

## Evaluation of a new yeast from Brazilian biodiversity, *Scheffersomyces shehatae* UMFG-HM 52.2, for pentose sugars conversion into bioethanol

### Avaliação de um nova levedura da biodiversidade brasileira, *Scheffersomyces shehatae* UMFG-HM 52.2, para a conversão de pentoses em bioetanol

Felipe Antonio Fernandes Antunes<sup>1</sup>; Thais Suzane dos Santos Milessi<sup>1</sup>; Anuj Kumar Chandel<sup>1</sup>; Vinicius P. Moraes<sup>1</sup>; Carlos Augusto Rosa<sup>2</sup>; Silvio Silvério da Silva<sup>1\*</sup>

#### ABSTRACT

Hemicellulose, a heterogeneous polymer of various sugars linked with xylose backbone, is the second most available carbohydrate polymer on Earth, after cellulose. Hemicellulose can be hydrolysed by thermochemical or enzyme mediated action into a mixed sugar solution primarily composed by xylose. Bioconversion of hemicellulose derived sugars into ethanol is an important criterion to get the satisfactory overall bioethanol yield from lignocellulosic materials. However, the development of hemicellulose conversion into ethanol requires additional challenges. Only few microorganisms are able to ferment pentose sugars into ethanol. Xylose-utilizing microorganisms have shown low rates of sugar assimilation, conversion and poor tolerance to ethanol. These factors often prevent their use in technological processes at large scale. In this line, the search for new microorganisms that efficiently assimilate pentoses, particularly xylose, is a priority within the context of the integral use of lignocellulosic biomass into ethanol. This study showed the improved fermentation of synthetic xylose into ethanol under batch cultivations by a new strain of *Scheffersomyces shehatae* (UMFG-HM 52.2), isolated from Brazilian Atlantic rain forest ecosystem. Ethanol production performance of this yeast was evaluated in synthetic medium (using supplemented synthetic xylose as carbon source) in 125mL Erlenmeyer flasks at 200 rpm contained 50 mL of medium incubated for 72 h at 30°C. After 18 h of incubation, ethanol yield ( $Y_{P/S}$ ) of 0.39 g/g and productivity (Qp) of 0.79 g/L was obtained. This study showed the potential of *S. shehatae* UMFG-HM 52.2 as a promising candidate in process to produce ethanol from pentose sugars.

<sup>1</sup> University of São Paulo – School of Engineering of Lorena - EEL-USP

<sup>2</sup> Federal University of Minas Gerais

\* Corresponding author [felipeantunes22@gmail.com](mailto:felipeantunes22@gmail.com); [silviosilverio@gmail.com](mailto:silviosilverio@gmail.com)

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#### RESUMO

A hemicelulose, polímero heterogêneo composto por diferentes monossacarídeos ligados em uma estrutura principal de xilose, depois da celulose, é o segundo carboidrato polimérico mais abundante na terra. A hemicelulose pode ser hidrolisada por meio termoquímico ou enzimático, em uma solução mista de açúcares, com a predominância de xilose. A bioconversão de açúcares derivados de hemicelulose em etanol é um critério importante para obter-se rendimentos satisfatórios na produção de bioetanol a partir de materiais lignocelulósicos. Entretanto, o desenvolvimento da conversão da hemicelulose em etanol requer desafios adicionais. Os microrganismos para a conversão de pentoses em etanol são poucos. Microrganismos fermentadores de xilose têm demonstrado baixos índices de assimilação e conversão de açúcares e baixa tolerância ao etanol. Esses fatores são importantes pois muitas vezes impedem a sua utilização em processos tecnológicos em larga escala. Neste contexto, a busca de novos microrganismos que assimilam eficientemente pentoses, particularmente a xilose, é uma prioridade dentro do contexto da utilização integral da biomassa lignocelulósica em etanol. Este estudo apresentou a fermentação de xilose em etanol sob cultivo em batelada por uma nova linhagem de levedura, *Scheffersomyces shehatae* (UMFG-HM 52.2), isolada da Mata Atlântica. A produção de etanol e o desempenho desta levedura foi avaliada em meio sintético (utilizando xilose sintética suplementada como fonte de carbono) em frascos Erlenmeyer de 125 mL a 200 rpm contendo 50 mL de meio incubado durante 72 h a 30 °C. Após 18 h de incubação, obteve-se rendimento de etanol ( $Y_{P/S}$ ) de 0,39 g/g e produtividade (Qp) de 0,79 g/L. Este estudo mostrou o potencial da levedura *S. shehatae* UMFG-HM 52.2 como um microrganismo promissor para o processo de produção de etanol a partir de pentoses.

