

Reuse of constructed wetland effluents for irrigation of energy crops

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ABSTRACT

The aim of this study was to evaluate biomass production of promising 'no-food' energy crops, *Vetiveria zizanioides* (L.) Nash, *Miscanthus × giganteus* Greef et Deu. and *Arundo donax* (L.), irrigated with low quality water at different evapotranspiration restitutions. Two horizontal subsurface flow (H-SSF) constructed wetland (CW) beds, with different operation life (12 and 6 years), were used to treat secondary municipal wastewaters for crop irrigation. Water chemical, physical and microbiological parameters as well as plant bio-agronomic characters were evaluated. The results confirm the high reliability of CWs for tertiary wastewater treatment given that the H-SSF1 treatment capacity remained largely unchanged after 12 years of operation. Average total suspended solids, chemical oxygen demand and total nitrogen removal for CWs were about 68, 58 and 71%, respectively. The *Escherichia coli* removal was satisfactory, about 3.3 log unit for both CW beds on average, but caution should be taken as this parameter did not achieve the restrictive Italian law limits for wastewater reuse. The average above-ground dry matter productions were 7 t ha⁻¹ for *Vetiveria zizanioides*, 24 t ha⁻¹ for *Miscanthus × giganteus* and 50 t ha⁻¹ for *Arundo donax*. These results highlight attractive biomass yield by using treated wastewater for irrigation with a complete restitution of evapotranspiration losses.

Key words | biomass for energy, constructed wetlands, evapotranspiration restitution, wastewater reuse

INTRODUCTION

Energy production from biomass of non-food crops is one of the most promising renewable sources in the short and middle term (Robbins *et al.* 2012). These energy sources are regarded as 'zero emission carbon' because the quantity of CO₂ released by combusting biomass does not exceed the amount that has been fixed previously by photosynthesis while the plants were growing (Hanegraaf *et al.* 1998). At the same time, the non-food energy crops may represent an interesting prospect for the agricultural sector in European Union countries characterised by food surplus and low incomes.

The perennial species, as *Arundo donax* (L.) and *Miscanthus × giganteus* Greef et Deu., are generating much interest in Europe. It has been shown that these species have high production levels in high resource environments (high inputs) (Angelini *et al.* 2009; Mantineo *et al.* 2009; Nasso *et al.* 2010; Borin *et al.* 2013; Barbera *et al.* in press), but there is little information on their ability to adapt to low input cultivation methods. Application of high fertilisers and

irrigation is generally required for a high and stable biomass production in energy crops (Barbera *et al.* 2009). *Arundo donax* is a perennial rhizomatous plant of grasslands and wetlands, belonging to the *Poaceae* family. It is native to the Mediterranean region, but it occurs over a wide range of climatic habitats (Lewandowski *et al.* 2003). Despite its C3 photosynthetic cycle, it has an elevated photosynthetic activity that allows the production of biomass similar to those obtained from C4 plants (Perdue 1958). Its growth starts from the rhizome in early spring and reaches in late autumn the average height of more than 5 metres, having a growing rate of about 5 centimetres per day in the right environments (Perdue 1958). *Miscanthus* is a perennial C4 grass endemic to East Asia that was introduced in Europe as an ornamental plant about 50 years ago (Lewandowski *et al.* 2003). *M. giganteus* is a sterile clone propagated by rhizome division or *in vitro* cultures (Angelini *et al.* 2009). The distinct advantage of this species is long productive age and biomasses use a renewable

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CR510 automatic weather station (Campbell Scientific, Inc., Logan, UT, USA). Kc ranged from 0.75 to 1.10 for *V. zizanioides* and *M. giganteus*, while for *A. donax* it varied from 1.00 to 1.30. The irrigation was applied from June to October in 2011 and 2012.

