

### Abstract

Wild type ethanologenic yeast species display low ethanol yields on xylose in lignocellulosic hydrolysate due to inhibitory effect of ethanol and other metabolic inhibitors. Nanofiltration was used to manipulate the feed in order to increase ethanol yield and productivity in the aforementioned system.

The hydrolysate achieved by enzymatic saccharification of chemically pre-treated biomass was subjected to nanofiltration and thereby split into two streams. The retentate stream was enriched in glucose while the permeate stream was enriched in xylose and depleted in metabolic inhibitors. The retentate and permeate streams were fermented by *Saccharomyces cerevisiae* and *Pichia stipitis* respectively to obtain maximum yields.

For a feed containing a low total sugar concentration, the permeate stream was subjected to an additional nanofiltration based concentration step to further increase the ethanol titer. For a feed containing a high total sugar concentration, the retentate stream was subjected to diafiltration to further increase the recovery of xylose. Overall ethanol yields between 0.39 and 0.43 were achieved on validation of the process with hydrolysate of sorghum bagasse.

### Introduction

- Bio-ethanol from lignocellulosic residue has been identified as one of the most significant energy resource for a sustainable future.
- Utilization of xylose (~30 % of biomass on dry-weight basis) in lignocellulosic hydrolysate is a challenge [1].
- Xylose utilising ethanologens suffer from low yields and productivities on xylose in the presence of metabolic inhibitors [1].
- Nanofiltration has previously been used for detoxification of hydrolysate [2], concentration and separation of different sugars [3].

### Materials and Methods

- Lignocellulosic hydrolysate procured from IICT, Hyderabad and simulated hydrolysate were used
- Optimization of nanofiltration operating parameters for maximal separation factor (Pentose/Hexose; inhibitors) and high permeate flux
- Adaptation of wild-type xylose utilizing yeast for inhibitor tolerance
- High cell density cultivation of yeast by microfiltration assisted cell recycle for enhanced productivity

