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The effect of the application of halotolerant microorganisms on the efficiency of a pilot-scale constructed wetland for saline wastewater treatment

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Abstract: In order to find the optimal design characteristics of constructed wetlands for saline wastewater treatment, halotolerant microorganisms, isolated from the water of the Sečovlje salterns, were inoculated into the media of a pilot-scale constructed wetland (CW). The purpose of this study was to examine the influence of different salinities on the efficiency of halotolerant microorganisms for the removal of pollutants in order to evaluate the possibility of their employment for saline wastewater treatment. The efficiency of ammonium removal (34.1 %) was the highest with 0 % NaCl in wastewater and slightly lower (31.8 %) when 2 g/dm^3 saccharose was added to aerated 1.5 % NaCl wastewater. The highest removal efficiency of chemical oxygen demand (COD) in the pilot-scale subsurface flow (SSF) CW was 83.6 % when saccharose (2 g/dm^3) was added to aerated 1.5 % NaCl wastewater. It was found that removal efficiency of the pilot-scale constructed wetland with inoculated halotolerant microorganisms showed a higher sensitivity to aeration and the presence of saccharose than to variation of the salinity of the wastewater. It can be concluded that halotolerant microorganisms, isolated from the Sečovlje salterns, are not sensitive to the changes in salinity and are, therefore, an alternative in the treatment of saline wastewater with a constructed wetland. However, with aeration their efficiency could be further improved.

Keywords: constructed wetlands; saline wastewater; halotolerant microorganisms.

INTRODUCTION

Seawater infiltration,[1] road deicing,[2,3] and landfill leachates,[4,5] as well as the chemical, petroleum, textile, leather and agro-food industries,[6] generate large amounts of saline wastewater. Consequently, pollution removal in hypersaline

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wastewater is likely to represent up to 5 % of the global wastewater treatment requirement.[7] The discharge of such wastewater affects aquatic life, water pot ability and agriculture. Thus, legislation is becoming more stringent and the treatment of saline wastewater, both for organic matter and salt removal, is nowadays compulsory in many countries. Saline effluents are conventionally treated through physico-chemical means, as biological treatment is strongly inhibited by salts. However, as the costs of physico-chemical treatments are particularly high, alternative systems for the treatment of organic matter from saline wastewater are nowadays increasingly the focus of research.[6] One of the alternative systems is constructed wetlands (CW). The use of this system is becoming very popular in many countries[8] but its application for saline wastewater treatment has not been studied extensively. Lin *et al.*[9] found that salinity played an important role in the growth of microorganisms, resulting in a switch of the microbial population when studying the effects of salinity on the degradation of atrazine in a subsurface flow (SSF) constructed wetland (CW). They found that increasing salinity depressed the activity of the microorganisms and, therefore, caused a poor degradation efficiency of the CW. Studies by Nitisoravut and Klomjek[10] also reported that the effect of salinity on biological oxygen demand (BOD) removal appeared to approach an exponential phase. 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.[9]

In order to maximize the efficiency CW treatment of saline wastewater and keep its area to a minimum, it is necessary to find the optimal CW design characteristics. One of the most important characteristics is the microorganisms, but usually they are sensitive to salinity. Several studies, conducted with conventional cultures of bacteria indicated that the following four common difficulties exist when treating saline and hypersaline wastes with organisms derived from freshwater and soil ecosystems:[11] limited extent of adaptations,[12–14] sensitivity to changes in ionic strength,[15,16] reduced degradation kinetics and high effluent suspended solids concentrations.[17] Several studies have shown[18–23] that utilization of salt-tolerant microorganisms in biological treatment could be a reasonable approach for the treatment of high salinity wastewater.[24] Although the number of studies dealing with the biological treatment of saline wastewater is increasing rapidly, not much is known regarding the application of halotolerant microorganisms in CW.

