Citric Acid from Citrus Processing Wastes

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INTRODUCTION

The development of a fermentation process for converting the carbohydrates of citrus wastes to citric acid is a new, although not necessarily novel, approach to disposal of liquid wastes in the citrus industry.

Approximately 100 million lbs of citric acid are produced annually in the U. S., primarily by fermentation. This fermentation has been a commercial process for many years and provides about 90 per cent of the world supply of citric acid. No commercially feasible method exists for producing this compound by chemical synthesis.

Citric acid is produced from various sugars by the mold *Aspergillus niger*, usually employing such cheap raw materials as beet and cane blackstrap molasses. Although citrus molasses is comparable in most respects to these types of molasses and is considerably cheaper, only one reported attempt (1), which was unsuccessful, has been made to utilize it in this manner. According to published analyses (2), citrus molasses contains all of the constituents required for fermentation by *Aspergillus niger*. A comparison of citrus and blackstrap molasses is given in Table I. Adding to the attractiveness of this picture is the fact that a ready market for citric acid already exists in Florida where various industries utilize approximately five million lbs of this compound annually.

An expanding citrus industry, and the phenomenal and continuous growth in population of the State of Florida are factors which could, predictably, lead to friction over the mounting problem of disposal of citrus canning plant wastes. In the early days of the industry, most of the canning plants were located outside of small Florida towns and were sufficiently remote from residential areas that they caused few problems. Now, however, extensive residential construction to accommodate the heavy influx of immigrants to Florida has resulted in engulfment of most of these plants by residential areas.

Canning plants which dump their wastes onto surrounding vacant land or release them directly to streams and lakes, now find themselves under ever increasing pressure from the public, and State and Federal agencies to dispose of their wastes in a less offensive manner.

PRESENT METHODS OF WASTE DISPOSAL

There is no uniform method in the citrus industry for disposal of cannery waste water. Probably the most common method is spray irrigation, in which the wastes,

TABLE I

COMPARISON OF CITRUS AND BLACKSTRAP MOLASSES (2)

	Citrus Molasses		Blackstrap	
Constituent	Commercial*	Clarified Laboratory**	Louisiana	Cuban
^{o}Brix	72.0	73.1	90.7	87.2
Sucrose	19.6	26.1	30.1	37.3
Reducing Sugars, Per Cent	22.9	24.9	26.4	16.6
Total Sugars, Per Cent	43.5	52.4	58.0	55.8
Ash, Carbonate, Per Cent	4.7	3.0	10.8	10.9
Protein (N x 6.25), Per Cent	4.1	3.6	1.6	2.1
pH	5.0	5.9	5.7	5.5

*Average of 36 samples
**Average of 16 samples

after a minimum of screening or settling, are sprayed through sprinklers upon land planted with cover crops. Liquid wastes too heavy or strong for disposal in this way, are trucked to remote sandy areas and dumped.

Some plants still use lagooning, with or without mechanical aeration, while others use trickling filters. Activated sludge is in limited useage and gives excellent results, although due to the strength of much of the wastes and intermittent operation of the canneries, care must be used to avoid overloading. A few plants, anticipating future strict regulation, have constructed, or are constructing, waste treatment plants of various types.

Regardless of the method of waste treatment, such operations, which exist only for the purpose of destruction of wastes, must be considered as a total financial liability to industry. On the other hand, if these wastes could be converted to some useful product there would be an opportunity to recover at least part of the cost of disposal or perhaps, ideally, offset costs with returns. It seems reasonable that removal of an appreciable part of the BOD of citrus wastes by fermentation to some product such as citric acid would not only reduce waste disposal problems but would provide a salable by-product.

The order of magnitude of the disposal problem in the citrus industry can easily be visualized when it is pointed out that last year (1966-67 season), 146 million boxes (90 lbs) of oranges alone were harvested in Florida, of which, more than 124 million boxes were processed in canneries. In more familiar terms than boxes, the quantity of oranges processed was more than 11.1 billion lbs, or in excess of 5.5 million tons. Processing of grapefruit and tangerines accounted for 2.4 billion lbs or 1.2 million tons. The total of all citrus fruit processed was 6.7 million tons, approximately equal to the entire deciduous tree fruit production of the United States. Consideration of these figures makes it rather obvious that the waste disposal problem in this industry is of fantastic proportions.

