UDC 543.138.3:547.477.1:664.15
ISSN 1330-9862 *original scientific paper*

(FTB-981)

Possibilities of Redox Potential Regulation in Submerged Citric Acid Bioprocessing on Beet Molasses Substrate

Marin Berovič[1,2]*, *Marjana Rošelj*[2] *and Mojmir Wondra*[3]

[1]Department of Chemical, Biochemical and Ecology Engineering, University of Ljubljana, Hajdrihova 19, SI-1115 Ljubljana, Slovenia

[2]National Institute of Chemistry, Ljubljana, Hajdrihova 19, SI-1115 Ljubljana, Slovenia

[3]Department of Food Technology, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia

Received: September 16, 1999
Accepted: May 17, 2000

Summary

In submerged citric acid production by *Aspergillus niger* on beet molasses substrate it has been found that for high yielding particular levels of the redox potential are of essential importance. Two maxima (260 and 280 mV) and two minima (180 and 80 mV) have to be obtained. Regulation of redox potential by chemical and physical methods was studied.

Bioprocessing could be significantly improved by appropriate redox potential regulation. Chemical methods comprising addition of oxidants and reductants did not block microbial growth, but evidently inhibited citric acid biosynthesis. The physical method of regulation, by variation of agitation and aeration, was found the most effective and appropriate for high yield in citric acid biosynthesis.

Key words: redox potential regulation, citric acid, beet molasses substrate

Introduction

In living organisms oxidation-reduction systems play such an intimate and essential part that life itself might be defined as a continuous oxidation-reduction reaction. It is not surprising, therefore, that theoretical speculations and experimental studies on oxidation and reduction processes in animals and plants have been actively pursued since the isolation of oxygen over 150 years ago *(1)*.

It becomes increasingly clear that redox potential is one of the most complex indicators of the physiological state of microbial cultures *(2)*. Just as the pH measurement represents a sum of all pH influencing componds, redox potential measurement represents a sum of all redox potential influencing compounds in bioprocessing broth *(3)*.

However, redox potential is a parameter that can give valuable information about the metabolism taking place in various aerobic and anaerobic microbial cultures *(4)*. Although insufficient attention was payed to this useful phenomenon in the past, there were some interesting and useful research studies that advocated the use of redox potential measurements for monitoring and controlling the dissolved oxygen *(5,6)*. At constant pH the relation between the redox potential and dissolved oxygen partial pressure can be simplified by logarithmic relation *(7,8)*.

In recent years much attention has been given to redox potential measurement and its uses especially in anaerobic bioprocesses *(9)*.

The importance of the redox potential measurements was also presented in articles on waste water bioprocessing, as in the case of propionate degrading *Methanospirillum* and *Methanocorpusculum* bacteria in a fluidized bed reactor, where degradation was inhibited

* Corresponding author; Phone: ++386 (0)1 1760 438; Fax: ++386 (0)1 1259 244; E-mail: marin.berovic@ki.si

at redox potential below –300 mV *(10)* or in anaerobic digestion in methanogenic bioprocessing in which volatile fatty acids were used as the substrate *(11).*

The redox potential measurement has also been found to be important in extremely thermophilic *Thermotoga* sp. bioprocessing where most of thermodynamic problems were associated with the relatively high redox potential *(12).*

In various aerobic processes the importance of the redox potential has been observed. In the case of the biochemical transformation of L-sorbose to 2-keto-L-gluconic acid by the mutant strain of *Pseudomonas (13)* it was found that the redox potential indicated the oxygen demand of the culture. During biosynthesis of antibiotics Levorin A and Levorin B produced by *Actynomyces levoris* it was found that at high redox potential compound A and at low redox potential compound B were produced *(14).*

For high product yields, the redox potential was also found to be a key parameter influencing the submerged citric acid biosynthesis on beet molasses substrate *(15,16).*

The importance of the redox potential was also very significant in bioprocessings with *Proteus vulgaris, Clostridium paraputrificium* and *Candida utilis (4), Lactobacilus sanfrancisco (17), Lactoccocus lactis (18) etc.* The results of fatty acids degradation by *Acetobacterium malicum* indicated the difference of redox potentials at which the electrons were released during oxydative pyruvate formation *(19).*

Xylitol formation by the recombinant *Saccharomyces cerevisiae* strain containing XYL1 gene of *Pichia stipatis*, was controlled by redox potential indication *(20).* In aceton-butanol bioprocessing by *Clostridium acetobutylicum* redox potential measurements were used in batch and continuous bioprocessing. A correlation between redox potential and a switch from an acidogenic to solventogenic metabolism was reported *(21).*

Although redox potential was studied in several bioprocesses, articles on regulation redox potential levels are more rare. In the experiments of Lengyel and Nyiri (22) and Patrick *et al. (23)* in various bioprocesses, the redox potential was regulated by oxidants. In experiments by Kjaergaard *(24)* and Kjaergaard and Joergensen *(25)* on *Bacillus licheniformis* reductants were used for maintainance of constant redox potential level, while in *Candida guilermondii* bioprocessing *(26)* the addition of *n*-paraffins was used as the carbon source to perform an iterative feeding of the substrate. Using addition of *n*-paraffins it was also possible to regulate cellular metabolism. The redox potential decreased after each addition of the carbon source due to increased metabolism of the increased amount of cell mass and to consequently increased oxygen demand, which for constant aeration implies a decrease in concentration of dissolved oxygen.

