Recycling Industrial Food Wastes for Lipids Production by Oleaginous Yeasts *Rhodospiridiobolus Azoricus* and *Cutaneotrichosporon Oleaginosum*.

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Research

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Abstract

Background

Microbial lipids have been emerging as a sustainable alternative to vegetable oils and animal fat to produce biodiesel and industrial relevant chemicals. The use of wastes for microbial processes can represent a way for upgrading low value feedstock to high value products, addressing one of the main goals of circular economy, the reduction of wastes by recycling. Two oleaginous yeasts, *Rhodospiridiobolus azoricus* and *Cutaneotrichosporon oleaginosum*, were used in this study to demonstrate the feasibility of the proposed approach.

Results

In this study wastes from industrial food processing, as pumpkin peels and syrup from candied fruits manufacture, were used for yeast cultivation and for lipids production. Evaluation of growth and sugar consumption revealed marked differences between the yeasts in capacity to utilize sucrose and glucose, the main sugars present in the feedstock. In particular, we observed an unexpected limitation in glucose metabolism on mineral media by *R. azoricus*. Both species showed ability to grow and accumulate lipids on media exclusively composed by undiluted pumpkin peels hydrolysate, and *R. azoricus* was the best performing. By a two-stage process carried out in bioreactor, this species reached a biomass concentration of 45 g/L (dry weight) containing 55% of lipids, corresponding to a lipid concentration of 24 g/L, with a productivity of 0.26 g/L/h and yield of 0.29 g lipids per g of utilized sugar. These values are close to the highest reported so far from organic wastes.

Conclusions

Wastes from industrial food processing were sufficient to completely support yeast growth and to induce lipid accumulation. This study provides strong evidence that the concept of valorisation through the production of lipids from the complete metabolism of nutrients present in agro-industrial wastes by oleaginous yeasts is promising for implementation of biotechnological processes in a circular economy contest.

Background

Nowadays, the market is facing an increasing demand of triacylglycerols (TAGs) due to their application in a variety of industrial sectors. Depending on their composition, TAGs can be used not only for production of biodiesel, but also of other high value-added products such as, to cite some, biosurfactants, detergents, solvents, lubricants, adhesives, cosmetics and degradable polymers (Metzger and Borscheuer, 2006; Foley et al. 2012; Koutinas et al. 2014; Liu et al. 2021; Zhang et al., 2021).

TAGs can be obtained by vegetable oils and animal fat, but their supply cannot be sustained only by these sources, due to the large land surfaces required for cultivation and the competition to food production (the so-called fuel vs food conflict). For these reasons, more sustainable alternatives are continuously sought, and, in this context, microbial lipids (or single cell oils, SCOs) are considered as promising ones. They show in fact a very similar composition to vegetable oils but, in contrast, can be produced independently from geographic location, season changes, climate and by faster and controllable processes (Dyer et al., 2008; Beopoulos et al., 2011; Zhang et al., 2021). In addition to chemical applications, microbial lipids can be used also as components for animal feed (Bélignon et al., 2016; Blomqvist et al., 2018).

