A C T A A L I M E N T A R I A P O L O N I C A

Vol. XV (XXXIX), No. 1 1989

JERZY CZUBA

ACETOBACTER BIOMASS YIELD IN RELATION TO GLUCOSE AND AMMONIA NITROGEN

Institute of Fermentation Industry, Warsaw

Key words: Acetobacter biomass, glucose, ammonia nitrogen, acetic acid fermentation.

Acetobacter biomass yield in relation to glucose and ammonia nitrogen during sumerged acetic acid fermentation of mash of ca 11% total concentration was determined. It was found that in these conditions biomass yield in relation to glucose equals ca 0.2, and to ammonia nitrogen ca 5 mg/mg.

Maximal, uninhibiting degree of glucose and ammonia nitrogen utilization were also determined. In the studied conditions they are ca 0.75 for glucose and ca 0.45 for ammonia nitrogen. These coefficients may be useful in determining optimal carbon and nitrogen doses for acetic acid fermentation.

INTRODUCTION

The studies of acetic acid fermentation now concern mainly the immobilization of bacteria cells [7], isolation, purfication and determination of enzyme activity of some Acetobacter strains [8, 13], also the construction of fermenters [9, 4], and the method of continuous fermentation [3].

However, scientific literature lacks information on the *Acetobacter* biomass yield in relation to carbon and to nitrogen sources during vinegar production. This prompted us to perform the present research.

In dripping methods of acetic acid fermentation it is impossible to relate the dosage of nutrients to the growth of bacteria biomass. The introduction of submerged fermentation [5, 6, 11] into acetic acid production enabled the determination of direct relationships between the biomass increment and utilization of fundamentals nutrients.

AIM AND SCOPE OF RESEARCH

The aim of this research was to determine the *Acetobacter* biomass yield in relation to glucose as carbon source and to ammonia nitrogen in conditions prevailing during the production of white vinegar over 10% in strength.

The scope of the research included observations of biomass increase fermentation course by various doses of glucose and di-ammonium phosphate as well as analysis of the contents of these medium components in the mash and the vinegar produced from it.

METHODS

MICROORGANISMS

The fermentation was conducted with *Acetobacters* taken from an industrial fermenter together with fermenting medium. Taxonomic studies conducted according to Bergey's Manual of Determinative Bacteriology [1] showed the presence of *A. aceti*, subspecies *orleanensis*, and *A. pasteurianus*, subspecies: *pasteurianus* and *lovaniensis*.

METHOD OF FERMENTATION

Laboratory fermenters of 1.8 dm^3 working capacity, equipped with self-sucking pipe agitators were used.

The mash contained 10% v/v of alcohol content dropped to 0.4-0.6% v/v, vinegar was removed from the fermenter, leaving about 0.9 dm^3 of it. The removed vinegar was replaced by 0.9 dm^3 of fresh mash. This operation was conducted during continuous aeration of the liquid contained in the fermenter. Fermentation took place at 29°C; aeration rate was 12.5 dm^3/dm^3 per hour.

At first the SGS nutrient used in the generator method of vinegar production [2], was applied. During experimental determination of bacteria biomass yield in relation to selected nutrients, the mash was supplemented with

- yeast autolysate — 3.5 cm^3cm/dm^3*
- $MgSO_4 \cdot 7H_2O$ — 300 mg/dm^3
- K_2SO_4 — 65 mg/dm^3
- NaCl — 75 mg/dm^3

and various doses of hydrated glucose and di-ammonium phosphate.

The biomass yield in relation to the tested medium component was calculated as the ratio of biomass increment during the fermentation cycle to the amount of the component utilized, using the formula

$$Y = \frac{2X_k - X_p}{C_b + C_p - 2C_k} \tag{1}$$

* Yeast autolysate was prepared from compressed baker's yeast. Yeast was mixed with water in 1 : 1 weight proportion and autolysed at 50-55°C for 2-3 days.

