Aalto-yliopisto
Kemian tekniikan korkeakoulu

CHEM-E3140 Bioprocess Technology II

Cell disruption methods

Group 1

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Abstract

Many biotechnologically produced compounds are intracellular and must be released from cells before recovery. The efficient recovery of products requires cell disruption, which can be achieved by using different methods and technologies, either mechanical or non-mechanical methods. The chosen technology depends on the product, cell type and scale. The cell disruption methods which are commonly used include the bead mill, sonication and French press. Other possible methods are the utilization of enzymes, detergents and osmotic shock. However, many of these techniques are viable only at laboratory scale due to increased consumption of energy, chemicals and water. Some of the techniques generate excess heat or the products are not stable in the given conditions. Other aspects that should be taken into consideration, in the design of the cell disruption process, are safety, health and environmental issues. In this report we are reviewing the most important cell lysis technologies and their advantages and disadvantages from a bioprocessing perspective.

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1. Introduction

Cell disruption is an essential part of biotechnology and the downstream processes related to the manufacturing of biological products. The disruption of cells is necessary for the extraction and retrieval of the desired products, as cell disruption significantly enhances the recovery of biological products. Cell disruption cannot be considered an isolated process, as it affects the physical properties of the cell slurry, thus indirectly influencing further downstream processes. Several types of cell disruption methods exist, as biological products may be extracellular, intracellular or periplasmic. Cell disruption methods can be categorised into mechanical methods and non-mechanical methods (Figure 1). Mechanical methods are divided into solid shear methods and liquid shear methods. Non-mechanical methods can be divided into physical methods, chemical methods and enzymatic methods. This report will discuss some methods from each category, as examples of the varying disruption methods available.

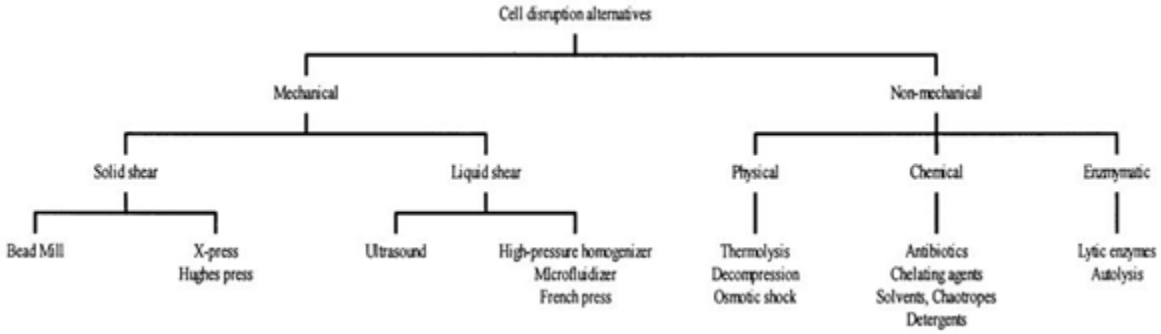

Figure 1. Methods of microbial cell disruption (Geciova J., 2002)

Different cells have different structures; hence they require different methods for disruption. Cell walls act as additional disruption deterrents, with yeast cells being particularly difficult to disrupt, as the cell wall limits the solvents access to the desired products. Other types of cells requiring disruption are bacterial cells, moulds, plant cells, mammalian cells and ground tissue. Bacterial cells may have different disruption methods, depending on whether they are gram positive or gram negative, as the amount of peptidoglycan and the presence of an envelope affect the overall process. Mammalian cells are the easiest to disrupt as they lack a cell wall, unlike plant cells, which are more difficult to disrupt.

The drying of the cell mass enhances disruption methods and may help bring down the costs. In some cases, more than one disruption method may be necessary to achieve full product recovery. Factors that influence the selection of disruption method include the susceptibility of the cells to disruption, product stability, the ease of extraction from the cell debris, the speed of the method and the cost of the method. Mechanical methods produce heat during the process, so additional cooling systems are required when using mechanical cell disruption methods.

Before cell disruption can take place, the cells must be separated from the culture medium. Secreted extracellular components need to be decreased and unutilized media components also need to be reduced. Ideally, the chosen cell disruption method is appropriate for the cells being disrupted, has a well understood mechanism, is sterilisable, containable and validated, with the possibility of automation. Other beneficial properties include a continuous and compact method, which is economical and efficient.

