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# Biodepolymerization of Polyamide Fibers Using *Yarrowia lipolytica* as Whole-Cell Biocatalyst

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**Abstract:** Polyamide is a thermoplastic polymer widely used for several applications, including cables in offshore oil and gas operations. Due to its growing annual production worldwide, this poorly biodegradable material has been a source of pollution. Given this scenario, the need has arisen to develop environmentally friendly techniques to degrade this waste, and biotechnology has emerged as a possible solution to mitigate this problem. This study aimed to investigate the potential of *Yarrowia lipolytica* to biodepolymerize polyamide fibers (PAF). Microbial cultures were grown in shaken flasks containing different concentrations of PAF (0.5 and 2 $g \cdot L^{-1}$) and in a bioreactor with and without pH adjustment. PAF mass loss was up to 16.8%, achieved after 96 h of cultivation in a bioreactor without pH adjustment. Additionally, NMR analyses revealed that the amorphous regions of PAF, which are more susceptible to depolymerization, were reduced by 6% during cultivation. These preliminary results indicate the biotechnological potential of *Y. lipolytica* to depolymerize PAF.

**Keywords:** biodepolymerization; Nylon; polyamide-6,6; *Yarrowia lipolytica*; adipic acid; hexamethylenediamine



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## 1. Introduction

Plastics are a ubiquitous class of synthetic polymer materials used in virtually all commercial and industrial sectors. In contrast to other materials, plastics offer advantages such as high flexibility, low maintenance requirements, lightness, weather resistance, low toxicity, and low price [1,2]. As a result of these advantages, the use of plastics is increasingly limitless and endless. Plastic waste can persist in the environment for decades due to its high recalcitrance, which supports its classification as non-biodegradable [3].

Polyamides are a versatile class of polymer, commonly called Nylons, and are a family of semicrystalline thermoplastics typically made from petroleum-derived feedstock [4]. Polyamides can be melt-processed into various shapes including membranes, films, and mostly fibers (i.e., fabrics). Polyamide-6,6 is one of the main types of polyamides produced for several applications such as electronics, food packaging, and mooring lines in the oil and gas industry [5,6]. It is synthesized by polycondensation of two initial monomeric units (adipic acid and hexamethylenediamine). Polyamide fibers (PAF) for mooring lines have been widely used in floating offshore oil and gas systems. PAFs are composed of carbon and nitrogen, releasing primary amines after hydrolysis [4–6]. After prolonged use in offshore applications, PAFs become waste and can result in severe ecological damage due to their recalcitrance for natural depolymerization [7,8]. Therefore, recycling these

materials to avoid environmental impacts and add value to the oil and gas business chain is of crucial relevance.

Given this context, plastic waste can persist in the environment for decades due to its high recalcitrance, which supports its classification as non-biodegradable. However, this paradigm has changed in recent years since enzymes and micro-organisms have been reported in the literature as biocatalysts able to break down the ester bounds of polymeric chains [8,9]. Enzymes that can hydrolyze poly(ethylene terephthalate) (PET) polyesters have been reported to be responsible for the bioconversion of these polymers into smaller molecules (e.g., intermediates, monomers, and oligomers). These enzymes include a group consisting of lipases, cutinases, esterases, and PETase. However, microbial PET depolymerization by micro-organisms that secrete these enzymes is also possible [10,11]. One example is the yeast species *Yarrowia lipolytica*, which has been applied for PET degradation due to its intense secretory activity and capacity to produce several bioproducts, such as organic acids and enzymes (e.g., lipases, esterases, proteases, and cutinases). This species can also assimilate several carbon sources, including hydrophobic substrates, such as n-alkanes and fatty acids [12,13]. Sales et al. [13] and Costa et al. [14] cultivated a wild-type strain of *Y. lipolytica* under submerged conditions to depolymerize PET, while Liu et al. [15] and Kosiorowska et al. [16] investigated using genetically-modified strains. Although enzymes and whole cells have been used as biocatalysts, microbial cells have the advantage of being prepared much more quickly and economically for industrial purposes due to their diversity and ease of manipulation [10]. Moreover, according to Fang et al. [17], the presence of the micro-organism in the depolymerization of plastic waste can increase the rate of hydrolysis compared to the free enzyme. Therefore, whole-cell biocatalysts provide a promising alternative to biocatalysts.

Although biocatalysts have been widely studied in the PET biodegradation process, studies on the depolymerization of polyamides remain almost unexplored. Some enzymes, such as manganese peroxidase from the white rot fungus and threonine hydrolase, have been reported to be able to hydrolyze polyamides and release the primary amines present in their structure [4,18,19]. Nevertheless, these enzymes are unsuitable due to their low catalytic activity and low efficiency in polymer biodegradation [4,20]. Thus, finding new biocatalysts is fundamental to enabling the efficient and sustainable degradation of polymer residues and monomer recovery.

Therefore, this study aimed to investigate the ability of the yeast *Y. lipolytica* to depolymerize PAFs from offshore systems in the oil and gas industry. For this purpose, a polyamide biodegradation study was carried out in submerged fermentation media with and without glucose. The catalytic activities of the lipase, esterase, and protease produced by the yeast were also evaluated.

## 2. Materials and Methods

### *2.1. Materials and Micro-Organism*

Agar, dextrose, universal peptone, and yeast extract were all purchased from Sigma-Aldrich (St. Louis, MO, USA). PAFs were obtained from the inner part of a mooring line used over 100 times in offloading procedures to transfer produced oil from offshore floating platforms of PETROBRAS to tanker cargo ships. The line was covered by a polyurethane coating (Figure 1). The filaments were manually cut into 1 cm pieces using a scissor for utilization in all assays in this study.

