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The Elimination of Pharmaceutical Agents with Microbiological Treatment from Municipal Sewage

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Abstract: Pharmaceutical agents accumulate in wastewater after consumption, but the conventional sewage treatment process is unable to remove them completely. The occurrence of certain compounds in the environment brings forth serious problems even at low concentrations. In this study, the microbiological elimination ability of four non-steroidal anti-inflammatory drugs (NSAIDs)—ibuprofen, naproxen, ketoprofen and diclofenac—were investigated under laboratory, pilot and plant conditions. Mixed cultures from environmental matrices presumably have the ability to reduce the concentration of target agents effectively. According to our analytical measurements, certain mixed cultures gained from natural habitats were even capable of reducing the amount of diclofenac efficiently, after being enriched to a 10^9 colony-forming unit—CFU/mL scale, and inoculated in adequate quantity. Target NSAIDs were detected at µg/L levels in both influents and effluents from wastewater treatment plants (WWTPs). The results showed that inoculated conventional activated sludge sewage treatment technologies have high efficiency for removing ibuprofen, ketoprofen and naproxen. The diclofenac-specific bacteria mix exhibited mild but positive removal efficiency compared to the control plant. This removal ability is also influenced by the hydraulic retention time (HRT).

Keywords: micropollutants; mixed cultures; non-steroidal anti-inflammatory drug (NSAID) removal; wastewater

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1. Introduction

In the near future, the lack of clean drinking water will be a huge risk factor worldwide [1]. Predictably, climate change magnifies the awaiting challenges we have to face. The sewer droughts in Europe push farmers to rely on irrigation systems to supply the amount of missing precipitation. As crop yields decline [2], effects spiral into many aspects of the food supply chain. The urgent need to utilize effluent water from treatment plants as a renewable material source gains even more focus.

Meanwhile, a large mass of medicaments are used for the treatment of diseases all over the world. In the European Union, there are more than 3000 different pharmaceutical substances available and their consumption has increased significantly in recent decades [3]. Most pharmaceuticals are degraded incompletely after application, and the metabolites, as well as the unchanged forms, are excreted and accumulate in sewage [4]. The measuring of active substances has become possible only in the last 15–20 years by the development of analytical technology and methods. Pharmaceutically active compounds are present in the environment in multicomponent mixtures. They are complex molecules with different functionalities, physico-chemical and biological properties [5].

Target NSAID components, especially diclofenac, are thoroughly investigated in the European Community [6]. The potential amount of diclofenac from WWTP effluents could easily reach up to 100 µg/L concentrations. Pairing with moderate sized 100 K personal equivalent (PE) treatment plants may result in many kilograms of unwanted pollution. In the recent proposal for a Directive of the European Parliament and of the

Council, concerning urban wastewater treatment (recast of 91/271/EEC) micro-pollutants and micro-plastics are highlighted as an elevated environmental risk. Centralized sewage treatment plants, according to this, should soon implement a fourth level of treatment technology to eliminate these pollutants, hence instigating technological developments to fulfill the necessary requirements.

Microbial degradation is one of the most important removal processes of organic pollutants [7]. Many pharmaceuticals are bio-transformed by organisms such as bacteria and fungi [8]. Many microorganisms can utilize them for metabolic functions and, in some cases, different microorganisms can cooperate to remove the pollutants [9]. The removal efficiency of pharmaceuticals in WWTPs fluctuates considerably and it is influenced by many factors [10]. Some compounds are not removed, some partly and some below the detection limit [11]. Other authors examined 43 different pharmaceutical substances in 72 influent and effluent samples [12]. They found that out of 32 agents in influent, 29 were also detectable in effluent. The remaining pharmaceutical residuals can enter the surface water and ground water [13]. The majority of them do not cause acute effects on the environment, but chronic effects are not well documented [14]. Although in recent years there have been dynamic developments in sewage treatment technologies, notably in advanced oxidation processes (AOPs) addressing complex organic compound degradation [15], still, conventional biological treatment processes are unable to remove the pharmaceutical residuals solely from sewage. Hence, combining AOPs with the already existing activated sludge method is an important step forward [16].

The most commonly used biological treatment in conventional WWTPs is the activated sludge process. The biological treatment is a combination of biodegradation, volatilization and adsorption. Enhancing biodegradation would be a sustainable method to remove pharmaceuticals via the activated sludge [9]. Finding and integrating the proper microorganism culture that could utilize these compounds via co-metabolism is pivotal. One study has already shown that pure culture isolated from environmental samples can be used to reduce the content of pharmaceuticals [17], but little research has been organized to examine the removal of pharmaceuticals by mixed cultures. Therefore, due to the lack of sufficient literature regarding the issue, we wanted to focus our investigation to find microorganism consortiums from natural habitats with the ability to eliminate the selected aforementioned NSAIDs in wastewater.

