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Removals of atenolol, gliclazide and prazosin using sequencing batch reactor

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ABSTRACT

Emergence of organic micropollutants, specifically pharmaceutical compounds (PhCs) in receiving water bodies possess a great threat towards our ecosystem presently and in future. By that, evaluating and monitoring the removal of PhCs, specifically those highly consumed in a certain area, is considerably critical in attempt to minimize discharge of PhCs in our waters. Therefore, this study assessed the removal mechanisms of three highly consumed PhCs in Malaysia, namely atenolol, gliclazide and prazosin, by considering the hydrolysis, adsorption and biodegradation mechanisms of the selected compounds. Moreover, the removal of these compounds was demonstrated in an aerobic sequencing batch reactor (SBR) system treating actual domestic wastewater added with the selected compounds. The detection of PhCs was conducted using using Ultra-High Performance Liquid Chromatography Quadrupole-Time-Of-Flight Mass Spectrometry (UHPLC/QTOF-MS), followed by investigation of microbial community in the sludge sample by next generation sequencing (NGS). The results highlighted that atenolol was highly biodegradable with 83% efficiency in SBR system. Meanwhile, both gliclazide and prazosin show moderate biodegradation efficiency at 41%. The results also demonstrated that gliclazide and prazosin exhibited recalcitrant behavior towards the biological treatment. In addition, prazosin was presumed to be hydrolyzed and exist as different chemical structure in the aqueous phase during treatment process. The microbial community analysis revealed *Mycobacterium* as one of the potential microbes in biodegradation of PhCs.

1. Introduction

The increasing global industrialization has resulted in the detection of emerging micropollutants in wastewater as well as receiving water bodies which typically come from various anthropogenic activities, such as healthcare procedures, research & developments processes and manufacturing of the compound itself [1]. Pharmaceutical compounds (PhCs) are classified as one of the emerging micropollutants, whereby its presence may disturb the aquatic flora and fauna in the receiving water bodies and possess risk to human health [2]. It was reported that although the PhCs present at low concentration, it was suspected to disrupt water safety and ecosystem balance in the long run [3]. A study on human consumption of PhCs reported that up to 75% of the consumed drugs were excreted from human body through urine and feces [4]. Eventually, these PhCs are released into the sewage treatment plant (STP) facilities. Therefore, it is crucial to focus on the highly consumed medications in a certain area as there are high probability of PhCs to be released into the local STPs and subsequently the receiving water bodies [3]. Moreover, in many cases local STPs are also receiving effluents from neighboring hospitals and pharmaceutical industries that may lead to the presence of pharmaceutical compounds in alarming concentrations [5]. Therefore, STP plays an important role at preventing PhCs occurrence in the environment. Till date, extensive research has been conducted to demonstrate the ability of wastewater treatment system to

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remove the detected pharmaceutical compounds in wastewater [6].

In Malaysia, highly consumed pharmaceuticals include medications for high blood pressure or anti-hypertensive and anti-diabetic medications [7]. Thus, this study focused on three types of PhCs, namely atenolol, gliclazide and prazosin. Atenolol was ranked 2nd in the most consumed medication in Malaysia, whilst gliclazide was ranked 7th and prazosin was the top 45 in the list. Due to the high consumption of these medication, previous reports have highlighted its detection in water bodies (i.e., river), for instance, atenolol (high blood pressure medication) was detected at 149–1410 ng/L, while gliclazide (anti-diabetic) and prazosin (anti-hypertensive) were detected at 22–130 ng/L and 14–525 ng/L, respectively [8]. The presence of these compounds in water bodies demonstrated the limitations of local STPs in treating these PhCs. This limitation may be caused by different characteristics of pharmaceutical compounds in wastewater that were affected by its physicochemical properties, such as solubility, polarity, volatility, etc. [9]. Depending on its characteristics, these compounds may be abiotically hydrolyzed, adsorbed into the sludge or biologically degraded into metabolites [10]. Thus, study on the fate of PhCs in wastewater treatment system is required to maximize the removal of these compounds in wastewater.

Several wastewater treatment technologies reportedly involved in removal of pharmaceutical compounds via biological approach, including conventional activated sludge treatment (CAS) [11], anoxic/oxic sequencing batch reactor (SBR) [12], membrane bioreactor (MBR) [13], constructed wetland and algal photobioreactor [14]. Nevertheless, SBR system gained its popularity among other wastewater treatment systems due to its numerous advantageous, including its less energy demand and smaller carbon footprint [15]. Furthermore, the ability of SBR system to operate both aerobic and anoxic conditions in a single tank is expected to enhance the removal of PhCs [16]. For instance, biodegradation of atenolol was achieved in aerobic condition, while biodegradation of sulfamethoxazole was found to be higher in anoxic condition [17]. However, the ability of SBR system in removing various pharmaceutical compounds, especially those majorly consumed in a certain country need to be further investigated.

