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. Cheese production industries worldwide generate more than 145 million tons of liquid whey per year. Generally, such dairy effluents may cause serious environmental pollution, if disposed of without any treatment. Hence, there is the need to treat this dairy effluent called whey by various cost effective processes. Since whey retains most of the nutrients of milk, the major component being lactose, it may serve as a promising substrate 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 thus help to protect the environment from serious damage.
1.1. Whey and its production

Whey is the liquid that remains after milk has been curdled and strained. It has a yellow/green color or sometimes even a bluish tinge, depending on the quality and type of milk used. It is a by-product of the manufacture of cheese or casein and has several commercial uses. Approximately 9 pounds of whey are produced for every 1 pound of cheese. Cheese may be produced through use of enzymes, such as rennet, that clot casein or addition of acid to lower the pH of the milk so that casein will precipitate (Wiley and Andrea, 2014). Some types of cheese use both methods to clot the casein. Sweet whey is manufactured during the making of rennet types of hard cheese like cheddar or Swiss cheese. Acid whey (also known as "sour whey") is a by-product produced during the making of acid types of dairy products such as cottage cheese or strained yogurt.

Whey drawn from curd that is clotted by rennet only will have a higher pH and is considered to be sweet whey. Whey drawn from curd that has formed through the use of acid (with or without added rennet) will have a lower pH and is referred to as acid whey.

1.1.1. Composition of whey

Whey represents 85%-95% of the milk volume and retains about 55% of the milk nutrients (Farizoglu et al., 2004). The composition of whey varies with the components in milk that is used for making cheese, the variety of cheese made, and the cheese-making process employed. The most abundant of these nutrients are lactose (45-50 g/ L), soluble proteins (6-8 g/ L), lipids (4-5 g/ L) and mineral salts (8%-10% of the dry extract).

![Figure 1.1 Distribution of milk components during cheese manufacture](Source: Smith, K. 2008. Dried Dairy Ingredients. Wisconsin Center for Dairy Research. p. 1-59)
Introduction

Distribution of milk components during manufacture of cheese has been shown in figure 1.1 and a comparative data of milk, acid whey and sweet whey composition has been shown in figure 1.2.

![Figure 1.2 Composition of milk, sweet and acid whey (Source: Smith, K. 2008. Dried Dairy Ingredients. Wisconsin Center for Dairy Research. p. 1-59)](image)

One of the major constituents of whey is lactose, a disaccharide composed of one glucose and one galactose molecule. The major whey protein, β-lactoglobulin, is remarkably stable to acids and proteolytic enzymes present in the stomach due to its very compact folding. The mineral salts are mainly NaCl and KCl (more than 50%), calcium salts (mainly phosphate) and others. Cheese whey also contains appreciable quantities of lactic and citric acid, non-protein nitrogen compounds (like urea and uric acid) and B group vitamins (Siso, 1996). Cheese whey also contains some heavy metals in low quantities such as Cd, Cr, Cu, Hg, Pb and Zn.

Cheese whey is characterized as a high polluting stream with high BOD and COD values in the range of 40,000–60,000 and 50,000–80,000 ppm, respectively (Saddoud et al., 2007). Lactose is mainly responsible for this high BOD and COD loading of whey. On the other hand, protein recovery reduces the COD of the whey by only about 12% (Farizoglu et al., 2004).

<table>
<thead>
<tr>
<th>Component</th>
<th>Milk</th>
<th>Sweet whey</th>
<th>Acid whey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total solids</td>
<td>12.5</td>
<td>6.5</td>
<td>6.5</td>
</tr>
<tr>
<td>Protein</td>
<td>3.5</td>
<td>0.8</td>
<td>0.7</td>
</tr>
<tr>
<td>Lactose</td>
<td>4.8</td>
<td>4.8</td>
<td>4.4</td>
</tr>
<tr>
<td>Ash</td>
<td>0.7</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Fat</td>
<td>3.5</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>—</td>
<td>0.1</td>
<td>0.5</td>
</tr>
<tr>
<td>Calcium</td>
<td>120</td>
<td>45</td>
<td>103</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>9</td>
<td>45</td>
<td>78</td>
</tr>
</tbody>
</table>
1.1.2. Whey products and their uses

The various products that can be produced from whey are concentrated or dry whey, demineralised whey, lactose (whey permeate), whey protein concentrate (WPC), whey protein isolate (WPI) and fermented whey products.

