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## Paracetamol biodegradation by activated sludge and photocatalysis and its removal by a micelle–clay complex, activated charcoal, and reverse osmosis membranes

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### ABSTRACT

Kinetic studies on the stability of the pain killer paracetamol in Al-Quds activated sludge demonstrated that paracetamol underwent biodegradation within less than one month to furnish *p*-aminophenol in high yields. Characterizations of bacteria contained in Al-Quds sludge were accomplished. It was found that *Pseudomonas aeruginosa* is the bacterium most responsible for the biodegradation of paracetamol to *p*-aminophenol and hydroquinone. Batch adsorptions of paracetamol and its biodegradation product (*p*-aminophenol) by activated charcoal and a composite micelle (octadecyltrimethylammonium)–clay (montmorillonite) were determined at 25°C. Adsorption was adequately described by a Langmuir isotherm, and indicated better efficiency of removal by the micelle–clay complex. The ability of bench top reverse osmosis (RO) plant as well as advanced membrane pilot plant to remove paracetamol was also studied at different water matrixes to test the effect of organic matter composition. The results showed that at least 90% rejection was obtained by both plants. In addition, removal of paracetamol from RO brine was investigated by using photocatalytic processes; optimal conditions were found to be acidic or basic pH, in which paracetamol degraded in less than 5 min. Toxicity studies indicated that the effluent and brine were not toxic except for using extra low energy membrane which displayed a half maximal inhibitory concentration (IC-50) value of 80%.

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Al-Quds sludge; biodegradation; paracetamol; micelle–clay complex; removal of pharmaceuticals; activated charcoal; membrane technology; toxicity; advanced oxidation techniques

## Introduction

The use of microorganisms suspended in activated sludge is the most widely used methodology for the treatment of wastewater. In this process, aeration basin is maintained by mechanical mixers or diffused air. The load of microorganisms is preserved by a continuous return of the settled biological flocks. As organic carbon is the most important energy source, heterotrophic bacteria dominate in activated sludge systems. Several variations of the activated sludge process have been developed in order to optimize carbon, nitrogen, and phosphorous removal from the wastewater.[1] Communities of prokaryotic microorganisms present in activated-sludge are responsible for most of the carbon and nutrient removal from sewage and thus represent

the core component of the biological wastewater treatment plant (WWTP). Consequently, a thorough knowledge of the ecology of the microbial community is required to reveal factors influencing the efficiency and stability of biological WWTPs and to develop promising strategies for improved process performance.[2] Therefore, the microbiological details, including the compositions of the microbial populations that are responsible for the mineralization processes, are very important in increasing the treatment efficiency.[1]

Microbial populations in WWTPs were frequently analyzed either by light microscopic observation [3,4] or by cultivation-dependent techniques.[5,6] These approaches led to the postulation of model organisms for the most important microbiologically driven processes in WWTPs.

[7] During the past decade, a variety of approaches were also developed and used to study bacterial diversity in WWTPs.[2] Extensive studies using culturable microorganisms that require their isolation and identification were performed. Characterization of bacterial populations in samples including raw wastewater by using commercially available identification kits was previously documented.[8–10]

The occurrence and fate of residues of pharmaceuticals in wastewater and the environment have attracted an increasing interest during the last decade.[11–13] The removal of many pharmaceuticals during municipal wastewater treatment has been found to be incomplete.[13–18] When they enter a WWTP, some pharmaceuticals are either partially retained in the sludge or metabolized to a more hydrophilic but still persistent forms and, therefore, pass through the WWTP and end up in the effluent.[19] As a result, residues of these compounds have been detected in surface waters at concentrations ranging from ng/L up to mg/L.[13,14,18,20,21] The reported removals of paracetamol in WWTPs vary from almost complete to 86% in municipal and 80% in hospital WTPs.[22–27] Concentrations ranging from several hundred nanograms up to 11.3 mg/L have been found in European WWTP effluents.[13,23,28] Other researchers found that paracetamol has been found at concentrations of up to 6 mg/L in European sewage treatment plant effluents, and up to 10 µg/L in natural waters in the USA.[29,30] Studies on the Tyne River in the UK and surface water in the USA showed that paracetamol concentration exceeded 65 µg/L in the former and 10 µg/L for the latter.[29] Rabiet et al. [28] detected 211 ng/L in well supplying drinking water in France.

Recently removal of aqueous paracetamol by electrochemical,[31] ozonation, H<sub>2</sub>O<sub>2</sub>-UV oxidation, and semiconductor photo catalysis has been reported for the degradation of paracetamol.[32]

Paracetamol is available in different dosage forms such as tablets, capsules, drops, elixirs, suspensions, and suppositories. Its saturated aqueous solution has a pH of about 6 and is stable with a half-life exceeding 20 years, but its stability decreases in acidic or alkaline conditions due to being slowly degraded, via a base- or acid-catalyzed hydrolysis of the amide bond, into acetic acid and *p*-aminophenol. Paracetamol is metabolized primarily in the liver, into nontoxic products via three metabolic pathways. Glucuronidation is believed to account for 40–66% of the metabolism of paracetamol, and sulfation (sulfate conjugation) may account for 20–40%.[33] N-hydroxylation and rearrangement, then glutathione conjugation, account for less than 15%. The hepatic cytochrome P450 enzyme system metabolizes paracetamol, forming a minor yet significant alkylating metabolite

known as NAPQI (N-acetyl-*p*-benzoquinone imine). NAPQI is then irreversibly conjugated with the sulfhydryl groups of glutathione. All mentioned three pathways yield final products that are inactive, non-toxic, and eventually excreted by the kidney. In the third pathway, however, the intermediate product NAPQI is toxic. NAPQI is primarily responsible for the toxic effects of paracetamol.[34]

Microbiological degradation of paracetamol was previously reported using a membrane bioreactor (MBR), inoculated with an enriched nitrifying bacterial culture. The MBR was found to be efficient in removing paracetamol continuously; after 16 days of operation, at a hydraulic retention time (HRT) of 5 days, more than 99.9% removal was obtained when supplying a synthetic WWTP effluent with paracetamol at a concentration of 100 µg/L as a sole carbon source. Two paracetamol degrading strains were isolated from the MBR biomass and identified as *Delftia tsuruhatensis* and *P. aeruginosa*.[35]

During the past seven years, we have been engaging in studying the stability of commonly used drugs in wastewater and methods for their removal. Our study aims were at first (i) to identify the most popular drugs prescribed in Palestine and Israel and preparing a ranking list of these drugs and (ii) to test the performance of the membrane plants toward the removal of these drugs from synthetic wastewater. In the first stage of this study, we have evaluated the most popular drugs and household chemicals in Palestine and Israel by executing two different statistical methods: (a) preparing questioners together with visits and interviews of pharmaceutical companies, physicians, pharmacists, and patients and (b) conducting a search on 5000 members of Kupaat Holeem Klalit. The study results revealed that paracetamol was among the top twenty pharmaceuticals that are mostly consumed in Israel and Palestine. Therefore, we sought to study its stability in wastewater and to remove it along with its metabolites by different advanced nanotechnology membranes, activated charcoal, and a micelle–clay complex.