This study aims to demonstrate the removal of selected PhCs, namely atenolol, gliclazide and prazosin, which were listed as the most consumed medications in Malaysia [7]. This study elaborates the removal mechanisms of selected PhCs while assessing its removal efficiency in laboratory scale SBR system. The SBR system has been utilized to treat domestic wastewater, and these selected compounds were added into the influent. The microbial community of the sludge samples from the SBR system was also performed to reveal the important microorganisms in removal of PhCs.

2. Methodology

2.1. Preparation of PhCs, wastewater and activated sludge samples

The pharmaceutical stock solution was prepared by diluting 1000 mg/L of each atenolol, gliclazide and prazosin in methanol. The stock solution was kept at −20 °C and diluted with ultrapure water for further experimental period. Meanwhile, wastewater and activated sludge samples were collected from local STP, whereby the STP has successfully applied the SBR technology into the full-scale treatment system. The collected wastewater and activated sludge samples were filtered through 1 mm sieve to remove large debris that may clog the tubing associated with the experimental setup. The collected wastewater sample was characterized in terms of COD, NH_3-N and total phosphorus (TP) as listed in Table 1. All samples were stored in 4 °C refrigerator prior to be used.

Table 1
Characteristics of collected wastewater sample.

Parameters	Concentration (mg/L)
Chemical Oxygen Demand (COD)	167 ± 28
Ammoniacal Nitrogen (NH_3-N)	26.1 ± 11.4
Total Phosphorus (TP)	23.5 ± 32.6
pH	7.27 ± 0.08

2.2. Analyses of removal mechanisms

Batch experiment was conducted to study the roles of sorption, biodegradation and hydrolysis in removal of selected PhCs by applying different experimental conditions. A total of three reactors with different inoculating conditions were utilized. First reactor (R-AS) was inoculated with activated sludge and wastewater samples to demonstrate the biodegradation, sorption and hydrolysis of the PhCs. Second reactor (R-ACS) was inoculated with autoclaved activated sludge and wastewater samples, demonstrating the sorption and hydrolysis process, whereby the biodegradation was eliminated by suppressing microbial growth and activities through autoclaving. Lastly, third reactor (R-C) contained solely wastewater sample to demonstrate the hydrolysis of PhCS. All wastewater samples were added with 1 mg/L of each atenolol, gliclazide and prazosin, respectively. The three reactors were incubated for 24 h under continuous aeration, in which samples were collected at 0 h, 8 h, 12 h and 24 h incubation time for the further analysis of PhCs.

2.3. Removal of selected PhCs in SBR system

An SBR column with working volume of 3 L was utilized in this study, whereby the reactor has been continuously operated for treatment of domestic wastewater and has exhibited stable treatment efficiency. Fig. 1 shows the schematic diagram and picture of the SBR column. The reactor was operated at 4 h cyclic time, comprising of 15 min feeding period, 58 min non-aeration, 140 min aeration period, 20 min settling phase, 5 min decanting and 2 min idle periods. During feeding phase, 1.5 L wastewater was introduced containing selected PhCs at 1000 μg/L atenolol and gliclazide, as well as 100 μg/L prazosin, respectively. The concentrations of PhCs were chosen in such way to allow reliable measurement of more than 90% decrease in concentration over the experimental period, following the detection limit of these PhCs [18]. Moreover, the two concentrations were selected by considering the concentrations of selected PhCs in wastewater samples of previous literature [8]. During aeration period, aeration was provided using air compressor through microsparger located at the bottom of the column. The aeration flow rate was controlled using an air flow meter to maintain concentration of dissolved oxygen (DO) between 2 and 4 mg/L. By the end of the cycle, 1.5 L effluent was decanted from the column and the effluent was subjected for further analyses.

2.4. Analytical methods

There were three main analytical procedure used in this study, i.e., analysis for biological nutrient content in wastewater samples, liquid chromatography analysis and sludge molecular analysis. The analysis of COD, NH_3-N and TP were conducted according to the Standard Methods for the Examination of Water and Wastewater [19].

