

1 Article

2 Intermittent aeration in a hybrid moving bed biofilm 3 reactor for carbon and nutrient biological removal

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10 Academic Editor: name

11 Received: date; Accepted: date; Published: date

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

24 **Keywords:** Advanced wastewater treatment; Intermittent Aeration; SND; kinetic tests.
25

26 1. Introduction

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

36 A possible solution is the introduction of new strategies and/or advanced wastewater treatment
37 technologies. Among the new strategies, the intermittent aeration can be an optimal solution. More
38 specifically, the intermittent aeration is the reduction of the aeration time of the biological reactor by
39 introducing periods without oxygen supply for the denitrification process [4]. In such a way, aerobic
40 and anoxic phases are periodically alternated simply through the tuning of aeration system (i.e. fixed
41 control of phase duration). In particular, the control strategy is addressed to complete nitrification in
42 the aerobic phase, and to reduce nitrate concentration during the anoxic phase in order to maximize
43 total nitrogen removal efficiency [4,5].

44 Although intermittent aeration can guarantee good quality effluent, there are some
45 disadvantages: it is very difficult to manage correctly it on the existing process or to improve it since
46 most processes target the oxidation ditch and need facilities related to selector, final clarifier and
47 return of sludge and treated water [6]. In this context, the vital parameters for good operation in these
48 processes are dissolved oxygen (DO) conditions depending on aeration/anoxic mixing, control of DO
49 and mixed liquor suspended solid (MLSS) concentrations. In fact, despite the anoxic and aerobic
50 conditions can be regulated alternatively for biological nitrogen removal, the diffusion of oxygen
51 inside of biomass flocs can be optimized [7].

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

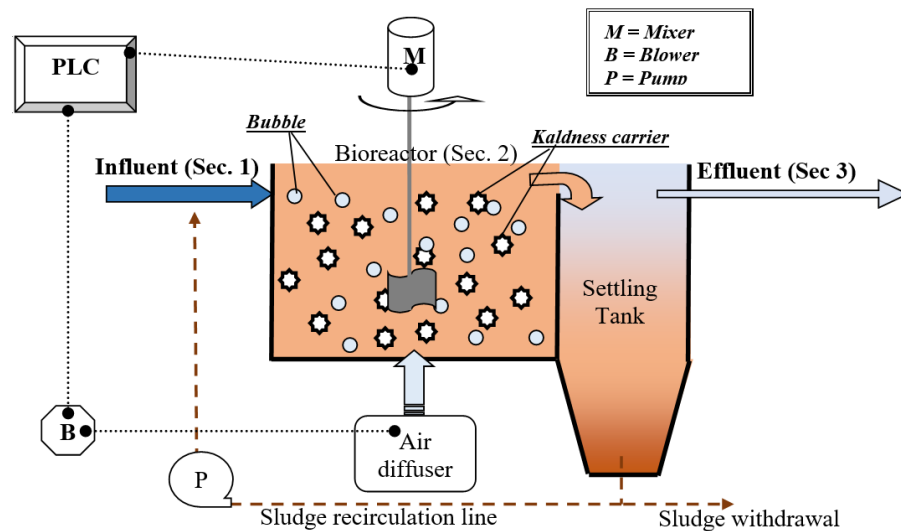
63 In order to better enhance carbon and nitrogen removal the combination of intermittent aeration
64 strategy and HMBBR can be a very attractive solution. In fact, the implementation of a simultaneous-
65 nitrification-denitrification (SND) process, directly in the bioreactor, seems to be the best choice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

70 Bearing in mind such considerations, the aim of the present study was to analyze the on/off
71 aeration cycle in a HMBBR pilot plant, in order to verify the advantages and the limitation of this
72 systems referring to carbon and nitrogen removal.
73

74 **2. Materials and methods**

75 *2.1. The Pilot Plant*

76 The study was conducted in a HMBBR laboratory scale plant, installed at the Laboratory of
77 Sanitary and Environmental Engineering of Enna University (Kore). In Figure 1 the layout of pilot
78 plant is shown.
79



80

81 **Figure 1** Schematic overview of the IA-MBBR pilot plant and carrier features

82 The pilot plant was fed with synthetic wastewater with an influent flow of 1 L·h⁻¹. The main
 83 compounds of the systemic mixture were: Sodium acetate, NH₄Cl, KPO₄. In particular, in order to
 84 control the influent concentration in different experimental periods, the dosage was changed.

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

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

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

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

105 More specifically, the experimental campaign was divided in four phases, each constituted by
 106 different "Time of aeration" (or so called "Aeration Time", t_a).

