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Valorization of Low-Cost Substrates for the Production of Odd Chain Fatty Acids by the Oleaginous Yeast *Yarrowia lipolytica*

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Abstract: Odd-chain fatty acids (OCFAs) have recently gained interest as target compounds in microbial production due to their diverse applications in the medical, pharmaceutical and chemical industries for the production of biofuels. *Yarrowia lipolytica* is a promising oleaginous yeast that has the ability to accumulate high quantities of fatty acids. However, the use of *Y. lipolytica* oils is still under research, in order to decrease the production costs related to the fermentation process and improve economic feasibility. In this work, sugar beet molasses (10–50 g/L) and crude glycerol (30 g/L) were used as the main carbon sources to reduce the processing costs of oil production from a genetically engineered *Y. lipolytica* strain. The effects of medium composition were studied on biomass production, lipid content, and OCFAs profile. Lipid production by yeast growing on molasses (20 g/L sucrose) and crude glycerol reached 4.63 ± 0.95 g/L of culture medium. OCFAs content represented 58% of the total fatty acids in lipids, which corresponds to ≈2.69 ± 0.03 g/L of culture medium. The fermentation was upscaled to 5 L bioreactors and fed-batch co-feeding increased OCFA accumulation in *Y. lipolytica* by 56% compared to batch cultures.

Keywords: *Y. lipolytica*; fermentation; odd chain fatty acids; industrial by-products; valorization; valuable compounds

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1. Introduction

With the current growing concerns about global warming and greenhouse effects, microbial oils are considered promising alternatives to fossil fuels. *Yarrowia lipolytica* is among the most suitable oleaginous yeasts for microbial oil production [1]. It is able to assimilate both hydrophilic and hydrophobic substrates to synthesize and store triacylglycerols (TAG) in the lipid bodies. *Y. lipolytica* has a well-studied metabolism and genome and has been engineered to accumulate large amounts of lipids, that may exceed 80% dry biomass basis [2].

Odd chain fatty acids (OCFAs), a kind of valuable lipid, have received particular interest for their applications in medical, agricultural, and fuel industries [3]. In particular, OCFAs composed of 15 and 17 carbon chain lengths have shown positive health effects [3]. It has been shown that the presence of pentadecanoic acid (C15:0) and heptadecanoic acid (C17:0) in serum was inversely associated with the risk of type 2 diabetes and cardiovascular diseases, and was a biomarker of obesity [4–6]. Moreover, a research study revealed that *cis*-9-heptadecenoic acid (C17:1) has an important anti-inflammatory effect and is an active compound against allergies and autoimmune diseases [7]. In addition, *cis*-9-heptadecenoic acid has shown antifungal activities, which may allow its usage as a biocontrol agent in the chemical and agricultural industries [8]. In addition, OCFAs contribute positively to the quality of biofuels, improving transesterification reactions or storage conditions [9].

Despite the wide range of applications for OCFAs, their presence in microbial oil is rare because microorganisms produce a greater proportion of even-chain fatty acids than

OCFAs. OCFAs are synthesized through the incorporation of propionyl-CoA in the initial condensation step of fatty acids biosynthesis. Propionyl-CoA is condensed to malonyl-CoA to form 3-oxovaleryl-ACP, a five-carbon compound which is the starting point for OCFAs synthesis. Then the elongation step takes place and 2 carbons are added in each cycle, which results in the production of OCFAs [10]. Propionyl-CoA is generated from propionate in yeast and bacteria. Previous research work studied the production of OCFAs from propionate supplementation in *Y. lipolytica* without genetic engineering [7,11], or in a genetically modified strain after deleting the PHD1 gene involved in the degradation of propionyl-CoA [9].

The main limitations for the industrialization of microbial oil production are their high production costs [12]. Usually, the cost of the culture media constitutes 50 to 80% of the total cost of the fermentation process [13]. Therefore, the use of low-cost substrates such as industrial or agricultural by-products has been investigated to decrease the cost of the process for single cell oil production. For instance, food-waste derived volatile fatty acids [14,15], palm oil mill effluent [16], sugarcane bagasse hydrolysate [17] and crude glycerol [18] have been used as low-cost substrates for lipid production in *Y. lipolytica*.

