Environ Monit Assess (2022) 194:881
https://doi.org/10.1007/s10661-022-10589-5

Nutrient and organic pollutants removal in synthetic wastewater by *Pseudomonas aeruginosa* and *Chryseobacterium* sp./biofilter systems

Nicoleta-Oana Nicula · Eduard-Marius Lungulescu · Gimi A. Rimbu · Andreea Culcea · Ortansa Csutak

Received: 4 April 2022 / Accepted: 11 August 2022 / Published online: 14 October 2022
© The Author(s), under exclusive licence to Springer Nature Switzerland AG 2022

Abstract Nutrient and organic pollution raise serious problems for aquatic ecosystems through the accumulation of organic carbon, the reduction of light penetration, and the loss of submerged aquatic vegetation. The over-enrichment of water with nitrogen and phosphorus leads to an imbalance in nutrient ratios, creating favorable conditions for toxic algal blooms, formation of oxygen-depleted water, etc. Thus, developing new technological solutions to reduce their amount is imperative. The present study investigates the capacity of *Pseudomonas aeruginosa* and *Chryseobacterium* sp. bacterial strains to form biofilm on solid support (biofilter), both individually and in tandem, using various analytical techniques. Also, the biofilm/biofilter systems' efficiency in removing nutrients such as nitrate, nitrite, ammonium, and phosphate ions from municipal wastewaters is assessed. The results showed a reduction of nutrient pollution of up to 91%, 98%, 55%, and 71% for nitrite, nitrate, ammonium, and phosphate ions. A reduction of about 78% of COD was also observed. The results were obtained in the absence of an additional aeration process, thus having a great potential for reducing total costs of wastewater treatment and developing ecological systems for wastewater management.

Keywords Pollutants removal · Municipal wastewater · Biofilter · *P. aeruginosa* · *Chryseobacterium* sp

N.-O. Nicula · E.-M. Lungulescu (✉) · G. A. Rimbu · A. Culcea
National Institute for R&D in Electrical Engineering ICPE-CA, 313 Splaiul Unirii, Bucharest 030138, Romania
e-mail: marius.lungulescu@icpe-ca.ro

N.-O. Nicula
e-mail: nicoleta.nicula@icpe-ca.ro

G. A. Rimbu
e-mail: gimi.rimbu@icpe-ca.ro

A. Culcea
e-mail: andreea.culcea@icpe-ca.ro

N.-O. Nicula
Faculty of Biology, University of Bucharest, 91–95 Splaiul Independentei, Bucharest, Romania

O. Csutak (✉)
Faculty of Biology, University of Bucharest, 1-3 Aleea Portocalelor, Bucharest, Romania
e-mail: ortansa.csutak@bio.unibuc.ro

Introduction

During the last decades, humanity has succeeded in disrupting the mechanism by which the planet ensures a balance in the water cycle in nature, with a sharp reduction in freshwater resources. Also, the development of the agro-industrial sector due to the increment in food requirement in the conditions of population growth, as well as the support and development of modern technologies, has led to an increase in water consumption. The amount of wastewater generated and discharged into the environment and the degree of contamination of soil, groundwater, and surface water have also risen, despite increasingly restrictive legislation for environmental protection (Rashid et al., 2021; Reid et al., 2019).

According to the Romanian National Institute of Statistics, in 2020, in Romania, the volume of adequately treated wastewater represented 65.4% of the total volume of wastewater generated, while at the European level, about 90% of urban wastewaters were collected and treated following the EU Waste Water Treatment Directive (EEA, 2021; INS, 2020).

In general, regardless of its source, wastewater may contain organic pollutants, suspended solids, nutrients (nitrogen, phosphorus), and heavy metals (cadmium, copper, chromium, nickel, lead, zinc). The loading with organic substances expressed by the parameters BOD_5 (Biochemical Oxygen Demand after 5 days) and COD (Chemical Oxygen Demand) is more evident in wastewater from household activities, at about 70.8%, respectively 63.1% (Daverey et al., 2019). In terms of nutrient loading, the highest values are also found in domestic wastewater: nitrogen (77.2%) and phosphorus (75.3%) (van Puijenbroek et al., 2019).

Various conventional or advanced techniques/methods are used to treat wastewater, in individual or integrated systems. The choice of treatment method depends on several factors, including the chemical composition of the water to be treated, the costs and duration of the process, or the additional impurities present in the wastewater (Wawrzkiewicz et al., 2019). Conventional methods (physical, chemical, mechanical methods (Srivastav et al., 2020; Xu et al., 2020)) have the disadvantage of high operating and maintenance costs, high energy consumption, large amounts of activated sludge generation, and a high global warming potential (through high greenhouse gas emissions) (Kotoula et al., 2020; Koutsou et al., 2018). A viable alternative to removing these disadvantages in the removal of micro-pollutants (Wang et al., 2018) is given by biological treatment systems, which are economically sustainable, environmentally friendly, low cost of operation, and can efficiently remove most pollutants from water (organic matter, nitrogen, and phosphorus) (Kotoula et al., 2020).

For the biological treatment of municipal waters, bacterial cultures, suspended or attached, are commonly used in activated sludge systems, drain filters, membranes, or biofilm reactors in mobile beds (MBBR) (Tchobanoglous et al., 2011), stabilization ponds, disposal systems of sludge, and aerobic reactors with biofilm (Von Sperling, 2007). MBBR is a biofilm-based reactor in which biofilms grow on the surface of small supports (bio support) as biomass carriers. These bio supports are designed to provide a large specific surface area for biofilm formation (Kanaujiya et al., 2019).

MBBR systems are considered to be a promising alternative to activated sludge treatment systems for reducing micro-pollutants, being simple, with a robust and compact design and high solid retention time, allowing microbial biomass formation, the possibility of aerobic and anaerobic microorganisms growth in the same reactor, high efficiency of wastewater treatment, relatively low production costs, etc. (Torresi et al., 2019). The disadvantage of MBBR systems is that they require aeration lines, regular monitoring of biomass activity, and a long time to start the process (Kanaujiya et al., 2019).

Various studies showed that certain bacterial species have a high capacity to degrade micro-pollutants in wastewater. Thus, *Pseudomonas aeruginosa* (Kanaujiya et al., 2019), *Pseudomonas putida* (Chen et al., 2017), *Acinetobacter* sp., *Artrobacter* sp., and *Bacillus thuringiensis* (Kanaujiya et al., 2019) degrade organic matter by adsorption processes on the surface of bacterial cells and their intracellular biotransformation/biodegradation, *Pseudomonas aeruginosa*, *Chromobacterium violaceum* (Nicula et al., 2022), *Thauera* sp., *Rhodanobacter* sp., and *Paracoccus* sp. (Cydzik-Kwiatkowska & Wojnowska-Baryla, 2015) degrade nitrogen compounds, while *Accumulibacter* sp., *Tetrasphaera* sp., *Dechloromonas*, *Candidatus Competibacter phosphatis*, *Pseudomonas aeruginosa*, and *Chromobacterium violaceum* (Cydzik-Kwiatkowska & Zielinska, 2018; Nicula et al., 2022) are involved in phosphate removal.

