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Detoxification of Pesticide-Containing Wastewater with Fe^{III}, Activated Carbon and Fenton Reagent and Its Control Using Three Standardized Bacterial Inhibition Tests

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Abstract: Discharge of toxic industrial wastewaters into biological wastewater treatment plants may result in inhibition of activated sludge bacteria (ASB). In order to find an appropriate method of detoxification, the wastewater of a pesticide-processing plant in Vietnam was treated with three different methods (Fe^{III}, powdered activated carbon (PAC), Fenton (Fe^{II}/H_2O_2)) analyzing the detoxification effect with the nitrification inhibition test (NIT), respiration inhibition test (RIT) and luminescent bacteria test (LBT). The heterotrophic ASB were much more resistant to the wastewater than the autotrophic nitrificants. The NIT turned out to be more suitable than the RIT since the NIT was less time-consuming and more reliable. In addition, the marine *Aliivibrio fischeri* were more sensitive than the nitrificants indicating that a lack of inhibition in the very practical and time-efficient LBT correlates with a lack of nitrification inhibition. With 95%, the Fenton method showed the highest efficiency regarding the chemical oxygen demand (COD) removal. Although similar COD removal (60–65%) was found for both the Fe^{III} and the PAC method, the inhibitory effect of the wastewater was reduced much more strongly with PAC. Both the NIT and the LBT showed that the PAC and Fenton methods led to a similar reduction in the inhibitory effect.

Keywords: bacterial inhibition tests; detoxification; EC_{50}; pesticides; wastewater treatment

1. Introduction

1.1. Motivation

The wide variety of industries and their complex production methods result in various types of wastewater containing toxic environmental chemicals such as plant protection agents (PPA), heavy metals, pharmaceuticals, etc. Toxic environmental chemicals are often persistent compounds with a high bioaccumulation potential [1,2]. Furthermore, chemical agents impair several vital metabolic processes of various organisms [3]. PPA, for example, contain highly bioactive compounds that are specifically used against certain organisms, i.e., pests or competitors. From the place of their application, PPA reach the surrounding environment in a wide variety of ways, in particular, soil, water and air [2]. PPA can be classified according to their mode of action, substance class and application. Insecticides (mainly halogenated hydrocarbons, pyrethroids, phosphoric acid esters and carbamates), herbicides (mainly phenoxycarboxylic acids, heterocyclic compounds, phosphonates) and fungicides (often organometallic compounds) are of the greatest importance worldwide [4].

Despite the knowledge about the risk associated with the use of PPA, their annual use has steadily increased since the 1920s [5].

If wastewater with toxic environmental chemicals is discharged directly into the environment, e.g., via the receiving water of a wastewater treatment plant (WWTP), it endangers human health and the natural environment [6]. After all, the human and ecotoxicological effects are insufficiently identified at present [6]. Industrial wastewater is usually treated together with urban wastewater in a municipal WWTP. However, the discharge of small amounts of highly toxic wastewater into a WWTP leads to a strong inhibition of, in particular, nitrifying bacteria in the activated sludge and thus to a considerable reduction of the purification capacity of the WWTP [7,8]. If the (eco)toxicologically and environmentally relevant compounds are known, reduction and avoidance strategies can be targeted at the emissions of compounds for which there is a strong need for action.

For this study, a toxic wastewater from a pesticide processing plant in an industrial zone in Can Tho, Vietnam, was investigated. This company specializes in mixing, processing and filling PPA. The PPA-polluted wastewater originating from washing and cleaning processes is subject to a strong inhibitory effect. In order to protect the WWTP biocoenosis, a pretreatment is therefore necessary. Within the scope of this work, three wastewater treatment methods (precipitation/flocculation (Fe^{III})), powdered activated carbon (PAC), Fenton (Fe^{II}/H_2O_2) were compared with each other with regard to their potential for the detoxification of wastewater containing pesticides.

In the literature, the toxicity of samples is usually described by tests based on the inhibition of light emission of *Aliivibrio fischeri* [9–11], the inhibition of ammonium oxidation or nitrate production of activated sludge bacteria [12,13], the inhibition of respiration of activated sludge bacteria [14–16] or even the inhibition of ATP luminescence of these bacteria [17]. However, most of these studies only show the inhibitory effect of individual substances added to a pure water matrix and are therefore not transferable to real wastewater. It is necessary to compare several bacterial inhibition tests with each other to find out which test is the most practical, meaningful and reliable for the type of wastewater examined. So far, such comparisons have mainly been carried out with a maximum of two tests [18–23]. Dalzell et al. [24] offer a very comprehensive comparison of five tests, but were also just using pure substances. Only few publications describe studies of the tests applied to real wastewater matrices, with mostly only one test based on the inhibition of respiration [25–27].

However, in order to be able to depict a complete picture of the reduction of the inhibitory effect by the different treatment methods mentioned above, three standardized bacterial inhibition tests, namely nitrification inhibition test (NIT), respiration inhibition test (RIT) and luminescent bacteria test (LBT) were therefore applied with regard to their applicability to pesticide-containing wastewater and thus as a possibility of a hazard assessment, e.g., for a WWTP.

1.2. Principle of Bacterial Inhibition Tests

In bacterial inhibition tests an inoculum (activated sludge or other bacteria) is exposed to different concentrations (dilution series) of a sample for a certain incubation period. The inhibition is determined via the change of a parameter, such as nitrate concentration (NO_3^-) (in the NIT), oxygen consumption (in the RIT) or light emission (in the LBT). The inhibitory effect of a test substance is demonstrated using a sigmoid dose-response curve, i.e., the inhibition is plotted against the concentration of the test substance in the associated batch. Key points are the concentrations of the substance, which cause an inhibition of x = 50%, 20% or 80% in comparison with the blind value, expressed as effective concentration (EC_x).

With respect to their nutrient requirements, i.e., their carbon source, bacteria are distinguished into heterotrophic and autotrophic microorganisms. Autotrophic microorganisms utilize inorganic carbon such as carbon dioxide (CO_2) or hydrogen carbonate (HCO_3^-), using energy from light (photoautotroph) or inorganic chemical reactions (chemoautotroph). Chemoheterotrophic microorganisms, on the other hand, utilize organically bound carbon to degrade organic compounds.

These reactions provide a high amount of energy, thus a small quantity of conversion is sufficient for rapid growth [23].

In WWTPs, the process of nitrification and denitrification is used. In nitrification, nitrificants (mainly the chemoautotrophic, aerobic microorganisms *Nitrosomonas* and *Nitrobacter*) convert ammonium (NH_4^+) to nitrite (NO_2^-) and nitrate (NO_3^-) [28]. Subsequently, nitrate is degraded to N_2 under anaerobic conditions by heterotrophic denitrifiers. Nitrification is, as compared to denitrification, the much more sensitive microbial process, which can be significantly disrupted by toxic substances such as *N*-allylthiourea [23]. Furthermore, autotrophic nitrificants are a limited group of bacteria that grow very slowly [7] because of their high sensitivity.

The marine and therefore halophilic bacteria *Aliivibrio fischeri*, used in the LBT, emit a natural light (bioluminescence) as a product of their metabolism, which can be detected photometrically. The luminescence is caused by enzymatic, energy metabolism dependent processes, the luciferin-luciferase system [29].