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

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

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

112 In the third and fourth phase (called "Phase III" and "Phase IV", respectively), the t_c was
 113 increased until 60 minutes and was varied the duration of the aeration time: in Phase III, the t_a was
 114 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
 115 minutes, with a ratio $t_a/t_c = 0.66$ (aeration time equal to 40 minutes and anoxic time of 20 minutes).

116 On the other hand, with the aim to evaluate also the influence of the influent organic matter, the
 117 OLR (organic loading rate) was gradually increased during the 200 days of experimentation, defining
 118 different "periods" in terms of OLR conditions. More specifically, the first two phases (Phase I and
 119 II) were constituted each by a Period (which lasted 50 days, coinciding with the duration of the
 120 phase). These periods were called "Period 0" and "Period 1", respectively, and were characterized by
 121 an average value of OLR of 1.4 $\text{kgCOD m}^{-3} \text{d}^{-1}$.

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

- 125 • in the Phase III, the periods called "Period 2A" and "Period 2B" were characterized by
 126 an OLR of 1.4 and 2.2 $\text{kgCOD m}^{-3} \text{d}^{-1}$, respectively;
- 127 • in the Phase IV, the periods called "Period 3A" and "Period 3B" were characterized by
 128 an OLR of 2.2 and 3.6 $\text{kgCOD m}^{-3} \text{d}^{-1}$, respectively.

129 All previous "Phases" (referred to different intermittent aeration strategies) and "Periods"
 130 (referred to different OLR) enabled to investigate the kinetics aspects and the performance of
 131 phenomena of organic matter removal, nitrification and denitrification with different strategy of
 132 aeration (continuous or intermittent), evaluating the best condition in terms of t_a/t_c ratio and OLR.

133 Furthermore, it should be specified that the variation of the NLR (nitrogen loading rate) was
 134 carried out gradually with a step-wise strategy and keeping the C/N ratio (always equal to 14): the
 135 strategy was to study the carbon and nitrogen removal phenomena during "in series" operation and
 136 without stressing the biomass previously selected (especially the biofilm). On the other hand, for all
 137 operational conditions, the concentration of influent phosphorus (as orthophosphate) was ensured,
 138 with a $\text{PO}_4\text{-P}$ concentration always greater than 1% of the OLR.

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

140 In Table 1, the main operational conditions are summarized.

141
142

Table 1 Operational condition

Phase	Period	Duratio [day]	Aeration	Average kgCOD m^{-3}	Average kgN m^{-3}	Aeration [min]	Anoxic [min]	Cycle [min]
I	0	50	continuous	1.4±0.1	0.1±0.01	<i>continuou</i>	-	-
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

143

144 2.2 Analytical methods

145 During plant management, the influent wastewater (section "Sec.1" of Figure 1), the mixed
 146 liquor in the biological reactor (section "Sec.2" of Figure 1) and the effluent flow (section "Sec.3" of
 147 Figure 1), were sampled meanly two times per week and samples were analyzed evaluating total and
 148 volatile suspended solids (TSS and VSS), soluble COD, $\text{NH}_4\text{-N}$, $\text{NO}_2\text{-N}$ and $\text{NO}_3\text{-N}$ [17]. Further, the
 149 dissolved oxygen (DO), pH and temperature (T) were daily measured using a handheld Multi 340i
 150 meter (WTW). It is worth noting that, the analyses of the suspended biomass in the mixed liquor
 151 section were carried out on the aerated sludge. More specifically, the analysis of total suspended
 152 solids (SS) and volatile (VSS) was carried out by filtering the sample with a 0.45 μm filter
 153 (GF/C). Once dried at 105 °, the residual content on the filter was evaluated and, after having

154 compared it to the volume of filtered sample, they were determined in mgSS/L. Similarly, the VSS_s
 155 were evaluated on the volatilized solid content, after incineration at 550 °C. On the other hand,
 156 biomass attached to carriers was measured by weighing 10–20 dried (105 °C, 1h) carriers from the
 157 reactors and 10–20 unused carriers. The biofilm concentration was evaluated as the difference
 158 between unused and used carriers multiplied by the number of carriers in one liter [18].

