Coagulation, flocculation and precipitation

Abstract

Fermentation is one of the most widely used reaction types to produce a wide range of chemical products. The downstream processing of a fermentation broth is a vital step to first isolate, and then purify, the wanted product. This report will focus on several chemical techniques within this; coagulation, flocculation and precipitation. Whilst coagulation and flocculation are involved in solid-liquid separation, precipitation is more often used later in purification, but the control of all three is highly important in achieving a success fermentation processing.

Background

Downstream processing is specifically for bioprocess purification. The main objective of the whole process is to separate, recover and purify products. Figure 1 presents the flowsheet of the basic steps of downstream processing. It contains first the removal of solids, then the primary purification stage, secondary purification and concentration, and final product isolation. The target of the solids removal stage for recovering the extracellular product is to separate and remove large particles with techniques such as centrifugation (P.F. Stanbury, 2017). Ultrafiltration, ion-exchange, liquid-liquid extraction and precipitation are some of techniques used within the primary isolation stage to fractionate or extract into fractions. After that, fractional precipitation or chromatographic techniques are used to purify and concentrate the product from any impurities. Afterwards, products are isolated by modification of flow-stream and drying may be needed as the final procedure (P.F. Stanbury, 2017).

This report focuses on three product separation techniques; coagulation, flocculation and precipitation. Mostly, coagulation and flocculation are applied in the solids removal stage, whilst precipitation is mainly in primary isolation stage.

Figure 1: Downstream purification process stages. Modified from (P.F. Stanbury, 2017)
Coagulation

Coagulation refers to the destabilization of a colloidal system by the action of additives (coagulants.) In the case of proteins, heat or mechanical stress cause a change in the charge of the system or the dispersed particles, enhancing their approximation and generation of micro-flocs. (Mazille & Dorothee, 2011)

The aggregation of particles, or generation of micro-flocs, is a fast process that takes only a few minutes. The process is driven by Van der Waal bonding forces and requires vigorous but short mixing and agitation in order to achieve its top efficiency. This guarantees that most of the particles or proteins are affected by the coagulant. (Mazille & Dorothee, 2011)

Two cases of coagulation can be distinguished; the chemical coagulation and the bio-coagulation (or denaturation) of proteins. The chemical coagulation usually refers to a change in the surface charge of the particles allowing particles to come closer. On the other hand, bio-coagulation refers to a change in the structure of, or the unfolding of, the protein followed by aggregation of proteins caused by coagulants, heat or vigorous mixing.

Chemical coagulation is widely used in waste water treatment. It allows the removal of colloidal particles and soluble substances which have length of 10 nm to 10 μm. (Koohestanian, et al., 2008). The most common coagulants for waste water treatment that neutralize the negative charge of colloids are: inorganics such as aluminum sulfate, aluminum chloride, polyamine chloride, ferric sulfate, ferrous sulfate and ferric chloride or organics like polyamines and PolyDADMAC (ChemTreat, 2016). Many different types of reactors are used, with the most common being: (EPA, 2002)

- Mechanical mixers in which rotating paddles, turbines or propellers do the agitation.
- Hydraulic mixers that use the turbulence of flow or baffles and diffusers in a vessel to mix the coagulant.
- Pump mixing using a pump to insert the coagulant in the most turbid location of the vessel or pipeline.

Coagulation of proteins is usually referred as denaturation. This process is widely used in downstream processing for removing proteins and tannins from beer and to create new products such as cheese or desserts like meringues.

Beer is produced from barley which contains proteins and tannins. Tannins give a bitter taste to beer and are usually attached to proteins, therefore they must be removed in order to achieve good taste and decrease the turbidity of the beer. When the beer broth is heated, the proteins unfold exposing their internal hydrophilic part, allowing the generation of new hydrogen bonds between proteins and so the generation of clogs. This process is called “Hot break” and is one of the main downstream processes for beer purification. (Braukaiser, 2009)
Coagulation is also used in cheese making. The casein milk proteins are unfolded due to the addition of lime, lactic acid or bacteria (remit), before the casein coagulates and traps fat between the new caseins bonds, producing cheese.

