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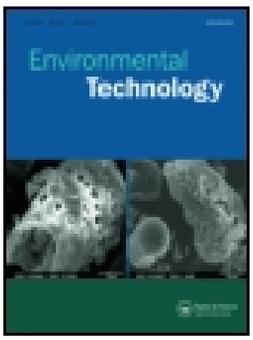

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RafikKaraman[1], Mustafa Khamis[2,3], Jehad Abbadi[4],Ahmad Amro[1], Mohannad Qurie[5],Ibrahim Ayyad[5], Fatima Ayyash[1], Omar Hamarsheh[4], Reem Yaqmour[4],Shlomo Nir[6], Sabino A. Bufo[7] and Laura Scrano[7], Sofia Lerman[8], Shirra Gur-Reznik[8], and Carlos G. Dosoretz[8]*

1. *Department of Bioorganic Chemistry, College of Pharmacy, Al-Quds University, Jerusalem, 20002, Palestine.*
2. *Department of Chemistry and Chemical Technology, College of Science and Technology, Al-Quds University, Jerusalem 20002, Palestine.*
3. *Department of Biology, Chemistry and Environmental science, College of Arts and Sciences, American University of Sharjah, Sharjah, UAE.*
4. *Department of Biology, College of Science and Technology, Al-Quds University, Jerusalem 20002, Palestine.*
5. *Centre for Chemical and Biological Analysis, Al-Quds University, Jerusalem 20002, Palestine.*
6. *Department of Soil and Water Sciences, The R.H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100, Israel.*

7.Crop Systems, Forestry and Environmental Sciences, University of Basilicata, Viadell'AteneoLucano 10, 85100, Potenza, Italy.

8. Faculty of Civil and Environmental Engineering, Technion-Israel Institute of Technology, Haifa 32000, Israel.

***Corresponding author:** R. Karaman (1958-), Professor, main research field: pharmaceuticals, design and stability. E-mail: dr_karaman@yahoo.com

Paracetamol biodegradation by activated sludge and photo-catalysis and its removal by a micelle-clay complex, activated charcoal and reverse osmosis membranes

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) (ODTMA)–clay (montmorillonite)) were determined at 25 ^{0}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 photo-catalytic processes; optimal conditions were found to be acidic or basic pH, in which paracetamol degraded in less than 5 minutes. Toxicity studies indicated that the effluent and brine were not toxic except for using extra low energy (XLE) membrane which displayed half maximal inhibitory concentration (IC-50) value of 80%.

Keywords: 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 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 wastewater-treatment plant, some pharmaceuticals are either partially retained in the sludge or metabolized to a more hydrophilic but still persistent forms and, therefore, pass through the wastewater treatment plant (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 are varying 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/Lin European sewage treatment plants (STP) effluents, and up to 10 µg/Lin natural waters in USA [29, 30]. Studies on the Tyne River in the UK and surface water in US 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_2O_2-UV oxidation, and semiconductor photo catalysis has been reported for 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 non-toxic products via three metabolic pathways. Glucoronidation 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 GSH 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 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 concentration of 100 µg/ L as a sole carbon source. Two paracetamol degrading strains were isolated from the MBR biomass and identified as *Delftiatsuruhatensis* and *Pseudomonas aeruginosa* [35].

During the past seven years we have been engaging in studying the stability of commonly used drugs in waste water 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 towards 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 Kupat 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 towards 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 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 wastewater treatment plant 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 from (Waters: USA), and a waters Micromass® Masslynx ™ detector with Photo diode array (PDA) (Waters 2996: USA). Data acquisition and control were carried out using Empower ™ software (Waters, USA). Analytes were separated on a 4.6 mm X 150 mm C18 XBridge ® column (5 µm particle size) used in conjunction with a 4.6 mm X 20 µm XBridge ™ C18 guard column. Microfilter was used with 0.45µm (Acrodisc® GHP, Waters, USA).

Paracetamol in 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 (MRM) 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) endcapped column (3 mm×10 cm, 2.6 µm pore size) was used with an isocratic 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^2) greater than 0.99 within the experimental concentration range. The limit of quantification (LOQ) and the limit of detection (LOD) 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 $\lambda_{max}$for each drug.

pH meter

pH values were recorded onpH meter model HM-30G: TOA electronics™ 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 0.45 μm filter, was determined on a multi TOC-VCPH analyzer (Shimadzu) using no dispersive infrared (NDIR) detection. The quantification limit for TOC was 0.2 mg/L.

Centrifuge and Shaker

Labofuge®200 Centrifuge was used, 230 V 50/60 Hz. CAT. No. 284811; made in Germany. Some of pharmaceuticals 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 (THF) 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 (OPA) was obtained from Riedel-De Haën (Germany).Microbiological growth media (MacConkey agar, Blood Agar, Eosin Methylene Blue Agar, Mannitol Salt Agar 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 96 wells microtiter plate following the manufacturer's protocol. The assay was performed by running a set of 8 double dilutions of the sample in question with provided assay buffer followed by an addition of hydrated culture of luminous bacteria. The level of light (in relative luminescence units - RLU) from the biosensors was recorded after 15 minutes 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 toxicity can be expressed as IC50 (inhibition concentration of 50%), defined as the minimal concentration of tested sample (in %) that results in 50% change of 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).

$$IC = \frac{RLU_{control} - RLU_{sample}}{RLU_{control}} \qquad \text{Eq. (1)}$$

Where, IC is the inhibitory concentration of the tested sample [%]; $RLU_{control}$ is the light level obtained in the negative control [RLU] and RLU_{sample} is the light level obtained in the sample [RLU].

