

Evaluation of herbaceous crops irrigated with treated wastewater for ethanol production

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Abstract

The competition for freshwater between agricultural, industrial, and civil uses has greatly increased in Mediterranean basin characterized by prolonged dry seasons. The aim of this study was to evaluate biomass production and the potential ethanol production of promising “no-food” herbaceous crops irrigated with low quality water at different ETc restitutions (0%, 50 and 100%). The research was carried out, in 2011 and 2012, in an open field near the full-scale constructed wetland (CW) municipal treatment plant located in the Eastern Sicily (Italy). The CW effluent has been applied in a experimental irrigation field of *Vetiveria zizanioides* (L.) Nash, *Miscanthus x giganteus* Greef et Deu. and *Arundo donax* (L.). Physical, chemical and microbiological analyses were carried out on wastewater samples collected at inlet and outlet of CW and pollutant removal efficiencies were calculated for each parameter. Bio-agronomical analysis on herbaceous species were made with the goal to evaluate the main parameters such as the plant dimension, the growth response and the biomass production. Biomass dry samples were processed with a three-step chemical pretreatment, hydrolysed with a mix of commercial enzymes and next fermented to obtain the yield of ethanol production. Average TSS, COD and TN removal for CW were about 74%, 67% and 68%, respectively. Although the satisfactory *Escherichia coli* removal, about 3.5 log unit for both beds on average, CW didn't

achieve the restrictive Italian law limits for wastewater reuse. As expected, irrigation was beneficial and the full ET replenishment increase the biomass productivity as compared to the other two treatment. The mean productivity of *Vetiveria zizanioides* and *Miscanthus x giganteus* were about 9, 26 and 38 t ha⁻¹ and 3, 7 and 12 t ha⁻¹ respectively in 0%, 50% and 100% ETc restitutions. *Arundo donax* gave higher values of dry biomass (78 t ha⁻¹ in 100% ETc restitution in 2011 season), and potential ethanol production (about 3,744 kg ha⁻¹). These results suggest the interest in the use of constructed wetland effluents for the irrigation of energy crops to obtain second generation ethanol, particularly in semiarid regions such as the Mediterranean area.

Introduction

The gradual depletion of petroleum-derived transportation fuels has focused attention on both renewable and environmentally friendly resources (Ge *et al.*, 2011), with ethanol from plants being a possible alternative (Sivakumar *et al.*, 2010). Ethanol produced from lignocellulosic biomass is one of the most suitable alternatives for partial replacements of fossil fuels because it provides energy that is renewable and less carbon intensive than gasoline. Bioethanol reduces air pollution and also contributes to mitigate climate change by reducing greenhouse gas emissions (Gnansounou, 2010).

The success of energy crops is closely linked to the production costs. For this reason it is preferable to grow perennial plants, because they need a limited soil management, and to adopt extensive cultivation systems. However, application of fertiliser and irrigation is generally necessary to achieve high crop productivity, especially in the Mediterranean zones, characterized by a chronic water shortage (Barbera *et al.*, 2009). In this context, the irrigation wastewater reuse is desirable because conserve water resources, reduce disposal of polluted effluents into surface water bodies enhances the economic benefits for farmers due to reduced need for fertilizer (Paranychianakis *et al.*, 2006) improve productivity of crops (Bedbabis *et al.*, 2010).

A viable solution for wastewater treatment could be represented by constructed wetland (CW) which are characterised by low O&M costs and by unskilled manpower requirements and represent environmentally sound alternatives to conventional wastewater treatment plants (Cirelli *et al.*, 2007)

This research activity evaluated: 1) the performances of a constructed wetland (CW) system located in Southern Italy, and its suitability for reusing the effluent; 2) the biomass production of herbaceous species when irrigated with CW effluent; 3) the ethanol yield of three herbaceous species.

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Key words: constructed wetland, wastewater reuse, herbaceous crops, evapotranspiration replenishment, ethanol.

