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Effect of Diclofenac Concentration on Activated Sludge Conditions in a Biological Wastewater Treatment Plant

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Abstract: Significant quantities of pharmaceutical substances enter biological wastewater treatment plants, where they interact with activated sludge microorganisms. An example of a pharmaceutical commonly used is the non-steroidal anti-inflammatory drug diclofenac (DCF). The presence of high concentrations of DCF in wastewater can disrupt nutrient removal processes, which are highly sensitive to external environmental factors. This paper discusses the effect of high DCF concentrations (1.04 mg/dm^3–12.5 mg/dm^3; 0.25 mg/gTS–3.0 mg/gTS) on the efficiency of nitrifying, denitrifying and phosphate-accumulating organisms in the wastewater treatment cycle. The condition of the activated sludge was assessed on the basis of the oxygen and nitrogen uptake rates values and the ability to biologically remove phosphorus compounds from the wastewater. The effect of DCF on the ability of methane-forming bacteria to produce biogas in the anaerobic digester was also investigated. None of the biochemical reactions of activated sludge were inhibited at applied DCF concentrations. A 33% reduction in biogas production was observed at a DCF dose of 0.0391 mg/gTS. Slight deviations from the typical course of biochemical transformation of ammonium compounds were recorded at a DCF concentration of 3 mg/gTS of sludge. However, in the concentration range studied, no negative effect of DCF, on the operation of the activated sludge, was found.

Keywords: pharmaceuticals; diclofenac; wastewater treatment; activated sludge; nutrient removing

Citation: Dereszewska, A.; Cytawa, S. Effect of Diclofenac Concentration on Activated Sludge Conditions in a Biological Wastewater Treatment Plant. *Water* **2023**, *15*, 1838. https://doi.org/10.3390/w15101838

Academic Editors: Aldona Dobrzycka-Krahel and Magdalena Bogalecka

Received: 31 March 2023
Revised: 24 April 2023
Accepted: 9 May 2023
Published: 11 May 2023

1. Introduction

The occurrence of pharmaceutical substances in the environment is a global problem. The extent of the risks associated with them and their impact on human health and biota is largely unknown. Micropollutants, including pharmaceuticals, antibiotics and hormones, can enter the aquatic environment from both diffuse and point sources [1–3]. In urbanized regions, wastewater treatment plants (WWTPs) play a key role in their dissemination [4,5]. For substances that have a particularly harmful impact on aquatic ecosystems, there are developed standards that specify maximum permissible values for pollution indicators. However, in WWTPs, there are no guidelines for the monitoring and disposal of pharmaceuticals. Currently, concentrations of pharmaceuticals in wastewater are not subject to testing at WWTPs. There are no regulated concentration thresholds in the legislation for these types of substances [6]. For this reason, the average daily concentration of drugs flowing into the sewage treatment plant is not known, nor is it known to what extent they affect the proper functioning of activated sludge.

According to published data, the main route for pharmaceuticals to enter the freshwater and marine environments is through wastewater discharges from municipal wastewater treatment plants [1–3]. It is estimated that WWTPs release about 1800 tons of pharmaceuticals into the Baltic Sea environment every year. The Baltic Sea ecosystem is more vulnerable to hazardous substances compared to other marine areas due to its low biodiversity and increased physiological stress caused by low salinity and low temperatures [7–9]. Water exchange in the Baltic Sea is slow, which means long retention times for persistent substances.

This makes the Baltic Sea ecosystem particularly sensitive to pharmaceutical pollution as well. Only a few of pollutants are removed during wastewater treatment processes with a high, over 90% efficiency, most are partially removed, or not at all [10]. Some of the drugs are removed in the process of biological degradation by activated sludge microorganisms, but many only through adsorption on its flocs [11,12]. Sewage sludge, along with adsorbed pharmaceuticals, is often managed for agricultural use, which poses a risk of spreading all of the contaminants it contains [13,14].

The monitoring of pharmaceutical substances in the environment is very limited, although surface and groundwater are monitored for selected substances under the Water Framework Directive [15,16]. The monitoring of water bodies exposed to hospital or veterinary wastewater discharges is also limited [17,18]. The synergistic effects of a mixture of various pharmaceuticals and other chemicals in the environment are also poorly recognized [19,20]. In order to improve the identification of priority substances for monitoring, particularly for new pollutants, the European Commission has established a Watch List [21]. The substances in it have been selected from those that may pose a significant risk to the aquatic environment and for which monitoring data are insufficient. The first Watch List created included diclofenac (DCF) [22].

Diclofenac (sodium 2- [2- (2,6-dichloroanilino)phenyl] acetate) is a polar pharmaceutical compound used in human and veterinary medicine to reduce inflammation and pain [23]. The structure of the DCF molecule is shown in Figure 1. It is one of the most widely sold painkillers in the world, used in both human and veterinary medicine [24–26].

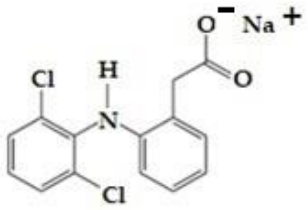

Figure 1. Molecular structure of diclofenac sodium salt.

