144007.
[43] Peng, H., Zhang, Y., Zhang, Q., Zhang, W., Li, M., Feng, J., Su, J., He, J., Zhong, M., 2022. Control of aeration time in the aniline degrading-bioreactor with the analysis of metagenomic: Aniline degradation and nitrogen metabolism. Bioresour Technol 344, 126281. https://doi.org/10.1016/j.biortech.2021.126281.
[44] Reguera, G., Kashefi, K., 2019. The electrifying physiology of Geobacter bacteria, 30 years on. Adv Microb Physiol. https://doi.org/10.1016/bs.ampbs.2019.02.007.
[45] Rollemberg, S.L.D., Barros, A.R.M., Firmino, P.I.M., dos Santos, A.B., 2018. Aerobic granular sludge: cultivation parameters and removal mechanisms. Bioresour Technol 270, 678–688. https://doi.org/10.1016/j.biortech.2018.08.130.
[46] Sathicq, M.B., Sabatino, R., Di Cesare, A., Eckert, E.M., Fontaneto, D., Rogora, M., Corno, G., 2022. PET particles raise microbiological concerns for human health while tyre wear microplastic particles potentially affect ecosystem services in waters. J Hazard Mater 429, 128397. https://doi.org/10.1016/j.jhazmat.2022.128397.
[47] Shen, N., Zhou, Y., 2016. Enhanced biological phosphorus removal with different carbon sources. Appl Microbiol Biotechnol 100 (11), 4735–4745. https://doi.org/10.1007/s00253-016-7518-4.
[48] Shi, Y.-J., Yang, L., Liao, S.-F., Zhang, L.-G., Liao, Z.-C., Lan, M.-Y., Sun, F., Ying, G.-G., 2021. Responses of aerobic granular sludge to fluoroquinolones: Microbial community variations, and antibiotic resistance genes. J Hazard Mater 414, 125527. https://doi.org/10.1016/j.jhazmat.2021.125527.
[49] Stevenson, K., Stallwood, B., Hart, A.G., 2008. Tire rubber recycling and bioremediation: a review. Bioremediation J 12 (1), 1–11. https://doi.org/10.1080/10889860701866263.
[50] Wagner, S., Hüffer, T., Klöckner, P., Wehrhahn, M., Hofmann, T., Reemtsma, T., 2018. Tire wear particles in the aquatic environment-a review on generation, analysis, occurrence, fate and effects. Water Res 139, 83–100. https://doi.org/10.1016/j.watres.2018.03.051.
[51] Wang, L., Luo, Z., Zhen, Z., Yan, Y., Yan, C., Ma, X., Sun, L., Wang, M., Zhou, X., Hu, A., 2020. Bacterial community colonization on tire microplastics in typical urban water environments and associated impacting factors. Environ Pollut 265, 114922. https://doi.org/10.1016/j.envpol.2020.114922.
[52] Wang, S., Gao, Y., Yan, K., You, L., Jia, Y., Dai, X., Chen, M., Diab, A., 2021. Effect of long-term aging on waste tire rubber and amorphous poly alpha olefin compound modified asphalt binder and its mixtures. Constr Build Mater 272, 121667.
[53] Wik, A., Dave, G., 2006. Acute toxicity of leachates of tire wear material to Daphnia magna—variability and toxic components. Chemosphere 64 (10), 1777–1784. https://doi.org/10.1016/j.chemosphere.2005.12.045.
[54] Wik, A., Dave, G., 2009. Occurrence and effects of tire wear particles in the environment–a critical review and an initial risk assessment. Environ Pollut 157 (1), 1–11. https://doi.org/10.1016/j.envpol.2008.09.028.
[55] Winkler, M.K.H., Kleerebezem, R., Khunjar, W.O., de Bruin, B., van Loosdrecht, M.C.M., 2012. Evaluating the solid retention time of bacteria in flocculent and granular sludge. Water Res 46, 4973–4980. https://doi.org/10.1016/j.watres.2012.06.027.
[56] Wolff, D., Krah, D., Dötsch, A., Ghattas, A.K., Wick, A., Ternes, T.A., 2018. Insights into the variability of microbial community composition and micropollutant degradation in diverse biological wastewater treatment systems. Water Res 143, 313–324. https://doi.org/10.1016/j.watres.2018.06.033.
[57] Xiang, L., Liu, C., Liu, D., Ma, L., Qiu, X., Wang, H., Lu, X., 2022. Antimony transformation and mobilization from stibnite by an antimonite oxidizing bacterium Bosea sp. AS-1. J Environ Sci 111, 273–281. https://doi.org/10.1016/j.jes.2021.03.042.
[58] Yang, M., Ma, F., Xie, Y., Li, L., Xue, Y., 2021. The SBR start-up performing simultaneous removal of organics, nitrogen and phosphorus from aniline wastewater: Pollutant removal efficiency and microbial community succession. Environ Pollut Bioavailab 33 (1), 104–112. https://doi.org/10.1080/26395940.2021.1924079.
[59] Ye, J., Cheng, R., Chen, Z., Fang, C., Shi, M., Yu, R., Xue, X., 2023. Stress response mechanism of wastewater biological nitrogen removal systems to environmentally realistic concentrations of tire wear particles: contribution of leachable additives. Bioresour Technol 387, 129610. https://doi.org/10.1016/j.biortech.2023.129610.
[60] Yin, Y., Zhang, Q., Peng, H., 2023. Retrospect and prospect of aerobic biodegradation of aniline: overcome existing bottlenecks and follow future trends. J Environ Manag 330, 117133. https://doi.org/10.1016/j.jenvman.2022.117133.
[61] Zhang, H.Y., Huang, Z., Liu, Y.H., Hu, L.X., He, L.Y., Liu, Y.S., Zhao, J.L., Ying, G.G., 2022. Occurrence and risks of 23 tire additives and their transformation products in an urban water system. Environ Int, 107715. https://doi.org/10.1016/j.envint.2022.107715.
[62] Zhang, X., Qu, Y., You, S., Ma, Q., Zhou, H., Zhang, L., Jing, J., Liu, L., 2018. Bioremediation of nitrogen-containing organic pollutants using phenol-stimulated activated sludge: performance and microbial community analysis. J Chem Technol Biotechnol 93 (11), 3199–3207. https://doi.org/10.1002/jctb.5676.
[63] Zhang, Z., Qiu, J., Xiang, R., Yu, H., Xu, X., Zhu, L., 2019. Organic loading rate (OLR) regulation for enhancement of aerobic sludge granulation: role of key microorganism and their function. Sci Total Environ 653, 630–637. https://doi.org/10.1016/j.scitotenv.2018.10.418.
[64] Zhong, X.Z., Li, X.X., Zeng, Y., Wang, S.P., Sun, Z.Y., Tang, Y.Q., 2020. Dynamic change of bacterial community during dairy manure composting process revealed by high-throughput sequencing and advanced bioinformatics tools. Bioresour Technol 306, 123091. https://doi.org/10.1016/j.biortech.2020.123091.

Supplementary Materials

Table S1 - Transitions from precursor ions to product ions

Compound	Precursor ion (m/z)	Product ion (m/z)	Fragmentor voltage (V)	Collision energy (V)	Cell accelerator voltage (V)	Retention time (min)
2-A-BTH	151	109	150	30	5	3.1
2-A-BTH	151	65	150	38	5	3.1
2-OH-BTH	152	124	140	22	4	5.4
2-OH-BTH	152	92	140	22	4	5.4
2-Me-S-BTH	168	135	135	28	5	6.8
2-Me-S-BTH	168	124	135	24	5	6.8
2-Me-S-BTH	168	109	135	32	5	6.8
2-Me-BTH	150	109	150	30	5	7.3
2-Me-BTH	150	65	150	30	5	7.3
5-Me-BTH	134	106	150	22	5	5.0
5-Me-BTH	134	79	150	22	5	5.0
6PPD	269	184	150	37	5	7.9
6PPD	269	107	150	29	5	7.9
6PPD	269	93	150	41	5	7.9
6PPD-Q	299	241	150	38	5	10.9
6PPD-Q	299	215	150	22	5	10.9
6PPD-Q	299	187	150	26	5	10.9
Aniline	94	77	100	23	5	1.5
Aniline	94	51	100	41	5	1.5
Aniline	94	50	100	53	5	1.5
BTH	136	109	150	23	5	6.5
BTH	136	77	150	23	5	6.5
BTH	136	65	150	38	5	6.5
DPG	212	195	150	20	5	4.2
DPG	212	119	150	24	5	4.2
DPG	212	94	150	24	5	4.2
DTG	240	133	150	25	5	5.1
DTG	240	116	150	33	5	5.1
HMMM	391	283	150	11	5	15.3
HMMM	391	253	150	23	5	7.1

HMMM	391	207	150	19	5	7.1
HMMM	391	177	150	27	5	7.1

Table S2. Total number of reads and α-diversity profiling in the collected biomass sample from the GSBRs

Sample	Number reads counts	Shannon	Chao	ACE	Simpson
Inoculum	28867	2.87	71.0	71.32	0.90
R1_21	45586	1.69	86.0	84.73	0.54
R1_42	33388	2.31	89.0	89.22	0.76
R1_84	92217	2.19	85.5	85.67	0.78
R2_21	85563	1.37	83.0	83.00	0.43
R2_42	30611	2.81	91.2	91.51	0.88
R2_84	39323	1.95	88.0	88.00	0.66
R3_21	22084	2.30	83.2	83.72	0.72
R3_42	23772	2.05	84.2	84.64	0.63
R3_84	42816	1.60	77.0	77.00	0.60
R4_21	24827	2.59	82.0	82.30	0.80
R4_42	31855	2.04	83.0	82.88	0.65
R4_84	38179	2.72	89.5	89.53	0.86

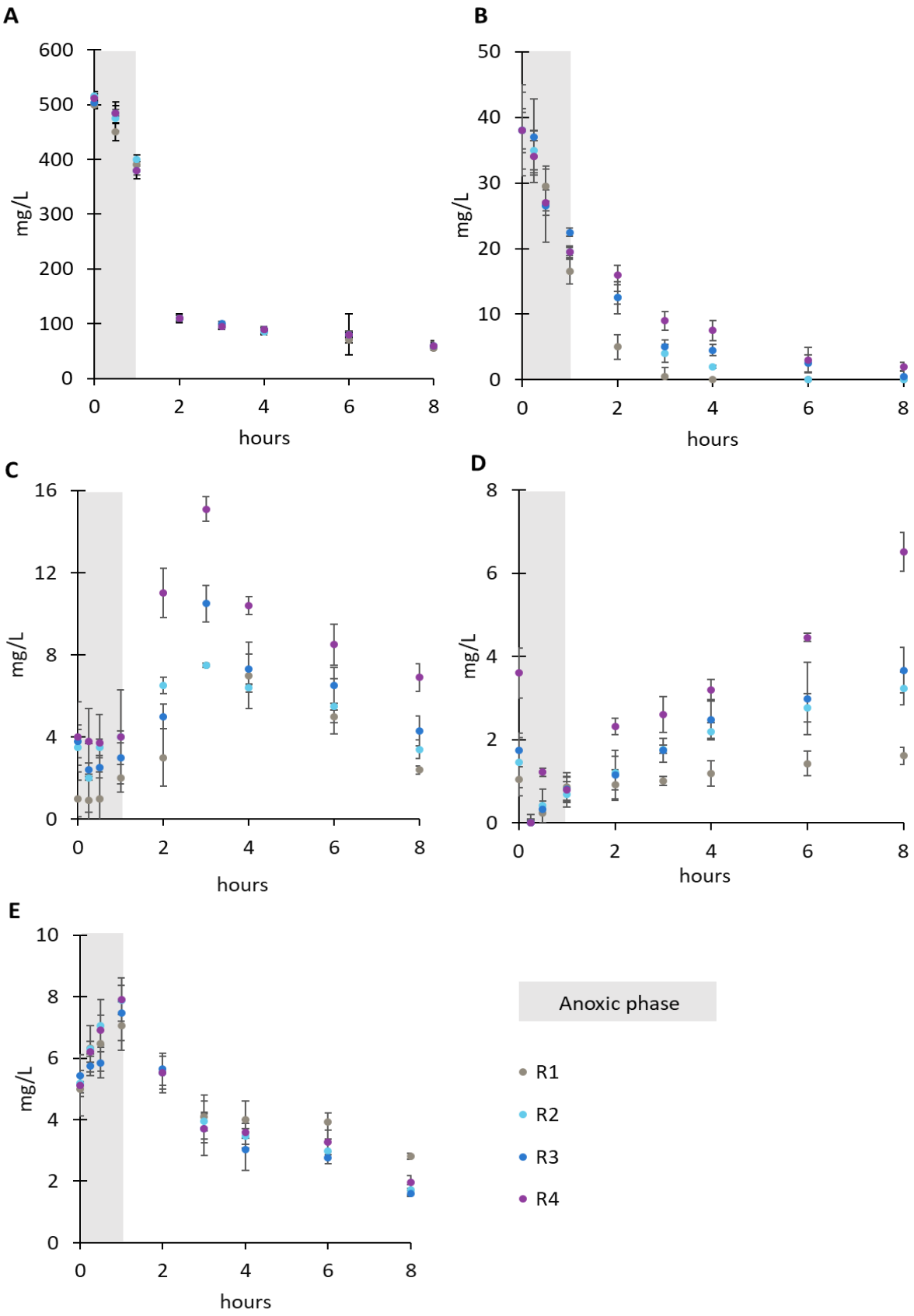

Fig. S1. The concentration of COD (A), N-NH_4(B), N-NO_2(C), N-NO_3 (D), P-PO_4 (E), during the cycle in GSBRs at the end of experiment

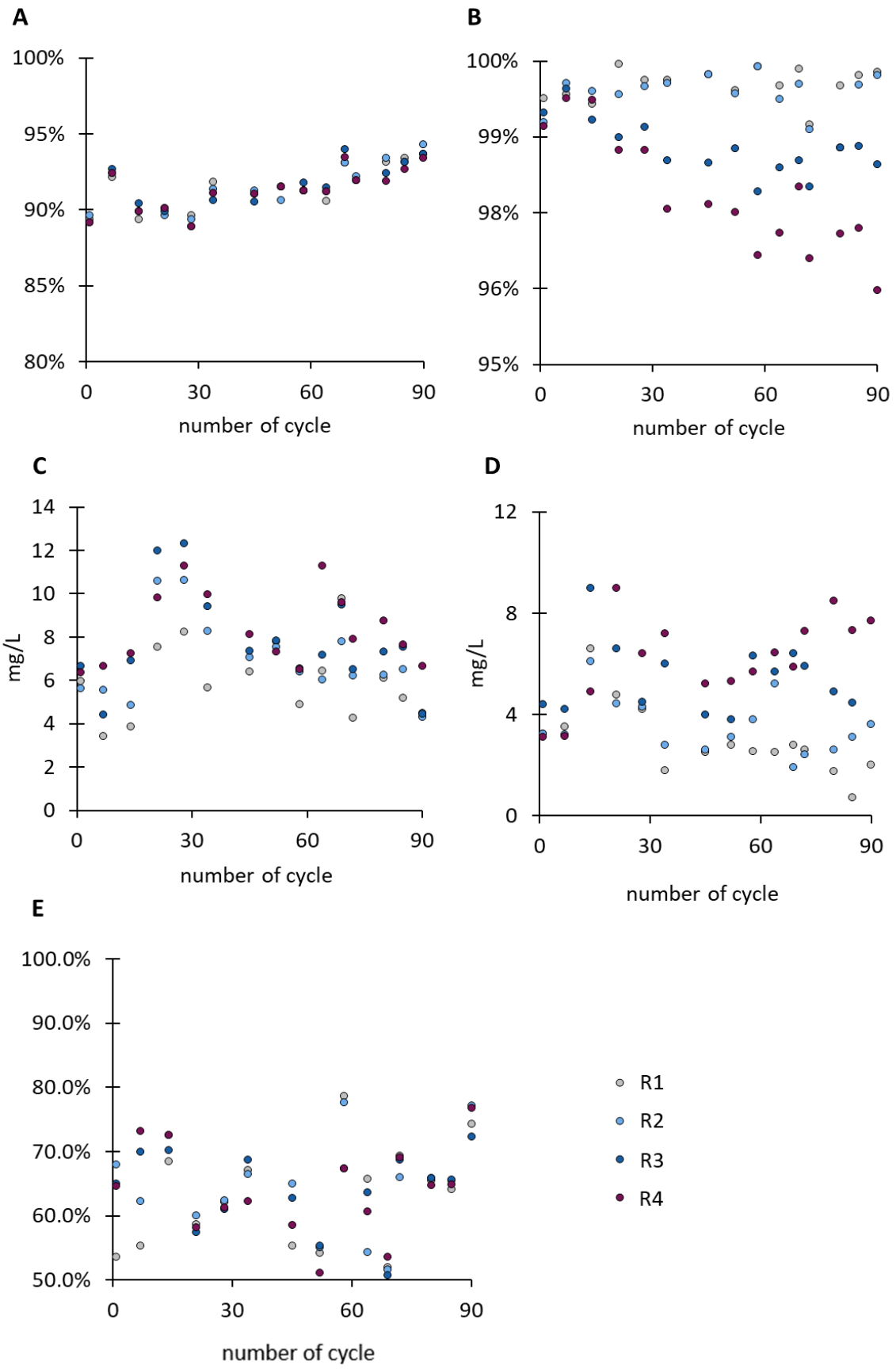

Fig. S2. Removal efficiency of COD (A), N-NH_4(B), P-PO_4 (E) and concentration of N-NO_2(C), N-NO_3 (D), during the experiment

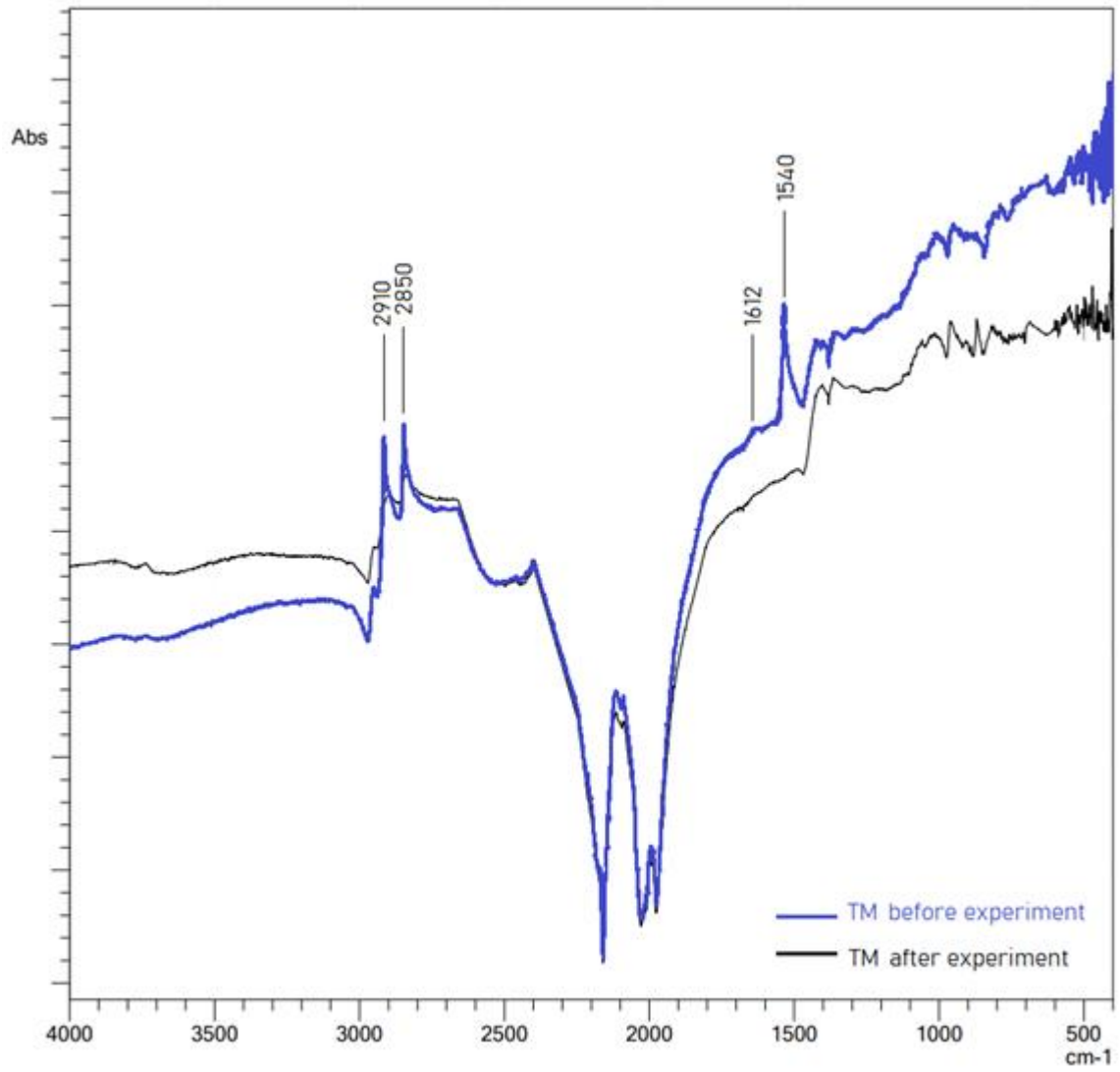

Fig. S3. FTIR analysis measurements of TM before and after aging in GSBRs

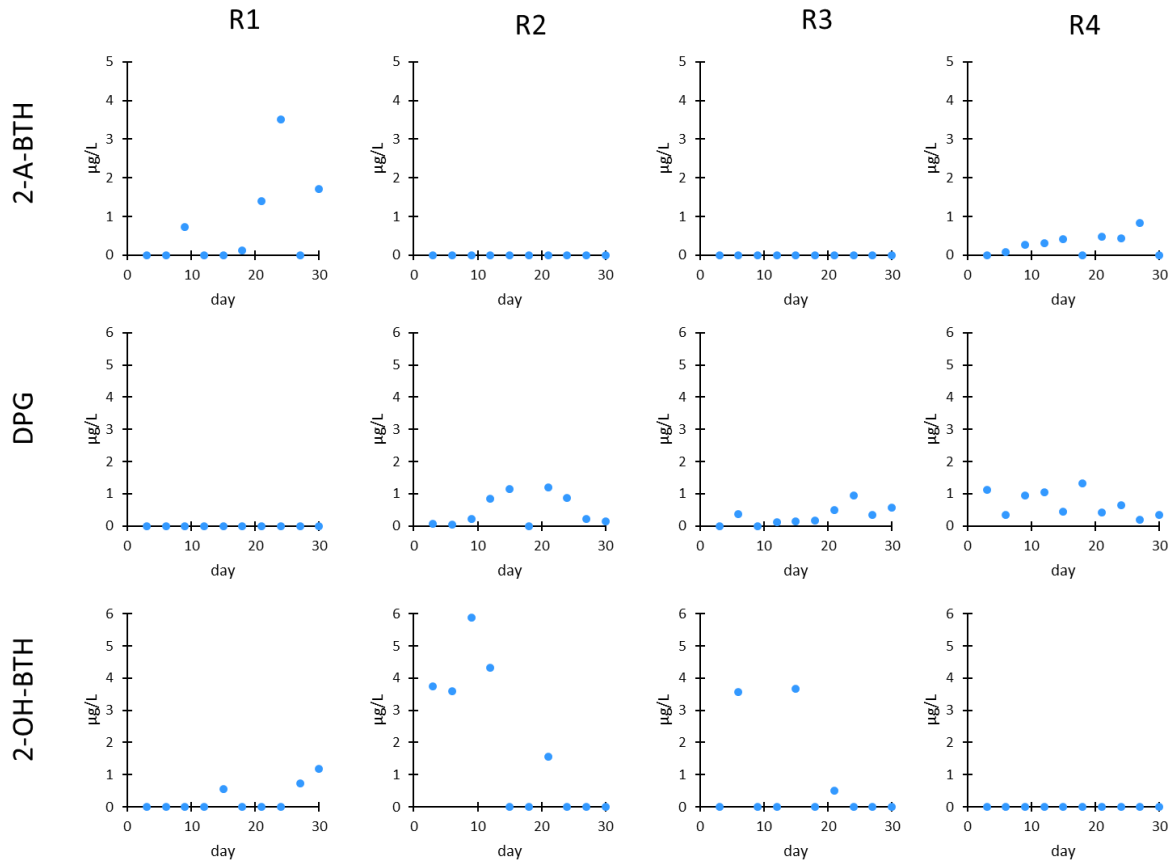

Fig. S4. Quantification of TM additives and their transformation products in treated wastewater from the reactors

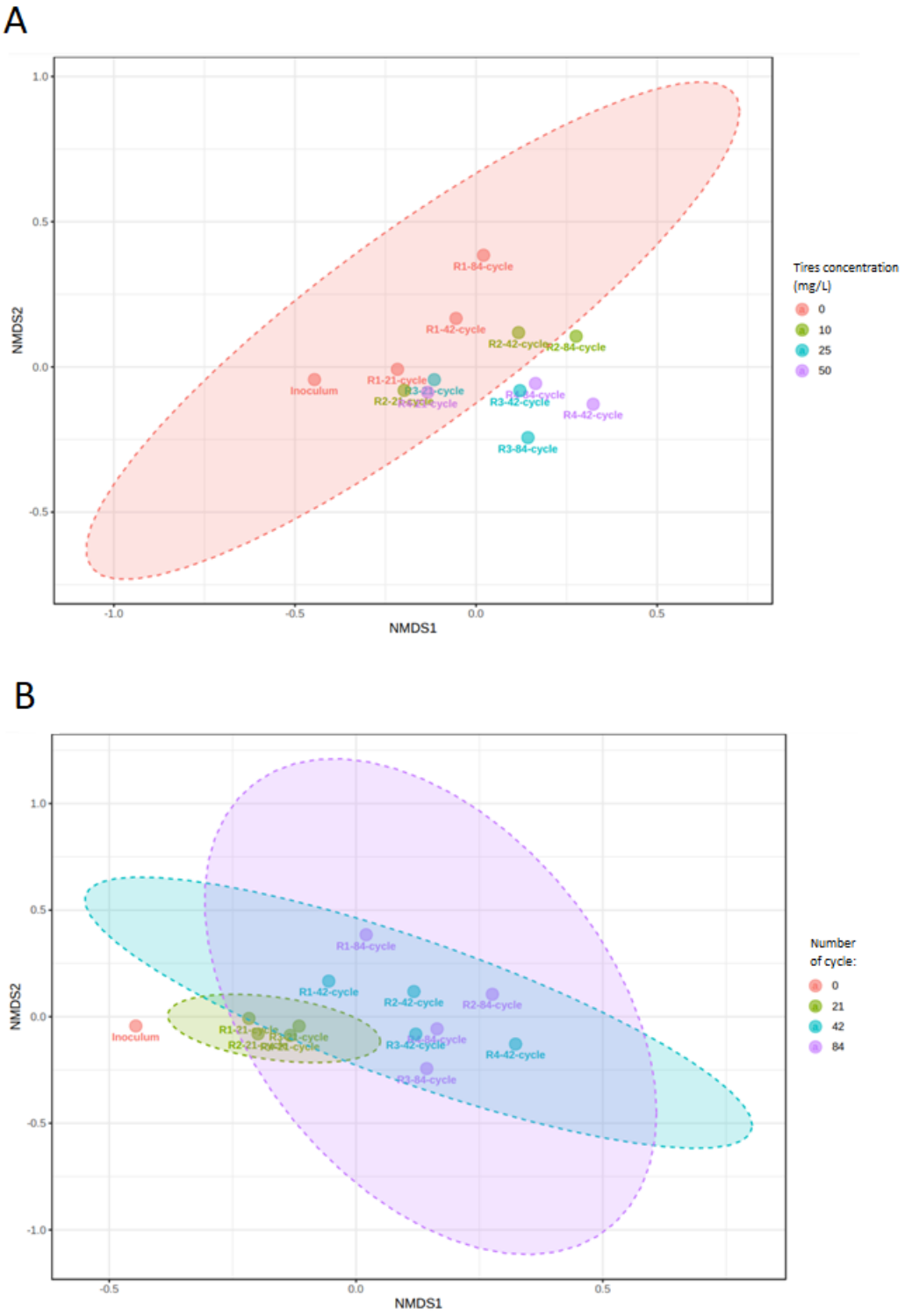

Fig. S5. β-diversity of microbial community composition of the biomass changed of dose of TM (A) and time of the experiment (B)