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Materials and methods

Experimental plans

The study was carried out in a full-scale constructed wetland treatment plant and in an open field of herbaceous species located in San Michele di Ganzaria (Eastern Sicily - Latitude 37° 16' North, Longitude 14° 25' East, altitude 350 m), a rural community of about 5,000 inhabitants. Constructed wetland consists of two Horizontal SubSurface Flow beds (H-SSF1 and H-SSF2) that receive part of secondary effluent (4 L/s) of the conventional wastewater treatment plant of village (Barbagallo *et al.*, 2011). H-SSF1 and H-SSF2 working in parallel and have an almost equal surface area (about 2,000 m²) but with different operation life: 12 and 6 years of functioning. The terminal section (190 m²) of H-SSF2 reed bed functions as a free water surface. Both reed beds was vegetated with *Phragmites australis*.

Wastewater treated by constructed wetlands has been used for irrigation of herbaceous crops. To this purpose, an experimental irrigation field of *Vetiveria zizanioides* (L.) Nash, *Miscanthus x giganteus* Greef et Deu. and *Arundo donax* (L.) was established. *V.zizanioides* and *M.giganteus* were transplanted in six plot of 9 m² (three repetitions for each species), in July 2008 and May 2009, respectively. *A.donax* was planted, in July 2008, on an area of about 1,000 m² divided in three blocks of about 330 m². All species were transplanted with a density of about 4 plants/m². The wastewaters were supplied by in-line labyrinth drippers system. A meteorological station was installed close to experimental plants, measuring rainfall, temperature, air moisture content, wind velocity, solar radiation and the evaporation. A CR510 automatic weather station (Campbell Scientific, Logan, UT) was installed close to the experimental site for the continuative measure of: rainfall, temperature, air moisture content, wind velocity, solar radiation and the evaporation.

Wastewater irrigation

The water volumes distributed were equal to 0% (S1 and S4), 50% (S2 and S5) and 100% (S3 and S6) of evapotranspiration losses (ETc) (Figure 1).

Wastewater irrigation scheduling was based on water balance equation (Eq. (1)):

$$I = ETc - P + Dp + Rf \quad (1)$$

Where I is irrigation water applied (mm), ETc, crop evapotranspira-

tion (mm) rate calculated as product of Penman-Monteith based reference evapotranspiration (ET₀) (ASCE-EWRI, 2004) and the FAO-56 crop coefficient (K_c). ET₀ reference data were determined by daily climatic factors using an on-site weather station. K_c ranging from 0.75 to 1.10 for *V.zizanioides* and *M.giganteus* while for *A.donax* varied from 1.00 to 1.30. The terms D_p and R_f represent deep percolation (mm) and runoff (mm), respectively. Since irrigation water was controlled, deep percolation and runoff were assumed negligible. The irrigations were realized from June to October 2011 and 2012.

Analysis

Wastewater analysis

Wastewater quality samples were collected at the inlet and outlet of H-SSF1 and H-SSF2. The following physicochemical parameters were evaluated according to APHA (1998) methods: electrical conductivity (EC), pH, total suspended solids (TSS) at 105°C, COD, total nitrogen (TN) NH₄-N, Total Nitrogen (TN) and PO₄-P. In order to evaluate the microbiological pollution *Escherichia coli* and *Salmonella* were also analyzed. *E. coli* was evaluated according to the standard methods (APHA, 1998) and *Salmonella* was examined according to the methodology reported in Barbagallo *et al.* (2003). For each CW were computed the percentage removal efficiencies, for physicochemical parameters, and the log reduction for microbiological parameters.

