SIMULTANEOUS REMOVAL OF ORGANIC MATTER, TOTAL NITROGEN AND PHOSPHOROUS IN A SINGLE CONTINUOUS FLOW ACTIVATED SLUDGE REACTOR OPERATED WITH SHORT AERATION CYCLES

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ABSTRACT

In a continuous activated sludge (AS) system treating urban wastewater operated with short aeration cycles the simultaneous removal of organic matter, total nitrogen and phosphorous has been attained. The pilot plant, with a reactor volume of 285 L, removed up to 90% of total and soluble COD, 90% of total N and 70% of phosphorous from the influent wastewater when operated with a hydraulic retention time of 8.6 hours, a sludge age of 16.8 days and a frequency of 15 / 30 minutes of On / Off aeration, respectively. The results here reported indicate that the sludge growing within the system is capable of simultaneous nitrification and denitrification within the reactor. It was also confirmed the existence of phosphorous accumulating microorganisms (PAO). This coexistence of all microbial species responsible for nitrification, denitrification and biological phosphorous removal within the reactor was also proved by means of activity assays and fluorescence in situ hybridisation (FISH) analysis.

This process represents a new bioreactor concept with the highest compacity wherein organic matter, total nitrogen and phosphorous are simultaneously removed. Further work is required for tuning and optimizing the process but the preliminary results here presented are quite promising for the complete development of this compact processes and its application to urban wastewater treatment and post-treatment of anaerobic effluents.

KEYWORDS

Activated sludge, control system, intermittent aeration, nutrient removal, wastewater.

INTRODUCTION

The abatement of organic matter and nutrients from wastewater has been conventionally attained through a sequence of biological processes usually comprising nitrification, denitrification, organic matter oxidation and biological phosphorous accumulation. There are significant differences on the chemical conditions that microorganisms need for conducting those bio-transformations. The most important differences are found on microorganisms responsible for removing nitrogen, which in its majority are nitrifiers (or ammonia oxidisers) and denitrifiers (or nitrite-nitrate reducers). Thus, most nitrifiers are strict aerobe autotrophic bacteria that use ammonia as energy source, inorganic carbon as carbon source and oxygen as electron sink, while most denitrifiers are facultative heterotrophic bacteria that use organic carbon as energy and carbon source and nitrite-nitrates as electron sink. (Loosdrecht and Jetten, 1998).

Moreover, the biological phosphorous removal (BPR) appears to add more complexity to the overall process of removing organic matter, total nitrogen and phosphorous from wastewater. BRP has been reported in systems when purging sludge containing microorganisms that have accumulated P-rich molecules (polyphosphates) as an internal energy pool. It is known the existence of aerobe heterotrophic bacteria that accumulate polyphosphates when exposed to alternating aerobic/anaerobic conditions. Also it has been
reported the capability of some denitrifying bacteria for doing so when exposed to alternating anoxic/anaerobic conditions (Kuba et al. 1996).

Thus far, the aforementioned sequence has been traditionally accepted as the most feasible option for removing organic matter and nutrients from wastewater. It has been conventionally conceived as a sequence of single bioprocesses, each of which operated under different conditions pursuing the only grow of the specific microorganisms. This sequence has been attained in continuous flow processes as a spatial sequence of different reactors or compartments connected by different lines and recirculations (Baeza et al. 2002). On the other hand, in discontinuous flow processes the sequence is defined as temporary changes on the operating conditions (Callado and Foresti, 2001; Gieseke et al., 2002). These different sequences have gave rise to different commercial processes, all of them demanding quite high investments.

More recently it has been reported a different approach for attaining the removal of organic matter and total nitrogen in single reactor either operated in continuous or discontinuous mode (Villaverde et al, 2000 and 2001). The strategy consists of merging the different phases described above in an overall phase wherein nitrifiers, denitrifiers and aerobe heterotrophs can coexist. The different microorganisms can grow within the same environment in which oxygen is introduced in a balanced manner through high frequency aeration cycles. The strategy of cycled aeration has been already tested using several reactors (Sasaki et al. 1993) or single reactors with long aeration cycles (Hao and Huang, 1996).

The present work moves a step further in the application of these processes operated with aeration cycles, with the aim of attaining not only the removal of organic matter and total nitrogen but also phosphorous in a single continuous flow reactor. The process here presented represents a new bioreactor concept with the highest compacity wherein organic matter, total nitrogen and phosphorous are simultaneously removed. Further work is required for tuning and optimizing the process but the preliminary results here presented are quite promising for the complete development of this compact processes and its application to urban wastewater treatment and post-treatment of anaerobic effluents.