Photo-catalytic experiments

Photo-catalysis experiments with UVA/TiO_2 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 mLglass reactors containing 180 mL of either water matrix spiked with 80 µg/ L of paracetamol. TiO_2 (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, 40W) emitting radiation between 300 and 420 nm with a maximum at 350 nm. UVA lamps were preheated for 20 min prior to 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 $NaHCO_3$ to facilitate TiO_2 removal, and kept in ice in the dark. Then samples were centrifuged during 20 min at 4000 rpm (Heraeus, Megafuge 1.0R centrifuge) and filtered by 0.45 μm syringe filter units (Millipore) prior to analysis. Light intensity was measured by ferrioxalateactinometry[38]and was found to be 990 $\mu W/cm^2$. The UV fluence was calculated multiplying the light intensity by the exposure time; it was found to be 3564 mJ/cm^2 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 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) were 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, Eosin Methylene Blue (EMB) to enhance gram negative bacteria, Mannitol salt

agar (MSA) to enhance the growth of Staphylococci. All plates were incubated at 36 °C for 24 h.

After the incubation period, inoculated plates were inspected for bacterial growth. Growth was observed in all plates except MSA plates which exclude *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 growth media was used for further identification using API-20E test kit for identification of enteric bacteria (bioMerieux, Inc.). The data obtained after the biochemical test were compared to the relevant catalogue to identify bacterial species in sludge samples.

Biodegradation of Paracetamol and p-aminophenol in Al-Quds wastewater.

Pseudomonas 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, 37].

Pure colonies of *Pseudomonas aeruginosa* were sub-cultured from the bacteria characterized in the sludge of Al-Quds wastewater treatment plant and streaked on the surface of twenty sterile fresh nutrient agar plates under complete sterile technique. The nutrient agar plates were incubated at 37ºC for 24 hours. The densely grown bacterial colonies were then extracted using sterile phosphate buffer having pH 7 by the aid of sterile glass rod under complete sterile technique and collected in sterile flasks obtaining a 100 mL of concentrated bacterial suspension of *Pseudomonas aeruginosa*.

200 mg of paracetamol or *p*-aminophenol were transferred to 2-liter brown bottle and were dissolved in 1L of sterilized wastewater (having COD and BOD of 232 and 178 mg/ L respectively). The *Pseudomonas aeruginosa* bacterial suspension harvested in sterile phosphate buffer was gradually added to the pharmaceutical-wastewater solution to reachoptical 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 x 10^6colony forming unit (cfu/ mL) of *Pseudomonas aeruginosa* according to a growth curve determined

previously for *Pseudomonas aeruginosa* (Log *Pseudomonas aeruginosa* count (cfu/mL) = 2.45 x 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 30ºC 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 0.5-0.7 (corresponding to 3.8 x 10^6- 1.2 x 10^7cfu/ml) and at the end of the experiment (14 days), the *Pseudomonas aeruginosa* population had a count of 2.0 x 10^5cfu/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 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

(a) *Stock solution:* Stock solution was prepared by dissolving paracetamol, or *p*-aminophenol standards in acetonitrile and water that was adjusted to pH 3.45 in 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 ($MgSO_4$), 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) in a ratio (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 a 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 a distilled water to a concentration of 500 mg/ L that was used in (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 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 10g/ L clay for 72h. 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 solution of 50 mg/ L as 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 two hours at room temperature, and then the content of the flask was centrifuged for 5 minutes and filtered using 0.45 µmmilliporefilters. A kinetic study of the extent of adsorption was determined by

taking 50 mL solution of 50 mg/ L paracetamol, or p-aminophenol, in 250 mL Erlenmeyer flask containing 0.5 g of micelle-clay complex then measuring the absorbance of the solution vs. 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 degree of selectivity. Trials involved different water matrixes to test the background effect of organic matter on 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: XLE (extra low energy), BW (brackish water), and SW (sea water). 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 laboratory-scale cross-flow cell system and in a pilot system. The laboratory-scale cross-flow cell system is comprised of two parallel channels of two cells in series each, holding 24 cm^2 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 a batch mode (full recycling of permeate and concentrate to the reservoir). All experiments were performed under controlled temperature at 21±2°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 µg/ L.

Samples for analyses were taken both from the feeding reservoir and the permeate channel of each individual cell. Experiments were run for 5 hours. 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).

$$R = \frac{C_F - C_p}{C_F} \times 100 \qquad \text{(eq. 2)}$$

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 the most effective in terms of resolution and permeation rates) was further tested under field conditions in Technion secondary effluent desalination-pilot plant with a nominal capacity of 10 m^3/h. The pilot plant comprises an ultrafiltration stage (~40 nm molecular weight cut off – MWCO, Dow) followed by a two stages-reverse osmosis system (8" and 4" BW Toray membranes, respectively). The recovery of the 1st-stage RO was 50% and the total recovery was about 85%. A schematic diagram of the desalination process is presented in Fig. S1 (supporting information). Experiments conducted in the pilot plant lasted 6 h and paracetamol was spiked at a concentration of 150 μg/ 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 morphological characterization of grown colonies and biochemical testing by API-20E test kit, the following bacterial species were isolated from Al-Quds University sludge samples: *Escherichia coli, Enterobactersakazakii, Citrobacterfreundii, Pseudomonas aeruginosa, Klebsiella pneumonia, Enterobacter cloacae*, *Enterobacteramnigenus, Enterobacteraerogenes, Salmonella spp., and Serratialiquefaciens*

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 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 degradation of paracetamol to *p*-aminophenol and acetate (Figure 2). The degradation was demonstrated by a disappearance of a peak characterized as the reactant (paracetamol) at a retention time of 3.92 minutes and a gradual appearance of a new peak at a retention time of 1.97 minutes characterized as a product (*p*-aminophenol). Figure 1illustrates a progress of paracetamol degradation as monitored by HPLC (Figures 2a and 2b). 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 minutes. The HPLC kinetic data obtained after one week monitoring was examined for linear correlation (figure 2c). 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 x $10^{-7}s^{-1}$.

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

Please insert Figure1, here

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Please insert Figure2b, here

Please insert Figure2c, here

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 *Pseudomonas aeruginosa* which was reported to have amidase enzymes that have the capability to catalyse breakdown of amide bonds in a variety of organic molecules.

Biodegradation of Paracetamol by Pseudomonas 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 NMR (for the degradation pathway see Figure 1). While the first metabolite reached its maximum around seven days, the second one increased until the end of the experiment (14 days) (Figure 2d). 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 (20mg/L) was incubated with *Pseudomonas aeruginosa* in the same manner and conditions as described for paracetamol. HPLC monitoring demonstrated that *p*-aminophenol underwent degradation to hydroquinone (Figure 2d). A complete biodegradation was achieved after 14 days of incubation with the bacteria.

