INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

Volume: 2: Issue-3: July-Sept -2011 IJABPT *ISSN 0976-4550*

EFFECT OF NITROGEN SOURCES ON MICROBIAL PRODUCTION OF XYLITOL

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ABSTRACT : Experimental investigation on batch fermentation of xylose to xylitol using *Candida parapsilosis NCIM-3323* has been carried out and the effect of different nitrogen sources on xylitol production has been investigated. It is noticed that Ammonium sulfate is best suitable compared to other sources used. The present work utilized five nitrogen sources namely Potassium Nitrate, Ammonium Chloride, Ammonium Sulfate, Ammonium Nitrate and Urea in the experiments for a fermentation period of 144 h at 30 ^{0}C at pH of 3.5 with initial xylose concentration of 60 g/l. The work was also carried out varying concentration of Ammonium sulfate, wherein it was found that 4 g/l of ammonium sulfate gives highest yield.

Key Words: Fermentation, *Candida parapsilosis*, nitrogen sources, microorganism, xylitol

INTRODUCTION

Xylitol is a five carbon sugar alcohol (1,2,3,4,5 pentahydroxy pentane) with a molecular formula, $C_5H_{12}O_5$. The sweetening property matching that of sucrose makes xylitol enjoy good application as sugar substitute for food processing industry. It produces perceived sensation of coolness in mouth as it comes in contact with saliva for its negative heat of solution, which makes it desirable in certain food products like beverages. The properties like prevention of dental cavity, non-sugar sweetening capability, adhesive property etc. makes it useful for several applications widely. More than forty yeasts have been identified to be effective in xylitol production. Out of these, yeasts that belong to *Candida* species are found to be very effective. Several investigators have reported work on effect of several variables on xylitol production using different types of yeasts. The present work deals with experimental investigation on the effect of nitrogen sources on microbial production of xylitol using *Candida parapsilosis*. Most of the earlier work relates to the use of microorganisms other than *Candida parapsilosis*. Also most of the earlier work dealt with variables other than nitrogen sources with different microorganisms. The work available on effect of nitrogen sources using *Candida parapsilosis* for xylitol production is very scarce. Hence the work finds importance due to the fact that it reports the best useful nitrogen source for *Candida parapsilosis* and also establishes its optimum concentration.

The nature and concentration of nitrogen source in the media influences the xylitol production and xylose utilization by the microorganism. Organic nitrogen nutrients like yeast extract have shown increased xylitol production compared to nitrogen salts (Saha & Bothast, 1997; Horitsu et al. 1992). Results of analyzing eight ammonium salts and four organic nitrogen sources used for xylitol production with *P. albertinsis* showed that ammonium acetate was most effective of all the salts and yeast extract is more suitable for xylitol production (Saha & Bothast, 1997). Winkelhausen and Kuzmanova (1998) observed increased xylitol production rate in *Candida tropicalis DSM 7524* when the medium contains 20 g/l yeast extract, while De Silva and Afschar (1994) observed for concentration higher than 15 g/l. In the case of *Candida guilliermandii* FTI 20037, the maximum yield of xylitol was obtained when concentration of yeast extract was 1 g/l (Silva et al., 1997). Sirsansaneeyakulu et al. (1995) reported improved cell growth, xylitol yield and productivity in *C. mongi* when the fermentation medium contained yeast extract and peptone. Vongsuvaulert and Tani (1989) observed highest productivities with *C. boidinii* when yeast extract was the nitrogen source.

Palnitkar and Lachke (1992) observed increased xylose utilization when an organic nitrogen source was in the media. Barbosa et al. (1990) also observed higher xylose consumption, but decreased xylitol production, when the medium contained 5 g/l yeast extract with *C. guillermondii*. Lu et al. (1995) studied the influence of aspargine, glucine, trader's protein, yeast extract, urea, casein hydrolyzate, NH_4NO_3, ammonium sulfate, ammonium chloride, $NH_4H_2PO_4$ and $NaNO_3$ as nitrogen sources for xylitol production with mutant *Candida species L-102*. A maximum xylitol production of 100 g/l was obtained using 114 g/l xylose when 3 g/l urea was used as nitrogen source. Barbosa et al. (1988) reported a higher xylitol yield with *C. guilliermondii* when urea was used in fermentation. De Silva and Afschar (1994) observed higher productivities in *C. guilliermondii* but not much difference in xylitol yield when urea replaced ammonium sulfate or ammonium chloride in the medium. Vandeska et al. (1995) observed increased xylitol yields and improved biomass production by *C. boidinii* when urea was used. Thus most of the earlier work was reported either organic sources like yeast extract, peptone or urea as best nitrogen sources for the respective microorganism used. The present work involves the search for suitable nitrogen source for xylitol production using *Candida parapsilosis NCIM-3323* and to establish its optimum concentration.

