CHAPTER 6

CONCLUSIONS

6.1 SUMMARY OF THE PRESENT WORK

The present investigation of Xylitol production through biotechnological methods led to following conclusions:

- Five xylose assimilating yeast strains were isolated from the total eighteen strains screened from sugarcane juice collected from Bannari Amman sugar’s limited, Sathyamangalam.

- All the isolates were identified based on 18S rRNA gene sequence analysis and compared the partial conserved sequences to draw phylogenetic relationship with other sequences through NCBI database. The five organisms are: *Candida tropicalis* isolate Balki1; *Zygoascus meyerae* isolate Balki2; *Zygoascus meyerae* isolate Balki3; *Candida parapsilosis* strain BKR1 and *Wickerhamomyces anomalous* strain BKR2. The conserved partial 18s rRNA sequences were deposited in the NCBI repository with Genbank accession numbers: KC415251, KC415252, KC415253, KC462059 and KC462060 respectively.

- Two yeast isolates namely *C. tropicalis* Balki1 and *C. parapsilosis* BKR1 produce xylitol which was confirmed by the FTIR, HPLC and NMR characterization.
- FTIR results reveal the number of stretched –OH residues and ascertains the presence of poly alcohols (xylitol)/branched sugars.

- HPLC method rapidly detects the xylitol at 4.9 min retention time as compared with the reference compound (xylitol from Sigma Aldrich, USA) by eluting the sample of interest in the isocratic elution columns.

- NMR studies confirmed the presence of xylitol in high composition and traces of unreacted xylose.

- Both the *C. tropicalis* Balki1 and *C. parapsilosis* BKRI were tested for the presence of enzyme, xylose reductase using NADPH assay. The NADPH assay reveals that the specific activity of xylose reductase by *C. tropicalis* Balki1 and *C. parapsilosis* BKRI were 191 and 390 U/μg respectively.

- Single factorial experiments were performed for the isolates *C. parapsilosis* BKRI and *C. tropicalis* Balki1 for the parameters such as pH, temperature and initial xylose concentration based on the literature survey.

- Xylitol yield was found to be maximum at optimum pH 4 and 5 for the isolates *C. parapsilosis* BKRI and *C. tropicalis* Balki1 respectively.

- Yield coefficient (Y_{p/s}) was estimated maximum at optimum temperature 30°C for both the strains. However the yield coefficient (Y_{p/s}) was high for *C. tropicalis* Balki1.
- Initial xylose concentration was optimized at 100 g/L for both the isolates.

- Fermentation studies were performed with the optimized values and found that the xylitol production as 4.25 and 4.13 mg/mL for *C. tropicalis* Balki1 and *C. parapsilosis* BKR1 respectively. The specific growth rate was found to be 0.02 h⁻¹ and 0.025 h⁻¹ for *C. tropicalis* Balki1 and *C. parapsilosis* BKR1 respectively. The *C. parapsilosis* BKR1 was chosen for further studies due to its nonpathogenic nature.

- Eleven medium components were screened for enhancing xylitol production through Plackett-Burman Design with the *C. parapsilosis* BKR1. According to the Pareto chart the significant medium components were identified as potassium dihydrogen phosphate, yeast extract, magnesium sulphate and xylose. The F and P value were calculated as 15.93 and 0.0013 for the model developed with adequate precision ratio of 20.67 using Design expert 7.0 software (Stat ease, USA).

- Response surface methodology was adopted for the optimization of significant medium components and process parameters using Design Expert software.

- Face Centered Central Composite Design (FCCCD) was successfully employed for experimental design of medium components optimization with high xylitol production. The medium components xylose (A), yeast extract (B),
potassium dihydrogen phosphate (C) and magnesium sulphate (D) were chosen based on Plackett-Burman design.

- The quadratic regression model developed using CCD for enhancing the xylitol productivity reveals the F value of 235.55 and P value less than 0.05 with a determination coefficient of 0.978. The adequate precision ratio obtained for the model was 39.116 which explains the goodness fit.

- The interaction effects of xylose & yeast extract (AB); xylose & potassium Dihydrogen phosphate (AC); yeast extract & potassium dihydrogen phosphate (BC) and potassium dihydrogen phosphate & magnesium sulphate (CD) were studied significantly using contour plots due to their capacity to influence the xylitol productivity.

