# SOME PRACTICAL AND THEORETICAL ASPECTS OF MOLD METABOLISM

O. E. MAY AND H. T. HERRICK

*Color and Farm Waste Division, Bureau of Chemistry and Soils, U. S. Department of Agriculture, Washington, D. C.*[1]

Detailed investigation of the metabolism of pure cultures of the lower fungi covers a period of forty years and may be dated from the researches of Wehmer concerning the production of oxalic and citric acids by species of Aspergillus, Penicillium and Citromyces. With gradual improvement in biological technic and with the establishment of order in the difficult classification of the various species of molds there has occurred a widespread interest in mycological chemistry which has been noticeably increased during the past decade. This work has resulted in the establishment of at least three industrial chemical processes in which the biochemical activities of molds are utilized. Various enzyme extracts are now obtained from molds on a large scale and are finding important application in different fields. Investigations concerning the composition of mold mycelia are being pursued in various laboratories and promise to yield results of considerable importance. This paper is limited, however, to a discussion of the production by molds of definite chemical products in the substrates upon which they are cultured.

## CITRIC ACID

The production of citric acid from sugars by molds was first investigated by Wehmer (1892) forty years ago. Since that time, particularly during the past fifteen years, the mycological formation of citric acid has been studied intensively (Bernhauer, 1932) and its industrial utilization has been successfully ac-

[1] Presented before 35th annual meeting of the Society of American Bacteriologists, Philadelphia, Dec. 27, 1933. 239th contribution from the Division.

complished. Production of citric acid in the United States by the mycological process is considerable, since large amounts are exported in addition to those required by domestic demand. Successful plant operation of the process has also been reported to be under way in Belgium, Czechoslovakia and Russia.

Recent investigations have brought forward new viewpoints of the chemical reactions leading to the formation of citric acid from sugars by molds and point to the possibility that the acid may result from a series of reactions analogous to those established in certain yeast and bacteria fermentations. Chrzaszcz and Tiukow (1930, 1932) have demonstrated the ability of certain Penicillia to produce citric acid along with succinic, fumaric and malic acids from ethanol and acetic acid and suggest the following series of reactions to explain the formation of these compounds:

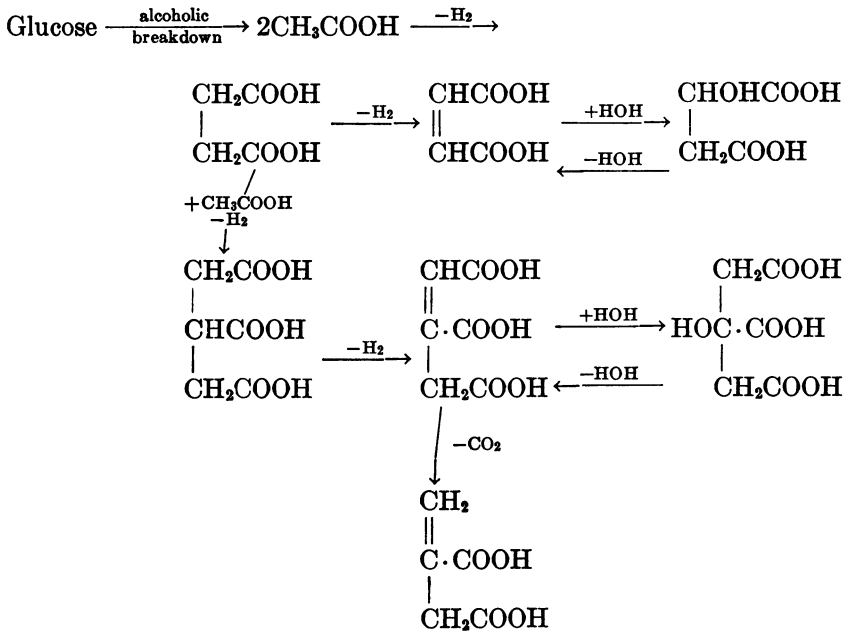
$$2CH_3COOH \xrightarrow{-H_2} \begin{array}{l} CH_2COOH \\ | \\ CH_2COOH \end{array} \xrightarrow{-H_2} \begin{array}{l} CHCOOH \\ \| \\ CHCOOH \end{array} \xrightarrow{+HOH}$$

$$\begin{array}{l} CHOHCOOH \\ | \\ CH_2COOH \end{array} \xrightarrow[-H_2]{+CH_3COOH} \begin{array}{l} \quad\quad CH_2COOH \\ \quad\quad | \\ HO\cdot C\cdot COOH \\ \quad\quad | \\ \quad\quad CH_2COOH \end{array}$$

In this connection it is of interest that the formation of succinic, malic and citric acids from acetic acid has been observed in oxygenated cultures of yeast (Wieland and Sonderhoff, 1932).

