Anaerobic Treatment Performance in Presence of Pharmaceutically Active Compounds

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Anaerobic Treatment Performance in Presence of Pharmaceutically Active Compounds

N.A.Z. Azizan[1], A. Yuzir*[1], F. F. Al-Qaim[2], and N. Abdullah[1]

[1] Malaysia-Japan International Institute of Technology (MJIIT), Universiti Teknologi Malaysia, Jalan Sultan Yahya Petra, 54100 Kuala Lumpur, Malaysia
[2] Chemistry Department, Faculty of Sciences for Women, University of Babylon, Hilla-Iraq, Iraq

*Corresponding author e-mail: muhdaliyuzir@utm.my

Abstract. Based on the occurrences of caffeine (CAF), gliclazide (GCZ) and prazosin (PRZ) in existing aerobic treatment processes as well as their persistency and potential risks to the environment, it is desirable to explore an alternative process to ensure complete removal of these compounds. Anaerobic process is widely known for its capability to efficiently degrade organic substrates present in wastewater, making it a viable option for the treatment of pharmaceutically active compounds. This study aims to examine the anaerobic treatment performance in the presence of pharmaceutical compounds. A batch experiment was conducted to assess the performance using synthetic wastewater and anaerobic digested sludge as inoculum at mesophilic condition of 37°C. Pharmaceutical analysis was then carried out using liquid chromatography-time of flight-mass spectrometry (LC-ToF-MS) instrument. Results shown that the anaerobic treatment performance was not affected in the presence of the three compounds. Overall, removal performance of the pharmaceutical compounds in descending order is PRZ > CAF > GCZ.

1. Introduction

Trace pharmaceuticals have been detected to be in ng/L to µg/L concentration in Malaysian waters, namely in treated wastewater effluent and receiving river stream. Among the tested pharmaceuticals, the concentration of stimulant caffeine was consistently higher than other pharmaceuticals [1-3]. In addition, first detection of anti-diabetic drug gliclazide and anti-hypertensive prazosin were discovered in Malaysian waters at significant concentration levels [1, 2]. The discovery of these pharmaceuticals are in correspondence to high consumption by local consumers [4, 5]. The previous studies found the trace pharmaceutical occurrences from sampling of treated effluent at the existing aerobic wastewater treatment plants in Malaysia [1-3]. As the current aerobic treatment systems were not designed to treat trace pharmaceuticals, this has resulted in incomplete removal of the trace compounds [6].

Concerns arose when caffeine, caffeine's metabolites and gliclazide were detected in aquatic species [7] and plants [8, 9], and discovered to have bioaccumulation potential [8-10]. Caffeine, gliclazide and prazosin may also form metabolites from treatment processes which have potential risks if discharged to the environment [10-13]. Even though the detections in the environment are deemed to be low, researchers agreed that these compounds are pseudo-persistent in the environment [14, 15] and presence of multiple pharmaceuticals in the environment might amplify the impact of the individual pharmaceutical [16]. Moreover, the toxicity impact may be more profound to directly impacted species like fish compared to mammals as they are continuously exposed to the trace pharmaceuticals.

Anaerobic treatment has been widely used in treating low to high organic wastewater such as domestic wastewater, industrial wastewater, leachate and so on [17]. The process also produces low final solids for disposal and potential methane production that can be recovered for energy usage [17-20]. The benefits of anaerobic process have led researchers to study on its potential in treating pharmaceutical compound. Positive results of pharmaceutical removal were observed for many pharmaceuticals. However, the degree of removal efficiencies varies for a different type of compounds [21-25]. There have also been cases whereby anaerobic treatment performance, namely COD removal and methanogenesis, was disrupted in presence of the micropollutants [26-28].