The aim of this study was to examine the efficiency of halotolerant microorganisms in correlation with wastewater salinity using a pilot-scale SSF CW. The high concentration of nutrients and organic matter in the salt ponds of the Sečovlje salterns, the high concentration of salt and its oscillation during rainstorms, designate the Sečovlje salterns as very interesting for the isolation of halotolerant microorganisms and their application in the media of constructed wetland for saline wastewater treatment. With this in mind, a pilot-scale SSF CW was designed, constructed and operated with halotolerant microorganisms, isolated from water of the Sečovlje salterns, inoculated in the media. Plants were not included in the pilot-scale SSF CW since the aim was to investigate the efficiency of halotolerant microorganisms in a sand/gravel/peat environment of varying salinity. It is known from the literature that plants accelerate the growth of microorganisms, especially in the area of the root environment,[25–28], but the employed halotolerant microorganisms were isolated from the water of the Sečovlje salterns and naturally they were not attached to the roots of plants. Changes in salinity also often occur in real CW and the aim was to investigate the efficiency of halotolerant microorganisms, isolated from the Sečovlje salterns, during Salinity shocks in the pilot-scale SSF CW.

EXPERIMENTAL

A pilot-scale SSF CW was constructed from three rectangular plastic tanks, each with the dimensions: length 0.77 m, width 0.16 m and depth 0.58 m, which were separated by 0.20–0.25 m long empty compartments on both sides (Fig. 1). The total length of the pilot-scale SSF CW was 2.99 m. Between the three soil-filled compartments, two perforated compartment walls were placed across the flow of the water. There was only water in these four narrow empty compartments. In every soil-filled compartment, there was a perforated pipe reaching to the bottom. The pipe ended with part of a plastic bottle from which a small plastic tube led to a glass bottle with a solution of barium hydroxide to catch carbon dioxide, as a measure of the microbial activity in the soil.[29–31] A perforated rubber pipe connected to an aquarium air pump, which was switched on only during the aeration treatment, was laid at the bottom of the pilot-scale SSF CW. The hydraulic retention time was determined by adding 0.1 % NaCl to the influent and the conductivity of the effluent was measured.[32]

The medium of the pilot-scale SSF CW was prepared as a mixture of sand (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 composition of the sand was: $CaCO_3$, 30.56 %; $MgCO_3$, 21.8 %; SO_3, 0.09 %; MnO_2, 0.02 %; TiO_2, trace; Fe_2O_3, 0.00 %; Al_2O_3, 0.00 % and SiO_2, 0.08 %. Peat, with the following characteristics: pH 3.5–4.0; organic matter, 35 % and total nitrogen, 0.4 %, was added to make up 10 % of the total volume. The final pH value of the medium mixture was 7.4.

The employed halotolerant microorganisms were isolated[33] from the active solar Sečovlje salterns in the autumn of 2005. The halotolerant microorganisms were cultivated for three months in synthetic wastewater (artificial wastewater (ART) medium)[34] and the optical density (*OD*) was measured at 600 nm to monitor their growth. The microorganisms were then stored at –20 °C. Before inoculation into the pilot-scale SSF CW, they were reactivated in the synthetic wastewater for one week. The reactivated culture (300 cm^3) was added to the first 115 dm^3 of the synthetic wastewater that was added into the pilot-scale SSF CW.The synthetic wastewater used throughout the study was composed of 130 mg/dm^3 yeast extract, 130 mg/dm^3 casein peptone, 130 mg/dm^3 meat extract, 317 mg/dm^3 CH_3COONH_4, 40 mg/dm^3 NH_4Cl, 24 mg/dm^3 K_2HPO_4, 8 mg/dm^3 KH_2PO_4, 100 mg/dm^3 $CaCO_3$, 100 mg/dm^3 $MgCO_3$, 40 mg/dm^3 NaCl and 5 mg/dm^3 $FeSO_4\cdot 7H_2O$. The volume of the wastewater in the pilot-scale SSF CW was 115 dm^3. The salinity of the synthetic wastewater was changed from no added NaCl to 1.5 % NaCl and to 3 % NaCl. When the measurements were realized with and without the aeration and with added saccharose in a concentration of 2 g/dm^3, as a source of organic carbon in the synthetic wastewater of pilot-scale SSF CW, the salt concentration in the synthetic wastewater was 1.5 % NaCl. When the synthetic wastewater contained 0 % and 3 % NaCl, the pilot-scale SSF CW was not aerated and no saccharose was added. Synthetic wastewater with 1.5 % NaCl was added to pilot-scale SSF CW together with halotolerant microorganisms and this salinity was maintained for two months with the wastewater being replaced weekly with fresh synthetic wastewater, without aeration. After this initial two-month period, the conditions in the pilot-scale SSF CW (salinity, aeration, and saccharose) were changed biweekly. In addition, the wastewater was replaced with fresh wastewater once a week. First, synthetic wastewater with 1.5 % NaCl was aerated for two weeks. Then synthetic wastewater with 3 % NaCl circulated for two weeks and after that, 1.5 % NaCl wastewater without aeration was circulated for a further two weeks. It was then replaced with 0 % NaCl synthetic wastewater for one week and finally with 1.5 % NaCl synthetic wastewater, which was aerated and contained 2 g/dm^3 saccharose, for two weeks. When changing the synthetic wastewater in the pilot-scale SSF CW, fresh synthetic wastewater was pumped into the first compartment at a flow of 1.7×10^{-6} m^3/s, while simultaneously, the old synthetic wastewater flowed out from the fourth/last compartment through the valve. Water was pumped into the pilot-scale SSF CW with an aquarium pump (Hydor Seltz L20 II). The same inflow and outflow were set with an aquarium pump and valves. After all of the fresh synthetic wastewater had been pumped into the pilot-scale SSF CW, the system was set to pump the water from the last compartment to the first one at a flow of 1.7×10^{-6} m^3/s, using an aquarium pump.