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

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

172 On the other hand, denitrification activity of the culture in the IA tank was also studied in batch
 173 tests. In these batch tests, the IA tank was operated as a batch reactor without aeration. At the
 174 beginning of the batch tests, the batch reactor was spiked with potassium nitrate (KNO₃) to result in
 175 different initial concentrations of nitrate-nitrogen (ranged from 30 to 40 mg·L⁻¹) in the batch tests.
 176 Each batch test was conducted after steady state of the IA process was achieved in the tank. The initial
 177 C/N ratio in the batch tests was maintained at the same value of influent in bench scale, by adding
 178 AcNa to the batch reactor. The batch reactor was then sealed and completely mixed with a magnetic
 179 stirrer. Mixed liquor samples (10 mL) were taken from the batch reactor at designated intervals of 15
 180 minutes for analyses of NO₃-N, NO₂-N and VSS to monitor the denitrification activity.
 181

182 3. Results and Discussion

183 In the following paragraphs the performance of the pilot plant in all the experimental periods
 184 have been discussed. In table 2, the values relating to the quality of the influent and effluent were
 185 previously shown (with the average removal efficiency in the period coupled).
 186
 187

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
	NH ₄	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
	NH ₄	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
	NH ₄	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
	NH ₄	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

	NH ₄	45 ± 3.9	1.4 ± 0.6	89 ± 1
	P _{TOT}	8.5 ± 0.1	5.3 ± 0.1	26 ± 2
3B	COD	1050 ± 70	55 ± 13	94 ± 2
	NH ₄	61 ± 5	0.35 ± 0.15	93 ± 2
	P _{TOT}	12 ± 0.5	6.3 ± 0.5	38 ± 3

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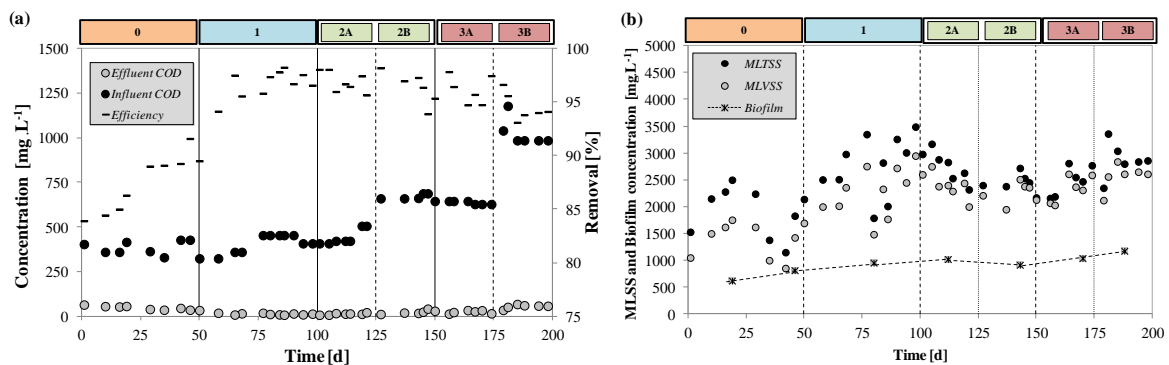
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3.1. COD removal and Biomass growth

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

192 High carbon performances were achieved in both aeration plant strategies (i.e. continuous and
 193 intermittent). More specifically, under all operating conditions, organics were removed satisfactory.

194 The organic removal performance increased slowly with the growth of attached biomass: the
 195 average organic removal increased from 81% to 87% during the continuous aeration period (Phase I),
 196 and 93-95% in the subsequent periods with intermittent aeration, despite the increase in OLR in the
 197 Period 2B and 3B.
 198



199

200 **Figure 2** COD concentration in the influent and effluent flow (a) and MLSS variation in the bioreactor (b)

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

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

213 On the other hand, during all periods of the “intermittent aeration”, in Phases II-IV, both the
 214 suspended biomass and biofilm concentration amounted to the almost constant values reached at the
 215 end of Period 0, with a constant SRT equal to 15 days. More specifically, the MLSS concentration was
 216 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
 217 Periods 2A-2B and 3 g·L⁻¹ in the Periods 3A-3B. By contrast, the relationship between the VSS and
 218 TSS grew during the experimental campaign, from 70 to 85%: probably, this phenomenon was due
 219 to mixed liquor seeding by the biofilm dethatched. A further increase (3-5%) was observed when the
 220 organic loading rate was increased during the Periods 2B and 3B.