To ensure compliance with future discharge requirements, upgrading existing wastewater treatment facilities by enhancing the biodegradation efficiency toward pharmaceuticals is a crucial step. In this study, we used the conventional method of microbial identification using culture-dependent and biochemical testing kits to qualitatively characterize the microbial populations in the activated sludge wastewater treatment process. In addition, inoculation of the wastewater polluted with paracetamol with specific characterized bacteria was investigated. Performance of \*\*advanced integrated membrane WWTP in terms of paracetamol and its

metabolites' removal from synthetic wastewater samples was also investigated.

## Materials and methods

### Instrumentation

#### High pressure liquid chromatography

High Pressure Liquid Chromatography (HPLC-PDA) system consisted of an alliance 2695 HPLC (Waters: USA), and a waters Micromass<sup>®</sup> Masslynx<sup>™</sup> detector with Photo diode array (PDA) (Waters 2996: USA). Data acquisition and control were carried out using Empower<sup>™</sup> software (Waters, USA). Analytes were separated on a 4.6 mm × 150 mm C18 XBridge<sup>®</sup> column (5 μm particle size) used in conjunction with a 4.6 mm × 20 μm XBridge<sup>™</sup> C18 guard column. Microfilter was used with 0.45 μm (Acrodisc<sup>®</sup> GHP, Waters, USA).

Paracetamol in reverse osmosis (RO) studies was tracked by liquid chromatography multiple stage-mass spectrometry (LC-MS/MS) (Agilent 1200 HPLC Hewlett Packard system), coupled with ion spray interface to an API 3200 (Applied Biosystems) triple quadrupole mass spectrometer. LC-MS/MS analyses were essentially performed according to Vanderford et al.[36] with some custom modifications. Electrospray ionization was used for the tracking together with multiple-reaction monitoring mode. The precursor ion was 152.1 (*m/z*) and the product ions were 110.1 and 65.0 (*m/z*). Other properties of the method were a dwell time of 150 ms, a retention time of 4 min, and the following voltages (V): DP-36, CE-21/39 and CXP-4. A Kinetex C-18 100A (Phenomenex) end-capped column (3 mm × 10 cm, 2.6 μm pore size) was used with an isocratic system of 95% of 0.1% (v/v) formic acid in water and 5% of pure methanol at a flow rate of 450 μL/min. The total duration of the method was 6 min. An injection volume of 25 μL was used and standard curves yielded confidence coefficients (*R*<sup>2</sup>) greater than 0.99 within the experimental concentration range. The limit of quantification and the limit of detection under these conditions (*S/N* ≥ 3) were 0.1 μg/L.

#### UV-spectrophotometer

The concentrations of samples were determined spectrophotometrically (UV-spectrophotometer, Model: UV-1601, Shimadzu, Japan) by monitoring the absorbance at λ<sub>max</sub> for each drug.

#### pH meter

pH values were recorded on pH meter model HM-30G: TOA electronics<sup>™</sup> and on Cyberscan Electrodes (PC 300 Series) (EUTECH Instruments, waterproof series).

#### Conductivity meter

The conductivity of the samples was determined using Cyberscan Electrodes (PC 300 Series) (EUTECH Instruments, waterproof series).

#### Total organic carbon

Dissolved organic carbon (DOC), defined as the total organic carbon (TOC) fraction that passes a 0.45 μm filter, was determined on a multi-TOC-V<sub>CPH</sub> analyzer (Shimadzu) using no dispersive infrared (NDIR) detection. The quantification limit for TOC was 0.2 mg/L.

#### Centrifuge and Shaker

Labofuge<sup>®</sup>200 Centrifuge was used, 230 V 50/60 Hz. CAT. No. 284811, made in Germany. Some of the pharmaceutical solutions were shaken with an electronic shaker (Bigbill shaker, Model No.: M49120-26, 220–240 V 50/60 Hz.) at 250 rpm.

#### Chemicals and reagents

All chemicals were of analytical grade. The clay used was Wyoming Na-montmorillonite SWY-2 obtained from the Source Clays Registry (Clay Mineral Society, Colombia, MO). Quartz sand (grain size 0.8–1.2 mm) was obtained from Negev industrial minerals (Israel). Octadecyltrimethylammonium (ODTMA) bromide was obtained from Sigma Aldrich. Paracetamol was obtained from Birzeit pharmaceutical company (Ramallah-Palestine). Activated Charcoal (12–20 mesh) was obtained from Sigma (Sigma Chemical Company, USA). De-ionized water was used to prepare all solutions. Methanol, acetonitrile, tetrahydrofuran, and water were both HPLC grade and purchased from Sigma Aldrich. Magnesium sulfate, hydrogen peroxide, and *p*-aminophenol were purchased from Sigma Aldrich. High purity diethyl ether (>99%) was purchased from Biolab (Israel); orthophosphoric acid was obtained from Riedel-De Haën (Germany). Microbiological growth media (MacConkey agar, Blood Agar, Eosin Methylene Blue (EMB) Agar, and Mannitol Salt Agar (MSA)) were obtained from Sigma-Aldrich company; API-20E test Kit was obtained from Biomerieux.

#### Toxicity microbiotests

Toxicity microbiotests for selected streams of the membrane separation experiments were performed applying TOX-SCREEN (CheckLight, Israel) kit assay in order to assess any remaining acute toxicity of paracetamol before and after the membrane separation.

The test was based on light-producing luminous bacteria applying a volume sample of 150 μL using a 96-well microtiter plate following the manufacturer's protocol.

The assay was performed by running a set of eight double dilutions of the sample in question with the provided assay buffer followed by the addition of a hydrated culture of luminous bacteria. The level of light (in relative luminescence units – RLU) from the biosensors was recorded after 15 min incubation in all dilutions, as well as in the negative and positive control samples with Infinite® M200 Pro multimode reader (Tecan).

The degree of a sample's toxicity can be expressed as IC<sub>50</sub> (inhibition concentration of 50%), defined as the minimal concentration of tested sample (in %) that results in 50% change in light output, as compared to light recorded in the clean reference water under defined assay conditions. The inhibitory concentration is calculated according to Equation (1).

$$IC = \frac{RLU_{\text{control}} - RLU_{\text{sample}}}{RLU_{\text{control}}}, \quad (1)$$

where IC is the inhibitory concentration of the tested sample (%);  $RLU_{\text{control}}$  is the light level obtained in the negative control (RLU); and  $RLU_{\text{sample}}$  is the light level obtained in the sample (RLU).