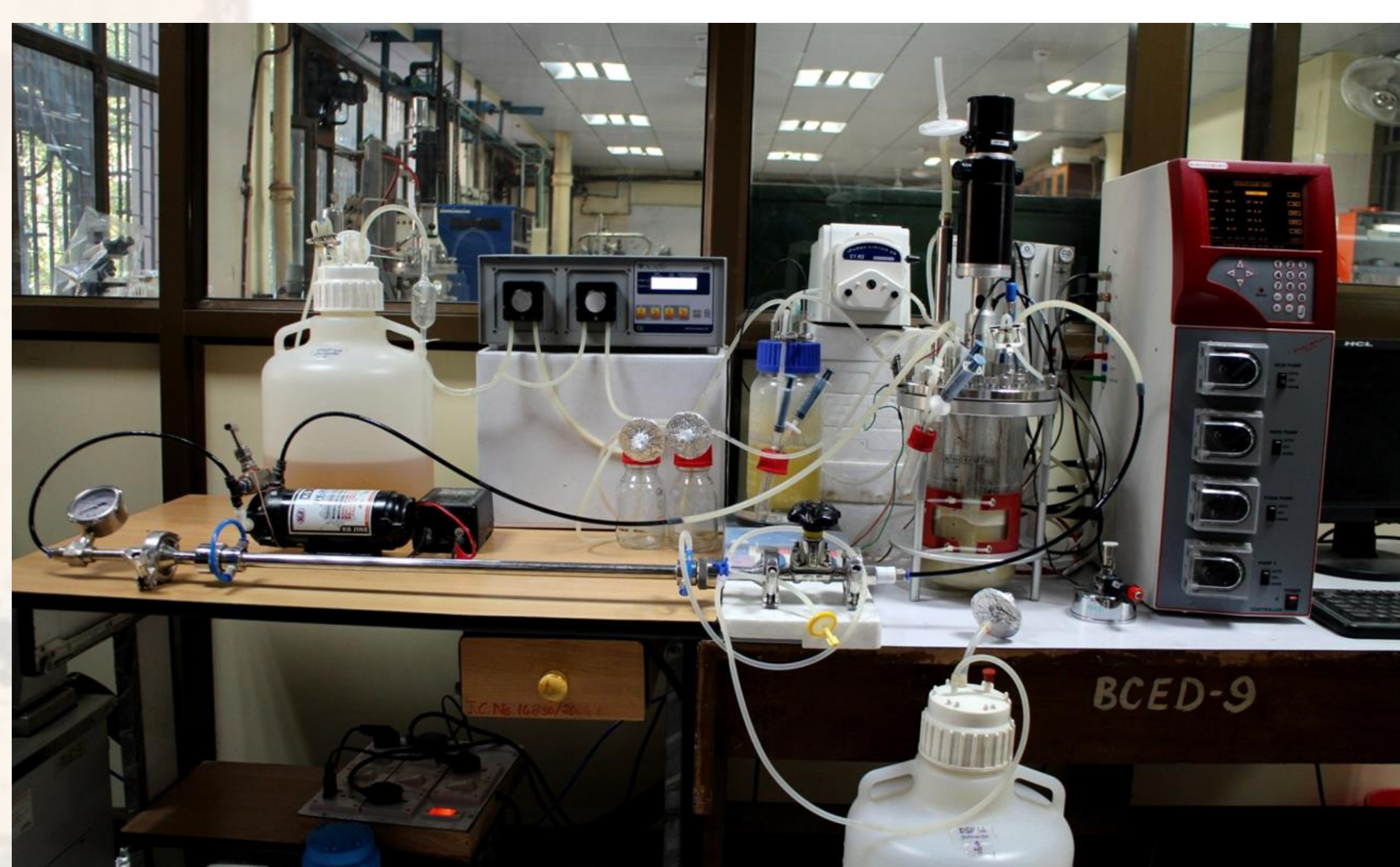


Figure 1: High cell density cultivation of *P. stipitis*

### Conclusions

- Nanofiltration can be used for manipulating the lignocellulosic hydrolysate in order to efficiently utilize xylose for ethanol production using wild-type yeast
- Ethanol titres greater than 30 g/l can be achieved
- Overall ethanol yield increased by as much as 28%
- Ethanol productivity increased by as much as 142% for a batch fermentation

### Industrial Significance

- Efficient process for production of second generation bioethanol from lignocellulosic biomass
- Enhanced utilization of xylose with a non-recombinant yeast
- High overall product yield, productivity and product titer

