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Degradation of olestra, a non caloric fat replacer, by microorganisms isolated from activated sludge and other environments

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Abstract

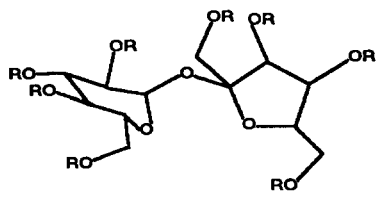
Olestra is a non-caloric fat substitute consisting of fatty acids esterified to sucrose. Previous work has shown that olestra is not metabolized in the gut and is excreted unmodified in human feces. To better understand the fate of olestra in engineered and natural environments, aerobic bacteria and fungi that degrade olestra were enriched from sewage sludges, soils and municipal solid waste compost not previously exposed to olestra. Various mixed and pure cultures were obtained from these sources which were able to utilize olestra as a sole carbon and energy source. The fastest growing enrichment was obtained from activated sludge and later yielded an olestra-degrading pure culture of *Pseudomonas aeruginosa*. This mixed culture extensively degraded both ^{14}C-fatty acid labeled olestra and ^{14}C-sucrose labeled olestra during 8 days of incubation. Longer-term incubation with pure cultures of *P. aeruginosa* demonstrated that >98% of ^{14}C-sucrose labeled olestra and >72% of ^{14}C-fatty acid labeled olestra was mineralized to CO_2 after 69 days. These results indicate that olestra degraders are present in environments not previously exposed to olestra and that olestra can serve as a sole carbon and energy source. Furthermore, a common bacterial species was isolated from activated sludge and shown to have the ability to degrade olestra.

Introduction

Olestra is comprised of octa-, hepta-, and hexa-esters of sucrose combined with naturally occurring fats and oils (Bergholz 1992a; Miller & Allgood 1993). Olestra possesses physical and organoleptic properties similar to common triglycerides and therefore can be used as a heat-stable replacement for dietary fat in a wide variety of foods. The physical properties of olestra are determined by the properties of the fatty acid side chains (Figure 1). For instance, olestra made predominately from unsaturated fatty acids will have a lower melting point than an olestra made predominately from saturated fatty acids.

Several studies have shown that olestra is not metabolized in the gastrointestinal (GI) tract of mammals, including humans (Bergholz 1992b; Glueck et al. 1980; Jandacek & Holcombe 1991; Nuck et al. 1994). Furthermore, studies in humans showed that olestra is not fermented by the intestinal microflora nor does it interfere with the normal processes of fermentation (Eastwood & Allgood 1995). Radiolabel absorption studies in rats have shown that olestra is essentially not absorbed from the GI tract (Miller et al. 1995). Studies with mice, rats, and dogs fed olestra at levels up to 10% of the diet for 20–24 months have provided confirmation that olestra is not significantly absorbed by examining potential target organs for the presence of olestra (LaFranconi et al. 1994; Miller et al. 1991; Wood et al. 1991).

Because it is not metabolized or absorbed, olestra will be excreted as a component of feces where it will reach wastewater treatment plants and enter the environment. Previous work has evaluated the environmental safety of olestra and has shown no adverse effects on the normal functioning of wastewater treatment plants, no adverse effects on plants and animals in the environment, and no adverse effects on soil physical properties (Allgood 1994a,b; Logan et al. 1993; McAvoy et al. 1996; Overcash et al. 1994). Olestra is removed



R = Fatty Acid Groups

Chemical Specifications for Olestra

	Specifications for Olestra	Typical Lot
Esterification		
Total Octa, Hepta, Hexa	97% min.	99.4
Octa	70% min.	72.6
Hexa	1% max.	0.3
Penta	0.5% max.	0.3
Fatty Acid Chain Length		
C12	1% max.	0.1
C14	1% max.	0.4
C16-C18	78% max.	94.2
C20 or Greater	20% max.	5.3
Saturation		
Total Unsaturated	83% max.	70.1
	25% min.	29.9