SCOs are produced by oleaginous microorganisms that possess the unique ability to accumulate lipids, especially TAGs, over 20% and up to 70% of the dry cell mass. Known oleaginous yeasts belong to the genera *Lipomyces*, *Yarrowia*, *Cryptococcus*, *Trichosporon* as well as *Rhodotorula* and *Rhodospiridium* (Ageitos et al., 2011; Chattopadhyay et al. 2021). Several parameters have been reported to influence lipid production, such as pH, oxygenation, temperature, phosphate and sulphate limitation, but the most crucial one is the ratio between carbon and nitrogen (C/N ratio) in the growth medium. Typically, when nitrogen level is low and the sugar content is still high (high C/N ratio), the carbon flux is diverted from acetyl-CoA (via citric acid) towards fatty acid biosynthesis (Ageitos et al., 2011; Vorapreeda et al., 2012). Remarkably, given their increasing role played in the field of microbial oils production, some oleaginous species have been engineered in order to by-pass strict metabolic regulation mechanisms, to enlarge their substrate range, as well as to modify their FAs composition (Tai and Stephanopoulos, 2012; Qiao et al., 2017; Donzella et al., 2019; Chattopadhyay et al. 2021). Oleaginous yeasts show ability to grow using a variety of carbon sources, including those present in waste streams from the agro-food sector (Huang et al., 2013; Qin et al., 2017). For example, yeast-based production of oils has been reported using, among others, a wide range of lignocellulose wastes such as dried sorghum stalks (Matsakas et al., 2015), cardon stalks (Tasselli et al., 2018), wastes generated from oil palm industries (Ahmad et al., 2019), mixed vegetable waste (Chatterjee and Mohan, 2018), but also cheese whey (Carota et al., 2017). The generation of agro-food wastes is constantly rising due to rapid growth of world economics and population. In this regard, waste management is becoming a major concern, triggering the idea that wastes can be turned into a resource. Hence, economy has been increasingly shifted from linear to circular, with the aim to pursuit the ambitious goal of zero waste by conversion of wastes into energy and high value products. Suitable feedstock is characterized by low or null cost on the market, and this represents one of the main reasons that increase attractiveness of recycling. On the other hand, one of the principal costs that need to be addressed is related to the transport of the waste from the site where it is generated. Another important aspect related with costs is that utilization of wastes for microbial cultivation often involves physical and/or chemical pretreatments as well as enzyme processes, that are necessary for the release of sugars available for metabolism. Toxic compounds, including organic acids, aldehydes, and phenols can be generated during harsh pretreatments and can inhibit the growth, but detoxification methods are again costly. Conversely, wastes-based media often do not require supplementary ingredients, because they result already rich in macro- and micro-nutrients necessary to support microbial growth. This can reduce the costs of the process.

In this paper we explored the valorization of wastes derived from the food industry, in particular pumpkin peels and a syrup derived from candied fruits manufacture. Italy is among the main world producers of pumpkins, being placed ninth according to the FAO (Food and Agriculture Organization of the United

Nations) statistics. By setting up a protocol of enzymatic hydrolysis we show the feasibility of producing yeast biomass rich in lipids using the oleaginous yeasts *Rhodospiridiobolus azoricus* (previously known as *Rhodospiridium azoricum*) and *Cutaneotrichosporon oleaginosum* (previously known as *Trichosporon oleaginosus*) cultivated on media derived entirely from pumpkin peel wastes. In order to increase lipid accumulation, a syrup derived from candied fruits manufacture was utilized to unbalance the C/N ratio in a two stage process carried out in bioreactor. This study provides strong evidence that the concept of valorization through the production of lipids from the complete metabolism of nutrients present in agro-industrial wastes by oleaginous yeasts is promising for implementation of biotechnological processes in a circular economy contest.