2. Materials and Methods

The studied components were chosen beforehand based on the amount of industrial production, population consumption and their persistence in WWTPs. The most produced acetylsalicylic-acid and para-acetaminophenol compounds are eliminated in multi-stage wastewater treatment technologies [18]. We examined four non-steroidal anti-inflammatory drugs—diclofenac, ibuprofen, naproxen and ketoprofen—which are also used in large quantities and generally present in wastewater [19]. Preliminary bacteria cultures were isolated from wastewater effluent (120), surface water (40) and soils (30) to form the initial baseline for further selection. In accordance with pure cultures, mixed cultures also have the capacity to eliminate pharmaceuticals presumably via co-metabolism [20]. The research assesses the series of experiments in which we have: (I) examined the ability of bacteria to remove the selected anti-inflammatories and analgesics in a laboratory environment; (II) determined if a pilot plant with activated sludge continuous feed technology utilizing regular municipal sewage displays significant removal efficiency with these mixed cultures; (III) tested the efficiency of these selected mixed bacteria cultures in existing WWTPs with conventional activated sludge technology that generally represents the mainstream technology in Hungary.

2.1. Laboratory Experiments

Samples were collected from influents and effluents and from surface waters. These samples were analyzed in Alkaloida Chemical Factory Ltd., Tiszavasvári, Hungary with

a High-Performance Liquid Chromatography (HPLC) system (Waters Alliance 2695) to determine the pharmaceutical agents' concentration. After that, the bacteria selection procedure started from WWTP effluent, surface waters and soil, with gradual steps. Samples of 50 µL were inoculated into 50 mL liquid Nutrient culture media (Meat extract (Merck KgaA, Darmstadt, Germany) 10 g/L, Peptone Bacteriological (Oxoid Ltd., Basingstoke, UK) 10 g/L, Sodium chloride (Reanal Ltd., Budapest, Hungary) 5 g/L) and also 50 µL was spread on each petri plate containing Nutrient Agar (Lab M Ltd., Heywood, UK) in triplicate, then incubated at 27 °C for 48 h. The enriched bacteria were collected from liquid media in the final volume of 3 mL after 6, 12, 18, 24 and 48 h; from solid media, the cultures were spread to slants. Plates were tested with colonies under 80 CFU. Colonies were separated according to shape, color or size. Enrichment and elimination processes were carried out with Rotofix 32A, spinning at 4000 rpm for 10 min.

2.2. Pilot and Field Experiments

The pilot plant was built in the WWTP of Nyíregyháza optimized for 10 m^3 per day capacity. It has an activated sludge system with excess sludge removal and can be operated with continuous and sequencing feed. The reactors of various functions encompass each other in a circular ring, creating a favorable hydraulic condition (Figure 1). The outer ring is the anoxic reactor, the middle is the aeration reactor and the inner is the secondary clarifier with 3.4, 3.58 and 1.2 m^3 volumes, respectively, yielding an overall 6.98 m^3 biological capacity. The pilot plant has internal recirculation between the oxic and anoxic reactor for nitrate removal, and settled sludge recirculation to allow initial mixing with the influent sewage.

The field experiments were carried out at the WWTPs of Ibrány as a control plant (W1), while Levelek (W2) and Újfehértó (W3) were inoculated. Average daily influents were 1208, 329 and 725 m^3 per day, respectively. The plants are equipped with mechanical, chemical and activated sludge biological treatment. The volumes of the overall biological reactors were 967 m^3, 750 m^3 and 1332 m^3, respectively.

The selected mixed cultures were enriched in an industrial fermenting unit into 1 m^3 scale utilizing halved nutrient suspension. The prepared suspensions were inoculated directly into the aeration reactors. In field and pilot experiments, sampling was performed of the influent and the effluent in accordance with ISO Guidance [21]. Additionally, excess sludge samples were collected from the pilot plant to further track NSAIDs. Samples were collected in 500 mL brown sample glasses with non-stick insert in triplicate, then delivered to the laboratory in a cooler box immediately and stored at 4 °C until the measurements.

Samples from the pilot plant and the field WWTPs were analyzed in the Chemical Research Center, Biomolecular Chemistry Institute, Liquid Chromatography Mass Spectrometry (LCMS) Laboratory of Hungarian Academy of Sciences, Budapest, Hungary with a HPLC-Q-Trap Mass Spectrometry/Mass Spectrometry (MS/MS) system (PerkinElmer PE 200 HPLC and Sciex 3200 Q Trap MS). The limits of quantification (LOQ) of diclofenac, naproxen, ketoprofen and ibuprofen are 0.5, 1.0, 0.2 and 0.7 µg/L, respectively.