221 3.2 Nitrification and denitrification phenomena

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

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

228 Concerning the nitrogen removal, as expected, the denitrification processes occurred only when
229 the intermittent aeration was performed. In general, a good nitrogen removal was reached in all IA
230 phases. The total nitrogen removal was mainly due to SND process in the reactor. In particular, when
231 the aeration phase was turned off, the DO in the reactor decreased from about 2-3 mg·L⁻¹ to 0 mg·L⁻¹
232 in few minutes and a sufficient carbon source was available due to the supplemented influent, which
233 was good for denitrification. In this context, it is interesting to underline that the nitrates totally
234 disappear in the Period 3B, when the intermittent aeration was performed with 40 minutes of t_a and
235 20 minutes of no-aeration (t_{na}) and, in particular, when OLR was equal to 3.6 kgCOD m⁻³ d⁻¹. In this
236 case, the total nitrogen concentrations in the effluent were kept less than 1-2 mg·L⁻¹. On the contrary,
237 during the period in continuous aeration, although the ammonia nitrogen was nitrified with an
238 efficiency > 60%, the removal of total nitrogen was negligible (<20% because there are not
239 denitrification).

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

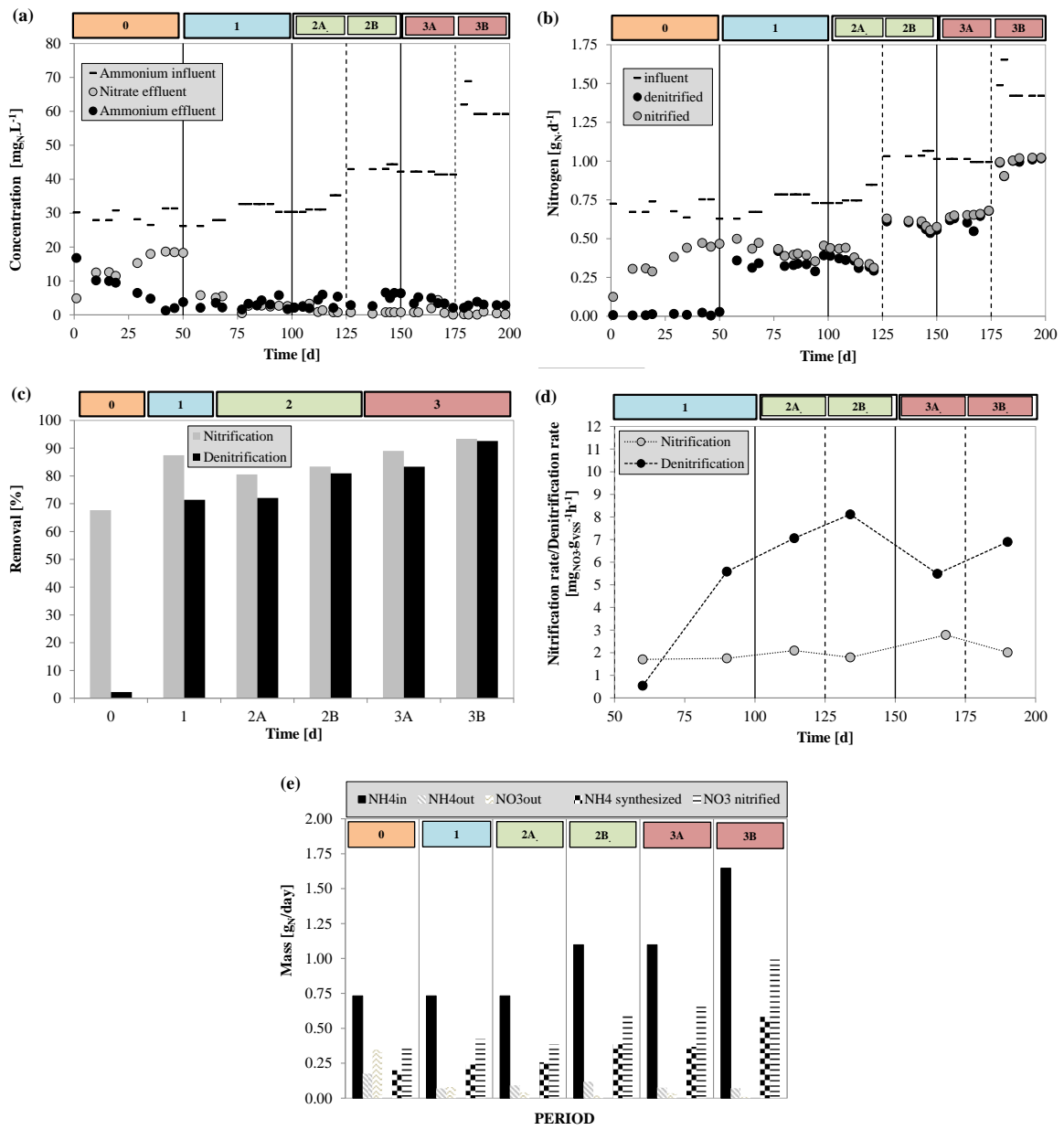
243 In the Period 0 the denitrification processes did not occurred, because the anoxic conditions were
244 never established. By contrast, the nitrification was already satisfactory (> 60%) because the
245 inoculation was carried out with activated sludge collected from a WWTP where the autotrophic
246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

