EOLI
Efficient Operation of Urban Wastewater Treatment Plants

Work Package 1

WP1 - POLIMI – Third year - mid term report

November 2004 – May 2005

Participating staff

Name	Background	Activities in EOLI
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Summary

Within the objectives of WP1, the POLIMI experimentation has included the operation and monitoring of the lab scale POLIMI-SBR and of the pilot scale ENEA-SBR.
The experimentation on the POLIMI-SBR has included the following main activities:

- the lab scale SBR fed on synthetic sewage was kept into operation and monitored. The SBR sludge was used to perform biomass activity tests to elucidate environmental conditions which may lead to nitrites build up. Various loading conditions were experimented. Several sludge inoculums were required to recover nitrites oxidation capacity.
- batch activity tests were performed, and are still in progress, with the MARTINA instrument to assess the activity of ammonium and nitrites oxidizers at various pH, DO, ammonium concentration and aeration patterns; main goal of these tests was to evidence factors which can favor ammonium oxidizers (AOB) over nitrite oxidizers (NOB), thus unbalancing the nitrification process. The elucidations of environmental conditions, which can lead to the main abnormal conditions observed during the POLIMI-SBR operation, are of interest to develop appropriate control strategies (WP 5) and supervision algorithms (WP7).
- batch biosorption tests were performed to confirm that this process is irrelevant for the POLIMI SBR, as assumed in the model developed within the WP2.
- the denitrification activity of the sludge of the POLIMI SBR was monitored regularly by pH-stat titration tests with the MARTINA sensor (see WP3 report).

The experimentation on the ENEA-SBR has included the following main activities:

- operation of a pilot SBR placed at the full-scale plant of Trebbo di Reno (Bologna) at different loadings, cycle durations and operating temperature;
- off-line tests operated with the manually operated titration unit (MARTINA) to simulate the modes of operation of the automatic titration unit (TITAAN), and, specifically:
    - monitoring of the maximum SBR nitrification activity;
    - evaluation of the SBR efficiency in nitrogen removal with maximum nitrification activity measurements.

The following report summarizes the main results of the above listed activities.

Performance of the lab-scale SBR (Polimi)

During the first semester of the third year of the EOLI project, the SBR reactor was continuously operated except for the period: 23/12/04 – 10/01/05 (Christmas holidays), during which the operation was stopped and the sludge was maintained in endogenous conditions at 4°C.

The lab scale SBR was fed and operated as described in the second year report. Due to the repeated appearance of nitrites in the effluent, the load has been frequently halved to increase oxygen availability and reduce ammonium load.

In order to follow the SBR performance, and, more specifically, the nitrification SBR capacity, the following parameters were periodically analyzed:

- biomass concentration (volatile suspended solids – VSS, total suspended solids – TSS), Figure ;
- effluent ammonium nitrate and nitrite concentrations, Figure ;

The COD effluent concentration was not measured continuously since, according to historical SBR data, its concentration was not varying significantly.

Notable events in the SBR conduction related to the period Nov 04-April 05 are reported in Table .

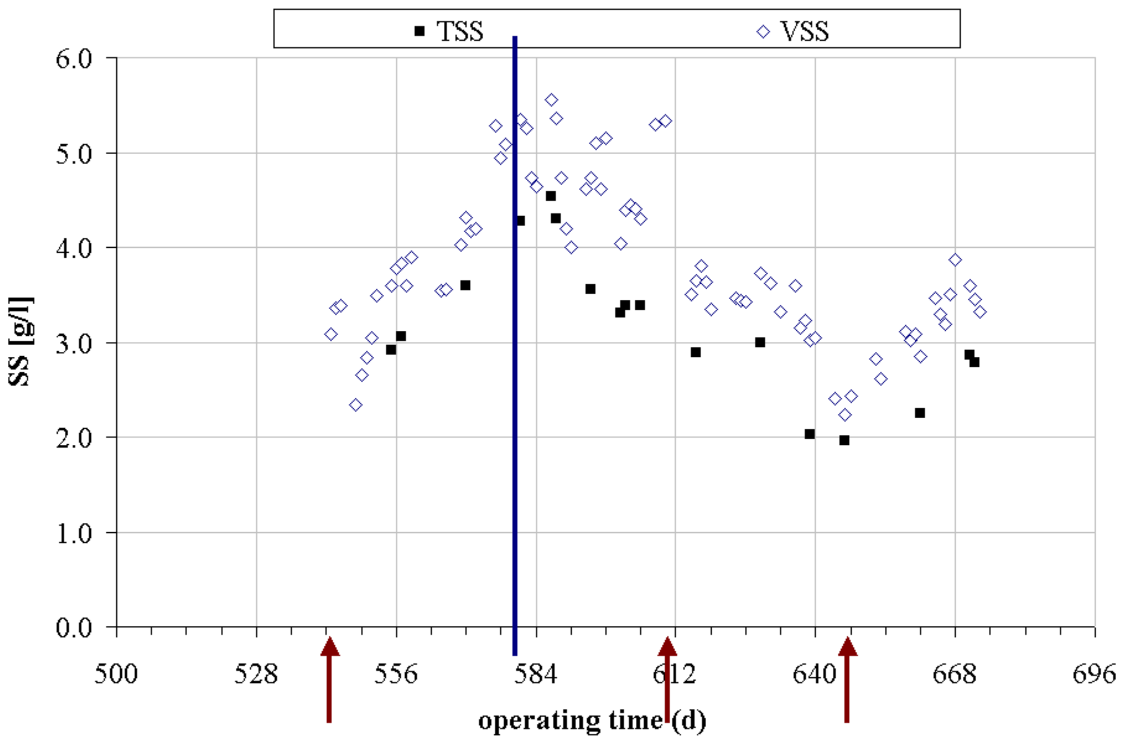

Figure 1: Suspended solids concentration in the lab-scale SBR sludge. Arrows indicate sludge inoculation; the continuous line indicates the Christmas stop.

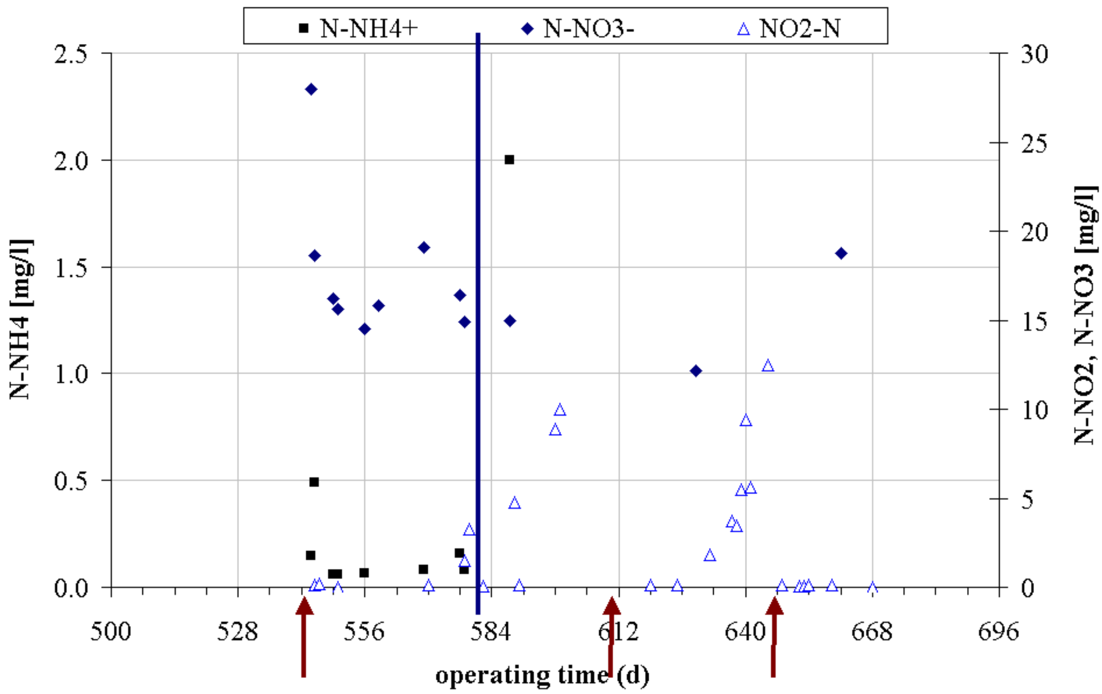

Figure 2: Concentrations of nitrogen forms in the lab-scale SBR effluent. Arrows indicate sludge inoculation; the continuous line indicates the Christmas stop.

Table 1: Polimi SBR relevant events.

operational time (d)	date	Relevant events
542	16/11/04	*SBR inoculated with 20 L of activated sludge at 3gVSS/L from Cantello (VA) WWTP.*
543	17/11/04	Load is halved
555	29/11/04	Fault in the electrical supply, no data log
558	2/12/04	New heating system
572	16/12/04	Problems in probes signals
573	17/12/04	No data logging during WE
579	23/12/04	SBR stopped for Christmas holiday, sludge stored at 4 ^oC
580	10/1/05	SBR reactivated, sludge kept in aeration for 24 h.
581	11/1/05	SBR loaded. Same load as before Christmas stop
588	18/1/05	Nitrites in the effluent. One night continuous aeration without load.
598	28/1/05	During the WE: continuous aeration without load
608	7/2/05	Continuous aeration without load
609	8/2/05	Continuous aeration without load
610	9/2/05	Continuous aeration without load
611	10/2/05	*SBR inoculated with 1/3 of activated sludge from Cantello (VA) WWTP. No load*
612	11/2/05	WE: half of the normal load
615	14/2/05	Half of the normal load
630	1/3/05	Due to tubes clogging, no load for 2 cycles
640	11/3/05	Very high nitrite effluent concentration. Aeration kept on; no load.
645	16/3/05	*Load is stopped. Supernatant decanted. SBR inoculated with 1/4 of activated sludge from Cantello (VA) WWTP. Addition of micronutrients in the influent.*
646	17/3/05	Half of the load.
658	29/3/05	Fault in temperature data log
659	30/3/05	Half of the load, pH was low (6.7-7.0), $NaCO_3$ added to increase pH

As far as *biomass concentration* is concerned, VSS varied between 2 and 4.5 g/L depending on sludge wastage.

As far as the nitrogen removal capacity is concerned, *ammonium oxidation* during the aerobic phase was almost always satisfactory. On the contrary, nitrites build up was periodically observed. Averagely, 3-4 weeks after each sludge inoculation, a sudden and continuous increase in the nitrite effluent concentration was observed, which could not be reverted by decreasing the loading rate nor by keeping the reactor aerated overnight.

