Zero-discharge wastewater treatment system for biomass production

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Abstract
Evapotranspirative willow system (EWS) is a zero-discharge wastewater treatment system where all influent water is used for growth of willows and evaporation. The EWS has no pollutant emissions to the environment and enables reuse of water and nutrients i.e. closing material flows. Willow clones used in EWS may significantly affect the performance of wastewater treatment plant, therefore the clones with high biomass production and resilience to permanent flooding, increased nutrient concentrations and salinity have to be selected. In the presented study a 27 m² pilot EWS was set up in November 2015 enabling to test three different willow clones from the Croatian selection namely 'V 052' (Salix alba var. calva x S. alba), 'V 093' (Salix alba x S. alba var. vitellina) x S. alba and 'V 160' (Salix alba) in three parallels. Stem height, diameter and number of shoots per stump were measured weekly in the first year of growth on site, along with water quality parameters and water level in the experimental beds during the start up operation.

There were no differences in stem height between the experimental clones; however, 'V 052' and 'V 093' have 0.16 and 0.26 m higher stems in control plots compared to the experimental ones, while 'V 160' experimental plants were in average 0.20 m higher compared to control ones. Experimental 'V 160' also developed more shoots compared to the controls and had significantly higher water demand. Between the examined clones, so far 'V 160' presents the most appropriate clone for the use in EWS; however further investigation will evaluate the efficiency of nutrient transfer from wastewater to wood biomass, water demand and the biomass yield for the selected clones.

Keywords
Closed material loop, wastewater reuse, evapotranspiration, willows, on-site wastewater treatment

INTRODUCTION
Evapotranspirative willow systems (EWS) enable wastewater treatment and recycling of water and nutrients through the willow biomass. They are most appropriate for on-site treatment of domestic wastewaters in the sites where requirements for wastewater discharge are strict or where soil infiltration is not possible. EWS are also attractive for users interested in biomass production for energy use (e.g. for house heating). EWS consists of impermeable bed with no outflow where all the water is used for plant growth and evaporation to the atmosphere. Due to no outflow EWS have no negative impact on the environment in terms of pollutant emissions.

According to Gregersen and Brix (2001) and Börjesson and Berndes (2006) the composition of domestic wastewater corresponds to the willows’ nutrient requirements; moreover, the wastewater can act as a fertiliser and can significantly increase the willow yield. However, the efficiency in pollutant uptake from wastewater to wood biomass and their accumulation in the system is poorly known but of crucial importance in designing and management of the system. Many studies have been done on short rotation willow coppice for wood biomass production which are irrigated with
partially treated wastewater and discharge the excess water to the underground or surface water bodies (Aronsson and Perttu, 2001; Dimitriou and Aronsson, 2011). Since these systems have a discharge; there is a need to evaluate the mass balance of nutrients in EWS with no discharge, i.e. the percentage of nutrients uptaken by the willows and the remaining in the sediment in relation to their amount in the influent wastewaters. In that way the amount of nutrients that can be removed from the system by harvesting wood biomass and the accumulation of nutrients in the system can be evaluated.

According to Swedish research, biomass production in short rotation willow coppice irrigated with wastewater is ca. 10 t DM ha\(^{-1}\) a\(^{-1}\) which means 50 kg of N ha\(^{-1}\) a\(^{-1}\) can be removed each year by harvesting. Moreover, more than 200 kg N ha\(^{-1}\) a\(^{-1}\) can be retained or removed due to denitrification and binding of N in the soil (Dimitriou and Aronsson, 2005). Similar to this, a US study on fertilized short rotation willow coppice reports annual biomass production of 15 to 22 t DM ha\(^{-1}\) a\(^{-1}\), which can remove 75 to 86 and 10 to 11 kg ha\(^{-1}\) a\(^{-1}\) of N and P, respectively (Adegbidi et al., 2001). On the other hand, a study made in Mediterranean climatic conditions reports that the aboveground biomass production of fertilized willow stand can reach up to 64 t DM ha\(^{-1}\) in two years after planting unrooted cuttings (Guidi et al., 2008), indicating that climate may have a significant effect on the system performance.

