The Role of Nitrogen in Multiorganism Strategies for Biosurfactant Production

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ABSTRACT

Production of large quantities of biosurfactants which are cost-competitive with surfactants of petrochemical origin requires the use of cost-free or cost-credit wastes as process feedstocks for microbial growth and biosurfactant synthesis. Several multiorganism strategies are suggested for improving biosurfactant yields from wastes. One such strategy involving co-culturing of lipogenic (oleaginous) microbes at one stage of the overall process was found imcompatible with the nitrogen requirements for regulation of lipogenesis. Other strategies are proposed which avoid conflicts in regulatory mechanisms. Emphasis is placed in these latter strategies on the uniqueness of municipal wastewater treatment sludges both to produce a cost-competitive biosurfactant and to offset the costs of high quality wastewater treatment.

INTRODUCTION

Microbially produced surfactants or biosurfactants (1) must compete with surfactants of petrochemical origin in three respects—cost, functionality and production capacity to meet the needs of the intended application. Whereas high production costs can be tolerated for biosurfactants used in low-volume, high-priced products (e.g., cosmetics, medicinals, etc.), high production costs are incompatible with those applications (e.g., enhanced oil recovery, EOR) which require high volumes of low-priced surfactants. As with all of biotechnology, the foci for reduction of biosurfactant production costs are the microbes (selected, adapted or engineered for high yields of product); the process (selected, designed and engineered for low capital and operating costs); the microbial growth substrate or process feedstock (selected for low cost), and the process by-products (minimized or managed as saleable products rather than treated and discarded as wastes).

In this paper, different strategies are examined for economical production of glycolipids of the type shown in Figure 1. These sophorose-containing glycolipids are the extracellular products of the yeast *Torulopsis bombicola* (2) and have been proposed for applications ranging from cosmetics formulation to recovery of petroleum crudes.

Objectives of the current studies are to define better those parameters which are instrumental in turning a technological success in biosurfactant production into an economic success for bulk production of multipurpose biosurfactants and to identify areas for further research.

MICROBES

Torulopsis bombicola (ATCC 22214) was selected for its ability to produce higher yields of biosurfactant than other biosurfactant-producing microbes. In suspended cell batch cultures of *T. bombicola*, final product concentrations of 67 g/l and product yields of 0.347 g of biosurfactant per g of substrate have been reported (3). A strain of *T. bombicola* has been isolated in Japan which can product up to 120-130 g/l (4). The second highest reported biosurfactant recovery is at concentrations of 2.1 g/l and product yields of 0.105 g/g from *Rhodococcus erythropolis* (5,6). The high product yield from *T. bombicola* is important for meeting both the cost and production capacity needed for high-volume industrial applications of low-cost biosurfactants.

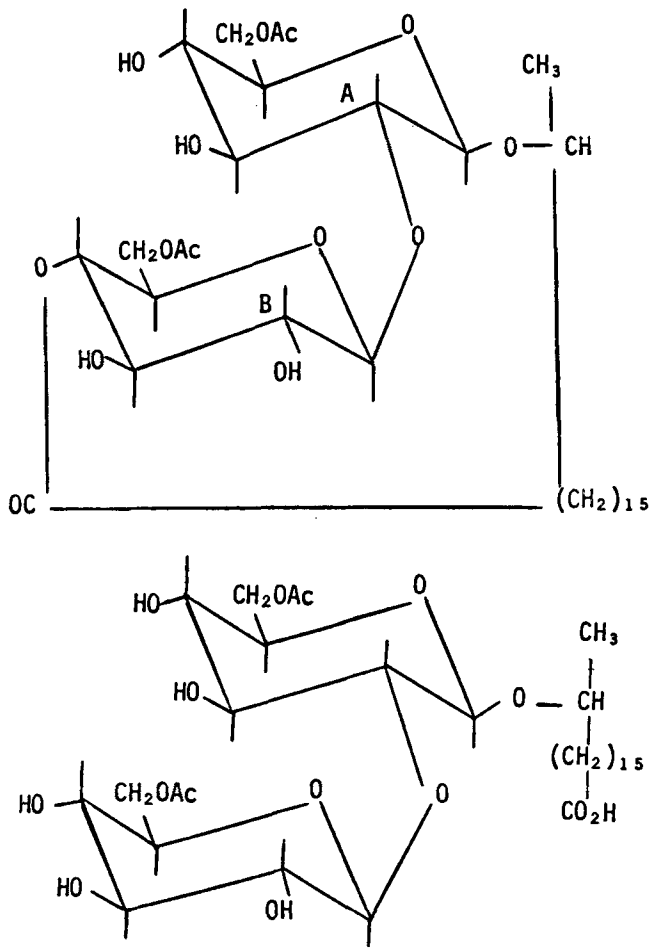


FIG. 1. Typical glycolipids produced by *Torulopsis* sp.

PROCESS

Part I: Biosurfactant Recovery

Biosurfactants are unlikely to be produced at low cost if extensive refining is required. Process development must, therefore, focus around biosurfactants (such as those produced by *T. bombicola*) which lend themselves best to simple, inexpensive process technologies by virtue, for example, of the surfactants being easily recovered from the fermentation broth by gravity separation of a surfactant-rich phase, and being obtained in their above separated form as relatively concentrated products which are free of major contamination (although surfactant mixtures could be tolerated).

