

CHAPTER IX. SINGLE-CELL PROTEIN AND ETHANOL PRODUCTION

A. Introduction

A1. EXPLANATION of the concept

Hydrolysis involves the use of waste materials as feedstock to produce single-cell protein and ethanol. Strictly speaking, two concepts are involved, the first of which is the production of a nutritious food for consumption by livestock or by humans. The second concept is the production of ethanol that can serve as a fuel in the production of energy. However, both concepts have a distinguishing characteristic -- namely, the use of a carbonaceous waste as the major source of carbon for the microorganisms that are involved.

The implementation of the first concept is a one-step process that consists of the use of waste as substrate in the culture of the single-cell microorganisms that collectively constitute an edible feedstuff that is highly nutritious for humans and livestock. Microorganisms that constitute the feedstuff are varieties or strains of the yeast, *Saccharomyces cerevisiae*, or of some other comparable species.

The implementation of the second concept is an integrated two-part process that consists first in the culture of microorganisms capable of fermenting sugars to ethanol, followed by harvesting the microorganisms and mixing them with sugar to produce ethanol. The microorganisms may be a particular yeast or bacterial species noted for its ethanol fermentation capability.

Although in the preceding paragraphs, reference is made to the concepts as one- or two-part processes, both must begin with a pre-treatment process in which the carbon in the waste is made available to the microorganisms. Pre-treatment is essential because, with rare exception, most of the carbon in waste is bound in highly complex molecules and, thus, is unavailable to all but a few highly specialised microorganisms. Fortunately, the bound carbon can be made accessible to the desired microorganisms through a process that disrupts the complex molecules -- namely, hydrolysis. Thus, hydrolysis is an essential step. Because of its importance, the greater part of this chapter is concentrated on hydrolysis and its various aspects.

A2. HISTORICAL development

The concept of expanding food and energy resources through the conversion of cellulose and complex carbohydrates in wastes into a single-cell protein feedstuff for humans and animal, or into ethanol, triggered an interest and subsequent research in the potential of hydrolysis in the 1940s [1]. These efforts became especially strong in the 1950s and persisted into the early 1960s. After a brief lag, interest and research revived in the late 1960s [2-4]. The interest originally was based on the fact that yeast constitutes a single-cell protein source that can serve as an important dietary supplement. Moreover, yeast also can produce ethanol through fermentation. Since the late 1970s, energy has begun to compete with and, indeed, surpass food in the hierarchy of popular concerns. The result is that now the interest is not so much in the conversion into a feedstuff as it is in the conversion into ethanol, a source of energy [5-7].

Sporadic interest in conversion of organic solid waste to ethanol continues as of this writing. In the early 2000s, the State of California (USA) analysed the production and economics of producing ethanol from several types of organic residues, including agricultural crop residues and

municipal solid waste [15]. The study found that large-scale production of ethanol from cellulosic biomass has not yet been demonstrated commercially.

A3. APPLICABILITY to developing countries

The principal source of cellulose and related complex carbohydrates in developing nations is agricultural residue; another source would be paper in municipal solid waste, although availability could be limited. This alternative to the management of some of the organic residues generated in economically developing countries may be too costly and sophisticated to be applicable to only but a few specific situations. Nevertheless, the concept is presented in this chapter for completeness.

B. Hydrolysis

B1. PRINCIPLES of hydrolysis

B1.1. Role of hydrolysis

As stated earlier, hydrolysis is an essential element in the waste to food and energy concepts, because it is through hydrolysis that the cellulose and carbohydrates in wastes are split into their constituent sugars. For example, the cellulose molecule may consist of more than 5,000 glucose units. The carbon in the glucose and other simple sugars is readily available to most microorganisms. Without the intervention of hydrolysis, the carbon in the cellulose and the complex carbohydrates are not available to microorganisms, particularly to those associated with single-cell protein or with ethanol fermentation. (The term “complex carbohydrates” will be referred to as “carbohydrates”.) It is through hydrolysis that the carbon in the glucose units that make up cellulose, and in the simple sugars that make up other carbohydrate molecules, are rendered available to yeasts and any other microorganisms that may be responsible for fermentation. (“Hydrolysis” often is termed “saccharification” when used in reference to the concept.)

B1.2. Factors

An especially influential factor in the hydrolysis of cellulosic waste is the ratio of crystalline to paracrystalline (amorphous) cellulose. The ratio has a major bearing on the practicality of using a particular waste as a feedstock to the process. The crystalline region of cellulose molecules is marked by a very closely packed structure and, hence, strong internal forces of attraction. On the other hand, the paracrystalline region is more randomly oriented. The high degree of order in the crystalline region renders the region more resistant than the amorphous (paracrystalline) region to hydrolysis. Therefore, the higher the ratio of crystalline to paracrystalline cellulose in a waste, the more difficult it is to hydrolyse the waste.

The surface-to-mass ratio of the waste particles exerts an important impact on hydrolysis, in that the smaller the particle, the more rapid is the physical or biological hydrolytic reaction. Another rate-related factor is the partial or complete masking of the cellulose molecules by lignin or some other resistant substance. The masking inhibits access of the hydrolytic mechanisms to the cellulose.

B1.3. Classification of methods of hydrolysis

The various methods of hydrolysis can be classified into three classes on the basis of the mechanism or process of splitting, i.e., disrupting cellulose and carbohydrate molecules. The classes are: chemical, physical-chemical, and enzymatic. In the literature, the terms “chemical

hydrolysis” and “acid hydrolysis” are often used synonymously. Even though physical disruption does not fully fit the classic definition of “hydrolysis”, in this chapter “acid hydrolysis” includes physical and physical-chemical disruption. Enzymatic hydrolysis is mostly biological in nature. Yet another class could be formed by integrating enzymatic hydrolysis with chemical hydrolysis.

