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D00293

United Nations Industrial Development Organization

ID

Distr.
LIMITED

ID/WG.50/13
26 November 1969

ORIGINAL: ENGLISH

Expert Working Group Meeting on the
Manufacture of Chemicals by Fermentation

Vienna, 1 - 5 December 1969

INDUSTRIAL CHEMICALS: ORGANIC SOLVENTS, ORGANIC ACIDS, MISCELLANEOUS PRODUCTS, MICROBIAL INSECTICIDES [1]

by

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id.69-6105

Historically, organic solvents and acids have been a dominant part of the fermentation industry. Today, the manufacture of the various organic solvents and acids that can be produced by fermentation are partly displaced by chemical synthesis. These include microbially produced acetone and butanol, ethanol from non-cerial substrates, lactic acid and acetic acid.

It is the purpose of this review to update the older processes and to present the current state of development of new fermentation products. The following fermentation products are discussed:

1. Organic solvents

1.1 Ethyl alcohol

1.2 Acetone-Butanol

1.3 2,3-Butanediol

1.4 Glycerol

1.5 Production of polyhydric alcohols with osmophilic yeasts.

2. Organic acids

2.1 Citric acid

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3.1 Dihydroxyacetone

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1. Organic solvents

1.1 Ethyl alcohol

Industrial ethyl alcohol, or ethanol, is the most widely used organic solvent in chemical industry, and, as these industries have expanded, so the demand for industrial alcohol has increased. Today, despite the rising cost of raw materials, the alcoholic beverage industries - distilled spirits, beer and wines - are increasing, but fermentation alcohol for industrial uses has steadily lost ground to processes for producing ethanol by chemical synthesis (Table 1). The raw materials used in the synthetic process are ethylene and natural gas. Countries possessing sources of natural gas or crude petroleum will obviously be able to manufacture the synthetic ethanol at low cost. Although the production of fermentation ethanol is favoured in countries possessing carbohydrate raw materials at low cost.

Fermentation methods

Ethanol can be produced from a variety of sugar-containing materials (see Table 1) by fermentation with yeasts. The several processes employed have been reviewed[1,2,3]. The raw material employed falls into two classes:

a) Those consisting mainly of fermentable sugar (molasses, sulfite liquors or whey).

b) Those in which the carbohydrate is predominantly a polysaccharide (starch or cellulose).

Table 1 Materials used for ethanol production[a)]

Raw material used	Ethanol Produced % of Total 1956	% of Total 1966	Millions Proof Gal 1956	Millions Proof Gal 1966
Grain and grain products	1.2	11.54	5.4	80.4
Molasses	25.5	1.59	126.7	11.0
Fruit	0.01	4.07	-	28.3
Sulfite liquors	1.32	0.90	6.5	6.2
Cellulose pulp; chemical and crude alcohol mixtures	0.52	0.12	2.5	0.8
Whey	0.09	0.06	0.4	0.4
From redistillation	2.12	4.11	10.5	28.6
Ethylene gas	9.80	18.29	48.6	127.6
Ethyl sulfate	59.34	59.32	294.4	413.8
Total	100.00	100.00	496.2	889.3

a) US Treasury Dept., Internal Revenue Service, Publication 67, 1956 and 1966

Ethanol tolerant strains of Saccharomyces cerevisiae are usually selected. They convert only hexose sugars including glucose, fructose, mannose, galactose, sucrose, maltose and raffinose to ethanol and carbon dioxide as expressed by the Gay-Lussac equation:

$$C_6H_{12}O_6 \longrightarrow 2\ C_2H_5OH + 2\ CO_2$$

The fermentation of whey needs a lactose-fermenting yeast S.fragilis or Torula cremoris[4]. Detailed reports on procedures for processing and fermenting the various carbohydrate-containing materials have been reviewed[3,5,6].

The commonest of the sugar raw materials is molasses, usually the by-product of sugar beet or sugar cane processing. The molasses containing about 55 % sugars are diluted with water to give a solution containing 15-20 per cent sugar, before nutrients such as ammonium sulphate and phosphates are added. The pH is adjusted to 4.0-4.5 and the fermenter is mixed with about 5 % by volume of vigorous yeast culture. Acidity increases are adjusted with ammonia and the temperature (28°-30°C) during the two-day batch fermentation is controlled with external water sprays or internal colling coils. The carbon dioxide which is envolved is collected for commercial use. In the batch fermentation vessels holding as much as 250 m^3 are used.

The fermented liquor containing 8 to 10 % of ethanol is distilled in continuous stills. Fusel oil - a mixture of higher alcohols - is obtained as a by-product of the distillation.

In countries with sizable paper pulp industries sulfite waste liquor is used as a raw material[7]. The liquor must be freed from excess sulphur dioxide before fermentation by passing a current of steam.

Starchy and cellulosic raw materials must be hydrolyzed to fermentable sugars before they can be utilized by the yeast. The first stage in a process is to cook the grain in order to gelatinize the starch. The hydrolysis can be effected in a number of ways: dilute acid may be used or high diastatic power barley malt followed by mashing. Methods based on the use of amylolytic enzymes from moulds have been developed. In the amylo-process, the mould (Aspergillus niger, A.oryzae or Rhizopus delemar are commonly used) is grown in an aerated mash for 24 hours at 38°C and then cooled to 30°-32°C before inoculation with yeast.