Figure 1. Chemical structure of olestra

during conventional wastewater treatment primarily by sorption to wastewater solids and settling in clarifiers (McAvoy et al. 1996). Studies with radiolabeled olestra have shown that it will be mineralized in activated sludge, although actual biodegradation during wastewater treatment will likely be minimal due to relatively short residence times (Allgood et al. 1994; McAvoy et al. 1996). Furthermore, it has been demonstrated that olestra will not have an adverse effect on the anaerobic digestion process (McAvoy et al. 1996). The rate and extent of mineralization in sludge-amended soil was determined for both a liquid (mostly C_{16-18} unsaturated fatty acid esters) and solid (mostly C_{22} saturated fatty acid esters) radiolabeled olestra, and olestra was found to have a biodegradation half-life of 10 and 88 days, respectively (McAvoy et al. 1996). These previous studies have demonstrated the efficient removal of olestra during wastewater treatment and the inherent ability of olestra to degrade in the soil environment following sludge application. The current work was conducted with liquid olestra to learn more about the diversity of organisms which could potentially degrade olestra.

The objectives of this study were to: 1) examine the distribution of microorganisms capable of using olestra as a sole carbon and energy source, 2) isolate mixed and pure cultures of olestra-degrading microbes, and 3) verify that olestra undergoes complete biodegradation and that intermediates do not accumulate during this process. The results provide a clearer understanding of olestra degradation and provide additional information for assessing the fate of olestra in the environment.

Methods

Chemicals

Non-radiolabeled liquid olestra was synthesized by the Procter and Gamble Co. (Cincinnati, OH) from sucrose and palmitic, oleic and linoleic fatty acid methyl esters. The fatty acid composition of the non-radiolabeled olestra was approximately 20%C_{16}, 35%$C_{18:1}$ and 35%$C_{18:2}$ based on gas chromatography. The degree of esterification was determined to be approximately 75% octa and 25% hepta-ester by normal-phase high performance liquid chromatography. ^{14}C-radiolabeled olestra was also synthesized by Procter and Gamble using ^{14}C-[U-sucrose] or ^{14}C-[1-fatty acid] and unlabeled constituents. For ^{14}C-[U-sucrose] olestra (specific activity 0.86 mCi/g), the significant components of starting fatty methyl esters were approximately 20%C_{16}, 4%C_{18}, 36%$C_{18:1}$ and 34%$C_{18:2}$. ^{14}C-[1-fatty acid] olestra (specific activity 0.68 mCi/g) was synthesized using approximately 25%$^{14}C_{18:1}$, 25%$^{14}C_{18:2}$ and 50%$C_{18:2}$. One hundred μg of olestra contains approximately 80 μg of carbon, assuming 100% octaester and 100% $C_{18:2}$ fatty acid. Using this estimate, 150 nCi of ^{14}C-[1-fatty acid] added to 5 ml of media would result in an addition of approximately 27 μg carbon/ml. Radiolabeled olestra was added to cultures as an aqueous dispersion with 10% type-III egg yolk lecithin (Sigma Chemical Co., St. Louis, MO). This mixture was heated to 50°C and sonicated prior to use to ensure a homogenous dosing solution. Sodium palmitate was purchased from the Aldrich Chemical Company, Inc. (Milwaukee, WI). High purity solvents were obtained from Burdick and Jackson (Muskegon, MI). Potassium hydroxide was purchased from the J. T. Baker Co. (Phillipsburg, NJ). Mercuric chloride was purchased from the Fisher Scientific Co. (Pittsburgh, PA) and was used for the creation of abiotic controls. Ultima Gold XR scintillation fluor (Packard Instrument Co., Meriden, CT) was used for liquid scintillation count-

Table 1. Description of olestra-degrading mixed cultures and isolates that demonstrated growth in bacterial media. All incubations were carried out aerobically with shaking at 22°C. All mixed cultures and isolates were able to grow on olestra as the sole carbon and energy source.

Mixed Culture	Initial Inoculum	Source	Isolates From Mixed Culture	Isolate Identification
AS1	Activated sludge	Sycamore WWTP[1] Cincinnati, OH	NA[2]	
AS2	Activated sludge	Western Regional WWTP Dayton, OH	Isolate #1	NA
			Isolate #2	NA
			Isolate #3	*Pseudomonas aeruginosa*
			Isolate #4	NA
AS3	Activated sludge	Western Regional WWTP Dayton, OH	NA	
SL	Soil	Summit Lake, WI	NA	
CB	Soil	Cedar Bog Urbana, OH	NA	

[1]Wastewater Treatment Plant.
[2]Not attempted.