2.3. Calculations and Applied Statistics

The removal efficiency of the target compound during sewage treatment was calculated according to Equation (1)

$$Removal(\%) = \frac{c_{inf} - c_{eff}}{c_{inf}} \times 100 \quad (1)$$

where c_{inf} is the concentration of the target compound in influent and c_{eff} in effluent. Statistical software Past version 2.17c was used for the statistical evaluation of the results. The significance of the differences between removal efficiencies was checked by Student t-test at a significance level of 0.05.

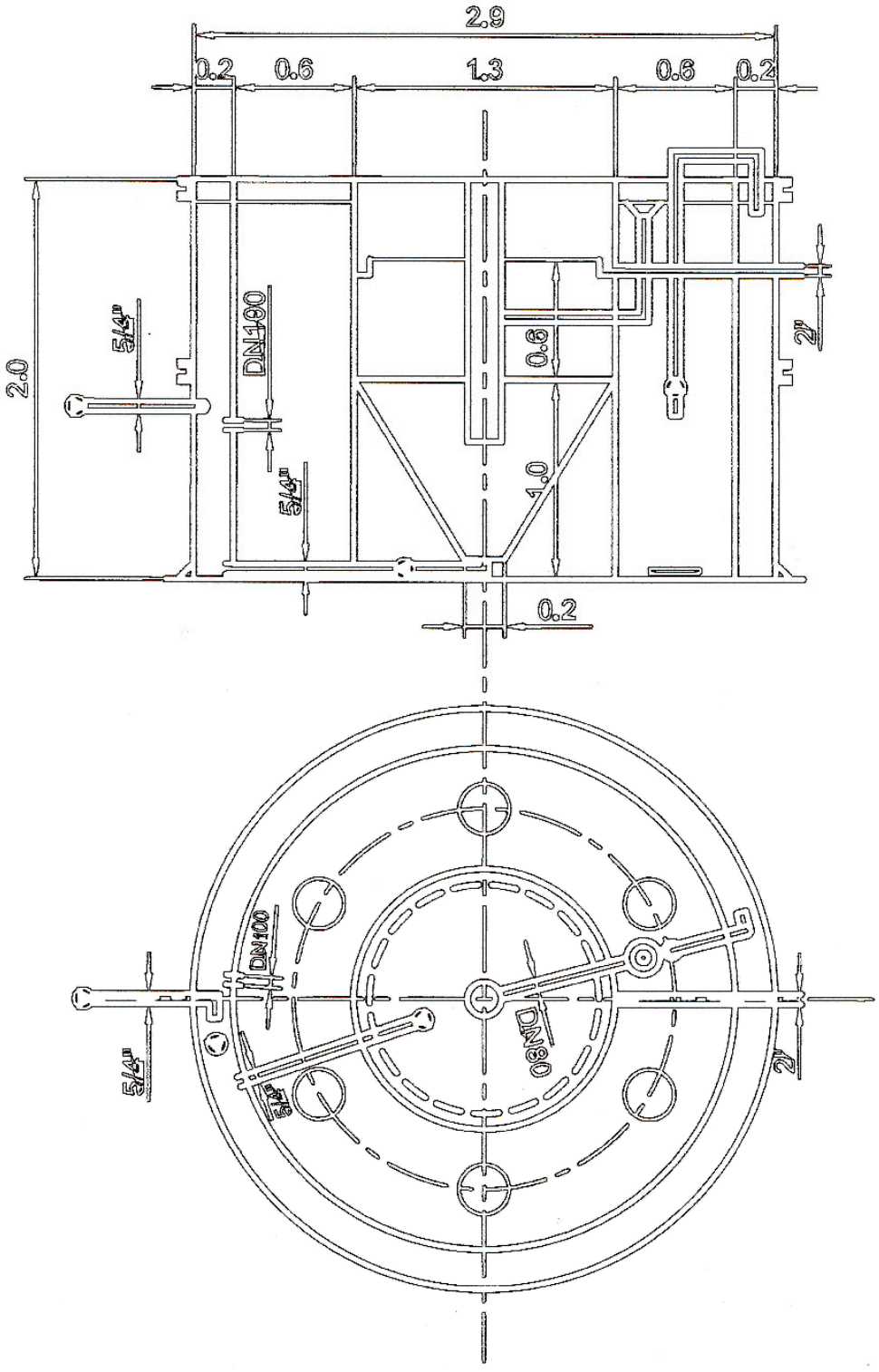

Figure 1. Schematic structure of the constructed experiment pilot plant; dimensions are expressed in: DN (mm); inch (″); meter (m).

