UNIVERSITY OF NOVA GORICA
GRADUATE SCHOOL

WATER SALINITY AND THE EFFICIENCY OF CONSTRUCTED WETLANDS

DISSERTATION

Milana Karajić

Mentors: prof. dr. Danijel Vrhovšek and prof. dr. Sidney A. Katz

Nova Gorica, 2015

UNIVERZA V NOVI GORICI
FAKULTETA ZA PODIPLOMSKI ŠTUDIJ

SLANOST VODE IN UČINKOVITOST RASTLINSKIH ČISTILNIH NAPRAV

DISERTACIJA

Milana Karajić

Mentorji: prof. dr. Danijel Vrhovšek in prof. dr. Sidney A. Katz

Nova Gorica, 2015

This dissertation is dedicated to my daughters Maša and Lana

…"No one has yet drunk a cup of honey
without mixing it with a cup of gall.
A cup of gall needs a cup of honey;
they are swallowed the easiest when mixed…"

P.P. Njegoš "The Mountain Wreath"

TABLE OF CONTENTS

ABBREVIATIONS AND SYMBOLS

ANAMMOX	Anaerobic ammonium oxidation
ANOVA	Analyses of variance
BOD_5	Biological oxygen demand for 5 days
CEC	Cation exchange capacity
COD	Chemical oxygen demand
CW	Constructed wetland
DO	Dissolved oxygen
ETS	Electron transport system activity
FFP	Free floating plants
F/M	Food/Microorganism ratio
FWS	Free water surface
HF	Horizontal flow
HRT	Hydraulic retention time
LECA	Light weight clay aggregates
MSW	Municipal solid waste
N	Nitrogen
NH_4	Ammonium
NO_3	Nitrate
NO_2	Nitrite
OD	Optical density
P	Phosphorus
PO_4	Phosphate
SBR	Sequencing batch reactor
SF	Surface flow
SS	Suspended solids
SSF	Subsurface flow
TKN	Total Kjeldal nitrogen
TN	Total nitrogen
TOC	Total organic carbon
Ptot	Total phosphorus
Q	Flow rate of wastewater
VF	Vertical flow
w/v	weight/volume

ABSTRACT

The focus of the thesis research was to investigate the influence of salinity on constructed wetland (CW) wastewater treatment. The thesis research was conducted at sub-surface flow (SSF) CW Dragonja which is situated on the Adriatic Coast, South-West Slovenia, and in the sand - gravel filter pilot plant for the treatment of a simulated saline wastewater. Results of this research suggested that increasing salinity at CW Dragonja negatively influenced the removal efficiency of almost all of the physical and chemical parameters investigated (ammonia, phosphate, nitrate, nitrite, COD and BOD_5), but the correlations were not significant. These weak coefficients of correlation mean that chloride ion concentration and conductivity can explain only small percent of the variability in removal efficiency of CW Dragonja. The possible presence of varying concentrations of pollutants in the landfill leachate, other than those investigated, may have had inhibitory influences on the microbiotic community in the CW Dragonja, and these inhibitory influences may have negatively impacted the treatment efficiency of CW Dragonja.

To investigate the process of saline wastewater treatment in the CW halotolerant microorganisms were innoculated into the substrate of the pilot plant, and the efficiency of wastewater treatment in the pilot plant was monitored. In order to investigate applications, survival and efficiency of halotolerant microorganisms in the pilot plant, the influence of 0%, 1.5% and 3% NaCl was investigated. The influences of aeration and aeration with 2 g/l of saccharose in synthetic wastewater were also investigated. Changes of pH, conductivity, redox potential, oxygen concentrations, concentrations of ammonium ions, chloride ions, phosphate ions, COD, BOD_5 and ETS activity, CO_2 concentrations in water and CO_2 concentrations in soil of the pilot plant were measured. It was found that removal efficiency of the pilot plant inoculated with halotolerant microorganisms was more influenced by aeration and the presence of saccharose (as organic carbon source) than by the variations in the salinity of the wastewater.

Keywords: constructed wetlands, saline wastewater, halotolerant microorganisms

POVZETEK

Raziskava teze je bila osredotočena na vpliv slanosti na učinkovitost rastlinske čistilne naprave. Raziskava je bila izvedena na rastlinski čistilni napravi Dragonja ki se nahaja na Jadranski obali, na jugo-zahodu Slovenije in na pilotskem modelu rastlinske čistilne naprave. Rezultati raziskave sugerirajo, da povečanje slanosti negativno vpliva na učinkovitost skoraj vseh spremljanih parametrov (amonijaka, fosfata, nitrata, nitrita, COD in BOD_5), ampak korelacija ni bila signifikantna. Majhni koeficienti korelacije pomenijo, da koncentracije kloridov in prevodnost lahko razložijo majhen delež variabilnosti učinkovitosti rastlinske čistilne naprave Dragonja. Mogoče ima prisotnost različnih koncentracij drugih polutantov v izcedni vodi, ki jih nismo spremljali v nalogi, inhibitorni vpliv na mikroorganizme v rastlinski čistilni napravi in tako negativno vpliva na učinkovitost rastlinske čistilne naprave Dragonja.

Za raziskavo procesa čiščenja slane odpadne vode so bili v substratu pilotskega modela rastlinske čistilne naprave inokulirani halotolerantni mikroorganizmi in spremljana učinkovitost čiščenja odpadne vode. Vpliv slanosti na učinkovitost, preživetje in uporabo halotolerantnih mikroorganizmov je bil preverjen pri 0%, 1.5% in 3.0% NaCl v odpadni vodi. Prav tako smo raziskali vpliv aeracije ter vpliv aeracije z dodano saharozo (2g/l) v sintetični odpadni vodi. V substratu pilotskega modela peščenega filtra rastlinske čistilne naprave smo spremljali spremembe v pH, prevodnosti, redoks potencialu, koncentraciji kisika, amonijevih ionov, kloridnih ionov, fosfatnih ionov, KPK, BPK_5 in ETS aktivnosti, koncentraciji CO_2 v vodi in koncentraciji CO_2. Ugotovili smo, da je na učinkovitost pilotskega modela, inokuliranega s halotolerantnimi mikroorganizmi, večji vpliv imela aeracija in prisotnost saharoze (kot organskega izvira ogljika), kot spremembe v slanosti vode.

Ključne besede: rastlinske čistilne naprave, slana odpadna voda, halotolerantni mikroorganizmi

LIST OF FIGURES

LIST OF TABLES

1 INTRODUCTION

The problem of high salinity in wastewater is present all over the world. Almost three quarters of the surface of the earth are covered by salt water and so it is not surprising that salts affect a large proportion of the world's land surface. Using seawater in the situations where high quality water is not essential such as toilet flushing has been practiced in arid and some coastal areas. The sewage resulting from such activities will have a high salt content. Seawater infiltration into the sewage system is also another cause of high salinity in the sewage from many regions. Many industries e.g. tanning, pickling, seafood canning, *etc*., are also discharging salty wastewater from their manufacturing processes (Panswad and Anan, 1999). Salt has also been used for decades to melt snow and ice from the road in order to improve traffic safety during winter. The reverse effect is that salt application has a range of environmental impacts (Lundmark, 2003). Pitt *et al.* (1999) concluded that chloride in stormwater would likely have a high groundwater contamination potential where road salts are used. Wastewaters from landfill leachate usually have high concentration of salts which fluctuates during the year. Such fluctuations could cause problems in the wastewater treatment processes (Zupančič Justin, 2006). The treatment of landfill leachete (Zupančič Justin, 2006; Bulc 2006, Bulc *et al.* 1997), road wastewater (Bulc and Vrhovšek, 1996; Lundmark, 2003), and tannery wastewater (Calheiros *et al.*, 2007, 2009a,b; Daniels, 2008) in constructed wetland (CW) has been reported.

In CW systems microorganisms have the main role in the removal of pollutants (Stottmeister *et al.*, 2003; Faulwetter *et al.*, 2009). It has been reported that reed beds are resilient to shock loadings, to feeding suspension and to climate variations (Calheiros *et al.*, 2007; Daniels, 2001). Lin *et al.* (2008) studied the effect of salinity on the degradation of atrazine in SSF CW (subsurface flow constructed wetland). Results indicated that microbial community played an important role in the atrazine degradation process and that salinity impacted on the growth of bacteria, resulting in a switch of the microbial community in SSF CW. Studies by Klomjek and Nitisoravut (2005) reported that the effect of salinity on BOD removal appeared to approach the exponential phase in the CW. The same restraining effect showed that salinity inhibited the metabolism of microorganisms in the wetland environment, which may be critical for the proper functioning and maintenance of the system. More recently, the study of Kaseva and Mbuligwe (2010) demonstrated that CW can be used as an option for improving the quality of tannery effluents, especially in the removal of chromium, with TDS levels around 10000 mg/l. Calherious *et al.*, 2010 concluded that CW with different plants performed well in terms of organic matter removal in the wastewater with the high salt content.

Conventional biological treatment systems are known as insufficient in removal of different pollutants from saline wastewaters, because of the adverse effects of salt on microbial flora. The reason for this is loss of activity of organisms in biological wastewater treatment operation due to plasmolysis in the presence of salt (Woolard and Irvine, 1995). Microorganisms requiring salt for growth are designated as halophilic. The intracellular salt concentration of halophilic and halotolerant microorganisms is low and they maintain an osmotic balance of their cytoplasm with the external medium by accumulating high concentration of various organic osmotic solutes (Margesin and Schinner, 2001). Therefore, utilization of salt tolerant microorganisms in biological wastewater treatment systems could be a solution for pollutants removal from saline wastewater (Kapdan and Erten, 2006). Lefebvre and Moletta (2006) also wrote that biological treatment is inhibited by high salt, but it has proved feasible to use salt-adapted microorganisms capable of withstanding high salinities and at the same time of degrading the pollutants that are contained in wastewater.

Several studies (Kargi and Dincer 1996; Kargi and Uygur 1996; Woolard and Irvine 1995; Tellez *et al.*, 1995; Yang 2000; Dan 2001) have shown that utilization of salt-tolerant microorganisms, such as halophilic bacteria or yeasts in biological treatment could be a reasonable approach for treatment of high salinity wastewater. In the literature was not found information about their application in CW, but good results of their application in other biological treatment applications give promise for CW applications.

The first part of this thesis was to demonstrate the efficiency of CW treating the landfill leachate with high salinity levels and the second to test the efficiency of halotolerant microorganisms which were taken from natural saltern environment under controlled laboratory conditions in sand-gravel filter pilot plant. Since little research has been reported exist about the influence of salinity on CW removal efficiency more empirical studies are needed. This thesis will contribute to the better knowledge about functioning of CW under different salinity levels and dependence of its efficiency to the variation in salinity. Further, the study is to author knowledge the first (or one of the rare) experimental mesocosmos study investigating the efficiency of halotolerant microorganisms in wastewater treatment and will contribute to the knowledge about suitability of that kind of microbial culture for the treatment of salt wastewater. Hence, the first chapter of the study analyzes the existing data of the CW gathered during long-term monitoring carried out by the municipal company in order to investigate the relationship between salinity and wastewater treatment efficiency. In the second part, the sand – gravel filter pilot plant CW was established, inoculated with halotolerant microorganism and the removal efficiency under different salinity conditions investigated.

1.1 Statement of thesis research objectives

Objectives:

1. To investigate the biochemical processes and treatment efficiency of constructed wetland exposed to salt water inputs.
2. To experimentally test the tolerance and efficiency of halotolerant microorganisms in the pilot plant exposed to different salt concentrations.

Working hypothesis:

1. Biochemical processes are repressed and CW treatment efficiency is reduced at elevated salinity of influent wastewater.
2. Halotolerant microorganisms are tolerant to variation in wastewater salinity and can efficiently remove the pollutants from the wastewater.

1.2 Thesis research plan

1. Perform analyses of data obtained during long term monitoring of water physico-chemistry in the Dragonja CW and search for the dependency of water treatment efficiency related to variation in salinity.
2. Experimentally test the efficiency of halotolerant microorganisms under different salinity conditions as follows:

    a. synthetic non aerated leachate in 1.5% sodium chloride,
    b. synthetic aerated leachate in 1.5% sodium chloride,
    c. synthetic carbon source fortified aerated leachate in 1.5% sodium chloride,
    d. synthetic non aerated leachate in 0% and 3% sodium chloride.

2 THEORETICAL BACKGROUND

2.1 General description of the Constructed Wetlands

Constructed wetlands (CWs) are wetlands designed to improve water quality. They use the same processes that occur in natural wetlands, but they have the flexibility of being constructed when and where they are needed. As in natural wetlands, vegetation, soil and hydrology are the major components. CWs consist of shallow (usually less than 1 m deep) ponds or channels which have been filled with sand and gravel mixture and planted with emergent, submerged or floating plants.

The first steps into developing the idea of using wetland plants for waste/sewage treatment in a very wide sense did Seidel K., 1967 and Kickuth R., 1984. Seidel K., 1978 was the very first with some trials and Kitckuth R., 1984 was the first to use a natural reed-wetland.

Different soil types and plant species are used in CWs (Haberl *et al.*, 2003). In the US, CWs systems are classified into two basic designs: free water surface (FWS) or surface flow (SF) systems and subsurface flow (SSF) systems. FWS treatment wetlands are densely vegetated and characterized by the type of vegetation used: (1) submerged macrophytes, (2) free floating macrophytes such as water lilies (*Nymphaea* spp.), lotus (*Nelumbo* spp.) and cowlily (*Nuphar* spp.), (3) macrophytes forming mats and (4) emergent macrophytes. Category systems (2) are still experimental, whereas (3) and (4) have currently only limited application. Free water systems may not offer many advantages over waste stabilization ponds. If mosquito breeding can be a serious problem in FWS systems then waste stabilization ponds should always be considered as an alternative (Ringuelet, 1983; Gray, 2004).

The climatic conditions in Europe limit the type of CWs that can be employed successfully throughout the year. For that reason, European CWs are almost exclusively SSF systems using emergent plant species commonly referred to as reed beds. A bed of soil or gravel 0.6 -1.0 m deep is used as a substrate for the growth of rooted emergent species. Settled wastewater flows through the substrate by gravity, either horizontally (Figure 1) or vertically (Figure 2), where it comes into contact with a mixture of facultative microorganisms living in association with the substrate and plant roots (Gray, 2004). SSF systems are normally monocultures of reeds that don't have permanent areas of open water, thus resembling a fen where the water movement is normally through the soil and not over the surface (Keddy, 2000).

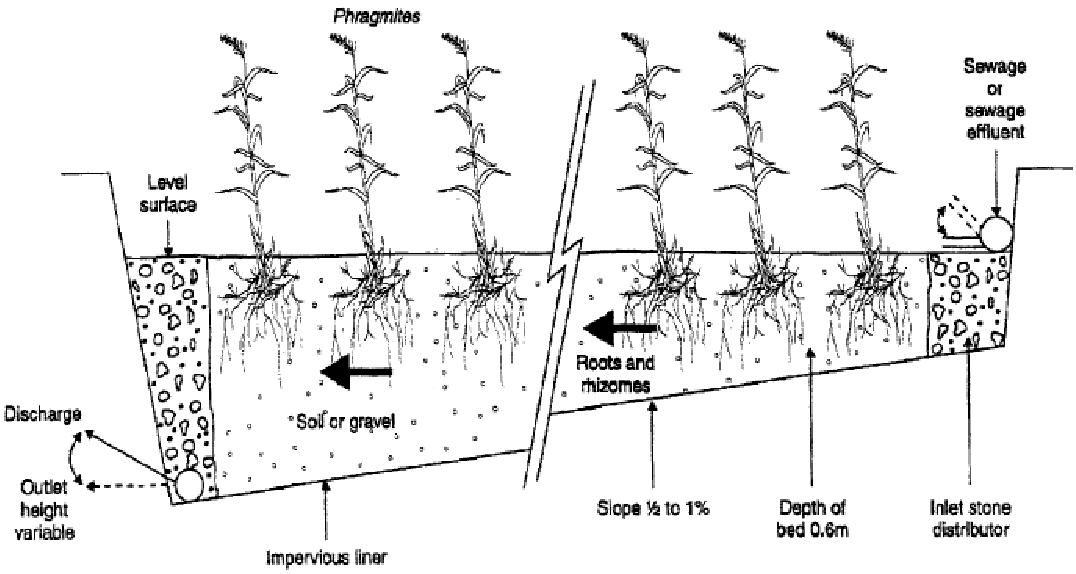


Figure 1: *Typical design of a horizontal flow reed bed treatment system (Cooper, 1990)*

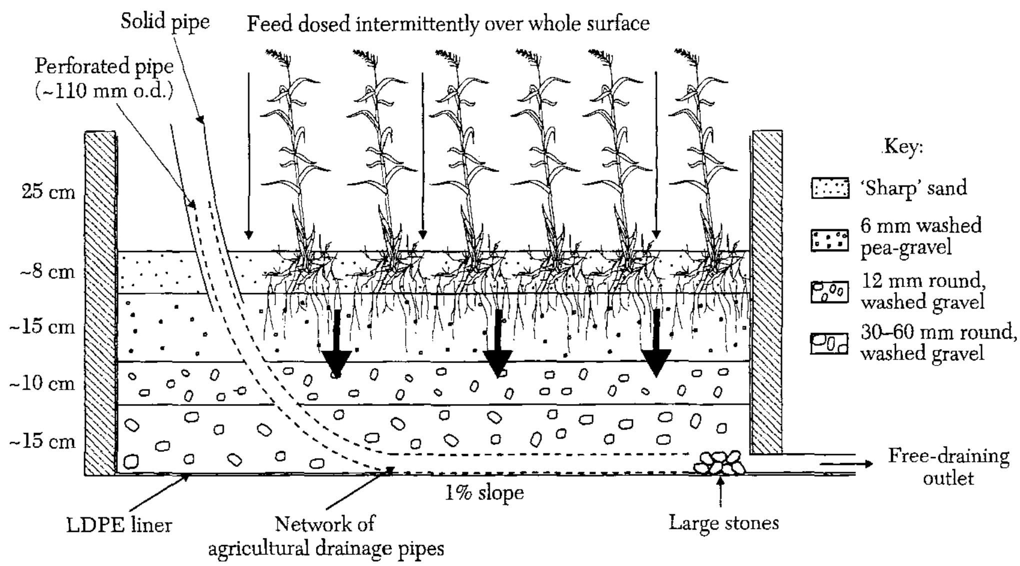


Figure 2: *Typical design of a vertical flow reed bed system (Cooper, 1999)*

In 1990s, increased demand for nitrogen removal from wastewaters led to more frequent use of Vertical Flow (VF) CWs which provide a higher degree of filtration, bed oxygenation and consequent removal of ammonia via nitrification. Horizontal

Flow (HF) CWs provide high removal of organics and suspended solids, but removal of nutrients is low. Removal of nitrogen is limited by anoxic/anaerobic conditions in the filtration beds which do not allow for ammonia nitrification. Phosphorus removal is restricted by the use of filter materials (pea gravel, crushed rock) with low sorption capacity. Various types of CWs may be combined in order to achieve higher treatment effect, especially for nitrogen. However, hybrid systems (Figure 3) are comprised most frequently of VF and HF systems arranged in a staged manner. HF systems can not provide nitrification, because of their limited oxygen transfer capacity. VF systems, on the other hand, do provide conditions favorable for nitrification. Little or no denitrification occurs in these systems. In hybrid systems (also sometimes called combined systems), the advantages of the HF and VF systems can be combined to complement the processes in each system producing an effluent low in BOD, which is fully nitrified and partly denitrified and hence has a much lower total-N outflow concentrations (Vymazal, 2005a,b).

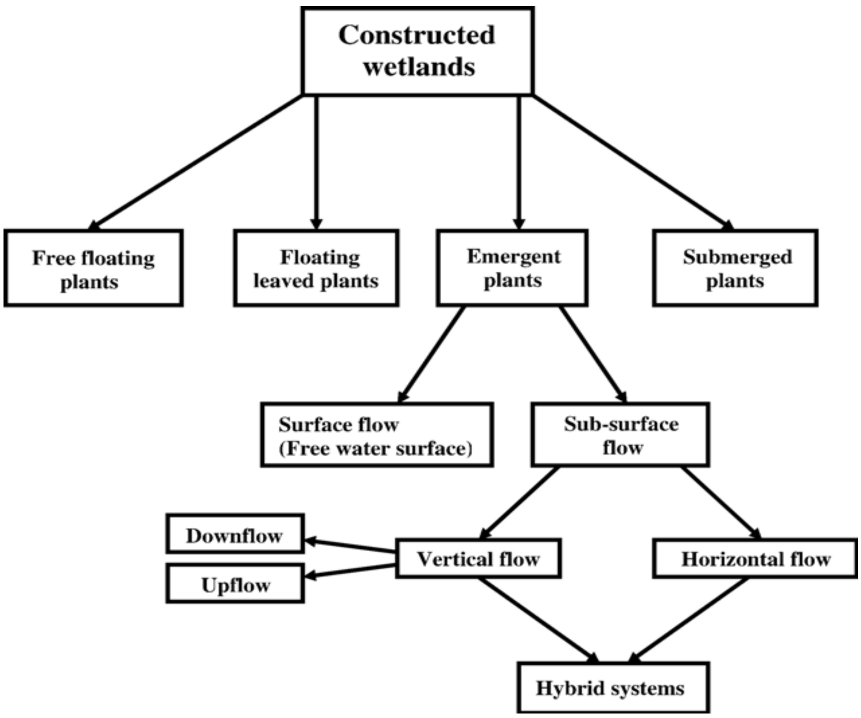


Figure 3: *Classification of CWs for wastewater treatment (Vymazal, 2001; 2007)*

Studies suggested that natural aeration of CWs did not meet the oxygen demand for treating pollutants (Brix H., 1994; Cottingham *et al.*, 1999; Wu *et al.*, 2001). It is necessary to reconsider the design of the constructed wetland systems for better performance in the cold seasons (Tao M. et al., 2010). Additional oxygen could be introduced into the wetland system through some design and manipulations such as the vertical-flow pre-treatment filter as hybrid system (Maehlum *et al.*, 1995; Vymazal, 2005a,b), frequent water level fluctuation (Austin *et al.*, 2003), air pipes (Sum *et al.*, 2006), passive air pumps (Admon *et al.*, 2005; Green *et al.*, 1998; Lahav *et al.*, 2001) and mechanical aeration in the gravel bed (Ouellet-Plamondon *et al.*, 2006; Nivala *et al.*, 2007; Yan *et al.*, 2006; Maltais-Landry *et al.*, 2007).

Nivala *et al*., 2014 presented adventages of aerated wetland technologies (Figures 4 and 5) that they have a reduced system area compared to traditional designs, can handle higher organic loading rates, have aerobic removal rates up to thousand times higher than conventional treatment designs, have excellent treatment efficiency even in cold climates, can be optimizes to increase treatment performance, are well adapted for use in developing countries (e.g., solar powered aeration). However, it remained unclear whether artificial aeration could improve the purification efficiency of constructed wetlands in the treatment of saline wastewater and how the redox environments influence the removal of pollutants.

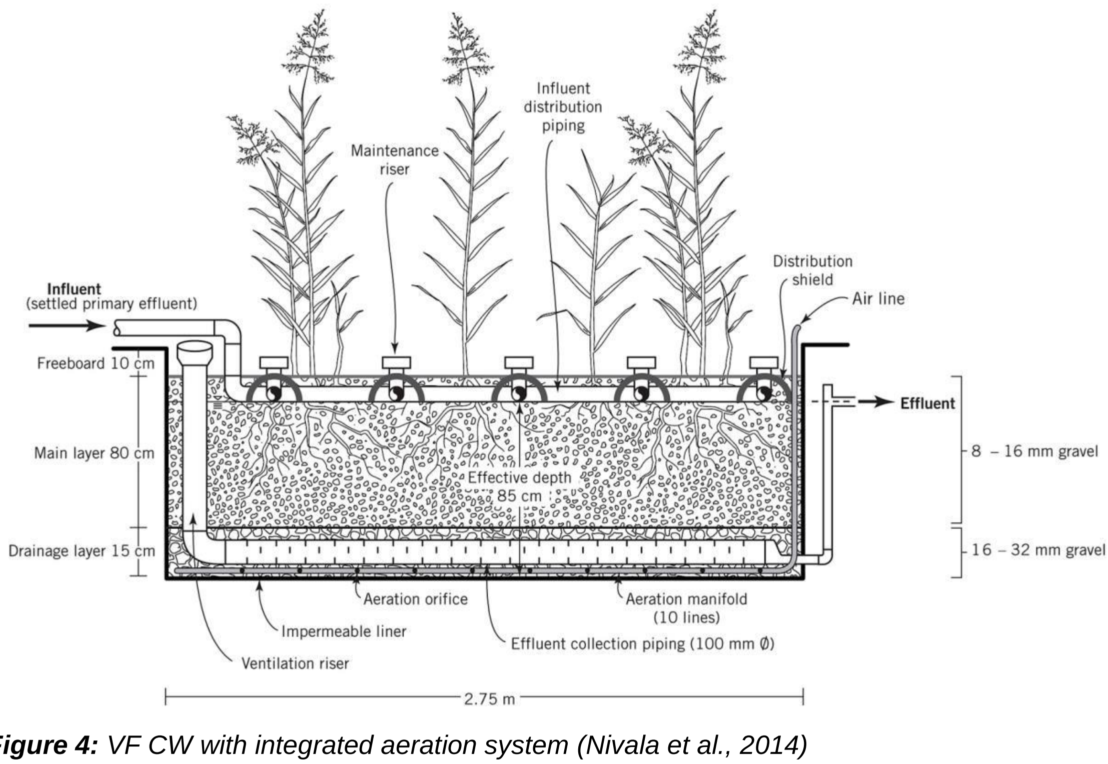


Figure 4: *VF CW with integrated aeration system (Nivala et al., 2014)*

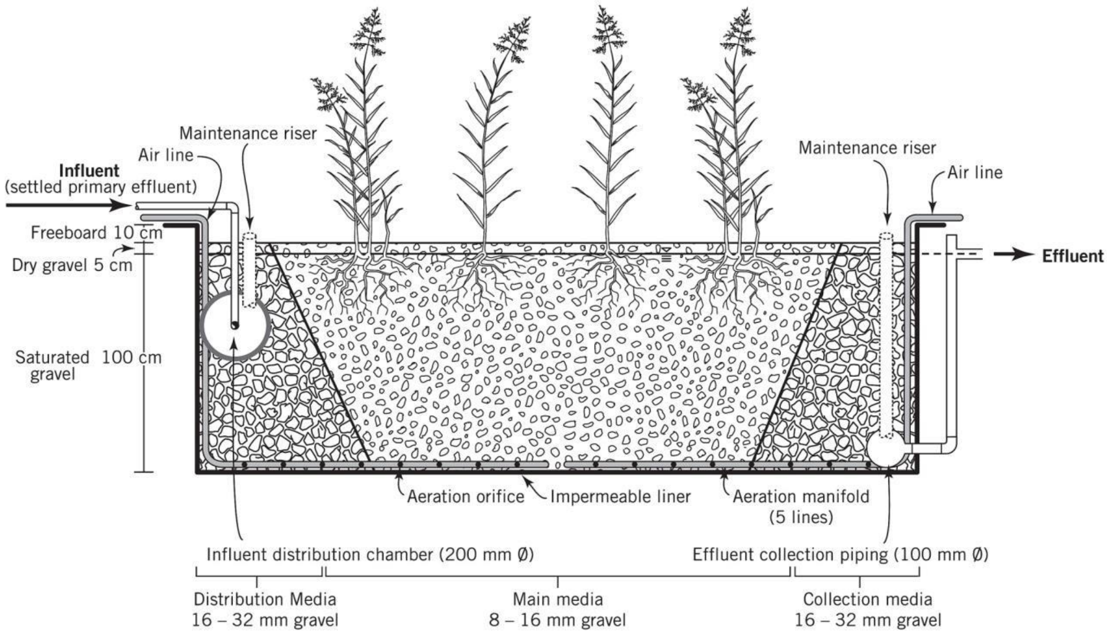


Figure 5: *HF CW with integrated aeration system (Nivala et al., 2014)*

In the 1970s and 1980s, CWs were nearly exclusively built to treat domestic or municipal sewage. Since the 1990s, the CWs have been used for all kinds of wastewater including landfill leachate, runoff (e.g. urban, highway, airport and agricultural), food processing (e.g. winery, cheese and milk production), industrial (e.g. chemicals, paper mill and oil refineries), agriculture farms, mine drainage and sludge dewatering (Vymazal, 2005a).

The processes in the CWs for freshwater treatments are relatively well known, but problems with high salinity of wastewater and efficiency of CWs require further investigation (Plaster, 2003; Novak and Trapp, 2005; Novak, 2005). Salt in wastewater influences all major parts of CW: plants, microorganisms and soils.

2.2 Theory Underlying Wastewater Treatment in a Constructed Wetland

The CW is a complex assemblage of water, substrate, plants (vascular and algae), litter (primarily fallen plant material), invertebrates (mostly insects larvae and worms) and an array of microorganisms (most important bacteria). Their treatment activity to purify wastewater relies upon natural microbial, biological, physical and chemical processes. The mechanisms that are available to improve water quality are therefore numerous and often interrelated. Those mechanisms include (Kadlec *et al.*, 2000):

1. Settling of suspended particulate matter;
2. Filtration and chemical precipitation through contact of the water with the substrate and litter;
3. Chemical transformation;
4. Adsorption and ion exchange on the surfaces of plants, substrate, sediment and litter;
5. Breakdown, transformation and uptake of pollutants and nutrients by microorganisms and plants;
6. Natural consumption and die-off of pathogens.

The chemistry of the water directly influences the biological process (such as microbial activity and photosynthesis of emergent plants). The physical features of the wetland also have a strong influence on both the chemical and biological processes. These three factors (chemical, physical and biological) are constantly in a state of flux and change (Kadlec and Knight, 1996).

2.2.1 Plants in constructed wetlands

The purification process in wetlands is complex. The most obvious biological component of the habitat, the emergent macrophytes, plays an important, role in the treatment of wastewater (Stephenson 1980; Nichols 1983; Gray 2004). The major removal mechanisms are bacterial and fungal transformations, and physical-chemical processes such as adsorption, precipitation and sedimentation (Chan *et al*., 1982). The plant rhizomes provide a stable surface for heterotrophic growth as well as enhancing sedimentation of solids by ensuring flocculation and maintaining near quiescent conditions. Plants have an effect on water quality that partially results from their effect on bacterial assemblages (Collins et al., 2004). The presence of plants in CWs enhances microbial diversity and activity and, additionally, the plant species root morphology and development seems to be a key factor influencing microbial plant interaction (Gagnon *et al.*, 2007). Emergent macrophytes dominate wetlands due to a hostile root environment, which has a restricted oxygen supply allowing facultative and anaerobic bacteria to flourish (Armstrong, 1982). The plants are able to translocate oxygen from their shoots to the roots making the rhizosphere (the root zone) an area where aerobic microorganisms can survive (Figure 6) (Mendelsson and Postek, 1982; Gray, 2004). Heterotrophic and nitrifying bacteria flourish with nitrates diffusing to oxygen limited zones (anoxic) of the wetland where they are removed from the system by denitrification (Sherr and Payne, 1978; Iizumi *et al.*, 1980; Gersberg *et al.*, 1986). Nutrients are trapped in wetlands, which are often referred to as nutrient sinks, not only in the sediment but in the actual plant biomass by nutrient uptake. Kadlec *et al.* (2000) concluded that macrophyte plants are essential in wetland treatment systems because they provide structure and a source of reduced carbon for the microbes that mediate most of the pollutant transformations that occur in wetlands. Organic matter accumulation in some wetlands is a direct or indirect result of the primary fixation of carbon from the atmosphere by plants. Wetland macrophytes further modify the texture, the hydraulic conductivity and the chemistry of the soil by the growth of plant roots and rhizomes. These plant structures initially serve as pathways for increased gaseous diffusion into and out of the wetland sediments. Gas-filled aerenchyma in wetland plants provides significantly less diffusional resistance, allowing some oxidation of soils in the immediate vicinity of the roots (rhizosphere) and the diffusion of carbon dioxide, hydrogen sulphide and even methane back to the atmosphere through the plants (Kadlec *et al.*, 2000). The term macrophyte includes vascular plants having visually observable tissues. The wetland macrophytes are the dominant structural component of most wetland treatment systems.

A number of articles have addressed the role of plants in remediation of contaminated soils and ground waters (Paterson *et al.*, 1990; Shimp *et al.*, 1993; Schnoor *et al.*, 1995; Simonich and Hites, 1995; Watanabe, 1997; Chang and Corapcioglu, 1998) and described how plants promote by various processes remediation of a wide range of chemicals at toxic waste sites. These processes include:

1. Modifying the physical and chemical properties of contaminated soils;
2. Releasing root exudates, thereby increasing organic carbon;

3. Improving aeration by releasing oxygen directly to the root zone, as well as increasing the porosity of the upper soil zones;
4. Intercepting and retarding the movement of chemicals;
5. Effecting co-metabolic microbial and plant enzymatic transformations of recalcitrant chemicals;
6. Decreasing vertical and lateral migration of pollutants to ground water by extracting available water and reserving the hydraulic gradient.

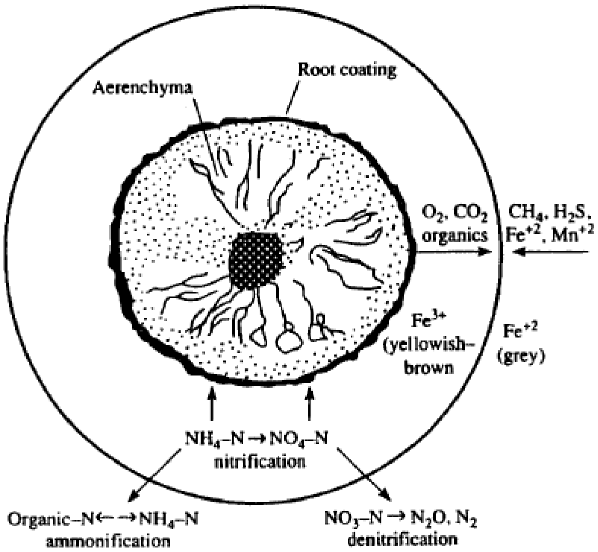


Figure 6: *Cross section of an oxidizing root growing in reduced sediment. The oxidized rhizosphere is depicted, along with some processes that occur as a result of this aerobic-anaerobic interface (Mendelsson and Postek, 1982)*

In many remediation projects, phytoremediation is seen as a final polishing step following the initial treatment of the high–level contamination. However, when contaminants are in low concentration, phytoremediation alone may be the most economical and effective remediation strategy (Jones, 1991; Susarala *et al.*, 2002).

Some of the factors affecting chemical uptake and distribution within living plants include:

1. Physical and chemical properties of the compound (e.g. water solubility, vapour pressure, molecular mass and octanol-water partition coefficient);
2. Environmental characteristics (e.g. temperature, pH, organic matter and soil moisture content); and
3. Plant characteristics (e.g. type of root system and type of enzymes).

Some of the mechanisms used by plants to facilitate remediation include: phytoextraction, phytopumping, phytostabilization, phytotransformation / degradation, phytovolatilization and rhizodegradation (Susarala *et al.*, 2002).
The choice of macrophytes is an important issue in CWs, as they must survive the potentially toxic effects of the effluent as well as its variability. Regionally abundant macrophyte species are adapted to the local climatic and edaphic conditions. However, their performance under the environmental conditions imposed by the wastewater, such as salinity, pH, dissolved oxygen (DO) and contaminant concentrations are usually unknown (Mufarrege *et al.*, 2011).

2.2.2 Soil in constructed wetlands

Soil (substrate) texture and structure are particularly important when water infiltration is a design factor. Soil structure refers to the aggregation of soil particles into clusters of particles referred to as peds. Well-structured soils with large voids between peds will transmit water more rapidly than structureless soils of the same texture. Even fine-textured soils that are well structured can transmit large quantities of water. Earth moving and related construction activity can alter or destroy the *in situ* soil structure and significantly change the natural permeability (Crites *et al.*, 2006). Wastewater is purified as it percolates through the soil by a range of physical, chemical and biological processes. Suspended materials, including microorganisms, are physically filtered out of solution as the water percolates through the upper soil layer. Organic material trapped in the soil is rapidly utilized by the high density of heterotrophs present. The humus, silt and clay particles which comprise the soil provide a very large surface area for ion-exchange with mineral ions, especially cations, becoming strongly bonded on to soil particles. In practice, ion exchange results in a high removal efficiency of metals, phosphate and ammonium ions from the water. Any nitrogen present is normally fully utilized by either plant uptake and subsequent harvest, the nitrification-denitrification process or volatilization (Gray, 2004).

Wetlands soils have a high trapping efficiency for a variety of chemical constituents. They are retained within the hydrated soil matrix by forces ranging from chemical bonding to physical dissolution within the water of hydration. The combined phenomena are referred to as sorption. A significant portion of the chemical binding is cation exchange, which is the replacement of one positively charged ion, attached to the soil or sediment, with another positively charged ion. The humic substances found in wetlands contain large numbers of hydroxyl and carboxylic functional groups, which are hydrophilic and serve as cation-binding sites. Wetlands are ideal environments for chemical transformations because of the range of oxidation states that naturally occur in wetland soils. Free oxygen decreases rapidly with depth in most flooded soils because of the metabolism of microbes that consume organic matter in the soil and through the chemical oxidation of reduced substances (Kadlec *et al.*, 2000).

Burchell *et al.* (2007) mentioned that organic matter addition can provide a carbon and nutrient source to the wetland and early in its development enhance denitrification and biomass growth. They found that increased organic matter addition, biosolids and dredged material blends significantly increased biomass growth in the second growing season when compared to no organic matter addition. Results of this research indicate that increased organic matter in the substrate would reduce the area required for in-stream CWs to treat drainage water in humid regions. It also serves as a demonstration of how dredged material can be used successfully in CWs, as an alternative to costly storage.

The choice of substrate in CWs is important as it serves as the support for the living organisms and provides storage for many contaminants. Its permeability affects the wastewater flow through the CW, and it is where chemical and biological transformations, by microorganisms and plants, occur (USEPA, 1995). The substrates can be natural, such as gravel, sand and organic materials including compost and waste material (USEPA, 1995; Korkusuz, 2005). Typical effective sizes of the media for subsurface flow CWs vary between 2 and 128 mm and porosity varies between 28% and 45% (USEPA, 2000). A porous media may be an interesting option since it provides greater surface area for treatment contact and for biofilm development (Calheiros *et al.*, 2008).

2.2.3 Microorganisms in constructed wetlands

These tiny organisms compete for sometimes limited and rapidly shifting supplies of energy-containing compounds and nutrients, and their growth and death have a very significant effect on the fate and transport of the majority of soil chemical constituents (Kadlec and Knight, 1996). Important transformations of nitrogen, iron, sulphur and carbon result from microbial processes. These microbial processes are typically affected by the concentrations of reactants as well as the redox potential and pH of the soil (Kadlec *et al.*, 2000).

Kadlec and Knight (1996) wrote that important chemical transformations in CWs are conducted by microbes. Functional parameters used to assess microbial activity include the rates of appearance or loss of chemical constituents of concern, such as dissolved oxygen (DO), ammonium ion, nitrate ion and organic carbon. One of the most common measurements of microbial activity is the BOD_5 test (APHA, 1990), which measures the amount of lost oxygen in a water sample under controlled temperature conditions (Kadlec and Knight, 1996).

Microbial assemblages can be found as a biofilm on substrate and root surfaces (Gagnon *et al*., 2007). Many parameters affect biofilm structure, especially nutrient availability or other environmental conditions (Kierek-Pearson and Karatan, 2005). Detailed knowledge about the microbial assemblages is needed to understand and explain the CWs functioning and thus the phytoremediation processes.

2.2.4 Biogeochemical processes in constructed wetlands

For better understanding of physical and chemical transformations and processes which occur in CWs, it is necessary to know more about biogeochemical processes in CW.

2.2.4.1 Oxygen

Because wetlands are associated with waterlogged soils, the concentration of oxygen within sediments and the overlying water is of critical importance. The rate of oxygen diffusion into water and sediment is slow and coupled with microbial and animal respiration leads to near anaerobic sediments within many wetlands (Moss, 1998). These conditions favour rapid peat build up, since decomposition rates and inorganic content of soils are low. Furthermore, the lack of oxygen in such conditions affects the aerobic respiration of plant roots and influences plant nutrient availability. Wetland plants have consequently evolved to have the ability to exist in anaerobic soils.

While the deeper sediments are generally anoxic, a thin layer of oxidised soil usually exists at the soil-water interface. The oxidised layer is important, since it permits the oxidised forms of prevailing ions to exist. This is in contrast to the reduced forms occurring at deeper levels of soil. The state of reduction or oxidation of iron, manganese, nitrogen and phosphorus ions determines their role in nutrient availability and also toxicity. The presence of oxidised ferric ion (Fe^{3+}) gives the overlying wetland soil a brown coloration, whilst reduced sediments have undergone 'gleying', a process by which ferrous iron (Fe^{2+}) gives the sediment a blue-grey tint.

Therefore, the level of reduction of wetland soils is important in understanding the chemical processes that are most likely to occur in the sediment and influence the overlying water column. The most practical way to determine the reduction state is by measuring the redox potential, also called the oxidation-reduction potential, of the saturated soil or water. The redox potential quantitatively determines whether a soil or water sample is associated with a reducing or oxidizing environment. Reduction is the release of oxygen (or hydrogen) and gain of an electron, while oxidation is the reverse; i.e. the gain of oxygen and loss of an electron. This is shown by equation (1), and explained in detail by Mitsch and Gosselink (2000).

$$E_H = E^0 - (RT/nF) \ln Q \qquad (1)$$

where,

E_H = redox potential (hydrogen ion scale);

E^0 = potential of reference (standard potential at 298ºK) (V);

R = gas constant = 8.314472 $JK^{-1}mol^{-1}$;

T = temperature (K);

n = number of moles of electrons transferred;

F = Faraday constant (the charge per a mole of electrons) = 9.6485309 x 10^4 ($Cmol^{-1}$ = $JV^{-1}mol^{-1}$);

Q = $\frac{C^c D^d}{A^a B^b}$, where the uppercase letters are the concentrations and the lowercase letters are the stoichiometric coefficients for the reaction: aA + bB → cC +dD.

Oxidation (and therefore decomposition) of organic matter (a very reduced material) occurs in the presence of any electron acceptor, particularly oxygen, although NO_3^-, Mn^{4+}, Fe^{3+} and SO_4^{2-} are also commonly involved in oxidation, but the rate will be slower in comparison with oxygen. A redox potential range between +400 mV and +700 mV is typical for environmental conditions associated with free dissolved oxygen. Below +400 mV, the oxygen concentration will begin to diminish and wetland conditions will become increasingly more reduced (<-400 mV).

Redox potentials are affected by pH and temperature, which influences the range at which particular reactions occur. The following thresholds are therefore not definitive:

1. Once wetland soils become anaerobic, the primary reaction at approximately +250 mV is the reduction of nitrate (NO_3^-) to nitrite (NO_2^-), and finally to nitrous oxide (N_2O) or free nitrogen gas (N_2).
2. At about +225 mV, Mn^{4+} is reduced to manganous compounds. Under further reduced conditions, ferric iron becomes ferrous iron between approximately +100 mV and -100 mV, and sulphates become sulphides between approximately -100 and -200 mV.
3. Under the most reduced conditions (<-200 mV) the organic matter itself and/or carbon dioxide will become the terminal electron acceptor. This results in the formation of low molecular weight organic compounds and methane gas (CH_4).

The redox capacity of a subsurface system can have important ecological and biogeochemical implications. We can consider two examples where redox capacity may control the biogeochemistry of a geologic system. The ecology of specific microbial communities is likely to be strongly dependent on the redox capacity. Geologic and aquatic systems that have high redox capacities provide relatively stable environments where specific microbial communities can flourish. As an example, consider a soil or sediment that has been contaminated with a crude oil. The addition of high concentrations of easily degradable aliphatic organic carbons will promote microbial activity, consuming oxygen and driving down the redox potential. This may limit the biodegradation of polycyclic aromatic hydrocarbons that generally require an aerobic environment for rapid biodegradation. A system with high redox capacity will be less affected than one with low redox capacity (Scholz, 2006).

2.2.4.2 Sulphur

In wetlands, sulphur is transformed by microbiological processes and occurs in several oxidation stages. Reduction may occur, if the redox potential is between -100 and -200 mV. Sulphides provide the characteristics "bad egg" odour of wetland soils. Assimilatory sulphate reduction is accomplished by obligate anaerobes such as *Desulfovibrio* spp. Bacteria may use sulphates as terminal electron acceptors in anaerobic respiration over a wide pH range, but utilization is highest around neutrality (Mitsch and Gosselink, 2000). The greatest loss of sulphur from freshwater wetland systems to the atmosphere is via hydrogen sulphide (H_2S). In oceans, however this is through the production of dimethyl sulphide from decomposing phytoplankton (Schlesinger, 1991). Oxidation of sulphides to elemental sulphur and sulphate can occur in the aerobic layer of some soils and is carried out by chemoautotrophic (e.g., *Thiobacillus* spp.) and photosynthetic microorganisms. *Thiobacillus* spp. may gain energy from the oxidation of hydrogen sulphide to sulphur, and by certain other species of the genus, by oxidation from sulphur to sulphate. Direct toxicity of free sulphide in contact with plant roots has been noted. There is a reduced toxicity and availability of sulphur for plant growth, if it precipitates with trace metals. For example, the immobilisation of zinc and copper by sulphide precipitation is well known. The input of sulphates to freshwater wetlands, in the form of Aeolian dust or as anthropogenic acid rain, can be significant. Sulphate deposited on wetland soils may undergo dissimilatory sulphate reduction by reaction with organic substrates. Protons consumed during this reaction generate alkalinity. This is illustrated by the increase in pH with depth in wetland sediments (Morgan and Mandernack, 1996). It has been suggested that this "alkalinity effect" can act as a buffer in acid rain affected lakes and streams (Rudd *et al.*, 1986; Spratt and Morgan, 1990).

In the present of light, photosynthetic bacteria, such as purple sulphur bacteria of salt marsh and mud flats, produce organic matter. This is similar to the familiar photosynthesis process, except that hydrogen sulphide is used as the electron donor instead of water (Scholz, 2006).

2.2.4.3 Carbon

Organic matter within wetlands is usually degraded by aerobic respiration or anaerobic processes (e.g., fermentation and methanogenesis). Anaerobic degradation of organic matter is less efficient than decomposition occurring under aerobic conditions.

Fermentation is the result of organic matter acting as the terminal electron acceptor (instead of oxygen as in aerobic respiration). This process forms low molecular weight acids (e.g., lactic acid), alcohols (e.g., ethanol) and carbon dioxide. Therefore, fermentation is often central in providing further biodegradable substrates for other anaerobic organisms in waterlogged sediments.

The sulphur cycle is linked with the oxidation of organic carbon in some wetlands, particularly in sulphur-rich coastal systems. Low-molecular weight organic compounds that result from fermentation (e.g., ethanol) are utilised as organic substrates by sulphur-reducing bacteria during the conversion of sulphate to sulphide (Mitsch and Gosselink, 2000).

Previous work suggests that methanogenesis is the principal carbon pathway in freshwater. Between 30 and 50% of the total benthic carbon flux has been attributed to methanogenesis (Boon and Mitchell, 1995; Scholz, 2006).

2.2.4.4 Nitrogen

Nitrogen has a complex biogeochemical cycle with multiple biotic/abiotic transformations involving seven valence states (+5 to −3). The compounds include a variety of inorganic and organic nitrogen forms that are essential for all biological life. The most important inorganic form of nitrogen in wetlands are the ammonium ion (NH_4^+), the nitrite (NO_2^-) ion and the nitrate (NO_3^-) ion. Gaseous nitrogen may exist as dinitrogen (N_2), nitrous oxide (N_2O), nitric oxide (NO_2 and N_2O_4) and ammonia (NH_3).

The various forms of nitrogen are continually involved in chemical transformations from inorganic to organic compounds and back from organic to inorganic (Figure 5). Some of these processes require energy (typically derived from an organic carbon source) to proceed, and others release energy that is used by organisms for growth and survival. All of these transformations are necessary for wetland ecosystems to function successfully, and most chemical changes are controlled through the production of enzymes and catalysts by the living organisms they benefit (Vymazal, 2007).

Ammonia volatilization is a physical and chemical process where ammonium-N is known to be in equilibrium between gaseous and hydrated forms. Reddy and Patrick (1984) pointed out that losses of NH_3 through volatilization from flooded soils and sediments are insignificant if the pH value is below 7.5 and very often losses are not serious if the pH is below 8.0.

Ammonification (mineralization) (Figure 7) is the process where organic N is biologically converted into ammonia. Ammonia is converted from organic forms through a complex, energy-releasing, multi-step, biochemical process. In some cases, this energy is used by microbes for growth, and ammonia is directly incorporated into microbial biomass. A large fraction (up to 100%) of the organic nitrogen is readily converted to ammonia (Kadlec and Knight, 1996).

Nitrification (Figure 7) is usually defined as the biological oxidation of ammonium ion to nitrate ion with nitrite ion as an intermediate in the reaction sequence. This definition has some limitations where heterotrophic microorganisms are involved but is adequate for the autotrophic and dominant species (Hauck, 1984). Nitrification has been typically associated with the chemoautotrophic bacteria, although it is now recognized that heterotrophic nitrification occurs and can be of significance. Nitrifiers prefer pH = 7.2 and higher (Paul and Clark, 1996). Nitrification is a chemoautotrophic process. The nitrifying bacteria derive energy from the oxidation of ammonia and/or nitrite and carbon dioxide is used as a carbon source for synthesis of new cells. Paul and Clark (1996) pointed out that Warrington, in 1878, at Rothamsted, United Kingdom, found that nitrification was a two-step process involving two groups of microorganisms. One microbial group oxidized ammonium-N to nitrite-N and another oxidized nitrite-N to nitrate-N (Paul and Clark, 1996; Schmidt *et al.*, 2001, 2003). The second step in the process of nitrification, the oxidation of nitrite to nitrate, is performed by facultative chemolitrotrophic bacteria which can also use organic compounds, in addition to nitrite, for the generation of energy for growth. In contrast with the ammonia-oxidizing bacteria, only one species of nitrite-oxidizing bacteria is found in the soil and freshwater, i.e., *Nitrobacter winogradskyi* (Grant and Long, 1981).

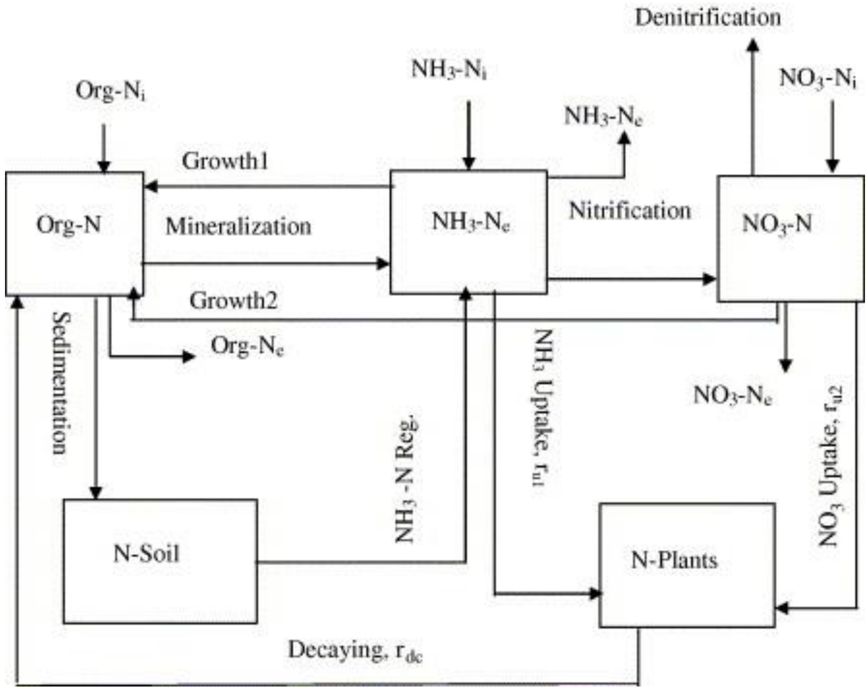


Figure 7: *Nitrogen transformation in horizontal subsurface flow constructed wetlands (Mayo and Bigambo, 2005)*

Denitrification (Figure 7) is most commonly defined as the process in which nitrate ion is converted into dinitrogen via intermediates nitrite ion, nitric oxide and nitrous oxide (Hauck, 1984; Paul and Clark, 1996; Jetten *et al.*, 1997). From a biochemical viewpoint, denitrification is a bacterial process in which nitrogen-oxygen species (in ionic and gaseous forms) serve as terminal electron acceptors for respiratory electron transport. Electrons are carried from an electron-donating substrate (usually, but not exclusively, organic compounds) through several carrier systems to a more oxidized form of nitrogen. The resultant free energy is conserved in ATP, following phosphorylation, and is used by the denitrifying organisms to support respiration. This reaction is irreversible, and occurs in the presence of available organic substrate only under anaerobic or anoxic conditions (Eh = +350 to +100 mV), where nitrogen is used as an electron acceptor in place of oxygen. More and more evidence is being provided from pure culture studies that nitrate ion reduction can occur in the presence of oxygen. Hence, in waterlogged soils nitrate ion reduction may also start before the oxygen is depleted (Kuenen and Robertson, 1987; Laanbroek, 1990). Diverse organisms are capable of denitrification. In an array are organotrophs, lithotrophs, phototrophs, and diazotrophs (Paul and Clark, 1996). Most denitrifying bacteria are chemoheterotrophs. They obtain energy solely through chemical reactions and use organic compounds as electron donors and as a source of cellular carbon (Hauck, 1984). The genera *Bacillus*, *Micrococcus* and *Pseudomonas* are probably the most important in soils; *Pseudomonas*, *Aeromonas* and *Vibrio* in the aquatic environment (Grant and Long, 1981). When oxygen is available, these organisms oxidize a carbohydrate substrate to CO_2 and H_2O (Reddy and Patrick, 1984). Aerobic respiration using oxygen as an electron acceptor or anaerobic respiration using nitrogen for this purpose is accomplished by the denitrifiers with the same series of electron transport system. This facility to function both as an aerobe and as an anaerobe is of great practical importance because it enables denitrification to proceed at a significant rate soon after the onset of anoxic conditions (redox potential of about 300mV) without change in microbial population (Hauck, 1984). Environmental factors known to influence denitrification rates include the absence of O_2, redox potential, soil moisture, temperature, pH value, presence of denitrifiers, soil type, organic matter, nitrate concentration and the presence of overlying water (Focht and Verstraete, 1977; Vymazal, 1995). Paul and Clark (1996) reported that the denitrifiers operate the best in the range $6.5 < pH < 7.5$.

Nitrogen fixation is the conversion of gaseous nitrogen (N_2) to ammonia. Nitrogen fixation requires nitrogenase, an oxygen-sensitive iron-, sulfur- and molybdenum-containing enzyme complex which also brings about the reduction of other substrates containing triple covalent bonds (e.g., nitrous oxide, cyanides or acetylene) (Stewart, 1973). In wetland soils, biological N_2 fixation may occur in the floodwater, on the soil surface, in aerobic and anaerobic flooded soils, in the root zone of plants, and on the leaf and stem surfaces of plants (Buresh *et al.*, 1980). It has been reported that greatest nitrogen-fixing activity in a flooded soils was found within the redox range from −200 to −260 mV (Buresh *et al.*, 1980). Fixation rates in wetlands receiving wastewater high in nitrogen are therefore probably much lower or essentially negligible compared to other nitrogen transformation processes (Vymazal, 2007).

Nitrogen assimilation refers to a variety of biological processes that convert inorganic nitrogen forms into organic compounds that serve as building blocks for cells and tissues. The two forms of nitrogen generally used for assimilation are ammonia and nitrate nitrogen. Because ammonia nitrogen is more reduced energetically than nitrate, it is preferable source of nitrogen for assimilation (Kadlec and Knight, 1996). In nitrate-rich waters, nitrate may become a more important source of nutrient nitrogen. Macrophyte growth is not the only potential biological assimilation process: microorganisms and algae also utilize nitrogen. Ammonia is readily incorporated into amino acids by many autotrophs and microbial heterotrophs. Nutrients are assimilated from the sediments by emergent and rooted floating-leaved macrophytes, and from the water in the free-floating macrophytes. The potential rate of nutrient uptake by a plant is limited by its net productivity (growth rate) and the concentration of nutrients in the plant tissue (Vymazal, 2007). Nutrient storage (standing stock) is similarly dependent on plant tissue nutrient concentrations, and also on the ultimate potential for biomass accumulation: that is, the maximum standing crop. Therefore, desirable traits of a plant used for nutrient assimilation and storage would include rapid growth, high tissue nutrient content, and the capability to attain a high standing crop (Reddy and DeBusk, 1987). In the literature, there are many reviews on nitrogen concentrations in plant tissue as well as nitrogen standing stocks for plants found in natural stands and CWs (e.g., Reddy and DeBusk, 1987; Vymazal, 1995; Vymazal *et al.*, 1999; Mitsch and Gosselink, 2000).

Ionized ammonia may be adsorbed from solution through a cation exchange reaction with detritus, inorganic sediments or soils. The adsorbed ammonia is bound loosely to the substrate and can be released easily when water chemistry conditions change. At a given ammonia concentration in the water column, a fixed amount of ammonia is adsorbed to and saturates the available attachment sites. When the ammonia concentration in the water column is reduced (e.g., as a result of nitrification), some ammonia will be desorbed to regain the equilibrium with the new concentration. If the ammonia concentration in the water column is increased, the adsorbed ammonia also will increase. If the wetland substrate is exposed to oxygen, perhaps by periodic draining, sorbed ammonium ion may be oxidized to nitrate ion (Kadlec and Knight, 1996). The Freundlich equation can be used to pilot plant ammonia sorption on the substrate (Sikora *et al.*, 1995). Ammonium ion (NH_4^+) is generally adsorbed as an exchangeable ion on clays, and chemisorbed by humic substances, or fixed within the clay lattice. It appears that these reactions may occur simultaneously. The rate and extent of these reactions are reported to be influenced by several factors, such as nature and amount of clays, alternate submergence and drying, nature and amount of soil organic matter, period of submergence and presence of vegetation (Savant and DeDatta, 1982). Some fractions of the organic nitrogen incorporated in detritus in a wetland may be eventually become unavailable for additional nutrient cycling through the process of peat formation and burial. The values of organic nitrogen burial have been reported for various natural wetlands, however, in CWs there are practically no data available (Vymazal, 2007).