Results And Discussion

Optimization of pumpkin peels pretreatments and quantification of components

Although acid hydrolysis is often used for saccharification of lignocellulosic feedstock, its use generally requires specialized equipment and it is also accompanied by several drawbacks, such as the generation of several inhibitory compounds like furfural and acetic acid, that can hamper the growth of microorganisms (Jönsson and Martin, 2016). Enzymatic hydrolysis was then considered more effective as pretreatment. For this purpose, a commercial preparation containing a blend of cellulases, β -glucosidases, and hemicellulases, suitable for the degradation of cellulose and hemicellulose to fermentable sugars, was used. To optimize conditions of enzyme concentration and time of hydrolysis suited for the pumpkin peels treatment, assays were carried out. Different amounts of enzymes blend were added to the biomass (details reported in Methods), and the reactions were monitored by quantifying the released glucose (Table 1). We observed that a minimum of 24 h was required to obtain a liquefied mixture, indicating that the action of the enzymes was probably not complete before this time. The addition of 2.25 μ L of enzyme solution *per* mL of mixture allowed the release of 23.6 g/L of glucose in 24 h (Table 1). Since glucose concentration did not increase after further 24 h, this time was considered optimal for hydrolysis. Therefore, these conditions (2.25 μ L/mL, 24 h) were selected for the following experiments. Interestingly, glucose and xylose were the only sugars released by the action of the enzymes. In fact, fructose and sucrose concentrations remained constant over the time at all the tested enzyme concentrations (fructose 5 g/L, sucrose 29 g/L). On the other hand, the addition of amylase did not result in an increase of glucose concentration, indicating that pumpkin biomass used in this study did not contain amylose, differently from that reported by Chouaibi and co-workers (Chouaibi et al., 2020). This can be due to differences in quality and provenience of the feedstock, that can greatly affect its final sugar composition. These factors can influence in fact the physical properties of the feedstock like density, moisture content, micro-morphology, and rheological aspects. Furthermore, chemical characteristics of the feedstock are also influenced, such as chemical composition, molecular functional groups percentage, and inorganic species presence (Kim et al., 2012; Yan et al., 2020). Sucrose was the principal sugar in our pumpkin waste, that was characterized by the presence of a tiny peel fraction. Sucrose is mostly present in the pulp, thus it can be found at different levels in the wastes, depending on the type of peeling operation adopted in processing. After centrifugation of the enzymatic hydrolysate, the liquid part obtained was characterized for its composition besides sugars (Table 2). In particular, quantification of acetic acid was done because it is known to be an inhibitor of cell growth. This compound was present at the same concentration detected in the non-treated sample. Probably, acetic acid was generated during the prior biomass storage by microorganisms already present in the pumpkin waste. Nitrogen (N) is an essential element for biomass production; nevertheless, lipid production is triggered by high C/N ratio. When the C/N ratio is low the cells invest nitrogen and carbon to produce biomass, whereas when the ratio is high the carbon is mainly directed to lipid production. In the pumpkin peels hydrolysate inorganic nitrogen (NH_4^+) was found only in traces. Total nitrogen, quantified using the Kjeldahl method, revealed that it contains mainly organic N, that accounted for 2.4 g/L. Table 2 reports the final concentrations of the assayed components of the pumpkin peels hydrolysate after 24 h of enzymatic digestion.

Analysis of growth and sugars utilization

The availability of a pumpkin peels hydrolysate containing a sugars mixture mainly consisting of sucrose, glucose and fructose lead us to evaluate first the capacity of *R. azoricus* and *C. oleaginosum* to grow in presence of all these sugars. Cultures were performed on mineral medium (YNB), in order to avoid the utilization of other carbon sources, that could mask their ability to consume the provided sugars. In this way, we could also obtain quantitative data, in terms of sugar consumption rates and biomass yield. The importance to obtain information about growth and carbon source utilization is particularly strong due to the considerable metabolic diversity among oleaginous yeasts. These parameters are in fact known as key factors for selecting suitable species and for developing efficient fermentation processes.

The cultivation of *R. azoricus* on mineral medium containing a mixture of sucrose, glucose and fructose showed that this yeast started to consume sucrose even in presence of the other two sugars (Fig. 1A). However, a limited capacity to utilize glucose was observed. Glucose in fact was slowly and partially assimilated during the growth, in contrast to fructose that was completely utilized within 48 h (Fig. 1A). As a consequence, a low amount of yeast biomass was reached after 94 h, corresponding to 4.3 g/L of dry weight. Based on these results, we decided to analyze the growth in presence of either glucose or fructose as sole carbon sources. On glucose (Fig. 1B), *R. azoricus* started to grow exponentially, but again we observed that this sugar was partially consumed and, also in this case, a low amount of dry weight was obtained. Cellular viability was then tested and, surprisingly, we found that 40% of cells were not viable after 72 h. These results in conclusion confirmed the impaired glucose assimilation observed in cultivation on the sugars mixture. By contrast, the cultivation on sole fructose revealed that this sugar does not cause the same early arrest of biomass production (Fig. 1C), allowing in fact to reach higher dry weight (9 g/L). By comparing the growth parameters (Table 3), we can see that the growth rate on glucose and on fructose containing media is rather similar, despite glucose consumption rate is higher than fructose. The main differences are found in terms of final amount of produced biomass and in biomass yield, indicating that fructose is metabolized in a more efficient way than glucose. These results were unexpected, because the growth of *R. azoricus* had been previously documented as occurring very efficiently on glucose-based media, especially for lipids production (Galafassi et al., 2012; Capusoni et al., 2017; Donzella et al., 2019). However, the media used for lipids production, containing yeast extract or corn steep, are not exclusively mineral as the one used in the present study. This prompted us to investigate if the addition of yeast extract to the medium containing the sugar mixture could help the cells, especially in glucose utilization. After 72 h (Fig. 1D), sucrose was completely hydrolyzed and glucose and fructose were depleted, leading to the production of 14 g/L of

biomass. These results demonstrate that enrichment of the medium by other nutrients, present in yeast extract, is essential for glucose metabolism and also improves sucrose consumption by this yeast species.