Flocculation

In a lot of ways, flocculation is very similar to chemical coagulation. Like coagulation, the chemical basis is that particles, generally cells, are kept apart in a suspension via electrostatic repulsion, due to negative charges on their surface (Talib et al. 2016). Flocculation can be thought of as aggregating the micro-flocs generated into much larger flocs that then settle on the base of the vessel. Hence both are generally involved in the same stage of the downstream processing – the initial solid-liquid separation.

One key area for flocculation is within centrifugations, which are commonly performed within downstream processing to separate the solids from the liquid via density differences. The settling rate of the cells is increased greatly with diameter, hence meaning that the larger the aggregate, the faster and more well defined the separation of it from the liquid (P.F. Stanbury, 2017).

Following centrifugations, one or more filtrations are generally performed. Here comes into play the second useful aspect of flocculation – that the larger flakes mean that there are few particles capable of blocking the filter. The strength of them also results in a filter cake that is easier to remove. On top of this, some systems, such as in microbial biomass production, are not compatible with filter aids. The use of flocculation helps to overcome this and keep the process working well (P.F. Stanbury, 2017).

An additional use of flocculation in downstream processing is for example in the harvesting of microalgae to produce biofuel. Due to the low cost of the final product, centrifugation is not performed to separate, nor are simple sedimentations or screenings because of the very small size of cells. Pre-flocculation to concentrate the cells is therefore vital, and can even allow separation by an extremely low cost gravity sedimentation (Vandamme et al. 2013).

To overcome the electrostatic repulsion between particles, flocculation, or clarification, agents often are required to be added. Generally, like for coagulants, ionic compounds such as aluminium sulfate and ferric chloride are regularly used to induce flocculation (Heitner 2000). Additionally, the cells may not first want to aggregate due to their varying shapes meaning that fitting together is
sterically difficult. Therefore, high molecular weight polymer bridges, particularly cationic ones, are also used extensively (P.F. Stanbury, 2017).

Within downstream processing, an example of used flocculation agents are chitosan and synthetic cationic polyacrylamide within the recovery of 1,3-propanediol from fermentation broth (Hao et al. 2006; Xiu & Zeng 2008). This is a different use for flocculation, being within the recovery and purification from cell debris, instead of the initial solid-liquid separation. Generally, the same compounds used as the coagulants also work for the flocculation in the solid-liquid separation stage.

The occurrence of flocculation is heavily dependent on several factors. These include the nature of the cells (or other separated component), as well as the medium itself, for example its own ionic character. Changing many different properties also influences flocculation – such as raising temperatures increasing the rate of it or a changing pH altering the ionic character of the cell surfaces and medium.

A different case of the use of flocculation is in the brewing of beer (Gassara et al. 2015). Here, flocculation of the yeast cells occurs naturally anyway within the fermentation broth, thus allowing the cells to be easily separated. The control of this is highly important; if it happens too early then the taste can be reduced, whilst if it doesn’t happen then yeasty deposits are left in the final beer product. The separated cells can also then be used in further fermentations, reducing costs.

The main additional use of flocculation is waste-water management; removing small particles contaminants, as well as pathogens (Ayekoe et al. 2016). It can also be used within the beneficiation of ores to remove inorganic particles from the waste streams, or also within the Bayer process for the inorganic residue from bauxite digestion. There are also many other cases where it is used. In these processes, other agents can be used, such as calcium hydroxide (lime) and even proteins like albumin (Heitner 2000).