**Palavras-chave:** *Scheffersomyces shehatae* UMFG-HM 52.2, Xilose, Etanol, Fermentação

## INTRODUCTION

Currently, petroleum fuels represent 80% of the worldwide energy consumed where 58% is allocated only to the transport sector (NIGAM; SINGH., 2011). There are several disadvantages linked to the use of fossil fuels such as the heavy dependency on gasoline, increased depletion of oil reserves and the emission of greenhouse gases into environment (MUSSATTO *et al.*, 2010; TSIGIE *et al.*, 2011). Use of ethanol as transportation fuel can greatly reduce the increased dependency on gasoline, once this production is environmental friendly. (HAMELINCK *et al.*, 2005). Among biofuels, bioethanol has gained prominence because it has a high octane rating, allowing wider limits of flammability and higher heat of vaporization, providing a higher compression ratio and shorter combustion than gasoline (BALAT; BALAT, 2009). It also contains high oxygen content (35% m/m), enabling cleaner combustion and contributing to the reduction of hazardous gases emissions in environment (GIRIO *et al.*, 2010).

Bioethanol produced from sugarcane juice or corn grains, so called “first generation production” is a primary renewable energy source in transportation sector in USA and Brazil (CARDONA, QUINTERO and PAZ, 2010.) However, since the raw material corresponds to 40% of the ethanol final price and because of the competence with food security concerns, the lignocellulosic biomass (such as sugarcane bagasse, corn stover, rice straw and wheat straw) has shown an inexpensive alternative of first generation ethanol as “second generation” ethanol production (ZALDIVAR; NIELSEN; OLSSON, 2001).

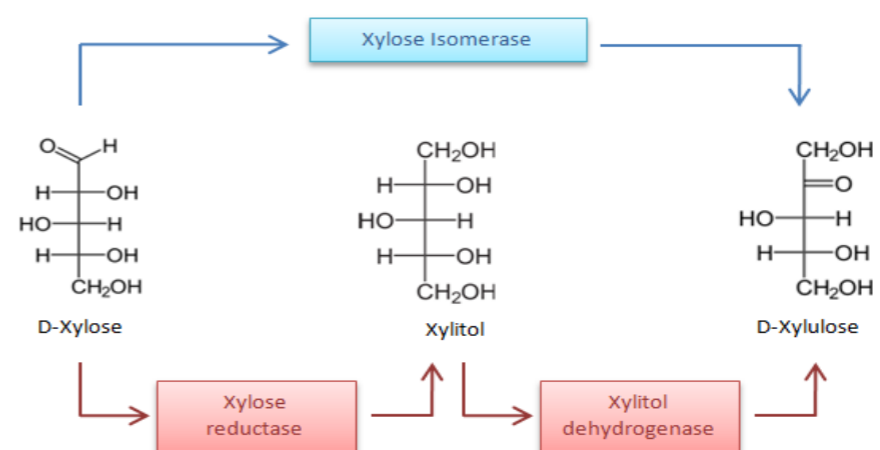
Despite the significant advantages of second generation ethanol, the process still required to be economically competitive with gasoline and first generation ethanol (STEPHEN; MABEE; SADDLER, 2012). In this context, it is relevant the study of the assimilation of hemicellulosic derived sugars into

ethanol, as hemicellulose is most promising carbon source on Earth after cellulose (HAN *et al.*, 2004).

Hemicellulose fraction can be solubilized into a mixed sugar stream by a pretreatment step. (CHANDEL *et al.*, 2013a). The hemicellulosic sugars solution is comprised of monomeric sugars such as xylose, glucose and arabinose in addition to some other compounds such as furans and phenolics which are usually toxic which need to be eliminated prior to use of sugar solution for ethanol production (CHANDEL *et al.*, 2013b; CARDONA; SANCHEZ; GUTIERREZ, 2009).

The xylose assimilation in microorganisms occurs by the pathways shown in Figure 1. After the entry of xylose in cell, the metabolism of D-xylose is initiated by a conversion first into D-xylulose catalyzed by the enzymes xylose reductase (XR) and xylitol dehydrogenase (XD) or by the action of the enzyme xylose isomerase (XI), depending on the microorganism. For example, yeasts like *Candida guilliermondii* and *Scheffersomyces stipitis* have XR and XD, while the fungus *Piromyces sp* has XI to assimilate xylose (KARHUMAA *et al.*, 2005).

