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Bioaugmentation Performance of a Bacterial Consortium for Moving Bed Biofilm Reactor (MBBR) Treating Municipal Wastewater †

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Abstract: A compatible microbial consortium with a high organic pollutant degradation ability, which includes a gram-positive *Brevibacillus parabrevis* B50 NCAIM B 001413 bacterial strain and a gram-negative *Pseudoxanthomonas mexicana* P32 NCAIM (P) B 001414 bacterial strain, was selected using high-throughput screening techniques. The compatible microbial consortium, encapsulated in alginate beds, was used to inoculate moving bed biofilm reactors from a small municipal wastewater treatment plant. The bioaugmentation performance of the inoculated consortium was evaluated by determining the water quality parameters before inoculation and one month after bioaugmentation treatment. The removal of organic matter was enhanced after treatment with the selected microbial consortium.

Keywords: organic matter removal; gram-positive—gram-negative bacterial consortium; alginate beads encapsulation; inoculation efficiency

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1. Introduction

Bioaugmentation of wastewater treatment intends to enhance efficiency in removing organic matter and pollutants by inoculation with selected microbial strains [1]. The inoculation efficiency also depends on the ability of the introduced bacteria to form protective biofilms [2]. The moving bed biofilm reactor (MBBR) is a wastewater treatment process that uses plastic carriers with a buoyancy close to water to support the development of the bioactive microbial biofilm [3,4]. This MMBR technology benefits by inoculation with bacterial strains with organic pollutant degradation ability and promoting biofilm formation [5–7].

This study describes the effect of applying a compatible microbial consortium with high organic pollutant degradation ability, which promotes biofilm formation, selected with a combination of high-throughput screening techniques [8–10], to enhance the efficiency of a small municipal wastewater treatment plant using MMBR technology. The bioaugmentation performance of the inoculated consortium was evaluated by determining the water quality parameters before inoculation and two weeks after inoculation. The collected water samples were analyzed following the guidelines specified in the Romanian "Normative regarding the establishment of pollutant load limits for industrial and urban wastewater when discharged into natural receivers —the NTPA-001:2002", that fulfills the European

Union Directive 91/271/EEC concerning urban waste-water treatment. This normative document sets essential regulatory limits for pollutant concentrations in wastewater discharges, ensuring the protection of natural water bodies. The analysis of the treated water demonstrated that the inoculation with the bioaugmentation consortium determined the compliance of wastewater discharges with legal requirements.

2. Materials and Methods

2.1. Biological Material

The biocompatible consortium was selected using a set of high-throughput screening techniques developed by our group [8–10] from the microbial strain collection of the National Institute for Research & Development in Chemistry and Petrochemistry—ICECHIM Bucharest. The gram-positive spore-forming bacteria Brevibacillus parabrevis B50 NCAIM B 001413 bacterial strain was isolated from soil and demonstrated a high organic matter degradation ability and capacity to produce manganese oxide, promoting the degradation of recalcitrant pollutants and quorum sensing (QS) signal autoinducer-2, AI-2 [11]. The gram-negative Pseudoxanthomonas mexicana P32 NCAIM (P) B 001414 bacterial strain was isolated by selective enrichment from a soil polluted with crude oil and demonstrated a high ability to produce biosurfactant and Acyl-homoserine lactones (AHL) QS signals.

2.2. Bioproduct Preparation and Bioaugmentation Treatment

The bacterial strains were separately cultivated on potato-dextrose broth supplemented by yeast extract from wine lees for 48 h, and biomass was harvested by centrifugation [12]. The biomass pellets were resuspended in phosphate saline buffer to reach 10^{10} ufc per mL and mixed in a 1:1 ratio. In the resulting suspension, 3 g of sodium alginate, with an average molecular mass of 40 kDa (Sigma-Aldrich, Merck Group, Darmstadt, Germany) per each 97 g of bacterial suspension, were added and dissolved by gentle stirring. The alginate suspension was prilled into calcium alginate beads using Encapsulator B-390 (Büchim Flawil, Switzerland), with a flow vibration nozzle, according to manufacturer instructions. The resulting beads were used to inoculate the first aeration tank of a small municipal wastewater treatment plant (Filipeștii-de-Pădure, Romania) that uses the MMBR technology according to the design of the DFR System (Bucharest, Romania). The used dose was 100 g beads per 1 m^3. The treatment was performed at the beginning of September 2021. Samples of discharged water from the wastewater treatment plant were taken before inoculation and one month after bioaugmentation treatment.