Anaerobic ammonium oxidation (ANAMMOX) is the anaerobic conversion of NO_2^- and NH_4^+ to N_2 (Mulder *et al.*, 1995). It was demonstrated that in the ANAMMOX process, nitrate ion was used as an electron acceptor. During further examination of this process indications were obtained that also nitrite could serve as a suitable electron acceptor for ANAMMOX process (van de Graaf *et al.*, 1995). More recently, it has become clear that nitrite ion is the key electron acceptor (Strous *et al.*, 1997). The detailed biochemistry of the process is still under investigation in laboratory experiments and wastewater treatment plants (e.g., Schalk *et al.*, 2000; Schmidt *et al.*, 2003; Strous and Jetten, 2004). Research is needed to better understand how the microbes and the ammonia oxidizing reactions compete in the ecology of varied wetland systems (Hunt *et al.*, 2005). Mechanisms that ultimately remove nitrogen from wastewaters include ammonia volatilization, denitrification, plant uptake (with biomass harvesting), ammonia adsorption, ANAMOX and organic nitrogen burial. Other processes (e.g., ammonification or nitrification) “only” convert nitrogen among various nitrogen forms but do not actually remove nitrogen from the wastewater. Also, not all the processes occur in all types of CWs (Table 1) and the magnitude of individual processes varies among types of CWs (Vymazal, 2007).

Table 1: *Potential magnitude of nitrogen transformations in various types of CWs (Vymazal, 2007)*

	FFP	**FWS**	**HF**	**VF**
Volatilization	**Low**	**Medium**	**Zero**	**Zero**
Ammonification	High	High	High	High
Nitrification	Low	Medium	Very low	Very high
Nitrate-ammonification	??	??	??	??
Denitrification	**Medium**	**Medium**	**Very high**	**Very low**
N_2 fixation	??	??	??	??
Microbial uptake	Low	Low	Low	Low
Plant uptake[a]	**Medium**	**Low**	**Low**	**Low**
Ammonia adsorption	Zero	Very low	Very low	Very low
Organic nitrogen burial	**Very low**	**Low**	**Low**	**Very low**
Fragmentation and leaching	??	??	??	??
ANAMMOX	??	??	??	??

Legend: [a] With harvest; Processes that ultimately remove total nitrogen from wastewater are in bold.
FFP- free-floating plants; FWS- free water surface; (HF) horizontal flow; (VF) vertical flow; CW- constructed wetland

When comparing inflow loading of CWs (Table 2) and aboveground standing stocks for emergent macrophytes, it is obvious that the amount of nitrogen removed via harvesting is quite low and usually does not exceed 10% of the inflow load for secondary treatment systems. When inflow loading is low (cca <100–200 gN/m^2yr) as in the case of tertiary treatment, then removal via harvesting may be important. It is also important to take into account the fact that standing stock is limited and does not increase with increasing loading rate in CWs for wastewater treatment. The results presented in Table 2 clearly indicate that singlestage CWs are not able to remove substantial amounts of total nitrogen unless it is achieved at the expense of a large treatment area and, therefore, hybrid systems may be a better solution when total nitrogen is the main target value (Vymazal, 2007).

Table 2: *Removal of ammonia-N, nitrate-N and TN in various types of CWs (mean values) (Vymazal, 2001, 2005 a,b, 2007)*

CW type		**Inflow**	**Outflow**	**Efficiency (%)**
	Concentration (mg/l)			
FWS	NH_4-N	12.9	5.8	55.1
	NO_3-N	5.6	2.2	60.7
	TN	14.3	8.4	41.2
HF	NH_4-N	38.9	20.1	48.3
	NO_3-N	4.4	2.9	38.5
	TN	46.6	26.9	42.3
VF	NH_4-N	55.0	8.7	84.2
	NO_3-N	0.7	24.4	
	TN	68.4	37.9	44.6

FWS- free water surface; HF – horizontal flow; VF - vertical flow; CW- constructed wetland

2.2.4.5 Phosphorus

Phosphorus in wetlands occurs as phosphate in organic and inorganic compounds. Free orthophosphate is the only form of phosphorus believed to be utilized directly by algae and macrophytes and thus represents a major link between organic and inorganic phosphorus cycling in wetlands. Another group of inorganic phosphorus compounds are linearly condensed and cyclic polyphosphates. Organically-bound phosphorus is present e.g., in phospholipids, nucleic acids, nucleoproteins, phosphorylated sugars or organic condensed polyphosphates (coenzymes, ATP, ADP) (Vymazal, 1995). Organic P forms can be generally grouped into easily decomposable P (nucleic acids, phospholipids, sugar phosphates) and slowly decomposable organic P (inositol phosphates, phytin) (Dunne and Reddy, 2005). Wetlands provide an environment for the interconversion of all forms of phosphorus. Soluble reactive phosphorus is taken up by plants and converted to tissue phosphorus or may become sorbed to wetland soils and sediments. Organic structural phosphorus may be released as soluble phosphorus if the organic matrix is oxidized. Insoluble precipitates form under some circumstances, but may re-dissolve under altered conditions (Kadlec and Knight, 1996). Phosphorus transformations in wetlands are: peat/soil accretion, adsorption/desorption, precipitation/dissolution, plant/microbial uptake, fragmentation and leaching, mineralization and burial. Thus, when evaluating a wetland ecosystem to retain P, all these components should be quantified. Richardson and Marshall (1986) found that soil adsorption and peat accretion control long-term phosphorus sequestration in wetlands. However, sorptions as well as storage in biomass are saturable processes, meaning they have a finite capacity and therefore can not contribute to long-term sustainable removal (Dunne and Reddy, 2005).

Most studies on phosphorus cycling in wetlands have shown that soil/peat accumulation is the major long-term phosphorus sink and that natural wetlands are not particularly effective as a phosphorus sink when compared with terrestrial ecosystems (Richardson, 1985). The sediment-litter compartment is the major P pool (>95%) in natural wetlands, with much lower plant pool and little in the overlying water (Verhoeven, 1986; Richardson and Marshall, 1986) with cycling between pools controlled by biological forces (i.e., microbes and plants). The peat accumulation rate in peatlands is very slow, with the world average of accretion being only 1 to 2 mm per year (Craft and Richardson, 1993). Adsorption refers to movement of soluble inorganic P from soil porewater to soil mineral surfaces, where it accumulates without penetrating the soil surface. Phosphorus adsorption capacity of a soil generally increases with clay content or mineral components of that soil (Rhue and Harris, 1999). The balance between P adsorption and desorption maintains the equilibrium between the solid phase and P in soil porewater. This phenomenon is defined as phosphate buffering capacity, which is analogous to the pH buffering capacity of a soil (Barrow 1983; Rhue and Harris, 1999). In organic soils P adsorption has been related to either high Al, Fe or Ca levels and the P sorption capacity of wetland soils may be predicted solely from the oxalate-extractable (amorphous) aluminum content of the soil (Richardson 1985). The sorption of P by soil is controlled by the concentration of phosphate in soil porewater and the ability of the solid phase to replenish phosphate into soil porewater. When soil particles become saturated with P, and soil porewater has low concentrations of P, there is a net movement of P from soil to soil porewater until an equilibrium between soil and soil porewater P concentrations is established. Sorption is generally described as a two-step process: 1) phosphate rapidly exchanges between the soil porewater and soil particles or mineral surfaces (adsorption), and 2) phosphate slowly penetrates into solid phases (absorption). Similarly, desorption of P can also occur in a two-step process (Dunne and Reddy, 2005). One of the proposed mechanisms for the release of phosphorus from soils upon submergence is the reductive dissolution of Fe (III) and Mn (IV) phosphate minerals (Patrick *et al.,* 1973). Anaerobic soils release more phosphate to soil solutions low in phosphate and sorbed more phosphate from soil solutions high in soluble phosphate than do aerobic soils (Patrick and Khalid, 1974). The difference in behavior of phosphate under aerobic and anaerobic conditions is attributed to the change brought about in ferric oxyhydroxide by soil reduction. The probably greater surface of the gel-like reduced ferrous compounds in an anaerobic soils results in more soil phosphate being solubilized when the solution phosphate is low and more solution phosphate being sorbed when the solution phosphate is high (Patrick and Khalid, 1974). Precipitation can refer to the reaction of phosphate ions with metallic cations such as Fe^{2+}, Al^{3+}, Ca^{2+} or Mg^{2+}, forming amorphous or poorly crystalline solids. These reactions typically occur at high concentrations of either phosphate or the metalloid cations (Rhue and Harris, 1999).

Microbial uptake of phosphorus is very fast, but the magnitude (amount stored) is very low. The uptake by microbiota (bacteria, fungi, algae, microinvertebrates, etc.) is likewise rapid because these organisms grow and multiply at high rates. It seems that the amount of microbial storage depends also on trophic status of the wetland. In less enriched sites the microbial uptake may store more phosphorus as compared to more eutrophic sites (Richardson *et al.*, 1997). Soil microorganisms participate in the solubilization of soil P. Of importance, and seldom recognized, is the amount of P that can be sequestered by the algal component of wetlands, especially in areas with open water. Vymazal (1995) pointed out that the role of algae in wetlands is mostly neglected despite the fact that algae can significantly influence the nutrient cycling in wetlands. In addition, attention is usually paid only to attached forms (periphyton) while the role of plankton in wetlands has been assessed only rarely. Algae and algal assemblages can affect phosphorus cycling either directly (uptake, release) or indirectly through photosynthesis-induced changes in water and soil/water interface parameters (pH, dissolved oxygen).

Most of the phosphorus is taken up by plant roots, absorption through leaves and shoots is restricted to submerged species but this amount is usually very low. Phosphorus uptake by macrophytes is usually highest during the beginning of the growing season (in most regions during the early spring), before maximum growth rate is attained (Boyd, 1969; Vymazal, 1995). Biomass increases, however, should not be counted as part of the long-term sustainable phosphorus removal capacity of wetlands (Kadlec and Knight, 1996). An important response to seasons is the translocation of nutrients within the plant. Prior to autumn senescence, the majority of important ions are translocated from shoot portions to the roots and rhizomes. These stored nutrients are used during early spring growth (Dykyjová and Květ, 1978; Garver *et al.*, 1988). Phosphorus storage in vegetation can range from short- to long-term, depending on type of vegetation, litter decomposition rates, leaching of P from detrital tissue, and translocation of P from above- to below-ground biomass.

It is clear that the extent to which particular mechanisms are involved depends on the type of CWs. Also, it has been well established that the mechanisms that remove P in CWs include only sorption on antecedent substrates, storage in biomass, and the formation and accretion of new sediments and soils (Kadlec and Knight, 1996). Peat/soil accretion is the major long-term phosphorus sink in wetlands but it could be effective only in treatment wetlands with high production of biomass and water overlying the sediment as it is the case of free water surface CWs with emergent vegetation. Adsorption and precipitation of phosphorus is effective in systems where wastewater comes in contact with the filtration substrate. It means that CWs with SSF have the major potential for phosphorus removal via these mechanisms. HF systems have higher potential adsorption and precipitation because the substrate is constantly flooded and there is not much fluctuation in redox potential in the bed. VF systems, where wastewater is fed intermittently, may not be as effective because the oxygenation of the bed may cause desorption and subsequent release of phosphorus. However, materials which are commonly used for SSF CWs i.e., washed gravel or crushed rock, usually provide very low capacity for sorption and precipitation. Recently, several filtration materials such as light weight clay aggregates (LECA) have been tested in CWs. The removal of phosphorus is very high (Vohla *et al.*, 2005; Jenssen and Krogstad, 2003) but it is important to realize that sorption and precipitation are saturable and the sorption decreases over time. Microbial uptake is considered in all treatment systems only as temporary storage of phosphorus with very short turnover rate. Phosphorus which is taken up by microbiota is released back to the water after the decay of the organisms. Similar to the removal of nitrogen, due high inflow loadings, the amount of P removed via harvesting of emergent macrophytes is low but could be important in CWs which have low inflow loading (cca <10–20 gP/m^{2}yr). Plant uptake is a more important route of phosphorus removal in systems with free-floating macrophytes. However, it is important to develop an efficient harvesting frequency in order to keep macrophytes at the optimum growth stage to ensure optimum phosphorus removal.

2.3 Comparison of Wastewater Treatment in a Constructed Wetland to Other Methods of Wastewater Treatment with Respect to Efficiency, Speed, Cost, Size

Though the same biological processes are the basis for most wastewater treatment systems (WWT), the number of technological solutions for achieving the goal probably is innumerable. The numbers of techniques are probably as many as there are sanitary engineers. However, the techniques may be categorized as follows:

1. soil filters and wetlands – terrestrial ecosystems working as natural filters; natural water courses, lakes, and wetlands; soils receiving irrigation wastewater; constructed wetland and ponds; soil or sand absorption systems; and trickling filters; and
2. treatment plants – rotating biological contactors; fluidized beds; and activated sludge systems including sequencing batch reactors (SBRs).

This array of techniques, presented in Table 3, describes the systems on a scale from natural ecosystems at one end to high-technology solutions at the other hand. In the choice of WWT to be used, many factors have to be considered like influent water characteristics, desirable effluent water quality, costs for building and maintenance, and population density and dimensioning (Pell and Worman, 2008).

Table 3: *Technologies for wastewater management (with relative costs, environmental impact and maintenance requirement) (UNEP, 2010)*

Wastewater management technologies

Technology	Capital cost	Operation & maintenance cost	Environmental impact
On-site technology			
Pit latrine	Low	Low	Pollution of groundwater
Composting toilet	Low	Low	Reuse of nutrients
Pour flush toilet	Low	Low	Pollution of groundwater
Improved on site treatment unit	Medium to high	Low to medium	Reuse of water and nutrients
Off-site technology			
Collection technology			
Conventional sewerage	High	High	Dependent on treatment
Simplified sewerage	Medium to high	Medium	Dependent on treatment
Settled sewerage	Medium	Low	Dependent on treatment
Treatment technology			
Activated sludge	High	High	Nutrients may need removal
Trickling filtration	Medium	Medium	Nutrients may need Removal
Lagoons	Low to medium (dependent on cost of land)	Low	Nutrients may need removal; aquaculture can be incorporated
Land-based treatment	Low to medium (dependent on cost of land)	Low to medium	Reuse of water and nutrients
Constructed wetland	Low to medium (dependent on cost of land)	Low	Amenity value
Anaerobic treatment	Medium	Medium	Produces biogas; further aerobic treatment needed

Even though Table 3 does not cover all available technologies, they represent major technologies for situations that are likely to be encountered. The Regional Overviews include technologies that are modifications or variations of the listed technologies or represent practices or advances in the regions (UNEP, 2010). Comparison of different small wastewater treatment technologies are shown in Table 4.

Table 4: *Comparison of different small wastewater treatment technologies (Stich and Hirschfeld 2010)*

	Activated sludge	**Trickling filter with aeration**	**MBR***	**SBR***	**WSB***	**CW***
Average capital costs (EUR)	6500	6400	7600	5100	5300	7100
Energy consumption (kWh) from producer	77-260	44-68	180	93-100	32-114	10
Energy consumption (kWh) in practice	170	90	151	116	98	9
Maintenance costs/year (EUR)	230	200	300	300	180	140
Required area (m^2)	<10	<10	<5,	<10	<10	30
Maintenance costs/average (EUR)	150	<100	300	250	150	30
Device management	Satisfactory	Good	Good	Satisfactory	Good	Very good
Frost protection	Safe partly	Safe	Safe	Safe partly	Good	Very good
Additional equipment to be installed in 3-compartments septic tank	Yes	No	No	Yes	Yes	Yes
Total score	Good	Good	Satisfactory	Satisfactory	Very good	Very good

*SBR-sequentch biological reactor; MBR-membrane biological reactor; CW-constructed wetland; WSB clean-fluid bed biofilm process (WSB, 2011)

Natural treatment systems differ from conventional wastewater treatment systems in terms of sustainability. They use natural renewable energy, rely on atmospheric diffusion and/or photosynthesis as the major source of oxygen, and are constructed using a minimum of man-made materials. They provide silent, normally odour free, robust treatment processes. However, they require much larger areas of land than conventional systems (Table 5). Apart from adequately treating the wastewater, the use of plants results in the production of excess biomass, which can be used for a variety of purposes such as energy production, animal feed, and even protein production, thus offsetting the cost of treatment (Gray, 2004). According to Gray (2004), natural treatment systems are: land treatments, macrophyte-based systems and stabilization ponds. CWs are one of the macrophyte-based systems.

***Table 5:** Comparison of natural treatment systems with conventional wastewater treatment systems (Gray, 2004)*

	Natural treatment system	*Conventional treatment system*
Energy	Renewable, natural (solar, kinetic wind energy)	Non-renewable, fossil fuel derived electricity
Action of treatment	Microorganisms, plants, soil	Microorganisms
Prime construction materials	Soil, liner possibly required	Concrete, steel, plastic
Aeration	Atmospheric diffusion and/or photosynthesis	Power intense mechanical aeration
Layout	Dispersed	Compact
Chemical usage	None/low	Moderate/high
Land area required	High	Low
Noise	None/very low	High
Odour	Low	Moderate/high
Aerosol formation	None	High
Costs: Capital	Moderate	High
Operational complexity	Low	High
Controlability	Low/moderate	High
Sludge production	Low, on-site disposal	High, off-site disposal
Sustainability	High	Low
Environmental impact	Low	Moderate
Typical use	Rural, small populations	Urban, large populations

In Table 6 could be seen energy requirements for different types of wastewater treatment plants. It could be recognise difference of energy utilization between conventional SSF CW and aerated SSF CW.

***Table 6:** Energy comparison different wastewater treatments (Kadlec and Wallace, 2009)*

System	*Hydraulic Load (m^3/d)*	*Energy Utilizitaion (kWh/m^3)*	*Reference*
SF CW	-	< 0.1	Brix, 1999
SSF CW	-	< 0.1	Brix, 1999
Facultative Lagoon + Rapid Infiltration	3.786	0.11	Campell and Ogden, 1999
Facultative Lagoon + Overland Flow	3.786	0.16	Crites *et al.*, 2006
Aerated SSF CW	5.500	0.16	Wallace *et al.*, 2006
Tidal Flow (Fill-and-Drain) CW	1.000	0.18	Maciolek and Austin, 2006
Carrousel Oxidation Ditch	3.786	0.51	U.S. EPA, 1996
Trickling Filter + Nitrogen Removal	3.786	0.61	Crites *et al.*, 2006
Activated Sludge + Nitrification	3.786	0.76	Campell and Ogden, 1999
Extended Aeration Package Plant	3.786	1.06	U.S. EPA, 1996
Sequencing Batch Reactor	303	1.13	U.S. EPA, 1996
Living Machine	3.786	1.51	U.S. EPA, 1996

Vymazal (2007) wrote that CWs can successfully treat diluted wastewater with low concentrations of organics (BOD_5). While activated sludge based treatment plants require some minimum concentrations of BOD_5 (50-80 mg/l) to keep the activated sludge in healthy conditions. HF SSF (horizontal subsurface flow) CWs can treat wastewater with BOD_5, concentrations well below 20mg/l. They cope well with water quality and quantity fluctuations and HF SSF CWs can operate intermittently. Therefore they can be used for summer houses, camping sites, seasonal restaurants and recreational areas. They require much less maintenance, but regular maintenance is absolutely necessary. HF SSF CWs is a robust technology that only rarely fails. Also they fit nicely into the landscape. On the other hand, there are some disadvantages. HF SSF CWs require more land than conventional treatment systems. If the HF SSF CW is designed for removal of organics and suspended solids, then the removal efficiencies for ammonia and phosphorus will be very low. But VF CWs provide a good removal of organics, suspended solids and ammonia. On the other hand, VF CWs provide little room for denitrifcation and therefore ammonia–N is usually only converted to nitrate-N. As a consequence, removal of total nitrogen is low, usually lower than in HF CWs. Removal of phosphorus is also low in VF CWs unless special filtration material with high sorption capacity is used. One such medium, LECA (light-expanded clay aggregates) is successfully used, for example, in Norway, Estonia and Portugal and can remove more than 90% of the phosphorus from the sewage. However the sorption capacity is saturable and, therefore, the filtration material must be either replaced when saturated or the volume of the bed must be very high in order to maintain the sorption capacity over a long period of time. Large volumes of special filtration material and the relatively high cost of these materials make these systems very expensive as compared to VF CWs with conventional filtration materials such as sand or gravel.

Zhou *et al.* (2009) reported a comparative study on CW and conventional wastewater treatments with three representative cases in Beijing. Considering the environmental and economic inputs and treated wastewater output based on energy, different characteristics of two kinds of wastewater treatments were revealed. The results showed that CWs are environmentally-benign, less energy-intensive despite the relatively low ecological waste removal efficiency and less costly in construction, operation and maintenance compared with the conventional wastewater treatment plants. In addition, energy analysis has shown the cyclic activated sludge system has the higher ecological waste elimination efficiency.

Zhou *et al.* (2009) concluded that it is difficult to decide which kind of treatment is the best to be adopted since complicated factors, such as the characteristics of inflows, locations and available capital, must be considered. In general, CWs with less investment in construction materials are appropriate for the small towns or villages where land prices are cheaper and the influents are rich in agricultural wastewater with high TN and TP concentrations. In the urban areas, cyclic activated sludge system is more popular due to the high land prices and severely polluted sewages.

2.4. Effects of Salinity on Wastewater Treatment in a Constructed Wetland

2.4.1 Source of Salinity in Influent to Constructed Wetland

High salinity wastewaters are usually generated from industries such as seafood processing, vegetable canning, pickling, tanning and chemical manufacturing. In addition, seafood processing factories located in arid zones use treated seawater or reused or recycled water in processing steps such as defrosting, butchering and washing raw materials. Thus, the effluent from these industries contains high salinity, which is approximately the same as that of seawater. In addition, the adoption of waste minimization techniques within these industries has led to reductions in waste volume, with corresponding increases in the concentrations of waste (Woolard and Irvine, 1995).

The seafood processing sector contributes serious organic pollution loads and high salinity to receiving waters. This feature leads to difficulties in biological treatment processes (Mendez *et al*., 1992). The fish processing industry including cooking, brine filling operations and dried salted fish preparation, normally produces wastewater high in organic matter, oil and grease, and salt content. Typical water consumption ranges from 18 to 74 m^3/ton of fish processed (Battistoni and Fava, 1994). This wastewater contains very high salt contents, ranging from 17g to 46 gNaCl/l (Dan 2000; Dan, 2001). Salt is used widely in vegetable canning processing to enhance flavor, to preserve, or for conditioning. Therefore this industry in general produces wastewater containing high salt content. Brine waste from fermenting pickles contains high salt content (3 to 20%) and extremely high organic concentrations (Joslyn and Timmsons, 1967).

Hypersaline wastewaters are also produced in significant quantities in chemical industries such as oil and gas production. These wastes contain organic compounds and high concentrations of salt (> 3.5%) (Dalmacija *et al*., 1996; Dan, 2001).

Tannery wastewaters are also characterized by high content of salts, and are often strongly alkaline with a high oxygen demand and a high content of chromium (Bajza and Vrcek, 2001). Nowadays chrome tanning is favoured by the majority of the leather industry because of the speed of processing, low cost, colour of leather and greater stability of the resulting leather (Hafez *et al.*, 2002). However, uptake of the chromium into the leather is not complete and relatively large amounts are found in the effluent. Estimates range from 2000–3000 mg/l (Bajza and Vrcek, 2001) to 3–350 mg/l (Vlyssides and Israilides, 1997). In the leather sector certain streams of effluents may contain as much as 80 g/l of NaCl (Lefebvre and Moletta, 2006). If these wastewaters are not treated before discharge they can cause serious environmental pollution. Treatment of these wastewaters is expensive, so many poorer countries only employ an initial treatment. Primary treatment may employ biological, oxidation or physico-chemical processes. These treatments though often leave chromium levels in the wastewater above the legal discharge limit for surfaces waters, cases reported in Brazil (Alves *et al.*, 1993) as well as in Slovenia (OG RS 35/96, 21/03). Therefore chromium treatment is often required. Ion exchange resins (Kocaoba and Akcin, 2002), reverse osmosis (Hafez *et al.,* 2002) and an electrolysis system (Vlyssides and Israilides, 1997) have all been investigated as methods of further purification. These methods, however, are expensive and are often not considered cost effective for small sized tanneries. The phytoremediation of soils polluted with tannery effluent using trees with tolerant mycorrhizal fungi has been investigated by Khan (2001) and is thought to have potential (Mant *et al.*, 2006). Constructed wetlands are a feasible technology for the treatment of tannery wastewater as an alternative to conventional biological systems (Daniels, 2001; Kucuk *et al*., 2003; Calheiros *et al*., 2007). Treatment of tannery wastewater in CW has been reported recently (Calheiros *et al*., 2007, 2009b; Daniels, 2008). More recently, Kaseva and Mbuligwe (2010) demonstrated that CW can be used as an option for improving the quality of tannery effluents, especially in the removal of chromium, with TDS levels around 10,000 mg/l.

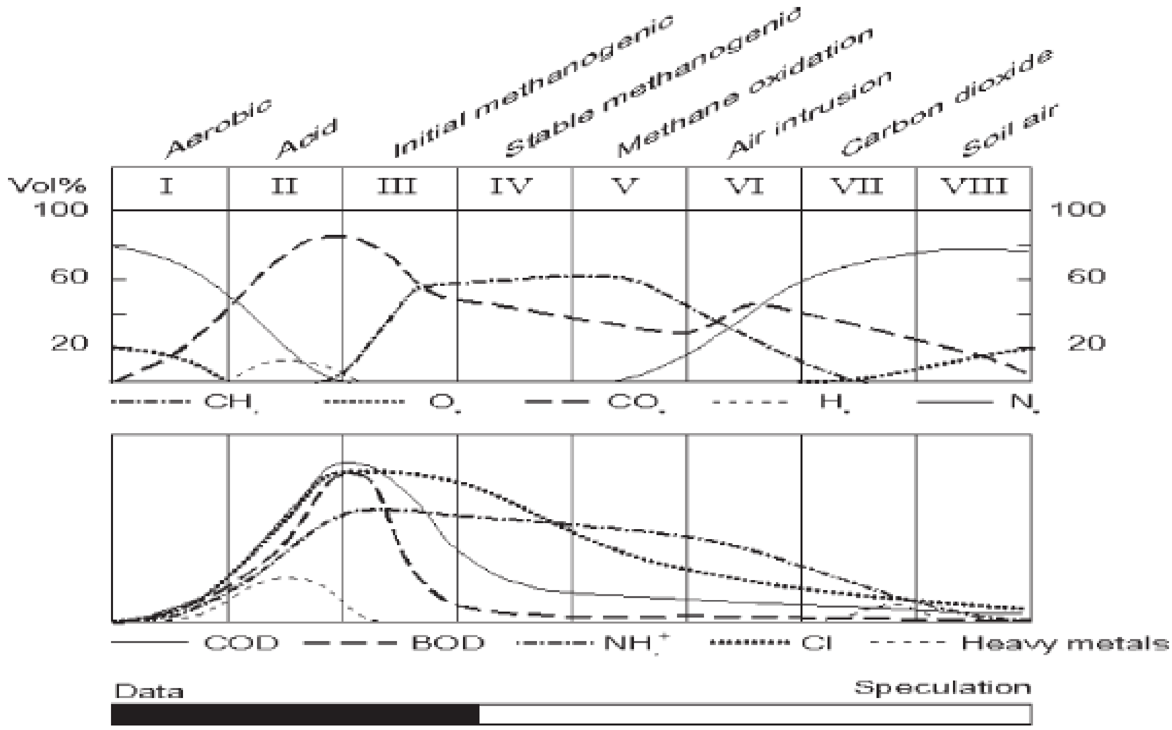
In coastal areas, when subsurface water rises, infiltration of saline water into sewers can result in high concentrations of chlorides and sulfates in wastewater. Therefore, large variation of salinity in domestic wastewater occurs normally in these areas. This can cause salt shocks or adverse effects on conventional biological treatment processes (Jones, 1991).

Salt has been used for decades to melt snow and ice from roads in order to improve traffic safety throughout winter. The negative effect is that salt application has a range of environmental impacts (Bulc and Vrhovšek, 1996; Lundmark, 2003). Pitt *et al*. (1999) concluded that chloride in storm water would likely have a high groundwater contamination potential where road salts are used.

High salinity is also found in landfill leachates. Pirbazari (1996) reported that the leachates from a domestic waste landfill (Los Angeles) and a hazardous waste landfill for chemical and petroleum waste (Niagara) had high concentrations of organic matter and high total dissolved solids (TDS).

Landfills were monitored for about 30 years by Kjeldsen *et al.* (2002). Throughout this period an increasing understanding of the complex series of chemical and biological reactions that take place with the burial of refuse in a landfill was developed. Figure 6 shows the gas and leachate composition as refuse decomposes. The figure is developed from the first description of the landfill phases given by Farquhar and Rovers (1973). The first four phases shown in the figure are referred to as the aerobic phase, the anaerobic acid phase, the initial methanogenic phase, and the stable methanogenic phase. Subsequent phases of decomposition, in which the waste cell begins to turn aerobic are based on theory and are somewhat speculative because no field data are available to document the onset of aerobic conditions (Christensen and Kjeldsen, 1995). This is due to the fact that most well monitored landfills are less than 30 years old and are still in the stable methanogenic phase. Figure 8 shows that high concentration of chloride ions, ammonium ions, COD and BOD in leachate could be observed, especially in acid and initial methanogenic phase (Kjeldsen *et al.*, 2002).

Mathewson and Mathewson (1998) concluded that a CW offered the most cost effective and environmentally sound technique to treat the landfill leachate.



Legend: COD = chemical oxygen demand; BOD = biological oxygen demand; NH_4^+= ammonium ions; Cl^- = chloride ions

Figure 8: *The lifetime of a landfill showing general trends in gas and leachate quality development (Kjeldsen et al., 2002)*

There are several experiences in Slovenia with treatment of MSW (municipal solid waste) landfill leachate in different types of CW (Table 7). Three of them have been monitored for longer periods (7-10 years). Generally, landfill leachates may contain very high concentrations of dissolved organic matter and inorganic macro components with the concentrations up to a factor 1000 to 5000 higher than concentrations found in groundwater (Kjeldsen *et al.*, 2002).

Table 7: *Characteristics of CW for leachate treatment in Slovenia (Bulc and Zupančič, 2007)*

CW for leachate treatment	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Avg. rainfall (mm/year)	1450	1450	1016	2215	900	843	1400	
The year of construction	1995	1996	1992	2004	1996	1995	1997	1989
Avg. hyd. load (cm/d)	0.5	0.7	3	4.6	2,3	3	1.6	4
CW area (m^2)	311	200	450	315	1000	600	600	600
Media type	washed gravel and sand 4-8 mm VF beds 4-16 mm HF beds		peat, soil, sand (1-8 mm) gravel (8-16 mm)	washed gravel, sand	sand and gravel (4-8, 8-16 mm) gravel 16-32 mm in the upper 10 cm of the beds	sand (1 - 8 mm) gravel (8 - 16 mm)		mixed gravel
Hydraulic conductivity	4.2×10^{-2} m/s VF beds 1.5×10^{-2} m/s HF beds			6.06×10^{-3} m/s 1st bed 3.48×10^{-3} m/s 2nd bed				5×10^{-4} m/s
Depth (m)	0.4-0.8	0.4-0.8	0.8	0.6	0.7	0.6-0.8	0.6-0.8	0.6
No of beds	3	3	2	2	6	2	4	1
Type of flow	SSF (VF-HF)	SSF (VF-HF)	SSF (HF)	SSF (HF)	SSF (HF/VF/HF)	SSF (HF/VF/HF)	SSF (HF/VF/HF)	SSF (HF)
Actual retention Time (d)	7.8	7.2	36		2			
Plant material	*Phragmites australis*						*Carex gracilis, Phragmites Australis*	*Phragmites Australis*
Monitoring period	1996-2003	1996-1997	1992-1999	1996 – 2000	2004-2005	1994-2004	2000-2001	1990-1993

Legend: (1) Old landfill site Ljubljana, (2) New landfill site Ljubljana, (3) Dragonja, (4) Ljubevč, (5) Ormož, (6) Ljutomer, (7) Gornji Grad, (8) Mislinjska Dobrava. SSF – subsurface flow, VF – vertical flow, HF – horizontal flow; Avg. = average.

Legend: (1) Old landfill site Ljubljana, (2) New landfill site Ljubljana, (3) Dragonja, (4) Ljubevč, (5) Ormož, (6) Ljutomer, (7) Gornji Grad, (8) Mislinjska Dobrava

CW Ljubevč is situated in the western part of Slovenia, CW Dragonja is beside the Adriatic coast in the south-western part and CW Barje in the closed landfill site at the capital city, Ljubljana, in the central part of Slovenia (Figure 9) (Zupančič *et al.*, 2005).

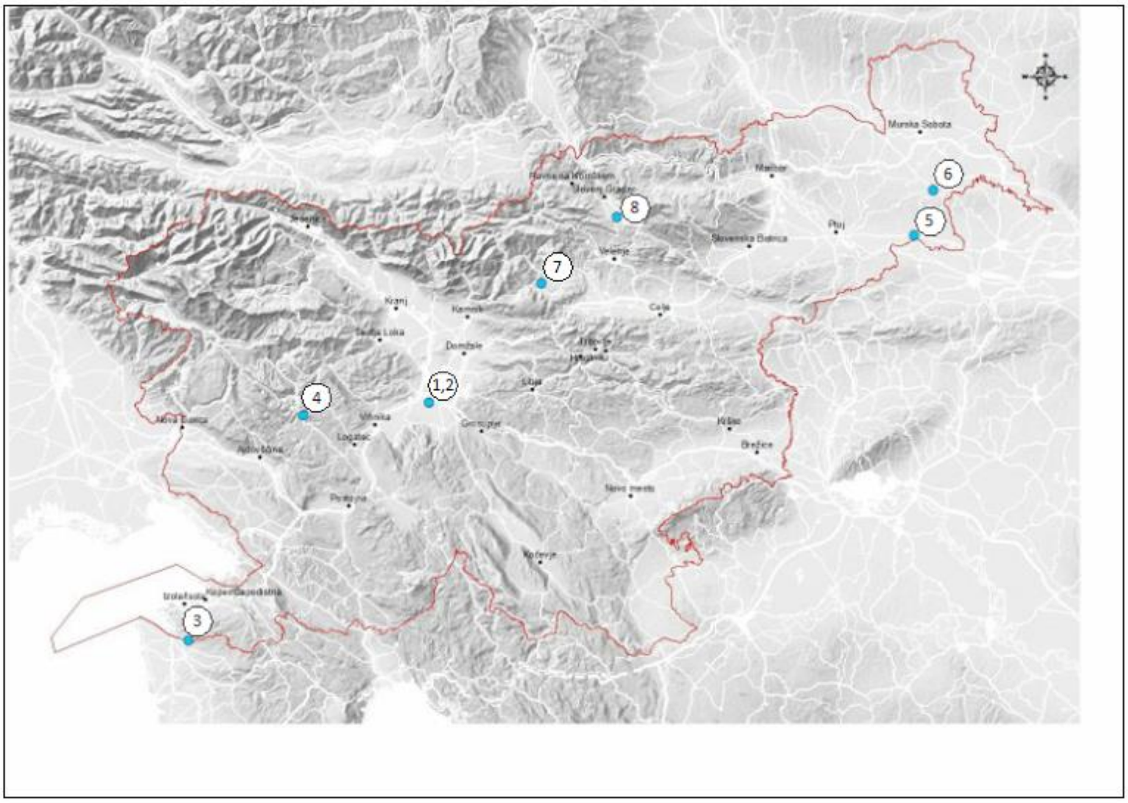


Figure 9: *Map of Slovenia showing the relative locations of CW for leachate treatmenta (Limnos, 2013)*

High values of COD, BOD, ammonium ion, chloride ion and electrical conductivity were also found in the Slovenian cases (Table 8), while there was only little or no phosphorous (Zupančič *et al.*, 2005). The concentration fluctuations were high and the performance of CWs varied with inflow leachate concentration. Very low total phosphorous (0,1-6,2 mg/l) concentrations additionally limited biological processes and had a negative influence on plant growth and overall treatment efficiency (Bulc, 1998).

Table 8: *The nine landfill sites with landfill leacahte composition of which CW were designed or/and constructed. (Ranges in mg/l unless otherwise stated) (Bulc and Zupančič, 2007)*

Landfill site	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
pH	6.8-8.2	7.6-8.1	7.9	6.4-7.4	7.3-9.7	7.8	7.4 - 7.9	6.9-9.2	7.8
Conductivity (mS/cm)	1.96-29.90	5.29-10.50	4.32-12.0		6.18-11.39	6.90-10.30	0.3 - 1.5	5.1	
Suspended solids (mg/l)	0.5-94	12-570	3-1309	14-660	41-1160	17-40	306	0.1-233	98-191
Settable solids (ml/l)	0.1-22	0.1-2.5			0.1-4.6	<0.1-0.8		0.5-1.5	1.4
COD (mg/l)	178-770	725-3250	247-1980	3540-1800	501-3300	250-110	514-1160	390-550	302-444
BOD_5 (mg/l)	10-300	87-1060	4.8-585	1350-10000	34-490	4-100	20 - 40	17-120	85-100
Adsorptive Organic Halogens (mg/l)					0.25-1.2	0.2-0.34	17 - 440	0.09-0.38	0.15-0.43
Tot Phenols (µg/l)	0.05	0.01	0.03	0.7-2.3	0.03-0.11	0.01	7.2 - 54	0.017	
Benzene, Toluene, Xylene (mg/l)					<1.0	<0.05		<0.01-0.015	
Volatile Chlorinated Hydrocarbons(mg/l)					<0.01				0.02-0.05
Mineral oils (mg/l)	1.604				<0.1			<1-3	0.1-1
Tensides (mg/l)					0.45-0.59	<0.1		2.31	
Formaldehyde(µg/l)	0.32	<0.01						<0.3	
Naphthalene(µg/l)			0.2						
Anthracene(µg/l)			0.58						
Polychlorinated biphenyls (µg/l)			0.016						
Pesticides (µg/l)			<0.001						
Nitrite (mg/l)	0.005 -1.8	0.005 -0.13	0-8.3	0.01	1-30	0.55	4.6	<0.2-0.75	
Nitrate (mg/l)	0.3-6.2	1.0-5.2	4.6-222		0.5-540	<0.5 - 30		1.3-6.28	0.18 - 0.50
Ammonium (mg/l)	275-753	225-600	58.6-581	90-410	85.4 - 378.9	450 - 730	172-1800	111-367	76.7 - 145
Tot. phosphorous (mg/l)	0.1-6.2	1.8-3.9	0.2-5.5		0.44-6.8	4.7			
Sulphate (mg/l)	0.2-86	490-665			92-340	420	87		
Sulphide (mg/l)	0.025-35	0.07-3.6	0.01-0.06			<0.05		0.05	0.03
Fluoride (mg/l)						0.4	0.05		
Cyanides (mg/l)			0.025			0.03		<0.02	
Chlorides (mg/l)	880-1515	570-960	710 -3124	170-680	296 -1994	600 - 1080	79 - 707	155 -746	166.1-366
Calcium (mg/l)	209	32.6				110	80		
Nickel (mg/l)						<0.05			0.02 - 0.05

1 Old landfill site of Ljubljana (40 ha); start of landfilling in 1967; deposited MSW, industrial wastes, thermoelectric ash; leachate treatment in CW; leachate analyses from 1997-2002.

2 New landfill site of Ljubljana (41 ha); start of landfilling in 1987, still in operation; deposited MSW, industrial wastes, thermoelectric ash; leachate treatment in CW and domestic wastewater treatment plant (WWTP); leachate analyses from 1996-1997.

3 Landfill site Dragonja (2.4 ha); start of landfilling in 1967, still in operation; deposited MSW, sewage sludge; leachate treatment in CW; leachate analyses from 1992-2002.

4 Landfill site Ljubevč (7.5 ha); start of landfilling in 1995, still in operation; deposited MSW, industrial wastes; leachate treatment in CW; leachate analyses from 2004-2005.

5 Landfill site Dobrava at Ormož (1.1 ha closed part – 2.4 ha final size); start of landfilling in 1985, still in operation; deposited MSW, industrial wastes, sewage sludge; leachate treatment in CW; leachate analyses from 2004-2005.

6 Landfill site Ljutomer (2.5 ha); start of landfilling in 1979; deposited MSW, industrial wastes (tannery, polyester, food processing industry), construction and demolition wastes; leachate treatment in CW; leachate analyses from 2002-2004.

7 Landfill site Gornji Grad (3 ha); start of landfilling in 1976; deposited mixed MSW, sewage sludge, wood, industrial wastes (textile); leachate treatment in CW; leachate analyses from 1997.

8 Landfill site Nova Gorica (31 ha); start of landfilling in 1980, still in operation; deposited mixed MSW, sewage sludge, industrial wastes; leachate treatment reverse osmosis; leachate analyses from 1995, 2000 – 2002.

9 Landfill site Tržič (5 ha); start of landfilling in 1972; deposited mixed MSW, sewage sludge, industrial wastes; leachate treatment municipal WWTP; leachate analyses from 2000 -2001.

The results have shown that CW systems could be a low-cost alternative for treatment of leachate from old landfill sites with relatively dilute leachate (Zupančič *et al.*, 2005). From Table 8 it could be noticed that CW Dragonja has a higher content of chloride ions in its wastewater than other CWs.

2.4.2 Effects of Salinity on Phragmites and Other Constructed Wetland Plants

Salinity has a negative effect on the growth of most freshwater plants. Usually, growth is gradually reduced as salinity increases above a threshold value, which varies from species to species (Plaster, 2003). Plants have been divided into two groups: the salt sensitive glycophytes and the salt-tolerant halophytes, although in reality one group merges into the other (Flowers and Flowers, 2005). While the most salt-tolerant plants (halophytes) can survive salinities about 55 mS/cm (seawater), growth of the most salt-sensitive plants (glycophytes) is severely limited at concentrations as low as 4 mS/cm (Plaster, 2003; Novak and Trapp, 2005). Under high-salinity irrigation, halophytes require a leaching percentage of 30-50% above consumptive use in order to flush excess salts below the root zone. This flushing is necessary because the build-up of salts in the root zone inhibits plant growth (Miyamoto *et al.*, 1996). However, this leaching fraction could result in significant discharge of N and P to an underlying groundwater aquifer if these nutrients are not efficiently absorbed by the soil-plant system. High concentrations of salts cause ion imbalance and hyperosmotic stress in plants. As a consequence of these primary effects, secondary stresses such as oxidative damage often occur.

The adaptations required to survive in salt-affected soils are the same in all plants. Such adaptations are at their extreme in halophytes, but can be found to differing degrees, in glycophytes. Salt tolerance depends upon (Flowers and Flowers, 2005):

1. morphology,
2. compartmentalization and compatible solutes,
3. regulation of transpiration,
4. control of ion movement,
5. membrane characteristics,
6. tolerance to high sodium/potassium ratios in the cytoplasm,
7. salt glands.

The plants used in CW wastewater treatment systems are all natural wetland species. They can be classified as submerged algae and macrophytes, floating macrophytes and emergent vegetation. Emergent species have a greater biomass than submerged or free floating species and are therefore able to store more nutrients per unit area of wetland (Greenway and Woolley, 1999). The Queensland experience (Greenway and Woolley, 1999) shows that a wide range of native species can live and thrive in sewage-effluent enriched constructed wetlands. Although emergent species have lower phosphorus and nitrogen tissue content than the free floating, submerged and aquatic creepers, biomass (i.e. nutrient storage capacity in plant tissue) are greater in the emergents. Harvesting shoot biomass in the emergents therefore removes more nitrogen and phosphorus per unit area of wetland (Greenway and Woolley, 1999). The most widely employed emergent aquatic macrophytes in SSF are *Phragmites australis* and *Typha latifolia*. *Gliceria maxima* and reed canary grass (*Phalaris arundinanacea*) are also used in Europe normally in combination with *Phragmites* spp., whereas in the USA, *Scirpus* spp. is popular. The efficiency of four emergent species *Typha domingensis, Typha orientalis, Phragmites australis and Scirpus validus* to treat the effluent from a poultry abattoir were compared by Finlayson and Chick (1983). All three genera were able to significantly reduce the suspended solids (83-89%) and turbidity (58-67%) of the effluent, whereas *Phragmites* and *Scirpus* were both able to oxygenate the anaerobic inflow. *Scirpus* was more effective than the other genera in reducing the concentrations of total nitrogen and phosphorus, and also reduced the concentration of sodium, potassium and chloride. Mant *et al*. (2006) suggested that *Penisetum purpureum* and *Brachiaria decumbens* may be suitable for phytoremediation of wastewaters containing chromium such as wastewater from tannery industry, while *Phragmites australis* is not suitable for the phytoremediation of chromium in a tropical environment. The research of Mant *et al*. (2006) indicates that a biological system such as the CW at Dragonja, using *Penisetum purpureum*, could achieve the same or similar levels of clean up as ion exchange (Kocaoba and Akcin, 2002) or reverse osmosis (Hafez *et al*., 2002; Mant *et al*., 2006). Klomjek and Nitisoravut (2005) studied growth of eight plant species under saline conditions in CWs. The most satisfactory plant growth and nitrogen assimilation were found for cattail (*Typha angustifolia*). These investigations also demonstrated that CWs were an option for improving the quality of saline wastewater. Brown *et al.* (1999) determined the feasibility of using three salt-tolerant plants (*Suaeda, Salicornia and*

Atriplex) as biofilters to remove nutrients from saline wastewater. They concluded that salt inhibited the growth rate, nutrient removal and volume of water that all three plants could process. The salinity of storm water may affect the plant growth rate and plant uptake through the toxic effects of both the sodium and chloride ions. Sodium ions may release cadmium from the sediment to the water, thereby increasing the cadmium concentration in the water (Greger *et al.*, 1995). In seawater, chloride commonly forms complexes with Zn, Cd, and to a lesser extent, Cu, but not with Pb, and thus the free ion concentration of the former metals will be reduced (Förstner, 1979; Wiliams *et al.*, 1994). Fritioff *et al.* (2005) investigated the effects of water temperature and salinity on the metal uptake (Cu, Zn, Cd and Pb) of two submersed plant species (*Elodea canadensis and Potamogeton natans*). They found that the salinity peaks in the winter may reduce the metal accumulation by plants by over 50%. Lymbery *et al.* (2006) found that growth rate of *Juncus kraussii* was affected by salinity and they found that salinity did not affect total nitrogen removal, but did reduce total phosphorus removal. Some macrophytes, such as *Salvinia herzogii De la Sota*, *Pistia stratiotes L.* and *Eichhornia crassipes (Mart.) Solms.*, could be more affected by high pH and salinity than by metal or sulphide concentration (Hadad *et al*. 2006; Maine *et al*. 2009). The adaptability of macrophytes to salinity is variable and species-dependent. Hester *et al*. (2001) studied salinity stress in different populations of *Panicum hemitomon Schult*., *Spartina patens (Aiton) Muhl*., and *Spartina alterniflora Loisel*. These authors found that plant morphology (size attributes) was strongly associated with salt tolerance in *Panicum hemitomon*, weakly associated in *Spartina patens*, and not associated in *Spartina alterniflora*. Highly salt-tolerant populations of *Spartina alterniflora* displayed the greatest ion selectivity (lower leaf Na^+/K^+ ratios), which was not displayed by the other two species. Studies of salinity and pH effects on the tolerance of *Typha* species are scarce. Macek and Rejmankova (2007) studied the response of emergent macrophytes to experimental nutrient and salinity additions, and found that *Typha domingensis Pers.* behaved as a typical competitor reducing only the plant height. Glenn *et al*. (1995) studied the effects of salinity on growth and evapotranspiration of *Typha domingensis* and found that a salinity value of 7–10 ppt (7,000–10,000 mg/l) would result in the deterioration of the macrophyte stands. Mufarrege *et al.* (2011) evaluated the response of two populations of *Typha domingensis* (plants from a CW and a natural wetland under different conditions of pH and salinity. They concluded that although *Typha* *domingensis* is not a halophyte species and does not possess anatomical structures to tolerate and excrete salts, it is capable of modifying its morphology in order to adapt to extreme conditions, such as the exposition to an industrial effluent. Because of its adaptive capacity, *Typha domingensis* is a good choice to treat wastewater of high pH and salinity, common characteristics of many industrial effluents.

Different populations of common reed (*Phragmites australis*) have wide ranges of salt tolerances (from 15 mS/cm to 80 mS/cm). Some data show 25% yield reduction at 17 mS/cm or 50% yield reduction at 50 mS/cm (Yensen, 2007; Novak and Trapp, 2005). Jing and Lin (2004) found that *Phragmites australis* can survive in a slightly saline CW while *Pennisetum alopecuroides* and *Miscanthus floridulus* can not. Kurata and Kiro (1991) showed that stands of *Phragmites communis (*which is used in older literature, but resembles a synonym to *Phragmites australis* (Cav.) Trin. ex Steud.) growing around the salt lake utilised up to 250 kg of nitrogen and 25 kg of phosphorus per hectare every year. *Phragmites australis* can survive under saline conditions, and it has often been observed to invade natural and constructed tidal wetlands (Lissner and Schierup, 1997; Chambers *et al.*, 1999; Yun *et al.*, 2003). Although *Phragmites australis* can acclimate to saline conditions by suppressing salt transport to shoots or through salt sequestration or exclusion (Flowers *et al.*, 1977), experimental evidence clearly indicates that high salinity levels impair their growth mainly through osmotic stress or ionic toxicity or both (Lissner *et al.*, 1999a, b; Burdick *et al.*, 2001). Choi *et al.* (2005), showed that increased salinity of rhizosphere water reduced the biomass accumulation of *Phragmites australis*. Kaseva and Mbuligwe (2010) found that a CW planted with *Phragmites mauritanus* was effective in removing chromium from a wastewater with a high content of dissolved solids (approx. 11 g/l). Calheiros *et al*. (2007) reported a successful establishment of *Phragmites australis* and *Typha latifolia* in CWs for tannery wastewater when compared to other plants, such as *Iris pseudacorus, Canna indica* and *Stenotaphrum secundatum*.

2.4.3 Effects of salinity on soil structure and properties

The chemical properties of soil affect growth, controls the removal of many waste constituents and influences the hydraulic conductivity of the soil profile. Sodium can affect the permeability of fine-textured soils by dispersing clay particles and thereby changing the soil structure that initially allowed water movement to a less permeable structure. The soil chemistry is a very important factor in the development and future maintenance of the vegetation (Table 9). The following tests are suggested for each of the major soil types on the site:

1. pH, cation exchange capacity (CEC), exchangeable sodium percentage (ESP) and electrical conductivity (EC);
2. plant available nitrogen (N), phosphorus (P), potassium (K) and lime or gypsum requirements for pH adjustment and maintenance.

The CEC of a soil is a measure of the capacity of negatively charged soil colloids to adsorb cations from the soil solution. This adsorption is not necessarily permanent because the cations can be replaced by others in the soil solution. These exchanges (except for excess sodium percentage in clay soils) do not significantly alter the structure of the soil colloids. The percentage of the CEC occupied by a particular cation is the percent saturation for the cation. The sum of the exchangeable hydrogen (H), sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg), expressed as a percentage of the total CEC, is the percent base saturation. Optimum ranges for percent base saturation of various crop and soil combinations have been identified. It is important for calcium (Ca^{2+}) and magnesium (Mg^{2+}) to be the dominant cations, rather than (Na^{+}) or potassium (K). Discharge of excess sodium (Na^{+}) ions to land disperses the soil particles and inhibits plant growth (Ellis, 1974) and as it accumulates, percolation is severely impaired and the tilth of the soil is reduced. In contrast, the divalent cations calcium (Ca^{2+}) and magnesium (Mg^{2+}) will rectify the effects of excess sodium.

Table 9: *Interpretation of Soil Chemical Tests (Crites et al., 2006; USEPA, 1981)*

Parameter and Test Result	Interpretation
pH of saturate soil paste	
< 4.2	Too acid for most crops
5.2-5.5	Suitable for acid tolerant crops
5.5-8.4	Suitable for most crops
> 8.4	Too alkaline for most crops
Cation exchange capacity (CEC) (mEq/100g)	
1-110	Limited adsorption (sandy soils)
12-20	Moderate adsorption (sit loam)
> 20	High adsorption (clay and organic soils)
Exchangeable cations	Desired range for good texture(as % of CEC):
Sodium	< 5
Calcium	60-70
Potassium	5-10
Exchangeable sodium percentage (ESP) (as%of CEC)	
< 5	Satisfactory
> 10	Reduced permeability in fine-texture soils
> 20	Reduced permeability in coarse-texture soils
Electrical conductivity (EC) (mmhos/cm)	
< 2	No salinity problems
2-4	Restricts growth of very sensitive crops
4-8	Restricts growth of very many crops
8-16	Only salt-tolerant crops will grow
> 16	Only a very few salt-tolerant crops will grow

The ratio between sodium and both calcium and magnesium is known as the sodium absorption ratio (SAR) which is used to characterize the suitability of a soil for plant growth (see Equation 2) (Bower *et al*., 1968; Rhoades J. D., 1968):

$$SAR = \frac{[Na^+]}{\sqrt{\frac{[Ca^{2+}]+[Mg^{2+}]}{2}}} \qquad (2)$$

The chemical symbols in equation (2) represent molar concentration of the corresponding ions.

The structure of clay soils can be damaged when there is an excess of sodium with respect to calcium and magnesium in the wastewater. The resulting swelling of some clay particles changes the hydraulic capacity of the soil profile. Irrigation of soil with wastewater leads to complex chemical changes, such as precipitation and oxidation-reduction. Immobilization of cations is enhanced at a soil water pH > 7 but acidic conditions lead to some mineral solubilisation and leaching. In conjunction with conductivity, which is used as a measure of total dissolved solids and hence the salinity hazard to crops, the SAR, which is a measure of the sodium or alkali hazard to crops, can be used to assess the suitability of wastewaters and effluents for irrigation (Gray, 2004). The cation distribution in the natural soil can be changed easily by the use of soil amendments such as lime or gypsum. The nutrient status of the soil is important if vegetation is to become a component in the treatment. Potassium is also important for proper balance with the other nutrients. The N, P and

K ratios for wastewaters and biosolids are not always suitable for optimum crop growth, and in some cases it has been necessary to add supplemental potassium (Crites *et al.*, 2006).

The humic substances found in wetlands contain large numbers of hydroxyl and carboxylic functional groups, which are hydrophilic and serve as cation binding sites. Other portions of these molecules are non polar and hydrophobic in character. The result is the formation of *micelles*, which are groups of humic molecules with their nonpolar sections combined in the centre and their negatively charged polar portions exposed on the surface of the *micelle* (Wershaw *et al.,* 1986). Protons or other positively charged ions may associate with these negatively charged sites to create electrical neutrality (Kadlec and Knight, 1996).

Biological, chemical and physical properties of wetland soils are interdependent. A long retention time is an important factor in many of the physico-chemical processes and also ensures maximum removal of pathogens. Metals present in wastewater become immobilized in the anaerobic mud, forming metallic sulphides. Release of carbon dioxide and methane from the anaerobic mud will also occur (Kadlec *et al.*, 2000). Microbial wetland fauna makes up a significant fraction of the organic carbon occurring in hydric soils. Soil microbial populations have significant influence on the chemistry of most wetland soils.

When assessing potential risks concerning metal mobility in recently created or restored wetlands, different physical–chemical substrate characteristics such as soil pH, CEC, redox status, carbonate, organic carbon and clay content must be taken into account. An important factor which can control metal mobility in flood control wetlands is closely linked to the prevalent hydrological regime. Flooded conditions minimized the availability of metals, while oxidation of the substrate seems to be associated with a rather rapid increase of metal availability and accumulation (Speelmans *et al.,* 2010).

In the study of Calheiros *et al.* (2008) the treatment of tannery wastewater by *Typha latifolia* CWs, established in different substrates, Filtralite NR3-8, Filtrolite MR3-8 (both are expanded clay aggregates) and fine gravel, was evaluated. They concluded that the three substrates were adequate for *Typha latifolia* development although there was higher propagation of *Typha latifolia* and higher removal of organic matter for the expanded clay aggregates when compared to the fine gravel. Overall, the expanded clay aggregates planted with *Typha latifolia* demonstrated significant higher percentage removals in terms of COD and BOD_5 when compared with the unplanted unit after long-term operation (Calheiros *et al.*, 2008).

2.4.4 Effects of Salinity on Microorganisms in Constructed Wetland

The complex organic and inorganic composition of many waste brines can make them difficult to treat biologically. Studies conducted with conventional (i.e. nonhalophilic) cultures of bacteria indicate that the following four common difficulties exist when treating saline and hypersaline wastes with organisms derived from freshwater and soil ecosystems (Woolard and Irvine, 1995):

1. Limited extent of adaptation. The general consensus among practitioners is that conventional cultures can not be effectively used to treat wastewater containing more than 3-5% of salt (Tokuz and Eckenfelder, 1978; Wong, 1992; Hockenbury *et al.*, 1978). It is also clear that any salt acclimation achieved is quickly lost when organisms are exposed to diluted ionic conditions (Kincannon and Gaudy, 1968; Doudoroff, 1940).
2. Sensitivity to changes in ionic strength. In general, shifts in salt concentration of 0.5-2% of salt will cause significant disruptions in the overall system performance. Rapid shifts in salt concentration typically cause more problems than gradual shifts (Kincannon and Gaudy, 1968; Lawton and Eggert, 1957). In certain cases, organisms acclimate to give satisfactory reactor performance under saline conditions (Lawton and Eggert, 1957; Kincannon and Gaudy, 1968). However, even with acclimated cultures adequate process performance requires constant ionic composition and rapid reductions in the salt content of the influent wastewater will cause additional upsets of the system (Lawton and Eggert, 1957). Equalization to stabilize salt content has been identified as a prime consideration in the design of facilities to treat saline wastes (Hockenbury *et al.*, 1978; Stover and Obayashi, 1991).

3. Reduced degradation kinetics. Degradation rates are sensitive to increased salt concentration.
4. High effluent suspended solids concentrations. Several researchers have speculated that salt induces cell lysis and reduces the populations of protozoa and filamentous organisms required for proper flocculation (Ludzack and Noran, 1965; Hockenbury *et al.*, 1978; Wong, 1992).

Studies on freshwater and terrestrially derived conventional treatment cultures indicate that these organisms are not genetically capable of surviving in hypersaline environments. Thus biological removal of organics from hypersaline wastes without dilution will require specialized microbes. Natural hypersaline environments contain complex and diverse communities of such organisms. These halotolerant and halophilic microbes are metabolically diverse and possess many of the same characteristics as conventional waste treatment cultures. Although nonhalophilic and marine bacteria can be found in these environments, it is the true halophiles that are of particular interest for hypersaline waste treatment (Woolard and Irvine, 1995).

