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Contents

NUMBER 1, JANUARY 1953

NUMBER 2, MARCH 1953

NUMBER 3, MAY 1953

CONTENTS OF VOLUME I

NUMBER 4, JULY 1953

NUMBER 5, SEPTEMBER 1953

NUMBER 6, NOVEMBER 1953

Effect of Alcohols on the Mycological Production of Citric Acid in Surface and Submerged Culture

I. Nature of the Alcohol Effect

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There has been a steady increase in the consumption of citric acid during the past 25 years. An estimate by Cochrane (1948) places annual manufacture at approximately 35 million pounds. Commercial production of citric acid is reputedly based on fermentation by *Aspergillus niger* of a suitable grade of beet syrup in shallow pans. A submerged process appears to be highly desirable, and many articles and patents (Perquin, 1938; Karow, 1942; Szücs, 1944; Waksman and Karow, 1946; Karow and Waksman, 1947; Shu and Johnson, 1948a, b; Perlman, 1949; Schweiger and Snell, 1949; and Snell and Schweiger, 1949) have appeared in this field. Proposed procedures all require a source of highly purified carbohydrate and in most cases also the use of oxygen for aeration. Large-scale production of citric acid in submerged culture has not been reported. Several improvements in the submerged culture process are needed. These include the use of less inoculum, greater fermentation speed, and the use of crude, cheap carbohydrate sources.

Investigations on these problems have been in progress for several years in the Fermentation Division of this laboratory. It was discovered in the course of the work that the use of low molecular weight alcohols—methanol, ethanol or isopropanol—as adjuncts to the culture medium greatly increased citric acid production in both surface and submerged culture.[2] Such use has made it possible to ferment directly crude carbohydrate substrates which other investigators have found necessary to purify, especially for use in submerged culture. This paper deals with the nature of the alcohol stimulation as related to the presence of trace elements as well as to initial acidity of the medium and to the quantity of inoculum used.

GENERAL METHODS

The strains of the *Aspergillus niger* group of molds used were obtained from the Northern Regional Research Laboratory's Collection.

Surface cultures consisted of 50 ml portions of medium inoculated with dry ungerminated spores in 200 ml Pyrex Erlenmeyer flasks.

Submerged cultures consisted of 100 ml of medium including alcohol adjuncts and inoculum in 300 ml flasks. The given alcohol was added to the cool sterile medium just prior to inoculation. Inoculations were made with a suspension of germinated spores. Flask cultures were shaken either on a Ross-Kirshaw machine at 150 rpm or on a Gump shaker at 200 rpm. All cultures were incubated at 30 C unless otherwise indicated.

Nutrient salts were of C. P. grade. The corn steep liquor was a commercial grade containing about 50 per cent solids. The glucose was the ordinary commercial variety known as Cerelose[3] or Clintose[3] containing about 10 per cent water and considered to be glucose monohydrate. The sucrose was a commercial grade of high purity. Absolute ethanol and a synthetic grade of methanol were used.

Measurements of pH were made with a glass electrode. The cultures were harvested by pouring the mycelium and fermented liquor onto a muslin cloth strainer. The mycelium was squeezed by hand and then placed back into the flask with 50 or 100 ml of distilled water for surface or submerged cultures, respectively. This mixture of water and mycelium was heated to boiling and again squeezed through the cloth strainer. The two lots of liquid were combined and made up to the desired volume. Aliquots were taken for volumetric titration with 0.1 N alkali. Phenolphthalein was used as indicator. The mycelium was dried to constant weight at 90 C. Citric acid was determined by the methods employed by Wells *et al.* (1936), and oxalic acid was determined as calcium oxalate precipitated with $CaCl_2$. Glucose was determined by the Shaffer-Hartmann (1921) method and sugars reported on an anhydrous basis. Sporulation was estimated under a scoring system where a value of 5 represents a uniform heavy crop of spores.

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[2] The April 10 issue of Chemical Abstracts, 1951, carries an abstract of a paper by Sakaguchi and Baba showing that ethanol and methanol slightly increased citric acid yields.

[3] The mention of products does not imply endorsement or recommendation by the Department of Agriculture over other products of a similar nature not mentioned.