1.3. Wastewater Treatment Methods for Detoxification

1.3.1. Precipitation/Flocculation via Fe^{III}

Depending on the pH, iron salts hydrolyze to poorly soluble metal hydroxides. This results in a decrease of the pH value. The precipitated metal hydroxides form separable flocks providing an adsorption surface for the compounds to be eliminated. These can then be separated from the treated wastewater, e.g., by means of sedimentation. Equation (1) shows the underlying pH-dependent equilibrium reaction of ferric iron (Fe^{III}).

$$Fe^{3+} + 3H_2O \rightleftharpoons Fe(OH)_3\downarrow + 3H^+ \quad (1)$$

The lower the pH value, the more the reaction equilibrium shifts to the left-hand side, which leads to the precipitate iron hydroxide dissolving again. In the presence of Fe^{III}, also a precipitation of phosphate frequently present in wastewater occurs (Equation (2)).

$$Fe^{3+} + {PO_4}^{3-} \rightleftharpoons FePO_4\downarrow \quad (2)$$

1.3.2. Adsorption via Powdered Activated Carbon (PAC)

PAC has a very large specific surface area (>1000 m^2/g). On this surface, e.g., organohalogens or dyes can be adsorbed. When PAC is used for wastewater treatment, it is homogenized with the wastewater and then separated again. Adsorption isotherms are typically modeled using the Langmuir (Equation (3), [30]) or Freundlich equation (Equation (4), [31]). Here, q is the load (mg adsorbate/g adsorbent) and c is the concentration of the adsorptive (mg/L) after a certain contact time. K_L, q_{max}, K_F and n are constants, which, inter alia, can be calculated with the help of the least squares method or by conversion into a linearized form and applying regression, e.g., as described by Ho et al. [32].

$$q = q_{max}\frac{K_L c}{1 + K_L c} \quad (3)$$

$$q = K_F c^{1/n} \quad (4)$$

1.3.3. Fenton Method (Fe^{II}/H_2O_2)

The Fenton method exploits the fact that hydrogen peroxide reacts in the presence of Fe^{2+} ions to the far more reactive hydroxyl radical (OH•) (Equation (5), [33]), which oxidizes the compounds to be eliminated. The Fenton reaction is most efficient in the low pH range since unreactive iron hydroxides precipitate at higher pH. In a mechanism similar to the Fenton reaction (Equation (6), [33]),

Fe^{2+} can again be formed with the release of a hydroperoxyl radical ($\bullet O_2H$) from Fe^{3+}, which is thus catalytically active.

$$Fe^{2+} + H_2O_2 \rightarrow OH\bullet + OH^- + Fe^{3+} \quad (5)$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + \bullet O_2H + H^+ \quad (6)$$

As a product of the Fenton reaction, ferric iron is formed in the long term, which can precipitate as $Fe(OH)_3$ (Equation (1)). This indeed reduces the hydroxyl radical formation but can also intensify the purification effect by additional adsorption of very stable compounds. Two variants of the Fenton process can be distinguished: The wastewater to be treated is immediately neutralized after the reaction time (e.g., 60 min) and the solids are subsequently separated (variant 1, as generally applied [34]) or the solids are separated after the reaction time at acidic pH, the sample is then neutralized and subsequently a sludge separation is performed again (variant 2).

2. Materials and Methods

2.1. Experimental Concept

Three different raw wastewater samples from a pesticide processing plant in Can Tho, Vietnam, were tested for their inhibitory effect by means of three standardized bacterial inhibition tests (NIT, RIT and LBT). Thus, in total nine inhibition tests with untreated wastewater samples were conducted. One of these raw wastewater samples was treated with three different wastewater treatment methods (Fe^{III}, powdered activated carbon, Fenton (Fe^{II}/H_2O_2)) with varying dosage concentrations to find out the most effective dosage quantity of each treatment method. Subsequently, for each treatment method, a further experiment was carried out on a larger scale with the most effective dosage quantity found from the first experiments. The treated samples of the experiment at larger scale were analyzed with the three bacterial inhibition tests. Thus, in total nine inhibition tests with treated wastewater samples were conducted. The overall objective was to compare these tests and to examine their applicability to these types of samples as well as the efficiency of the treatment methods on the inhibitory effects on different microorganisms. Furthermore, the raw sample and the treated samples were examined for various parameters such as pH, chemical oxygen demand (COD), o-PO_4^{3-}-P, total P and turbidity.

2.2. Chemicals and Reagents

Bidistilled water was produced on-site by means of an ion exchanger (Seradest SD 2000, ELGA LabWater, Celle, Germany) and a downstream filter unit (Seralpur PRO 90 CN, Seral Reinstwassersysteme, Ransbach-Baumbach, Germany). Sodium bicarbonate (100%), ammonium sulfate (p.a.), *N*-allylthiourea (p.a.), peptone (>80%), meat extract, urea (p.a.), calcium chloride dihydrate (p.a.), magnesium sulfate hexahydrate (100%), magnesium chloride hexahydrate (p.a.), potassium hydrogen phosphate (p.a.), potassium chloride (p.a.), iron chloride hexahydrate (p.a.), sulfuric acid (p.a.) and sodium hydroxide (p.a.) were purchased from MERCK (Darmstadt, Germany). Sodium chloride (100%) was purchased from VWR International (Radnor, PA, USA), iron sulfate heptahydrate (100%) from Sigma Aldrich (St. Louis, MO, USA) and 30% hydrogen peroxide solution from AppliChem (Darmstadt, Germany). Powdered activated carbon Norit SAE Super was obtained from Cabot Corporation (Boston, MA, USA).

2.3. Bacterial Inhibition Tests

2.3.1. Preparation of Activated Sludge

The activated sludge was taken from the effluent of the aeration tank of a WWTP on the site of the institute in Büsnau, Germany. The pollution load of this WWTP corresponds to approximately 10,000 inhabitants. The activated sludge sample was washed to remove nitrification inhibitors or nitrate (NO_3^-) adhering thereto and influencing the following bacterial inhibition tests. At a speed

of 4000 rpm and a temperature range of 3–10 °C, the sludge was centrifuged for 10 min (Sorvall RC-5B SuperSpeed centrifuge (Thermo Fisher Scientific, Waltham, MA, USA) with cooling unit). The supernatant was then decanted off and the sludge was resuspended with an equivalent portion of nitrate-free drinking water and recentrifuged. After these two washing procedures, the sludge was resuspended again, adjusted to the reference volume, and thus the required activated sludge concentration (dry substance concentration) of approximately 3 g/L was established. Until the next day, the washed activated sludge was stirred and aerated by means of a compressed air distributor (Optimal pump, SCHEGO Schemel & Goetz, Offenbach, Germany). The dry substance (DS) content of the washed activated sludge was determined directly before the test. For this purpose, a specific volume of the sludge was filtered through a polyethersulfone membrane (0.45 µm pore size, Sartorius Stedim Biotech, Göttingen, Germany). The dry weight increase of this membrane divided by the filtered volume corresponds to the dry substance.