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

Materials and Methods

Microorganism

Aspergillus niger strain A60 (NRRL 2270) was used through all experiments. Conidia of 7-day-old cultures from malt agar slants incubated at 30 °C were used as inocula for the bioprocess. The initial spore concentration of the inoculum in all experiments was $5 \cdot 10^7$ conidia mL^{-1}. The innoculum spore concentration was controlled by the spectrometric method *(27,16).*

Media composition

The production substrate based on beet molasses included 12.5 % of the total reducing sugar. The optimal initial concentrations of $K_4Fe(CN)_6$, H_3PO_4 and pH were determined by previous experiments in shaken cultures. $K_4Fe(CN)_6$ was added to the production substrate in two portions *(28).* The bioprocess was carried out at 30 °C. Decrease of the bioprocessing broth volume, caused by evaporation and sampling, was taken into account in all experiments.

Bioreactor

All experiments were performed in a 18-liter laboratory Stirred Tank Reactor (STR) (Bioengineering AG, Switzerland). The working volume of the microbial culture was 15 L. The reactor was equipped with a sterilized Ingold pH electrode, redox electrodes and Industrial Lab $p(O_2)$ electrode, together with automatic foam control and temperature control unit. For on-line measurements Intec pH meter M-7822N and redox meter 005–300000 were used. On-line oxygen partial pressure measurements were performed by polarographic electrode Industrial Lab MFG 509 with IL amplifier Type 531. All experiments with chemical regulation of redox potential were conducted at constant aeration Q_g = 1 L L^{-1} min^{-1} and agitation N = 500 min^{-1}.

Analytical methods

Citric acid was determined by the Sanfran-Denstadt method *(27).* Biomass was separated from fermentation broth by filtration and, after drying at 105 °C to the constant weight (mass), gravimetrically determined. For total reducing substances concentration the Fehling method was used *(27).*

Results

It was found out earlier that to achieve high yield of citric acid the redox potential should follow the typical profile containing two maxima (at 260 and 280 mV) and two minima (at 180 and 80 mV) during the course of bioprocessing *(15,16).* Typical experiment for high-yield citric acid production is shown in Fig. 1.

Based on this finding, the main aim of this work was to develop a method for redox potential regulation and a process regulation tool that provide the essential redox levels needed for high citric acid production. Two different methods were tested: chemical, using oxidants and reductants referred in some other processes *(26,28)* and the physical method, based on simultanous regula-

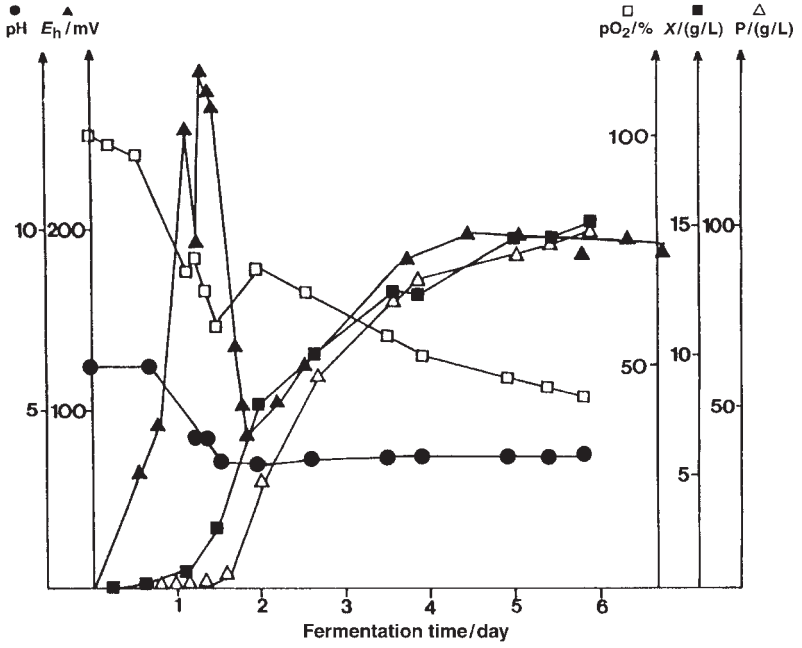

Fig. 1. Process parameters of high citric acid yielding bioprocessing; ▲ E_h redox potential, ❑ $p(O_2)$ dissolved oxygen partial pressure, ● pH, ■ *X* biomass, Δ *P* citric acid

