Evaluation of sequencing batch reactor (SBR) and sequencing batch biofilm reactor (SBBR) for biological nutrient removal from simulated wastewater containing glucose as carbon source

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Abstract In general, conventional activated sludge (ASP) or enhanced biological phosphorus removing (EBPR) sludge has been used as seed culture for developing EBPR sludge and the time reported for development varies from months to year. In the present study cow-dung has been used as seed culture and EBPR sludge was developed within 36 days. The developed EBPR sludge has been used to evaluate the performance of sequential batch reactor (SBR) and sequential batch biofilm reactors (SBBR) for simultaneous nitrogen and phosphorus removal from synthetic wastewater containing glucose as carbon source. Three reactors were operated, SBR-1 containing only suspended biomass, SBBR-2 and SBBR-3 containing 5% and 10% polyurethane foam (PUF) media respectively along with suspended biomass. In all the reactors phosphorus removal was nearly the same and was more than 80%. In all the three reactors greater than 90% nitrification was achieved. Nitrogen removal in SBR-1 was 48% and in SBBR-2 and SBBR-3 it was more than 62%. On line monitoring of oxidation-reduction potential (ORP), pH and phosphorus during a cycle indicated that ORP and pH can be useful for real time control and optimization of the process.

Keywords Cow dung; enhanced biological phosphorus removal; glucose; sequential batch reactor; sequential batch biofilm reactor

Introduction
Biological phosphorus removal in biofilm reactors has been shown to be an efficient alternative to EBPR in activated sludge systems. Biological phosphorus and nitrogen removal in biofilm processes have a potential advantage compared to suspended growth processes because of less vulnerability to sludge loss and also biofilm processes are more compact. Further, these biofilm processes offer the possibility of achieving simultaneous nitrification and denitrification due to the prevailing anoxic zone in the biofilm near the attached surface during the aeration phase. Due probably to the aforementioned reasons, the sequencing batch biofilm reactor (SBBR) has been developed for simultaneous removal of nitrogen and phosphorus (Shin and Park, 1991; Gonzalez-Martinez and Wilderer, 1991; Morgenroth and Wilderer, 1998). Morgenroth and Wilderer (1999) have evaluated the mass transfer limitations for oxygen and organic substrate in SBBR and found that there was only a minor effect on overall phosphorus removal. Frequent back-washing maintained a thin active biofilm which in turn achieved efficient phosphorus removal. However, Falkenstoft et al. (2001) has mentioned about the possible diffusion limitations hampering the biological phosphorus removal in the biofilm. Hence, it is likely that biofilms may have an advantage in removing nitrogen but may have limitations for phosphorus removal. Therefore it seems that a combination of suspended growth and biofilm (attached growth) in a sequencing batch reactor might be advantageous for simultaneous removal of nitrogen and phosphorus.

There are various reports on the development of bio-P from activated sludge and it takes
several months to a year for enrichment of bio-P (Appeldoorn et al., 1992; Converti et al., 1993; Cech and Hartman, 1993; Belia and Smith, 1997; Dabert et al., 2001). The start up period can possibly be considerably reduced by bioaugmentation with EBPR sludge or pure biological phosphorus removing culture (Belia and Smith, 1997; Dabert et al., 2001). However, only activated sludge or EBPR sludge has been used and the authors have not come across any report on the use of any other seed culture for development of EBPR.

In this study the seed material used was cow-dung to develop EBPR. The main advantage of using cow-dung is the ease of availability, transportation and storage, whereas the EBPR sludge may not be available everywhere. There have been reports claiming that EBPR could not be accomplished with glucose as organic substrate and therefore to address this issue, in this study glucose has been used as carbon source to induce and maintain EBPR. In view of the aforementioned objectives, the experimental protocol was initiated by developing bio-P (EBPR) culture using cow-dung as seed material with synthetic wastewater containing glucose as carbon source in the SBR. Then, the EBPR sludge so developed was used to evaluate the efficacy of SBR and SBBRs.

