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EFFECT OF YEAST EXTRACT CONCENTRATION ON MICROBIAL PRODUCTION OF XYLITOL

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Abstract- The work involves an experimental study on the effect of initial yeast extract concentration on microbial production of xylitol from xylose using *Candida Parapsilosis* NCIM-3323 and the optimum values have been established. The initial yeast extract concentration was varied in the range of 3-8 g/l at a temperature of 30 ^{o}C, pH of 3.5 and initial xylose concentration of 60 g/l. It is observed that maximum production of xylitol is obtained for an initial yeast extract concentration of 4 g/l. It is seen that maximum xylitol concentration of 17.96 g/l, maximum xylitol yield of 0.299 g/g, maximum xylitol volumetric productivity of 0.748 g/g.h and maximum specific productivity of 0.0125 g/g.h were obtained for an initial extract concentration of 4 g/l.
Key words - Batch fermentation, biomass, *Candida Parapsilosis*, volumetric productivity,specific productivity, yield, xylitol, xylose

Introduction

Xylitol is a naturally occurring sugar with wide application like its use in food processing industry as a substitute for sugar due its sweetening power. It causes sensation of coolness in mouth which is desirable in certain food products like beverages. It is used for prevention of dental caries. Xylitol easily metabolises in human body independent of insulin and produces the same amount of energy signifying its application to diabetic food.

Various forest and agricultural materials rich in hemicelluloses have been used as a raw material in xylitol manufacture. Hemicelluloses are chemically a xylan, a long polysaccharide molecule consisting of D-xylose units. In the manufacturing process of xylitol, the xylan molecules are first hydrolysed into D-xylose .The latter is chemically reduced to xylitol which is then separated and xylitol is crystalised. It demands pure D-xylose. The entire process is complicated and demands great engineering skills and experience. Xylitol can also be produced by means of batch fermentation utilizing D-xylose.

Microbial conversion of D-xylose into xylitol is governed by several factors like pH, initial xylose concentration, nitrogen sources like initial yeast extract concentration etc. The present work involves the study on the effect of initial yeast extract concentration on the microbial production of xylitol from D-xylose using *Candida parasilosis* NCIM-3323.

It is noticed in the earlier work that best productivity was obtained when the media were supplemented with yeast extract as a nitrogen source using *C. boidinii* [1] and in the experiments with *C. mogii* [2] improved growth of biomass, cell yield and xylitol productivity was reported by the supplementation of fermentation medium with yeast extract and peptone.

MATERIALS AND METHODS

Microorganism

The yeast *Candida Parapsilosis* NCIM-*3323* on Agar slants was procured from National Chemical Laboratory, Pune has been used for the present study. The culture was preserved in a refrigerator at 4 ^{0}C by periodic subcultures on agar slants.

Preparation of subcultures

Subcultures of yeast are prepared once in a month using agar slants. The chemicals are required per 100 ml of distilled water for slant preparation are given in Table 1.The medium was poured upto ⅓ rd of the sterilized test tube kept at an angle of 30 degrees and cooled to solidify the medium. After attaining room temperature, they were exposed to UV light for 30-40 minutes. The slants were then inoculated with a strain of yeast and kept at 30 degrees for 2 days. After the colonies have developed, they were stored at 4 ^{0}C in a refrigerator.

Seed culture and fermentation medium

Seed culture medium for fermentation was prepared from prepared stock cultures. Composition is given in Table 2. Seed culture was grown in 100 ml of the medium in a 250 ml conical flask at 100 rpm on an incubator shaker for 24 h at 30 ^{0}C. To prevent the reaction with xylose, $(NH_4)_2SO_4$ was sterilized separately in an autoclave for 15 minutes at 15 psi

pressure and 120 ^{0}C temperature to kill the undesirable microorganisms. pH was adjusted to 6 with acetic acid and KOH and they were exposed to
UV light for 30-40 minutes to kill the remaining undesirable microorganisms. A loopful of organisms is used to inoculate the medium.
Batch fermentation was performed in 500 ml conical flask in an incubator shaker at 100 rpm for 7 days by transferring known volume of inoculum into the fermentation medium. The medium for fermentation is prepared by taking medium composition as given in Table 3. Batch fermentation studies were made varying initial yeast extract composition.

