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Lipid Accumulation in an Oleaginous Yeast (*Candida* 107) Growing on Glucose Under Various Conditions in a One- and Two-Stage Continuous Culture

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Lipid accumulation and fatty acid composition in *Candida* 107 have been studied using a two-stage continuous culture system in which the first vessel was run under carbon-limited conditions and then the entire output was passed into a second vessel, where lipid accumulation was stimulated by adding only glucose. Maximum lipid accumulation (28% of yeast [dry weight]) occurred for a volume ratio of vessel 1 to vessel 2 of 3:5, with 30 g of glucose per liter being added to vessel 2 operated at 25°C with an aeration rate of between 0.1 and 1.0 volume of air/volume of medium per min. Although the maximum specific rate of lipid formation (0.05 g of lipid/g of yeast per h) was higher than in a nitrogen-limited, single-stage system, the efficiency of lipid formation was much less and never exceeded 14 g of lipid produced per 100 g of glucose consumed. The fatty acid composition was not significantly altered in either the two-stage or single-stage culture (nitrogen-limited) systems by changes in growth temperature (from 19 to 33°C) or aeration rates (0.05 to 1.0 volume of air/volume of medium per min); or, in the two-stage system, by changes in the residence time of the yeast in the second vessel (from 3.2 to 24.4 h), or, in the single-stage system, by changes in pH (from 3.5 to 7.5). Only when the concentration of glucose entering vessel 2 of the two-stage system was less than 30 g/liter did significant changes in the fatty acids occur. Thus, although a two-stage continuous culture system allows lipid accumulation to be separated from the growth phase, it offers no practical advantages over a single-stage system as a means of producing microbial oils and fats.

We have shown that lipid accumulation occurs in an oleaginous yeast during continuous culture using nitrogen-limited medium (7). Under these conditions the amount of lipid accumulated can equal the concentration attained in the batch culture, provided the growth rate of the organism is held at about one-third of its maximum.

The use of continuous culture in evaluating the potential of microorganisms as a source of oils and fats is, therefore, beneficial, but it can also help to assess the influence of various growth conditions on lipid accumulation in an unequivocal manner. In this latter respect, there have been several studies in which effects such as dissolved oxygen, temperature, substrate concentration, etc., upon the lipid composition of yeasts have been examined (1, 2, 3, 9). Unfortunately, however, none of these studies has involved a recognized oleaginous (lipid-accumulating) microorganism. The accumulation of lipid in such organisms may well be subject to different constraints, particularly as the majority of lipid accumulation occurs after cell multiplication has finished (11, 14). Therefore, to study the effects that various growth parameters may have on lipid accumulation, we found it necessary to separate the two phases of yeast development and accordingly used a two-stage continuous culture system: in the first stage, growth occurred without lipid accumulation, i.e, by using carbon-limited medium; in the second stage, lipid accumulation was encouraged by feeding only glucose as an extra nutrient and thus allowing the yeast to grow nitrogen limited. By varying the conditions only in the second stage we hoped to see what, if any, were the primary influences upon lipid accumulation. At the same time, we ascertained whether the same variables produced similar effects when applied to lipid accumulation occurring in a single-stage continuous culture system. We believe this to be the first time that such effects have been examined in a oleaginous yeast growing in continuous culture and that this is also the first occasion that lipid

accumulation has been demonstrated in a yeast growing in a two-stage continuous culture system.

MATERIALS AND METHODS

Organism and growth. *Candida* 107 was grown in a one-stage continuous culture system under nitrogen-limited conditions as previously described (7) with a dilution rate of 0.10/h, the temperature at 30°C, the pH at 5.5 and the aeration rate at 1 volume of air/volume of medium per min. The medium contained (g/liter) KH_2PO_4, 7.0; Na_2HPO_4, 2.0; $MgSO_4 \cdot 7H_2O$, 1.5; NH_4Cl, 1.5; yeast extract, 1.5; $CaCl_2 \cdot 6H_2O$, 0.2; $FeCl_3 \cdot 6H_2O$, 0.1; biotin, 0.001; $ZnSO_4 \cdot 7H_2O$, 0.001; the pH was adjusted to 5.5 with HCl. Glucose was at 30 g/liter unless otherwise stated.

