Continuous Culture Fermentation of Whey Permeate to Produce Microbial Oil[1]

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ABSTRACT

The oil-accumulating yeast, *Candida curvata* D, was grown in Cheddar cheese whey permeate in continuous culture at various dilution rates. The amount of lipid obtained per hour was greatest at a dilution rate of .05 h^{-1}. Continuous culture fermentations yielded lipids of consistent composition except for the fastest dilution rate examined (.2 h^{-1}). The lipid produced at this dilution rate contained less oleic acid and more linoleic acid than did lipid produced at slower dilution rates and resembled the lipid found in exponential phase cells in batch culture. When lipid production rates in batch and continuous culture systems were compared, the continuous culture system was much more efficient. This increased efficiency might also be seen on an industrial scale and could make the fermentation of whey permeate to produce oil more economically feasible.

INTRODUCTION

For a number of years, we have studied the fermentation of cheese whey and whey permeate to produce microbial oil. We have isolated four yeast strains able to grow on lactose and accumulate large quantities of intracellular lipid (9). The lipid accumulated is largely triglyceride with a composition similar to that of cocoa butter (8). Using the most efficient lipid producer, *Candida curvata* D, we have demonstrated that a culture may accumulate up to 60% of its dry weight as lipid, that whey permeate is a better substrate than whey for oil production, and that permeate obtained from the production of various types of cheese may be used as a substrate (6, 9).

We have conducted our studies using batch culture fermentations. Continuous culture fermentation, however, is potentially more efficient, more cost effective, and able to produce a product of consistent yield and composition (2). Ratledge and his colleagues have used continuous culture systems to study lipid accumulation in a number of yeasts (1, 4, 5, 10).

Evans and Ratledge (3) recently tested *C. curvata* D in continuous culture in a semidefined laboratory medium with five different carbon sources. They found that lactose supported the highest biomass and lipid yield and that lipid composition was consistent.

We report here on the continuous fermentation of whey permeate by *C. curvata* D to produce triglyceride oil.

MATERIALS AND METHODS

Organism

Candida curvata D was used in all experiments. Stock cultures were stored on malt extract agar slants at 4°C. Inocula for fermentations were grown in sterile whey permeate for 18 h, with shaking at 30°C.

Permeate Production

Cheddar cheese whey was obtained from the pilot plant of the Food Technology Department, Iowa State University. The whey was filtered at room temperature through a Romicon hollow fiber ultrafiltration system, Model XM50 (Romicon, Inc., Woburn, MA), with a 50,000 M_r nominal retention size. Permeate was frozen immediately in 5-liter batches and stored at −20°C until needed.

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Fermentation Conditions

Permeate was sterilized by autoclaving and the sterility of the incoming medium was monitored. Fermentations were run in a 350-ml working volume bench top fermenter (Bioflow Model C30, New Brunswick Scientific Co., New Brunswick, NJ) with accessory pH controller (Model pH-40, New Brunswick) and dissolved oxygen controller (Model DO-50, New Brunswick). All fermentations were run at 30°C with pH maintained at pH 5.4 ± .2. Aeration rate was 1 vol/vol/min, and agitation rate was 500 to 600 rpm. Dissolved oxygen concentration was maintained at or above 80% saturation throughout the fermentation. The dilution rate (D, medium vol/vessel vol/h) was altered by varying the rate of medium addition by means of a peristaltic pump.

Analyses

Samples were obtained from the effluent from the fermenter vessel. After each change of dilution rate, the system was allowed to run until at least six complete changes of medium had passed through the vessel to ensure that steady state conditions had been attained, and cell count and dry weight values were monitored throughout this period. At least three samples were obtained for each dilution rate. Values for the measured parameters were stable after four changes of medium had passed through the fermenter vessel.

Analyses for cell dry weight, cell counts, medium nitrogen, chemical oxygen demand (COD), lipid content, and lipid composition were performed as described previously (6, 9). Values presented are the averages of two to six replicates. Replicate samples were in close agreement with each other. Statistical analyses were performed as described by Mood and Graybill (7).

RESULTS AND DISCUSSION

Fermentation Characteristics

The high carbon:nitrogen ratio of whey permeate makes it an excellent substrate for lipid production (9). The permeate used in the present study had an initial COD of 45,700 mg/liter and 400 mg/liter initial nitrogen. Lipid accumulation is favored at low dilution rates in continuous culture, because lipid production exceeds the production of nonfat cell constituents at low growth rates (4). Therefore, we operated the continuous culture fermentation of whey permeate at slow dilution rates. Results are summarized in Table 1. For comparison, data from a typical batch fermentation are also included.

