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Lipid production from *Arundo donax* grown under different agronomical conditions

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ABSTRACT

Hydrolysates of Giant reed (*Arundo donax*) biomass from three different agronomical conditions were used to grow the oleaginous yeast *L. Starkey*. The agronomical conditions affected the cellulose fraction of biomass, the amount of inhibitors generated during the acid hydrolysis, and the triglyceride yield after the yeast fermentation. Yet, the composition of triglycerides was not affected.

Different approaches were developed to reduce the effect of inhibitors. The preliminary dilution of hydrolysates was studied, obtaining the highest values of biomass and lipid yields with a 50% dilution. Alternatively, the hydrolysates were pre-treated by adsorption and overliming. The latter pre-treatment gave the best results. A third approach was offered by the use of pre-adapted yeasts, that were able to grow in the presence of raw hydrolysates.

The composition of the microbial triglycerides was compatible with the production of a biodiesel suitable for use as automotive fuel.

1. Introduction

Biodiesel is a renewable, biodegradable, non-toxic fuel, potentially alternative to the petroleum-based diesel. Though a growing interest has been devoted to biodiesel in the last years, there are some factors still limiting its market penetration. As a matter of facts, the feedstock materials used for the so-called 1st generation biodiesel (i.e. vegetable oils and animal fats) cannot satisfy the demand for biodiesel at the current rate of consumption. In addition, the relatively high cost of these materials, accounting for about 75% of the biodiesel price, makes biodiesel more expensive than the mineral diesel [1]. The highest yields of seeds (3–5 t ha^{-1}) and oils (1.5–2.5 t ha^{-1}) are achieved growing the more productive crops (i.e. sunflower) in plainy, deep and fertile soils, mostly in Turkey [2], France [3] and Italy [4], under conditions of high input (high fertilization rates and full irrigation). This implies relevant socio-economic and ethical questions: gross revenue per hectare of high input sunflower (2–3000 dollars ha^{-1}) is not competitive with the alternative crops for high fertility environments (15–20,000 dollars ha^{-1} for vegetables and fruits) and therefore a biofuel crop expansion in these lands is not reasonably predictable. In more marginal lands, the sunflower productivity, such as that of other oil crops, is too low to make profitable their cultivation: seed and oil yields fall to 1–2 t ha^{-1} and 0.5–1.0 t ha^{-1} respectively, and gross revenue unlikely overcome 1000 dollars ha^{-1}.Furthermore, considering the actual increase of world population and the consequent food demand, is it sustainable and ethically acceptable to threaten food security by using croplands for biofuel crops in place of food crops? The use of edible oils to produce biodiesel is threatening food supplies and biodiversity, causing social and environmental problems in different developing countries.

In order to overcome these problems, a main requirement is the availability of cheaper sources of triglycerides, requiring only a limited use of fertile soil [1]. Lignocellulosic biomasses offer a viable option to obtain renewable energy, reducing the requirement of fertile soils. An efficient technology for processing lignocellulosic

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biomasses could open new perspectives as regards the biofuel production, for different reasons:

- A large range of waste biomasses can be recycled, such as non-food parts of crops (stems, leaves and husks), forest products, and also industry wastes (woodchips, skin and pulp from fruit pressing, etc.);
- Suitable non-food crops (switchgrass, jatropha, miscantus, etc.) can be cultivated in partially-fertile soils, not used for agriculture, to obtain both vegetable oil (to produce biodiesel according to the traditional method) and lignocellulosic biomasses for biofuel production;
- Since cellulose and hemicelluloses are the main component of plants, the yield of feedstock biomasses per unit area is significantly increased until 40 t d.m. ha^{-1} [5].

So far, much attention has been directed towards the exploitation of lignocellulosic biomasses for the production of bioethanol. In the recent years, a different approach has emerged based on the use of the lignocellulose hydrolysates to grow oleaginous microorganisms [6–11]. Oleaginous microorganisms are able to produce more than 20% of their weight in the form of lipids, prevalently made of triacylglycerols. In particular, oleaginous yeasts are attracting increasing interest due to their simpler cultural requirements. As a matter of facts, they only require nitrogen limiting conditions and the presence of a carbon source in excess to accumulate lipids [12–14]. The basic physiology of lipid accumulation in the oleaginous yeasts has been well studied [15].