For growth in two-stage continuous culture, the first vessel was as previously specified (7) and contained 1.2 to 1.5 liters of medium. The second vessel was a similar unit with a maximum working volume of 3.5 liters (model MF105, New Brunswick Scientific Co. Inc., New Brunswick, N.J.). The units were connected so that the entire output of vessel 1 was pumped directly into vessel 2. The holdup time of yeast in the connecting tubing was less than 1 min. The composition of the medium for the first stage was carbon limited, i.e., with the above medium modified with NH_4Cl at 3 g/liter and glucose at 12 g/liter. Glucose, usually to give 30 g/liter, was the only nutrient added into the second stage which then ran nitrogen limited. Temperature, pH, and aeration rates for both vessels were as above except when otherwise stated. The dilution rate of vessel 1 was usually 0.15/h. Steady-stage conditions were held in vessel 1 without change during the course of an experiment; after a change in the steady-state conditions in vessel 2, at least five changes of the medium occurred before samples were taken on 2 consecutive days. If biomass and lipid concentrations did not agree, further samples were taken until they did.

The increase in biomass (or lipid) per liter of medium in vessel 2 was calculated as $x' - xf \cdot f'^{-1}$, where x and x' are the biomass (or lipid) concentrations (grams per liter) entering and leaving vessel 2 at flow rates f and f' respectively. The growth rate of the yeast in the second stage was calculated as $\mu' = f'x' - fx/V_2x'$, where V_2 is the volume of medium in vessel 2.

Calculations concerning the specific rate of lipid production were carried out as previously described (7). All calculation relating to yields, rates, etc., in vessel 2 were made for what happened in that vessel only.

Analytical measurements. All determinations were as previously described (7).

RESULTS

Effect of residence time in vessel 2 upon the accumulation of lipid in two-stage culture. Maximum lipid accumulation in one-stage continuous culture of *Candida* 107 requires the yeast to be grown at a dilution rate equal to one-third the value of the maximum growth rate (7). Therefore, in adapting a one-stage system to a two-stage system, the first criterion to be decided was the optimum residence time for the yeast in the second-stage vessel. In all cases, growth in the second vessel was limited by the supply of nitrogen. The amount of nitrogen being carried over from vessel 1 was reduced as low as possible without rendering the culture in vessel 1 nitrogen limited.

Using a constant input of yeast, the residence time for the yeast in vessel 2 was varied either by altering the volume within the vessel, or by adding a dilute solution of glucose. The results are presented in Table 1. In all cases, the rate of adding glucose into vessel 2 was varied so as to maintain a steady-stage concentration of glucose of 30 g/liter had there been no consumption of glucose in the second stage. Some growth occurred in vessel 2 at all dilution rates as the increase in yeast (dry weight) was always more than the amount of lipid being synthesized (Table 1). This was probably due both to NH_4Cl (approximately 0.15 g/liter) which was carried over from vessel 1, and, as this amount would have only increased the biomass by about 1 g/liter, also by intracellular pools of amino acids or similar compounds within the cells which entered vessel 2. This latter phenomenon was noted by Brown and Rose (3) after growth of *Candida utilis* also under glucose-limited conditions, and, if this was also occurring with *Candida* 107, protein synthesis, etc., could take place. Therefore, complete abolition of growth in vessel 2 would appear impossible even if NH_4Cl had been carried over, but growth is clearly far less than usual in a corresponding single-stage process.

Lipid accumulation did not begin in yeast that was held in vessel 2 until the dilution rate was 0.11/h or less and declined when the dilution rate became less than 0.08/h. Thus, lipid accumulation only occurs to an appreciable extent over a fairly narrow range of dilution rates. The amount of lipid produced, even at the optimum dilution rate, did not approach that previously attained in a single-stage culture (7), although the maximum specific rates of lipid production were approximately equal in the two situations.

The fatty acids of the total lipid (Table 1) were similar in proportion to those in the lipid entering vessel 2 and hardly varied in composition at any dilution rate. This was surprising in view of the range of dilution rates used (from 0.32 to 0.04/h) and the range of lipid contents of the yeast that resulted (from 25 to 6%). Such changes in dilution rate had, however, brought about changes in fatty acid composition of the

TABLE 1. *Effect of residence time on lipid accumulation and fatty acid composition of Candida 107 growing in a two-stage continuous culture*

