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BIODEGRADATION CHARACTERISTICS
OF
SUCROSE ESTERS

by

Seng Nio Yap

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A thesis submitted to the Faculty and the Board of Trustees of the Colorado School of Mines in partial fulfillment of the requirement for the degree of Master of Science.

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A B S T R A C T

Rates of ultimate biodegradation of a surface active sugar derivative, sucrose monolaurate, were studied under various conditions using pure and C^{14} -tagged compound. The rates were measured in terms of CO_2 production using ethanolamine as the CO_2 absorber. The results show that the sugar ester is readily biodegradable in synthetic media with river bacteria, river water, and in sludge under aerobic and anaerobic conditions. In synthetic media the biodegradability of the sugar ester is almost comparable to that of glucose, sucrose, and lauric acid, and is superior to the surfactants used in domestic detergents, C_{12} LAS and tergitol 15-S-9.

In the activated sludge, the rate of CO_2 production from the sugar ester is almost as fast as the rate of glucose and sucrose, except at the very early period of incubation.

Complete ultimate biodegradation of the sugar ester is readily achieved in river water and in sludge under aerobic and anaerobic conditions at $27^{\circ}C$.

Studies on temperature effect using synthetic media have shown that temperatures within the range of 15° to $27^{\circ}C$ have little effect on rate. At $5^{\circ}C$, the rate decreases substantially. However,

the rate of the conversion of the 1-C on laurate to CO_2 is quite rapid though much slower than at 15° and 27°C .

Studies on various kinds of sucrose esters with fatty acids chain length ranging from C_{12} to C_{22} show that the ester with C_{12} fatty acid (sucrose monolaurate) displays the highest biodegradation rate. The ester with C_{14} fatty acid, and the mixed ester with C_{16} and C_{18} fatty acids show a slightly slower rate than sucrose monolaurate. A mixed ester of C_{18} , C_{20} , and C_{22} fatty acids shows a very slow rate of biodegradation.

Studies on the mechanism of the degradation of sucrose mono-fatty acid esters using sucrose monolaurate (1-C^{14} on laurate) have shown that the degradation proceeds essentially via hydrolysis of the ester linkage.

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A C K N O W L E D G M E N T S

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I N T R O D U C T I O N

Most household and institutional detergent residues find their way into waste treatment plants and ultimately into natural water. The household detergents available commercially contain an organic surfactant, a builder, usually tripolyphosphate compounds, a filler, and such miscellaneous compounds as optical bleach, perfume, etc. In many sewage treatment plants surfactants and other organic materials are degraded by bacterial oxidation, after which the effluent water is discharged into a stream or river. The bacterial oxidation is accomplished by an activated sludge process. The sludge is a bacterial floc which develops naturally upon aeration of any concentrated nutrient solution under nonsterile conditions. The vigorous aeration in the activated sludge process makes it a good foam generator if surfactant components are present. Excessive foam might lower the plant treatment efficiency. Therefore, surfactants that are resistant to bacterial oxidation or oxidized only at a slow rate may cause difficulties to sewage treatment plants, and will also contribute to river pollution. Because the use of easily biodegradable surfactants in detergents will largely eliminate this problem, a study of the biodegradability of surfactants is of interest to the detergent industry as well as to others concerned with waste-water treatment and water supply.

The study of surfactant biodegradation has assumed importance as a consequence of the chemical revolution which occurred in the detergent industry during the 1950's. At that time the replacement of soap in cleaning and laundry formulations by synthetic detergents became technically and economically feasible as a result of the commercial development of alkylbenzenesulfonate (ABS), a surfactant superior to soap in hard water and also more economical. In a very few years the changeover was essentially complete in the United States, ABS becoming the surfactant in major use. But, unfortunately, this surfactant residue had caused heavy foam in sewage treatment plants and also began to be noticeable in wastewater, treated sewage, and receiving waters because of its resistance to biodegradation.

One solution to the problem of foaming was the development of more biodegradable surfactants, among which the anionic surfactant linear alkylbenzenesulfonate (LAS) comprises the bulk of the present day market. Nonionic surfactants which contain ethylene oxide polymers also comprise a significant amount of the total volume of surfactants produced. Since the use of these two types of surfactant (anionics and nonionics) far exceeded that of all other surfactants in household detergent formulations, biodegradation studies on these anionic and nonionic surfactants were required. A comprehensive discussion of biodegradation studies and of test methods available can be found in Swisher's recent book (1). Methods for biodegradation studies of nonionic surfactants is also described in The Soap and Detergent Association Scientific and Technical Report (2). A review of biodegradation studies on nonionics can be found in Schick (3).

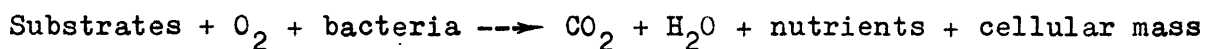
There is no concise definition of biodegradation. In the simplest way biodegradation can be defined as the destruction of chemical compounds by the biological action of living organisms. In the biodegradation of surfactants, the living organisms of interest are the microorganisms present in the various environments receiving our wastewater, among which bacteria are the most important.

The best guidelines to date in the formulation of definitions of biodegradation are the following, as set forth by a special committee on Standard Methods of the Water Pollution Control Federation(4):

Primary Biodegradation: Biodegradation to the minimum extent to change the identity of the compound.

Environmental Acceptable Biodegradation: Biodegradation to the minimum extent necessary to remove such undesirable properties of the compound as foamingness and/or toxicity.

Ultimate Biodegradation: Biodegradation to inorganic end products possibly specified nonuniquely by the reaction of the form:



The reason a concise definition on biodegradation is wanting is that the process is dependent upon a very complex set of variables, as described by Wayman in his recent paper (5); among them are:

1. Concentration and chemical structure of the substrate;
2. Type of the biological system under consideration in terms of aerobic and anaerobic organisms, their viability, and acclimation characteristics of viable species to substrates;
3. Concentration and composition of the organisms;
4. Dispersion and/or coagulation aspects of the system under study;

5. Other physical and chemical factors such as agitation rate, pH, temperature, growth promoters or inhibitors; and
6. Analytical method.

Therefore, the significance attached to the results obtained in studies on biodegradation is dependent upon the intended use.

Methods of assessing biodegradation are arbitrary at best.

Among the types of generalized test employed to date may be generally classified The River Die-Away, Trickling Filter, Activated Sludge, Oxygen-Uptake, CO₂ Generation, Bacterial Growth, and Field Test Results. Some of these methods have legal significance while others are at least condoned by the government (6).

The River Die-Away Method : This method has been discussed in many recent papers (7,8,9). The method involves the inoculation of a source of culture in river water with added surfactant with subsequent measurement of the decay of the surfactant. For anionic surfactants, the measurement is done using the standard methylene blue active substance (MBAS) method. For nonionic surfactants containing polyoxyethylene polymers, cobalt thiocyanate is used. River Die-Away method has been employed in aerobic and anaerobic studies (10).

Standard Method in the United Kingdom : The British Standing Technical Committee (6) has adopted a standard method similar to the River Die-Away test, but with some improvements. A solution containing 10 mg per liter of surfactant is added to BOD dilution water inoculated with 30 mg of air-dried activated sludge per liter. The solution is stirred gently in the dark at 20°C for three weeks. Samples are removed daily and the surfactant concentration remaining

is determined by the Longwell and Maniece methylene blue method (11). This method has also been applied to nonionics using foam measurement and chromatographic techniques (12,13).

S D A Procedure: The Soap and Detergent Association (SDA) has promulgated a test procedure for both ABS and LAS(14) involving both a presumptive and confirmatory test. In the presumptive test a compound's biodegradability is tested with respect to a 90% limitation. Microorganisms are inoculated into a Shake Culture Flask containing a well defined chemical growth medium of the following composition: 3.0 g NH_4Cl ; 1.0 g K_2HPO_4 ; 0.25 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; 0.25 g KCl ; 0.002 g $\text{FeSO}_4 \cdot 6\text{H}_2\text{O}$; and 0.30 g yeast extract in 1.0 liter of distilled water. The concentration of the surfactant is 30 mg per liter. The temperature is 25°C. Two 72-hour adaptive transfers are made prior to the 8-day test. One ml of the 72-hour culture is transferred to 100 ml of fresh medium plus surfactant. The flasks are stoppered with cotton plug and aerated by a reciprocating shaker. Samples are taken at zero time and on the 7th and 8th days. If the biodegradation exceeds 90%, as measured by the MBAS method, no further testing is warranted. If it is less than 80%, it is considered not to be biodegradable. If it falls within 80 to 90%, its biodegradability must be confirmed. In the confirmation test the surfactant must be at least 90% biodegradable to pass. The test employs a modification of the Semi-Continuous Activated Sludge Test.

The Official German Test Method (15,16): This procedure employs the continuous Activated Sludge Procedure and is an official and legal test method under the German law. The law requires that the surfactant tested must meet a standard biodegradability of at least

80% . Sewage containing 20 mg per liter of test surfactant is fed into a 3-liter aeration chamber with overflow into a 2-liter sedimentation chamber. Three hours residence time is allowed to conform to sewage plant practice. Sludge is recirculated from the settler to the aerator by means of airlift. The clarified overflow from the sedimentation chamber, essentially sewage effluent, is composited and analyzed daily for residual surfactant content by the standardized methylene blue analytical method, and the percent reduction of MBAS input is calculated. The daily determinations are repeated over a test cycle of 21 days.

The Bunch-Chambers Method (17): This method permits continuous acclimatization of the organism to the test substrate. Ninety ml of BOD dilution water containing 5.0 mg of yeast extract and 2.0 mg or other suitable amount of test compound is inoculated with 10 ml of settled sewage. For each test material, three of the above described systems are prepared in three separate flasks. The flasks are stored loosely capped or with cotton plug for 7 days. Weekly transfers of 10 ml are made from each flask to another containing 90 ml of BOD dilution water, yeast extract, and surfactant for 3 weeks. Each subculture is analyzed at the end of 7 days for the amount of test substrate remaining.

Warburg Oxygen Uptake Method (18,19): This method involves the inoculation of organisms and surfactant into a system of constant volume and in the presence of an excess of oxygen. One measures the amount of oxygen depletion in a closed system as a function of time. Any CO_2 evolved in the process is absorbed in a KOH well.

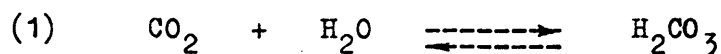
Carbon Dioxide Generation Method: In this method the biodegradability of a compound is measured in terms of the rate of CO_2 generation. The test substance is incubated in BOD dilution water or other growth media plus bacteria. CO_2 -free air is passed through the system with constant flow rate to remove any CO_2 that is generated in the system. The CO_2 is trapped in a CO_2 absorber, such as $\text{Ba}(\text{OH})_2$ solution (20,21,22); NaOH solution (20); phenethylamine(23); hyamine (24); and ethanolamine (25,26). By sampling the gas stream as a function of time, the rate of CO_2 production during the biological oxidation can be determined. Any of the CO_2 absorbers mentioned above may be used to determine C^{14}O_2 and CO_2 , but the organic amines dissolved in methanol are preferable when both C^{14}O_2 and CO_2 have to be determined. In this case the absorption can be accomplished in a counting vial, and the radioactivity can be determined directly by adding a scintillation solvent to the absorption vial. The total amount of CO_2 can be determined by boiling the amine carbonate with HCl , the CO_2 liberated being adsorbed in a known amount of $\text{Ba}(\text{OH})_2$ solution. The excess of $\text{Ba}(\text{OH})_2$ is then back titrated with standard HCl .

Bacterial Growth Method: In this method the biodegradability of the test compound is determined by measuring the rate of bacterial growth and substrate disappearance. Numerical values are assigned using a mathematical model which relates the rate of bacterial growth to the substrate consumption (27).

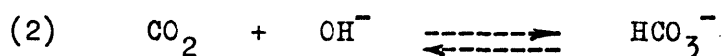
The analytical methods mostly used to follow the course of the degradation of anionic and nonionic surfactants have certain short-

coming : The measurement of foaming properties (2,12,13,28,29) and surface tension (30,31) reveal only a change in surface activity, which is not necessarily correlated with the extent of biodegradation process. The cobalt thiocyanate colorimetric method (2,32,33,34) for surfactants containing ethylene oxide polymers, provides at best only information on the degradation of the ethylene oxide chain. The MBAS method for anionic surfactants (35,36,37) measures any materials containing a single strong anionic center strong enough to form a stable salt with the methylene blue cation, and at the same time containing a hydrophobic group sufficiently lipophilic to enable the salt to be extracted to an organic layer, from the aqueous phase. Not specific, and subjected to many interferences, it is not quite suitable for sewage analysis. Many methods, such as IR (2,38) and TLC (2,12,13) require an isolation procedure prior to actual measurement, which introduces some uncertainty as to the completeness of the extraction of intermediates. The oxygen-uptake methods (1,p.140; 18,19,39) have some uncertainty caused by endogenous respiration: In the absence of added food, endogenous respiration of living bacteria is continually taking place, resulting from their continuing oxidation of those neighboring cells that have died. Thus, the O_2 taken up by the system includes not only that for the oxidation of the test compound but also whatever is utilized in the endogenous respiration as well. Customarily, a blank is run in conjunction with the test sample, the O_2 consumption being subtracted. But, as the blank and fed samples constitute different environments for bacteria, the endogenous process may not be parallel from the one to the other, and the net results might therefore be in error. This uncertainty might reach up to as high as

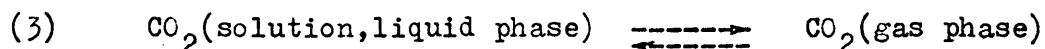
20%, depending on the conditions of the test. The O_2 -uptake method can not be applied as such to mixtures of test compounds with ordinary foods, such as occur in sewage, where the oxygen consumption for the food would mask that for the test compound. The CO_2 generation method is also uncertain due to endogenous respiration as described in the O_2 -uptake method; but, this uncertainty can be overcome easily by employing C^{14} -substrate. However, there are other disadvantages of the method that have to be considered: In the CO_2 generation method the rate of CO_2 produced from the aqueous biological system is measured in terms of the CO_2 trapped from the gas stream. But, in any aqueous solution containing CO_2 , reactions of the molecular CO_2 with water and hydroxide ion will occur



followed by fast interconversions among the carbonate system, H_2CO_3 , HCO_3^- , and $CO_3^{=}$.



Because the solution is in contact with the gaseous phase, another reaction, the transfer of molecular CO_2 between phases, also occurs



The relative significance of the above reactions will depend on pH as well as other factors. Reaction (2) becomes significant only at a higher pH. Miller and others (40) have studied the rate of the above reactions in sea water. By using the rate constants they

obtained, they have shown that, at pH 8.2, the hydroxide-ion reaction carries about 48% of the CO_2 transferred, compared to 52% for hydration-dehydration.

From the above description it is obvious that, when CO_2 is purged from an aqueous biological system, the rate of the CO_2 removal from the system depends upon the variables: pH, temperature, flow rate of the air stream passing through the system, etc., as well as the rate of CO_2 production itself. Under identical conditions the amount of CO_2 being purged will be proportional to the rate of CO_2 production. The CO_2 generation method is therefore useful for comparing the rates of biodegradation of different substrates at the same conditions. However, at pH greater than 7 such as in river water (pH about 8) a considerable amount of CO_2 will be retained in solution as HCO_3^- ; hence, the rate and the extent of biodegradation measured as CO_2 trapped from the gas stream will differ significantly from the actual values. In such cases, the solution should be sampled in conjunction with the sampling of the gas stream, and the amount of CO_2 and HCO_3^- in solution determined.

The CO_2 generation method has many advantages over others: It can provide information on whether or not the substrate is completely biodegradable to CO_2 and H_2O , while other methods merely measure the disappearance of the original compound. This method is also more accurate and sensitive, when C^{14} -substrate is used. For C^{14}O_2 determination, use of organic amine in methanol would make it an extremely quick and easy method. The CO_2 generation method might also provide information on mechanism when substrate with characteristic tagging is used. This method can be used to study C^{14} -compound in a batch

sludge system without any significant interference.

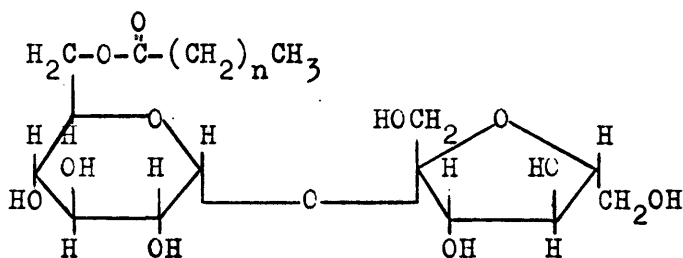
Though the $C^{14}O_2$ generation method is probably the most reliable, it has not been used in the biodegradation studies of LAS. Most of the analytical studies of biodegradability of LAS used the MBAS and O_2 -uptake method (1,p.340). Therefore, though the biodegradabilities of various kinds of LAS have been intensively studied, the extent of LAS undergoing ultimate biodegradation is not very well known. Ryckman's (41) study of 2-sulfophenyloctane (-decane, and -dodecane) using Warburg respirometer, MBAS, IR, and the formation of inorganic sulfate, showed that complete degradation of the LAS was achieved. On the other hand, Borstlaps (42) in studying the intermediates obtained from biodegradation of LAS-DOBS C300 and LAS-DOBS JN, found a considerable amount of intermediates which showed no significant change after the first few weeks up to 18 weeks.

Although the complete oxidation of LAS to CO_2 and H_2O is still in question, LAS comprises the bulk of the present day market. This is mainly due to its excellent detergency, low price, and more readily biodegradable as compared to ABS. Though LAS is said to be biodegradable, the rate of degradation is rather slow. Therefore, the development of more readily biodegradable detergents with competitive price and detergency is desirable.

Recently, attention was drawn to a surface active sugar derivative: the sucrose fatty acid esters, with fatty acids ranging from C_{12} to C_{20} . It has been demonstrated that the sucrose ester series performed at least as well and in some instances better in detergency than the standard anionic surfactants; the saturated esters gave generally better performance than the unsaturated esters(43). These

sugar esters might be expected to undergo ultimate biodegradation very readily, because both of the esters' components, sucrose and fatty acids, are known to be readily degraded to CO_2 and H_2O .

The preferred structural formula of the mono-ester is:



Sucrose esters differ in many respects from most surfactants. They are neutral and non-irritating even to mucous tissues, and proved to be non toxic and digestible (44). Isaac and Jenkins (45) have shown that sucrose esters are fermented rapidly. Kulovana and Pitter (46) have studied the biodegradation of various kinds of sucrose esters in an activated sludge system. The biodegradability was determined by measuring the biological oxygen demand using a respirometer. They reported that sucrose esters are degraded at a faster rate than LAS. Wayman and Robertson (10) indicated that sucrose esters possess most desirable properties in aerobic and anaerobic environments, claiming that under aerobic conditions the sugar esters seem to offer the most promising results among the various classes of surfactants in the curtailment of water pollution from detergent residues in both surface and ground water.

Because the discharge of crude or partially treated sewage into a river constitutes by far the commonest form of river pollution, the study of biodegradation of surfactants by river bacteria is as important as the study of biodegradation in an activated sludge system. Very little work has been done concerning biodegradation of sucrose

esters by river bacteria. Weil and Stirton(47) had studied the biodegradation of sucrose monopalmitate in river water. The biodegradability was determined by measuring the change in surface tension of the solution. This method was not very reliable because the organic substances present in the river water and the biodegradation intermediates might interfere; quite frequently, bacteria could produce surface active compounds. A study of biodegradation using the CO_2 generation method, especially using C^{14} -substrate, would provide more reliable information.

It was the purpose of this work to study the biodegradation of sucrose esters by river bacteria in synthetic media and river water, using the CO_2 generation method. Ethanolamine in methanol was used as the CO_2 absorber. Sucrose monolaurate(C^{14} UL sucrose)diluted with chromatographic-purified sucrose monolaurate was used in most of the biodegradation studies. Sucrose monolaurate (1- C^{14} on laurate) was used in studying the mechanism. The rates of C^{14}O_2 and total CO_2 production were determined. Among the variables studied were the effect of temperature, flow rate of the air stream, ester concentration, and kinds of esters. In addition, the biodegradation of sucrose monolaurate in activated sludge and anaerobic sludge was also studied. For comparison, experiments were performed with the following biodegradable compounds: sucrose (C^{14} UL), glucose (C^{14} UL), lauric acid, LAS and tergitol 15-S-9*.