1.1.2.1. Concentrated/dry whey

Whey is concentrated and dried for several reasons such as to reduce cost for storage and transportation or to induce crystallization of lactose. For concentration and drying of whey, four different methods are used: conventional hot roller milk driers; heating until a concentrated liquid was obtained, cooling to solidification, and then extruding in a tunnel; two-stage steam heating; and a combination of spray drying and rotary drum drying (Gillies, 1974). Concentrated whey is whey where a portion of the water has been removed leaving all other constituents in the same relative proportions. Dry whey is fresh whey that has been pasteurized and contains all constituents, except water, in the same proportions as found in the original whey. The drying of whey may be seen as a continuation of the concentration of whey in order to produce a stable low moisture product for functional and nutritional end uses. This concentrated or dried whey can be used as human food, animal feed and for coatings.

1.1.2.2. Demineralized whey

Processes such as precipitation, ion exchange, electrodialysis and membrane filtration may be used to remove the minerals. The salts in whey have a significant effect on its taste and may hamper the use of whey in food products. In delactosed whey (mother liquor), lactose content is reduced to 50%, protein content is increased, and along with this the mineral content is also increased to 20% on total solids. This makes the taste of mother liquor more unfavourable for applications in human food, a problem that may be solved by desalting. Demineralized whey has applications in infant food.

1.1.2.3. Lactose (Whey permeate)

Milk sugar lactose can be purified from cheese whey or permeate by crystallization. Whey or permeate is concentrated until the solubility of lactose is exceeded and lactose crystals form. The crystals then are washed to remove impurities and dried. Lactose is a disaccharide, that is, a
Introduction

carbohydrate made up of two sugar molecules. The monosaccharides or sugar molecules that comprise lactose are glucose and galactose. The chemical name for lactose is 4-O-β-galactopyranosyl-D-glucopyranose.

However, the use of lactose in foods is restricted by its low solubility and intolerance. In the pharmaceutical industry, lactose is used as excipient for most tablet drugs because it is inert, non-hygroscopic, and available with high purity and having good binding properties (Fox, 2009).

Different commercial products derived by chemical transformation of lactose are made (e.g. galacto-oligosaccharides, lactulose lactitol lactosucrose, lactobionic acid, gluconic acid, lactosyl urea, lactosyl monolaurate and tagatose).

Hydrolized lactose solutions possess greater sweetening power than lactose and have application in both confectionary and ice-cream industries, replacing saccharose or starch syrup (Siso, 1996).

1.1.2.4. Whey protein concentrate (WPC) and whey protein isolates (WPI)

Whey is a rich source of a number of proteins. Nowadays whey ultrafiltration (UF) and diafiltration (DF) are standard operations in the dairy industry that allow protein recovery without significant loss of their functional properties and with a low salt content, making it suitable for human consumption (Pouliot, 2008).

The product whey protein concentrate (WPC) contains between 50 - 85% protein on a dry basis, while whey protein concentrates with protein contents on a dry basis greater than 90% are referred to as whey protein isolates (WPI) with very small amounts of lactose and fat. Whey proteins are high quality proteins with a protein efficiency ratio (PER) of 3.4, higher than casein (2.8) and similar to egg albumin (Siso, 1996). Whey proteins represent between 15 and 22% of the proteins in milk. The major protein fractions with defined molecular weights of whey are: immunoglobulins (Igs), mainly immunoglobulin-G (IgG), bovine serum albumin (BSA), α-lactalbumin (α-La), and β-lactoglobulin (β-Lg). Besides these, whey contains also numerous minor proteins, called low abundance proteins, such as lactoferrin (LF), lactoperoxidase (LP), proteose peptone (PP), osteopontin (OPN), lisozyme (LZ), among others; LF and LP are the
most abundant minor proteins (Santos et al., 2012). Major characteristics of whey proteins are shown in Table 1.1.