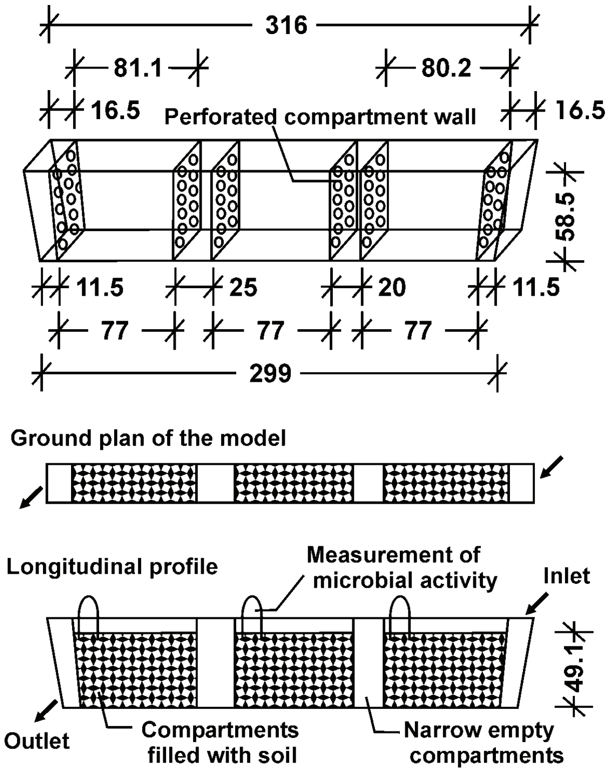

Fig. 1. Pilot-scale SSF CW with dimensions (cm).

Effluents from all four compartments of the pilot-scale SSF CW were analyzed daily for the first fifteen days after inoculation and subsequently less frequently. Samples of the treated synthetic wastewater were then taken on the first, third and seventh day after adding fresh synthetic wastewater. Water samples were taken from each water compartment. The efficiency of the pilot-scale SSF CW was assessed based on the difference between the ammonium, phosphate and *COD* concentrations at the influent and the effluent.[32] The concentrations of ammonium, phosphate and *COD* were measured using a LF2400 Windaus photometer, Germany. To determine the ammonium, phosphate and COD concentrations, the Windaus "Ready mixed cuvette test kit", Cat. No. 3773900, "Aquanal test kit", Cat. No. 3745100, and "CSB-Fertigkuvetten Type 1500", Cat. No. 804691826, were used, respectively. The pH value, the oxygen and carbon dioxide concentrations and the redox potential were also measured. The redox potential, pH and oxygen concentration were measured in all four water compartments with a WTW Sonde Multi 350*i*/SET, Wissenschaftlich, Germany, every second day. The COD value was measured in the first and last compartment. All the mention parameters were measured according to APHA.[35]

The carbon dioxide concentration in the soil[29–31] and the ETS activity (electro transport system activity)[36] were measured in order to determine the microbial activity. The measurements of the ETS activity and CO_2 concentration were realized using an Ocean Optics USB2000 spectrometer, USA. The ETS activity and carbon dioxide concentration in the soil were measured in all three compartments every second day of the experiments.