Pseudomonas aeruginosa is a gram-negative mobile proteobacterium, optionally anaerobic, grows in a normal and lower oxygen atmosphere, in various conditions and temperatures, and can aggregate into biofilms (Thi et al., 2020). The species can decompose substances, including hydrocarbons, to reduce various compounds with nitrogen and heavy metals from residual water (An et al., 2020; Kamika & Momba, 2013). Although these bacteria have a high degree of pathogenicity and high resistance to antimicrobial compounds, they are widely used in many biological wastewater treatment processes. However, this pathogenic risk of *Pseudomonas aeruginosa* can be is easily controlled by the use of effluent disinfection methods before being discharged into the environment (Zhang et al., 2020).

Chryseobacterium sp. is a gram-negative aerobic bacteria, yellow-pigmented, rod-shaped, spore-free, with a high ability to reduce phosphorus, organic carbon (COD), and $NH4^+$-N in wastewater (Kämpfer et al., 2003; Kundu et al., 2014; Mahto & Das, 2022). Also, it has a high capacity to reduce volatile suspended solids (VSS) and, consequently, reduce the amount of activated sludge from biological wastewater treatment processes by up to 50% (Kang et al., 2022; Wang et al., 2021). Kang et al. (2022) showed that *Chryseobacterium* sp. acquires a high resistance to different types of antibiotics in wastewater treatment.

Our study aimed to determine the ability of two bacterial strains belonging to *Pseudomonas aeruginosa* (*Pa*) and *Chryseobacterium* sp. (*Cs*) species to efficiently remove organic matter, nitrate, nitrite, phosphate, and ammonium ions from synthetic municipal wastewater. The growth of the bacterial biofilm on the surface of a biosupport and the elimination of the mentioned pollutants were made individually for each strain, but also in tandem, by eliminating the continuous aeration process. This approach could represent a viable solution to reduce municipal wastewater treatment costs.

Materials and methods

Culture conditions and wastewater cell suspensions preparation

The bacterial strains *Pseudomonas aeruginosa* ATCC 27,853 (*Pa*) and *Cryseobacterium* sp. (*Cs*) (Microbial Collection of the Department of Genetics, Faculty of Biology, University of Bucharest) were grown in plates filled with Muller Hinton agar medium (MH) (Sigma-Aldrich, USA), followed by incubation (CO_2 Incubator InCuSave MCO 215 LIT, Scientific Instruments, Athens, Greece) at 37 °C for 24 h.

Bacterial growth curve

The bacterial growth, both in synthetic wastewater and MH broth, was evaluated from the growth curves. These curves were obtained from the graphical representation of the optical density values at 600 nm (Tecan Genios Microplate Reader, Baldwin Park, NC, USA) as a function of time (hourly, for 24 h). Each sample consisting of 400 μL synthetic wastewater or MH broth was inoculated with 0.5 McFarland standard concentration of bacterial strains (*Pa*, *Cs*, *Pa* + *Cs*). Bacterial growth in synthetic wastewater, under experimental conditions (24 °C, continuous agitation of 10 h/day, pH: 7–8), was evaluated by optical density measurements, at 600 nm, at 24, 48, 120, 240, and 360 h. Previously, individually weighed, six polypropylene biofilters (height: 16 mm; diameter: 22 mm; specific surface: 400 m^2/m^3; PROFLEX MBBR MEDIA, code MB1622, Novara Invest SRL, Bihor, RO) were immersed in the volume of synthetic wastewater (250 mL) in plastic containers. To keep the microorganisms in suspension and to facilitate the formation of the biofilm on the surface of the solid support, the water containers were gently shaken at 100 r.p.m. with an orbital stirrer (DLAB SKL-0180-PRO, Rowland, USA) for 5 h/day. The synthetic wastewater was made by adapting the recipe presented in the reference (Corpuz et al., 2021): 200 mg/L D-glucose, 200 mg/L Sucrose, 70 mg/L yeast extract, 66.73 mg/L $(NH_4)_2SO_4$, 10.91 mg/L NH_4Cl, 4.43 mg/L KH_2PO_4, 21 mg/L $MgSO_4 \cdot 7H_2O$, 21 mg/L $MnSO_4 \cdot H_2O$, 30 mg/L $NaHCO_3$, 19.74 mg/L $CaCl_2$, 0.14 mg/L $FeCl_3 \cdot 6H_2O$, 468 mg/L $NaNO_3$, and 546 mg/L $NaNO_2$.

Evaluation of the ability to form biofilm on biofilter

In order to evaluate the growth of each bacterial strain on biofilter, and their synergistic effect, the adhesion capacity to the solid substrate was evaluated both by gravimetric measurements of biofilm/biofilter systems (at 24, 48, 120, 240, and 360 h) and spectrophotometric by O.D. measurements (600 nm), using wash waters obtained by ultrasonic treatment of biofilm/biofilter systems in sterile distilled water (20 mL). The biofilm viability assessment was performed both quantitatively, by O.D. measurements at 570 nm, and qualitatively, by resazurin testing (Sigma-Aldrich, Saint Louis, USA), as follows: 400 μL of distilled water (from ultrasonic treatment) and 20 µL of 1% resazurin were placed in microtiter plates, which were subsequently placed on the shaker with orbital shaking (DLAB SKL-0180-PRO, Rowland, USA) for 1 h at 300 rpm. The presence of viable cells in the wash water was highlighted by color changing from blue to pink.

Evaluation of the physico-chemical parameters of the synthetic water

The physico-chemical parameters of the synthetic wastewater, inoculated with the two bacterial strains (independently and in tandem), were evaluated by measurements of temperature, pH (pH meter HI10832, Hanna Instruments, Ins., Woonsocket, USA), conductivity (Multiparameter HI2020-01 Edge, Hanna Instruments, Ins., Woonsocket, USA), and dissolved oxygen meter (WTW OXI 340i / SET, Labstuff.eu, Weilheim in Oberbayern, Germany) after 24, 48, 120, 240 and 360 h.

Removal of some pollutants in synthetic wastewater

The removal capacity of organic substances was assessed by determining the COD according to the standard ISO 6060 (ISO6060, 1989) using the Gerhardt type Chemical Oxygen Analyzer (C. Gerhardt GmbH & Co., Germany).

The reduction capacity of nitrite, nitrate, ammonium, phosphate, and iron ions was evaluated photometrically, using standard methods and reagents (Hanna, 2016), adapted for Hanna HI83300 Multiparameter Photometer equipment (Hanna Instruments, Ins., Woonsocket, USA).

Statistical analysis

All analyses were performed in triplicates and the results were presented as mean ± standard deviation (SD) of three independent experiments. The statistical significance was analyzed by Student's *t*-test. A value of *p* less than 0.05 was considered significant. The statistical analysis was performed with Microsoft Excel 2019 software.

Results and discussion

Bacterial growth in MH and synthetic wastewater media under controlled conditions

For each bacterial strain used in this study, growth curves were recorded in MH broth culture medium (Fig. 1a) and synthetic wastewater (Fig. 1b) for 24 h at 37 °C.