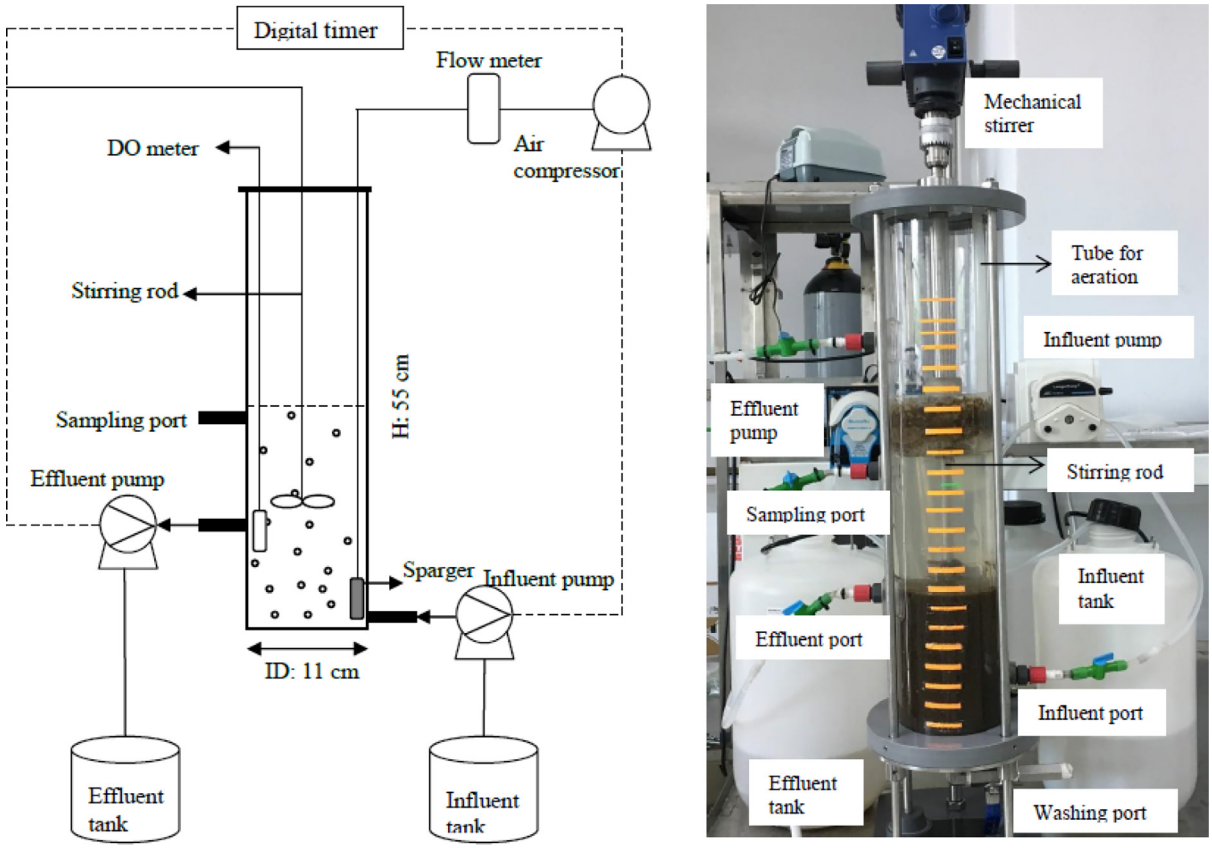

Fig 1. Schematic diagram and picture of laboratory scale SBR system used in this study.

The quantification of PhCs were conducted using Ultra-High Performance Liquid Chromatography Quadrupole-Time-Of-Flight Mass Spectrometry (UHPLC/QTOF-MS) system. Separation of 10 µL samples injected was performed using Thermo Scientific C18 column (AcclaimTM Polar Advantage II, 3 × 150 mm, 3 µm particle size) on an UltiMate 3000 UHPLC system (Dionex) equipped with a vacuum degasser, a quaternary pump, and an autosampler. Gradient elution was performed at flow rate of 0.3 mL/min and 40 °C column temperature using a mobile phase of 0.1% formic acid in water (A) and 100% acetonitrile (B) with 25 min total run time. The elution started at 5% B and was then linearly increased to 60% B at min 3, further increased to 97% B at min 6, and then kept isocratic until min 11. Then, the elution was returned to its starting conditions. High resolution mass spectrometry of targeted compound (atenolol, gliclazide and prazosin) was carried out using a MicroTOF QIII Bruker Daltonic using an ESI positive ionization with the following settings of capillary voltage, 4500 V; nebulizer pressure, 1.2 bar; drying gas, 8 L/min at 200 °C. The mass range was set at 50–1000 *m/z*. The accurate mass data of the molecular ions, provided by the TOF analyzer, were processed by Compass Data Analysis software (Bruker Daltonik GmbH). Each compound having limit of detection (LOD) of 30 ug/L (atenolol) and 20 ug/L (prazosin and gliclazide) with percentage recovery of >100% [6,8].