After the inoculation made on March 16, no further nitrite accumulation was observed. Probably, sludge withdrawn in springtime was more active and enriched in nitrifying organisms; furthermore, micronutrients have been added since these compounds are present in real sewage, but they are lacking in the synthetic one. Also, a more constant sludge retention time may have played a role in biological stability of the system. Periodical sludge withdrawals of one liter once a day may cause a perturbation. In fact, NOB activity often got worse and worse after these extractions and might have come to NOB progressive washout. Therefore, the sludge age is now controlled much more carefully and sludge is extracted regularly in small amounts (0.25 L at each cycle).

Performance and operation of the pilot-scale SBR (ENEA)

During the period November 2004 – May 2005, the ENEA pilot plant has been operated mainly to test the on-line titrator prototype TITAAN (developed within the WP3 - Hardware sensors development) and to experience the operation of a plant fed with real sewage during wintertime. The process configuration was the same reported in the previous EOLI reports, and it can be seen in Figure ; the duration of the whole cycle and of the single phases were modified several times during the experimentation, on the basis of the efficiency achieved by the plant.

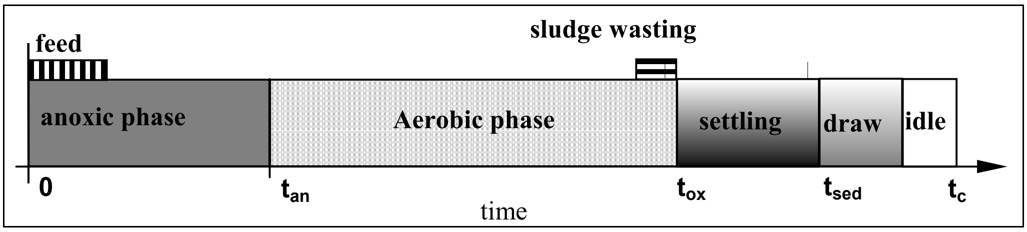

Figure 3: pilot SBR cycle

The pilot SBR was operating properly at the end of November 2004, but immediately after the 2nd EOLI annual meeting in Milan an accidental massive loss of sludge occurred. Therefore, sludge was withdrawn from the full scale treatment plant and was added to the pilot-scale SBR in order to restore the proper working conditions rapidly. Moreover, two air heating devices have been installed by the pilot plant in order to keep the temperature of the process at 15°C (atmospheric temperature had often dropped below zero for several days), allowing nitrifiers to grow adequately. In fact, the maximum growth rate for autotrophs at 15 °C is reported to be roughly half of the rate at 20 °C (Melcer et al., 2003).
On day December the 14th, TSS concentration in the pilot-scale SBR exceeded 4 g/L; automatic purge of excess sludge was started and set in order to keep a SRT of 30 days instead of 21 days (as it was previously) to cope with the reduced nitrifiers growth rate due to the lower temperature in the reactor.

Table 2 – Phase configuration 26th November 2004 – 3rd January 2005 – 4 cycles/day

Phase	Duration
Fill	20 min
anoxic reaction (fill included)	1 h 20 min
aerobic reaction	3 h 20
settle	40 min
decant	20 min
Idle	40 min

During Christmas holidays, the influent loading pipe froze and broke, so that operation of the plant was forcedly stopped on the 23rd of December.

Then the plant was emptied completely and a new inoculum of fresh sludge was added on the 12th of January 2005, after insulating the loading pipe (about 15 meters long) and keeping water moving in a closed loop to prevent freezing of still water. In spite of these efforts, a heavy snowfall caused the freezing of the circuit and the plant wasn't fed from the 17th to the 20th of January.

The operation was re-started again, but the plant was not able to nitrify efficiently. Therefore, it was decided to reduce the number of cycles per day and to increase the aerated fraction of the cycle. The theoretical SBR volumetric load was decreased from 0.288 to 0.216 kgBOD/(m^3*day), as calculated by assuming 172 mgBOD/L as the annual average influent BOD concentration. In Table , the new phases configuration with an 8-hour cycle are reported.

Table 3 - Phases configuration from the 20th January 2005 – 3 cycles/day

Phase	Duration
Fill	20 min
anoxic reaction (fill included)	1 h 30 min
aerobic reaction	4 h 30 min
settle	1 h
decant	20 min
Idle	40 min

Despites the last modification, the performance of the pilot plant didn't improve as expected.

In figures 4, 5, 6, and 7 the main parameters monitored during the period November '04 – May '05 are reported. Nitrification instability is evident, but nitrites did not accumulate, according to the previous working period of the pilot SBR (June – October '04). However, high nitrites effluent concentrations were detected occasionally, but this event was always associated to high effluent concentrations of ammonia. Some examples are: 21/12/04 - ammonium 7 mgN/L, nitrite 1.2 mgN/L; 18/01/05 - ammonium 8.9 mgN/L, nitrite 2 mgN/L. In other words, it appears that the process was negatively affected by nitratation (operated by AOB) and not by nitritation (operated by NOB).

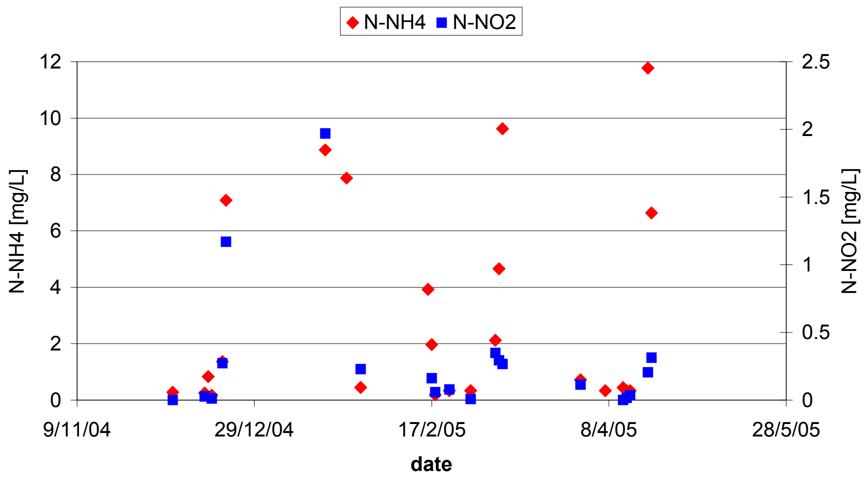

Figure 4 - Ammonium and nitrous nitrogen in the SBR effluent

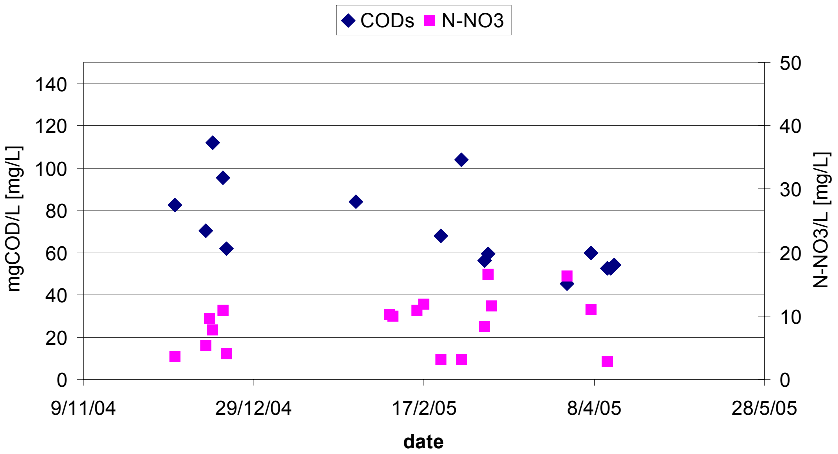

Figure 5 - Soluble COD and nitric nitrogen in the SBR effluent

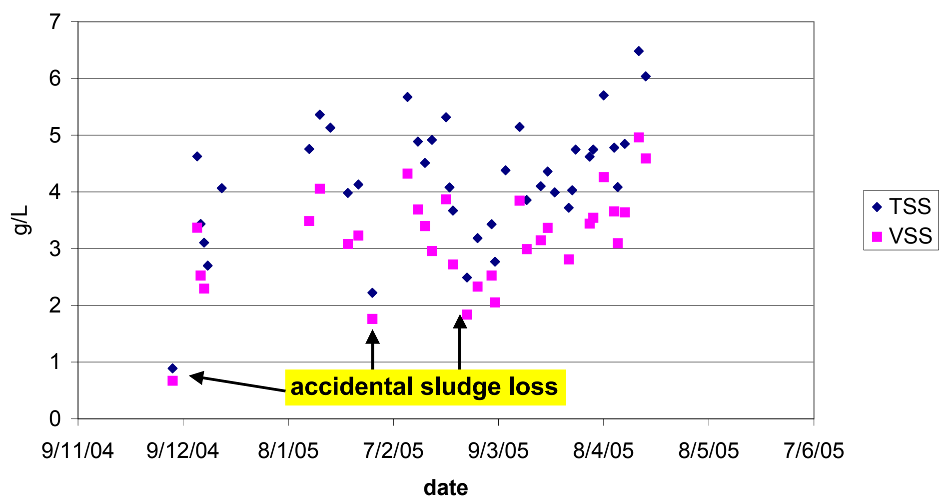

Figure 6 – TSS and VSS evolution in the SBR

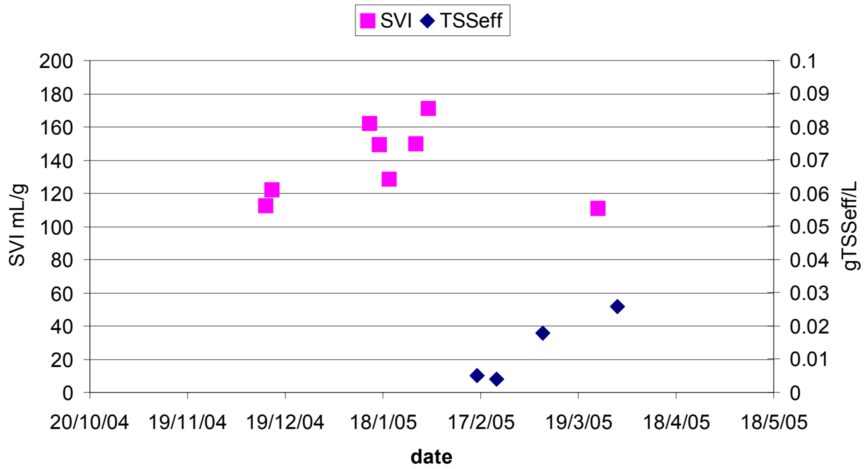

Figure 7 - SVI and effluent TSS

Some settling problems were detected, as shown by SVI values: low temperatures and plant malfunctions have had negative impacts on this parameter. At present, SVI is decreasing and settling problems are not expected in the next few months.

