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Extended summary

Advanced continuous biological process via nitrite for the anaerobic supernatants treatment

Curriculum: materials, water and soil engineering

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Abstract. The wastewater obtained from the anaerobic zootechnical digestors presents high values of Total Nitrogen (TN), mainly as Ammonia, salts and a low COD/TN ratio. According with the European Directive 91/676/EEC the nutrients removal must be considered. A pilot scale experimentation was designed to observe the optimal conditions for the removal of the nitrogen from zootechnical anaerobic supernatants following the nitrite pathway: an innovative technology that may yield up a reduction in aeration and COD requirements preventing the complete oxidation of ammonium and retaining the reaction into nitrite formation. The pilot plant operated with a continuous influent flow and was characterized by alternative oxic and anoxic phases automatically regulated by a control device processing the online signals of dissolved oxygen (DO) and oxidation reduction potential (ORP) probes. After a preliminary start-up in high COD/TN ratios conditions, evolved to the operative optimization, the second and the third phases were characterized, respectively, by a NLR of 0.16 and 0.43 $kgNm^{-3}d^{-1}$. Every phase was divided into sub-periods with limiting COD/TN ratios from 5 to 3 and TKN/NH_4-N ratios from 1.2 to 2.

The results showed elevated removal rates, even with a COD/TN ratio equal to 3. Ammonium and total nitrogen decrement percentages were higher than 85% in all the periods with limiting COD/TN values. The different nitrogen effluent forms varied from 9 to 80 as mgNH_4-N/l and from 12 to 180 as mgTN/l. This behavior was justified by the kinetic batch experiments, that demonstrated the high specialization of the biomass in nitrogen removal by the nitrite pathway: the AUR tests showed high ammonium oxidation rates (on average 0.133 kgNH_4-$N_{oxidized}$kgMLVSS^{-1}d^{-1}) and a nitrosation contribution to the nitrification higher than 65%, while NUR tests verified that the nitrite reduction represents the 81% of the whole denitrification rate.

Keywords. Anaerobic supernatants, Automatic control, Nitrite pathway, Nitrogen removal, Zootechnical wastewater.

1 Problem statement and objectives

The wastes obtained from the anaerobic zootecnical digestors present high values of Total Nitrogen (TN), mainly as Ammonia, salts and a low COD/TN ratio [1]. These characteristics have to be considered for the choice of the hypothetical solution as removal and/or recover of the nutrients according with the European Directive 91/676/EEC. The traditional biological nitrogen removal process involves the oxidation of ammonium to nitrate, followed by reduction with an organic carbon source to nitrogen gas. In this pathway nitrite is an intermediate of both steps. A complete nitrogen removal could be achieved preventing the complete oxidation of ammonium, retaining the reaction into nitrite formation. The retention of this reaction can be achieved limiting the dissolved oxygen concentration (DO) in the reactor below 2 mg/l [2]. The further step must be the denitritation of nitrite to nitrogen gas. This nitrite pathway may yield up to a 25% reduction in aeration and 40 % reduction in COD requirements. In the last years many examples of a fully functional nitritation process have been treated in literature as Sharon and Anammox processes [3] [4]. The Sharon process is based on partial ammonium oxidation operated by autotropic bacteria followed by reduction in anoxic environment, while Anammox process consist in a partial ammonium oxidation operated by a different kind of autotrophic bacteria (Anammox bacteria), that are able to oxidize ammonium using nitrite as electron acceptor.

This work is finalized to experiment on a pilot plant (Fig.1) a biological alternate oxic and anoxic process, continuously fed, to remove nitrogen from zootecnical wastes and to discover the optimal working conditions. The biological process is designed to operate with a continuous influent flow and is characterized by alternate aerobic and anoxic phases automatically regulated by a control device processing the online signals of dissolved oxygen (DO) and oxidation reduction potential (ORP) probes. The online signals are closely related with the concentration of the nitrogen forms and the device switch from aerobic to anoxic phase and vice versa when the signal identify the total consumption of ammonium (aerobic phase) or nitrite and nitrate (anoxic phase). Statistical software controls and optimizes the process checking the time lengths of both phases and defining the end-reason that led the automatic control device to change phase (set point maximum time, maximum DO and/or ORP, optimal condition).

