UNESCO-IHE
Institute for Water Education

Universidad del Valle

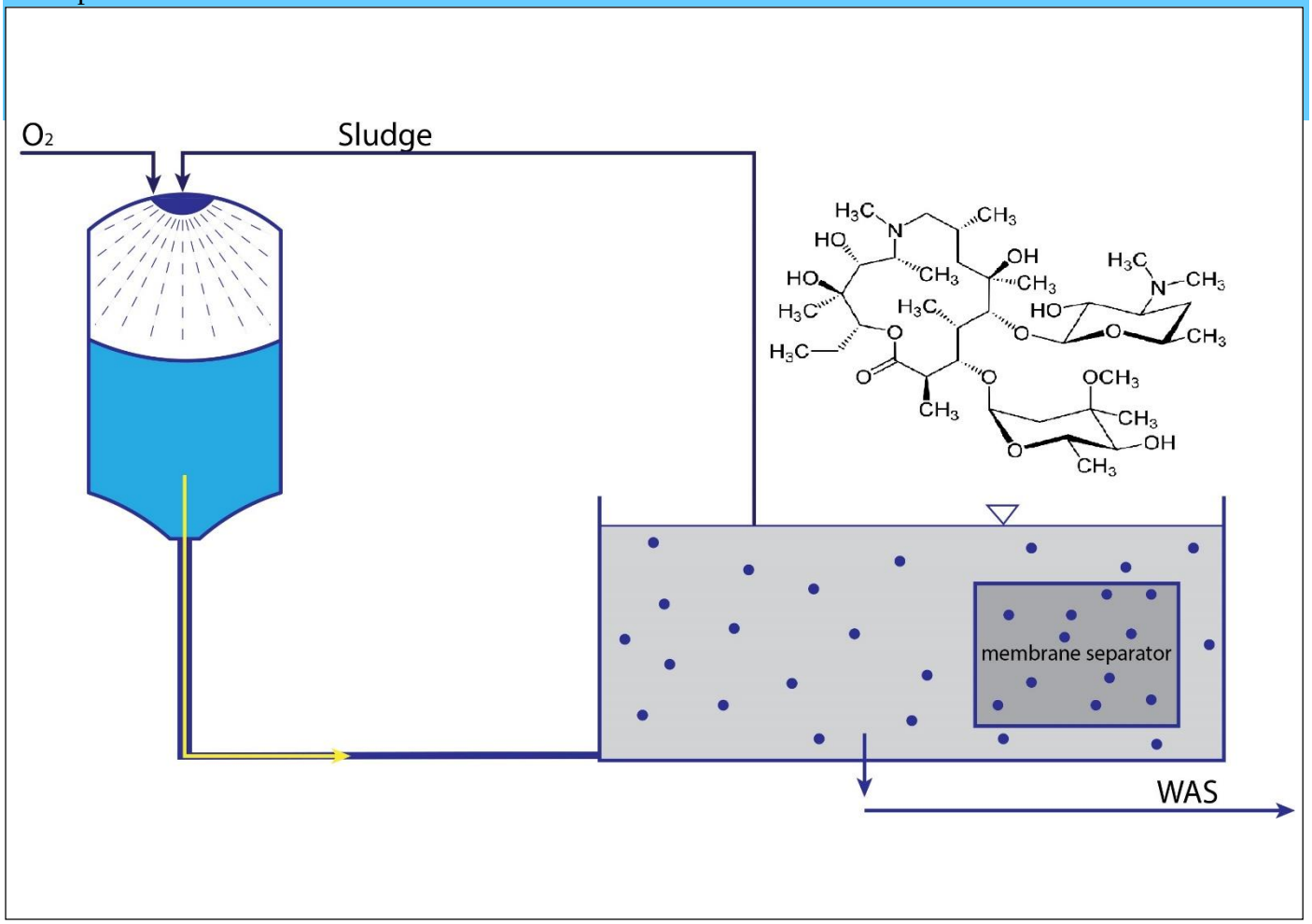
FACULTY OF FOOD TECHNOLOGY AND BIOTECHNOLOGY
PBF
UNIVERSITY OF ZAGREB - CROATIA
1956

Influence of supersaturated oxygen transfer technology on membrane fouling and azithromycin removal in a membrane bioreactor

Giovanna Guarinoni Ardito

MSc Thesis UWS-SE CALI 2016-11

April 2016



Influence of supersaturated oxygen transfer technology on membrane fouling and azithromycin removal in a membrane bioreactor

Master of Science Thesis
by
Giovanna Guarinoni Ardito

Supervisor
Prof. Carlos Madera, PhD, Msc (Univalle University)

Mentors
Tineke Hooijmans, PhD, Msc (UNESCO-IHE)
Hector García Hernández, PhD, MSc (UNESCO-IHE)
Marin Matošić, PhD, MSc (University of Zagreb)

This research is done for the partial fulfilment of requirements for the Master of Science double degree at the UNESCO-IHE Institute for Water Education, Delft, the Netherlands and Univalle University, Cali, Colombia.

Delft
April 2016

Abstract

Conventional aeration systems as fine and coarse bubble diffusers present low oxygen transfer efficiency at high MLSS concentrations. At these conditions, the use of the SDOX unit has been proven to have better oxygen transfer efficiency, reducing costs related to energy for providing aeration (Bilal, 2013).
Moreover, current design for wastewater treatment plants does not consider the removal of micro-pollutants. Membrane bioreactors have been applied successfully to achieve higher and more consistent micro-pollutant removal. This technology is facing some research and development challenges. Among these challenges, membrane fouling is one of the most serious problems.

The aim of this research was to evaluate the effect of SDOX over: biological processes, sludge characteristics, azithromycin removal and membrane fouling in a membrane bioreactor.

Five experiments were carried out changing some operational conditions. Each experiment consisted in the operation of a continuously fed MBR supplying oxygen with two different technologies. In first instance with bubble diffusers aerators distinguishing two phases: acclimatization (for eventual initial shocks) and operation. Secondly it was operated using SDOX unit, where also two phases took placed.

The effect of SDOX on sludge characteristics was pronounce for all the experiments. The particle size decreased (44-64) %, while the DSVI increased. It is interesting to notice that the EPSc measured presented the highest values for Experiments 2 and 3. Moreover, for these experiments the difference in operational conditions(HRT) seems to have a bigger impact in sludge characteristics than SDOX. Regarding the effect of SDOX over azithromycin removal efficiency, it was proven that it affected negatively the overall removal efficiency of azithromycin. For both experiments a drop was observed after its connection. Finally, the effect of SDOX over membrane fouling needs to be analyzed in two phases. For effluents which do not contain azithromycin, no incidence of SDOX over membrane fouling was found. Thus, in view of it possible application, more research is needed to evaluate the fouling due to cake decreasing the shear rate. In contrast, for effluents spiked with azithromycin a higher deterioration of the membrane was caused by the operation of the membrane with SDOX as the aeration technology.

Acknowledgements

I would like to extend my gratitude to ANII and Bill and Melinda Gates Foundation for granting me the opportunity to do my master studies.
I would like to express my gratitude to my supervisor Prof. Carlos Madera and my mentors: Dr. Marin Matošić, Prof. Tineke Hooijmans, Dr. Héctor García for their guidance and valuable comments during my research.
I would like to thanks Ruđer Bošković Institute, Division for Marine and Environmental Research, Laboratory for Analysys and Biogeochemistry of Organic Compounds, for their collaboration and technical support.
My special thanks for the Faculty of Food Technology and Biotechnology of Zagreb, the entire third floor and the Water Technology Laboratory staff: Vlado, Josip, Sangyeob and Marin. Thanks for the patience, the knowledge, the time and the affection.
I would also like to thanks to the staff of Biotechnology of Flavor Research, Udelar. My special thanks to Laura for the encouragement, and the support.
In addition, I would like to thanks my family and friends from Uruguay. For making me feel loved all these 21 months besides the distances.
Finally, I would like to thanks all my new friends from Colombia and Delft. Specially Cecilia, Analia, Pablo, Guillermo, Nicolas and Rodrigo. For all the shared moments that I will never forget.

Abbreviations

AE	Aeration efficiency
AZI	Azithromycin
AZI-TP	Azithromycin transformation product
CAS	Conventional activated sludge
BD	Bubble diffuser
COD	Biochemical oxygen demand
DO	Dissolved oxygen
DSVI	Diluted sludge volumetric index
EPSc	Extracellular polymeric substances carbohydrate fraction
EPSp	Extracellular polymeric substances protein fraction
HTR	Hydraulic retention time
J	Flux
K	Permeability
MBR	Membrane bioreactor
MLSS	Mixed liquor suspended solid
MLVSS	Mixed liquor volatile suspended solid
OTR	Oxygen transfer rate
OUR	Oxygen uptake rate
PLC	Programmable logic controller
QTOFMS	Quadrupole time of flight mass spectrometry
SDOX	Supersaturated dissolved oxygen
SMP	Soluble microbial products
SRT	Sludge retention time
TMP	Transmembrane pressure
TSS	Total suspended solid
UHPLC	Ultrahigh performance liquid chromatography
VSS	Volatile suspended solid
WWTP	Wastewater treatment plant

Table of Contents

List of Figures

List of Tables

CHAPTER 1

Introduction

1.1. Background

In the last decades municipal wastewater treatment was designed to remove organic matter and nutrients, currently efforts are focussing on the removal of micro-pollutants and other hazardous substances.
Membrane bioreactors (MBR), in which membranes are applied in case of a biological wastewater treatment system for biomass separation, provide many advantages over conventional treatment. Some of them are: (i) operation under higher mixed liquor suspended solids (MLSS) concentration, which reduces footprint of the plant, (ii) operation at longer sludge retention time (SRT), decreasing the sludge production in the system and a (iii) high-quality effluent, from the use of microfiltration and ultrafiltration membranes for separation of biomass from treated water.
Besides current applications in municipal and industrial wastewater treatment, potential application areas include nitrate removal from drinking water, removal of endocrine disrupting compounds from water and wastewater streams, enhancing biofuels production via membrane assisted fermentation and gas deliver or extraction.(Yang, et al., 2006)
Many studies show that the discharge of treated effluent from WWTPs is a major pathway for the introduction of micro-pollutants to surface water. WWTPs act as primary barriers against the spread of micro-pollutants but the removal efficiency varies depending on the contaminant and other conditions. Advanced treatment processes, such as, membrane bioreactors can achieve higher and more consistent micro-pollutant removal.(Luo, et al., 2014)
However, membrane fouling in MBR restricts their widespread application because it reduces productivity and increases maintenance and operating costs.(Chang, et al., 2002)
Furthermore, aeration is one of the major cost items for all aerobic treatment systems. Air is usually provided with coarse and fine bubble diffusers, but their efficiency is negatively affected by MLSS. Innovations are required in the field of aeration to overcome the disadvantages of conventional aeration systems. This research aims to explore the performance of a pilot scale MBR system operating with different oxygen supply systems including bubble diffusers and concentrated oxygen delivery systems (SDOX).
Previous experiments conducted by Bilal (2013), showed that the SDOX unit was effective to deliver dissolved oxygen at concentrations of MLSS higher than 30 g/L. Moreover, Bilal (2013) has noticed a reduction in the floc size, which can be attributed to the exposure of microorganisms to alternate periods of high pressure.
Other experiments carried out by Librán-Vázquez (2015), showed that sludge from an MBR operated for four days with bubble diffusers presented higher total resistance to membrane filtration than sludge from an MBR which was acclimatized for the SDOX system. It was also

concluded that fouling after acclimatization of the MBR to the SDOX aeration system was attributed more to membrane fouling than to cake formation.
In this research, the effects of the reduction in floc size and the particle size distribution over membrane fouling will be assessed. Due to SDOX exposure, generation of extracellular polymers by bacteria would take place. This compounds are well known because of its high fouling properties(Meng, et al., 2009), for this reason they will be measure and correlated to changes on membrane permeability.
Simultaneously, the influence of SDOX system over azithromycin removal efficiency will be evaluated extracting and quantifying this compound in samples from the influent and samples from the permeate.

1.2. Problem definition

Conventional aeration systems as fine and coarse bubble diffusers present low oxygen transfer efficiency at high MLSS concentrations. At this conditions, the use of the SDOX unit has been proven to have better oxygen transfer efficiency, reducing costs related to energy for providing aeration (Bilal, 2013).
Moreover, current design for wastewater treatment plants does not consider the removal of micro-pollutants. Membrane bioreactors has been applied succesfully to achieve higher and more consistent micro-pollutant removal. This technology is facing some research and development challenges. Among these challenges, membrane fouling is one of the most serious problems.
A large number of recent publications (Lin, et al., 2014) indicate that the biomass supernatant (SMP) and extracellular polymeric substances (EPS) are major components affecting MBR fouling.
However, any possible correlation between the application of the SDOX system and EPS has not yet been determined.
The current research will assess the effects of the SDOX unit on the operation of an MBR, evaluating the performance of solid/liquid separation at two different concentrations of MLSS. Moreover, the effect of SDOx on membrane fouling and removal efficiency of azithromycin will be studied as well.

1.3. Research Questions

- What is the influence of SDOx system on membrane fouling in a membrane bioreactor operated at two different concentrations (10 and 20 g/L) of MLSS.

- What is the influence of SDOx system in a membrane bioreactor over azithromycin removal efficiency

CHAPTER 2

Research Objectives

This chapter presents the general and specific objectives of the research.

2.1. Main objective

The main objective of this research is to evaluate the effect of using a concentrated oxygen delivery system SDOX unit over solid liquid separation processes, focus on membrane fouling and azithromycin removal in an MBR.

2.2. Specific objectives

Evaluate the performance of a pilot scale MBR system in terms of fouling when it is operated with bubble aerators and with SDOX unit.

Evaluate membrane filtration differences when performing MBR with bubble aerators and SDOX unit at different MLSS concentrations.

Evaluate the influence of SDOX unit in a MBR over azithromycin removal efficiency

CHAPTER 3

Literature Review

This chapter presents a literature review of membrane bioreactors, membrane fouling, aeration technologies, and emergent contaminants.

3.1. Membrane bioreactors

Membrane bioreactor (MBRs) are combinations of activated sludge process and membrane filtration units for biomass retention. The reactor is operated similar to a conventional activated sludge process but without the need for a secondary clarifier. Reliable biomass retention enables MBRs to operate with high mixed-liquor suspended solids (MLSS) concentrations, which reduces the reactor size, despite the relatively long solids retention times (SRTs). Moreover, MBRs have a small overall plant footprint because of the modestly sized bioreactors and the absence of external clarifiers or filters. As a result the product water quality is significantly higher than that generated by conventional treatment, obviating the need for a further tertiary disinfection process.

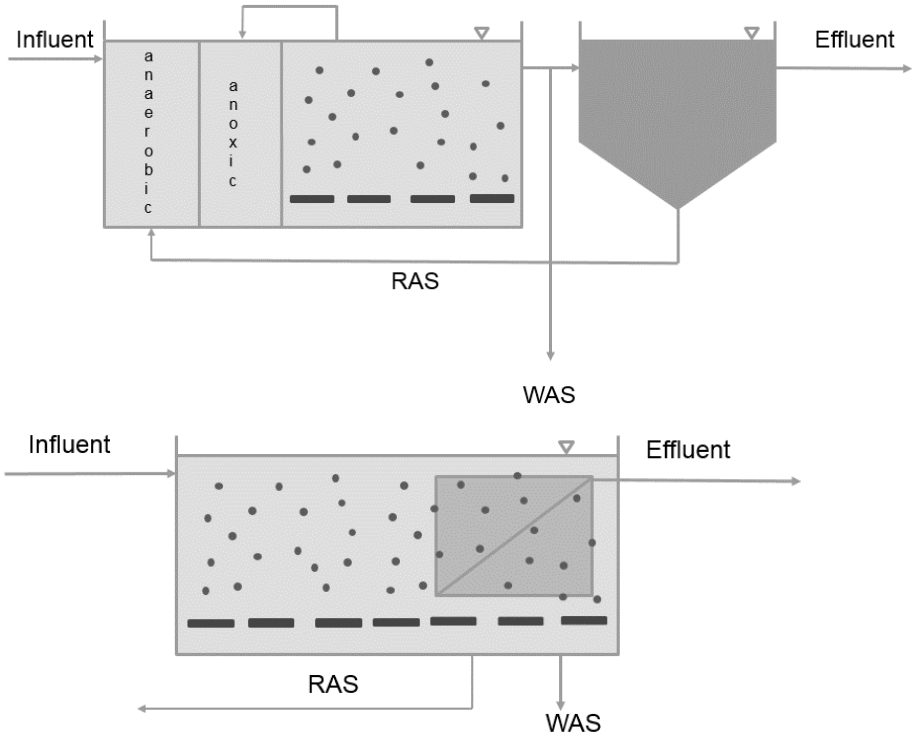


Figure 3-1 *(a) Above: CAS (b) Under: MBR systems.*

Adapted from (Daigger, et al., 2005)

3.2. Membrane fouling

Membrane fouling represents the main limitation for MBRs. It is caused by the interactions between sludge suspension and membrane, where the type of membranes used play key roles in membrane fouling(Shen, et al., 2015). Numerous parameters that influence fouling must be considered since fouling phenomena have been shown as a complex interaction of hydrodynamics (orthogonal and parallel to the membrane surface), mass transfer, biological state, and prevailing compounds (Kraume and Drews, 2010)
Significant advances in understanding fouling of individual components such as bacteria, yeast, proteins, and colloids have occurred in microfiltration and ultrafiltration. While some broad trends for simple colloids are valid for macromolecules (the most commonly studied of which are proteins), the labile nature of proteins and range of polydispersity of naturally occurring macromolecules such as polysaccharides and humic substances add a particular complexity to the fouling mechanisms. In addition, the interaction between the suspended colloids or those in the deposited "cake" in a mixed species environment has the potential to significantly change the nature of the foulant layer in terms of resistance and reversibility.(Le-Clech, et al., 2006)
Membrane fouling can be classified according to: (i) relative location to the membrane structure, (ii) characteristics of the foulants (biological or chemical) and (iii) attachment strength of the fouling materials. A description of membrane fouling classification can be found Table 3-1.

Table 3-1 ***Membrane fouling classification***

Relative location to the membrane structure	Concentra-tion polarization (CP)	Accumulation of solutes or particles in a thin liquid layer adjacent to the membrane surface. Inherent phenomenon of membrane filtration
	External fouling or fouling layer	Deposition of particles, colloids and macromolecules on the membrane surfaces.
	Internal fouling or pore blocking	Adsorption and deposition of solutes and fine particles within the internal structure of membranes, e.g. pore narrowing or blocking.
Biological and chemical characteristi cs of membrane foulants	Biofouling	Deposition and growth of microorganisms on membrane surfaces.
	Organic	Deposition of proteins, polysaccharides, humic acids and other organic substances (either soluble or colloidal) originated from feed water or microbial secretion
	Inorganic	Chemical precipitation of inorganic crystals and/or biological precipitation of inorganic-organic complexes

Attachment strength of fouling materials	Reversible	Loose attachment of fouling materials to membrane surfaces, which can be removed by physical cleaning method, e.g., relaxation, a strong shear force or backflush.
	Irreversible	Strong matrix of fouling layer with solutes which cannot be removed with physical methods. , e.g., formation of gel layer, pore narrowing or blocking
	Residual	Cannot be removed by chemically enhanced backflush or maintenance cleaning but can be removed by recovery cleaning
	Irrecoverable	It is not readily removed by typical chemical cleaning, permanent fouling builds up over a number of years and might ultimately determine membrane life

Adapted from (Wang, et al., 2014)

3.2.1. Extracellular polymeric substances

Extracellular polymeric substances (EPS) are the construction materials for microbial aggregates such as biofilms, flocs and activated sludge liquors, which can be located at or outside the cell surface. Soluble EPS which are also called Soluble Microbial Products (SMP) are components that are released into solution from substrate metabolism (usually with biomass growth) and biomass decay (Meng, et al., 2009). Different classes of macromolecules belong to this groups such as polysaccharides, proteins, nucleic acids, phospholipids and other polymeric compounds. Due to its heterogeneous and changing nature, EPS can form a highly hydrated gel matrix in which microbial cells are embedded. Therefore, they can be responsible for the creation of a significant barrier to permeate flow in membrane processes. Finally, bioflocs attached to the membrane can play a major nutrient source during the biofilm formation on the membrane surface (Le-Clech, et al., 2006)

3.3. Transmembrane pressure and permeability

By definition, Transmembrane pressure (TMP) is the difference between feed and permeate side pressure. Considering the pressure drop along a membrane module, it can be written as Equation 3-1:

Equation 3-1 *Transmembrane pressure*

$$TMP = \frac{P_{feed} + P_{retentate}}{2} - P_{permeate}$$

However, often only one pressure transducer on the permeate side is used and the feed pressure is taken as the initially recorded pressure before permeation.(Drews, 2010)

Permeability (K) can be calculated then with the following equation:

Equation 3-2 *Permeability*

$$K = \frac{J}{TMP}$$

Where:
J: Flux

3.4. Aeration technologies

The aeration in MBRs is generally provided by fine bubble aerators, used to keep the content of the aerobic tank well mixed and provide oxygen to the biomass. In addition, in submerged MBRs, coarse bubble aerators situated under the membrane modules are used to scour and/or gently agitate the membranes in order to control membrane fouling (Germain and Stephenson, 2005).
Aeration systems are used to transfer oxygen into the liquid media. Oxygen is used in aerobic processes for growth, maintenance and in other metabolic routes. The concentration of dissolved oxygen in the suspension of microorganisms, depends on the oxygen transfer rate OTR from the gas to the liquid phase, and on the rate of its consumption by the microorganism, the oxygen uptake rate (OUR) (García-Ochoa, et al., 2010). Biomass characteristics, influent characteristics, aeration system, tank geometry and operational conditions are known to have an effect on the oxygen transfer. In relation to biomass characteristics, particle concentration, particle size and viscosity are three parameters interrelated with aeration. The aeration intensity affects the particle size and the viscosity, while solids concentration modifies the viscosity.
Oxygen transfer rate is affected by other factors as temperature and pressure and can be calculated using the following equation:

Equation 3-3 *Oxygen transfer rate.*

$$OTR = K_L a\ (DO - DO_{sat})\ x\ V \qquad (KgO/h)$$

Where:
$K_L a$: liquid − side mass transfer coeficient (h)
DO: dissolved oxygen in water (kgO_2/m^3)
DO_{sat}: dissolved oxygen in water at saturation kgO_2/m^3)
V: water volume (m^3)
Another parameter used to define the oxygen transfer in biological aerated system is the Alpha - factor (α), which can be calculated by the following equation:

Equation 3-4 *Alpha - factor.*

$$\alpha = \frac{\alpha \mathrm{SOTE}}{\mathrm{SOTE}}$$

Where:
SOTE: oxygen transfer efficiency at standards conditions (20°C, 1 atm)(%)
α SOTE: oxygen transfer efficiency in process water at standard conditions (%)

3.4.1. Coarse-bubble aerators

Coarse-bubble systems have been applied for membrane scouring because they increased turbulence and hence shear forces. They are characterized by having a very turbulent nature and a less severe surfactant interfacial accumulation. One of the advantages of coarse bubble diffusers is that due to their high turbulence they are less affected by fouling and scaling than other types of aeration systems. Coarse-bubble aerators are characterized by low oxygen transfer efficiency because due to the big size of the bubbles they travel rapidly through the water column.

3.4.2. Fine-bubble systems

Fine bubbles diffusion has been used for biomass aeration due to the enhanced oxygen transfer. The most common bubble diffusers are fine pores because of it higher aeration efficiency. One of the disadvantages is that these systems require periodic cleaning and have a lower alpha factor than coarse bubble diffusers. Fine-pore diffusers systems have high oxygen transfer efficiency as smaller bubbles result in more bubble surface area per unit volume and greater oxygen transfer exchange. The aeration systems characteristics applied in MBR are described in the following table:

Table 3-2 Aeration systems in MBRs

	Fine bubbles	Coarse bubbles
Bubble size	2-5 mm	6-10 mm
OTE (percentage of O_2 transfer per m depth)	3-10 %	1-3 %
Mechanical component	Air blower	Air blower
Diffuser type	Ceramic or membrane diffuser disk, come or tube	Steel or plastic disk or tube
Shear rate	Bubble velocity α d^2 (stokes Law) Small bubbles sizes provide lower velocity and hence smaller shear forces	Bubble velocity, and so shear, is higher than fine bubbles aeration since the larger bubbles rise faster than small bubbles

Adapted from (Judd and Judd, 2006)

3.4.3. SDOx technology

SDOx technology works by saturating a side-stream of a process with oxygen and re-injecting the supersaturated solution back into the main flow for an effective mixing and distribution of it in the process.(Librán-Vázquez, 2015)
First, oxygen is injected into a pressurized saturation chamber. Then, water is sprayed through the pressurized oxygen and is instantly supersaturated with dissolved oxygen, up to 350 mgDO/L is obtained. This supersaturated stream is rapidly reintroduced into the treatment reactor. Since the SDOX pre-dissolves oxygen into a side-stream of the flow, no bubbles are formed, allowing the oxygen injected to remain at the desired depth with no bubbles rising through the water column. (BlueInGreen, 2015)
Figure 3-2 illustrates how the SDOX works.

