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Evaluation and Identification of Key Economic Bottlenecks for Cost-Effective Microbial Oil Production from Fruit and Vegetable Residues

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Abstract: Intensive horticultural systems for the production of vegetables in greenhouses represent one of the main industries generating organic waste, as those that do not meet the quality standards for the fresh market or the processing industry are discarded. This highlights the importance of using these residues as raw material for other applications, such as bioenergy and bioproducts production, within the framework of a bio-based economy that maximizes the utilization of biomass resources in a sustainable manner. In this work, the microbial oil production from discarded pepper using the oleaginous yeast *Cryptococcus curvatus* was evaluated. Overall, a total lipid accumulation of 16.8 g/L was achieved with a fatty acid profile suitable to produce biodiesel. The lipid yield obtained was 0.12 g/g sugars. In addition, experimental results were used to assess the techno-economic feasibility of a proposed microbial oil plant using the software Aspen Plus. This plant yields approximately 96 kg of microbial oils/ton dry discarded pepper, with an estimated Minimum Selling Price of 7 €·kg^{-1}. These figures point out the necessity of increasing the yield of microbial oil production and considering the utilization of possible by-products, such as mannitol and cell debris, to improve the economic performance of the process.

Keywords: lipid; agri-food residues; oleaginous yeast; microbial oil; techno-economic analysis

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1. Introduction

The biorefinery concept, linked to the bio-based economy, has the general objective of integrating different processes for converting biomass into bioenergy and bioproducts of interest, getting waste/emission reduction, minimizing the exploitation of new resources, and valuing from feedstocks to by-products and final product according to the European Energy Research Alliance [1]. Inexpensive carbon sources and the use of optimized processes can lead to an increase in cost-effectiveness because of the reduction of economic and energy costs. One of the different strategies to achieve these objectives is the search of new sources of low-cost raw materials.

The importance of the fruit and vegetable sector in Europe is reflected in the magnitude of the total annual output value estimated, which was 60.7 billion euros in 2021. About 58% of this value corresponded to fresh vegetables. In 2020, the European Union countries (EU-27) produced 2.90 million tons of peppers and around 50% came from Spain [2]. In addition, more than 85% are produced under glass or accessible cover. The fruit and vegetable sector and, in particular, the horticultural intensive type systems dedicated to the production of greenhouse vegetables, represent one of the main industries generating organic waste. One of the organic residues generated in large volumes is the sorted pepper fruits that do not meet grade standards for either the fresh market or the processing industry. These fruits being commonly damaged, diseased, too small, and/or misshapen are among

the major discarded reasons. About 7–10% of peppers produced are sorted depending on crop type and market situation [3]. In the region of Almeria (Spain)—an area with a high concentration of greenhouses—the estimated volume of waste produced in 2021 accounted to 73,000–100,000 tons. These vegetable wastes traditionally ended up in landfills or were utilized for compost production [4]. However, nowadays, these residues are transferred to external waste management companies and authorized recycling plants for their treatment and recovery process, implying an extra cost to the farms. This highlights the importance of employing greenhouse crop residues as raw material for other applications, such as renewable energy sources, thus giving them a new added value [5]. Therefore, an attractive alternative to using this waste, rich in organic matter, is the production of biofuels and high-added value bio-products in a biorefinery concept.

Among the most attractive and promising feedstocks for biofuel production are microbial oils obtained from oleaginous organisms such as yeasts, microalgae, fungi, and bacteria [6]. Yeasts have certain advantages over the others mentioned, such as a short life cycle compared to fungi, a more suitable size for harvesting compared to bacteria, no dependence on light and climate unlike microalgae, as well as greater robustness in tolerating inhibitory compounds and growing using a diversified range of sugars and substrates [7]. These microorganisms can accumulate up to 70–80% of intracellular lipids per dry weight [8] and the fatty acid profile obtained presents an adequate composition for biodiesel manufacturing [9,10], as the oil obtained is rich in saturated and monounsaturated fatty acids [10].

In this study, the production of microbial oil for its subsequent transformation into biodiesel using fruits and vegetable residues, specifically discarded pepper waste (DPW), as feedstock is evaluated. The oleaginous yeasts *Cryptococcus curvatus* were selected for lipid production, as this yeast was previously used for this purpose [11]. The evaluation of the economy of new processes is essential to assess its competitiveness in the current market and to identify the main bottlenecks of the process [12]. Therefore, the present work also presents a techno-economic evaluation of the microbial oil production from discarded pepper residues using the experimental results obtained in our laboratory. For this purpose, the proposed processing plant was first modelled by the process simulation software Aspen Plus. The resulting simulation data were used as a basis for studying the economic viability of microbial oil production system.