2 Research planning and activities

The pilot plant (Fig.1) consists in a CSTR fed with anaerobic supernatants of a civil WWTP (60000 PE) mixed with zootechnical digested and methanol, as external source of carbon for the denitrification phase. The plant reactor is a stainless steel tank (volume of 70 L), furnished with mixer, blower, probes (pH, DO, ORP and MLSS) and secondary settler. A 100 L storage tank keeps the influent mix of anaerobic supernatants and zootechnical digested. The influent mix is pumped with a loading pump inside the reactor at regular times (HRT of 3.7 d). Methanol is pumped inside the reactor at the beginning of every anoxic cycle. The secondary settler clarifies the water from the reactor: the settled sludge is pumped back to the reactor while the clarified water is conveyed into a final tank.

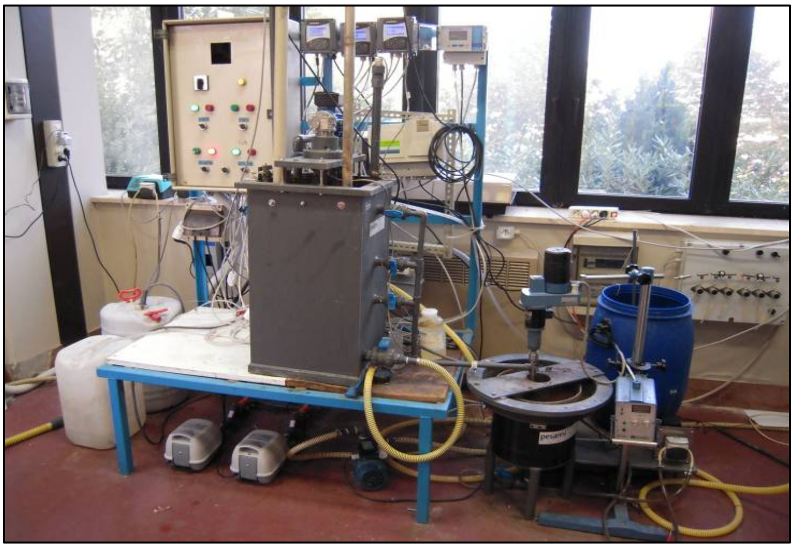

Figure 1. The pilot plant

The experimentation began on April 2010. Regular laboratory analysis and the interpretation of the probes online signals allowed to control and manage the biological process. The laboratory analysis have been made at the influent and effluent water for the parameters of pH, alkalinity, ammonia, COD, TKN, TSS, inorganic cations and anions, while for the biological sludge from the reactor were analyzed MLSS and MLVSS. These characterizations were implemented by kinetic studies in batch and in the reactor to obtain the nitrification and denitrification rates. All the chemical and physical concentrations were monitored according with Standard Methods [5]. The ammonia (AUR) and nitrite or nitrate (NUR) uptake rates were performed in separate batch tests (600 mL), on the basis of Kristensen method [6], to define the nitrogen removal performances and the nitrites formation.

The work has been divided in three phases: every phase was characterized by different nitrogen loading rates (NLR) and divided into more sub-periods with limiting COD/TN ratios. The phase 1 was dedicated to the optimization of the biological process: a biomass has been inoculated inside the reactor, filled with the anaerobic supernatant, and made grow on the optimal conditions for its specialization in the nitrogen removal via nitrite. An high COD/TN ratio was provided to support the biomass growing.

The phase 1 was divided into 3 sub-periods:

- Period 1: Startup
- Period 2: Biomass growing
- Period 3: Optimization of the nitrogen removal

The phase 2 was characterized by a nitrogen loading rate of 0.16 $kgNm^{-3}d^{-1}$. In order to rise the nitrogen influent concentration a zootechnical waste was added to the anaerobic digested supernatant.

The phase 2 was divided into 4 sub-periods, with a COD/TN ratio gradually lower:

- Period 1: Increase of the nitrogen load and optimization
- Period 2: COD/TN equal to 5
- Period 3: COD/TN equal to 4
- Period 4: COD/TN equal to 3

The phase 3 was characterized by an higher nitrogen loading rate, obtained with a bigger volume of zootechnical waste added to the anaerobic supernatant, equal to 0.43 $kgNm^{-3}d^{-1}$. In the last period of this phase a different zootechnical waste was added, in order to test the removal efficiencies at different TKN/N-NH_4 ratios.
The phase 3 was divided into 4 sub-periods, with a COD/TN ratio gradually lower:

- Period 1: COD/TN equal to 3
- Period 2: COD/TN equal to 3.5
- Period 3: COD/TN equal to 4
- Period 4: TKN/NH_4-N equal to 2