The molecular analysis was performed to determine the important microbial groups during biodegradation of the selected PhCs. These included the extraction of genomic DNA (gDNA), polymerase chain reaction (PCR) to amplify the targeted DNA sequence and Next Generation Sequencing (NGS) to investigate the microbial community of the activated sludge sample in SBR column before and after the addition of PhCs in the system. The extracted gDNA were subjected to PCR to amplify targeted 16 s rRNA genes in V3 and V4 region by using forward primer (5′-CCTAYGGGRBGCASCAG) and reverse primer (3′- GGACTACNNGGGTATCTAAT). The PCR products were then used for library preparation. The library preparation and NGS were conducted using Illumina Miseq (USA).

3. Results and discussions

3.1. Removal mechanisms of selected PhCs

The removal of atenolol, gliclazide and prazosin in batch experiments was performed to investigate different removal mechanisms that might be involved in removal of PhCs, namely hydrolysis, sorption and biodegradation of PhCs [9]. Through abiotic degradation, pharmaceuticals can undergo hydrolysis process where the PhCs react with wastewater and form a new compound. The hydrolysis of PhCs depends on the pH value of the compounds and the surrounding aqueous condition [10]. Other than that, the PhCs also likely to undergo sorption process where the compounds adhere on the surface of sludge or incorporated into the sludge. Meanwhile, biodegradation is a process whereby the microorganisms transform or alter the structure of chemical compound through metabolic or enzymatic activity [20]. The mechanisms of PhCs removal are highly dependent on the physicochemical properties of the compounds, which include solubility, polarity, volatility dissociation, partition coefficient, etc., whereby the PhCs may be able to undergo either all mechanisms (i.e., hydrolysis, sorption and biodegradation) or only one or two of the processes.

In this study, reactor R-AS containing both activated sludge and wastewater samples demonstrated all mechanisms, i.e., biodegradation through microbial activities in sludge samples, sorption through the attachment to microbial cells and hydrolysis in

wastewater sample. Meanwhile, reactor R-ACS eliminated the biodegradation mechanism by suppressing the microbial growth and activities, but retaining the sorption mechanisms to the inoculated sludge and hydrolysis in the wastewater sample. Autoclaving was selected to inhibit the microbes as the procedure provide complete inactivation of the sludge, although changes on sludge structure may be observed [21]. In addition, reactor R-C, which only contained wastewater sample represented the hydrolysis process in wastewater as there are no possibilities of biodegradation and sorption processes [22]. Thus, the concentrations of the PhCs due to biodegradation and sorption mechanism was calculated using the following equation:

$$Biodegradation = (R - AS) - (R - ACS) - (R - C)$$

$$Sorption = (R - ACS) - (R - C)$$

Meanwhile, the removal efficiency of PhCs was calculated as:

$$Removal\ efficiency(\%) = \frac{C_f - C_i}{C_i} x100\%$$

where C_i is the initial concentration of compounds and C_f is the final concentration at designated time intervals.

Fig. 2(a) illustrates the removal of atenolol in different conditions. Atenolol was not removed in R-ACS and R-C which indicated that atenolol was not sorbed to the sludge and neither hydrolyzed in wastewater. Meanwhile, in R-AS, up to 90% of the atenolol was successfully removed. Hence, this study demonstrated that the main removal mechanism of atenolol in aerobic condition was based on biodegradation. This result was aligned with previous study by Stevens-Garmon et al. that reported atenolol has a low potential to be absorbed into sludge as it is less hydrophobic at pH 7 [23].

Fig. 2(b) highlights the removal of gliclazide and it was found that 63% and 34% of gliclazide was removed in R-AS and R-ACS, respectively. Meanwhile, the removal of gliclazide in R-C was neglected as it was not significant. Determination of the biodegradation percentage of gliclazide was calculated by evaluating the differences of remaining concentration in all reactors. Hence, the biodegradation of gliclazide was demonstrated at 29% after 24 h of incubation and the sorption of gliclazide was reported at 34%. The biodegradation of gliclazide in this study was higher compared to previous study that reported 15% of gliclazide degradation after 22 days of experimental period [18]. Removal of gliclazide may be related to the transformation of the parent compound to carboxyl and hydroxyl metabolites [24]. The findings in this study suggested that removal of gliclazide may be achieved in activated sludge system, however prompting for more investigation.