Nitrification instability was investigated further. Figure 8, 9 and 10 show on-line data collected during the night cycle of the 16th February; feed time was around 2:00 AM, with low organic and nitrogenous loads. It appears clearly that, at the end of the aerobic phase, endogenous conditions are not reached, since DO concentration doesn't reach the saturation value. Also, reduced compounds as ammonium nitrogen are still present in the reactor (ORP value reaching 0 mV only at the end of the aerobic phase). pH and DO curves might support the hypothesis that a certain amount of organic matter might have been oxidized during the first half of the aerobic phase: in fact DO concentration is always very low, as it should be if heterotrophs exert a high oxygen consumption; the pH decrease due to nitrification can be observed only after about one hour of aeration. When aeration is on, pH in the reactor is affected by several processes: it increases as CO_2 is stripped off, it decreases because of acidity produced by nitrification and, if acetate is present, it increases because of acetic acid consumption by heterotrophs. By observing the trend of pH in Figure 8 it is evident that the last two processes are predominant over nitrification in the first part of the aerobic phase. The DO trend can be explained by considering that DO is a limiting substrate both for heterotrophs and autotrophs, but the first group of bacteria may take up substrate faster at low concentrations (the DO half-saturation constant value for heterotrophs is reported to be half than that of autotrophs, Henze et al., 1986). Therefore, at very low DO concentrations, nitrifiers activity could be slowed down severely, as oxygen is almost completely taken up by heterotrophs.

In the first period of operation of this plant (June – October '04), COD was almost completely removed during the anoxic phase by means of nitrates as electron acceptors. Most probably, poor nitrification limited the nitrates availability at the beginning of the anoxic cycle; this, in turn, caused a limited removal of the organic matter trough denitrification.

This is confirmed by Figure , which shows the trend of the redox potential in the two days before the cycle of the 16th February - 2 AM. At the end of aerobic phase, ORP never reached positive values: this means that the sludge was not fully nitrifying and, as a consequence, few nitrates were available at the end of the cycle. On the contrary, depletion of nitrates during the anoxic phase was completed in each of the cycles in Figure , as indicated by the appearance of the bending point χ (i.e.: end of denitrification), as reported by Mauret et al., 2001.

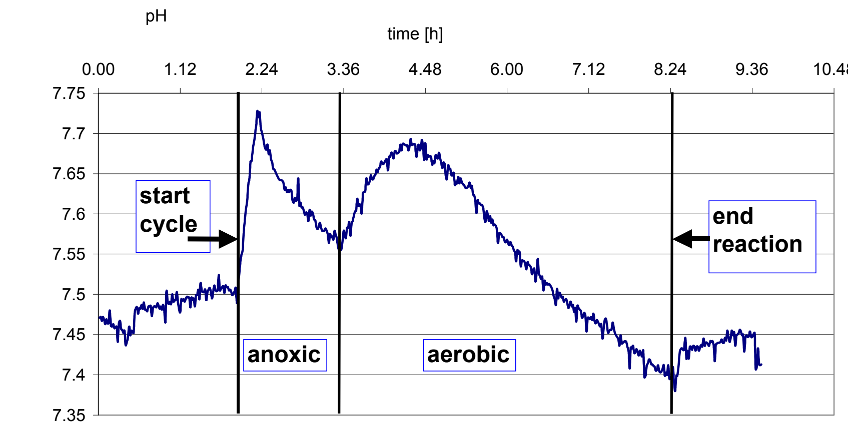

Figure 8 – Profile of pH for the night cycle performed on 16/02/05 (start at 2 AM)

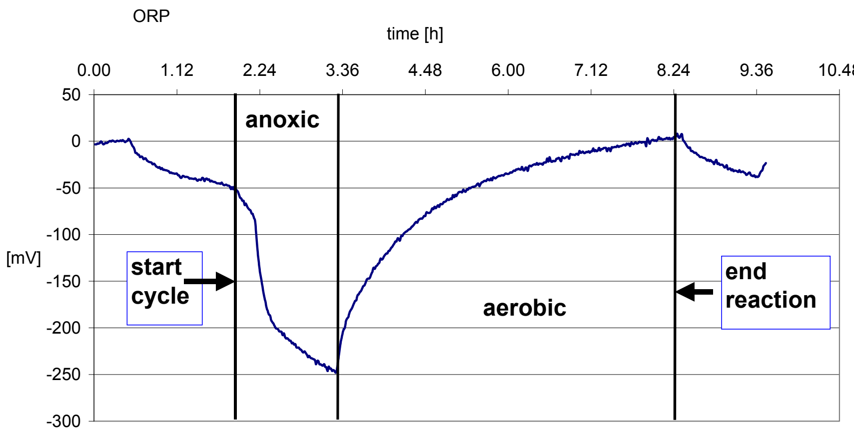

Figure 9 - Profile of ORP for the night cycle performed on 16/02/05 (start at 2 AM)

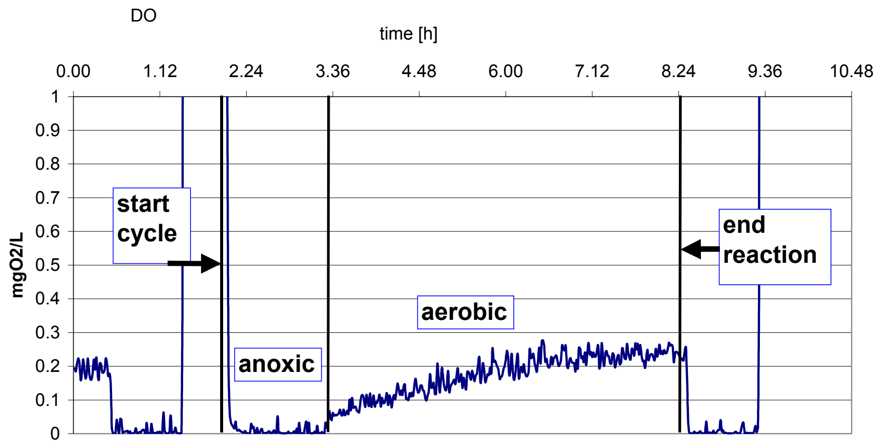

Figure 10 - Profile of DO for the night cycle performed on 16/02/05 (start at 2 AM)

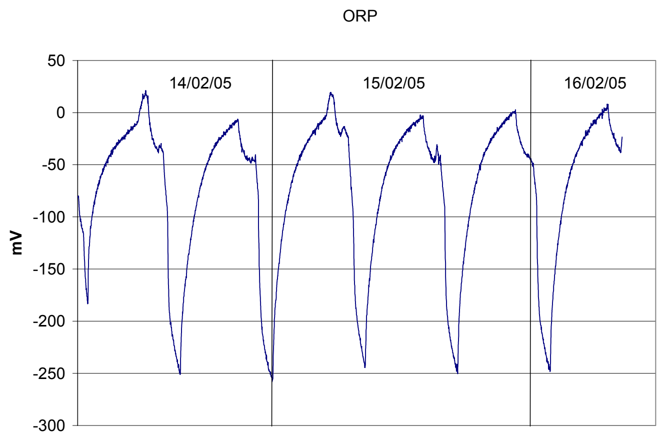

Figure 11 - Profile of ORP trend during the 2 preceding days of the night cycle 16/02/05: ORP value almost doesn't arise over 0 mV (symptom of incomplete nitrification)

On the 28th of February, 30% of the biomass in the SBR was lost because of a fault in the plant-managing program, but the previous biomass concentration was recovered in about 10 days. However, in the following days insufficient nitrification performance was shown by on-line data and activity bioassays. All results were confirmed by laboratory analysis on the effluent.
From all data, it appears clearly that the increase in the aerobic fraction was not sufficient to improve nitrification in the reactor. Therefore, for the whole month of March it has been decided to favor the growth of autotrophs by limiting that of heterotrophs. The loading rate was then reduced to a quarter by operating 1 cycle per day instead of 4. When nitrification improved, the load was gradually increased. However, winter temperature prevented a rapid quick recovery of the nitrification performance. In Bologna, the winter 2004-05 has been quite rigid, with abundant snowfalls and freezing air temperatures during the night (-10° C for some periods). These conditions severely affected the management of the pilot plant and it has been really difficult to keep the temperature of the reactor constantly above 15 °C. The lack of an automatic temperature control system obliged to manual adjustments of the heaters, which were not really suitable for such a climate.

From the profiles of on-line data collected on March 20th, improvement of nitrification process can be detected (Figure 12, 13 and 14, cycle duration = 12 hours). In the peak-load cycle (the second in the graphs, starting at 10 AM) DO concentration reaches saturation before the end of the cycle and ORP reaches positive values too. These data mean that nitrification improved in time, but the total efficiency is not yet satisfactory.

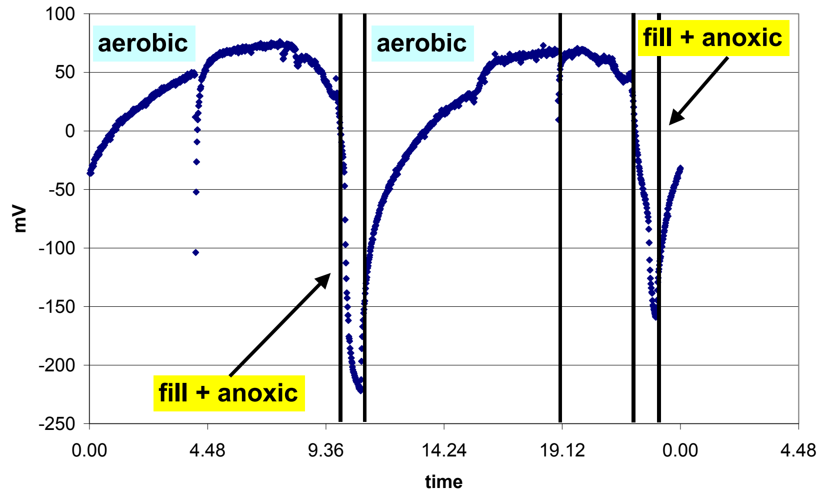

Figure 12 – ORP profile during the peak load cycle on the 20th March (12 hours cycle)