2. Materials and Methods

2.1. Raw Material

The raw material used in this study has its origin in the horticultural sector of Spain. Among the different agri-food residues generated, DPW has been selected as a low-cost raw material to obtain a suitable microbial culture medium. The DPW was cut and crushed using a Danamix TR/bM-330 industrial blender for homogenization. After homogenization, the solid and liquid fractions were collected in a basket centrifuge (RTL2BD COMTEIFA). The liquid fraction was used as a culture medium after sterilization by filtration (Nalgene Rapid Flow^{TM} 0.22 µm).

2.2. Microbial Oil Production

The oleaginous yeast *Cryptococcus curvatus* CL6032 from Biobanco Nacional del Instituto de Salud Carlos III (BBN-ISCIII) was used in this study for the production of microbial oil. The liquid fraction of the DPW has been selected as the culture medium, as indicated in the previous section. Yeast cells were propagated in yeast extract-peptone-dextrose (YPD) medium containing 20 g/L glucose, 20 g/L peptone, and 10 g/L yeast extract. One loop of yeast cells was transferred to 50 mL YPD into 250-mL Erlenmeyer baffled flasks and incubated in a rotary shaker at 26 °C and 180 rpm for 24 h. Then, cells were harvested by centrifugation (3000× *g*, 5 min), washed once with sterile water, and inoculated into the corresponding fermentation media at an OD_{600nm} of 1–1.5. Fermentation was carried out in 0.5 L Applikon bioreactors (Applikon, Delft, The Netherlands) to better control

the conditions established with a culture volume of 0.25 L. The parameters set for lipid accumulation were 28 °C, pH 6, 1 vvm, and variable stirring to maintain dissolved oxygen at a 20% (*v/v)* from air saturation concentration. Samples were periodically collected to monitor the optical density by spectrophotometry at λ = 600 nm. The cell biomass was also measured by dry cell weight (DCW) using a pre-weighed 0.22 µm nitrocellulose membrane to filter the cells and a microwave (750 W for 10 min) to dry cell biomass. The sugar consumption was analyzed by HPLC, as described below in Section 2.3.1.

A pulse-feeding strategy was selected as the cultivation mode with a pure glucose solution. Glucose source pulses were added when sugar levels in the culture broth were relatively low or an increase of dissolved oxygen was observed. Two glucose pulses were added during the test to have a final concentration in the medium of 50 g/L per pulse. For this purpose, 50 mL of fermented medium were first collected to analyze sugars and cell biomass concentration and the intracellular lipid content. Then, the same volume of a concentrated pure glucose solution was added to have the final aforementioned glucose concentration. After 143 h of fermentation, all the cell biomass was collected by centrifugation at 5000× *g* and 4 °C for 15 min, washed with sterile water, and freeze-dried to proceed with the lipid extraction protocol.

2.3. Analysis

2.3.1. Raw Material and Culture Medium Composition

The carbohydrate content of the DPW fractions was analyzed following methods described elsewhere [13]. Sugar composition of the raw material and pulses, as well as the monitoring of sugar consumption by the yeast, was analyzed using HPLC Waters Alliance 2695 equipment, with a refractive index detector (detector 2414) equipped with a Carbo Sep CHO 782 chromatographic column (Transgenomic, Omaha, NE, USA) at a temperature of 70 °C. Degassed ultrapure water with 0.5 mL/min flow was used as a mobile phase. The determination of the nitrogen content in the medium was carried out according to the Kjeldahl method.

2.3.2. Lipids and Fatty Acid Profile Determination

To determine the total percentage of lipids accumulated in the cells, a cell lysis and lipid extraction protocol with 3.2 mL of HCl 4M at 55 °C for 2 h and a mixture of chloroform/methanol (2:1 *v/v*) at 20 °C for 3 h, respectively, were applied. Finally, after centrifugation at 2000× *g* for 15 min, the lipids were collected in the chloroform phase that was evaporated under a nitrogen stream and were quantified using the gravimetric method (data are expressed as g lipid/100 g dry cell biomass) [14].

For the analysis of the fatty acid profile and its quantification, a gas chromatograph (Agilent 7890A) was used, equipped with a flame ionization detector (FID) and a split injector. A polysiloxane capillary column DB-23 (Agilent, Santa Clara, CA, USA), length 30 m and 25 mm id, the split ratio was 1/20, the injector temperature was 250 °C, and the detector temperature was 280 °C, respectively. This analysis was carried out following the determination of total lipids as fatty acid methyl esters (FAME) protocol previously described by NREL [15].

2.4. Techno-Economic Assessment

2.4.1. Process Description

Based on the experimental results obtained in this work, a microbial oil production plant design using DPW as raw material is proposed. According to the amount of DPW produced in Almeria, the projected annual capacity of the proposed plant is 100,000 tons per year of DPW. Figure 1 shows a simplified diagram, including the main stages of the proposed microbial oil production process.