Arguments abound regarding the relative superiority of one or the other particular class. Nevertheless, currently physical-chemical approaches are in the ascendancy in terms of attention. As will become apparent in the succeeding sections of this chapter, all classes depend upon complex technology, equipment, and highly qualified personnel. As mentioned previously, these complexities sharply limit the applicability of hydrolysis in developing countries.

B2. ACID hydrolysis

Basically, acid hydrolysis is a process in which the cellulosic fraction of a waste is suspended in an acidified aqueous medium that is maintained under pressure at an elevated temperature. It shares with other hydrolysis systems the general substrate and operational factors described in the preceding section. Other factors of particular significance to acid hydrolysis are liquids-to-solids ratio, acid concentration, and temperature. The rate of acid hydrolysis increases with increase in liquids-to-solids ratio.

Minimum particle size is determined by economic practicality, because energy and monetary costs of size reduction increase almost exponentially when the intended particle size is less than 5 cm. The permissible upper limit of the liquids-to-solids ratio also is determined by economic practicality. The consensus apparently places the upper ratio at 10 parts liquid to 1 part solids. Cost of acid, percentage of acid recovery, and rate of acceleration of corrosion establish the maximum permissible acid concentration. For sulphuric acid, the concentration would be about 0.5% in most situations. Yield of sugar is highest at the higher temperature levels and acid concentrations.

B2.1. System design

Acid hydrolysis can be carried out on either a batch basis or a continuous basis. Not unexpectedly, the batch approach is more appropriate for smaller operations, i.e., processing on the order of 120 Mg or less per day. In a batch operation, the entire hydrolysis process takes place in a single reactor. It proceeds in a sequence of steps: hydrolysis, flash vaporisation, neutralisation, and centrifugation. A 110 Mg/day operation would be based on two 70 Mg/day reactors operating in parallel, and involve the use of the same storage tanks and same centrifuge [3].

As in a batch system, the process steps in a continuous system are hydrolysis, flash vaporisation, neutralisation, and centrifugation. In a continuous system, however, a series of reactors is involved, the design of which differs from that of the single reactor in a batch operation. Each reactor in the series is followed by a screw press. The first reactor is designed to hydrolyse only the hemicellulose fraction of the waste. Sugars released by the hydrolysis are harvested by passing the reactor discharge through the screw press. Liquid discharge from the press contains the sugars. Sugars in the alphacellulosic fraction are in the solids (pulp) residue from the press. These sugars are obtained by re-acidifying the pulp and then passing it successively through a second and a third reactor. These two reactors are designed to hydrolyse the alphacellulose fraction of the waste.

The yield of sugars produced in acid hydrolysis is equal to about 35% to 45% of the incoming cellulose.

B2.2. Recent developments in technology

Most of the recent developments in hydrolysis seem to centre on the improvement and broadening of conventional chemical hydrolysis. This is accomplished by way of conditioning cellulosic and carbonaceous waste components, particularly fibres, such that sugar recovery efficiency is substantially improved. Thus, one approach involves exploding cellulosic fibres through the application of liquid anhydrous ammonia to biomass under pressure at 30° to 80°C for a few minutes, and then rapidly releasing the pressure. This blows individual fibres apart, thereby greatly increasing the surface area and accessibility of the cellulosic component. The ammonia is removed and the resulting material can be hydrolysed by weak acid or enzymes. Another innovation involves the use of a coaxial feeder and extruder to process biomass at 250°C and 3.2 MPa pressure. The exploded product is a moist fibre that is partially hydrolysed to permit easy fermentation. In a third innovation, concentrated acid (sulphuric, hydrochloric, or hydrofluoric acid) at 140° to 160°C results in approximately a 90% conversion of cellulose to sugar. (Compensating for the use of weaker acid by elevating the temperature to 180° to 200°C results in the production of undesirable byproducts, and sugar conversion drops to 50% to 60%.)

B3. ENZYMATIC hydrolysis

B3.1. Principles

In keeping with the descriptive term, “enzymatic”, hydrolysis of cellulosic and carbonaceous wastes is accomplished through the agency of the enzyme, cellulase. The process is essentially biological in that the hydrolytic enzyme is produced by microorganisms genetically capable of synthesising it. Cellulase is an enzyme that specifically splits cellulose molecules into their constitutive sugars (hexoses and pentoses).

B3.2. Types of cellulases and their relative effectiveness

B3.2.1. Constitutive vs. induced

The presence of cellulase is continuous in some microbes and is continuously synthesised. It is not continuously present in certain other microbes, and its synthesis must be triggered, i.e., induced by an external stimulus, usually the presence of cellulose or other reducing agent. Enzymes in the first class are termed “constitutive”, and those in the second class are termed “induced”.

B3.2.2. Extracellular vs. intracellular cellulase

The microbial origin of cellulase necessitates a two-stage process in enzymatic hydrolysis. In stage-1, cellulases are produced and harvested. In stage-2, the harvested enzymes are introduced into a waste. In the waste, the enzymes split, i.e., hydrolyse, cellulose and carbohydrate molecules into fermentable sugars, which are then harvested. Generally, the harvested sugars are used as the carbon source in ethanol fermentation.