Bio-agronomical analysis

In the giant reed parcel were identified nine square sampling areas, of one meter side each, where the bio-agronomical survey and sampling activity were carried out during the experimental period. While in the *V.zizanioides* and *M.giganteus* were defined sampling areas of about 4 m² in the centre of each plot. In the sampling area, bio-agronomical analysis on tested species were made with the goal to evaluate the main parameters such as the plant dimension, the growth response and the biomass production. Plant samples for the evaluation of productivity were taken in December 2011 and 2012. Biomass dry weight was determined by drying plant tissue samples in a thermo-ventilated oven at 65 °C until constant weight was reached.

At time of harvest, dry biomass samples of each species have been sent to the Biotechnical Laboratory ENEA in Trisaia in order to characterize each species in terms of fiber (hemicellulose, cellulose and lignin). Afterwards all of them were processed with a three-step chem-

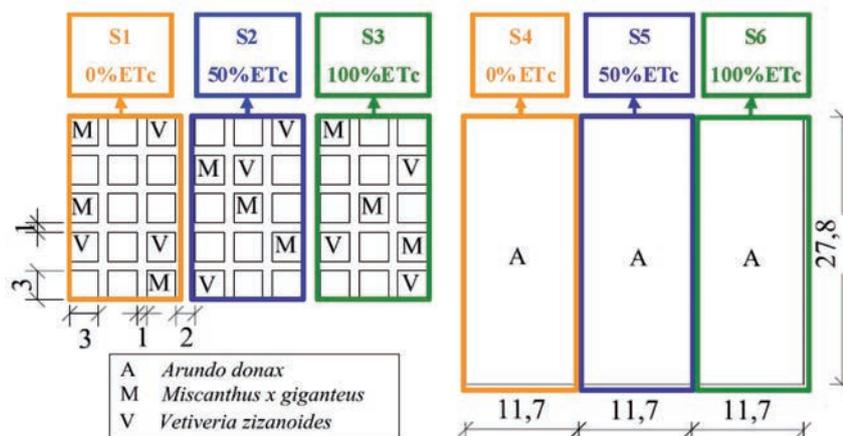


Figure 1. Layout of experimental plant for herbaceous crops.

ical pretreatment to recover most of cellulose and make the biomass more accessible (Borin *et al.*, 2011). This pretreated material was then hydrolysed with a mix of commercial enzymes and next fermented to obtain for each studied plant the yield of ethanol production.

Results and discussion

Constructed wetland performance

During the whole observation period the values of pH and EC of CWs influent and effluent were similar and varied in the range 6.9–7.8 and 1.1–1.7 mS/cm, respectively.

Table 1 shows the average concentrations of the chemico-physical and microbiological parameters in and out of the CWs during the investigation period. The respective mean removal efficiencies are shown in Figure 2.

About 100% and 47% of TSS analysed samples in CWs influent resulted out, respectively, of legislation limits for wastewater reuse (D.M. 185/2003) and for wastewater discharge in water bodies (D.lgs. 152/2006). In the H-SSF1 and H-SSF2 effluent, average TSS concentrations ranged from 6 to 9 mg/L and from 8 to 12 mg/L, respectively. The TSS Italian limit for wastewater irrigation reuse was only exceeded by 17% (H-SSF1) and 18% (H-SSF2) of the samples.

In both effluents, COD and TN concentrations were always below the limits imposed by the D.lgs. 152/2006 and D.M. 185/2003, highlighting average removal efficiencies, respectively, of about 55 and 67% in H-SSF1 and 61 and 74% in H-SSF2. Also the average NH₄-N and PO₄-P removal in H-SSF1 (63% and 26%) was lower than in H-SSF2 (71% and 48%). This results could be explained by ability of algae and microphytes to remove the nutrient elements directly on the open water surface at the end of H-SSF2.

The performance was good for *Salmonella* removal, which was never detected in the effluent of CWs. During the 2012 observation period the *E.coli* concentration in the HSSF2 effluent showed an average decrease of 3.5 log units and 3.0 log units in the H-SSF1 effluent. Only 40% of total samples matched the limit of *E.coli* fixed by D.M. 185/2003. However, the *E.coli* concentration in the H-SSF1 and H-SSF2 effluent (always equal or less 10³ UFC/100 mL) ensure that health-based targets

proposed by the WHO (2006) are matched particularly if drip irrigation is used.