MICROBIAL GROWTH SUBSTRATE (PROCESS FEEDSTOCK)

For maximal yields of glycolipid biosurfactant from *T. bombicola*, two substrates (sugar and lipid or hydrocarbon) are required. Using inexpensive and commercially available

substrates (molasses and soybean oil), it was calculated (3) that the substrates alone would place the biosurfactant production cost at $0.96 CDN/kg ($1.3 CDN = $1 US). The total production cost was suggested to be $2.75 CDN/kg (3). Even if sugars from Jerusalem artichoke were used, we have calculated the cost of substrate to contribute $0.83 CDN/kg to the total production cost of the biosurfactant. The price of commercial surfactants of the nonionic alcohol ethoxylate and alkylphenol ethoxylate types for use in EOR has been estimated at $1.41 to $1.57 CDN/kg (7). One recent analyst (8) has suggested the availability of commercial surfactants for EOR at a price as low as $0.86 CDN/kg, although this may be overly optimistic. Clearly, substrate costs contribute significantly to the overall cost of biosurfactant production, and contribute 60-70% to a maximal cost of $1.40 CDN/kg which would be necessary to make biosurfactants cost-competitive with the ethoxylates.

Substrates for microbial growth and biosurfactant production must be reduced in cost. Perhaps in an economic atmosphere dominated by petroleum and petrochemical prices, the best way currently to reduce substrate costs for biotechnology is to use wastes which are either free or carry a cost credit for environmental benefit. Table I summarizes, by chemical type, the substrates for biosurfactant production and the advantages, disadvantages and possibilities of using both refined and waste substrates. The requirement of two substrates for maximal glycolipid production by *T. bombicola*, the desirability of using waste substrates, and the considerations in Table I would suggest the following strategies for utilization of waste substrates:

(a) Select a waste substrate which has both carbohydrates and lipids.
(b) Select a waste which is either lipid- or carbohydrate-rich, and transport it to and blend it with a waste of the opposite nature.
(c) Select a waste which is carbohydrate-rich and microbially convert part of the carbohydrate to lipid as needed for biosurfactant production.

Strategy (a) involves the difficulty of finding a waste with just the right balance of carbohydrate and lipid to make the waste an optimal substrate for *T. bombicola*. Strategy (b) involves the likelihood of having to transport at best one of the wastes to be blended and hence increased energy input and cost of the final biosurfactant. Strategy (c) involves the use of additional microbes other than *T. bombicola* (a multiorganism strategy) and hence increased complexity of the process.

PROCESSES AND BY-PRODUCTS

Part II: Microbial Processes for Biosurfactant Production from Waste Substrates

Table II illustrates 5 different process strategies for glycolipid production and the contribution of purchased feedstock to the final biosurfactant production cost when both, one or none of the substrates is purchased commercially as opposed to being obtained from waste. The cost of waste substrate is assumed to be zero.

Processes I, II and III illustrate the general concepts described in the previous section, i.e., the use of commercial or waste substrates which are rich in sugars and/or lipids. Process III of Table III illustrates one multi-microorganism strategy in which the appropriate lipogenic (oleaginous) bacteria or yeast (M) and the appropriate lipogenic algae (A) are co-cultured to produce microbial single cell oil in the form of triglycerides. The microbial triglycerides and the same sugar used to feed the lipogenic microbes are used by *T. bombicola* (T) to produce glycolipid.

This multiorganism strategy (Process III) is illustrated more completely in Figure 2. Some of the lipogenic microbes which could be used are shown in Table III. A list of wastes on which lipogenic microbes have been grown has been prepared previously (9) and includes rice hull hydrolysate, starch waste liquors, whey, domestic waste and potato processing wastes. Process III would produce a 600% increase in biosurfactant yield from sugar alone compared

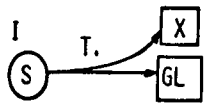
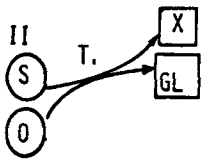
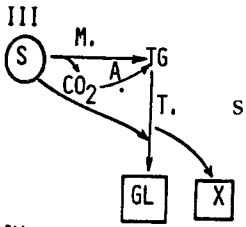
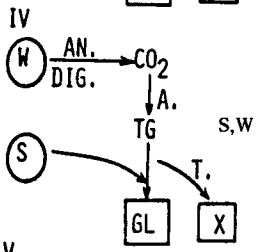
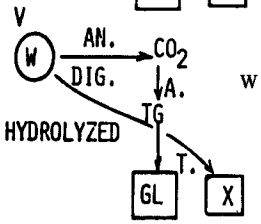
TABLE I

Feedstocks for Microbial Surfactant Production

Feedstock	Advantages	Disadvantages
Carbohydrates	Abundant in most geographic regions from biomass resources (silvaculture, agriculture). Present (sometimes with lipids) in wastes which might have a cost credit.	Lowest conversion efficiency of all substrates to biosurfactants unless lipids or hydrocarbons are provided along with the carbohydrates.
Hydrocarbons	Abundant in some geographic regions from petroleum resources. High yields of glycolipid biosurfactants when provided with carbohydrates.	Cost of biosurfactant production from non-waste hydrocarbons is tied to the cost of petroleum. Seldom present together with carbohydrates in wastes. Use of hydrocarbon wastes for biotechnology has been studied little.
Triglycerides, fatty acids		
Seed oil and animal fat	Abundant in some geographic regions from agricultural resources. High yields of glycolipid biosurfactants when provided with carbohydrates. Present (sometimes with carbohydrates) in wastes which might have a cost credit.	Cost of biosurfactant production from non-waste seed-oils and animal fats is tied to the real positive costs of these substrates.
Microbial oil (single cell oil, SCO)	Potential of being produced from lipid-poor carbohydrate-containing wastes which may have a cost credit. High yields of glycolipid biosurfactants when provided with carbohydrates.	Research needed for multi-organism strategies for biosurfactant production Process definition Process regulation Process economics