Harvesting is facilitated by the fact that the cellulases involved in enzymatic hydrolysis are synthesised extracellularly by the cellulolytic microorganisms that produce them. Because they are extracellular, the cellulases are in the culture medium. If necessary, they can be extracted from the medium.

Some cellulolytic bacteria synthesise their enzymes intracellularly. For example, the cytophage have their enzymes system bound in the cell wall or membrane. With such an arrangement, hydrolysis depends upon the existence of a close contact between the cellulose and the cell wall

or membrane. Access to the bound enzyme would necessarily be by way of disrupting the individual microbes. Obtaining a cell-free enzyme extract would be an expensive operation.

B3.2.3. Enzymatic systems

The various cellulolytic enzyme (cellulase) systems can be divided into two groups -- namely, C_1 and C_X . The C_1 groups are effective on highly crystalline forms of cellulose (e.g., cotton fibre). They split crystalline cellulose into linear anhydroglucose. The anhydrous glucose chains are then split into soluble carbohydrates by C_X enzymes. This sequence has an important bearing on rate of hydrolysis of cellulosic waste because the first step in the hydrolysis must be the splitting of crystalline, i.e., resistant forms into simpler forms that are accessible by a wider array of enzymes. Thus, the higher the concentration of C_1 enzymes and hence the greater the concentration of microbes that synthesise them, the faster is the rate of hydrolysis. The fungus, *Trichoderma reesei*, has long been recognised as being an especially active synthesiser.

B3.3. Factors

The factors discussed in this section are specific to enzymatic hydrolysis. Chief among them are: 1) concentration of inducing agent (i.e., reducing sugar), 2) concentration of hydrolysis product (glucose), and 3) pre-treatment of waste.

B3.3.1. Concentration of inducing agent

The required concentration of cellulose is minute, i.e., about 0.5%. Activity usually is assured because cellulose generally is found in minute amounts with cellulose. However, it should be noted that cellulase production is repressed and activity is curtailed at cellulose concentrations greater than about 1.9%.

B3.3.2. Concentration of hydrolysis product

Cellulase formation also is inhibited and repressed in the presence of high concentrations of glucose. Inhibition resulting from a concentration of cellulose above the critical level can be counteracted by simultaneously imposing an inhibitory situation. It has been reported [7] that the concentration of enzymatic hydrolysis reducing sugars increases with the increase in concentration of solids in the substrate. The report states that using a 25% solids charge of compression-milled paper and a 10 IU/g enzyme-to-substrate ratio, it is possible to produce a reducing sugar syrup that has a concentration of 11%. Practical ethanol production is possible with such a syrup.

B3.3.3. Pre-treatment

Ideally, pre-treatment at a reasonable cost decreases cellulose crystallinity, disrupts the physical structure of lignin, and curtails cellulose polymerisation. The various proposed forms of pre-treatment may involve one or all of following three major steps: particle size reduction, heating, and perhaps, chemical treatment.

Most pre-treatment methods are based on the assumption that the cellulosic waste has been separated from the municipal solid waste stream and that all contaminants have been removed to the maximum extent permitted by economic feasibility.

Maintaining the temperature of the cellulose at 218°C during milling renders the inner surface of the cellulose more accessible and modifies the structure of the cellulose. Structure is modified

through the oxidation that takes place during heating. Cellulose can be heated either in a rotary kiln or preferably in an indirect-heat calciner dryer.

One of the several proposed forms of pre-treatment involves the induction of mild swelling and the partial solubilization of lignin through exposure to an alkali (e.g., NaOH) [4]. Succeeding the exposure to alkali is a period of air oxidation, which depolymerises the cellulose to a lower degree of crystallinity.

Another proposed form of pre-treatment calls for exposure to steam and compression milling (two-roll). Steaming is done by exposing moist solids to temperatures of 195° to 200°C for 15 to 30 min in a pressure vessel. The problem is that, although steaming increases the reactivity of agricultural residues and hardwoods, urban wastes lose 40% of their reactivity.

Another of the several proposed methods calls for compression or two-roll milling of newspaper for 6 to 10 min. Apparently, this innovation results in substantial increases in rates of enzymatic hydrolysis and yields of sugar. Another benefit attributed to compression milling is an increase in bulk density of the paper great enough to permit slurries of 20% to 30% to be used in hydrolysis [4]. The treatment is equally effective with all types of cellulosic materials. The additional expenditure of energy involved in compression milling reportedly is less than 0.60 kWh/kg newspaper.

B3.4. Technology

Advances in the technology of enzymatic hydrolysis of urban solid wastes took place in the 1970s. Since then, advance has been very slow and largely confined to refinements in equipment. Thus, the basic technology current in the 1970s is, with minor modifications, pertinent to present conditions. One of the more active centres of research into practical application of hydrolysis to municipal solid waste, i.e., the paper fraction, was at the Berkeley campus of the University of California (UC), and is fairly typical [5,6]. Hence, the process developed there is used to exemplify hydrolysis technology and the complexities associated with it.

The UC process incorporates the following five major steps: 1) feedstock preparation, 2) enzyme production, 3) the actual hydrolysis, 4) collection of the sugar (glucose) product, and 5) drying the residue. The entire flow pattern is diagrammed in Figure IX-1.

