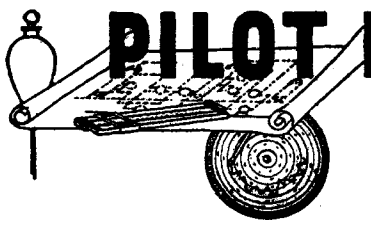

Itaconic Acid by Fermentation with Aspergillus Terreus

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UNSATURATED carboxylic acids have found widespread commercial uses, especially in the field of synthetic resins and plastics. Itaconic acid, an unsaturated dicarboxylic acid with interesting commercial possibilities, may be produced by the dehydration or pyrolysis of citric acid (*2, 3, 37, 41*), by the decarboxylation of aconitic acid (*1, 35*), and by the fermentation of sugar solutions with molds (*21, 42, 43, 45*).

Research on its production by fermentation has been conducted at the Northern Regional Research Laboratory. Early laboratory studies evaluated various strains of *Aspergillus terreus* for use in surface culture fermentations for producing itaconic acid from glucose solutions (*29–31, 33*). Submerged and agitated cultures were studied later (*26–28, 34*). The project has culminated with pilot plant scale evaluation of the fermentation and recovery processes.

The properties of itaconic acid are such that it might be used in much larger quantities than at present if it were available at reasonable cost. Esters of itaconic acid may be polymerized, or the esters may be copolymerized with other materials. The polymerized esters may be used as adhesives, plastics, elastomers, and surface coatings, while copolymers yield a variety of products including rubberlike masses, castings, resins of excellent strength and flexibility, impregnating and waterproofing compounds, and coatings of good electrical insulation and chemical resistance (*4, 6–19, 22–25, 33, 38–40, 44*). Ester of itaconic acid and high molecular weight alcohols have shown promise for use as vinyl plasticizers, while colorless polymeric plasticizers have been prepared by heating dibutyl itaconate with air or peroxides (*5*). There is some possibility that synthetic polyelectrolyte soil conditioners similar to Krilium may be prepared from itaconic acid.

This paper describes pilot plant experiments in which itaconic acid was produced by fermentation of corn sugar with *Aspergillus terreus* NRRL 1960 and also experiments dealing with the recovery methods used to obtain crystalline itaconic acid. This pilot plant evaluation was undertaken to find suitable operating conditions for the process on a commercial scale. An approximate cost estimate is included, based on engineering data obtained from the fermentation and recovery investigations.

Pilot Plant Experiments Were Run in 300- and 600-Gallon Fermentors

Fermentations were conducted in two fermentors, one of 300-gallon capacity constructed of Type 302 stainless steel and the other of 600-gallon capacity constructed of Type 347 stainless steel. The latter is provided with a 60-gallon seed tank constructed of Type 347 stainless steel.

The 300-gallon fermentor is 13 feet tall, 24 inches in diameter, and is equipped with a half jacket. A propeller-type blade is mounted on a horizontal shaft 15 inches above the dished bottom of the fermentor. Agitator speed is variable from 100 to 250 r.p.m. The temperature of the contents may be maintained within 0.5° F. of the desired temperature. A pipe cross sparger introduces sterile air to the fermentor immediately below the agitator. The air is sterilized by passing it through a column 10 inches in diameter containing 8 feet of 10–24 mesh activated carbon.

The 600-gallon fermentor, $7\frac{1}{2}$ feet tall and 48 inches in diameter, is equipped with a jacket and dished heads. Agitation is provided by a turbine-type agitator mounted on a vertical shaft with a speed range of 77 to 230 r.p.m. The agitator turbine rotates inside a wide circular deflecting shield. The temperature of the contents is maintained in the same manner as in the 300-gallon fermentor. Air entering the fermentor through a circular sparger 16 inches in diameter constructed of $1\frac{1}{2}$-inch pipe is directed against a target mounted below the turbine. The air is sterilized by passing it through a column 16 inches in diameter packed with 8 feet of 10–24 mesh activated carbon. Air is not prehumidified.

The 60-gallon seed tank is 4 feet tall, 22 inches in diameter, and is jacketed. Agitation is supplied by a two-bladed propeller mounted at the bottom of a top-entering shaft. Agitator speed is variable from 90 to 250 r.p.m., and automatic temperature control is provided. Sterile air is introduced by a $\frac{3}{4}$-inch pipe sparger near the bottom of the tank.

Foaming of the medium during fermentation is controlled by the addition of antifoam automatically.