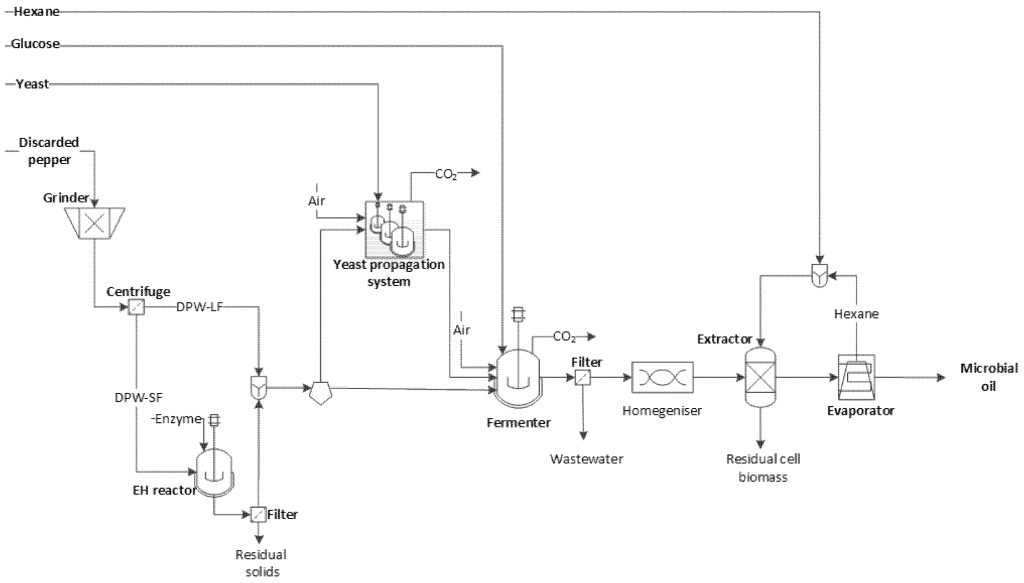

Figure 1. Simplified diagram of the microbial oil production plant based on discarded peppers.

First, in the processing plant, the DPW raw material is crushed. The resulting stream is then subjected to a solid–liquid separation in a decanter centrifuge, resulting in a solid fraction (DPW-SF) containing mainly glucans and a liquid fraction (DPW-LF) with glucose and fructose as the main components. Then, the solid fraction undergoes an enzymatic hydrolysis (EH) process at 20% (*w*/*w*) solid loading, producing a sugar-rich media stream. This process is carried out at 50 °C with 150 mg/g of glucans of cellulase enzyme blend (Sigma Aldrich, Saint Louis, MI, USA) for 72 h, reaching an EH yield of 65.0% of the theoretical [16,17]. After HE, the resulting stream is filtered to remove the residual insoluble solids and mixed with the DPW-LF stream. Then, 10% of the resulting stream is used for cell propagation using the oleaginous yeast *C. curvatus* CL6032. The rest of the liquid stream obtained is then subjected to fermentation process to microbial oil production. This fermentation process is operated in a fed-batch mode with the addition of pure glucose (1.7 g glucose/g sugars in DPW) at 28 °C and 0.4 vvm for 140 h. The cell propagation phase is carried out in batch mode for 24 h using the same conditions as the fermentation phase. After fermentation, cells are harvested and mechanically disrupted in a homogenizer. Subsequently, the stream obtained is subjected to an extraction process with hexane (25% (*w*/*w*) yeast in hexane), followed by an evaporation process in order to collect the microbial oils and recover the hexane [18]. Approximately 67.3% lipid recovery yield is achieved [18]. Finally, the hexane stream is recirculated into the extraction unit.

2.4.2. Data Acquisition

The microbial oil production system was modelled in Aspen Plus v12.0 (Aspen Technology Inc., Bedford, MA, USA) for obtaining all the data required for the techno-economic evaluation. Physical property data for cellulose, hemicellulose components, and enzymes were taken from the literature [19]. The Non-Random Two Liquid (NRTL) thermodynamic model was used.

The simulation of the main units of the processing plant is described below. The enzymatic hydrolysis unit was modelled using a stoichiometric block (RStoic block). The microbial oil production in the fermentation reactor was also simulated based on a stoichiometric approach. For this purpose, a series of stoichiometric reactions representing the microbial growth and the microbial oil accumulation phases were implemented [20]. The yeast propagation process was simulated similarly to the fermentation reactor, but in this case, only the reactions associated with the microbial oil growth phase were considered. The reactions considered to model the fermentation and yeast propagation processes are summarized in Table 1.

Table 1. Reactions included in the simulation model.