*Yarrowia lipolytica* wild strain IMUFRJ 50682 [21] was used as a microbial biocatalyst for all assays. Cells were stocked at 4 °C after 24 h growth in YPD medium (1% yeast extract, 2% peptone, 2% dextrose; $w/v$) with 2% agar ($w/v$) for solidification.


**Figure 1.** Oil and gas mooring line used in this work, which is made of polyamide fibers (PAF).

### *2.2. Maximum Non-Inhibitory Concentration (MNIC) Assay*

The reference method (M27-A2) for broth dilution antifungal susceptibility testing (Clinical and Laboratory Standards Institute, Berwyn, PA, USA) was used to determine the tolerance of *Y. lipolytica* towards adipic acid and hexamethylenediamine, as previously reported [22]. Stock solutions of adipic acid (10 $g \cdot L^{-1}$) and hexamethylenediamine (200 $g \cdot L^{-1}$) were prepared for two-fold serial dilution in 96-well microplates containing YP medium.

### *2.3. Microbial Depolymerization of PAF by Y. lipolytica in Erlenmeyer Flasks*

Yeast cells were pre-inoculated in YPD medium and grown at 29 °C, 160 rpm, for 72 h. Then, 250 mg of cells were harvested and transferred to 400 mL of YPD or YP medium (without 2% dextrose) in 1 L Erlenmeyer flasks. Two different PAF concentrations were used: 0.5 and 2 $g \cdot L^{-1}$. A control condition without the addition of PAF was also included, as well as a control with PAF and without cell inoculation. All tested conditions were carried out in duplicated under orbital agitation (Innova® 42 Incubator Shaker by New Brunswick Scientific, Edison, NJ, USA) at 250 rpm, 29 °C.

### *2.4. Microbial Depolymerization of PAF by Y. lipolytica in Bioreactors*

*Y. lipolytica* cultivation was carried out in a stirred tank bioreactor (Multifors 2 by Infors HT, Bottmingen, Switzerland) equipped with a 1 L vessel and two Rushton impellers. Yeast cells (500 mg) were inoculated in 500 mL of YP medium (500 mL) supplemented with 0.5 $g \cdot L^{-1}$ of PAF. Two different conditions were tested: (1) controlling pH at 7.0 ± 0.2 with the addition of sodium hydroxide solution (0.5 M), and (2) no pH control. Reactions were carried out at 500 rpm, 29 °C, and 1 $L.min^{-1}$ aeration rate.

### *2.5. PAF Characterization*

Solution-state Nuclear Magnetic Resonance (NMR) samples were prepared by dissolving the filaments in phenol:1,1,2,2-tetrachloroethane-d2 60:40 $v/v$ at 80 °C to guarantee complete solubilization of 30 mg polyamide in 0.5 mL solvent mixture. The $^{1}H$-$^{13}C$ NMR gradient-selected Heteronuclear Multiple Bond Correlation (gHMBC) spectra were performed on Agilent INOVA-300 (7.05 Tesla of magnetic field) equipped with a 5 mm inverse

multinuclear z-gradient probe head, operating at 299.98 MHz for proton and 75.44 MHz for carbon. The long-range coupling constant of 8.0 Hz was used to set delays in the pulse sequence. Spectra were acquired with 512 (2 × 256) F1 increments, 32 transients per increment, relaxation delay of 1.5 s, acquisition time of 0.128 s, with a $^{1}$H-$^{13}$C J-evolution delay of 80 ms. A sine bell processing was applied to both $^{1}$H and $^{13}$C dimensions and zero filled to 1 K data points to have a matrix of 1 K × 1 K real data points.

Solid-state NMR (ssNMR) analyses were performed on a Bruker Avence NEO 500 spectrometer (11.75 Tesla of magnetic field) applying carbon-13 cross polarization with magic angle spinning ($^{13}$C CP/MAS experiment) at room temperature on a 2.5 mm probe head. A ramp CP pulse sequence was used, with high power $^{1}$H decoupling (SPINAL method) during the acquisition of the free induction decay (FID). Other acquisition parameters were 15 kHz of MAS rate; 3.8 µs of $^{1}$H pulse duration (π/2 pulse); 5 s of recycle delays; 50 ms of acquisition time; 50 kHz of spectral width; 2048 recorded transients; and 2 ms of contact time. The spectra were obtained by Fourier transform of the FIDs and were externally referenced by tetramethylsilane (TMS), using adamantane as a secondary reference (signals at 29.46 and 38.50 ppm). Spectra were processed with 20 Hz exponential multiplication.

The infrared spectroscopy analysis of the samples was collected using a Nicolet iS 50 FTIR spectrometer with a diamond crystal and ATR accessory. The measurements were made at a resolution of 4 $cm^{-1}$ and 32 scans.

### *2.6. Analytical Methods*

Cell growth measurement was determined based on a standard curve generated by the correlation between dry cell weight and optical density at 570 nm (DR6000 UV-VIS Spectrophotometer by Hach, Loveland, CO, USA). The pH monitoring was performed using an 827 pH Lab Meter (Metrohm, Zofingen, Switzerland).

Protease activity was monitored by azocasein hydrolysis using an adapted assay protocoled by Sales et al. [12], which was based on the methodology reported by Charney and Tomarelli in 1947 [23]. One enzyme unit (U) was defined as the amount of enzyme that leads to an increment of 0.01 unit in absorbance per minute. Esterase and lipase activities were determined using 2.5 mM of p-nitrophenyl butyrate and p-nitrophenyl laurate for each enzyme, respectively, according to the methodology reported by Pereira-Meirelles et al. [11]. Reactions were carried out in 96-well microplates containing 100 mM sodium phosphate buffer (pH 7.0). The release of p-nitrophenol was detected at 410 nm over 10 min. One unit of enzyme activity is defined as the quantity of enzyme that catalyzes the release of 1 µmol of p-nitrophenol per minute. For all protocols cited above to determine enzymatic activities, a Multiskan™ GO Microplate Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to obtain absorbance measurements.