The strains showed different growth behaviors in terms of growth phases (i.e., the lag phase, exponential growth, and stationary, respectively). Thus, depending on the growing medium used, the two strains have different lag phase periods of approx. 6–8 h in MH medium, respectively 4–6 h

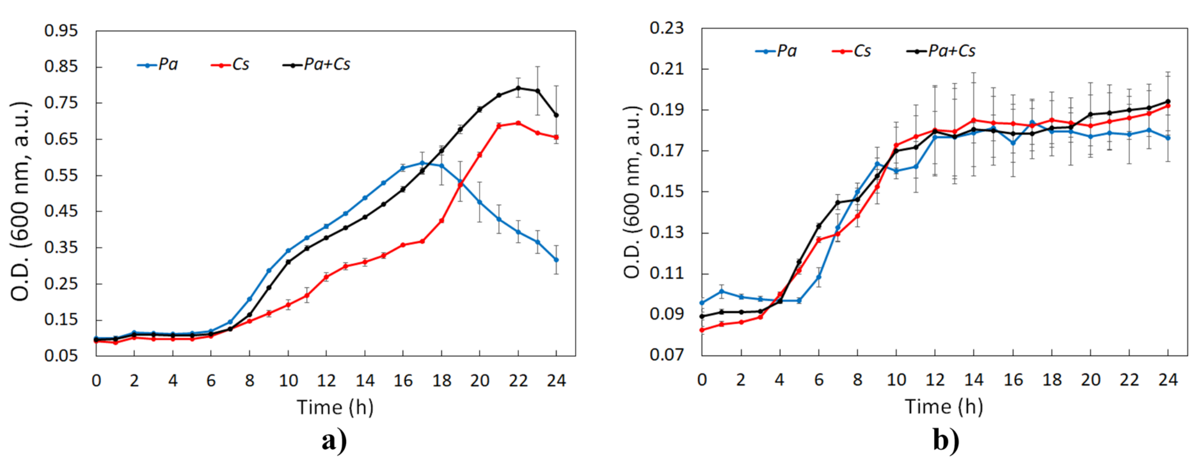

Fig. 1 Bacterial growth in **a** MH medium and **b** synthetic wastewater

in synthetic wastewater. The comparative analysis of growth curves of individual vs. mixed culture revealed that the duration of the lag phase increases in the direction of $Pa < Pa + Cs \leq Cs$ in the case of the MH environment, respectively $Cs \leq Pa + Cs < Pa$, in the case of synthetic wastewater. In both culture media, *Chryseobacterium* sp. appears to have a two-stage exponential growth phase (diauxic growth), between which there is a lag phase. This phase is higher in the case of MH medium, compared to synthetic wastewater, probably due to the organic composition of the medium. In the case of the synthetic wastewater environment, the behavior is explained by the presence of glucose and sucrose and their different metabolization over time by *Chryseobacterium* sp. (Chu & Barnes, 2016). Also, in the case of the MH medium, the reaching of the stationary phase takes place after approx. 17 h for *Pa*, 21 h for *Cs*, and approx. 22 h for $Pa + Cs$. In the mixed system, the delaying in the reaching of the stationary phase is due to contribution of *Cs* strain, but also to competition with *Pa* for nutrients, respectively. In synthetic wastewater, the stationary phase is reached after approx. 10 h for all three types of systems. The explanation might reside in the composition and concentration of nutrients (Nicula et al., 2022) or the different C/N ratios (Kundu et al., 2014) of the two media used. In the case of synthetic wastewater, the abundance of compounds with nitrogen, sulfur, and phosphorus can stimulate bacterial growth and long-term viability, as suggested by the length of the stationary phase.

It should also be noted that, according to the recorded absorbance (O.D.) values, during the 24 h of growth under controlled conditions, the density of bacterial culture was up to 3 times higher in MH medium than in synthetic wastewater.

Similar results, obtained in various academic studies, showed a lag phase of 6–8 h for *Pseudomonas aeruginosa* (Zhu et al., 2019) and 3–5 h for *Chryseobacterium* sp. (Kundu et al., 2014). In our previous study (Nicula et al., 2022), we showed that *Pseudomonas aeruginosa* has an adaptation phase of 2–4 h in synthetic wastewater used in aquaculture, respectively, less than 2 h in Luria Broth (LB) culture medium, which demonstrates, once again, the influence of medium composition on the growth behavior of bacteria.

Bacterial growth in wastewater synthetic wastewater under experimental conditions

The growth of bacterial strains was monitored by optical density measurements performed on synthetic wastewater samples taken at different periods (Fig. 2). The growth of both individual and combined cultures showed similar trends: a period of adaptation to the experimental conditions of approximately 24 h, followed by a log phase up to approximately 120 h, after which the optical density values began to decrease. This effect can be due either to the decrease of the cellular viability of bacterial cultures or, instead, to cell migration on the solid support leading to an enhanced amount of biofilm.

During the experiments, changes in the physicochemical parameters of the synthetic water were observed (Table 1), closely correlated with bacterial growth: slight increase in ambient temperature within the first 48 h of the experiment, constant increase of electrical conductivity throughout the whole experiment, and a decrease in the amount of dissolved oxygen.

An interesting aspect, observed after the first 24 h from the beginning of the experiment, was given by a sharp decrease of the pH values of the inoculated synthetic wastewater from 7.4 to 6.5 for *Pa*, 5.9 for *Cs*, and 5.4 for the mixed cultures ($Pa + Cs$). The results could be explained by the strains' metabolic activity, which can lead to the formation of organic acids in the environment, especially by metabolizing the phosphate ions (Paul & Sinha, 2015). To continue the experiment, adjusting the pH value with a solution of