TABLE II

Microbial Processes for Glycolipid Production

Process	Substrates	Contribution of Feedstock to Process Cost ($cdn/kg) Purchased Substrate S,O	Waste Substrate	S ±O	±O S	S,O	Yield (g/100 g Feedstock) Overall	Per Purchased Feedstock S,O /S/ O
I 	S	–		4.59	0	–	3	-/ 3/-
II 	S,O	0.83		0.21	0.64	0	36	36/70/74
III 	S			0.83	0	–	18	-/18/-
IV 	S,W	–		0.21	0	–	6	-/70/-
V 	W	–		–	–	–	4	-/ - /-

T = *Torulopsis bombicola*
M = Lipogenic bacteria or yeast
A = lipogenic algae
An.Dig. = Anaerobic Digestion
W = Complex Waste

S = Sugar
O = Oil
TG = Triglyceride
GL = Glycolipid
X = Biomass

with a single organism strategy (Process I), which produces only 0.032 g biosurfactant/g sugar (10).

Key to the ability to use Process III and hence to utilize a single substrate (waste or commercial) is the ability to co-culture the lipogenic algae with other lipogenic microbes to allow for efficient use by the algae of the respiratory CO_2 evolved by the other microbes. Whether or not lipogenic algae, bacteria and/or yeast can be optimally co-cultured for lipogenesis depends on whether the nutritional and environmental requirements for lipid synthesis by the different co-cultured microbes are sufficiently similar.

Nitrogen has been documented as a regulator of lipogenesis in yeast (11-15) and algae (16). Figure 3 shows the metabolic pathways for synthesis of lipids from glucose and the site of the rate-limiting step for lipogenesis by yeast (13,15) under nitrogen limiting conditions. Under such conditions, there are diminished nitrogen-dependent metabolic activities such as protein and nucleic acid biosyntheses. The energy charge of the cell therefore increases, resulting in higher ATP and lower AMP. Metabolism of isocitrate is AMP-dependent, and consequently, both isocitrate and citrate accumulate in the mitochondria. The citrate is transported out of the mitochondria to the cytoplasm and eventually to the outside of the cell if citrate is not utilized in the cytoplasm. ATP:citrate lyase is key to cytoplasmic utilization of citrate through generation of acetyl-CoA for lipid synthesis. The activity of ATP:citrate lyase increases upon nitrogen depletion as does the concentration of malic enzyme which converts malate to pyruvate.

Whether similar enzyme changes occur in lipogenic algae and bacteria has not been documented. However, the nitrogen concentrations, which regulate whatever changes do alter lipid storage rates, would determine the compatibility for co-culturing of lipogenic algae with lipogenic bacteria and/or yeast. Figures 4 and 5 show the rates of lipid production and biomass accumulation by *Chlorella* and *Arthrobacter AK19* in response to nitrogen levels in the medium. Exponentially growing cells were cultured on media shown

TABLE III

Some Intracellular Lipid Accumulating Microbes (from 9) Which Could be Used to Produce Triglyceride Precursors to Biosurfactants

		Substrate	Total lipid as a % biomass (% neutral lipid)	Lipid yield (g lipid/100 g sugar)
Lipomyces lipofer	yeast	glucose	60-65 (80)	22.0
Rhodotorula gracilis	yeast	glucose	55-65 (75)	16.0
Chlorella vulgaris	algae	CO_2	30 (65)	–
Chlorella pyrenoidosa	algae	CO_2	65-75 (70)	–
Chlorella sorokinana	algae	CO_2	45 (80)	–
Arthrobacter AK19	bacteria	glucose	80 (90)	14.0

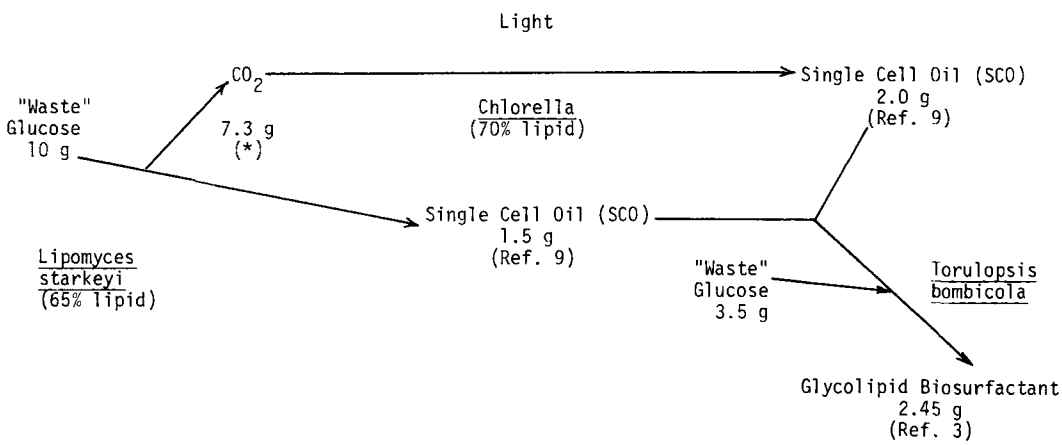


FIG. 2. A multiorganism strategy for glycolipid production from simple waste using co-cultured lipogenic algae *(Chlorella)* and lipogenic yeast *(Lipomyces)* to produce the lipid substrate for *Torulopsis* biosurfactant production. Lipogenic bacteria could be substituted for the lipogenic yeast. Yields are based on the references cited and 50% biomass in substrate utilization where indicated by an asterisk (*).

in Table IV and then transferred to media with various nitrogen levels.

