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GROWTH OF ACETOBACTER BIOMASS AND PRODUCT FORMATION DURING ACETIC FERMENTATION

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Key words: Acetobacter, vinegar production, biomass growth, rate of product formation

The effect of acetic acid concentration on specific rate of Acetobacter biomass growth and specific rate of product formation was analyzed during production of ca 10% vinegar. The biomass growth rate decreased while the specific rate of product formation increased with the increase of acetic acid concentration.

INTRODUCTION

Both the product and the substrate of acetic fermentation significantly affect the activity and growth of Acetobacter biomass [1, 2, 9-14, 16-18]. the absence of alcohol in the medium, particularly at low acetic acid concentrations, may lead to further oxidation of acetic acid to carbon dioxide and water [9].

Nanba, Tamura and Nagai [18] found that small quantities of acetic acid (10 g/dm^3) increase the specific rate of bacteria growth and shorten the lag-phase. This phenomenon is particularly apparent in media with low ethanol concentrations.

In media with acetic acid concentrations of over 20 g/dm^3, the growth of biomass of the investigated bacteria was severely inhibited, being almost completely arrested when the acetic acid content reached 40-50 g/dm^3 and ethanol concentration was 5-75 g/dm^3.

Ethanol in the medium also inhibits bacteria growth. In media lacking acetic acid, ethanol clearly inhibited bacteria biomass growth at concentrations of over 50 g/dm^3. In media with acetic acid bacterial biomass growth was inhibited already at ethanol concentration of 5 g/dm^3 [18]. The oxidation of ethanol to acetic acid and of the latter to carbon dioxide and water is significantly affected by glucose. Its absence clearly curbs these processes [9].

The quoted experiments were conducted mostly in conditions differing greatly from those prevailing in industrial production of spirit vinegar with acetic acid content of 10 of more g/100 cm^3. In these experiments a complete arrest of bacteria growth and activity was observed in media containing upwards of 4-6 g of acetic acid in every 100 cm^3 of medium [1, 15, 17, 18] although the studied bacteria were isolated from industrial fermenters.

These results confirm the findings of Conner and Allgeier [3] that acetic acid bacteria in laboratory cultures on slants and dishes lose their technologically useful properties. There are very few publication dealing with the growth and activity of Acetobacters during industrial production of vinegar with acetic acid content of about 10 g/100 cm^3.

Hromatka et al. [13] introduced the concept of total wort concentration being the sum of acetic acid concentration (in g/100 cm^3) and alcohol concentration (in % vol.). Theoretically, 1 cm^3 of ethanol yields about 1 g of acetic acid, hence the total wort concentration determines the maximum attainable acetic acid concentration. Fermentation intensity was observed to differ considerably depending on the total medium concentration [10, 13].

Ebner [7] produced vinegar of 14.3 g acetic acid per 100 cm^3 attaining an acidification rate of 3.8 g/100 cm^3/24 h, while Greenshields [8] obtained vinegar with 5 g acetic acid per 100 cm^3 at acidification rate of 18 g/100 cm^3/24 h.

In the production of distilled vinegar, as a rule containing upwards of 10% acetic acid, fermentation takes place in unsterile conditions. Neither the fermented wort, nor the fermenters, nor the air are sterilized, and only generally accepted standards of hygiene are observed.

The inoculum for new fermentation cycles are bacteria contained in the vinegar remaining in the fermenter after the previous production cycle. Provided the equipment works well, the quality of raw materials is adequate and unvarying, and there is no human error, this process may continue unbroken for many years. It may be assumed that the conditions inside the fermenters, particularly the high acetic acid concentration, are sufficient safequards against the development of alien microflora.

METHODS

In our experiments we strove to create conditions maximally resembling those in industrial production of vinegar containing about 10 g acetic acid per 100 cm^3. In particular, we maintained the same acetic acid and ethyl alcohol concentrations, and used the same bacteria cultures.

Unlike in experiments by other authors who used pure Acetobacter cultures, pre-bred on laboratory media, we used bacteria transferred from an industrial fermenter to a microtechnological-scale apparatus according to the procedure applied in preparing the industrial process, without first transplanting them to a laboratory medium.

The studies were performed in microtechnological scale in conditions of periodic submerged acetic fermentation in 1.8-dm^3 fermenters with automatic-suction pipe mixers. When ethanol was fermented off to 0.4-0.6% vol., 900 cm^3 of the medium was replaced with fresh wort with 10% vol. ethanol and 1 g/100 cm^3 acetic acid, augmented with the necessary nutrient components [4-6].