The degree of utilization of the medium component as the ratio of the amount of the component utilized to the amount introduced, was calculated according to the formula:

$$\varphi = \frac{C_b + C_p - 2C_k}{C_b} \tag{2}$$

The fermentation rate coefficient — unit production of acetic acid (JPK) — was calculated according to the formula:

$$JPK = \frac{120}{t}(2k_k - k_p - k_b) \tag{3}$$

Denotations:

Y — biomass yield in mg of cell dry mass/mg,
φ — degree of utilization of tested medium component,
X — concentration of bacteria biomass in mg of cell dry mass/dm^3,
C_i — concentration of medium component in mg/dm^3,
k_i — concentration of acetic acid in % w/v,
t — duration of the analyzed fermentation cycle in hours,

indexes denote the kind of the analyzed sample:

k — vinegar obtained during the analyzed fermentation cycle,
p — vinegar obtained during the preceding fermentation cycle,
b — mash used during the considered fermentation cycle.
120 — coefficient derived from the formula:

$$\frac{24 \cdot 900}{1.8 \cdot 100}$$

ANALYTICAL METHODS

Fermentation was controlled by analysing acetic acid and ethyl alcohol content in the fermenting medium.

Acidity was determined by titrating 1 cm^3 of the substrate with 0.1 n NaOH solution against phenolphthalein. Alcohol content was determined using the Semichon-Flanzy method.

In order to assess the utilization of the medium components, the contents of reducing substances and ammonia nitrogen were determined in mash and vinegar. The reducing substances, glucose and maltose, were determined using the Steinhoff and Schoorl methods.

Bacteria biomass content was determined in the obtained vinegar using the nephelometric method.

RESULTS AND DISCUSSION

SGS COMPONENTS UTILIZATION

Several dozen fermentation cycles were performed with various doses of the SGS nutrient. For each new dose, the fermentation was conducted until the process stabilized fully. Mean values were calculated from three successive cycles, differing in concentration of bacteria biomass (X) and fermentation rate (JPK) by 10 % at most. Table 1 presents the dependence of those values on the nutrient dose.

Table 1. Effect of varying doses (D) of SGS nutrient on bacteria biomass concentration in vinegar (X) and fermentation rate (JPK)

D g/dm^3	X mg/dm^3	JPK $g/dm^3/d$
1.6	104	28.5
3.1	158	41.6
3.4	173	49.3
3.5	179	54.6
3.6	184	52.0
3.7	186	53.8
4.0	184	53.6
4.5	182	50.7

A 3.5 g/dm^3 dose of the SGS nutrient was found not to inhibit fermentation rate and it was used in the subsequent fermentation cycles. Table 2 presents glucose, maltose and ammonia nitrogen contents in mash and vinegar during subsequent stabilized fermentation cycles.

Table 2. Glucose, maltose and ammonia nitrogen content in mash and vinegar. SGS nutrient dose — 3.5 g/dm^3. Concentration (C) in mg/dm^3

Glucose			Maltose			Ammonia nitrogen		
C_b	C_k/C_p	φ	C_b	C_k/C_p	φ	C_b	C_k/C_p	φ
1068	418	0.608	743	614	0.173	216	188	0.130
1089	452	0.585	704	605	0.141	212	186	0.123
1072	428	0.599	726	624	0.140	204	179	0.122
1069	450	0.578	753	662	0.121	208	179	0.139
1058	445	0.579	741	633	0.146	218	189	0.133
1091	420	0.615	715	602	0.158	204	180	0.118
1075	436	0.595	728	614	0.156	215	187	0.130
1082	439	0.594	737	625	0.152	209	186	0.110
1076	436	0.594	731	622	0.148	211	184	0.126

RESULTS AND DISCUSSION

SGS COMPONENTS UTILIZATION

Several dozen fermentation cycles were performed with various doses of the SGS nutrient. For each new dose, the fermentation was conducted until the process stabilized fully. Mean values were calculated from three successive cycles, differing in concentration of bacteria biomass (X) and fermentation rate (JPK) by 10 % at most. Table 1 presents the dependence of those values on the nutrient dose.

Table 1. Effect of varying doses (D) of SGS nutrient on bacteria biomass concentration in vinegar (X) and fermentation rate (JPK)

D g/dm^3	X mg/dm^3	JPK $g/dm^3/d$
1.6	104	28.5
3.1	158	41.6
3.4	173	49.3
3.5	179	54.6
3.6	184	52.0
3.7	186	53.8
4.0	184	53.6
4.5	182	50.7

A 3.5 g/dm^3 dose of the SGS nutrient was found not to inhibit fermentation rate and it was used in the subsequent fermentation cycles. Table 2 presents glucose, maltose and ammonia nitrogen contents in mash and vinegar during subsequent stabilized fermentation cycles.

