



## BIOLOGICAL SCIENCES

# Hydrolysis of sweet potato (*Ipomoea batatas* (L.) Lam.) flour by *Candida homilentoma* strains: effects of pH and temperature using Central Composite Rotatable Design

ANA LETÍCIA S. COELHO, AGELLES A. ARRAES, THIAGO LUCAS DE ABREU-LIMA & SOLANGE CRISTINA CARREIRO

**Abstract:** The current study focuses on the evaluation of culture parameters on the enzymatic hydrolysis of *Ipomoea batatas* (L.) Lam flour by *Candida homilentoma* strains. A 2-factor-5-level CCRD was used to evaluate the effect of pH and temperature on the hydrolysis process. For the S-47 strain, pH and both studied parameters were significant at 48 h and 96 h, respectively. Regarding S-81 strain, temperature was the only factor affecting the process, at 96 hours. The regression models were significant, and no lack of fit was observed for them.

**Key words:** amylases, *Candida homilentoma*, reducing sugar, statistical design, starch, sweet potato flour.

## INTRODUCTION

Fossil fuels have boosted the progress of human civilization over the last century. However, the current depletion of oil reserve allied to environmental problematic, such as global warming, impels governments and research centers to seek alternative fuels (Ledesma-Amaro et al. 2015, Pothiraj et al. 2015, Wang et al. 2016).

Ethanol is one of the most biofuel used in the world and, as their demand increases, the production from non-conventional raw sources, including starched-based materials, has been encouraged (Lareo et al. 2013, Omidvar et al. 2016, Rathore et al. 2016). Nevertheless, the production cost of these biofuels - with exception to corn, which has mature technology developed by the USA - remains a challenge, especially in relation to hydrolysis process, where the conversion of polysaccharides

into monosaccharides takes place (Gao et al. 2012, Vicktor et al. 2013, Padmanaban et al. 2015).

The use of sweet potato (*Ipomoea batatas* (L.) Lam.) as raw material for ethanol production can reduce the costs of process (Padmanaban et al. 2015), since this tuberous root contains several nutrients, such as carbohydrates, some amino acids, besides minerals (Ca, Fe, Mg, K, P, Na and Zn) and vitamins (Hayek et al. 2013). Moreover, Pereira et al. (2017) reported that it is possible to obtain a greater quantity of fermentable sugars from sweet potato than other common crops, such corn, for example. In addition, sweet potato flour has some advantages over fresh one, such as: (i) higher sugar concentration; (ii) energy saving due to lesser manipulation of material and (iii) less viscous wort (Lareo et al. 2013).

Nonetheless, the cost of amylases has been recognized as limiting factor for expanding the production of ethanol from starched-based

sources (Pereira et al. 2017). In this sense, Pereira et al. (2017) reported that the use of crude extracts instead of purified enzymes could be a solution to such problem. However, due to the lack of experimental studies in this field, optimization tests should be firstly carried out.

In this context, we can highlight two parameters that deserve attention: pH and temperature. Scriban (1985) states that enzymes are very sensitive to  $H^+$  concentration in reaction medium, because pH modifies the active site of the enzymes, thus, preventing the binding of substrate. The increase of temperature in the reaction environment provides more collisions between enzyme and substrate, accelerating the reaction kinetic; but could also break intramolecular bonds of the enzymes, resulting in the protein denaturation (Hayek et al. 2013, Sandri et al. 2015).

Furthermore, Mehta et al. (2016) reported that the interaction between pH, temperature and time of incubation, strongly influences the metabolism of microorganisms, which consequently affect the production of enzymes.

Thus, Central Composite Rotatable Design (CCRD) can be considered an important tool to optimize independent factors that influences the enzyme production/activity, maximizing the yield and productivity (Premalatha et al. 2015, Selvan et al. 2016, Liu & Peng 2017).

Therefore, the current study aimed to investigate the influence of pH and temperature on the enzymatic hydrolysis of sweet potato flour by two *Candida homilentoma* strains, under submerged culture.