The chlorinated benzene ring in the DCF structure significantly reduces its biological degradation [27]. The primary human hepatic metabolites of DCF are 4′-hydroxydiclofenac and 5-hydroxydiclofenac [6,28]. DCF is a weak acid and is largely dissociated at a pH suitable for both marine and freshwater environments. It is readily metabolized after oral use, but its assimilation is lower after dermal application. A relatively large part of the DCF in the effluent may come from topical application. Most of the dose of drugs dermally applied does not pass through the body and is therefore not converted [3,24,29].

DCF belongs to the group of non-steroidal anti-inflammatory drugs (NSAIDs). DCF is one of the drugs with well-documented negative effects on wildlife. The use of DCF for veterinary purposes nearly destroyed vulture populations in Southeast Asia [30,31]. In the marine environment, the presence of DCF is found in fish and otters, among others [3,32]. At high concentrations (50–100 mg/dm^3), it has a negative effect on the growth of Gram-positive and Gram-negative bacteria by inhibition of DNA synthesis [33]. Paje et al. [34] presented that biofilms composed of bacterial and algal populations lost about 70% of their total initial biomass after 4 weeks of exposure to DCF at a concentration of 100 g/dm^3. Relatively little information is available on the effects of DCF on activated sludge microorganisms. The studies by Felis et al. indicated a significant toxic effect of diclofenac on microorganisms present in activated sludge. The EC_{50} parameter determined for DCF is 6.7 mg/dm^3, which is lower than the toxic concentration for other commonly present non-steroidal anti-inflammatory drugs (NSAID) ibuprofen (EC_{50} = 12.6 mg/dm^3) and naproxen (EC_{50} = 11.1 mg/dm^3) [35]. In other studies, DCF toxicity has been observed at higher concentration values (EC_{50} = 23 ± 4 mg/dm^3), indicating significantly less toxicity of this drug [36]. The differences in the reported toxicity ranges of DCF may be due to the interaction of the drug with other pollutants flowing into the activated sludge in the wastewater.

Most of the processes currently used to treat wastewater only remove a small fraction of the micropollutants. Therefore, a significant amount of them are commonly found in treated wastewater. Thus, WWTPs play a significant role in the entry of some NSAIDs into surface waters [3,6]. Pharmaceuticals, including NSAIDs, can exert a wide range of stimulating and inhibiting effects on biochemical processes in activated sludge—including nutrient removal processes. The effects of some anti-inflammatory drugs, commonly found in the influent of WWTPs (e.g., ibuprofen, triclosan, paracetamol, diclofenac), on activated sludge are described in the literature, but the information on DCF is very selective and poor [37–39].

In the process of wastewater treatment with activated sludge, exposed to stress caused by the presence of NSAIDs, a decrease in total nitrogen removal, an increase in microbial biodiversity and an increase in extracellular polymeric substances production have also been observed [40]. Studies have also indicated that the presence of NSAIDs (including DCF) in wastewater does not significantly affect the wastewater treatment, and the accumulation of DCF in activated sludge should not negatively affect the quality of compost produced from sludge. Indeed, DCFs have been shown to be biodegradable both in the soil environment and during sludge composting. DCF is rapidly mineralized in a variety of agricultural soils (at a concentration of 0.1 g/g, it has a half-life of about 5 days) [41–43].

The average concentrations of DCF in wastewater reported in the scientific literature widely vary and range from 2 ng/dm^3 to 2500 ng/dm^3 [24]. In wastewater tested at Poland's WWTPs, the highest value that was recorded in the influent wastewater was 7.7 µg/dm^3 [44]. Although DCF is soluble in water [45], its removal efficiency in WWTPs is not satisfactory [3]. Activated sludge removes about 50% of the inflowing DCF at a concentration of 50 µg/dm^3. The efficiency drops to about 15% when the concentration of DCF has increased tenfold. Therefore, an increase in the concentration of DCF in the wastewater causes a significant reduction in removal in the WWTP. The degree of DCF reduction in WWTPs depends on the technology used. The DCF removal efficiency in conventional WWTPs has been estimated at an average of 34% and 50% in membrane bioreactors [46,47]. Unlike other anti-inflammatory drugs, DCF is not effectively removed either during the activated sludge process or in the filter bed [48,49]. In a conventional activated sludge WWTP, about half of the influent DCF load passes with the effluent into the ecosystem. An increase in the biodegradability of DCF in activated sludge is observed in the presence of readily available carbon compounds (e.g., glucose), which is influenced by co-metabolic processes [19]. The removal of DCF is also favored by a seasonal increase in temperature that promotes microbial activity. Therefore, up to a twofold increase in DCF removal efficiency is observed in the summer compared to the winter [50]. In the Baltic Sea catchment area, the average concentration of DCF in WWTPs effluent was reported to be around 2510 ng/dm^3 [5]. Higher concentrations of DCF in treated wastewater compared to raw wastewater have been repeatedly observed. This demonstrates the ability of DCF to temporarily accumulate on sludge flocs [6,51].

