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Enthalpy content as a function of lipid accumulation in *Rhodotorula glutinis*

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Abstract Microcalorimetry has been demonstrated to be a suitable on-line method for monitoring the lipid production phase of oleaginous yeasts. The choice of lipid extraction method for the oil accumulated by oleaginous yeasts is highly important both for accuracy when quantifying the lipid level and determining the fatty acid composition. The energy content of *Rhodotorula glutinis* increased from 23.0 kJ/g to 30.6 kJ/g dry biomass during the lipid-accumulating phase and was directly correlated to the analysed level of lipids, when an alkaline hydrolysis extraction method was used. Consequently, bomb-calorimetric measurements of the energy content were shown to be an indirect method of quantifying the lipid content in oleaginous yeasts. The fatty acid composition remained rather constant during the batch growth of *Rh. glutinis* with approximately 70% unsaturated C18 fatty acids. The high energy content as well as the fatty acid composition of *Rh. glutinis* makes this yeast a better candidate for use as aquaculture feed compared with the commonly used *Saccharomyces cerevisiae*.

Introduction

Oleaginous yeast are characterized by their efficient conversion of sugars to oil, if grown under conditions limited by a component other than carbon (Ratledge and Boulton 1985). The non-oleaginous Baker's yeast and some *Candida* species are frequently used as feed additives in animal farming, mainly as a cheap source of proteins and vitamins or as probiotics (Chapman 1988; Cole et al. 1992; Fuller 1973). The accumulation of lipids in oleaginous yeasts adds one more feature, *i.e.* a source of lipids, which makes them potentially useful in aquaculture feed.

In fish farming, baker's yeast (*Saccharomyces cerevisiae*) is the species almost exclusively used as feed so far. A lipid composition including certain long and unsaturated fatty acids and a high energy content have been shown to be of utmost importance for fish larvae survival (Hove and Grahl-Nielsen 1991; Lubzens et al. 1985; Watanabe et al. 1982). However, *S. cerevisiae* contains low amounts of long and unsaturated fatty acids and the energy content is not particularly high (Hunter and Rose 1972; Larsson et al. 1993; Ratledge 1989). Therefore, in several experiments using baker's yeast as feed for small animals (*eg. Artemia* or *Brachionus*), later used for feeding fish larvae, the media for growth have been supplemented with lipids (Appelbaum 1977; Imada 1979; Mahnken et al. 1980), alternatively yeasts have been sprayed with lipids in order to increase the nutritional value. In contrast *Rhodotorula glutinis*, an oleaginous yeast that has been reported to produce lipids in quantities up to 72% of the biomass (w/w) (Ratledge 1989), would provide a package of important nutrients including a high energy content.

Different factors affect the level and composition of lipids accumulated by oleaginous yeasts. These factors are medium composition (Evans and Ratledge 1983, 1984; Hansson and Dostalek 1986; Ykema et al. 1988; Zhelifonova et al. 1983), physicochemical factors such as pH, temperature and oxygen pressure (Granger et al. 1992; Hansson and Dostalek 1986; Prapulla et al. 1992; Rattray et al. 1975) and mode of cultivation as well as growth rate (Evans and Ratledge 1983; Gill et al. 1977; Granger et al. 1992; Prapulla et al. 1992). In addition to intracellular lipids, extracellular lipids have also been examined and models for their oleaginous biochemistry (Ratledge 1988, 1989)and process optimi-

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zation (Prapulla et al. 1992; Ykema et al 1986, 1988)have been proposed. However, no rapid on-line method for continuous monitoring of the onset and cessation of lipid accumulation has, to our knowledge, been demonstrated neither has the relation between the level of intracellular triacylglycerols and energy content per weight biomass been examined in oleaginous yeast. Furthermore, an accurate, rapid and reproducible qualitative and quantitative extraction of intracellular lipids is difficult because of the rigid yeast cell wall (Bagchi and Dutta 1979; Moon and Hammond 1978; Sobus and Holmlund 1975), resulting in pronounced variations in reported yeast lipid data. Also a few indirect methods have been described, *e.g.* NMR (Moreton 1989)and lipid-specific dyes (Cooksey et al. 1987); however these have usually been rough and suitable for screening of oleaginous yeasts but not for accurate quantification.