2. Mechanical physical methods

The main principle of the mechanical disruption methods, is that the cells are being subjected to high stress via pressure, abrasion with rapid agitation with beads, or ultrasound. (Geciova, 2002) Some methods of disruption are cavitation, shearing, impingement, or combination of those. Intensive cooling of the suspension after the treatment is required in order to remove the heat that was generated by the dissipation of the mechanical energy. Some high-pressure methods can only be applied in laboratory scale, such as French press and Hughes press. For industrial use, the bead mill and high-pressure homogenizer, are suitable. (APV, 2009)

2.1 Bead mill

Bead mills have been originally used in the paint industry, and have been adapted for cell disruption in both small scale and large scale production. (Geciova J., 2002). It is an efficient way of disrupting different microbial cells as different designs have been developed. The main principle requires a jacketed grinding chamber with a rotating shaft, running in its center (figure 2). Agitators are fitted with the shaft, and provide kinetic energy to the small beads that are present in the chamber. That makes the beads collide with each other. The choice of bead size and weight is greatly dependent on the type of cells. The diameter can affect the efficiency of cell disruption in relation of the location of the desired enzyme in the cell. The increased number of beads increases the degree of disruption, due to the increased bead-to-bead interaction. The increased number of beads, however, also affects the heating and power consumption. An optimal condition for bead load is considered between 80 and 85% of the free volume. The discs run at a speed of 1500-2250 rpm. Glass beads with a diameter greater than 0.5 mm are considered best for yeast cells, and diameter lesser than 0.5 mm is optimal for bacterial cells. (APV, 2009) The process variables are: agitator speed, proportion of the beads, beads size, cell suspension concentration, cell suspension flow rate, and agitator disc design. (Chisti Y., 1986)

Main issues related to bead mills, are the high temperature rises with increase of bead volume, poor scale-up, and most importantly, there is a high chance of contamination. (Harrison S., 1991)

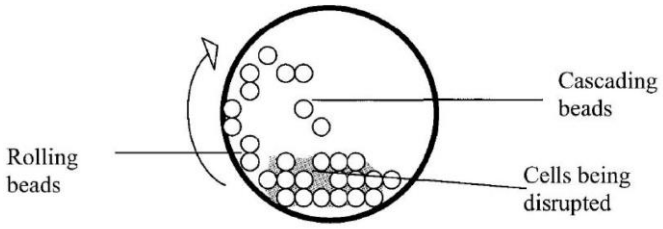

Figure 2. The basic principle of a bead mill

$$\ln\left(\frac{R_m}{R_m - R}\right) = kt$$

K is a function of the rate of agitation (1500-2250 rpm), cell concentration (30-60% wet solids), beads diameter (0.2-1.0 mm), and temperature. Where R - protein released (kg protein/kg biomass), Rm - max protein available, k -rate constant and is a function of temperature. (Chisti Y., 1986)

2.2 Ultrasound

Ultrasonic disruption is caused by ultrasonic vibrators that produce a high frequency sound with a wave density of about 20 kHz/s (figure 3). A transducer then converts the waves into mechanical oscillations through a titanium probe, which is immersed into the cell suspension. Such a method is used for both bacterial and fungal cell disruption. Bacterial cell can be disrupted in 30 to 60 sec, and yeast between 2 and 10min. This method is usually used in combination with a chemical method (mostly lysis). (Harrison S., 1991)

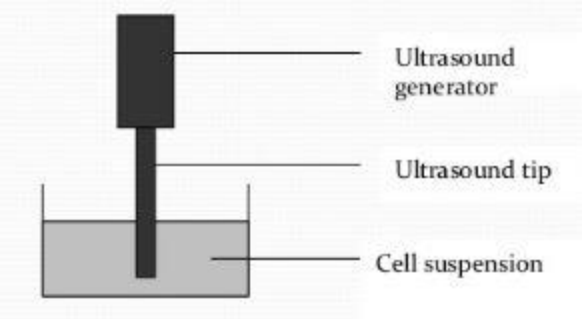

Figure 3. Schematic representation of ultrasonic disruption

Sonication can be very effective in small scale work; however, upscaling is very poor. It has high energy requirements, as well as high health and safety issues, due to noise. It is not continuous. (Chisti Y., 1986)