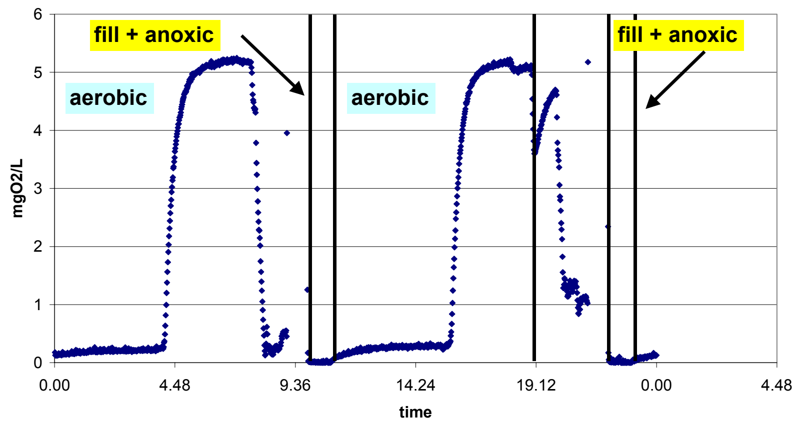

Figure 13 - DO profile during the peak load cycle on the 20th March (12 hours cycle)

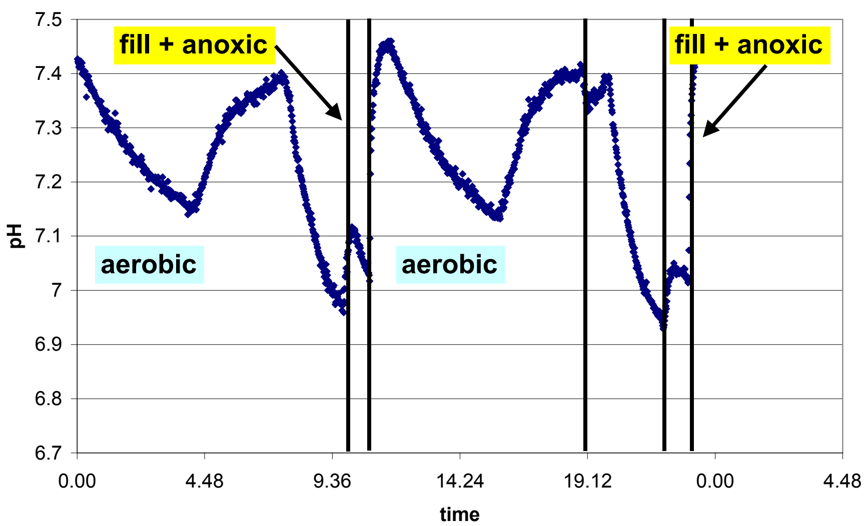

Figure 14 - pH profile during the peak load cycle on the 20th March (12 hours cycle)

Starting from the end of March, 3 cycles per day were restored (phases configuration in Table) achieving quite good nitrogen removal efficiencies. Figure 15 shows DO and ORP trends for the peak-load cycle on April the 5th. It appears that during this cycle denitrification had not been completed and nitrates were not completely depleted at the end of the anoxic phase. However, nitrification was completed after 3.5 hours of aeration.

Table 4 - Phase configuration from 31/03/05 – 3 cycles/day

Phase	Duration
Fill	20 min
anoxic reaction (fill included)	1 h
Aerobic reaction	5 h 30
Settle	40 min
Decant	20 min
Idle	30 min

From April 11, the initial daily volumetric load was applied to the plant (600 L/day) with 4 cycles per day (see Table 1). Up to now (April 22nd) the performance is acceptable, but some incomplete nitrification occurs during peak-load cycles (e.g.: Figure 16).

Table 1 - Phase configuration from 11/04/05 – 4 cycles/day

Phase	Duration
fill	20 min
anoxic reaction (fill included)	45 min
aerobic reaction	3 h 45
settle	45 min
decant	20 min
idle	25 min

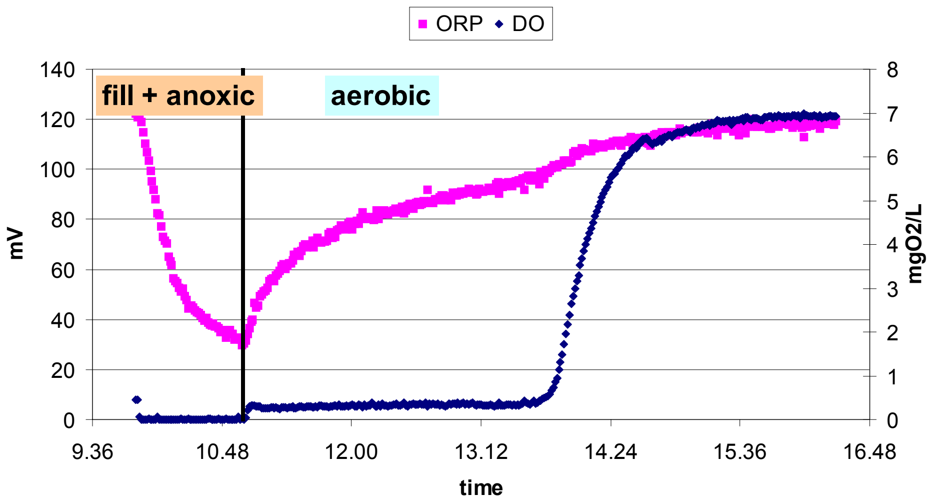

Figure 15 - ORP and DO trends for peak load cycle 05/04/05 - 8 h cycle –

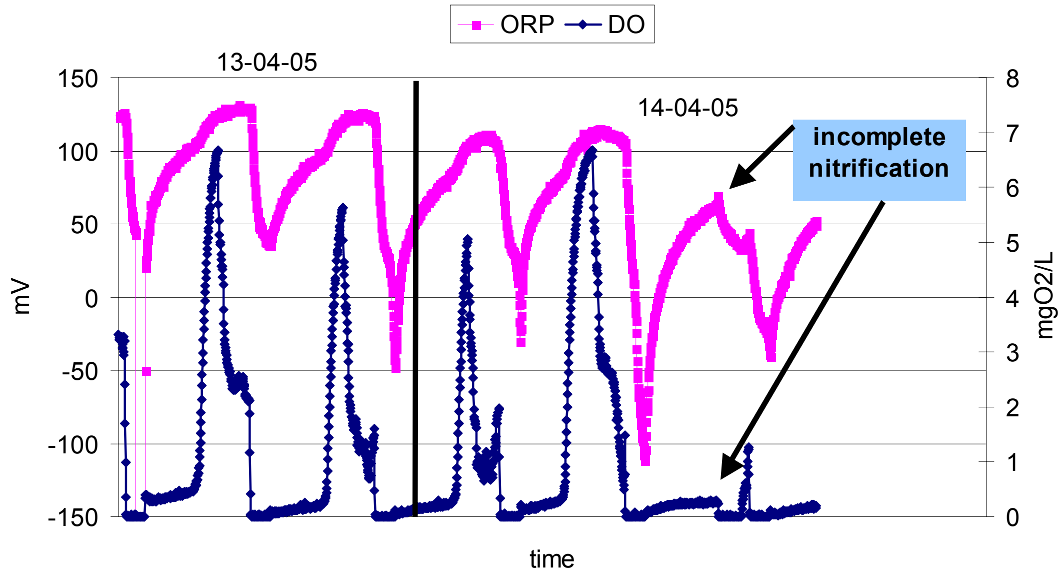

Figure 16 –ORP and DO trends on 13th – 14th April '05 – 6 h cycle -

Off-line pH-DO-stat tests to monitor ENEA - SBR nitrification performances

Due to a delay in the development of the on-line titrator TITAAN (POLIMI-ENEA main task in WP3), data gathered from this device at this time are not sufficient for process evaluation on the pilot-scale SBR located at Trebbo di Reno (Bologna). Hence, some off-line titration tests have been performed with the MARTINA laboratory titrator and used to simulate the elaborations that should be performed by the TITAAN to evaluate the performance of the reactor and to isolate some process faults which occurred during the operation of the pilot-scale plant.

Maximum SBR nitrification activity monitoring

pH-DO-stat tests have been performed in our lab using the titrator MARTINA to evaluate the maximum nitrification rate of the sludge and to understand the evolution of the SBR autotrophic biomass activity. Tests were performed by following the procedure that has been developed for the on-line titrator TITAAN and that is defined as "Mode 3" in the EOLI POLIMI WP3 2nd annual report (Milan, November 2004). All experiments were performed under non-limiting oxygen concentrations (DOstat = 8 mgO_2/L), pH = 8.3 and at the controlled temperature of 20.5°C. Data are reported in figure17 in terms of maximum specific nitrification activity, calculated as average values from a pH- DO-stat test.
Figure 17 shows that nitrification was very poor in December '04 (2.6 mgN-NH4/(gVSS*h)). Few days after the restart with new sludge, the test was repeated and the specific activity was at its lowest value, most probably because of the cold temperature (about 8°C) in the aeration basin of the full-scale plant since November 04. Tests were repeated periodically and allowed us to detect the improvement of the nitrification process in time. The comparison with on-line and analytical data confirms that nitrification efficiency slowly increased with time (from 14/01/05 – restarting of the plant), according to the data reported below.

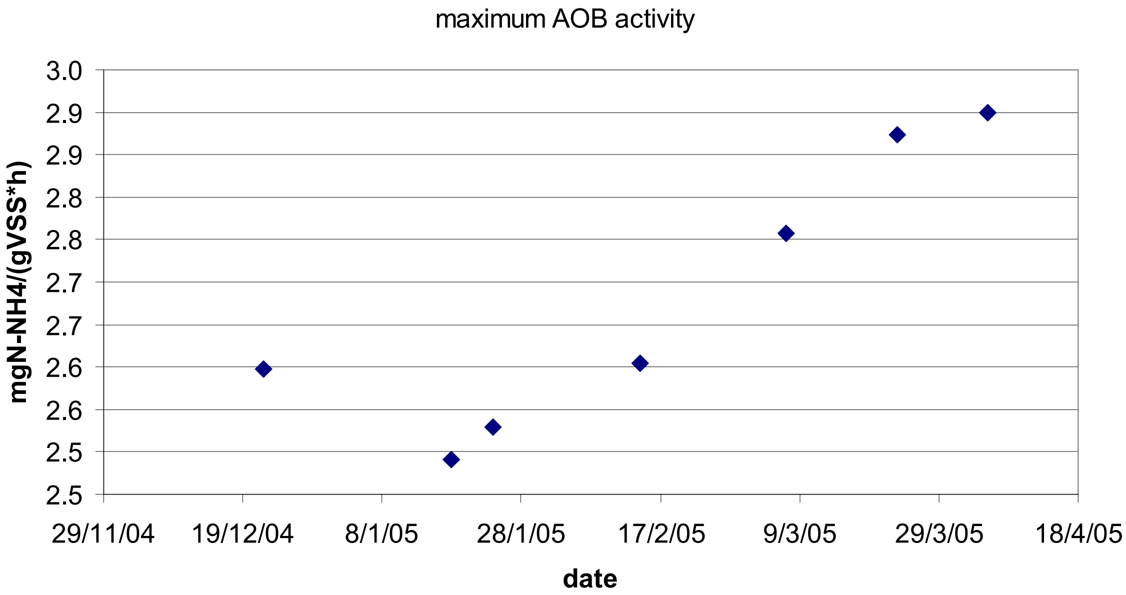

Figure 17 – Maximum specific nitrification activity (AOB) for SBR sludge, estimated with off-line pH-DO-stat tests at 20.5°C

Analytical determinations on effluent samples didn't reported any nitrite accumulation in the reactor; this means that NOB activity was never lower than AOB activity, confirming that the first step of nitrification has been the main problem for the SBR pilot plant.

