Present chapter depicts an overall conclusion that can be drawn on the entire thesis work. The overall outcome of the study related to ethanol production from de-proteinated whey has been discussed here. The present work aims to study the separation and purification of lactose from whey, a dairy by-product and its use as a substrate in the production of bio-ethanol, a “green technology” which will help to serve the dual purpose of reducing the pollution load on environment due to the discharge of this highly polluting whey stream and meeting the world’s fuel demand in this face of ongoing depletion of fossil fuels. Finally, this chapter states the future scope of the present work to improve the production of ethanol from whey lactose and its application at the industrial level.
9.1 Conclusions of the present research work

The present study has been done on the ethanol production from lactose recovered from whey, a dairy by-product and is being characterized as a highly polluting waste stream due to its huge production all over the world and its high BOD and COD loading.

Different membrane systems such as microfiltration, ultrafiltration, nanofiltration and reverse osmosis play an important role in the recovery of valuable components of whey. Microfiltration of raw whey was done as a pretreatment step in order to remove the suspended casein and fat particles and thus help to reduce the membrane fouling during subsequent stages of ultra and nanofiltration processes. Ultrafiltration and nanofiltration were done and lactose and proteins were successfully separated from whey. Fouling tendency of the microfiltration, ultrafiltration as well as nanofiltration has been removed to a great extent using Hollow Fiber Membrane configuration due to cross flow action and as such the membranes could be re-used for several times for effective separation of those components. FTIR analysis revealed that the quality of both the lactose and protein was not affected after freeze-drying process. It was observed that up to 90% of lactose and 80% of protein recovery could be achieved using the advanced separation technology. While using a different cross flow ultrafiltration module termed as Vivaflow 50, and fitted with 5kDa molecular weight cut-off (MWCO) PES membrane, proteins and lactose were recovered in retentate and permeate respectively and a 43.33% enhancement of protein concentration was achieved after attainment of a VCF of 2 corresponding to a TMP=2.5 bar. Kinetic study of lactose hydrolysis using β-Galactosidase was done and the reaction velocity of enzymatic hydrolysis was found to be maximum at 30°C while the \( K_m \) value was seen to be lowest at 40°C. The activation energy of the hydrolysis reaction was calculated using Arrhenius equation. Maximum value of catalytic efficiency (0.6427 sec\(^{-1}\)) was obtained at 30°C.

In order to increase the lactose concentration obtained in the ultrafiltration permeate, which will ultimate help to increase the ethanol productivity, nanofiltration was done and different lactose concentrations were obtained by adjusting the Volume Concentration Ratio at 2, 3 and 4 respectively. Highest lactose concentration obtained was 9% (w/v). Lactose solutions were hydrolyzed for 6 hours using β-Galactosidase to obtain different glucose concentrations at 30°C and then fermented using \( S. \) cerevisiae. It has been seen that an ethanol concentration of 5.8g/ L
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was achieved using deproteinated whey as feed. From this dilute ethanol solution, 99.9 percent pure ethanol could be produced by adopting proper separation technique.

In order to get maximum ethanol production using *S. cerevisiae* from whey lactose, the sugar needs to be hydrolyzed under optimized conditions. Response surface methodology was found to be effective in optimizing and determining the interactions among process variables for lactose hydrolysis in free and immobilized mode. A maximum lactose hydrolysis of 88% in 6 hours for free mode and 72% in 8 hours in immobilized mode were achieved under optimized conditions predicted by response surface methodology. The enzyme immobilized in calcium alginate beads could be re-used successfully for several cycles of lactose hydrolysis and thus can help in reducing the economic cost of the process.

Cheese whey powder (CWP) is a concentrated form of cheese whey containing high lactose content and therefore, can be an interesting alternative to cheese whey or whey permeate for obtaining high ethanol production. Deproteinized CWP was used as fermentation medium for production of ethanol in presence of *K. marxianus* NCIM 3217. Temperature and pH for the fermentation process were optimized at 35°C and 4.5 respectively. The effect of initial lactose concentration of the feed CWP on ethanol production was studied under optimized pH and temperature and it was found that 200 g/L was the critical substrate concentration above which both ethanol and biomass production decreased which might be due to decrease in membrane fluidity and enzyme activity leading to reduced yeast growth and product formation. For all the models, significance of correlation ($R^2$) values were close to unity, which depict that the proposed models fitted the experimental data very well. Biomass yield, product yield and ethanol productivity were all found to be highest for initial lactose concentration of 200 g/L.

9.2 Future scope of the work

The present study deals with ethanol production from lactose recovered and purified from whey. However, there are several scopes that can be explored further and has been summarized as follows:

- While using *Saccharomyces* sp., lactose needs to be hydrolyzed to glucose using β-Galactosidase enzyme. If the enzyme can be immobilized in a membrane having
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appropriate MWCO such that glucose can pass into the permeate solution, then glucose can be produced and purified in a single step which can lead to better ethanol production.

- K.marxianus is the yeast employed that can directly consume the lactose recovered from whey. However, in order to enhance the ethanol tolerance and lactose-to-ethanol conversion yields of K.marxianus, metabolic engineering and system biology toolboxes can be used that are becoming increasingly widespread. Some strategies such as intergeneric yeast fusants (K.marxianus plus Saccharomyces fragilis), co-culture approaches or using mutant strains of K. marxianus can be explored for better ethanol production from whey lactose using K. marxianus.

- Tolerance to high temperatures, ethanol and high concentrations of sugar are important features for microorganism producers of ethanol. Thus, more promising microbial strains (bacteria as well as yeasts) tolerant to these stresses should be isolated from the waste streams, characterized and used either in pure or mixed cultures for fermentation of whey lactose.

- The use of continuous stirred reactor can be explored and studied in details coupled with ethanol recovery from the fermentation vessels. Yeasts can be immobilized or recycled to keep the fermentation rates high.

- Simultaneous ethanol formation and separation from the fermentation vessel can be investigated to overcome product inhibition. Instead of distillation, more economical ethanol separation methods. Pervaporation may be very useful in the process of ethanol separation from fermentation medium.

- Economic feasibility and scaling-up of the process of ethanol production from whey lactose can be invested in order to establish its industrial significance, especially in India.

As the present work deals with ethanol production from lactose recovered and purified from whey, a dairy waste stream, using microbial strains, lot of scope is there to improve the performance of the membrane systems applied in the separation process as well as fermentation technology, to ensure its' applicability at the industrial level.