Analytes concentrations were determined by high-performance liquid chromatography (HPLC), as previously reported [14]. An LC-20 HPLC instrument (Shimadzu, Kyoto, Japan) was equipped with an Aminex HPX-87 column (300 × 7.8 mm) with a pre-column with cation-exchange resin (Bio-Rad Laboratories Ltd., Hercules, CA, USA). The mobile phase consisted of 5 mM $H_2SO_4$ at a flow rate of 0.6 mL·$min^{-1}$. The refractive index detector was utilized at 55 °C, oven temperature was kept at 55 °C, and the injection volume was 20 µL.

PAF morphology was analyzed by scanning electronic microscopy (SEM). Circa 0.5 $cm^3$ was removed from each sample, mounted on an aluminum conductive support, and metalized with a thin layer of Ag. Then, it was adhered to an aluminum sample holder and analyzed using a JEOL 7100F scanning electron microscope (JEOL, Tokyo, Japan), operating in a high vacuum at 1 kV.

## 3. Results and Discussion

### *3.1. PAF Characterization*

HMBC is a bidimensional NMR technique, which has been used for resonance assignments because it provides a wealth of structural information that is particularly helpful

in the characterization of complex molecules. It plays a keyhole by connecting molecular fragments through their carbon–proton coupling usually via two or three bonds and typically gives the final answers concerning a structural problem. In Figure S1, the complete attribution of NMR signals can be seen, confirming the structure of polyamide-6,6 in the PAF samples. Table 1 summarizes key information for spectral assignments of the polymer backbone.

**Table 1.** gHMBC correlations of PAF sample.

| $\delta_C$ (ppm) | $\delta_H$ (ppm) | gHMBC ($^1$H-$^{13}$C) |
|---|---|---|
| 24.9 | 2.45 | $^2J_{H4\text{-}C5}$ |
| 24.9 | 1.99 | $^2J_{H5\text{-}C5}$ |
| 26.0 | 3.51 | $^3J_{H1\text{-}C3}$ |
| 26.0 | 1.71 | $^2J_{H2\text{-}C3}$ |
| 26.0 | 1.50 | $^2J_{H3\text{-}C3}$ |
| 28.7 | 3.51 | $^2J_{H1\text{-}C2}$ |
| 28.7 | 1.50 | $^2J_{H3\text{-}C2}$ |
| 175.1 | 6.35 | $^2J_{NH\text{-}C6}$ |
| 175.1 | 3.51 | $^3J_{H1\text{-}C6}$ |
| 175.1 | 2.45 | $^2J_{H4\text{-}C6}$ |
| 175.1 | 1.99 | $^3J_{H5\text{-}C6}$ |

### *3.2. Maximum Non-Inhibitory Concentration (MNIC) Assay for PAF Monomers*

One factor that might be limiting the depolymerization of polyamide-6,6 is the toxicity of the monomers released during the process (i.e., adipic acid and hexamethylenediamine) to the yeast. Based on the MNIC assay in this study, *Y. lipolytica* was able to grow at all concentrations of adipic acid tested (up to 5 $g{\cdot}L^{-1}$), so it was not possible to determine a maximum non-toxic concentration. The pH of the culture medium became more acidic as the concentration of adipic acid increased, reaching a pH of 4.5, but the acidity of the culture medium did not influence the growth of the yeast since *Y. lipolytica* can grow under acidic conditions [24]. This aspect of the yeast is very relevant to both the polyamide depolymerization process and the adipic acid production process, as acid stress and low cell tolerance to adipic acid itself have resulted in low yields and titers of microbially produced adipic acid [25].

On the other hand, concentrations above 1.5 $g{\cdot}L^{-1}$ of hexamethylenediamine are toxic to *Y. lipolytica*. This toxicity may be associated with the pH of the culture medium, which became very alkaline (>8.2) at concentrations above 1.5 $g{\cdot}L^{-1}$ and this may have influenced the viability of the yeast and not just the effect of the toxicity of the compound itself.

### *3.3. Microbial Depolymerization of PAF by Y. lipolytica in Erlenmeyer Flasks*

Initially, *Y. lipolytica* was cultivated in YPD medium supplemented with 0.5 and 2.0 $g{\cdot}L^{-1}$ of PAF and cell growth profiles are shown in Figure 2. Kinetic growth profiles of the experimental conditions were similar to the control condition (without PAF); however, it is possible to observe a slight increase in the maximum cell concentration in the presence of 0.5 $g{\cdot}L^{-1}$ of PAF compared to the control. Glucose was completely consumed after 30 h of cultivation when the growth deceleration phase began in all experimental conditions (Figure 2a,c,e).