2.3.2. Nitrification Inhibition Test (NIT)

The NIT was carried out according to ISO 9509 [35]. Usually up to eight batches were prepared for this purpose: one blind batch, one reference batch and up to six test batches (Figure 1). 25 mL of a nutrient solution consisting of 2.65 g/L ammonium sulfate and 5.04 g/L sodium bicarbonate (buffer) and corresponding volumes of the test sample and bidistilled water (a total of 100 mL) were added to each 500 mL Erlenmeyer flask. The test sample had to be adjusted to a pH of 7.6 ± 0.1 in advance with H_2SO_4 and NaOH. In the reference batch, 2.5 mL of a 1.16 g/L *N*-allylthiourea solution (reference inhibitor) were added instead of the test sample. The blind batch did not contain any test sample. Together with 125 mL of nitrifying activated sludge (see Section 2.3.1) the final volume in each batch was 250 mL. The Erlenmeyer flasks were clamped in brackets in a water bath at a temperature of 22 °C being three-quarters below water level. With Pasteur pipettes, connected to compressed air distributors, the batches were aerated with room air and kept in diffuse light. Care was taken that the sludge was suspended. After a test period of 4 h, the aeration was switched off and each batch was filtered through a folded filter (Whatman 597 1/2 with a pore size of 4–7 µm) into a glass tube. The nitrate and nitrite contents of all batches were then determined as an indicator of the nitrification activity. The nitrification rate in the blind batch was always between 2.0 and 6.5 mg N/(g DS·h) and was thus within the specified range according to ISO. The nitrification inhibition I_N was determined according to Equation (7). Here, ρ_b, ρ_t and ρ_r are the sum of the nitrate and nitrite concentrations (in mg/L) in the blind (b), the test (t) and the reference batch (r).

$$I_N = (\rho_b - \rho_t)/(\rho_b - \rho_r) \times 100\% \quad (7)$$

2.3.3. Respiration Inhibition Test (RIT)

The RIT was performed according to ISO 8192 [36]. On the day of the measurement, a series for the determination of inhibition of total respiration (I_T) as well as a series for the determination of inhibition of heterotrophic respiration (I_H) were prepared. Up to seven batches were prepared for each series: one blind batch each and up to six test batches per sample (Figure 1). In every 1 L beaker, 16 mL of synthetic wastewater (composition see Figure 1) and corresponding volumes of the test sample and bidistilled water (both 234 mL in total) were added. To the blind batch and the test batches of the series for the determination of the I_H, also 2.32 mL of a 2.5 g/L *N*-allylthiourea solution (ATU, reference inhibitor) were pipetted. These mixtures had to be adjusted to a pH of 7.5 ± 0.5 with H_2SO_4 and NaOH. 250 mL of activated sludge (see Section 2.3.1) was added to the batches within intervals of 20 min. The final volume was thus 500 mL per batch. With the addition of the activated sludge, aeration began for 30 min (incubation). Subsequently, the test batches were successively transferred to Karlsruhe bottles on magnetic stirrers and the change in oxygen consumption, the respiration rate (mg O_2/(L·min)), was determined. In order to determine the oxygen consumption by the test sample, which cannot be attributed to microbial respiration, an abiotic control

batch was added to the series for the determination of the I_T. In this batch, bidistilled water was added instead of the activated sludge. The inhibition of total respiration (I_T), heterotrophic respiration (I_H) and autotrophic respiration (I_A) could be calculated by means of Equations (8)–(10). In this case, R_{TB} and R_{HB} are the respiratory rates in the blind batches and R_T and R_H are the respiratory rates in the test batches with (H) and without ATU addition (T).

$$I_T = ((R_{TB} - R_T)/R_{TB}) \times 100\% \quad (8)$$

$$I_H = ((R_{HB} - R_H)/R_{HB}) \times 100\% \quad (9)$$

$$I_A = (1 - (R_T - R_H)/(R_{TB} - R_{HB})) \times 100\% \quad (10)$$

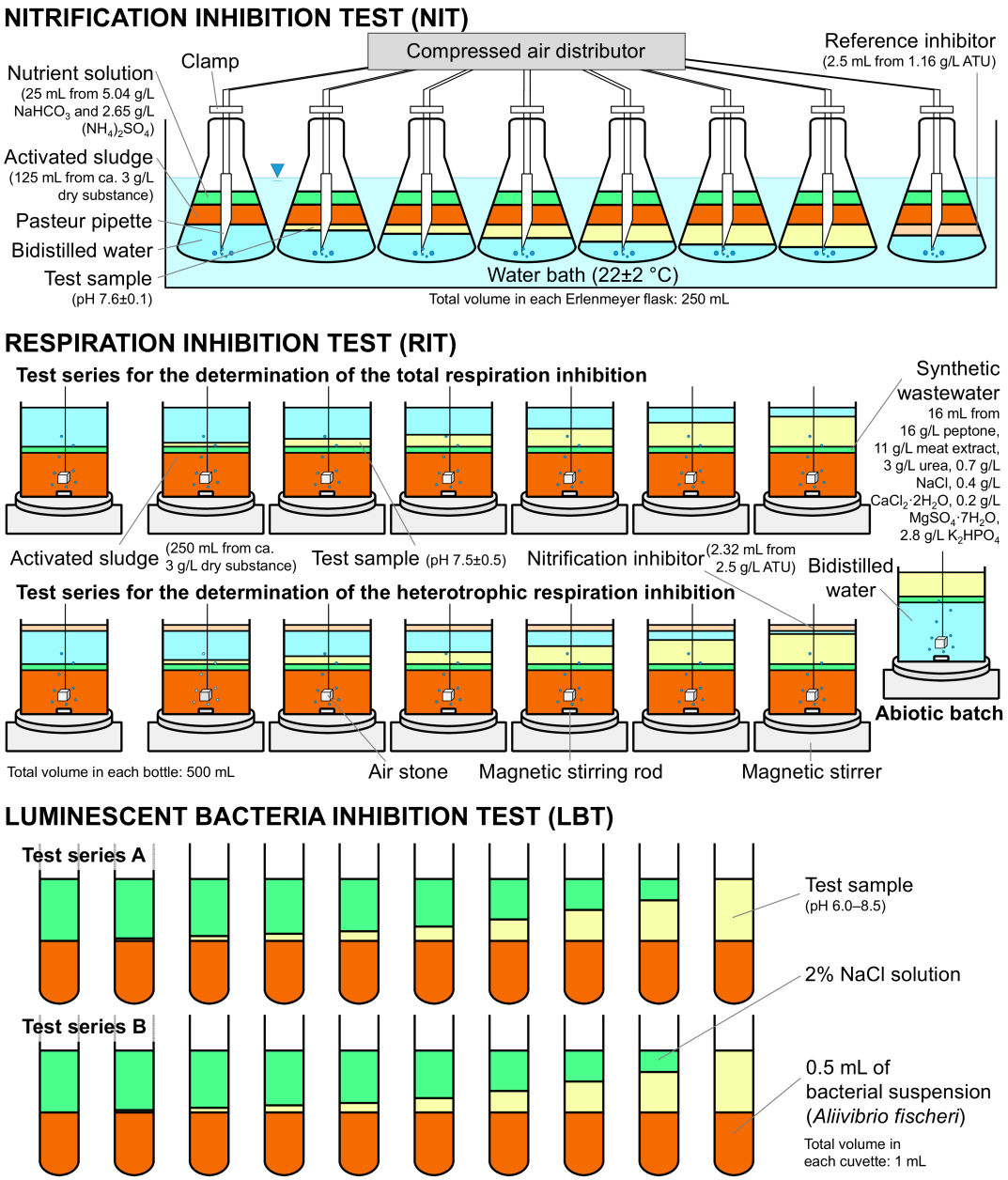

Figure 1. Construction of the bacterial inhibition tests used in this work (ATU: *N*-allylthiourea).