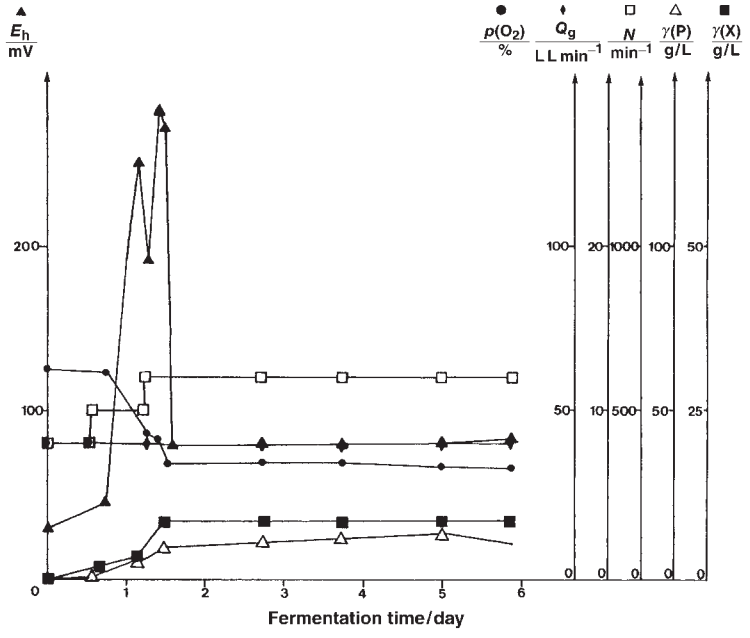

Fig. 2. Process parameters of citric acid bioprocessing at chemical regulation of redox potential using 0.1 % sodium sulphite as reductant and 0.1 % hydrogen peroxide as oxidant; ▲ E_h redox potential, ● $p(O_2)$ dissolved oxygen partial pressure, ■ *X* biomass, Δ *P* citric acid, ◆ Q_g aeration rate, ❑ *N* impeller speed

tion of agitation and aeration. The results of the most typical runs conducted under different experimental conditions are discussed and presented in Figs. 2–7.

Chemical methods

In the first part of our experiments, for redox potential regulation 0.1 % hydrogen peroxide, as oxidant and 0.1 % sodium sulphite as reductant, were used. The results of a typical experiment are presented in Fig. 2.

The first redox peak was reached at 260 mV. At this level no oxidant was added. Typical microbial growth forms were exhibited by boulbous cells. After the first maximum the first redox minimum at 180 mV was initiated by the addition of 80 mL of the solution containing 0.1 % of sodium sulphite. After this addition, the micro-

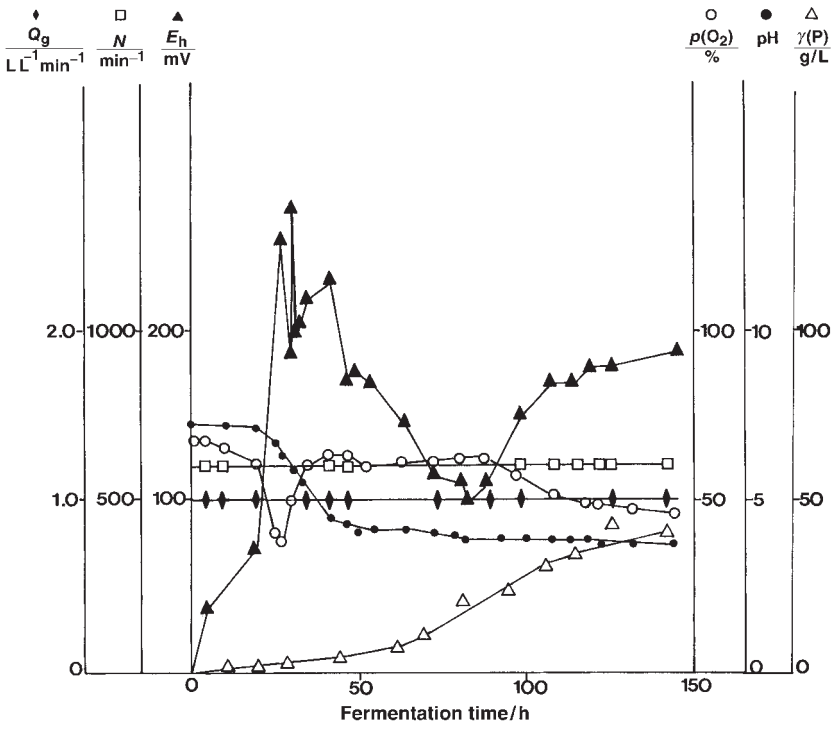

Fig. 3. Process parameters of citric acid bioprocessing at chemical regulation of redox potential using 20 % glucose as reductant and 0.1 % hydrogen peroxide as oxidant; ▲ E_h redox potential, ❍ $p(O_2)$ dissolved oxygen partial pressure, ◆ Q_g aeration rate, ❑ N impeller speed, Δ P citric acid, ● pH