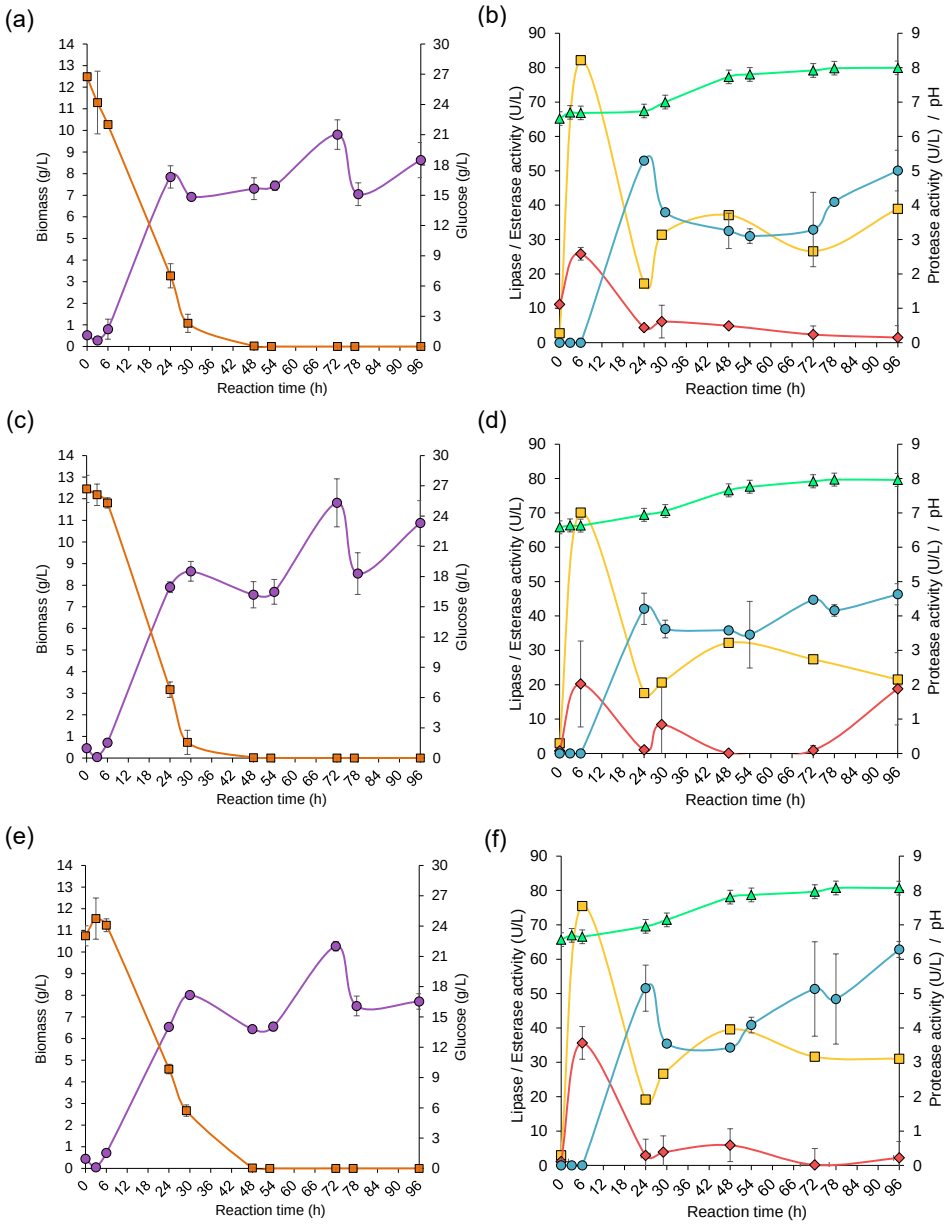


**Figure 2.** Cultivation of *Y. lipolytica* in YPD medium supplemented with PAF at 28 °C, 250 rpm. All conditions were carried out in duplicate: negative control (**a**,**b**), 0.5 g·L$^{-1}$ (**c**,**d**), or 2 g·L$^{-1}$ (**e**,**f**) of PAF. Biomass (purple circles) and glucose (orange squares) concentrations, pH (green triangles), lipase (red diamonds), esterase (yellow squares), and protease (blue circles) activities.

Several enzymes have been described as potential nylonases, such as amidases, cutinases, hydrolases, and proteases [26,27]. Lipase, esterase, and protease activities during depolymerization of PAF by *Y. lipolytica* were assayed (Figure 2b,d,f). The experiments carried out were similar in all conditions, with maximum lipase and esterase activity occurring around 6 h of cultivation and, after this period, there was a decline in activity, while protease activity began. This fact coincides with glucose exhaustion from the medium.

Insignificant weight loss (~1.7%) was observed for the control condition, which consisted of YP medium without cells. However, biodegradation of 0.5 and 2 g·L$^{-1}$ PAF by *Y. lipolytica* in YPD medium resulted in weight losses of 3.3% and 1.9%, respectively, after 96 h.

ssNMR spectra (Figure 3) were used for the assessment of PAF crystallinity determination. The spectral region of aliphatic carbons, in special around 42 ppm, which is related to amorphous methylene groups directly bonded to nitrogen in the polymer chain, reveals the degree of crystallinity of semicrystalline polyamide-6,6.