Vessel	Dilution rate (per h)	Residence time (h)	Specific growth rate (per h)	Biomass (g/liter)	Biomass increase (g/liter)[a]	Rate of biomass synthesized (g/liter per h)	Biomass yield (g of yeast/100 g of glucose)[b]	% Lipid in biomass	Total lipid produced (g/liter)	Actual increase in lipid (g/liter)	Lipid yield (g of lipid/g of glucose)[b]
1[d]	0.15	6.67	0.15	6.07		0.91	50.5	9.5	0.57		4.8
2	0.316	3.16	0.184	5.34	3.11	0.98	35.5	10.4	0.55	0.35	4.0
	0.15	6.67	0.089	8.85	3.17	0.47	31.0	11.9	1.05	0.57	5.6
	0.11	9.1	0.056	11.59	5.92	0.65	34.0	18.1	2.09	1.6	9.1
	0.098	10.2	0.059	13.04	7.84	0.77	36.6	25.4	3.31	2.92	13.6
	0.080	12.5	0.054	15.08	10.12	0.81	37.4	24.2	3.65	2.91	10.7
	0.053	18.9	0.025	11.07	5.22	0.28	22.3	11.0	1.22	0.61	2.7
	0.041	24.4	0.023	15.46	9.40	0.38	26.8	6.3	0.97	0.4	1.1

Vessel	Rate of lipid synthesized (g/liter per h)	Specific rate of lipid production (g of lipid/g of biomass per h)	Relative proportion of fatty acids in lipid (%, wt/wt)								
			14:0	16:0	16:1	18:0	18:1	18:2	18:3	22:0 + 24:0	Δ/mol[c]
1[d]	0.086	0.014	T[e]	21.5	1.5	7.5	40.0	23.5	T	2.5	0.89
2	0.11	0.035	T	22.9	2.5	8.8	37.6	24.5	2.1	T	0.90
	0.085	0.027	T	26.5	2.4	6.9	37.8	22.2	2.1	T	0.90
	0.18	0.031	T	28.9	1.5	7.0	41.5	16.3	1.8	1.1	0.81
	0.29	0.037	T	28.4	2.0	6.1	42.3	21.0	T	T	0.86
	0.23	0.020	T	26.4	2.8	6.4	39.9	19.8	2.1	T	0.88
	0.03	0.006	T	27.4	2.5	6.9	38.2	21.0	2.0	T	0.88
	0.016	0.002	T	31.0	2.1	7.0	36.0	20.0	1.0	T	0.81

[a] Calculated as given in Materials and Methods.
[b] For vessel 2, this refers to glucose added only to that vessel.
[c] Calculated as by Kates and Baxter (10).
[d] Average values pertaining in vessel 1, acting as feed vessel, over course of run; biomass ±0.5 g/liter; lipid percent, ±1.5.
[e] T, Trace.

lipid during single-stage cultivation (7).

In the ensuing work, the volume ratio of vessel 1/vessel 2 was kept at about 3:5 with respective dilutions rates of 0.15/h and 0.1/h, which was the optimum indicated for maximum lipid accumulation. The effects of various growth conditions on lipid formation in vessel 2 were then studied. The effects of these conditions on lipid accumulation in a single-stage culture were determined for comparison.

Effect of glucose concentration. Previous work (7) established that when NH_4Cl was at 1.5 g/liter, glucose at 30 g/liter gave a high lipid content of the yeast but without being completely exhausted. Although glucose was, therefore, used at this concentration in subsequent work, its concentration in the incoming medium was found to influence markedly not only the amount of lipid synthesized, which was to be expected, but also the fatty acid composition of that lipid (Table 2).

In both one- and two-stage culture, lipid accumulation was maximal for glucose at about 30 g/liter. In the single-stage system, conditions were not optimal for maximum lipid accumulation which requires a dilution rate of about 0.06/h (7) and, furthermore, with only 10 and 20 g of glucose/liter, growth was both carbon and nitrogen limited. In both systems, increasing the concentration of glucose produced less unsaturation in the fatty acids which was more marked in the two-stage system.

