Effect of Aeration and Carbon Dioxide on Cell Morphology of *Candida utilis*

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The effect of CO₂ availability on cell size, shape, and aggregation in continuous cultures of *Candida utilis* was studied in minimal medium with glucose or ethanol as the sole carbon and energy source. Enrichment with CO₂ was achieved (i) by using the substrate with more C atoms, (ii) by using pure oxygen and thus decreasing aeration intensity at the same dissolved-oxygen concentration, or (iii) by adding CO₂ to the aeration gas. The cells were always of yeast shape, and no filaments were formed. In cultures with a biomass concentration above 6 g (dry weight) per liter, no cell aggregates were observed. In cultures with a lower biomass, the daughter cells failed to separate from the parent cells and formed aggregates with thickened walls. The average cell number per aggregate was found to be higher, and the average protoplast volume lower, under conditions of probable CO₂ limitation. Simultaneously, the ratio of total dry weight to wet weight of protoplasts was considerably higher, indicating an increased share of wall or extracellular material. The possible effect of the observed morphological changes for maintaining a suitable concentration gradient of CO₂ around the cell is discussed.

The yeast *Candida utilis*, which is able to grow on ethanol as the sole carbon and energy source, is a promising organism for single-cell protein production. Its growth in continuous culture has been extensively studied in both one-stage (9, 16) and multistage (21-24) continuous culture in order to determine the conditions of optimal yield and productivity. The organism is variable in size and shape, and under particular cultivation conditions cell aggregates have been observed. For several other organisms, transition from the single-cell phase of growth to the formation of filaments or aggregates was found to be affected by CO₂ deficiency (2, 27).

During utilization of substrates with a low number of C atoms, the importance of carboxylation reactions increases (18; S. Haarasilta and E. Oura, Abstr. Euchem Conference on Metabolic Reactions in the Yeast Cell in Anaerobic and Aerobic Conditions, 1977), especially at higher growth rates. When high dilution rates and high substrate concentrations in the inflow are used, vigorous aeration must be maintained in the fermentors to avoid oxygen limitation. Under such conditions, the CO₂ evolved is greatly reduced and CO₂ limitation is likely to occur, especially at low biomass concentration.

In this study, we investigated the morphological characteristics and cell dry mass of *C. utilis* in three experiments, each enabling comparison of cultivation conditions with different CO₂

availability: (i) growth on two substrates, glucose and ethanol, with different numbers of C atom; (ii) growth on ethanol with different aeration intensities (using air or pure oxygen at the same dissolved-oxygen concentration); and (iii) growth on ethanol at low biomass concentration under air or CO₂-enriched air aeration.

MATERIALS AND METHODS

Organism. *C. utilis* strain 136 from the Collection of the Department of Fermentation Chemistry and Technology (Institute of Chemical Technology, Prague) was used.

Cultivation medium. Liquid medium modified on the basis of Light (13) was used in two concentrations (1 and 2 in Table 1). Initial pH was 4.5.

Cultivation equipment. Apparatus I used for experiments 1 and 3 was a conical glass vessel, mixed by aeration and medium fed by a peristaltic pump (developmental workshops of the Czechoslovak Academy of Sciences); the level of medium was maintained by an overflow system. CO₂ in experiment 3 was fed by the same pump, and its percentage in the aeration gas was checked before the experiment by recording the pH in 10-μmol solution of bicarbonate saturated with the particular aeration gas. The vessel was filled with 50 ml of a 24-h batch culture in medium no. 1 with 20 g of ethanol per liter.