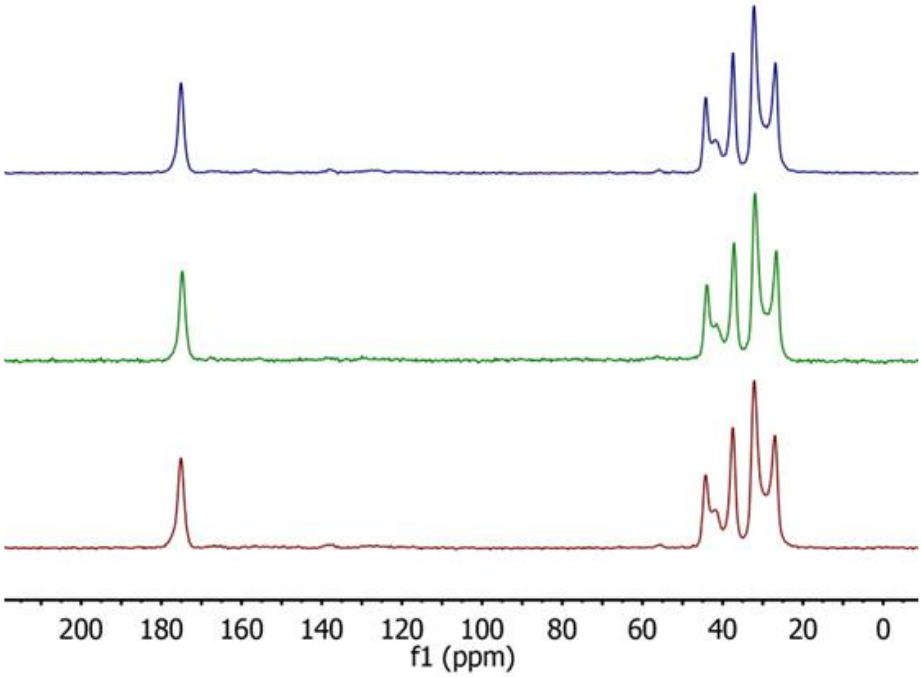


**Figure 3.** $^{13}$C CP/MAS NMR spectra of PAF after incubation in YPD medium: 2 g·L$^{-1}$ (blue), 0.5 g·L$^{-1}$ (green), control (red).

The detailed comparison of $^{13}$C CP/MAS spectra in the aliphatic region (Figure S2) reveals that the amorphous peaks of methylene groups directly bonded to nitrogen are decreasing in the cultivation media samples compared to the control sample. The chemical shift behavior of these peaks around 42 ppm (amorphous phase) and 45 ppm (crystalline phase) can be understood based on the γ-gauche effect. The crystalline state has a trans zig-zag structure and the amorphous carbons are undergoing rapid transitions between these conformations.

After careful deconvolution of all $^{13}$C CP/MAS spectra (Figures S3–S5) in the aliphatic spectral region, it was possible to compare the crystallinity of the control concerning the samples that came from cultivation media. An increasing order of crystallinity could be established: Control < 0.5 g·L$^{-1}$ < 2 g·L$^{-1}$, indicating a depolymerization process directed to the amorphous phase in the cultivation media samples, since the crystalline phase imposes physical and mechanical barriers for biodepolymerization (Table 2). Similar findings are reported for biodepolymerization of PET, in which enzymes preferentially attack amorphous regions due to the higher accessibility of polymeric chains to the catalytic site [28].

**Table 2.** Contents of crystalline and amorphous phases in PAF samples after cultivation of *Y. lipolytica* in YPD medium.

| Sample | Crystalline Phase (%) | Amorphous Phase (%) |
|---|---|---|
| Control | 49.5 | 50.5 |
| 0.5 g/L | 53.0 | 47.0 |
| 2.0 g/L | 55.5 | 44.5 |

The PAF remaining at the end of the cultivations in YPD medium were also analyzed by infrared spectroscopy. The spectra of these samples and the control PAF (raw, as received, not subjected to the microbiological process) are shown in Figure 4.

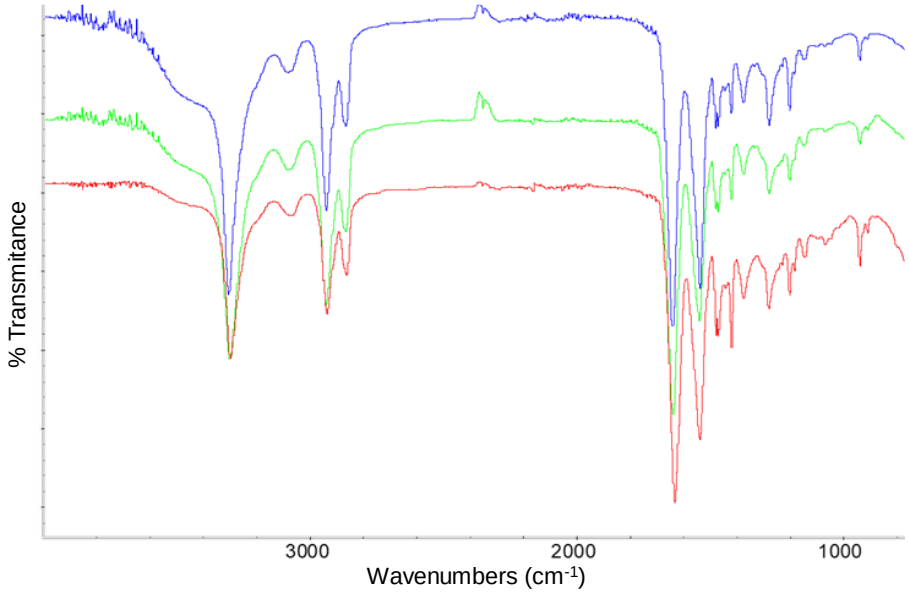


**Figure 4.** Infrared spectra of polyamide fiber samples at the end of the fermentations in flasks in YPD medium: 2 g·L$^{-1}$ (red), 0.5 g·L$^{-1}$ (green), control (blue).

The absorption bands at 3298 cm$^{-1}$ and 2934 cm$^{-1}$ correspond to N-H and C-H stretching vibration, respectively, due to amino and methylene groups. The IR bands at 1636 cm$^{-1}$ and 1539 cm$^{-1}$ are attributed to the stretching of the C=O bond and the bending of the N-H bond of the amide, respectively. Low intensity absorptions are also observed around 1200 cm$^{-1}$, also associated with the amide groups (-CONH-) of polyamide-6,6 [29], confirming the results observed by NMR.