Wastewater quality samples were taken monthly at the inlet and outlet of H-SSF1 (from April to September 2011 and from March to September 2012) and H-SSF2 (from April to September 2011 and from March to October 2012). The following physicochemical parameters were evaluated according to APHA (1998) methods: pH, electrical conductivity, total suspended solids (TSS) at 105 °C, chemical oxygen demand (COD), NH₄-N, total nitrogen and PO₄-P. In the samples collected during the 2012 sampling campaign, also the microbiological characteristics of wastewater were analysed. In particular, *Escherichia coli* was analysed according to *Standard Methods* (APHA 1998) while the *Salmonella* was examined according to Cirelli et al. (2007). Bacterial numbers measured in CFU per 100 mL were transformed into log₁₀. For each CW were computed the percentage removal efficiencies according to IWA Specialist Group on Use of Macrophytes in Water Pollution Control (2000) for physicochemical parameters, and the log reduction for microbiological parameters.

For *A. donax*, nine square sampling areas, of 1 metre side each, were selected where the bio-agronomical survey and sampling activity were carried out during the experimental period. For *V. zizanioides* and *M. giganteus*, sampling areas of about 4 m² were defined in the centre of each plot. In the sampling areas, bio-agronomical analysis on tested species was made with the goal of evaluating the main parameters such as the plant dimension, the growth response and the biomass production. Plant samples for the evaluation of productivity and heating values 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. Each sample was evaluated for the lower heating value (LHV) determined by subtracting the heat of vaporisation of the water vapour from the higher heating value, evaluated directly with the Mahler bomb (SN 3472, SDM, Torino, Italy) (ASTM 2009). For each irrigation regime, the energy output (EO), multiplying the dry biomass yield for the LHV, was evaluated.

Over both research years, the water use efficiency index (WUE) was calculated as the ratio between dry biomass production (g m⁻²) at the final harvest and the water used by the crop (L m⁻²), determined by adding together water supplied by irrigation and precipitation during the vegetative period (Mantineo et al. 2009). Also the energy water use

efficiency index (EWUE) was estimated as the ratio between EO and the water used by the crop.

Statistical analysis

The results were analysed using analysis of variance and Student–Newman–Keuls tests, after verifying the homogeneity of the variances using Bartlett's test (Statistica, v. 10, StatSoft, Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

CW performance

During the study period, the pH values of the CW influent and effluent were similar and slightly alkaline ranging from 6.9 to 7.8. Also the electrical conductivity did not show significant differences between the influent and effluent, with values from 1.1 to 1.7 mS/cm.

Table 1 summarises mean chemical and microbiological characteristics in and out of the CWs during the observation period and the respective mean removal efficiencies.

The mean TSS concentration in the CW influent ranged between 42 and 63 mg L⁻¹ with 100% of TSS samples being outside the Italian legislation limit (10 mg/L) for wastewater irrigation reuse (Decree of Italian Environmental Ministry, M.D. 185/2003), whereas only 17% (H-SSF1) and 18% (H-SSF2) of wastewater samples collected at the outlet of CWs exceeded the TSS concentration of 10 mg L⁻¹. The mean TSS concentration in the H-SSF2 effluent was higher than the values observed in the H-SSF1 effluent. This could be explained by alga growth occurring in the free water surface area at the end of the H-SSF2, which increases TSS concentration in the effluent. The growth of algae in H-SSF2 had a positive impact on the removal efficiency of nutrient, showing higher values than those of H-SSF1. In particular, the mean reductions of NH₄-N and PO₄-P in the H-SSF1 and H-SSF2 were about 63 and 26% respectively, whereas in H-SSF2 the mean removal efficiencies were about 71% for NH₄-N and 48% for PO₄-P, respectively. However, in the H-SSF1 and H-SSF2 effluents the mean concentrations of chemical–physical parameters, with the exception of PO₄-P, were not significantly different (Table 1).