Cultures Were Incubated at 93° F. for 48 Hours, with Aeration

Stock cultures of *Aspergillus terreus* NRRL 1960 are carried on malt agar of the following composition:

	%
Malt extract	2.5
Peptone	0.1
Dextrose	2.0
Agar	2.0

The medium used in the preparation of the inoculum is the same as that used for the fermentation:

	%
Glucose (monohydrate)	6.60
$(NH_4)_2SO_4$	0.27
$MgSO_4.7H_2O$	0.08
Corn steep liquor (as received)	0.18

For spore production, a Kolle flask containing malt agar is inoculated with *A. terreus* NRRL 1960. After 2 weeks at 86° F. a plentiful supply of spores is present. About 3 square cm. of spores are scraped from the surface to inoculate 750 ml. of sterile medium in a small flask. Contents of the flask are aerated for 48 hours at 93° F., and are used to inoculate 20 to 40 gallons of sterile medium in the seed tank.

Cultures in the seed tank are incubated at 93° F. for 48 hours at atmospheric pressure, with aeration at the rate of 1/2 to 1 volume of air per minute per volume of medium, and are adequate in volume to inoculate the 200- and 400-gallon fermentations.

Medium for Inoculum and Fermentations Contained about 6% Anhydrous Glucose; Sterilization Was Batchwise and Continuous

Most of the fermentations were carried out with medium containing about 6.0% anhydrous glucose and of the same nutrient composition as the inoculum medium. Sterilization was usually carried out batchwise with indirect steam. The pH of the medium was usually adjusted before batch sterilization to avoid loss of ammonia. After the medium was held at an elevated temperature for 30 to 60 minutes, it was cooled to 95° F., while a positive air pressure was maintained. For continuous sterilization the medium was pumped from a mixing tank to a steam jet heater at a constant rate. The steam heated the medium instantaneously to the desired temperature, after which the hot medium passed through insulated holding coils to a cooler, and thence to the fermentor. The medium was inoculated immediately. No pH adjustment was necessary with continuous sterilization.

Fermentations were usually conducted under 10 to 15 pounds per square inch gage pressure, with rates of aeration up to 1/2 volume per minute. Agitation was varied from 75 to 175 r.p.m., corresponding to an approximate net power input that varied from 0.2 to 2.0 hp. per 1000 gallons.

The course of the fermentation was followed by determining the decrease in reducing sugars (*36*), and itaconic acid production by titration with alkali. Final determination of the itaconic acid present was made by the bromine absorption method (*20*).

The following factors influencing the formation of itaconic acid by fermentation of corn sugar with *Aspergillus terreus* NRRL 1960 were investigated on a pilot plant scale: Methods of sterilization, antifoam agents, medium composition, inoculum, fermentation pressure, aeration and agitation, fermentation temperature, and pH adjustment. Conduction of the fermentation in a semicontinuous manner was also investigated.

Medium sterilization was accomplished successfully by both batch and continuous methods. Batch sterilization by indirect steam was conducted at 240° to 250° F. for 30 minutes at a pH of 5.0 or lower to prevent loss of ammonia. Continuous sterilization was conducted at 300° F. for 5 minutes with no pH adjustment, the natural pH of the medium being about 6.5. Indirect steam was used in preference to direct steam during batch sterilization to prevent iron particles from entering the medium, since iron is extremely toxic to this fermentation. Either continuous or batch sterilization with direct steam may be employed if the steam line is well trapped.

Since it was necessary to control foaming during the fermentation, a suitable antifoam was necessary. Soybean oil and a solution of 0.75% octadecanol in 95% ethanol were used. When soybean oil was used the rate of fermentation was reduced considerably. The octadecanol antifoam was used successfully, although excessive amounts caused some diminution in the rate of acid production.

Reduced Amounts of Ammonium Sulfate or Steep Liquor in Medium Slowed Fermentations

The concentrations of sugar, steep liquor, magnesium sulfate, and ammonium sulfate were varied in an effort to determine suitable conditions for high itaconic acid yields with satisfactory rates of fermentation. When the content of anhydrous glucose was in the range of 9 to 10% the initial fermentation rate was good, but it decreased rapidly, so that the over-all rate and the yield were low. Attempts to increase the over-all acid production rate in these media containing high sugar concentrations by further addition of nutrients or inocula after 90 hours were unsuccessful. Best results were obtained in media containing about 6.0% anhydrous glucose. Comparable results were also obtained in previous laboratory work by Nelson *et al.* (*34*). Results of typical experiments are shown in Table I.