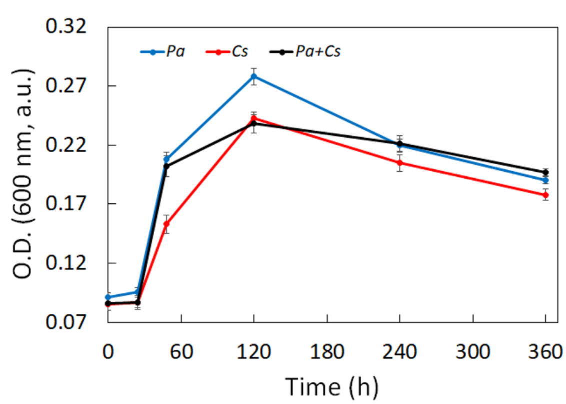

Fig. 2 Growth of bacteria strains during experiments

Table 1 Evolution of physico-chemical parameters of the synthetic wastewater

Strains/parameters	Initial synthetic wastewater	Inoculated synthetic wastewater					
		0 h	**24 h**	**48 h**	**120 h**	**240 h**	**360 h**
Pseudomonas aeruginosa							
Temperature (°C)	23.4 ± 0.4	23.4 ± 0.2	23.8 ± 0.3	23.8 ± 0.2	23.6 ± 0.2	23.6 ± 0.3	23.6 ± 0.2
pH	7.4 ± 0.1	7.3 ± 0.1	6.5 ± 0.2*	7.3 ± 0.1	7.3 ± 0.2	7.6 ± 0.3	7.5 ± 0.3
Conductivity (μS/cm)	333 ± 10	332 ± 12	301 ± 15	320 ± 7	341 ± 15	371 ± 26	1075 ± 58
Dissolved Oxygen (mg/L)	6.3 ± 0.3	6.3 ± 0.2	5.1 ± 0.4	4.9 ± 0.3	4.4 ± 0.2	3.58 ± 0.3	3.02 ± 0.9
***Chryseobacterium* sp.**							
Temperature (°C)	23.4 ± 0.4	23.5 ± 0.2	24.0 ± 0.3	23.7 ± 0.3	23.7 ± 0.2	23.6 ± 0.3	23.6 ± 0.2
pH	7.4 ± 0.1	7.3 ± 0.1	5.9 ± 0.2*	7.3 ± 0.1	7.3 ± 0.2	7.6 ± 0.3	7.5 ± 0.3
Conductivity (μS/cm)	333 ± 10	333 ± 7	314 ± 7	310 ± 11	361 ± 12	499 ± 31	1078 ± 35
Dissolved Oxygen (mg/L)	6.3 ± 0.3	6.3 ± 0.2	4.9 ± 0.5	4.2 ± 0.3	3.8 ± 0.2	3.38 ± 0.3	2.90 ± 0.1
***Pseudomonas aeruginosa* + *Chryseobacterium* sp.**							
Temperature (°C)	23.4 ± 0.4	23.6 ± 0.3	24.3 ± 0.4	23.8 ± 0.3	23.7 ± 0.3	23.8 ± 0.3	23.8 ± 0.2
pH	7.4 ± 0.1	7.3 ± 0.2	5.4 ± 0.2*	7.4 ± 0.2	7.4 ± 0.2	7.7 ± 0.3	7.8 ± 0.3
Conductivity (μS/cm)	333 ± 10	331 ± 13	304 ± 5	270 ± 15	370 ± 15	509 ± 27	1087 ± 38
Dissolved Oxygen (mg/L)	6.3 ± 0.3	6.3 ± 0.1	4.4 ± 0.3	3.9 ± 0.4	3.4 ± 0.2	3.0 ± 0.3	2.72 ± 0.4

*The pH was adjusted to 7.4 by the dropwise addition of 0.6 N NaOH

0.6 N NaOH, dropwise, to a value between 7.0 and 8.0 was necessary. After this adjustment, the pH of the solutions remained unchanged until the end of the experiment.

Evaluation of biofilm formation capacity

Bacteria have a remarkable ability to attach to various surfaces, to form well-organized aggregates, sometimes made up of communities of different species, called biofilms. *Pseudomonas aeruginosa* is a bacterial species used as a model microorganism to study biofilms, being able to adapt to different environments and surfaces (Chang, 2018).

Biofilm formation occurs in several stages, starting from the adsorption of macromolecules and nutrients on the surface, followed by the initial cell transport, adhesion, and irreversible attachment. Colonization and growth of the biofilm occur through the formation of microcolonies and the production of extracellular polymeric substances (Flemming et al., 2016). Biofilm reactors provide the necessary conditions for the reproduction of microorganisms and the use of organic matter dissolved in water as a food source. Organic matter is bioconverted into bacterial biomass that must be separated by sedimentation, thus forming activated sludge (rich in bacterial cultures) (Samer, 2015).

The biofilm formation capacity of individual and combined strains was evaluated by quantitative gravimetric (Table 2) and spectrophotometric determinations by O.D. measurements. The O.D. analysis (Fig. 3a) was performed after ultrasonically treating the biofilm/biofilter system for 30 s, which was sufficient for the detachment of the bacterial biofilm from the surface of the filter, without affecting viability, as it results from the resazurin cell viability test (Fig. 3b).

Table 2 Quantitative determination of the formed biofilm

Strains	Biofilm mass (mg)				
	24 h	**48 h**	**120 h**	**240 h**	**360 h**
Ps	4.66 ± 1.24	7.00 ± 0.82	61.33 ± 2.05	147.00 ± 7.34	159.32 ± 10.42
Cs	3.00 ± 0.81	8.67 ± 0.47	73.22 ± 4.02	163.66 ± 4.28	178.98 ± 7.94
Ps* + *Cs	2.47 ± 0.41	10.67 ± 0.94	74.00 ± 5.71	161.00 ± 8.20	172.25 ± 6.36

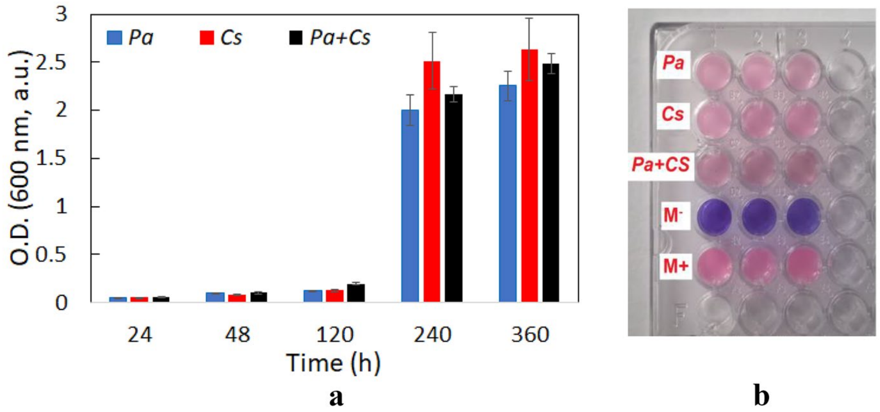

Fig. 3 **a** O.D. measurements of wash waters (after ultrasonic treatment). **b** Testing cells' viability with resazurin after 360 h. The pink color indicates the presence of viable cells in wash water

The bacterial biofilm showed a continuous development on the biofilter, up to 240 h, followed by a smaller growth (of maximum 10%) for the next 120 h (Table 2). In the first 24 h of the experiment, the amount of biofilm formed by *Pa* is 36% higher than *Cs* and 47% compared to the combined system (*Ps*+*Cs*), respectively. After 48 h, the amount of biofilm formed by *Pa* is constantly lower than in the other two bacterial systems.

It is known that bacterial biofilm adhesion properties on different substrates are species-specific, depending on the substrate's surface properties and biofilm-substrate interactions (Nicula et al., 2022). The different behavior of the three bacterial cultures during the first hours of biofilm formation on the solid support can be explained by the different mechanisms of bacterial adhesion to the surface of the biofilter, but also the different development capacities of the two studied strains (Sundell & Wiklund, 2011; Thi et al., 2020). Biofilm formation by *Pa* is a cyclic process that occurs through the (i) initial adhesion of bacteria to the biofilter surface (i.e., association with the biofilter surface and production of extracellular polymeric substances such as proteins, polysaccharides, lipids), followed by (ii) irreversible attachment and cell division, the (iii) formation of microcolonies and cell–cell interaction, the (iv) formation of "mushroom" type structures, and the (v) formation of cavities in the biofilm matrix and cell dispersion (Thi et al., 2020). According to Basson et al. (2008), the absence of biofilm-associated structures (e.g., fimbriae, pili, flagella) suggests that the biofilter surface hydrophobicity and, both, autoaggregation and coaggregation capacity of *Chryseobacterium* sp. play an essential role in biofilm formation. The adhesion mechanism involves the formation of bacteria microcolonies or multilayered biofilm on the biofilter surface due to their high affinity to hydrophobic substances.