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 to the two metabolites, respectively.

Please insert Figure 2d, here

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, reverse osmosis and spiral wound, activated carbon and micelle-clay filters. We are also presenting here results of removal of paracetamol and its metabolite, *p*-aminophenol in suspension by a micelle-clay complex in comparison with activated carbon. The micelles of the organic cation octadecyltrimethylammonium (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 adsorption of the same organic cation as monomers [43]. The micelle-clay is ideally suited for the adsorption of anionic organic molecules e.g., 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):

$$\frac{C_e}{Q_e} = \frac{1}{K * Q_{max}} + \frac{C_e}{Q_{max}} \qquad \text{Eq. (3)}$$

Where:

C_e: equilibrium concentration of (solute, e.g., paracetamol) (mg/L).

Q_e: equilibrium mass of adsorbed paracetamol (or *p*-aminophenol) per gram of absorbent (mg/g).

K: Langmuir affinity constant (L/mg).

Q_{max}: 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 Langmuir adsorption isotherm as shown by the plot of Ce\Qe versus Ce (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}● 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}● 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 the charcoal. The values of the products Q_{max}x K in the case of the micelle-clay complex are similar for both paracetamol and the metabolite.

Please insert Figure3a, here

Please insert Table 1, here

Kinetic study of paracetamol and p-aminophenol

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

Please insert Figure3b, here

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 lab scale at the background of UPW (ultra-pure water) and TE (tertiary effluents). At the pilot plant the experiments were held using tertiary effluents and a BW membrane. The results are shown in Table 2 and in Figure4.

Please insert Table 2, here

Please insert Figure 4, here

These results show that tight nonporous membranes (BW and SW) are required for 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 Figure4; 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 microbiotests

Toxicity tests were performed on membrane separation experiments at 3 points: before spiking paracetamol (in order to evaluate the toxicity of the effluent matrix), after spiking and after 5 hours 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≥95). Results of the toxicity tests can be seen in Figure5.

Please insert Figure5, here

As shown in Figure5, 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≈100. The only exception was the test that was performed using XLE membrane and tertiary effluents that showed an initial toxicity of IC-50=80±3 but this value was due to the matrix background and not paracetamol itself.

UVA/TiO_2 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. Results are presented on Figure6.

Please insert Figure6, here

As presented in Figure6the optimal pH ranges are the acidic and the basic ones. Paracetamol degraded in 5 min in those conditions when using pure water. In neutral conditions it took 20 min for the 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. Results are presented in Figure7.

Please insert Figure7, here

As shown in Figure7, 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 photo catalytic 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 towards the removal of paracetamol. As shown in the table, previous reported experiments demonstrated poor capability of activated carbon filters towards removing paracetamol, presumably due to the hydrophilic nature of the drug. Furthermore, 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 for improving 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 Al-Quds activated sludge wastewater treatment plant. The results indicated that Al-Quds University activated sludge reactor contained the following micro-organisms: *Escherichia coli, Enterobactersakazakii, Citrobacterfreundii, Pseudomonas aeruginosa, Klebsiella pneumonia, Enterobacter cloacae*, *Enterobacteramnigenus, Enterobacteraerogenes, Salmonella spp., and Serratialiquefaciens.* Paracetamol was found to undergo selective biodegradation by *Pseudomonas aeruginosain* 14 days. The biodegradation products were *p*-aminophenol and hydroquinone as identified by ^{1}H-NMR and LC-MS.

Desalination by applying tight non-porous membranes (i.e. RO) appears as a reliable tool for removal of pharmaceuticals, including low molecular weight congeners such as paracetamol, and 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 bea preferable choice for water restoration and principally for water recycle conservation in a sustainable agriculture. Implementation of advanced oxidation technologies for the treatment of desalination brines can aid in achieving a 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. Kinetics of adsorption and the adsorption parameters strongly suggest that integrating clay-micelle filters within wastewater treatment plant isa promising technology for efficient removal of the studied pharmaceuticals and their degradation products.

Acknowledgements

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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 [a]		
		K(L/mg)	Q_{max}(mg/g)	Q_{max}●K(L/g)
Paracetamol	Micelle-clay complex	0.033±0.006	185.2±9.7	6.1
	Charcoal	0.035±0.0.07	129.9±1.7	4.5
p-aminophenol	Micelle-clay complex	0.46±0.06	15.3±0.22	7.0

[a]Valuesof R^2 were above 0.99 in all cases. The conditions and results of the Langmuir adsorption analysis are given in Figure 3a.

Table 2: Rejection (%) of paracetamol in different matrixes in three different RO membranes in lab scale vs. pilot scale[a]

	Lab- scale		Pilot Scale
	Pure water	Tertiary effluent	Tertiary effluent

SW	95±2	98±1	---
BW	95±2	96±1	89±1
XLE	88±2	94±8	---

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

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 wastewater treatment plants	96-100[48]
Activated carbon	65[49]
Continuous Packed-Bed Photoreactor Configuration	42[50]
laboratoryscale membrane bioreactor (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]

List of Figures

Figure S1. Schematic process diagram of the secondary effluents-desalination pilot plant at NirEtzion

Figure 1: Schematic pathway for a degradation of paracetamol in Al-Quds sludge and by *Pseudomonas aeruginosa* bacteria.

Figure 2a: Chromatogram showing the hydrolysis of paracetamol after 2 days in presence of wastewater at 25° C.

Figure 2b: Chromatogram showing the hydrolysis of paracetamol after 7 days in presence of wastewater at 25° C.

Figure 2c: First order kinetic plot for the biodegradation of paracetamol in Al-Quds activated sludge at 25 ^{0}C.

Figure 2d: Biodegradation curve for paracetamol (blank points) and *p*-aminophenol (filled points) within 14 Days incubation. In activated sludge of 100 mg/ L initial paracetamol concentration and `T= 25 ^{0}C. Data represent average of triplicate measurements.