MATERIAL AND METHODS

Pilot plant

The pilot plant used throughout the experimentation period consists of a conventional activated sludge reactor and a secondary settling tank (Figure 1). Both, the reactor and settling tanks had a cylindrical geometry and were made of polyethylene with volumes of 285 and 187 liters, respectively. The pilot was operated as a conventional AS process in terms of values of hydraulic and sludge retention times and the recycling rate of sludge to the reactor. The only difference with conventional AS processes was that the oxygen was introduced through short aeration cycles. In this sense, since aeration was intermittent, the reactor was provided with a stirring device that maintained the sludge mixed while the aeration was shut down.

Figure 1. Scheme of the activated sludge pilot plant operated with cycled aeration throughout the experimentation.
The pilot treated domestic wastewater coming from the nearest sewage collector. The wastewater was previously treated in a sieve of 1 mm and a primary decanter placed outside the lab. The water entering the pilot had an averaged composition typical of an urban wastewater of medium strength (Table 1). The reactor operated always at an ambient temperature of around 20 Celsius degrees.

**Table 1. Average composition of wastewater entering the reactor**

<table>
<thead>
<tr>
<th></th>
<th>Variation range</th>
<th>Mean value</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD_{total} (mg O_2 / L)</td>
<td>450-1500</td>
<td>600</td>
</tr>
<tr>
<td>COD_{soluble} (mg O_2 / L)</td>
<td>180-450</td>
<td>250</td>
</tr>
<tr>
<td>TKN (mg N / L)</td>
<td>45-80</td>
<td>60</td>
</tr>
<tr>
<td>N-NH_4 (mg N / L)</td>
<td>40-70</td>
<td>45</td>
</tr>
<tr>
<td>PO_4^3- (mg P / L)</td>
<td>5-10</td>
<td>8</td>
</tr>
</tbody>
</table>

**Control system**

The most critical aspect of the process from the technical point of view was the operation and maintenance of the aeration system in order to have a good control of the oxygen that is transferred into the liquid media. Two aeration systems were used for aerating the mixed liquor, initially it was mounted a submerged ejector that gave rise to severe disruption of the microbial flocs resulting in very poor settling properties of the sludge. In consequence, a conventional aeration system of blower and diffusers was installed. The system transferred enough dissolved oxygen (DO) into the mixed liquor allowing for the formation of largest microbial flocs that showed very good settling properties. The aeration control system was built using a commercial software, Labview 5.1, that allowed for the continuous monitoring of all parameters through an easy-to-use interface on the PC. It was also possible to have total control of all mechanical and electrical devices and to modify the set points of the control aeration system.

**Table 2.** Different aeration control strategies followed throughout the experimentation. The time values are expressed in minutes and correspond to the time span under which the specified condition set into brackets is satisfied. The set point of cero DO (DO = 0 mg/L) in practice corresponds to the detection limit of the DO sensor.

<table>
<thead>
<tr>
<th></th>
<th>Period I</th>
<th>Period II</th>
<th>Period III</th>
<th>Period IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control parameters</td>
<td>DO and time</td>
<td>DO and time</td>
<td>DO, ORP and time</td>
<td>time</td>
</tr>
<tr>
<td>Aeration system</td>
<td>Submerged ejector</td>
<td>Blower + diffusers</td>
<td>Blower + diffusers</td>
<td>Blower + diffusers</td>
</tr>
<tr>
<td>Aeration Set points</td>
<td>On</td>
<td>[DO = 0 mg/L AND (t (DO = 0) = 10')]</td>
<td>[DO = 0 mg/L AND (t (DO = 0) = 10')]</td>
<td>[DO = 0 mg/L ] AND (breakpoint on d(ORP)/dt)</td>
</tr>
<tr>
<td></td>
<td>Off</td>
<td>DO = 3 mg/L OR (t (air on) = 20’)</td>
<td>DO = 3 mg/L OR (t (air on) = 20’)</td>
<td>DO = 3 mg/L OR (t (air on) = 20’)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t (air off) = 30’</td>
<td>t (air on) = 15’</td>
<td>t (air off) = 30’</td>
</tr>
</tbody>
</table>

In Table 2 the different control strategies adopted throughout the experimentation are described in terms of the control parameters used, their set points and duration. Four periods can be differentiated according to the control. The selection of the different control strategies was done after evaluating the information gathered on-line and direct observation of the process performance in terms of removal percentages of organic matter, total nitrogen and phosphorous. For such purpose the pilot plant was equipped with sensors for recording on-line the values of all parameters of significance (Jeon et al, 2001), such as DO, pH, ORP, temperature, and concentration of NO_3^-, NO_2^-, PO_4^3- y NH_4^+ in the effluent.