3. Results

3.1. Laboratory Experiments

The preliminary selected 190 bacteria cultures were examined. First, we stress tested them with elevated concentrations of NSAID (ketoprofen and diclofenac 25–50 mg/L, naproxen and ibuprofen 20–40 mg/L). The prepared cultures were incubated for 3, 6, 9, 12, 18 and 21 days with continuous shaking at 27 °C in liquid Nutrient media containing

NSAIDs in a mentioned concentration then examined their viability with culturing after spread. The 170 isolated cultures—approximately 90% of them—could withstand and grow on artificial media. In the next step, the remaining cultures were tested with 50 mL preliminarily collected wastewater influent samples, to check if they could tolerate raw sewage. We added bacteria enriched in liquid media to the raw sewage and shook for 24 h. Then, we inspected their viability in solid nutrient media by comparing with the control influent. This test resulted in fewer, but potentially viable, pure and mixed bacteria cultures. As an outcome, altogether 120 pure and 30 mixed cultures were isolated with the potential ability to eliminate NSAID agents. The selection procedure is summarized in Figure 2.

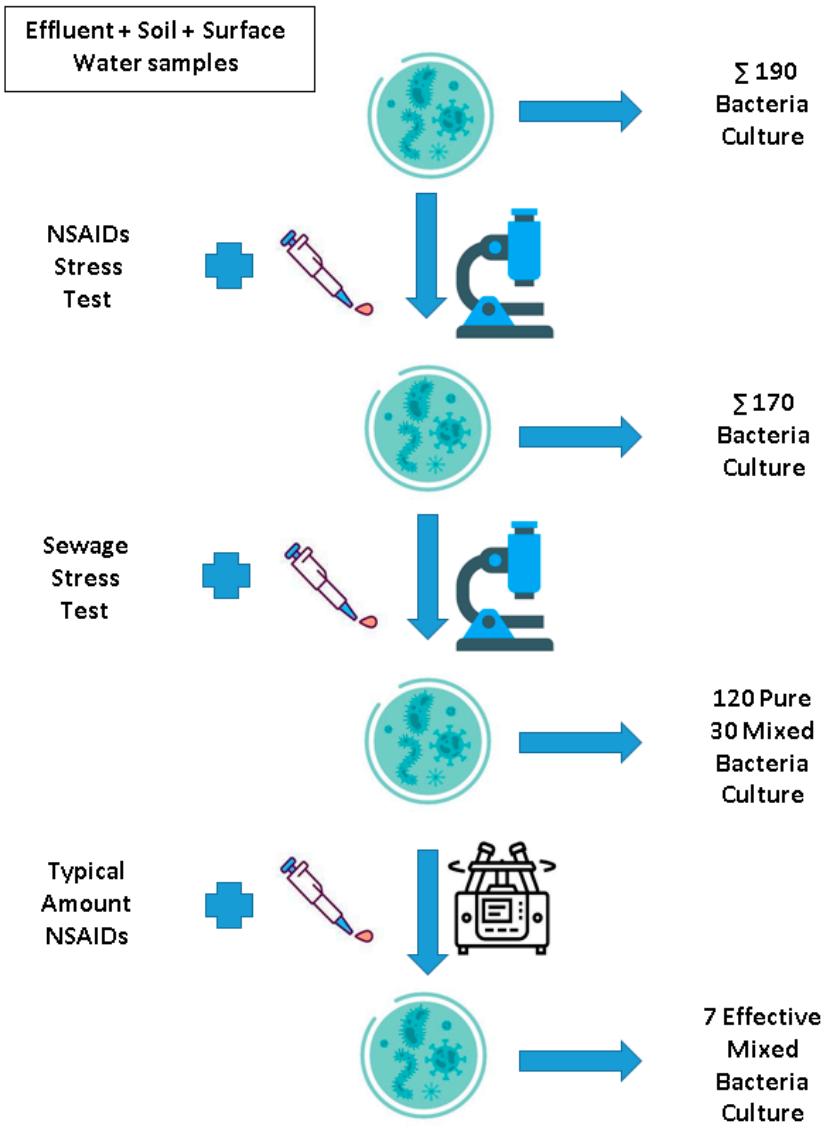

Figure 2. Schematic process of microbial culture selection.

In the next phase, we wanted to identify if the bacteria cultures could eliminate the NSAID agents per se, or if the pharmaceutical agent presence was indifferent in their metabolism, hence retention occurring by easily reversible accumulation or surface adsorption. The cultivated cultures were again exposed to target components, but on a lower, adjusted concentration (ibuprofen 30 µg/mL, naproxen 30 µg/mL, diclofenac 7.5 µg/mL, ketoptofen 3 µg/mL). From previous measurements executed at Budapest City Southern-Pest WWTP and Nyíregyháza City WWTP influent [22], we already knew that the target agents represented more typical amounts in raw wastewater (ibuprofen 8.31 µg/L, naproxen 5.9 µg/L, diclofenac 2.53 µg/L, ketoprofen 2.68 µg/L).