Analysis

Xylitol was analyzed according to the method given in European Pharmacopeia Supplement 2000 (page No. 1237). Dry cell weight was estimated using 0.22 micron filter paper by taking the difference between initial and final weights of filter paper. Reducing sugar (Xylose) content of the medium was estimated spectrophotometrically at 540 nm using dinitrosalicylic acid reagent [3].

Results and discussion

The present work involves the effect of initial yeast extract concentration on xylitol production from xylose using *Candida parasilosis* NCIM-3323. To assess the effect of initial yeast extract concentration on xylitol production, experiments were carried out in 500ml erlenmeyer flasks with 350ml of the fermentation medium with medium composition given in Table 3 except initial ammonium sulphate concentration and initial KH_2PO_4 concentration. In this study initial yeast extract concentrations are varied as 3 g/l, 4 g/l, 5 g/l, 6 g/l, 7 g/l, 8 g/l. Initial pH of the samples was adjusted to 3.5, fermentation temperature was set at 30 ^{o}C, initial xylose concentration of 60 g/l, initial ammonium sulphate concentration of 8 g/l, initial KH_2PO_4 concentration of 7 g/l, were used. Samples were inoculated with 10 ml, of seed culture of 20 h grown cells of *candida parasilosis* NCIM-3323 in the liquid medium as given in Table 2. Samples were fermented for 168 h in an incubator shaker at 100 RPM. 10 ml broth was withdrawn for every 24 h and analyzed for xylitol and biomass dry weight.
From the results it is seen that maximum xylitol concentration of 17.96 g/l , maximum xylitol yield of 0.299 g/g, maximum xylitol volumetric productivity of 0.748 g/gh, maximum xylitol specific productivity of 0.0125 g/gh were obtained for an initial yeast extract concentration of 4 g/l. Maximum biomass concentration of 1.71 g/l, maximum biomass yield of 0.0285 g/g, maximum biomass volumetric productivity of 0.0687 g/g, maximum biomass specific productivity of 0.00114 g/gh were obtained for an initial yeast extract concentration of 6 g/l. “Figs. (1) - (3)” represent the effect of initial yeast extract concentration on volumetric productivity, specific productivity and yield of xylitol, while “Figs. (4) – (6)” represent the effect of initial yeast extract concentration on volumetric productivity, specific productivity and yield of biomass. It is seen in the present work that maximum productivity and yield of xylitol and biomass is obtained for an initial yeast extract concentration of 4 g/l and 6 g/l respectively.

References

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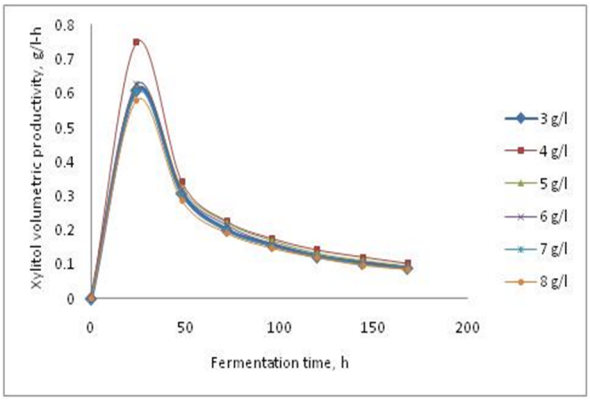

Fig. 1 Effect of initial yeast extract concentration on volumetric productivity of xylitol

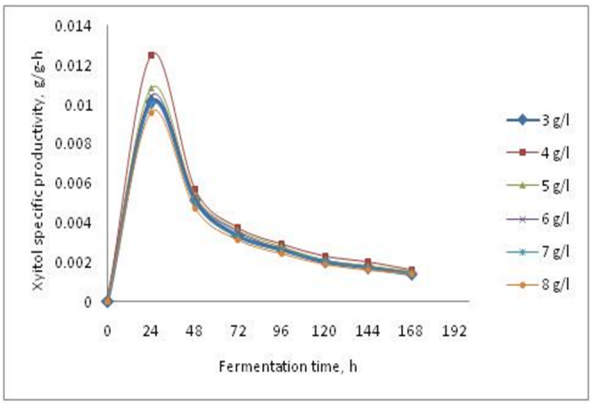

Fig. 2 Effect of initial yeast extract concentration on specific productivity of xylitol