This paper discusses the effects of a wide range of DCF concentrations on the condition of the activated sludge of a biological WWTP. The novelty of the work is the study of the effects of very high concentrations of DCF, which is a high-environmental risk drug, on organisms responsible for the removal of nutrients in the WWTP and the protection of the marine environment. The aim of the study is: (i) to determine the effect of DCF on the bacteria responsible for the biological processes of nitrification, denitrification and dephosphatation; (ii) to assess the condition of the activated sludge, by analyzing the uptake rates of oxygen, ammonium and nitrogen, as well as the values and changes in phosphate concentration; (iii) to assess the effect of DCF on biogas production in the closed digester digestion process.

2. Materials and Methods

2.1. Materials

A commercial anti-inflammatory drug 'DicloDuo' from PharmaSwiss, containing 75 mg of the pharmaceutical per tablet, was used as a source of DCF.

Sludges: primary, activated and from digester, as well as raw sewage for the study, were directly taken from the 'Swarzewo' WWTP. The biological unit of this WWTP is an anaerobic/aerobic sequencing batch reactor (SBR) with biological nutrient removal enhanced by chemical phosphorus precipitation. The WWTP treats around 7000 m^3 of municipal wastewater per day (average values: BOD = 550 [mgO_2/dm^3]; BOD/COD = 0.45). A more detailed composition of the inflowing wastewater has been characterized in other studies [6,44]. DCF concentration in raw sewage varies from 0.6 $\mu g/dm^3$ (in summer) to 7.7 $\mu g/dm^3$ (in winter). The removal rate of DCF in WWTP averages 60%.

The activated sludge from the biological reactor was collected from the SBR reactor. The total dry matter content (TS) in the sludge was 5.85 g/dm^3.

Sludge from the fermentation chamber of the 'Swarzewo' WWTP was used to measure the effect of DCF on the methane-forming potential of sewage sludge. Total dry matter content in the sludge was 27.8 g/dm^3 (16.4 g/dm^3 VS). The chamber was fed with primary sludge with a TS of 23 g/dm^3 and VS = 19.6 g/dm^3 (85.2% of TS).

2.2. Methods

The aim of the study was to assess the effect of DCF on activated sludge under conditions as close as possible to those of the WWTPs. It is known that the use of synthetic wastewater in testing can lead to significant differences in process-scale results. Synthetic wastewater differs from raw sewage in their compositions and characteristics, especially in terms of alkalinity, BOD, COD/BOD and C/N/P ratio [52]. Therefore, the raw wastewater and sludge used in the study were directly taken from the 'Swarzewo' WWTP process line. After collection, they were kept under refrigeration so that the wastewater and sludge of the stable composition were dosed throughout the experimental series. The amount of sludge collected and the technical possibilities of storing it under refrigeration were only sufficient to perform two analyses, and only two results were obtained for individual measuring points. This discrepancy was not placed on the graph as the differences between them were small (within the range of 0.4–5%) and the graphs showed a uniform trend of change. The arithmetic mean of two measurements was plotted on each graph. In the case of long-time measurement series, the test material (background) was taken twice, which was recorded in the test results (Series 1 and Series 2).

The study of the biochemical activity of activated sludge under the influence of diclofenac was carried out in a 20 dm^3 model reactor equipped with a stirrer, an aeration system and sensors (O_2, pH, NO_3, NH_4, temperature). A schematic of the aerobic reactor used in the study is shown in Figure 2a. The reactor has conditions suitable for nitrification, denitrification and dephosphatation processes. An amount of 13 dm^3 of activated sludge, taken from the SBR chamber, was conditioned for 24 h (aeration, maintaining oxygen levels at 3 mg/dm^3). After the conditioning process, 5 dm^3 of raw wastewater was added, containing an appropriate dose of DCF tablets dissolved in water. When individual doses of the drug were introduced into the reactor with activated sludge and wastewater, DCF concentrations were in the range of 0 to 12.5 mg/dm^3 (up to 3.0 mg/gTS). The mixture was continuously stirred to ensure constant conditions in the reactor. First, a one-hour period was provided under anaerobic/anoxic conditions (to observe the activity of denitrifying and PAO). Then, aerobic conditions (with an oxygen concentration of about 3 mg/dm^3) were applied through continuous aeration. This stage allows observation of the activity of nitrifying bacteria. The entire testing process lasted 24 h, similar to the real wastewater treatment cycle at the 'Swarzewo' WWTP. Samples for analysis (OUR, PO_4 concentration) were taken before DCF addition ('background'), 5 min after addition ('start'), after the anaerobic phase ('1 h anaerobic'), after 3 h of aeration ('3 h aerobic') and after the entire 24-h cycle ('24 h cycle').