Lin *et al.* (2008) found that increased salinity affected the growth of some bacteria which resulted in changes in their relative numbers in the microbial community. This apparent impact showed salinity impacted the metabolism of microorganisms critical to the proper functioning and maintenance of the system. Previously Nitisoravut and Klomjek (2005) proposed salt had a negative impact on biological degradation rates of organic compounds in wastewater, and they attributed the reduced rates of BOD removal to the inhibition of microbial enzymes and microbial growth.

The effects of salinity on the removal of dissolved organic carbon and nutrients from municipal wastewater by constructed mangrove microcosms planted with *Aegiceras corniculatum* were investigated by Wu *et al.*, 2008. During a four-month wastewater treatment experiment, the treatment efficiency was reduced by high salinity, and the removal percentages of dissolved organic carbon, ammonia-N and inorganic N dropped, from 91% to 71%, from 98% to 83% and from 78% to 56%, respectively, when salinity was increased from 0 to 30 parts per thousands (ppt).

The studies of Faulwetter *et al*., 2009 and Truu *et al.*, 2009 in different types of CWs provided an overview on microbial communities and factors affecting microbial activity in such systems, but with no reference to the influence of high salinity content in such communities. The work by Lefebvre and Moletta (2006) constitutes an attempt to describe the microbial communities involved in the aerobic and anaerobic treatment of hypersaline tannery wastewater.

More recently two studies were published (Calheiros *et al.,* 2009a; Calheiros *et al.,* 2010) which focused on the diversity of bacterial communities from two series of two stage HF CWs treating tannery wastewater. In the both studies CWs were polishing treatment of treating tannery wastewater. Conductivities in inlet water were 16.96 ± 0.21 mS/cm (Calheiros *et al.,* 2010) and 7.47 ± 0.64 mS/cm (Calheiros *et al.,* 2009a).

In the first study (Calheiros *et al.,* 2009a) several series were separately planted with *Typha latifolia* and *Phragmites australis* in expanded clay aggregates and operated for 31 months. Both series were effective in removing organic matter from the inlet wastewater, however, based on batch degradation experiments it seems that biodegradation was limited by the recalcitrant properties of the wastewater.
In the next study (Calheiros *et al.,* 2010), each series was planted with *Arundo donax* or *Sarcocornia* sp. in a substrate composed by expanded clay and sand. They concluded that:

1. The high salt content in the wastewater did not jeopardize the removal efficiency of organic matter in the CW;
2. The clustering analysis suggested that a diverse and distinct bacterial community inhabits each CW series;
3. The type of plant seems to have a major effect on the established bacterial communities; and
4. CW with different plants performed well in terms of organic matter removal, which may be due to the high bacterial diversity found within the systems.

However, conventional biological treatment systems are known as insufficient for removal of COD from saline wastewaters because of the adverse effects of salt on microbial flora. The reason for this is loss of activity of organisms in biological wastewater treatment operations due to plasmolysis in the presence of salt. Microorganisms requiring salt for growth are designated as halophilic. The intracellular salt concentration of halophilic and halotolerant microorganisms is low and they maintain an osmotic balance of their cytoplasm with the external medium by accumulating high concentration of various organic osmotic solutes (Margesin and Schinner, 2001). Therefore, utilization of salt tolerant microorganisms in biological wastewater treatment systems could be a solution for COD removal from saline wastewater (Kapdan and Erten, 2006). Lefebvre and Moletta (2006) also wrote that biological treatment is inhibited by high salt, but it has proved feasible to use salt-adapted microorganisms capable of withstanding high salinities and at the same time of degrading the pollutants that are contained in wastewater. The application of salt-tolerant microorganisms on sludge was found in the literature (Kargi and Dincer, 1996; Kargi and Uygur 1996; Woolard and Irvine 1994; Woolard and Irvine 1995; Dincer and Kargi 1999) but there is not much information about their use in CW.

The results of Koprivnikar (2008) showed that it is feasible to use inocula of enriched and salt adapted microorganisms for the treatment of organically contaminated wastewaters with elevated salt concentrations.

In CW systems, microorganisms have the main role in the removal of pollutants (Stottmeister *et al.*, 2003; Faulwetter *et al.*, 2009). Although data about applications of halotolerant microorganisms in conventional biological processes was found in the literature (Thanh and Simard 1971; Kargi and Dincer,1996; Kargi and Uygur 1996; Woolard and Irvine 1994; Woolard and Irvine 1995; Tellez *et al.* 1995; Hinteregger and Streichsbier 1997; Dincer and Kargi 1999; Yang, 2000; Nishihara, 2001; Simard and Thanh, 1973) there is almost no evidence about their applications to low-cost systems as CWs. Microorganisms from the Sečovlje Salterns have high viability under saline conditions, because of that they have great potential to improve treatment of saline wastewaters in CW.

2.5 The Sečovlje Salterns and Their Microorganisms

2.5.1 Description of the Sečovlje Salterns

The Sečovlje salterns (geographic coordinates: X: 396423; Y: 35216) are the most northerly located solar salt production system on the Adriatic Sea coast (Figure 9). Dating from the 12th century, the salterns are located in southeast part of Piran Bay along the estuary of the Dragonja River. Climatic conditions there are sub-Mediterranean.

2.5.2 Operation of the Sečovlje Salterns

Salt is collected only during the salt production season (May-September). The evaporating seawater is routed to ponds, where first calcite precipitates, followed by gypsum and NaCl (Butinar *et al.*, 2005; Schneider, 1995). During the salt harvesting season the evaporation of seawater is strongly enhanced by offshore winds, which cool the evaporating surface so that the brine temperature rarely exceeds 32 ºC (Gunde-Cimerman *et al*., 2000). In saltern ponds is eutrophic concentrated brine. The crystallizer sedimentation surface is stabilized with a firm stromatolitic mat, allowing only manual salt collection. Outside the season of salt production the salinity of the water mainly remains below 5% (w/v) NaCl. During the salt production season, it varies from 3-32% NaCl (Butinar *et al.*, 2005). The crystallizers do not have the typical red brine coloration. The solar energy collection is enhanced by dark colored microbial mat at the bottom of the pond, while strong offshore winds act to aid evaporation and cool the temperature of the brine. To prevent possible damage from rainfall, the salt is harvested almost continuously, sometimes as soon as it starts to crystallize at 27% of total salts concentration. The physical and chemical parameters of water sampled from the Adriatic saltern ponds are presented in Table 10 (Butinar *et al.*, 2005). The pH in all ponds was 7.2 from the beginning of December to mid-February. It then increased and remained between 7.4 and 8.2. The temperature of the brine was around 0ºC in winter and increased up to a maximum of 26ºC in July. Conditions in the environment were most extreme during the peak of salt production by the end of August. In the crystallization pond (Table 10, fourth pond) the NaCl concentration increased to 30% and water activity decreased to 0.72, while in the other tested ponds (Table 10, first to third pond) salinities ranged from 13% to 24% and water activities from 0.89 to 0.93. As salinity increased, oxygen decreased from values as high as 10.3 mg/l down to 0.5 mg/l in August. Outside the salt production season, oxygen concentrations in all ponds ranged from 8.7 to 16.0 mg/l. Due to occasional high values of nutrients during the season, the chemical oxygen demand was between 255 and 1890 mg/l. Although concentrations of nitrogen and phosphorus were generally below 0.1 mg/l, two peaks of nitrogen were observed: the first one at the beginning of the season, at salinity 8–10% (0.3–1.7 mg Nitrogen/l), and the second one at salinity 21–25% (8.2-13.2 mg Nitrogen/l). Simultaneous with the nitrogen peak in August, phosphorus concentrations reached a peak of 0.3 mg/l (Butinar *et al.*, 2005).

Usually, characteristic for saline wastewater besides high salinity is salinity oscillation, which represents a major problem for microorganisms for wastewater treatment and at the same time high concentration of organic matter and nutrients. The high concentration of nutrients and organic matter in salt ponds of the Sečovlje salterns (Table 10), high concentration of salt and their oscillation during rainstorms, represent the Sečovlje salterns as very interesting for the isolation of halotolerant microorganisms and their application to saline wastewater treatment.

Table 10: *Physical and chemical characteristics with their seasonal ranges in hypersaline water of selected ponds in the Adriatic salterns (Butinar et al., 2005)*

	First pond	Second pond	Third pond	Fourth pond
Salinity (%)	3-13	5-21	4-24	10-30
Water activity (a_w)	0.89-0.96	0.79-0.90	0.77-0.90	0.72-0.93
O_2 (mg/l)	4.9-10.8	3.4-7.0	2.1-6.1	0.5-9.3
N (mg/l)	<0.1-5.7	<0.1-7.5	0.1-9.2	<0.1-13.2
P (mg/l)	0.01-0.09	0.05-0.14	0.01-0.08	0.04-0.3
BOD_5 (mg/l)	11-328	5-730	<1-24	<1-73
COD (mg/l)	255-1250	680-1630	600-1570	560-1890
Cl^- (g/l)	9.1-104	12.7-128	19.5-151	9.4-184
K^+ (g/l)	0.38-3	0.55-2.5	0.43-2.9	0.46-4.8
Na^+ (g/l)	9.7-82	14.5-72.4	11.3-82.4	12.1-95
SO_4^{2-} (g/l)	0.39-13.6	0.79-13.5	2.7-18.2	0.47-26.1

2.5.3 Characteristics of the Microorganisms from the Sečovlje Salterns

Hypersaline waters of a saltern crystallizer at salinities at or near saturation are one of the most extreme environments with respect to the sodium chloride concentration.

The microbial diversity of this type of hypersaline environment has been extensively studied, focusing on the use of both molecular ecological (Benlloch *et. al.*, 2002; Benlloch *et. al.*, 2001; Bowman *et. al.*, 2000; Ochsenreiter *et. al.*, 2002; Anton *et al*., 2000; Pašić *et. al*., 2005) and cultivation-based methods (Bolhuis *et. al.*, 2004; Burns *et. al.*, 2004).

The aim of Koprivnikar (2008) was to prepare inocula, containing consortia of microorganisms from salterns at Sečovlje, adapted to elevated salt concentrations conditions. They used an enrichment media with 1.5 % NaCl for enrichment of the desired microorganisms. They also compared wastewater treatment efficiency of microorganisms from salterns that are adapted to media conditions and those that are inoculated directly from salterns. They found adapted microorganisms to be more efficient that those taken directly from the salterns in treating the wastewater. Molecular analysis gave the insight to bacterial and fungal community dynamics during the enrichment process. Phylogenic classification of microorganisms, selected because they demonstrated successful adaptation to salty media, showed bacterial genera *Marinobacter*, *Halomonas* and *Idiomarina* and fungal genera *Saccharomyces*, *Candida*, *Didymella* and *Acremonium* to be the most successful in salty media. Bacterial genera *Pseudomonas*, *Aeromonas*, *Alishewanella*, *Vibrio* and fungal genera *Saccharomyces*, *Candida*, *Phoma*, *Coniothyrium* and some others were found to be the most successful in salty media, inoculated with activated sludge. Among microorganisms that showed successful reproduction in treatment plant pilot plants were bacteria, related to the genera *Halomonas, Acinetobacter* and *Comamonas*, and fungi, related to *Aureobasidium, Kabatiella, Phaeosphaeria, Didymella, Phoma, Dimorphospora* and *Helicodendron*. The results of Koprivnikar (2008) research showed that it is feasible using inoculums of enriched and salt adapted microorganisms for treatment of organically contaminated waters elevated salt concentrations.

2.6 The kinetics of a biochemical process

The kinetics of all biochemical processes are related to temperature through the Arrhenius relationship (e.g. Fogler, 2005). For a given catalytic or microbial system, with given chemical components, this relationship is shown:

$$k = A \exp[-E_a/RT] \quad (3)$$

k - the rate constant as a function of temperature;

A - the pre-exponential factor;

E_a - the activation energy in J/mol;

R - the gas constant = 8.314 J/(molK);

T is the absolute temperature in K.

3 MATERIAL AND METHODS

3.1 Description of Constructed Wetland Dragonja

SSF CW Dragonja is located in the Adriatic Coastal area of Slovenia, near Sečovlje (Figure 10). The CW was built in January 1992 with the intention of purifying the leachate from the adjacent municipal solid waste landfill thereby mitigating the direct pollution of the surrounding agricultural land and groundwater.

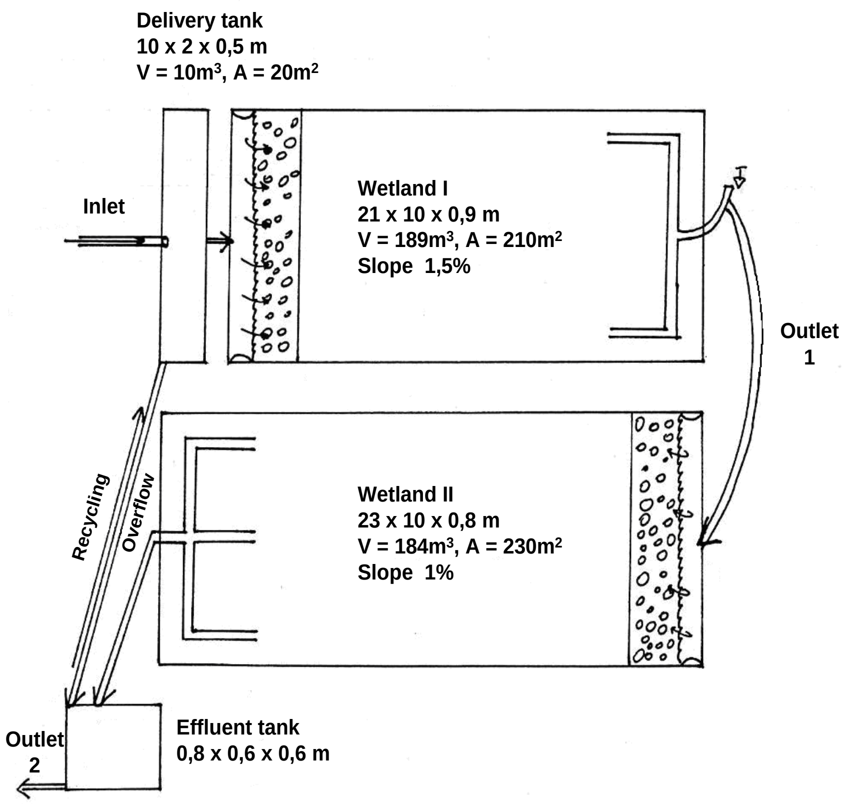
Figure 10: *Map of Dragonja landfill and Sečovlje salterns*

Collected leachate was treated in the CW and released into the municipal sewage system for further treatment. The CW with a horizontal subsurface flow consists of two cells (Figure 11) having a total surface area of 450 m^2, with a bottom slope of 1-1.5% and a depth of 80-90 cm. The cells are filled with a peat, soil, sand and gravel mixture and planted with common reeds (*Phragmites australis*) (Pavčič, 1995; Bulc 1998). The system was isolated from the groundwater by clay (10 cm), PEHD foil (2 mm) and clay (20 cm). Inflow and outflow can be regulated by valves and expected flow was 5-10 l/min. In the year 2007, flow was set to 6.3 l/min. Ground water had no influence on the system, but there was an influence of rainwater and evapotranspiration.


Figure 11: *Constructed wetland Dragonja. (left) CW Dragonja in the phase of construction. (right) First cell of CW Dragonja*

In order to follow the efficiency of wastewater treatment a monitoring program was set up by the government and carried out by the municipality company Okolje Piran d.o.o. (Danijela Kleva Švagelj, 2006) under supervision of the Institute of Public Health Koper. The monitoring started in 1992, when the CW was built and countinues until today.

From 1992 to the end of the year 2000 measurements were made at the location of wastewater influent and at the effluent 2 (Figure 12) of the CW, eleven times per year. From 1992 to 1997 monitoring also included the analysis of effluent 1 (Figure 12). The frequency of measurements was in accordance with the OG RS 35/96, 21/03 and 7/2000 regulations. At the end of the year 2000, the legislation was changed (OG RS 7/2000) and measurements were made only at the effluent 2, four times per year from the years 2001 to 2006 (Danijela Kleva Švagelj, 2006). During 2007, measurements were done four times per year at the influent and effluent 2 (effluent 2) of the CW with a constant flow of 6.3 l/min.



Legend: Influent was taken at Inlet; Effluent 1 was taken at Outlet 1; Effluent 2 was taken at Outlet 2.

Figure 12: *Scheme of CW Dragonja*

3.1.1 Measurements of parameters of Constructed Wetland Dragonja

Measurements of physical and chemical parameters of wastewater (BOD_5, COD, ammonium ion, nitrate ion, nitrite ion, total nitrogen, pH, temperature, conductivity, chloride ion, total phosphorus, iron) were made according to standard methods (APHA, 1990) (Table 11) in the laboratory of the Institute of Public Health, Koper (Danijela Kleva Švagelj, 2006). Temperature and pH were measured *in situ*.

Table 11: *Methods for measurements of parameters of CW Dragonja (Danijela Kleva Švagelj, 2006)*

Parameter	**Method**
Temperature	DIN 38 404-C4-2
pH	ISO 10523
$BOD_{5\ years}$	ISO 5815-1
COD	ISO 15705
Ammonium ion	ISO 10304-2
Nitrate ion, nitrite ion, phosphate ion and chloride ion	ISO 10304-2
Total nitrogen	DIN 38409-H28
Total phosphorus	ISO 6878
Conductivity	ISO 7888
Iron	ISO 15586

Data from CW Dragonja for the years 1992 to 2000 were used to calculate CW efficiency and the relations between parameters and efficiency.

3.1.2 Data analysis of CW Dragonja

Principal component analysis (PCA) based on a correlation matrix was used to examine the variation in chemical parameters measured in the landfill wastewater at the influent and effluent of CW. The entire data set was not used in the analysis, since all the considered parameters were measured simultaneously in only 58 samples. The dates, where missing values appeared were not considered in the PCA analysis and the parameters with many missing values were also discarded from the analysis. The CANOCO software package (Braak and Šmilauer, 2002) was applied.

Student t test for CW Dragonja was carried out to compare wastewater concentrations in influent and effluent. It was calculated using Microsoft Excel (version 2007). The Pearson correlation coefficient between parameters was also calculated using MS Excel.

A two-way ANOVA was used for statistical analysis of pH, COD, $BOD_{5,}$ total phosphorus, ammonium ion, nitrite ion, nitrate ion and total nitrogen of CW Dragonja in correlation to the concentrations of chloride ions and location (influent and effluent). Concentrations of chloride ions were divided into six classes: class 1<500 mg/l; class 2: 500-1000 mg/l; class 3: 1000-1500 mg/l; class 4: 1500-2000 mg/l; class 5: 2000-2500 mg/l; class 6>2500 mg/l. The Tukey test was used to conduct multiple comparisons of means honestly significant difference (HSD) when ANOVA results were significant (Fowler *et al.*, 1998). Statistical analyses were performed using R statistical software (R version 2.5.0) (Wessa, 2007). The term »statistically significant« is used when statistical tests gave a p value of <0.05, »highly significant« for $p<0.01$ and »very highly significant« for $p<0.001$.

3.2 Pilot plant gravel - sand filter for saline wastewater

Treatment of synthetic wastewater with a laboratory pilot plant gravel - sand filter for saline wastewater under conditions of different salinities, inoculated with halotolerant microorganisms from the Sečovlje salterns, was assesed to test the applicability of halotolerant microorganisms which were meant to improve the treatment efficiency of saline wastewater.

3.2.1 Description of pilot plant gravel - sand filter for saline wastewater

The laboratory pilot plant (Figure 13 and 14) was constructed and inoculated with halotolerant microorganisms to investigate its efficiency with different concentrations of salt in synthetic wastewater. Efficiencies at different salinities were evaluated in the same pilot plant sequentially. Consequently, treatments with different salinities in the pilot plant were not independent. We wanted to exclude any contamination of the pilot plant with other microorganisms, thus we did not treat the pilot plant with other microorganisms before starting. Figure 13 shows a schematic of the pilot plant. It was constructed from three rectangular tanks made of plastic, with the following dimensions: length 0.77 m, width 0.16 m and depth 0.58 m, respectively, separated with 0.20-0.25 m long empty compartments at both ends. The front side of the pilot plant was made out of Plexiglas™. The total length of the pilot plant was 2.99 m. During the experiments, the Plexiglas™ side was covered with dark plastic material to obstruct light. Between the compartments filled with soil, there were perforated compartment walls placed across the flow of water. Through these four narrow empty compartments water flowed at a constant rate of 100 ml/min. The samples for water analyses were taken at the bottom of compartments filled only with water – empty compartments on Figure 14. In every one of the soil filled compartments, there was a perforated pipe which reached to the bottom of the compartment. The pipe ended with a part of a plastic bottle and from each bottle a small plastic tube led to a glass bottle with a solution of barium hydroxide to absorb carbon dioxide as a measure of microbial activity in the soil.

A perforated rubber pipe was laid at the bottom of the pilot plant. It was connected to an aquarium air pump that was turned on only during the aeration treatment.

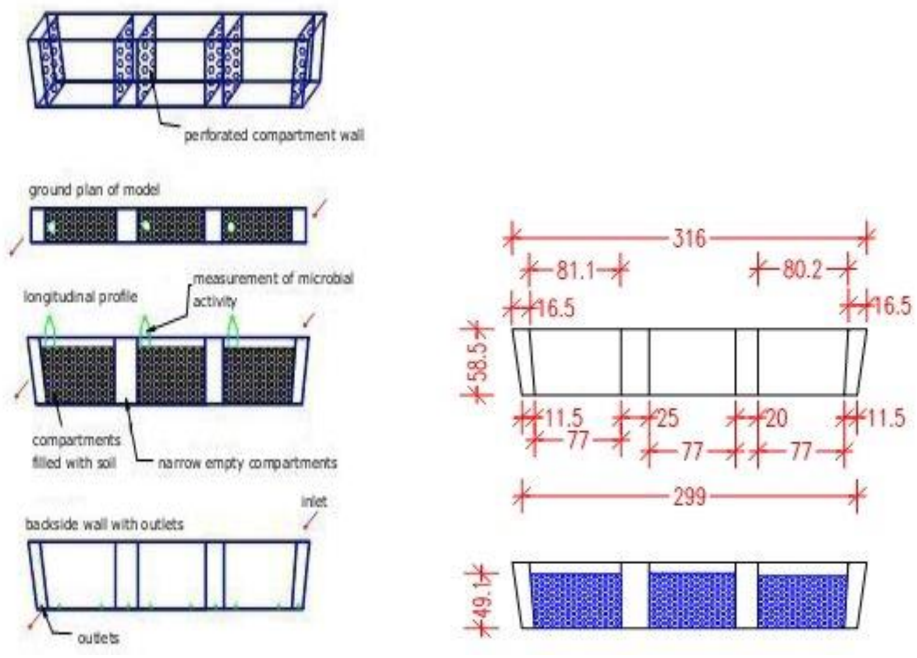


Figure 13: _Pilot plant with dimensions_

Figure 14: *Pilot plant*

3.2.2 Soil

Soil was prepared as mixture of limestone and peat. The sand was prepared from particles of different sizes: 1-4 mm (30%; 0.12 m^3) 4-8 mm (60%; 0.24 m^3) and 8-16 mm (10%; 0.04 m^3). The chemical content of the limestone was: $CaCO_3$ 30.56%, $MgCO_3$ 21.8%, MnO_2 0.02%, TiO_2 in trace, Fe_2O_3 0.00, Al_2O_3 0.00, SiO_2 0.08%. Peat was added in 10% of the total volume with the following characteristics: pH = 3.5-4, organic matter 35%, total nitrogen 0.4%.

The final pH value of the soil mixture was 7.4.

The pilot plant did not contain plants because the aim was to investigate the efficiency of halotolerant microorganisms in the sand/gravel/peat environment with variations of salinity. Different plant species may have an influence on the number of bacteria on roots. Vymazal *et al.* (2001) reported that there were significantly higher bacteria counts on *Phragmites australies* roots when compared to the roots of *Phalaries arundinacea.* Similar results were found by Calheiros *et al.* (2010). It is known from the literature that plants accelerate the growth of microorganisms, especially in the area of the root environment (Armstrong, 1982; Mendelsson and Postek, 1982; Kadlec, 2000; Gray, 2004), but the halotolerant microorganisms in this research were isolated from water of the Sečovlje salterns and naturally they were not attached to the roots of plants. However, it could be assumed that the presence of plants would increase the efficiency of microorganisms used.

3.2.3 Wastewater composition

In order to minimize the impact of the variations in the landfill leachate composition on the research, a synthetic leachate having a constant composition was used in this research. The composition was the same as ART (Kosjek *et al.*, 2007; Reasoner and Geldreich, 1985) components (Table 12). The volume of the water in the pilot plant was 115 l.

Table 12: *Synthetic wastewater composition (Kosjek et al., 2007; Reasoner and Geldreich,1985)*

Component (Producer)	ART
	mg/l
Yeast extract (Bio Merieux, France)	130
Casein peptone (Difco, Michigan, USA)	130
Meat extract (Merck, Darmstadt, Germany)	130
CH_3COONH_4(Merck, Darmstadt, Germany)	317
NH_4Cl (Merck, Darmstadt, Germany)	40
K_2HPO_4 (Kemika, Zagreb, Croatia)	24
KH_2PO_4 (Kemika, Zagreb, Croatia)	8
$CaCO_3$ (Merck, Darmstadt, Germany)	100
$MgCO_3$ (Merck, Darmstadt, Germany)	100
NaCl (SolanaTuzla)	40
$FeSO_4 \cdot 7H_2O$ Sigma-Aldrich, St.Louis, Missouri, USA	5

Synthetic wastewater with a volume of 115 l was prepared in a plastic container (the green container seen in Figure 14) from which it was pumped into the first compartment of the pilot plant, while the old synthetic wastewater flowed out of the fourth compartment. The flow rate of the wastewater (Q) was constant (100 ml/min). It was pumped with an aquarium water pump (Hydor Seltz L20 II) and its flow was regulated with a valve. When all of the 115 l fresh synthetic wastewater was in the pilot plant pilot plant, the aquarium water pump was placed into the last compartment of the pilot plant. The wastewater was then circulated from the last compartment to the first for six days with the same and constant Q (100 ml/min) using the aquarium water pump. On the seventh day, the contents of the pilot plant were changed with fresh synthetic wastewater.

3.2.4 Microorganisms

Halotolerant microorganisms were isolated from three locations of active Slovenian solar Sečovlje salterns in the September of 2005. Samples were collected in sterile vessels, transported to the laboratory at ambient temperature and maintained at a temperature of 4ºC until used. The details of their isolation have been reported by Koprivnikar (2008). Briefly, 50 ml of each sample were filtered with suction through 0.45 µm filters. Under sterile conditions, the filters containing the microorganisms were transferred to flasks containing 300 ml of sterilized ART in 1.5 % NaCl (Kosjek *et al.,* 2007; Reasoner and Geldreich, 1985) (Table 11). Halotolerant microorganisms were cultivated for two months. Optical density (OD) at 600 nm was measured to monitor their growth. The microorganisms were then stored at -20°C. Before inoculation into the pilot plant they were reactivated on the same media (300 ml ART with 1.5% NaCl) for one week. 300 ml of reactivated culture was added to the first 115 l of synthetic wastewater that was added into the pilot plant.

3.2.5. Experimental design

The pilot plant effluents in all four compartments were analysed daily for the first fifteen days following inoculation and after that less frequently. Samples of the treated synthetic wastewater were taken on the first, third and seventh day after adding fresh synthetic wastewater. Water circulated in the pilot plant for seven days. Every seventh day 115 l of treated synthetic wastewater were removed from the pilot plant and replaced with fresh synthetic wastewater. After that, synthetic wastewater with halotolerant microorganisms and 1.5% NaCl were added. This salinity was maintained for two months with wastewater being replaced with fresh water once per week, without aeration. After this initial period, conditions in the pilot plant were changed every two weeks, with wastewater being replaced with fresh water once per week. First, 1.5% NaCl was aerated for two weeks (Figure 15). Then synthetic wastewater with 3% NaCl without aeration circulated for two weeks, and after that 1.5% NaCl wastewater without aeration circulated for another two weeks. It was then replaced with 0% NaCl without aeration in the synthetic wastewater for one week and finally with 1.5% NaCl in the synthetic wastewater that was aerated and contained 2 g/l of saccharose, for two weeks. Synthetic wastewater was changed, fresh synthetic wastewater was pumped into the first compartment with a flow of 100 ml/min whilst at the same time the old synthetic wastewater poured out through the valve from the fourth/last compartment. Water was pumped into the pilot plant with an aquarium pump. The same inflow and outflow were set with an aquarium pump and valves. After all of the fresh synthetic wastewater was pumped into the pilot plant, the system was set to pump the water from the last compartment to the first one, using an aquarium pump with a flow of 100 ml/min. The microbial community in this kind of a system needs time to acclimate to new experimental conditions, but in this experimental study after the initial period of two months, we did not wait for the microorganisms to acclimate. Instead measurments were carried out continuously to follow the adaptation and efficiency of halotolerant microorganisms during changes of salinity. These changes of salinity often occure in a real CW, and we wanted to investigate the efficiency of halotolerant microorganisms isolated from the Sečovlje salterns, during salinity shocks. The efficiency of the pilot plant was assessed throughout this period. All parameters were measured first, third and seventh day.

The method with conductivity was used for estimation of hydraulic retention time (HRT) (Bulc, 1998). A volume of 1 g/l NaCl was added to the influent at time 0 (t_0) and then conductivity was continuously measured at effluent of compartment 4 (final compartment). The difference between the time when conductivity has maximum and t_0 was estimated to be the retention time. The estimated retention time was 1 day and 5 hour. The water flow was constant all the time during experiments on the pilot plant.

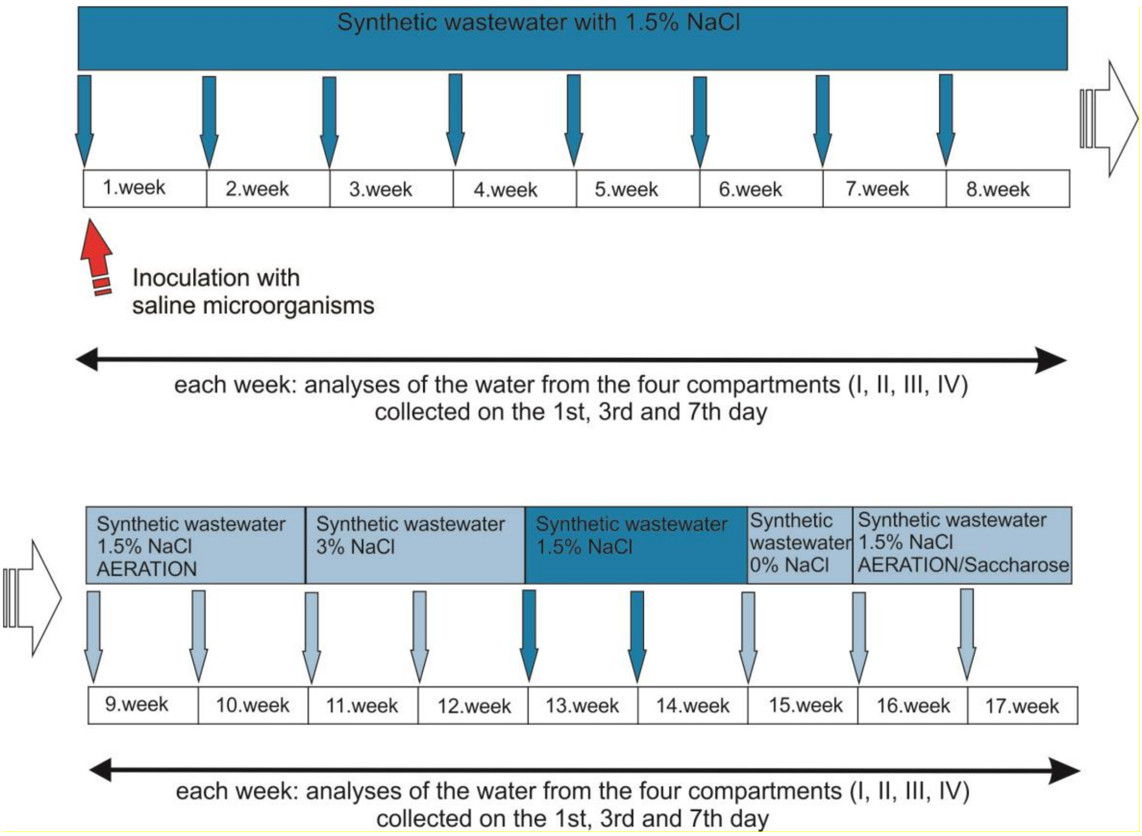


Figure 15: *Experimental design of pilot plant*

3.2.6 Analytical methods

3.2.6.1 Measurements of temperature, pH, conductivity, redox potential and oxygen

The pH value, temperature, conductivity, redox potential and oxygen were measured. The parameters were determined according to APHA methods (1990). Temperature, pH, conductivity, redox potential and oxygen concentration were measured with WTW Sonde Multi 350i/SET, Wissenschaftlich, Germany.

3.2.6.2 Measurements of nutrients and chloride ions

The efficiency of the pilot plant was assessed on the basis of difference between ammonium ion, nitrate ion, nitrite ion, chloride ion, phosphate ion, oxygen and carbon dioxide concentrations and COD at the influent and the effluent. The parameters were determined according to APHA methods (1990). Concentrations of ammonium ion, nitrate ion, nitrite ion, chloride ion, phosphate ion and COD were measured using a LF2400 Windaus photometer, Germany. To determine ammonium ion concentration the »Ready mixed cuvette test kit«, cat. no. 3773900, was used, for nitrate ion the "Aquanal test kit" cat. no. 800320204 and "Refill aquanal kit" cat. no. 3744800 were used, for nitrite ion the "Mischreagenzien-test nitrit" cat. no. 800320207, for chloride ion the "Photometer-reagenz aquanal" cat. no. 800320210 and for phosphate ion the "Aquanal test kit" cat. no. 3745100 were used. All kits were supplied by Windaus, Germany. Water samples for analysis were taken from each water compartment.

3.2.6.3 Measurements of microbial activity

The carbon dioxide in the soil, ETS activity (electro transport system activity) and optical density were measured in order to determine the microbial activity. Measurements of ETS activity and OD were done using an Ocean Optics USB2000 spectrometer, USA. Measurements of biomass were done according to the Sluiter *et al.* (2005) method. ETS activity and carbon dioxide in soil were measured in all three compartments with soil.

3.2.6.3.1 Released carbon dioxide in soil

The carbon dioxide released in soil, as result of microbiological activity, was measured and calculated according to methods published in the literature (Hanstveit, 1992; Venosa *et al.*, 1992; Thomas, 1995; Heath, 1998; Zupančič, 2001). Carbon dioxide was captured for 24 h in a $Ba(OH)_2$ solution (80 mg / 250 ml distilled water) where CO_2 was transformed to $BaCO_3$ (equiation 4).

$$Ba(OH)_2 + CO_2 \rightarrow BaCO_3 + H_2O \qquad (4)$$

The remaining $Ba(OH)_2$ was titrated with 0,05 M HCl until the first point of the colour change from pink to colourless (pH = 8.3 -10) (equation 5)

$$Ba(OH)_2 + 2HCl \rightarrow BaCl_2 + 2H_2O \qquad (5)$$

Then total $BaCO_3$ was determined by titration to a second point marked by a change in colour from yellow to brown-yellow (indicator, methylorange, pH = 2.9 – 4.6, equation 6)

$$BaCO_3 + 2\ HCl \rightarrow BaCl_2 + H_2O + CO_2 \qquad (6)$$

All samples were titrated with 0,05 M HCl to the phenolphtalein end point (V_1) and then to the methylorange end point (V_2). From the difference between V_2 and V_1 the volume of 0.05 M HCl equivalent to the CO_2 was obtained V (ml) which was put in the following formula (7) and released CO_2 were calculated:

$$V\ (ml) * 0.05 * 44/24 = mg\ CO_2/h \qquad (7)$$

The equipment is shown in Figure 9. There were perforated pipes which were glued with green plastic bottles in each compartment. From each of the green plastic bottles there was a pipe which was connected with glass bottle containing the $Ba(OH)_2$ solution. The glass bottles were evacuated during CO_2 capture, with white plastic pipes connected to a suction pump.

3.2.6.3.2 Electron transport system (ETS) activity

The ETS activity (respiratory activity or INT reduction capacity) was measured spectrophotometrically as the rate of reduction of iodonitrotetrazolium chloride (INT) to formazan and converted to oxygen utilised per wet mass per hour using equation (8) below (Trevors, 1984). The ETS activity was considered to be an indicator of the microbial community's respiration capacity.

$$\text{ETS activity } (\mu l\ O_2/gh) = (Abs^{490\ nm} * V_r) / (S * t * 1.42 * V_t) \qquad (8)$$

$Abs^{490\ nm}$ – absorption of isobutanol fraction
V_r – final reaction volume
S – sample size (mass of soil)
t – incubation time
1.42 – factor of conversion to oxygen volume (Kenner and Ahmed, 1975)
V_t – volume of isobutanol

3.2.6.3.3 Optical density (OD)

Optical density (OD) of wastewater was measured spectrophotometricaly at 600 nm to determine the microbial cell density in the wastewater using Lambda Bio instrumentation (Lambda Bio, UV/VIS; Perkin Elmer Corp., Analytical Instruments, Connecticut, USA). One ml of wastewater was transferred to a cuvette for measurement.

Measurement of OD means measurement of reduction of light (photons) intensity when it travels through the matter. If sample contains particles which absorb light, the intensity of light traveling through matter is decreased. For measurement cells density in liquid medium OD is measured at 600 nm, because at this wavelength cells but not other particles in the medium reduce the intensity of light (Koprivnikar, 2008).

3.2.7 Data analysis of pilot plant

3.2.7.1 Statistical analyses

Student t test and analysis of variance (f test) were performed to compare different salinity levels, aeration-nonaeration and saccharose addition. Due to the nature of the data (high variability) significance level of $p = 0.05$ was chosen. Significant result, at the 95% probability level, tells us that our data are good enough to support a conclusion with 95% confidence. All statistics were calculated using the Statistical Package for Social Sciences (SPSS) version 18.0 for Windows (SPSS Inc., Chicago, IL, U.S.A.).

The correlation coefficient (R) and determination coefficient (R^2) were calculated using the MS Microsoft Excel program. The removal and reduction percentage statistics (mean value, standard deviation, minimum and maximum values) for COD, ammonia, phosphate and conductivity were also calculated using this program.

3.2.7.2 The kinetics of processes in pilot plant

The reaction rate constant (k) of selected biochemical parameters as a function of temperature was calculated using the following equation based on steady state perspective (Schnoor, 1996):

$$k = (c_{out}/c_{in}-1)/t_d \tag{9}$$

k - the reaction rate constant;
c_{out} – concentration at finally effluent;
c_{in} - concentration at influent;
t_d – hydraulic detention time;

where:

$$t_d = V/Q \tag{10}$$

V- volume of pilot plant;

Q - flow in pilot plant.

$$k_{T1} = k_{T2} \exp (E_{act}/RT_1T_2 (T_1 - T_2)) \tag{11}$$

In modeling environmental systems usually temperature range is from 0 to 35°C and under these conditions equation (11) simplifies to equation (12) because E_{act}/RT_1T_2 is approximately constant:

$$k_{T1} = k_{T2}\theta^{(T1-T2)} \tag{12}$$

θ – constant temperature coefficient (Schnoor, 1996).

The impact of temperature on the process duration was investigated in the range of 14-26°C. Kinetic equations were applied for the calculation of the k (9) and θ (12) for the most important biochemical parameters from pilot plant (COD, ammonium and phosphate) (Schnoor, 1996).

4 RESULTS AND DISCUSSION

Landfill leachates may contain dissolved organic matter and inorganic macro components at concentrations higher by factors of 1000 to 5000 than the concentrations found in groundwater (Kjeldsen *et al.*, 2002). High values of COD, BOD, ammonium ion, chloride ion and electrical conductivity were also found in leachates from Slovenian landfills, while there was only little or no phosphorous (Zupančič *et al.*, 2005). The concentration fluctuations were large and the performance of CWs varied with regard to inflow leachate concentration. The results reported by Zupančič *et al*. (2005) have shown that the CW system could be a low-cost alternative for leachate treatment from old landfill sites with leachates having lower concentrations of contaminants. Table 9 shows that CW Dragonja has the highest concentration of chloride ions at the influent and at the effluent of any of the CWs in Slovenia. One of the reasons is probably that leachates from the Dragonja Landfill normally have higher content of chloride ions. Also, CW Dragonja is located near the coast and the wind from the sea does transmit certain amounts of salt. Because CW Dragonja lies near the Adriatic Coast, it was chosen as a site to investigate the influence of wastewater salinity on CW efficiency.

4.1 Results of CW Dragonja

Physical and chemical parameters from the CW at the Dragonja landfill were measured monthly between 1992 and 2007 by the Institute of Public Health and analyzed in this work in order to investigate the influence of salinity on the CW efficiency.

4.1.1 The variability of physical and chemical parameters in CW Dragonja

4.1.1.1 The temperature, chloride , conductivity, pH and oxygen

Water temperatures at CW Dragonja ranged from 1°C during winter to 28°C during summer months with a mean 17°C at influent and 15°C at effluent 2 (Figure 16). The temperature did not significantly differ between influent and effluent 2 (Student t-test on log transformed data, $t=0.758$, $p=0.45$) (Table 13). Many individual wetland processes, such as microbially mediated reactions, are affected by temperature.

Response is much greater to changes at the lower end of the temperature scale (< 15ºC) than at the optimal range (20 to 35ºC). That means that all processes regulating organic matter decomposition and all nitrogen cycling reactions (mineralizations, nitrification and denitrification) are affected by temperature (Kadlec and Reddy, 2001).

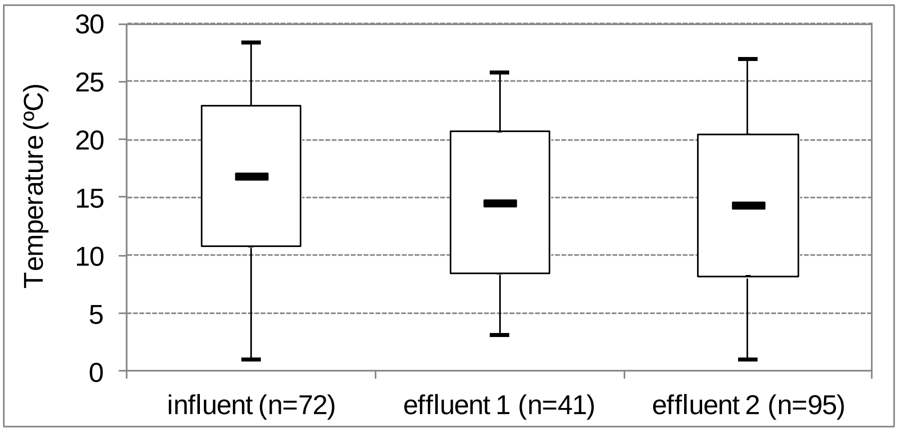


Figure 16: *Mean water temperatures (middle line) with standard deviation (box) and minimum (lower line) and maximum values (upper line) at CW Dragonja from 1993 to 2007 measurements.*

The changes in concentration of chloride ions and conductivity over time are shown in Figures 17 and 18. The greatest decreases in concentration of chloride ions and conductivity were at effluent 2. With higher conductivity at the influent, there was also higher conductivity at the effluents. This trend was similar to that with the concentration of chloride ions. This demonstrates that CW Dragonja reduces salinity.

Leachate from municipal landfills is typically enriched in Na^+, K^+, Ca^{2+}, Mg^{2+}, Sr^{2+}, Cl^-, HCO_3^{2-}, NH_4^+, Fe, and ^3H (Baedeker and Back 1979; Hackley *et al.*, 1996). Mean chloride ione concentrations in the influent was 1918 mg/l. It corresponds to a salinity of approximately 0.3% NaCl. Mean chloride ione concentrations in effluent 2 was 1338 mg/l (Table 13). Chloride ione concentrations in influent was significantly lower than in effluent 2 (Student t-test, t=1.659, p<0.001).

Mean conductivity in influent was 10 mS/cm, in the efluent 1 8.1 mS/cm, and in effluent 2 6 mS/cm (Table 13). It was significantly lower in effluent 2 (Student t-test, t=7.274, p<0.0001).

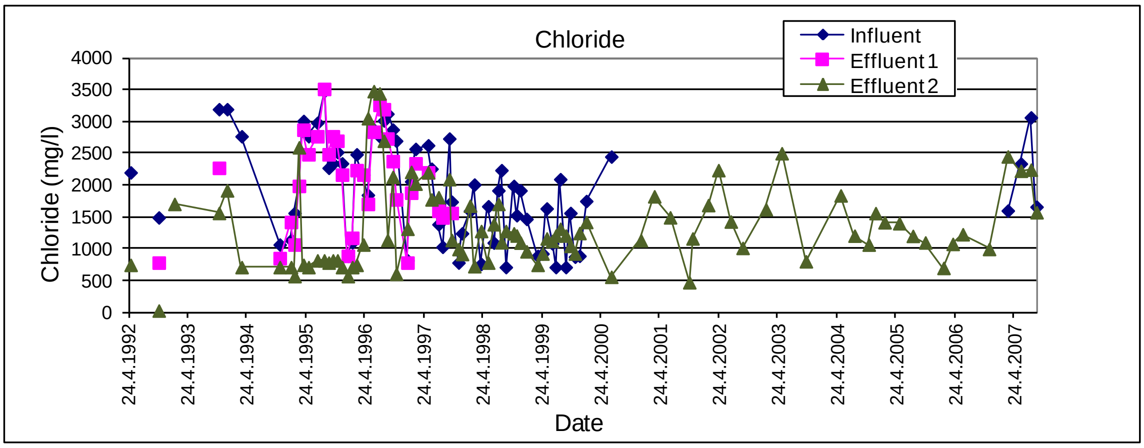


Figure 17: *The variations of chloride concentrations in the influent, the effluent 1 and the effluent 2 of CW Dragonja over time*

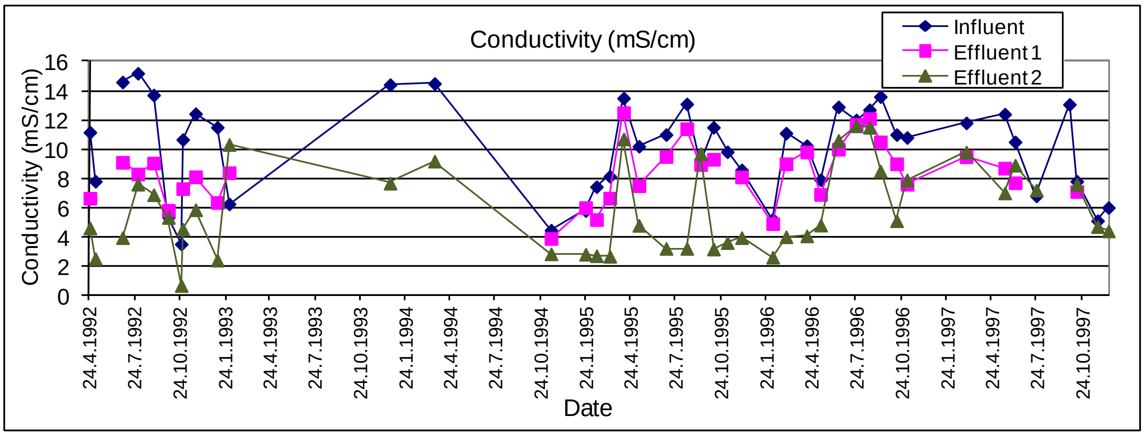


Figure 18: *The variations of conductivity in the influent, the effluent 1 and the effluent 2 of CW Dragonja over time*

According to Figure 19 some variations of pH occurred in CW Dragonja, but generally pH was neutral. The range of pH in the influent was from 7.1-8.5 and in the effluent 2 from 6.45-8.2. Nivala *et al.* (2007) investigated a SSF CW for landfill leachate and also found that pH values were generally neutral, even when significant nitrification occurred, indicating that the wetland system had a pronounced buffering capacity. A pH range of approximately 7.5-8.5 is optimal for the nitrification processes (Platzer, 1996). The influent average value of pH 7.9 means that the landfill is in the methanogenic phase (Christensen *et al.*, 2001). The maximum pH was 8.5 at the influent of CW, and the minimum pH was 6.5 at the effluent 2. The pH in SSF CW Dragonja decreased by 6.5%. These results, showing that pH of the wastewater decreases as a result of H^+ ion release when ammonium ion is oxidized, were also in the accordance with the conclusions of Villaverde *et al.* (1997). pH was significantly lower in effluent 2 in comparison to influent (Student t-test, t= 7.704, p<0.001)

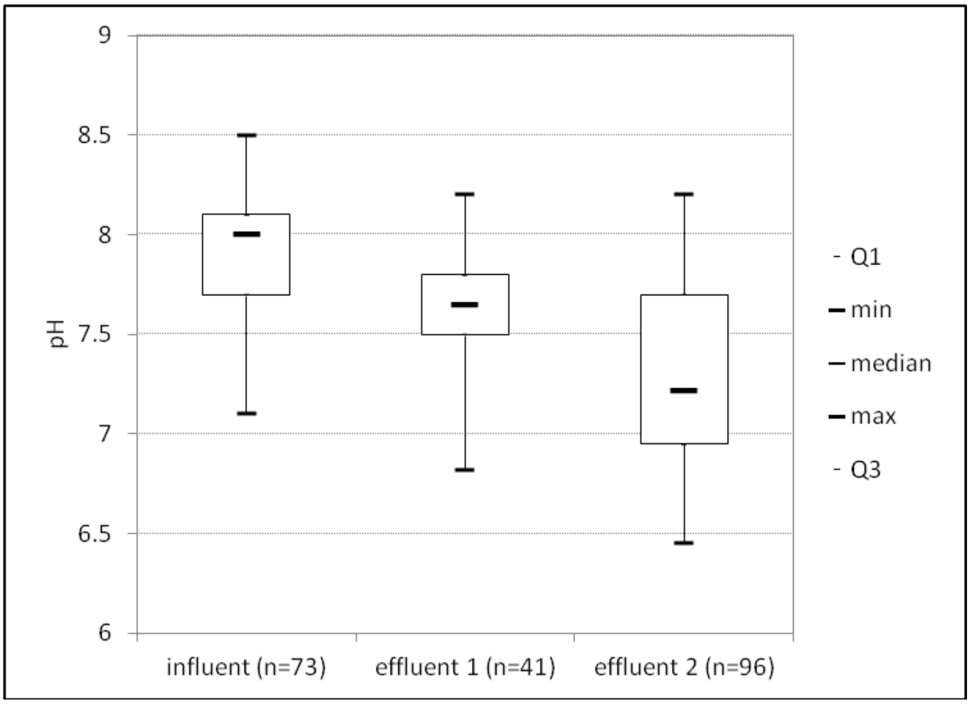


Figure 19: *Median (middle line) values for pH with first and third quartile (box), and minimum (lower line) and maximum (upper line) at CW Dragonja from 1993 to 2007*

HF SSF CW Dragonja as well as other HF SSF CWs can be considered as a mainly anaerobic system (Baptista, 2003; Vymazal, 2007). The average oxygen value in the influent wastewater of CW Dragonja was 1.53 mg/l and was not significantly different from the effluent 2 (Student t-test, t=-0.328; p=0.373). The minimum value was 0.2 mg/l (Figure 20). The oxygen content decreased through the CW, mainly because oxygen was consumed during the degradation processes. This was in accordance with Bulc (2006), who also noticed oxygen consumption during the processes in CW. The occurrence of anaerobic reactions is in agreement with the redox potential measurements that are on the average usually under -100 mV for all water depths (Garcia *et al.*, 2003); values higher than -100 mV were measured near the water surface while lower oxygen concentrations were measured near the bottom of CW. Results for the removal of ammonium, nitrate, nitrite and phosphate ions are also in accordance with anaerobic conditions in the CW.

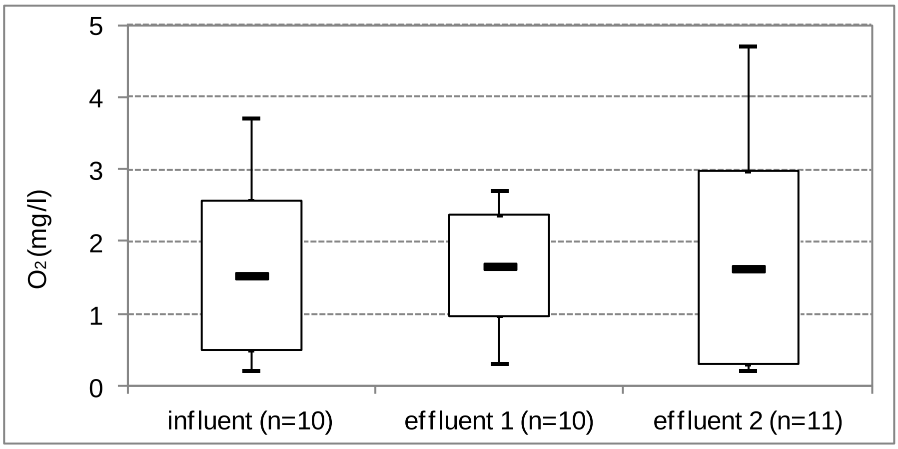


Figure 20: *Mean (middle line) values for oxygen concentrations with standard deviation (box), minimum (lower line) and maximum (upper line) at CW Dragonja from 1993 to 2007 measurments*

Table 13: *Means and variances for measured parameters at CW Dragonja from 1993 to 2007*

	Mean	**Variance**	**Sample size**
Temperature			
influent	17	39	65
effluent 1	13	44	30
effluent 2	15	27	35
Chloride ion			
influent	1918	594887	56
effluent 1	1207	118409	28
effluent 2	1338	919980	26
Conductivity			
influent	10	9	41
effluent 1	6	7	6
effluent 2	6	9	35
Oxygen			
influent	1.53	1.09	9.00
effluent 1	/		1.00
effluent 2	1.56	1.88	8.00

4.1.1.2 The nutrients in CW Dragonja

Figure 21 shows the variability of COD at the influent and at the effluents of CW Dragonja. The mean value of COD at the influent was 1001mg/l and at the effluent 2 was 473 mg/l (Table 15). From Figure 21 it is apparent that higher concentration of COD at the influent means higher concentration at the effluent 2 of CW. It can also be seen that fluctuations at the influent COD are more intensive, but effluent 2 COD does not vary as greatly.

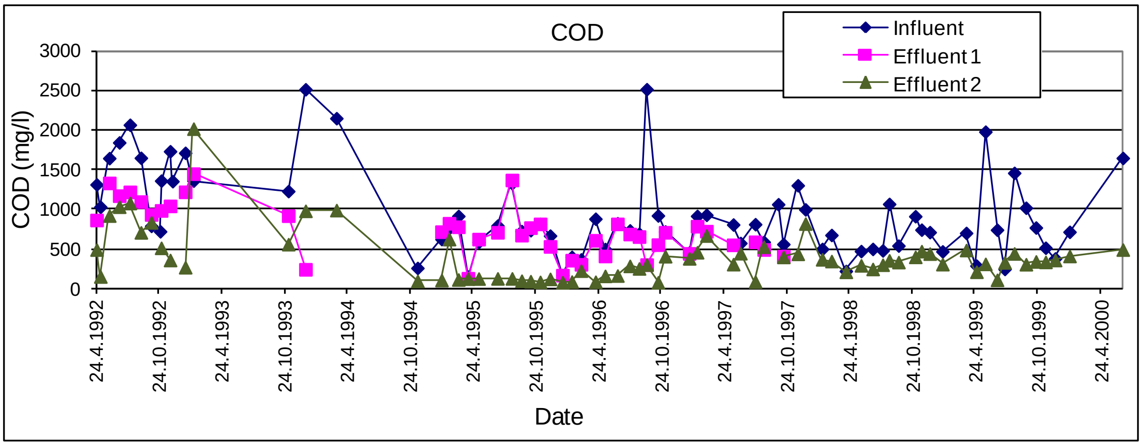


Figure 21: *The variations of COD concentrations in the influent, the effluent 1 and the effluent 2 of CW Dragonja over time*

From the changes of BOD_5 shown in Figure 22, it is obvious that the influent concentrations of organic matter were higher than those at the effluents. Mean values at the influent were 110 mg/l and at effluent 2 was reduced to 42 mg/l (Table 15).

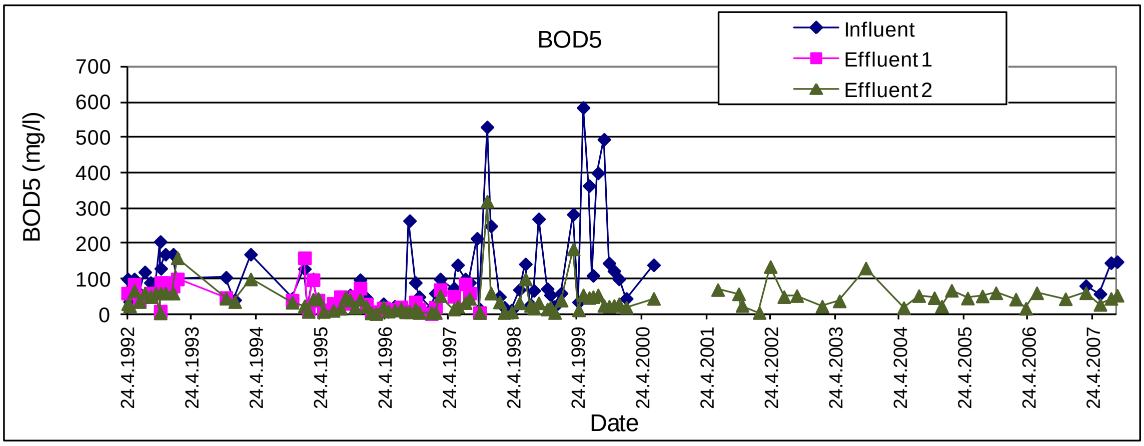


Figure 22: *The variations of BOD_5 concentrations in the influent, the effluent 1 and the effluent 2 of CW Dragonja over time*

Changes in the ammonium ion concentration at the influent and at the effluents are shown in Figure 23. The reduction of ammonium ion concentration at the effluent 2 can be seen as evidence of nitrification in the CW. Mean values at the influent were 383 mg/l and at the effluent 2 it was reduced to 138 mg/l (Table 15).