Table 2. Description of olestra-degrading mixed cultures and isolates which demonstrated growth in fungal media. All incubations were carried out aerobically without shaking at 22°C. All mixed cultures and isolates were able to grow on olestra as the sole carbon and energy source.

Mixed culture	Initial inoculum	Source	No. of isolates indentified from mixed culture	Isolate identification
AS1-F	Activated sludge	Sycamore WWTP[1] Cincinnati, OH	2	*Trichoderma virde*
				Monocillium indicum
AS2-F	Activated sludge	Western Regional WWTP Dayton, OH	1	*Cladosporium castellani*
CB-F	Soil	Cedar Bog Urbana, OH	1	*Verticillium albo-atrum*
COM-F	Compost	Procter and Gamble Municipal Solid Waste Cincinnati, OH	1	*Verticillium albo-atrum*

[1] Wastewater Treatment Plant

ing. All other chemicals used were reagent grade or better.

Enrichment cultures

Descriptions of the cultures obtained in this study are found in Tables 1 (bacterial) and 2 (fungal). Enrichments were carried out in sterile Erlenmeyer flasks capped with cotton plugs at 22°C and a pH of 7.1 and 5.5 for bacteria and fungi, respectively. Samples (1 g) were taken from a variety of habitats and locations and incubated aerobically with 0.3 g olestra or sodium palmitate in 50 ml of mineral salts media (MSM). The substrates, which are water insoluble, were added directly or sonicated with lecithin before addition to ensure a homogenous dosing solution. The salts medium used was a modification of that described by Leadbetter and Foster (Leadbetter & Foster 1958), with a ten-fold increase in sodium phosphates to improve buffering capacity and prevent significant pH shifts during incubation. Bacterial incubations were carried out aerobically on gyratory shakers (125 rpm), while fungal enrichments were incubated quiescently. All enrichment cultures were repeatedly transferred to fresh media and eventually grown solely on olestra. Cultures which exhibited increased turbidity and showed visual changes in the olestra were continuously transferred to fresh media with olestra as the substrate. One culture, AS2, exhibited faster growth on olestra than any of the other cultures. It was initially derived from activated sludge, initially grown on palmitic acid and subsequently transferred to olestra as a sole carbon and energy source.

Isolation of a pure olestra-degrading bacterial culture

An aliquot of an actively growing AS2 culture was serially diluted onto trypticase soy agar plates (TSA, Difco, Detroit, MI) and incubated at 22°C. Representative colonies were used to inoculate test tubes containing 15 ml of trypticase soy broth. Isolates were then incubated at 22°C with shaking (125 rpm). Once turbid, these cultures were again streaked onto TSA plates and grown in mineral salts medium with olestra as the sole carbon and energy source.

Isolate identification

AS2 Isolate #3 was identified by independently evaluating its cellular fatty acids (MIDI, Newark, OK) and ability to utilize various carbon sources using Biolog plates (Biolog, Inc., Hayward, CA). These evaluations were conducted by Microcheck, Inc. (Northfield, VT) and Analytical Services, Inc. (Essex Junction, VT), respectively. The remaining bacterial isolates were not identified. Microcheck, Inc. also identified the fungal cultures by microscopic evaluation of morphological features or by fatty acid analysis.

Olestra mineralization and metabolite analysis - mixed bacterial culture

AS2 was grown for 6 days in 1100 ml of sterile MSM with 0.75 ml of olestra in a 4 L Erlenmeyer flask. The cells were harvested by centrifugation (10,410 x g, 15 min, room temperature), washed with approximately 100 ml of 1:10 dilution of MSM without nitrogen and resuspended in 400 ml of this medium. Half of the suspension was autoclaved and amended with 0.1% $HgCl_2$ to serve as abiotic controls. Five ml subsamples of the live and abiotic cultures were transferred to 250 ml biometer flasks containing 110 to 170 nCi of ^{14}C-[sucrose] olestra or ^{14}C-[fatty acid] olestra. Five ml of 0.3 N KOH were placed in the sidearms as the trapping solution. At various times over the 200 h incubation period, 1 ml of KOH was removed, combined with 10 ml of Ultima Gold XR scintillation fluor, and $^{14}CO_2$ production was determined by liquid scintillation counting (LSC) using a Beckman 7800 scintillation counter (Fullerton, CA). At the end of the incubation period, the culture was acidified by the addition of 50 μl of 6 N HCL in order to volatilize $^{14}CO_2$ into the headspace of the flask. The flask was left overnight to trap this $^{14}CO_2$.