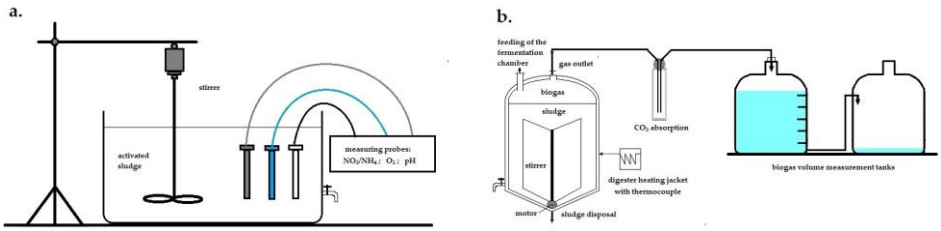

Figure 2. Diagrams of experimental models: (**a**) aerobic reactor; (**b**) fermentation.

The effect of DCF on the respiratory activity of activated sludge was determined by the oxygen uptake rate (OUR) test. The respiratory activity of activated sludge after introducing wastewater with a certain dose of the drug was compared with the respiratory activity of activated sludge after introducing wastewater, with the same composition, without the addition of the drug.

During the experiments, the concentrations of nitrogen compounds were measured every 5 min using a nitrogen probe installed in the model reactor. On that basis, the correctness of nitrogen transformations was controlled—ammonium nitrogen oxidation (nitrification process) and nitrate nitrogen reduction (denitrification process).

Methane fermentation was carried out in a 50 dm^3 model fermentation reactor filled with sludge from the digester of the 'Swarzewo' WWTP. In the studies conducted in the model fermentation chamber, the same operating conditions were maintained as in the digester used in the 'Swarzewo' WWTP process line. During the tests, the temperature was maintained at 38 ± 1 °C, and the pH was 7.3 ± 0.2. A constant stirring mode was used in the chamber. The volume of biogas produced was measured in a system consisting of a graduated (to 0.5 dm^3) tank filled with water and a reserve tank (Figure 2b). The system worked on the principle of connected vessels, and the biogas produced pushed excess water into a collection tank. To calculate the methane content, the CO_2 present in the biogas was absorbed in a scrubber filled with calcium hydroxide. Continuous measurements of the composition of biogas produced in the 'Swarzewo' WWTP showed that the presence of other gases was negligible (<0.1%) and could be disregarded.

In the first stage of the study, the parameters obtained during the fermentation of 1 dm^3 of pharmaceutical—free primary sludge(background—sample "PS") were determined. This process was carried out twice. The volume of biogas produced was measured after 1, 2, 4 and 7 days of fermentation. In both trials, gas production was negligible after 4 days, and it was assumed that the fermentation process had proceeded to completion.

Then, successive kilogram portions of primary sludge with DCF (dissolved in 50 cm^3 water) were added at 7-day intervals. With each week, the dose of the pharmaceutical was increased. Subsequent doses of DCF were respectively: 75 mg; 75 mg, 150 mg, 200 mg. During the period of the experiment, the fermentation sludge in the model chamber was not replaced (only 1 kg of sludge was discharged, balancing the addition of sludge feeding the reactor). Therefore, there was an accumulation of the drug from 0.012 to 0.073 mg/gTS of digestion sludge in the reactor.

2.3. Analytical Methods

Phosphate concentrations were measured using commercial assays from Merck (Spectroquant® 114752, 114542 and 114543, respectively). All colorimetric analyses were performed using a Spectroquant Vega 400 spectrophotometer (Merck, Darmstad, Germany).

The dry weight of samples (TS) was determined according to standard methods [53].

The pH was measured using a WTW Multi-720 pH meter.

Microorganism activity was measured using the oxygen uptake rate (OUR) measurement [54,55]. The activated sludge (alone or mixed with DCF) was aerated in a 1 dm^3 vessel to a dissolved oxygen level above 6 mg/dm^3. The OUR index was expressed as the decrease in dissolved oxygen (mg O_2) per unit time (h) per gram of activated sludge dry weight (g TS).

The nitrate uptake rate (NUR) was expressed as a decrease in nitrate nitrogen ($mg_{N\text{-}NO3}$) per unit time (h) per gram of activated sludge dry weight (g TS). The ammonia uptake rate (AUR) was expressed as a decrease in ammonium nitrogen ($mg_{N\text{-}NH4}$) per unit time (h) per gram of activated sludge dry weight (g TS) [56,57].

The activity of PAO was measured based on the measurement of phosphate concentration in the activated sludge: released in the anaerobic phase and bound in the aerobic phase.

3. Results and Discussion

3.1. Respirometric Measurements

The study of the effect of DCF on the respiratory activity of sludge was performed in two series, differing in the composition of the raw wastewater (S1 and S2) added to the activated sludge and the content of DCF in it. In the first series, three measurements were made, with the addition of: raw wastewater S1 alone, wastewater containing 75 mg DCF (1 mgDCF/gTS) and wastewater containing 150 mg DCF (2 mgDCF/gTS). In the second series, four measurements were made, with the addition of: raw wastewater S2 alone, wastewater containing 18.75 mg DCF (0.25 mgDCF/gTS), wastewater containing 37.5 mg DCF (0.5 mgDCF/gTS) and wastewater containing 225 mg DCF (3 mgDCF/gTS). The results of the oxygen uptake by the activated sludge are shown in Figure 3.