Herbaceous crops: biomass and ethanol productivity

Environmental conditions and irrigation volumes

During the two growing seasons, the daily minimum air temperatures ranged from -4.3 to 22.2°C and the maximum from 7.1 to 43.3°C with average seasonal values of 18.8 °C (2011) and 19.9°C (2012). Total rainfall from March to October was 406 mm in 2011 while in 2012 it was only 163 mm (Figure 3), with 179 and 197 days without rain, respectively.

The higher temperature associated with lower precipitation in 2012 irrigation period compared to the same period in 2011 generated the significantly different ET_c values. In particular, irrigation water volumes applied in *V.zizanioides* and *M.giganteus* crops were 250 mm and 480 mm (2011 season) and 380 mm and 780 mm (2012 season) at plots

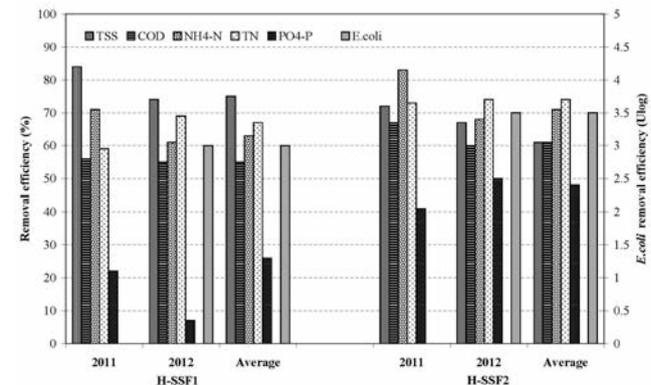


Figure 2. CWs removal efficiency of TSS, COD, NH₄-N, TN, PO₄-P and *E. coli* during the observation period

Table 1. Comparison between average pollutant wastewater concentration in the influent (In) and effluent (Out) of H-SSF1 and H-SSF2 with the Italian wastewater discharge limits into surface waters (D.Lgs. 152/2006) and for agriculture reuse (D.M. 185/2003).

Parameters	2011				2012				Average				Law Limits	
	H-SSF1		H-SSF2		H-SSF1		H-SSF2		H-SSF1		H-SSF2		D.Lgs. 152/06	D.M. 185/03
	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out		
TSS (mg/L)	63	9	63	12	42	6	43	8	46	6	47	9	35	10
COD (mg/L)	97	33	97	19	56	19	59	17	63	21	66	18	125	100
NH ₄ -N (mg/L)	24	5	24	3	12	3	13	3	14	4	15	3	-	2
TN (mg/L)	30	11	30	7	28	7	29	6	29	7	29	6	15	35
PO ₄ -P (mg/L)	4.4	3.5	4.4	2.6	3.3	2.7	3.1	1.3	3.6	2.8	3.3	1.5	-	-
<i>E.coli</i> (Ulog/100 mL)	-	-	-	-	5.4	2.4	5.4	1.9	5.4	2.4	5.4	1.9	3.7	1.7*

*Maximum value to be detected in 80% samples for natural treatment systems.

with, respectively, 50% and 100% restitution of ETc. While in *A. donax* plots the irrigation water applied, during spring/summer 2011 and 2012, were 300 mm (50% ETc) and 600 mm (100% ETc) and 480 mm (50% ETc) and 960 mm (50% ETc), respectively.

Crop growth and biomass yield

The increase of ETc restitution, from 0% to 100%, positively influenced the plant higher and, consequently, the aboveground biomass production for all tested species (Table 2) while no significant difference were highlighted for different irrigation thesis. Biomass water content at harvest was lowest in *M. giganteus*, varying from 33 to 38%, compared to *A. donax* (50-56%) and *V. zizanioides* (62-65%). This could be explained with the life cycle of plants: *V. zizanioides* and *A. donax* are active during most of the wintertime in the Mediterranean environment whereas the *M. giganteus* stems dry up completely during winter.