2.3 French press and high pressure homogeniser

In a French press, or high pressure homogenization, the cell suspension is drawn through a valve into a pump cylinder (figure 4). Then it is forced under pressure of up to 1500 bar, through a narrow annular gap and discharge valve, where the pressure drops to atmospheric. Cell disruption is achieved due to the sudden drop in pressure upon the discharge, causing the cells to explode. This method is one of the most widely known and used methods. It is mostly used for yeast cells. It is a vital unit in the dairy production industry, for milk homogenization. (Middleberg A., 1995) By operating the press at higher pressures, the number of passes of the slurry through it can be decreased in order to obtain the desired degree of disruption. However, the operating pressure may be limited due to the deactivation of certain heat-sensitive proteins, which may increase the number of passages required. Hence, protein release is dependent on several factors: temperature, intracellular location of the enzymes, number of passes, and operating pressure. The process is dependent on biomass concentration. (APV, 2009)

The French press is a small scale method, whereas the homogenizer can be applied to a large scale production. Homogenisers can vary in design and has a high amount of solids, up to 50% of the feed. Heat generation is also high – 1.5ºC/1000 psi. (Geciova J., 2002)

$$-\frac{dR}{dN} = k'R.$$

Correlation between protein release and number of passes.

Protein release (R) is first order with respect to the number of passes (N). The dependence of protein release on operating pressure (P 400-600 bar) can be expressed as a function of the pressure raised to an exponent. (Middleberg A., 1995)

$$\ln\left(\frac{R_m}{R_m - R}\right) = kNP^a$$

The results can be combined in the correlation, where R is protein released (kg protein/kg biomass), Rm is max protein available, k is rate constant and is a function of temperature, a is the pressure exponent. (Middleberg A., 1995)

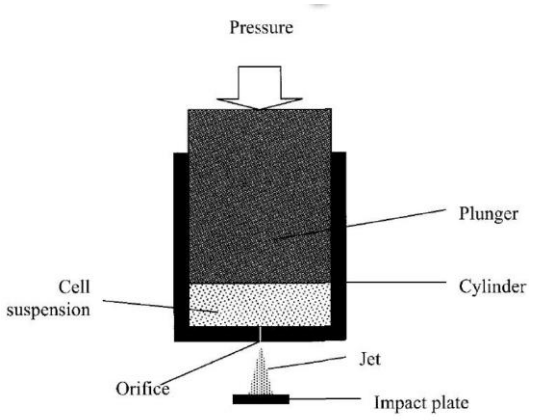

Figure 4. Schematic representation of the basic principle of a French press.

3. Non-mechanical physical methods

3.1 Thermolysis

Thermolysis has shown potential in becoming more common in large scale production. Periplasmic proteins in G(-) bacteria are released when the cells are heated up to 50ºC. Cytoplasmic proteins can be released from *E.coli* within 10min at 90 ºC. Improved protein release has been obtained after short high temperature shocks, than when at longer temperature exposures at lower values. Unfortunately, the results are highly unreliable, as the protein solubility changes with temperature fluctuations. (Middleberg A., 1995)

Freezing and thawing of a cell slurry can cause the cells to burst due to the formation and melting of ice crystals. Gradual freezing, leading to the formation of larger crystals, can cause an extensive damage to the cell. By combining this method with cell grinding, this technique has shown great results. However, it is very costly, and restricted to small-scale laboratories. Some reports have also shown loss of enzyme activity. (Harrison S., 1991)

3.2 Decompression

During explosive decompression, the cell suspension is mixed with pressurized subcritical gas for a specified time, depending on the cell type. The gas enters the cell and expends on release, causing the cell to burst. Decompression has been used in small scale laboratories for the disruption of *E.coli.* The technique has shown promising results with yeasts, where it has the advantage that supercritical CO_2 is able to extract off-flavours that are caused by lipid components. This technique is proving to be promising, being gentle on the cells, resulting in large debris that are easier to remove in order to obtain the desired product. Downsides, however, include its low efficiency and its high dependency on pressure release and time of contact between the cell suspension and the gas. Decompression chamber is shown in figure 5. (Harrison S., 1991)

Figure 5. Decompression chamber

3.3 Osmotic shock

The proper functionality of cell's processes usually requires strictly defined chemical conditions. This means e.g. that cell's internal pH or salt concentrations should not deviate significantly from the optimal values. The optimal conditions and ability to withstand suboptimal conditions are species specific. Cells have an ability to actively control the internal conditions but sudden and major changes in cell's surrounding environment might lead to extreme shock which results in cell death and disruption.