Process	Reaction
Microbial growth	C6 sugars + 4.3 O_2 + 0.35 amino acid → yeast + 4.1 CO_2 + 5.2 H_2O
Microbial growth	C5 sugars + 3.3 O_2 + 0.35 amino acid → yeast + 3.1 CO_2 + 4.2 H_2O
Microbial oil accumulation	13.5 C6 sugars + O_2 → $C_{57}H_{104}O_6$ + 24 CO_2 + 29 H_2O
Microbial oil accumulation	12.5 C6 sugars +2.5 O_2 → $C_{51}H_{98}O_6$ + 24 CO_2 + 26 H_2O
Microbial oil accumulation	13.5 C6 sugars +2.5 O_2 → $C_{57}H_{98}O_6$ + 24 CO_2 + 32 H_2O
Microbial oil accumulation	14 C6 sugars +2.5 O_2 → $C_{57}H_{110}O_6$ + 27 CO_2 + 29 H_2O

In this model, the yeast cell biomass was defined as $C_4H_{6.5}O_{1.9}N_{0.7}$ and its physical properties were calculated according to Popovic [21]. As a nitrogen source in the microbial growth phase, all the required nitrogen was considered to be present in the raw material in the form of organic nitrogen. In this simulation, this component was modelled as an amino acid, specifically defined as lysine. The elemental composition of triglycerides was defined based on the primary fatty acids obtained in the lipid profile. All these triglycerides were defined considering each acid as the only fatty acid of each triglyceride: oleic acid-based triglyceride ($C_{57}H_{104}O_6$), palmitic acid-based triglyceride ($C_{51}H_{98}O_6$), linoleic acid-based triglyceride ($C_{57}H_{98}O_6$), and stearic acid-based triglyceride ($C_{57}H_{110}O_6$).

Based on the results of the mass and energy balances generated in the simulation, the capital and operating costs were calculated using the software Aspen Economic Analyzer V12.0 (Aspen Technologies, Inc., Bedford, MA, USA). To evaluate the economic viability of the process, the Net Present Value (NPV) and the minimum selling price (MSP) were calculated. The NPV (Equation (1)) is an effective parameter for determining whether a project is potentially profitable ($NPV > 0$) or unprofitable ($NPV < 0$). The MSP is defined as the minimum price at which the profitability of a project is zero, i.e., there is no profit or losses, and is estimated as the value at which the NPV equals zero.

$$NPV = \sum_{t=0}^{n} \frac{C_t}{(1+i)^t} \quad (1)$$

where t, n, i, and C_t indicate the project year, project lifetime, discount rate, and net cash inflow–outflows in year t, respectively. The analysis was made in euros for a 20-years life period, and depreciation expenses were calculated using the straight-line method. The interest rate and the income tax were fixed at 10% and 25%, respectively [22]. Table 2 shows raw material, chemicals, utilities, and labor costs.

Table 2. Market prices of the raw material, chemicals, utilities, and labor.

Compound	Value	Unit	Comment
DPW	−6.0	€/t	[23]
Enzyme	284.0	€/t	[24]
Process Water	1.3	€/m^3	[25]
Glucose	384.0	€/t	[26]
Hexane	491.5	€/t	[27]
Cooling Water	3.9	€/m^3	[28]
Electricity price	80.0	€/MWh	[29]
Steam	14.1	€/GJ	[28]
Labor cost	2874.0	€/worker·month	[30]

3. Results and Discussion

3.1. Raw Material Composition

Table 3 shows the composition of the DPW, the raw material used in microbial oil production, which was the starting point for calculating yields in the different stages proposed in this study. The chemical composition of this material had a high percentage of extractable compounds (61.4%), of which 94.2% are extractable compounds in water,

with sugars as the main element. These extractable compounds were mainly made up of fructose (28.5%) and glucose (18.1%). The total extractable sugars in the DPW account for 50.7%. The content of structural carbohydrate analyzed was 11.0%, glucans being the major component, followed by mannans. These carbohydrates could be used by performing a hydrolysis stage with enzymatic catalysts and then used as a carbon source in the culture of oleaginous yeasts. The DPW presents a total of 62.8 g of potential sugars per 100 g of dry DPW. Therefore, this material could be used to produce microbial oil in a biotransformation process mediated by oleaginous yeast. It should be noted that 80% of potential sugars are presented in the aqueous extract.

Table 3. Discarded pepper waste (DPW) composition. Data expressed as percentage (*w*/*w*) on dry weight (DW) basis.

Component	% (*w*/*w*)
Organic solvent-extract	3.6 ± 0.1
Aqueous extract:	57.8 ± 1.2
Glucose	18.1 ± 0.5
Fructose	28.5 ± 0.0
Sucrose	2.2 ± 0.1
Galactose	0.6 ± 0.0
Xylose	0.4 ± 0.1
Arabinose	0.2 ± 0.0
Mannose	0.7 ± 0.0
Glucan	5.6 ± 0.12
Xylan	1.4 ± 0.1
Galactan	1.1 ± 0.0
Arabinan	0.7 ± 0.0
Mannan	2.2 ± 0.1
Acetyl groups	0.9 ± 0.2
Acid-insoluble lignin	17.1 ± 0.5
Whole Ash	5.5 ± 0.1

In order to recover the sugars contained in the extractable fraction, a simple fractionation was carried out, which combines a mechanical stage by crushing and homogenization and a separation by centrifugation. This resulted in two separate fractions—the DPW-LF and DPW-SF fraction. Table 4 includes the composition of both fractions. The available sugars in the DPW-LF fraction were approximately 60 g/L (glucose and fructose). The nitrogen concentration was approximately 1.7 g/L.