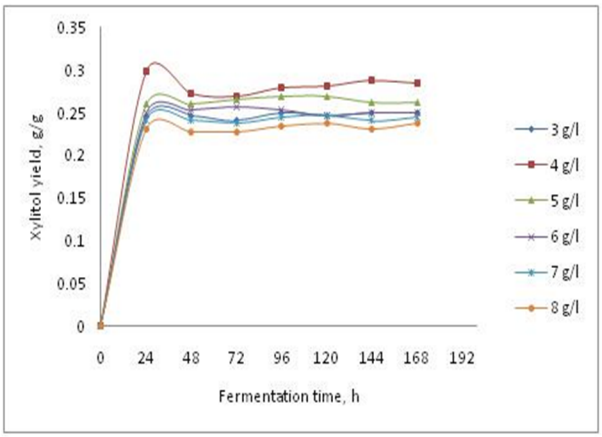

Fig. 3 Effect of initial yeast extract concentration on yield of xylitol

Table 1. *Chemicals required for slant preparation*

Component	Malt extract	Glucose	Yeast extract	Peptone	Agar
Composition, g/l	0.3	1.0	0.3	0.5	2.0

Table 2. *Seed culture preparation for fermentation*

Component	D-xylose	Yeast extract	$MgSO_4.7H_2O$	KH_2PO_4	$(NH_4)_2SO_4$
Composition, g/l	10	2	0.4	5	2

Table 3. *Fermentation medium composition for fermentation studies*

Component	D-xylose	Yeast extract	$MgSO_4.7H_2O$	KH_2PO_4	$(NH_4)_2SO_4$
Composition, g/l	60	2	0.4	5	2

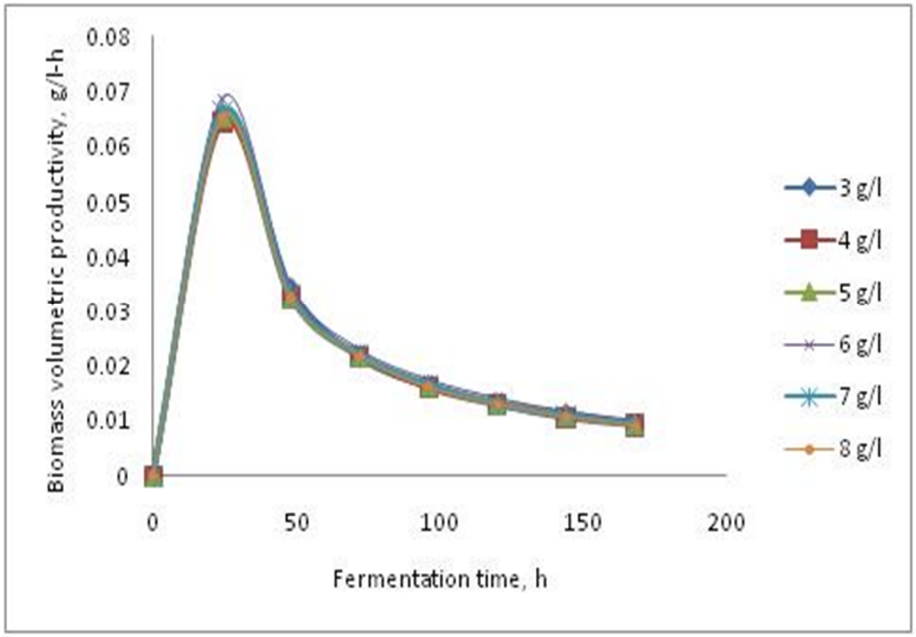

Fig. 4 Effect of initial yeast extract concentration on volumetric productivity of biomass