TYPES OF CITRUS WASTES

Getting rid of solid wastes from citrus processing plants cannot be considered a real disposal problem. These wastes, consisting of pulp, peel, and seeds, instead of constituting a financial liability, are so efficiently utilized in a variety of ways that the processors actually realize substantial profits from marketing them as by-products. Most of this type of waste is converted to dried peel feed for cattle (or dried citrus pulp as it is known locally) which is probably one of the most outstanding examples of converting a huge waste disposal problem to a money making operation. The demand for dried pulp is consistently good with the result that a given year's production is generally exhausted prior to commencement of production in the following year. In addition, some of the components of this waste are removed during feed manufacture and processed to yield other by-products such as seed oil, flavonoids, pectin, and d-limonene.

As a consequence of the manufacture of dried pulp as a means of disposal of solid citrus wastes, a sugar-containing liquid, called press liquor, is produced. This press liquor creates a second disposal problem, which will be considered shortly.

The process used for producing dried pulp involves several steps which will be described briefly. The peel residue from the cannery is moved by conveyors to a hammer mill or shredder where it is chopped into small pieces. Lime is added and mixed thoroughly with the peel to degrade the pectin and promote release of the bound juices. The peel then goes through a pug mill and continuous presses where approximately 40 per cent of the bulk of the peel is removed as press liquor. This press liquor is evaporated in multi-stage evaporators to 71 - 72^{o} Brix molasses, while the solid material is dried in rotating driers to yield dried pulp feed. This dried feed is sold in 100-lb bags or in bulk, while citrus molasses is sold by the ton and is usually transported by tanker trucks.

The production of cirtus molasses cannot be considered a profitable operation since production costs and market prices are frequently about the same (about $14.00 - 16.00 per ton). Above the only justification for the manufacture of molasses is that it provides a means for disposing of press liquor and other concentrated wastes at break-even prices or, occasionally, a small profit. Most of this molasses is sold as a cattle feed. It is hoped that this unfavorable situation can be altered by better utilization of citrus molasses in a variety of fermentations, including the production of citric acid. Several of the characteristics of citrus press liquor and molasses which may suggest some of their potentialities are shown in Table II.

PRESENT STATUS OF THE FERMENTATION

It should be pointed out that this study has been underway for only one year and, as a consequence, much remains to be done. The information that has been obtained to date will be presented, however, as well as immediate expectations.

This project was designed to utilize both citrus canning plant waste water and citrus molasses. To avoid toxicity due to the high concentrations of detergents and alkalies present in clean-up waters, segregation of plant waste water is necessary. The preferred waste waters for fermentation use would include those of high BOD content which impose the greatest load on usual disposal operations. Examples of these wastes are shown in Table III. Fermentable sugars such as glucose, fructose, and sucrose account for roughly 70 per cent of the BOD of these liquids.

Although the wastes described have high BOD content, they are too dilute for use as fermentation substrates. Citrus molasses, by contrast, is much too concentrated for fermentation. Since it is desired to consume as much waste water as possible, the obvious solution is to use such waste water for dilution of molasses to the proper concentration for fermentation.

TABLE II

APPROXIMATE COMPOSITION OF CITRUS PRESS LIQUOR AND MOLASSES (2)

Constituent	Average Values Press Liquor	Molasses
pH	5.7	5.0
^{o}Brix	10.1	72.0
Sucrose, Per Cent	2.4	20.5
Reducing Sugars, Per Cent	4.2	23.5
Total Sugars, Per Cent	6.6	45.0
Protein (N x 6.25), Per Cent	0.5	4.1
Pentosans, Per Cent	0.3	1.6
Citric Acid, Per Cent	0.8	4.0
*Ash, Per Cent	0.7	4.7

*K, Ca, Na, Mg, Fe, P, Mn, Cu, Si, S, B

The optimum sugar concentration for this fermentation has been determined to be about 17 per cent total sugar, or approximately 20^{o} Brix, therefore, it is estimated that 600-700 gals of waste water could be utilized in diluting 175 gals (one ton) of molasses to the desired level. This has the added advantage of utilizing quantities of citrus molasses for which additional outlets are needed. At the present stage of development of this fermentation, tap water, rather than waste water, was used to dilute the molasses to the desired ^{o}Brix since it was less difficult to maintain a uniform substrate.

A problem that may be anticipated in using raw waste water as a diluent is the presence of peel oil which is toxic to many microorganisms. This oil is absent from molasses since it is volatilized and removed during the processing. Flash heat treatment of waste water prior to use should resolve this problem when such water is later substituted for tap water.

