ACCUMULATION OF LIPID BY *RHODOTORULA GLUTINIS* IN CONTINUOUS CULTURE

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SUMMARY

Rhodotorula glutinis accumulated 35% (w/w) lipid when grown nitrogen-limited in a chemostat at a dilution rate (D) of 0.02^{-1}. At D = 0.10 h^{-1}, the lipid content was only 15% (w/w). Dual limitation of nitrogen and phosphate increased neither the amount of lipid produced nor the lipid yield (~14g lipid per 100g glucose consumed). The fatty acid composition was unchanged by the growth rate.

INTRODUCTION

Opportunities for using micro-organisms as sources of oils and fats are being explored in several laboratories (Ratledge, 1978). We have previously shown that lipid accumulation can be achieved by single-stage, continuous culture of a suitable yeast provided the culture is run at a slow growth rate with nitrogen-limited medium (Gill *et al.*, 1977; Hall and Ratledge, 1977). We have now extended this work to *Rhodotorula glutinis* (formerly *R. gracilis*) which is one of the archetypal oleaginous yeasts (*c.f.* Woodbine, 1959).

METHODS

Rhodotorula glutinis NCYC 154G was grown at pH 5.5 in continuous culture in a 2-litre vessel using either nitrogen-limited (glucose-excess) medium or glucose-limited medium. Lipid was extracted using chloroform/methanol (2:1 v/v) from moist, freshly-harvested cells after their disruption in a French pressure chamber. Methyl esters of the constitutent fatty acids, formed by transmethylation of the total lipid with sodium methoxide, were analysed by gas liquid chromatography. All experimental procedures are detailed in Gill *et al.* (1977).

RESULTS

Rhodotorula glutinis was grown at 28°C and maintained at a variety of steady states between dilution rates (D) of 0.02 h^{-1} and 0.10 h^{-1}. Wash-out occurred at 0.12 h^{-1} under both carbon- and nitrogen-limited conditions. As in our previous observations with *Candida* 107,

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appreciable lipid accumulation occurred only under nitrogen-limited conditions with the amount of lipid within the cells increasing some 2.5-fold as the dilution rate was decreased from the maximum to the minimum. The maximum lipid content of the yeast, 35% (w/w), was lower than that attained during a batch culture of the yeast on the same nitrogen-limited medium in the same fermenter. Here a 48% lipid content was attained after 46 h growth. However, as a lower biomass was attained in the batch culture (11g from 30g glucose) than in continuous culture, the overall lipid yields (g lipid per 100g glucose consummed) were not significantly different between batch and continuous culture (Fig. 1).

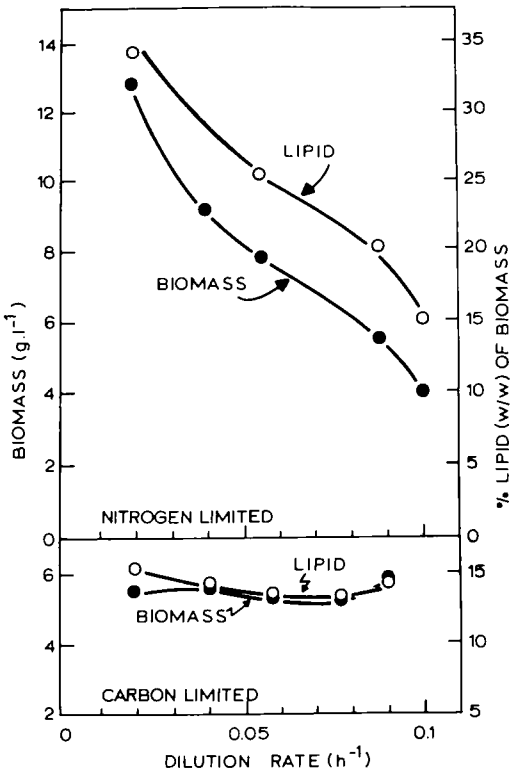

Fig. 1. Biomass production and lipid accumulation in *R. glutinis* growing in continuous culture. Nitrogen-limited medium contained (g litre^{-1}) glucose 30, NH_4Cl 1.5 and yeast extract (11% N content) 1.5; carbon-limited medium contained glucose 12, NH_4Cl 3.0 and yeast extract 1.5.

