# Effect of the start-up length on the biological nutrient removal process

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### Abstract

In this work, a study of the start-up stage of a Biological Phosphorus Removal (BPR) process was carried out.

To do this, SBR alternating anaerobic and aerobic conditions were used with conventional activated sludge and fed with synthetic influent at the beginning of each cycle. During the length of the cycles (4 h) the most important parameters were determined.

The main results obtained were that during the first days of the start-up stage the phosphorus removal percentage increased more quickly that those of the COD and ammonium-nitrogen. However, it was necessary to extend the start-up stage to at least 30 days in order to obtain the maximum phosphorus removal percentages and a stable Phosphorus Accumulating Organisms (PAO) population, whereas the maximum COD and ammonium-nitrogen removal percentages were obtained in about ten days.

Once the start-up period had finished the ratio P released to COD stored was determined, obtaining a value of about 0.14, which is significantly lower than the theoretical value (0.55), suggesting the presence of a significant GAO population.

*Keywords: activated sludge, biological phosphorus removal, biological nitrogen removal, start-up stage.* 

# 1 Introduction

Biological Nutrient Removal (BNR) processes are being widely used to remove nutrients, Nitrogen and Phosphorous, as well as organic compounds contained in



wastewater, due to the EU stringent Phosphorus and Nitrogen standards for treated wastewater discharged on sensitive areas (Table 1) and its low cost compared with Coagulation-Flocculation processes.

Parameter	Population Equivalent 10000 to 100000	Population Equivalent >100000	Minimum Reduction
Total Phosphorus	$2 (mg \cdot L^{-1})$	$1 (mg \cdot L^{-1})$	80%
Total Nitrogen	$15 (mg \cdot L^{-1})$	$10 (mg \cdot L^{-1})$	70% - 80%

 Table 1:
 Phosphorus and Nitrogen limits in treated water (EU directive).

Phosphorous and Nitrogen compounds have been reported to be the limiting nutrient in eutrophication, for this reason the BNR processes have been extensively investigated. For the simultaneous removal of Nitrogen and Phosphorus from wastewater, the biological reactor must alternate between at least three environments, anaerobic, anoxic and aerobic.

Under anaerobic conditions, Phosphorus Accumulating Organisms (PAO) readily take up biodegradable organic carbon substrates [1-4], and store them as Polyhydroxyalcanoate (PHA), the energy required for this anaerobic process is obtained from the hydrolysis of intracellular polyphosphates and the glycolysis of glycogen coupled to the release of orthophosphate to the liquid [5].

Under anoxic conditions, the denitrification of heterotrophs is the main reaction, where heterotroph microorganisms convert nitrate into atmospheric nitrogen, in this process nitrate is used as the oxidizing agent [6] (eqn (1)).

$$A_{red} + NO_3^- \rightarrow A_{ox} + 0.5N_2$$
(1)

Finally under aerobic conditions, PAO use the PHA for generating energy for growth, glycogen synthesis, and phosphate uptake, where the PAO take up of phosphate is more than that released during the anaerobic phase. Another important reaction is the nitrification of ammonium nitrogen compounds into nitrites and eventually nitrites to nitrates [7] (eqns (2-3)).

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$$\operatorname{NH}_{4}^{+} + 3 \quad \operatorname{O}_{2} \to 2\operatorname{NO}_{2}^{-} + 2\operatorname{H}_{2}\operatorname{O} + 4\operatorname{H}^{+} + \Delta\operatorname{H}_{\operatorname{nitrosomonas}}$$
 (2)

$$2NO_{2}^{-} + O_{2} \rightarrow 2NO_{3}^{-} + \Delta H_{nitrobacter}$$
(3)

Under aerobic conditions, the aerobic oxidation of organic compounds contained in wastewater also takes place, here symbolized by the average composition  $C_{18}H_{19}O_9N$ , during growth of PAO and heterotrophs microorganisms [7] (eqn (4)).

$$C_{18}H_{19}O_{9}N + H^{+} + 17,5O_{2} \rightarrow 18CO_{2} + 8H_{2}O + NH_{4}$$
 (4)

Usually, the start-up of the BPR processes are carried out by feeding the wastewater to the bioreactor of the Wastewater Treatment Plant (WWTP), previously inoculated with activated sludge, in a fed-batch mode. During this stage, all the exposed processes take place independently and it is very



interesting to determine how the start-up takes place in order to evaluate how to proceed in the start-up stage of a full-scale WWTP.