Then, glucose was removed from the culture medium to induce the yeast to use PAF as a carbon source and cell growth was monitored during 96 h of cultivation of *Y. lipolytica* in YP medium supplemented with 0.5 and 2.0 g·L$^{-1}$ of PAF (Figure 5c,e).

The kinetic growth profile of the control and the condition containing 2.0 g·L$^{-1}$ of PAF are similar. Both conditions reached the growth deceleration phase around 48 h and the final biomass concentration was approximately 7.0 g·L$^{-1}$. However, the condition containing 0.5 g·L$^{-1}$ of PAF showed slower growth, and only after 72 h of cultivation it achieved the maximum biomass concentration of 8.7 g·L$^{-1}$, while the other conditions reached the maximum biomass concentration around 54 h of cultivation. Similarly, to the YPD medium assay, the maximum lipase and esterase activities were at 6 h, although these activities had been higher in YP assays (Figure 5b,d,f). This fact was also observed previously in another study of our group and the best hypothesis is because lipase production by *Y. lipolytica* undergoes repression in glucose presence and may be related to the scarcity of carbon sources and more prominent use of nitrogen ones, like peptone and yeast extract, used in this work, that may favor the production of these enzymes [14,30].

Under these conditions, it was possible to observe a mass loss of PAF similar to the tests on YPD medium, which showed indexes almost twice as high in the condition with 0.5 g·L$^{-1}$ compared to the control, while there was no significant difference in the condition containing 2.0 g·L$^{-1}$ of PAF.

SEM was used to investigate the morphological changes in PAF after its incubation with microbes, corroborating evidence of the polymer's biodegradation. Figure 6 shows signs of degradation of the PAF surface, whether through erosion in stretches, roughness, or darkening of the material. The incidences of these signs were more intense in the fibers

submitted to contact with yeast than in the control condition (without cells), which showed more solid deposits (possibly fouling).

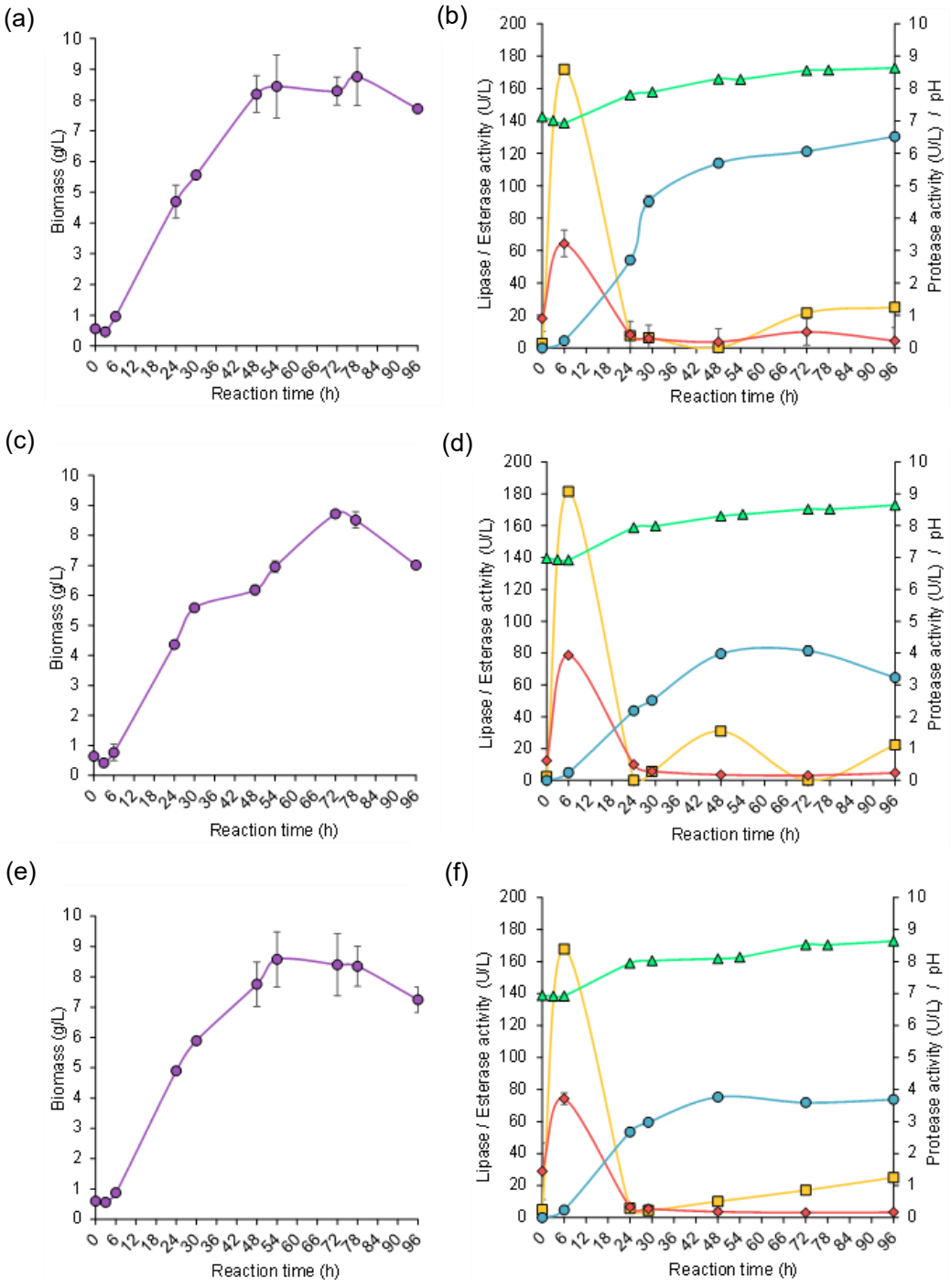


**Figure 5.** Cultivation of *Y. lipolytica* in YP medium supplemented with PAF at 28 °C, 250 rpm. All conditions were carried out in duplicate: negative control (**a**,**b**), 0.5 g·L$^{-1}$ (**c**,**d**), or 2 g·L$^{-1}$ (**e**,**f**) of PAF. Biomass concentrations (purple circles) pH (green triangles), lipase (red diamonds), esterase (yellow squares), and protease (blue circles) activities.