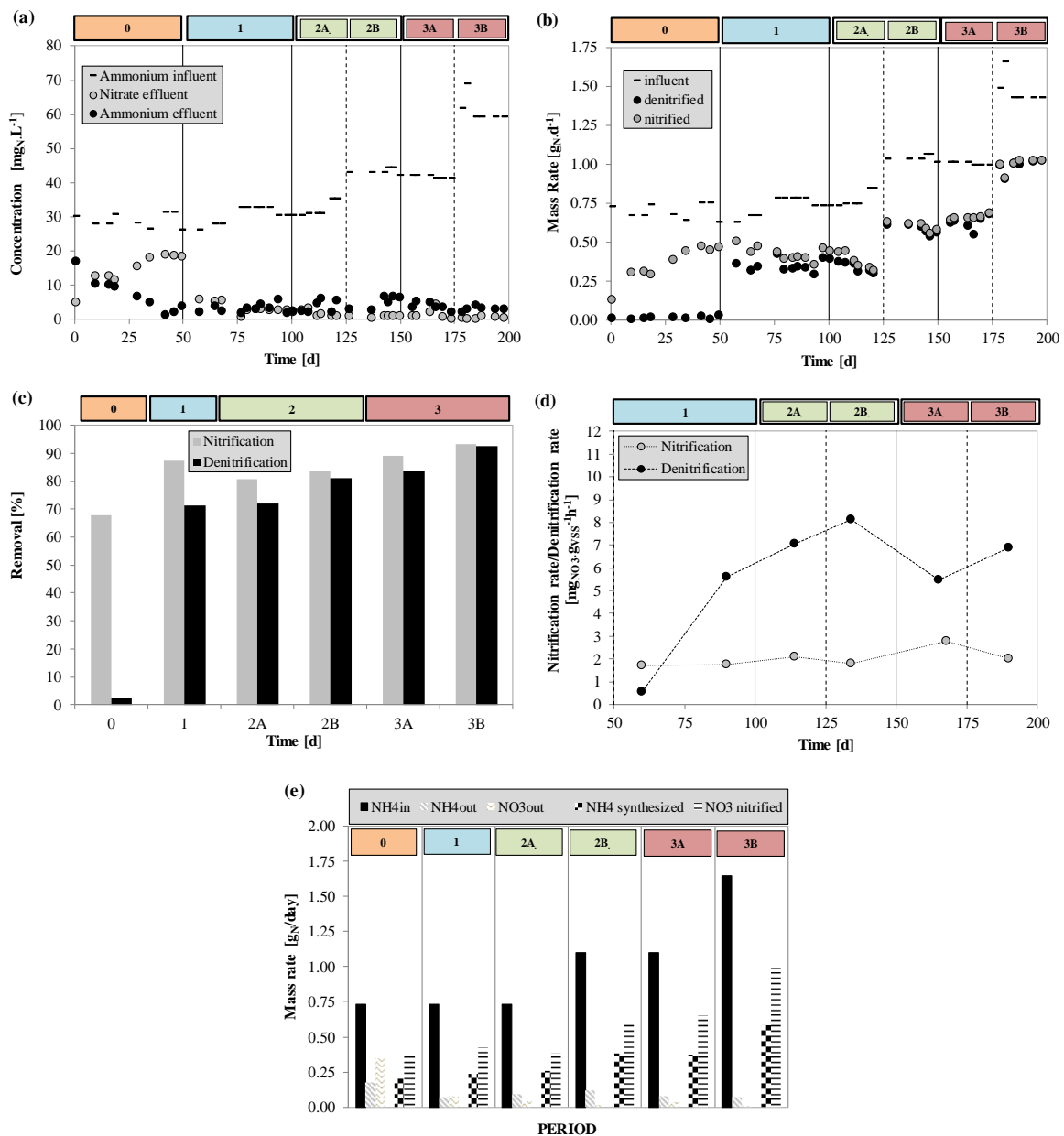
- 248 • In the Period 1, the nitrification performance further increased, because the "slow-growing
249 biomass" continues to grow. The denitrification reached satisfactory values of removal (70%)
250 because the phases of "no-aeration" guarantee the anoxic conditions, which were absent in the
251 period to continuous aeration.
- 252 • In Phase III, similarly for both Periods 2A and 2B, the performance of nitrification did not
253 change, despite the duration of the aeration stage was doubled compared to the previous period
254 (from 15 to 30 minutes): this is probably due to the fact that the t_a/t_c ratio was maintained at a
255 value of 0.5. Probably in this context, the aerobic autotrophic bacteria have not particular benefit
256 from the increase in continuous aeration period due to the identical duration of the period where
257 the oxygen was absence. On the contrary, it seems that the longer "stopping" in the bioreactor
258 aeration caused a slightly decrease in the nitrification. As for the denitrification, in the Period
259 2A the pilot plant shows a behavior similar to performance the "nitrification" in the transition
260 from Period 0 to the Period 1: the denitrification remains almost constant at 70% value, because
261 the denitrified bacteria were not favorited by the increase of no-aerated phase, due to the not
262 perfect balance in duration between aerated and non-aerated phases (similarly to what
263 happened for autotrophic bacteria). On the other hand, in the Period 2B the increase of substrate
264 availability (mainly in terms of biodegradable carbon) improved the denitrification performance
265 of heterotrophic bacteria, that are less limited by the organic substrate.
- 266 • In Phase IV, the new cycle conditions improved the overall performance of both nitrogen
267 transformation phenomena. The performance of nitrification greatly increased (from 83% to 86%
268 in Period 3A and 91% in Period 3B) despite the total cycle time was maintained at 60 minutes.
269 More specifically, in the Period 3A and 3B, in fact, the duration of the aeration phase was
270 increased at the expense of that of "no-aeration" (t_a/t_c ratio is now of 0.66): this has contributed
271 to favor the kinetics of autotrophic bacteria, but did not alter substantially the kinetics of
272 denitrifying bacteria (which have guaranteed almost constant performance). By contrast, the