Precipitation

Precipitation is a process that is used for product purification or recovery, especially in the primary purification. Figure 3 shows the basic mechanism of precipitation in a simple way (Diana Romanini, 2013). It can be attached to different stages for the product recovery process (P.F. Stanbury, 2017). Meanwhile, precipitation can also be used as a partial process for enrichment and concentration. Thus, the operating volume of the following process is lower than it was previously. Through the precipitation stage, products can be obtained directly or by further technique after a cell lysate (P.F. Stanbury, 2017). The mechanism of precipitation is that soluble compounds in solution become insoluble due to different chemical reaction parameters, e.g. pH. For instance, ethanol precipitation is an approach for protein precipitation by adding ethanol as an anti-solvent.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{precipitation.jpg}
\caption{The basic mechanism of precipitation (Diana Romanini, 2013)}
\end{figure}
To perform the precipitation process, there are several agents in use.

1. Agents that aim to change solution pH conditions to precipitate products (P.F. Stanbury, 2017). The principle of this agents is that pH is changed to reach the compound isoelectric point, at which point no overall charge is present in the solution and solubility decreased.

2. Salts such as sodium sulfate are one agent in protein product recovery and fractionation, which is called salting-out also. (P.F. Stanbury, 2017) Salts added into solution bond stronger with water than protein, leading to protein precipitation (Michael L. Shuler, 2002). There is an equation which explains the relationship between solubility of proteins in solution and ionic strength, as listed below:

\[
\log \frac{S}{S_0} = -K's(I)
\]

where solubility (S, g/l) works as function of solution ionic strength (I). \(S_0\) is the protein solubility when I=0. \(K's\) is the salting-out constant value, which depends on temperature and pH of solution (Michael L. Shuler, 2002). A plot of this is shown in Figure 4. The basic mechanism of precipitation (Diana Romanini, 2013) illustrates the relationship of ion concentration and protein solubility. The equation below explains the connection of ionic molar concentration and ionic charge with ionic strength, where \(C_i\) is the ionic molar concentration and \(Z_i\) is ionic charge: (Michael L. Shuler, 2002)

\[
I = 12C_iZ_i^2
\]

\[\text{Figure 4 Effect of inorganic salts on solubility of a typical protein (Michael L. Shuler, 2002)}\]

Protein solubility is decreasing logarithmically along with ionic strength.

3. An organic solvent is added into a solution, resulting in precipitation (P.F. Stanbury, 2017). This method can precipitate proteins also. The principle of this method is to precipitate proteins by reducing the dielectric constant of the solution (Michael L. Shuler, 2002). One application of using organic solvents to precipitate protein is that of an active enzyme—pectinase precipitation. Chilled acetone or ethanol is used as the organic addition (S.A. Singh, 1999).

4. Polymers are also a possible precipitation agent (P.F. Stanbury, 2017). Non-ionic polymers such as polyethylene glycol (PEG) are normally used and those polymers work as organic solvents.
5. In addition to cell aggregation, polyelectrolytes can also be applied in compounds precipitation (P.F. Stanbury, 2017).

6. Triazine dyes, which are called protein binding dyes, bind to some categories of proteins and perform the precipitation process (P.F. Stanbury, 2017).

7. Affinity precipitants are the most studied and utilized precipitation agents nowadays, since these agents can bind and precipitate proteins selectively (P.F. Stanbury, 2017).

8. Temperature is not actually an agent, but it can lead to selective precipitation phenomenon in the purification step for various thermostable products and in the deactivation of cell proteases (Michelle Y.T. Nga, 2006).

Conclusion

Coagulation, flocculation and precipitation are three processes involved in the purification and separation of products from a fermentation broth. Coagulation and flocculation are both used within the solid-liquid separation stage of the downstream processing, with coagulation being the faster initial process. The stability of the colloidal suspensions is broken by coagulation, forming micro-flogs, before flocculation helps increase the size of the grouped particles to aid sedimentation.

Precipitation is more generally used within purification and concentration, later in the process. It can also be within the solid-liquid separation however if the products are dissolved in solution.

The use and control of all three techniques is important for efficient downstream processing.

References


P.F. Stanbury, A. W. S. H., 2017. The recovery and purification of fermentation products. 出处: F.