Sugar beet molasses is a low-cost byproduct produced by the sugar industry [19]. In addition to sucrose, molasses contains nitrogen, vitamins, organic acids and minerals that make it an interesting substrate for cell growth during fermentation [20,21]. The composition of sugar beet molasses has been previously studied and described [22]. Authors have shown that the major compounds present in sugar beet molasses are sucrose (60.9% of DM), proteins (13.5%), ash (11.7%), and organic acids including lactic acid (4.5%) and pyrocarbonic acid (2.77%) [22]. Phenolic compounds such as vanillin, kaempferol and hydroxybenzaldehyde were also found in sugar beet molasses [23]. Crude glycerol is obtained in large quantities from biodiesel production. It is estimated that 100 kg of crude glycerol is generated during the production of 1 ton of biodiesel [24]. The possible use of crude glycerol in fermentation media has been studied to identify an opportunity for its valorization, because the purification process of crude glycerol is energy- and time-consuming [25]. Crude glycerol solution contains, in addition to glycerol, methanol, water, sodium salts of fatty acids, fatty acid esters, and sodium chloride. The percentages of impurities in crude glycerol vary depending on the oil source, trans-esterification process, and catalyst concentration [26].

The purpose of this study was to investigate the effect of sugar beet molasses and crude glycerol as low-cost substrates for the production of OCFAs by fermentation, using an engineered strain of *Y. lipolytica*. The production of OCFAs was upscaled to a 5-L bioreactor, both in batch and fed-batch modes.

2. Materials and Methods

2.1. Yarrowia Lipolytica Strain

The oleaginous yeast strain *Y. lipolytica* JMY7877 was engineered from OCFA-producing strain, JMY7780 (Δphd1 Δmfe1 Δtgl4 pTEF-DGA2) [27]. Two gene expression cassettes—invertase SUC2 from *Saccharomyces cerevisiae* (JME2347, JMP62-LEU2 ex-pTEF-ScSUC2-Tlip2) and hexokinase HXK1 from *Y. lipolytica* (JME2103, JMP62- URA3 ex-pTEF-YlHXK1-Tlip2)–were introduced to the genome of *Y. lipolytica* JMY7780 resulting in the JMY7877 strain, able to assimilate sucrose. Details about strain construction could be found in [28].

To prepare a stock cell culture, yeasts were inoculated from an agar plate (1 colony) into an Erlenmeyer flask (0.5 L) with 0.1 L of a medium containing sucrose 20 g/L, yeast extract 10 g/L, and peptone 20 g/L, and incubated in a shaker at 28 °C, 170 rpm agitation, for 24 h. Na_2HPO_4 (0.05 M) and KH_2PO_4 (0.05 M) were added to the media to regulate the pH to 6. After 24 h, the cell culture was mixed with 50% sterilized pure glycerol (*w*/*v*) and stored at −80 °C in cryotubes. The experimental set-up is represented in Figure 1.

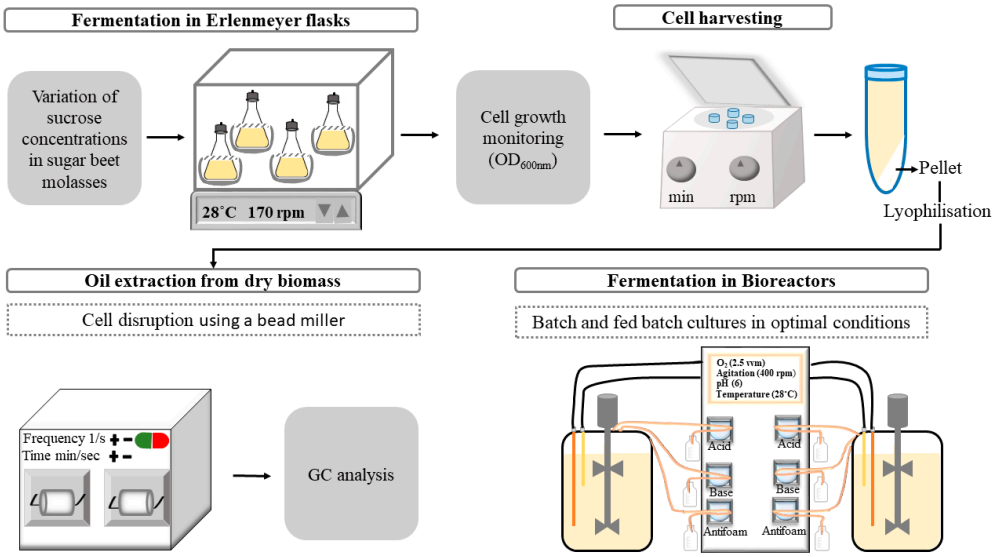

Figure 1. Experimental set-up for the production of OCFAs in *Y. lipolytica*.