- FCCCD experimental results deliver a maximum xylitol yield of 0.56 g/g and the levels of significant factors in modified minimal medium with xylose as sole carbon source were found to be xylose – 104.69 g/L, yeast extract – 4.12 g/L, potassium dihydrogen phosphate – 2.84 g/L, magnesium sulfate – 2.09 mg/L respectively.

- Box-Behnken Design (BBD) experimental design was successfully employed for the optimization of process parameters with enhanced xylitol production using modified minimal medium. The process parameters chosen were agitation (A); pH (B); temperature (C) and inoculum level (D) based on the single factorial optimization results.

- The quadratic regression model developed using BBD for enhancing the xylitol productivity showed the F value of 90.08 and P value less than 0.05 with a determination
coefficient of 0.9787. The adequate precision ratio obtained for the model was 29.33 which explain the goodness fit.

- The interaction effects of pH & inoculum level (BD) and temperature & inoculum level (CD) were studied significantly using contour plots for maximum xylitol production.

- The xylitol yield was not differed much with the various agitation values reveals an important fact that the shake flask cultures doesnot vary with xylitol production whatsoever the rpm was fixed. Agitation shall be considered as a significant parameter for xylitol production under bioreactor studies.

- Experimental results showed a maximum xylitol yield of 0.53 g/g for the Box-Behnken design. Levels of agitation, temperature, pH and inoculum in modified minimal medium with xylose as sole carbon source were 107.4 rpm, 29.9°C, 5.04 and 1.02 mL, respectively.

- Validation studies in the laboratory shake flask for 5 days results a maximum xylitol production of 15 mg/mL at 72 h with a xylitol yield and productivity were 0.84 g/g and 0.175 g/L.h, respectively. The fermentation behavior was also plotted to show the variation in the biomass, xylose consumption and xylitol productivity.

- Corncob was chosen to be best source due to its high hemicellulose content. The maximum xylose (37.2%) was extracted from the corncob using the dilute acid, vacuum concentration and activated carbon treatment. Infact, other pretreatment methods yield less xylose content.
Xylitol production in laboratory-scale bioreactor was carried out with modified minimal medium with xylose as sole carbon source and corn cob hemicellulosic hydrolyzates were studied.

Fermentation studies reveal that the maximum xylitol production was 8.25 mg/mL at 72 h with a xylitol yield of 0.41 g/g and productivity of 0.115 g/L.h for an initial xylose concentration (20 g/L) in modified minimal medium.

Similarly, in the corncob hemicellulosic hydrolyzate medium, maximum xylitol production was 12.92 mg/mL at 48 h with a xylitol yield of 0.34 g/g and productivity of 0.269 g/L.h for an initial xylose concentration (37.2 g/L).

Xylose consumption, biomass and xylitol production were also plotted for comparison between the CHM and MMM.

Volumetric mass transfer coefficient (kL,a) was estimated as 44.6 h⁻¹ for the *C. Parapsilosis* strain BKR1 in the corn hydrolyzate medium using the dynamic gassing method. The specific oxygen uptake rate was 0.0123 DO% . L / g cells.s

Bioreactor was constructed with the specifications as mentioned in the table 5.20. The aspect ratio was 2:1 with two flat blade Rushton turbine and baffles. A sparger was mounted and connected to a compressor for air supply.

The power requirement for the designed bioreactor was estimated to be 1.017 kW based on volumetric mass transfer correlation and dimensionless numbers.

Fermentation behavior for the designed STR was performed for three days. Maximum xylitol production was 5.32 g/L.
obtained at 48 hours with a xylitol yield of 0.289 g/g and productivity of 0.11 g/L.h for initial xylose concentration of 37 g/L.

6.2 FUTURE WORK

In the presented work, designed stirred tank reactor yields a less quantity of Xylitol compared to the laboratory - scale bioreactor. However, the following research opportunities are opened by this presented work which shall be explored in future.

- The xylitol production will be assessed in the hydrolyzates of sugarcane bagasse with *C. parapsilosis* BKR1.
- Improvement of *C. Parapsilosis* BKR1 to adapt & enhance the Xylitol production in a commercial medium.
- Development of recombinant strain incorporated with chromosome integrated xylose reductase gene under strong promoter for enhanced xylitol productivity.