Table I. Effect of Glucose Concentration on Itaconic Acid Production

(All fermentations conducted at 95° F., 100–125 r.p.m., and 12–15 lb./sq. inch gage)

Run No.	Original Glucose, %	Final Glucose, %	Inoculum, %	Air[a]	Yield[b]	Average Production Rate[c]
18	10.00	1.26	1	1/10	45.2	0.29
19	9.32	0.73	1	1/16	40.8	0.31
9	5.94	0.05	1	1/17	53.5	0.36
7	5.97	0.10	2	1/17	49.1	0.42
3	5.88	0.32	1	1/20	50.6	0.40
82	8.98	0.73	10	1/4	57.5	0.68
68	6.18	0.06	10	1/4	60.0	1.10
81	5.20	0.06	12 1/2	1/4	56.8	1.45

[a] Volumes of air per minute per volume of medium.
[b] Grams of itaconic acid produced per 100 grams of anhydrous glucose supplied.
[c] Expressed as ml. 0.1 *N* NaOH per hour for a 10-ml. sample.

In experiments with a medium containing 6.0% anhydrous glucose, 0.18% steep liquor, and 0.27% ammonium sulfate, it was found that the concentration of magnesium sulfate could be reduced to 0.075% without affecting the fermentation. Whenever the concentrations of ammonium sulfate or steep liquor were reduced, slow fermentations resulted. Replacement of ammonium sulfate with urea or ammonium nitrate resulted also in low fermentation rates and in low yields. Concentrations of steep liquor employed were in the range of 0.18 to 0.25%. If the fermentation rate was slow with a new batch of steep liquor, the steep concentration was increased until the normal rate was again reached.

Several attempts were made to replace the refined glucose with other carbohydrate sources—cane and beet molasses and hydrol. In these experiments no significant amounts of itaconic acid were produced. It is possible that these carbohydrate sources might

Table II. Effect of Inoculum on Itaconic Acid Production

(All fermentations conducted at 95° F., and 10–15 lb./sq. inch gage)

Run No.	Inoculum, %	Original Glucose, %	Final Glucose, %	Air[a]	Agitator Speed, R.P.M.	Yield[b]	Average Production Rate[c]
48	1	6.18	1.93	1/4	100	32.7	0.24
46	10	6.00	0.70	1/4	100	50.5	0.38
59	1	6.09	0.60	1/4	125	45.6	0.41
60	10	6.15	0.05	1/4	125	64.2	1.08
70	5	5.95	0.04	1/8	125	61.0	0.77
75	11	6.06	0.04	1/8	125	60.5	1.55

[a] Volumes of air per minute per volume of medium.
[b] Grams of itaconic acid produced per 100 grams of anhydrous glucose supplied.
[c] Expressed as ml. 0.1 *N* NaOH per hour for a 10-ml. sample.

be employed if changes were made in medium composition and operating conditions or by pretreatment of the sirup.

Table III. Production of Itaconic Acid by Semicontinuous Fermentation Procedure

(All fermentations conducted at 95° F., and 10–15 lb./sq. inch gage, with 1/4 volume aeration and 125 r.p.m. agitation)

Cycle	Original Glucose, %	Batch Yield[a]	Average Production Rate[b]
1	5.20	56.8	1.45
2	4.04	62.7	1.50
3	4.49	58.7	1.08

[a] Grams of itaconic acid produced per 100 grams of anhydrous glucose supplied.
[b] Expressed as ml. 0.1 *N* NaOH per hour for a 10-ml. sample.

Only 1% by volume of inoculum was needed for seed development, and 10% was used in the more successful fermentations. Results of typical pilot plant fermentations in which the percentage of inoculum was varied from 1 to 11 are given in Table II.

It may be possible to conduct this fermentation in a continuous fashion. One experiment was carried out in a semicontinuous manner. After 225 gallons of medium containing 5.2% glucose and 12.5% inoculum had been fermented completely, 150 gallons of the liquor were withdrawn and replaced with 150 gallons of sterile sugar-nutrient medium. After the latter medium had been completely fermented, the procedure was repeated. In each case satisfactory fermentation rates and yields were obtained. Results of these tests showed a slightly higher production rate for the semicontinuous fermentation compared with the normal batch fermentation with a 72-hour total cycle including cleaning, steaming, filling, and emptying. Results of these experiments are listed in Table III.

