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JERZY CZUBA

INCREASE OF ACETOBACTER BIOMASS AND ACIDIFICATION RATE IN SUBMERGED FERMENTATION

Institute of Fermentation Industry, Warszawa

Key words: acetic acid fermentation, *Acetobacter biomass*, submerged fermentation.

Bacteria growth and acidification rate during submerged acetic acid fermentation were investigated. It was demonstrated that in the considered conditions the functions of bacteria biomass increase and acidification rate are linear.

INTRODUCTION

The method of submerged acetic acid fermentation is being ascribed to Hromatka [1] and Haeseler [2]. Hromatka and co-workers are responsible for a number of practical findings applicable in the technology of the process [1, 3-5].

In experimental studies of *Acetobacter* respiration intensity during submerged fermentation at 9.5% total concentration* of the medium, the rate of acidity η ranged from 8.65 to 12.94 [g/100 cm^3] d^{-1}, and biomass concentration was 17-25.6 mg cell dry mass per 100 cm^3. Mean respiration rate, resulting from the obtained acidification rates, was of the order of 7750 cm^3 O_2 per g cell dry mass and hour, i.e. ca. 10.5 $mg \cdot mg^{-1} \cdot h^{-1}$.

In subsequent studies concerning the effect of temperature on the course of fermentation (11% total concentration in the medium) [5], the biomass content was not analysed, Hromatka [5] reports only the maximum acidification rates and the time of acidification rate doubling defined as

$$D = \frac{(t_2-t_1)\log 2}{\log \eta_2 - \log \eta_1}$$

where t — time, η — acidification rate.

* Total concentration is the combined concentration of acetic acid (in g/100 cm^3) and of ethyl alcohol (in % vol).

In the optimum temperature range of 24.3-29.7°C, the rate of acidification η was 13.08 for the lower end of this range, and 11.04 [g/100 cm^3] d^{-1} for the upper end; the corresponding times of acidification rate doubling were 4.72 and 4.37 h, respectively.

The first apparatus for submerged acetic acid fermentation is the Acetator, manufactured by Frings (FRG) according to Ebner's patent [6]. Similar equipment is also produced in the U.S.A. (Cavitator) [7], Austria (Acetator) [8], and Switzerland (Vinegator) [9]. Aside from the Cavitator which is used mainly for continuous acetic acid fermentation of low-concentrated mashes, all these apparatuses are used in the production of vinegar containing upwards of 10 g acetic acid per 100 cm^3 by semi-continuous fermentation consisting in periodic replacement of part of the fermented mash at continuous aeration of the fermented medium In most cases, 50% of the working capacity of the fermenter is replaced. When the fermented alcohol concentrations is 0.2-0.5% vol, half of the fermenter content is pumped out (this is the so called crude vinegar) and replaced with fresh mash containing 10-12% vol of ethyl alcohol and 1.0-1.2 g CH_3COOH/100 cm^3. Changes in acidity and bacteria biomass concentration during fermentation in a Frings Acetator are illustrated in Fig. 1.,

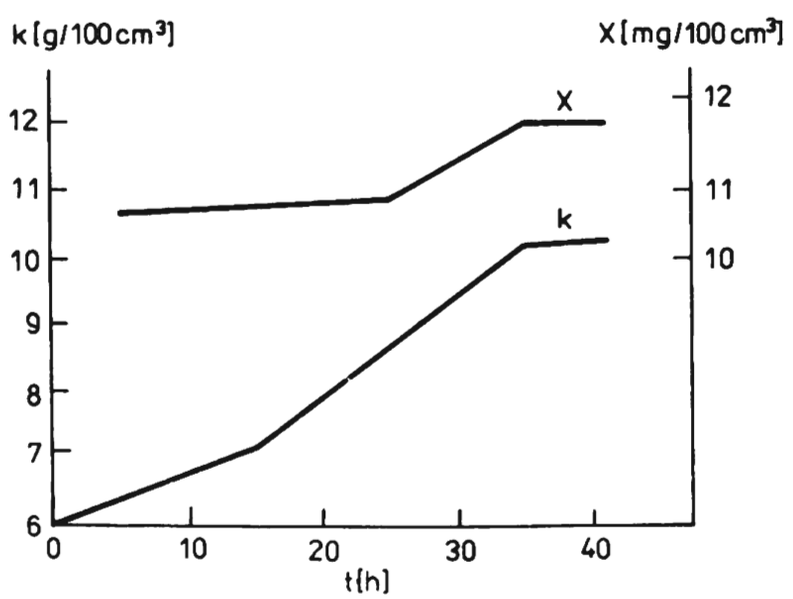

Fig. 1. Changes of acidity k and bacteria biomass concentration X during a typical fermentation in an Acetator manufactured by Frings (FRG)

