Intermittent aeration in a hybrid moving bed biofilm reactor for carbon and nutrient biological removal

Gaetano Di Bella 1; Giorgio Mannina 2,3

1. University of Enna “Kore” (ITALY); gaetano.dibella@unikore.it
2. Department of Engineering, University of Palermo (ITALY); giorgio.mannina@unipa.it
3. College of Environmental Science and Engineering, Tongji University, 1239 Siping Road, Yangpu District, Shanghai 200092, China

* Correspondence: giorgio.mannina@unipa.it; Tel.: +39 09123896556

Abstract: The paper presents an experimental study on a lab scale Hybrid Moving Bed Biofilm Reactor with intermittent aeration. Specifically, a comparison between two different operating conditions were analyzed: continuous and intermittent aeration. Both continuous and intermittent aeration were monitored and compared in order to get the best operational conditions. The intermittent aeration campaign was sub-divided in 3 phases with different duration of alternation of aerobic and anoxic times and Organic and Nitrogen Loading Rates. The efficiency of N-removal improved by 70% during the intermittent aeration. The best condition was observed with 40 minutes of aeration and 20 minutes of no-aeration; organic loading rate of 2.2 kgCODm⁻³d⁻¹ and nitrogen loading rate of 0.25 kgNm⁻³d⁻¹; under these operational conditions the removal efficiencies for carbon and nitrogen were 93% and 90%, respectively. The derived results provide the basis for WWTP upgrade in order to meet stricter effluent limits at low energy requirements.

Keywords: Advanced wastewater treatment; Intermittent Aeration; SND; kinetic tests.

1. Introduction

Conventional Activated Sludge (CAS) plants for wastewater treatment have several limitations related to high production of excess sludge, large surface area demand and low flexibility. In addition, their upgrading generally involves the use of multiple tanks (anoxic, aerobic) in order to obtain a complete nutrient removal. In the last few years there has been a growing attention regarding the receiving water body quality state [1]. In this context, several wastewater treatment plants need to be upgraded in order to meet stricter effluent limits. CAS upgrading requires additional space that may not be available near the existing treatment plants and, whenever the space is available, large capital investments are needed in crowded metropolitan areas [2]. For this reason, in recent years the recurrence to other innovative systems for wastewater treatment is increased [3].

A possible solution is the introduction of new strategies and/or advanced wastewater treatment technologies. Among the new strategies, the intermittent aeration can be an optimal solution. More specifically, the intermittent aeration is the reduction of the aeration time of the biological reactor by introducing periods without oxygen supply for the denitrification process [4]. In such a way, aerobic and anoxic phases are periodically alternated simply through the tuning of aeration system (i.e. fixed control of phase duration). In particular, the control strategy is addressed to complete nitrification in the aerobic phase, and to reduce nitrate concentration during the anoxic phase in order to maximize total nitrogen removal efficiency [4,5].
Although intermittent aeration can guarantee good quality effluent, there are some disadvantages: it is very difficult to manage correctly it on the existing process or to improve it since most processes target the oxidation ditch and need facilities related to selector, final clarifier and return of sludge and treated water [6]. In this context, the vital parameters for good operation in these processes are dissolved oxygen (DO) conditions depending on aeration/anoxic mixing, control of DO and mixed liquor suspended solid (MLSS) concentrations. In fact, despite the anoxic and aerobic conditions can be regulated alternatively for biological nitrogen removal, the diffusion of oxygen inside of biomass flocs can be optimized [7].

Regarding the adoption of new advanced wastewater treatment technologies for plant upgrading, hybrid moving bed biofilm reactor (HMBBR) can be of great interest [8,9,27,28]. HMBBR can be adopted to upgrade existing overloaded activated sludge plants without building new tanks. Regarding the traditional biological suspended biomass systems, HMBBRs are characterized by the jointly of both suspended and attached biomass. The latter grows attached on small carrier elements that move freely along with the water in the reactor [10]. The developed biofilm increases the total biomass, as well as the pollutant removal rate. In addition, the hybrid reactor improves the removal of various types of substances since different species of bacteria, particularly the slow growers (such as nitrifiers), are able to grow in the biofilm. Interesting advantages of HMBBRs, especially looking at the traditional fixed bed biofilm reactor (biofilters), regard the low head losses, no filter channeling and no need of periodic backwashing [11].