Figure 3a: Langmuir isotherms for the removal of paracetamol by charcoal (squares), and by a micelle- clay complex (diamonds), and *p*-aminophenol by a micelle- clay complex (triangles).Adsorbent dosage was 5g/L; Concentrations of paracetamol and *p*-aminophenol varied between 1 to 10 mg/L; T= 25 ^{0}C. Data represents average of triplicate measurements.

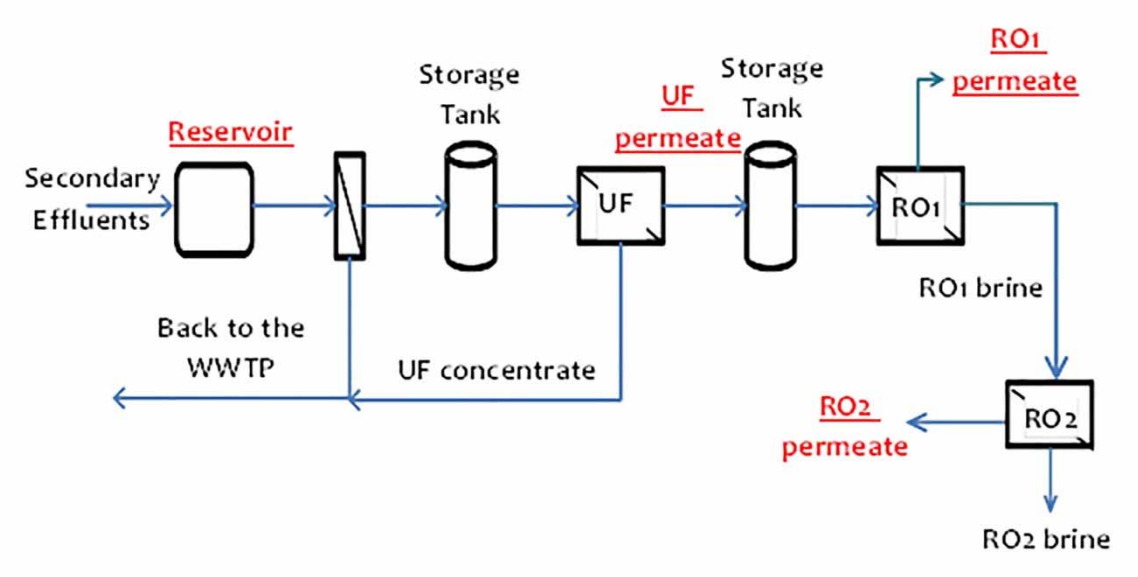
Figure 3b: Effect of contact time (min) on the removal of paracetamol (squares) and *p*-aminophenol (diamonds), by micelle-c lay complex. Initial concentrations for both paracetamol and *p*-aminophenol were 50 mg/L. Adsorbent dosage was 5g/L and T= 25 ^{0}C. Data represents average of triplicate measurements.

Figure 4: Rejection (%) of paracetamol by different matrixes in three different RO membranes in 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.

Figure 6: UVA/TiO_2 oxidation of paracetamol at different pH values. Symbols represent actual data. Lines are fit to 1st order kinetics. Rate equations are shown in graph.

Figure 7: UVA/TiO_2 oxidation of paracetamol in different water matrixes. Symbols represent actual data. Lines are fit to 1st order kinetics. Rate equations are shown in graph.



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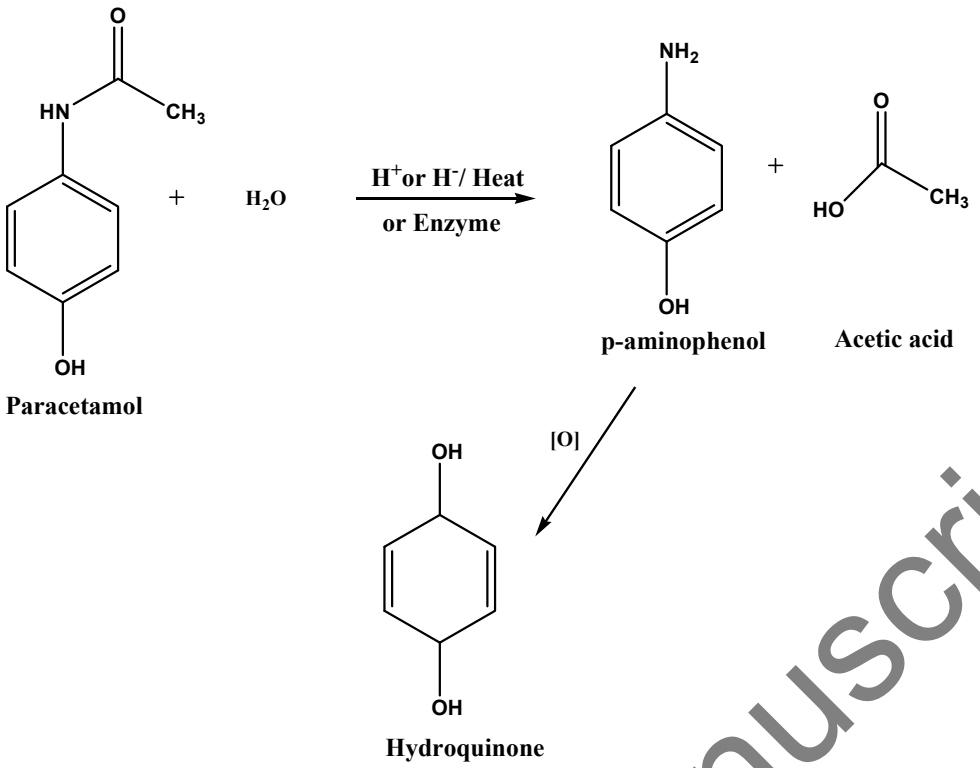