Table 4. DPW-LF and DFW-SF fraction composition.

DPW-LF Composition	Concentration, g/L	DPW-SF Composition	%, *w*/*w*
Glucose	24.3 ± 0.8	Organic solvent-extract	10.8 ± 2.4
Xylose	0.4 ± 0.0	Aqueous extract:	23.0 ± 2.2
Galactose	0.4 ± 0.1	Sugars	7.3 ± 3.7
Arabinose	0.1 ±0.0	Glucan	21.3 ± 1.1
Mannose	0.4 ± 0.2	Xylan	2.3 ± 0.1
Fructose	35.7 ± 1.5	Galactan	2.7 ± 0.1
Sucrose	0.5 ± 0.2	Arabinan	0.7 ± 0.0
Mannitol	0.4 ± 0.0	Mannan	2.1 ± 0.2
Total nitrogen	1.7 ± 0.0	Acetyl groups	0.9 ± 0.2
		Acid-insoluble residue	17.1 ± 0.5
		Whole Ash	3.1 ± 0.0
		Total Nitrogen	2.0 ± 0.3

3.2. Microbial Oil Production

The accumulation of microbial oil was favored with the fed-batch strategy by adding glucose pulses, as presented in Table 5. The DPW-LF supported yeast growth, as can be seen in Figure 2, reaching a biomass concentration of more than 38 g/L of DCW within 24 h and more than 40 g/L of DCW at the end of the process. Sugars were depleted after 21 h of cultivation. At that time, a pulse of glucose was added to continue with the process following the fed-batch strategy (Figure 2). This step was repeated before complete glucose depletion at 53 h to avoid lipid consumption by the yeast, since it may use the lipid as a carbon and energy source in the absence of sugars, and therefore lipid accumulation would be reduced [31]. During the fed-batch process, mannitol was also found in the cultivation media, as depicted in Figure 2, reaching up to 28 g/L. The literature reports the ability of other yeasts to produce mannitol, such as the oleaginous yeast *Yarrowia lipolytica* [32], but it had not been reported in *C. curvatus* previously.

Table 5. Lipid content and lipid production by *C. curvatus* at the start and end of the test and before the two glucose pulses added.

	Lipid Content (%, *w*/*w*)	Lipid Production (g/L)
Initial	7.0	0.2
Before the first glucose pulse	8.6	3.3
Before the second glucose pulse	14.3	6.2
Final	32.6	16.8

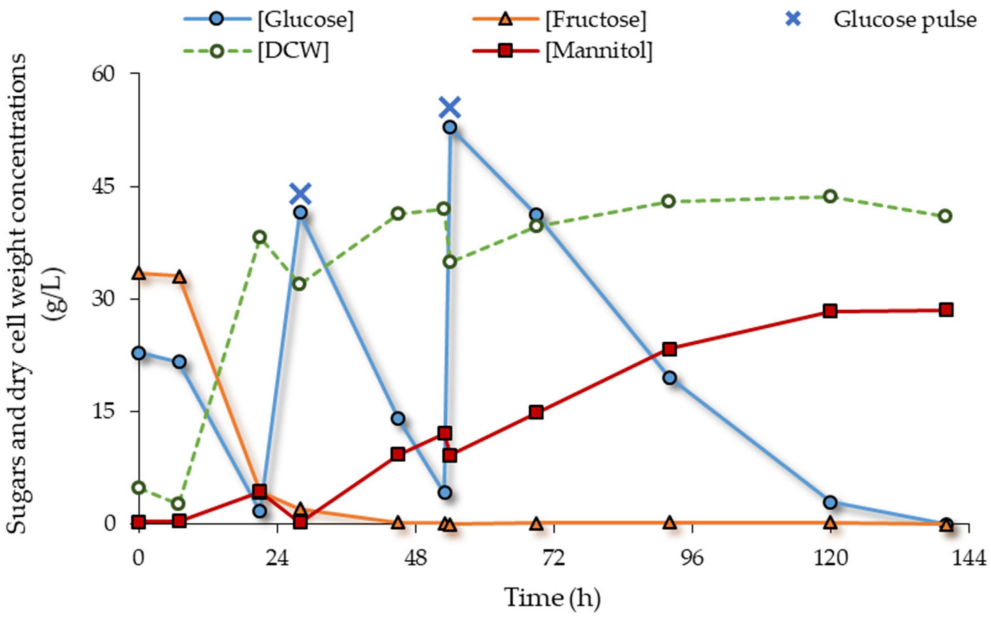

Figure 2. Time course of sugars and cell biomass during the fed-batch culture by *C. curvatus* on DPW-LF through glucose pulses.

