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Optimization Of Paddy Straw Saccharification Using A-Amylase From Aspergillus Tamarii

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Abstract

Stable enzymes under a wide range of environmental conditions are requiredbio-based industries. Extremophiles recently roused more attention as the potential source of these enzymes,one of themcalledα-amylase. α-Amylases are the earliest and powerful starch hydrolysing enzymes that restoresynthetic hydrolysis of starch in industries. It releasessugars i.e glucose, maltose and little amount dextrin by cleaving the α-(1,4)-D-glucosidicbond instarch and other similar polysaccharides. It representsaround 30% of the overall enzyme market. α-amylase applied in a variety of biotechnologicalapplications such as in starch hydrolyzing, surfactants, textile, pulp, and drug industries.This study aimed to optimize paddy straw saccharification by solid substrate fermentation α-amylaseproduction from Aspergillus tamarii fungus. The optimum of saccharification showed for pH 6.0, 12%(w/v) of substrate concentration and enzyme activity of 10 U/g of paddy straw.

Keywords: Paddy straw**,** saccharification,α-amylase, Aspergillus tamarii fungus.

Introduction

Enzymes havepowerful biological catalysts whichhasten themetabolic operations of a bio-activities(Aleemet al., 2018). Microbial enzymes are attracting a lot of interest because of its high stability, low cost, accessibility,efficiency and ecological sustainability(Singh et al., 2019). The α-amylase considers a vitalenzyme that breakdown the α-1,4-glycosidicbonds of starch polymers and usedin bioprocess(Rathouret al., 2020).

Amylases have a variety of industries like food, fabric, surfactants, paper,medicinal and renewablesindustries(Paul et al., 2021). Amylase found in plants, animals,and microorganisms. Microbial amylases becomemore popular because of their capacity to serve typical industrial demands(Rana et al., 2013; Bozicet al., 2017).Among the microbe that make amylase, Aspergillus tamariidiscovered as one of thehighest industrially amylaseproducers (Mohan et al., 2019).

α-amylasesdivided into two categories depending on their capacity to hydrolyze: liquefying and saccharifyingα-amylases which mayhydrolyze30-40%and 50-60% of the starch,respectively(Janeceket al., 2014).

Hence, the goals of this work were to produce α-amylase from solid substrate fermentation using Iraqi fungal isolate Aspergillus tamarii and measure of saccharificationoptimization bypaddy straw from fermentable sugarproduction.

Materials and Methods

Paddy Straw

Paddy straws were taken from Iraqipaddyprocessing plant. The paddy straws grounded using a blender after choppedabout1-2 cm in length. Later, thepowderedpaddy straws then sieved and gathered in the 0.4-1.00 mm range.

Microorganisms

The microorganism used in this study was a fungal isolate, obtained from Agricultural Engineering Sciences College- University of Baghdad, recultured on PDA medium at 28℃ for 5days and used for further study.

α-AmylaseProduction using SSF

α-amylasewas produced in a 250 mLErlenmeyer flask which contains 0.2% NaCl, 0.4% yeast extract and 0.8% peptone and added to 10 g of paddy straw flour and then sterilized at for 15 min 121°C.The substrate was then loaded with 8% v/w of spore suspension Aspergillus tamarii which reported of 1×10^6spore/mL and grown for 4 daysat room temperature. Then, theenzyme separeted by putting of refrigerated distilled water(100 mL) and rotating for 30 min at 200 rpm.After that, the mixtures was filtrated by cheese cloth and the supernatant was then taken in the next study

α-amylase Assay

The activity of α-amylase determined by calculating the reducing sugars releasedfrom starch(Bernfeld, 1995). Thesolutionincluding starch(0.5%) mixedin acetate buffer (pH 6.0) and added to diluted α-amylase which incubation at 80°C for 30 min. Then, the released reducing sugars evaluatedbydinitrosalicylic acid reagent(DNSA). One α-amylase unit described as the amount of enzyme neededto release of 1mmol of reducing sugars per milliliter per minute.

