UNIVERSITY OF LJUBLJANA
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Master study of CHEMICAL ENGINEERING

THE IMPACT OF SELECTED EUTECTIC SOLVENT TO BIOLOGICAL WASTEWATER TREATMENT PLANT

MASTER THESIS

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Declaration

I declare that I am the author of the submitted master thesis.

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Ljubljana, February, 2014

Content

Acknowledgement

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Abstract

In the recent years, there has been intensive development of non-hazardous solvents and reaction media, able to replace common organic compounds which are possible aquatic contaminants due to their toxicity, volatility and persistency. As a result, eutectic solvents (ES) were introduced and they are used in different processes (bio-transformations, separation). These eutectic solvents (ES) are generally composed of two or three cheap and safe components that are capable of self-association, to form a eutectic mixture with a melting point lower than that of each individual component. However, they could spread widely by wastewaters and affect large areas in terms of chronic toxicity, bioaccumulation, etc. The benefits and drawbacks of these components are currently being discussed.

The biological treatment is often used due to its reliability, simplicity and high cost-effectiveness as well providing many advantages in terms of biodegradable matter and nitrogen compounds removal. However, the efficiency of biological processes is strongly limited because of refractory or inhibitory compounds in wastewaters. Aerobic treatment based on activated sludge process, have been widely studied and adopted.

The aim of this research focuses on the determination of the impact caused by the addition of selected ES to the aerobic WWTP. We selected the ES based on choline chloride and malonic acid. First, its toxicity to microorganisms of activated sludge was determined by measurement of inhibition of oxygen consumption of activated sludge. It helped to identify the initial concentration of ES in the influent to the pilot WWTP. The experiment was started with setting up pilot aerobic WWTP, feed by synthetic municipal wastewater and its efficiency was monitored by several parameters. After steady-state operation of WWTP was achieved, the selected amount of ES was added in the influent and treatment efficiency was monitored continuously. The amount of the ES was increased to find the highest concentration of the component still possible to treat in the biological wastewater treatment plant. At the end, it has been seen that although the toxicity of ES to microorganisms was so high, the impact to the biological treatment plant at the same concentrations was negligible, obtaining good treatment efficiency in the pilot WWTP.

Symbols

APHA	American Public Health Association
AS	Activated sludge
AWWA	American Water Works Association
ATU	Allylthiourea
BOD	Biochemical oxygen demand ($mg \cdot L^{-1}$)
BOD_5	Biochemical oxygen demand after 5 days ($mg \cdot L^{-1}$)
CAS	Chemical Abstracts Service
CC	Chlorine chloride
COD	Chemical oxygen demand ($mg \cdot L^{-1}$)
COD_{IN}	Chemical oxygen demand in influent ($mg \cdot L^{-1}$)
COD_{OUT}	Chemical oxygen demand in effluent ($mg \cdot L^{-1}$)
D	Diameter (dm)
DCF	Dichlorophenol
DOC	Dissolved organic carbon ($mg \cdot L^{-1}$)
Dt	Biodegradation (%)
E	Treatment efficiency (%)
ES	Eutectic solvent
FAO	Food and Agriculture Organization
F/M	Food to microorganism ratio ($kg_{BOD5} \cdot kg_{AS}^{-1} \cdot d^{-1}$)
H	Height (dm)
HBD	Hydrogen bond donor
Het	Heterotrophic microorganisms
HRAS	Hi-Rate activated sludge
HRT	Hydraulic retention time (h)
IC	Inorganic carbon ($mg \cdot L^{-1}$)
I	Inhibition of oxygen uptake of total microorganisms (%)
I_H	Inhibition of oxygen uptake of heterotrophic microorganisms (%)
IL	Ionic liquid
I_N	Inhibition of oxygen uptake ok nitrifying microorganisms (%)
IN	Influent
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
M	Metals ($mg \cdot L^{-1}$)
m	Mass of activated sludge (g)
MA	Malonic acid
MLTSS	Mixed liquor total suspended solids (kg)
MO	Total microorganisms
Nit	Nitrifying microorganisms
NH_4^+-N	Nitrogen in the form of ammonium ($mg \cdot L^{-1}$)
NO_2^--N	Nitrogen in the form of nitrite ($mg \cdot L^{-1}$)
NO_3^--N	Nitrogen in the form of nitrate ($mg \cdot L^{-1}$)
OCb	Oxygen consumption in the blank ($mg \cdot L^{-1}$)
OCs	Oxygen consumption in the sample ($mg \cdot L^{-1}$)
OUT	Effluent
Q	Flow of the influent ($L \cdot h^{-1}$ or $m^3 \cdot h^{-1}$)
R	Oxygen consumption rate in the blank ($mg \cdot g^{-1} \cdot h^{-1}$)
RBC	Rotating biological contactor
R_{HA}	Rate of oxygen uptake for the abiotic control. (with ATU) ($mg \cdot g^{-1} \cdot h^{-1}$)
R_{HB}	Rate of oxygen uptake for the blank (with ATU) ($mg \cdot g^{-1} \cdot h^{-1}$)
R_{HS}	Rate of oxygen uptake for each sample (with ATU) ($mg \cdot g^{-1} \cdot h^{-1}$)
Rs	Specific respiration ratio ($mg \cdot g^{-1} \cdot h^{-1}$)
R_{TA}	Rate of oxygen uptake for the abiotic control (no ATU) ($mg \cdot g^{-1} \cdot h^{-1}$)
R_{TB}	Rate of oxygen uptake for the blank (no ATU) ($mg \cdot g^{-1} \cdot h^{-1}$)

R_{TS}	Rate of oxygen uptake for each sample (no ATU) ($mg \cdot g^{-1} \cdot h^{-1}$).
SLR	Sludge loading rate ($kg_{BOD5} \cdot kg_{AS}^{-1} \cdot d^{-1}$)
SS	Suspended solids ($mg \cdot L^{-1}$)
SVI	Sludge volume index ($mL \cdot g^{-1}$)
TDS	Total dissolved solids ($mg \cdot L^{-1}$)
TOC	Total organic carbon ($mg \cdot L^{-1}$)
TS	Total solids ($mg \cdot L^{-1}$)
UK	United Kingdom
UWWTPs	Urban wastewater treatment plants
VOC	Volatile organic compound ($mg \cdot L^{-1}$)
V	Volume of aeration tank (L or m^3)
Vs	Volume of activated sludge sampled. (mL)
Vss	Volume settled activated sludge (mL)
VSS	Volatile suspended solids ($mg \cdot L^{-1}$)
VU	Sedimentation of activated sludge ($mL \cdot L^{-1}$)
WEF	Water Environmental Federation
WFD	Water Framework Directive
WTW	Wissenschaftlich-Technische-Werkstätten
WW	Wastewater
WWTP	Wastewater treatment plant
X	Concentration of activated sludge ($mg \cdot L^{-1}$ or $g \cdot L^{-1}$)
ρ_1	Dissolved oxygen concentration in the beginning in the blank ($mg \cdot L^{-1}$)
ρ_2	Dissolved oxygen concentration at the end in the blank ($mg \cdot L^{-1}$)
Δt	Time interval between two measurements (min)
$30minEC_{50}$	Conc.(Vol.%) which inhibits oxygen consumption by 50% (in 30min)
$30minEC_{20}$	Conc. (Vol.%) which inhibits oxygen consumption by 20% (in 30min)

1. Introduction

Wastewaters are always a priority issue for the protection of the environment. They could have negative impact to the aquatic biota and human health if they are discharged to the surface water without any treatment. There are many disasters caused by the contamination of the rivers because of the wastewater discharges directly in the aquatic environment, above all if we go back to the 19th century, when population was ignoring consequences of the wastewater discharges.

One example could be the rupture of one tank full of activated sludge and wastewater in the Guadiamar river (in Spain), polluting the river with heavy metals such as cadmium, lead, zinc and copper. It affected an area of 4.634 hectares, contaminating 2.703 hectares with activated sludge and 1.931 with acidic water. The river pollution affected cultivation lands and forests. Harvests were no longer suitable for consumption, causing financial problems for farmers in the area. Fishes died and birds too, as a result of the consumption of polluted fish. It took one whole month for the river water to recover to its original state. This occurred in April 25 in 1998 (Lenntech, 2014). However, this is just an example of some disasters related to the untreated water.

As a result, wastewater treatment plants appeared on 19th century, being the solution for the removal of the pollutants in wastewater. Nowadays, one of the fundamental part in the wastewater treatment plant (WWTP) is the biological treatment. Most well known systems of wastewater treatment are processes with activated sludge, which removes dissolved organic and inorganic substances, and coagulates colloidal and nonsettled solids (Orhon et al., 2009; Ford, 2009). However, many of these substances can cause some impact to the biological treatment. Scientists are constantly researching new chemical and sometimes these compounds are rapidly available in the market, without almost any environmental impact assessment. Consequently, industries use them for their processes and then generate wastewater that needs to be treated in the WWTP. As a result, this WWTP could be affected and its treatment efficiencies could be reduced. Through this thesis, this situation is reflected, in particular with a selected eutectic solvent (ES), which has been studied for its advantages and drawbacks in some applications. The compatibility of the ES with the biological treatment in the WWTP was studied through toxicity and biodegradability assessment proceeded by simulation study in a pilot WWTP.

2. The aim of the thesis

The aim of the master thesis is focused on the determination of the impact of selected eutectic solvent (ES) to the aerobic wastewater treatment plant (WWTP). The efficiency of the WWTP before and after the addition of ES will be compared. Three hypotheses were addressed:

1st hypothesis: Eutectic solvent selected is toxic to aerobic microorganisms of activated sludge. For assessment of the toxicity, measurement of inhibition of oxygen consumption of activated sludge was employed, because some recent publications highlighted that ES may be toxic for microorganisms.

2nd hypothesis: ES are generally biodegradable. The biodegradation of the selected eutectic solvent will be determined by the measurement of oxygen consumption in a closed respirometer.

3rd hypothesis: ES affects the biological treatment in WWTP. For assessment of the impact, biological treatment will be simulated in pilot WWTP. It is necessary to obtain daily data about all the parameters and efficiency of the pilot WWTP (7 L of volume) feed by synthetic wastewater without any addition of ES. Parameters such as pH, temperature, dissolved oxygen concentration, wastewater flow rate, concentration of the activated sludge in the system, sludge volume index, sedimentation of activated sludge, chemical oxygen demand (COD), biological oxygen demand (BOD), total organic carbon (TOC), will be measured. When steady-state operation of WWTP will be achieved, the selected amount of ES will be added in the influent and treatment efficiency will be monitored continuously, similarly like before addition of ES. The amount of ES will be increased. At the end, the effect of ES to WWTP will be determined.

3. Theoretical background

3.1. Definition of Wastewater

Wastewater (WW) is the liquid that is collected after industrial, commercial, agriculture or domestic activities. Depending on the origin of this residue and the contaminants present, it is possible to find some different classifications. However, most wastewaters can be divided into these general categories (Roš, 1993):

Domestic wastewater: Results of discharges from households as well as public buildings and other facilities, including water for street cleaning and fire fighting, and also wastewater from small local industries connecting to the sewerage system.

Commercial wastewater: Coming from commercial businesses, small industries operations, and other public facilities usually connected to the common sewerage system.

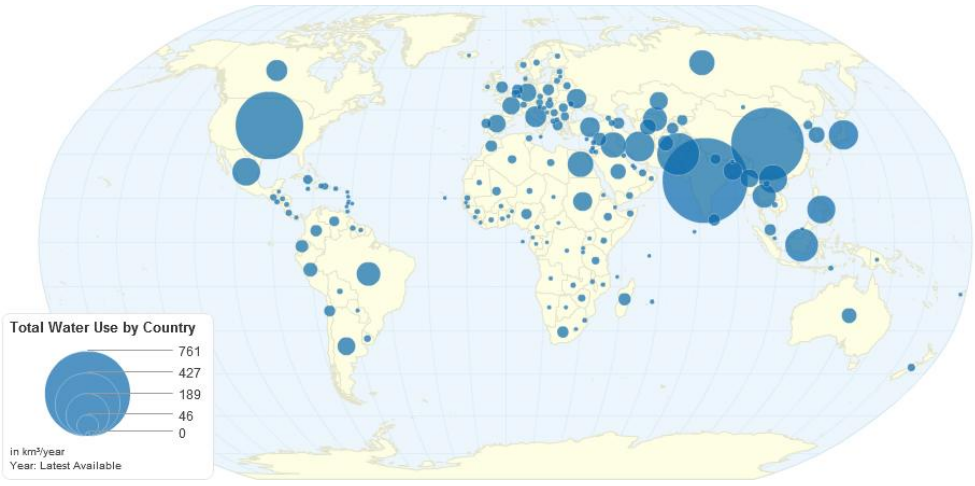
Industrial wastewater: Produced by large industrial plants of all kinds.

Agricultural wastewater: From livestock production, and from plant and animal processing operations.

Seepage water (Foreign water): From managed drainages and drainage pipelines, the artificial lowering of the groundwater level and groundwater that leaks into sewerage systems, through pipelines and other installations.

Rainwater: Including all forms of precipitation-rain, snow, hail and fog.

In the following map (Picture 1) the amount of water used per inhabitant all over the world is presented.



Picture 1: Total water used by country in $km^3 \cdot year^{-1}$ according to Food and Agriculture Organization (FAO) of the United Nations 2010 (AQUASTAT, 2011).

The map (Picture 1) shows total water withdrawal for agricultural, industrial and municipal purposes by country. Water withdrawal is the quantity of water removed from available sources for use in any purpose. Water drawn-off is not necessarily entirely consumed and some portion may be returned for further use downstream.

The sum of the total water withdrawal used in over the world (by all the countries) is **3905.86 $km^3 \cdot year^{-1}$** (Picture 1). This calculation includes the municipal water withdrawal, the industrial water withdrawal and agricultural water withdrawal.

In this calculation, renewable freshwater resources as well as potential over-abstraction of renewable groundwater or withdrawal of fossil groundwater and eventual use of desalinated water or treated wastewater are included. Otherwise, other categories of water use are not included, such as water for cooling of power plants, mining, recreation, navigation, fisheries, etc., which are sectors that are characterized by a very low net water consumption rate.

It is important to indicate that the data in Picture 1 are not registered in the same year in all of the countries but all of it was calculated between 1995 and 2009. As a result, the countries where the consumption of water is higher are China, India and United States, with quantities of 554 $km^3 \cdot year^{-1}$ (2005), 761 $km^3 \cdot year^{-1}$ (2010) and 478 $km^3 \cdot year^{-1}$ (2005), respectively. The total world water withdrawal, estimated almost 4000 $km^3 \cdot year^{-1}$, is actually wastewater that must be treated (AQUASTAT, 2011).

3.2. Parameters to determine the quality of water

About 75% of suspended solids and 40% of filterable solids in wastewater are organic matters. These organic compounds usually consist of combinations of carbon, hydrogen and oxygen, and, in some cases, nitrogen. Also other elements such as sulfur, phosphorus and iron may be present. The main groups of organic substances in the wastewater are proteins (40-60%), carbohydrate (25-50%), fat and oil. With the measurements of some physical and chemical parameters the amount of pollutants presented in wastewater can be determined (Rabah, 2014).

3.2.1. Physical parameters

Temperature, color and odor: These parameters are more common to determinate the quality of the potable water. Color, for example, may be related to the presence of iron or manganese. Temperature affects taste and odor perceptions. Corrosion and incrustations, which, in turn, affect color, taste and odor, can be directly related to pH (Ontario, 2006).

Acidity: Acidity of water is the quantitative capacity to react with a strong base to a designated pH. Acidity is a measure of an aggregate property of water and can be interpreted in terms of specific substances only when the chemical composition of the sample is known (APHA, AWWA, WEF, 2005).

Alkalinity: Measures the amount of alkaline compounds in the water, such as carbonates, bicarbonates and hydroxides. These compounds are natural buffers that can remove excess hydrogen, or H^+ ions (Murdoch et al., 1991).

Conductivity: Electrical conductivity is a measure of the ability of water to conduct electricity. Pure water does not conduct electricity, so that means that we can measure the conductivity which results from impurities present in the water. It is an indirect measure of the presence of inorganic dissolved solids such as chloride, nitrate, sulfate, phosphate, sodium, magnesium, calcium, iron and aluminum. The presence of these substances increases the conductivity of a body of water. Organic substances like oil, alcohol, and sugar do not conduct electricity very well, and thus have a low conductivity in water (Murdoch et al., 1991).

3.2.2. Chemical parameters

Biological oxygen demand – BOD_5 ($mg \cdot L^{-1}$): This parameter measures the mass concentration of dissolved oxygen consumed under specified conditions by the microorganisms. It is biochemical oxidation of organic matter in wastewater. The incubation time is 5 days (ISO 5815-1, 2003).

Chemical oxygen demand - COD ($mg \cdot L^{-1}$): This parameter measures the mass concentration of oxygen equivalent to the amount of dichromate consumed by dissolved and suspended matter when a wastewater sample is treated with that oxidant under defined conditions (ISO 6060, 1989).

Total organic carbon - TOC ($mg \cdot L^{-1}$): This parameter measures the organic carbon present in wastewater in different fractions (based on oxidation states). The different fractions are defined as dissolved organic carbon (DOC) and inorganic carbon (IC). DOC is the fraction that passes through a 0.45 µm pore-diameter filter. TOC is the sum of DOC and IC (ISO 8245, 1989).

Total dissolved solids - TDS ($mg \cdot L^{-1}$): Solids that cannot be filtered include dissolved solids, colloidal solids, and very small suspended particles (Masters, 1998).

Total suspended solids – TSS ($mg \cdot L^{-1}$): Total solids than can be removed by a membrane filter (having a pore size of about 1.2 µm) (Masters, 1998).

Total solids – TS ($mg \cdot L^{-1}$): Is the sum of suspended and dissolved solids (Masters, 1998).

Nitrogen – N ($mg \cdot L^{-1}$): Nitrogen occurs in natural wastewaters in various forms, including nitrate (NO_3^-), nitrite (NO_2^-), and ammonia (NH_3). NO_3^- is the essential nutrient for many photosynthetic autotrophs and has been identified as the growth limit nutrient. It can be found in relatively high concentrations where it is relatively nontoxic to aquatic organisms. When nitrate concentrations become excessive, however, and other essential nutrient factors (phosphates) are present, the eutrophication can become a problem. Ammonia is the least stable form of nitrogen and thus difficult to measure accurately. NH_3 is one of the most important

pollutants in the aquatic environment because of its relatively high toxicity. It is discharged in large quantities in industrial, municipal and agricultural wastewaters. Nitrite is less stable and usually present in much lower amounts than nitrate. Nitrite is extremely toxic to aquatic life, however, is usually present only in trace amounts in most natural freshwater systems because it is rapidly oxidized to nitrate. The last form to find nitrogen is as organic nitrogen. It is the byproduct of living organisms. It includes such natural materials as proteins and peptides, nucleic acids and urea, and numerous synthetic organic materials (APHA, AWWA, WEF, 2005; Rand and Petrocelli 1985).

Phosphorus – P ($mg \cdot L^{-1}$): Phosphorus is often the limiting nutrient for plant growth, meaning it is in short supply relative to nitrogen. Phosphorus usually occurs in nature as orthophosphate (PO_4^{3-}) and it contributes to the alkalinity of the water and to the eutrophication phenomenon (Murdoch et al., 1991).

Metals - M ($mg \cdot L^{-1}$): Wastewater contain different metals and in different concentrations. Some of them may be either beneficial or toxic, depending on concentration. "Heavy metals" are the most toxic to aquatic organisms in this group. Some examples of heavy metals are: copper (Cu), iron (Fe), cadmium (Cd), zinc (Zn), mercury (Hg), and lead (Pb) (APHA, AWWA, WEF, 2005).