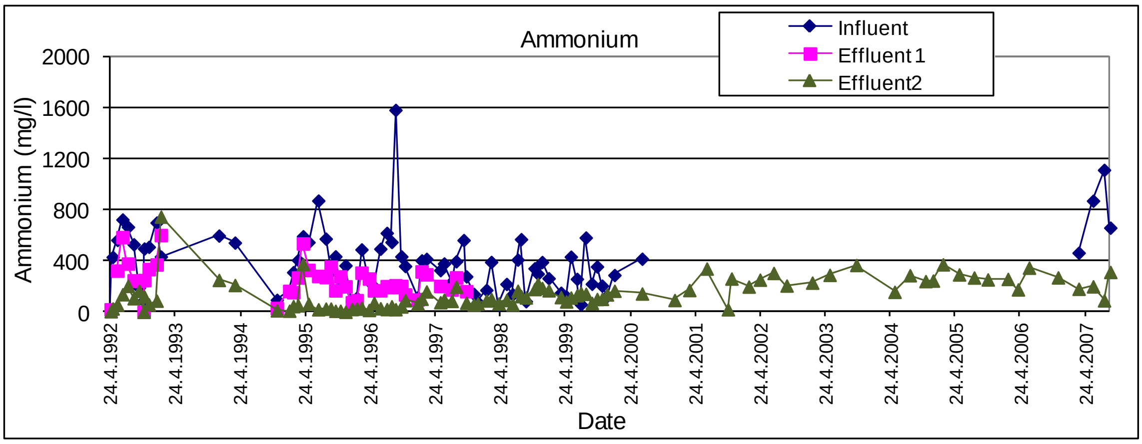


Figure 23: *The variations of ammonium ion concentrations in the influent, the effluent 1 and the effluent 2 of CW Dragonja over time*

From Figure 21, 22 and 23 could be noticed that values from effleuent 2 in the period 2001 to 2007 were, at least in part, higher than between 1995 and 1999/2000. The reason for that is connection new part of landfill to CW Dragonja at the beginning of 2000 year.

Figure 24 presents ammonium, nitrate and nitrite ions variations in the effluents. It can be seen that concentrations of ammonium ions are mostly higher then concentrations of nitrate and nitrite ions in the effluents. This is expected, because concentrations of ammonium were higher then concentrations of nitrate and nitrite (Table 15). The reduction of ammonium ion concentration at the effluent 2 can be seen as evidence of nitrification in the CW. Mean influent concentration was 383 mg/l and at the effluent 2 it was reduced by 50% (Table 15). However, the effluent values for ammonium ion concentrations were still higher than the permitted values (OG RS 35/96, 21/03, 7/00). Removal efficiency of ammonium ion was lower than the removal efficiency for organic matter (COD and BOD_5). Similarly, Bulc and Zupančič (2007) found that compared with the organic matter, ammonium ion is more difficult to remove in CW as nitrification bacteria are autotrophic microorganisms that have a slow respiration rate and require a considerable amount of oxygen to function. Removal of ammonium ion in CW, from a highly concentrated landfill leachate, is therefore extremely complicated, because the main treatment mechanisms of nitrification and aerobic removal of organic material are oxygen-limited processes. They involve a series of physical, chemical and biological processes such as adsorption inside the bed matrix, filtration, precipitation, sedimentation, ion exchange, plant sorption and microbiological reactions (Connolly *et al.*, 2004). Studies have shown that plant-mediated oxygen-transfer rates are very small relative to the oxygen demand exerted by the wastewater under common loading conditions. As a result, many current wetland designs neglect plant-mediated oxygen transfer altogether (Balizon *et al.*, 2002; Scholz and Xu, 2002; Scholz, 2006), leading to the development of enhanced treatment systems that are capable of providing sufficient oxygen transfer for nitrification and removal of organic material, introducing oxygen to the system through frequent water level fluctuation, passive air pumps or direct mechanical aeration of the water in the gravel bed (Nivala *et al.*, 2007). Therefore, when CW Dragonja is renovated, installation of an additional system for aeration of wastewater or construction of the hybrid CW system will be strongly recommended to improve CW performance.

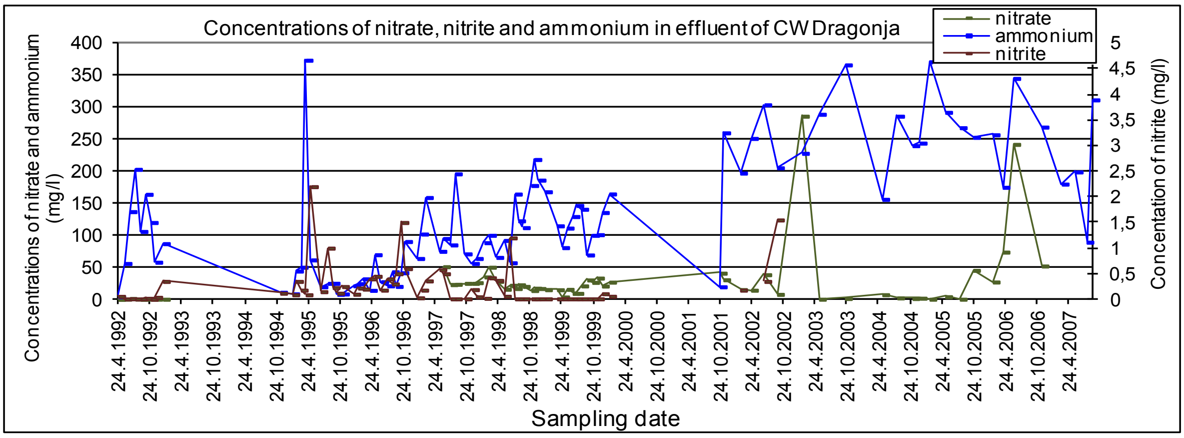


Figure 24: *The variations of nitrate, nitrite and ammonium ion concentrations in the effluent 2 of CW Dragonja over time*

In CW Dragonja phosphorus concentrations were very small to non-detectable. Very low phosphorus concentrations additionally limited biological processes and had a negative influence on the plant growth and overall treatment efficiency (Bulc, 1998). Figures 25 and 26 show variations of phosphate and total P concentrations. In general the influent concentrations were higher than the effluents.

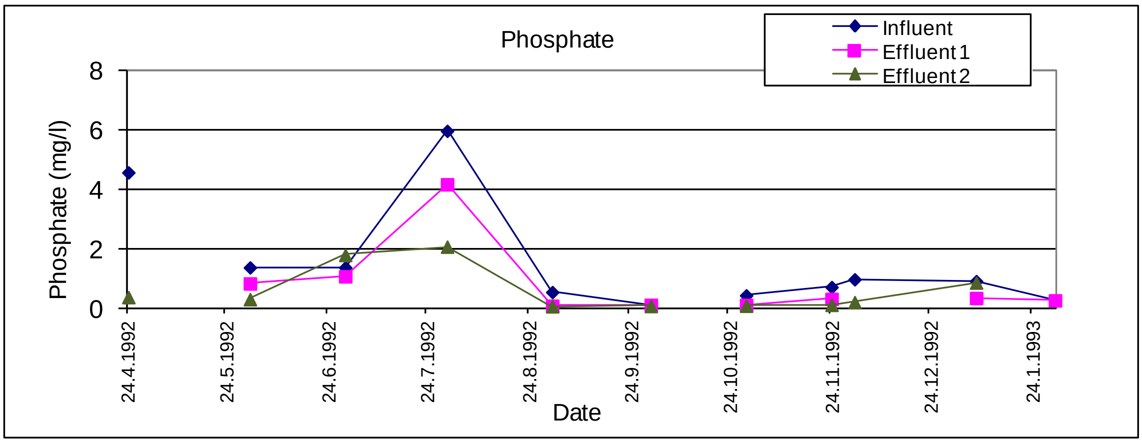


Figure 25: *The variations of phosphate concentrations in the influent, the effluent 1 and the effluent 2 of CW Dragonja over time*

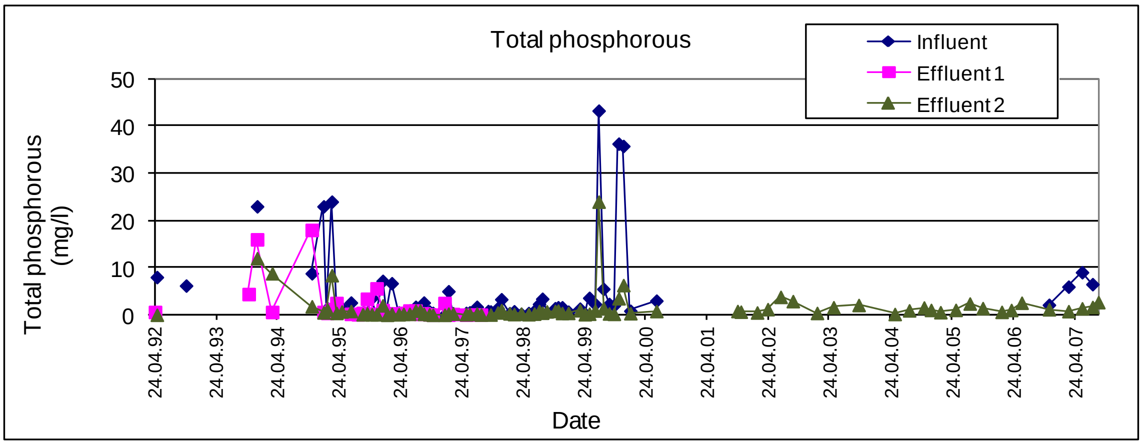


Figure 26: *The variation of total phosphorus concentrations in the influent, the effluent 1 and the effluent 2 of CW Dragonja over time*

In general, influent water chemistry variability to CW Dragonja was extremely high as calculated by PCA as shown in Figure 27. PCA on the water chemistry at the inflow explained 57.9 % of the variance in the data by the first two ordination axes (Axis 1: 37.1%; Axis 2: 20.9%, Figure 27). The first axis was primarily explaining positive gradient in ammonium, COD, and chloride ions, while the second axis explained primarily a gradient in BOD_5. There was no strong gradient in salinity across the time and salinity was moderately correlated with ammonium. Other studies also shown that landfill leachates are highly variable in composition mostly influenced by a number of waste- and site-specific factors, such as refuse composition, age of the landfill and climate (Hernandez *et al.*, 1999).

Comparison of water chemistry between samples taken in the influent and effluent 2 revealed the separation of those two groups on the basis of ammonium and Total Klejdahl Nitrogen (TKN) (Figure 28).

The first PCA axis explained 38.5 % and the second axis 18.6 % of the variability in the data. The second axis was in the positive correlation with nitrate and nitrite ions. Similarly, high variability between parameters in effluent 2 and influent of CW was found in other studies (e.c. Song *et al.*, 2006, Campell and Ogden, 1999). The positive correlation with nitrate and nitrite ions was found in Poe *et al*., 2003.

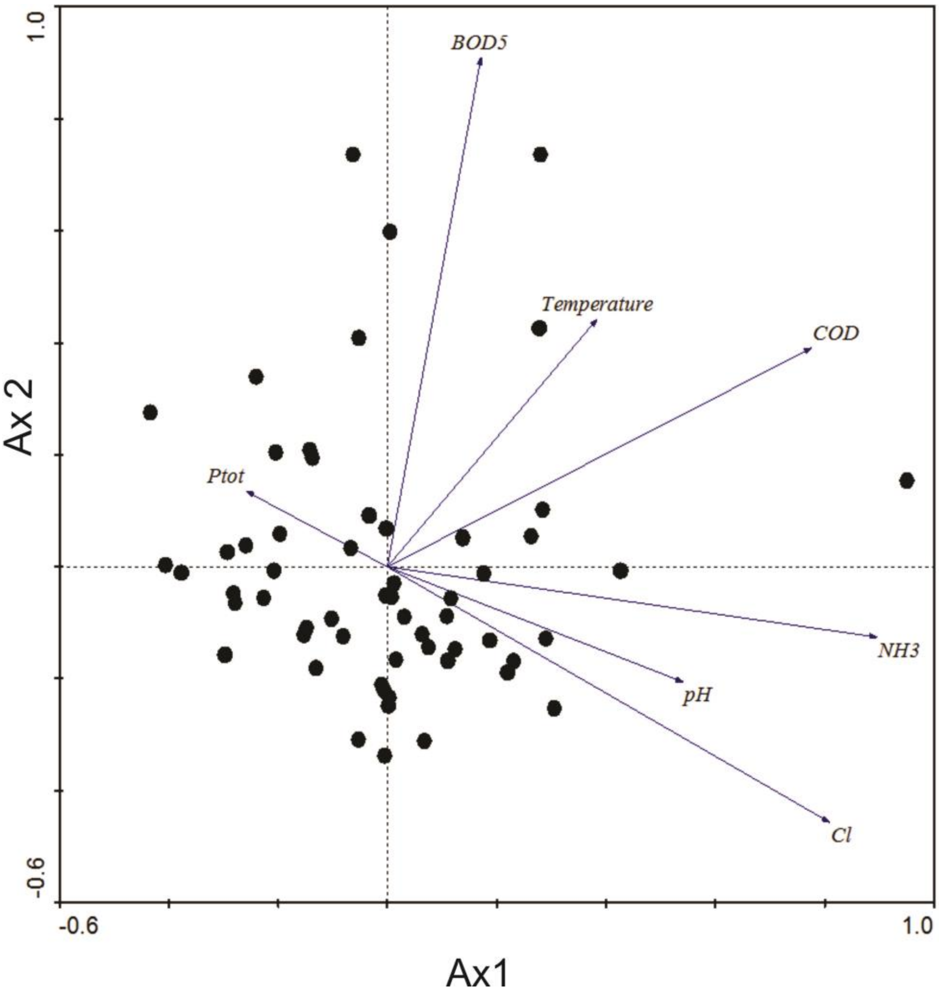


Figure 27: *PCA ordination diagram with black dots representing water samples taken at the inflow of CW Dragonja on 58 occasions from 1992 to 2000 and arrows indicating chemical parameters measured in the wastewater on those sampling occasions*

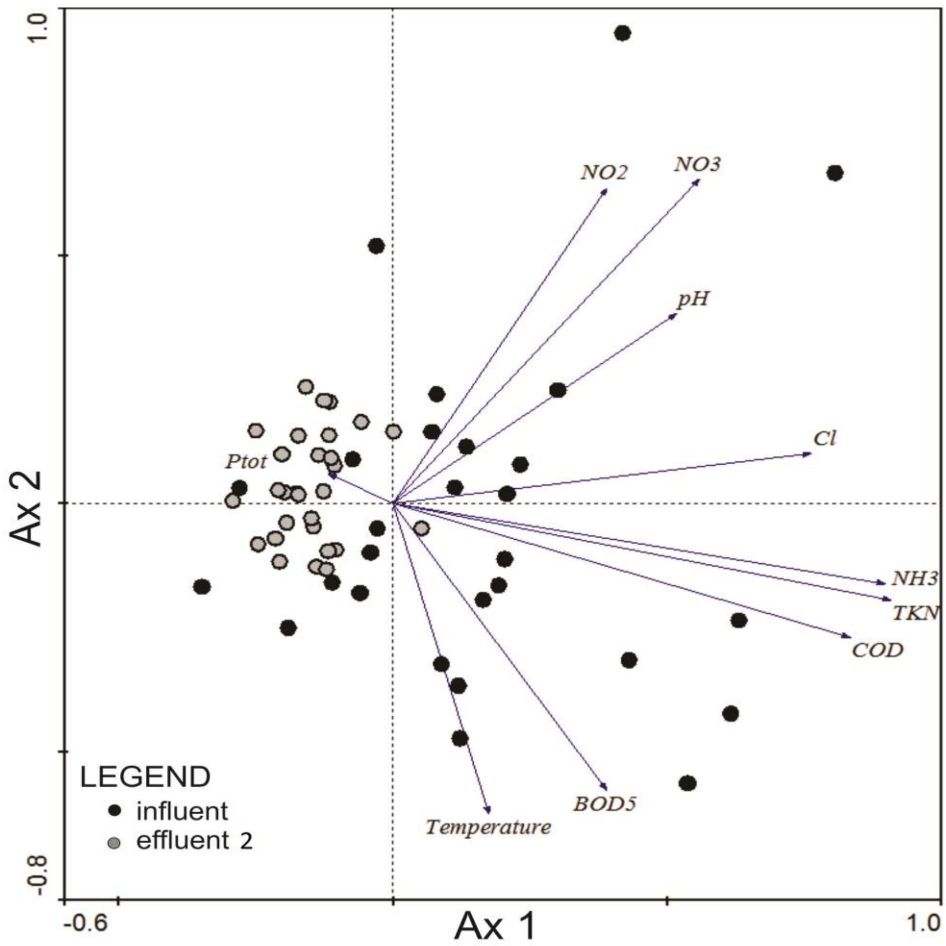


Figure 28: *PCA ordination diagram with black dots representing water samples taken at the inflow of CW Dragonja and grey dots representing samples taken at the effluent 2 on 57 occasions from 1997 to 2000 and arrows indicating variability in chemical parameters*

The PCA shows that variabilities of other parameters in the influent wastewater to CW Dragonja are high. Beacuse of that influence of different salinity could not be separate clearly from influence of other parameters on the removal efficiency of nutrients.

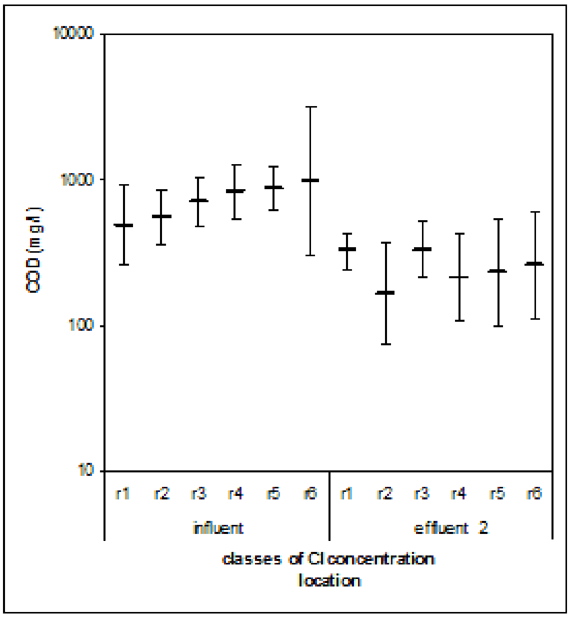
The two-way ANOVA with the location of the measurement (influent/effluent 2) and salinity as the factors was used to test the significance of those two factors for the difference in COD, BOD_5, total P, total N and the concentrations of ammonium, nitrite, and nitrate ions at CW Dragonja. Concentrations of chloride ions were divided into six classes: C-1 < 500 mg/l; C-2 500-1000 mg/l; C-3 1000-1500 mg/l; C-4 1500-2000 mg/l; C-5 2000-2500 mg/l; and C-6 > 2500 mg/l (Table 14, Figure 29 and 30). Low p-values ($p<0.05$) indicated that concentrations of chloride ions affect concentrations of nitrogen compounds (ammonium, nitrite, and nitrate ions and total N). This is in accordance with the results of previous investigators (Dahl *et al.*, 1997; Dincer and Kargi, 1999; Panswad and Anan, 1999) who found that concentrations of chloride ions affected processes such as nitrification and denitrification in biological wastewater treatment. All measured parameters were significantly different between influent and effluent 2, indicating that during wastewater flow through CW, significant reduction of parameters like COD, BOD_5, total P, NH_4^+, NO_2^-, NO_3^- and total N, occurred. Concentrations of chloride ions and location (influent or effluent 2) significantly affected pH, ammonium ion concentrations and total nitrogen.

Table 14: *A summary of two-way ANOVA for comparing parameters of CW Dragonja with location (influent and effluent 2) and concentrations of chloride ions as factors*

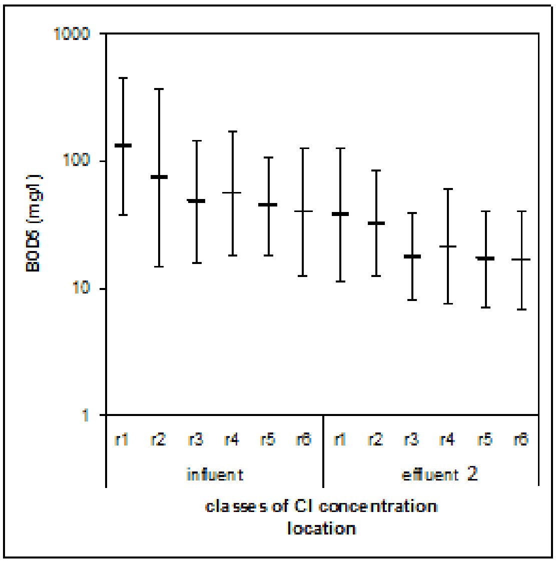
	Location	**Cl**	**Location and Cl**
COD	***	NS	NS
BOD_5	***	NS	NS
total P	***	NS	NS
NH_3	***	*	***
NO_2	*	***	NS
NO_3	*	**	NS
total N	***	**	*

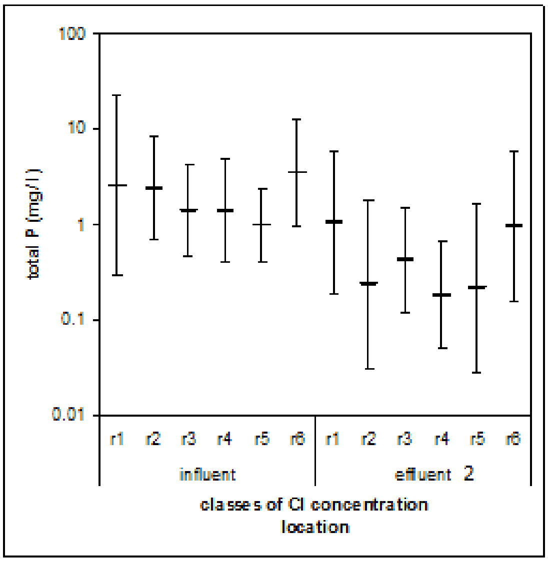
Note: Location=influent or effluent; Concentrations of Cl was devided in 6 classes: Cl1<500 mg/l; Cl2: 500-1000 mg/l; Cl3: 1000-1500 mg/l; Cl4: 1500-2000 mg/l; Cl5: 2000-2500 mg/l; Cl6>2500 mg/l; NS=not significant; p<0.05=*; p<0.01**; p<0.001***

On the basis of the data from CW Dragonja, it can not be concluded that high effluent 2 pollutant concentrations are caused by the high salinities of the water. The correlation coefficients between salinity and several parameters (BOD_5, COD, PO_4^{3-}, NO_3^-, NH_4^+) as well as the correlation coefficients with the removal efficiencies (Annex A1: Table 18 and 19; Annex A2: Table 20) are very weak suggesting that varying chloride concentration can explain only a small percent of the variability in pollutant removal.



a) $p_l = 0.000$; $p_c = 0.204$; $p_{lxc} = 0.094$



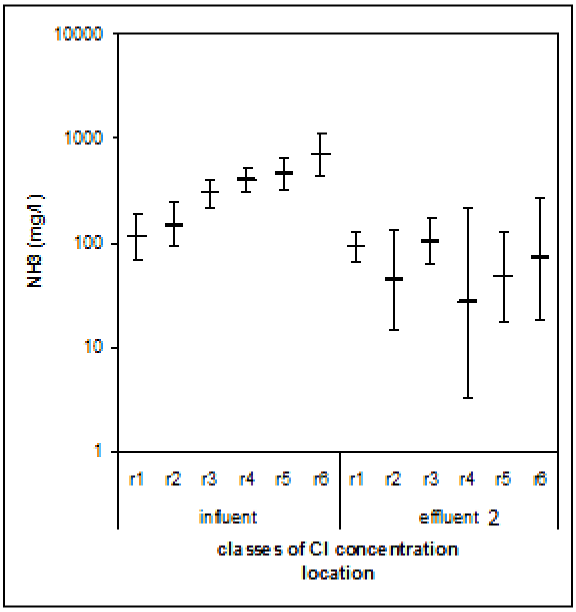


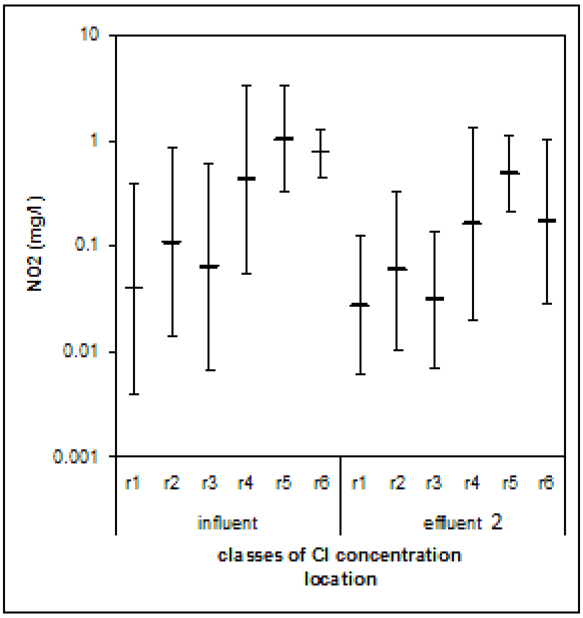
b) $p_l = 0.000$; $p_c = 0.050$; $p_{lxc} = 0.995$

c) $p_l = 0.000$; $p_c = 0.065$; $p_{lxc} = 0.723$

Note: Concentrations of Cl was devided in 6 classes: Cl1<500 mg/l; Cl2: 500-1000 mg/l; Cl3: 1000-1500 mg/l; Cl4: 1500-2000 mg/l; Cl5: 2000-2500 mg/l; Cl6>2500 mg/l. Location=influent or effluent 2

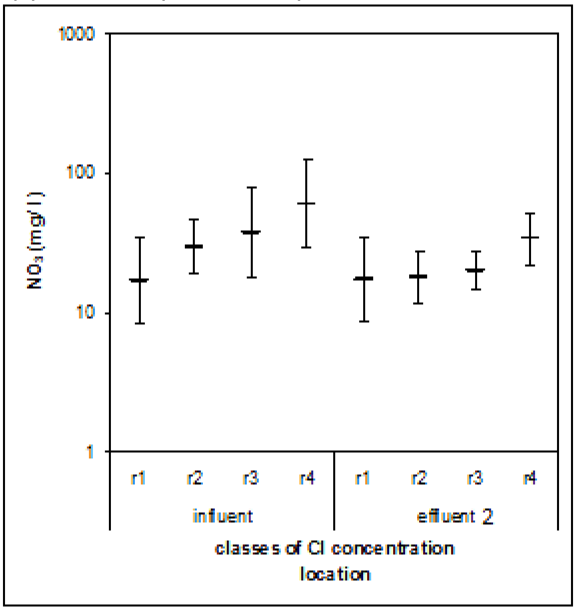
Figure 29: *Mean values (±SD) for physical and chemical parameters: a) COD, b) BOD_5, c) total P with different classes of concentration of chloride ions (c) and location (l) as factors for two-way ANOVA*

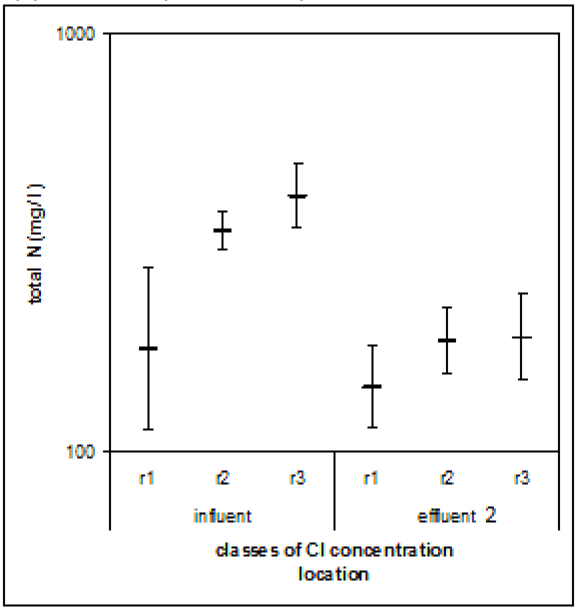




a) $p_l = 0.000$; $p_c = 0.020$; $p_{lxc} = 0.000$

b) $p_l = 0.028$; $p_c = 0.000$; $p_{lxc} = 0.978$





c) $p_l = 0.014$; $p_c = 0.002$; $p_{lxc} = 0.368$

d) $p_c = 0.000$; $p_l = 0.000$; $p_{lxc} = 0.042$

Note: Concentrations of Cl was devided in 6 classes: Cl1<500 mg/l; Cl2: 500-1000 mg/l; Cl3: 1000-1500 mg/l; Cl4: 1500-2000 mg/l; Cl5: 2000-2500 mg/l; Cl6>2500 mg/l. Location=influent or effluent 2

Figure 30: *Mean values (±SD) for physical and chemical parameters: a) ammonium, b) nitrate c) nitrite and d) total N with different classes of concentration of chloride ions (c) and location (l) as factors for two-way ANOVA*

The correlation coefficients are probably the consequence of changes to flow rate and concentrations of pollutants at the influent due to the rain or drought. A further reason could also be a complex composition of landfill leachate where many other pollutants could be present and have greater inhibitory influences on the removal efficiencies than does salinity, thereby hindering investigation of salinity influence. For this reason, we used a synthetic wastewater (leachet) in our experiments with pilot plant to research the influence of salinity on the efficiency under constant and controlled conditions.

4.1.1.3 Salinity and nutrients in CW Dragonja

In general, from the literature review, high salinities have negative effects on organic matter and nitrogen removal (Kargi and Dincer, 1996; Panswad and Annan, 1999; Glass and Silertsein, 1999; Intrasungkha *et al.*, 1999). Panswad and Anan (1999) found that nitrification is the process most sensitive to sudden increases in salinity. Therefore, concentrations of NH_3, NO_3, COD, BOD_5 and chloride ions were correlated at the effluent of CW Dragonja. It is evident from Figures 31, 32, 33 and 34 that with higher concentration of chloride ions at the effluent 2, the concentration of ammonium ions, nitrate ions, COD and BOD_5 also increases.

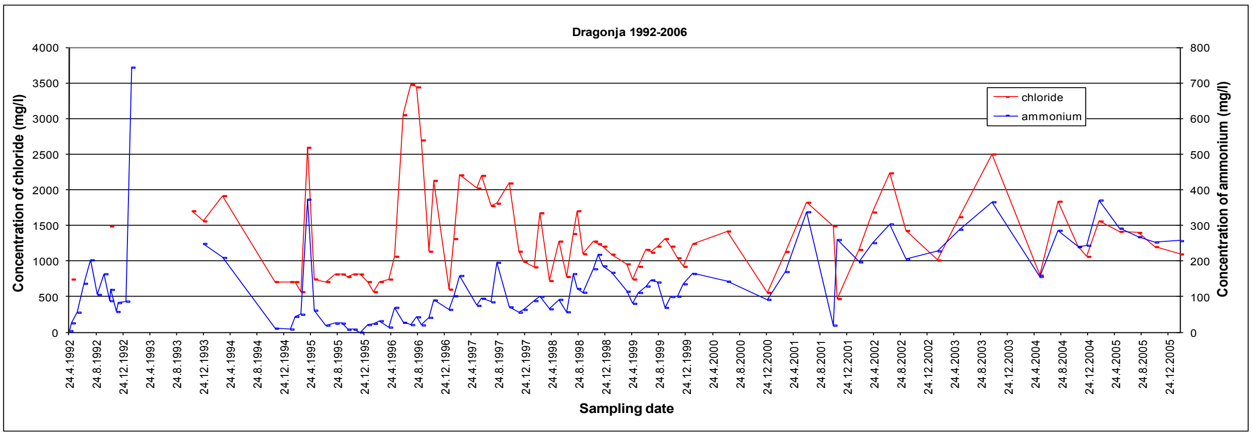


Figure 31: *The concentrations of chloride and ammonium ions in the effluent 2 of CW Dragonja over time*

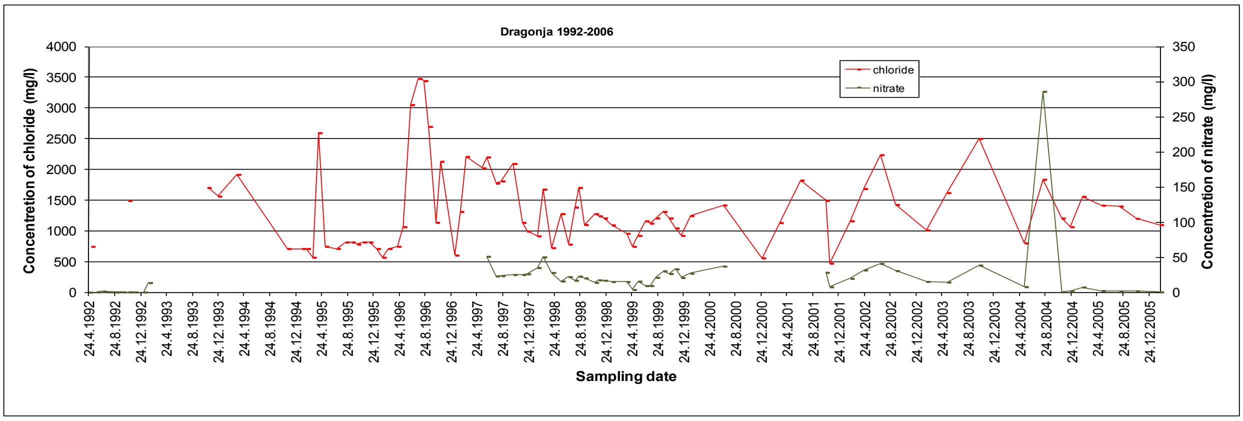


Figure 32: *The concentrations of chloride ions and nitrate in the effluent 2 of CW Dragonja over time*

Although the reduction of ammonium ion concentration, COD and BOD_5 in the CW could be difficult because the complex mixture of organic compounds at the influent could be resistant to biological degradation, and available carbon could limit denitrification, Figures 33 and 34 show increases in the concentrations of organic matter (in terms of COD and BOD_5) with increased chloride ion concentrations at the effluent 2.

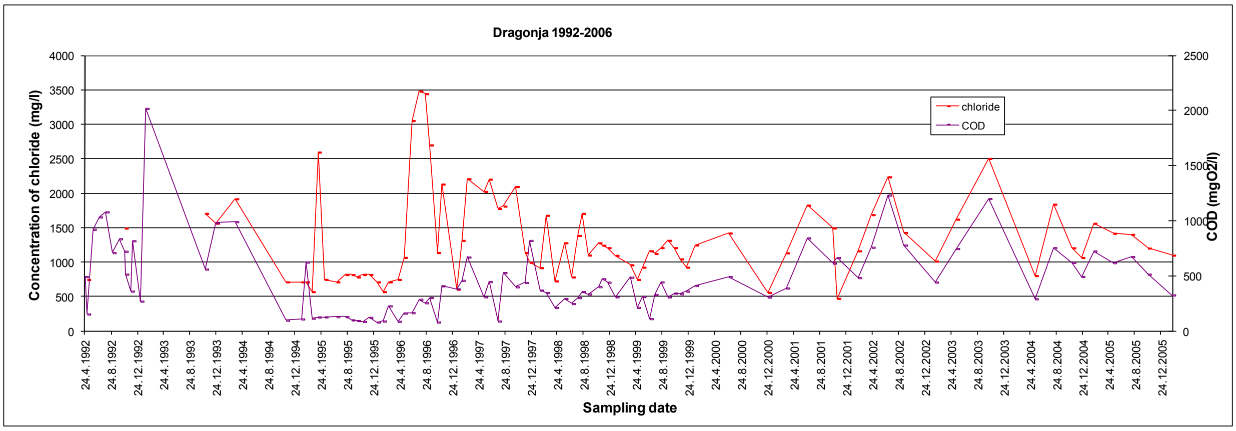


Figure 33: *The concentrations of chloride and COD in the effluent 2 of CW Dragonja over time*

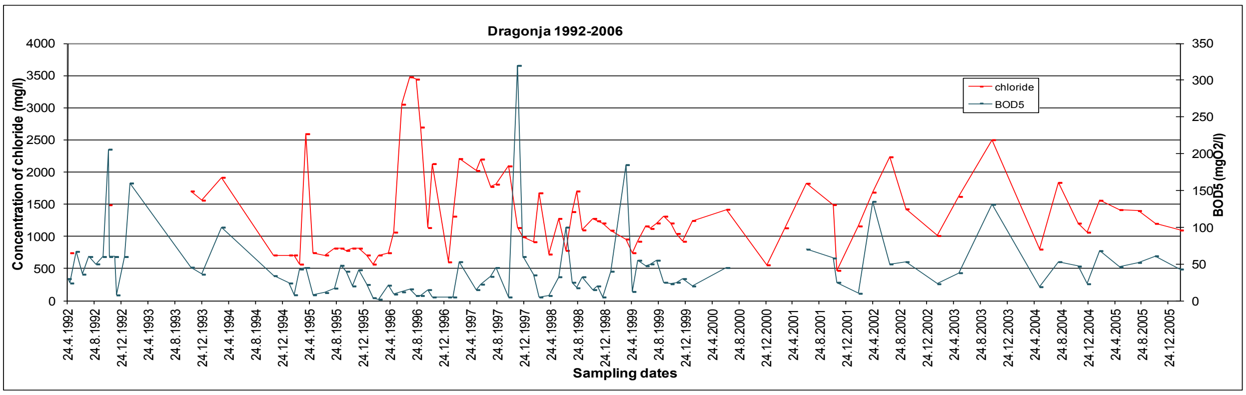


Figure 34: *The concentrations of chloride ions and BOD_5 in the effluent 2 of CW Dragonja over time*

Lin, *et al* (2008) reported a similar observation. They found increased salinity affected the growth of some bacteria which resulted in changes in their relative numbers in the microbial community. This apparent impact showed salinity impacted the metabolism of microorganisms critical to the proper functioning and maintenance of the system. Previously Nitisoravut and Klomjek (2005) proposed salt had a negative impact on biological degradation rates of organic compounds in wastewater, and they attributed the reduced rates of BOD removal to the inhibition of microbial enzymes and microbial growth.

4.1.1.4 The removal efficiency of CW Dragonja

The influent and effluent 2 concentrations and percent removal statistics (mean value, standard deviation, minimum and maximum values) for BOD_5, COD, TN, NH_4-N, NO_3-N, NO_2-N, Cl, conductivity, total P and PO_4 are presented in Table 18. The greatest removal efficiency among the various constituents was for the total phosphorus (68%), orthophosphate (64%), COD (53%), BOD_5 (52%) and NH_4^+-N (50%) (Table 15). Negative removal efficiency (i.e., production) for nitrate ion and nitrite ion would be an expected result of nitrification wherein a portion of the ammonia in the influent wastewater was-nitrified into nitrate and nitrite ions which gave higher concentrations of those two parameters at the effluent 2 compared to the influent. CW Dragonja is HF SSF CW, where anaerobic conditions prevail and the process of denitrification is very intensive, ammonification is high, but nitrification level is very low (Table 15) (Vymazal, 2007). Consequently, final removal efficiencies of nitrate and nitrite ions were negative. Negative removal efficiencies are therefore probably the result of low concentration of oxygen in CW Dragonja and of small quantity of easily accessible organic compounds. Since most dentrifying bacteria are chemoheterotrophs, it suggests that the carbon source was insufficient for denitrificators. They obtain energy solely through chemical reactions and use organic compounds as electron donors and as a source of cellular carbon (Hauck, 1984).

All parameters varied substantially in concentrations and removal efficiency during the operation period as shown by the relative high values for the standard deviations (Table 15). This variability occur because the microorganisms and plants, responsible for removal efficiency depend on temperature, dilution of wastewater, retention time and salinity of wastewater which varied especially during rain and temperature changes (Akratos and Tsihrintzis, 2007). During rain, wastewater is diluted and salinity decreased, but in the summer during higher temperatures with higher evaporation and low precipitation, salinity in the wastewater increases (Sartaj *et al.,* 1998). Similarly, Bulc (1998) concluded for CW Dragonja that during the rain, wastewater is diluted and salinity decreased, but during dry periods the situation is reversed.

The mean value of reductions in conductivity and chloride ion concentrations were 39% and 24%, respectively (Table 15). Similarly, Bulc (2006) reported 35% reduction in the concentration of chloride ions. Reduction of salinity could be explained by assuming the adsorption of salt ions, mostly sodium and chloride ions, on porous media of CW. This reduction of salinity by SSF CW as a low cost technology is interesting for consideration in reuse of wastewater, because in most cases, salinity is a major problem for reuse purposes such as irrigation (Zupančič-Justin, 2006).

Table 15: *The influent and the effluent concentrations and removal efficiencies in each unit of CW Dragonja*

	Influent	Effluent 1	Effluent 2	Removal (%) Effluent 1	Removal (%) Effluent 2
Mean of **Conductivity (mS/cm)**	10.0	8.1	6.0	21	**39**
SD	3.3	2.3	2.9	13	29
Min	3.5	2.1	0.7	0.6	*Neg.*
Max	18.0	13.0	12.0	45	81
Mean of **Cl (mg/l)**	1918	2051	1338	4.3	**24**
SD	778	775	654	18	38
Min	710	781	27	*neg.*	*Neg.*
Max	3479	3514	3479	48	49
Mean of **COD (mg/l)**	1001	703	473	21	**53**
SD	727	346	332	17	30
Min	151	99	79	0	*Neg.*
Max	5220	2018	1450	88	98
Mean of **BOD_5 (mg/l)**	110	45	42	30	**52**
SD	118	33	42	61	35
Min	2	2	2	*neg.*	*Neg.*
Max	585	160	320	96	98
Mean of **PO_4 (mg/l)**	1.6	0.7	0.6	47.3	**64**
SD	1.9	1.3		22	92
Min	0.1	0.1		21	*Neg.*
Max	6.0	4.2		81	88
Mean of **total P (mg/l)**	4.90	1.90	1.40	46	**68**
SD	9.00	4.20	3.10	27	29
Min	0.03	0.02	0.00	*neg.*	*Neg.*
Max	43	18	24	96	98
Mean of **NH_4 (mg/l)**	383	239	138	32	**50**
SD	258	135	116	35	46
Min	11.00	0.14	0.00	*neg.*	*Neg.*
Max	1579	600	744	60	70
Mean of **NO_2 (mg/l)**	0.800	0.880	0.300	*neg.*	***Neg.***
SD	1.400	2.000	0.550	312	466
Min	0.002	0.002	0.001	*neg.*	*Neg.*
Max	8.2	12.5	3.2	85	88
Mean of **NO_3 (mg/l)**	33	7.6	36	39	***Neg.***
SD	43	12	74	117	1574
Min	0.26	0.03	0.01	*neg.*	*Neg.*
Max	222	35	424	99	99
Mean of total **N (mg/l)**	327		170		**36**
SD	159		39		44
Min	80		99		*neg..*
Max	688		248		75

Legend: neg.= negative

The mean removal efficiency of COD was 53% and the mean removal efficiency of BOD_5 was 52% (Table 15). Similarly, another previous study of landfill leachate CW showed that removal efficiency for COD was 50% and for BOD_5 59% (Bulc, 2006).

This is in accordance with our results, which showed higher removal efficiency for BOD_5 (53%) than for COD (50%).

Removal efficiency of total phosphorus and phosphate were 68% and 64%, respectively (Table 15). Total phosphorus and phosphate are mainly removed by plant uptake and adsorption on porous media (Kadlec and Knight, 1996). Low removal efficiency could be explained with the fact that reducing conditions (i.e. lack of oxygen, anaerobic conditions) can lead to solubilisation of minerals and release of phosphorus (Reed *et al.*, 1995; Kadlec and Knight, 1996). Bulc (2006) also noticed that concentration of total phosphorus at the effluent increased by the amount of precipitation.

Mean influent concentration of ammonium was 383 mg/l and at the effluent 2 it was reduced by 50%. However, the effluent 2 values for ammonium ion concentrations were still higher than the permitted values (OG RS 35/96, 21/03, 7/00). Removal efficiency of ammonium ion was lower than the removal efficiency for organic matter (COD and BOD_5). Similarly, Bulc and Zupančič (2007) found that compared with the organic matter, ammonium ion is more difficult to remove in CW as nitrification bacteria are autotrophic microorganisms that have a slow respiration rate and require a considerable amount of oxygen to function. Removal of ammonium ion in CW, from a highly concentrated landfill leachate, is therefore extremely complicated, because main treatment mechanisms of nitrification and aerobic removal of organic material are oxygen-limited processes. They involve a series of physical, chemical and biological processes such as adsorption inside the bed matrix, filtration, precipitation, sedimentation, ion exchange, plant sorption and microbiological reactions (Connolly *et al.*, 2004). Studies have shown that plant-mediated oxygen-transfer rates are very small relative to the oxygen demand exerted by the wastewater under common loading conditions. As a result, many current wetland designs neglect plant-mediated oxygen transfer altogether (Balizon *et al.*, 2002; Scholz and Xu, 2002; Scholz, 2006) leading to the development of enhanced treatment systems, capable of providing sufficient oxygen transfer for nitrification and removal of organic material, introducing oxygen to the system through frequent water level fluctuation, passive air pumps or direct mechanical aeration of the water in the gravel bed (Nivala *et al.*, 2007). Therefore, when CW

Dragonja is renovated, installation of an additional system for aeration of wastewater or construction of the hybrid CW system will be strongly recommended to improve CW performance.

Negative removal efficiency for nitrate could be noted in Table 15, similarly as in Bulc (2006). This indicates intensive nitrification of ammonium ions occurred at the CW Dragonja while denitrification level was very low, consequently concentration of the nitrate ion increased. The average value of total nitrogen removal in CW Dragonja during two years (from 1998 to 2000 year) of monitoring was 36%, therefore nitrogen removal was more efficient than the negative nitrate ion and nitrite ion removal but less then ammonia removal. It confirms that total nitrogen removal alongside denitrification as major removal mechanisms were plant uptake, ammmonia volatilization, amonia adsorption and organic nitrogen burial. This is in accordance with Vymazal (2007), who wrote that other processes (e.g. ammonification or nitrification) »only« convert nitrogen among various nitrogen forms, but do not actually remove nitrogen from the wastewater.

A value of 36% for total nitrogen removal is in accordance with Vymazal (2007) who wrote that total removal of nitrogen in HF SSF CW is around 40% and in various types of CW it varies between 40-50%. Although the removal efficiency of ammonia had a mean value of 50%, the effluent 2 values were above the permitted values for outflow into recipient (OG RS 47/05, 7/00).

4.2 Results of pilot plant studies

The studies on freshwater and terrestrially derived conventional treatment microbial cultures indicate that these microorganisms are not genetically capable of surviving in hypersaline environments (Woolard and Irvine, 1995; Lefebvre and Moletta, 2006). Thus biological removal of organics from saline wastewater without dilution would require specialized microbes. Natural hypersaline environments contain complex and diverse communities of such organisms. These halotolerant and halophilic microbes are metabolically diverse and possess many of the same characteristics as conventional waste treatment cultures. Although nonhalophilic and marine bacteria can be found in these environments, it is the true halophiles that are of particular interest for treating hypersaline wastewater (Woolard and Irvine, 1995). The application of salt-tolerant microorganisms to conventional biological processes has been mentioned in the literature, but there is little information about their application in low-cost technology such as CW.

The high concentrations of nutrients and organic matter and the high concentration of salt and its oscillations during rainstorms make the Sečovlje salterns (Table 11) very interesting for the isolation of halotolerant microorganisms and their application for saline wastewater treatment.

In order to determine applicability, survival and efficiency of halotolerant microorganisms isolated from the Sečovlje salterns for saline wastewater treatment in CW with variable wastewater salinity, a pilot plant was constructed and used (Figures 12 and 13). Shorter applications of other salinity was in order to imitate actual conditions and salinity shocks.

4.2.1 Characteristics of pilot plant

4.2.1.1 Influence of different salinities

4.2.1.1.1 Influence of different salinities on temperature, conductivity, chloride, pH, redox and oxygen

Air temperature in the experimental room, where the pilot plant was located, slowly increased from 14 °C at the beginning of the experiment to 23 °C at the end (Annex C1: Figures 89, 90 and 91). This was due to the fact that the pilot plant was located in a non-temperature controlled room and that experiments started in the spring. Water temperature increased from 14 °C to 26.7 °C (Figure 35). Generally, the influence of temperature on the operation of CW is not high, but precipitation does have a strong influence (Bulc, 2006; Maehlum, 1998). In an earlier study, Bulc (2006) concluded that the influence of temperature on CW operation was imperceptible, although degradation of organic material, ammonification, nitrification and denitrification are known as temperature dependent processes (Platzer 1996; Kadlec and Knight, 1996). Similarly, Maehlum (1998) determined that the removal efficiency of CW (ammonium, nitrate, nitrite, COD) in winter months could be the same as in summer months due to greater solubility of oxygen and to physical processes which can sometimes dominate over microbial degradation. The temperature of the water was also higher at the end of the experiment than at the beginning, in accordance with the trend in rising air temperature. A slow increase of water temperature can be attributed to the slow increase of air temperature. However, the water temperature was always several degrees (3-4 °C) higher than the air temperature (Annex C1: Figures 92 and 93).

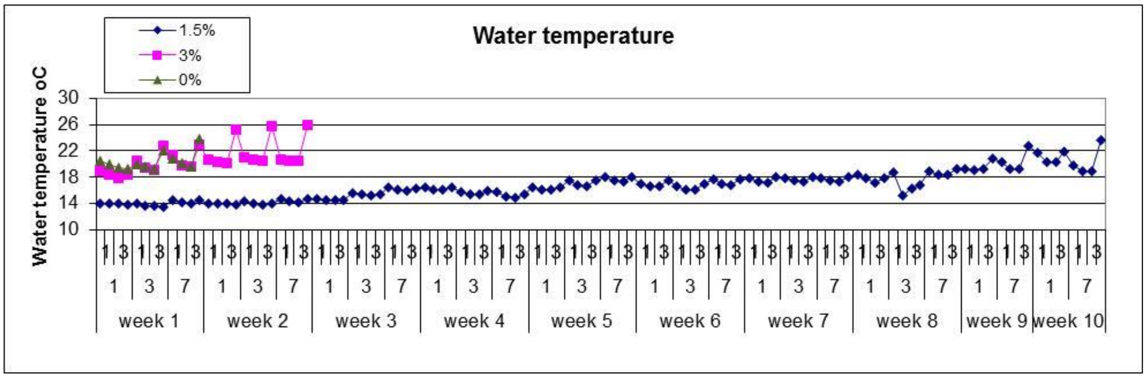


Figure 35: *Water temperature during operation period (measured on 1st, 3rd and 7th day of the week in all four compartments), at different salinities (0% NaCl, 1.5% NaCl and 3% NaCl)*

Chloride concentrations (Figure 36) and conductivity increase with increasing salinity (%NaCl) in wastewater. In the experiment with the application of 0% NaCl the concentration of chloride ions increased after one day. The reason could be the release of the chloride ions from the soil as a consequence of previous experiments with higher NaCl concentrations (1.5% and 3%) and the same could be noticed for the conductivity (Annex C2: Figures 94, 95 and 96).

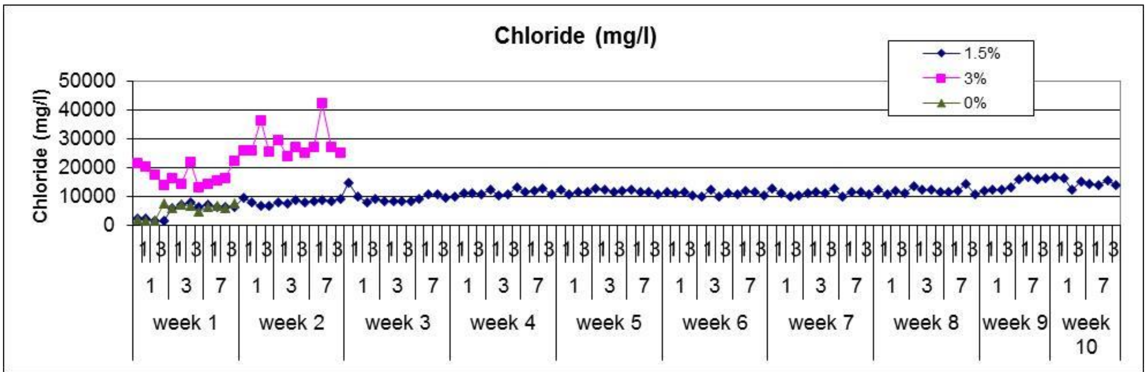


Figure 36: *Variation of chloride concentrations during operation period (measured on 1st, 3rd and 7th day of the week) in all four compartments,at different salinities (0% NaCl, 1.5% NaCl, 3% NaCl)*

Denitrifiers operate best in the range 6.5 < pH < 7.5, whilst nitrifiers prefer pH = 7.2 and higher (Paul and Clark, 1996). In the pilot plant pH was around 8.3 varying from 7.7 to 8.5 without aeration (Figure 37). The pH values for nitrification were optimal in the pilot plant and CW Dragonja (Figure 19), with a slightly higher than optimal pH for denitrifiers. Reddy and Patrick (1984) pointed out that losses of NH_4^+-N through volatilization from flooded soils and sediments are insignificant if the pH value is below 7.5 and very often losses are not serious if the pH is below 8.0. Large losses of NH_4^+-N through volatilization from the pilot plant are unlikely because the pH did not exceed 8.5. Nivala *et al.* (2007) investigated SSF CW for landfill leachate and found that pH values were generally neutral, even when significant nitrification occurred, indicating that the wetland system had a pronounced buffering capacity. In CW Dragonja range of pH was from 7.1 to 8.5 at influent. The pH of shallow flood water is greatly affected by the total respiration activity of all the heterotrophic organisms (Vymazal, 2007). In the study of Baere *et al.* (1984), the pH dropped significantly after each shock treatment with high concentration of NaCl. The lowest pH was at 3% NaCl (Annex C3: Figure 97).

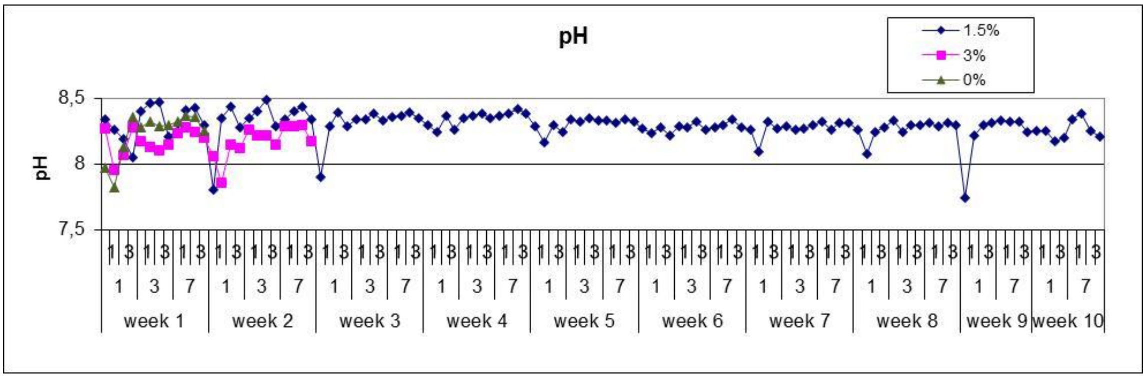


Figure 37: *Variation of pH during operation period (measured on 1st, 3rd and 7th day of the week) in compartments 1, 2, 3, and 4, at different salinities (0% NaCl, 1.5% NaCl, 3% NaCl)*

Because wetlands are associated with waterlogged soils, the concentration of oxygen within sediments and the overlying water is of critical importance. The rate of oxygen diffusion into water and sediment is slow, and this (coupled with microbial and animal respiration) leads to near anaerobic sediments within many wetlands (Moss, 1998). The deeper sediments are generally anoxic, a thin layer of oxidized soil usually exists at the soil-water interface. This oxidized layer is important, since it permits the oxidized forms of prevailing ions to exist. This is in contrast to the reduced forms occurring at deeper levels of soil. The state of reduction or oxidation of iron, manganese, nitrogen and phosphorus ions determines their role in nutrient availability and also toxicity. The concentration of oxygen in the CW pilot plant was most frequently below 0.4 mg/l (Figure 38), except during the first days of the cycle when fresh synthetic wastewater was added. Obviously available oxygen was quickly consumed by microorganisms. After the first day, there was a lack of oxygen but otherwise, the pilot plant gravel - sand filter for saline wastewater had similar conditions regarding oxygen as the wetlands. Salinity however did not have an influence on the oxygen concentrations, which were very low at all salinities (Annex C4: Figure 98).

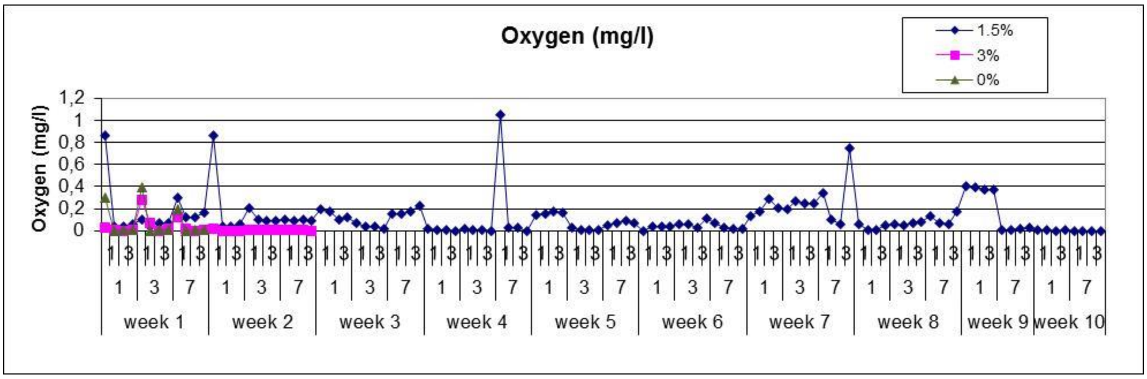


Figure 38: *Variation of oxygen concentration during operation period in the first two weeks (measured on 1st, 3rd and 7th day of the week) in compartments 1, 2, 3, and 4, at different salinities (0% NaCl, 1.5% NaCl, 3% NaCl)*

Redox potential was around -90 to -60 mV, which means confirms the anaerobic conditions in the CW pilot plant (Figure 39). Redox potential did not depend on salinity.