**Table 1.1 Characteristics of whey proteins**

<table>
<thead>
<tr>
<th>Proteins</th>
<th>Concentration (g/L)</th>
<th>MW, Da</th>
<th>Isoelectric point</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-lactoglobulin (β-Lg)</td>
<td>3.2</td>
<td>18277</td>
<td>5.4</td>
</tr>
<tr>
<td>α-lactalbumin (α-La)</td>
<td>1.2</td>
<td>14175</td>
<td>4.4</td>
</tr>
<tr>
<td>Bovine serum albumin (BSA)</td>
<td>0.4</td>
<td>66267</td>
<td>5.1</td>
</tr>
<tr>
<td>Immunoglobulin G</td>
<td>0.7</td>
<td>150000</td>
<td>5.8</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>0.1</td>
<td>80000</td>
<td>7.9</td>
</tr>
<tr>
<td>Lactoperoxidase</td>
<td>0.03</td>
<td>70000</td>
<td>9.6</td>
</tr>
<tr>
<td>Glycomacropeptides</td>
<td>1.2</td>
<td>67000</td>
<td>-</td>
</tr>
</tbody>
</table>

Whey proteins have a high nutritional value, due to the high content of essential amino acids, especially sulfur-containing ones. Moreover, whey proteins have functional properties (e.g., high solubility, water absorption, gelatinization, and emulsifying capacities) essential in food application. Whey proteins are globular molecules with a substantial content of α-helix motifs, in which the acidic/basic and hydrophobic/hydrophilic amino acids are distributed in a fairly balanced way along their polypeptide chains. Individual proteins are produced by ion exchange/chromatographic methods. Each whey protein has unique attributes for nutritional, biological, and food ingredient applications; otherwise, individual milk proteins exhibit better functionality than in their native protein mixtures (Huffman and Harper, 1999). Benefits of WPC and WPI in food applications include its high protein and amino acid content; low calorie, fat, and sodium content; lack of pathogens, toxic compounds, and antinutritional factors; good
emulsification capacity; compatibility with other ingredients; ready availability; and the perception that it is a “natural” product.

The enzymatic hydrolysis of whey proteins increases their solubility in water and modifies their functional properties (Gauthier et al., 1993). These hydrolyzates are being used as protein supplements for infant formula, athletes and bodybuilders. Whey proteins are also been recently used in the production of iron propionate, an antianaemic preparation.

1.1.2.5. Whey fermentation products

For many years, cheese whey was used in different bioconversions; for examples the microbial biomass production for animal feed supplement (Kosikowsky, 1979), biogas production using anaerobic methanogenic bacteria (Siso, 1996), bioethanol production by Kluyveromyces marxianus (Sansonetti et al., 2009a) or recombinant Saccharomyces cells (Guimarães et al., 2008), hydrolized lactose solution in sweeteners and dietary supplements production (Siso, 1996). Therefore, at present, it can be very interesting and promising to consider again the possibility to use cheese whey and, particularly, glycidic component because of new demands, as bioplastic synthesis (poly-hydroxyalkanoates -PHA- and polylactate acid -PLA-), antimicrobial peptides (bacteriocins), enzymes and esopolisaccharides (EPS).

1.2. Different membrane processes and their applications in dairy industry

Membrane filtration is a separation process which separates a liquid into two streams by means of a semi-permeable membrane. The two streams are referred to as retentate (portion that does not cross the membrane or is “retained” i.e. the larger molecules) and permeate (portion that crosses the membrane or “permeates” i.e. the smaller molecules). By using membranes with different pore sizes, it is possible to separate specific components of milk and whey. Depending on the application in question, the specified components are either concentrated or removed/reduced. Membrane filtration can basically be divided into 4 main technologies: Microfiltration (MF), Ultrafiltration (UF), Nanofiltration (NF) and Reverse Osmosis (RO).

1.2.1. Microfiltration

Microfiltration is a low pressure-driven (10 to 50 psig) membrane filtration process, which is based on a membrane with an open structure allowing dissolved components to pass while
most non-dissolved components are rejected by the membrane. In the dairy industry, microfiltration is widely used for bacteria reduction and fat removal in milk and whey as well as for protein and casein standardisation, especially of cheese milk. Another application for the technology is for removal of natural synthetic organic matter to reduce fouling potential. MF can be used as a pretreatment to RO or NF to reduce fouling potential.