To examine the disappearance of olestra and the formation of metabolites, five ml of washed cells were transferred to 20 ml sterile screw cap vials and amended with 110 to 170 nCi of ^{14}C-[sucrose] olestra or ^{14}C-[fatty acid] olestra. Five replicates were prepared for each treatment. The vials were capped with Whatman GF/C glass fiber filters (Whatman International Inc., Maidstone, England) to allow oxygen permeation and incubated at 22°C on a gyratory shaker (125 rpm). At various times, a vial from each treatment was acidified with 15 μl of 6 N HCl and immediately flash frozen in a bath of acetone and dry ice. The frozen cultures were lyophilized overnight using a Vitris Unitop 600L lyophilizer (The Virtis Co., Gardiner, NY) and extracted four times with 5 ml portions of a 1:1 solution of hexane and diethyl ether (v/v). This was performed to separate the radiolabeled components from the culture matrix (which may interfere with the TLC) and to concentrate the radiolabel prior to analysis. These culture extracts, which contained radiolabeled parent and metabolites, were separated by thin layer chromatography (TLC) using 20 cm x 20 cm Whatman silica gel (150 Å, 250 μm) plates with preabsorbant strips and a mobile phase consisting of heptane:toluene:methanol (56:43:2). The plates were scanned using a BIOSCAN System 200 Imagining Scanner and software (BIOSCAN, Washington, D.C.). Selected samples of extracted solids were sequentially extracted to quantitate the amount of radiolabel that had been incorporated into the major constituents of microbial biomass (carbohydrates, lipids, nucleic acids and proteins) using the procedure of Carpenter (Carpenter 1977). Finally, the extracted residue was combusted using a Packard Model 307 Sample oxidizer and analyzed for radioactivity.

Olestra mineralization - bacterial single species

AS2 Isolate #3 was grown on olestra in MSM as described above for 5 days. Cells were harvested, washed, resuspended and dead controls were prepared as described previously. One hundred and fifty to 200 nCi of ^{14}C-[fatty acid] olestra or ^{14}C-[sucrose] olestra was combined with the resuspended pellet in biometer flasks and $^{14}CO_2$ production was determined over a 69 d period as described earlier.

Results and discussion

Cultures

The initial environmental survey indicated that organisms capable of utilizing olestra for growth are prevalent in a number of different habitats. Table 1 lists the bacterial mixed cultures and individual isolates that exhibited growth on olestra. These cultures were included based upon visual observations of increased turbidity and physical changes in the olestra growth substrate. Upon dosing, olestra formed clear, oily droplets on the surface of the medium. During incubation, the droplets became dispersed and microscopic examination revealed heavy colonization by the microorganisms. In most cases, no olestra could be observed in these cultures after a period of time.

As shown in Table 1, bacteria from two separate wastewater treatment plants and two distinct soil samples possessed the ability to utilize olestra as a sole carbon and energy source. This is significant for three reasons. First, these compartments are the major sites where disposed olestra will eventually reside. Thus, the recruitment of microbes from other habitats should not be a requirement for olestra degradation. Secondly, several pure strains of bacteria in activated sludge degrade olestra, demonstrating that the degradation of olestra is not likely dependent on the activity of multiple bacterial species. Lastly, the fastest growing isolate obtained from the Dayton, OH activated sludge culture (Table 1, AS2 Isolate #3) was identified as *Pseudomonas aeruginosa*. *P. aeruginosa* is commonly found in wastewater and is widely distributed in nature, including soil and water (Breed & Murray 1957; Rosebury 1962). Thus, an olestra-degrading bacterial species is very likely to be present in all areas of the environment where olestra may enter, including soil, domestic sewage and natural water systems. Therefore, the current bacterial composition of environments where olestra may enter likely have the ability to mineralize olestra despite having never been exposed to it.