In order to better enhance carbon and nitrogen removal the combination of intermittent aeration strategy and HMBBR can be a very attractive solution. In fact, the implementation of a simultaneous-nitrification-denitrification (SND) process, directly in the bioreactor, seems to be the best choice for biological nutrient removal (in terms of space required) [12]. Several researchers with intermittent aeration strategy have been investigated [13-15]. However, there are only few studies dealing with intermittent aeration in MBBR plants, especially for hybrid configuration, which, as far as authors are aware, are virtually absent [16].

Bearing in mind such considerations, the aim of the present study was to analyze the on/off aeration cycle in a HMBBR pilot plant, in order to verify the advantages and the limitation of this systems referring to carbon and nitrogen removal.

2. Materials and methods

2.1. The Pilot Plant

The study was conducted in a HMBBR laboratory scale plant, installed at the Laboratory of Sanitary and Environmental Engineering of Enna University (Kore). In Figure 1 the layout of pilot plant is shown.
The pilot plant was fed with synthetic wastewater with an influent flow of 1 L-h-1. The main compounds of the systemic mixture were: Sodium acetate, NH₄Cl, KPO₄. In particular, in order to control the influent concentration in different experimental periods, the dosage was changed.

The bioreactor was inoculated with activated sludge collected by the full-scale wastewater treatment plant of Enna (Italy). In order to optimize the process (mainly in terms of hydraulic performance) several operational condition changes have been carried out during the initial phase of the experimentation.

The biological phase was operated inside a bench-scale reactor designed to allow the development of suspended activated sludge and biofilm on free floating plastic carriers followed by a settlement unit. The setup consisted of a feeding tank (100 L), a bioreactor (7.5 L), where intermittent aeration occurs (equipped with air diffusers and mixer), and a settling tank (3.5 L). The biological reactor was filled with the KaldnesTM K1 carriers with a 33% filling ratio, corresponding to a specific surface area in the reactor of 150 m²·m⁻³. The typical characteristic of Kaldnes™ K1 carriers are: diameter = 9.1 mm, height = 7.2 mm, density = 0.95 kg·L⁻¹, porosity = 0.05. The "effective biofilm growth surface" and "carrier density" in the reactor are 95 m²·m⁻³ and 300 m²·m⁻³ respectively [9].

In order to control the influent flow and sludge recirculation (from settling tank to bioreactor) the plant was equipped with 2 peristaltic pumps. For the start-up phase, 10 L of activated sludge, drawn from the aeration tank of the wastewater treatment plant of Enna (IT), was inoculated directly into the bioreactor.

Overall, the experimental campaign lasted 200 days: after cultivation in batch mode (about 30 days), the operational conditions were changed with the aim to compare the results of different strategies in "continuous aeration" (CA) and "intermittent aeration" (IA) mode. A Programmable Logic Controller (PLC) regulated the on/off aeration cycles.

More specifically, the experimental campaign was divided in four phases, each constituted by different “Time of aeration” (or so called “Aeration Time”, tₐ).

The first phase, called "Phase I", lasted 50 days in CA condition.

The subsequent phases lasted 50 days and were characterized by intermittent aeration.

The second phase, called “Phase II”, was characterized by a total duration of the cycle (t_c) of 30 minutes, which the 50% of it was in aerated condition (both aeration time and anoxic time of 15 minutes each).

In the third and fourth phase (called “Phase III” and “Phase IV”, respectively), the t_c was increased until 60 minutes and was varied the duration of the aeration time: in Phase III, the t_a was 30 minutes with a t_a/t_c ratio equal to 0.5 (similarly to Phase II), in Phase IV, the t_a was increased to 40 minutes, with a ratio t_a/t_c = 0.66 (aeration time equal to 40 minutes and anoxic time of 20 minutes).
On the other hand, with the aim to evaluate also the influence of the influent organic matter, the OLR (organic loading rate) was gradually increased during the 200 days of experimentation, defining different “periods” in terms of OLR conditions. More specifically, the first two phases (Phase I and II) were constituted each by a Period (which lasted 50 days, coinciding with the duration of the phase). These periods were called “Period 0” and “Period 1”, respectively, and were characterized by an average value of OLR of 1.4 kgCOD m⁻³ d⁻¹.

