Research Article

Biodegradation and utilization of waste cooking oil by *Yarrowia lipolytica* CECT 1240

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Biodegradation of waste cooking oil and its application as lipase production inducer in cultures of *Yarrowia lipolytica* CECT 1240 have been investigated, both in shake flasks and a bench-scale bioreactor. The ability of this strain to degrade the spent oil was evaluated by monitoring COD throughout the cultures, and a remarkable decrease was recorded (almost 90% decrease in oil COD after 3 days in bioreactor). Moreover, the addition of waste cooking oil to the medium led to a significant augmentation in extracellular lipase production by the yeast, compared to oil-free cultures. This confirms the suitability of the studied residue as an inducer of lipase biosynthesis, which is a very interesting fact, from an economic standpoint. These results were confirmed when a fed-batch strategy was proposed. Finally, some properties of the crude enzyme were studied, and compared to the enzymes obtained when non-used oil was added to the medium.

Practical application: Nowadays the search of new strategies to valorize wastes from the food and agro industries is attracting a great scientific interest due to the important advantages offered from an economic and environmental point of view. For this reason, the yeast *Yarrowia lipolytica* CECT 1240 is proposed for degrading waste cooking oils. This approach entails also another benefit in terms of lipolytic enzyme synthesis, since the addition of used up oils has a lipase inducer effect. The enormous interest of lipases is reflected in the number of applications that they present, which justify the research work proposed here. The process was successfully carried out both in shake flasks and a bench-scale bioreactor, allowing producing high levels of lipolytic activity at the same time that the COD was diminished up to nearly 90%.

Keywords: biodegradation / cooking oil / lipase / waste / *Yarrowia lipolytica*

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1 Introduction

Large amounts of cooking oils, subjected to high temperatures (160–200 °C) for relatively long periods of time, are daily produced throughout the world. As a result of the harsh conditions, oxidation of fatty acids and their subsequent transformations may cause unpleasant flavors and odors, which severely limits long-term repeated use [1, 2]. Some procedures have been proposed to eliminate free fatty acids and extend oil usability [3]; however, they are seldom utilized. Disposal of this kind of polluting wastes has become an increasingly distressing matter. The immiscibility of oil in water, albeit facilitating separation by flotation, hinders transportation through pipelines, disposal, and biodegradation. When poured down drains, they result in coating and eventual occlusion of drainage and sewage pipes [4].

From an economic standpoint, the development of feasible ways for reutilization of waste edible oils and fats would be highly desirable. Traditionally, they have been used for soap fabrication, as additives in domestic animals feedstock, or for other creative applications such as the fabrication of an

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Abbreviation: COD, chemical oxygen demand; **OMW,** olive mill wastewater

inexpensive cleaning agent for soot and carbon residues on machine parts and firearms [5]. However, most recent research contributions on this topic focus on the utilization of waste vegetable oils as raw material for the production of biodiesel [6], an alternative environmentally friendly fuel for Diesel engines composed of monoalkyl esters of long chain fatty acids. Utilization of waste oil would help to reduce production costs for this combustible, therefore contributing to resolve one of the main obstacles to its extensive utilization [1, 7–9]. Additionally, the use of spent vegetable oil as diesel engine fuel, following thermal cracking to reduce its viscosity, has been proposed [10].

Biodegradation of vegetable oils has been studied mainly in relation to their incorporation (after chemical modification) to the formulae of lubricants and greases [11], or the treatment of accidental oil spills [12–14]. A wide number of microorganisms, both aerobic and anaerobic, are able to break up vegetable oils, although chemical characteristics of the oil could determine its biodegradability [12]. Vegetable oil biodegradation occurs in a similar way both in the presence or absence of oxygen, through hydrolysis of triglycerides and beta oxidation of released fatty acids. High oil concentrations may inhibit anaerobic degradation under methanogenic conditions, although this may be solved if iron-reducing conditions are induced [14]. The design of strategies involving microorganisms, more specifically yeasts to simultaneously degrade waste oils and obtain high added-value products would be an interesting endeavor, both from an economic and environmental angle [15, 16].