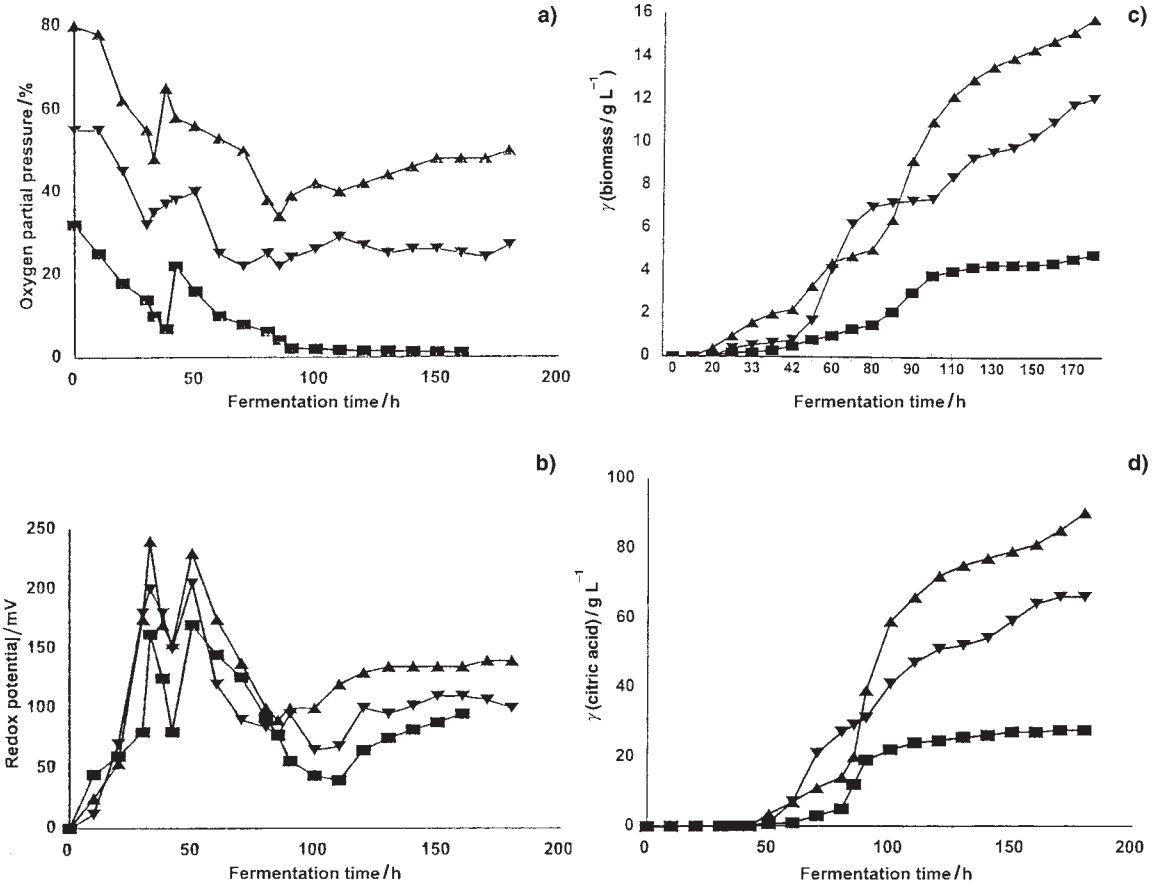

Fig. 4. Oxygen partial pressure (a), redox potential (b), biomass (c) and citric acid (d) time courses at aeration rates Q_g and impeller speed N; ■ Q_g = 0,4 L L^{-1} min^{-1}, N = 300 min^{-1}; ▼ Q_g = 0.6 L L^{-1} min^{-1}, N = 500 min^{-1}; ▲ Q_g = 1.0 L L^{-1} min^{-1}, N = 700 min^{-1}

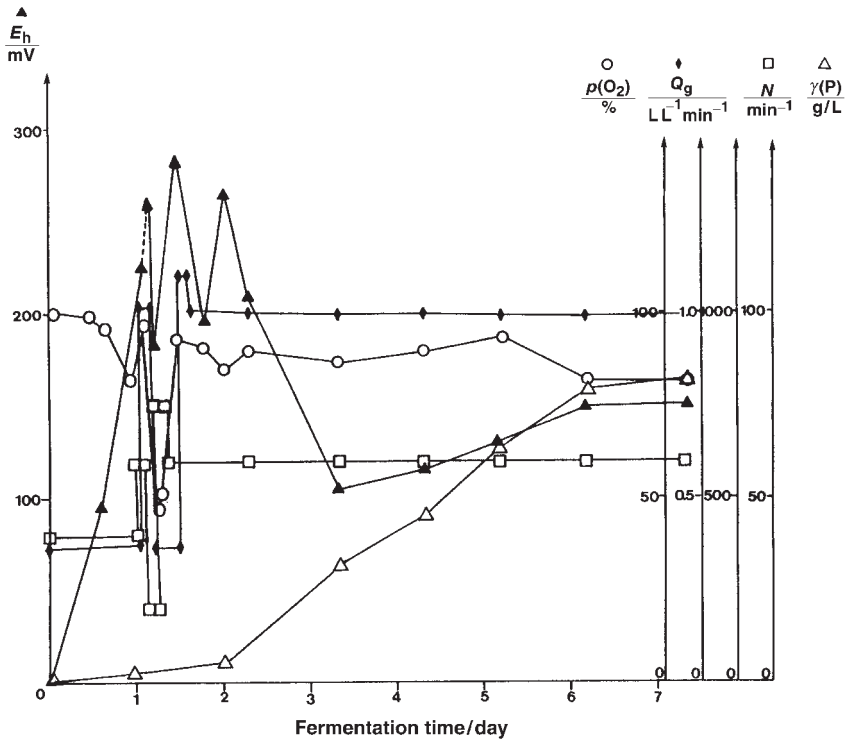

Fig. 5. Process parameters of citric acid yielding bioprocessing at physical regulation; ▲ E_h, redox potential, ❍ $p(O_2)$, dissolved oxygen partial pressure, ◆ Q_g aeration rate, ❑ N impeller speed, Δ P citric acid

bial growth turned from bulbous to filamentous hyphae growth forms. At the second redox maximum, at 280 mV, obtained by addition of 20 mL of 0.1 % solution of hydrogen peroxide, filamentous hyphal aggregates were the dominant growth form. The second redox minimum was obtained by the addition of 180 mL of sodium sulphite solution. After this, the microbial form did not change. Furthermore, the shear field in the substrate caused the formation of filamentous hyphal aggregates which were present up till the end of bioprocessing. This period was also followed by unchanged redox potential of 80 mV.

Although this method was well suited to the course of the optimised levels of the redox potential curve, the microbial growth after addition of sodium sulphite turned completely to low productive filamentous growth forms, with significantly reduced biosynthesis of citric acid. The addition of the reductant did not actually stop microbial growth, but it turned it into an unproductive growth form. After 168 h the average amounts were: 5.5 g/L dry biomass, ($Y_{X/S}$ = 9.5 %) and 12.8 g/L citric acid ($Y_{P/S}$ = 22.0 %).