3 Analysis and discussion of main results

The present study evaluated the removal efficiencies of the biological treatment for anaerobic digested supernatants, in a pilot scale reactor continuously supplied.
The experimentation highlighted that the nitrogen removal via nitrite is an applicable solution: inside the pilot plant, fed with different fractions of anaerobic digested supernatants, was possible to inoculate and grow a biomass able to remove all the nitrogen forms.
The average influent characterization highlighted an increasing TN concentration (Tab.1): from an average value equal to 200 mg/l during the first phase, to an average value equal to 1600 mg/l in the third phase. In all the periods, except the last period of the third phase, the NH_4-N contribution to the TN concentration was higher than 75%. The NO_2-N and NO_3-N influent concentrations were negligible.
In the last period of the third phase the NH_4-N contribution to the TN rose to 50%.
The influent COD concentration (Tab.1), calculated as the sum of the contribution of the COD of the anaerobic wastes and the COD of methanol, guaranteed the correct COD/TN ratios in every period of the experimentation: during the optimization phase an high COD/TN ratio was provided to support the biomass growth, while in the other periods the ratio was limiting, between 3 and 5.

Table 1. Average influent characterization

Phase		Alka-linity	COD tot	NH_4-N	TKN	NO_2-N	NO_3-N	TN	Cl^-	PO_4-P
	COD/TN	Mg/l	Mg/l	Mg/l	Mg/l	Mg/l	Mg/l	Mg/l	Mg/l	Mg/l
Process optimization	4.8	1672	1552	236	316	0	1	319	251	12
	14.0	1197	2446	206	231	0	1	232	287	10
	8.0	935	1388	163	168	0	0	168	507	26
Nitrogen loading rate 0.16 $kgNm^{-3}d^{-1}$	5.9	2149	3562	514	622	1	8	633	576	32
	5.0	1960	3249	514	655	0	1	656	358	29
	4.1	2491	2406	469	610	1	2	613	464	26
	3.4	2433	2023	419	545	1	1	547	506	33
Nitrogen loading rate 0.43 $kgNm^{-3}d^{-1}$	3.3	5939	6644	1423	1740	2	3	1742	2514	113
	3.6	3941	7901	1240	1582	0	17	1598	4920	328
	4.0	1922	6232	1097	1468	6	6	1477	1648	48
	3.9	4631	4576	730	1489	12	3	1503	818	32

The NLR analysis evidence the increasing nitrogen loading ratio, from 0.06 to 0.43 $kgNm^{-3}d^{-1}$ (Fig.2). In every phase the NLR was higher than the average value of civil wastewater treatment plant, equal approximately to 0.05 $kgNm^{-3}d^{-1}$.

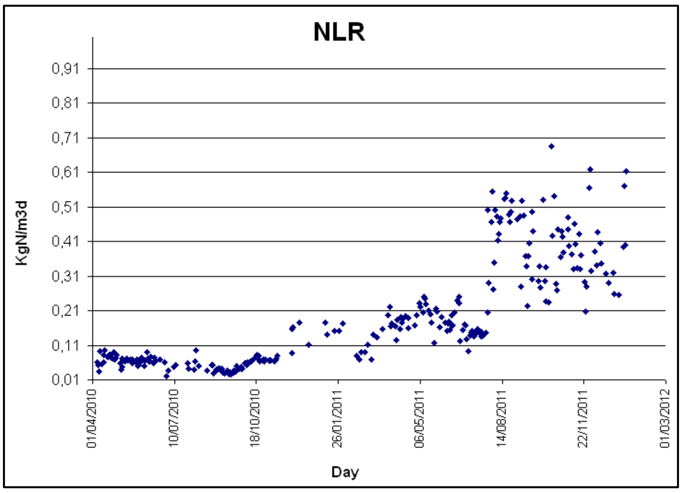

Figure 2. Nitrogen loading rates (NLR)

The biomass concentration showed a rapid growth in the optimization phase, from 0 to 4000 mgMLSS/l, followed by a regular increase in the second and third phase but interrupted by frequent losses of biomass in the effluent water (Fig.3). The losses of biomass were due to the presence of a cationic polyelectrolyte in the anaerobic supernatant, that caused the flocculation of the biomass in the secondary settler and its leak in the effluent water. The average value of MLSS concentration in the second and third phase was respectively about 7100 mg/l and 11000 mg/l. The MLVSS/MLSS ratio rose quickly in the optimization phase to 0.88 and progressively decreased during the experimentation to 0.65 because of the entry of inert substances with the influent waste (Fig.3).

