# Flash hydrolysis of yeast (*Saccharomyces cerevisiae*) for protein recovery

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## Abstract

Protein-rich spent yeast is a waste by-product of brewing and other fermentation industry. A continuous-flow hydrothermal treatment called 'flash hydrolysis' was deployed for protein recovery and yeast disposal. A feed slurry with 1-15 wt% yeast was hydrolyzed at temperatures ranging between 160-280 °C for a very short residence time of 10±2 s. Using 10 wt% yeast at 240 °C, 66.5% carbon, 70.4% nitrogen and 61.0% overall yeast biomass was solubilized in liquid hydrolysate. The liquid hydrolysate in which 63.1% of analyzed amino acids in yeast feed were solubilized, was tested as nutrient for cultivation of *E. coli* in a continuous bioreactor. The steady-state *E. coli* concentration was 1.18 g $L^{-1}$ and 0.93 g $L^{-1}$ using liquid hydrolysate and commercial yeast extract, respectively. Finally, the kinetic parameters for yeast solubilization (reaction order, activation energy and pre-

exponential factor) were found to be 0.86, 21.3 kJ $mol^{-1}$ and 19.36 $[L\ g^{-1}]^{n-1}\ s^{-1}$, respectively.

**Keywords:** Yeast, Flash Hydrolysis, Amino Acid, Nutrient Recycle, Reaction Kinetics

## 1. Introduction

Beer is one of the most popular beverages in the world. In 2019, its global production was 1.9 billion hL with annual market growth of about 1.4%. The brewing process produces three main by-products, namely spent grain, spent yeast and residual hops. Spent yeast is the second largest by-product of the brewing industry after spent grain and its yield is 1.7 to 2.3 g $L^{-1}$ of beer produced [1]. During fermentation (brewing), yeast cells multiply many times depending on the process conditions to produce significantly greater amount of yeast. The harvested yeast, also called spent yeast, is a tan colored, thick and viscous slurry with solid concentration of 3-15 wt% [1,2]. Although, it has some low-value application as animal feed, it is generally considered an organic waste. Its disposal is a concern as the techniques that are generally used such as incineration and landfills, have environmental impacts [3].

The soluble contents of yeast biomass obtained by disrupting and removing the yeast cell membrane is called yeast extract (YE) [4,5]. Only a fraction of the large quantities of spent yeast produced during beer manufacturing is reused as inoculum and thus the rest can be a cost-effective raw material for YE production [6,7]. The high protein content in yeast represents a good source of essential amino acids, which should be part of the diet as neither humans nor other mammals can synthesize them [8,9]. The amino acid

43 composition of YE depends on the cell wall disruption method and following processes
44 [5,8,10–12]. Amino acids in yeast have potential to serve as dietary supplement with
45 health benefits. For example, the non-proteinogenic amino acid γ-aminobutyric acid
46 (GABA) in yeast stimulates immune cells and can prevent diabetes [13]. Products like
47 glutamic acid in the extract can be used as flavor enhancer [14] or the extract can be heat-
48 processed further to form typical YE flavors [15]. YE can be widely used in the food
49 industry, since the European Parliament regulation has classified it as a natural flavor [16],
50 and it has also been assigned a "generally recognized as safe" (GRAS) status [11,17]. YEs
51 are also added to the wort in the brewing industry as a nitrogen source to compensate for
52 inadequate nitrogen supply to the yeast starter culture [18–20]. The quality and type of
53 amino acids in YE play a role in development of flavor in the brew [21].

54 YE can be mainly produced using non-mechanical and mechanical processes. Various
55 non-mechanical disruption methods like autolysis, plasmolysis [22,23] in organic salt
56 solution or non-polar organic solvent, acid or alkali catalyzed hydrolysis, or enzymatic
57 hydrolysis [17,24] are applied to produce YE. Methods other than enzymatic autolysis
58 generate wastewater with chemicals. Enzymatic autolysis is relatively environmental
59 friendly but requires long process time and it is cost intensive at larger scale [3].
60 Commercially, autolytic or plasmolytic methods are primarily used [25] to achieve the
61 highest possible extract yield [12]. Other than cell membrane lysis, another objective
62 during autolysis can be the enzymatic hydrolysis of proteins into amino acids or the
63 splitting of RNA to form flavor-enhancing 5'-nucleotides [4,25]. When thermally sensitive

substances like enzymes or specific cell wall components like β-glucan are to be extracted, mechanical disruption methods such as cell mills are used [19,25,26].

Lamoolphak et al. [3] examined hydrothermal decomposition of baker's yeast into proteins and amino acids. For a batch reaction of 20 min in water at 250 °C, 78% of yeast was solubilized and protein produced was found to be 0.16 mg/mg dry yeast. Also, the hydrolysis products were tested as nutrient source for yeast cultivation and the growth rate was found to be comparable with commercial YE at same concentration. In a study using hog hair, subcritical water at 250 °C for 30 min was reported as a viable process for extraction of amino acids. Longer reaction time resulted in decomposition of hydrolyzed amino acids to ammonia [27]. Also, during hydrothermal reaction of biomass with longer residence time, the hydrolyzed carbohydrates and proteins react further to produce undesired compounds [28].

Hydrothermal treatment of yeast to produce proteins and amino acids can be developed further using flash hydrolysis (FH). In this context, we report a novel approach for protein extraction from yeast using a chemicals-free subcritical water based continuous-flow FH process. FH, where wet biomass slurry is subjected to high temperature for a very short residence time (8-12 s) has been a proven technology for the fractionation of algae components like proteins and lipids [28]. Spent yeast slurry produced during brewing can be economically fractionated through a continuous FH process without any dilution or concentration. FH can not only be a solution for disposal of spent yeast but also provide a revenue stream by recovering valuable components. To the

best of our knowledge, a scalable continuous-flow process characterized by short residence time (10±2 s) used in this study, has not been reported so far.