Bernhauer, Böckl and Siebenauger (1932) have extended and modified these concepts and have suggested that the formation of ethanol and acetic acid by molds is preceded by an alcoholic breakdown of the sugar according to Neuberg's scheme. This view has been supported by the isolation of methyl glyoxal from sodium hexose diphosphate cultures of citric-acid-producing Aspergilli (Suthers and Walker, 1932). Bernhauer and Siebenauger (1931) have suggested that citric acid is formed from acetic acid by way of aconitic acid in a manner analogous to the succinic-fumaric-malic acid relationships established in enzyme studies of certain

bacteria, and they have cited experimental evidence that citric-acid-producing organisms can not only transform aconitic acid to citric acid but can also bring about the reverse reaction. While aconitic acid has not been isolated from sugar cultures of molds it is of interest that itaconic acid has been so recovered in appreciable yields (Kinoshita, 1931). The following series of reactions expresses the mechanism by which citric acid may be formed according to Bernhauer's concept:




The formation of fumaric and succinic acids by molds is of considerable interest and significance. Butkewitsch and Federoff (1929, 1930) have suggested that succinic acid is formed from acetic acid which in turn is produced by oxidation of ethanol or dismutation of acetaldehyde arising from the breakdown of sugars, basing their statements on experiments carried out with species of Mucor. Fusaria as a rule produce ethanol in appreciable quantities when grown under the usual conditions of surface culture on glucose solutions (Anderson, 1924; Birkinshaw

et al., 1931; Letcher and Willaman, 1928) and there is some evidence that its formation follows the usual course found in yeast fermentations (Anderson, Everett and Adams, 1933; White and Willaman, 1928). Unpublished work carried out in the Color and Farm Waste Division with certain species of Fusarium has demonstrated that, if these organisms are grown submerged in well-aerated glucose solutions containing $CaCO_3$, appreciable quantities of succinic acid are formed together with noticeable amounts of acetaldehyde and acetic acid. The quantity of ethanol produced under these conditions is much less than in surface cultures. In this case succinic acid is produced definitely at the expense of the glucose, probably according to the mechanism proposed by Butkewitsch and Federoff.

The close relationships obtaining in the formation of citric, fumaric, malic and succinic acids and their direct connection with ethanol and acetic acid indicate a common mechanism governing their formation up to a certain point. The preponderance of one or more end-products probably depends on the nature of the enzymic complexes of the particular organism and on the cultural conditions which determine which complex will function most effectively.

## GLUCONIC ACID

Although not discovered among the products of mold metabolism until 1922, gluconic acid has since been recognized as a commonly-occurring substance in glucose cultures of molds (Bernhauer, 1932). Rather detailed studies of the oxidation of glucose to gluconic acid, including semi-plant scale investigations, have been carried out in the Color and Farm Waste Division (May et al., 1929). The process is operated in much the same manner as that used for the production of citric acid. Shallow aluminum pans are used for culture vessels and yields of around 60 per cent of theory are readily obtained from 20 to 25 per cent commercial glucose solutions. To overcome the obvious disadvantages inherent in the use of shallow pans some studies have been made of the production of gluconic acid by submerged mold growths in aerated glucose solutions. When the

oxidation is carried out under increased air pressures and in the presence of $CaCO_3$, yields of gluconic acid from 80 to 90 per cent of theory are obtained from 20 per cent commercial glucose solutions.

The mechanism governing the formation of gluconic acid is apparently one of simple oxidation. Although the possibility of a Cannizzaro reaction, involving the production of a molecule each of gluconic acid and mannitol from two molecules of glucose, has been pointed out (Raistrick and Rintoul, 1931), the enzyme studies of Müller (1929) indicate that the acid is formed by direct oxidation or dehydrogenation of glucose.

### KOJIC ACID

The production of kojic acid from sugars by species of the Aspergillus flavus-oryzae groups is an oxidation process that could be developed readily on an industrial scale in a manner similar to that employed for gluconic and citric acids should sufficient demand warrant it. Yields around 50 per cent of theory may be obtained from 20 to 25 per cent solutions of commercial glucose (May et al., 1931). Kojic acid may be formed by molds from a variety of carbon sources, ranging from starch and inulin to glycerol and dihydroxy acetone. Yields of 30 per cent have been obtained from the last-mentioned compound (Challenger, Klein and Walker, 1931)). As yet no completely acceptable mechanism has been suggested to explain its formation, and it is possible that an initial series of reactions are involved which are similar to those encountered in the production of citric and succinic acids. The flavus-oryzae group of Aspergillus is capable of producing ethanol under certain conditions of culture, indicating that these organisms possess the enzymic system required for the alcoholic breakdown of hexoses. It is of interest that the formation of kojic acid from mannitol by certain acetic acid bacteria has been reported (Takahashi and Asai, 1933).