Average COD and TN removal for H-SSF1 (55 and 67% respectively) and H-SSF2 (61 and 74% respectively) were similar despite the different ages of operation. In both effluents, COD and TN concentrations were always below the

Table 1 | Mean influent (In) and effluent (Out) wastewater concentrations and mean pollutant removal efficiencies (R) throughout 2011 and 2012 monitoring period in H-SSF1 and H-SSF2 (standard deviation in brackets)

Year	CW	TSS		COD		NH ₄ -N		TN		PO ₄ -P		E. coli							
		In	Out	R (%)	In	Out	R (%)	In	Out	R (%)	In	Out	In	Out					
		(mg L ⁻¹)	(mg L ⁻¹)	(%)	(mg L ⁻¹)	(mg L ⁻¹)	(%)	(mg L ⁻¹)	(mg L ⁻¹)	(%)	(mg L ⁻¹)	(mg L ⁻¹)	log unit	R					
2011	H-SSF1	63 ^{ab}	9 ^c (3)	84 (5)	97 ^a (77)	33 ^c (11)	56 (24)	24 ^a (21)	5 ^c (3)	71 (14)	30 ^{ab} (15)	11 ^b (1)	59 (24)	4.4 ^{ab} (0.1)	3.5 ^b (0.1)	22 (2)	ND	ND	
	H-SSF2	63 ^{ab}	12 ^c (6)	72 (27)	97 ^a (77)	19 ^d (7)	67 (34)	24 ^a (21)	3 ^c (1)	83 (12)	30 ^{ab} (15)	7 ^b (2)	73 (20)	4.4 ^{ab} (0.1)	2.6 ^c (0.3)	41 (6)	ND	ND	
2012	H-SSF1	42 ^b	34 ^c (3)	74 (26)	56 ^b (30)	19 ^d (7)	55 (30)	12 ^b (13)	3 ^c (2)	61 (27)	28 ^{ab} (23)	7 ^b (4)	69 (19)	3.3 ^b (2.0)	2.7 ^c (2.0)	7 (53)	5.4 ^a (0.3)	2.4 ^b (1.3)	3.0 (1.3)
	H-SSF2	43 ^b	8 ^c (4)	67 (29)	59 ^b (31)	17 ^d (9)	60 (32)	13 ^b (14)	3 ^c (2)	68 (27)	29 ^{ab} (24)	6 ^b (4)	74 (19)	3.1 ^{bc} (2.0)	1.3 ^d (1.0)	50 (27)	5.4 ^a (0.3)	1.9 ^b (0.6)	3.5 (0.4)

ND, not determined.
In each parameter mean concentrations followed by different superscripts are significantly different ($p < 0.05$).

limits imposed by the Italian regulation for wastewater reuse (100 mg L⁻¹ for COD and 35 mg L⁻¹ for TN).

During the observation period the *E. coli* concentration in the H-SSF2 effluent showed an average decrease of 3.5, and 3.0 log units in the H-SSF1 effluent. Only 40% of total samples matched the limit for *E. coli* (50 CFU per 100 mL) fixed by Italian legislation for wastewater reuse. However, the *E. coli* concentration in the H-SSF1 and H-SSF2 effluent (always equal to or less than 10³ CFU per 100 mL) ensures that health-based targets proposed by the WHO (2006) are met, particularly if drip irrigation is used. Furthermore, the performance was good for *Salmonella* removal, which was never detected in the CWs' effluents.

Energy crop productivity

The field was subjected to a typical Mediterranean semi-arid climate with dry summers and mild winters. Total rainfall from March to October was 406 mm in 2011, while in 2012 it was only 163 mm (Figure 2), with 179 and 197 days without precipitations, respectively. Along the 2011 and 2012 growing seasons, the daily average air temperature was 18.8 and 19.9 °C, respectively. The higher temperature associated with lower precipitation, higher values of SR and low air moisture content in the 2012 irrigation period compared to the same period in 2011 generated significantly different ET_c values. Consequently, the irrigation volumes were significantly higher in 2012 than in 2011. In particular, irrigation water volumes applied to *V. zizanioides* and *M. giganteus* crops were 250 and 480 mm (2011 season) and 380 and 780 mm (2012 season) at plots with, respectively, 50 and 100% restitution of ET_c, whereas in *A. donax* plots the following irrigation water was applied: 300 mm (50% ET_c) and 600 mm (100% ET_c) during spring/summer 2011, and 480 mm (50% ET_c) and 960 mm (50% ET_c) during the 2012 season.

