Biomass and Bioenergy 107 (2017) 191–197

Contents lists available at ScienceDirect

Biomass and Bioenergy

journal homepage: www.elsevier.com/locate/biombioe

Research paper

Chemical composition and yield of rhizome biomass of *Arundo donax* L. grown for biorefinery in the Mediterranean environment

Simona Proietti[a], Stefano Moscatello[a], Massimo Fagnano[b,**], Nunzio Fiorentino[b], Adriana Impagliazzo[b], Alberto Battistelli[a,*]

[a] Institute of Agro-Environmental and Forest Biology (IBAF), National Research Council (CNR), via Marconi 2, 05010, Porano, TR, Italy
[b] Department of Agricultural Sciences, University of Naples Federico II, Via Università 100, 80055, Portici, Naples, Italy

ARTICLE INFO

Keywords:
Giant reed
Biofuel
Non-structural carbohydrate
Cellulose
Hemicellulose
Lignin

ABSTRACT

The contribution of the rhizome to productivity of fermentable sugars and the detailed composition of rhizomes were analyzed in three mature stands of *Arundo donax* L. cultivated in three locations of variable fertility in the South of Italy. Although the average yearly aboveground dry biomass and rhizome amount showed large and significant differences among sites, (15.3 and 2.6 Mg ha^{-1} year^{-1} of rhizomes in the most and less productive sites respectively), rhizomes of all sites had more than 30% of the dry matter (DM) as non-structural carbohydrates (NSC). Sucrose and starch were the most abundant NSC but measurable amounts of glucose, fructose, galactose and of the valuable trisaccharide raffinose were also present. The amount of NSC in rhizomes affected their content of dry mater, and water extractives. The ash content also varied significantly among cultivation sites; the highest amount was recorded in rhizomes of the most productive site (Acerra). The abundance in cell wall components of rhizomes was similar to that of published values for the above ground biomass. The present results demonstrate that NSC content in rhizomes of mature stands is a conserved trait. Hence, rhizome biomass, thanks to its quantity and high fermentable sugars content, should be considered as a relevant fraction of the *A. donax* crop product whose utilization can increase the productivity and the environmental fingerprint of this crop, in view of its biomass utilization in biorefinery.

1. Introduction

The implementation of biorefinery plants is only possible with an adequate supply of feedstock that do not compete with food production systems. This has fostered research on species able to grow in area were food production is not a benchmark. *Arundo donax* L., is one of the species that has recently received attention as a potential biomass producer in low input non-food systems for the temperate and hot zones. *A. donax* is a perennial rhizomatous wetland grass, widespread all over the world; it shows a very high adaptability to abiotic and biotic stresses as drought, flood [1], salinity [2] and pests, maintaining an elevated potential for biomass yield [3]. *A. donax* growth is very fast during spring and summer in Mediterranean environments, resulting in the accumulation of large amounts of biomass even with minimal agricultural input such as irrigation, fertilization and phytosanitary treatments [4,5]. This plant could be suitable also for the cultivation of marginal areas, where the use of other species is disadvantageous. Trials carried out in Italy have shown that *A. donax* could be a very good feedstock for bioenergy production due to high yield potential [6,7]. Aboveground average dry biomass yield of 38 Mg ha^{-1} year^{-1} was recorded during growth from year 2–12 after planting [6], while Mantineo et al. [5] reported about the same production from the first three years of *A. donax* growth. A. donax was also able to grow in degraded or contaminated soils [8–10] or soils subjected to accelerated erosion [11]. This species was shown to have a favorable environmental impacts in marginal lands, due to carbon storage within the soil [12, 13; it was also suggested as being suitable in projects of phytoremediation of polluted soils [14].