The course of fermentation presented in the Figure departs from that ascertained by Hromatka and co-workers [1, 3-5]. It is thus possible that modern fermenters for submerged acetic acid fermentation significantly restrict the potential capabilities of the microflora active in this process.

AIM AND SCOPE OF RESEARCH

The aim of the research was to determine the dynamics of submerged acetic acid fermentation performed in conditions prevailing during production of vine-

gar over 10% in strength. The studies concerned the rate of growth of the biomass of bacteria active in the process, and the fermentation rate.

MATERIAL AND METHODS

MICROORGANISMS

Fermentation was started with *Acetobacter* microorganisms taken from an industrial fermenter together with fermenting mash.

FERMENTATION

The process was conducted on a micro-technological scale in fermentors of 1.8 dm^3 working capacity, equipped with self-suction agitators (1450 r.p.m). When alcohol content dropped to 0.4-0.6% vol, the crude vinegar was removed (0.9 dm^3 of it was left in the fermentor) and immediately replaced with 0.9 dm^3 of fresh mash containing 10% vol ethanol and 1 g acetic acid per 100 cm^3. The mash also contained suitable nutritive components. The experimental fermentation was carried out at 29°C and by an air flow amounting to 12.5 $dm^3\ dm^{-3}\ h^{-1}$.

Also industrial-scale experiments using Frings acetators were performed.

PROCESS CONTROL

Acidity and bacteria biomass concentration in the fermenting medium were checked every several hours. Acidity was determined by titration, and biomass content by nephelometry. Temperature, agitating speed and air flow were monitored and modified, if necessary. Alcohol content was determined in the final stages of fermentation in order to prevent its excessive decrease; the Semichon-Flanzy method was used here.

RESULTS AND DISCUSSION

MICROTECHNOLOGICAL-SCALE EXPERIMENTS

Several dozen fermentations were performed in the described conditions. Table 1 contains results of ten successive fermentation cycles of more or less identical course. The last of these cycles was subjected to detailed analysis, and the obtained results are collected in Table 2. For these results, the good approximation of function X (t) is a straight line described by the formula $X = 0.5180\ t + 8.8360$, or, generally, $X = \alpha t + X_0$, where X — bacteria biomass content at time t (in mg/100 cm^3 as cell dry mass), X_0 — biomass content at $t = 0$, α — linear coefficient of biomass increase (in mg/100 cm^3/h), t — time (in h). The correlation coefficient for the analysed values was 0.9980, and the confidence interval

Table 1. Analysis results for succesive fermentation cycles (t — fermentation time in h, k — acidity of the obtained vinegar (in g CH_3COOH/100 cm^3), X — bacteria biomass concentration in the obtained vinegar (in mg cell dry mass/100 cm^3)

Fermentation No.	t	k	X
1	17.5	10.10	18.12
2	18.0	10.25	18.00
3	17.5	10.15	17.90
4	18.5	10.31	19.82
5	18.5	10.32	20.10
6	18.0	10.11	18.21
7	18.0	10.15	19.32
8	18.0	10.25	20.03
9	18.0	10.01	17.90
10	18.0	10.24	18.08
mean	18.0	10.19	18.75
confidence interval ±	0.24	0.072	0.678

Table 2. Changes in acidity and biomass content during fermentation (means from three analyses)

Fermentation time t (h)	Acidity k (g CH_3COOH/100 cm^3)	Biomass content X (mg dry mass/100 cm^3)
1.5	5.55	9.54
2.5	5.67	10.32
4.5	5.95	10.83
6.5	6.33	12.40
8.5	6.81	13.20
11.5	7.69	14.91
18.0	10.24	18.09

for coefficient α was ± 0.0377.* Logarithmic and exponential functions were also considered, and for them the correlation coefficients were lower (0.9916 and 0.9631, respectively).