### Photocatalytic experiments

Photocatalysis experiments with UVA/TiO<sub>2</sub> were performed to evaluate the potential of oxidation of paracetamol remaining in the RO brines after the separation of paracetamol. The runs were carried out at room temperature in 500 mL glass reactors containing 180 mL of water matrix spiked with 80 µg/L of paracetamol. TiO<sub>2</sub> (Anastase, P25, Degussa) at 1 g/L was added and stirred prior to irradiation to achieve thermodynamic adsorption equilibrium. The reactors were placed in a dark chamber on a magnetic stirrer to facilitate mixing and illuminated from the top with a UVA lamp (Eversun, 40 W) emitting radiation between 300 and 420 nm with a maximum at 350 nm. UVA lamps were preheated for 20 min prior to the runs. Samples of 3 mL were taken every 5 min in the first hour and once per hour for every remaining hour of the experiments. Total sampling volume per reactor was restricted to 10% of the initial volume (18 mL). Each condition was as tested in three parallel reactors to cope with this restriction. The samples were immediately supplemented with an excess of NaHCO<sub>3</sub> to facilitate TiO<sub>2</sub> removal, and kept in ice in the dark. Then the samples were centrifuged for 20 min at 4000 rpm (Heraeus, Megafuge 1.0R centrifuge) and filtered by 0.45 µm syringe filter units (Millipore) prior to the analysis. Light intensity was measured by ferrioxalate actinometry [37] and was found to be 990 µW/cm<sup>2</sup>. The UV fluence was calculated by multiplying the light intensity by the exposure time; it was found to be 3564 mJ/cm<sup>2</sup> per hour of experiment. The duration of the runs

was 1 h for pure water and 6 h for brines taken from the RO1 and RO2 stages in the pilot plant. The pure water experiments were also carried out in three ranges of pH (pH = 3.5, 7.0, and 10.0) to determine the optimal pH for the experiments.

## Methods

### Characterizing microbial community

Sludge samples were obtained from the activated sludge process treatment plant located at Al-Quds University. Sampling was performed in duplicate using clean and sterile glass bottles; the samples were transferred directly to the laboratory for immediate processing. Five milliliters of sludge were transferred from each bottle to clean and sterile test tubes (labeled 'centrifuged'), centrifuged, and then 2 mL of the supernatant was used in a culture process. Two milliliters of the sample (from both groups, original samples and centrifuged) was streaked on the top of the following culture media: Nutrient Agar which enhances the growth of all bacteria spp., MacConkey agar to enhance the growth of Gram-negative bacteria and characterize lactose fermenters from non-lactose fermenters, Blood agar as enriched, differential media to detect fastidious bacteria and its hemolytic activity, EMB to enhance Gram-negative bacteria, and MSA to enhance the growth of Staphylococci. All plates were incubated at 36°C for 24 h.

After the incubation period, the inoculated plates were inspected for bacterial growth. Growth was observed in all plates except MSA plates, which excludes *Staphylococcus* spp. in the sludge samples. Cultures of new samples on MSA were repeated to confirm the negative growth.

Morphological characterization of colonies grown on all types of growth media was done and reported; a typical colony of different types observed on the growth media was used for further identification using the API-20E test kit for the identification of enteric bacteria (bioMérieux, Inc.). The data obtained after the biochemical test were compared to the relevant catalogue to identify bacterial species in the sludge samples.

### Biodegradation of paracetamol and p-aminophenol in Al-Quds wastewater

*P. aeruginosa* was chosen to be used in paracetamol biodegradation since this bacterium was detected in Al-Quds wastewater and was reported to have amidases responsible for the degradation of amides.[35,38]

Pure colonies of *P. aeruginosa* were subcultured from the bacteria characterized in the sludge of Al-Quds WWTP and streaked on the surface of 20 sterile fresh NA plates under complete sterile technique. The NA



plates were incubated at 37°C for 24 h. The densely grown bacterial colonies were then extracted using sterile phosphate buffer having pH 7 by the aid of a sterile glass rod under a complete sterile technique and collected in sterile flasks, obtaining 100 mL of concentrated bacterial suspension of *P. aeruginosa*.

About 200 mg of paracetamol or *p*-aminophenol was transferred to 2-L brown bottle and was dissolved in 1 L of sterilized wastewater (having COD and BOD of 232 and 178 mg/L, respectively). The *P. aeruginosa* bacterial suspension harvested in sterile phosphate buffer was gradually added to the pharmaceutical–wastewater solution to reach optical densities of 0.5 in the pharmaceutical–wastewater–bacterial suspension (at 545 nm using the sterile wastewater as a blank), which corresponds to  $3.8 \times 10^6$  colony forming unit (cfu/mL) of *P. aeruginosa* according to a growth curve determined previously for *P. aeruginosa* ( $\text{Log } P. aeruginosa \text{ count (cfu/mL)} = 2.45 \times \text{optical density} + 5.35$ ) and confirmed by counting the bacterial population using the standard plate pour technique. The inoculated pharmaceutical–wastewater solution was placed in a water bath at 30°C for 14 days with continuous mixing with the aid of aeration. The bacterial count in the reactor was maintained during the period of the biodegradation experiment at an optical density between 0.5 and 0.7 (corresponding to  $3.8 \times 10^6$ – $1.2 \times 10^7$  cfu/ml), and at the end of the experiment (14 days), the *P. aeruginosa* population had a count of  $2.0 \times 10^5$  cfu/ml.

Paracetamol was extracted from the biological degradation reactor after 1, 2, 4, 7, 10, and 14 days. The extraction was performed using chloroform, and then liquid chromatography was performed to determine the concentrations of the resulting metabolites. At the end of the incubation period, the reaction mixture was extracted separately with three different solvents: chloroform, ethyl acetate, and hexane. The organic solvent was dried using anhydrous magnesium sulfate and filtered. The filtrate was evaporated to dryness. The dry residue was subjected to silica gel column chromatography for the separation of the products (metabolites).

### Sample and standards preparation for HPLC

- Stock solution*: Stock solution was prepared by dissolving paracetamol, or *p*-aminophenol standards in acetonitrile and water that was adjusted to pH 3.45 at a ratio of 10:90 to a concentration of 200 mg/L that was used in (b).
- Calibration curves*: A 500 mL stock solution of 1:1 mixture of paracetamol or *p*-aminophenol, with a final concentration of 100 mg/L, was prepared in the same manner as for (a). The diluted solutions

that were prepared from the stock solution were 10, 20, 40, 60, 80, and 100 mg/L. Then, each diluted solution was extracted three times with ether or chloroform. The ether or chloroform extracts were combined, dried on anhydrous magnesium sulfate ( $\text{MgSO}_4$ ), filtered, and evaporated. For HPLC-PDA analysis, the dried residue after solvent evaporation was dissolved with a mixture of water: acetonitrile (water adjusted to pH 3.45 using dilute *o*-phosphoric acid) at a ratio of 90:10 v/v and was injected to the HPLC apparatus.

### Chromatographic conditions for the separation of paracetamol and *p*-aminophenol

The optimal HPLC conditions that were efficient for the separation of paracetamol and *p*-aminophenol were C-18 as the separation column, a mixture of water: acetonitrile (water pH adjusted to 3.45 using dilute *o*-phosphoric acid) (90:10 v/v) as a mobile phase, flow rate of 1.0 mL/minute, and UV detection at a wavelength of 247 nm.

