CHAPTER 3:
Aims and Objectives
3. Aims and Objectives
Dairy industry, like most other agro-industries, generates strong waste waters characterized by high biological oxygen demand (BOD) and chemical oxygen demand (COD) concentrations representing their high organic content. Whey is one such waste stream that is being generated by cheese/casein manufacturing industries. Generally, such dairy effluents will create a negative impact on the environment, if disposed off without any treatment. The intensive industrialization in the dairy industry and the ever increasing attention towards understanding the pollution problems have made the traditional outlet of whey such as its use as an animal feed, as a fertilizer, or simply as dumping it into the rivers or drains very problematic. Hence, there is the need to treat this dairy effluent called whey by various cost effective and environmental friendly processes. Whey contains components of high nutritive value such as lactose, protein, vitamins and minerals. Therefore, instead of treating whey as a “waste”, it can be treated as a “cheap resource” which can be processed to produce wide range of commercial products. Recent developments in membrane filtration have provided exciting new opportunities for large-scale protein and lactose fractionation from whey. Since whey retains the sugar called lactose (4.5-5%), it may be extracted and concentrated from whey and therefore, serve as a promising feedstock for production of bio-ethanol using suitable microbial strains. The use of this bio-ethanol, a clean bio-fuel, may help to reduce the dependency on petroleum based system and help in the diminution of greenhouse gas emissions, a prime reason for global climate change. Thus, the recovery of lactose from whey and its conversion to bio-ethanol by fermentation process will aid in meeting the fuel demand of the world and can be used as a “green technology” to meet the waste disposal challenge.

3.1. Scope of the work
The main aim of the present work was to study the feasibility of bio-ethanol production from lactose recovered from whey. In the present study, two types of microbes, Saccharomyces cerevisiae and Kluyveromyces marxianus have been used for the fermentation process. Following few paragraphs will be devoted to brief on the chapters presented in this whole thesis.

Chapter 4 presents an ultrafiltration process to separate lactose and protein with high yield and purity from whey using hollow fiber module. Ultrafiltration in a diafiltration mode was used in
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In order to improve the yield of protein in the retentate, which was then freeze-dried to get the end product in dried form. Nanofiltration of the permeate stream from ultrafiltration was done to concentrate the lactose part and was similarly freeze-dried. The performance of both ultrafiltration and nanofiltration was characterized in terms of permeate flux. The influence of transmembrane pressure on both ultrafiltration and nanofiltration membranes was studied.

In Chapter 5 an attempt was made to carry out ultrafiltration (UF) of pretreated casein whey in a cross-flow module fitted with 5kDa molecular weight cut-off polyethersulphone (PES) membrane to recover whey proteins in the retentate and lactose in the permeate. Effects of processing conditions, like transmembrane pressure on permeate flux and rejection were investigated and reported. Enzymatic kinetic study for lactose hydrolysis was carried out at 3 different temperatures ranging from 30°C to 50°C using β-galactosidase enzyme.

In Chapter 6, the work investigated the recovery of lactose from casein whey, a dairy effluent using four stage discontinuous diafiltration using a cross-flow ultrafiltration module fitted with 5kDa polyethersulfone membrane. The dilute lactose solution, recovered in the permeate was concentrated by using nanofiltration (NF) membrane in a NF module. Different lactose concentrations were obtained by adjusting the Volume Concentration Ratio at 2, 3 and 4 respectively during NF operation. The lactose solutions thus obtained were subsequently hydrolyzed to glucose at 30°C at a pH 7 using β-galactosidase enzyme. Anaerobic fermentation of the glucose solutions thus obtained was carried out in incubator shaker maintained at a temperature of 37°C and at a pH of 5.5. Ethanol concentration was determined using potassium dichromate analytical method and was reported.

In Chapter 7, the study was aimed to optimize the process of lactose hydrolysis using free and immobilized β-galactosidase to produce glucose and galactose. For Saccharomyces cerevisiae to be used in the fermentation of lactose recovered from whey, the sugar must be hydrolyzed to glucose in an optimized manner. Response surface methodology (RSM) by central composite design (CCD) was employed to optimize the degree of hydrolysis by varying the independent parameters in free and immobilized modes.

In Chapter 8, deproteinized cheese whey powder (CWP), a dried and concentrated form of cheese whey, was used as fermentation medium for ethanol production using Kluyveromyces
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marxianus strain NCIM 3217 in batch experiments. At first temperature and pH for the fermentation process were optimized and then at optimized conditions of temperature and pH, the effect of various initial lactose concentrations (150 g/ L, 200 g/ L, 250 g/ L) of the feed CWP on ethanol production was studied and the modelling was done using unstructured, kinetic models to depict the importance of yeast growth, product formation and substrate utilization for all the three lactose concentrations during the fermentation process.

Chapter 9 is a briefing on the outcome of the whole job and an elaboration on the future scope that can be explored based on the present work.
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3.2. Work-progress representation

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Activity</th>
<th>Time in Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Literature survey, collection of casein whey sample, characterization of whey</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>MF, UF, NF of casein whey to recover and purify lactose and protein using HFM and Viva Flow modules, performance analysis of these modules</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Lactose hydrolysis using free β-Galactosidase enzyme to glucose and galactose, comparative study with enzyme entrapped in calcium alginate beads, enzyme kinetic study, optimization using RSM</td>
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<tr>
<td>4</td>
<td>Batch fermentation of hydrolyzed whey lactose using <em>Saccharomyces cerevisiae</em></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Batch fermentation of cheese whey powder (CWP) with high lactose concentrations using <em>Kluyveromyces marxianus</em>, kinetic modelling and optimization of the fermentation process</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Data analysis and preparation of manuscripts for possible publication</td>
<td></td>
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</tbody>
</table>

*Figure 3.1.* Year-wise graphical presentation on the progress of the whole research activity.
3.3. Technical Programme (Flow Chart)

The work plan of the present study has been shown in the following flow chart (figure 3.2).

**Figure 3.2.** Work plan of the whole research work.