Osmotic shock is a technology which can be utilized in biotechnical applications to cause cell lysis. In this technology, cells are first exposed to either high or low salt concentration. Then the conditions are quickly changed to opposite conditions which leads to osmotic pressure and cell lysis (figure 6). The reason for that is that water quickly flows from low salt concentration conditions towards conditions with high salt concentration. Thus, if the cells are first exposed to high salt concentration solution, water flows into cell after exposure to low salt concentration. As a result, pressure in cell increases and cell explodes (Stanbury et al. 2016). Conversely, if cell are exposed to high salt concentration (~1 molar solution) after exposure to low concentration, water flows out of the cell which leads to cell disruption.

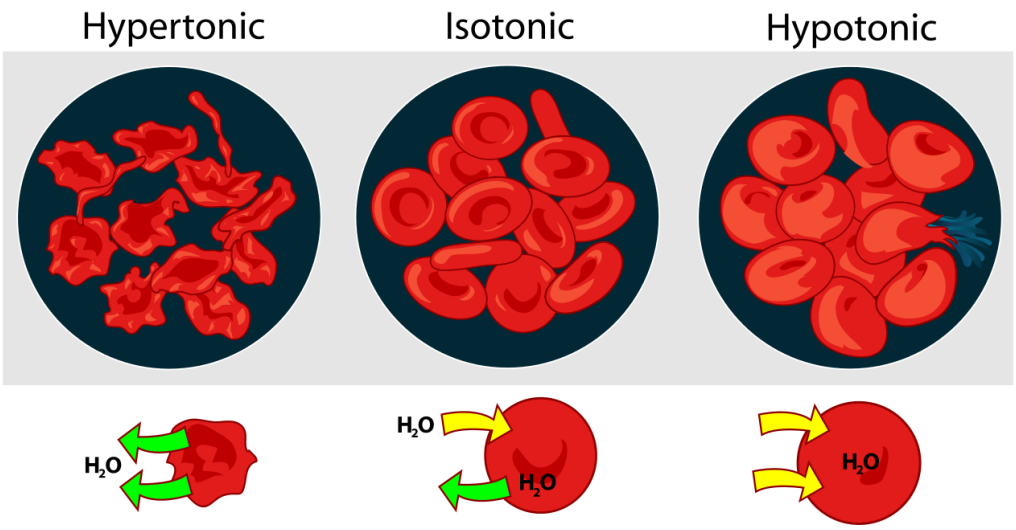

Figure 6. Osmotic shock. Exposure of cells to either high or low salt concentration causes cell disruption.

Osmotic shock is not commonly used method for cell disruption because of its low efficiency. The efficient disruption would commonly require for example enzymatic pre-treatment to weaken the cells. In addition, this technology requires addition of high amounts of salts and water usage is high. Also product may be diluted which increases downstream processing costs.

4. Non-mechanical chemical and enzymatic methods

In addition to physical and mechanical methods, several chemical methods for cell disruption exist. These methods rely on utilization of chemical substances or enzymes in disruption process. The mechanisms of actions are multiple, but the most widely used methods act by destroying the cell wall by enzymes, osmotic pressure, or by interfering or precipitating cell wall proteins. In addition, several disruption methods can be combined to achieve desired efficiency. The alternative strategies are reviewed in more detail below.

4.1 Detergents

Detergents that are used for disrupting cells are divided into anionic, cationic and non-ionic detergents. The common thing for all detergents is that they directly damage the cell wall or membrane, and this will lead to release of intracellular content (figure 7). One of the most commonly used anionic detergent is sodium dodecyl sulfate (SDS) which reorganizes the cell membrane by disturbing protein-protein interactions (Thermo Fisher Scientific | Detergents for Cell Lysis and Protein Extraction). Another commonly used compound for cell lysis is Triton X100, which is non-ionic detergent. Its mechanism of action is to solubilize membrane proteins (Harrison 2011). In addition to these chemical compounds, for example cationic detergent ethyl trimethyl ammonium bromide can also be used for cell disruption. It is speculated that it acts on cell membrane lipopolysaccharides and phospholipids (Stanbury et al. 2016).