Evaluation of SBR nitrogen removal efficiency with maximum nitrification activity measurements

SBR nitrogen removal efficiency has been estimated for some tests either with lab analysis or by means of mass balances based on the maximum nitrification activity measured with off-line pH-DO-stat tests.

The following formula has been used to evaluate nitrogen removal efficiency:

$$\eta_{N-titration} = \frac{Act_{AOB} \cdot X \cdot V_{reaction} \cdot time_{reaction}}{TKN_{infuent} \cdot V_{influent}} \cdot \frac{DO_{SBR}}{Ks_{AUT} + DO_{SBR}} \cdot \frac{1}{\theta^{(T°C-20°)}}$$

where:

Act_{AOB} is the maximum specific nitrification rate [mgN/(gVSS*h)] estimated on pure ammonia as substrate with pH-DO-stat tests at 20.5°C, pH=8.3, DO=8 mgO_2/L;

X is the VSS concentration in the SBR reactor during the aerobic phase (gVSS/L);

$V_{reaction}$ is the active volume of the reactor during aerobic phase (L);

$V_{influent}$ is the volume of sewage treated during the cycle (L);

$TKN_{influent}$ is the concentration of nitrogen of the sewage treated during the cycle (mgN/L);

$time_{reaction}$ is the duration of aerobic phase (h);

DO_{SBR} is the DO average concentration during the aerobic phase (DO is measured on-line; mgO_2/L);

Ks_{AUT} is the DO half-saturation constant for autotrophs during aerobic phase; the default value, assumed for the calculations, is 0.4 mgO_2/L according to Henze et al., 1986;

$\theta^{(T°C-20°)}$ is the temperature corrective factor (assuming $\theta = 1.08$) used to relate the activity measured in the laboratory at controlled temperature, to the real plant temperature (T°C).

In the following Table nitrogen removal efficiencies are reported for several peak-load cycles:

Table 5 - Nitrogen removal efficiency

Date	$TKN_{influent}$	$N\text{-}NH_{4\text{-}effluent}$	$\eta_{Analytical}$	$\eta_{pHDOstat\ titration}$
10/06/2004	78.8	0.485	98%	-
26/06/2004	78	0.51	98%	-
21/09/2004	52.6	0.67	96%	-
28/09/2004	86.8	0.05	99%	-
07/10/2004	93	1.06	96%	-
08/03/2005	74.1	4.659	**79%**	***68%***
09/03/2005	73.6	9.625	**56%**	***45%***

Table 6 – Parameters used to evaluate nitrogen removal efficiency with pH-DO-stat tests

Date	Maximum AOB activity (by pH-DO-stat tests)	Temperature In the SBR	X (VSS in SBR)	$time_{reaction}$	$V_{reaction}$	DO_{SBR}	Ks_{AUT}	$\eta_{pHDOstat\ titration}$
	mgN/(gVSS*h)	°C	gVSS/L	[min]	[L]	mgO_2/L	mgO_2/L	
08/03/2005	2.75	20	2.8	220	500	0.46	0.4	**68%**
09/03/2005	2.75	20	2.8	220	500	0.22	0.4	**45%**

Data reported above show how big was the reduction of nitrogen removal efficiency in cycles performed in 2005 compared to the first ones. For the two cycles in which we estimated both $\eta_{analytical}$ and $\eta_{pHDOstat\ titration}$ it is important to observe that the mass balance underestimates the total amount of nitrogen oxidized in the reactor. This can be explained by the relevant number of parameters needed to estimate the amount of oxidized nitrogen and their relative uncertainties, that are not negligible (for instance VSS concentration, temperature in the lab and in the reactor, titrants concentration and so on). Moreover, the ammonium uptake for heterotrophic synthesis is not taken into account. Anyhow, a positive or negative trend of evolution of the process can be easily detected on the basis of activity rates estimated by a pH-DO-stat test.

The maximum AOB activity was measured with a single pH-DO-stat test performed on March 7, assuming that for the 2 following days this parameter should have not varied significantly.

Very different efficiencies have been reported on March the 8th compared to March the 9th, in spite that the nitrogen loading rate were almost identical in the 2 days, and the same can be stated for the organic loading in terms of total COD and filtered COD (as detected by means of lab-analyses). Probably, in this case, a partial inhibition of the autotrophic biomass occurred, due to the presence of some toxicants in the sewage, having acute effects on AOB.

References

Henze, M., Grady Jr, C. P. L., Gujer, W., Marais, G. v. R., Matsuo, T. (1986). Activated sludge model no 1. International Water Association, formerly International Association on Water Pollution Research and Control. Scientific and Technical Reports No 1. ISSN: 1010-707X.

Mauret M., Ferrand F., Boisdon V., Spérandio M. and Paul E. (2001) Process using DO and ORP signals for biological nitrification and denitrification : validation of a food-processing industry wastewater treatment plant on boosting with pure oxygen Water Science & Technology, Vol. 44 No 2-3 pp 163–170.

Melcer H., Dold P. L., Jones R., Bye C. M., Takacs I., Stensel H. D., Warren Wilson A., Sun P., Bury S. (2003) M. Methods for wastewater characterization in activated sludge modeling, Water Environment Research Foundation

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Abnormal Conditions in POLIMI SBR

As stated earlier, the main abnormal conditions experienced frequently in the POLIMI lab-scal SBR was the sudden and irreversible nitrite build up, as it occurred several times before.

Factors which affect NOB/AOB activity

The aims of this experimentation were:

- to evaluate the effect of some environmental conditions on the activity of ammonium and nitrite oxidizing bacteria
- to identify conditions which can favor AOB over NOB, thus possibly leading to an unbalanced nitrification in the SBR.

pH, free ammonia, DO and aeration patterns are reported in the literature to have a relevant effect of both groups of nitrifying bacteria. However, very wide ranges can be found on the relevance of these effects. Therefore, batch tests were conducted to point out which of these aspects was indeed relevant for the SBR sludge.

Materials and Methods

Nitrification activity was measured by pH-DO-stat titration performed with the laboratory titrator developed within this project (MARTINA instrument). The principle of operation and the experimental procedure applied to measure AOB and NOB activities has been already described in previous reports (see Deliverable 3.2).
Sludge samples were drawn either from the lab-scale POLIMI SBR or, when the latter was suffering for poor nitrification, from a full scale WWTP whose sludge was also used for the SBR inoculation. However, even this latter sludge was occasionally showing a low nitrification capacity, which could be due to the low temperature at which the WWTP was operated during the sampling period (January and February).

Tests to assess the effect of DO, pH and NH_3 concentration on the activity of AOB and NOB were performed as follows: a 0.5 L sludge sample was poured into the MARTINA reactor. An initial set of environmental parameters was fixed. A first activity test was performed by adding the appropriate substrate for the bacteria group whose activity had to be assessed (ammonium or nitrite, 5 mgN/L). When the added nitrogen had been fully oxidized, all parameters were kept constant but the one whose influence was under study, which was increased or decreased. The substrate was spiked again to assess the nitrification activity under the new condition. In some of the tests, substrate was supplemented a third time, after having made a third change in the parameter under study. The maximum nitrification activity measured after each substrate spike was then plotted against the varied parameter.

Tests to assess the effect of the aeration pattern on the decay rate were performed as follows. A batch of 8 L of sludge was sampled and kept under aeration overnight to reach endogenous conditions. Nitrification (AOB and NOB) activity was measured. The sludge was then split into four batches, all continuously mixed, but characterized by different aeration patterns:

Batch A (anoxic): not aerated;
Batch B (aerobic): continuously aerated;
Batch C (12h): aeration was cyclically kept on for 12 h and off for the following 12 h.
Batch D (1h): aeration was cyclically kept on for 1 h and off for the following hour.

All batches were kept under endogenous conditions at room temperature (19-22 ^{o}C). Daily, pH was measured in all batches and corrected to remain in the range 7.5-8.0. Nitrification activity, VSS, ammonium, nitrites and nitrates concentration were determined after 4 and 7 days.

Results

First, the effect of dissolved oxygen on nitrification activity was determined. Tests conditions were the following:

sludge concentration = 2-3 gVSS/L,
pH was adjusted to optimal, i.e.: 8.3 for the AOB test and 7.5 for the NOB test,
temperature = 21°C
DO = varied between 1.0 and 7.5 mg/L. Note that, during DO-stat titration, the DO concentration oscillates around the set point value of approximately ±0.25 mg/L, therefore, set-point values lower than 1.0 could not be tested precisely.

AOB and NOB activity against DO is plotted in Figure, together with the fitting Monod curve, whose best-fit parameters were estimated to be:

AOB: maximum activity = 3.0 mgN/gVSS/h; half saturation constant = 0.1 mgO_2/L
NOB: maximum activity = 2.6 mgN/gVSS/h; half saturation constant = 0.4 mgO_2/L

These results confirm the higher affinity of AOB for oxygen, as it as been reported in previous experimentations. Barnes e Bliss (1983) indicated a range for the DO half saturation constant of 0.25-0.5 mg/L for AOB and of 0.34-2.5 mg/L for NOB. Hanaki et al. (1990) found that AOB activity did not vary significantly at DO-concentration below 0.5 mgO_2/L, while the NOB activity halved. However, being the DO level during the aerobic react phase of the POLIMI SBR normally above 1 mgO_2/L, DO limitation does not seem to be a major cause for nitrite build-up in this reactor.