An important advantage offered by the application of the oleaginous microorganisms stems from their ability to produce aerobically lipids from residual organic matters, with no addition of expensive nutrients. So far, in order to optimise the cost of the process, as well as to increase its environmental benefit, different residual materials have been tested as possible nutrients for the oleaginous yeasts, such as sewage sludge [16], primary effluent wastewaters [17], sugar cane bagasse [9], tomato waste [18], glycerol [19], olive-mill wastewater [20] or other sources [6,13,14]. Recently, lignocellulosic residues, such as agriculture stover, forest residues and energy plants, have been increasingly considered as starting materials for biodiesel production [7–10,21,22].

In this study, we demonstrate that the oleaginous yeast *Lipomyces starkeyi* can be grown in the presence of lignocellulose hydrolysates obtained from different samples of *Arundo donax* (Giant reed), a widely distributed perennial grass. Currently, the culms of *A. donax* represent an interesting source of cellulose for producing paper [23], second generation ethanol or biopolymers [24]. Furthermore, the residual lignin content (15–20%) can be interesting for compost production or other high value materials such as lignin-based resin coatings and composites [23]. The *A. donax* is considered a promising crop for industrial fuel production, due to the high biomass productivity, the ability to be intensively cultivated and the adaptability to different climatic and soil conditions. *A. donax* is known to produce high lignocellulosic biomass yields in marginal lands such as polluted soils [25] and salinized soils [26]. Perennial crops, such as *A. donax*, may guarantee an efficient protection of hilly soils subjected to accelerated erosion [27,28]. This is of particular importance, as the climate changes are increasing the rain erosivity in the soil tillage period of annual crops, causing catastrophic soil losses until to 250 t ha^{-1} [29].

The microbial lipids from *L. starkeyi* can be used as an alternative feedstock for the synthesis of biodiesel. Specific attention has been devoted to the cellular growth inhibitors generated during the acid pre-treatment production, still hindering a full industrial application of the process [10,21,30]. *L. starkeyi* are able to degrade carbohydrates in soil and ensilage using extracellular carbohydrolases, and contribute to the biodegradation of herbicides [31]. They have already been used to produce lipids [16,20,22], as they may offer significant amounts of microbial oils with little reutilisation of the stored lipids [32].

The proposed approach may allow a more efficient exploitation of the land area. As a matter of fact, from cultivation of oleaginous plants commonly used for 1st generation biodiesel (e.g. sunflower) a lipid yield of not more than 1–2 t ha^{-1} is obtained [2–4]. On the contrary, the yield of lignocellulosic biomasses obtained from *A. donax* is usually 20–40 t ha^{-1} [5]. Assuming a biomass-to-sugars yield of 55% [33] and sugar-to-lipid yield of 14% [9], a total lipid yield of 1.5–3.1 t ha^{-1} can be estimated. In addition, the cultivation of *A. donax* can be adapted to marginal lands [25,26], offering good yields also with low input cropping systems [5].

2. Materials and methods

2.1. Microorganisms and culture medium

The oleaginous yeasts *L. starkeyi* were obtained from the collection of the Dipartimento di Biologia Vegetale of the Perugia University (Italy). The microorganisms were kept on potato dextrose agar (Sigma) at T = 5 °C and cultivated in a synthetic N-limiting medium, containing (g/L): KH_2PO_4, 1.0; $MgSO_4$ $7H_2O$, 0.5; $(NH_4)_2SO_4$, 2.0; yeast extract, 0.5; glucose, 70.0. The growth was carried out under aerobic conditions at 30 °C on a rotary shaker at 160 rpm (Minitron, Infors HT, Switzerland).

2.2. Lignocellulosic biomasses

A. donax was grown in open field condition in plainy and hilly areas of Southern Italy. The filed site were:

(a) Torre Lama experimental station, University of Napoli, (40°37′N, 14°58′E, 30 ma.s.l.), here identified as TL.
(b) Centro Rotary experimental station, University of Napoli, (40°92′N, 15°13′E, 717 ma.s.l.) here identified as CR.