The development of continuous processes for hydrolysis, mashing and fermentation has been successful studied. A continuous ethanol fermentation using starchy materials[8,9] is described, but a continuous process using molasses is technical easier[10,11]. Continuous fermentation may bring with it a loss of yield due to infection of the mash with undesirable microorganisms. In this case the acidification of the mash is helpful or the use of penicillin or Sodium-pentachlorophenolat[12].

1.2 Acetone-Butanol

There are several closely related fermentations in which acetone and alcohols occur as endproducts. The organisms having been employed on a commercial scale for producing these solvents are nearly all species of the anaerobic spore-forming bacterium Clostridium. Breakdown of sugars by these bacteria gives a variety of endproducts. Important fermentations are:

Acetone-Butanol

Butanol-Isopropanol

Acetone-Ethanol

The microbial production of acetone-butanol was one of the first large-scale process being developed and was pioneered by Weizmann[13]. Today, uses of both acetone and butanol grew and synthetic processes were developed. Competition between the fermentation and synthetic process has become very acute. Demand for acetone and butanol is still rising owing to the rapid expansion of the chemical industries. The most important factors in the acetone-butanol fermentation process are the costs of the carbohydrate and of the energy. The process is particularly suitable in countries where both carbohydrate and coal or fuel are cheap. More than 60 per cent of the production costs for a factory of reasonable size (10 000 - 20 000 tons per year) are on molasses and the costs of coal for steam are raising to about 15-20 per cent of the total costs[14,15,16]. The acetone-butanol fermentation has been reviewed by several workers[17,18,19].

Despite the rigorous precautions that are taken to exclude unwanted microorganisms from the acetone-butanol fermentation plant, infections are sometimes encountered and are usually lactic acid organisms. Infection with bacterial viruses or bacteriophages has proved much more trouble and can lead to the death of the culture in the fermenter in just a few hours. Strains of Cl.acetobutylicum can be immunized against phages by serial transfer through media containing the virus. The phages that attack strains of Cl. acetobutylicum are strain-specific, so that fermentations having become infected with phages can be re-inoculated with an immune strain of the same bacterium.

Many kinds of sugar-containing raw materials may be used by the saccharolytic acetone-butanol bacteria, the favoured ones are cane molasses or corn mashes. Sucrose, glucose, beet molasses, citrus molasses and sulfite liquors may also be used as sources of sugars, but there is no report of any of these materials having been used on a commercial scale.

The following short description of the fermentation process is based on the report by Beesch[18]. Molasses is diluted with water to give a sugar concentration of 5-7 per cent. The mash is cooked and sterilized at 107°C and charged to sterile fermenters after cooling to 31°C. Following inoculation with 3 per cent seed culture from a 24 hr culture tank of Clostridium saccharo-acetobutylicum or other suitable strains of Cl.acetobutylicum. In the final fermenter,