A. donax showed the highest values of dry biomass, with a mean value of about 50 t ha⁻¹, followed by *M. giganteus* (-42%) and *V. zizanioides* (-85%). From first to the second year *Arundo donax* biomass dry yield decreased about 39% due to the reduction (about -50%) of the stalks density. The biomass yield of these species in the tested environment, has been higher (on average +20-30%) than recorded by other authors in two long-term experimental field carried out in Central (Angelini *et al.*, 2009) and Southern Italy (Mantineo *et al.*, 2009). These differences in yield performance can be linked to density planting (twice more than the other investigations).

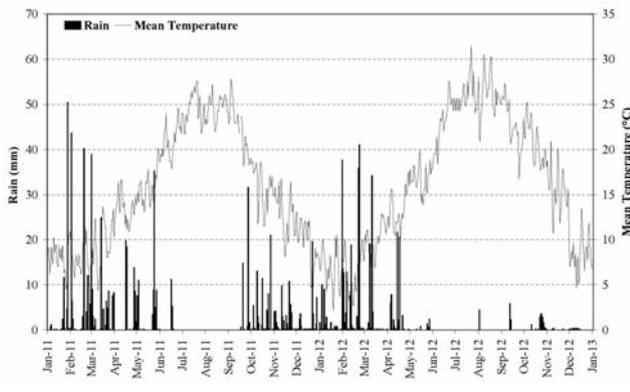


Figure 3. Precipitation and average air temperature series from January 2011 to December 2012.

The yield of *V. zizanioides* determined in this experiment showed values ranging between 2.6 to 16.6 t ha⁻¹, that were comparable to those obtained in other similar field experiments carried out in North Italy ranging between 10 to 12 t ha⁻¹ (Monti *et al.*, 2005).

With regard to the 2012 yield of *M. giganteus*, it was observed that the high summer temperatures associated with an extended period without significant rainfall events, induced an early senescence state in *M. giganteus* plants without irrigation supply with subsequent dry biomass production failures. These environmental conditions have also influenced the yields of irrigated thesis decreased by 33% (50% ETc) and 31% (10% ETc) than 2011 season. However, the average total biomass produced in the different irrigation treatments was comparable than recorded by other authors in experimental sites with similar climatic and crop management characteristics (Angelini *et al.*, 2009; Mantineo *et al.*, 2009; Zub and Brancourt-Hulmel, 2010).

Ethanol yield

The preliminary study on the chemical composition of biomasses investigated highlighted higher cellulose and hemicellulose concentrations in *A. donax* and *M. giganteus* while the *V. zizanioides* showed lower lignin content (Table 3). These fiber compositions of dry biomass samples were according with the data reported in the Phyllis database (<http://www.ecn.nl/phyllis/single.html>) and the BioBIB database (<http://www.vt.tuwien.ac.at/biobib/>). We can suppose that the *A. donax* and *M. giganteus* are the most suitable candidates for the production of fermentable sugars and ethanol by means of the appropriate pretreatment, hydrolysis and fermentation. Infact, the cellulosic and hemicellulosic fractions, are the most important biomass components that hydrolysates produce glucose subsequently fermented into ethanol (Currelli *et al.*, 2002).

Despite the lower concentrations of cellulose and hemicellulose *V. zizanioides* showed higher fermentation yield (glucose percentage fer-

Table 3. Chemical composition of the herbaceous crops.