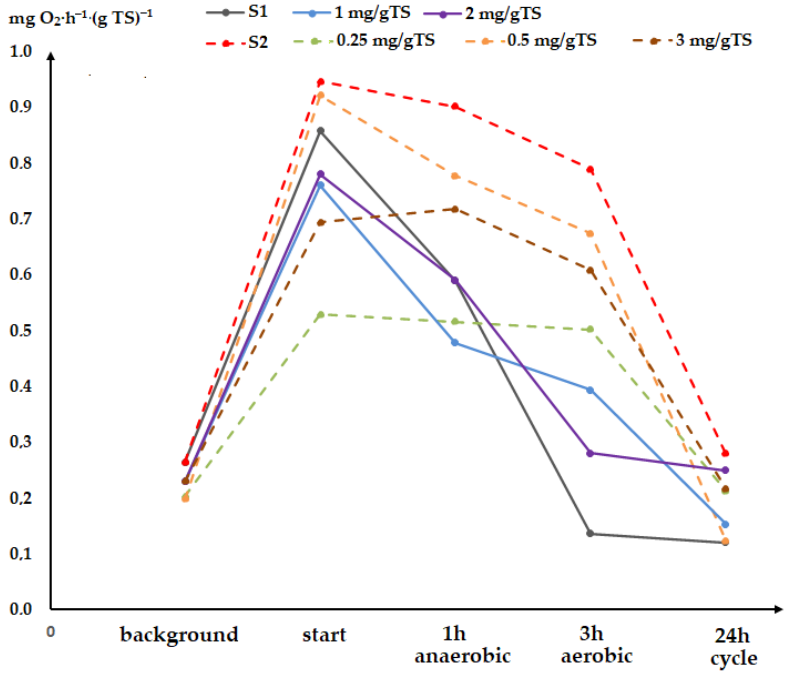

Figure 3. OUR of activated sludge in wastewater with diclofenac vs. duration of treatment.

Respirometry can be used in WWTPs as a toxicity test and used to detect substances that inhibit the purification processes. The presence of toxic substances in the wastewater results in a decrease in OUR compared to the graph characterizing respiration of the background [54]. Respirometric measurements showed that, in the range of concentrations used in the experiment, DCF does not negatively affect the metabolism of activated sludge. The initial OUR value for the activated sludge used in the series was for S1 and S2, respectively: 0.26 and 0.18 mg $O_2 \cdot h^{-1} \cdot (gTS)^{-1}$. The high nutrient content supplied in the inflowing sewage of the S1 series causes an increase in the rate of oxygen consumption by the sludge to 0.95 mg O_2 $h^{-1} \cdot (gTS)^{-1}$, and for sewage of the S2 series, an increase to 0.85 mg O_2 h^{-1} $\cdot (gTS)^{-1}$. As the wastewater treatment process progressed, the OUR values decreased (regardless of the DCF concentration), reaching at the end of the experiment a level of respiratory activity close to the background activity. Although the graphs differ in the course of changes, nothing indicates poisoning of the activated sludge. There is also no

dependence of the course of the OUR graphs on the size of the DCF dose. The occurring changes primarily depend on the composition of the wastewater used for the tests (S1, S2).

Although the respirometric test did not show a toxic effect of the DCF on the activated sludge, the environmental impact of DCF should not be underestimated. Respirometry tests for toxicity detection are very useful, since results are quickly received; however, for a more quantitative description of the toxic effect, it is preferably used in combination with EC_{50} measurements. The EC_{50} concentration represents the acute toxicity of compounds to various aquatic organisms. If EC_{50} is higher than 100 mg/dm^3, it is assumed that the tested compounds are not toxic. Values of EC_{50} for DCF range from 0.23–224 mg/dm^3 [58,59]. Some species in the marine environment are adversely affected by DCF at concentrations as low as $\leq$1 µg/dm^3 [60–63]. Among others, it exhibits chronic toxicity to phytoplankton and benthos [46]. Long-term exposure to DCF, with concentrations exceeding 5 µg/dm^3, causes kidney damage and gill lesions in rainbow trout. According to the literature, long-term exposure especially threatens juvenile fish [64,65]. It should also be noted that pharmaceuticals have significant chronic toxicity, can accumulate in organisms and their harmfulness often increases in correlation with other substances present in the environment [32,47,66,67]. The free flow of DCF through WWTPs, without toxic effects on activated sludge, and thus, undetected at the treatment plants, threatens biological life in the Baltic Sea.

Assuming: Respirometry of activated sludge in the presence of DCF and without the drug is comparable. The concentrations used were up to 1500 times higher than the values recorded in the raw sewage at the 'Swarzewo' WWTP. No symptoms of reduction in the activated sludge activity were observed even at high doses of diclofenac.