After initial transient responses, the rates of lipid storage, non-lipid biomass accumulation and the medium nitrogen levels were determined. For both the lipid-storing algae and bacteria, a similar profile is observed. There is an optimum nitrogen concentration for lipid storage by both organisms, but this optimum concentration differs by nearly 16-fold. Below the optimum concentration of nitrogen, the lipid synthesis rate decreases but non-lipid biomass accumulation rate increases for both organisms, particularly the bacterium. The tendency for increased lipid synthesis rates with decreasing nitrogen in the medium is similar to the behavior of yeast and likely represents a response to primary events such as reduction in protein and nucleic acid synthesis, resulting subsequently in reduced AMP, increased citrate in the cytoplasm, possibly increased level of ATP:citrate lyase activity and henced increased lipogenesis. The decreased rate of lipid storage at very low nitrogen levels may reflect a decrease in de novo synthesis of enzymes specifically involved in lipid synthesis (in addition to enzymes of lower glycolysis and TCA cycles). A decrease in any or all of the enzymes for lipogenesis, lower glycolysis or TCA cycle may shunt the flow of carbon into glycogen storage or extracellular polymer production and result in the increased non-lipid biomass observed at very low nitrogen levels.

A time delay has been observed (1) between release of citrate into the cytoplasm and the increased activity of ATP: citrate lyase. It has not yet been determined whether de novo enzyme synthesis is part of the regulatory mechanism (e.g., synthesis of ATP:citrate lyase) or merely necessary for replacement of glycolytic, TCA, etc. enzymes. However, in either case the implication for batch culturing of lipogenic organisms is that a time frame for optimal lipid storage exists. This time frame is between the time when the nitrogen levels signal induction of lipogenesis and the time when the nitrogen levels preclude de novo synthesis of the regulatory enzymes or replacement of other essential enzymes. This time frame is controlled by the rate of nitrogen decrease in the culture and is therefore a function of the biomass concentration at the time of induction (Figure 6). Lower biomass concentrations at the time of induction would slow the consumption rate and prolong the time for induction of regulatory enzymes and/or optimal lipid

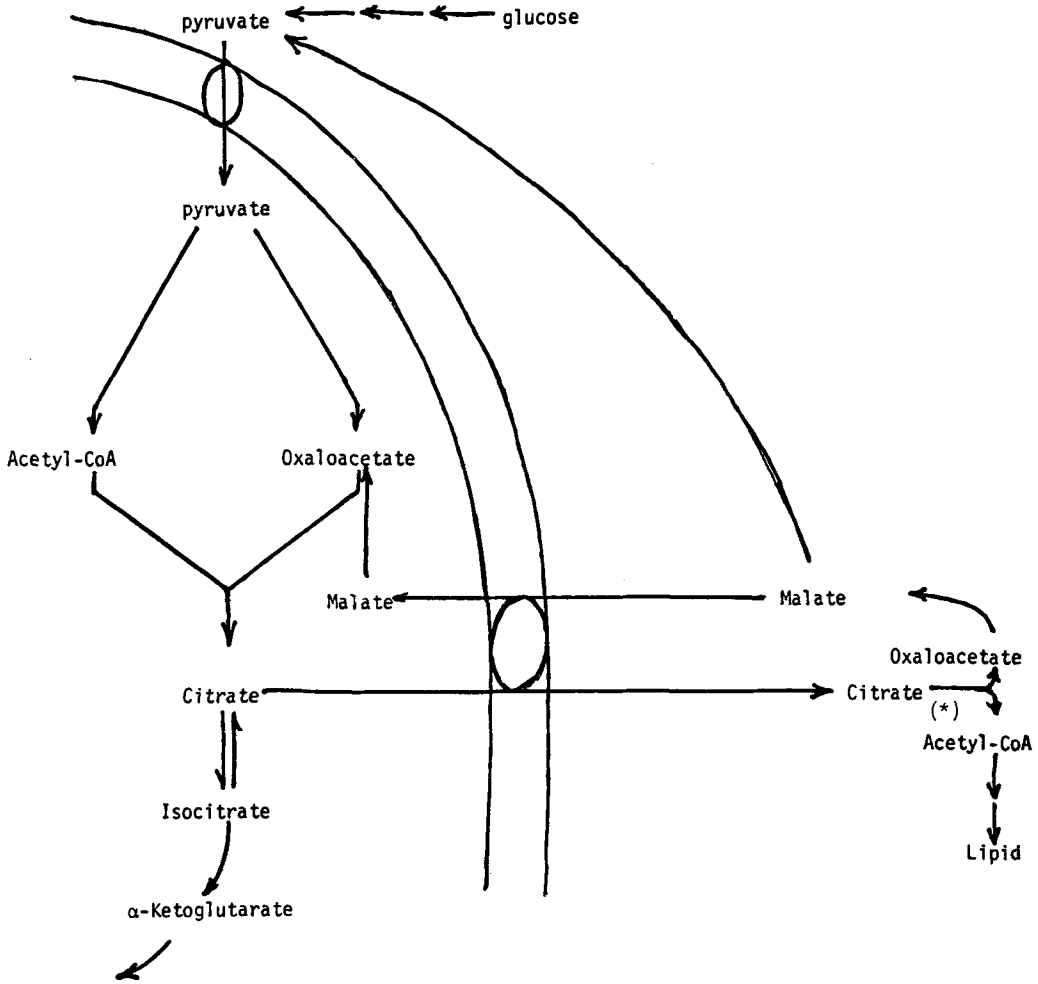


FIG. 3. Metabolic pathways of lipid synthesis from glucose showing the location (*) of ATP:citrate lyase, the rate limiting enzymatic step in lipogenesis by yeast (13,15).

storage rates. The use of a slowly metabolized nitrogen source such as proline may have a similar effect of increasing the time frame for induction and/or lipogenesis.