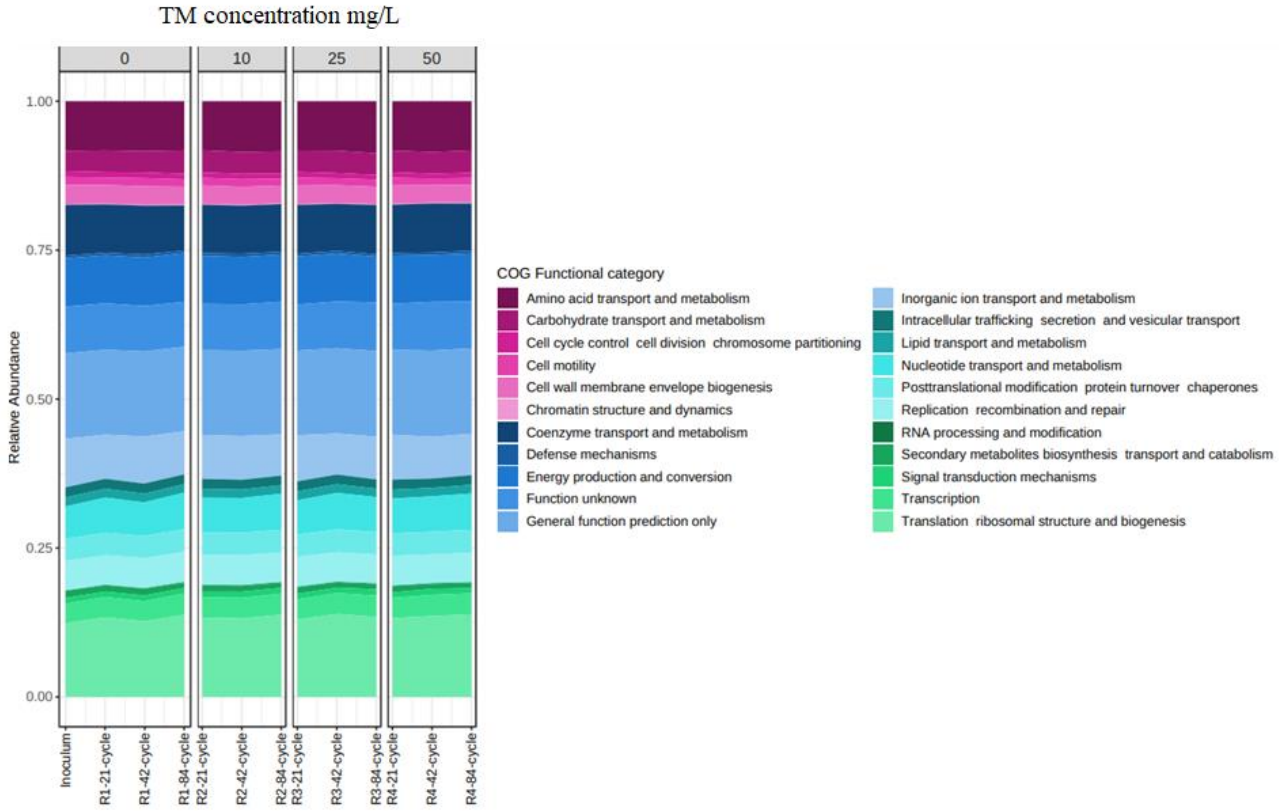

Fig. S6 Bacterial functional prediction based on COG-level functional categories

Olsztyn, 4.04.2024

(miejscowość, data)

Piotr Jachimowicz

(imię i nazwisko kandydata)

Inżynieria środowiska, górnictwo i energetyka

(dyscyplina naukowa)

Przewodniczący Rady Naukowej Dyscypliny

inżynieria środowiska, górnictwo i energetyka

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prof. dr hab. inż. Marcin Dębowski

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Oświadczam, że mój wkład merytoryczny w przygotowanie w pracy:

Jachimowicz, P., Peng, R., Hüffer, T., Hofmann, T., Cydzik-Kwiatkowska, A. (2024). Tire materials disturb transformations of nitrogen compounds and affect the structure of biomass in aerobic granular sludge reactors. Journal of Hazardous Materials, 133223.

polegał na: udziale w zaplanowaniu koncepcji badań, opracowaniu metodyki, wykonaniu części eksperymentalnej, opracowaniu i interpretacji wyników, graficznym opracowaniu wyników, pozyskaniu funduszy na prowadzenie badań oraz przygotowaniu pierwszej wersji manuskryptu.

Piotr Jachimowicz

(podpis kandydata)

Vienna, 27.08.2024

(miejscowość, data)

Ruoting Peng

(imię i nazwisko)

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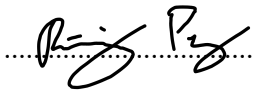

(signature)

Olsztyn, 16.04.2024

(miejscowość, data)

dr. hab. Thorsten Hüffer

(imię i nazwisko kandydata)

Przewodniczący Rady Naukowej Dyscypliny

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Uniwersytetu Warmińsko-Mazurskiego w Olsztynie

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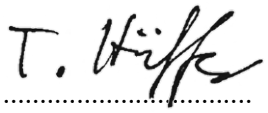

.....

(Dr. Thorsten Hüffer)

Olsztyn, 16.04.2024

(miejscowość, data)

prof. dr. Thilo Hofmann

(imię i nazwisko)

Przewodniczący Rady Naukowej Dyscypliny

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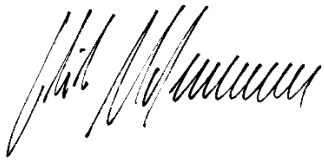

Vienna, April 16, 2024

Prof. dr. Thilo Hofmann

Olsztyn, 4.04.2024

(miejscowość, data)

prof. dr hab. inż. Agnieszka Cydzik-Kwiatkowska

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A. Cydzik-Kwiatkowska

(podpis)

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Microplastics in granular sequencing batch reactors: Effects on pollutant removal dynamics and the microbial community

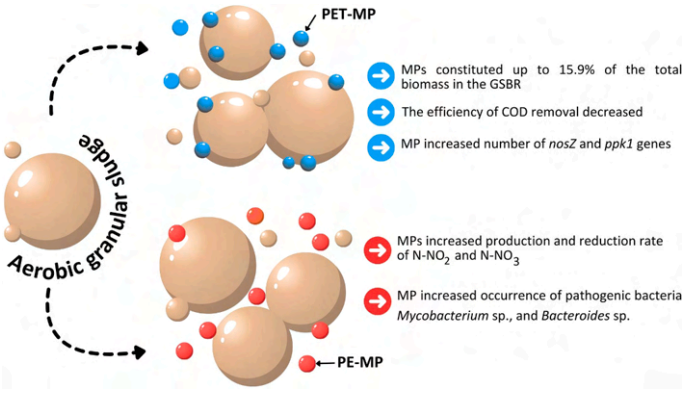
Piotr Jachimowicz *, Weronika Mądzielewska, Agnieszka Cydzik-Kwiatkowska

Department of Environmental Biotechnology, University of Warmia and Mazury in Olsztyn, Sloneczna 45G, 10-709 Olsztyn, Poland

HIGHLIGHTS

- Microplastic type affects efficiency and rate of pollutant removal by aerobic granular sludge
- PET microplastic exhibit greater biomass affinity than PE microplastic
- The type of microplastic shapes the microbiome of aerobic granules
- Microplastic, especially PET, dosages affected *nosZ* and *ppk1* gene abundances

GRAPHICAL ABSTRACT



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Polyethylene terephthalate
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Real-time PCR
Metagenomic
Biological wastewater treatment

ABSTRACT

This study investigated the relationship between microplastic (MP) presence and pollutant removal in granular sludge sequencing batch reactors (GSBRs). Two types of MPs, polyethylene (PE) and polyethylene terephthalate (PET), were introduced in varying concentrations to assess their effects on microbial community dynamics and rates of nitrogen, phosphorus, and organic compound removal. The study revealed type-dependent variations in the deposition of MPs within the biomass, with PET-MPs exhibiting a stronger affinity for accumulation in biomass. A 50 mg/L dose of PET-MP decreased COD removal efficiency by approximately 4 % while increasing P-PO_4 removal efficiency by around 7 % compared to the control reactor. The rate of nitrogen compounds removal decreased with higher PET-MP dosages but increased with higher PE-MP dosages. An analysis of microbial activity and gene abundance highlighted the influence of MPs on the expression of the *nosZ* and *ppk1* genes, which code enzymes responsible for nitrogen and phosphorus transformations. The study also explored shifts in microbial community structure, revealing alterations with changes in MP dose and type. This research contributes valuable insights into the complex interactions between MP, microbial communities, and pollutant removal processes in GSBR systems, with implications for the sustainable management of wastewater treatment in the presence of MP.

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1. Introduction

Wastewater treatment plants (WWTPs) in Europe use a combination of secondary and tertiary treatment processes to ensure that wastewater is treated to a high standard before it is discharged into the environment. In European Union countries in 2020, 68.9 % of the population was connected to tertiary-level treatment and 12.9 % to secondary-level treatment [1]. WWTPs are primarily designed to remove nutrients such as organic materials, phosphorus, and nitrogen from wastewater. They are not specifically designed to remove emerging contaminants, a newer class of pollutants that have become a concern in recent years. Emerging contaminants in wastewater include pharmaceuticals, personal care products, and microplastics (MPs) and have raised concerns due to their potential effects on public health and the environment [2-4].

MPs, defined as plastic particles with a size diameter of less than 5 mm, can enter WWTPs through various sources, including domestic and industrial wastewater. According to Franco et al. [3], the concentration of MPs in the influent of an urban WWTP was found to be 645 MP particles/L, while an industrial WWTP showed a higher concentration of up to 1567 MP/L. Once in a WWTP, MPs adhere to sludge, which creates a threat of MP spread when sludge is used as fertilizer or disposed of on land [5]. Currently, membrane technology is highly effective in removing microplastics from wastewater, nearly completely preventing their release into the aquatic environment. However, as mentioned, this does not solve the issue entirely, as microplastics accumulate in sludge. Additionally, membranes are still quite costly to operate.

The presence of MPs in WWTPs has raised concerns about their potential impact on the efficiency of the treatment process [6-8]. Li et al. [9], used five common types of MPs and tested them at 0, 1000, 5000, and 10,000 particle/L concentrations in wastewater in biological reactors with activated sludge. The MPs had a detrimental effect on the rate of ammonia oxidation, while their effect on nitrite oxidation was minimal. The addition of MPs, particularly polyvinyl chloride and polyester at concentrations of 5000 particles/L, increased denitrification efficiency. Gan et al. [10] reported that when the polystyrene MP concentration was increased from 0 mg/L to 200 mg/L, the specific nitrogen removal rate and the activity of the enzymes were inhibited. The inhibition of the specific nitrite reduction rate and the specific nitrate reduction rate was most pronounced at an MP concentration of 100 mg/L, and that of nitrite reductase and nitrate reductase at a concentration of 50 mg/L.

Aerobic granular sludge (AGS) has emerged as a promising technology for wastewater treatment due to its compactness, high biomass concentration, and tolerance to toxic compounds. AGS is a type of microbial aggregate formed through the self-immobilization of microorganisms under certain conditions. AGS is composed of microorganisms and extracellular polymeric substances (EPS), which are complex biopolymers produced by microorganisms. EPS plays a crucial role in AGS formation and function providing structural support and helping to retain microorganisms [11]. The microbiological communities present in AGS display an ability to adapt to the presence of MPs in wastewater [8]. Jachimowicz et al. [12] revealed that the presence of tire-derived MPs caused a shift in EPS-producing bacterial communities towards ones resistant to micropollutants (genera *Rubrivivax, Ferruginibacter*, and *Xanthomonas*). This shift also moved metabolic activities towards increased biodegradation and DNA repair processes.

The study presented here aimed to evaluate how different dosages of MP affect the removal efficiency of nutrients in granular sequencing batch reactors (GSBRs). An additional aim was to visualize changes in the activity of key genes related to nitrification, denitrification, and phosphorus removal, as well as the microbial community structure in AGS exposed to MP. While some research has investigated the effects of MP on activated sludge systems, there is a notable gap in understanding their effects on AGS. This study focused on analyzing the removal rates of compounds by AGS and microbial activity throughout the GSBR cycle, something that has not been extensively studied to date. What sets this research apart from others is the use of technological and molecular biology tools (real-time PCR, metagenomics) to comprehensively visualize the changes induced by the presence of MPs. This approach aims to gain holistic insights into how AGS behaves in the presence of MPs, allowing the operating conditions of the treatment system to be optimized. In addition, understanding the removal rates of compounds can highlight potential bottlenecks in the treatment process, facilitating the development of more effective treatment strategies. Overall, this information could significantly contribute to improving the efficiency and performance of AGS in wastewater treatment processes affected by MP.

2. Materials and methods

2.1. GSBRs setup

The study involved two experimental runs, in each run four GSBRs were operated (Fig. S1). The reactors had a diameter of 10 cm and a height of 50 cm, a working volume of 3.0 L, and were operated at a wastewater exchange ratio of 60 %. GSBR cycle lasted 8 h, including an anoxic phase (60 min), an aeration phase (400 min), a settling phase (10 min), and a feeding/discharging phase (5 min each). During the aeration phase, the air was supplied with a superficial velocity of 0.8 cm/s (120 L/h). The inoculum sludge was obtained from the control reactor used in a previous study [12]. Although the concentration of MP in AGS was not measured, the sludge age of about 30 days suggested that the MP accumulated during the 9-month period were flushed out with the biomass. This conclusion was based on the fact that only synthetic wastewater, which did not contain MP, was used in the reactor.

The synthetic wastewater composition, simulating municipal wastewater, included NH_4Cl – 76.1 mg/L, $Na_2HPO_4{\cdot}12\ H_2O$ – 46.2 mg/L, NaCl – 10.1 mg/L, KCl – 4.7 mg/L, $CaCl_2{\cdot}2\ H_2O$ – 4.7 mg/L, $MgSO_4{\cdot}7\ H_2O$ – 16.7 mg/L, $NaHCO_3$ – 243.3 mg/L, Na_2CO_3 – 162.2 mg/L, $FeCl_3{\cdot}6\ H_2O$ – 0.2 mg/L, $ZnSO_4$ – 0.2 mg/L, $MnSO_4{\cdot}H_2O$ – 0.2 mg/L, $CuSO_4$ – 0.2 mg/L [13]. Sodium acetate was sources of organic carbon in wastewater. The influent wastewater had pollutant concentrations of 600–700 mg COD/L, 50–70 mg N-NH_4/L, and 10–16 mg TP/L. In all GSBRs, at least 200 cycles were conducted (about 2 months of reactor operation) and the treatment efficiency of the GSBRs was considered stable if the range of changes in the effluent characteristics did not exceed 5–10 % within 7 days. After the confirmed adaptation period of the AGS (full granulation), the addition of MP commenced.

The MPs dosed to GSBRs were chosen based on their common occurrence in the environment and their widespread use in plastic products [14]. These polymers were polyethylene (PE-MP), and polyethylene terephthalate (PET-MP). The PET polymers were acquired in powder form from Goodfellow (UK), and PE in powder form from ALDRICH (USA). The supplier-provided densities for MP-PE and MP-PET were 0.94 and 1.3 g/mL, respectively. The particle size distribution was 40–48 μm, and $<$ 300 μm for MP-PE and PET, respectively. Due to the differences in sizes between MP-PE and MP-PET, the GSBR with PET and PE were analyzed separately. This approach ensured an accurate assessment of the research findings.

In each experimental run, either PE-MP or PET-MP was added to three reactors labeled PET1, PET10, and PET50 at concentrations of 1, 10, and 50 mg/L. In the case of GSBRs dosed with PE (at identical MP concentrations as for PET), these reactors were labeled PE1, PE10, and PE50. In each run, one reactor, labeled PET0 and PE0, served as a control and was fed with MP-free wastewater. It should be noted that the same experimental conditions prevailed in both runs, with the only difference being the type and size of MP added to the reactors.

2.2. Physicochemical analyses

In the influent and effluent of the GSBR, N-NH_4, N-NO_2, N-NO_3, P-PO_4, and COD concentrations were measured. The measurements were carried out according to APHA [15] standards. The changes in pollutant

concentrations over time were measured in duplicate during the GSBR cycles (at the last cycles of the experiment).

2.3. Biomass analysis and MP detection

The total mass of MPs present in the sludge was calculated for the entire GSBRs volume. Mixed liquor suspended solids (MLSS) in the GSBRs, were determined by the gravimetric method using HE53 Moisture Analyzer (Mettler Toledo). Following the experiment, biomass samples were promptly collected, frozen, and stored at − 20 °C. Upon thawing, the sludge samples were homogenized (based on wet weight) for extraction. 100 mL of sludge was combined with 900 mL of deionized water and 1.2 g of sodium chloride (to achieve saturation) in an Erlenmeyer flask. This mixture was stirred for 15 min and allowed to settle for 2 h. The upper water layer was then filtered using a 40 μm sieve within a vacuum filtration unit. This extraction process was carried out in triplicate, collecting all extracts within the sieve. Subsequently, the sieve in the filtration unit underwent rinsing with over 600 mL of distilled water to eliminate any salt residues. The collected extract in the sieve, along with the bottom layer, underwent a series of treatments. Initially, a 300 mL hydrogen peroxide solution (H_2O_2, 30 %) was applied overnight to eliminate and break down organic materials. The resulting mixture was then poured into 900 mL of distilled water, vacuum-filtered through glass fiber filters, rinsed multiple times with deionized water, and finally dried in a desiccator for 3 days. Additionally, Fourier-transform infrared spectroscopy (FTIR) analysis was conducted to confirm the presence of PET and PE, with the methodology described in detail in Jachimowicz et al. [12].

2.4. Real-time PCR

Real-time PCR was used to quantify the absolute abundance of specific genes in the biomass collected from various time points (1, 2, 4, and 8 h) during the GSBR cycle. The targeted genes included: the *16 S rRNA* gene (total bacterial activity), an ammonia monooxygenase (*amoA* gene), nitrite reductases (*nirK* and *nirS* genes), nitrous oxide reductase (*nosZ* gene), and polyphosphate kinase (*ppk1*) (Table S1).

Biomass samples were obtained on the final days of the experimental run (two replications) and preserved in fenozol at − 20 °C until RNA extraction using a Total RNA kit (A&A Biotechnology) following the manufacturer's protocol. The concentration of extracted RNA was measured using NanoDrop Lite (Thermo Scientific). Equal amounts of RNA (500 μg) from each sample replicate were utilized as templates for creating first-strand complementary DNA (cDNA) employing the RevertAid™ H Minus First Strand cDNA Synthesis Kit (Fermentas).

For accurate gene copy number analysis, standard curves were constructed using plasmids carrying cloned inserts (PCR products of known sizes). For cloning, the Clone Jet PCR cloning kit (Thermo Scientific) and chemically competent *Escherichia coli* 109 (Promega) were used. The standard curve for 16 S rDNA was constructed using isolated genomic DNA from *Escherichia coli* strain JM109 (Promega), which contains copies of the 16 S rDNA gene. The presence of inserts in all clones was confirmed using PCR with suitable primers (For detailed information see Rusanowska [16].

Real-time PCR for each sample was performed in quadruplicate following thermal profiles outlined in the supplementary materials (Table S1). The reaction mixture for assessing microbial activity consisted of 10 μL of Power SYBR Green PCR Master Mix (Applied Biosystems), the particular primers (see Table S1.), 0.5 μL of cDNA, and water to achieve a final volume of 20 μL. Gene amplification was conducted using the 7500 Real-Time PCR System (Applied Biosystems). After amplification, a dissociation stage was conducted to confirm the melting temperature of the PCR products. Additionally, the products underwent electrophoresis alongside the GeneRuler™ 100 bp DNA Ladder Plus (Fermentas) molecular marker to determine their molecular mass.

2.5. Metagenome and bioinformatics analysis

To evaluate the influence of various types and doses of MP on the composition of the microbial community in the GSBRs, samples of biomass and wastewater (10 mL) were collected from the reactors after 180 cycles. These samples were preserved at − 20 °C until DNA extraction. The DNA extraction was performed using the FastDNA® SPIN Kit for Soil (MP Biomedical). DNA from the three replications in each reactor was combined. The quality and quantity of DNA were assessed using a NanoDrop spectrophotometer (Thermo Scientific) and agarose electrophoresis.

The amplified DNA samples were processed using primers 515 F/806 R (GTGCCAGCMGCCGCGGTAA/GGACTACHVGGGTWTCTAAT), targeting the V4 region of the bacterial 16 S rDNA gene [17]. The MiSeq Illumina Platform at the Research and Testing Laboratory (USA) was utilized for sequencing. Chimeric sequences were removed using UCHIME in *de novo* mode [18]. Reads failing to meet quality criteria or falling below half the anticipated length were excluded. The quality criteria involve merging paired reads using a method similar to PANDAseq, with a minimum Phred score of 3. Quality filtering is performed by truncating at the first base with a Phred score below 16. Reads are then trimmed to fixed lengths to avoid terminal gaps. Dereplication identifies unique sequences and records their abundance. Singletons are discarded to improve specificity. OTU clustering using UPARSE-OTU sorts sequences by decreasing abundance, updates OTUs, and discards chimeric reads. These steps ensure accurate sequence analysis by eliminating errors and reducing spurious OTUs. Subsequently, the UPARSE algorithm was employed to group the remaining sequences into operational taxonomic units (OTUs, [19]).

Taxonomic classification was accomplished by aligning the centroid sequence of each cluster with the NCBI database using the USEARCH global alignment algorithm. The resultant sequences underwent bioinformatic analysis employing the Microbiome Analyst tool for comprehensive microbiome data analysis [20].

2.6. Statistical analysis

Calculations and statistical analyses were done using Statistica 13. To evaluate statistically significant differences in pollutant concentrations, ANOVA testing was used. For multivariate comparisons, Tukey's HSD posthoc testing was performed. The abundance of various species of bacteria was correlated with the dose of MP using Pearson correlation analysis. In conducting our study, we established a significance level of $p < 0.05$.

3. Results and discussion

3.1. Type-dependent variations of MP deposition in biomass

The deposition of MPs in biomass exhibited type-dependent variations (Fig. 1A). The PET-MPs demonstrated a higher affinity for accumulating within the biomass structure, with concentrations ranging from 121 to 159 mg MP/g MLSS. Furthermore, the concentration of PET-MPs in biomass increased proportionally with the dosage of MPs in wastewater. In contrast, the amount of deposited PE-MPs was consistent (approximately 30 mg MP/g MLSS) across all reactors. Our prior investigations [21,22] revealed that PE was associated with biomass washout from the reactor, resulting in negligible alterations in biomass concentration within the reactors. Considering those results along with the findings of our present study, it can be inferred that only limited amounts of PE can be incorporated into biomass, likely due to its density. The density of MPs plays a critical role in influencing their behavior during wastewater treatment. In the presence of raw high-solids influent, MPs with lower densities tend to float or settle when entangled in solid flocs [23]. Our experiment was conducted in GSBRs, with an air diffuser grid located at the bottom of the reactor, which can also

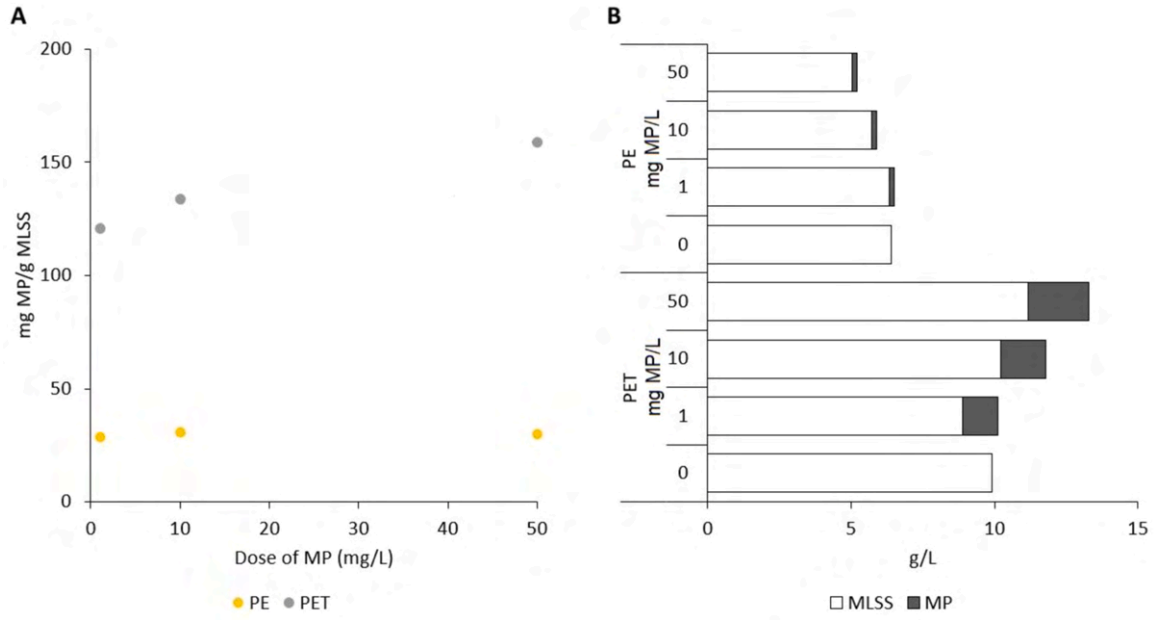

Fig. 1. The concentration of MPs (A) and MLSS (B) in GSBRs.

influence the buoyancy of lightweight plastics such as PE. According to Xu et al. [24], air microbubbles come into contact with MPs suspended in the wastewater, forming a 'bubble-MPs' complex with an overall lower density than the density of water.