For subsequent time intervals there was calculated the acidification rate η, originally introduced by Hromatka

$$\eta = \frac{k_2 - k_1}{t_2 - t_1} 24,$$

i.e. the mean increment of acidity (in g/100 cm^3) between times t_1 and t_2, expressed as mean increment per day (24 h). It was assumed that the momentary acidification rate at the calculated mean level will be attained half-way into the period for

* The confidence level in all statistical calculations was 0.95.

which it was calculated, i.e. at time $t = 0.5\ (t_1 + t_2)$. Table 3 gives the calculated acidification rates and assumed times of their attainment.

Table 3. Acidification rate during fermentation

Fermentation time t (h)	Acidification rate η $\eta[(g/100\ cm^3)\ d^{-1}]$
2.00	2.88
3.50	3.36
5.50	4.56
7.50	5.76
10.00	7.04
14.75	9.42

Function η (t), similarly as X (t), turned out to be linear. The straight line equation for the given values is of the form $\eta = 0.5263\ t + 1.7099$ or, generally, $\eta = \alpha' t + \eta_0$, where η — acidification rate at time t, η_0 — acidification rate at time $t = 0$, α' — linear coefficient of acidification rate increase. The correlation coefficient for the analysed values was 0.9988, and the confidence interval for coefficient α' was ± 0.0354. The correlation coefficient for the logarithmic function was lower — 0.9825.

Basing on the confidence intervals for the determined values of α and α', it may be assumed that they are equal, and hence that the lines X (t) and η (t) are parallel.

Taking the mean values of α and α', we get two basic equations describing the dynamics of submerged acetic acid fermentation in conditions of unlimited bacteria biomass growth:

$$X = \alpha t + x_0 \tag{1}$$

$$\eta = \alpha t + \eta_0 \tag{2}$$

For the values given in Table 2 and 3, the mean value of α is 0.522.

Equation (2) requires a separate analysis. Dividing both sides of the equation by 24 and taking into consideration that

$$\frac{\eta}{24} = \frac{dk}{dt}$$

we get the following differential equation

$$\frac{dk}{dt} = \frac{\alpha}{24} t + \frac{\eta_0}{24}$$

After transformation and integration of both sides of this equation in the interval from $t = 0$ to $t = t$, and from k_0 to k, we get the quadratic equation

$$\frac{\alpha}{48} t^2 + \frac{\eta_0}{24} t - (k - k_0) = 0 \tag{3}$$

in which the new symbol k denotes acidity at time and k_0 acidity at time $t = 0$ t.

Using this equation it is possible to calculate the time needed for an acidity increase from k_0 to k, namely

$$t = \frac{\sqrt{\eta_0^2 + 48\alpha(k-k_0)} - \eta_0}{\alpha} \tag{4}$$

as well as the acidity increase after a given time

$$k - k_0 = \frac{1}{48}\alpha t^2 + \frac{1}{24}\eta_0 t. \tag{5}$$

The values of α and η_0 must, of course, be know from a previous fermentation cycle provided that the course of the process is regular, or determined in the given cycle by analysing acidity and biomass content.

Subtracting equations (1) and (2) by sides and carrying out transformations, we get

$$x - x_0 = \eta - \eta_0 \tag{6}$$

This means that in conditions of unlimited aeration the figure for bacteria biomass increment expressed in mg/100 cm^3 is equal to the figure for acidification rate increase expressed in [g/100 cm^3] d^{-1}.

In turn, dividing both sides of equation (6) by X and assuming that $\eta/X = a$, we get

$$a = \frac{x - x_0 + \eta_0}{x}$$

where a stands for biomass activity in g CH_3COOH per mg cell dry mass during 24 h.

Using the above relations it is possible to calculate the bacteria activity at any moment of fermentation. In the analysed fermentation cycle, the initial biomass activity was 0.1935 g mg^{-1} d^{-1}, and the final activity was 0.6059 g mg^{-1} d^{-1}. In the last phase of the fermentation cycle maintained in the described conditions, the activity of bacteria was almost three times greater than directly after the replacement of crude vinegar with fresh mash.

The changes of X, η and a during the analysed cycle are illustrated in Fig. 2.