Apparatus II for experiment 2 was a fermentor described previously (20) with mechanical mixing. The dissolved-oxygen concentration was kept constant at the required level. The basic constant aeration rate covered about 90% of the gas flow rate; the rest of the flow rate was regulated by parallel periodic aera-

TABLE 1. *Composition of cultivation media*

Medium	Amt per liter of distilled water	
	1	2
Na ₂ HPO ₄ ·12H ₂ O	1.94 g	1.26 g
NaH ₂ PO ₄ ·2H ₂ O	3.35 g	3.18 g
NH ₄ Cl	7.70 g	0.50 g
K ₂ SO ₄	3.85 g	0.25 g
Solution B	1.0 ml	0.1 ml
Solution C	1.0 ml	0.1 ml
Solution B, MgSO ₄ ·7H ₂ O	587.6 g	
Solution C ^a		
CaCl ₂ ·6H ₂ O	41.85 g	
FeCl ₃ ·6H ₂ O	51.54 g	
MnCl ₂ ·4H ₂ O	18.77 g	
ZnSO ₄ ·7H ₂ O	13.62 g	
CuSO ₄ ·5H ₂ O	2.17 g	
H ₃ BO ₃	0.58 g	
Na ₂ MoO ₄ ·2H ₂ O	4.62 g	
CoCl ₂ ·6H ₂ O	4.34 g	

^a Amount per liter of 3 M HCl.

tion controlled by a dissolved-oxygen analyzer. Cultivation was started from a batch inoculum as described previously (20). Other details are given in Table 2.

Biomass concentration. In experiment 2, the dry weight in grams per liter (*X*) was determined gravimetrically (drying for 1 h at 72°C and 2.5 h at 105°C). In experiments 1 and 3, the biomass was monitored by absorbance (*A*) at 500 nm. For estimating dry weight, the regression $X = 0.645A$ was used ($r^2 = 0.896$; standard error of the estimate = 0.305). The regression was computed from 25 data covering the range 0.49 to 1.9 g/liter of *X* determined on glass fiber filters (GFC Whatman) (E. Králíčková, diploma thesis, Institute of Chemical Technology, Prague, 1979).

Dry weight values divided by cell number represent the mean cell dry mass (*M*) in picograms.

Microscopic examination. In samples preserved by formaldehyde (final concentration, 4%) the total number of cells per milliliter and the number of cell aggregates and single cells were determined by using a Bürker hemacytometer. A volume of 0.05 mm³ containing at least 500 cells was examined. The mean size of the cell aggregate was estimated as the mean number of cells per one aggregate. The lengths and widths of 50 or 100 cells from a sample were measured in a projection microscope (Visopan; Reichert, Vienna). Cell volumes and surfaces were calculated, assuming the cell shapes to be rotation ellipsoids. All the measurements were for protoplasts only and did not include the wall or extracellular material.

The total wet weight of protoplasts (*B*, in grams per liter) was estimated from cell counts and volumes, assuming a specific gravity of 1.1.

The age distribution according to the number of scars and the percentage of dead cells was examined with fluorochrome (3).

RESULTS

In all experiments (Table 2), the cells were often observed not to separate after budding,

TABLE 2. *Characteristics of three experiments*

Expt no.	Apparatus	Determination										Cultivations	Duration of each cultivation (td)
		Working vol (ml)	Dilution rate (h ⁻¹)	Medium no.	Substrate	Inflow substrate concn (g/liter)	Aeration (ml/min)	CO ₂ added in aeration gas (%)	Regulation of O ₂ concn	Regulation of pH			
1 (glucose vs ethanol)	I	300	0.15	2	Glucose or ethanol	0.5	400	0	None	None	2 parallel	27	
2 (air vs oxygen)	II	1,500	0.28	1	Ethanol	17.5		0	At 5 concn	At 4.5	10 in series	7	
3 (air vs air + CO ₂)	I	300	0.18	2	Ethanol	0.5	1,000	0.5 for 17 tD, then 0.1	None	None (2.3-2.6)	2 parallel	33	

forming branched aggregates. The size of the aggregates depended on the cultivation conditions. The only series with separated single cells and negligible aggregation were the four cultures aerated by pure oxygen and the air-aerated culture using 5 mg of O₂ per liter in experiment 2 (Fig. 1).