According to the results, 107 pure cultures could grow and survive. However, none of them were able to eliminate target NSAID components on their own. In parallel, the 30 mixed cultures were prepared, from which 7 mixed cultures after 48 h of incubation could eliminate the pharmaceutical agents to a significant extent (Table 1). Bacteria survival and the removal capability were the main driving forces in the selection process. In the end phase, the removal abilities expressed as percentages remained pivotal; hence, the results below 10% removal effectiveness were excluded from the analysis and marked as ineffective. Measurements were confirmed by HPLC from the supernatant of the test conical flasks. The HPLC graphs showed not only the concentrations of the pure agents and their metabolites, but also all the organic and carrier components that were altogether part of the pharmaceutical.

Table 1. Measured average removal efficiency with standard deviations (SD) of the overall test NSAID agents with the effective microbial combination.

∑ NSAID Agent Tests	Removal Efficiency ± SD %	Microbial Combination
Ibuprofen I1-18	39 ± 3.6	I10, I15, I14
Naproxen A1-12	21 ± 4.8	A9, A10
Diclofenac D1-12	58 ± 14	D3
Ketoprofen K1-12	70 ± 11	K8

The elimination pathway was theoretically suspected to occur because of the co-substratum mechanism according to a previous investigation [23], in which the microorganisms depend on each other to transform complex organic structures. However, metabolites were not tracked and identified individually. In the experiment, the initial NSAID concentrations were theoretically higher than we would normally expect from sewage in Hungary, but still, removal rates were significant.

3.2. Pilot Experiments

The inoculation was carried out in two separate flasks. Totals of 40 L of diclofenac specific bacteria culture, and 20 L of the other three bacteria culture in 1:1:1 ratio were added directly to the aeration reactor. Bacteria concentration was in 10^9 CFU/mL scale. Throughout the investigation period, the pilot plant received settled influent with the following properties: NH_4-N = 53 ± 12 mg/L, chemical oxygen demand (COD) = 491 ± 208 mg/L, pH 7.37 ± 0.27 with (SD), respectively. The reactor temperature decreased gradually in the autumn period from 20 °C to 16 °C. Samples were taken 15 times during the 11-week period. Influent sewage NSAID concentration averaged between 4.3 and 18.4 μg/L, 9.8 and 27.5 μg/L and 0.1 and 3.9 μg/L for ibuprofen, naproxen and ketoprofen, respectively. The results in certain cases were below the quantification limit. To address this issue in the interpretation, we valued them as half of their quantification. Results were calculated from the paired influent effluent and sludge measurements; percentages were determined according to the mentioned equation. Removal efficiencies averaged and deviated at the end of the experiment at 96.4 ± 1.4%, 97.1 ± 0.9% and 63 ± 43% with (SD), respectively. These results suggest that after inoculation the components were eliminated with excellent efficiency. It was viable to keep up the results long term; hence, it holds the promising possibility of protecting our recipient water bodies from these pharmaceutical agents. Diclofenac, however, demonstrated different results. Influent concentration ranged between 0.5 and 3.7 μg/L. Despite the higher amount of initial inoculum, it was possible to detect negative efficiency. Consequently, another 40 L of diclofenac-specific bacteria was added to the aeration reactor after three weeks from the start. From this, we expected to boost the performance of removal. Until the very end of the experiment, removal ability averaged and deviated at 8.2 ± 28%, showing a limited positive potential.

To complement the research, we tracked NSAID agents in the sludge itself. Due to suspected co-metabolism, the awaited process was expected to transform the primary agents into metabolites. The excess sludge's liquid and dry phases were separated and

measured. The liquid phase can be interpreted as an effluent. Therefore, this result yielded similar concentrations and retention abilities with the correlated effluent samples. On the other hand, the dry matter content exhibited a different phenomenon, especially in the case of diclofenac (Figure 3). Due to the coherently low concentration of ketoprofen in the sludge as well, metabolic processes remained uninterpretable. Ibuprofen and naproxen target agents were only present in subtle or zero concentrations compared to the influent, thus suggesting almost complete elimination. According to this experiment, elimination occurs until this point of the treatment process. Diclofenac, however, was in a much higher concentration in the dry matter compared to the influent, hence showing potential accumulation in the sludge.