It should be mentioned that the concentration of MLSS in the GSBRs varied (Fig. 1B). In the PET reactors, the concentration ranged from 8.87 to 11.18 g MLSS/L, while in the PE reactors, the concentration was much lower, ranging from 5.04 to 6.40 g MLSS/L. With the highest dose of PET-MPs, it PET constituted 15.9 % of the total biomass in the reactor. The concentration of MLSS in the reactors affects the amount of MP retained in the biomass. As reported by Nguyen et al. [25], there is a significant positive correlation between MLSS concentration and MP retention. MPs agglomerated in biomass also affect the volume of generated sludge that needs to be managed. Wei et al. [26] reported that waste sludge production can increase by up to 9 %.

3.2. Removal of organic matter (COD) and phosphorus in GSBRs

The efficiency of COD removal in the GSBRs varied depending on the concentration of MP in the wastewater (Fig. 2A). The COD removal efficiency in the GSBRs was influenced by the presence of PET-MPs in the wastewater. The average COD removal efficiency during the experiment was highest in PET0, reaching 85.5 % ± 4.7 %. As the concentration of dosed PET increased in the reactor, the efficiency of COD removal decreased to 84.4 % ± 5.3 %, 83.1 % ± 7.4 %, and 81.6 % ± 7.5 % in PET1, PET10, and PET50, respectively. Similar results were observed by Zhang et al. [27]. Granular sludge exhibited good tolerance for low concentrations (15 MP/L) of PET-MPs, but high concentrations (75–300 MP/L) significantly inhibited granular sludge activity, resulting in lower COD removal efficiency. In our study, the dosage of PE-MP did not exert a significant effect on COD removal efficiency, which varied between 87.6 % in PE10 and 89.4 % in PE1.

COD removal in the GSBR cycle followed pseudo-first-order kinetics.

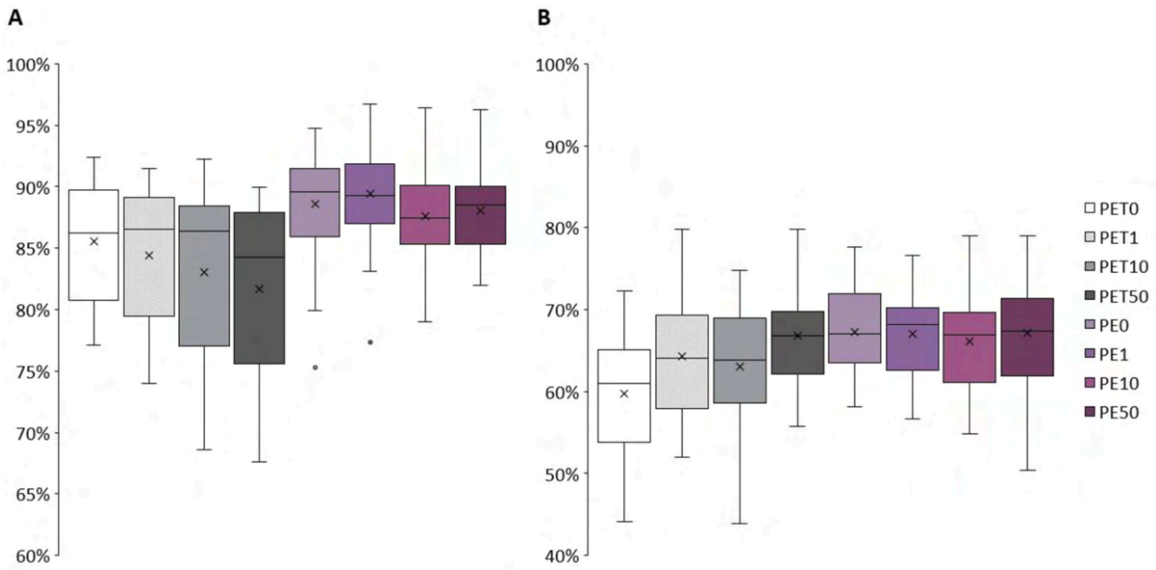

Fig. 2. Average removal efficiency of COD (A) and P-PO_4 (B) in GSBRs (n = 27).

The COD removal rate (Table 1) varied depending on MP dosage. With PET-MP, the removal rate increased from 5.45 mg/(g MLSS•h) in PET0 to 5.96 mg/(g MLSS•h) in PET1, and then sharply declined to 5.05 and 4.50 mg/(g MLSS•h) in PET10 and PET50, respectively. In contrast, with PE-MP, the COD removal rate correlated linearly ($r = 0.89$) with MP dosage, increasing from 8.16 mg/(g MLSS•h) in PE0 to 10.24 mg/(g MLSS•h) in PE50.

The presence of MP also affected the efficiency of $P\text{-}PO_4$ removal in GSBRs (Fig. 2B). In PET-MP reactors, significant changes in $P\text{-}PO_4$ removal efficiency were observed, with removal efficiencies of 64.3 % ± 8.1 %, 63.1 % ± 7.0 %, and 66.9 % ± 6.1 % for doses of 1, 10, and 50 mg/L, respectively, and an efficiency of 59.8 % ± 7.7 % in the control reactor. The analysis of changes in $P\text{-}PO_4$ removal efficiency during the experiment suggests potential adaptation of the microbial community to external carbon sources, possibly due to the leaching of carbon compounds from the MP. Parveen et al. (2023) reported that both PET-MP and PE-MP can serve as sources of dissolved organic carbon (DOC) released into the aquatic environment at levels ranging from 35.2 to 108.6 mg-C/L per g MP. The duration of exposure plays a significant role, with PET release of 40.7 mg C/L after 3 days increasing to 108.6 mg C/L after 7 days. These findings, along with those of our research, suggest that PET-MP accumulation in biomass supports $P\text{-}PO_4$ removal from wastewater. In reactors with MP-PE, the efficiency of $P\text{-}PO_4$ removal ranged from 66.1 % to 67.3 %, with no significant differences observed between various MP doses.

In the anoxic phase of the PET run, the release kinetics of $P\text{-}PO_4$ followed zeroth-order kinetics and changed significantly with different MP dosages (Table 1). For PET-MP, it increased from 0.29 mg/(g MLSS•h) in PET0 to 0.45 mg/(g MLSS•h) in PET50. Similarly, in GSBRs dosed with PE-MP, removal rates increased from 0.29 to 0.38 mg/(g MLSS•h) in PE0 and PE50, respectively. In the aerobic phase, in reactors dosed with PET, the removal rate was significantly higher than in the PET0 reactors (0.12 vs. 0.09 mg/(g MLSS•h)). The removal rate also increased with PE-MP dosage, from 0.13 to 0.17 mg/(g MLSS•h) for PE0, and PE50, respectively. The increased removal rate in reactors with MP could be attributed to $P\text{-}PO_4$ sorption on the surface of MP [28].

3.3. Nitrogen compounds in GSBRs

Increasing the dosage of MP only had a statistically significant effect on the efficiency of $N\text{-}NH_4$ removal in the case of PET-MPs ($r = 0.67$, $p < 0.05$). The efficiency of $N\text{-}NH_4$ removal in the PET-MP GSBRs ranged from 97.0 % ± 3.9 % in PET0 to 94.9 % ± 4.4 % in PET50. The correlation between PE dosage and $N\text{-}NH_4$ removal was not statistically significant, and the removal efficiency remained close to 95 % in this series (Fig. 3A).

The rate of $N\text{-}NH_4$ removal decreased with increasing MP doses from 0.38 mg/(g MLSS•h) in PET0 to 0.32 mg/(g MLSS•h) in PET50 (Table 1). Previous reports have indicated that MP can inhibit nitrification activity by affecting the functions of ammonia-oxidizing bacteria [9]. However, in the series where PE-MP was dosed, it was observed that, at high concentrations of MP (10–50 mg/L), the removal rate of $N\text{-}NH_4$ was higher. This increased removal of $N\text{-}NH_4$ may be attributable to the ability of PE to rapidly sorb $N\text{-}NH_4$ on its surface [29].

This study revealed that $N\text{-}NH_4$, $N\text{-}NO_2$, $N\text{-}NO_3$, and $P\text{-}PO_4$ concentrations in the GSBR cycle followed zeroth-order kinetics (Table 1). In the effluent from reactors dosed with PET, the dosage did not have a noticeable effect on the $N\text{-}NO_2$ concentration (Fig. 3B). However, in the effluent from PE reactors, there was a noticeable correlation between the MP dosage and the $N\text{-}NO_2$ concentration in the effluent ($r = -0.77$, $p < 0.05$). The highest concentration was recorded in PE0 (1.94 ± 0.7 mg/L) and the lowest in PE50 (1.60 ± 0.74 mg/L). Rozman et al., [30] reported that the accumulation of MP in constructed wetlands resulted in changes in nutrient cycling, particularly increased removal of $N\text{-}NO_2$, which could have significant effects on water quality.

The effects of the two MPs on the rates $N\text{-}NO_2$ production and reduction differed. With PET-MP, there were no significant differences in production rate at different MP dosages, which ranged from 0.29 to 0.33 mg/(g MLSS•h) in PET0, PET10, and PET50, and reached 0.38 mg/(g MLSS•h) in PET1. The rate of $N\text{-}NO_2$ reduction was 0.17 mg/(g MLSS•h) in PET0 and 0.15 mg/(g MLSS•h) in PET50. With PE-MP, in contrast, both the production and reduction rates increased with the dosage of MP. The $N\text{-}NO_2$ production rate ranged from 0.38 mg/(g MLSS•h) in PE0 to 0.49 mg/(g MLSS•h) in PE50, and the $N\text{-}NO_2$ removal rate ranged from 0.17 to 0.24 mg/(g MLSS•h) in PE0 and PE50, respectively.

The dosage of MP affected the concentrations of $N\text{-}NO_3$ in the treated wastewater, but this influence depended on the type of MP (Fig. 3C). In the case of PET-MP, the concentration of $N\text{-}NO_3$ significantly decreased with the MP dosage ($r = -0.55$, $p < 0.05$) from 3.74 ± 1.49 mg/L in PET0 to 2.10 ± 1.02 mg/L in PET50. A potential reason for the increased efficiency of $N\text{-}NO_3$ removal in reactors with more MP might be the release of additional carbon compounds from PET, which could aid the denitrification process [31]. In the case of PE-MP, the $N\text{-}NO_3$ concentration was significantly higher than in reactors with PET-MP, ranging from 7.22 mg/L to 7.50 mg/L ($F=9.99$, $p < 0.05$). However, the correlation between PE-MP dosage and the concentration of $N\text{-}NO_3$ was not statistically significant.

With PET-MP, there was not a significant correlation between the MP dosage and the $N\text{-}NO_3$ production rate. The production rate was higher in PET1 (0.30 mg/(g MLSS•h)) than in PET0 (0.26 mg/(g MLSS•h)). With PET10 and PET50, the production rate was either similar to (0.26 mg/(g MLSS•h)) or lower than (0.24 mg/(g MLSS•h)) that in PET0. It is worth noting that the $N\text{-}NO_3$ reduction rate was significantly higher ($p < 0.05$) in the GSBRs to which PET-MP was dosed (0.38 to 0.41 mg/(g MLSS•h)) than in the PE reactors. In the case of the PE-MP GSBRs, there were significant positive correlations between the MP dosage and the rates of production and reduction of NO_3 ($p < 0.05$).

Table 1
Kinetic changes of removal COD, $P\text{-}PO_4$ and nitrogen compounds in GSBRs mg/(g MLSS•h).

MP type	PET				PE			
	PET0	PET1	PET10	PET50	PE0	PE1	PE10	PE50
rCOD	5.43 ± 0.44	5.96 ± 0.42	5.05 ± 0.33	4.50 ± 0.35	8.16 ± 0.95	8.41 ± 0.88	8.96 ± 0.79	10.24 ± 0.74
rP-PO_4 (anoxic)	0.29 ± 0.02	0.40 ± 0.04	0.40 ± 0.02	0.45 ± 0.02	0.29 ± 0.03	0.27 ± 0.02	0.35 ± 0.03	0.38 ± 0.01
rP-PO_4 (aerobic)	0.09 ± 0.01	0.12 ± 0.01	0.12 ± 0.02	0.12 ± 0.01	0.13 ± 0.01	0.15 ± 0.02	0.15 ± 0.01	0.17 ± 0.01
rN-NH_4	0.38 ± 0.02	0.41 ± 0.05	0.35 ± 0.02	0.32 ± 0.02	0.58 ± 0.05	0.58 ± 0.03	0.64 ± 0.04	0.73 ± 0.04
rN-NO_2*	0.33 ± 0.02	0.38 ± 0.06	0.33 ± 0.03	0.29 ± 0.03	0.38 ± 0.03	0.39 ± 0.04	0.43 ± 0.02	0.49 ± 0.04
rN-NO_2**	0.17 ± 0.01	0.18 ± 0.02	0.16 ± 0.02	0.15 ± 0.01	0.17 ± 0.02	0.19 ± 0.03	0.21 ± 0.01	0.24 ± 0.01
rN-NO_3*	0.26 ± 0.03	0.30 ± 0.04	0.26 ± 0.01	0.24 ± 0.00	0.65 ± 0.03	0.66 ± 0.04	0.71 ± 0.06	0.81 ± 0.04
rN-NO_3**	0.33 ± 0.01	0.41 ± 0.02	0.38 ± 0.02	0.39 ± 0.02	0.39 ± 0.02	0.40 ± 0.01	0.44 ± 0.02	0.49 ± 0.02

Two different kinetics were observed during the operational cycle of the reactor: * - production rate * * - removal rate.

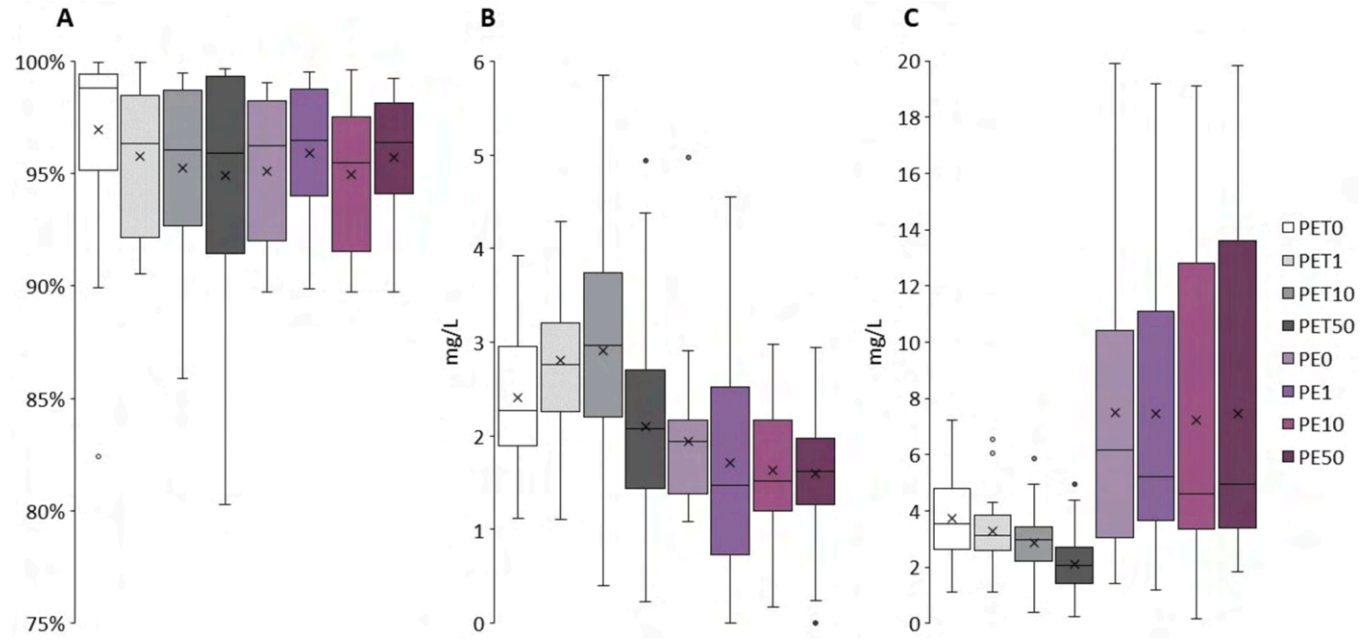

Fig. 3. The removal efficiency of N-NH_4 in the GSBRs (A) and concentration of N-NO_2 (B) and N-NO_3 (C) in the GSBRs' effluents (n = 27).

3.4. Microbial activity

The dosage of PET-MP significantly influenced the transformation of some pollutants in wastewater and affected the abundance of certain key genes responsible for nitrogen and phosphorus transformation, while PE-MPs had a lesser effect (Fig. 4). For GSBRs with PET-MP, the *16 S rRNA* gene copy numbers ranged from 3.27×10^6 to 4.11×10^9. The copy numbers for the *16 S rRNA* gene were higher in GSBRs with PE-MP, from 2.85×10^8 to 4.78×10^9.

The dosage of MPs did not negatively affect the abundance of *amoA* genes. In GSBRs dosed with PET-MP, the expression of *amoA* genes ranged from 1.66×10^3 to 4.49×10^4. It is noteworthy that the abundance of these genes in these GSBRs increased at the 2-hour and 4-hour time points of the cycle, when aerobic conditions prevailed and intensive nitrification took place. The abundance of *amoA* copies showed a positive correlation with both N-NO_2 ($r = 0.58$, $p < 0.05$) and N-NO_3 ($r = 0.56$, $p < 0.05$) concentrations in the GSBR cycle. This indicates the proper progress of the nitrification process, as ammonia was sequentially converted into nitrites by organisms possessing *amoA* genes [32]. For the experiments involving PE-MP, the highest quantities of *amoA* genes were detected in PE0 (3.27×10^4-1.38×10^5) and PE50 (1.15×10^4-1.31×10^5). However, the gene count was significantly lower in PE1 (4.59×10^3-5.98×10^4) and PE10 (6.22×10^3-2.63×10^4).

The dosage did not correlate significantly with the abundance of the *nirK* gene, nor did the abundance differ significantly depending on the type of MP. In the PET-MP GSBRs, *nirK* abundance ranged from 4.01×10^3 to 3.21×10^5; an increase in gene copy number was observed in the majority of GSBRs (except for PET1) during the aerobic phase (2–8 h), regardless of the MP dose. In the GSBRs where PE was dosed, the *nirK* gene copy numbers fluctuated between 1.23×10^4 and 1.37×10^8. In the case of PE0, PE1, and PE10, the number of copies of this gene tended to increase with time. However, in PE50, this trend was entirely reversed, and the number decreased with time. Zhang et al., [33] reported a decrease in the abundance of *nirK* genes with an increase in the MP dose that was possibly associated with the small size and relatively large surface area of the MPs. In the PET-MP GSBRs in the present study, the abundance of *nirS* ranged from 2.05×10^3 in PET0 to 1.23×10^7 in PET1. With PET-MP, the highest expression of the *nirS* gene was observed at the beginning of the GSBR cycle, just after substrate dosing. Then, in PE0, PE1, and PE10, the number of *nirS* gene copies significantly decreased. This may result from the presence of NO_2 in the reactor, which can be utilized by denitrifiers. Li et al. [34] reported that the abundance of the *nirS* gene could be easily influenced by environmental parameters.

A noticeable trend was observed in the PET-MP GSBR cycle at 0, 1, and 10 mg MP/L - over time, the number of *nirS* gene copies decreases while the number of *nirK* gene copies increases. Two kinds of nitrite reductase genes (*nirK* and *nirS* genes) can be involved in denitrification. Since there are no organisms known that carry both nitrite reductase genes, these two enzymes are considered mutually exclusive [35]. This indicates a competition between two groups of bacteria responsible for nitrite reductase using different genes [36].

The number of *nosZ* gene copies varied throughout the operational cycle of GSBRs, ranging between 3.69×10^3-5.45×10^5 in the GSBRs with PET-MP and 4.96×10^3-5.44×10^5 in the GSBRs with PE-MP. The dosage of PET-MPs positively affected($r = 0.53$, $p < 0.05$) the abundance of *nosZ* gene copies in the cycle. In PET50, an increase in the gene copy number over time was observed, showing a 22-fold increase in the 8th hour of the cycle compared to the 1st hour of the cycle. The study by Wang et al. [37] highlighted the influence of aged MPs on sediment nitrogen levels, showcasing a dose-response effect. Over the initial 10 days, higher concentrations of aged MPs notably enhanced denitrification (abundance of the *nosZ* gene).

There was a correlation between the copy number of the *nosZ* and *ppk1* genes ($r = 0.97$, $p < 0.05$) PET-MP GSBR. The reason behind such a correlation could be attributed to the substantial presence of microorganisms known as denitrifying phosphorus-accumulating organisms. These organisms can perform both phosphorus and nitrogen removal simultaneously by utilizing N-NO_3 as the electron acceptor and they often prevail in AGS structure [38]. The correlation between *nosZ* and *16 S DNA* ($r = 0.53$, $p < 0.05$) can be attributed to the fact that microorganisms capable of denitrification are widely distributed across various bacterial and archaeal taxa. As highlighted by Wigginton et al. [39], samples collected from denitrification reactors and aerobic tanks across 38 WWTPs revealed the presence of *nosZ* gene in the denitrification reactor in 92 % of the systems and aerobic reactors in 82 % of systems. This widespread occurrence indicates the prevalence of denitrifiers in both aerobic and anoxic tanks at WWTP. In the case of PE-MP, no significant changes were observed between the MP dose and the number of *nosZ* gene copies. The highest *nosZ* gene abundances were

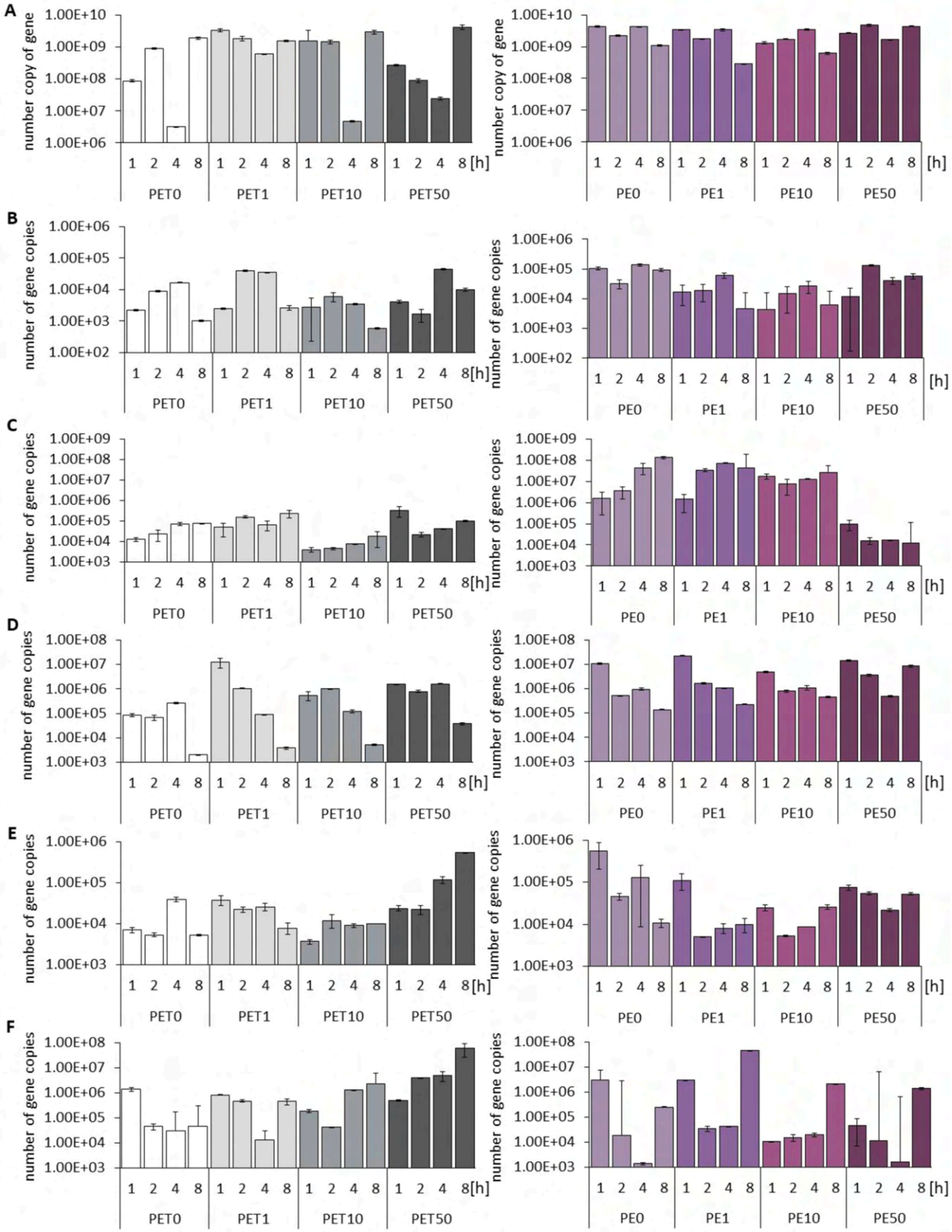

Fig. 4. Changes in the abundance of 16 S rDNA (A), *amoA* (B), *nirK* (C), *nirS* (D), *nosZ* (E), and *ppk1* (F) genes in biomass during the cycle of GSBR cycle.

in PE0 and the highest gene expression was observed in the 1st hour of the GSBRs cycle.

The dosage of PET-MP in reactors significantly influenced the abundance of the *ppk1* gene ($r = 0.51$, $p < 0.05$). In the case of PET0 and PET1, the highest number of the *ppk1* gene was observed during the first hour (anoxic phase) of the cycle (8.19×10^5 - 1.38×10^6); these values decreased in subsequent hours. However, in PET10 and PET50, the *ppk1* gene copy number increased towards the end of the cycle (4–8 h), reaching 2.24×10^6 - 6.07×10^7 gene copies. The rise in *ppk1* gene activity might be attributed to phosphorus accumulation by microorganisms that can actively uptake orthophosphates under aerobic conditions using organics stored in preceding anaerobic phases [40]. Additionally, a significant correlation was found between the abundance of *ppk1* and *16 S r*DNA genes ($r = 0.55$, $p < 0.05$) in the GSBR where PET-MP was dosed with wastewater. This increase in abundance during the cycle might be due to the proliferation of organisms capable of accumulating phosphorus, which could constitute 15–20 % of the entire bacterial population in WWTP [41]. In the GSBRs where PE-MP was dosed, the abundance of *ppk1* genes varied from 1.34×10^3 to 4.59×10^7 gene copies, no correlations were found between the dosage and the abundance of *ppk1* genes.