Soil texture was similar (Silty-Clay and poor in organic matter) in the two sites, with higher carbonate content in CR (Table 1). The meteorological conditions of the two sites were significantly different, with more severe water deficit in plainy site (Fig. 1). In TL, reference evapotranspiration was 2.9 mm/day on the average, total rainfalls were 810 mm/year, and water deficit was measured from March to September (−617 mm). In CR, reference evapotranspiration was 2.3 mm/day on the average and total rainfalls were 1672 mm/year, and water deficit was measured only from May to August (−137 mm).

Table 1
Soil physico-chemical features of the two sites.

	Torre Lama (TL)		Centro rotary (CR)	
Soil layer (cm)	0–30	30–60	0–30	30–60
Sand (%)	46.5	41.5	37.0	36.8
Silt (%)	22.3	28.5	24.3	25.0
Clay (%)	31.7	30.0	38.2	37.7
Texture class	SiCL	SiCL	SiCL	SiCL
pH	7.4	7.5	8.1	8.3
Total carbonate (%)	0.0	0.0	16.0	17.4
Organic matter (%)	1.24	1.11	1.09	0.95
Nitrogen (g/kg)	0.92	0.62	0.93	0.81
C/N ratio	7.87	10.4	6.77	6.87
K_2O (mg/kg)	430	432	350	362
P_2O_5 (mg/kg)	32	25	19	13

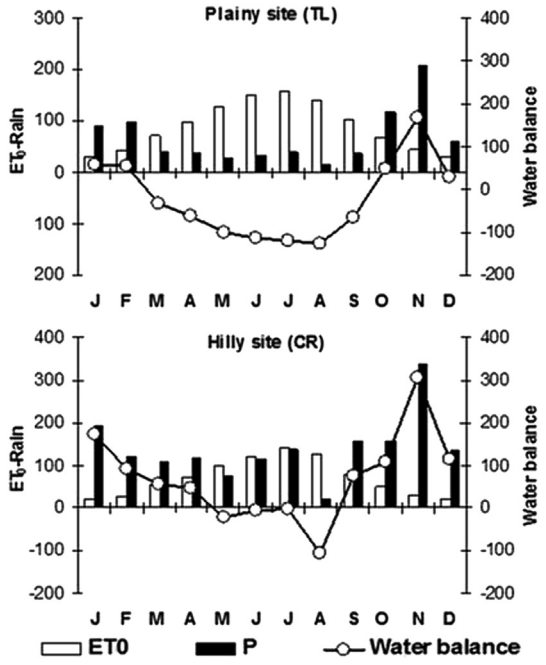

Fig. 1. Monthly values of precipitations (P), evapotranspiration (ET0) and water balance (mm per month) in the cultivation sites.

In TL samplings were made at the harvest (December 2010) of the 3rd year of crop cycle (planting was made in March 2008) and two water regimes were compared: rainfed *vs*. full irrigation (100% restitution of evapotranspiration).

In CR samplings were made at the harvest (January 2011) of the 8th year of crop cycle (planting was made in March 2004). In order to obtain realistic results, *A. donax* plants were grown in the hilly site only under rainfed conditions (no water resources are available for irrigation in hilly and mountain croplands of Southern Italy).

In both the sites, transplanting was made from rhizomes with a plant density of 1 × 1 m, and nitrogen fertilization was made at the end of rainy period (April) with a low dose (100 kg N ha−1 from urea).

2.3. Hydrolysis of lignocellulosic biomasses

Oven-dried stems of *A. donax* (3 g) were suspended in a 5% (v/v) solution of H_2SO_4 (30 ml). Preliminary experiments showed this to be the optimal acid concentration to maximize the fermentable sugars yield. When adopting higher concentrations of H_2SO_4, a lower yield was obtained, as sugar molecules underwent further degradation reactions. The biomass suspended in acid was autoclaved at 121 °C for 20 min [18], neutralized to pH 6.5 by addition of saturated KOH solution and filtered with filter paper. When necessary, the hydrolysate suspension was lyophilized, and subsequently redissolved in fermentation media to obtain the required concentration of sugars.