RESULTS

The pH in pilot-scale SSF CW was around 8.3, varying from 7.7 to 8.5 without aeration; therefore it was optimal for nitrification and slightly higher than optimal for denitrifiers.[37] In the case where the wastewater contained 2 g/dm^3 saccharose, the pH varied from 6.4 to 8.1, thus being optimal also for denitrifiers. The concentration of oxygen in the pilot-scale SSF CW was mostly lower than 0.4 mg/dm^3, except during the first days of the cycle when fresh synthetic wastewater was added. The oxygen concentrations without aeration were less than 1.5 mg/dm^3, but with aeration and without saccharose it increased. The redox potential was around –90 to –60 mV, which means anaerobic conditions existed in the pilot-scale SSF CW. With aeration, the redox potential increased to +20 mV, however the conditions were still anoxic.

After the second week of inoculation, an increase of the ETS activity in 1.5 % NaCl without aeration was noticed and it remained between 2×10^{-9} and 4×10^{-9} dm^3O_2 g^{-1} h^{-1}. The ETS activity, as a measure of the respiration capacity of the microbial community, increased only in aerated 1.5 % NaCl wastewater with 2 g/dm^3 of added saccharose. The ETS activity was the lowest with 0 % NaCl without aeration and the highest with 3 % NaCl.

During the first two weeks of the inoculation period with 1.5 % NaCl, the concentrations of carbon dioxide in the water and soil were lower than later. The carbon dioxide concentrations in the water and soil were similar in all concentrations of salt (0, 1.5 and 3 % NaCl). With 2 g/dm^3 of saccharose in the aerated wastewater, the concentrations of carbon dioxide were the highest during the first day of measurements in the water and in the soil and then decreased.

For all salinity conditions (0, 1.5 and 3 % NaCl), the most significant reduction of the ammonium concentration was registered after seven days. The best removal efficiency was observed in wastewater in the absence of NaCl (Table I). In 1.5 % NaCl wastewater, the removal efficiency was reduced with or without aeration in comparison to that of wastewater in absence of NaCl. However the combination of aeration and saccharose significantly increased the removal efficiency to the same level as that of wastewater in the absence of NaCl. Under high salinity conditions (3 % NaCl), the ammonium removal efficiency was equivalent to the one in wastewater containing 1.5 % NaCl.

After inoculation, the phosphate removal efficiency was around 50 % in the wastewater with 1.5 % NaCl without aeration. Changes in salinity (reduction to 0 % or increase to 3 % NaCl) led to a reduced removal efficiency, especially in the case of the lower NaCl concentration (Table I). Aeration alone and in combination with saccharose addition, also had a negative impact on the phosphate removal efficiency.

The concentration of COD decreased most on the last (seventh) day of every cycle. The final concentrations of COD on the last day for all salt concentrations (0, 1.5 and 3 % NaCl) were approximately the same (Table I), leading to the conclusion that salinity did not affect the removal efficiency of COD. Aeration increased the COD removal efficiency and when saccharose was added to aerated wastewater, the concentration of COD first increased, however the greatest reduction of COD was also achieved under these conditions.

DISCUSSION

Reddy and Patrick[38] pointed out that losses of ammonium through volatilization from flooded soils and sediments are insignificant if the pH value is below 7.5 and very often the losses are not serious if the pH is below 8.0. According to pH, high losses of ammonium through volatization are not to be expected in the pilot-scale SSF CW. In the study of Baere *et al.*,[39] the pH dropped significantly after each shock treatment with a high concentration of NaCl. Also in the presented experiment, the lowest pH was registered with 3 % NaCl.

Carbon dioxide is a product of microbial metabolism and the obtained results confirmed earlier suggestions that saccharose as an energy source and aeration would stimulate the growth of the halotolerant microorganisms, which were isolated from the Sečovlje salterns, thus increasing the amount of produced carbon dioxide. This increase probably caused the drop in pH under these conditions. However, since the carbon dioxide concentration decreased on a third day, the amount of added saccharose was obviously enough only for about three days of intensive metabolism. After the third day the pH also started to rise and on the seventh day it was at the same level as for the other conditions.