273 latter were helped by the increase in the carbonaceous substrate only during the Period 3B, so
274 the denitrification performances increased from 81% (Period 3A) to over 90% (COD
275 concentration increased from about 650 mg/L, in the period 3A, to about 1050 mg/L, in the Period
276 3B).

277
278 In order to complete Nitrogen-nitrogen removal discussion, the figure 3(e) summarizes the mass
279 balance of nitrogen forms. According with what described above, the graph clearly shows that:

- 280 • nitrified nitrogen increases over time (from Period 0 to period 3B), depending on the
- 281 stabilization of the nitrifying bacteria and the optimization of the operating conditions:
- 282 • the nitrogen lost by cell synthesis is in the range 33-38% approximately;
- 283 • in almost all experimental periods, the removal of total nitrogen is attributable to a maximum of
- 284 65% to the net nitrification of ammonia nitrogen and to 35% of cell assimilation.
- 285





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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), and specific rate (d) of nitrification/denitrification process (d) and Nitrogen-nitrogen Mass-mass balance (e).

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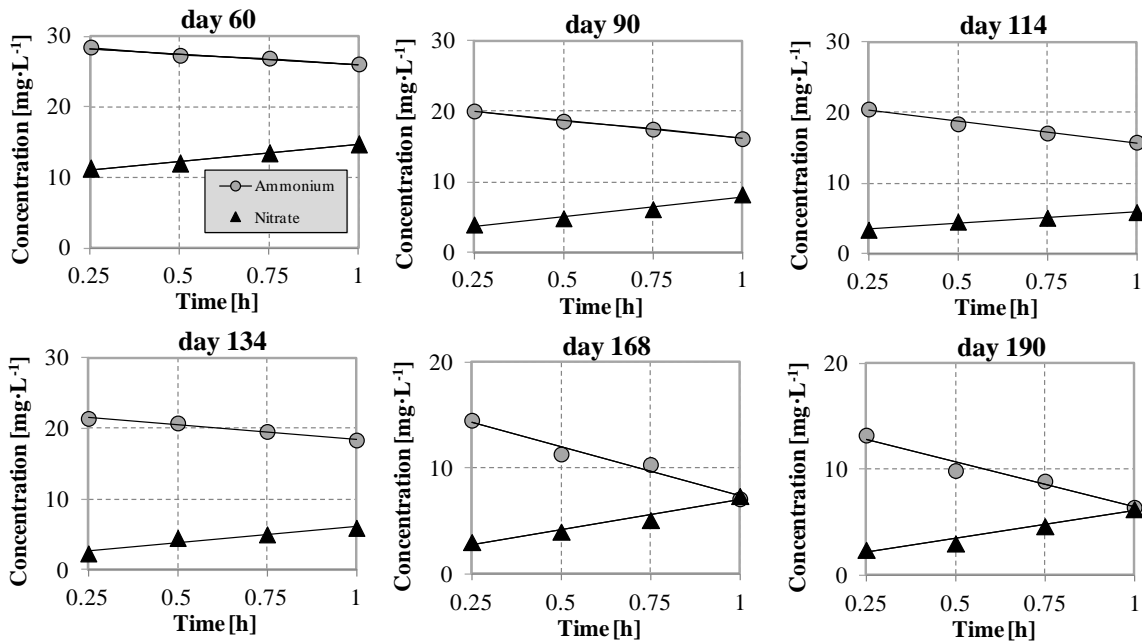
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The results observed in terms of performance were confirmed by the specific removal of nitrate and ammonium, shown in the Figure 3d (in terms of specific rate of nitrification and denitrification). The data reported in the graphs were calculated on 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:

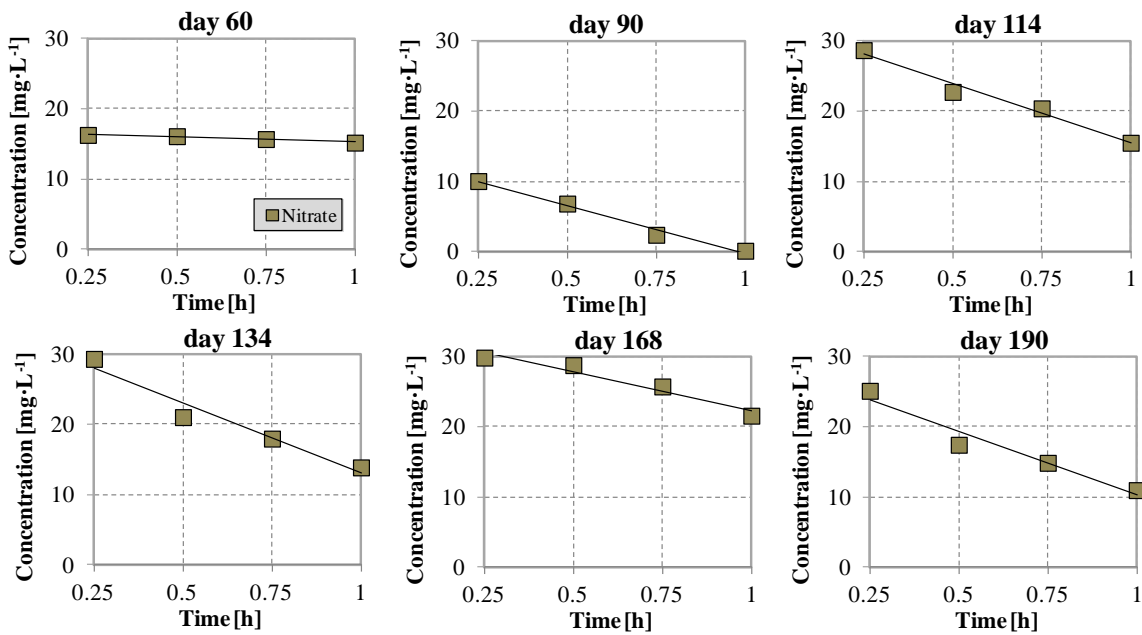
- $1.7 \pm 0.5 \text{ mgNO}_3\text{-N}_{\text{nitrified}} \cdot \text{gSS}^{-1} \cdot \text{h}^{-1}$ and $5.58 \pm 0.8 \text{ mgNO}_3\text{-N}_{\text{denitrified}} \cdot \text{gSS}^{-1} \cdot \text{h}^{-1}$ in the Period 1 (at the end of the period),
- $2.09 \pm 0.15 \text{ mgNO}_3\text{-N}_{\text{nitrified}} \cdot \text{gSS}^{-1} \cdot \text{h}^{-1}$ and $7.05 \pm 1.4 \text{ mgNO}_3\text{-N}_{\text{denitrified}} \cdot \text{gSS}^{-1} \cdot \text{h}^{-1}$ in the Period 2A,
- $1.79 \pm 0.21 \text{ mgNO}_3\text{-N}_{\text{nitrified}} \cdot \text{gSS}^{-1} \cdot \text{h}^{-1}$ and $8.11 \pm 1.4 \text{ mgNO}_3\text{-N}_{\text{denitrified}} \cdot \text{gSS}^{-1} \cdot \text{h}^{-1}$ in the Period 2B,
- $2.78 \pm 0.4 \text{ mgNO}_3\text{-N}_{\text{nitrified}} \cdot \text{gSS}^{-1} \cdot \text{h}^{-1}$ and $5.48 \pm 0.2 \text{ mgNO}_3\text{-N}_{\text{denitrified}} \cdot \text{gSS}^{-1} \cdot \text{h}^{-1}$ in the Period 3A,
- $2.01 \pm 0.3 \text{ mgNO}_3\text{-N}_{\text{nitrified}} \cdot \text{gSS}^{-1} \cdot \text{h}^{-1}$ and $6.88 \pm 0.4 \text{ mgNO}_3\text{-N}_{\text{denitrified}} \cdot \text{gSS}^{-1} \cdot \text{h}^{-1}$ in the Period 3B.