The last phases were characterized each by two periods. More specifically, both Phase III and IV were constituted by two periods lasted 25 days where the influence of gradual increase of OLR was analyzed:

- in the Phase III, the periods called “Period 2A” and “Period 2B” were characterized by an OLR of 1.4 and 2.2 kgCOD m⁻³ d⁻¹, respectively;
- in the Phase IV, the periods called “Period 3A” and “Period 3B” were characterized by an OLR of 2.2 and 3.6 kgCOD m⁻³ d⁻¹, respectively.

All previous “Phases” (referred to different intermittent aeration strategies) and “Periods” (referred to different OLR) enabled to investigate the kinetics aspects and the performance of phenomena of organic matter removal, nitrification and denitrification with different strategy of aeration (continuous or intermittent), evaluating the best condition in terms of tₐ/tᵣ ratio and OLR.

Furthermore, it should be specified that the variation of the NLR (nitrogen loading rate) was carried out gradually with a step-wise strategy and keeping the C/N ratio (always equal to 14): the strategy was to study the carbon and nitrogen removal phenomena during “in series” operation and without stressing the biomass previously selected (especially the biofilm). On the other hand, for all operational conditions, the concentration of influent phosphorus (as orthophosphate) was ensured with a PO₄-P concentration always greater than 1% of the OLR.

Finally, the pilot plant operated under the condition of 11 hours of HRT and 15 days of SRT.

In Table 1, the main operational conditions are summarized.

| Phase | Period | Duration | Aeration | Average COD | Average VSS | Anoxic | Cycle |
|-------|--------|----------|----------|-------------|-------------|--------|-------|
| I     | 0      | 50       | continuous | 1.4±0.1    | 0.1±0.01    | -      | -     |
| II    | 1      | 50       | intermittent | 1.4±0.1    | 0.1±0.01    | 15     | 15    | 30    |
| III   | 2A     | 25       | intermittent | 1.4±0.1    | 0.1±0.01    | 30     | 30    | 60    |
|       | 2B     | 25       | intermittent | 2.2±0.1    | 0.15±0.01   | 30     | 30    | 60    |
| IV    | 3A     | 25       | intermittent | 2.2±0.1    | 0.15±0.01   | 40     | 20    | 60    |
|       | 3B     | 25       | intermittent | 3.3±0.1    | 0.24±0.01   | 40     | 20    | 60    |

2.2 Analytical methods

During plant management, the influent wastewater (section “Sec.1” of Figure 1), the mixed liquor in the biological reactor (section “Sec.2” of Figure 1) and the effluent flow (section “Sec.3” of Figure 1), were sampled mainly two times per week and samples were analyzed evaluating total and volatile suspended solids (TSS and VSS), soluble COD, NH₄-N, NO₂-N and NO₃-N [17]. Further, the dissolved oxygen (DO), pH and temperature (T) were daily measured using a handheld Multi 340i meter (WTW). It is worth noting that, the analyses of the suspended biomass in the mixed liquor section were carried out on the aerated sludge. More specifically, the analysis of total suspended solids (SS) and volatile (VSS) was carried out by filtering the sample with a 0.45 μm micron filter (GF/C). Once dried at 105 °, the residual content on the filter was evaluated and, after having...
compared it to the volume of filtered sample, they were determined in mgSS/L. Similarly, the VSSs were evaluated on the volatilized solid content, after incineration at 550 °C. On the other hand, biomass attached to carriers was measured by weighing 10–20 dried (105 °C, 1 h) carriers from the reactors and 10–20 unused carriers. The biofilm concentration was evaluated as the difference between unused and used carriers multiplied by the number of carriers in one liter [18].

Finally, in order to analyze the physiological conditions of the biomass and the kinetic behavior, the activated sludge functional microorganism groups (nitrifiers and heterotroph denitrifies) were characterized through determinations of maximum specific ammonia utilization rate (AUR) and nitrate utilization rate (NUR) test [19].