Others: It is important to determinate the concentration of chloride, calcium, potassium, etc.

Usually, legislation contains requirements for effluent limits for all these parameters in water, to prevent the pollution of the environment.

3.3. Pollutants in wastewater

These constituents described above, need to be removed from the wastewater, prior discharging. Obviously, their concentrations are depending upon the origin of the wastewater. In general, the pollutants of a typical domestic wastewater are (Anderson, 2009):

- Organic matter: Fats, oils and carbohydrates.
- Nitrifying compounds: Organic nitrogen in terms of proteins, urea and ammonium.
- Phosphate.
- Inorganic Matters: Chloride, metals.

In the following table (Table 1) the major constituents of a typical domestic wastewater are presented.

Table 1: Typical constituents of domestic wastewater (Falcone, 2013).

Constituent/Parameter	**Concentration ($mg \cdot L^{-1}$)**		
	Strong	Medium	Weak
Total solids	1200	700	350
Dissolved solids (TDS)	850	500	250
Suspended solids	350	200	100
Nitrogen (as N)	85	40	20
Phosphorus (as P)	20	10	6
$Chloride^-$	100	50	30
Alkalinity (as $CaCO_3$)	200	100	50
Grease	150	100	50
BOD_5	300	200	100

It is difficult to determinate average values for the major constituents contained in industrial wastewater, because they depend on the nature of the industry. In the following table (Table 2) there is a conventional characterization of different industrial wastewaters.

Table 2. Conventional characterization of different industrial wastewaters (Orhon et al., 2009).

Industrial water	Parameter ($mg \cdot L^{-1}$)								
	Total COD	**Sol. COD (**)**	**BOD_5**	**Total N**	**NH3-N**	**Total P**	**TSS**	**VSS(*)**	**alkalinity**
Textile									
Denim processing	2400	1700	/	35	5.6	34	500	70	530
Polyester knit fabrics	1985	1485	/	27	1.7	9	213	22	960
Cotton knit fabrics	2310	2185	/	24	/	4.5	135	80	/
Acrylic processing	1900	1590	/	72	/	4.2	90	43	/
Cotton woven fabric	1240	1176	680	144	/	2.2	/	/	/
Yogurt&buttermilk processing	1500	/	1000	63	/	7.2	191	/	/
Tannery									
Raw water	4180	1495	/	250	/	/	2070	/	/
Primary effluent	2255	1290	/	215	160	5.9	770	470	1420
Chemical effluent	1090	1035		197	160	1.8	150	55	1140
Meat processing									
Integrated meat	7230	5500	3180	/	67	3.3	910	850	/
Poultry (chicken, turjey, etc)	2690	1700	1595	343	207	30	418	/	/

(*)VSS: The amount of total suspended solids burned off at 550 ± 50°C ($mg \cdot L^{-1}$) (CPWA, 2003).
(**) Soluble COD: Samples are filtered through a 0.45 mm filter before analysis (Orhon et al., 2009).
(/) Data not available.

In domestic wastewaters, solids (total, suspended and dissolved solids) are the predominant pollutants. Then, the BOD_5 and alkalinity are the following parameters with higher concentration in domestic wastewater. In case of industrial wastewaters, all parameters usually have higher concentrations, in comparison to a typical domestic wastewater. Meat processing wastewaters have the higher concentration of total COD ($mg \cdot L^{-1}$), BOD_5 and N with values of 7230 $mg \cdot L^{-1}$, 3180 $mg \cdot L^{-1}$ and 343 $mg \cdot L^{-1}$, respectively. On the other hand, tannery industry has the higher concentration of total solids, with a value of several thousands in the raw water.

Nitrogen and phosphorus could be presented in low concentration in both industrial and domestic wastewaters.

3.4. The necessity of wastewater treatment

Without a wastewater treatment the hazard for the environment, human health and aquatic organisms can be serious. Consequences of the pollution caused by wastewaters are summarized as (USGS Water Science School, 2013):

- Degradation of organic matter and debris cause consumption of dissolved oxygen and thus risk for aquatic organisms.

- Phosphorus and nitrogen (including ammonia), can cause eutrophication, or over-fertilization of receiving waters, being toxic to aquatic organisms, promote excessive plant growth, reduce available oxygen, harm spawning grounds, alter habitat and lead to a decline in certain species.

- Chlorine compounds and inorganic chloramines can be toxic to aquatic invertebrates, algae and fish.

- Bacteria, viruses and disease-causing pathogens can pollute beaches and contaminate shellfish populations, leading to restrictions on human recreation, drinking water consumption and shellfish consumption.

- Metals, such as mercury, lead, cadmium, chromium and arsenic can have acute and chronic toxic effects on species.

- Other substances such as some pharmaceutical and personal care products, primarily entering the environment in wastewater effluents, may also pose threats to human health, aquatic life and wildlife.

For such reasons, it is necessary to avoid any contamination of the environment by wastewaters and thus wastewaters have to be treated to remove all these pollutants. Treated wastewater can be then returned back to the watercourse and use again.

3.5. Historical overview of wastewater treatment

To know the beginning of the wastewater treatment, we need to go back, in the middle of the 19 century, when the population didn’t know anything about the damage that the polluted water could cause (Anderson, 2009).

During that century, several epidemics of waterborne diseases such as cholera and typhoid fever ravaged throughout Europe. The emerging knowledge of the role of microorganisms and sanitary systems for the spreading of diseases resulted in the construction of sewer systems in several large cities. In the final of the 19 century, the vast population increase in urbanized areas lead to severe pollution of rivers and lakes, creating a demand for wastewater treatment (Anderson, 2009).

First wastewater treatment plant was applied in Europe, consisted mainly of the primary treatment, i.e. screens, grits, strainers and settling tanks. In UK, the leading nation on wastewater treatment in that time, constructed of full-scale biological treatment plant employing biofilm technique (trickling filter), it was operated as early as the 1880 (Anderson, 2009).

The secondary treatment was established in Europe during the first half of the 20 century, introducing the activated sludge process.

Around 1950, the incentive for wastewater treatment switched from disease prevention to prevention of eutrophication, as the nutrients nitrogen and phosphorus started to attract attention. However, until 1970s tertiary treatment used for nutrient removal was not generally incorporated in the treatment procedure in Europe (Anderson, 2009).

Since that moment, the increased amounts of wastewater, stricter discharge regulations and lack of space in urbanized areas in the modernized society accelerate the development of alternative methods for biological wastewater treatment, trying to improve the process, looking for low cost processes with high efficiency and low maintenance (Anderson, 2009).

3.6. Legislative requirements

With the development of the industrial processes and technology, the environmental associations started to concern about the amount of wastewater produced and the quantity of discharge from industries without any treatment. Nowadays several legislative requirements exist, with the maximum values and the minimum percentage of reduction for all the pollutants commented before.

One of the most important directive accepted to establish common wastewater requirements all over the Europe is called The Water Framework Directive (WFD). It was created to achieve good qualitative and quantitative status of all water bodies and contains information, studies and requirements for drinking water, bathing water, industrial wastewater, agriculture wastewater, etc. (Water Framework Directive, 2014).

On 21 May, 1991 the Council Directive 91/271/EEC (Council Directive, 1991) concerning urban wastewater treatment was adopted with the objective to protect the environment from the adverse effects of urban wastewater discharges and discharges from certain industrial sectors. Some of the directive requirements for the urban wastewater treatment plants are presented in the following tables (Table 3, Table 4).

Table 3: Requirements for discharges from urban water treatment plants subject to articles 4 and 5 of the Council Directive 91/271/CEE of 21 may 1991 (Council Directive, 1991).

Parameter	**Concentration**	**Minimum percentage of reduction ([1])**	**Reference method of measurement**
Biochemical oxygen demand (BOD_5 at 20 ºC) without nitrification ([2])	25 $mg \cdot L^{-1}$ O_2	70-90 40 Under Article 4 (2)	Homogenized, unfiltered, undecanted sample. Determination of dissolved oxygen before and after 5-day incubation at 20 ºC ± 1 ºC, in complete darkness. Addition of a nitrification inhibitor.
Chemical oxygen demand (COD)	125 $mg \cdot L^{-1}$ O_2	75	Homogenized, unfiltered, undecanted sample Potassium dichromate
Total suspended solids	35 $mg \cdot L^{-1}$ ([3]) 35 under Article 4 (2) (more than 10000 p.e.) 60 under Article 4 (2) (2000-10000 p.e.)	90 ([3]) 90 Under Article 4 (2) (more than 10000 p.e.) 70 under Article 4 (2) (2000-10000 p.e.)	- Filtering of a representative sample through a 0.45 µm filter membrane. Drying at 105 ºC and weighing. - Centrifuging of a representative sample (for at least five minutes with mean acceleration of 2800 to 3200 g), drying at 105 ºC and weighing.

([1]) Reduction in relation to the load of the influent

([2])The parameter can be replaced by another parameter: Total organic carbon (TOC) or Total oxygen demand (TOD) if a relationship can be established between BOD_5 and the substitute parameter.

([3]) This requirement is optional.

Analyses concerning discharges from lagooning shall carried out on filtered samples, the concentration of total suspended solids in unfiltered water samples shall not exceed 150 $mg \cdot L^{-1}$.

The minimum percentage of reduction (in relation to the load of the influent) it is 70-90% in case of BOD_5, and 75% of COD. Total suspended solids have the strictest requirement, with a 90% of reduction, in relation to the load of the influent. To fulfill these requirements, a really efficient process is needed, that results in treated wastewater with maximum 25 $mg \cdot L^{-1}$ O_2 of BOD_5, 175 $mg \cdot L^{-1}$ O_2 of COD, and 35 $mg \cdot L^{-1}$ of total suspended solids.

In some sensitive areas, where the probability of eutrophication problems is high, a control of nitrogen and phosphorus concentration is required too (Table 4).

Table 4. Requirements for discharges from urban wastewater treatment plants to sensitive areas which are subject to eutrophication. One or both parameters may be applied depending on the local situation. The values for concentration or for the percentage of reduction shall apply (Council Directive, 1991).

Parameter	**Concentration**	**Minimum percentage of reduction ([1])**	**Reference method of measurement**
Total phosphorus	2 $mg \cdot L^{-1}$ (10000-100000 p.e.) 1 $mg \cdot L^{-1}$ (more than 100000 p.e.)	80	Molecular absorption spectrophotometry
Total nitrogen ([2])	15 $mg \cdot L^{-1}$ (10000-100000 p.e.) ([3]) 10 $mg \cdot L^{-1}$ (more than 100000 p.e.)	70-80	Molecular absorption spectrophotometry

([1]) Reduction in relation to the load of the influent.
([2])Total nitrogen means the sum of Kjeldahl nitrogen (organic and ammonium nitrogen) nitrate-nitrogen and nitrite-nitrogen.
([3]) Requirements for nitrogen may be checked using daily averages when it is proved. In this case, the daily average must not exceed 20 $mg \cdot L^{-1}$ of total nitrogen for all the samples when the temperature from the effluent in the biological reactor is superior or equal to 12 ºC. The conditions concerning temperature could be replaced by a limitation on the time of operation to take account of regional climatic conditions.

The minimum percentage of reduction (in relation to the load of the influent) is 80% in case of phosphorus and 70-80% for nitrogen.

3.7. Actual situation

Currently, industries and population in general are more concerned about the problem of the amount of wastewater generated. In the following figures (Figure 1, Figure 2) the difference in wastewater treatment between 1990 and 2009 in Europe is presented.

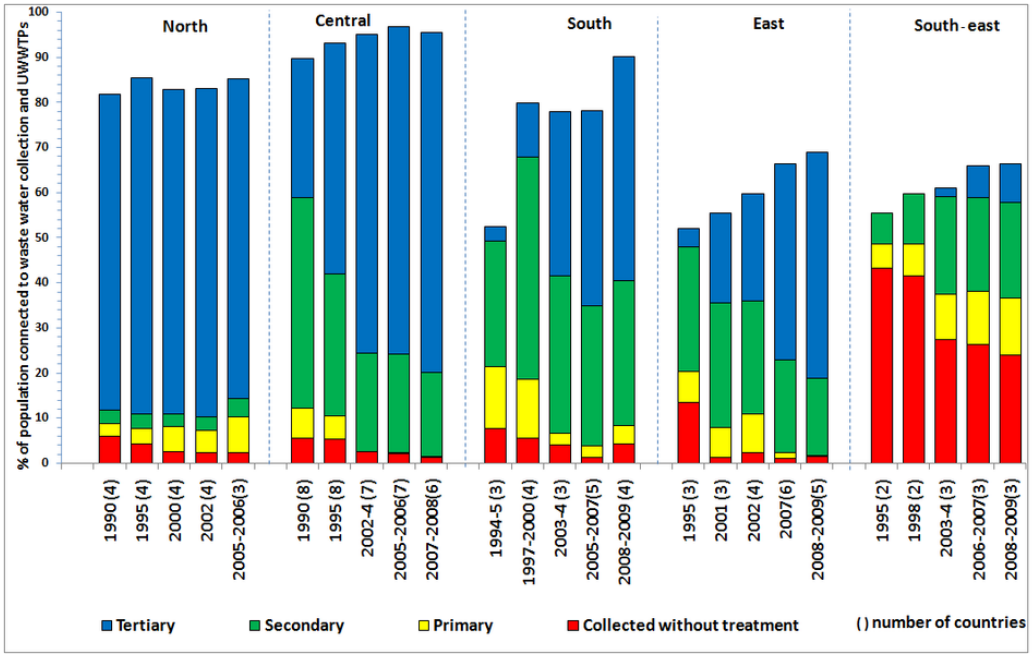


Figure 1: Changes in wastewater treatment in Europe between 1990 and 2009. (European Environment Agency (A), 2013).

North: Norway, Sweden, Finland and Iceland.

Central: Austria, Denmark, England & Wales, Scotland, the Netherlands, Germany, Switzerland, Luxembourg and Ireland.

Southern: Cyprus, Greece, France, Malta, Spain and Portugal.

East: Czech Republic, Estonia, Hungary, Latvia, Lithuania, Poland, Slovenia, Slovakia.

South Eastern: Bulgaria, Romania and Turkey.

Wastewater treatment in all parts of Europe has improved during the last 15 to 20 years. Generally, the amount of wastewater collected without treatment is reduced and the use of tertiary treatment increased because of the necessity to obtain water with a high quality or with specific requirements. Figure 1 shows a satisfied evolution in Central, South and East European countries and a good statistics in case of North European countries, with not many changes between 1990 and 2006. On the other hand, countries like Bulgaria, Romania or Turkey were the countries with less evolution in wastewater treatment and the highest ratio of wastewater collected without any treatment.

In the Figure 2 the evolution in wastewater treatment in some of the Eastern European countries is discussed separately.

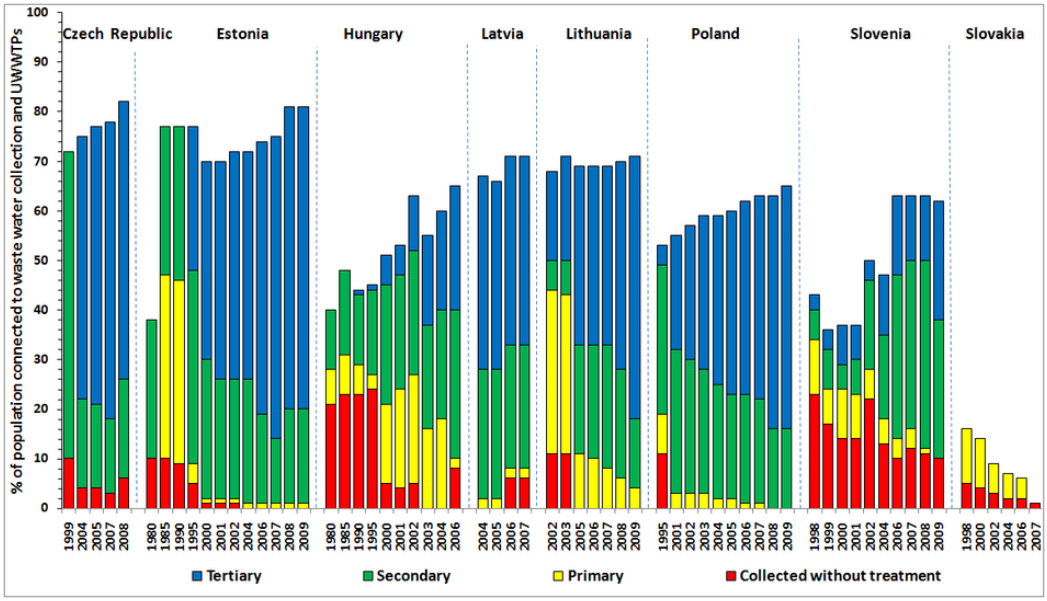


Figure 2: Changes in wastewater treatment in Eastern European countries between 1980 and 2009 (European Environmental Agency (B), 2013).

Estonia and Czech Republic were two countries with a satisfying evolution in wastewater treatment. Poland had good statistics too, with a 0% of wastewater collected without treatment. In the opposite side, Slovenia had the highest ratio of untreated water and the worst situation is showed in Slovakia, where the use of secondary and tertiary treatment did not increase.

3.8. Biological treatment of WW

It is well known, that the most common way of removal of organic, nitrogen or phosphorus compounds from wastewater are biological processes. It is possible to achieve high elimination efficiency of these pollutants with a relatively low capital and operational costs, in comparison to physicochemical or chemical processes.

Wastewater treatment process is a highly complex system with large disturbances, which are controllable with difficulties mainly due to mass and hydraulic load variations. Consequently, it is difficult to maintain the WWTP in a steady state. Biological wastewater treatment, in particular, represents the removal of organic matter in a reactor.

During the biological treatment under aerobic conditions, the organic matter is used as a source of food for microorganisms, suspended organics are removed by being enmeshed in the biological activated sludge flocks (biomass), colloidal organics are partially absorbed and entrapped by the activated sludge and a portion of the soluble organics are sorbed by the activated sludge flocks. Consequently, 85% removal of the total COD can be achieved after 10 to 15 minutes of activated sludge-wastewater contact. In addition, a portion of organic compounds (about 50% in term of organic carbon) is oxidized to CO_2 and H_2O and approximately 60% or energy content in wastewater organics is consumed for

synthesis of new biomass. The rest (about 40%) represents reaction heat loss (Derco et al., 1999).
In the next section (3.9) all the different processes in a wastewater treatment plant are explained with more details.

3.9. WWTP treatment

Wastewater treatment plants are usually designated to provide primary, secondary, or advanced treatment, depending on the degree of treatment required (Figure 3). Primary treatment plants utilize physical processes, such as screening and sedimentation, to remove pollutants that will settle, float, or that are too large to pass through simple screening devices. Primary treatment typically removes about 35% of the BOD and 60% of the suspended solids. While the most visibly objectionable substances are removed in primary treatment and some degree of safety is provided, the effluent still has enough BOD to cause oxygen depletion problems and enough nutrients, such as nitrogen and phosphorus, to accelerate eutrophication. In secondary treatment, the physical processes that make up primary treatment are augmented with processes that involve the microbial oxidation of pollutants. When properly designed and operated, secondary treatment removes about 90% of the BOD and 90% of suspended solids. However, typically no more than half of the nitrogen content and one-third of the phosphorus content are removed during secondary treatment. In special circumstances, advanced treatment (previously called tertiary treatment) may be required. This last treatment is varied and specialized, depending on the nature of the pollutants that must be removed (Masters, 1998; Metcalf, 1995).