The purpose of the present investigation has been (a) to evaluate the method of microcalorimetry as a tool for continuous on-line monitoring of lipid accumulation in *Rh. glutinis*, (b) to analyse the energy content as a function of the lipid accumulation in *Rh. glutinis* and (c) to use bomb calorimetry for indirect analysis of the lipid content. This work is part of an on-going project investigating the biology and use of yeast for aquaculture (Andlid et al. 1993; Vazquez-Juarez et al. 1993, 1994).

Materials and methods

Organisms and growth conditions

Two strains of *Rh.* glutinis: Rh2 isolated from an estuary of the Swedish west coast (Norkrans 1966) and CCUG 22164 (obtained from CCUG Department of Clinical Bacteriology S-413 46 Göteborg, Sweden), were compared with respect to the amount and composition of acyl lipids formed. Cultivation was performed in a bioreactor (LKB 1601 Ultroferm, Sweden) in which 2.5 l of yeast nitrogen base (YNB) without ammonium sulphate and amino acids (Difco), supplemented with glucose (20 or 30 g/l) and ammonium sulphate (0.618 g/l), was inoculated with 10 ml 24-h preculture grown in YNB without amino acids supplemented with glucose (10 g/l). The agitation rate was 300 rpm, aeration 5 l sterile air/min, temperature 30° C and the pH was controlled at 4.5. During growth, the rate of heat production (dQ/dt) was monitored continuously. Samples were withdrawn at selected intervals for the determination of dry weight, extracellular ammonium concentration and lipid content.

Microcalorimetry

The rate of heat production (dQ/dt) was measured continuously by connecting the fermentor to a flow calorimeter (Bioactivity Monitor LKB 2277 Thermometric, Järfälla, Sweden) of the heat-conduction type (Suurkuusk and Wadsö 1982). The microcalorimeter was operated at 30° C. The flow-through cell and the connecting tubes were sterilized with 70% ethanol and then rinsed with sterile culture medium. The culture was pumped at a rate of 80 ml/h (micro Perpex pump, LKB 2132) from the fermentor to the calorimeter. Before enterering the calorimeter the cell suspension was met by a flow of water-saturated and sterile air (40 ml/h). After passing the calorimeter the flow was returned to the growth vessel. This experimental set-up yielded an effective volume of the flow-through cell of 0.34 ml. For details see (Larsson et al. 1991).

Dry weight determinations

Culture samples (2×8 ml) were centrifuged for 5 min at 3500 *g*, washed twice with deionized water, dried for 24 h at 105°C, and allowed to cool in a desiccator before weighing.

Determination of extracellular concentration of ammonium

Culture samples (2×8 ml) were centrifuged for 5 min at 3500 *g* to remove cells. The supernatants were frozen immediately and stored in the freezer (–20° C). Ammonium was analysed using enzyme combination kits (Biochemica Test Combination, Boehringer Mannheim GmbH, Mannheim Germany).

Lipid analyses

Three different extraction methods were compared for both strains. *Bligh and Dyer* (Bligh and Dyer 1959) procedure with different modifications (extraction time for up to 10 h, high solvent/sample ratio, homogenization with glass beads and lyophilization), *KOH/ethanol method* and *Hexane/ethanol method.*

KOH/ethanol method

A 10-ml sample of cell suspension was centrifuged for 8 min at 3000 *g* and washed once in 0.9% NaCl. The cell pellet or 25 mg freeze-dried cells was used. An internal standard (C17:0) and 5 ml KOH in ethanol (2.14 M KOH in 12% ethanol v/v) were added and the sample was heated to 70° C for 2 h in a sealed tube. HCl was used to adjust the pH to approximately 3. The liberated fatty acids of the acyl lipids were extracted twice with 5 ml hexane, pooled and evaporated under a flow of nitrogen gas. The fatty acids of the lipids were converted to methyl esters using 2.5% HCl (gaseous) in dry methanol (Liljenberg and Kates 1985).

Hexane/ethanol method

A 10-ml sample of cell suspension was washed once in 0.9% NaCl. An internal standard (C17:0) was added to the pellet and the sample was extracted for 20 min stepwise with (a) 5 ml ethanol, followed by (b) 5 ml ethanol:hexane (1:1) and finally (c) 5 ml hexane. Between each step the samples were centrifuged and undissolved material was exposed to the next solvent. The three solvent fractions were pooled and the fatty acids of the lipids were converted to methyl esters as above.