### Adsorption studies of paracetamol and *p*-aminophenol onto micelle–clay complex and charcoal

#### Sample and standards preparation

(a) *Stock solution*: Each stock solution of paracetamol or *p*-aminophenol standard was prepared in a separated two volumetric flask by dissolving the compound in distilled water to a concentration of 500 mg/L that was used in (b).

(b) *Calibration curves*: Each 500 mL stock solution of each compound with a final concentration of 500 mg/L prepared in (a) was used to prepare the following diluted solutions: 1, 2, 4, 6, 8, and 10 mg/L. Then, the absorption of each solution of each of the tested compounds was determined using a UV-spectrophotometer.

#### Micelle–clay complex preparation

The micelle–clay complex was prepared as described elsewhere.[39] Briefly, the micelle–clay complex was prepared by stirring 12 mM of ODTMA with 10 g/L clay for 72 h. The suspensions were centrifuged for 20 min at 15000 g, supernatants were discarded, and the complex was lyophilized.

#### Batch adsorption studies

Batch adsorption experiment was carried out for a solution of 50 mg/L of the initial concentration of paracetamol or *p*-aminophenol. The experiment was performed

in 250 mL Erlenmeyer flasks containing 0.5 g micelle–clay complex. The flask was shaken in an electric shaker for 2 h at room temperature, and then the content of the flask was centrifuged for 5 min and filtered using 0.45  $\mu\text{m}$  Millipore filters. A kinetic study of the extent of adsorption was determined by taking 50 mL solution of 50 mg/L paracetamol, or *p*-aminophenol, in a 250 mL Erlenmeyer flask containing 0.5 g of the micelle–clay complex and then measuring the absorbance of the solution versus time.

### Membrane separation experiments

**Bench-scale experiments.** The aim of this part of the study was to evaluate the rejection ability of nonporous membranes, RO of different degrees of selectivity. Trials involved different water matrixes to test the background effect of organic matter on the separation and adsorption of paracetamol. Membranes tested included a wide range of tight types. Flat sheet thin film composite membranes of increasing selectivity were used (courtesy of Filmtec, Dow Chemical Co., USA), as follows: extra low energy (XLE), brackish water (BW), and seawater. Manufacturer characteristics and tested characteristics of the membranes as well as details of the applied membrane pressures were adapted from Gur-Reznik et al. [40]. Membrane separation experiments were carried out in both a laboratory-scale cross-flow cell system and a pilot system. The laboratory-scale cross-flow cell system comprises two parallel channels of two cells in series each, holding 24 cm<sup>2</sup> filtration area-flat sheet membranes. The filtration apparatus was custom built, so all the parts of the system, except the cells that were made of polycarbonate, were made of stainless steel. The system was operated in batch mode (full recycling of permeate and concentrate to the reservoir). All experiments were performed under controlled temperature at  $21 \pm 2^\circ\text{C}$  by means of a heat exchanger located in the feeding tank and connected to a water chiller system. Pressures and flow rates were adjusted manually and recorded. Paracetamol was spiked to the system at a concentration of 120  $\mu\text{g/L}$ .

Samples for analyses were taken from both the feeding reservoir and the permeate channel of each individual cell. Experiments were run for 5 h. Reported average rejections were determined based on individual compound concentrations in the membrane feed (reservoir) and permeate measured at the end of the experimental run (Equation (2)).

$$R = \frac{C_F - C_P}{C_F} \times 100, \quad (2)$$

where  $R$  is the rejection of the compound in the solute

(%),  $C_F$  is the concentration of the compound in the feed (ppb), and  $C_P$  is the concentration of the compound in the permeate (ppb).

### Pilot plant experiments

The ability of BW-type membranes (found to be the most effective in terms of resolution and permeation rates) was further tested under field conditions in the Technion secondary effluent desalination pilot plant with a nominal capacity of 10 m<sup>3</sup>/h. The pilot plant comprises an ultrafiltration stage ( $\sim 40$  nm molecular weight cut-off – MWCO, Dow) followed by a two-stage RO system (8" and 4" BW Toray membranes, respectively). The recovery of the first-stage RO was 50% and the total recovery was about 85%. A schematic diagram of the desalination process is presented in Figure S1 (supporting information). Experiments conducted in the pilot plant lasted 6 h and paracetamol was spiked at a concentration of 150  $\mu\text{g/L}$ .

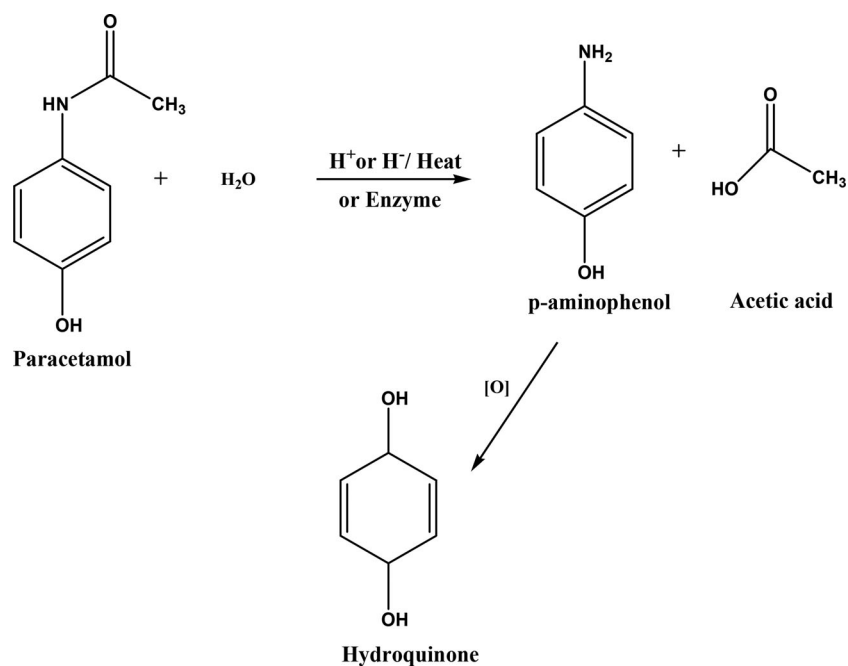
## Results and discussion

### Characterization of bacteria in Al-Quds sludge

According to the conventional culture method using selective and differential growth media followed by the morphological characterization of grown colonies and biochemical testing by using the API-20E test kit, the following bacterial species were isolated from Al-Quds University sludge samples: *Escherichia coli*, *Enterobacter sakazakii*, *Citrobacter freundii*, *P. aeruginosa*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Enterobacter amnigenus*, *Enterobacter aerogenes*, *Salmonella* spp., and *Serratia liquefaciens*.