More specifically, to determine nitrification activity in the IA process, batch nitrification tests were conducted with the culture from the IA tanks (after the 60th day). A 3-L glass bottle was used as the batch reactor. Two and half liters of mixed liquor from an IA tank were introduced into the batch reactor for each batch nitrification test. Ammonium chloride (NH₄Cl) was added into the batch reactor. The ratio of the influent C/N at the beginning of each batch test was maintained the same as that in the influent to the continuous flow IA tanks, by adding methanol to the batch reactor. The batch reactor was well mixed with a magnetic stirrer, and continuously aerated through a diffusing stone with an airflow rate of 500 mL·min⁻¹. Six samples (10 mL each) were drawn at designated intervals of 15 minutes and analyzed for NH₄-N, NO₃-N, NO₂-N and VSS.

On the other hand, denitrification activity of the culture in the IA tank was also studied in batch tests. In these batch tests, the IA tank was operated as a batch reactor without aeration. At the beginning of the batch tests, the batch reactor was spiked with potassium nitrate (KNO₃) to result in different initial concentrations of nitrate-nitrogen (ranged from 30 to 40 mg·L⁻¹) in the batch tests. Each batch test was conducted after steady state of the IA process was achieved in the tank. The initial C/N ratio in the batch tests was maintained at the same value of influent in bench scale, by adding AcNa to the batch reactor. The batch reactor was then sealed and completely mixed with a magnetic stirrer. Mixed liquor samples (10 mL) were taken from the batch reactor at designated intervals of 15 minutes for analyses of NO₃-N, NO₂-N and VSS to monitor the denitrification activity.

### Table 2  Influent and effluent qualities

| Period | Parameter | Influent | Effluent | Removal |
|--------|-----------|----------|----------|---------|
|        |           | Average concentration [mg/L] | Average concentration [mg/L] | Average [%] |
| 0      | COD       | 380 ± 26 | 47 ± 12  | 87 ± 4  |
|        | NH₄       | 30 ± 4.5 | 14 ± 4.5 | 67 ± 11 |
|        | P_{TOT}   | 5.5 ± 0.3| 4.2 ± 0.1| 14.3 ± 2|
| 1      | COD       | 380 ± 35 | 13 ± 5   | 96 ± 2  |
|        | NH₄       | 30 ± 1.5 | 3.5 ± 1.7| 87 ± 3  |
|        | P_{TOT}   | 5.5 ± 0.8| 1.6 ± 0.25| 65 ± 5 |
| 2A     | COD       | 380 ± 54 | 13 ± 4   | 97 ± 1  |
|        | NH₄       | 30 ± 2.5 | 1.7 ± 1.6| 80 ± 10 |
|        | P_{TOT}   | 5.5 ± 0.7| 2.6 ± 0.1| 47 ± 2  |
| 2B     | COD       | 640 ± 31 | 24 ± 18  | 95 ± 3  |
|        | NH₄       | 45 ± 0.5 | 0.6 ± 0.2| 83 ± 5  |
|        | P_{TOT}   | 8.5 ± 0.3| 1.6 ± 0.5| 71 ± 6  |
| 3A     | COD       | 640 ± 11 | 24 ± 3   | 96 ± 2  |

3. Results and Discussion

In the following paragraphs the performance of the pilot plant in all the experimental periods have been discussed. In table 2, the values relating to the quality of the influent and effluent were previously shown (with the average removal efficiency in the period coupled).
3.1. COD removal and Biomass growth

In Figure 2, the results of organic removal performance and biomass growth, in terms of COD concentration and MLSS variation trend in the bioreactor, are shown.

High carbon performances were achieved in both aeration plant strategies (i.e. continuous and intermittent). More specifically, under all operating conditions, organics were removed satisfactory. The organic removal performance increased slowly with the growth of attached biomass: the average organic removal increased from 81% to 87% during the continuous aeration period (Phase I), and 93-95% in the subsequent periods with intermittent aeration, despite the increase in OLR in the Period 2B and 3B.