## MATERIALS AND METHODS

### Microorganism

The two *Candida homilentoma* strains (codified as S-47 and S-81) used in this study were isolated from the nests of leaf-cutting ants (*Acromyrmex*

*balzani*) of the Brazilian Cerrado (savanna), and were deposited in the microbial Culture Collection Carlos Rosa at the Federal University of Tocantins, Brazil.

### Inoculum Preparation

For the inoculum preparation, S-47 and S-81 strains were reactivated by streaked method onto Sabouraud-glucose nutrient agar (2% glucose (w/v), 1% peptone (w/v), 1.8% agar (w/v), 0.5% yeast extract (w/v), 0.02% chloramphenicol (w/v)), and then incubated in a biological incubator, Biochemical Oxygen Demand (BOD), for 48 h at 25°C. Thereafter, a loop of fresh culture was transferred from the agar plates to 200 mL of Sabouraud-glucose broth (5% glucose (w/v)), which was incubated in an agitador shaker (Tecnal) at 200 rpm, for 24 h at 30°C.

The biomass was separated by centrifugation (centrifuge Model 280R Excelsa 4 FANEM) at 10000g for 30 minutes, the supernatant was discarded and biomass was further used in the hydrolysis experiments.

### Cultivation Media

The sweet potato flour used as a substrate in the current study was donated by the Laboratory of Renewable Energy Production Systems (Laboratório de Sistemas de Produção de Energias Renováveis (LASPER)) of UFT. For culture medium preparation, the starched-based material was firstly passed through a sieve (10 mesh) to obtain a fine and homogeneous flour. Sodium acetate buffer at different pH values (10mM; 4.5- 6.5) were prepared according to the experimental design proposed (Table I), and subsequently used as liquid phase of slurry consisting of 2% (w/v) of sweet potato starch (flour) and 0.5% (w/v) of yeast extract. The mixture was sterilized by autoclaving at 121°C (1 atm) for 20 minutes.

**Table I. Experimental ranges and levels of temperature and pH.**

Code	Variable	Low axial (-1.42)	Low factorial (-1)	Center (0)	High factorial (+1)	High axial (+1.42)
X <sub>1</sub>	pH	4.5	4.8	5.5	6.2	6.5
X <sub>2</sub>	Temperature (°C)	30	33	40	47	50

### Submerged Culture Process

The biomass obtained as described above (subitem inoculum preparation) was suspended in 10 mL of sterile buffer sodium acetate 10mM (app. 10<sup>6</sup> cells.mL<sup>-1</sup>) for each pH value tested. Then, the suspension was transferred to Erlenmeyer flasks (250 mL) containing 90 mL of sterile culture medium (subitem cultivation media). The flasks were incubated in thermostatic bath at different temperatures from 30 to 50°C, as shown in Table I. Aliquots of 5 mL of the samples were taken at 0 (blank experiment), 24, 48 and 96 hours. Thereafter, the culture medium were centrifuged at 10000g for 10 minutes, and the supernatant was used for analysis of the reducing sugars. All runs were conducted in triplicate.

### Central Composite Rotatable Design and Statistical Analysis

Table I shows the experimental design matrix, with variables range and levels. The operational parameters – pH and temperature – were examined at five different levels (- α, -1, 0, +1, +α) by Central Composite Rotatable Design (CCRD).

In order to clarify the influence of variables and the significance of mathematical models, an analysis of variance (ANOVA p<0.1) was performed, using software R Project for Statistical Computing version R-2.15.2 (R Development Core Team 2011).