3.5. Microbial structure

The number of reads for individual samples ranged from 37,174 to 76,822. Alpha diversity was assessed using three indices: Chao1, ACE, and Shannon (Table 2). Concerning the Chao1 index, the value increased with PET dosage (from 135.9 to 154.7) but notably dropped at a dosage of 50 mg/L (135.9). For PE-MP, the highest value reached 123 in PE0 and then declined across all reactors with MPs, stabilizing at similar levels (109−112). ACE index mirrored the changes of Chao1 across all GSBRs. Regarding the Shannon index, alpha-diversity remained stable (3.29–3.37) for the PET-MP run. However, in GSBRs dosed with PE-MP, diversity was highest in PE0 (2.84) but declined with increasing PE-MP dosage (2.63–2.54). Across the PE-MP run, a consistent decrease in alpha diversity was observed across all indices with increasing MP dosage.

Microbiological communities in GSBRs were influenced by the MP dose (Fig. 5A). At the phylum level in reactors dosed with PET, *Proteobacteria* (51.5–72.6 %), *Bacteroidetes* (19.2–41.0 %), *Verrucomicrobia* (2.0–5.7 %), and *Chlamydiae* (0.1–4.3 %) dominated the microbial community. In GSBRs with PE dosing, the dominant phyla were *Proteobacteria* (44.9–54.0 %), *Bacteroidetes* (34.9–45.6 %), *Acidobacteria* (2.8–10.5 %), and *Cyanobacteria* (2.4–5.8 %). *Proteobacteria* was the dominant phylum in the sewage treatment process, containing most of the functional bacteria involved in nitrogen and phosphorus removal [42]. The other dominant phylum was *Bacteroidetes*, which are well-known degraders of organic matter [43].

The most abundant microorganisms at the genus level (Fig. 5B) in GSBRs included *Acinetobacter* (0.30–4.71 %), *Aquimonas* (1.38–11.76 %), *Arenimonas* (0.46–8.88 %), *Brevundimonas* (0.33–5.75 %), *Ferruginibacter* (0–4.69 %), *Flavobacterium* (0.01–5.75 %), *Geobacter* (0.01–3.34 %), *Hyphomonas* (2.19–9.77 %), *Microcystis* (0.05–5.75 %), *Nitrosospira* (0.56 %−14.46 %), *Paracoccus* (0.95–5.59 %), *Pseudoxanthomonas* (3.25–16.65 %), *Woodsholea* (5.28–11.61 %), *Xanthomonas* (0.46–3.97 %), and others (8.37–22.84 %). Unclassified microorganisms comprised 21.29–47.69 % of the entire microbial community within AGS.

Table 2
Total number of reads and α-diversity profiling in the collected biomass sample from the GSBRs.

Sample name	Chao1	ACE	Shannon	Number of reads
PET0	139.1 ± 2.3	140.1 ± 5.2	3.29	76,18
PET1	145.5 ± 1.8	147.8 ± 5.7	3.37	68,710
PET10	153 ± 2.9	154.7 ± 5.7	3.3	76,729
PET50	135.9 ± 1.2	139.1 ± 5.7	3.36	37,171
PE0	123 ± 2.9	124.4 ± 5.3	2.84	42,421
PE1	112.2 ± 2.9	114.9 ± 5.3	2.62	52,570
PE10	109.1 ± 3.6	110.1 ± 5.2	2.54	55,661
PE50	109.1 ± 1.4	112.1 ± 5.2	2.63	47,119

An analysis was conducted to link individual microorganisms (genus level) with the technological data obtained in the respective GSBRs (PET vs. PE dosage), visualizing differences in microbial communities (Fig. 5C). In GSBR dosed with PET-MP, abundances of microorganisms belonging to genera *Rhizobium* ($r = 0.97$), *Zoogloea* ($r = 0.97$), *Nitrosospira* (0.95), *Chitinophaga* ($r = 0.94$), *Geobacter* ($r = 0.94$), *Aminobacter* ($r = 0.93$), *Phreatobacter* ($r = 0.92$), *Flexibacter* ($r = 0.91$), *Runella* ($r = 0.84$), *Hydrogenophaga* ($r = 0.83$), *Hyphomicrobium* ($r = 0.82$), *Sphingomonas* ($r = 0.82$), *Ideonella* ($r = 0.81$), *Thiobacillus* ($r = 0.78$), *Phenylobacterium* ($r = 0.77$), *Bosea* ($r = 0.77$), *Ectothiorhodospira* ($r = 0.76$), *Ferruginibacter* ($r = 0.76$), *Stenotrophomonas* ($r = 0.74$), *Acidovorax* ($r = 0.71$), *Pseudoxanthomonas* ($r = 0.70$), and *Hyphomonas* ($r = 0.70$) correlated with MP dose. Meanwhile, in the GSBRs dosed with PE-MP, significant correlations were observed between the abundances of microorganisms belonging to genera *Arenimonas* ($r = 0.94$), *Bergeyella* ($r = 0.90$), *Cloacibacterium* ($r = 0.86$), *Azoarcus* ($r = 0.82$), *Rheinheimera* ($r = 0.82$), *Microcystis* ($r = 0.81$), *Paracoccus* ($r = 0.80$), *Pseudoclavibacter* ($r = 0.80$), *Leucobacter* ($r = 0.78$), *Aquimonas* ($r = 0.77$), and *Nitrospira* ($r = 0.77$), and MP dose. Among the mentioned microorganisms, many play crucial roles in the cycling of organic compounds, nitrogen, and phosphorus. *Nitrosospira* sp. and *Nitrospira* sp. are classified as bacteria oxidizing ammonium and nitrite nitrogen, respectively [44]. On the other hand, genera *Zoogloea*, *Hydrogenophaga*, *Hyphomonas*, *Acidovorax*, *Azoarcus*, *Hyphomicrobium*, *Paracoccus*, *Rhizobium*, and *Flexibacter* are denitrifiers [12,44,45,33]. Additionally, *Pseudoxanthomonas* sp. and *Hyphomicrobium* sp. are organisms capable of phosphate accumulation in WWTP [33,46]. In addition, it should be noted that some of the microorganisms such as *Acidovorax*, *Hydrogenophaga*, *Bosea*, Ideonella, *Sphingomonas* and *Chitinophaga*, found in both communities, have been described by other researchers for their ability to degrade plastics [47,21,22,12,48].

In the case of GSBR with PET-MP, the microbial genera whose proportion in the microbial community increased with the MP dose (Fig. 6) included *Bacteroides* ($r = 0.98$), *Singulisphaera* ($r = 0.98$), *Gemmata* ($r = 0.98$), *Ectothiorhodospira* ($r = 0.98$), *Devosia* ($r = 0.98$), *Dechloromonas* ($r = 0.97$), *Woodsholea* ($r = 0.95$), *Microcystis* ($r = 0.95$), *Rhodoplanes* ($r = -0.95$), *Beijerinckia* ($r = -0.98$), *Falsirhodobacter* ($r = -0.99$) and others ($p < 0.05$). Meanwhile, in the case of GSBR with PE-MP similar correlation was observed for the genera *Mycobacterium* ($r = 0.98$), *Oscillochloris* ($r = 0.98$), *Fusobacterium* ($r = 0.98$), *Lactobacillus* ($r = 0.98$), *Stenotrophomonas* ($r = 0.95$), *Bacteroides* ($r = 0.95$), *Rhizobium* ($r = -0.95$), *Phreatobacter* ($r = -0.95$), *Woodsholea* ($r = -0.97$), and *Ectothiorhodospira* ($r = -0.99$).

In both GSBR runs, the abundance of *Bacteroides* sp. Bacteria positively correlated with the MP dosage. *Bacteroides* sp. Are classified as potentially pathogenic bacteria and are known to occur in the planktonic form in wastewater [49]. The correlation between the occurrence of pathogenic *Mycobacterium* sp. and the dose of PE in GSBR also raises concerns about potential health risks associated with the presence of these microorganisms in the WWTP [50]. Increased PET-MP dosage stimulated the growth of *Singulisphaera* sp. which may cause filamentous bulking in AGS [51]. *Gemmata* sp. and *Dechloromonas* sp. are microorganisms known for their ability to degrade aromatic compounds. This observation may indicate the leaching or release of chemical compounds from PET, leading to their degradation by these microorganisms [52]. Obligate denitrifier *Woodsholea* sp. showed a positive correlation with the PET-MP dose, while a negative correlation was observed with the PE-MP dose. Therefore, the enrichment of *Woodsholea* sp. in the system can be attributed to the anoxic environment created within dense and intact granules [53]. The increase in the abundance of *Devosia* sp. may be linked to the colonization of MPs trapped within the structure of AGS.

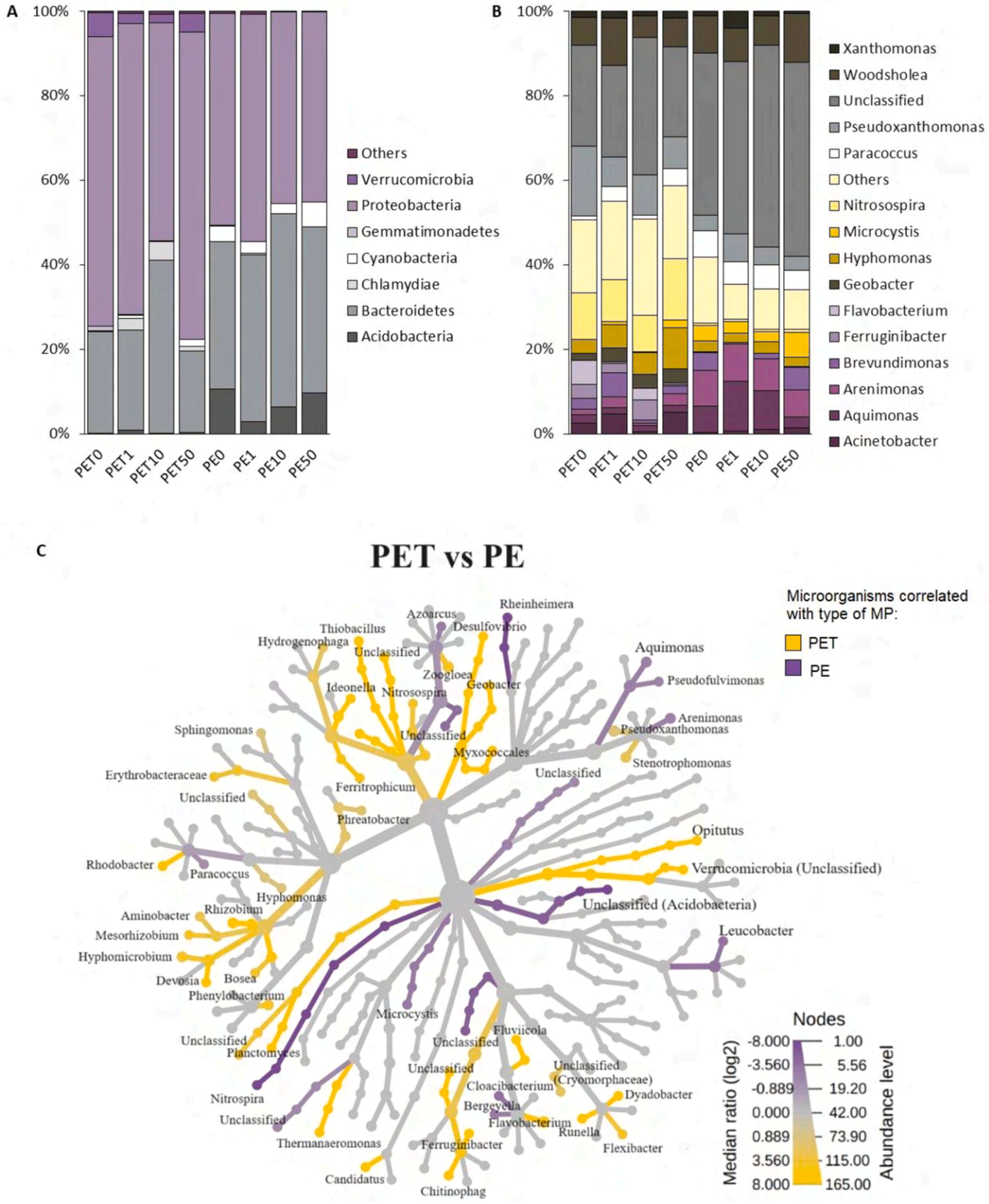

Fig. 5. The most abundant microorganisms (phylum – A, and genus -B level) and dendrogram of cluster analysis, comparison between PET and PE microbial community.

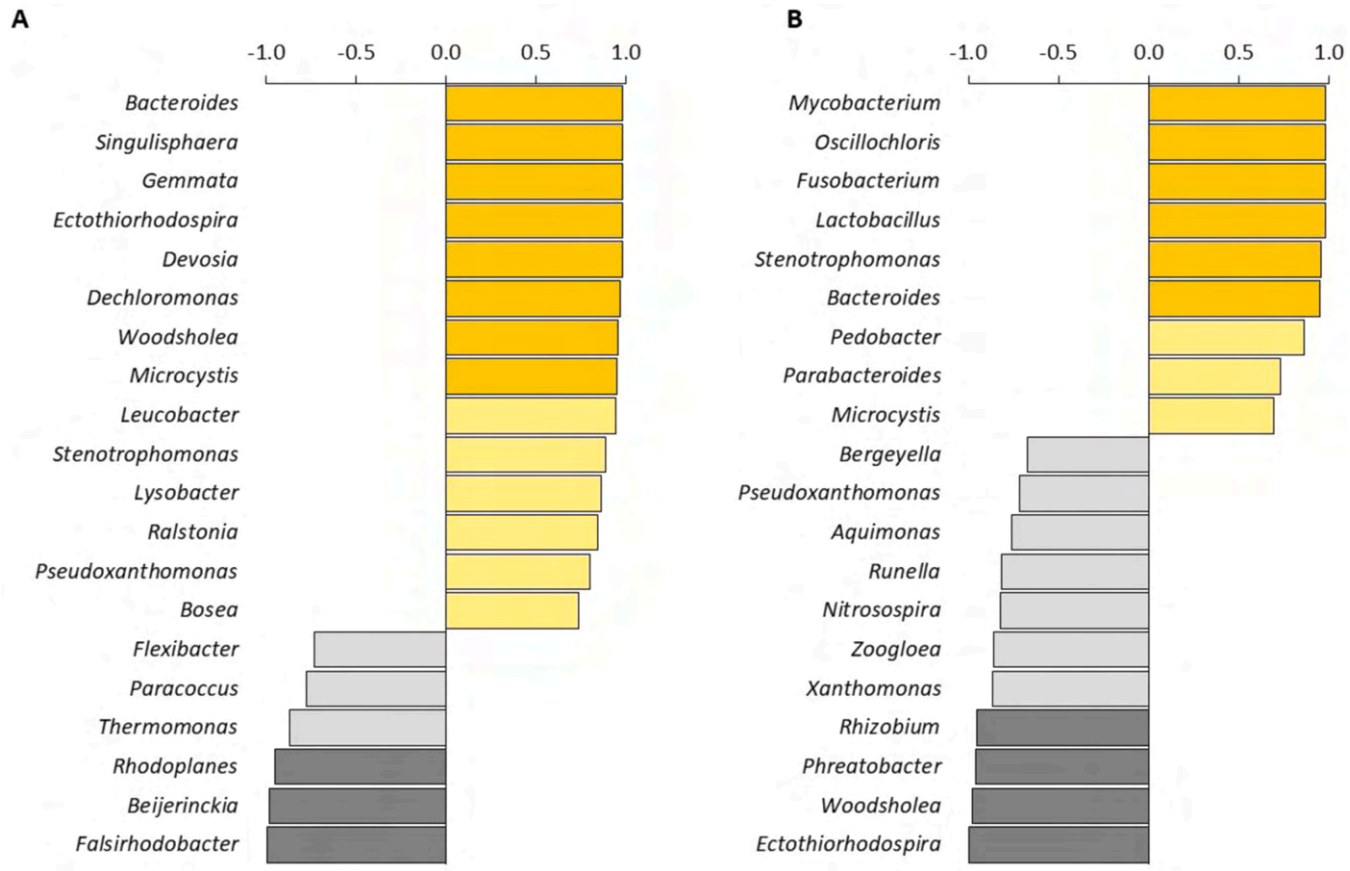

Fig. 6. Microorganisms most strongly correlated with the dose of PET-MPs (A) and PE-MPs (B).

As demonstrated by Tagg et al. [54] in their experiment on microbial colonization of MPs, the occurrence and growth of *Devosia* sp. in MP biofilms, while being completely absent in natural particles, may indicate the presence of a plastic-specialized taxon. The increase in *Microcystis* sp. in response to higher doses of both PET-MP and PE-MP may pose operational challenges to the biological part of the WWTP. Sun et al. [55] found that all cyanobacterial cells could be removed without damage into settled sludge under optimum conditions through coagulation/flocculation and sedimentations, but cell lysis and intracellular toxin release might be caused by the increase of sludge settling time. Additionally, this could potentially contribute to the transit of mycotoxins into the aquatic environment, raising concerns about water quality [56]. Despite AGS being considered a technology resistant to micropollutants, the presence of MP induces changes in the microbial community that may translate into operational issues. Therefore further studies on the effect of different MP on AGS microbiome composition and activity are necessary.

4. Conclusion

As a result of extensive studies on the effect of MP in GSBR systems on the removal of organic pollutants, nitrogen, and phosphorus, significant knowledge has been gained on the different effects of different types of MP. It was found that the deposition of MP in biomass depends on MP type, with PE-MPs showing a constant amount of deposited particles, while PET-MP shows a tendency to accumulate. The presence of MP significantly influenced the efficiency of organic matter, phosphorus, and nitrogen removal and also affected the activity of microorganisms, mostly nitrogen-transforming bacteria. Microbiological analysis revealed that reactors with PET-MP and PE-MP dosing differed in microbial structure and that certain microorganisms were correlated with the type of MP. Further research in this area is essential to better understand the mechanisms of the effects of MP on GSBR reactors and to develop effective strategies for contaminant removal in the presence of MP.

Environmental implication

The presence of microplastics (MPs) in wastewater is a major environmental concern. Our research shows that MPs not only accumulate in biomass but also interfere with the efficiency of wastewater treatment processes in granular sludge aerobic reactors (GSBR). This highlights the hazardous nature of MPs, as they affect the dynamics of pollutant removal and microbial community activity. Understanding these interactions is critical to addressing the environmental problems posed by MP in wastewater treatment systems. By identifying the effects of MP on GSBR performance, our work helps development of strategies to mitigate their negative effects and thus improve the effectiveness of wastewater treatment processes.

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CRediT authorship contribution statement

Weronika Mądzielewska: Investigation, Formal analysis, Data curation. **Piotr Jachimowicz:** Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Agnieszka Cydzik-Kwiatkowska:** Writing – review

& editing, Supervision, Methodology, Conceptualization.

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

The data that has been used is confidential.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2024.135061.

References

[1] European Environment Agency, 2020. Urban waste water treatment. ⟨https://www.eea.europa.eu/publications/urban-waste-water-treatment-for⟩.
[2] Finckh, S., Beckers, L.M., Busch, W., Carmona, E., Dulio, V., Kramer, L., et al., 2022. A risk based assessment approach for chemical mixtures from wastewater treatment plant effluents. Environ Int 164, 107234. https://doi.org/10.1016/j.envint.2022.107234.
[3] Franco, A.A., Arellano, J.M., Albendín, G., Rodríguez-Barroso, R., Quiroga, J.M., Coello, M.D., 2021. Microplastic pollution in wastewater treatment plants in the city of Cádiz: abundance, removal efficiency and presence in receiving water body. Sci Total Environ 776, 145795. https://doi.org/10.1016/j.scitotenv.2021.145795.
[4] Osińska, A., Korzeniewska, E., Harnisz, M., Felis, E., Bajkacz, S., Jachimowicz, P., et al., 2020. Small-scale wastewater treatment plants as a source of the dissemination of antibiotic resistance genes in the aquatic environment. J Hazard Mater 381, 121221. https://doi.org/10.1016/j.jhazmat.2019.121221.
[5] Cydzik-Kwiatkowska, A., Milojevic, N., Jachimowicz, P., 2022. The fate of microplastic in sludge management systems. Sci Total Environ, 157466. https://doi.org/10.1016/j.scitotenv.2022.157466.
[6] Dai, H.H., Gao, J.F., Wang, Z.Q., Zhao, Y.F., Zhang, D., 2020. Behavior of nitrogen, phosphorus and antibiotic resistance genes under polyvinyl chloride microplastics pressures in an aerobic granular sludge system. J Clean Prod 256, 120402.
[7] Qin, R., Su, C., Liu, W., Tang, L., Li, X., Deng, X., et al., 2020. Effects of exposure to polyether sulfone microplastic on the nitrifying process and microbial community structure in aerobic granular sludge. Bioresour Technol 302, 122827.
[8] Zheng, X., Han, Z., Shao, X., Zhao, Z., Zhang, H., Lin, T., et al., 2022. Response of aerobic granular sludge under polyethylene microplastics stress: physicochemical properties, decontamination performance, and microbial community. J Environ Manag 323, 116215.
[9] Li, L., Song, K., Yeerken, S., Geng, S., Liu, D., Dai, Z., et al., 2020. Effect evaluation of microplastics on activated sludge nitrification and denitrification. Sci Total Environ 707, 135953. https://doi.org/10.1016/j.scitotenv.2019.135953.
[10] Gan, Y., Gong, B., Huang, X., Fang, F., Peng, T., Liu, Z., 2024. Response of aerobic granular sludge under acute inhibition by polystyrene microplastics: activity, aggregation performance, and microbial analysis. Environ Pollut 349, 123923.
[11] De Kreuk, M.K., Kishida, N., van Loosdrecht, M.C.M., 2007. Aerobic granular sludge – state of the art. Water Sci Technol 55 (8-9), 75–81. https://doi.org/10.2166/wst.2007.244.
[12] Jachimowicz, P., Peng, R., Hüffer, T., Hofmann, T., Cydzik-Kwiatkowska, A., 2023. Tire materials disturb transformations of nitrogen compounds and affect the structure of biomass in aerobic granular sludge reactors. J Hazard Mater, 133223. https://doi.org/10.1016/j.jhazmat.2023.133223.
[13] Coelho, M.A.Z., Russo, C., Araujo, O.Q.F., 2000. Optimization of a sequencing batch reactor for biological nitrogen removal. Water Res 34 (10), 2809–2817. https://doi.org/10.1016/S0043-1354(00)00010-5.
[14] Sun, J., Dai, X., Wang, Q., Van Loosdrecht, M.C., Ni, B.J., 2019. Microplastics in wastewater treatment plants: detection, occurrence and removal. Water Res 152, 21–37. https://doi.org/10.1016/j.watres.2018.12.050.
[15] APHA, A., (2012) Standard methods for examination of water and wastewater. Washington: American Public Health Association, Standard Methods. ISBN 978–087553-013–0.
[16] Rusanowska, 2015. Diversity and abundance of denitrifying bacteria in aerobic granular sludge [Ph.D. thesis]. University of Warmia and Mazury in Olsztyn.
[17] Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., et al., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci USA 108, 4516–4522. https://doi.org/10.1073/pnas.1000080107.
[18] Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics. https://doi.org/10.1093/bioinformatics/btr381.
[19] Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods 10 (10), 996–998. https://doi.org/10.1038/nmeth.2604.
[20] Dhariwal, A., Chong, J., Habib, S., King, I.L., Agellon, L.B., Xia, J., 2017. MicrobiomeAnalyst: a web-based tool for comprehensive statistical, visual and meta-analysis of microbiome data. Nucleic Acids Res 45 (W1), W180–W188. https://doi.org/10.1093/nar/gkx295.
[21] Jachimowicz, P., Jo, Y.J., Cydzik-Kwiatkowska, A., 2022. Polyethylene microplastics increase extracellular polymeric substances production in aerobic granular sludge. Sci Total Environ 851, 158208. https://doi.org/10.1016/j.scitotenv.2022.158208.
[22] Jachimowicz, P., Nosek, D., Cydzik-Kwiatkowska, A., 2022. Chemical and microbiological changes on the surface of microplastic after long term exposition to different concentrations of ammonium in the environment. Sci Total Environ 830, 154784. https://doi.org/10.1016/j.scitotenv.2022.154784.
[23] Carr, S.A., Liu, J., Tesoro, A.G., 2016. Transport and fate of microplastic particles in wastewater treatment plants. Water Res 91, 174–182. https://doi.org/10.1016/j.watres.2016.01.002.
[24] Xu, Z., Bai, X., Ye, Z., 2021. Removal and generation of microplastics in wastewater treatment plants: a review. J Clean Prod 291, 125982. https://doi.org/10.1016/j.jclepro.2021.125982.
[25] Nguyen, P.D., Tran, Q.V., Le, T.T., Nguyen, Q.H., Kieu-Le, T.C., Strady, E., 2023. Evaluation of microplastic removal efficiency of wastewater-treatment plants in a developing country. Vietnam Environ Technol Innov 29, 102994.
[26] Wei, W., Huang, Q.S., Sun, J., Dai, X., Ni, B.J., 2019. Revealing the mechanisms of polyethylene microplastics affecting anaerobic digestion of waste activated sludge. Environ Sci Technol 53 (16), 9604–9613. https://doi.org/10.1021/acs.est.9b02971.
[27] Zhang, Y.-T., Wei, W., Huang, Q.-S., Wang, C., Wang, Y., Ni, B.-J., 2020. Insights into the microbial response of anaerobic granular sludge during long-term exposure to polyethylene terephthalate microplastics. Water Res 179, 115898. https://doi.org/10.1016/j.watres.2020.115898.
[28] Yu, H., Liu, M., Gang, D., Peng, J., Hu, C., Qu, J., 2022. Polyethylene microplastics interfere with the nutrient cycle in water-plant-sediment systems. Water Res 214, 118191. https://doi.org/10.1016/j.watres.2022.118191.
[29] Li, X., Jiang, X., Song, Y., Chang, S.X., 2021. Coexistence of polyethylene microplastics and biochar increases ammonium sorption in an aqueous solution. J Hazard Mater 405, 124260. https://doi.org/10.1016/j.jhazmat.2020.124260.
[30] Rozman, U., Klun, B., Kalčíková, G., 2023. Distribution and removal of microplastics in a horizontal sub-surface flow laboratory constructed wetland and their effects on the treatment efficiency. Chem Eng J 461, 142076. https://doi.org/10.1016/j.cej.2023.142076.
[31] Negi, D., Verma, S., Singh, S., Daverey, A., Lin, J.-G., 2022. Nitrogen removal via anammox process in constructed wetland – a comprehensive review. Chem Eng J 437, 135434. https://doi.org/10.1016/j.cej.2022.135434.
[32] Yu, H., Xie, B., Khan, R., Yan, H., Shen, G., 2019. The changes in functional marker genes associated with nitrogen biological transformation during organic-inorganic co-composting. Bioresour Technol, 122197. https://doi.org/10.1016/j.biortech.2019.12219.
[33] Zhang, B., Huang, S., Wu, L., Guo, Y., Shi, W., Lens, P.N., 2023. Micro (nano) plastic size and concentration co-differentiate the treatment performance and toxicity mechanism in aerobic granular sludge systems. Chem Eng J 457, 141212. https://doi.org/10.1016/j.cej.2022.141212.
[34] Li, F., Li, M., Shi, W., Li, H., Sun, Z., Gao, Z., 2017. Distinct distribution patterns of proteobacterial nirK-and nirS-type denitrifiers in the Yellow River estuary, China. Can J Microbiol 63 (8), 708–718. https://doi.org/10.1139/cjm-2017-0053.
[35] Fan, H., Bolhuis, H., Stal, L.J., 2015. Denitrification and the denitrifier community in coastal microbial mats. FEMS Microbiol Ecol 91 (3), fiu033. https://doi.org/10.1093/femsec/fiu033.
[36] Che, Y., Liang, P., Gong, T., Cao, X., Zhao, Y., Yang, C., et al., 2017. Elucidation of major contributors involved in nitrogen removal and transcription level of nitrogen-cycling genes in activated sludge from WWTPs. Sci Rep 7 (1), 44728. https://doi.org/10.1038/srep44728.
[37] Wang, X., Zhang, X., Yao, C., Shan, E., Lv, X., Teng, J., et al., 2023. Impact of aged and virgin microplastics on sedimentary nitrogen cycling and microbial ecosystems in estuaries. Sci Total Environ 878, 162977. https://doi.org/10.1016/j.scitotenv.2023.162977.
[38] Yun, G., Lee, H., Hong, Y., Kim, S., Daigger, G.T., Yun, Z., 2019. The difference of morphological characteristics and population structure in PAO and DPAO granular sludges. J Environ Sci 76, 388–402. https://doi.org/10.1016/j.jes.2018.06.003.
[39] Wigginton, S., Brannon, E., Kearns, P.J., Lancellotti, B., Cox, A., Loomis, G.W., et al., 2018. Nitrifying and denitrifying bacterial communities in advanced nitrogen-removal onsite wastewater treatment systems. J Environ Qual 47 (5), 1163–1171. https://doi.org/10.2134/jeq2018.03.0116.
[40] Nguyen, H.T., Le, V.Q., Hansen, A.A., Nielsen, J.L., Nielsen, P.H., 2011. High diversity and abundance of putative polyphosphate-accumulating Tetrasphaera-related bacteria in activated sludge systems. FEMS Microbiol Ecol 76 (2), 256–267. https://doi.org/10.1111/j.1574-6941.2011.01049.x.
[41] Mielczarek, A.T., Nguyen, H.T.T., Nielsen, J.L., Nielsen, P.H., 2013. Population dynamics of bacteria involved in enhanced biological phosphorus removal in Danish wastewater treatment plants. Water Res 47 (4), 1529–1544. https://doi.org/10.1016/j.watres.2012.12.003.

