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Study The Factors Effecting For Xylitol Production

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Abstract: Xylan papyrus was used as a base material for the bio-conversion of xylose into xylitol by the yeast Candida tropicalis, and xylitol was used by using the HPLC technique. xylitol was produced under different conditions and factors, the optimum temperature was 30 ° C, the best source of nitrogen was yeast extract, the best concentration of xylose was 60%, the optimum pH was 5.5 and the best ventilation speed was 150 rpm, so the highest concentration of xylitol was 50.8 mg / ml. using the above conditions.

Keyword: Xylitol, Submerged fermentation, Candida tropicalis.

INTRODUCTIO

MATERIAL AND METHODS

Microorganisim:
Candida tropicalis yeast was obtained from the Iranian Biological Resource Center (IBRC) and activated using a medium (YPG) consisting of yeast extract: 10 g/l, Peptone: 10 g/l, glucose: 70 g/l, and incubation at 28 ° C (Kurtzman and Fell, 1998). the pH was adjusted to 6 with a dilute solution of hydrochloric acid (HCl), and when using a solid medium, an agar was added at a ratio of 15 g/l.

Procedure method
The production medium was inoculated with 10 ml /100 ml of the medium from the *Candida tropicalis* yeast suspension prepared above, containing 10^6 cells / ml. The flasks were incubated in a vibrating incubator at 150 rpm at 30 ° C for 48 hours.

xylitol extraction
Xylitol was extracted by central centrifugation at 20374 xg for 30 minutes at 4 ° C, and the filtrate was used to detect xylitol (West, 2009).
Diagnosis of xylitol with High Performance liquid chromatography
HPLC technique was used in the diagnosis of xylitol produced by the yeast *Candida tropicalis*. This method was followed in the process of qualitative and quantitative determination of xylitol, as the following conditions were followed :
A highly performance liquid chromatography device belonging to the Chemistry Lab / Department of Food Sciences / College of Agriculture / Ferdowsi University was used to quantitatively and qualitatively estimate xylitol using the Eurokat H column with dimensions 8 x 300 mm and the Refractive Index (RI) for alcohol sugars, and the volume of the injected sample was 20 µl.

The test was performed using the mobile phase of 100% sulfuric acid aqueous solution (pH 2.0 ± 0.1). The separation was carried out at room temperature and with a flow velocity of 0.15 ml / min.

Factors effecting for xylitol production

Several conditions were studied to determine the optimum, including the optimum temperature, the optimal nitrogen source, the optimum xylose concentration, the optimum aeration speed, and the optimum pH.

Optimum temperature

The decanters containing 100 ml of the aforementioned prepared production medium were inoculated with 10 ml of suspensions of *Candida tropicalis* (1 ml contains 10^6 cells). The flasks were incubated in a vibrating incubator of 150 rpm at different temperatures that included (20, 25, 30, 35 and 40) C. For 48 hours.

The optimum source of nitrogen

The yeast extract was replaced in the production medium with several organic sources with the same concentration, including Peptone and Urea. The flasks were inoculated and incubated, taking into account the optimal conditions obtained in the previous experiment.

Optimum xylose concentration in the production medium

The base material (papyrus xylose) was added to the production medium at different concentrations (5, 10, 20, 30, 50, 60, 70 and 80%), and the production process was carried out in the same manner mentioned above, taking into account the optimal conditions obtained from previous experiments.

Optimum ventilation speed

The flasks were incubated with a vibrating incubator at different speeds that included (50, 100, 150, 200 and 250) revolutions / minute, taking into account the optimal conditions obtained in the previous experiments.

The optimum pH

The initial pH of the production medium was adjusted to (3.5, 4.5, 5.5, 6.5 and 7.5). The flasks were inoculated and incubated under the optimal conditions obtained in the previous experiments.

Xylitol was administered with a high-efficiency liquid technology at the end of each of the above trials.