In all the other cultures, the aggregate size usually was between 5 and 20 cells. The smallest aggregates were observed after prolonged incubation on glucose (experiment 1, Fig. 2) and on ethanol with CO₂ enrichment (experiment 3, Fig. 3). In experiment 2, in air-aerated cultures, an indirect relation between X and aggregate size was found, except with the lowest O₂ concentration tested (Fig. 1). In experiment 1 on ethanol, the aggregate size increased gradually from 16 mean retention times (tD) on, and after 27 tD the aggregates were so dense that the cell num-

FIG. 1. Cultivation on ethanol (dilution rate = 0.28 h⁻¹; inflow substrate concentration = 17.5 g/liter): values after 7 tD at various dissolved-oxygen concentrations (x axis). Closed symbols, aeration by pure oxygen; open symbols, aeration by air. Abbreviations: X , dry weight; M , mean cell mass; v , mean protoplast volume; n , mean cell number per aggregate.

FIG. 2. Cultivation on glucose (closed symbols) and ethanol (open symbols). Dilution rate = 0.15 h⁻¹; inflow substrate concentration = 0.5 g/liter. Abbreviations: A , absorbance; others as in Fig. 1.

bers could only be roughly estimated (Fig. 2). Under comparable cultivation conditions (i.e., experiment 3, cultivation on ethanol without CO₂ enrichment), which differed only in having higher aeration intensity, the aggregate size varied between 11 and 18 during 33 tD and showed no further increase.

The sizes and shapes of individual cells varied in all experiments within similar ranges. The variation of individual cell sizes in one sample also was comparable for all experiments judging from similar variation coefficients. The mean cell lengths and widths usually correlated. However, after prolonged incubation in both series of experiment 1 and in series without CO₂ enrichment in experiment 3, there was an increase in

ratio of length to width (Tables 3 and 5). The smallest cells (below $10 \mu\text{m}^3$) were found in experiment 1 in both series after 20 tD and in experiment 3 in the series without CO_2 enrichment. In the same experiment under CO_2 enrichment, the average volumes increased during cultivation to values comparable to those from experiment 2 (Table 4), and toward the end they reached the maximum values found (about $25 \mu\text{m}^3$).

Judging from absorbance or dry weight, steady states were achieved in all experiments after 7 to 10 tD without greater fluctuations, except in the ethanol-fed culture in experiment 1. In experiment 3 with 0.5% CO_2 enrichment, both the absorbance and cell counts were constantly lower by 50% than in the culture without CO_2 addition. From 17 tD on, the CO_2 percentage was lowered to 0.1%. Subsequently absorbance and cell counts increased, and within 5 tD they reached a new steady-state value that was slightly higher than in the other culture (Fig. 3).

Cell counts and cell sizes varied more than

FIG. 3. Cultivation on ethanol (dilution rate = 0.18 h^{-1} ; inflow substrate concentration = 0.5 g/liter). Closed symbols, aeration by air enriched with CO_2 ; open symbols, aeration by air. Arrow indicates the time of change in CO_2 percentage from 0.5 to 0.1. Abbreviations are as in Fig. 2.

TABLE 3. Characteristics of protoplast sizes and cell mass in experiment 1 (average of 50 cells)

Parameter ^a	Substrate ^b	Value after given tD			
		16.3	19.9	23.5	27.2
Length	a	3.1	3.0	3.0	3.1
	b	3.4	3.1	3.1	2.9
Length/width	a	1.54	2.11	2.26	2.13
	b	1.72	2.26	2.13	2.13
C_v	a	53.2	49.0	57.5	
	b	72.6	55.9	58.2	55.3
v/s	a	0.53	0.42	0.41	0.42
	b	0.59	0.41	0.43	0.41
X/B	a	0.79	1.55	1.47	
	b	0.41	0.87	0.91	0.77

^a C_v , Coefficient of variation of volume (v) (standard deviation as percentage of the mean); s , surface.