3.2. Dephosphatation, Nitrification and Denitrification

The process of biological phosphorus removal is carried out in two stages: in anaerobic conditions, phosphorus is released from the sludge into the solution (supernatant), then, in the aerobic phase, phosphorus is absorbed from the solution and accumulated in the sludge bacterial cells. Samples for the analysis of phosphate concentration in the supernatant (after filtering the activated sludge) were taken at the same time intervals as the samples for OUR measurement: before adding sewage ('background'), 5 min after adding sewage containing DCF ('start'), after the anaerobic phase ('1 h anaerobic'), after 3 h of sludge aeration ('3 h aerobic') and after the entire 24-h cycle ('24 h cycle'). Changes in the phosphate concentration in the activated sludge are shown in Figure 4:

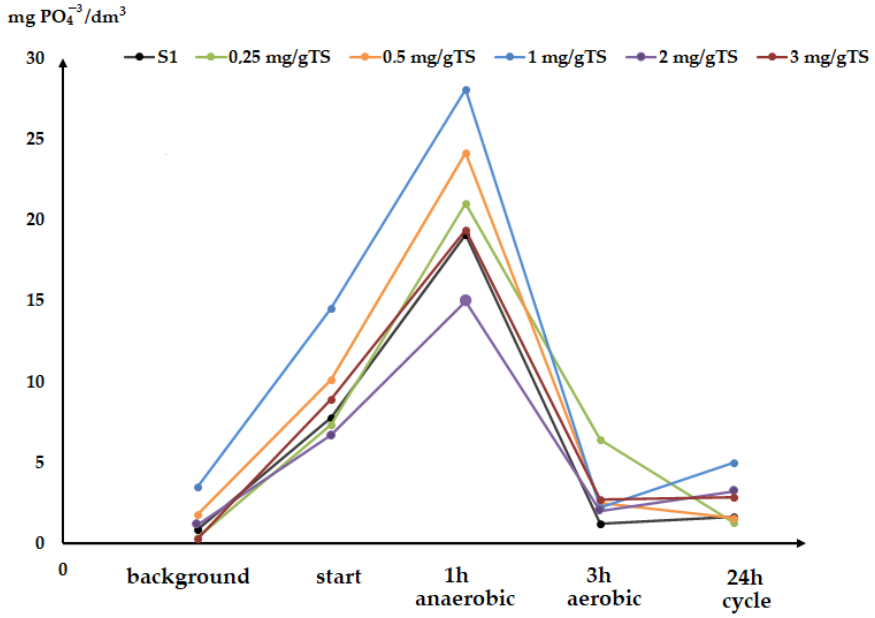

Figure 4. Changes of phosphate concentration in supernatant vs. duration of treatment.

Exposure to DCF at concentrations ranging from 0.25 mg/gTS to 3.0 mg/gTS did not result in any apparent interference with the anaerobic release and aerobic uptake of phosphorus. These processes also do not depend on the concentration of DCF. Disproportionately to DCF concentrations, the rates of phosphate release in the anaerobic phase (8.30 mgPO_4/h–14.08 mgPO_4/h) and phosphate absorption in the aerobic phase (4.32 mgPO_4/h–7.22 mgPO_4/h) of phosphates by PAO also change. The final slight increase in phosphates is typical of the wastewater mineralization process without an influx of new medium (new portion of wastewater) and is associated with the activated sludge mineralization.

Information on the effect of DCF on PAO is lacking. However, two other NSAIDs, triclosan and ibuprofen, are known to have a negative effect on this group of bacteria. Triclosan, at a concentration of 0.1 mg/dm^3, inhibits the growth of certain bacterial strains and reduces phosphorus removal efficiency in activated sludge environments. With long-term exposure to triclosan, the growth of polyphosphate accumulating organisms (PAOs) is inhibited [37]. In the study by Liu et al. [38], this inference was not confirmed. Long-term dosing of triclosan at a ten-fold higher concentration (1 mg/dm^3) did not negatively affect the processes of nutrient removal from wastewater, while the impact of ibuprofen caused a significant decrease in the microbial diversity of the activated sludge. As can be seen, researchers have obtained widely divergent results, confirming the need for a detailed study of the effects of single drugs on individual strains. Particularly as disturbed dephosphatation in wastewater treatment poses a threat to surface waters, especially to the Baltic Sea, susceptible to eutrophication processes.

During the experiments, the concentrations of nitrogen compounds were measured. Based on the indications of the nitrogen probe, graphs of changes in the concentration of ammonium nitrogen (Figure 5) and nitrate nitrogen (Figure 6) were made.

The reaction rate for the oxidation of ammonium nitrogen compounds varies from 0.0086 (sample 1 mg/TS) to 0.0223 (sample 3 mg/TS). Reaction rates do not significantly deviate from background rates, except for the 3 mg/TS sample, for which the highest rate was recorded (see AUR values in Table 1). In the remaining samples, the course of nitrification is typical for nitrifying bacteria. In all reactions, ammonium nitrogen is completely oxidized to nitrate. This confirms the proper operation of nitrifying bacteria.