The detailed description begins with step-2, because step-1 (feedstock) was essentially covered in the preceding section, Pre-Treatment. The first of the two stages that constitute step-2, enzyme production, involves fungal growth followed by enzyme synthesis. Separation of the enzyme solution is the second stage. Fungal growth is accomplished by using standard industrial fermenters and a medium, and applying cultural conditions that favour the growth of the desired fungus (e.g., *Trichoderma*). Among these conditions are: 1) a medium that includes 0.3% superphosphate, 0.5% glucose, and the essential trace elements; and 2) a dilution rate of 0.2 per day. The medium should be sterilised. Sterilisation can be done by way of steam injection or heat exchange. Within the first stage, pure cellulose is introduced into the rapidly growing culture to induce enzyme synthesis. The introduction of pure cellulose initiates enzyme synthesis [8].

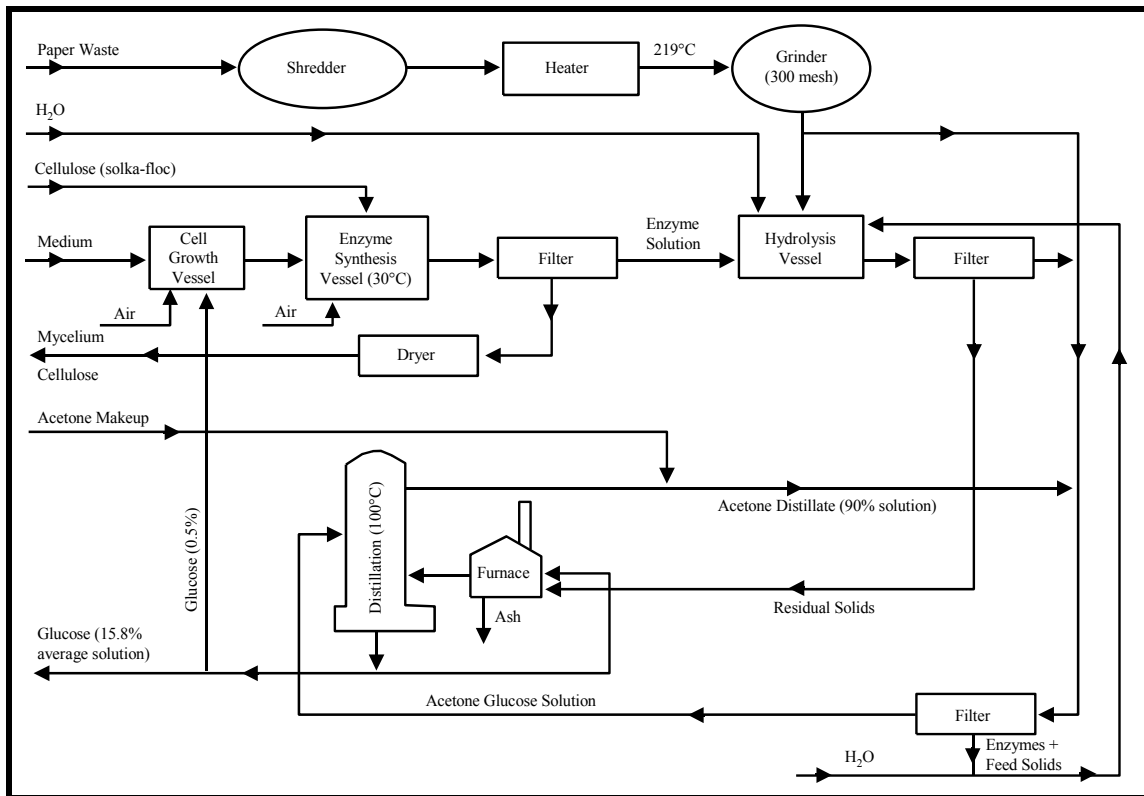


Figure IX-1. Diagram of the UC hydrolysis process

Separation of the enzyme is the second of the two main stages of step-2 of the UC process. The three methods available for separation are ultracentrifugation, precipitation by adding ammonium sulphate, and precipitation by adding acetone. Ultracentrifugation is very costly. Of the two precipitation methods, only the acetone method is practical because it is not always possible to separate the ammonium sulphate from the precipitate. A disadvantage of the acetone method is a 14% loss of enzymatic activity with each reuse of the enzyme solution. The precipitate (cellular material and unhydrolyzed cellulose) is removed from the enzyme solution by pressure filtration. The precipitate may then be dried and used as a cattle feedstuff [9,10].

B4. PRODUCTION system

Hydrolysis is the step in which the cellulose waste to be hydrolysed is introduced. Introduction is by way of suspending the waste in the enzyme solution produced in step-1. The enzymes in the solution catalyse the conversion of the cellulose into sugars.

B4.1. Specifications

The following specifications and conditions are taken from References 5 and 6. They are: 1) solids concentration of suspension, 11.5%; 2) retention time, 40 hr; and 3) suspension temperature, 50°C (this renders conditions relatively aseptic). Solids remaining in the suspension after hydrolysis is completed are removed by passing hydrolysed effluent through a pre-coated vacuum filter. The solids residue can be burned and the resulting heat energy used to generate steam and distil acetone from the effluent. Acetone from the distillation system (combined with a small makeup stream) is added to the aqueous enzyme-glucose solution in a volumetric ratio of 2:1. An almost complete precipitation of protein results. The precipitate may contain as much as 85% of the original enzyme activity. The enzyme solution is recovered by means of a pressure filter and returned to the hydrolysis units. Acetone is recovered by passing the filtrate through acetone distillation columns. Heat for the distillation columns comes from the combustion of the

residual solids. The distillate is 90% acetone. The glucose solution remaining after the distillation contains only a trace of acetone. About 1% of the glucose solution is returned to the first fermentation stage, and the remaining 99%, which is a 5% to 6% solution of reducing sugar, constitutes the final plant product.

