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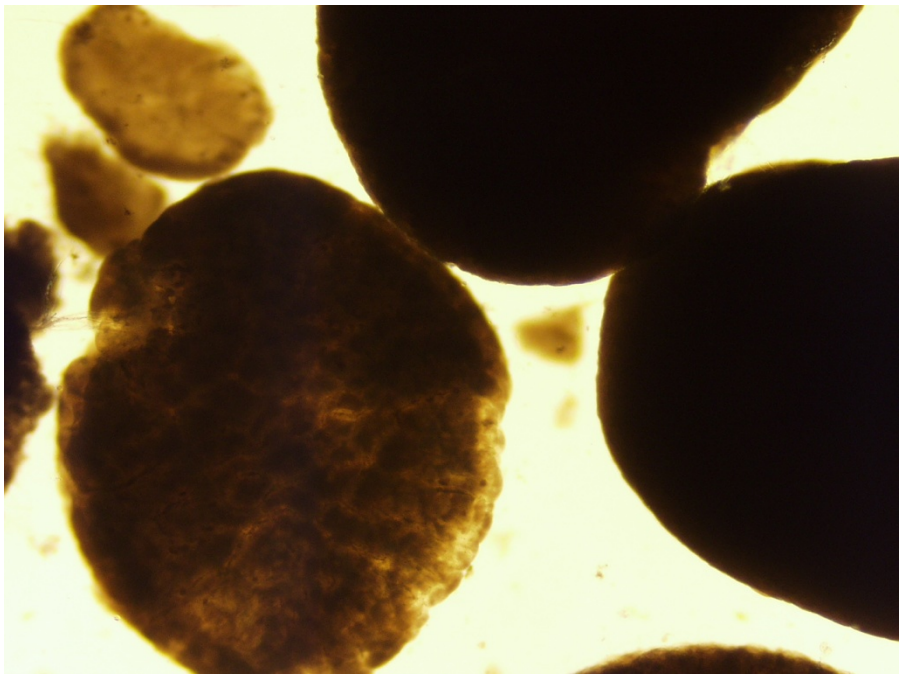

Laboratory study on granular sludge nutrient removal for wastewater treatment

Master of Science Thesis in the Master's Programme Infrastructure and Environmental Engineering

MASSIMO ROSSETTO

Department of Civil and Environmental Engineering
Division of Water Environment Technology
CHALMERS UNIVERSITY OF TECHNOLOGY
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Nomenclature

CMTR	Completely Mixed Tank Reactor
COD	Chemical Oxygen Demand
CODIN	Reactor Influent COD concentration
DO	Dissolved Oxygen
Ft	Reactor Feeding Time
MLSS	Mixed Liquor Suspended Solids
N-NH_3	Ammoniacal Nitrogen
N-NH_4^+	Ammonium Nitrogen
NH_3	Free Ammonia
OHO	Organic Heterotroph Organism
OrgN	Organic Nitrogen
ORP	Oxydation-Reduction Potential
PAO	Polyphosphate Accumulating Organism
PHB	Polyhydroxybutyrate
R1	Reactor 1
R2	Reactor 2
R3	Reactor 3
SBR	Sequencing Batch Reactor
SOUR	Specific Oxygen Uptake Rate
SST	Secondary Settling Tank

SVI	Sludge Volume Index
TN	Total Nitrogen
TOC	Total Organic Carbon
V_o	Reactor Operative Volume
VSS	Volatile Suspanded Solids
WWTP	Wastewater Treatment Plant

Chapter 1

Introduction

1.1 Problems related to polluted waters

With the term wastewater treatment, generally all the physical and biological treatments that have the aims to remove contaminants from industrial and domestic polluted water are considered. The objectives are to produce a treated effluent, that normally is discharged into rivers, lakes or seas and a solid sludge rich in nutrients, suitable for disposal or reuse.

There are many reasons to treat wastewater: to safeguard the men's health from the pathogenic bacteria present in the uncleaned water, to prevent the life of aquatic plant and fishes, to avoid the eutrophication development in rivers and lakes.

Usually the contaminants mainly removed are: organic substances, nitrogen, phosphorous and solid particles. Discharging organic matter lead to oxygen consumption; heterotrophic bacteria present in the aquatic environment use the organic matter as substrate to grow and hence consuming oxygen. If the oxygen consumption rate is greater than the capability of the atmospheric oxygen to dissolve in water, critical conditions can be reached. In this conditions many living organism die.

Nitrogen and phosphorous are the main responsible for the eutrophication process. A great algae growth in lakes and rivers leads to two main problems. The first is an aesthetic pollution that is not so important, because it doesn't give problems to the aquatic ecosystem. The second is more important: in fact when the algae die, they become biomass which can be degraded by the heterotrophic bacteria leading to the problems described above.

Finally the removal of the solids is important to reduce the concentration of bacteria discharged. Normally in the wastewater a lot of different pathogenic bacteria are dissolved, so to prevent the men's health it is good to discharge an effluent with low concentration of solids.

1.2 Biological nutrient removal

With different conditions and different types of bacteria it is possible to remove nutrients and pollutants dissolved in the wastewater. Here some informations about the biological removal of organic substances, nitrogen and phosphate are given.

1.2.1 Organic matter

The concentration of carbon substances in the wastewater, can be expressed with different parameters. In this work COD and TOC were used.

The COD, chemical oxygen demand, measure the amount of oxygen necessary to chemically oxidize organic material using potassium dichromate in acid solution. So it is an indirect measurement of the organic matter, based on the assumption that the higher the organic content and the higher is the oxygen request to the oxidation process and so higher the COD value.

The TOC, total organic carbon, is the amount of carbon bound in an organic compound. It is different compared to COD since it measure the quantity of carbon present in a molecule.

The organic matter can be biologically degraded both under anaerobic and aerobic conditions. The anaerobic degradation take place thanks to different processes, bacteria and enzymes which transform the organic matter into carbon dioxide and methane. The aerobic degradation is an oxidation that transforms the organic matter in carbon dioxide and water. In wastewater treatment plants (WWTPs) for urban and industrial wastewater treatment aerobic degradation is generally preferred and instead the sludge stabilization is usually carried out with an anaerobic degradation, in order to recover energy by the methane production.

1.2.2 Nitrogen

Different forms of nitrogen can be found in wastewater and the biological degradation can be performed with different condition and by different types of organisms.

The organic nitrogen consists of a complex mixture of compounds including amino acids, sugars and proteins. In wastewater application this form of nitrogen is usually neglected, because it undergoes a biological process, ammonification, in the sewer system and is totally converted to ammonia before arriving at WWTP.

Nitrogen in the form of ammonia is divided in free ammonia nitrogen (N-NH_3) and saline ammonia nitrogen (N-$NH_4{}^+$). The two compounds are both dissolved in water, and they coexist in equilibrium, as described by the following reaction:

$$NH_3 + H^+ \rightarrow NH_4^+ \tag{1.1}$$

As show in figure 1.1 free ammonia concentration increase with pH increasing. Free ammonia is a poison compound for the bacteria, especially for nitrifiers; this is the reason why for nitrogen biological removal it's better to have a pH in the range of 7.5 - 8.5, where the free ammonia concentration is lower than 20%.

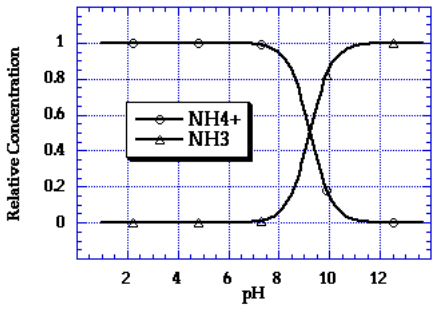


Figure 1.1: Ammonium equilibrium

Finally there is nitrogen in the form of nitrate (N-NO_3^-) and in form of nitrite (N-NO_2^-). The sum of these forms of nitrogen is called total nitrogen (TN):

$$TN = OrgN + N - NH_3 + N - NH_4^+ + N - NO_3^- + N - NO_2^- \tag{1.2}$$

Biological nitrogen removal is performed by two different processes: nitrification and denitrification. In the first ammonium is converted to nitrite and then to nitrate by two different steps, as described by equations 1.3 and 1.4:

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2 + 4H^+ + 2H_2O \tag{1.3}$$

$$2NO_2 + O_2 \rightarrow 2NO_3^- \tag{1.4}$$

Ammonium oxidation into nitrite is performed by ammonia oxidizing bacteria, mainly Nitrosomonas, instead the nitrite conversion into nitrate is performed by Nitrobacter. Both steps are oxidation reactions, so the processes can take place only in aerobic environment.

Denitrification is the nitrate reduction to nitrogen gas. The process is carried out by heterotrophic bacteria, Nitrosomonas, in anoxic environment. The steps involved during denitrification are described by equation 1.5:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \tag{1.5}$$

1.2.3 Phosphate

In the biological phosphorous removal, the influent dissolved phosphorous is accumulated into cell biomass ad then removed with sludge wasting. The bacteria involved are the polyphosphate accumulating organisms (PAOs).

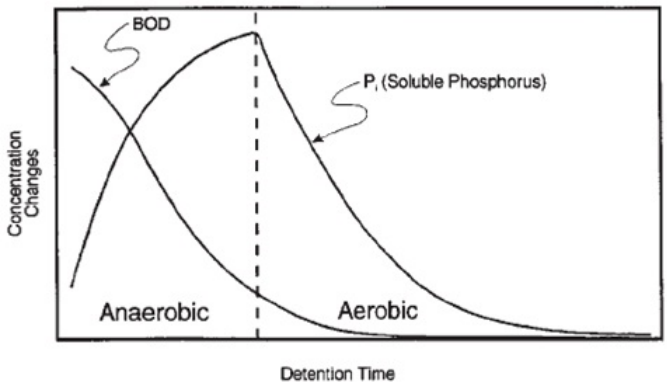


Figure 1.2: BOD and phosphate concentration trend during aerobic and anaerobic conditions

During anaerobic conditions, PAOs, assimilates acetate produced by the fermentation of the biodegradable soluble COD and produces polyhydroxybutyrate (PHB) storage products using the stored polyphosphate as energy source. At the same time there is a release of orthophosphate. So during anaerobic condition in the cell biomass the concentration of PHB increases and that of polyphosphate decreases.

During the aerobic phase the stored PHB is used as energy source and as carbon source for new cell growth. The energy released from the PHB oxidation is used to incorporate dissolved phosphate in the bacteria cell. It results a decreasing on the dissolved phosphate concentration.

The process are described in Fig. 1.2.

1.3 Wastewater treatment plants

Nowadays wastewater treatment plants are usually divided in two different lines: the water line and the sludge line. The first present all the treatments processes related to the pollutant removal from the influent water. Generally it includes three stages:

1. Primary treatment removes material that will either float or readily settle out by gravity. It includes the physical processes of screening, comminution, grit removal, and sedimentation.

2. Secondary treatment removes the suspended and dissolved material. It includes the biological reactors where there is the nutrients removal and the secondary settling tank where the suspended solids settle and so they are removed.

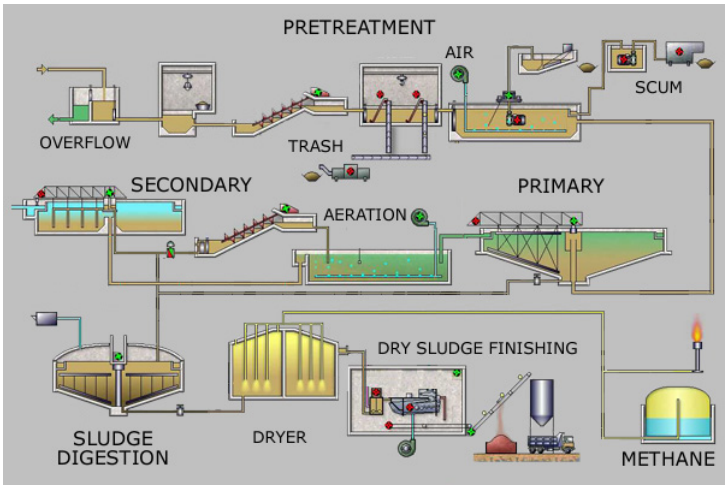


Figure 1.3: Wastewater treatment plant scheme

3. Tertiary treatment includes all treatments of the effluent with the aim to perform a more efficient pollutants removal. A common process in this stage is the disinfection, where the pathogen bacteria are killed with UV ray or ozone.

The sludge line comprises all the processes to dehydrate and stabilize the sludge formed during the process. The aim is to create a sludge with a low concentration of organic substances and with a low percentage of water.

In figure 1.3 is presented a common process scheme. Focusing on the biological nutrient removal, the most common technology used is activated sludge. It is a biological sludge, continuously mixed and kept in suspension in the aeration tank, containing an active biomass capable to stabilize wastewater in aerobic conditions. Here two main processes take place: COD removal and ammonium conversion into nitrate.

The aeration tank is followed by a secondary settling tank (SST). The purpose of SST is to permit the settling of the sludge in order to remove the particulate COD and the PAOs containing the phosphate from the effluent, and to control and set the best solids concentration in the aeration tank. To ensure a good settleability in the sludge, the target is to reach a sludge volume index (SVI) with a value between 100 and 120 ml/g. Greater values indicate a bad settleability and if the SVI exceed 150 ml/g the sludge has a very bad settleability and generally it contains filamentous bacteria that create a net inside the sludge and they don't allow the sedimentation of VSS.

To ensure a complete nitrogen removal and an enhanced phosphorous removal, other reactors can be added to the aerated one. Usually an anoxic step follow the aeration tank in order to degrade the nitrite and nitrate formed during the process of nitrification, and an anaerobic reactor is placed as first reactor, to induce the

phosphorous release from the PAOs.

From this brief description it is easy to understand that the wastewater treatment plants need a lot of space to be built and also it must be taken in account that an increasing inhabitants number and on the economical activity leads to the necessity of enlargement the existing plant. Unfortunately it is not always possible to have all the space needed and so it is useful to find alternative technologies for biological removal of nutrients.

The aim of this work is a study of granular sludge. Applying this technology to an sequential reactor, it is possible to perform COD, phosphorous, ammonia and nitrate removal in the same reactor, and so the space needed for the WWTP construction is less than that necessary for a plant using activated sludge. Furthermore the granular sludge has a better settleability, enabling sludge settling in the biological reactor. Hence it is not necessary to have a secondary settling tank.

1.4 Granular sludge

Granular sludge is a biological sludge formed by granules that are composed of microbial cells embedded in extracellular polymeric substances. This polymeric matrix is mainly formed by proteins, polysaccharides, humic acids and lipids. As a results, the granules, has a very dense and compact structure composed of different types of bacteria: heterotrophs (OHOs), nitrifiers, denitrifiers and phosphate accumulating organisms (PAOs), and so is possible to remove organic compounds, ammonia, nitrates, nitrites and phosphates simultaneously. Furthermore the granules large size permit them to settle very rapidly, so it is more easy to separate the sludge from the treated effluent.

Many studies have shown that granules growth can be obtained by using different carbon source: glucose, acetate, ethanol, phenol, synthetic wastewater and with different organic loads [1]. However the types of carbon source and the organic load affect the micro structure, the shape and the size of the granules [2].

To obtain a granular sludge with an excellent settleability it is also important to set a short settling time [3], as this allows to selects only the granules with a fast settling and permit the washout of the solids with slower settling. So it is very important to set the right settling time for the aerobic granulation; Tay et al. [4] demonstrated that mature granules can settle in 1 min.

The granule structure is determined by the hydrodynamic shear force as well. The bacteria secrete more extracellular polysaccharides as the shear force increase and finally the granules structure becomes more strong and more compact [4, 5]. The hydrodynamic behavior depends on the type of reactor used. In an SBR the air and liquid upflow create an homogeneous circular flow where the bacterial aggregates

are constantly subject to an hydraulic attrition. In a completely mixed tank reactor (CMTR), instead, the microbial aggregates are subject to varying shear force and random collision; this lead to flocs formation with irregular shape and size, and the granules only occasionally can form [1, 6]. This is the reason why the aerobic granulation column SBR are chosen.

In comparison with a traditional activated sludge, the aerobic granulation in an SBR presents some advantages:

- stability and flexibility: the SBR can be adapted to fluctuating nutrients load and is also able to treat wastewater with toxic compounds
- higher biomass retention that allow to treat wastewater with very high substrates load
- excellent settleability that enables biological nutrients removal and the secondary settling phase in the same tank with requires less space needed for the construction of the WWTP

Chapter 2

Aims and objectives

This work is dealing with aerobic granular sludge studied in three column sequencing batch reactors for wastewater treatment. The aim was to assess the growth of the granules when the reactors were seeded with activated sludge and using reject water, collected at the Rya WWTP and acetate solutions with different concentrations as carbon source. Also the characteristics of the granular sludge were performed to monitor the volatile suspended solids concentration and the sludge volume index development , settling velocity as well as granule structure by microscopic observation.

Chapter 3

Material and methods

3.1 System description

As show in figure 3.1 the system tested was composed of three column SBR fed with: reject water, a solution of sodium acetate and a solution containing phosphate and micro nutrients.