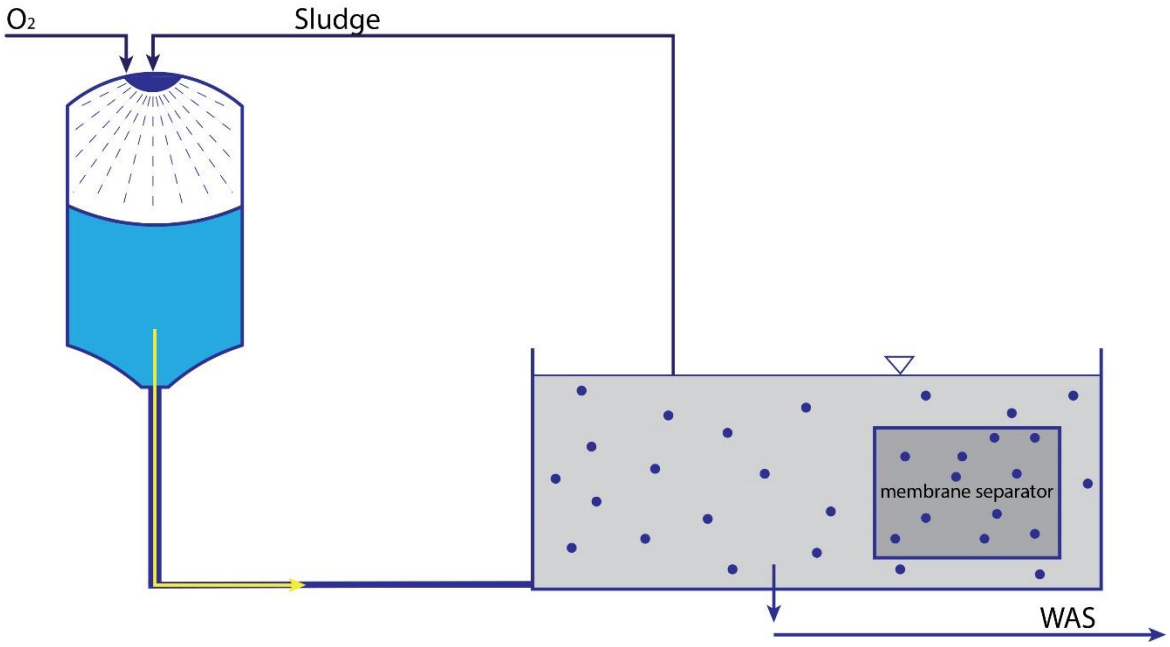


Figure 3-2 *MBR with SDOx unit*

Some of the advantages of SDOx system are:

- High efficiency DO delivery resulting in lower operating costs
- Increased flexibility over where DO is delivered so critical locations can be treated
- No degassing so the DO delivered to the water remains bio-available
- The SDOX can be used for long-term treatment to provide excess oxygen for natural removal of organic pollutants
- Ability to direct inject into the main process flow piping eliminates the need for large concrete basins typically required for bubble diffusion – and, uses ~40% less O_2 than typical bubble diffusion applications
  (BlueInGreen, 2015)

3.5. Emergent contaminants

Emerging contaminants, also termed as, micropollutants consist of a vast and expanding array of anthropogenic as well as natural substances. These include pharmaceuticals, personal care products, steroid hormones, industrial chemicals, pesticides and others compounds. This group of compounds are present in waters at trace concentrations, ranging from a few ng/L to several μg/L.

Current wastewater treatment plants (WWTPs) are not specifically designed to eliminate emergent contaminants. As a result, many compounds have been found in different water bodies (groundwater, drinking water, waste water).

The occurrence of micropollutants in the aquatic environment have been frequently associated with a number of negative effects, including short-term and long-term toxicity, endocrine disrupting effects and antibiotic resistance of microorganisms(Luo, et al., 2014).Some countries or regions have adopted regulations for a small number of micropollutants (eg nonylphenol and derivatives). However, other micropollutants such as pharmaceutical are not included in the list of regulated substances yet. (Luo, et al., 2014)

3.5.1. Pharmaceuticals

Pharmaceutical are one of the groups of emerging contaminants more versatile and reported to date. Among different harmful effects, a special attention has been paid to the assessment of environmental risks associated with the widespread occurrence of antimicrobials in the aquatic environment. One of the key issues is the possible importance of the aquatic route for the spreading of antibiotic resistance. One strategy to limit proliferation of resistant bacteria is to reduce the exposure to antimicrobials by improving their removal from wastewater. (Senta, et al., 2011)

On previous studies conducted by Larsson et al. (2007) high concentration of pharmaceuticals where found in the aquatic environment as a result of the wastewater discharge from pharmaceutical production facilities. Fluoroquinolone ciprofloxacin was detected in receiving ambient waters at extremely high concentration, reaching into mg per liter range (Larsson, et al., 2007). Similar problem was reported in Croatia in a small water course that received combined wastewater effluents from the baker's yeast factory and production of macrolide antibiotic azithromycin. (Senta, et al., 2011)

3.5.2. Azithromycin

Azithromycin is an antibiotic useful for the treatment of a number of bacterial infections. Azithromycin prevents bacteria from growing by interfering with their protein synthesis. It binds to the 50S subunit of the bacterial ribosome, thus inhibiting translation of mRNA. This compound is an acid-stable antibiotic which half-life allows a large single dose to be administered and yet maintain bacteriostatic levels in the infected tissue for several days. Following a single dose of 500 mg, the apparent terminal elimination half-life of azithromycin is 68 hours. Biliary excretion of azithromycin, predominantly unchanged, is a major route of elimination. Over the course of a week, about 6% of the administered dose appears as unchanged drug in urine.

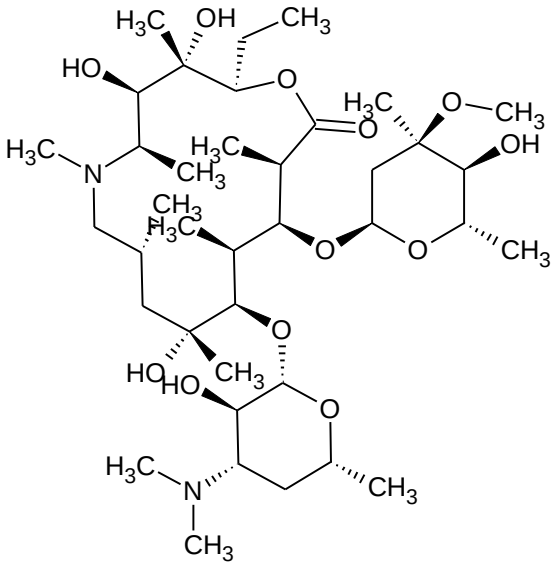


Figure 3-3 *Azithromycin*

Because of the physico-chemical properties mentioned above it can be noticed that is really difficult to remove this compound in a conventional wastewater treatment. Couple with it widespread prescription makes it important to evaluate technologies to remove this kind of pollutants.

Studies by (Senta, et al., 2011)showed an efficient removal of macrolide antibiotics using MBR which was strongly affected by the hydraulic retention time. The impact of other parameters on removal efficiency was not pronounced. High elimination efficiencies were obtained at different compositions of synthetic wastewater, with drastically changing carbon and nitrogen loads (Senta, et al., 2011)

CHAPTER 4

Experimental Approach

During this research, the influence of SDOX unit over biological processes, sludge characteristics, membrane fouling and azithromycin removal was evaluated. In order to asses it, the following five experiments were carried out using conventional aeration system and SDOX unit. This chapter presents a description of the experimental setups, all the parameters that were measured and the media for performing the experiments

4.1. Experiments

In this research, five experiments were carried out by operating a pilot MBR of 23 L continuously. The following chart summarizes some of the parameters for each experiment.

Table 4-1 *Summary experiments.*

Experiment	MLSS (g/L)	Flux (L/h.m^2)	Bubble diffusers acclimatization (days)	Bubble diffusers operation (days)	SDOX acclimatization (days)	SDOX operation (days)	Influent spiked with azithromycin
1	10	15	-	5	-	5	NO
2	10	25	4	2	3	3	NO
3	10	25	3	5	2	4	YES
4	20	15	3	3	3	3	NO
5	20	15	3	3	3	3	YES

SDOX's effect over biological processes was evaluated by studying several constituents such as: COD, nitrate, ammonia, and phosphate in the influent and permeate.
On the other hand, in order to evaluate its effect over sludge characteristics other parameters were studied. DSVI, particle size distribution and extracellular polymeric substances were measured and compared for the different experiments.
Its effect over membrane fouling was assessed by calculating the loss in permeability after operation. Besides, during the experiments transmembrane pressure was recorded constantly. The results were compared with the results obtained by operating the same system with conventional aeration.
Finally, the influence of SDOX over azithromycin removal was evaluated by extracting it from sludge samples, and measuring its concentration in the influent and effluent. Moreover, some biotransformation products of azithromycin were measured, identified and quantified.

4.2. MBR Layout

This section presents the two setups used in the experiments. Figure 4-1 shows the layout for the experiments carried out with buble diffusers. The picture presents: influent and effluent pumps, the membrane, the bubble diffuser, the pressure gauge and the PLC.

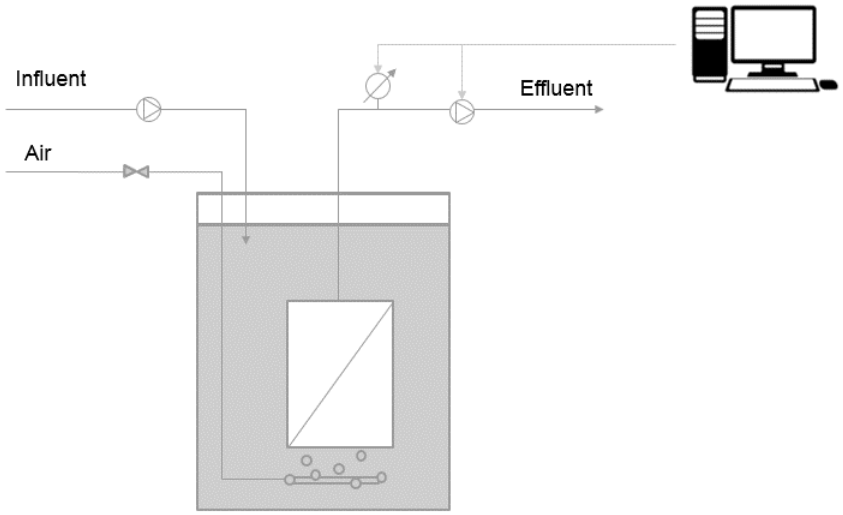


Figure 4-1 *System layout of MBR with conventional aerators.*

Figure 4-2 illustrates the layout for the experiments carried out with SDOX unit. The influent, effluent and recirculation pumps, membrane bioreactor, SDOX discharge valve, SDOX unit, pressure gauge and PLC are shown.

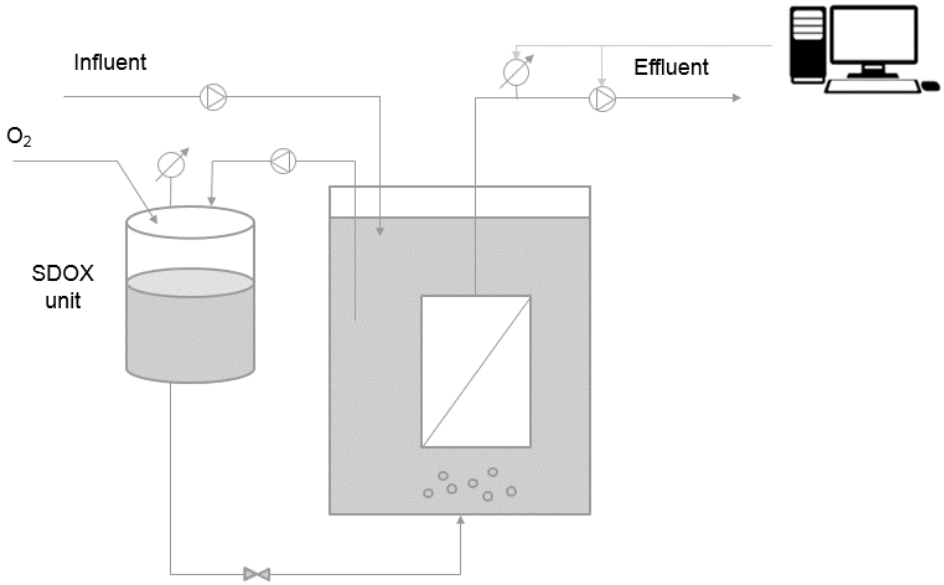


Figure 4-2 *System layout of MBR with SDOx system.*

4.3. Procedure

For the startup of the reactor, the sludge used as inoculum was collected from a full scale wastewater treatment plant. It was sampled from the aeration basin at a concentration of 3.5 g/L. Afterwards it was sieved through a 0.9 µm sieve to eliminate bigger particles that could obstruct the valves or pumps in the system. Finally, it was concentrated by gravity settling to achieve a concentration of 10 g/L for experiments 1,2 and 3. Moreover, for experiments 4 and 5 a final concentration of 20 g/L MLSS was required.

Experiments 1, 2 and 3

A continuous MBR system was assessed operating it with two different aeration technologies: bubble diffusers and SDOX unit. Each system was operated at a MLSS concentration of 10 g/ L. The lab scale system was composed by a vessel of 31 L volume, operated at a volume of 23 L. The reactor was fed with synthetic wastewater described in 4.6., at a flow rate of 40 L/day or 66 L/day depending on the desired flux.
The influent was prepared in order to reach a concentration of biochemical oxygen demand that allows a MLSS concentration of 10 g/L inside the reactor. The SRT was fixed at 20 days. The oxygen demand for the reactor was 76,7 g O_2/day and was calculated with Ekamas steady state model as described in Appendix A (Henze, 2008).
DO in the tank, pH, TMP were registered every day. VSS, TSS, DSVI and proteins were measured three times a week. Regarding to the effluent quality COD, NO_3, NH_4, PO_4 were measured regularly.
Membrane fouling was evaluated by measuring changes in the TMP on a daily basis. Furthermore, the loss in permeability after operation was also calculated following the procedure described in 4.13. The removal efficiency for azithromycin was assessed by extracting and quantifying the compound and its possible metabolites from samples in the influent and in the permeate. Following the procedure described in 4.13.

For each experiment fresh sludge was collected from the wastewater treatment plant and concentrated to a desired concentration. Firstly, it was exposed to bubble diffusers for a period of 6-8 days, followed by the exposure to SDOX aeration during the last 6-8 days of the experiment.(Figure 4-3)

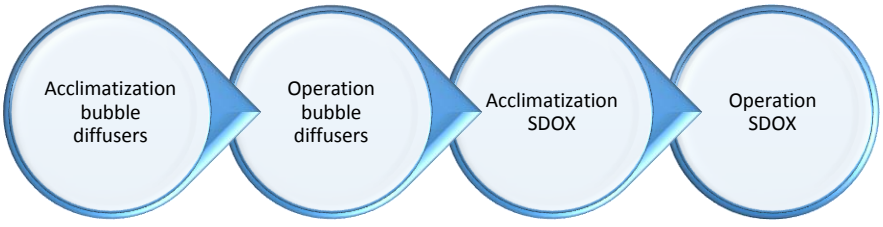


Figure 4-3 *Experimental phases.*

In order to be able to ensure that the differences observed in the systems that were compared were due to influence of SDOX, all the parameters were controlled. Enough food, aeration and mixing were provided for the microorganisms. Furthermore, an acclimatization phase before each operation phase was defined.

Acclimatization phase: For practical purposes, the end of this phase was assumed when values of removal for COD in the permeate were higher than 95%. It lasted 3-5 days depending on the experiment.

Operation phase: was defined after each acclimatization phase. It lasted between 2-5 days for each experiment. A full description was shown in Table 4-1 Summary experiments.

The first day of each phase the membrane was changed and the initial permeability was measured in demineralized water. Membranes used were completely new membranes as well as membranes already used that were cleaned with a chlorine solution before operation. The last day of operation remain permeability in water was measured in order to calculate the loss in permeability.

Experiments 4 and 5

For these experiments influent was prepared and fed in order to reach a concentration of biochemical oxygen demand that allows a MLSS concentration of 20 g/L inside the reactor. A full description is found in Appendix A.
The oxygen demand for the lab scale system was of 86.6 g O_2/day and was calculated with Ekamas steady state model as described in Appendix A.
The K_{LA} for the system was measured as described in Appendix A, the OTR was 61.8 g O_2/day. As a result, a second diffuser was needed to achieve DO concentration above 2 mgO_2/L during the operation.

The differences between Experiments (1,2,3) and Experiments (4, 5) were:

-MLSS concentration
-Fluxes
-Azithromycin concentration
A full description is shown in Table 4-1 Summary experiments.

4.4. Membrane bioreactor

A pilot Kubota MBR of a total volume of 31 liters (14 × 22 × 103) cm was used (Figure 4-4). The MBR had three immersed flat sheet membranes modules XJ3 by Kubota. The membranes were made of chlorinated polyethylene with a filtration area of 0.11 m^2 each and an average pore size of 0.4 µm. The system was fed with the synthetic wastewater described in 4.6. Organics components were autoclaved and added by gravity using a medical infusion set with a valve to regulate the flow. Inorganic components of synthetic wastewater were prepared in a 200 L tank and pumped into the system by a Lab Metering Pump (FMI PM6014 RHV). Pump flow rate was controlled by a level sensor located inside the reactor in order to achieve a

constant level. During each experiment, three membranes were placed inside the reactor. However, filtration was conducted only through the one located in the middle while the others two served as a barrier to ensure good air scouring. Air scouring was conducted by pumping air at ten centimeters under the membranes through a diffuser connected to a HP80 air blower. For experiments 1,2 and 3 this was enough to provide oxygen to the biomass. For experiments 4 and 5 a second diffuser was needed in order to achieve DO concentrations above 2 mg/L which was located outside of the outer membranes in order to maintain constant membrane scouring rate. A gauge band Ashcroft 2274, with a measuring range from -1 to 1 Bar was connected to the membrane in operation. Moreover, the digital pressure gauge was connected to a PLC which was recording the pressure constantly during the operation. At the bottom of the MBR was located a discharge valve for waste sludge removal to control the SRT. The same valve was open during the SDOX experiments and discharge from the SDOX vessel was pumped through that pipe back to the bioreactor.

Figure 4-4 *MBR and SDOX unit.*

4.5. SDOX unit

The SDOX unit was a 4 liters vessel which was filled with 2.5 L of sludge. It was continuously filled with a NEMA 4X IP66 continuous tubing pump, and the discharge from the vessel was intermittent through a ball valve whose opening and closing was performed by using pressure provided by a compressor. The discharge valve was programmed to open one millisecond each 34 seconds and ensure a constant level inside the pressure vessel. The approximate volume of one discharge was 102 mL. The SDOX vessel was also connected to a pressurized tank with pure oxygen which maintained the pressure inside the SDOX vessel at 5 Bars. Pressure vessel had therefore a headspace filled with pure oxygen at five bars.

4.6. Media

The synthetic wastewater consisted of stock solutions A and B (Experiments 1, 2 and 4) or stock solutions A, B and C (Experiments 3 and 5).

Solution A. Organics source

A solution with glucose, acetate and peptone (Table 4-2) was prepared weekly with the appropriate concentration of biochemical oxygen demand in order to keep constant MLSS concentration in the reactor. Concentration and flow rate was estimated using steady state system equations (Henze, 2008) This solution was autoclaved to ensure stability. In Experiments 1,2 and 3 500 mL of this solution was provided daily to the biomass. For experiments 4 and 5, 1000 mL was added.

Table 4-2 *Organics source.*

	Glucose	**Acetate**	**Peptone**	**Total**
$[COD]_{final}$ (mg/L)	1123.5	819	931	**2873.5**

Solution B. Inorganics nutrients and trace elements source

To provide the micronutrients and inorganics compounds seven stock solutions were prepared separately to avoid precipitation. The stock solutions were added in a 200 L tank and diluted with demineralized water. This solution was prepared each three days. A summary of the components for each liter of inorganics is summarize in the following Table 4-3 Inorganics source.

Table 4-3 *Inorganics source.*

Compound	M element (g/mol)	Concentration of the element in synthetic ww (mg/L)
$NaHCO_3$	61.0	536.00
$MgSO_4.7H_2O$	24.3	36.00
$CaCl_2$	40.1	60.00
NH_4Cl	14.0	35.00
KH_2PO_4	31.0	11.76
$FeCl_3.6H_2O$	55.8	0.21
$NiSO_4.7H_2O$	58.7	0.11
$ZnSO_4.7H_2O$	65.4	0.21

$Na_2MoO_4.2H_2O$	96.9	0.01
$CuSO_4$	63.5	0.20
$MnCl_2.4H2O$	54.9	0.70
$CoCl_2.6H2O$	58.9	0.10
H_3BO_3	10.8	0.01

Solution C. Azithromycin

During experiments 3 and 5 appropriate volume of a stock of azithromycin in ethanol was also added to the 200 L tank together with Solution B.
Because these two experiments were performed at different fluxes, different concentrations of azithromycin were added to ensure the same daily load. The influent was spiked with 1 mg/L and 1,65 mg/L of azithromycin for experiments 3 and 5 respectively.

4.7. Sludge thickening

The sludge needed to be thickened in order to achieve the required experimental concentration. Sludge was allowed to settle in a container (Figure 4-5), and the supernatant was removed in order to concentrate the sludge in the desired concentration. However, for high concentrations of sludge the sludge needed to be filtered using membranes with a pore size of 0.45 μm until the desired concentration was achieved.

Figure 4-5 *Sludge concentration.*

4.8. COD, Total Suspended Solids

COD was measured by SM- 5220D
Suspends solids (TSS) and volatile suspended solids (VSS) concentrations were determined according to standard methods for examination of water and wastewater SM-2540D.

4.9. Nitrate, ammonia and phosphate

Nitrate was measured using Nitrate Test. Merck set number 1.09713.0001, analogous to DIN 38405-9.
Ammonia was measured according to ISO 7150-1, based on Indophenol Blue method.
Phosphate was measured using Spectroquant method Merck set number 1.14848.0001, analogous to DIN EN ISO 6878.

4.10.Sludge Volume Index

Sludge volume index (SVI) is typically used to monitor settling characteristics of activated sludge. For addressing settleability a sample was taken every day and the settleability was determined by performing DSVI tests.
Settleability test were done by calculating the sludge volume index (SVI). The procedure for calculating the SVI is described as follows:
1.0 L of sample was placed in a settling column and the solids were distributed by covering the top of it and inverting the cylinder three times. A stirring rod was inserted and the stopwatch was started maintaining the temperature equal to the temperature that is the reactor operating. The volume of the suspension was measured a different time intervals and then the settled sludge volume in millimeters was reported for that time interval.

Equation 4-1 *Sludge volume index*

$$SVI = \frac{settled\ sludge\ volume\ (mL/L)\ \times\ 1000}{X_{dil}(mg/L)}$$

4.10.1. Diluted Sludge Volume Index

The Diluted Sludge Volume Index (DSVI) is defined as the volume (mL) occupied by 1g of sludge after 30 min of settling in a 1-litre unstirred measuring cylinder with the condition that 150<DSV_{30}<250 mL/L. (Figure 4-6)

Equation 4-2 *Diluted sludge volume index.*

$$DSVI = \frac{DSVI_{30}\ (mL/L)\ \times\ 1000}{X_{dil}(mg/L)}$$

Where:
$DSVI_{30}$: settled volume (mL) of sludge after 30 minutes of settling after the necessary number of dilutions have been made to obtain 150<DSV30<250 ml/l in a 1 liter unstirred cylinder
X_{dil}: MLSS concentration (g/L) in the test cylinder after the necessary dilutions have been done.

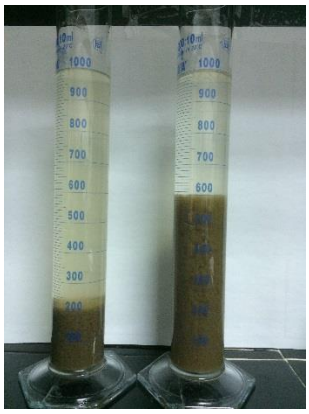


Figure 4-6 *DSVI test.*

4.11. Particle Size Distribution

Particle size distribution was measured using a Mastersizer 2000 (Figure 4-7).
Mastersizer 2000 measures and represents the volume-weighted distributions which is a statistical distribution of particles of different sizes. The technique it uses is a static light scattering, more specifically laser diffraction. Laser diffraction measures particle size distributions by measuring the angular variation in the intensity of the light scattered as a laser beam passes through a dispersed particulate sample. Large particles scatter light at small angles relative to the laser beam and small particles scatter light at large angles. The angular scattering intensity data is then analyzed to calculate the size of the particles responsible for creating the scattering pattern, using the Mie theory of light scattering. The particle size is reported as a volume-equivalent sphere diameter.