Previous research had only included gliclazide removal in the constructed wetlands process [29]. While knowledge on degradation of prazosin were limited to removal via electrochemical process [11] and bacteria isolation [12]. Considering the occurrence of gliclazide and prazosin as emerging contaminants from aerobic treatment process and the potential of anaerobic process to biodegrade pharmaceutical compounds, this study aims to examine the anaerobic treatment performance in the presence of caffeine, gliclazide and prazosin. To the best of knowledge, this is the first study that includes gliclazide and prazosin biodegradation in anaerobic treatment. Caffeine is included in this study as it is considered a reliable anthropogenic biomarker based on its stability in the environment [30, 31] and its biodegradability under anaerobic condition [24, 32].

2. Materials and methods

2.1. Chemicals and reagents

Pure standards (≥99%) of caffeine (CAF), gliclazide (GCZ) and prazosin hydrochloride (PRZ) were purchased from Sigma Aldrich (USA), while HPLC-grade methanol was obtained from Merck (USA). Ultrapure water was supplied from Thermo Scientific Smart2Pure (Sweden). A mixed standard stock solution of the three pharmaceutical compounds (1000 mg/L) was prepared in methanol and stored at -20°C.

Synthetic wastewater was formulated by mixing peptone (800 mg/L), glucose $C_6H_{12}O_6$ (2720 mg/L), yeast extract (560 mg/L), calcium chloride $CaCl_2$ (40 mg/L), magnesium sulfate $MgSO_4$ (40 mg/L), ammonium chloride NH_4Cl (320 mg/L), iron (II) sulfate $FeSO_4$ (32 mg/L) and potassium dihydrogen phosphate KH_2PO_4 (60 mg/L). Sodium bicarbonate $NaHCO_3$ is also added to regulate the pH between 6.5 to 7.5. All compositions are reagent grade purchased from Merck (USA) except for yeast extract (Difco, USA).

2.2. Batch study

Batch experiments were conducted to assess anaerobic treatment performance under mesophilic condition. Initially, anaerobic digested sludge to be used as inoculum was sampled from the existing municipal wastewater treatment plant located in Kuala Lumpur. This plant operates the anaerobic process in mesophilic condition. Prior to commencement of the experiment, the inoculum was warmed to 37°C in incubator overnight. The experiments were then carried out by adding 1 mg/L of the pharmaceuticals (CAF, GCZ and PRZ) to a mixture of synthetic wastewater and inoculum (50:50 v/v) in 250mL air-tight glass bottles for up to 90 days. To ensure a complete anaerobic condition i.e. no presence of oxygen in the reaction, nitrogen gas was purged into the sample bottles for 5 minutes before the bottles were sealed with butyl rubber stopper and incubated at 37°C in waterbath. All bottles were wrapped with aluminium foil to minimise the effect of photodegradation. As an experimental control, the abiotic effect was observed by spiking mixed pharmaceuticals in ultrapure water, while sorption effect was assessed by spiking mixed pharmaceuticals in an autoclaved mixture of synthetic wastewater and inoculum. The same concentration of pharmaceuticals at 1 mg/L was spiked in the control experiments. Samplings were conducted in duplicate at Day 0, 7, 14, 30, and 90.

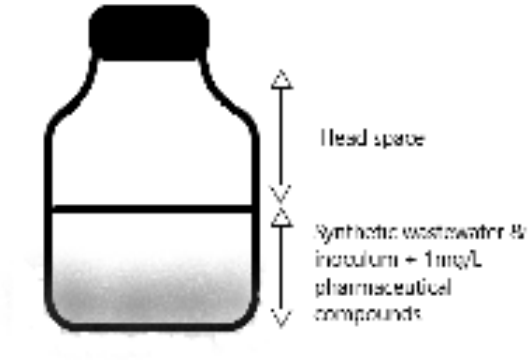

Figure 1. Conceptual diagram of batch experiment for anaerobic treatment of pharmaceutical compounds

2.3. Analysis of samples
Sample analysis is divided into two: anaerobic process performance and analysis of pharmaceuticals.