2.4. Fermentation in hydrolysates of lignocellulosic biomasses

A fixed volume (150 mL) of *Arundo donax* hydrolysate (ADH) was inserted, without external organic supplement, in conical flask of 500 mL, and inoculated by 2 mL of microorganism suspension, obtained dissolving 5 loops of solid culture in 8 mL of physiological solution. The flasks were incubated in an rotary shaker at an agitation rate of 160 ± 5 rpm and T = 30 °C.

2.5. Lipid extraction and measurement

Methanol (5.0 mL) and chloroform (2.5 mL) were added to 200 mg of dry biomass and vortexed 5 s. Subsequently, the cells were disrupted for 12 min in an Ultrasonic Homogenizer (Omni Ruptor 250, USA) at 50% power and 90% pulser. The cells were then filtered off with Whatman no.1 filter paper. The solvent-lipid mixture was then placed in 50 mL centrifuge tubes and centrifuged for 10 min at 2000 rpm (Rotanta 460R, Hettich, USA) at 20 °C. The lower layer was then transferred to a pear-shape flask with Pasteur pipette. Again, 10 mL of 10% (v/v) methanol in chloroform were added to the residue, a new centrifugation was carried out, and the lower phase was added to that from the first extraction. The solvent in the pear-shape flask was evaporated to dryness (BÜCHI Rotavapor R-200, Switzerland) and the extracted weight was finally recorded after drying at 105 °C for 1 h.

2.6. Biomass analysis

The biomass concentration could not be measured by optical density determination, due to the darkness of the medium. In order to evaluate the dry biomass weight, the biomass was centrifuged at 6000 rpm for 10 min and dried at 105 °C until constant weight.

2.7. Chemical analyses

Cellulose, hemicellulose and lignin were measured according to the method of Ververis et al. [23].

The fatty acids composition was determined by GC analysis on a Shimadzu GC 17/3 gas-chromatograph equipped with a flame ionisation detector, following the method adopted by Li and co-workers [12].

The TOC measurements were carried out with a TOC-$V_{CSH/CSN}$ (Shimadzu, Japan), upon suitable dilution of a culture medium sample. The TOC values were obtained subtracting the IC (inorganic carbon) value from the TC (total carbon) value.

The concentration of reducing sugars was measured following the method of Nelson-Somogyi [34].

Potential inhibitor compounds were analysed by HPLC (LC2010c), equipped with a refractive index detector (RID-10A, Shimadzu, Japan), following the method adopted by Chen and co-workers [21].

2.8. Detoxification of ADH

The biomass growth inhibitors generated in the course of the hydrolysis were partially removed by overliming, increasing the pH of the hydrolysate to 10.0 by addition of $Ca(OH)_2$. After 1 h, the hydrolysate was filtrated under vacuum, acidified to pH 5,5 with 5 M H_2SO_4 and filtrated again after 1 h for precipitate removal.

Alternatively, the inhibitors were removed by adsorption. The hydrolysate was first neutralized with NaOH to pH 6.5, and then treated with activated charcoal at a weight ratio of 0.05. The hydrolysates were then incubated overnight at 30 °C and 160 rpm, then filtrated to remove the adsorbent. Finally, pH was adjusted to 6,5 with $Ca(OH)_2$ or 5 M H_2SO_4.

2.9. Statistical analysis

All experiments have been carried out adopting a minimum of three replicate tests.

Table 2
A. donax: conditions of treatments and cultivation parameters.

Treatment	Site	Water	Temperature	Biomass yield			Basal Ø	Height	Culms	Culms
				t ha^{-1}f.w.	t ha^{-1}d.w.	% d.m.	Mm	m	n. m^{-2}	%
TL100w	Torre Lama	Irrigated	High	48.7	26.4	54.2	19.5	3.8 a	7.5 b	85.4
TL0w	Torre Lama	Rainfed	High	50.8	24.0	47.2	19.6	3.7 a	10.8 b	88.9
CR	Centro Rotary	Rainfed	Low	42.7	20.9	48.2	21.3	2.8 b	24.5 a	92.6
			Average	47.4	23.8	49.9	20.2	3.4	14.3	89.0
			Significance	n.s.	n.s.	n.s.	n.s.	0,05	0,01	n.s.