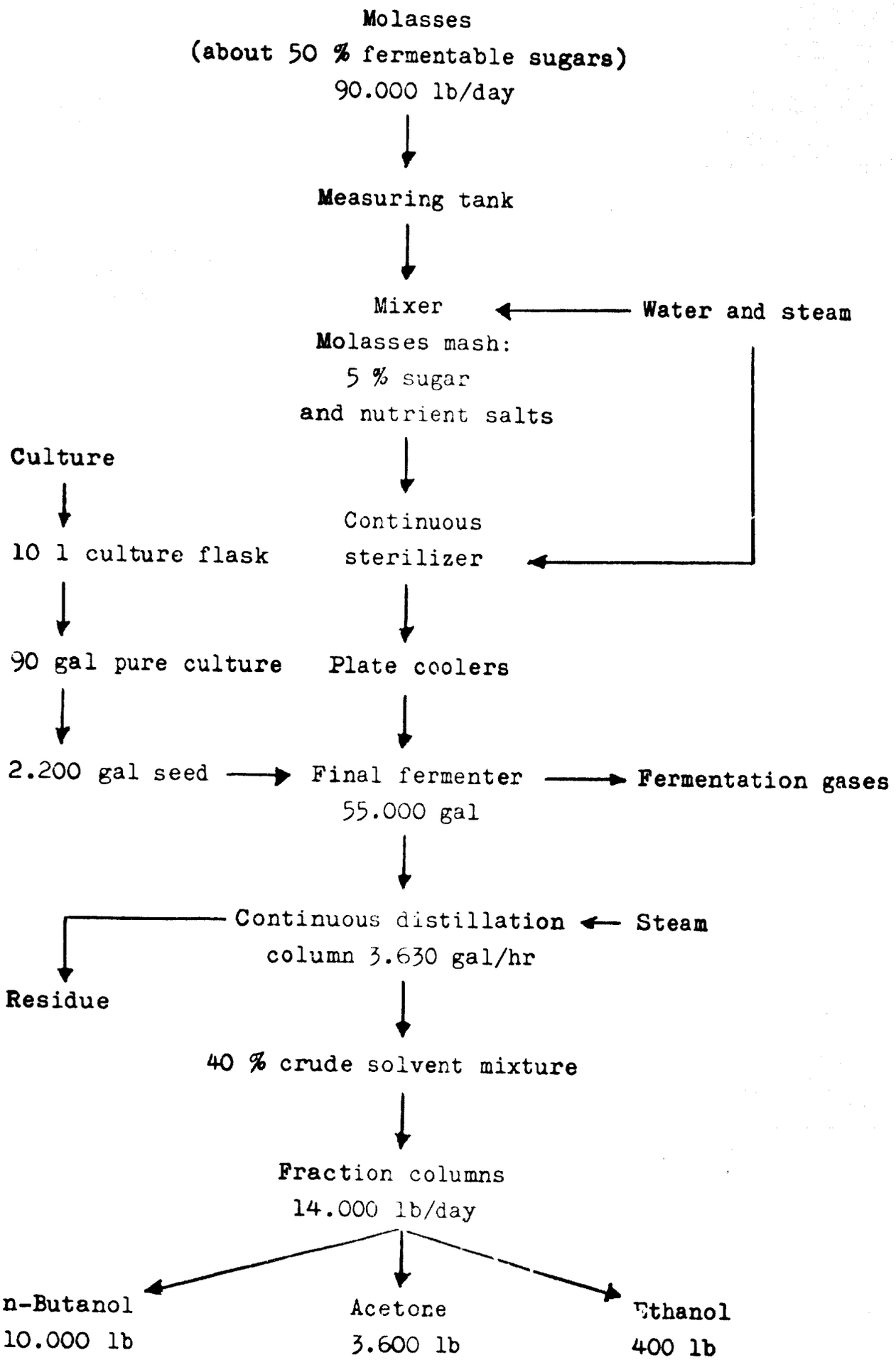
ammonium hydroxide is usually added stepwise to hold the pH above 5.1 to 5.3 after the fermentation has reached about 16 hrs of age. After fermentation has proceeded for 42 to 48 hours, the fermented beer containing 1.7 to 2.4 per cent of solvents is stripped in a continuous-type still. The crude mixture is separated by fractional distillation.

The most important products formed in the acetone-butanol fermentation are n-butanol, acetone, ethanol, carbon dioxide, hydrogen and riboflavin-containing feeds from the fermentation residue. Figure I shows a flow diagram for a modern acetone fermentation of molasses[20].

Yields, based on sucrose, range from 29 % to 33 %. The individual solvents in the mixture vary from 68 % to 73 % for n-butanol, 26% to 32 % for acetone and 1 % to 3 % for ethanol.

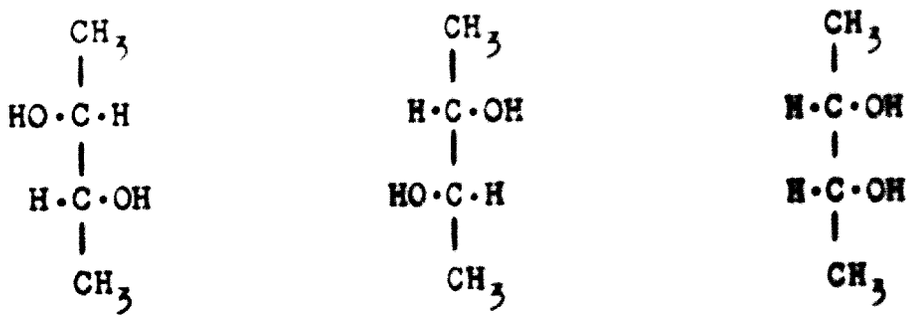
In the last time was successfully carried out continuous acetone-butanol fermentation on a laboratory scale, using both one-stage and multi-stage systems[21,22]. One of the principal drawbacks to the batch fermentation - the toxicity of butanol to the bacterium - has been overcome in this continuous process by employing high dilution rates. In the continuous process the yield of solvents produced was 33 % to 35 %, which was at least as good as in a control batch fermentation. These results indicate that a continuous process for the acetone-butanol fermentation may be adopted by industry in countries having inexpensive carbohydrate materials at hand.

Figure 1 Flowsheet



1.3 2,3-Butanediol

There are three stereoisomeric forms of 2,3-butanediol, all of which are produced by bacterial fermentation. The structural formulas are shown below:



D(-)-2,3-Butanediol (Levorotatory form) | L(+)- (Dextrorotatory form) | meso

A number of species or strains of bacteria classified in the genera Aerobacter, Aerobacillus, Aeromonas, Serratia, and Bacillus possess the ability to produce 2,3-butanediol. Only two organisms, A.aerogenes and B.polymyxa have been shown to be potentially useful in the industrial production of 2,3-butanediol. A mixture of the L(+)- and meso forms of 2,3-butanediol is produced by strains of A.aerogenes[23]. The D(-)-form of 2,3-butanediol is produced characteristically by B.polymyxa[24].