2.3. Analysis of the Water Samples

The water samples were analyzed following NTPA-001:2002, the European Union Directive 91/271/EEC, and corresponding standards [13,14]. pH measurements were performed using a calibrated pH meter equipped with a glass electrode (Seven Compact with InLab® Viscous Pro-ISM electrode, Mettler Toledo, Columbus, OH, USA), according to ISO 10523:2008 [13]. Electrical conductivity was determined using a calibrated conductivity meter (model E-45, Phoenix Instrument, Garbsen, Germany). The sample was directly measured without dilution, in conformity with ISO 7888:1985 [14]. Chloride concentration was determined using titration with silver nitrate ($AgNO_3$) and sodium chromate, with ISO 9297:2001 [15] as a guide. The chemical oxygen demand (COD) analysis was conducted using the sealed tube method. The sample was treated with a dichromate solution in the presence of a silver catalyst, as in the ISO 6060:1996 standard [16]. The biochemical oxygen demand (BOD5) was determined by incubating the sample for five days at 20 °C in an incubator (Algaetron A230, Photon Systems Instruments, Drásov, Czech Republic). Dissolved oxygen levels were measured before and after the incubation with an oxygen meter HI98193 (Hanna Instruments, Nusfalau, Romania), and the BOD5 value was calculated according to SR EN 1899:2003-2 [17]. Total suspended solids (TSS) content was determined by filtering a known sample volume through a pre-weighed glass fiber filter. The filter was then dried in a laboratory oven (UE200 Memmert, Buechenbach, Germany). The glass filter increase in weight corresponded to the TSS concentration, according to ISO 11923:1997 [18]. The residue at 105 °C was determined by evaporating a determined sample volume at 105 °C until constant weight (using an analytical balance, model MS105DU, Mettler Toledo, Columbus, OH, USA). The residue remaining after evaporation represented the non-volatile solids in the sample -STAS 9187-84 [19]. Extractable organic substances were determined by extracting the sample with an appropriate solvent (petroleum ether or hexane), followed by gravimetric analysis SR 7587:1996 [20]. Total nitrogen analysis was performed according to SR EN ISO 11905:2003 [21] as the total nitrogen from the forms of free ammonia, ammonium, nitrites, nitrates, and nitrogenous organic compounds that can be converted into nitrates under oxidative conditions achieved with sodium peroxodisulfate. Quantification was performed with a spectrometer (Ocean FX® UV-Vis spectrometer Ocean Insights, Orlando, FL, USA). The biodegradable synthetic detergents were analyzed using the spectrophotometric methods specified in SR EN 903:2003 [22], as the index of active substances on methylene blue (MBAS). Total phosphorus was determined by the ammonium molybdate spectrometric method, involving the reaction of phosphorus with ammonium molybdate to form a blue complex, following ISO 6878:2008 [23].

3. Results and Discussion

The determined water quality parameters are illustrated in the following figures, compared with the thresholds established by the NTPA-001:2002 and the European Union Directive 91/271/EEC. Figure 1 represents pH, electrical conductivity, and chloride values, and Figure 2 illustrates COD, BOD 5, and total suspended solids values.

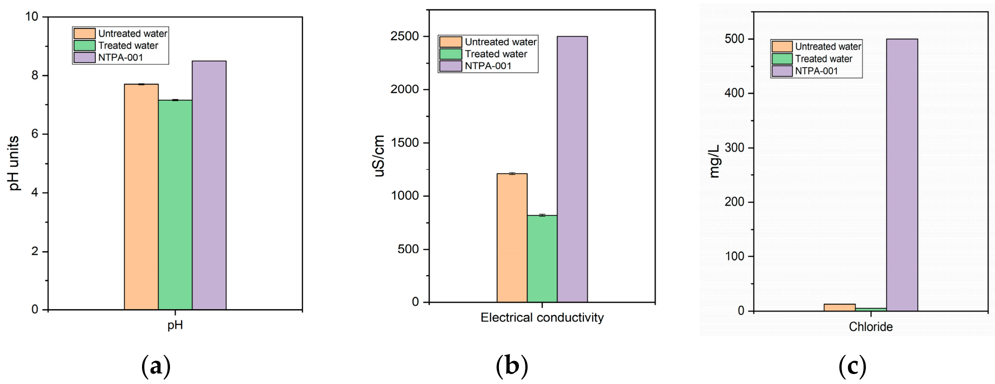


Figure 1. pH (**a**), electrical conductivity (**b**), and chloride values (**c**), determined for initial samples from untreated water, samples after bioaugmentation treatment, and maximum threshold values.