Materials and methods

Development of EBPR sludge

A laboratory scale SBR with working volume of 5 L was operated for the enrichment/development of bio-P (EBPR) organisms using cow dung as seed material. Seed material was prepared by sieving the cow dung through a 355-micron sieve (Mesh No.: BS No. 44 ASTM, 0.355 mm). The prepared slurry taken was 1% weight/volume. The synthetic feed was prepared in tap water with the carbon source as glucose. The chemical oxygen demand (COD) to phosphorus ratio in the influent was maintained in the range of 15 to 30 and in general average COD was 800 mg/L. Other nutrients and micronutrients in the influent were added according to Shin and Park (1991).

The reactor was operated in SBR mode with feed, anaerobic, aerobic, settling and decanting phases. In each cycle, synthetic wastewater was added at the beginning of anaerobic phase followed by the aerobic and settling phase. Supernatant (2.5 L) was withdrawn at the end of the settling phase and replaced with the same amount of fresh synthetic wastewater. The SBR cycles had 11.5 hours each of anaerobic and aerobic phase and 1 hour of settling, decanting and fill phase.

Operation of SBR and SBBRs

Evaluation of performance of SBR and SBBRs was done by operating, three 2.5 L (working volume) laboratory scale reactors. The seed culture used in the three reactors was the EBPR sludge. Two reactors were filled with polyurethane foam (PUF) media of size 1 cm × 1 cm × 1 cm. The media added was 5% and 10% of liquid volume for SBBR-2 and SBBR-3 respectively. On day 29 the PUF media was added to SBBR-2 and SBBR-3. The three reactors were operated with the same influent synthetic wastewater concentration and operational strategy. The COD to phosphorus ratio in the influent was maintained in the range of 50 to 65. The average COD in the influent was 1000 mg/L and ammonia nitrogen was between 35 to 40 mg/L. The reactors were operated for 11.5 hours in each of the anaerobic and aerobic phases and 1 h for settling, decanting and fill phase. In each cycle 1.25 L of supernatant was decanted after settling followed by the addition of fresh synthetic wastewater.

Analytical methods

Water quality parameters monitored were, mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), COD, phosphorus, ammonia nitrogen, nitrite-nitrogen, nitrate nitrogen, pH and oxidation-reduction potential (ORP). All the chemical
Results and discussion

Development of bio-P (EBPR) from cow-dung

During enrichment/development of bio-P organisms no sludge was wasted to facilitate biosolids accumulation. One of the characteristic features of EBPR sludge is uptake of organic carbon with concomitant release of phosphorus in the anaerobic phase and uptake of phosphorus in the aerobic phase (Henze et al., 1995). This feature has been termed as phosphorus removal yield (PRY) by Dabert et al. (2001) and is used for ensuring the EBPR sludge development. The PRY is estimated by calculating the percentage of phosphorus accumulated during the aerobic phase as: \((\text{phosphorus at the end of anaerobic stage} - \text{phosphorus at the end of aerobic stage}) \times 100)/\text{phosphorus at the end of anaerobic stage.}\)

From Figure 1 it can be observed that up to day 35 the PRY was in the range of 7.5% to 55% which increased to more than 80% on day 36 and then onwards remained more than 75% indicating the development of stable EBPR sludge.

Figure 1 also presents temporal variation of COD removal after the anaerobic phase and phosphorus in the effluent. It can be observed that from day 1 to day 20 the COD removal varied between 16 to 86%. The variations in phosphorus release (0 to 17%) and uptake (0 to 45%) were observed in the same period. From day 20 to day 35, COD removal was in the range of 75 to 85% and the phosphorus removal was in the range of 40 to 50%. From day 36 onwards good and stable removal of phosphorus (>75%) and simultaneous phosphorus release was observed. A probable reason for the rapid change in phosphorus removal from 50% on day 35 to more than 75% on day 36 might be due to, the enzyme system responsible for biological phosphorus removal developed on the day 36 and induced bio-P (EBPR) mechanism in the mixed culture of cow-dung. The observed behavior is in agreement with the observations of Ubukata and Takii (1994).