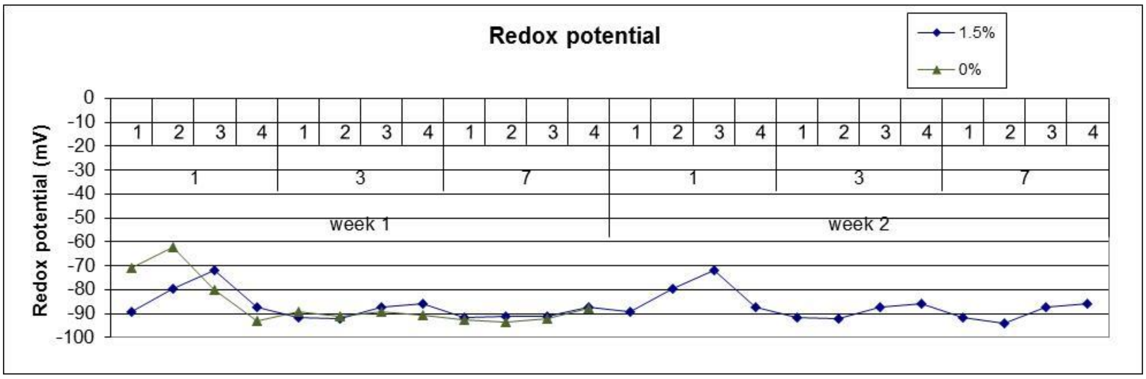


__Figure 39:__ Variation of redox potential during operation period in two weeks (measured on 1st, 3rd and 7th day of the week) in all four compartments, at different salinities (0% NaCl, 1.5% NaCl, 3% NaCl)

4.2.1.1.2 Influence of different salinites on nutrients in pilot plant

The COD decreased most on the last (seventh) day of every cycle (Figure 40). The final COD, on the last day in the last compartment for all salt concentrations (0%, 1.5% and 3% NaCl) was approximately the same. These results indicated that different salt concentrations did not have high influence on the organic matter removal (in term of COD) in CW pilot plant with inoculated halotolerant microorganisms (Annex C5: Figure 99).

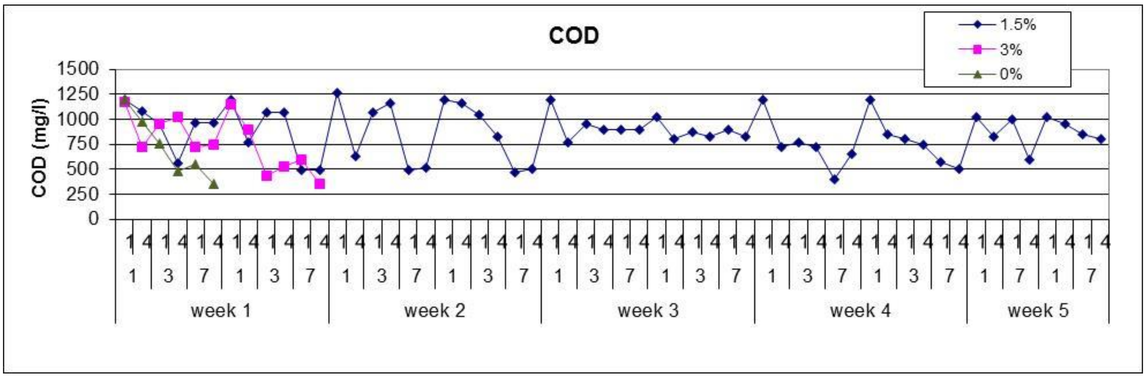


__Figure 40:__ Variation of COD concentrations during operation period (measured on 1st, 3rd and 7th day of the week) in compartments 1 and 4, at different salinities (0% NaCl, 1.5% NaCl, 3% NaCl)

It can be noticed that at all concentrations of NaCl (0%, 1.5% and 3%) after day 7, the last compartment had the highest reduction of ammonium ion concentration (Figure 41). Fluctuations of ammonium ion concentrations could be noticed between compartments during the same day, probably due to the processes occurring in each compartment (Annex C6: Figure 100). Generally, the anaerobic conditions in the pilot plant were responsible for the low removal of ammonium ion, because oxidation by autotrophic bacteria of ammonia to nitrite and then nitrite to nitrate (nitrification process) would require aerobic conditions., contrary to Dahl *et al.* (1997) who found that inhibition of the nitrifiers in response to a rapid increase of chloride concentration with conventional microorganisms. Pilot plant results indicated that removal of ammonium ions was not influenced by salt concentrations in our pilot plant inoculated with halotolerant microorganisms. These results were in accordance with Kargi and Dincer (1996), who found that adverse effects of high salt concentrations were significantly alleviated by using salt-tolerant microorganisms.

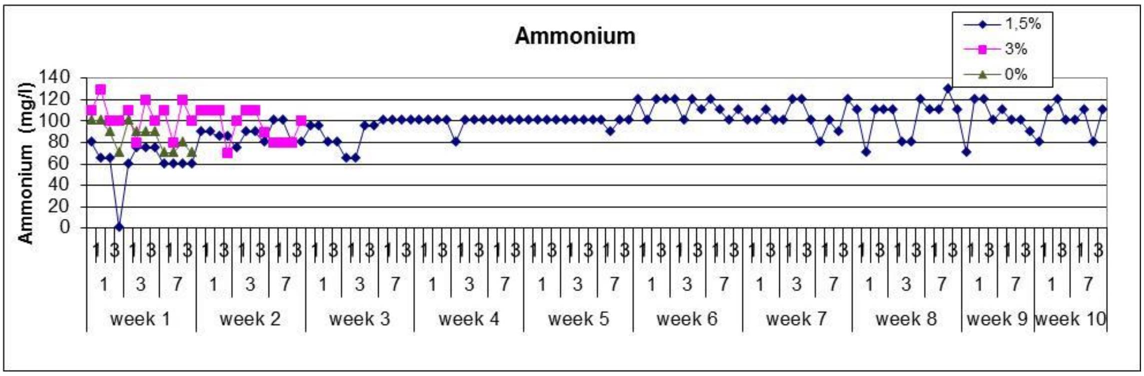


Figure 41: *Variation of ammonium concentrations during operation period (measured on 1st, 3rd and 7th day of the week in all four compartments) at different conditions: different salinities (0% NaCl, 1.5% NaCl, 3% NaCl)*

It can be observed that phosphate ion changes similarly in wastewater with 1.5% (after second week of inoculation) and 3% NaCl (Figure 42), but a slightly higher concentration of phosphate ion was detected at 0% NaCl in wastewater (Annex C7: Figure 101). This indicates that with 0% saline wastewater, phosphate ion was washed out of the solid phase at the bottoms of the chambers. This is in accordance with the study of Bulc (2006) and with the results from CW Dragonja (Figure 25), where it was found that phosphate ions were washed out during periods of heavy rain. However, an increase in salinity to 3% NaCl did not substantially change phosphate removal.

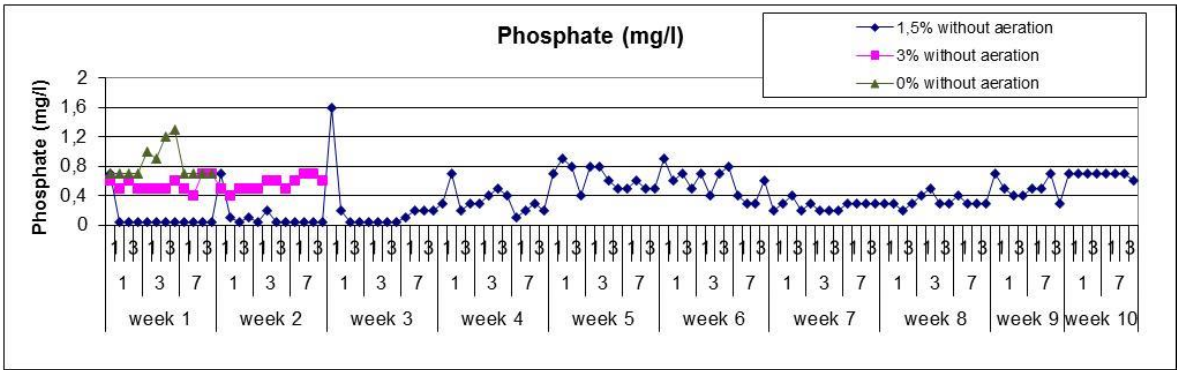


Figure 42: *Variation of phosphate concentrations during operation period (measured on 1st, 3rd and 7th day of the week) in all four compartments, at different salinities (0% NaCl, 1.5% NaCl, 3% NaCl)*

4.2.1.1.3 Influence of different salinites on microbial activity in pilot plant

Measurements of optical density at 600 nm were employed to monitor microbial activity. The highest density was found at 0% and 3% NaCl at the beginning of experiments, but after the first day it was not dependent upon salt concentration and optical density at 600 nm was similar to that of the medium on the first day of the experiment (Figure 43).

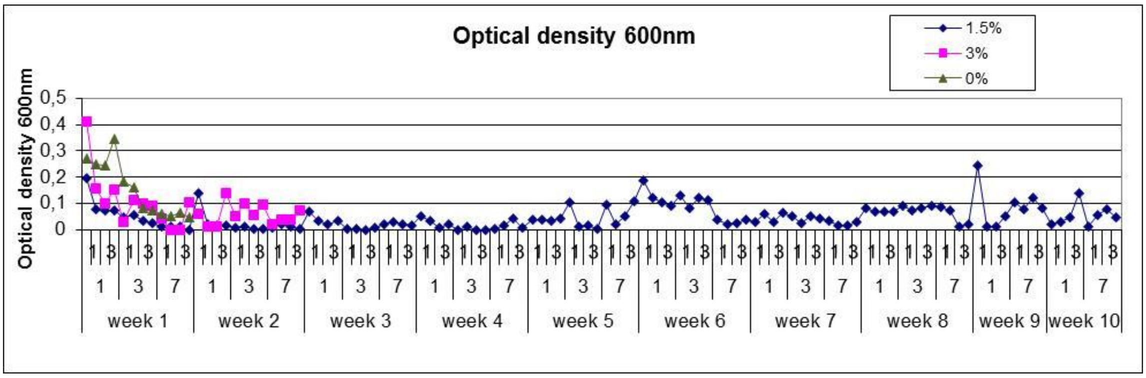


Figure 43: *Variation of optical density at 600nm during operation period (measured on 1st, 3rd and 7th day of the week) in all four compartments, at different salinities (0% NaCl, 1.5% NaCl, 3% NaCl)*

Those results indicated that halotolerat microorganisms required three days for adaptation to new salt concentrations in pilot plant. After that, optical density was the similar in all concentrations of salt (0%, 1.5% and 3% NaCl) (Annex C8: Figure 102). This means that halotolerant microorganisms are not sensitive to the changes in salt concentrations as are the conventional microorganisms. However, higher optical density in the first few days could also be explained by assuming stimulation of microbial growth with the introduction of new nutrients into the system. It was recognised that optical density at 600 nm rises in weeks six, nine and ten, but no explanation exists at present.

In addition to the optical density measurements, microbiological activity was monitored by measurements of ETS activity (Figure 44). After the second week, increase of ETS activity in synthetic wastewater containing 1.5% NaCl was observed (Annex C9: Figure 103). Subsequently, the ETS activity more or less stayed at that level. As ETS activity is a measure of microbial activity it could be concluded that halotolerant microorganisms were adapted and inoculated into the system after two weeks under these conditions. In the following weeks there is a trend that towards seven day ETS increasing, but it was not identical throughout the whole series of weeks. ETS activity as a measure of microbial community respiration capacity showed higher ETS activity at 3% NaCl, but settled at the same level as that of the 1.5% NaCl in the second week. From this it could be concluded that halotolerant microorganisms isolated from the Sečovlje salterns and inoculated into pilot plant were not affected by 3% salinity. Lin *et a*l. (2008) reported that salinity impacted the growth of bacteria resulting in a change in the composition of the microbial community in the CW pilot plant that was inoculated with conventional culture of bacteria, but in this study, ETS activity was not reduced at 3% salinity. Hence, the conclusion could be that the same microorganisms were active at 1.5% and 3% NaCl, thus they were not sensitive to the sudden increase in salinity.

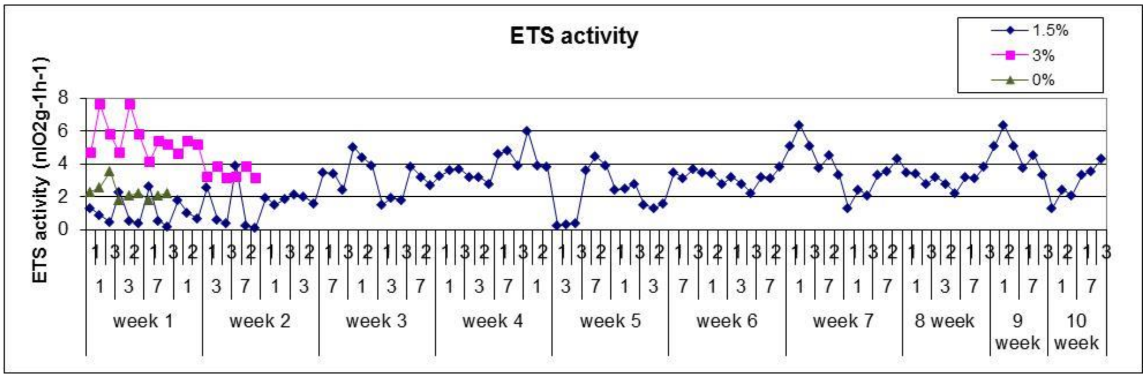


Figure 44: *Variation of ETS activity during operation period (measured on 1st, 3rd and 7th day of the week) in tanks 1, 2 and 3, at different salinities (0% NaCl, 1.5% NaCl, 3% NaCl)*

Measurements of carbon dioxide concentrations were used as a third indicator of microbial activity in the CW pilot plant. It has been observed that concentrations of carbon dioxide in the water and soil change similarly (Figure 45 and Figure 46). It can be observed that during the first two weeks of the inoculation period with 1.5% NaCl, concentrations of carbon dioxide in the water and soil were lower than later. An increase in the production of carbon dioxide after the second week of inoculation suggests that after the second week halotolerant microorganisms were adapted to conditions in the pilot plant and were successfully inoculated. This confirms the conclusions about the time of acclimation, obtained on the basis of ETS activity. Carbon dioxide concentrations in the water and soil were similar in all concentrations of salt (0%, 1.5% and 3% NaCl) showing that the metabolism of halotolerant microorganisms was not affected by the changes of salt concentrations. Therefore, halotolerant microorganisms from the Sečovlje salterns can be considered as insensitive to salinity changes.

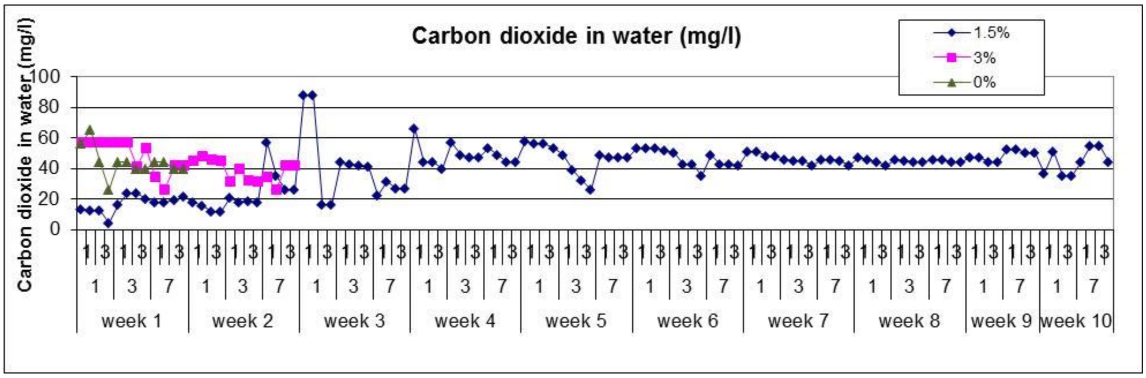


Figure 45: *Variation of carbon dioxide concentrations in water during operation period (measured on 1st, 3rd and 7th day of the week) in all four compartments, at different salinities (0% NaCl, 1.5% NaCl, 3% NaCl)*

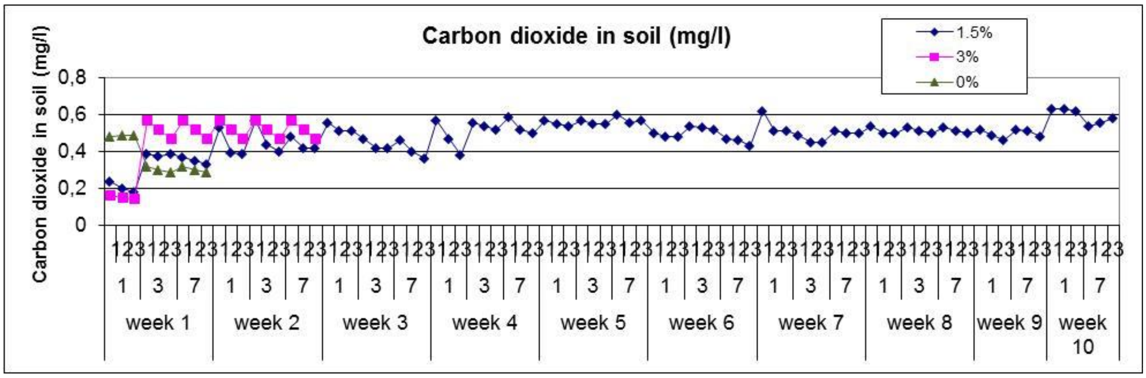


Figure 46: *Variation of carbon dioxide concentrations in soil during operation period (measured on 1st, 3rd and 7th day of the week) in tanks 1, 2 and 3, at different salinities (0% NaCl, 1.5% NaCl, 3% NaCl)*

Comparison of parameters between different salinities have shown significant differences for chloride, optical density, ammonium, phosphate and carbon dioxide in substrate when comparing 1.5 and 0 % salinity; for chloride, pH, phosphate, oxygen and ETS activity when comparing 1.5 and 3 %; and for chloride, optical density, ammonium, phosphate and ETS activity when comparing 0 and 3 % salinity (Annex C11: Table 23 and 24). The results indicate that concentrations of phosphate were the most sensitive for changes in salinity. Accumulated salt in substrate can disturb adsorption and precipitation of phosphate in substrate (Hu *et al*., 2005). COD in CO_2 in water was not sensitive on salinity variations.

From the results for CW pilot plant with changes in salinity from 0% NaCl to 3% NaCl, it can be seen that salinity did not affect the microflora. The limiting factor for removal efficiency is the available oxygen. A low level of oxygen lowers the redox potential. The anaerobic conditions then influence nitrification and total nitrogen removal, COD and ETS activity. Therefore to further increase removal efficiency of halotolerant microorganisms, some aeration should be included in the system. Since not many differences were observed in the removal efficiency with differing salinities, it can be concluded that halotolerant microorganisms, isolated from the Sečovlje salterns, are not sensitive to the changes in salinity and are therefore, as already described for the use of halotolerant microorganisms in conventional treatment, an alternative in the treatment of saline wastewater by CW.

Some abiotic parameters show a rather similar and reproducible variation over the seven day periods. In contrast, the nutrient parameters vary at a minimum range for several weeks, but then – either in the middle or towards the end of the ten weeks – variation increases, but keeping a low variation range in other periods again. This effect is different for different parameters regarding the weekly periods of higher variation. This is a phenomenon so far unexplained, but certainly was recognised.

4.2.1.2 Influence aeration and supplemented carbon sources (saccharose)

4.2.1.2.1 Influence of aeration and supplemented carbon sources (saccharose) on pH, oxygen and redox potential in pilot plant

Organic matter within wetlands is usually degraded by aerobic respiration or anaerobic processes (e.g., fermentation and methanogenesis). Anaerobic degradation of organic matter is less efficient than decomposition occurring under aerobic conditions. Fermentation is the result of organic matter acting as the terminal electron acceptor (instead of oxygen as in aerobic respiration). This process forms low molecular weight acids (e.g., lactic acid), alcohols (e.g., ethanol) and carbon dioxide. Therefore, fermentation is often central in providing further biodegradable substrates for other anaerobic organisms in waterlogged sediments (Mitsch and Gosselink, 2000). Although the importance and role of the microfauna community in conventional wastewater treatment processes have been extensively documented in conventional activated sludge systems, such as removal of dispersed bacteria (Curds, 1963), presence of toxic compounds (Puigagut *et al.*, 2005; Madoni *et al.*, 1998), organic load (Salvado and Gracia, 1993), sludge health (Madoni, 1994), quality parameters (Martin-Cereceda *et al.*, 2002; Al-Shahwani and Horan, 1991), and the effluent quality in terms of BOD_5 (Salvado *et al.*, 1995), there is a lack of information concerning the role of such microorganisms in natural treatment systems such as SSF CW (Puigagut *et al.*, 2007). Currently no information is available on the effect soluble or particulate organic matter has on the SSF CW removal efficiency, although an excessive amount of suspended solids is considered to be one of the major factors related to the clogging of the granular medium (Langergraber *et al.*, 2003). Some possibility of mechanical realization and energy consumption of aerated CW are published by Nivala *et al*., 2014 and Tao M, 2010. It is known that organic content, aeration and salinity affect the performance of wastewater treatment processes, and it was considered important to investigate how their different combinations influence the efficiency of SSF CW.

It can be seen that without added saccharose changes of pH were similar and varied from 7.7 to 8.5 (Figure 47). The pH values for nitrification were optimal in the pilot plant, but slightly higher than optimal for denitrifiers. In the case where wastewater contained 2.0 g/l saccharose, pH varied from 6.4 to 8.1, and pH values were optimal also for denitrifiers.

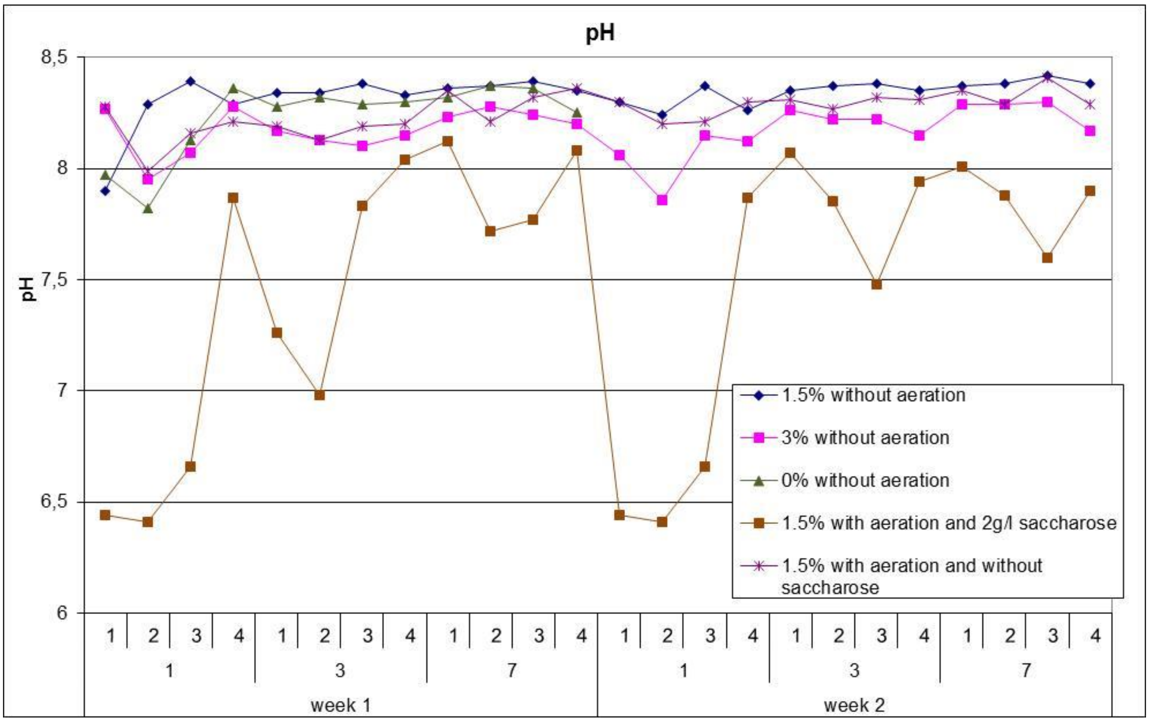


Figure 47: *Variation of pH during operation period (measured on 1st, 3rd and 7th day of the week) in compartments 1, 2, 3, and 4, at different salinities (0% NaCl, 1.5% NaCl, 3% NaCl), aeration (with or without) and with aeration and 2 g/l saccharose*

Oxygen concentrations without aeration were less than 1.5 mg/l, but with aeration, and without saccharose, the concentration of dissolved oxygen rose to 6.5 mg/l (Annex C12: Figure 106). When 2.0 g/l saccharose was added to synthetic wastewater, oxygen was consumed during the degradation processes. The availability of this energy source (saccharose) was also confirmed by a higher production of carbon dioxide during that period (Annex C10: Figure 104, 105).

With aeration the redox potential had positive values, while without aeration it was negative (Figure 48). During aeration the redox potential was not constant and decreased gradually from the first to the seventh day. Although the pilot plant was aerated and the redox potential had positive values, conditions were still anoxic (<100 mV). This means that aeration should be increased in the pilot plant gravel - sand filter for saline wastewater by adding air pumps in each compartment to achieve oxic conditions.

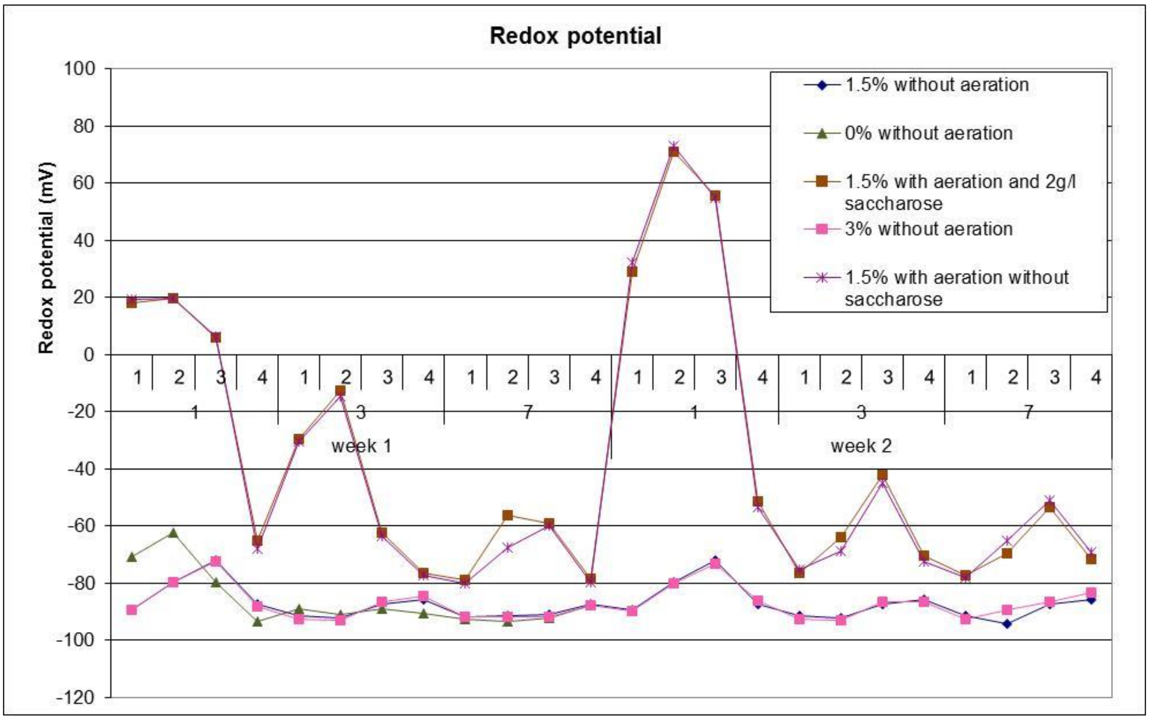


Figure 48: *Variation of redox potential during operation period (measured on 1st, 3rd and 7th day of the week) in compartments 1, 2, 3, and 4, at different salinities (0% NaCl, 1.5% NaCl, 3% NaCl), aeration (with or without) and with aeration and 2 g/l saccharose*

4.2.1.2.1 Influence of aeration and supplemented carbon sources (saccharose) on nutrients in pilot plant

It can be observed that the COD was higher when 2.0 g/l saccharose was added, but at the same time the greatest reduction of COD was achieved at these conditions (Figure 49). Final COD values in second week, in the last compartment, on the last day were the same for all conditions. This is a consequence of added saccharose and aeration which stimulate growth of microbes and metabolism, using organic matter as a source of energy.

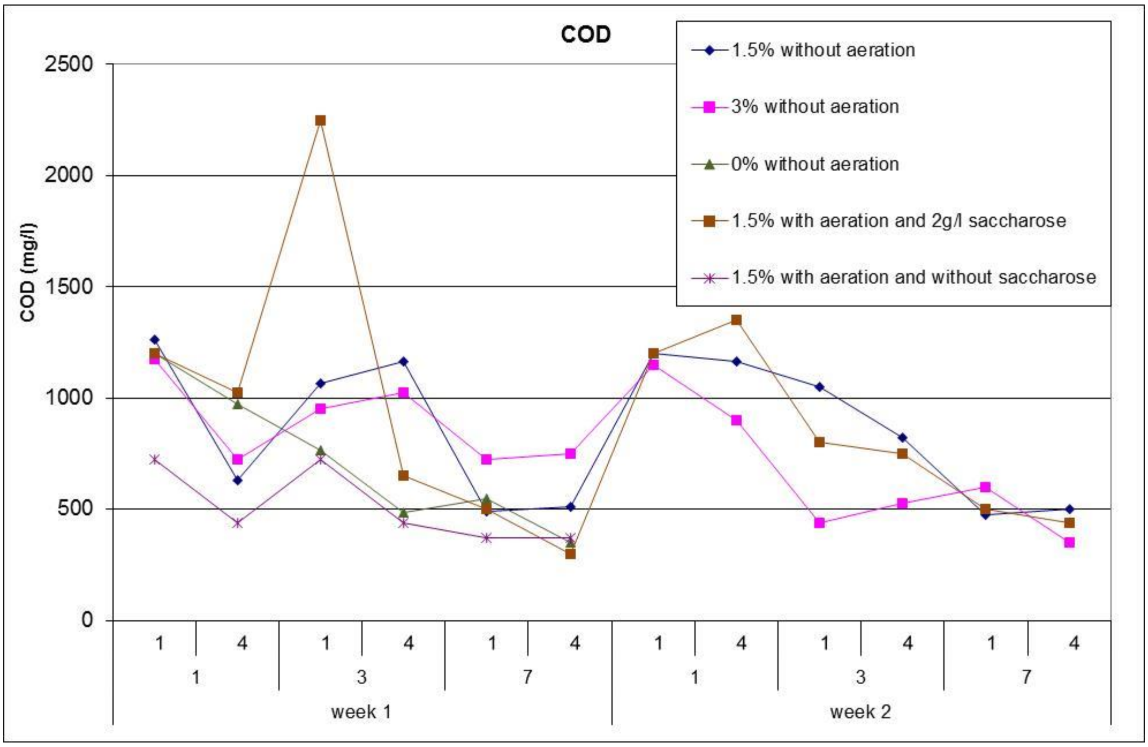


Figure 49: *Variation of COD concentrations during operation period (measured on 1st, 3rd and 7th day of the week) in compartments 1, 2, 3, and 4, at different salinities (0% NaCl, 1.5% NaCl, 3% NaCl), aeration (with or without) and with aeration and 2 g/l saccharose*

Ammonium ion concentrations were found to vary between compartments during the same day as well as within a compartment on a day-by-day basis. When experiments were compared, it could be noticed that with 1.5% NaCl with and without areation, lower concentration of ammonium ion was detected in the case of aeration in the second week, last day, first compartment (Figure 50). This indicated that aeration supports the process of nitrification. This is in accordance with the literature (Hauck, 1984; Kadlec and Knight, 1996; Paul and Clark, 1996), where it was explained that nitrification is the aerobic process where the ammonium ion is aerobically converted to nitrite ion and finally to nitrate ion. Similarly, Vymazal (2007) concluded that the ability of HF CW to nitrify ammonia is very limited under the prevailing anaerobic conditions. This also in agreement as, at the same salinity, higher removal of ammonium ion removal occurred with aeration of the pilot plant gravel - sand filter for saline wastewater.

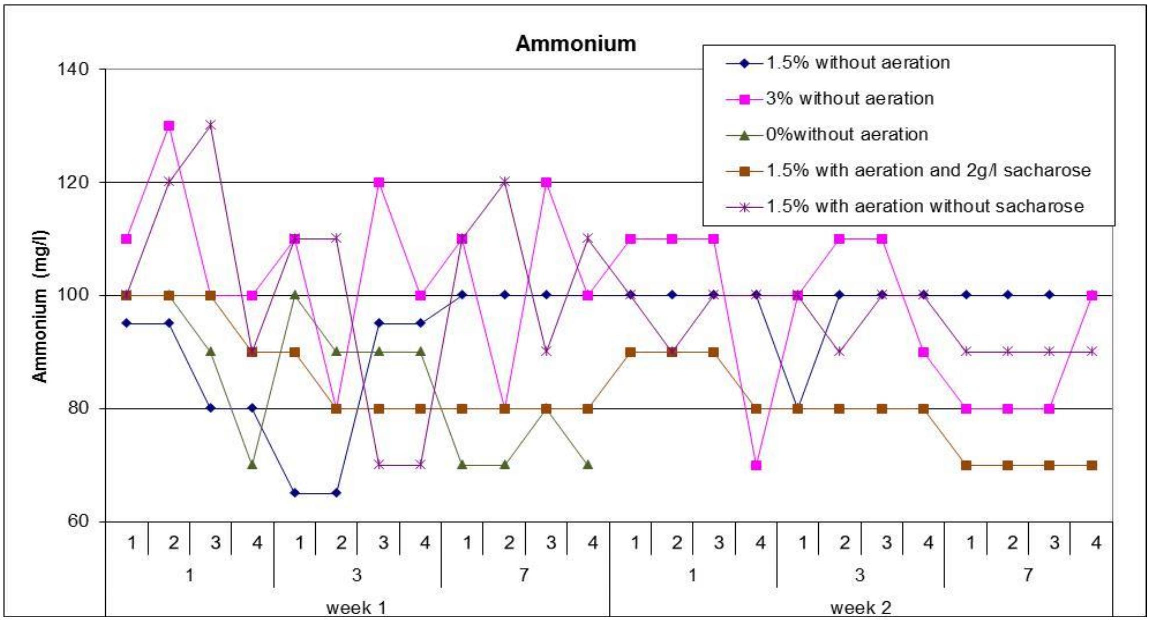


Figure 50: *Variation of ammonium ion concentrations during operation period (measured on 1st, 3rd and 7th day of the week) in compartments 1, 2, 3, and 4, at different salinities (0% NaCl, 1.5% NaCl, 3% NaCl), aeration (with or without) and with aeration and 2 g/l saccharose*

Various aeration systems have been recommended for introducing oxygen into SSF CW. Among these are: frequent water level fluctuation (tidal-flow) (Austin *et al.*, 2003; Behrends *et al.*, 1996; Zoeller and Byers, 1999), passive air pumps (vertical-flow) (Green *et al.*, 1998) or direct mechanical aeration of the water in the gravel bed (horizontal-flow) (Dufay, 2000; Flowers, 2002; Wallace, 2001), which was improved by Nivala *et al.* (2007). When comparing the same conditions for 1.5% NaCl wastewater with aeration, with and without 2.0 g/l saccharose, it can be noticed that there was lower concentration of ammonium ion in the fourth compartment, on the last day of the second week, in the experiment with the saccharose in the wastewater. These results are in accordance with–Vymazal (2007), who reported that some degradation processes require energy (typically derived from an organic carbon source) to proceed, and others release energy that is used by organisms for growth and survival. It suggests that added saccharose stimulates growth of the microbial community and nitrification, because saccharose serves as a source of energy.

The highest concentration of phosphate throughout the experiment was measured in the wastewater with 0% added NaCl (Figure 51). These results indicated phosphates that had accumulated there in earlier experiments, were washed out of the soil. This is in accordance with earlier observations (Figure 42).

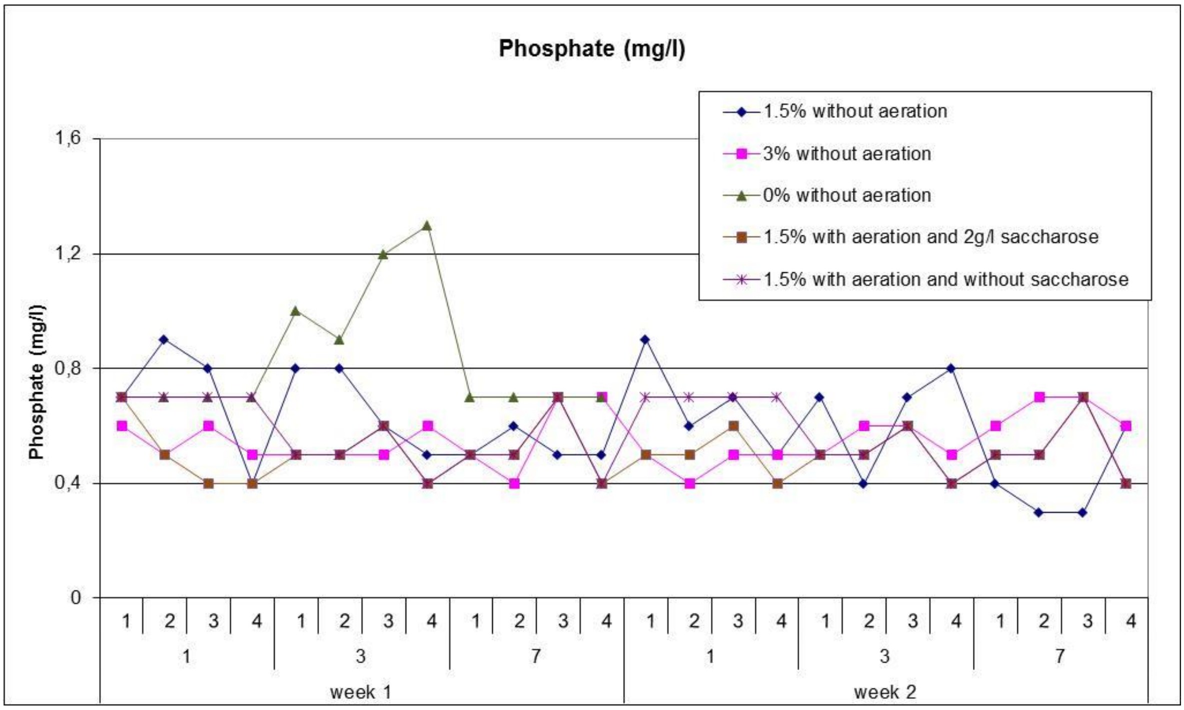


Figure 51: *Variation of phosphate concentrations during operation period (measured on 1st, 3rd and 7th day of the week) in compartments 1, 2, 3, and 4, at different salinities (0% NaCl, 1.5% NaCl, 3% NaCl), aeration (with or without) and with aeration and 2 g/l saccharose*

It was noticed that the ETS activity was highest in experiments with aerated wastewater containing saccharose. This indicates that saccharose and aeration stimulate growth and metabolism of halotolerant microorganisms, which were isolated from the Sečovlje salterns. The same was confirmed with the results from the optical density measurements and measurements of the carbon dioxide concentrations in soil and water. Added saccharose together with aeration stimulates growth of halotolerant microorganisms. As a consequence of their metabolism, the processes of nitrification and COD removal were higher, because saccharose acts as a source of energy. Also aeration only, without added saccharose at the same salinity, produced higher nitrification and higher COD removal in the pilot plant gravel - sand filter for saline wastewater than without aeration, but lower than with both aeration and added saccharose.

Aeration significantly influent on phosphate, ammonium and ETS activity (Annex C13: Table 25 and 26). Mostly all parameters are influenced with aeration and added carbon sources (saccharose). The presence of saccharose in the aerated system influences more parameters than does aeration of the system without saccharose. This implies saccharose has a greater influence than dose aeration.

4.2.1.2.3 Influence of aeration and supplemented carbon sources (saccharose) on microbial activity in pilot plant

In the aerated wastewater containing 2.0 g/l of saccharose, the concentrations of carbon dioxide in water in all four compartments were the highest (Figure 52) on the first day of the measurements.

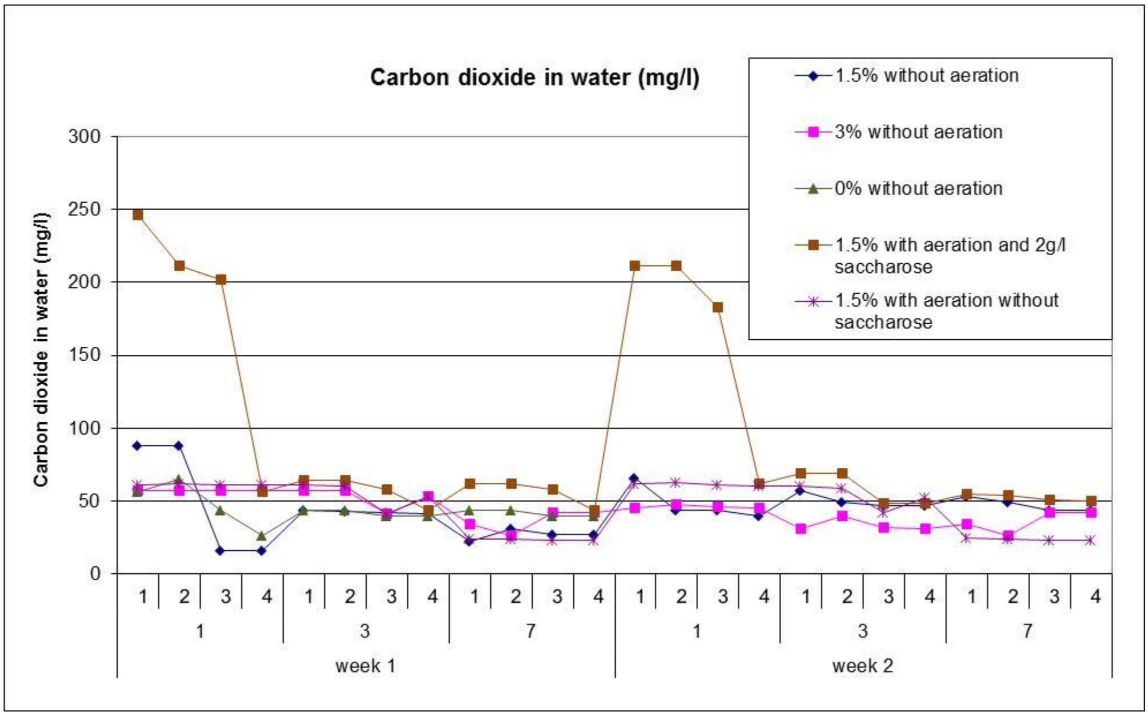


Figure 52: *Variation of carbon dioxide concentrations in water during operation period (measured on 1st, 3rd and 7th day of the week) in compartments 1, 2, 3, and 4, at different salinities (0% NaCl, 1.5% NaCl, 3% NaCl), aeration (with or without) and with aeration and 2 g/l saccharose*

Concentrations of carbon dioxide in the soil were the highest at the beginning of the cycle measurement under the same conditions of aeration and saccharose concentration (Figure 53). Because carbon dioxide is a product of microbial metabolism, results confirm earlier suggestions that saccharose and aeration stimulate growth of the halotolerant microorganisms isolated from the Sečovlje salterns, thus increasing the amount of carbon dioxide produced.

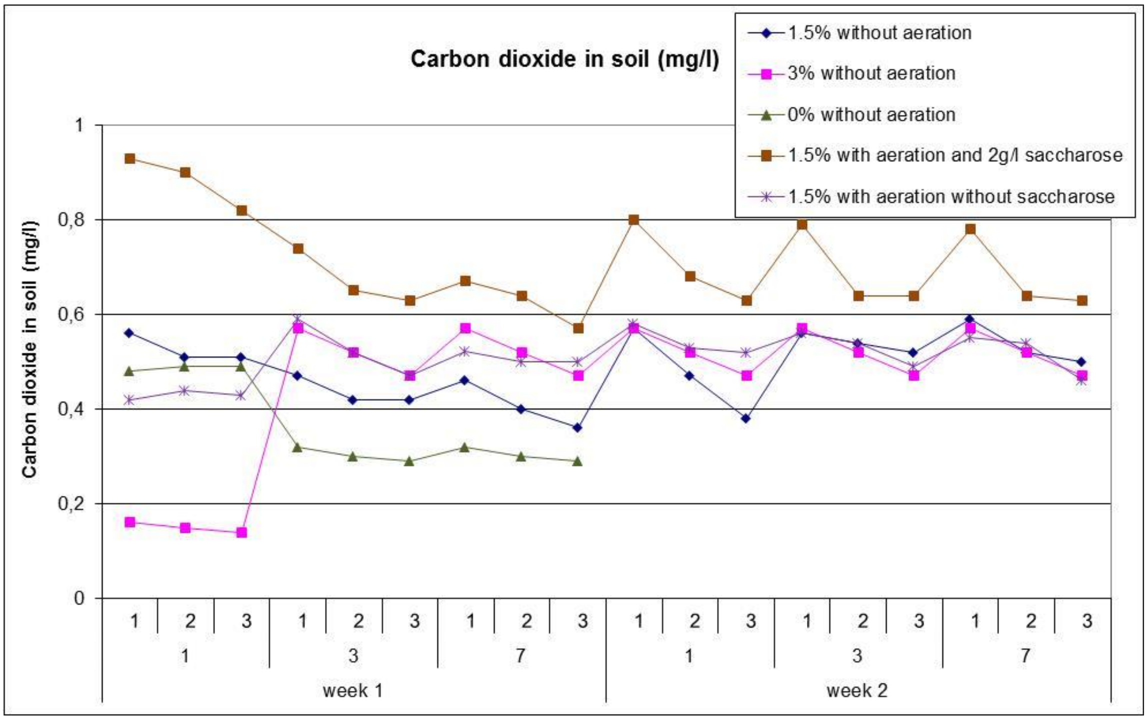


Figure 53: *Variation of carbon dioxide concentrations in soil during operation period (measured on 1st, 3rd and 7th day of the week) in compartments 1, 2, 3, and 4, at different salinities (0% NaCl, 1.5% NaCl, 3% NaCl), aeration (with or without) and with aeration and 2 g/l saccharose*

ETS activities were lowest at 0% NaCl without aeration (Figure 54). Comparisons of ETS activities in the second week, last day, last compartment, showed the significantly higher values ($p<0{,}001$; $F=-4{,}882$) in the presence of saccharose and aeration. This indicates that saccharose and aeration stimulate growth of halotolerant microorganisms isolated from the Sečovlje salterns. These results are in accordance with the study of Burchell *et al.* (2007), where it was found that addition of organic matter to the soils, used for in-stream CW, significantly increased biomass growth when compared to the addition of inorganic matter.

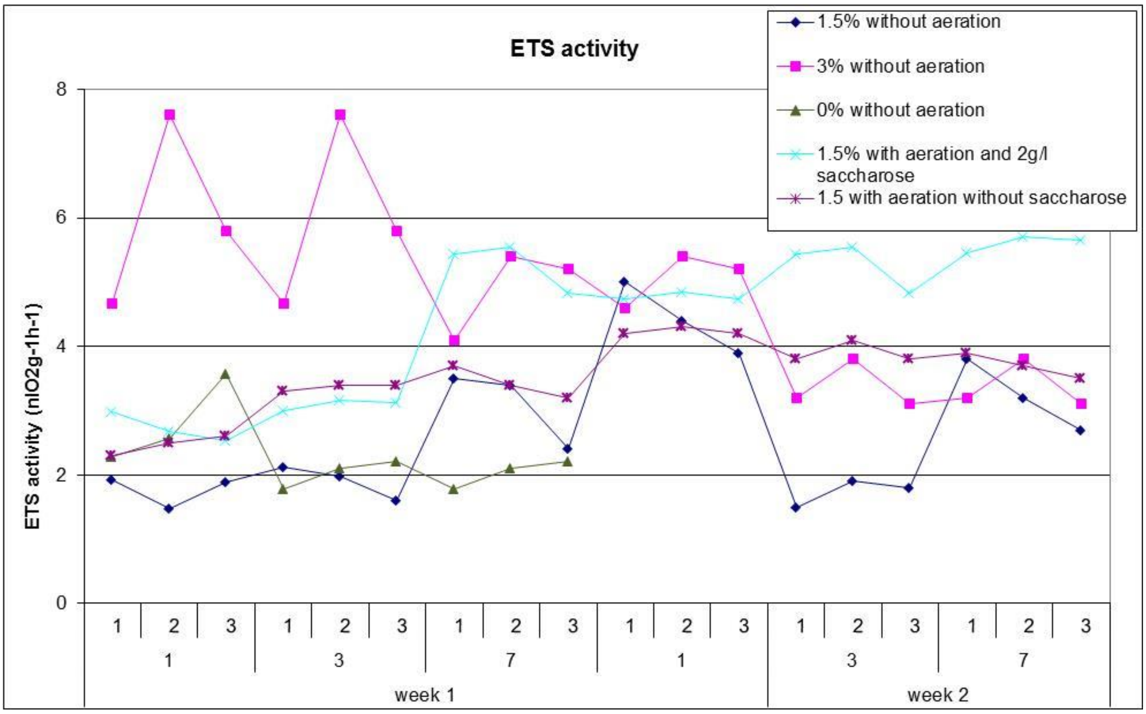


Figure 54: *Variation of ETS activity during operation period (measured on 1st, 3rd and 7th day of the week) in compartments 1, 2, 3, and 4, at different salinities (0% NaCl, 1.5% NaCl, 3% NaCl), aeration (with or without) and with aeration and 2 g/l saccharose*

The changes of optical density (Figure 55) where the highest optical densities were observed in the case where 2.0 g/l saccharose was added to aerated wastewater. Similar results were found for ETS activity (Figure 54). These observations are attributed to the addition of saccharose and aeration of the chambers in the pilot plant which stimulate growth of microbes.

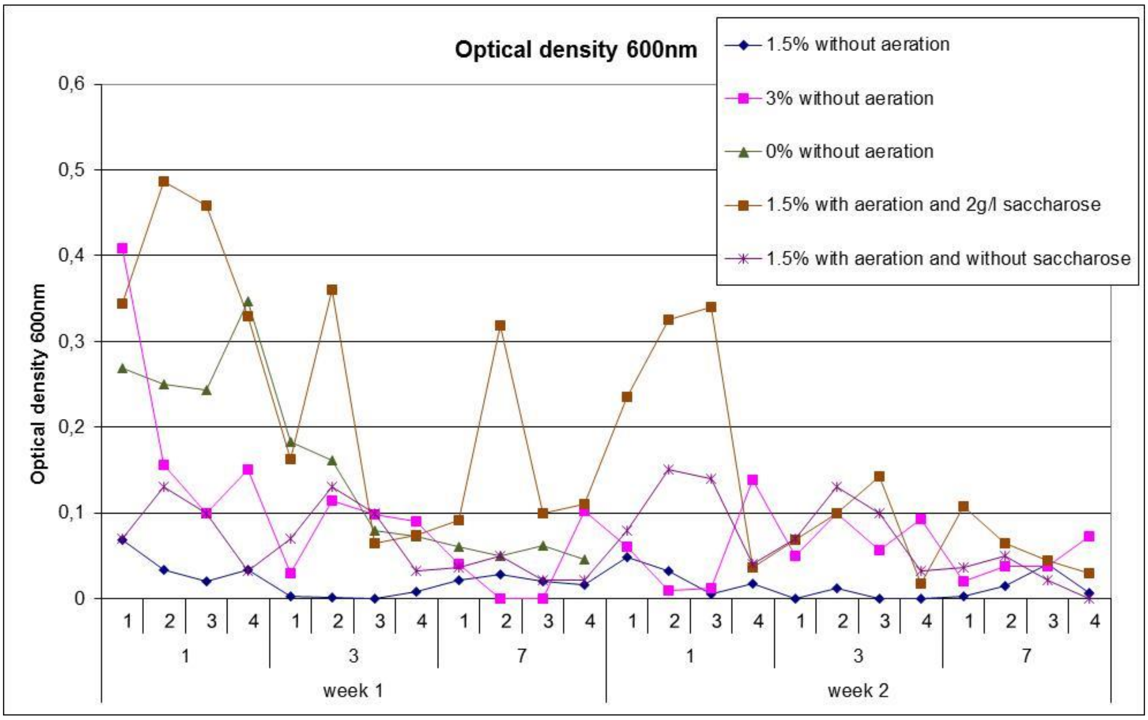


Figure 55: *Variation of optical density at 600 nm during operation period (measured on 1st, 3rd and 7th day of the week) in compartments 1, 2, 3, and 4, at different salinities (0% NaCl, 1.5% NaCl, 3% NaCl), aeration (with or without) and with aeration and 2 g/l saccharose*

4.2.1.3 Correlations between parameters of pilot plant

Graphs of correlations between removal efficiency (ammonium ion concentrations, COD and phosphate) and concentrations of chloride ions of pilot plant were made from the data in the fourth compartment for the seventh day (Figures 56 to 58).

For removal of ammonium ions, there is better efficiency at low chloride ion concentrations. Above a chloride concentration of approximately 8000 mg/l, efficiencies change very little (Figure 56). For COD removal it can be said that chloride ion concentrations do not influence the removal efficiency, since the slope of the trendline is $64.51e^{-1E-05x}$ (Figure 57). Those results indicated that different salt concentrations do not have an influence on the organic matter removal (COD) in pilot plant inoculated with halotolerant microorganisms. For phosphate removal similar as for ammonium it appears that above the chloride concentration of approximately 8000 mg/l efficiencies change very little (Figure 58).

All correlations (Figures 56, 57 and 58) between removal efficiency (ammonium ions, COD and phosphate) and concentrations of chloride ions have low values, which indicated that the relationship between removal efficiency of the pilot plant inoculated with halotolerant microorganisms and concentrations of NaCl is weak.

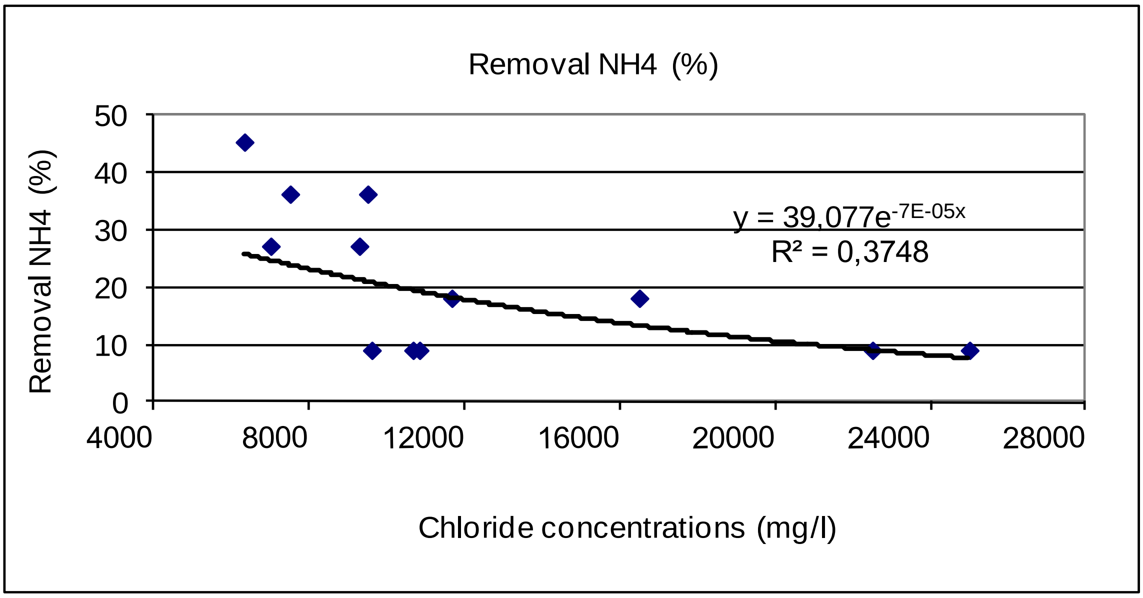


Figure 56: *Correlation between removal efficiency of NH4 (%) and chloride concentrations at the pilot plant in the fourth compartment on the seventh day*

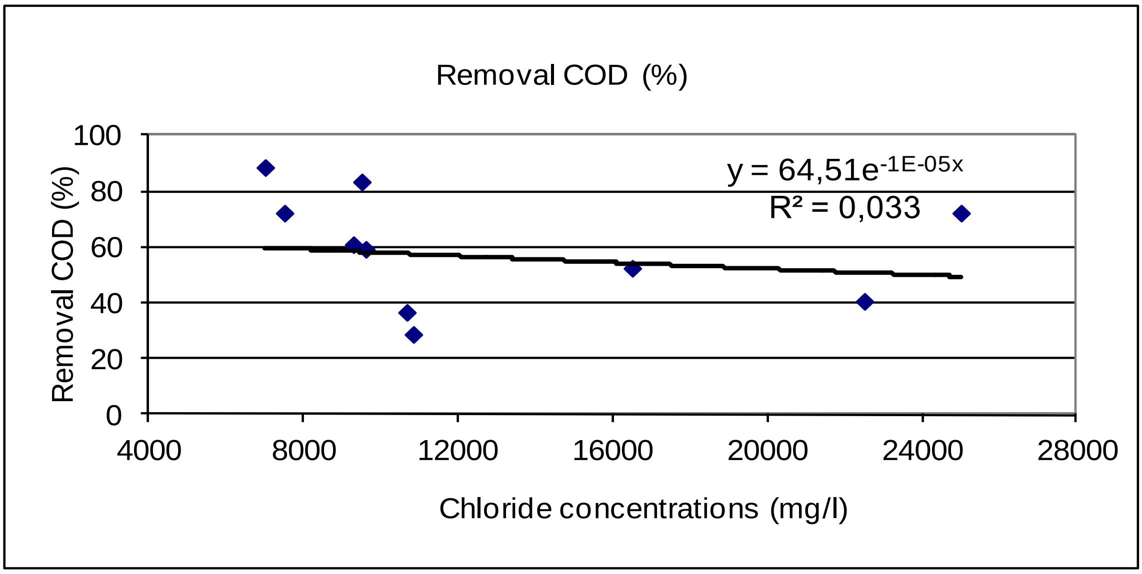


Figure 57: *Correlation between removal efficiency of COD (%) and chloride concentrations at the pilot plant in the fourth compartment on the seventh day*

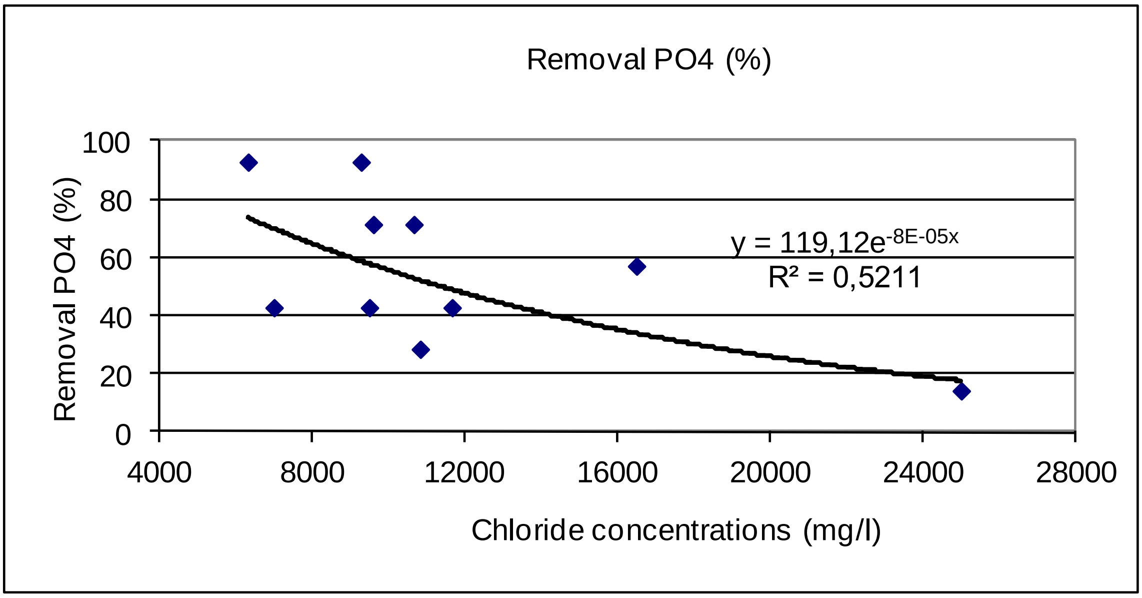


Figure 58: *Correlation between removal efficiency of PO4 (%) and chloride concentrations at the pilot plant in the fourth compartment on the seventh day*

Figures 59 and 60 show correlations of ammonium ion removal efficiencies with the redox potential and COD in fourth compartment on the seventh day. It could be seen that removal efficiencies for ammonium ion and COD increase with increasing redox potential. The correlation coefficients are not high. The low redox potential could explain the ammonium ion removal. COD removal had higher dependency on redox potential, which confirms previous conclusions that COD removal is more dependent on aeration than on salinity.