2.3.1. Pharmaceutical analysis
Gas chromatography-thermal conductivity detector (GC-TCD) Clarus® 690 GC (Perkin Elmer, USA) instrument was used to analyse the biogas composition. For each sample, 5 mL headspace gas was drawn from each sample bottle using an air-tight syringe and taken for loop injection. Nitrogen as a carrier gas in the system was operated at 30 mL/min. The column temperature was set at 170°C while the detector temperature at 200°C. COD analysis was analysed using Hach High Range Plus Reagent vials (USA) with reactor Hach DRB 200 and DR6000 spectrophotometer. pH meter OHAUS Starter 3100 (USA) was used to monitor pH and temperature. Total suspended solids (TSS) and volatile suspended solids (VSS) were assessed according to Standard Methods [33].

2.3.2. Pharmaceutical analysis
Analysis of pharmaceutical concentration was carried out using liquid chromatography coupled with time-of-flight mass spectrometry (LC-ToF-MS) instrumentation. Mobile phases for the analysis were 0.1% of formic acid in water (A) and acetonitrile (B). Flowrate was set to 0.3 mL/min at column temperature of 40°C. Each sample was pre-treated by centrifuging at 10000rpm for 5 min. The samples were then filtered with 0.45 µm nylon membrane filter (Thermo, USA) and subsequently filtered four times with 0.2 µm GHP filter (Waters, USA). Filtered samples were then transferred to glass vials before analysis.

Sample aliquots of 5µL were directly injected to C18 column 3µm, 3mm x 150mm (Thermo Scientific) in UltiMate 3000 UHPLC system (Dionex, USA). Gradient elution began at 5% of B for 1 min and increased to 60% of B for the next 2 min. The elution then further increased to 97% of B over 3 min and remained isocratic for 5 min. Next, the elution returned to its initial condition for 9 min and equilibrated for 5 min. Mass spectrometry was then performed using MicroTOF QIII Bruker Daltonic (Germany) at ESI positive ionisation mode with the following settings: capillary voltage of 4500V, nebuliser pressure at 1.2 bar, and drying gas of 8 L/min at 200°C. Mass range was set between 50 to 1000 m/z.

3. Results and discussions
The formulated synthetic wastewater has the following characteristics: pH 7.01, total COD 6400 mg/L, soluble COD 3800 mg/L, BOD 1142 mg/L and MLSS 33 mg/L. The wastewater was subsequently diluted to achieve soluble COD of 1127 ± 138 mg/L. Anaerobic digested sludge which was used for the inoculum has the following characteristics: pH 6.86, total COD 6300 mg/L, soluble COD 390 mg/L, MLSS 12067 mg/L and MLVSS 8833 mg/L.

Throughout the experiment, pH of the mixture maintained within neutral range, at 7.03 ± 0.29. Figure 2 shows the graphical performance of overall COD removal. The removal of COD in the first seven days was only 3.61 ± 2.41% with respect to the initial COD concentration. The performance then significantly improved on Day 14 to more than 40% removal. By Day 30 onwards, more than 90% of COD was successfully removed from the anaerobic process, achieving COD concentration as low as 33 mg/L.

Low COD removal in the first seven days may be due to high availability of soluble organics from hydrolysis stage as well as an active fermentation process, as per recorded by previous studies [34, 35]. Conversion of COD to methane gas was also the highest at this time, indicating active methanogenesis activity in the process. Methane gas production was the highest on Day 30 at 55.5 ± 0.52% which correlates with the highest COD removal. Consequently, as the availability of soluble COD decreases, methane gas composition also decreases. Biomass activity may still convert residual COD to methane, in soluble form instead of gas [28]. At the same time, the composition of CO_2 did not exceed 26% of the biogas composition in the experiment, as shown in Figure 3.