2.3.4. Luminescent Bacteria Test (LBT)

The LBT was carried out according to ISO 11348-3 (process with freeze-dried bacteria *Aliivibrio fischeri*) [37]. The frozen luminescent bacteria were first reconstituted with cold water and mixed with diluent (20 g/L sodium chloride, 2.035 g/L magnesium chloride hexahydrate and 0.30 g/L potassium chloride) (test suspension). The test sample had first to be adjusted to pH 6.0–8.5 with H_2SO_4 and NaOH and salified to a concentration of 20 g/L NaCl. In a thermoblock with thermostat (LUMIStherm® LANGE) tempered to 15 ± 1 °C, 2% NaCl solution was used to prepare dilutions of this test sample in two rows, each with 10 cuvettes (A and B series, each dilution in duplicate) as shown in Figure 1. Each of the two rows contained a blind batch without the test sample. For every batch, the initial luminous intensity LI_0 of the test suspension (0.5 mL) was first determined photometrically (luminometer LUMIStox® LANGE) and immediately thereafter 0.5 mL of the corresponding test sample dilution was added. After a 30 min incubation time, the luminous intensity LI_{30} was determined for each batch. In order to determine the inhibition of luminous intensity I_L, the color-corrected luminous intensity values of each batch, which were output by the luminometer, were computed according to Equation (11). In the results chapter, the numbers are shown as average values of the replicate batches with the associated standard deviation in the form of error bars.

$$I_L = (LI_{30} - LI_0)/LI_0 \times 100\% \quad (11)$$

2.3.5. Calculation of the Effective Concentration (EC)

The bacterial inhibition (I) was applied over the test sample concentration (C). The resultant curve generally resembles a sigmoid function that can be represented by the modified Weibull equation (Equation (12)) as already applied successfully in this form, e.g., by Backhaus et al. [38] and Gendig et al. [14]. The parameters m_1 (location parameter), m_2 (slope parameter), m_3 (saturation value, usually 100%) and m_4 (baseline parameter) were determined using the least squares method. The effective concentration EC_x (EC_{20}, EC_{50} or EC_{80}) at the inhibition I_x (=20, 50 or 80%) was then calculated using Equation (13).

$$I = m_3 - m_4 \cdot e^{-e^{m_1 + m_2 \cdot \log_{10}(C)}} \quad (12)$$

$$EC_x = 10^{\frac{-\ln\left[\ln\left(\frac{m_3 - I_x}{m_4}\right)\right] - m_1}{m_2}} \quad (13)$$

2.4. Analytical Methods

The photometric determination of nitrate was carried out according to ISO 7890-1 [39]. This method is based on the fact that nitrate ions react in sulfuric and phosphoric acid solution with 2,6-dimethylphenol to 4-nitro-2,6-dimethylphenol, which can be photometrically measured at a wavelength of 338 nm. The photometric determination of nitrite was carried out according to ISO 6777 [40]. This process is based on the fact that at a pH of 1.9 nitrite ions form a pink dye with 4-aminobenzosulfonamide, orthophosphoric acid and *N*-(1-naphthyl)-1,2-ethylenediamine dihydrochloride, which can be photometrically detected at a wavelength of 540 nm. A Jasco V-550 spectrophotometer (Jasco Labor Datentechnik, Groß-Umstadt, Germany) was used for these detections. The oxygen content was measured online with the USB probe WTW Multi 3430 (WTW, Weilheim, Germany). In doing so, care was taken that no gas phase surrounded the probe, so that it was surrounded tightly with the sample to be tested. The pH was measured using the Greisinger probe GMH 5550 (Greisinger, Regenstauf, Germany). The turbidity was determined with the device WTW Turb 430 IR (WTW, Weilheim, Germany). The chemical oxygen demand (LCK 614, LCK 344), o-PO_4^{3-}-P (LCK 349, LCK 348), total N_b (LCK 138, LCK 238) and total Fe (LCK 320) were determined using Hach cuvette rapid tests (Hach, Berlin, Germany). The determination of total P was carried out according to ISO 6878 (molybdenum blue method) [41]. In one raw sample, pesticides were determined using GC-MS (gas chromatograph Hewlett Packard (Palo Alto, CA, USA) 5890N Series II, Hewlett Packard

5972 Series mass selective detector, column: Varian VF-Xms, length: 30 m, diameter: 0.25 mm, film thickness: 0.25 µm). Prior to the analysis, an internal standard was added to the sample, which was liquid-liquid extracted (dichloromethane) and evaporated to 100 µL.

2.5. Experiments with Pesticide-Containing Wastewater

2.5.1. Wastewater Samples

A total of three raw wastewater samples (A, B and C) were taken from a collecting basin on the premises of a pesticide processing plant in Can Tho, Vietnam, at intervals of several months. This plant produces solid mixtures and solutions of numerous pesticides and mixes these with additives such as emulsifiers, wetting agents, softeners, defoamers or surfactants. In this plant, pesticides are not synthesized. The wastewater accrues at rinsing and mixing processes, which is why it also contains large amounts of detergents, and it is characterized by an oily surface, which indicates impurities with gasoline and oil. A sample with a COD of 9 g/L was sent to Germany (after sampling, the sample was immediately stored in an isolated container and sent to Germany by express freight, therefore, transportation took no more than 36 h) and diluted with bidistilled water by a factor of 1:10. This dilution was necessary to provide sufficient volume for an extensive experimental program (all degradation/adsorption experiments described in Sections 2.5.2–2.5.4). It was important that the sample did not lose too much inhibitory effect due to the dilution. Thus, the main goal of the experiments, the comparison of the three bacterial inhibition tests among these samples, was not disturbed by the dilution. After dilution, the sample (hereinafter referred to as sample A) had the following composition: 900 mg/L COD, pH 6.0, 71.2 mg/L total P, 5.02 mg/L o-PO_4^{3-}-P, 0.551 mg/L NO_3^--N, 0.137 mg/L NO_2^--N, 84.5 mg/L total N_b, 3.76 mg/L total Fe, 107 NTU (turbidity). From sample B (2.5 g/L COD, pH 6.5, 227 mg/L total N_b, 235 mg/L total P) and sample C (11.9 g/L COD, pH 6.1, 820 mg/L total N_b, 330 mg/L total P), only the inhibitory effect was analyzed by means of the three bacterial inhibition tests (Sections 2.3.2–2.3.4) and no treatment was applied. All samples were stored at 5 °C. Possible pesticides contained in the wastewater are mentioned and discussed in Section 3.1.

2.5.2. Experiments Regarding Precipitation/Flocculation via Fe^{III}

Eight 100 mL bottles were filled each with 50 mL of raw sample A and placed on a magnetic stirrer. Once all the batches had been mixed with the appropriate volume of Fe^{III} stock solution (4.36 g/L iron chloride hexahydrate) to obtain Fe^{III} concentrations between 0 and 180 mg/L, the batches were stirred at 200 rpm for 10 min. Meanwhile, the pH was measured. Subsequently, the samples sedimented over 15–24 h. The next day, the supernatant of all the batches was transferred into new bottles, its pH was measured, and then adjusted to pH 7 with H_2SO_4 and NaOH. Subsequently, the batches were again subjected to sedimentation of any new precipitates for 15–24 h. The COD concentration was then determined in all supernatants. For a second experiment, 10 L of raw sample A was treated according to this procedure. The dosed Fe^{III} concentration in this case was 81 mg/L. The treated sample was analyzed for various parameters such as total P, total N_b, total Fe, and for their bacterial inhibitory effect using the three inhibition tests (Sections 2.3.2–2.3.4).