Figure 4-7 *Mastersizer 2000.*

4.12.Extracellular polymeric substances

Extraction method

According to the procedure mentioned by (Le-Clech, et al., 2006)

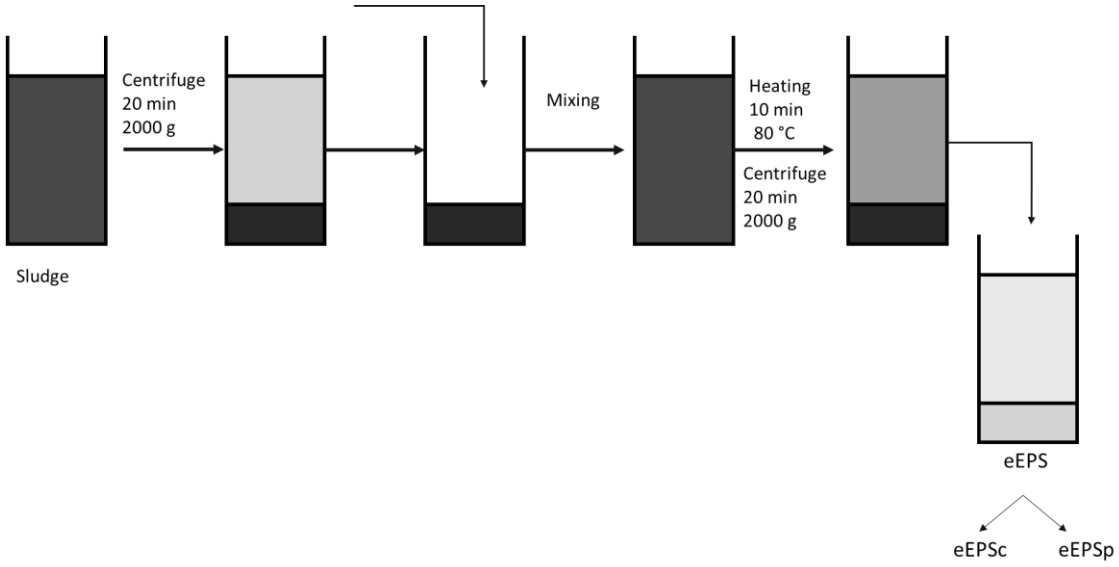


Figure 4-8 *Proposed method for EPS extractions and measurements.*

Extracted from (Le-Clech, et al., 2006)

The solution containing eEPS were then characterized by its relative content of protein (eEPSp) and carbohydrate (eEPSc), measured by photometric methods, (Lowry, et al., 1951) and (Dubois, et al., 1956) respectively.

EPS quantification, protein fraction (Lowry method)

The following solutions were prepared:
Reagent A: $NaCO_3$ (Fluka, Lot 139781933008295) 2% in 100 mL NaOH 0.1 N.
Reagent B: $CuSO_4.5H_2O$(Merck, Lot 2790) 0.5 % in 1 % sodium tartrate.
Reagent C: 50 mL Reagent A + 1mL Reagent B (This solution was discard after one day)
Reagent D: Folin Reagent Diluted (1:3) (Kemika, Lot 13927)
A stock solution of Bovine Serum Albumin (Sigma Aldrich, Lot #SLBM3734V) was prepared each time proteins were measured.

The solution to be measured was prepared transferring 0.2 mL of sample or standard solution into a 4.0 mL glass tube. After, 1 mL Reagent C was added and mixed followed by 10 minutes of incubation at room temperature in the dark. Afterwards, 0.3 mL of Reagent D was added.

The reaction took place in the dark during 30 minutes. The samples were transfer to a micro cuvette and absorbance was recorded at λ=750 nm.

EPS quantification, carbohydrate fraction (Phenol sulfuric acid method)

The solution to be measure was prepared transferring 0.9 mL of sample or standard solution into a 7.0 mL glass tube. After, 0.45 mL of phenol (Sigma Aldrich, Lot #MKBV9724V) was added, followed by 2.0 mL of sulfuric acid (Kemika, Lot 1816501). Solutions were mixed and incubated for 20 minutes in a water bath at 30 ºC. Afterwards, absorbance was measured for each sample at λ= 490 nm.(Figure 4-9)

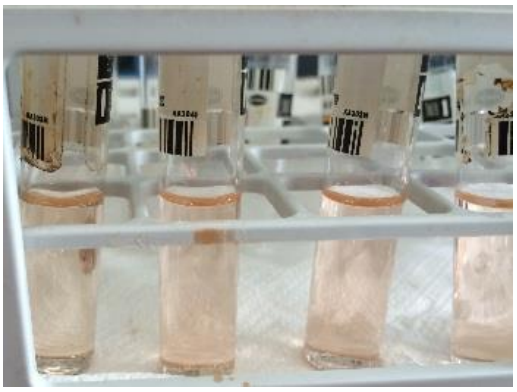


Figure 4-9 *EPSc quantification.*

4.13. Azithromycin measurement

Sampling

On a daily basis were collated samples of influent and effluent which were frozen at -4 ºC for subsequent quantification (Figure 4-10).
On the other hand, samples of sludge were also taken daily. The extraction of azithromycin was made by centrifugation of 15mL of sludge at 4500 g during 20 minutes. The supernatant was completely transferred to a measuring cylinder, and the sludge was transferred quantitatively to a glass container with four millilitres of methanol (J.T. Baker, Lot 1602302801). The samples were kept at -4 ºC for subsequent quantification (Figure 4-10).

Figure 4-10 *Influent, effluent, supernatant and sludge samples.*

UHPLC-QTOFMS

The analysis of the samples, including both azithromycin and its potential transformation products, was performed using ultrahigh-performance liquid chromatography (UHPLC)coupled to quadrupole-time-of-flight mass sprectrometry (QTOFMS)(Figure 4-11). UHPLC separation was performed using a Waters Acquity UPLC system (Waters Corp., Milford, MA, USA), equipped with binary solvent delivery system and autosampler. The chromatographic separations employed a column (50 mm x 2.1 mm) filled with 1.7 µm BEH C18 stationary phase (Waters Corp., Milford, MA, USA). Binary gradients at a flow rate of 0.4 mL/min were applied for the elution. The eluents A and B were 0.1 % HCOOH in water and 0.1 % HCOOH in acetonitrile, respectively. The analysis was performed in positive ionization mode (PI) by applying a following gradient: the elution started at 5% B and, after a 1 minute of isocratic hold, the percentage of B was linearly increased to 50 % in 8 minutes. Te total run-time, including column conditioning to reach initial conditions, was 10 minutes.
The mass spectrometry was performed on a QTOF Premier instrument (Water Micromass, Manchester, UK) using an orthogonal Z-spray-electrospray interface. Drying gas and nebulizing gas was nitrogen, while argon was used as a collision gas in MS-MS experiments. The desolvation gas flow was set to 700 L/h at a temperature of 300 ºC. The cone gas flow was adjusted to 25 L/h, and the source temperature to 120ºC. The capillary and cone voltages in were 3500 V and 30 V, respectively. The instrument was operated in V mode with TOFMS data being collected between m/z 50-1000, applying collision energy of 4 eV. All spectra were recorded using extended dynamic range (DRE) option in order to correct for possible peak saturations and the data were collected in the centroid mode with a scan time of 0.08 seconds and interscan time of 0.02 seconds. In order to ensure maximum accuracy and reproducibility of the system, all acquisitions were carried out using an independent reference spray via the lock spray interface. Leucine encephaline (m/z 554.2615) was applied as a reference mass.
The data were processed using the MassLynx software incorporated in the instrument.

Figure 4-11 *UHPLC-QTOF MS*

4.14. TMP and permeability

A vacuum gauge was placed for measuring transmembrane pressure during the experiments. For each experiment, membrane fouling was assessed by evaluating membrane permeability. For measuring permeability of clean membrane, the MBR was filled with demineralized water, and TMP was measured for different fluxes. Finally, the permeability was calculated as the slope of the linear plot of Flux vs. TMP. The same procedure was followed to measure permeability in demineralized water of the fouled membrane. The difference between the permeability for the fouled membrane and the initial permeability was the loss of permeability after operation.

To be able to evaluate the changes in the system over time, the data collected (TMP) was normalized to the same temperature using the following equation:

Equation 4-3 *Normalization permeability.*

$$K_{20} = \frac{K_T \times \mu^T}{\mu^{20}}$$

Where:
K_{20}: Permeability at 20 º C.
K_T: Permeability at temperature, T.
μ^T: dynamic viscosity of permeate at temperature, T.
μ^{20}: dynamic viscosity of permeate at 20 º C.

CHAPTER 5

Results and discussion

This chapter presents a description and discussion of the results for each of the five experiments.

5.1. Influence of SDOX on biological processes

During Experiment 1 the MLSS concentration inside the reactor was between 11-13 g/L(Figure 5-1), the VSS was between 9-11 g/L, the DO level was between 5-7 mg/L while pH values were between 7.0 and 8.0.

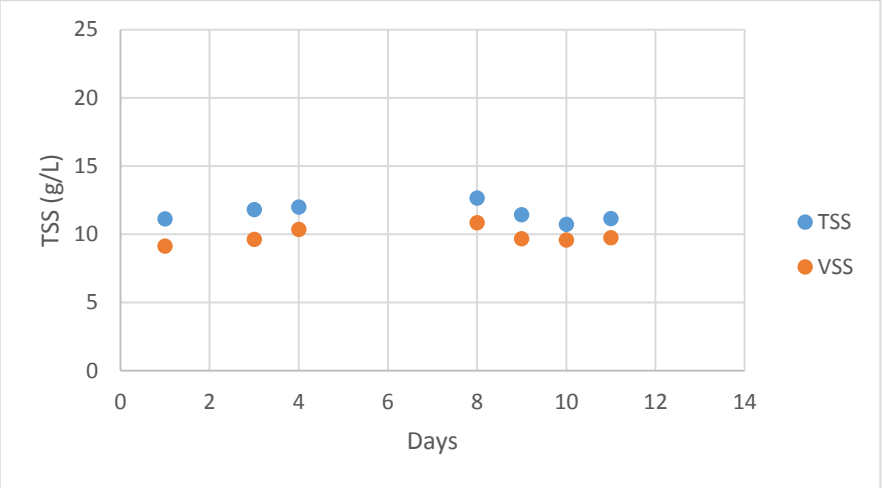


Figure 5-1 *TSS Experiment 1.*

Experiment 1 was performed as a first trial. The main objective of this experiment was to get a constant system. Several difficulties were faced in order to find tuning of the 5 different flows. Among other difficulties most serious were: malfunction of the sensor for the pump feeding the inorganics solution, SDOX vessel leakage, and other leakages in different connections. For this reason, this experiment lasted for ten days, 5 days of operation with bubble diffusers and five days of operation with SDOX. During the experiment the average COD removal was 97% and as it was expected an increase in COD was observed after the unit of SDOX was connected (Figure 5-2). This can be due to sludge flocs breakage after high pressure exposure. Similar observation was previously reported by Bilal (2013) and Librán-Vázquez (2015).

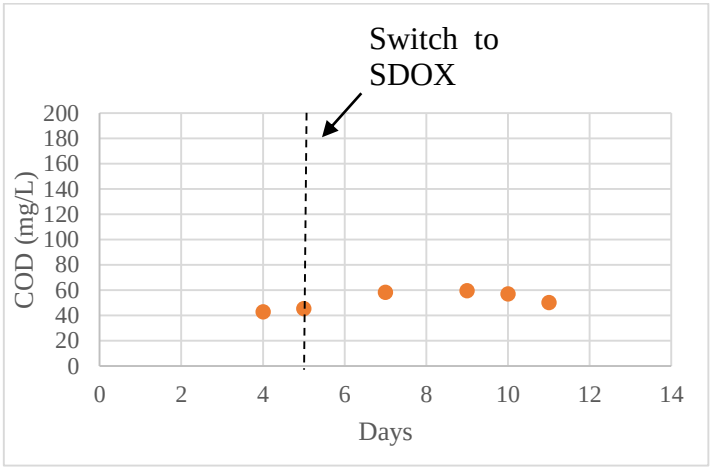


Figure 5-2 *Permeate COD - Profile. Experiment 1.*

For experiments 2,3 4 and 5 was followed the procedure described in 4.3, where an acclimatization phase and an operation phase where defined for each aeration technology.
Acclimatization phase: this phase was included in order to avoid results influenced by bacteria acclimatization to the new conditions. Due to the drastic change of environment in a short period of time, bacteria may have a behavior which does not reflect reality. This can affect the quality of the effluent as well as membrane fouling
Operation phase: was defined after each acclimatization phase.

For Experiment 2, MLSS was between 10-11 g/L (Figure 5-3), the MLVSS was between 8-9 g/L, the DO level was between 6-9 mg/L while pH values were between 7.0 and 8.0. The average COD removal was 96% with again higher values the day after SDOX was connected. However, the increase in COD was not so pronounce and the average COD removal for SDOX phase was also 96% (Figure 5-4).

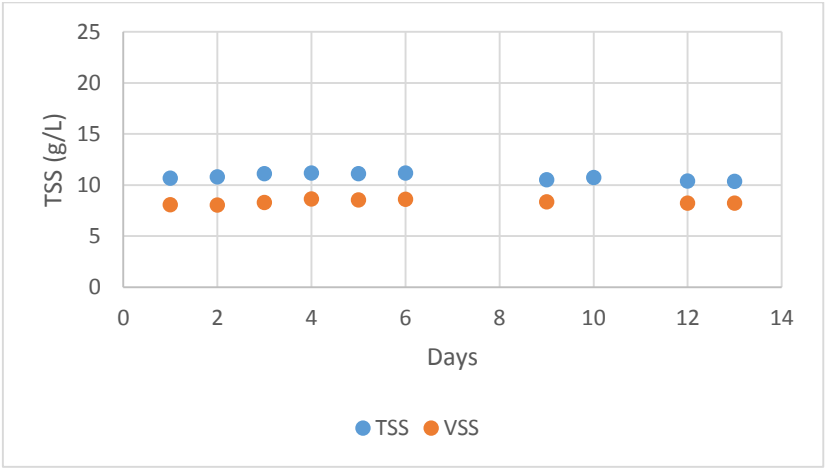


Figure 5-3 *TSS Experiment 2.*

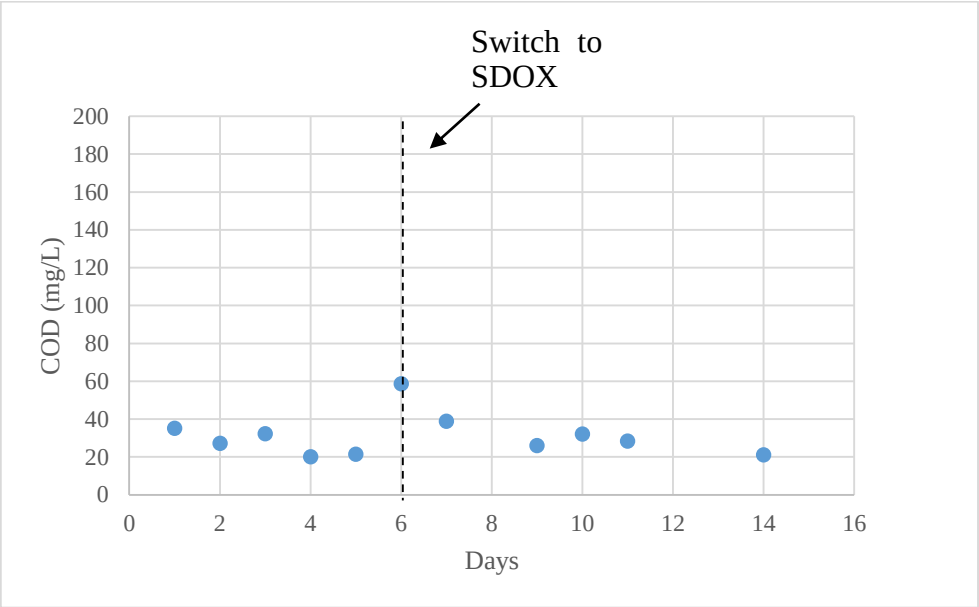


Figure 5-4 *Permeate COD-Profile. Experiment 2.*

As it can be seen in (Figure 5-5) after one day of operation nitrification was achieved, ammonia in the effluent was almost zero during the whole experiment, presenting the highest value 0.5 mg NH_4-N/L after SDOX connection. Even though, nitrifiers growth more slowly, there was no need to wait because they were already present in the sludge used as inoculum. However, the first day nitrate in the effluent was lower than the rest of the days as probably bacteria needed some time to acclimatize to the new conditions.

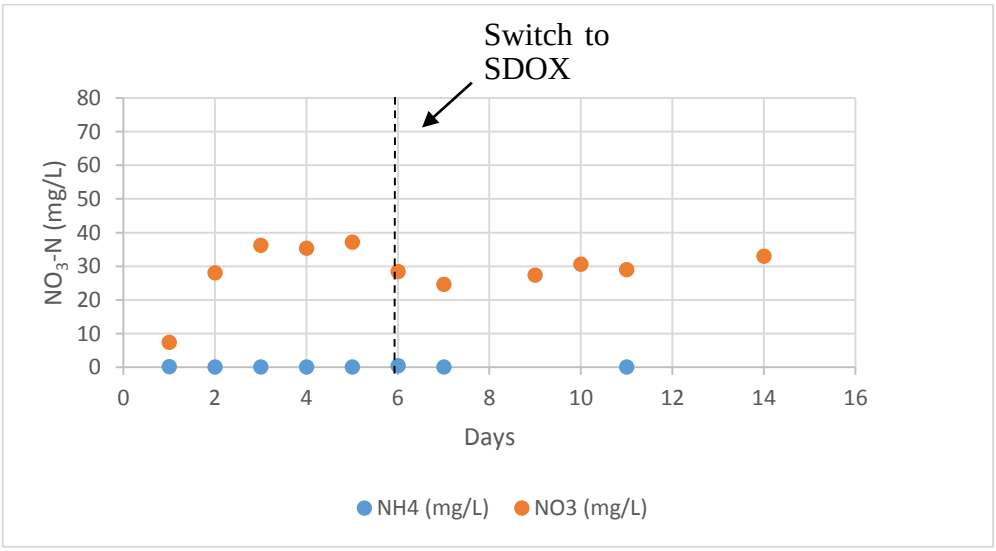


Figure 5-5*.Permeate N-Profile. Experiment 2.*

Regarding to phosphate in the effluent, it followed the same trend as nitrate. Presenting higher uptake at the beginning of the experiment and at the end.(Figure 5-6)

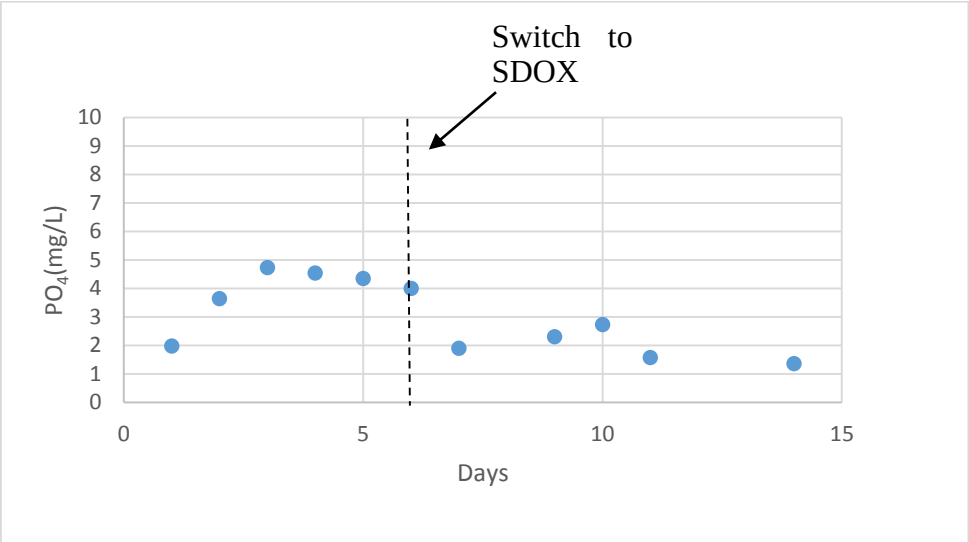


Figure 5-6. *Permeate P-Profile. Experiment 2.*

For Experiment 3 MLSS was between 9-11 g/L (Figure 5-7), the MLVSS was between 7-10 g/L, the DO level was between 7-9 mg/L while pH values were between 7.0 and 8.0. The average COD removal was 93%. The average COD removal was slightly lower for SDOX phase than for bubble diffusers (Figure 5-8). This observation can be explained by the fact that bacteria were dying inside the reactor due to the addition of the antibiotic. One reason could be that a fraction of the antibiotic is adsorbed between the flocs of sludge. Once the SDOX start working it was release due to the breakage of the flocs. Then some bacteria that could grow previously because they were strategically located become exposed to higher doses of antibiotic.

Another reason could be that after some days the amount of the antibiotic inside the reactor adsorbed on the sludge was higher which caused the death of a wider spectra of microorganisms.

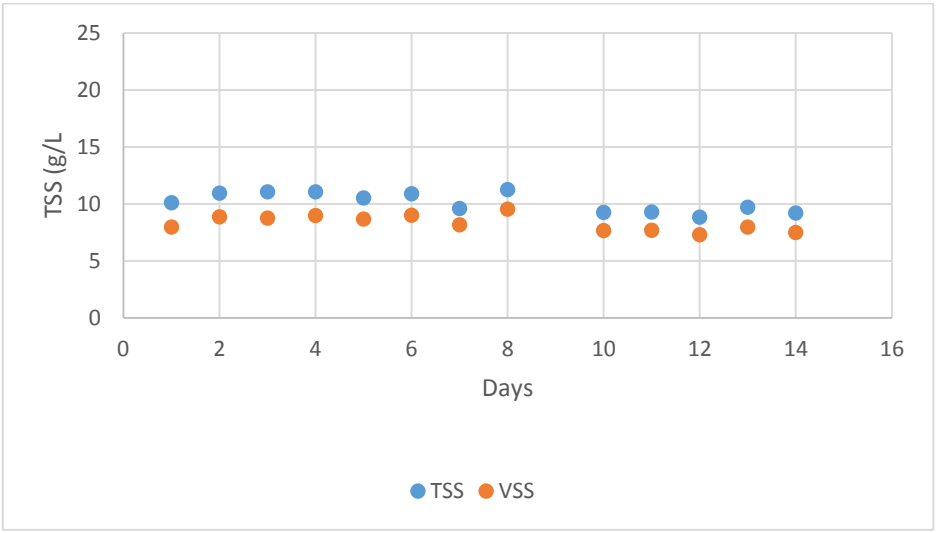


Figure 5-7 *TSS Experiment 3.*

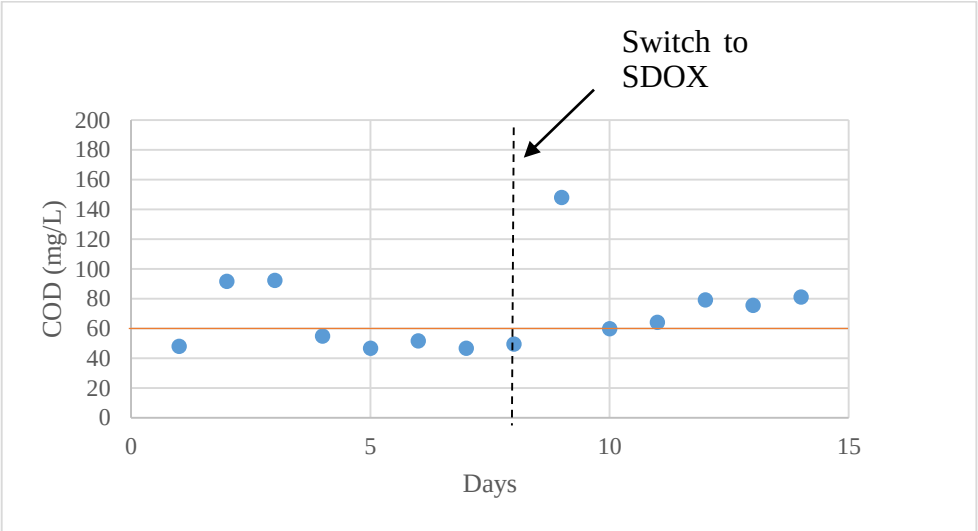


Figure 5-8 *Permeate COD-Profile. Experiment 3.*

Regarding to ammonia and nitrate from Figure 5-9 can be conclude that nitrification was achieved, ammonia in the effluent was almost zero (<0.5 mg NH_4-N/L) during the whole experiment. An increase in nitrate was observed after switching the aeration system which can be attributed to lysis of bacteria and release of nitrogen from cells into bulk liquid which was then nitrified.