As dilution rate decreased, the percentage of COD reduction, total cell dry weight, and the amount of lipid produced all increased. Total cell numbers were fairly constant at a concentration of approximately one billion cells per milliliter at all dilution rates. The nonfat cell dry weight per liter was also relatively constant. At slower growth rates, the culture, already limited by the low nitrogen in the amount of nonfat cell mass it could produce, was able to convert additional carbon to cellular lipid. The higher biomass yields produced at lower dilution rates can thus be attributed to higher lipids with correspondingly higher percentage of utilization of available carbon in the permeate. At the slowest dilution rate tested, the amount of carbon used and the amounts of cell dry weight and lipid produced were equivalent to the results obtained in batch culture.

Lipid Production Rate

If this fermentation is to be used on an industrial scale for the production of microbial lipid, the key parameter to be determined is the lipid production rate, i.e., how much lipid can be produced per unit volume of permeate per hour. As can be seen in Table 1 and Figure 1, although the amount of lipid produced per liter of permeate was greatest at the slowest dilution rate tested, the greatest lipid production rate (grams of lipid per liter per hour) was observed at a dilution rate of .05 h^{-1}. The more rapid throughput of material at this dilution rate more than compensated for the lower concentration of lipid per liter obtained in comparison with the slower dilution rate.

Lipid production rates for batch fermentations can be calculated by dividing the lipid yield by the number of hours needed to run a complete fermentation. The optimum amount of lipid is produced in 72 h in a batch fermentation of whey permeate (6). Start-up and shutdown procedures, including fermenter cleaning

TABLE 1. Growth, lipid production, and substrate utilization by *Candida curvata* D in continuous culture in whey permeate.

Dilution rate	% COD[1] reduced	Cell number	Dry weight	Nonfat dry weight	% Lipid per dry weight	Lipid	Lipid production rate
		(organisms/ml)	(g/liter)			(g/liter)	(g/liter/h)
.02	94^{a}	ND[2]	14.2^{a}	6.9^{ab}	51^{a}	7.20^{a}	.144
.05	92^{a}	1.5×10^{9a}	13.1^{b}	7.5^{a}	45^{ab}	5.83^{b}	.306
.1	65^{b}	1.0×10^{9b}	8.7^{c}	6.1^{b}	32^{b}	2.75^{c}	.285
.2	29^{c}	7.9×10^{8b}	6.4^{d}	5.8^{ab}	10^{c}	$.6^{d}$	.124
Batch	95^{a}	1.3×10^{9b}	13.8^{a}	6.3^{ab}	55^{a}	7.59^{a}	.095

^{a,b,c,d}Numbers not sharing the same letter are significantly different ($P<.05$).

[1]Chemical oxygen demand.

[2]Not determined.

and sterilization, media preparation, and inoculation, would add at least 8 h to each fermentation cycle. Thus, given typical lipid yield values as presented in Table 1, the lipid production rate for a batch fermentation system is .095 g/liter/h. The maximum lipid production rate obtained in continuous culture compared very favorably to this value. These results agree with those of Evans and Ratledge (3), who reported that lipid production rate by *C. curvata* D in semidefined laboratory media may be four to five times faster in continuous than in batch culture.

The continuous fermentation of whey seems to be very close to economic feasibility. According to Fournier and Glatz (Department of Chemical Engineering, Iowa State Univ., unpublished report), the total production costs for operating a 464,000-liter fermenter in this process for 20 h would be about $2,000. Such a plant should produce 2773 kg lipid and 3273 kg nonlipid cell mass in that time. If the nonlipid portion is about 20% protein and worth about as much as soy protein (about $.44/kg), it should be valued at $288. The residual COD typically requires $.11/kg for disposal. There would be 1661 kg of COD remaining, so this amounts to $183. We have considered the permeate to have zero value and have taken no credit for the savings realized by not needing to dispose of the original COD. Thus, to break even an oil price of about $.68/kg would be needed. Prices for typical vegetable oils have been about $.55/kg. Most methods of permeate disposal do well to break even, and this fermentation process seems close to the break-even point. If *C. curvata* oil should be more valuable than soy or palm oil, or if greater efficiency could be achieved in the fermentation, the process could show a profit.