With respect to the multiorganism strategy for biosurfactant production, it seems evident that:

1—Co-culturing of lipogenic algae with lipogenic bacteria or yeast to produce the lipid substrate for biosurfactant synthesis is unlikely to produce optimal yields of the lipids unless species can be found with very similar nitrogen requirements for induction and sustenance of optimal lipogenesis. It probably is better to employ biosurfactant production strategies which preclude conflicts in process regulation, rather than to attempt to coordinate similarly regulated systems.

2—Batch culturing practices for microbial synthesis of the lipid substrate are likely to produce lower lipid yields than continuous culturing, especially when high biomass concentrations are used within the reactors.

The objective of Process III of Table II was to obtain a higher yield of biosurfactant from a single substrate through use of lipogenic organisms which convert part of the single sugar substrate to the co-required lipid substrate. When a commercial sugar substrate is used, algae were to be co-cultured with the other lipogenic microbes to increase recovery of the sugar carbon as lipid and hence to keep the feedstock costs low. If, however, the sugar is a cost-free waste, then the loss of sugar carbon as CO_2 is tolerable and lipogenic yeast or bacteria in the absence of algae could be used to synthesize the lipid substrate for biosurfactant production.

Not all potential waste substrates are abundantly rich in sugars which could be partly converted to lipids and partly used directly for biosurfactant production. Processes IV and V of Table II illustrate routes for utilizing complex wastes (e.g., municipal waste) for biosurfactant production. Key to these process strategies is the use of anaerobic digesters to provide CO_2 for lipogenic algae. The sugar substrate for biosurfactant production is provided from an external source (Process IV) or obtained by hydrolysis of complex components (e.g., cellulose) within the waste (Process V).

One example of Process V is illustrated in more detail in Figure 7. It should be noted that municipal sludge has 5-30% lipid, 4-8% cellulose and 3-7% hemicellulose. The necessity for the algal production of triglycerides will depend on the ratio of lipids to total utilizable sugars which will be liberated upon sludge hydrolysis as discussed below.

When municipal waste is used, several advantages exist:

1—Only one feedstock is used in the process so that transportation charges are minimized, especially if biosurfactant is produced at the waste treatment site.

2—The feedstock is a complex waste with a cost credit for environmental benefit. Part of the cost of benefit will almost always be borne by the users (residents and industry) who created the wastewater which must be treated and, therefore, the selling price of biosurfactant must not totally cover the cost of feedstock production.

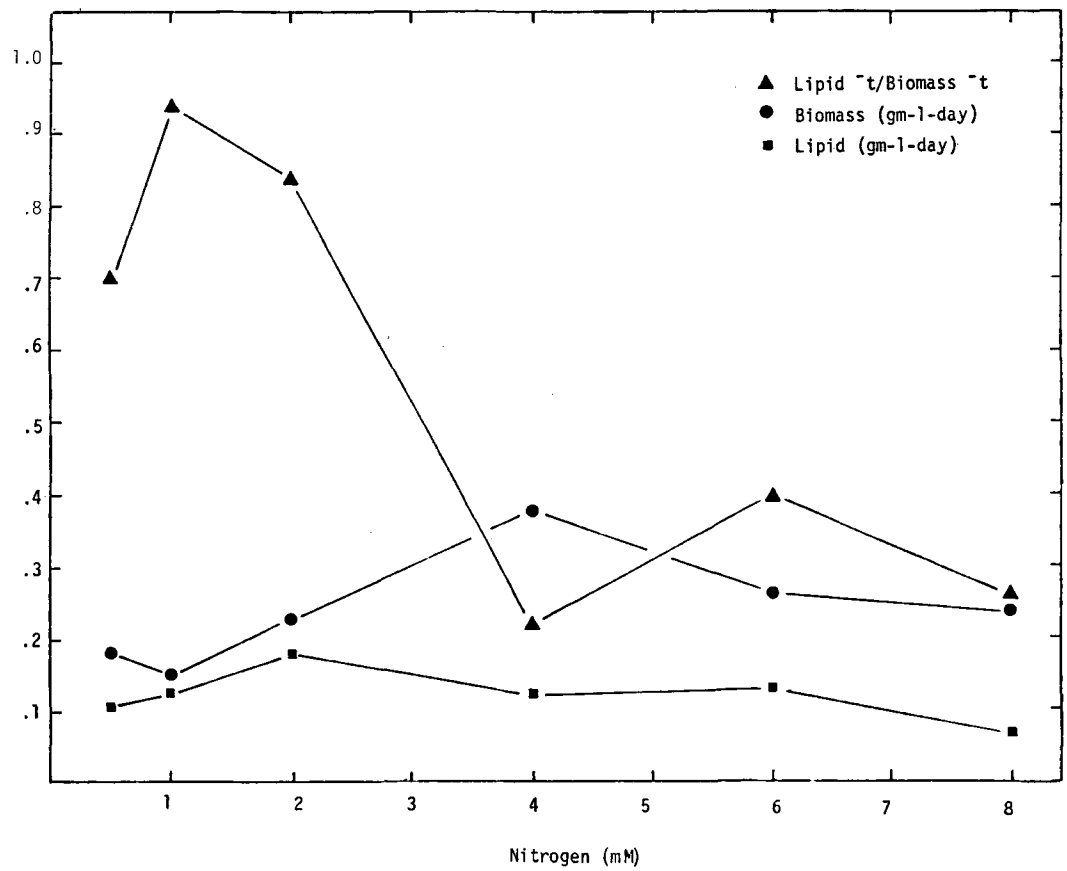


FIG. 4. Nitrogen dependence of the rates of both lipid storage and biomass accumulation by the algae *Chlorella sorokiniana*.