Optimization of Paddy Straw Saccharification

The hydrolysis of paddy straw flour(raw starch) was achieved in a 100 mLErlenmeyer flask that contains of paddy straw flour (10%)with acetatebuffer(0.05 M)at pH 6 which then added to 10 mL of α-amylase(paddy straw 8U/g). After that, the contentincubated for 4 days, 150 rpm at 45°C. Each day the samplewas taken.

1. pH:

The hydrolysis process started with pH values of 5.5, 6.0, 6.5, and 7. A 0.05 M acetate buffer was used to alter the pH. The temperature was 45°C, the substrate concentration was 10%, and the enzyme concentration was 8 U/g of paddy straw.

2. Substrate concentration:

For the hydrolysis process, different substrate concentrations of 6 %, 8 %, 10 %, and 12 %were employed. The pH was set at 45°C, and the enzyme concentration was 8 U/g of paddy straw, as determined by a prior experiment.

3.Enzyme concentration:

The hydrolysis procedure was carried out using enzyme doses of 4, 6, 8, and 10 U/g of paddy straw, respectively. The pH and substrate concentration were determined by a prior experiment to be optimal. At 45°C, the step was completed.

Results and Discussion

Under SSFthe α-amylaseproduction was isolated.Thebenefits of SSF compared to submerged fermentation (SmF), likehigher yield, easy approach, small cost, lesser energy demandand lowersewage production, and greaterquality recovery(Asgheret al., 2006). About 4 days of SSF,the activity of α-Amylasereported of 43.22 U/g usingpaddy straw.

A researcher Mohan et al., (2019) showed the enzyme activity of α-amylase from Aspergillustamarii was higher. Maximum α-amylase produced by Aspergillusnigerof potato peel waste(Angelia et al.,2019).

In figure(1), the released concentration of fermentable sugar in all the pH values raised at two days but the maximum was 2.70 g/L at pH 6.0 while in all concentrations of substrate, the released concentration of fermentable sugar was elevated at two days but higher was 3.80 g/L at 12% of substrate concentration (figure2). On the other hand, the released concentration of fermentable sugar in all enzyme concentrations obtained at two days, but superior was 3.75 g/L in enzyme concentration(10U/g paddy straw)Figure(3).

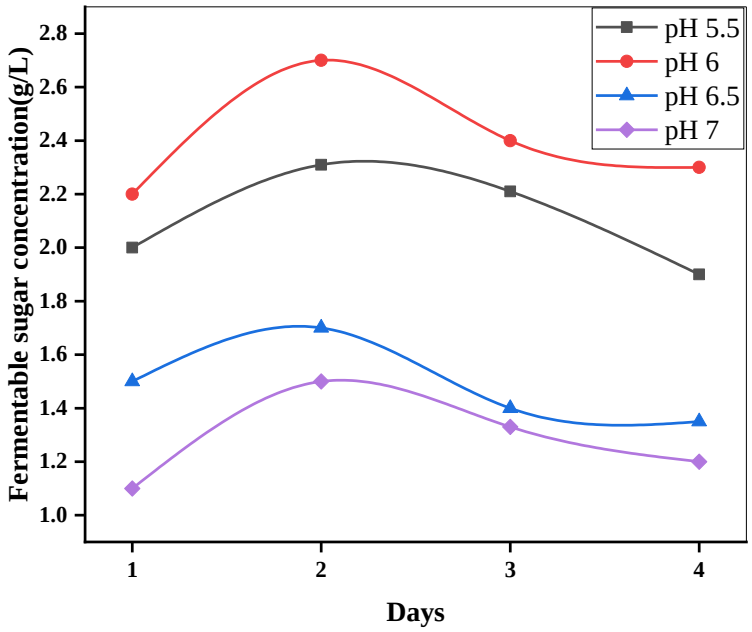

Fig. 1. The impact of variable pH on thepaddy strawsaccharificationthroughα-amylase production at 45°C with an enzyme concentration of 8 U/g of paddy straw and asubstrate concentration (10%).