For all of these advantages, giant reed is considered a very interesting non-food crop as source of biomass for the production of second-generation biofuel, bioenergy and other high value products for the chemical industry [6,15–18] with no competition for land with food crops. This has fostered research and improved the knowledge regarding yield and chemical characteristics of aboveground part of plant. However, most studies examining the potential of *A. donax* as a biomass producer for biorefinery have taken into account only the aboveground

Abbreviations: DM, Dry matter; NSC, Non-structural carbohydrates

* Corresponding author.
** Corresponding author.
E-mail addresses: fagnano@unina.it (M. Fagnano), alberto.battistelli@ibaf.cnr.it (A. Battistelli).

http://dx.doi.org/10.1016/j.biombioe.2017.10.003
Received 10 March 2017; Received in revised form 29 September 2017; Accepted 6 October 2017

biomass production [16]; with the exception of Nassi o Di Nasso et al. [19] who quantified also rhizome biomass accumulation.

Rhizomes are modified stems that grow perpendicularly to the gravitational force, normally underground, and can produce stems and root from nodes, function as storage sites of reduced carbon and mineral nutrients, and affect plant survival under stressful conditions [19–21]. *A. donax* can produce large quantities of rhizomes that represent a relevant portion of its total crop biomass. Nassi o Di Nasso et al. [19] showed a rhizome dry biomass yield of 16 Mg $ha^{-1}year^{-1}$, for a three years old crop. Giant reed translocates nutrients to the rhizome at the end of the growing season [22], and remobilize them for regrowth of the new shoot at the beginning of the vegetation season, reducing fertilizers requirements. A number of studies have been carried out to estimate the seasonal dynamics of above and belowground resources allocation in rhizomatous perennial grasses such as *Miscanthus*, *Panicum* and *Arundo* [23,24], but the focus of existing studies was mineral and nitrogen partitioning. The present study is of particular importance as the carbohydrate content and partitioning in addition to the cell wall chemical characteristics of *A. donax* rhizome, similar to other rhizomatous grasses, are lacking. *A. donax* plantations can produce biomass for as long as 10–15 years, thereafter the yield declines dramatically and the crop stand must be removed. Upon removal of a giant reed plantation, or eventually with partial harvests during the plantation period, the rhizome could be used as a feedstock for bioenergy production or as a raw material for green chemistry applications, e.g. for the production of molecules of interest such as xylobiose and tetra- and penta-saccharides [25].

The aims of this work were: i) to evaluate the contribution of rhizome to total biomass yield by stands cultivated in sites of the Mediterranean area of different fertility; ii) to evaluate the quality of such rhizome biomass in terms of NSC (fermentable carbohydrates) and structural components. Our work highlights the potential role of rhizome biomass in increasing productivity of fermentable sugars; hence affecting the profitability and environmental fingerprint of *A. donax* cultivation for green chemistry and helping to improve the sustainability of biomass based agro-industrial systems in the Mediterranean area.

2. Materials and methods

2.1. Biomass sampling

Giant reed rhizomes were collected from 3 experimental fields located in marginal land areas of the Campania Region, Southern Italy: Acerra, (40° 59′N, 14° 20′E, 26 m a.s.l.), from a 6 year stand (2009–2014) in polluted soils of a Vesuvius plain; Sant'Angelo dei Lombardi (40° 55′ N, 15° 07′ E, 700 m a.s.l.) from an 11 year stand (2004–2014) in a hilly area of Southern Apennine subjected to accelerate erosion; and Bellizzi (Torre Lama) (40° 37′N, 14° 58′E, 30 m a.s.l), from a 7 year stand (2008–2014) in a coastal plain subjected to soil salinization. Details of cropping systems are reported in Fiorentino et al. [9], Fagnano et al. [11] and Impagliazzo et al. [26], respectively. In all sites the same low input cropping system was adopted with low doses on N fertilizers and no irrigation. Physico-chemical characteristics of the soils of the three locations are reported in Table 1.

Table 1
Soil characteristics in the tree cultivation sites. N-tot, total nitrogen; SOM, soil organic matter; N-NO_3, nitric nitrogen; N-NH_4, ammonia nitrogen.