Table 2 shows fungal isolates enriched from activated sludge, soil and compost that exhibit growth on olestra. As with the bacteria, these isolates represent microorganisms from diverse environments capable of utilizing olestra as a sole growth substrate. These fungal isolates are representative of common genera prevalent in nature. Members of the genus *Trichoderma* are saprophytes that are commonly found in soils (Atlas & Bartha 1987). *Cladosporium* species

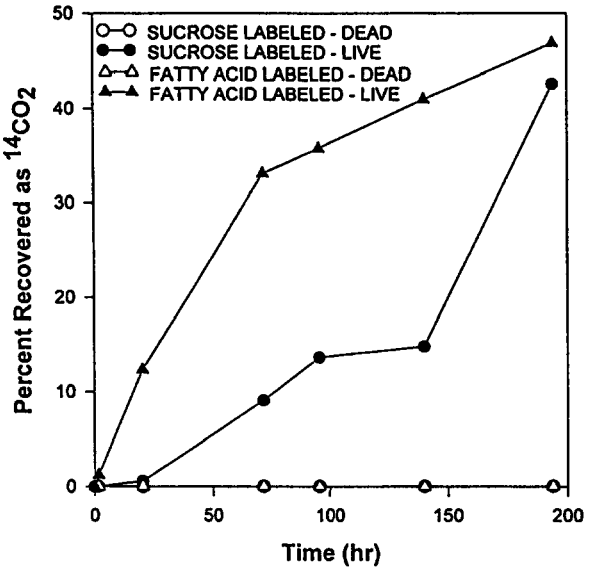


Figure 2. Mineralization of ^{14}C-[sucrose] olestra and ^{14}C-[fatty acid] olestra by the mixed culture AS2.

are the major constituent of the atmospheric fungal community at concentrations ranging from 10^1 to 10^4 m^{-3} (Atlas & Bartha 1987). *Cladosporium* and other airborne fungi are widely dispersed by gravity and precipitation. *Monocillium indicum* and *Verticillium albo-atrum* are commonly encountered in soil and plant materials, respectively (Gilman 1945; McGinnis 1980). The utilization of olestra by these common and widely-dispersed species indicates that olestra degrading microorganisms are present in a number of environments with widely differing characteristics.

Olestra mineralization and metabolite analysis - mixed bacterial culture

Since olestra could serve as a sole carbon and energy source for AS2, both the primary biodegradation and the mineralization of olestra by this culture was quantified (Figure 2). In less than 200 h, more than 40% the ^{14}C-[fatty acid] or ^{14}C-[sucrose] olestra was recovered as $^{14}CO_2$. However, the rate of $^{14}CO_2$ production varied with the location of the radiolabel. The olestra labeled in the fatty acid moiety was rapidly converted to $^{14}CO_2$ without a lag phase. Mineralization of the sucrose moiety, however, was preceded by a short lag phase followed by rapid and extensive $^{14}CO_2$ production. These results are consistent with the removal of

Table 3. Recovery of radiolabeled parent, metabolites, CO_2 and biomass from AS2 cultures incubated with ^{14}C-olestras as a function of time and position of the radiolabel in the olestra

Test material treatment	Hours of incubation	Percentage of added radioactivity				
		Parent	Polar metabolite	Other	Biomass	CO_2
^{14}C-[U-sucrose] Olestra						
Biotic	0	97.2	0.0	0.0	1.2	0.0
Abiotic	0	92.1	0.0	0.0	2.6	0.0
Biotic	1.7	81.1	6.2	0.0	0.8	0.0
Abiotic	1.7	92.7	0.0	0.0	ND[1]	0.0
Biotic	140	8.0	20.1	0.9	25.5	14.8
Abiotic	140	82.0	3.4	2.2	ND	0.1
Biotic	192	5.2	11.3	0.0	17.3	42.6
Abiotic	192	75.7	2.4	3.8	1.5	0.1
^{14}C-[1-fatty acid] Olestra						
Biotic	0	88.7	0.0	5.2	1.2	0.0
Abiotic	0	80.8	0.0	5.4	2.3	0.0
Biotic	1.7	67.4	5.9	5.4	4.9	1.2
Abiotic	1.7	72.8	2.6	7.9	ND	0.0
Biotic	140	2.6	4.3	3.2	3.3	41.0
Abiotic	140	63.2	10.2	5.5	ND	0.0
Biotic	192	1.2	2.3	2.3	15.8	46.9
Abiotic	192	57.1	6.9	6.2	4.8	0.1

[1]ND - Not determined

fatty acids groups preceding the transport and mineralization of the sucrose.