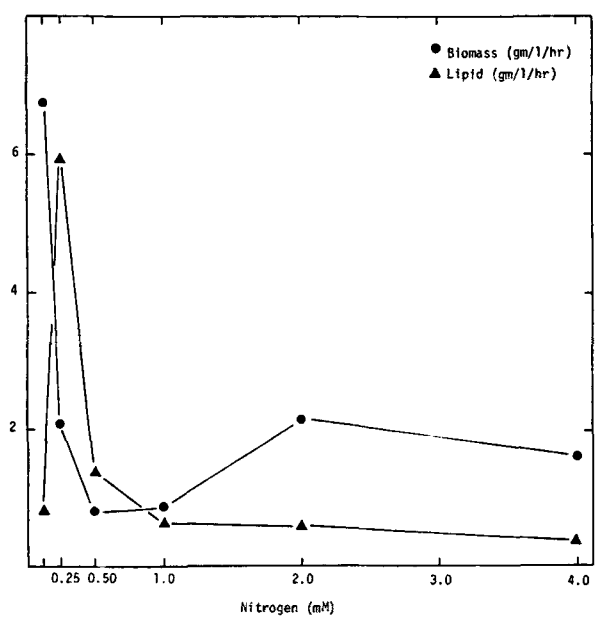


FIG. 5. Nitrogen dependence of the rates of both lipid storage and biomass accumulation by the bacterium *Arthrobacter* AK19.

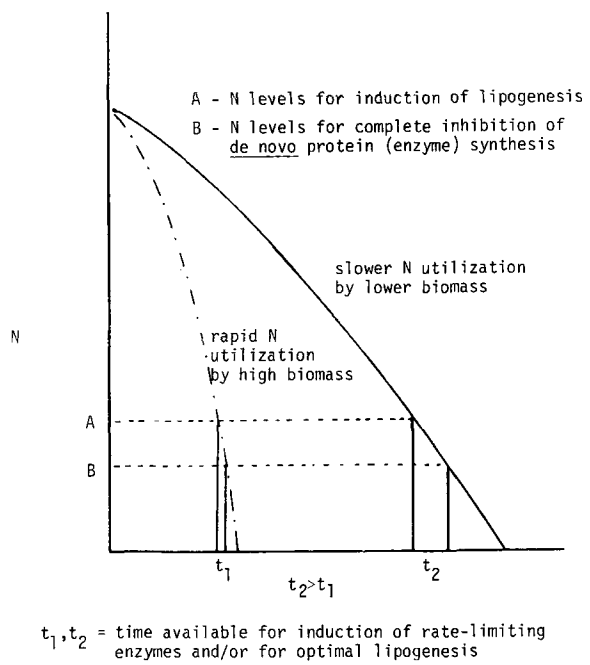


FIG. 6. A proposed influence of nitrogen on lipogenesis in batch cultures.

3—The feedstock (activated sludge from conventional wastewater treatment) already is centrally collected (unlike agricultural field residue, forestry trimming waste, etc.) and, in some communities, the anaerobic digester and biogas recovery and utilization facilities already are in place.

4—The feedstock is available year-round and with a relatively constant composition (depending primarily on the industrial input).

5—Energy requirements of the process are provided internally by the produced methane.

6—The process is relatively simple. For example, the hydrolysis unit simultaneously releases oil from the algae and glucose from sludge cellulose; glycolipid is recoverable from the fermentation medium by gravitational settling;

TABLE IV

Culture Media and Conditions for Algae and Bacteria Used in the Nitrogen Dependence Studies

	Chlorella sorokiniana (ATCC 22521)		*Arthrobacter* AK19 (ATCC 27779)	
Salts media	KNO_3	2 mM	KNO_3	10 mM
	Na_2HPO_4	1 mM	$MgSO_4$	5 mM
	$MgSO_4$	.3 mM	$CaCl_2$	.4 mM
	$CaCl_2$	.1 mM	Na_2HPO_4	5 mM
	Tris	5 mM	KH_2PO_4	5 mM
	EDTA	.170 mM	Trace element studies (18)	
	KOH, 85%	.470 mM		
	H_3BO_3	.185 mM		
	$FeSO_4$	.018 mM		
	$ZnSO_4$	.03 mM		
	$MnCl_2$	.008 mM		
	$CuSO_4$	.006 mM		
	$Co(NO_3)_2$	.002 mM		
Carbon supply	CO_2	(5% in N)	glucose	(0.2 M)
Nitrogen supply	.003 M for maintenance, variable for limiting studies		.005 M for maintenance, variable for limiting studies	
Culture conditions	Shake flasks with 50 ml culture on a rotary shaker at 200 rpm; temperature 23 C; 300 foot candles for algae cultures (light intensity).			