This study aims at optimizing the temperature and feed concentration for recovery of proteins from yeast through FH. To investigate the possible application of the YE obtained by FH as a medium for bacterial cultivation, the liquid hydrolysate obtained under the optimal experimental conditions was used as nutrient for *E. coli* cultivation, and the performances compared with those of a commercial product. Finally, the kinetics of yeast solubilization reaction were determined based on the experimental results.

## 2. Material and methods

'Red Star Active' (Milwaukee, WI, USA) dry yeast was used as a model for brewer's spent yeast in FH experiments. The yeast was composed of lipids (6 wt%), carbohydrates (33 wt%) and proteins (50 wt%). Hydrochloric acid (HCl) and sodium hydroxide (NaOH) were purchased from Alfa Aesar (Ward Hill, MA, USA). Amino acid standard H, eluent chemicals for Ion Chromatography (IC) and standard for elemental composition (2,5-Bis(5-tert-butyl-benzoxazol-2-yl)thiophene) were purchased from Thermo Scientific (Waltham, MA, USA). Organic carbon (1000 ppm) and nitrogen (100 ppm) standards were procured from Ricca Chemical Company (Arlington, TX, USA). For *E. coli* growth experiments, the strain (ATCC 25922) was obtained from ATCC (Manassas, VA, USA), while commercial YE for media was procured from Sigma Aldrich (St. Louis, MO, USA). De-ionized water was used for all the experiments unless otherwise specified.

### 2.1 Flash hydrolysis

106 The schematic of the FH setup is shown in Fig. 1. The unit consists of pumping system,
107 tubing, reactor, induction heating and control system, quenching zone and back pressure
108 regulator (BPR). The induction heating and control system supplied by GH Induction
109 Atmospheres (Rochester, NY, USA) could provide up to 5 kW of power. A LEWA (Holliston,
110 MA, USA) EcoFlow diaphragm metering pump used in this study is capable of delivering
111 concentrated yeast slurry at appropriate flow rates to maintain desired residence time in
112 the reactor and generating appropriate pressures for subcritical water conditions. For ease
113 of construction, the reactor and tubing were made of the same high-pressure tube
114 supplied by High Pressure Equipment (Erie, Pennsylvania, USA). A tubular reactor with
115 internal diameter of 0.31'' (7.9 mm) was selected to meet the required residence time.
116 The 16'' (40.6 cm) long tube which was wound by the induction heater coil was considered
117 as the reaction zone. An Omega (Norwalk, CT, USA) TJ36 thermocouple located at the end
118 of the reaction zone was inserted inside the tubular reactor through a junction to measure
119 the reaction temperature. The design pressure for the tubes and connectors was 137.9
120 MPa, which gave sufficient safety margin when operating at pressure of 10.3±0.15 MPa.
121 The quenching zone was designed to utilize chilled water to lower the slurry temperature
122 to below 100 °C, so the output hydrolysate would remain in liquid phase at collection
123 point. A dome loaded BPR was used in the FH setup, manufactured specially for research
124 by Equilibar (Fletcher, NC, USA).

125 Yeast slurry of 1, 5, 10, and 15 wt% was made using dry yeast and DI water. It was then
126 pumped at a flow rate of 95 mL $min^{-1}$ to maintain the residence time of 10±2 s in the

reactor. The pressure of 10.3±0.3 MPa which was higher than the vapor pressure of water in the temperature range of study was maintained using BPR and then the induction heater was switched on. The desired temperature of 160, 200, 240 or 280 °C was maintained in the reactor using induction heater power control system. The deviation in reaction temperature was <10 °C during all the runs. After the system reached steady state in terms of flowrate, pressure and temperature, the hydrolysate was collected at the outlet of the reactor. The hydrolysate was made up of liquid fraction (liquid hydrolysate) and solid fraction (solid residue). At a given temperature and yeast feed concentration, the system was operated for 10 min. The residence time of the reactor was calculated using eq. 1, where *V* is the reactor volume (mL), *F* is the volumetric flow rate of pumps (mL $min^{-1}$), $\rho_{pump}$ is the density of water at pump conditions (g $mL^{-1}$), and $\rho_{P,T}$ is the density of water at reactor conditions (i.e., pressure and temperature).

$$t = \frac{V}{F(\frac{\rho pump}{\rho_{P,T}})} \quad (1)$$

The solubilization of components (carbon, nitrogen and amino acids) in liquid hydrolysate was calculated using eq. 2 and solid residue was calculated using eq. 3.

$$Solubilization\ (\%) = \frac{Component\ in\ liquid\ hydrolysate\ (g/l)}{Component\ in\ feed\ (g/l)} \times 100 \quad (2)$$

$$Solid\ residue\ (\%) = \frac{Solids\ in\ hydrolysate\ (g/l)}{Solids\ in\ feed\ (g/l)} \times 100 \quad (3)$$

All FH experiments were performed in duplicate and the reported results are the average of two values.