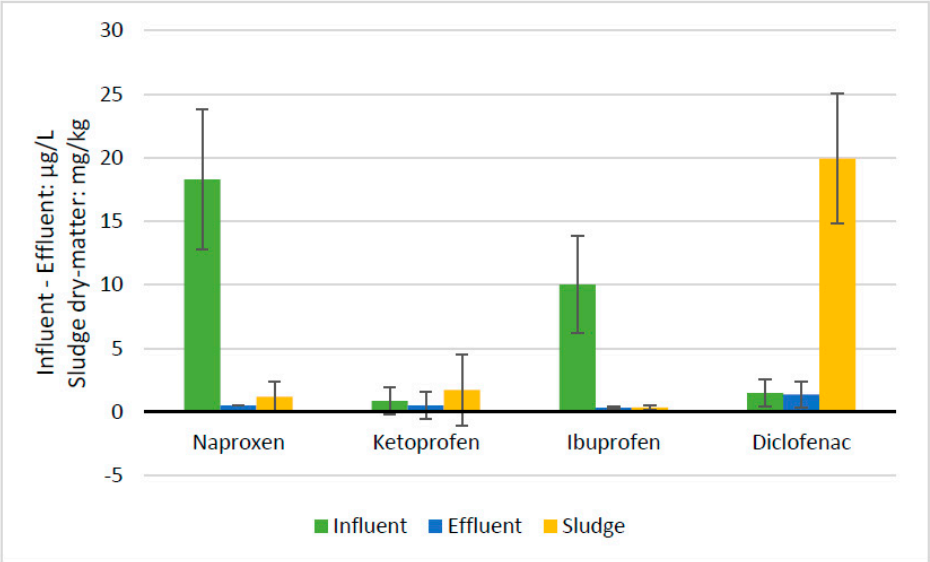

Figure 3. Average NSAID concentrations with standard deviation bars in pilot plant, where the target agents in the sludge content are expressed: mg/kg of the dry matter, in the influent and effluent: µg/L.

3.3. Field Experiments

The removal percentages of selected NSAIDs in the W1, W2 and W3 treatment plants were evaluated. The control plant W1 received no inoculations. From this plant, altogether 14 samples were taken and analyzed in 11 weeks. In the effluent water, we could barely detect these pharmaceutical agents, thus yielding above 75% elimination of naproxen, ketoprofen and ibuprofen. Diclofenac, which is considered to be the most difficult compound to degrade in sewage, mostly remained in its original concentration in the effluent. In some cases, there were even higher concentrations than in the influent, thus resulting in negative values. Confirming this, in the case of diclofenac, the control plant W1 achieved $-45.4 \pm 74\%$ (SD) removal efficiency (Figure 4). While it seems very unnatural, the high range of fluctuation can be explained with the comparably lower concentration of the compound; hence, small concentration deviations yield major percentages. The negative tendency was theoretically suspected because of the desorption process. Without the proper bacterial community, the limited initial elimination could be addressed to weak adsorption as a consequence of short retention time. Between the sampling intervals, unmeasured quantities could enter and linger, then later re-pollute the WWTP due to the weak chemical bonds, leading to an unintentional vicious circle of the target agent.

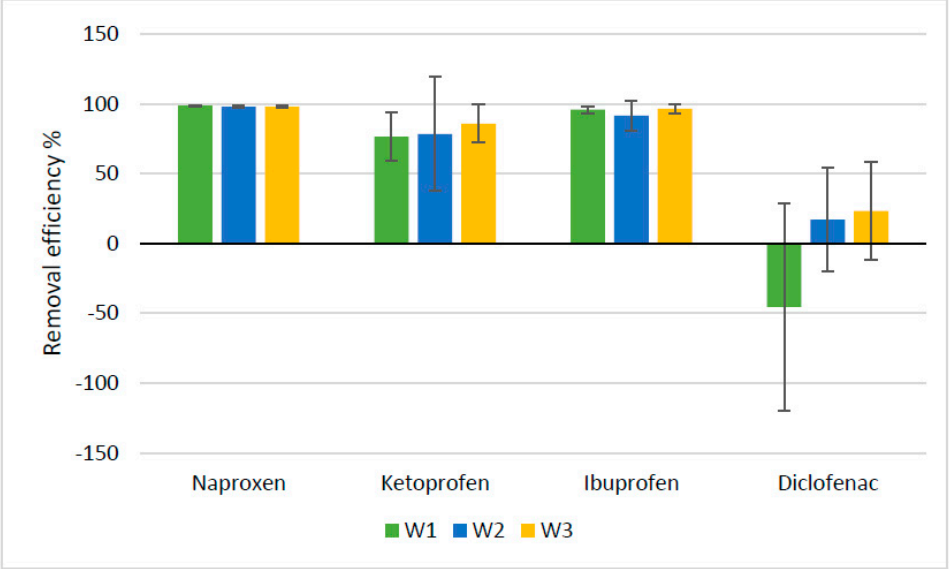

Figure 4. Removal efficiencies and their standard deviation bars (%) in the control and inoculated WWTPs with the preselected effective mixed bacteria culture. W1 = Ibrány as control, W2 = Levelek, W3 = Újfehértó.