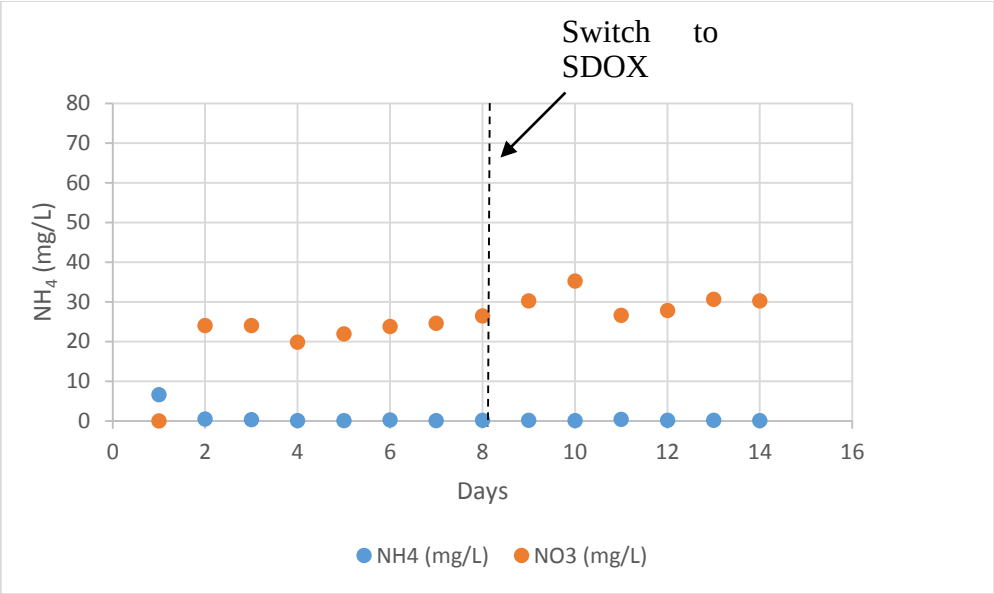


Figure 5-9 *Permeate N-Profile. Experiment 3.*

From (Figure 5-10) can be observed that phosphate was released after day 8 which contributes for the explanation of increase of cell lysis after SDOX was introduced.

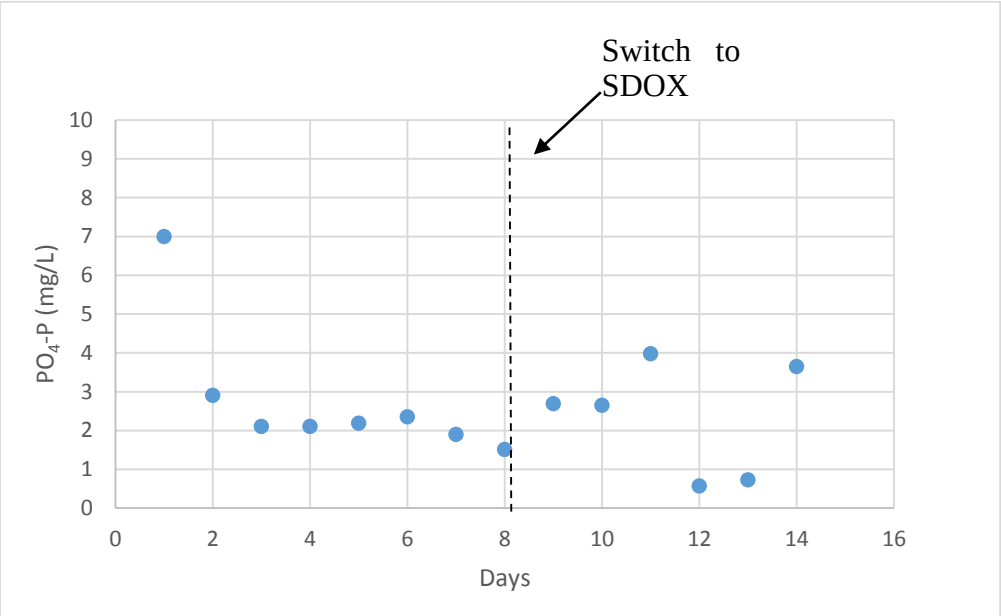


Figure 5-10 *Permeate P-Profile. Experiment 3.*

Experiment 4 presented MLSS values between 19-22 g/L (Figure 5-11), the VSS was between 15-18 g/L, the DO level was between 3-5 mg/L while pH values were between 7.0 and 8.0. The average COD removal was 96%. Even though the removal efficiency is similar to Experiment 1,2 and 3 the COD in the effluent was higher, an average of 117mgO_2/L (Figure 5-12). This difference can be explained due to the higher MLSS concentration inside the reactor. The death regeneration process in which cells result in generation of some soluble biodegradable substrate available could have contributed to a higher value of COD in the effluent.

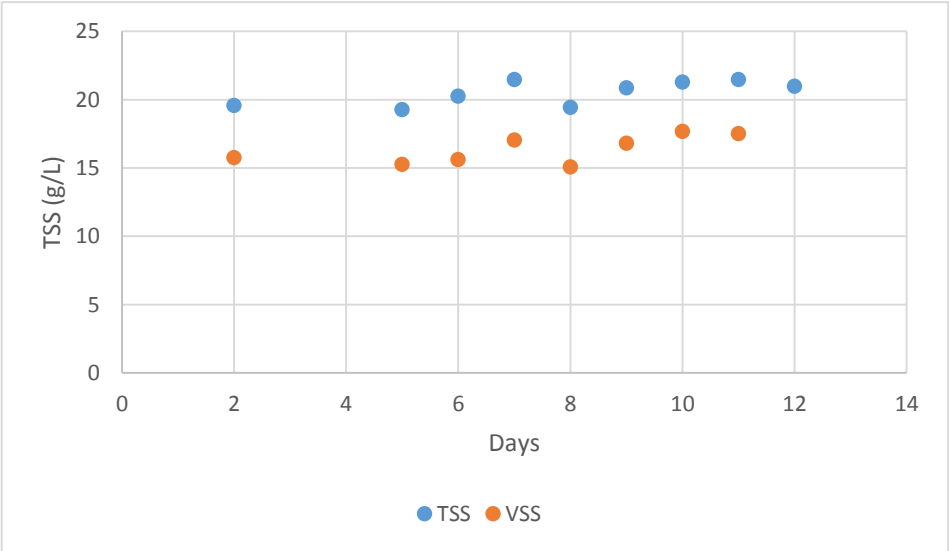


Figure 5-11 *TSS Experiment 4.*

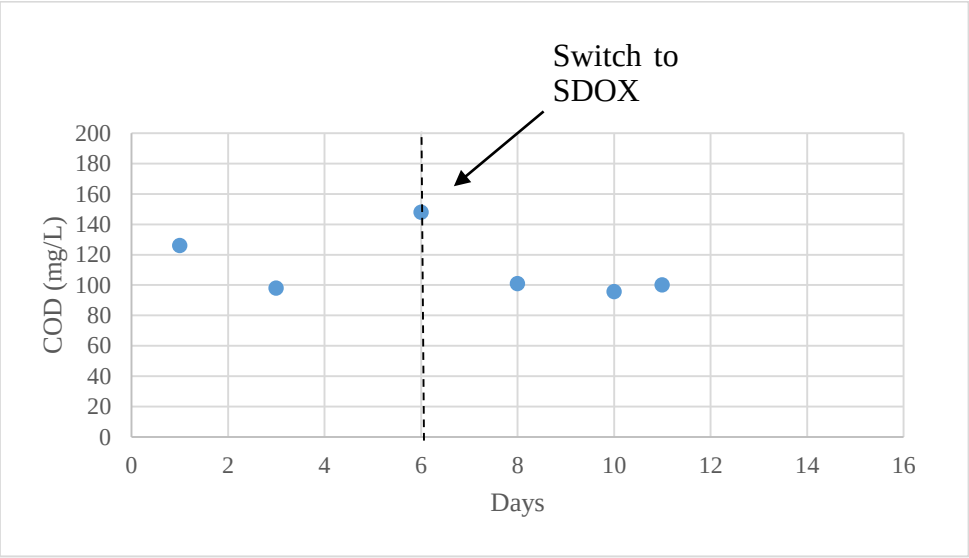


Figure 5-12 *Permeate COD-Profile. Experiment 4.*

In relation to nitrate as it is shown in (Figure 5-13) an increase was observed after switching the aeration. This was followed by a decrease in the last days of experiment. Moreover, it was also noticed that the last day of the experiment DO values were lower. It may be that high MLSS concentration clogged the diffusers by the end of the experiment which probably affected the oxygen supply during some hours and as a result nitrification.

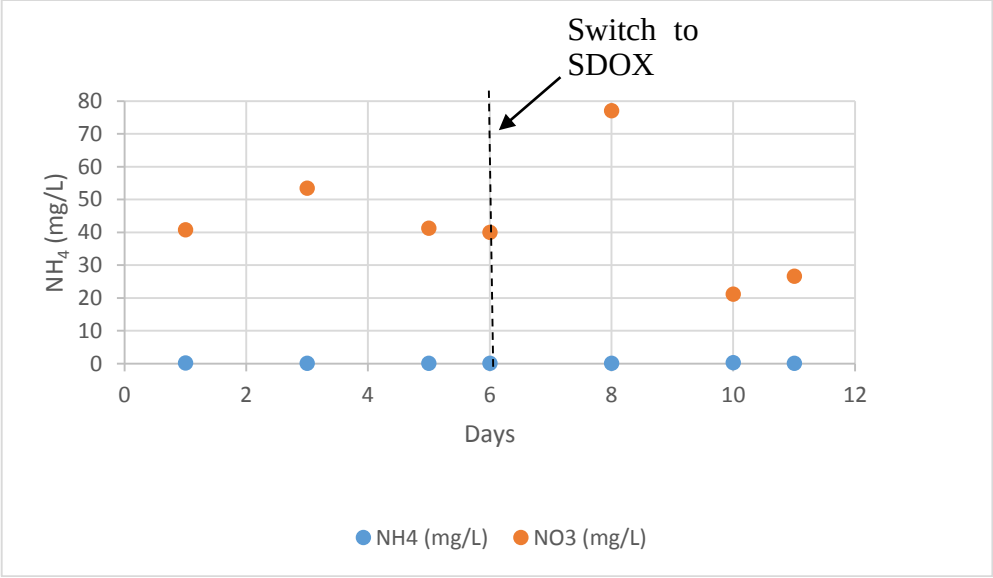


Figure 5-13 *Permeate N-Profile. Experiment 4.*

Regarding to phosphate , the same trend as for nitrate was observed.(Figure 5-14)

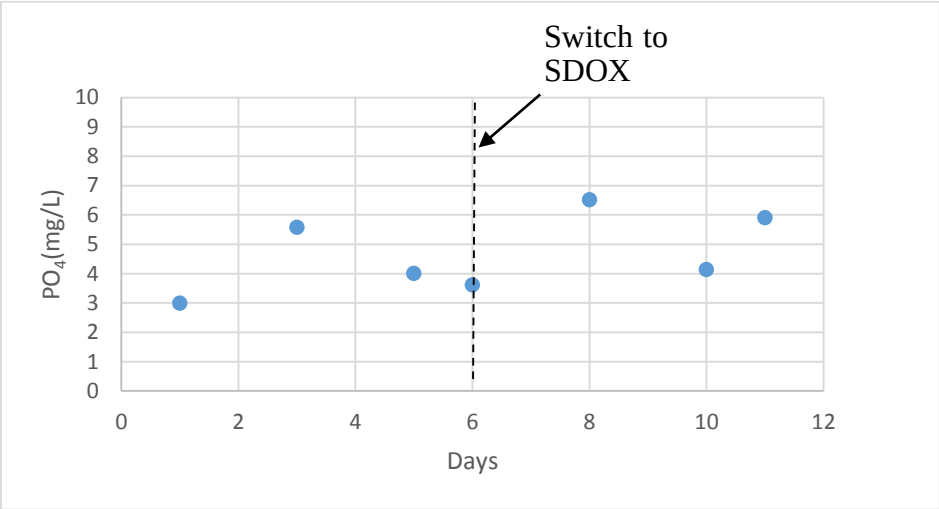


Figure 5-14 *Permeate P-Profile. Experiment 4.*

During Experiment 5 the MLSS concentration inside the reactor was between 20-22 g/L (Figure 5-15), the VSS was between 14-17 g/L, the DO level was between 2-5 mg/L while pH values were between 7.0 and 8.0. The average COD removal was 96%. The average concentration of COD in the effluent was 117mgO_2/L. Higher than for Experiments 1,2 and 3 and similar to Experiment 4. As it was mentioned before it could have occurred due to lysis. As it was observed in Experiment 3, the average COD removal was slightly lower for SDOX phase than for bubble diffusers. (Figure 5-16)

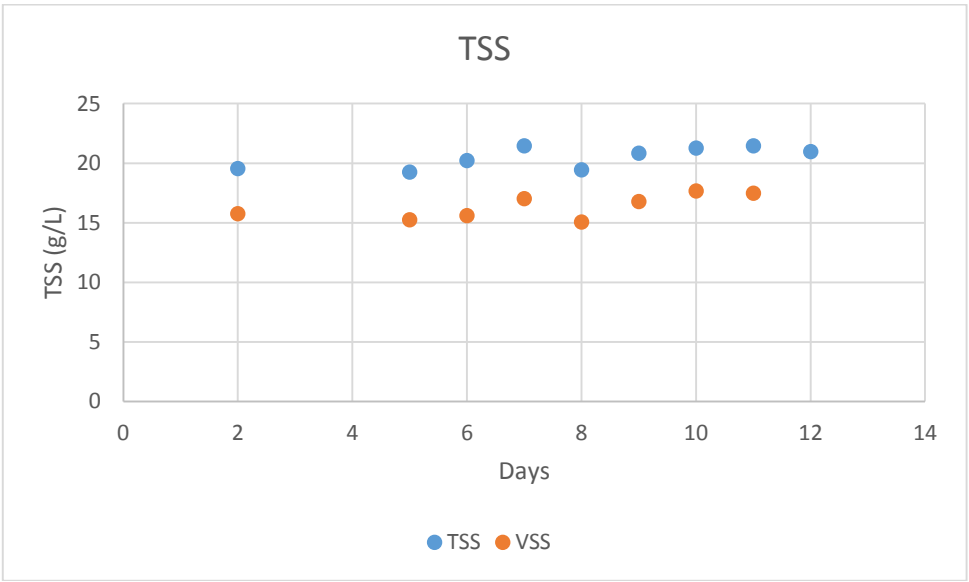


Figure 5-15 *TSS Experiment 5.*

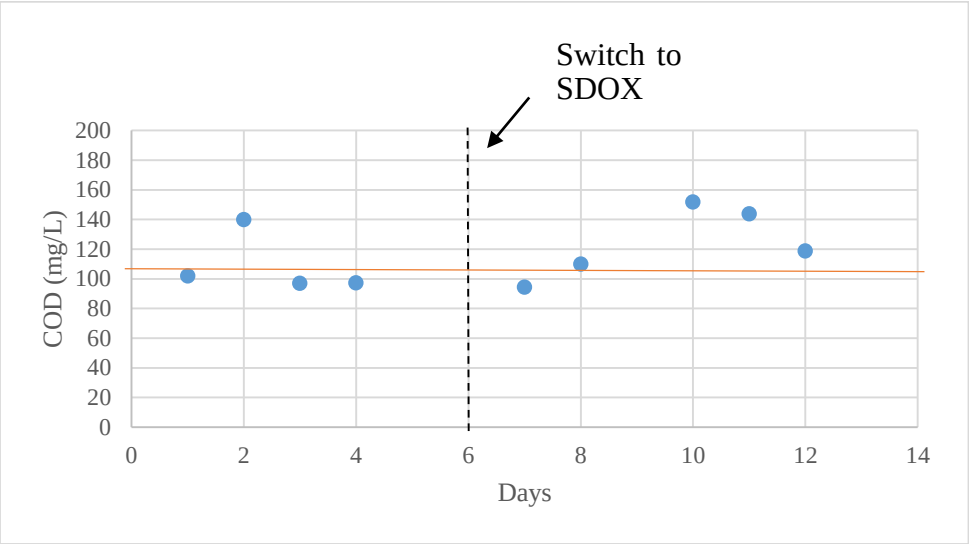


Figure 5-16 *Permeate COD-Profile. Experiment 5.*

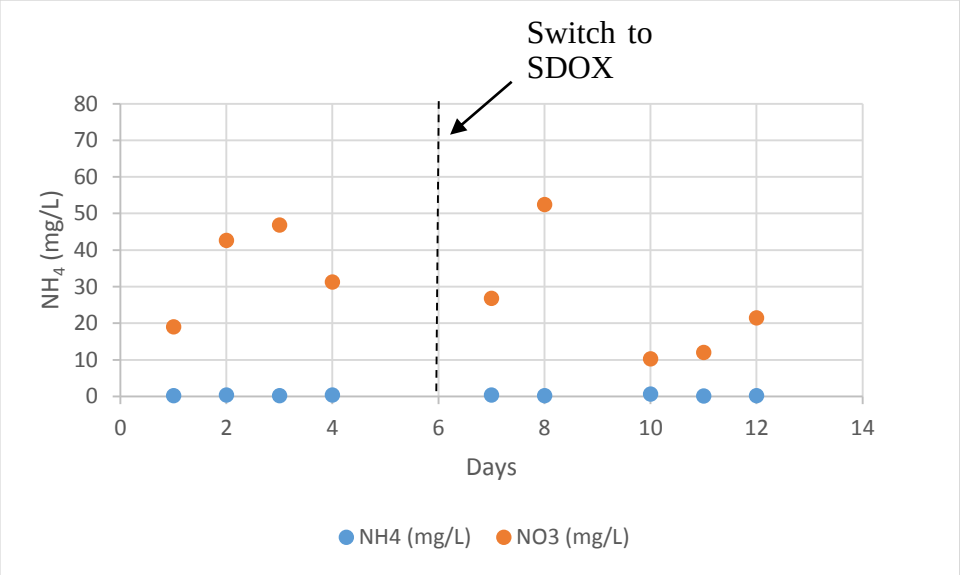


Figure 5-17 *Permeate N-Profile. Experiment 5.*

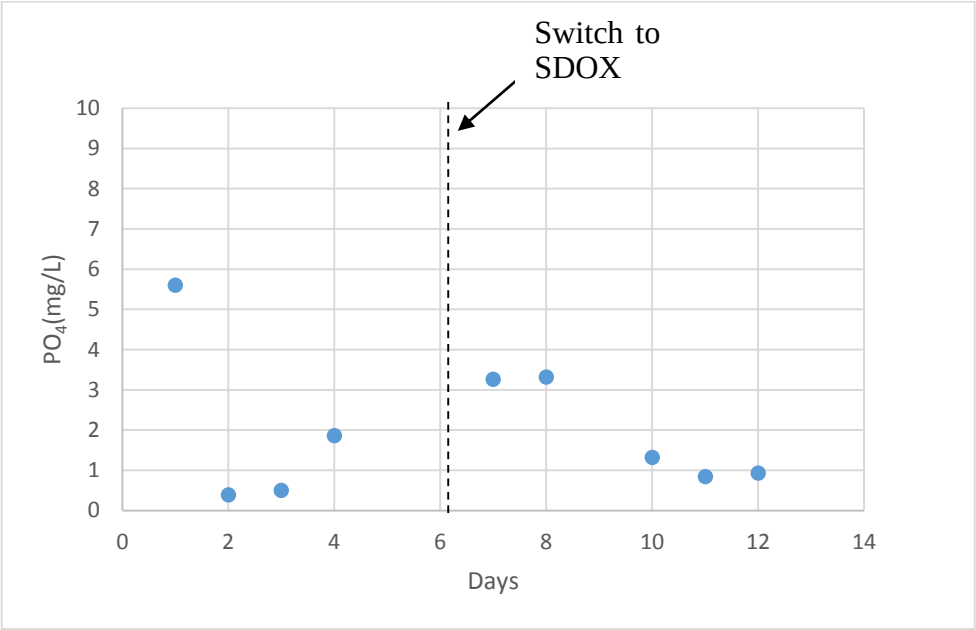


Figure 5-18 *Permeate P-Profile. Experiment 5.*

In conclusion, the effect of SDOX over biological processes was most pronounce immediately after its connection. In general, for these experiments higher values of COD, nitrate and phosphorous were observed in the permeate. This was followed by a recovery in the removal efficiency reaching values slighter lower than for bubble diffusers. It needs to be highlighted that for Experiment 3 and 5 in which azithromycin was added a bigger difference was observed as azithromycin influences bacterial metabolism and performance in treatment.

5.2. Influence of SDOX in sludge characteristics

The main purpose of this experiment was to evaluate how the characteristics of biomass was affected as a function of the exposure time by the high pressure conditions set by the SDOX aeration system

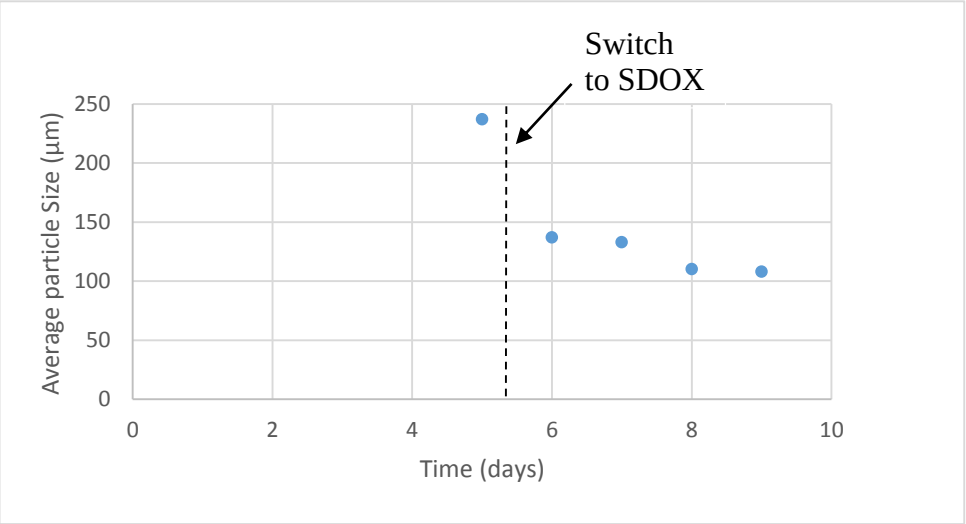


Figure 5-19 *Average particle size.. Experiment 1.*

As it can be seen in Figure 5-20 the last day of operation for bubble diffusers the average particle size was 237 µm. On the other hand, the last day of operation of SDOX unit the average particle size was 108 µm. The overall reduction was 54%. The highest dropped was registered the following day after switch to SDOX with a reduction of 42%. Furthermore, after SDOX started the particle size distribution was more uniform. As it is shown in Appendix B. the graph for bubble diffuser had a wider range of particles present than the one for SDOX.

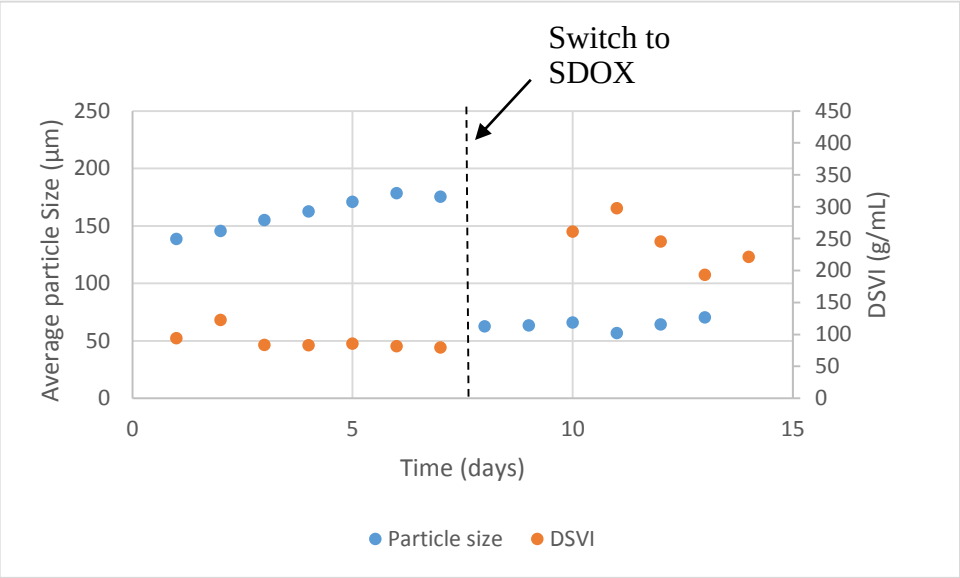


Figure 5-20 *Average particle size. Experiment 2.*

During the bubble diffuser phases for Experiment 2 the average particle size increased every day as it can be observed in Figure 5-20. An overall increase of 21 % was observed. This can be attributed to the presence of sticky substances which promote the bonding of sludge particles to make bigger flocs. Also, in the MBR the flocs were probably exposed to less shear than in full scale WWTP. The sludge was taken from the aeration basin where it had been constantly pumped with return from secondary settler. As in Experiment 1, the highest drop was the day after SDOX was connected, presenting a reduction of 64 % in the average particle size. The same results were previously observed by Librán-Vázquez (2015).
Regarding to DSVI, it presented constant values during bubble diffusers operation. However, when the aeration was switched, DSVI increased conforming particle size decrease and slower settling as smaller particles settle slower according to Stoke's law.