## 2.2 Analyses of flash hydrolysis products

After each experiment, a mixture of liquid and solid products was recovered and separated by vacuum filtration using mixed cellulose esters (MCE) membrane disc filters (0.22 µm). The solid fraction was washed using DI water and dried at 105 °C to determine percentage of yeast solubilized. The liquid hydrolysate was analyzed for total organic carbon (TOC) and total nitrogen (TN) composition using Shimadzu TOC/TN analyzer (Suzhou, China). The gaseous products, which were appreciably low in the temperature range of study, were vented without analysis. At select reaction conditions, solid residue composition was further analyzed using Flash 2000 Elemental Analyzer (EA) by Thermo Scientific (Bremen, Germany) and Cary 630 Fourier Transform Infrared Spectroscopy (FTIR) (Santa Clara, CA, USA). At these select reaction conditions, liquid fraction was also further analyzed for amino acid composition using IC (Dionex ICS-5000 AAA-Direct$^{TM}$ equipped with an AminoPac PA10 column and column guard) supplied by Thermo Scientific (Waltham, MA, USA). A calibration curve was generated using an external amino acid standard for quantification. Free amino acids in the liquid hydrolysate were analyzed by directly running the samples through IC, whereas the peptides in the liquid hydrolysate were acid hydrolyzed to free amino acids before IC analysis. The amino acid composition of yeast was also analyzed by acid hydrolyzing the biomass to free amino acids and then running the samples through IC. The acid hydrolysis of liquid hydrolysate and yeast was done using 6N HCl at 110 °C for 18 h, followed by neutralization using 6N NaOH before IC analysis. Other than histidine, all 16 amino acids in Amino Acid Standard H used for the IC

analysis, could be accurately quantified. Therefore, histidine was only qualitatively analyzed.

### 2.3 Nutrients recycle for *E. coli* cultivation

The liquid hydrolysate produced at select FH condition was freeze-dried for preservation. This freeze-dried powder was called FH YE and was further tested as a nutrient (nitrogen) source for *E. coli* growth. Continuous experiments were carried out in chemostat mode to assess the YE exploitation at steady state. The strain was precultured overnight in standard lysogeny broth (LB) medium, containing 10 g $L^{-1}$ of peptone and 5 g $L^{-1}$ of commercial YE and 5 g $L^{-1}$ of NaCl. Under sterile condition, about 5 mL of this culture was used as inoculum for cultivation tests. Cultivation tests were carried out in continuous reactors, comparable to a perfectly mixed continuous stirred-tank reactor (CSTR), with a working volume of 50 mL. The reactors were composed of sterilized glass bottles, where mixing was provided by magnetic stirring, coupled with filtered air bubbling, which also provided the required oxygen, at an average flow of 2 L $h^{-1}$. The temperature was maintained at 37 °C using a thermostatic bath. Fresh medium was continuously provided by means of a Sci-Q 400 peristaltic pump supplied by Watson Marlow (Falmouth, UK) at a constant volumetric flow rate of 28 mL $d^{-1}$ from a sterilized, external, stirred glass bottle. An overflow tube was placed on the opposite side of the fresh medium inlet, ensuring the same outlet volumetric flow rate, thus keeping a constant culture volume. The resulting residence time, calculated as the ratio between the reactor volume and the flowrate, was 1.8 h. Optical density at 600 nm was measured every hour to observe the establishment of

188 a steady state. After the steady state was reached, it was maintained for about 4 h, and at
189 least 5 samples were taken during this time, to measure the biomass concentration in
190 terms of dry weight (DW). DW was measured by filtering under vacuum 10 mL of
191 previously harvested cells, with a 0.22 µm filter. The filter was then dried for 2 h at 100 °C
192 in a laboratory oven.

193 Four different culture media were tested 1) standard LB, used as a control, with
194 commercial YE; 2) LB with FH YE; 3) commercial YE only; and 4) FH YE only. The
195 formulations are reported in Table 1. The amount of FH YE supplied, with respect to the
196 commercial YE, was adjusted based on the nitrogen content (data certified by the
197 manufacturer). Student's T test was performed in order to ascertain statistically significant
198 differences among the tested conditions in terms of average biomass concentration
199 reached at steady state. The level of statistical significance was taken at $p < 0.05$.

200 **Table 1.** Composition of media used in the *E. coli* cultivation tests

| | **Peptone ($g\ L^{-1}$)** | **Commercial YE ($g\ L^{-1}$)** | **FH YE ($g\ L^{1}$)** | **NaCl ($g\ L^{-1}$)** |
|---|---|---|---|---|
| **LB (control)** | 10 | 5 | -- | 10 |
| **LB FH** | 10 | -- | 8.2 | 10 |
| **Commercial YE** | -- | 10 | -- | 10 |
| **FH YE** | -- | -- | 16.3 | 10 |

201

202 **2.4 Yeast solubilization kinetics**

The experimental results obtained at different temperatures (160-280 °C) and yeast feed concentrations (1-15 wt%) were used to retrieve the yeast solubilization kinetics in the FH process. The overall solubilization reaction, lumping all the water-soluble products into one since insoluble products were separated and gaseous products were vented, can be summarized as:

$$X \rightarrow Products \quad (4)$$

where *X* represents yeast. The rate of yeast solubilization (g $L^{-1}$ $s^{-1}$) can be expressed as:

$$r_x = -kc_x^n \quad (5)$$

where *k* is the kinetic constant ([L $g^{-1}$]$^{n-1}$ $s^{-1}$), $c_x$ the yeast concentration (g $L^{-1}$) and *n* is the order of reaction. The kinetic constant can be expressed as a function of temperature, according to the Arrhenius law (eq. 6).