The more versatile of the two organisms, in a variety of suitable substrate, is B.polymyxa. This species is actively diastatic and fermentation may be run using starchy raw materials. A.aerogenes, however, is not diastatic and can ferment only sugars. The economies of the 2,3-butanediol

fermentation limit the carbohydrate sources to the various types of molasses, the cerial grains, or industrial waste liquors. The following substrates have been successfully used for 2,3-butanediol fermentation[25]: Citrus press juice, citrus molasses, blackstrap molasses, beet molasses and sulfite waste liquor. A.aerogenes is usually capable of fermenting considerably higher concentrations of sugar than B.polymyxa. The optimal conditions for the 2,3- butanediol fermentations are described[3,25].

One of the greatest problems of the 2,3-butanediol fermentation is the economical recovery of the product from the fermentation process. The major difficulties are due to its high boiling point (180°-184°C), solubility in water and the presence of dissolved and solid constituents of the fermentation mash. Methods for the isolation of 2,3-butanediol are continuous solvent extraction with, for example, ethyl acetate or working with a counter current steam-stripping column.

The stereoisomeric 2,3-butanediols have potential uses as solvents, moisteners and softeners in the pharmaceutical industry. A promising future for the 2,3-butanediols may be seen in the expanding plastics industry, especially for the production of linear polyesters.

1.4 Glycerol

In industry, glycerol is prepared by the saponification of fats and oil, whereas synthetic glycerol is produced from allylchloride, acrolein and propylene oxide. Processes for the production of glycerol by fermentation are the sulfite process developed by Connstein and Lidecke[26]; the Cocking and Lilly process, using a mixture of sulfite and bisulfite[27]; the alkaline process[28] and the production of glycerol by osmophilic yeasts[29,30]. Reviews of the production of polyhydric alcohols are given[31,32].

In the first three processes the basic fermentation medium contains 10 per cent of a fermentable sugar (beet- or cane-molasses) and nutrient salts. The medium is inoculated with a culture of Saccharomyces cerevisiae and maintained at the optimum temperature at 30°C for 48 to 60 hours. It is important to control the temperature of the fermentation. The yeast may be used repeatedly, if purified between fermentations.

The basis of the sulfite process is the addition of 3 per cent of sodium sulfite to the medium for the fixation of acetaldehyde. In the absence of sodium sulfite the intermediary acetaldehyde is reduced to ethanol during the fermentation of sugars by yeasts. In the presence of sodium sulfite the acetaldehyde is fixed and can not serve as hydrogen acceptor, the intermediary dihydroxyacetonephosphate acts as the main hydrogen acceptor under reduction to glycerol. On the basis of sucrose fermented, approximately

20 to 25 % of glycerol, 30 % of ethanol and 50 of acetaldehyde were obtained by Connstein and Lüdecke.

The Cocking-Lilly process is a modification of the sulfite process. Mixtures of normal sulfites and bisulfites are added to the fermentation medium, so that the process is running under mild acid conditions. The fermentation time is shorter than in the normal sulfite process.

The Eoff process. A nutrient solution containing a sugar is inoculated with a selected yeast[33] or a UV-mutant of S.cerevisiae and incubated at 30 to 32°C. An alkaline reacting compound, for example sodium carbonate, is stepwise added to the fermenting medium in amounts up to 5 %. Glycerol yields of 10.5 to 24.2 % and corresponding ethanol yields of 36 to 27 % were obtained by fermentation of molasses in the presence of sodium carbonate[35].

1.5 Production of polyhydric alcohols with osmophilic yeasts.

The polyhydric alcohols are produced in good yield by a group of yeasts and yeast-like organisms whose outstanding characteristic is their tolerance of high concentrations of sugars or salts. The polyhydric alcohols commonly produced have been glycerol, erythritol, arabitol and mannitol. Osmophilic yeasts were found to produce up to 0.6 g polyols/g glucose utilized. The literature concerning the biochemistry, physiology and especially the factors influencing the product-

ion of polyhydric alconols has been reviewed by Spencer[32]. The results obtained support the view that fermentation of glycerol or the other polyhydric alcohols possess a real economic production chance.

2. Organic acids

Various organic acids may accumulate in cultures of micro-organisms as a result of the dissimilation of sugars. These acids may represent endproducts from the anaerobic break-down of sugars (e.g. propionic acid and lactic acid), or they may appear in growing cultures following the incomplete oxidation of sugars (e.g. citric acid and gluconic acid). Of the various organic acids that can be produced by fermentation, only citric, itaconic and gluconic acids have not been challenged by chemical synthesis. The annual world production of citric acid is believed between 200.000.000 and 220.000.000 pounds and of gluconic acid between 30.000.000 and 40.000.000 pounds.

2.1 Citric acid

The development of citric acid fermentation process may be divided in two processes. The first process, that of surface fermentations using Aspergillus niger cultures, was reported in 1917[36]. The modern process, that of submerged fermentation using also strains of A.niger, was developed in 1949[37]. The aspects of the citric acid fermentation process have been mentioned in several reviews[38,39,40].