In EWS willow trees are used; however the studies on short rotation coppice have also investigated the performance of poplars. The later were shown to have lower biomass production yields, lower evapotranspiration and less accumulation of nutrients compared to willows (Guidi et al. 2008; Pistocchi et al., 2009). In terms of willows, appropriate clones have to be selected since there are significant differences between them regarding growth, nitrogen, salt tolerance and water use efficiency (Weih and Nordh, 2002; Rosso et al., 2013). From previous research in phytoremediation it is know that autochthonous plant species are more resistant and productive compared to fast growing clones acquired in other climatic environments (Zupančič Justin et al., 2010). The willow species or clones to be used in EWS should have high biomass production, high water uptake, salt tolerance and be adapted to permanent flooding and increased nutrient concentrations in wastewater. However, and according to Guidi et al. (2008), transpiration depends mainly on development stage, plant nutritional status and climate characteristics rather than on the willow species.

Different species of Salix sp. have been investigated in short rotation willow coppice treated with landfill leachate: S. purpurea (Justin and Zupančič, 2009), S. alba, S. nigra (Alker et al., 2003) and S. amygadalina (Bialowiec et al., 2007). S. viminalis was studied in EWS (Gregersen and Brix, 2001) and S. matsudana, S. jessoensis, S. fragilis and S. alba were studied in terms of biomass production for production of bioenergy (Rosso et al., 2013). The latter study showed the highest biomass production for clone SE03-001 (S. babilonica x S. alba) x S. matsudana f. lobatoglandulosa. The results of these studies are difficult to compare since the willows were grown under different conditions regarding water and nutrient availability and different climate.

In presented study the selected hybrids of S. alba are investigated, namely ‘V 052’ (Salix alba var. calva x S. alba), ‘V 093’ (Salix alba x S. alba var. vitellina) x S. alba and ‘V 160’ (Salix alba). Two of the clones were also under investigation for biomass production in Croatia. According to Kajba and Andrić (2014) the mean biomass production of non-fertilized willow stands in the first and second successive two-year rotations at age 2/3 and 2/5 were higher for willow clone ‘V 093’ (18.5 t DM ha\(^{-1}\) a\(^{-1}\) and 23.5 t DM ha\(^{-1}\) a\(^{-1}\)) in comparison to ‘V 052’ (10.4 t DM ha\(^{-1}\) a\(^{-1}\) and 19.3 t DM ha\(^{-1}\) a\(^{-1}\)).
This study presents preliminary results of EWS start up in terms of willow growth and differences between the clones during the first vegetation season. Nutrient balance will be calculated and biomass production and evapotranspiration will be monitored in order to evaluate the performance and efficiency of EWS under sub-Mediterranean climate.

MATERIALS AND METHODS
The 27 m² pilot EWS is located in Ajdovščina, Slovenia, next to a municipal wastewater treatment plant (WWTP) and is in operation since March 2016. The pilot plant consists of nine impermeable beds (each 3 m long, 1 m wide and 1.8 m deep), filled with local soil up to 1.5 m. A pilot EWS was designed based on willow systems that are in operation in Denmark. Design modifications were done according to the higher, intense and time concentrated precipitation of sub-Mediterranean climate, i.e. inclination of the beds’ top soil layer towards a rainwater drainage pipe installed on the surface at the end of each bed. Besides this, a clay layer is integrated 0.1 m underneath the soil surface in order to reduce the percolation of rainwater into the bed. Each bed of EWS is planted with three willow trees resulting in plant density of 1 m⁻¹ (Figure 1). Three willow clones are being tested, each in three beds (parallels) distributed in a Latin square: 'V 052' (Salix alba var. calva x S. alba), 'V 093' (Salix alba x S. alba var. vitellina) x S. alba, and 'V 160' Salix alba. The clones were produced by the forest nursery Topolje (forest administration Osijek) in eastern Croatia and provided at age 1/1 years. The seedlings were planted in November 2015 and cut back to 10 cm above ground in February 2016. Each bed is equipped with an inlet pipe and piezometers which enable monitoring of water level in the bed. The inflow water is mechanically pre-treated municipal wastewater from adjacent WWTP and is added to the beds according to the plants’ needs. The amount of inflow water is controlled by a flow meter and manually operated valve. The pilot EWS is equipped with time domain reflectometer (TDR) probes to measure soil water content at four different depths: in top soil layer (0.05 m below the surface), in clay layer (at the depth of 0.15 m), in the root zone (at the depth of 0.5 m) and the deepest layer (at the depth of 1 m) in order to evaluate water use and its effect on plant growth. There are no outlet pipes in the beds.