Reducing sugar (RS) production was selected as the dependent variable (Y), whereas pH and temperature were selected as the independent variables. The mathematical model to predict

the response variable was fitted as a quadratic polynomial equation, as shown in equation (1).

$$Y = \zeta_0 + \sum_{i=1}^n \zeta_i X_i + \sum_{i=1}^n \zeta_{ii} X_i^2 + \sum_{i,j=1}^n \zeta_{ij} X_i X_j \quad (1)$$

The terms X<sub>i</sub> and X<sub>j</sub> represent the independent variables; whereas, ζ<sub>0</sub>, ζ<sub>i</sub>, ζ<sub>ii</sub> and ζ<sub>ij</sub> correspond to the regression coefficients for the central, linear, quadratic, and interaction terms of the model, respectively.

### Analytical Methods

The total reducing sugar was measured by the 3,5-dinitrosalicylic acid (DNS) method as described by Miller (1959). For this, sweet potato flour (1% (w/v)) was dispersed in sodium citrate buffer (50 mM, pH 5.0), which was employed as a starch solution. To 500 μL of crude enzyme extract, 500 μL of the starch solution was added, and the reaction mixture was incubated for 30 minutes at 50°C. Afterwards, 2 mL of DNS reagent was transferred to the test tubes, and the mixture was boiled for 5 minutes. Then, the reaction was stopped, by immersion of tubes in ice, and the resulting mixture was diluted by adding 10 mL of distilled water.

The reducing sugar concentration in the supernatant was spectrophotometrically determined by BioSpectro spectrophotometer at 540 nm.

Cell viability was employed to investigate the number of healthy cells in a sample, i.e., it determines the number of living or dead cells,

based on a total cell sample. Thus, aliquots of the reaction solution were taken at 0 and 96 h to determine the cell viability (%) using a Neubauer chamber and methylene blue 0.1% (w/v) reagent.

## RESULTS AND DISCUSSION

Table II displays the effects pH and temperature, at each factor level, on the enzymatic hydrolysis of sweet potato flour.

For S-47 strain, the ANOVA ( $p < 0.1$ ) results showed that pH and temperature have no influence over the dependent variable at the first 24 hours (Table III). It means that both parameters in study have the same effect on enzyme activity and production. At this condition, the reducing sugar production values ranged from 69.6 mg.L<sup>-1</sup> (run number 4) to 592.8 mg.L<sup>-1</sup> (run number 1). After 48 hours of incubation, pH was the only significant parameter, while at 96 hours, the statistical analysis showed that linear and quadratic terms of temperature, quadratic term of pH and interaction of both parameters can significantly influence ( $p < 0.01$ ) the hydrolysis process.

S-81 strain demonstrated similar performance as S-47 at 24 and 48 hours (Table IV). The data (Table IV) also indicated that at 96 hours the hydrolysis by S-81 strain was positively affected by temperature.

Indeed, experiments conducted at 47°C led to the highest quantification of RS; 379.1 mg.L<sup>-1</sup> and 541.3 mg.L<sup>-1</sup> for assays number 3 and 4, respectively (Table I). However, at 50 °C (Table I) the reducing sugar production decreased (227.5 mg.L<sup>-1</sup>), which can be due to an unsuitable environment for enzyme production and/or activity.

The effects and p-value of reaction parameters are summarized in Table IV.

The coefficients of regression were calculated and the following equation could be established considering the statistically significant terms, pH ( $X_1$ ) and temperature ( $X_2$ ).

(S-47) 48 hours:

$$Y = 154.277 - 89.110 X_1^2 \quad (2)$$

(S-47) 96 hours:

$$Y = 141.498 + 29.405 X_2 - 68.058 X_1^2 + 27.924 X_2^2 + 101.219 X_1 X_2 \quad (3)$$

(S-81) 96 hours:

**Table II. Production of reducing sugars (mg.L<sup>-1</sup>) by *Candida homilentoma* strains using Central Composite Rotatable Design (CCRD).**