Species	Extractives (%)	Hemicellulose (%)	Cellulose (%)	Lignin (%)	Ashes (%)
<i>Arundo Donax</i>	14,13±0,13	33,57±0,57	43,69±0,30	8,68±0,25	0,56±0,04
<i>Miscanthus x giganteus</i>	12,35±0,38	34,21±1,24	46,14±0,58	6,14±0,62	1,15±0,02
<i>Vetiveria zizanioides</i>	18,98±0,33	39,77±0,45	34,88±0,46	5,39±0,04	0,80±0,11

Table 2. Mean values of density, height, moisture content and dry biomass evaluated of the herbaceous crops in December 2011 and 2012.

Species	Irrigation thesis	Plant density (n. plant/m ²)		Height (cm)		Moisture content (%)		Dry biomass (t/ha)	
		Dec -11	Dec-12	Dec -11	Dec-12	Dec -11	Dec-12	Dec -11	Dec-12
<i>Arundo donax</i>	0% ETc	22	12	419	392	56	51	48.3	25.6
	50% ETc	22	14	475	437	56	50	59.5	40.9
	100% ETc	24	15	498	453	50	49	78.5	48.5
<i>Miscanthus giganteus</i>	0% ETc	83	-	119	-	38	-	19.6	-
	50% ETc	85	73	179	168	37	51	30.6	20.6
	100% ETc	91	80	276	229	33	52	44.6	30.6
<i>Vetiveria zizanioides</i>	0% ETc	4	4	96	91	65	69	2.6	3.7
	50% ETc	4	4	128	136	62	66	3.9	10.8
	100% ETc	4	4	169	168	63	66	7.0	16.6

mented into ethanol) and ethanol production than those obtained for *A.donax* and *M.giganteus* (Table 4). These results could be mainly ascribed to the higher lignin content in *A.donax* and *M.giganteus* biomasses that has reduced the accessibility of cellulose to enzymes decreasing the hydrolysis rate (Currelli *et al.*, 1997).

However, since the aboveground biomass yield per hectare is much higher for *A.donax*, the average ethanol yield per unit of cultivated area (2,400 Kg ha⁻¹) was about 1.1 times the mean ethanol yield calculated for *M.giganteus* (2,190 Kg ha⁻¹) and 3.4 times the average values of *V.zizanoides* (710 Kg ha⁻¹).

Conclusions

The results of the experimental activities show that constructed wetlands are able in removing the main chemical and physical pollutants from the secondary effluent of urban wastewaters treatment plant. However, *Escherichia coli* (mean removal efficiency of about 3.3 log unit) in CWs effluents didn't respect the Italian standard for wastewater reuse but complied with the WHO guidelines. In this case stabilisation reservoirs would be used coupling the CWs and would therefore be a reliable and economic solution to further reduce the microbiological load in treated wastewater (Barbagallo *et al.*, 2003b).

The results highlight attractive biomass yield and an interesting crop energy capability by using treated wastewater for irrigation. The aboveground dry matter production, as expected, was positively affected by the full ET replenishment. The higher performance observed under 100% ETc restitution regime indicates that any effort to maximize and stabilize herbaceous biomass production might be subordinated to an adequate water supply. However, the *A.donax* showed significant biomass production even in the absence of irrigation with an average value of about 37 t ha⁻¹.

The biomass and ethanol yield analysis showed highest values for *A.donax* and *M.giganteus*. This suggest the technical feasibility of extensive cultivation of these species on marginal land in the semiarid regions such as the Mediterranean area in order to maximise agricultural profits.

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Table 4. Glucose and ethanol concentration obtained, respectively, by the hydrolysis and fermentation processes and fermentation and ethanol yields.

Species	Glucose (g/L)	EtOH yield (g/L)	Fermentation production (%)	Ethanol g(EtOH)/100g(S.S.)
<i>Arundo donax</i>	13,7±0,4	4,1±0,4	58,9±3,8	4,8±0,4
<i>Miscanthus giganteus</i>	21,1±1,6	6,2±0,9	57,6±3,8	7,5±0,4
<i>Vetiveria zizanoides</i>	21,4±3,5	9,1±0,5	84,0±9,2	9,6±2,6

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