When *C. oleaginosum* was cultivated on mineral medium containing the same mixture of sugars (glucose, fructose and sucrose) we observed a very different behavior. The cells initially grew by using available glucose and fructose, that were depleted after 30 h (Fig. 2A). Sucrose was then hydrolyzed, indicating that the presence of monosaccharides delayed sucrose utilization. In this culture, the dry weight continued to increase until 120 h, and in fact a higher amount of biomass was obtained (12 g/L), in comparison to *R. azoricus* (Table 3). The same behavior was observed by cultivating the cells on medium containing only glucose as carbon source (Fig. 2B): also in this case the biomass continued to increase in parallel with glucose utilization. By comparing the results obtained in terms of final biomass concentration and yield (Table 3), we can conclude that on mineral media *C. oleaginosum* is able to use glucose in a much more efficient way than *R. azoricus*. However, in this case, the addition of yeast extract to the medium containing the mixture did not improve sucrose utilization (Fig. 2C), demonstrating that enrichment of the medium by other nutrients does not contribute to improve sucrose metabolism.

The limited ability to metabolize glucose by *R. azoricus* and its early cellular death could lead to think to a phenomenon of glucose toxicity. In mammalian cells under hyperglycemic conditions, activation of the aldose reductase pathway causes redox unbalance and induction of oxidative stress, upregulating glucose toxicity pathways, as non-enzymatic glycation and disruption of mitochondrial respiratory chain (Tang et al. 2012). Aldose reductases are present in yeasts and have been studied mainly for the production of sugar alcohols (Park et al., 2016; Jagtap et al., 2019), but their role in regulation of sugar metabolism has been scarcely investigated.

However, it is noteworthy that this metabolic inability occurs only when *R. azoricus* grow on mineral media, and is removed by the addition of yeast extract, which is a rich source of nutrients. This reinforces the necessity of studies that carefully analyze growth parameters in order to identify the basis of metabolic diversity among oleaginous yeasts, as recently reported (Brandenburg et al., 2021; Chmielarz et al., 2021). This kind of information is often lacking because mineral media are not suitable for industrial bioprocesses.

Lipids production from pumpkin peels waste-based medium

With the aim of developing fermentative processes for the production of lipids from pumpkin wastes, shaken flask cultures of *R. azoricus* and *C. oleaginosum* were set up using pumpkin peels hydrolysate-based medium.

Cultivation of yeasts was carried out on medium (pH 5.5) containing nutrients exclusively derived from pumpkin peels hydrolysate (Table 2). After 42 h of *R. azoricus* cultivation, we observed that all the sugars were completely consumed, by producing 27.4 g/L of dry weight (Fig. 3A). Acetic acid was also metabolized, as we previously reported by this species (Galafassi et al., 2012). Biomass dry weight further increased, reaching the concentration of 28.1 g/L after 64 h, probably due to the use of other undetected carbon sources present in the pumpkin peels hydrolysate (likely free amino-acids). This yeast biomass contained 39% of lipids, that corresponds to a concentration of 11 g/L, with a lipid yield of 0.22 (calculated on the consumed sugar).

When *C. oleaginosum* was cultivated on the same medium, glucose and fructose were exhausted after 65 h, but sucrose was only partially hydrolyzed (Fig. 3B). This indicates a slower utilization of this sugar, in comparison to *R. azoricus*, and in agreement with the results showed in Fig. 2C. In addition, a lower level of lipids was obtained, representing 29% of yeast dry weight, that corresponds to a concentration of 7 g/L after 94 h. However, lipid yield was similar to that of *R. azoricus*.