In further experiments water solution of 0.1 % hydrogen peroxide as oxidant and 20 % of glucose as a reductant were used. In Fig. 3 a typical example of such experiments is presented. The first maximum at 260 mV was obtained at 25th h. In this period the bulbous cell agglomerates appeared.

After that the regulation of redox potential started. The first redox minimum at 180 mV was obtained at 30th h by addition of 275 mL of 20 % glucose in sterile demineralized water. The second maximum at 280 mV was reached soon after the addition of 26 mL of 0.1 % hydrogen peroxide. This phase was characterized by small spherical pellets with short and thin peripherial hyphae. The second mimimum of 200 mV appeared soon after the second peak. Soon after, the regulation was stopped. As the result of the uncontrolled process the third maximum at 240 mV appeared. In this phase mycelium growth in the pellet form with thin and long peripherial hyphae appeared. After that the third redox level at 100 mV appeared, and in the continuation it increased to 180 mV at the end of the bioprocess.

By use of these agents the regulation of the redox potential of the bioprocessing broth was made possible, the optimal course was well fitted and the additions of both compounds did not inhibit the developement of productive pellets. Mycelial pellets with thick and long peripherial hyphae were a typical morphology feature. After 168 h the average amounts of 11.1 g/L dry biomass ($Y_{X/S}$ = 13.5 %) and 40 g/L citric acid ($Y_{P/S}$ = 48 %) were obtained.

Physical methods

Physical parameters, such as temperature, bioreactor head-space pressure, agitation and aeration, strongly influenced the volumetric oxygen transport coefficient in the liquid phase. It is increasing by lowering the temperature and by increasing bioreactor head-space pressure, agitation and aeration. Higher oxygen partial pressure strongly affects the increase of the gradient between oxygen partial pressure in the liquid phase and that in microbial cell.

The total redox potential of the measured system is defined by Nernst equation:

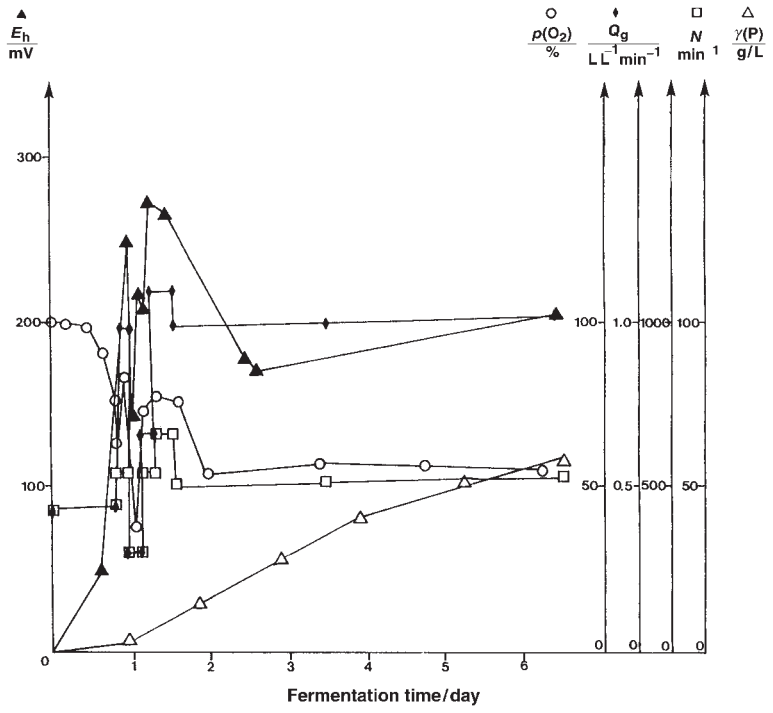

Fig. 6. Process parameters of citric acid yielding bioprocessing at physical regulation; ▲ E_h, redox potential, ❍ $p(O_2)$, dissolved oxygen partial pressure, ◆ Q_g aeration rate, ❑ N impeller speed, Δ P citric acid

$$E_h = E_o + (RT/n\,F) \cdot \ln (a_{oxidants}/a_{reductants}) \qquad /1/$$

The increasing activity of oxidants in the cells is also related to the activity of oxydants and oxygen partial pressure in liquid phase. It could strongly influence metabolic and enzyme activities in the microorganism *(29)*.

Citric acid bioprocessing is an oxygen sensible process. Various redox potential levels, biomass and citric acid amounts were obtained at various levels of oxygen partial pressure in the liquid phase Figs. 4 a-d.

As the experiments with oxidants and reductants did not give any significant results concerning citric acid formation, a physical methods such as a regulation of agitation and aeration were used. The possibilities of fitting the optimum levels of the whole redox potential curve and separated levels were tested.

First, the possibilities of regulation of both redox maximum and the first redox minima were tested (Fig. 5).