### GALLIC ACID

This acid has long been known to be produced by the action of molds on tannin. In the modern process clear tannin extract

is sterilized and inoculated with a pure culture of a species of Aspergillus. The solution is aerated and agitated so that the organism develops in a submerged condition and hydrolysis of the tannin proceeds rapidly (Calmette, 1900). Fernbach (1900) and Potterin (1900) independently of each other demonstrated that the enzyme tannase could be separated from *Aspergillus niger* and could then be used to hydrolyze solutions of tannin to gallic acid.

## INDUSTRIAL ALCOHOL

The amylo process for the saccharification of starch, in which a species of Mucor is usually employed, is used to some extent in distilleries instead of the customary malting process. An account of the operation of the amylo process has been published recently (Owen, 1933). The cooked starch is inoculated with the organism and the mash is aerated and agitated. Hydrolysis proceeds rapidly and usually within twenty-four hours has reached a point which permits inoculation with yeast. Alcohol recovery follows the usual distillery practice.

The work of Raistrick and his associates (1931) deserves special mention in any discussion of mold metabolism. These investigators have surveyed systematically the biochemistry of more than two hundred species of molds and have isolated and identified many new compounds resulting from the metabolism of glucose (Birkinshaw and Raistrick, 1933; Charles et al., 1933; Oxford and Raistrick, 1933; Raistrick and Simonart, 1933). As space is too limited for any detailed account of this work, the interested reader is referred to the original publications for particular phases of these investigations.

## REFERENCES

ANDERSON, A. K. 1924. Minn. Studies in Plant Science, no. **5**, 237.

ANDERSON, A. K., EVERETT, E. L., AND ADAMS, P. D. 1933. Jour. Agr. Res. **46**, 473.

BERNHAUER, K. 1932. Die Oxydative Gärungen. Berlin.

BERNHAUER, K., BÖCKL, N., AND SIEBENÄUGER, H. 1932. Biochem. Ztschr. **253**, 39.

BERNHAUER, K., AND SIEBENÄUGER, H. 1931. Biochem. Ztschr. **240**, 232.

BIRKINSHAW, J. H., CHARLES, J. H. V., RAISTRICK, H., AND STOYLE, A. R. M. 1931. Phil. Trans. Roy. Soc. **220** (B), 93.

BIRKINSHAW, J. H., AND RAISTRICK, H. 1933. Biochem. Jour. **27**, 370.

BUTKEWITSCH, W., AND FEDEROFF, M. W. 1929. Biochem. Ztschr. **206**, 440; **207**, 302.

BUTKEWITSCH, W., AND FEDEROFF, M. W. 1930. Biochem. Ztschr. **219**, 87, 106.

CALMETTE, A. 1900. Ger. Pat. 129, 164.

CHALLENGER, F., KLEIN, L., AND WALKER, T. K. 1931. Jour. Chem. Soc. London, 16.

CHARLES, J. H. V., RAISTRICK, H., ROBINSON, R., AND TODD, A. R. 1933. Biochem. Jour. **27**, 499.

CHRZASZCZ, T., AND TIUKOW, D. 1930. Biochem. Ztschr. **218**, 73; **222**, 245.

CHRZASZCZ, T., TIUKOW, D., AND ZAKOMORNY, M. 1932. Biochem. Ztschr. **250**, 254.

FERNBACH, A. 1900. Compt. Rend. **131**, 1214.

KINOSHITA, K. 1931. Bot. Mag. Tokyo **45**, 60.

LETCHER, H., AND WILLAMAN, J. J. 1928. Phytopathology **16**, 941.

MAY, O. E., HERRICK, H. T., MOYER, A. J., AND HELLBACH, R. 1929. Ind. Eng. Chem. **21**, 1198.

MAY, O. E., MOYER, A. J., WELLS, P. A., AND HERRICK, H. T. 1931. J. Am. Chem. Soc. **53**, 774.

MÜLLER, D. 1929. Biochem. Ztschr. **205**, 111.

OWEN, W. L. 1933. Ind. Eng. Chem. **25**, 87.

OXFORD, A. E., AND RAISTRICK, H. 1933. Biochem. Jour. **27**, 634.

POTTERIN, H. 1900. Compt. rend. **131**, 1215.

RAISTRICK, H., *et al.* 1931. Phil. Trans. Roy. Soc. **220** (B), 1–367.

RAISTRICK, H., AND RINTOUL, W. 1931. Phil. Trans. Roy. Soc. **220** (B), 9.

RAISTRICK, H., AND SIMONART, P. 1933. Biochem. Jour. **27**, 628.

SUTHERS, A. J., AND WALKER, T. K. 1932. Biochem. Jour. **26**, 317.

TAKAHASHI, T., AND ASAI, T. 1933. J. Agr. Chem. Soc. Jap. **9**, 369.

WEHMER, C. 1892. Beitrage zur Kenntnis einheimischer Pilze. Hannover and Leipsig.

WHITE, M. G., AND WILLAMAN, J. J. 1928. Biochem. Jour. **22**, 583.

WIELAND, H., AND SONDERHOFF, R. 1932. Annalen. **499**, 213.