Table 2. Glucose, maltose and ammonia nitrogen content in mash and vinegar. SGS nutrient dose — 3.5 g/dm^3. Concentration (C) in mg/dm^3

Glucose			Maltose			Ammonia nitrogen		
C_b	C_k/C_p	φ	C_b	C_k/C_p	φ	C_b	C_k/C_p	φ
1068	418	0.608	743	614	0.173	216	188	0.130
1089	452	0.585	704	605	0.141	212	186	0.123
1072	428	0.599	726	624	0.140	204	179	0.122
1069	450	0.578	753	662	0.121	208	179	0.139
1058	445	0.579	741	633	0.146	218	189	0.133
1091	420	0.615	715	602	0.158	204	180	0.118
1075	436	0.595	728	614	0.156	215	187	0.130
1082	439	0.594	737	625	0.152	209	186	0.110
1076	436	0.594	731	622	0.148	211	184	0.126

The obtained results, especially the degree of utilization of the studied medium components (glucose — 0.594, maltose — 0.148, amonia nitrogen — — 0.126) indicate that:

— glucose is a much better carbon source for Acetobacters than maltose,

— it is possible to substantially lower the dose of the studied medium components.

In the successive experiments, maltose was not used as a carbon source, and it was attempted to determine bacteria biomass yield in relation to glucose and ammonia nitrogen as well as maximal utilization of these medium components which do not limit fermentation, Acetobacter biomass yield in relation to glucose and degree of glucose utilization.

The composition of the medium was used as given in Methods, with the difference that 1 g/dm^3 of di-ammonium phosphate served as nitrogen source and glucose was used in doses varying from 1.9 to 1.0 g/dm^3.