* Tergitol 15-S-9 is a polyoxyethylene alcohol surfactant which is a major ingredient in some of the present-day liquid dishwashing detergents. The biodegradability of a similar compound polyoxyethylene stearyl alcohol had been studied very recently by Nooi and others (48) using a similar CO_2 generation method; the biodegradability was found to be comparable to slightly branched alkylbenzenesulfonate.

M A T E R I A L S A N D M E T H O D S

The materials, experimental equipment, the procedure for biodegradation studies, and the analysis of carbon dioxide are described in the following section.

M a t e r i a l s

Reagents

All materials not specifically discussed below were "analytical grade" materials.

Sucrose Monolaurate: FG, SEL-1, FG sample # 8264, Colonial Sugar Company (C. S. C.); COD = 1.73 g/g *

Sucrose Monomyristate: Purified grade, SEM-1, P-1 sample #8266, C. S. C.; COD = 1.80 g/g *

Sucrose Mono-Stearate, Arachidate, Behenate: Refined, SESAB-1, R-1 sample # 8574, C. S. C.; COD = 2.03 g/g *

Nitto Ester: Contained 70% sucrose palmitate, 30% sucrose stearate; 70% monoester and 30% diester. P-1570, Dai-Nippon Sugar MFG Co LTD; COD = 1.87 g/g *

Pure Sucrose Monolaurate and Sucrose Monomyristate: These pure esters were obtained by purifying the above sucrose monolaurate and

* COD = Chemical oxygen demand determined according to the method described in the Standard Methods for Examination of Water and Wastewater (35,p.495).

sucrose monomyristate using absorption chromatographic method described by Hiroyuki Mima (49). Sixty to 200 mesh chromatographic grade silica gel was used as the absorbent; 25% absolute pure ethyl alcohol in spectra AR benzene was used as the eluent. The size of the column: 1 x 26 in.

Sucrose Monolaurate (C¹⁴ UL sucrose): Specific activity = 8.09 x 10⁶ dpm/mg; radio purity = 99%; International Chemical & Nuclear Corporation (I. C. N. C.).

Sucrose Monolaurate (1-C¹⁴ on laurate): Sp activity = 3.98 x 10⁶ dpm/mg; radio purity = 99%; I. C. N. C.

Sucrose (C¹⁴ UL): Sp activity = 6.2 x 10⁷ dpm/mg; radio purity = 99%; I. C. N. C.

Glucose (C¹⁴ UL): Sp activity = 4.8 x 10⁷ dpm/mg; New England Nuclear.

Tergitol 15-S-9: mol.wt = 625, COD = 2.10 g/g*; Union Carbide Corporation.

LAS: Lot No 1-1; linear alkylate sulfonate, contained 60.8% LAS; 36.1% Sodium sulfate; 0.4% free oil; 2.7% water; Eq wt = 348; Soap and Detergent Association.

Lauric Acid: "Eastman grade," Eastman Organic Chemicals.

Ethanolamine in Methanol: This was prepared by dissolving ethanolamine (OR, Mallinckrodt) in absolute methanol in the volume ratio of 20 to 80, in a CO₂-free glove bag. This solution was stored in a 25-ml burette assembly-automatic, with 2-l bottle reservoir, and protected from the atmospheric CO₂ by CaCl₂ tubes filled with ascarite.

* see page 14

Ba(OH)₂ 0.025 N: This solution was prepared by dissolving 7 g Ba(OH)₂ in 1800 ml of distilled water (previously boiled to reduce the amount of dissolved CO₂). It was stored in the same way as the ethanolamine in methanol.

Scintillation Solvent: Four g PPO and 100 mg POPOP in 1 liter toluene (Scintill AR, Mallinckrodt). For radioactivity determination, 10 ml of this solution was added to 0.5 ml sample plus 10 ml methanol.

Synthetic Media: Stock solution of synthetic media was prepared by dissolving 5.75 g Na₂HPO₄; 3.0 g KH₂PO₄; 0.5 g NH₄Cl; 2.0 ml of 22.5 g/l MgSO₄ · 7H₂O; 2.0 ml of 0.25 g/l FeCl₃ · 6H₂O; 0.5 ml of 27.5 g/l CaCl₂ in 4 liters of distilled water. pH = 7.0 ± 0.1. After being sterilized at 250°F for 15 min, this solution was stored. For biodegradation studies, 250 ml of this solution was used for each 1250 ml culture.

Stock Solution of Substrate: Accurately weighed test substrate was dissolved in distilled water and diluted to the mark in a 500-ml volumetric flask. This solution was sterilized by filtering it through a 0.15-μ millipore filter under slightly reduced pressure. It was then stored in a well stoppered sterile Erlenmeyer flask. For biodegradation studies in river water and activated sludge, 1200 ppm of stock solutions were prepared; 25 ml of the solution (measured by a volumetric pipet) was used to make 1500 ml of 20 ppm solution. Solutions of sucrose esters were always freshly prepared to avoid hydrolysis. Solutions of Nitto ester and sucrose mono-stearate, arachidate, behenate were previously heated below 70°C for about 45 min to bring them into solution before filtration.

The C¹⁴-tagged sucrose esters were diluted 40 to 100 times by

the chromatographic purified sucrose monolaurate. The sucrose($C^{14}UL$) was diluted about 300 times by GW pure sugar. The glucose($C^{14}UL$) was diluted about 100 times by reagent grade glucose.

Seeds

S_6 : Isolated from Clear Creek, close to Illinois St. in Golden, Colorado. It was grown in Bacto B_3 nutrient broth and then stored in nutrient agar in the refrigerator.

S_{13} : Isolated from South Platte River, under 46th Ave. Viaduct in Denver, Colorado. It was grown and stored in the same way as S_6 .

S_0 : Isolated from the activated sludge of the sewage treatment plant of Coors Adolph Co in Golden, Colorado. Ten ml of the sludge was added to 90 ml synthetic media containing about 20 ppm of the test substrate. The culture was grown at room temperature. Weekly transfers of about 5 ml were made from the culture to another flask containing 95 ml of synthetic media and 20 ppm of test substrate. For biodegradation studies at lower temperature, the cultures were grown at the desired temperature for about one week prior to the biodegradation studies. For tergitol and LAS the cultures were aerated continuously. With sucrose ester growth was so easy that aeration was not necessary.

S_6 and S_{13} were acclimatized to the test substrate in the same way as S_0 , unless otherwise stated. At the first inoculation of the seeds from the agar slant, 1 mg of yeast extract was added to 100 ml of the culture. Two to 3 day-old bacteria were used to inoculate the solution used in the biodegradation studies.

River Water, Activated Sludge, and Anaerobic Sludge

River Water: The water from Clear Creek and South Platte River were taken from the same places as described in S₆ and S₁₃. The water was freshly sampled. After the suspended materials had been allowed to settle for about 2 hours, the decanted water was filtered through glass wool. CO₂-free air was bubbled through it for about 2 hours to purge most of the CO₂ from solution. The pH of the water before and after the CO₂ was purged was 7.9 and 8.4 respectively. One thousand four hundred and seventy five ml of the river water was used for each study.

Activated Sludge and Anaerobic Sludge: The activated sludge was taken from the activated sludge of Coors' sewage treatment plant, and the anaerobic sludge from the anaerobic digester. In order to simulate sewage treatment conditions, the sludges were used immediately after they were taken, without any filtration or decantation. The sludges were shook vigorously before pouring into each incubation flask. The amount of activated sludge and anaerobic sludge used for each incubation flask was 875 ml and 150 ml respectively.

A p p a r a t u s a n d P r o c e d u r e

The apparatus and procedure for biodegradation studies, which consisted of aerobic and anaerobic studies, and the apparatus and procedure for analysis of carbon dioxide are described.

A. Aerobic Biodegradation Studies

Incubation Apparatus: The incubation unit, consisting of incubation flask, condenser, and absorption vessel is illustrated in Figure 1a. A 2-l wide-mouth Erlenmeyer flask was used as the incubation

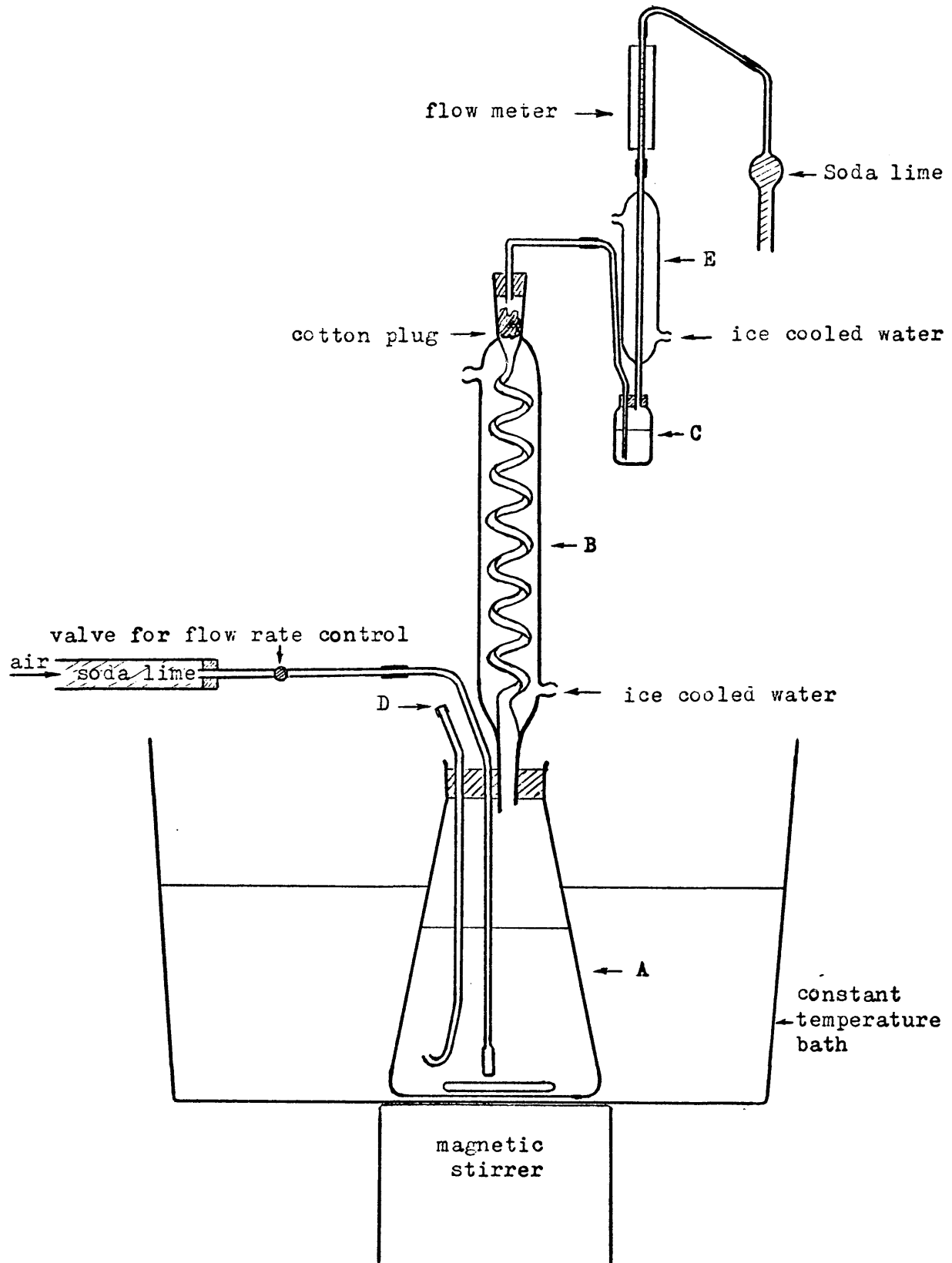


Figure 1a. Incubation apparatus for aerobic studies.

flask (A). An 8-in. Graham coiled reflux condenser (B) cooled by ice water was used as a cooler. A liquid scintillation counting vial was used as the absorption vessel (C). The incubation flask was covered by a # 13 rubber stopper equipped with the Graham condenser, a 40-to 60- μ fritted glass tubing connected to CO₂-free air*, and a thick-wall 2.5-mm ID glass tubing. The upper end of the tube is covered by a rubber lid (D). A 2.5-in. teflon-coated magnetic stir bar was used for agitation. The incubation flask was immersed in a 18-in. plastic constant temperature bath. A magnetic stirrer was set under the bath. The absorption vessel was covered by a # 1 rubber stopper equipped with a 0.3-mm ID thick-wall capillary tube and a 5-in. reflux condenser (E). The capillary tube was connected to the Graham condenser by a teflon and glass tubing. The condenser (E) was connected to a flow meter which was connected to a CaCl₂-tube containing soda lime.

The constant temperature bath could hold 4 incubation flasks; 2 baths were prepared for these studies so that 8 experiments could be run at the same time.

Procedure: The condenser and the incubation flask and its contents were sterilized at 250°F for 15 min. (except for the activated sludge experiments). Then 1250 ml of nutrient solution containing 10 to 40 ppm of the test substrate was introduced into the incubation flask. A blank without any test substrate was always run in conjunction with the test samples. In the case of synthetic media where the untagged CO₂ had to be determined, CO₂-free air with a flow rate of about 20 ml per min was passed through the system for about 15 hours to purge the CO₂ from the system. After the temperature of the solu-

* N₂ and O₂ gas in the ratio of 4 to 1, filtered by a 1x20-in. soda lime tube.

tion was attained, the flow rate of the air stream was adjusted to about 8 to 10 ml per min (unless otherwise stated). Then 2.5 ml of acclimatized bacteria suspension (contained about 10^7 bacteria per ml as determined by the pour plate method) was injected to the incubation flask through the rubber lid D. The empty absorption vessel was replaced by an absorption vessel containing 11 ml of ethanolamine in methanol. In the case of activated sludge and river water, the temperature of the system was adjusted before the substrate was added; the air stream was trapped immediately after the substrate was introduced.

The air stream was sampled as a function of time. Sampling was performed as follows:

The absorption vessel was pulled down from its stopper so that the capillary tube was just above the absorbing solution. The capillary tube was rinsed with enough methanol to bring the absorbing solution back to the original volume. The absorption vessel was then removed and tightly closed, being replaced immediately by a new vial containing absorbing solution. This operation was performed in about 10 seconds to avoid as much contamination as possible from atmospheric CO_2 . The amount of CO_2 from the incubation system which was carried by the gas stream during the replacement of the absorption vial could be neglected.

For the determination of C^{14}O_2 in the absorption vial, 10 ml of scintillation solvent was added to the absorption vial under a stream of CO_2 -free air. The counting efficiency for C14 in this solution was about 60%. The counting was done using a Beckman LS-133 liquid scintillation counter. It was followed by subsequent determination

of total CO_2 whenever necessary.

In some of the experiments, in conjunction with the sampling of the air stream, the solution was also sampled to determine the amount of the CO_2 and HCO_3^- remaining in the solution. The sampling and determination of CO_2 and HCO_3^- (applied only to carbon-14) was performed as follows:

Twenty five ml of the solution were sampled from the incubation flask through the rubber lid D by means of a hypodermic syringe. The solution was introduced into a dry 25-ml volumetric flask. After the flask was filled to the mark, 1 drop of saturated HgCl_2 solution was immediately added to inhibit metabolism. The solution was then introduced into a 125-ml Erlenmeyer flask. The volumetric flask was rinsed 3 times with 10 ml distilled water. The Erlenmeyer flask was provided with a rubber stopper equipped with an inlet and an outlet glass tubing. The inlet tube was connected to an air source and the outlet tube to a CO_2 absorbing system. Two ml of 3 N HCl was injected through the rubber stopper, and the CO_2 purged. The radioactivity found in the absorbing solution was related to the amount of C^{14}O_2 and $\text{HC}^{14}\text{O}_3^-$ in solution.

At the end of the biodegradation experiments HCl was injected into the incubation flask to acidify the solution to pH 1; the CO_2 was then purged and determined.

To obtain radio-active material balance, the radioactivity remaining in the solution at the end of the experiments was determined. The sludge was homogenized by an ultrasonic device before sampling.

B. Anaerobic Biodegradation Studies

Incubation Apparatus: The apparatus used for anaerobic studies was similar to that of the aerobic studies, except that a 250-ml Erlenmeyer flask was used for the incubation flask, and it was provided with an electrode to measure the E.M.F. of the solution (see Figure 1b). As the purging gas, oxygen-free N_2 gas* was used.

Procedure: The procedure was similar to that of the activated sludge described in the aerobic studies, except for a few cases mentioned below. Before the test substrate was added to the sludge, oxygen-free gas was passed through the system. If the E.M.F. of the sludge indicated a value below -200 mv, 5 ml of 1200 ppm of the test surfactant solution (measured by a volumetric pipet) was added. The gas stream was immediately trapped.

C. Determination of Total Carbon Dioxide

Apparatus: The apparatus used in the determination of total CO_2 was similar to that of the incubation apparatus. It consisted of reaction flask, cooler, and absorption vessel as illustrated in Figure 1c. A 500-ml flat-bottom bottle was used as the reaction flask (A). A 12-in. Graham coiled reflux condenser was used as the cooler (B). A 23 x 150-mm test tube was used as the absorption vessel (C).

* The N_2 gas was passed through a series of three 500-ml gas washing bottles with fritted glass tubing, each bottle containing granular Zn metal and chromous acid prepared as follows: 75 g of 20-mesh metallic Zn treated with 50 ml of 3 N HCl and stirred vigorously for 30 sec. Then 2.5 ml of saturated $HgCl_2$ solution diluted to 50 ml was added, and the mixture stirred for 3 min; the Zn was washed by decantation and placed in the washing bottle. Fifty g of $CrK(SO_4)_2 \cdot 12H_2O$ dissolved in 250 ml of water was added to the washing bottle together with 10 ml of 5 N H_2SO_4 . The O_2 in the solution was purged, and the solution was left to stand over night.

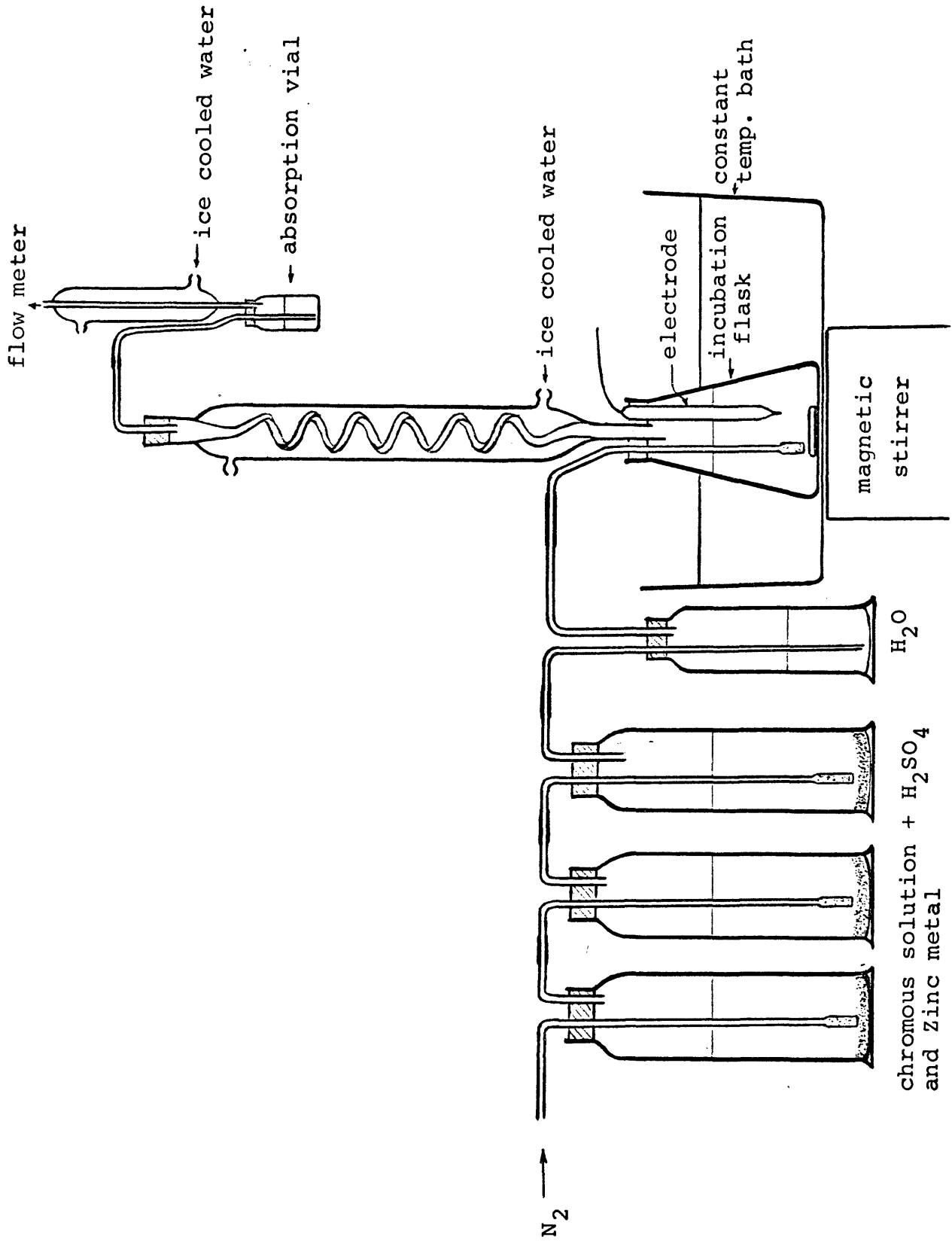


Figure 1b. Incubation apparatus for anaerobic studies.