Once the biofilm was growth, the invariance of the data of organic removal performance confirms the results reported by other authors concerning the intermittent aeration strategy applied to other installations [20,21]. Therefore, the HMBBR under intermittent aeration showed that the growth of heterotrophic biomass as biofilm is a further advantage in terms of COD removal and biological process stability.

In this context, it is important to underline that the growth of the biofilm on the physical supports occurred mainly during the Phase I, with a “continuous aeration”. More specifically, the concentration of the biofilm reached at the end of the Period 0 (namely, around 1 g∙L⁻¹) was maintained almost constant during the sub-sequential intermittent aeration phases. In fact, the biological stress operated by alternating the aerated and non-aerated stages can limit the biofilm growth. To confirm such a fact, during the Phase IV (in both Period 3A and 3B), when the length of aeration period was increased, it seems that the biofilm growth starts again.

On the other hand, during all periods of the “intermittent aeration”, in Phases II-IV, both the suspended biomass and biofilm concentration amounted to the almost constant values reached at the end of Period 0, with a constant SRT equal to 15 days. More specifically, the MLSS concentration was maintained at about 2.5-3 g∙L⁻¹ during the Period 1 and 2A, and slightly less than 2.5 g∙L⁻¹ in subsequent Periods 2A-2B and 3 g∙L⁻¹ in the Periods 3A-3B. By contrast, the relationship between the VSS and TSS grew during the experimental campaign, from 70 to 85%; probably, this phenomenon was due to mixed liquor seeding by the biofilm dethatched. A further increase (3-5%) was observed when the organic loading rate was increased during the Periods 2B and 3B.

|       | NH₄ | P⁰TOT  | COD  | NH₄ | P⁰TOT  |
|-------|-----|--------|------|-----|--------|
| 3B    | 45 ± 3.9 | 8.5 ± 0.1 | 1.4 ± 0.6 | 89 ± 1 |
|       | 1050 ± 70 | 55 ± 13 | 94 ± 2 |
|       | 61 ± 5 | 0.35 ± 0.15 | 93 ± 2 |
|       | 12 ± 0.5 | 6.3 ± 0.5 | 38 ± 3 |

Figure 2 COD concentration in the influent and effluent flow (a) and MLSS variation in the bioreactor (b)
3.2 Nitrification and denitrification phenomena

Regarding the nitrogen removal, Figure 3 reports the nitrogen data nitrified and denitrified.

As shown in Figure 3a, the nitrates were produced after the 10th day, confirming the growth of autotrophic biomass. More specifically, the nitrification process improved in time, according to data shown in Figure 3b and 3c, because the autotrophic organisms grew in both suspended and attached form. In general, the optimum nitrification has been reached when the longest aeration phase was applied (Phase IV), confirming an average nitrification greater than 90%.

Concerning the nitrogen removal, as expected, the denitrification processes occurred only when the intermittent aeration was performed. In general, a good nitrogen removal was reached in all IA phases. The total nitrogen removal was mainly due to SND process in the reactor. In particular, when the aeration phase was turned off, the DO in the reactor decreased from about 2-3 mg L\(^{-1}\) to 0 mg L\(^{-1}\) in few minutes and a sufficient carbon source was available due to the supplemented influent, which was good for denitrification. In this context, it is interesting to underline that the nitrates totally disappear in the Period 3B, when the intermittent aeration was performed with 40 minutes of t\(_a\) and 20 minutes of no-aeration (t\(_n\)) and, in particular, when OLR was equal to 3.6 kg COD m\(^{-3}\) d\(^{-1}\). In this case, the total nitrogen concentrations in the effluent were kept less than 1-2 mg L\(^{-1}\). On the contrary, during the period in continuous aeration, although the ammonia nitrogen was nitrified with an efficiency > 60%, the removal of total nitrogen was negligible (<20% because there are not denitrification).

More detailed findings, confirming the general behavior described in the previous figures, can be deduced from Figure 3c. In particular, the analysis of the average performance of nitrification and denitrification in each period demonstrates other important results.

In the Period 0 the denitrification processes did not occurred, because the anoxic conditions were never established. By contrast, the nitrification was already satisfactory (> 60%) because the inoculation was carried out with activated sludge collected from a WWTP where the autotrophic bacteria were present, furthermore, the pilot plant was conducted with high SRT, in order to favor the biofilm growth on the carrier.