Early experiments were carried out at atmospheric pressure, while other variables were being studied. Later, the other variables were held constant while the fermentations were conducted under a positive pressure of 10 to 15 pounds per square inch gage. A substantial increase in the rate of acid production was noted. An increase of pressure to 30 pounds per square inch gage did not change the fermentation rate appreciably. Increase in pressure resulted in a decrease of foaming, with antifoam consumption decreasing accordingly. Results of typical experiments are shown in Table IV.

Table IV. Effect of Fermentation Pressure on Itaconic Acid Production

All fermentations conducted at 93° F., with 1% inoculum, 1/4 volume aeration, and 100 r.p.m. agitation)

Run No.	Fermentor Pressure, Lb./Sq. Inch Gage	Original Glucose, %	Final Glucose, %	Yield[a]	Average Production Rate[b]
30	0	5.94	0.05	44.4	0.32
31	0	6.09	0.08	50.3	0.43
33	12	6.12	0.06	55.1	0.79
28	15	5.61	0.08	52.0	0.72
27	30	6.03	0.10	50.8	0.68

[a] Grams of itaconic acid produced per 100 grams of anhydrous glucose supplied.
[b] Expressed as ml. 0.1 *N* NaOH per hour for a 10-ml. sample.

During the course of the experiments, many variations in aeration and agitation were studied. Since there are physical differences in the two fermentors, the optimum conditions are different for each.

Optimum conditions were found to be about 1/4 volume of air in each fermentor, with agitator speed of about 125 r.p.m. in the large fermentor and about 115 r.p.m. in the small fermentor. These conditions apply to batches of around 200 gallons. When 400 gallons were fermented in the 600-gallon fermentor, the amount of air needed for a satisfactory fermentation was only 1/8 volume.

In general, the fermentation rate was increased as the rate of agitation was increased, accompanied by a corresponding increase in the antifoam requirement. However, a point was finally reached where increased agitation was accompanied by such large antifoam requirement that an inhibitory effect set in which reduced the fermentation rate. At very high rates of agitation foaming was uncontrollable with 0.75% octadecanol in 95% ethanol.

The fermentation rate increased as the aeration was increased up to about 1/4 volume. A further increase in aeration did not increase the rate. High rates of aeration required excessive amounts of antifoam and resulted in lowering of the fermentation rate in some instances. It is assumed that the large amounts of antifoam required inhibited the fermentation in the same manner as was encountered with excessive agitation.

Results of typical experiments in which aeration and agitation were varied are shown in Table V.

Table V. Effect of Aeration and Agitation on Itaconic Acid Production

(All fermentations conducted at 93° F., and 12–15 lb./sq. inch gage with 1% inoculum)

Run No.	Air[a]	Agitator Speed, R.P.M.	Original Glucose, %	Final Glucose, %	Yield[b]	Average Production Rate[c]
1	1/20	100	6.15	0.18	45.3	0.28
7	1/16	100	5.97	0.10	49.1	0.42
28	1/4	100	5.61	0.08	52.0	0.72
33	1/4	100	6.12	0.06	55.1	0.79
20	1/2	100	6.21	0.43	45.0	0.55
21	1/2	100	6.00	0.04	50.4	0.85
33	1/4	100	6.12	0.06	55.1	0.79
47	1/4	100	6.03	0.05	58.8	0.84
51	1/4	115	6.40	0.05	56.4	0.90
53	1/4	115	6.03	0.07	57.9	1.21
45	1/4	125	6.06	0.06	60.0	0.43
43	1/4	150	6.65	3.07[d]	..	0.62

[a] Volumes of air per minute per volume of medium.
[b] Grams of itaconic acid produced per 100 grams of anhydrous glucose supplied.
[c] Expressed as ml. 0.1 *N* NaOH per hour for a 10-ml. sample.
[d] Foamed uncontrollably and was discontinued.

Fermentations at 95° F. with Medium pH of 5.0 Gave Highest Acid Production Rate

Fermentations were conducted with temperatures ranging from 93° to 103° F. Above 98° F. the enzyme system did not develop properly, so that fermentation rates were extremely low. Best results were obtained at 95° F. Results of typical experiments are shown in Table VI.