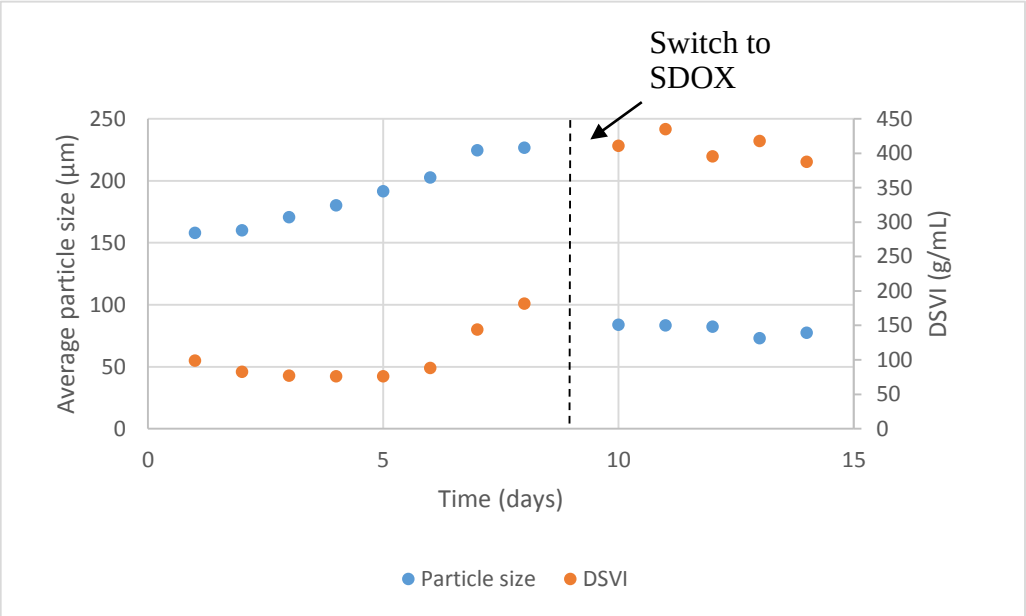


Figure 5-21 *Average particle size. Experiment 3.*

Figure 5-21 shows the results of the average particle size distribution and DSVI for Experiment 3. During the bubble diffuser phases the average particle size increased daily. The last day of operation, an overall increase of 30 % was observed. In the same way as in Experiment 1 and 2, after the start-up of SDOX was observed the highest drop in the average particle size, with a reduction of 63 %.

In relation to DSVI, during the first five days of operation for bubble diffusers, it was constant. However, the last three days it duplicated. A possible explanation is that bacteria were dying due to the antibiotic. Furthermore, in this experiment a lot of foam was observed as it can be seen in Figure 5-22. Many studies have reported that foaming problems may be attributed to filamentous bacteria. Microthrix parvicella is the most frequently reported microorganism responsible of bulking and foaming problems (Rossetti, et al., 2005).

On the other hand, Agridiotis, et al. (2006), suggested that the relationship between foaming and filamentous bacteria does not always explain its formation. Moreover, he pointed out that the interrelationship between foaming and the surface characteristics of activated sludge can be a solution to understand and solve this problem.

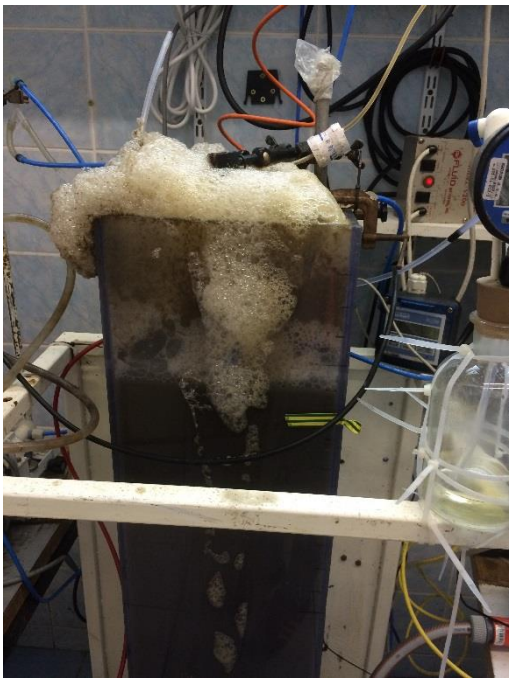


Figure 5-22 *Foaming. Experiment 3.*

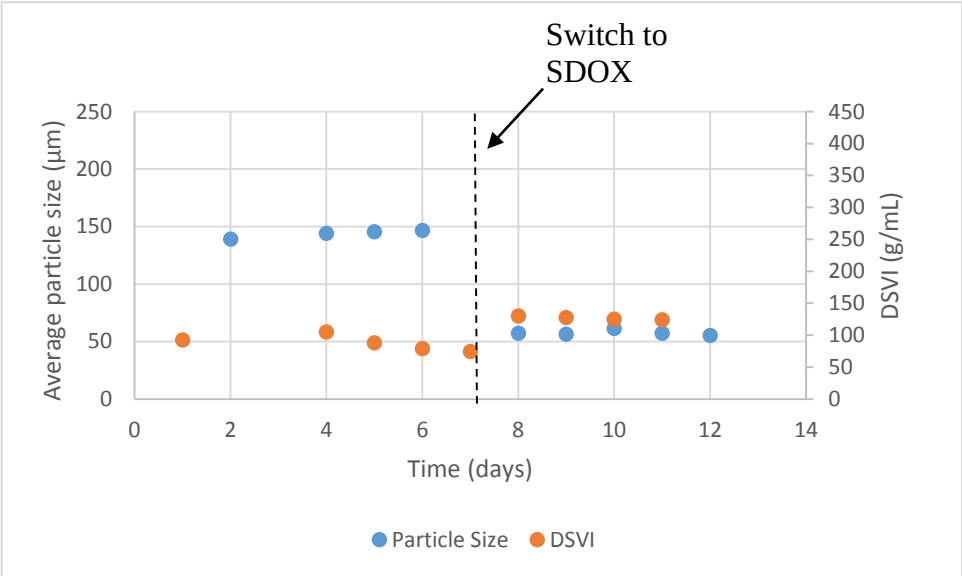


Figure 5-23 *Average particle size. Experiment 4.*

On the other hand, in Experiment 4 the average particle size during bubble diffusers operation was constant as it is shown in Figure 5-23. Since the MLSS concentration inside the reactor was higher it was expected that average particle size increased more than for Experiments 1, 2 and 3. However, MLSS was not the only modification for Experiment 4, flux was also changed. The drop in the average particle size after the aeration was changed was 61 %.

Concerning to DSVI, even though there was an increase of 42.9 %, it was not as remarkable as the previous shown in Figure 5-20 and Figure 5-21 which show an increase of 51,7 %, 69 % for Experiment 1 and 2 respectively.

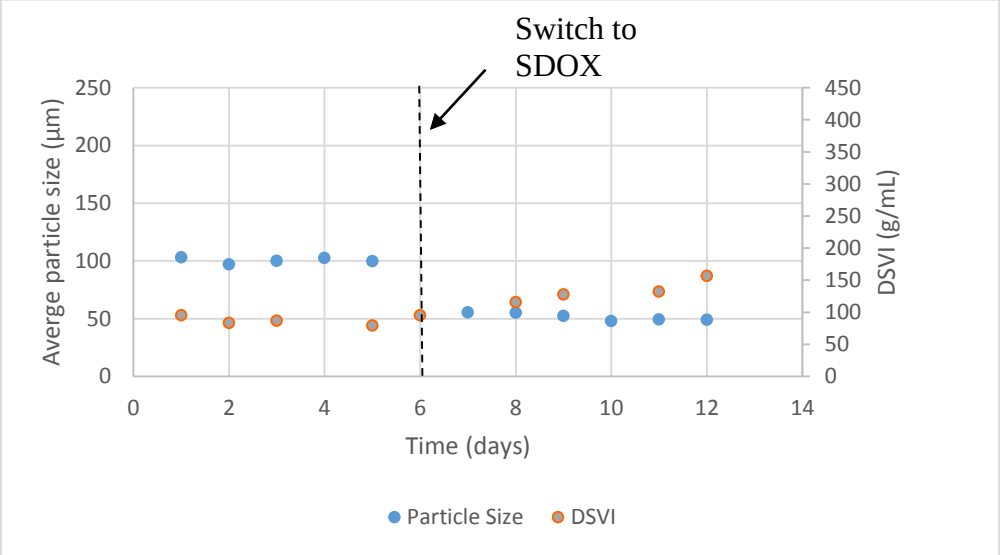


Figure 5-24 *Average particle size. Experiment 5.*

Finally, in Figure 5-24 can be observed the results for Experiment 5. The average particle size was constant during bubble diffusor operation. Furthermore, after SDOX was connected a decrease of 44 % was seen.

Table 5-1. *EPSp and EPSc fractions summary.*

		EPSp (mg/g)	**EPSc (mg/g)**
Experiment 2	BD	145.2	23.9
	SDOX	112.2	21.3
Experiment 3	BD	> 156.2	>25.0
	SDOX	> 156.2	>25.0
Experiment 4	BD	50.2	31.8
	SDOX	47.3	34.1
Experiment 5	BD	69.4	37.9
	SDOX	54.6	36.6

According to Kim, et al. (2001) breakage of microbial flocs due to pump shear leads to the flux decrease because it induces the decrease in floc size and release of EPS into the activated sludge reactor.

In this study, after SDOX unit was connected a decreased in particle size was observed for each experiment. However, this was not correlated with an increase in EPS content as shown in Table 5-1.

Other studies, carried out by Meng, et al. (2007) pointed out the influence of HRT on membrane fouling. A lower HRT would result in high EPS concentration. This was observed during this research. Experiments 2 and 3 had an HRT= 8 h and presented the highest EPS concentration. While for Experiments 4 and 5 HRT was 14 h and lower values for EPS were observed.
Meng, et al. (2007) also observed that the low HRT could cause excessive growth of filamentous bacteria in sludge suspension. It led to more release of EPS, higher sludge viscosity and irregular shaped flocs.

To conclude, the effect of SDOX on sludge characteristics was assessed. For all the experiments the particle size decreased (44-64) %, presenting the biggest drop the day after SDOX connection. The decrement in the particle size was correlated with a higher DSVI for all of the experiments. However, significant differences in settling were observed for similar reduction in particle size. Experiments 2 and 3 presented the highest increase of DSVI. It is interesting to notice that also the EPSc measured presented the highest values for Experiments 2 and 3. Moreover, for these experiments the difference in operational conditions(HRT) seems to have a bigger impact in sludge characteristics than SDOX.

5.3. Influence of SDOX on azithromycin removal

Results showed that unaltered azithromycin and only one biotransformation product were detected (Figure 5-25). The biotransformation product corresponded to phosphorylated-azithromycin (Figure 5-26) . Terzic, et al. (2011) already reported this compound. According to Wright (2005), phosphoryl-transfer is an enzymatic strategy for antibiotic inactivation, more specifically macrolides. Besides, Terzic, et al. (2011) showed that significant amount of the transformation product was enhanced after 40 days. In contrast, for this research bacteria did not need an adaptation period. Since day 1, for both experiments the metabolite reached high concentrations.

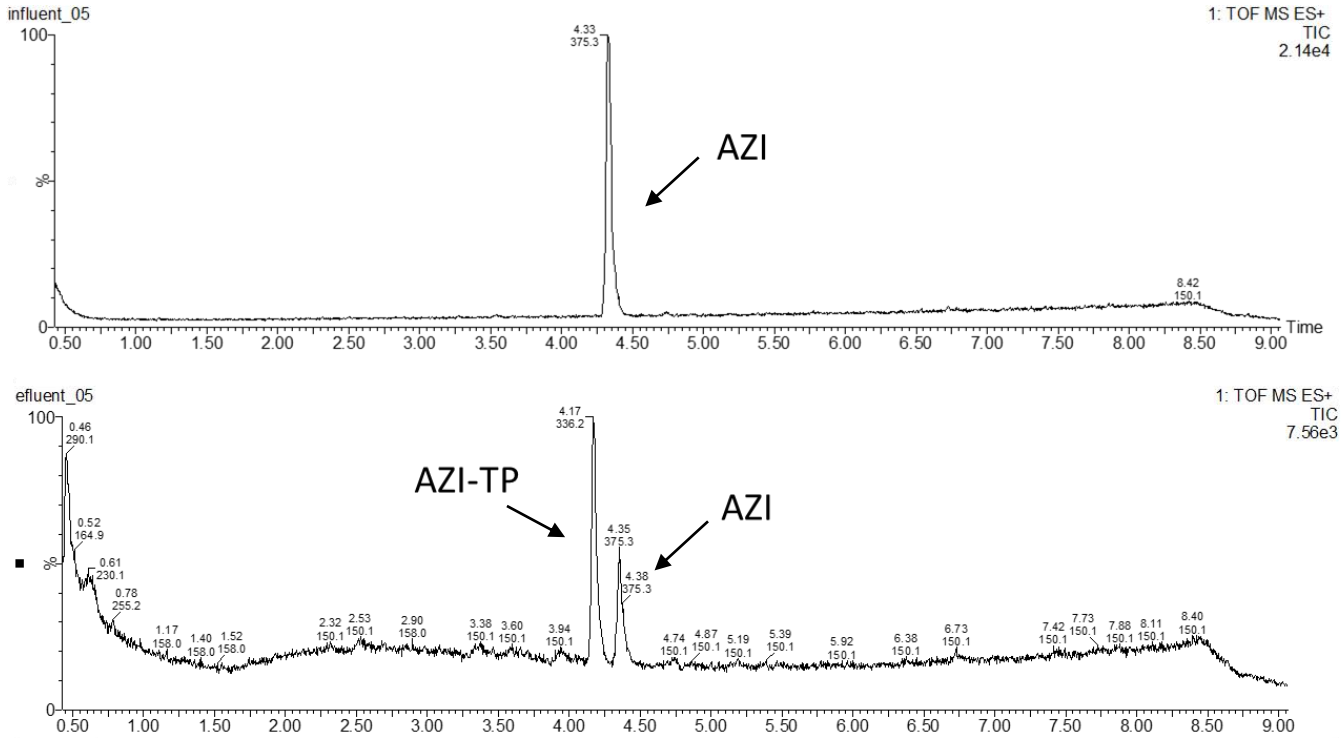
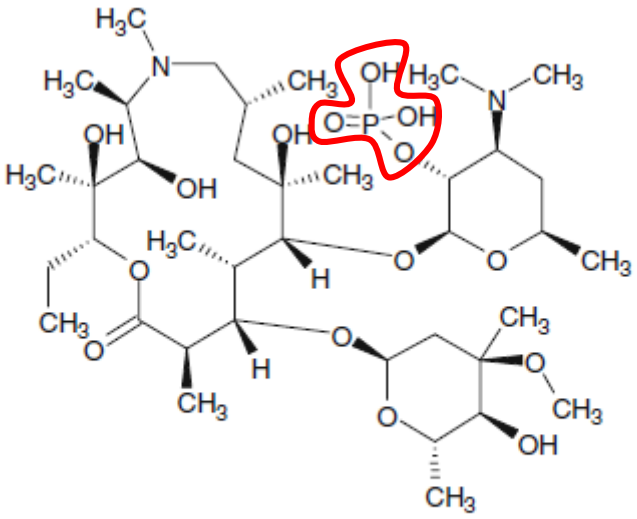


Figure 5-25 *Example of total ion chromatograms (TIC) of influent and effluent samples.*



AZI TP

Figure 5-26 *Azithromycin biotransformation product.*

There are two process that need to be taken into account to evaluate the removal of azithromycin in the MBR. Firstly, the biotransformation of the compound and secondly the adsorption of it

into the sludge. Volatilization for macrolides can be ignored due to it really low Henry constant (Suarez, et al., 2010).
Senta, et al. (2011), reported removal efficiencies of macrolides strongly dependent on hydraulic retention time. They achieved (70-80) % and (40-50) % removal for HRT=16h and HRT= 8h respectively. In this study, for Experiment 3 (Figure 5-27) an average removal of 57% was achieved operating the reactor with bubble diffusers at HRT= 8h. However, after the aeration system was switched to SDOX the removal decreased significantly. One possible explanation is that the removal due to adsorption was higher for bubble diffusers. While particle size was increasing as it was shown in (Figure 5-21) azithromycin could be caught between the layers of the flocs of sludge. However, Senta, et al. (2011) proved that only 0.33 % of the removed azithromycin was adsorbed in the sludge.
Notwithstanding, the shock effect induced by SDOX was clearly observed in samples taken (36-40) h after it connection. The worst removal was on Day 10 with an overall removal of 28 % and the average removal after SDOX was connected was 40%. Even though the system recovered, it did not achieve the same removal efficiency as bubble diffusers.

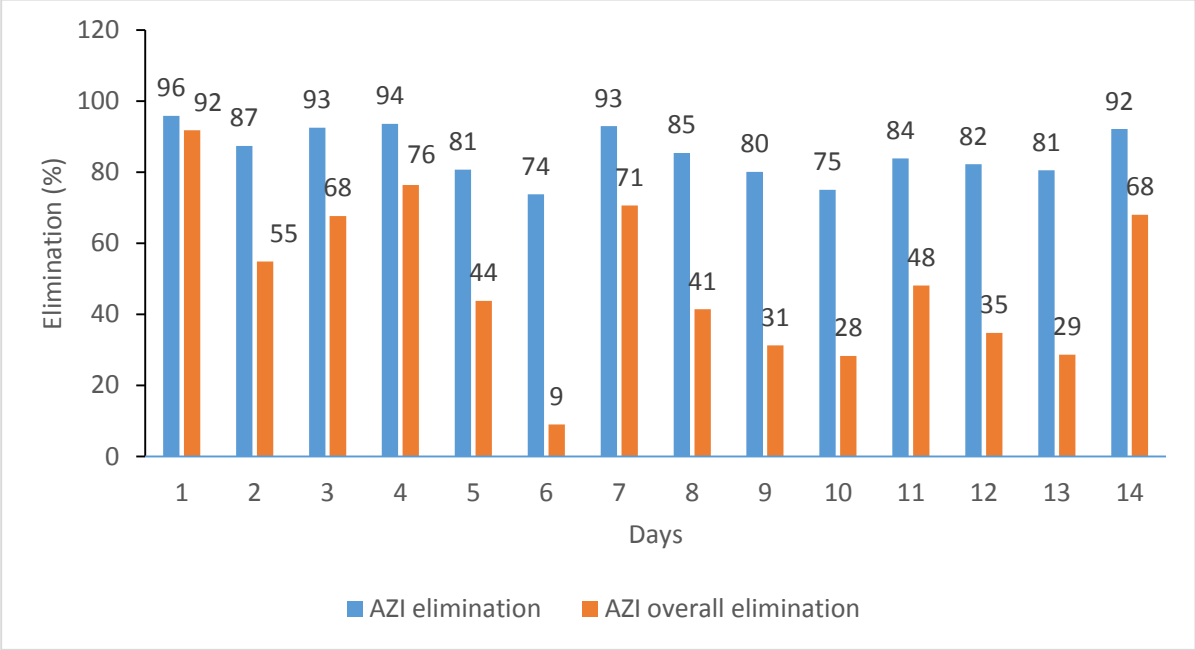


Figure 5-27 *Azithromycin removal efficiency. Experiment 3.*

In the same way, Experiment 5 showed consistent results supporting the trend observed in Experiment 3(Figure 5-28). An average removal of 76 % was achieved operating the reactor with bubble diffusers at HRT= 14h. As for Experiment 3, the shock effect induced by SDOX was observed in samples taken (36-40) hours after it connection. The average removal dropped 26 % reaching an average value of 50 % removal.
Both, Experiment 3 and Experiment 5 presented the same overall effect of SDOX treatment on azithromycin removal, however a higher removal efficiency was observed for experiment 5. Since HRT and MLSS were different, further investigation is needed to explain the mechanism. Quantifying the amount of azithromycin in the solid phase can bring a better understanding of this mechanism.

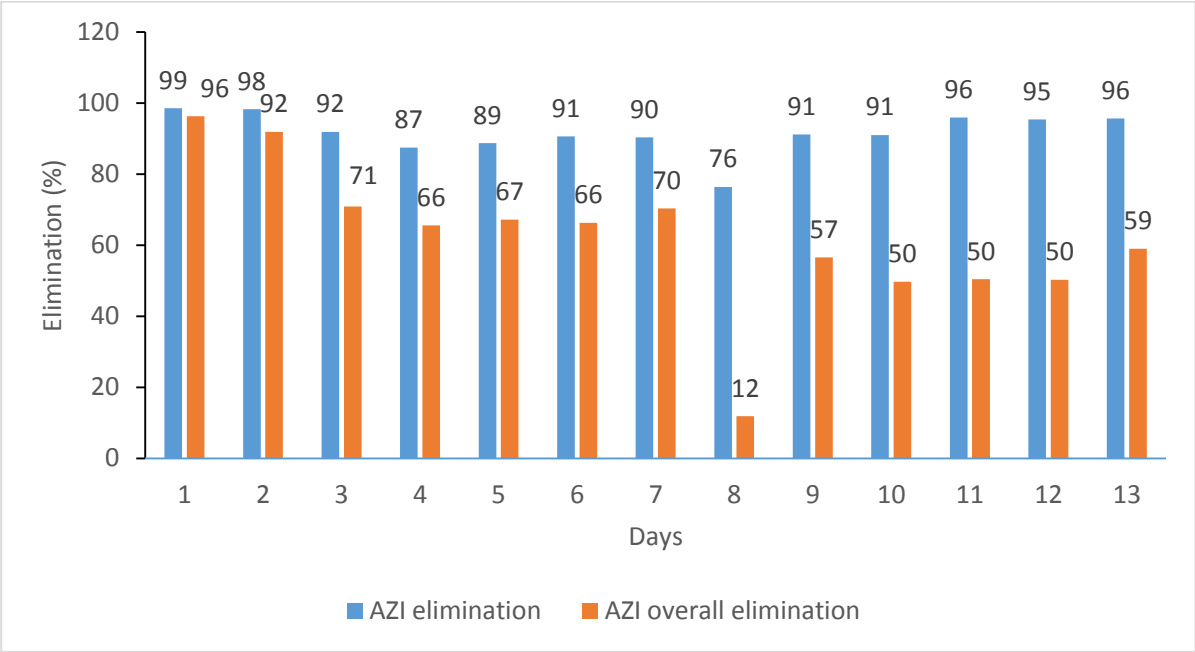


Figure 5-28 *Azithromycin removal efficiency. Experiment 5.*

To summarize, MBR showed consistent result in the removal of azithromycin, which could have been affected by MLSS concentration or HRT. In relation to SDOX, it was proven that it affected negatively the overall removal efficiency of azithromycin. For both experiments a drop was observed after its connection.

5.4. Influence of SDOX on membrane fouling

This section presents the results of the assessment of the influence of SDOX over membrane fouling for the five different experiments performed.

Figure ***5-29*** shows the pressure recorded by the PLC during Experiment 1. It can be observed that the TMP for the operation with bubble diffuser was low and constant. On the other hand, the TMP registered for the operation with SDOX was constant during the whole experiment and slightly higher than for bubble diffusers.

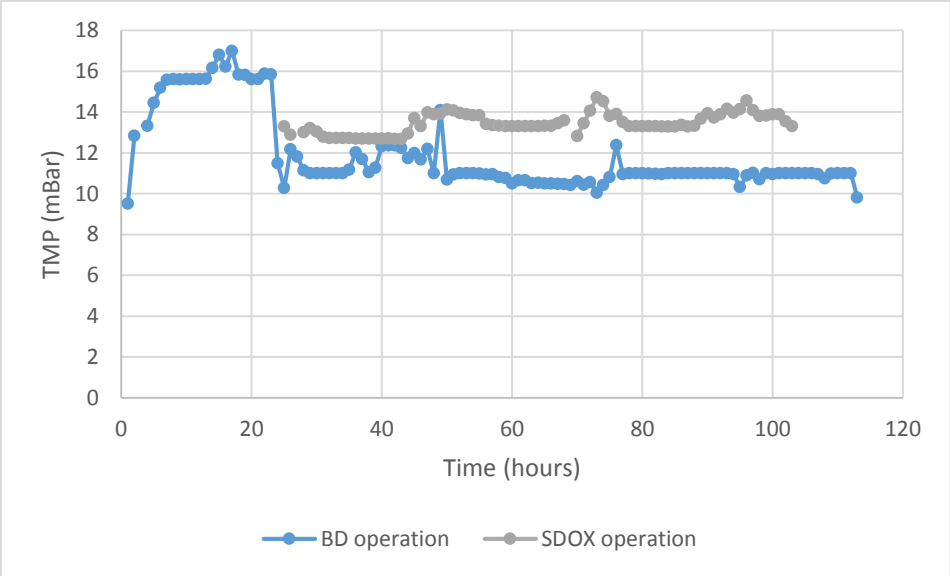


Figure 5-29 *TMP-Profile. Experiment 1.*

The particle size is an important property in membrane fouling in MBRs. Some studies observed that smalls sludge flocs could more easily adhere to the membrane surface, therefore fouling the membrane more.(Lin, et al., 2011)
However, Shen, et al. (2015) suggested that floc size had no apparent effect on membrane pore clogging fouling.
In this research, the average particle size and its distribution changed after the aeration was switched to SDOX (Figure 5-19). Nonetheless, for Experiment 1 it did not cause any significantly higher fouling rate. As it can be observed in (Figure 5-30) the lost in permeability after operation for both aeration systems was similar. For bubble diffusers the lost was 87 % while for SDOX the lost was 82 %.