The lipid yield in continuous culture with nitrogen-limited medium was maximal, at 14.4, at the lowest dilution rate and steadily declined to approximately half this value, 7.3, at the highest dilution rate. The specific rate of lipid production was approximately constant at

Table 1. Fatty acid composition of *Rh. glutinis* grown in continuous culture under various conditions. Composition of media as given in Fig. 1.

Growth conditions:	Nitrogen-limited medium				Carbon-limited medium			
Dilution rate (h^{-1}):	0.06	0.06	0.06	0.06	0.02	0.04	0.055	0.09
Temperature (°C):	22.5	25.0	28.0*	30.0	28.0	28.0	28.0	28.0
Major fatty acids**:	Relative % of fatty acids in lipid							
16:0	2	4	7	2	tr	tr	tr	tr
16:0	12	12	13	16	15	15	14	13
17:0	6	5	3	2	1	1	1	2
18:0	3	4	6	7	2	3	4	5
18:1	26	30	35	39	21	23	24	32
18:2	25	24	23	24	39	38	38	34
18:3	5	4	3	1	1	1	1	1
20:0	18	17	10	9	19	16	16	11
Δ/mole†	0.95	0.93	0.94	0.91	1.03	1.05	1.05	1.06

tr = trace

* The fatty acid composition at 28°C was unaffected by the dilution rate Similar relative propositions were found at all dilution rates from 0.02 h^{-1} to 0.10 h^{-1}.

** Trace amounts of $C_{12:0}$, $C_{13:0}$, $C_{14:0}$, $C_{15:0}$, $C_{15:1}$, $C_{16:1}$, $C_{16:2}$ were seen in most but not every instance.

† No. of double bonds per mole of lipid = (1 x no. of monoenoic acid + 2 x no. of dienoic acids + 3 x no. of trienoic acids) ÷ 100 (see Kates and Baxter, 1963).

0.015g lipid produced/g biomass h^{-1} from D = 0.04 h^{-1} and above; at D = 0.02 h^{-1} this rate was halved. This pattern was similar to that previously observed with *Candida* 107 though the maximum specific rate of lipid formation in that yeast was higher (up to 0.04g lipid/g biomass h^{-1}).

With carbon-limited medium, specific rates of lipid production in *R. glutinis* were directly proportional to the dilution rate and varied from 0.003 at D = 0.02 h^{-1} to 0.013g lipid/g biomass h^{-1} at D = 0.09 h^{-1}. Biomass yields under both carbon- and nitrogen-limited growth reached 50% at maximum growth rates. This value decreased with nitrogen-limited cultures to 42% as the dilution rate dropped to its minimum value (0.02 h^{-1}).

The fatty acid composition of the lipid of the yeast grown under nitrogen-limited conditions remained constant at all dilution rates. Under carbon-limited conditions, slight changes in the relative pro-

portions of the fatty acids were seen as the diltuion rate altered; the principal fatty acids affected were oleic acid (18:1) and arachidic acid (20:0) which varied inversely. However, because of smaller changes in the proportion of linoleic acid (18:2), the overall degree of unsaturation of the lipid, measured as Δ/mole (Kates and Baxter, 1963), remained constant (Table 1).

The relative proportions of fatty acids in nitrogen-limited cultures, although not affected by the dilution rate, were influenced by the growth temperature, a feature which was not seen previously with *Candida* 107 (Hall and Ratledge, 1977). Again oleic and arachidic acids were the principal fatty acids affected (Table 1). In spite of arachidic acid reaching 18% of the total acids, the degree of unsaturation of the lipid was virtually unchanged in going from 22.5°C to 30°C mainly because of compensating changes in the amounts of linolenic acid (18:3) being produced. Thus, although the composition of the fatty acids can be changed, the effects are not radical enough to alter the fluidity of the lipid as measured by the Δ/mole parameter.