- In the Period 1, the nitrification performance further increased, because the "slow-growing biomass" continues to grow. The denitrification reached satisfactory values of removal (70%) because the phases of "no-aeration" guarantee the anoxic conditions, which were absent in the period to continuous aeration.

- In Phase III, similarly for both Periods 2A and 2B, the performance of nitrification did not change, despite the duration of the aeration stage was doubled compared to the previous period (from 15 to 30 minutes): this is probably due to the fact that the \(t_{a}/t_{n}\) ratio was maintained at a value of 0.5. Probably in this context, the aerobic autotrophic bacteria have not particular benefit from the increase in continuous aeration period due to the identical duration of the period where the oxygen was absence. On the contrary, it seems that the longer "stopping" in the bioreactor aeration caused a slightly decrease in the nitrification. As for the denitrification, in the Period 2A the pilot plant shows a behavior similar to performance the "nitrification" in the transition from Period 0 to the Period 1: the denitrification remains almost constant at 70% value, because the denitrified bacteria were not favorited by the increase of no-aerated phase, due to the not perfect balance in duration between aerated and non-aerated phases (similarly to what happened for autotrophic bacteria). On the other hand, in the Period 2B the increase of substrate availability (mainly in terms of biodegradable carbon) improved the denitrification performance of heterotrophic bacteria, that are less limited by the organic substrate.

- In Phase IV, the new cycle conditions improved the overall performance of both nitrogen transformation phenomena. The performance of nitrification greatly increased (from 83% to 86% in Period 3A and 91% in Period 3B) despite the total cycle time was maintained at 60 minutes. More specifically, in the Period 3A and 3B, in fact, the duration of the aeration phase was increased at the expense of that of "no-aeration" (\(t_{a}/t_{n}\) ratio is now of 0.66); this has contributed to favor the kinetics of autotrophic bacteria, but did not alter substantially the kinetics of denitrifying bacteria (which have guaranteed almost constant performance). By contrast, the
latter were helped by the increase in the carbonaceous substrate only during the Period 3B, so the denitrification performances increased from 81% (Period 3A) to over 90% (COD concentration increased from about 650 mg/L, in the period 3A, to about 1050 mg/L, in the Period 3B).

In order to complete nitrogen removal discussion, the figure 3(e) summarizes the mass balance of nitrogen forms. According with what described above, the graph clearly shows that:

- nitrified nitrogen increases over time (from Period 0 to period 3B), depending on the stabilization of the nitrifying bacteria and the optimization of the operating conditions;
- the nitrogen lost by cell synthesis is in the range 33-38% approximately;
- in almost all experimental periods, the removal of total nitrogen is attributable to a maximum of 65% to the net nitrification of ammonia nitrogen and to 35% of cell assimilation.
The results observed in terms of performance were confirmed by the specific removal of nitrate and ammonium, shown in the Figure 3d (in terms of specific rate of nitrification and denitrification).

The data reported in the graphs were calculated on the basis of the individual tests of AUR and NUR, reported in Figures 4 and 5. More specifically, the analysis of AUR and NUR test confirms that both nitrification and denitrification activities were satisfactory, with velocities of nitrification and denitrification respectively equal to:

- 1.7±0.5 mgNO₃⁻N nitrified·gSS⁻¹·h⁻¹ and 5.58±0.8 mgNO₃⁻N denitrified·gSS⁻¹·h⁻¹ in the Period 1 (at the end of the period),
- 2.09±0.15 mgNO₃⁻N nitrified·gSS⁻¹·h⁻¹ and 7.05±1.4 mgNO₃⁻N denitrified·gSS⁻¹·h⁻¹ in the Period 2A,
- 1.79±0.21 mgNO₃⁻N nitrified·gSS⁻¹·h⁻¹ and 8.11±1.4 mgNO₃⁻N denitrified·gSS⁻¹·h⁻¹ in the Period 2B,
- 2.78±0.4 mgNO₃⁻N nitrified·gSS⁻¹·h⁻¹ and 5.48±0.2 mgNO₃⁻N denitrified·gSS⁻¹·h⁻¹ in the Period 3A,
- 2.01±0.3 mgNO₃⁻N nitrified·gSS⁻¹·h⁻¹ and 6.88±0.4 mgNO₃⁻N denitrified·gSS⁻¹·h⁻¹ in the Period 3B.