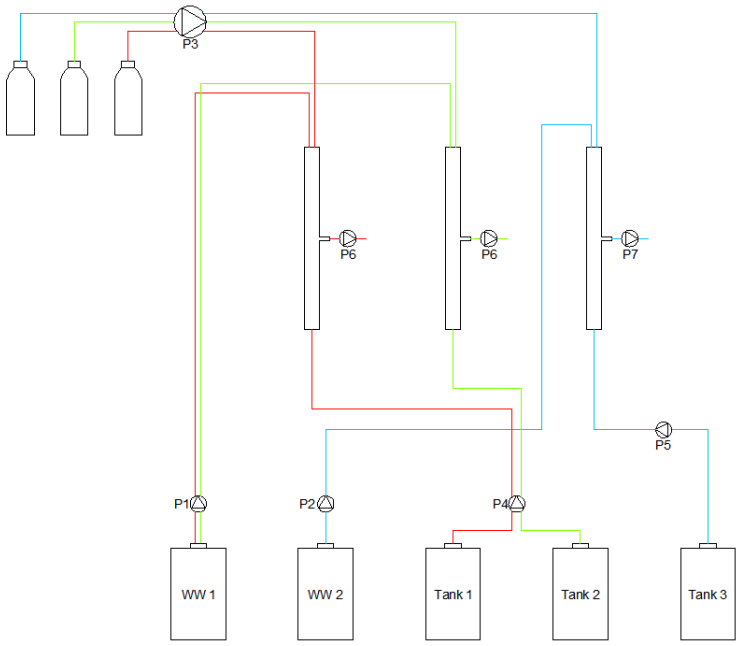


Figure 3.1: System design

The reject water, contained in two vessels, was put in two fridges to prevent nutrient degradation during the storage period. Every week the reject water was substituted with a new batch. Pump 1 fed reactor 1 (R1) and reactor 2 (R2) with reject water contained in vessel 1 and pump 2 fed reactor 3 (R3) with reject water contained in vessel 2.

With pump 3, sodium acetate solution was pumped in the reactors.

Finally phosphate and micro nutrients solution was prepared in 100 l tank. Pump 4 fed R1 and R2 and pump 5 fed R3.

Different conditions were set for the three reactors, preparing different concentration for both the solution, that of acetate and that contained in the 100 l tank and setting different flows for reject water and for phosphates solutions. Furthermore the operational period was divided in two run: during the first run the condition were set as shown in table 3.1a, and during the second run the nitrogen load in R1 and R2 was increased, while in R3 were maintained the same conditions.

Unfortunately because of the variation in reject water concentrations, the expected conditions were not exactly reached in the two runs. In table 3.1 expected and obtained results are reported.

Furthermore it was observed that the pumps were not able to maintain a constant flow. It tend to decrease with the decreasing of the water level in the vessels containing reject water and in the big tanks containing the phosphate and micro nutrients solution.

During second run, the idea was to increase the nitrogen load keeping the first run nutrients ratio, but a mistake in the chemicals concentration calculation was done, so different ratio were obtained because of the low COD concentration that was fed to the reactors. However the goal was to observe what will happen in the reactors after increasing nitrogen load, and the wrong calculation doesn't affect the objective pursuit.

Reactor	COD : N : P	Reactor	COD : N : P
Expected conditions		Obtained Conditions	
1	100 : 5 : 1	1	100 : 6 : 0.9
2	100 : 10 : 1	2	100 : 11 : 1.4
3	100 : 20 : 1	3	100 : 20 : 1.3

(a) During first run

Reactor	COD : N : P	Reactor	COD : N : P
Expected conditions		Obtained Conditions	
1	100 : 22 : 4	1	100 : 38 : 5
2	100 : 60 : 6	2	100 : 97 : 8
3	100 : 20 :1	3	100 : 28 : 1.4

(b) During second run

Table 3.1: Expected and obtained conditions

3.1.1 Reactors

The tested reactors are built in acrylic glass, and they have a total volume of 3.67 l. The inner diameter is d_{in}=0.06 m and the total height is h_r=1.32 m. The air inlet

is place on the reactor bottom and it has a diameter d_{air}=0.04 m; above is placed a circular sparger stone with the diameter d_s=0.05 m. The withdrawal tube is at the height of h_w=0.63 m.

During operational period the reactors were filled with conditions reported in table 3.2.

Reactor	height reached (m)	Volume (l)
1	1.12	3.17
2	1.03	2.91
3	1.09	3.08

(a) First run

Reactor	height reached (m)	Volume (l)
1	1.12	3.17
2	1.12	3.17
3	1.09	3.08

(b) Second run

Table 3.2: Operational reactor levels

To set the pump flows the pump curves were made. For each pump rate the time necessary to fill a volume of 250 ml were measured and then the pump flows in ml/min were calculated. The test were performed with the pipe diameters of 6.4 and 8 mm. Knowing the pumps flow with different pipe diameters and with different pump rates, were chosen the right combination to reach a final operational volume close to 3.05 l. In appendix A the calculation done in order to decide the pipe diameter used and the pump rate set are reported.

The reactors work in cycles. Each cycle take 4 hours to be complete and they are composed by the following phase:

1. Feeding: the reactors are filled with reject water, acetate solution and phosphate and micro nutrients solution. The feeding time is set to 4 minutes

2. Anaerobic phase: it takes 55 minutes

3. Aerobic phase: oxygen is provided with compressed air; the time for this step varies and is increased with the decreasing of settling time, in order to maintain constant the cycle time.

4. Settling: during this phase the sludge is able to settle down to the reactor bottom. The settling time is set at 30 minutes at the beginning of test, and while the granules form it is gradually decreased reaching a final value of 2 minutes.

5. Withdrawal: it takes 4 minutes, and during this final stage the reactors are partially emptied. The exchange ratio was 43%.

3.2 Feeding

3.2.1 Reject water

The reject water used was collected at the Göteborg WWTP and changed every week. In order to diminish the nutrient concentration, the reject was diluted 2 times, so the vessels were filled with 15 l of reject water and 15 l of distilled water. In table 3.3 are reported the nutrient concentration for all the reject water that was fed to the reactors (diluted concentrations) during operational time.

Date	Unfiltered						
	TOC (mg/l)	TN (mg/l)	COD (mg/l)	N-NH_3 (mg/l)	PO_4^{3-} (mg/l)	N-NO^{3-} (mg/l)	N-NO^{2-} (mg/l)
2011-03-13			910	660	5		
2011-03-18			990	560	20.4		
2011-03-25			860	420	17.3	1.7	0.9
2011-04-04			920	640	17.4	2	0.3
2011-04-08	370.6	659.6	620	620	13.8	3.6	0.7
2011-04-15			1000	720	17.8	1.9	0.07
2011-04-22	423.3	739.1	1060	820	5.2	2.8	0.79
2011-04-29	495.7	744	877				
2011-05-06	392.6	720	695	720	17.7	2.2	0.06
2011-05-13	383.7	697.3	679	740	14.1	3	0.22
2011-05-20	458.3	718	811	600	16.3	2.8	0.07
2011-05-27	230.7	621.6	408	580	11.6	2.7	0.05
2011-06-03	237.5	680	420	520	15.2	3.6	1.44
2011-06-10	368.1	689.3	652	660	19.5	4.1	0.17
2011-06-13	372.3	746.4	659	660	18.4	5.2	0.09
2011-06-17	388	701	687	740	15.3	3.3	0.37
2011-06-23	494.5	706.9	875	1000	17.8	5	0.12
2011-06-29	380.1	651.6	673	1200	18.6	2.8	0.73
2011-07-06	440.5	742.5	780	780	13.5	3.5	0.27

Table 3.3: Diluted reject water composition

3.2.2 Solutions

To ensure enough COD concentration in all reactors, three different sodium acetate solutions were prepared. The solutions were putted in bottles with a total volume of 2l. A little amount of hydrochloric acid was put in the bottles in order to reach a solution pH equal to 5. This conditions avoid a too high pH value into the reac-

tors. The acetate solutions concentration was calculated to reach the nutrient ratio described in table 3.1, taking in account the reject COD concentration too.

In the same manner the phosphate and micro nutrients concentration were calculated since the aim was to get the same concentration of the ions Mg, Ca, K and Fe in the reactors. These solutions was put in tank with a total volume of 100 l, and the chemicals added are the following:

- potassium phosphate dibasic
- magnesium sulfate hepta-hydrate
- calcium chloride
- iron sulfate hepta-hydrate

Reactor	Mass (g)	Conc. (g/l)	COD (g/l)
1	160	80	62.4
2	111	55.5	43.3
3	119	59.5	46.4

(a) First Run

Reactor	Mass (g)	Conc. (g/l)	COD (g/l)
1	97	48.5	37.9
2	9.5	4.75	3.8

(b) Second Run

Table 3.4: Acetate solution

Compound	R1 (mg/l)	R2 (mg/l)	R3 (mg/l)
$MgSO_4 \cdot 7H_2O$	13.8	18.8	21.5
$CaCl_2$	16.5	22.5	25.8
$FeSO_4 \cdot 7H_2O$	11	15.0	17.2
K_2HPO_4	88	82.0	101

(a) First Run

Compound	R1 (mg/l)	R2 (mg/l)
$MgSO_4 \cdot 7H_2O$	20	27.8
$CaCl_2$	24	33.3
$FeSO_4 \cdot 7H_2O$	16.1	22
K_2HPO_4	455	440

(b) Second Run

Table 3.5: Tank Concentrations

In appendix B are explained the calculation done to decide the quantity of chemicals to add in each reactor every cycle.

3.2.3 Influent nutrient concentration

The reactor influent COD were calculated in the following way:

$$COD_{in} = \frac{Ft}{V_o} \cdot (Q_r \cdot COD_r + Q_{Ac} \cdot COD_{Ac}) + 0.57 \cdot COD_p \tag{3.1}$$

where

- Ft: reactor feeding time (min)
- V_o: operative reactor volume equal to 3.05 l
- Q_r: reject flow (ml/min)
- COD_r: reject water COD concentration (mgCOD/l)
- Q_{Ac}: acetate flow (ml/min)
- COD_{Ac}: Sodium acetate solution COD concentration (mgCOD/l)
- COD_p: COD concentration left in the reactor from the previous cycle (mgCOD/l)

The final part of the formula takes into account the COD concentration left in the reactor after the withdrawal. In fact during this stage, only 43% of reactors total volume was washed out, while 57% remain inside.

The formula used to calculate influent ammonia, nitrate, nitrite and phosphate have the same form.

3.3 Analysis

Different analysis were made to evaluate: the nutrient removal efficiency, the sludge concentration, the SVI and the bacteria activity. The analysis performed:

- Effluent analysis
- Sludge Analysis
- Test on Specific Oxygen Uptake Rate (SOUR)
- Cycle analysis
- pH and redox potential measurements
- dissolved oxygen measurement

3.3.1 Effluent analysis

With these analyses the nutrient concentration in the effluent were measured, to be able to calculate the removal efficiency for all the three reactors.

The analysis were performed on filtered sample with a colorimetric method using the Hach portable data logging spectrophotometer DR/2010 at a wavelength of 890 nm. For each parameters different sample dilution was prepared, in order to reach the machine reading range.

The tests were made on filtered samples. To filter the sample a vacuum filtration installation and filter papers with a porosity of 0.45 μm were used.

3.3.1.1 COD content

To measure the COD 2 ml of sample was placed in a small tube containing a digestion solution of sulfuric acid and mercury. The methods is applicable to sample with a COD concentration in a range of 0 - 150 mg/l. After the tube was boiled two hours at 150°C and cooled it was analyzed with Hach machine.

3.3.1.2 Nitrogen in form of ammonia

To measure the N-NH_3 a two step reaction was performed. First 10 ml of sample react with ammonium salicylate and then with ammonium cyanurate. The change in the sample color, from yellow to green, indicates the presence of ammonia in the sample. After the reaction occurred, the concentration of ammonia nitrogen was measured with Hach machine. For this parameter a blank was prepared with deionized water.

3.3.1.3 Nitrogen in form of nitrate and nitrite

The measurements on nitrates was made by adding Nitraver nitrate reagent in a tube containing 10 ml of sample. The blank was prepared with 10 ml of the same sample without adding reagent. After the reaction occurred, the presence of nitrate was indicated by an orange color in the sample.

The same procedure is followed for nitrogen in form of nitrite with the difference that Nitriver nitrite reagent was used. The presence of nitrite in the sample was indicated by a pink color.

3.3.1.4 Phosphate

The phosphate analysis was performed adding Phos Ver 3 reagent to 10 ml of sample. The blank was prepared with 10 ml of the same sample without adding reagent. After the reaction occurred, with the presence of phosphate the sample assumes a blue coloration.

3.3.2 Sludge analysis

The aim of this analysis is to measure the sludge volatile suspended solids (VSS) concentration to be able to calculate the SVI and to measure the granules settling velocity.

The VSS concentration is measured to monitor the formation of new biomass: it was expected to have an increase in the solids concentration during the time. The VSS concentration is measured applying the standard methods [7]. In a small cup a filter paper was placed. After weighting 5ml of sludge was filtered, and then it was dried in oven at 105°C to measure the mixed liquor suspended solids (MLSS). Finally the filter paper was placed in the furnace at 500°C to obtain the mass of volatile solids in 5ml of sludge. The VSS concentration is obtained dividing the measured solids mass with the sample volume.

To calculate the SVI, firstly 100ml of sludge was put in a graded cylinder and then I measured the volume of sludge settled in 30 minutes. By dividing the obtained value with the VSS, the sludge volume index (SVI) was calculated.

An example on how to calculated the MLSS, VSS concentrations and the SVI is reported in Table 3.6.

Mass Cup + Filter paper (g)	Mass Cup + Filter paper after 105°C (g)	Mass Cup + Filter paper after500°C (g)	Sample volume (ml)	Sludge volume after 30 min settling (ml/100ml)
27.7361	27.74367	27.71809	5	22

$$MLSS = \frac{27.74367\text{g} - 27.7361\text{g}}{5\text{ml}} \cdot 1000^{\text{ml}}/_{\text{l}} = 1.514^{\text{g}}/_{\text{l}}$$

$$VSS = \frac{27.74367\text{g} - 27.73809\text{g}}{5\text{ml}} \cdot 1000^{\text{ml}}/_{\text{l}} = 1.116^{\text{g}}/_{\text{l}}$$

$$SVI = \frac{220^{\text{ml}}/_{\text{l}}}{1.116^{\text{g}}/_{\text{l}}} = 197^{\text{ml}}/_{\text{g}}$$

To measure the settling velocity, from a sludge sample one granules was carefully picked. First the diameter was measured by a caliber and then the granule was put in a graded cylinder filled with water of ambient temperature. As the granule is in the water it starts to settle down, and then to calculate the settling velocity the time needed to travel through a known distance is measured. The ratio between the known distance and the time represent the settling velocity.

3.3.3 Specific Oxygen Uptake Rate (SOUR)

This test was performed to check the activity of the heterotrophic and nitrifying bacteria, according to the method described by Surmacz-Gorska et all. [8]

In a bottle 250ml of reject water and a known volume of granule were placed. Then with an oxygen meter the dissolved oxygen concentration was measured every 15 seconds. After the oxygen concentration was diminished of 2 mg/l, in the bottle 100 μl of allylthiourea solution was added and the dissolved oxygen concentration was continued to be registered every 15 seconds.

The slope of the lines obtained plotting the oxygen concentrations against the time in minutes, represents the oxygen uptake rate, i.e. the rate at which the oxygen is consumed by bacteria.

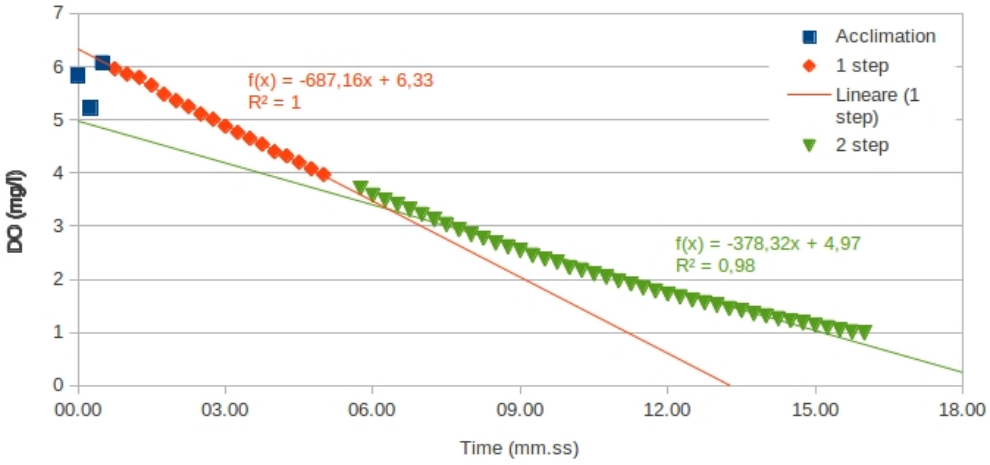


Figure 3.2: Plot example obtained with the OUR test (ATU added at the beginning of step 2).