A rearrangement of fatty acids occurred in the yeast that was held in the second stage of the two-stage system without any addition of glucose. Although there was no net synthesis or catabolism of lipid (or biomass), the proportion of linoleic acid in the fatty acids increased from

TABLE 2. *Effect of glucose concentration on lipid accumulation and fatty acid composition of Candida 107 growing at constant dilution rate in one- and two-stage continuous cultures*

Culture	Concn of glucose added (g/liter)	Glucose consumed (g/liter)	Biomass (g/liter)	Biomass increase (g/liter)	Rate of biomass synthesized (g/liter per h)	Biomass yield (g of yeast/100 g of glucose used)[a]	% Lipid in biomass	Total lipid produced (g/liter)	Lipid increase (g/liter)	Rate of lipid synthesized (g/liter per h)	Lipid yield (g of lipid/100 g of glucose)[a]
One-stage[c]	10	10	4.6		0.46	46.4	9.4	0.43		0.043	4.3
	20	20	9.0		0.90	45.2	17.6	1.6		0.16	8.0
	30	29.5	12.4		1.24	42.0	15.4	3.15		0.315	10.5
	40	30.5	11.1		1.11	36.3	23.6	2.62		0.26	6.5
Two-stage[e]	0	0	5.1	−0.4	0	0	9.1	0.46	0		
	10	10	8.7	3.6	0.4	35.9	17.6	1.52	0.95	0.10	9.5
	20	20	11.3	6.5	0.71	32.5	22.7	2.55	2.01	0.22	10.0
	30	26.5	10.9	6.5	0.74	24.4	28.6	3.1	2.57	0.29	8.5
	40	27.5	9.8	5.8	0.67	21.0	26.2	2.55	2.01	0.23	5.75

Culture	Specific rate of lipid production (g of lipid/g of yeast per h)	Relative proportion of fatty acids in lipid (%, wt/wt)									Δ/mol[b]
		12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	22:0 + 24:0	
One-stage[c]	0.009	T[d]	0.5	26.5	2.6	7.2	32.6	28.3	1.0	T	0.95
	0.018	T	T	28.5	1.0	7.6	38.6	23.1	1.0	T	0.89
	0.025	T	T	29.8	1.0	8.0	40.1	20.2	1.0	T	0.85
	0.024	T	T	31.6	T	8.1	40.6	18.0	1.0	T	0.80
Two-stage[e]		T	1.2	21.3	1.4	4.8	26.4	41.8	1.4	1.0	1.17
	0.025	T	2.4	24.5	4.1	8.5	31.7	26.3	2.3	T	0.95
	0.034	T	1.3	28.0	T	7.8	48.2	9.5	2.3	2.0	0.75
	0.033	T	1.2	26.0	T	7.5	45.1	12.3	2.7	3.0	0.78
	0.040	T	1.0	26.0	1.2	7.9	47.4	11.4	1.9	3.0	0.77

[a] See Table 1.
[b] See Table 1.
[c] One-stage culture run at D = 0.1/h. N.B. This was not the feed vessel for vessel 2 in the two-stage system.
[d] T, Trace.
[e] Two-stage culture: vessel 1, glucose limiting at 12 g/liter, D = 0.155 h/lipid content at 11.5% ± 2%, fatty acid composition as given in Table 1. Vessel 2, D from 0.103/h (no glucose added) to 0.116/h (40 g of glucose added per liter). Ratio of volumes, vessel 1:vessel 2 = 0.667; μ for vessel 2 = 0.05 to 0.06/h.

24% in the incoming yeast to 42% in the outgoing yeast. In all other cases, no such rearrangement was evident, suggesting that in the absence of a carbon source dehydrogenation of lipids may be stimulated.

Effect of temperature. An increase in the growth temperature of an organism often brings about an increase in the proportion of saturated fatty acids in the lipid and has been reported often as a major influence on lipid composition (15). *Candida* 107 has a maximum growth temperature of about 34°C and could be maintained at a dilution of at least 0.1/h between 19 and 33°C in a single-stage chemostat, although the biomass concentration was adversely affected at the extremes of temperature (Table 3). The amount of lipid synthesized, however, showed little variation. In the two-stage system the biomass concentration remained constant between 22 and 32.5°C, though lipid accumulation reached a maximum at 25°C. In the one-stage system lipid accumulation was highest at 30°C (Table 3).