Fig. 2(c) shows the removal of prazosin by various mechanisms in all respective reactors. It was worth noting that removal effi-

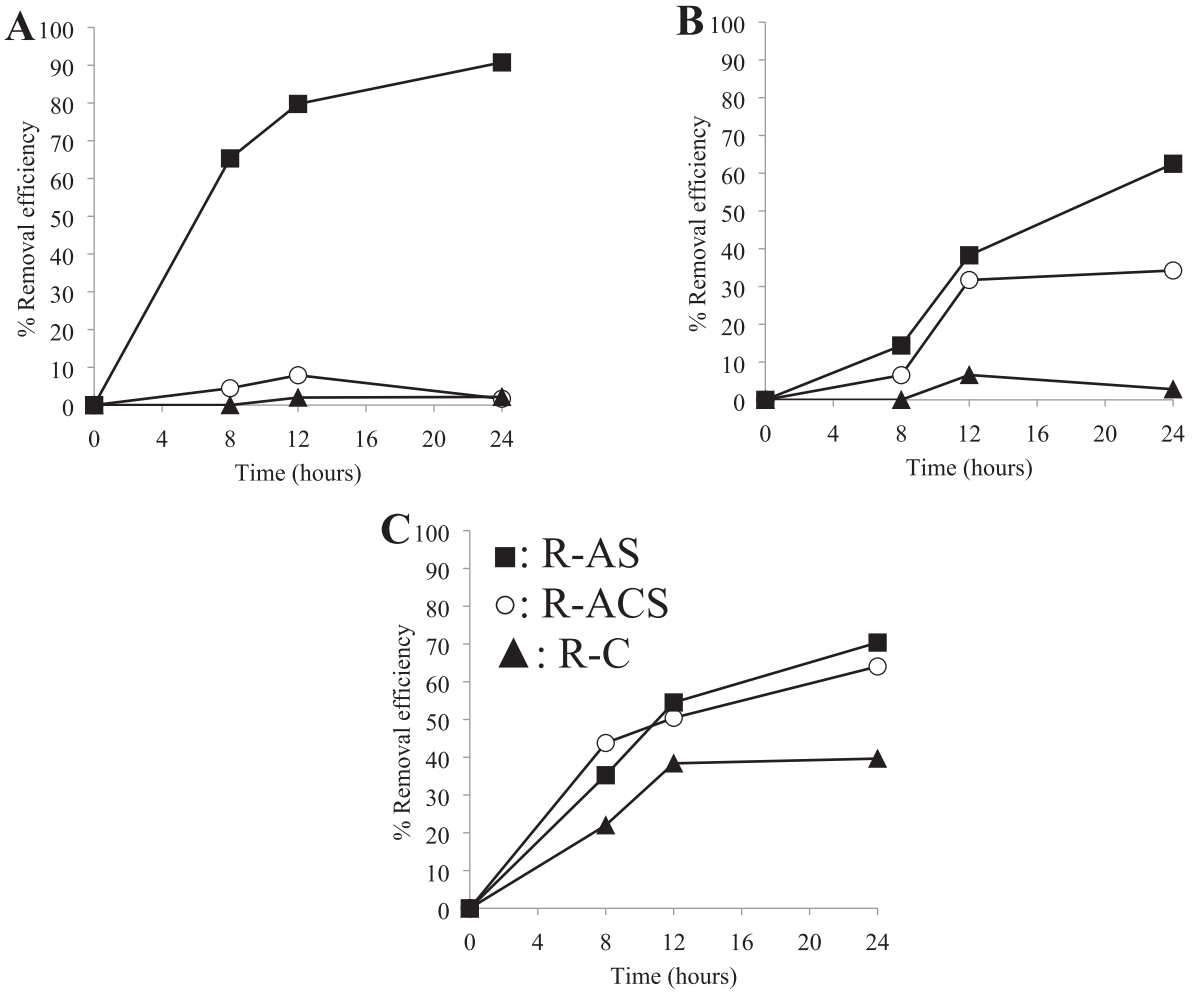

Fig. 2. Removal efficiency of (a) atenolol (b) gliclazide and (b) prazosin under different experimental conditions, namely R-AS containing activated sludge, R-ACS containing autoclaved sludge sample and R-C containing only wastewater.

ciency of prazosin in R-C, which was due to hydrolysis was relatively high at 40% which may be attributed to its low solubility in aqueous phase [25]. Meanwhile, the sorption of prazosin was calculated and was found at 24%. After accounting the sorption and hydrolysis of prazosin, the biodegradation percentage was reported at only 6%. Nevertheless, there has been limited study on the biodegradation or removal of prazosin in wastewater and fate of prazosin in wastewater remained unknown.

In summary, the removal mechanisms of atenolol, gliclazide and prazosin in this study is represented in Fig. 3. Atenolol exhibited highest biodegradability at 90% biodegradation percentage, followed by gliclazide at 29% and prazosin at 6%. Removal of gliclazide may also be achieved through sorption process at 34%, while removal of prazosin was mainly due to hydrolysis process at 40%, respectively. The results also demonstrated the recalcitrant characteristics of gliclazide and prazosin as its removal efficiency was only reported at less than 70% after considering all mechanisms of biodegradation, sorption and hydrolysis.