Gas chromatography

The methyl esters of the fatty acids were analyzed on a Hewlett Packard (USA) 5890 gas chromatograph with flame ionization detector. The column, a 30×0.32-mm (international diameter) DB225 (J and W Scientific, USA) fused-silica column (0.25 μm film thickness) was temperature-programmed from 170° C (held for 20 min) to 195° C at 5° C/min (held until termination of run). Injector and detector temperatures were maintained at 210° C. The flow was 1.8 ml/min. Split injection was used.

Growth conditions for the determination of elemental composition and heat of combustion

Rh. glutinis CCUG 22164 was grown as batch cultures in a 15 l bioreactor (14 l working volume, model L1523, Bioengineering, Wald, Switzerland) at a controlled pH of 4.5, a temperature of 30° C, an agitation rate of 700 rpm and an aeration rate of 300 l/h for the first 16 h of growth, thereafter the aeration rate was 100 l/h. The preculture was grown for 48 h in a 1-l shake flask containing 140 ml. All of this was used as inoculum for the bioreactor. Samples were withdrawn after 16, 46, 90 and 161 h of growth for determination of biomass and ammonium concentrations and for determination of elemental composition and enthalpy content of the cells.

Preparation of samples for determination of enthalpy of combustion and elemental composition

Cells were harvested by centrifugation at 5000 *g*, washed twice with deionized water and freeze-dried under vacuum as previously described (Gurakan et al. 1990). The flasks were opened and the freeze-dried cells were spread on petri dishes at least 12 h before analysis in order to equilibrate with the ambient humidity.

Determination of elemental composition, heat of combustion, ash and residual water content

Enthalpy of combustion (ΔH_x) measurements were made with an adiabatic calorimeter (model, IKA C400, Janke & Kunkel, Germany) using 0.5 g freeze-dried cells compressed to a tablet. Calibration of the calorimeter, preparation of tablets and conditions of operation were according to Gurakan et al. (1990).

The elemental composition of freeze-dried cell samples was determined using a C, H, N analyzer (model 2400, Perkin-Elmer, Norwalk, USA) as previously described (Gurakan et al. 1990). Results for ΔH_x and the elemental composition are all presented in terms of ash- and water-free biomass.

The residual water content of the freeze-dried cell samples was determined by drying for 24 h at 105° C and the ash content was determined by heating to 600° C for 6h.

Results

Fatty acid composition and yield

Three extraction procedures, based on previously described methods (Moon and Hammond 1978) were compared using both strains of *Rh. glutinis*. *Rh glutinis* Rh2 were found to be a low-lipid-producing strain. The total level of fatty acids ranged from 7.5% to 14% during a batch culture (Fig. 1). These values were obtained using alkaline (KOH/ethanol) hydrolysis for lipid extraction. The problem of accurate determination of lipids in yeasts was demonstrated by comparison with the hexane/ethanol and Bligh and Dyer extraction methods, which showed a limited extraction capacity ranging from 10% to 25% of the amount fatty acids obtained by the KOH/ethanol method. The Bligh and Dyer method was used with several modifications, *e.g.* large solvent volumes, long extraction time, homogenization and lyophilization. However, the lipid yield was always highest with the method based on alkaline hydrolysis, showing that even chloroform/methanol extraction was insufficient for measuring the total lipids in this yeast. Furthermore, the experimentally obtained fatty acid composition was strongly dependent on the method used (Fig. 2). When alkaline hydrolysis was used, C18:1 and C18:2 constituted approximately 50% and 15%, respectively, of the total lipids extracted, while the hexane/ethanol method yielded an apparent fraction of approximately 30% for both of them (Fig. 2). Figure 3 shows the composition of the fatty acids of the high-

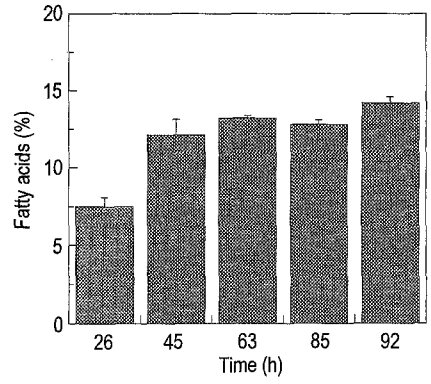

Fig. 1 Intracellular level of fatty acids of *Rh. glutinis* Rh2. The intracellular fatty acids were extracted at different times during batch growth with a limited nitrogen source and with glucose as the carbon and energy source. The values are presented as the percentage of fatty acids (g) per unit of biomass (g)