In early experiments using sulfuric acid for pH adjustment, the fermentation medium was sterilized at about pH 3.5, then lowered to pH of 1.9 to 2.1 before inoculation, the total required

Table VI. Effect of Fermentation Temperature on Itaconic Acid Production

(All fermentations conducted at 12 lb./sq. inch gage, with 1% inoculum and 1/4 volume aeration)

Run No.	Temp., ° F.	Agitator Speed, R.P.M.	Original Glucose, %	Final Glucose, %	Yield[a]	Average Production Rate[b]
47	93	100	6.03	0.05	58.8	0.84
49	93	100	6.09	0.06	57.1	0.79
51	95	115	6.40	0.05	56.4	0.90
53	95	115	6.03	0.07	57.9	1.21
36	98	100	5.97	0.05	56.0	0.80
42	98	100	6.15	3.45[c]	..	0.06
40	103	100	6.09	3.57[c]	..	0.08

[a] Grams of itaconic acid produced per 100 grams of anhydrous glucose supplied.
[b] Expressed as ml. 0.1 *N* NaOH per hour for a 10-ml. sample.
[c] Discontinued because of slow fermentation rate.

amounting to about 0.15% by weight concentrated sulfuric acid. When difficulty was encountered in recovering crystalline itaconic acid because of excess darkening and polymerization, the amount of sulfuric acid for pH adjustment was reduced to about 0.03%. In this procedure the pH of the medium was adjusted to 5.0 or below before sterilization, and no further acid addition was made. Finally, it was decided to eliminate sulfuric acid in order to facilitate crystal recovery, and itaconic acid itself was used to lower the pH of the medium before batch

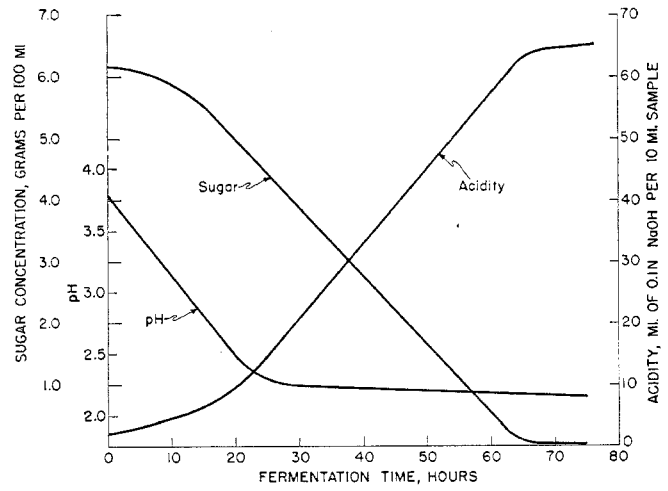

Figure 1. Changes in Medium during Fermentation

sterilization. About 0.05% of itaconic acid reduced the pH to 5.0 or below. This was sufficient to prevent loss of ammonia during batch sterilization. The increase in the starting pH from 2.0 to 4.0 to 5.0 did not slow the fermentation; in fact, the average rate of acid production was increased. Typical results are listed in Table VII.

Contamination of the medium by microorganisms was prevented by adherence to pure culture technique throughout the process. Because of the low pH developed during the fermentation, very little trouble was caused by contamination. Contamination by *Aspergillus niger* in the inoculum occurred in only two of a total of more than 80 pilot plant fermentations. In each case acid production stopped abruptly, although sugar utilization continued.

In several experiments the air supply was interrupted for periods of from 15 to 60 minutes. In each case the enzyme system was destroyed and the conversion of glucose to itaconic acid decreased to a negligible value. It was possible, however, to generate a new enzyme system by adding nutrients. After a proliferation period of about 20 hours, glucose was again converted to itaconic acid, although at a somewhat lower rate.

Crystallization of the Acid from Concentrated Solution Proved Best Recovery Method

Preliminary experiments were conducted to determine the simplest and most economical method of recovering itaconic acid from the fermented liquors. Solvent extraction with butyl and isoamyl alcohols was abandoned because of excessive esterification and polymerization during solvent removal. Solvent extraction may be possible with ethyl ether, ethyl acetate, or amyl acetate, although the solubility of the acid in these compounds at room temperature is below 3% by weight. Recovery of the acid by crystallization from concentrated solution in water was chosen because of the method's simplicity and the availability of suitable equipment.

After the fermentation was completed, the beer was filtered through a recessed-plate filter press. Air was then blown through the press. The mycelial mat peeled easily from the filter cloths. No filter aids were necessary.

The filtered liquor, after concentration to about one thirteenth its original volume in a 55-gallon stainless steel kettle, contained more than 40% itaconic acid. Evaporation under a vacuum of 15 inches proved satisfactory for both color of product and foam control. The kettle was heated with indirect steam at about 5 pounds per square inch gage. Some difficulty was encountered in emptying the kettle because of crystals deposited on the interior walls and coils. An evaporator employing forced circulation should prevent this crystal accumulation.