**Figure 3** NH₄-N and NO₃-N concentration in the influent and effluent (a), and specific mass nitrogen denitrified and nitrified (b), efficiency of removal (c), (d) and specific rate (d) of nitrification/denitrification process (d) and -(e) Nitrogen-nitrogen Mass-mass balance (e).
It is important to underline that the specific nitrate formation rates were in the range 1.7–2.8 mgNO₃-N gSS⁻¹ h⁻¹, which were within literature range (namely, 0.78–7. mgNO₃⁻¹-Nitrified gSS⁻¹ h⁻¹). [22]. Similar results were derived for the specific denitrification rates or specific ammonium oxidation rates (2.76 - 9.05 mgNO₃-N denitrified gSS⁻¹ h⁻¹).

The overall AUR data reported in Figure 3d also show that the nitrification activity remained meanly constant for all periods, with small increase in the Period 3A. Contrarily, the rate of denitrification only improves from Period 1 to Period 2B. In the Period 3A the denitrification rate decreases slightly, due to the reduction of the no-aerated time. However, it is important to underline that the denitrification was generally improved when a greater OLR was applied for the specific Period (2B and 3B).

Figure 4 AUR test

Figure 5 NUR test
3.3 Phosphorus removal

In general, if an anaerobic phase was not planned, the removal of phosphorus is almost exclusively due to the metabolic needs of the biomass. Nevertheless, in the case of IA operation without automatic control, the analysis of phosphorus removal can emphasize the real conditions of anoxia and anaerobic conditions that occur during the process, especially when a biofilm is present in the bioreactor. In particular, if the removal of phosphorus exceeds 10-15%, it is possible to deduce that there is a concrete contribution of phosphorus accumulating organisms in phosphorus removal [23,24]. The phenomenon is due to the development of an anaerobic layer periodically formed in the biofilm during the no-aerated period.

In Figure 6 the phosphorus concentration and the performance of removal were shown.

![Graph](image)

**Figure 6** Total phosphorus concentration in the influent and effluent (a) and removal efficiency (b)

In general, the data reported in Figure 6 show that during the CA period the phosphorous removal is due only to the metabolic needs. On the other hand, during the IA period the phosphorous removal increased. Nevertheless, in this case, as shown by the data reported in Figure 6b, it is evident the performance difference between Periods 1-2A-2B and Periods 3A-3B.

In Periods 1, 2A and 2B, the high performance of P removal confirms that the anoxic denitrification of nitrates probably competes with the anaerobic metabolism of Poly-P (PHB storage and P release), which subsequently used the P released in the anaerobic phase. So, probably during the time of non-aerated phase both the anoxic and anaerobic phase occur. During the Periods 3A and 3B this phenomenon is attenuated and the non-aerated time is largely used for the anaerobic denitrification, with reduction of the total performances of phosphorous removal by Poli-P.

Finally, the analysis of Figure 6b also shows that the increase in the carbon load, in both Periods 2B and 3B, favors the anaerobic metabolism operated by the phosphorus accumulating bacteria: this is due to an improvement in the polyhydroxybutyrate (PHB) storage as uncontrolled effect of the greater organic matter concentration during the competitive phase carried out by denitrifying bacteria and polyphosphate-accumulating organisms (PAOs) [25, 26].

4. Conclusions

The HMBBR process was implemented with intermittent aeration to regulate SND process. When treating a regular wastewater, the TN were approximately 90%, and their concentrations in the unfiltered effluent were generally less than 5 mg/L. In particular, after an integrated analysis of different operational condition, the best IA period was obtained by alternating 40-minute of aeration with 20 minutes of non-aeration.

The combination of intermittent aeration and biofilm-suspended biomass in the reactor played a critical role in the success of the process to achieve enhanced nutrient removal and energy saving. Therefore, the derived results offer a very useful database for real WWTPs aimed at establishing a good compromise between strict effluent quality and energy consumption.
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