2.5.3. Experiments Regarding Adsorption via PAC

Different amounts of PAC were added to nine 100 mL bottles and moistened with 500 µL of bidistilled water to facilitate subsequent homogenization with the sample. Subsequently, 50 mL of raw sample A (resulting in 0–1800 mg/L PAC) were added to all the bottles and each mixture was stirred on a magnetic stirrer at a speed of 400 rpm for 30 min. Subsequently, a portion of each batch was membrane-filtered (polyethersulfone membrane with a 0.45 µm pore size) and the COD concentration in the filtrate was determined. For a second experiment, 10 L of raw sample A was treated with

1125 mg/L PAC according to this procedure. The treated sample of this second experiment was analyzed as described in Section 2.5.2.

2.5.4. Experiments Regarding Fenton Method (Fe^{II}/H_2O_2)

Various amounts of iron sulfate heptahydrate were added to six 100 mL bottles. Next, 50 mL of raw sample A acidified to pH 2.5 by means of H_2SO_4 were added to each bottle, various volumes of hydrogen peroxide (333 g/L H_2O_2) were added and stirred on a magnetic stirrer at a speed of 400 rpm for 60 min. The concentrations dosed were 0–574 mg/L Fe^{II} and 0–1913 mg/L H_2O_2, which corresponds to 0–100% of the stoichiometrically required amount of oxidant according to COD of the raw sample [42]. In each batch, the ratio of Fe^{II} to H_2O_2 was 0.3 g/g and 0.18 mol/mol, respectively. The pH was sporadically checked during stirring. Subsequently, the batches sedimented over 15–24 h. The next day, the supernatant of all batches was transferred into new bottles, its pH was measured and adjusted to 7 with H_2SO_4 and NaOH. Thereupon, the batches sedimented again over 15–24 h. Then, the COD of each supernatant was analyzed. For a second experiment with 10 L of raw sample A, an Fe^{II} concentration of 309 mg/L and an H_2O_2 concentration of 1030 mg/L (corresponds stoichiometrically to 54%) was applied. The treated sample of this second experiment was analyzed as described in Section 2.5.2.

3. Results and Discussion

3.1. Raw Wastewater Samples

Figure 2 summarizes the inhibitory effects of the three raw wastewater samples on luminescent bacteria, autotrophic nitrificants and heterotrophic activated sludge bacteria. For all samples, with EC_{50} values between 3.9 and 17.7 mL/L, the luminescent bacteria were found to be the most sensitive. The EC_{50} values for nitrificants were between 17.3 and 480 mL/L. The heterotrophic organisms, on the other hand, proved to be very resistant, but not to the extent that they could process the raw samples as a nutrient (no significant negative values). Sample C was the sample with the highest inhibitory effect. However, it is also expected that the sample with the highest concentrations of COD, N and P will also have the highest concentrations of pesticides. The proportion of abiotic respiration as measured in the RIT was always <7% of the blind value and thus insignificant.

In addition to solvents such as xylene, methanol and cyclohexanone, the pesticide company also uses other additives. These are, for example, surface-active surfactants, defoamers, buffer substances, softeners and emulsifiers. Furthermore, cleaning agents or soap residues could also have entered the company's wastewater since it mainly consists of rinsing water. However, the majority of the mentioned substances, above all the solvents [25,43,44], are readily biodegradable. It must therefore be assumed that the inhibitory effect is mainly due to the pesticides in the wastewater. Sample B was analyzed for pesticides by GC-MS. Thus, pesticides were found even in the ppm-range (25.0 mg/L fenobucarb, 17.5 mg/L dimethoate, 3.5 mg/L butachlor, 0.7 mg/L diazinon, 0.27 mg/L isoprothiolane, 68 µg/L chlorpyrifos-ethyl, 65 µg/L ethoprophos, 55 µg/L endosulfan, 7 µg/L propanil, 5 µg/L 2,4-D) and partly above their water solubility limit. Glyphosate, the most commonly used product of the plant, is not detected by means of GC-MS, but is certainly present at very high concentrations in the wastewater (see high P concentrations). Further, warfarin, thiosultap-Na, mancozeb, bromadiolone, abamectin and copper oxychloride belong to products frequently used in the plant. The tropical heat could also have contributed to the partial degradation of the compounds in the wastewater. The resulting degradation products may be even more toxic than the actual active substance itself. For example, 1.2 mg/L of 2-sec-butylphenol (degradation and intermediate product of fenobucarb), 0.12 mg/L of 3,4-dichloroaniline (degradation product of propanil) and 0.17 mg/L of 2,4-dichlorophenol (degradation product of 2,4-D), were found in raw sample B. Both latter degradation products (0.65 mg/L EC_{50} of 3,4-dichloroaniline vs. 20.8 mg/L EC_{50} of propanil; 1.24 mg/L EC_{50}

2,4-dichlorophenol vs. 128 mg/L EC_{50} 2,4-D) have a verified stronger inhibiting effect on fluorescent bacteria than their starting materials [45].

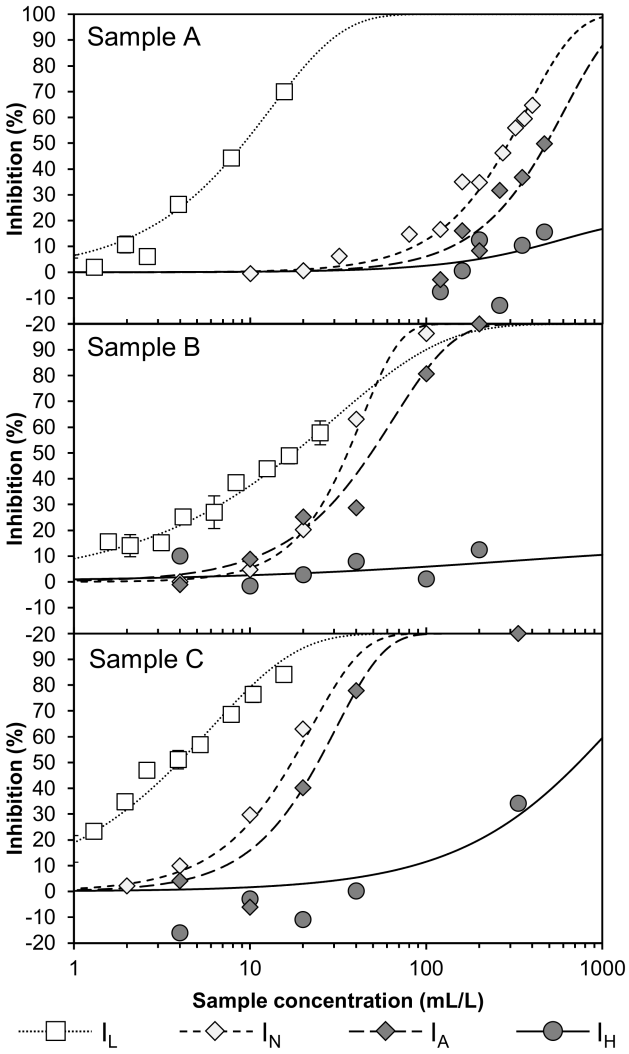

Figure 2. Bacterial inhibition (I) as a function of the sample concentration of three raw wastewater samples from a pesticide processing plant in Vietnam. I_L: Luminescent bacteria test; I_N: Nitrification inhibition test; I_A (autotrophic), I_H (heterotrophic): Respiration inhibition test. Sample A: 0.9 g/L COD, sample B: 2.5 g/L COD, sample C: 11.9 g/L COD.

