Chapter 2

Scope of the present work

In many situations the process water of dairy effluents (excluding cleaning in place steps with chemical agents) is mainly composed of diluted milk and whey in a ratio depending upon the type of product (cheese, dried milk products, milk, butter etc.) of the plant. Typically whey only causes problems when it is large (greater than 10%) portion of the flow to a treatment plant. Thus it is not only a serious environmental problem, but also an unused waste of potentially valuable food nutrients, and thus warrants recovery for the prevailing conditions of developing countries like India. Thus search has begun for new methods to use whey. In recent years, there has been a widespread and increasing interest throughout the world in creating new channels of utilization of by-products of dairy industry especially the whey. Moreover, economical disposal of by-products is an essential criterion for profitable dairying. However, frequently used option to exploit the world wide enormous amount of whey is ultra filtration to produce a protein concentrate. During the manufacture of whey protein concentrates, a permeate with almost same lactose content present in untreated whey is formed as a by-product. Lactose content up to 50 g/l corresponds to a COD between 40,000- and 60,000 mg/l, which may disrupt the biological process of sewage-disposal plants (Ghaly and Singh, 1989). These problems could be overcome by further use of whey permeate as a substrate for microorganisms for biotechnological production of valuable compounds. Chemicals such as colouring matter, flavouring agents and aroma compounds, biodegradable polymers, bio insecticides, organic acids and alcohols, many food ingredients such as amino acids and vitamins and many protein products including food enzymes also can be made from whey fermentation. However, only few of them will have commercial significance. There are many factors need to be considered in selecting a fermentation process for whey permeate utilization. Thus with proper uses of whey, the waste product which is presenting a major disposal problem to the dairy industry can be changed in to a number of useful and profitable products. Hence further
developments in whey fermentation will prove to be a profitable effort for the dairy industry.

2.1 Production of organic acids

Several organic acids with food uses like lactic acid, citric acid, lactobionic acid, gluconic acid, itaconic acid, acetic acid and propionic acid can be obtained from whey by different micro-organisms and processes (Hobman, 1984; Blanc and Goma, 1989; Roukas and Kotzekidou, 1991; Zayed and Zahren, 1991; Chiarini et al., 1992; Colomban et al., 1993; Fourier et al., 1993 and Nortan et al., 1994). Most of them find applications in specialty chemicals. For example, lactic acid can be used to produce propylene oxide, biodegradable poly lactic acid polymers, and propylene glycol or acrylic fibres, making the lactic acid market of prime importance (about 27 million kg per year)(Datta et al., 1998). The techniques for the fermentation of whey to lactic acid have been well known for some years and few developments appeared in the recent years. For lactic acid the worldwide production in 2002 was estimated at 40,000 t per year and the price was estimated at about $2/ kg. Recent interest in biodegradable polymer could lead to enhanced demand for lactic acid. It is currently used as both food additive and an individual chemical. Homo fermentative lactic bacteria from the genera of lactobacillus and streptococcus are usually used in the fermentation process for lactic acid production from whey. Among the organic acids, citric acid is the only acid produced commercially by fermentation. Much of the world’s supply of citric acid comes from fermentation of molasses. As there are few manufacturers of citric acid in the world, some dairy developed countries could have the potential of using whey permeate as a feedstock for the manufacture of this product. Hence in the present work, production of lactic acid and citric acid from whey has been taken up.

2.2 Production of ethanol

It has been suggested by several workers that the lactose source being directly fermentable and present as a negative valued waste stream would serve as an excellent feedstock for ethanol fermentation. This process normally uses lactose-fermenting
yeasts such as *Kluyveromyces fragilis* or *Pseudotropicalis* coupled with traditional fermentation system. The market for ethanol exists already. Ethanol is competitive with gasoline, or with the currently used gasoline additives. Ethanol and protein by-products from an integrated whey-based facility are almost profitable for the owner.