Technology Readiness Level: 3/5

### Results

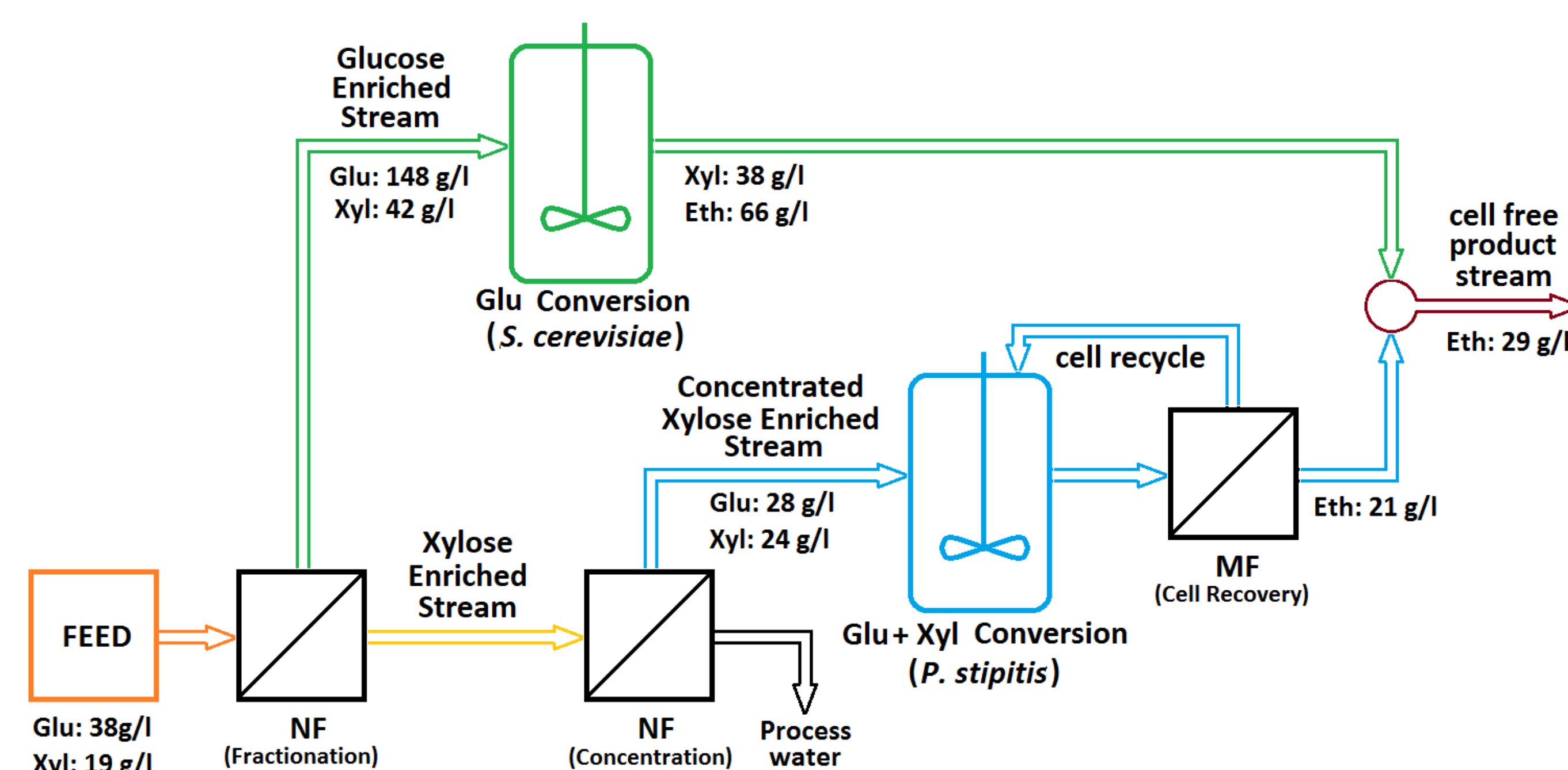


Figure 2: Schematic for ethanol fermentation of feed containing low total sugar concentration. A second concentration stage was used for increasing ethanol titre.