### Biodegradation of paracetamol in Al-Quds sludge

As mentioned in the introduction section, aqueous solutions of paracetamol are quite stable at room temperature. The half-life of paracetamol solutions (pH 6) lasts over 20 years; however, upon heating, or under acidic or basic conditions its stability decreases and it degrades to furnish *p*-aminophenol and acetic acid (Figure 1). This hydrolysis reaction was found to be carried out by enzymatic cleavage or by microwave-assisted alkaline hydrolysis of amide bond. Stability studies of paracetamol in purified water at room temperature demonstrated that paracetamol was completely stable after incubation for more than one month due to the high energy needed to overcome the barrier for the cleavage of its amide bond. On the other hand, HPLC monitoring results of Al-Quds University sludge solutions containing paracetamol (at room temperature) revealed a gradual



**Figure 1.** Schematic pathway for the degradation of paracetamol in Al-Quds sludge and by *P. aeruginosa* bacteria.

degradation of paracetamol to *p*-aminophenol and acetate (Figure 2). The degradation was demonstrated by the disappearance of a peak characterized as the reactant (paracetamol) at a retention time of 3.92 min and a gradual appearance of a new peak at a retention time of 1.97 min characterized as a product (*p*-aminophenol). Figure 1 illustrates a progress in paracetamol degradation as monitored by HPLC (Figure 2(a) and 2 (b)). Characterization of the product, *p*-aminophenol, was confirmed by injecting a standard solution of *p*-aminophenol to HPLC that gave only one peak at a retention time of 1.97 min. The HPLC kinetic data obtained after one week of monitoring were examined for linear correlation (Figure 2(c)). A linear correlation was obtained when  $\ln$  [paracetamol] was plotted against time with a correlation coefficient  $R^2 = 98$ . The pseudo-first-order reaction rate constant was found to be  $8.5 \times 10^{-7} \text{ s}^{-1}$ .

The degradation of paracetamol in Al-Quds University sludge might be due to the presence of a variety of bacteria-containing enzymes having the potential to catalyze amide linkage hydrolysis or/and heavy metals which can play a role as catalyst for the breakdown of paracetamol amide linkage.

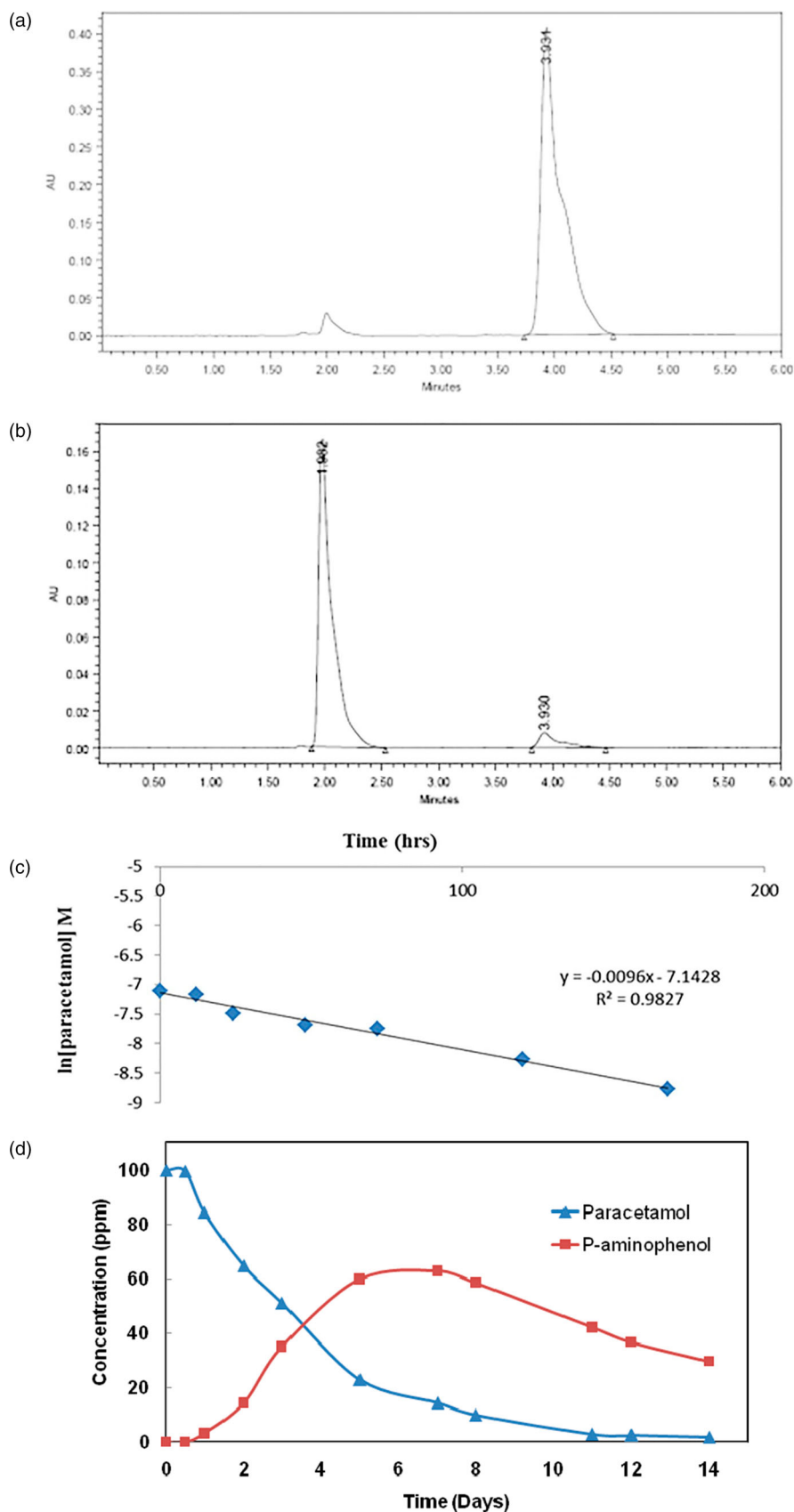
In order to shed light on the source of paracetamol degradation, the bacteria in Al-Quds University sludge were isolated and characterized. Among the bacterial species found in the sludge was *P. aeruginosa*, which was reported to have amidase enzymes that have the

capability to catalyze the breakdown of amide bonds in a variety of organic molecules.

### Biodegradation of paracetamol by *P. aeruginosa*

In the first set of experiments, paracetamol was used as a single substrate at a concentration of 10 mg/L. As deduced by HPLC determinations, paracetamol was degraded within 14 days. During paracetamol degradation, two new metabolites were detected and tentatively identified by means of LC-MS and nuclear magnetic resonance (NMR) (for the degradation pathway, see Figure 1). While the first metabolite reached its maximum around 7 days, the second one increased until the end of the experiment (14 days) (Figure 2(d)). Metabolite 1 was characterized as *p*-aminophenol and the second metabolite was identified as hydroquinone. Metabolite 2 remained stable throughout the experimental period of 14 days. Transformation of paracetamol via metabolite 1 to metabolite 2 was confirmed by running a second set of experiments by which a standard solution of *p*-aminophenol (20 mg/L) was incubated with *P. aeruginosa* in the same manner and conditions as described for paracetamol. HPLC monitoring demonstrated that *p*-aminophenol underwent degradation to hydroquinone (Figure 2(d)). A complete biodegradation was achieved after 14 days of incubation with the bacteria.