The plot shows two different lines: the orange represents the oxygen consumption due to heterotrophs and nitrifiers, the green instead represents the consumption due only to heterotrophs. By measuring the VSS concentration in the bottle, and dividing this value with the OUR, it is possible to calculate the Specific oxygen uptake rate (SOUR).

The first analysis was made by adding 30 ml of suspended granules into 300 ml of reject water, but the results obtained did not show any difference between the first step, where both heterotrophs and nitrifiers work, and the second step where nitrifiers were inhibited. So the problem could due by two reasons:

1. the allylthiourea was not able to inhibit the nitrifiers, for the lower concentration or because it was too old

2. the granules concentration was too low, and the nitrifiers activity was not appreciable if compared to the heterotrophic (OHOs) activity

To solve the problem, first an OUR analysis was made with a synthetic wastewater, in order to understand if allylthiourea solution work well, and then the OUR analysis was performed by increasing the granules concentration in the bottle.

3.3.4 Cycle analysis

The cycle analysis was made to verify that all the nutrient degradation processes took place during the aerobic and anaerobic stage. During the anaerobic stage, from each reactor were collected samples every 10 minutes, instead during the aerobic phase, the samples were collected every 15 minutes.

The nutrients concentration were measured to observe their variation, in the reactors, during the time.

3.3.5 DO, pH and redox potential measurements

pH and redox potential can be used as a tool to understand the processes going on in the reactors. The pH variation is due mainly to organic matter degradation, nitrification and denitrification.

During the anaerobic phase, the fermentation processes are responsible for the more complex organic matter conversion, into alcohol and acetic acid. In this step some H^+ ions are released, leading to a lower pH values on the contrary, denitrification produces alkalinity leading to increased pH.

During aerobic phase, nitrification alkalinity consumption decrease pH values, in fact, as happens during anaerobic degradation of organic compounds, some hydrogen ions are produced. Some studies [9, 10] show that in this stage, it is possible to have a pH decreasing until reaching a minimum called "ammonia valley", corresponding to the point where ammonia conversion into nitrite and nitrate is stopped. After this point the pH increase thanks to the phosphorous uptake and to CO_2 stripping.

The oxidation-reduction potential (ORP) estimates if an environment has a reductive or an oxidant behavior. This parameter is used to observe if nitrification and denitrification processes take place in the reactor. In fact nitrification is responsible for the nitrate and nitrite accumulation inside the reactors, these compounds raise ORP because they release bound oxygen. On the contrary denitrification lower the water redox potential, consuming nitrate and nitrite.

However ORP is a powerful tool only during the anaerobic phase; in fact during aerobic phase it is obvious there is an ORP increment: the oxygen provided creates a more oxidative ambient.

ORP, pH and DO measurements were done by putting three different electrodes, one for each parameter, inside the reactors. The measurements were done only few centimeter below the water top level. As the sludge settled during anaerobic phase,

the reactions took place in the bottom of the reactor, therefore the actual values may not have been measured due to incomplete mixing of the reactor contents.

Chapter 4

Results and discussion

Granules start to appear in all three reactors after one month from the reactors startup. They continue to growth during all the experimental period. Figure 4.1 depicts the average diameter measured in the three reactors. R1 show granules with diameter varying between 1mm and 8 mm. From the microscopic investigation the granules appear to have regular spherical shape (figure 4.2). The bigger granular show a slight irregular boundary. In R2 the granules exhibit a diameter varying between 1 and 6 mm and a more spherical shape and regular boundary than R1. R3 show the biggest granules, their diameter vary between 4 to 14 mm and they have not a uniform spherical shape, furthermore the boundary is not regular.

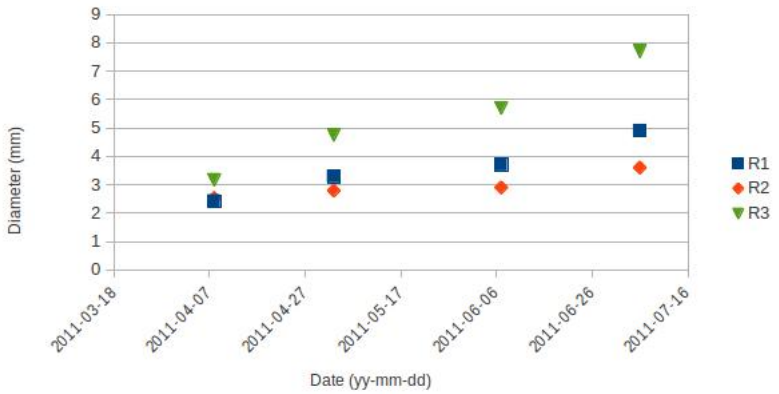
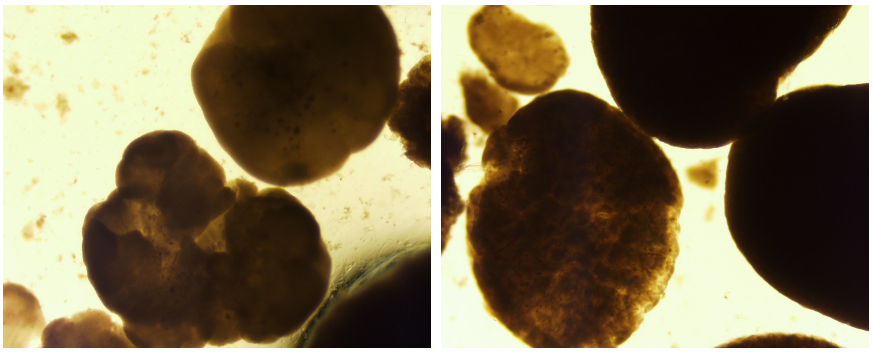
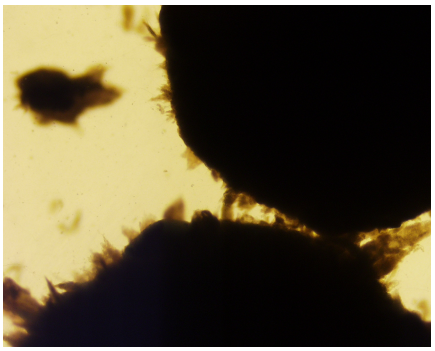


Figure 4.1: Granule dimension

After observing the granular sludge growth, the different analyses were started as described before. The obtained results are reported below.



(a) R1 (b) R2



(c) R3

Figure 4.2: Granules Picture

4.1 Effluent analysis

4.1.1 COD

As show in figure 4.3, 4.4 and 4.5 COD is removed from the beginning of the operational time in each reactor. The gray line in the plots divide the results obtained in the first run, from the results obtained in the second run.

In R1 the removal efficiency remains constant during all the operational time, around a value of 80%; in R2 the removed COD increase during the time and become constant after one month from the reactor start up reaching a value close to 100%; in R3 at the beginning the removal efficiency is not stable and it varies a lot, it become stable after three months around a value of 60%.

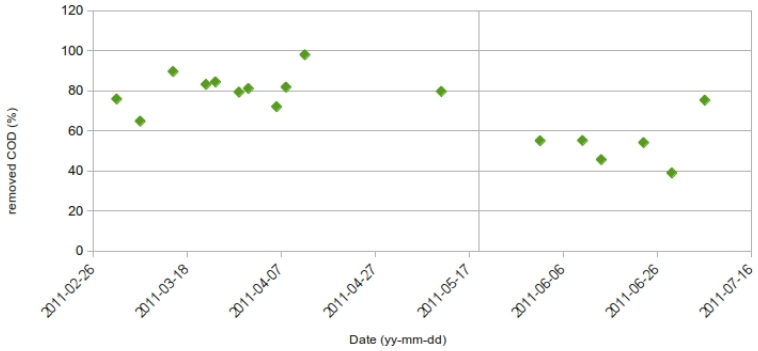


Figure 4.3: COD removal efficiency in R1

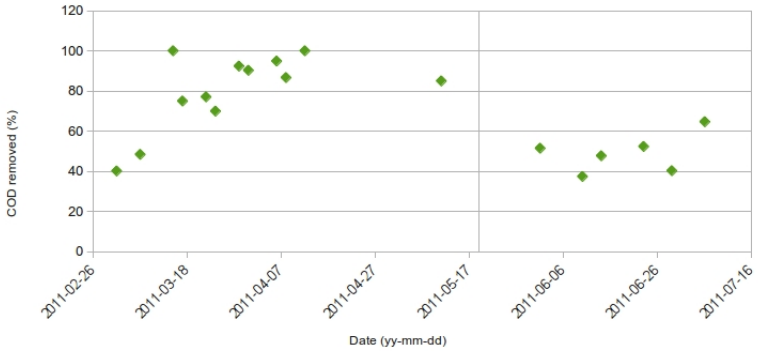


Figure 4.4: COD removal efficiency in R2

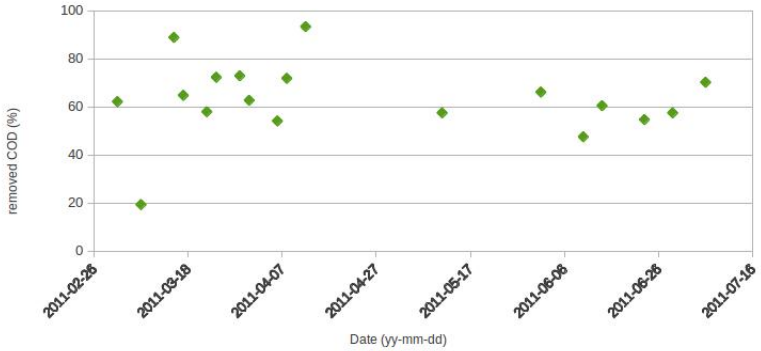


Figure 4.5: COD removal efficiency in R3

Figure 4.6, 4.7 and 4.8 compares the influent and effluent COD in all three

reactors. It is interesting to notice that increasing the influent COD in the first two reactors, the effluent COD remains stable below the 200 mg/l in R1 and around 100 mg/l in R2. The increase in COD load is due to a mistake preparing the acetate solution, so a greater quantity of readily biodegradable COD entered in the reactors, that it is easily to degrade for the heterotrophs. This explain why with an higher COD load, the effluent concentration remain constant. In R3 the effluent COD concentration is less stable, if compared to the other two reactors, however the average effluent COD it is around 300 mg/l.

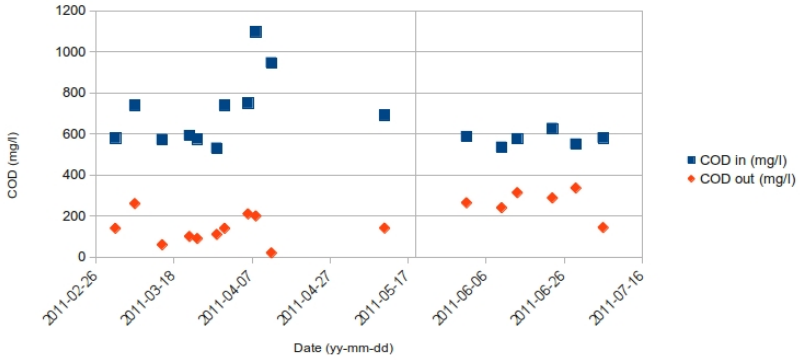


Figure 4.6: Influent and effluent COD in R1

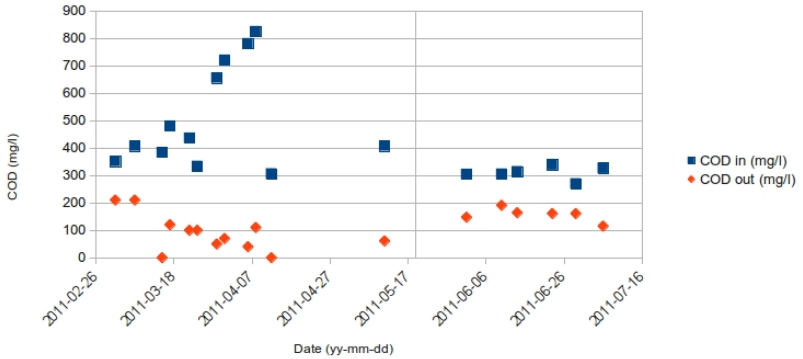


Figure 4.7: Influent and effluent COD in R2

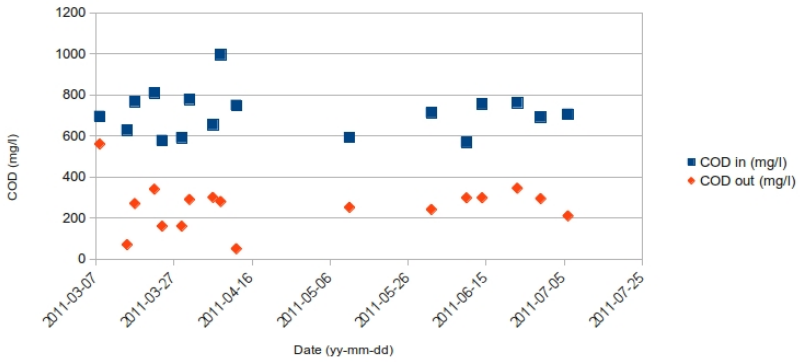


Figure 4.8: Influent and effluent COD in R3

After changing the conditions in R1 and R2 the removal efficiency decreased a bit; it was around 50% for both reactors. Increasing the ammonia load, lead to increase of the reject water flows and to keep the COD concentration constant, the acetate concentration were decreased. So in the second part of the test the readily biodegradable COD is less than in the first part, and this is the reason for the decreased COD removal efficiency. From figure 4.6 and 4.8 it is possible to see that the effluent COD concentration increased: the values are 250 mg/l and 150 mg/l for R1 and R2 respectively.

4.1.2 Nitrogen

4.1.2.1 Reactor 1

Looking at figure 4.9 it seems that ammonia removal starts from the beginning of the operational time, firstly with a very low efficiency and then with an higher one.

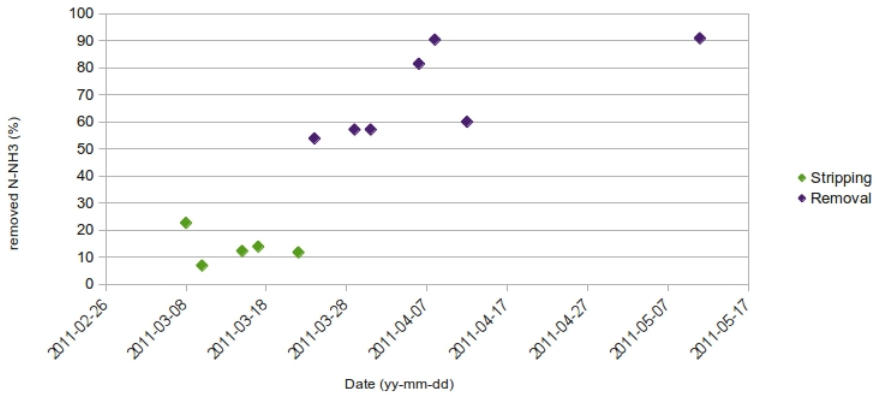


Figure 4.9: Ammonia removal efficiency for R1

As explained in equations 1.3 and 1.4 an increase in nitrate and nitrite concentration was expected if nitrification process take place. Figures 4.10 and 4.11 show that in the first 20 days, the effluent nitrate and nitrite concentrations are constant during the time and very close to 0 mg/l.