Table 1 presents the reported time period for development of EBPR for different seed sludge, carbon source and cycle time. It is evident from the table that time required for development of EBPR with cow-dung as seed sludge is much less than the reported time period with glucose as the carbon source.

![Figure 1](https://iwaponline.com/wst/article-pdf/48/3/73/423169/73.pdf)
**Table 1** Startup time for the EBPR development stated in literature

<table>
<thead>
<tr>
<th>Innoculum seed</th>
<th>Carbon source in feed composition</th>
<th>Cycle time (h)</th>
<th>Startup period (d)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBPR sludge</td>
<td>Sodium acetate and glucose</td>
<td>6/8/12</td>
<td>84</td>
<td>Gonzalez-Martinez and Wilderer (1991)</td>
</tr>
<tr>
<td>ASP sludge</td>
<td>Glucose</td>
<td>12</td>
<td>60</td>
<td>Shin and Park (1991)</td>
</tr>
<tr>
<td>EBPR sludge</td>
<td>Acetic acid and glucose</td>
<td>6</td>
<td>42</td>
<td>Appeldoorn <em>et al.</em> (1992)</td>
</tr>
<tr>
<td>ASP sludge</td>
<td>Acetic acid and glucose</td>
<td>–</td>
<td>app. 90</td>
<td>Cech and Hartman (1993)</td>
</tr>
<tr>
<td>ASP sludge</td>
<td>Sodium acetate</td>
<td>12</td>
<td>57</td>
<td>Conerti <em>et al.</em> (1993)</td>
</tr>
<tr>
<td>ASP sludge</td>
<td>Glucose</td>
<td>8</td>
<td>70–80</td>
<td>Belia and Smith (1997)</td>
</tr>
<tr>
<td>ASP sludge</td>
<td>Acetic acid</td>
<td>6</td>
<td>33</td>
<td>Dabert <em>et al.</em> (2000)</td>
</tr>
<tr>
<td>Cow dung</td>
<td>Glucose</td>
<td>24</td>
<td>36</td>
<td>Present study</td>
</tr>
</tbody>
</table>

Note: ASP: Conventional activated sludge process

**SBR versus SBBR performance**

Figure 2 shows from day 16 onwards the variation of phosphorus as well as COD along with volatile solids in SBBR-2. Similar trends were observed in the other two reactors (SBR-1 and SBBR-3). The EBPR sludge developed was used in the three reactors to observe the effectiveness of bioaugmentation in reducing the startup time. The SBRs attained 50% phosphorus removal on the 3rd day (data not shown). However, due to accidental addition of sodium sulfate (2.52 g/L) instead of sodium bicarbonate from day 4 to day 34 the reactor performance deteriorated and led to poor phosphorus removal. After stoppage of sulfate addition, the system recovered quite rapidly and from day 35 onwards EBPR was observed. Possibly bio-P organisms survived in the system containing sulfate and the rapid recovery of EBPR was observed. PUF media were added to SBBR-2 and SBBR-3 on day 29 and after a week biomass attachment on the media was observed. In all the reactors phosphorus removal was in the range of 80 to 90% which continued up to day 50. Based on the mass balance of phosphorus uptake and biomass growth, it is estimated that sludge had approximately 9.7% phosphorus.

Table 2 shows the variation of parameters of the three reactors on day 50. The COD removal in the anaerobic phase was in the range of 75 to 80% in all the three reactors. All the three reactors were performing EBPR since there was phosphorus release and COD uptake in the anaerobic phase and in the subsequent aerobic phase, phosphorus was taken.