$$k = A \cdot \exp\left(-\frac{E_a}{RT}\right) \quad (6)$$

with *A* being the pre-exponential factor, $E_a$ the activation energy (kJ $mol^{-1}$), *R* the ideal gas constant (8.314 J $mol^{-1}$ $K^{-1}$) and *T* the temperature in K. The reaction kinetic parameters (*A*, $E_a$ and *n*) were regressed based on experimental data, considering the material balance of a Plug-Flow reactor (PFR). According to the experimental set-up used, isothermal conditions and steady-state assumption were considered. The material balance can be written as:

$$\frac{dc_x}{dz} = \frac{1}{v} r_x = \frac{A \cdot \exp\left(-\frac{E_a}{RT}\right) c_x^n}{v} \quad (7)$$

where $z$ is the length co-ordinate of the reactor (cm), and $v$ is the velocity inside the tube (cm $s^{-1}$), calculated as the ratio between the volumetric flow rate and the reactor cross-sectional area. The material balance was solved from $z = 0$ ($c_x = c_{x,0}$) to $z = L$, using ode23 in MATLAB®. The fminsearch function was used to minimize the sum of squared errors (SSE, eq. 8) between experimental and calculated values of the outlet biomass concentration, $c_{x,L}$.

$$SSE = \sum (c_{x,L,calc} - c_{x,L,exp})^2 \quad (8)$$

## 3. Results and discussion

### 3.1 Flash hydrolysis

As reported in Fig. 2, the impact of yeast concentration in the feed on solubilization of carbon and nitrogen was not substantial at all investigated temperatures. However, higher yeast concentration in feed resulted in a lower yeast solubilization at 240 and 280 °C. This could be due to differences in heat transfer (radial temperature gradient) within the slurry of different yeast concentration. By increasing the reaction temperature from 160 to 240 °C, solubilization of carbon and nitrogen steadily increased. The change in carbon and nitrogen solubilization with increase in reaction temperature from 240 to 280 °C was not as substantial as the change with increase in reaction temperature from 200 to 240 °C, suggesting the occurrence of saturation. Whereas the overall biomass solubilization steadily increased with increase in temperature from 160 to 280 °C, as shown in Fig. 2C. Operation of the FH system at 15 wt% yeast feed was challenging due to higher deviation in reaction temperature and frequent clogging of the BPR. Therefore, for higher accuracy,

243 FH products obtained at different temperatures (160-280 °C) using 10 wt% yeast feed
244 were further analyzed using IC, EA and FTIR. The overall biomass solubilization using 10
245 wt% yeast feed at 240 and 280 °C and residence time of 10±2 s was 61.0% and 78.3%,
246 respectively. This was comparable to the value of 78% solubilization in a batch
247 hydrothermal reaction carried out at 250 °C for a considerably higher reaction time of 20
248 min using the same yeast concentration, while it was significantly higher than the 32%
249 obtained through autolysis after 19 h as reported by Lamoolphak et al. [3]. Compared to a
250 batch hydrothermal process, continuous-flow FH with a short residence time could
251 possibly have lower capital and operating cost due to smaller equipment size and lower
252 energy requirement, in terms of scale-up at industrial scale.

253 The nitrogen solubilization trend which is an indirect representation of protein
254 solubilization shown in Fig. 2B indicated that increasing temperature of FH beyond 240 °C
255 did not substantially improve nitrogen solubilization, which is also observed in amino acid
256 profile of the acid hydrolyzed liquid hydrolysate shown in Fig. 3. The total average
257 deviation in amino acid solubilization reported was less than 6% for duplicate
258 experiments. Decrease in solubilization of some amino acids like alanine, threonine, lysine,
259 serine, aspartate and cystine at 280 °C indicate partial degradation. Except for cystine,
260 solubilization of all other amino acids analyzed increased with increase in temperature
261 from 160 to 240 °C. Solubilization of cystine was complete at 160 °C but it decreased with
262 increase in temperature and reached the lowest 3.2% at 280 °C. Cystine could be
263 completely solubilized at 160 °C as it accounted for only 0.5% of total amino acids

analyzed in yeast. Also, due to its low concentration, a small degradation with rise in reaction temperature significantly affects its solubilization percentage compared to other amino acids. The transformation of amino acids under hydrothermal condition occurs through decarboxylation to produce carbonic acid and amines and deamination to produce ammonia and organic acids. The ratio and extent of deamination to decarboxylation differs depending on the type of amino acid and severity of experimental conditions. These undesired reactions which cause amino acid degradation can be significantly reduced by using FH compared to conventional batch hydrothermal reactions with longer residence time [28,29]. The IC analysis of liquid hydrolysate before acid hydrolysis showed presence of free amino acids in very low concentrations (<5% solubilization), arginine being the only exception. The solubilization of free arginine increased from 17.0% to 37.4% with increase in temperature from 160 to 280 °C. The total solubilization of all the amino acids analyzed in yeast at 160, 200, 240 and 280 °C was 21.4, 34.4, 63.1 and 60.3%, respectively.