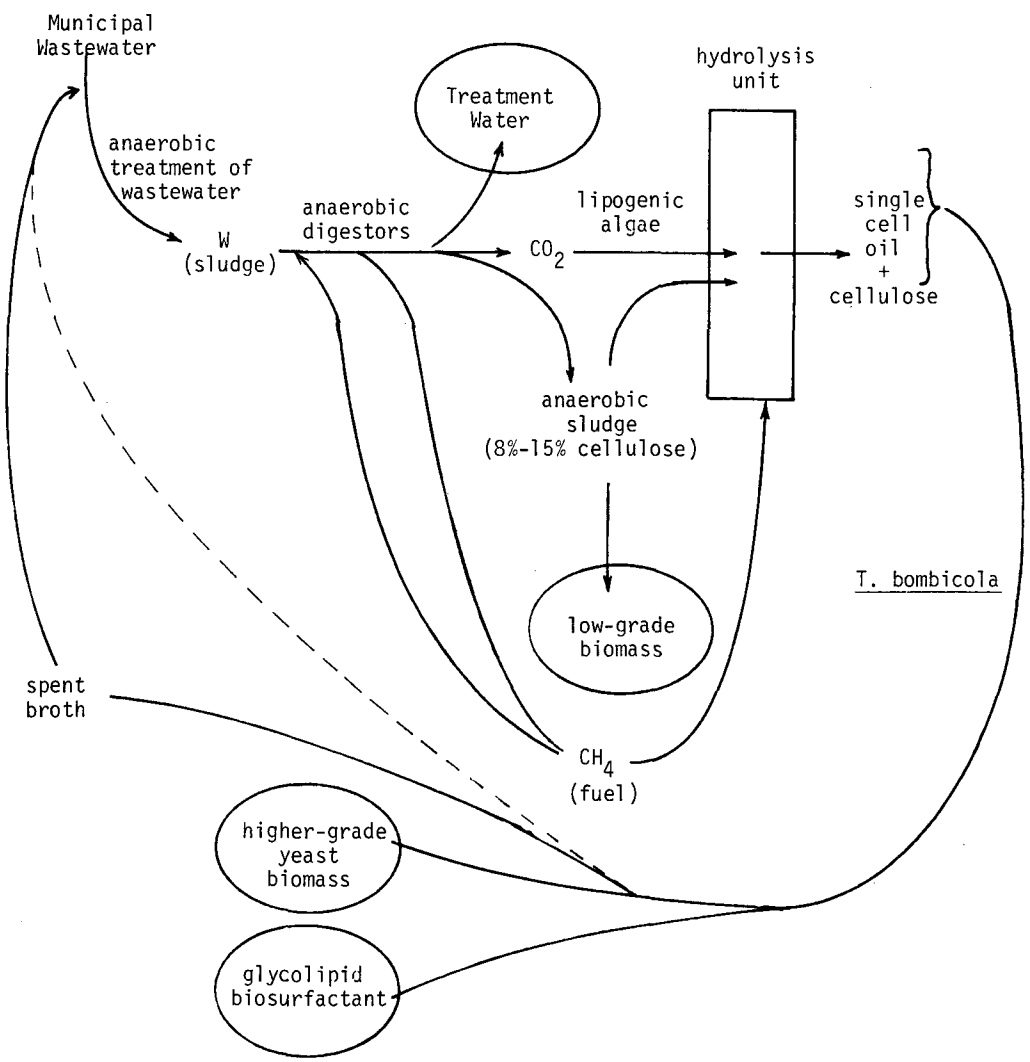


FIG. 7. Multiorganism strategy for glycolipid production from complex wastes using anaerobic digestion to produce the CO_2 substrate for lipogenic algae which in turn produces the lipid for *Torulopsis* biosurfactant production.

Torulopsis biomass from biosurfactant production can be recycled to the front end of the entire process for treatment or separated from the medium and sold as a yeast-rich feed supplement; gaseous by-products (CO_2, CH_4) are or can be used in the process, and 4 final products are obtained (treated water, high-grade and low-grade biomass, biosurfactant) with the latter products having sales value to help offset the water treatment costs.

The yields (g product/100 g feedstock) for biosurfactant production from complex wastes (Table II) are noticeably lower; however, in defense of these strategies, two points should be mentioned. First, the waste is cost-free or carries a cost credit, and therefore loss of feedstock carbon as by-product CO_2 is not a severe problem to feedstock economics. Some of the CO_2 could be salvaged via algal routes when this is deemed desirable. Second, the lower yield is partly explained by some of the feedstock carbon appearing as by-product CH_4. However, as this by-product serves the energy requirements of the process, it is not wasted.

In a community of 100,000 people using conventional and sophisticated tertiary treatment of wastewater, about 5 metric tons of sludge might be produced per day with a daily cost to that community of $3,700 (17). Process V (ignoring sludge lipids, using algae to produce lipids and assuming 8% cellulose in the sludge) might minimally produce 155 kg surfactant per day with a value of $217 ($1.40 CDN/kg), and approximately 12500 m^3 of methane which has a value of $175/day in support of the entire wastewater treatment and surfactant production process. The surfactant alone contributes 5.8% of the cost for the most sophisticated of wastewater treatments (involving tertiary treatment for phosphate removal) and perhaps as high as 37% for the least sophisticated treatment technologies. If the selling price of the biosurfactants can be higher than $1.40/kg (due to eventual increases in oil and petrochemical costs), then the contribution to reducing the costs of wastewater treatment also will be increased.

The above calculation using sludge assumed that the lipids for biosurfactant production would come from algae using anaerobically produced CO_2. However, as mentioned above, sludge already has lipid content of its own. If these lipids were used and the excess sugar needed to utilize the higher lipid content of some sludges were provided by hydrolysis of newspapers (an already collected waste in some communities), then the biosurfactant yields would be as shown in Table V, depending on whether algal lipid supplementing of the sludge lipids was used. As can be seen, supplementing with algal lipid will increase significantly the biosurfactant production from sludges containing a low lipid content.