**Figure 1** Mechanistic pathway showing the xylose assimilation by microorganisms possessing xylose isomerase and xylose reductase plus xylitol dehydrogenase.



After conversion to D-xylulose, for ethanol excretion, metabolism continues with the phosphorylation reaction catalyzed by the enzyme xylulokinase that converts that compound to xylulose 5-P. Further, xylulose 5-P is metabolized by glycolytic pathway intermediates such as glyceraldehyde 3-P and fructose 6-P by phosphopentose pathway. Both compounds are converted into pyruvate by the glycolytic pathway, which leads to ethanol by two sequential reactions (by decarboxylation of pyruvate decarboxylase enzyme, which converts pyruvate to acetaldehyde and ethanol by reduction to alcohol dehydrogenase) (TOIVARI *et al.*, 2001; JEFFRIES; JIN, 2004; JEFFRIES, 2006).

Thus, an important factor for the microbial production of ethanol from xylose is the kind of microorganism and their levels of enzymatic activity responsible for the efficient assimilation and metabolism of xylose. The yeast *Saccharomyces cerevisiae* is the most commonly microorganism used in industrial ethanol production, however, this yeast is not able to assimilate pentoses (JEFFRIES, 2006; CANILHA *et al.*, 2012). The search for new microorganisms that naturally has this feature is still a challenge to the viability of bioethanol production, since among the known microorganisms that naturally fermenting pentoses only 1% of them are capable of converting xylose to ethanol (HAHN-HAGERDAL *et al.*, 2007). Among the yeasts with this feature, *Scheffersomyces stipitis* (LI *et al.*, 2012) has shown promising ethanol yields. Further studies should be conducted for the mass bio-prospecting of new xylose sugar assimilating microorganisms with the obvious potential of xylose conversion into ethanol.

In this context, the present work aims to evaluate the potential of new strain of *S. shehatae*, UMFG-HM 52.2, isolated from Brazilian biodiversity for the ethanol production from xylose sugar.

## MATERIALS AND METHODS

### Microorganism and inoculum preparation

Cells of *Scheffersomyces shehatae* UMFG-HM 52.2, kindly provided from the culture collection of Federal University of Minas Gerais (UFMG,) were used for the fermentation of synthetic xylose solution. Strains were maintained on YPD (yeast extract, peptone, dextrose and agar) plates and stored at 4 °C. The inoculum was prepared by transferring a loopful of strains from the slant into 125 ml Erlenmeyer flasks containing 50 ml of synthetic medium composed by 30.0 g/l of xylose, 10.0 g/l of yeast extract and 20.0 g/l of peptone. The flasks were incubated in a rotatory shaker (Innova 4000 Incubator Shaker, New Brunswick Scientific, Enfield, CT, USA) at 200 rpm and 30 °C for 24 h. Following 24 h growth, broth was centrifuged for 10 min at 3000 rpm and inoculum solution was prepared corresponding to 0.5 g/l cells (d. wt).

### Fermentation

The fermentation assays were carried out in 125 ml Erlenmeyer flask containing 50 ml of synthetic xylose solution (37 g/L) (supplemented with Media defined by Parekh *et al.*, (1986) (5g/L of ammonium sulfate, 3g/L of yeast extract and 3g/L of malt extract) and initial cell concentration (0.5 g/l) of *S. shehatae* UFMG 52.2. Flasks were incubated in a rotator shaker (Innova 4000 Incubator Shaker, New Brunswick Scientific, Enfield, CT, USA) at 200 rpm and 30 °C for 72 h.

### Analytical methods

Xylose and ethanol concentrations were analyzed by HPLC (Schimadzu LC-10 AD (Kyoto, Japan) with column equipped with BIO-RAD Aminex HPX-87H (300 9 7.8 mm) coupled to a detector of refractive index RID-6A, with eluent 0.01 N sulfuric acid at a flow rate of 0.6 ml min<sup>-1</sup> and column

temperature of 45 °C. The samples were filtered through Sep Pak C18 filter before passing at HPLC. The standard chemicals were purchased from Sigma Aldrich (St. Louis, MO).