In this context, the aim of this work was to study the influence of the start-up stage of a BNR process paying special attention to the COD, Nitrogen and Phosphorus removal processes.

# 2 Material and methods

#### 2.1 Inoculum

Activated sludge from a conventional activated sludge full-scale WWTP was used as inoculum for the batch experiments. Before the start of the experiments, the activated sludge was aerated for five hours in order to ensure the complete consumption of all the substrates contained in the wastewater.

#### 2.2 Synthetic wastewater

The wastewater used for the cultivation of microorganisms was prepared daily and was a synthetic wastewater that simulated the characteristics of real urban sewage. The composition of the synthetic wastewater and its characteristics are shown in Table 2.

Component	Concentration	Parameter	Concentration
Glucose (g·m <sup>-3</sup> )	161.0	$COD (g \cdot m^{-3})$	320.0±15.0
Soybean Peptone (g·m <sup>-3</sup> )	161.0	$BOD_5 (g \cdot m^{-3})$	210.0±10.0
$(NH_4)_2SO_4(g \cdot m^{-3})$	74.2	$BOD_u (g \cdot m^{-3})$	250.0±15.0
$KH_2PO_4 (g \cdot m^{-3})$	44.5	Total Nitrogen (g·m <sup>-3</sup> )	32.6±3.0
NaHCO <sub>3</sub> (g·m <sup>-3</sup> )	115.0	Organic Nitrogen $(g \cdot m^{-3})$	16.1±3.0
$MgSO_4 \cdot 7H_2O~(g \cdot m^{-3})$	50.0	Total Phosphorus (g·m <sup>-3</sup> )	10.1±0.7
$CaCl_2 (g \cdot m^{-3})$	30.0	pН	7.2±0.1
$(NH_4)_2 Fe(SO_4)_2$ (g·m <sup>-3</sup> )	3.0		

Table 2:Wastewater composition and characteristics.

### 2.3 Batch experiments

Three identical SBRs, with a reaction volume of 2.4 L per reactor, were used. The SBRs were operated continuously in cycles of 4 h. Each cycle consisted of several phases: influent addition (5 min), anoxic–anaerobic reaction (75 min), aerobic reaction (135 min), waste sludge withdrawal (1 min), settling (20 min) and effluent withdrawal (5 min). The temperature and pH of each reactor were



constantly maintained at 20°C and 7.2, respectively, and dissolved oxygen during aerobic stages was set to saturation. The sludge retention time was reduced stepwise from 40 to 6.7 d. Further details concerning the SBR experimental set-up can be found in previous publications [8].

### 2.4 Analytical methods

Samples were taken and immediately filtered through a Millipore glass fibre filter (pore size 0.45 µm). Following this, filtration samples were analyzed for the following parameters:  $S_{COD}$ ,  $S_{PT}$  and  $S_{N,NH4}$ . In addition, XSS, VSS and  $X_{PT}$ were determined in the activated sludge. All analyses of wastewater and activated sludge were performed in accordance with the methods described in the literature [9].

#### 3 **Results and discussion**

### 3.1 COD removal

The COD removal from the wastewater was investigated during the start-up stage. The results indicate that, from the beginning of the start-up stage, the COD removal was always more or less the same, about 90%.

The negligible effect of the start-up stage over the COD removal could be explained by taking into account that from the beginning there was a sufficient concentration of activated sludge, which consumed the COD mainly during the aerobic stage. Although the Nitrogen and Phosphorus removal processes were associated with COD removal, the amount of COD removed by these processes was negligible compared to the COD removed under aerobic conditions. Comparing the COD concentration in the effluent wastewater and the inert COD in the influent wastewater, it was concluded that most of the effluent COD corresponded to the inert fraction, which indicated that the process was very stable and achieved a very good COD removal efficiency [10].