In conclusion, these results indicate that the pumpkin peels waste represents a complete source of nutrients for yeast cultivation, allowing to obtain high concentrations of biomass. In addition, this medium also sustains lipid production (11 g/L by *R. azoricus* and 7 g/L by *C. oleaginosum*).

Two-stage process in bioreactor for lipids production

Based on these results, we decided to develop a fermentative process for lipids production by using the yeast *R. azoricus*, that exhibited the best performance on pumpkin wastes-based medium. Usually, the process for lipid production is performed in two stages, the first carried out at low C/N ratio to produce high amount of cell biomass, and the second by feeding nutrients at high C/N ratio to trigger lipid accumulation (Ageitos et al., 2011).

The cultivation in bioreactor, under controlled conditions of oxygen and pH, resulted in the production of 30 g/L of biomass, already containing 37% of lipids, after 46 h of cultivation (Fig. 4). At this point, sucrose and glucose were depleted, whereas fructose was still present. In order to increase lipid content, we decided to feed the culture by using another industrial food waste coming from the production of candied fruits (a syrup from mango processing). This waste contains in fact high concentration of glucose and fructose, but does not contain nitrogen, making it a perfect C source for our purpose to unbalance the C/N ratio of the medium. After further 44 h of process, all glucose and part of fructose were metabolized by the yeast (Fig. 4). Sugars were converted into lipids, allowing the yeast biomass to reach a lipid content of 55% of dry weight. The lipid concentration after 90 h of process was 24 g/L (Fig. 4), that corresponded to a lipid productivity of 0.26 g/L/h. Based on the sugars utilized the lipid yield was 0.29.

These results represent a very promising starting point for developing a lipid production process. Similar results were reported by Slininger and co-workers (2016), that developed a very efficient two-stage process on undetoxified lignocellulose hydrolysate, by identifying top-performing lipids producing yeasts, and also close to the highest reported by Cho and Park (2018) from other organic wastes. Moreover, by enriching the initial medium with nitrogen we can increase the biomass production during the first step, reaching higher dry weight, that in the second step can account for higher final lipid concentration. Previously, by the same oleaginous species we reported a two stage process on glucose-based medium, performed by feeding nitrogen in the first step and glucose in the second step, that resulted in the production of 80 g/L of yeast biomass containing 41% of lipid, and corresponding to a final lipid concentration of 33 g/L (Capusoni et al., 2017).

In conclusion, by the exclusive use of food wastes (pumpkin peels and candied fruits syrup), *R. azoricus* was able to efficiently grow and produce lipids with high productivity and yield.

Conclusions

This proof-of-concept study has shown the feasibility to produce yeast biomass and lipids in an economical way using a medium based on pumpkin wastes, demonstrating a possible process of upgrading low value industrial food wastes to high value products. The use of two inexpensive residues, i.e. pumpkin peels and syrup from candied fruits processing, for sustaining microbial oil production has been never reported before. They can therefore represent a renewable and low-cost feedstock to fulfil nutrients requirement of yeasts. Lipids can be extracted and used to produce biofuels and chemicals. Yeast biomass can in turn be used as a high-quality animal feed ingredient, because it is considered a well-balanced source of protein, and can provide also vitamins (mainly the B group) (Øverland et al., 2013). In conclusion, one of the main goals of circular economy, which is the reduction of wastes by recycle, could be addressed.

Methods

Strains, media and growth conditions

The yeast strains used in this work are wild type strain DBVPG 4620 reclassified as *Rhodospiridiobolus azoricus* (Capusoni et al., 2017) and wild type strain ATCC 20509 classified as *Cutaneotrichosporon oleaginosum* (Gujari et al., 2011). For long-term storage, yeast strains were maintained at -80 °C on 15% (v/v) glycerol.

YPD medium contained 10 g/L yeast extract (Biolife, Italy) 20 g/L peptone (Biolife, Italy) and 20 g/L glucose (Sigma Aldrich, Italy).

YNB medium contained Yeast Nitrogen Base 0.17 g/L, (Difco BD, Italy), and 0.1 M MES hydrate (Sigma Aldrich, Italy) to maintain pH 6. Sugars (sucrose, glucose, fructose from Sigma Aldrich, Italy) were added individually at 50 g/L, or in mixture to simulate the pumpkin peels hydrolysate composition.