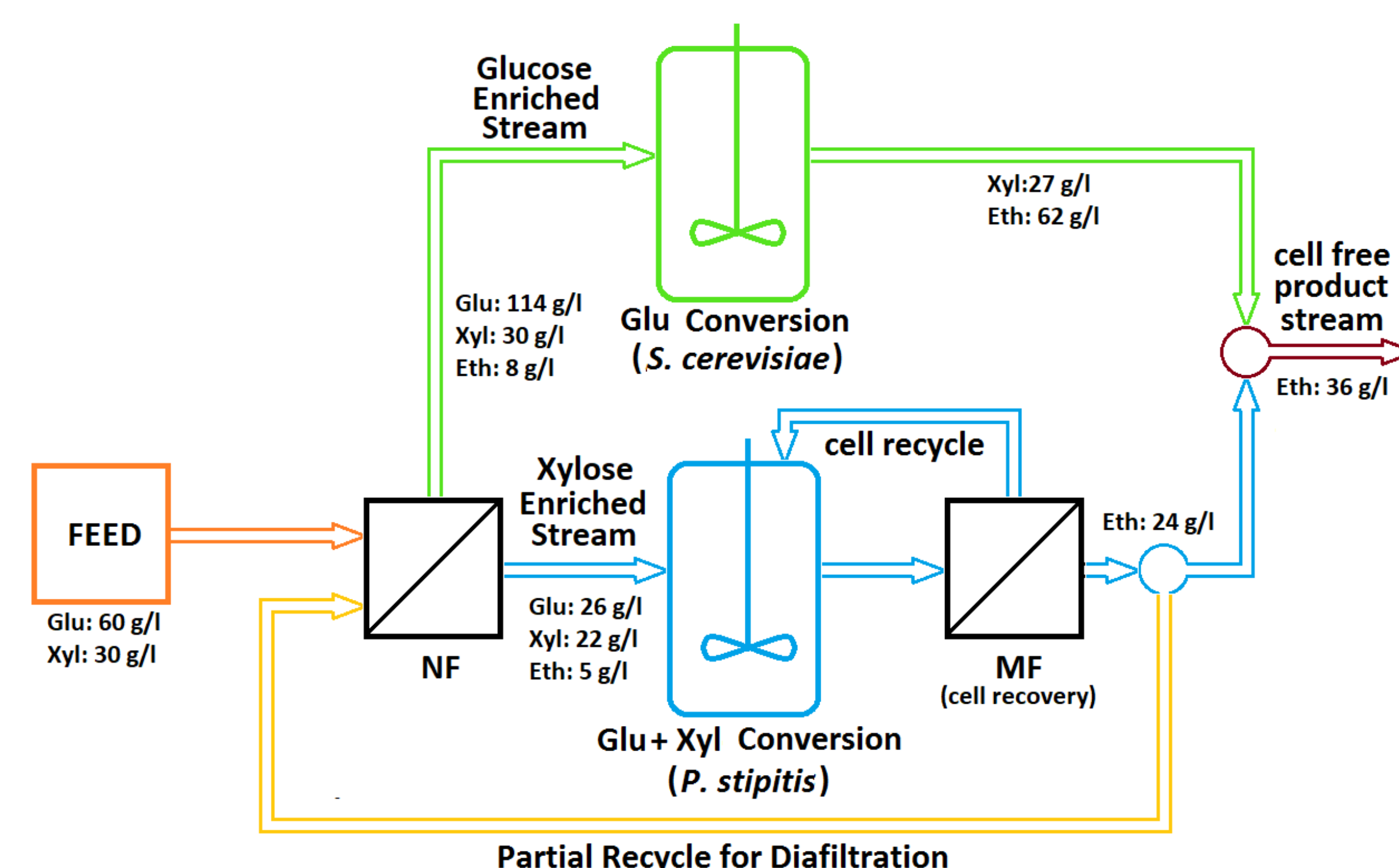


Figure 3: Schematic for ethanol fermentation of feed containing high total sugar concentration. A Diafiltration stage was used for increasing xylose recovery.

Table 1: Comparison of overall yield and product concentration achieved in IITD process with conventional process

| Feed Characteristics                                                | Low Total Sugar Concentration:<br>38 g/l Glucose + 19 g/l Xylose |                   | High Total Sugar Concentration:<br>60 g/l Glucose + 30 g/l Xylose |                   |
|---------------------------------------------------------------------|------------------------------------------------------------------|-------------------|-------------------------------------------------------------------|-------------------|
|                                                                     | Conventional Process                                             | IIT Delhi Process | Conventional Process                                              | IIT Delhi Process |
| Overall Ethanol Yield on Glucose *                                  | 92%                                                              | 91%               | 92%                                                               | 92%               |
| Overall Ethanol Yield on Xylose *                                   | 16%                                                              | 53%               | -                                                                 | 52%               |
| Overall Ethanol yield *                                             | 66%                                                              | 78%               | 61%                                                               | 78%               |
| Ethanol Concentration                                               | 20 g/l                                                           | 29 g/l            | 28 g/l                                                            | 36 g/l            |
| Process time for batch fermentation with adapted <i>P. stipitis</i> | 60 hrs                                                           | 36 hrs            | 60 hrs                                                            | 36 hrs            |
| * % of theoretical yield                                            |                                                                  |                   |                                                                   |                   |

### References

- [1] A. Madhavan, A. Srivastava, A. Kondo and V.S. Bisaria, "Bioconversion of lignocellulose-derived sugars to ethanol by engineered *Saccharomyces cerevisiae*," Critical Reviews in Biotechnology, vol. 32, no. 1, pp. 22-48, 2012.
- [2] S.K. Maiti, Y.L. Thuyavan, S. Singh, H.S. Oberoi and G.P. Agarwal, "Modeling of the separation of inhibitory components from pretreated rice straw hydrolysate by nanofiltration membranes," Bioresource Technology, vol. 114, pp. 419-427, 2012.
- [3] K. Sasaki, Y. Tsuge, D. Sasaki, T. Hasunuma, T. Sakamoto, Y. Sakihama, C. Ogino and A. Kondo, "Optimized membrane process to increase hemicellulosic ethanol production from pretreated rice straw by recombinant xylose-fermenting *Saccharomyces cerevisiae*," Bioresource Technology, vol. 169, pp. 380-386, 2014.

### Acknowledgement