Since the FTIR spectrum was similar for solid residues produced in duplicate experiments, only one set of spectra is reported as an example. As reported in Fig. 4, the lipid absorbance (C-H stretch) at wavelength 2920 and 2853 $cm^{-1}$ intensifies with increase in temperature. With rising reaction temperature, the biomass solubilization increases, and thus biochemical components other than lipids are selectively solubilized, as observed by Garcia-Moscoso et al. [28] in case of algae FH. Absorbance linked to amide I (C=O stretch) and amide II (N-H and C-N vibrations) at 1637 $cm^{-1}$ and 1526 $cm^{-1}$ respectively,

285 slightly rose with increase in reaction temperature from 160 to 240 °C. The absorbance
286 was maximum at 280 °C which suggests higher degree of protein-derived insoluble
287 product formation at 280 °C. Under hydrothermal conditions (>250 °C), Maillard reactions
288 occur between carbonyl group of carbohydrates and amine group of proteins or amino
289 acids resulting in the formation of dark brown high-molecular-weight material called
290 melanoidins [30,31]. This nitrogenous material with low solubility results in processing
291 difficulties like fouling of process equipment, as observed in FH at 240 and 280 °C using 15
292 wt% yeast feed. The absorbance due to β-glucans at 1031 $cm^{-1}$ and 990 $cm^{-1}$ was found to
293 be similar in yeast and solid residue produced at reaction temperatures other than 280 °C.
294 The absorbances linked to glucans at 280 °C nearly disappeared, indicating higher degree
295 of carbohydrate hydrolysis at 280 °C.

296 In Table 2, it is shown that the nitrogen content in the solid residue produced at 160 °C
297 was higher compared to yeast, as only 23.7% nitrogen was solubilized when yeast
298 solubilization was 31.2%. The C/N ratio in solid residue increased from 5.8 to 6.8 at 240 °C
299 due to selectively higher solubilization of proteins in hydrolysate and accumulation of
300 lipids and other non-protein compounds in solid residue, which was also observed in the
301 FTIR spectrum. Further increase in temperature up to 280 °C, caused decrease in solid
302 residue formation from 39.0% to 21.7%, whereas the increase in nitrogen solubilization
303 was only 5%. This suggests a possible formation of insoluble proteinaceous/nitrogenous
304 degraded material from solubilized protein and amino acids at 280 °C which was also
305 revealed by the decrease in solubilization of some amino acids in the liquid hydrolysate

and the increased absorbance due to amides in FTIR spectrum. The sharp increase in solid residue carbon content at 280 °C, suggests the occurrence of carbonization reaction [3] and reduction in O/C ratio [32] due to higher reaction temperature.

**Table 2.** Elemental analysis of yeast and solid residues produced using 10 wt% yeast feed and FH reaction temperature 160, 200, 240 and 280 °C

| | N (%) | C (%) | H (%) | C/N |
|---|---|---|---|---|
| **Yeast** | 7.0±0.03 | 44.7±0.14 | 6.9±0.00 | 6.4 |
| **160 °C** | 8.4±0.02 | 48.8±0.24 | 7.4±0.14 | 5.8 |
| **200 °C** | 7.7±0.14 | 48.9±0.38 | 7.4±0.11 | 6.3 |
| **240 °C** | 7.5±0.03 | 50.8±0.07 | 7.5±0.03 | 6.8 |
| **280 °C** | 12.0±0.07 | 56.9±0.23 | 7.4±0.05 | 4.8 |

Overall, using 10 wt% yeast feed, FH at 280 °C helped lower solid residue formation but had slight negative effect on overall protein solubilization. Hence, FH of yeast at 240 °C was found to be the optimum temperature for protein solubilization, as it could also result in lower operating costs compared to 280 °C.

### 3.2 Nutrients recycle for *E. coli* cultivation

The FH YE used in the *E. coli* growth experiments was obtained at 240 °C and 10 wt% yeast feed. A comparison in terms of composition with the commercial product was made, revealing a difference in macronutrient content. In particular, the commercial YE contains

11 wt% of nitrogen, as certified by the manufacturer for the specific lot used. The information about carbon content was not available, but literature reports an average content of about 40 wt% [33]. The composition of the FH YE showed a lower nitrogen content (6.7 wt%), but a slightly higher carbon content (47 wt%). With the aim of assessing the possible exploitation of FH YE as nitrogen source, the media were formulated normalizing the final nitrogen content, as reported in Table 1, also to account for the main aim of recycling protein and nitrogen thanks to FH. Continuous cultivation was chosen as culturing method, in order to allow cells to acclimate to the new medium composition. Results of average biomass concentration at steady state are reported in Fig. 5: remarkably, the biomass concentration obtained using the FH YE was significantly higher than that produced with the LB (control). This suggests not only that the flash hydrolysate can be used as alternative source of nitrogen, but it is also beneficial for growth, possibly due to the higher carbon content provided. To ascertain this hypothesis, a second set of experiments was carried out, without providing peptone (which itself is a nitrogen source) but doubling the concentration of YE to avoid possible nutrient limitations that may cause the culture washout in continuous reactors. The average biomass concentration reached was found not to be significantly different than that obtained in LB medium, confirming that carbon and nitrogen are indeed supplied in excess in the standard media composition. Also, in this case, an increased biomass concentration was observed when providing FH YE as sole source of nutrient compared to commercial YE. Thus, it appears that the liquid hydrolysate from FH is able not only to provide

341 macronutrients, but possibly other micronutrients (vitamins) that result in an improved
342 overall bacterial growth. Phenolic compounds were not analyzed in this study; however, it
343 has been previously reported that the liquid hydrolysate generated through conventional
344 hydrothermal reactions with longer residence time than FH have higher concentration of
345 phenolic compounds. This limits its application as nutrient media due to the inhibitory
346 effect of phenolic compounds on microbial growth [28]. YE is commonly used as a source
347 of micronutrients, co-factors, vitamins and minerals, but the exact composition is not
348 always available, making comparison of the two products difficult. On the other hand,
349 from an overall mass balance perspective, FH YE seems to be a promising alternative as
350 cultivation media. As a drawback, it should be mentioned that the turbidity of the medium
351 composed by FH YE was found to have increased, which is a critical point when applying
352 optical density methods to assess growth.