The reaction flask was provided with a # 10 rubber stopper equipped with the Graham condenser and a 5-mm ID glass tubing which was connected to a source of CO₂-free air*. A 3/4-in. teflon-coated magnetic stir bar was used for agitating the reaction mixture. The reaction flask was put on a magnetic stirrer hot plate. The absorption vessel was provided with a 3-hole # 4 rubber stopper equipped with a 40-to 60- μ fritted glass tubing connected to a 3-way stopcock which was connected to the top of the Graham condenser; a 10-ml burette; and a glass and tygon tubing connected to a tube containing soda lime. The absorption vessel was provided with a 1/2-in. teflon-coated magnetic stir bar.

Procedure: The reaction flask was filled with 15 ml of 3 N HCl and 60 ml of distilled water. The flask was stoppered and a stream of CO₂-free air with a flow rate of about 200 ml per min was passed through the system to purge any CO₂ present in the system. Then the vial containing CO₂ and ethanolamine was introduced into the reaction flask immediately after the lid was opened. The flow rate of the air stream was reduced to about 30 ml per min. The fritted glass tubing was then dipped into the absorption vessel containing 20 ml of 0.025 N Ba(OH)₂ solution and 3 drops of 0.1% phenolphthalein. The reaction mixture was boiled gently for 15 min. (After the mixture was boiling, the flow rate of the air stream was increased to 50 to 70 ml per min.) Then the excess of Ba(OH)₂ was back titrated slowly with 0.05 N HCl. After the end point was nearly reached, the flow rate of the air stream was reduced to about 20 ml per min, and the Ba(OH)₂ adsorbed on the fritted glass tubing was washed out as follows: The stopcock(F)

* Compressed air passed through soda lime and Ba(OH)₂ solution.

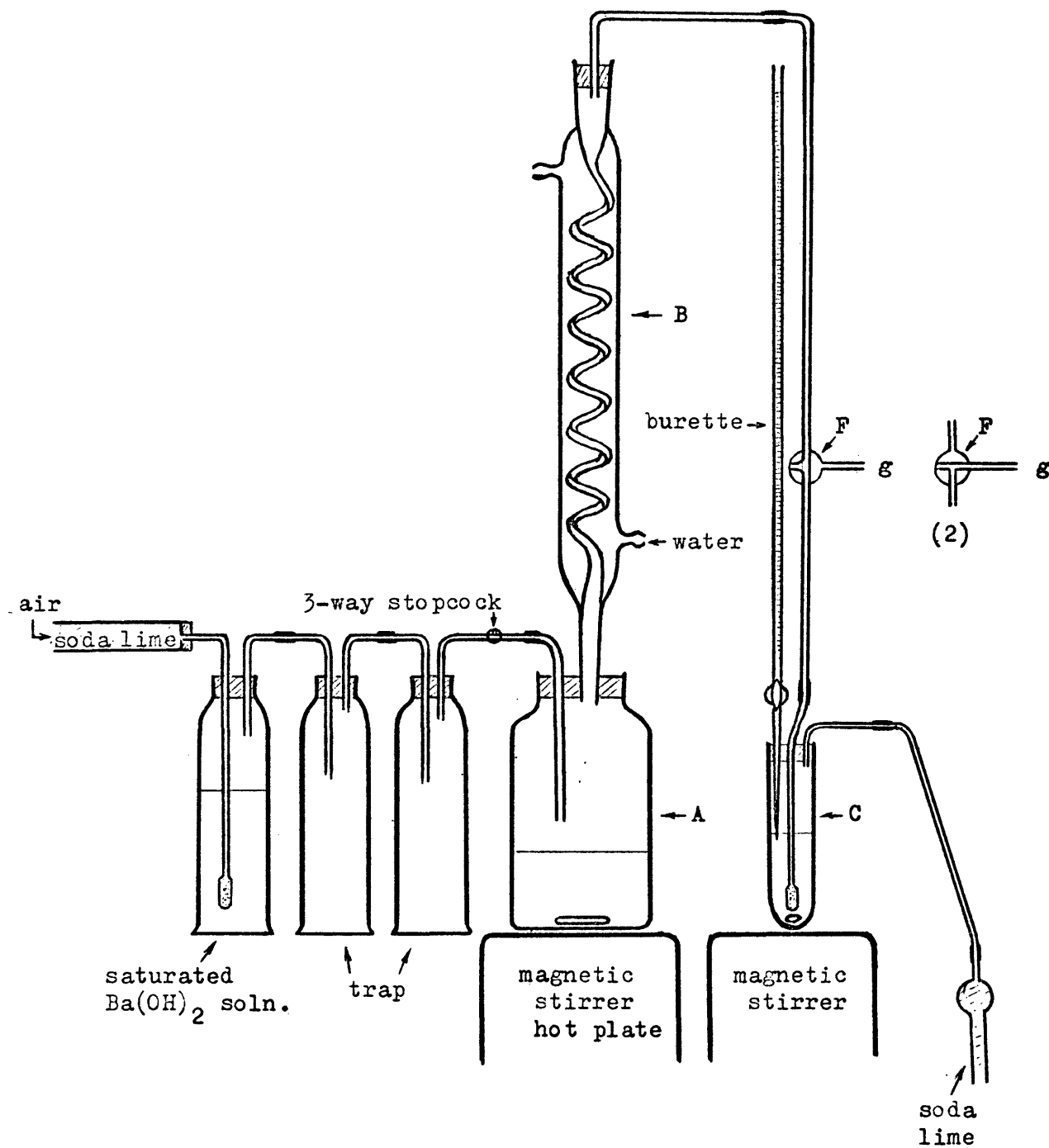


Figure 1c. Apparatus for the determination of CO_2 .

was turned to position 2. About 2 ml of the solution from the absorption vessel was drawn into the fritted glass tubing through g. Then the stopcock was turned back to the original position. The solution in the fritted glass tubing blew back to the absorption vessel and the color of the indicator turned to dark pink again; one or 2 drops of HCl was needed to bring the color back to light pink. The washing of the fritted glass tubing was repeated 5 times. Then the HCl was added drop by drop until the color of the indicator just disappeared. The washing and the titration near the end point should be done quickly, because the pink color will soon reappear, and the results will thus be too low. With enough practice this method did not produce an error in excess of 5% as long as the amount of CO₂ did not go below 2 mg. For CO₂ of about 1 mg, the error may attain a value of 10% or higher. For CO₂ of about 5 mg or more the error was about 2% or less. Known amount of Na₂CO₃ was used as the CO₂ source in testing the accuracy of this method.

The method used in these studies was checked against glucose (C¹⁴ UL) diluted with reagent grade glucose; the total CO₂ and C¹⁴O₂ generated were determined as a function of time (see Appendix I).

Biodegradation Studies Performed

- A. Preliminary examination of the biodegradability of sucrose monolaurate.
 - A-1. Pure sucrose monolaurate in synthetic media with different seeds, S₆, S₁₃, and S₀.
 - A-2. Pure sucrose monolaurate in synthetic media compared to glucose.

- A-3. Sucrose monolaurate (C^{14} UL sucrose) in synthetic media compared to sucrose (C^{14} UL).
- A-4. Pure sucrose monolaurate in synthetic media compared to lauric acid.
- B. The effect of flow rate of the air stream on rate of CO_2 production.
- C. The effect of ester concentration on rate of CO_2 production.
- D. The effect of temperature on rate of CO_2 production.
- E. Sucrose esters with fatty acids of different chain length ($C_{12}-C_{22}$).
- E-1. Pure sucrose monolaurate and sucrose monomyristate.
- E-2. Commercial sucrose monolaurate; sucrose monomyristate; sucrose mono-stearate, arachidate, behenate; and Nitto ester.
- F. Sucrose monolaurate (C^{14} UL sucrose) in river water from Clear Creek; sucrose monolaurate (C^{14} UL sucrose) and sucrose monolaurate (1- C^{14} on laurate) in South Platte River water.
- G. Sucrose monolaurate in activated sludge.
- G-1. Sucrose monolaurate (C^{14} UL sucrose) and sucrose monolaurate (1- C^{14} on laurate).
- G-2. Sucrose monolaurate (C^{14} UL sucrose) compared to glucose (C^{14} UL) and sucrose (C^{14} UL).
- H. Sucrose monolaurate (C^{14} UL sucrose) and sucrose monolaurate(1- C^{14} on laurate) in anaerobic sludge.
- I. Sucrose monolaurate in synthetic media compared to C_{12} LAS and tergitol 15-S-9.
- J. Study on the mechanism using sucrose monolaurate(1- C^{14} on laurate) in synthetic media.

RESULTS AND DISCUSSION

Results of Biodegradation Studies in Terms of CO₂ Generation

A. Preliminary Examination of the Biodegradability of Sucrose Monolaurate

A-1. Pure Sucrose Monolaurate in Synthetic Media with Different Seeds.

The results are shown in Figure 2a and Appendix II, table 2a.

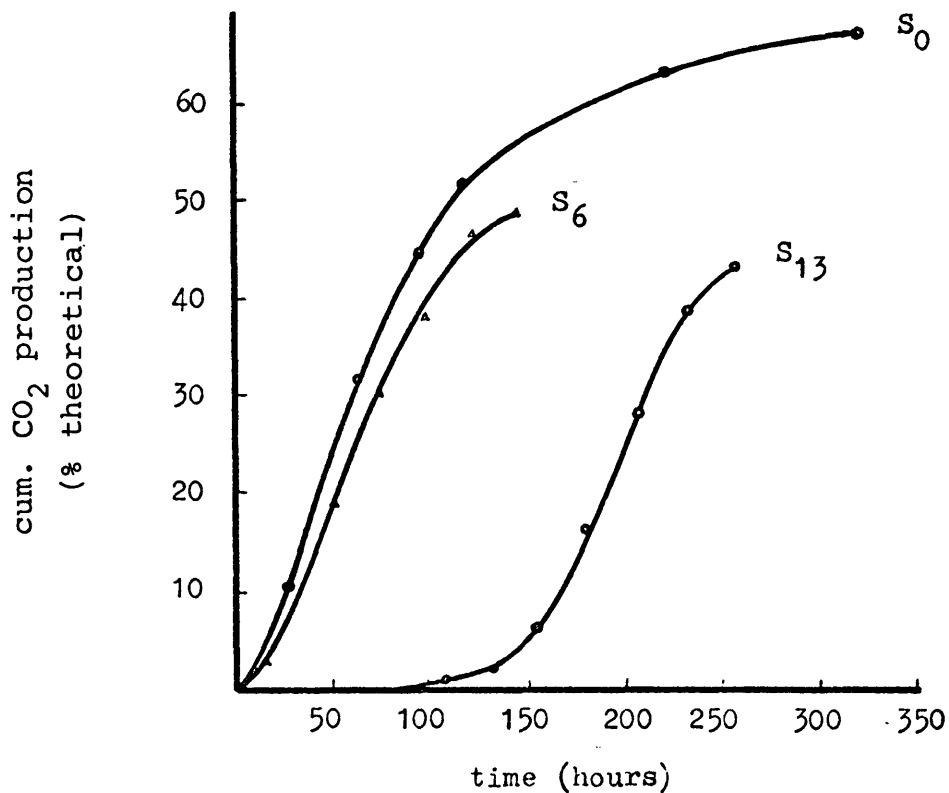


Figure 2a. Rate of CO₂ production from the degradation of sucrose monolaurate in synthetic media with S₆, S₁₃, and S₀; at 20 ppm, 27°C, pH 7.1

A-2. Pure Sucrose Monolaurate and Glucose in Synthetic Media.

The results are shown in Figure 2b and Appendix II, table 2b.

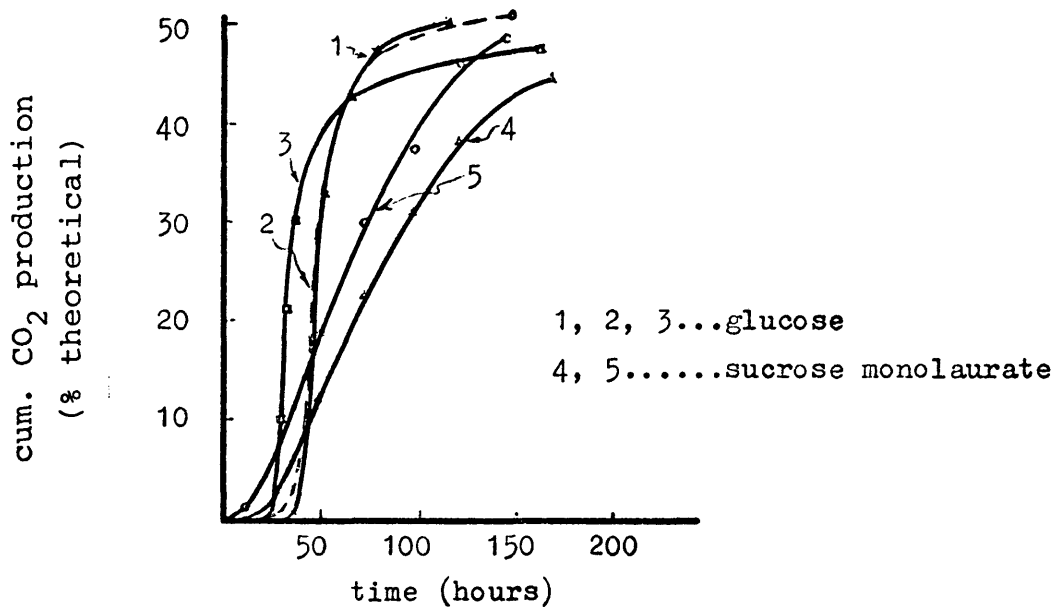


Figure 2b. Rate of CO₂ production from the degradation of sucrose monolaurate and glucose in synthetic media with S₆, at 20 and 23 ppm respectively; 27°C, pH 7.1

A-3. Sucrose Monolaurate (C¹⁴ UL sucrose) and Sucrose (C¹⁴ UL) in Synthetic Media.

The results are shown in Figure 2c and Appendix II, table 2c.

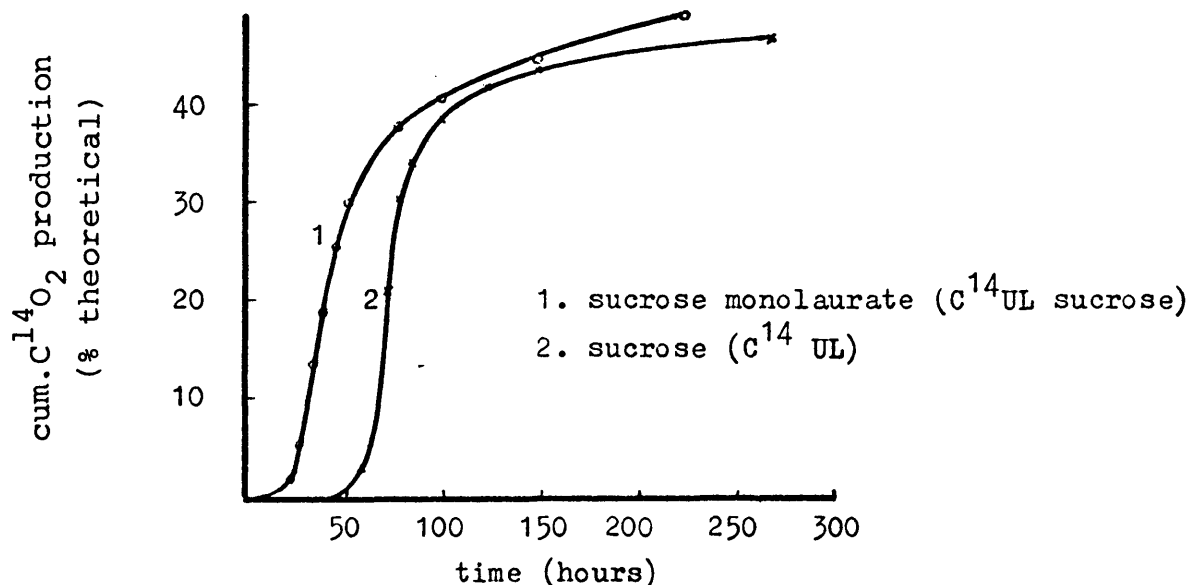


Figure 2c. Rate of C¹⁴O₂ production from the degradation of sucrose monolaurate (C¹⁴UL sucrose) and sucrose (C¹⁴UL) in synthetic media with S₆, at 20 ppm, 27 C, pH 7.0

A-4. Sucrose Monolaurate (C^{14} UL sucrose) and Lauric Acid in Synthetic Media.

In this experiment the rate of biodegradation of the laurate portion of the sucrose ester was compared to lauric acid. Because sucrose monolaurate (C^{14} UL laurate) was not available for this work, sucrose monolaurate (C^{14} UL sucrose) was used. The amount of $C^{14}O_2$ and total CO_2 (tagged and untagged) was determined. The amount of CO_2 produced from the laurate portion was obtained by difference (total $CO_2 - C^{14}O_2$; see table 2d in Appendix II). The results are shown in Figure 2d.

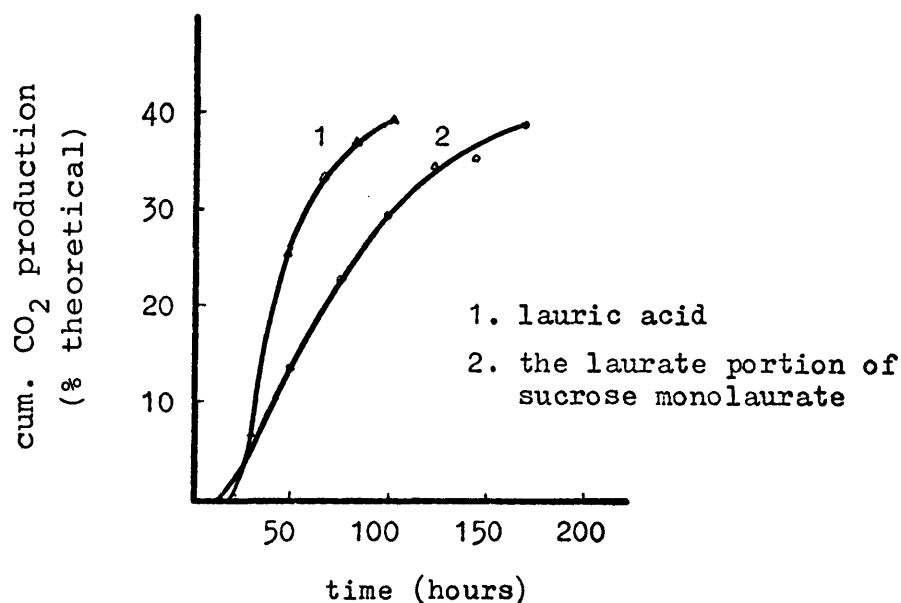


Figure 2d. Rate of CO_2 production from the degradation of lauric acid and the laurate portion of sucrose monolaurate in synthetic media with S_6 , at 20 ppm, $27^\circ C$, pH 7.0

Figures 2a - d show the biodegradability of sucrose monolaurate. Figure 2a shows the results of biodegradation of purified sucrose monolaurate by seed isolated from different natural media. The results have shown that S_6 (from Clear Creek) produces behavior similar to S_0 (from activated sludge). Hence, river water bacteria can easily biodegrade sucrose monolaurate. However, certain types of river bacteria (S_{13} from South Platte River) apparently require different adaptation periods when cultured in synthetic media containing sucrose ester, even though the bacteria were grown in the same media containing the ester prior to experiment. This indicates that biodegradation studies employing seeds isolated from different river water are difficult to compare from study to study.