Crystallization of the hot, concentrated acid solution was carried out in a 30-gallon open-topped hemispherical kettle, equipped with a jacket and variable speed agitator. The jacket is connected with a temperature control system which allows crystallization to proceed at any rate desired.

Separation of itaconic acid crystals from the mother liquor was accomplished by centrifugation in a Tolhurst centrifuge equipped with a 26-inch basket. The crystals were washed with a minimum amount of cold water. They were scraped out of the basket and dried in a vacuum tray dryer under 26 inches of vacuum at 125° F.

The first mother liquor from the Tolhurst centrifugation was returned to the evaporator for further concentration to about half its volume under the same conditions as the first concentration. Experiments conducted to determine the best crystallization conditions for the second crop of crystals showed very little difference. The shortest crystallization time (16 hours) and cooling with 60° F. water were chosen for all succeeding crystallizations.

The second crop of crystals was removed by centrifugation in he same manner as the first. The second mother liquor was discarded, while the second crop of crystals was returned to be mixed with the filtered beer of a succeeding fermentation. In this way most of the impurities were discarded, and the crystal purity of the first crop was maintained in the range of 96 to 97%.

Nine 150-gallon batches of filtered beer containing between 3.6 and 4.0% itaconic acid were processed as described. The first four batches were processed to bring the system into equilibrium. Of the 238 pounds of itaconic acid contained in the remaining five batches, 206 pounds were recovered in the form of light tan crystals of 96% purity, containing 0.1% moisture—a net re-

Table VII. Effect of Initial pH and of pH Adjustment with H_2SO_4 or Itaconic Acid on the Production of Itaconic Acid

(All fermentations conducted at 93–95° F., and 10–15 lb./sq. inch gage, with 1/4 volume aeration and 100–125 r.p.m. agitation)

Run No.	H_2SO_4, %	Itaconic Acid, %	pH before Inoculation	Inoculum, %	Original Glucose, %	Final Glucose, %	Yield[a]	Av. Production Rate[b]
28	0.15	..	2.1	1	5.61	0.08	52.0	0.72
33	0.15	..	2.2	1	6.12	0.06	55.1	0.79
47	0.03	..	4.1	1	6.03	0.05	58.8	0.84
51	0.03	..	4.0	1	6.40	0.05	56.4	0.90
53	..	0.05	4.9	1	6.03	0.07	57.9	1.21
46	0.19	..	2.2	10	6.00	0.72	50.5	0.38
34	0.16	..	2.1	10	6.12	0.05	52.9	0.50
52	0.03	..	4.9	10	6.09	0.71	53.5	0.95
50	0.03	..	4.1	10	6.15	0.14	63.5	1.20
62	..	0.05	4.2	10	6.00	0.05	61.1	1.13
68	..	0.05	4.5	10	6.18	0.06	60.0	1.10

[a] Grams of itaconic acid produced per 100 grams of anhydrous glucose supplied.
[b] Expressed as ml. 0.1 *N* NaOH per hour for a 10-ml. sample.