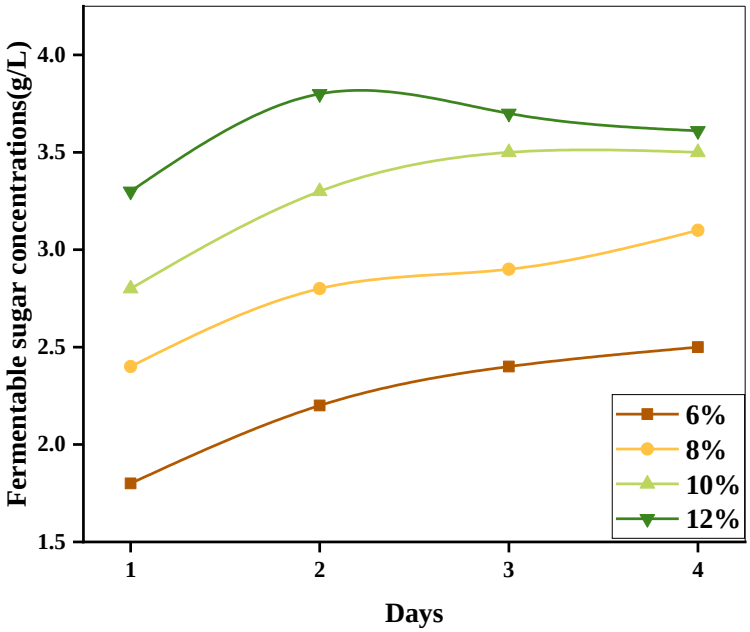

Fig.2. The impact of variable substrate concentration on thesaccharification of paddy straw byα-amylase production at pH 6.0 and 45°C with an enzyme concentration of 8 U/g of paddy straw.

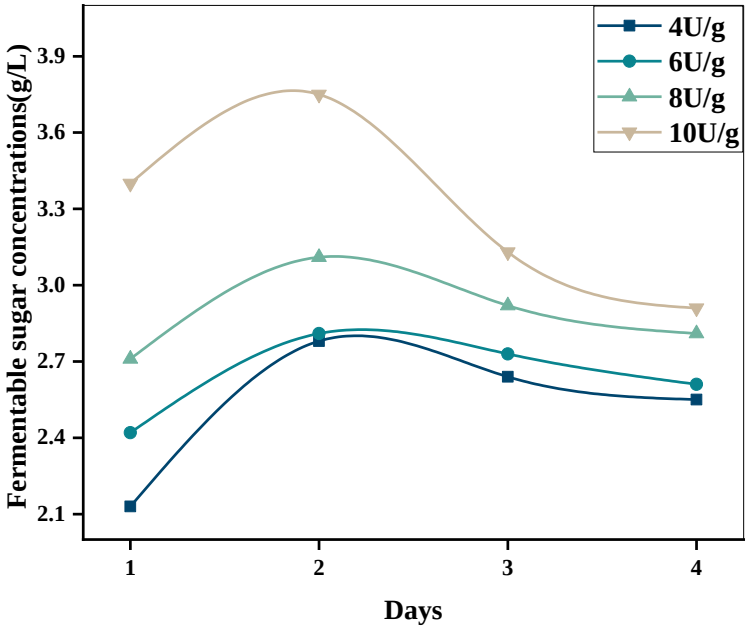

Fig. 3. The impact of variable enzyme concentration on thesaccharification of paddy straw byα-amylase production at pH 6.0 and 45°C with a substrate concentrationof 12%.

The activity of enzyme is normally related toenzyme concentration; because the enzymeconcentration is normallylower than the substrate, the enzymeislimitiedwith saturation in concentrations of substrate. Thus,at high concentrations of substrate, activity no longerchanges with increasing substrate.No matter how much more enzyme or substrate is supplied, when a saturation value of substrate or enzyme is established, there is no further rise in enzyme production.This even indicates that, there at saturation value, no amount of extra substrate or enzyme will affect the enzyme reactions.(Yang et al., 2006; Chen et al., 2008).

Conclusion

α-Amylaseisolated by SFFwas 43.22 U/gof paddy strawfrom Iraqi isolate Aspergillus tamarii. According to the results of the study, the best conditions of paddy straw hydrolysiswere pH 6.0, 12% of substrate concentration, and 10 U/g of paddy straw enzyme concentration. As a result, this enzyme may be employedin ethanol production industry.

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