Samples were also extracted and analyzed by TLC to confirm the disappearance of parent olestra and to examine radiolabeled metabolites present in the culture media. This analysis revealed two major peaks in the samples: parent olestra at R_f 0.85 and a polar metabolite at R_f 0.05. In the biotic treatments, the disappearance of parent was concurrent with the uptake of radioactivity into biomass, evolution of $^{14}CO_2$ and the appearance of a transient polar metabolite (Table 3). Levels of both parent ^{14}C-[sucrose] and ^{14}C-[fatty acid] olestra decreased rapidly over the course of the incubations. After 192 hours, only 5.2% of added ^{14}C-[sucrose] olestra and 1.2% of ^{14}C-[fatty acid] olestra parent could be recovered from the biotic treatments. In comparison, 75.7% of added parent ^{14}C-[sucrose] olestra and 57.1% of ^{14}C-[fatty acid] olestra were recovered after 192 hr from the abiotic treatments. Early in the experiment, recovery of parent was high for all treatments, exceeding 90% for ^{14}C-[sucrose] olestra and 80% for ^{14}C-[fatty acid] olestra. As time progressed, the recovery of parent decreased in the abiotic controls. This loss occurred in the absence of $^{14}CO_2$ production, suggesting that it was not biologically mediated and may have been due to sorption to the glassware. A polar peak of radiolabeled material did appear at the TLC origin, suggesting that chemical hydrolysis and radiolysis of the ^{14}C-test materials may have contributed to this loss.

Concurrent with the loss of the ^{14}C-[sucrose] olestra in the biotic samples was the appearance of a single polar peak with an R_f of 0.05 prior to any significant incorporation into biomass or production of $^{14}CO_2$. This material did not co-chromatograph with any fatty acid standard. Within 1.7 hrs, approximately 6% of the added radioactivity was converted to this material, and after 140 hrs, 20% of the initial radioactivity was localized in this peak. Subsequently, the level of radioactivity in this peak declined by nearly one-half with the evolution of a significant quantity of $^{14}CO_2$. A material with a similar R_f was observed at low levels in the later samples of the abiotic control as well as in the ^{14}C-[fatty acid] olestra biotic and abiotic treatments. Given that this metabolite appeared with both ^{14}C-[sucrose] and ^{14}C-[fatty acid] olestra, it likely contained both carbohydrate and fatty acid moieties. Furthermore, the observation that a similar

Table 4. Incorporation of ^{14}C-[fatty acid] and ^{14}C-[sucrose] olestra into the various biomass fractions of the mixed bacterial culture, AS2.

Test material treatment	Hours of incubation	Percentage of added radioactivity recovered					
		Carbohydrates	Lipids	Nucleic acids	Proteins	Combusted solids	Total incorporation into biomass
^{14}C-[U-sucrose] olestra							
Bioactive	0	0.1	0.3	0.1	0.6	0.1	1.2
	1.7	0.1	0.4	0	0.2	0.1	0.8
	139	3.3	11.5	5.1	2.2	3.5	25.5
	192	7.3	2.2	5.9	1.5	0.4	17.3
Abiotic control	0	0.2	1.6	0.1	0.6	0.1	2.6
	192	0.2	0.5	0.2	0.5	0.1	1.5
^{14}C-[1-fatty acid] olestra							
Bioactive	0	0.2	0.7	0.1	0.1	0.1	1.2
	1.7	1.6	1.5	0.1	0.5	1.2	4.9
	139	0.7	0.6	1.0	0.6	0.4	3.3
	192	3.6	8.8	1.0	1.9	0.5	15.8
Abiotic control	0	0.2	1.6	0.1	0.3	0.1	2.3
	192	0.5	2.2	0.1	1.5	0.4	4.8