Effect of aeration rate. The formation of unsaturated acids is an oxygen-dependent process (12), and low aeration rates, producing low dissolved oxygen levels, therefore may affect the fatty acid composition of a lipid. This effect would be particularly relevant if an oleaginous yeast was growing at a high cell density (say, greater than 50 g/liter) which, by having a high oxygen demand, may induce conditions of oxygen-limited growth. An aeration rate of 0.1 volume of air/volume of medium per min, or less, was insufficient to maintain the biomass at its usual density in the single-stage system which

TABLE 3. *Effect of temperature on lipid accumulation and fatty acid composition of Candida 107 growing at constant dilution rate in one- and two-stage continuous cultures*

Culture	Temp (°C)	Biomass (g/liter)	Biomass increase (g/liter)	Rate of biomass synthesized (g/liter per h)	Biomass yield (g of yeast/100 g of glucose)[a]	% Lipid of biomass	Total lipid produced (g/liter)	Lipid increase (g/liter)	Rate of lipid synthesized (g/liter per h)	Lipid yield (g of lipid/100 g of glucose)[a]	Specific rate of lipid production (g of lipid/g of yeast per h)
One-stage[c]	19	7.6		0.76	37.9	17.9	1.36		0.14	6.7	0.018
	24	10.9		1.09	44.3	15.5	1.69		0.17	6.9	0.016
	27	11.1		1.11	45.1	16.6	1.84		0.18	7.5	0.016
	30	10.3		1.03	43.6	21.5	2.21		0.22	9.4	0.021
	32.8	4.6		0.46	38.3	14.0	0.64		0.064	5.3	0.014
Two-stage[e]	22	12.4	7.16	0.71	33.4	18.1	2.24	1.79	0.18	8.4	0.025
	25	13.0	7.7	0.81	35.4	27.0	3.5	3.0	0.31	13.7	0.054
	27.5	13.3	7.7	0.80	38.6	20.5	2.72	2.24	0.23	11.2	0.030
	30	12.9	7.8	0.83	36.5	21.0	2.71	2.26	0.24	10.6	0.031
	32.5	12.5	7.3	0.76	34.6	19.5	2.43	1.93	0.20	9.1	0.027

Culture	Relative proportion of fatty acids in lipid (%, wt/wt)										Δ/mol[b]
	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	22:0	24:0	
One-stage[c]	T[d]	T	28.2	2.3	6.0	42.0	19.3	0.6	0.4	1.2	0.85
	T	0.7	22.6	1.5	7.2	37.7	21.3	2.0	2.3	4.7	0.88
	T	T	25.6	1.0	8.5	40.3	20.4	1.8	T	2.4	0.88
	T	T	22.6	2.5	4.6	41.7	21.2	1.5	2.2	2.9	0.91
	T	0.9	24.4	1.8	7.3	41.8	18.9	1.7	3.0		0.87
Two-stage[e]	T	T	26.7	2.9	3.9	42.7	15.0	2.2	1.5		0.82
	T	T	23.5	1.6	7.7	48.0	8.9	2.4	2.0		0.75
	1.0	1.0	29.0	2.0	8.1	41.0	15.3	1.9	T		0.79
	T	T	28.0	2.0	7.0	42.6	14.9	2.3	T		0.81
	T	T	30.0	1.7	8.3	43.0	15.9	2.0	T		0.81

[a] See Table 1.

[b] See Table 1.

[c] One-stage culture: D = 0.1/h; other conditions as given in Materials and Methods. Note: This was not the feed vessel for vessel 2 in the two-stage system.

[d] T, Trace.

[e] Two-stage culture: vessel 1, glucose limiting at 12 g/liter; D = 0.015/h; temperature, 30°C; biomass, 5.9 ± 0.2 g/liter; lipid content, 9.1 ± 0.5%; fatty composition as given in Table 1. Vessel 2, D = 0.1/h; glucose added at 30 g/liter. Ratio of volumes of vessel 1:vessel 2 = 0.632; μ for vessel 2 = 0.05 to 0.06/h.

therefore decreased with a concomitant drop in lipid accumulation (Table 4). Surprisingly, no such changes occurred in the two-stage system where the yeast maintained its concentration at this low aeration rate and lipid accumulation was as great at the lowest oxygen levels as at the highest. Neither in the one- nor two-stage system did a restricted air supply have a significant effect on the synthesis of unsaturated acids. (Deprivation of oxygen was confirmed at the lowest aeration rates in both systems by using a dissolved oxygen electrode.)

Effect of pH. The effect of pH on lipid accumulation has not been as extensively studied as other parameters, but, from what little information is available (11, 15), it probably has little effect on fatty acid composition. The effect of pH was therefore studied only in a one-stage system (Table 5) and predictably, within the range of pH values at which *Candida* 107 can grow, pH had little influence either on the amount of lipid produced or its fatty acid composition. Optimum yields of biomass and lipid were obtained at pH 5.5.