Treatment plant W2 was tested for 11 samples over 11 weeks, and was inoculated twice. First, 1000 L was added, containing a 1:1:1:1 ratio of selected mixed bacteria cultures. After 3 weeks, the second inoculation was executed with 2000 L, of which 1000 L contained purely diclofenac-specific mixed bacteria culture, and 1000 L in a 1:1:1 ratio of naproxen-, ibuprofen- and ketoprofen-specific mixed bacteria culture. The analytical results from the influent sewage showed a wide range of pharmaceutical concentrations (naproxen 15–64 µg/L, ketoprofen 0.8–2.3 µg/L, ibuprofen 5–16 µg/L). Again, the removal efficiencies were calculated from the paired influent and effluent measurements according to the aforementioned equation. We calculated the removal efficiencies after microbiological treatment, resulting in 98.1 ± 1%, 78.4 ± 40% and 91.6 ± 11% with (SD), respectively. Regarding diclofenac, the concentration of influents and effluents varied highly. The influent concentrations were between 1.8 and 15 µg/L. Because of this, it was possible to calculate over 80% efficiencies. This implied impressive promises of enhancement. However, in the long term, calculated results declined to an average of 17.1 ± 37 (SD)%.

In the case of W3, the plant was also inoculated twice during the experiment, but altogether 15 samplings were performed throughout 11 weeks. The first dosage consisted of 2000 L of diclofenac-specific bacteria, and a 2000 L mix of ketoprofen-, naproxen- and ibuprofen-specific bacteria. Later, the plant was inoculated again with 2000 L diclofenac-specific bacteria culture. The influent sewage contained pharmaceutical residues in the following ranges: naproxen 17–82 µg/L, ketoprofen 0.5–18.1 µg/L, ibuprofen 10–24 µg/L. Meanwhile, in the case of W3, pre-settled influent sewage characteristics were obtained with the following results: NH_4-N 79 ± 14 mg/L, COD 483 ± 51 mg/L, pH 6.83 ± 0.19 with (SD), respectively; temperature gradually decreased from 22 °C to 13 °C in the period. The calculated efficiencies peaked high (naproxen 98.0 ± 1%, ketoprofen 85.9 ± 13%, ibuprofen 96.6 ± 3.3% with SD, respectively), correlating with the results of W2. Diclofenac residues in the influent sewage varied greatly between 1.7 and 13.6 µg/L. Removal efficiency reached up to 80% again in certain cases. Unfortunately, despite the second inoculation, which was supposed to further enhance biodegradation, the summarized result averaged at 23.3 ± 35% (SD) in the end.

Comparing the removal efficiencies of W2 and W3 with W1 using Student *t*-test, we found the following tendencies at *p*-level of 0.05. Naproxen and ibuprofen retention were unaffected by the inoculation. Most interestingly, diclofenac in both inoculated plants showed higher average removal efficiencies: W2 = +17.1 ± 37% (SD), W3 = +23.3 ± 35%

(SD), compared to the $-45.4 \pm 74\%$ (SD) of control plant W1. Statistically, both of them were significant: W2 $p = 2.3 \times 10^{-4}$ (n = 11), W3 $p = 5.6 \times 10^{-6}$ (n = 14). Ketoprofen elimination was successfully enhanced at the plant of W3 compared to the control plant, ($85.9 \pm 13\%$ (SD) vs. $76.6 \pm 17\%$ (SD)), yielding significant treatment with $p = 0.0384$ (n = 12).

4. Discussion

The complete palette of NSAID elimination remains unsolved, despite the invested efforts to attain a viable technology. It is, however, clear that the last effluent point of municipal wastewater treatments should be somehow involved. Microbial affinity is a key element to halt target compound concentrations in the environment. The vast complex living entities in the reactors grant a multi-cultural space with the unique ability to incorporate a wide range of surplus bacteria and fungi. It became clear that bacteria alone could not eliminate modern molecules [24]. Previous studies have summarized that naproxen and ibuprofen could be eliminated altogether from effluent water. Ketoprofen showed some affinity to be further degraded toward biphenyls, similarly to diclofenac, confirmed with HPLC and LC-MS measurements [25]. Finding the adequate bioreactor composition is pivotal in the complete degradation of ketoprofen. In contrast, diclofenac is way too persistent for standard activated sludge technologies. In the sediment region of surface waters, smaller concentrations are rapidly degraded, while in larger quantities it becomes toxic. Mixed bacteria cultures have already proved to be a potential option to increase the elimination of pharmaceuticals and personal care products [9,25]. Isolating and connecting new microbial chain-transformation to the already known structures could be evidently beneficial.

The pilot plant design was copied from the local 120K PE large WWTP of Nyíregyháza City, with the base goal to mimic its reactor ratios and treatment steps. The constant sewage feed derived from the already mechanically treated sewage of the parent WWTP; hence, the reflux of the overshadowing sludge digester's decant could certainly deliver surplus micropollutants. Regardless, all the target agents except diclofenac were successfully eliminated in the pilot plant. A simple anoxic/oxic reactor with an almost 1:1 ratio could instigate elimination, since important biological oxygen demand (BOD_5) concentration was available because of the lack of an anaerobic selector. High concentrations of readily available carbon sources are known to be important to eliminate diclofenac [26]. The microbial treatment appeared to be somewhat effective; thus, lesser concentrations could pollute the recipient water bodies. However, according to this, complete retention was impossible.