3.2. Removal of pharmaceutical compounds in SBR system

The removal efficiencies of atenolol, gliclazide and prazosin in the SBR system throughout 30 days of experimental period are illustrated in Fig. 4. The average removal efficiencies of atenolol, gliclazide and prazosin were at 83%, 41% and 41%, respectively. The highest removal efficiency was demonstrated for atenolol, aligned with the previous discussion in Section 3.1. Removal efficiency of atenolol reached 81% after three days of SBR operation and found to be steadily increased throughout the experimental period. The highest atenolol removal efficiency was at 88% on day 30. The results were significantly better than previous reports achieving 63% removal efficiency by applying both anoxic and aerobic phases [17]. However, up to 89% atenolol removal efficiency was reported in fully aerobic SBR system with DO concentration more than 2 mg/L [17]. Meanwhile, only 73% atenolol removal efficiency was recorded under microaerobic condition with DO concentration of 0.3 mg/L [17]. It was highlighted that atenolol removal efficiency was better in aerobic conditions with high DO concentration, thus, modification of aeration strategy and DO monitoring could be implemented in future studies to further increase the atenolol removals [26,27]. In addition, atenolol removal was found to be related with successful nitrification, which occurred in aerobic conditions, whereby nitrification process aids to co-metabolically oxidize atenolol [28]. The high abundance of ammonia oxidizing bacteria (AOB) was also related with high atenolol removal efficiency. Thus, atenolol was concluded as non-recalcitrant in biological wastewater treatment process. This study has demonstrated good atenolol removal efficiency in SBR system comparable with previous literatures as summarized in Table 2.

On the other hand, removal efficiency of gliclazide increased from 25% to 36% from day 3 to day 6 of experimental period. The efficiency then further increased to 49% on day 18, but slightly decreased to 47% towards the end of experiments. Gliclazide was likely bound to the organic phases based on its lipophilicity and solubility properties [24]. Previous study described that gliclazide would most probably accumulate in organic matter and the tissues of living organisms rather than remain in aqueous phase in the environment. Therefore, gliclazide was expected to be absorbed to the sludge rather than biodegraded, as mentioned earlier. When sorption happened, there was probability that desorption may occur [24]. Desorption process may be beneficial as it may allow higher biodegradation rate of the compound in the aqueous phase [24]. In this study, desorption occurred on day 12 throughout the sudden decreased of gliclazide removal efficiency. This study has recorded higher removal efficiency of gliclazide as compared to different experimental conditions, achieving less than 20% gliclazide removal efficiency [18]. Nevertheless, the average gliclazide removal efficiency was reported at 39% after 30 days of experimental period, indicating the recalcitrant properties of this compound in biological wastewater treatment system. However, future studies focusing on sorption process of gliclazide may be conducted to fully understand the mechanisms. Moreover, the detection of gliclazide in activated sludge sample may be performed to detect the attached compound and assess the possible compounds recovery.

Removal efficiency of prazosin after six days of experiments was found to be highest at 46% and remained constant throughout the study. Prazosin removal was reported to be mainly from hydrolysis and sorption process, while it was found to be recalcitrant to biodegradation process [29]. Therefore, the prazosin removal efficiency was not increased throughout the experimental period. Although previous study has reported the roles of *Bacillus* sp. in biodegradation of prazosin, there has been no conclusive evidence of its efficiency in SBR system [30]. However, another study utilizing electrochemical process has demonstrated up to 77% prazosin removal in wastewater as shown in Table 2. Thus, prazosin removal might be challenging in biological wastewater treatment process, but utilization of physical or chemical treatment process by the end of wastewater treatment system may be applied to fully removed prazosin from the wastewater.

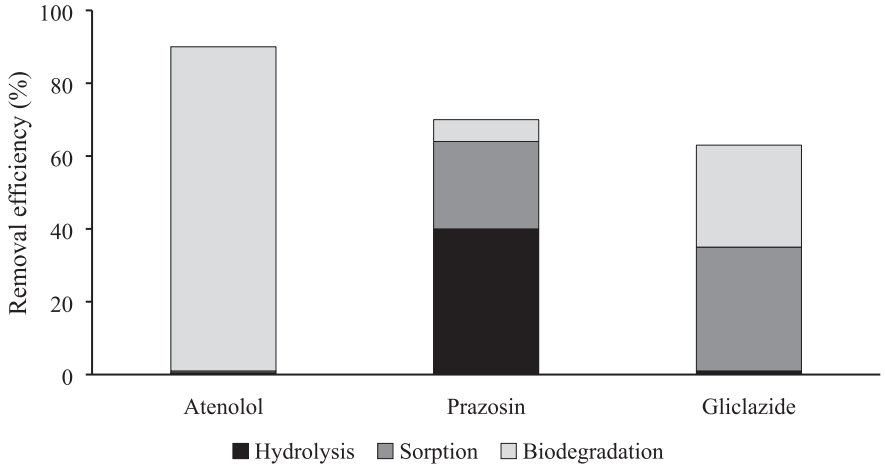

Fig. 3. Removal percentage of atenolol, gliclazide and prazosin based on the hydrolysis, sorption and biodegradation of the pharmaceutical compounds.