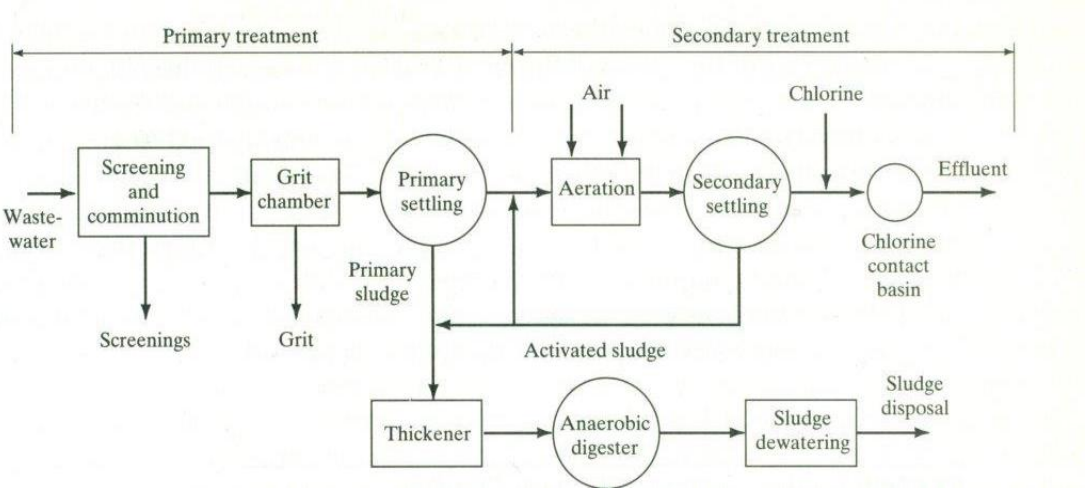


Figure 3. Schematic of a wastewater treatment facility providing primary and secondary treatment using the activated sludge process (Masters, 1998).

3.9.1. Primary treatment

It starts with a simple screening. Screening removes large floating objects that might otherwise damage the pumps or clog small pipes. After that, the wastewater passes into a grit chamber, where it is held for a few minutes. The retention time is chosen to be long enough to allow sand, grit, and another heavy material to settle out but is too short to allow lighter, organic materials to settle. These only heavier materials are usually no offensive and, after washing, can be easily disposed in a landfill (Masters, 1998).

When the wastewater leaves the grit chamber, passes to a primary settling tank (sedimentation basin or primary clarifier) where the flow rate is reduced sufficiently to allow most of the suspended solids to settle out by gravity. After a retention time of 3 or 4 hours, 50% to 65% of suspended solids and 25% to 40% of BOD are removed. The solids that settle (primary activated sludge) are removed and scum that floats to the top of the tank (Masters, 1998).

3.9.2. Secondary treatment: biological treatment

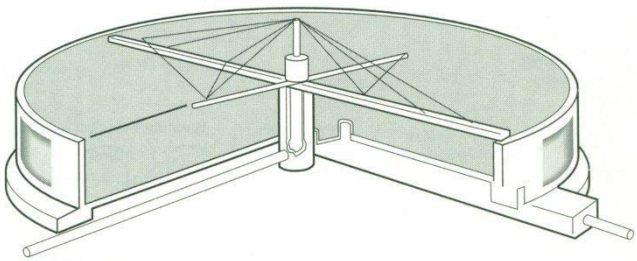
The main aim of the secondary treatment is to provide additional BOD and suspended solids removal. There are three different commonly used approaches, all of them take advantage of the ability of microorganisms to convert organic matter into stabilized, low energy compounds. Tricking filter (and its variations) and the activated sludge process are carried out after primary treatment, obtaining about 90% of the suspended solids and BOD removal (Masters, 1998).

Trickling Filters

It was first used in 1892 and consists of a rotating distribution arm, which sprays liquid wastewater over a circular bed of “first size” rocks or other coarse material (Picture 2). Spaces, which contain the air circulation, maintain the aerobic conditions (Masters, 1998).

The size of the openings is such that there is no actual filtering taking place, so the name trickling filter is not really an appropriate name. Instead, the individual rocks in the bed are covered by a layer of biological film that absorbs and consumes the pollutants trickling through the bed. This biofilm consists mainly of bacteria, but it can include fungi, algae, protozoa, worms, insect, larvae and snails. The accumulating biofilm periodically slides off individual rocks and is collected at the bottom of the filter, together with the treated wastewater, and passed on to the secondary settling tank, where it is removed. Recycling of the effluent seems to be beneficial, because it enables more effluent organic removal, and it also provides a way to keep the biological biofilm from drying out and then dying during the low-flow conditions (Masters, 1998).

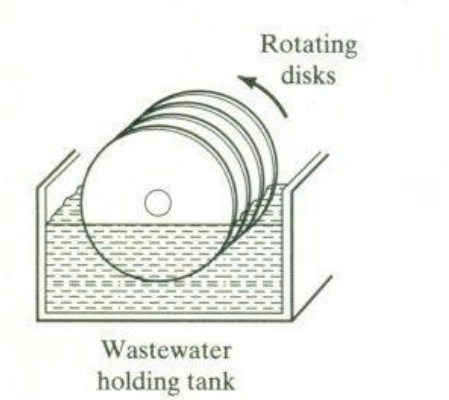
Plastic media became popular as a replacement of rocks, because the same volume can be designed to achieve greater surface areas for biofilm growth, and their lightness allows much deeper beds. Sometimes, these filters with plastic materials are called biofilters (Masters, 1998).



Picture 2. Cross section of a tricking filter (Masters, 1998)

Rotating Biological Contactor (RBC)

They consist of a series of closely spaced, circular, and plastic disks, which are attached to a rotating horizontal shaft (Picture 3). 40% of each disk is submersed in a tank, which contains the untreated water. The biomass film that grows on the surface of the disks moves into and out of the wastewater as the RBC rotates. While the microorganisms are submerged in the wastewater, they absorb organics; while they are rotated out of the wastewater, they are supplied with needed oxygen (Masters, 1998).



Picture 3. RCB cross section (Masters, 1998)

Activated Sludge Process

It was developed later than trickling filter. With this system, more BOD is removed and it is less sensitive to the temperature changes than the trickling filter. In addition, the design costs are lower. Figure 3 presents the wastewater treatment following this system, called activated sludge process. As indicated before, the biological process is achieved in the aeration tank, which receive effluent from the

primary clarifier and also, receives a mass of recycled biological organisms from the secondary settling tank, known as activated sludge. Air or oxygen is pumped into the tank which contains and agitation system. All is mixed between 6 and 8 hours and after that, the mixed liquor flows into the secondary settling tank, where the solids, mostly biomass, are separated from the liquid. To maintain the proper bacterial population in the aeration tank, a portion of those solids is returned and the remainder needs to be processed and disposed off (Masters, 1998).

3.9.3. Biodegradation of wastewater

As it is described before, the biodegradation of wastewater is necessary to remove organic, nitrogen or phosphorus compounds. The process is carried out by microorganisms (Activated sludge), which remove carbon and nutrients from wastewaters with various metabolic and respiratory processes. These microorganisms are mainly prokaryotes, fungi, protozoa, algae and rotifers. Commonly prokaryotes species in wastewater are *Alpha-, Beta- and Gammaprotebacteria, Bacteroidetes* and *Actinobacteria*. The bacteria in a wastewater treatment plant comprise both heterotrophic and autotrophic organisms (Anderson, 2009).

Heterotrophic group of microorganisms is the predominant, and under aerobic conditions, are responsible for oxidation of biodegradable organic material resulting in production of carbon dioxide, water, ammonia and new biomass. On the other hand, biological nitrogen removal is achieved by a combination of nitrification, the oxidation of ammonia to nitrite, and denitrification, the reduction of nitrate to nitrogen gas. These processes are presented in the following reactions, /1/ to /7/ (Anderson, 2009; Ziembińska, 2011). In the biological treatment system, normally these three aerobic processes occur simultaneously (Pitter and Chudoba, 1990). E represents energy (reaction /1/ to /3/):

- Oxidation of organic substances (Dissimilatory Process):

$$C_xH_yO_z + (x + y/4 - z/2)O_2 \rightarrow xCO_2 + y/2H_2O + E \qquad /1/$$

- Synthesis of biomass (Assimilatory Process):

$$\mathrm{n(C_xH_yO_z) + nNH_3 + (x + y/4 - z/2 - 5)O_2 \rightarrow}$$

$$(C_5H_7NO_2)_n + n(x-5)CO_2 + n(y-4)/2H_2O + E \qquad /2/$$

- Endogenous respiration (Autooxidation):

$$(C_5H_7NO_2)_n + 5nO_2 \rightarrow 5nCO_2 + 2nH_2O + nNH_3 + E \qquad /3/$$

The formula $(C_5H_7NO_2)_n$ represents generalized description of cell material, with regard to mutual ratios of the most important elements constituting cell tissue, obtained from experimental studies.

The presence of nitrogen and phosphorus is necessary to produce a new biomass. The optimal BOD_5 : N : P ratio is 100 : 5 : 1., and other micronutrients, like heavy metals, etc. are also needed.

Ammonium nitrogen is usually removed by transformation to nitrite or nitrate in aerobic environment. Inorganic carbon, i.e. carbon dioxide is utilized by chemoautotrophic nitrification bacteria (*Nitrosomonas, Nitrobacter*) in synthesis of new microorganisms. The energy-yielding two step oxidation of ammonia to nitrate is generally represented as follows (Arundel, 2000):

Nitrosomonas $2\mathrm{NH_4^+} + 3\mathrm{O_2} \rightarrow 2\mathrm{NO_2^-} + 4\mathrm{H^+} + 2\mathrm{H_2O}$ /4/

Nitrobacter $2\mathrm{NO_2^-} + \mathrm{O_2} \rightarrow 2\mathrm{NO_3^-}$ /5/

Total reaction $NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O$ /6/

After nitrification, nitrate and nitrite produced can be removed in anoxic conditions (less than 0.5 $mg \cdot L^{-1}$ of dissolved oxygen, level practically zero). In this medium they are reduced and in the end, nitrogen gas is produced. Organic carbon source is necessary for this process, called denitrification.

Two models of nitrate reduction can occur in biological reactors: assimilating and dissimilating or denitrification (Arundel, 2000). Assimilating nitrate reduction involves the reduction of nitrate to ammonia for use in cell synthesis. It occurs independently of oxygen concentration and when ammonium nitrogen is not available. On the other hand, dissimilating nitrate reduction or denitrification is coupled to the respiratory electron chain and involves the reduction of nitrate to nitrite to nitric oxide to nitrous oxide to nitrogen:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \qquad /7/$$

Another compound that needs to be removed is phosphate, and this process is carried out with a combination of an aerobic and anaerobic processes. Phosphate normally is removed by adding a coagulant, usually alum $[Al_2(SO_4)_3]$ or lime $[Ca(OH)_2]$. The reaction with alum is /8/:

$$Al_2(SO_4)_3 + 2PO_4^{3-} \rightarrow 2AlPO_4\downarrow + 3SO_4^{2-} \qquad /8/$$

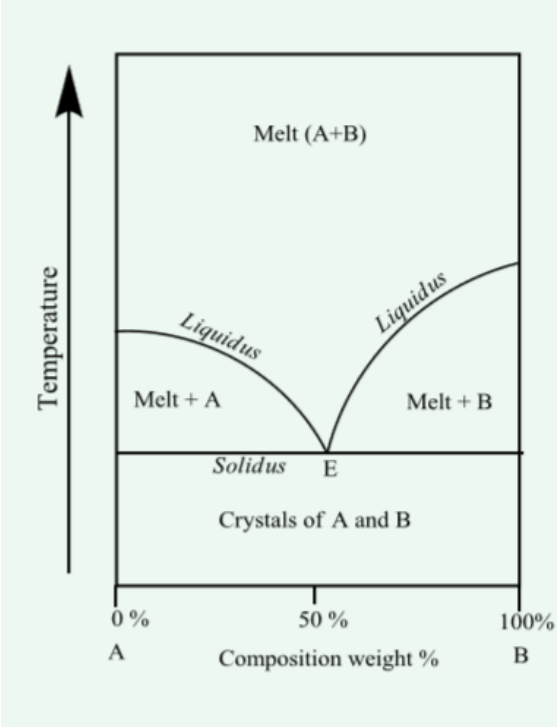
Alum is sometimes added to the aeration tank, when the activated sludge is used, to avoid the usage of additional equipment (Masters, 1998).

3.10. Eutectic solvents

The interest in eutectic solvents (ES) has been increased enormously in the last decades. They are potentially attractive to replace common organic compounds which are possible aquatic contaminants due to their toxicity, volatility and persistency. Actually, they receive also the name of “green solvents “due to their chemical inertness with water, easy synthesis and biodegradability, among other things. However, some studies demonstrate that ES presents also some drawbacks. Next, all the characteristics, advantages and disadvantages are discussed with more details (Haerens et al., 2009; Zhang et al., 2012).

3.10.1. Definition and structure of Eutectic Solvents

Eutectic solvents (ES) are generally composed of two or three cheap and safe components that are capable of self-association, often through hydrogen bond interactions, to form a eutectic mixture with a melting point lower than that of each individual component (Picture 4). The lower melting point of ES was induced by the hydrogen bond that combines two organic molecules forming super-molecules. This hydrogen bond decreases the electrostatic attraction between the hydrogen and organic molecules leading to a decrease in the melting point. The term ionic mixtures for these components were adopted although it shares many similarities with ionic liquid (IL), but eutectic solvents, are liquids containing both ions and neutral molecules (Abbot, 2014; Zhang et al., 2012).



Picture 4. Properties of ES when two components (A and B) are mixed (Abbot, 2014).

Nowadays many different eutectic solvents has been synthesized. Their physical properties are significantly affected by the structure of the hydrogen bond donors, since their characteristics are govern by the hydrogen bond between the molecules. Eutectic solvents (ES) are eutectic-based IL and comprise bulky cations and smaller anions that are bound to hydrogen bond donor (HBD) or a metal halide. They are usually produced using quaternary ammonium salts $R1R2R3R4N+X^-$ complexed with metal halide (Type 1), hydrated metal halide (Type 2) or HBD such as acids, amides and alcohols (Type 3) (Taubert, 2013).

The most commonly used quaternary ammonium salts in synthesizing ES are choline iodide, choline chloride and chlorcholine choline, which react with any compound from amides, amines, carboxylic acids, alcohols and metal halides (Shanti, 2011). In Table 5 and Table 6 common compounds used for synthesis of eutectic solvents are presented.

Table 5. Common organic salts in eutectic solvents (Bajc, 2014).

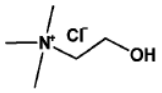
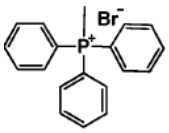
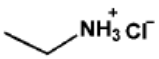
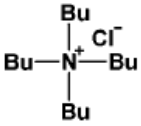
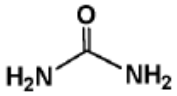
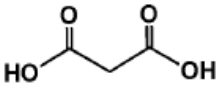
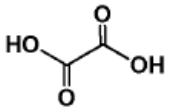
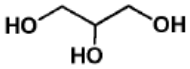
	Structural formula	Compound form
ORGANIC SALT		Choline chloride (ChCl)
		Methyltriphenylphosphonium bromide
		Ethylammonium chloride
		Tetrabutylammonium chloride

Table 6. Common donors of hydrogen bond in eutectic solvents (Bajc, 2014).

	Structural formula	Compound Name
DONOR OF HYDROGEN BOND		Urea
		Malonic acid
		Oxalic acid
		Glycerine

3.10.2. Use of the Eutectic Solvents

In the recent years, there has been intensive development of non-hazardous solvents and reaction media, able to replace common organic compounds which are possible aquatic contaminants due to their toxicity, volatility and persistency. As a result, eutectic solvents (ES) were introduced and they are used in laboratory and large scale in different processes (bio-transformations, separation, etc.). They present excellent characteristics, listed below (Abbot, 2014; Zhang et al., 2012; Hayyan et al., 2012):

- Very cheap due to low cost of raw materials.
- Simple preparation procedures: The components of eutectic can be easily mixed and converted to ionic liquid without further purification steps and no medium being required during the synthesis.
- Safe to be use because most of the formulations are non-toxic.
- Biodegradable nature making it to be more environmental friendly. This is also one of the reasons of being called “green solvents”.
- Good biocompatibility since quaternary ammonium salts such as chlorine chloride was used as an additive in chicken feed.
- Very low volatile organic compound (VOC) compared to ordinary solvent.
- Sustainable.
- Non-flammable and non-reactive to water.

Because of these excellent properties, several applications of ES as replacements of conventional organic solvents in biological reactions have been reported. In

addition they have been used in the development of organic dyes for solar cells, glycerol extraction from biodiesel and electrodeposition and extraction of metals (Taubert, 2013).

Although they present a lot of advantages, some recent publications exposed that ES are not convenient for some chemical processes due to the lack of their corrosivity and toxicological data. In most countries, it is necessary the registration by the appropriate authority, before any chemical product it is legally supplied, sold or used. The ES are not covered by Regulation (EC) NO 1907/2006, called REACH Directive, eventhough some companies manufacture them and put them on the market. The registry contains components of ES only (Hayyan et al., 2012).

Hayyan et al. (2012) examined toxicity of choline chloride based ES with four hydrogen bond donors including glycerine, ethylene glycol, triethylene glycol and urea. In their study, the ES tested had no toxic effect on any of the bacteria studied. However, the cytotoxicity of the tested ES was much higher than that of their individual components, indicating that their toxicological behavior is different. They suggested that the toxicity and cytotoxicity of ES varied according to the structure of the components and exposed the necessity for careful use of the terms non-toxicity and biodegradability of ES (Hayyan et al., 2012).

4. Experimental

For evaluation of the impact of the selected eutectic solvent (ES) to the activated sludge (AS) in WWTP, function of a pilot WWTP prior and after the addition of ES was monitored. The experimental research included the synthesis of ES, which was based on chlorine chloride (CC) and malonic acid (MA). Then the determination of toxicity to microorganisms of activated sludge was determined, as well as the biodegradability in common environmental condition. Finally simulation of ES removal in WWTP was run in a pilot plant.

The pilot experiment started with setting up the pilot aerobic WWTP (V = 7 L) by synthetic municipal wastewater and the efficiency was monitored. The pilot WWTP was monitored by pH, temperature, dissolved oxygen concentration, wastewater flow rate, concentration of the activated sludge in the system, sludge volume index, sedimentation of activated sludge (*APHA, AWWA, WPCF, 2005*), COD (*ISO 6060, 1989*), BOD (*ISO 5815-1, 2003*), TOC (*ISO 8245, 1989*), etc. After the achievement of the steady-state operation of WWTP, the selected amount of ES was added in the influent and treatment efficiency was monitored continuously. The amount of the ES was increased to find the highest concentration of the component still possible to be treated in the biological wastewater treatment plant.

4.1. Synthesis of selected eutectic solvent

The ES is composed from choline chloride with malonic acid in a molar ratio of 1:1. 28.65 g of choline chloride and 21.35 g of malonic acid is needed for the preparation of the sample. The experiment starts with a simple mixing of components at 500 rpm and approximately 70 ºC. When the mixture starts to be liquid, it is possible to increase the rpm to 1000 min^{-1}. The ES solution is ready after 3 or 4 hours and it is a liquid with a high viscosity and a density of 1.232 $g \cdot mL^{-1}$. The density was measured at 20 ºC with a pycnometer (Capacity of 5 mL; Carl Stuart Co., Leek, UK).

