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Redox Potential in Submerged Citric Acid Fermentation

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Summary. The role and importance of the redox potential phenomena in submerged citric acid production are discussed. The redox potential of the fermentation broth is the result of oxydo-reduction processes where the metabolic activity of the microorganism *Aspergillus niger* plays the most significant role. The course of the redox curve for a good yielding citric acid production is presented and interpreted. The experiments of submerged citric acid production were carried out on beet molasses treated with potassium hexacyanoferrate and inoculated with *A. niger* spores.

Introduction

In living organisms redox systems play such an intimate and essential part that life itself might be defined as a continuous oxydo-reduction process (Hewitt 1950). It can be concluded that the reactions determining the redox potential in microbial media are complex and almost unknown. However, the redox potential is still a parameter which can give valuable information about the metabolism taking place in microbial cultures especially those continuously supplied with oxygen (Kjaergaard 1977). Some workers have advocated the use of redox measurements for monitoring and controlling the dissolved oxygen (Lengyel and Nyiri 1965; Wimpenny 1970). The reason for popularity of redox potential measurements is probably because it appears simple, i.e. requiring only a polished platinum electrode and a calomel reference electrode in conjunction with a recorder (Jacob 1970).

Redox potential determinations are also possible with use of redox dyes (Knight and Field 1930), but their use is not recommended because of the possible toxicity of some of them and of only semiquantitative results (Hill 1973).

In different aerobic processes the importance of the redox potential has been observed. In the case of biotechnological transformation of l-sorbose to 2-keto-l-gluconic acid by a mutant strain of *Pseudomonas*, Tengerdy 1971 found that the redox potential indicated the oxygen demand of the culture. It was demonstrated (Ishizaki et al. 1974) that the redox potential during the microbial production of inosine depended on the pH value, the dissolved oxygen concentration, the equilibrium constant, and on the oxydo-reduction potential in the liquid. During the biosynthesis of the antibiotics levorin A and levorin B produced by *Actinomyces levoris* it was found (Sukharevich et al. 1970) that at high redox potential values, compound A is produced and at low ones compound B.

Although the redox situation in the fermentation broth is reflected in the redox potential values measured, its characteristics cannot be generalized and for every particular microbial process the role of the redox potential should be studied.

Materials and Methods

Aspergillus niger, strain A60 (NRLL 2270) was used throughout all experiments. Keeping conditions and inoculum preparation were the same as previously reported (Jernejc et al. 1982). The initial spore concentration in the fermenter was 4.10^7 conidia/l. Deluted beet molasses (12,5% reducing sugar) was used as substrate.

The optimal addition of $K_4(Fe(CN)_6)$ was determined for every molasses sample by experiments in shaken cultures. It was added in two parts, before sterilization (primary additive) and after it (secondary additive).

Submerged citric acid fermentations were carried cut in a 5 l "Bioengineering AG" laboratory fermenter at a temperature of 30 °C. The stiring speed was 600 rpm, the aeration rate l/v/v/min. The redox potential measurements were carried out with a polished re-

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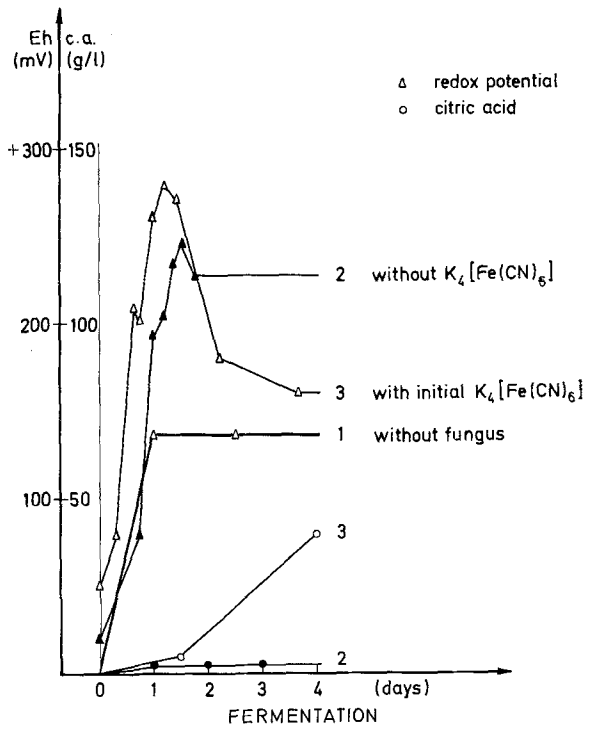