MATERIALS AND METHODS

Culture

Candida parapsilosis-NCIM - 3323, obtained from National Chemical Laboratory, Pune on agar slants was preserved in a refrigerator at a temperature of 4 ^{0}C by periodic subculture on agar slants.

Stock Culture

Subcultures of yeast are prepared once in a month using agar slants. The following chemicals are required for 100 ml of distilled water for slant preparation:

Component	Malt extract	Glucose	Yeast extract	Peptone	Agar
Composition	0.3 g	1 g	0.3 g	0.5 g	2 g

All the above chemicals were weighed and dissolved in 100 ml of distilled water. Agar-agar does not dissolve readily in distilled water and hence the solution was heated until agar-agar dissolved completely. pH of the solution was adjusted to 6.4 – 6.8 with KOH and Acetic acid. All the chemicals were mixed and sterilized in an autoclave for 15 minutes at a temperature of 120 ^{0}C and a pressure of 15 psi.

The medium for slant preparation was poured upto 1/3rd of the sterilized test tube kept at an angle of 30 ^{0} to provide more surface area of the nutrients for the growth of micro-organism and cooled to solidify the medium. After the slants have reached the room temperature, they were exposed to UV light for 30-40 minutes to destruct unwanted microorganism. Then the slants were inoculated with the strain of yeast. The slants were kept at 30 ^{0} for a period of two days. After the colonies developed, the slants were stored at 4 ^{0}C.

Seed culture medium and fermentation medium preparation

Seed culture medium for fermentation was prepared from the prepared stock cultures with composition as given below:

Component	D-Xylose	Yeast extract	$MgSO_4.7H_2O$	KH_2PO_4	$(NH_4)_2SO_4$
Composition, g/l	15	2	0.4	5	2

Seed culture was grown in 100 ml of the medium in a 250 ml conical flask, at 100 rpm in a labline incubator shaker for 24 h at 30 ^{0}C. To prevent the reaction with xylose, ammonium sulfate was sterilized separately in an autoclave for 15 minutes at a pressure of 15 psi and a temperature of 120 ^{0}C to kill the undesirable microorganisms.

The pH was adjusted with acetic acid or KOH and they were exposed to UV light for 30-40 minutes to kill the remaining undesirable microorganisms. Loopful of organisms are used to inoculate the medium.

Fermentation Conditions

The batch fermentation was performed in 500 ml conical flask in an incubator shaker at 100 rpm for 5 days by transferring 10 ml of inoculum into the fermentation medium. The medium for fermentation is prepared by taking medium composition as given in the table above except xylose concentration. Xylose concentration used is 60 g/l at a temperature of 30 ^{0}C and pH of 3.5 for 144 h fermentation period.

Analysis

Xylitol produced was found by analysis according to the method given in European Pharmacopoei Supplement 2000 (Pg No. 1237). Biomass weight was estimated using 0.22 micron filter paper by taking the difference of initial and final weights of filter paper. The reducing sugar (xylose) content of the medium was estimated spectrophotometrically at 540 nm using Miller's method (1959) with dinitrosalicylic acid reagent.

RESULTS AND DISCUSSION

Yeast cells have a nitrogen content of around 10 % of their dry weight and are capable of utilizing a range of inorganic and organic sources of nitrogen for incorporation into the structural and functional nitrogenous components of the cell (Walker, 1998). Although yeasts cannot fix molecular nitrogen, simple inorganic nitrogen sources such as Ammonium Sulfate are widely utilized as nitrogen source in yeast growth media since it also provides a source of assimilable Sulfur. Some yeast can also grow on nitrate as well as urea as a source of nitrogen. A variety of other organic nitrogen compounds such as amino acids, peptides, and amines can also provide the nitrogenous requirements of the yeast cells.