DISCUSSION OF RESULTS

We observed changes in biomass concentration (X) and in acidification rate (η) [5] during periodic submerged acetic fermentation of wort (11% total concentration) with unlimited aeration and nutrient supply.

We found that for these conditions, good approximations of the function of biomass concentration and time, and the function of acidification rate and time are straight lines, this being indicated by correlation coefficients close to unity (0.9980 and 0.9825).

The following equations of these functions were formulated for the exemplary fermentation cycle:

$$x_t = 0.5180\ t + 8.8360,$$

or generally:

$$x_t = \alpha_1\ t + x_o;$$

and:

$$\eta_t + 0.5263\ t + 1.7099,$$

or generally:

$$\eta_t = \alpha_2\ t + \eta_o.$$

The course of fermentation in this cycle is described in the Table.

Table. Fermentation cycle course

t	k	X	μ	η	q
1.5	5.55	9.54	0.0543	2.499	10.91
2.5	5.67	10.32	0.0502	3.026	12.22
4.5	5.95	10.83	0.0478	4.078	15.69
6.5	6.33	12.40	0.0418	5.131	17.24
8.5	6.81	13.20	0.0392	6.183	19.52
11.5	7.69	14.91	0.0347	7.762	21.69
18.0	10.24	18.09	0.0286	11.183	25.76

Notation:
t — time (h)
k — acetic acid content (g/100 cm3)
x — bacteria biomass concentration (mg/100 cm^3) as dry cell mass
μ — specific rate of biomass growth (h^{-1})
η — acidification rate (g/100 cm^3/24 h)
q — specific rate of acetic acid formation (mg/mg/h)
α_1 — linear coefficient of bacteria biomass growth (mg/100 cm^3/h)
α_2 — linear coefficient of acidification rate growth (g/100 cm^3/h)
Indices : t — at time t; o — at time 0

The linear with respect to time bacteria biomass increment observed in these studies [5] indicates that the specific biomass growth rate decreases in time, and hence that during periodic submerged acetic fermentation the bacteria are in the reduced growth phase. The specific growth rate in conditions of the described experiments may be defined as $\mu_t = \alpha_i\ x_t^{-1}$.

The data in the Table reveal a statistically significant dependence of specific rate of bacteria biomass growth on acetic acid content in the medium (Figure). This dependence is described by the exponential function

$$\mu_t = 0.013 \exp (7.633\ k_t^{-1})$$

(correlation coefficient 0.989 at confidence level of 0.95). the function shows that increasing acetic acid concentration inhibits bacteria biomass growth in an exponential manner, and this result shows that the findings of Bar et al. [1] that Acetobacter growth is inhibited exponentially are also valid in the acetic acid concentrations range found in the production of vinegar containing upwards of 10 g of this acid per 100 cm^3.

Muraoki et al. [16] demonstrated that the reduction of acetic fermentation intensity observed in media of pH > 3.1 is due entirely to undissociated acetic acid particles. In media of pH < 3.1 this inhibition is the result of the presence of both undissociated acetic acid particles and hydrogen ions.

These findings are a probable explanation of the differences between detailed dependences in this range presented in this work and in publications describing studies with low concentrations of acetic acid [1, 18].

The absence of the exponential growth phase 3 in bacteria active in the production of vinegar containing about 10 g acetic acid per 100 cm^3 may be seen as due to both low pH of the medium (pH 3.1 corresponds to acetic acid concentration of 6.5 g/100 cm^3) and high concentration of undissociated acetic acid particles.

In the available literature the highest specific rate of Acetobacter biomass growth (0.5 h^{-1}) was reported by Nanba, Tamura and Nagai [18] who obtained it by culturing bacteria on a medium with about 1 g acetic acid per 100 cm^3. When this concentration was increased to 4-5 g/100 cm^3, the bacteria ceased to grow.

The maximum specific rate of bacteria biomass growth in experiments by Bar et al. [1] — 0.108 h^{-1} — was attained in a medium with 1.5 g acetic acid/100 cm^3. This rate dropped to about 0.01 h^{-1} when acetic acid content in the medium was increased to about 4 g/100 cm^3, that is to a level below the lowest concentrations used in industrial production of ca. 10% vinegar.