The first redox maximum at E_h = 240 mV appeared after 23 h (Q_g = 0.4 L L^{-1} min^{-1}, N = 400 min^{-1}). As the level of 240 mV was too low for the further process development, the optimal level of 260 mV was obtained by increasing the aeration rate Q_g to 1 L L^{-1}min^{-1} and agitation to 600 min^{-1}. By further reducing the aeration rate Q_g to 0.35 L L^{-1} min^{-1} and agitation to 200 min^{-1} after 30 h the first redox minimum at E_h = 180 mV was obtained. After this step, aeration was increased again to 1.2 L L^{-1} min^{-1} and agitation to 700 min^{-1}. After desired level of the second peak the aeration and agitation were reduced to 1.0 L L^{-1} min^{-1} and 600 min^{-1} and they were maintained unchanged until the end of bioprocess. The change in aeration and agitation induced the start of uncontrolled second minimum at 195 mV, which appeared after 38 h. As the reduction of aeration and agitation was too small, the level of the second minimum was too high and in the following process it jumped out of the control. Therefore the third redox potential peak at 260 mV appeared after 48 h. At these unregulated conditions, another 27 h were needed to obtain the minimum of 100 mV. Bioprocessing ended after 168 hours, with long and thin peripheral hyphae pellets. The average amounts of 11.2 g/L dry biomass ($Y_{X/S}$ = 10.1 %) and 62.5 g/L citric acid ($Y_{P/S}$ = 56.2 %) were obtained. (Fig. 5).

In this group of experiments regulation of the first redox minimum and both maxima were studied.

The first redox maximum at E_h = 260 mV was induced after 22 h by Q_g = 1.0 L L^{-1} min^{-1} and N = 600 min^{-1}. By reducing the aeration rate Qg to 0.3 L L^{-1} min^{-1} and agitation to 300 min^{-1}, after 24 h the first redox minimum at E_h = 140 mV was obtained. The second maximum level at E_h = 210 mV appeared after 26 h. By increasing of agitation to 700 min^{-1} and aeration to 1.0 L L^{-1} min^{-1} the correct level at 280 mV for the second maximum was obtained after 33 h. In further course the redox potential was not maintained, so it stayed constant at the same aeration and agitation rates. It decreased to the second minimum at E_h = 170 mV after 53 h. After the second maximum aeration and agitation were reduced to 1.0 L L^{-1} min^{-1} and 500 min^{-1}. Compact spherical pellets with thick and long peripheral hyphae were typical morphology forms which dominated until the end of the bioprocess. The bioprocessing conditions, aeration and agitation stayed constant until the end of the bioprocess after 168 h, when 72.5 g/L of citric acid ($Y_{P/S}$ = 58.0 %) and biomass 12.2 g/L ($Y_{X/S}$ = 9.7 %) were obtained (Fig.6).

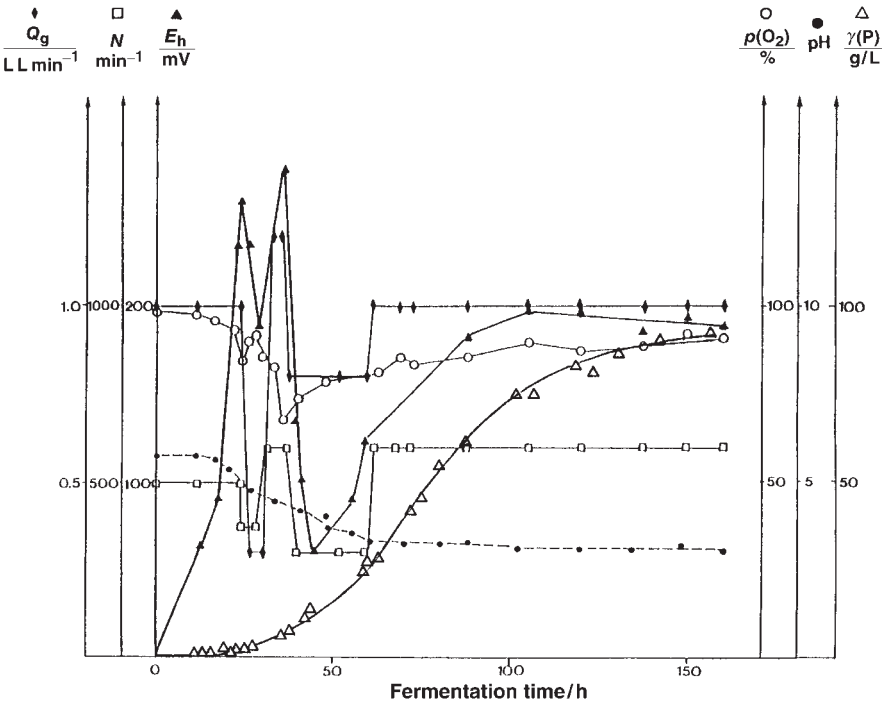

Fig. 7. Process parameters of citric acid yielding bioprocessing at physical regulation; ▲ E_h redox potential, ❍ $p(O_2)$, dissolved oxygen partial pressure, ◆ Q_g aeration rate, ❑ N impeller speed, Δ P citric acid

In the last group of experiments an attempt was made to fit the whole optimised redox levels profile by simultaneous regulation of aeration and agitation during the whole bioprocess. The first redox maximum E_h = 260 mV appeared after 24 h using aeration rate Q_g = 1.0 L L^{-1} min^{-1} and agitation N = 500 min^{-1}. By further reduction of the aeration rate Q_g to 0.4 L L^{-1} min^{-1} and agitation to 400 min^{-1}, the first redox minimum at E_h = 180 mV was obtained. The second maximum at E_h = 280 mV was obtained after 35 h of the bioprocess by increasing agitation from 400 to 600 min^{-1} and aeration rate from 0.3 to 1.2 L L^{-1} min^{-1}.