3.2. Experiment Regarding Precipitation/Flocculation via Fe^{III}

Figure 3 summarizes the results from a test series in which raw sample A was treated with three different wastewater treatment methods. It becomes clear that the treatment with Fe^{III} led to a decrease in the pH value with increasing dosage concentration (Figure 3a), asymptotically approaching a value of approximately 2.5. No significant COD removal was seen up to an Fe^{III} concentration of 45 mg/L. Sludge precipitated between 60 and 120 mg/L Fe^{III}, which contributed to almost 70% COD removal. At higher Fe^{III} concentrations, however, no sludge was formed, so that no COD removal occurred. A similar progression has already been observed for other organically contaminated wastewaters with high complexing agent content [46].

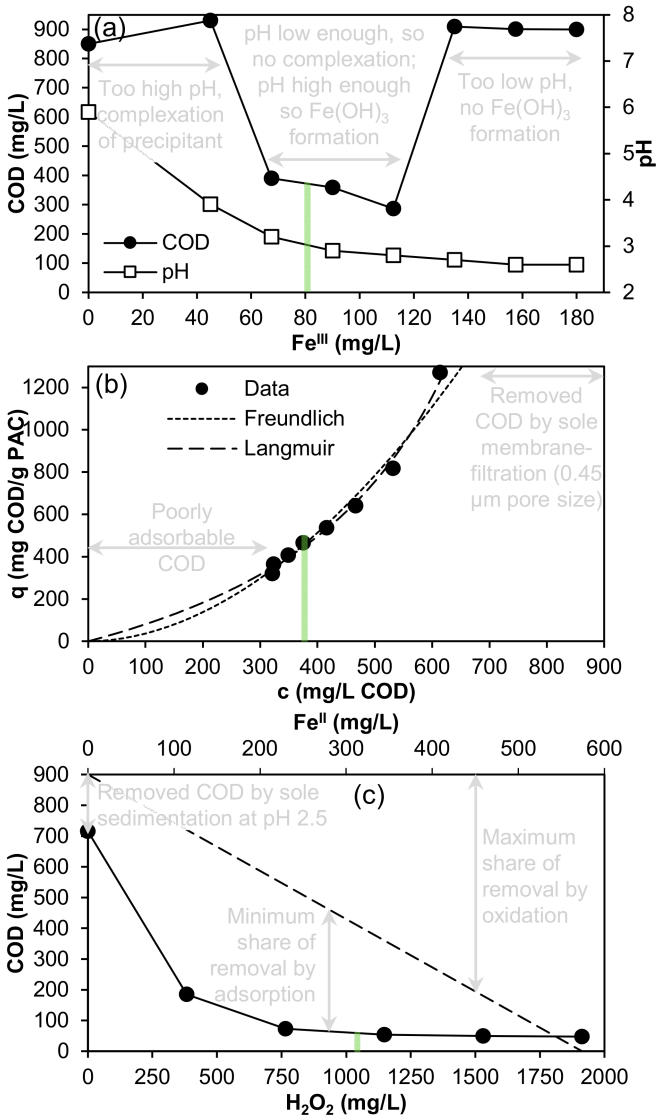

Figure 3. COD removal in the course of the treatment of raw wastewater sample A (pH 6, 0.9 g/L COD) with three different methods as a function of the reagents on a scale of 50 mL. Vertical green markings represent the dosages used for the experiment shown in Figure 4. (**a**) Treatment with $FeCl_3$ for 10 min. The COD was measured in the supernatant after neutralization. The stated pH value corresponds to that in the supernatant before neutralization, but differed from the one directly after the addition of Fe^{III} only marginally. (**b**) Treatment with PAC for 30 min. The COD was measured in the filtrate after membrane filtration (0.45 µm pore size). (**c**) Treatment with Fenton reagent at pH 2.3–2.5 for 60 min. The COD was determined in the supernatant after neutralization.

Both complex formation and precipitation of iron hydroxide are pH-dependent processes. Independent of each other, the higher the pH value, the more complexes are formed or the precipitation of iron hydroxide occurs. Furthermore, the dosage of Fe^{III} causes a pH decrease (Equation (1)). The absence of COD removal at low Fe^{III} concentrations may be attributable to the complexing of the precipitant with complexation agents present in the wastewater. Glyphosate, e.g., can undergo complexes with Fe^{3+} or $Fe(OH)_2{}^+$ at the pH values observed (>3.5) up to 45 mg/L Fe^{III} [47]. In the dosage range of 60–120 mg/L Fe^{III}, in which precipitation and COD removal were observed, the adjusted Fe^{III} concentration exceeded the complex binding capacity. Also, the pH was lower than 3.5, which reduced the probability of complexation. Thus, the precipitation of $Fe(OH)_2$ and

$Fe(OH)_3$ could occur [48]. At higher Fe^{III} concentrations, the buffering capacity of the wastewater was no longer sufficient to maintain the pH in a range adequate for the precipitation of iron hydroxide in the dosed Fe^{III} concentration range [49]. The neutralization increased the efficiency of the complexing agents and consequently no sludge formation was found in the second sedimentation phase for all batches. For the experiment on a larger scale, a dosage concentration of 81 mg/L Fe^{III} was chosen (Section 3.5). This was high enough that precipitation and thus COD removal occurred and low enough so that the sludge volume did not become too high.

3.3. Experiment Regarding Adsorption via PAC

Figure 3b shows the COD loads on PAC from the adsorption experiment at room temperature and without prior pH adjustment (pH 6.0). In addition, both the Freundlich (K_F = 0.006, n = 0.527, r^2 = 0.981) and the Langmuir isotherms (q_{max} = −695.3, K_L = 0.001, r^2 = 0.936), calculated according to Ho et al. [32] by regression analysis on the basis of the obtained data, are plotted. The 30 min contact time was chosen since a preliminary experiment had shown that a contact time of 5 min was already sufficient for the equilibrium state. It is striking that the two adsorption isotherms resemble the progression of a square function. This is because the PAC was separated by membrane filtration. This membrane filtration already contributed to a COD removal of 200 mg/L even without PAC dosing (COD in the filtrate: 700 mg/L). Thus, even with the lowest possible concentrations of PAC, a residual COD concentration of >700 mg/L could have never been achieved. This asymptotic approach to c = 700 mg/L COD is better illustrated by the Langmuir isotherm in the loading range 0–1300 mg/g than by the Freundlich isotherm, although the latter has a higher r^2. Furthermore, both adsorption isotherms cannot accurately depict whether a certain proportion of the organic components would remain in solution even at the highest PAC concentrations. For this, however, the PAC concentrations tested were also not high enough. For example, with a complex matrix as found in raw sample A, it is possible that some organic compounds such as glyphosate have such a strong polarity that an affinity to PAC is not present [50]. For the larger scale experiment (Section 3.5), a dosage concentration of 1125 mg/L PAC was chosen because higher concentrations caused too low loadings and lower concentrations caused too little COD removal.