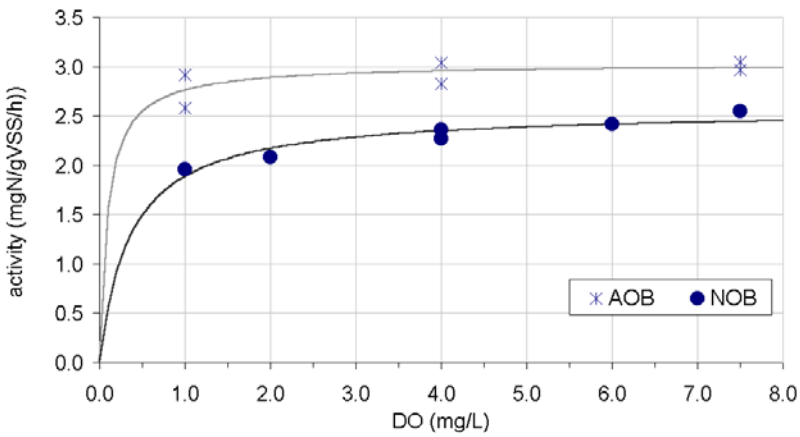


Figure18: Effect of dissolved oxygen on the activity of NOB and AOB.

Secondly, the effect of pH on nitrification activity was studied (Figure). Tests conditions were the following:

range of sludge concentration during the experiments = 2.0-3.8 gVSS/L,

pH = varied between 7.5 and 8.3 which is the usual range within which the pH of the POLIMI SBR remains during the aerobic react phase.

temperature = 21°C

DO = 7.5 mg/L

As for the NOB activity, a decreasing trend with pH can be observed. However, this reduction (from 3.42±0.39 mgN/gVSS/h at pH 7.5 to 2.88±0.57 mgN/gVSS/h at pH 8.3, average reduction of 16%) seems to be moderate. On the contrary, AOB activity increased with pH from 1.47 mgN/gVSS/h at pH 7.5 to 2.31 mgN/gVSS/h at pH 8.3. However, the sludge sample which was used to evaluate the effect of pH on AOB activity showed a low nitrification activity compared to the one used to assess the effect of low DO levels. Therefore, a second trial is planed in the next weeks to confirm the observed trend.

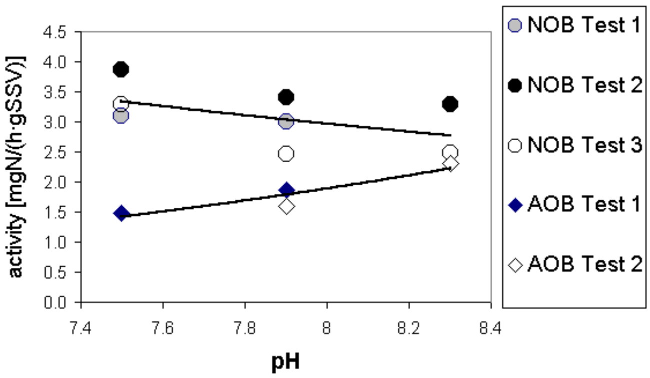


Figure 19: Effect of pH on the activity of AOB and NOB.

The third evaluation, still in progress, is aimed at assessing the effect of the free ammonia concentration on the NOB activity. Actually, this bacteria group is known to be inhibited by free ammonia levels as low as 1 mgN/L (Anthonisen et al., 1976), which are easily exceeded in batch fed reactors such as SBRs.

To assess NOB activity of mixed cultures (such as activated sludge samples) in the presence of a constant ammonia concentration, an AOB selective inhibitor is needed to avoid concomitant oxidation of ammonium to nitrite. ATU is known to fully inhibit AOB and to be ineffective on NOB. However, the sensitivity of activated sludge samples to organic compounds may vary widely according to their specific growth conditions. Therefore, a preliminary test (Figure) was aimed to verify the non-inhibitory effect of ATU to NOB. A sludge sample under endogenous conditions was spiked with nitrites (5 mgN/L) before and after the addition of 15 mg/L of ATU. These two activity values were found to be very close, therefore no ATU inhibition was observed. It is worth noting that, after ATU inhibition, a reduction of the endogenous titration rate was clearly observed, indicating that ammonification, and therefore a slow nitrification was taking place in the sludge sample, which was as high as 0.2 mgN/gVSS/h.

Tests to assess the effect of increasing free ammonia concentrations will be performed in the near future.

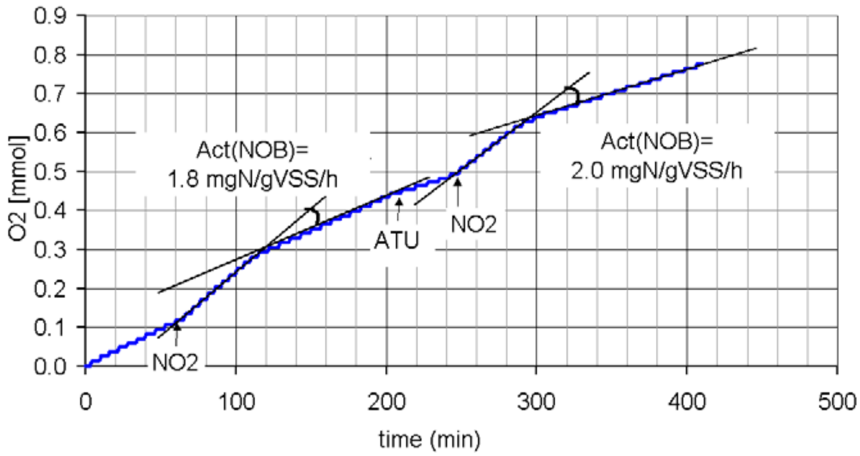
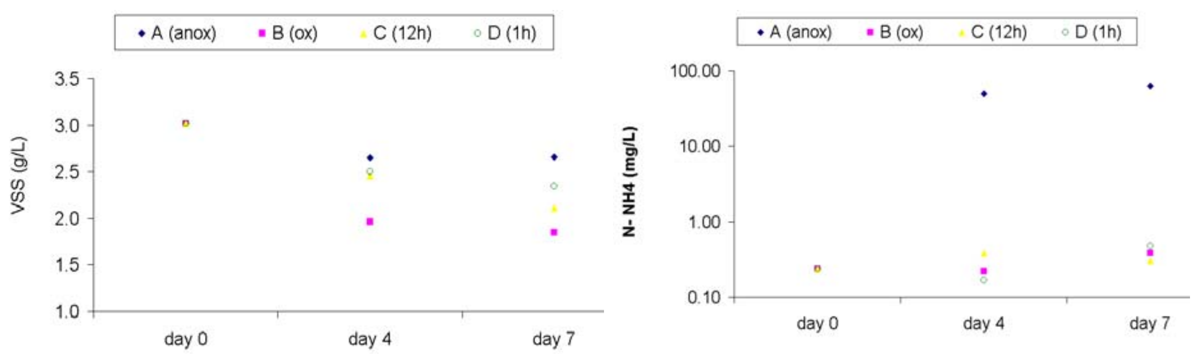


Figure 20: DO-stat titration curve for the assessment of the effect of ATU on NOB activity.

One test has also been performed to assess the effect of the aeration pattern on the decay rate of activated sludge bacteria. In the first graph in Figure , the concentration of VSS in time is presented. The decay constant (k_d, Table 7) for heterotrophic bacteria can be estimated by fitting the VSS versus time data with a decreasing exponential function:
$VSS(t) = VSS(0) \cdot e^{-kd \cdot t}$

Table The decay process was found to be faster under aerobic conditions, while the lowest value was that measured under constant anoxic conditions. Conversely, batch D (1h) showed a slow biomass decay than batch C (12h), confirming that prolonged aerobic periods accelerate the biodegradation of the death biomass.
As for the nitrogen forms (see Figure), the ammonium released from biomass lysis was accumulating under anoxic conditions. On the other hand, under aerobic conditions the ammonium released was oxidized to NOx –by nitrifying bacteria–, which accumulated in solution being denitrification prevented by the redox condition. For batch A and B, the theoretical total nitrogen concentration could be calculated by assuming the N release per mass VSS oxidized. Table 8 reports the comparison between experimental values and expected theoretical ones, calculated by assuming a specific N release of 0.11 gN/gVSS (as obtained by applying ASM1 most common stoichiometric parameters). A lower ammonium accumulation would be expected in both batches. Nitrogen containing compound may had been adsorbed on sludge flocs in the beginning of the test, which may have led to a higher N release. As for batch C and D, a N mass balance cannot be obtained, since N was removed by the alternation of nitrification and denitrification, as suggested by the much lower N content for these batches.



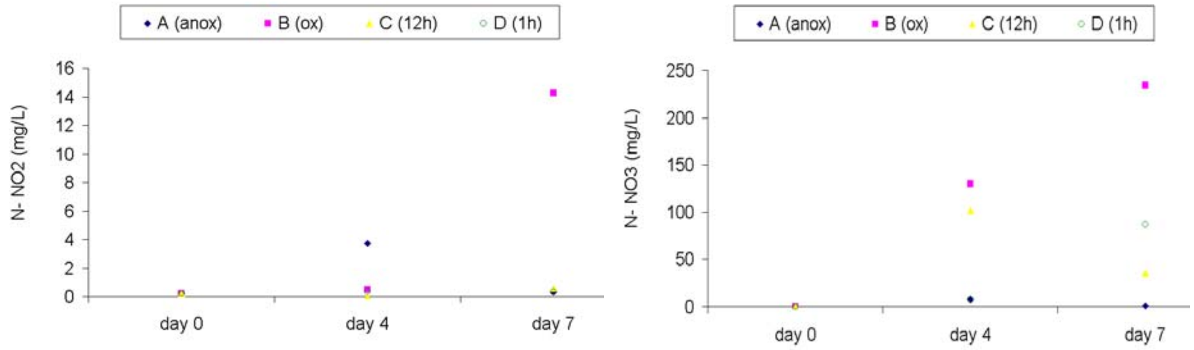


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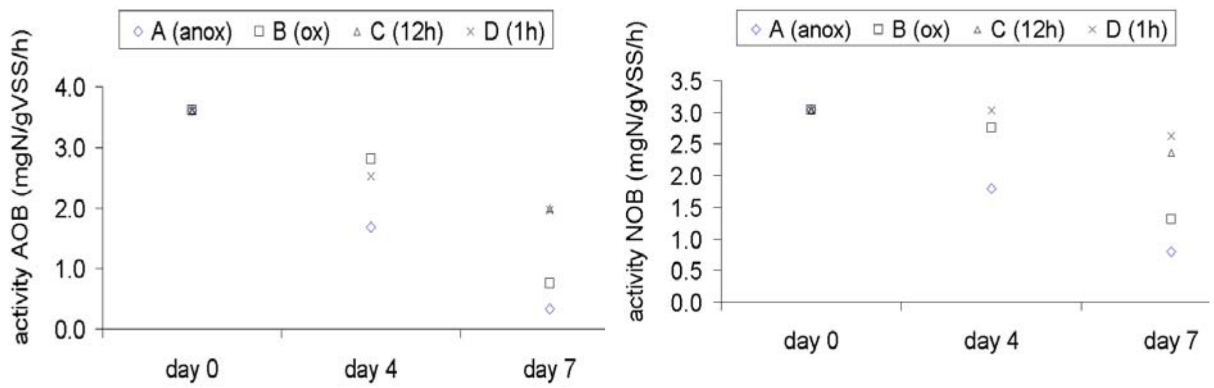
Figure 21: VSS and nitrogen forms for endogenous sludge samples kept under various redox conditions.

