Phosphorus uptake kinetics in a biofilm sequencing batch reactor

C. Nava-Ramírez, S. González-Martínez

Abstract Using data from previously reported experimental work, theoretical kinetic analysis of the aerobic uptake of phosphate is presented. The data obtained in biofilm sequencing batch laboratory reactor was adjusted with polynomial regression to fit a curve during the uptake phase and then analyzed with two kinetic models: Classical Michaelis–Menten and one considering the enzymes involved have allosteric character. Data from nine experimental runs, every one under different operational conditions, were analyzed. The results show that the experimental values used fit in the model of an allosteric enzyme or enzyme-complex with four-binding sites. Based on this consideration, a catalytic model of sequential interactions was obtained which allows the interpretation of the kinetic mechanisms involved in the process. The calculated phosphorus uptake rate values do not show significant differences from the experimental ones.

1 Introduction

Phosphorus and carbon removal in wastewater treatment systems can be achieved by changing the conditions of the microorganisms from aerobic to anaerobic in an alternating mode. In order to develop better design and operation procedures, the knowledge of the microbiology and kinetic relations involved in the anaerobic and the aerobic phases is essential and are not yet well understood.

The main problem to develop a kinetic model is the lack of knowledge of the biochemical reactions involved. The consequences are that most of the kinetic models are empirically developed for one of the aerobic or anaerobic phases and do not allow the analysis of the system as a whole. This is understandable when considering that the biochemical reactions involved during the anaerobic phase (phosphate release) are completely different from the ones taking place during the aerobic phase (phosphate uptake). Phosphate (PO₄³⁻) release is a reaction inside the cell to recover energy that makes phosphate a waste product. Because the release and uptake reactions involve different enzymes, it is not possible to develop one model for the whole cycle: Every phase has to be considered enzymatically independent form each other. Figure 1 shows the typical behavior of a treatment cycle in a biofilm SBR operated to remove phosphates from wastewater. During the initial anaerobic phase, PO₄ is released and carbonaceous material (COD) is stored inside the cell while, in a subsequent aerobic phase, the previously released PO₄ is captured and the stored organic material is processed.

Enzymes catalyze all the biochemical reactions, therefore, in the strict sense, it is possible to define any biological processes using models based on enzyme kinetics. Classical enzyme kinetics are based on the Michaelis–Menten model, which is used to describe the kinetics of substrate removal and expresses the relationship between substrate concentration and specific consumption rate. This model assumes that the enzymes have multiple but independent substrate binding sites and its analysis is made with hyperbolic functions. However, if the binding of a substrate molecule produces structural changes in the enzyme molecule which result in changes in vacant site affinity, the rate curve does not adjust to Michaelis–Menten’s classic kinetics and the enzyme is classified as allosteric [1]. Allosteric mechanisms are those which are more common in biochemical systems [2, 3]. The function of allosteric enzymes is related to processes of metabolic regulation. There are two types of models that explain allosteric mechanisms: That of sequential interaction and that of concerted symmetry. The sequential interaction model assumes sequential or progressive changes in vacant site affinity, as these are occupied in turn. The models proposed by Hill, Adair, Pauling, [1] and [4, 5] are examples of this type.

In the concerted symmetry model, proposed by Monod, it is considered that the binding of a substrate molecule with the enzyme produces simultaneous changes at all the binding sites of the enzyme macromolecule [1]. There are various attempts to describe the anaerobic PO₄ release process [6–8] but, for the aerobic PO₄ uptake, there is none to describe the mechanisms involved in the process.

The main objective of this research project is to describe the kinetics of the phosphate uptake during the aerobic phase in a biofilm reactor operated under the sequencing batch procedure.

2 Method

This study bases the kinetic analysis in the experimental results reported by [9, 10] for a biofilm laboratory-scale...
batch reactor. The reactor was operated under the fill and draw procedure consisting in four different steps in each cycle: Fill, anaerobic phase, aerobic phase, and draining. Mixing was guaranteed using a recirculation pump with a volume exchange rate of 6 min. Figure 2 shows a diagram of the reactor. The experimental sequence is shown in Table 1.

The reactor was fed with a mixture of sodium acetate, glucose and peptone, and the concentration adjusted to a COD of 300 mg/l. The runs indicated in Table 1 were monitored for PO₄ and COD, obtaining curves as the ones shown in Fig. 1. The data (experimental curves) was adjusted with polynomial functions. The order of the polynomial was chosen according to significant values for statistic parameters as Student’s t-test, correlation coefficient and standard error.

The determination of the constants involved in the Michaelis–Menten model was based on the methods proposed by Lineweaver-Burk, Hanes-Woolf, Woolf-Augustinson-Hofstee, and Eadie-Scatchard [11]. To obtain an allosteric model, the following methodology was involved: (a) Determination of the number of enzymes binding sites. The definition of these parameters was made using Lineweaver-Burk and Hill’s diagnostic tests. (b) For the definition of the catalytic model, the method indicated by [2] was used.

### 3 Results
Initially, intent was made to adjust all graphs to the same polynomial degree, considering that the same enzyme process was involved. However, the use of this criterion was not statistically justified. Third degree polynomial functions were selected for the 6- and 8-h cycles, while second degree was used for the 12-h cycle. According to Table 1, Figures 3, 4 and 5 show the experimental values and polynomial adjusted curves of the nine runs. The values of substrate concentration (S), rate (v), and correlation coefficient (r) are indicated in the figures. The duration of the anaerobic phase is expressed in percent of the cycle duration.

In Figures 3a–c, 4a and c, during the first 30 min of the aerobic phase, a sigmoid behavior is observed. The slope

![Fig. 1](image1.png)

**Fig. 1.** Typical behavior of the phosphate and COD removal sequence in a sequencing batch reactor (from [9], modified)

![Fig. 2](image2.png)

**Fig. 2.** Experimental reactor [10]

### Table 1. Cycle and phases duration for the operation of the reactor

<table>
<thead>
<tr>
<th>Cycle duration (h)</th>
<th>6</th>
<th>8</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of the anaerobic phase (in % of the total cycle duration)</td>
<td>30</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

![Fig. 3](image3.png)

**Fig. 3.** Experimental and calculated PO₄-P uptake for the 6-h cycle. a 30% Anaerobic phase; b 45% anaerobic phase; c 63% anaerobic phase