RESULTS AND DISCUSSION

Optimum temperature

The rate of growth and the production of xylose depends on the temperature, because the cell's ability to isolate xylose from the medium or the environment, which is regulated by its attraction to the transporter proteins in the membrane, and the efficiency of the internal enzymes that regulate the stimulation of xylose depends on the temperature (Tamburini *et al*, 2015). the temperature of 30 ° C used in the past in the production of xylitol by the yeast *Candida tropicalis* from papyrus xylose, where it is noted that the amount of xylitol produced, amounting to 8.3 mg / ml, is observed with a decrease in the quantity produced with the rise and fall of temperatures below 30 ° C, while the amount of xylitol produced was 5.93 mg/ml at 25 C and 7.11 mg/ml at 35 C. Tamburini *et al*., (2015) emphasized that a decrease in temperature means that any substance obtained by effective transport (such as xylose) becomes increasingly less readily available in *Candida* yeasts. On the other hand, in

the event of a rise in temperature, the limits of The increase is imposed by thermal inhibition of the important cellular components, and the researcher indicated that there is a clear increase in xylitol productivity in the thermal range between 29 and 34, noting that temperatures higher than 35 ° C and less than 28 ° C have a decrease in productivity. Ghindea *et al*, (2010) found that the best temperature for the production of xylitol in yeast is around 30 ° C, with little significant differences between them, noting that despite the growth of yeasts at temperatures higher than 37 ° C, there is a clear decrease in the amount of xylitol. Albuquerque *et al*., (2014) indicated that the highest yield of xylitol obtained using .produced *K. marxianus* yeast was at a temperature of 40 ° C, where the amount of xylitol produced was 7 g / l, while it reached a temperature of 30 ° C 4.5 g / l. Barathikannan and Agastian, (2016) that the best temperature for xylitol production from *Candida tropicalis* is 30 ° C.

The optimal source of nitrogen

The results obtained in the highest amount of xylitol produced was when using the yeast extract, which represents the source of nitrogen in the original medium, and thus the amount of xylitol produced was approximately 8.4 mg / ml, while urea gave the amount of xylitol reached 7.2 mg/ml and peptone 6.12 mg/ml. Ghindea *et al*., (2010) confirmed that yeast extract and urea are among the most nitrogenous sources that had a positive effect in giving a good opportunity to produce xylitol from yeast, and several studies confirmed that these sources have catalytic effects on some strains of *C. boidinii*.

There are many sources that have studied the effect of urea as a source of nitrogen in the production of xylitol, as Rodrigues *et al*., (2011) studied the effect of using urea and ammonium sulfate as a source of nitrogen for the production of xylitol from *Pichia stipitis* YS-30, as it was added to the decomposed corn feed, and it was observed when The use of urea showed an increase in xylitol compared to ammonium sulfate, and Ko *et al*., (2008) found the advantage of replacing the yeast extract with urea when producing xylitol. Zhang *et al*., (2012) evaluated xylitol produced from *C. athensis* yeast using fortified vegetable residues. They noted that the yeast possessed a high ability to convert xylose into xylitol, and this study indicated that urea is one of the most supportive nitrogen sources in this field. When Hongzhi *et al*., (2011) found that ammonium sulfate and yeast extract gave a significant result when producing xylitol by using *Candida tropicalis*, compared to other sources, which included ammonium nitrate, peptone, and urea.

Optimum xylose concentration in the production medium

The highest xylitol productivity was obtained from papyrus xylose by using *C. tropicalis* yeast when using xylose at a concentration of 60% as the amount of xylitol produced 49.8 mg/ml, the concentration 50% was 44.3 mg/ml and concentration 70% was 47.8 mg/ml, compared to other concentrations that included (20,30,40,80)%, and Ghindea *et al*., (2010) indicated that the concentration of xylose is one of the factors affecting the growth of yeast and the fermentation process, as the initial concentration of xylose affects the production of xylitol. the microorganisms that can grow under high osmotic pressure by producing a large amount of xylitol in high concentrations of xylose. Studies on *C. tropicalis* strains have confirmed that high concentrations of xylose and the optimal ventilation rate give significant cellular growth at the beginning of the fermentation process, which leads to the development .of Xylitol production process

Meyrial *et al*, (1991) and Nolleau *et al*., (1991) found that a higher concentration of more than 200 g / liter stimulated xylitol production, while lower concentrations less than 50 g / l led to lower production, and higher productivity. It is obtained using medium levels of xylose (60-200) g/l, and this depends on the type of microorganism, and it has been emphasized that starting with high levels of carbon sources leads to gradually inhibition of fermentation, and a

large amount of xylose will be consumed to obtain sufficient energy for breathing. And ATP production, to counterbalance the high osmotic pressure produced by the high concentration of the substrate.
Tamburini *et al*., (2015) found that the highest xylose yield was obtained when the xylose concentration ranged between 60-80 g/l. Barathikannan and Agastian (2016) confirmed that the xylose concentration is one of the critical factors for the growth and fermentation process.