As for nitrification activity, Figure reports AOB and NOB activity and their ratio for the four batches. Aeration patterns which led to the lower net decay were those which have been cyclically aerated, while both fully aerobic and fully anoxic conditions led to a more relevant reduction in the nitrification capacity of the sludge. Faster decreases in nitrification rate under aerobic conditions may be due to “aerobic digestion”, that is hydrolytic activity by heterotrophic bacteria, although nitrification of the ammonium released by dead biomass takes place also. On the other hand, under fully anoxic conditions, although the hydrolytic activity is expected to be lower, nitrifying bacteria cannot grow because of the lack of oxygen. Moreover, in all batches, the ratio between NOB and AOB activity resulted to increase with time. Unexpectedly, the redox condition which lead to the higher NOB over AOB activity ratio was the anoxic one. Finally, data indicate that none of the experimented aeration pattern brought to a NOBactivity/AOBactivity lower than 1, which would cause nitrites build up. Cyclic aeration seems to be the condition which equally affects the net decay rate of AOB and NOB.
More tests are needed to confirm these observations.



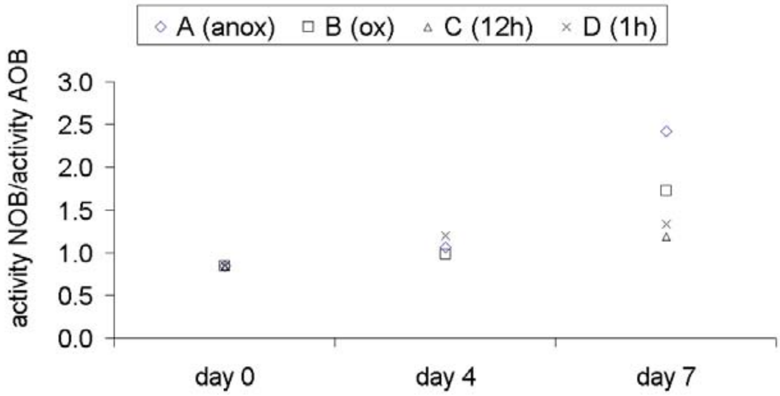


Figure 22: AOB and NOB activities for endogenous sludge samples kept under various redox conditions.

Table 7: decay constant (k_d) for heterotrophic bacteria for different aeration patterns, calculated on the VSS disappearance.

Batch	A (anox)	B(aerobic)	C(12h)	D(1h)
k_d (d^{-1})	0.02	0.08	0.05	0.04

Table 8: Comparison between theoretical and measured nitrogen release for batch A and B.

Batch	A (anoxic)		B(aerobic)	
N content	Experimental	Theoretical	Experimental	Theoretical
day 4	61	42	131	118
day 7	64	41	249	131

Conclusions and perspectives

According to the experimental results, none of the studied environmental conditions (DO level, pH and aeration pattern) was found to significantly favor AOB over NOB activity. However, more experiments and repetitions are needed to confirm this observation. The effect of free ammonia still has to be tested.

No final conclusions can be drawn on the environmental conditions which can cause and justified the frequent nitrites build up experienced in the POLIMI-SBR.

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Bliss, PJ; Barnes, D (1986). Modelling nitrification in plant-scale activated sludge. Water Sci. Technol.18(6), 139-148.

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Batch experiments for the study of the biosorption phenomenon

Batch tests were performed on sludge samples, drawn from the SBR, to which various amounts of COD were initially spiked. Two organic sources were tested: a synthetic wastewater made of peptone and meet extract (first experimental campaign, E1) and a suspension of dry milk serum (second experimental campaign). The evolution of the soluble COD concentration was then followed in time.
In order to discriminate the relative contribution of denitrification and adsorption to the overall COD removal, the following redox conditions were tested: strict anaerobic (AN), anoxic (AX) and anaerobic followed by anoxic (AN+AX).

Material and Methods

Activated sludge samples used for this experimentation came from a lab-scale SBR fed on the synthetic wastewater described in Table . Main design and loading parameters of the SBR are listed in Table .
Sludge samples underwent the following procedure before they were used in adsorption tests. A batch of sludge (approximately 1 L) was sampled from the SBR at the end of the aerobic phase. Nitrates concentration was measured and acetate was added according to a COD/N ratio of 4-5 in order to ensure complete denitrification. An overnight mixed reaction was then allowed. The following day, biomass total and suspended solids were measured (VSS and TSS). To remove excess acetate, the sludge was aerated during 15 min. Then, nitrogen gas was bubbled during 5 min to ensure strictly anaerobic conditions. Finally, 250 mL of sludge were transferred into 500 mL-volume Erlenmeyers, which were bubbled with nitrogen gas for further 5 min and then sealed to prevent oxygen transfer to the liquid phase. During the time course, 20 mL- mixed liquor samples were drawn and filtered on a 0.45 μm membrane, for soluble COD determination (Hach-Lange spectrophotometric kits). In anaerobic tests, nitrite and nitrate concentrations (Hach-Lange spectrophotometric kits) were also measure at time 2 min and 60 min.
Experimental conditions of both experimental campaigns are summarised in Table.

Results

Synthetic wastewater as the organic carbon source

In Figure , soluble COD concentrations assessed in all anaerobic adsorption tests are reported. Table 1 summarises the percentage on the remaining COD for the same tests, while mean values are shown in Figure .

Table 9: Experimented organic carbon sources.

Composition synthetic WW (mg/l)	
Peptone	457
Meat extract	313
NaCl	20
$CaCl+2H_2O$	16
$MgSO_4+7H_2O$	6
K_2HPO_4	80
CH_3COONa	128
COD	900
TKN	100
Composition solution of dry milk serum (mg/l)	
Dry milk serum	1000
COD	1000

Table 10: Main design parameters of the lab-scale SBR.

Maximum working volume	20 L
Volumetric exchange ratio	6 L
Number of cycle per days	4
Cycle phases	Anoxic load (10 min)
	Anoxic react (45 min)
	Aerobic react (4 h 45 min)
	Settling (37 min)
	Draw (23 min)

Table 11: Experimental conditions of both experimental campaigns (E1 and E2).

Experiment	Batch	Initial COD concentration (mg/l)	Biomass concentration (mgVSS/L)	F/M (gVSS/gCOD)	pH (initial-final)	Redox conditions
E1 – Run 1	1	255	3060	0.08	8.0-8.1	AN
	2	910	3060	0.30	8.0-8.1	AN
E1 – Run 2	3	172	3595	0.05	8.3-8.2	AN
	4	306	3595	0.09	8.1-8.2	AN
	5	612	3595	0.17	8.2-8.1	AN
E1 – Run 3	6	284	3555	0.08	8.1-8.0	AN
	7	284	3555	0.08	7.9-8.2	AX
	8	284	3555	0.08	7.9-8.2	AN+AX
E2 – Run 1	9	425	3096	0.065	8.1-8.0	AN
	10	1227	3096	0.19	8.1-7.9	AN
E2 – Run 2	11	240	3005	0.08	8.2-8.2	AN
	12	240	3005	0.08	8.2-8.2	AX
	13	240	3005	0.08	8.2-8.2	AN+AX

Table 12: Percentage of the remaining COD during the course of anaerobic tests with synthetic influent as organic carbon source.

Batch	Initial COD concentration (mg/L)	Remaining COD (%)						
		2 min	10 min	30 min	45 min	50 min	60 min	75 min
1	255	98	97	64	79		79	
2	910	98	94	93	92		93	
3	172	102	101	87	81		-	
4	306	92	86	83	78		73	
5	612	99	94	86	81		78	
6	284	102	97	89		81	76	69

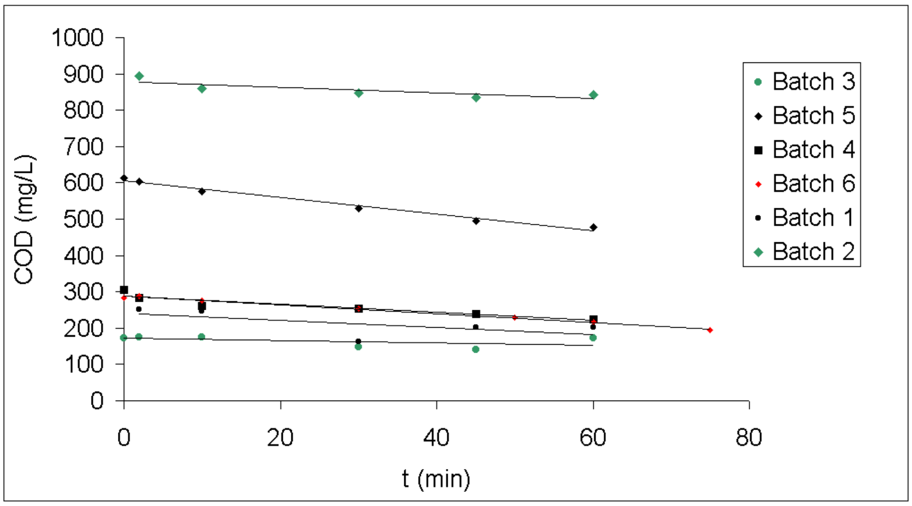

Figure 23: COD evolution during the course of anaerobic tests with synthetic influent as organic carbon source.

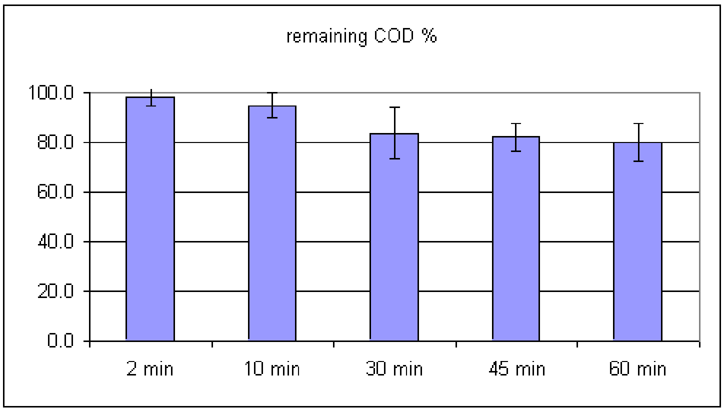

Figure 24: Mean percentage on the remaining COD during the course of anaerobic tests with synthetic wastewater as organic carbon source.