Fig. 1. Redox potential measurements and citric acid formation by *Aspergillus niger*. (1) non-inoculated sugar beet molasses medium + $K_4(Fe(CN)_6)$; (2) inoculated sugar beet molasses medium without $K_4(Fe(CN)_6)$; (3) inoculated sugar beet molasses medium with $K_4(Fe(CN)_6)$

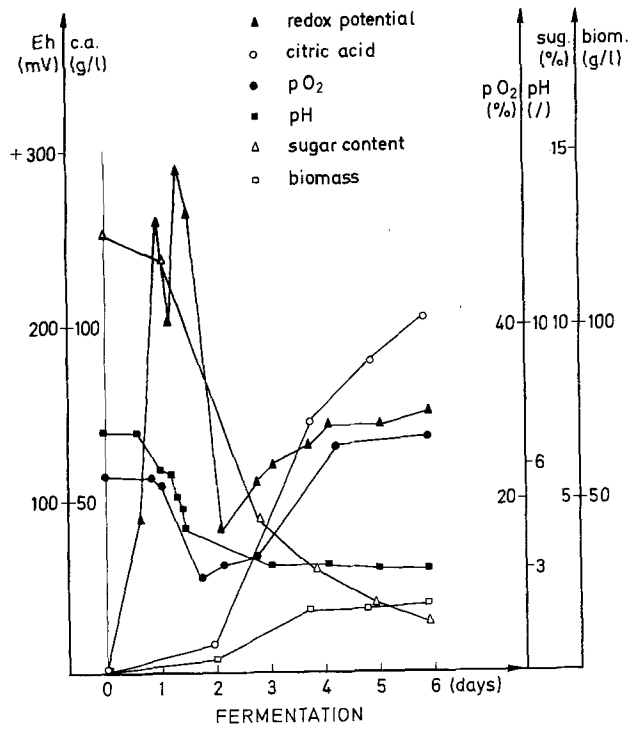

Fig. 2. Normal citric acid fermentation and redox potential: Experimental measurements of redox potential (Eh), dissolved oxygen partial pressure (P_{O_2}) P_{O_2}, pH, biomass (biom), citric acid (c.a.) and sugar (sug.) as described in text

dox electrode Pt-4865 (Ingold) and an amplifier 005-300000 (Intec, Stuttgart), the pH measurements with an electrode 465-35Kl (Ingold) and a pH meter M-7822N (Intec, Stuttgart). For the measurements of the O_2 partial pressure in the fermentation substrate a polalographic electrode (Industrial Lab.) and an "oxygen amplifier" (Instrumentation Lab., Type 531) were used. Citric acid was determined titrimetrically, biomass after drying at 105 °C, reducing sugar content by the Fehling method.

Results and Discussion

The redox potential in citric acid production media is of complex nature. As a sugar source beet molasses was used which contains different organic substances and several metal-ions. The balance of the latter in the fermentation broth is of essential importance for citric acid biosynthesis. The addition of $K_4(Fe(CN)_6)$ to the substrate causes the formation of metal-ion complexes which regulate the ion balance of the substrate (Clark et al. 1965).

The redox potential studies in beet molasses media, treated with $K_4(Fe(CN)_6)$ as primary and secondary additives (Cimerman et al. 1980) showed that in fermentation with high citric acid yields certain values of redox potential had to be reached.

The redox potential of a sterile aerated and uninoculated substrate with $K_4(Fe(CN)_6)$ addition is shown in Fig. 1, curve 1. Typical redox curves were observed in experiments using inoculated beet molasses without any addition of potassium hexacyanoferrate (Fig. 1, curve 2) and only with the primary addition of this compound (Fig. 1, curve 3) in the presence of *Aspergillus niger*. The effect of the microorganism itself on the redox potential changes in the substrate can be obviously seen.

The absence of potassium hexacyanoferrate in the substrate significantly influences the morphology of the production strain. Growth was diffused and did not show the productive pellet form and citric acid was produced only in traces.

The characteristics of a successful submerged citric acid fermentation on beet molasses are presented in Fig. 2. The redox potential curve is significant for the optimal process. It is characterized by two typical peaks with the characteristics of 260 mV after 24 h of incubation and 280 mV after 36 h and minimum of 200 mV after 28 h between these values.