B4.2. Capital equipment requirements

The capital equipment requirements for a hydrolysis plant have been reported for a 9.1 Mg/day processing capacity [5,6]. The requirements are summarised in Table IX-1. The plant would produce about 0.3 Mg/day of dry fungal mycelium-cellulose mixture and 8.3 Mg/day of glucose in the form of a 5.3% syrup. The distribution of the capital costs among the major processing stages of the system is itemised in Table IX-2. It should be noted that the lists presented in Tables IX-1 and IX-2 do not take into consideration the capital requirements for acetone recovery. Moreover, the percentage of the total capital investment for cellulose pre-treatment, especially for particle size reduction (milling), is unrealistically low.

C. Single-cell protein

Selection of an appropriate microorganism is essential to the success of any single-cell protein production undertaking. The microorganism must be one that is edible and can serve as a feedstock for humans and/or livestock. Of course, its culture must be technologically and economically feasible. To satisfy the second condition: 1) the organisms must grow rapidly and vigorously; 2) culture of the organism should involve the use of relatively simple growth units and inexpensive nutrient sources (e.g., commercial crop fertilisers); 3) ideally, the organism could be grown in open culture, or at least as an enrichment culture; and 4) because single-cell protein production is only marginally economically feasible, the least “permissible” condition is the need to culture the organism under sterile conditions, i.e., as a completely pure culture. However, competition with other organisms is eliminated in sterile culture and rapidity of growth is thereby increased. Moreover, contamination with possibly toxic organisms is avoided.

Most of the work on single-cell protein production has been focused on the yeast, *Candida utilis* (*Torula utilis*). The yeast meets most of the requirements named in the preceding paragraph. Not only is the yeast easily grown, it also is a good food and fodder yeast. Although sterility is necessary, purity of culture is not essential.

The high nucleic acid content of bacterial proteins renders them less desirable as feedstuff for man and animal. Additionally, some groups of bacteria are characterised by the possession of endotoxins. The endotoxins could be incorporated in the feedstuff product. There is also a possibility that certain bacterial feedstuffs can promote allergenic reactions in humans who handle or ingest them. Finally, the much smaller size of bacteria makes them more difficult to harvest than yeasts.

Table IX-1. Major equipment requirements for a 9.1 Mg/day hydrolysis plant

Item	Size/Capacity
Fermenters (4)	197 m ³
Hydrolysis vessel	263 m ³
Filter 1	2.23 m ² surface
Filter 2	8.36 m ² surface
Air filter	0.22 SCMS
Shredder	454 kg/hr
Heater	454 kg/hr
Grinder	817 kg/hr
Dryer 1	13.9 m ² surface
Dryer 2	118 m ² surface
Heat exchanger 1	7.9 m ²
Heat exchanger 2	4.7 m ²
Heat exchanger 3	18.6 m ²
Heat exchanger 4	2.8 m ²
Air compressor	0.234 SCMS
Medium supply tanks (2)	37.9 m ³
Fermenter motors (4)	10 hp
Hydrolysis unit motor	20 hp
Medium supply motors (2)	5 hp
Solids feeder	817 kg/hr
Screw conveyors (2)	454 kg/hr
Centrifugal pumps (3)	189 L/min

Source: Reference 5.

Table IX-2. Distribution of fixed capital costs for a 9.1 Mg/day hydrolysis plant

Item	(%)
1. Cellulose pre-treatment	11.1
Shredding	
Heating	
Grinding	
2. Enzyme production	52.6
Fermentation	
Air and medium sterilisation	
Medium supply system	
3. Cellulose hydrolysis	12.4
4. Cellulose recycle and product recovery	23.9
Filtration	
Drying	
Total	100

Source: Reference 5.

C1. INDIRECT vs. direct production

The relation of single-cell protein production to the reclamation of useful nutrient elements in waste is by way of the utilisation of sugars formed through hydrolyzation of cellulosic substances in municipal waste. However, a separate hydrolysis step may be bypassed by culturing the yeast directly on the cellulosic waste. For convenience, in this presentation, the two approaches are respectively designated by the terms “indirect” and “direct”.

C1.1. Indirect production

The production of *C. utilis* is an example of the indirect approach. The sequence of events in the production is diagrammed in Figure IX-2.

With respect to nutritional requirements, the sugars (glucose) satisfy the carbon needs. The other required essential nutritional elements are nitrogen, phosphorus, and potassium, which must come from an external source. Usually, nitrogen is added as an ammonium compound (e.g., ammonium sulphate); a phosphate is used for phosphorus; and a potassium sulphate or hydroxide compound for potassium. Generally, it is not necessary to add the essential trace elements.

Principal cultural conditions are a temperature at 20° to 35°C; and O₂, about 1.02 kg/kg cell mass-produced. The necessarily aerobic conditions are attained by continuously agitating the culture. The volume of air applied to meet the oxygen demand would be a rate of about 120 millimoles O₂ absorbed per L-hr (3.84 g/L-hr). The yield to be expected at such a rate is 3.66 g yeast per L-hr.

Under proper cultural conditions, the yield of the cell mass should be from 45% to 55% of the sugar consumed [3]. The production rate under continuous conditions depends upon a combination of cell mass and hydraulic detention time (culture volume/volume feed medium/day). Maximum cell concentration is a function of the hydrolysate sugar concentration multiplied by the sugar conversion efficiency of the yeast.