Note: different letters indicate differences significant per $P \leq 0.05$.

3. Results and discussion

3.1. Biomass yield of A. donax

The cultivation trials of *A. donax* were carried out in two different sites (Table 1), following three different treatments, as shown in Table 2. The results in Table 2 also indicate that the biomass yield did not change significantly, confirming the high adaptability of this species to drought (TL0) and to low temperatures (CR). In the less favourable conditions (CR), the reduction of plant height was compensated by the increased number of culms per m^2. The *A. donax* did not show significant differences between irrigated (TL100w) and rainfed conditions (CR and TL0w), because the water deficit in the hilly site (CR) during the summer was very limited, while in the plainy site (TL) the deep root system and the high water retrieval capacity [35] allowed the water uptake also from the water table, that in that zone is time-invariant and about 1.0–1.5 m deep. The composition of the biomass samples obtained from each treatment is reported in Table 3 (columns a–c), in terms of cellulose, lignocelluloses and lignin content.

3.2. Growth of L. Starkeyi in the presence of A. donax hydrolysates

All the samples of *A. donax* were hydrolysed following a conventional procedure (see Materials and methods). The concentrations of reducing sugars in the *A. donax* hydrolysates (ADH) were 83,7 g/L, 81,2 g/L and 80,8 g/l respectively for the samples TL100w, TL0w, CR.

When using raw ADH without any external organic supplement to culture *L. starkeyi*, no appreciable cellular growth was observed, and correspondingly no significant reduction of TOC and sugars was detected. This result was clearly due to the higher concentration of inhibitors.

The yeasts were cultured in water mixtures of ADH, containing ADH fractions of 25%, 50% and 75% respectively. The growth curves obtained with hydrolysates of the sample TL100w, reported in Fig. 2a, show that a longer lag phase was recorded as more concentrated ADH are used. The maximum biomass concentration was reached in the presence of 50% ADH. A poor growth was observed in the presence of 75% ADH, showing that at this concentration the effect of the inhibitors is still significant. The concentration of reducing sugars decreased until the complete exhaustion in the tests carried out with 25% and 50% ADH (Fig. 2b), whereas the sugar conversion in the presence of 75% ADH was incomplete. The TOC levels, reported in Fig. 2c, were in all cases reduced until a final asymptotic level was reached, that was higher in the presence of the more concentrated medium. This result indicates that a fraction of the organic components of the hydrolysate could not be metabolized by the yeasts.

Qualitatively similar profiles of biomass, reducing sugars and TOC were obtained growing hydrolysates from samples TL0w and CR. (data not shown). The final values of biomass and lipid concentration of *L. Starkeyi* obtained using different hydrolysates (after a 50% dilution) are compared in Table 3 (columns d–f).

The highest values of both parameters were obtained using the hydrolysate from the sample TL100w. A possible explanation of this result stems from the higher cellulose content of this sample (column a in Table 3). However, it is known that both the cellular growth and the lipid synthesis may be inhibited by some

Table 3
Effect of different cultivation treatments on the composition of the lignocellulosic biomass and on the composition of *L. starkeyi* grown in the presence of the hydrolysate ADH 50%.

Treatment	Composition of lignocellulosic biomass			Composition of *Lipomyces starkeyi*		
	(a) Cellulose, %	(b) Hemicell., %	(c) Lignin, %	(d) Biomass conc., g/L	(e) Lipid fraction, %	(f) Lipid conc., g/L
TL100w	46.2	23.2	16.2	9.99	19.7	197
TL0w	43.8	23.5	18.9	7.81	19.5	152
CR	42.6	22.8	18.7	7.50	16.7	125

ADH 50% = water-hydrolysate mixture containing an hydrolysate fraction of 50%.