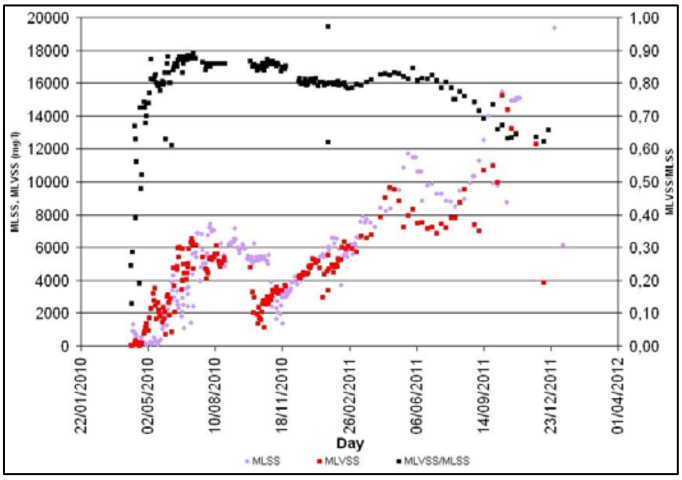

Figure 3. MLSS and MLVSS concentrations and MLVSS/MLSS ratios

The effluent water was characterized for the same parameters of the influent waste (Tab.2), and the removal efficiencies were calculated from the concentration values (Tab.3).
During the first and second phase, featured by a medium TN load, the NH_4-N concentration progressively decreased (Tab 2) and its removal efficiency rose (Tab.3): in the limiting COD/TN periods were registered NH_4-N removal efficiencies higher than 97%. This result confirm the biomass specialization in nitrogen removal with limiting COD concentrations.
In the third phase an average NH_4-N concentration equal to 70 mg/l (Tab.2) corresponded to removal efficiencies higher than 90%: an higher nitrogen loading rate did not affect the excellent ammonia removal registered in the previous phase.

Table 2. Average effluent characterization

Phase	COD/TN	Alka-linity	COD tot	NH_4-N	TKN	NO_2-N	NO_3-N	TN	PO_4-P
		Mg/l	Mg/l	Mg/l	Mg/l	Mg/l	Mg/l	Mg/l	Mg/l
Process optimization	14	473	391	31	67	1	2	70	3
	8.0	458	261	91	96	2	14	110	15
Nitrogen loading rate 0.16 $kgNm^{-3}d^{-1}$	5.9	805	357	80	110	1	53	163	16
	5.0	635	204	13	17	0	1	18	15
	4.1	362	176	10	11	1	71	83	30
	3.4	498	189	9	10	0	1	12	16
Nitrogen loading rate 0.43 $kgNm^{-3}d^{-1}$	3.3	888	2145	59	80	0	14	94	40
	3.6	1162	2030	77	124	1	12	105	61
	4.0	571	1801	77	106	12	28	151	33
	3.9	2420	2133	62	158	61	10	181	8

The effluent concentrations of NO_2-Nand NO_3-N were generally low, except for the the second period of the second phase, where NO_3-N concentrations equal to 71 mgN/l (Tab.2) evidenced problems in the denitrification phase, solved with the application of a longer denitrification time.
The high NO_3-N concentration weakly influenced the TN removal efficiency: in the second phase, after the optimization period, the TN removal efficiencies were included between 82% and 98%. In the third phase the application of an high nitrogen loading rate and a different TKN/NH_4-N ratio (4th period) did not affected the TN removal efficiency, always higher than 86% (Tab.3).
The COD removal efficiency in the first and second phase was higher than 80%. In the third phase the efficiency decreased to an average value equal to 67%: the decrease was not caused by a lower removal capacity of the process but by the presence of the cationic polyelectrolyte in the anaerobic supernatant that caused the leak of the biomass from the secondary settler. Later experimental works demonstrated that the addition of a further cationic polyelectrolyte can improve the settleability of the effluent water.