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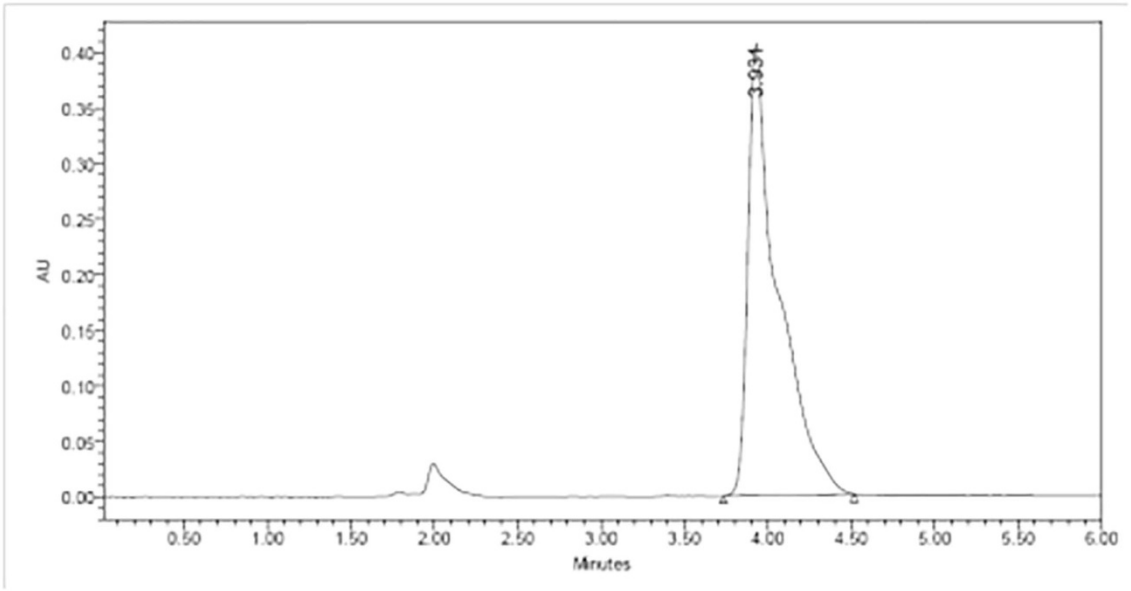
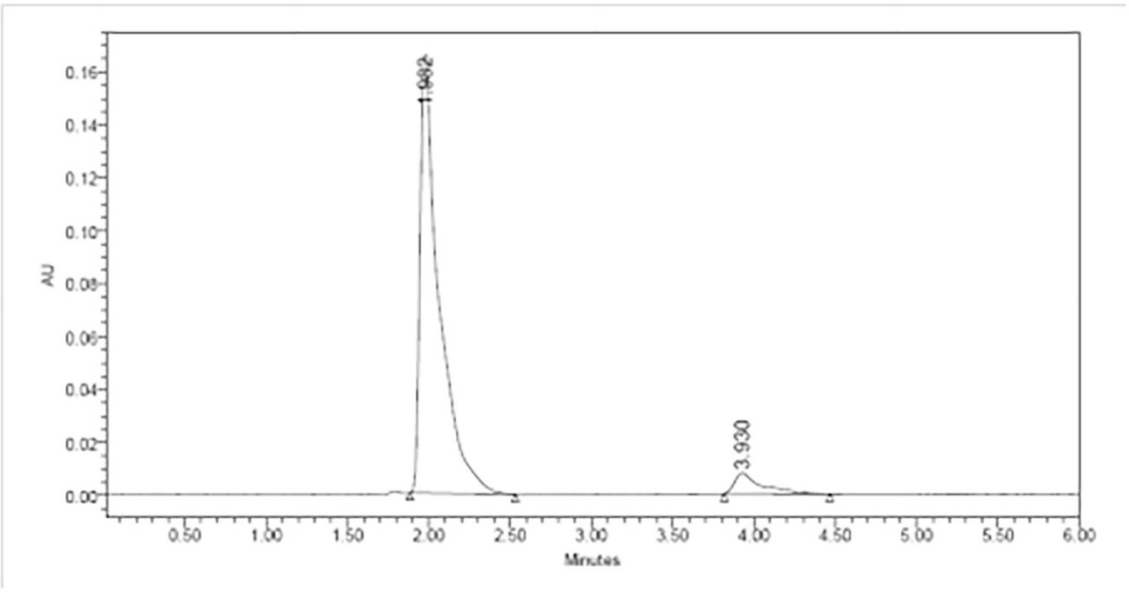


Figure 2a.



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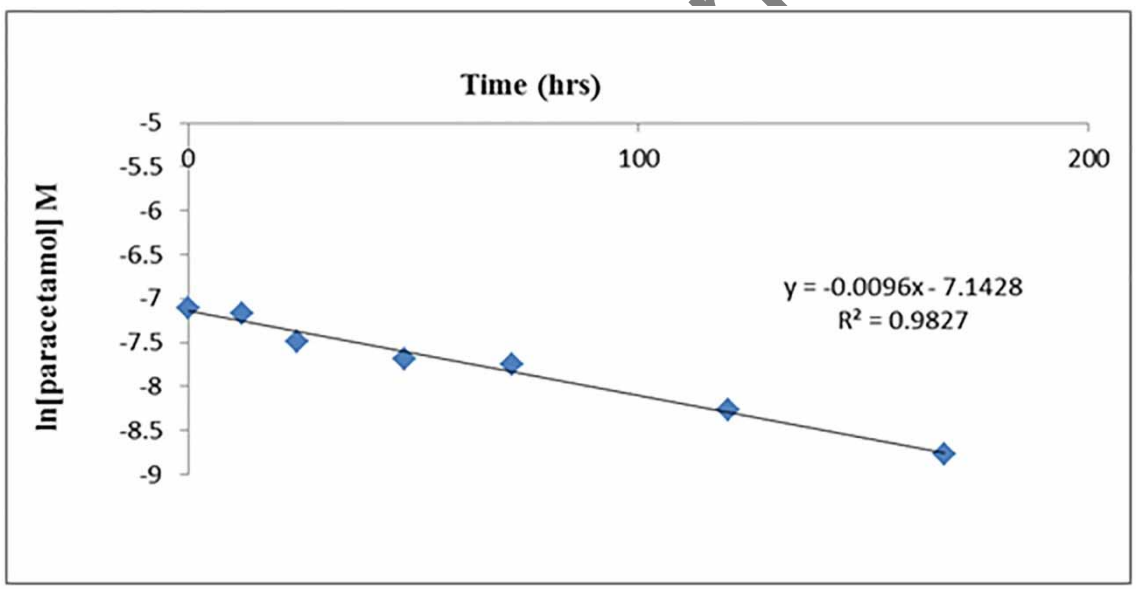