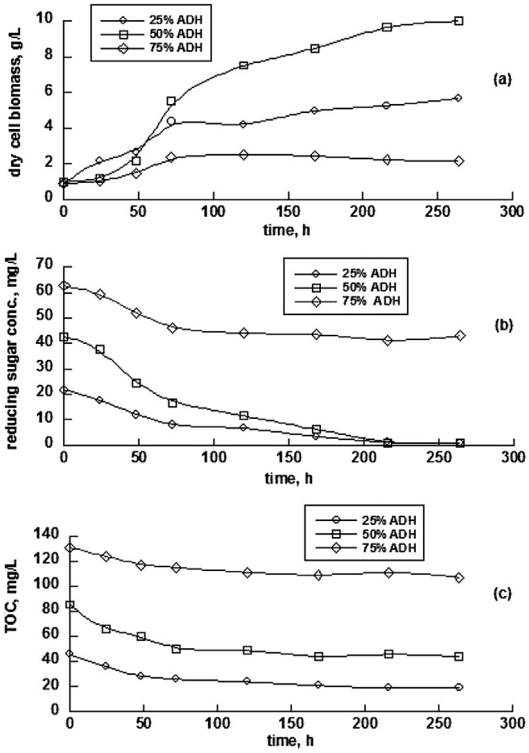

Fig. 2. Time-profiles of different variables in the course of the *L. Starkeyi* growth, in the presence of the hydrolysates of the *A. donax* sample TL100w. (a) Dry biomass, (b) Reducing sugars, (c) Total Organic Carbon (TOC).

Table 4
Concentration (g/L) of potential inhibitors in raw hydrolysates of *A. donax*.

Compound	Treatment		
	TL100w	TL0w	CR
Acetic acid	6.22	7.22	7.10
Levulinic acid	1.44	1.21	1.23
Formic acid	1.67	1.80	1.88
Furfural	0.1	0.38	0.35
5-HMF	0.73	1.06	1.13
Vanillin	0.035	0.041	0.055
Hydroxybenzaldeyde	0.056	0.101	0.088

5-HMF = 5-hydroxymethylfurfural.

degradation products produced in the course of the hydrolysis [19,20]. These compounds are generated from the degradation of sugars (e.g. furfural from xylose and 5-hydroxymethylfurfural), lignin (e.g. vanillin, syringaldehyde, and 4-hydroxybenzaldehyde) and other components of the lignocelluloses (e.g. acetic acid from acetyl groups, formic acid from xylose oxidation, and levulinic acid from glucose oxidation) [36]. The concentrations of some potential inhibitors in hydrolysates of different samples of *A. donax* are reported in Table 4. The data show that all these inhibitors, with the exception of the levulinic acid, are less concentrated in the hydrolysate of the sample TL100w. Consequently, the higher levels of biomass and lipid concentration obtained using the sample TL100w can be explained also taking into account the reduced concentration of inhibitors.

3.3. Pre-treatments to remove inhibitors

In order to remove the degradation products generated by the hydrolysis treatment, the ADH were preliminary treated following 3 different protocols, namely: (a) overliming treatment with concentrated $Ca(OH)_2$, (b) neutralization with NaOH, followed by adsorption on active carbons, (c) overliming followed by neutralization and adsorption.

The biomass concentration–time profiles shown in Fig. 3 were obtained growing *L. starkeyi* in the presence of hydrolysates subjected to these protocols. The experimental data demonstrate that both the adsorption and the overliming allow the biomass growth in the presence of undiluted hydrolysate. However, the adsorption with active carbon yielded best results in comparison to the overliming. Coupling both methods did not improve significantly the results obtained with active carbons. The profiles obtained with samples TL0w and CR in the same tests were qualitatively similar.