Choline chloride is a quaternary amine salt, it dissociates in water into the corresponding positively charged quaternary hydroxyl alkylammonium ion and the negatively charged chloride ion (OECD SIDS, 2004).

The structural formula of CC is presented in Figure 4. The main characteristics of CC are described in the Table 7.

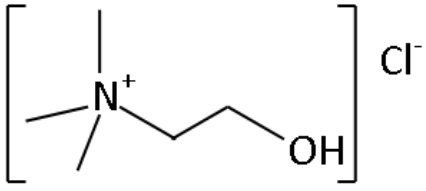


Figure 4. Structural formula of choline chloride.

Table 7. Main characteristics of choline chloride (CC).

CAS Number:	67-48-1
IUPAC Name	Ethanaminium, 2-hydroxy-N,N,N-trimethyl-, chloride
Molecular formula:	$C_5H_{14}NO.Cl$
Molecular weight:	139.63 g·mole^{-1}
Melting point:	247 °C
Solubility in water (20ºC):	650 g·L^{-1}

CC is also known as non-toxic organic salt, biodegradable and a low cost. It is one of the most common compound to synthesize different eutectic solvents (Abbott, 2014). Some applications of choline chloride based ES are: Electrodeposition of Zinc-tin alloys, synthesis of polyoxometalate based hybrids and preparation of zeolite (Hayyan et al., 2013).

Malonic acid is a carboxylic acid (Figure 5). Carboxylic acids donate hydrogen ions if a base is present to accept them. They react in this way with all bases, both organic (for example, the amines) and inorganic. Their reactions with bases, called "neutralizations", are accompanied by the generation of substantial amounts of heat. Neutralization between an acid and a base produces water plus a salt (Chemical Books, 2014).

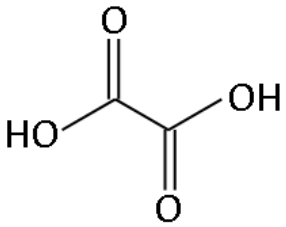


Figure 5. Structural formula of Malonic acid (MA).

The main characteristics of MA are described in the Table 8.

Table 8. Main characteristics of malonic acid (NCBI, 2014).

CAS Number:	144-62-7
IUPAC Name	Propanedioic acid
Molecular formula:	$C_3H_4O_4$
Molecular weight:	90.03 g·mole^{-1}
Melting point:	189.5 °C
Solubility in water (20ºC):	90 g·L^{-1}

Malonic acid is used as building block in chemical synthesis, specifically to introduce the molecular group -CH_2-COOH (Chemical Books, 2014).

When the eutectic solvent is synthesized, it is characterized by a eutectic temperature of 10 °C (Figure 6).

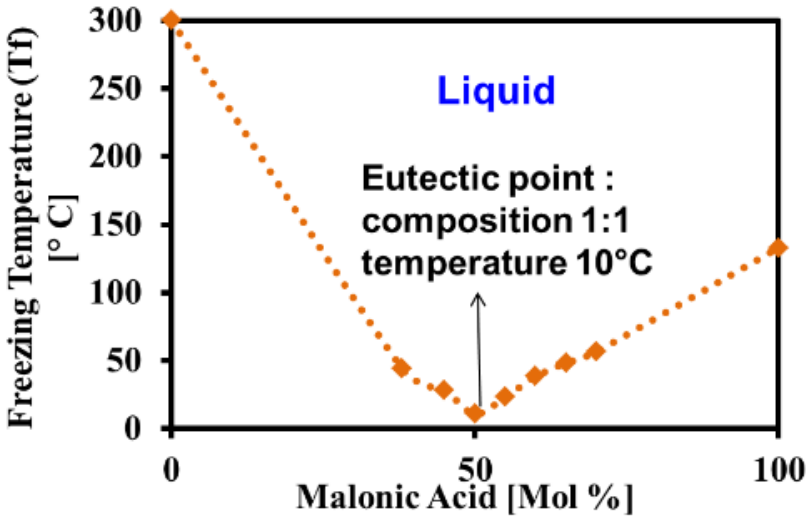


Figure 6. Phase diagram for choline chloride/malonic acid (CC/MA) system (Taubert, 2013).

ES composed of CC and MA has been shown to be very effective. To mention one example, some recent studies shown that the ES based on CC/MA is effective for the removal of post etch residues, which contains significant amount of cooper based inorganic materials (Taubert e al., 2014).

4.2. Pilot WWTP

The principle of the WWTP is based on the biological treatment by activated sludge (chapter 3.9.2). In the next chapter, the description of the pilot WWTP is presented.

4.2.1. Description

The pilot wastewater treatment used in this study is presented in Picture 5.

Picture 5. Pilot WWTP, with the different important parts, identified by numbers:

1: Influent tank (synthetic municipal wastewater)
2: Tubes
3: Pump
4: Aeration tank with membrane for separation
5: Effluent (treated wastewater)
6: Digital and analogical temperature and airflow display
7: Flow meter

The Influent tank (1) has a capacity of 20 L and contains the synthetic municipal wastewater. When the system works, this wastewater flows through the tube (2) and is transported by the pump (3) to the aeration tank (4). Water flow rate is controlled with the flow meter (7) by an analogical system. The aeration tank contains the activated sludge, which was obtained from the Central Municipal Treatment Plant of Ljubljana City (CČN, Zalog, Ljubljana); it was collected in aeration basin, and transported to the laboratory. This Aeration tank has a capacity of 7 liters. It has a temperature sensor, a membrane inside and the aeration system in the bottom. Good aeration conditions in the reactor are controlled in the digital and analogical temperature and airflow display (6), which shows the air flow rate in $L \cdot min^{-1}$. When the biological treatment is completed, the effluent (5) leaves the aeration basin through the membrane and goes to the sewage system through a tube. The temperature of the system is controlled digitally (in ºC) (6).

4.2.2. Preparation of synthetic wastewater sample

Synthetic wastewater was prepared according to ISO 11733 (ISO 11733, 2004). The Influent tank contained 10 L of municipal synthetic wastewater, which was prepared every day. The composition of the wastewater was:

1) Peptone solution: 0.18 $g \cdot L^{-1}$ of peptone. It is derived generally from animal liver. It contains vitamins, fat, proteins, amino acids, etc. Basically, the protein is source of carbon for the microorganisms and thus, they can grow up in the reactor.

2) Inorganic solution: Its function is to introduce the necessary minerals to microorganisms. The composition is described in Table 9.

Table 9. Composition of the inorganic solution.

Salt	**Concentration ($g \cdot L^{-1}$)**
CON_2H_4	30
NaCl	7
$CaCl_2 \cdot 2H_2O$	4
$MgSO_4 \cdot 7H_2O$	2
K_3PO_4	28

3) Tap Water.

The WWTP was feed 40 days with this synthetic municipal wastewater to achieve constant operation. Then 0.006 Vol.% of ES was introduced for 8 days, 0.012 Vol.% for 9 days, 0.015 Vol.% for 7 days, 0.020 Vol.% for 6 days and the last concentration added was 0.030 Vol.% for 6 days.

4.2.3. Daily controlled parameters in WWTP

4.2.3.1. Temperature

In the reactor, the control of the temperature is very important. It must be constant and between 19 to 22 ºC. This range of temperature is the optimum for the microorganisms to work in an efficient way and reproduce as well. If the temperature reaches more than 37 ºC the microorganisms could not work well and if it is less than 18 ºC, they don't have the optimum conditions for fast growth. It was controlled with a temperature sensor in the reactor.

4.2.3.2. Air flow rate

The air flow rate was set up between 1 and 2 $L \cdot min^{-1}$ to allow transformation of organic matter by organisms and maintain appropriate aerobic conditions (>2 $mg \cdot L^{-1}$) (Arundel, 2000).

4.2.3.3. Wastewater flow rate

The maintenance of a constant wastewater flow rate in the system is important to prevent perturbations in the system (variations in the efficiency, retention time, etc). It was controlled with a flow meter.

4.2.3.4. Concentration of dissolved oxygen

To check the aerobic conditions in the reactor it is necessary to measure the concentration of dissolved oxygen in the WWTP. The suitable concentration of dissolved oxygen must be higher than 2 $mg \cdot L^{-1}$. With these values the microorganisms can develop and perform the biological reactions. This parameter was measured with an oxygen sensor, which converts the measurement of the partial pressure of dissolved oxygen to $mg \cdot L^{-1}$ through the Henry's constant implemented in the device. (WTW pH/Oxi 340i) (Arundel, 2000; Doran, 2013).

4.2.3.5. Agitation

If there is not an appropriate agitation in the system, the activated sludge starts to settle to the bottom of the reactor. Furthermore, the agitation is important to ensure the good distribution of the oxygen, microorganisms and organic matter in the tank volume. The agitation in WWTP was maintained by the aeration system, which it was analogically controlled (Picture 5).

4.2.3.6. pH in the influent

The pH in the influent must be between 6 and 8 (Arundel, 2000). Because of the addition of the selected ES, which contains a strong acid, it was necessary to regularly check this parameter. The pH was checked with pH indicators.

4.2.3.7. Control of activated sludge

Concentration of activated sludge

It is necessary to maintain appropriate balance between the amount of microorganisms and the quantity of organic matter to treat. The optimum concentration of activated sludge (AS) is approximately 2-3 $g \cdot L^{-1}$ (Arundel, 2000), and a value lower or higher than the optimum pose problems in aeration and also in the settlement of the AS. The concentration of AS was obtained after filtration of 100 mL of activated sludge (removed from the aeration tank) through filtration paper. Next, filtration paper and activated sludge were dried at 105 ºC for 90 minutes and weighted.

$$X = \frac{m \cdot 1000\ mL}{Vs} \qquad [1]$$

X = Concentration of activated sludge ($mg \cdot L^{-1}$)
m = Mass of activated sludge (g)
Vs = Volume of activated sludge sampled (mL)

Sedimentation of activated sludge (VU):

Is a parameter for routine control of biological treatment plant using activated sludge. It is based on determination of volume of 1 L of AS settled after 30 minutes.

$$VU = \frac{Vss}{Vs} \qquad [2]$$

VU = Sedimentation of activated sludge ($mL \cdot L^{-1}$)
Vss = Volume settled of activated sludge (mL)
Vs = Volume of the activated sludge sampled (1000 mL)

Sludge volume index (SVI):

Is the volume in milliliters occupied by 1 g of a suspension after 30 minutes of sedimentation. SVI is typically used to monitor settling characteristics of activated sludge and other biological suspensions (APHA, AWWA, WEF, 2005). SVI should be between 50 and 150 $mL \cdot g^{-1}$ (Arundel, 2000).

$$SVI = \frac{VU}{X} \qquad [3]$$

SVI = Sludge volume index ($mL \cdot g^{-1}$)
VU = Sedimentation of activated sludge ($mL \cdot L^{-1}$)
X = Concentration of activated sludge ($g \cdot L^{-1}$)

4.2.3.8. Hydraulic retention time (HRT)

It is the time that the wastewater stays in the biological reactor. It is calculated following the expression (Arnaldos, et al., 2012).

$$\mathrm{HRT} = \frac{\mathrm{V}}{\mathrm{Q}} \qquad [4]$$

$$V = \frac{\pi \cdot D^2}{4} \cdot H \qquad [5]$$

HRT = Hydraulic retention time (h)
V = Volume of the aeration tank (L)
Q = Flow of the influent ($L \cdot h^{-1}$)
D = Diameter of the aeration tank (dm)
H = Height of the aeration tank (dm)

The hydraulic retention time must be long enough to remove the requisite proportion of BOD from the wastewater. In a conventional activated sludge system, the HRT should be between 5 and 14 hours (Davies, 2005).

4.2.3.9. F/M ratio

This is the relationship between the load of BOD (or microorganism 'food', in $kg \cdot day^{-1}$) entering the aeration tank, and the mass of microorganisms in the aeration tank available to treat the incoming BOD. This is therefore known as the Food to Mass ratio (F/M ratio), also often referred to as the Sludge Loading Rate (SLR).

$$\mathrm{F/M\ ratio} = \frac{\mathrm{BOD_5} \cdot \mathrm{Q}}{\mathrm{X} \cdot \mathrm{V}} \cdot \frac{24}{1000} \qquad [6]$$

F/M ratio = Food to microorganisms ratio ($kg_{BOD5} \cdot kg_{AS}^{-1} \cdot d^{-1}$)
BOD_5 = Biochemical oxygen demand in influent after 5 days ($mg \cdot L^{-1}$)
Q = Flow of the influent ($m^3 \cdot h^{-1}$)
X = Concentration of the activated sludge ($g \cdot L^{-1}$)
V = Volume of the aeration tank (m^3)

We determined the F/M ratio considering the chemical oxygen demand in the influent (COD_{IN}) results instead of the BOD_5 results, due to the fact that BOD_5/COD ratio for the synthetic WW prior to the addition of ES was close to one (very good biodegradability) as well as it stayed close to one after the addition of ES. We estimated that calculation using COD instead of BOD_5 was justified.

The typical F/M ratios for different biological systems are presented in Table 10.

Table 10: Activated sludge process ranges for F/M ratio control (Lee Mishoe, 1999).

Process range names	**Name type of the WWTP**	**F/M range**
Extended aeration	Extended aeration Sequencing batch reactors Race track or orbital ditch	0.02-0.07 $kg_{BOD5} \cdot kg_{MLTSS}^{-1}$
Standard activated sludge	Conventional activated sludge Contact stabilization Step aeration Complete (or homogenous) mix Others used with nutrient removal	0.11-0.23 $kg_{BOD5} \cdot kg_{MLTSS}^{-1}$
Hi-Rate activated sludge (HRAS)	based on desired removal (75 to 69% efficiency)	0.45-4,53 $kg_{BOD5} \cdot kg_{MLTSS}^{-1}$

4.2.3.10. Treatment Efficiency

The treatment efficiency is one of the most important general parameters, giving information of the quantity of organic and inorganic matter that is removed from the wastewater. It is determined from the results of the COD, following the expression:

$$E\ (\%) = \frac{COD_{IN} - COD_{OUT}}{COD_{IN}} \cdot 100 \qquad [7]$$

E = Treatment Efficiency (%)
COD_{IN} = Chemical oxygen demand in influent ($mg \cdot L^{-1}$)
COD_{OUT} = Chemical oxygen demand in effluent ($mg \cdot L^{-1}$)

The treatment efficiency can be calculated also by DOC or using BOD_5 parameter. For a typical WWTP the BOD removal is expected to be higher than 90% (Arundel, 2000).

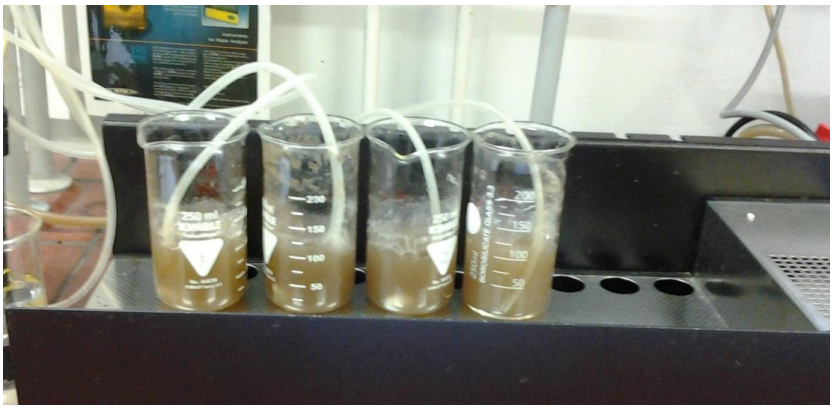
4.3. Analytical methods

ES characteristics**,** the efficiency of WWTP and pyshico-chemical parameters of the influent and the effluent in the WWTP were evaluated following the standard procedures. First of all, the toxicity test by activated sludge (ISO 8192, 2007) and the evaluation of aerobic biodegradability of ES in aqueous medium by determination of oxygen demand in a closed respirometer (ISO 9408, 1999) were

evaluated. Analytical control of influent and effluent and monitoring of the treatment procedures included BOD_5 (biochemical oxygen demand (ISO 5815/1, 2003), COD (chemical oxygen demand, ISO 6060, 1989)), DOC (dissolved organic carbon), IC (inorganic carbon) (Shimadzu TOC 5000A Analyzer, 1998, ISO 8245, 1989)) and nitrogen as organic, ammonium (ISO 7150/1, 1984) and nitrate determination. After the addition of ES, the concentration of chloride was also evaluated (APHA, AWWA, WEF, 2005).

4.3.1. Toxicity test by activated sludge (ISO 8192, 2007)

In the presence of biodegradable substances, activated sludge (AS) consumes oxygen at a higher rate than in their absence, depending on, among other factors, on concentration of the microorganisms. The addition of a toxic concentration of a test material results in a decrease in the oxygen consumption rate. The measurements of the oxygen consumption are determined with an oxygen sensor and the percentage inhibition of the oxygen consumption should be estimated by comparison of the results of a control mixture containing no test material (Blank test). Two parallel systems with and without N-Allylthiourea (ATU, inhibitor for nitrification process) were carried out. Because experiments are conducted twice, with and without ATU added, the inhibitory effect on oxygen uptake by all activated sludge microorganisms (without ATU added) as well as to heterotrophic microorganisms (with ATU) can be measured (Picture 6). The difference between these two measurements give the nitrification and thus inhibitory effect to nitrifying microorganisms can be also measured.



Picture 6. Determination of the toxicity of ES to activated sludge.

The test was conducted with different concentrations of ES (0.018 Vol.%, 0.029 Vol.% and 0.040 Vol.%). Another sample with 0.040 Vol.% did not contain activated sludge, to evaluate if the eutectic solvent degraded abiotically. Oxygen consumption rates were measured after 30 minutes of incubation. It was measured for each sample every 30 seconds during 5 minutes. Consistency of activated sludge was assessed in preliminary test with 3,5-dichlorophenol. Concentration of AS was 1500 $mg \cdot L^{-1}$ (Table 11).

Table 11. Composition of the samples in the test with 3,5-dichlorophenol.

Sample name	3,5-dichlorophenol (mL)	Inorg. solution (mL)	Peptone solution (mL)	AS volume (mL)	Total volume (mL)
Blank	/	3.2	3.2	44	100
3 mg·L^{-1} DCF	0.3	3.2	3.2	44	100
5 mg·L^{-1} DCF	0.5	3.2	3.2	44	100
10 mg·L^{-1} DCF	0.1	3.2	3.2	44	100

In Table 12 the composition of the samples in the test with ES and ATU is presented.

Table 12. Composition of the samples in the test with ES and ATU.

Sample name	ES Conc. (Vol. %)	Inorg. solution (Table 14) (mL)	Peptone solution (Table 13) (mL)	AS Volume (mL)([1])	ATU (mL) ([2])	Total volume (mL)
Blank	/	3.2	3.2	28	1	100
0.018 Vol.%	0.018	3.2	3.2	28	1	100
0.029 Vol.%	0.029	3.2	3.2	28	1	100
0.040 Vol.%	0.040	3.2	3.2	28	1	100
0.040 Vol.% Abiotic	0.040	3.2	3.2	/	/	100

([1]) The volume of activated sludge (AS) to take depends upon how much concentrated is the activated sludge used. In the test concentration of AS should be 1.5 g·L^{-1}. With the concentration required and the concentration of the AS that was prepared, the volume necessary to fulfill the requirement was calculated and in this experiment it was 28 mL).

([2]) The test was conducted twice to calculate the average. Each time was done with and without ATU and its concentration was 11.6 mg·L^{-1}.

(/) Not added.

In Table 13 and Table 14, the composition of the peptone solutions and the inorganic solution are presented.