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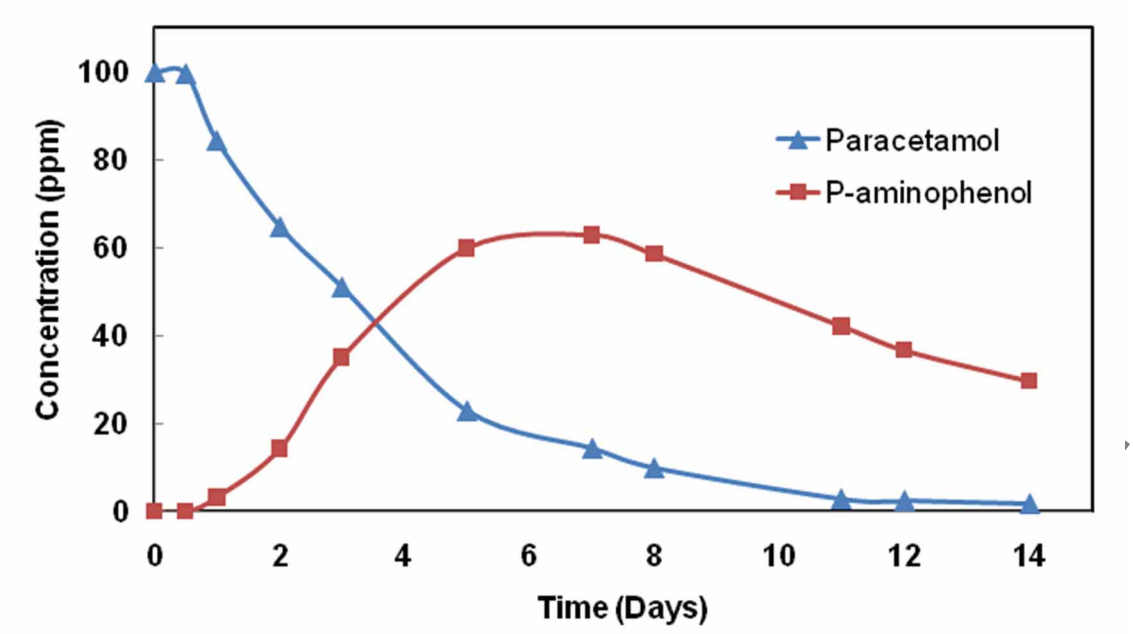

Figure 2d.

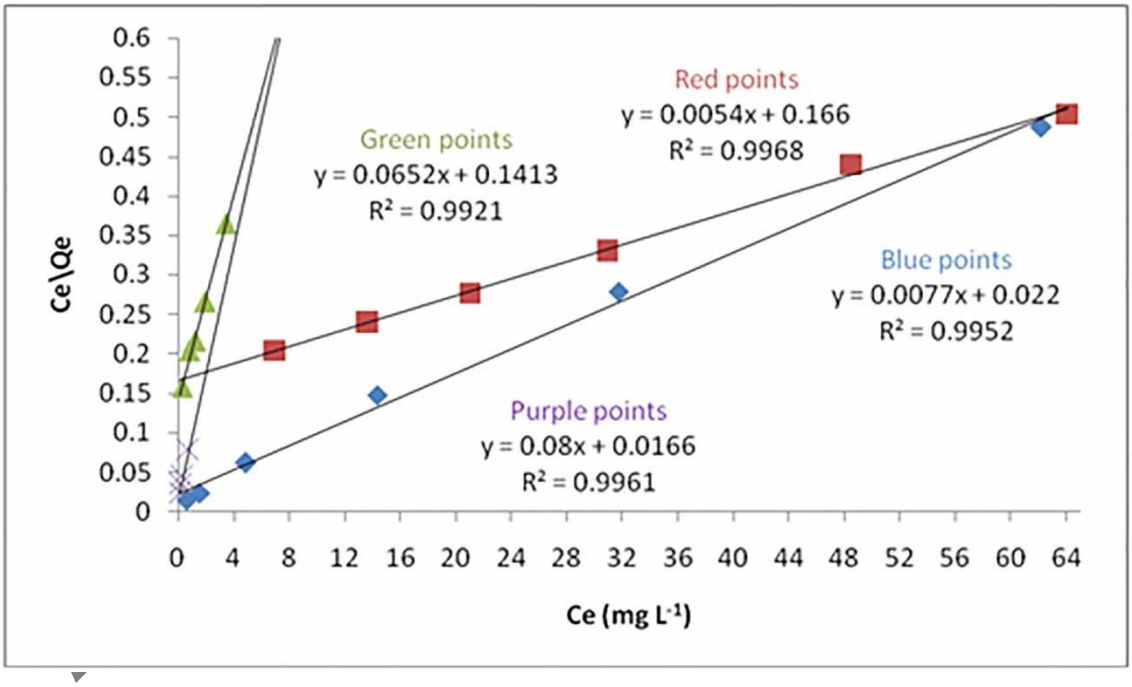

Figure 3a.