2.2. Fermentation Conditions

2.2.1. Shake Flask Experiments

The fermentation media was composed of sugar beet molasses (10–50 g/L of sucrose) and crude glycerol (30 g/L) as main carbon substrates, yeast extract (1 g/L) as organic nitrogen substrate, ammonium chloride (0.5 g/L) as inorganic nitrogen substrate, sodium acetate (20 g/L) as precursor of acetyl-CoA, sodium propionate (5 g/L) as precursor of propionyl-CoA, and saline solution (1×). The composition of 1000× concentration saline solution with a final volume of 1 L was the following: H_3PO_4 85% liquid- 107 g, KCl- 20 g, NaCl- 20 g, $MgSO_4 \cdot 7H_2O$- 27 g, $ZnSO_4 \cdot 7H_2O$- 7.7 g, $MnSO_4 \cdot H_2O$- 0.47 g, $CoCl_2 \cdot 6H_2O$- 0.3 g, $CuSO_4 \cdot 5H_2O$- 0.6 g, $Na_2MoO_4 \cdot 2H_2O$- 0.094 g, H_3BO- 0.3 g and water up to 1 L. To evaluate the impact of propionate as precursor of OCFA, a fermentation experiment was performed under the same conditions, using 20 g/L sucrose in molasses, and without propionate. The initial concentration of sucrose in sugar beet molasses, determined by high performance liquid chromatography (HPLC) according to the method detailed in Section 2.3, was 800 g/L, and dilutions from 10 to 50 g/L were performed in water. Since complex components (molasses, crude glycerol, and yeast extract) were used in the medium, the content of nitrogen was determined using the Kjeldahl method. To estimate the C/N ratio, the amounts of carbon in sucrose, sodium acetate, sodium propionate, glycerol and yeast extract (35%), and the contents of nitrogen in ammonium chloride, molasses (3%) and yeast extract (65%) were used. The C/N ratio varied from 21 to 24, depending on the concentration of molasses, which is the optimum range for lipid production in *Y. lipolytica* [9,29,30]. Cultures were first performed in 0.5 L Erlenmeyer flasks each containing 0.1 L of culture medium, and inoculated by adding 2 mL of a stock cell culture. The flasks were placed in a rotary shaker at 28 °C, 170 rpm agitation, for 160 h. Samples were taken periodically from each flask to monitor cell growth. They were diluted 20 times in ultrapure water, and the optical density (OD) was measured at 600 nm using a Thermo BioMate 3 Spectrophotometer. A non-inoculated medium was used for the blank of the spectrophotometer. In addition, for fatty acid analysis, 1 mL of each sample was centrifuged for 10 min at 13,000 rpm (Micro Star 12, VWR, Rosny-Sous-Bois, France), and the obtained pellets were washed with 1 mL ultrapure water to remove medium traces. The washed pellets were then lyophilized for three days (Alpha 1–4 LD Plus, Martin Christ, Osterode am Harz, Germany) and used for lipid extraction and fatty acid analysis.

2.2.2. Bioreactor Experiments

The selected concentration of sugar beet molasses giving the maximal cell density (OD_{600nm}) and OCFAs content was upscaled to a 5-L bioreactor (Biostat B Plus, Sartorius Stedim Biotech, Göttingen, Tüßling, Germany). The bioreactors and the culture media (4.75 L) were sterilized separately at 121 °C for 20 min using a HMC 110V autoclave (HMC Europe GmbH, Germany). The media were then introduced into 5-L twin bioreactors equipped with pH, temperature and oxygen probes. After cooling down to 28 °C, the media were saturated with oxygen and their pH was automatically calibrated to 6 using NaOH (1 M) and H_2SO_4 (1 M). Then, 0.25 L of a mid-exponential phase pre-culture (cultivated in 1 L Erlenmeyer flask in a rotary shaker at 28 °C, 170 rpm agitation, and during 24 h) was added to inoculate the medium. The temperature was maintained at 28 °C with a double jacket and circulating cooling water, the agitation speed was fixed at 400 rpm and the air flow was adjusted to 2.5 vvm. Samples were taken periodically from the bioreactors to determine the cell density and lipid content, as described in Section 2.2.1. After centrifugation, the supernatants were stored at −20 °C to determine the substrate consumption kinetics using HPLC. At the end of fermentation (160 h), microbial biomass was harvested by centrifugation at 4600 rpm for 20 min at 4 °C. The obtained pellets were washed with ultrapure water to remove medium traces and lyophilized for three days.