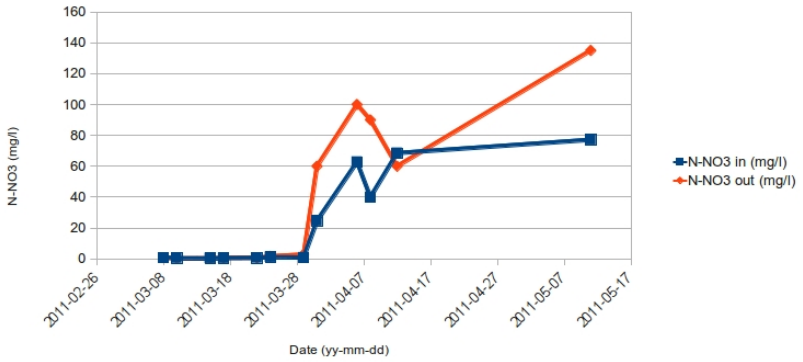


Figure 4.10: Influent and effluent nitrate concentration in R1

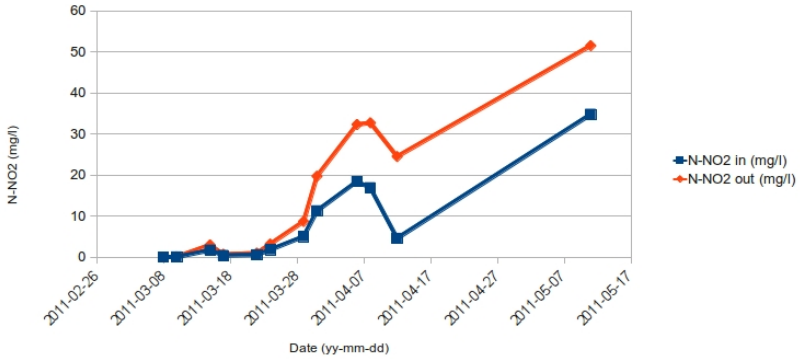


Figure 4.11: Influent and effluent nitrite concentration in R1

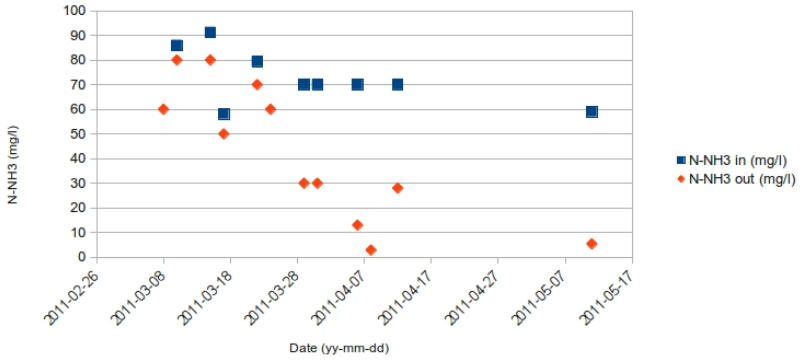


Figure 4.12: Influent ammoniacal nitrogen vs effluent ammoniacal nitrogen in R1

The nitrification process starts on 24th of March when nitrite concentration starts to increase. But only the first nitrification step take place, the nitrate concentration in fact remains at a value of 0 mg/l. The nitrite conversion to nitrate starts on the 31st of March, figure 4.10 show that from this day the nitrate concentration starts to increase. However the oxidation rate of ammonia is greater than nitrite oxidation rate, as demonstrated by the nitrite accumulation in the reactor. This is due to the greater sensitiveness of Nitrobacter than Nitrosomonas. According to Yang at all. [11], the free ammonia inhibition threshold is 10-150 mg/l for Nitrosomonas and 0.1-4 mg/l for Nitrobacter; in this case the threshold are different because the ammonia conversion to nitrite starts with a concentration of ammonia equal to 85 mg/l, and the nitrite oxidation starts with a ammonia concentration equal to 36 mg/l. In any case, is confirmed the greater sensitiveness of Nitrobacter. These values were obtained calculating the free ammonia concentration starting by the ammoniacal

nitrogen concentration measured, as descripted in apendix D. Ammonia removal efficiency increase over the time and reaches value around 90% with an effluent concentration less than 12 mg/l, as show in figure 4.12. The initial ammonia removal is not due to the process of nitrification, but to the ammonia stripping during the aeration phase and to nitrogen utilization for the new biomass growth. Figure 4.10 and 4.11 show that from 6th of April both nitrite and nitrate concentration starts to decrease. The nitrite decrease is due to the nitrite oxidation into nitrate, instead the nitrate decrease which suggest that denitrification take place too; but in the last analysis they increase again. There can be two reasons to this: or denitrification and nitrite oxidation is stopped by some poisonous substances present in the reject water used in the last analysis or the initial decreasing of both the parameters were a results of the lower influent concentration.

Increasing the ammonia load nitrification continues to go on; as shown in figure 4.13 the effluent ammonia concentration is in the range of 50 - 100 mg/l and the removal efficiency increase over the time, passing from an initial value of 30% to a final value of 90%.

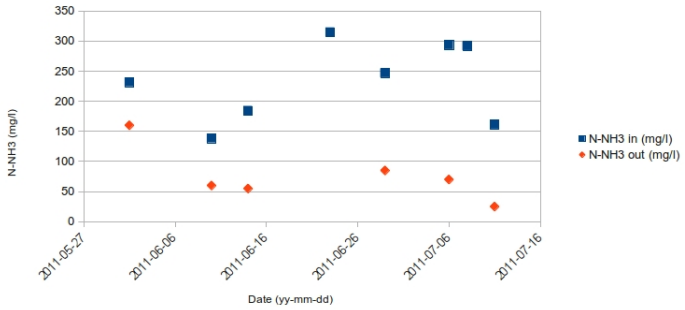


Figure 4.13: Influent and effluent ammoniacal nitrogen in R1 during second run

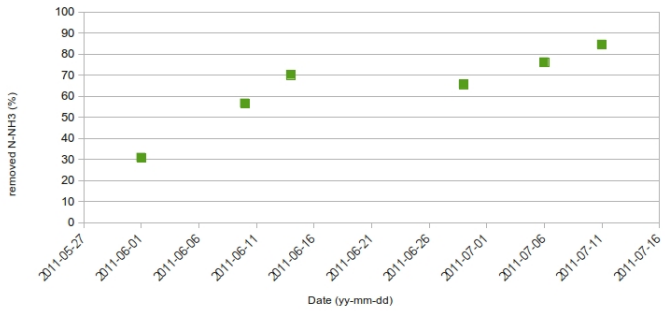


Figure 4.14: Ammonia removal efficiency in R1 during second run

As results of this process, nitrite concentration initially increase reaching the values of 300 mg/l and then decrease reaching the minimum value of 60 mg/l, indicating that also the nitrite oxidation take place. In the last analysis the effluent nitrite concentration increase again, the reason is that the influent concentration increases. The same fate is observed for nitrate: an initial increasing followed by a decreasing, so denitrification occurs, and then again an increasing, due to the greater influent concentration.

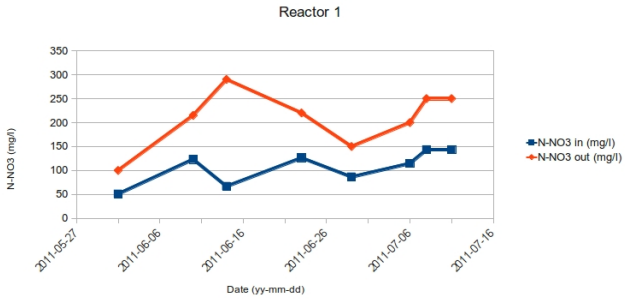


Figure 4.15: Influent and effluent nitrate concentration in R1 during second run

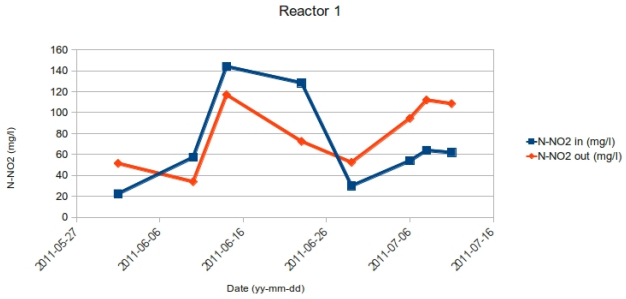


Figure 4.16: Influent and effluent nitrite concentration in R1 during second run

During the second run the total nitrogen was analyzed. As said before the total nitrogen is the sum of the organic nitrogen, and the nitrogen in form of ammonia, nitrate and nitrite. The organic nitrogen concentration is neglected because usually organic nitrogen concentration is converted into ammonia before arriving at the wastewater treatment plant. So the formula for total nitrogen become:

$$TN = N - NH_3 + N - NH_4^+ + N - NO_3^- + N - NO_2^- \tag{4.1}$$

If only nitrification take place the ratio between influent and effluent total nitrogen is equal to 1, because the total load of nitrogen doesn't decrease, but there is only the conversion of the ammonia nitrogen into nitrate and nitrite. On the contrary, if

denitrification take place too, the ratio must increase reaching values greater than 1, because the nitrogen concentration decrease, due to the conversion of nitrate in nitrogen gas that escape from the reactor.

Figure 4.17 show the TN ratio over the time during the operational time after increasing the load of ammonia.

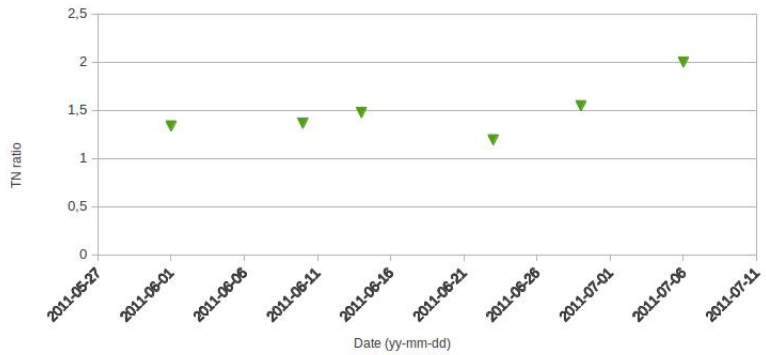


Figure 4.17: TN ratio in R1 during second run

It can be notice that while the concentration of nitrate and nitrite increase, so only the nitrification take place in the reactor, the ratio value is very close to 1, and after that the nitrate concentration decrease and the ratio value start to increase too, and it takes values greater than 1, as expected.

4.1.2.2 Reactor 2

In the beginning nitrification does not take place in R2 and a small amount of ammonia is removed due to the stripping during the aeration phase and to the consumption of nitrogen for the biomass growth. The ammonia starts to be converted in nitrite from the 22nd of March. The conclusion is supported by the percentage of ammonia removed that, as show in figure 4.19, starts to increase reaching values very close to 100% and the effluent ammoniacal nitrogen concentration that decrease, reaching values very close to zero.

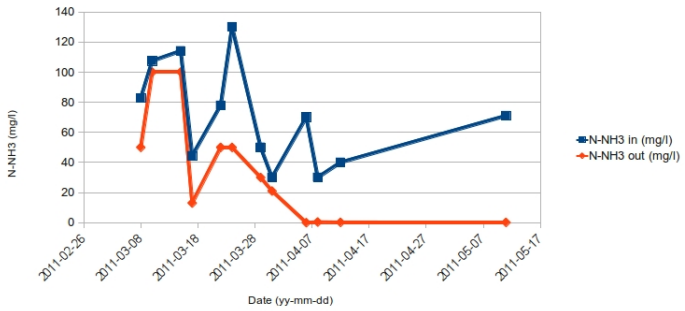


Figure 4.18: Influent and effluent ammoniacal nitrogen in R2

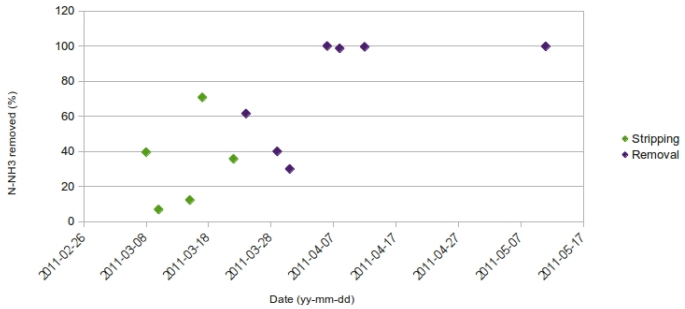


Figure 4.19: Ammonia removal efficiency for R2

With the beginning of nitrification, nitrite concentration increase passing from values close to 0 mg/l to 16 mg/l. The rising trend, indicates that till the 29$^{\text{th}}$ of March, the nitrite oxidation is not complete, the process rate increase after this date, as demonstrated by figure 4.21, were it is possible to observe the decrease in nitrite concentration that reach values lower than 3 mg/l in the last analysis. The same fate is observed for the nitrate. As happens for R1, denitrification take place with free ammonia concentration lower than 36 mg/l.

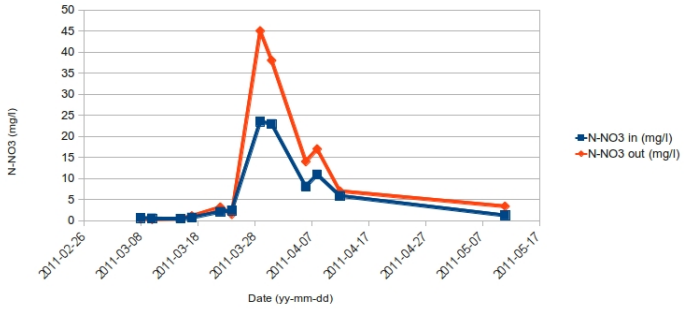


Figure 4.20: Influent and effluent nitrate in R2

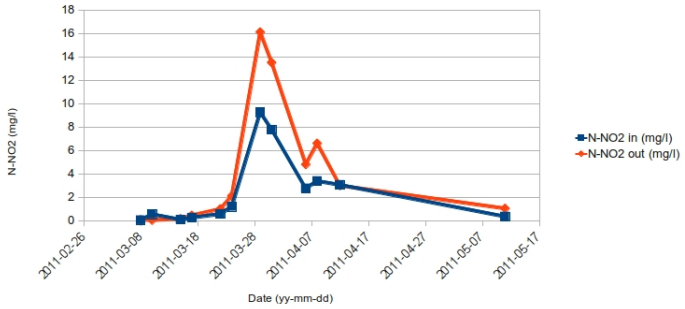


Figure 4.21: Influent and effluent nitrite in R2

By increasing the ammonia load, at first there is an increase in effluent ammoniacal nitrogen concentration, but after it starts to decrease, reaching a final value below 50 mg/l. The bacteria take a bit more than 10 days to acclimate on the new condition. The ammonia removal efficiency increase over the time, to become stable for some days and then increase again reaching final values in the range of 70 - 80%.

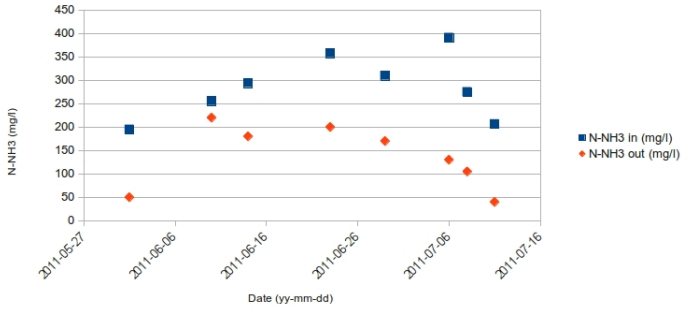


Figure 4.22: Influent vs effluent ammoniacal nitrogen during second run

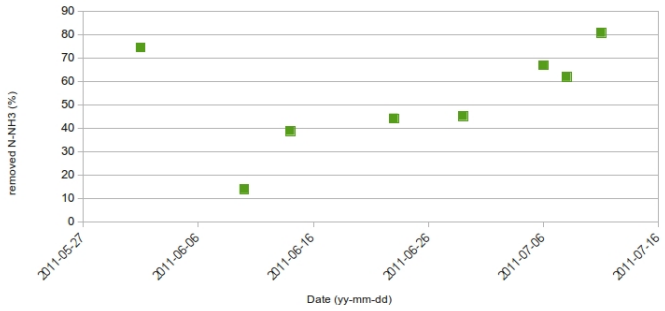


Figure 4.23: Ammonia removal efficiency in R2 during second run

As expected with the decrease in ammonia effluent concentration there is an increase in nitrate and nitrite concentration followed by a decrease. So the situation does not change and both nitrification and denitrification take place inside the reactor.

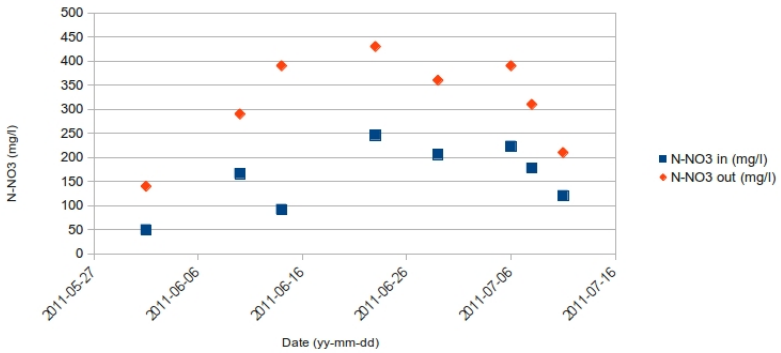


Figure 4.24: Influent and effluent nitrate concentration in R2 during second run

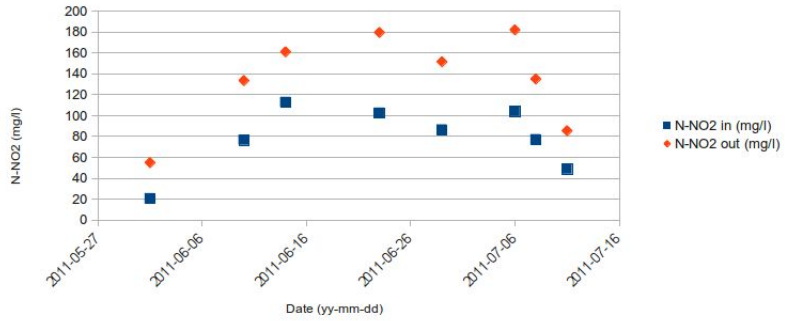


Figure 4.25: Influent and effluent nitrite concentration in R2 during second run

The conclusions are supported by the ratio between the influent and effluent total nitrogen: that pass from values close to 1 to values greater than 1, demonstrating a decrease in nitrogen load in the effluent.