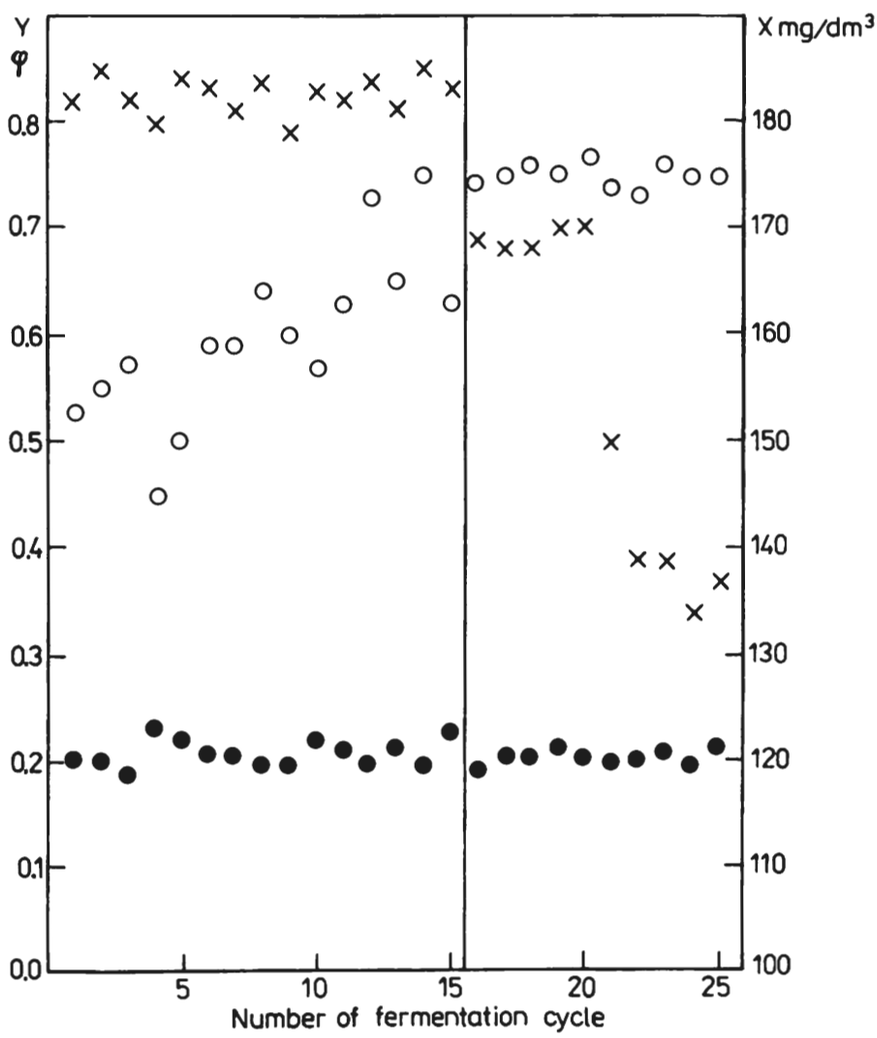

Fig. 1. Acetobacter biomass yield Y (•) in relation to glucose, degree of its utilization (o) and bacteria biomass concentration in vinegar X (x) at decreasing glucose doses

Fig. 1 presents the observed concentration of biomass X and the coefficients Y and φ calculated according to formulas (1) and (2) in the successive fermentation cycles.

With the lowering of glucose dose the utilization degree increased from ca 0.5 to ca 0.75.

Using the limiting dose of di-ammonium phosphate, the mean value of biomass yield in relation to ammonia nitrogen was $Y = 5.19 \pm 0.20$ (confidence coefficient 0.95), and the nitrogen utilization degree $\varphi = 0.445 \pm 0.024$ (confidence coefficient 0.95).

VERIFICATION OF THE COEFFICIENTS

In order to verify the coefficients, 15 fermentation cycles with various doses of glucose and di-ammonium phosphate were conducted. The glucose and di--ammonium phosphate doses were calculeted according to the formula:

$$D = \frac{100\,X_k}{p\,Y\,\varphi} \tag{4}$$

in which:

D — nutrient component dose (mg/dm^3),

p — content of analyzed compound or element in the source of it (%),

X_k — the assumed concentration of bacteria biomass in vinegar (mg/dm^3),

Y and φ — the determined biomass yield and degree of utilization of the analyzed compound or element.

In the first series of experiments (15 fermentation cycles) the glucose and di-ammonium phosphate doses were calculated assuming $X_k = 100$ mg/dm^3, in the second $X_k = 250$ mg/dm^3, and in the third — doses adjusted to bacteria biomass concentration determined in the second series, i.e., 201 mg/dm^3. The first the cycles of each series were rejected, as it was the period of stabilizing conditions of fermentation. Table 3 presents fermentation results of the last five cycles of each series.

Table 3. Fermentation with use of calculated doses of glucose and di-ammonia phosphate

Assumed concentration of bacteria biomass X_z (in mg/dm^3)	100		250		201	
Dose of nutrient component (in mg/dm^3) hydrated glucose diammonia phosphate	722 204		1804 510		1450 410	
No of fermentation cycle	X	JPK	X	JPK	X	JPK
11	112	31.20	196	63.80	204	64.7
12	115	35.0	200	62.9	202	63.8
13	110	34.5	213	62.0	196	63.6
14	106	34.3	198	63.8	215	62.9
15	107	34.5	200	63.8	199	64.9
Mean	110	33.9	201	63.3	203	64.0
Confidence interval ×	5	1.9	8	1.0	9	1.0

The glucose and di-ammonium phosphate dose calculated for the assumed bacteria biomass concentration $X_k = 250$ mg/dm^3 allowed to determine the maximal achievable bacteria biomass concentration in conditions of semi-continuous submerged fermentation during production of ca. 10% vinegar. This concentration is 200 mg/dm^3. The results obtained in the first and third series of experiments confirmed the determined coefficients values.

110 ± 5 mg/dm^3 was obtained with the assumed bacteria biomass concentration of $X_k = 100$ mg/dm^3, and 203 ± 9 mg/dm^3 with the assumed concentration of 201 mg/dm^3.

CONCLUSION

It was determined that during acetic fermentation of spirit mash of ca 11% total concentration, Acetobacter biomass yield equals 0.2 mg of cell dry mass per 1 mg of utilized glucose, and ca 5 mg cell dry mass per 1 mg of ammonia nitrogen utilized from $(NH_4)_2HPO_4$.

Maximal utilization degress of glucose and ammonia nitrogen from $(NH_4)_2HPO_4$ unlimiting bacteria growth are 0.75 and 0.45, respectively.

The coefficients determined during experiments may find application in establishing rational, unlimiting doses of glucose (carbon source) and di-ammonium phosphate (nitrogen source).