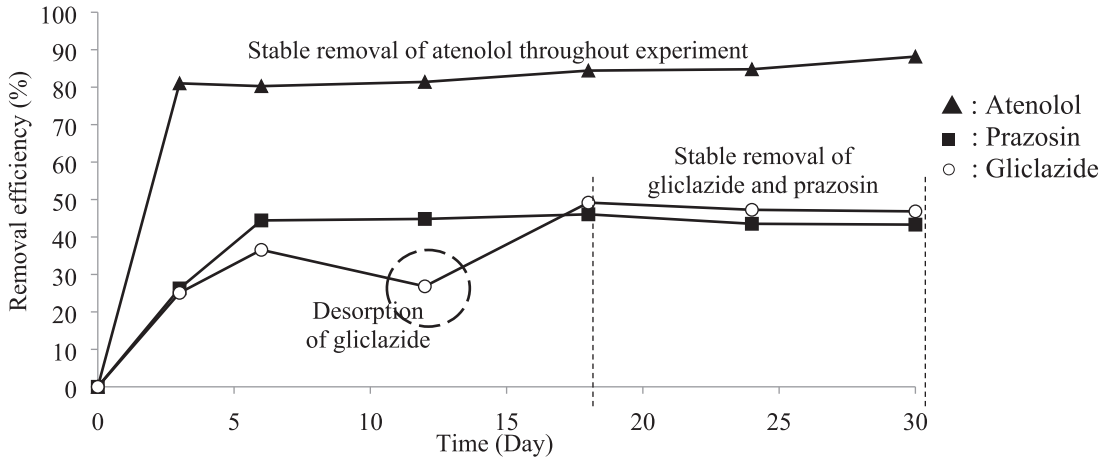

Fig. 4. Removal of atenolol, gliclazide and prazosin in SBR system for 30 days of experimental period.

Table 2
Removal efficiencies of atenolol, gliclazide and prazosin in this study as compared to previous reports.

Compounds	Removal efficiencies	System	Reference
Atenolol	83%	SBR system: anoxic and aerobic phase	This study
	89%	SBR system: full aerobic	[17]
	63%	SBR system: anoxic and aerobic phase	[17]
	80%	Microalgae based photobioreactor	[28]
Gliclazide	39%	SBR system: anoxic and aerobic phase	This study
	15%	Batch reactor	[18]
	18%	Manometric respiratory test	[18]
Prazosin	41%	SBR system: anoxic and aerobic phase	This study
	77%	Electrochemical process	[29]

3.3. Microbial community in SBR system

The activated sludge samples on initial (sample I) and final days (sample II) of experimental period were subjected for analysis of microbial community. A total of 391 OTUs were detected on the initial sludge sample while a 425 OTUs were detected after 30 days of experimental period. The presence of PhCs in the feeding of SBR system has increased the microbial richness and diversities in the activated sludge. The major groups of phyla detected were Proteobacteria, Actinobacteria, Bacteroidetes, Chloroflexi and Patescibacteria. Proteobacteria were dominant in both samples with a relative abundance of 30% and 31% in sample I and II, respectively. In sample I, Chloroflexi (19%), Bacteroidetes (17%), Actinobacteria (17%) and Patescibacteria (6%) were the most common after Proteobacteria. Other phyla, such as Acidobacteria, Verrucomicrobia and Nitrospirae were detected at 3%, while Firmicutes was 1%. In the meanwhile, in sample II, Actinobacteria was detected at 22% after Proteobacteria followed by Bacteroidetes (20%), Chloroflexi (13%), Patescibacteria (7%), Verrucomicrobia (3%), and Acidobacteria (2%). Nitrospirae and Firmicutes were