A fed-batch fermentation strategy was tested to improve OCFA accumulation in *Y. lipolytica*. The fermentation conditions and substrates concentrations were the same as for the bioreactors performed in batch mode. The only modification was the supplementation of the culture media by pure sucrose after 72 h fermentation, to reach 20 g/L.

2.3. Substrates Consumption Analysis

The concentrations of sucrose, glucose and fructose (released in the medium by the action of invertase), glycerol, sodium acetate, and sodium propionate were determined by HPLC using an Agilent 1260 Infinity II equipped with an Aminex HPX-87H column coupled to a Refractive Index (RI) detector. The mobile phases used were sulfuric acid (5 mM) for the quantification of sodium acetate and sodium propionate, and water for the quantification of sugars and glycerol. The flow rate was adjusted to 0.6 mL/min and the column temperature was set at 35 °C. The samples were filtered before injection using 0.45 µm filters, diluted in ultrapure water (for sugars and glycerol quantification) or in sulfuric acid (5 mM) (for sodium acetate and sodium propionate quantification) and injected with a volume of 10 µL. Identification and quantification were achieved using a standard curve for each substrate.

2.4. Lipid Extraction and Fatty Acid Analysis

2.4.1. Lipid Extraction from Dry Biomass

The pellets obtained after freeze-drying were used for lipid extraction and analysis. The method used for lipid extraction was adapted from a previous study [31], with some modifications. An amount of ≈30 mg of dry biomass was weighed and placed into a 2-mL screw tube in the presence of two stainless steel beads (4.9 mm diameter). Then, 100 µL of 5 mg/mL of internal standard dodecanoic acid (C12:0), prepared in a mixture of cyclohexane: isopropanol (2:1, *v*:*v*), was added. Extraction was performed by adding 900 µL of cyclohexane:isopropanol (2:1, *v*:*v*) and milling for 5 min at 30 Hz using a bead miller (Retsch MM400, Haan, Germany). The mixture was centrifuged for 15 min at 13,000 rpm using a MicroStar12 centrifuge (Micro Star 12, VWR, Rosny-Sous-Bois, France) and the supernatant was transferred to a 10 mL glass tube. The extraction was repeated twice by adding 1 mL of cyclohexane:isopropanol (2:1, *v*:*v*). The supernatants resulting from the three extractions (3 mL) were evaporated under nitrogen flow at 60 °C and the recovered oil was subjected to transesterification.

2.4.2. Preparation of Fatty Acid Methyl Esters (FAMEs) for Gas Chromatography (GC) Analysis

Transesterification was performed in glass tubes (13 × 100 mm) containing the extracted lipids after solvent evaporation. To each tube, 0.3 mL toluene (Carlo Erba, Val-de-Reuil, France) and 1 mL methanolic HCl (3 M) (Sigma Aldrich, Saint-Quentin-Fallavier, France) were added. The tubes were flushed with nitrogen to avoid oxidation, tightly closed, and placed in an oven at 80 °C for 2 h. To stop the transesterification reaction, 0.5 mL of sodium bisulfite (Acros Organics, Fisher Scientific France, Illkirch, France) (5% *w*/*v* in water) was added and the tubes were vortexed for 10 s. The extraction of FAMEs was performed by adding 1.7 mL of cyclohexane (Carlo Erba, France) followed by vortexing for 1 min. After centrifugation for 5 min at 2000 rpm, 100 µL were taken from the upper phase containing FAMEs, evaporated under nitrogen flow, and resuspended in 1 mL cyclohexane for GC analysis.

The fatty acid composition of lipids was analyzed using GC apparatus equipped with a capillary column (60 m × 320 µm × 0.25 µm) and coupled to a flame ionization detector (GC-FID Agilent Technologies 7890A, Les Ulis, France). The GC oven was initially set at 150 °C for 2 min, elevated to 220 °C at the rate of 1.5 °C/min, and then held at this temperature for 30 min. Helium was used as carrier gas at a flow rate of 30 mL/min. Samples (2 µL) were injected manually using a 5 µL Hamilton syringe. FAMEs were identified according to their retention time by comparison to commercial standards of *cis*-9-heptadecenoic acid (Cayman Chemical Company, Ann Arbor, MI, USA), *cis*-10-nonadecenoic acid (Merck, Fontenay Sous Bois, France), methyl nonadecanoate (Merck, France), and Supelco 37 FAME mix (Merck, France) containing among others *cis*-10-heptadecenoic acid.