Meanwhile, target compounds may transfer to the dry sludge content. Along with the elimination tendencies, the removal pathway is observed to be potentially irreversible, except for diclofenac. While receiving foreign decant fluxes from the parent WWTP's anaerobic digesters, which are generally known to store all four target NSAID agents [27], especially diclofenac could be very recalcitrant. Technically, the dewatering decant flow connected after primary settling, but before the attached pilot plant. Also, it is important to note that primary settling is only moderately effective as an elimination process for pharmaceuticals [28]. The pilot plant excess sludge reluctantly depleted all the source compounds from the polluted influent except diclofenac. The generally calculated approximate HRT could not get any higher than 14 h in continuous feed, and according to previous studies that is a crucial parameter. Alternatively, it was only enough to slightly increase its properties to enhance retention via adsorption successfully. However, the gradual enzymatic process was missing from the bacteria community in which the diclofenac compound could be degraded into metabolites completely [23]; consequently, it continuously appeared in the sludge.

Wastewater treatment plants in certain scenarios, even without specific microorganisms, are capable of effectively removing ibuprofen, naproxen, ketoprofen and diclofenac to some extent [29]. Evidently, we found in the control plant the same results: 96.0%, 98.9%, 76.6% and −45.4, respectively. These numbers fit into previous reports suggesting the re-

moval efficiency of ibuprofen, naproxen, ketoprofen and diclofenac varying between 60 and 100%, 40 and 98%, 51 and 100% and −2 and 60%, respectively [30]. The observed retention of diclofenac sheds light on how vulnerable the treatment process is. While, in two samples, subtle removal occurred, it was outweighed by the overall negative tendencies. Excess sludge removal, pre-thickening and dewatering at the very end of the process can reverse the progress. Since no clear major degradation occurs, only weak adsorption, diclofenac could certainly accumulate in the sludge. Also, via the sludge decant water it could return to the beginning of the entire treatment at an elevated concentration due to desorption. The control plant could not effectively eliminate the complex molecule structure.

Microbial inoculation in W2 and W3 resulted in significant diclofenac removal compared to the control plant. Presumably, due to the enhanced microbial activity more steps of transformation occur, enabling a stronger connection to the sludge flocks. Observing the inoculation quantity as a function of the influent quantity, it is important to state that W2 and W3 received higher ratios of bacteria regarding naproxen, ketoprofen and ibuprofen = 1.77 L/m^3 and 0.92 L/m^3, respectively, versus the pilot plant of 0.66 L/m^3. Presumably knowing the difficulties of diclofenac removal, this ratio was 3.80 L/m^3 and 5.52 L/m^3 for W2, W3 and 8 L/m^3 for the pilot plant. Still, a higher ratio did not imply better results. However, possible HRT was more than double in both inoculated plants compared to the control and pilot plant because all the plants were operated with the same principles: 1:1 ratio of influent and sludge feed, 1:2 of internal denitrification ratio. The available reactor-size/daily-influent-quantity ratio allow longer interaction with the microbial community. This coincides with the reported diclofenac retention and HRT correlation [31]. Thus, higher HRT is definitely encouraged in order to visualize the elimination effectiveness of the mixed bacteria culture. Interestingly, none of the plants were equipped with anaerobic tanks similar to the pilot plant, which are reported to effectively enhance biotransformation despite the higher carbon demands. That step could reasonably open up new microbial elimination steps [32] along with the technically increased sludge retention time.

Obtaining favorable results even with diclofenac is promising for micropollutant treatment in sewage utilizing microbial enhancement. The effluents contaminated the recipient water bodies to a lesser extent. These results showed us a possible sustainable solution for elimination and some hope for meeting the increasing demand for irrigation with effluent water in the agriculture sector. Selecting the suitable sludge-composting processes should be carefully addressed in affected areas to decrease target NSAID agent exposure [33].

5. Conclusions

Combining different sources of bacteria from environmental matrices resulted in certain mixed cultures that were capable of reducing the concentration of selected pharmaceutical agents under laboratory conditions verified by analytical measurements. Pilot plant experiments showed promising elimination results. In the field test, WWTPs, except for diclofenac, could yield above 75% removal efficiencies for ketoprofen, naproxen and ibuprofen. Selected mixed bacteria cultures decreased the concentration of diclofenac in both pilot and field experiments significantly, while HRT suspected to remain an important parameter. Significant removal differences were observed between the control and one of the inoculated plants for ketoprofen. Supplying the proper mixed bacteria culture achieved favorable results in the removal of target NSAID compounds. However, diclofenac accumulated in the excess sludge; hence, the right combination was still missing. Consequently, a sustainable, all-round biological elimination for selected agents remained unanswered.

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