Utilization of waste feedstocks for biosurfactant (or other bioproduct) production has the potential of reducing the production costs of the biosurfactants to the level of being competitive with similarly functioning petrochemicals, and of improving the economics of waste treatment. In the case of wastewater treatment, the costs of producing high quality treated waste (Table V) could be reduced by 40-45%. At a time when the quality of drinking and recreational waters and the necessity of countering a dependence upon petroleum are pointedly before us, it is appropriate that further biotechnology research be devoted to defining processes, to assessing the regulatory factors operating in these processes and to examining the economics for production of bioproducts from recycled wastes.

This paper is the first stage in such research towards biosurfactant production and is the forerunner to a more complete process description and economic evaluation.

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TABLE V

Biosurfactant Production From Municipal Wastewater Treatment Sludges

Process	Kg biosurfactant d^{-1} per population of 10^5 (i.e., per 5 metric tons sludge)	Selling price of glycolipid biosurfactant (at $1.40 CDN/kg) as a % of high-quality waste treatment operating costs (including tertiary treatment costs)
V (ignoring sludge lipids and using only algal lipids produced from anaerobic digestor CO_2)	155	5.8
V (or IV) (except that algal lipids are replaced by sludge lipids (5%-30%) and newsprint is added to high-lipid sludges to provide enough sugars)	175-1050	6.6-40
V (or IV) (using both sludge lipids and algal lipids produced from anaerobic digester CO_2; newsprint added as needed for high lipid wastes)	420-1220	16-46

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Biomodification of Fats and Oils: Trials with *Candida lipolytica*[1]

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ABSTRACT

Various oil-accumulating yeasts were tested for their ability to produce lipase and live on fats and oils as carbon sources. Of these, *Candida lipolytica* seemed most promising, and the possibility was explored of modifying fats and oils by fermenting them with *C. lipolytica* and extracting the modified oil deposited in the yeast cells. Oxygen was required for the growth of yeast on fats and oils, but unless the oxygen level was controlled at a low value after cell populations peaked, most of the substrate oil was converted to citrates rather than accumulating as oil. Oil accumulation by *C. lipolytica* from a corn oil substrate was slightly depressed by excess nitrogen in the medium. The yeasts were able to use about 18 g/l of oil in 72 hr. At substrate oil levels greater than 18 g/l, the dry yeasts were 60% oil, and about 45-57% of the substrate oil was recovered as yeast oil. The fatty acid composition of the yeast oil was quite similar to that of the substrate oil under optimum conditions of deposition. Sterols, but not tocopherols, were transferred from the substrate to the yeast oil. *Candida lipolytica* oil was high in free fatty acids. The greatest potential for biomodification by fermentation with *C. lipolytica* seems to be in altering glyceride structure.

INTRODUCTION

Fat and oil technologists have several techniques available for modifying compositions of raw materials, namely, blending fats and oils from diverse sources, hydrogenation, fractional crystallization, interesterification and plant breeding (1,2). These techniques make available a wide range of lipid products and have made it possible to substitute fats and oils for each other in many instances. But some advantageous techniques are missing. For example, we have as yet no good way to remove the linolenic acid selectively from an oil. We cannot reverse the hydrogenation reaction and convert tallow into an oil rich in linoleic acid. We have only limited ability to modify glyceride structure.

We look with envy on biological systems with their precise control of chain length, unsaturation and glyceride composition, and we ponder the possibility of using enzymes to modify lipids, but these systems seem discouragingly complex and fragile. There is, of course, a kind of biomodification that we have been using for years. We convert oils in feed grains into animal fats. This is not very efficient, but it is known that microorganisms do bioconversions quite efficiently. Usually, when fermentations are considered in regard to fats and oils, they have been used to convert carbohydrates to oils, but microorganisms are known that will use fats and oils as carbon sources and also will accumulate oil (2,3,4).

These experiments were conducted to explore the possibility of biomodifications of fats and oils by the yeast, *Candida lipolytica.* Fermentation conditions were optimized, and the yeast oil that accumulated was examined.

Methods

Yeast cultures were obtained from the collection of the Iowa State University Food Technology Department; from the USDA Northern Regional Research Center, Peoria, IL, and from Dr. C. Ratledge, University of Hull, England. The cultures were maintained on agar slants containing 0.3% yeast extract, 0.3% malt extract, 0.5% peptone (Difco Laboratories, Detroit, MI) and 1% glucose.

Selection for organisms able to use fat as a carbon source and accumulate fat was made on plates containing 5% corn oil, 0.67% yeast nitrogen base (Difco) and 2% agar. Colonies were stained with Oil red O (Pfaltz and Bauer, Inc., Flushing, NY) by saturating an isopropanol solution with the dye, diluting this with an equal volume of water and using it as a wet-mount medium. To test for lipase production, tributyrin agar plates were prepared (5), and the cell pastes to be tested were added to wells cut in the plate and incubated for 48 hr at 37 C. The agar was inspected for clear zones around the wells.

Fermentations were done in a 750-ml fermenter (Multigen, New Brunswick Scientific Co., New Brunswick, New Jersey) with a 450-ml working volume. Air flow and agitation rates could be controlled. The pH was controlled by a TTT2 automatic titrator (Radiometer, Copenhagen, Denmark) with the addition of 3 N NaOH. Dissolved oxygen was measured with an electrode prepared according to Johnson et al. (6) and recorded with a DO-50 dissolved oxygen recorder (New Brunswick). Fermentations were at 30 C,

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