The second peak coincides with the end of the exponential growth phase and the end of pellet formation. The decrease of the redox potential to 80 mV after 52 h can be compared with the finding that microbiological biosynthetic reactions proceed more favourably at redox potential values near the minimum of the redox curve of a particular culture involved (Tengerdy 1961). This was also found to be true for citric acid production (Hewitt 1950; Matkowitz and Kovac 1957). It seems that low redox po-

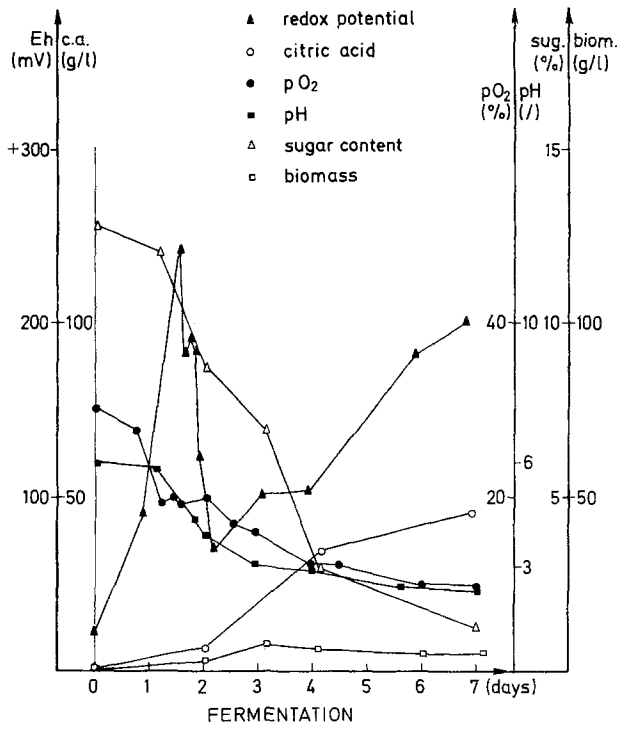

Fig. 3. Low-yielding, abnormal citric acid fermentation. Parameter measurements as in Fig. 2 and as described in text

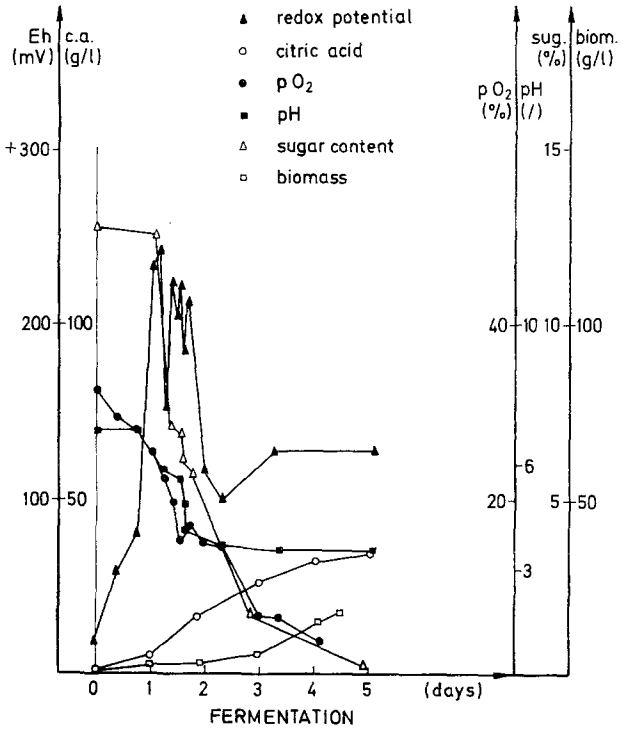

Fig. 5. Citric acid fermentation and effect of contamination on the course of the redox potential. All measurements as described in Fig. 2 and in text