The results shown in Figure 2b indicate that the two test of sucrose monolaurate run under conditions similar to that of glucose exhibit similar behavior. It is of interest to note that the bacteria were readily acclimatized and grown on the surfactant. This behavior is also shown in Figure 2c, where the rate of degradation of the ester was compared to sucrose. In 50 hours about 30% of the sucrose portion of the ester was converted to CO_2 , while the production of CO_2 from the free sucrose just started. The faster degradation of the ester was probably due to the ability of the bacteria to sorb the sucrose ester much better than glucose or sucrose. Wayman and Burt (27) in studies on sorption of various kinds of surfactant onto bacterial surface have found a large amount of sucrose monolaurate was sorbed by bacteria (in the order of mg per g bacteria).

In Figure 2d the curve showing the rate of degradation of the laurate portion of the ester exhibits similar behavior with that of lauric acid, though the degradation of the laurate is somewhat slower than that of the free lauric acid.

In all of the experiments the rates of the degradation reached a plateau at about 40% to 50% of the theoretical amount of CO_2 production; this does not mean that the biodegradation was incomplete. Bunch (50) has pointed out that in the biodegradation of glucose a plateau is reached when about 40% of the theoretical O_2 has been absorbed. At this stage all of the glucose has disappeared, part of it having been oxidized to CO_2 and H_2O , and the rest converted to new cells or protoplasm. Under the condition of the standard BOD test this stage is reached in about 36 hours. Upon further aging, with no further addition of substrate, the system will resume oxygen uptake to about 70% of the theoretical amount in 5 days in the case of glucose. This results from the further oxidation of protoplasmic components. On the basis of these observations it has been proposed that any product showing oxygen uptake over 40% to 45% of the theoretical amount should be considered essentially degraded (51). Thus sucrose monolaurate, which biodegradation rate reached a plateau at CO_2 production of about 40% to 50% of the theoretical (similar to that of glucose and sucrose), could be considered as being completely biodegradable. The S_0 -curve in Figure 2a does not reach a plateau at about 50% of the theoretical amount of CO_2 production; this might be due to rapid endogenous respiration.

B. The Effect of Flow Rate of the Air Stream on Rate of CO₂ Production

As previously indicated, the rate of CO₂ purged from a solution of a biological system will depend upon the rate of metabolic production and its subsequent removal as related to flow rate. In order to see the effect of flow rate, experiments were run with the same incubation system but with different flow rates. The CO₂ was purged and trapped, and the solution was analyzed for CO₂ and HCO₃⁻ as a function of time. To obtain high accuracy, C¹⁴ compound was used as substrate. The results are shown in Figure 3a-d and Appendix II, table 3.

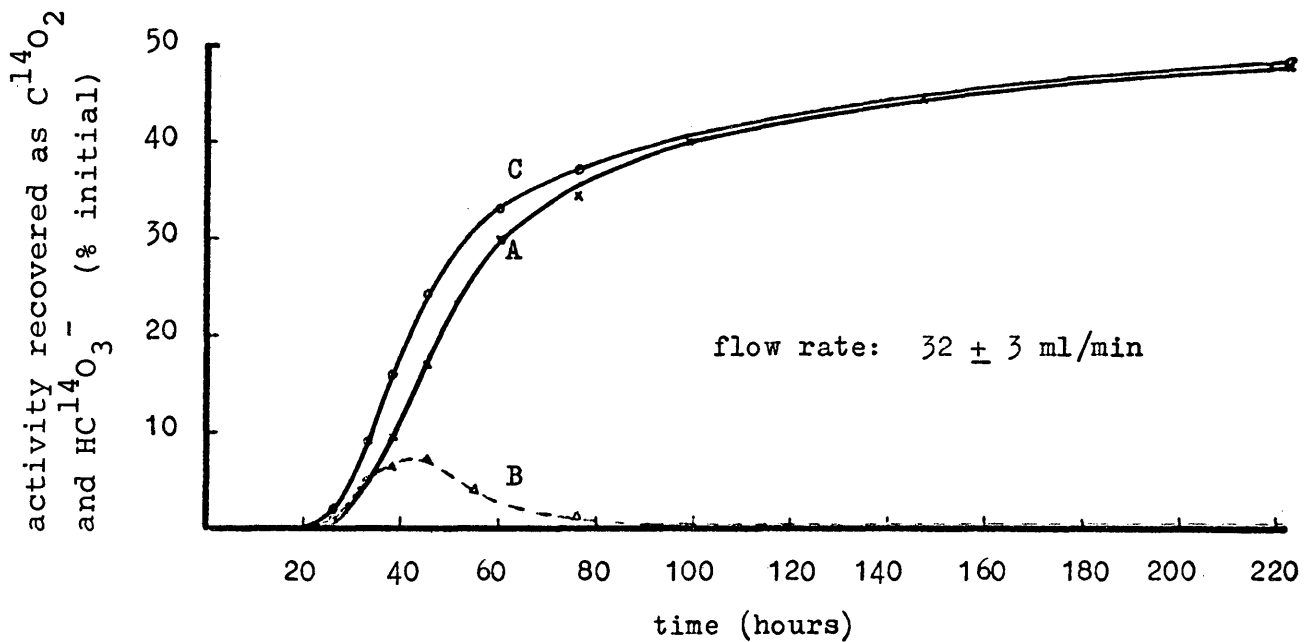


Figure 3a. Rate of C¹⁴O₂ production from the degradation of sucrose monolaurate₂ (C¹⁴UL sucrose) in synthetic media with S₆, at 20 ppm, 27°C, pH 7.0; volume of the incubation flask: 21; volume of the solution: 1250 ml.

In figure 3a and 3b curve A represents the C¹⁴O₂ produced from solution and sorbed in ethanolamine; curve B shows the amount of

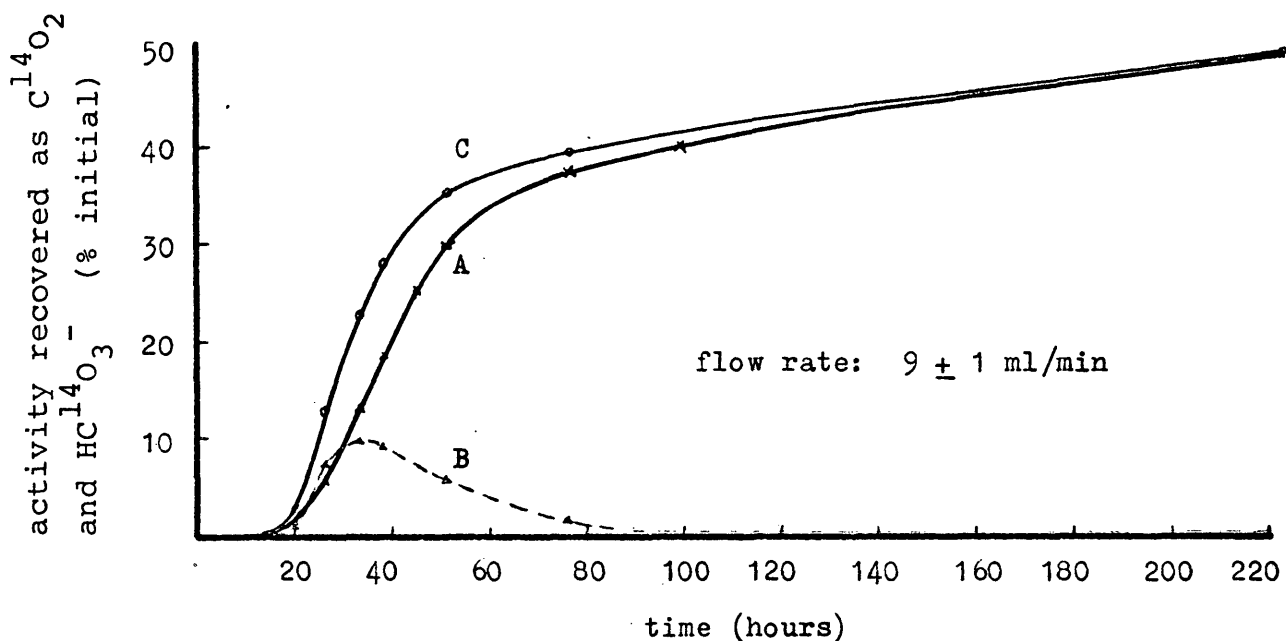


Figure 3b. Rate of $C^{14}O_2$ production from the degradation of sucrose monolaurate ($C^{14}UL$ sucrose) in synthetic media with S_6 , at 20 ppm, 27°C, pH 7.0

$C^{14}O_2$ and $HC^{14}O_3^-$ in solution as a function of time; curve C is the sum of A and B. The data indicate that significant errors result from ignoring the $CO_2(aq)$ and the CO_2 converted to HCO_3^- . The figures have shown that when the rate of CO_2 production reached the maximum, the air stream was unable to remove immediately all of the CO_2 being produced. As a result, CO_2 was built up in the solution. The apparent rate (the rate measured in terms of CO_2 trapped in ethanolamine) was therefore lower than the actual rate. As time passed the rate of CO_2 production decreased, and the CO_2 which has been built up in the solution was slowly removed. At this time the apparent rate became greater than the actual rate (The deviation of the apparent rate from the actual rate is shown by curves A and C). When the rate of CO_2 production reached a plateau, there was practically no CO_2 remaining in solution; at this time the apparent rate was about equal to the actual rate.

For a given rate of CO_2 production the life time of CO_2 in solution will determine the slope of the apparent rate, and the life time of CO_2 in solution will depend upon the flow rate of the air stream. However, though the flow rate has an effect on the life time of CO_2 in solution, it is not significantly different between rate of 9 to 32 ml per min, as shown in Figure 3c-d.

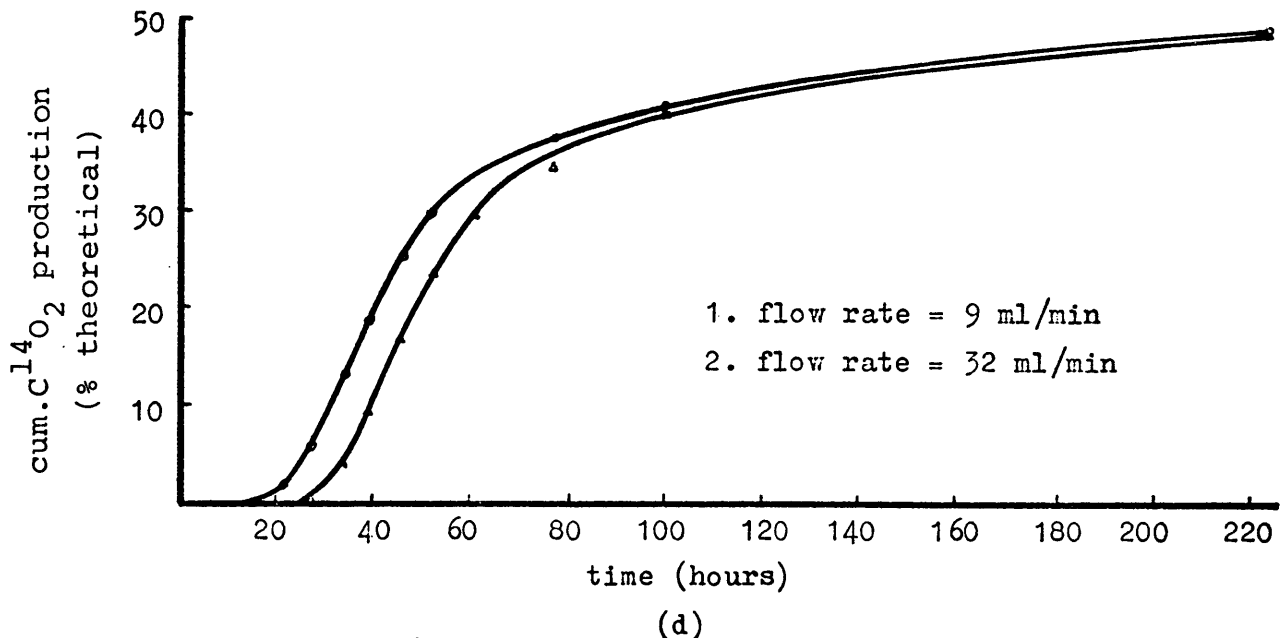
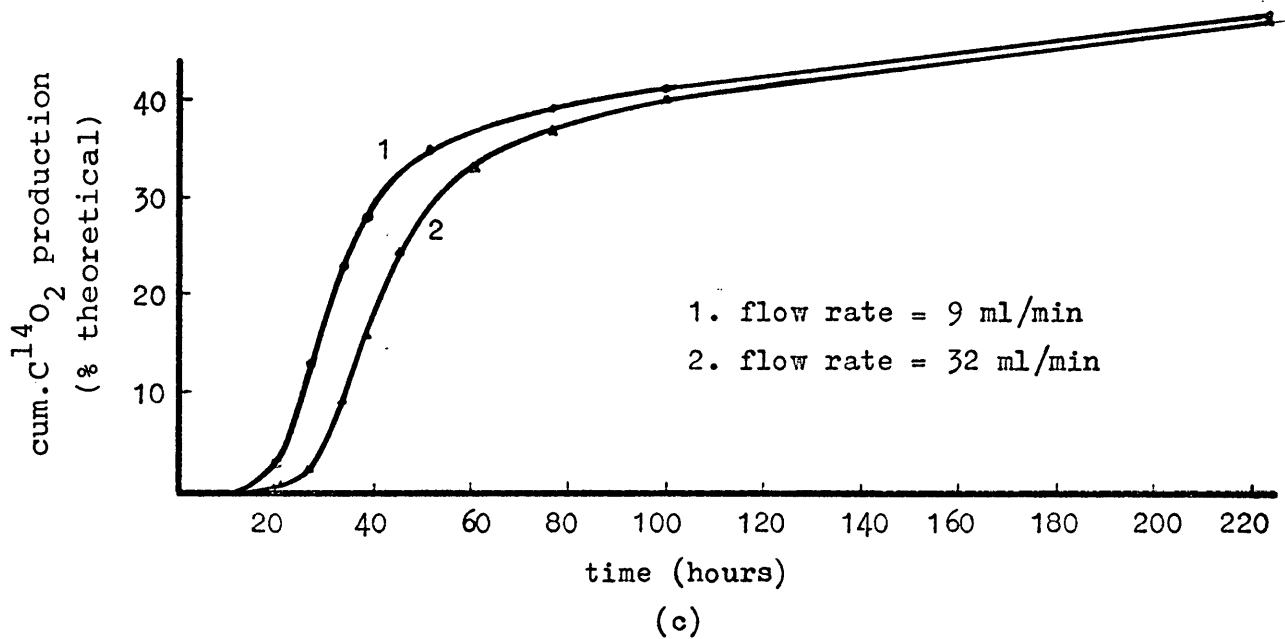


Figure 3c-d. Rate of C^{14}O_2 production from the degradation of sucrose monolaurate (C^{14}UL sucrose) in synthetic media, at 27°C , pH 7.0
 3c. actual rate; 3d. apparent rate.

The use of a very high flow rate or a small incubation flask might minimize the deviation of the apparent rate from the actual rate. However, there are certain disadvantages to be considered. The foaming problem might be serious and the absorption of CO_2 might not be quantitative at a very high flow rate; and the use of a small incubation flask might impair the accuracy of the determination of the untagged CO_2 .

Consequently, a flow rate of about 9 ml per min was utilized for the biodegradation studies. In studies at pH 7, the measurement of the apparent rate seemed adequate. In fact, for most of our purposes only information regarding relative rates was needed. However, at higher pH values (about pH 8) both the air stream and the solution should be sampled.

C. Effect of Surfactant Concentration on Rate of CO_2 Production using Sucrose Monolaurate (C^{14} UL sucrose)

It is well known that the rate of biodegradation depends on substrate concentration. Two different tests were run on sucrose monolaurate (C^{14} UL sucrose). The rate of C^{14}O_2 production and total CO_2 production are shown in Figure 4a-d and Appendix II, table 4.

In both instances the rate of C^{14}O_2 and total CO_2 production increased with an increase in concentration from 10 to 42 ppm.

Though the study was made employing synthetic media, similar results would be predictable for river water and activated sludge tests.

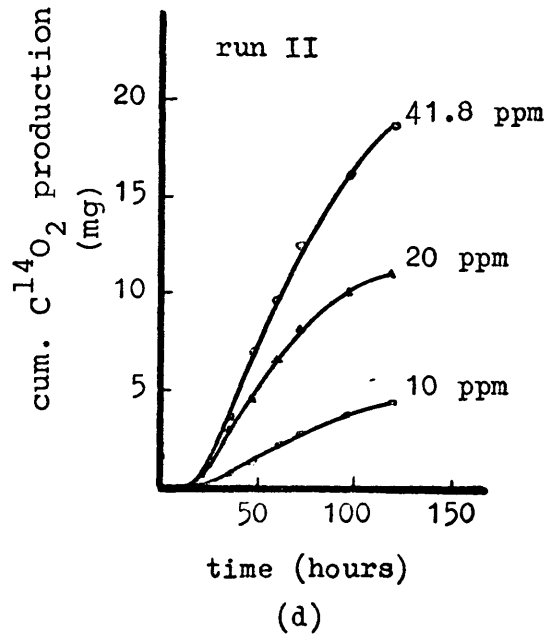
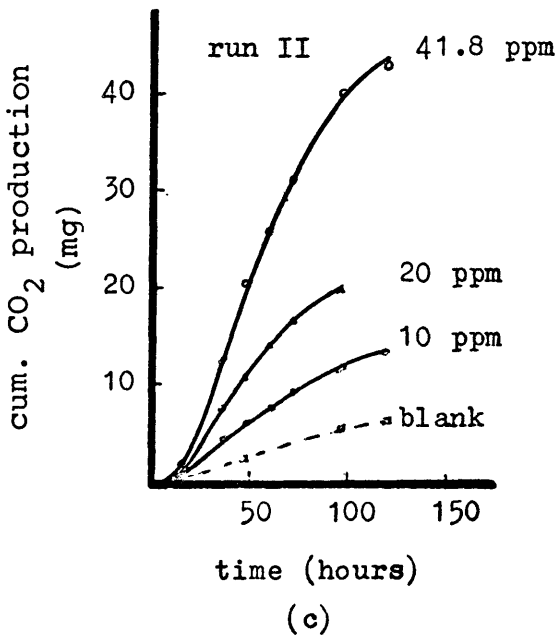
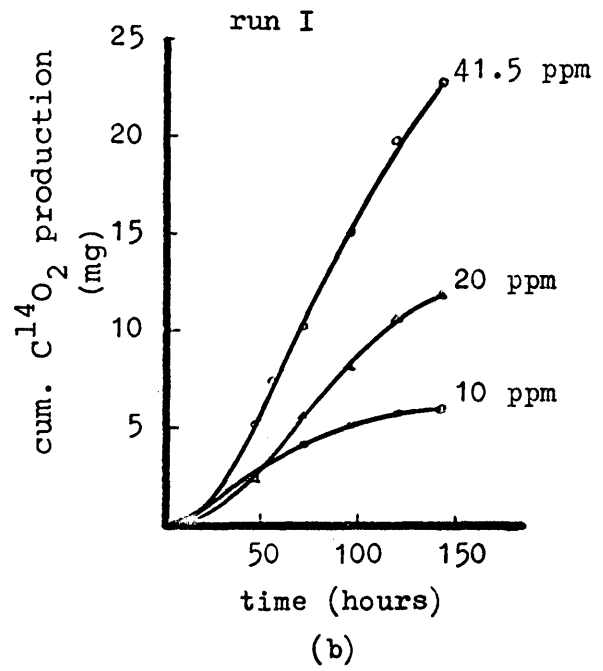
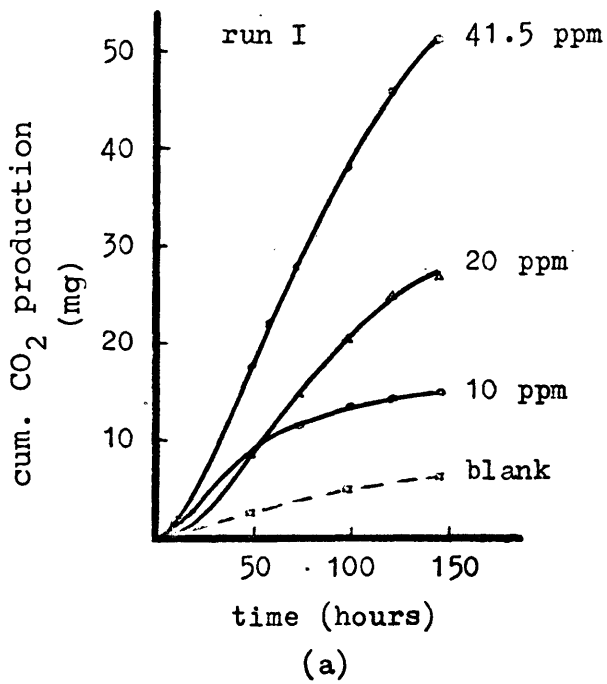


Figure 4a-d. Rate of CO₂ production from the degradation of sucrose monolaurate (C¹⁴UL sucrose) at various concentration, in synthetic media with S₆, at 27°C, pH 7.0

D. Effect of Temperature on Rate of CO₂ Production

In general, biodegradation studies on surfactants are performed at 20° to 30°C. This is not adequate for biodegradation studies in natural water where the temperature varies according to the season. Surfactants which are readily degraded at 25°C might not be degraded at low temperature; for example, at 27° and 15°C, about 27% and 12% of the carbon of tergitol 15-S-9 was converted to CO₂ in 15 days; at 5°C, about 1% of the theoretical amount was produced in 22 days (52). Temperature might greatly affect the rate as well as the extent of biodegradation.