353 **3.3 Yeast solubilization kinetics**

354 The values of the kinetic parameters were obtained from the regression. The reaction
355 was found to be of order 0.86, with an activation energy of 21.3 kJ $mol^{-1}$ and pre-
356 exponential factor of 19.36 $[L\ g^{-1}]^{n-1}\ s^{-1}$. The goodness of the fitting can be inferred from
357 the plots of Fig. 6, where the result of the regressed model is represented together with
358 experimental points in the whole range investigated (Fig. 6A), and calculated values are
359 plotted against experimental ones, showing that points are well aligned along the bisector
360 (Fig. 6B). In addition, standardized residuals are all comprised within the interval [-2, 2],

except for one data point (160 °C and 15 wt% yeast feed), with most of them being between -1 and 1.

The kinetic parameters obtained could further be used for design of commercial scale FH setup. In particular, the kinetic model can be applied to assess the best operating conditions of a real scale plant in terms of optimum operating temperature: a tradeoff temperature can be selected, balancing energy required for heating and gain in reaction conversion. A further technoeconomic analysis will also assess the costs of the process proposed, related to best operating temperature. Additional information that can be obtained by the modeling approach are related to the best residence time, that has a role in the design of a full-scale reactor for yeast biomass hydrolysis.

## 4. Conclusions

A continuous hydrothermal process for recovery of nutrients from yeast was developed whereby 66.5% carbon, 70.4% nitrogen and 61.0% overall yeast was solubilized at 240 °C. Flash hydrolysis with residence time of 10±2 s can serve as waste disposal and nutrient recovery technique for yeast slurry (1-15 wt%) without dilution or cost-intensive drying. The liquid hydrolysate from flash hydrolysis could be used as nutrient for cultivation of *E. coli* without growth inhibition. The kinetic parameters, reaction order (0.86), activation energy (21.3 kJ $mol^{-1}$) and pre-exponential factor (19.36 $[L\ g^{-1}]^{n-1}\ s^{-1}$) could further be used for scale-up of flash hydrolysis setup.

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**Figure captions**

**Figure 1.** Schematic of the flash hydrolysis setup

**Figure 2.** [A] Solubilization of carbon, [B] solubilization of nitrogen and [C] solid residue after FH of 1, 5, 10 and 15 wt% yeast feed at reaction temperature 160, 200, 240 and 280 °C

**Figure 3.** Amino acid solubilization in liquid hydrolysate generated using 10 wt% yeast feed and FH reaction temperature 160, 200, 240 and 280 °C, analyzed after acid hydrolysis (amino acids split between three plots [A], [B] and [C])

**Figure 4.** FTIR spectrum of solid residues produced using 10 wt% yeast feed and FH reaction temperature 160, 200, 240 and 280 °C

**Figure 5.** Average *E. coli* concentration using standard LB media with commercial YE (light grey) and FH YE (dark grey) compared to media containing only commercial YE (light grey) and FH YE (dark grey). Error bars refer to standard deviation and letters show statistically significant differences with $p < 0.05$

**Figure 6.** (A) Model (lines) and experimental results (dots) of outlet yeast (solid residue) concentration as a function of inlet feed concentration at different temperatures and (B) calculated (line) against experimental values (dots) of outlet yeast (solid residue) concentration

**Figure 1.**

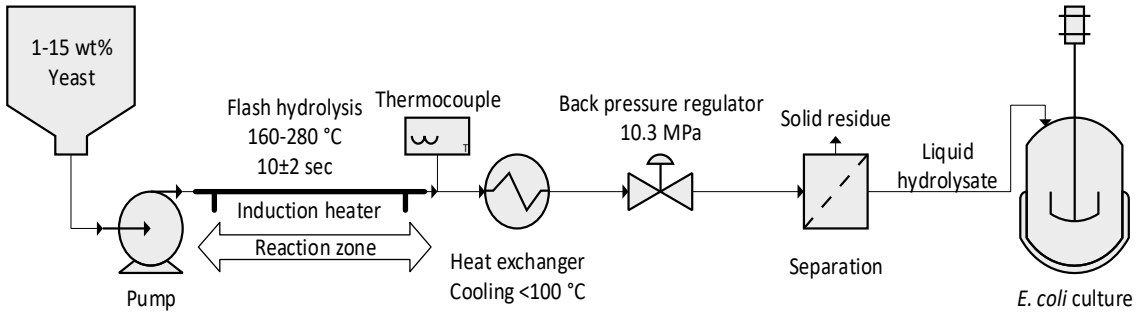

1-15 wt%
Yeast
Flash hydrolysis
160-280 °C
10±2 sec
Thermocouple
Back pressure regulator
10.3 MPa
Solid residue
Liquid
hydrolysate
Induction heater
Reaction zone
Pump
Heat exchanger
Cooling <100 °C
Separation
E. coli culture