[42] Wang, Q., Li, Y., Liu, Y., Zhou, Z., Hu, W., Lin, L., et al., 2022. Effects of microplastics accumulation on performance of membrane bioreactor for wastewater treatment. Chemosphere 287, 131968. https://doi.org/10.1016/j.chemosphere.2021.131968.

[43] Thomas, F., Hehemann, J.H., Rebuffet, E., Czjzek, M., Michel, G., 2011. Environmental and gut bacteroidetes: the food connection. Front Microbiol 2, 93. https://doi.org/10.3389/fmicb.2011.00093.

[44] Lu, Z., Li, Z., Cheng, X., Xie, J., Li, X., Jiang, X., et al., 2023. Treatment of nitrogen-rich wastewater by mixed aeration combined with bioaugmentation in a sequencing batch biofilm reactor: Biofilm formation and nitrogen-removal capacity analysis. J Environ Chem Eng 11 (2), 109316. https://doi.org/10.1016/j.jece.2023.109316.

[45] 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.

[46] Yuan, Q., Gong, H., Xi, H., Xu, H., Jin, Z., Ali, N., et al., 2019. Strategies to improve aerobic granular sludge stability and nitrogen removal based on feeding mode and substrate. J Environ Sci. https://doi.org/10.1016/j.jes.2019.04.006.

[47] Bahl, S., Dolma, J., Singh, J.J., Sehgal, S., 2021. Biodegradation of plastics: a state of the art review. Mater Today Proc 39, 31–34. https://doi.org/10.1016/j.matpr.2020.06.096.

[48] Yuan, J., Ma, J., Sun, Y., Zhou, T., Zhao, Y., Yu, F., 2020. Microbial degradation and other environmental aspects of microplastics/plastics. Sci Total Environ 715, 136968. https://doi.org/10.1016/j.scitotenv.2020.136968.

[49] Galafassi, S., Sabatino, R., Sathicq, M.B., Eckert, E.M., Fontaneto, D., Dalla Fontana, G., et al., 2021. Contribution of microplastic particles to the spread of resistances and pathogenic bacteria in treated wastewaters. Water Res 201, 117368. https://doi.org/10.1016/j.watres.2021.117368.

[50] Li, Q., Hu, Y., Kou, D., Yang, W., Tang, W., Chen, Q., et al., 2022. Factors impacting microplastic biofilm community and biological risks posed by microplastics in drinking water sources. Water Air Soil Pollut 233 (6), 179. https://doi.org/10.1007/s11270-022-05649-y.

[51] He, Q., Zhang, J., Gao, S., Chen, L., Lyu, W., Zhang, W., et al., 2019. A comprehensive comparison between non-bulking and bulking aerobic granular sludge in microbial communities. Bioresour Technol 294, 122151. https://doi.org/10.1016/j.biortech.2019.122151.

[52] Ran, Z., Zhu, J., Li, K., Zhou, L., Xiao, P., Wang, B., 2018. Study on the membrane bioreactor for treating surfactant wastewater. J Water Clim Change 9 (2), 240–248. https://doi.org/10.2166/wcc.2018.046.

[53] Gao, W., Hu, Y., Jiao, X., Gao, M., Wang, X., 2021. Recovery of structure and activity of disintegrated aerobic granular sludge after long-term storage: effect of exogenous N-acyl-homoserine lactones. Chemosphere 281, 130894. https://doi.org/10.1016/j.chemosphere.2021.13.

[54] Tagg, A.S., Sperlea, T., Labrenz, M., Harrison, J.P., Ojeda, J.J., Sapp, M., 2022. Year-long microbial succession on microplastics in wastewater: chaotic dynamics outweigh preferential growth. Microorganisms 10 (9), 1775. https://doi.org/10.3390/microorganisms10091775.

[55] Sun, F., Pei, H.Y., Hu, W.R., Ma, C.X., 2012. The lysis of *Microcystis aeruginosa* in $AlCl_3$ coagulation and sedimentation processes. Chem Eng J 193, 196–202. https://doi.org/10.1016/j.cej.2012.04.043.

[56] Romanis, C.S., Pearson, L.A., Neilan, B.A., 2021. Cyanobacterial blooms in wastewater treatment facilities: significance and emerging monitoring strategies. J Microbiol Methods 180, 106123. https://doi.org/10.1016/j.mimet.2020.106123.

Table S1. Primers and conditions applied in real-time PCR

Gene	Primer set	Primer concentration	Thermal Profile	Reference
16S rRNA	519F/907R	100 nM	94˚C/15 s, 50˚C/45 s, 60˚C/45 s	Lane, (1991)
amoA	amoA1F/amoA2R	100 nM	94˚C/15 s, 50˚C/45 s, 60˚C/45 s	Rotthauwe et al., (1997)
nirK	F1aCu/R3Cu	150 nM	94˚C/15 s, 55°C/1 min, 60°C/1 min	Throbäck et al., (2004)
nirS	cd3aF/R3cd	150 nM	94˚C/15 s, 53°C/1 min, 60°C/1 min	
nosZ	NosZ-F/NosZ1622R	200 nM	94˚C/15 s, 60˚C/2.5 min	
ppk1	870F/1002R	200 nM	94˚C/15 s, 51°C/1 min, 60°C/1 min	He et al., (2007)

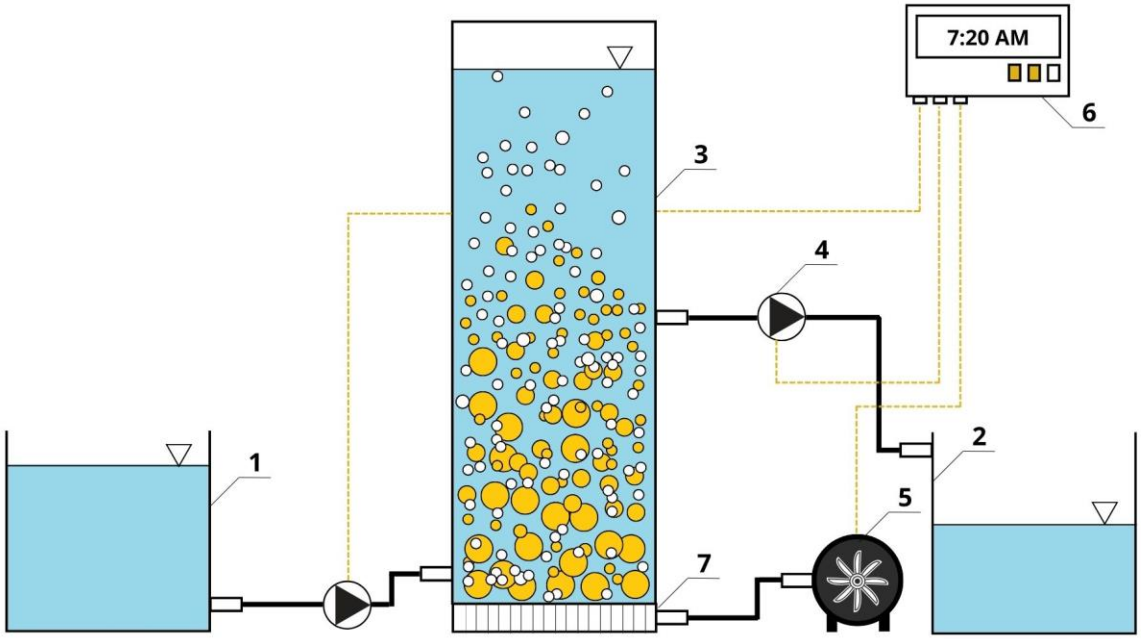

Figure S1. Scheme of the GSBR; 1 - raw wastewater tank, 2 – purified wastewater tank, 3 - GSBR, 4 - peristaltic pump, 5 – air compressor, 6 - control system, 7 - fine bubble diffuser.

Olsztyn, 24.08.2024

(miejscowość, data)

Piotr Jachimowicz

(imię i nazwisko kandydata)

Inżynieria środowiska, górnictwo i energetyka

(dyscyplina naukowa)

Przewodniczący Rady Naukowej Dyscypliny

prof. dr hab. inż. Marcin Dębowski

Uniwersytetu Warmińsko-Mazurskiego w Olsztynie

OŚWIADCZENIE

autora rozprawy doktorskiej

Oświadczam, że w pracy pod tytułem:

Jachimowicz, P., Mądzielewska, W., & Cydzik-Kwiatkowska, A. (2024). Microplastics in granular sequencing batch reactors: Effects on pollutant removal dynamics and the microbial community. Journal of Hazardous Materials, 476, 135061.

Mój wkład merytoryczny w jej przygotowanie polegał na: udziale w zaplanowaniu koncepcji badań, opracowaniu metodyki, wykonanie części eksperymentalnej, opracowaniu i interpretacji wyników, graficznym opracowaniu wyników, pozyskaniu funduszy na prowadzenie badań oraz przygotowaniu pierwszej wersji manuskryptu

.....

(podpis kandydata)

Olsztyn, 24.08.2024

(miejscowość, data)

Weronika Mądzielewska

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Mój wkład merytoryczny w jej przygotowanie polegał na uczestnictwie w pozyskaniu i opracowaniu interpretacji wyników z zakresu biologii molekularnej.

Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez Pana Piotra Jachimowicza jako część rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów naukowych opublikowanych w czasopismach naukowych. Oświadczam, iż samodzielna i możliwa do wyodrębnienia część ww. pracy wykazuje indywidualny wkład kandydata Pana Piotra Jachimowicza polegający na:

udziale w zaplanowaniu koncepcji badań, opracowaniu metodyki, wykonanie części eksperymentalnej, opracowaniu i interpretacji wyników, graficznym opracowaniu wyników, pozyskaniu funduszy na prowadzenie badań oraz przygotowaniu pierwszej wersji manuskryptu.

Weronika Mądzielewska

(podpis)

Olsztyn, 24.08.2024

(miejscowość, data)

prof. dr hab. inż. Agnieszka Cydzik-Kwiatkowska

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OŚWIADCZENIE

współautora

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Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez Pana Piotra Jachimowicza jako część rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów naukowych opublikowanych w czasopismach naukowych. Oświadczam, iż samodzielna i możliwa do wyodrębnienia część ww. pracy wykazuje indywidualny wkład kandydata Pana Piotra Jachimowicza polegający na:

udziale w zaplanowaniu koncepcji badań, opracowaniu metodyki, wykonanie części eksperymentalnej, opracowaniu i interpretacji wyników, graficznym opracowaniu wyników, pozyskaniu funduszy na prowadzenie badań oraz przygotowaniu pierwszej wersji manuskryptu.

A. Cydzik-Kwiatkowska

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(podpis)

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Chemical and microbiological changes on the surface of microplastic after long term exposition to different concentrations of ammonium in the environment

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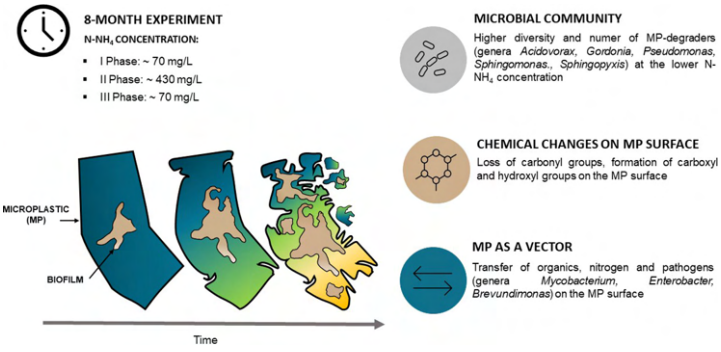
HIGHLIGHTS

- Biomass from a WWTP is a source of microorganisms with PET-degradation potential.
- A high concentration of N-NH_4 inhibited the growth of MP-degraders in biofilm.
- Independent of the N-NH_4 concentration, MP was a vector of pollutants and pathogens.
- Environmental conditions changed the chemical structure of the MP surface.

GRAPHICAL ABSTRACT



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ABSTRACT

The increasing production of plastic in the world has resulted in the widespread pollution of the environment with microplastics (MP). MP enter facilities such as wastewater treatment plants or landfills characterized by various ammonium concentrations. The aim of this study was to determine the structure of the microbial community on MP surfaces at various concentrations of ammonium nitrogen, and in particular, to identify microorganisms capable of polyethylene terephthalate (PET) degradation. Moreover, changes in the chemical characteristics of the MP surface resulting from microbial activity were also investigated, and the potential of MP to serve as a vector for pollutants was determined. The tests were carried out in a reactor filled with PET for a period of 260 days. The experiment was carried out in 3 phases: in I and III phase, the concentration of N-NH_4 was about 70 mg/L, while in II phase, it was about 430 mg/L. On the MP surface, biofilm-forming microorganisms from the genera *Rhodococcus*, *Pseudomonas* and *Xantomonas* were identified at the lower ammonium concentration. At this concentration, MP-degraders belonging to genera *Acidovorax*, *Gordonia*, *Pseudomonas*, *Sphingomonas*, and *Sphingopyxis* were identified in the biofilm. At the higher N-NH_4 concentration, the biomass was enriched with bacteria from genera *Nitrosospira*, *Nitrosomonas* and *Terrimonas*, and the number of microorganisms with the potential to degrade MP decreased. Analysis of the MP surface during the experiment has showed the loss of carbonyl groups and formation of carboxyl and hydroxyl groups, which indicated the degradation of MP. Independent of the ammonium concentration in the environment, MP was a carrier of pathogenic microorganisms from the genera *Mycobacterium*, *Enterobacter* and *Brevundimonas*.

1. Introduction

In 2019, global production of plastics reached 368 million metric tons, having recorded annual increases of 3–6% over the previous 10 years. Those plastics are used in various industries. In Europe, about 40% of

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plastics are used for single-use products in packaging. In the European Union, 29.1 million metric tons of plastic waste were collected in 2018, of which 42.6% was recovered for energetic purposes, 32.5% was recycled, and 24.9% was landfilled (PlasticsEurope, 2018).

Microplastics (MPs) are plastic particles with a diameter less than 5 mm. Depending on their origin, primary and secondary, MPs are distinguished (Jaikumar et al., 2019). Primary MPs are designed to be of microscopic size and are manufactured for specific industrial or domestic applications (e.g. cosmetics). Secondary MPs are formed by mechanical, biological or chemical degradation of larger plastic particles (Auta et al., 2017). Both primary and secondary MPs can be transported to aquatic environments in treated sewage, rainwater, surface runoff or landfill leachate.

Monitoring of MP transformations in landfill leachate or wastewater treatment plants (WWTP) is important because it allows to assess the risk associated with MP as a pollutant vector and to estimate the effectiveness of MP decomposition. In leachate samples from 12 different landfills, MP particles concentration varied from 0.42 to 24.58 MP/L. The most common types of MP were polyethylene (PE, 35%), polypropylene (PP, 35%) and polyethylene terephthalate (PET, 6%) in the form of fragments (58.6%), flakes (22.87%) and fibers (14.81%) (He et al., 2019). The large specific surface area of MP and the presence of biofilm favor sorption of such pollutants as antibiotics, hormones, pesticides, heavy metals or organic compounds (Atugoda et al., 2020; Wang et al., 2020; Selvam et al., 2020, Li et al., 2018). The nutrients adsorbed on the surface of the MP can be decomposed and released into the surrounding water column favoring eutrophication (Y. Zhang et al., 2020).

Wastewater and leachate are characterized by a wide range of concentrations of organic and nitrogen compounds, which effect the development of specific microbial communities and thus the potential for plastic degradation. The ammonium concentration in leachate from different landfills may vary from 39 to 10,250 mg/L (Tatsi and Zouboulis, 2002; Marttinen et al., 2003), and depends e.g. on the landfill age (Kulikowska and Klimiuk, 2008). In WWTPs, the concentration of ammonium in municipal wastewater usually ranges from 50 to 70 mg/L (Henze et al., 1997; Świątczak and Cydzik-Kwiatkowska, 2018a, 2018).

Large specific surface area and polarity of MP promote its colonization by planktonic microorganisms capable of adhesion and creating a biofilm (Chen et al., 2019). The composition and structure of biofilm are determined by temperature, nutrient availability, hydrodynamic conditions, and also chemical composition of the MP. Feng et al. (2020) showed that microorganisms from the *Rhodobacteraceae*, *Cellvibrionaceae* and *Flavobacterium* families dominated in the biofilm on the polyvinyl chloride (PVC) surface, while polyamide (PA) was mainly inhabited by microorganisms from the *Sphingomonadaceae*, *Rhodobacteraceae*, *Vibrionaceae* and *Mirotrichaceae*. The formation of biofilm affects the buoyancy of MP (sinking in water column).

Some environmental microorganisms inhabiting polymers are able to degrade them and use them as a source of energy and carbon. Gram-negative bacterium *Ideonella sakaiensis*, isolated from sewage sludge, was able to degrade PET at 30 °C using: PET hydrolase (PETase) and mono (2-hydroxyethyl) terephthalate hydrolase (MHETase). PET was initially broken down with PETase-mediated hydrolysis to mono-(2-hydroxyethyl) terephthalic acid and then with MHETase to ethylene glycol and terephthalic acid (Yoshida et al., 2016). The degradation rate of PET film can be as high as 22.5 mg μmol/(L PETase·day) (Ma et al., 2018).

The factor that strongly affects the composition of the biofilm microbiome is the concentration of N-NH_4 (Q. Zhang et al., 2020). In the studies of Chen et al. (2018) it was shown that at 255 mg N-NH_4/L in wastewater in the aerobic granules, *Thauera* sp. (18.6%) and *Actinomycete* sp. (9.72%) predominated, while at 505 mg N-NH_4/L, *Rhodospirillaceae* (11.88%) and *Leadbetterella byssophila* (12.8%) were most numerous. Increasing concentration of N-NH_4, lowered efficiency of N-NH_4 removal (74.1 vs 98.0). At low concentration of N-NH_4 (<50 mg/L) in environment the most dominated nitrifying microorganisms such as *Thauera* sp., *Dokdonella* sp., and *Aeromonas* sp.

In this study, a long-term experiment was conducted in a reactor operated in continuous mode, as opposed to the commonly conducted short-term experiments that use batch tests. The objective was to investigate the development and changes of the microbial community on the surface of MP at different concentrations of ammonium nitrogen, and, in particular, to identify microorganisms capable of degradation of PET. The study also assessed how MP to serves as a vector transporting nutrients and pathogens to the aquatic environment. This research fills a knowledge gap concerning the degradation of MP and the ecology of microorganisms in environments with moderate and high concentrations of ammonium nitrogen, such as landfill leachate or wastewater treatment plants, which are important sources of MP.

2. Materials and methods

2.1. Microplastic

The MP was created by grinding PET plastic bottles collected from the premises of Waste Management Facility in Olsztyn (53° 47′ N, 20° 32′ E). The selected bottles came from one producer from the domestic market, which ensured the homogeneity of the sample. In the initial processing stage, the bottles were rinsed, paper labels were removed, then bottles were cut into small square pieces (5 cm × 5 cm). The material was ground on an automatic grinder SM 2000 (Retsch) to particles with a diameter of 4.6 ± 0.74 mm.

2.2. Substrate

Liquid carbon free basal medium (LCFBM) contained 0.7 g of KH_2PO_4, 0.7 g of K_2HPO_4, 0.7 g of $MgSO_4{\cdot}7\ H_2O$, 1.0 g of NH_4NO_3, 0.005 g of NaCl, 0.002 g of $FeSO_4{\cdot}7\ H_2O$, 0.002 g of $ZnSO_4{\cdot}7\ H_2O$ and 0.001 g of $MnSO_4{\cdot}H_2O$ per 1000 mL of deionized water (Yang et al., 2014). The concentration of total nitrogen in the medium was 514.5 ± 17.3 TN mg/L. The medium was dosed in appropriate amounts to maintain the concentration of ammonium nitrogen in the reactor at the level of about 70 and 430 mg N-NH_4/L, in I and II phase, respectively. To create an ecological niche in the reactor favoring the appearance of microorganisms capable of MPs degradation, no organic carbon sources were added with wastewater.

2.3. Reactor setup

The sealed cylinder-shaped reactor (Fig. 1), with 100 cm high, 50 cm in diameter, was filled up to 20% of the reactor height with MP. The experiment was conducted for 260 days. The MP in the reactor was sprinkled by sprinklers placed in the upper part of the reactor. LCFBM was dosed thrice a week (at regular intervals). One-time sprinkling lasted 15 min and consisted of circulating 5 L of water with peristaltic pumps 8 times a day. Samples were taken from the bottom of the reactor at regular intervals for physico-chemical and molecular analyses. Air was dosed through a perforated grate at the bottom of the reactor at a rate of 20 L/min. There were probes in the reactor for online measurement of humidity, temperature and oxygen concentration. During the experiment, the average temperature was 38.2 ± 1.1 °C. The operation of the reactor was controlled by the controller. Three phases were distinguished in the experiment: I - biofilm adaptation and development (day 0–46; ~70 mg N-NH_4/L), II - adaptation to a high concentration of N-NH_4 (day 46–86; ~430 mg N-NH_4/L), III - adaptation to low concentration of N-NH_4 (day 86–260; ~70 mg N-NH_4/L). Because the removal rate of ammonium in each reactor cycle was low, the average concentration of ammonium in the reactor between the LCFBM dosing was about 70 and 430 mg N-NH_4/L.

As inoculum, plastic particles (1 cm × 1 cm) incubated with 200 mg of activated sludge from the Municipal Wastewater Treatment Plant in Olsztyn "Łyna" (53° 47′ N, 20° 27′ E) in 1 L of LCFBM for 30 days at 37 °C was used.