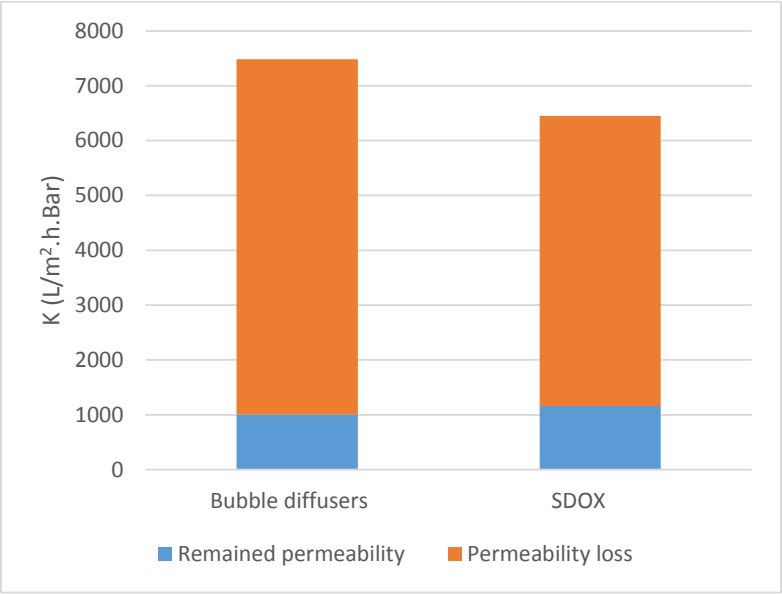


Figure 5-30 *Membrane permeability. Experiment 1.*

Figure 5-31 and Figure 5-32 show the results obtained for Experiment 2. In this experiment, which was performed under a flux of 25 L/h.m^2 after 24 hours of acclimatization phase for bubble diffusers the pressure was increasing gradually. Afterwards the pressure stabilized in 125 mBar. However, during the operation period for bubble diffusers, the pressure increased really fast. After two days of operation high fouling was observed. The pressure abruptly increased and the system could not be operated anymore.

This observation differs from Experiment 1 and were caused by higher flux in Experiment 2. Much faster fouling in the operational phase with bubble diffusers than in acclimatization phase was possibly caused by release and accumulation of fouling substances from the acclimatization phase remained in the bioreactor also in the operation phase.

According to Le-Clech, et al. (2006) SMP have a big impact on membrane performance. During filtration these substances can interact with the membrane through different mechanisms: adsorption on the surface, blockage of the pores and or formation of a gel structure which can provide nutrients for biofilm formation.

On the other hand, during the SDOX acclimatization (1) TMP increased really fast. After 12 hours, it reached the same values as bubble diffusers operation phase. However, during the first night of acclimatization of the SDOX connected to the MBR, several problems were experienced. The tubing of the pump that recirculate the sludge from the reactor to the SDOX vessel broke. As a consequence, half of the sludge came out of the reactor. The system operated with this problem during a couple of hours, while the sludge was being accidentally collected in the effluent tank. Next morning, the sludge was settled and returned to the reactor. The membrane was changed and the acclimatization phase for SDOX (2) started. Coming up next, in the SDOX acclimatization (2) the pressure was quite low for three days. This was unexpected, since in all the previous phases of this experiment, the pressure was medium or high. One reason could have been that after the incident only the sludge was returned to the reactor while the supernatant was discharged. Supernatant may have contained high concentration of SMP, which explains the high TMP recorded in acclimatization phase (1).

Notwithstanding, in the SDOX operation phase was observed an increased in the pressure once again. After the first day of operation, the pressure was stabilized in 180 mBar.
At the end of this experiment, an additional experiment was performed to clarify the observed differences in Experiment 2. In order to do this, the sludge was washed as it is described below. Half of the sludge was taken out of the reactor. It was settled by gravity and the supernatant was removed. Then, the sludge was put into the reactor again and the volume inside the reactor was completed with water to reached 23 L. The system was operated during three days at the same flux (25 L/h.m^2) to evaluate the effect of washing the sludge. It was observed that TMP was really low. This proved that the low values of pressure in the SDOX acclimatization phase (2) were due to the unintentional washing of the sludge, which leaded disappearance of the fouling substances after the sludge was collected and returned into the reactor.

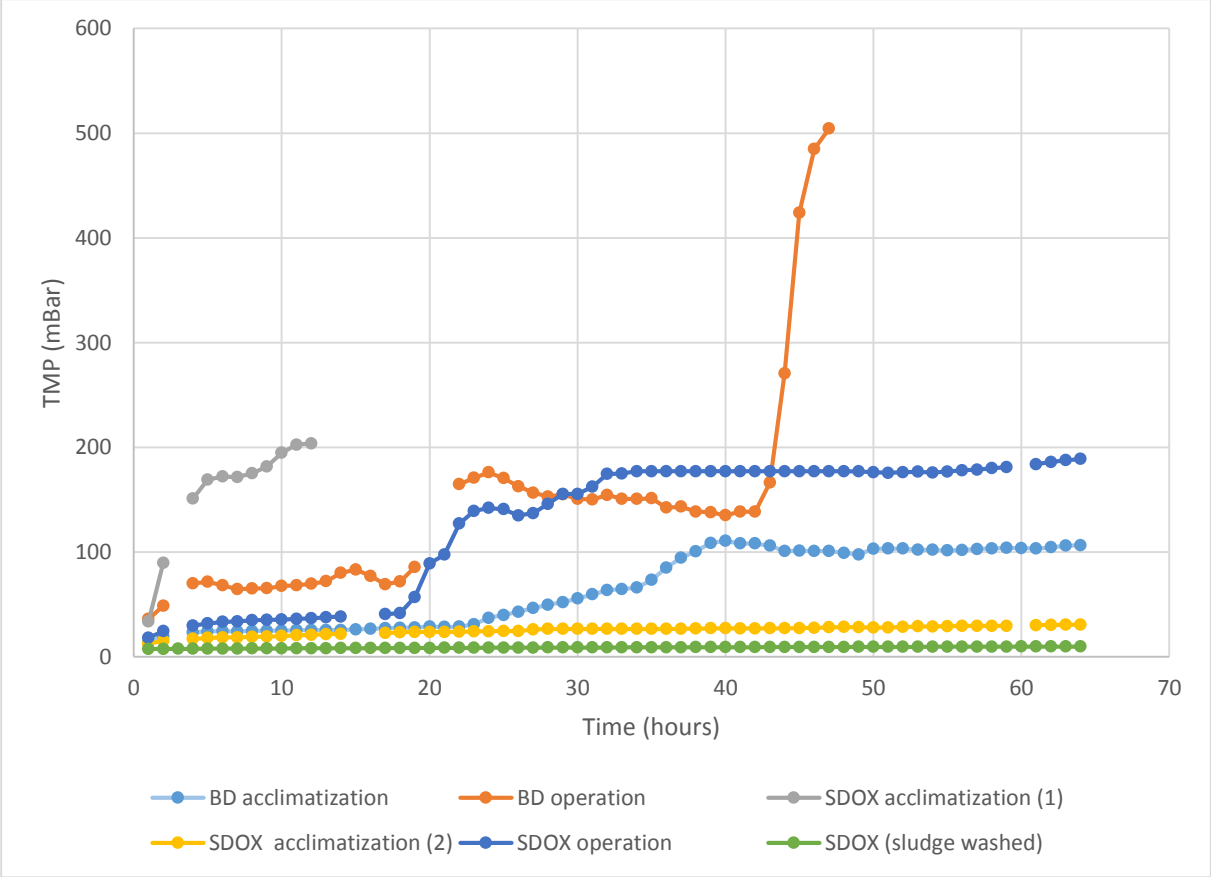


Figure 5-31 *TMP-Profile. Experiment 2.*

From the permeability assessment it can be observed the same fact (Figure 5-32). The remained permeability for bubble diffuser and SDOX operation phase was comparable and low. The lost in permeability was 86% and 94% respectively. However, the operation phase for bubble diffusers lasted one day less due to high TMP values. It is also important to highlight that the initial permeability for bubble diffusers operation phase was lower than the initial permeability for the other phases of this experiment. This can be attributed to some chemical product that

new membranes have for protection. It should have been removed before starting the experiments.
On the other hand, the lost in permeability for SDOX acclimatization (2) phase, and SDOX with sludge washed phase was 84% and 79% respectively. The remain permeability for the mentioned phases was higher than the remain permeability for bubble diffuser and SDOX operation phase.

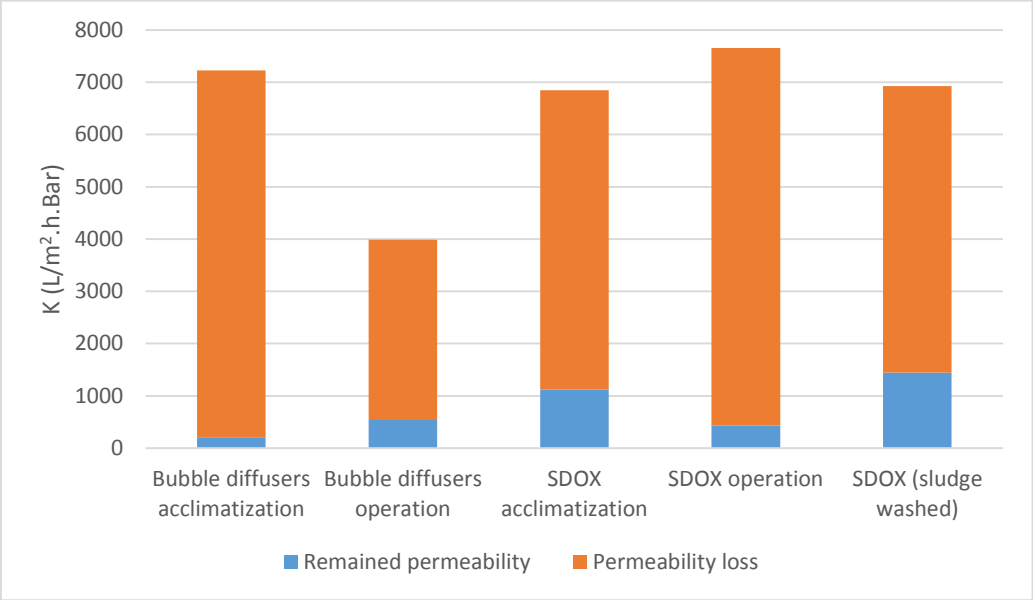


Figure 5-32 *Membrane permeability. Experiment 2.*

During Experiment 3, the TMP for bubble diffusers phases was low and constant. However, when SDOX was connected and immediate increment in TMP was observed. Due to the high initial fouling was not possible to reach a constant flux of 25 L/m^2. h. After two days of acclimatization the membrane was changed for a new one. As it can be seen in graph (Figure 5-33) , the new membrane also fouled very fast.
This fact can also be observed in the permeability assessment (Figure 5-34). The remained permeability for SDOX was lower than the remained permeability for bubble diffusers. The lost in permeability were 63%, 87%, 96%, and 94% for bubble diffusers acclimatization, bubble diffusers operation, SDOX acclimatization and SDOX operation respectively.

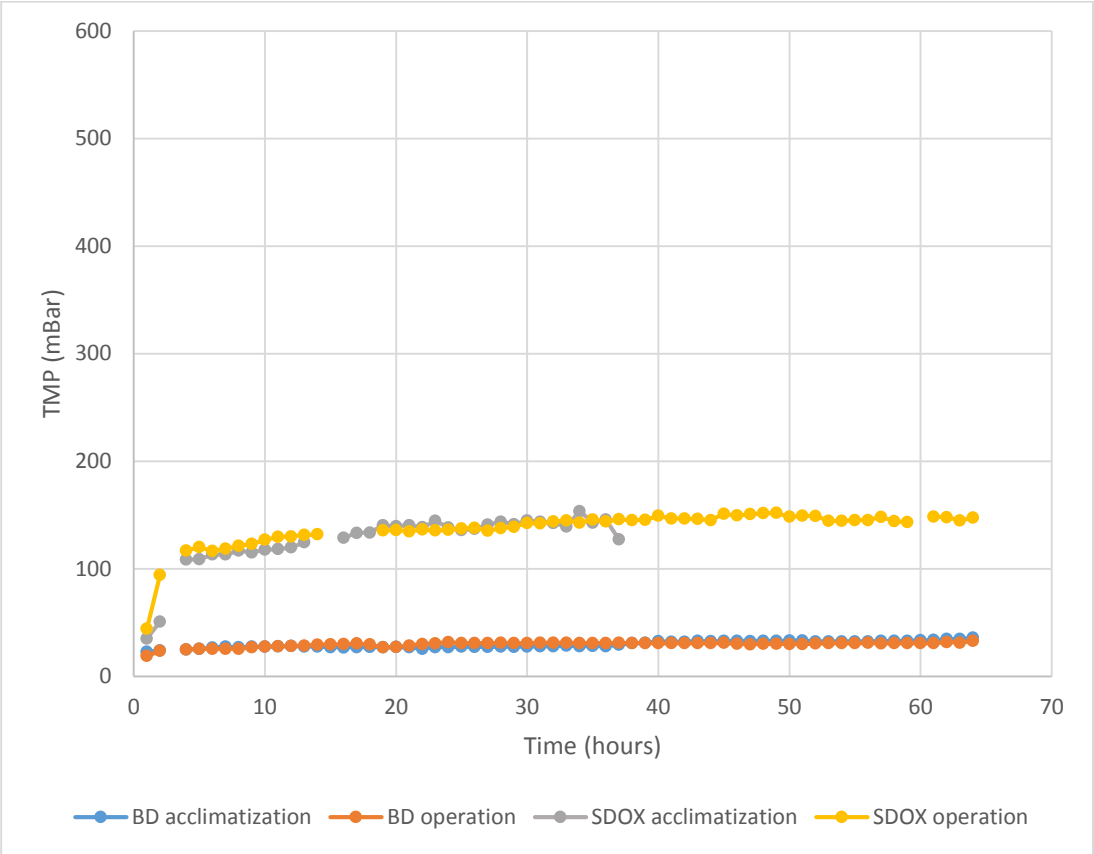


Figure 5-33 *TMP-Profile. Experiment 3.*

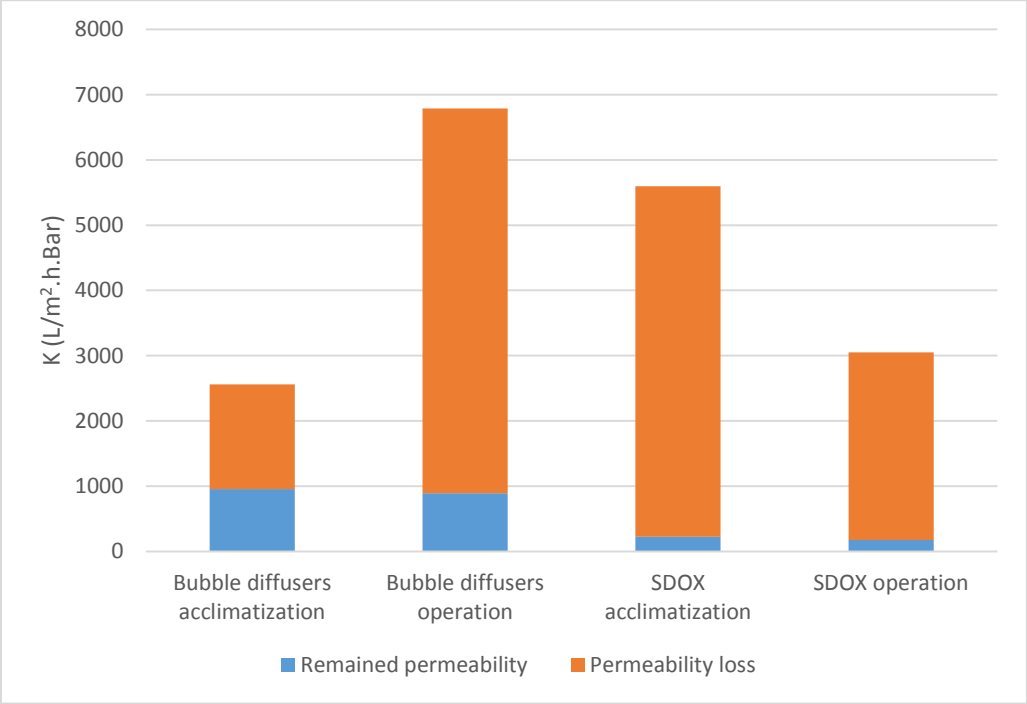


Figure 5-34 *Membrane permeability. Experiment 3.*

For this experimental conditions, SDOX presented a higher negative effect over the membrane. The TMP raised immediately after it connection and the percentage of the lost permeability was higher for both acclimatization and operation period.
From Figure 6-37 it can be observed that during Experiment 4, TMP was low and constant for each phase (6-12 mBar)(Figure 5-35). No big differences where noticed between the systems. However, there was a slighter decrease in TMP during operation phases.
In relation to permeability, the lost was 68%, 74%, 74% and 68% for bubble diffusers acclimatization, bubble diffusers operation, SDOX acclimatization and SDOX operation respectively. As it can be noticed in (Figure 5-36) there was no significant difference in the remain permeability for all the phases.

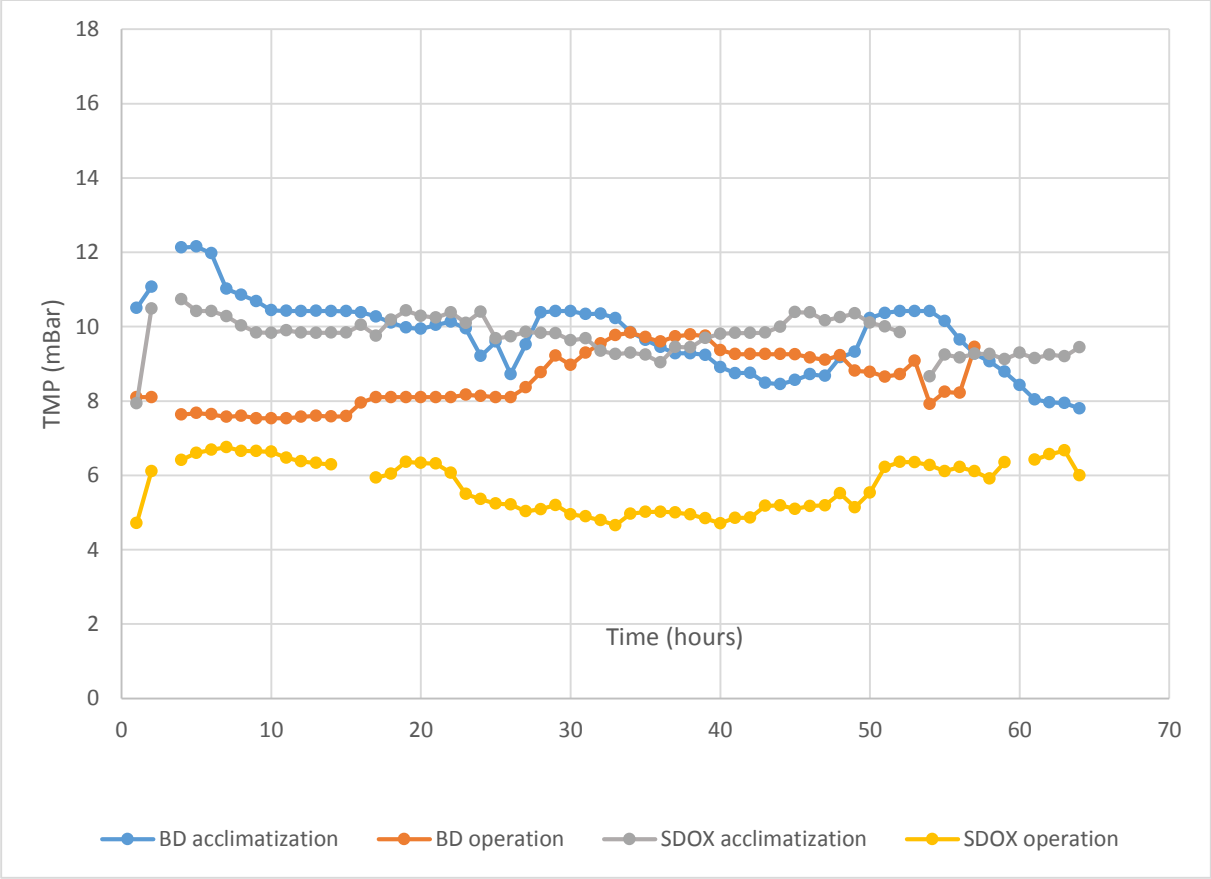


Figure 5-35 *TMP-Profile. Experiment 4.*

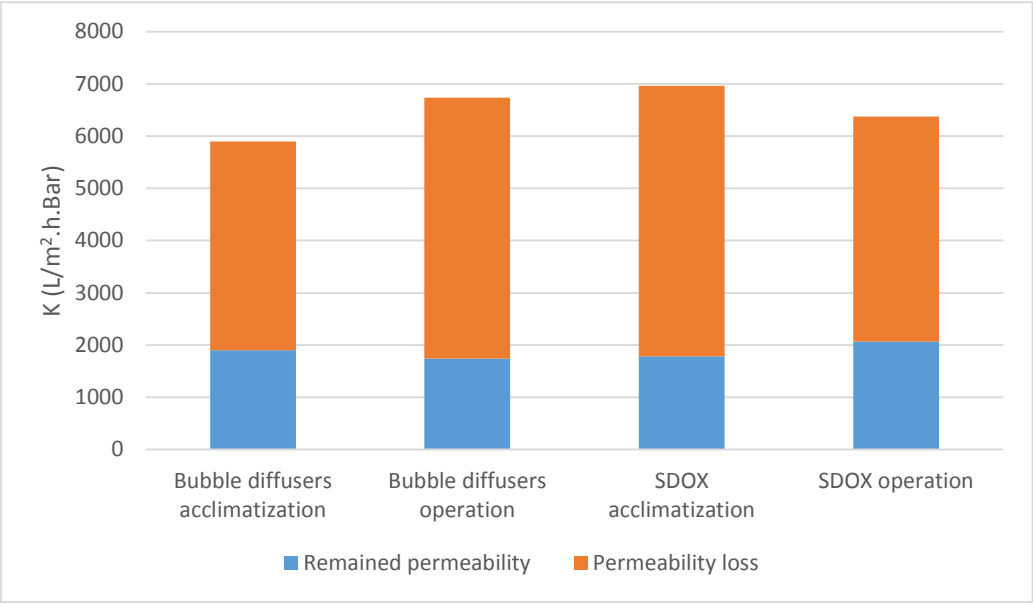


Figure 5-36 *Membrane permeability. Experiment 4.*

TMP-Profile and permeability for Experiment 5 are shown in Figure 5-37 and Figure 5-38. TMP was low and constant for each phase. No big differences where noticed between the systems. However, there was a slighter decrease in TMP during operation phases.
When SDOX was connected and immediate increment in TMP was observed, followed by a decrement and stabilization for operation phase.
The remain permeability for SDOX was lower than the remain permeability for bubble diffusers. The lost in permeability were 63%, 59%, 78%, and 78% for bubble diffusers acclimatization, bubble diffusers operation, SDOX acclimatization and SDOX operation respectively. This fact was also observed in Experiment 3.