All successful procerses for citric acid production need selected strains for high yields and suitability for use in inoculum development. The special strains of A.niger with these desirable characteristics have been developed through mutation hybridization techniques. Synthetic media have been used in many studies on citric acid production by strains of A.niger in either submerged culture or surface culture. These studies have been helpful in determining the roles of certain trace elements including iron, zinc, manganese and copper. The yields of citric acid obtained from different media containing raw sugar were inversely proportional to the iron content of the sugar. Copper proved to be useful in counteracting the effects of iron. A second improvement was the observation that the addition of alcohols or esters to the citric acid process reversed the inhibitory effects of metallic ions including zinc, iron and manganese on citric acid production.

Decationized solutions of cane molasses, high-test molasses, beet molasses, ground corn, wheat starch, glucose or sucrose are suitable for us as carbohydrate sources. The molasses is diluted to about 20 to 25 % sugar concentration. The high sugar concentration is believed to inhibit formation of acids other than citric acid. Treatment of the molasses with ferrocyanide, followed by filtration, reduce the dissolved iron content. Nutrients which may be supplied to the mash are ammonium nitrate or urea, $MgSO_4$ at about 0.1 % and KH_2PO_4 at 0.1 to 0.2 % and the pH is adjusted

to the range of 2 to 3. The low pH and the extreme sensivity to iron makes it necessary to use plastic coating iron vessels or stainless steel fermenters. The temperature during the process is held between 27 to 33°C and an aeration rate of 0.5 to 1.0 vol per vol per min in the submerged culture is used. After 7 to 12 days the yield, based on glucose, ranges from 70 to 90 per cent.

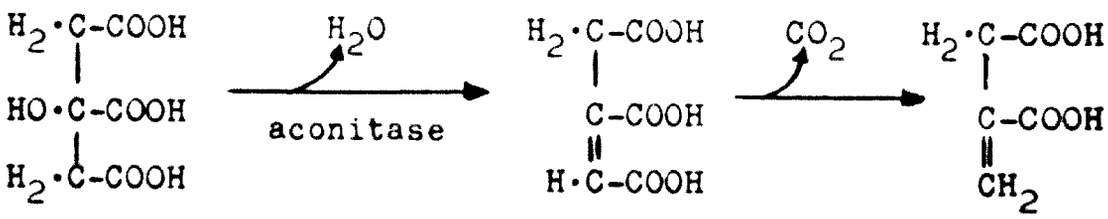
The recovery of citric acid from the fermentation liquor is carried out by precipitation with $Ca(OH)_2$ and the calcium citrate is filtered, the residue treated with sulfuric acid. The dilute solution is further purified and evaporated in a circulating vacuum granulator. citric acid so prepared can be recristallized from water.

Citric acid is one of the most widely used acidulants in the food, pharmaceutical and cosmetic industries. Large quantities of the acid are used in the preparation of soft drinks, desserts, jams and frozen fruits. In recent years, increasing amounts of citric acid have been employed as chelating and sequestering agents. These properties permit its wide industrial use in electroplating, in leather tanning and in refining of old oil wells from iron.

2.2 Itaconic acid

Unlike citric acid, itaconic acid is not an intermediate in any of the major metabolic pathways involved in carbohydrate metabolism. Experiments using isotopically labelled

substrates and enzymatic studies supposed that citric acid and cis-aconitic acid are the precursors of itaconic acid in Aspergillus terreus fermentations[41].



citric acid cis-aconitic acid itaconic acid

Itaconic acid is formed by A.terreus and A.itaconicus in surface culture or in submerged fermentation The itaconic acid fermentation by A.terreus is sensitive to the iron ion content[42], although that by A.itaconicus is reported not to be sensitive[43].Alkaline earth metal salts, copper and zinc were found to stimulate the conversion of carbohydrate to itaconic acid. Cane molasses, raw sugar or cane juice were used in the fermentation medium. The temperature is maintained at 35 to 40°C and the pH at 2.2 to 3.8. Best conversion rates were obtained when high inoculum levels were used. After three days the final itaconic acid concentration is about 85 g per liter. A continous process was successfully operated yielding 60 % itaconic acid[44]. At the end of the fermentation, the mycelium is filtered off and the solution is demineralized by successive passage through cation and anion exchange resin bed[45]. One recrystallization from water gives the refined grade of itaconic acid.

With a conjugated double bond and two carboxyl groups, itaconic acid is a very reactive substance, it can be polymerized or co-polymerized with other monomers to give valuable plastics. Esters of itaconic acid are also utilized for the production of resins. In addition, itaconic acid can be used in manufacturing detergents.

2.3 Gluconic acid

Microbial production of gluconic acid is an important and expanding industry. Almost all of the gluconic acid used industrially is produced by fermentation processes. Gluconic acid is obtained from many microorganisms, particularly by bacteria of the Acetobacter and Pseudomonas genera and by moulds of the Penicillium and Aspergillus genera. At present time the commercial production of gluconic acid or gluconates is based on the submerged culture of A.niger in glucose media, stirred and aerated under superatmospheric pressure[46,47]. The kinetics of the gluconic acid fermentation from glucose have been studied[48]. D-Gluconolactone was found as an intermediate in the fermentation and accumulated at times in large amounts.