2.4. Physico-chemical analyses

Physico-chemical analyses of the inflow and outflow from the reactor were performed in 3 replications and included COD, N-NH_4, N-NO_2, N-

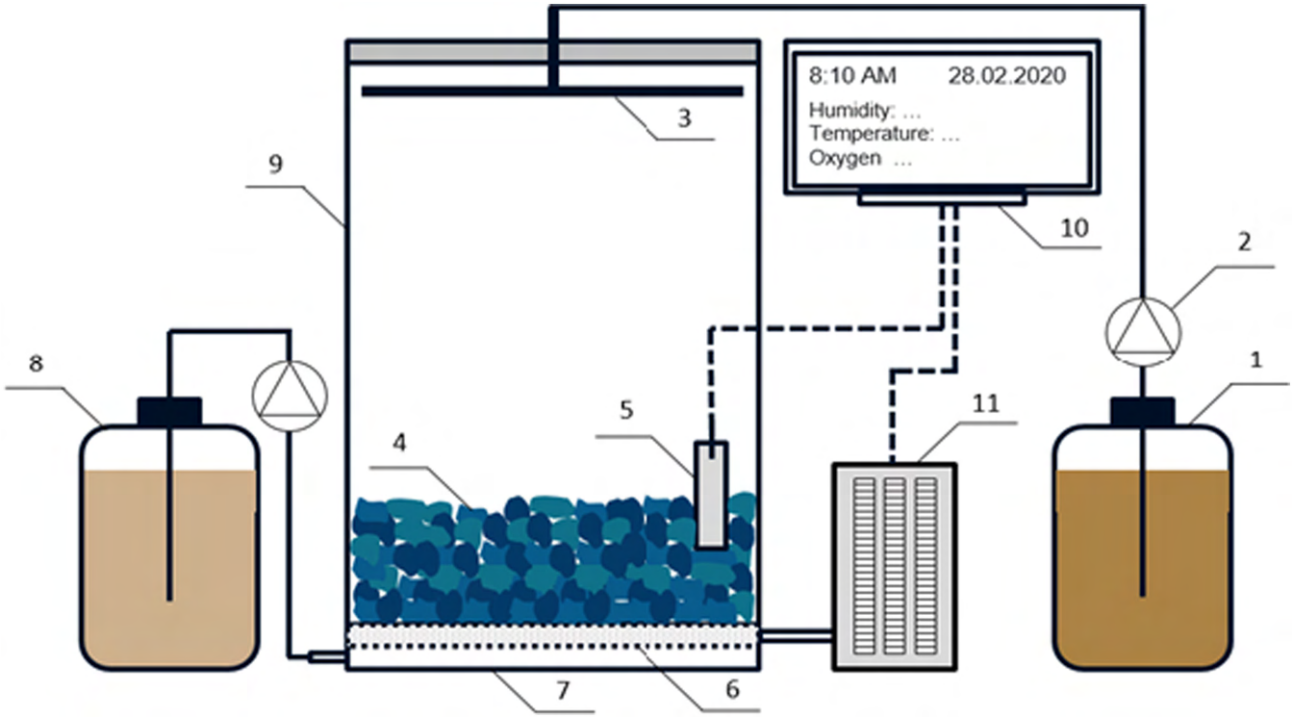


Fig. 1. Diagram of the reactor: 1 - tank with the nutrient solution, 2 - pump, 3 - sprinkler, 4 - MP, 5 - analytical probes (humidity, temperature, oxygen concentration), 6 - aeration grate, 7 - receiving cuvette, 8 - outflow tank, 9 - reactor, 10 - controller, 11 – compressor.

NO_3 (cuvette testes, Hach Lange, Poland) and pH (TitroLine Easy, Donserv). The kinetics of the rate of changes of nitrogen compounds after introducing a fresh portion of the substrate to the reactor was also determined.

To determine the impurities retained on the plastic surface, MP sample was taken from the reactor from a depth of 10 cm. MP was dried with a compressed air stream and stored in tightly closed glass Petri dishes at room temperature until the analyses were performed. COD and TN were determined according to APHA (1992). The results obtained for the control sample, which was pristine MP used as an input to the reactor, were subtracted from the obtained results. The biofilm biomass was removed by ultrasound treatment (10 min at 25 kHz) followed by centrifugation for 30 min at 13,000*g*. Then, the biomass was measured according to APHA (1992). Changes in MP mass during the experiment was assessed by weighting of 100 particles of pristine MP and 100 particles of MP after the experiment (biomass from MP surface was removed by ultrasound treatment as described above).

Batch tests were carried out to determine $N\text{-}NH_4$ sorption on MP. The tests were conducted in 50 mL capacity glass beakers with synthetic wastewater with initials concentration of 70 and 430 mg $N\text{-}NH_4$/L. The concentrations of MP were 10%, 2.5% and 0.5% weight/volume (w/v). The samples were shaken at 150 RPM for 24 h at ambient temperature in a mechanical shaker (TB51, Laboshake). During the tests, the ammonium concentrations in the wastewater were measured in regular time intervals.

2.5. Chemical and morphological changes of MP surface

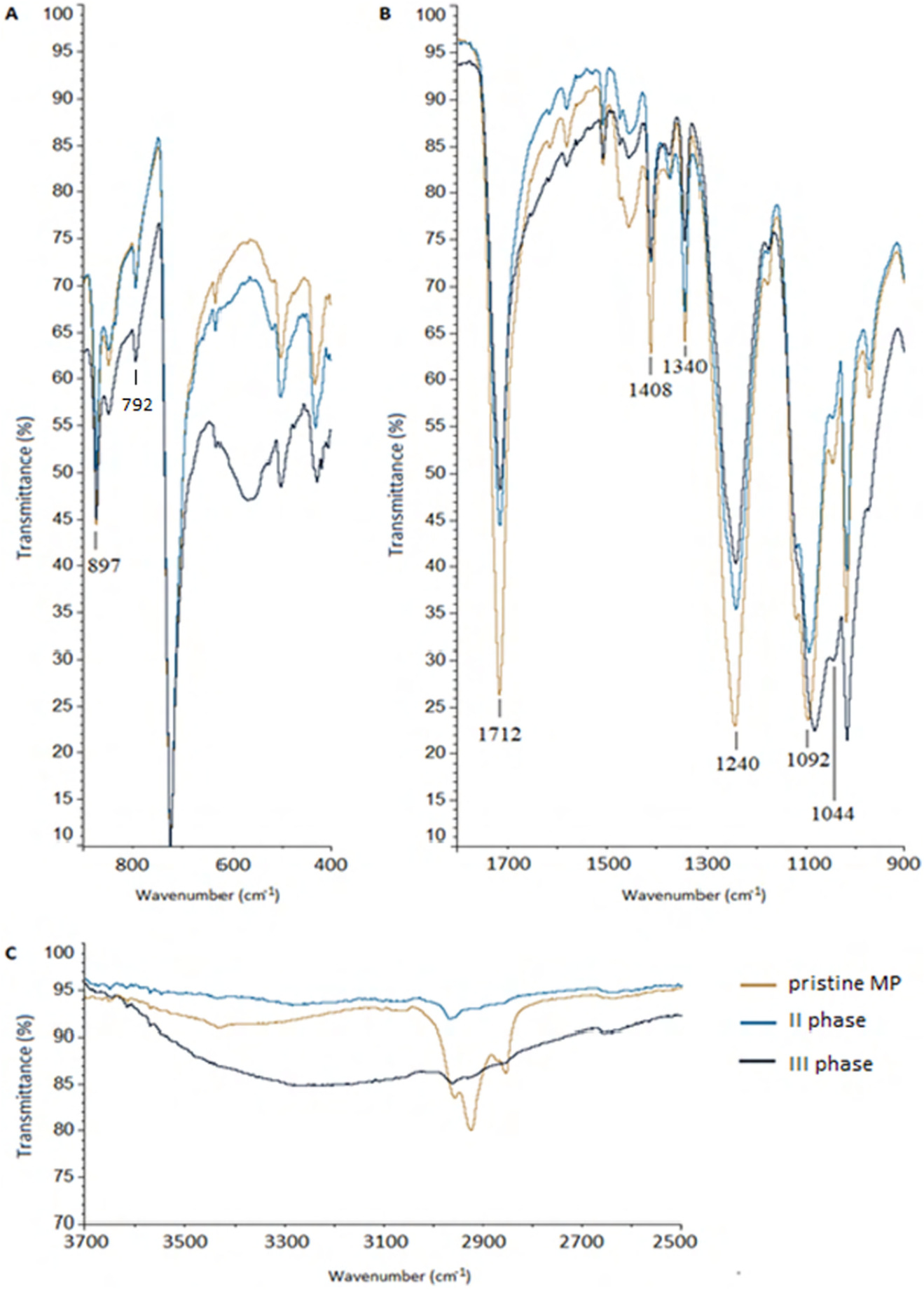
To analyze changes in chemical bonds on the PET surface during the experiment, MP constituting the feed to the reactor (pristine MP) and MP after 74 (II phase) and 243 (III phase) days of the experiment were analyzed using Fourier Transform Infrared Spectroscopy (FTIR). The MP surface was washed with ethanol before analysis to avoid the influence of biomass on FTIR results. A spectrometer Nicolet 6700 (Thermo Scientific) equipped with an attenuated total reflection (ATR-FTIR) diamond crystal attachment was used to establish the reference and MP surface spectra. Measurements were taken with resolution of 4 cm^{-1} in wavenumber ranges from 4000 to 400 cm^{-1} (32 scans per sample). Three different points i.e., the central point and two different edge points of each samples were examined. Selected spectra from sample were compared with their reference spectra from the library. Background spectra were regularly measured against air with the same setting as described above. Between measurement, the ATR-FTIR crystal was cleaned with ethanol to avoid cross-contamination. In addition, spectroscopic characterization of the morphological changes of MP surfaces was performed using a Quanta FEG 250 scanning electron microscope (SEM, Quanta FEG 250, FEI).

2.6. Microbiome analysis

For microbiological analyses, 40 mL of MP with the surrounding supernatant was taken into sterile falcon at the 0, 16, 30, 74, 243 day of the experiment. Each sample was centrifuged for 15 min at 8000 rpm in the MPW-380 centrifuge (MPW). About 500 μL of the liquid mixed with biomass were taken from the bottom of the falcon and used for DNA isolation. The isolation was carried out in 3 replications using the FastDNA® SPIN Kit for Soil (MP Biomedical); the DNA from replicates was mixed prior further analyses. The quantity and quality of DNA was assessed with the use of NanoDrop spectrophotometer (Thermo Scientific) and agarose electrophoresis. The samples were then amplified using primers 515F/806R (GTGCCA GCMGCCGCGGTAA/GGACTACHVGGGTWTCTAAT) targeting the V4 region of the bacterial 16S rDNA gene (Caporaso et al., 2011) and sequenced using MiSeq Illumina Platform in Research and Testing Laboratory (USA). The obtained sequences were analyzed bioinformatically (Heberle et al., 2015; Świątczak and Cydzik-Kwiatkowska, 2018a). The sequences have been deposited in the NCBI Sequence Read Archive (SRA) as the experiment entitled “Chemical and microbiological changes on the surface of microplastic after long term exposition to different concentrations of ammonium in the environment” (Accession: PRJNA782808).

2.7. Data analysis

To observe the relationship between the physico-chemical parameters, the correlation matrix was made in RStudio version 1.2.5042 using the “corrplot” package (Wei et al., 2017). The heat map analysis of relative


A
B
C
Transmittance (%)
Wavenumber (cm-1)
897
792
1712
1408
1340
1240
1092
1044
pristine MP
II phase
III phase

abundance of microorganisms in samples was performed with RStudio using the "heatmap.2" packages (Warnes et al., 2016). The Shannon-Wiener biodiversity index (Hill, 1973) was also determined at a genus level.

3. Results and discussion

3.1. Analysis of chemical and morphological changes of MP surface

Long-term operated reactor was used in the study to ensure stable conditions for the growth of microorganisms, including MP-degraders, and to reproduce the conditions that occur during waste landfilling, such as a long exposure of MP to microbial activity. Such long-term exposure cannot be obtained in short-term batch tests.

PET sample was analyzed by ATR-FTIR (Fig. 2) and visible differences were noted between pristine and environmentally exposed MPs.

The bands at 792 cm^{-1} (Fig. 2A) and 1408 cm^{-1} (Fig. 2B) are assigned to CH in-of-plane and out-plane deformation of aromatic ring. For the 792 cm^{-1} band, the transmittance decreased during the experiment while for 1408 cm^{-1} the opposite tendency was observed. The intensity of those bands informs about changes in thickness of PET (Sammon et al., 2000) and, in our study, indicates the decrease in the PET mass (8.21 ± 0.68 vs 7.64 ± 0.55 mg per MP particle). The band 897 cm^{-1} is attributed to -CH_2 rocking of the ethylene glycol unit in *gauche* forms. For this band a decrease in transmittance was observed in the sample from the III phase of the experiment.

Significant change in the intensity of the band at 1044 cm^{-1} (Fig. 2B), corresponding to *gauche* conformer of the asymmetric stretching of the ester (CO) of ethylene glycol unit (Forestier et al., 2020), was observed between pristine MP sample and MP from III phase of the experiment. In our study transmittance of band 1092 cm^{-1} (Fig. 2B), assigned to the ester (CO) stretching in *gauche* forms was higher in pristine MP in comparison to MP form II phase of the experiment. On the other hand it decreased, in the case of samples from the III phase of the experiment. Moreover, an increase in 1081 cm^{-1} band (Fig. 2B) was observed.

The band at 1340 cm^{-1} (Fig. 2B) that corresponds to -CH_2 wagging of trans conformation of the ethylene glycol units is widely used in the literature to identify the change in crystallinity of PET. In our study, the increase in the intensity of this band with time indicates that the PET structure changed from crystalline to more amorphous, which is more prone to degradation (Mecozzi and Nisini, 2019). The band at 1240 cm^{-1} corresponded to the vibrations of ester (CO) groups in trans form and its intensity increased with the time of the experiment. The change in trans form here also agrees well with the step-wise increase in intensity observed for band 1340 cm^{-1}.

The degradation of MP surface increased with time as can be deduced from transmittance at 1712 cm^{-1} (Fig. 2B), corresponding to carbonyl stretching (C = O). The increase in this band's intensity is attributed to loss of carbonyl groups, which is thought to affect other bands by increasing their intensity, as carbonyl is part of the ester groups and a substituent group of the aromatic ring. Similar trends were observed on the PET surface exposed to activity of bacterial consortia able to degrade hydrocarbons (Denaro et al., 2020).

Spectra changes in the 2800–3500 cm^{-1} (Fig. 2C) region were observed that can be attributed to oxidation of artificial polymer surface (Brandon et al., 2016). In this region -OH and -CH vibration were found which are useful for identification of degradation based on the formation of carboxylic and hydroxyl groups. During the experiment, visible change in PET colour was also observed from blue to yellowish (Supplementary materials, Fig. S1) which also indicated polymer oxidation (Rieckmann and Volker, 2003).

SEM analysis showed morphological changes on the MP surface (Fig. 3). The pristine MP had a homogeneous, glossy surface. The exposition of MP to the environmental factors caused the surface to deform and increased its roughness, and cracks and damage to the surface were visible. Ioakeimidis et al. (2016) investigated the degradation potential of plastic bottles of different ages that were found in an aquatic environment. Their SEM results showed that, with longer ages of PET exposure, the PET surface was cracked and uneven, which was similar to observations in our study. Our SEM results regarding the damage on the MP surface were in agreement with the results obtained by FTIR. Loss of carbonyl groups and formation of carboxyl and hydroxyl groups observed in FTIR was in agreement with increasing degradation of MP surface observed in SEM.

3.2. Transformation of nitrogen compounds and sorption of pollutants

The concentration of pollutants in the effluent depended on the phase of the experiment (Table 1). The efficiency of ammonium nitrogen removal was much higher in I and III phases (18.1–23.7%) than in the II phase (8.4%). However, the total amount of ammonium removed per reactor volume was higher at the higher ammonium concentration in the environment (about 36 mg N-NH_4/L and 17 mg/L at 430 N-NH_4 mg/L and 70 N-NH_4 mg/L, respectively). This higher ammonium removal can result from a higher nitrifier abundance in the biofilm (see point 3.3). Similar observations were reported in a study conducted in lab-scale wastewater treatment systems (Świątczak and Cydzik-Kwiatkowska, 2018b). There was also a visible increase in nitrification activity at lower concentrations of ammonium nitrogen in the reactor. In a study by Qin et al. (2020) in granular sludge reactors spiked with polyether sulfone (PES), it was observed that rate of N-NH_4 removal decreased in the presence of MP in the reactor.

In the case of the COD concentration in the outflow (Supplementary materials, Fig. S2), its value significantly decreased in the III phase of the experiment and negatively correlated with the concentration of biofilm biomass accumulated on the MP surface. This indicates that the microbial community was adapted to environmental condition, and the biomass incorporated chemical substances generated during MP degradation. In the present study, the rate of ammonium nitrogen removal in the reactor at the end of the experiment was low (0.86 mg N-NH_4/(L·h)).

A positive correlation (Supplementary materials, Fig. S3) was found between the temperature in the reactor and the COD concentration in the effluent ($r = 0.87$, $p < 0.05$). During the presented experiment, the average temperature (38.2 ± 1.1 °C, Supplementary materials – Fig. S4) was maintained in the temperature range typical of landfills (35–45 °C), which promoted a high rate of polymer degradation (Hanson et al., 2009; Pirzadeh et al., 2007). Supplying air into the reactor containing biofilm-covered MP allowed microorganisms to efficiently oxidize plastic compounds (Lee et al., 2020). A negative correlation was observed between the air flow and both the temperature ($r = -0.82$, $p < 0.05$) and the humidity ($r = -0.85$, $p < 0.05$), resulting from cooling and drying of the reactor due to air movement. Buxbaum (1968) has shown that humidity positively affects the rate of PET hydrolysis.

In the reactor, MP and cell lysis were the only sources of organics for microbial growth. The average biomass concentration on the MP surface was 2.07 mg/g MP in the I phase of the experiment, and it increased to 2.32 mg/g MP in the III phase. There was no correlation, between the concentration of COD in the effluent and the amount of COD retained on the MP surface ($r = 0.67$, $p > 0.05$), but a significant correlation was found between the concentration of N-NH_4 in the environment and the TN sorbed on the MP surface ($r = 0.96$, $p = 0.01$). At the end of the experiment, the amount of COD retained on the MP surface (Supplementary materials, Fig. S5), including COD retained in the biofilm, was 1510.89 mg/g MP.

The batch test of sorption of ammonium on MP surface was carried out (Supplementary materials, Fig. S6). At concentration of 70 mg N-NH_4/L, the highest sorption capacity was 26.6 mg N-NH_4/(g MP·day) and ammonium was sorbed at a rate of 1.11 mg N-NH_4/h. At the concentration of

Fig. 2. ATR-FTIR comparative spectra of the surface of the MP-PET. For better visualization, enlarged excerpts of the ATR-FTIR comparative spectra are given at wavenumbers (A) 400–900 cm^{-1}, (B) 9000–1800 cm^{-1}, (C) 2500–3700 cm^{-1}.

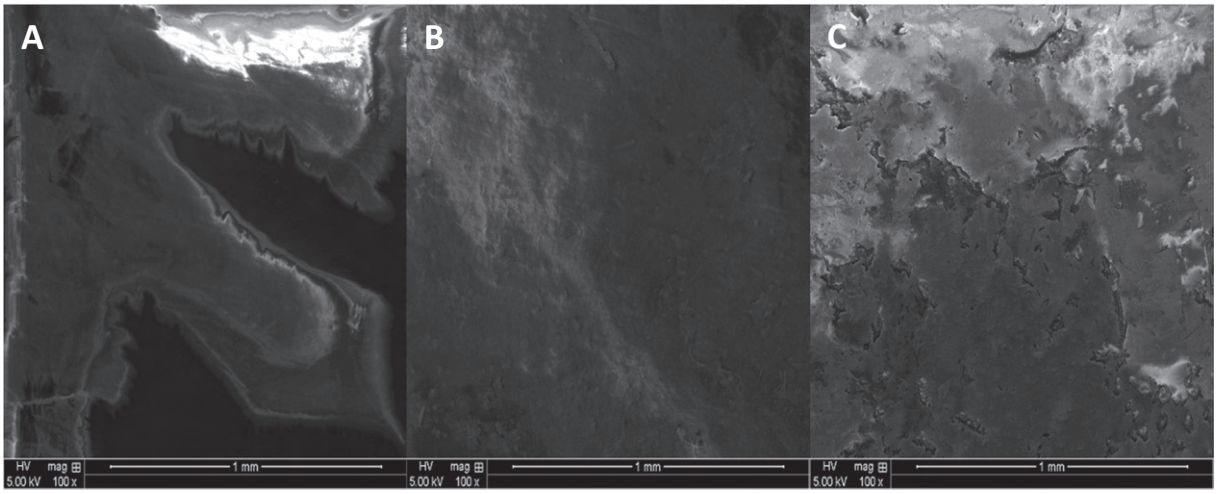


Fig. 3. SEM visualizations of the surface topography of the MP during experiment: (A) pristine MP, (B) II phase, (C) III phase.

430 mg N-NH_4, the highest sorption capacity was 204.0 mg N-NH_4/(g MP·day) and ammonium was sorbed at a rate of 8.52 mg N-NH_4/h. A lower m/v ensured more effective sorption of N-NH_4 due to higher accessibility of active centres on the MP surface. At a higher w/v ratio, the centres may be blocked by the overlapping plastic particles, which disturbs sorption. Properties of polymers influence their sorption capacity. For example, the decreased polymer crystallinity (noted during the experiment) increases capacity and rate of sorption of the contaminants from environment (Mato et al., 2001).

In the presented study, MP was exposed to variable ammonium concentrations for a long period of 260 days. The time of exposure of MP to environmental conditions may result in increased sorption of pollutants (Supplementary materials, Fig. S5). Liu et al. (2018) examining the sorption capacity between pristine and aged MP showed that a significant difference in the adsorption behavior between this two types of MP resulted from the content of oxygen-containing functional groups, which increased sorption of ciprofloxacin on aged MP. The sorption on aged PS and PVC was more than 123.3% and 20.4% higher, respectively, than on the corresponding pristine MP. The highest concentration of TN in the presented experiment was observed on day 74 (448.9 mg/g MP). Previous study showed that sorption of NH_4 onto PE occurred through a surface adsorption mechanism and that a sorption rate constant was 0.08 g/(mg·min) (Li et al., 2020). In this study, the measurement of ammonium removal efficiency was carried out in a period of stable reactor performance. In this period, active sites on MP surface were saturated with ammonium ions and dynamic sorption and desorption took place. Therefore, the sorption capacity was not taken into account when calculating the efficiency of ammonia removal. On the basis of the obtained experiment results, it can be concluded that MP is capable of transporting large amounts of nitrogen compounds, contributing in enrichment of surface waters with nutrients, which may induce algae blooms (Peng et al., 2017).

In our research, the pH in the reactor was kept at a level of 7.43 ± 1.11, which favored the sorption of pollutants on MP. Surface charge of MP affects the sorption behaviours (Xu et al., 2018). MPs are always negatively charged in alkaline solution and the surface of polymers is protonated or positively charged in acid solution (Wang et al., 2015). Therefore, alkaline environments would conduce to the sorption of cations on MPs, due to the electrostatic attraction.

3.3. Microbiome

Molecular analysis of biofilm was conducted with the use of MiSeq Illumina Platform. The total number of sequencing reads in all samples was 150,356 and ranged from 19,956 (II phase) to 4443 (inoculum). In the 16S rRNA gene libraries obtained from inoculum at a phylum level, *Proteobacteria* (80.86%) and *Bacteroidetes* (14.17%) predominated (Fig. 4). In the I phase, the percentage share of *Proteobacteria* decreased from 80.86% to 47.96% while *Bacteroidetes* from 14.71% to 7.51%; simultaneously a significant increase in *Actinobacteria* abundance was observed (from 0.60% to 28.55%). At 16th day of the experiment, an intensive growth of *Firmicutes* (14.30%) was observed in the reactor. In the II phase, *Proteobacteria* (47.03%), *Nitrospirae* (18.33%) and *Bacteroidetes* (7.22%) were the most numerous while at the end of the experiment *Proteobacteria* (54.15%) and *Actinobacteria* (30.74%) predominated.

Number of sample-specific OTUs varied from 3 to 18 – the highest number of specific OTUs was observed in the phase biofilm development (I phase, Fig. 5). In this phase also the alpha-diversity, expressed by Shannon-Wiener index, was the highest reaching 2.96 in the 30th day of the experiment (Fig. 4). This increase can be related to the biofilm growth and competition between various microorganisms in its structure. The lowest microbial diversity was observed at the highest N-NH_4 load in the II phase of the experiment.

Table 1
Physico-chemical indicators during the phases of the experiment.

Physico-chemical indicator	Units	Sampling point	Phase		
			I (n = 4)	II (n = 7)	III (n = 20)
N-NH_4	mg/L	Influent	67.65 ± 10.06	426.29 ± 168.69	72.95 ± 17.41
		Effluent	54.93 ± 19.06	390 ± 186.57	53.60 ± 21.47
N-NO_2		Influent	0	0	0
		Effluent	3.25 ± 0.27	0.31 ± 0.04	2.56 ± 0.21
N-NO_3		Influent	119.37 ± 5.76	557.35 ± 40.13	128 ± 8.45
		Effluent	106.02 ± 6.12	525.50 ± 52.22	137 ± 9.73
COD		Effluent	212.33 ± 102.26	248.42 ± 89.62	111.39 ± 39.82
Biofilm	mg/g MP	Reactor	2.07	2.11	2.32

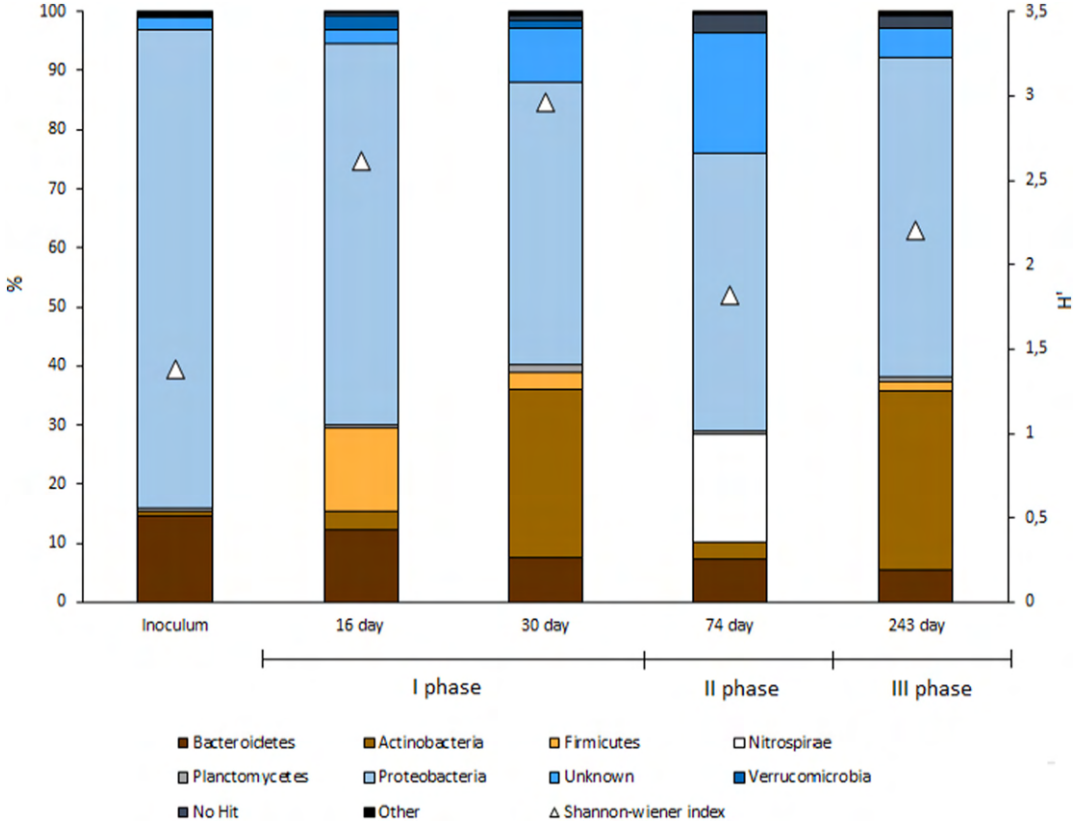


Fig. 4. Bar chart depicting relative abundance (%) of phyla in MP biofilm and a value of Shannon-Wiener index in the samples.