TABLE I. Removal efficiencies over time (%); S.D. = standard deviation; aer. = aeration; d1 = first day of circulating wastewater; d3 = third day of circulating wastewater; d7 = seventh day of circulating wastewater; Min. = minimum removal efficiency; Max. = maximum removal efficiency

Quantity	1.5 % NaCl without aer.			1.5 % NaCl with aer.			1.5 % NaCl with aer. + saccharose[a]			0 % NaCl without aer.			3 % NaCl without aer.		
	d1	d3	d7	d1	d3	d7	d1	d3	d7	d1	d3	d7	d1	d3	d7
NH_4-N															
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
Min.	0	0	0	0	0	0	9.1	18.2	27.3	9.1	9.1	27.3	0	0	0
Max.	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															
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
Min.	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
Max.	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
PO_4															
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
Min.	-128.6	-14.3	0	0	14.3	0	0	14.3	0	0	-85.7	0	14.3	14.3	0
Max.	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

[a] c (saccharose) = 2 g/dm^3

Obviously, the available oxygen was quickly consumed by the microorganisms and after the first day there was a lack of oxygen, making the conditions in the pilot-scale SSF CW similar to those in wetlands.[40] Salinity, however, did not influence the oxygen concentrations, which were very low for all the studied salinities. The low concentrations of oxygen resulted in the redox potential being independent of salinity and hence the registered variations were the same in different cycles and for different salinities. Conversely, the redox potential changed under conditions of aeration, when the amount of oxygen was increased and consequently the redox potential also. However, even when the pilot-scale SSF CW was aerated and the redox potential had positive values, the conditions were still anoxic (< 100 mV). This means that the pilot-scale SSF CW should be more aerated, with more air pumps in each compartment to achieve oxic conditions in order to increase the efficiencies of the removal of pollutants. The presence of plants usually enhances the aeration of CW but based on the results obtained in this study additional aeration is proposed. When 2 g/dm^3 saccharose was added to the synthetic wastewater, oxygen was consumed during degradation processes and also aeration could not import enough oxygen to the pilot-scale SSF CW.

As the ETS activity is a measure of microbial activity, it could be concluded that the halotolerant microorganisms were adapted and inoculated into the system after two weeks under the employed conditions (1.5 % NaCl). Also, the increased production of carbon dioxide in the first two weeks and the stable production after the second week of inoculation confirm that the halotolerant microorganisms had adapted to the conditions in the pilot-scale SSF CW after the second week and were successfully inoculated. From the results of the ETS activity at 3 % salinity, it could be concluded that the halotolerant microorganisms isolated from the Sečovlje salterns and inoculated into the pilot-scale SSF CW were neither affected by 3 % salinity nor by a drop of salinity, since the ETS activity at 0 % NaCl was similar to that with 1.5 % NaCl. The increase in the ETS activity with aerated 1.5 % NaCl wastewater containing saccharose means that the number of microorganisms increased due to the aeration and the presence of the additional energy. Lin *et al.*[9] reported that salinity impacted the growth of bacteria resulting in a switch of the microbial community in a pilot-scale SSF CW that was inoculated with a conventional culture of bacteria. However, in the present case, the ETS activity was not reduced by 3 % salinity, hence the conclusion could be that the same microorganisms were active at 1.5 % and 3 % NaCl, thus they were insensitive to the sudden increase in salinity.

Generally, anaerobic conditions in a pilot-scale SSF CW cause low removal of ammonium because the oxidation of ammonia to nitrite and then of nitrite to nitrate (nitrification process) occurs under aerobic conditions by autotrophic bacteria. Contrary to Dahl *et al.*,[41] who found inhibition of the nitrifiers in the case of a rapid increase in the chloride concentration with conventional microorganisms, the results obtained in this study indicate that the removal of ammonium ions was not influenced by increased salt concentrations in the case of the inoculated halotolerant microorganisms. These results are in accordance with Kargi and Dincer,[18] who found that the adverse effects of high salt concentrations were significantly alleviated by the use of salt-tolerant microorganisms. Vymazal[42] concluded that the ability of a horizontal flow CW to nitrify ammonia is very limited because anaerobic conditions usually exist. This is in accordance with the present results where, at the same salinity, high removal of ammonium did not occur even with aeration of the pilot-scale SSF CW because the conditions were still anoxic. To aerate the system of SSF CW, different aeration systems for the introduction of oxygen were suggested, such as frequent water level fluctuation (tidal-flow),[43–45] passive air pumps (vertical-flow)[46] or direct mechanical aeration of the water in the gravel bed (horizontal-flow),[47–49] which was improved by Nivala *et al.*[50] The lower concentration of ammonium found in the experiment with saccharose were also in accordance with the study of Vymazal,[42] who reported that some degradation processes require energy (typically derived from an organic carbon source) to proceed, and others release energy, which can be used by organisms for growth and survival. This suggests that the added saccharose stimulated the growth of the microbial community and also the nitrification process because saccharose acts as a source of energy.