As expected, irrigation was in general beneficial and full ET replenishment (100%) increased the biomass productivity as compared to the other two treatments (0 and 50% ET_c). In particular, the increase of irrigation volumes positively influenced the plant height and, consequently, the above-ground biomass production for all tested species (Table 2), while no significant differences were highlighted for plant density in the different irrigation regimes. 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 by the life cycle of plants: *V. zizanioides* and *A. donax* are active during most of the winter-time in the Mediterranean environment, whereas the *M. giganteus* stems dry up completely during winter.

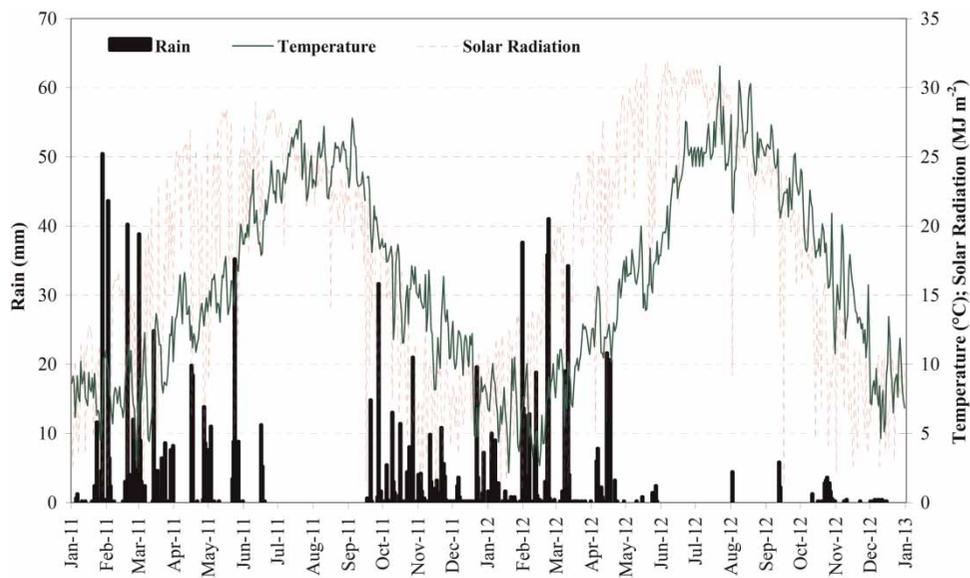


Figure 2 | Rainfall, solar radiation and air temperature series from January 2011 to December 2012.

A. donax showed a dry biomass mean value (about 50 t ha^{-1}) of about two and seven times higher than that showed by *Miscanthus × giganteus* and *Vetiveria zizanioides*. *Arundo*'s total aerial dry matter produced in the second growing season was significantly lower (i.e. 39% lower) than that obtained in the first one due to the reduction (about 50%) of the stalk 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 studies 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).

The average dry biomass yield of *V. zizanioides* showed values ranging between 2.6 and 16.6 t ha^{-1} , which were comparable to those obtained in other similar field experiments carried out in Northern Italy, ranging between 10 and 12 t ha^{-1} (Monti et al. 2005).

With regard to the 2012 yield of *M. giganteus*, we 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 negatively influenced the dry mass yields of irrigated plots, with a decrease of 33% (50% ETc) and 31% (100% ETc) from the 2011 to the 2012 season. However, the average total biomass produced in the different irrigation treatments was comparable to that recorded by other authors in experimental sites with similar climatic and crop management

characteristics (Angelini et al. 2009; Mantineo et al. 2009; Zub & Brancourt-Hulmel 2010).