The preferred conditions for the gluconic acid production are an original glucose concentration between 38 to 45 %, aeration rates of 1.0 to 1.5 vol of air per min per vol of fermentation broth, air pressure on the fermenter of 30 psig and a high degree of agitation, at a temperature of 33 to 34°C. In a fermenter with a contactor agitation

system the aeration rate can be decreased to 0.2 to 0.4 vol of air per min per vol fermentation broth, yielding 95 to 97 % gluconic acid. Fermenters could be inoculated with vegetative mycelium or with mycelium freshly separated from a previous fermentation. The gluconic acid process is a relatively short fermentation (36 to 40 hours), the batch turn around time is therefore an appreciable portion of the total processing cycle. As a consequence, continuous fermentation can be an important consideration in devising a cheaper process if a new plant is to be built.

In view of the increasing importance of sodium gluconate as an industrial sequestering agent, the main part of gluconic acid produced is recovered as sodium gluconate. Many important applications have been found for gluconic acid and its derivatives in the food, feed, pharmaceutical and industrial fields[46].

3. Miscellaneous Products

3.1 Dihydroxyacetone

Dehydrogenation of glycerol with selected strains of Acetobacter results in the formation of dihydroxyacetone. The microorganisms used in this process are A.suboxydans, A.xylinum and A.aceti. The need for major amounts of dihydroxyacetone stimulated studies toward a commercially fermentation[49,50,51]. Optimum yield of dihydroxyacetone (95 to 97 %) could be achieved with Acetobacter suboxydans

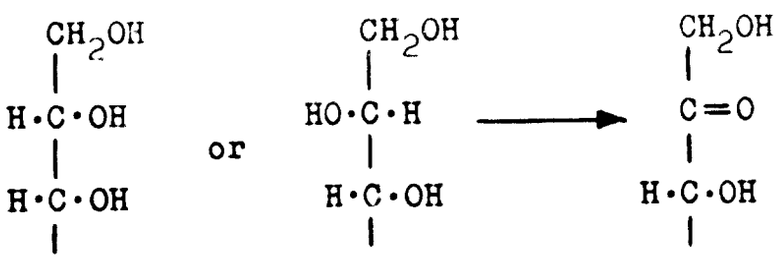
in submerged aerated and agitated fermentation at 30°C at about 25 hours with a mash containing 10 % glycerol, 0.5 % brewers yeast, 0.5 % corn steep liquor and 0.5 % KH_2PO_4. The pH is adjusted to 5.5 .

A mutant of Brevibacterium fuseum oxidized glucose to dihydroxyacetone.

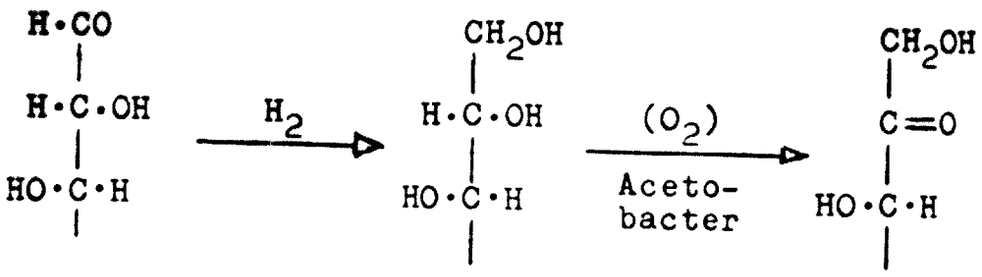
Crystalline dihydroxyacetone is obtained by ion exchange procedure[52]. Dihydroxyacetone is used in the cosmetic industry as a sun-tanning agent.

3.2 Sorbose, Fructose

The oxidation of polyhydric alcohols by Acetobacter species is a well-known process. The Bertrand rule[53,54] states that species are able to oxidize a secondary alcohol group of a polyhydric alcohol to a ketone when its position lies between a primary and a secondary alcohol group.



Sorbose, an important intermediate in the synthesis of ascorbic acid, is produced by the oxidation of D-sorbitol in accordance of the Bertrand's rule.



D-Glucose D-Sorbitol L-Sorbose

The fermentation conditions for this oxidative process were similar to those for gluconic acid production. Sorbitol solutions of 20 to 30 % concentration with 0.5 % yeast extract or 0.3 % corn steep liquor were found to be suitable substrates for the fermentation. Nearly quantitative conversion of a 30 % solution of sorbitol to sorbose during 45 hours at 30°C is obtained. The oxidation of sorbitol to sorbose in continuous fermentation in good yield was described by Müller[55]. Sorbose is recovered by adding of charcoal and filter-cel to the broth, filtered and concentrating the filtrate at 60°C under vacuum to a sirup. Crystallized sorbose is the result by cooling the sirup to 15°C.

