Full Length Research Paper

Removal of Glyphosate from Water: Applying Coupled Sequencing Batch Reactor (SBR)-Adsorption Method

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Wastewater discharged from Glyphosate manufacturing is a major environmental concern due to its complicated treatment process. In this study, the performance of a sequencing batch reactor (MSBR)-adsorption process treating Glyphosate wastewater was investigated. Operation results from SBR process showed that effluent’s chemical oxygen demand (COD) removal efficiency, total suspended solids (TSS) and total phosphorus (TP) were 40 %, 105 mgL⁻¹ and 55 mgL⁻¹, respectively. However, the result of TP concentration did not meet the discharge limitation. The SBR effluent was then undergone an adsorption process using palm kernel shell-based activated carbon (PKS-AC). Minimum adsorbent dosage of 7 gL⁻¹ was needed to further reduce TP concentration to discharge limitation of 2 mgL⁻¹.

Key words: Glyphosate; biodegradation; sequencing batch reactor (SBR); adsorption; palm kernel shell

1. INTRODUCTION

Glyphosate [N-(Phosphonomethyl)glycine] is a nonselective herbicide. Glyphosate acid and its salts are moderately toxic compounds in environmental protection agency (EPA) toxicity class II. The half-life of Glyphosate in soil is between 3-174 days. Glyphosate herbicide was first developed for use in agricultural purposes in the early 1970s. Table 1 shows the chemical structure of Glyphosate.

Glyphosate is essentially nontoxic to mammals and birds, but fish and invertebrates are sensitive to the herbicide. It was reported that Glyphosate was lethal to amphibians under natural conditions (Relyea, 2005). The mortality rate due to Glyphosate poisoning is reported to be between 7.5% and 16.7% (Motojyuku et al., 2008). Environmental Protection Agency (EPA) found 1 mgL⁻¹ as the safe level of Glyphosate concentration in drinking water (EPA, 2006).

One modification of conventional biological method e.g. activated sludge is sequencing batch reactor (SBR). SBR is used for wastewater effluent less than 20000 m³d. A SBR, basically, is similar to an activated sludge process, with this difference that aeration, sedimentation and clarification are achieved in a single batch reactor. Although SBR is not novel it has been successfully used for a variety of hazardous and high strength industrial wastewater, such as winery, tannery and petrochemical. SBR process possesses the unique ability of extending and shortening the react phase or handling intermittent influent (Kulkarni, 2012).

### Table 1: Chemical name and chemical structure of Glyphosate and its salt.

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>Chemical structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glyphosate parent acid</td>
<td>( \text{HO} - \text{C} - \text{N} - \text{P} - \text{O}^- \text{H} )</td>
</tr>
<tr>
<td>Glyphosate salt</td>
<td>( \text{HO} - \text{C} - \text{N} - \text{P} - \text{O}^- \text{H}_2 )</td>
</tr>
</tbody>
</table>
Previous studies reported that Glyphosate can be converted to aminomethylphosphonic acid (AMPA) using a variety of bacterial strains from soil, water or activated sludge, but only a small amount of AMPA degrades to further metabolites. As a result, the AMPA accumulated in industrial reactor contains Glyphosate waste (McAuliffe et al., 1990). In this study, an attempt is made to remove Glyphosate and its metabolites from aqueous solution by using coupled SBR-adsorption method. The final Glyphosate concentration, chemical oxygen demand (COD) and total phosphorus (TP) are considered as key values for performance of the coupled SBR-adsorption method.

2. MATERIALS AND METHODS

2.1 Sequencing batch reactor (SBR)-adsorption set-up

The schematic diagram of experimental set-up is shown (Figure 1). The SBR-Adsorption unit consisted of a 5.0 L glass beaker with 4.0 L practical volume. The SBR was sealed including ports for feeding, discharging, sample collection, supplying air and dissolved oxygen (DO) and pH probe. Feeding and discharging were accomplished by using a peristaltic pump. The SBR was operated at the ambient temperature of around 28 ± 2 °C. Solution was being mixed using mechanical stirrer at a speed of 250 rpm. During the experiments dissolved oxygen (DO) of 3 mgL⁻¹ was maintained.

When the performance of SBR has stabilized, i.e. effluent parameters COD and Glyphosate concentration reached to a steady state, at the end of each cycle, the clarified solution was discharged from the top of the SBR. The effluent passed through a filter paper (Advantec, No.5B) to avoid clogging the palm kernel shell-based activated carbon (PKS-AC).