Removal of pollutants from municipal synthetic wastewater

The synthetic wastewater used in this study has been modified to comprise both organic pollutants and nutrients such as nitrate, nitrite, phosphate, ammonium (Fig. 4a–e), and iron ions.

COD removal

Chemical Oxygen Demand (COD) is the mass concentration of oxygen that represents the equivalent to the amount of dichromate consumed by dissolved and suspended matter when a sample (water or sludge) is treated with a specific oxidant under defined conditions (ISO6060, 1989). In our case, organic substances introduced into synthetic wastewater (e.g., glucose, sucrose, and yeast extract) lead to a value of COD of about 537 ± 10.1 mg/L (Fig. 4a).

The ability to reduce COD was dependent on the bacterial strains. Thus, *Chryseobacterium* sp. demonstrated, consistently throughout the experiments, a higher COD reduction capacity, reaching up to 73% after 360 h, compared to only 56% for *Pseudomonas aeruginosa*. Interestingly, the use of the two strains together seemed to have a synergistic effect in reducing COD, the efficiency of COD elimination being up to 7% higher than that observed in the case of *Chryseobacterium* sp.

The results obtained in the reduction of COD correlate very well with those recorded when quantifying

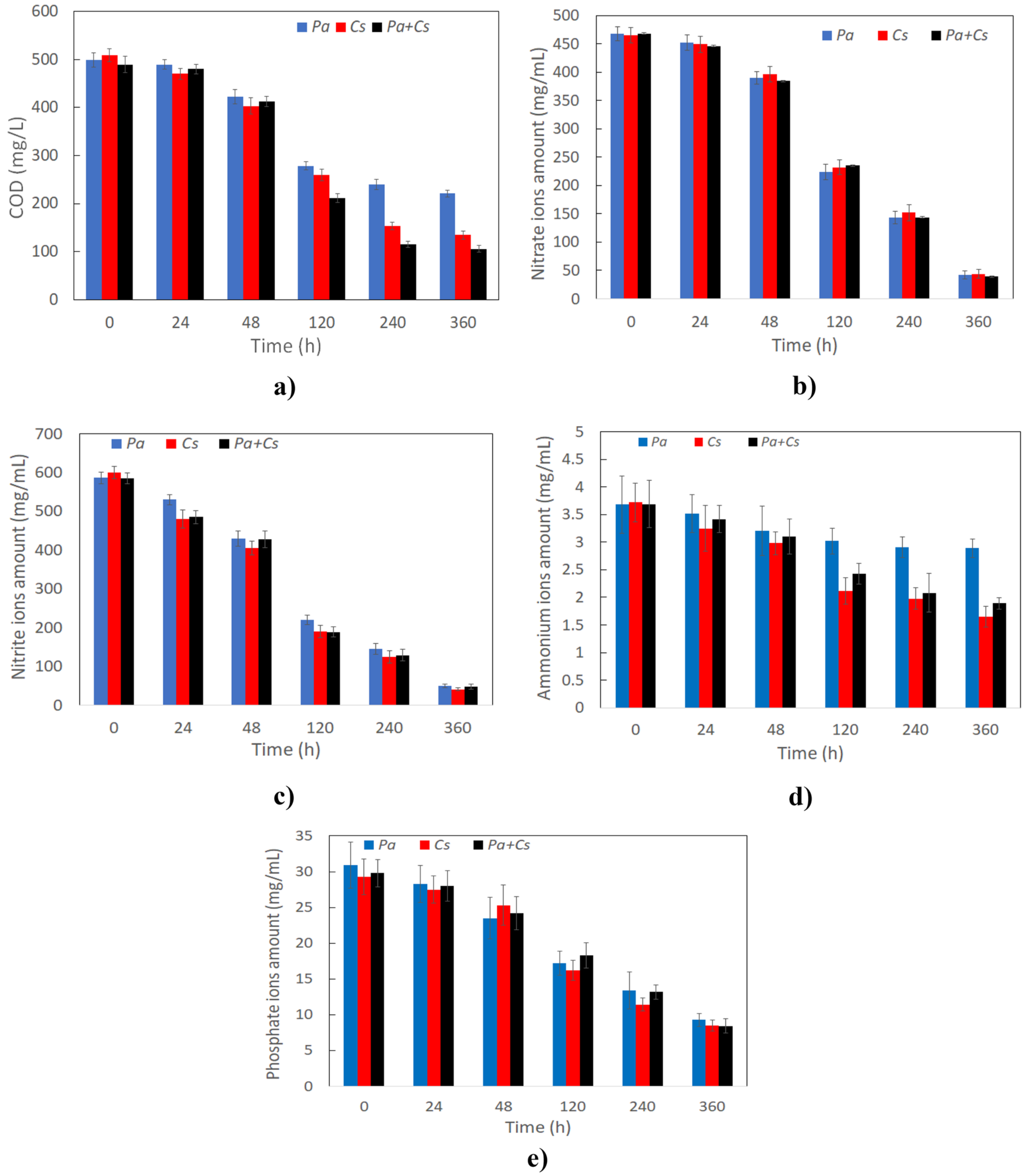

Fig. 4 Evolution of COD and nutrients amount during biofilm/biofilter treatment: **a** COD, **b** nitrate ions, **c** nitrite ions, **d** ammonium ions, **e** phosphate ions

the amount of biofilm formed on biofilters. Consequently, an augmented biofilm formation leads to a higher COD reduction capacity.

Moreover, the availability of COD in wastewater contributes to increased efficiency of nitrogen and phosphorus removal, as microorganisms used in denitrification processes and phosphate reduction use organic carbon in these processes (Ogata et al., 2016).

Removal of nutrients from synthetic wastewater

Nitrate, nitrite, and ammonium ions

Nutrient pollution consists of an over-enrichment of water with nitrogen and phosphorus. It produces the eutrophication of the aquatic ecosystems with adverse effects, such as accumulation of organic carbon, reduction of the light penetration, and loss of submerged aquatic vegetation. Also, it causes an imbalance of nutrient ratios, creating favorable conditions for toxic algal blooms and the formation of oxygen-depleted water (OECD, 2012).

Figure 4b–e compares the ability to reduce nitrate, nitrite, ammonium, and phosphate ions in the synthetic wastewater, by the biofilm (of the two bacterial strains studied, individually and in the mixture)/biofilter systems. As it can be seen, nitrate and nitrite ions reduction is only slightly influenced by the bacterial species, compared to the case of ammonium and phosphate ions.

In the case of nitrate and nitrite ions, a maximum reduction of up to 91% and 98%, respectively, was determined, for all three biofilm/biofilter systems, by the end of the experiment. Regarding the reduction of ammonium ions, the most effective results, up to 55%, were observed for the strain *Chryseobacterium* sp., compared to only 25% for *Pseudomonas aeruginosa*.