Table 13. Composition of Peptone solution.

Compound	**Quantity**
Peptone (g)	20
Destilled water	Filled to 1000 mL

Table 14. Composition of inorganic solution.

Compound	**Structural Formula**	**Quantity (g)**
Sodium chloride	NaCl	0.7 g
Calcium chloride dihydrate	$CaCl_2.2H_2O$	0.4
Magnesium sulfate heptahydrat	$MgSO_4.7H_2O$	0.2
Potassium phosphate	K_2HPO_4	2.8
Destilled water	H_2O	Filled to 1000 mL

With the values of concentration of oxygen for the blank, the total rate of oxygen uptake in the blank (R) is calculated following the equation [8]:

$$R = \left(\frac{\rho_1 - \rho 2}{\Delta t}\right) \cdot 60 \qquad [8]$$

R = Oxygen consumption rate in the Blank ($mg \cdot g^{-1} \cdot h^{-1}$)
ρ_1 = Oxygen concentration in the beginning in the Blank ($mg \cdot L^{-1}$)
ρ_2 = Oxygen concentration at the end in the Blank ($mg \cdot L^{-1}$)
Δt = Time interval between two measurements (min)

The specific respiration ratio (Rs) is expressed as the amount of oxygen consumed per dry weight of activated sludge per hour and it is obtained with the equation [9]:

$$Rs = \frac{R}{X} \qquad [9]$$

Rs = Specific respiration ratio ($mg \cdot g^{-1} \cdot h^{-1}$)
R = Oxygen consumption rate in the Blank ($mg \cdot g^{-1} \cdot h^{-1}$)
X = Concentration of activated sludge ($g \cdot L^{-1}$)

Oxygen concentration values ($mg \cdot L^{-1}$) are plotted versus time (min) for each sample and the linear trendline is obtained. The percentage inhibition (I, %) of the total oxygen consumption at each concentration tested is given by the equation [10] :

$$I(\%) = \left[1 - \left(\frac{R_{TS} - R_{TA}}{R_{TB}}\right)\right] \cdot 100 = \left[1 - \left(\frac{R_T}{R_{TB}}\right)\right] \cdot 100 \qquad [10]$$

I = Inhibition of oxygen uptake of total microorganisms (%)
R_{TS} = Rate of oxygen uptake for each sample (no ATU) ($mg \cdot g^{-1} \cdot h^{-1}$)
R_{TA} = Rate of oxygen uptake for the abiotic control (no ATU) ($mg \cdot g^{-1} \cdot h^{-1}$)
R_{TB} = Rate of oxygen uptake for the blank (no ATU) ($mg \cdot g^{-1} \cdot h^{-1}$)

Similarly, the percentage inhibition of heterotrophic oxygen uptake (I_H), at each concentration is obtained following the equation [11]:

$$I_H(\%) = \left[1 - \left(\frac{R_{HS} - R_{HA}}{R_{HB}}\right)\right] \cdot 100 = \left[1 - \left(\frac{R_{HS}}{R_{HB}}\right)\right] \cdot 100 \qquad [11]$$

I_H = Inhibition of oxygen uptake of heterotrophic microorganisms (%)
R_{HS} = Rate of oxygen uptake for each sample (with ATU) ($mg \cdot g^{-1} \cdot h^{-1}$)
R_{HA} = Rate of oxygen uptake for the abiotic control. (with ATU) ($mg \cdot g^{-1} \cdot h^{-1}$)
R_{HB} = Rate of oxygen uptake for the blank (with ATU) ($mg \cdot g^{-1} \cdot h^{-1}$)

Finally, the percentage inhibition of oxygen uptake due to nitrification (I_N) at each concentration is given by the equation [12]:

$$I_N(\%) = \left[1 - \left(\frac{R_{TS} - R_{HS}}{R_{TB-} R_{HB}}\right)\right] \cdot 100 \qquad [12]$$

I_N = Inhibition of oxygen uptake of nitrifying microorganisms (%)
R_{TS} = Rate of oxygen uptake for each sample (no ATU) ($mg \cdot g^{-1} \cdot h^{-1}$)
R_{HS} = Rate of oxygen uptake for each sample (with ATU) ($mg \cdot g^{-1} \cdot h^{-1}$)
R_{TB} = Rate of oxygen uptake for the blank (no ATU) ($mg \cdot g^{-1} \cdot h^{-1}$)
R_{HB} = Rate of oxygen uptake for the blank (with ATU) ($mg \cdot g^{-1} \cdot h^{-1}$)

The obtained inhibition (%) for each sample is plotted versus logarithm of concentration of the sample (Vol.%) and the 30minEC_{50} and 30minEC_{20} values are obtained. 30minEC_{50} and 30minEC_{20} are the concentration which inhibits the oxygen consumption by 50% and 20% respectively. 30minEC_{20} is also usually referred to as the first reliably measured inhibition toxicity test with activated sludge.

4.3.2. Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium by determination of oxygen demand in a closed respirometer (ISO 9408, 1989)

Biodegradation is the decay or breakdown of organic compounds that occurs when microorganisms use an organic substance as a source of carbon and energy. During degradation, extracellules from microorganisms break down complex compounds yielding smaller molecules or short chains, which are smaller enough to pass the semi-permeable outer bacterial membranes, and then to be utilized as carbon and energy sources. When the end products are CO_2, H_2O or CH_4, the degradation is called mineralization. The process of biodegradation under aerobic condition can be expressed as /9/:

$$C + O_2 \rightarrow CO_2 + H_2O + C_{remainder} + C_{biomass} + \text{minerals} + E \qquad /9/$$

The amount of carbon substrate, that is assimilated or mineralized, normally depends on the type of microorganism that was used for degradation, the substrate, temperature and other environmental factors as well.

Activity and survival of microorganisms in the environment are affected by many factors. Important factors for biodegradation are nutrients, availability of oxygen, temperature and pH. Nutrients like N, P, and O like co-substrate are needed for optimal growth of heterotrophic microorganisms. Deficit of nutrients in medium inhibits microorganisms growth and lowers the rate of biodegradation of organic carbon.

The aim of this test is to know the biodegradability of ES. ES was the sole source of carbon and energy in the medium. The inoculated medium was stirred in a closed flask and the consumption of oxygen was determined either by measuring the amount of oxygen required to maintain a constant gas in the respirometer flask or by measuring the change in pressure in the apparatus. Evolved CO_2 was absorbed in potassium hydroxide (KOH) in the test vessel. The degradation was followed over a period of 28 days by determining the consumption of oxygen. Incubation was placed in the dark and at constant temperature (20ºC).

When the results of the oxygen consumption ($mg \cdot L^{-1}$) were obtained from each barometric bottle, the biodegradation (%) was calculated following the equation [13].

$$D_t = \frac{OC_s - OC_b}{COD} \cdot 100 \qquad [13]$$

D_t = Biodegradation (%)
OC_s = Oxygen consumption in the sample in time t ($mg \cdot L^{-1}$)
OC_b = Oxygen consumption in the blank in time t ($mg \cdot L^{-1}$)
COD = Chemical oxygen demand of the sample ($mg \cdot L^{-1}$)

In the Blank sample (without ES) the oxygen consumption of microorganisms for themselves (endogenic respiration) was monitored. In addition, the reference compound was also needed to check the activity of inoculum. If it is correct, it must degrade more than 70% in 14 days (ISO 9408, 1989). In Table 15 the composition of the mixture tested is presented.

Table 15. Composition of the blank, ES samples and reference compound in 1000 mL.

	Blank 1	**Sample 1**	**Sample 2**	**Reference**
Activated sludge (mL)	5.0	5.0	5.0	2.5
ES (µL)	/	0.6 (*)	0.6	/
ATU sol.(11.6 $g \cdot L^{-1}$) (mL)	/	/	4	/
Nutrient A (mL) ($^{+}$)	10	10	10	10
Nutrient B (mL) ($^{+}$)	1	1	1	1
Nutrient C (mL) ($^{+}$)	1	1	1	1
Nutrient D (mL) ($^{+}$)	1	1	1	1
Sodium acetate (mg)	/	/	/	106
COD ($mg \cdot L^{-1}$)	(**)	100	100	100
Total volume (mL)	1000	1000	1000	500

(/) Not added.

(*) COD of ES is 1700000 $mg \cdot L^{-1}$. Value calculated to obtain COD = 100 $mg \cdot L^{-1}$.

(**) Depend upon characteristics of activated sludge.

($^{+}$) Description is given in Table 16.

If NH_4^+ is present in the sample, the consumption of oxygen for nitrification can occur and thus the ATU (inhibitor nitrification) was added to sample 2 to prevent this process (Table 15).

The following table (Table 16) shows the composition of the nutrient medium.

Table 16. Composition of the nutrient medium.

solution	Substante	m (g)
A	KH_2PO_4	8.5
	K_2HPO_4	21.75
	$Na_2HPO_4.2H_2O$	33.4
	NH_4Cl	0.5
	Water	1000 ml
B	$MgSO_4.7H_2O$	22.5
	Water	1000 ml
C	$CaCl_2$	27.5
	Water	1000 ml
D	$FeCl_3.6H_2O$	0.25
	Water	1000 ml

4.3.3. Chemical oxygen demand (ISO 6060, 1989)

This method measures the amount of oxygen equivalent required in samples which contains organic matter susceptible to be oxidized with strong oxidants, usually potassium dichromate. The principle it is based on refluxing the sample in a strongly acidic solution with a known excess of potassium dichromate. After digestion, the remaining unreduced potassium dichromate is titrated with ferrous ammonium sulfate to determine the quantity of potassium dichromate consumed and the oxidizable organic matter is calculated in terms of oxygen equivalent ($mg \cdot L^{-1}$).

4.3.4. Biochemical oxygen demand after n days (ISO 5815/1, 1989)

The test measures the oxygen required for the biochemical degradation of organic material (carbonaceous demand) and the oxygen demand used to oxidize inorganic material, such as sulfides and ferrous ions. In the method, the concentration of oxygen is measured prior and after incubation period (5 days). BOD_5 is computed from the difference between initial and final concentration of oxygen ($mg \cdot L^{-1}$) of the sample.

4.4.3. Dissolved organic carbon (ISO 8245, 1989)

The carbon in wastewater is composed as a variety of organic and inorganic compounds and they are summarized as total carbon (TC) content. Total organic carbon (TOC) presents all organic compounds in the sample. Dissolved organic carbon (DOC) is the fraction of TOC that passes through a 0.45 µm pore-diameter filter. To determine this parameter, the sample must be filtered and a microportion of it is injected into a heated reaction chamber packed with an oxidative catalyst at 680ºC. As a result, the water is vaporized and the organic carbon is oxidized to CO_2 and H_2O. The CO_2 from oxidation of organic and inorganic carbon is transported in the carrier-gas stream and it is measured by means of a non-dispersive infrared analyzer. Two measures are determined, TOC and inorganic carbon (IC), and DOC is calculated as a difference between both values.

4.3.5. Determination of concentration of nitrite, nitrate, ammonium (APHA, AWWA, WEF, 2005)

In a conventional wastewater, nitrite, nitrate, ammonium nitrogen and organic nitrogen are present. These three first forms ($N\text{-}NO_2^-$, $N\text{-}NO_3^-$, $N\text{-}NH_4^+$) are determinate by spectrometric methods, according to the standard procedure.

The concentration of ammonium nitrogen ($N\text{-}NH_4^+$) is determined by a reaction of ammonium with sodium salicylate and sodium citrate, in the presence of sodium dichloroisocyanurate and in alkaline media. The reaction generates a yellow-green

compound which is analyzed spectrophotometrically at 655 nm (ISO 7150/1, 1984).

The Nitrite nitrogen ($N\text{-}NO_2^-$) is determined through formation of a reddish purple azo dye produced in a acid media, by coupling diazotized sulfanilamide with N-(1-naphtyl)-ethylendiamine dihydrochloride (NED). According to the standard procedure, (APHA, AWWA, WEF, 2005) the measurement of the absorbance is at 543 nm.

Nitrate nitrogen ($N\text{-}NO_3^-$) is reduced to nitrite in the presence of cadmium copper granules in a glass column. The nitrite produced is determined by the same procedure described above.

4.3.6. Determination of Chloride (APHA, AWWA, WEF, 2005)

Chloride ion (Cl^-) is one of the major inorganic anion contained in wastewaters. Chlorides are titrated with mercuric nitrate because of the formation of soluble, slightly dissociated mercury chloride. In an acid media, diphenylcarbazone indicates the titration endpoint by formation of a purple complex, according to the standard method (APHA, AWWA, WEF, 2005).

5. Results and Discussion

To determine the impact of the selected ES to microorganisms of activated sludge, toxicity and biodegradability tests were conducted. Consequently, simulation of aerobic treatment in pilot aerobic biological WWTP was performed.

5.1. Toxicity of eutectic solvent

Toxicity test with 3.5 dichlorophenol confirmed good sensitivity of the activated sludge. 30 minEC_{50} was in the required range (2-25 $mg \cdot L^{-1}$) obtaining a value of 3.52 $mg \cdot L^{-1}$. The activated sludge was suitable for further testing.

In Table 17 the results of oxygen concentration for all the samples without ATU are presented. The Oxygen consumption rate in the blank (R) ($mg \cdot g^{-1} \cdot h^{-1}$) was calculated with the equation [8] and the value obtained was 31.08 $mg \cdot g^{-1} \cdot h^{-1}$. Then Specific respiration ratio (Rs) ($mg \cdot g^{-1} \cdot h^{-1}$) was determined with the equation [9] obtaining 20.72 $mg \cdot g^{-1} \cdot h^{-1}$ (more than 20 $mg \cdot g^{-1} \cdot h^{-1}$ as requested by ISO 8192, (2007). This confirms appropriate efficiency of the activated sludge used.

Table 17. Dissolved oxygen concentration values ($mg \cdot L^{-1}$) for all the samples in the first test without ATU.

	Concentration of dissolved oxygen ($mg \cdot L^{-1}$)				
Time (min)	**Blank**	**0.018 Vol.%**	**0.029 Vol.%**	**0.040 Vol.%**	**0.040 Vol.% abiotic (**)**
0.0	8.89	9.44	9.70	9.50	9.95
0.5	8.65	9.29	9.80	9.64	9.97
1.0	8.40	9.16	9.80	9.61	9.99
1.5	8.17	9.03	9.83	9.51	9.99
2.0	7.90	8.91	9.79	9.52	10.01
2.5	7.62	8.78	9.69	9.51	10.01
3.0	7.37	8.65	9.60	9.46	10.01
3.5	7.09	8.51	9.51	9.51	10.01
4.0	6.83	8.38	9.44	9.41	10.01
4.5	6.57	8.28	9.44	9.43	10.02
5.0	6.30	8.16	9.40	9.42	10.02
Slope (RT) (*)	0.5213	0.256	0.0882	0.0345	/

(*) RT = Oxygen uptake rate for blank and different concentrations (0.018-0.040 Vol.%) of the ES.
(**) No oxygen consumption.
(/) Not determined.

In Figure 7 Dissolved oxygen concentration values for total microorganisms ($mg \cdot L^{-1}$) were plotted versus time (min) for each sample and the linear trendlines were obtained.

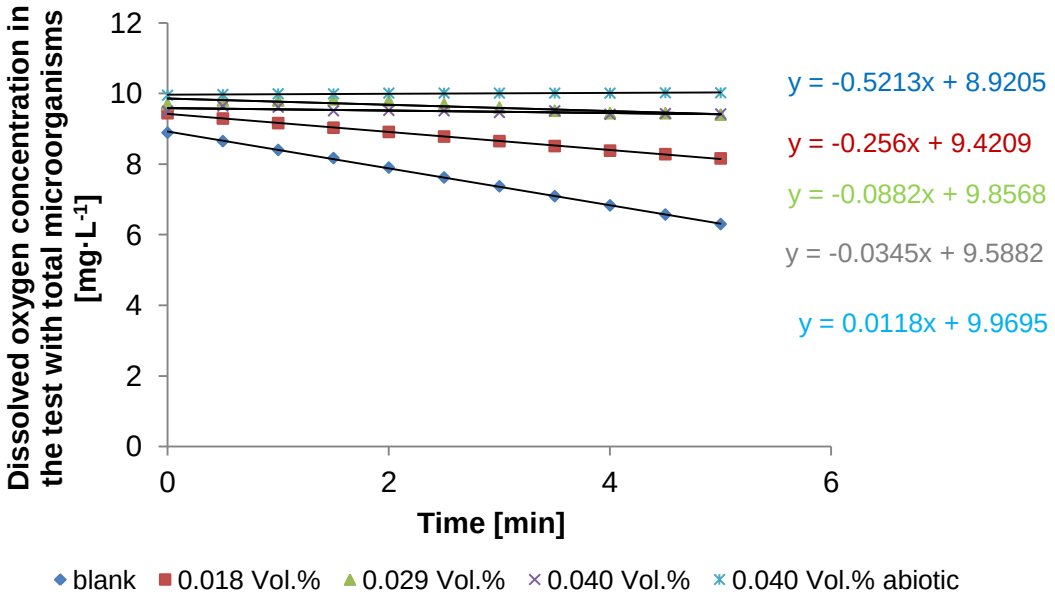


Figure 7. Dissolved oxygen concentration ($mg \cdot L^{-1}$) in the test with total microorganisms vs. time (min) in the first toxicity test with activated sludge.

The oxygen consumption rate was lower when the concentration (Vol.%) of ES was higher, because the microorganisms were affected by the toxicity of ES. As a result, the Inhibition must increase, when the concentration of ES is increased. Inhibition for each sample was calculated following the equation [10].

Next, we represented the Inhibition of oxygen uptake of total microorganisms vs. logarithm of concentration of ES (Vol.%) (Table 18).

Table 18. Inhibition of oxygen uptake of total microorganisms and the logarithm of concentration of ES (first toxicity test).

Sample	Conc. (Vol.%)	Log conc.	Inhibition, I (%)
0.018 Vol.%	0.018	-1.745	51
0.029 Vol.%	0.029	-1.538	83
0.040 Vol.%	0.040	-1.398	93

The obtained inhibition (%) for each sample was plotted versus logarithm of concentration of the sample (Figure 8) and the 30minEC_{50} and 30minEC_{20} values were obtained.

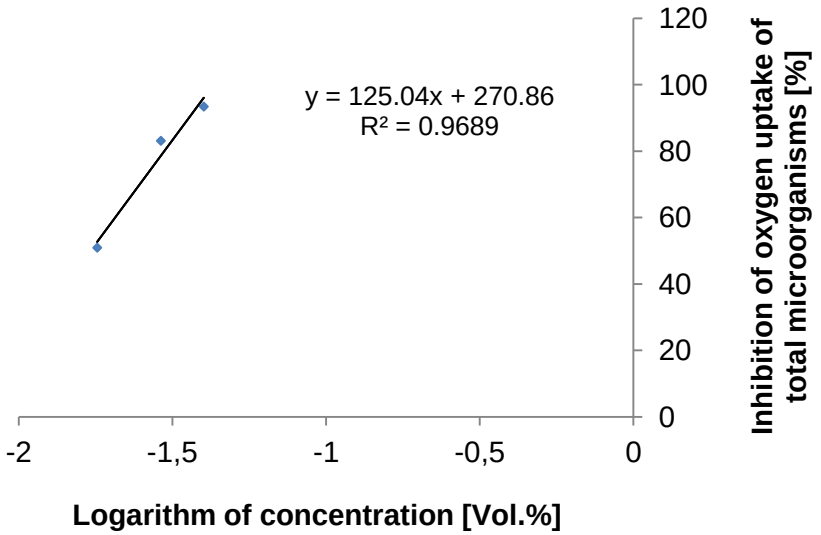


Figure 8. Inhibition of oxygen uptake of total microorganisms vs. log. of concentration of ES (first toxicity test).