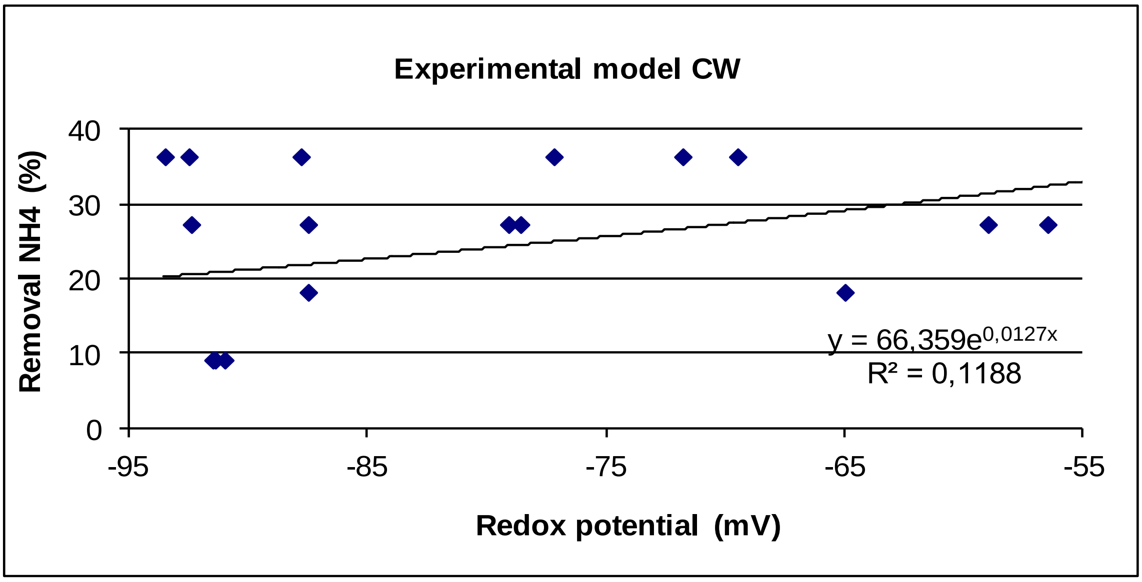


Figure 59: *Correlation between removal efficiency of NH4 (%) and redox potential concentrations at the pilot plant in the fourth compartment on the seventh day*

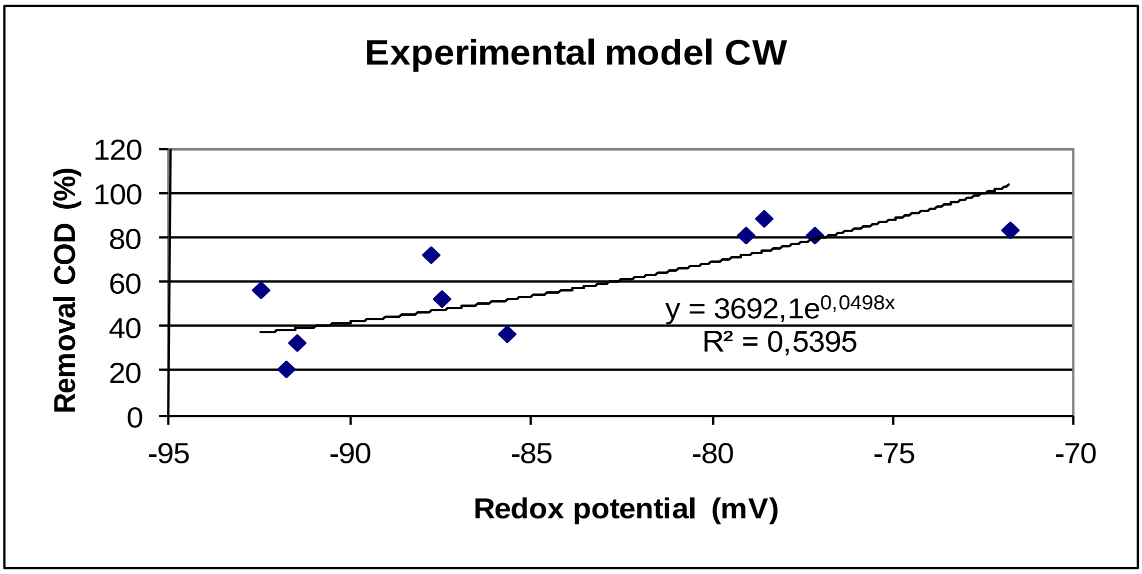


Figure 60: *Correlation between removal efficiency of COD (%) and redox potential concentrations at the pilot plant in the fourth compartment on the seventh day*

Correlation coefficients between physical and chemical parameters and conductivity or concentration of chloride ions were not significant. Pearson correlation shows that correlation between concentrations of ammonium and chloride ions were statistically significant ($p<0.01$) as well as between concentrations of chloride ions and BOD_5 ($p<0.01$), but Pearson correlation of other parameters with chloride ions was not statistically significant. Pearson correlation between conductivity and other parameters was not statistically significant, except for correlation between the concentrations of ammonium ions and chloride ions ($p<0.001$) (Annex B: Table 21 and 22).

Concentrations of chloride ions and ammonium ions correlated, but correlations between removal efficiency of ammonium ions and concentrations of chloride ions (Figure 56) were not significant (R^2=0.317). But it was the highest R^2 when it was compared with other correlations between removal efficiency and concentration of chloride ions (Figures 57 and 58).

4.2.1.4 Kinetics of some biochemical procesess in pilot plant

4.2.1.4.1 Reaction kinetics

Consider reaction:

Organic waste + $O_2 \rightarrow$ end products (13)

The organic waste can be quantified as BOD, COD or constituents such as TKN, NH_4^+ and PO_4^{3-}.

A rate law can be written:

Reaction rate = r = - k [COD] [O_2] (14)

Since the pilot plant are open to the atmosphere, it can be assumed O_2 not be rate limiting and, thus, will incorporated in the rate coefficient, k, yielding:

Reaction rate = r = - k [COD] (15)

This is first-order system. The k_r is the k that is modified by the Arrhenius Equation.

4.2.1.4.2 Bioreaction model

Bioreactor
(considered completly mixed)

Mass

ΔMass

Volume

Mass Rate In $\longrightarrow$ [Bioreactor] $\longrightarrow$ Mass Rate out (16)

Mass balance around the Bioreactor would be:

[Mass Rate In] - [Mass Rate Out] ± [Mass Rate of Change] = Accumulation Rate(17)

If the system is considered to be at Steady–State (no shock loads) the Accumulation rate becomes zero.

Making the following Definitions:

[Mass Rate In] = Q C_{in} (18)

[Mass Rate In] = Q C (19)

[Mass Rate of Change] = V r (20)

where C_{in} = Input concentration [mg/l];

C = Output and internal concentration [mg/l];

V = System volume [l];

Q = Input and Output flow rates [l/day];

r = reaction rate (from Reaction Kinetics) [mg/lday].

and substituing into the Mass Balance Equation (17):

$$Q\,C_{in} - Q\,C \pm r\,V = 0 \quad (21)$$

is obtained.

Substituing Equation 15 in general terms for the reaction rate,

$$r = -\,k\,C \quad (22)$$

$$Q\,C_{in} - Q\,C \pm r\,V = 0 \quad (23)$$

$$Q\,C_{in} - Q\,C - k\,C\,V = 0 \quad (24)$$

note $V/Q = t_d$ (25)

t_d = hydraulic detention time of system [days] substituing and solving for C:

$$C = C_{in}\,[1/(1 + k\,t_d)] \quad (26)$$

This equation can be used to simulate the Steady-State System behavior in the pilot plant:

Pilot plant

C_{in},Q → $\boxed{}$ → C_{out},Q (27)

Rate coefficients, k were calculated, employing Equation 22, using C_{out} data of seventh day from last fourth compartment. We use C_{out} of seventh day from last fourth compartment, because water circulated in pilot plant seven days (Figure 15). Arrhenius plots of reaction rate constant at any temperature for ammonium (Figure 61), COD (Figure 62) and phosphate (Figure 63) are shown. Activation energies (Ea) for the reaction were obtained from the slope (Schnoor, 1996):

$$\text{Slope} = -Ea/R \tag{28}$$

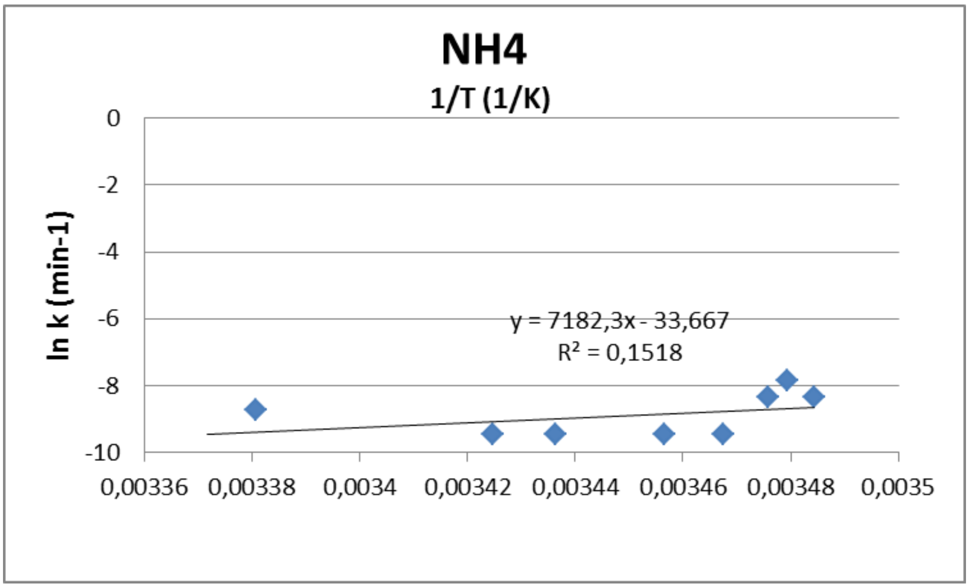


Figure 61: *The graphic plot of ln k versus 1/T during ammonium removal*

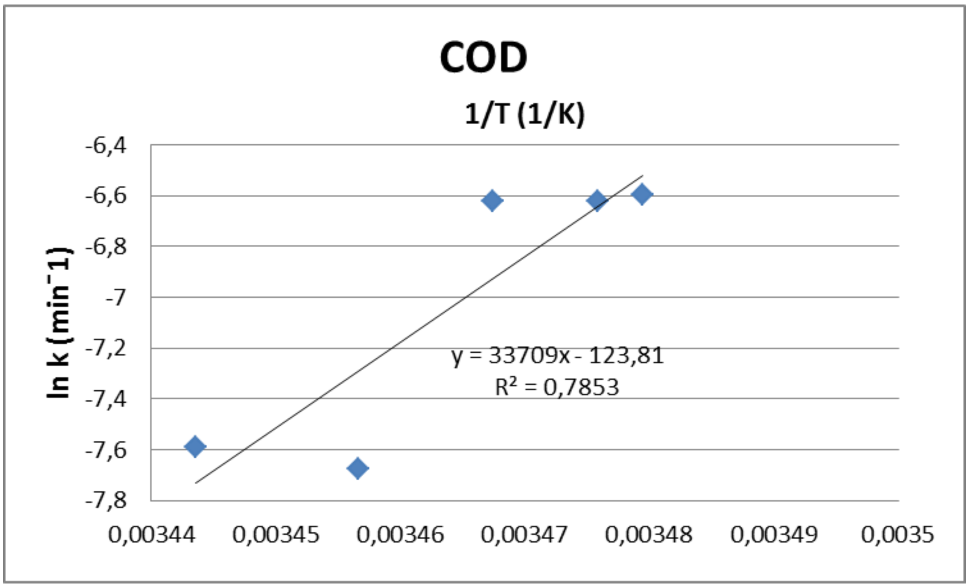


Figure 62: *The graphic plot of ln k versus 1/T during COD removal*

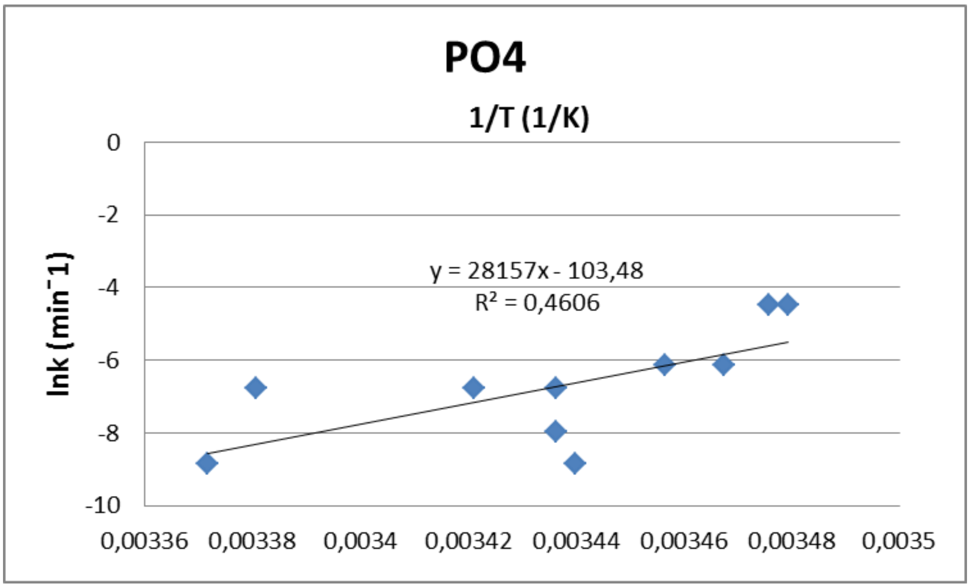


Figure 63: *The graphic plot of ln k versus 1/T during phosphate removal*

θ was calculated according equations 11 and 12. Results are shown in Table 16.

Table 16: *Calculated temperature coefficients*

Patrameters	**Θ**
Phosphate	1.00033
Ammonium	0.999
COD	1.0004

Some authors have found that θ is always close to one (Reed and Brown, 1995; Kadlec and Reddy, 2001; Stein *et al*., 2006), which means that there is little, if any, influence of temperature on k, implying that Ea is low or even null (Freire *et al*., 2009). Metcalf and Eddy (2003) stated that E_{act}/RT_1T_2 is constant as most wastewater treatment operations and processes are carried out at or near the ambient temperature. Our experimental results (Table 16) are in agreement with literature (Kadlec and Reddy (2001); Freire *et al*., 2009) where were found that θ varied from 0.9 to 1.015 for surface flow systems.

4.2.2 The removal efficiency of pilot plant

The concentrations and percent of removal statistics (mean value, standard deviation, minimum and maximum values) for NH_4^+-N, COD, conductivity, and PO_4^{3-} of the pilot plant influent and effluent are presented in Table 17. Removal efficiency of ammonium ion (34.1%) was the highest with 0% NaCl in wastewater and slightly lower (31.8%) with aeration when 2 g/l saccharose was added in wastewater containing 1.5% NaCl. This observation that the removal efficiency of SSF CW is rather low for COD and ammonia, usually <70% and <30%, respectively, was reported previously (Garcia *et al.*, 2006). The highest removal efficiency of COD in the pilot plant was 83.6% when saccharose (2 g/l) was added in 1.5% NaCl aerated wastewater. Removal efficiencies for both parameters (COD and ammonia) were higher in the pilot plant inoculated with the halotolerant microorganisms than in the study of Garcia *et al.* (2006). Removal efficiencies of both parameters (COD and NH_4^+-N) did not change significantly between the different salinities, but indicate that they were more dependent on aeration and the presence of sugar as an organic carbon source than they were on salinity.

Table 17: *Statistics of overall the influent and the effluent concentrations and removal efficiencies over time*

	Removal efficiency (%)														
	1.5% NaCl without aeration			1.5% NaCl with aeration			1.5% NaCl with aer. + 2 g/l sacchharose			0% NaCl without aeration			3% NaCl without aeration		
	d1	d3	d7	d1	d3	d7	d1	d3	d7	d1	d3	d7	d1	d3	d7
NH4-N(mg/l)															
Mean	14.4	13.3	17.0	6.8	14.8	17.0	15.9	26.1	31.8	18.2	15.9	34.1	6.8	7.9	15.9
S.D.	17.7	13.5	16.4	8.7	14.5	16.4	6.4	3.2	4.9	12.9	4.5	4.5	12.6	10.2	12.6
Minimum	0	0	0	0	0	0	9.1	18.2	27.3	9.1	9.1	27.3	0	0	0
Maximum	99.7	45.4	45.4	18.2	36.4	45.4	27.3	27.3	36.4	36.4	18.2	36.4	36.4	37.3	27.3
COD(mg/l)															
Mean	30.4	29.1	44.3	54.2	54.2	70.6	42.1	58.0	83.6	14.0	50.6	64.4	22.0	41.9	52.1
S.D.	12.7	12.7	16	15.9	15.9	1.1	23.4	28.7	3.6	12.6	15.5	11.2	17.1	23.4	14.5
Minimum	7.9	7.9	20.9	42.9	42.9	70.6	16.0	15.1	81.1	5.1	39.7	56.5	7.1	19.0	40.7
Maximum	50.2	55.7	68.2	65.5	65.5	70.6	61.3	75.5	88.7	22.9	61.6	72.3	42.9	65.4	72.3
Conductivity															
Mean	4.2	6.2	1.4	2.9	3.0	2.6	19.2	25.6	27.4	-6.3	-31.4	-36.8	11.2	12.0	11.4
S.D.	17.9	16.1	18.3	0.4	0.2	0.2	13.4	9.4	9.3	66.5	7.6	1.3	12.6	6.5	6.1
Minimum	-34.3	-37.5	-38.0	2.6	2.9	2.2	8.5	17.0	17.3	-102.0	-37.5	-37.8	0	5.7	5.5
Maximum	71.6	44.9	34.0	3.3	3.3	2.9	48.0	37.4	36.4	43.4	-20.5	-35.0	38.3	18.4	18.4
PO4															
Mean	30.8	55.1	54.3	28.6	28.6	25.0	0	28.6	25	0	-57.1	0	26.8	23.2	12.5
S.D.	49.0	36.7	30.2	20.2	10.8	16.6	0	10.8	16.6	0	26.1	0	9.1	7.4	16.1
Minimum	-128.6	-14.30	0	0	14.3	0	0	14.3	0	0	-85.7	0	14.3	14.3	0
Maximum	92.9	92.9	92.9	42.9	42.9	42.9	0	42.9	42.9	0	-28.6	0	42.9	28.6	42.9

Legend:
S.D. = standard deviation; aer.=aeration
d1= first day of circulating wastewater; d3= third day of circulating wastewater; d7= seventh day of circulating wastewater

The changes of conductivity in the pilot plant, as seen in Table 17, are a consequence of changes in salinity and the mixing of the fresh synthetic wastewater with residual material from the previous experiment in the first compartment. At 0% NaCl there was an increase in conductivity after the first day and for wastewater with 3% NaCl there was a reduction of conductivity after the first day, when wastewater was mixed.

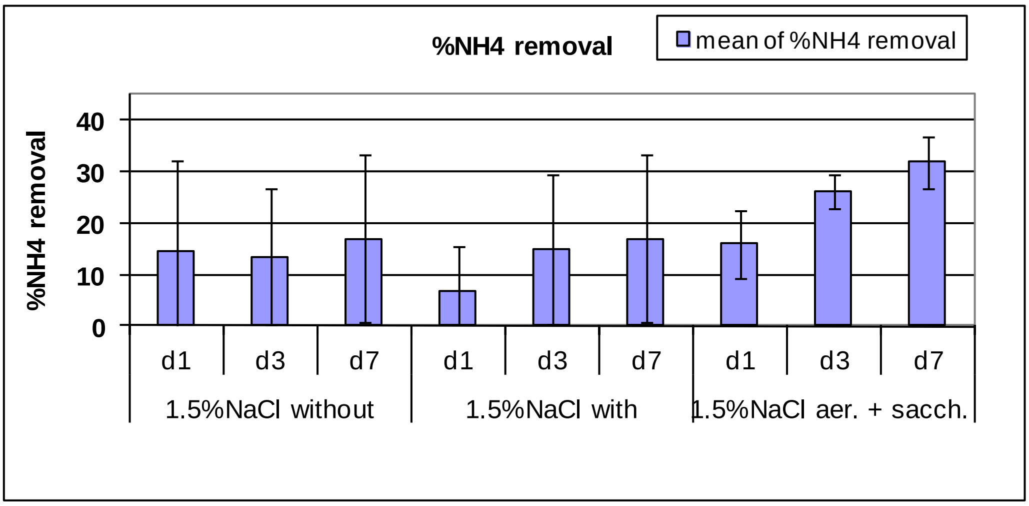


Figure 64: *Mean values ± (SD) of percent removal of ammonium over time by pilot plant in the same salinity (1.5% NaCl) without aeration, with aeration and with aeration plus 2g/l sacharose*

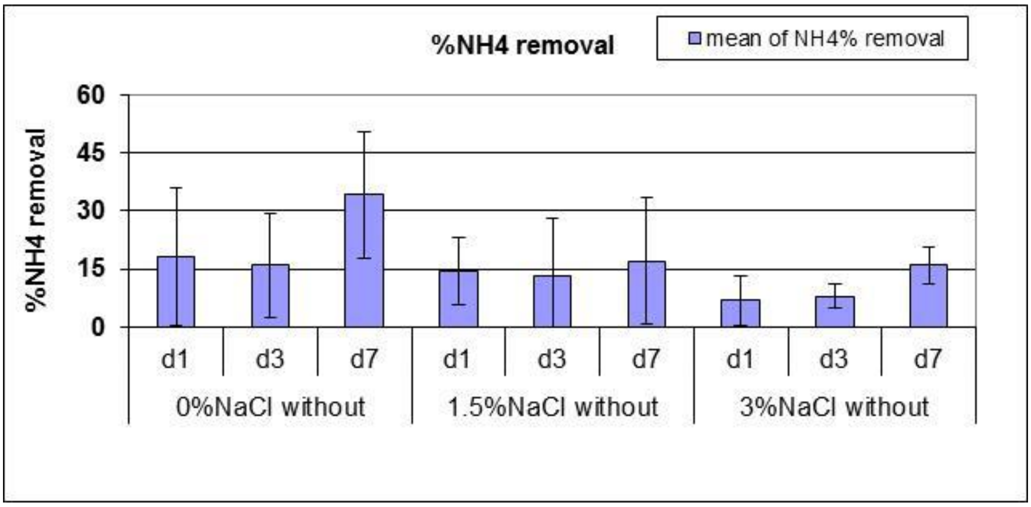


Figure 65: *Mean values ± (SD) of percent removal of ammonium over time by pilot plant in different salinities*

Figures 64 and 65 show that the highest ammonium ion removal efficiency (34.1%) was in wastewater containing no sodium chloride and 31.8% when 2 g/l of saccharose was added in aerated wastewater containing 1.5% sodium chloride. When comparing different salt concentrations, mean values for ammonium ion removal (Table 17) also indicate best efficiency at 0% NaCl (34.1%) followed by 1.5% salinity (17.0%) and 3% salinity (15.9%). However, differences were not statistically significant, indicating that the halotolerant microorganisms used in these experiments were not affected by salinity. Aeration and aeration in the presence of saccharose slightly increased the rate of ammonium ion removal. The decreased nitrification can be attributed to the anaerobic conditions (redox potential < 100 mV) even with the aeration of the pilot plant.

Lefebvre and Moletta (2006) reported that biological treatment is inhibited by high salt concentrations, but it has been proved feasible to use salt-adapted microorganisms, capable of withstanding high salinities and at the same time degrading the pollutants that are contained in wastewater. Therefore, utilization of salt tolerant microorganisms in biological wastewater treatment systems could substantially enhance COD removal from saline wastewater (Kapdan and Erten, 2006).

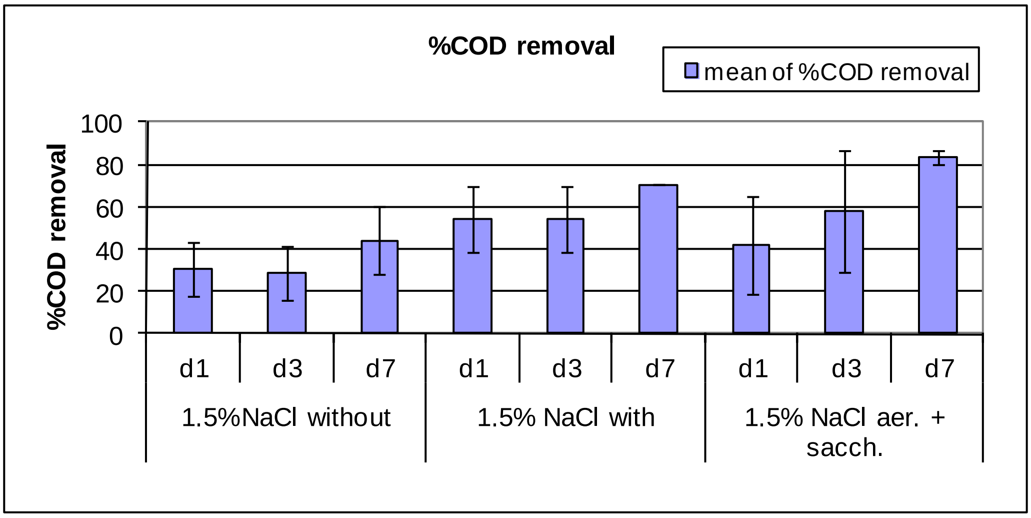


Figure 66: *Mean values ± (SD) of percent removal of COD over time by pilot plant in the same salinity (1.5% NaCl) without aeration, with aeration and with aeration plus 2g/l sacharose*

Removal efficiencies of COD in the pilot plants with 1.5% NaCl wastewater were the highest with aeration and 2 g/l saccharose (Figure 66). In the situation of 1.5% NaCl in wastewater, it was noticed that higher removal efficiencies were achieved with aeration compared to nonaerated wastewater.

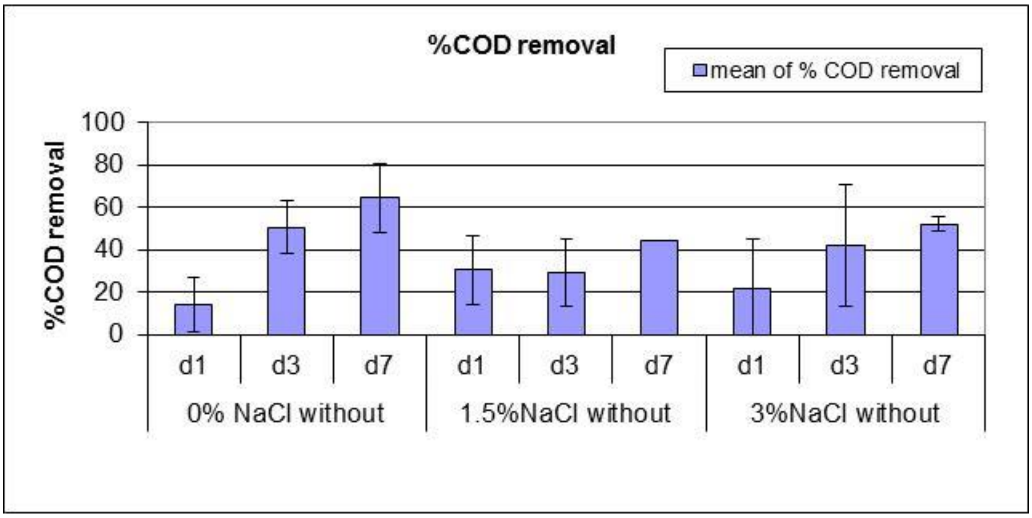


Figure 67: *Mean values ± (SD) of percent removal of COD over time by pilot plant in different salinities*

When comparing different salt concentrations (Figure 67), mean values for COD removal indicate the best efficiency at 0% NaCl (64.4%) followed by 3% salinity (52.1%) and 1.5% salinity (44.3%). Since differences were not statistically significant, results indicate that the halotolerant microorganisms were not affected by salinity. This confirms our hypothesis that halotolerant microorganisms can improve the treatment of saline wastewater in CW. This means that aeration also improved the processes of organic matter decomposition. Removal efficiencies of pilot plants inoculated with halotolerant microorganisms did not depend on variations in salinity but much more on aeration and the presence of saccharose, as already shown.

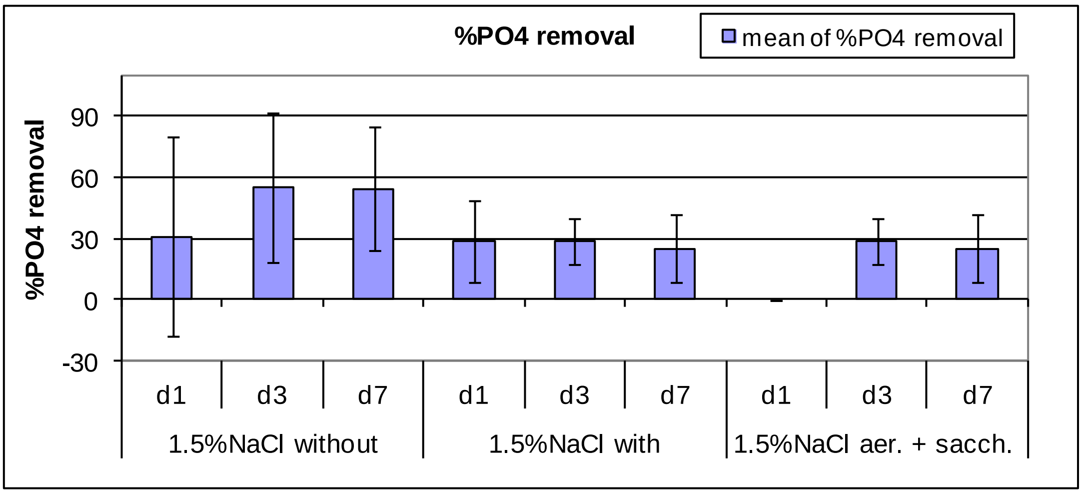


Figure 68: *Mean values ± (SD) of percent removal of phosphate over time by pilot plant in the same salinity (1.5% NaCl) without aeration, with aeration and with aeration plus 2g/l sacharose*

The major phosphorus removal processes are sorption, precipitation and peat/soil accretion. However, the soil accretion, the only non-saturable process, occurs only in FWS CWs. Removal of phosphorus in all types of CWs is low unless special substrates with high sorption capacity are used. Removal of total phosphorus varied between 40% and 60% in all types of CWs depending on CWs type and inflow loading (Vymazal, 2007). From Figures 68 and 69 it can be noticed that the highest removal efficiency of phosphate was with 1.5%NaCl without aeration and it had a mean value 54.3% (Table 17).

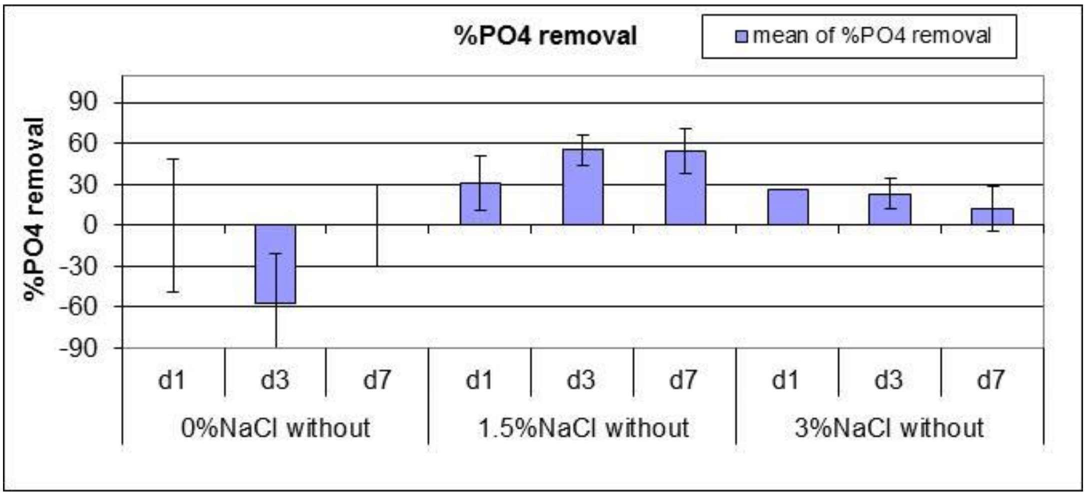


Figure 69: *Mean values ± (SD) of percent removal of phosphate over time by pilot plant in different salinities*

This is probably the result of the sorption of the phosphate ions on the substrate of the pilot plant gravel - sand filter for saline wastewater in the period of acclimation, since in the later weeks with 1.5% NaCl, concentrations of phosphate were similar to the 3% NaCl (Annex C7: Figure 101). Therefore, salinity had little, if any, influence on phosphate removal. When wastewater containing no added sodium chloride was added to the pilot plant, phosphate removal efficiency was first negative, and after 7 days it became 0%. This indicates phosphates were first washed out due to the initial decrease in salinity, and subsequently the phosphate concentration returned to its original value. The increase in phosphate concentration after reduction of salinity is also in accordance with the study of Bulc (2006) and results from CW Dragonja (Figure 25), where it was found that total phosphorus concentration in the effluent increased after periods of rain.

From Figures 70 and 71 it can be noticed that COD removal efficiency (%) was higher than the removal efficiency of NH_4^+-N and that aeration and an additional source of carbon improved COD removal more than they improved NH_4^+-N removal.

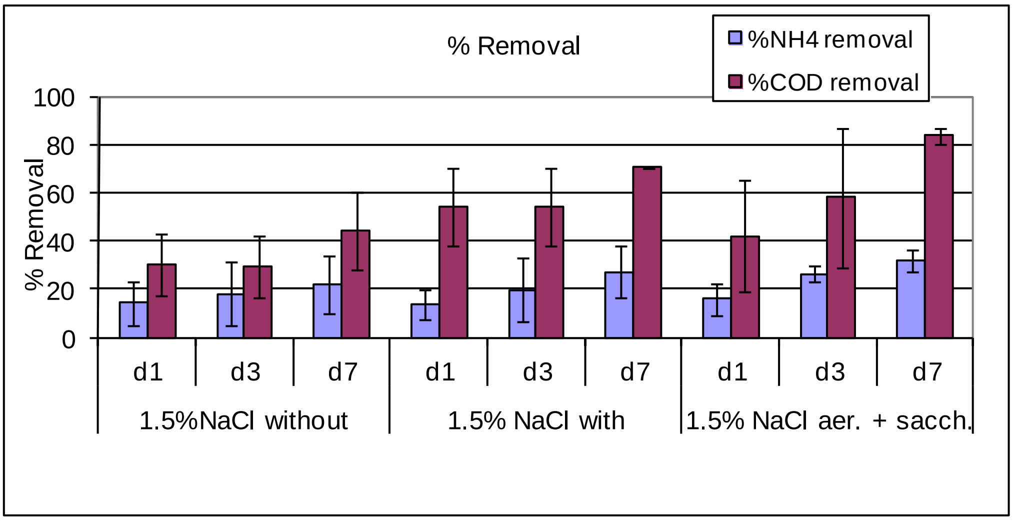


Figure 70: *Variation of percent removal of ammonium (%NH_4-N) and COD (%) over time in pilot plant in the same salinity (1.5% NaCl) without aeration, with aeration and with aeration plus 2g/l sacharose*

Microorganisms were able to use additional oxygen and saccharose for better degradation of the organic material however redox potential was still too low to improve NH_4^+-N removal efficiency.

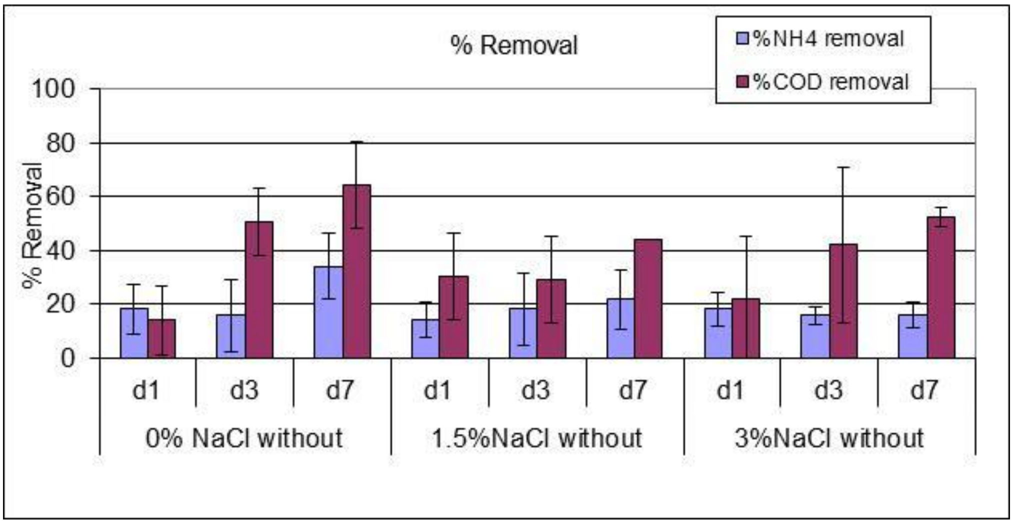


Figure 71: *Variation of percent removal of ammonium (%NH_4-N) and COD (%) over time in pilot plant in different salinities*

Salinity itself did not exert a substantial influence on COD and NH_4^+-N removal efficiencies. The measurements of microbial activity halotolerant microorganisms as

ETS (Figure 55), OD (Figure 54) and carbon dioxide in water and soil (Figures 52 and 53) also show little if any influence from salinity. These measurements provide additional evidence that microbial activity was not influenced by salinity. Consequently the halotolerant microorganisms isolated from the Sečovlje salterns are appropriate for use in saline wastewater SSF CW.

Figures 72 to 75 show mean values ± (SD) of the removal efficiency (%) of ammonium (NH_4^+-N) ions over time (1st, 3rd and 7th day) through compartments. It could be seen that in all compartments except in thirth the highest ammonium (NH_4^+-N) removal was with 0% salinity, but very close to 1.5% NaCl with aeration and added saccharose. Similar results are observable in Table 17.

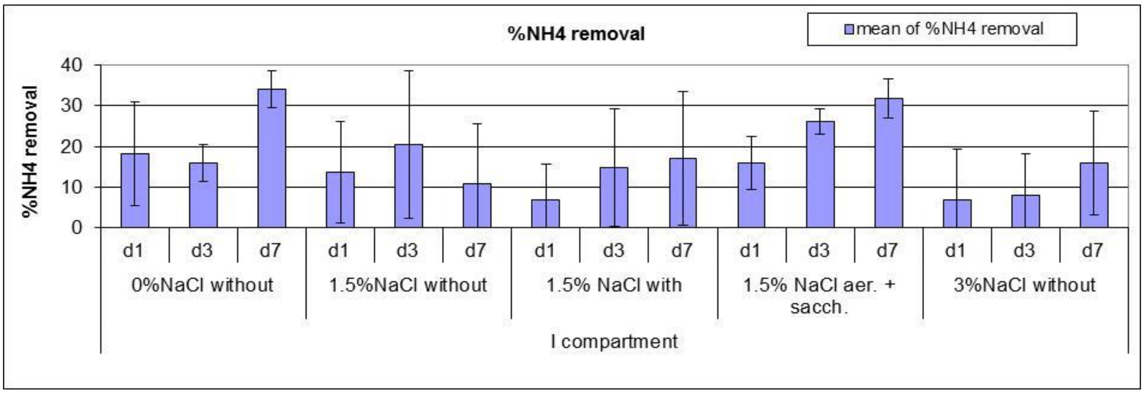


Figure 72: *Mean values ± (SD) of percent removal of ammonium (NH4+-N) over time through compartments I by pilot plant in different conditions*

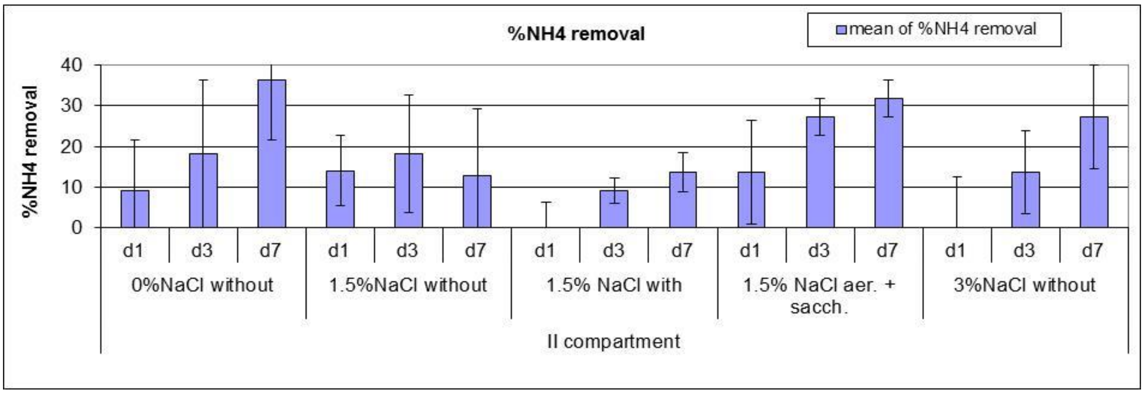


Figure 73: *Mean values ± (SD) of percent removal of ammonium (NH4+-N) over time through compartment II by pilot plant in different conditions*

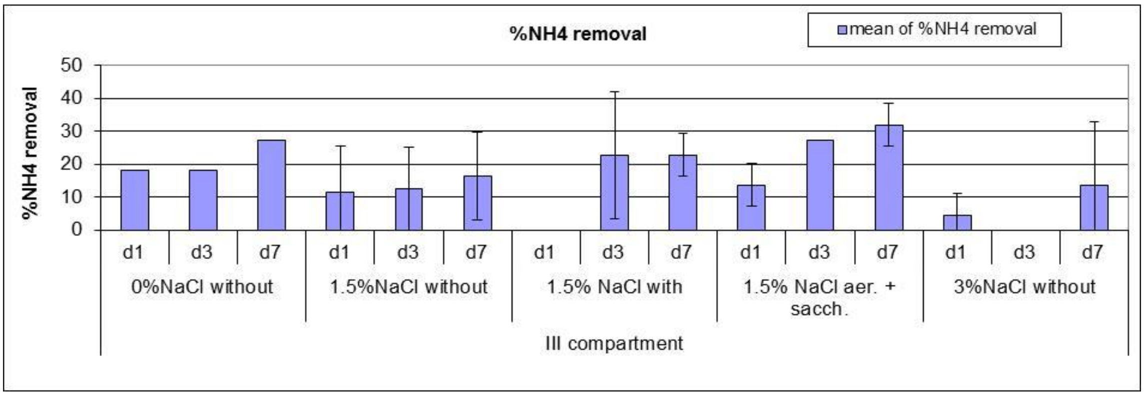


Figure 74: *Mean values ± (SD) of percent removal of ammonium (NH4+-N) over time through compartment III by pilot plant in different conditions*

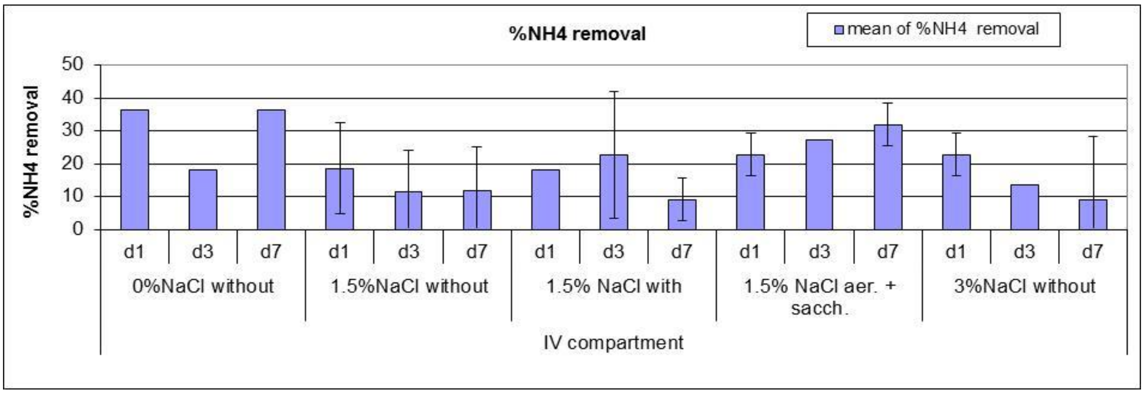


Figure 75: *Mean values ± (SD) of percent removal of ammonium (NH4+-N) over time through compartment IV by pilot plant in different conditions*

Figure 76 shows percent removal efficiency (%) of ammonium (NH_4^+-N) by pilot plant in fourth compartment on seventh day. It was finally compartment and finally day of circle of circling water in pilot plant. It shows similarly with Figures 72, 73, 74, 75 and Table 17 that the highest removal efficiency (%) of ammonium (NH_4^+-N) was with 0% NaCl.

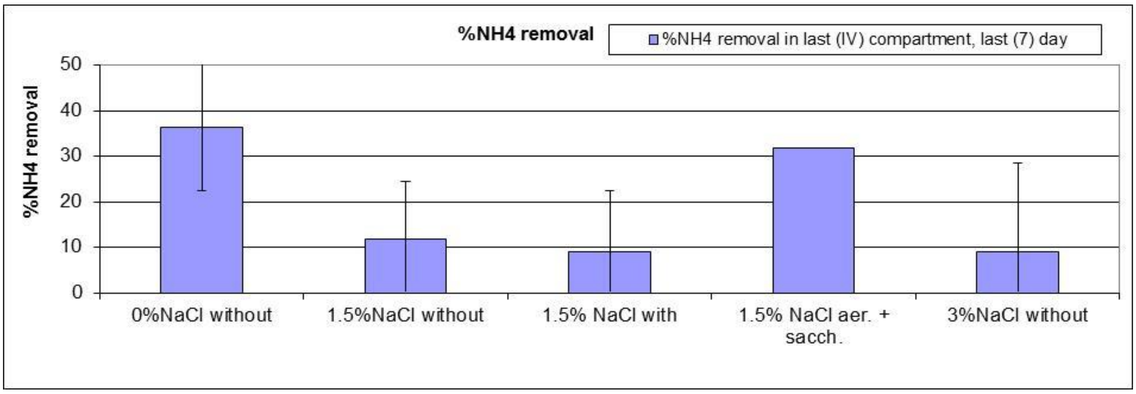


Figure 76: *Mean values ± (SD) of percent removal of ammonium (NH4+-N) by pilot plant in fourth compartment, seventh day*

Figures 77 and 78 show mean values ± (SD) of the removal efficiency (%) of COD over time (1^{st}, 3^{rd} and 7^{th} day) through compartments. From both figures it could be seen that the highest removal efficiency (%) of COD was in 1.5% NaCl with aeration and saccharose, similarly as in Table 17. Figure 79 presents the percent removal of COD by pilot plant in fourth compartment, seventh day. It can be seen that the highest removal efficiency (%) of COD was in 1.5% NaCl with aeration and saccharose as in Figures 77 and 78. In the last compartment, the final day's removal efficiency (%) of COD was similar between 1.5 NaCl with aeration and 0% salinity.

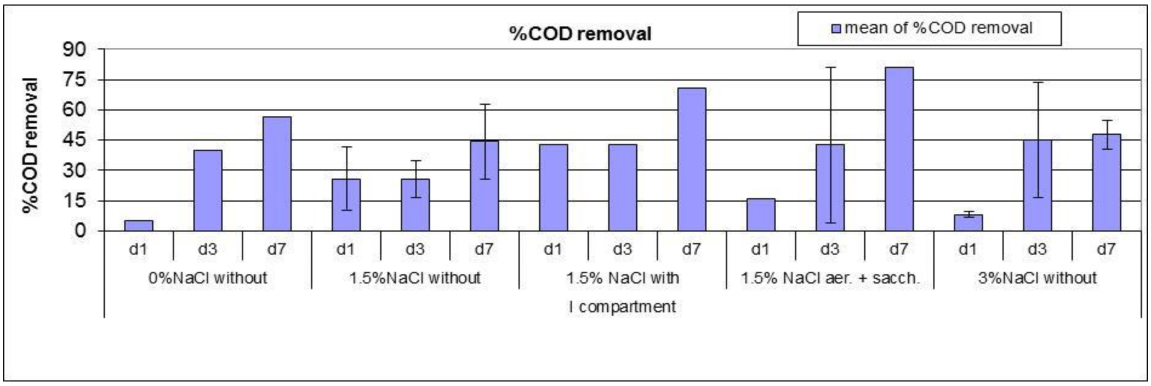


Figure 77: *Mean values ± (SD) of percent removal of COD over time through compartment I by pilot plant in different conditions*

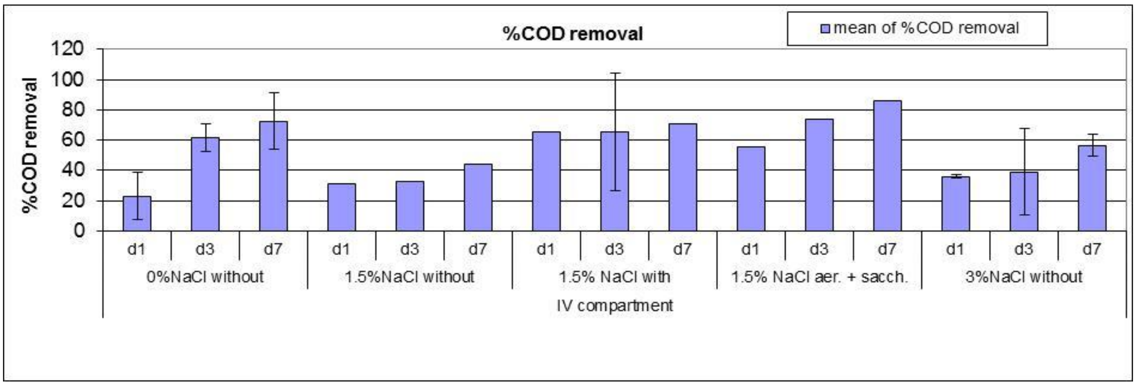


Figure 78: *Mean values ± (SD) of percent removal of COD over time through compartment IV by pilot plant in different conditions*

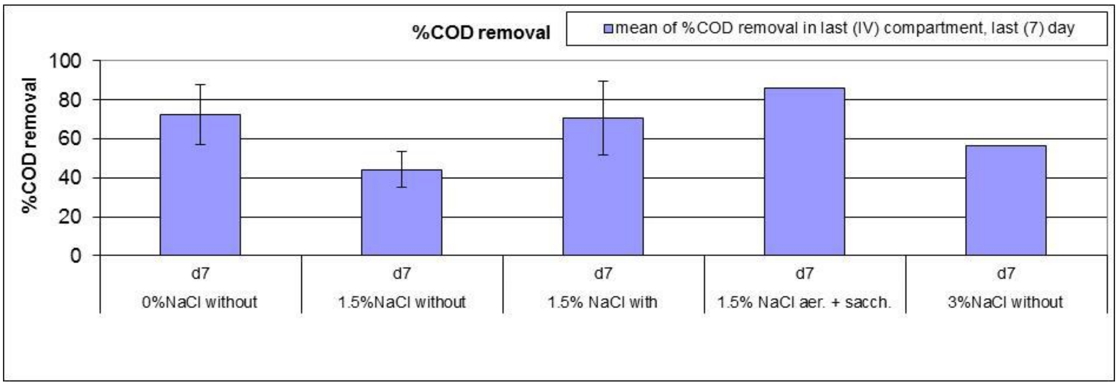


Figure 79: *Mean values ± (SD) of percent removal of COD by pilot plant in fourth compartment, seventh day*

Figures 80, 81, 82 and 83 present the removal efficiency of phosphate ions through the compartments. It can be seen that the highest removal efficiency (%) of phosphate was in 1.5% NaCl without aeration in all four compartments. In all compartments removal was negative with 0% NaCl, because it was wash out between compartments. In the last compartment, the final day's removal efficiency (%) of phosphate was the highest with 1,5% NaCl without aeration (Figure 84).

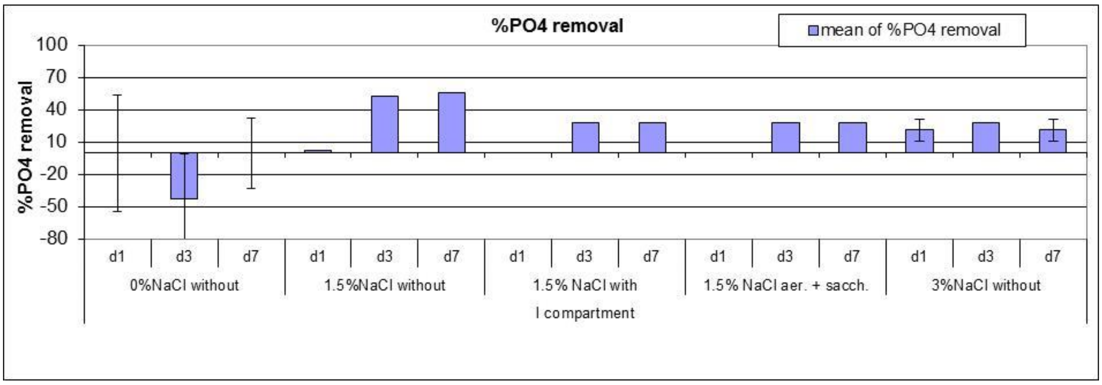


Figure 80: *Mean values ± (SD) of percent removal of phosphate over time through compartment I by pilot plant in different conditions*

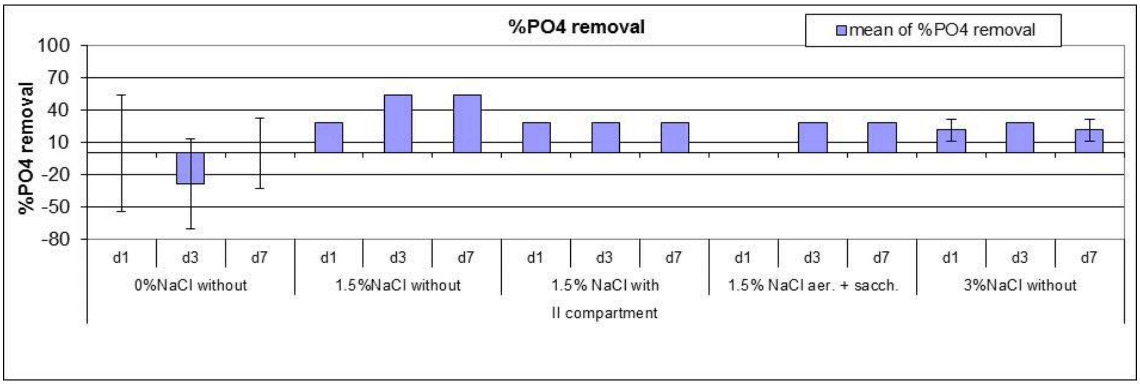


Figure 81: *Mean values ± (SD) of percent removal of phosphate over time through compartment II by pilot plant in different conditions*

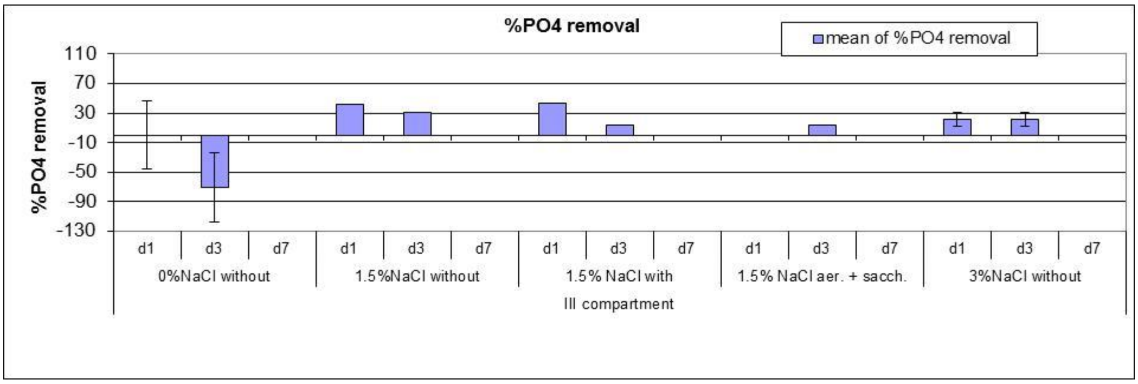


Figure 82: *Mean values ± (SD) of percent removal of phosphate over time through compartment III by pilot plant in different conditions*

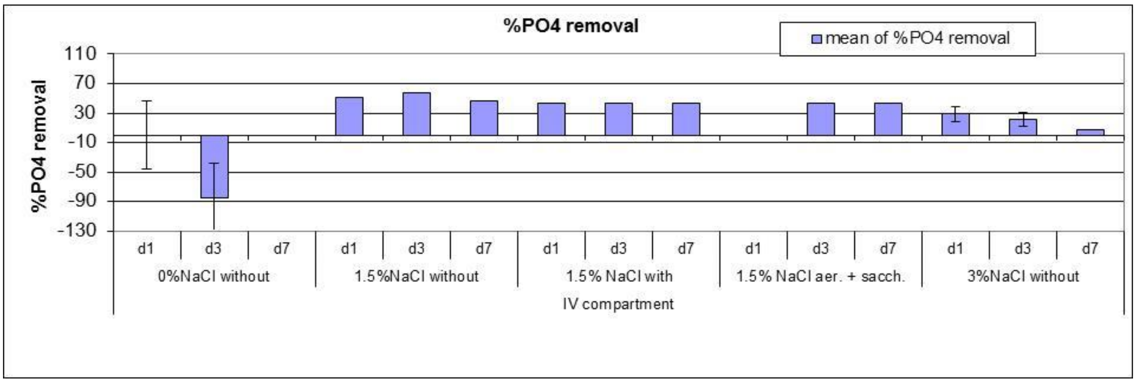


Figure 83: *Mean values ± (SD) of percent removal of phosphate over time through compartments IV by pilot plant in different conditions*

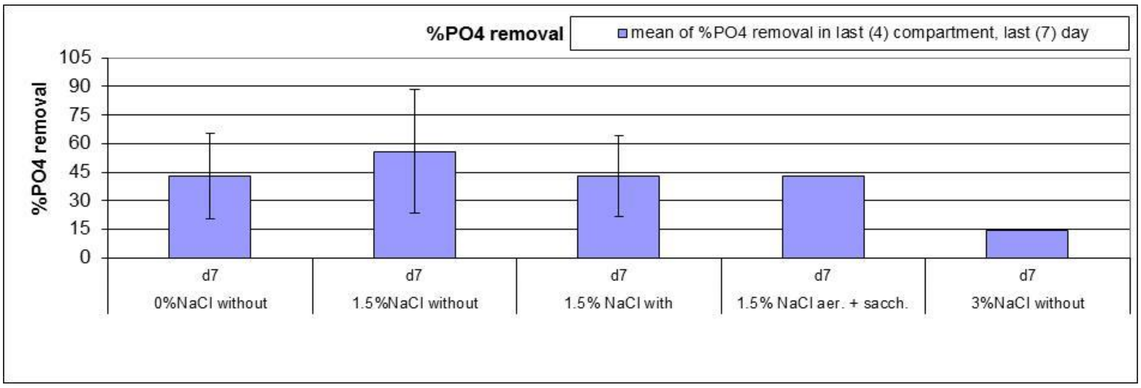


Figure 84: *Mean values ± (SD) of percent removal of phosphate by pilot plant in fourth compartment, seventh day*

From Figures 85 and 86 variations of removal efficiency (%) of phosphate and ammonium ions through the compartments with different salinity of wastewater, with aeration and without and also with aeration, when 2 g/l saccharose was added, in wastewater could seen. It could be noticed that removal efficiencies, of both parameters, are higher with higher number of compartment. Removal efficiencies were the highest in the last (fourth) compartment.