**Figure 2.**

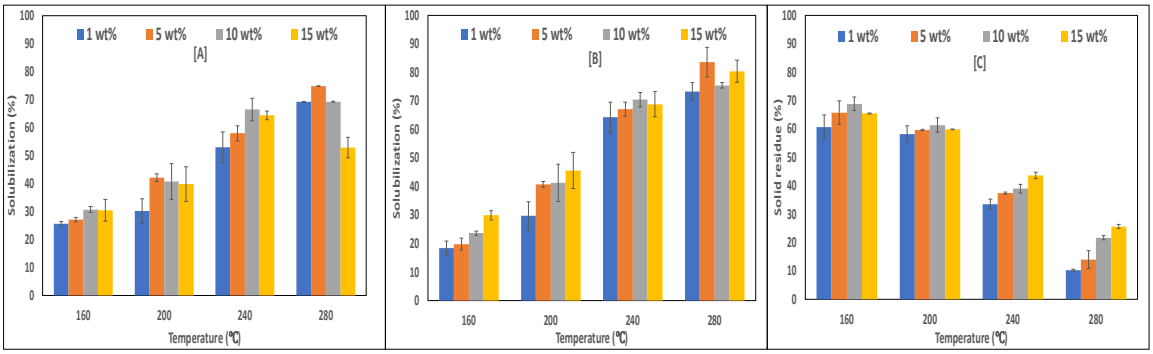

1 wt%
5 wt%
10 wt%
15 wt%
[A]
[B]
[C]
Solubilization (%)
Solid residue (%)
Temperature (℃)
160
200
240
280

**Figure 3.**

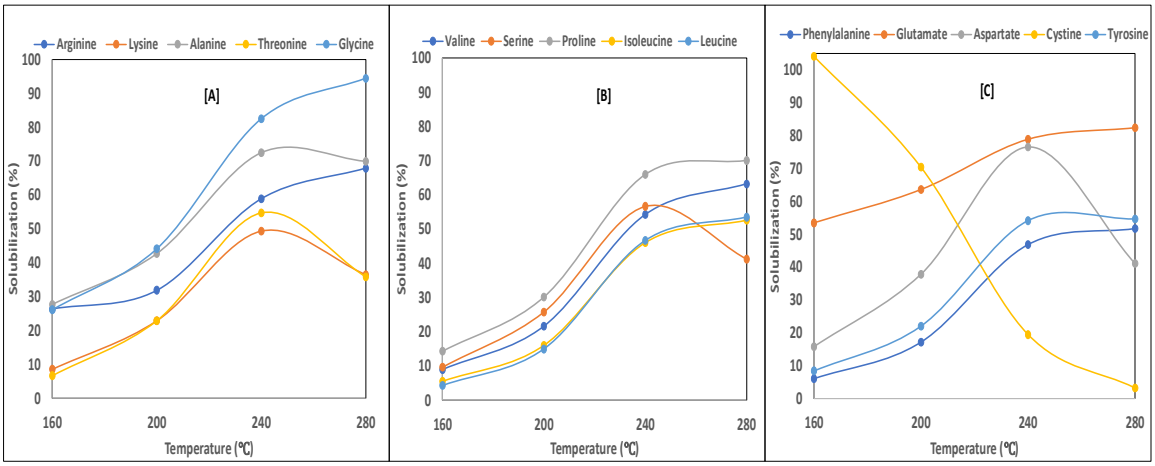

Arginine
Lysine
Alanine
Threonine
Glycine
[A]
Solubilization (%)
Temperature (℃)
Valine
Serine
Proline
Isoleucine
Leucine
[B]
Solubilization (%)
Temperature (℃)
Phenylalanine
Glutamate
Aspartate
Cystine
Tyrosine
[C]
Solubilization (%)
Temperature (℃)

**Figure 4.**

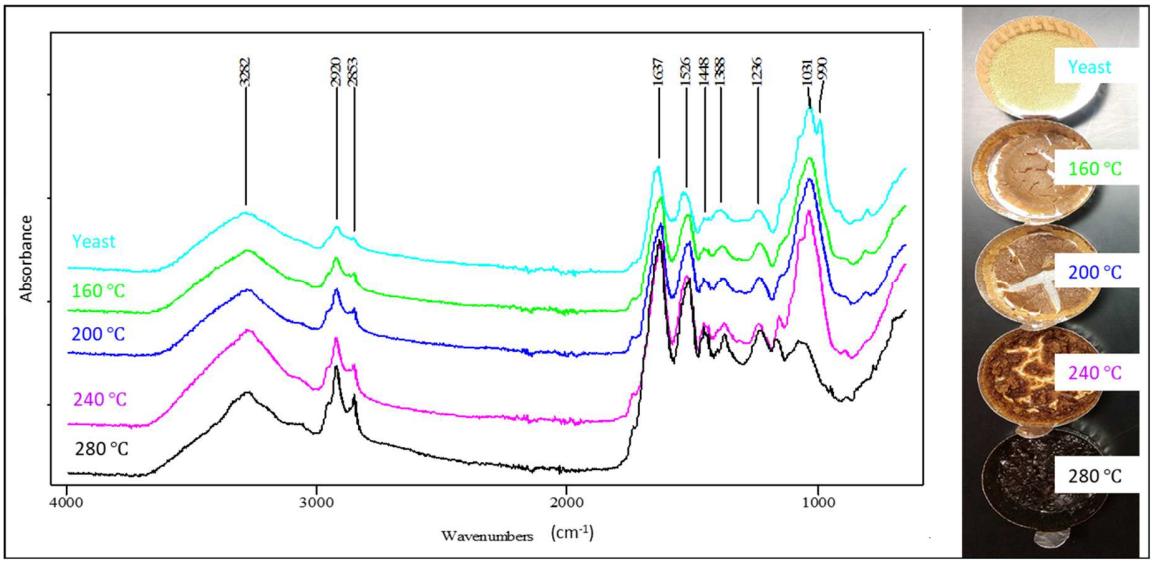

3282
2920
2853
1637
1526
1448
1388
1236
1031
930
Yeast
160 °C
200 °C
240 °C
280 °C
Absorbance
4000
3000
2000
1000
Wavenumbers (cm-1)
Yeast
160 °C
200 °C
240 °C
280 °C