When crude vinegar is replaced by fresh mash, there takes place a relatively rapid change of conditions of growth of the biomass of bacteria active in the process. The change consists in a reduction of acidity, and increases in the concentration of alcohol and nutritive components. On the one hand, the bacteria are subjected to a kind of shock brought about by abruptly changing conditions, and on the other they are provided with possibilities of cell regeneration after the adverse conditions prevailing in the final stages of the previous fermentation cycle. The activity of bacteria drops by more than three times. In time, as fermentation progresses, the bacteria cells regenerate attaining a higher level of activity.

In contrast to research by Hromatka and co-workers, there was no logarithmic phase of bacteria growth or acidification rate increase in the investigated fermentation conditions. However, the obtained results confirmed the significant

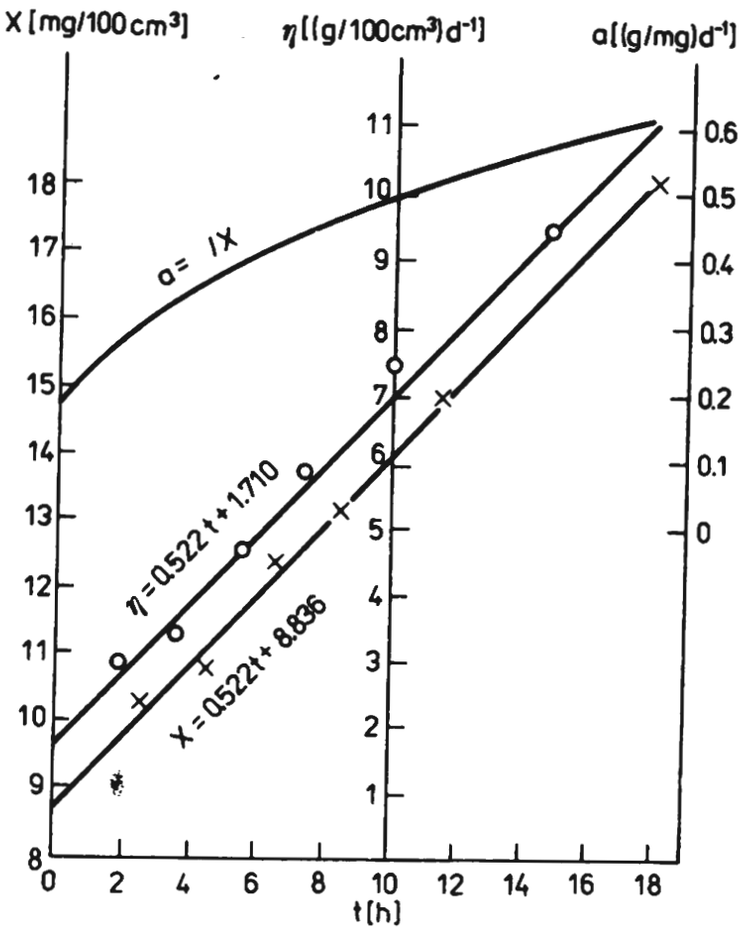

Fig. 2. Changes in biomass concentration X, acidification rate η and bacteria activity a in conditions of unlimited growth

effect of the medium's total concentration (the factor determining the maximal strength of the obtained vinegar) on the course of fermentation.

At lower total concentrations and inoculum additions of the order of 20% of the fermented medium Hromatka et al. [1] observed the generally recognized phases of microorganisms growth, namely the lag-phase, the log-phase and the phase of retarded growth. During semi-continuous fermentation, when 50% of the fermenter content is retained for the next fermentation cycle, the inoculum also amounted to 50%.

The ca. 11% total concentration of the medium, i.e. an acidity of over 10 g/100 cm^3 in the final phase of the succesive fermentation cycles, probably creates conditions preventing the logarithmic phase of bacteria biomass growth.

The coefficient of bacteria growth α defined here may be expressed as dX/dt. The specific rate of biomass increase may thus be represented in the form $\mu = \alpha/X$. As it was found, the value of α remains constant while biomass content increases linearly with time. The value of specific growth rate defined as

$$\mu = (\alpha x/\alpha' t)x^{-1}$$

tends to decrease. In the initial phase of the analysed fermentation cycle, the value of μ may be put at α/X_0, or 0.06 h^{-1}, and in the final phase at α/X_{18}, or 0.03 h^{-1}.