The comparison of microbial communities at the genus level in the individual phases of the experiment showed that 27 OTUs (10.71%) of all OTUs (252) were present in the biomass regardless of the phase of the experiment with a percentage share exceeding 0.5% (*Mycobacterium*, *Bradyrhizobium*, *Rhodopseudomonas*, *Hyphomicrobium*, *Mesorhizobium*, *Sphingomonas*, *Nitrosomonas*, *Nitrosospira*, *Enterobacter*, *Dokdonella* and *Rhodanobacter*) (Fig. 6). There was a significant difference between the composition of inoculum and samples collected during the experiment. There were 49 OTUs (19.44%) that occurred only during the experiment among which sequences belonging to genera *Acidovorax*, *Aquabacterium*, *Bacillus*, *Bdellovibrio*, *Brevibacillus*, *Gordonia*, *Hydrogenophaga*, *Ideonella*, *Pseudomonas*, *Pseudoxanthomonas*, *Rhodococcus*, *Sphingopyxis*, *Streptomyces*, *Terrimonas*, *Thermoactinomyces* and *Thermomonas* were the most numerous.

The chemical composition and morphology of the MP surface affect the formation of the biofilm. During biofilm formation increased production of extracellular polymeric substance (EPS) is observed (Hossain et al., 2019). The EPS secreted by microorganisms ensure stronger biofilm adhesion to plastic surface and excreted EPS and enzymes have a significant impact on biodeterioration of polymer surface. In the presented study, the genera in biofilm able to produce EPS were *Rhodococcus*, *Pseudomonas* and *Xantomonas*. The presence of *Pseudomonas* sp. was especially important for biofilm development and colonization of the PET plastic because microorganisms belonging to this genus possess type IV pili. Those pili are spindly, fibrous organelles and are involved in bacterial movement on solid surfaces through a twitching motility, as well as bacterial attachment to host cells or other surfaces (Vague et al., 2019). *Xantomonas* sp. is known to adhere to polystyrene in the microtiter plates and form dense and uniform biofilms in vitro. These bacteria also release factors that attract the cells adhered to a surface and that may favor the deposition of other bacteria which multiply and promote biomass increase (Guerra et al., 2018).

Because synthetic polymers are not soluble in aqueous solutions, biofilm producing bacteria may degrade such materials more efficiently than planktonic strains (Gilan and Sivan, 2013). Microorganisms on the surface of plastic create an ecological niche indicating potential metabolic adaptation, i.e. adhesion, chemotaxis, resistance to toxic additives contained in plastic and the ability to degrade it (Roager and Sonnenschein, 2019). In our research, at a low concentration of ammonium in the environment, in the biofilm microorganisms capable of MP degradation were identified, such as *Acidovorax*, *Aneurinibacillus*, *Aquabacterium*, *Bacillus*, *Brevibacillus*, *Enterobacter*, *Gordonia*, *Hydrogenophaga*, *Ideonella*, *Pseudomonas*, *Rhodococcus*, *Rhodopseudomonas*, *Sphingomonas*, *Sphingopyxis*, *Streptomyces* and *Thermoactinomyces* (Yuan et al., 2020; Bahl et al., 2021). *Acidovorax* sp. and *Ideonella* sp. produce PETase. Literature data indicate low concentration of ammonium in the environment favors presence of MP-degraders. Lu et al. (2020) showed that increasing ammonium concentration in the environment from 56 to 130 mg/L decreased the abundance of microorganisms belonging to genera *Gordonia* (15% vs 3%) and *Rhodococcus* (80% vs 20%). Additionally, enzymes produced by *Acidovorax* sp. were qualified by Joo et al. (2018) as type IIb enzymes with disulfide bond formation, which are highly active to decompose PET. Stains of *Brevibacillus* and *Aneurinibacillus* isolated from waste management landfills and WWTP showed effective degradation towards LDPE, HDPE and PP. The percentages of weight reduction for strips of LDPE, HDPE and PP at 50 °C were 58.21 ± 2%, 46.6 ± 3% and 56.3 ± 2%, respectively (Skariyachan et al., 2018).

Some strains of *Pseudomonas* sp. produce enzymes such as serine hydrolases, esterases, and lipases, which assist in plastic degradation (Bhardwaj et al., 2013), and are able to degrade plastic rapidly. For example, a *Pseudomonas* sp. strain isolated from a plastic waste disposal site contributed to a 15% weight loss of HDPE after a 30-day incubation (Balasubramanian et al., 2010), and another *Pseudomonas* isolate degraded over 20% of PE after 30 days (Kathiresan, 2003). In the research of McCormick et al. (2016) it was shown that *Pseudomonas* sp. and *Aquabacterium* sp. were 8.7 and 14.5 times more abundant on MP in the water environment than on organic material. In addition, studies of the biofilm consisting of a mixture of *Pseudomonas* sp. and *Bacillus* sp. strains showed that they grew

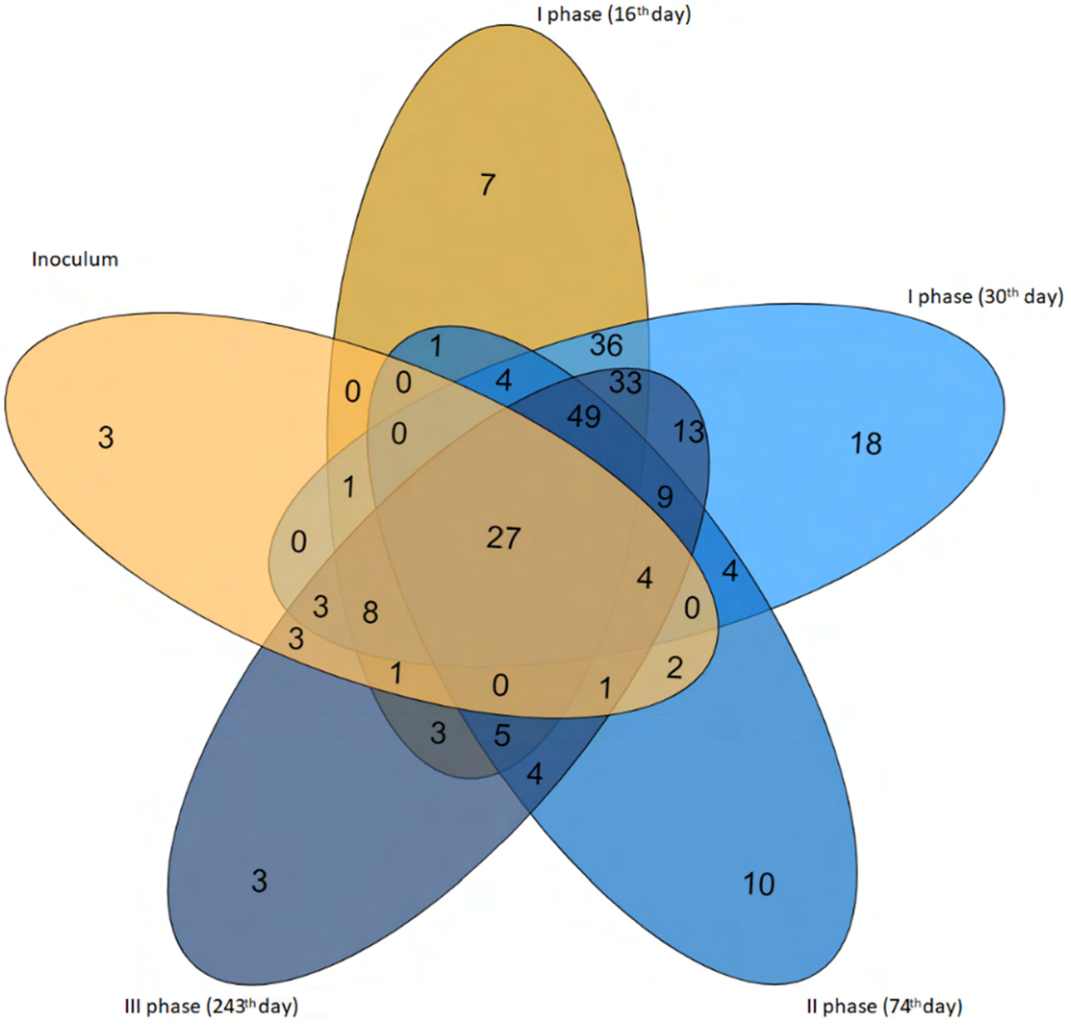


Fig. 5. Venn diagram showing the unique and shared OTUs in the biomass samples during the experiment.

synergistically in the presence of PET and used its degradation products, such as bis(2-hydroxyethyl) terephthalic acid (BHET), as sole sources of carbon. Enzymes secreted from microbial consortium converted BHET to the metabolically usable monomers of terephthalic acid (TPA) and ethylene glycol. Draft genomes provided evidence that the strains can metabolize TPA and ethylene glycol, the building blocks of PET polymers, due to cooperation and ability to cross-feed in the environment with a limited nutrient availability (Roberts et al., 2020).

The results of Kumar et al. (2020) also indicated that *Rhodococcus* sp. can grow on TPA as the sole carbon substrate and are able to degrade the PET structure. After incubation for 132 h, 30.52% weight loss of the PET film was observed as a result of activity of *Rhodococcus* sp.

Many authors point to the degradability of MP by bacteria capable of decomposing hydrocarbons, including *Gordonia* sp. Kwapisz et al. (2008) observed that *Gordonia* sp. displayed considerably higher activity in hydrocarbon degradation in the medium supplemented with nitrate than in the culture enriched with ammonium ions, which may explain the decrease of *Gordonia* sp. presence in our experiment from 1.45% in I phase to 0.07% in II phase with a high N-NH_4 concentration. Foght et al. (1999) investigated the role of the N source on bacterial degradation of aromatic hydrocarbons (PAH) and observed that nitrate did not affect the pH, whereas ammonium amendment led to progressive acidification, accompanied by an inhibition of the degradation of PAHs. In the present study, another genus able to degrade aromatic and halogenate hydrocarbons and thus important for bacterial MPs degradation was *Sphingopyxis* sp. The highest abundance of *Sphingopyxis* sp. was observed at lower ammonium concentrations in the reactor.

Although a large number of MP-degrading genera were observed, it should be emphasized that their relative abundances were low and changed over time (Fig. 6).

The studies showed that the high concentration of N-NH_4 inhibited the growth of microorganisms capable of MP degradation but increased relative abundance microorganisms from the genera *Nitrosospira* (15.77%), *Nitrosomonas* (8.43%), *Terrimonas* (2.24%) and *Methyloversatilis* (0.76%). In the studies of Wang et al. (2016) the intensive development of *Methyloversatilis* was observed at a 60–1000 mg N-NH_4/L, while *Nitrosospira* and *Nitrosomonas* occurred in the full range of the analyzed concentrations (30–1000 mg N-NH_4/L). The microbiological community changed significantly at 120 mg N-NH_4/L and, as in our research, *Sphingobium* sp. and *Dokdonella* sp. disappeared from the biofilm.

Potentially pathogenic microorganisms developed in the surface of MP, included genera *Enterobacter*, *Pseudomonas*, *Mycobacterium*, *Sphingopyxis*, *Aquabacterium* and *Brevundimonas*. Even despite the low numbers of potential pathogens associated with MP, the possible long-term effect of MP colonization should be considered. Plastic fragments represent more stable surfaces than wood or most other natural particles, which might lead to more durable biofilms (Oberbeckmann et al., 2018). Plastic biofilms might stay intact longer and transport pathogen-containing biofilms further compared to natural surfaces (Hoellein et al., 2014).

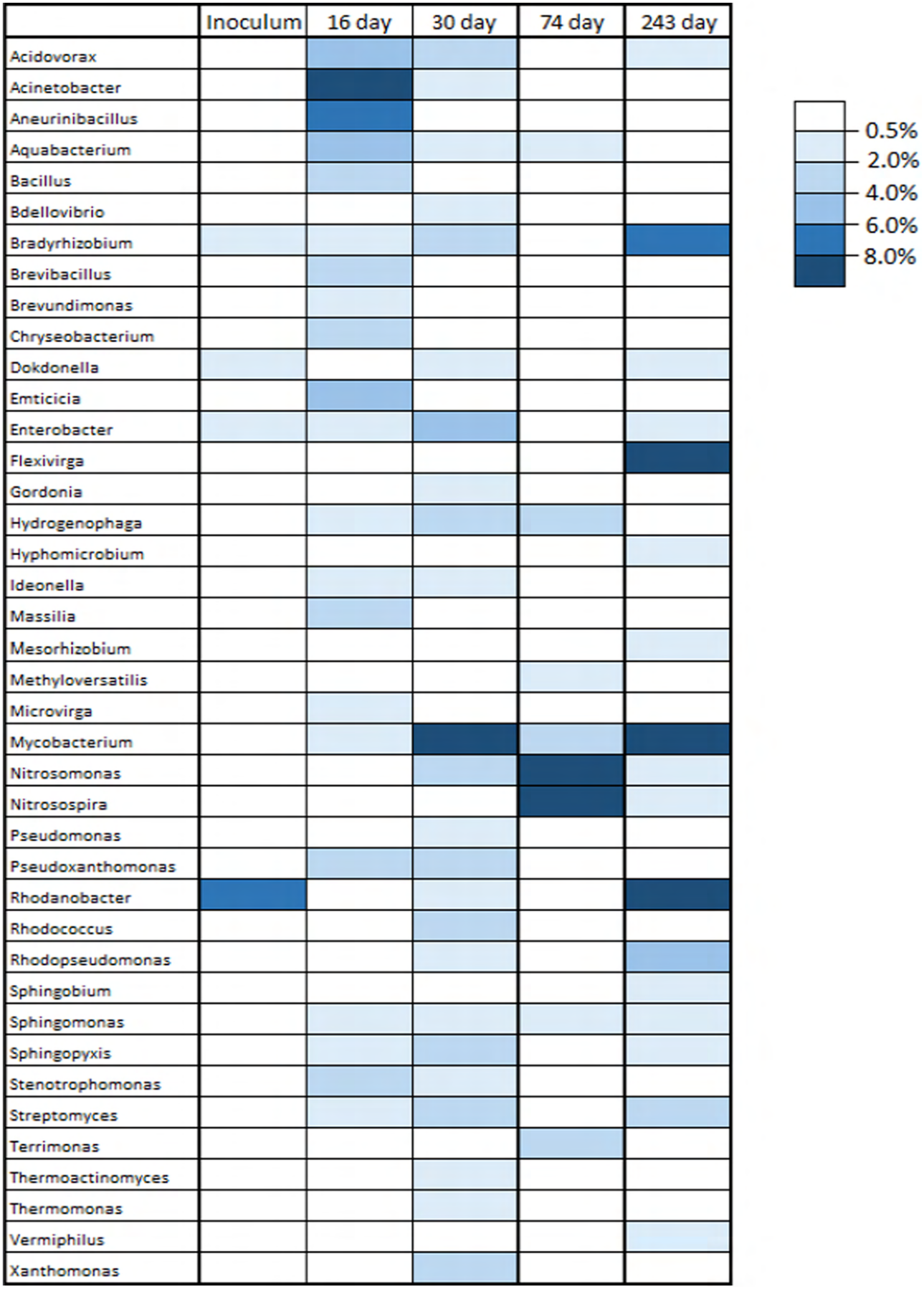


Fig. 6. The most abundant bacterial genera.

4. Conclusion

This study showed that the microbial community on MP surface varies greatly depending on the environmental conditions in the reactor. At a low ammonium nitrogen concentration in the environment, the diversity of microorganisms capable of using PET as a carbon source (e.g., *Acidovorax*, *Gordonia*, *Pseudomonas*, *Sphingomonas* and *Sphingopyxis*) was high, although their relative abundance was low. Raising the concentration of N-NH_4 in the environment increased the abundance of *Nitrosospira*, *Nitrosomonas* and *Terrimonas* in the community and decreased the abundance of microorganisms capable of MP degradation. It was also found that MP is a vector that transfers organic compounds, nitrogen and pathogenic microorganisms (*Mycobacterium* sp., *Enterobacter* sp. and *Brevundimonas* sp.), thus posing a threat to water reservoirs and human health. SEM and FTIR analyses indicated that the MP surface was degraded and that MP structure gradually changed from crystalline to amorphous.

CRediT authorship contribution statement

Conceptualization: PJ, ACK;
Data curation: PJ;
Formal analysis: PJ;DN
Funding acquisition: PJ, ACK;
Investigation: PJ;
Methodology: PJ;
Project administration: ACK, PJ;
Resources: PJ, ACK;
Software: PJ; DN
Supervision: ACK;
Validation: PJ; DN
Visualization: PJ;
Roles/Writing – original draft: PJ;
Writing – review & editing: PJ, ACK.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.154784.

References

APHA, 1992. Standard Methods for the Standard Methods for the Examination of Water and Wastewater. 18th ed. American Public Health Association (APHA), American Water Works Association (AWWA), Water Pollution Control Federation (WPCF), Washington, DC, USA.

Atugoda, T., Wijesekara, H., Werellagama, D., Jinadasa, K., Bolan, N., Vithanage, M., 2020. Adsorptive interaction of antibiotic ciprofloxacin on polyethylene microplastics: implications for vector transport in water. Environ. Technol. Innov. 19, 100971. https://doi.org/10.1016/j.eti.2020.100971.

Auta, H., Emenike, C., Fauziah, S., 2017. Distribution and importance of microplastics in the marine environment: a review of the sources, fate, effects, and potential solutions. Environ. Int. 102, 165–176. https://doi.org/10.1016/j.envint.2017.02.013.

Bahl, S., Dolma, J., Singh, J.J., Sehgal, S., 2021. Biodegradation of plastics: a state of the art review. Mater. Today: Proc. 39, 31–34. https://doi.org/10.1016/j.matpr.2020.06.096.

Balasubramanian, V., Natarajan, K., Hemambika, B., Ramesh, N., Sumathi, C.S., Kottaimuthu, R., Rajesh Kannan, V., 2010. High-density polyethylene (HDPE)-degrading potential bacteria from marine ecosystem of gulf of MannarIndia. Letters in Applied Microbiology 51 (2), 205–211. https://doi.org/10.1111/j.1472-765X.2010.02883.x.

Bhardwaj, H., Gupta, R., Tiwari, A., 2013. Communities of microbial enzymes associated with biodegradation of plastics. J. Polym. Environ. 21 (2), 575–579. https://doi.org/10.1007/s10924-012-0456-z.

Brandon, J., Goldstein, M., Ohman, M., 2016. Long-term aging and degradation of microplastic particles: comparing in situ oceanic and experimental weathering patterns. Mar. Pollut. Bull. 110 (1), 299–308. https://doi.org/10.1016/j.marpolbul.2016.06.048.

Buxbaum, L.H., 1968. The degradation of poly (ethylene terephthalate). Angew. Chem. Int. Ed. Engl. 7 (3), 182–190. https://doi.org/10.1002/anie.196801821.

Chen, G., Huang, J., Tian, X., Chu, Q., Zhao, Y., Zhao, H., 2018. Effects of influent loads on performance and microbial community dynamics of aerobic granular sludge treating piggery wastewater. J. Chem. Technol. Biotechnol. 93 (5), 1443–1452. https://doi.org/10.1002/jctb.5512.

Chen, X., Xiong, X., Jiang, X., Shi, H., Wu, C., 2019. Sinking of floating plastic debris caused by biofilm development in a freshwater lake. Chemosphere 222, 856–864. https://doi.org/10.1016/j.chemosphere.2019.02.015.

Caporaso, G., Lauber, L., Walters, W., Berg-Lyons, D., Lozupone, C., Turnbaugh, P., Fierer, N., Knight, R., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences of the United States of America 108 (Suppl), 4516–4522 10.1073/.

Denaro, R., Aulenta, F., Crisafi, F., Di Pippo, F., Viggi, C., Matturro, B., Tomei, P., Smedile, F., Martinelli, A., Lisio, V., Venezia, C., Rossetti, S., 2020. Marine hydrocarbon-degrading bacteria breakdown poly (ethylene terephthalate)(PET). Sci. Total Environ. 749, 141608. https://doi.org/10.1016/j.scitotenv.2020.141608.

Feng, L., He, L., Jiang, S., Chen, J., Zhou, C., Qian, Z.J., Hong, P., Sun, S., Li, C., 2020. Investigating the composition and distribution of microplastics surface biofilms in coral areas. Chemosphere 126565. https://doi.org/10.1016/j.chemosphere.2020.126565.

Foght, J., Semple, K., Gauthier, C., Westlake, D., Blenkinsopp, S., Sergy, G., Wang, Z., Fingas, M., 1999. Effect of nitrogen source on biodegradation of crude oil by a defined bacterial consortium incubated under cold, marine conditions. Environ. Technol. 20, 839–849. https://doi.org/10.1080/09593332008616879.

Forestier, E., Guigo, N., Combeaud, C., Billon, N., Sbirrazzuoli, N., 2020. Conformational change analysis of poly (ethylene 2, 5-furandicarboxylate) and poly (ethylene terephthalate) under uniaxial stretching. Macromolecules 53 (19), 8693–8703. https://doi.org/10.1021/acs.macromol.0c00691.

Gilan, I., Sivan, A., 2013. Effect of proteases on biofilm formation of the plastic-degrading actinomycete rhodococcus ruber C208. FEMS Microbiol. Lett. 342 (1), 18–23. https://doi.org/10.1111/1574-6968.12114.

Guerra, M., Malafaia, C., Macedo, A., Silva, M., Mariano, R., Souza, E., 2018. Biofilm formation by xanthomonas campestris pv. Viticola affected by abiotic surfaces and culture media. Tropical. Plant Pathol. 43 (2), 146–151. https://doi.org/10.1007/s40858-017-0190-0.

Hanson, J., Yeşiller, N., Oettle, N.K., 2009. Spatial and temporal temperature distributions in municipal solid waste landfills. Journal Environmental Engineering 136, 804–814. https://doi.org/10.1186/s12859-015-0611-3.

Heberle, H., Meirelles, G., da Silva, F., Telles, G., Minghim, R., 2015. InteractiVenn: a web-based tool for the analysis of sets through Venn diagrams. BMC Bioinformatics 16, 169.

He, P., Chen, L., Shao, L., Zhang, H., Lü, F., 2019. Municipal solid waste (MSW) landfill: a source of microplastics?-evidence of microplastics in landfill leachate. Water Res. 159, 38–45. https://doi.org/10.1016/j.watres.2019.04.060.

Henze, M., Harremoes, P., Arvin, E., la Cour Jansen, J., 1997. Wastewater treatment. Biol. Chem. Process. 2 rev.

Hill, M.O., 1973. Diversity and evenness: a unifying notation and its consequences. Ecology 54, 427–432. https://doi.org/10.2307/1934352.

Hoellein, T., Rojas, M., Pink, A., Gasior, J., Kelly, J., 2014. Anthropogenic litter in urban freshwater ecosystems: distribution and microbial interactions. PLoS ONE 9, e98485. https://doi.org/10.1371/journal.pone.0098485.

Hossain, M., Jiang, M., Wei, Q., Leff, L.G., 2019. Microplastic surface properties affect bacterial colonization in freshwater. J. Basic Microbiol. 59 (1), 54–61. https://doi.org/10.1002/jobm.201800174.

Ioakeimidis, C., Fotopoulou, K.N., Karapanagioti, H.K., Geraga, M., Zeri, C., Papathanassiou, E., Galgani, F., Papatheodorou, G., 2016. The degradation potential of PET bottles in the marine environment: an ATR-FTIR based approach. Sci. Rep. 6 (1), 1–8. https://doi.org/10.1038/srep23501.

Jaikumar, G., Brun, N.R., Vijver, M., Bosker, T., 2019. Reproductive toxicity of primary and secondary microplastics to three cladocerans during chronic exposure. Environ. Pollut. 249, 638–646. https://doi.org/10.1016/j.envpol.2019.03.085.

Joo, S., Cho, I., Seo, H., Son, H., Sagong, H., Shin, T., Choi, Kim, K., 2018. Structural insight into molecular mechanism of poly (ethylene terephthalate) degradation. Nature communications 9 (1), 1–12. https://doi.org/10.1038/s41467-018-02881-1.

Kathiresan, K., 2003. Polythene and plastics-degrading microbes from the mangrove soil. Rev. Biol. Trop. 51 (3–4), 629–633.

Kulikowska, D., Klimiuk, E., 2008. The effect of landfill age on municipal leachate composition. Bioresour. Technol. 99 (13), 5981–5985. https://doi.org/10.1016/j.biortech.2007.10.015.

Kumar, V., Maitra, S.S., Singh, R., Burnwal, D.K., 2020. Acclimatization of a newly isolated bacteria in monomer tere-phthalic acid (TPA) may enable it to attack the polymer polyethylene tere-phthalate (PET). J. Environ. Chem. Eng. 8 (4), 103977. https://doi.org/10.1016/j.jece.2020.103977.

Kwapisz, E., Wszelaka, J., Marchut, O., Bielecki, S., 2008. The effect of nitrate and ammonium ions on kinetics of diesel oil degradation by Gordonia alkanivorans S7. Int. Biodeterior. Biodegradation 61 (3), 214–222. https://doi.org/10.1016/j.ibiod.2007.08.002.

Lee, Y.K., Murphy, K.R., Hur, J., 2020. Fluorescence signatures of dissolved organic matter leached from microplastics: polymers and additives. Environ. Sci. Technol. 54 (19), 11905–11914. https://doi.org/10.1021/acs.est.0c00942.

Li, X., Jiang, X., Song, Y., Chang, S.X., 2020. Coexistence of polyethylene microplastics and biochar increases ammonium sorption in an aqueous solution. J. Hazard. Mater. 124260. https://doi.org/10.1016/j.jhazmat.2020.124260.

Li, J., Zhang, K., Zhang, H., 2018. Adsorption of antibiotics on microplastics. Environ. Pollut. 237, 460–467. https://doi.org/10.1016/j.envpol.2018.02.050.

Liu, G., Zhu, Z., Yang, Y., Sun, Y., Yu, F., Ma, J., 2018. Sorption behavior and mechanism of hydrophilic organic chemicals to virgin and aged microplastics in freshwater and seawater. Environ. Pollut. 246, 26–33. https://doi.org/10.1016/j.envpol.2018.11.100.

Lu, L., Wang, G., Yeung, M., Xi, J., Hu, H.Y., 2020. Shift of microbial community in gas-phase biofilters with different inocula, inlet loads and nitrogen sources. Process Biochem. 91, 57–64. https://doi.org/10.1016/j.procbio.2019.11.032.