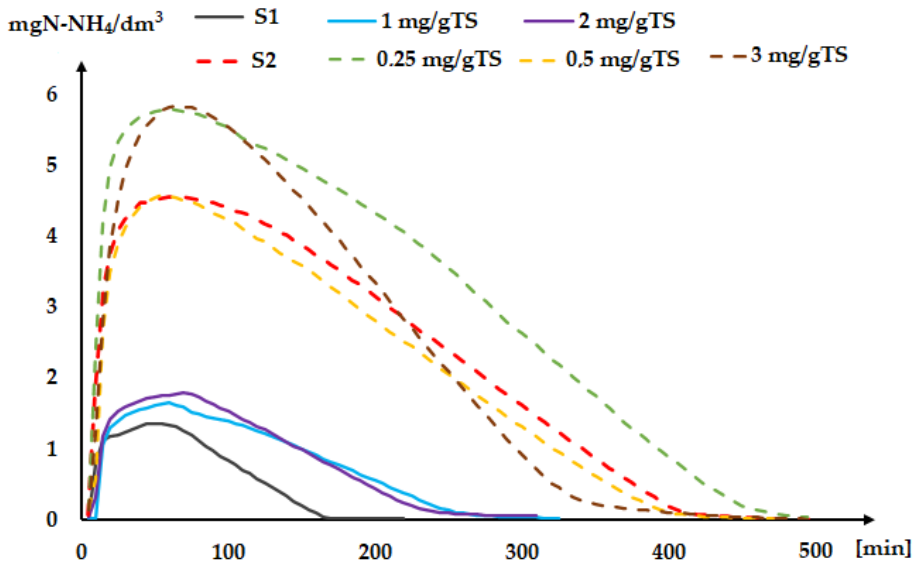

Figure 5. Changes in N-NH4 concentration in activated sludge with diclofenac vs. duration of treatment.

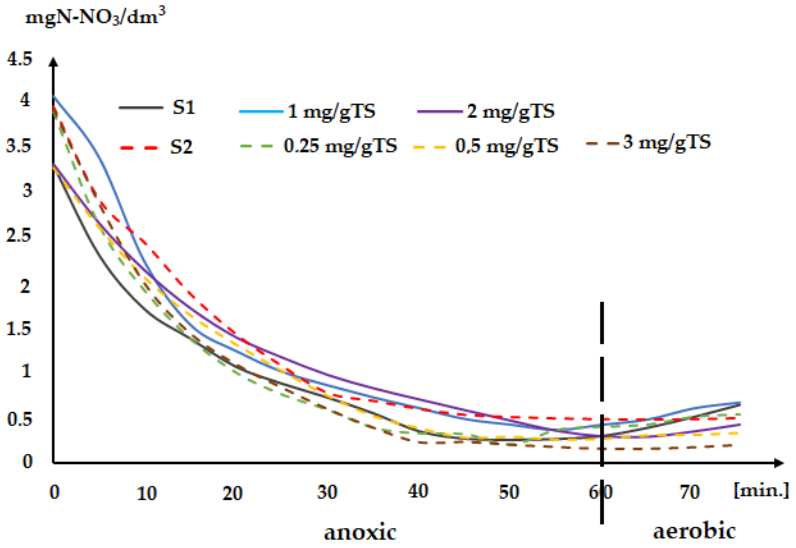

Figure 6. Changes of N-NO_3 concentration in activated sludge with diclofenac vs. duration of treatment.

Table 1. Comparison of the values of AUR and NUR.

Sample	S1	1 mg/gTS	1 mg/gTS	S2	0.25 mg/gTS	0.5 mg/gTS	3.0 mg/gTS
AUR	0.013	0.009	0.010	0.015	0.016	0.014	0.022
NUR	0.671	0.079	0.061	0.076	0.086	0.066	0.082

At this stage of the study, no detailed analysis of the kinetics of the process was carried out, so it is difficult to explain the increase in the nitrification rate at the highest dose of DCF. The oxidation of the ammonium ion is a two-step reaction (oxidation of N-NH_4 to N-NO_2 and then N-NO_2 to N-NO_3). Two different groups of bacteria are responsible for these reactions, while the sensor detects the combined result of metabolism involving both groups. Perhaps there was a dominance of one of these groups. Such a phenomenon, under the influence of ibuprofen, was observed in the study by Liu et al. [38]. Stress caused by drug dosage induces changes in the proportion of denitrifying bacteria—*Denitratisoma* i *Hyphomicrobium* strains multiply while the development of *Nitrospira* is partially inhibited. Thus, the effect of drugs from the NSAIDs group on activated sludge can also affect the inhibition of nitrification and denitrification processes in activated sludge. Such changes are caused, for example, by a dose of ibuprofen or paracetamol of 250 mg/dm^3 [38,39,67].

In our study, at the highest dose of the drug, a marked slowing of the process in the final phase of nitrification (after 320 min of the experiment) is also observed. It may hypothetically indicate a poisoning of the nitrifying bacteria of the first oxidation step (from N-NH_4 to N-NO_2). However, such a conclusion requires more detailed biochemical analyses, combined with microbiological analysis.

Figure 6 shows the process of nitrate reduction to gaseous nitrogen (denitrification) under anoxic conditions.