The % of TAG was calculated according to Equation (1):

$$\% \text{ TAG} = \left(\frac{\text{Total fatty acids corrected peak areas} * \text{mass of the internal standard (mg)}}{\text{Peak area of the internal standard} * \text{microbial dry weight (mg)}} \right) \times 100 \quad (1)$$

2.5. Statistical Analysis

All experiments and measurements were repeated three times, and average and standard deviations of data were calculated. One-way analysis of variance (ANOVA) was used to determine the significant differences using Excel software (Microsoft Excel 2016 MSO 16.0.4266.1001).

3. Results and Discussion

3.1. Impact of Sugar Beet Molasses and Crude Glycerol on Cell Growth

In this study, sugar beet molasses and crude glycerol were used as the main carbon substrates for yeast growth and lipid production, in order to develop a cost-effective fermentation medium. The growth kinetics obtained by varying the concentrations of sucrose in molasses from 10 to 50 g/L are shown in Figure 2a. The maximal cell density (OD_{600nm}) obtained during the stationary phase was significantly different ($p < 0.05$) when the concentration of sucrose in molasses increased from 10 g/L (OD_{600nm} = 16) to 20 g/L (OD_{600nm} = 20). This corresponds to an increase in microbial dry weight from 11 to 16 g of dry biomass/L of medium (Figure 2b). However, a further increase in this concentration did not cause any significant improvement in the cell density nor in microbial dry weight ($p > 0.05$). Moreover, when the concentration of sucrose in molasses increased from 20 g/L to 40 g/L and then to 50 g/L, an increase in the lag (adaption) phase duration was observed, and longer time was required to reach maximal cell density. Some previous works have studied the impact of molasses on the growth of microorganisms. An increase in the duration of the lag phase was observed during the fermentation of *Clostridium butyricum* W5 when the concentration of sugar in molasses was increased from 20 to 120 g/L [32]. According to the authors, this result was associated with the high osmotic pressure of the medium due to the high sucrose concentrations in sugar beet molasses. In another work, a toxic effect of molasses on the cell growth has been reported in a fermentation medium containing 10% molasses [33]. It was concluded that increasing the concentration

of molasses from 12% to 16% could increase the concentration of phenolic compounds in the fermentation medium, which could present an inhibitory effect on cell growth [34]. The current work also studied the impact of crude glycerol, compared to pure glycerol, on cell growth. Cultures using pure glycerol instead of crude glycerol did not show any significant difference in growth kinetics (data not shown), which indicates that crude glycerol can be used as carbon substrate instead of pure glycerol, reducing thereby the fermentation cost.