The present work utilizes five nitrogen sources namely Potassium Nitrate, Ammonium Chloride, Ammonium Sulfate, Ammonium Nitrate and Urea in the experiments for a fermentation period of 144 h at 30 ^{0}C at pH of 3.5 with initial xylose concentration of 60 g/l. It is noticed that xylitol production is more for Ammonium Sulfate compared to other sources used. Hence Ammonium Sulfate is best suitable source of nitrogen for xylitol production among the sources studied. Figs. 1-3 represent the effect of nitrogen source on volumetric productivity, specific productivity and yield of xylitol while Figs. 4-6 indicate those of biomass. The figures indicate higher productivity and yield for Ammonium Sulfate.

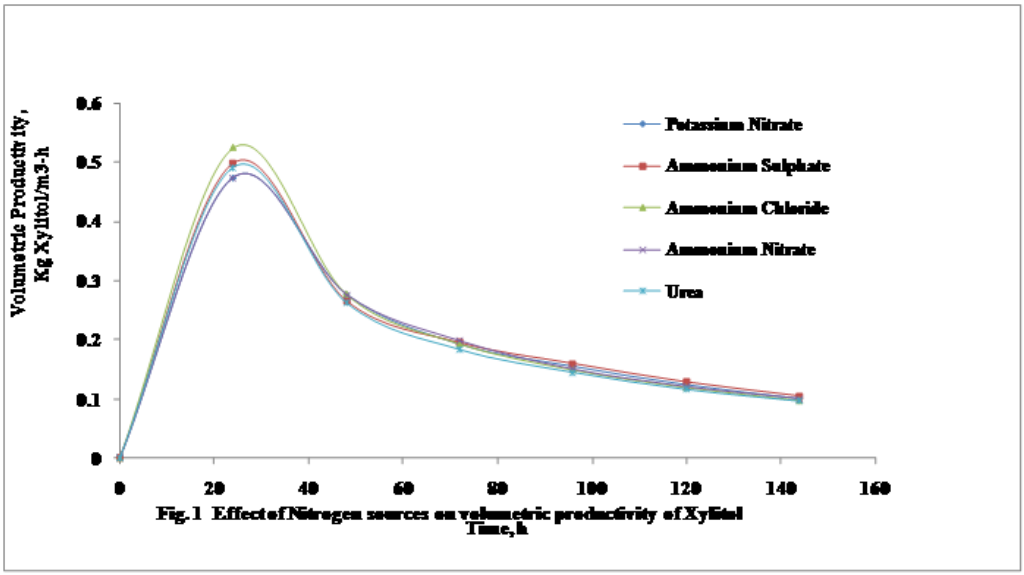

Fig.1 Effect of Nitrogen sources on volumetric productivity of Xylitol