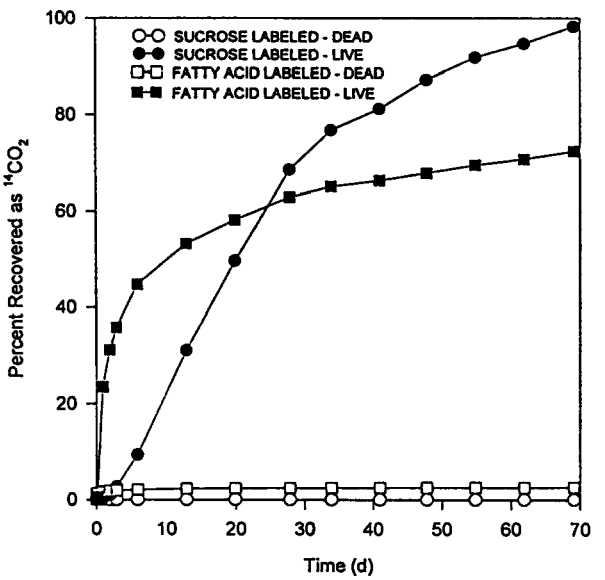


Figure 3. Mineralization of radiolabeled olestra by AS2 Isolate #3 (*Pseudomonas aeruginosa*).

material was formed under abiotic conditions suggested that this material was a lower ester olestra.

Incorporation into biomass was determined by sequentially extracting the solvent extracted cells with a series of reagents to recover various components of biomass. Table 4 shows the results of these analyses. Less than 5% of the radiolabel added to the abiotic controls was recovered in the biomass fractions. The majority was present in the lipid fraction and presumably was parent not recovered in the initial solvent extraction. With the biotic samples incubated with ^{14}C-[sucrose] olestra, 25% of the added radiolabel was incorporated into biomass after 139 hrs. Most of the incorporation was in the lipid and nucleic acid fractions. After 192 hrs, total incorporation was 17.3% and was largely centered in the carbohydrate and nucleic acid fractions. In the case of the ^{14}C-[fatty acid] olestra, significant incorporation (16%) was only observed in the 192 hr sample, where it was concentrated in the lipid fraction.

These combined observations suggest that olestra is initially converted to a more polar material such as a lower ester olestra, which is subsequently broken down into its substituent carbohydrate and fatty acid components, which are incorporated into biomass and mineralized to CO_2. These data agree with the hypothesis that the initial step of olestra metabolism is the cleavage of the fatty acid moieties by esterases. The metabolite that was created during metabolism was transient and did not accumulate in the medium.

The previous experiment utilized a mixed bacterial culture and was relatively short in duration. A longer term mineralization experiment was conducted with *Pseudomonas aeruginosa* (Isolate #3 from culture AS2, Figure 3). More than 98% of the added ^{14}C-[sucrose] olestra was recovered as $^{14}CO_2$ over the 69 d incubation period and more than 72% of the added ^{14}C-[fatty acid] olestra was recovered as $^{14}CO_2$ within the same time frame. Consistent with the previous mixed culture experiments, the mineralization of the ^{14}C-[sucrose] olestra lagged behind that of the ^{14}C-[fatty acid] olestra, further supporting the removal of the fatty acid moieties prior to the degradation of the sucrose moiety. These data demonstrate that the complete degradation of olestra can be carried out by a single bacterial species and that essentially all of the olestra in this system was bioavailable for degradation.

Conclusions

Olestra can serve as a sole carbon and energy source for a wide range of environmentally relevant mixed and pure cultures of bacteria as well as several fungi. Further experiments demonstrated that both the primary and ultimate degradation of olestra occurs relatively rapidly and does not generate any persistent metabolites. The ubiquitous species, *Pseudomonas aeruginosa*, was isolated from activated sludge and was shown to degrade >98% of olestra over a 69 d period. These observations indicate that common microorganisms indigenous to sewage treatment plants and soils are capable of degrading olestra, despite the fact that these organisms have not been previously exposed to olestra. The data firmly demonstrate the inherent biodegradability of olestra and are consistent with previous work demonstrating mineralization in sewage and soil.

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