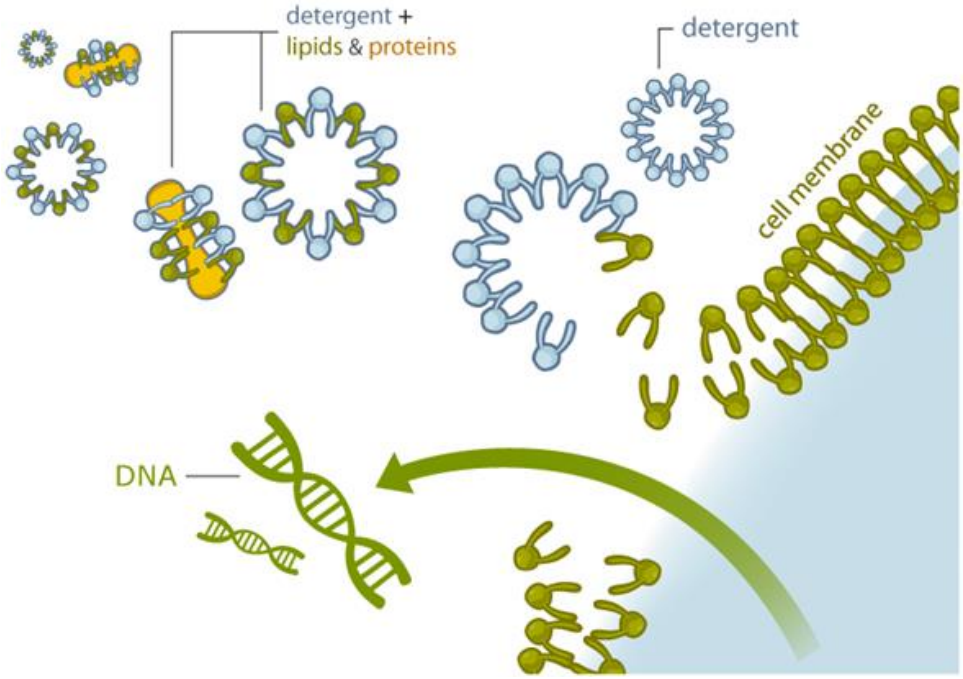

Figure 7. Cell disruption with detergents. Detergents interact with cell membrane compounds which will lead to disassembly of cell membrane.

The disadvantage of using detergents for cell lysis is that many proteins will be denatured in lysis process. Detergents may also disturb subsequent downstream processing steps. Thus additional purification step may be required after cell lysis, which limits their utilization in large scale

processes. However, detergents are commonly used for cell lysis in laboratory for example once DNA, RNA or proteins are extracted from cells.

4.2 Solvents

One additional method for chemical cell disruption is the utilization of chemical solvents. Solvents which can be used for cell lysis include for example some alcohols, dimethyl sulfoxide, methyl ethyl ketone or toluene (Stanbury et al. 2016). These solvents extract cell wall's lipid components which leads to release of intracellular components. This method can be used with wide range of production organisms but the problem can be that some proteins are denatured. However, the advantage is that by the choice of solvent, it might be possible to select the relished product. This method is not generally applied in large scale processes.

In addition to solvents, cell lysis can be achieved by hydrolysing the cell wall by alkali compound (pH 10.5-12-5). Disadvantage of this method is that chemical costs for neutralization of alkali are high. In addition, the product may not be stable in alkali conditions.

4.3 Enzymes

Another strategy to achieve cell lysis is to use digestive enzymes which will decompose the microbial cell wall (figure 8). Different cell types and strains have different kind of cell walls and membranes, and thus the used enzyme depends on microbe. For example, lysozyme is commonly used enzyme to digest cell wall of gram positive bacteria. Lysozyme hydrolyzes β-1-4-glucosidic bonds in the peptidoglycan (Crapisi et al. 1993). The cell wall of gram negative bacteria differs from the cell wall of gram positive bacteria so lysozyme is not very efficient in the case of gram negative cell wall.