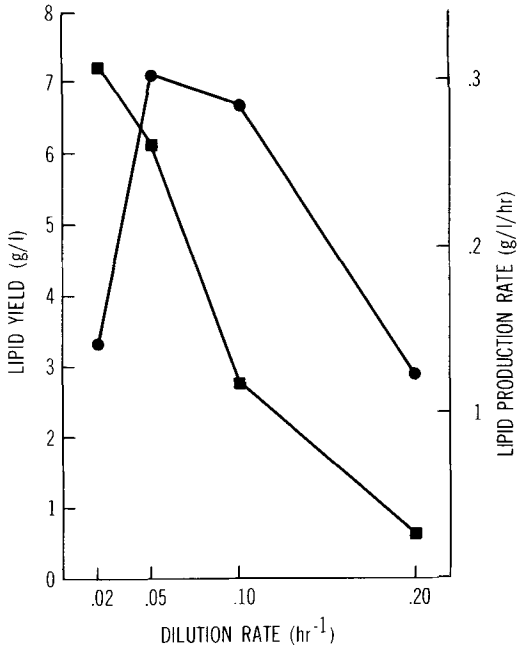

Figure 1. Effect of dilution rate (D) on lipid production by *Candida curvata* D in continuous culture fermentations of whey permeate (■ lipid yield (g/liter), • lipid production rate (g/liter/h).

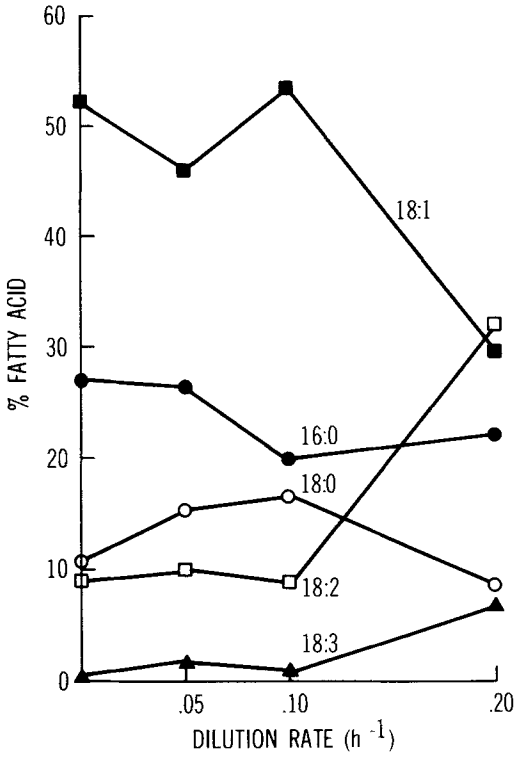

Figure 2. Effect of dilution rate (D) on the fatty acid composition during the fermentation of whey permeate by *Candida curvata* D (● 16:0, ○ 18:0, ■ 18:1, □ 18:2, ▲ 18:3).

Fatty Acid Composition

The composition of the lipid produced at various dilution rates was analyzed to determine if the composition was stable and if the lipid was different from that synthesized in batch culture. Results are presented in Figure 2.

At slow dilution rates, the lipid composition was very similar to that previously reported for stationary phase cells grown in whey permeate (8). The lipid was mainly triglyceride with palmitic (16:0) and oleic (18:1) acids being the predominant fatty acids. However, at the most rapid dilution rate examined, oleic acid was greatly reduced and linoleic acid (18:2) was greatly increased. The lipid composition at this rapid dilution rate more closely resembled the composition of the lipid in exponentially growing cells in batch culture in whey permeate (8). The lipid composition has also been shown to vary considerably during exponential growth in whey permeate and to become stabilized when the cells reached stationary phase (8). In contrast, Evans and Ratledge (3) reported that the lipid composition of *C. curvata* D varied during batch culture growth on lactose in a semidefined medium but was very stable in continuous culture growth in the same medium in a dilution rate range between .02 and .3 h^{-1}. Perhaps there is some factor in whey permeate that influences the fatty acid composition of rapidly growing cells.

CONCLUSIONS

Continuous fermentation of whey permeate to produce lipid seems to be more efficient and potentially more economical than batch fermentation. Further work is needed with a scaled-up large volume continuous fermentation system to investigate the applicability of this process to an industrial situation.

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