In this work PKS-AC form (KD Technology, Malaysia) was used as adsorbent. PKS-AC in granular form with mesh sizes (based company information), i.e. 0.60 –1.70mm, was used. PKS-AC was in the laboratory grade and used directly as received from the supplier. Table 2 represents the characteristics of PKS-AC.

The effect of pH on aminomethylphosphonic acid (AMPA) adsorption onto PKS-AC was studied by adjusting the pH of synthetic solution of AMPA with diluted HCl or NaOH solution in the range 2.0 to 10. The experiments were carried out using 50 mgL⁻¹ AMPA and 1 gL⁻¹ PKS-AC as adsorbate and adsorbent, respectively. The adsorption process (post-treatment) was carried out at different PKS-AC dosages of 1, 3, 5, 6 and 7 gL⁻¹ and pH of 2.5. The experiments were accomplished at the mechanical stirrer speed of 150 rpm for 6 h. the adsorption process was followed by a 30 min settling step to separate PKS-AC from solution. For each cycle, the old adsorbent particles were displaced by new granule PKS-AC. To separate the adsorbent from the AMPA solution, the sample was passed through the filter paper.

![Fig.1: Schematic diagram of a SBR-Adsorption unit.](image)
Table 2: Characteristics of PKS – based activated carbon.

<table>
<thead>
<tr>
<th>PKS AC</th>
<th>Average particle size (mm)</th>
<th>( S_{BET} ) (m(^2)/g)</th>
<th>( V_{micro} ) (cm(^3)/g)</th>
<th>( V_{meso} ) (cm(^3)/g)</th>
<th>( V_{total} ) (cm(^3)/g)</th>
<th>( D_{micro} ) (Å)</th>
<th>( D_{meso} ) (Å)</th>
<th>( \rho ) (Kg/L)</th>
<th>Ash content</th>
</tr>
</thead>
<tbody>
<tr>
<td>PKS</td>
<td>0.60-1.70</td>
<td>1237</td>
<td>0.47</td>
<td>0.09</td>
<td>0.595</td>
<td>5.3</td>
<td>24.8</td>
<td>0.924</td>
<td>&lt;6%</td>
</tr>
</tbody>
</table>

2.2 Start-up

Herbicide Glyphosate was used to simulate the industrial wastewater. The SBR was seeded with prepared mixed bacteria. To enhance the bacterial growth, Glyphosate was diluted with distilled water containing inorganic nutrients i.e. \( K_2HPO_4 \); \( KH_2PO_4 \); (NH\(_4\))SO\(_4\) according to the minimum nutrient requirement BOD:N:P ratio of 100:5:1 (Metcalf and Eddy, 2003). The system was being aerated, mixed and fed with organic loading rate (OLR) of 57 mg CODL\(^{-1}\)d\(^{-1}\) until the biomass concentration of 500 mgL\(^{-1}\) was obtained. During this process no biomass was added or withdrawn. The cycle of one SBR run was 24 h with the following stages: Filling; aeration and settling-drawing. The SBR was fed applying aerated fill method. At the filling stage the SBR was filled within 60 min, then aeration continued for 21 h. This was followed by settling-drawing of 2 h.

At the end of the adsorption process the values of Glyphosate concentration, COD, total suspended solids (TSS), total phosphorus (TP) and turbidity were measured. All experiments were duplicated. Measurement of COD was performed by passing solution through a 0.45 µm filter.

2.3 SBR performance

A phosphate buffer was used to maintain the pH of solution at 6.5 during the filling and aeration stages. Operating parameters are presented in Table 3. To obtain the desired food/bio-mass (F/M) ratio, total suspended solid (TSS) and to maintain a vial bacterial system, the sludge retention time (SRT) was maintained by wasting the excess sludge from SBR. To investigate the effect of different parameters i.e. F/M ratio and OLR batches with varying parameters were prepared. These parameters were calculated by using the Equations 1-4.

\[
Q = f .N \\
OLR = \frac{Q .COD_{in}}{V} \\
HRT = \frac{V}{Q} \\
F/M = \frac{OLR}{MLVSS}
\]

where, \( Q \) is influent volume, \( f \) is influent volume per cycle, \( N \) is number of cycle per day and \( V \) is volume of SBR.