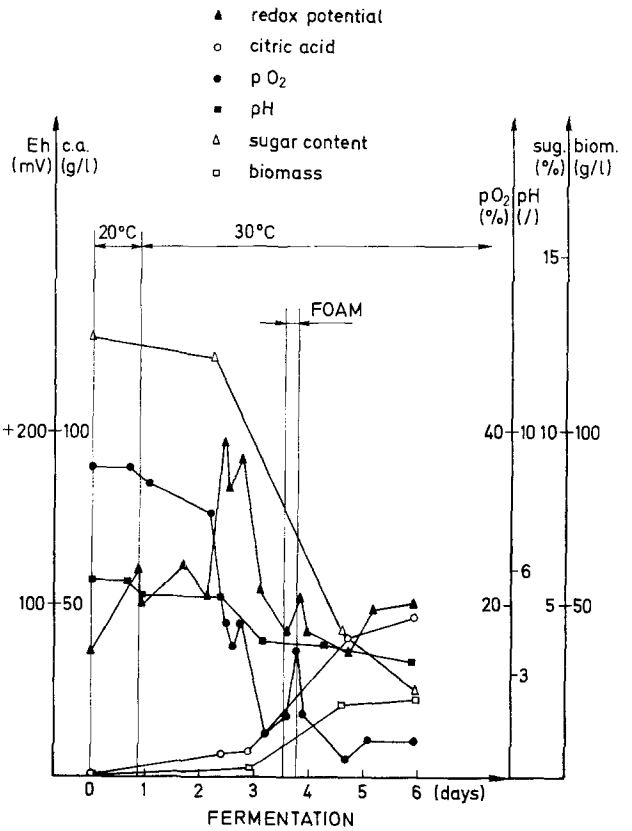

Fig. 4. Effect of a temperature shift on the citric acid fermentation with Aspergillus niger and sugar beet molasses medium. Experimental measurements as in Fig. 2 and as described in text

tentials (80 mV) are connected with the start of product formation (Tengerdy 1961).

In comparison Fig. 3 presents the characteristics of an unsuccessful fermentation which is also reflected by the course of the redox potential curve. The second peak is low and almost negligable (190 mV). Growth was diffused and the citric acid production minimal.

The effect of a temperature change is as well reflected in the course of the redox potential. Fig. 4 presents the data from an experiment started at an initial temperature of 20 °C. The temperature was changed after 20 h to 30 °C. The effect of this change can be significantly seen from the redox curve. In the same experiment foaming caused a loss of 1,000 ml substrate (89 h) which was also correlated with the formation of a new peak of the redox curve.

Another interesting change of the redox potential curve was caused by the occurrence of an unexpected infection of the substrate (Fig. 5). Microscopical control after 40 h of incubation revealed an abundant bacterial infection in the substrate.

It can be concluded that for an effective citric acid production, the values of redox potential peaks are important, as well as their time relationships. Deviations of ± 10 mV do not effect the results, but larger changes allow one to predict low yields of the product. The most critical time for the submerged citric acid production is the period of the first 36 h of fermentation. From the course of

the redox potential curve of this time interval, the success of the whole process can be predicted. This period is also indicated by changes in pH and pO_2, but only the redox potential curve is significant. The present method of redox potential measurements offers an effective and quick indication by means of which it is possible to estimate whether the process will be successful or not. If the continuous measurement of the redox potential is employed, it can save time, energy and costs with regard to the large scale production of citric acid.

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References

Cimerman A, Škafar S, Johanides V (1980) Yu Pat 35,459

Clark DS, Ito K, Thymchuk P (1965) Effect of K-ferrocyanide on the Chem. Composition of Molasses Mash Used in Citric Acid Fermentation Biotechnol Bioeng 7:269–278

Hewitt LF (1950) Oxidation-Reduction Potentials in Bacteriology and Biochemistry. ES Livingstone Ltd, Edinburg

Ishizaki A, Subai H, Hirose Y (1974) Basic Aspects of Electrode Potential Change in Submerged Fermentation. Agr Biol Chem 38:2399–2406

Jacob HE (1970) Redox Potential, Methods in Microbiology vol. 2/IV. Accademic Press, London New York

Jernejc K, Cimerman A, Perdih A (1982) Citric Acid Production in Chemically Defined Media by Aspergillus niger. Eur J Appl Microbiol Biotechnol 14:29–33

Kjaergaard L (1977) The redox Potential: Its Use and Control in Biotechnology. Adv Biochem Eng 7:131–150

Lengyel ZL, Nyiri L (1965) An Automatic Aeration Control System for Biosynthetic processes. Biotechnol Bioeng 7:91–100

Matkovics B, Kovatcs E (1957) Untersuchung der Zitronensäure Production und der Potentialveränderung in Oberflächen und Tiefkulturen. Naturwissenschaften 44:447–451

Sukharevich VI, Yakovleva EP, Tsyganov VA, Svezova NN (1970) The Effect of Aeration and Redox Potential of the Medium on Biosynthesis of Levorin A and B. Mikrobiologiya 39:981–985

Tengerdy RP (1961) Redox Potential Changes in the 2-keto-l gluconic Acid Fermentation – II. Relationship between Redox Potential and Product Formation. J Biochem Microbiol Technol Eng 3:255–260

Wimpenny JWT (1969) The Effect of Eh (redox potential) on regulatory processes in facultative anaerobes. Biotechnol Bioeng 11(4):623–629

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