The second redox minimum at 80 mV was obtained by reducing aeration from 1.2 to 0.3 L L^{-1} min^{-1} and agitation rate from 600 to 200 min^{-1}. After maintaining this condition for three hours, agitation and aeration were changed back to 1.0 L L^{-1} min^{-1} and 600 min^{-1} and it was kept unchanged until the end of the bioprocess. At the end redox potential rose to 180 mV.

Table 1. The average results of all four group of experiments

	Examples of experiments				
	I Fig. 2	II Fig. 3	III Fig. 5	IV Fig. 6	V Fig. 7
t/h	168	168	168	168	168
$\gamma(S)/(g/L)$	67.7	42.8*	13.9	0.2	0.0
$\gamma(X)/(g/L)$	5.5	11.1	11.2	12.2	14.7
$\gamma(P)/(g/L)$	12.8	40.0	62.5	72.5	105.0
$Y_{P/S}$/%	22.0	48.0	56.2	58.0	84.0
$Y_{X/S}$/%	9.5	13.5	10.1	9.7	11.7

* reduced off 55 g/L added glucose

The mycellia morphology was similar to previous experiments including small compact pellets with short and thick peripherial hyphae, a highly productive form.

The bioprocess ended after 168 h with a yield of 105 g/L of citric acid ($Y_{P/S}$ = 84.0 %) and 14.7 g/L of biomass ($Y_{X/S}$ = 11.7 %) (Fig. 7).

In Table 1 the average results of all four groups of experiments are presented.

Discussion

For high citric acid yield during the bioprocess on beet molasses several phases are important.

The first phase is characterized by fast germinated conidia, at the begining, and at the end, by bulbbous cells, when the top of the first redox peak at 260 mV was reached. This phase indicated an increased respiration and growth of mycelia, followed with a significant increase in redox potential from 0 to 20 mV up to 260 mV.

After the first peak, in the second phase, a period of inhibition followed. In this period a significant decrease in respiration appeared, indicated by increased oxygen partial pressure profile (Fig.1) and followed by the first redox minimum at 180 mV after 28 h. This phase could be initiated by the presence of increased concentration of fructose obtained by derived of succrose hydrolysis.

At the begining of the third phase, typical for the second redox peak at 280 mV after 32 h microbial growth turned to spherical pellets, which are the productive form of citric acid.

In the fourth phase, after a large drop of redox potential to the second minimum at 80 mV after 36 h, abundant citric acid production started. As it was reported earlier *(13)*, at the lowest redox potential level the start of citric acid accumulation was indicated. Significantly low redox potential reveals the reducing state of the complex redox system of the bioprocessing broth, where the respiratory enzyme system signifies strong metabolic activity. It seems that start of citric acid biosynthesis *(30)*, as well as some other microbiological reactions, proceed favorably at the redox potential near the minimum of the redox curve of the particular culture involved *(1,13,25,31)*. This was found to be true also in riboflavin bioprocessing *(32).*

Using a chemical agent, sodium sulphite and hydrogen peroxide, the experiments showed that additional flux of sodium sulphite did not block microbial growth totally, but it significantly inhibited the microbial physiology and therefore citric acid biosynthesis.

Regulation of the redox potential by using hydrogen peroxide and glucose was a more suitable combination. This method favours formation of small spherical pellets with thin and long peripheral hyphae, that represented low citric acid productive growth forms. If regulation was stopped soon after the second maximum and the start of citric acid biosynthesis, the whole process jumped out of the ideal line and the new, the third maximum (at 240 to 270 mV) appeared.

The use of physical method, by regulating the aeration and agitation, seems to be more applicable and useful. Controlling the first maximum and the first minimum by simultaneous change of aeration and agitation, it was found that by stopping further aeration and agitation control at the level of the second minimum, the whole process escaped out of control continuing towards undesired levels. In this part a continuous maintenance of both parameters was necessary.

Similar results were obtained also at the fourth group of experiments, where after the second redox maximum the aeration and agitation stayed unchanged until the end of bioprocess. Although in this case spherical pellets with thick and long peripheral hyphae were obtained, fermentation ended with low yields of citric acid and biomass.

Finally, the last group of the experiments gave the appropriate answer to the problem. In this case regulation of the redox potential course proceeded over the whole range of the critical redox potential levels, fitting the optimal course by simultaneous step changes of aeration and agitation. In this case growth of *Aspergillus niger* in the most productive form (spherical pellets with thick and short peripheral hyphae) was obtained which resulted in citric acid yield in the range of 100 g/L in average.

Conclusions

For high citric acid yield in submerged bioprocess on beet molasses, two maxima (at 260 and 280 mV) and two minima (at 180 and 80 mV) of the redox potential were found to be optimal. These findings are also in good agreement with the results of Wimpenny and Neckelen *(2)*, who found that the most significant enzymes of the Krebs cycle relevant for citric acid biosynthesis are active at redox potential level from 200 to 300 mV.

Regardless of the chemical agens referred *(26,28)*, results in this paper do not confirm the use of oxidants and reductants as regulators of the redox potential during the citric acid bioprocess.

The best results for redox estimation were obtained using the physical method. This method was based on regulating too low/high levels of redox potential by step variation of aeration and agitation. It is a simple and practical approach based on changing the gradient of oxygen transfer in the bioprocessing substrate, which influences changes in intracelluar oxygen concentration, and therefore also the physiology of the cell. It regulates oxygen activity in bioprocessing broth by promoting or reducing the enzymatic activity in various stages of biosynthesis and it also does not bring any additional flux of redox regulating substances to the process.

