Lactulose, a functional food ingredient, also known as galactofructose, has been used in a wide variety of foods as a bifidus factor for intestinal regulation. It does not occur naturally, therefore, it is not hydrolyzed by mammalian digestive enzymes (Dahlqvist and Gryboski, 1965; Ruttloff et al., 1967) and ingested lactulose passes from both stomach and small intestine without degradation. In the colon, large numbers of bacteria metabolize lactulose and consume as their own food. In doing so, these bacteria produce lactic acid, acetic acid, and formic acid as well as carbon dioxide gas. These acids biochemically draw fluid into the bowel that softens the stool, this leads to the laxative effect of lactulose.

Lactulose has received attention from the past decades owing to its importance in both pharmaceutical and food sectors. It is used medicinally as a treatment for portal systemic encephalopathy, chronic constipation, and hyperammonenemia, complications of liver disease, tumour prevention, immunology, anti-endotoxic effects, maintain blood glucose and insulin level (Schumann, 2002; Schuster-Wolff-Bühring et al., 2010). In food industries, it is applied in a wide variety of foods as a bifidus factor or as a functional ingredient for intestinal regulation. Lactulose has also some properties with desirable effects in food products such as flavour enhancing properties, favourable browning behaviour, excellent solubility in water, etc. In addition to providing useful modifications to food flavour and physico-chemical characteristics, it also has beneficial effects on the consumer’s health. It can be used as a sweetener for diabetic patients, as a sugar substitute in various liquids or dried food preparations such as confectionery.
products, beverages, infant milk powders, bakery products, yoghurts, dairy desserts, etc., which are routinely manufactured for old people (Crittenden and Playne, 1996; Strohmaier, 1998). Apart from its food and pharmaceutical applications, lactulose is also considered as prebiotic because it stimulates the growth of health-promoting bacteria in the gastrointestinal tract, such as bifidobacteria (*Bifidobacteria bifidum, B. adolescentis, B. infantilus, B. longum*) and lactobacilli (*Lactobacillus acidophilus, L. casei, L. bulgaricus, etc.*) and at the same time inhibits pathogenic bacteria such as *Salmonella* (Schumann, 2002).

The prebiotic nature of lactulose has increased the awareness among the people and boosts its demand day by day. To fulfill the increasing demand, the commercial production of lactulose is needed. Recently, lactulose is synthesized at commercial level by the alkaline isomerization of lactose molecules in which a ketose is formed from an aldose molecule by regrouping the glucose residue to the fructose molecule (Aider and Halleux, 2007). However, this method has several drawbacks, such as a high level of lactulose degradation, considerable amounts of inorganic catalysts and formation of coloured by-products in the reaction mixture, which lead to difficulty in waste management and product purification.

In contrast to chemical synthesis, enzymatic synthesis of lactulose generally produces few by-products that avoid the need for protection/deprotection chemistry, and it affects environment at very low impact. Technological importance of enzymatic synthesis of lactulose has aroused keen interest among researchers during the last decade. The enzyme β-galactosidase is broadly used to prepare lactose-hydrolyzed milk products including flavoured milk, cheese, yoghurt, etc. for lactose-intolerant or lactase-deficient individuals. Beside these, it has several applications in biotechnology, pharmaceutical, nutraceuticals and food industries (Carpio et al., 2000; Guven et al.,
β-Galactosidase can be used to synthesize lactulose from lactose through a transgalactosylation reaction, using fructose as a galactosyl acceptor (Hua et al., 2010; Guerrero et al., 2011). It is a well known enzyme for transgalactosylation reaction and synthesis of lactose based derivatives including lactulose.

The enzyme β-galactosidase can be obtained from a wide variety of sources such as micro-organisms, plants and animals. However, according to their sources, their properties differ markedly (Panesar et al., 2006). Enzymes of plants and animals origin are of little commercial value but several microbial β-galactosidases have technological interest too. Further, micro-organisms offer a number of advantages such as easy handling, higher multiplication rate and high production yield over other available sources of this enzyme. Among micro-organisms, bacteria, yeast, and moulds are main sources of this enzyme. The most widely used microbial sources are Kluyveromyces sp. and Aspergillus sp.

The mesophile yeast Kluyveromyces has been reported to be the most important source for the production β-galactosidase due to its dairy environmental habitat and extraordinary lactose hydrolysis activity (Gekas and Lopez-Leiva, 1985). However, the industrial applications based on enzymatic hydrolysis processes are being hindered, due to intracellular location of yeast enzyme, which makes its extraction difficult and expensive. Therefore, the use of whole cells as a source β-galactosidase is an effective way to lower the β-galactosidase production cost because complex purification is not necessary. However, the permeability barrier of the cell envelope for substrates and products often causes very low reaction rates in whole cells especially yeast cells (Kondo et al., 2000). Therefore, use of permeabilized cells could be an interesting alternative. This technique can be helpful in developing a low cost technology for lactose hydrolysis to produce lactose-free milk products and has recently attracted
interest for the production of prebiotics like galacto-oligosaccharides and lactulose via the transgalactosylation reaction (Onishi and Yokozeki, 1996; Lee et al., 2004; Panesar et al., 2006). Furthermore, the use of immobilization technology in the production of lactulose is of significant importance from economic point of view. Immobilization has been found to be the convenient method to make reuse of cells, higher cell densities in bioreactors, high yields of immobilization and easy recovery of reaction products (Brodelius and Vandamme, 1987).

Immobilization is the restriction of cell mobility within a defined space with retention of its catalytic activity, which can be used repeatedly and continuously. Different methods such as physical entrapment within porous matrix, encapsulation, adsorption or attachment to a pre-formed carrier and crosslinking have been used for the immobilization of microbial cells. Among these, entrapment is the most commonly used method for the immobilization of microbial cells (Song et al., 2005). In entrapment methods of immobilization, microbial cells are enclosed in a porous polymeric matrix, which allow the diffusion of substrates to the cells and of products away from the cells. Thus, in view of the above, immobilized cell technology can also be used for the production of lactulose.

In addition to commercial available lactose, lactulose can also be synthesized by using whey (rich source of lactose) as a raw material, making the production process more economical (Song et al., 2013a). Dairy industry produces a large quantity of whey (dairy by-products), which requires a particular attention for their disposal due to the dissolved sugars, proteins, fats, and residues of additives. Approximately 85% of the total milk used for manufacturing cheese/paneer is discarded as whey. Most of the milk plants do not have proper treatment system for the disposal of whey and dumping of whey constitutes significant loss of potential food and energy as whey retains about
55% of total milk nutrients (Marwaha and Kennedy, 1988). Its disposal as a waste product possesses high-strength wastewater pollution problems for surrounding environment due to its high biological oxygen demand and chemical oxygen demand (Abboud et al., 2010). Thus, the cost-effective disposal and utilization of whey has become increasingly important to the dairy industries. To overcome this problem, a better alternative is to utilize the whey as raw material for processing into value added product like lactulose, which may contribute wholly or partially to the cost of dairy processing industry.

To the best of our knowledge, literatures on use of whole cells for the production of lactulose from whey are fragmentary. Additionally, the production of lactulose from whey using permeabilized immobilized cells system has not been reported previously. In view of the above, the present work has been carried out to fulfill the following objectives:

1. Isolation and screening of yeast cells for β-galactosidase production.
2. Media and process optimization for β-galactosidase production.
4. Immobilization of permeabilized yeast cells on different matrices.
5. Production of lactulose using immobilized permeabilized yeast cells.
6. Production of lactulose from whey using immobilized permeabilized yeast cells.