Table 3. Removal efficiencies

Phase	COD/TN	E% NH4-N	E% TN	E% COD
		%	%	%
Process optimization	14	83	65	81
	8.0	59	54	91
Nitrogen loading rate 0.16 $kgNm^{-3}d^{-1}$	5.9	69	74	90
	5.0	97	98	94
	4.1	98	82	92
	3.4	98	98	91
Nitrogen loading rate 0.43 $kgNm^{-3}d^{-1}$	3.3	95	94	71
	3.6	93	93	65
	4.0	91	88	69
	3.9	92	86	63

The automatic regulation of the control device, that process the online signals of dissolved oxygen (DO) and oxidation reduction potential (ORP) probes (Fig.4), allowed the instauration of the ideal condition for the selection of a biomass specialized in removal of nitrogen by the nitrite pathway: the DO concentration ranged between 0 mg/l in the anoxic phase and 2 mg/l in the aerobic phase (Fig.4). Sporadic peak of 3 mgOD/l were registered because of difficulties in the control of the air flow rate in the reactor.
The oxidation reduction potential (ORP) ranged between +300mv in the nitrification phase and -100 mv in the denitrification phase and guaranteed the correct aerobic and anoxic conditions in the reactor (Fig.4).

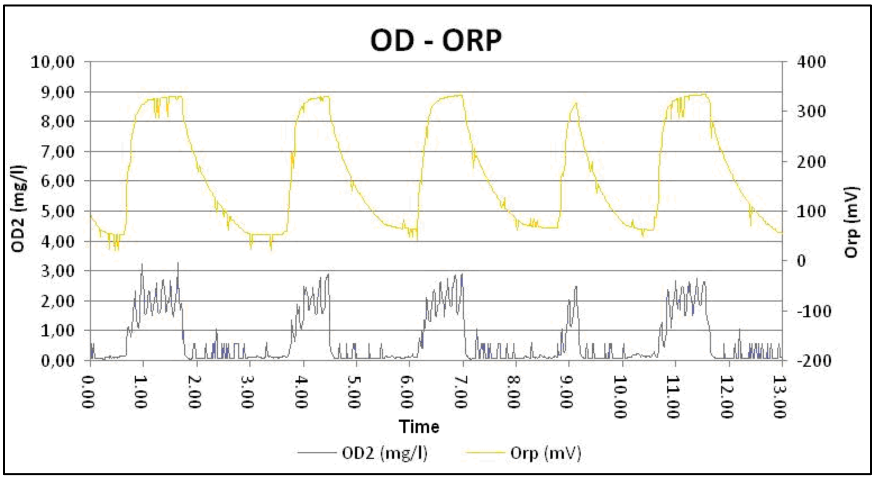

Figure 4. OD and ORP signals

The nitrogen removal rates were higher than the typical average rates of civil wastewater treatment plants: the AUR tests made in the second phase showed an average ammonium oxidation rate equal to 0.107 $kgNH_4\text{-}N_{oxidized}kgMLVSS^{-1}d^{-1}$, that further increased in the third phase to 0.149 $NH_4\text{-}N_{oxidized}kgMLVSS^{-1}d^{-1}$(Tab.4).
The test proved that the biomass is specialized in the TN removal via nitrite: after the optimization period the nitrite formation rate was higher and in some cases double than the nitrate formation. During the limiting COD periods the nitrosation contribution to the nitrification was in the average equal to 80% (Fig.5).

Table 4. Kn values obtained from AUR tests, normalized at 20°C

Phase	COD/TN	kgN-NOx/kg VSS*d	kgN-NO2/kg VSS*d	kgN-NO3/kg VSS*d		
		NOx-N	NO_2-N	NO_3-N	NO_2-N/Nox-N	NO_3-N/N-NOx
Nitrogen loading rate 0.16 kgNm^{-3}d^{-1}	5.9	0.075	0.010	0.065	0.13	0.86
	5.9	0.084	0.059	0.025	0.71	0.30
	5.0	0.094	0.075	0.020	0.80	0.21
	5.0	0.127	0.090	0.037	0.71	0.29
	4.1	0.175	0.116	0.062	0.65	0.35
	3.4	0.087	0.064	0.026	0.71	0.29
Nitrogen loading rate 0.43 kgNm^{-3}d^{-1}	3.3	0.181	0.168	0.013	0.93	0.07
	4.0	0.192	0.175	0.018	0.91	0.09
	3.9	0.073	0.058	0.015	0.79	0.21