In all those experiments where this method was applied and high reproducible citric acid production up to 110 g/L, with $Y_{P/S}$ = 84 %, was obtained.

References

1. L. F. Hewitt: *Oxidation-Reduction Potentials in Bacteriology and Biochemistry* 6th ed., Livingstone Edinburgh (1950).
2. J. W. T. Wimpenny, D. K. Neckelen, *Biochim. Biophys. Acta, 253* (1971) 352–359.
3. A. Kramli, S. Szabo, *Acta Biol. Acad. Sci. Hung. 6* (1959) 171–185.
4. L. M. Balakireva, V. M. Kantere, I. L. Rabotnova, *Biotechnol. Bioeng. Symp. 4* (1974) 769–780.
5. A. Ishizaki, H. Shibai, Y. Hirose, *Agr. Biol. Chem. 38* (1974) 2399–2405.
6. M. K. Radjai, R. T. Hatch, T. W. Cadman, *Biotech. Bioeng. Symp. 14* (1984) 657–672.
7. H. E. Jacob, *Biotech. Bioeng. Symp. 4* (1974) 781–788.
8. K. Memmert, C. Wandrey, *Proceedings of 4th European Congress on Biotechnology 3* (1987) 153–157.
9. S. Beck, B. Schink, *Arch. Microbiol. 163* (1995) 182–187.
10. B. Heppner, G. Zellner, H. Diekmann, *Appl. Microbiol. Biotechnol. 36* (1992) 810–816.
11. M. Peck, D. P. Chynoweth, *Biotechnol. Bioeng. 39* (1992) 1151–1160.
12. P. H. Janssen, H. W. Morgan, *FEMS Microbiol. Lett. 96* (1992) 213–218.
13. R. P. Tengerdy, *Biotechnol. Bioeng. 3* (1961) 255–272.
14. V. I. Sukharevich, E. P. Yakovljeva, V. A. Tsyganov, N. N. Shvezova, *Mikrobiologya, 39* (1970) 981–985.
15. M. Berovič, *Ph.D. Thesis*, University of Ljubljana (1986).
16. M. Berovič, A. Cimerman, BHRA, *3rd International Congress on Bioreactor and Bioprocess Fluid Dynamics*, A. Nienow (Ed.), Cambridge, MEP Publications (1993) pp. 533–545.
17. P. Stolz, G. Bocker, R. F. Vogel, W. P. Hammes, *FEMS Microbiol. Lett. 10* (1993) 237–242.
18. P. Vonktaveesuk, I. Tonokawa, A. Ishizaki, *J. Ferm. Bioeng. 77* (1994) 508–512.
19. J. Strochaker, B. Schink, *FEMS Microbiol. Lett. 90* (1991) 83–88.
20. J. Hallborn, M. F. Gorwa, N. Meinander, M. Penttila, S. Keranen, B. Hanhagerdal, *Appl. Microbiol. Biotechnol. 42* (1994) 326–333.

21. S. Penguin, G. Goma, P. Delorme, P. Soucaille, *Appl. Microbiol. Biotechnol. 42 (*1994) 611–616.
22. Z. L. Lenyel, L. Nyiri, *Biotechnol. Bioeng. 7* (1961) 91–100.
23. W. H. Patrick Jr., B. G. Williams, J. T. Moragham, *Soil Sci. Amer. Proc. 37* (1973) 331–332.
24. L. Kjaergaard, *Eur. J. Appl. Microbiol. 2* (1976) 215–220.
25. L. Kjaergaard, B. B. Joergensen, *Biotechn. Bioeng. Symp. 9* (1979) 85–94.
26. S. Y. Huang, C. S. Wu, *J. Ferment. Technol. 52* (1974) 818–827.
27. D. Mamilović, *M.Sc. Thesis*, University of Zagreb (1990).
28. A. Cimerman, S. Škafar, V. Johanides, *YU Patent P2481/74* (1974).
29. D. E. F. Harrison, *J. Appl. Chem. Biotechnol. 22* (1972) 417–440
30. B. Matkovicz, E. Kovacz, *Naturwissenschaften*, *44* (1957) 447–448.
31. L. Kjaergaard, *Adv. Biochem. Eng. 7* (1977) 131–141.
32. L. Kjaergaard, B. B. Joergensen, *5th International Bioprocessing Symposium*, H. Dellweg (Ed.), Berlin (1976) pp. 24–30.

Mogućnosti regulacije redoks-potencijala tijekom submerzne proizvodnje limunske kiseline na podlozi od melase iz šećerne repe

Sažetak

Određena razina redoks-potencijala bitna je za velike prinose limunske kiseline proizvedene s *Aspergillus niger* na melasi od šećerne repe. Za dobar prinos potrebna su dva maksimuma (pri 260 i 280 mV) i dva minimuma (pri 180 i 80 mV) redoks-potencijala. Regulacija redoks-potencijala ispitivana je kemijskim i fizikalnim postupcima. Odgovarajućom regulacijom redoks-potencijala moglo se bitno poboljšati iskorištenje u bioprocesu koji je imao malo iskorištenje. Kemijski postupci, što obuhvaćaju dodatak oksidansa i reducensa, ne sprječavaju rast mikroorganizama, ali očito inhibiraju biosintezu limunske kiseline. Najdjelotvorniji i najprikladniji bio je fizikalni postupak regulacije redoks-potencijala, koji se sastojao u promjeni miješanja i aeracije, kako bi se postigli veliki prinosi u biosintezi limunske kiseline.