The concentration of free cells was determined by turbidimetry using spectrophotometer (Beckman DU 640 B Fullerton, CA) at wavelength of 600 nm and correlated with the dry weight of cells (g/l) through a calibration curve. The measurements were made on diluted cell suspensions, after centrifugation, washing and re-suspension of cells in distilled water.

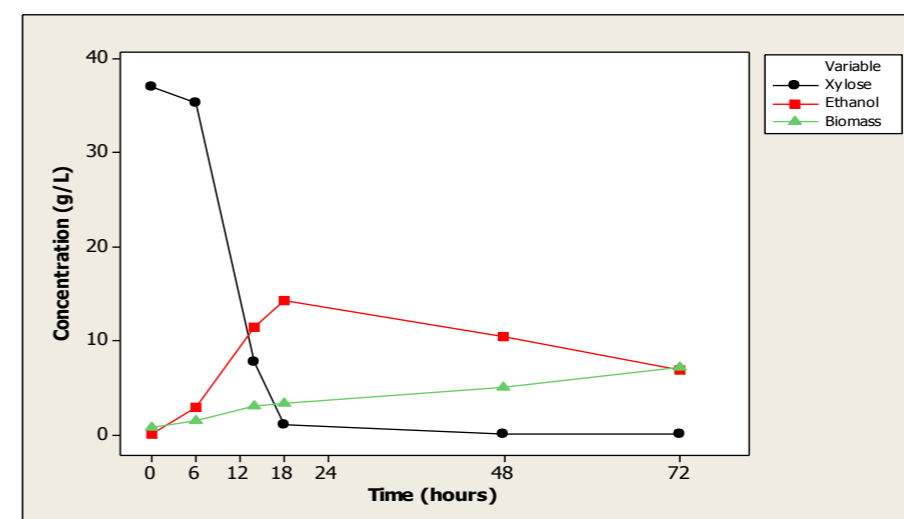
## RESULTS AND DISCUSSION

For second generation ethanol production from lignocellulosic materials, the search for promising microorganisms capable of fermenting pentose sugars is a key factor for the economic ethanol production. Xylose assimilating yeasts has shown better ethanol productivity and yield than other microorganisms such as bacterias (OLSSON; HAHN-HÄGERDAL, 1996). This characteristic is due the large size of yeast cells, ability to grow easily in different pH, low nutritional requirements and greater resistance to contamination. The fermentative profile of the xylose conversion into ethanol by this fermenting pentose yeast *S. shehatae* UMFG-HM 52.2 was evaluated. The results are presented in Figure 2.

It was verified that ethanol and biomass associate concentration increased in first hours of fermentation. After 18 hours of fermentation reaction, maximum ethanol production (14.2 g/L) was achieved with almost 100% of xylose consumption. This behavior showed the high feasibility of this yeast for xylose conversion into ethanol. According to Joshi et al. (2011), the strain *S. shehatae* is able to convert pentoses as hexoses into ethanol and other value-added products with high yields. In preliminary studies with this yeast in the 1980s, *S. shehatae* showed higher ethanol

yield than other pentose-fermenting yeasts studied (DU PREEZ; VAN DER WALT, 1983; DU PREEZ *et al.*, 1986). After 18 h of incubation, ethanol concentration was found to be decreased. This behavior can be attributed to the exhaustion of xylose in the media and the utilization of the ethanol by cells as a carbon source for cell maintenance. In this work, we highlight the feasibility of fast sugars consumption of this yeast. It is evident that the biomass growth also became slow after this time in comparison with the growth of cells using xylose as carbon source.

**Figure 2** Fermentative profile of the synthetic xylose supplemented (5g/L of ammonium sulfate, 3g/L of yeast extract and 3g/L of malt extract) conversion into ethanol by *Scheffersomyces shehatae* UMFG-HM 52.2 (200 rpm and 30 °C for 72h). Assays were performed in replicates. Standard deviation was within 5 %.



During fermentation of pentose sugars from synthetic mediums and lignocellulosic hydrolysates, the use of ethanol as a carbon source after sugar depletion is a common characteristic of *S. shehatae* (Chandel et al. 2007). Taking into consideration this characteristic of this microorganism, it is required to terminate the fermentation assay at this time after attaining



maximum ethanol levels. Table 1 shows the fermentation kinetic parameters for the ethanol production by *S. shehatae* UMFG-HM 52.2.