^b a, Ethanol; b, glucose.

absorbance or X values, especially in air-aerated, ethanol-fed cultures. Consequently, the M also varied within a broad range in these cultures (15 to 30 in experiment 1, 8 to 28 in experiment 2, and 8 to 26 in experiment 3), with considerable fluctuations during prolonged cultivation. On the other hand, both the glucose-fed culture and the oxygen-aerated and CO_2 -enriched ethanol-fed cultures showed consistently lower and rather stable M values in the range of 8 to 10 pg (experiments 1 and 2) or 11 to 18 pg (experiment 3).

The X/B ratio was lowest in the oxygen-aerated cultures of experiment 2 (below 0.50) and did not exceed 1.00 in the glucose-fed culture (experiment 1) and ethanol-fed culture with 0.1% CO_2 (experiment 3). In the other cultures, higher values of X/B and wide fluctuations were observed. The high X/B ratio indicated that the dry weight included a considerable share of cell wall or envelope material. The negative stain of cells from ethanol-fed air-aerated cultures did not show any capsular envelopes. However, on scanning electron micrographs, the cell aggregates appeared to be embedded in amorphous matter without apparent structure.

In Table 6, the ranges of morphological characteristics were compared for the cultivations with and without pH regulation. The ranges were similar for both types of cultivations, with a slight trend toward higher values of M and X/B at suboptimal pH.

The percentage of dead cells and the age distribution (Table 7) was investigated in experiment 3. These values were similar in both series, and the age distribution approached the theoretical value (3). The oldest cells observed (<1% of total) had six to seven mother scars. Like dead cells, they were always found in the middle of the aggregates. The low percentage of dead

TABLE 4. Characteristics of protoplast sizes and cell mass in experiment 2 at different oxygen concentrations/aeration conditions (average of 100 cells)

Parameter ^a	Aeration ^b		Values after 7 tD				
O ₂ concn (mg/liter)	a		0.89	2.98	4.47	4.84	5.44
	b	0.43		2.84	4.25		5.67
Length	a		3.4	3.5 ^c	3.2	3.7	3.7
	b	4.3		3.6	3.7		3.3
Length/width	a		2.13	2.0 ^c	1.88	2.18	2.31
	b	2.87		1.89	1.95		2.06
C _v	a		52.3	48.9	43.6	42.6	62.0
	b	84.1		66.1	73.2		58.7
v/s	a		0.48	0.50 ^c	0.48	0.50	0.48
	b	0.50		0.58	0.58		0.48
X/B	a		0.48	1.33 ^c	1.56	0.94	0.99
	b	0.38		0.42	0.34		0.46

^a See Table 3.^b a, Aerated by air; b, aerated by pure oxygen.^c Average of two cultures.

TABLE 5. Characteristics of protoplast sizes and cell mass in experiment 3 (average of 100 cells)

Parameter ^a	Aeration ^b	Value after given tD					
		11.7	16.1	20.0	24.5	28.6	33.1
Length	a	3.4	3.5	3.8	3.7	3.6	3.1
	b	3.7	4.1	4.5	4.5	4.7	5.1
Length/width	a	2.0	2.10	2.53	2.64	2.57	2.38
	b	2.31	2.21	2.81	2.36	2.61	2.38
C _v	a	37.9	50.4	43.3	42.6	68.3	49.5
	b	38.5	42.7	45.4	62.2	52.0	49.5
v/s	a	0.48	0.46	0.46	0.41	0.42	0.40
	b	0.48	0.52	0.49	0.57	0.53	0.55
X/B	a	0.88	0.93	0.75	1.83	1.91	0.80
	b	0.99	0.51	0.62	0.44	0.72	0.53

^a See Table 3.^b a, Aerated by air; b, aerated by air enriched with CO₂.

cells indicated that low pH values did not adversely affect viability.

DISCUSSION

The values of CO₂ supply or CO₂ tension around the cells could not be estimated from our data. Several techniques are available for measuring CO₂ tension in the bulk liquid phase (28) and such measurements would permit a more rigorous study of these phenomena. However, the CO₂ concentration in the cell environment is different from that in the liquid phase. As for oxygen, the CO₂ concentration gradient around and within the cell depends both on the aeration intensity and gas transfer coefficient and on the cell morphology (shape, volume-to-surface ratio, aggregation, thickness of envelopes) and cell or biomass concentration. Even if there is no morphological diffusion barrier for CO₂, the concentration gradient around the cell may depend on the ratios of the respective rates: CO₂ production rate and CO₂ demand by the cells and liquid-to-gas transfer rate.