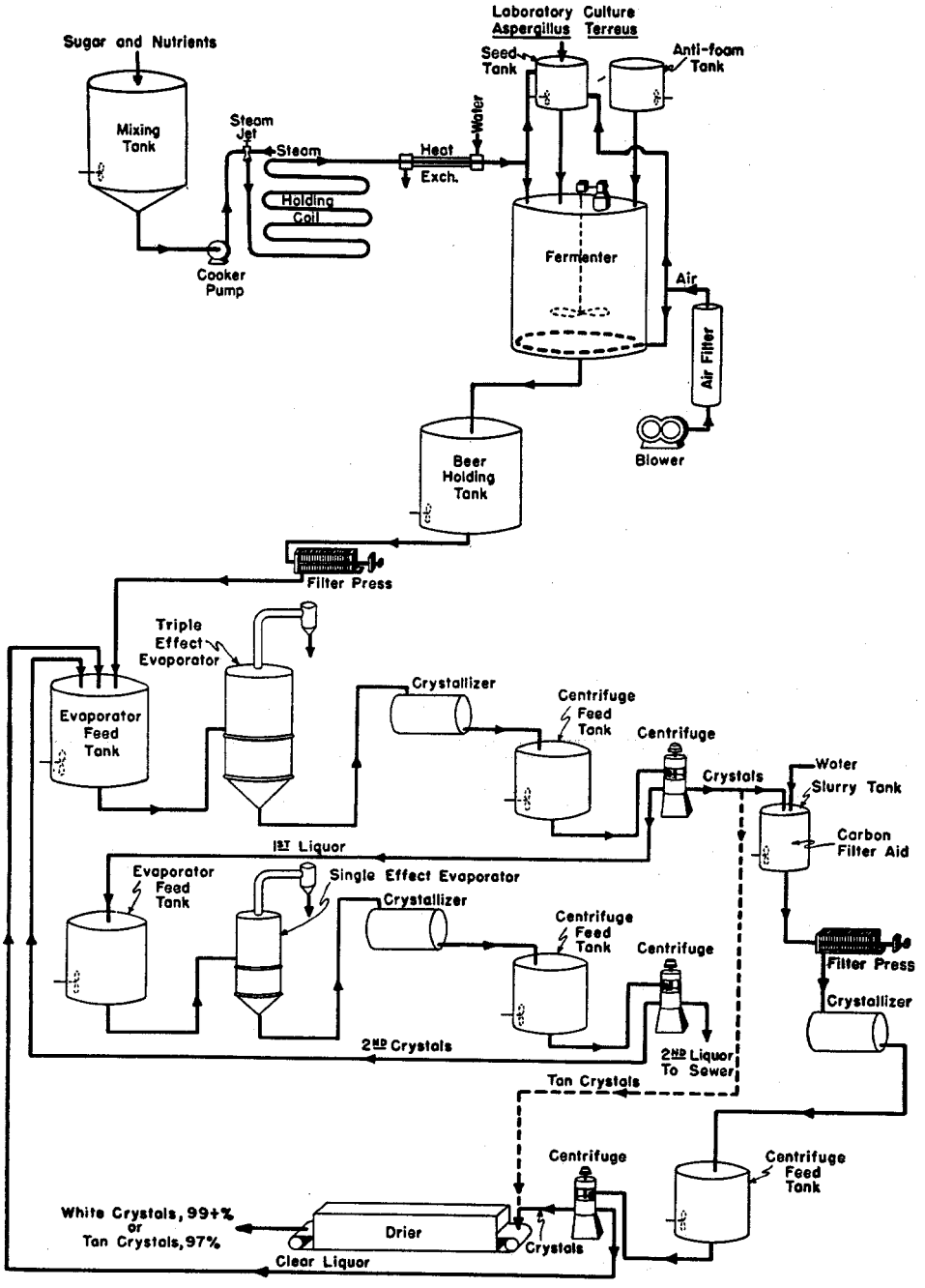

Figure 2. Flow Sheet for Itaconic Acid Production by Fermentation

covery of 86.5%. Although some mechanical losses were encountered, this recovery compares favorably with laboratory experiments on 20-gallon batches of filtered beer, which indicated a recovery of 91% of the total itaconic acid in the form of 97% tan crystals.

Since no further acid could be recovered by crystallization from the second mother liquor, the liquor was discarded in these tests, although some acid undoubtedly might be recovered from it by other procedures.

Decolorization of the tan crystals with carbon was necessary to obtain a white crystalline product. The tan crystals were dissolved in enough hot water to make a 25% solution. Darco G-60 carbon amounting to 2% by weight of the acid was added, and the solution was agitated for 30 minutes. Filter aid was added in an amount equal to the weight of the carbon, and the mixture was filtered in a steam-jacketed Sparkler filter. The clear filtrate with two hot water washings of the carbon cake was pumped to the crystallizing kettle. The washed carbon cake was discarded. The liquor was crystallized overnight at a final temperature of about 60° F. The crystals, recovered by centrifugation, were dried in a vacuum tray dryer to 0.1% moisture. They analyzed 99+% purity and 0.1% moisture. Eighty per cent of the acid in the crude crystals was recovered as white crystals, 1% was lost with the decolorizing carbon, and the remainder in the mother liquor was recycled in the recovery process.

Since crude itaconic acid exhibits a marked tendency to sublime, it may also be possible to purify the crude product on a commercial scale by sublimation.

All equipment contacting the itaconic acid was constructed of stainless steel.