The higher concentration of phosphate with 0 % NaCl indicates that phosphate was washed out with 0 % saline wastewater. This is in accordance with the study of Bulc,[5] in which it was found that all the phosphorus was washed out during precipitation.

As was found by Akratos and Tsihrintzis,[8] the organic removal efficiencies were significantly higher than those for nitrogen and phosphorus removal. This is the case in most wetland systems and it is probably the consequence of nitrogen and phosphorus removal requiring longer hydraulic retention times.

When comparing different salt concentrations, the mean values for COD removal showed the best efficiency with 0 % NaCl (64.4 %) followed by 3 % salinity (52.1 %) and 1.5 % salinity (44.3 %), indicating that the used microorganisms were not affected by salinity and could therefore improve the saline wastewater treatment process in CW. In the case of 1.5 % NaCl in the wastewater, it was noticed that higher removal efficiencies were achieved with aeration compared to non-aerated wastewater. This means that aeration improves organic matter decomposition processes and that they were hindered by lack of oxygen. The removal efficiencies of COD in the pilot-scale SSF CW with 1.5 % NaCl wastewater were the highest with aeration and 2 g/dm^3 saccharose addition, but the final COD concentration was same as without added saccharose, which therefore did not help in lowering the final COD concentration but did help in increasing the ammonium removal efficiency. The removal efficiencies of the pilot-scale

SSF CW with inoculated halotolerant microorganisms did not depend on variations of the salinity but did depend much more on aeration and the presence of saccharose. Additionally, the experiment indicated that the inoculated halotolerant microorganisms in the pilot-scale SSF CW were tolerant to salinity variations.

The found removal efficiencies of ammonium and COD confirmed the study of Garcia *et al.*[51] that the removal efficiency of an SSF CW is rather low for COD and ammonia, usually < 70 % and < 30 %, respectively. They indicated that the efficiencies were more dependent on aeration and the presence of sugar as an organic carbon source. This was also confirmed by the variation of the percent removal of ammonium and COD with time. The process of aeration of the pilot-scale SSF CW did not improve the removal efficiency for ammonium, but improved the COD removal efficiency by 26 %, when experiments with the same salinity (1.5% NaCl), with or without aeration, were compared. This confirms the results of Scholz,[52] who claimed that the ammonium removal efficiency is more dependent on the aerobic conditions than COD removal. A higher removal efficiency was found with aeration of the pilot-scale SSF CW, but not as much as expected. This could be explained by the low redox potential in the pilot-scale SSF CW, meaning that the aeration was not adequate.

The fact that ETS and reduction of COD were the highest in the presence of saccharose and aeration shows that saccharose and aeration stimulated the growth and metabolism of the halotolerant microorganisms. These results are in accordance with the study of Burchell *et al.*,[53] in which it was found that the addition of organic matter to the soils used for an in-stream CW significantly increased biomass growth when compared to the addition of inorganic matter. The same final concentrations of COD for all conditions are a consequence of the added saccharose and aeration, which stimulated the growth of the microbes and metabolism by using the organic matter as a source of energy. Since the COD concentrations were similar at all conditions with varying salinity, the COD removal efficiency was not influenced by salinity changes.

The relatively high values of the standard deviation of the removal efficiencies (Table I) for ammonium and phosphate are the consequence of high variations of the removal efficiency during the operation period. Akratos and Tsihrintzis[8] explained that variations occur because the bacteria for nitrogen are less efficient at low temperatures. Similarly, the water temperature was not constant in the pilot-scale CW employed in the present study. It changed from 14 to 26 °C. The negative values of the phosphate removal efficiencies and lack of oxygen in the pilot-scale CW confirmed that phosphate is mainly removed by adsorption on the porous media[32] and that reducing conditions (*i.e.*, lack of oxygen) can lead to solubilization of minerals and release of dissolved phosphorus.[32,54] The COD removal efficiencies were relatively stable during the entire operation under all conditions. This can be seen from the relatively low standard deviation values of the removal efficiencies for COD, compared to standard deviation of ammonium and phosphate. Similarly, the study by Akratos and Tsihrintzis[8] found that the removal efficiencies for COD were stable, while ammonium and phosphate removal efficiencies were not observed in their pilot-scale CWs.