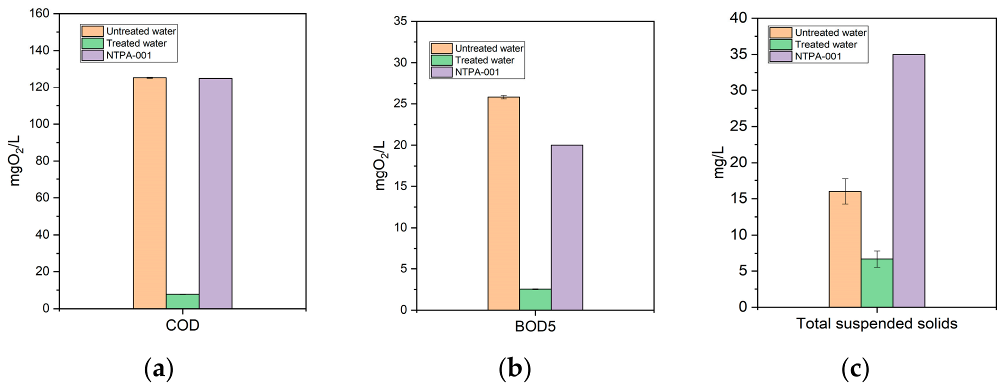


Figure 2. Chemical oxygen demand (COD, **a**), biochemical oxygen demand (BOD5, **b**), total suspended solid (**c**), determined for initial samples from untreated water, samples after bioaugmentation treatment, and maximum threshold values.

The pH values for both untreated (7.71) and treated (7.15) samples were within the acceptable range of 6.5 to 8.5, meeting the NTPA-001 criteria. Similarly, the electrical conductivity values (1210, respectively, 820 ± 10 µS/cm) remained below the specified maximum threshold (2500 µS/cm), indicating compliance with the standard. Chlorides are a significant water quality parameter that measures the concentration of chloride ions (Cl^-) in water. The measured values in samples before treatment (12.8 mg/L) and after treatment (5.3 mg/L) were below the permissible limits set by the NTPA-001 standard [24].

COD is an essential parameter used to assess the organic content and pollution level in water. On the water sample before the bioaugmentation treatment, the COD measured was 125.33 mg/L, being at the superior limit of the NTPA-001, while after the treatment, the water COD value was reduced to 7.7 mg/L.

BOD5 indicates the level of organic matter pollution in the water and helps assess its overall health and the potential for self-purification. The substantial decrease in BOD5 between the water samples taken before the inoculation (25.8 mg O_2/L) and after the inoculation (2.5 mg O_2/L) suggests a successful bioaugmentation. The high level of BOD5 is most probably due to the COVID-19 pandemic conditions, which affected the functionality of wastewater treatment plant, due to high level of disinfectants and other organic pollutants [25,26]. Bioaugmentation with robust bacterial strains, with high organic degradative ability, seems to re-balance the functionality of the wastewater treatment plant.

Total suspended solids (TSS) represent the concentration of particles and solid matter that remain in the water after filtration and drying at 105 °C. After the application of the treatment, the TSS value decreased by 57% from the initial value (16 mg/L), down to 7 mg/L. The values were both under the NTPA-001.

Figure 3 illustrates residues at 105 °C, extractible organic substances, total nitrogen, determined for initial samples from untreated water, samples after bioaugmentation treatment, and maximum thresh-old values.