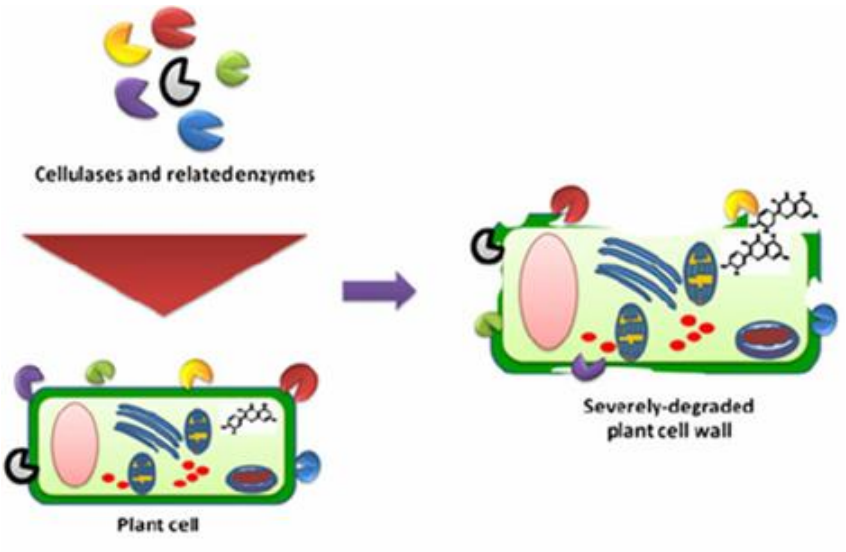

Figure 8. Enzymatic cell disruption concept. Enzymes degrade the cell wall components which will lead to release of intracellular compounds.

The cell wall of yeast and fungi differs significantly from the cell wall bacteria. One commonly used enzyme mixture for degradation of cell wall of yeast and fungi is Zymolyase. It has for example β-1,3 glucanase and β-1,3-glucan laminaripentao-hydrolase activities (Zymolyase | Yeast lytic enzyme). In addition, the enzymes that are commonly used for degradation of cell wall of yeast and fungi include different cellulases, pectinases, xylanases and chitinases.

The utilization of enzymes in cell lysis process is one of the gentlest methods (Harrison 2011). However, the enzyme's high price and limited availability limits their utilization in large scale processes. In addition, the added enzyme may complicate downstream processing (e.g. purification). However, these drawbacks could be minimized by immobilization of enzymes (Crapisi et al. 1993).

3. Conclusion

Certain mechanical methods are only viable at a laboratory scale, due to their cost-effectiveness and scale-up difficulty. Mechanical methods are well suited for industrial scale, and are the most popular disruption methods in use. High energy requirements and high pressure requirements are disadvantages of mechanical methods. Many variables ranging from required apparatus to optimal materials affect the efficacy of mechanical methods. Methods like ultrasound may offer significant energy savings when compared to solid shear mechanical methods. The difficulty of sterilization and cleaning procedures makes mechanical methods susceptible to contamination though. Mechanical methods, like sonication, have severe health and safety issues, resulting from noise.

Mechanical and physical methods have specific condition requirements, including pressure requirements and temperature requirements. These conditions need to be strictly monitored as they may affect protein release, protein solubility and cause undesirable effects in the products. Changing temperatures, used in thermolysis, may cause cells to burst or may damage cells with the formation of crystals. Temperature variation also affects the activity of enzymes and may alter three-dimensional structures, resulting in unforeseen consequences. In addition to the previously mentioned issues, a major problem with physical methods is their high cost.

Chemical methods are risky to use for the disruption of sensitive cells, as the used solvents and detergents can cause protein denaturation, damaging the final product. A significant issue related to chemical methods is the removal and recovery of the chemical disrupter, making chemical methods highly applicable at a laboratory scale. Chemical methods also have low efficacy, making them more expensive and less useful as disruption methods. The high consumption of solvents and water makes chemical methods environmentally unfriendly. On the contrary enzymatic methods are gentle, with fewer side effects, yet high costs make them impractical.

In conclusion, disruption methods are a necessary part of any industrial production line, offering many benefits for the enhancement of product recovery. Purity and sterility requirements have significant impact on the choice of the disrupter, as do the properties and the structure of the cell. The properties of the recovery product are also significant; as certain methods may damage or lower the quality of the final product. Economic factors and product quality are the deciding factors affecting process design. Further studies need to be conducted on different method options and technological development may lead to new, innovative techniques.

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