Microbial lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) are considered as extremely useful biocatalysts due to their numerous potential applications, such as food and detergent manufacture and specific synthesis of fine chemicals or chiral drugs [17]. They catalyze both hydrolysis and synthesis reactions (*i.e.*, esterification, transesterification), and are usually stable in organic solvents and at relatively high temperatures [18–21]. Many microorganisms are able to produce extracellular lipases, although nutritional and physico-chemical factors (*i.e.*, temperature, pH, nitrogen, and carbon sources, presence of lipids, inorganic salts, agitation, oxygen concentration) strongly influence the levels of enzyme expression [17, 22]. Although the addition of vegetable oil to the culture medium has been repeatedly used to trigger microbial lipase production, the possibility of using waste cooking oil has not been evaluated so far. If successful, this approach could help to diminish lipase production costs, and also contribute to lessen the environmental problem posed by used frying oils.

The lipase production ability of several strains of *Yarrowia lipolytica* has been investigated [23–35]. Some researchers have proposed the utilization of this yeast to reduce contamination in olive mill wastewater (OMW). They obtained promising results in terms of COD reduction, and a significant decrease in polyphenol content [36]. In previous works, our group indicated rather high levels of extracellular lipase in submerged and solid-state cultures of *Y. lipolytica* CECT 1240 [27, 28]. The influence of several lipids and surfactants on biomass and lipolytic enzyme biosynthesis was examined, and the best results were obtained when vegetable oils were added to the culture medium.

In this work, the ability of *Yarrowia lipolytica* CECT 1240 (ATCC 18942) to grow on a liquid medium supplemented with waste cooking oil has been investigated. The variation in chemical oxygen demand (COD) has been monitored throughout the cultures, in order to assess the viability of the process as a decontamination method. Also, the levels of lipase production have been determined to evaluate the efficiency of the spent oil as lipolytic enzyme production inducer.

2 Materials And Methods

2.1 Microorganism and media

Yarrowia lipolytica CECT 1240 (ATCC 18942) was grown on peptone-malt extract agar plates. The medium consisted of (g dm^{-3}, in distilled water): glucose 10, peptone 5, yeast extract 3, malt extract 3, and agar 20. Plates were incubated at 30 °C for 7 days and stored at 4 °C between transfers.

Submerged cultures were carried out using a basal medium [23] prepared in distilled water, containing (per dm^3): 20 g glucose, 2 g urea, 1 g KH_2PO_4, 0.5 g $MgSO_4{\cdot}7H_2O$, 0.1 g $CaCl_2$, 0.1 g NaCl, 0.5 mg H_3BO_3, 0.2 mg $FeCl_3{\cdot}4H_2O$, 0.4 mg $ZnSO_4{\cdot}7H_2O$, 0.4 mg $MnSO_4{\cdot}H_2O$, 0.1 mg KI, 0.04 mg $CuSO_4{\cdot}5H_2O$, 200 μg thiamin, 8 μg D-biotin. The medium without urea and vitamins was sterilized at 121 °C for 20 min. After cooling, the vitamins and urea previously sterilized by microfiltration (0.22 μm) were added to the basal medium.

2.2 Culture conditions

First, the microorganism was grown in 0.250 dm^3 Erlenmeyer flasks containing 0.15 dm^3 of basal medium, in order to obtain the inoculum for oil degradation and lipase production. A volume of 0.01 dm^3 of 0.9% NaCl solution was added to a previously grown agar plate, and 10^{-3} dm^3 of the resulting suspension was added to the liquid medium as inoculum. The flasks were incubated in an orbital shaker (New Brunswick, Innova 4400) at 30 °C, 150 rpm, for 48 h.

Flask cultures for the investigation of waste oil biodegradation and lipase production were carried out in 0.25 dm^3 Erlenmeyer flasks containing 0.15 dm^3 of basal medium, to which a fixed concentration (1–30 g dm^{-3}) of waste cooking oil was added. This oil was collected in local restaurants and was mainly composed by oleic acid (31%) and linoleic acid (55%), with a free fatty acid content of 5.2%. The culture medium was emulsified for 7 min at 995 $rad{\cdot}s^{-1}$ (Homogeniser IKA T25) before autoclaving. The flasks were inoculated 2% v/v with the freshly prepared cultures of *Yarrowia lipolytica*, and incubated in an orbital shaker at

150 rpm and 30 °C. They were capped with cellulose stoppers, which permit passive aeration. Some experiments were also performed on the same medium with only 5 g dm^{-3} glucose and 20 g dm^{-3} oil. Control cultures were carried out without addition of waste oil.