Figure 1. Pilot plant design (letters A, B, C define three different willow clones).

The pilot EWS is positioned perpendicular to a dominant wind direction with the purpose of increasing evapotranspiration. Besides this, the plant is located on a plane area deprived of higher vegetation and shading which additionally increases wastewater usage. With an aim of avoiding the borderline effect of a relatively small pilot plant, willows were planted also along each side of the
EWS. The clones planted outside of the experimental beds receive only rainwater and are monitored as control trees.

The volume and quality of inflow wastewater is monitored (BOD, COD, PO₄-P, TP, NH₄-N, NO₃-N, TN) as well as dissolved oxygen, pH, electric conductivity and temperature of the influent and water in the experimental beds. Willow height, number of shoots and diameter 20 cm above coppice and water level in the beds are measured weekly since March 2016. Biomass production will be analysed at the end of growing season when all the trees will be harvested. Aboveground willow biomass and soil were analysed for nutrient and heavy metal content at the beginning of experiment and will be compared to the analyses at the end of first growing season. Volumetric soil water content is measured via TDR probes twice per day.

The temperature, precipitation and duration of daily solar radiation are measured on site. According to climatic data obtained from Slovenian Environmental Agency the average yearly precipitation at the location of pilot EWS for the period from 2006 to 2015 was 1428 mm and the potential ET for the same period was 943 mm per year showing a net positive water balance for the area. System evapotranspiration will be calculated from components defining water balance.

RESULTS

The paper presents the results of willows growth in a pilot EWS during the first four months of operation (March – June 2016). The nine experimental beds of EWS were filled with municipal wastewater at the beginning of experiment. The wastewater had the following characteristics: 330 mg/L BOD₅, 680 mg/L COD, 8.5 mg/L TP, 6.5 mg/L PO₄-P, 40 TN, 20 NH₄-N, 1.2 NO₃-N. The EC, pH and concentration of dissolved oxygen during monitoring period did not differ significantly between the beds with different clones (Table 1) showing similar growing conditions for all willows.

<table>
<thead>
<tr>
<th>Unit</th>
<th>'V 093'</th>
<th>'V 160'</th>
<th>'V 052'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electric conductivity</td>
<td>mS/cm</td>
<td>2.98±0.85</td>
<td>2.73±0.38</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>7.8±0.4</td>
<td>7.8±0.4</td>
</tr>
<tr>
<td>Dissolved O₂</td>
<td>%</td>
<td>54±18</td>
<td>64±21</td>
</tr>
<tr>
<td>T</td>
<td>°C</td>
<td>15±2.5</td>
<td>15±2.4</td>
</tr>
</tbody>
</table>

Table 1. Physical and chemical parameters of water in the experimental beds of EWS with three willow clones.

All willows planted in EWS and around it (the control trees) successfully sprouted. From February 2016 when the they were cut back, the willows in experimental beds have grown 1.69±0.20, 1.71±0.19 and 1.72±0.28 m, for ‘V 052’, ‘V 160’ and ‘V 093’, respectively (Figure 2). No significant differences between the clones were observed. The control willows of ‘V 052’ and ‘V 093’ were 0.16 and 0.26 m higher compared to the plants in experimental beds, showing an inhibitory effect of wastewater or permanent flooding to the growth of these clones. In contrary, the clone ‘V 160’ in experimental beds was in average for 0.20 m higher compared to the control ‘V 160’ showing better tolerance to the conditions in the EWS. In case of ‘V 160’, the plants in experimental beds showed better growth compared to control trees since the beginning of monitoring (experimental plants always higher compared to control), while in ‘V 093’, the control plants were higher for all monitoring period. Moreover, the difference in height between test and control clones is increasing. The effect of wastewater on ‘V 052’ is not consistent, since the control trees were lower compared to experimental ones in May, however in June, the growth of experimental trees was delayed.
The number of shoots showed similar results like plant growth for ‘V 093’ and ‘V 160’ (Figure 3), namely ‘V 160’ responded positively to the growth in beds permanently flooded with wastewater (number of shoots is higher in experimental plants compared to the control ones) and ‘V 093’ responded negatively since number of shoots is higher in control plants. While height of ‘V 052’ showed mixed response to wastewater, the number of shoots in ‘V 052’ was higher in experimental compared to control plants showing higher biomass production in conditions of permanent flooding with wastewater. Both ‘V 160’ and ‘V 052’ developed around nine shoots in experimental beds and significantly lower number in control plants (4-5), while ‘V 093’ developed higher number of shoots in control plants (9) and lower in experimental ones (6). Stem diameter for all experimental clones was 1.8 cm. No differences between the clones and the controls were observed.