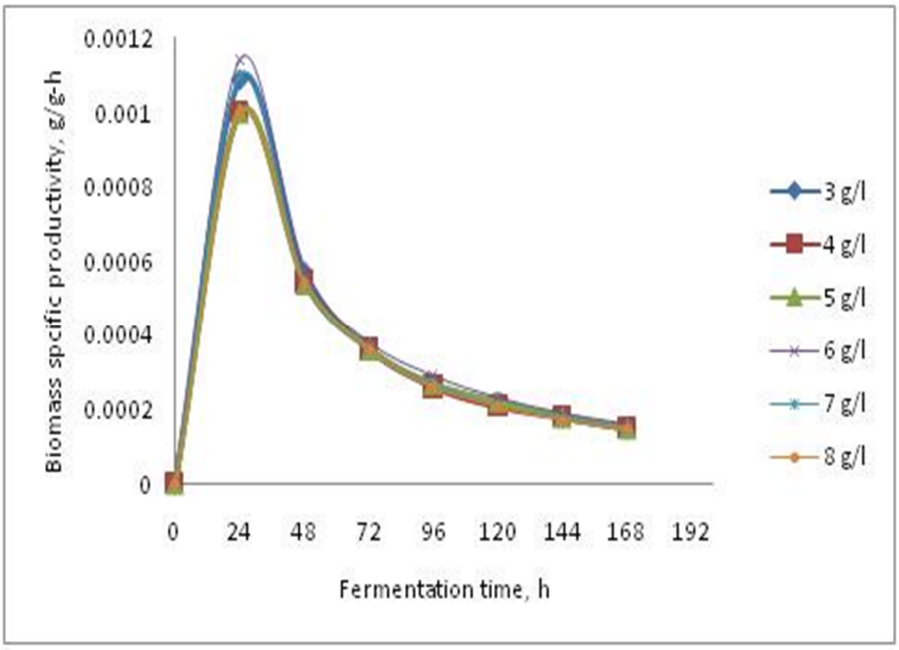

Fig. 5 Effect of initial yeast extract concentration on specific productivity of biomass

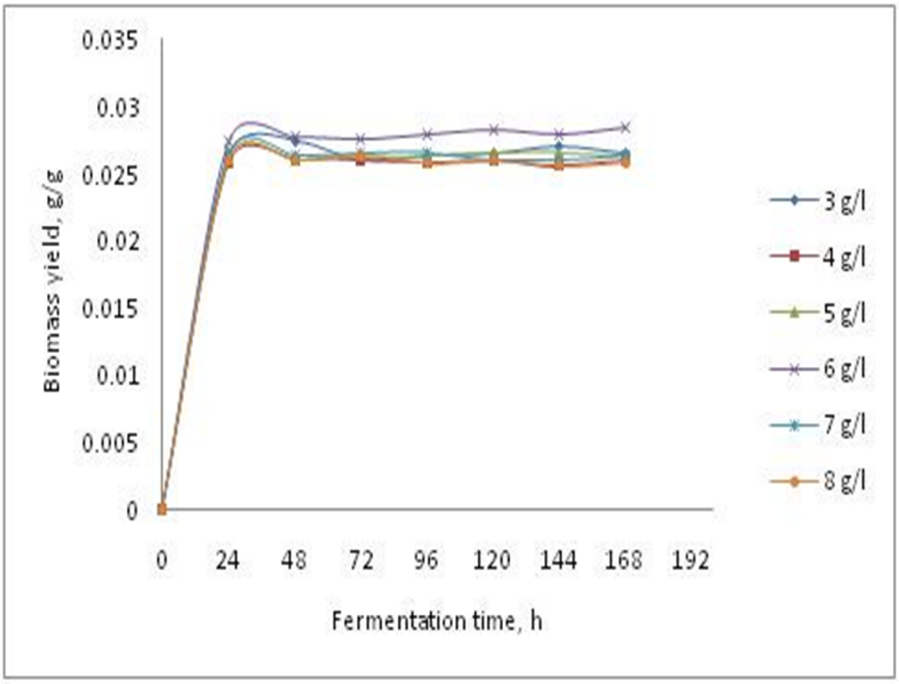

Fig. 6 Effect of initial yeast extract concentration on yield of biomass