SBR was fed by a fresh solution every day and any new set up was operated to reach a steady state condition for at least 3 cycles (Chan et al., 2010). The steady state was assessed by measuring the percentage of COD removal.

2.4 Analytical methods

The measured parameters COD, biological oxygen demand (BOD), sludge volume index (SVI), mixed liquor suspended solid (MLSS) and mixed liquor volatile suspended solid (MLVSS) were carried out according to standard methods (Baird, 2005).

Table 3: Operating parameters of SBR.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>F/M ratio (gCODgMLVSS(^{-1})d(^{-1}))</td>
<td>0.13-0.4</td>
</tr>
<tr>
<td>HRT (d)</td>
<td>1-3</td>
</tr>
<tr>
<td>Influent Glyphosate (mgL(^{-1}))</td>
<td>300</td>
</tr>
<tr>
<td>SRT (d)</td>
<td>10</td>
</tr>
<tr>
<td>TP (mgL(^{-1}))</td>
<td>55</td>
</tr>
</tbody>
</table>
The measurement of TP was carried out by forming phosphomolybdate complex that was reduced to intensely colored molybdenum blue by the ascorbic acid. The absorbance of blue complex was measured by a spectrophotometer (GENEYES 10 UV-USA) at 880 nm.

The Glyphosate was measured by the method described before (Nourouzi et al., 2011). The morphology of PKS-AC particles coated with Au was observed by scanning the electron microscope (SEM) at an accelerated voltage of 15 kV (S-3400N, Hitachi).

3. RESULTS AND DISCUSSION

3.1 SBR performance

To assess the performance of SBR for treatment of Glyphosate aqueous solution, the reactor was operated in a sequencing batch mode for a 24 h cycle. After starting up, Glyphosate degrading bacteria began to degrade the target compound. A statistical analysis (ANOVA) was carried out on the effluent COD of SBR. The P values (> 0.05) of COD at five last cycle of SBR implied that there was no significant difference in final COD and the system reached to a stable state.

The SBR was conducted at OLR of 57 to 170 mg COD L⁻¹ d⁻¹. Corresponding Glyphosate concentration in influent was 300 mg L⁻¹. The BOD:COD ratio corresponding to the influent was approximately 0.23. The values of MLSS and MLVS both represent the concentration of microorganisms in the system. To obtain the volatile fraction of suspended solid the ratio of MLVSS:MLSS was calculated. The results showed that the value of fraction was more than 0.95. Table 4 represents the resulting SBR effluent parameters after the biological treatment (Figure 2). The presented data are the average of three measurements.
The MLVSS value for a SBR system and conventional load ranges from 2000 to 4000 mgL$^{-1}$ (Corbitt, 1990). In this work, the value of MLVSS was lower than 2000 mgL$^{-1}$ with the corresponding F/M ratio ranging between 0.08 and 0.2 g COD g MLVSS$^{-1}$d$^{-1}$. It can be observed from Figure 2 that the MLVSS concentration increased slowly from 500 to constant value of 1130 mgL$^{-1}$ with the average growth yield of 0.06 mgbiomass mgCOD$^{-1}$. The low growth yield of Glyphosate degrading bacteria can be attributed to its metabolite pathway. The major metabolites and bacterial degradation pathway of Glyphosate is shown (Figure 3). Microorganisms can utilize Glyphosate as carbon source and lead to the production of aminomethylphosphonic acid (AMPA) (Solomon et al., 2007). Almost all Glyphosates convert to AMPA in the presence of inorganic phosphate but only few percentage of AMPA degrades to further metabolites. Thus, the bacterial growth was limited by the availability of a carbon source.
The results declared that in all three HRT of 1, 2 and 3 d, approximately all Glyphosates were converted to their metabolites while about 40 percent of COD removal was achieved as shown in Table 4 and Figure 2. The percentage of COD removal at the initial stage was 35 % and it increased to 40 % with the increase of MLVSS from 500 to 680 mgL\(^{-1}\). Later on, the percentage of COD removal decreased to 33 % with the decrease of HRT from 3 to 1 d. The percentage of COD removal again increased to 40 % as the experiment progressed (approximately after 17 number of cycle) with the increase of MLVSS concentration to 800 mgL\(^{-1}\). The same results were observed when HRT decreased from 2 to 1 d. It can be concluded that the gradual increase of MLVSS concentration compensated the reduction of HRT and enhanced the COD removal.