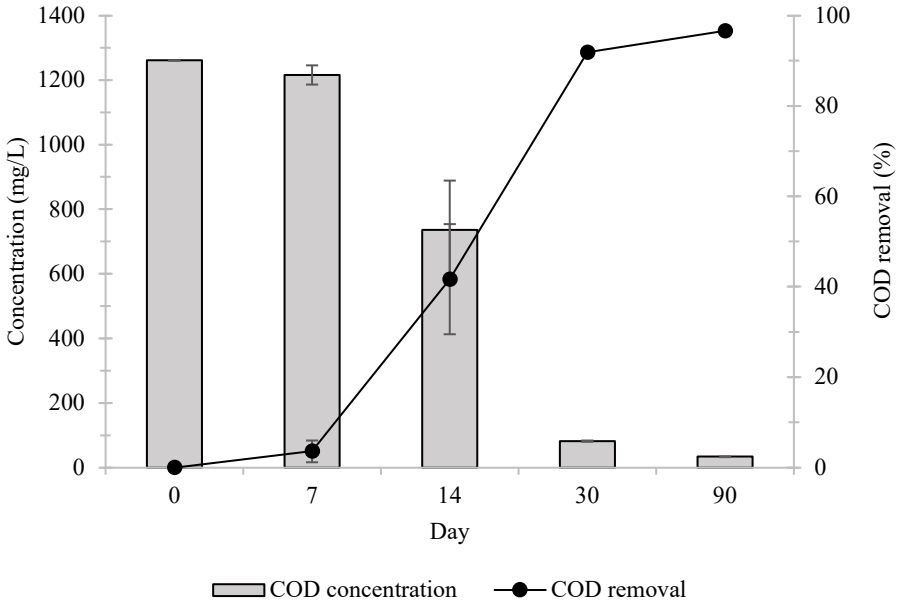

Figure 2. COD removal performance throughout experiment

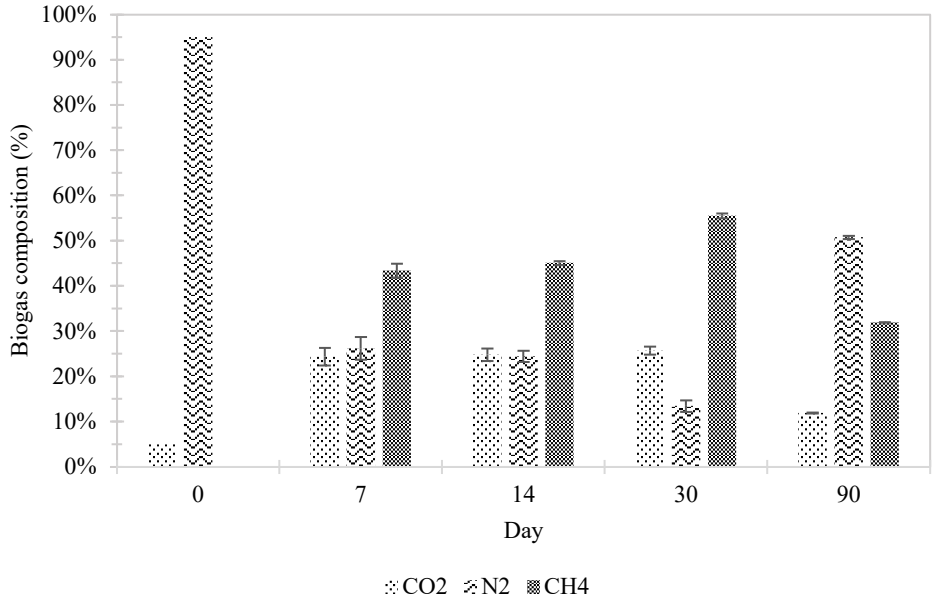

Figure 3. Biogas composition throughout the experiment

From the analysis of the standards, retention times for CAF, GCZ and PRZ are consistent at 6.2, 8.0 and 6.0min. The mass spectrometry of these compounds at its retention times can be seen in Figure 4. Limit of detection for the pharmaceutical compounds are 50 µg/L for CAF and 30 µg/L for GCZ and PRZ. Calibration curves for all three compounds have good linearity ($R^2 > 0.96$). Results of initial