Runs	$X_1$	$X_2$	S-47			S-81		
			24h	48h	96h	24h	48h	96h
1	4.8	33	592.8	118	180.8	0.089	0.02	239.95
2	6.2	33	514.6	20.3	ND	ND	ND	21.18
3	4.8	47	ND	ND	ND	0.037	ND	379.1
4	6.2	47	69.6	58.1	224	ND	ND	541.3
5	4.5	40	77.6	32.6	8.8	ND	ND	297.1
6	6.5	40	114.8	ND	ND	ND	ND	286.8
7	5.5	30	137	230	130	49.4	67.06	152.6
8	5.5	50	194.5	266.8	265.9	610.05	574.7	227.5
9	5.5	40	200.6	206.8	160.9	220.6	202.9	262.05
10	5.5	40	118.6	170.6	140.6	135.8	113.8	185.3
11	5.5	40	93	86.8	123	114.7	118.2	165.8

ND: not detectable.

$$Y = 204.71 + 95.31 * X_2 \quad (4)$$

The signs of the coefficient terms of models define the correlation between the related parameter (pH and/or temperature) and its response. The positive sign indicates that, as the value of one effect changes, the RS production changes in the same direction; whereas, for the negative sign, the response variable presents opposite behavior (Soleymani et al. 2015, Boudechiche et al. 2017).

The ANOVA was employed in order to evaluate the significance of regression models represented in equations 2, 3 and 4. The data summarized in Table V indicate that all regression models could be considered significant ( $Pr(>F) < 0.05$ ) to represent the correlation between RS production and dependent variables. Furthermore, based in the values of  $Pr(>F)$ , it can be inferred that a slightly discrepancies obtained in the response can be attributed to noise (Soleymani et al. 2015).

It could be seen that there is no lack of fit for models ( $Pr(>F) > 0.05$ ), which is an indication that experimental data obtained are in good agreement with regression models. Besides, according to Severo Júnior et al. (2007), the regression is considered statistically significant and predictive when F-value ( $F_{\text{tab}} / F_{\text{calc}}$ ) is greater than 4.

The three-dimensional (3D) response surface (Figure 1) was plotted using Protimiza

Experimental Design Software, for analysis of the S-47 strain data at 96 h, aiming to obtain optimal condition (s) resulted from the interaction of response (Y RS production) with experimental levels for each variable, pH ( $X_1$ ) and temperature ( $X_2$ ).

Therefore, considering the results of the statistical analysis, the optimal pH condition – codified data pH (-0.0389 at 48 h) and (-0.1547 at 96 h) – for the S-47 strain was observed around center level (pH = 5.4). Indeed, as observed in Table I, the maximum RS production 266.8 mg.L<sup>-1</sup> (run number 8- 48h) and 265.9 mg.L<sup>-1</sup> (run number 8- 96h) was obtained at pH 5.5. Galdino et al. (2011) reported that the alpha-amylase produced from yeasts usually present optimal activity in a pH range of 4.0 and 6.0. Besides, the authors described similar results (optimum pH at 5.5) for Amy1 enzyme - an alpha-amylase produced from *Cryptococcus flavus* and expressed in *Saccharomyces cerevisiae*. Therefore, our findings (optimal amylases activity at pH 5.0) corroborate with data present in the literature.

Amid et al. (2014) claimed that the results obtained for pH parameter can be related with isoelectric point (IP) of amylases, which in general is around 4.7. The enzyme produced from *C. homilentoma* S-47 showed better hydrolysis efficiency at pH 5.4. This fact can be justified based on the reduction of electrostatic repulsion forces, which can lead to maximum

**Table III. Main effect (t-value) and p-value of pH and temperature for S-47 *Candida homilentoma*, using CCRD.**

Coefficients variables	t value	Pr(> t )	t value	Pr(> t )	t value	Pr(> t )
	S-47 (24 hours)		S-47 (48 hours)		S-47 (96 hours)	
Intercept	1.148	0.303	3.873	0.0117 <sup>a</sup>	8.816	0.000312 <sup>a</sup>
pH (L)	0.075	0.943	-0.439	0.6787	0.390	0.712850
T (L)	-1.617	0.167	-0.143	0.8920	2.998	0.030172 <sup>a</sup>
pH (Q)	0.225	0.831	-3.086	0.0273 <sup>a</sup>	-5.850	0.002067 <sup>a</sup>
T (Q)	0.563	0.563	0.900	0.4094	2.400	0.061602 <sup>a</sup>
pH*T	0.738	0.738	1.129	0.3102	7.282	0.000764 <sup>a</sup>

<sup>a</sup>Statistically significant variables (p < 0.1); L-linear term; Q-quadratic term.