The final biomass concentrations and triglyceride yields measured after the growth in the presence of the pre-treated hydrolysates are reported in Table 5(a). These results indicate that the lipid fractions in the yeasts are substantially similar to those

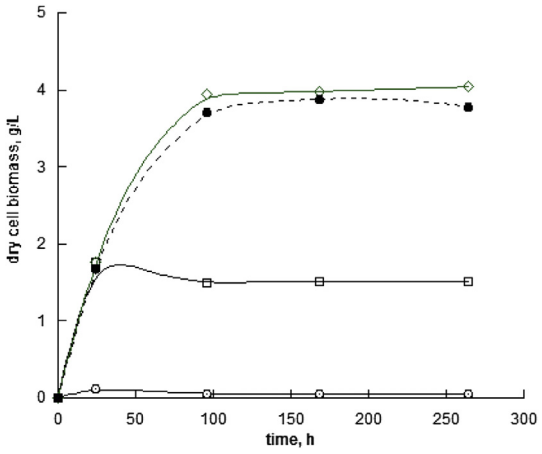

Fig. 3. Growth curves of the *L. Starkeyi* growth in the presence of hydrolysates from the treatment TL100w. Preliminary treatments: no treatment (○), overliming (□), active carbon (◊), overliming + active carbon (●).

observed in the previous tests (see Table 3), and, whatever the pre-treatment protocol, are significantly affected only by the sample of *A. donax* used. Even in this case, the hydrolysates obtained from the sample TLw100 offered better performances both in terms of biomass concentration and lipid fraction.

3.4. Yeast adaptation to inhibitors

An alternative method to overcome the effect of inhibitors is offered by the adaptation of the *L. Starkey* in the presence of hydrolysates at progressively higher concentration. To this scope, three consecutive 10-day growth cycles were carried out. After the first cycle, carried out in the presence of 50% ADH, the biomass was collected and partly used to as inoculum for the second cycle, carried out with the 75% ADH. Similarly, the biomass obtained after the second cycle was used to inoculate a 100% ADH medium. The experimental results shown in Fig. 4, obtained with sample TLw100, indicate that the pre-adapted *L. Starkey* were able to grow in the presence of 100% ADH (i.e. undiluted raw hydrolysates). Similar results were obtained with samples TL0w and CR.

The final values of biomass concentration and triglyceride yield, obtained with adapted yeasts in the presence of hydrolysates of different samples of A. donax, are reported in Table 5(b). The lipid fractions in the yeasts showed a slight increase as subsequent cycles were carried out, though they were mainly affected by the sample of *A. donax* used. Again, the best values of biomass concentration and lipid fraction were obtained in the presence of hydrolysates of the sample TLw100.

Table 5
Biomass and lipid concentrations of *L. starkeyi*.

	Biomass conc. g/L			Lipid fraction, %			Lipid conc. g/L		
(a) Effect of preliminary treatments of the raw hydrolysate (ADH 100%)AC = adsorption with active carbon. OL = overliming.									
Treatment	**AC**	**OL**	**AC + OL**	**AC**	**OL**	**AC + OL**	**AC**	**OL**	**AC + OL**
TL100w	4.04	1.51	3.78	19.9	19.7	19.8	80.4	29.7	74.8
TL0w	3.56	1.45	3.60	19.6	19.8	19.5	69.8	28.7	70.2
CR	3.27	1.56	3.18	16.8	16.6	16.6	54.9	25.9	52.8
(b) Effect of subsequent adaptation cyclesof *L. starkeyi*in the presence of ADH 50% (cycle I), ADH 75% (cycle II), and ADH 100% (cycle III).									
Cycle	**I**	**II**	**III**	**I**	**II**	**III**	**I**	**II**	**III**
TL100w	9.31	6.21	5.34	19.7	19.9	19.9	183	124	106
TL0w	9.11	5.94	5.20	19.5	19.8	19.9	178	118	103
CR	8.99	5.97	5.03	16.7	16.8	17.1	150	100	86.0

ADH 50%, ADH 75% = water-hydrolysate mixtures containing hydrolysate fractions of 50% and 75% respectively.
ADH 100% = raw hydrolysate.