**Figure 2.** (a) Chromatogram showing the hydrolysis of paracetamol after 2 days in the presence of wastewater at 25°C. (b) Chromatogram showing the hydrolysis of paracetamol after 7 days in the presence of wastewater at 25°C. (c) First-order kinetic plot for the biodegradation of paracetamol in Al-Quds activated sludge at 25°C. (d) Biodegradation curve for paracetamol (blank points) and *p*-aminophenol (filled points) within 14 days of incubation. In activated sludge of 100 mg/L initial paracetamol concentration and  $T = 25^\circ\text{C}$ . Data represent average of triplicate measurements.

It is indicated that the identification of metabolites 1 and 2 was confirmed by injecting authentic samples of *p*-aminophenol and hydroquinone into HPLC that gave identical peaks for the two metabolites, respectively.

### Adsorption studies of paracetamol and *p*-aminophenol onto micelle–clay complex and charcoal

Our previous investigations on the removal of pharmaceuticals from water or sludge have employed a variety of membranes such as hollow fiber, RO and spiral wound, activated carbon, and micelle–clay filters. We are also presenting here the results of the removal of paracetamol and its metabolite, *p*-aminophenol, in suspension by using a micelle–clay complex in comparison with activated carbon. The micelles of the organic cation ODTMA interact with negatively charged clay (montmorillonite) at optimal ratios.[39,41,42] The complex has a very large surface area per weight; it includes large hydrophobic parts and has an excess of a positive charge, about half of the exchange capacity of the clay. X-ray diffraction, electron microscopy, and adsorption experiments indicated that the material characteristics of the micelle–clay complex are different from those of an organo–clay complex, which is formed by the adsorption of the same organic cation as monomers.[43] The micelle–clay is ideally suited for the adsorption of anionic organic molecules, for example, herbicides and dissolved organic matter (DOM),[39] certain antibiotics,[41] as well as certain inorganic anions, such as perchlorate.[42] Filters filled with a micelle–clay complex mixed with sand were employed to study the ability of the mixture adsorbents in removing a variety of pharmaceuticals.[44]

### Adsorption isotherms

The adsorption of paracetamol and its hydrolysis product *p*-aminophenol onto a micelle–clay complex and charcoal was analyzed by the Langmuir equation (Equation (3)):

$$\frac{C_e}{Q_e} = \frac{1}{K \cdot Q_{\max}} + \frac{C_e}{Q_{\max}}, \quad (3)$$

where  $C_e$  is the equilibrium concentration of the solute (e.g. paracetamol) (mg/L).  $Q_e$  is the equilibrium mass of adsorbed paracetamol (or *p*-aminophenol) per gram of adsorbent (mg/g).  $K$  is the Langmuir affinity constant (L/mg).  $Q_{\max}$  is the maximum amount of solute adsorbed per gram of micelle–clay complex, or charcoal (mg/g).

As in all our previous studies on pharmaceuticals, their adsorption is well described by the Langmuir adsorption isotherm, as shown by the plot of  $C_e/Q_e$  versus  $C_e$  (Figure 3). The correlation coefficient values ( $R^2$ ) obtained by the two adsorbents were in the range 0.9921–0.9968.

The calculated  $Q_{\max}$  and  $K$  values for the removal of the pharmaceuticals (Table 1) are 185.2 mg/g and 0.033 L/mg, respectively. The corresponding values in the case of charcoal are  $Q_{\max} = 129.9$  mg/g and  $K = 0.035$  L/mg. In this case, the maximal number of adsorption sites is about 50% larger in the case of the micelle–clay complex, whereas the affinity constant (at the given temperature) of charcoal is 6% larger. The adsorbed amounts according to the Langmuir equation are mostly dependent on the product of  $Q_{\max} \cdot K$ , which can be fixed more reliably, whereas fitting to the data can be obtained by choosing larger  $K$  and smaller  $Q_{\max}$  values or vice versa. Hence we added these quantities in Table 1. The ratio of the value of  $Q_{\max} \cdot K$  between the micelle–clay and charcoal for paracetamol is 1.36.

In the case of *p*-aminophenol, we only present the results for the micelle–clay complex, due to fortuitously large errors in the case of charcoal. The values of the products  $Q_{\max} \times K$  in the case of the micelle–clay complex are similar for both paracetamol and the metabolite.

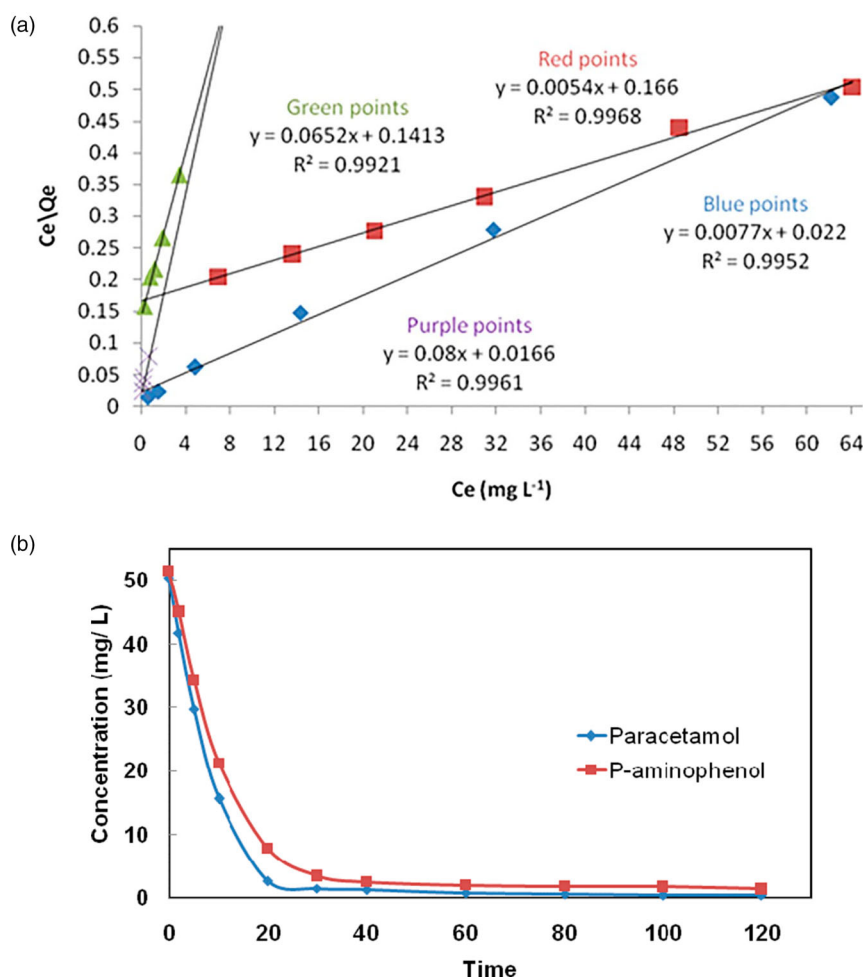
### Kinetic study of paracetamol and *p*-aminophenol

Figure 3(b) demonstrated a decrease in both paracetamol and *p*-aminophenol concentrations as function of time. This result reveals that the micelle–clay complex is efficient for paracetamol and *p*-aminophenol removal with an optimum contact time of 30 min.