**Table 1** Fermentative parameters ( $Y_{P/S}$ ,  $Y_{X/S}$ ,  $Q_p$ ,  $Q_x$  and xylose consumption) of *S. shehatae* UMFG-HM 52.2 after 18 hours of synthetic xylose supplemented fermentation (5.0 g/L of ammonium sulfate, 3.0 g/L of yeast extract and 3.0 g/L of malt extract).

	Ethanol Yield $Y_{P/S}$ (g/g)	Biomass Yield $Y_{X/S}$ (g/g)	Ethanol Productivity $Q_p$ (g/L.h)	Biomass Productivity $Q_x$ (g/L.h)	Xylose Consumption (%)
Synthetic medium	0.39	0.07	0.79	0.18	97.34%

The fermentation of synthetic xylose supplemented media showed ethanol yield ( $Y_{P/S}$ ) and biomass yield ( $Y_{X/S}$ ) of 0.39 g/L and 0.07 g/L, respectively. According to Skoog and Hahn-Hägerdal (1990), the oxygen availability plays an important role for yeast metabolism, interfering directly on cell growth, redox balance and xylose transport. Silva, Mussato and Roberto (2010) studied ethanol production from rice straw hydrolysate by the yeast *Pichia stipitis* and observed a deviation of metabolism towards biomass production with the increase of aeration. According to Mussato and Roberto (2010), the carbon source consumption is divided by the yeast into biomass and ethanol production. This behavior of the yeast is strongly influenced by available oxygen. This metabolic behavior can be clearly seen with the comparison between  $Y_{P/S}$  and  $Y_{X/S}$  values. In the present work,  $Y_{P/S}$  was significantly higher than  $Y_{X/S}$ , which shows that the amount of distributed oxygen was satisfactory for the yeast metabolism and properly directed to ethanol synthesis and not for biomass growth. Besides the carbon source and aeration, yeast requires substances for the cellular synthesis and energy, such as nitrogen source. In the present work,

nitrogen source was added in the synthetic medium as proposed by PAREKH et al. (1986).

Bioconversion of pentose sugars and hemicellulosic hydrolysates into ethanol has been studied by several researchers under different fermentation conditions. Our results can be fairly compared with the existing results as shown in Table 2.

**Table 2** Comparison of the results from present work with some existing studies

Reference	Carbon Source	Microorganism	$Y_{P/S}$ (g/g)	$Q_p$ (g/L.h)
Present Work	Synthetic Medium	<i>S. shehatae</i> UMFG-HM 52.2	0.39	0.79
Chandel et al. (2007)	Sugarcane bagasse	<i>S. shehatae</i> NCIM 3501	0.30	0.21
Canilha et al. (2010)	Sugarcane bagasse	<i>S. stipitis</i> DSM 3651	0.30	0.13
Ferreira et al. (2011)	Sugarcane bagasse	<i>S. stipitis</i> UFMG-IMH 43.2	0.19	0.13
Sun and Tao (2010)	Rice straw	<i>S. shehatae</i> CICC 1766	0.31	0.17
Ge et al. (2011)	Synthetic Medium	<i>S. shehatae</i> HDYXHT-01	0.41	0.55
Scordia et al. (2012)	<i>Arundo donax</i> L.	<i>S. stipitis</i> CBS6054	0.33	0.17

This results of ethanol yield and productivity shows the potential of the new yeast- *S. shehatae* UFMG-HM 52.2 for ethanol production. For ethanol performance comparing among these microorganisms, it must be considered that the results were achieved in different cultivation conditions, methods and source of carbon profile. Moreover, we observed fast conversion of xylose into ethanol (0.79 g/L.h) by *S. shehatae* UFMG-HM 52.2, which is an interesting feature for large scale biorefinery operations.

## CONCLUSION

This study showed the potential of *S. shehatae* UFMG-HM 52.2 for the conversion of synthetic xylose supplemented into ethanol under batch fermentation conditions. Medium composition (5.0 g/L of ammonium sulfate, 3.0 g/L of yeast extract and 3.0 g/L of malt extract) and aeration conditions (200 rpm) in fermentation assays were adequate for the desired level of ethanol production. The results obtained in the current study are quite comparable with the earlier reports wherein the other strains of *S. shehatae* were employed. The interesting feature of *S. shehatae* UFMG-HM 52.2 is the fast conversion of xylose into ethanol with high productivities which could be useful for large scale bio-refinery operations.

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