**Table IV. Main effect (t-value) and p-value of pH and temperature for S-81 *Candida homilentoma*, using CCRD.**

Coefficients variables	t value	Pr(> t )	t value	Pr(> t )	t value	Pr(> t )
	S-81 (24 hours)		S-81 (48 hours)		S-81 (96 hours)	
Intercept	1.621	0.166	1.604	0.170	3.549	0.0164 <sup>a</sup>
pH (L)	0.00	1.00	0.00	1.00	-0.251	0.8116
T (L)	1.683	0.153	1.635	0.163	2.704	0.0426 <sup>a</sup>
pH (Q)	-1.694	0.151	0.736	0.495	1.359	0.2321
T (Q)	0.648	0.546	-1.708	0.148	0.151	0.8862
pH*T	0.00	1.00	0.00	1.00	1.907	0.1149

<sup>a</sup>Statistically significant variables ( $p < 0.1$ ); L-linear term; Q-quadratic term.

interaction of enzymes with the surrounding buffer, thereby increasing their activity.

Meanwhile, for the strain S-81, the pH did not show any significant effect, proving the stability of enzyme at pH ranges proposed in the present study.

Xu et al. (2016) point out that in the starch ethanol industry it is necessary to use microorganisms and enzymes that maintains the high performance even at acid pH, because this specific condition is maintained during the fermentation process. In this context, Ullah et al. (2012) gave a brief explanation about unfavorable conditions caused by weak organic acids on the microbial growth. In this regard, the authors observed that acid dissociation inside *S. cerevisiae*, specifically in the cytosol, probably cause a stress condition that results in oxidative damage and perturbation of the plasma membrane. Beyond that, the growth inhibition of *S. cerevisiae* observed when yeasts are exposed to extreme acidity conditions is connected with ATP (adenosine triphosphate) depletion, because phosphofructokinase - a key enzyme of glycolysis - is sensitive to low pH.

In contrast, the statistical analysis revealed that, at 96 hours, the production of RS by S-47 strain was dependent of the temperature and interactive factor of both dependent variables (t-value 7.282). Regarding S-81 *C. homilentoma*, the results showed a positive effect promoted

by temperature on the RS production. Therefore, it can be assumed that the use of higher temperatures tends to favors the reducing sugar production.

For both cases, the best condition for temperature was estimated around 50 °C. In fact, according to literature data (Vicktor et al. 2013) the optimal temperature for enzymatic degradation of starch materials, using alpha-amylases and glucoamylases, range from 40 to 60°C.

Betiku et al. (2013) evaluated the optimal conditions for enzymatic hydrolysis of sweet potato peels. The authors proposed a predictive model from which it was possible to determine the conditions for maximum conversion of the substrate into glucose, these being, 56.4 °C,  $\alpha$ -amylase dose of 1% (v/v) and hydrolysis period of 60 minutes. Oliveira et al. (2015) demonstrated that amylases produced by *Candida parapsilosis* and *Candida glabrata* maintaining their structure and catalytic activity under experimental conditions of 50 °C and 1 hour, although maximum activity was recorded at 60 °C.