Under our experimental protocol, three com-

binations of cultivation conditions were examined, permitting at all times a comparison of two cultures with different CO₂ availability. This comparison was not so clear in experiment 2 (Table 5, Fig. 1). At the lowest O₂ concentration tested, aeration by air (though more intense than by pure oxygen) apparently was still too low and inefficient to remove CO₂. Moreover, the concentration of biomass considerably differed in the two series compared except for a narrow range of O₂ concentrations (Fig. 1, top).

During prolonged incubation in experiments 1 and 3, morphological differences between the two compared cultures increased and were not stable even after 20 to 30 tD. However, no wash-out or regular increase in cell counts occurred, and the absorbance values were stable. Fluctuations in cell counts and sizes under constant external conditions may be explained by a feedback mechanism, as examined and modeled by Krambeck et al. (12) for a bacterial strain growing on methane as the sole carbon and energy source.

In glycerol-fed cultures of *C. utilis*, cell morphology was examined at different pH values (14). At the suboptimal pH of 2.3, cells were shorter than at pH 4.5, they had thicker walls, and 10% were in aggregates (compared with only 7% at pH 4.5). In our experiments, the morphological differences between two series (with similar pH) of the same experiment were greater than the above-mentioned differences, and no consistent difference between the cultures at pH 2.1 to 2.6 and pH 4.5 was found (Table 6). Irrespective of pH, each experimental series with lower CO₂ availability differed from the other in the following typical characteristics: (i) higher degree of cell aggregation, (ii) higher X/B ratio indicating a higher share of wall or extracellular material in the total biomass, (iii) in most cases,

TABLE 6. Range of morphological characteristics in experiments without (1 and 3) and with (2) pH regulation

Expt no.	pH	Parameter				
		Length	Length/width	Vol	M	X/B
1, 3	2.1–2.6	2.9–5.1	1.54–2.81	9.2–28.3	9.4–30.2	0.41–1.91
2	4.5	3.3–4.3	1.89–2.87	14.9–23.5	7.9–28.7	0.34–1.56

TABLE 7. Percentage of 500 cells without mother scars and dead cells

Category	Aeration ^a	Values after given tD		
		23	27	31
No mother scars	a	43.1	46.0	48.4
	b	45.3	43.0	46.2
Dead cells	a	5.8	3.9	2.4
	b	5.1	5.2	3.2

^a See footnote b, Table 5.

lower protoplast volumes. The only exceptions were the cultures with biomass concentrations round 6 g/liter with small aggregates, but still higher X/B ratios in air-aerated than in oxygen-aerated cultivations (experiment 2). Apparently, at this cell density the CO₂ level is high even with rather high aeration. Consistent with our data, cell aggregation was mentioned, but not quantified (5), for *Saccharomyces cerevisiae* in continuous culture after increased aeration.

As a consequence of biotin deficiency, aggregation of cells was also found in *Arthrobacter globiformis* (4). In yeasts, deficiencies of meso-inositol, inositol, or biotin are known to influence the abscission process of daughter cells (1). Biotin deficiency of *S. cerevisiae* under certain conditions resulted in the formation of abnormally thick and multilayered cell walls (7, 8). The cell aggregates of *C. utilis* in our culture with lower CO₂ availability were of similar appearance, and the protoplasts in the aggregates were separated by thick wall layers. The effect of biotin deficiency on morphological changes in different microorganisms is often comparable to the effect of CO₂ deficiency (11).

During our experiments under low CO₂ availability, no true pseudomycelium was observed, although the effect of CO₂ deficiency on filament formation was regularly observed in various dimorphic organisms (27) and the effect of biotin deficiency on pseudomycelium growth of *C. albicans* was examined (17).