1.2.2. Ultrafiltration

Ultrafiltration is a medium pressure-driven (30 to 100 psig) membrane filtration process. Ultrafiltration is based on a membrane with a medium-open structure allowing most dissolved components and some non-dissolved components to pass, while larger components are rejected by the membrane. In the dairy industry, ultrafiltration is used for a wide range of applications such as protein standardisation of cheese milk, powders, fresh cheese production, protein concentration and decalcification of permeates as well as lactose reduction of milk. During ultrafiltration of whey, the retentate consists of protein, fat, and insoluble salts whereas lactose, soluble minerals, and much of the water are in the permeate.

1.2.3. Nanofiltration

Nanofiltration is a medium to high pressure-driven (150 to 600 psig) membrane filtration process. Generally speaking, nanofiltration is another type of reverse osmosis where the membrane has a slightly more open structure allowing monovalent ions to pass through the membrane. Divalent ions are to a large extent rejected by the membrane. In the dairy industry, nanofiltration is mainly used for special applications such as partial demineralisation of whey, lactose-free milk or volume reduction of whey. Pushing water through the smaller membrane pores of NF requires a higher operation pressure than either MF or UF.

1.2.4. Reverse osmosis

Reverse Osmosis is a high pressure-driven membrane filtration process which is based on a very dense membrane. In principle, only water passes through the membrane layer. In the dairy industry, reverse osmosis is normally used for concentration or volume reduction of milk and whey, milk solids recovery and water reclamation.
Introduction

For highly concentrating lactose from milk and whey, NF and RO are very useful processes. Application of membrane processes for separation of different components of milk/whey has been shown in figure 1.3.

**Figure 1.3** Membrane processes applied for separation of different whey particles (Source: Wisconsin Center for Dairy Research, www.cdr.wisc.edu)

There are mainly two types of filtration processes: Dead-end and cross-flow (or tangential flow). In dead-end filtration, the feed flow is perpendicular to the membrane whereas in cross-
flow, the feed flow is parallel to the membrane. Cross-flow is favoured over dead-end system since it causes less deposition on membrane surface and has additional benefit of sweeping any particles that are adsorbed on to the surface.

1.2.5. Modes of membrane operation

Different modes of membrane operation have been shown in the table 1.2

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch Processing</td>
<td>Retentate stream is recycled to achieve desired final product concentration. Advantages are lower capital costs, less complex system, higher average flux, flexibility and disadvantage is longer residence time</td>
<td>![Schematic presentation]</td>
</tr>
<tr>
<td>Continuous Processing</td>
<td>Used for processing large volumes and an important advantage is</td>
<td></td>
</tr>
</tbody>
</table>

9
decreased residence time

**Single pass**

- Feed passes through system once (no recycle)
- Shortest residence time of any processing mode
- Needs large membrane area

**Recirculation (feed and bleed)**

- Retentate removed at same rate fresh feed is added
- Lower flux because of constant high solids

**Diafiltration (DF)**

- Used to increase percentage concentration of a retained component
- Water added to retentate during UF/ MF to remove additional permeate species
- Process of adding water to the retentate to reduce the concentration of permeable solids in the retentate. Diafiltration water is added at a rate that equals the rate of removal of permeate in continuous diafiltration.
Introduction

- Whey/milk reduces amount of lactose in the retentate (thereby increasing the percentage of protein in the retentate).
- Diafiltration process may be continuous or discontinuous.

Process of adding water to the retentate to reduce the concentration of permeable solids in the retentate. The volume of retentate is reduced through filtration and water then added to dilute the retentate to a certain volume. The retentate then is processed again by filtration. The process of repetitive dilution followed by filtration is known as discontinuous diafiltration.
1.3. Ethanol

1.3.1. Properties and applications of ethanol

As demand for the limited global supply of non-renewable energy resources increases, the price of oil and natural gas will keep on increasing. As a result, production of ethanol from renewable carbohydrate raw materials for use as an alternative liquid fuel has been a possible attractive solution. The use of ethanol as a fuel for internal combustion engines, either alone or in combination with other fuels, has been given much attention mostly because of its possible environmental and long-term economical advantages over fossil fuel.

Ethanol is a flammable, colorless liquid with a special odor. Ethanol contains a hydroxyl group, -OH, bonding to a carbon atom (CH₃CH₂OH). It’s boiling and melting points are 78.5°C and -114.1°C respectively and has a density of 0.789 g ml⁻¹ at 20°C (Ethanol, http://en.wikipedia.org/wiki/Ethanol).