It can be clearly observed that after 30 min of contact the COD biosorption levels around the 15-20% of the initial amount. No evident correlation can be observed between F/M and biosorption levels.

As for Run 3, the comparison of COD and nitrogen evolution under different redox conditions is presented in Figure . The equivalent nitrogen (Neq) is calculated as: Neq = $N\text{-}NO_3^- + 0.6\cdot N\text{-}NO_2^-$; as suggested by Sözen and Orhon (1999). From the Neq uptake rate, denitrification rates (r_D) were calculated for Batch 7 (AX) and Batch 8 (AN+AX):
r_D(Batch 7) = 24 $mgN_{eq}\ g^{-1}VSS\ h^{-1}$
r_D(Batch 8) = 10 $mgN_{eq}\ g^{-1}VSS\ h^{-1}$
While the value obtained for Batch 8 is within a usual range for the denitrification of the rbCOD fraction (literature values ranging from 1 to 20, as reviewed in Kujawa and Klapwijk, 1999), the value in batch 7 seems to be slightly above the usual range.
Moreover, COD/N ratio were estimated as the ratio between r_D and the COD uptake rate for the same batches:
COD/N(Batch 7) = 9
COD/N(Batch 8) = 11
Commonly this ratio is estimated as: COD/N = $2.86/(1-Y_H)$, therefore, assuming the average value for the COD/N ratio of 10, the observed Y_H results to be 0.71. The latter value is slightly higher than the values most usually found for anoxic heterotrophic yields (0.4-0.6 as reviewed in Muller et al., 2003). A higher COD/N ratio can be explained by considering that the COD uptake is due to both denitrification and biosorption.

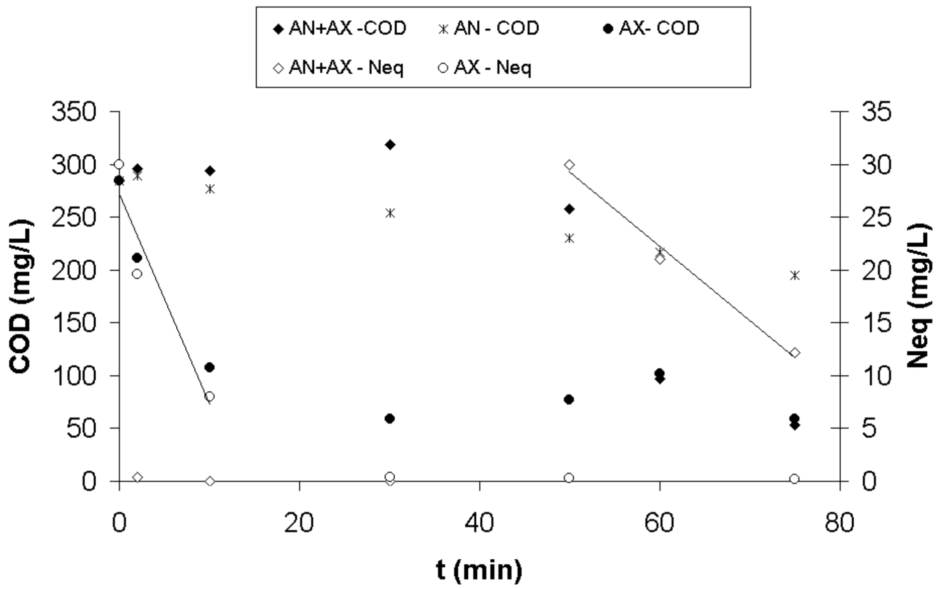

Figure 25: COD and nitrate evolution during the course of AN, AX and AN+AX tests with synthetic influent as organic carbon source.

Solution of milk serum as the organic carbon source

In Figure 26, soluble COD concentrations assessed in all anaerobic adsorption tests are reported. Table 13 summarises the percentage of the remaining COD for the same tests, while mean values are shown in Figure . Again, no clear correlation can be observed between the initial F/M and the extent of biosorption. The fraction of "biosorbed" organic substrate levelled out after 30 min of contact. Averagely, a fraction of 15-20% of the initial organic carbon was absorbed. The biosorption behaviour of the sludge resulted to be very similar to the one observed with the synthetic WW as carbon source (see comparison in Figure).

Table 13: Percentage on the remaining COD during the course of anaerobic tests with milk serum as organic carbon source.

Batch	Initial COD concentration (mg/L)	Remaining COD (%)					
		2 min	10 min	30 min	50 min	60 min	75 min
9	425	84.2	83.5	80.7			
10	1227	97.9	89.4	88.1			
11	240	86.2	87.3	84.7	86.5	85.4	81.7

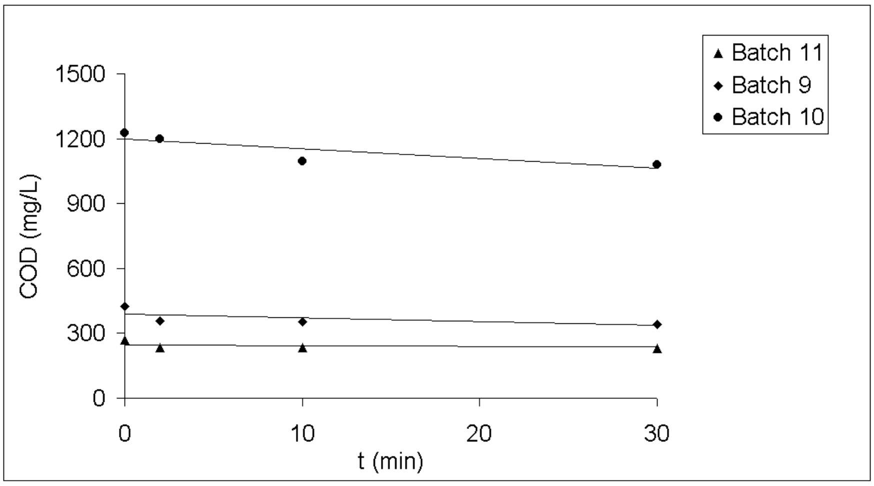

Figure 26: COD evolution during the course of anaerobic tests with milk serum as organic carbon source.

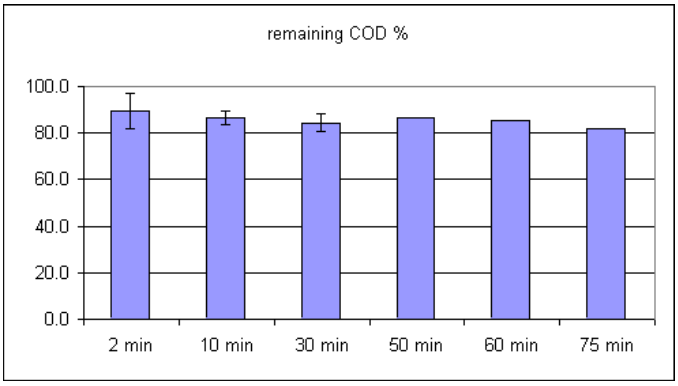

Figure 27: Mean percentage on the remaining COD during the course of anaerobic tests with milk serum as organic carbon source.

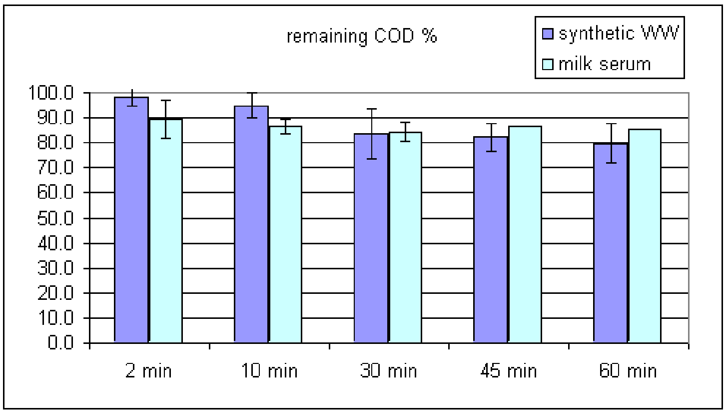

Figure 28: comparison between the biosorption behaviour of the sludge with two different organic carbon sources under anaerobic conditions.

As for Run 2, the sludge behaviour under anoxic conditions was found to be very different from the one observed in the presence of the synthetic WW. In Figure , COD and Neq evolutions for batches 11 (AN), 12 (AX) and 13 (AN+AX) are shown. No significant difference was found among the COD trends in the three batches, suggesting that no denitrification took place. This hypothesis is supported by the negligible Neq decrease in both batches 12 and 13.
Apparently, the sludge grown on the synthetic feed could not efficiently use the milk serum as carbon source for denitrification, without proper acclimation.

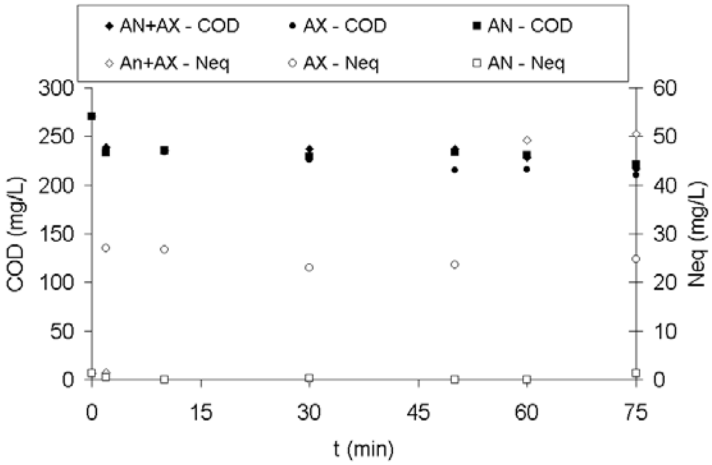

Figure 29: COD and nitrate evolution during the course of AN, AX and AN+AX tests with milk serum as organic carbon source.

Conclusions

The biosorption behaviour of the sludge used in the experimentation resulted to be similar in all tests and limited to a fraction ranging from 15 to 20% of the initial amount of COD added. No correlation between biosorption and initial F/M was observed within the experimented range (0.05-0.19 gCOD/gVSS). Biosorption resulted to be substantially concluded within the first 10 min of contact. The extent of biosorption was similar with both the organic carbon sources tested (synthetic feed and milk serum). Finally, the sludge was found to denitrify efficiently if the synthetic feed was used (note that this was the usual feed of the SBR from which the sludge was withdrawn); on the contrary no denitrification took place when milk serum was fed as carbon source.

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Future developments

WP1 activities at POLIMI and ENEA in the next semester will only be devoted to integration with other task groups. In particular, more tests on biosorption and on factors affecting NOB/AOB activity will be performed.