**Chemical and microbial analysis**

Throughout the experimentation samples of the mixed liquor were collected for determining those parameters that were not determined and registered on-line. These parameters were the chemical oxygen
demand (COD), the total Kjeldhal nitrogen (TKN) and the suspended solids (SS) volatile (VSS) and total (TSS). These analysis were done according to Standard Methods (1995).

The sampling of the mixed liquor allowed for direct observation of the sludge flocs under the microscope. Also the specific activity of nitrifiers, denitrifiers and aerobe heterotrophs was experimentally determined through activities conducted according to Villaverde et al. (2001). Moreover, we conducted the analysis of the sludge for microbial species that accumulated phosphorous. These analysis included indirect analysis using of Poly-P stains and direct determination through fluorescence in-situ hybridization (FISH) techniques, using the beta and gamma probes.

RESULTS AND DISCUSSION

Reactor performance

The pilot plant was continuously operated for more than nine months. During the different operation periods the hydraulic and sludge retention times (HRT and SRT) were varied within the ranges of 6 to 12 hours and 6 to 17 days, respectively. Both, the HRT and SRT were adjusted within those ranges in each period in order to achieve the best removal percentages of organic matter, total nitrogen and phosphorous. The best results obtained for the four periods are presented in Table 3.

| Table 3. Operation conditions and results obtained throughout the experimentation. |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| Duration (d)                    | 108             | 33              | 46              | 80              |
| HRT (h)                         | 9.5             | 7.0             | 7.5             | 8.5             |
| SRT (d)                         | 12.3            | 12.3            | 12.3            | 16.8            |
| COD\textsubscript{1} removal (%)| 70-90           | 70-90           | 80-90           | 80-90           |
| COD\textsubscript{2} removal (%)| 50-80           | 80-90           | 70-90           | 80-90           |
| Total N removal (%)             | 20-30           | 30-40           | 50-60           | 80-90           |
| P removal (%)                   | 10-15           | 20-30           | 60-80           | 50-70           |

The main characteristics of the four operational periods are the following:

Period I. It is an acclimation period wherein it is aimed the initial coexistence of nitrifiers, denitrifiers, aerobe heterotrophs and Phosphorous Accumulating Organisms (PAO). The aeration device built in the plant was a submerged ejector that although it provided good mixing it produced high shear stress to microbial flocs. This shear stress resulted in poor settling properties of the sludge and the loss of part of the biomass. In general although this period did not show good nitrification nor a significant P-uptake, it allowed for the initial establishment of the different microorganisms (nitrifiers, denitrifiers, PAO…).

Period II. We replace the former aeration device by a new system formed by a blower and diffusers. This resulted in a rapid response of the floc structure and an increase of the biomass within the reactor, the wash-out problem was overcome. In this period the removal percentage of organic matter increased up to 80% and total nitrogen was removed up to 40%. This clearly indicated the coexistence of the different microbial species responsible for the removal of organic matter and nitrogen within the microbial aggregates. PAO did not appear to be significantly present in the system during this period.

Period III. During this period the tuning of the control system was attained in order to increase the removal percentage of carbon, nitrogen and phosphorous. In this sense a new control parameter, the oxidation-reduction potential (ORP) was incorporated to the control algorithm. After a few assays the system started to remove more efficiently nitrogen (up to 60%), which indicated that the ORP might play an important role on the optimization of this simultaneous nitrification and denitrification process taking place in a single reactor with cycled aeration. Moreover this new strategy also revealed the significant increase of
P-accumulating activity within the system and P was 70% removed. After conducting more experiments we could not obtain any further improvement on the removal of nitrogen.

Period IV. After thorough evaluation of the results obtained on the other periods, we concluded that the coexistence of all these microorganisms can be attained by following the response of the system to changes on the dissolved oxygen concentration. However the optimization of the simultaneous removal processes of carbon, nitrogen and phosphorous by interpreting the meaning of changes on DO, ORP, time… and some other parameters, it appeared to be very difficult to attain by a simple control system. Also we did not consider the option of moving to a more advanced control system of the type of neural network or artificial intelligence since it will mean a handicap to further applications of a process whose simplicity is one its better advantages. In consequence, we decided to change the control strategy. Instead of regulate the dosage of oxygen as a function of the microbial growth (followed through the on-line determination of several parameters as it was done in Periods I, II and III) we decided to regulate the microbial growth by imposing a stress force. Thus far, we imposed a time-regulated aeration to force microorganisms to grow as a function of the different media conditions (concentration of different substrates…). It was soon observed that the coexistence of the different species was not only maintained but also we obtained a better removal of total nitrogen, which means that the coupling of nitrifiers and denitrifiers is better attained through an aeration frequency based on fixed periods of aeration and non-aeration. The removal of organic matter and phosphorous was also maintained at fair values of up to 90 and 70%, respectively.