The higher reduction capacity of nitrite ions than nitrate ions can be attributed to the mechanism of elimination of ammonium ions by converting NH_4^+-N to NO_3^--N (Liu et al., 2016), which provides an additional intake of nitrate ions to the initially entered quantity. This behavior suggests that the ability to remove nitrate ions may be greater than the one reported in the present study, including both initially and converted NO_3^--N amount.

In a previous study (Nicula et al., 2022), the reduction efficiency of *Pseudomonas aeruginosa* in nitrate, nitrite, and ammonium ions in synthetic aquaculture wastewater was up to 86%, 87%, and 10%, respectively. It should be noted that the lower reduction efficiency of these ions can be explained by the presence in the synthetic wastewater of a concentration of antibiotic (Oxytetracycline) of 0.2 µg/mL. Wei et al. (2021) also evidenced that *Pseudomonas aeruginosa* exhibits both nitrification and denitrification activity under heterotrophic aerobic conditions, simultaneously or independently, depending on the availability of nitrogen sources. In the simultaneous presence of the three types of nitrogen ions, as in our case, the reduction of NH_4^+-N ions took precedence over nitrite and nitrate ions. A similar mechanism, heterotrophic nitrification-aerobic denitrification, has also been shown for certain *Chryseobacterium* sp. (Qiao et al., 2020), demonstrating a reduction capacity of up to 98% for nitrite, nitrate, and ammonium ions after 15 days of the experiment, which confirms our results.

On the other hand, although the availability of dissolved oxygen in synthetic water decreased during the experiment (Table 1), *Pseudomonas aeruginosa*, being optionally anaerobic, was able to use nitrate and nitrite ions as electron acceptors of the respiratory chain. Therefore the soluble ions NO_3^--N and NO_2^--N were converted to gaseous nitrous oxide (N_2O) or N_2 (Vo et al., 2020).

In the case of *Chryseobacterium* sp., the reduction of COD (Fig. 4a) in wastewater (up to 73%) and the correlation with the reduction of NH_4^+-N (up to 55%) recommend this species for alternative bio-treatment processes of wastewater, based on the simultaneous elimination of organic content and ammoniacal nitrogen.

Phosphate ions

In the case of phosphate ions, the reduction efficiency of the studied bacterial strains was not influenced by the belonging to a particular species, being up to about 70% at the end of the experiment, the results being similar to those obtained in the previous study (66% reduction by *Pseudomonas aeruginosa*), in the presence of contaminated water supplemented with antibiotics (Nicula et al., 2022). The presence of NO_3^--N ions acts as an electron acceptor in reducing phosphate ions, especially in conditions of limited access to molecular oxygen (Liu et al., 2016). In their study, Benammar et al. (2015) reported a phosphate ion reduction efficiency of approx. 81% by a strain of *Pseudomonas* isolated from activated sludge samples, taken from an aerobic area of a wastewater treatment plant. The removal efficiency has been improved by using an additional carbon source as a substrate for microbial growth, proving thus that the efficiency of phosphate ion reduction is dependent on the nature of the carbon source (Benammar et al., 2015). Also, the ability to reduce phosphate ions by the studied strains can be related to phosphatase activity (Paul & Sinha, 2015).

Iron

In general, iron is a nutrient that contributes to the optimal growth of bacteria, being an essential element for the biosynthesis of amino acids and pyrimidines, electron transport, DNA synthesis, and others (Banin et al., 2005; Bonneau et al., 2020; Kim et al., 2009).

In our case, after the end of the experiment (360 h), the results showed a reduction in the amount of Fe in synthetic wastewater, compared to the initial value, up to 97–99% for the system inoculated with *Pa*, 92–97% for *Cs*, and 93–98% for the mixed culture, respectively. The high reducing capacity of *P. aeruginosa* is probably due to multiple systems involved in iron detection and capture from the environment and iron cellular acquisition and storage regulation. Iron also makes an essential contribution to the formation of *Pseudomonas* sp. biofilms (Banin et al., 2005).

These experimental results demonstrate that high performance in reducing organic pollutants and nutrients is achieved by using simple bacterial biofilm/solid support systems. These results are obtained at the laboratory level, so it is necessary to perform experiments on an industrial scale and use real municipal wastewater. Subsequently, it is intended to create a cascade treatment system for municipal wastewater (e.g., bacterial biofilm/solid support—yeast—MFC) for extensive treatment of these waters and to study the influence of certain parameters (e.g., temperature, water flow, organic content) on the treatment efficiency.

Conclusions

The bioremediation efficiency of municipal wastewaters was evaluated using synthetic wastewater media inoculated with two bacterial strains commonly found in wastewater, *Pseudomonas aeruginosa* and *Chryseobacterium* sp., in terms of removal of COD and different nutrients (nitrate, nitrite, phosphate, ammonium, iron).

The bacterial strains biofilm/filter systems, individual or mixed cultures, used in this study showed a reduction of up to 91% for NO_3^--N, 98% for NO_2^--N, 55% for NH4+-N, and 70% percent for PO_4^{3-} ions. A reduction of COD of about 73% was observed for *Chryseobacterium* sp., 55% for *Pseudomonas aeruginosa*, and 78% for the mixed culture. Also, removing iron ions up to 99% was recorded due to bacterial metabolism.

The results provide solid evidence for implementing the tested biofilm/filter systems in large-scale treatments of municipal wastewaters, with a significant positive impact on aquatic life, in a sustainable energy manner, with low maintenance and implementation costs.

Acknowledgements We thank Lecturer dr. Robertina Ionescu from the Department of Genetics, Faculty of Biology, University of Bucharest, for kindly providing us with the bacterial strains.

Author contribution Nicoleta-Oana Nicula: conceptualization, methodology, investigation, data curation, resources, writing—original draft preparation, writing—review and editing, supervision. Eduard-Marius Lungulescu: methodology, investigation, writing—original draft preparation, writing—review and editing, project administration, funding acquisition; Gimi A. Rimbu: writing—review and editing, funding acquisition; Andreea Culcea: investigation, writing—review and editing; Ortansa Csutak: conceptualization, writing—original draft preparation, writing—review and editing, supervision.

Funding The financial support was provided by the Ministry of Research, Innovation and Digitization through contracts: PN 19310101-46 N/2019 and project number 25PFE/30.12.2021 – Increasing R-D-I capacity for electrical engineering-specific materials and equipment regarding electromobility and "green" technologies within PNCDI III, Programme 1.

Declarations

Conflicts of interest The authors declare no competing interests.