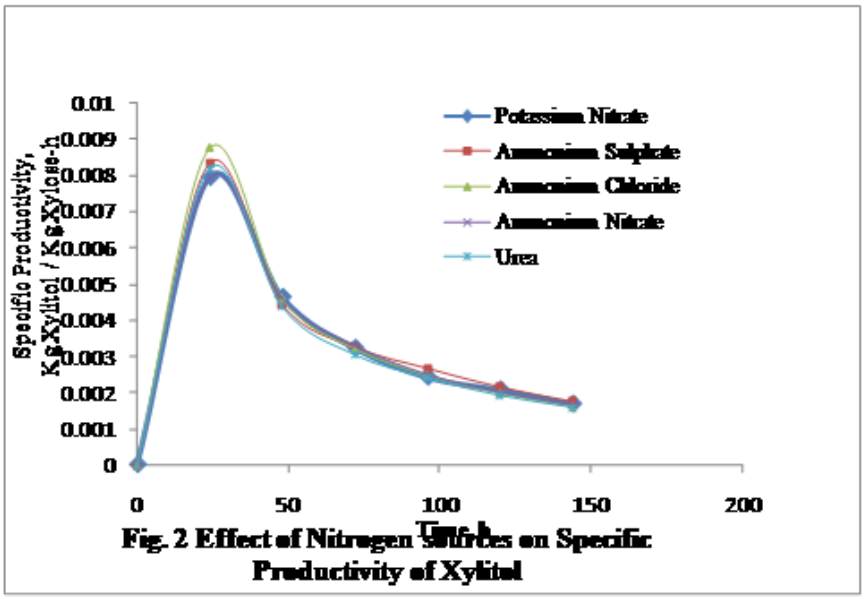

Fig. 2 Effect of Nitrogen Sources on Specific Productivity of Xylitol

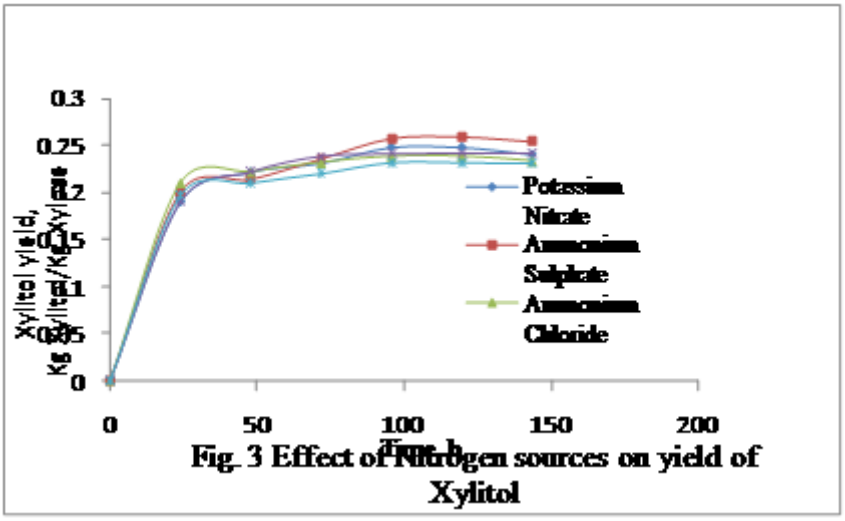

Fig. 3 Effect of Nitrogen sources on yield of Xylitol

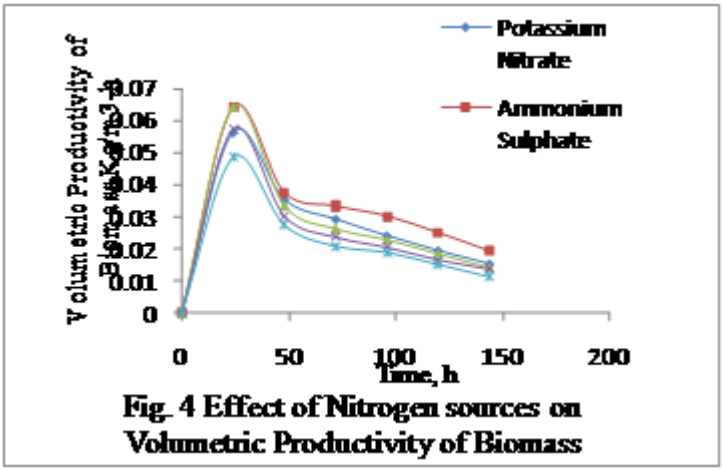

Fig. 4 Effect of Nitrogen sources on Volumetric Productivity of Biomass

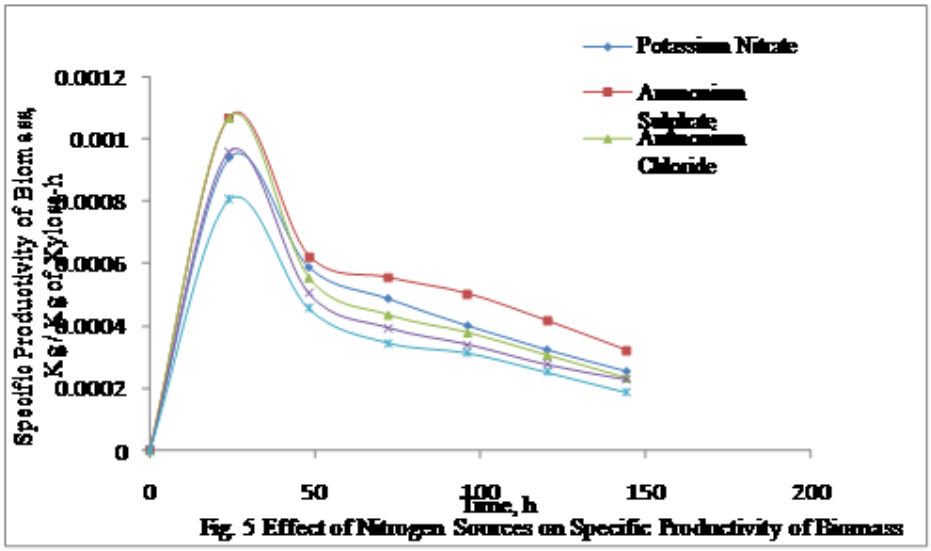

Fig. 5 Effect of Nitrogen Sources on Specific Productivity of Biomass

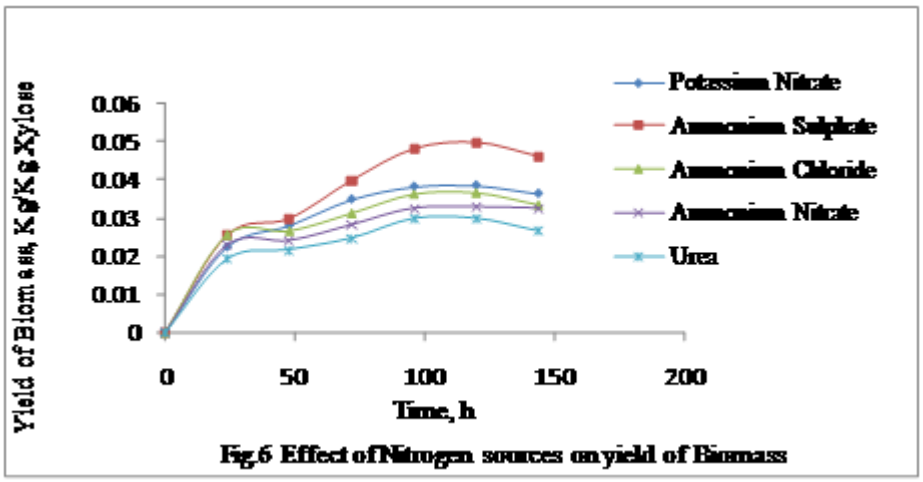

Fig.6 Effect of Nitrogen sources on yield of Biomass

The work is extended by performing experiments with different concentrations of Ammonium Sulfate to know the optimum concentration of Ammonium Sulfate. Concentrations of 4, 6, 8, 10 g/l of Ammonium Sulfate are used in the experimental work wherein it was found that xylitol production is highest at 4 g/l of Ammonium Sulfate as seen from Fig. 7.