Simultaneous removal of C, total N and P in a single continuous flow reactor

As it was expected the organic carbon was efficiently removed throughout all periods. The major effort was put on achieving simultaneous nitrification and denitrification and also on assuring the growth of P-accumulating organisms (PAO) within the reactor. The simultaneous removal of C, N and P was observed at its major intensity during the fourth period, in which air was introduced into the reactor at a fixed-time frequency (Table 2).

Regarding the removal of nitrogen, the presence of nitrates and nitrites in the liquid was very scarce and their concentration levels were always under 1 mgN/L. We sampled the mixed liquor in order to conduct specific activity assays, which revealed a strong activity of nitrifiers and denitrifiers within the sludge. Moreover, the absence of nitrites and nitrate in the liquid clearly indicated the existence of an amensalism interaction between nitrifiers and denitrifiers, according to which the oxidized forms of nitrogen produced by nitrifiers (i.e. nitrite or nitrate) were immediately reduced by denitrifiers. Moreover, speaking in kinetic terms the overall nitrogen removal process is limited by the nitrification process, i.e. by the kinetics of nitrite and nitrate formation. These observations are in accordance with previous results obtained with this type of reactor treating anaerobic effluents (Villaverde et al. 2001).

Also some interaction between the removal processes of N and P could be observed. From data shown in Table 3, the improvement on the efficiency of the removal of nitrogen from 60% in period III up to 90% in period IV, it was associated with a slight decrease in the efficiency of the removal of phosphorus from 80 to 70%, respectively. These differences can be explained by an intrinsic effect of the different SRT and the aeration control used during both periods, but also by the existence of microbial interactions between the microorganisms responsible for the removal of nitrogen and phosphorous.

However the idea of removing P biologically in one single continuous flow reactor was quite striking. In this sense we followed the concentrations of DO, nitrite, nitrate and phosphates during a complete aeration cycle, i.e. during the aeration and non-aeration steps, and we observed that P was accumulated during the aeration phase, which indicated the presence of PAO aerobes in the reactor. Also it was observed that P-release took place in the non-aeration phase of the cycle once oxygen and nitrate were completely absent in the media. These observations are in concordance with the mathematical models of biological phosphorus removal (Ekama and Wentzel,1999), according to which PAO aerobes accumulated P in oxic conditions and released P when exposed to anaerobic conditions, i.e. total absence of oxygen and nitrate.
Moreover, in order to demonstrate the presence of bioP-activity, we proceeded to the examination of the suspended solids of the mixed liquor by using Poly-P stain, FISH probes and microscopy techniques. The assays with Poly-P stain (Figure 2) clearly revealed the presence of Poly-P granules in the microbial flocs indicating the existence of P-accumulating activity although no information was obtained on either the chemical or biological origin of those granules. Thus far, we proceed for the analysis of the sludge for the presence of some PAO using FISH probes. We looked for some well know aerobic P-accumulating microorganisms such as *Proteobacteria*, we searched for alpha (α), beta (β) and gamma (γ) groups of *Proteobacteria* using FISH probes. The positive results with β (Figure 3) and γ probes indicated the presence in the microbial flocs of β and γ-*proteobacteria*, which confirm the presence of PAO responsible for the P-removal process taking place in the reactor.

![Figure 2](image1.png)  
**Figure 2.** Microscope photograph showing the stained Poly-P cristals (dark spots) within the microbial floc.

![Figure 3](image2.png)  
**Figure 3.** Microscope photograph of a floc with bacteria stained in green (light color) with a FISH probe specific for β-*Proteobacteria*.

These results probed the existence of bioP-removal process within the reactor. This is a quite unique scenario since the Enhance Biological Phosphorous Removal (EBPR) processes that operate in a continuous mode usually consist of several reactors/compartmentts (Farchill *et al.* 1993; Ekama and Wentzel, 1999).

### Table 4. Performance of the system in the period IV that lasted 77 days during which the hydraulic and solids retention times (HRT and SRT) were varied in order to achieved the highest removal percentages of C, N and P.