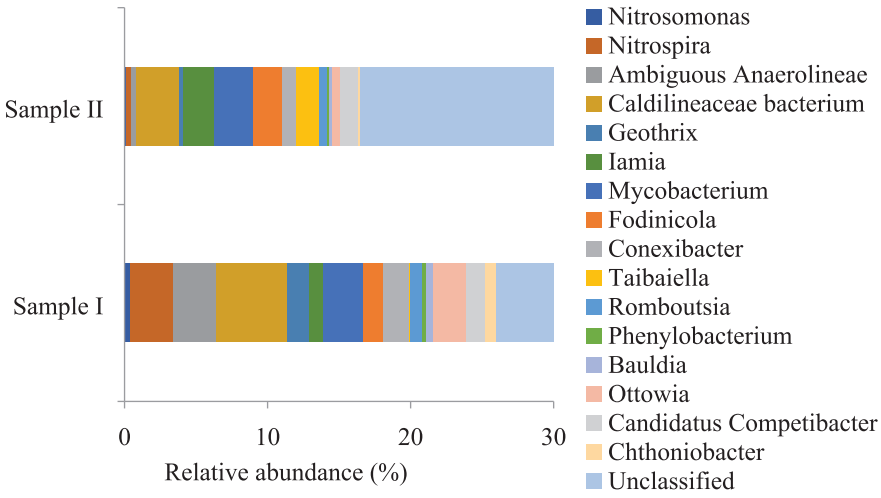

Fig. 5. Classification of microbial community in sludge samples from SBR system on day-0 (sample I) and day-30 (sample II) of reactor operation.

detected higher than 1% in sample I, but, were detected at 0.7% (Firmicutes) and 0.4% (Nitrospirae) in sample II.

At genus level (Fig. 5), the results highlighted the increased of relative abundance of *Iamia*, *Fodinicola* and *Taibaiella* in the sample II, indicating that they may facilitate the biodegradation of atenolol, gliclazide and prazosin. Relative abundance of *Iamia* was increased from 1% to 2.2% in sample I and II, respectively. In addition, *Fodinicola* was increased from 1.4% to 2.2% in sample I and II, while *Taibaiella* was increased significantly from 0.1% to 1.6% in sample I and II, respectively. However, further analyses are required to identify their roles in biodegradation process. Among other species that survived under presence of PhC were *Mycobacterium* and *Rombutsia*, demonstrating their resistance towards these compounds. *Mycobacterium* was commonly found in the wastewater treatment plant and it was reported to be able to degrade aromatic hydrocarbons and nitrogen-containing heterocycles, such as morpholine [31].

In addition, the other bacteria that were reported to be able to degrade complex chemical compounds, including *Geothrix*, *Phenylobacterium* and *Ottowia* were also detected in the activated sludge sample. *Geothrix* was found to be able to reduce ferric iron [Fe(III)] in the aromatic hydrocarbons degradation, while, *Phenylobacterium* was reported to be able to degrade chloridazon which is an active ingredient of the herbicide Pyramin. Additionally, *Ottowia*, which is a bacterium that can utilize carboxylic acids and amino acids as substrates was reported to be able to assist phenol degradation. The three genera of bacteria, *Geothrix, Phenylobacterium* and *Ottowia*, presence in the sample might contribute to degradation of pharmaceutical compounds studied which are atenolol, gliclazide and prazosin. However, further investigation is required in order to understand and recognize the specific bacteria which assist the breakdown and degradation of pharmaceutical compounds specifically atenolol, gliclazide and prazosin.

4. Conclusions

This study has successfully demonstrated the removal mechanisms of atenolol, gliclazide and prazosin in which these compounds were analyzed for biodegradation, sorption and hydrolysis mechanisms. In addition, the removal efficiency of the selected PhCs in laboratory scale SBR system was elucidated. Atenolol was found to be biodegradable and achieved 88% removal efficiency in laboratory scale SBR system. Gliclazide was moderately removed and show persistency behavior toward biological treatment with only 41% removal in SBR. Meanwhile, prazosin was removed at 41% in SBR. The results highlighted that atenolol was biodegradable in activated sludge. Meanwhile, gliclazide was found to be sorbed into or onto the sludge while prazosin seems to be hydrolysed and exist as different structure in the aqueous phase. Biodegradation of gliclazide and prazosin was found to be relatively low, demonstrated the recalcitrant characteristics of these compounds in biological treatment process. Analysis of the microbial community shows that the relative abundance of functional bacteria for nitrification and denitrification decreased after addition of PhCs. Among functional bacteria detected after addition of PhCs were *Iamia*, *Fodinicola*, *Taibaiella* and *Mycobaterium* that may contribute to the biodegradation of selected pharmaceutical compounds.

CRediT authorship contribution statement

Yasmin Munirah Mat Zaini: Investigation, Writing – original draft, Visualization. **Laila Dina Amalia Purba:** Investigation, Writing – original draft, Visualization. **Norhayati Abdullah:** Writing – review & editing, Supervision, Project administration. **Ali Yuzir:** Writing – review & editing, Supervision, Funding acquisition. **Koji Iwamoto:** Writing – review & editing, Supervision. **Shaza Eva Mohamad:** Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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