303 It is important to underline that the specific nitrate formation rates were in the range 1.7–2.8
 304 $\text{mgNO}_3\text{-N gSS}^{-1}\text{h}^{-1}$, which were within literature range (namely, $0.78\text{--}7. \text{mgNO}_3\text{-N}_{\text{nitrified}}\text{gSS}^{-1}\text{h}^{-1}$). [22].
 305 Similar results were derived for the specific denitrification rates or specific ammonium oxidation
 306 rates ($2.76\text{--}9.05 \text{mgNO}_3\text{-N}_{\text{denitrified}}\text{gSS}^{-1}\text{h}^{-1}$).

307 The overall AUR data reported in Figure 3d also show that the nitrification activity remained
 308 meanly constant for all periods, with small increase in the Period 3A. Contrarily, the rate of
 309 denitrification only improves from Period 1 to Period 2B. In the Period 3A the denitrification rate
 310 decreases slightly, due to the reduction of the no-aerated time. However, it is important to underline
 311 that the denitrification was generally improved when a greater OLR was applied for the specific
 312 Period (2B and 3B).
 313



314
 315 **Figure 4** AUR test



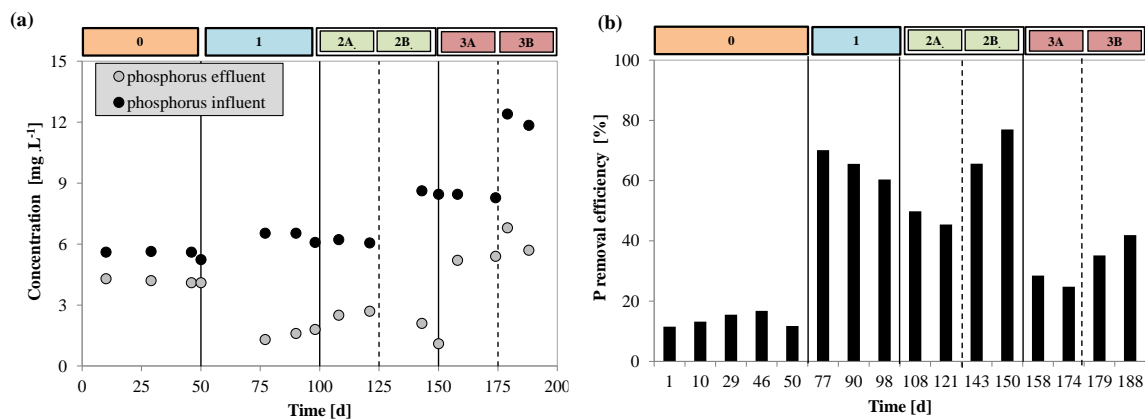
316
 317 **Figure 5** NUR test

318

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.



330

331 **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].

347

348 4. Conclusions

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

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

358 **Acknowledgements** The authors want to thank Eng. Maria Gabriella Giustra for their precious help
359 with pilot plant operation.
360

361 **"Author Contributions:** Di Bella Gaetano, conceived and designed the experiments concerning
362 biological Treatment; Giorgio Mannina conceived and designed the experiments concerning
363 biological Treatment, supervised the work and co-wrote this paper.
364

364

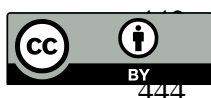
365

366 **Conflicts of Interest:** Declare conflicts of interest or state "The authors declare no conflict of interest."
367 Authors must identify and declare any personal circumstances or interest that may be perceived as
368 inappropriately influencing the representation or interpretation of reported research results. Any role
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370 in the writing of the manuscript, or in the decision to publish the results must be declared in this
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372 in the collection, analyses, or interpretation of data, in the writing of the manuscript, and in the
373 decision to publish the results".

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