In our previous work with *Candida* 107 (Gill *et al.*, 1977) the effect of phosphate limitation on lipid accumulation was examined. Although this has a detrimental effect on lipid levels in *Saccharomyces cerevisiae* (Ramsay and Douglas, 1979), it had little effect on those in *Candida* 107. However, dual limitation of nitrogen and phosphate induced a high lipid content in *Candida* 107 growing at its maximum dilution rate. Dual limitations of nitrogen and phosphate were therefore also tried with *R. glutinis*. With phosphate decreased to 0.5g KH_2PO_4 per litre (instead of 9.0g KH_2PO_4/Na_2HPO_4 per litre) and nitrogen as for other nitrogen-limited medium at 1.5g NH_4Cl per litre, lipid accumulation was slightly increased to 38% of the biomass at the lowest dilution rate (0.02 h^{-1}). Biomass yield was, however, decreased to 34% at this dilution rate with a concomitant low lipid yield of 13%. At higher dilution rates, the amount of lipid accumulated dropped (as in Fig. 1) to 18.2% at D = 0.10 h^{-1} and thus the pattern seen with *Candida* 107 was not repeated here. Although the biomass yield of *R. glutinis* increased up to 46% at maximum dilution rate, the lipid yield dropped to 8.4% principally, of course, due to the decline in lipid content of the cells.

The fatty acid composition of cells grown with phosphate and nitrogen dual limitation again did not vary with the growth rate and

the composition was similar to that given in the appropriate column in Table 1.

DISCUSSION

The performance of *R. glutinis* as an oleaginous yeast in continuous culture was not exceptional. The overall pattern of lipid accumulation followed the general pattern found previously with *Candida* 107 in that the greatest accumulation was with nitrogen-limited medium being supplied at a low dilution rate. Although the amount of lipid accumulated was almost as high as in *Candida* 107, i.e. about 35% of the biomass, this was not as high as had been achieved in batch-culture (see Kessell, 1968). However it is probably not possible to produce a biomass with much more than 40% lipid in continuous culture as under such conditions there is usually an efficient utilization of the carbon substrate, giving biomass yields of about 50%, with the maximum lipid yield not exceeding 20% (Ratledge, 1978). Thus from 100g of substrate one would obtain optimally 50g biomass containing 20g lipid. Higher concentrations of lipid in a micro-organism can thus only be achieved to the detriment of the biomass yield and though this can clearly be accommodated in batch culture it is unlikely to be realized in continuous culture unless some unusual combination of nutrient limitations is devised.

The fatty acids of the lipid of *R. glutinis* showed no changes with the growth rate under nitrogen-limited conditions; those of *Candida* 107 did show some changes under such conditions but under carbon-limited conditions considerable changes in the fatty acid patterns occurred in both yeasts as the dilution rate was varied. The fatty acids from *R. glutinis* are distinct from those of *Candida* 107; in the former yeast there is a much higher degree of unsaturation, the principal acids being oleic (35%), linoleic (23%) and palmitic (13%) whereas in *Candida* 107 the major acids were oleic (35%), palmitic (35%) and stearic (14%) with linoleic acid at about 10%.

The usefulness of *R. glutinis* for a single cell oil (SCO) process may seem unlikely in view of the slow dilution rate at which such a culture would have to be grown to achieve high lipid contents. However, as we have already pointed out elsewhere (Ratledge and Hall, 1977), such cultures can be run at very high cell densities (say up to 50 or 60g l^{-1}) as the oxygen demand during lipid accumulation will be very low (a) because of the low growth rate *per se* and (b) lipid being chemically more

reduced than sugar requires no oxygen for its synthesis (the synthesis in fact consumes reducing equivalents within the cell, further sparing the need for oxygen). Output from a fermenter producing SCO could therefore be the same as from one producing SCP. The price difference between an SCO like that from *R. glutinis* and that of a yeast SCP may be sufficient to warrant consideration of SCO as a viable commercial process.

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