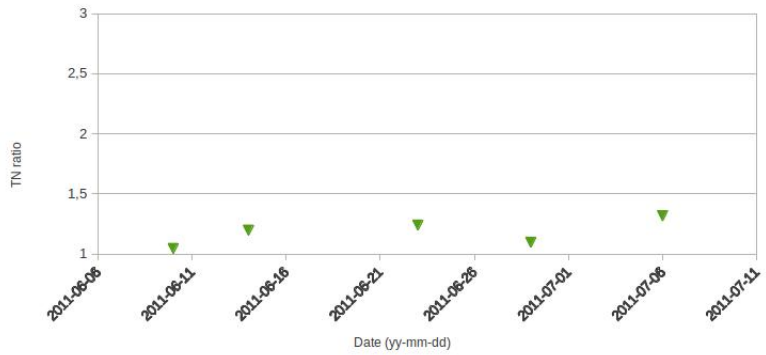


Figure 4.26: TN ratio in R2 during second run

4.1.2.3 Reactor 3

From the beginning R3 worked with a high ammonia load. As shown in figure 4.27 the nitrification and denitrification process take more time to start compared to the other two reactors.

Nitrification starts at the beginning of May this which is demonstrated by the increasing in nitrate and nitrite effluent concentration (figure 4.28 and 4.29 show both nitrate and nitrite curves). From the collected data the percentage of removed ammonia does not increase when the nitrification process starts, but it remain around a value of 20%, only at the end of the operational time a removal percentage greater than 50% is registered. Probably this trend is due to the high ammonia load, that

inhibits the nitrifiers which needs some more time to the acclimation. However even if the process starts, the level on effluent ammoniacal nitrogen remains high, with an average value greater than 150 mg/l.

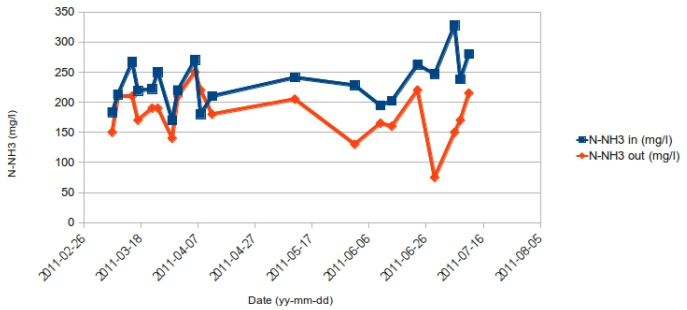


Figure 4.27: Influent vs effluent ammoniacal nitrogen in R3

Denitrification instead, starts the 6$^{\text{th}}$ of July as demonstrated by the figure 4.28, where it can be notice the decreasing on influent and effluent nitrate. The nitrite trend are similar in exception for the concentrations that are a bit loer (figure 4.29). The decreasing of nitrogen load, due to the process of denitrification, is demonstrated by the ratio between the effluent and influent total nitrogen, that increase reaching final values greater than one.

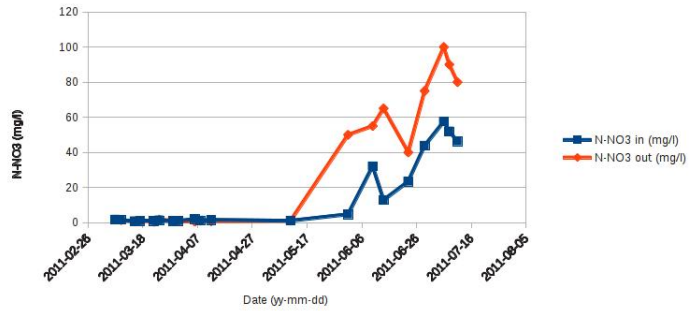


Figure 4.28: Influent and effluent nitrate in R3

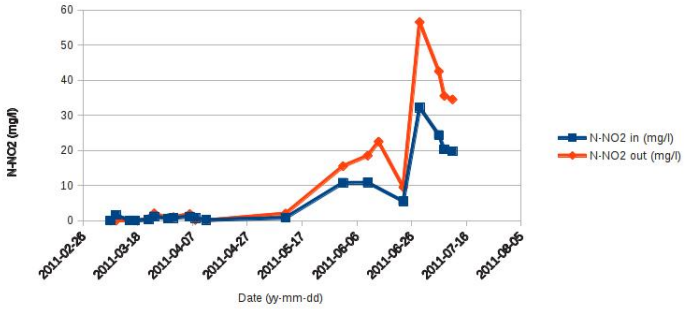


Figure 4.29: Influent and effluent nitrite in R3

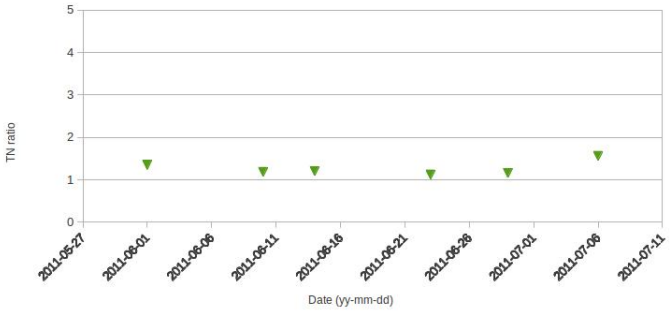


Figure 4.30: Total nitrogen ratio on R3

4.1.3 Phosphate

In R1 the phosphate removal efficiency is not greater than 20%, in exception for the analysis performed between the 7th and the 27th of April. According to P.H. Jones et al.[12] increasing the COD load, leads to a greater release of phosphate, by PAOs, during the anaerobic phase and a greater uptake during the aerobic phase. The increase of the phosphorous removal is due to the increase on the COD influent concentration due to a mistake preparing the acetate solution.

The same happen for R2, increasing the COD load increase the phosphorous removal, but in R2, it can be notice that the values obtained are less stable than in R1 (figure 4.32).

Finally in R3 the removed percentage of phosphorous is more stable, around a value of 50%. Probably the more stable trend in R3 is due to the higher COD load. In fact for this reactor the average influent COD concentration is 700 mg/l, in the other two reactors it is a bit lower: 600 mg/l for R1 and 400 mg/l for R2.

However in all three reactors a little phosphorous removal is observed.

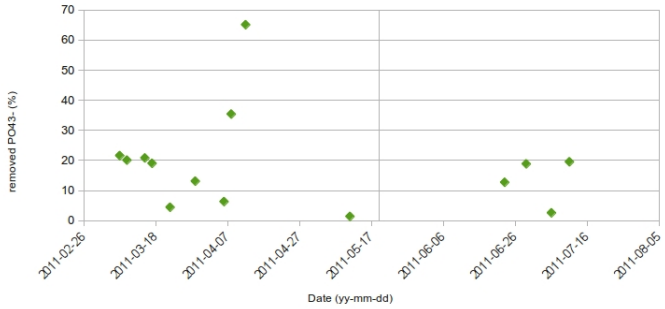


Figure 4.31: Phosphate removal efficiency in R1

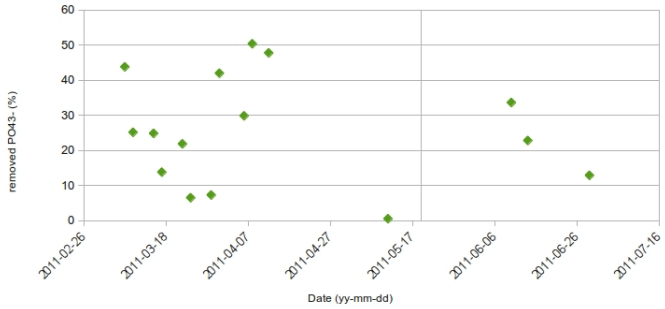


Figure 4.32: Phosphate removal efficiency in R2

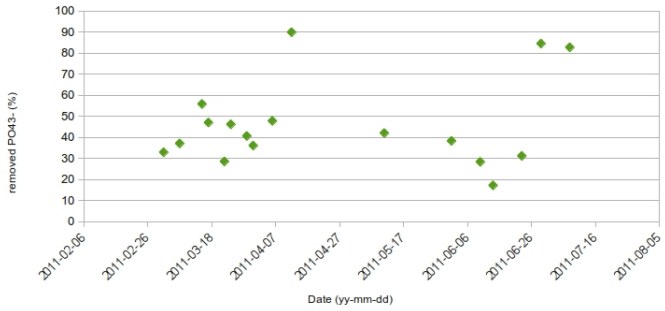


Figure 4.33: Phosphate removal efficiency in R3

4.1.4 Effluent analysis summary

From the results obtained by the effluent analysis, it can be said that COD and phosphate biological removal occur from the beginning of the analysis. Concerning

the COD removal in the first operational period, R1 and R2 have a good efficiency near the 100%, whereas R3 seems to have an efficiency of 60%, furthermore reached in a longer period respect the other two reactors. Increasing the ammonia load, the efficiency of R1 and R2 decrease to around a value of 50%, this can be due to the higher ammonia levels in reactors that is a toxic compound for the bacteria. So probably a certain amount of heterotrophs were inhibited.

Concerning the phosphate, the best reactor is R3, in fact it performed the best with a removal efficiency of more than 50% and in more stable manner. R1 and R2 however perform some phosphorous biological removal but lower compared to R3.

Nitrification take more time to start, probably due to a greater sensitivity of nitrifying bacteria. However with the low ammonia levels R1 and R2 performed better a good ammonia removal than in R3 that from the beginning had a greater ammonia load, the removal efficiency is very low. An interesting increase is observed only at the end of the operational time. Increasing the ammonia load the nitrification process continues to going on but R1 has a greater ammonia removal then R2. Probably this is can be due to the greater biomass decreasing in R2 respect in R1, and to the higher ammonia load in R2 during the second run.

Denitrification take place in all three reactors. It is reasonable that denitrification take more time to take place compared to the other processes, because the nitrate concentration at the beginning was close to 0 mg/l in each reactor. Increasing the load, nitrification, starts in R1 too.

Finally, it is interesting that in R1 and R2 only with ammonia concentration lower than 30 mg/l, denitrification take place and the rate of nitrite oxidation increase. It should be taken as inhibition levels, but by increasing the ammonia load, the two process continue to work. It can be concluded that the concentration of 36 mg/l can be inhibiting threshold to the nitrification process; after the processes has started, the nitrifyers and denitrifyers are able to continue even if the ammonia concentration is greater than 36 mg/l.

4.2 Sludge analysis

4.2.1 Volatile Suspended Solids

The VSS concentration increase in all three reactors from the beginning of the operational time till the 12^{th} of April. After this date the oxygen uptake rate analysis was started by removing a great quantity of sludge from the reactors.

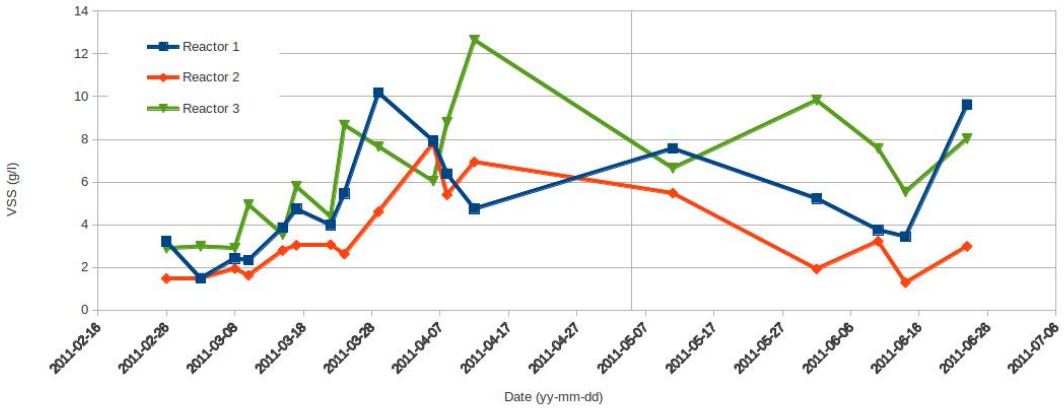


Figure 4.34: VSS concentration

In the first part the VSS concentration increase, indicating that new biomass forms in the reactors. It was expected to have an initial increase in the solids concentration, and then a stabilization around a certain value. However the expectations were not confirmed by the data, due to the sludge removal necessary for the oxygen uptake analysis. This explains the decreasing sludge concentration shown in figure 4.34.

A strange trend is observed in all three reactors, in fact the VSS concentration start to decreasing from the 29^{th} and 24^{th} of March and from the 6^{th} of April respectively for R1, R3 and R2. On 24^{th} of March a bit more was started to be removed from the reactors to perform the OUR analysis, decreasing the sludge age and giving less biomass concentration in both reactors.

The results show that granular sludge is able to ensure higher biomass concentration, in fact before starting to remove a lot of sludge from reactors to perform the OUR test, the VSS concentration reached in the reactors were: 10 g/l, 12 g/l and 8 g/l for R1, R2 and R3 respectively. The results are similar to those obtained by Arrojo et al. and Liu et al.[13, 14].

4.2.2 Sludge Volume Index

As said before the granular sludge has a better settleability than the more traditional activated sludge. This characteristic is explained by the SVI. In figure 4.35 the SVI values calculated during the operational time are reported.

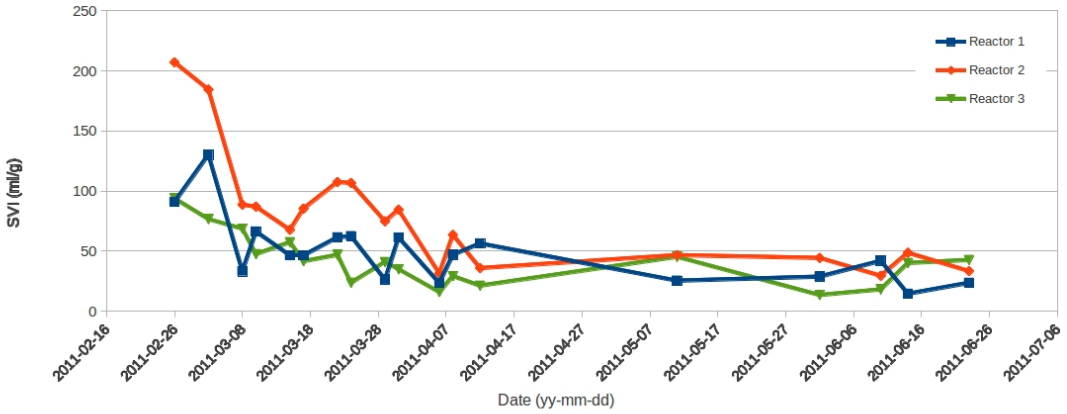


Figure 4.35: Sludge volume index

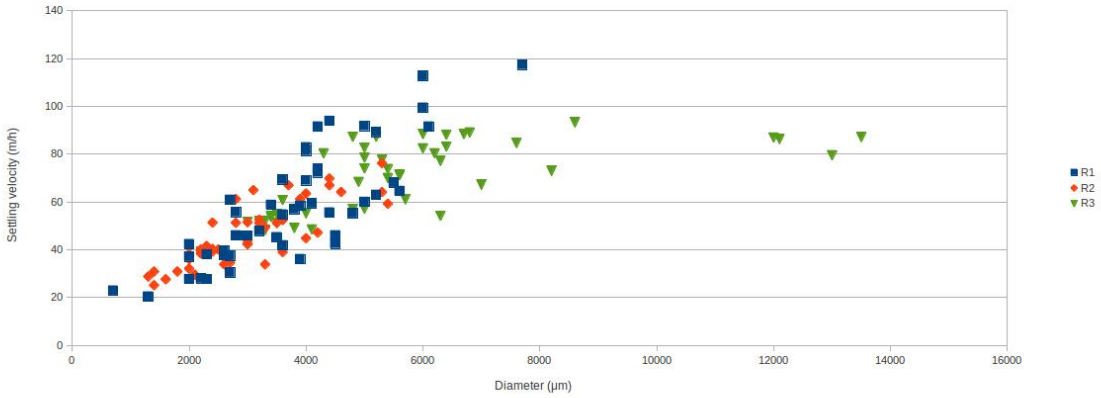


Figure 4.36: Settling Velocity

The SVI values decrease in the first two months and then stabilize at values below the 50 ml/g for all three reactors, demonstrating the better settleability of granular sludge. The obtained values were compared with some other works it was noticed the the obtained results were similar [13, 14, 16, 17].

4.2.3 Settling velocity

Different studies shown that granules' settling velocity varies in a wide range, usually greater than 30 - 70 m/h [15]. The settling velocity measured in this study varies in the range between 20 - 120 m/h, 25 - 80 m/h and 60 - 90 m/h in R1, R2 and R3, respectively. Figure 4.36 show the measured settling velocity as function of granules diameter.

The settling velocity measured are greater than average settling velocity of bioflocs activated sludge (usually less than 10 m/h), demonstrating that granular sludge can settle in shorter time. The settling velocity appear to be reduced at very large diameters in R3. They were observed to be hollow.

4.3 Oxygen uptake rate test

As anticipated in section 3.3 at the beginning there were some problems with this test; the results did not show any difference between the two steps (with and without allylthiourea added). To understand if the problem was due to the allylthiourea solution, a test performed with a synthetic wastewater was made. The synthetic water composition is summarized in table 4.1. The compounds concentration were set to ensure a C:N:P ratio equal to 100:5:1.