The lipid content at time 0 h was 7.0% (*w*/*w*) of the total DCW, representing the lipid content that these cells naturally present in their membranes and organelles since they have not yet started the accumulation phase. Before the first pulse (21 h), the lipid content was 8.6% (*w*/*w*) of DCW, which increases to 14.3% (*w*/*w*) of DCW before the second pulse of glucose (53 h) and to 32.6% (*w*/*w*) of DCW at the end of the assay (143 h), reaching 16.8 g/L of lipids (Table 5). Under the nitrogen-limited condition and in the absence of other nutrient sources, the addition of a carbon source to the medium triggers the lipid biosynthesis in this oleaginous yeast [33–35].

Concerning FAME content in yeasts, a maximum of 29.2% was reached at the end of the assay. Oleic (C18:1), palmitic (C16:0), and linoleic (C18:2) acids represented 90% of the total

fatty acid profile at the end of the experimental trial (Table 6). This fatty acid composition is similar to vegetable oils with potential for biodiesel production (Figure 3), with a profile composed predominantly of 16 and 18 carbon atoms [31] (Table 6). The balance between long-chain fatty acids, saturated and unsaturated, indicates an appropriate composition in the profile in terms of biodiesel properties. The saturated ones improve the oxidative stability and cetane index and the unsaturated ones improve the properties of cold flow and pour point [36].

Table 6. Fatty acid composition represented as % (*w*/*w*) of the total lipid produced by *C. curvatus* before and after the fermentation process and before each glucose pulse.

	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3
Initial	11.1	0.0	9.9	30.1	35.2	13.4
Before the first glucose pulse	15.2	0.0	6.7	50.2	21.7	6.1
Before the second glucose pulse	22.3	0.0	8.0	53.5	13.2	3.0
Final	27.4	1.3	5.1	50.7	13.3	2.2

C16:0 (Palmitic acid), C16:1 (Palmitoleic acid), C18:0 (Stearic acid), C18:1 (Oleic acid), C18:2 (Linoleic acid), C18:3 (Linolenic acid).

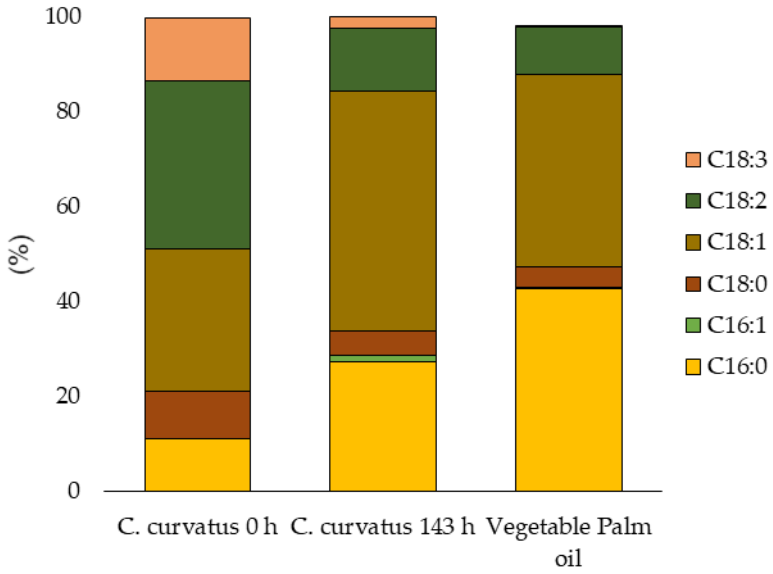

Figure 3. Comparison between the fatty acid profile of the lipids produced by *C. curvatus* at 0 h and 143 h, and the profile resulting from vegetable palm oil. Data source for vegetable palm oil: Demirbas [37].

In order to compare the lipid yield achieved in this work with other works in the literature, Table 7 summarizes the results of lipid production by different strains of *C. curvatus* growing on other low-cost carbon sources.

The lipid yield obtained in this work was 0.12 g/g sugars. This lipid yield was higher than that obtained by Carota et al. [9], growing *C. curvatus NRRLY* on orange peel waste and under batch culture conditions. However, as can be noted in Table 7, this result is lower than others reported in the literature, with *C. curvatus* cultivated in fed-batch strategy. For instance, a lipid yield of 0.18 g/g sugars was obtained with *C. curvatus ATCC 20509* grown in organosolv-pretreated corn stover enzymatic hydrolysate [39], and in deproteinized cheese whey and wine lees hydrolysate [40]. The lower yield in lipids compared to other studies may be due to the high yield in cell biomass concentration reached in this work (0.43 g DCW/g sugar), along with the coproduction of process by-products, such as mannitol. In contrast, this process can benefit from avoiding complex pretreatments to obtain free sugars from these types of raw material, since it is mainly composed of monomeric sugars as previously discussed.