### 3.2 Nitrogen removal

Figure 1 shows the influence of the start-up length on the nitrogen removal from the wastewater.

In this Figure it can be seen that the increased length of the start-up stage favoured nitrogen removal. This could be explained due to acclimatisation of the activated sludge to the new environmental conditions and the growth of the microorganisms involved in the nitrification-denitrification processes. As can be seen in this Figure, in about 10 d, the maximum nitrogen removal was obtained. The maximum increase in the nitrogen removal percentage was about a  $35\% d^{-1}$ .

# **3.3 Phosphorus removal**

The results obtained for phosphorus removal with the length of the start-up stage are presented in Figure 2.





Figure 1: Ammonium nitrogen removal during the start-up stage.



Figure 2: Phosphorus removal during the start-up stage.

In this Figure, it was observed that, during the first days, the phosphorus concentration in the effluent was reduced quickly, which could be explained because of the increase of activated sludge concentration and the growth of the microorganism involved in the phosphorus removal mechanisms (mainly the PAO organisms). Once stationary phosphorus removal had been reached, about 15 days after the beginning of the experiment, the effluent concentration was about 1 mg L<sup>-1</sup>. At the beginning of the start-up stage, the maximum increase in the nitrogen removal percentage was about a 40% d<sup>-1</sup>.

In order to examine the biological phosphorus mechanisms in depth, the profiles of the COD and the phosphorus during batch cycles carried out at different moments of the start-up stage were determined. These data are presented in Figures 3 and 4.







Figure 4: Phosphorus profiles during different batch cycles.

These results obtained for the COD profiles indicate that at the beginning of the operation almost no COD was accumulated during the anaerobic stage, which indicates the absence of microorganisms able to store substrates inside the cell. This situation is typical in activated sludge from pure aerobic processes. 10 days later, a significant COD amount was stored under anaerobic conditions, but this storage was even increased after 30 days of operation. These observations indicate that the development of a culture containing a significant amount of organisms involved in the accumulation of substrates under anaerobic conditions is slower than that of the typical microorganisms present in the activated sludge. In order to correlate this information with the interesting parameter, the phosphorus removal, the phosphorus profile was also determined (Figure 4).

First of all, this information was used to evaluate the evolution of the phosphorus release and storage. On this basis the phosphorus profile during the three cycles was correspondent to the observation made for the COD profile. That is, the higher the start-up length, the higher the phosphorus release under anaerobic conditions and therefore removal. However, the authors of this work considered it interesting to determine the P release to COD store ratio obtained under anaerobic conditions (Table 2).

Start-up Length (d)	$[S_{PT} released/S_{DQO} stored] (mg S_{PT}/mg S_{COD})$ Under anaerobic conditions	
0	0.08	
10	0.10	
31	0.14	

This information was considered to be interesting because the higher the value, usually near 0.5 mg/mg, the higher PAO population on the activated sludge, which is the best situation. Moreover, low values of these ratios will supply very interesting information about the development of the microorganisms' ability to store COD but not P, usually called Glycogen Accumulating Organisms (GAO) bacteria, which is undesirable in BNR reactors because of competition with the PAO bacteria for the available COD substrate. In our case the ratio presented a low value, which indicated the significant presence of GAO bacteria.

# 4 Conclusions

The main conclusions of this study are shown below:

- The COD removal can be achieved without significant problems from the beginning of the start-up period.
- The biological nitrogen removal needs a period of acclimatisation of about 10 days to reach the best removal yields.
- The biological phosphorus removal is the most complicated process to be adequately initiated during the start-up stage, requiring at least 30 d to be reach the optimum removal yields



• One of the most important inconveniences during the start-up stage of the phosphorus removal is the development of a bacterial population able to store COD under anaerobic conditions, but without the ability to store P under aerobic conditions.

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