Yeast cells were cultivated at 28°C in a rotary shaker at 150 rpm in bluffed flasks with an air-liquid ratio of 1:5. Cells from pre-cultures (inoculated from glycerol stocks) grown overnight on YPD were harvested by centrifugation (5000 rpm/2300 rcf, 10 min in Eppendorf 5415D centrifuge) and inoculated at OD₆₆₀ 0.1 in YNB medium or at OD₆₆₀ 0.4 in pumpkin peels hydrolysate-based medium. Cell growth was monitored by measuring the increase of optical density at 660 nm (OD₆₆₀) using a spectrophotometer (Eppendorf, Italy).

Preparation of pumpkin peels hydrolysate

Fresh pumpkin peels (from Azienda Agricola Paratorosso, Settala, Milano) were oven-dried over-night at 70 °C and milled with a laboratory grinder Bimby TM 3300 to obtain a fine powder. The powder was stored at 4 °C in a tightly closed jar.

Small scale enzymatic hydrolysis was carried by suspending the peel powder (solid loading of 12% (w/v), in distilled water. The mixture was then sterilized in autoclave (Cavallo S.r.l., Milan, Italy) at 0.5 atm, 112 °C for 30 min. After cooling to 50 °C, the mixture was adjusted at pH 5.5 added with different amounts (1.12, 2.25, 5.5, 11.25, 22.5 µL/mL) of Cellic CTec2 enzyme cocktail (Novozymes, Denmark) (hydrolytic activity >1150 U/mL) and incubated at 50 °C in an oil bath under magnetic stirring.

At different times, 0.5 mL aliquots were collected, and sugar content was analyzed by enzymatic assay. The higher scale hydrolysis was carried out in a 2-L bioreactor (Applikon Biotechnology, The Netherlands). The bioreactor filled with the reaction mixture (650 mL) was autoclaved for 1 hour at 1 atm, 121 °C. Temperature was set at 48 °C, agitation at 650 rpm and pH at 5.5. To control bacterial contamination, antibiotics were added (Ampicillin 100 µg/mL, Kanamycin 10 µg/mL and Chloramphenicol 5 µg/mL). The hydrolysis was started by the addition of enzymes (1.46 mL of Cellic CTec2). After 24 h, the hydrolysate was centrifuged in sterile tubes at 5000 rpm/3214 rcf in Eppendorf 5804R centrifuge for 1 h, and the liquid phase stored at -20 °C.

Sugars and acetic acid determination

The concentrations of sugars and acetic acid were determined by employing commercial enzymatic kits (K-GLUHK, K-SUFRG, K-XYLOSE by Megazyme Ltd, Ireland and 10148261035 by R-Biopharm AG, Germany, respectively). All the assays were performed in triplicate and standard deviations varied between 1 and 5%. The biomass and product yields were calculated as the ratio between the total amount of biomass or products and the total amount of consumed sugars.

Nitrogen determination

Inorganic nitrogen was determined by employing a commercial enzymatic kit (10542946035 by R-Biopharm AG, Germany).

Total nitrogen concentration in culture supernatants was determined by Kjeldahl method using a SpeedDigester K-376 and a KjelMaster K-375 (Buchi Italia).

Dry weight determination

Cells were collected from the medium (1 mL or 2 mL of cell culture) by centrifugation (5 min at 13200 rpm/16100 rcf in Eppendorf 5415D centrifuge). The pellets were dried overnight at 105°C. In the case of pumpkin peels hydrolysate, the dry weight of the pellet present at T₀ was determined and subtracted to the value of the following measurements.

Lipid determination

Lipid content was determined via the sulfo-phospho-vanilline colorimetric method (Spinreact, Spain) on the washed cell pellets corresponding to approximately 30 OD, suspended in 0.5 mL of cold redistilled water. The assays were performed in triplicate and standard deviations varied between 1 and 5%.

Fed batch cultivation

Fed-batch cultures were performed in a 2-L bioreactor (Applikon Biotechnology, The Netherlands), with a starting volume of 600 mL. Pumpkin peel hydrolysate was used as medium for yeast cultivation. Temperature was set at 30 °C, the air inlet at 1 vvm and the agitation at 500 rpm.