<table>
<thead>
<tr>
<th>Changes on HRT and SRT and duration</th>
<th>Parameters adjusted</th>
<th>Removal percentages</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HRT (h)</td>
<td>SRT (d)</td>
</tr>
<tr>
<td>1st change (20 days)</td>
<td>8.8</td>
<td>12.3</td>
</tr>
<tr>
<td>2nd change (20 days)</td>
<td>8.5</td>
<td>16.8</td>
</tr>
<tr>
<td>3rd change (37 days)</td>
<td>6.5</td>
<td>5.7</td>
</tr>
</tbody>
</table>

Regarding the effect of the HRT and SRT over the simultaneous removal of organic matter, total nitrogen and phosphorous (Henze *et al.* 2002), it can be observed in Table 4 the different removal percentages of C, N and P recorded during Period IV for different values of HRT and SRT. Also, in Figures 4 and 5 there are presented the experimental values of total N and phosphorous entering and leaving the reactor during this period together with the removal percentage of total N and phosphorous, respectively. It must be indicated that during the whole period the aeration was switched on and off following the same time sequence of 15 minutes of aeration and 30 minutes of non-aeration. Keeping this in mind, we operated for HRT and SRT values of 8.8 hours and 12.3 days, respectively. Under these conditions we obtained good removal percentages of C, N and P removal (see Table 4). With the aim of improving these results we increased the sludge age up to 16.8 days maintaining the HRT (of 8.5 h), with the purpose of allowing for a higher growth of nitrifiers. The response of the system was positive and we removed up to 90% of total nitrogen although the removal percentage of phosphorous decreased slightly. This indicated that the different microbial consortia growing within the reactor was in a complex equilibrium and that traditional criteria must be applied with special care when aiming the optimization of the process.
Afterwards we decided to decrease both, the HRT and SRT down to values of 6.5 hours and 5.7 days, respectively. The main purpose was to evaluate the robustness of the process, in terms of its capability for removing total N and P under very low HRT and SRT values. The system maintained a good removal percentage of organic matter and showed certain lack of stability regarding the removal of nitrogen and phosphorous (Figure 4 and 5), the reactor was able to remove total N and P at percentages that varied between 60-80 and 30-60, respectively.

**Figure 4.** Total nitrogen entering and leaving the reactor and its removal percentage during period IV. Total nitrogen is determine as the sum of ammonia, organic nitrogen, nitrite and nitrate. The vertical lines crossing the graph indicate the different changes on HRT and SRT values according to Table 4.

**Figure 5.** Phosphorous entering and leaving the reactor and its removal percentage during period IV. The vertical lines crossing the graph indicate the changes on HRT and SRT values according to Table 4.

CONCLUSIONS

A conventional activated sludge process has been adapted for the simultaneous removal of organic matter, total nitrogen and phosphorus, by means of implementing short aeration cycles that regulated the amount of dissolved oxygen available for microbial growth. The main conclusions that can be withdrawn are the following:
1. Throughout a single aeration cycle the dissolved oxygen was made to fluctuate between 0 and 3 mg/L causing the variation of most important chemical parameters, which modulated the activity and growth of the different microorganisms. The aeration control strategies were based on different control parameters such as dissolved oxygen, ORP and time. All of them allowed for the coexistence of the different microorganisms responsible for the removing of C, N and P.

2. The best results were obtained when operating with a fixed time sequence of On and Off aeration, more specifically cycles of 15 minutes of aeration and 30 minutes of non aeration. Under these conditions and operating with HRT of 8.5 hours and SRT of 16.8 days the system removed up to 90% of total and soluble COD, up to 90% of total nitrogen and up to 70% of phosphorous.

3. The removal process of nitrogen was controlled by the nitrification, i.e ammonia oxidation, since nitrate and nitrite levels were scarcely detected within the mixed liquor.

4. The removal process of phosphorous was conducted by PAO aerobes that accumulated and released P in aerobic and anaerobic conditions, respectively. This was also demonstrated by using Fluorescence In-Situ Hybridization (FISH) techniques, it was demonstrated the presence of beta and gamma Proteobacteria, a well known P-accumulating microorganism.

The results here presented prove that conventional processes can be easily upgraded for nutrient removal by a simple control of substrate/oxygen addition in order to attain the coexistence of nitrifiers, denitrifiers and PAO within the reactor. The application of these On/Off aeration strategies to those processes may upgrade them for the removal of nutrients without any major construction investments or space requirements.

ACKNOWLEDGEMENTS

This research was financially supported by the Ministry of Science and Technology of Spain through projects 1FD97-2175 and PPQ2000-1127, and the AGBAR Foundation.

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