The obtained results demonstrate that it is possible to achieve biomass concentration and acidification rates comparable to those reported by Hromatka et al. [1] with the total concentration of the medium around 11%. The studies repor-

ted here show that in fermentation cycles carried out in the described conditions, the specific rate of *Acetobacter* biomass respiration varies with time. Given the fact that the specific respiration rate is a measure of bacteria activity, the changes of the two magnitudes will be analogous, as will be the factors causing them.

Considering the momentary acidification rates and biomass concentrations or momentary activities, and recalling that *Acetobacter* must use up 1 M of molecular oxygen to produce 1 M of acetic acid, one may define the specific rate of respiration in Acetobacter microorganisms as

$$Q = \frac{\eta}{x} \frac{M_{O_2}}{M_{CH_3COOH}} \frac{1000}{24} \quad (\text{mg}O_2/\text{mg dry mass})\text{h}^{-1}.$$

Hence, at the beginning of the analysed fermentation cycle, the specific respiration rate was 4.3 mg mg^{-1} h^{-1}, and in the final phase it was 13 mg mg^{-1} h^{-1}.

Hromatka and Ebner [1] put the mean specific rate of *Acetobacter* respiration for the given fermentation at 10.5 mg mg^{-1} h^{-1}. The respective figure for the fermentation cycle analysed in this research is 10.36 mg mg^{-1} h^{-1}, i.e. it is virtually the same as in Hromatka's experiments.

INDUSTRIAL-SCALE EXPERIMENTS

The presented theory of acetic acid fermentation in conditions of unlimited bacteria growth indicates possibilities of improving fermentation rate by removing the oxygen barrier occuring in the currently produced submerged fermentation equipment.

The measure of acetic acid fermentation rate in industrial practice is the so called unit conversion, or the number of dm^3 of alcohol processed in the fermenter during 24 h, per 1 m^3 of the fermenter's working capacity. In the types of fermenters enumerated above, this index, in conditions analogous to those investigated here, is 25 dm^3 m^{-3} d^{-1} on average.

A Frings Acetator of 24 m^3 nominal working capacity was used in an experiment consisting in a relative increase of aeration intensity. When 16 m^3 of the fermenter capacity were filled, the unit conversion was of the order of 40 dm^3 m^{-3} d^{-1}.

A 50% increase of aeration intensity resulted in an analagous increase of fermentation rate. This was achieved, of course, with an increased dose of nutrients.

Another experiment, this time with a Frings Acetator of unaltered working capacity of 12 m^3, involved the use of an additional blower which increased air flow by about 35%, from the nominal 4 m^3 m^{-3} h^{-1} to 5.4 m^3 m^{-3} d^{-1}. In this experiment the mean annual unit conversion was 30.4 dm^3 m^{-3} d^{-1}, compared with 22 dm^3 m^{-3} d^{-1} achieved in previous years. The improvement in unit conversion was of the same order as the increase in aeration intensity. In this case too the nutrients dose was higher.

Fig. 3 illustrates the changes in acidification rate during a single fermentation cycle in the described conditions. As can be seen, there is a distinct threshold of

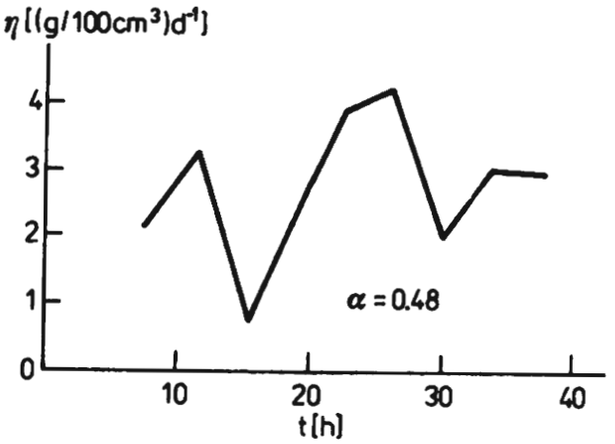

Fig. 3. Changes in acidification rate in an Acetator manufactured by Frings (FRG) with an additional blower and unlimited supply of nutrients

acidification rate increase after which there occurs a sharp breakdown of fermentation. Three such thresholds occur in the cycle depicted in Fig. 3.