Dissolved oxygen concentration was measured by AppliSens oxygen probe (Applikon Biotechnology, The Netherlands), starting from 100% of saturation. Foam formation was controlled by the addition of a silicon antifoaming agent (Sigma 204). The pH, measured by AppliSens pH electrode (Applikon Biotechnology, The Netherlands), was automatically adjusted and maintained at 5.5 by adding 5 M KOH or 10% (v/v) solution of H₂SO₄. Syrup from candied fruits (mango) manufacture (SVZ, Industrial Fruit & Vegetable Ingredients, Breda, the Netherlands), diluted to glucose 199 g/L and fructose 296 g/L, was supplied after 46 h in order to unbalance the C/N ratio.

Declarations

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Author contributions

S. D. and I. S. contributed at the same extent to the study, for conceptualization, data curation, analysis and writing the draft. A. F.: investigation, analysis; L.P: contribution to discussion of the results; C. C.: conceptualization, supervision of investigation, writing the manuscript.

Availability of data and materials

The datasets used and/or analysed during the current study are available from

the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare that they have no competing interests

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Tables

Table 1. Glucose concentration obtained after 24 h and 48 h of enzymatic digestion performed on pumpkin peel in small scale (2 mL).

Cellic CTec2 (μL/mL)	24 h- Glucose (g/L)	48 h- Glucose (g/L)
-	3.9±0.3	4.1±0.5
1.12	17.2±0.4	19.2±0.3
2.25	23.6±0.5	23.8±0.6
5.5	22.3±0.5	22.7±0.8
11.25	21.8±0.7	20.8±0.6
22.5	21.1±0.6	19.3±0.7

Table 2. Concentration of the main components of pumpkin peel hydrolysate (after 24 h of enzymatic digestion, large scale). The grams of each compound released per gram of biomass are also indicated.

Medium component	Concentration (g/L)	g/g _{biomass}
Glucose	18±1.5	0.15
Sucrose	29±1.8	0.24
Fructose	5±0.6	0.04
Xylose	0.4±0.08	0.003
Acetic acid	0.9±0.2	0.0075
Nitrogen	2.4±0.3	0.02

Table 3. Growth parameters of *R. azoricus* and *C. oleaginosum* cultivated in shaken flasks on YNB medium added with different sugars.

<i>R. azoricus</i>						<i>C. oleaginosum</i>				
	max (h ⁻¹)	q (mmol _{sug} /g _{dw} /h)	Biomass yield (g _{dw} /g _{sug})	Biomass (g/L)	Consumed sugars (g/L)	max (h ⁻¹)	q (mmol _{sug} /g _{dw} /h)	Biomass yield (g _{dw} /g _{sug})	Biomass (g/L)	Consumed sugars (g/L)
Glucose	0.21±0.02	3.40±0.09	0.20±0.04	4.10±0.24	20±1.00	0.22±0.09	3.21 ±0.21	0.33±0.09	12.00±0.51	36±1.3
Fructose	0.23±0.03	2.51±0.17	0.32±0.07	9.01±0.21	28±1.50	ND	ND	ND	ND	ND
Glu+Fru+Suc	0.22±0.02	ND	0.26±0.09	4.30±0.12	34±1.22	0.26±0.08	ND	0.21±0.06	12.06±0.46	33±0.97

ND: not determined

Figures

Figure 1

Cultivation of *R. azoricus* on YNB medium containing: 30 g/L of sucrose, 15 g/L of glucose and 5 g/L of fructose (A); 50 g/L of glucose (B); 50 g/L of fructose (C); 30 g/L of sucrose, 15 g/L of glucose, 5 g/L of fructose and 1 g/L of yeast extract.

Figure 2

Cultivation of *C. oleaginosum* on YNB medium containing: 30 g/L of sucrose, 15 g/L of glucose and 5 g/L of fructose (A); 50 g/L of glucose (B).

Figure 3

Cultivation of *R. azoricus* (A) and *C. oleaginosum* (B) in shaken flasks on pumpkin peel hydrolysate-based medium.

Figure 4

Cultivation of *R. azoricus* in 2-L bioreactor on pumpkin peel hydrolysate-based medium.