Table 7. Metrics of lipid production by several *C.curvatus* strain growing on low-cost carbon source.

Yeast	Culture Mode	Carbon Source	Lipid (g/L)	Lipid (*w*/*w*)	Lipid Yield (g/g)	Reference
NRRLY-1511	Batch (shake flasks)	Orange peel waste	1.74	15	0.09	[9]
ATCC 20509[a]	Fed-batch (Bioreactor)	Wheypermeate	26.0 [b]	35	-	[38]
ATCC 20509	Fed-batch (Bioreactor)	Corn stover enzymatic hydrolysates	31.3	61.7	0.18	[39]
ATCC 20509	Fed-batch (Bioreactor)	Deproteinized cheese whey and wine lees hydrolysate	33.1	49.6	0.18	[40]
ATCC 20509	Fed-batch (Bioreactor)	Crude glycerol	31.2–32.9 [c]	44.2–52.9	0.21–0.26[c]	[41]
CL6032	Fed-batch (Bioreactor)	Discarded pepper	16.8	32.6	0.12	This work

[a] *Apiotrichum curvatum;* [b] Estimated based on [39]; [c] Estimated based on [42].

3.3. Techno-Economic Assessment Results

Considering the model described in Section 2.4, the amount of microbial oil produced, along with the material and energy inputs obtained from the process simulation, are shown in Table 8. The amount of microbial oil obtained for a 300 tons per day DPW biomass plant is 3.5 tons per day. These values correspond to a total yield of 96 kg of microbial oils per ton of dry DPW raw material. In a similar study performed by Koutinas et al. [20], higher microbial oil yields (approximately 230 kg per ton of dry raw material) were obtained. However, these results are not comparable since that study used pure glucose as the sole raw material, and assumed higher glucose to lipids conversion yields, 0.23 g of lipids/g of sugars compared to the 0.12 g of lipids/g of sugars obtained in this study, with DPW as raw material and a pulse-feeding strategy.

Table 8. Main material and energy inputs and outputs of the microbial oil process studied.

Compound	Amount	Unit
DPW	100,000	t/year
Enzyme	68	t/year
Process Water	9096	m^3/year
Glucose	8496	t/year
Hexane	257	t/year
Cooling Water	220,250	GJ/year
Steam	3673	GJ/year
Electricity	44,063	MWh/year
Microbial oil	1153	t/year

With the aim of evaluating the economic feasibility of the proposed microbial oil production system, both total project costs (including installed equipment costs and other costs such as field expenses, project contingencies, engineering costs, etc.) and operating costs were calculated from the simulation data. A total installed equipment cost of €30.3 million and a total project cost of €48.4 million were obtained.

The annualized operating cost obtained for the microbial oil production plant is presented in Table 9. Operating cost includes all material (DPW raw material, chemical compounds, enzymes, and water), utilities, depreciation expense, labor cost, maintenance and administrative cost, and insurance. The utilities consumed were identified as the most significant contributor to the total operating cost, accounting for approximately 35% of the cost. This high contribution is due to the electricity consumed in the process, mainly associated with the agitation and aeration of the fermenters and yeast propagation reactors necessary to produce the microbial oil. Similar results have been found by other

authors [18,20], who identify the bioconversion section as the major contributor to the electricity consumption of the process. Depreciation expense and inputs also show significant contributions with shares of 19% and 17% of total operating cost, respectively.

Table 9. Annualized operating cost for the microbial oil process studied.

	Cost	Unit
Inputs	2.1	M€/year
Utilities	4.4	M€/year
Depreciation expense	2.4	M€/year
Labor cost	1.8	M€/year
Maintenance	1.5	M€/year
Administration and insurance	0.3	M€/year
Total operating cost	12.6	M€/year

Figure 4 shows the contribution of each input category to the total input cost. Total input costs are dominated by the cost of the pure glucose added to the fermenters, accounting for approximately 94% of the total input cost. The remaining inputs contribute individually with percentages below 5%.

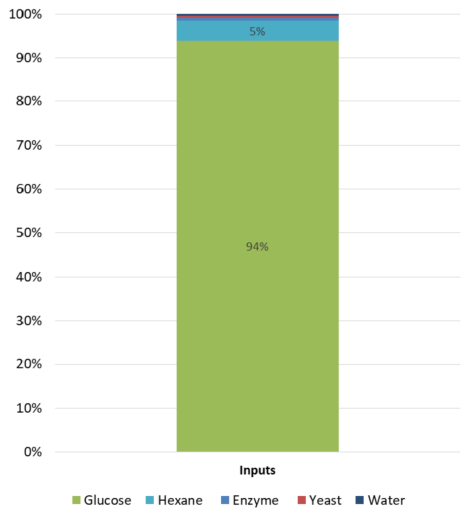

Figure 4. Share of each input category to the total input cost.