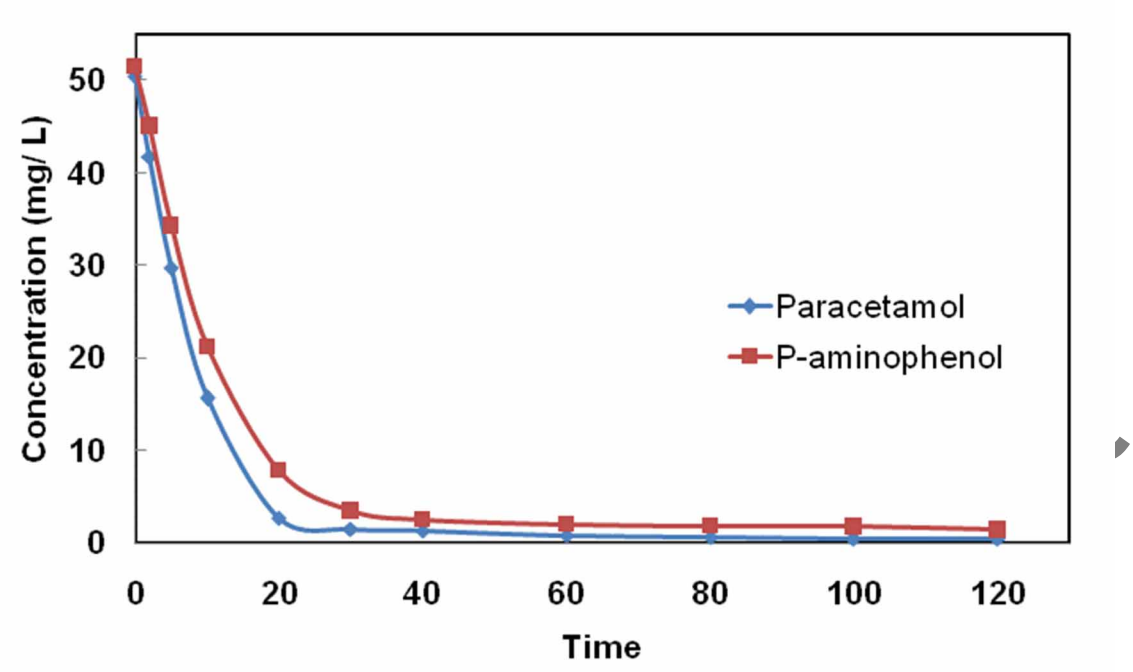

Figure 3b.

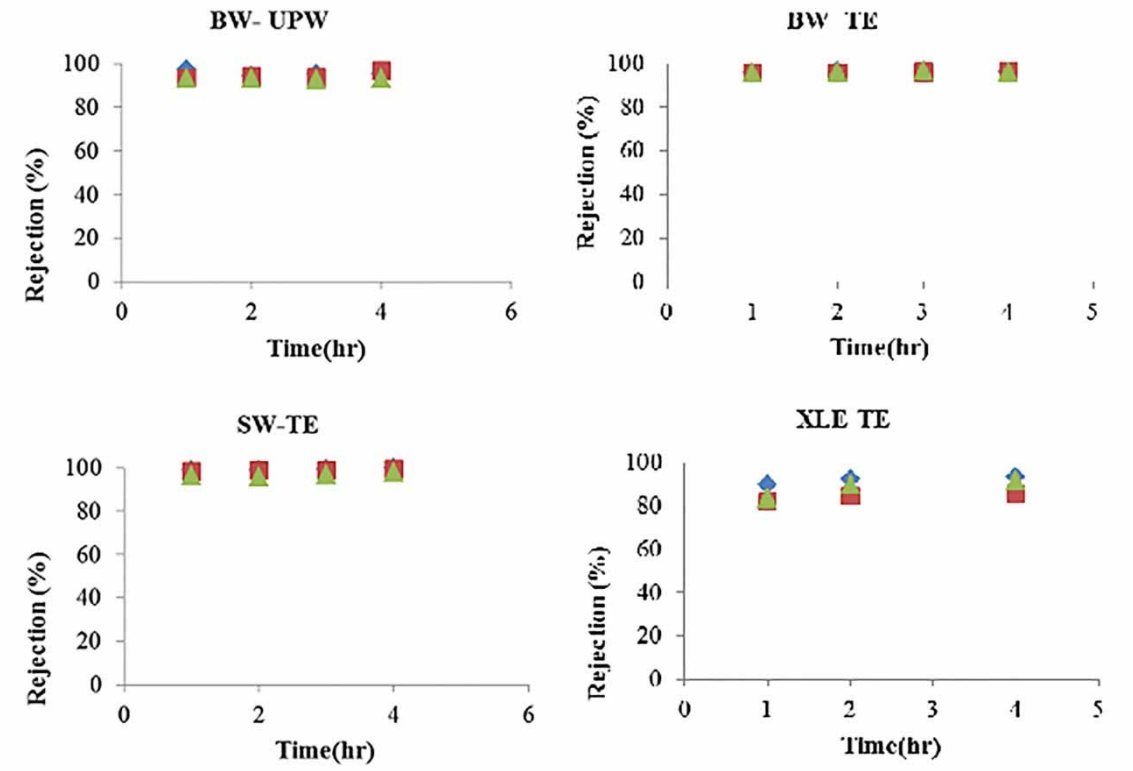

Figure 4.