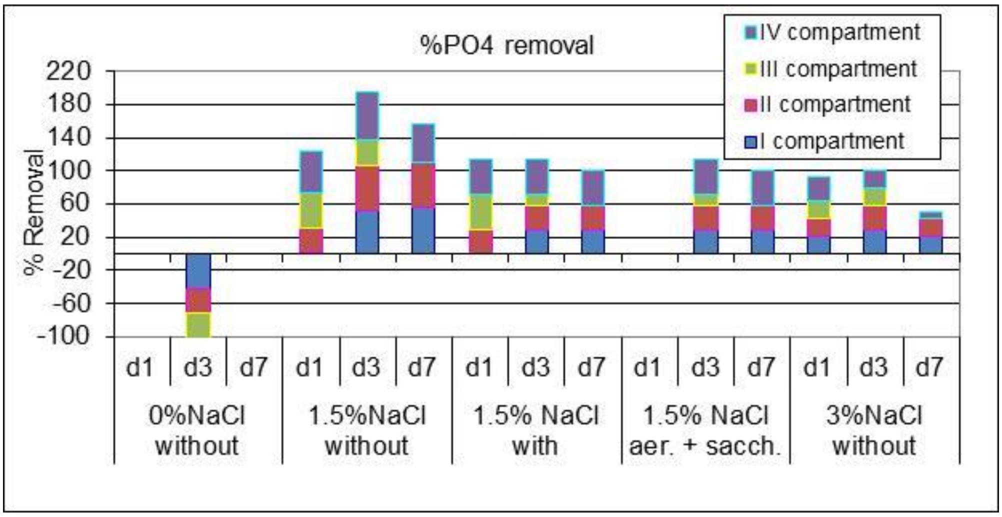


Figure 85: *Variations of percent removal of phosphate over time through compartments by pilot plant in different conditions*

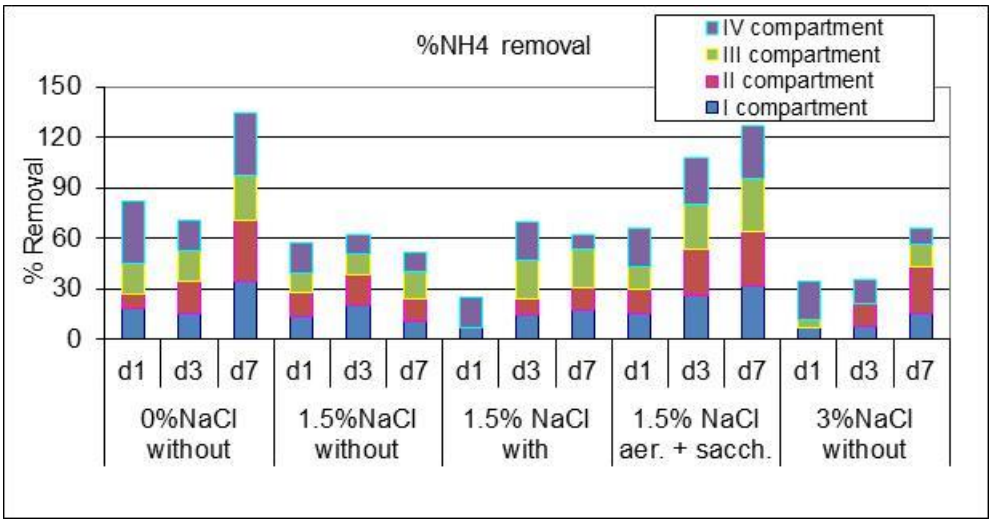


Figure 86: *Variations of percent removal of NH4+-N over time through compartments by pilot plant in different conditions*

Figure 87 shows variations of COD removal in the first and fourth compartments with different wastewater salinities, with aeration and without and also with aeration when 2 g/l saccharose was added to the wastewater. Both compartments had similar removal efficiencies, as expected, since wastewater circulated from the fourth to the first compartment. Larger differences can only be noticed for the first day of experiments, when wastewater was not yet changed in each compartment of the pilot plant.

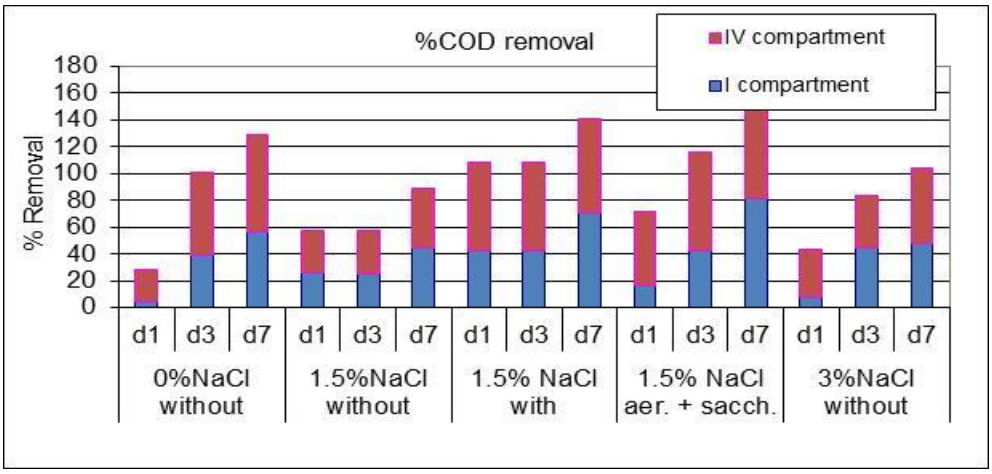
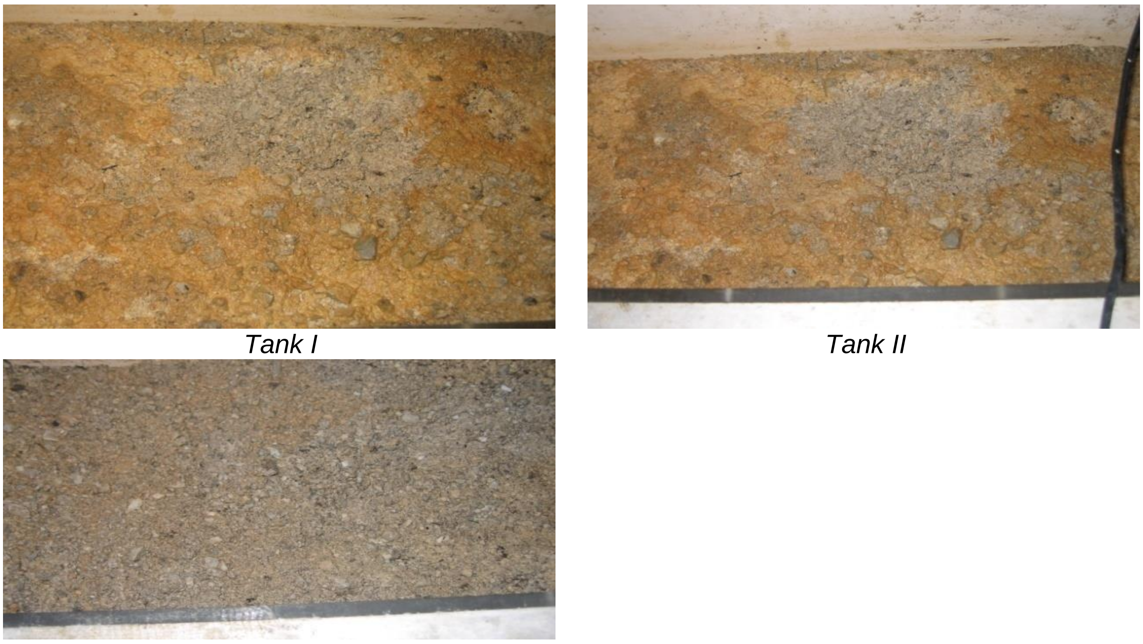


Figure 87: *Variations of percent removal of COD over time through compartments by pilot plant in different conditions*

Aeration of the pilot plant did not improve removal efficiency for ammonium ion, but improved COD removal efficiency to 26%, when experiments at the same salinity (1.5%NaCl), with and without aeration, were compared. This confirms Scholz's (2006) observation that COD removal efficiency is more dependent on aerobic conditions than ammonium ion removal. Higher removal efficiency was found with the aeration of the pilot plant, but not as much as expected. This could be explained by the low redox potential in the pilot plant. Although the sand - gravel filter pilot plant gravel - sand filter for saline wastewater was aerated, conditions were anoxic (<100 mV). This indicates that the pilot plant should be aerated more using additional air pumps in each compartment to get oxic conditions and then possibly achieve higher removal efficiency.

At the end of the experiment, 0.15 g of biomass was found in 1 g of soil from the pilot plant. At the beginning of the experiment, there was 0.11 g biomass/g soil. This means that biomass increased by 0.04 g/g of soil in the course of the experiment. From Figure 88 it is apparent that the biomass was mostly accumulated near the influent (I tank) and then gradually less in the other two tanks. Degradation of incoming sewage is completed at the distance in the pilot plant. Similar results were reported earlier by Kadlec and Knight (1996); and by Garcia *et al.* (2005). The way that microfauna was distributed along the experimental system according to the sort of organic matter supplied was probably due to differences in organic matter retention and accumulation within the SSF CW. In this sense, it is well known that in subsurface flow constructed wetlands the particulate organic matter is mostly retained near the influent (Kadlec and Knight, 1996; Garcia *et al.*, 2005).



Tank I *Tank II*

Tank III

Figure 88: *Appearance of soil in pilot plant at the end of experiment*

Legend: Brown parts are product of biodegradation and accumulation of orrganic matter; grey parts are solid

5 CONCLUSIONS

Increased wastewater salinity reduced SSF CW Dragonja removal efficiency, but not significantly. Removal efficiency of ammonium ion, nitrate ion, total phosphorus, BOD_5 and COD were reduced with higher influent conductivity, but coefficients of correlation were low. These weak coefficients of correlation mean that chloride concentration and conductivity can explain only a small percent of the variability in removal efficiency. The variability in the treatment efficiency of CW Dragonja may be attributed to the presence of varying concentrations of toxic materials other than those determined in the influent and listed in Table 14, thus possibly inhibiting the treatment process.

It can be concluded that in the pilot plant gravel - sand filter for saline wastewater inoculated with halotolerant microorganisms, removal efficiency did not depend so much on the variation of salinity but much more on the aeration and presence of saccharose, making oxygen and energy source the factors that limited removal efficiencies.

Results of redox potential and ammonia removal indicate that the pilot plant should be aerated more using additional air pumps in each compartment to get oxic conditions and then possibly achieve higher removal efficiency.

Results show that microbial respiration was the most intensive when fresh synthetic wastewater was added to the pilot plant and when the synthetic wastewater contained 2 g/l saccharose.

Results for ETS activity, as a measure of microbial activity, lead to the conclusion that two weeks after inoculation into pilot plant for saline wastewater the halotolerant microorganisms had adapted to the new conditions.

It was observed that the changes in phosphate ion concentration in wastewater with 1.5% (after second week of inoculation) were similar to those in wastewater with 3% NaCl, but a slightly higher concentration of phosphate ion was detected at 0% NaCl in wastewater. This indicates that with 0% saline wastewater, phosphate from previous experiments was washed out of the substrate. An increase in the salinity to 3% NaCl did not substantially change phosphate removal.

In the absence or presence of saccharose, the pH in the pilot plant varied between 7.7 and 8.5. The variations were not significant.

Results obtained in the pilot plant confirm our hypothesis that halotolerant microorganisms could survive in saline wastewater, that they could be effective during variability of wastewater salinity and that they could improve wastewater treatment of saline water in the pilot plant gravel - sand filter for saline wastewater.

No example of application of halotolerant microorganisms to CW or a sand-gravel pilot plant was detected in literature available at present. The results from the pilot plant show that use of halotolerant microorganisms could be effective during variability of wastewater salinity and improve the efficiency of its treatment. Special attention should be paid to the aerobic/anaerobic conditions because anerobic conditions strongly hinder ammonium ion removal regardless of salinity, while COD removal is not sensitive to changes in salinity.

5.1 Specific contribution of PhD

Very little information on somewhat similar approaches is reported in literature so far, especially regarding the long-term interpretation of data sets from Dragonja CW, and dedicated test series using the innovative experimental set-up. It was definitely shown in this thesis that halotolerant microorganisms can enhance COD removal in CW or sand-gravel type of treatment facilities, and that this type of organisms showed no sensitivity to variable, as well as higher salinity.

6 REFERENCES

Admon S., Tarre S., Sabbah I., Lahav O., Beliavski M., Green M. 2005. Treatment of presettled municipal wastewater using a passively aerated vertical bed. Environ. Eng. Sci, 22: 707;

Akratos C. S., Tsihrintzis V. A. 2007. Effect of temperature, HRT, vegetation and porous media on removal efficiency of pilot-scale horizontal subsurface flow constructed wetlands. Ecological engineering, 29: 173–191;

Al-Shahwani S. M., Horan N. J. 1991. The use of protozoa to indicate changes in the performance of activated sludge plants. Water Research, 25: 633–638;

Alves M. M., Gonzalez Beca C. G., Guedes De Carvalho R., Castanheira J. M., Sol Pereira M. C., Vasconcelos L. A. T. 1993. Chromium removal in tannery wastewaters polishing by *Pinus sylvestris* bark. Water Research, 27, 8: 1333–1338;

Anton J., Rosselo-Mora R., Rodriguez-Valera F., Amman R. 2000. Extremely halophilic bacteria in crystallizer ponds from solar salterns. Appl. Environ. Microbiol., 66: 3052-3057;

APHA. 1990. Standard Methods for Examination of Water and Wastewater. 17^{th} edition. Washington DC;

Armstrong W. 1982. Water-logged soils. In: Environment and plant ecology. Etherington J. R. (ed.), John Wiley, Chichester: 290-330;

Austin D. C, Lohan E., Verson E. 2003. Nitrification and denitrification in a tidal vertical flow wetland pilot. Proceedings of the water environment technical conference 2003. Water Environment Federation. Los Angeles, California;

Baedeker M. J., Back W. 1979. Hydrogeological processes and chemical reactions at a landfill. Ground Water, 17, 5: 429–437;

Baere D. L. A., Devocht M., Van Assche P., Verstraete W. 1984. Influence of high NaCl and NH_4Cl salt levels on methanogenic associations. Water Res., 18, 5: 543-548;

Bajza Z., Vrcek I. V. 2001. Water quality analysis of mixtures obtained from tannery waste effluents. Ecotoxicology and Environmental Safety, 50: 15–18;

Balizon M. E., Dolmus R., Quintana J., Navarro Y., Donze M. 2002. Comparison of conventional and macrophyte-based systems for the treatment of domestic wastewater. Water. Sci. Technol, 45: 111-116;

Baptista J. D. C. 2003. Microbial communities in subsurface flow wetlands. In: The Use of Aquatic Macrophytes for Wastewater Treatment in Constructed Wetlands.

Dias V., Vymazal J. (ed.). Articles of the 1st International Seminar, Lisboa, Portugal: 265–276;

Barrow N. J. 1983. On the reversibility of phosphate sorption by soils. J Soil Sci, 34: 751–758;

Battistoni P., Fava G. 1994. Fish processing wastewater treatment requirements by line production changes. Wat. Sci. Tech., 29, 9: 111–119;

Behrends L., Sikora F., Coonrod H., Bailey E., Bulls M. 1996. Reciprocating subsurface-flow constructed wetlands for removing ammonia, nitrate, and chemical oxygen demand: potential for treating domestic, industrial, and agricultural wastewaters. Proceedings of WEFTEC '96; the 69th annual conference and exposition of the water environment federation. Alexandria, Virginia;

Benlloch S., Acinas S. G., Anton J., Lopez- Lopez A., Luz S. P. Rodriguez-Valera F. 2001. Archeal biodiversity in crystallizer ponds from a solar saltern: culture versus PCR. Microbiol. Ecol., 41: 12-19;

Benlloch S., Lopez- Lopez A., Casamayor E. O., Ovreas L., Goddard V., Daae F. L., Smerdon G., Massana R., Joint I., Thingstad F., Pedros-Alio C., Rodriguez-Valera F. 2002. Procaryotic genetic diversity throughout the salinity gradient of a coastal solar saltern. Environ. Microb., 41: 12-19;

Bolhuis H., Poele E. M., Rodriguez-Valera F. 2004. Isolation and cultivation of Walsby's square archaeaon. Environ. Microbiol.: 1287-1289;

Boon P. I., Mitchell A. 1995. Methanogenesis in the sediments of an Australian freshwater wetland: comparison with aerobic decay and factors controlling methanogenesis. FEMS Microbiol. Ecol., 18, 3: 174-190;

Bower C.A., Ogata G., Tucker J.M. 1968. Sodium hazard of irrigation waters as influenced by leaching fraction and by precipitation or solution of calcium carbonate. Soil Sci. 106: 29-34;

Bowman J. P., McCammon S. A., Rea S. M. Mc Meekin T. A. 2000. The microbial composition of three limnologicaly disparate hypersaline Antarctic lakes. FEMS Microbiol. Lett., 183: 81-88;

Boyd C. E. 1969. Production, mineral nutrient absorption, and biochemical assimilation by *Justicia americana* and *Alternanthera philoxeroides*. Arch Hydrobiol, 66:139–160;

Brix H. 1994. Functions of macrophytes in constructed wetlands. Wat. Sci. Tech., 29: 71;

Brix H. 1999. How 'green' are aquaculture, constructed wetlands and conventional wastewater treatment systems? Wat. Sci. Tech., 40, 3: 45-50;

Brown J. J., Glenn E. P., Fitzsimmons K. M., Smith S. E. 1999. Halophytes for the treatment of saline aquaculture effluent. Aquaculture, 175: 255-268;

Bulc T., Vrhovšek D. 1996. Čiščenje otpadnih voda s cestišč z rastlinskimi čistilnimi napravami. V: Voda in ceste. Strokovno posvetovanje, Slovensko društvo za hidravlične raziskave, DRC, Novo Mesto: 73-78;

Bulc T. 1998. The efficiency of landfill leachate treatment with different types of constructed wetlands. Dissertation Thesis, University of Ljubljana, Biotechnical Faculty, Department for Biology;

Bulc T. 2006. Long term performance of a constructed wetland for landfill leachate treatment. Ecological engineering, 2, 6: 365–374;

Bulc G. T., Zupančič J. M. 2007. Sustainable Solution for Lanfill Leachate with use of Phytoremediation. In: Landfill Research Trends. Velini A. A. (ed.)., 4: 33;

Burchell M. R., Saggs R. W., Lee C. R., Broome S., Chescheir G. M., Osborne J. 2007. Substrate Organic Matter to improve nitrate removal in Surface-Flow Constructed Wetlands. J Environ Qual, 36: 194-207;

Burdick D. N., Buchsbaum R., Holt E. 2001. Variation in soil salinity associated with expansion of Phragmites australis in salt wetlands. Environ. Exp. Bot., 46: 247–261;

Buresh R. J., Casselman M. E., Patrick J. W. H. 1980. Nitrogen fixation in flooded soil systems. Adv Agron, 33: 149–192;

Burns D. G., Camakaris H. M., Janssen P. H., Dyall-Smith M. 2004. Combined use of cultivation-dependent and cultivation-independent methods indicates that members of most haloarchael groups in an Australian crystallizer ponds are cultivable. Appl. Environ. Microbiol., 79: 5258-5265;

Butinar L., Zalar P., Frisvad J. C., Gunde-Cimerman N. 2005. The genus Eurotium – members of indigenous fungal community in hypersaline waters of salterns. FEMS Microbiology Ecology, 54: 491-498;

Calheiros C. S. C., Rangel A. O. S. S., Castro P. M. L. 2007. Constructed wetland systems vegetated with different plants applied to the treatment of tannery wastewater. Water Res., 41, 8: 1790–1798;

Calheiros C. S. C., Rangel A. O. S. S., Castro P. M. L. 2008. Evaluation of different substrates to support the growth of *Typha latifolia* in constructed wetlands treating tannery wastewater over long-term operation. Bioresource Technology, 99: 6866–6877;

Calheiros C. S. C., Duque A. F., Moura A., Henriques I. S., Correia A., Rangel A. O. S. S., Castro P. M. L. 2009a. Changes in the bacterial community structure in two-stage constructed wetlands with different plants for industrial wastewater treatment. Bioresource Technology, 100: 3228–3235;

Calheiros C. S. C., Rangel A. O. S. S., Castro P. M. L. 2009b. Treatment of industrial wastewater with two-stage constructed wetlands planted with *Typha latifolia* and *Phragmites australis*. Bioresource Technology, 100: 3205–3213;

Calheiros C. S.C., Teixeira A., Pires C., Franco A. R., Duque A. F., Crispim L. F. C., Moura S. C., Castro P. M. L. 2010. Bacterial community dynamics in horizontal flow constructed wetlands with different plants for high salinity industrial wastewater polishing. Water Research, 44: 5032-5038;

Campell C.S., Ogden M.H. 1999. Constructed Wetlands in the Sustainable Landscape is the first book to integrate aesthetic design and planning issues with the technical aspects of wetlands engineering. John Wiley and Sons, New York: 43-56.

Caselles-Osorio A., Garcia J. 2007. Effect of physico-chemical pretreatment on the removal efficiency of horizontal subsurface-flow constructed wetlands. Environmental Pollution, 146: 55-63;

Chambers R. M., Meyerson L. A., Saltonstall K. 1999. Expansion of Phragmites australis into tidal wetlands of North America. Aquat. Bot., 64: 261–273;

Chan E., Bursztynsky T. A., Hantzsche N., Litwin T. J. 1982. The use of Wetlands for Water Pollution Control. Municipal Environmental Protection Agency. Washington;

Chang Y., Corapcioglu M. Y. 1998. Plant-enhanced subsurface bioremediation of non-volatile hydrocarbons. J. Environ. Eng, 112: 162-169;

Christensen T. H., Kjeldsen P. 1995. Landfill emissions and environmental impact: An introduction. In: Sardinia' 95, Fifth International Landfill Symposium Proceedings. Christensen T. H., Cossu R., Stegmann R. (ed.), CISA, Cagliari, Italy, 3;

Christensen T. H., Kjeldsen P., Bjerg P. L., Jensen D. L., Christensen J. B., Baun A., Albrechtsen H. J., Heron G. 2001. Biogeochemistry of landfill leachate plumes. Applied Geochemistry, 16: 659-718;

Choi W. J., Ro H. M., Chang S. X. 2005. Carbon isotope composition of *Phragmites australis* in a constructed saline wetland. Aquatic Botany, 82: 27–38;

Chu L. M., Cheung K. C., Wong M. H. 1994. Variations in the chemical properties of landfill leachate. Environmental Management, 18, 1: 105-117;

Collins B., McArthur J. V., Sharitz R. R. 2004. Plant effects on microbial assemblages and remediation of acidic coal pile runoff in mesocosm treatment wetlands. Ecological Engineering, 23: 107-115;

Connolly R., Zhao Y., Sun G., Allen S. 2004. Removal of ammoniacal-nitrogen from an artificial landfill leachate in downflow reed beds, Process Biochemistry, 39: 1971-1976;

Cooper P. F. 1990. European design and operations guidelines for reed bed treatment systems. Prepared by EC/EWPCA Emergent Hydrophyte Treatment Systems Expert Group, Water Research Centre, Swindon, UK;

Cooper P. F. 1999. A review of the design and performance of vertical-flow and hybrid reed treatment systems. Water Science and Technology, 40, 3: 1-9;

Cottingham P.D., Davies T. H., Hart B. T. 1999. Aeration to promote nitrification in constructed wetlands. Environ. Tech, 20: 69;

Craft B. C., Richardson C. J. 1993. Peat accretion and phosphorus accumulation along a eutrophication gradient in the northern Everglades. Biogeochemistry, 22: 133–156;

Crites R. W., Middlebrooks E. J., Reed S. C. 2006. Natural Wastewater Treatment Systems. Taylor and Francis Group, CRC Press. New York;

Curds C. R. 1963. The floculation of suspended matter by *Paramecium caudatum*. J. Gen. Microbiol., 33: 357–363;

Dahl C., Sund C., Kristensen G. H., Vredenbregt L. 1997. Combined Biological Nitrification and Denitrification of High Salinity Wastewater. Wat. Sci. Tech., 36, 2-3: 345-352;

Dalmacija B., Karlovic V., Tamas Z. and Miskovic D. 1996. Purification of high salinity wastewater by activated sludge process. Wat. Res., 30, 2: 295-298;

Dan N. P. 2000. Special Study: Saline wastewater survey and trends of study on biological treatment. AIT, Bangkok, Thai Land;

Dan N. P. 2001. Biological treatment of high salinity wastewater using yeast and bacterial systems. Dissertation. Asian Institute of Technology. School of Environment, Resources and Development Bangkok, Thailand: 27-90;

Daniels, R., 2001. Enter the root-zone: green technology for the leather manufacturer, part 2. World Leather. 14, 5: 52–54;

Daniels R. P. 2008. Giving nature a chance. S&V African Leather, 2, 5: 17-21;

Danijela Kleva Švagelj. 2006. Data of the Public company Okolje Piran d.o.o. Monitoring did Institute of Public Health Koper in the period between 1992 to 2007. (personal source, April 2006);

DIN 38 404-C4-2. Bestimmung der Temperature;

DIN 38409-H28. Determination of total nitrogen - TN;

Dincer A. R., Kargi F. 1999. Salt Inhibition of Nitrification and Denitrification in Saline Wastewater. Environ. Tech., 20, 1147-1153;

Dufay J. A. 2000. Patent: Constructed wetlands remediation system. New Mexico 6,159,371;

Dunne E. J., Reddy K. R. 2005. Phosphorus biogeochemistry of wetlands in agricultural watersheds. In: Nutrient management in agricultural watersheds: a wetland solution. Dunne E. J., Reddy R., Carton O. T. (ed.). Wageningen Academic Publishers. Wageningen, The Netherlands: 105-119;

Doudoroff M. 1940. Experiments on the adaptation of *Escheria coli* to Sodium Chloride. J. Gen. Physiology, 23: 585-611;

Dykyjová D., Květ J. 1978. Pond littoral ecosystems: structure and functioning. Springer-Verlag. Berlin;

Ellis B. G. 1974. The Soil as a Bological Filter. EPA660/2-74-002. US Environmental Protection Agency;

Faulwetter J. L., Gagnon V., Sundberg C., Chazarenc F., Burr M. D., Brisson J., Campera A. K., Stein O.R. 2009. Microbial processes influencing performance of treatment wetlands: a review. Ecological Engineering 35, 987-1004;

Farquhar C. J., Rovers F. A. 1973. Gas production during refuse decomposition, Water Air Soil Pollut.: 2, 483;

Finlayson C. M., Chick A. J. 1983. Testing the potential of aquatic plants to treat abbatoir effluent. Water Research, 17: 415-422;

Flowers D. A. 2002. Patent: process and system for enhanced nitrogen removal in a wetland wastewater treatment facility. United States 6,447,682;

Flowers T. J., Flowers S. A. 2005. Why does salinity pose such a difficult problem for plant breeders? Agricultural Water Management, 78: 15-24;

Flowers T. J., Troke P. F., Yeo A. R., 1977. The mechanism of salt tolerance in halophytes. Ann. Rev. Plant Physiol., 28: 89–121;

Freire F. G., Davies L. C., Vacas A. M., Novais J. M., Martins-Dias S. 2009. Influence of operating conditions on the degradation kinetics of an azo-dye in a vertical flow constructed wetland using a simple mechanistic model. Ecological Engineering, 35: 1379–1386;

Fritioff A., Kautsky L., Greger M. 2005. Influence of temperature and salinity on heavy metal uptake by submersed plants. Environmental Pollution, 133: 265-274;

Focht D. D., Verstraete W. 1977. Biochemical ecology of nitrification and denitrification. Adv Microb Ecol, 1: 135–214;

Fogler H. S. 2005. Elements of Chemical Reaction Engineering. Prentice Hall. USA;

Förstner U. 1979. Metal transfer between solid and aqueous phases. In: Metal Pollution in the Aquatic Environment. Förstner U., Wittman G. T. W. (ed.). Springer-Verlag, Berlin: 211-224;

Fowler J., Cohen L., Jarvis P. 1998. Practical statistics for field biology. Chichester, John Wiley & Sons;

Gagnon V., Chazaren F., Comeau Y., Brisson J. 2007. Influence of macrophyte species on microbial density and activity in constructed wetlands. Water Science and Technology, 56, 3: 249-254;

Garcia J., Aguirre P., Barragan J., Mujeriego R., Matamoros V., Bayona J. P. 2005. Effect of key design parameters on the efficiency of horizontal subsurface flow constructed wetlands. Ecological Engineering, 25: 405–418;

Garcia J., Capel V., Castro A., Ruiz I., Soto M. 2006. Anaerobic biodegradation tests and gas emissions from subsurface flow constructed wetlands. Bioresour. Technol. doi:10.1016/j.biortech.2006.10.016

Garcia J., Ojeda E., Sales E., Chico F., Piriz T., Aguirre P., Mujeriego R. 2003. Spatial variations of temperature, redox potential and contaminants in horizontal flow reed beds. Ecol. Eng., 21: 129–142;

Garver E. G., Dubbe D. R., Pratt D. C. 1988. Seasonal patterns in accumulations and partitioning of biomass and macronutrients in *Typha* spp. Aquat Bot, 32: 115–127;

Gersberg R. M., Elkins B. V., Lyon S. R., Goldman C. R. 1986. Role of aquatic plants in wastewater treatment by artificial wetlands. Water Research, 20: 363-368;

Glass C., Silerstein J. 1999. Denitrification of high-nitrate, highsalinity wastewater. Water Res, 30, 1: 223–229;

Glenn E., Thompson L. T., Frye R., Riley J., Baumgartner D. 1995. Effects of salinity on growth and evapotranspiration of *Typha domingensis Pers*. Aquat Bot, 52:75–91;

Grant W. D., Long P. E. 1981. Environmental microbiology. Blackie and Son, Glasgow;

Gray N. F. 2004. Biology of Wastewater Treatment. 2nd edition. Vol. 4. Imperial College Press. London: 641- 733;

Green M., Friedler E., Safrai I. 1998. Enhancing nitrification in vertical flow constructed wetland utilizing a passive air pump. Water Res, 32, 12: 3513–3520;

Greenway M., Woolley A. 1999. Constructed wetlands in Queensland: Performance efficiency and nutrient bioaccumulation. Ecological Engineering, 12: 39–55;

Greger M., Kautsky L., Sandberg T. 1995. A tentative pilot plant of Cd uptake in *Potamogeton pectinatus* in relation to salinity. Environmental and Experimental Botany, 35, 2: 215-225;

Gunde-Cimerman N., Zalar P., De Hoog S. and Plemenitaš A. 2000. Hypersaline waters in salterns – natural ecological niches for halophilic black yeasts. FEMS Microbiol. Ecol., 32: 235–240;

Haberl R., Grego S., Langergaber G., Kadlec R.H., Cicalini A.R., Dias S. M., Novais J. M., Aubert S., Gerth A., Thomas H., Hebner A. 2003. Constructed wetlands for Treatment of Organic Pollutants. J. Soils & Sediments.3, 2: 109-124;

Hackley K. C., Liu C. L., Coleman D. D. 1996. Environmental isotope characteristics of landfill leachates and gases.Ground Water, 34, 5: 827–836;

Hadad H. R., Maine M. A., Bonetto C. 2006. Macrophyte growth in a pilot-scale constructed wetland for industrial wastewater treatment. Chemosphere. 63, 10:1744–1753;

Hafez A. I., El-Manharawy M. S., Khedr M. A. 2002. RO membrane removal of unreacted chromium from spent tanning effluent. A pilotscale study, Part 2. Desalination, 144: 237–242;

Hanstveit A. O. 1992. Biodegradibility of petroleum waxes and beeswax in an adapted CO_2 evolution test. Chemosphere, 25: 605-620;

Hauck R. D. 1984. Atmospheric nitrogen chemistry, nitrification, denitrification, and their relationships. In: The handbook of environmental chemistry, the natural environment and biogeochemical cycles. Hutzinger O. (ed.). Springer-Verlag, Berlin 1: 105–127;

Heath E. 1998. Mikrobiološka razgradnja nekaterih poliaromatskih ogljivodikov in identifikacija njihovih produktov. Doktorska disertacija. Univerza v Ljubljani. Fakulteta za kemojo in kemijsko tehnologijo: 57-59;

Hernandez A. J.,Adarve M.J., Gil A., Pastor J. 1999. Soil salination from landfill leachates: Effects on the macronutrient content and plant growth four grassland species. Chemosphere. 38, 7: 1693-1711;

Hester W. M., Mendessohn I. A., McKee K. L. 2001. Species and population variation to salinity stress in *Panicum hemitomon*, *Spartina patens*, and *Spartina alterniflora*: morphological and physiological contraints. Environ Exp Bot. 46: 277–297;

Hinteregger C., Streichsbier F. 1997. *Halomonas* sp., an moderately halophilic strain, for biotreatment of saline phenolic wastewater. Biotechnol Lett, 19: 1099–1102;

Hockenbury M. R., Burstein D., Jamro E. S. 1978. Total Dissolved Solids Effects on Biological Treatment. In Proceedings of the 32nd Industrial Waste Conference. Purdue University, Lewis Publishers; Chelsea;

Hu C., Zhang T. C. ,Huang Y. H. , Dahab M. F., Surampalli R. 2005. Effects of Long-Term Wastewater Application on Chemical Properties and Phosphorus Adsorption Capacity in Soils of a Wastewater Land Treatment System. Environ. Sci. Technol., 39, 18: 7240–7245;

Hunt P. G., Poach M. E., Liehr S. K. 2005 Nitrogen cycling in wetland systems. In: Nutrient management in agricultural watersheds: a wetland solution. Dunne E. J., Reddy K. R., Carton O. T. (ed.). Wageningen Academic Publishers, The Netherlands: 93-104;

Iizumi H., Haattori A., McRoy C. P. 1980. Nitrate and nitrite in interstitial waters of eelgrass beds in relation to the rhizosphere. Journal of Experimental Marine Biology and Ecology, 47: 191-201;

Intrasungkha N., Keller J., Blackall L. 1999. Biological nutrient removal efficiency in treatment of saline wastewater. Water Technol, 39, 6:183–190;

ISO 5664. Water quality - Determination of ammonium –Distilation and titration method;

ISO 5815-1. Water quality – Determination of biochemical oxygen demand after n days (BODn) – Part 1 Dilution and seeding method with allylthiourea addition;

ISO 6878. Water quality – Determination of phosphorus – Ammonium molybdate spectrometric method;

ISO 7888. Water quality – Determination of electrical conductivity;

ISO 10304-2. Water quality – Determination of dissolved anions by liquid chromatography of ions. Determination of bromide, chloride, fluoride, nitrite, nitrate, phosphate and sulfate;

ISO 10523. Water quality-Determination of pH;

ISO 15586. Water quality – Determination of trace elements using atomic absorption spectrometry with graphite furnace;

ISO 15705. Water quality - Determination of the chemical oxygen demand index (ST-COD)- Small scale – tube method;

Jenssen P. D., Krogstad T. 2003. Design of constructed wetlands using phosphorus sorbing lightweight aggregate (LWA). In: Constructed wetlands for wastewater treatment in cold climates. Mander Ü., Jenssen P. D., (ed.). Southampton, UK: WIT Press, 259–271;

Jetten M. S. M., Logemann S., Muyzer G. M., Robertson L. A., DeVries S., Van Loosdrecht M. C. M. 1997. Novel principles in the microbial conversion of nitrogen compounds. Antonie van Leeuwenhoek, 71: 75–93;

Jing S. R., Lin Y. F. 2004. Seasonal effect on ammonia nitrogen removal by constructed wetlands treating polluted river water in southern Taiwan. Environ Pollut., 127, 2: 291-301;

Jones K. C. 1991. Organic Contaminants in the Environment. Elsevier Applied Science. New York;

Joslyn A., Timmons B. 1967. Salt-Use in Food Processing. In: Fundamentals of Food Processing Operations: Ingredients, Methods and Packaging. Heid J. L., Joslyn M.A. (ed.). The Avi Publishing Co., England: 25-32;

Kadlec R. H., Knight R. L. 1996. Treatment wetland. Boca Raton. Lewis Publishers, CRC Press, Inc. Florida, USA: 29-33;

Kadlec R. H., Knight R. L., Vymazal J., Brix H., Cooper P., Haberl R. 2000. Constructed Wetlands for Pollution Control: Processes, Performance, Design and Operation. Scientific and Technical Report No. 8, International Water Association, IWA Publishing, London;

Kadlec R. H., Reddy K. R. 2001. Temperature Effects in Treatment Wetlands. Water Environment Research, 73, 5: 543-557;

Kapdan I. K., Erten B. 2006. Anaerobic treatment of saline wastewater by *Halanaerobium lacusrosei*. Process Biochem;

Kadlec R. H., Wallace S., D. 2009. Treatment Wetlands. Taylor and Francis Group, CRC Press. New York, 2: 830-840;

Kargi F., Uygur A. 1996. Biological Treatment of Saline Wastewater in an Aerated Percolator Unit Utilizing Halophilic Bacteria. Env. Tech., 17: 325-320;

Kargi F., Dincer A. R. 1996. Enhancement of biological treatment performance of saline wastewater by halophilic bacteria. Bioprocess Eng, 15: 51–58;

Kaseva M. E., Mbuligwe S. E. 2010. Potential 365 of constructed wetland systems for 366 treating tannery industrial wastewater. Water Sci. Technol. 61,4: 1043-1052;

Keddy P. A. 2000. Wetland Ecology: Cambridge University Press, Cambridge, UK: 12- 22;

Khan A. G. 2001. Relationships between chromium biomagnification ratio, accumulation factor, and mycorrhizae in plants growing on tannery effluent-polluted soil. Environmental International, 26: 417–423;

Kickuth R.1984. Das Wurzelraumverfahren in der praxis. Landschaft and Stadt, 16: 145-153;

Kierek-Pearson K., Karatan E. 2005. Biofilm development in bacteria. Adv. Appl. Microbiol., 57: 79–111;

Kincannon D. F., Gaudy A. F. 1968. Response of Biological Waste Treatment Systems to Changes in Salt Concentration. Biotechnol. Bioeng., 10: 483-496;

Kjeldsen P., Barlaz M. A., Rooker A. P., Baun A., Ledin A., Christensen T. H. 2002. Present and Long-Term Composition of MSW Landfill Leachate: A Review. In: Critical Reviews in Environmental Science and technology, 32, 4:297-336;

Klomjek P., Nitisoravut S. 2005. Constructed treatment wetland: a study of eight plant species under saline conditions. Chemosphere, 58: 585–593;

Kocaoba S., Akcin G., 2002. Removal and recovery of chromium and chromium speciation with MINTEQA2. Talanta, 57: 23–30;

Koprivnikar M. 2008. Development of salt concentration inoculums for biological treatment of wastewater salt content. Graduation thesis, University of Ljubljana;

Korkusuz, E.A., 2005. Manual of Practice on Constructed Wetlands for Wastewater Treatment and Reuse in Mediterranean Countries. Report, MED-REUNET II Support Programme (EC Project No: INCO-CT-2003-502453). Agbar Foundation, Spain.
</http://medreunet. org//>;

Kosjek T, Heath E., Kompare B. 2007. Removal of pharmaceutical residues in a pilot wastewater treatment plant. Anal Bioanal Chem, 387:1379–1387;

Kucuk O. S., Sengul F., Kapdan I. K. 2003. Removal of ammonia from tannery effluents in a reed bed constructed wetland. Water Sci. Technol., 48, 11–12: 179–186;

Kuenen J. G., Robertson L. A. 1987. Ecology of nitrification and denitrification. In: The nitrogen and sulphur cycles. Cole J. A., Ferguson S. J. (ed.) Cambridge University Press. Cambridge, UK: 162–218;

Kurata A., Kiro T. 1991. Water Quality Aspects in Guidelines of Lake Management. Lake Shore Management. International Lake Environment Committee and United Nations Environment Program;

Laanbroek H. J. 1990. Bacterial cycling of minerals that affect plant growth in waterlogged soils. Aquat Bot, 38: 109–25;

Lahav o., Artzi E., Tarre S., Green M. 2001. Ammonium removal efficiency using a novel unsaturated flow biological filter with passive aeration. Wat. Res., 35: 397;

Langergraber G., Haberl R., Laber J., Pressl A. 2003. Evaluation of substrate clogging processes in vertical flow constructed wetlands. Water Sci. Technol., 48, 5: 25–34;

Lawton G. W., Egger C. V. 1957. Effect of High Sodium Chloride Concentration on Trickling Filter Slimes. J. Water Pollut. Control Fed., 37: 1404;

Lefebvre O., Moletta R. 2006. Treatment of organic pollution in industrial saline wastewater: A literature review. Water research, 40: 3671-3682;

Limnos 2013.
http://www.limnos.si/publikacije.php

Lin T., Wen Y., Jiang L., Li J., Yang S., Zhou Q. 2008. Study of atrazine degradation in subsurface flow constructed wetland under different salinity. Chemosphere. doi:10.1016/j.chemosphere.2007.11.059;

Lissner J., Schierup H. H. 1997. Effects of salinity on the growth of *Phragmites australis*. Aquat. Bot., 55, 247–260;

Lissner J., Schierup H. H., Comin F. A., Astorga V. 1999a. Effect of climate on the salt tolerance of two *Phragmites australis* populations. Part I: growth, inorganic solutes, nitrogen relations and osmoregulation. Aquat. Bot.,64: 317–333;

Lissner J., Schierup H. H., Comin F. A., Astorga V. 1999b. Effect of climate on the salt tolerance of two *Phragmites australis* populations. Part II: diurnal CO_2 exchange and transpiration. Aquat. Bot., 64: 335–350;

Ludzack F. J., Noran D. K. 1965. Tolerance of high salinity by conventional wastewater treatment process. Journal WPCF, 37, 10: 1404-1416;

Lundmark A. 2003. Predicting environmental impact of deicing salt – a pilot plantling approach. Dept. of Land and Water Resources Engineering, KTH;

Lymbery A. J., Doupé G. R., Bennett T., Starcevich M. R. 2006. Efficiency of a subsurface-flow wetland using the estuarine sedge *Juncus kraussii* to treat effluent from inland saline aquaculture. Aquacultural Engineering. 34, 1: 1-7;

Macek P., Rejmankova E. 2007. Response of emergent macrophytes to experimental nutrient and salinity additions. Funct Ecol. 21:478–488;

Maciolek D., Austin D. 2006. Low Energy Biological Nitrogen Removal by Cation Exchange, Thin Film Oxygen Transfer, and Heterotrophic Nitrification in Sequencing-Batch, Packed-Bed Reactors. Proceedings of the Water Environment Federation, WEFTEC: Session 21 through Session 30, 23:1560-1582;

Madoni P. 1994. A sludge biotic index (SBI) for the evaluation of the biological performance of activated sludge plants based on the microfauna analysis. Water Res., 28: 67–75;

Madoni P., Gorbi G., Taje E. 1998. Toxic effect of chemical disinfection of wastewater on freshwater ciliates. Acta Protozool., 37: 221–225;

Maehlum T., Jenssen P. D., Warner W. S. 1995. Cold-climate constructed wetlands. Water Sci. Technol., 32: 95;

Maehlum T. 1998. Wetlands for treatment of landfill leachates in cold climates. In: Proceedings of the Constructed Wetlands for the Treatment of Landfill Leachates.

Mulamoottil G., McBean E. A., Rovers F. (eds.). CRC Lewis Publishers, New York, USA: 33–46;

Maine M. A., Sune N., Hadad R. H., Sanchez G. C., Bonetto C. 2009. Influence of vegetation on the removal of heavy metals and nutrients in a constructed wetland. J Environ Manag. 90: 355–36;

Maltais – Landry G., Chazarenc F., Comeau Y., Troesch S. 2007. Effects of artificial aeration, macrophyte species, and loading rate on removal efficiency in constructed wetland mesocosms treating fish farm wastewater. J. Environ. Eng. and Sci., 6: 409;

Mant C., Costa S., Williams J., Tambourgi E. 2006. Phytoremediation of chromium by pilot plant constructed wetland. Bioresource Technology, 97: 1767–1772;

Margesin R., Schinner F. 2001. Potential of halotolerant and halophilic microorganisms for biotechnology. Extremophiles, 42: 73–83;

Martın-Cereceda M., Zamora J., Perez-Uz B., Guinea A. 2002. Ciliate communities of rotating biological contactor biofilms: a multivariate approach. system. Appl. Microbiol., 25: 301–313;

Martin C. D., Johnson K. D., Moshiri G. A. 1999. Performance of a constructed wetland leachate treatment system at the Chunchula landfill, Mobile County, Alabama. Water Science and Technology. 40, 3: 67-74;

Mathewson C.C., Mathewson H.A. 1998. Designing a wetland for wastewater treatment – a truly interdisciplinary effort. In: International Association for Engineering Geology and the Environment, International Congress. Vancouver. 5, 8;

Mayo A. W. and Bigambo T. 2005. Nitrogen transformation in horizontal subsurface flow constructed wetlands. In: Physics and Chemistry of the Earth, Parts A/B/C. Nhapi I., Holch W., Mazvimavi D., Mashauri D., Jewitt G., Mudege N., Swatuk L.A., Beukman R. (ed.). 30, 11-16: 623 – 992;

Mendelsson I. A., Postek M. T. 1982. Elemental analysis of deposits on the roots of *Spartina alterniflora* Loisel. American Journal of Botany, 69: 904-912;

Mendez R., Omil F., Soto M., Lema J. M. 1992. Pilot plant studies on the anaerobic treatment of different wastewaters from a fish-canning factory. Wat. Sci. Tech., 25, 1: 37-44;

Metcalf and Eddy. 2003. Revised by Tchobanoglous, G., Burton, F.L., Stensel, H.D., Wastewater Engineering—Treatment Reuse, 4th ed. McGraw-Hill Series in Civil and Environmental Engineering, New York: 55–56;

Mitsch W. J., Gosselink J. G. 2000. Wetlands. John Wiley & Sons. New York, USA, 3: 920;

Miyamoto S., Glenn E. P., Olsen M. W. 1996. Growth water use and salt uptake of four halophytes irrigated with highly saline water. J. Arid Environ, 32: 141-159;

Morgan M. D., Mandernack K. W. 1996. Biogechemistry of sulphur in wetland peat following 3.5 Y of artificial acidification (HUMEX). Environ. Intern., 22, 5: 605-610;

Moss B. 1998. Ecology of Fresh Waters. Blackwell Science. Oxford, UK, 3;

Mufarrege M. M. Di Luca G. A. Hadad H. R. Maine M. A. 2011. Adaptability of *Typha domingensis* to high pH and salinity. Ecotoxicology. 20: 457–465;

Mulder A., van de Graaf A. A., Robertson L. A., Kuenen J. G. 1995. Anaerobic ammonium oxidation discovered in a denitrifying fluidized bed reactor. FEMS Microbiol Ecol, 16:177–184;

Nichols D. S. 1983. Capacity of natural wetlands to remove nutrients from wastewater. Journal of the Water Pollution Control Federation, 55, 5: 495-505;

Nishihara E. 2001. Technical details of the yeast cycle system, Personal Communication and Company Process Catalog;

Nitisoravut S., Klomjek P. 2005. Inhibition kinetics of salt-affected wetland for domestic wastewater treatment. Water Research 39: 4413–4419;

Nivala J., Hoos M.B., Cross C., Wallace S., Parkin G. 2007. Treatment of landfill leachate using an aerated, horizontal subsurface-flow constructed wetland. Science of the Total Environment, doi:10.1016/j.scitotenv.2006.12.030;

Nivala J., Aubron T., Bernhard K., Mosig P., Afferden M., Wallace S., Müller R. 2014.
Performance and dimensioning of actively aerated treatment wetland systems. DWA-Workshop, Postdam, Germany;

Novak J. 2005. Fitoremedijacija slanih območij. Seminarska naloga. Univerza v Lubljani. Fakulteta za gradbeništvo in geodeziju.: 91-95;

Novak J., Trapp S. 2005. Growth of Plants on TBT-contaminated Harbour Sludge and Effect on TBT Removal. ESPR-Environ SCI & Pollut Res., 12, 6: 332-341;

Ochsenreiter T., Pfeifer F., Schleper C. 2002. Diversity of archae in hypersaline environments charachterized by molecular-phylogenetic and cultivation studies, Extremophiles. 6.: 247-274;

OG RS 35/1996, 21/2003: Official Gazette of the Republic of Slovenia. Decree on the Emission of Substances and Heat in the Drainage of Wastewater from Pollution Sources, 35/1996, amendment 21/2003;

OG RS 47/2005: Decree on the emission of substances and heat in the discharge of wastewater into waters and public sewage system. Official Gazette of the Republic of Slovenia;

OG RS 7/2000. Decree on the emission of substances in the discharge of landfill effluent. Official Gazette of the Republic of Slovenia;

Oman C., Hynning P. A. 1993. Identification of organic compounds in municipal landfill leachates. Environ. Pollut., 80: 265–271;

Ouellet – Plamondon C., Chazarenc F., Comeau Y., Brisson J. 2006. Artificial aeration to increase pollutant removal efficiency efficiency of constructed wetlands in cold climate. Eco. Eng., 27: 258;

Panswad T., Anan C. 1999. Specific oxygen, ammonia and nitrate uptake rates of a biological nutrient removal process treating elevated salinity wastewater. Bioresource Technol; 70: 237–243;

Pavčič M. 1995. Constructed Wetland for Landfill Leachate Treatment at Dragonja. Graduation thesis. University of Ljubljana, Biotechnical Faculty, Biology Dep.: 2-25;

Pašić L., Bartual G., Poklar Ulrih S., Grabnar N., Herzog Velikonja N. 2005. Diversity of halophilic archaea in the crystallizers of an Adriatic solar saltern. FEMS microbiology, ecology; 54: 491-498;

Paterson S., Mackay D., Tam D., Shiu W. Y. 1990. Uptake of organic chemicals by plants: a review of processes, correlations and mdels. Chemosphere, 21: 297-331;

Patrick J. W. H., Gotoh S., Williams B. G. 1973. Strengite dissolution in flooded soils and sediments. Science, 179: 564–566;

Patrick J. W. H., Khalid R. A. 1974. Phosphate release and sorption by soils and sediments: effect of aerobic and anaerobic conditions. Science, 186: 53–55;

Paul E. A., Clark F. E. 1996. Soil microbiology and biochemistry. Academic Press. San Diego, California, 2: 340 -345;

Pell M., Wörman A. 2008. Biological Wastewater Treatment Systems. In: Encyclopedia of Ecology, 1: 426-441;

Pirbazari J. 1996. Hybrid membrane filtration process for leachate treatment. Wat. Res., 30: 2691-2706;

Pitt R., Clark S., Field R. 1999. Groundwater contamination potential from stormwater infiltration practices. Urban Water, 1: 217-236;

Plaster E. J. 2003. Soil pH and Salinity. In Soil Science and Management, 4: 173-195;

Platzer C. 1996. Enhanced nitrogen elimination in subsurface flow artificial wetlands—a multi stage concept. In: Proceedings of the Fifth International Conference on Wetland Systems for Water Pollution Control, Vienna, Austria, September 15–19: I/7-1–I/7-9;

Poe A. C., Piehler M. F., Thompson S. P., Paerl H. W. 2003. Denitrification in a constructed wetland receiving agricultural runoff. Wetlands. 23, 4: 817-826;

Puigagut J., Salvado H., Garcıa J. 2005. Short-term harmful effects of ammonia nitrogen on activated sludge microfauna. Water Res., 39: 4397–4404;

Puigagut J., Salvado H., Garcıa J. 2007. Effect of soluble and particulate organic compounds on microfauna the community in subsurface flow constructed wetlands. ecological engineering, 29: 280–286;

Reasoner D. J., Geldreich E. E. 1985. A new medium for the enumeration and subculture of bacteria from potable water. Appl. Environ. Microbiol. 49,1: 1-7;

Reddy K. R., Patrick W. H. 1984. Nitrogen transformations and loss in flooded soils and sediments. CRC Crit Rev Environ Control, 13: 273–309;

Reddy K. R., DeBusk W. F. 1987. Nutrient storage capabilities of aquatic and wetland plants. In: Aquatic plants for water treatment and resource recovery. Reddy K. R., Smith W. H. (ed.). Orlando, Florida: Magnolia Publishing: 337–357;

Reed S. C., Middlebrooks E. J. and Crites R. W. 1995. Natural Systems for Waste Management and Treatment . 2nd edition. McGraw-Hill, New York, USA;

Reed S. C., Brown D. S. 1995. Subsurface flow wetlands - a performance evaluation. Water Environ. Res., 67: 244–248;

Rhue R. D., Harris W. G. 1999. Phosphorus sorption/desorption reactions in soils and sediments. In: Phosphorus biogeochemistry in subtropical ecosystems. Reddy K. R., O'Connor G. A., Schelske C. L. (ed.). CRC Press, Boca Raton, Florida: 187–206;

Rhoades J.D., Krueger D.B., Reed M.J., 1968. The effect of soil-mineral weathering on the sodium hazard of irrigation waters. Soils Sci. Am. J. 32: 643-647;

Richardson C. J. 1985. Mechanisms controlling phosphorus retention capacity in freshwater wetlands. Science, 228:1424–1427;

Richardson C. J., Marshall P. E. 1986. Processes controlling movement, storage, and export of phosphorus in a fen peatland. Ecol Monogr,56: 279–302;

Richardson C. J., Qian S. S., Craft B. C., Qualls R. G. 1997. Predictive pilot plants for phosphorus retention in wetlands. Wetlands Ecol Manag, 4: 159–175;

Ringuelet R. 1983. Un bel example d’epuration des eaux domestiques pousse jusqu’a la decontamination fecale avec des methodes naturalles douces et rustiques: le basin versant de l'etang de Salces-Leucate. Techniques et Sciences Municipales: Eau, 4: 187-201;

Robinson H. D., Grantham G. 1988. The treatment of landfill leachates in on-site aerated lagoon plants: experience in Britain and Ireland. Wat. Res., 22, 6: 733-747;

Rudd J. W. M., Kelly C. A., Louis V. S., Hesslein R. H., Furutani, Holoka M. H. 1986. The role of sulfate reduction in long term accumulation of organic and inorganic sulfur in lake sediments. Limnol. Oceanogr., 31, 6: 1281-1291;

Salvado H., Gracia M. P. 1993. Determination of organic loading rate of activated sludge plants based on protozoan analysis. Water Res., 27, 5: 891–895;

Salvado H., Gracia M. P., Amigo J. M. 1995. Capability of ciliated protozoa as indicators of effluent quality in activated sludge plants. Water Res., 29, 4: 1041–1050;

Sartaj M., Fernandes L., Castonguay N. 1998. Treatment of leachate from a landfill receiving industrial, commercial, institutional, and construction, demolition wastes in an engineered wetland. Proceeding: Constructed wetlands for the treatment of landfill leachates. Mulamoottil; G., McBean E. A., Rovers F. (ed.). CRC Lewis Publishers, NY, USA: 165 – 174;

Savant N. K., DeDatta S. K. 1982. Nitrogen transformations in wetland rice soils. Adv Agron, 35: 241–302;

Schalk J., de Vries S., Kuenen J. G., Jetten M. S. M. 2000. Involvement of a novel hydroxylamine oxidoreductase in anaerobic ammonium oxidation. Biochemistry, 39: 5405–5412;

Schlesinger W. H. 1991. Biogeochemistry: An Analysis of Global Change. Academic Press. San Diego, USA;

Schmidt I., Zart D., Bock E. 2001. Effects of gaseous NO_2 on cells of *Nitrosomonas eutropha* previously incapable of using ammonia as an energy source. Antonie van Leeuwenhoek, 79: 39–47;