Ma, Y., Yao, M., Li, B., Ding, M., He, B., Chen, S., Zhou, X., Yuan, Y., 2018. Enhanced poly (ethylene terephthalate) hydrolase activity by protein engineering. Engineering 4 (6), 888–893. https://doi.org/10.1016/j.cej.2019.01.033.

Marttinen, S.K., Kettunen, R.H., Rintala, J.A., 2003. Occurrence and removal of organic pollutants in sewages and landfill leachates. Sci. Total Environ. 301 (1–3), 1–12. https://doi.org/10.1016/S0048-9697(02)00302-9.

Mato, Y., Isobe, T., Takada, H., Kanehiro, H., Ohtake, C., Kaminuma, T., 2001. Plastic resin pellets as a transport medium for toxic chemicals in the marine environment. Environ. Sci. Technol. 35 (2), 318–324. https://doi.org/10.1021/es0010498.

McCormick, A., Hoellein, T., London, M., Hittie, J., Scott, J., Kelly, J., 2016. Microplastic in surface waters of urban rivers: concentration, sources, and associated bacterial assemblages. Ecosphere 7 (11), e01556. https://doi.org/10.1002/ecs2.1556.

Mecozzi, M., Nisini, L., 2019. The differentiation of biodegradable and non-biodegradable polyethylene terephthalate (PET) samples by FTIR spectroscopy: a potential support for the structural differentiation of PET in environmental analysis. Infrared Phys. Technol. 101, 119–126. https://doi.org/10.1016/j.infrared.2019.06.008.

Oberbeckmann, S., Kreikemeyer, B., Labrenz, M., 2018. Environmental factors support the formation of specific bacterial assemblages on microplastics. Front. Microbiol. 8, 2709. https://doi.org/10.3389/fmicb.2017.02709.

Pirzadeh, E., Zadhoush, A., Haghighat, M., 2007. Hydrolytic and thermal degradation of PET fibers and PET granule: the effects of crystallization, temperature, and humidity. J. Appl. Polym. Sci. 106 (3), 1544–1549. https://doi.org/10.1002/app.26788.

Peng, Y., Liu, L., Jiang, L., Xiao, L., 2017. The roles of cyanobacterial bloom in nitrogen removal. Sci. Total Environ. 609, 297–303. https://doi.org/10.1016/j.scitotenv.2017.03.149.

PlasticsEurope, 2018. Plastics – The Facts 2017. PlasticsEurope, Avenue E. van Nieuwenhuyse 4/3, 1160 Brussels. Belgium.

Rieckmann, T., Volker, S., 2003. Poly(ethylene terephthalate) polymerization – mechanism, catalysis, kinetics, transfer and reactor design. Modern Polyesters, Chemistry and Technology of Polyesters and Copolyesters. Wiley Series in Polymer Science. John Wiley and Sons, Ltd, West Sussex (UK), pp. 31–116 https://doi.org/10.1002/0470090685.ch2.

Roager, L., Sonnenschein, E.C., 2019. Bacterial candidates for colonization and degradation of marine plastic debris. Environ. Sci. Technol. 53 (20), 11636–11643. https://doi.org/10.1021/acs.est.9b02212.

Roberts, C., Edwards, S., Vague, M., León-Zayas, R., Scheffer, H., Chan, G., Swartz, N., Mellies, J., 2020. Environmental consortium containing pseudomonas and bacillus species synergistically degrades polyethylene terephthalate plastic. Msphere 5 (6). https://doi.org/10.1128/mSphere.01151-20.

Sammon, C., Yarwood, J., Everall, N., 2000. An FT–IR study of the effect of hydrolytic degradation on the structure of thin PET films. Polym. Degrad. Stab. 67 (1), 149–158. https://doi.org/10.1016/S0141-3910(99)00104-4.

Selvam, S., Jesuraja, K., Venkatramanan, S., Roy, P., Jeyanthi Kumari, V., 2020. Hazardous microplastic characteristics and its role as a vector of heavy metal in groundwater and surface water of coastal South India. J. Hazard. Mater. 123786. https://doi.org/10.1016/j.jhazmat.2020.123786.

Skariyachan, S., Patil, A., Shankar, A., Manjunath, M., Bachappanavar, N., Kiran, S., 2018. Enhanced polymer degradation of polyethylene and polypropylene by novel thermophilic consortia of Brevibacillus sps. and Aneurinibacillus sp. screened from waste management landfills and sewage treatment plants. Polym. Degrad. Stab. 149, 52–68. https://doi.org/10.1016/j.polymdegradstab.2018.01.018.

Świątczak, P., Cydzik-Kwiatkowska, A., 2018a. Performance and microbial characteristics of biomass in a full-scaleaerobic granular sludge wastewater treatment plant. Environ. Sci. Pollut. Res. 25, 1655–1669. https://doi.org/10.1007/s11356-017-0615-9.

Świątczak, P., Cydzik-Kwiatkowska, A., 2018b. Treatment of ammonium-rich digestate from methane fermentation using aerobic granular sludge. Water Air Soil Pollut. 229 (8), 1–12. https://doi.org/10.1007/s11270-018-3887-x.

Tatsi, A.A., Zouboulis, A.I., 2002. A field investigation of the quantity and quality of leachate from a municipal solid waste landfill in a Mediterranean climate (Thessaloniki, Greece). Adv. Environ. Res. 6 (3), 207–219. https://doi.org/10.1016/S1093-0191(01)00052-1.

Qin, R., Su, C., Liu, W., Tang, L., Li, X., Deng, X., Wang, Chen, Z., 2020. Effects of exposure to polyether sulfone microplastic on the nitrifying process and microbial community structure in aerobic granular sludge. Bioresource Technology 302, 122827. https://doi.org/10.1016/j.biortech.2020.122827.

Vague, M., Chan, G., Roberts, C., Swartz, N., Mellies, J., 2019. Pseudomonas isolates degrade and form biofilms on polyethylene terephthalate (PET) plastic. BioRxiv 647321. https://doi.org/10.1101/647321.

Wei, T., Simko, V., Levy, M., Xie, Y., Jin, Y., Zemla, J., 2017. Package 'corrplot'. Statistician 56 (316), e24.

Wang, Z., Luo, G., Li, J., Chen, S.-Y., Li, Y., Li, W.-T., Li, A.-M., 2016. Response of performance and ammonia oxidizing bacteria community to high salinity stress in membrane bioreactor with elevated ammonia loading. Bioresour. Technol. 216, 714–721. https://doi.org/10.1016/j.biortech.2016.05.123.

Wang, F., Shih, K.M., Li, X.Y., 2015. The partition behavior of perfluorooctanesulfonate (PFOS) and perfluorooctanesulfonamide (FOSA) on microplastics. Chemosphere 119, 841–847.

Wang, C., Zhao, J., Xing, B., 2020. Environmental source, fate, and toxicity of microplastics. J. Hazard. Mater. 124357. https://doi.org/10.1016/j.chemosphere.2014.08.047.

Warnes, M.G.R., Bolker, B., Bonebakker, L., Gentleman, R., Huber, W., 2016. Package 'gplots'. Various R Programming Tools for Plotting Data.

Xu, B., Liu, F., Brookes, P., Xu, J., 2018. The sorption kinetics and isotherms of sulfamethoxazole with polyethylene microplastics. Mar. Pollut. Bull. 131, 191–196. https://doi.org/10.1016/j.marpolbul.2018.04.027.

Yang, J., Yang, Y., Wu, W.M., Zhao, J., Jiang, L., 2014. Evidence of polyethylene biodegradation by bacterial strains from the guts of plastic-eating waxworms. Environmental Science & Technology 48 (23), 13776–13784. https://doi.org/10.1021/es504038a.

Yoshida, S., Hiraga, T.K., Takehana, I., Taniguchi, H., Yamaji, Y., Maeda, K., Toyohara, K., Miyamoto, Y., Kimura, K., Oda, A., 2016. Bacterium that degrades and assimilates poly (ethylene terephthalate). Science 351, 1196–1199. https://doi.org/10.1126/science.aad6359.

Yuan, J., Ma, J., Sun, Y., Zhou, T., Zhao, Y., Yu, F., 2020. Microbial degradation and other environmental aspects of microplastics/plastics. Sci. Total Environ. 715, 136968. https://doi.org/10.1016/j.scitotenv.2020.136968.

Zhang, Y., Liang, J., Zeng, G., Tang, W., Lu, Y., Luo, Y., Xing, W., Tang, N., Huang, W., Ye, S., 2020. How climate change and eutrophication interact with microplastic pollution and sediment resuspension in shallow lakes: A review. Science of The Total Environment 705, 135979. https://doi.org/10.1016/j.scitotenv.2019.135979.

Zhang, Q., Chen, X., Zhang, Z., Luo, W., Wu, H., Zhang, L., Zhang, X., Zhao, T., 2020b. Performance and microbial ecology of a novel moving bed biofilm reactor process inoculated with heterotrophic nitrification-aerobic denitrification bacteria for high ammonia nitrogen wastewater treatment. Bioresour. Technol. 315, 123813. https://doi.org/10.1016/j.biortech.2020.123813.

Chemical and microbiological changes on the surface of microplastic after long term exposition to different concentrations of ammonium in the environment

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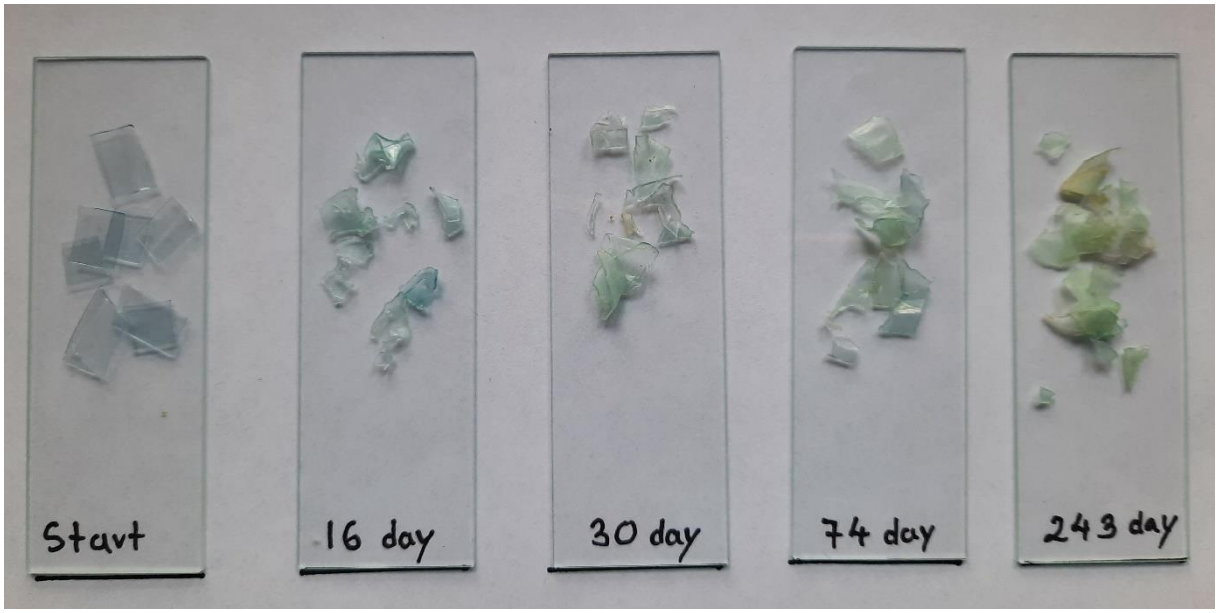

Figure S1. PET morphological changes during the experiment

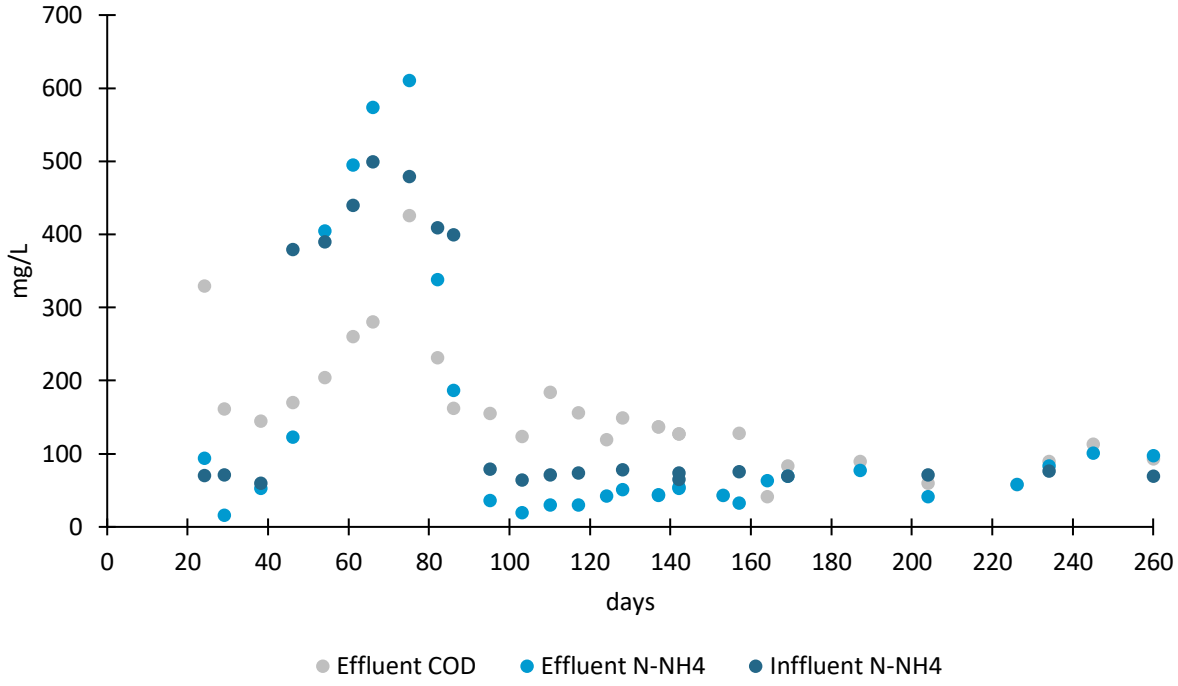

Figure S2. Concentration of ammonium nitrogen and COD in the reactor

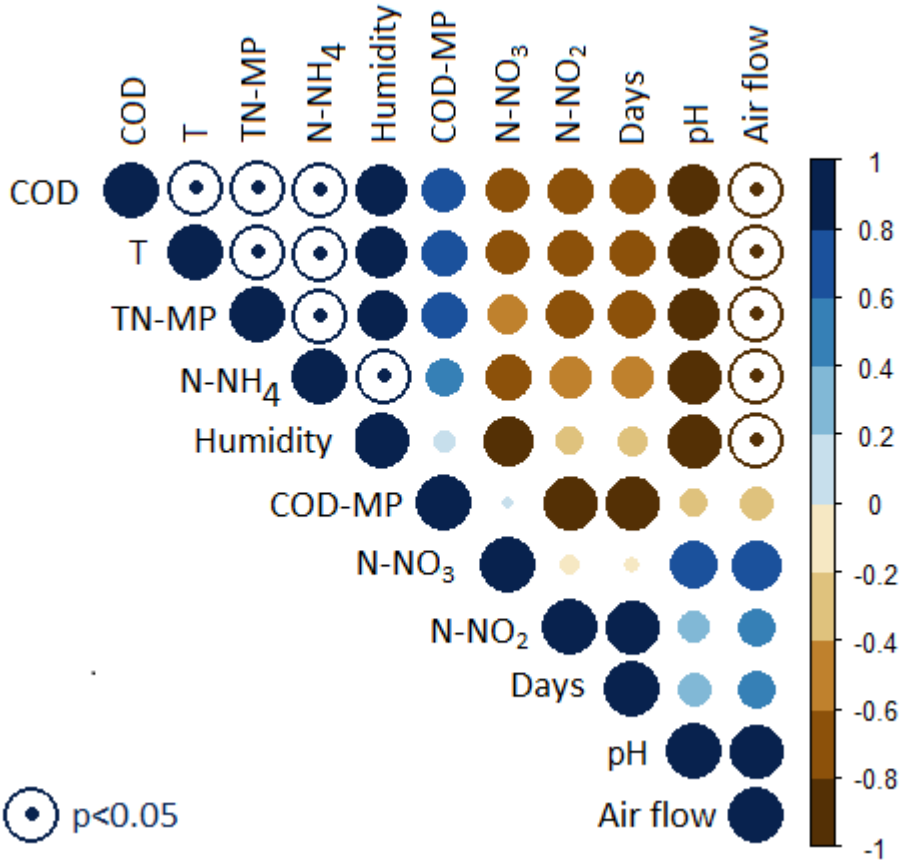

Figure S3. Correlation between concentration of physico-chemical parameters in reactor

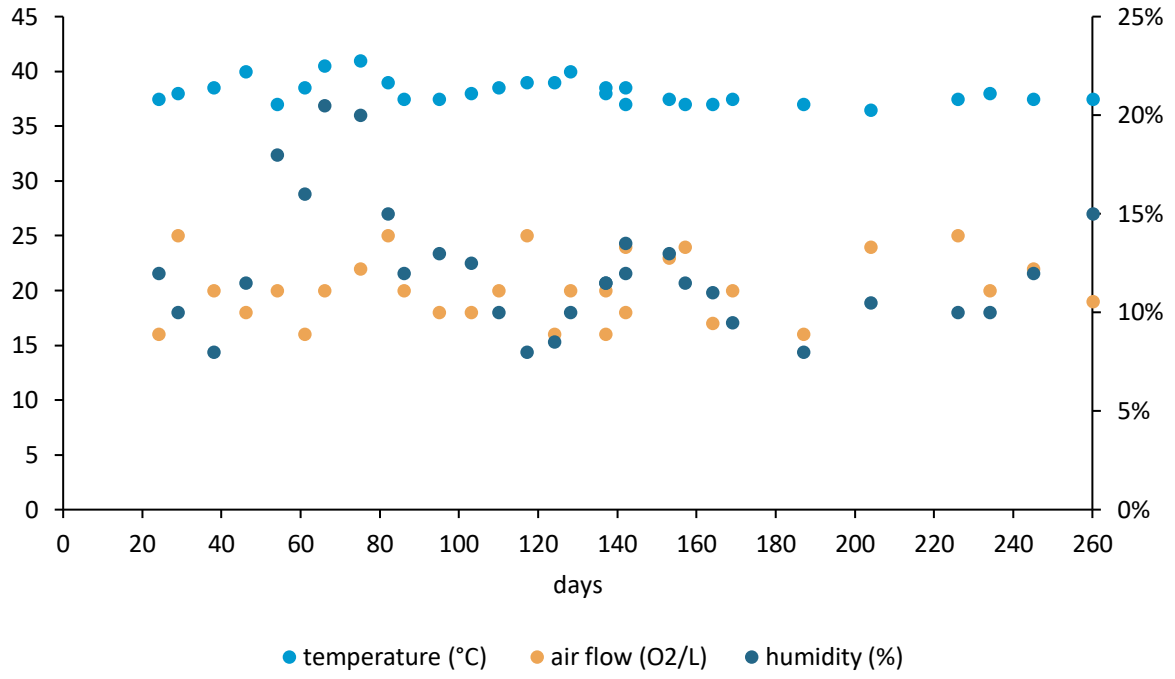

Figure S4. Temperature, humidity and air flow in reactor during experiment

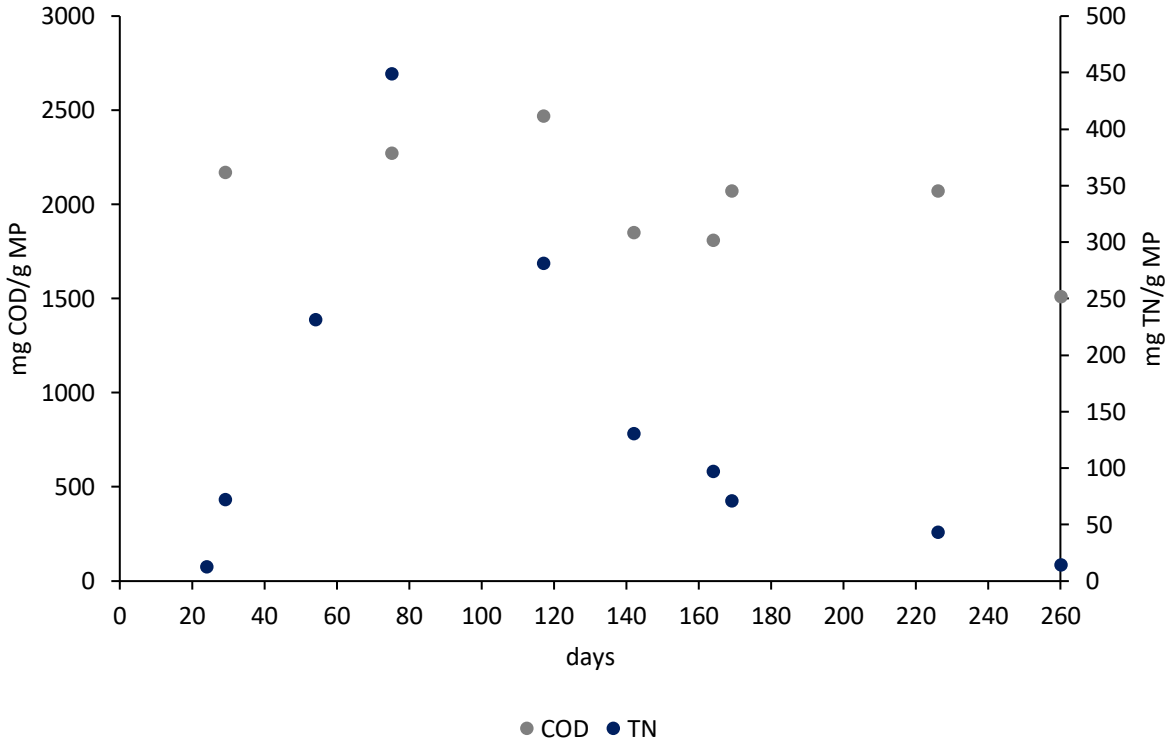

Fig. S5 COD and TN sorbed on MP surface during the experiment

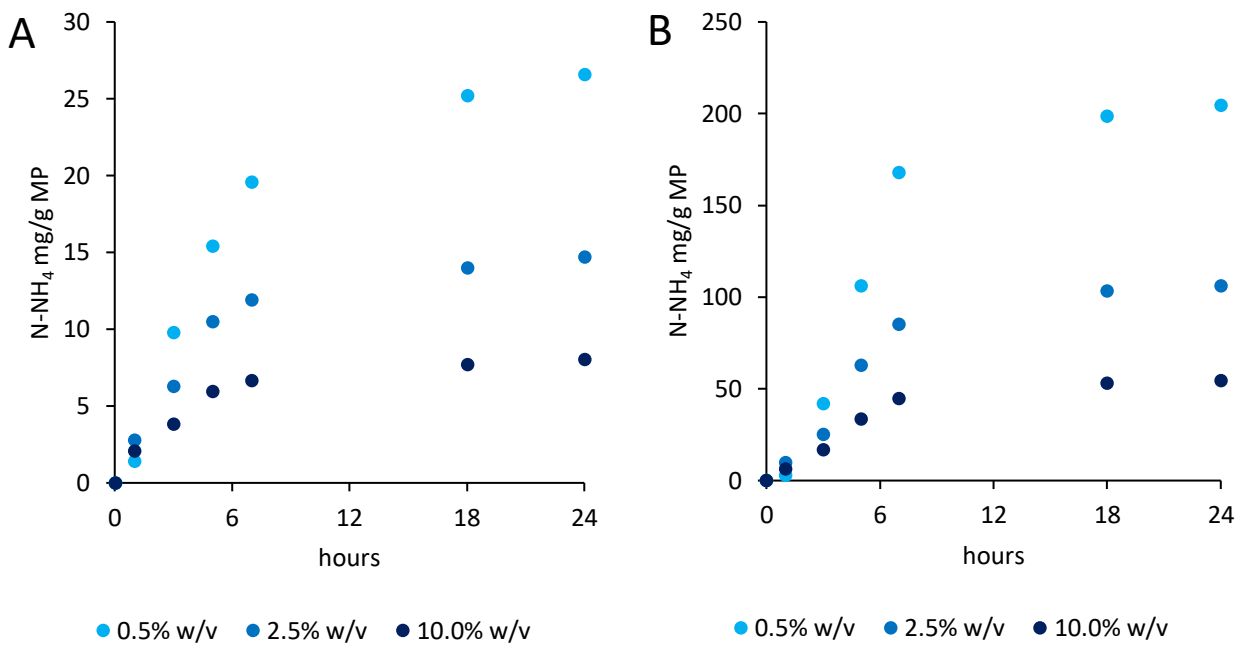

Fig. S6 Sorption of N-NH_4 at MP in batch experiments at initial concentration: (A) 70 mg N-NH_4/L, (B) 430 mg N-NH_4/L,

Olsztyn, 4.04.2024

(miejscowość, data)

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Jachimowicz, P., Nosek, D., Cydzik-Kwiatkowska, A. (2022). Chemical and microbiological changes on the surface of microplastic after long term exposition to different concentrations of ammonium in the environment. Science of The Total Environment, 830, 154784.

polegał na: udziale w zaplanowaniu koncepcji badań, opracowaniu metodyki, wykonaniu części eksperymentalnej, opracowaniu i interpretacji wyników, graficznym opracowaniu wyników oraz przygotowaniu pierwszej wersji manuskryptu.

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Olsztyn, 4.04.2024

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Mój wkład merytoryczny w jej przygotowanie polegał na: uczestnictwie w pozyskaniu i opracowaniu interpretacji wyników technologicznych.

Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez Pana Piotra Jachimowicza jako część rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów naukowych opublikowanych w czasopismach naukowych. Oświadczam, iż samodzielna i możliwa do wyodrębnienia część ww. pracy wykazuje indywidualny wkład kandydata Pana Piotra Jachimowicza polegający na:

udziale w zaplanowaniu koncepcji badań, opracowaniu metodyki, wykonanie części eksperymentalnej, opracowaniu i interpretacji wyników, graficznym opracowaniu wyników oraz przygotowaniu pierwszej wersji manuskryptu.

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Jachimowicz, P., Nosek, D., Cydzik-Kwiatkowska, A. (2022). Chemical and microbiological changes on the surface of microplastic after long term exposition to different concentrations of ammonium in the environment. Science of The Total Environment, 830, 154784.

polegał na: udziale w zaplanowaniu koncepcji badań, pozyskaniu funduszy na prowadzenie badań oraz korekcie artykułu przed złożeniem do wydawnictwa.

Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez Pana Piotra Jachimowicza jako części rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów naukowych opublikowanych w czasopismach naukowych. Oświadczam, iż samodzielna i możliwa do wyodrębnienia część w/w pracy wykazuje indywidualny wkład kandydata Pana Piotra Jachimowicza polegający na:

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