With the inhibition results, we saw that at a really low addition of ES, the inhibition was high. That means that the ES was very toxic for the microorganisms, obtaining a value of 93% in the sample with 0.040 Vol.% of ES.

30minEC_{50} value is the concentration which inhibits the oxygen consumption by 50%. We also calculated 30minEC_{20}, which inhibits the oxygen consumption by 20%, to know the estimated first quantity of ES to add in the WWTP. The 30minEC_{20} was selected as appropriate value, because 20% of inhibition is usually referred to as the first reliably measured inhibition in toxicity test with activated sludge (Rand, 1995). Using the equation from linear trendline from Figure 8, 30minEC_{50} and 30minEC_{20} were calculated. 30minEC_{50} was 0.017 Vol.% and 30minEC_{20} was 0.010 Vol%.

The test has been done in the same way with ATU to determine the impact of the ES to the heterotrophic microorganisms. In Table 19 the measurement of the dissolved oxygen concentration ($mg \cdot L^{-1}$) during 5 minutes of the test, for all of the samples, is presented.

Table 19. Dissolved oxygen concentration values ($mg \cdot L^{-1}$) and inhibition results for all the samples in the first test with ATU:

	Concentration of dissolved oxygen ($mg \cdot L^{-1}$)				
Time (min)	**Blank**	**0.018 Vol.%**	**0.029 Vol.%**	**0.040 Vol.%**	**0.040 Vol.% abiotic (**)**
0.0	9.52	9.86	10.53	9.76	10.00
0.5	10.00	9.91	10.51	9.85	10.03
1.0	9.12	9.87	10.49	9.73	10.13
1.5	8.99	9.79	10.47	9.97	10.18
2.0	8.77	9.69	10.45	9.98	10.23
2.5	8.69	9.63	10.43	9.99	10.27
3.0	8.57	9.54	10.42	9.99	10.28
3.5	8.44	9.52	10.40	9.96	10.29
4.0	8.27	9.42	10.38	9.73	10.31
4.5	8.10	9.33	10.36	9.67	10.32
5.0	7.97	9.26	10.35	9.79	10.30
Slope (RH) (*)	0.3491	0.1338	0.0364	0.0105	/

(*) RH = Oxygen uptake rate for blank and different concentrations (0.018-0.040 Vol.%) of the ES.

(**) See comment in Table 17.

(/) Not determined.

In Figure 9 dissolved oxygen concentration values in the test with heterotrophic microorganisms ($mg \cdot L^{-1}$) were plotted versus time (min) for each sample and the linear trendlines were obtained.

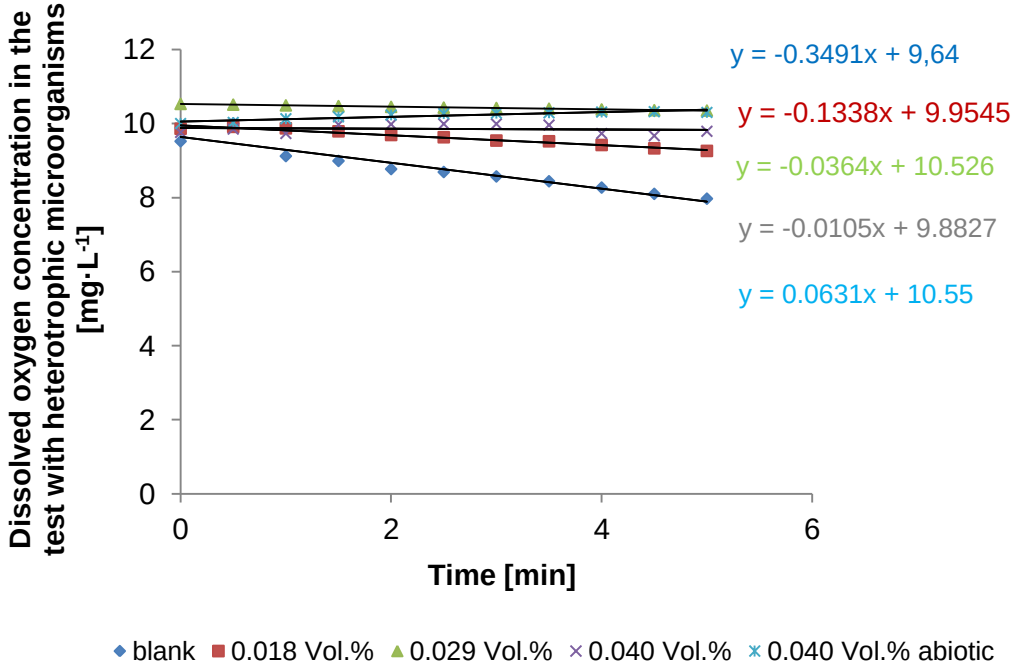


Figure 9. Dissolved oxygen concentration in the test with heterotrophic microorganisms ($mg \cdot L^{-1}$) vs. time (min) in the first toxicity test with activated sludge.

The results of the Inhibition of heterotrophic microorganisms (equation [11]) were higher when the concentration of ES was increased. It has been concluded that the ES caused a negative impact to heterotrophic microorganisms.

The results of the inhibition of oxygen uptake of heterotrophic microorganism (I_H) and the logarithm of concentration of ES are presented below (Table 20).

Table 20. Inhibition of oxygen uptake of heterotrophic microorganisms and the logarithm of concentration of ES (first toxicity test).

Sample	Conc. (Vol.%)	Log conc.	Inhibition, I_H (%)
0.018 Vol.%	0.018	-1.745	62
0.029 Vol.%	0.029	-1.538	90
0.040 Vol.%	0.040	-1.398	97

The obtained inhibition of heterotrophic microorganisms (%) for each sample was plotted versus logarithm of concentration of the sample (Figure 10) and the 30minEC_{50} values were obtained.

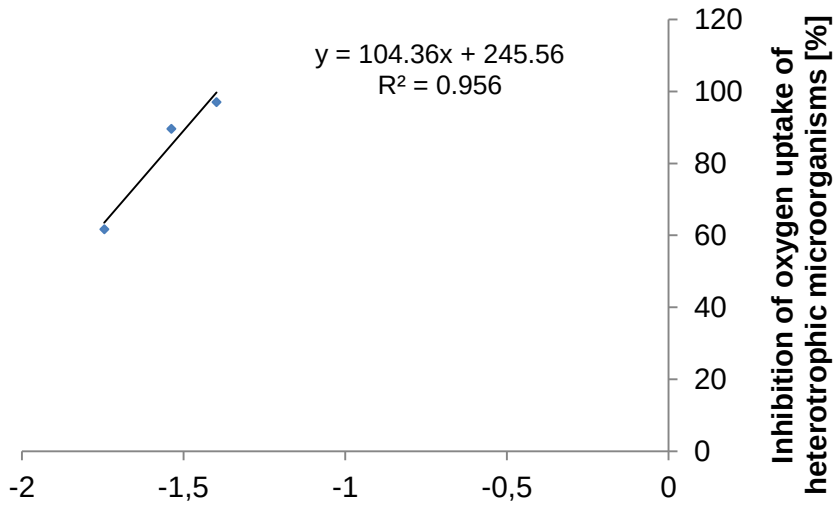


Figure 10. Inhibition of oxygen uptake of heterotrophic microorganisms vs. log. of concentration of ES (first toxicity test).

Using the equation from linear trendline (Figure 10), 30minEC_{50} and 30minEC_{20} were calculated. 30minEC_{50} was 0.0134 mg·L^{-1} and 30minEC_{20} was 0.007 mg·L^{-1}.

After finishing the characterization of total and heterotrophic microorganisms we calculated the impact to nitrifying microorganisms. I_N (%) was calculated with the equation [12], obtaining the results presented, in Table 21.

Table 21. Inhibition of oxygen uptake of nitrifying microorganisms and the logarithm of concentration of ES (first toxicity test).

Sample	Conc. (Vol.%)	Log conc.	Inhibition, I_N (%)
0.018 Vol.%	0.018	-1.745	29
0.029 Vol.%	0.029	-1.538	70
0.040 Vol.%	0.040	-1.398	86

* No reliable measurement of inhibition (%).

The obtained inhibition (%) for each sample was plotted versus logarithm of concentration of each sample (Figure 11) and 30minEC_{50} and 30minEC_{20} values were obtained.

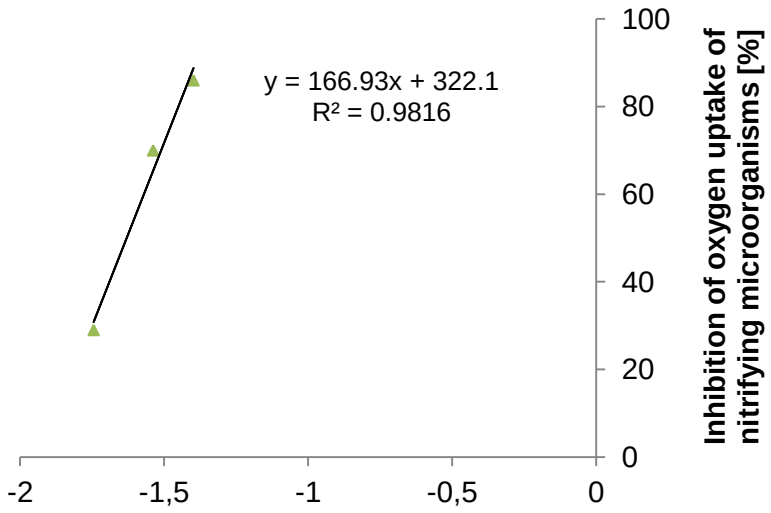


Figure 11. Inhibition of oxygen uptake of nitrifying microorganisms vs. log. of the concentration of ES (first toxicity test).

With the results inhibition of nitrifying microorganims, we could see that the inhibition of the heterotrophic microorganisms was higher than for the nitrifying microorganisms. 30minEC_{50} was 0.023 Vol.% and 30minEC_{20} value was 0.015 Vol.%.

To minimize the experimental error, we decided to run the test twice, obtaining the average values. The oxygen consumption rate in the Blank (R) ($mg \cdot g^{-1} \cdot h^{-1}$) was calculated again with the equation [8] and the value obtained for this second test was 27.12 $mg \cdot g^{-1} \cdot h^{-1}$. Then Specific respiration ratio (Rs) ($mg \cdot g^{-1} \cdot h^{-1}$) was determined again with the equation [9] obtaining 18.08 $mg \cdot g^{-1} \cdot h^{-1}$ (less than 20 $mg \cdot g^{-1} \cdot h^{-1}$ as requested by ISO 8192, 2007). The results obtained in the second test could be not reliable due to the lower activity of AS. However, because of the toxicity of 3.5-DCF was in the required range, we assumed that the obtained results could be considered.

In Table 22 and Table 23 all results are collected (1st test and 2nd test) for the Inhibition, 30minEC_{50} and 30minEC_{20} values for total (Total), heterotrophic (Het) and nitrifying (Nit) microorganisms.

Table 22. Inhibition (%) of oxygen consumption in both toxicity test with the average obtained.

	Inhibition (%)								
Conc. (Vol.%)	**0.018**			**0.029**			**0.040**		
MO	**Total**	**Het**	**Nit**	**Total**	**Het**	**Nit**	**Total**	**Het**	**Nit**
1st test	51	62	29	83	90	70	93	97	86
2nd test	35	49	20	38	28	/	65	61	70
Avg	43	55	25	33	59	70	79	79	78

(/) Not determined reliable.

Table 23. 30minEC_{20} and 30minEC_{50} in both tests and the average obtained:

	30minEC values (Vol.%)					
	30minEC_{20}			**30minEC_{50}**		
MO	**Total**	**Het**	**Nit**	**Total**	**Het**	**Nit**
1st test	0.010	0.007	0.015	0.017	0.013	0.023
2nd test	0.013	0.003	0.018	0.031	0.020	0.029
Avg	0.012	0.005	0.017	0.024	0.016	0.026

After both toxicity test carried out we concluded that the selected eutectic solvent was very toxic for the microorganisms, being more toxic for the heterotrophic than for the nitrifying microorganisms. With the 30minEC_{20} of total microorganisms average value we obtained the first concentration of ES to add in the synthetic wastewater (0.012 Vol.%). To ensure that the ES would not cause a high negative impact in the WWTP we decided to add the 50% of the 30minEC_{20} value, so the first concentration in the WWTP was 0.006 Vol.%.

5.2. Biodegradability of eutectic solvent

The biodegradation of ES was measured each day, during 28 days. In Table 24 the oxygen consumption ($mg \cdot L^{-1}$) results for ES samples and the blank are presented.

Table 24. Oxygen consumption ($mg \cdot L^{-1}$) for the blank, ES samples (with and without ATU) and the reference compound.

	Oxygen Consumption ($mg \cdot L^{-1}$)					
Time (Days)	Blank (no ATU)	Blank (no ATU)	Blank avg. (no ATU)	ES (no ATU)	ES (with ATU)	Reference compound
0	0	0	0	0	0	0
1	2	2	2	0	2	14
2	5	5	5	18	20	38
3	14	14	14	48	49	48
4	13	13	13	48	49	48
5	14	13	13	48	49	48
6	13	13	13	48	49	47
7	15	15	15	48	49	48
8	16	16	16	48	49	47
9	17	17	17	48	49	47
10	18	18	18	48	49	48
11	18	18	18	48	49	48
12	18	18	18	48	49	48
13	18	18	18	48	49	48
14	18	18	18	48	49	48
15	18	18	18	48	49	48
16	18	18	18	48	49	48
17	18	18	18	48	49	48
18	18	18	18	48	49	48
19	18	18	18	48	49	48
20	18	18	18	48	49	48
21	18	18	18	48	49	48
22	18	18	18	48	49	48
23	18	18	18	48	49	48
24	18	18	18	48	49	48
25	18	18	18	48	49	48
26	18	18	18	48	49	48
27	18	18	18	48	49	48
28	18	18	18	48	49	48

With the equation [13] we calculated the biodegradation of ES and the reference compound. At the end, biodegradation (%) was represented vs. time (days) (Figure 12).

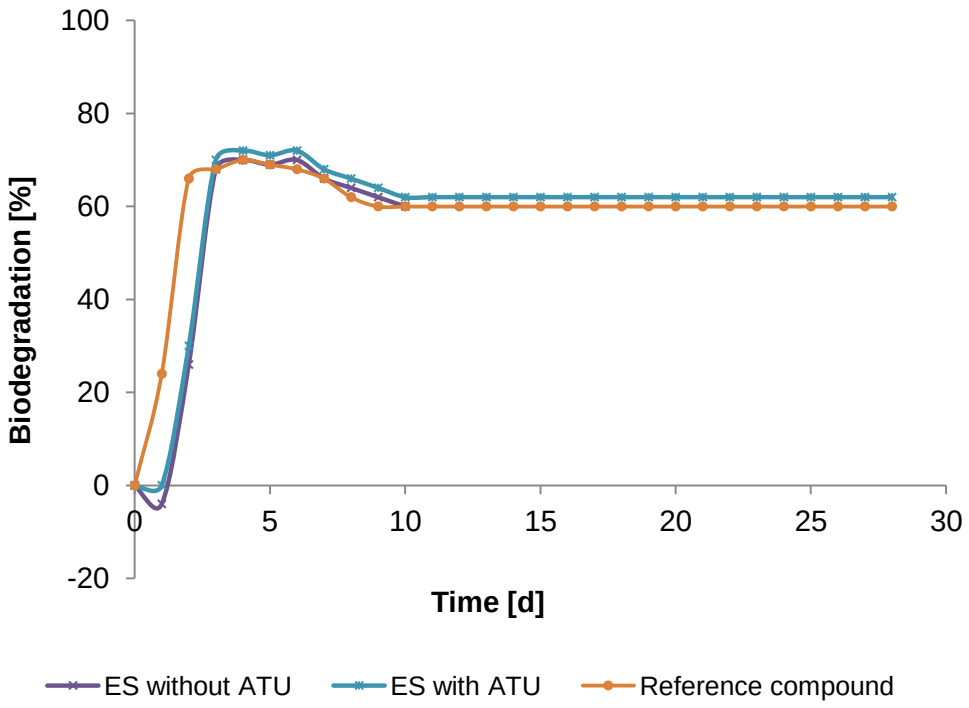


Figure 12. Biodegradation (%) of ES (with and without ATU) and the reference compound vs. time (days).

The biodegradation (%) of the reference compound must be more than 70% in 14 days to say that the activity of the inoculum used and all other parameters of the test are suitable. As we obtained this percentage in 4 days, we could say that the test results were reliable. In Figure 12 we can see that ES is biodegradable, because it degraded 70% in 4 days. The both curves for ES (with and without ATU) are more or less equal, that means that the nitrification did not interfere in the biodegradation. It seems that in the first days (1st and 2nd day) microorganisms had been affected by the toxicity of ES but then they adapted to the substance.

5.3. Treatment in the WWTP

Next, all the results obtained during the monitoring of WWTP (with and without ES) are presented.

5.3.1. Daily control of parameters

During the experimental work, good conditions for the WWTP were verified every day (not included weekends or holidays). Factors as temperature, air flow rate, wastewater flow rate, and dissolved oxygen concentration should maintain constant if there is no impact of eutectic solvent in WWTP. In Figure 13 the daily monitoring of temperature (ºC) and dissolved oxygen concentration (mg·L^{-1}) is presented.

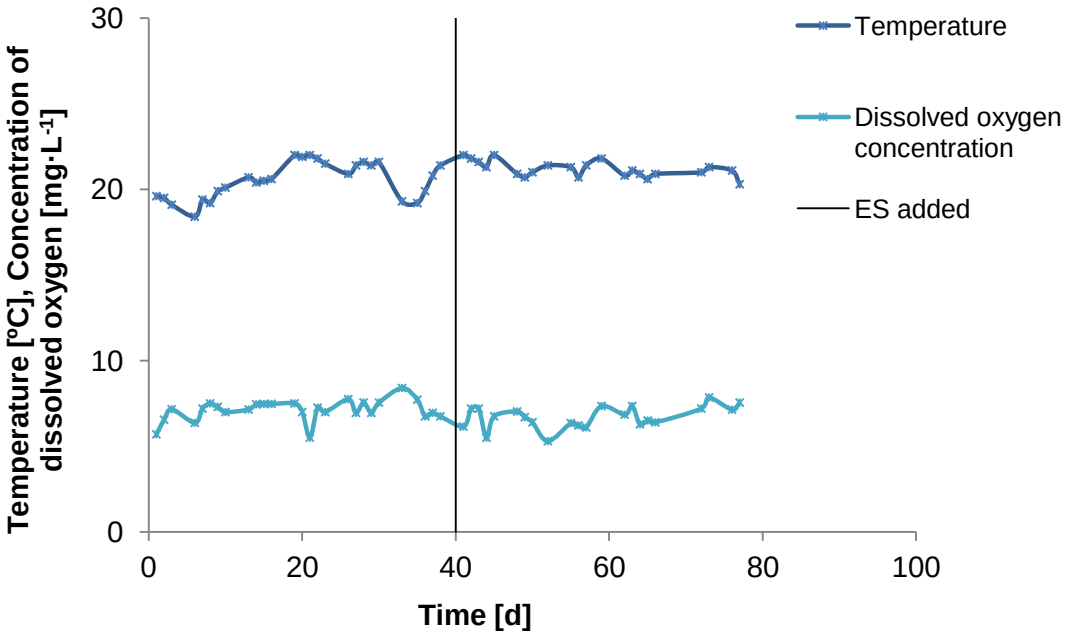


Figure 13. Daily registration of temperature (ºC) and dissolved oxygen concentration (mg·L^{-1}) in the WWTP.