3.4. Experiment Regarding Fenton Method (Fe^{II}/H_2O_2)

The results of the experiment treating the pesticide-containing raw sample A acidified to pH 2.5 and stirred for 60 min in the presence of $FeSO_4$ and H_2O_2, then left to sediment, neutralized and sedimented again are depicted in Figure 3c. Experiments with wastewaters high in complexing agent concentrations—and raw sample A was such a wastewater—had shown that this variant is preferable over the variant with direct neutralization after the 60-min contact time [42]. Thus, the COD was reduced with an increasing dosage quantity to an asymptotically approximated minimum value of approximately 50 mg/L. The pH decreased only slightly to a minimum value of 2.3 (not shown in Figure 3c). The distance between the initial COD concentration and the dashed line in Figure 3c illustrates the maximum proportion of COD removal that can be attributed to mere oxidation. Since the line representing the COD progression is almost entirely below the dashed line, thus, further elimination processes must have occurred in addition to the oxidation. Essentially, these are attributable to adsorption to precipitates of Fe^{III} (e.g., $Fe(OH)_3$ and $FePO_4$) occurring during the reaction by oxidation of Fe^{II} by H_2O_2. Even in the case of a lack of Fenton reagent dosage, thus in the case of mere sedimentation at pH 2.5, a COD removal of around 200 mg/L occurred. This removable concentration of solids had already been demonstrated in the experiment with PAC (membrane-filtration of the raw sample without PAC dosage led to 200 mg/L less COD in the filtrate), which illustrates that the solids contained in the wastewater sedimented well at pH 2.5, whereas they did not do this at the original pH of the sample (pH 6.0) (see Figure 3a at 0 mg/L Fe^{III}). Such facilitated sedimentation of organic components at pH 2.5 is possibly due to the fact that the functional groups of the organic compounds are predominantly protonated, thus have a lower charge and

a weaker repulsion occurs; much more, these compounds may attract and agglomerate to heavier flocks. Furthermore, complexing agents in the wastewater lose their efficiency at such low pH values, so that, e.g., cations are no longer kept in solution and can precipitate. The COD of approximately 50 mg/L, which was not removed even with stoichiometric H_2O_2 dosage, indicates organic compounds that cannot be precipitated, adsorbed or oxidized at room temperature even by OH hydroxyl radicals. In other studies, the Fenton process has already proven to be successful with regard to the degradation of pesticides in wastewater. However, such degradation is not always associated with complete mineralization. Huston and Pignatello [51], for instance, showed that despite intensive treatment of aqueously dissolved glyphosate with the Photo-Fenton method for 120 min, although no glyphosate could be found, 65% total organic carbon remained in solution and approx. 30% P were still organically bound. For the larger scale experiment (Section 3.5), concentrations of 309 mg/L Fe^{II} and 1030 mg/L H_2O_2 were chosen since higher concentrations did not result in any higher COD removal.

3.5. Experiment Regarding Fe^{III}, PAC and Fe^{II}/H_2O_2 on a Larger Scale

Table 1 summarizes various parameters before and after the treatment of raw sample A with Fe^{III} (precipitation/flocculation), PAC and Fe^{II}/H_2O_2 (Fenton reagent). All the treated samples showed a very weak turbidity and were colorless. The organics detected as COD in the treated samples were therefore in dissolved form. Phosphorus compounds (most of the phosphorus was organically bound or condensed phosphates) were significantly removed by the precipitation/flocculation method and the Fenton method, whereas with the PAC method no P-removal was achieved. This shows that the phosphorus compounds in the wastewater had to be composed predominantly of polar organophosphates, such as ethoprophos, polyphosphates from detergents or phosphonates such as glyphosate (other phosphonates are also present in detergents). Such compounds tend to adsorb onto polar iron hydroxide sludge [52]. On PAC, which surface is preferred by non-polar compounds [53], therefore, no accumulation of polar phosphorus compounds occurred. The Fenton method also showed significantly higher efficiency with respect to the COD removal (95%) compared to the other two methods. The difference between the Fe^{III} method and the PAC method with respect to the COD removal was very low (60–65%).

Table 1. Comparison of different parameters of raw sample A before and after treatment with 81 mg/L Fe^{III}, 1125 mg/L PAC and Fenton reagent (309 mg/L Fe^{II}, 1030 mg/L H_2O_2 at pH 2.3–2.5) on the scale of 10 L batches.

Parameter	Raw Sample	Fe^{III}	PAC	Fenton
Turbidity (NTU)	107	0.98	0.80	0.61
Color	greenish	colorless	colorless	colorless
pH	6.0	7.0	6.0	7.0
COD (mg/L)	903	318	354	50.3
Total P (mg/L)	71.2	19.3	70.4	<0.50
o-PO_4^{3-}-P (mg/L)	5.17	1.12	5.16	<0.50
EC_{20} (LBT) (mL/L)	3.1	16.7	179	652
EC_{50} (LBT) (mL/L)	9.2	79.5	478	886
EC_{80} (LBT) (mL/L)	20.5	253	990	>1000
EC_{20} (NIT/RIT) (mL/L)	139/228	137/260	701/399	>1000/>1000
EC_{50} (NIT/RIT) (mL/L)	293/481	380/395	900/>1000	>1000/>1000

Figure 4 shows the results from the inhibition tests applied to the raw sample and the three treated batches with regard to the inhibitory effect on luminescent bacteria, nitrificants and heterotrophic bacteria. The key EC values are summed up in Table 1. The mean effect concentrations found in the case of the NIT and RIT were in a similar range. However, the scattering of the inhibitory effects observed with the RIT was far more pronounced, which shows that this measurement method is very error-prone. Heterotrophic bacteria were not significantly inhibited by the raw sample (see Section 3.1), so that the treatment with the three methods did not cause a significant change

regarding this type of bacteria. The Fe^{III} method only caused a slight reduction in the inhibitory effect on luminescent bacteria. Nitrification-inhibiting compounds were obviously not sufficiently removed by this method. The already mentioned very similar COD removal (60–65%) by the Fe^{III} and the PAC method consequently did not correlate with the detoxification efficiency. Thus, the PAC method reduced the inhibitory effect of the pesticide-containing wastewater more significantly then the Fe^{III} method. Therefore, nitrification-inhibiting compounds were better removed by PAC, the less-polar adsorbent. The PAC and the Fenton method, therefore, proved to be very good methods for the complete detoxification of pesticide-containing wastewater with regard to nitrifying activated sludge bacteria. The very good detoxification effect of the Fenton process and the activated carbon process has already been demonstrated in several experiments with pesticides dissolved in pure water [54,55]. In particular, organochlorine compounds such as 2,4-D and propanil, which were present in the wastewater discussed here, exhibited very high loadings between 110 and 410 mg/g on activated carbon [56,57]. Thus, it can be assumed that these or similar compounds were well removed in the here-discussed experiments, too, leading to the observed good detoxification effect. In addition, the Fenton method has the advantage that both non-polar compounds can be degraded by OH radicals and rendered "harmless" and that stable polar compounds can be removed by the adsorption on Fenton sludge. Thus, for example, non-polar phosphorus compounds that are not removed by the Fe^{III} method can also be transformed into inorganic phosphate, which is then discharged as insoluble $FePO_4$ with the Fenton sludge.