DISCUSSION

The changes that occur in the composition of a microorganism due to variations in growth conditions reflect the changes which have been brought about in the metabolic status of that organism. The composition of a given constituent may, however, change either as a direct consequence of an alteration in the environment or as an indirect consequence of a change in some other chemically unrelated constituent. These two types of influence on the composition of a constituent of a microorganism might be separated if the growth phase of the organism does not coincide with the synthesis of that constituent. Such is the case with lipid

TABLE 4. *Effect of aeration rate on lipid accumulation and fatty acid composition of Candida 107 growing at constant dilution rate in one- and two-stage cultures*

Culture	Aeration rate (vol of air/vol of medium per min)	Biomass (g/liter)	Biomass increase (g/liter)	Rate of biomass synthesized (g/liter per h)	Biomass yield (g of yeast/100 g of glucose used)[a]	% Lipid in biomass	Total lipid produced (g/liter)	Lipid increase (g/liter)	Rate of lipid synthesized (g/liter per h)	Lipid yield (g of lipid/100 g of glucose)[a]
One-stage[c]	0.05	5.3		0.53	32.1	10.4	0.55		0.055	3.3
	0.1	9.4		0.94	39.5	19.7	1.85		0.185	7.8
	0.5	10.3		1.03	44.6	23.9	2.46		0.246	10.8
	1.0	10.6		1.06	45.3	21.4	2.26		0.226	10.7
Two-stage[e]	0.05	13.7	8.0	1.12	35.8	22.1	3.03	2.46	0.34	10.9
	0.1	13.9	8.4	1.17	37.9	25.1	3.5	2.9	0.41	13.06
	0.5	12.7	7.3	1.02	39.7	21.5	2.7	2.26	0.32	12.28
	1.0	11.7	6.5	0.91	40.1	21.4	2.5	2.04	0.28	12.7

Culture	Specific rate of lipid production (g of lipid/g of yeast per h)	Relative proportion of fatty acids in lipid (%, wt/wt)									
		12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	22:0 + 24:0	Δ/mol[b]
One-stage[c]	0.010	T[d]	T	27.1	1.8	11.9	39.1	18.9	1.0	T	0.82
	0.020	T	T	25.9	2.1	8.6	42.6	18.6	1.5	T	0.86
	0.024	T	T	25.2	2.0	7.3	41.3	22.9	1.0	T	0.92
	0.021	T	T	24.1	2.0	8.6	41.7	21.3	2.0	T	0.92
Two-stage[e]	0.042	1.1	1.3	24.1	2.0	5.9	45.0	14.9	2.5	2.0	0.84
	0.049	T	1.5	24.5	2.7	8.6	41.6	15.7	2.4	2.0	0.83
	0.044	1.0	1.4	29.1	2.1	7.9	40.4	15.1	2.0	1.0	0.79
	0.043	T	1.3	25.7	2.0	7.6	43.7	15.9	2.0	1.0	0.83

[a] See Table 1.
[b] See Table 1.
[c] One-stage culture was run at $D = 0.1$/h. Note: This was not the feed vessel for vessel 2 in the two-stage system.
[d] T, Trace.
[e] Two stage culture: conditions as given in Table 3. Ratio of volume of vessel 1:vessel 2 = 0.63; μ for vessel 2 = 0.05 to 0.06/h.

formation, and, by following this under a variety of conditions in a two-stage continuous system, we hoped to ascertain what were the primary influences on lipid and fatty acid synthesis. Having completed this study we are, however, unable to make such an evaluation because of the unexpected constancy of the fatty acid composition of the lipid under most growth conditions. As *Candida* 107 is the only organism to have been examined in such a manner, we do not know whether this is a characteristic of the organism or a widely occurring phenomenon among oleaginous microorganisms. The former is probably the case, however, as many nonoleaginous yeasts readily alter their fatty acid profiles with changes in temperature, aeration rate, and pH (8, 14, 15). There is unlikely to be any biochemical reason why the majority of oleaginous yeasts should behave differently.