pharmaceutical compounds concentrations shown low recovery of CAF, GCZ and PRZ (20%, 43% and 11% respectively) from the initial wastewater analysis. Low recovery may be attributed by matrix effect from the interference of other components within the wastewater [36], especially since the initial soluble COD is considered high. Sample pre-concentration may be necessary to minimise the matrix effect and achieve better recovery for the three compounds in the future works.

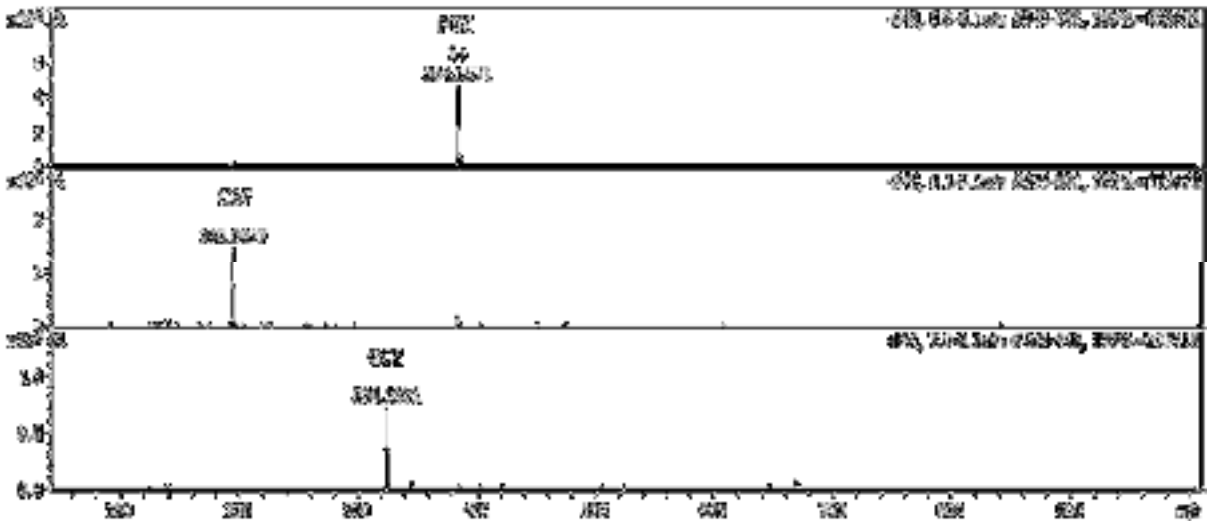

Figure 4. Mass spectrometry of PRZ (5.9 min), CAF (6.2 min) and GLZ (8.0 min)