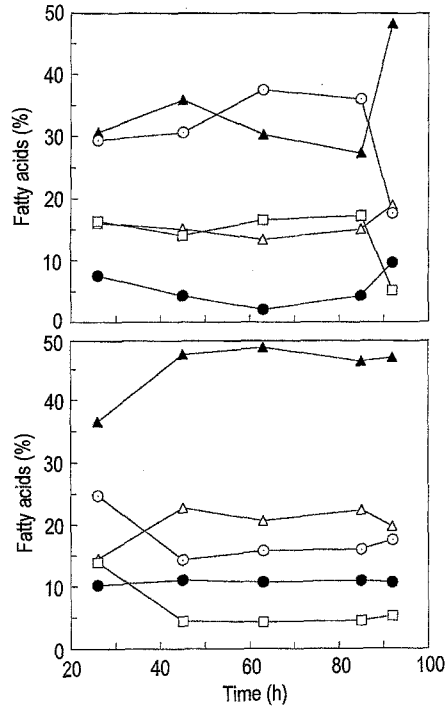

Fig. 2 Comparison of intracellular fatty acid composition obtained by using hexane/ethanol extraction (*upper panel*) and KOH/ethanol extraction (*lower panel*). The fatty acids were extracted during batch growth of *Rh. glutinis Rh2* with a limited supply of nitrogen and with glucose as the carbon and energy source. C16:0 (△), C18:0 (●), C18:1 (▲), C18:2 (○), C18:3 (□). The levels of the different fatty acids are presented as percentages of the total amount of fatty acids

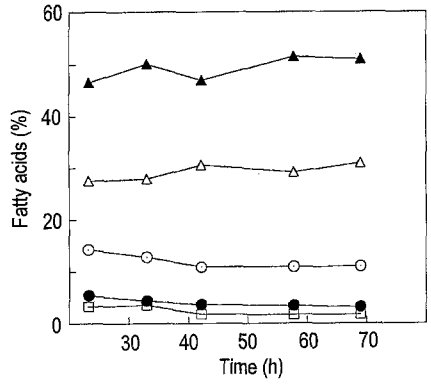

Fig. 3 Fatty acid composition during batch growth of *Rh. glutinis* CCUG 22164 with a limited supply of nitrogen and with glucose as the carbon and energy source. The fatty acids were extracted by KOH/ethanol extraction. C16:0 (△), C18:0 (●), C18:1 (▲), C18:2 (○), C18:3 (□). The levels of the different fatty acids are presented as percentages of the total amount of fatty acids

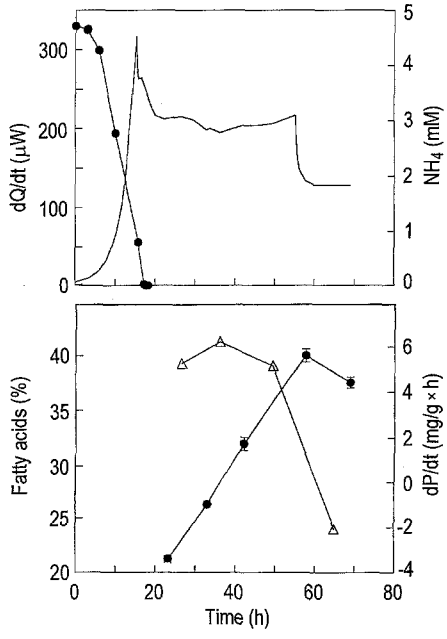

Fig. 4 Changes in measured parameters during batch growth of *Rh. glutinis* CCUG 22164 with a limited supply of nitrogen and with glucose as the carbon and energy source. *Upper panel:* heat production rate, d*Q*/d*t*, (–) and extracellular concentration of ammonium (●). *Lower panel*: intracellular concentration of fatty acids (●) extracted with KOH/ethanol and lipid production rate, d*P*/d*t*; (Δ). The *error bars* show the min/max values of the duplicates

lipid-producing strain CCUG 22164 during batch growth. The relative amounts of fatty acids did not change much during the growth period, with oleic acid (C18:1) accounting for approximately half of the pool; 70% of the total fatty acids was unsaturated C18 fatty acids. The maximum value of accumulated total fatty acids for this strain, during prevailing conditions, was 46.2% (alkaline hydrolysis extraction) of the dry biomass (Table 3). If corrected for glycerol, the triacylglycerols correspond to 51.6% of the dry biomass (calculated from the average fatty acid molecular mass of 264 g/mol, based on gas chromatographic determinations).