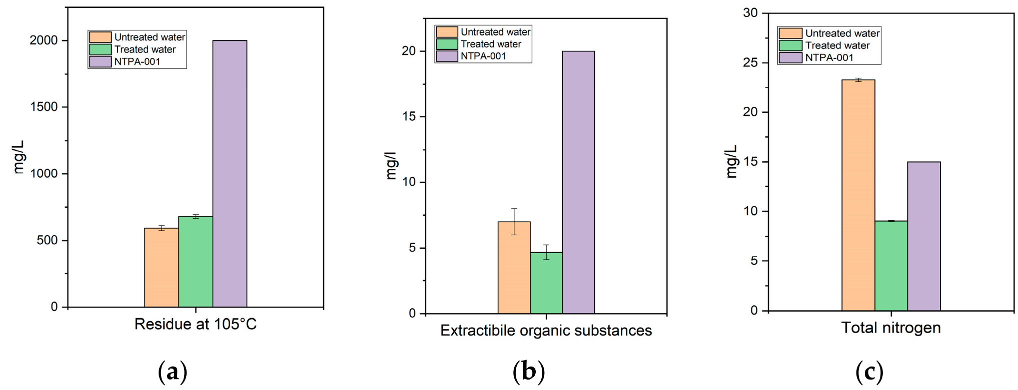


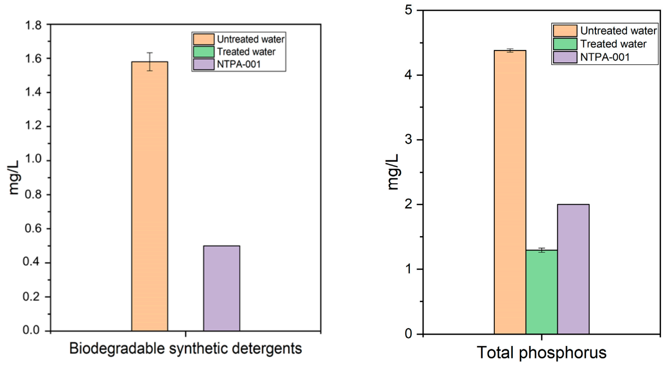
Figure 3. Residues at 105 °C (**a**), extractible organic substances (**b**), total nitrogen (**c**), determined for initial samples from untreated water, samples after bioaugmentation treatment, and maximum threshold values.

Residue at 105 °C represents the sum of all the organic and inorganic substances dissolved in the water. After the treatment, a slight increment occurred (680 mg/L vs. 593 mg/L), but remained within the acceptable limit. Most probably, the increase residues at 105 °C is due to enhanced mineralization of organic matter—and the enhancement of the mineralization process is supported by the finding related to BOD5.

The threshold for extractable organic substances, as indicated by the NTPA-001 standard [24], is 20 mg/L. Both water samples were well below this limit, and after the treatment, a 33% lower value was obtained (4.8 mg/L), indicating that the treatment process effectively reduced the concentration of extractable organic substances in the water.

On the first water sample (untreated), the total nitrogen concentration in the water samples taken before bioaugmentation treatment was 23.3 mg/L, higher than the 15 mg/L NTPA-001 threshold. This value correlates with the higher level of BOD and probably also resulted from the overloading of wastewater treatment plants with organic pollutants during the COVID-19 pandemic, as already mentioned [25,26]. After the treatment, this parameter decreased to 9.04 mg/L.

Figure 4 represents the biodegradable synthetic detergents and total phosphorus, determined for initial samples from untreated water, samples after bioaugmentation treatment, and maximum threshold values according to regulations in force.



(**a**) (**b**)

Figure 4. Biodegradable synthetic detergents (**a**), and total phosphorus (**b**), determined for initial samples from untreated water, samples after bioaugmentation treatment, and maximum threshold values.

Biodegradable synthetic detergents negatively impact the environment due to their foaming effect, contribution to eutrophication, and altered salinity and pH [27]. In the water sample taken before bioaugmentation treatment, the value was 1.58 mg/L, more than three times over the NTPA-001 limit [24]. Synthetic detergent consumption (including soaps) was much higher during the COVID-19 pandemic and significantly influenced wastewater treatment plants [18]. Notably, after the treatment, the concentration of biodegradable synthetic detergents was below the detection limit (<0.2 mg/L) in the treated samples, indicating effective treatment to remove these substances. The application of bacteria from *Bacillus* and *Pseudomonas* genera (*sensu lato*) was recently proposed as a sustainable approach for synthetic detergent degradation and environmental protection [28].

Total phosphorus represents the sum of all forms of phosphorus present in the water, including dissolved and particulate phosphorus compounds. The concentration of total phosphorus in the water sample taken before bioaugmentation treatment was found to be 4.38 mg/L, 2.4 times over the NTPA-001 limit (2 mg/L). Such an increase in phosphorus concentration is probably also related to the increased detergent utilization [29]. In the treated water sample, the total of phosphorus decreased to 1.29 mg/L, indicating that the treatment process effectively reduced the total phosphorus concentration in the water.

Overall, the results indicate the performance of the bacterial consortium used as inoculant for the bioaugmentation of a small wastewater treatment plant using MBBR technology.

4. Conclusions

The bioaugmentation treatment with the bacterial consortia, which includes a gram-positive *Brevibacillus parabrevis* B50 NCAIM B 001413 bacterial strain and a gram-negative *Pseudoxanthomonas mexicana* P32 NCAIM (P) B 001414 bacterial strain, proved to be effective in the re-equilibration of the main functions of wastewater treatment plant unbalanced due to higher level of organic contaminants resulting from specific conditions of the COVID-19 pandemic. The data presented in this work assess the efficiency of the bioaugmentation treatment that ensures compliance with environmental regulations to safeguard water resources.

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