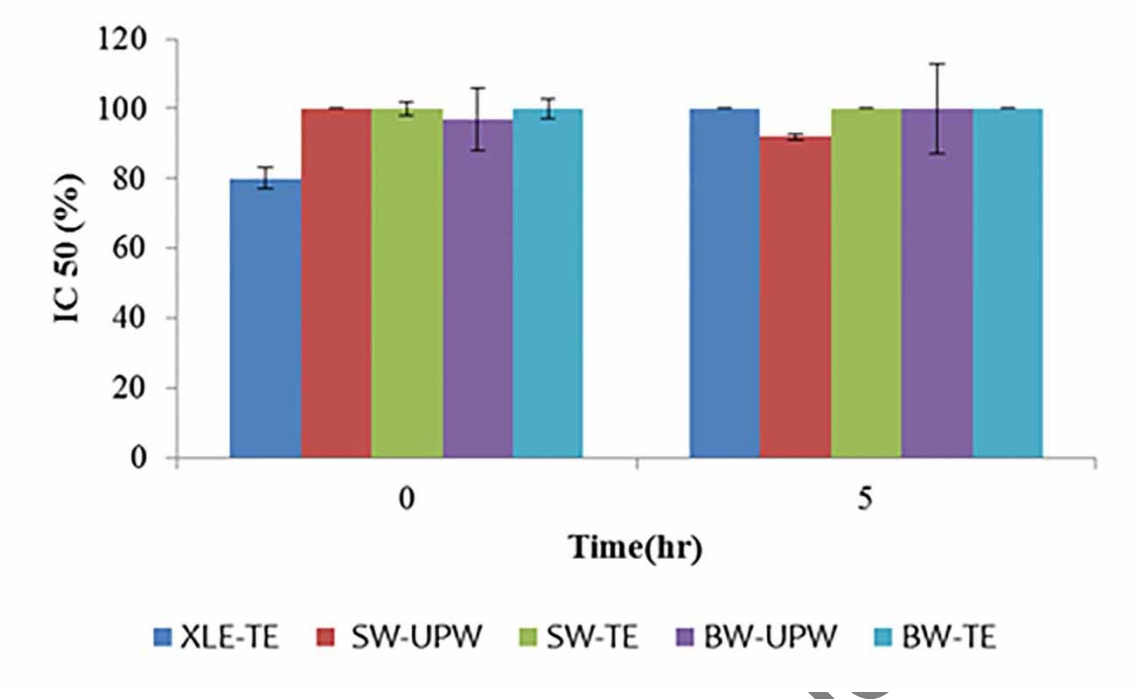

Figure 5.

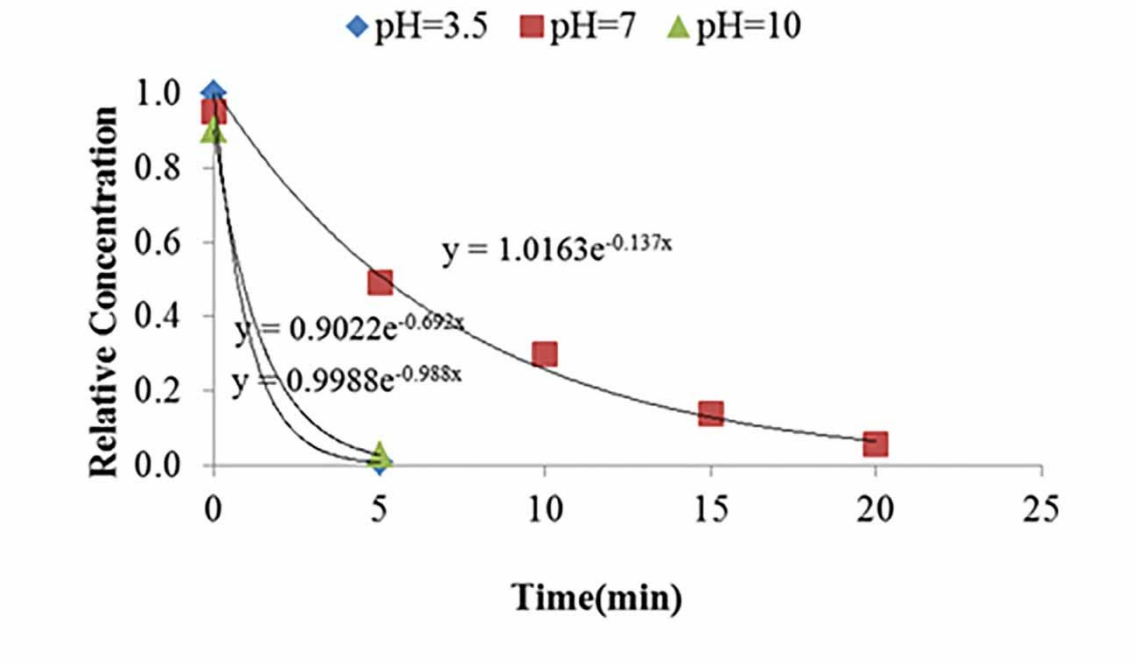

Figure 6.

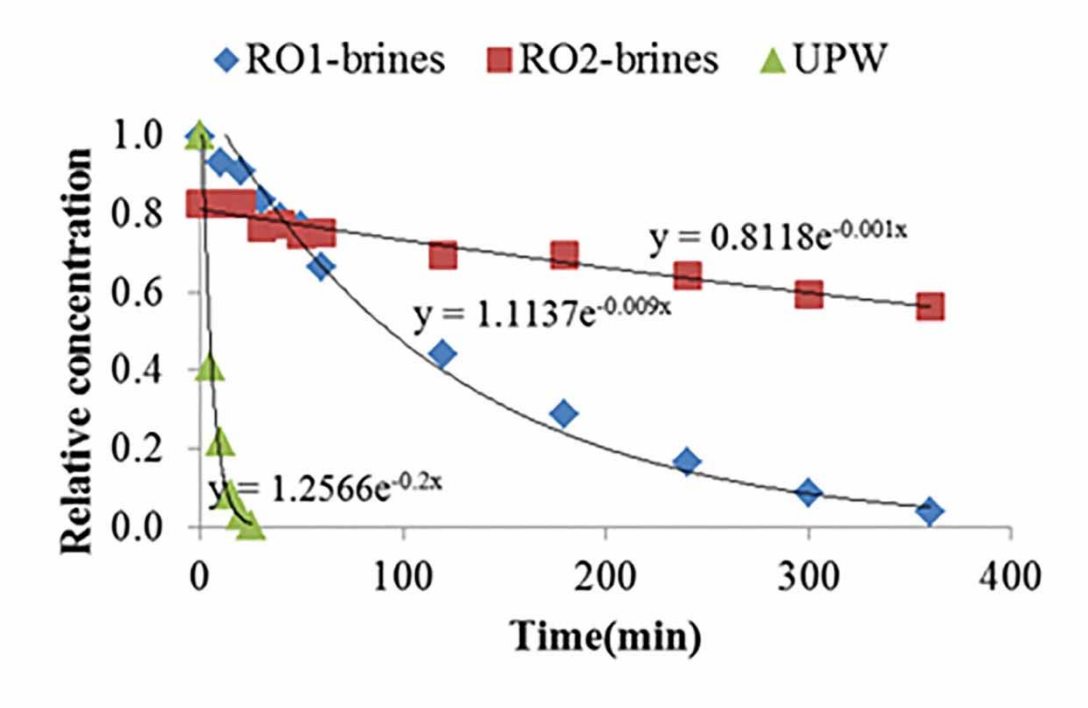

Figure 7.