Temperature was always between 19 and 21 ºC. The measurement of the dissolved oxygen concentration was made with an oxygen sensor, and values were always between 6 and 8 mg·L^{-1}. However, there is one value of dissolved oxygen concentration on day 21 that decreased to 5.5 mg·L^{-1}. The reason is that we increased the peptone concentration in wastewater 1.5 times (adding 2.7 g in 10 L, instead of 1.8 g) It has been done due to the fact, that F/M was a bit low according to the literature data (Arundel, 2000) and we wanted to increase it to improve the treatment efficiency. As a result, we obtained worst efficiency in the WWTP, not so good concentration of dissolved oxygen, etc. because organic load in pilot system seemed to be too high. After 5 days it had been reduced again to initial concentration (1.8 g of peptone per 10 L of wastewater). It can be concluded that ES degraded more easily in comparison to peptone because the addition of ES did not affect these parameters.

Air flow rate and wastewater flow rate were also measured daily. The results are shown in Figure 14.

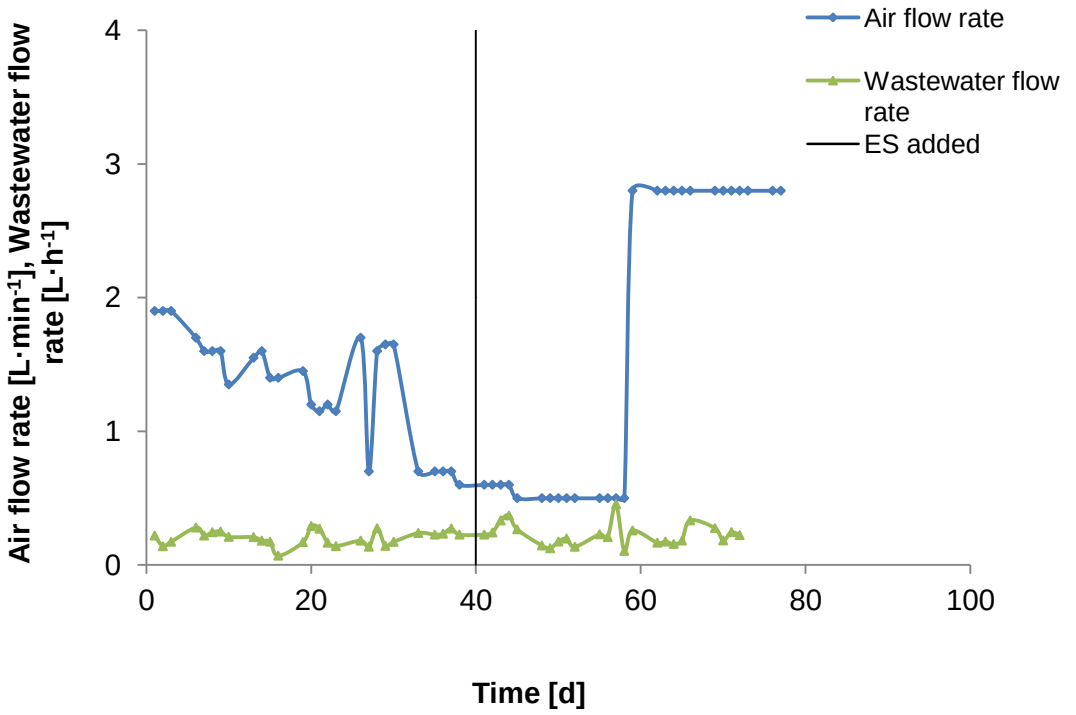


Figure 14. Daily monitoring of air flow rate ($L \cdot min^{-1}$) and wastewater flow rate ($L \cdot h^{-1}$) in the WWTP.

Air flow rate was approximately between 2 and 1 $L \cdot min^{-1}$ during the first 40 days. Then we had problems to maintain the aeration system in the WWTP and air flow rate started to decrease. Consequently, we needed to change the aeration system and put additional external aeration system, to increase the air flow rate to 2.5-3.0 $L \cdot min^{-1}$. The control of wastewater flow rate was difficult because of the regulator system, because we could not measure the water flow rate directly and we needed to calculate the wastewater flow rate as the wastewater pumped in the WWTP in one day. The values were always between 0.16 and 0.25 $L \cdot h^{-1}$ and the addition of ES did not affect these parameters.

5.3.2. Control of activated sludge

Concentration of activated sludge, sedimentation of activated sludge (VU) and sludge volume index (SVI) were determined frequently to evaluate the impact of selected eutectic solvent to the microorganisms growth and sedimentation characteristics. When activated sludge (AS) was removed, visual parameters as smell, formation and shape of flocks were observed.

In the following figures (Figure 15 and Figure 16) the monitoring of all these parameters during the experimental work is presented.

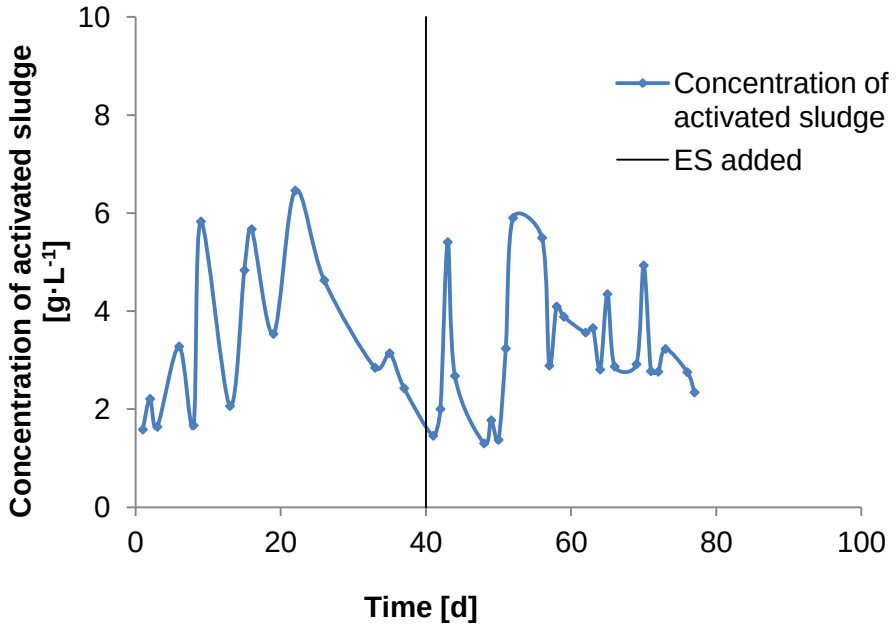


Figure 15. Concentration of AS ($g \cdot L^{-1}$) in the aeration tank vs. time (days).

The concentration of AS ($g \cdot L^{-1}$) in the reactor was always between 2-6 $g \cdot L^{-1}$. We calculated it using equation [1] and the variability of the results could be because of the sedimentation of the AS in the bottom of the aeration tank. This phenomenon could occur when there is not good agitation in the aeration basin. Consequently, the concentration of AS was slightly different if the sample of AS that was taken from the middle, the top or the bottom of the aeration tank. Sometimes, when the concentration was lower, we added some new activated sludge in the aeration tank, to maintain the optimum concentration (2-3 $g \cdot L^{-1}$, Arundel, 2000). The addition of ES did not affect the AS and any of the parameters related (SVI, VU).

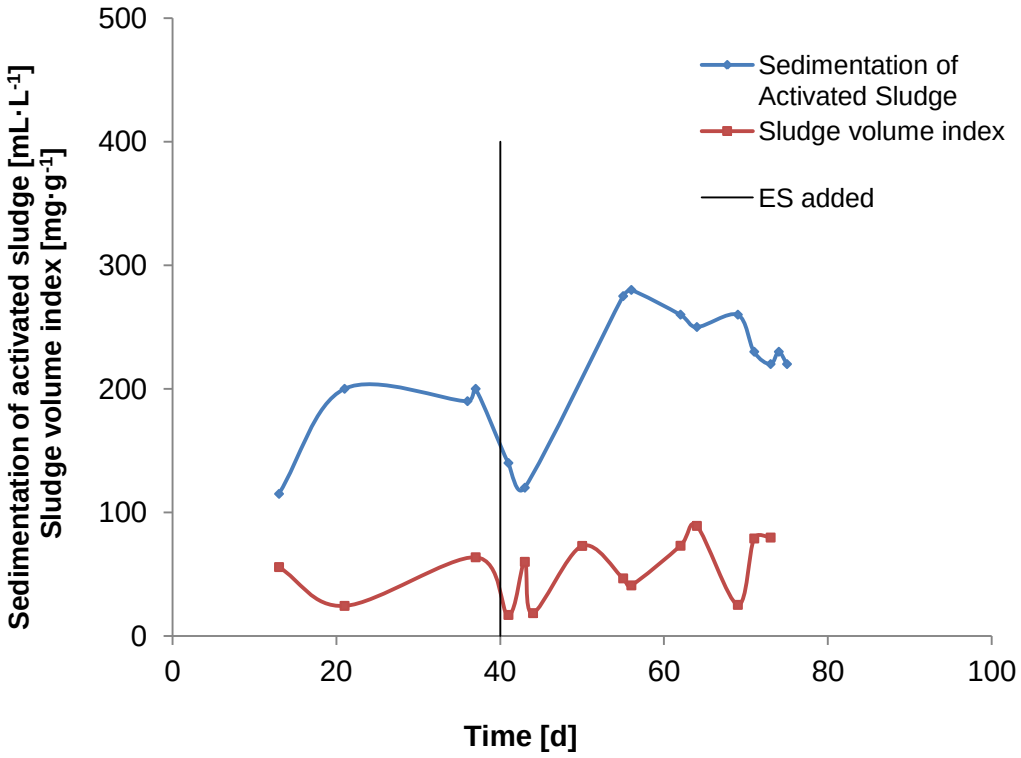


Figure 16. Sedimentation of AS (VU) and Sludge Volume Index (SVI) in the aeration tank vs. time (days).

We started to calculate these parameters approximately two weeks after starting with the daily monitoring of the WWTP and after that, we determined them twice to three times per week. The values of sedimentation of AS (VU) were between 190-280 $mL \cdot L^{-1}$ almost all of the time (equation [2]). Only two times, lower values were obtained (100 $mL \cdot L^{-1}$ approximately). Sludge volume Index (SVI) was calculated with equation [3] and had more variability in the results, with values between 20 and 80 $mL \cdot g^{-1}$. The variability of both parameters could be because of the variability in the results of the concentration of AS (Figure 15). However the addition of ES did not affect these parameters.

5.3.3. Hydraulic Retention time

Hydraulic Retention time (HRT) was calculated following the equation [4]. In the following table (Table 25) the average for all the parameters needed for calculation of the HRT are presented.

Table 25. Average of all of the parameters needed to calculate Hydraulic retention time (HRT) in the aeration tank.

Conc. of ES (Vol.%)	Avg. Flow in the influent (Q) ($L \cdot h^{-1}$)	Avg. Volume of aeration tank (V)* (L)	Avg. HRT (h)
Without ES	0.2074	6.79	**32.74**
0.006	0.2666	6.79	**25.47**
0.012	0.2291	6.79	**29.64**
0.015	0.2583	6.79	**26.28**
0.020	0.1574	6.79	**43.30**
0.030	0.2757	6.79	**24.62**

* The height (H) of the AS was 21.6 cm. The diameter of the aeration tank was 20 cm. We calculated the volume of the aeration tank following the equation [5].

HRT did not change with the addition of ES. It was always between 26 and 30 hours, except one value that was 40 hours. The differences between the values are caused because of the variability of water flow rate, which, as we commented before, it was difficult to regulate and maintain constant. As a result, the values are not comparable to values of common wastewater treatment plant, which normally have a HRT between 4 and 15 hours (Davies, 2005).

5.3.4. F/M Ratio

F/M ratio was calculated following the same criteria as HRT, calculating the average for all of the parameters needed. F/M ratio was obtained following the equation [6]. In the Table 26 the results are presented.

Table 26. Average of the parameters needed to determine F/M ratio in the aeration tank.

Conc. of ES (Vol.%)	Avg. Flow in the influent (Q) ($m^3 \cdot h^{-1}$)	Avg. Vol. Aeration tank (V) (m^3)	Avg. Conc. AS (X) (g/L)	Avg. COD IN ($mg \cdot L^{-1}$)	Avg. F/M ratio ($kg_{COD} \cdot kg_{AS}^{-1} \cdot d^{-1}$)
Without ES	0.0002	0.0068	4.77	212	**0.03**
0.006	0.0003	0.0068	2.14	208	**0.09**
0.012	0.0002	0.0068	3.44	314	**0.07**
0.015	0.0003	0.0068	3.65	314	**0.08**
0.020	0.0002	0.0068	3.57	343	**0.05**
0.030	0.0003	0.0068	2.91	393	**0.13**

Results are very variable. F/M ratio was between 0.03 and 0.13 $kg_{COD} \cdot kg_{AS}^{-1} \cdot d^{-1}$. In this case it is difficult to classify in a specific process range (Table 10) but it seems that in the beginning it was comparable to the conditions in Extended Aeration process (F/M between 0.02 and 0.07 $kg_{COD} \cdot kg_{AS}^{-1} \cdot d^{-1}$). After the addition of 0.030

Vol.% the conditions in the system changed to Standard Activated Sludge process (F/M between 0.11 and 0.23 $kg_{COD} \cdot kg_{AS}^{-1} \cdot d^{-1}$). F/M ratio increased when we added ES but it did not increase linearly because of all the changes in the system (variability of concentration of AS, etc).

5.3.5. Treatment efficiency

The COD was one of the most important parameters, because it was used for the monitoring of treatment efficiency.

We also calculated the COD of ES, obtaining 1,648,000 $mg \cdot L^{-1}$ in the first determination and 1,804,000 $mg \cdot L^{-1}$ for the second time. We obtained an average of 1,726,000 ± 78,000 $mg \cdot L^{-1}$. The determination of COD was necessary for the biodegradability test and for the estimation of the amount of concentration of ES to add in the WWTP.

In Figure 17 the values of COD in Influent (COD_{IN}) and Effluent (COD_{OUT}) before and after the addition of ES are presented.

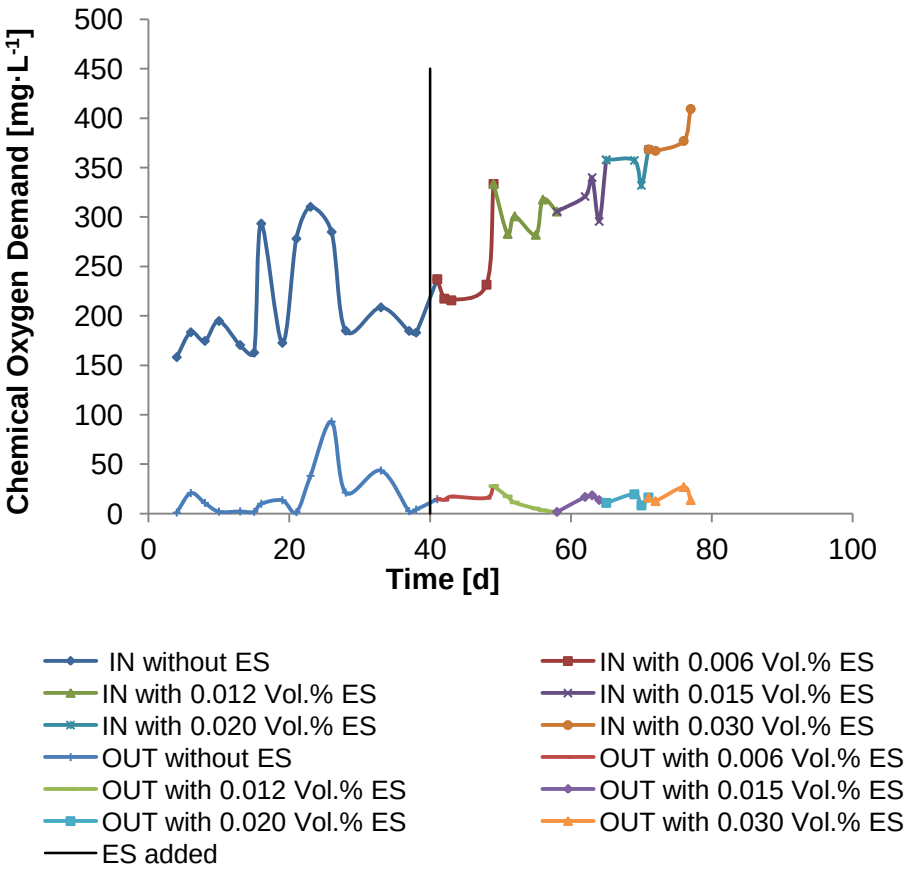


Figure 17. Chemical oxygen demand in influent (IN) and effluent (OUT) of the WWTP with the addition of ES vs. time (days).

As the Figure 17 shows, COD in influent (IN) was increasing with the quantity of Eutectic solvent added. The system started with a COD IN between 160 and 250

mg·L^{-1} and it was increasing with the time. The concentration of eutectic solvent was higher every week, with values of 0.006 Vol.% in the beginning, and afterwards was increased to 0.012 Vol.%, 0.015 Vol.% and 0.020 Vol.%. The concentration of 0.030 Vol.% of eutectic solvent was added at the last week of the experimental work, obtaining values of COD of 350-400 mg·L^{-1}.

Although the quantity of organic matter to treat was higher every week, the COD in the effluent (COD_{OUT}) did not change significantly, with values between < 5 mg·L^{-1} and 100 mg ·L^{-1} as the maximum.

Treatment efficiency on the process was calculated (equation [7]) .The COD removal efficiency (%) vs. time (days) is presented in Figure 18.

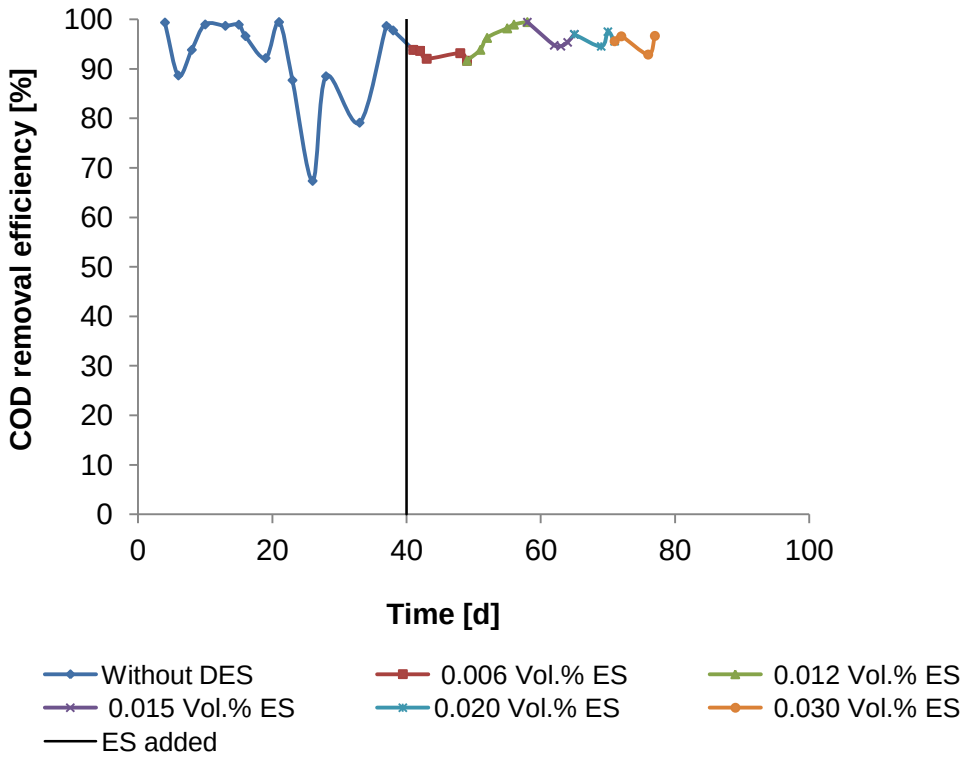


Figure 18. COD removal efficiency (%) in the WWTP with the addition of ES vs. time (days).