The sludge volume index (SVI) is obtained by measuring the volume of 1 g of sludge after 30 minutes of settling. Practically, the SVI is an important factor in the design process and the assessment of sludge settleability of system. A typical SVI value ranges between 80 and 150 mLg\(^{-1}\) (Grady et al., 1999). In this work, as F/M ratio decreased from 0.2 to 0.08 gCODgMLVSS\(^{-1}\)d\(^{-1}\) the value of SVI increased from 50 to 70 mLg\(^{-1}\), which remained satisfactory. It can be observed that SVI is affected by the F/M ratio. As the concentration of MLVSS in SBR increased the F/M ratio decreased. The results demonstrated a negative relation between SVI value and F/M ratio over the given range of experimental data.

### 3.2 Effect of OLR on COD removal

An operational parameter OLR is considered as both the liquid flow rate and the pollution concentration. OLR is used in the SBR design as it bears reactor and operational characteristic. OLR has a significant effect on the removal of COD and BOD in the activated sludge system. It was shown that an increase of OLR increased the COD removal due to the enhanced growth of bacteria by providing more organic nutrients. On the other hand, high concentration of organic substrate can inhibit bacteria activities due to the increase of toxicity effect of substrate (Chan et al., 2010).

In order to investigate the effect of OLR on Glyphosate and COD removal, the SBR was operated with two different OLR of 280 and 400 mgCODL\(^{-1}\) corresponding to the Glyphosate concentration of 500 and 700 mgL\(^{-1}\), respectively. Table 5 represents the resulting SBR and effluent parameters using different OLRs. It is observed that the concentration of Glyphosate in effluent didn’t change but the percentage of COD removal demonstrates a 26 and 30 % reduction in COD removal for the OLR of 280 and 400 mgCODL\(^{-1}\), respectively. The low MLVSS obtained by applying OLR over the given range is contributed to the inhibitory effect of a higher Glyphosate concentration. On the other hand, the microbial system needs a sufficient biomass to maintain a biological activity and low COD uptake by bacteria which cannot provide the required nutrients (Pholchan et al., 2008).

### 3.3 Pre-filtration

The presence of suspended matters is one of the major concerns in the adsorption process. Suspended particles can clog the pore volume of adsorbent and consequently decrease removal efficiency. The typical floc size in biological system is in the range 10-100 μm. Thus, a filter with minimum pore size of 10 μm is required to separate majority of suspended solids from effluent. In this work, a 4 μm filter was used for pre-filtration process. Table 4 indicates that the concentration of TSS in discharged effluent from the SBR ranged between 80 to 130 mgL\(^{-1}\). The porosity of PKS-AC after treating the SBR effluent with and without pre-filtration is shown (Figure 4). Distinction between photos shows how suspended particles impacted the porosity of PKS-AC. In pre-filtered system, remaining particles didn’t affect the performance of PKS-AC while the surface of PKS-AC was fully covered by bacteria when the SBR effluent wasn’t filtered. The results emphasized that pre-filtration was necessary to prevent the clogging of the pores in PKS-AC as shown in Table 6.

**Table 5: Characteristic of SBR effluent after biological treatment applying different OLR.**

<table>
<thead>
<tr>
<th>OLR</th>
<th>280 (mgCODL(^{-1}))</th>
<th>400 (mgCODL(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glyphosate (mgL(^{-1}))</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>COD removal (%)</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>MLVSS (mgL(^{-1}))</td>
<td>780</td>
<td>700</td>
</tr>
</tbody>
</table>
3.4 Adsorption experiment

The adsorption of APMA from a synthetic solution onto PKS-AC was investigated (data are not presented). Adsorption isotherm models such as Langmuir, Freundlich and Redlich-Peterson were used to describe the adsorption of AMPA by PKS-AC. The results indicated that the Langmuir adsorption isotherm model best fitted the experimental data. The maximum adsorption capacity of PKS-AC was found to be 34 mg g⁻¹.