Optimum ventilation speed
The presence of oxygen is an important factor in the destruction of xylose by the yeast, and it has been proven that proper ventilation ensures a complete metabolism of xylose into xylose, and the level of oxygen required for xylose metabolism depends on the type of microorganism, El-Baz *et al*., (2011).
The best ventilation was obtained with a number of cycles of 150 rpm, which is similar to the number of cycles applied in the previous experiments, and thus there was no increase in the amount of xylitol produced than in the previous experiment. This is 49.8 mg / ml, while gave 100 rpm the amount of xylitol was 45 mg/ml and 200 rpm was 39.2 mg/ml.
Barathikannan and Agastian (2016) emphasized that ventilation is the key to the production of xylitol from yeast, and demonstrated the importance of balancing the carbon source between cell growth and xylitol. production
Albuquerque *et al*., (2014) indicated the importance of the redox imbalance in the production of xylitol, which causes the difference in the enzymes' preference for the enzymatic conjugates represented by NADPH in the case of Xylose reductase and NAD + in the case of Xylitol dehydrogenase.
Gimenes *et al*., (2002) indicated that the metabolism of xylose in yeasts and filamentous fungi takes two steps: the first step is to reduce xylose to xylitol by NADPH, depending on the enzyme Xylose reductase. Then, in the second step, xyloseol is converted to xylulose by the enzyme NAD + -Linked xylitolrogenase. The behavior of xylose fermentation within yeasts varies depending on the availability of oxygen. In bio-ventilation, the produced NADH is re-oxidized in the respiratory chain, where oxygen is the final receiver of the electrons, and when the oxygen concentration decreases, the electron transport system becomes unable to re-oxidize all produced NADH, which leads to its accumulation inside the cells, causing the balance of oxidation, which leads to an increase in the formation of xylitol and reduces its conversion to Xylulose, but in anaerobic conditions, no transformation occurs due to the limited enzymatic accompaniments inside the cell, which leads to the suspension of the reaction due to the lack of NAD +, in addition to the rate of ventilation and initial concentration. Both xylose affect the activity of the enzyme Xylose reductase, and in order for xyloseol to be produced optimally, it is necessary to ensure that the ideal ventilation rate is reached to restore NADH oxidation to the required levels (Corona *et al*, 2016).
Mussatta and Roberto, (2003) found that the best xylitol yield from *C. guilliermondii* FTI was 0.84 g when using an incubator with 300 rpm incubators using dissolved rice straw medium.

Optimum pH
The highest amount of xylitol produced was 50.8 mg /ml at pH 5.5, while the quantity produced decreased to 37.54 and 39.29 mg / ml at pH numbers 4.5 and 6.5, respectively. Barathikannan and Agastion, (2016) indicated that the best pH for xylitol production from *D. hansenii's* yeast was 5.5 while it was 4.5-5.6.7 for *C. parapsilosis*, *C. guilliemondii* and *C. boidinii*, respectively, Guaman Burneo *et al*, (2015) stated that a pH between 4.5-7.0 is the best range for xylitol production from Candida species, and that the decrease in pH leads to inhibition of yeast growth due to acetate formation and thus affects xylitol productivity.

Cheng *et al*., (2009) noted that when increasing the pH of the production medium from 4.5 to 6.0, it led to an increase in the amount of xylitol produced.
Tamburini *et al*., (2015) indicated that the optimum pH for xylitol production from *Candida tropicalis* is 5.5, while Mohamad *et al*. (2009) found that the pH of xylitol production from *C. tropicalis* was 4.0.

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