An effect of cell aggregation on the CO₂ concentration gradient in the immediate surroundings of the cells is probable. Loomis (15) assumed the changes occurring in cell morphology to be an adaptation for obtaining a desirable CO₂ concentration gradient. The thickening of the en-

velope between the protoplast and the external medium probably acts in a similar way.

At low CO₂ availability, though the total biomass might be similar to that formed with CO₂ enrichment, the proportion of protoplasts decreased considerably. The percentage of protein in yeast cell walls is usually about 10 (25), and so the protein production in CO₂-deficient cultures would be less efficient at a reasonable biomass yield. The protein content of *C. utilis* on ethanol may vary considerably due to various cultivation conditions (21, 24), and a rather wide range (30 to 50%) of dry weight was observed at the same yield of 0.6. Analogous with the above assumptions, an effect of increasing oxygen transfer coefficient on the decrease of cell protein of glucose-fed *Klebsiella aerogenes* in batch culture was observed (19).

Individual cell volumes or cell masses are known to increase and cell length/width ratios to decrease with increasing growth rate (26), especially near $\mu_{\max}$ (6). In our experiments, the ranges of morphological characteristics observed at different growth rates were quite comparable. The rates examined were about 0.3 to 0.6 of $\mu_{\max}$. In this region, apparently, the volumes are independent of growth rates, as previously found for other yeast species (10).

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LITERATURE CITED

1. Bartnicki-Garcia, S., and I. McMurrough. 1971. Biochemistry and morphogenesis of yeasts, p. 441–491. In A. H. Rose, and J. S. Harrison (ed.), *The yeasts*, vol. 2. Academic Press, Inc., New York.
2. Bartnicki-Garcia, S., and W. J. Nickerson. 1962. Induction of yeastlike development in *Mucor* by carbon dioxide. *J. Bacteriol.* 84:829–840.
3. Beran, K., I. Málek, E. Streiblová, and J. Lieblova. 1967. The distribution of the relative age of cells in yeast populations, p. 57–68. In E. O. Powell, C. G. T. Evans, R. E. Strange, and D. W. Tempest (ed.), *Microbial physiology and continuous culture*, 3rd International Symposium. Elliott Bros A. Yeoman Ltd., Liverpool.