Ethanol fuel is an alternative to gasoline. It can be combined with gasoline in any concentration. Anhydrous ethanol, that is, ethanol without water, can be blended with gasoline in varying quantities to reduce the consumption of petroleum fuels, as well as to reduce air pollution. In the US, tolerance of ethanol depends on the individual vehicle. In Brazil, ethanol-powered and flexible-fuel vehicles are capable of running on hydrated ethanol, an azeotrope of ethanol and water. In addition, flexible-fuel vehicles can run on any mixture of hydrated ethanol and gasoline, as long as there is at least 20% ethanol. Ethanol can also be used to power fuel cells and to produce biodiesel. Ethanol as a much cleaner fuel has major advantages over gasoline. Ethanol is a renewable and biodegradable energy source with less greenhouse effect as compared to gasoline. Ethanol blends contain more oxygen resulting cleaner burning in engines and help to operate with optimal performance. Ethanol blends reduce hydrocarbon, nitrogen oxide (up to %20 with high level ethanol blends), carbon dioxide (100% on a full life cycle basis), volatile organic carbon compound (with high level ethanol blends 30%) emissions affecting on depletion of ozone layer. Sulphur dioxide, particulate matter (PM), cancer-causing benzene and butadiene (more than 50%) emissions are reduced by using ethanol blends (Hansen et al., 2005).
Introduction

Ethanol is widely used for sanitizing, cleaning and as a solvent. Also it's an additive of perfumes, paints, spirits, foodstuffs, antiseptics and fuels. Ethanol is also vital for the chemicals, pharmaceuticals, disinfectants, adhesives, cosmetics, detergents, explosives, inks, hand cream, plastics and textile industries. Ethanol is used directly as fuel or as an octane-enhancing gasoline additive. Ethanol as a much cleaner fuel has major advantages over gasoline. Ethanol is a renewable and biodegradable energy source with less greenhouse effect as compared to gasoline. Thus ethanol has tremendous applications in chemical, pharmaceutical and food industries in the form of raw material, solvent and fuel. The annual production of industrial ethanol is about four million tonnes, 80% of which is produced by fermentation (Zafar and Owais, 2006). Ethanol is obtained by aerobic/anaerobic fermentation of sugars by an appropriate microorganism. A number of different substrates as well as several microorganisms have been tested. One substrate that is very promising is lactose, which is the main sugar in milk and represents an enormous underutilized waste product from all the different kinds of cheese whey produced by the dairy industry. Lactose can, under proper operating conditions, be either hydrolyzed and fermented or directly fermented giving ethanol as the main product. The commercial enzymes used for lactose hydrolysis are β-galactosidases of diverse origins. Yeast and fungal enzymes have the greatest commercial interest.

1.3.2. Fermentation processes for ethanol production

Alcoholic fermentation, also referred to as ethanol fermentation, is a biological process in which organic molecules such as sugar are converted into cellular energy and thereby produce ethanol and carbon dioxide as metabolic waste products. Because yeasts perform this conversion in the absence of oxygen, alcoholic fermentation is considered an anaerobic process. Yeast produces ethanol when it respires anaerobically and ultimately the ethanol will kill the yeast. Fermentation occurs under special conditions requiring specific pH, oxidation-reduction potential (ORP), temperature, dissolved oxygen and nutrients, which need to be closely monitored.

1.3.2.1 Microbial growth phases during fermentation process

As the cells in batch fermentation grow, they follow a particular growth curve (Figure 1.4). The growth curve contains four distinct regions known as phases. They are as follows:
Introduction

- **Lag phase**: When a particular microorganism is introduced into a selected growth medium, the medium is inoculated with the particular microorganism. Growth does not occur immediately, but takes a little while. This is the period of adaptation of the cells to their new environment, called the lag phase.

- **Exponential Phase/logarithmic growth phase**: It is the second major phase of microbial growth in a batch ferment fermentation process where the cells have adjusted to their new environment. The cells are dividing at a constant rate resulting in an exponential increase in the number of cells present.

- **Stationary phase**: The third major phase of microbial growth is the stationary phase. It occurs when the number of cells dividing and dying is in equilibrium and can due to depletion of one or more essential growth nutrients, accumulation of toxic growth associated by by-products or stress associated with the induction of a recombinant gene.