Temperature is a physical parameter that plays an important role on the growth of microorganisms and production of metabolites by them (Deb et al. 2013). Previous studies (Amid et al. 2014, Homaei et al. 2016) reported that thermostable enzymes have some advantages

**Table V. ANOVA for regression models for pH and temperature.**

<b>S-47 (48 hours)</b>					
<b>Source</b>	<b>DF</b>	<b>Sum Square</b>	<b>Mean Square</b>	<b>F-value</b>	<b>Pr (&gt;F)</b>
Model	2	62636	31317.9	6.5779	0.0398
Residuals	5	23805	4761.1		
Lack of fit	3	16228	5409.3	1.4277	0.4372
Pure error	2	7578	3788.8		
R <sup>2</sup>	0.7455				
<b>S-47 (96 hours)</b>					
<b>Source</b>	<b>DF</b>	<b>Sum Square</b>	<b>Mean Square</b>	<b>F-value</b>	<b>Pr (&gt;F)</b>
Model	2	41007	20504	26.5284	0.0021767
Residuals	5	3864	773		
Lack of fit	3	3145	1048	2.9144	0.2658145
Pure error	2	719	360		
R <sup>2</sup>	0.9584				
<b>S-81 (96 hours)</b>					
<b>Source</b>	<b>DF</b>	<b>Sum Square</b>	<b>Mean Square</b>	<b>F-value</b>	<b>Pr (&gt;F)</b>
Model	2	73605	36803	3.6862	0.01038
Residuals	5	49919	9984		
Lack of fit	3	44741	14914	5.76	0.1515
Pure error	2	5178	2589		
R <sup>2</sup>	0.7211				

**DF: degree freedom.**

in industrial process, since the use of high temperatures facilitates the production process of biofuels, because it improves the solubility of substrate, reduce the viscosity of reaction mixture, contamination and external cooling costs, beyond favor kinetic reaction.

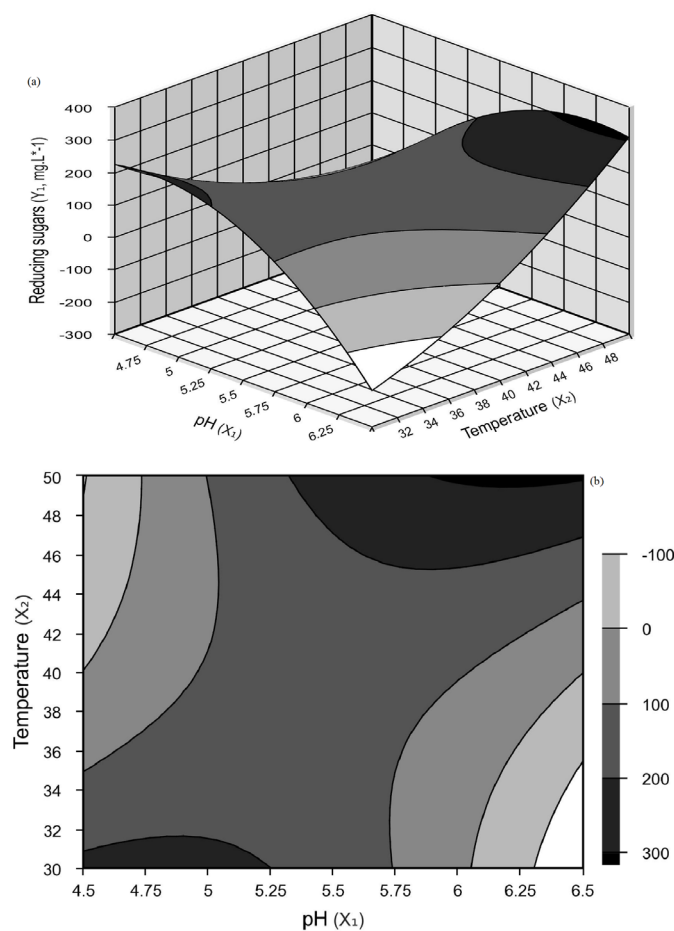
In this case, Sahnoun et al. (2015) highlight some relevant parameters for control/monitoring of temperature during hydrolysis process, namely: water content, heat conductivity, nature of the substrate used and solid layer particles.