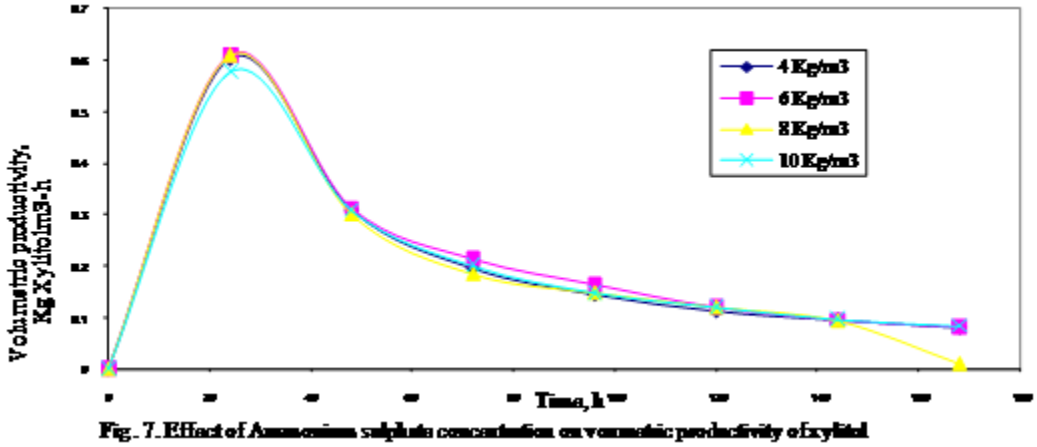

Fig. 7. Effect of Ammonium sulphate concentration on volumetric productivity of xylitol

CONCLUSION

The work involves experimental investigation on the effect of nitrogen sources on xylitol production by batch fermentation using *Candida parapsilosis*, wherein it was found that Ammonium Sulfate is best suitable source among the sources studied namely, Potassium Nitrate, Ammonium Chloride, Ammonium Sulfate, Ammonium Nitrate and Urea. The work is also extended using different concentrations of Ammonium Sulfate. It was found that 4 g/l of Ammonium Sulfate gives highest productivity of xylitol. It can be concluded that Sulfur also contributes to the growth of *Candida parapsilosis*.

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