CONCLUSIONS

Salinity changes in the studied pilot-scale SSF CW did not have a strong influence on the removal efficiency of pollutants, mainly because the processes were affected by a lack of oxygen and energy.

According to the increase in the carbon dioxide concentration and ETS activity, the microorganisms required about two weeks to establish a stable population.

Salinity affects the process of ammonium removal, which was more effecttive in the absence of salinity. However, in saline wastewater, the NaCl concentration had no impact on the removal efficiencies. The process of ammonium removal in the pilot-scale SSF CW was affected by the available energy, which could be seen from the increase in the removal efficiency when saccharose was added. According to the literature, the process is strongly dependant on the available oxygen, but since the pilot-scale SSF CW was not aerated sufficiently with the employed aeration system, the removal efficiencies for ammonium were low.

A higher concentration of phosphate was detected with 0 % NaCl in wastewater. This indicates that phosphate was washed out under these conditions.

Aeration and saccharose addition increased the COD removal efficiency but the final COD concentrations were the same, therefore the additional source of energy is not as important in this case as for ammonium removal. The COD removal efficiency was affected by lack of oxygen but salinity did not have an influence.

The results obtained from the pilot-scale SSF CW show that the use of halotolerant microorganisms can improve the efficiency of saline wastewater treatment. Special attention should be paid to the aerobic/anaerobic conditions because anaerobic conditions strongly hinder COD removal regardless of salinity. In addition, ammonium removal is not sensitive to changes in salinity but care should be taken about aeration and also about providing the energy required for ammonium removal. Plants and their rhizosphere are players in the aeration of ecosystems and therefore provide better conditions for aerobic microorganisms. Thus, the use of plants, preferably halotolerant varieties in association with halotolerant microorganisms could improve removal efficiencies in the treatment of wastewater. However, the obtained results show that aeration alone is not sufficient and that parameters other than aeration (*e.g.*, sugar addition) should be included in the design of SSF CWs with halotolerant microorganisms to improve saline wastewater treatment.

И З В О Д

УТИЦАЈ ПРИМЕНЕ ХАЛОТОЛЕРАНТНИХ МИКРООРГАНИЗАМА НА ЕФИКАСНОСТ ЕКСПЕРИМЕНТАЛНОГ СИСТЕМА ПОЉА ЗА БИОЛОШКО ПРЕЧИШЋАВАЊЕ СЛАНЕ ОДПАДНЕ ВОДЕ

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У циљу проналажења оптималних карактеристика система поља за биолошко пречишћавање слане одпадне воде, халотолерантни микроорганизми, изоловани из воде солане Сечовље, били су инокулирни у медијум експерименталног система поља за биолошко пречишћавање. Циљ рада био је да се испита утицај различитог салинитета на ефикасност пречишћавања слане одпадне воде са халотолерантним микроорганизмима, са намером да се оцене могућности примене халотолерантних микроорганизама за пречишћавање слане одпадне воде. Ефикасност одстрањивања амонијум јона (34,1 %) била је највећа са 0 % NaCl у одпадној води и мало нижа (31,8 %) када је 2 g/dm^3 сахарозе било додато у озрачену одпадну воду са 1,5 % NaCl. Највећа ефикасност смањења хемијске потрошње кисеоника (КПК) у експерименталном систему поља за биолошко пречишћавање са подповршинским током износила је 83,6 % када је сахароза (2 g/dm^3) била додата у озрачену одпадну воду са 1,5 % NaCl. Ефикасност експерименталног система поља за биолошко пречишћавање са инокулираним халотолерантним микроорганизмима показује већу осетљивост на озрачивање и присуство сахарозе, него на варијацију салинитета одпадне воде. Може се закључити да халотолерантни микроорганизми, изоловани из солане Сечовље, нису осетљиви на промене салинитета и због тога су алтернатива у третману слане одпадне воде системом поља за биолошко пречишћавање. Њихова ефикасност може бити унапређена озрачивањем.

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