The significance of pH\*T effect (Table III) imply that the increase of temperature and pH levels, simultaneously, increase the reducing sugar production. This result can be justified based on the fact that the cytoplasmic pH of *Candida* spp. depend not only of external pH,

but is also related to temperature, yeast specie, and supplementation/availability of nutrients in the medium (Stewart et al. 1989).

Similarly, Nawaz et al. (2017) claimed that the production of enzymes is related to the kind of yeast, and is regulated by physiological, biochemical, and nutritional requirements of these microbial isolates. Thus, metabolism-mediated enzyme production is mostly influenced by temperature, pH, incubation time, carbon and nitrogen sources, inoculum size, etc. Besides, growth conditions differ due to genetic diversity of microbial strains and need to be optimized in order to achieve high yield in the enzyme production (Nawaz et al. 2017).

Abdel-Rahman et al. (2016) investigated the influence temperature and pH on the growth and



**Figure 1.** Effect of temperature and pH on the enzymatic hydrolysis of sweet potato flour by *C. homilentoma* (S-47) strain under submerged culture: (a) response surface and (b) contour plots.

biological features of *Candida membranifaciens* HA19, *Candida tropicalis* HA147 and *Candida sake* HA124. The results demonstrated that amylases production and activity fluctuates due to variations in culture parameters.

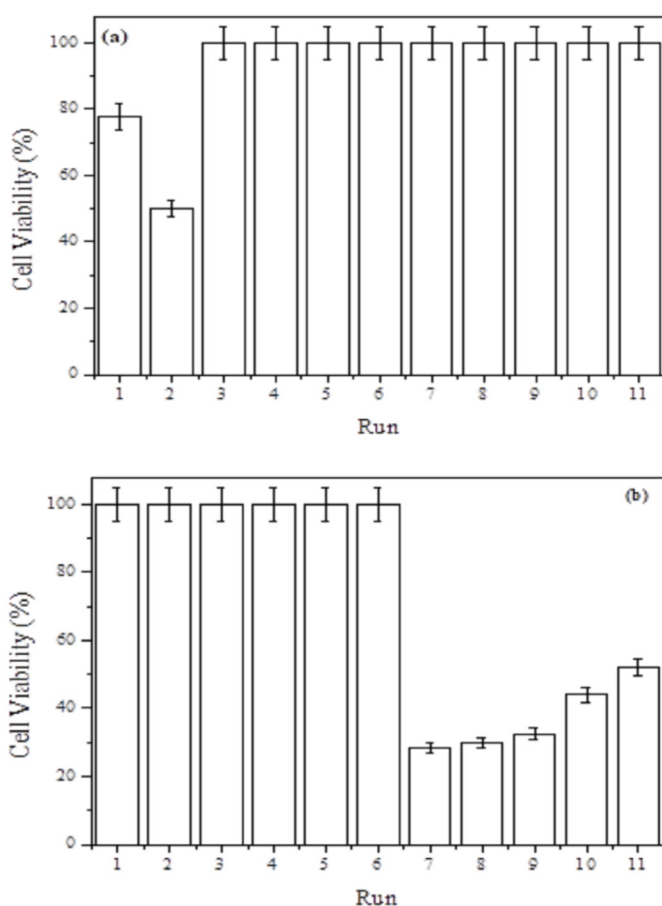
Cell viability is a technique used to investigate the number of healthy cells in a sample, that is, it determines the number of living or dead cells, based on a total cell sample. Thus, aliquots of the reaction solution were taken at 0 and 96 h to determine the cell viability (%), using a Neubauer chamber and methylene blue 0.1% (w/v) reagent.

The percentage of healthy cells was determined by viable cell count analysis at 96 hours (Figure 2). The viability of S-47 *C. homilentoma* cells (Figure 2a), decreased by

about 77.6% and 50% for assays number 1 and 2, respectively, while for the other conditions, it remained approximately at 100%, compared to the control condition (0 hour).