![Figure 2](https://iwaponline.com/wst/article-pdf/48/3/73/423169/73.pdf)
The average phosphorus removal in all the reactors was more than 80%. The ratio of phosphorus release to glucose uptake was 0.035 P-mol/C-mol for SBR-1, 0.037 P-mol/C-mol for SBBR-2 and 0.043 P-mol/C-mol for SBBR-3 which is much less when compared to the reported data (0.25 to 0.75 P-mol/C-mol) with acetate as carbon source (Smolders et al., 1994). The lower ratio observed is in agreement with the biochemical model proposed by Smolders et al. (1994) as glucose metabolism requires less ATP (18 mmol/g(dw)) to be metabolized than acetate (158 mmol/g(dw)) and therefore release of phosphorus in the anaerobic phase would be less when glucose is used.

In all the reactors, greater than 90% nitrification was achieved and nitrogen removal in the SBBRs was about 62%, and 48% in SBR-1. It may be that in SBR-1 the population of nitrifiers, denitrifiers and bio-P organisms must be coexisting and denitrification took place only in the anaerobic phase but in the SBBRs the denitrifiers present in the interior of the biofilm on PUF participated in denitrification (nitrate removal) in the aerobic phase also. Thereby nitrogen removal achieved in SBBR-2 and SBBR-3 was slightly higher than SBR-1.

On-line sensor values (pH and ORP) help in identifying process parameters, which might be useful for monitoring and real-time control purposes and possible optimization of the SBR cycle (Chang and Hao, 1996). ORP has been demonstrated to be a useful tool for indicating the biological state of a system. The pH values of a biological system respond to microbial reactions and often provide a good indication of the ongoing biological reactions. Figure 3 presents the typical profiles of on-line sensor values for a cycle in the three reactors.

The ORP curve displayed a sharp decrease within a few hours in the anaerobic phase with the corresponding phosphorus release. There was a sharp increase in ORP value in the aerobic phase, which reached a maximum during the phosphorus uptake. Sharp decrease in pH was observed at the beginning of the anaerobic phase mainly due to the phosphorus release and fermentation byproducts. These are typical trends as observed by Chang and Hao (1996) and Lee et al. (2001). Rapid increase in pH occurred at the beginning of aerobic stage, primarily due to the stripping of carbondioxide out of the system. Subsequent decrease of pH in the aerobic stage was probably due to the release of H\(^+\) from nitrification and eventual formation of a valley (Lee et al., 2001). The variation in ORP had a shift in SBR-1 and the minimum value of ORP during the anaerobic phase achieved in SBBR-2 and SBBR-3 was lower than SBR-1. Also the phosphorus release in the SBBRs was higher than SBR and this may be due to participation of biomass present on the PUF media in the phosphorus removal.

In the first 3.5 hour, the anaerobic phosphorus release rate was in the range of 0.31 to 0.46 mg P/gm VSS. h and the phosphorus uptake rate was in the range of 0.48 to
0.74 mg P/gm VSS.h in the three reactors. The phosphorus uptake rate in SBBR-2 and SBBR-3 was more in the initial hours but at the end of the aerobic phase phosphorus removal remains the same. This was evident from the ORP profiles in the three reactors (Figure 3). The phosphorus release and uptake rates observed are less when compared to literature values (Brdjanovic et al., 1998; Merzouki et al., 2001), which might be due to use of glucose in the present study.

Preliminary microbiological examination of EBPR sludge showed the predominance of Gram +ve cocci.

**Conclusions**

The present study has demonstrated that EBPR sludge could be developed in a short period of 36 days with cow dung as seed material. Glucose can be used as substrate to induce and maintain the EBPR process in the SBR and SBBR. Biological phosphorus and nitrogen removal can be achieved in SBBR, which consists of PUF media and suspended biomass and the results were comparable with SBR containing only suspended biomass. SBBRs in comparison to SBR have an edge in nitrogen removal, however the performance is the same with reference to phosphorus removal for the experimental conditions employed in the present study.

**References**