The SBR effluent properties listed in Table 4 showed that biological treatment of Glyphosate solution did not meet the discharged limitation of 2 mg L⁻¹ total phosphorus (TP). The biodegradation of Glyphosate was via AMPA pathway and the bacteria used inorganic phosphorus as a phosphorus source (Moneke et al., 2010). TP includes inorganic phosphate and organic phosphorus. Subsequently, only few inorganic phosphates were added as a supplementary nutrient and the value of TP was due to the phosphorus of Glyphosate compound. In this work, the adsorption method was used as a polishing treatment. The PKS-AC was used to remove the undegraded metabolite (specially AMPA) and produce final effluent which met the discharge limitation of 2 mg L⁻¹ TP.

3.4.1 Effect of pH

The pH of solution is one of the most important parameters affecting the adsorption process (Cho et al., 2006). The residual TP varied with different
initial pH (Figure 5). The minimum residual TP was at pH 2.5 and it increased with further increase in the pH. No removal of TP was observed for pH greater than 8. The results declared that the adsorption process was highly dependent on pH of the solution, which affected the surface charge of the PKS-AC. The carbon activated at high temperature, consists of carboxyl, phenolic, alcoholic and quinone groups, which results in a negative charge surface that adsorbs H+ and exhibits a positive zeta potential (Sotelo et al., 2002; Aksu and Kabasakal, 2004). The chemical structure of main Glyphosate metabolite AMPA, is presented in Figure 3. The functional group of phosphate is negatively charged at low pH and possesses affinity to the positively charged surface. The pHpzc can be used to explain the effect of pH. When the pH of solution is below the pHpzc, the surface charge of the adsorbent is positive but it is negative when the pH is above the pHpzc (Radovic et al., 1997). Nourouzi et al., (2009) reported that the pHpzc of PKS-AC was 8.1. Thus, it was expected that when the pH value of the solution was below the pHpzc (pH< 8.1), the PKS-AC surface became positively charged and exhibited an anion adsorption capacity. Therefore, when the pH of solution was decreased, the residual TP also decreased due to the positive surface charge of the adsorbent. When the pH of solution was increased the density of positive charge sites decreased and the adsorption of negatively charged compounds decreased as well as due to the repulsive force between adsorbent and adsorbate.

3.4.2 Effect of PKS-AC dosage

The effect of PKS-AC dosage on the removal of TP was investigated. The results were used to determine the minimum dosage of PKS-AC for meeting discharge limitation of 2 mgL⁻¹ TP. The removal of TP was as a function of PKS-AC dosage (Figure 6). The experiments were conducted at a constant initial TP concentration of 55 mgL⁻¹. The residual TP decreased from 55 to 2 mgL⁻¹ with the increase of PKS-AC dosage from 1 to 7 gL⁻¹. The reduction in the residual TP with increase of dose of PKS-AC was due to the fact that the number of functional sites increased with increasing dosage of PKS-AC. As a result, the residual TP was controlled by the availability of functional sites (Subha and Namasivayam, 2008). The polarity or solubility of the compound which is the determinant of affinity of solute to solid or liquid phase is related to the structure of molecule. In other words, the solubility of the adsorbate has a negative relation with its adsorption onto carbon (Kim et al., 2006). With regard to the high solubility of AMBA, the adsorption capacity of AMPA onto PKS-AC could be affected by its solubility.

The performance of coupled SBR-adsorption method was assessed by measuring parameters i.e. Glyphosate concentration, COD, TSS, turbidity and TP. The values of parameters are listed in Table 7.
4. Conclusion

The removal of herbicide Glyphosate in water by using the coupled SBR-adsorption method was investigated. The results showed that Glyphosate was almost completely converted to its metabolites with COD efficiency of 40%. Except for the TP, all parameters in the SBR effluent i.e. Glyphosate concentration, COD and TSS, met the discharge limitation except for TP.

It was found that parameters of HRT and F/M affected the COD removal. The percentage of COD removal decreased when HRT decreased. On the other hand, the percentage of COD removal increased when F/M decreased. In this work, the maximum MLVSS concentration of 1130 mgL$^{-1}$ was obtained with the OLR of 170 mgCODLd$^{-1}$.

The results revealed that the biodegradation of Glyphosate was not adequate to meet discharge limitation of 2 mgL$^{-1}$ TP. Therefore, the SBR effluent was further treated by using the PKS-AC adsorption. The minimum PKS-AC dosage of 7 gL$^{-1}$ was found necessary for the reduction of TP concentration to 2 mgL$^{-1}$.

Acknowledgment

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