C1.2. Direct production

Direct production differs from indirect production in that organisms are cultured upon unhydrolyzed wastes. Indirect production involves two discrete steps (hydrolysis and cell production); whereas in direct production, the two steps are neither spatially nor always temporally discrete. Although of necessity, the steps are sequential (hydrolysis must precede utilisation for cellular growth; both may involve the same microorganism). In other words, an organism can degrade a cellulosic molecule and utilize the constituent sugars to synthesise cellular mass. All sequences are not occurring simultaneously and, collectively, they constitute a single unit process. Therefore, at least some of the microorganisms must be cellulolytic, i.e., capable of breaking down cellulose molecules. Preferably, most should be cellulolytic. A disadvantage is the inability to use submerged culture in the absence of special adaptations.

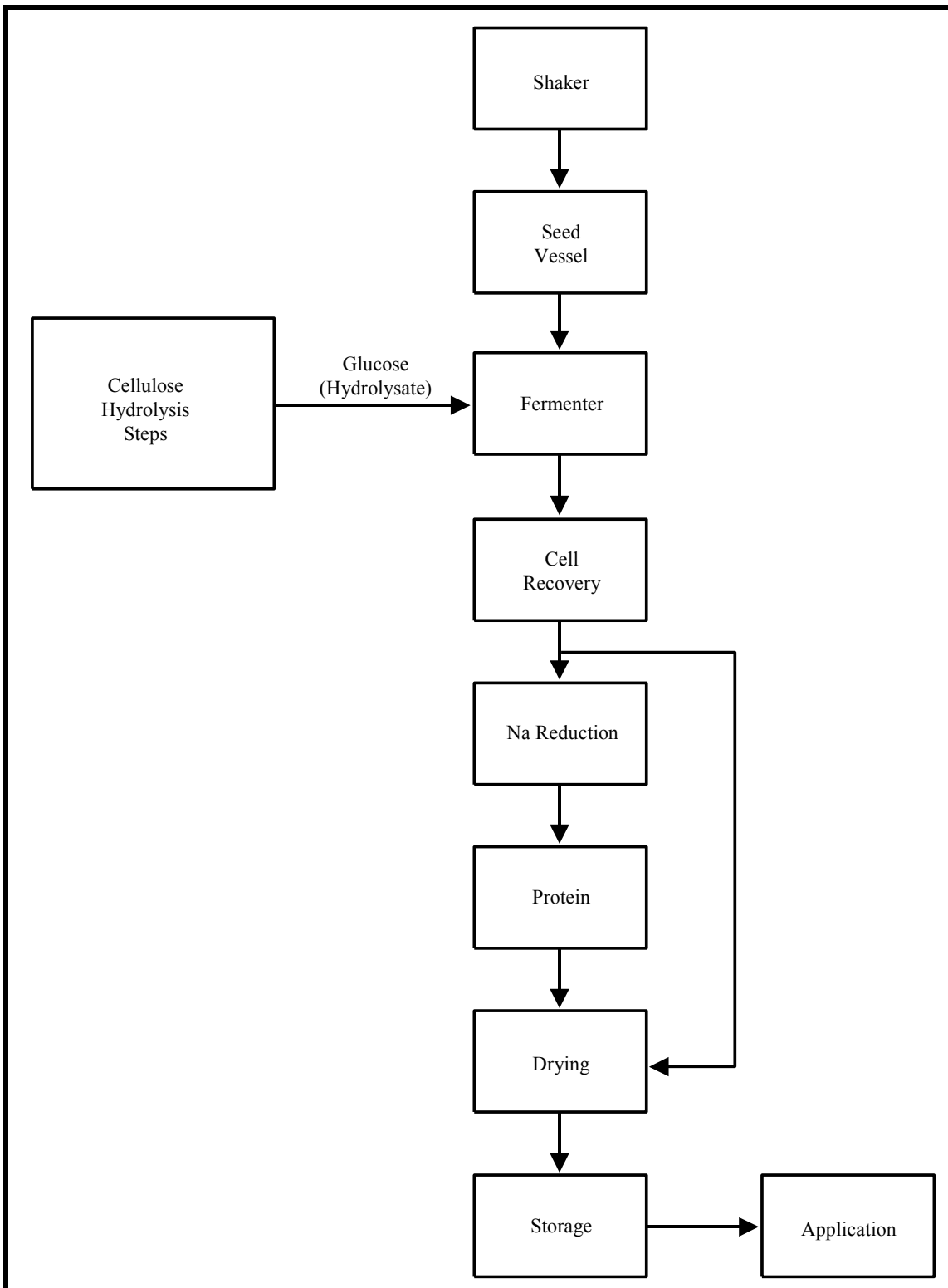


Figure IX-2. Indirect production of single-cell protein

Most of the experience with single-cell production from waste has been at the laboratory- and pilot-scale levels and has been with paper and bagasse. Paper is from 40% to 80% cellulose, 20% to 30% lignin, and 10% to 30% hemicellulose and xylosans. Bagasse is the residue remaining after the juice has been extracted from sugar cane by milling. Inasmuch as the studies were limited to laboratory- and pilot-scale levels, projections and estimates based on the studies must be considered in that light.

Among the cellulolytic microorganisms that have been studied are the yeasts, *C. utilis* and *Myrothecium verrucaria*, and the bacteria, *Cellulomonas flavigena* [3,11].