In order to evaluate and compare the characteristics of the produced enzymes, additional flask cultures were performed, using the basal medium with 5 g dm^{-3} glucose and 10 g dm^{-3} olive oil, sunflower oil and waste-cooking oil, respectively.

Bioreactor cultures were carried out in a 5-L stirred tank bioreactor (Biostat B, Braun, Germany), containing 3 L of basal medium with 5 g dm^{-3} glucose and 30 g dm^{-3} waste cooking oil. Airflow and agitation speed were 1 L min^{-1} and 400 rpm, respectively.

Experiments were done in duplicate and samples were analyzed in triplicate. The values in the figures correspond to mean values with a deviation less than 15%.

2.3 Sample preparation

In all cases, samples (whole flask content or 10 mL sample in flask and bioreactor cultures, respectively) were centrifuged for 10 min at 5000 g and 5 °C. The supernatant was recovered, homogenized at 9500 rpm for 1 min (Homogenizer IKA T25), and utilized for COD and lipolytic activity determination. The solid residue was thoroughly washed, and resuspended in distilled water for biomass determination.

2.4 Analytical methods

Lipolytic activity was determined spectrophotometrically using *p*-nitrophenyl myristate (pNPM) as substrate. The reaction medium consisted of 10^{-4} dm^3 sample, 8×10^{-4} dm^3 50 mmol dm^{-3} Tris–HCl buffer (pH 7.2) containing 40 mM $CaCl_2$, and 10^{-4} dm^3 pNPM 25 mmol dm^{-3} dissolved in ethanol. The hydrolysis reaction was allowed to proceed for 20 min at 30 °C and afterwards stopped by adding 2.5×10^{-4} dm^3 of 2 mmol dm^{-3} Na_2CO_3. The mixture was kept on ice for at least 15 min before being centrifuged (5000 g, 10 min). The absorbance of the supernatant was monitored at 400 nM in a Unicam Helios β (Thermo Electron Corp.) spectrophotometer. Under alkaline conditions, the molar extinction coefficient was 17.215 ± 0.002 $mmol^{-1}$ cm^{-1}. One activity unit was defined as the amount of enzyme that produced 1 μmol of *p*-nitrophenol per min under standard assay conditions. The activities were expressed in U dm^{-3}.

Biomass concentration was measured via turbidimetry at 600 nm, and the obtained values were converted to g cell dry wt dm^{-3} using a previously determined calibration curve.

The COD was determined following standard method UNE 77004 [37]. Oil-associated COD in the cultures was calculated by substracting the COD obtained in control cultures, to which oil had not been added.

2.5 Enzyme characterization

Enzyme activity-pH profiles were determined by measuring lipolytic activity on *p*-nitrophenyl myristate, as indicated above, using different buffer solutions in the reaction mixture: citrate-phosphate for pH 4, phosphate for pH 6-7, Tris–HCl for pH 8–9. Enzyme activity-temperature profiles were obtained by determination of lipolytic activity by the described method, at pH 5.0 and different incubation temperatures (30–60 °C). The results were expressed as percentages, referred to the maximum activity value.

Enzyme stability toward pH was determined by incubating samples in suitable buffer solutions at 30 °C, and measuring residual lipolytic activity after 24 h. Enzyme thermostability was assessed by incubation of samples in a pH 5.0 buffer at several temperatures, and measuring residual lipolytic activity after 1 h.

3 Results And Discussion

There are several end-uses for waste frying oils, such as the production of soaps or of energy by anaerobic digestion, thermal cracking, and more recently, the production of biodiesel. The development of a procedure allowing simultaneous biodegradation and reutilization of waste cooking oils, while producing a high added-value product would be of great interest, both from an economic and environmental point of view. In this work, used up vegetable frying oil has been added to the culture medium of *Yarrowia lipolytica* CECT 1240, a lipase producer. The level of oil biodegradation has been assessed, as well as the ability of this residual material to act as lipase production inducer by the microorganism.