Also noteworthy is the value of coefficient α at times of acidification rate increase. The maximal value of this coefficient was 0.48 between hours 15.5 and 19.5 of fermentation, being lower but similar to the respective value obtained in the microtechnological-scale experiments (0.522).

CONCLUSIONS

1. In microtechnological-scale experiments with semi-continous acetic acid fermentation of mashes with ca 11% total concentration, in conditions of unlimited *Acetobacter* growth, the increases of bacteria biomass and of acidification rate were found to be linear during the fermentation cycle. The general equations of these functions with respect to time are

$$X = \alpha t + X_0 \text{ and } \eta = \alpha t + \eta_0.$$

2. The changes of acidity during the fermentation cycle in the investigated microtechnological-scale conditions may be described with the following quadratic equation:

$$k - k_0 = \frac{\alpha}{48} - t^2 + \frac{\eta_0}{24} t$$

3. The figure for *Acetobacter* biomass increment expressed as mg/100 cm^3 is identical with the figure for acidification rate increase expressed as [g/100 cm^3] d^{-1}: $X\text{-}X_0 = \eta\text{-}\eta_0$.

4. The results indicate possibilities of significant intensification of submerged acetic acid fermentation, and the findings of industrial-scale research substantiate the conclusions derived from microtechnological-scale experiments.

5. The findings reported here confirm the need to design fermenters which do not limit bacteria biomass growth during the production of vinegar of acidity in excess of 10% by the method of semi-continuous acetic acid fermentation.

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Author address: 02-532 Warszawa, Rakowiecka 36.

J. Czuba

WZROST BIOMASY *ACETOBACTER* ORAZ SZYBKOŚĆ KWASZENIA W WARUNKACH WGŁĘBNEJ FERMENTACJI

Instytut Przemysłu Fermentacyjnego, Warszawa

Streszczenie

W pracy badano przebieg półciągłej, wgłębnej fermentacji octowej w fermentorach mikrotechnicznych i przemysłowych. Obserwowano wzrost biomasy *Acetobacter* oraz kształtowanie się szybkości kwaszenia w czasie cyklu fermentacyjnego.

Stwierdzono, że przebieg fermentacji w acetatorze firmy Frings, przedstawiony na rys. 1, znacznie odbiega od wyników badań twórców wgłębnej metody fermentacji octowej Hromatki i współpr. Hromatka i współpr. stwierdzili obecność logarytmicznej fazy wzrostu bakterii i szybkości kwaszenia, podczas gdy w trakcie ponad 40 h fermentacji w acetatorze obserwuje się bardzo powolny przyrost biomasy oraz kwasowości.

W skali mikrotechnicznej, w warunkach stężeń kwasu octowego i alkoholu występujących w procesie produkcji octu o mocy 10%, przeprowadzono kilkadziesiąt cykli fermentacyjnych. Czas trwania, końcowe stężenie kwasu octowego i biomasy bakterii w dziesięciu kolejnych cyklach fermentacyjnych przedstawiono w tab. 1. W tab. 2 podano wyniki oznaczeń kwasowości i zawartości biomasy podczas jednego z przedstawionych w tab. 1, wyrównanych cykli fermentacyjnych. W tab. 3 podano wyliczone dla poszczególnych odcinków czasu szybkości kwaszenia. Na rys. 2 przedstawiono funkcje szybkości kwaszenia (η) i stężenia biomasy (X) względem czasu. Analiza regresji tych zmiennych wykazała, że równania liniowe o następujących postaciach:

$$X = \alpha t + X_0$$
$$\eta = \alpha t + \eta_0$$

są dobrymi przybliżeniami tych funkcji. Współczynnik korelacji dla funkcji stężenia biomasy (X) względem czasu (t) wyniósł 0,9980, a dla funkcji szybkości kwaszenia względem czasu η (t) — 0,9988. Uzyskane wyniki świadczą o nie wykorzystywaniu potencjalnych możliwości bakterii kwasu octowego w obecnie budowanych fermentorach. Kształtowanie się szybkości kwaszenia w acetatorze firmy Frings wyposażonym w dodatkową dmuchawę przedstawiono na rys. 3.