Roels given, among others, results of experiments on biomass yield of different microorganisms in relation to glucose as the sole source of carbon and energy [12]. He gives the values of Y_{SX} from 0.44 for Bacterium HR, to 0.77 for Trichoderma viride — the mean value: 0.61 mol dry mass/mol substrate.

Taking into account the general biomass formula ($CH_{1.8}O_{0.5}H_{0.2}$) it is possible to calculate the obtained in this work value of biomass yield in relation to glucose as 0.98 mol dry mass/mol glucose.

The biomass yield value, much higher than the one given by Roels may be explained by the fact that during acetic acid fermentation there are two carbon compounds in the medium: glucose and ethanol. Ethanol is oxidized to acetic acid and constitutes the main energy source for bacteria, while carbon contained in glucose is used as the main bacteria biomass component.

LITERATURE

1. Buchanan R. E., Gibbons N. E.: Bergey's Manual of Determinative Bacteriology. The Williams and Wilkins Corp., Baltimore 1974, 251, 276.
2. Czuba J.: Badania nad opracowaniem optymalnego składu pożywki dla bakterii fermentacji octowej przy zastosowaniu metody powierzchni efektywności czynników. Doctor thesis. Warszawa, SGGW 1976.
3. Ghommidh C., Cutayar J. M., Navarro J. M.: Biotechnology Letters 1986, **8** (1), 13.
4. Greenshields R. N., Smith E. L. Process Biochemistry 1974, **9** (3), 11.

5. Haeseler G.: Brantweinwirtschaft 1949, 75, 17.
6. Hromatka O., Ebner H.: Enzymologia 1949, **12**, 369.
7. Mori A.: Process Biochemistry 1985, **20** (3), 6[illegible].
8. Muraoka H., Watabe Y., Ogasawara N., Takahshi H.: J. Ferment Technol. 1982, **60** (1), 41.
9. Müller F.: Process Biochemistry 1978, **13** (11), 10
10. Peppler H. J.: Microbial Technology. Reinhold Publ. Corp. New York, Amsterdam, London 1967, 344.
11. Patent USA No. 3, 445, 245 (1969).
12. Roels J. A.: Energetes and kinetics in biotechnology. Elsvier Biomedical Press, Amsterdam, New York, Oxford 1983, 30.
13. Walisch S.:.Acta Microbiol. Polonica 1982, **31** (1), 29.

Manuscript received: January 1988
Authors address: 02-532 Warszawa, Rakowiecka 36

J. Czuba

WYDAJNOŚĆ BIOMASY ACETOBACTER W STOSUNKU DO GLUKOZY I AZOTU AMONOWEGO

Instytut Przemysłu Fermentacyjnego, Warszawa

S t r e s z c z e n i e

Badano wydajność biomasy Acetobacter w stosunku do glukozy i azotu amonowego w warunkach fermentacji octowej brzeczki o sumarycznym stężeniu 11%. Początkowo stosowano pożywkę SGS, której nie ograniczającą dawkę ustalono na 3,5 g/dm^3 (tab. 1). Podczas fermentacji z użyciem tej dawki pożywki oceniano stopień wykorzystania glukozy, maltozy i azotu amonowego (tab. 2). Stwierdzono niewielkie wykorzystanie maltozy i azotu. W dalszych doświadczeniach zmodyfikowano tak skład pożywki, że wyeliminowano z niej maltozę. Przy stosowaniu nie ograniczającej dawki fosforanu dwuamonowego stosowano zmienne, coraz niższe dawki glukozy. Wydajność biomasy w stosunku do glukozy utrzymywała się na poziomie ok. 0,2 mg suchej masy komórkowej na 1 mg glukozy, przy stałym wzroście stopnia wykorzystania glukozy — do ok. 0,75 (rys. 1).

W kolejnym doświadczeniu dawkę glukozy utrzymywano na stałym nie ograniczającym poziomie, a stosowano zmienne, malejące dawki fosforanu dwuamonowego. Wydajność biomasy w stosunku do azotu amonowego utrzymywała się na względnie stałym poziomie ok. 5 mg suchej masy komórkowej na 1 mg wykorzystanego azotu przy stałym wzroście jego wykorzystania do ok. 0,45 (rys. 2). W końcowej fazie doświadczeń obserwowano wyraźne obniżanie się zawartości biomasy bakterii w odfermentowanym podłożu.