In order to determine the temperature effect on biodegradation of sucrose esters, duplicate tests were run on pure sucrose monolaurate in synthetic media in the temperature range of 5° to 27°C. The results are shown in Figure 5a and Appendix II, table 5a.

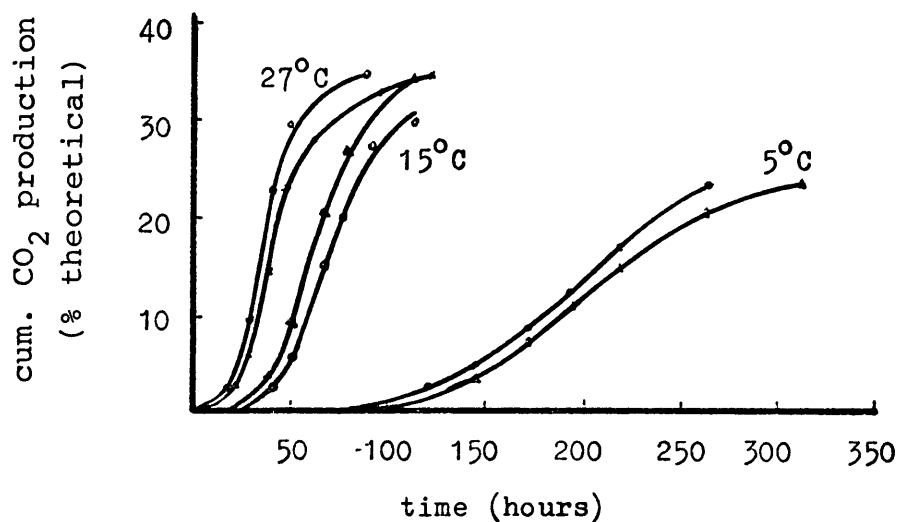


Figure 5a. Rate of CO₂ production from the degradation of sucrose monolaurate at various temperature in synthetic media with S₆, at 20 ppm, pH 7.1

The rate shows little difference in the temperature range of 15° to 27°C. However, at 5°C there is a very significant difference in the rates. This is most likely the result of poor growth and low enzymatic activity at 5°C. Though the degradation of sucrose monolaurate at 5°C is relatively slow, it is much higher than tergitol 15-S-9 at the same temperature.

In addition to the above experiments the effect of temperature on the sucrose and laurate portion was studied employing sucrose monolaurate (C^{14} UL sucrose) and sucrose monolaurate (1- C^{14} on laurate). The results are shown in Figure 5b and 5c, and in Appendix II, table 5b.

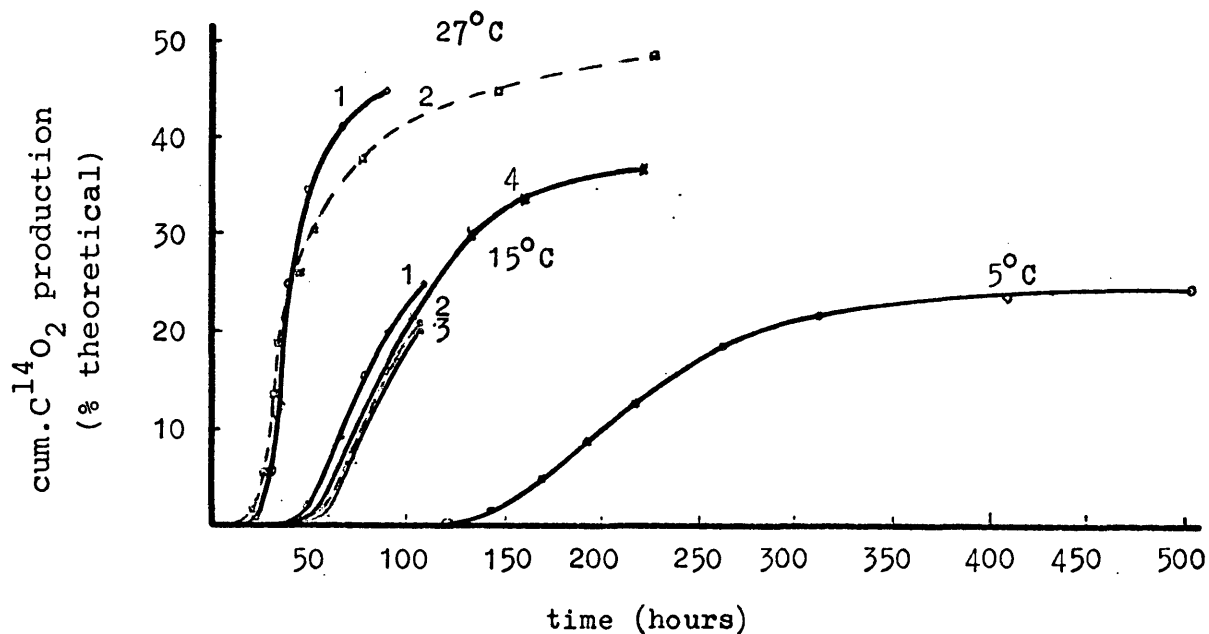


Figure 5b. Rate of $C^{14}O_2$ production from the degradation of sucrose monolaurate(C^{14} UL sucrose) in synthetic media with S_6 , at 20 ppm, pH 7.1

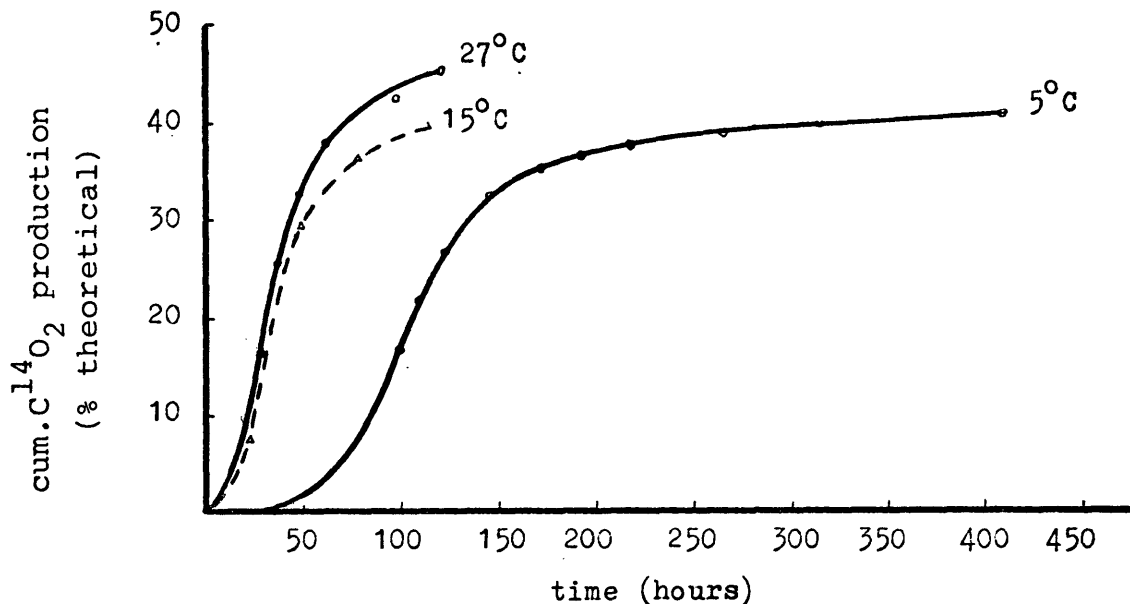
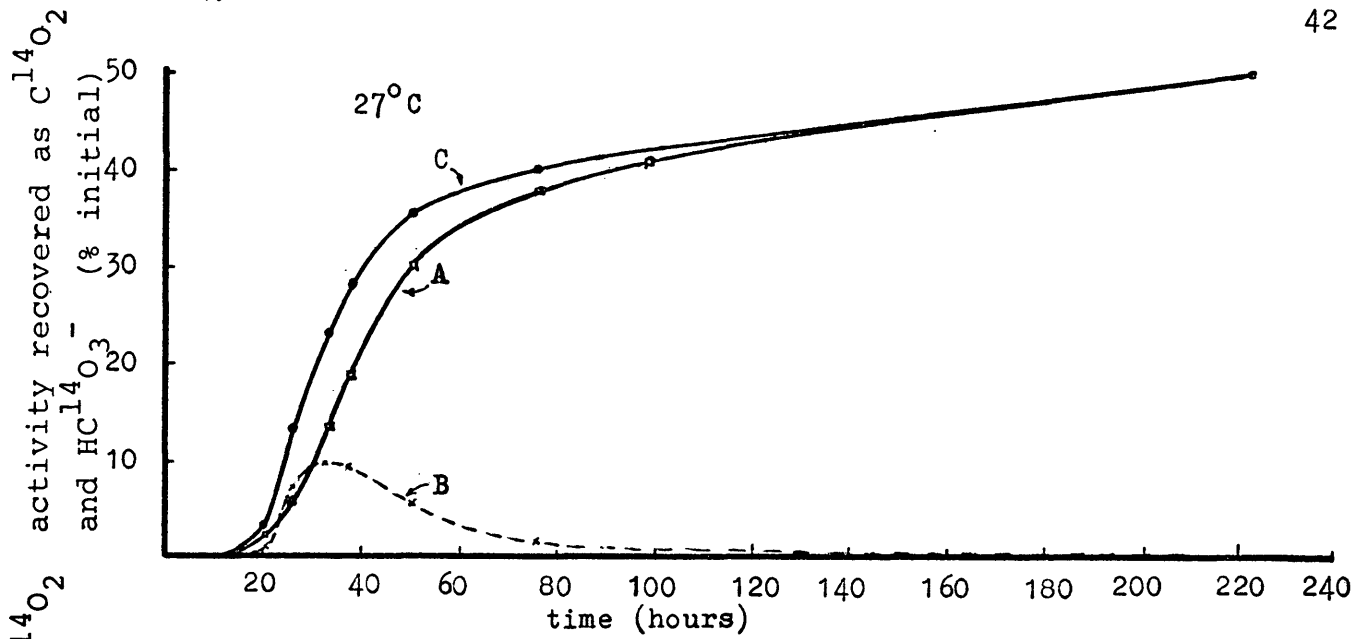


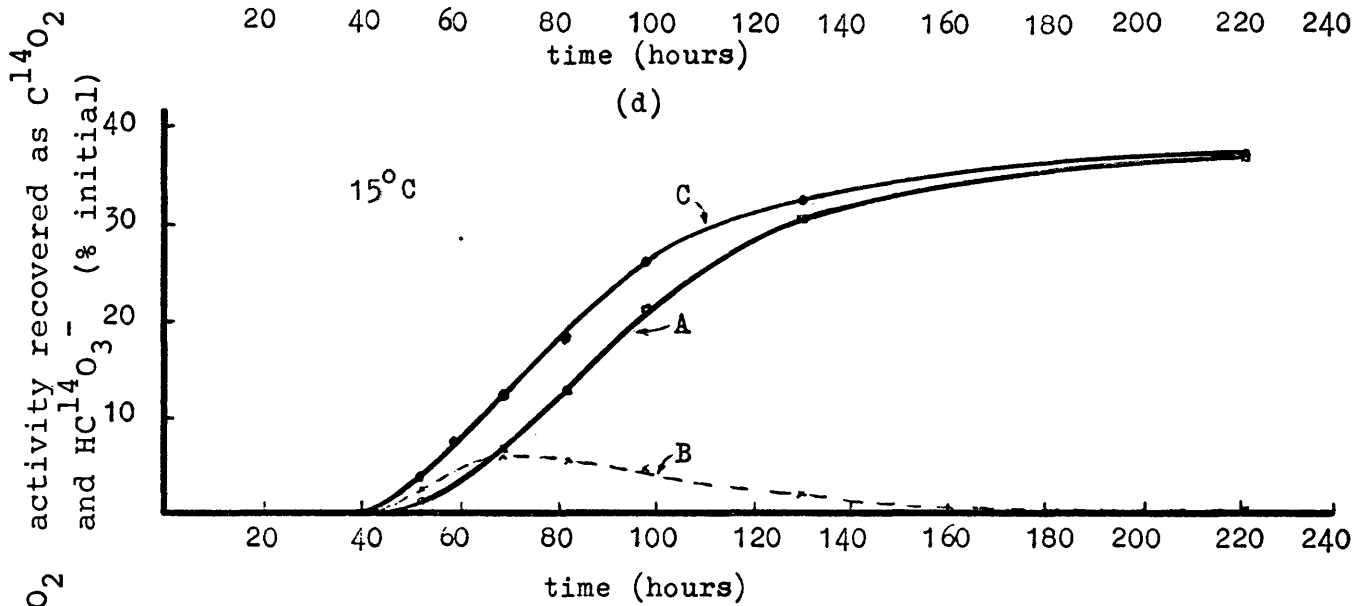
Figure 5c. Rate of $C^{14}O_2$ production from the degradation of sucrose monolaurate $^2(1-C^{14}$ on laurate) in synthetic media with S_6 , at 20 ppm, pH 7.1

The effect of temperature is rather pronounced for the sucrose portion of the ester. The excellent precision of the results is indicated for the four tests run at 15°C. At 27°C sucrose is very rapidly metabolized by the bacteria. At 5°C the rate decreases substantially. The lag phase is longer as the temperature decreases even for preacclimatized bacteria.

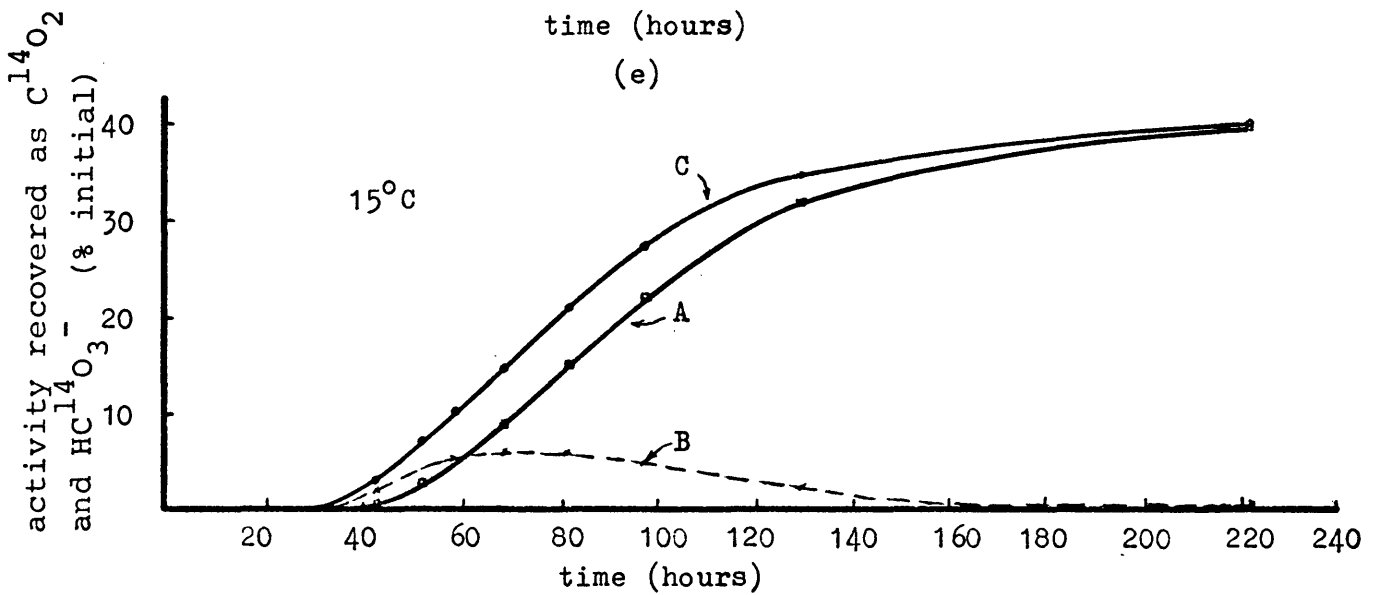
The temperature effect on the laurate portion is not as pronounced as observed for the sucrose. The rates in the range of 15°C to 27°C are nearly the same. At 5°C the rate of conversion of the $1-C^{14}$ to $C^{14}O_2$ is quite rapid, though much slower than at 15°C and 27°C. In contrast to the sucrose portion, the rates of CO_2 production from $1-C^{14}$ at 5°C to 27°C reach plateaus at almost the same level. Apparently the enzymes which are responsible for ultimate degradation of the fatty acid are little affected by temperature.



(d)



(e)



(f)

Figure 5d-f. Rate of $C^{14}O_2$ production from the degradation of sucrose monolaurate ($C^{14}UL$ sucrose) in synthetic media, pH 7.0, at 27° and $15^\circ C$. Curve A represents the $C^{14}O_2$ produced from solution and sorbed in ethanolamine; curve B shows the amount of $C^{14}O_2$ and $HC^{14}O_3^-$ in solution as a function of time; curve C is the sum of A and B.

Figure 5d-f (and table 5c in Appendix II) indicate the results of the biodegradation study on sucrose monolaurate (C^{14} UL sucrose) at 27° and $15^{\circ}C$, where $C^{14}O_2$ remaining in solution was considered. Though there is a difference between the apparent and the actual rates, the variation is not marked (see Figure 5g and 5h).

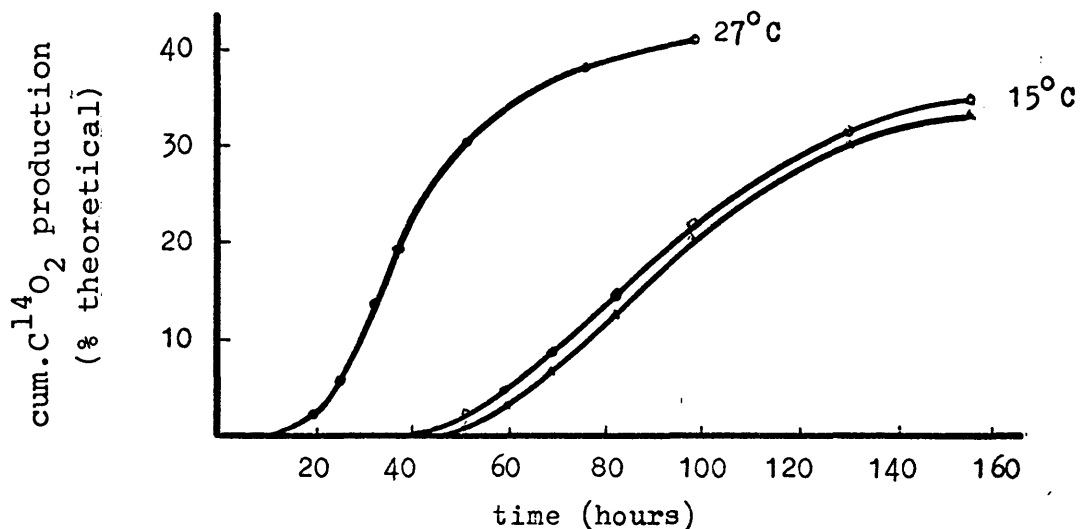


Figure 5g. Apparent rate of $C^{14}O_2$ production from the degradation of sucrose monolaurate (C^{14} UL sucrose) in synthetic media, pH 7.0

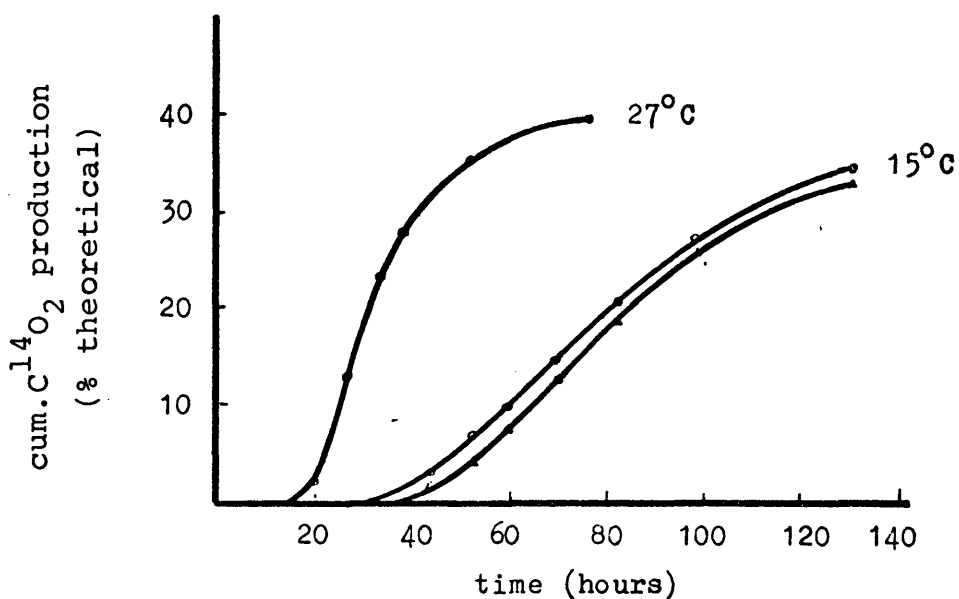


Figure 5h. Actual rate of $C^{14}O_2$ production from the degradation of sucrose monolaurate (C^{14} UL sucrose) in synthetic media, pH 7.0

E. Sucrose Esters with Fatty Acids of Different Chain Length (C₁₂-C₂₂).

E-1. Pure Sucrose Monolaurate and Sucrose Monomyristate in Synthetic Media.