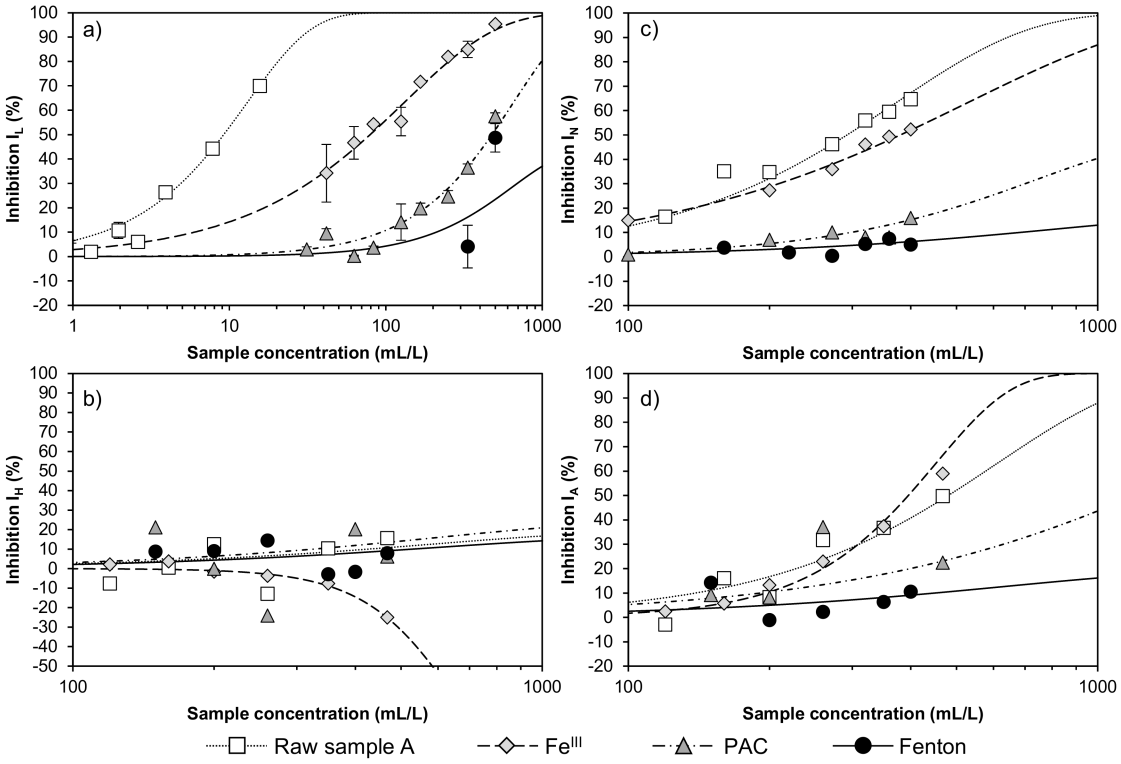

Figure 4. Inhibition of luminescent bacteria (**a**); heterotrophic activated sludge bacteria (**b**); nitrifying activated sludge bacteria (**c**,**d**) as determined by the nitrification inhibition test (**c**) and the respiration inhibition test (**b**,**d**) as a function of the sample concentration of sample A before and after treatment with precipitation/flocculation (81 mg/L Fe^{III}), 1125 mg/L PAC and Fenton reagent (309 mg/L Fe^{II} and 1030 mg/L H_2O_2 at pH 2.3–2.5) at the scale of 10 L.

In Table 2, the NIT, RIT and LBT are compared in terms of time, effort and sensitivity of the bacteria. In summary, the LBT is the least time-consuming and least labor-intensive method and uses the most

sensitive bacteria as inoculum. It must therefore always be considered whether it is absolutely necessary to carry out a test with activated sludge bacteria (especially in the case where usable activated sludge is not available, e.g., when the centralized WWTP is under construction and activated sludge is not available), since the effect concentrations determined by the practical luminescent bacteria test (an experienced technician can carry out 6–10 determinations per day) are always lower than those of the RIT and NIT. Thus, it was found that the type of wastewater investigated with an EC_{50} of around 500 mL/L on luminescent bacteria could be assumed to have no significant nitrification inhibition. However, it must always be taken into account that when using the LBT, the inhibitory effect of a test sample on the activated sludge can be overestimated. The NIT, on the other hand, can be used to predict operational disturbances at the wastewater treatment plant much more precisely.

Table 2. Comparison of three bacterial inhibition tests in terms of workload, applicability and reliability.

Parameter	NIT	RIT	LBT
ISO	9509	8192	11348
Sensitivity	High	Low–High *	Very High
Use of activated sludge	Yes	Yes	No
Nitrification inhibition	Yes	Yes	No
Heterotrophy inhibition	No	Yes	No
Reliability	Good	Little	Very good
Sample volume	Up to 1 L	Up to 1 L	mL-range
Personnel expenses	Extensive	Extensive	Low
Total working time [a,b]	5.5 h	4.25 h (2.75 h) [c]	1.5 h
Sample preparation [a,b]	0.75 h	1.25 h (1 h) [c]	0.75 h
Measurement [a,b]	4.00 h	3 h (1.75 h) [c]	0.75 h
Analysis [a]	0.75 h	-	-

* High in the case of autotrophic respiration and low in the case of heterotrophic respiration, [a] For experienced laboratory personnel, [b] Values in parentheses if only total respiratory inhibition is determined, [c] Incubation time of the sludge of 30 min.

4. Conclusions

Three standardized bacterial inhibition tests (nitrification inhibition test, respiration inhibition test, luminescent bacteria test) were compared with respect to their applicability to wastewater containing pesticides. Compared to autotrophic nitrificants, heterotrophic activated sludge bacteria were much more resistant to this type of wastewater. In order to examine the inhibitory effect of pesticide-containing wastewater on activated sludge, the NIT is therefore preferable to the RIT, as the RIT is much more labor-intensive than the NIT, fewer sensitive organisms are used as inoculum and the results are even less reliable. The marine *Aliivibrio fischeri* (luminescent bacteria), on the other hand, proved to be significantly more sensitive than the autotrophic activated sludge bacteria. This means that a lack of inhibition on luminescent bacteria is highly likely to lead to a lack of inhibition on nitrifying bacteria. Furthermore, three wastewater treatment methods were also compared in terms of their effectiveness with regard to the potential detoxification of pesticide-containing wastewater. The Fenton method (Fe^{II}/H_2O_2) showed a significantly higher efficiency in terms of COD removal compared to the precipitation/flocculation (Fe^{III}) and PAC methods. Similar COD removal by Fe^{III} and PAC did not correlate with detoxification efficiency. The PAC method reduced the inhibitory effect of the pesticide-containing wastewater much more than the precipitation/flocculation process. Both the NIT and the LBT showed that the precipitation/flocculation method had the lowest detoxification efficiency and the PAC and Fenton method led to a similar reduction in the inhibitory effect of the wastewater.

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