### Membrane separation experiments

Membrane separation studies of the selected model compounds were performed with RO flat sheet membranes of decreasing selectivity/increasing permeability in a laboratory scale in the background of ultrapure water and tertiary effluents (TE). At the pilot plant, the experiments were held using TE and a BW membrane. The results are shown in Table 2 and in Figure 4.

These results show that tight nonporous membranes (BW and SW) are required for the effective removal of synthetic organic compounds such as paracetamol, since the rejections of looser nonporous membranes (XLE type) are matrix and compound dependent. It appears that when the membrane gets fouled, the rejection increases because the fouling layer serves as another barrier to the compounds as can be seen in



**Figure 3.** (a) Langmuir isotherms for the removal of paracetamol by charcoal (squares), by a micelle–clay complex (diamonds), and *p*-aminophenol by a micelle–clay complex (triangles). Adsorbent dosage was 5 g/L; Concentrations of paracetamol and *p*-aminophenol varied between 1 and 10 mg/L;  $T = 25^\circ\text{C}$ . Data represent the average of triplicate measurements. (b) Effect of contact time (min) on the removal of paracetamol (squares) and *p*-aminophenol (diamonds), by a micelle–clay complex. Initial concentrations for both paracetamol and *p*-aminophenol were 50 mg/L. Adsorbent dosage was 5 g/L and  $T = 25^\circ\text{C}$ . Data represent the average of triplicate measurements.

**Table 1.** Langmuir adsorption parameters  $K$  and  $Q_{\max}$  for the adsorption of paracetamol and *p*-aminophenol onto a micelle–clay complex and charcoal.

Pharmaceuticals	Adsorbents	Langmuir sorption parameters <sup>a</sup>		
		$K(\text{L}/\text{mg})$	$Q_{\max}(\text{mg}/\text{g})$	$Q_{\max}K(\text{L}/\text{g})$
Paracetamol	Micelle–clay complex	$0.033 \pm 0.006$	$185.2 \pm 9.7$	6.1
	Charcoal	$0.035 \pm 0.007$	$129.9 \pm 1.7$	4.5
<i>p</i> -Aminophenol	Micelle–clay complex	$0.46 \pm 0.06$	$15.3 \pm 0.22$	7.0

<sup>a</sup>Values of  $R^2$  were above 0.99 in all cases. The conditions and results of the Langmuir adsorption analysis are given in Figure 3(a).

Figure 4; lower right panel for the case of the loosest membrane, XLE. The pilot scale results also show high rejection of paracetamol using a BW membrane, as shown in Table 2.

### Toxicity microbioassays

Toxicity tests were performed on membrane separation experiments at three points: before spiking

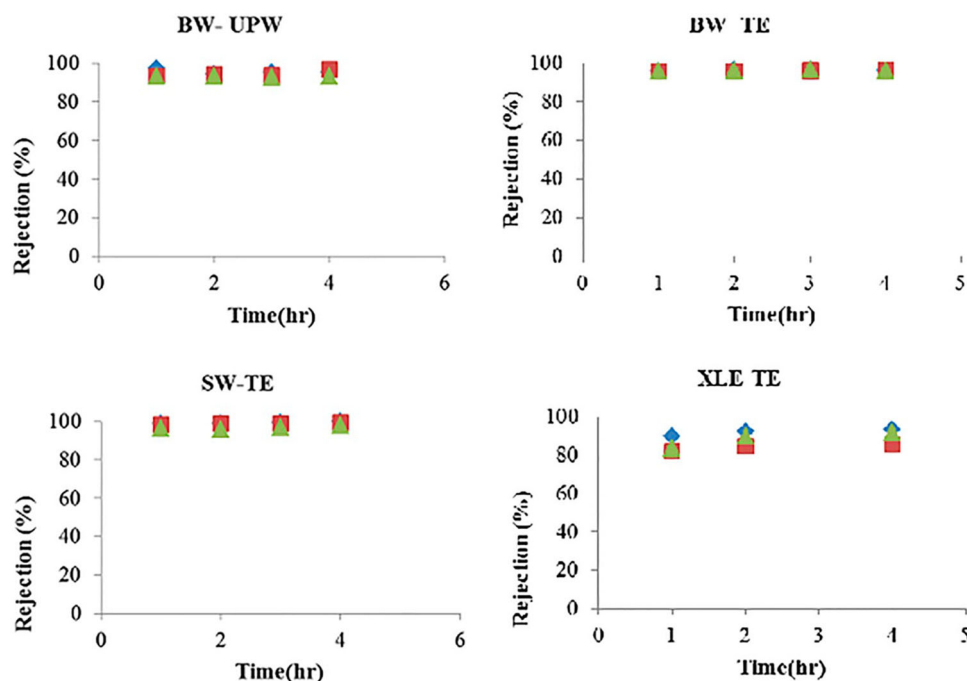
**Table 2.** Rejection (%) of paracetamol in different matrixes in three different RO membranes on a laboratory scale versus pilot scale.

	Laboratory scale		Pilot scale TE
	Pure water	TE	
SW	95 ± 2	98 ± 1	–
BW	95 ± 2	96 ± 1	89 ± 1
XLE	88 ± 2	94 ± 8	–

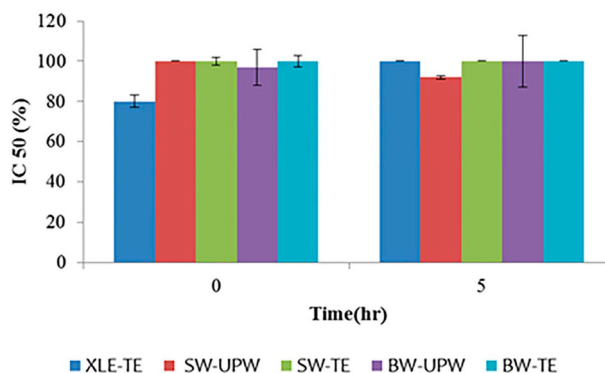
Notes: Conditions are given in Methods. Paracetamol was spiked to the system at a concentration of 120 and 150 µg/L in laboratory and pilot scales, respectively.

paracetamol (in order to evaluate the toxicity of the effluent matrix), after spiking, and after 5 h of membrane separation. The toxicity was assessed using half maximal inhibitory concentration (IC-50). Most of the effluents before the spike showed negligible toxicity at the concentration studied ( $IC_{50} \geq 95$ ). Results of the toxicity tests can be seen in Figure 5.

As shown in Figure 5, most of the solutions did not show any toxicity before and after the membrane separation at an initial concentration of 120 ppb of paracetamol with an  $IC_{50} \approx 100$ . The only exception was the test that was performed using XLE membrane and TE that showed an initial toxicity of  $IC_{50} = 80 \pm 3$ , but this value was due to the matrix background and not paracetamol itself.