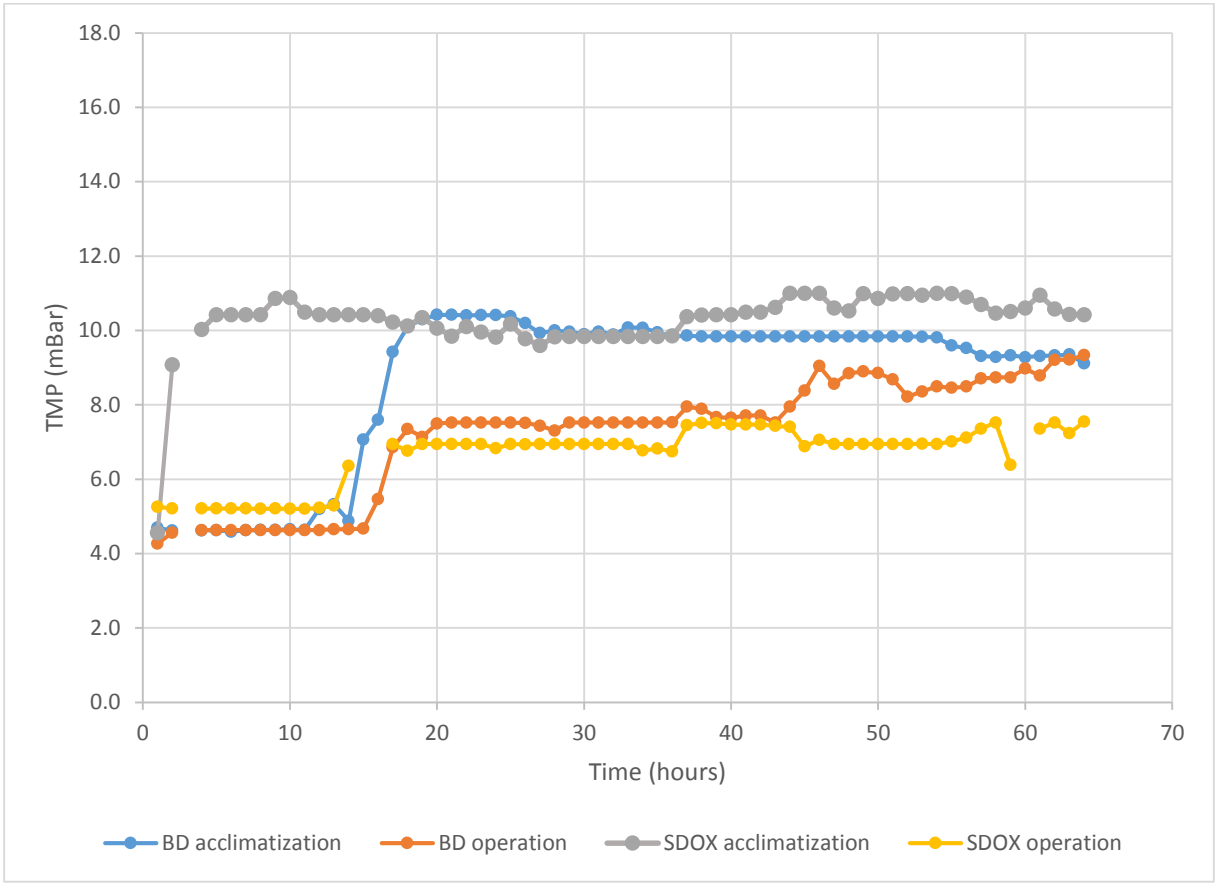


Figure 5-37 *TMP-Profile. Experiment 5.*

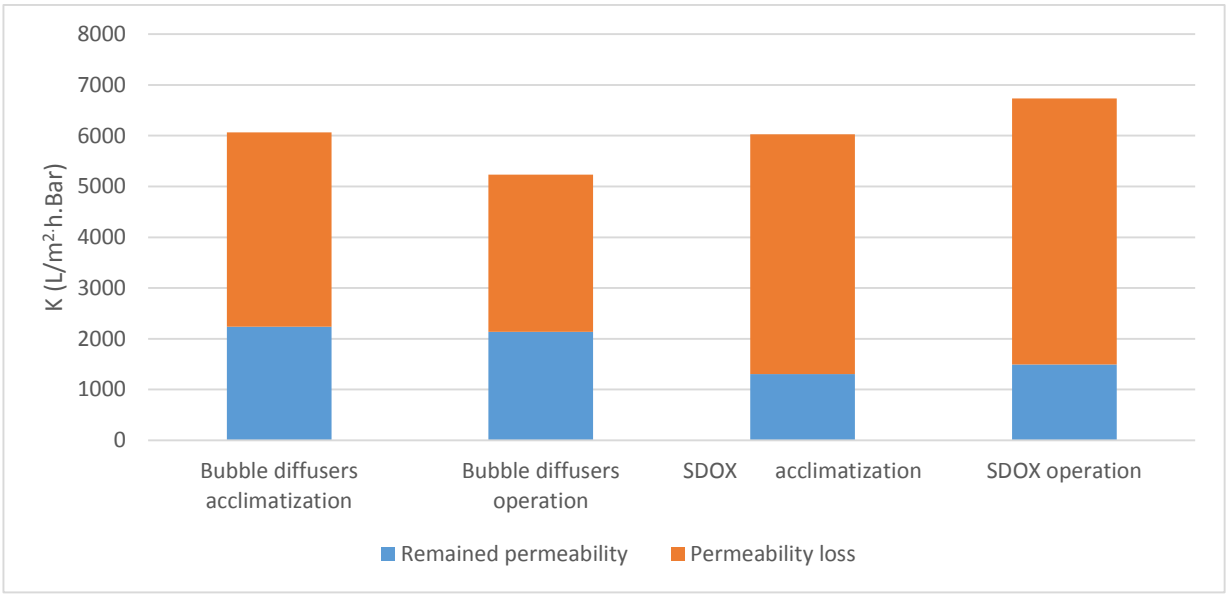


Figure 5-38 *Membrane permeability. Experiment 5*

To sum up, the effect of SDOX over membrane fouling needs to be analyzed in two phases. For effluents which does not contain azithromycin, no incidence of SDOX over membrane fouling

was found. In contrast, for effluents spiked with azithromycin a negative effect over membrane fouling was caused by SDOX.

CHAPTER 6

Conclusions

In conclusion, the effect of SDOX over biological processes was not very significant. Despite higher values of COD, nitrate and phosphorous in the effluent after the initial shock, the system recovered after one day. It needs to be highlighted that for Experiment 3 and 5 in which azithromycin was added to the synthetic wastewater, a bigger difference was observed.

As a consequence of using SDOX as the aeration technology in MBRs a big change in the sludge characteristics was observed. Particle size distribution decreased (44-64) % while the DSVI increased. However, the decrease in particle size did not correspond with higher fouling rate than the fouling rate observed for bubble diffusers. Changes in operational parameters seemed to have a bigger impact on the concentration of EPS, therefore in the fouling rate.

Nevertheless, the effect of SDOX over membrane fouling needs to be analyzed in two phases. For effluents which do not contain azithromycin, no significant influence of SDOX over membrane fouling was noticed. However, further investigations are needed in order to establish the influence of SDOX in cake formation when the shear rate is reduced in order to save energy.

On the other hand, for effluents spiked with azithromycin, a negative effect of SDOX over membrane fouling was observed. A higher deterioration of the membrane performance was observed for experiments with azithromycin when SDOX was used than with bubble diffusers.

Finally, MBR operated with bubble diffusers showed consistent results in the removal of azithromycin (57-76) % depending on the operational conditions. In contrast, the removal efficiency dropped to (40-50) % after SDOX was connected. In conclusion, a negative effect over removal efficiency was noticed with SDOX technology. To have a better understanding of the mechanisms implied, quantification of azithromycin in the solid phase is necessary.

References

Agridiotis V, Forster CF, Balavoine C, Wolter C, Carliell-Marquet C (2006) An examination of the surface characteristics of activated sludge in relation to bulking during the treatment of paper mill wastewater. Water and Environment Journal 20: 141-149 DOI 10.1111/j.1747-6593.2005.00008.x

Bilal Z (2013) Novel Alternative for Aeration in the Treatment of Wastewater by Membrane Bioreactors Operated at High Biomass Concentrations in the Context of Emergency Sanitation. UNESCO-IHE, Delft

BlueInGreen (2015) SDOX - Dissolved Oxygen System

Chang IS, Clech PL, Jefferson B, Judd S (2002) Membrane fouling in membrane bioreactors for wastewater treatment. Journal of Environmental Engineering 128: 1018-1029 DOI 10.1061/(ASCE)0733-9372(2002)128:11(1018)

Daigger GT, Rittmann BE, Adham S, Andreottola G (2005) Are membrane bioreactors ready for widespread application? Environmental Science and Technology 39: 399A-406A

Drews A (2010) Membrane fouling in membrane bioreactors—Characterisation, contradictions, cause and cures. Journal of Membrane Science 363: 1-28 DOI 10.1016/j.memsci.2010.06.046

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith P (1956) Colorimetric method for determination of sugars and related substances. Anal chem 28: 350-356

García-Ochoa F, Gomez E, Santos VE, Merchuk JC (2010) Oxygen uptake rate in microbial processes: An overview DOI 10.1016/j.bej.2010.01.011

Germain E, Stephenson T (2005) Biomass characteristics, aeration and oxygen transfer in membrane bioreactors: Their interrelations explained by a review of aerobic biological processes DOI 10.1007/s11157-005-2097-3

Henze M (2008) Biological Wastewater Treatment: Principles, Modelling and Design IWA Pub.

Judd S, Judd C (2006) The MBR book

Kim JS, Lee CH, Chang IS (2001) Effect of pump shear on the performance of a crossflow membrane bioreactor. Water Research 35: 2137-2144 DOI 10.1016/S0043-1354(00)00495-4

Kraume M, Drews A (2010) Membrane bioreactors in waste water treatment - Status and trends. Chemical Engineering and Technology 33: 1251-1259 DOI 10.1002/ceat.201000104

Larsson DG, de Pedro C, Paxeus N (2007) Effluent from drug manufactures contains extremely high levels of pharmaceuticals. J Hazard Mater 148: 751-755 DOI 10.1016/j.jhazmat.2007.07.008

Le-Clech P, Chen V, Fane TAG (2006) Fouling in membrane bioreactors used in wastewater treatment. Journal of Membrane Science 284: 17-53 DOI 10.1016/j.memsci.2006.08.019

Librán-Vázquez B (2015) Influence of concentrated oxygen delivery technology in solid/liquid separation of aerobic systems. UNESCO-IHE, Delft

Lin H, Zhang M, Wang F, Meng F, Liao BQ, Hong H, Chen J, Gao W (2014) A critical review of extracellular polymeric substances (EPSs) in membrane bioreactors: Characteristics, roles in membrane fouling and control strategies. Journal of Membrane Science 460: 110-125 DOI 10.1016/j.memsci.2014.02.034

Lin HJ, Gao WJ, Leung KT, Liao BQ, Lin HJ (2011) Characteristics of different fractions of microbial flocs and their role in membrane fouling. Water Science and Technology 63: 262-269 DOI 10.2166/wst.2011.047

Lowry OH, Rosebourgh NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193: 265-275

Luo Y, Guo W, Ngo HH, Nghiem LD, Hai FI, Zhang J, Liang S, Wang XC (2014) A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. Science of the Total Environment 473-474: 619-641 DOI 10.1016/j.scitotenv.2013.12.065

Meng F, Chae SR, Drews A, Kraume M, Shin HS, Yang F (2009) Recent advances in membrane bioreactors (MBRs): Membrane fouling and membrane material. Water Research 43: 1489-1512 DOI 10.1016/j.watres.2008.12.044

Meng F, Shi B, Yang F, Zhang H (2007) Effect of hydraulic retention time on membrane fouling and biomass characteristics in submerged membrane bioreactors. Bioprocess and Biosystems Engineering 30: 359-367 DOI 10.1007/s00449-007-0132-1

Rossetti S, Tomei MC, Nielsen PH, Tandoi V (2005) "microthrix parvicella", a filamentous bacterium causing bulking and foaming in activated sludge systems: A review of current knowledge. FEMS Microbiology Reviews 29: 49-64 DOI 10.1016/j.femsre.2004.09.005

Senta I, Matošić M, Jakopović HK, Terzic S, Ćurko J, Mijatović I, Ahel M (2011) Removal of antimicrobials using advanced wastewater treatment. Journal of Hazardous Materials 192: 319-328 DOI 10.1016/j.jhazmat.2011.05.021

Shen LG, Lei Q, Chen JR, Hong HC, He YM, Lin HJ (2015) Membrane fouling in a submerged membrane bioreactor: Impacts of floc size. Chemical Engineering Journal 269: 328-334 DOI 10.1016/j.cej.2015.02.002

Suarez S, Lema JM, Omil F (2010) Removal of Pharmaceutical and Personal Care Products (PPCPs) under nitrifying and denitrifying conditions. Water Research 44: 3214-3224 DOI 10.1016/j.watres.2010.02.040

Terzic S, Senta I, Matosic M, Ahel M (2011) Identification of biotransformation products of macrolide and fluoroquinolone antimicrobials in membrane bioreactor treatment by ultrahigh-performance liquid chromatography/quadrupole time-of-flight mass spectrometry. Analytical and Bioanalytical Chemistry 401: 353-363 DOI 10.1007/s00216-011-5060-x

Wang Z, Ma J, Tang CY, Kimura K, Wang Q, Han X (2014) Membrane cleaning in membrane bioreactors: A review. Journal of Membrane Science 468: 276-307 DOI 10.1016/j.memsci.2014.05.060

Wright GD (2005) Bacterial resistance to antibiotics: Enzymatic degradation and modification. Advanced Drug Delivery Reviews 57: 1451-1470 DOI 10.1016/j.addr.2005.04.002

Yang W, Cicek N, Ilg J (2006) State-of-the-art of membrane bioreactors: Worldwide research and commercial applications in North America. Journal of Membrane Science 270: 201-211 DOI 10.1016/j.memsci.2005.07.010

Appendices

Appendix A Membrane Bioreactor dimensioning

MLSS 10 g/L

The flow (Q) was calculated in order to achieve a flux of 15 L/m^2.h in a membrane with an area (A) 0.11 m^2.

$$Q = F \times A = 15\ L/m^2.h\ \times\ 0.11\ m^2 = 1.6\ L/h \sim 40\ L/d$$

$$Q = 0.04\ \ m^3/d$$

The lab scale systems volume, MLSS concentration and SRT were fixed in 20 L, 10 g/L and 20 days respectively.
Then the COD concentration was calculated to maintain a sludge concentration of 10 g/L

Reactor VSS mass

$$MX_{BHv} = \frac{Q_i S_{bi} Y_{hv} SRT}{1 + \ b_H SRT} = \frac{0.04 * 1250 * 0.45 * 20}{1 + 0.24 * 20} = 78\ gVSS$$

$$MX_{EHv} = \ f_H b_H MX_{BHv} SRT = 0.2 * 0.24 * 78 * 20 = 74\ gVSS$$

$$MX_{IV} = \ Q_i X_{IV} SRT = 0.04 * 10.3 * 20 = 8\ gVSS$$

$$MX_N = \frac{Q_i NO_x Y_a SRT}{1 + \ b_A SRT} = \frac{0.04 * 28 * 0.12 * 20}{1 + 0.08 * 20} = 1\ gVSS$$

$$MX_{bio} = MX_{BHv} + MX_{EHv} + MX_N = 78 + 74 + 1 = 153\ gVSS$$

$$MX_V = MX_{bio} + MX_{IV} = 153 + 8 = 161\ gVSS$$

Reactor TSS mass

$$MX_T = MX_v + MX_{IO}$$

$$MX_{IO} = Q_i X_{ioi} SRT + f_{iOHO} MX_{BHv} = 0.04 * (250 - 200) * 20 + 0.15 * 78 = 52\ gISS$$

$$MX_T = 161 + 52 = 213\ gTSS$$

For a reactor volume 20 L, and a total biomass in the reactor of 213 g, the MLSS concentration in the reactor will be,

$$X_T = \frac{MX_T}{V} = \frac{213}{20} = 10.6\ g/L$$

The sludge production will be,

$$FX_T = \frac{MX_T}{SRT} = \frac{212.8}{20} = 11\ gTSS/day$$

The flow waste will be,

$$Q_w = \frac{FX_T}{X_T} = \frac{11}{10.6} = 1\ L/day$$

The oxygen requirements will be,

$$FO_c = Q_i S_{bi} - f_{cv}\left[\frac{MX_{BHv} + MX_{EHv}}{SRT}\right] + FO_n$$

$$FO_c = 0.04 * 1250 - 1.48\left[\frac{78 + 74}{20}\right] + 4.57 * 28 * 0.04 = 43.3\ gO_2/d$$

MLSS 20 g/L

In this phase the lab scale systems volume, the wastewater characteristics and the flow will be the same. In order to achieve a MLSS concentration of 20 g/L the load will be changed. The SRT will be 20 days.

Reactor VSS mass

$$MX_{BHv} = \frac{Q_i S_{bi} Y_{hv} SRT}{1 + b_H SRT} = \frac{0.04 * 2500 * 0.45 * 20}{1 + 0.24 * 20} = 155\ gVSS$$

$$MX_{EHv} = f_H b_H MX_{BHv} SRT = 0.2 * 0.24 * 155 * 20 = 149\ gVSS$$

$$MX_{IV} = Q_i X_{IV} SRT = 0.04 * 20.3 * 20 = 16.2\ gVSS$$

$$MX_N = \frac{Q_i NO_x Y_a SRT}{1 + b_A SRT} = \frac{0.04 * 56 * 0.12 * 20}{1 + 0.08 * 20} = 2.1\ gVSS$$

$$MX_{bio} = MX_{BHv} + MX_{EHv} + MX_N = 155 + 149 + 2.1 = 306.2\ gVSS$$

$$MX_V = MX_{bio} + MX_{IV} = 306.2 + 16.2 = 322.4\ gVSS$$

Reactor TSS mass

$$MX_T = MX_v + MX_{IO}$$

$$MX_{IO} = Q_i X_{ioi} SRT + f_{iOHO} MX_{BHv} = 0.04 * (250 - 200) * 20 + 0.15 * 155 = 63.3\ gISS$$

$$MX_T = 322.4 + 63.3 = 385.7\ gTSS$$

For a reactor volume 20 L,

$$X_T = \frac{MX_T}{V} = \frac{385.7}{20} = 19.3\ g/L$$

The sludge production will be,

$$FX_T = \frac{MX_T}{SRT} = \frac{385.7}{20} = 19\ gTSS/day$$

The flow waste will be,

$$Q_w = \frac{FX_T}{X_T} = \frac{19}{19.3} = 1\ L/day$$

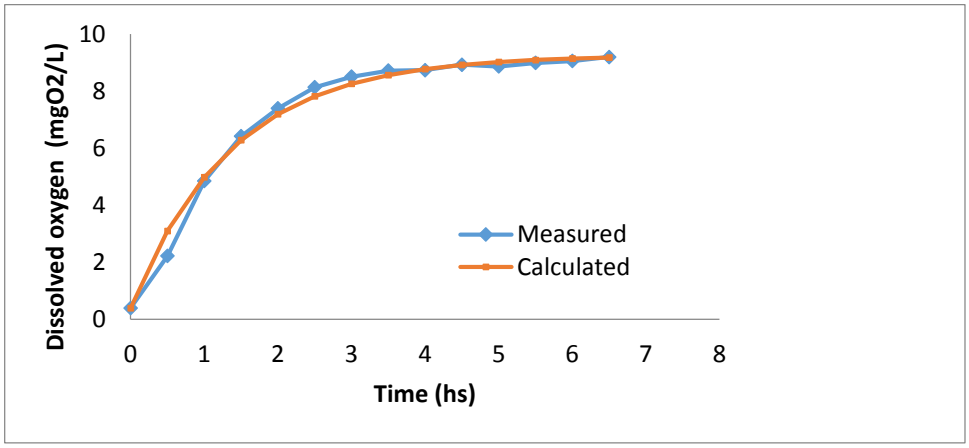
The oxygen requirements will be,

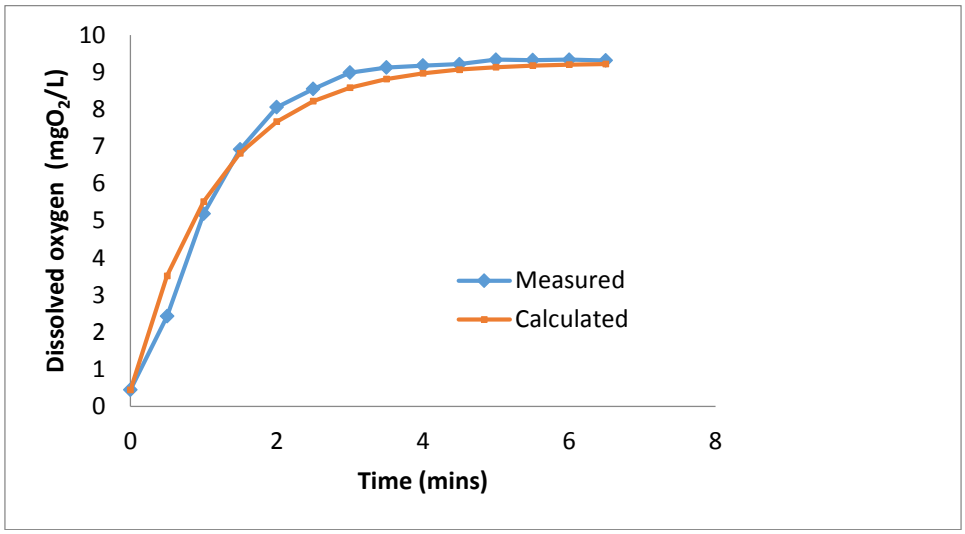
$$FO_c = Q_i S_{bi} - f_{cv}\left[\frac{MX_{BHv} + MX_{EHv}}{SRT}\right] + FO_n$$

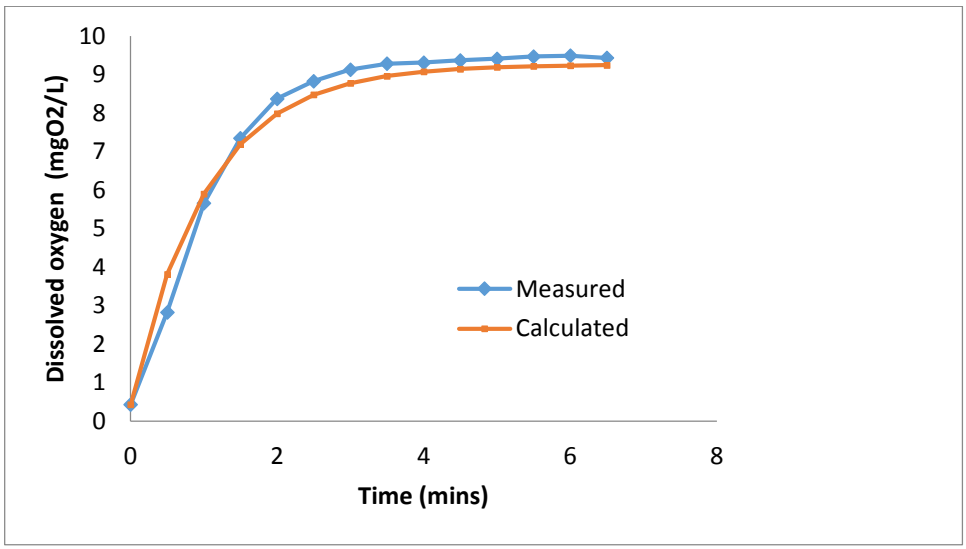
$$FO_c = 0.04 * 2500 - 1.48\left[\frac{155 + 149}{20}\right] + 4.57 * 56 * 0.04 = 86.6\frac{gO_2}{d}$$

Oxygen transfer rate

K_{LA} in clean water was calculated using the gassing out method with nitrogen.







AVG (min)	**0.8711**
STDV	**0.1227**

$$\alpha = \frac{K_{LA\,,sludge}}{K_{LA,clean\ water}}$$

According to (Muller et al., 1995) an α=0.5 was assumed

$$K_{LA\,,sludge} = 0.5 \times 0.87 = 26.3\ h^{-1}$$

Using the following equation OTR was estimated

$$OTR = K_{LA\,,sludge} \times (DO_{sat} - DO) \times V$$

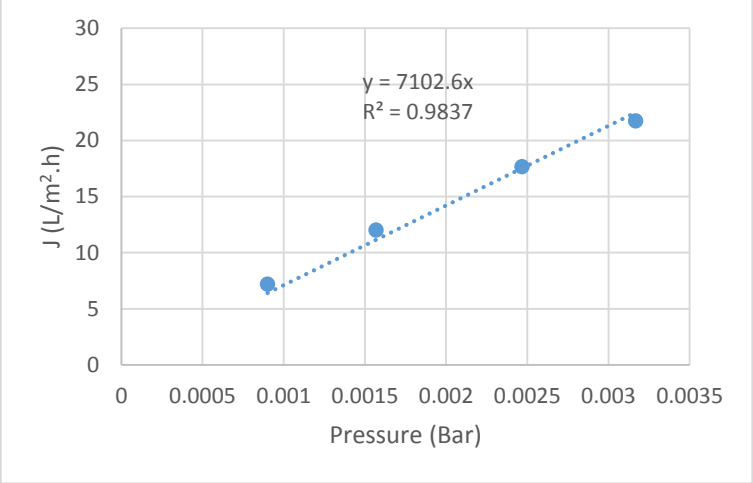
$$OTR = 26.3 \times (9.26 - 5.0) \times 23\ L$$

$$OTR = 61.8\ gO_2/d$$

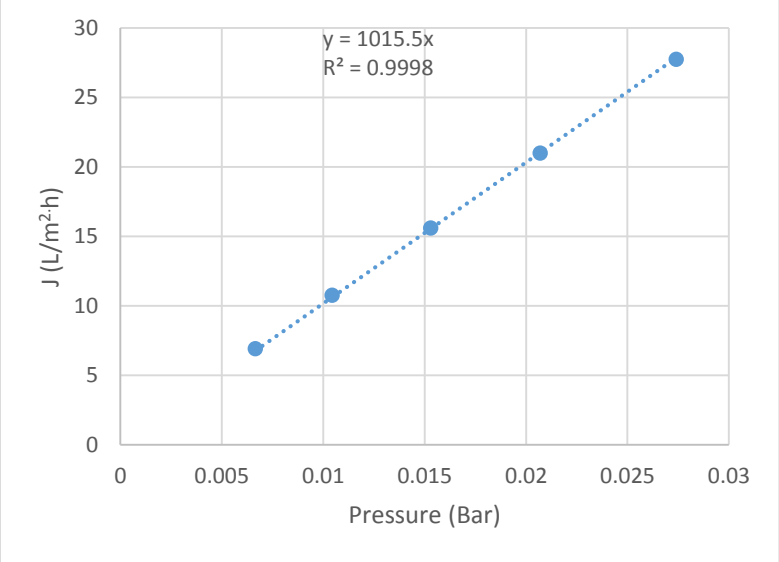
Appendix B Permeability

For measuring permeability of clean membrane, the MBR was filled with demineralized water, and TMP was measured for different fluxes. Starting the measurement from fluxes around 10 $L/m^2.h$. and increasing it until reach a flux of 25 $L/m^2.h$. To increase the flux in each step the flow of permeate was modified. After TMP reached a constant value it was registered and the flux was increased again. This procedure was done by duplicate for each membrane. Finally, the permeability was calculated as the slope of the graph Flux vs. TMP. The same procedure was followed to measure permeability in demineralized water of the fouled membrane. The difference between the permeability for the fouled membrane and the initial permeability is the loss in permeability after operation.