The high values of specific rate of Acetobacter biomass growth given above exceed several times the values obtained in our studies. Bear in mind however that the bacteria biomass growth in these studies, although increasingly slower, took place during the entire technological process in acetic acid concentrations ranging from 5.42 to over 10 g/dm^3. This shows that the bacteria population we used was much more resistant to acetic acid than Acetobacter cultures used by other authors.

The dependence of the specific rate of acetic acid formation, defined as

$$q_t = (1000\ \eta_t)/(24\ X_t),$$

on the acetic acid content in the medium turned out to be quite different. We demonstrated experimentally that the specific rate of product formation

increased with the increase of acetic acid concentration in the medium (Figure). A good approximation of this dependence is the function:

$$q_t = 44.069\text{-}175.454\ k_t^{-1}$$

(correlation coefficient 0.974 at confidence level 0.95).

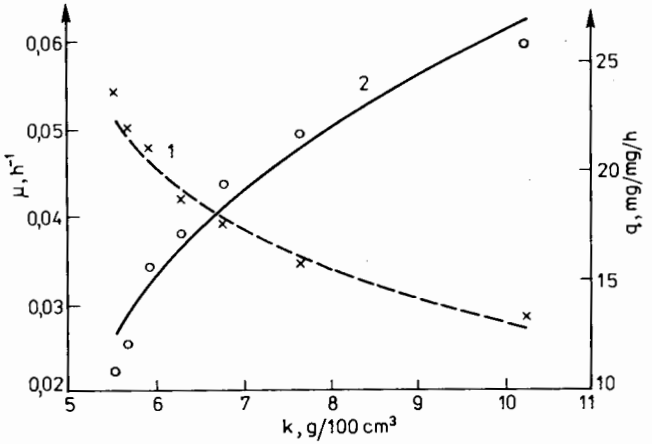

Figure. Dependence of specific rate of biomass growth (μ) and specific rate of product formation (q) on acetic acid content (k) in fermenting liquid 1 — μ, 2 — q

During aeration of the medium subjected to acetic fermentation, a portion of the biomass passes to the atmosphere in the form of aerosol. Such bacteria biomass losses may burden the presented dependences with error when the biomass content is small. However, such an error should not have bearing on the direction of the observed transformations and on the practical applicability of the obtained results. Proof of this may be the improvement of fermentation in industrial installations after partial elimination of aeration intensity restrictions. In microtechnological scale with no limitations on aeration (air flow of 12.5 $dm^3/dm^3/h$) the acetic acid production in each particular case was around 60 $kg/m^3/24$ h [4, 6]. When air flow was increased in industrial conditions from 4 to 6 $dm^3/dm^3/h$, the amount of acetic acid produced increased from about 23 to about 37 $kg/m^3/24$ h [5].

CONCLUSIONS

1. Increasing acetic acid content during fermentation was found to inhibit bacteria biomass growth, similarly as in experiments of other authors with different ranges of acetic acid concentration. The dependence between specific rate of biomass growth and acetic acid content is represented by the exponential function

$$\mu_t = 0.013 \exp (7.633\ k_t^{-1}).$$

2. Unlike in the case of specific rate of biomass growth, increasing concentration of acetic acid in the medium was found to boost the specific rate of product formation in keeping with the formula

$$q_t = 44.069\text{-}175.454\ k_t^{-1}.$$

3. Bacteria collected together with the medium from an industrial fermenter were more resistant to high concentrations of acetic acid than pure Acetobacter cultures pre-bred on laboratory media.

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WZROST BIOMASY ACETOBACTER I TWORZENIE PRODUKTU PODCZAS FERMENTACJI OCTOWEJ

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Streszczenie

Analizowano wpływ stężenia kwasu octowego na właściwą szybkość wzrostu biomasy bakterii rodzaju Acetobacter oraz właściwą szybkość tworzenia produktu podczas procesu otrzymywania octu spirytusowego o mocy ok. 10%. W miarę wzrostu stężenia kwasu octowego w fermentującej cieczy (k) obserwowano obniżanie się właściwej szybkości wzrostu biomasy (μ), jednocześnie obserwowano wzrost właściwej szybkości tworzenia produktu (q). Dobrymi przybliżeniami tych zależności, przedstawionych na wykresie były funkcje:

$$\mu_t = 0.013 \exp (7.633\ k_t^{-1})$$

oraz

$$q_t = 44.069\text{-}175.454\ k_t^{-1}$$