4. Chan, E. C. S. 1964. Morphological aberration of *Arthro-bacter globiformis* cells due to biotin deficiency. *J. Bacteriol.* **87**: 641-651.
5. Cowland, T. W., and D. R. Maule. 1966. Some effects of aeration on the growth and metabolism of *Saccharomyces cerevisiae* in continuous culture. *J. Inst. Brew.* **72**:480-488.
6. Dean, A. C. R., and P. L. Rogers. 1967. The cell size and macromolecular composition of *Aerobacter aerogenes* in various systems of continuous culture. *Biochim. Biophys. Acta* **42**:267-274.
7. Dixon, B., and A. H. Rose. 1964. Observations on the fine structure of *Saccharomyces cerevisiae* as affected by biotin deficiency. *J. Gen. Microbiol.* **35**:411-419.
8. Dunwell, J. L., F. Ahmad, and A. H. Rose. 1961. Changes in the polysaccharide composition of yeast resulting from biotin deficiency. *Biochim. Biophys. Acta* **51**:604-607.
9. Herbert, D. 1976. Stoichiometric aspects of microbial growth, p. 1-30. In A. C. R. Dean, D. C. Ellwood, C. G. T. Evans, and J. Melling (ed.), *Continuous culture*, vol. 6: Applications and new fields. E. Horwood, Chichester.
10. Johnston, G. C., C. W. Ehrhardt, A. Lorincz, and B. L. A. Carter. 1979. Regulation of cell size in the yeast *Saccharomyces cerevisiae*. *J. Bacteriol.* **137**:1-5.
11. Jordan, H. V., and A. Howell. 1965. Nutritional control of cellular morphology in an aerobic actinomycete from the hamster. *J. Gen. Microbiol.* **38**:125-130.
12. Krambeck, C., H. J. Krambeck, and J. Overbeck. 1977. Autonomous growth fluctuations of the methane oxidizing bacterial strain M 102 in batch and continuous culture. *Arch. Microbiol.* **115**:119-126.
13. Light, P. A. 1972. Influence of environment on mitochondrial function in yeast, p. 509-526. In A. C. R. Dean, S. J. Pirt, and D. W. Tempest (ed.), *Environmental control of cell synthesis and function*, 5th International Symposium. Academic Press, Inc., New York.
14. Lirova, S. A., E. Schuhmann, K. Strunk, M. P. Khovrychev, and I. L. Rabotnova. 1978. The effect of pH values of the medium unfavourable for growth on physiological, morphological and cytological characteristics of the chemostat culture of *Candida utilis*. *Mikrobiologiya* **47**:414-423. (In Russian).
15. Loomis, W. F. 1961. Cell differentiation. A problem in selective gene activation through self-produced micro-environmental differences of carbon dioxide tension, p. 509-525. In T. W. Goodwin and O. Lindberg (ed.), *Biological structure and function*. Academic Press, Inc., New York.
16. Machek, F., F. Štros, A. Prokop, and L. Adámek. 1976. Production and isolation of protein from synthetic ethanol, p. 135-145. In A. C. R. Dean, D. C. Ellwood, C. G. T. Evans, and J. Melling (ed.), *Continuous culture*, vol. 6: Applications and new fields. E. Horwood, Chichester.
17. Nickerson, W. J., and Z. Mankowski. 1953. Role of nutrition in the maintenance of the yeast—shape in *Candida*. *Am. J. Bot.* **40**:584-592.
18. Oura, E., and S. Haarasilta. 1976. The capacity of baker's yeast to assimilate CO₂, p. 123-125. In A. L. Kairanto, E. Riihimäki, and P. Tarkka (ed.), *Proceedings of the 2nd National Meeting on Biophysics and Biotechnology*. Espoo, Finland.
19. Páca, J., and M. Fabiánková. 1976. Effect of external conditions of facultative anaerobic bacteria cultivations on changes in cell proteins. *Sci. Pap. Inst. Chem. Technol. Prague E* **46**:67-93.
20. Páca, J., and V. Grégr. 1977. Growth and metabolic activity of *Klebsiella aerogenes* in a chemostat culture. *J. Ferment. Technol.* **55**:213-223.
21. Páca, J., and V. Grégr. 1977. Growth characteristics of *Candida utilis* on volatile substrate in a multistage tower fermentor. *Biotechnol. Bioeng.* **19**:539-554.
22. Páca, J., and V. Grégr. 1979. Effect of dilution rate on growth characteristics of *Candida utilis* and in multistage tower fermentor. *J. Ferment. Technol.* **57**:227-232.
23. Páca, J., and V. Grégr. 1979. Effect of P_{O₂} on growth and physiological characteristics of *Candida utilis* in a multistage tower fermentor. *Biotechnol. Bioeng.* **21**:461-477.
24. Páca, J., and V. Grégr. 1979. Growth characteristics of *Candida utilis* in a multistage culture system. *Enzyme Microb. Technol.* **1**:100-106.
25. Phaff, H. J. 1971. Structure and biosynthesis of the yeast cell envelope, p. 135-210. In A. H. Rose and J. L. Harrison (ed.), *The yeasts*, vol. 2. Academic Press, Inc., New York.
26. Veldkamp, H. 1976. *Continuous culture in microbial physiology and ecology*. Meadowfield Press, Durham.
27. Wimpenny, J. W. T. 1969. Oxygen and carbon dioxide as regulators of microbial growth and metabolism, p. 161-197. In P. Meadow and S. J. Pirt (ed.), *Microbial growth*, 19th Symposium of the Society for General Microbiology. Cambridge University Press, Cambridge.
28. Yagi, H., and F. Yoshida. 1977. Desorption of carbon dioxide from fermentation broth. *Biotechnol. Bioeng.* **19**:801-819.