The fatty acid profile of *Candida* 107, however, is not immutable. Changes in the growth rate and the choice of a limiting nutrient produce considerable variations in the proportions of the fatty acids (7) as do changes in the growth substrate (17) and, as shown here, the concentration of glucose in the incoming medium. The influence of different concentrations of glucose on fatty acid composition has been observed previously by Johnson et al. (9) and Babij et al., (1) who both worked with *C. utilis*; however, they observed opposite effects. The former found that linoleic acid decreased in proportion from 54 to 29% as the glucose concentration increased, whereas the latter workers found, under similar conditions, that linoleic acid increased from 5 to 25% of the total fatty acids. Our findings, though not as marked, are similar to those of Johnson et al. (9).

The content of lipid in *Candida* 107 was adversely affected by low aeration rates in the single-stage culture, but not in the two-stage system. The rate of biomass production in vessel 2 of the two-stage system, though higher

TABLE 5. *Effect of pH on lipid accumulation and fatty acid composition of Candida 107 growing at dilution rates of 0.1/h in a one-stage continuous culture*

pH	Biomass (g/liter)	Rate of biomass synthesized (g/liter per h)	Biomass yield g of yeast/100 g of glucose)	% Lipid in biomass	Lipid produced (g/liter)	Rate of lipid synthesized (g/liter per h)	Lipid yield (g of lipid/100 g of glucose)	Specific rate of lipid production (g of lipid/g of yeast per h)	Relative proportion of fatty acids in lipid (%, wt/wt)								
									14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	22:0 + 24:0	Δ/mol[a]
3.5	10.4	1.04	39.3	18.4	1.9	0.19	7.2	0.018	0.7	29.2	1.0	7.4	41.6	16.3	1.3	1.0	1.5	0.79
4.5	11.9	1.19	44.7	20.1	2.4	0.24	8.9	0.020	0.5	28.6	1.6	8.6	43.1	14.7	1.2	1.0	T	0.78
5.5	12.2	1.22	45.6	22.6	2.76	0.276	10.1	0.022	1.0	29.8	1.0	6.4	39.6	19.6	1.4	1.3	T	0.85
6.5	11.6	1.16	43.1	22.0	2.55	0.25	9.3	0.022	T[b]	33.0	1.0	8.1	39.7	16.4	1.0	T	T	0.77
7.5	11.8	1.18	38.6	19.5	2.30	0.23	7.5	0.019	T	29.1	2.0	6.9	40.4	18.9	1.0	1.6	T	0.83

[a] See Table 1.
[b] T, Trace.

than in the single-stage unit, was not limited by the low supply of air which presumably reflects a decrease in oxygen demand due to some alteration in metabolism. Presumably, as growth was unaffected, this did not retard lipid production. Respiratory processes are thus clearly connected with lipid synthesis which concurs with the conclusion of Einsele et al. (5) that the respiratory efficiency within *Candida tropicalis* controls the rates of synthesis of cell components. Rogers and Stewart (16) concluded similarly after observing a decrease in fatty acid content of *Candida parapsilosis* at low oxygen concentrations without a significant alteration in the degree of unsaturation. Numerous workers, however, have found that oxygen-limited growth does decrease the synthesis of unsaturated acids in a variety of yeasts (4, 13, and references therein).

The failure of *Candida* 107 to alter its fatty acid profile with changes in temperature seems unusual. Although Brown and Rose (3) found only slight variations in the overall degree of unsaturation of the fatty acids from *C. utilis* in going from 30 to 15°C, this was due to the not inconsiderable changes in concentrations of linolenic acid being counter-balanced by opposite variations in linolenic acid concentrations. Other workers, however, have reported changes in fatty acid profiles in oleaginous microorganisms grown at different temperatures (6, 18), but their studies did not use chemostat cultures which are essential to eliminate alterations in the growth rate of organisms which, in turn, may also cause the fatty acids to change in composition. Similar considerations apply to the effect of pH on lipid formation and fatty acid composition of oleaginous yeasts (11, 19).

The highest specific rate of lipid formation was attained in the two-stage system, although in these experiments the one-stage system was not run at the optimum dilution rate (0.06/h) for lipid production. Even so, with a specific rate of lipid formation of 0.5 g/g of yeast recorded on more than one occasion, this was significantly higher then found previously with the one-stage system by Gill et al. (7). At no time, however, did the efficiency of lipid formation approach that of 22 g of lipid per 100 g of glucose consumed found previously with single-state cultivation (7). We conclude, therefore, that even though following lipid formation in a two-stage continuous culture has much to offer in the understanding of the conditions affecting lipid and fatty acid formation, it confers no practical advantage over a single-stage system as a means of producing microbial oils and fats.

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