Compound	Mass (g)	V (l)	Concentration (g/l)
CH_3COONa	0.502	0.5	1.00
NH_4Cl	0.084	0.5	0.17
K_2HPO_4	0.024	0.5	0.05

Table 4.1: Synthetic water composition

As shown in figure 4.37 using a synthetic water the slope of the line changed after adding allylthiourea, so this demonstrates that the inhibiting solution works well.

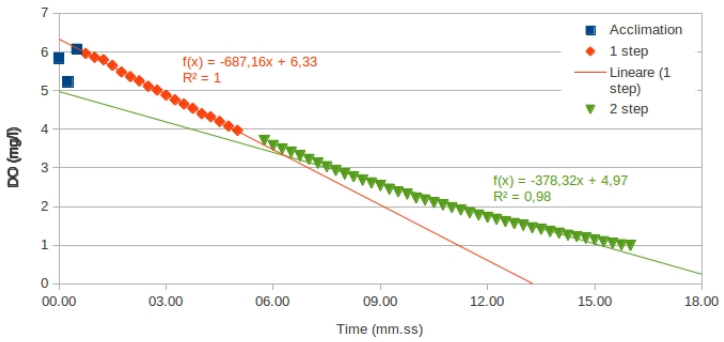


Figure 4.37: OUR analysis with synthetic water for R1

The slope of the line indicates the bacteria consumption of oxygen, in the first step both OHOs and nitrifiers are active, and so the slope must be greater, in absolute value, than the slope in the second step where only the OHOs act.

This positive results suggest that taking 30 ml of suspended granules, gives a too low concentration, so it was decided to repeat the OUR test taking 30 ml of settled granules and diluted reject water taken from the vessels contained in the fridges. The results obtained were positive, as shown in figures 4.38, 4.39 and 4.40, in all three reactors it's possible to notice a difference in the line slope between the step 1 and 2.

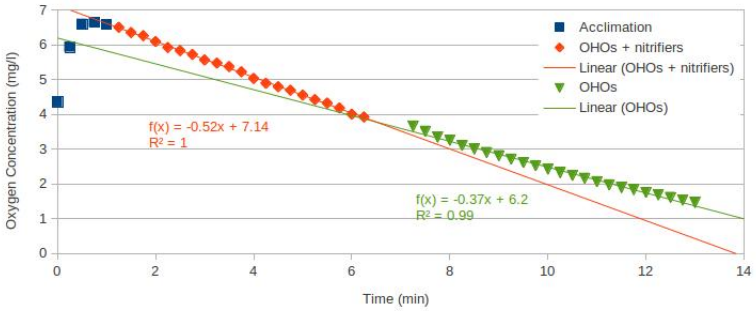


Figure 4.38: OUR test with 30 ml of settled granules for R1

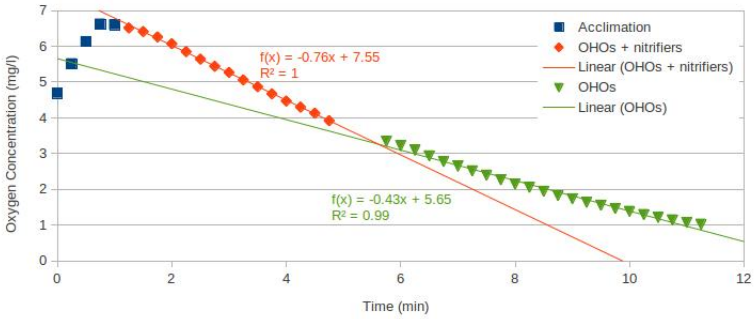


Figure 4.39: OUR test with 30 ml of settled granules for R2

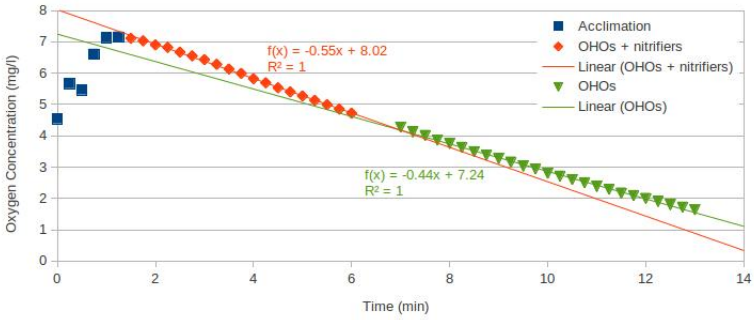


Figure 4.40: OUR test with 30 ml of settled granules for R3

A test was performed with different volumes of settled granules (5 ml, 10 ml, 20 ml, 30 ml, 40 ml and 50 ml). The expectation was to obtain a SOUR curve that starts with a value very close to 0 $mgO_2/l \cdot h$ and then increase till reach a stable value. The idea is that there is a limit granules concentration under which the nitrifiers activity is not appreciable if compared to that of heterotrophs. After that the limit concentration was found, the volume of granules was increased in order to obtain the optimal concentration that permit to nitrifiers to have the maximum activity.

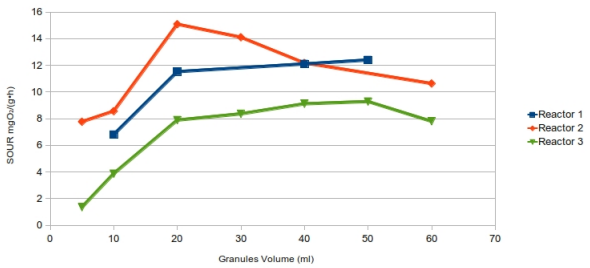


Figure 4.41: SOUR due to OHOs and Nitrifiers

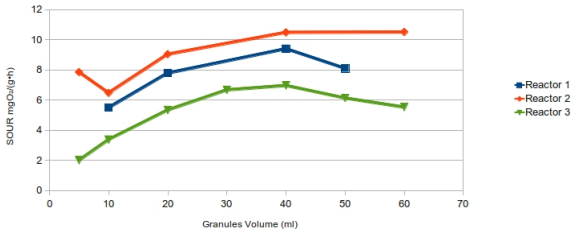


Figure 4.42: SOUR due to OHOs

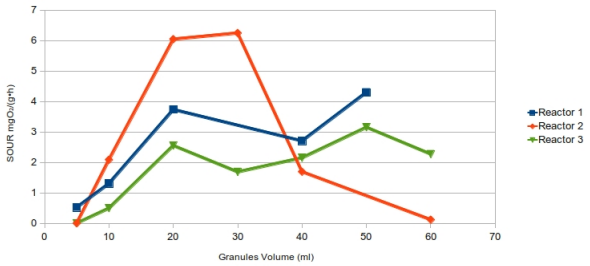
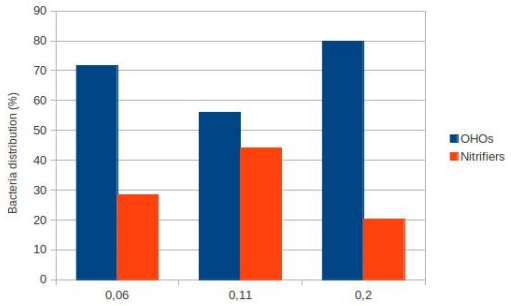


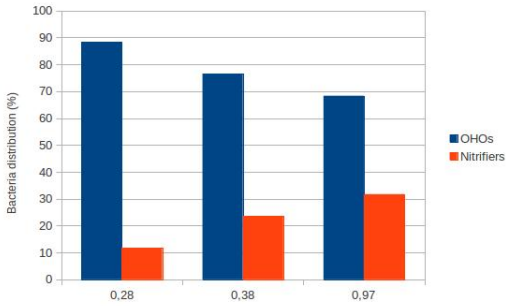
Figure 4.43: SOUR due to Nitrifiers

As shown in figure 4.41 and 4.42 the bacteria activity reach an optimal point with a granules volume greater than 20 ml, in fact after this point the SOUR become stable indicating that the bacteria work at the maximum rate. 20 ml of granules correspond to a VSS concentration of 2.35 g/l, 2.42 g/l and 3.69 g/l for reactor 1, 2 and 3 respectively. Taking a look at the plot that show only the nitrifiers activity, it can be notice, that for R1 and R3, the nitrification process become appreciable with a granules volume greater than 20 ml, but for R2, the process of nitrification is appreciable only in a small range, comprised in volume of 20 - 30 ml. Probably the decrease in the SOUR curve in R2, is mainly due to some error performing the VSS measurements. In fact when the granules become mature, it is very difficult to take a sample with an uniform solids concentration so it was believed that the two last points in the nitrifiers SOUR curve, are wrong points.

Figure 4.44a shows the bacterial distribution in the reactors. Yang et al. [18, 19], show that increasing the ratio between nitrogen and COD inside the reactor, the OHOs activity distribution decreases, while that of nitrifiers increases; this supports the hypothesis reported in biofilm reactors that once the organic carbon is reduced, the nitrifying bacteria would lose their competitive disadvantages and become a more important component of the biofilm or aerobic granules. A similar trend is observed comparing the granules growth in R1 and R2, where the initial N/COD ratio were 0.06 and 0.11 respectively. On the contrary, in R3 that has an average N/COD ratio equal to 0.23, the highest value for heterotrophic activity and the lowest value for the nitrifying activity can be observed. This result is reasonable, if it is assumed that in the first running period nitrification did not take place in R3.



(a) First run

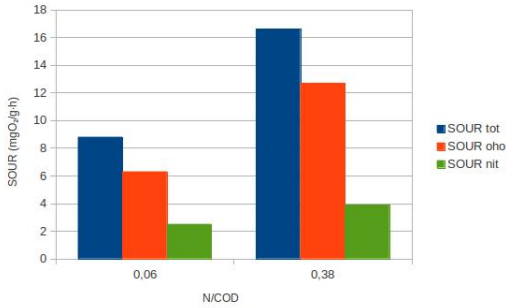


(b) Second run

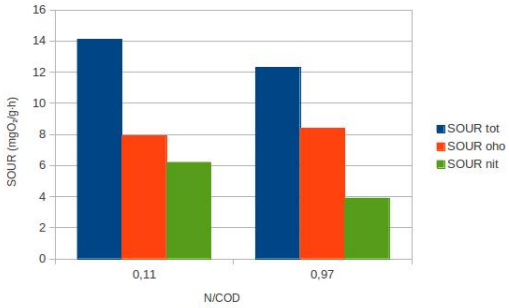
Figure 4.44: Bacterial activity distribution

The trend is different during second run, as observed in figure 4.44b, when the nitrification process took place in all three reactors.

Figures 4.45 compares the total, nitrifying and heterotrophic activity specific oxygen uptake rate during first run, with the conditions during second run. In R1 the increases of ammonia load leads to an increase of the bacterial activity for both nitrifiers and heterotrophs. So starting with a N/COD ratio equal to 0.06 and then increasing it to a value of 0.38 does not inhibit the bacteria. In R2 the N/COD ratio, changed from 0.11 to 0.97; the results show a decreasing bacterial activity due to the high ammonia that inhibited both the heterotrophs and nitrifiers, as explained by Yang et al. [11].



(a) R1



(b) R2

Figure 4.45: SOUR variation

In R3 the same conditions were kept. However, two OUR tests were made to observe if something changed during the time of the operation. At the end of operational times, the nitrifiers activity remained constant, while the heterotrophs

SOUR increase.

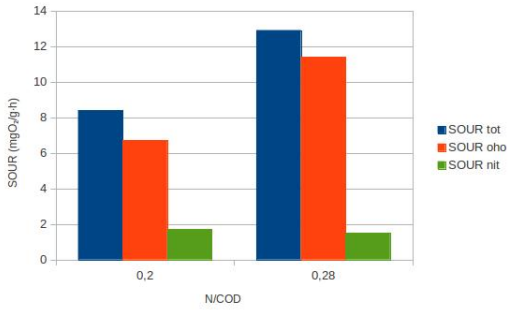


Figure 4.46: SOUR variation in R3

From the OUR test it can be observed that the granules growth in all three reactors are able to perform both the process of COD degradation and nitrification, and the bacterial activity goes on at relatively high load of ammonia too. The only case were the bacteria results to be partially inhibited, is on R2 with a N/COD ratio equal to 0.97.

4.4 Cycle analysis

The cycle analyses were performed on 2011/04/14. From the effluent analysis data it can be notice that after this date, the process of nitrification took place in R1 and R2, denitrification took place only in R2, the phosphate and organic matter removal took place in all three reactors.

4.4.1 TOC

Figures 4.47, 4.48 and 4.49 show the fate of TOC in the three reactors during a cycle. The gray line in the plot, identify the end of anaerobic and the beginning of aerobic phase.

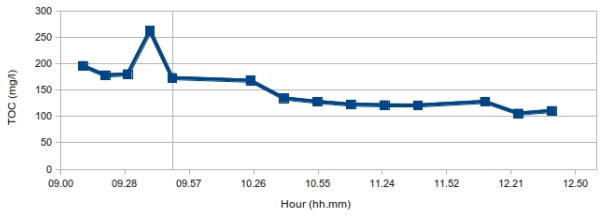


Figure 4.47: TOC trend in R1

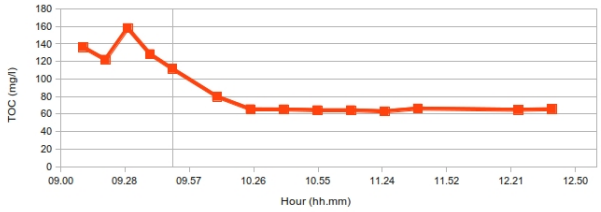


Figure 4.48: TOC trend in R2

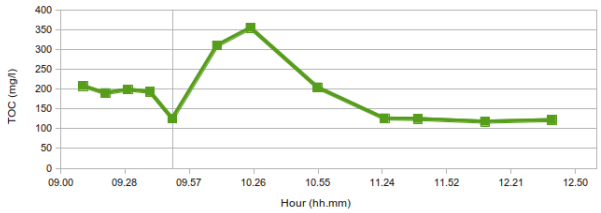


Figure 4.49: TOC trend in R3

In all three reactors the TOC concentration decrease over time supporting the observation made for the effluent data, that in all three reactors the organic substances removal take place. However there is a small difference, in fact from the effluent data R2 is that with the greater removal capability, followed by R1 and then from R3. The same conclusion can made looking the specific oxygen uptake rate due only to heterotrophs.

Table 4.2 report the influent, effluent TOC and the calculated removal efficiency.

Reactor	TOC in (mg/l)	TOC out (mg/l)	Efficiency (%)
1	446	110	75
2	209	65	69
3	376	120	68

Table 4.2: TOC removal efficiency during cycle analysis

The influent TOC values reported, are greater than values which can be extrapolated by previous plot. The data reported in the table are calculated taking in account the acetate, reject and previous cycle TOC. In the plot is not possible to read the right TOC influent concentration because during anaerobic phase the water is not properly mixed, so the values reported in the plot for this phase are not reliable.

The calculated efficiency, however, are similar to that obtained during the effluent analysis in the same period for R1 and R3. R2 has a lower efficiency, probably some problems during the feeding stage occurred, leading to have a lower influent nutrient concentration from reject water. This hypothesis is confirmed by the nitrate analysis, where it was noticed that a very low concentration of these compounds too.

4.4.2 Nitrogen

As for the effluent analysis in this section the simultaneous trend of ammonia and nitrate for each reactor is considered. The nitrate is only considered for R1 and R2, because in the third reactor the concentration during the cycle was very low.

4.4.2.1 Reactor 1

As can be notice in figure 4.50 for R1 during the aerobic phase starts ammonia degradation. It is confirmed by ammoniacal nitrogen concentration that pass from an initial value of 50 mg/l to a final value equal to 20 mg/l.

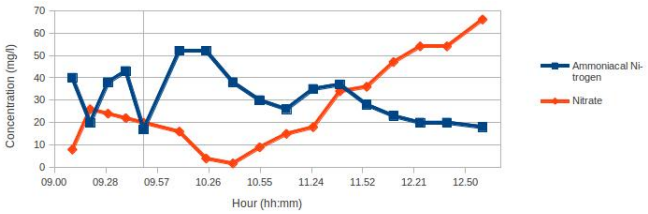


Figure 4.50: Ammoniacal nitrogen and nitrate trend in R1

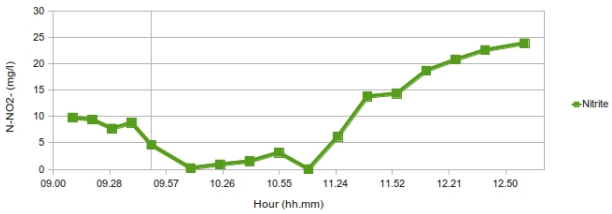


Figure 4.51: Nitrite trend in R1

At the same time both the concentration of nitrate and nitrite increased, indicating ammonia conversion. Nitrate pass from initial value of 20 mg/l to final value

of 70 mg/l (figure 4.50) instead nitrite pass from initial value of 5 mg/l to final value close to 25 mg/l (figure 4.51). The accumulation of nitrite show that nitrification process is not complete, otherwise a decrease of this parameter should be observed and the nitrate accumulation demonstrate that denitrification process does not take place. These conclusion are confirmed by total nitrogen trend figure 4.52; it remains constant with a value that fluctuate between 60 and 50 mg/l demonstrating that the total nitrogen concentration does not diminish.