A. donax showed the highest WUE compared to *M. giganteus* and *V. zizanioides* due to its higher yields (Table 2). With the increase of water irrigation volume the *A. donax* reduced the WUE in both investigation periods, with WUE values ranging from 7.8 to 11.9 g L^{-1} , in 2011 investigation period, and from 4.3 to 15.7 g L^{-1} in the second season. These values were generally higher than those obtained in other experimental field studies (ranging between 0.93 and 7.63 g L^{-1}) carried out in Southern Italy (Mantineo et al. 2009). For *M. giganteus*, there were no significant differences between WUE values determined for the different irrigation treatments in the same year. Similar values were reported by Mantineo et al. (2009), in the Mediterranean environment, with two different irrigation treatments (75 and 25% of maximum evapotranspiration restoration), while Beale et al. (1999) and Clifton-Brown & Lewandowski (2000) in Central Europe have observed WUE values between 7.8 and 13.4 g L^{-1} . This could be explained by the higher crop evapotranspiration rate occurring in the Mediterranean environment compared to Central Europe. In the second year, the WUE values of *A. donax* and *M. giganteus* were rather low due to the reduced total yield. Finally, the WUE of *V. zizanioides* was low (mean value of about 1.3 g L^{-1}) due to the lowest yield compared to the other two crops.

The mean LHV determined for *A. donax*, *M. giganteus* and *V. zizanioides* were 7.21 , 10.09 and 4.95 MJ kg^{-1} , respectively. As expected LHVs were reduced with

Table 2 | Mean values of density, height, moisture content, dry biomass, WUE index, EO and EWUE evaluated for the herbaceous crops in December 2011 and 2012 (standard deviation in brackets)

Species	Irrigation regime	Plant density (no. plant m ⁻²)		Height (cm)		Moisture content (%)		Dry biomass (t ha ⁻¹)		WUE (g L ⁻¹)		EO (GJ ha ⁻¹)		EWUE (KJ L ⁻¹)	
		Dec 11	Dec 12	Dec 11	Dec 12	Dec 11	Dec 12	Dec 11	Dec 12	Dec 11	Dec 12	Dec 11	Dec 12	Dec 11	Dec 12
<i>Arundo donax</i>	0% ETc	22 ^a (1)	12 ^b (2)	419 ^{cd} (40)	392 ^d (51)	56 ^a (3.0)	51 ^{ab} (2.0)	48.3 ^c (4.8)	25.6 ^c (3.0)	11.9 ^b (1.2)	15.7 ^a (1.8)	348 ^c (35)	185 ^c (21)	86 ^b (8.5)	113 ^a (13.1)
	50% ETc	22 ^a (1)	14 ^b (1)	475 ^{ab} (5)	437 ^c (26)	56 ^a (1.6)	50 ^b (1.6)	59.5 ^b (1.5)	40.9 ^d (5.3)	8.4 ^c (0.2)	6.4 ^{cd} (0.8)	429 ^b (10)	295 ^d (38)	65 ^c (1.6)	54 ^c (7.1)
	100% ETc	24 ^a (1)	15 ^b (1)	498 ^a (45)	455 ^{bc} (10)	50 ^b (0.5)	49 ^b (1.3)	78.5 ^a (5.7)	48.5 ^c (3.7)	7.8 ^c (0.6)	4.3 ^d (0.3)	566 ^a (41)	350 ^c (27)	64 ^c (4.6)	37 ^d (2.8)
<i>Miscanthus giganteus</i>	0% ETc	85 ^{ab} (1)	-	119 ^d (3)	-	38 ^b (2.1)	-	19.6 ^c (1.1)	-	4.8 ^{ab} (0.3)	-	197 ^c (11)	-	49 ^a (2.7)	-
	50% ETc	85 ^{ab} (3)	73 ^c (8)	179 ^c (27)	168 ^c (21)	37 ^b (3.0)	51 ^a (3.5)	30.6 ^b (0.6)	20.6 ^c (1.7)	4.7 ^{ab} (0.1)	3.8 ^{bc} (0.3)	308 ^b (6)	208 ^c (17)	47 ^a (0.9)	38 ^b (3.1)
	100% ETc	91 ^a (1)	80 ^{bc} (7)	276 ^a (26)	229 ^b (46)	33 ^b (2.2)	52 ^a (1.3)	44.6 ^a (4.2)	30.6 ^b (2.7)	5.0 ^a (0.5)	3.2 ^c (0.3)	450 ^a (43)	309 ^b (27)	51 ^a (4.8)	33 ^b (2.9)
<i>Vetiveria zizanioides</i>	0% ETc	4 (0)	4 (0)	96 ^c (3)	91 ^c (5)	65 ^{ab} (0.4)	69 ^a (1.2)	2.6 ^c (0.6)	3.7 ^d (0.3)	0.6 ^c (0.1)	2.3 ^a (0.2)	13 ^d (3)	18 ^d (1)	3 ^b (0.7)	11 ^a (0.8)
	50% ETc	4 (0)	4 (0)	128 ^b (10)	136 ^b (10)	62 ^b (1.7)	66 ^{ab} (0.7)	3.9 ^d (0.9)	10.8 ^b (0.8)	0.6 ^c (0.1)	2.0 ^{ab} (0.1)	19 ^d (4)	53 ^b (4)	3 ^b (0.7)	10 ^a (0.7)
	100% ETc	4 (0)	4 (0)	169 ^a (10)	168 ^a (9)	63 ^b (2.0)	66 ^{ab} (1.7)	7.0 ^c (3.0)	16.6 ^a (2.9)	0.8 ^c (0.3)	1.8 ^b (0.3)	55 ^c (15)	82 ^a (15)	4 ^b (1.7)	9 ^a (1.5)