90% of BOD removal is the minimum removal considered acceptable in an efficient treatment with AS system (Arundel, 2000). The results, calculated on the basis of COD in this research, were always between 90 and sometimes almost 100%. Just two measurements were less that 90%, with values between 75 and 85%. This was before the addition of ES and the reason could be the same as we explained in the results for dissolved oxygen concentration (chapter 5.3.1), because of the concentration of peptone, that was for a few days 1.5 times higher. The addition of ES did not affect treatment efficiency; it was constant during all experimental work.

5.3.6. Biological oxygen demand (BOD_5)

We analyzed also the Biological oxygen demand after 5 days (BOD_5) before and after the addition of ES in the WWTP. The results are shown in Figure 19.

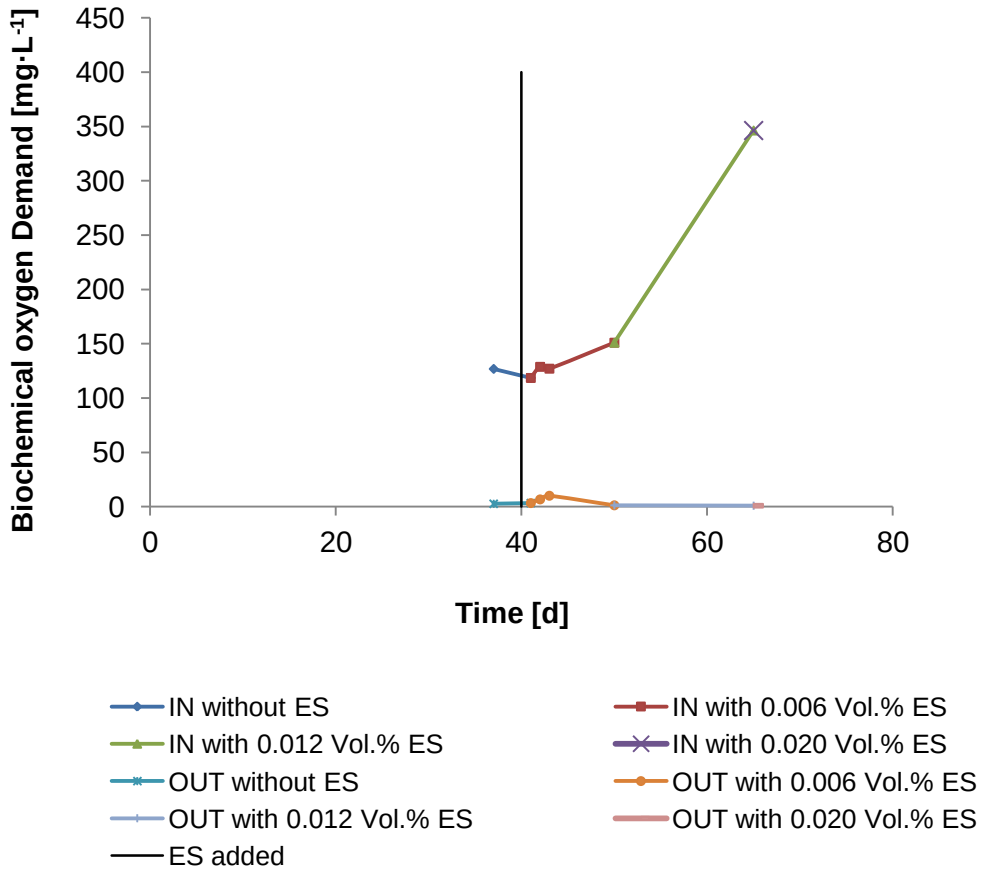


Figure 19. Biochemical oxygen demand in influent (IN) and effluent (OUT) of the WWTP with the addition of ES vs. time (days).

We started to measure BOD_5 after 35 days of the WWTP pilot monitoring. We did not obtained reliable data when we measured this parameter with a concentration of 0.030 Vol.% of ES. As a result, in Figure 19 there are no results for this concentration. The influent values were between 126 $mg \cdot L^{-1}$ without ES and it increased to 346 $mg \cdot L^{-1}$ with a concentration of 0.020 Vol.% of ES. With the data obtained we can conclude that BOD_5 ($mg \cdot L^{-1}$) in the influent increased more quickly with the addition of ES than the chemical oxygen demand. However, for the BOD_5 in the effluent, the values were always between 2 $mg \cdot L^{-1}$ and 10 $mg \cdot L^{-1}$, and there were no difference when we started with the addition of ES. Therefore it can be concluded that ES did not affect BOD_5 removal efficiency.

5.3.7. DOC removal

We started to determine the DOC parameter 35 days after the beginning of the WWTP monitoring. Results obtained in DOC tests are close to the COD results explained before. The DOC in the influent (IN) increased with the time, because of the addition of eutectic solvent. However, DOC in the effluent (OUT) was always low (Figure 20), showing that the efficiency, according to the minimum percentage considered acceptable in an efficient treatment with AS system (Arundel, 2000) for the DOC removal was optimal all the time (Figure 21).

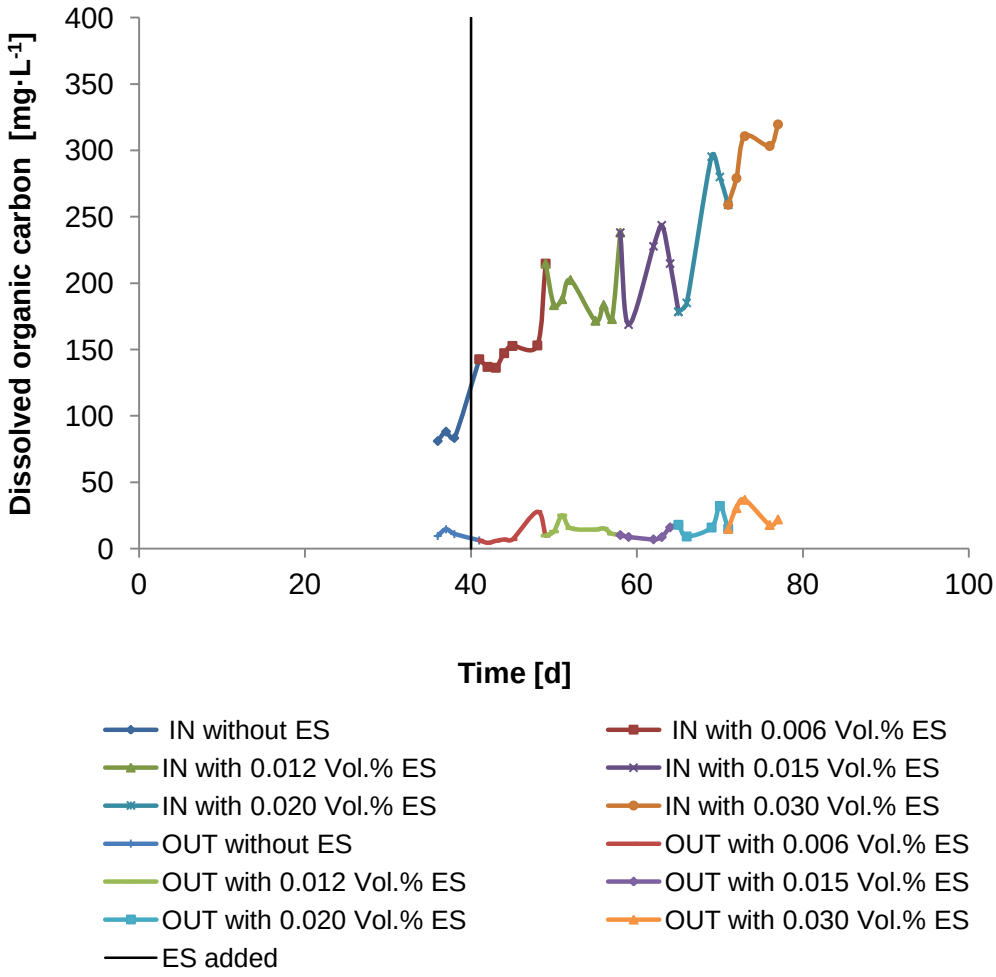


Figure 20. Dissolved organic carbon (DOC) in influent (IN) and effluent (OUT) of the WWTP with the addition of ES vs. time (days).

For the influent, DOC had values between 150 and 400 mg·L^{-1}, depending on the quantity of ES in the wastewater. COD in effluent (OUT) was always between < 4 and 40 mg·L^{-1}, but there were no difference when eutectic solvent was added. As a result, DOC efficiency shows always values between 80 and 97% (Figure 21).

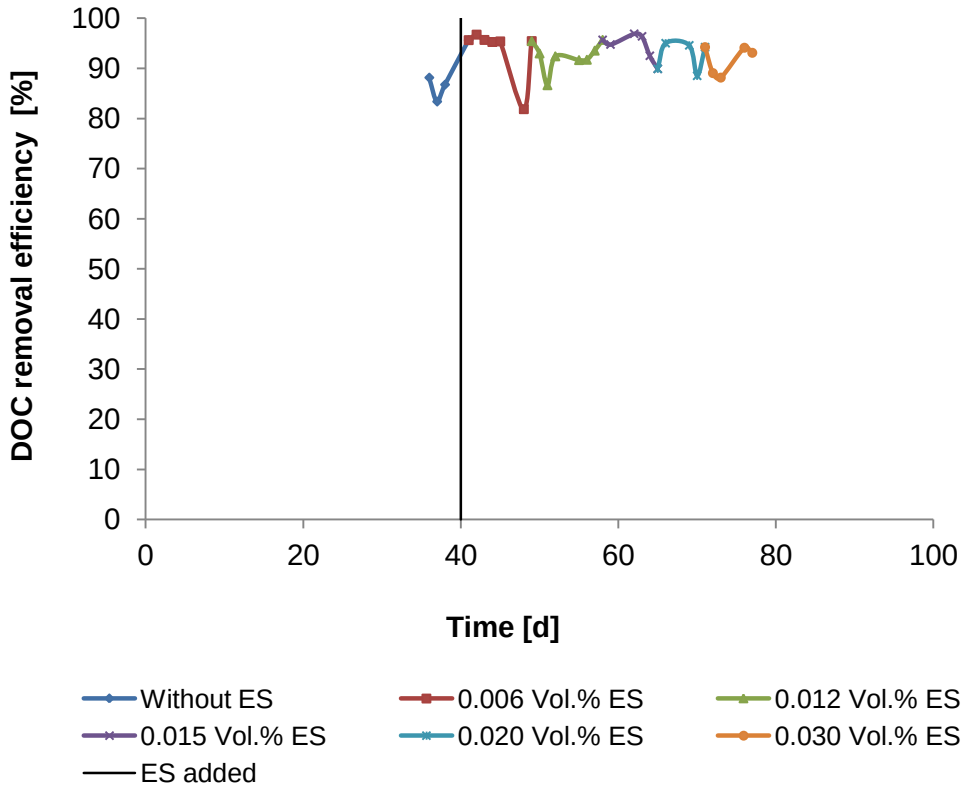


Figure 21. DOC removal efficiency (%) of the WWTP with the addition of ES vs. time (days).

5.3.8. Nitrite, nitrate and ammonium concentration

NO_4^+-N, NO_3^--N and NO_2^--N in $mg \cdot L^{-1}$ were present in influent (synthetic municipal WW) when we started to add ES in the system. We determined all of them three times, for three different concentration of ES (0.015 Vol.%, 0.020 Vol.% and 0.030 Vol.%). In Figure 22 the amount of the nitrogen in these three different forms in the influent and in the effluent vs. the concentration of ES (Vol.%) is presented.

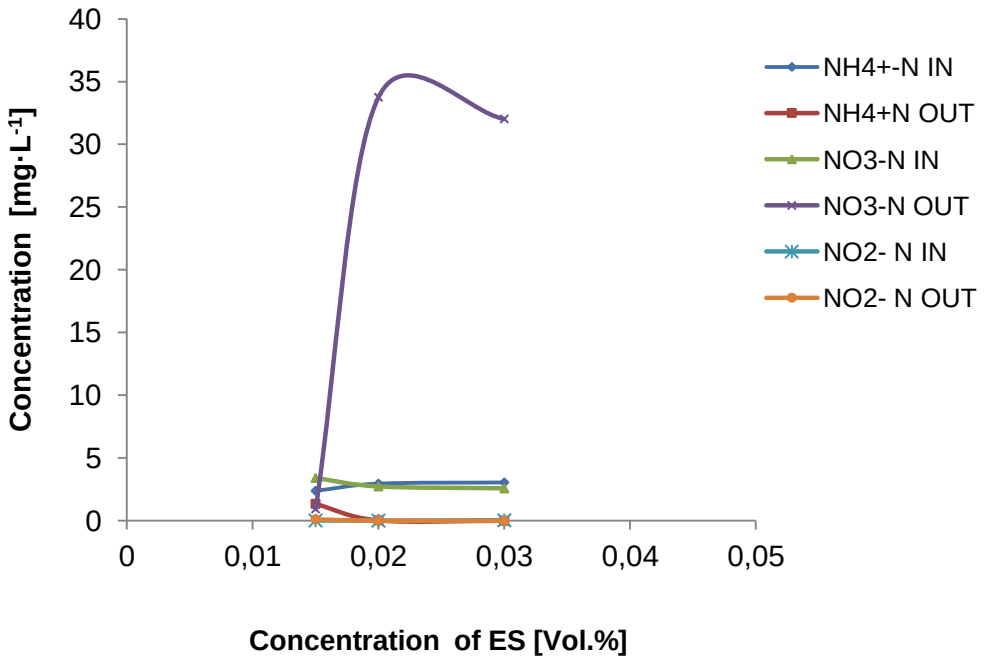


Figure 22. Amount of nitrogen in three different forms in the influent (IN) and the effluent (OUT) of the WWTP vs. the concentration of ES (Vol.%).

Concentrations of nitrite in the influent and in the effluent were very low, between 2.5 and 3.4 mg·L^{-1} for the influent (IN) and 2.3 and 3 mg·L^{-1} for the effluent (OUT). There was no difference when the concentration of ES was increased. However, nitrate had so much different values when we determined the concentration of nitrate in the effluent, which contained a different concentration of ES. It starts with a value of 0.93 mg·L^{-1} when the concentration of ES in wastewater was 0.015 Vol.% and it went up to 33 mg·L^{-1} when the concentration of ES was 0.030 Vol.%. The reason could be that the organic nitrogen, which selected ES contained (Figure 4) started to degrade and peptone added in synthetic municipal wastewater as well. This could be a good hypothesis because the good biodegradability of ES was showed (chapter 5.2). With these concentrations of nitrate in the effluent it has been observed that we need a denitrification process after the treatment in pilot WWTP. With the NH_4^+-N results, we could ensure a good nitrification process, because the concentration of NH_4^+-N in the influent was 2.37 mg·L^{-1} (with 0.015 Vol.% ES), 2.95 mg·L^{-1} (for 0.020 Vol.% ES) and 3.05 mg·L^{-1} (for .0.030 Vol.% ES) and it was reduced in the effluent until 1.34 mg·L^{-1} (for 0.015 Vol.% ES), 0.02 mg·L^{-1} (for 0.020 Vol.% ES) or less than 0.01 mg·L^{-1} (for 0.030 Vol.% ES).

5.3.9. Concentration of Chloride

High quantity of chloride anion in wastewater could interfere in the biological treatment in a negative way. It is known that high salinity cause cell plasmolysis and death of microorganisms due to the increase of osmotic pressure and reduce the quantity of the filamentous bacteria that play a part in a mechanical integrity

and the structure of the flocks. Recent studies show that protozoans have a limited resistance to salinity shocks and they normally die in 24h with a concentrations of NaCl higher than 40 $g \cdot L^{-1}$ (Lefebure and Moletta, 2006). For that reason, it was necessary to ensure that the concentration of chloride in the wastewater did not exceed the value. The determination of chloride was done when we added the first concentration of ES in the wastewater (0.006 Vol.%).

The concentration of chloride obtained in the influent (IN) was 65 $mg \cdot L^{-1}$ and in the effluent (OUT) was 35 $mg \cdot L^{-1}$. Normally when the concentration of chloride in wastewater is less than 10 $g \cdot L^{-1}$ is considered nonpolluted wastewater (in terms of chloride). Water which passed some clorification processes obtains levels of chlorides between 40 to 63 $mg \cdot L^{-1}$ (García-Vargas, 2012). We compared these values with our results and we concluded that there were no salinity problems in the pilot WWTP. Although the concentration of ES, which contain Cl^- (Figure 4) was increased every week, we considered that it was no necessary to determine the chlorides concentration anymore, because it was so low with a concentration of 0.006 Vol.% and the following concentrations were not so high to assume that we could exceed the concentration of 10 $g \cdot L^{-1}$.

6. Conclusion

Eutectic solvents (ES) are called “green solvents “due to their low price, chemical inertness with water, easy synthesis and biodegradability among other things. However, some recent studies shown that they can be toxic. As a result, they could impact the biological treatment in the WWTP. The selected eutectic solvent in this study was based on choline choride and malonic acid and the following hypotheses were determined.

The toxicity of ES was determined by the measurement of inhibition of oxygen consumption of activated sludge (*ISO 8192, 2007*). We showed that the selected eutectic solvent was very toxic for the microorganisms, being more toxic for the heterotrophic microorganisms than for the nitrifying microorganisms. Therefore, the **1st hypothesis** “Eutectic solvent selected is toxic to aerobic microorganisms of activated sludge”, is confirmed.

The biodegradation of chlorine chloride-malonic acid ES was tested by the measurement of oxygen consumption in a closed respirometer (ISO 9804, 1999) and we determined that it was degraded more than 70% in 4 days. Therefore, the **2nd hypothesis** “ES are biodegradable”, is confirmed.

We monitored the pilot WWTP without ES (40 days) and after the addition of ES (33 days) during 77 days. After the addition of ES, the COD, DOC, BOD_5 and F/M ratio increased in the influent quickly but the treatment efficiency was higher than 90% during the experiment, the impact on the WWTP was minimal. Therefore, **the 3rd hypothesis** “ES affects the biological treatment in WWTP” is refused. However, it is important to remark that this hypothesis it is only refused with concentrations from 0.006 Vol.% to 0.030 Vol.% of selected eutectic solvent based on chlorine chloride and malonic acid. As a result, with a higher concentration of the ES, the treatment of the WWTP could be affected and the impact could be different too if the ES tested would be a different compound.

At the end, the thesis combines the monitoring of the biological treatment in the WWTP with the addition of the new solvents, called eutectic solvents. I would like to express that the monitoring of the biological treatment in the WWTP requires a complex approach and it can be affected by many factors. Moreover, there are so many different eutectic solvents and it is difficult to define their characteristics in a general term. It is needed to carry on with an intense research to know exactly their effects on a biological treatment in the WWTP.

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