The Crystalline Product Has Been Used Successfully for Forming Esters and as an Antioxidant

Analysis of the experimental results shows that itaconic acid may be prepared successfully from glucose by subjecting it to fermentation with the mold *Aspergillus terreus* NRRL 1960. Composition of the production and inoculum media are the same:

	%
Glucose monohydrate	6.60
$(NH_4)_2SO_4$	0.27
$MgSO_4.7H_2O$	0.08
Corn steep liquor (as received)	0.18

Media may be sterilized continuously at 300° F. for 5 minutes, or batchwise at 250° F. for 30 minutes. If batch sterilization is employed, the pH of the medium should be reduced to 5.0 with itaconic acid in order to prevent loss of ammonia during heating. Inoculum may be brought up in 1% stages, but 5 to 10% of inoculum should be used in the production fermentor for maximum rate and yield. A fermentation temperature of 95° F. is satisfactory, together with aeration at the rate of 1/8 volume of air per minute per volume of medium, pressure in the range of 10 to 20 pounds per square inch gage, and moderate agitation in the range of 1 to 2 h.p. per 1000 gallons. A suitable antifoam is 0.75% octadecanol in 95% ethanol. After 48 to 72 hours of fermentation, the maximum amount of acid will be present, equal to a yield of about 60 grams of acid per 100 grams of anhydrous glucose supplied.

Changes in pH, sugar concentration, and acid content of a typical fermentation (run 60) are shown in Figure 1.

Recovery of itaconic acid from the beer is accomplished by filtering the beer, concentrating the filtrate to less than one tenth

Table VIII. Estimated Investment Costs of Plants for Producing Itaconic Acid

	Tan Acid, Purity 97%[a]	White Acid, Purity 99+%[b]
Land and improvements	$ 35,000	$ 35,000
Building, 390,000 cu. ft.	200,000	...
Building, 420,000 cu. ft.	...	215,000
Equipment, delivered	440,000	530,000
Installation of equipment	110,000	130,000
Piping, wiring	110,000	130,000
Other construction costs	105,000	125,000
Contingencies, engineering, and contracting fees	300,000	335,000
Total plant cost	$1,300,000	$1,500,000

[a] Annual capacity, 3,000,000 pounds of 97% itaconic acid.
[b] Annual capacity, 2,910,000 pounds of 99+% itaconic acid.

its volume, crystallizing, centrifuging the first crop of crystals, concentrating the first mother liquor to one half its volume, and recovering a second crop of crystals as before. The second crop of crystals is recycled with filtered beer to be concentrated. In this manner, 90% of the total acid may be recovered as light tan crystals of 96 to 97% purity. Further purification to a white product of 99+% purity requires treatment in concentrated solution with 2% of decolorizing carbon, based on the weight of crystals treated, followed by filtration, crystallization, centrifugation, and drying.

Because of the low pH encountered during fermentation and recovery, all equipment used must be constructed of corrosion resistant materials.

A flow sheet for estimating production costs of the process under the conditions described is shown in Figure 2. Table VIII lists the land, building, and equipment necessary for plants producing approximately 3,000,000 pounds per year of light tan itaconic acid with purity of 97%, or white itaconic acid with purity of 99+%. The total estimated plant investments are $1,300,000 and $1,500,000, respectively. Estimated production costs for these plants are given in Table IX. These amount to 28.0 cents per pound for 97% itaconic acid, or 30.9 cents per pound for 99+% acid, with corn sugar at 7.5 cents per pound. These are plant production costs only; they do not include administrative and selling expenses.

Table IX. Estimated Plant Production Cost of Itaconic Acid

	Tan Acid, Purity 97%		White Acid, Purity 99+%	
	Annual cost[a]	Cost per pound	Annual cost[b]	Cost per pound
Raw materials	$483,400	$0.161	$496,100	$0.171
Utilities	76,400	0.025	76,400	0.026
Labor and supervision	66,500	0.022	77,400	0.026
Maintenance	57,950	0.019	67,500	0.023
Fixed charges	157,250	0.053	182,500	0.063
	$841,500	$0.280	$899,900	$0.309

[a] Annual capacity, 3,000,000 pounds of 97% itaconic acid.
[b] Annual capacity, 2,910,000 pounds of 99+% itaconic acid.

During the course of the developmental work, more than 3000 pounds of itaconic acid were produced by fermentation, and more than 500 pounds were recovered in crystalline form. The crystalline material were used successfully for forming esters with a variety of monohydric and polyhydric alcohols, polymers of these esters, and copolymers with methyl methacrylate. The pure acid was also successfully used as an antioxidant for soybean oil, in which the oil was stabilized through removal of metallic oxidation catalysts by metal chelation.

The strain of *Aspergillus terreus* employed in this investigation may be obtained from the Culture Collection Section of the Northern Regional Research Laboratory.

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