Cultures

Stock cultures of known citric acid producing strains of *A. niger* were obtained from the Northern Regional Research Laboratory, Peoria, Ill., and the American Type Culture Collection, Washington, D. C. Although these standard cultures appeared to be satisfactory for this fermentation, it was felt that isolation of additional cultures from natural sources might result in obtaining better adapted acid-producing strains of greater vigor. Twenty-five isolates were made from moldy citrus fruits using potato dextrose agar. These cultures were inoculated into the standard molasses medium and grown under controlled cultural conditions. Fourteen of these isolates were found to be good producers of citric acid. Several of the cultures produced greater quantities of citric acid, more rapidly, than the best of the stock cultures. A number of these cultures have been selected for later irradiation with ultraviolet light in an attempt to obtain mutants with enhanced citric acid productivity.

TABLE III

BOD CONTENT OF CITRUS PROCESSING WASTE WATERS (5)

Operation	mg/l BOD
Fruit Washing	500
Juice Extraction	500-1500
Juice Evaporator Condenser Water	0-500
Molasses Evaporator Condenser Water	0-500
Sectionizing	5,000- 10,000
Essential Oil Recovery	20,000- 45,000
Peel Bin Drip	60,000-120,000
Press Liquor	60,000-120,000

Optimum Temperature

The optimum temperature for production of citric acid by this mold is 28-30 C. This temperature range was used throughout this study.

Sugar and Nitrogen Content of the Fermentation Medium

Dilute molasses fermentation media were screened for suitability for the fermentation by using 300 ml quantities of medium in one-liter flasks with shaking at 150 rpm at 30 C. It was attempted throughout to obtain an adequate fermentation medium with a minimum of pretreatment or supplementation. The optimum sugar concentration of the substrate was found to be around 15-17 per cent. A rapid method of obtaining this approximate concentration was to dilute the molasses to 20^{o} Brix as determined by measurement with a refractometer. Naturally, for more specific determination of total sugar content, it was necessary to use somewhat better methods. A colorimetric method (6) was found to be satisfactory for this purpose.

Since excessive nitrogen has been shown to promote mycelial growth to the detriment of acid production (3) (a medium giving optimal growth of the mold does not provide good citric acid production), the natural nitrogen content of the diluted molasses (1-2 per cent) was considered to be adequate.

Growth Factors

Most investigators have found that *A. niger* does not require organic growth factors for citric acid production (3). Since molasses already contains a number of vitamins (2) and free amino acids (4), none were added to the medium.

Toxic Additives

Fermentation media containing certain toxic compounds were screened for suitability in the manner previously mentioned. Citric acid production by *A. niger* is stimulated when selected toxic agents are added to the medium. These agents are generally considered to be inhibitors of specific respiratory enzymes which results in accumulation of citric acid. The usual compounds employed were potassium ferrocyanide or methanol. The former agent, at a concentration of 0.3 per cent w/v, was found to be more effective than methanol at a variety of concentrations, although the latter compound showed some stimulatory effect.

Aeration and Oxygenation

The citric acid fermentation is a highly aerobic process, therefore provision was made for aerating the substrate during fermentation. Preliminary laboratory-scale fermentations were shaken vigorously to aerate the medium. The quantity of citric acid produced under these conditions was considerably less than expected which suggested that insufficient oxygen was reaching the culture. Since an increased rate of shaking was impractical due to size of the fermentation vessels, sparging with pure oxygen at a rate of 0.5 scfm during shaking was attempted.

Oxygenation in this manner, when commenced immediately after inoculation of the medium, inhibited development of the cultures and resulted in low acid production. By contrast, if the culture was permitted to develop for 24-48 hrs with only shaking, and then shaken during oxygenation for an additional 24-48 hrs, acid production was stimulated.

This delayed oxygenation treatment reduced the fermentation time from seven days to three-five days and doubled the quantity of acid produced. While this was promising, analysis of the fermented medium revealed that a considerable amount of sugar remained unconverted to citric acid. Work now in progress indicates that vigorous mechanical stirring before and during oxygen sparging, rather than shaking alone, will be necessary for the proper level of oxygenation, and therefore, maximum acid production.

Effect of Trace Elements

Reports in the literature are contradictory upon whether trace elements stimulate or inhibit citric acid production. The general consensus appears to be that citric acid accumulation occurs when the medium is deficient in one or more of the following: phosphate, maganese, iron, zinc, and possibly copper. The average values of trace elements present in citrus molasses are shown in Table IV. It should be noted that these analyses were obtained using 72° Brix molasses and that the content of these elements is probably no more than 25 per cent of the indicated values when molasses is diluted to 20° Brix. Two of these ions, iron and phosphate, appear to be important in the citric acid fermentation. Media which contained appreciable quantities of these elements consistently gave poor yields of citric acid (3). Apparently such ions are required for the normal growth of