3.1 Shake-flask cultures

Biodegradation of waste cooking oil in submerged cultures of *Yarrowia lipolytica* CECT 1240 has been investigated. Initially, the yeast was grown in Erlenmeyer flasks on a liquid medium to which a certain amount (20 g dm^{-3}) of the spent oil was added, and the variation in COD after 6-days culture time was compared to that observed in control cultures (no added oil). Two different initial glucose concentrations (5 and 20 g dm^{-3}) in the culture medium were considered, as model concentrations providing different levels of cell growth [38]. The aim of this experiment was to find out if carbon source depletion could further oil consumption by the microorganism as reported Carvalho *et al.* [39]. A glucose consumption rate of 1.8 g dm^{-3} day^{-1} was observed throughout the cultivation period (6-days). In the culture with 5 g dm^{-3} of glucose, total glucose depletion was found after 3 days. Nevertheless, 8 g dm^{-3} of glucose remains in the media carried out with 20 g dm^{-3} after the culture time. The results confirmed the ability of *Y. lipolytica* CECT 1240 to metabolize waste cooking oil, since around 80 and 50% decrease in

oil-associated COD was observed at the end of the cultivation time with 5 and 20 g dm^{-3} of glucose, respectively. These data suggest that glucose depletion furthers consumption of the waste cooking oil by the microorganism.

The highest biomass levels yielded (9 g dm^{-3}) were obtained for the culture containing 20 g dm^{-3} of glucose. The values are lower than those reported by Corzo and Revah and Deive *et al.* [23, 38], which reported maximum values of biomass of 30 and 17 g dm^{-3}, respectively, when they supplemented the basal medium with olive oil. This fact indicates that the waste cooking oil composition might exert a considerable influence at cell metabolism level. On account of the higher degradation levels attained with low glucose concentration, 5 g dm^{-3} initial glucose was added to the culture medium in all subsequent experiments.

Afterwards, the microorganism was grown in shake flasks to which different concentrations of waste oil were added (1–30 g dm^{-3}). COD evolution during the cultures is shown in Fig. 1. The importance of evaluating initial lipidic concentration has already been stressed by Papanikolau *et al.* [40], who reported that stearin (trioctadecanoin, or a glyceryl ester of stearic acid) concentrations higher than 10 g dm^{-3} were adequate for lipid accumulation in *Y. lipolytica*. Deive *et al.* [38] determined that 3 days was the minimum time required to reach the stationary phase by *Y. lipolytica* CECT 1240 in analogous environmental conditions. Therefore, samples were taken out at 3 and 6 days, to have representative values of degradation. Final degradation close to 100% was attained for the lowest oil concentrations (1 and 5 g dm^{-3}), while around 80% of the waste oil was metabolized after 6 days culture in the other cases. As it has been previously reported in several research works [40, 41, 15] fatty acid composition of the fatty substrate can be playing a major role in the lipid uptake by *Y. lipolytica*, explaining thus the lower percentages of degradation at high lipidic concentrations. Cell growth could also be affected by this variable. Additionally, the values are comparable to those obtained by Lanciotti *et al.* [36]. They analyzed the COD reduction after 3 days of cultivation by 62 strains of *Y. lipolytica*, and obtained that just three strains were able to provide about 30–40% of COD reduction after 3 days of cultivation. Hence, the results obtained here support the suitability of the studied strain to treat waste cooking oil.

Additionally, extracellular lipolytic activity was measured in the culture medium (Fig. 2) and significant enzyme concentrations were found in experiments supplemented with waste cooking oil. Domínguez *et al.* and Deive *et al.* [27, 28, 38] pointed out the necessity of the addition of vegetable oils in order to trigger the lipase synthesis by this specific strain, and the possibility of using waste oil instead of non-used oil is confirmed in this work. This fact is of interest, because it entails the valorization of a residue as enzyme inducer. Maximum enzyme activity increased with oil concentration up to 5 g dm^{-3}, then decreased, but in all cases enzyme production was higher when waste oil had been added to the culture medium. It should be emphasized that a limitation of the lipase production has been reported in other investigations when increased concentrations of oil were used in the medium, due to oxygen transfer limitations [42]. On the other hand, some authors have described decreased lipase concentrations as oil (*i.e.*, carbon source) was depleted in the culture medium [35, 38].