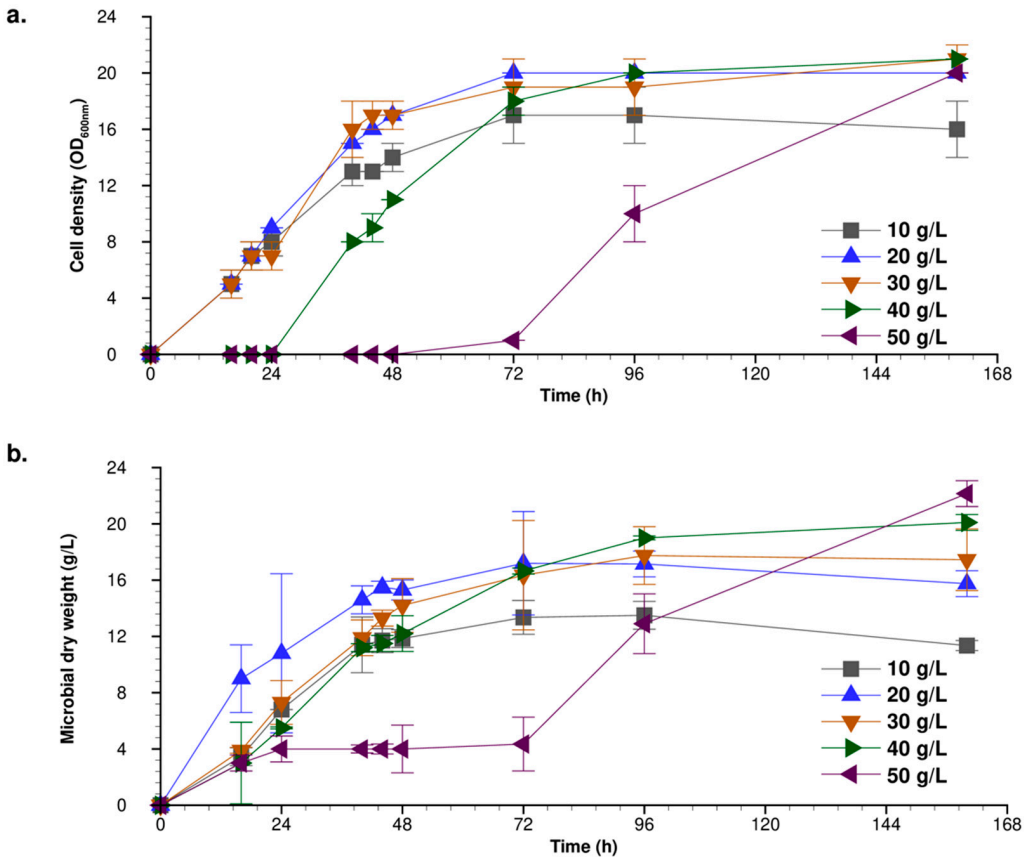

Figure 2. Growth kinetics of *Y. lipolytica* for different sucrose concentrations in sugar beet molasses. (**a**) Kinetics using cell density (OD_{600nm}), (**b**) kinetics using microbial dry weight (g/L).

3.2. Evaluation of Lipid Production in Erlenmeyer Flasks

Lipid and OCFA contents, with the lipid profiles, obtained after 160 h of fermentation for different sucrose concentrations in molasses (10–50 g/L) are presented in Figure 3. For all concentrations of sugar beet molasses, the obtained lipid contents were higher than that accumulated in the wildtype strain W29 (1.30 g/L) after 192 h of fermentation, cultivated on 60 g/L glucose [35]. The GC analysis of the extracted lipids showed that OCFAs represented more than 50% of total fatty acids in *Y. lipolytica*. C17:1 was the major OCFA accumulated in *Y. lipolytica* with no significant differences ($p > 0.05$) between all concentrations of sugar beet molasses. The presence of propionate in the medium was necessary for OCFAs synthesis, since only 7% of OCFAs were accumulated in the absence of propionate compared to 58 % in the presence of propionate. The use of propionic acid has increased the production of OCFAs, in particular C17:1, up to 37% of total fatty acids in *Candida* sp., *Torulaspora delbrueckii*, *Kluyveromyces polysporus*, and *Trichosporon cutaneum* [36]. In another study, the production of OCFAs by *Rhodococcus* sp. YHY01. was optimized in a synthetic medium containing glycerol, propionate, and ammonium chloride (0.32%, 0.76%, 0.040% *w*/*v*, respectively) [37]. It was shown that this strain accumulated 85% *w*/*w* of OCFAs in the order of C17:0 > C15:0 > *cis*-10-C17:1 > 10Me-C17:0 > C19:0 > *cis*-10-C19:1. Compared with a recent study [27], where the same strain and the same ratio

of acetate:propionate were used, but adding glucose as a carbon source, the lipid profile was similar to that found in the current work. However, the percentage of C17:1 obtained using sugar beet molasses as a carbon source was higher, accounting for 38% of the total fatty acids, compared to that obtained after 120 h (32%) in the other study [27]. In addition to heptadecenoic acid (C17:1), other OCFAs were produced in lower amounts, including pentadecanoic acid (C15:0), heptadecanoic acid (C17:0), and nonadecanoic acid (C19:0; C19:1). No significant differences ($p > 0.05$) were observed in the content of OCFAs when the concentration of sucrose in molasses varied between 10 and 50 g/L. These results indicate that *Y. lipolytica* could grow on sugar beet molasses and produce lipids with high content of OCFAs.