The economic feasibility of the proposed plant was evaluated by estimating the MSP of the microbial oil and comparing this value with that of conventional vegetable oils and other values reported in the literature. According to Section 2.4.1, the microbial oil MSP is estimated as the value at which the NPV equals zero. For the present modelled plant with a production of 1153 t/year of microbial oil, the estimated MSP of the microbial oil was approximately 17 €/kg. This value is substantially higher than the price for common vegetable oils (1–2 €/kg) [42] and exceeds the price estimated in other similar models presented in the literature. For instance, Koutinas et al. [20] obtained a price of approximately 5.2 €/kg using pure glucose as raw material. Parsons et al. [42] reported a MSP between 4 and 14 €/kg depending on the scale of the plant (100–10,000 t/year) and the raw material used (sucrose or wheat straw). However, these studies assumed an overall sugar to microbial oil yield of about 0.23–0.28 g/g of sugars, which is approximately 2–2.5 times higher than the present model. These results point to the necessity of increasing the yield of microbial oil production. To evaluate this point, the influence of microbial oil yield on the MSP is illustrated in Figure 5. In this process, to achieve an MSP lower than 7 €/kg, it would be necessary to reach microbial yields close to the theoretical.

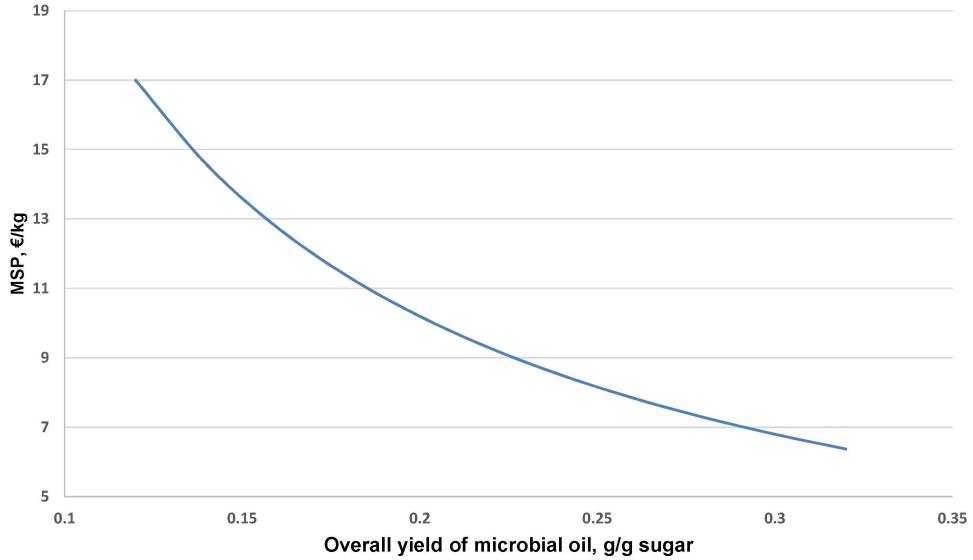

Figure 5. Influence of overall microbial yield on the microbial oil MSP.

Although the plant presented does not show a high economic performance compared to other studies in the literature, it is important to remark that the production of microbial oils from residual feedstocks, such as discarded vegetables and fruits, is in the early stages of research. Therefore, these bioprocesses have a wide margin for improvement. In addition, other high value-added bioproducts not considered in this economic analysis, such as the use of cell debris for animal feeding and/or the coproduction of mannitol, could be obtained, contributing to the economy of this process from a biorefinery perspective and significantly improving the profitability of the plant.

4. Conclusions

In this work, the microbial oil production from discarded pepper using the oleaginous yeast *Cryptococcus curvatus* was evaluated. Through this process, the production of lipids with a fatty acid profile suitable for use in biodiesel together with other valuable compounds, such as mannitol, was achieved. The software Aspen Plus was successfully applied using the experimental results to obtain the mass and energy balance needed for the techno-economic evaluation of the microbial oil plant proposed. Although the MSP of microbial oil obtained in the proposed plant is quite high, the use of fruits and vegetables residues represent a novel approach in this kind of processes that can be further optimized. The electricity consumed in the process, associated with the agitation and aeration of the fermenters and yeast propagation reactors, along with the cost of the pure glucose added to the fermenters during the pulses, are identified as the main contributors to the operations cost. These results point out the need to increase the yield of microbial oil production, to the search for a more economical alternative carbon source to pure glucose, and to consider the utilization of possible by-products, such as mannitol and cell debris, to improve the economic performance of the process.

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