Fructose. The oxidation of D-mannitol to D-fructose by A.suboxydans was studies in detail by Peterson et al.[56]. They were able to convert solutions of up to 24 per cent mannitol to fructose, with yields up to 100 per cent. In addition, the concentration of fructose in the final broth could be raised to 35 per cent by batchwise addition of solid mannitol to a fermentation originally containing

20 per cent mannitol.

An interesting production of fructose was described by Holstein and Holsing[57]. In this process, sucrose is inverted to form a mixture of glucose and fructose. The inverted mixture is inoculated with an active culture of selected species of Acetobacter, Penicillium or Aspergillus under conditions which result in the oxidation of the glucose to gluconate but leave the fructose intact. The gluconic acid produced can be separated from fructose by ion exchange resins.

The main application of fructose is as the constituent of parenteral solutions for intravenous infusion. These solutions offer many advantages over other carbohydrates. Fructose is often chosen for infusion of shock patients, it provides a quickly available source of energy. The superior sweetness of fructose should permit its substitution in foods for larger quantities of sucrose, thereby affecting a saving in calories.

3.3 Polysaccharides

Many microorganisms are known to produce extracellular polysaccharides (see reviews 58,59,60), some of which are potential pharmaceutical and industrial important, but the only polysaccharides having been produced on a commercial scale are certain dextrans and xanthan gums. Microbial polymers have not been fully exploited to date and many microbial polysaccharides are awaiting investigations.

Dextran. Species of bacteria from a number of genera, including Leuconostoc, Streptococcus, Streptobacterium, Acetobacter and Betabacterium possess the ability to produce dextrans under certain fermentation conditions. Plant production of dextran was carried out with only two strains, Leuconostoc mesenteroides and L.dextranicum. Inoculum and fermentation media containing 10 % sucrose, 0.25 % yeast extract, 0.5 % corn steep liquor, 0.5 % K_2HPO_4, 0.1 % NaCl, 0.02 % $MgSO_4$ and the important trace elements iron and manganese. The medium is continuous sterilized at 142°C. The inoculum is increased in several stages. The final fermentation is carried out at 25°C in an agitated fermenter and a very low aeration rate. The starting pH value of 6.5 decreases to pH 4.5 during 24 hours. At this time no sucrose is left and the maximum viscosity of the fermentation broth is attained. The dextrans are precipitated by addition of methanol, redissolved in pyrogen-free water at 60-70°C and reprecipitated. A controlled hydrolysis of the native dextran following to give dextrans of the required molecular size. The microbial process for the production of dextran from sucrose according to the overall equation:

$$n \text{ sucrose} \longrightarrow (\text{glucose})_n + n \text{ fructose}$$

therefore, the theoretical yield of dextran should be about 47 per cent.

Enzymatic synthesis of dextran. An alternative process of production of microbial dextran is to extract the extra-

cellular transglycosidase enzyme, the dextransucrase and use this to polymerize sucrose in vitro. Medium for the production of dextransucrase consisted of 2 % sucrose, 2 % corn steep liquor and mineral salts. After inoculation with L.mesenteroides the temperature is maintained at 24°C. and the pH at 6.7. The time required for optimal dextransucrase production is about 6 to 8 hours. The culture medium is clarified and the clear broth could be used directly or the dextransucrase could be precipitated with methanol at a pH of 5.1. The enzymatic process is obtained by adding to the culture filtrate up to 10 % sucrose, adjusting the pH to 5.1 and holding the temperature at 30°C for 8 hours. On completion of the reaction, the dextrans are precipitated with 40-50 per cent methanol. Under these conditions the dextran having a molecular weight of up to 100 millions. The production of low molecular weight dextrans is possible by increasing the concentration of sucrose and adding primers, preferable primers are hydrolyzed dextrans.

Numerous applications have been found for these dextrans, but their commercial importance is based primarily upon their value as blood plasma extenders; secondly as molecular sieves after modification with epichlorohydrine. These crosslinked dextrans are insoluble in water and serve for gel filtration techniques.

Xanthan gum. A number of biosynthetic heteropolysaccharides have been suggested for commercial production. At this time, only one of these gums has reached commercial production on

a substantial scale namely the xanthan gum produced by a mutant of Xanthomonas campestris. It was shown that these heteropolysaccharides might prove to be valuable industrial gums in the pharmaceutical, cosmetic, food and textiles industries.

Xanthan gum with a molecular weight of about one million contains D-glucose, D-mannose and D-glucuronate in the molar ratio of 2.8:3.0:2.0[61]. This substance is partially acetylated and it contains pyruvate. The production of xanthan gum is carried out in a two day fermentation with dextrose, sucrose or cruder forms of carbohydrates as substrates. A protein supplement and inorganic nitrogen source are necessary for efficient xanthan production. The fermentation required a pH range of 6 to 7.5, a temperature of 28 to 31°C and a very high aeration rate under strong sterile conditions. For standard products, the xanthan gum is recovered from the broth by precipitation with an alcohol or as an insoluble salt.

4. Microbial insecticides

The use of microorganisms as insecticides is one of the novel fermentation developments. About 1000 insect pathogens belonging to the bacteria, fungi, protozoa, rickettsiae or viruses are described[62,63]. Of these pathogens bacteria and viruses offer the best chance for development into practical microbial insecticides because of their specifity and effectiveness against many economically important insects.