Figure 6 (and table 6 in Appendix II) show the rate of CO₂ production from degradation of purified sucrose monolaurate and monomyristate. Approximately 54% of the theoretical amount of CO₂ was produced from sucrose monolaurate in 15 days, and about 43% from sucrose monomyristate over the same period of time. In order to determine the rate at which bacteria acclimatized to the two substrates, the seed was not pre-acclimatized as indicated for the other experiments. Though the extended amount of time (compare with previous study of sucrose monolaurate) indicates the importance of the acclimatization of the seed to the substrate prior to testing, the short lag phase shown in Figure 6 indicates that the seed was easily acclimatized to both substrates. With respect to the difference in chain length, the ester with C₁₂ fatty acid displayed a slightly higher rate of biodegradation than the ester with C₁₄ fatty acid.

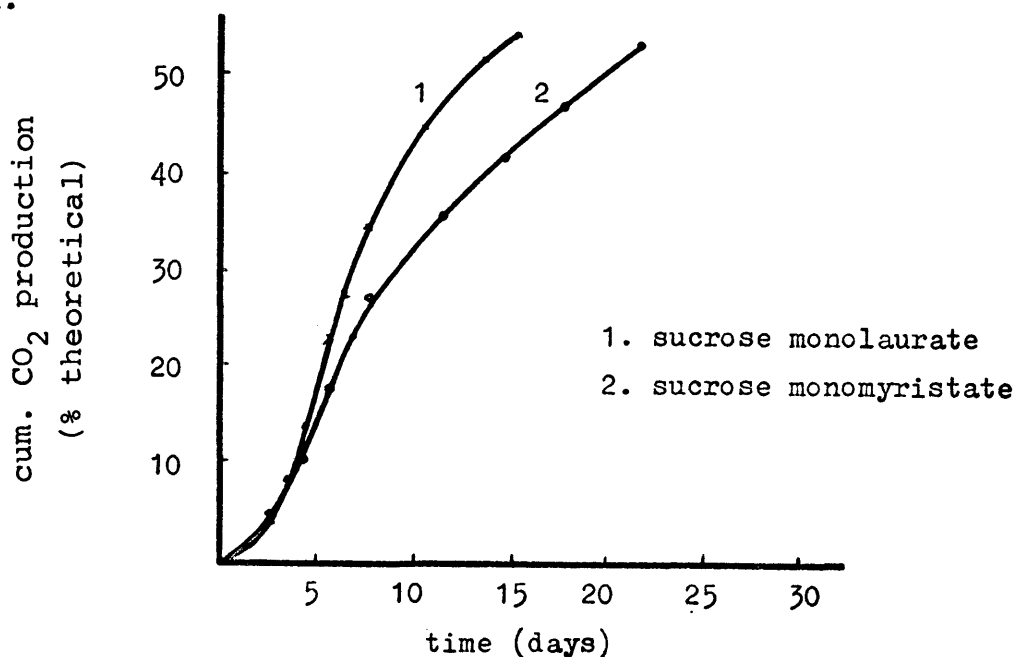


Figure 6. Rate of CO₂ production from the degradation of sucrose monolaurate and sucrose monomyristate in synthetic media with S₆, at 20 ppm, 27° C, pH 6.9

E-2. Commercial Sucrose Monolaurate; Sucrose Monomyristate; Sucrose Mono- Stearate, Arachidate, Behenate; and Nitto Ester in Synthetic Media.

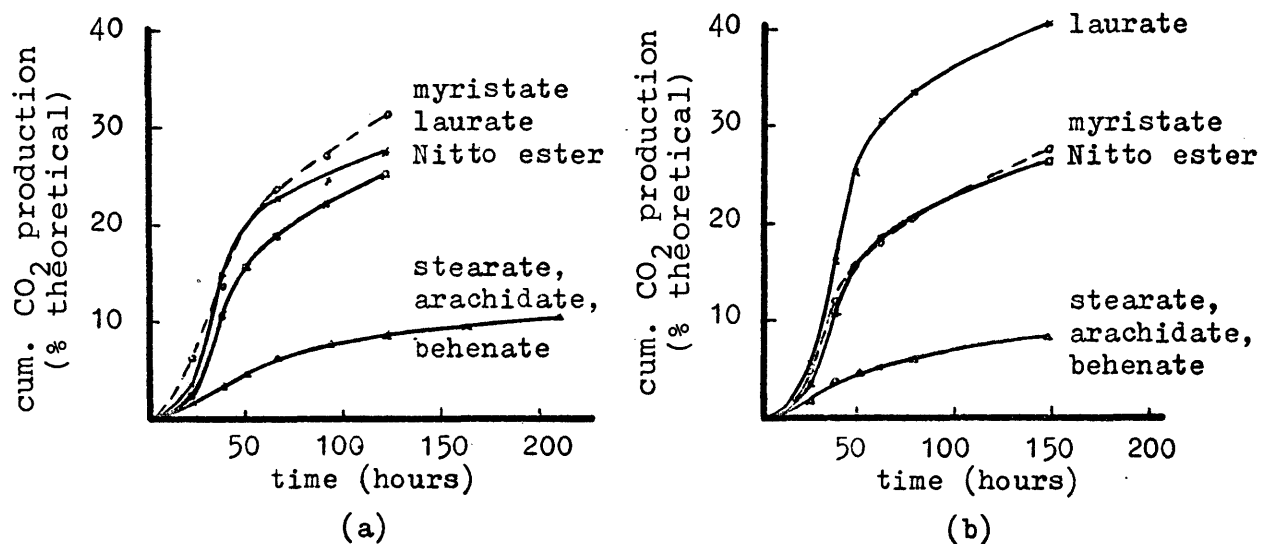


Figure 7. Rate of CO₂ production from the degradation of commercial sucrose monolaurate; sucrose monomyristate; sucrose mono-stearate, arachidate, behenate; and Nitto ester in synthetic media with S₆, at 12 ppm, 27°C, pH 7.1

Figure 7 (and table 7 in Appendix II) show the results from two runs on a number of commercially available types of sucrose esters. Nitto ester is a commercially available Japanese formulation comprised of a mixture of 70% sucrose palmitate and 30% sucrose stearate consisting of 70% monoester and 30% diester. The S₆ was preacclimatized to each substrate prior to the test. The results indicate that sucrose monolaurate, monomyristate, and Nitto ester were degraded at similar rates, though the rate of sucrose monolaurate seems to be slightly faster. As the chain length of the fatty acids increased further, the rate of CO₂ production dropped. The rate of sucrose mono-stearate, arachidate, behenate was only about 1/3 of that of sucrose monomyristate.

The results from the above experiments utilizing pure sucrose monolaurate and sucrose monomyristate, commercial sucrose monolaurate, sucrose monomyristate, and sucrose mono- stearate, arachidate, behenate, have shown that the longer the chain length of the fatty acids, the slower the biodegradation. This is in agreement with the results obtained by Loer and Roth (53) who found that the rate of aerobic biodegradation of fatty acids decreased as the chain length increased.

There are some sources of error which will be discussed with respect to experiment E-2.

1. The monoesters used were not pure; they contained small amounts of free sucrose and fatty acid as well as diester and polyester. However, for our practical purposes the results obtained above may be considered adequate, since we are dealing with biodegradation studies of sucrose esters regarding their uses in detergent formulation.

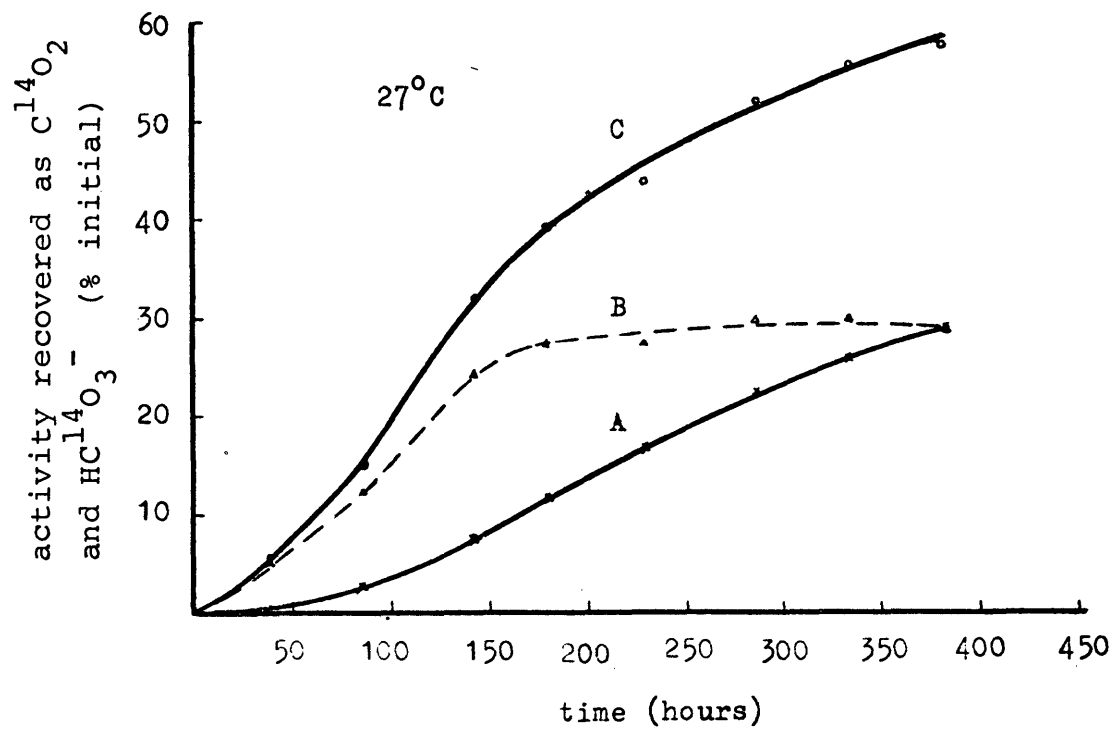
2. The sucrose monolaurate and sucrose monomyristate are gummy, they may not be wholly homogeneous; random sampling might produce error. This might be the reason for the relatively low reproducibility of the sucrose monolaurate and sucrose monomyristate tests compared to those of sucrose mono- stearate, arachidate, behenate, and Nitto ester, which are fine powder.

3. Because of the low solubility of sucrose monomyristate, sucrose mono- stearate, arachidate, behenate, and Nitto ester, very low esters concentration was used (12 ppm). As a result, the experiment could not be considered accurate. However, the much slower biodegradation rate of sucrose mono- stearate, arachidate, behenate compared to the lower esters was quite obvious.

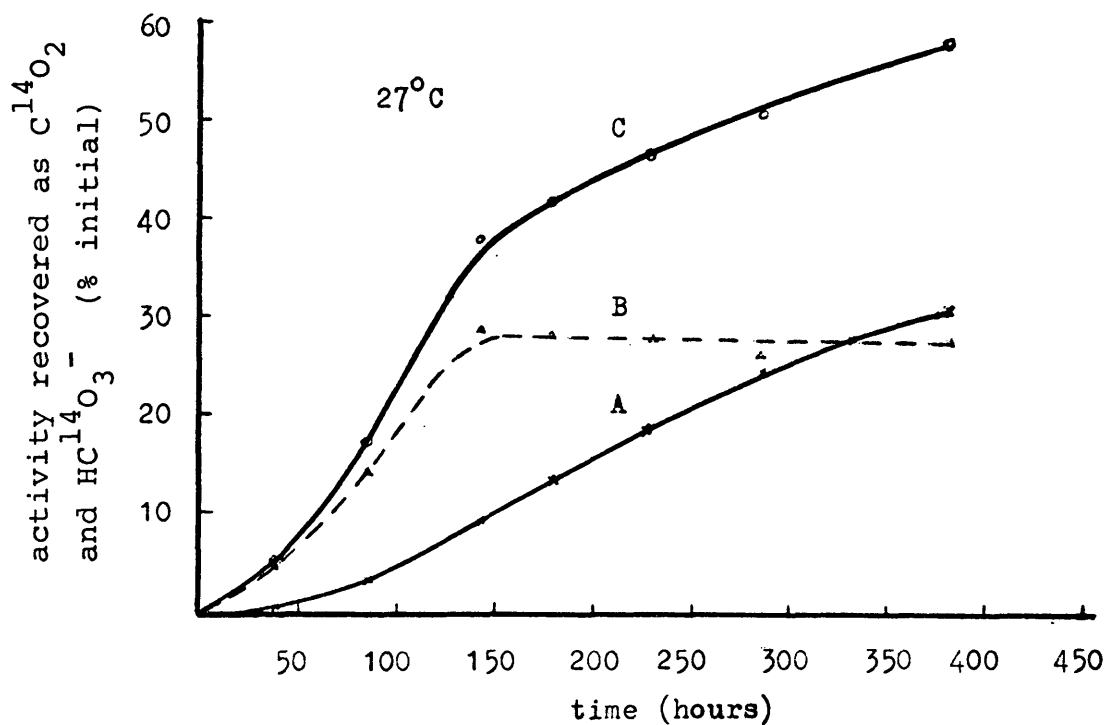
F. Sucrose Monolaurate in River Water from Clear Creek and South Platte River

In studying the biodegradation of sucrose esters in river or sewage water where other organic substrates are present, use of C^{14} sucrose esters is required. Because sucrose ester (C^{14} UL) was not available, sucrose monolaurate (C^{14} UL sucrose) or sucrose monolaurate (1- C^{14} on laurate) was used. Figure 8a-c (and table 8a in Appendix II) show the rate of $C^{14}O_2$ production from degradation of sucrose monolaurate (C^{14} UL sucrose) in river water from Clear Creek, taking into account the amount of $C^{14}O_2$ and $HC^{14}O_3^-$ in solution. Curve A represents the $C^{14}O_2$ produced and purged from the solution and sorbed in ethanolamine. Curve B shows the amount of $C^{14}O_2$ and $HC^{14}O_3^-$ in solution as a function of time. Curve C is the sum of curve A and B. About 40% of the theoretical amount of $C^{14}O_2$ was generated in 7 days at 27°C; and about the same amount was produced in 14 days at 15°C. The rate decreased after about 35 to 40% of the theoretical amount of $C^{14}O_2$ was generated, but it did not reach a plateau, as found in the synthetic media. In river water the extent of sucrose monolaurate to undergo ultimate biodegradation seems to be higher than in synthetic media.

In South Platte water both sucrose monolaurate (C^{14} UL sucrose) and (1- C^{14} on laurate) were studied. The amount of $C^{14}O_2$ and $HC^{14}O_3^-$ in solution was not determined; hence, the actual rate of $C^{14}O_2$ production was not known. However, the river water was acidified at the end of the test, and the $C^{14}O_2$ liberated was trapped and determined. By comparing the apparent rate of $C^{14}O_2$ production in South Platte water shown in Figure 8d and table 8b in Appendix II, with that of



(a)



(b)

Figure 8a-b. Rate of $C^{14}O_2$ production from the degradation of sucrose monolaurate ($C^{14}UL$ sucrose) in river water from Clear Creek, at 12 ppm, $27^\circ C$, pH 8.4 - 8.3

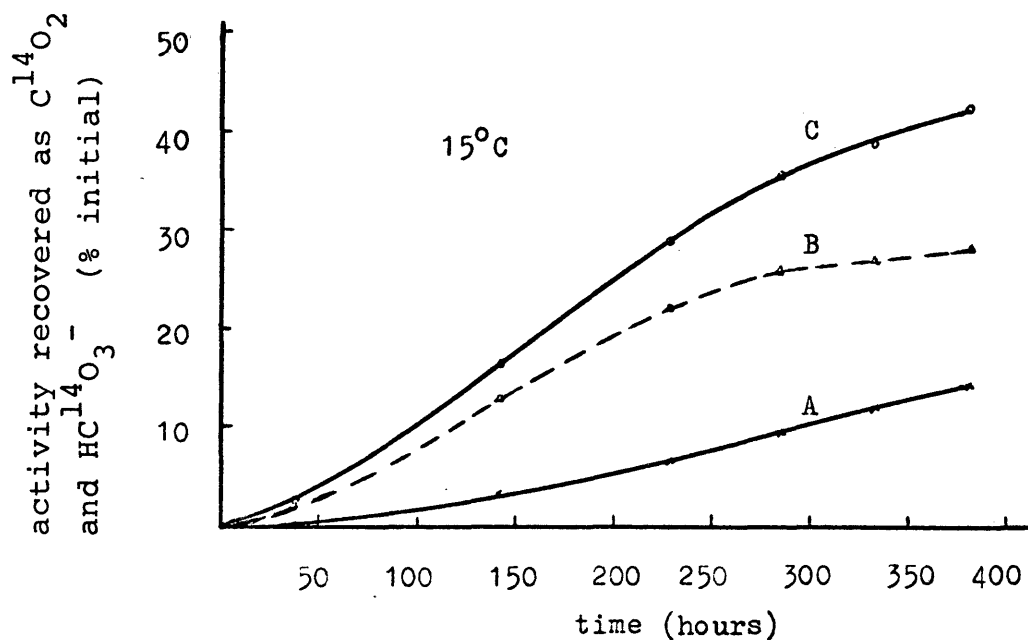


Figure 8c. Rate of $C^{14}O_2$ production from the degradation of sucrose monolaurate ($C^{14}UL$ sucrose) in river water from Clear Creek, at 12 ppm, $15^\circ C$, pH 8.4 - 8.3

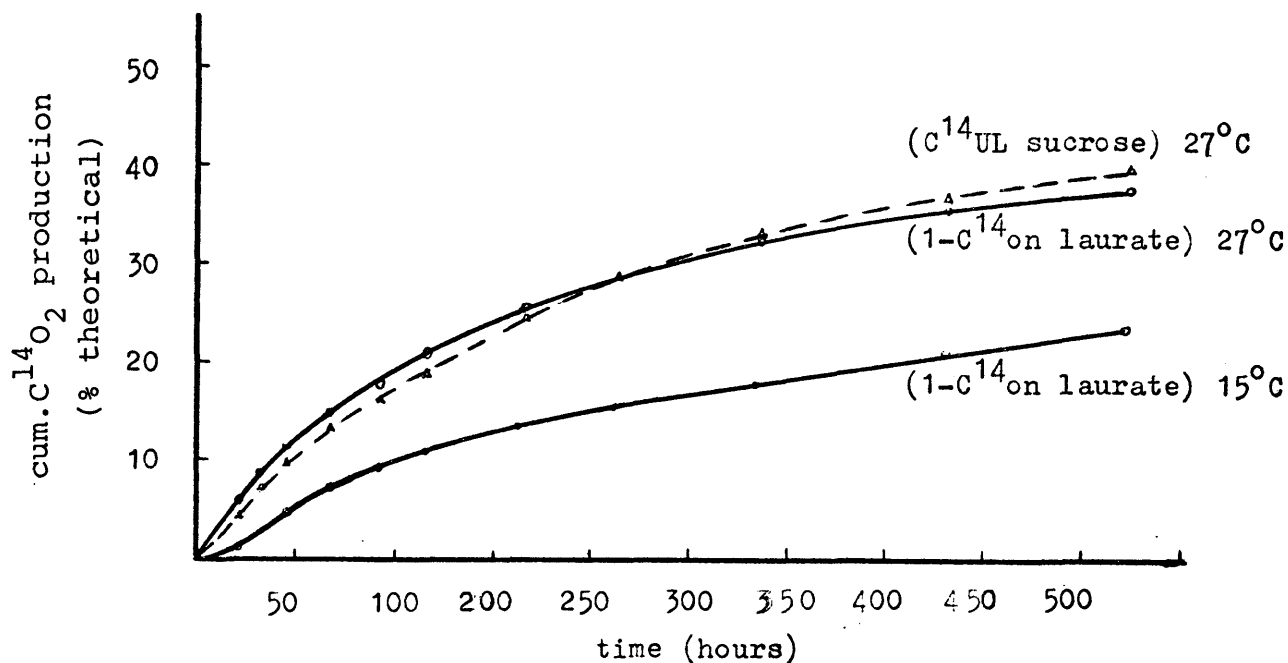


Figure 8d. Apparent rate of $C^{14}O_2$ production from the degradation of sucrose monolaurate ($1-C^{14}$ on laurate) and sucrose monolaurate ($C^{14}UL$ sucrose) in river water from South Platte River at 20 ppm, 27° and $15^\circ C$, pH 8.4 - 8.3

Clear Creek, it can be seen that the actual rate of $C^{14}O_2$ production in South Platte water was much higher. By the end of 20 days incubation at $27^\circ C$ in South Platte water, a total amount of about 92% of theoretical amount of $C^{14}O_2$ was produced from sucrose monolaurate ($C^{14}UL$ sucrose) after acidification of the river water (see Appendix II, table 8b); while in Clear Creek water, only about 60% was produced in the same period of time (obtained by extrapolation of curves C in Figure 8a and 8b). The ultimate biodegradation seems to be highly dependent upon the bulk composition of the river water. Clear Creek was sampled up-stream from the Coors sewage effluent and South Platte was sampled down-stream from the sewage plant effluent. IT CAN BE CONCLUDED THAT BIODEGRADATION STUDIES EMPLOYING DIFFERENT BULK COMPOSITION OF RIVER WATER RESULTS IN DIFFERENT RATES OF BIODEGRADATION.