Monitoring of lipid production by microcalorimetry

Microcalorimetry was used for direct monitoring of the initiation and cessation of lipid accumulation. This method can be used for monitoring growth as well as it gives simultaneous information about the total metabolic activity and about metabolic shifts (Gustafsson 1991). Figure 4 shows the rate of heat production by a population of *Rh. glutinis* CCUG 22164 grown in a medium inducing lipid accumulation. The first maximum at 15 h is correlated to the exhaustion of nitrogen and thereby the onset of triacylglycerol accumulation. The rate of lipid production was fairly constant with values of approximately 6 mg g^{-1} h^{-1} throughout the whole lipid production phase (Fig. 4). The cessation of the lipid production phase after 55 h was, in this experiment, determined by the exhaustion of glucose and was detected on-line by the microcalorimeter. At this point the maximum amount of fatty acids (40% w/w) was obtained (the glucose concentration of 20 g/l used in this experiment was below the concentration yielding maximum amounts of lipids, *cf.* Table 3). This was followed by respiration of internal lipids as demonstrated by a decrease to 37% (w/w) fatty acids of the dry biomass at 70 h.

Elemental composition

There were quite dramatic changes in the elemental composition of *Rh. glutinis* CCUG 22164 as a consequence of lipid accumulation (Table 1). After 16 h of growth, before any significant lipid production took place, the cellular composition was similar to that previously reported for yeasts (Larsson et al. 1993) with a carbon and nitrogen content of approximately 50% and 10% (g/g), respectively. Concomitant with an increased level of lipids there was an increase in carbon and hydrogen content, together with a decrease in nitrogen and oxygen content of the cells. In addition, accumulation of lipids also resulted in a more reduced biomass (Table 1).

Energy content

The energy content (ΔH_x) of *Rh. glutinis* CCUG 22164 increased as a consequence of an increased amount of intracellular lipids (Tables 2, 3). When expressed per gram of biomass the increase was dramatic. To our knowledge, the value of 30.59 kJ/g obtained after 161 h of growth (Table 2) is the highest enthalpy of combustion ever reported for any microorganism. The other

Table 1 Elemental composition, molar mass (M_x) relative to C and degree of reduction (γ_x) of *Rh. glutinis* CCUG 22164 during batch growth with a limited supply of nitrogen and with glucose as the carbon and energy source. Percentages of C, H, N and O were calculated as g element/g ash- and water-free biomass ±SD, $n=4$. γ_x was calculated as $4C+H-2O-3N$, where C, H, O and N denote the atomic coefficients of the elements in the biomass

Time (h)	C (%)	H (%)	N (%)	O (%)	Formula	M_x (g/mol C)	γ_x
16	51.55±0.18	7.37±0.07	9.96±0.04	31.13±0.26	$CH_{1.70}O_{0.45}N_{0.17}$	23.31	4.80
46	58.42±0.11	9.05±0.09	2.25±0.03	30.28±0.18	$CH_{1.85}O_{0.39}N_{0.03}$	20.54	5.07
90	62.03±0.07	9.84±0.13	1.61±0.06	26.52±0.17	$CH_{1.89}O_{0.32}N_{0.02}$	19.32	5.25
161	63.79±0.23	10.09±0.26	1.17±0.04	24.95±0.47	$CH_{1.88}O_{0.29}N_{0.02}$	18.83	5.30

Table 2 Enthalpy of combustion (ΔH_x), ash and residual water content of *Rh. glutinis* CCUG 22164 during batch growth with a limited supply of nitrogen and with glucose as the carbon and energy source

Time (h)	Residual water (%)[a]	Ash (%)[a]	ΔH_x (kJ/g)[b]	ΔH_x (kJ/mol C)
16	4.37±0.03	7.04±0.00	22.97±0.06	535
46	2.85±0.01	4.18±0.02	27.21±0.14	559
90	2.42±0.01	3.35±0.01	29.59±0.07	572
161	2.10±0.04	2.91±0.02	30.59±0.25	576