In a study that involved the culture of *M. verrucaria* on a substrate composed of ball-milled newspaper, a yield of crude protein amounting to 1.42 g/L was obtained [11]. A pilot-scale study involved the application of a system such as is diagrammed in Figure IX-3 [12,13]. The organism used in the investigation was *C. utilis*. The bagasse was pre-treated because experience had shown that without pre-treatment, the soluble carbohydrate content of untreated bagasse is only about 2%; whereas after treatment, it is almost 18%. Pre-treatment reduces the cellulose crystallinity of the bagasse from almost 50% to only 10%. As stated earlier, pre-treatment generally takes one or a combination of the following forms: fine milling and exposure to moderately elevated temperature under either acid or alkaline conditions.

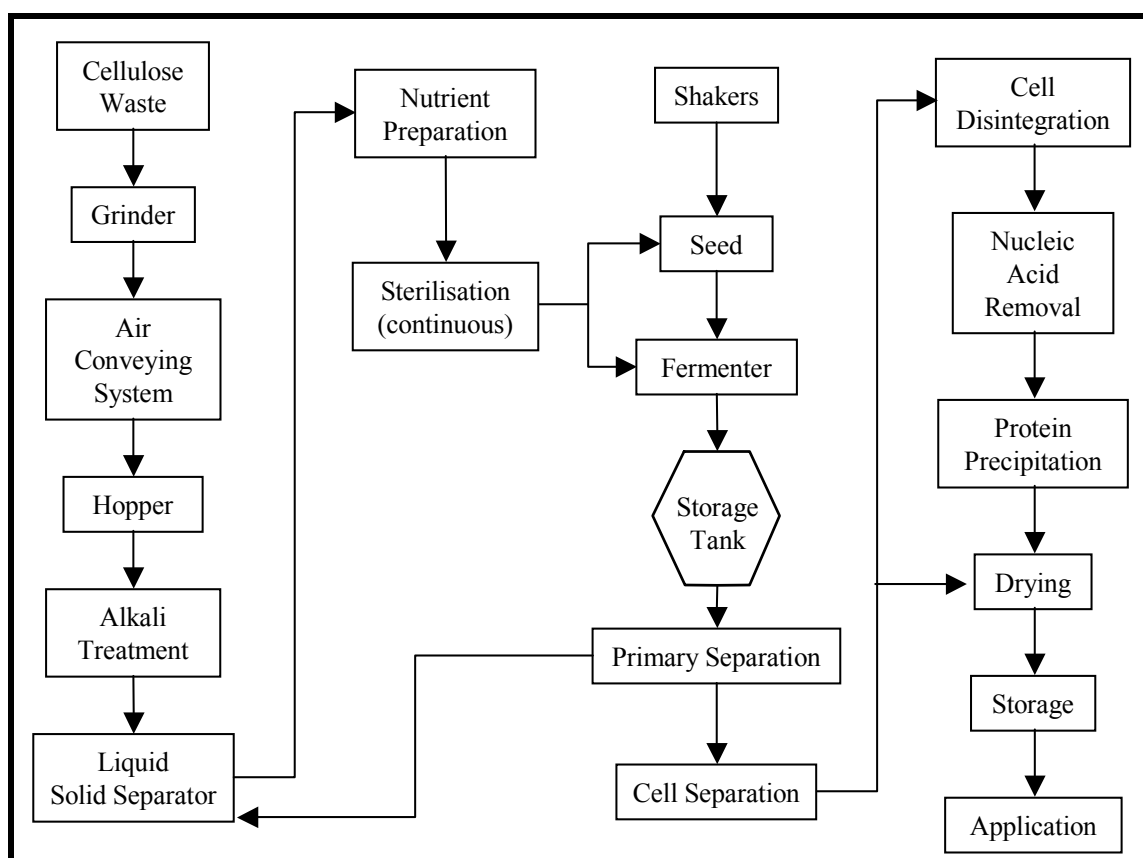


Figure IX-3. Direct production of single-cell protein by US Army Natick Lab

The bacteria *C. flavigena* and *C. uda* constituted the product in a pilot study in which the feedstock was bagasse [4]. The study confirmed the need to pre-treat bagasse -- specifically, alkaline pre-treatment. Moreover, in the study, extent of conversion of feedstock to cell mass was very modest despite a continuous fermenter efficiency of 75% and an approximate 90% solubilization of bagasse. Supplementary nutritional needs could be supplied by fertiliser and industrial chemicals. From 50% to 55% of the product is crude protein that has a good amino acid balance [14].

Another pilot-scale study involved a mixed culture of *Cellulomonas* and *Alcaligenes faecalis*. The cell density was 6.24 g/L. The crude protein composition was as follows (in g/100 g protein): arginine, 9.21; histidine, 2.30; isoleucine, 4.74; leucine, 11.20; lysine, 6.84; methionine, 1.86; phenylalanine, 4.36; tyrosine, 2.67; threonine, 5.37; and valine, 10.71.

C2. HARVESTING

Harvesting usually is done in two main stages: a concentration stage and a concentrate processing stage.

C2.1. First-stage concentration

This stage results in the formation of a concentrate that has a sludge-like consistency and is in need of further processing. The need for the concentration step arises from the relatively low concentration of cells and large volumes of material that must be processed. The sludge (concentrate) is dewatered and dried. The concentration step is beset with many and grave difficulties due to the microscopic size and the physical characteristics of the cells, as well as their modest monetary value. The several technologies available for accomplishing the concentration step can be grouped into the categories of screening, filtration, settling (sedimentation), and centrifugation.