Schmidt I., Sliekers O., Schmid M., Bock E., Fuerst J., Kuenen J. G., 2003. New concepts of microbial treatment process for the nitrogen removal from wastewaters. FEMS Microbiol Rev, 27: 481–492;

Schneider J. 1995. Eindunstung von Meerwasser und Salzbildung in Salinen. Kali Steinsalz, 11: 325–330;

Schnoor J. L., Licht L. A., McCutcheon S. C., Wolfe N. L., Carreira L. H., 1995. Phytoremediation of organic and nutrient contaminants. Environ. Sci. Technol., 29: 318-323;

Schnoor J. L. 1996. Chemical reaction kinetics. In: Environmental modeling. Schnoor J. L. (ed.) John Wiley and Sons. USA: 94-98;

Scholz M. 2006. Wetlands systems to control urban runoff. Univrsity of Edinburgh, UK: 91-110;

Scholz M., Xu J. 2002. Comarison of constructed reed beds with different filter media and macrophytes treating urban run stream water contaminated with lead and copper. Ecol. Eng., 18: 385-390;

Sherr B. F., Payne W. J. 1978. Effect of *Spartina alterniflora* root-rhizome system on salt marsh soil denitrifying bacteria. Applied Environmental Microbiology, 35: 724-729;

Shimp J. F., Tracy J. C., Davis L. C., Lee E., Huang W., Erickson L. E., Schnoor J. L. 1993. Beneficial effects of plants in the remediation of soil and groundwater contaminated with organic materials. Crit. Rev. Environ. Sci. Technol., 23: 41-77;

Seidel K. 1967. Aufnahme und Umwandlung organischer Stoffe durch die Flechtbinse. Wasser und Abwasser, 6: 138-139;

Seidel K. 1978. Gewässerreinigung durch höhrere Pflanzen. The cleansing of bodies of water by higher plants. In: Garten und Landschaft 88, H. 1: 9-17;

Sikora F. J., Zhu T., Behrend L. L., Steinberg S. L., Coonrod H. S. 1995. Ammonium removal in constructed wetlands with recirculating subsurface flow: removal rates and mechanisms. Water Sci Technol, 32, 3: 193–202;

Simard R.E., Thanh N. C. 1973. Biological treatment of domestic sewage by yeast and fungi. AlChE Symposium Series;

Simonich S. L., Hites R. A. 1995. Organic pollutant accumulation in vegetation. Environ. Sci. Technol., 29: 2905-2914;

Sluiter A., Hames B., Ruiz R., Scarleta C.,Sluiter J., Templeton D. 2005. Determination of Ash in Biomass. Biomass Analysis Technology. Laboratory Analitical Procedure;

Song Z., Zheng Z., Li J., Sun X., Han X., Wang W., Xu M. 2006. Seasonal and annual performance of a full-scale constructed wetland system for sewage treatment in China. Ecological Engineering. 26, 3, 1: 272-282;

Speelmans M., Lock K., Vanthuyne D.R.J., Hendrickx F., Laing G. D., Tack F.M.G., Janssen C.R. 2010. Hydrological regime and salinity alter the bioavailability of Cu and Zn in wetlands. Environmental Pollution, 158: 1870–1875;

Spratt H. G., Morgan M. D. 1990. Sulfur cycling in a cedar dominated freshwater wetland. Limnol. Oceanogr., 35, 7, 1586-1593;

Stein O. R., Biederman J. A., Hook P. B., Allen W. C. 2006. Plant species and temperature effects on the *k–C** first-order model for COD removal in batch-loaded SSF wetlands. Ecological Engineering., 26: 100–112;

Stephenson M. 1980. The Environmental Requirements of Aquatic Plants. Publication No65. Appendix A. The state Water Resources Control Board, Sacramento, California;

Stewart W. D. P. 1973. Nitrogen fixation. In: The biology of the blue-green algae. Carr N. G., Whitton B. A. (ed.). University of California Press. Berkeley, California: 260–278;

Stich G. and Hirschfeld W.M. 2010. Dezentrale Lösungen zur Abwasserentsorgung im ländlichen Raum.
http://www.wsb-clean.de/de/91_wsb-magazin.php (10.6.2011)

Stottmeister U., Wießner A., Kuschk P., Kappelmeyer U., Kastner M., Bederski O., Muller R.A., Moormann H. 2003. Effects of plants and microorganisms in constructed wetlands for wastewater treatment. Biotechnology Advances, 22: 93-117;

Strous M., van Gerven E., Kuenen J. G., Jetten M. 1997. Effects of aerobic and microaerobic conditions on anaerobic ammonium-oxidizing (anammox) sludge. Appl Environ Microbiol, 63: 2446–248;

Strous M., Jetten M. S. M. 2004. Anaerobic oxidation of methane and ammonium. Annu Rev Microbiol, 58: 99-117;

Stover E. L., Obayashi 1991. High TDS Wastewater Treatability Study-Design Considerations. In: 45^{th} Purdue Industrial Waste Conference Proceedings, Lewis, Chelsa, MI: 281-286;

Sum Y. B., Feng J. W., Tian Y. C., Li S., Tie J. X., Zhang J. B., Yuan S. J. 2006. Treatment of rural domestic sewage with self-aeration subsurface constructed wetland. Acta Scientiae Circumstantiae, 26: 404;

Susarala S., Medina V. F., McCutcheon S. C. 2002. Phytoremediation: An ecological solution to organic chemical contamination. Ecological Engineering, 18: 647-658;

Tanner C. C., Kadlec R. H., Gibbs M. M., Sukias J. P. S., Nguyen M. L. 2002. Nitrogen processing gradients in subsurface-flow treatment wetlands — influence of wastewater characteristics. Ecological Engineering, 18: 499–520;

Tao M., He F., Xu D., Li M., Wu Z. 2010. How Artificial Aeration Improved Sewage Treatment of an Integrated Vertical-Flow Constructed Wetland. Polish J. of Environ., 19, 1: 183-191;

Tellez G. T., Nirmalakhandan N., Gardea-Torresdey J. L. 1995. Evaluation of biokinetic coefficients in degradation of oilfield produced water under varying salt concentrations. Wat. Res., 29, 7: 1711-1718;

Ter Braak C. J. F., Šmilauer P. 2002. CANOCO, version 4.5;

Thanh N.C., Simard R.E. 1971. Biological Treatment of Wastewater by Yeasts. Journal WPCF, 45, 4: 674-680;

Thomas K. V. 1995. Characterization and Environmental Effects of Unresolved Cpmplex Mixtures o Hydrocarbones. Ph.D. Thesis. University of Plymouth, UK;

Tokuz R. Y., Eckenfelder W. W. 1978. The Effects of F/M on an Activated Sludge System Treating High Salinity Wastewater. In Proceedings: 33rd Purdue Industrial Waste Conerence. Lewis Publishers, Chelsea;

Trevors J. T. 1984. The measurement of electron transport system (ETS) activity in freshwater sediment. Water Research, 18, 5: 581-584;

Truu M., Juhanson J., Truu J. 2009. Microbial biomass, activity and community composition in constructed wetlands. Science of the Total Environment, 407, 13: 3958-3971;

UNEP. 2010. Division of Technology, Industry, and Economics. International Source Book On Environmentally Sound Technologies for Wastewater and Stormwater Management.
http://www.unep.or.jp/ietc/Publications/TechPublications/TechPub-15/main_index.asp (1.6.2011)

USEPA. 1981. Process Design Manual: Land Treatment of Domestic wastewater. EPA 6125/1-811-018. Center for Environmental Research Information, US. Environmental Protection Agency, Cincinnati, OH;

US EPA. 1996. Evaluation of Alternative Wastewater Treatment Technologies. An interim process evaluation of the AEES “Living machine”, Frederick County, MD, Report no. 832-B-96-002;

USEPA 1995. A Handbook of Constructed Wetlands, A Guide To Creating Wetlands For: Agricultural Wastewater, Domestic Wastewater, Coal Mine Drainage, Stormwater In The Mid-Atlantic Region, Volume1, General Considerations. EPA: 903b95004a. United Sates Environmental Protection Agency, USA;

USEPA 2000. Wastewater Technology Fact Sheet – Wetlands: Subsurface Flow. EPA: 832-F-00-023. United Sates Environmental Protection Agency, USA;

van de Graaf A. A., Mulder A., de Bruijn P., Jetten M. S. M., Robertson L. A., Kuenen J. G. 1995. Anaerobic oxidation of ammonium is a biologically mediated process. Appl Environ Microbiol, 61:1246–1251;

Venosa A. D., Haines J. R., Nisamaneepong W., Govind R., Pradhan S., Siddique B. 1992. Efficacy of commercial products in enhancing oil biodegradation in closed laboratory reactors. Journal of Industrial Microbiology, 10: 13-23;

Verhoeven J. T. A. 1986. Nutrient dynamics in minerotrophic peat mires. Aquat Bot, 25:117–137;

Villaverde S., Encina P. A. G., Polanco F. F. 1997. Influence of pH over nitrifying biofilm activity in submerged biofilters. Wat. Res., 31: 1180-1186;

Vlyssides A., Israilides C. J. 1997. Detoxification of tannery waste liquors with an electrolysis system. Environmental Pollution, 97, 1–2: 147–152;

Vohla C., Põldvere E., Noorvee A., Kuusemets V., Mander Ü. 2005. Alternative filter media for phosphorus removal in a horizontal subsurface flow constructed wetland. J Environ Sci Health, 40: 1251–1264;

Vymazal J. 1995. Algae and element cycling in wetlands. Lewis Publishers, Chelsea, Michigan: 698-700;

Vymazal J., Dušek J., Květ J. 1999. Nutrient uptake and storage by plants in constructed wetlands with horizontal subsurface flow: a comparative study. In: Nutrient cycling and retention in natural and constructed wetlands. Vymazal J. (ed.). Backhuys Publishers. Leiden, The Netherlands: 85-100;

Vymazal J., Balcarová J., Dousŏvá H., 2001. Bacterial dynamics in the subsurface constructed wetland. Water Science and Technology, 44, 11/12: 207-209;

Vymazal J. 2005a. Constructed wetlands for wastewater treatment. Ecological Engineering, 25: 475- 477;

Vymazal J. 2005b. Horizontal subsurface flow and hybrid constructed wetlands systems for wastewater treatment. Ecological Engineering, 25: 478- 490;

Vymazal J. 2007. Removal of nutrients in various types of constructed wetlands. Science of the Total Environment, 380: 48-65;

Wallace S. D. 2001. Patent: system for removing pollutants from water. Minnesota 6,200,469 B1;

Wallace S., Higgins J., Crolla A., Kinsley C., Bachand A., Verkuijl S. 2006. High-rate Ammonia Removal in Aerated Engineered Wetlands. In: 10th IWA International Conference on Wetland Systems for Water Pollution Control, September 23-29 2006, Lisbon, Portugal: 255-262;

Watanabe M. E. 1997. Phytoremediation on the brink of commercialization. Environ. Sci. Technol. 31: 182- 186;

Wershaw R. L., Thorn K. A., Pincney D. J., MacCarthy P., Rice J. A., Hemond H. F. 1986. Application of membrane pilot plant to secondary structure of humic materials in peat. In: Peat and Water. Fuchsman C. H. (ed.). Elsevier, New York: 133-157;

Wessa P. 2007. Pearson Correlation in Free Statistics Software, Office for Research Development and Education.
http://www.wessa.net/rwasp_correlation.wasp/ (5.5.2008)

Wiliams T. P., Bubb J. M., Lester J. N. 1994. Metal accumulation within salt marsh environments: a review. Marine pollution Bulletin, 28, 5: 277-290;

Witten I.H., Frank E. 2000. Data mining: Practical Machine Learning Tools and Techniques with Java Implementations. Morgan Kaufmann Publishers;

Wong J. M. 1992. Biotreatment of Contaminated Groundwater with High Organics and Salinity Contents. In: Procesings of the 46th Annual Purdue Industrial Waste Conerence. Lewis Publishers, Chelsea;

Woolard C. R., Irvine R. L. 1994. Biological treatment of hypersaline wastewater by a biofilm of halophilic bacteria. Water. Environ. Res., 66:230–235;

Woolard C. R., Irvine R. L. 1995. Response of a periodically operated halophilic biofim reactor to changes in salt concentration. Wat. Sci. Tech., 31, 1: 41-50;

WSB. 2011.
http://www.wsb-clean.de/en/3_process.htm (10.6.2011)

Wu, Y., Tam, N.F.Y., Wong, M.H., 2008. Effects of salinity on treatment of municipal wastewater by constructed mangrove wetland microcosms. Marine Pollution Bulletin 57, 727-734;

Wu M. Y., Franz E. H., Chen S. 2001. Oxygen fluxes and ammonia removal efficiency efficiencies in constructed treatment wetlands. Water Environ. Res., 73: 661;

Yan L., Wang S. H. Luo W. G., Huang J., Zhong Q. S. 2006. Study on the oxygen condition in subsurface flow wetlands in operation. Environ. Sci., 27: 2009;

Yang L., Lai C. T., Shieh W. K. 2000. Biodegradation of dispersed diesel fuel under high salinity conditions. Wat. Res., 34, 13: 3303-3314;

Yensen N. P. 2007. Halophyte DataBase – Salt-tolerant plants and their uses. http://www.ussl.ars.usda.gov/pls/caliche/Halophyte.query (1.6.2008)

Yun S. I., Choi W. J., Choi Y. D., Lee S. H., Yoo S. H., Lee E. J., Ro H. M., 2003. Distribution of heavy metals in soils of Shihwa tidal freshwater wetlands. Korean J. Ecol., 26: 65–70;

Zhou J.B., Jiang M.M., Chen B., Chen G.Q. 2009. Emergy evaluations for constructed wetland and conventional wastewater treatments. Communications in Nonlinear Science and Numerical Simulation. 14, 4: 1781-1789;

Zoeller K. E, Byers M. E. 1999. Patent: wastewater treatment system. Kentucky 5,897,777. October 3, 1997;

Zupančič M. 2001. Vloga substrata pri odstrnjivanju kovinskih ionov v pilot plantu rastlinske čistilne naprave. Magistarsko delo. Univerza Ljubljana, Biotehniška fakulteta: 49-51;

Zupančič J. M., Bulc G. T., Vrhovšek D., Bukovec P, Zupančič M., Zrimec A., Berden Zrimec M. 2005. Slovenian experience: MSW landfill leachate treatment in constructend wetlands and leachet recycling on landfill cover vegetated with trees. In Sardinia 2005 Proceedings of Tenth International Waste Management and Landfill Symposium. Cagliari, Italy;

Zupančič-Justin M. 2006. Sledenje onesnaževal v sistemu predčiščenja in vračanja izcedne vode pri sanaciji odlagališča komunalnih odpadkov. Doktorska disertacija. Univerza Ljubljana, Biotehniška fakulteta: 83-123.

7 ANNEXES

ANNEX A1

Correlations between parameters of wastewater from CW Dragonja

Table 18: *The correlation coefficients between parameters of wastewater from CW Dragonja*

	1/R	COD	BOD_5	P tot	NH_4	NO_2	NO_3	Cl	Fe
1/R		0.32	0.05	0.02	**0.44**	-0.04	**0.44**	0.90	0.09
COD			**0.41**	0.24	**0.72**	-0.12	0.07	0.33	0.19
BOD_5				0.16	0.29	-0.05	0.01	0.03	0.29
P tot					0.15	-0.10	-0.05	-0.01	0.36
NH4						-0.11	0.09	0.25	**0.41**
NO_2							0.98	-0.04	-0.27
NO_3								0.33	-0.36
Cl									-0.12

Best correlations between different parameters are shown in bold.
Description of table: 1/R - conductivity [mS/cm]; P tot – total phosphorus [mg/l]; Cl - chloride [mg/l]; NH_4 – ammonium [mg/l]; NO_2 – nitrite [mg/l]; NO_3 – nitrate [mg/l]

Table 19: *The coefficients of determination between parameters of wastewater from CW Dragonja*

	1/R	COD	BOD_5	P tot	NH_4	NO_2	NO_3	Cl	Fe
1/R		0.10	0.00	0.00	0.20	0.00	0.19	0.82	0.01
COD			0.17	0.06	**0.51**	0.01	0.00	0.11	0.04
BOD_5				0.02	0.09	0.00	0.00	0.00	0.08
P tot					0.02	0.01	0.00	0.00	0.13
NH4						0.01	0.01	0.06	0.16
NO_2							0.17	0.00	0.07
NO_3								0.11	0.13
Cl									0.01

Best correlations between different parameters are shown in bold
Description of table : 1/R - conductivity [mS/cm]; P tot – total phosphorus [mg/l]; Cl - chloride [mg/l]; NH_4 – ammonium [mg/l]; NO_2 – nitrite [mg/l]; NO_3 – nitrate [mg/l]

ANNEX A2

Correlations between removal efficiency of wastewater from CW Dragonja

Table 20: *The correlation coefficients (R) and determination coefficients (R2) between removal efficiency of NO3%, total P%, BOD_5%, NH_3%, COD% and conductivity of CW Dragonja*

Removal efficiency (%)	Corellation coefficient (R)	Determination coefficient (R^2)
Parameter	Conductivity	Conductivity
NO_3%	-0.273	0.075
Total P%	-0.226	0.051
BOD_5%	-0.108	0.012
NH_3%	-0.002	3.24×10^{-6}
COD%	-0.006	3.65×10^{-5}

ANNEX B

Correlations between parameters of pilot plant

Table 21: *Corellation coefficient (R) between the parameters of the pilot plant*

	T°C w.	pH	NH4	NO3	NO2	PO4	Cl	O2	uS/cm	OD	CO2w	CO2s	ETS	COD	BOD5
pH	-0.30														
NH4	-0.00	0.09													
NO3	-0.02	0.04	0.03												
NO2	-0.22	-0.04	-0.10	0.04											
PO4	0.46	-0.34	0.05	-0.01	0.11										
Cl	0.36	0.04	0.21	-0.03	-0.10	0.13									
O2	0.18	0.03	-0.06	-0.03	0.00	-0.04	-0.09								
uS/cm	0.28	0.10	0.25	0.00	0.00	0.05	**0.89**	-0.09							
OD	0.24	**-0.59**	-0.05	0.03	0.05	**0.49**	-0.06	-0.08	-0.10						
CO2w.	0.30	**-0.85**	0.06	-0.02	-0.04	0.37	0.03	-0.02	-0.02	**0.53**					
CO2s.	0.45	**-0.54**	0.04	-0.02	-0.11	0.25	0.10	-0.06	0.03	0.24	**0.60**				
ETS	**0.55**	-0.26	0.12	-0.02	-0.17	0.21	0.36	-0.08	0.37	0.16	0.23	0.26			
COD	-0.22	**-0.65**	-0.04	-0.03	0.28	0.22	-0.12	-0.13	-0.05	0.43	0.49	0.32	-0.10		
BOD5	0.45	**-0.39**	-0.15	-0.07	-0.09	0.17	-0.27	**0.47**	-0.36	0.16	0.40	0.40	0.15	0.12	
redox	0.02	-1.00	0.06	0.00	0.00	0.09	0.02	**0.61**	-0.03	0.50	**0.90**	**0.65**	0.16	**0.86**	0.38

Table 22: *Determination coefficient (R2) between parameters of pilot plant*

	T°C w.	pH	NH4	NO3	NO2	PO4	Cl	O2	uS/cm	OD	CO2w	CO2s	ETS	COD	BOD5
pH	0.09														
NH4	0.00	0.01													
NO3	0.00	0.00	0.00												
NO2	0.05	0.00	0.01	0.00											
PO4	0.21	0.12	0.00	0.00	0.01										
Cl	0.13	0.00	0.04	0.00	0.01	0.02									
O2	0.03	0.00	0.00	0.00	0.00	0.00	0.01								
uS/cm	0.08	0.01	0.06	0.00	0.00	0.00	**0.79**	0.01							
OD	0.06	0.35	0.00	0.00	0.00	0.24	0.00	0.01	0.01						
CO2w.	0.09	**0.72**	0.00	0.00	0.00	0.14	0.00	0.00	0.00	0.28					
CO2s.	0.20	0.30	0.00	0.00	0.01	0.06	0.01	0.00	0.00	0.06	0.36				
ETS	0.30	0.07	0.01	0.00	0.03	0.04	**0.13**	0.01	0.135	0.03	0.05	0.07			
COD	0.05	**0.43**	0.00	0.00	0.08	0.05	0.01	0.02	0.00	0.19	0.24	0.10	0.01		
BOD5	0.20	0.15	0.02	0.00	0.01	0.03	0.07	0.22	0.131	0.02	0.16	0.16	0.02	0.00	
redox	0.00	1.00	0.00	0.00	0.00	0.01	0.00	**0.37**	0.00	0.25	0.81	**0.42**	0.03	0.70	0.14

ANNEX C1

Variations of temperature during experiments in pilot plant

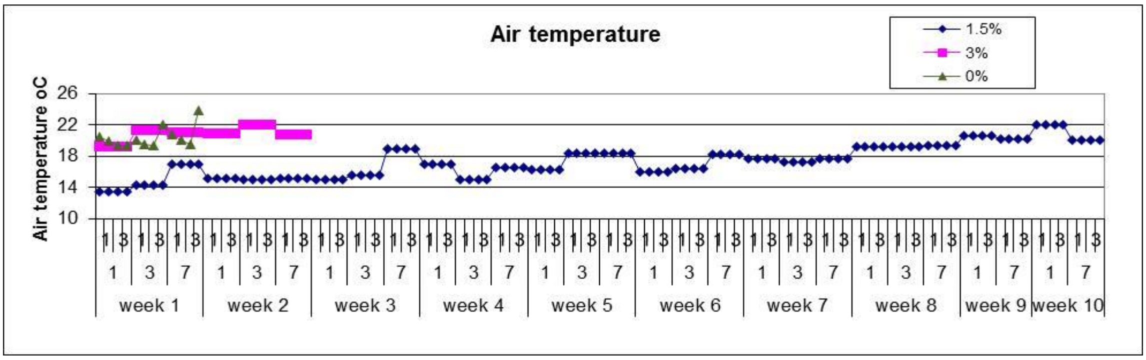


Figure 89: *Air temperature during operation period (measured on 1st, 3rd and 7th day of the week in all four compartments) at different salinities (0% NaCl, 1.5% NaCl, 3% NaCl)*

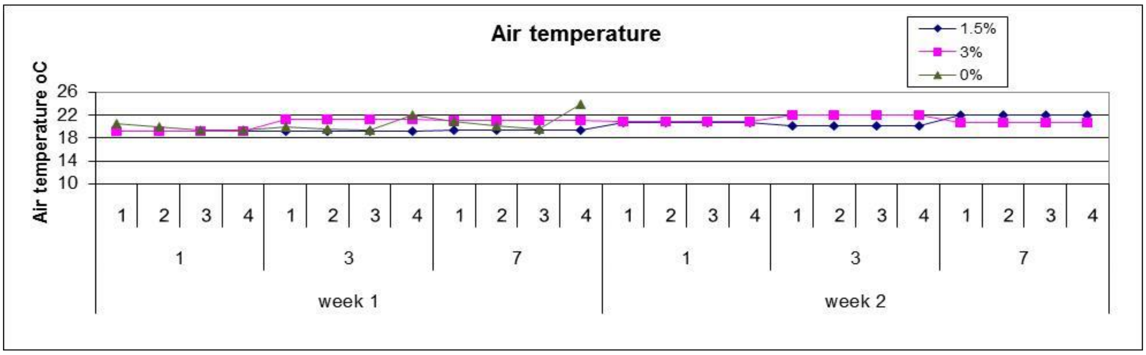


Figure 90: *Air temperature during operation period in the first two weeks (measured on 1st, 3rd and 7th day of the week in all four compartments) of different salinities (0% NaCl, 1.5% NaCl and 3% NaCl)*

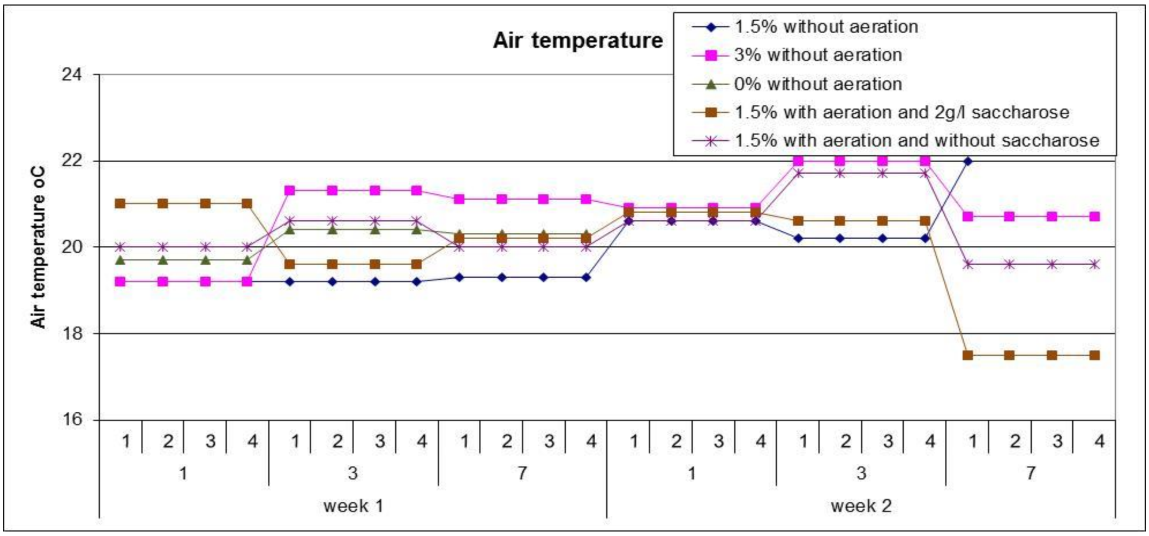


Figure 91: *Air temperature during operation period (measured on 1^{st}, 3^{rd} and 7^{th} day of the week in all four compartments) at different conditions: different salinities (0% NaCl, 1.5% NaCl, 3% NaCl), aeration (with or without) and with aeration and 2 g/l saccharose*

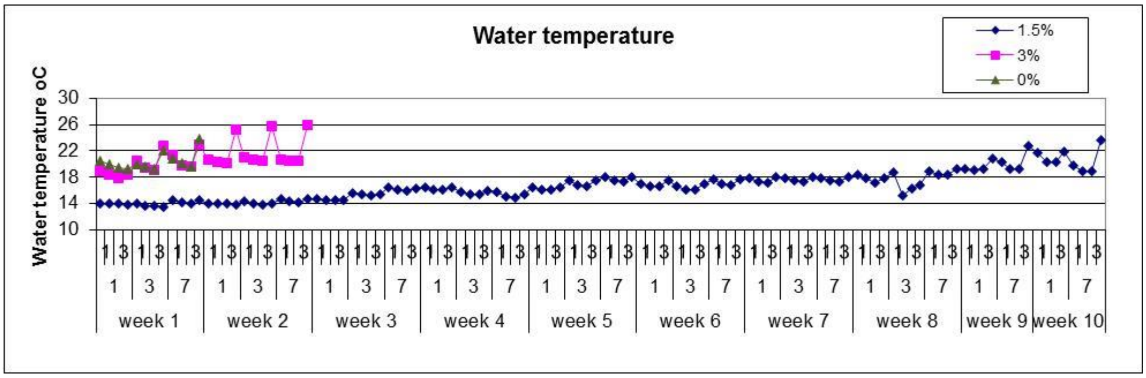


Figure 92: *Water temperature during operation period in the first two weeks (measured on 1st, 3rd and 7th day of the week in all four compartments) of different salinities (0% NaCl, 1.5% NaCl and 3% NaCl)*

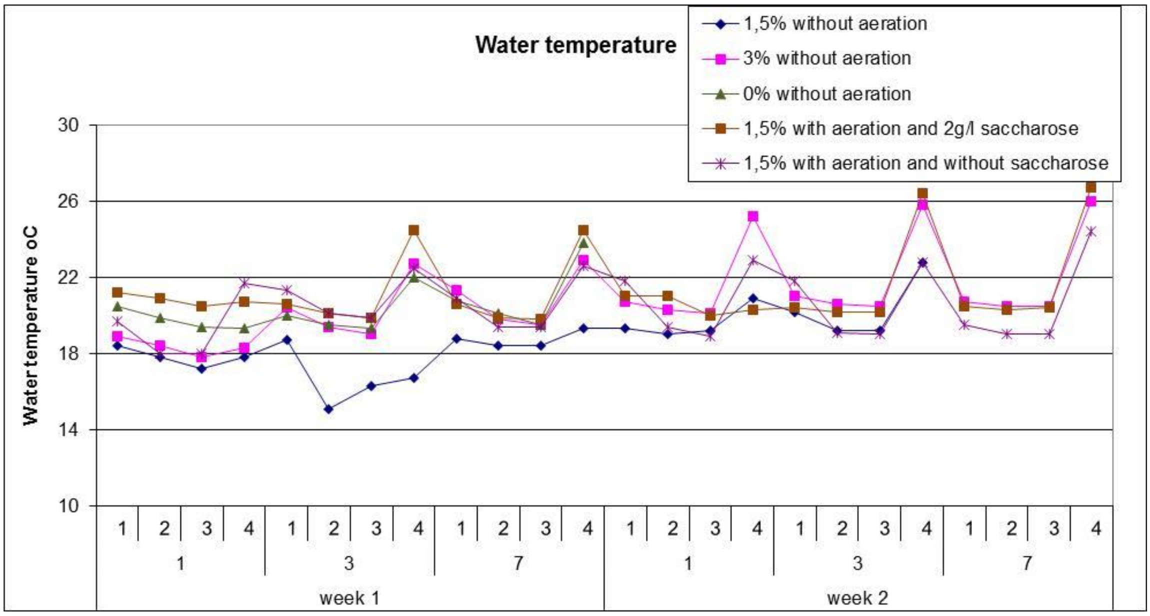


Figure 93: *Water temperature during operation period (measured on 1st, 3rd and 7th day of the week in all four compartments) at different conditions: different salinities (0% NaCl, 1.5% NaCl, 3% NaCl), aeration (with or without) and with aeration and 2 g/l saccharose*

ANNEX C2

Variations of chloride and conductivity during experiments in pilot plant

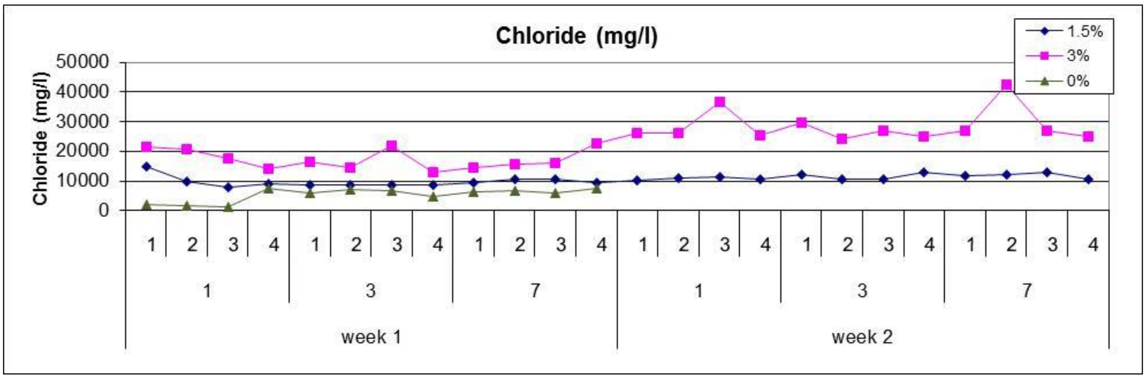


Figure 94: *Variation of chloride concentrations during operation period in the first two weeks (measured on 1st, 3rd and 7th day of the week) in all four compartments, at different salinities (0% NaCl, 1.5% NaCl, 3% NaCl)*

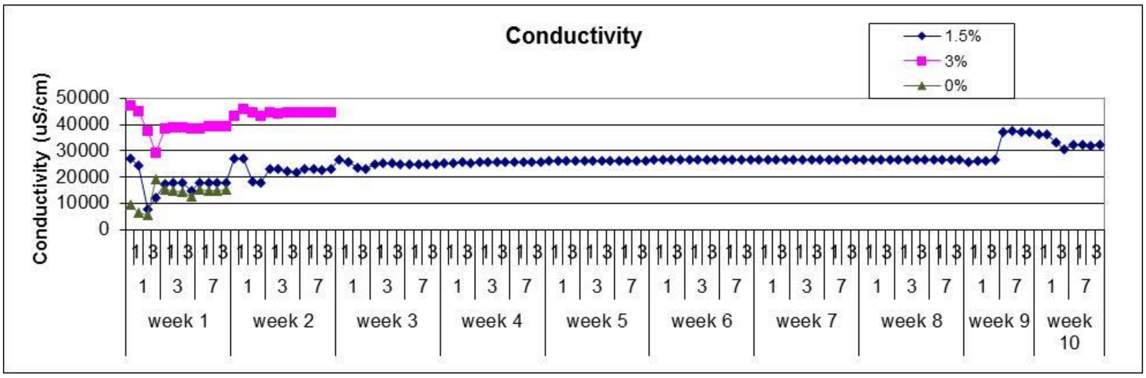


Figure 95: *Variation of conductivity during operation period (measured on 1st, 3rd and 7th day of the week) in all four compartments, at different salinities (0% NaCl, 1.5% NaCl, 3% NaCl)*

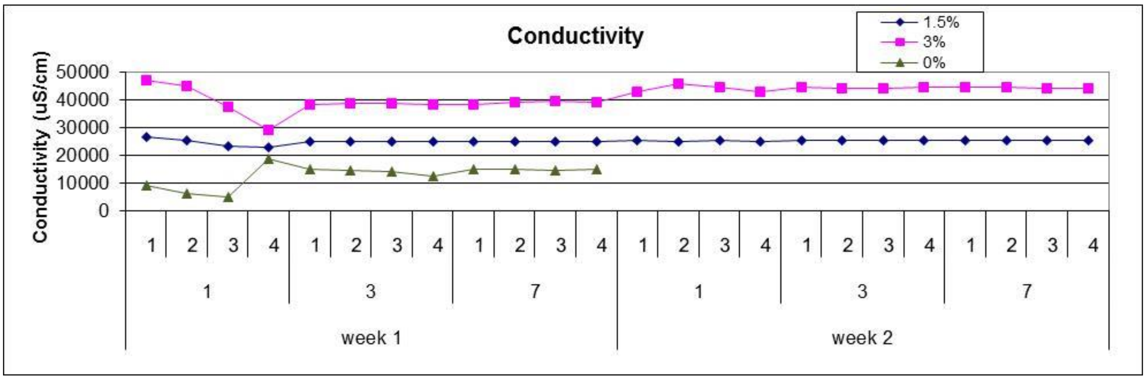


Figure 96: *Variation of conductivity during operation period in the first two weeks (measured on 1st, 3rd and 7th day of the week) in all four compartments, at different salinities (0% NaCl, 1.5% NaCl, 3 NaCl)*

ANNEX C3

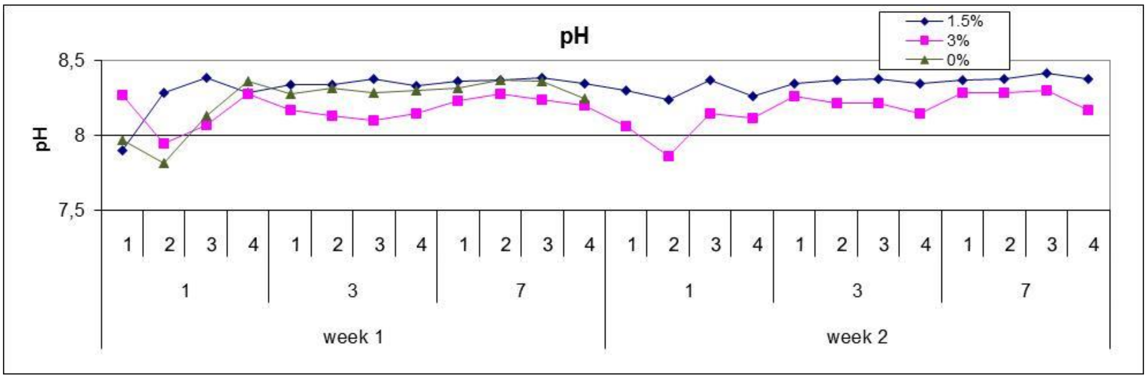


Figure 97: *Variation of pH during operation period in the first two weeks (measured on 1st, 3rd and 7th day of the week) in compartments 1, 2, 3, and 4, at different salinities (0% NaCl, 1.5% NaCl, 3% NaCl)*

ANNEX C4

Variations of oxygen and redox potential during experiments in pilot plant

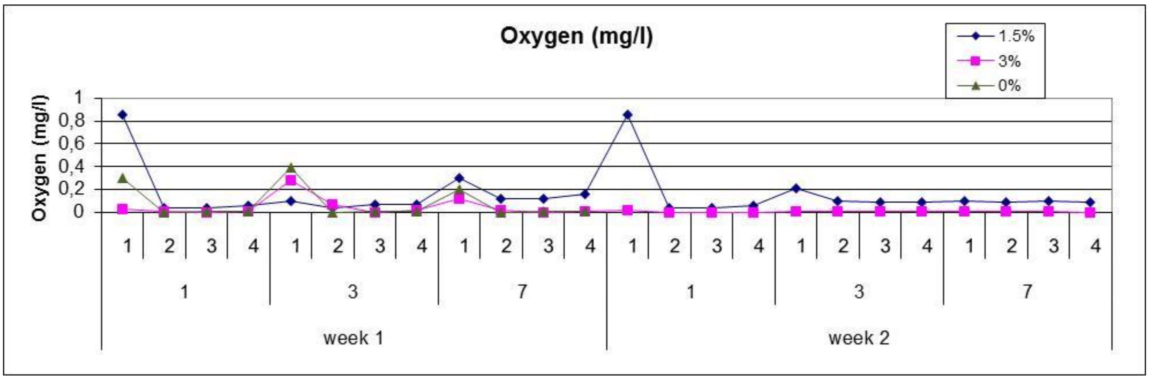


Figure 98: *Variation of oxygen concentration during operation period in two weeks (measured on 1st, 3rd and 7th day of the week) in compartments 1, 2, 3, and 4, at different salinities (0% NaCl, 1.5% NaCl, 3% NaCl)*

ANNEX C5

Variations of COD during experiments in pilot plant

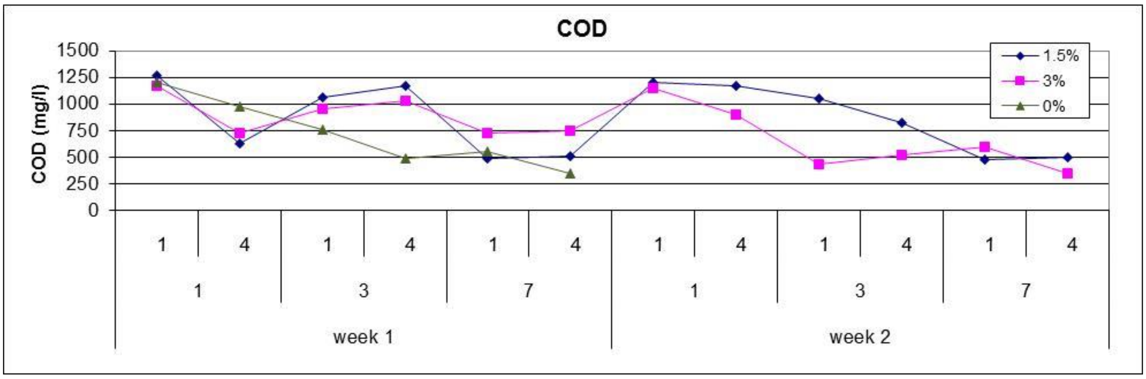


Figure 99: *Variation of COD concentrations during operation period in the first two weeks (measured on 1st, 3rd and 7th day of the week) in compartments 1 and 4, at different salinities (0% NaCl, 1.5% NaCl, 3% NaCl)*

ANNEX C6

Variations of ammonium during experiments in pilot plant

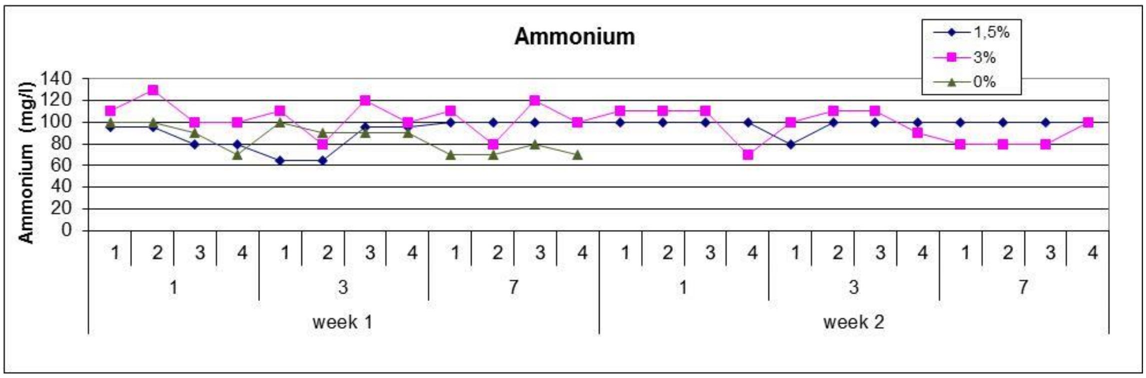


Figure 100: *Variation of ammonium concentrations during operation period in the first two weeks (measured on 1st, 3rd and 7th day of the week in all four compartments) at different conditions: different salinities (0% NaCl, 1.5% NaCl, 3% NaCl)*

ANNEX C7

Variations of phosphate during experiments in pilot plant

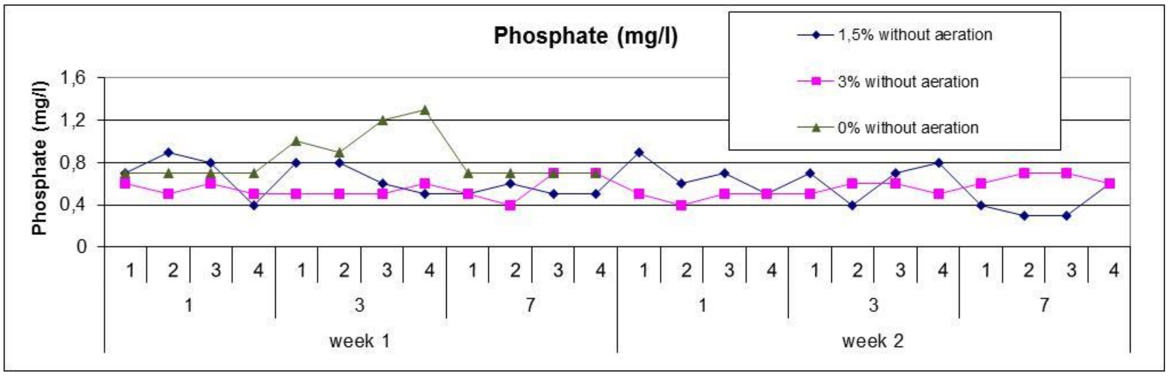


Figure 101: *Variation of phosphate concentrations during operation period in the first two weeks (measured on 1st, 3rd and 7th day of the week) in all four compartments, at different salinities (0% NaCl, 1.5% NaCl, 3% NaCl)*

ANNEX C8

Variations of OD during experiments in pilot plant

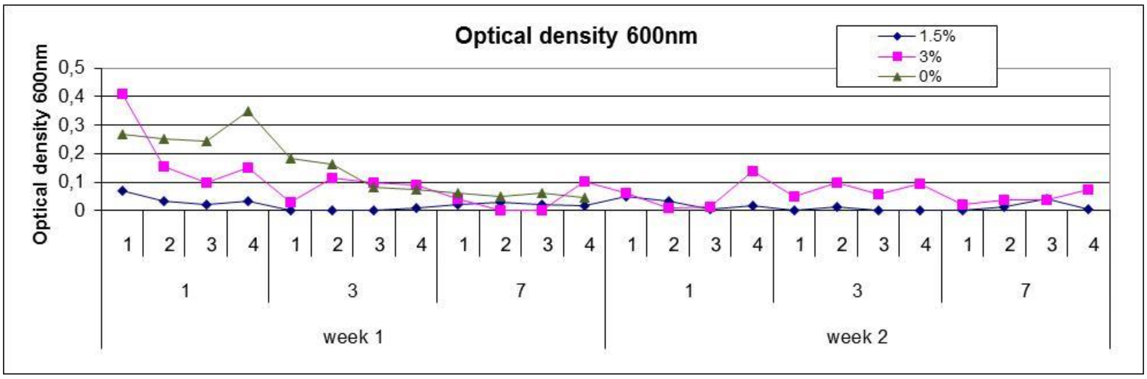


Figure 102: *Variation of optical density at 600 nm during operation period in the first two weeks (measured on 1st, 3rd and 7th day of the week) in all four compartments, at different salinities (0% NaCl, 1.5% NaCl, 3% NaCl)*

ANNEX C9

Variations of ETS activity during experiments in pilot plant

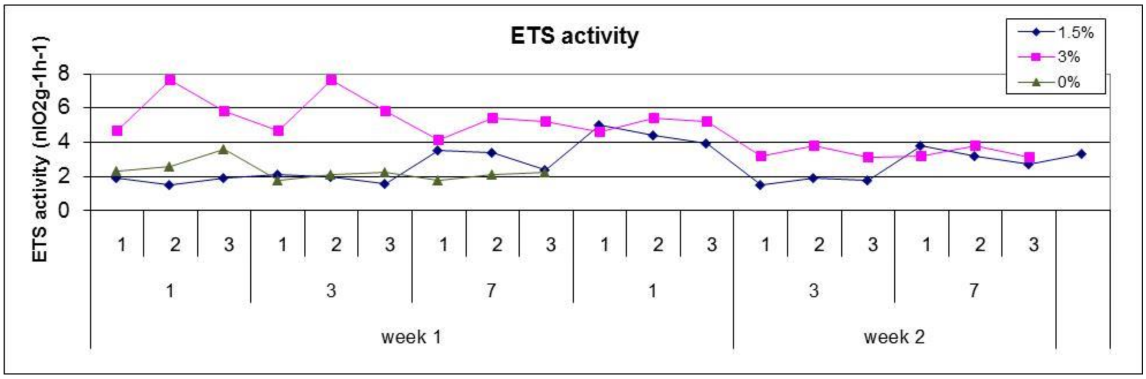


Figure 103: *Variation of ETS activity during operation period in the first two weeks (measured on 1st, 3rd and 7th day of the week) in tanks 1, 2 and 3, at different salinities (0% NaCl, 1.5% NaCl, 3% NaCl)*

ANNEX C10

Variations of carbon dioxide during experiments in pilot plant

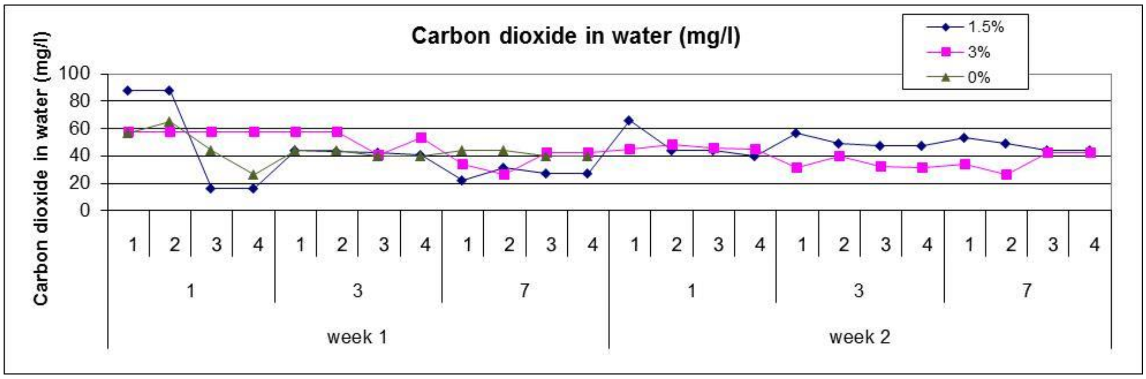


Figure 104: *Variation of carbon dioxide concentrations in water during operation period in the first two weeks (measured on 1st, 3rd and 7th day of the week) in all four compartments, at different salinities (0% NaCl, 1.5% NaCl, 3% NaCl)*

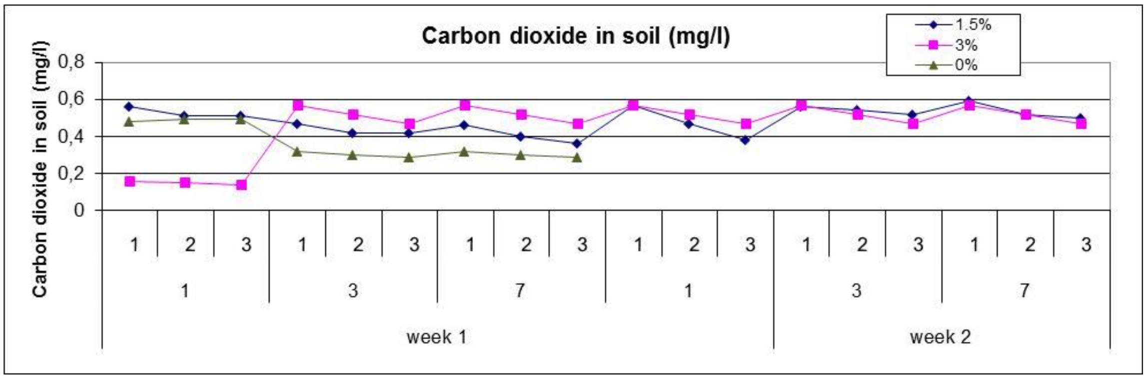


Figure 105: *Variation of carbon dioxide concentrations in soil during operation period in the first two weeks (measured on 1st, 3rd and 7th day of the week) in tanks 1, 2 and 3, at different salinities (0% NaCl, 1.5% NaCl, 3% NaCl)*

ANNEX C11

Results of T and F- test made on data of pilot plant between different salinities

Table 23: *Results of T - test where salinity was main factor*

Results of Student t test								
Salinity	**1,5**	**0,0**		**1,5**	**3,0**		**0,0**	**3,0**
	t	p		t	p		t	p
Cl	5,230	**< 0,001**		-6,212	**< 0,001**		-10,893	**< 0,001**
COD	1,229	0,224		0,442	0,660		-0,982	0,343
OD	-16,080	**< 0,001**		-2,014	0,054		2,163	**< 0,05**
pH	1,238	0,239		4,895	**< 0,001**		1,053	0,308
NH4	3,322	**0,003**		-0,089	0,930		-2,997	**0,005**
PO4	-7,712	**< 0,001**		-4,490	**< 0,001**		6,812	**< 0,001**
O2	1,551	0,128		3,185	**< 0,01**		1,245	0,235
CO2 water	-1,187	0,251		-1,313	0,196		0,084	0,933
CO2 mg/h substrat	3,944	**< 0,001**		0,755	0,459		-1,742	0,094
ETS activity (nlO2g-1h-1)	1,432	0,165		-5,661	**< 0,001**		-6,730	**< 0,001**

Table 24: *Results of F - test where salinity was main factor*

Results of F test						
Salinity	**1,5**	**0,0**	**1,5**	**3,0**	**0,0**	**3,0**
	F	*p*	*F*	*p*	*F*	*p*
Cl	22,472	0,000	2,299	0,012	0,102	0,000
COD	1,010	0,578	1,838	0,150	1,820	0,202
OD	0,187	0,000	0,292	0,000	1,566	0,176
pH	0,402	0,008	0,983	0,451	2,449	0,034
NH4	5,531	0,002	3,577	0,000	0,647	0,229
PO4	1,684	0,170	3,909	0,000	2,322	0,043
O2	10,982	0,000	57,649	0,000	5,249	0,000
CO2 water	2,487	0,047	2,058	0,024	2,058	0,024
CO2 mg/h substrat	0,888	0,354	0,350	0,001	0,395	0,092
ETS activity (nlO2g-1h-1)	7,496	0,002	1,161	0,381	0,155	0,006

ANNEX C12

Variations of oxygen during experiments in pilot plant with aeration

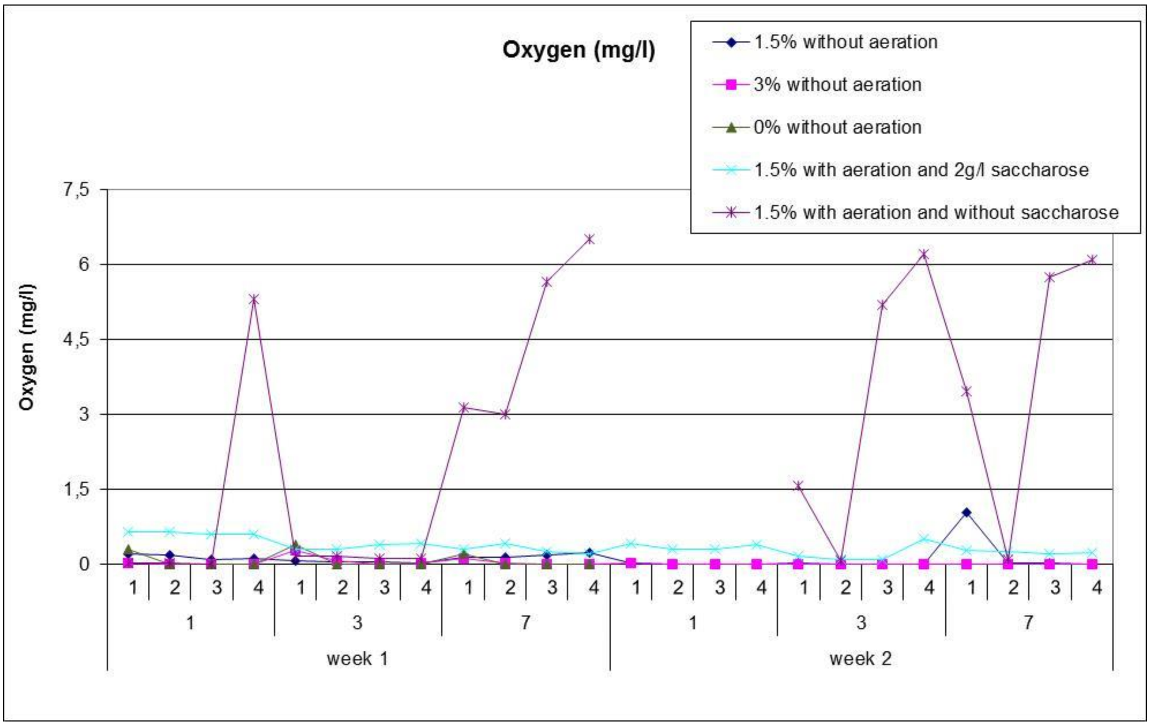


Figure 106: *Variation of oxygen concentration during operation period (measured on 1st, 3rd and 7th day of the week) in compartments 1, 2, 3, and 4, at different salinities (0% NaCl, 1.5% NaCl, 3% NaCl), aeration (with or without) and with aeration and 2 g/l saccharose*

ANNEX C13

Results of T and F- test made on data of pilot plant including aeration and saccharose as main factor

***Table 25:** Results of T - test where aeration and saccharose are main factor*

Results of Student t test	NO AER NO SAC	AER NO SAC	NO AER NO SAC	AER SAC	AER NO SAC	AER SAC
	t	p	t	p	t	p
Cl	-0,447	0,655	2,598	**p<0,05**	7,611	**p<0,001**
COD	1,875	0,066	-1,079	0,302	-1,942	0,073
OD	-1,970	0,060	-4,538	**p<0,001**	-1,696	0,097
pH	2,125	**p<0,05**	5,733	**p<0,001**	5,367	**p<0,001**
NH4	0,563	0,575	5,149	**p<0,001**	3,623	**p<0,001**
PO4	-3,798	**p<0,001**	-4,080	**p<0,001**	-1,313	0,196
O2	-3,808	**p<0,001**	-7,557	**p<0,001**	3,479	**p<0,01**
CO2 water	0,521	0,603	-3,868	**p<0,001**	-3,860	**p<0,001**
CO2 mg/h substrat	-1,738	0,090	-9,607	**p<0,001**	-8,133	**p<0,001**
ETS activity (nlO2g-1h-1)	-4,015	**p<0,01**	-4,882	**p<0,001**	-2,398	**p<0,05**

***Table 26:** Results of F - test where aeration and saccharose are main factor*

Results of F test	NO AER NO SAC	AER NO SAC	NO AER NO SAC	AER SAC	AER NO SAC	AER SAC
	F	*p*	*F*	*p*	*F*	*p*
Cl	95,703	0,000	35,811	0,000	0,374	0,011
COD	1,611	0,264	0,168	0,000	0,104	0,003
OD	0,085	0,000	0,095	0,000	1,127	0,389
pH	1,028	0,494	0,020	0,000	0,019	0,000
NH4	2,788	0,003	10,369	0,000	3,719	0,001
PO4	2,402	0,009	1,673	0,078	0,696	0,196
O2	0,031	0,000	3,297	0,001	262,150	0,000
CO2 water	1,237	0,434	0,046	0,000	0,037	0,000
CO2 mg/h substrat	31,196	0,001	0,709	0,153	0,023	0,000
ETS activity (nlO2g-1h-1)	9,813	0,009	1,599	0,137	-4,882	0,000

ACKNOWLEDGMENTS

I would like to express my gratitude to my mentor prof. dr. Danijel Vrhovšek, for giving me the chance to pursuit this project and for all the support during my work. I am very gratefull to my second mentor prof. dr. Sidney A. Katz for all suggestions, advices and support. I would also like to thank to all great people in Limnos d.o.o. who supported me during my work.

I would also like to thank dr. Nataša Mori for the great help on my work and for the availability and patience for it. I would also like to thank dr. Aleš Lapanje and his team for supporting during my work on The Institute of Physical Biology. I would like to thank dr. David and dr. Vojč Kocman for constructive suggestions during my work. I would also like to thank to Ms. Danijela Kleva from JP Okolje Piran for her help about constructed wetland Dragonja.

I would like to thank Committee Members prof. dr. Christopher G. Uchrin, prof. dr. Gregor A. Janauer and doc. dr. Barbara Čenčur Curk for their time, interest, and constructive suggestions.

Concerning entities, I would like to thank Ad Futura, Scientific and Educational Foundation of the Republic Slovenia, that gave finantial support to my Phd study.

I would like to thank Dejan Štebih and his family.

I would like to thank, my mother Anka for all help, support and pations during my work. I would like to my father Marko, my brother Milan and his family Dragana and Marko, prof. dr Marina Bujko for always believing in my work and for all the support and encouragement, without wich, none of this could be possible.

I would like to express special great gratitude to my daughters Maša and Lana for all love, support and patience during my work.