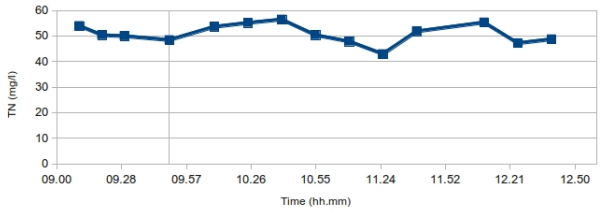


Figure 4.52: Total nitrogen trend in R1

4.4.2.2 Reactor 2

In R2 during the cycle analysis it was noticed that ammoniacal nitrogen concentration was very low. As was said before (section 4.4.1) this was due to problems with the reject feeding pump which pumped in too little reject. However the results obtained confirm the processes observation made looking at the effluent data.

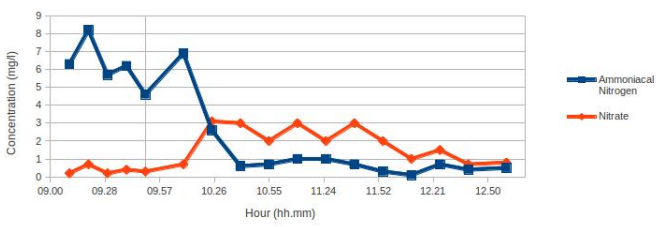


Figure 4.53: Ammoniacal nitrogen and nitrate trend in R2

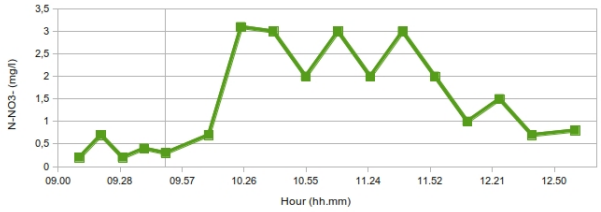


Figure 4.54: Nitrite trend in R2

As aerobic phase starts, the ammoniacal nitrogen is rapidly converted in nitrate and nitrite passing from an initial value of 7 mg/l to a final value below 1 mg/l. At the same time the nitrate and nitrite start to increase. From 10.25 am to 11.40 am the two compounds seems to have a constant concentration, some small variation can be observed, probably due to some accidental errors preparing the diluted samples to analyze. After 11.40 am, both the parameters start to decrease. The nitrite reach a concentration very close to 0 mg/l, indicating that nitrification take place in complete manner, instead the nitrate reach a concentration below 1 mg/l showing that denitrification take place too.

In R2 both nitrification and denitrification start, and this is confirmed by the trend of total nitrogen. It decrease over the time, as showed in figure 4.55.

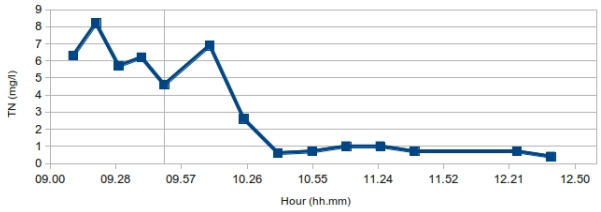


Figure 4.55: Total nitrogen trend in R2

4.4.2.3 Reactor 3

In this reactor neither nitrification nor denitrification take place.

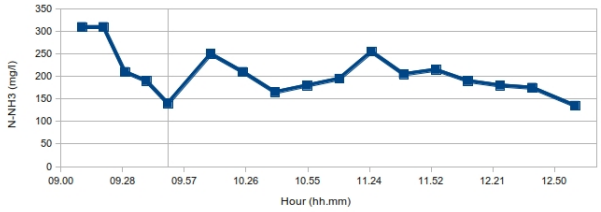


Figure 4.56: Ammoniacal nitrogen trend in R3

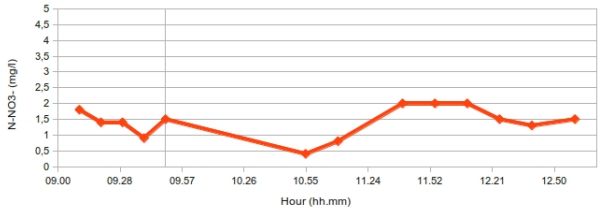


Figure 4.57: Nitrate trend in R3

Figure 4.56 shows that at the beginning of aerobic phase the ammoniacal nitrogen concentration is 250 mg/l. During the time this value had some variation and in the end it seems to decrease indicating an ammonia removal. Unfortunately the nitrate concentration doesn't increase, or better it does not in appreciable manner to justify the starting of nitrification. The observation is confirmed by the total nitrogen that remain constant, around a value of 200 mg/l.

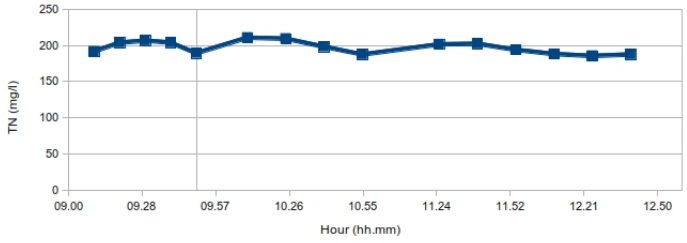
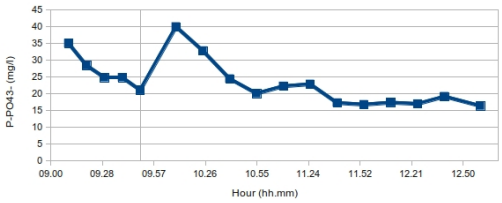


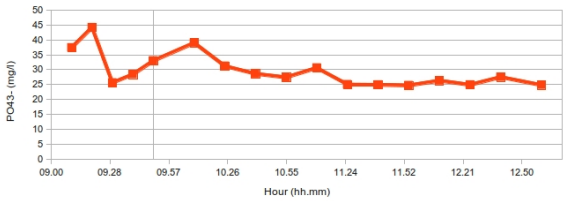
Figure 4.58: Total nitrogen trend in R3

4.4.3 Phosphate

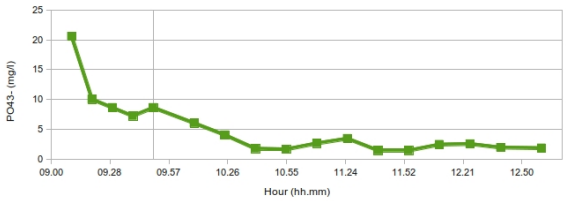
As show in figure 4.59 the phosphorous removal take place in all three reactors.



(a) R1



(b) R2



(c) R3

Figure 4.59: Phosphorous trend

It was expected to be able to observe an increasing in phosphate concentration during the anaerobic phase, due to release of these compounds by the PAOs, and a decrease during the aerobic phase, due to the PAOs uptake. On the contrary all the plots show a phosphate decrease during the anaerobic phase. The main problem is poor mixing during the anaerobic phase, in fact inside the reactors there were not any mechanism able to ensure a complete mixing. Instead during the aerobic phase the continuous air flow ensure a complete mixing. Hence it is hard to assess what happened during the anaerobic phase.

4.5 pH and redox potential (ORP)

4.5.1 First Run

Before nitrification starts, all the three reactors show a similar pH path: a decreasing during the anaerobic phase, due to the fermentation processes and production of compounds such as alcohol and acetic acid, and a slow increase during aerobic phase due to the CO_2 stripping and to phosphate uptake.

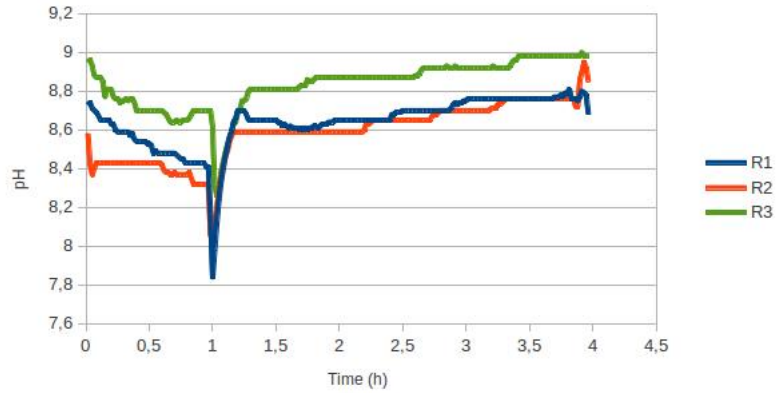


Figure 4.60: pH trend in R1, R2 and R3 before nitrification had started

As nitrification starts, in all three reactors the pH decrease during aerobic phase. As sad before nitrification produces hydrogen ions and so the pH goes down. Figure 4.62 show ORP trend for the three reactors. In R1 the nitrate accumulation increase the redox potential thanks to the bound oxygen added from the ammonia conversion into nitrite and nitrate. Instead even if nitrification start, in R2 and R3, during anaerobic phase, the ORP continues to decrease. For R2 There is no explanation, it seems that the maximum nitrate concentration reached, 45 mg/l, is not able to create a more oxidative environment. Instead in R3, the ORP measurements were done in a period were the nitrate and nitrite concentration were very low, even if nitrification was started, and so the redox potential does not increase.

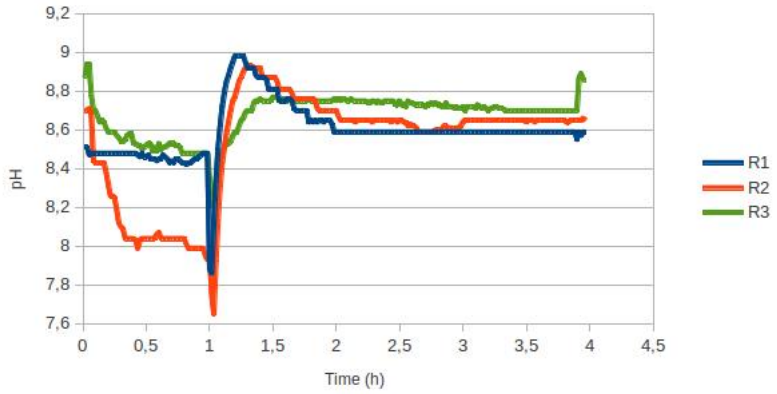


Figure 4.61: pH trend after nitrification starts

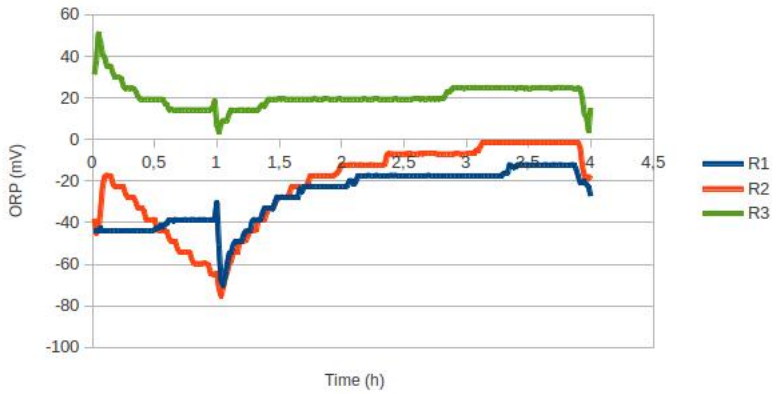


Figure 4.62: ORP trend after nitrification starts

When denitrification starts the results obtained are similar for R1 and R2, while no data are available for pH in R3. The pH is constant during anaerobic phase. So the pH decreasing due to the fermentation bioproduct is balanced by the pH increase due to denitrification. During aerobic phase, instead, pH in R1 decrease for all the length period, in R2 it firstly decrease reaching a minimum. This point is called "ammonium valley" and it correspond to the point were ammonia conversion in nitrite and nitrate is stopped. After this point the pH increase a bit reaching a constant value (a bit greater than 8.5) corresponding to the end of phosphorous uptake. ORP trend is similar for all reactors, and it decrease during anaerobic phase. Denitrification convert nitrate in nitrogen gas, so bounded oxygen is removed and is formed a more reductive environment.

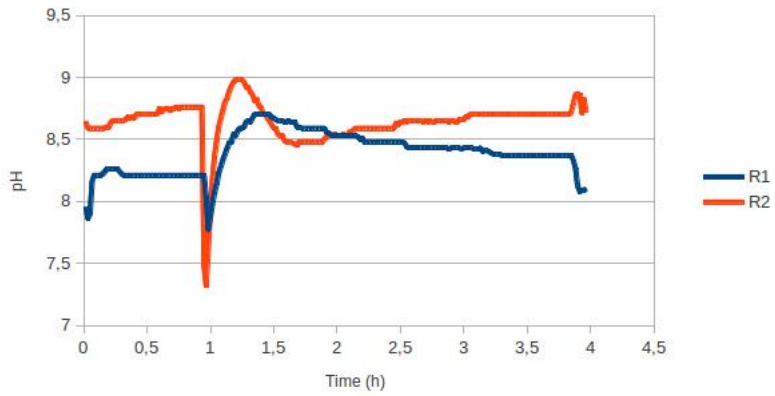


Figure 4.63: pH trend when denitrification starts

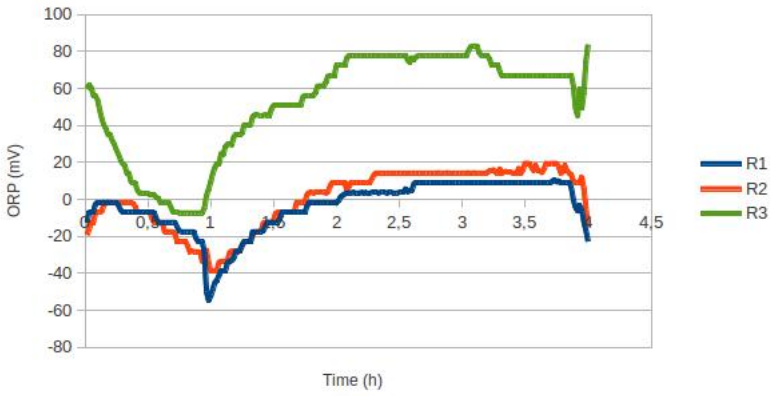


Figure 4.64: ORP trend when denitrification starts

4.5.2 Second run

During second run the pH trend and the TN ratio, greater than 1, suggest that both nitrification and denitrification continues to go on in R1 and R2, so the processes are not inhibited by the increase in ammonia load. On the contrary the ORP increase suggests that at the beginning of the second run there is a nitrate accumulation as confirmed by the effluent data. It was believed that denitrification does not stop, but probably the higher ammonia does not affect nitrification rate, but it lowered the denitrification rate. So denitrifiers bacteria are able to degrade only a little part of nitrate produced by ammonia degradation, and this explain the increase in ORP even though denitrification take place.

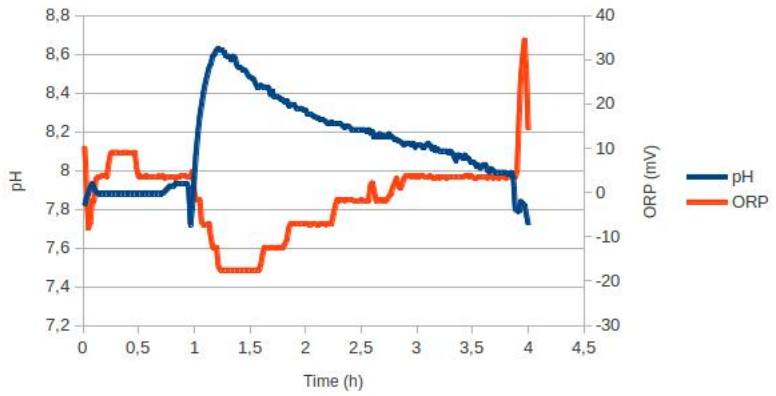


Figure 4.65: pH and ORP profile in R1 during second run

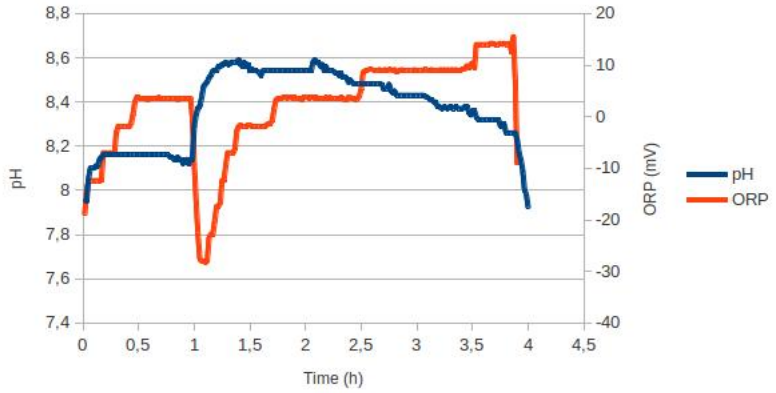


Figure 4.66: pH and ORP trend in R2 during second run

During second run R2 is not able to complete nitrification, in fact the pH plot doesn't show the "ammonium valley" reached during first run.