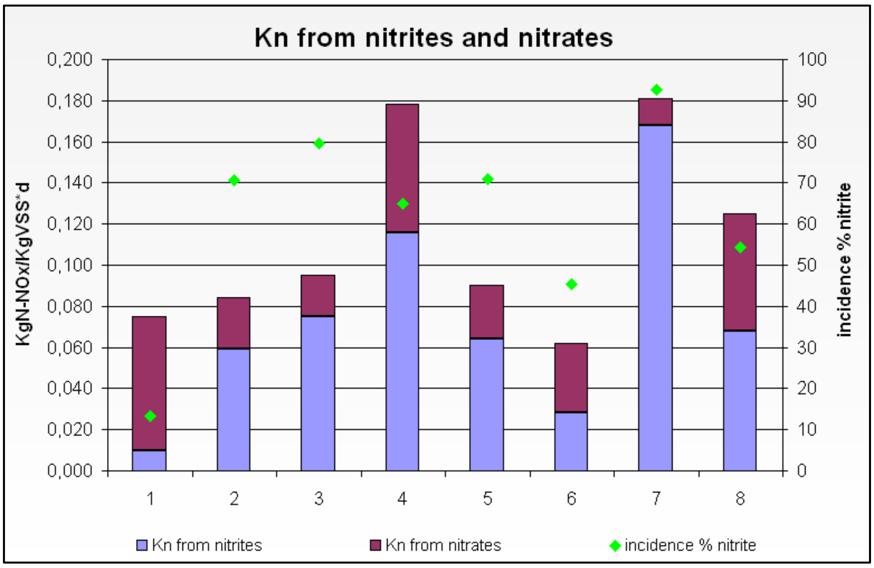

Figure 5. Kn from nitrites and nitrates and percentage contribution of nitrites to the nitrification

In the anoxic phase high denitrification rates were registered (Tab.5): in the second phase to evaluate the Kd was used a standard laboratory method that ruled the addiction of potassium nitrate in a batch reactor. The method however was not appropriate and returned a low Kd value, because the biomass was specialized in the nitrogen removal via nitrite. In the third phase the standard method was changed: in the reactor was added a sodium nitrite solution. This new method allowed a more realistic estimation of the Kd values, that highlighted an average denitrification rate equal to 0.183 kgNOx-$N_{reduced}$kgMLVSS^{-1}d^{-1} (Tab.5).

Table 5. Kd values obtained from NUR tests, normalized at 20°C

Phase	COD/TN	kgN-NOx/kg VSS*d NOx-N	kgN-NO_2/kg VSS*d NO_2-N	kgN-NO_3/kg VSS*d NO_3-N	NO_2-N/Nox-N	NO_3-N/N-NOx
Nitrogen loading rate 0.16 $kgNm^{-3}d^{-1}$	5.9	0.038	0.000	0.038	0.00	1.00
	5.9	0.011	0.000	0.011	0.00	1.00
	4.0	0.057	0.002	0.055	0.04	0.96
	3.4	0.007	0.000	0.007	0.00	1.00
Nitrogen loading rate 0.43 $kgNm^{-3}d^{-1}$	3.3	0.141*	0.124*	0.017*	0.88*	0.12*
	4.0	0.108*	0.087*	0.021*	0.81*	0.19*
	4.0	0.092	0.000	0.092	0.00	1.00
	4.0	0.335*	0.331*	0.004*	0.99*	0.01*
	3.9	0.107*	0.106*	0.001*	0.99*	0.01*

*N-source: Sodium nitrite

4 Conclusions

The aim of the experimentation was to test the nitrogen removal by the nitrite pathway in a pilot plant, fed with a continuous influent flow of a mix of anaerobic and zootechnical digested supernatants. The removal rates were very high in all the phases, even with COD/TN ratios limiting for the denitrification phase. After the optimization periods the ammonium and total nitrogen removal efficiencies were higher than 82%. The increment of the nitrogen loading rate to 0.43 $kgTNm^{-3}d^{-1}$, coupled with limiting COD/TN ratios and the application of a TKN/NH_4-N ratio equal to 2, did not affect in the excellent performances obtained at lower loading conditions. This behaviour was justified by the kinetic batch experiments made after the optimization periods, that demonstrated the elevated specialization of the biomass in nitrogen removal by the nitrite pathway: the AUR tests showed high ammonium oxidation rates (up to 0.073 $kgNH_4$-$N_{oxidized}$ kg $MLVSS^{-1}d^{-1}$) and a nitrosation contribution to the denitrification higher than 65%, while NUR tests performed with addition of $NaNO_2$ showed nitrites oxidation rates up to 0.087 $kgNO_2$-$N_{reduced}$ kg $MLVSS^{-1}d^{-1}$.

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