[a] ±min/max, $n=2$
[b] Ash- and water-free biomass ±SD, $n=3$

Table 3 Comparison between measured and calculated (from ΔH_x measurements) values of lipid content of *Rh. glutinis* CCUG 22164 during batch growth with a limited nitrogen supply and with glucose as the carbon and energy source

Time (h)	ΔH_x (kJ/g)	Fatty acids[a] (%)	Lipids[b] (%)	Lipids[c] (%)
16	22.97	8.3	9.3	9.3
46	27.21	32.9	36.9	35.9
90	29.59	37.3	41.7	50.8
161	30.59	46.2	51.6	57.1

[a] Measured content of total fatty acids (g fatty acids/g dry biomass)
[b] Measured values, corrected for glycerol, see Results
[c] Calculated using the equation:
$\Delta H_{X1}=\Delta H_{lip}\cdot x_{lip}+\Delta H_{X0}(1-x_{lip})$, where ΔH_{X1} = heat of combustion of biomass (kJ/g), ΔH_{X0} = heat of combustion of biomass with a basal concentration of triglycerides (in this case 9.3% lipids and 22.97 kJ/g), kJ/g, ΔH_{lip} = heat of combustion of triglycerides, 38.91 kJ/g, x_{lip} = weight fraction of triglycerides in biomass above the basal concentration (g/g)

strain, Rh2, produced low amounts of lipids, ranging from 7.5% to 14% (Fig. 1). This was reflected in the ΔH_xvalues of Rh2, ranging from 20.78 (±0.01) kJ/g to 21.90 (±0.00) kJ/g.

Discussion

Much effort has been expended to optimize aquaculture feed, including the use of yeasts (Dendrinos and Thorpe 1987; Frolov et al. 1991; Imada 1979; Kitajima et al. 1980). Usually yeast is given alone or together with phytoplankton to animals like *Brachionus* or *Artemia,* which later constitute the food for fish larvae (most fish larvae require live foods (Dendrinos and Thorpe 1987)). When given to *Artemia* or *Rotifers*, the total level of lipids and the fatty acid composition in the yeast are reflected in those of the animal fed on them (Dendrinos and Thorpe 1987; Frolov et al. 1991). Animals in general can not synthesise C18:2 and C18:3 fatty acids and are therefore dependent on an intake via the food. In one of the few studies where marine yeast have been added as feed for fish and scallop larvae there was an increased growth rate as compared to when baker's yeast or mixed food without marine yeast was used (Iwasaki and Kamiyta 1977; Kadowaki et al. 1980). *Rh. glutinis*, belonging to a genus frequently isolated from marine environments, shows a fatty acid profile (Figs. 1, 3) that would be suitable for feeding fish larvae. The specific need differs between marine and fresh water fish (and species), and a combination of yeast and algae may be needed for marine fish. *Rh. glutinis* has a high level of C18:1 and a relatively high level of C18:2 and C18:3α compared to different strains of baker's yeast (Gelinas et al. 1990; Hunter and Rose 1972; Wilson and McLeod 1976). The fatty acids taken in via the food serve, for example, as necessary components of cell membranes and as precursors of prostaglandins. Salmonids are able to elongate and desaturate C18:3 to C20:5 and C22:6, whereas strictly marine fish have a more limited capacity for this (Owen et al. 1975). In one investigation, 70% of the C18:3 from the diet was later present in C22:6 in rainbow trout (Owen et al. 1975).

The energy content of the feed in aquaculture is another crucial point, especially in the start-feeding of larvae. Nagata and Whyte (1992) pointed out that algae, frequently used as food for rotifers, provides important fatty acids but often not enough energy. Our results show that the energy content of *Rh. glutinis* per weight of biomass was unusually high (Table 2) as a result of lipid storage. The enthalpy content of non-oleaginous yeast is only between 20 kJ/g and 24 kJ/g (Larsson et al. 1993). An important feature that follows from the cultivation of oleaginous yeasts is that, provided a controlled fermentation process is used, it is possible to

choose a certain level of lipids and thereby energy, depending on the duration of the oil-accumulating phase.