4.6 Dissolved oxygen

The dissolved oxygen values show a similar trend for both R1 and R2. As depicted in figure 4.67 and 4.68, during the anaerobic phase there is an oxygen consumption, and it reach rapidly a concentration equal to 0 mg/l. During the aerobic phase, the oxygen concentration increase rapidly in the first minutes, and then it decrease a bit. As described by the cycle analysis data, the greater amount of organic matter and ammonia is degraded in a very short time. Here, probably, the oxygen consumption

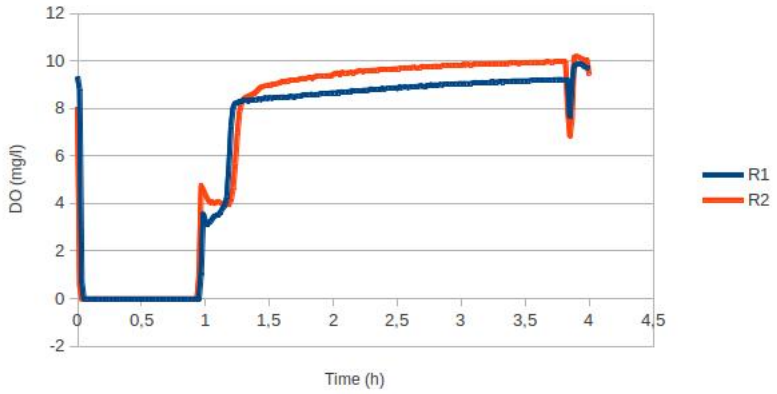


Figure 4.67: DO in R1 and R2

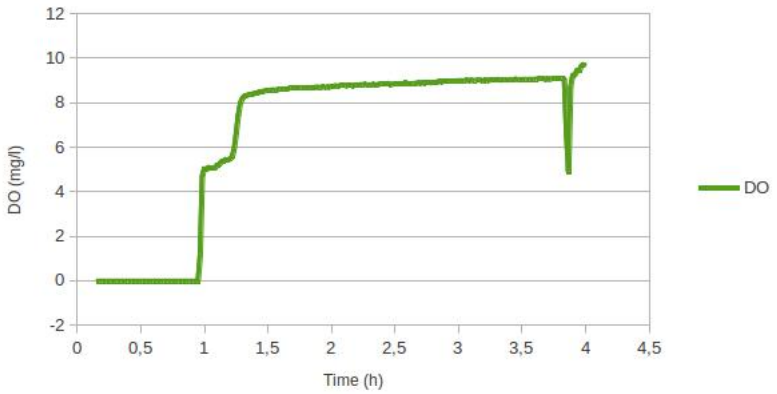


Figure 4.68: DO in R3

rate is higher than re-oxygenation rate and then the oxygen concentration decrease. Once the greater amount of COD and ammonia degradation is completed, the oxygen concentration increase again, reaching saturation value.

In R3 the same trend during the first minutes of aerobic phase was not observed: oxygen concentration does not decrease, but increase with a lower slope. The higher ammonia load slows the bacteria activity, and so the consumption oxygen rate is less than the re-oxygenation rate even if there are a great substrate concentration too.

Chapter 5

Conclusions

Granules appear after one month after the reactors start-up. The different nutrient conditions affect mainly the granular sludge biological properties, and to a minor degree the granules' shape and size. In R2 the smallest granules with a more spherical shape and the best nutrient removal efficiencies were obtained, probably thanks to their greater specific area. In R1 and R3 the granules were bigger with a less uniform shape.

COD degradation can achieve very high efficiency close to 90%, but ammonia concentration strongly inhibits heterotrophic bacteria. In fact, in R3 that starts with higher ammonia concentration, the average COD removal efficiency calculated is 60%. During the second run COD removal efficiency decrease both in R1 and R2. This is due to the higher ammonia concentrations and to the lower readily biodegradable COD pumped into the reactors.

Nitrifiers and denitrifiers bacteria are more sensitive to ammonia condition, however starting with a load comprised in the range of 0.22 - 0.25 $^{kgN}/_{m^3 \cdot d}$ permit ammonia and nitrate degradation in relative short time. The denitrification process is not going on with a very high efficiency, probably it could be enhanced by adding a second anaerobic phase in the cycle, following the aerobic ones. In this manner the nitrate produced during the aerobic stage can be degraded in the same cycle. Starting with higher ammonia load nitrification and denitrification start too, but in longer time and with a very low efficiency. During the second run the process continue to going on, but with a lower efficiency.

OUR tests support the conclusion made above. Nitrification and denitrification process take place in short time with N/COD ratio less than 0.15. With the higher ratio obtained in R3, the two processes need more time to start, as the distribution of nitrifiers is minor then in the other two reactors. In R1 going from an N/COD ratio equal to 0.12 to 0.67 does not inhibit bacterial activity and both nitrifiers and heterotrophs continues the nutrient removal processes. At the beginning of the second run ammonia degradation efficiency diminish a bit and then it raise

again reaching values close to that obtained in the first run. Probably the initial decreasing is due to the new conditions to which the bacteria need to acclimate. On the contrary in R2 going from a N/COD ratio equal to 0.15 to 1.74, the activity of both the bacteria decrease. This mean that the process efficiency diminish, as demonstrated by the effluent analysis. In the first run ammonia degradation is close to 100%, during second run it falls down at value close to 80%. In R3, as nitrification starts, the ammonia removal efficiencies reach values close to 70% at the end of the work time.

Phosphorous removal takes place too, but it is not stable and its efficiency depends on the readily biodegradable COD concentration: as the concentration is higher, the greater the removal percentage.

The increased ammonia load of R1 and R2 show that granular sludge is less sensitive to shock load, however it should be interesting to see what will happen with changing the nutrient load conditions more frequently, for example starting with a low load, then increase it and eventually decrease it.

Granular sludge exhibit greater physical properties compared to activated sludge: solid biomass retention, settleability and settling velocity are greater, as described before. Thanks to the greater settleability, granular sludge is less prone to a solids wash out during the last cycle phase.

To improve nitrogen removal, pH and ORP profile can be used to set the right operational regime for the aerobic and anaerobic phase. During anaerobic stage, ORP decrease as denitrification goes on, when all the nitrate are converted to nitrogen gas, the ORP profile change, and it decrease with higher slope. When this point is reached, denitrification is completed, so the anaerobic phase duration can be set in order to reach this point. Aerobic phase duration instead can be set by looking the pH. As nitrification goes on, pH diminish reaching the ammonia valley. Then pH increase again till a constant value corresponding to phosphorous uptake completion. Aerobic phase duration can be set at the time needed to obtain a pH constant value.

To ensure more homogeneous condition during anaerobic phase a mixing system can be integrated in the reactors, for example a magnetic stirrer. In this study during anaerobic phase there was no mixing system, so uniform conditions were not achieved in the reactors, and all the biological reactions take place at the bottom, where the granules were settled. This condition is reflected in the pH, DO and ORP data: they are not so precise inasmuch the electrodes for measuring these parameters were placed on the top of reactors.

Chapter 6

Appendix

6.1 Appendix A - Flow calculation

Here the decision criteria to select the pump flows are reported. Tables 6.1 report the data obtained in the test made for drawing the pump curves.

For R1 a pipe diameter of 6.4 mm and a pump rate of 10 rpm was chosen for reject water and a pipe diameter of 8 mm and a pump rate of 60 rpm for nutrients concentration contained in the 100 l tank. At these conditions the reject water flow (Q_r) is 46 ml/min and the tank flow (Q_t) is 293.3 ml/min. Considering the feeding time, 4 min, it is possible to calculate the operational volume (V_o) and the height reached by the water in R1 (h_o). V_w represent the water volume pumped in the reactor during feeding phase. The operational volume is the sum of the water volume and the volume of water left in the reactor from the previous cycle.

$$V_w = (Q_{rej} + Q_t + Q_{Ac}) \cdot F_t = \frac{(46 + 293.3 + 7.5)^{ml}/_{min} \cdot 4min}{1000\frac{ml}{l}} = 1.39l$$

$$h_o = \frac{V_w \cdot 4}{\pi \cdot d_r^2} + h_w = \frac{1.39l \cdot 4}{\pi \cdot 0.6^2 dm^2} \cdot 10^{cm}/_{dm} + 63cm = 112cm$$

$$V_o = \frac{\pi \cdot d_r^2 \cdot h_o}{4} = 3.17l$$

The calculation explain the conditions in R1: every cycle the reactor was filled to a level of 1.12 m corresponding to a volume of 3.17 m^3.I done the same calculation for the other reactors obtaining the value reported in table 6.1.

Rate	Time (s)	Volume (ml)	Flow (ml/min)
10	120	92	46.0
10	120	92	46.0
20	120	160	80.0
20	120	158	79.0
40	60	150	150.0
40	60	146	146.0
60	60	218	218.0
60	60	219	219.0
80	50	240	288.0
80	50	233	279,6
100	30	175	350.0
100	30	174	348,0
120	30	210	420.0
120	30	214	428.0

(a)

Rate	Time (s)	Volume (ml)	Flow (ml/min)
10	120	128	64.0
10	120	124	62.0
20	120	207	103.5
20	120	212	106.0
40	60	200	200.0
40	60	200	200.0
60	45	220	293.3
60	45	220	293.3
80	30	191	382.0
80	30	192	384.0
100	25	223	535.2
100	25	220	528.0
120	20	214	642.0
120	20	211	633.0

(b)

Table 6.1: Pump flows with a pipe diameter of 6.4 mm (a) and of 8 mm (b)

6.2 Appendix B - Chemicals calculation

The total amount of COD in R1 is the sum between the COD concentration in reject water and in the acetate solution. The goal is to calculate the mass of acetate to add in the bottle in order to achieve a COD:N:P ratio in R1 equals to 100:5:1. The COD concentration entering in R1 every cycle is:

$$COD_{in} = \frac{(Q_r \cdot COD_r + Q_{Ac} \cdot COD_{Ac}) \cdot Ft}{V_o}$$

$$COD_{Ac} = \frac{M_{Ac}}{V_b} \cdot \beta$$

The therm β indicates the ratio between the grams of oxygen necessary for degrade 1 g of sodium acetate. From stoichiometry 2 moles of oxygen are necessary to degrade 1 moles of acetate, so:

$$CH_3COONa + H_2O + 2O_2 \rightarrow NaOH + 2CO_2 + 2H_2O$$

$$\beta = \frac{2 \cdot MW_{O_2}}{MW_{Ac}}$$

The only nitrogen source is the nitrogen in form of ammonia, nitrate and nitrite in the reject water. From the analysis on reject water (tab. 3.2) it can be notice that the ammonia concentration is greater than the other two forms of nitrogen, so with a simplification the influent nitrogen concentration is calculated as the influent ammonia due to the reject water.

Finally the influent phosphorous is the sum of the ortophosphate concentration in reject water and in the tank:

$$P = \frac{(Q_r \cdot PO_{4,r}^{3-} + Q_t \cdot PO_{4,t}^{3-}) \cdot F_t}{V_o}$$

$$PO_{4,t}^{3-} = f_{PO_4^{3-}} \cdot \frac{M_P}{V_t} = \frac{MW_{PO_4^{3-}}}{MW_P} \cdot \frac{M_P}{V_t}$$

Varying the sodium acetate mass and the potassium phosphate dibasic mass, the value that permit to have a C:N:P ratio close to 100:5:1 was chosen. The same calculations were made for the other two reactors.

Symbols:

- M_{Ac} sodium acetate mass added (g)
- V_b volume of the bottle containing acetate solution (2 l)
- MW_{O_2} oxygen molar weight (g/mol)
- M_{Ac}sodium acetate molar weight (g/mol)
- $PO_{4,r}^{3-}$ phosphate ions concentration in reject water (mg/l)
- $PO_{4,t}^{3-}$ phosphate ions concentration in the phosphate and micro nutrients solution (g/mol)

- M_P mass of potassium phosphate dibasic added in the tank (g)
- V_t tank volume (100 l)
- $MW_{PO_4^{3-}}$ phosphate ions molar weight (g/mol)
- MW_P potassium phosphate dibasic molar weight (g/mol)

6.3 Appendix C - TOC / COD conversion

From April the organic matter using the TOC parameter was begun to be used. To have a data continuity, a model was built to find relationship between TOC and COD. In the reject water analysis data of 8^{th} and 22^{th} of April , both the parameter were estimated and then it was noticed that in reject water the ratio between COD and TOC was equal to 1.77.

Assuming a COD removal percentage equal to TOC removal percentage, it is possible to write:

$$\frac{COD_{in} - COD_{out}}{COD_{in}} = \frac{TOC_{in} - TOC_{out}}{TOC_{in}}$$

$$1 - \frac{COD_{out}}{COD_{in}} = 1 - \frac{TOC_{out}}{TOC_{in}}$$

$$\frac{COD_{out}}{COD_{in}} = \frac{TOC_{out}}{TOC_{in}} \tag{6.1}$$

Calling B the second term of eq. 6.1, it becomes:

$$\frac{COD_{out}}{COD_{in}} = B \tag{6.2}$$

Considering that:

- influent COD can be calculated by eq. 6.3:

$$COD_{in} = \frac{Ft}{3.05} \cdot (Q_{Ac} \cdot COD_{Ac} + Q_{rej} \cdot COD_{rej}) + 0.57 \cdot COD_p \tag{6.3}$$

- the ratio between COD and TOC in the acetate solution, calculated by stoichiometry is 2.67:

$$\frac{COD_{Ac}}{TOC_{Ac}} = 2.67 \tag{6.4}$$

- as sad before, the ratio between COD and TOC in reject water is 1.77:

$$\frac{COD_{rej}}{TOC_{rej}} = 1.77 \tag{6.5}$$

By substituting eq. 6.4 and 6.5 in eq. 6.3, influent COD can be written as:

$$COD_{in} = \frac{Ft}{3.05} \cdot (2.67 \cdot Q_{Ac} \cdot TOC_{Ac} + 1.77 \cdot Q_{rej} \cdot TOC_{rej}) + 0.57 \cdot COD_p \quad (6.6)$$

then substituting eq. 6.6 in eq. 6.2 and calling A the first term of eq. 6.6, I obtain:

$$\frac{COD_{out}}{\frac{Ft}{3.05} \cdot (2.67 \cdot Q_{Ac} \cdot TOC_{Ac} + 1.77 \cdot Q_{rej} \cdot TOC_{rej}) + 0.57 \cdot COD_p} =$$

$$= \frac{COD_{out}}{A + 0.57 \cdot COD_p} = B \quad (6.7)$$

Knowing, from the analysis, the reactor influent and effluent TOC, the reject water TOC and, from stoichiometry, the acetate TOC, it is possible to convert the TOC parameter in COD, by the following formula:

$$COD_{out} = \frac{AB}{1 - 0.57B}$$

$$COD_{in} = COD_{out} \cdot \frac{TOC_{in}}{TOC_{out}}$$

Thanks to some effluent and reject analysis, where both COD and TOC measurements, were taken, a qualitative and quantitative analysis on the model efficiency was performed. As show in fig. 6.1 the COD measured values are very close to the concentration calculated by the model, an exception for the last point where there is an high absolute error.

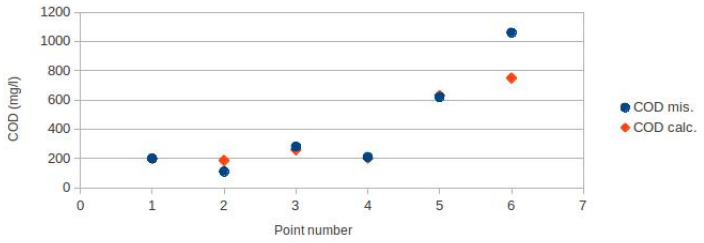


Figure 6.1: COD measured vs COD calculated

To estimate in quantitative manner the model attainability a model efficiency test was performed. This test is similar to the linear regression coefficient R^2, and show the variation around the perfect overlap line. It is calculated by the following

formula:

$$EF = 1 - \frac{\sum(y_{i,m} - y_{i,c})}{\sum(y_{i,m} - \bar{y})}$$

where:

1. $y_{i,m}$ is the i$^{\text{th}}$measured value
2. $y_{i,c}$ is the i$^{\text{th}}$calculated value
3. $\bar{y}$ is the average measured value

If the model is perfect EF take a value equal to 1, as the model attainability diminish, the test result is lower than one. In this case the test gives a result of 0.83, so the model gives results with a low error.

6.4 Appendix D - Ammoniacal nitrogen conversion in free ammonia

Considering that ammoniacal nitrogen is the ammonia nitrogen fraction, the conversion was made using the following equation:

$$NH_3 = \frac{MW_{NH_3}}{MW_N} \cdot N - NH_3$$

where:

1. NH_3 is the calculated concentration of free ammonia (mg/l)
2. MW_{NH_3} is the free ammonia concentration molar weight
3. MW_N is the nitrogen molar weight
4. N-NH_3 is the measured concentration of ammoniacal nitrogen

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