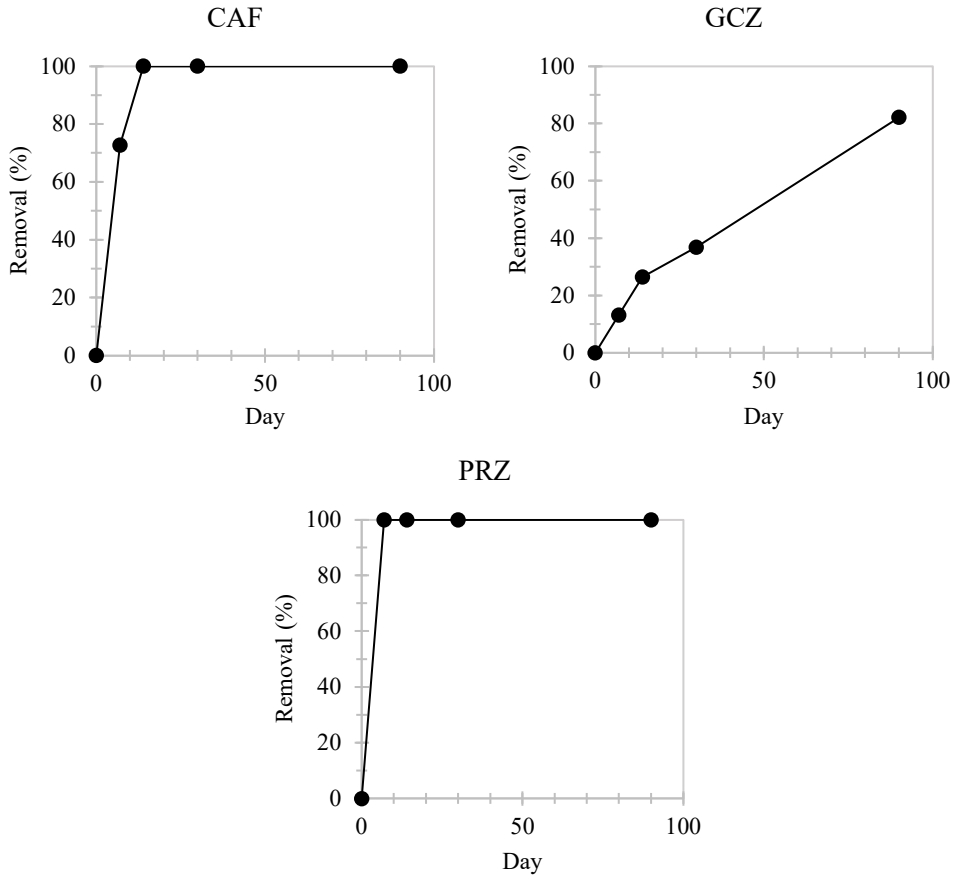

Figure 5. Removal efficiencies of CAF, GLZ and PRZ

Graphical representation of removal for the pharmaceutical compounds can be referred to Figure 5. Good removal of CAF and PRZ were observed with PRZ at higher removal rate compared to CAF. In relation to the anaerobic process, it is most likely that biodegradation of CAF and PRZ corresponded to the active stage of methanogenesis, especially for PRZ, as rapid utilisation of soluble organics for methane conversion were recorded between Day 0 to Day 14. Biodegradation of CAF is consistent with other studies [32, 37] and based on its hydrophilic characteristics [38], CAF is most likely biodegraded in this study than sorbed to solid phase. This also supports the feasibility of this compound as reference compound for this study. With respect to PRZ, rapid removal of this compound may be also due to biodegradation and biotransformation to metabolites. Relation can be made to the findings by Mohd Mohsi et al. (2019) which discovered the potential of *Bacillus spp.* in the biodegradation and biotransformation of PRZ in hospital wastewater [12]. GCZ removal took a longer time and show almost a linear trend compared to the other two compounds. At the end of the experiment, up to 83% of GCZ could be removed while concentrations of CAF and PRZ were well below detection limit. Persistency of GCZ has been recorded by Petrie et al. (2018) whereby GCZ is still present in the final effluent even after 12 months of treatment in horizontal sub-surface flow constructed wetlands, but the compound was not detected in the sludge [29].

4. Conclusions

From the batch experiment, it can be stated that COD removal achieved in this study is excellent considering the high initial COD concentration. The highest COD utilisation is also consistent with the surge of methane production. These results indicate that anaerobic treatment performance is not affected by the presence of the pharmaceutical compounds at the concentration level introduced in the process. Overall, removal performance of the pharmaceutical compounds in descending order is PRZ > CAF > GCZ. While PRZ has rapid removal in the first seven days, GCZ was observed to biodegrade at a slower rate and still not completely removed even after 90 days of reaction. To the best of knowledge, this

study is the first study that reports on the removal of PRZ and GCZ under anaerobic mesophilic condition. CAF has also proven to be a good biomarker for this study.

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