References

An, Q., Deng, S., Xu, J., Nan, H., Li, Z., & Song, J. -L. (2020). Simultaneous reduction of nitrate and Cr(VI) by Pseudomonas aeruginosa strain G12 in wastewater. *Ecotoxicology and Environmental Safety, 191*, 110001. https://doi.org/10.1016/j.ecoenv.2019.110001

Banin, E., Vasil, M. L., & Greenberg, E. P. (2005). From the cover: Iron and Pseudomonas aeruginosa biofilm formation. *Proceedings of the National Academy of Sciences, 102*(31), 11076–11081. https://doi.org/10.1073/pnas.0504266102

Basson, A., Flemming, L. A., & Chenia, H. Y. (2008). Evaluation of adherence, hydrophobicity, aggregation, and biofilm development of Flavobacterium johnsoniae-like isolates. *Microbial Ecology, 55*(1), 1–14. https://doi.org/10.1007/s00248-007-9245-y

Benammar, L., Menasria, T., Ayachi, A., & Benounis, M. (2015). Phosphate removal using aerobic bacterial consortium and pure cultures isolated from activated sludge. *Process Safety and Environmental Protection, 95*, 237–246. https://doi.org/10.1016/j.psep.2015.03.011

Bonneau, A., Roche, B., & Schalk, I. J. (2020). Iron acquisition in Pseudomonas aeruginosa by the siderophore pyoverdine: An intricate interacting network including periplasmic and membrane proteins. *Scientific Reports*. https://doi.org/10.1038/s41598-019-56913-x

Chang, C. -Y. (2018). Surface sensing for biofilm formation in Pseudomonas aeruginosa. *Frontiers in Microbiology, 8*, 2671–2671. https://doi.org/10.3389/fmicb.2017.02671

Chen, Y., Zhang, C., & Li, Y. (2017). Ultrasonic-assisted biodegradation of endocrine disrupting compounds in soil by Pseudomonas putida: The importance of rhamnolipid for intermediate product degradation. *Chemical Research in Chinese Universities, 33*(2), 179–186. https://doi.org/10.1007/s40242-017-6281-0

Chu, D., & Barnes, D. J. (2016). The lag-phase during diauxic growth is a trade-off between fast adaptation and high growth rate. *Scientific Reports, 6*(1), 25191. https://doi.org/10.1038/srep25191

Corpuz, M. V. A., Borea, L., Senatore, V., Castrogiovanni, F., Buonerba, A., Oliva, G., Ballesteros, F., Zarra, T., Belgiorno, V., Choo, K. -H., Hasan, S. W., & Naddeo, V. (2021). Wastewater treatment and fouling control in an electro algae-activated sludge membrane bioreactor. *Science of the Total Environment, 786*, 147475. https://doi.org/10.1016/j.scitotenv.2021.147475

Cydzik-Kwiatkowska, A., & Wojnowska-Baryla, I. (2015). Nitrogen-converting communities in aerobic granules at different hydraulic retention times (HRTs) and operational modes. *World Journal of Microbiology and Biotechnology, 31*(1), 75–83. https://doi.org/10.1007/s11274-014-1766-1

Cydzik-Kwiatkowska, A., & Zielinska, M. (2018). Microbial composition of biofilm treating wastewater rich in bisphenol A. *Journal of Environmental Science and Health, Part a, Toxic/hazardous Substances & Environmental Engineering, 53*(4), 385–392. https://doi.org/10.1080/10934529.2017.1404326

Daverey, A., Pandey, D., Verma, P., Verma, S., Shah, V., Dutta, K., & Arunachalam, K. (2019). Recent advances in energy efficient biological treatment of municipal wastewater. *Bioresource Technology Reports, 7*, 100252. https://doi.org/10.1016/j.biteb.2019.100252

EEA. (2021). *European Environment Agency: Waste water treatment improves in Europe but large differences remain*. Retrieved February 15, 2022 from https://www.eea.europa.eu/highlights/waste-water-treatment-improves-in

Flemming, H. -C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S. A., & Kjelleberg, S. (2016). Biofilms: An emergent form of bacterial life. *Nature Reviews Microbiology, 14*(9), 563–575. https://doi.org/10.1038/nrmicro.2016.94

Hanna. (2016). *Hanna Instruments HI83300 Instruction Manual* Hanna Instruments Inc., Woonsocket, Rhode Island, 02895, USA. Retrieved March 22, 2022 from https://www.haines.com.au/mwdownloads/download/link/id/222/

INS. (2020). *National Institute of Statistics: Romania - Water distribution and wastewater disposal in 2020*. Retrieved February 15, 2022 from https://insse.ro/cms/sites/default/files/field/publicatii/distributia_apei_si_evacuarea_apelor_uzate_in_anul_2020.pdf

ISO6060. (1989). ISO 6060:1989 Water quality — Determination of the chemical oxygen demand.

Kamika, I., & Momba, M. N. B. (2013). Assessing the resistance and bioremediation ability of selected bacterial and protozoan species to heavy metals in metal-rich industrial wastewater. *BMC Microbiology, 13*(1), 28. https://doi.org/10.1186/1471-2180-13-28

Kämpfer, P., Dreyer, U., Neef, A., Dott, W., & Busse, H. J. (2003). Chryseobacterium defluvii sp. nov., isolated from wastewater. *International Journal of Systematic and Evolutionary Microbiology, 53*(Pt 1), 93–97. https://doi.org/10.1099/ijs.0.02073-0

Kanaujiya, D. K., Paul, T., Sinharoy, A., & Pakshirajan, K. (2019). Biological treatment processes for the removal of organic micropollutants from wastewater: A review. *Current Pollution Reports, 5*(3), 112–128. https://doi.org/10.1007/s40726-019-00110-x

Kang, M., Yang, J., Kim, S., Park, J., Kim, M., & Park, W. (2022). Occurrence of antibiotic resistance genes and multidrug-resistant bacteria during wastewater treatment processes. *Science of the Total Environment, 811*, 152331. https://doi.org/10.1016/j.scitotenv.2021.152331

Kim, B. J., Park, J. H., Park, T. H., Bronstein, P. A., Schneider, D. J., Cartinhour, S. W., & Shuler, M. L. (2009). Effect of iron concentration on the growth rate of Pseudomonas syringae and the expression of virulence factors in hrp -inducing minimal medium. *Applied and Environmental Microbiology, 75*(9), 2720–2726. https://doi.org/10.1128/aem.02738-08

Kotoula, D., Iliopoulou, A., Irakleous-Palaiologou, E., Gatidou, G., Aloupi, M., Antonopoulou, P., Fountoulakis, M. S., & Stasinakis, A. S. (2020). Municipal wastewater treatment by combining in series microalgae Chlorella sorokiniana and macrophyte Lemna minor: Preliminary results. *Journal of Cleaner Production, 271*, 122704. https://doi.org/10.1016/j.jclepro.2020.122704

Koutsou, O. P., Gatidou, G., & Stasinakis, A. S. (2018). Domestic wastewater management in Greece: Greenhouse gas emissions estimation at country scale. *Journal of Cleaner Production, 188*, 851–859. https://doi.org/10.1016/j.jclepro.2018.04.039

Kundu, P., Pramanik, A., Dasgupta, A., Mukherjee, S., & Mukherjee, J. (2014). Simultaneous heterotrophic nitrification and aerobic denitrification by *Chryseobacterium* sp. R31 isolated from abattoir wastewater. *Biomed Research International, 2014*, 436056. https://doi.org/10.1155/2014/436056

Liu, H., Wang, Q., Sun, Y., Zhou, K., Liu, W., Lu, Q., Ming, C., Feng, X., Du, J., Jia, X., & Li, J. (2016). Isolation of a non-fermentative bacterium, Pseudomonas aeruginosa, using intracellular carbon for denitrification and phosphorus-accumulation and relevant metabolic mechanisms. *Bioresource Technology, 211*, 6–15. https://doi.org/10.1016/j.biortech.2016.03.051