G. Sucrose Monolaurate in Activated Sludge from Coors Treatment

Plant

G-1. Sucrose Monolaurate ($C^{14}UL$ sucrose) and Sucrose Monolaurate ($1-C^{14}$ on laurate).

Figure 9a (and table 9a in Appendix II) show the rate of $C^{14}O_2$ production from sucrose monolaurate ($C^{14} UL$ sucrose) in activated sludge. About 41% to 46% of the sucrose portion of the ester (with initial concentration of 10.5 and 40 ppm) was oxidized to CO_2 in 30 hours at $27^\circ C$. Figure 9b (and table 9a in Appendix II) show the rate of $C^{14}O_2$ production from sucrose monolaurate ($1-C^{14}$ on laurate) in activated sludge. About 44% of the $1-C$ of the laurate was oxidized to CO_2 in 30 hours at $27^\circ C$.

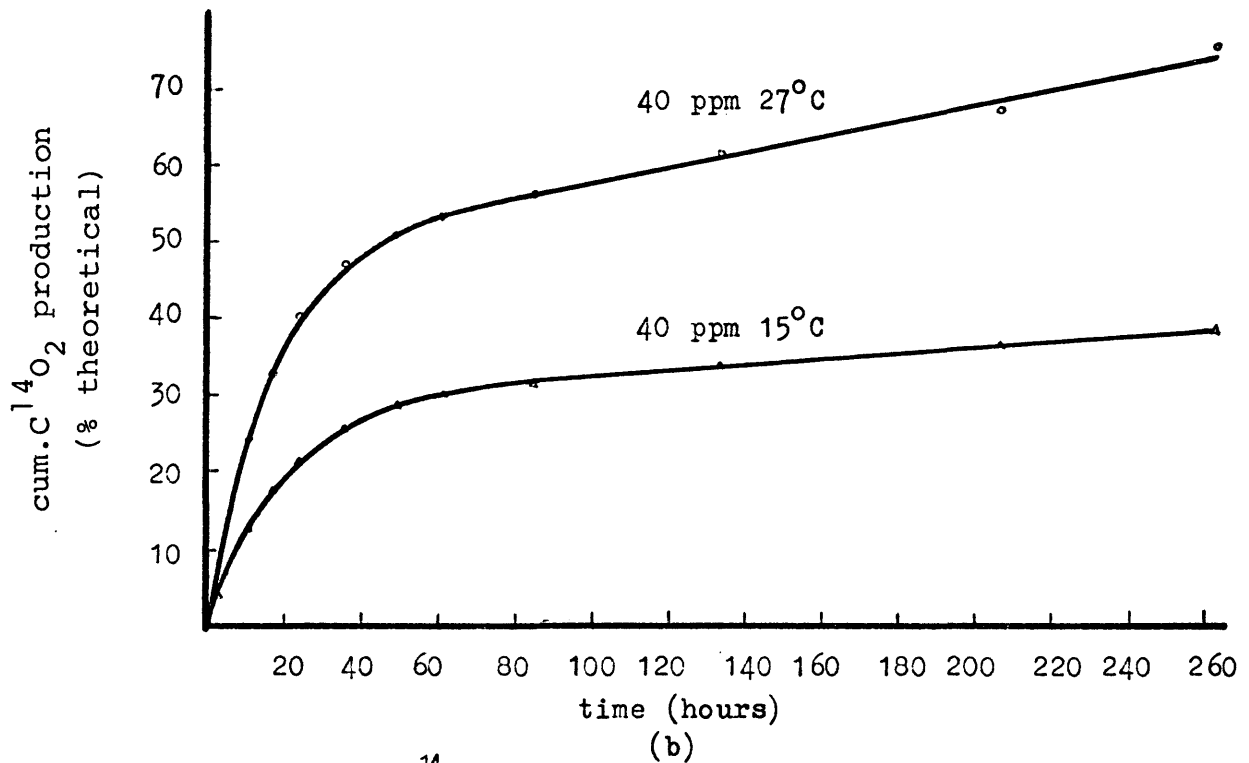
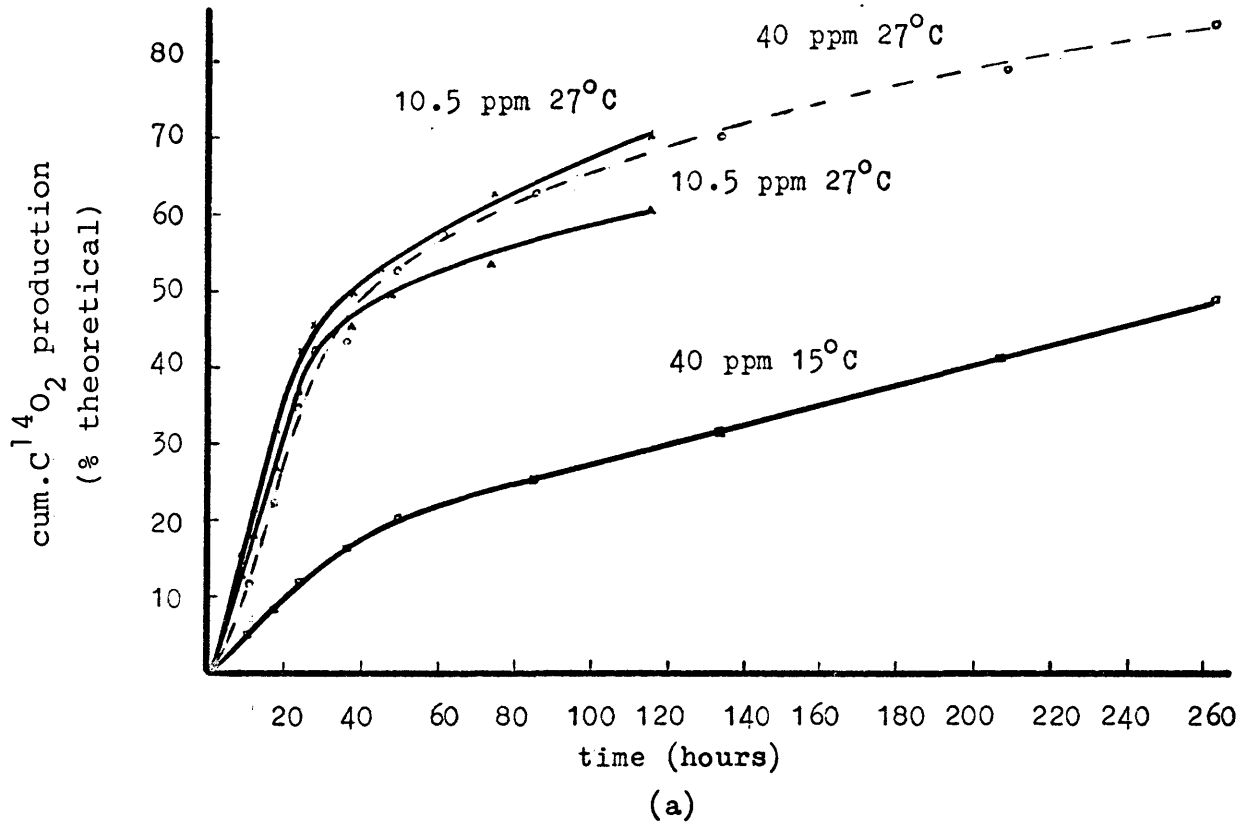


Figure 9a-b. Rate of $C^{14}O_2$ production from the degradation of sucrose monolaurate in activated sludge, pH 7.0 - 7.2
 9a. sucrose monolaurate (C^{14} UL sucrose)
 9b. sucrose monolaurate (1- C^{14} on laurate)

The above results indicate that both of the ester's components, sucrose and laurate*, were rapidly oxidized to CO_2 in the batch sludge system at 27°C . However, at 15°C , the rate was much slower; this is inconsistent with the results obtained from the study of temperature effect in synthetic media, which show that temperature ranging from 15° to 27°C had little effect on rate. The much slower rate in the sludge at 15°C compared to 27°C might be due to several factors, among which are:

1. The sludge which was originally at about 20°C was not preacclimatized to 15°C prior to the test.

2. The rate of biodegradation of a given substrate is dependent upon the amount of viable cells as well as upon other factors. In the test employing synthetic media cell growth is governed by the amount of added substrate (sucrose monolaurate) and the amount of nutrient; in sludge, growth is governed by other substrates which are present in much higher concentration. It is very likely that in the sludge there are ample organic substrates which are readily utilized by bacteria at 20°C , but not easily utilized at 15°C . As a result, when the sludge is brought from 20°C to 15°C , the steady state which is already achieved at 20°C is disturbed, resulting in a decrease of growth. Consequently, the rate of biodegradation of all substrates decreases considerably.

At 27°C , about 88% of the sucrose portion and 79% of the 1-C of the laurate was oxidized to CO_2 in 11 days. These results again indicate that complete ultimate biodegradation of sucrose monolaurate could be easily achieved.

* Though the data indicate 1-C on laurate, the ease of the degradation of the rest of the carbon atoms of the fatty acid can be predicted (see β -oxidation mechanism of fatty acids on page 68).

G-2. Sucrose Monolaurate (C^{14} UL sucrose) compared to glucose (C^{14} UL) and Sucrose (C^{14} UL).

Because mono- and disaccharides are known to be excellent carbon sources for sewage bacteria, glucose and sucrose were studied in contrast to sucrose monolaurate (C^{14} UL sucrose) with respect to their $C^{14}O_2$ generation rates in activated sludge. The results of the test are shown in Figure 9c and Appendix II, table 9b.

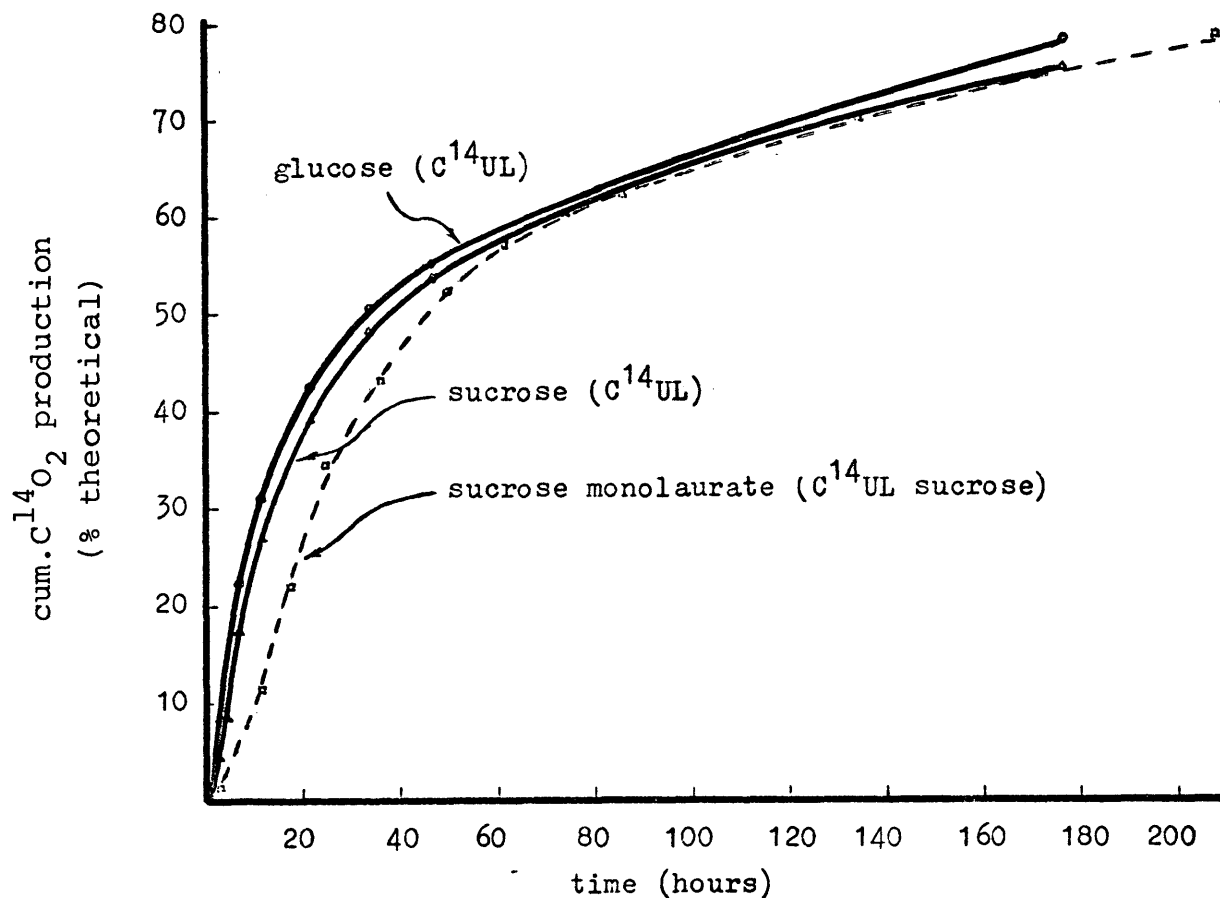


Figure 9c. Rate of $C^{14}O_2$ production from the degradation of sucrose monolaurate ($C^{14}UL$ sucrose), glucose ($C^{14}UL$), and sucrose ($C^{14}UL$) in activated sludge, at 40 ppm, $27^{\circ}C$, pH 7.0 - 7.2

Figure 9c indicates that at the early period of incubation the rate of $C^{14}O_2$ production from sucrose monolaurate was slower than that of glucose and sucrose. After about 10 hours the degradation proceeded at almost the same rate. About 50% of the theoretical amount of $C^{14}O_2$ was produced by the three substrates within 45 hours. These results lead to the conclusion that when sucrose monolaurate is continually fed and treated in a sewage treatment plant, the sucrose monolaurate will be removed and converted to CO_2 and H_2O at about the same rate as sucrose and glucose.

According to Swisher (54) an activated sludge system with a continuous operation of 3 to 6 hours retention time may be equal or superior to a 24-hour semicontinuous operation, in biodegradation. Therefore, a biodegradation study showing 50% degradation within 45 hours in a batch Die-Away method, in an efficient continuous sewage treatment plant, may be expected to take place in less than 6 hours, which is a normal retention time for a sewage treatment plant.

H. Sucrose Monolaurate (C^{14} UL sucrose) and Sucrose Monolaurate ($1-C^{14}$ on laurate) in Anaerobic Sludge.

Biodegradation is much slower under anaerobic conditions than when there is an abundance of air; the reactions are thermodynamically less favored and the processes are less efficient. Nevertheless, two important areas of sewage treatment involve anaerobic systems: septic tanks and anaerobic digesters; study of surfactant behavior under related conditions is likewise important.

In this experiment the biodegradability of sucrose monolaurate under anaerobic conditions was studied using anaerobic sludge taken

from Coors anaerobic digester [similar to work done by Manganeli (55) in studying the persistence and effect of an alkylaryl sulfonate in sludge digestion]. The results are shown in Figure 10 and Appendix II, table 10.

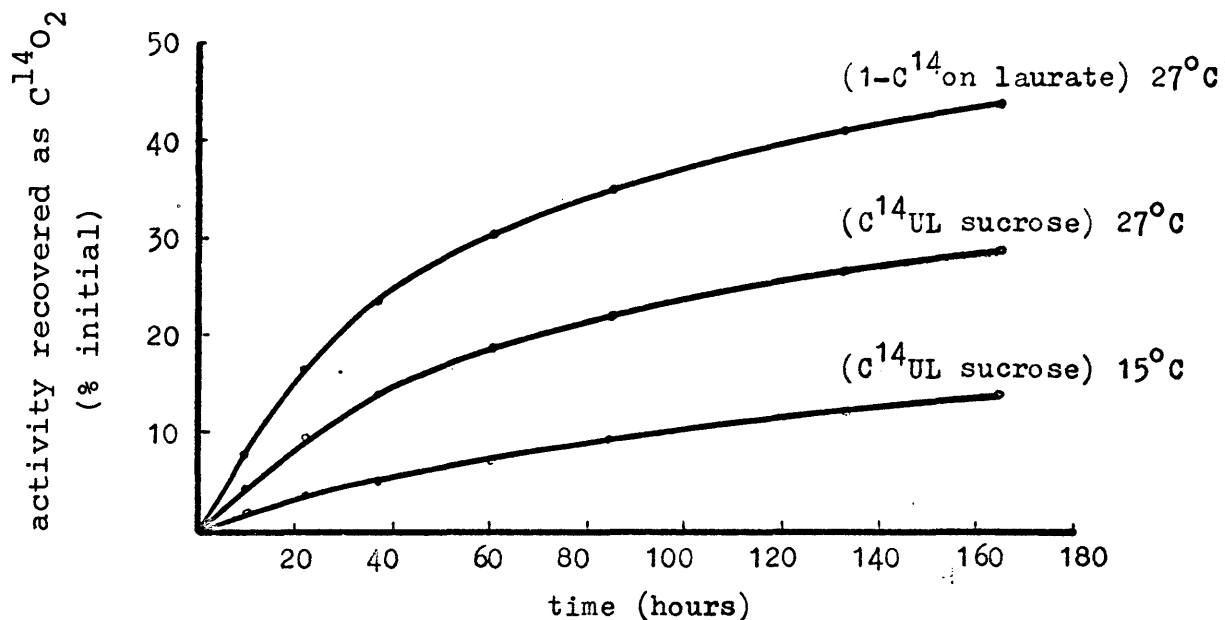


Figure 10. Rate of $C^{14}O_2$ production from the degradation of sucrose monolaurate ($C^{14}UL$ sucrose) and sucrose monolaurate ($1-C^{14}$ on laurate) in anaerobic sludge, at 40 ppm, pH 8.