The water usage in experimental beds was in line with the most efficient growth of ‘V 160’, namely
a total water volume used between March and June 2016 was 982 L for ‘V 160’, while ‘V 093’ and ‘V 052’ used only 585 and 512 L, respectively.

**DISCUSSION**

The preliminary results of testing three willow clones for biomass production in EWS have so far shown that ‘V 160’ is better adapted to permanent flooding and/or increased nutrient concentrations of wastewater compared to ‘V 093’ and ‘V 052’. The ‘V 160’ experimental plants were higher and had more shoots compared to controls. Numerous studies have shown that the composition of wastewater matches to the nutrient requirements of willows; moreover, wastewater can act as a good fertiliser (e.g. Gregersen and Brix, 2001; Börjesson and Berndes, 2006; Justin and Zupančič, 2009) which was shown in this study for ‘V 160’ but not for other clones. Willow yield increase due to irrigation with wastewater can be as high as 30-100% in a wastewater fed system compared to commercial rainfed willow cultivation (Börjesson and Berndes, 2006). According to this, Curneen and Gill (2014) report the highest biomass and evapotranspiration in willow cultivars receiving septic tank effluent compared to the systems fed with secondary treated effluent and rainwater. Higher biomass production of wastewater irrigated willows is shown in increased stem diameter and height of plants compared to non-irrigated plants (Guidi et al., 2008). In this study no differences in stem diameter were observed between the clones in experimental beds neither between the experimental and control plants. However, the willows are in the first half of their first vegetation season and differences can appear latter on.

Better growth of ‘V 160’ in experimental beds compared to controls can also be the result of higher water availability in the beds compared to controls. Comparing the water usage, the results have shown that ‘V 160’ has higher water demand compared to ‘V 052’ and ‘V 093’, therefore the ‘V 160’ plants in control plots could have lower growth due to lower water availability (note that the controls were only fed with rain). This is in accordance with Börjesson and Berndes (2006) who pointed out that not only nutrients increase the biomass yield but also water availability. However permanent flooding has negative effects on growth of terrestrial plans, even on willows which are proved to be resistant to high soil water content. Guidi and Labrecque (2009) have shown that permanent flooding had negative effects on most growth parameters in willows except the number of shoots per plant and root biomass; however, in this study no negative effects were observed for ‘V 160’ up to now.

Further differences in biomass production and nutrient uptake are expected at the end of vegetative season, when biomass will be harvested and analysed for nutrients and dry matter content as shown by Guidi et al. (2005) with a positive correlation between water consumption and N and P uptake. Attention will be given also to ‘V 052’ which had similar biomass production as ‘V 160’ when comparing only the plants in experimental beds, despite the controls of ‘V 052’ had even higher biomass production.

So far ‘V 160’ seems to be the most appropriate clone for on-site wastewater treatment in EWS due to high biomass production and due to the highest water usage. Higher evapotranspiration and water usage is a beneficial characteristic when designing EWS since the area footprint of a treatment plant can be smaller. Consequently, also the costs of construction can be reduced. The selection of appropriate willow clone turned out to be an important design parameter in EWS planning since there are significant differences between willow clones as reported also by other studies (e.g. Weih and Nordh, 2002; Kajba and Andrić, 2014).
Zero-discharge systems for wastewater treatment such as EWS enable wastewater and nutrient reuse. They have no negative impact on the environment in terms of pollutant emissions, because all the water and nutrients are used for processes in the system; moreover, EWS enable production of biomass as a renewable energy resource. Wastewater reuse has a great potential for a positive shift towards circular economy, where all materials are recycled.

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