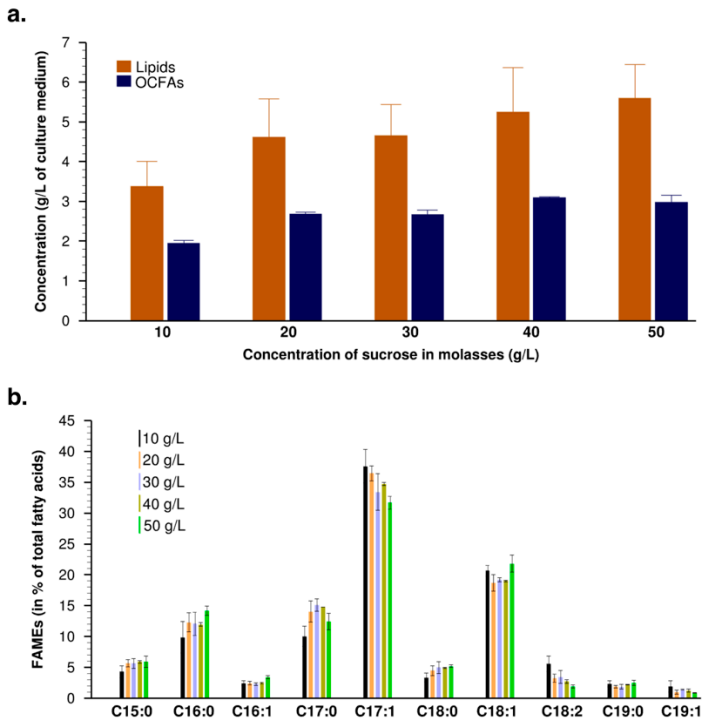

Figure 3. (**a**) Total lipid content in *Y. lipolytica* cultivated on different sucrose concentrations in sugar beet molasses (10–50 g/L), (**b**) lipid profiles obtained by GC-FID, depending on sucrose concentration in sugar beet molasses (10–50 g/L).

3.3. Evaluation of Lipid Production in Bioreactors

According to the above-mentioned observations and to statistical analysis, the increase in sugar concentration in molasses from 10 to 20 g/L significantly increased the values of OD_{600nm} ($p < 0.05$) in the stationary phase. However, lipid content and OCFA content obtained after 160 h of fermentation were not significantly different when the concentration of sucrose in molasses varied from 10 to 50 g/L ($p > 0.05$). For this purpose, fermentations in continuous stirred-tank bioreactors (5 L culture medium) were conducted using a sucrose concentration of 20 g/L. After 160 h of fermentation, the measured cell density (OD_{600nm}) was 18.90 ± 0.23 in the Erlenmeyer flasks and 18.19 ± 1.20 in the bioreactors. The microbial dry weight obtained from bioreactors was 16 g of dry biomass/L of medium. The total lipid content after 160 h of fermentation in 5-L bioreactors was 3.84 ± 0.50 g/L of the culture medium, with more than 50% of OCFAs in the total fatty acids. The concentrations of substrates were determined during fermentation in 5-L bioreactors up to 160 h (Figure 4). HPLC results show that sucrose was completely hydrolyzed into 10 g/L glucose and 10 g/L fructose after 24 h of fermentation. The strain showed more affinity to glycerol than to sugars, as the concentrations of glucose and fructose remained constant during the first 48 h of fermentation, whereas glycerol was rapidly consumed. The concentration of glucose started to decrease after 72 h and was totally consumed after 96 h. Fructose was

not consumed simultaneously with glucose and its concentration remained relatively high (10 g/L) until 96 h. A similar behavior to glycerol was observed for sodium acetate, where the concentration decreased rapidly after 48 h of fermentation, while the consumption of propionate started after 96 h, reaching 1.89 g/L in the medium after 160 h of fermentation.

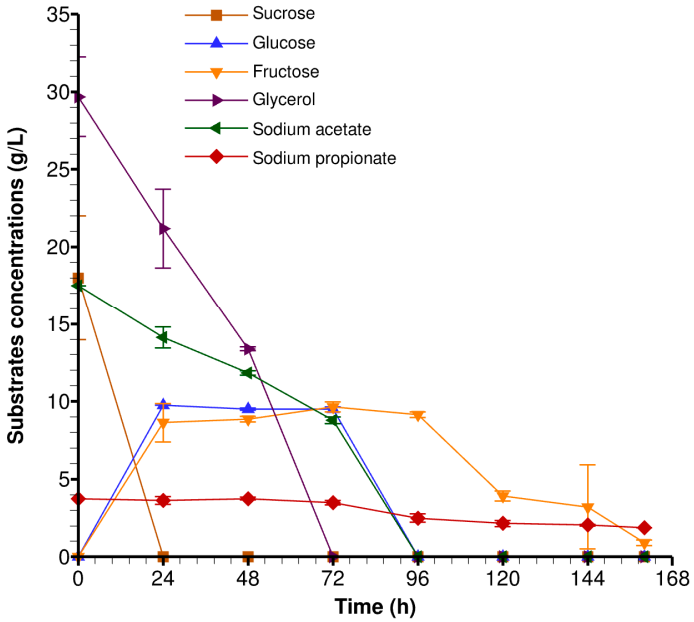

Figure 4. Substrates consumption during the batch fermentations conducted in 5-L bioreactors using a sucrose concentration of 20 g/L in sugar beet molasses.