Also, a linear relationship could be established between the decrease in COD rate (mg $O_2 \ldots dm^{-3}$ day^{-1}) and the oil concentration, as it can be seen in Fig. 3. Higher contents of waste cooking oil led to higher decrease in COD, which seems

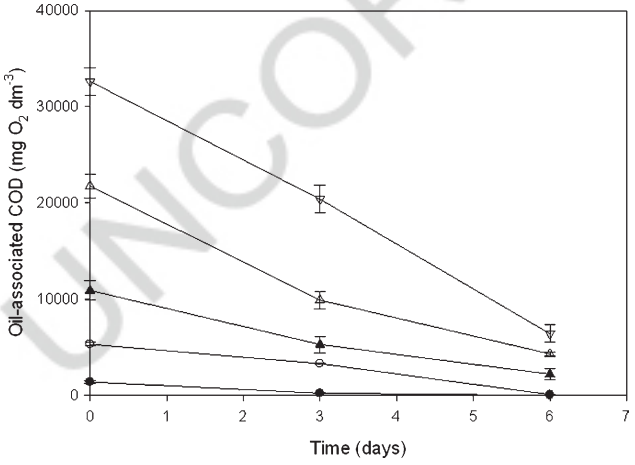

Figure 1. Evolution of oil-associated COD in shake-flask cultures of *Yarrowia lipolytica* CECT 1240 on basal medium containing 5 g dm^{-3} glucose and different concentrations of waste cooking oil, at 30 °C and 150 rpm: (•) 1 g dm^{-3}, (○) 5 g dm^{-3}, (▲) 10 g dm^{-3}, (△) 20 g dm^{-3} and (▽) 30 g dm^{-3}.

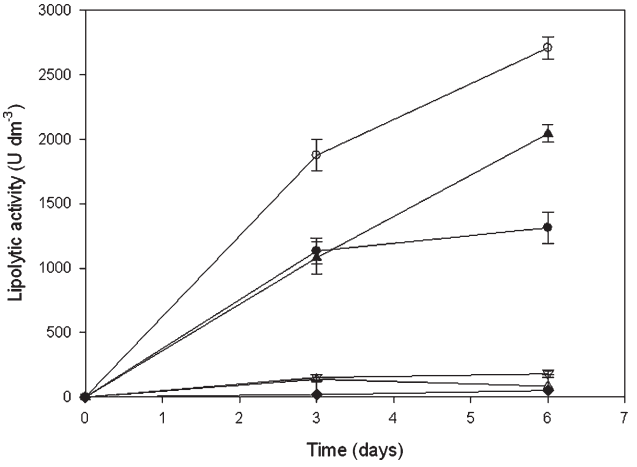

Figure 2. Lipolytic activity in shake-flask cultures of *Yarrowia lipolytica* CECT 1240 on basal medium containing 5 g dm^{-3} glucose and different concentrations of waste cooking oil, at 30 °C and 150 rpm: (◆) No added oil, (•) 1 g dm^{-3}, (○) 5 g dm^{-3}, (▲) 10 g dm^{-3}, (△) 20 g dm^{-3} and (▽) 30 g dm^{-3}.

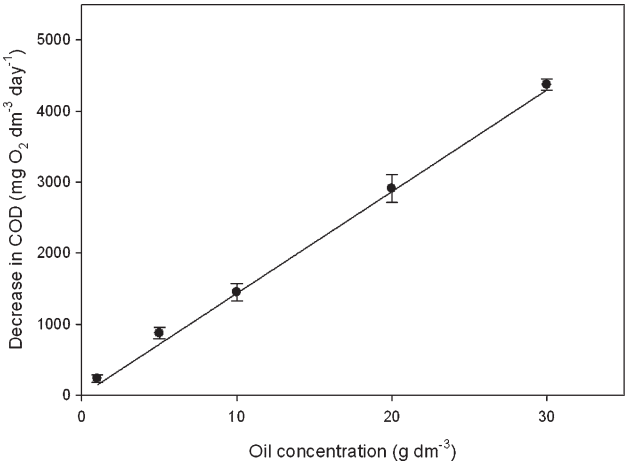

Figure 3. Linear relationship between decrease in COD and waste cooking oil concentration in shake-flask cultures of *Yarrowia lipolytica* CECT 1240 on basal medium containing 5 g dm^{-3} glucose, at 30 °C and 150 rpm (COD = 143.18 · [Oil concentration]).