In this context it ought to pay attention to all phases from production of an insect pathogen to its use in the field. Many of the problems encountered in this field are concerned not with the pathogen itself but with adequate coverage and stability of the microbial material in situ.

At the present time, only two bacterial insecticides are produced commercially. Bacillus popilliae, which causes the milky disease of the Japanese beetle , is produced only in larvae of Japanese beetles. Such an unlikely production process is necessary because B.popilliae does not sporulate readily outside of the insect host, it is an obligate pathogen. Bacillus thuringiensis, the causative agent of fatal diseases in many lepidopterous insects, is produced by conventional fermentation technique[64]. B.thuringiensis is a facultative pathogen and needs not an insect host. From the practical standpoint, it is advantageous, to develop commercial bacterial insecticides from facultative pathogens. The production of the sporeforming B.thuringiensis on an industrial scale has been described[64,65]. Safety to human, other vertebrates and plants has been conclusively demonstrated for B.thuringiensis but has not been established for other pathogens of insects.

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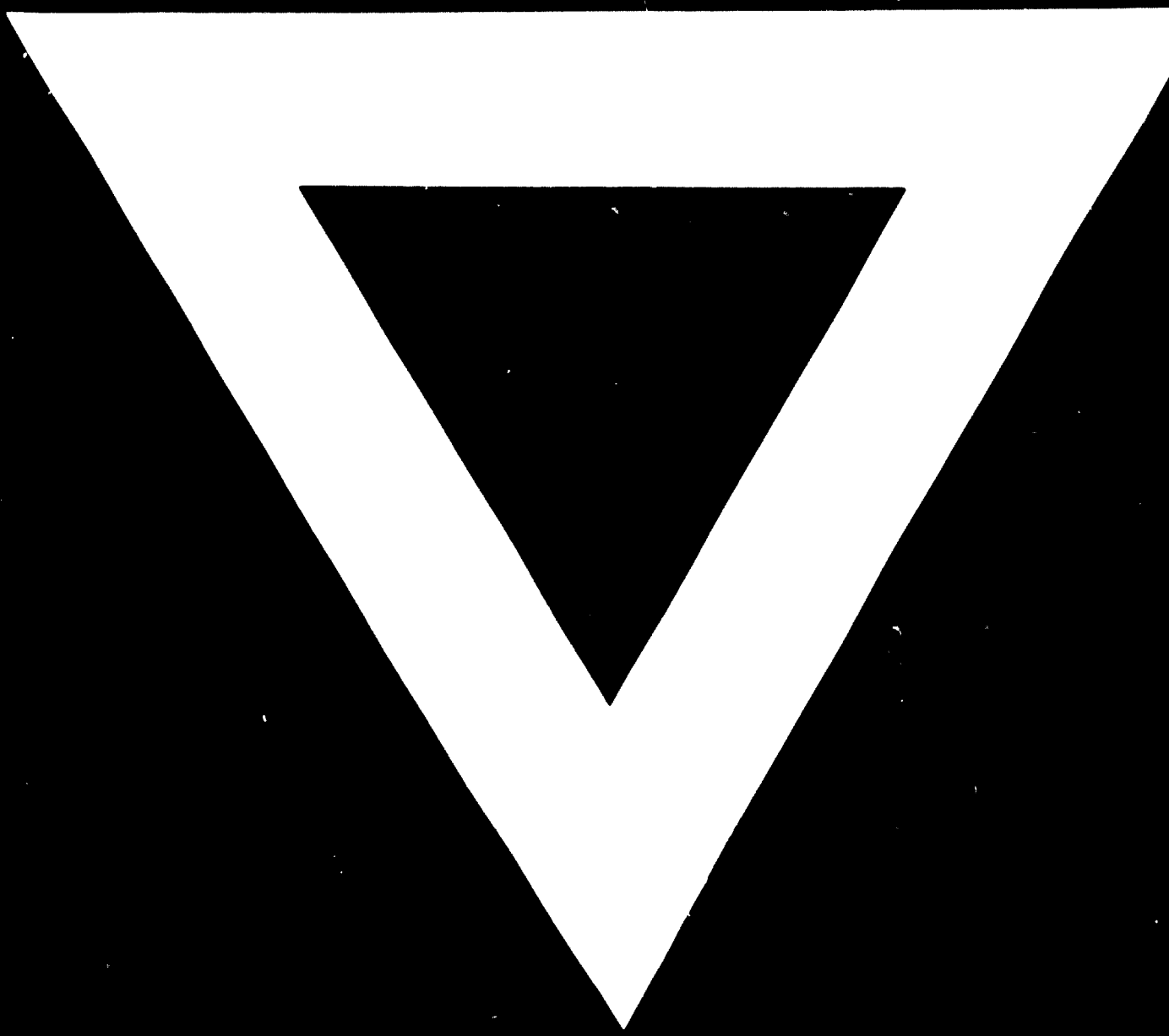

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