TABLE IV

TRACE ELEMENTS IN 72° BRIX CITRUS MOLASSES (2)

	Per Cent		Per Cent
Potassium	1.1	Manganese	0.002
Calcium	0.8	Copper	0.003
Sodium	0.3	Silica	0.04
Magnesium	0.1	Sulphur	0.17
Iron	0.04	Boron	0.0006
Phosphorus	0.06		

A. niger, which prevents accumulation of citric acid. Removing or decreasing the iron and phosphate content of the media by absorption with cation exchange resins, or chemical treatment, resulted in markedly improved acid production. Although molasses seemed to be naturally deficient in these ions, it seemed desirable to further insure the elimination of iron by addition of potassium ferrocyanide to remove it from the medium. Addition of this compound was found to stimulate citric acid production when used at a rate of 0.3 per cent w/v. Ferrocyanide may have a secondary effect in addition to removal of iron, which may be inhibition of certain respiratory enzymes resulting in an accumulation of citric acid. Methanol has also been frequently mentioned in the literature (3) as exerting a toxic effect upon *A. niger*, perhaps in a manner similar to ferrocyanide, however, this compound had only a slight stimulatory effect on acid production in citrus molasses media.

Cationic exchange resins have been used successfully by other workers for purification of beet and cane molasses substrates prior to use in the citric acid fermentation (3). It seemed desirable to determine whether this would have similar effects with citrus molasses even though metals were present in only small amounts (Table IV). Samples of 20° Brix molasses were passed through columns of cation exchange resins and compared with untreated samples by fermentation under standard conditions. The treated molasses yielded somewhat more citric acid than untreated molasses, although the total time required for completion of fermentation was the same (seven days) with both types of sample.

Pretreatment of citrus molasses with ferrocyanide or cation exchange resins, or preferably both, is required to make it a suitable substrate for this fermentation.

Miscellaneous Pretreatment of Citrus Molasses

To determine whether the pectin constituent of molasses adversely affected citric acid production, comparative fermentations were run using depectinized and ordinary molasses. The enzyme, pectinol, was used for removal of pectin. No difference in citric acid production was obtained between these two types of molasses indicating that the small amount of pectin present in citrus molasses had little effect upon the fermentation.

Samples of molasses were also treated with activated charcoal prior to fermentation to determine the effect of this treatment on citric acid production. Although this part of the study is incomplete at present, there are indications that such treatment stimulates production of citric acid, probably due to absorption of unknown toxic factors. It is expected that this pretreatment will be particularly important in purifying waste citrus press waters to be used as the diluent for molasses. An additional effect, of perhaps great significance in later processing to recover citric acid, is the fact that charcoal absorption effectively decolorized the normally dark molasses medium. This suggests that it may be more advantageous to decolorize the substrate prior to fermentation rather than attempting to decolorize the citric acid during recovery operations.

SUMMARY AND FUTURE OUTLOOK

Laboratory-scale fermentation tests have shown that citrus molasses is a suitable substrate for citric acid production by *A. niger*. The optimum total sugar concentration of 15-17 per cent, was obtained by diluting the molasses with tap water to 20° Brix. Other required or desirable treatments included, adsorption with cationic exchange resins to remove contaminating metals and addition of 0.3 per cent potassium ferrocyanide to the medium to insure removal of iron. Optimum growth conditions included a temperature range of 28-30 C, vigorous shaking during the first 24 hrs of growth, followed by oxygen sparging during the next 24-48 hrs of growth. The usual fermentation time under these conditions was three-five days. It was suggested that increased efficiency in agitation during fermentation may be necessary. The maximum production of citric acid obtained under the conditions described was approximately 25 per cent of theoretical.

Upon the basis of the work thus far completed, it seems reasonable to predict that citric acid production by fermentation of citrus molasses and other liquid citrus cannery wastes is entirely feasible. The process presents excellent possibilities for consumption of appreciable quantities of both citrus molasses and selected canning plant waste waters, with a proportional reduction in the load on waste treatment facilities.

Calculation of the yield of citric acid which might reasonably be expected by fermentation of these citrus wastes indicates that with yields of 50-70 per cent of theoretical, approximately 450-630 lbs of citric acid could be produced per ton of citrus molasses. This quantity of product would have a gross value of $135.00 - 189.00. By contrast, citrus molasses at last report was selling for only $14.00 - 16.00 per ton. Fermentation of citrus molasses to citric acid would be a much more profitable method for disposing of this citrus waste than marketing it as cattle feed. Less obvious, but still significant, would be the additional savings realized through eliminating waste treatment of the waste waters consumed during the process.

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