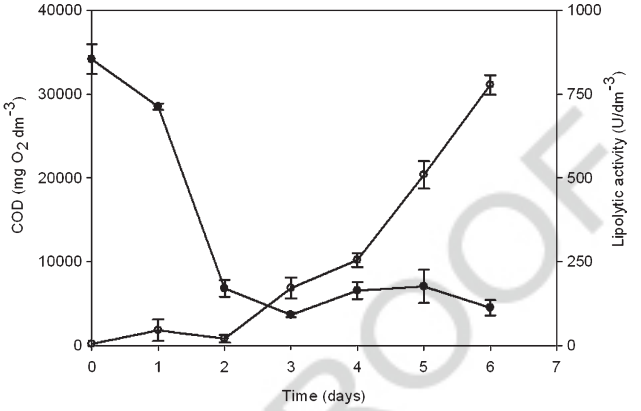

Figure 4. COD and lipolytic activity in *Yarrowia lipolytica* CECT 1240 cultures carried out in a stirred tank bioreactor (400 rpm, 1 dm^3 min^{-1} airflow), on basal culture medium containing 5 g dm^{-3} glucose and 30 g dm^{-3} waste cooking oil: (•) COD, (○) Lipolytic activity.

to indicate that COD reduction is not solely associated with lipase production, but also with the synthesis of other metabolites during the biological process. Following this behavior, Lanciotti *et al.* [36] also described different trends for COD reduction and lipolytic enzyme activity in cultures of several yeasts.

An additional promising application of this strain could be the treatment of other oil-based wastes, such as OMW. The residual oil that OMW contains in relatively large quantities (from 0.3 to 3.5 g dm^{-3} depending on the extraction process efficiency) makes this waste a potentially suitable medium for lipase production, as it has been recently reported [43–45].

3.2 Bioreactor cultures

Taking into account the results achieved in flask cultures, the efficiency of the studied strain for waste cooking oil degradation and lipase production was assessed in a bench scale bioreactor. A medium containing the highest oil concentration (30 g dm^{-3}) and lowest amount of glucose (5 g dm^{-3}) previously assayed was employed, and COD and lipolytic activity were monitored during the cultivation time (6 days). The results are shown in Fig. 4. More rapid oil consumption was recorded in the bioreactor, compared to flask cultures. Almost 90% of initial COD was eliminated in just 3 days, taking into account that glucose consumption rate is 10 g dm^{-3} day^{-1}. This degradation level is similar to that reported by Scioli and Vollaro [46], which indicated about 80% COD reduction after carrying out the biological process in a 3.5 L fermenter. The same value was also obtained by De Felice *et al.* [47] using *Y. lipolytica* 20255. On the other hand, lipolytic enzyme levels progressively increase from day 2 onwards, attaining values close to 800 U dm^{-3} after 6-days culture time.

In view of the rapid COD reduction and high lipase production levels observed, the feasibility of a fed-batch strategy was assessed, as a means to further the usefulness of the culture for COD reduction and waste cooking oil valorization. Deive *et al.* [38] had previously assayed the culture of *Y. lipolytica* CECT 1240 in fed-batch and continuous mode, and obtained very promising results. Thus, 30 g of waste cooking olive oil were added after 6 days of cultivation, and COD and enzyme activity levels were monitored afterwards. The main results are presented in Table 1. The studied strain was capable of reducing COD values nearly by 50% within 24 h of oil addition. Also, further increase in enzyme activity was observed. Thus, the microorganism was able to degrade additional waste cooking oil while producing lipolytic enzymes after a new addition of the residue, although at a lower rate than before. These results support the interest of the studied strain, and its potential for the treatment of oily wastes.

Moreover, it is noteworthy that the maximum lipolytic enzyme production attained in the bioreactor culture (927 U dm^{-3}) was more than 5-fold higher than that detected in flask cultures when an analogous culture medium was used. This fact might be related to the specific hydrodynamic conditions inside the bioreactor, in terms of agitation and aeration. On the one hand, an increased transfer area is furthered by forming small sized bubbles which are dispersed in the culture medium, increasing their residence time on the liquid. On the other hand, a good agitation favors turbulence which leads to a decrease on the thickness of the liquid layer on the gas/liquid interface, reducing the resistance to oxygen mass transfer. This statement is in agreement with the conclusions reported by Lopes *et al.* [48], who recorded an increase of 96% in lipase activity values when used a 5-bar air pressure instead of 1-bar pressure during the culture of *Yarrowia*

Table 1. COD reduction and lipolytic activity production during fed batch operation in *Yarrowia lipolytica* CECT 1240 cultures carried out in a stirred tank bioreactor (400 rpm, 1 dm^3 min^{-1} airflow). Oil addition: 30 g waste cooking oil added at day 6.