To achieve high lipid accumulation in oleaginous microorganisms, the fermentation medium should contain excess carbon substrate and a limiting amount of nitrogen [33]. Therefore, 20 g/L of pure sucrose were added after 72 h of fermentation, to be hydrolyzed to 10 g/L glucose and 10 g/L fructose. The main reason for adding pure sucrose instead of molasses is to avoid the presence of nitrogen in the fermentation medium. The availability of nitrogen in the medium results in the nutritional resources being directed to biomass growth instead of lipid accumulation. However, when nitrogen is limited, the enzyme AMP deaminase is activated and results in a decrease in the mitochondrial AMP concentration, which is accompanied by an increase of accumulated citrate in mitochondria. Citrate is then transferred to the cytoplasm and converted to acetyl-CoA by ATP-citrate lyase [38]. Compared to batch cultures, the addition of pure sucrose resulted in an increase of the contents of total lipids and OCFAs by 49% and 56%, respectively (Figure 5). Furthermore, C17:1 content significantly increased by 61% compared to batch cultures. The percentage increase in lipid content was close to that obtained in a previous study [9], where 50.35% increase in total lipid by *Y. lipolytica* Δphd1 was observed after the addition of glucose and propionate at four time points during fermentation. However, for OCFA content, a 56% increase was obtained in the current study, compared to only 12.64% in the work of Park et al. [9]. Other previous studies have also showed that fed-batch fermentation enhanced the production of lipids by the obese strain *Y. lipolytica* JMY3501 cultivated on glucose [39], and by the strain *Y. lipolytica* BCC64401 cultivated on sugarcane bagasse hydrolysates [40]. The fed-batch fermentation conditions can be further optimized by testing various feeding rates of carbon sources to improve lipid and OCFA contents.

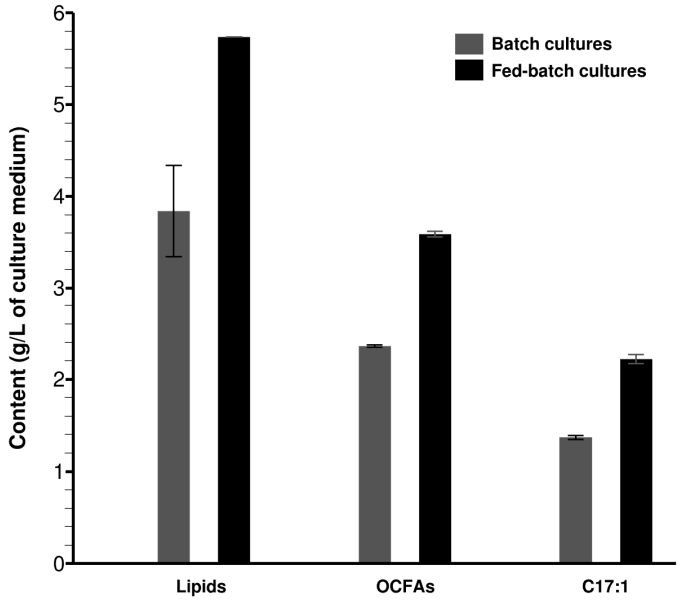

Figure 5. Lipids, OCFAs and C17:1 contents obtained after 160 h of fermentation by *Y. lipolytica* in 5-L bioreactors (batch and fed batch cultures).

4. Conclusions

In this study, it was shown that a genetically modified *Y. lipolytica* strain produced OCFAs using sugar beet molasses and crude glycerol as the main carbon sources. Therefore, microbial oil may be produced using low-cost by-products, thus providing a means to decrease the cost of fermentation. Total lipid and OCFA contents obtained after 160 h of fermentation in 5-L bioreactors using 20 g/L of sucrose were 3.84 ± 0.50 g/L g/L in sugar beet molasses and 2.36 ± 0.06 g/L of culture medium. Under the same fermentation conditions, C17:1 was the major OCFA produced, and yielded 1.37 ± 0.02 g/L of culture medium. In addition, a fed-batch culture in 5-L bioreactors further improved the production of total lipids, OCFAs, and C17:1 by 49%, 56%, and 61%, respectively. OCFAs are unusual lipids presenting several potential applications in different industrial fields. The improvement of fed-batch strategies by optimizing various feeding rates of carbon sources may lead to a further increase in OCFA contents in *Y. lipolytica*.

Author Contributions: M.K.: conceptualization, funding acquisition, project administration, supervision, writing—review and editing; S.E.K.: methodology, formal analysis, data curation, writing—original draft preparation; M.K. and S.E.K.: validation, investigation, resources, visualization. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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