Though it is possible to find several work related to the search of microorganisms with the capacity of producing ethanol directly from lactose, up to now, *Kluyveromyces fragilis* is the microorganism of choice for most commercial plants (Castillo, 1990). In batch fermentation *K. fragilis* utilizes more than 95% of lactose of unconcentrated whey with a conversion efficiency of 80-85% of theoretical value of 0.538 kg ethanol/ kg lactose consumed (Mawson, 1994). The number of microorganisms able to metabolize lactose directly is limited, but also they are inhibited by moderate sugars and ethanol concentrations (Moulin and Galzy, 1984). *Saccharomyces cerevisiae*, the yeast most used in wine and beer fermentations, lacks the lactose permease system (the membrane of lactose carrier that controls the entry of sugar into cells), as well as the intra cellular enzymes for lactose hydrolysis, β-galactosidase, thus rendering it unable to ferment lactose directly into ethanol (Russel, 1986; Castillo, 1990). One interesting alternative consists of the hydrolysis of lactose by β-galactosidase from another microorganism and subsequent fermentation by *Saccharomyces cerevisiae* (Champagne and Goulet, 1988). This process can be developed in two steps or in only one step with mixed cultures or with the enzyme and yeast co-immobilized (Buyukgungor, 1987; Axelsson *et al*., 1991). Another alternative that is currently being intensively studied consists of achieving, by recombinant DNA techniques, the expression of the genes that code for the β-galactosidase and lactose permease system of *K. lactis* in *Saccharomyces cerevisiae* (Russel, 1986; Sreekrishna and Dickson, 1985; Farahnak *et al*., 1986). In this way, *S. cerevisiae* could be developed directly on cheese whey producing fermentation products (Porro *et al*., 1992). However, the recombinant yeasts elaborated up to this point in time are very slow growing and have reduced in genetic stability, so yields are low even in specially designed bioreactors. (Jeong *et al*., 1991). Hence in the present work, co-culturing of *Saccharomyces cerevisiae* MTCC 170 with *Kluyveromyces marxianus* MTCC 1388 as a means of enhancement of ethanol production from whey has been attempted with success.
2.3 Single cell protein (SCP) production

Microbial biomass has been produced commercially from whey since the 1940s. The development and operation of several pilot-scale and commercial plants in France, the USA, Germany and Austria has been reported (Sienkiewicz and Riedel 1990; Mawson, 1994). Industrial microbial biomass production from cheese whey for use as a food started in France at Fromageries Le Bel around 1958 (the patent of the process is dated 1955) (Yves, 1979; Moulin and Galzy, 1984). The biomass is primarily used as an animal-feed supplement but also in human foods (Mawson, 1994). The production by Fromageries Le Bel of about 2300 t/year of SCP has been reported, and the product has been used for more than 10 years in human dietetic nutrition. The origin of the raw material favours SCP’s acceptability for human consumption (Olsen and Allerman, 1991).

An industrial process for producing bakery yeast (Saccharomyces cerevisiae) from cheese whey was adopted by the Nutrisearch Company in 1983 in Kentucky. The process consists of lactose hydrolysis in cheese whey by immobilized lactase, followed by glucose-galactose fermentation (Castillo, 1990). Whey permeate has also been used for yeast protein (Moon et al., 1978; El-Samragy et al., 1988; Champagne et al., 1990, Ganesh et al., 1996). Of all the whey valorization process, (concentration, drying and constituent extraction), the production of yeast proteins is particularly interesting because of the increasing demand for lysine-rich proteins. Industrial production of yeasts from deproteinized whey has been successful for a long time and, therefore confirms the importance of this process of valorization (Lembke et al., 1975; Hernandez et al., 1978). However the utilization of ‘crude’ whey (i.e. whey that has not been deproteinized, diluted or sterilized) to produce yeasts has not been much developed until now, for this reason it is necessary to select a new yeast strain and its culture on crude whey have been carried out in the present study along with other treatments.
2.4 Objectives

Until recently, in India scanty report is available for the treatment of dairy effluents including whey for the production of citric acid, lactic acid and ethanol. Therefore an attempt has been made for realization of economic potential of whey and dairy processed water, which have been considered as waste, for the recovery of valuable products such as citric acid, lactic acid and ethanol using appropriate microorganisms.

With this background, the following objectives have been framed.

1. Production of citric acid using *Aspergillus niger* NCDC 55 from whey and dairy effluents.
2. Production of lactic acid using *Lactobacillus bulgaricus* NCDC 068 from whey and dairy effluents.
3. Production of ethanol using yeast cultures namely *Saccharomyces cerevisiae* MTCC 170 and *Kluyveromyces marxianus* MTCC 1388 from whey.
4. Production of microbial biomass (SCP) to satisfy the increasing needs of protein from whey and dairy effluents.