- **Death/decline phase**: It is the fourth major phase of microbial growth. The rate of cells dying is greater than the rate of cells dividing.

*Figure 1.4 Microbial growth curve (Source: Shuler and Kargi, 2002, Bioprocess Engineering Basic Concepts, 2nd ed., Prentice Hall, Upper Saddle River, NJ)*
1.3.2.2 Lactose to ethanol process

The starting compound in the fermentation pathway is pyruvate, which is usually produced from sugar substrates, such as glucose and lactose through the glycolysis pathway. In alcoholic fermentation, pyruvate is first transformed in acetaldehyde by pyruvate decarboxylase, with the production of a carbon dioxide molecule and then into ethanol by the enzyme alcohol dehydrogenase (Figure 1.5). From a molecule of glucose, 2 molecules of ATP, the “molecular unit” of energy inside the cells, are produced.

\[ C_6H_{12}O_6 \text{ (glucose)} \rightarrow 2 \text{CH}_3\text{CH}_2\text{OH} \text{ (ethanol)} + 2 \text{CO}_2 + 2 \text{ATP} \]

Lactose transformation into ethanol is a process that involves both glycolisis and alcoholic fermentation. Since glycolysis starting point is a molecule of glucose, lactose molecules have to be firstly broken into the two monosaccharide units, glucose and galactose. The latter sugar is then converted into glucose by the action of three different enzymes, known as the Leloir pathway. The two molecules of glucose are then oxidized in the glycolysis pathway, in which we have the production of a total of 4 ATP molecules and 4 molecules of pyruvate per lactose molecule. The pyruvate is then reduced into ethanol and carbon dioxide with a 1:1 stochiometry. The overall mechanism of lactose to ethanol transformation has been shown in figure 1.6.

![Figure 1.5 Glucose to ethanol pathway (Source: Cellular Metabolism And Fermentation, http://www2.estrellamountain.edu/faculty/farabee/BIOBK/BioBookGlyc.html)](image-url)
1.3.2.3 Modes of fermentation process

There are basically three modes of fermentation process: (1) Batch fermentation process. (2) Fed batch fermentation process and (3) Continuous fermentation process (Figure 1.7). The mode of operation is dictated by the type of product being produced.

- **Batch fermentation**: Batch culture is a closed culture system, which contains limited amount of nutrient medium. In the batch fermentation process, the entire medium is
removed from the fermentation vessel. The vessel is then thoroughly washed, cleaned and the new batch is started only thereafter. The bioreactor is initially loaded with fresh medium and inoculated with selected microorganism. During the growth period, no medium is added or removed. The biomass, nutrients and products concentrations change continuously in time. During the batch fermentation process, various physiological states of the microorganism are observed (lag, exponential, stationary, death phases).

The best advantage of batch process is the optimum levels of product recovery. The disadvantages are the wastage of unused nutrients, peaked input of labour and time lost between batches.

- **Fed batch fermentation**: When a batch culture is subsequently fed with fresh nutrient medium without removing the growing microbial culture, it is called fed-batch culture. In the absence of outlet flow, the volume in the bioreactor will increase linearly. The nutrients are added in several doses to ensure that there are not surplus nutrients in the fermenter at any time. Surplus nutrients may inhibit microorganism growth. By adding nutrients little by little, the reaction can proceed at a high production rate without getting overloaded. The best way to control the addition of the feed is monitoring the concentration of the nutrient itself in the fermenter or reactor vessel.

The main advantages of the fed batch fermenter are: the extension of the exponential growth phase and production of metabolites of interest, the production of high biomass and product concentrations and the reduced inhibition by the substrate.

- **Continuous fermentation**: Exponential growth in batch fermentation may be prolonged by adding of fresh medium to the vessel. In the continuous fermentation process, the added medium displaced an equal volume of culture from the vessel and the end products are continuously removed. If medium is fed continuously with such a culture at a suitable rate, a steady state is eventually achieved i.e., the formation of new biomass by the culture is balanced by the loss of cells from the vessel. Thus, under steady state conditions, the specific growth rate is controlled by the dilution rate, which is an experimental variable.
The steps involved in a typical fermentation process have been shown in figure 1.8.

**Figure 1.8** Steps in a typical fermentation process (Source: Díaz-Montaño, D.M., 2013)