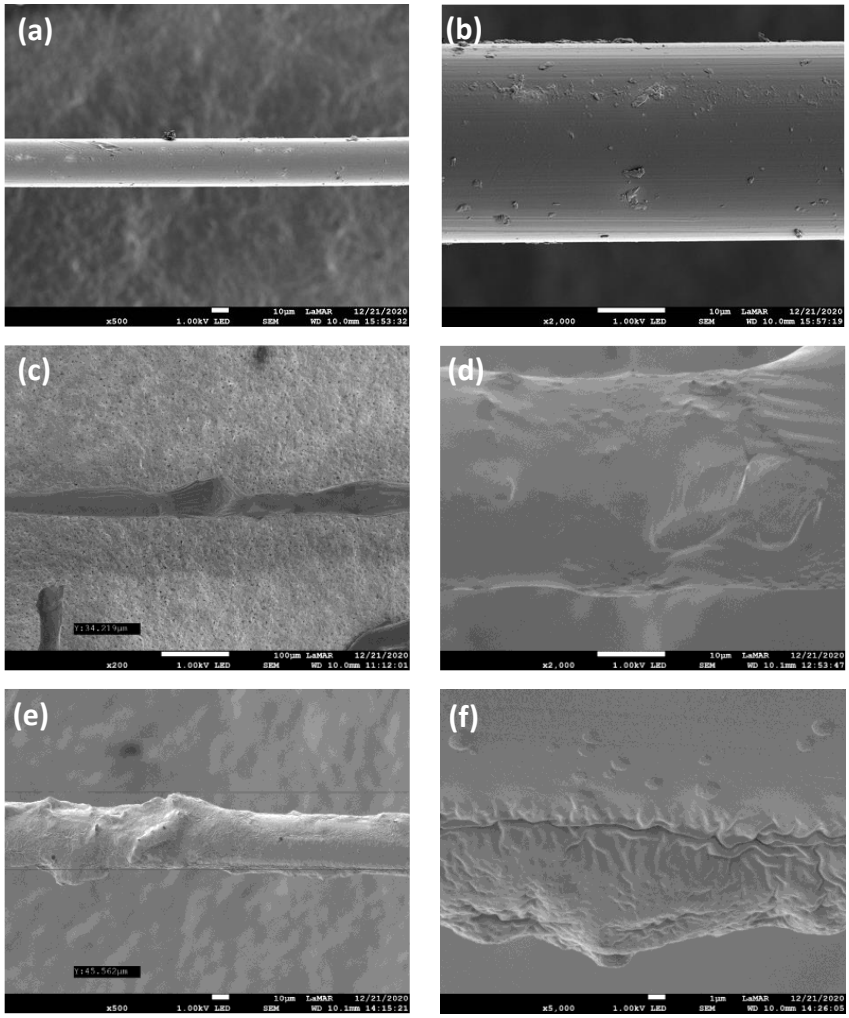


**Figure 6.** SEM images of 0.5 g·L$^{-1}$ of PAF in different media: (**a**,**b**) YP medium without cells (control); (**c**,**d**) cultivation in YPD medium with cells after 96 h; (**e**,**f**) cultivation in YP medium with cells after 96 h.

### *3.4. Microbial Depolymerization of PAF by Y. lipolytica in Bioreactors*

Based on the results of depolymerization of PAF by *Y. lipolytica* in flasks, an experiment was carried out in a bioreactor with YP medium supplemented with 0.5 g·L$^{-1}$ of PAF with and without pH adjustment (at 7.0) (Figure 7). In the condition in which there was no pH adjustment, two deceleration phases of growth could be observed, between 24–48 h and 54–78 h (Figure 7a). This kinetic profile was not observed in the condition in which the pH was adjusted; however, both conditions reached cell concentration values close to 13.2 and 11.3 g·L$^{-1}$ at the end of cultivation with and without pH adjustment, respectively.

Regarding enzyme activity, lipase activity was higher than esterase activity in bioreactor assays, unlike the observed in flasks in YP medium. This is because lipase production can be influenced by different environmental factors, such as carbon and nitrogen sources, medium pH, temperature, dissolved oxygen concentration, and the presence of inducers [31]. The oxygen availability for the micro-organisms is one of the most important factors and oxygen transfer is more efficient in bioreactors than in flasks.

Different authors have found an optimum lipase activity at pH 7 for *Y. lipolytica* [30,32,33]. In the condition with pH adjustment at 7, there were two peaks in lipase activity: at 6 h, during the start of the growth acceleration phase when the activity was 151 U·L$^{-1}$, and at 36 h, during the growth deceleration phase when the activity was 187 U·L$^{-1}$, while in the condition without pH adjustment, there was only a maximum lipase activity of 186 U·L$^{-1}$

at 6 h (Figure 7b,d). A similar profile with two peaks of lipase activity was also observed by Brígida et al. [30] using the same strain.