Besides the fatty acid composition and high energy content there are other properties of *Rhodotorula* yeasts that make them potentially suitable as aquaculture feed, *e.g.* (a) the presence of carotenoids serving as pigmentation, antioxidants and vitamins (Moore et al. 1988; Nelis and De Leenheer 1990; Partali et al. 1985) (b) a buoyancy and size matching small animals (Appelbaum 1977; Coutteau et al. 1992), (c) the production of extracellular bioemulsifiers (Johnson et al. 1992) (possible to harvest as a by-product), which often are desirable in food preparation and (d) the ability of the yeast to grow on pentoses (Höfer et al. 1971) as well as hexoses. In fact, Evans and Ratledge (1983) have shown a higher lipid yield in *Rh. glutinis* when grown on xylose compared to growth on other sugars.

Methodological key questions addressed in this work were, will the use of microcalorimetry enable continuous on-line monitoring of oil production by microorganisms and may the indirect method of enthalpy of combustion measurements provide a tool for convenient and rapid lipid quantification? As shown previously, the microcalorimeter is useful in monitoring different physiological states (Blomberg et al. 1988). In this study, three clearly distinguishable phases were seen (Fig 4): a) non-nutrient-limited growth, where the largest contribution of the increased heat production rate was due to an increased biomass concentration, b) starvation of nitrogen, which causes a drop in the heat production rate since the metabolism is changed by the onset of lipid accumulation, which continues until c), the energy depletion causes a sharp drop in the heat production rate. In other words, microcalorimetry provides a method that continuously monitors on-line the lipid production phase and thereby its onset and cessation.

It is a difficult task to quantify the lipid content of yeast cells accurately. In the majority of published works the extracted lipids have been dried and weighed, a method we found unsatisfactory in terms of reproducibility. During growth of an oleaginous yeast in excess of carbon and energy while nitrogen is limiting, the conversion of sugars results in neutral lipids, principally triacylglycerols (Ratledge and Boulton 1985). Up to 92% of the total lipid fraction are triacylglycerols. Therefore, instead of gravimetrically determining total lipids, accumulated lipids were determined from fatty acid data in this study. Errors due to uncertainty in the nature of the material weighed, *i.e.* the presence of proteins etc., are thereby excluded (Fig. 4, Tables 1–3). Furthermore, classical extraction procedures (*e.g.* Bligh and Dyer 1959) have been used with limited success (Kessel 1968; Moon and Hammond 1978), which is in agreement with our results. The correspondence of the analysed fatty acid content, obtained by KOH/ethanol extraction, and the enthalpy of combustion values (Table 3) points to the reliability of this extraction method, compared to ethanol/hexane and chloroform/methanol extractions. In addition, this implies that the most reliable data on fatty acid composition are achieved by using the KOH/ethanol extraction method. There are also sterols and other lipids in oleaginous yeasts. However, the correlation between lipid content, measured as total fatty acids, and energy content of the cells showed that acyl lipids constitute the major part of the lipid fraction.

This investigation shows that measurements of enthalpy of combustion provide an alternative way of quantifying the lipid concentration of cells. We assume that $\Delta H_x = 22.97$ kJ/g (Table 3) is a representative value for this yeast when the lipid concentration is at a basal level (Table 1). Usually the enthalpy of combustion values are very insensitive to changes in growth rate, growth phase, limiting substrate and environmental conditions (Larsson et al. 1993). Therefore, it seems reasonable to assume that the increase in ΔH_x during batch growth of *Rh. glutinis* (Table 3) under these conditions is solely due to an accumulation of lipids. On the basis of these assumptions the concentration of triacylglycerols may be calculated using the equation:

$$\Delta H_{X1} = \Delta H_{lip} \times X_{lip} + \Delta H_{X0}(1 - x_{lip})$$

where ΔH_{X0} (kJ/g) is the value obtained with a basal level of lipids, ΔH_{X1} (kJ/g) is the measured value, ΔH_{lip} (kJ/g) is the enthalpy of combustion for triacylglycerols (38.91 kJ/g) and x_{lip} (g/g) is the weight fraction of triacylglycerols above the basal level. As shown in Table 3, the calculated and measured lipid concentrations agree fairly well.

The present work has focused on the value of yeasts as a nutritional source in aquaculture. Several properties, of which some were investigated in this work, make it attractive to introduce oleaginous yeast such as *Rh. glutinis* as feed in aquaculture, which will be evaluated in forthcoming feeding experiments. Methodologically it was shown that microcalorimetry may be a useful method for on-line control of oil-producing yeast and that bomb calorimetry may be used as an indirect method for lipid quantification.

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