Mahto, K. U., & Das, S. (2022). Bacterial biofilm and extracellular polymeric substances in the moving bed biofilm reactor for wastewater treatment: A review. *Bioresource Technology, 345*, 126476. https://doi.org/10.1016/j.biortech.2021.126476

Nicula, N. -O., Lungulescu, E. -M., Ieropoulos, I. A., Rimbu, G. A., & Csutak, O. (2022). Nutrients removal from aquaculture wastewater by biofilter/antibiotic-resistant bacteria systems. *Water, 14*(4), 607. https://doi.org/10.3390/w14040607

OECD. (2012). *Agriculture's impact on aquaculture: Hypoxia and eutrophication in marine waters*. Retrieved March 22, 2022 from https://www.oecd.org/greengrowth/sustainable-agriculture/49841630.pdf

Ogata, F., Ueda, A., Tanei, S., Imai, D., & Kawasaki, N. (2016). Simultaneous removal of phosphate and nitrite ions from aqueous solutions using modified soybean waste. *Journal of Industrial and Engineering Chemistry, 35*, 287–294. https://doi.org/10.1016/j.jiec.2016.01.007

Paul, D., & Sinha, S. N. (2015). Biological removal of phosphate using phosphate solubilizing bacterial consortium from synthetic wastewater: A laboratory scale. *EnvironmentAsia*, *8*(1).

Qiao, Z., Sun, R., Wu, Y., Hu, S., Liu, X., Chan, J., & Mi, X. (2020). Characteristics and metabolic pathway of the bacteria for heterotrophic nitrification and aerobic denitrification in aquatic ecosystems. *Environmental Research, 191*, 110069. https://doi.org/10.1016/j.envres.2020.110069

Rashid, R., Shafiq, I., Akhter, P., Iqbal, M. J., & Hussain, M. (2021). A state-of-the-art review on wastewater treatment techniques: The effectiveness of adsorption method. *Environmental Science and Pollution Research, 28*(8), 9050–9066. https://doi.org/10.1007/s11356-021-12395-x

Reid, A. J., Carlson, A. K., Creed, I. F., Eliason, E. J., Gell, P. A., Johnson, P. T. J., Kidd, K. A., MacCormack, T. J., Olden, J. D., Ormerod, S. J., Smol, J. P., Taylor, W. W., Tockner, K., Vermaire, J. C., Dudgeon, D., & Cooke, S. J. (2019). Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biological Reviews, 94*(3), 849–873. https://doi.org/10.1111/brv.12480

Samer, M. (2015). Biological and chemical wastewater treatment processes. In. InTech. https://doi.org/10.5772/61250

Srivastav, A. L., Patel, N., & Chaudhary, V. K. (2020). Disinfection by-products in drinking water: Occurrence, toxicity and abatement. *Environmental Pollution, 267*, 115474. https://doi.org/10.1016/j.envpol.2020.115474

Sundell, K., & Wiklund, T. (2011). Effect of biofilm formation on antimicrobial tolerance of Flavobacterium psychrophilum. *Journal of Fish Diseases, 34*(5), 373–383. https://doi.org/10.1111/j.1365-2761.2011.01250.x

Tchobanoglous, G., Franklin, L., Burton, E. C., & Stensel, H. D. (2011). Wastewater engineering treatment and reuse (Fourth Edition).

Thi, M. T. T., Wibowo, D., & Rehm, B. H. A. (2020). Pseudomonas aeruginosa Biofilms. *International Journal of Molecular Sciences, 21*(22), 8671–8696. https://doi.org/10.3390/ijms21228671

Torresi, E., Tang, K., Deng, J., Sund, C., Smets, B. F., Christensson, M., & Andersen, H. R. (2019). Removal of micropollutants during biological phosphorus removal: Impact of redox conditions in MBBR. *Science of the Total Environment, 663*, 496–506. https://doi.org/10.1016/j.scitotenv.2019.01.283

van Puijenbroek, P. J. T. M., Beusen, A. H. W., & Bouwman, A. F. (2019). Global nitrogen and phosphorus in urban waste water based on the Shared Socio-economic pathways. *Journal of Environmental Management, 231*, 446–456. https://doi.org/10.1016/j.jenvman.2018.10.048

Vo, C. -D. -T., Michaud, J., Elsen, S., Faivre, B., Bouveret, E., Barras, F., Fontecave, M., Pierrel, F., Lombard, M., & Pelosi, L. (2020). The O2-independent pathway of ubiquinone biosynthesis is essential for denitrification in Pseudomonas aeruginosa. *Journal of Biological Chemistry, 295*(27), 9021–9032. https://doi.org/10.1074/jbc.ra120.013748

Von Sperling, M. (2007). *Wastewater characteristics, treatment and disposal*. IWA publishing.

Wang, N., Chen, X., Ji, Y., Yan, W., Chui, C., Liu, L., & Shi, J. (2021). Enhanced sludge reduction during swine wastewater treatment by the dominant sludge-degrading strains Chryseobacterium sp. B4 and Serratia sp. H1. *Bioresource Technology, 330*, 124983. https://doi.org/10.1016/j.biortech.2021.124983

Wang, Y., Qiu, L., & Hu, M. (2018). Application of yeast in the wastewater treatment. *E3S Web of Conferences, 53*, 04025. https://doi.org/10.1051/e3sconf/20185304025

Wawrzkiewicz, M., Polska-Adach, E., & Hubicki, Z. (2019). Application of titania based adsorbent for removal of acid, reactive and direct dyes from textile effluents. *Adsorption-Journal of the International Adsorption Society, 25*(3), 621–630. https://doi.org/10.1007/s10450-019-00062-0

Wei, R., Hui, C., Zhang, Y., Jiang, H., Zhao, Y., & Du, L. (2021). Nitrogen removal characteristics and predicted conversion pathways of a heterotrophic nitrification–aerobic denitrification bacterium, Pseudomonas aeruginosa P-1. *Environmental Science and Pollution Research, 28*(6), 7503–7514. https://doi.org/10.1007/s11356-020-11066-7

Xu, R., Qin, W., Tian, Z. S., He, Y., Wang, X. M., & Wen, X. H. (2020). Enhanced micropollutants removal by nanofiltration and their environmental risks in wastewater reclamation: A pilot-scale study. *Science of the Total Environment*, *744*. <Go to ISI>://WOS:000573548400001.

Zhang, X., Yan, J., Luo, X., Zhu, Y., Xia, L., & Luo, L. (2020). Simultaneous ammonia and Cr (VI) removal by Pseudomonas aeruginosa LX in wastewater. *Biochemical Engineering Journal, 157*, 107551. https://doi.org/10.1016/j.bej.2020.107551

Zhu, K., Chen, S., Sysoeva, T. A., & You, L. (2019). Universal antibiotic tolerance arising from antibiotic-triggered accumulation of pyocyanin in Pseudomonas aeruginosa. *PLoS Biology, 17*(12), e3000573. https://doi.org/10.1371/journal.pbio.3000573

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.