	Before oil addition	After oil addition
Maximum lipolytic activity (U dm^{-3})	778	927
Lipolytic enzyme production rate (U dm^{-3} day^{-1})	129.7	21.3
Maximum COD reduction (%)	89.4	51.6
Maximum COD reduction rate (mg O_2 dm^{-3} day^{-1})	10 182	5454

lipolytica. Also, an efficient agitation could improve the contact between the microorganism and the oil, thus facilitating its utilization. However, the relevance of the strain selected must be taken into account since, contrarily to the results presented here, Alonso *et al.* [49] concluded that increased agitation and aeration rates negatively affected lipase production by another strain of *Y. lipolytica*.

In summary, the studied biological system appears as a promising tool for the treatment and valorization of oily residues from the food industry, such as waste cooking oil.

3.3 Enzyme characteristics

Some characteristics of the crude extracts (*i.e.*, cell-free concentrated extracellular liquid) were assayed in order to evaluate if the use of waste cooking oil as carbon source and lipase inducer, instead of non-used oil, could alter the main catalytic properties of the synthesized enzymes. Thus, shake flask cultures of *Y. lipolytica* CECT 1240 were carried out in culture media supplemented with waste cooking oil, non-used olive oil and non-used sunflower oil. Identical environmental conditions were employed in all cases, and the enzyme crude solutions were recovered at the end of the cultures.

First, the influence of temperature and pH on lipase activity was assessed in the enzyme solutions obtained from the abovementioned cultures. The selected operating conditions were a temperature range of 30–60 °C and a pH varying between 4 and 9, respectively. As it can be seen in Fig. 5, the enzyme extracts showed maximum activity at pH values around 7.0, as a number of microbial lipases described in previous reports, such as those from *Bacillus* sp, *Acinetobacter* sp., and a mutant *Yarrowia lipolytica* strain [50, 51, 26]. The activity-pH profiles were rather close for the three enzyme solutions assayed, and more so for those obtained in waste cooking oil and sunflower oil-supplemented cultures. Since sunflower oil is commonly used as commercial frying oil in our geographical area, it would be reasonable to expect a certain degree of similarity between the effects of both oils, related to their basic composition.

As for enzyme thermoactivity, a crucial feature in industrial biocatalysis, all the crude enzymes showed maximum activities at temperature values between 30 and 40 °C, and the activity-temperature profiles were also quite similar. These values of thermoactivity are typical for lipases obtained from mesophilic microorganisms, and have been found for other strains of this yeast, such as *Y. lipolytica* 681 [23].

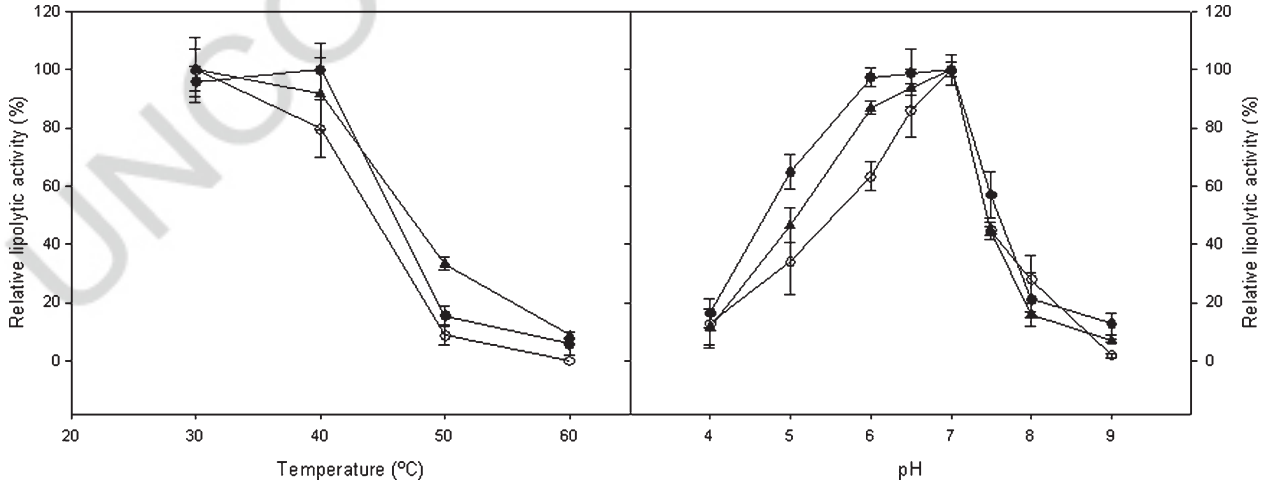

Figure 5. Influence of pH and temperature on lipolytic enzyme activity for different *Yarrowia lipolytica* CECT 1240 enzyme extracts: (•) Cultures with sunflower oil, (○) Cultures with olive oil, (▲) Cultures with waste cooking oil.