**Figure 4.** Rejection (%) of paracetamol by different matrixes in three different RO membranes on a laboratory scale. See also Table 2. pH values of 3.5, 7, and 10 are indicated by diamonds, squares, and triangles, respectively.

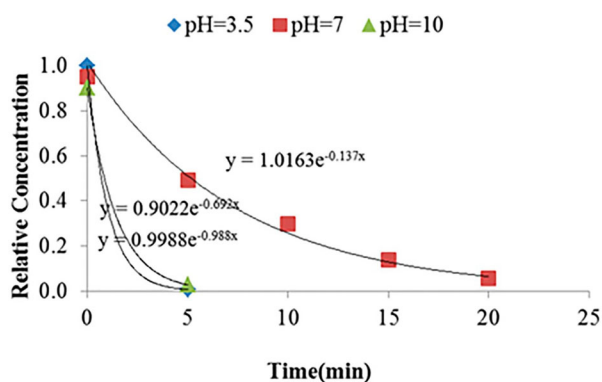


**Figure 5.** Paracetamol toxicity at 120 ppb before and after membrane separation.

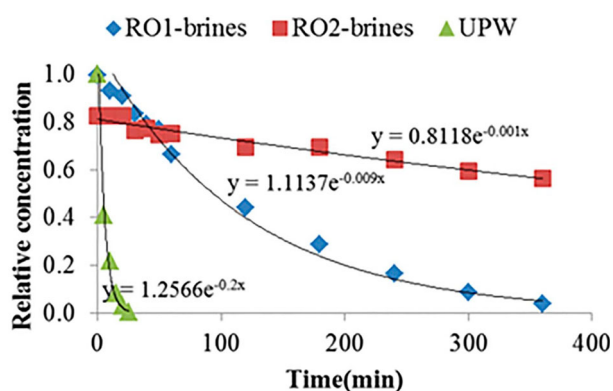
### UVA/TiO<sub>2</sub> experiments

The purpose of these experiments was to address the problem of high concentrations of paracetamol in the brines after the RO membrane separation. First, the effect of pH on paracetamol oxidation in pure water was tested to determine the optimal working conditions. The initial concentration of paracetamol was 80 ppb. The results are presented in Figure 6.

As presented in Figure 6, the optimal pH ranges are the acidic and the basic ones. Paracetamol degraded in 5 min in those conditions when using pure water. In



**Figure 6.** UVA/TiO<sub>2</sub> oxidation of paracetamol at different pH values. Symbols represent actual data. Lines are fit to first-order kinetics. Rate equations are shown in graph.



**Figure 7.** UVA/TiO<sub>2</sub> oxidation of paracetamol in different water matrices. Symbols represent actual data. Lines are fit to first-order kinetics. Rate equations are shown in graph.

neutral conditions, it took 20 min for paracetamol to degrade. Despite those results, it was decided to use the neutral range of pH due to its economic benefit – no need to use chemicals to change the pH level. The experiment was then performed using pure water versus two types of brines – RO1 and RO2 brines, and lasted for 6 h. The results are presented in Figure 7.

As shown in Figure 7, paracetamol in RO1 brines degraded almost completely during the 6 h of the experiment, whereas in pure water it degraded in only 20 min. Paracetamol degradation in RO2 brines went only up to

40% in 6 h. This is due to the quenching effect as described by Azerrad et al. [45]. The RO2 brines are much more concentrated and have much more organic matter and alkalinity that compete with paracetamol for the free radicals. For this reason the oxidation is less efficient. The reaction was found to match a first-order reaction, with confidence coefficients ( $R^2$ ) greater than 0.98. Also, the photocatalytic degradation of paracetamol has many products according to Yang et al. [46] since the reaction is not specific. This should be considered when applying this method since some of the products of paracetamol degradation might have some degree of toxicity.

### Paracetamol removal efficiency by different methods

Table 3 lists the efficiency of different methods toward the removal of paracetamol. As shown in the table, previous reported experiments demonstrated poor capability of activated carbon filters toward removing paracetamol, presumably due to the hydrophilic nature of the drug. Furthermore, the uptake of paracetamol by different plants revealed poor absorption of the drug from its aqueous solutions into different parts of the plants. On the other hand, tertiary treatment wastewater plants equipped with nano-membranes such as hollow fiber, spiral wound, and RO proved to be quite efficient in removing paracetamol [19,47–53].

This study has shown that the efficiency of ODTMA micelle–clay filter in removing paracetamol is comparable to that of RO membrane (100% removal). Therefore, it is suggested that an integration of ODTMA clay–micelle complex filters in existing WWTPs may be helpful in improving the removal efficiency of recalcitrant residues of hydrophilic pollutants such as paracetamol and that the integration of micelle–clay filters in sewage treatment systems can be a promising technology.

### Conclusions

The conventional method of microbial identification using culture-dependent and biochemical testing kits was employed to qualitatively characterize the microbial populations in the Al-Quds activated sludge WWTP. The results indicated that the Al-Quds University activated sludge reactor contained the following microorganisms: *E. coli*, *E. sakazakii*, *C. freundii*, *P. aeruginosa*, *K. pneumoniae*, *E. cloacae*, *E. amnigenus*, *E. aerogenes*, *Salmonella* spp., and *S. liquefaciens*. Paracetamol was found to undergo selective biodegradation by *P. aeruginosa* in 14 days. The biodegradation products were *p*-

**Table 3.** Removal (%) of paracetamol using different methods.

Removal method	Percentage removal
Sugar cane bagasse (SCB); natural adsorbent	45 [47]
Vegetable sponge (VS); natural adsorbent	40 [47]
Tertiary WWTPs	96–100 [48]
Activated carbon	65 [49]
Continuous Packed-bed photoreactor configuration	42 [50]
Laboratory-scale MBR	80 [19]
Activated carbon from different sources	60–97 [51]
Photocatalysis with various electron acceptors	92–99 [52]
Al-Quds tertiary treatment plant	100 [53]



aminophenol and hydroquinone as identified by <sup>1</sup>H-NMR and LC-MS.

Desalination by applying tight nonporous membranes (i.e. RO) appears as a reliable tool for the removal of pharmaceuticals, including low molecular weight congeners such as paracetamol, and the generation of high-quality effluents, regardless of the water matrix. The BW-type membranes, providing acceptable rejections of both organic matter and inorganic species with reasonable fluxes, appear to be a preferable choice for water restoration and principally for water recycle conservation in sustainable agriculture. Implementation of advanced oxidation technologies for the treatment of desalination brines can aid in achieving sustainable wastewater reuse, with the added value of a reduced treatment volume.

Adsorption studies using two low-cost adsorbents, charcoal and clay–micelle complex, revealed that both adsorbents are efficient in removing the pharmaceutical together with their biodegradation products. The adsorption isotherms were found to fit Langmuir isotherms and the adsorption parameters were evaluated. The kinetics of adsorption and the adsorption parameters strongly suggest that integrating clay–micelle filters within WWTPs is a promising technology for the efficient removal of the studied pharmaceuticals and their degradation products.

### Disclosure statement

No potential conflict of interest was reported by the authors.

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