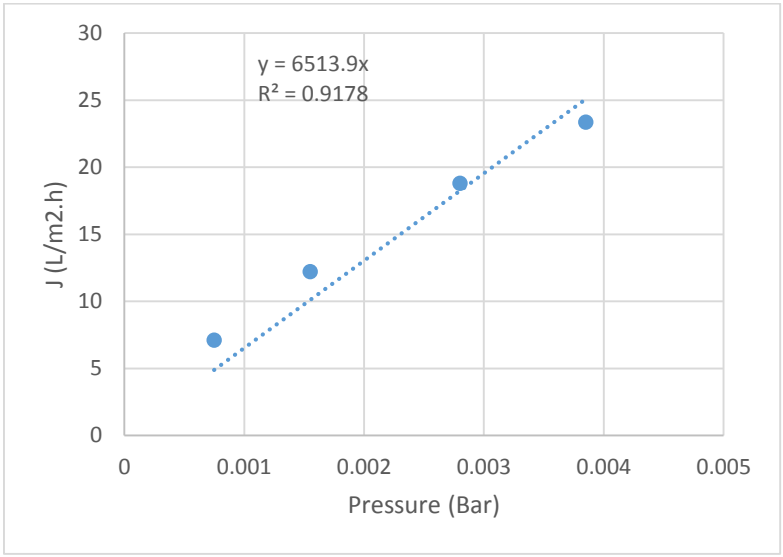
Initial permeability in water (Experiment 1, Bubble diffusers)



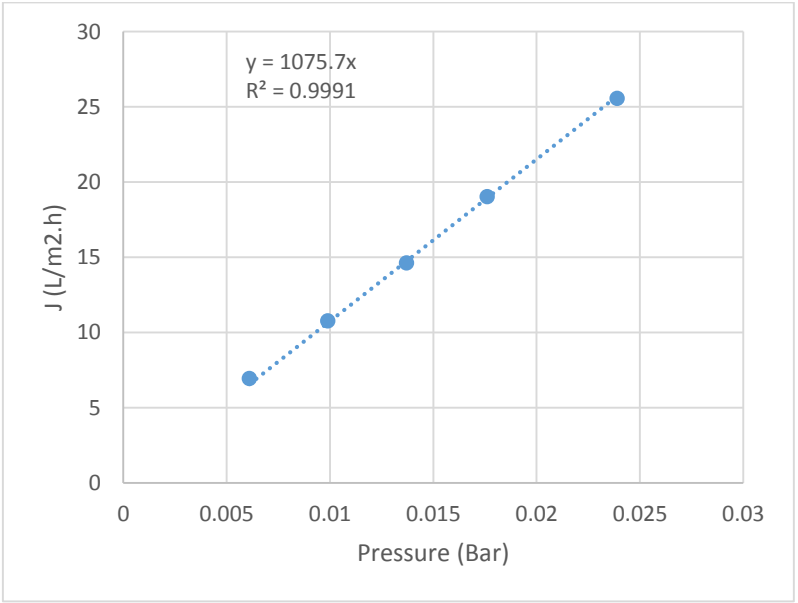
Permeability after operation in water (Experiment 1, Bubble diffusers)



Initial permeability in water (Experiment 1, SDOX)



Permeability after operation in water (Experiment 1, SDOX)



The permeability measured was then normalized to 20 ºC.

$$K_{20} = \frac{K_T \times \mu^T}{\mu^{20}}$$

	Bubble diffusers	SDOX
Temperature (ºC)	17.9	20.4
Initial Permeability in water ($L/m^2.h.bar$)	7483.9	6449.9
Permeability after operation in water ($L/m^2.h.bar$)	1006.4	1159.4
% Lost Permeability	**86.6**	**82.0**

The same procedure was done for each phase of each experiment.

Appendix C Particle size distribution

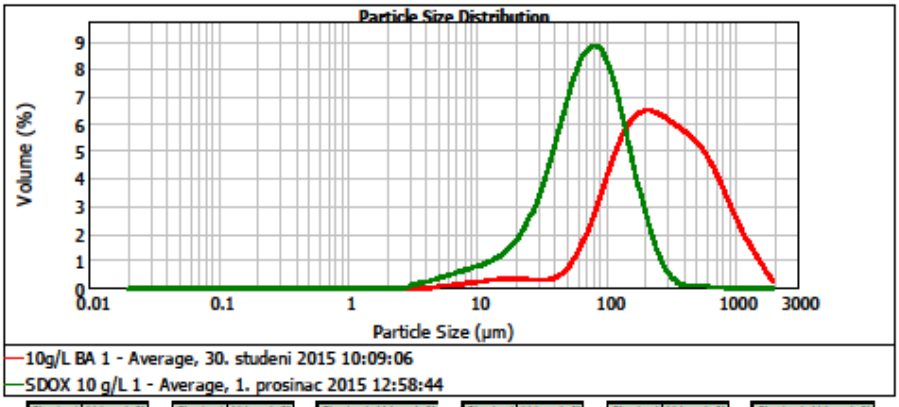
Experiment 1

MASTERSIZER 2000

Result Analysis Report

Sample Name: SDOX 10 g/L 1 - Average	**SOP Name:** Voda - 2000s	**Measured:** 1. prosinac 2015 12:58:44	
Sample Source & type:	**Measured by:** Fermenter 1	**Analysed:** 1. prosinac 2015 12:58:46	
Sample bulk lot ref:	**Result Source:** Averaged		
Particle Name: Activated sludge	**Accessory Name:** Hydro 2000S (A)	**Analysis model:** General purpose	**Sensitivity:** Enhanced
Particle RI: 1.500	**Absorption:** 0	**Size range:** 0.020 to 2000.000 um	**Obscuration:** 11.63 %
Dispersant Name: Water	**Dispersant RI:** 1.330	**Weighted Residual:** 0.536 %	**Result Emulation:** Off
Concentration: 0.0725 %Vol	**Span :** 1.933	**Uniformity:** 0.614	**Result units:** Volume
Specific Surface Area: 0.14 m²/g	**Surface Weighted Mean D[3,2]:** 42.964 um	**Vol. Weighted Mean D[4,3]:** 84.763 um	

d(0.1): 22.660 um **d(0.5):** 71.418 um **d(0.9):** 160.714 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	0.88	120.226	6.01	1258.925	0.00
0.011	0.00	0.120	0.00	1.259	0.00	13.183	0.99	138.038	4.89	1445.440	0.00
0.013	0.00	0.138	0.00	1.445	0.00	15.136	1.15	158.489	3.75	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	0.00	17.378	1.38	181.970	2.88	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.00	19.953	1.70	208.930	1.76	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.00	22.909	2.14	239.883	1.05	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.00	26.303	2.70	275.423	0.56	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.08	30.200	3.41	316.228	0.26	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.15	34.674	4.23	363.078	0.12	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.22	39.811	5.13	416.869	0.06	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.28	45.709	6.04	478.630	0.06	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.36	52.481	6.87	549.541	0.06	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.43	60.256	7.53	630.957	0.05	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.51	69.183	7.91	724.436	0.01	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.59	79.433	7.98	831.764	0.00	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.68	91.201	7.65	954.993	0.00	10000.000	
0.091	0.00	0.955	0.00	10.000	0.76	104.713	6.98	1096.478	0.00		
0.105	0.00	1.096	0.00	11.482		120.226		1258.925	0.00		

Operator notes:

Malvern Instruments Ltd.
Malvern, UK
Tel :- +[44] (0) 1684-892456 Fax +[44] (0) 1684-892789

Mastersizer 2000 Ver. 5.60
Serial Number : MAL1039333

File name: Voda
Record Number: 397
23.3.2016 13:32:29

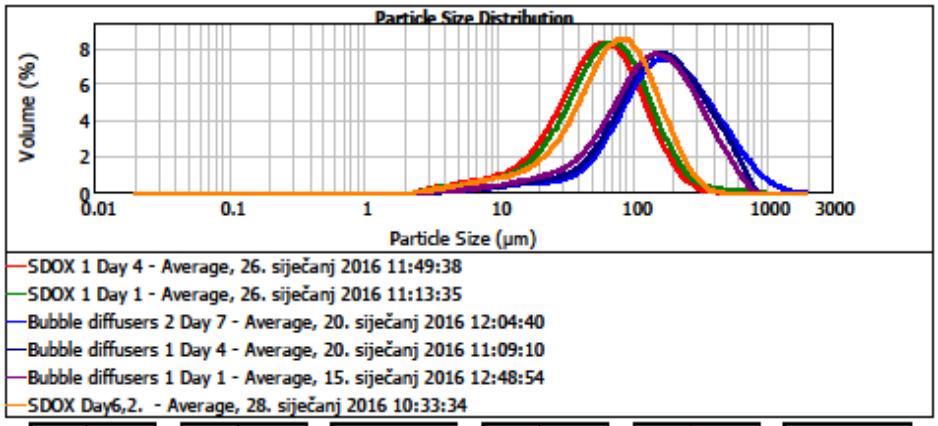
Experiment 2

MASTERSIZER 2000

Result Analysis Report

Sample Name: SDOX Day6,2. - Average	**SOP Name:** Voda - 2000s	**Measured:** 28. siječanj 2016 10:33:34	
Sample Source & type:	**Measured by:** Unknown	**Analysed:** 28. siječanj 2016 10:33:36	
Sample bulk lot ref:	**Result Source:** Averaged		
Particle Name: Activated sludge	**Accessory Name:** Hydro 2000S (A)	**Analysis model:** General purpose	**Sensitivity:** Enhanced
Particle RI: 1.500	**Absorption:** 0	**Size range:** 0.020 to 2000.000 um	**Obscuration:** 24.44 %
Dispersant Name: Water	**Dispersant RI:** 1.330	**Weighted Residual:** 0.677 %	**Result Emulation:** Off
Concentration: 0.1606 %Vol	**Span :** 2.002	**Uniformity:** 0.623	**Result units:** Volume
Specific Surface Area: 0.142 m²/g	**Surface Weighted Mean D[3,2]:** 42.315 um	**Vol. Weighted Mean D[4,3]:** 86.818 um	

d(0.1): 22.183 um **d(0.5):** 73.231 um **d(0.9):** 168.818 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	0.87	120.226	6.18	1258.925	0.00
0.011	0.00	0.120	0.00	1.259	0.00	13.183	0.99	138.038	5.19	1445.440	0.00
0.013	0.00	0.138	0.00	1.445	0.00	15.136	1.15	158.489	4.13	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	0.00	17.378	1.38	181.970	3.07	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.00	19.953	1.70	208.930	2.15	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.00	22.909	2.12	239.883	1.37	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.05	26.303	2.66	275.423	0.79	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.11	30.200	3.32	316.228	0.39	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.18	34.674	4.08	363.078	0.11	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.24	39.811	4.91	416.869	0.02	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.31	45.709	5.75	478.630	0.00	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.39	52.481	6.52	549.541	0.00	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.46	60.256	7.15	630.957	0.00	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.53	69.183	7.56	724.436	0.00	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.61	79.433	7.68	831.764	0.00	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.69	91.201	7.48	954.993	0.00	10000.000	
0.091	0.00	0.955	0.00	10.000	0.77	104.713	6.98	1096.478	0.00		
0.105		1.096		11.482		120.226		1258.925	0.00		

Operator notes:

Malvern Instruments Ltd.
Malvern, UK
Tel :- +[44] (0) 1684-892456 Fax +[44] (0) 1684-892789

Mastersizer 2000 Ver. 5.60
Serial Number : MAL1039333

File name: Voda
Record Number: 683
23.3.2016 13:40:27

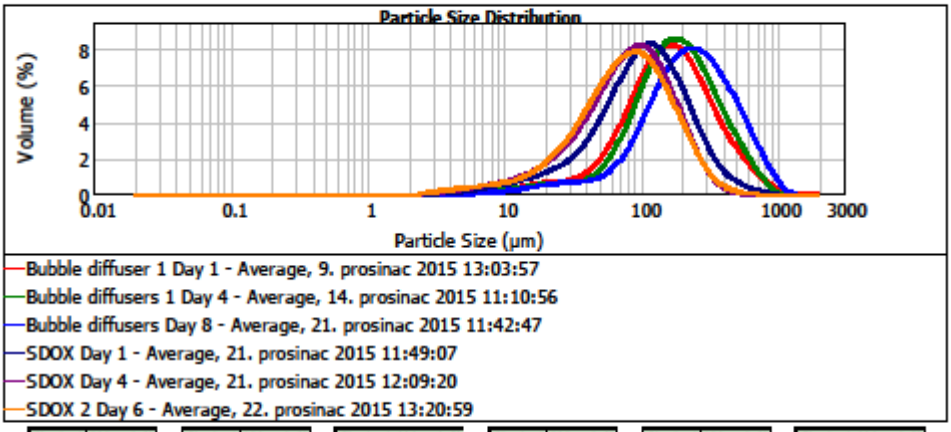
Experiment 3

MASTERSIZER 2000

Result Analysis Report

Sample Name: SDOX 2 Day 6 - Average	**SOP Name:** Voda - 2000s	**Measured:** 22. prosinac 2015 13:20:59	
Sample Source & type:	**Measured by:** Fermenter 1	**Analysed:** 22. prosinac 2015 13:21:00	
Sample bulk lot ref:	**Result Source:** Averaged		
Particle Name: Activated sludge	**Accessory Name:** Hydro 2000S (A)	**Analysis model:** General purpose	**Sensitivity:** Enhanced
Particle RI: 1.500	**Absorption:** 0	**Size range:** 0.020 to 2000.000 um	**Obscuration:** 28.07 %
Dispersant Name: Water	**Dispersant RI:** 1.330	**Weighted Residual:** 0.606 %	**Result Emulation:** Off
Concentration: 0.2016 %Vol	**Span :** 2.169	**Uniformity:** 0.683	**Result units:** Volume
Specific Surface Area: 0.133 m²/g	**Surface Weighted Mean D[3,2]:** 45.224 um	**Vol. Weighted Mean D[4,3]:** 97.055 um	

d(0.1): 23.732 um **d(0.5):** 78.331 um **d(0.9):** 193.617 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	0.74	120.226	6.29	1258.925	0.00
0.011	0.00	0.120	0.00	1.259	0.00	13.183	0.90	138.038	5.55	1445.440	0.00
0.013	0.00	0.138	0.00	1.445	0.00	15.136	1.11	158.489	4.69	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	0.00	17.378	1.39	181.970	3.76	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.00	19.953	1.74	208.930	2.88	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.00	22.909	2.18	239.883	2.04	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.00	26.303	2.69	275.423	1.37	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.06	30.200	3.28	316.228	0.84	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.12	34.674	3.93	363.078	0.48	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.18	39.811	4.60	416.869	0.26	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.22	45.709	5.26	478.630	0.11	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.32	52.481	5.92	549.541	0.05	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.37	60.256	6.47	630.957	0.02	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.42	69.183	6.87	724.436	0.00	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.47	79.433	7.09	831.764	0.00	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.54	91.201	7.07	954.993	0.00	10000.000	
0.091	0.00	0.955	0.00	10.000	0.63	104.713	6.80	1096.478	0.00		
0.105	0.00	1.096	0.00	11.482		120.226		1258.925	0.00		

Operator notes:

Malvern Instruments Ltd.
Malvern, UK
Tel :- +[44] (0) 1684-892456 Fax +[44] (0) 1684-892789

Mastersizer 2000 Ver. 5.60
Serial Number : MAL1039333

File name: Voda
Record Number: 573
23.3.2016 13:37:23

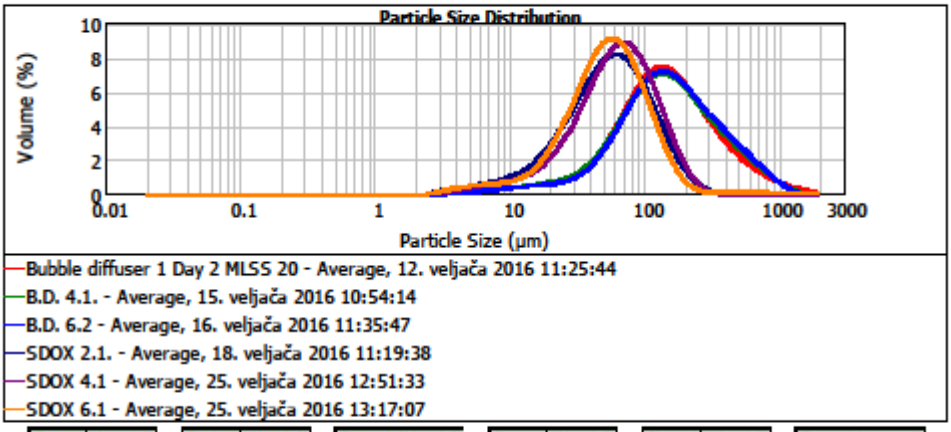
Experiment 4

MASTERSIZER 2000

Result Analysis Report

Sample Name: SDOX 6.1 - Average	**SOP Name:** Voda - 2000s	**Measured:** 25. veljača 2016 13:17:07	
Sample Source & type:	**Measured by:** Fermenter 1	**Analysed:** 25. veljača 2016 13:17:09	
Sample bulk lot ref:	**Result Source:** Averaged		
Particle Name: Activated sludge	**Accessory Name:** Hydro 2000S (A)	**Analysis model:** General purpose	**Sensitivity:** Enhanced
Particle RI: 1.500	**Absorption:** 0	**Size range:** 0.020 to 2000.000 um	**Obscuration:** 22.51 %
Dispersant Name: Water	**Dispersant RI:** 1.330	**Weighted Residual:** 0.689 %	**Result Emulation:** Off
Concentration: 0.1204 %Vol	**Span :** 1.868	**Uniformity:** 0.689	**Result units:** Volume
Specific Surface Area: 0.172 m²/g	**Surface Weighted Mean D[3,2]:** 34.974 um	**Vol. Weighted Mean D[4,3]:** 68.729 um	

d(0.1): 19.787 um **d(0.5):** 53.424 um **d(0.9):** 119.587 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	0.87	120.226	3.39	1258.925	0.04
0.011	0.00	0.120	0.00	1.259	0.00	13.183	1.13	138.038	2.32	1445.440	0.03
0.013	0.00	0.138	0.00	1.445	0.00	15.136	1.51	158.489	1.46	1659.587	0.02
0.015	0.00	0.158	0.00	1.660	0.00	17.378	2.06	181.970	0.82	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.00	19.953	2.76	208.930	0.42	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.00	22.909	3.63	239.883	0.20	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.00	26.303	4.61	275.423	0.12	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.08	30.200	5.64	316.228	0.11	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.16	34.674	6.63	363.078	0.12	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.25	39.811	7.47	416.869	0.13	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.32	45.709	8.05	478.630	0.14	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.39	52.481	8.30	549.541	0.14	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.45	60.256	8.18	630.957	0.13	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.49	69.183	7.65	724.436	0.10	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.53	79.433	6.81	831.764	0.08	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.57	91.201	5.75	954.993	0.05	10000.000	
0.091	0.00	0.955	0.00	10.000	0.62	104.713	4.58	1096.478	0.04		
0.105	0.00	1.096	0.00	11.482	0.71	120.226		1258.925			

Operator notes:

Malvern Instruments Ltd.
Malvern, UK
Tel :- +[44] (0) 1684-892456 Fax +[44] (0) 1684-892789

Mastersizer 2000 Ver. 5.60
Serial Number : MAL1039333

File name: Voda
Record Number: 751
23.3.2016 13:42:38

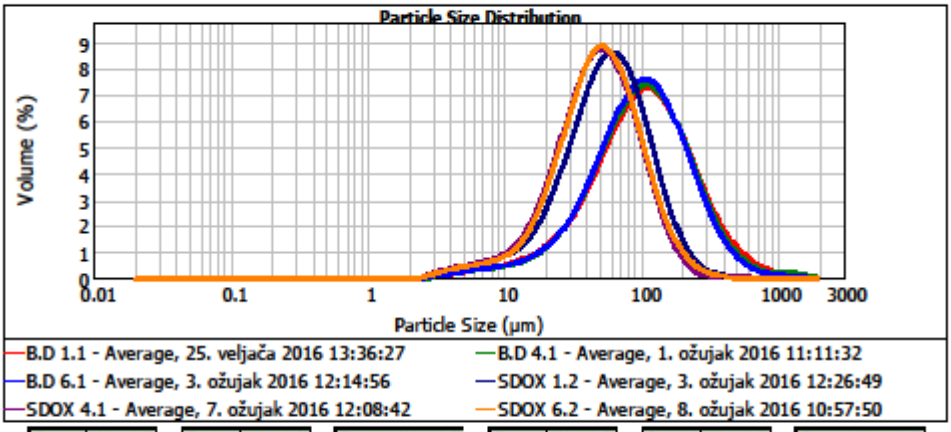
Experiment 5

MASTERSIZER 2000

Result Analysis Report

Sample Name: SDOX 6.2 - Average	**SOP Name:** Voda - 2000s	**Measured:** 8. ožujak 2016 10:57:50	
Sample Source & type:	**Measured by:** Fermenter 1	**Analysed:** 8. ožujak 2016 10:57:52	
Sample bulk lot ref:	**Result Source:** Averaged		
Particle Name: Activated sludge	**Accessory Name:** Hydro 2000S (A)	**Analysis model:** General purpose	**Sensitivity:** Enhanced
Particle RI: 1.500	**Absorption:** 0	**Size range:** 0.020 to 2000.000 um	**Obscuration:** 19.53 %
Dispersant Name: Water	**Dispersant RI:** 1.330	**Weighted Residual:** 0.821 %	**Result Emulation:** Off
Concentration: 0.0974 %Vol	**Span :** 1.985	**Uniformity:** 0.635	**Result units:** Volume
Specific Surface Area: 0.181 m²/g	**Surface Weighted Mean D[3,2]:** 33.198 um	**Vol. Weighted Mean D[4,3]:** 61.103 um	

d(0.1): 18.300 um **d(0.5):** 49.549 um **d(0.9):** 116.676 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	1.07	120.226	3.04	1258.925	0.00
0.011	0.00	0.120	0.00	1.259	0.00	13.183	1.42	138.038	2.19	1445.440	0.00
0.013	0.00	0.138	0.00	1.445	0.00	15.136	1.91	158.489	1.50	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	0.00	17.378	2.55	181.970	0.98	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.00	19.953	3.34	208.930	0.65	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.00	22.909	4.26	239.883	0.39	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.00	26.303	5.23	275.423	0.24	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.08	30.200	6.19	316.228	0.15	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.16	34.674	7.03	363.078	0.09	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.25	39.811	7.65	416.869	0.02	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.31	45.709	7.98	478.630	0.00	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.37	52.481	7.94	549.541	0.00	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.43	60.256	7.57	630.957	0.00	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.48	69.183	6.90	724.436	0.00	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.53	79.433	6.02	831.764	0.00	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.59	91.201	5.02	954.993	0.00	10000.000	
0.091	0.00	0.955	0.00	10.000	0.69	104.713	4.00	1096.478	0.00		
0.105	0.00	1.096	0.00	11.482	0.84	120.226		1258.925	0.00		

Operator notes:

Malvern Instruments Ltd.
Malvern, UK
Tel :- +[44] (0) 1684-892456 Fax +[44] (0) 1684-892789

Mastersizer 2000 Ver. 5.60
Serial Number : MAL1039333

File name: Voda
Record Number: 839
23.3.2016 13:43:58