**Figure 5.**

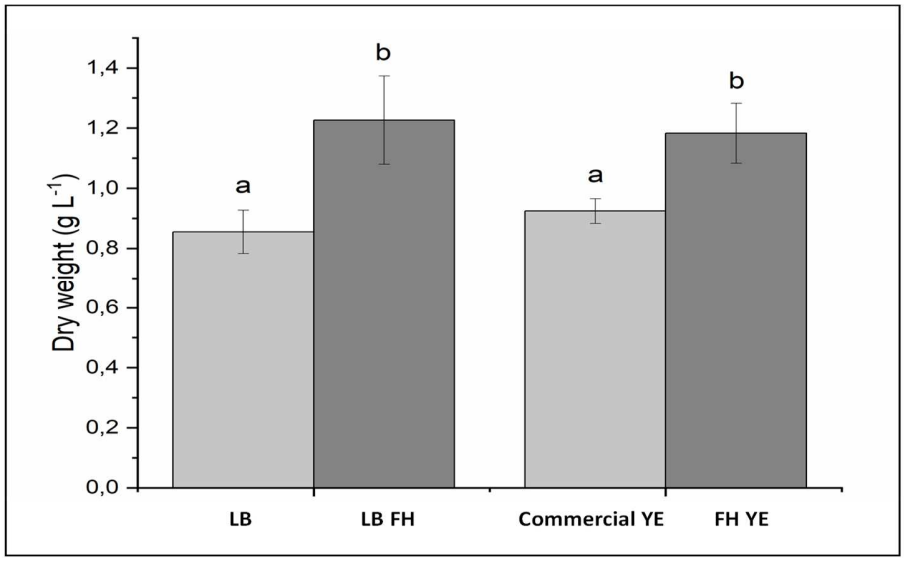
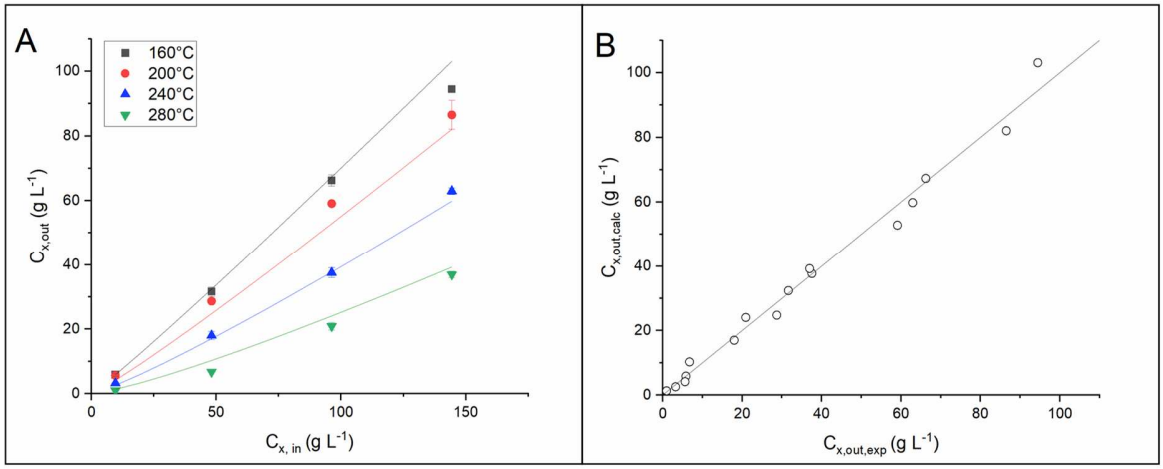
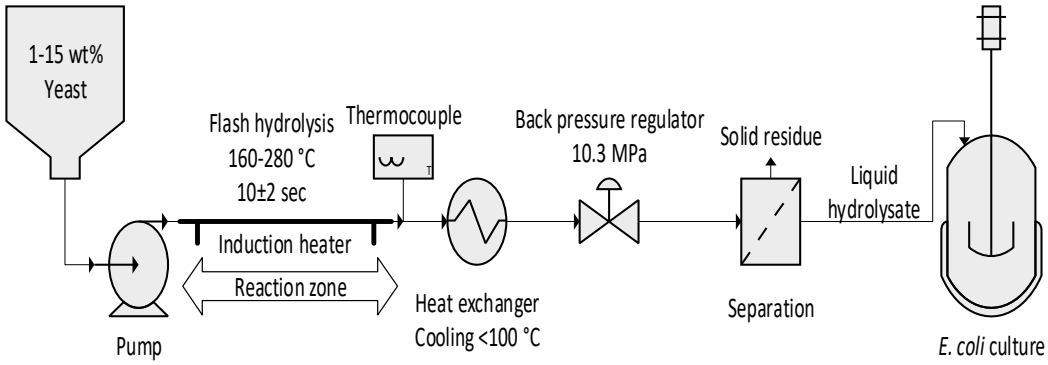

Dry weight (g L-1)
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0,0
a
b
a
b
LB
LB FH
Commercial YE
FH YE

**Figure 6.**


A
160°C
200°C
240°C
280°C
$C_{x,out}$ (g $L^{-1}$)
$C_{x,in}$ (g $L^{-1}$)
B
$C_{x,out,calc}$ (g $L^{-1}$)
$C_{x,out,exp}$ (g $L^{-1}$)


1-15 wt%
Yeast
Flash hydrolysis
160-280 °C
10±2 sec
Thermocouple
Back pressure regulator
10.3 MPa
Solid residue
Liquid
hydrolysate
Induction heater
Reaction zone
Heat exchanger
Cooling <100 °C
Separation
Pump
E. coli culture