	Sand %	Silt %	Clay %	N-tot %	SOM %	$\frac{N-NO3}{N-NH4}$ratio	pH
Acerra	55.7	27.5	16.7	0.16	2.64	1.25	7.7
Bellizzi	34.2	26.1	39.6	0.09	1.57	0.55	7.8
S. Angelo dei Lombardi	36.9	24.6	38.0	0.08	1.03	0.40	8.2

Aerial biomass was harvested every year at the end of winter and dried at 70 °C to constant weight. Underground biomass samples were collected during winter, from sample areas of 1 m^2, washed, weighted and dried at 70 °C until to constant weight. Two subsamples of 1 kg were collected from each plot (3 replicates x 3 sites), immediately frozen with dry ice, and sent to the laboratory for the chemical characterization.

2.2. Analysis of NSC

Soluble carbohydrates (glucose, fructose, sucrose, raffinose) and starch in the biomass were extracted for analysis as in Moscatello et al. [27]. In brief, 10 mg of dry material was extracted in 1 ml of 50% ethanol/water at 80 °C for 45 min. Soluble sugars were recovered in the supernatant after centrifugation at 16000*g* for 5 min. Starch in the pellet was hydrolyzed enzymatically to glucose after washing and autoclaving. The supernatant containing soluble carbohydrates, and the glucose solution obtained after starch hydrolysis were filtered on 0.2 μm nylon filters (GE-Whatman, Maidstone, UK), then analyzed by high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (Thermo Scientific™ Dionex™ ICS-5000, Sunnyvale, CA U.S.A.) consisting of a dual pump system (quaternary analytical pump plus isocratic pump), a pulsed amperometric detector, and an analytical CarboPac PA20 column (3 mm × 150 mm) with guard column. The detection cell contained a gold working electrode (1.0 mm in diameter) and an Ag/AgCl reference electrode. Pulsed amperometric detection was carried out with the following waveform: E1 = +0.10 V (t1 = 0.4 s), E2 = −2.00 V (t1 = 0.01 s), E3 = +0.60 V (t = 0.01 s), E4 = −0.10 V (t = 0.06 s). The integration range began at 0.2 s and ended at 0.4 s. The electrical signal was integrated in nanocoulomb (nC). Runs were carried out at 30 °C using an injection valve with a 5 μl injection loop. Mobile phase was NaOH at a flow rate 0.5 ml min^{-1}. Sugars were eluted under the following conditions: 0–12 min, 10 mM; 12–25 min, 80 mM; 25–39 min, 240 mM; 39–55 min, 10 mM. Regeneration of amperometric electrode was done with a post-column addition of concentrated sodium hydroxide (300 mM) using a secondary pump at a flow rate of 0.25 ml min^{-1}. The carbohydrate standard solutions were prepared using HPLC grade reagents (Sigma, Steinheim, Germany).

2.3. Analytical methods for the determination of chemical composition of rhizome

Chemical analyses were performed in accordance to the standard biomass analytical methods provided by the National Renewable Energy Laboratory (NREL) as detailed in Santi et al. [28]. The collected samples were milled with a MF10 IKA mill (Werke GmbH & Co. KG) and sieved to screen particles until 500 μm in size. After milling, the samples were oven dried for 24 h at 45 °C and then used for the determination of the biomass chemical composition. In brief, the relative moisture was determined by placing the samples at 105 °C for 24 h in a ventilated oven. The ash content was quantified after ignition of dried samples at 575 ± 25 °C for 24 h using a muffle furnace equipped with a ramping program (Nabertherm P300 Germany).

A two-step extraction was used to quantify extractives in the rhizome biomass samples. Approximately 1.5 g of dried sample was placed in cellulose extraction thimbles, 100 mm length by 25 mm diameter (GE-Whatman, Maidstone, UK). The first step was performed with water, by extraction in 25 ml of deionized water at 50 °C for 30 min. This step was repeated 4 times to a final extraction volume of 100 ml for each sample. The second step was performed, using 100% ethanol on the water insoluble material, following the same procedure described for the first step. Water and ethanol extracts were quantified gravimetrically; the water soluble components were freeze-dried, while the ethanol extractable material was dried using a rotavapor (VV 2000 Heidolph Instruments, Schwabach, Germany).