In all samples, the process proceeds at a similar speed. NUR values are collected in Table 1. Denitrification does not proceed to the complete removal of nitrates, it remains at a concentration of about 0.5 mg/dm^3, which may be influenced by the lack of organic carbon supply and the low concentration of N-NO_3, slowing down the reaction [40].

Comparison of phosphate changes and denitrification graphs indicates that nutrient-competing strains of phosphate and denitrifying bacteria under experimental conditions do not have an inhibitory effect on each other. According to the literature, the release of phosphates should begin after the end of the anoxic phase and the decrease in nitrate [68,69]. However, in the experiment conducted, phosphate was also released during the first hour

in which anaerobic conditions were maintained (concentration O_2 < 0.2 mg/dm^3), despite nitrate concentrations in the range of 0.5–4.5 mg/dm^3.

Assuming: The presence of DCF in the wastewater does not affect the WWTP's operation in terms of nutrient removal. Phosphorus metabolism in activated sludge, both its release and uptake processes, proceeds without interference. The only deviations from the typical courses of biochemical reactions were recorded in the second phase of nitrification of nitrate formation, with DCF concentrations of 3 mg/gTS.

3.3. Fermentation

The basic raw material used for the production of biogas in the fermentation chambers of the WWTP is primary sludge mixed with excess sludge from the biological treatment stage. The volume of biogas produced in the 'Swarzewo' WWTP by 1 Mg of dry organic matter of such a feed mixture is 184 m^3 on average and contains about 60% of methane (own research). Pharmaceuticals contained in the primary sludge and accumulating as a result of the adsorption process on excess sludge flocs may have a negative effect on methane-forming microorganisms. In order to determine the effect of DCF on the ability to produce biogas in digesters, the biogas potential (BP [m^3/MgTS]) of primary sludge was measured and the biogas potential of a mixture of primary sludge with different doses of DCF was measured. The results are summarized in Table 2. The fermentation sludge in the model chamber was not replaced during the experiment. For this reason, taking into account the poor biodegradability and good adsorption capacity of DCF, it was assumed that concentrations of supplied DCF concentrations accumulate in the fermentation sludge environment.

Table 2. Biogas potential for primary sludge without and in the presence of diclofenac.

Accumulation DCF in Sludge	Biogas Potential (BP)	Methane Content in Biogas
[mg DCF/g TS]	[m^3/Mg TS]	[%]
0	119.1	61
0.0065	149.4	60
0.0130	209.1	60
0.0262	125.7	62
0.0391	79.7	61

In the last measurement, a 33% decrease in biogas production was observed at the high cumulative concentration of DCF in the digester sludge (0.0391 mg/g TS). The concentrations of the pharmaceutical used in the study were very high, and are not found in municipal wastewater. Thus, it can be assumed that the presence of DCF in the fermented sludge is not the cause of the inhibition of biogas production.

4. Conclusions

In conclusion, the results presented show that the presence of DCFs in wastewater does not affect the operation of the WWTP in terms of nutrient removal and methane generation. Methane-producing bacteria show the greatest sensitivity to DCF. At a dose of 0.0391 mg DCF/gTS, there is a 33% reduction in biogas production. When DCF is applied at a concentration of 12.5 mg/dm^3 (3.0 mg/gTS), changes in the nitrification process occur, but detailed biochemical studies are required to explain this phenomenon. However, the observed changes do not lead to inhibition of the nitrification process. It should be noted that the concentrations at which changes were observed are 1500 times higher than the values recorded in the 'Swarzewo' WWTP influent.

The results show that if there are very high concentrations of DCFs in the wastewater, they will not be detected at the WWTP. The lack of disturbances in the treatment process and the lack of monitoring of the concentration of this drug in the effluent will not raise concerns about the composition of the raw effluent or the quality of the treated effluent. Thus, due to the low removal rate of DCF on activated sludge flocs, WWTPs may be a

source of continuous uncontrolled DCF inflow to the Baltic Sea and contamination of its waters.

Author Contributions: A.D. and S.C.—conceptualisation, methodology, formal analysis, investigation, resources, data curation, writing—original draft preparation, review and editing, visualisation, supervision. All authors have read and agreed to the published version of the manuscript.

Funding: The paper presents results developed in the scope of the research project "Monitoring and analysis of the impact of selected substances and materials in terms of environmental protection", supported by Gdynia Maritime University (project grant no. WZNJ/2023/PZ/10).

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Abbreviations

List of abbreviations used in the article:

AUR	ammonia uptake rate
BOD	biological oxygen demand
C/N/P	carbon/nitrogen/phosphorous
COD	chemical oxygen demand
DCF	diclofenac
EC_{50}	the concentration representing the acute toxicity of the compound to various aquatic organisms
OUR	oxygen uptake rate
NSAID	non-steroidal anti-inflammatory drug
NUR	nitrogen uptake rate
PAO	phosphate accumulating organisms
SBR	anaerobic/aerobic sequencing batch reactor
TS	total solids
VS	volatile solids
WWTP	wastewater treatment plant

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