The decrease of cell viability (Figure 2) can be due to inhibition by hydrolysis products and/or depletion/availability of substrate. Indeed, Yun et al. (2015) reported that *Candida homilentoma* did not assimilate some carbon sources, such as maltose. Moreover, reducing sugar are not the only product released by  $\alpha$ -amylase in the fermentation broth, oligosaccharides can also be formed, acting in this case as an inhibitor of the strains (Paludo et al. 2018). Furthermore, enzymes and yeasts could act under different optimal conditions (Masiero et al. 2014).





**Figure 2. Viability of *C. homilentoma* strains (a) S-47 and (b) S-81, determined by methylene blue staining and Neubauer chamber count.**

Our results suggest that pH levels for assays number 1 (4.8) and 2 (6.2) (Table I) and the temperature of 33°C created an adverse condition for yeasts strains. According to data available in the literature, the oscillations of pH can affect the performance of plasma membrane ATPase, responsible for the transmembrane H<sup>+</sup> gradient, which are considered a driving force for nutrient transportation (Imai & Ohno 1995). Besides, intracellular pH triggers cell responses, such as induction of heat shock proteins; and also regulates the key enzymes involved in glycolysis and gluconeogenesis cycles. Therefore, the intracellular pH can be considered one of the most important factors that impact yeast physiology (Imai & Ohno 1995).

According to Sandri et al. (2015), the decrease of pH in the culture environment can induce catabolic repression and growth inhibition.

Undoubtedly, yeast viability is considered an important aspect to be controlled during alcoholic fermentation. In this regard, the high cell viability is desirable; as well as the use of microorganism in recycling mode. This last condition is unavailable when it comes to the production of ethanol from sweet potato, because fibrous residue present in the vinasse confine the yeast cells. In this case, fresh inoculum should be prepared at each new cycle of fermentation, keeping the viability high (Pavlak et al. 2011).

In this work, we evaluated the influence of pH and temperature on the hydrolysis of

sweet potato flour by *Candida homilientoma* strains. Thus, for future studies, we propose the analysis of other parameters, such as nitrogen source, inorganic salt ions and cell/enzyme concentrations, beyond the application of immobilized catalytic system, that could improve the performance of hydrolysis process using sweet potato flour as a substrate.

## CONCLUSIONS

The results obtained in this work showed that both *C. homilientoma* strains demonstrated a promising performance on the hydrolysis of sweet potato flour. The production of reducing sugars by S-47 strain was statistically affected by pH and temperature. Regarding S-81 *C. homilientoma*, the data show that the temperature was considered a significant parameter for the hydrolysis of substrate, and the optimal pH values could be eventually establish in a new range of study. The cell viability at the end of the hydrolysis process was kept in 100% for most of the assays. Although some experiments indicate the reduction of viability, the enzymatic process was kept satisfactory.

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#### ANA LETÍCIA S. COELHO<sup>1</sup>

<https://orcid.org/0000-0001-6370-0782>

#### AGELLES A. ARRAES<sup>2</sup>

<https://orcid.org/0000-0001-6031-1028>

#### THIAGO LUCAS DE ABREU-LIMA<sup>2</sup>

<https://orcid.org/0000-0003-4222-4128>

#### SOLANGE CRISTINA CARREIRO<sup>2</sup>

<https://orcid.org/0000-0003-4651-072X>

<sup>1</sup>Universidade Federal de Santa Catarina/UFSC, Departamento de Engenharia Química e Engenharia de Alimentos, Trindade, 88040-900 Florianópolis, SC, Brazil

<sup>2</sup>Universidade Federal do Tocantins/UFT, Programa de Graduação em Engenharia de Alimentos, Avenida NS 15, ALCNO 14, 77001-090 Palmas, TO, Brazil

Correspondence to: **Ana Letícia Silva Coelho**  
E-mail: [alscoelho@uft.edu.br](mailto:alscoelho@uft.edu.br)

#### Author contributions

ALS Coelho performed data curation and wrote the manuscript draft; AA Arraes performed data curation and formal analysis; TL Abreu-Lima, and SC Carreiro performed manuscript editing and revised the language.