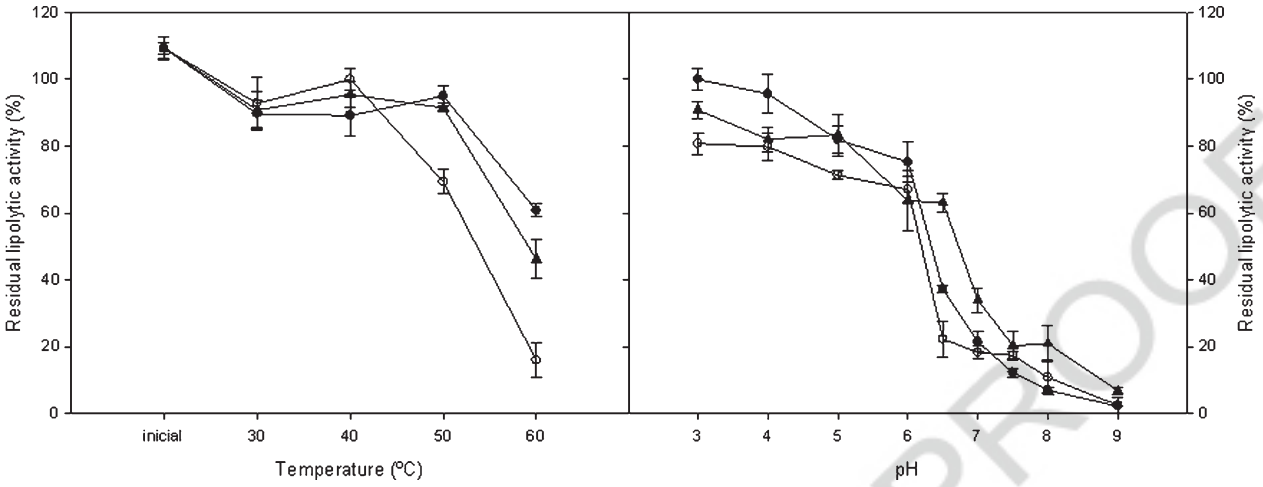

Figure 6. Influence of pH and temperature on lipolytic enzyme stability for different *Yarrowia lipolytica* CECT 1240 enzyme extracts: (•) Cultures with sunflower oil, (○) Cultures with olive oil, (▲) Cultures with waste cooking oil.

Thermal stability is another important property, often investigated during the evaluation of enzyme characteristics and applicability. The deactivation of the three enzyme extracts was assessed after 24 h incubation at 30 °C and different pH values ranging from 3.0 to 9.0. The results are shown in Fig. 6. The observed profiles follow a similar trend. All the enzyme solutions show the highest stability in acidic environments, while a dramatic fall in stability is registered at pH values over 6.5.

Regarding thermal stability (at pH 5), the enzymes produced in cultures supplemented with sunflower oil and waste-cooking oil appeared to be more resistant to thermal deactivation than that obtained from olive oil-supplemented cultures (Fig. 6). This effect is noticeable at temperatures above 40 °C. Some authors have reported the production of different lipolytic isoenzymes by strains of *Y. lipolytica*, and they identified the genes associated with their production [52, 34]. Those isoenzymes showed different biochemical properties. In the present case, the culture conditions (*i.e.*, type of added oil) could lead to variation in the isoenzymes ratio in the enzyme extracts, and this could possibly explain the differences observed between their properties.

4 Conclusions

The necessity of new strategies to valorize wastes from the food and agro industries supports the interest of the present work. The potential application of *Yarrowia lipolytica* CECT 1240 to degrade waste cooking oils is confirmed, as well as the efficiency of this residue to promote the production of lipolytic enzymes by the strain. The process was successfully carried out both in shake flasks and a bench-scale bioreactor, allowing producing high levels of lipolytic activity at the same time that the COD was diminished up to nearly 90%. Besides, the efficient degradation of repeated waste oil charges was ascertained. Also, the catalytic potential of the crude enzymes obtained in cultures supplemented with waste cooking oil was assessed, and they compared favorably with those obtained when non-used oils were added to the culture medium.

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The authors have declared no conflict of interest.

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