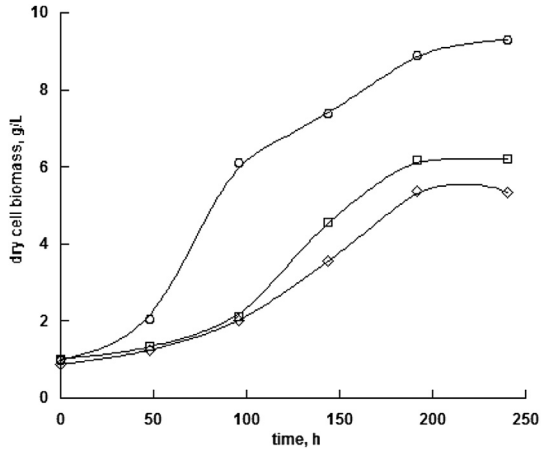

Fig. 4. Growth curves of the *L. Starkeyi* during subsequent adaptation cycles: cycle I, in the presence of ADH 50% (○); cycle II, in the presence of ADH 75% (□); cycle III, in the presence of ADH 100% (◊). ADH 50%, ADH 75% = water-hydrolysate mixtures containing hydrolysate fractions of 50% and 75% respectively. ADH 100% = raw hydrolysate.

3.5. Triglyceride composition

The fatty acids distribution in the lipids extracted from the yeasts was measured, using samples of *L. Starkey* cultured in the presence of different hydrolysates (Table 6).

In a microbial oil, higher concentrations of unsaturated fatty acids improve cold properties (as they reduce the cloud point, the pour point, and the cold filter plugging point). Nevertheless, unsaturated fatty acids reduce the oxidation stability [37]. It has been suggested that oils with high content of monounsaturated fatty acids and low in unsaturated and polysaturated fatty acids could accomplish both these antagonistic requirements [38,39]. However, a microbial oil containing fractions of saturated and monounsaturated fatty acids that are both close to 50% is considered a reasonable compromise between oxidative stability and cold flow properties [40,41]. In this view, the composition of the microbial oils obtained within this study, containing an oleic acid fraction in the range 45–50%, and a saturated acids fraction above 40% (as reported in Table 6), is compatible with the production of a biodiesel offering excellent performances as automotive fuel.

The fatty acid distribution (Table 6) was not significantly affected by the agronomical conditions of the *A. donax*. On the contrary, the pre-treatment of the hydrolysate, as well as the use of pre-adapted yeasts, leaded to slight increases in the oleic acid concentration.

Table 6
Distribution (%) of fatty acids in the triglycerides obtained under different experimental conditions.

Hydrolysed sample	ADH 50% (TL100w)	ADH 50% (TL0w)	ADH 50% (CR)	ADH 50% (TL100w) after active carbon ads.	ADH 100% (TL100w) with adapted *L. starkeyi*
Myristic acid C14:0	2	1.8	1.9	1.7	1.9
Palmitic acid C16:0	23.7	24.1	23.8	22.3	22.7
Palmitoleic acid C16:1	<1	<1	<1	<1	<1
Stearic acid C18:0	16.3	16.2	16.3	15.8	16.0
Oleic acid C18:1	46.5	45.8	46	49.5	48.7
Linoleic acid C18:2	6.5	6.7	6.6	6.3	6.4
Linolenic acid C18:3	1.6	1.8	1.8	1.5	1.5
Arachidonic acid C20:4	<1	<1	<1	<1	<1

ADH 50% = water-hydrolysate mixtures containing an hydrolysate fractions of 50%. ADH 100% = raw hydrolysate.

4. Conclusions

This study demonstrated that it is possible to produce triglycerides from *Arundo donax* cultivated in marginal lands, to reduce the competition with food crops for fertile lands, and to offer a sustainable way to produce renewable energy (biodiesel) or building blocks for biopolymers.

The conditions of the agronomical treatments affected the composition of the lignocellulosic biomass, and eventually the lipid and biomass yields obtained by yeast grown in the hydrolysates.

Preliminary dilution or pre-treatment of hydrolysates, as well as yeasts pre-adaptation by progressive increases of the hydrolysate concentration, were effective in reducing the effect of the microbial growth inhibitors.

The composition of the microbial triglycerides was suitable to obtain a biodiesel with reduced tendency to oxidation and good cold performance.

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