C2.1.1. Screening and filtration

Screening and filtration are discussed under a single heading because they share a common characteristic: separation of particles (cells) depends upon the difference between the size of the particles and that of the openings (screen) or pores (filter medium). The problem is that the screen or filter medium becomes clogged before a workable “cake” can be accumulated.

C2.1.2. Settling

Their small size, low specific gravity, density, and low settling velocity render concentration by sedimentation impractical. The settling velocity of yeast cells is approximately 1.1×10^{-5} cm/sec.

Significant advances in settling tank design and operation may enhance settling to a point at which it becomes a feasible option. Another approach to settling or a modification is to induce floc formation and thereby promote settling to a level at which it might be practical. Floc formation can be induced by altering the surface charge of yeast cells such that they agglomerate into floc particles. Surface charge can be altered by introducing a polymer flocculant (either anionic or cationic) into the suspension. Alteration can also be accomplished by passing the suspension through an ion exchange column.

C2.1.3. Centrifugation

Centrifugation is an effective concentration method. Unfortunately, it is expensive in terms of equipment and power, and requires skilled personnel. A high-velocity rotor is necessary because of the microscopic size and low specific gravity and density of the cells and viscosity of the medium. A putative advantage is that the two separation stages can be accomplished in a single operation.

C2.2. Second stage - concentrate (sludge) processing

Treatment consists of dewatering and drying. Flash drying is a good approach. It is rapid and is amenable to mass production and is successfully used in food and feedstuff preparation. Moreover, it removes threats to human and animal health posed by chance pathogens. Other options include pressure filtration and vacuum drying, such as is used in sewage sludge conditioning.

C3. EQUIPMENT requirements and costs

The data listed in Table IX-3 provide an indication of the equipment that might be required in commercial production of single-cell protein. The table includes data that indicate the relation of the cost of individual types of equipment to the total cost. The basic flows for minimum-size versions of direct and indirect production of single-cell protein are presented in Table IX-4.

Table IX-3. Equipment requirements and relative costs for the production of single-cell protein

Cost Element	Direct Production		Indirect Production	
	Equipment (500-L base)	% of Total Cost	Equipment (1,200-L base)	% of Total Cost
Pre-treatment line	6 units	18	5 units	15
Sterilisation system	2 units	3	2 units	3
Enzyme production line	--	--	5 units ^a	11
Syrup manufacturing line	--	--	4 units	3
Cell (metabolite) line	2 units ^b	22	2 units ^c	11
Cell recovery line	2 units	5	2 units	5
Protein recovery	3 units	7	3 units	6
Drying distillation	1 unit	10	1 unit	10
Instrumentation - interface		15		18
Computer hardware - program (software)		20		18
Totals		100		100

Source: Reference 2.

^a 350-L seed vessel; 1,200-L fermenter; two storage tanks; one ultrafiltration system.

^b 100-L seed vessel; 500-L fermenter.

^c 550-L seed vessel, 1,200-L fermenter.

Table IX-4. Basic annual flows of a minimum-size commercial single-cell protein plant

Parameter	Direct Production (Mg)	Indirect Production (Mg)
Raw material input	8,100	17,000
Intermediate output	--	6,000
SC output	1,200	1,200
Liquid flow rate for cell separation	20,000	20,000
Average cell mass (g/L) ^a	25	25

Source: Reference 12.

^a Assuming 20% loss during recovery.

D. Ethanol production

The relation of ethanol production to hydrolysis is through the use of the glucose released in the hydrolysis of a cellulosic waste [16,17]. In other words, the substrate for ethanol production is the sugar produced in the hydrolysis of the cellulosic fraction of the waste feedstock. The sugar serves as the carbon source for the microbes responsible for ethanol fermentation.

Expressed over-simply and hence very loosely: “Ethanol fermentation is accomplished by culturing strains of microbes capable of converting the carbon (C) in the glucose molecule to the C in ethanol”. Several strains and varieties of bacteria and fungi have been identified as being capable of fermenting sugars to ethanol. Among the fungi are a few varieties of *Rhizopus* and yeast forms of *Aspergillus*, *Penicillium*, and “Fungi imperfecti”. Perhaps the best known and most thoroughly explored are selected varieties and strains of the yeast, *Saccharomyces cerevisiae*. (Because *S. cerevisiae* is representative, it is used in the succeeding paragraphs to typify ethanol fermentation technology.)

In contrast to the aerobic conditions required in hydrolysis, ethanol fermentation takes place only under anaerobic conditions regardless of type of microorganism. Hydrolysate sugars are readily fermented by *S. cerevisiae*. The conversion efficiency reportedly is 83%, and the gross energy for the conversion of newsprint to ethanol is 47% for the hydrolysis step, and 37% for the waste to ethanol step (exclusive of credit for waste heat) [6]. The net efficiencies are 34% and 24%, respectively.

Inasmuch as practical experience in the use of cellulosic solid waste for ethanol production has been negligible, reliance must be had on information gathered in past laboratory- and pilot-scale studies. Consequently, projections and extrapolations reported in the literature are largely conjectural. This limitation, however, applies only to the use of hydrolysate sugars as an ethanol fermentation substrate. The technology of ethanol fermentation of substrates other than cellulosic solid wastes not only is well developed, it also is being vigorously continued because of the significance of ethanol as an energy source. Documentation of the advance also continues apace. Even with respect to cellulosic wastes, the uncertainty and scarcity of the literature is more applicable to the preparation of the feedstock and its hydrolysates than to the fermentation of the hydrolysates. Thus, one can justifiably conclude that conventional fermentation technology should be suitable for the fermentation of the hydrolysis sugars.

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