In each parameter and in each species, values followed by different superscripts are significantly different ($p < 0.05$).

increasing biomass moisture contents (Table 2). However, despite the highest LHV highlighted for *M. giganteus*, *A. donax*, thanks to its higher biomass yield, was the investigated species with the highest EO. The EWUE has a similar trend to WUE for all investigated species. The EO values are comparable to those obtained by other authors (Mantineo et al. 2009), despite the lower LHV results due to the higher biomass moisture contents.

CONCLUSIONS

The CWs located in San Michele di Ganzaria (Sicily) have proved to be efficient in removing the main chemical and physical pollutants from the secondary effluent of an urban wastewaters treatment plant. The results confirm the high reliability of CWs for tertiary wastewater treatment given that the H-SSF1 treatment capacity remained largely unchanged after 12 years of operation. However, *E. coli* (mean removal efficiency of about 3.3 log unit) in CW effluents did not meet the Italian standard for wastewater reuse but complied with the WHO guidelines. In this case stabilisation reservoirs could 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. 2003).

The results highlight the potential in the use of CW effluents for the irrigation of high biomass herbaceous production. In particular, *Arundo donax* (full irrigated) was the most productive species with maximum dry biomass yield of about 78 t ha⁻¹, about 43 and 79% higher than maximum productivity detected, respectively, for *Miscanthus × giganteus* and *Vetiveria zizanioides*. The semi-arid climate conditions, with no effective rainfall during the spring–summer period, have certainly influenced above-ground biomass productions, which were consistently higher in plots with full ET replenishment. However, *Arundo donax* showed significant biomass production even in the absence of irrigation, with an average value of about 37 t ha⁻¹. Furthermore, for this species the WUE, which accounts for the enhancing of the water distributed as irrigation and precipitation, indicates that the least irrigated crop makes the best use of water supplied with irrigation. The higher biomass moisture contents, which negatively affected the LHV and OE values, proved that the biomass must be harvested in the dry period at the end of the vegetative cycle (i.e. late winter). In conclusion, *Arundo donax* and *Miscanthus × giganteus* could be represent a profitable solution to the possible cultivation, for energy purposes, of

marginal land in the semi-arid regions such as the Mediterranean area, overcoming some problems of the sustainability of energy crops. Finally, the availability of treated wastewater for irrigation would provide, with a complete restitution of ETc, biomass yields and EOs significantly higher than those obtained without irrigation or with 50% ET restoration.

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