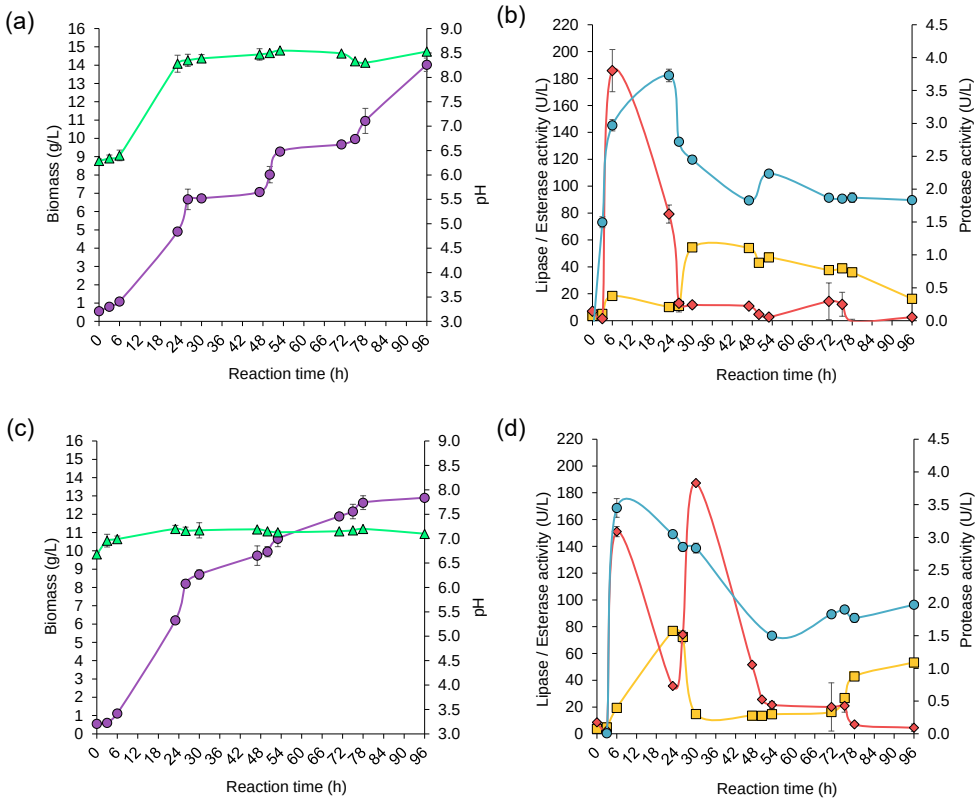


**Figure 7.** Cultivation of *Y. lipolytica* in YP medium supplemented with 0.2 g·L$^{-1}$ of PAF at 28 °C, 250 rpm, in bioreactors without pH control (**a**,**b**) or controlled at pH 7 (**c**,**d**). Biomass concentrations (purple crosses), pH (green triangles), lipase (red diamonds), esterase (yellow squares), and protease (blue circles) activities.

The maximum esterase activity was 78 U·L$^{-1}$ at 23 h in the condition with pH adjustment and, in the period from 30 to 72 h, the activity stabilized at around 14 U·L$^{-1}$ and, after this period, increased again (Figure 7d). On the other hand, in the condition without pH adjustment, the maximum esterase activity was 54 U·L$^{-1}$ at 30–46 h and, after this period, there was a decrease in activity (Figure 7b). In addition, protease activity was anticipated in the first few hours of bioreactor cultivation compared to experiments in flasks.

The changes that occurred due to the action of the yeast on the PAF were evaluated quantitatively by measuring the weight loss of the polymer. In the bioreactor, there was a more significant loss of mass of the PAF than in flasks, with a 17% and 13% decrease in weight in the conditions without and with pH adjustment after 96 h, respectively. These findings support the hypothesis of the yeast's ability to biopolymerize PAF.

Other authors have reported higher percentages of mass loss from polyamide-6,6, but extended the experiments over many days, from 35 to 40 days [34,35]. Tiwari et al. [35] obtained a 21% reduction in the weight of polyamide-6,6 after 40 d of biodepolymerization by *Achromobacter xylosoxidans*. Tiwari et al. [34] obtained a mass loss of polyamide-6,6 of 22% after 35 d of biodegradation evaluation with *Bacillus brevis*. However, Sudhakar et al. [36] obtained a weight reduction of only 7% using the bacterium *Bacillus cereus* in the biodepolymerization of polyamide-6,6 for 3 months.

## 4. Conclusions

The biorecycling of synthetic polymers has been a field of study intensively explored in recent years to develop a greener route alternative to tackle plastic pollution, such as polyamide waste. In this work, *Y. lipolytica* showed to be a promising biocatalyst to deconstruct PAF, although degradation extension is still slow for real-world implementation. Lipase, esterase, and protease enzymatic activities were tracked down during cultivations, but no clear induction of their production was observed in the presence of PAF. Therefore, further studies are necessary to elucidate the enzymes involved in polyamide chain breakdown by enzymatic machinery produced by this yeast, as well as to increase depolymerization yields.

**Supplementary Materials:** The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/fermentation10050239/s1, Figure S1. Labeled polyamide-6,6 chemical structure and gHMBC NMR spectrum expanded regions of PAF. X = impurity; Figure S2. Aliphatic region of $^{13}$C CP/MAS NMR spectra of PAF: Control < 0.5 g·L$^{-1}$ < 2 g·L$^{-1}$; Figure S3. Deconvoluted aliphatic region of $^{13}$C CP/MAS NMR spectrum of PAF control; Figure S4. Deconvoluted aliphatic region of $^{13}$C CP/MAS NMR spectrum of the sample with 0.5 g·L$^{-1}$ of PAF; Figure S5. Deconvoluted aliphatic region of $^{13}$C CP/MAS NMR spectrum of the sample with 2 g·L$^{-1}$ of PAF.

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