In anaerobic digestion methane and carbon dioxide are the normal metabolic end products (1, p.188). Methane fermentation of one mole of hexose will produce 3 moles CO_2 and 3 moles CH_4 (see page 67). Thus, if a uniformly labeled hexose undergoes complete methane fermentation, 50% of its original activity will be found as $C^{14}O_2$, and 50% as $C^{14}H_4$. In the results of anaerobic study of sucrose monolaurate ($C^{14}UL$ sucrose) shown in Figure 10, about 29% of the original activity was recovered as $C^{14}O_2$ in 7 days at 27°C. This result indicated that about 58% of the theoretical amount of $C^{14}O_2$ was generated. At 15°C about 14% of

the original activity was recovered as $C^{14}O_2$ in the same period, which means that about 28% of the theoretical amount of $C^{14}O_2$ was produced.

In methane fermentation of fatty acids, odd numbered carbon atoms of fatty acid will be converted to CO_2 , while the even numbered carbon atoms will be converted to CH_4 (56). Therefore, from methane fermentation of sucrose monolaurate (1- C^{14} on laurate), one might expect 100% of the original activity to be recovered as $C^{14}O_2$. Figure 10 shows that about 44% of the initial activity of sucrose monolaurate (1- C^{14} on laurate) was recovered as $C^{14}O_2$ in 7 days at 27°C.

The results of this experiment have shown that under anaerobic conditions sucrose monolaurate is readily biodegradable. In fact, the actual rate of biodegradation was even higher than that shown in Figure 10; because the pH of the sludge was about 8 - 8.5, a considerable amount of CO_2 remained in solution. After acidification of the sludge at the end of the test (165 hours), an additional 19% of the initial activity (see table 10) was recovered as $C^{14}O_2$ from the test of sucrose monolaurate (C^{14} UL sucrose) at 27°C; 16.1% from sucrose monolaurate (C^{14} UL sucrose) at 15°C; and 22.7% from sucrose monolaurate (1- C^{14} on laurate) at 27°C. Thus, from sucrose monolaurate (C^{14} UL sucrose) at 27°C, 47.9% of the initial activity was recovered as $C^{14}O_2$, which means 95.8% of the theoretical amount of $C^{14}O_2$ was generated. From sucrose monolaurate (C^{14} UL sucrose) at 15°C, 30.0% of the initial activity was recovered as $C^{14}O_2$ (60.0% of the theoretical amount). From sucrose monolaurate (1- C^{14} on laurate) at 27°C, 66.6% was recovered as $C^{14}O_2$ (66.6% theoretical amount).

I. Sucrose Monolaurate in Synthetic Media in Contrast to C₁₂ LAS and Tergitol 15-S-9

The previous results have indicated that sucrose monolaurate is readily biodegradable in synthetic media, natural water, and in sludge under aerobic and anaerobic conditions at 27°C. In this experiment the biodegradability of sucrose monolaurate was compared to that of domestic LAS (with average alkyl chain of C₁₂) and tergitol 15-S-9, which is a major ingredient in some of the present-day liquid dish-washing detergents. Figure 11 and Appendix II, table 11, show the results of this study.

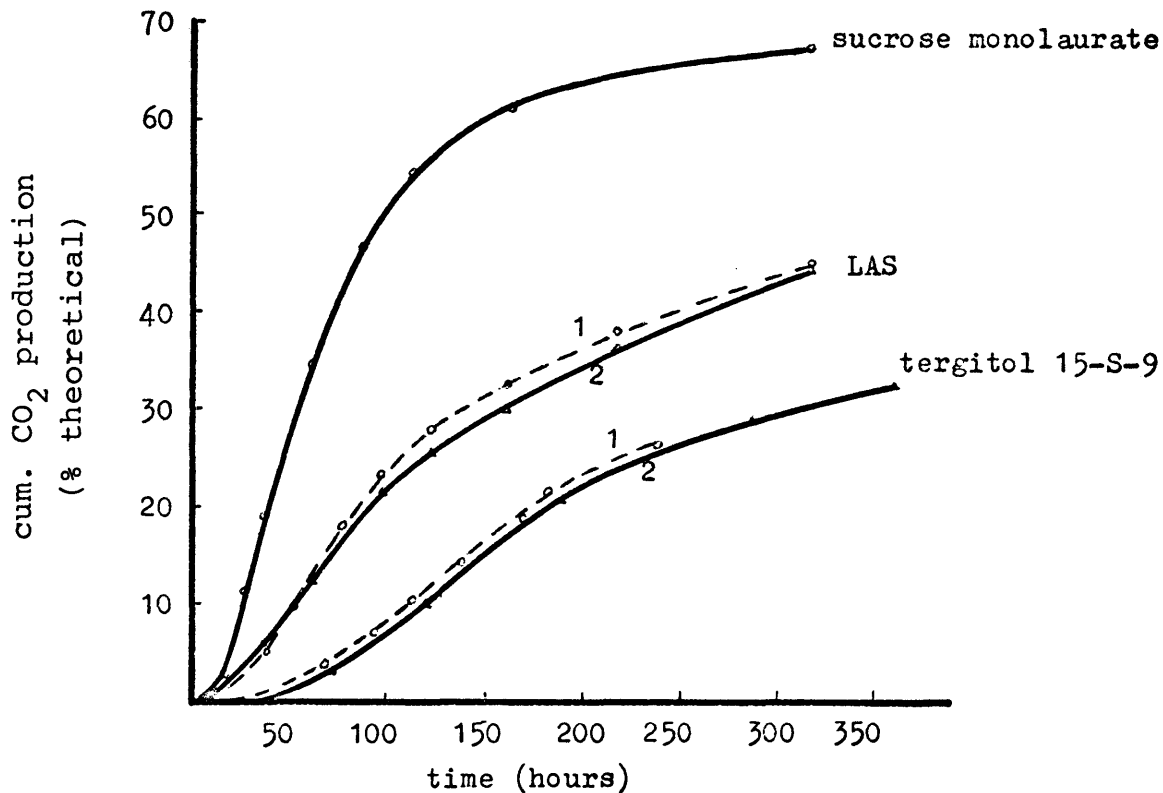


Figure 11. Rate of CO₂ production from the degradation of sucrose monolaurate, LAS and tergitol 15-S-9 in synthetic media with S₀, at 20 ppm, 27°C, pH 7.0

The rate of CO_2 production from degradation of sucrose monolaurate was superior to either that of LAS and tergitol 15-S-9. Approximately 50% of the sucrose monolaurate was oxidized to CO_2 in 98 hours (about 4 days), whereas only 23% and 7% of LAS and tergitol 15-S-9, respectively were oxidized to CO_2 in the same time period. These results indicate that sucrose monolaurate possesses superior biodegradation qualities to domestic LAS and tergitol 15-S-9.

J. Study on the Mechanism of Biodegradation of Sucrose Ester using
Sucrose Monolaurate (1-C¹⁴ on laurate)

Though sucrose esters are known to be biodegradable, the mechanism through which degradation takes place in microorganisms has not been thoroughly studied. In animals and humans, sucrose esters are hydrolyzed to fatty acids and sucrose (57). It is reasonable to believe that in microorganisms, the degradation might take place in the same fashion. Kulovana and Pitters (46) from their study on biodegradation of sucrose esters in activated sludge indicated (without direct proof) that sucrose esters were hydrolyzed to fatty acids and sucrose, followed by subsequent degradation of the sucrose and fatty acid separately. The purpose of this part of the experimental work was to study the mechanism of the degradation of sucrose esters employing river bacteria.

Based on the generally-known metabolic pathways of sucrose, glucose, straight-chain fatty acids and linear alkyl chain, a similar mechanism for the break down of sucrose esters seems to be reasonable. Two possible mechanisms were considered:

(1) The break-down of sucrose esters is via hydrolysis of the ester linkage forming fatty acids and sucrose, followed by degradation of the fatty acids and sucrose individually.

(2) The break-down begins with ω -oxidation from the end of the alkyl chain of the fatty acid, without hydrolyzing the ester linkage.

expect a high rate of $C^{14}O_2$ production at the early period of incubation. This $C^{14}O_2$ generation will then slow down while the untagged CO_2 production increases. If the degradation takes place according to the second possibility described above, then the untagged CO_2 will be generated first.

The results of this study are shown in Figure 12a-d and Appendix II, table 12.

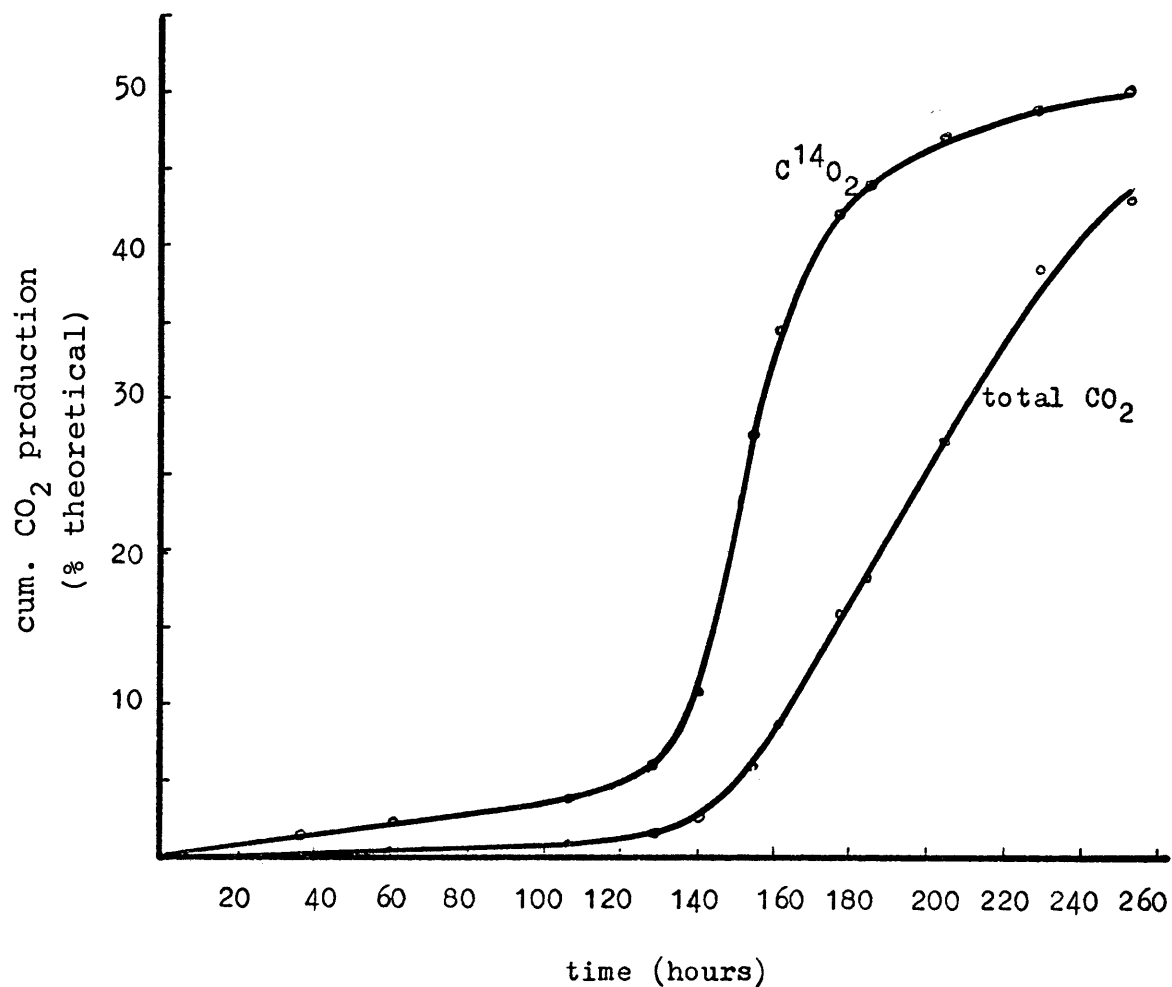
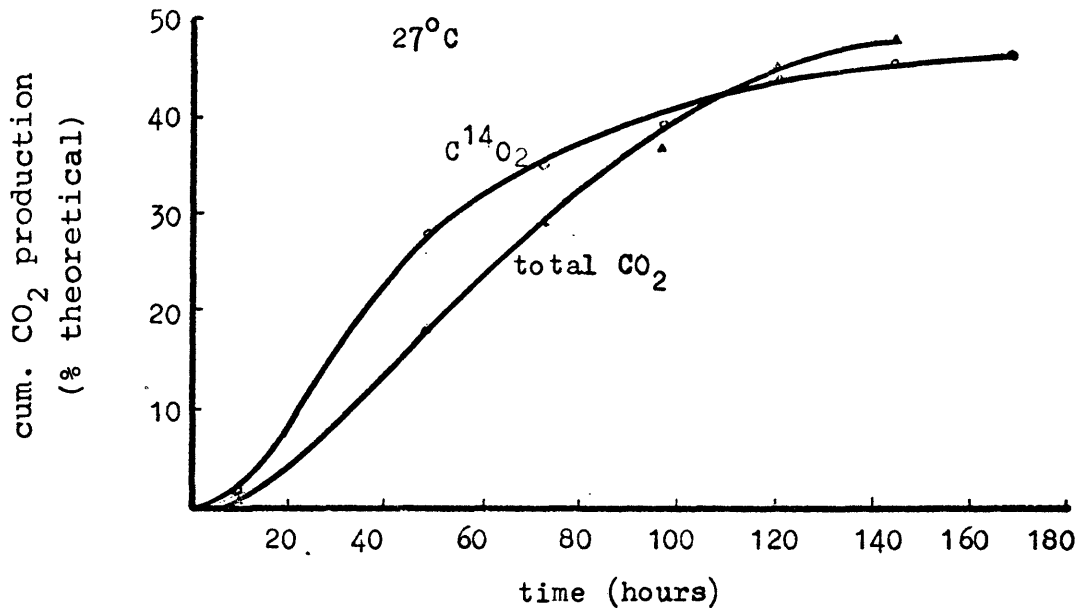
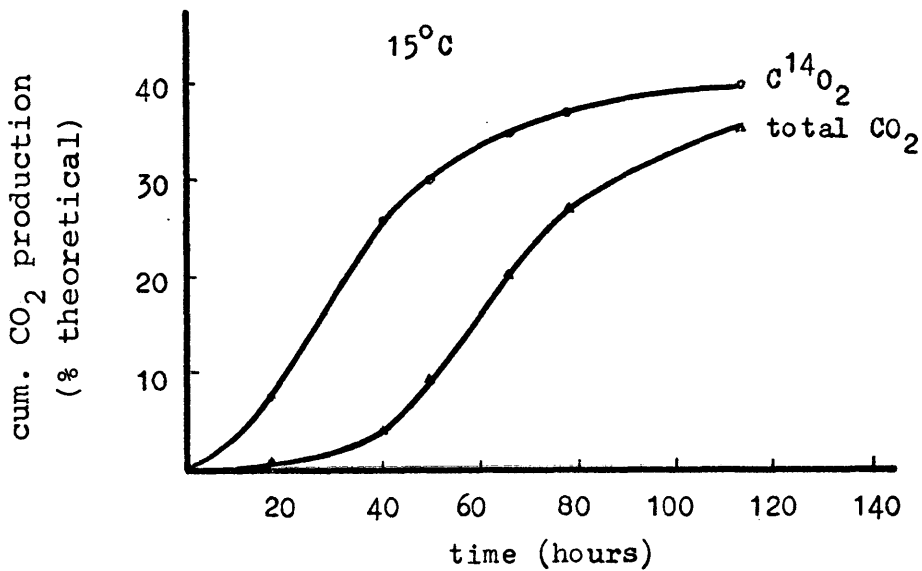


Figure 12a. Rate of CO_2 production from the degradation of sucrose monolaurate (1- C^{14} on laurate) in synthetic media with S_{13} , at 20 ppm, $27^\circ C$, pH 7.1



(b)



(c)

Figure 12b-c. Rate of CO_2 production of the degradation of sucrose monolaurate (1- C^{14} on laurate) in synthetic media with S_6 , at 20 ppm, pH 7.1

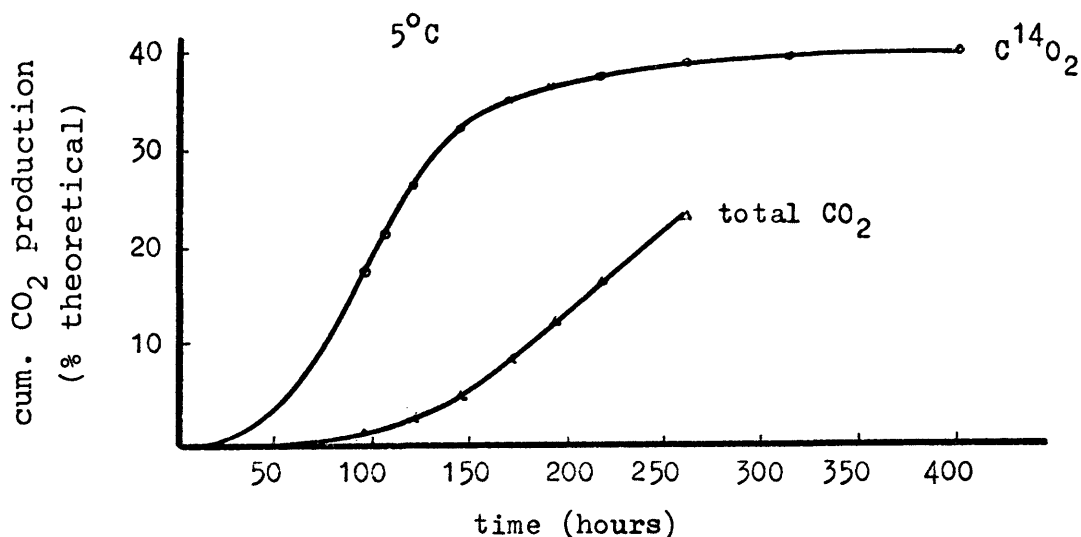


Figure 12d. Rate of CO_2 production from the degradation of sucrose monolaurate ($1-C^{14}$ on laurate) in synthetic media with S_6 , at 20 ppm, pH 7.1

In Figure 12a through 12d $C^{14}O_2$ was generated soon after the solution was inoculated. The rate suddenly increased, and then leveled off while the rate of the total CO_2 production gradually increased. With S_6 this behavior was more readily indicated at lower temperature.

From the above results it can be concluded that the degradation of sucrose esters takes place essentially via hydrolysis of the ester linkage. These observations then suggest that the hydrolysis of the ester linkage is the first step of the degradation process. It seems quite reasonable to infer that the products of hydrolysis, sucrose and fatty acid, will be degraded according to the general metabolic pathways which have been described elsewhere (such as in 58,59,60). A brief summary of the degradation pathways, gathered from the above references, is shown in Figure 12e-h.

Figure 12e shows the pathway of sucrose to pyruvic acid. Sucrose is hydrolyzed to fructose and glucose. The fructose is then oxidized

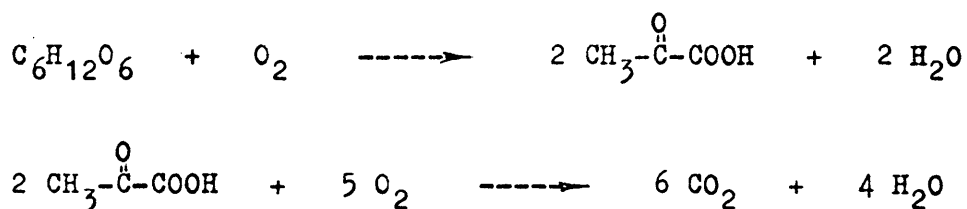
to pyruvic acid via fructose-1-phosphate. There are three different pathways for the oxidation of glucose:

- (1) Pantose phosphate pathway
- (2) Embden-Meyerhof glycolytic pathway
- (3) Entner-Dondoroff pathway

About 70 to 90% of glucose is oxidized according to the Embden-Meyerhof pathway. Up to the step of pyruvate this pathway can operate under anaerobic as well as aerobic conditions.

This step is followed by oxidation of the pyruvic acid to CO_2 and H_2O as shown in Figure 12f. No oxygen is taken up in this oxidation; instead, water is added and dehydrogenated several times. The hydrogen is picked up by the appropriate coenzymes and brought to the reaction with oxygen in the respiratory chain.

A complete oxidation of 1 mole glucose via the Embden-Meyerhof pathway and tricarboxylic acid cycle in conjunction with respiratory chain will produce 6 moles of CO_2 and 6 moles of H_2O :



Under anaerobic conditions the metabolic end products of biodegradation are normally CO_2 and CH_4 (1, p.188;56). Maximum free energy is released when the final end products are CO_2 and CH_4 . A scheme of the conversion of glucose to CO_2 and CH_4 summarized from (58,59) is shown in Figure 12g.

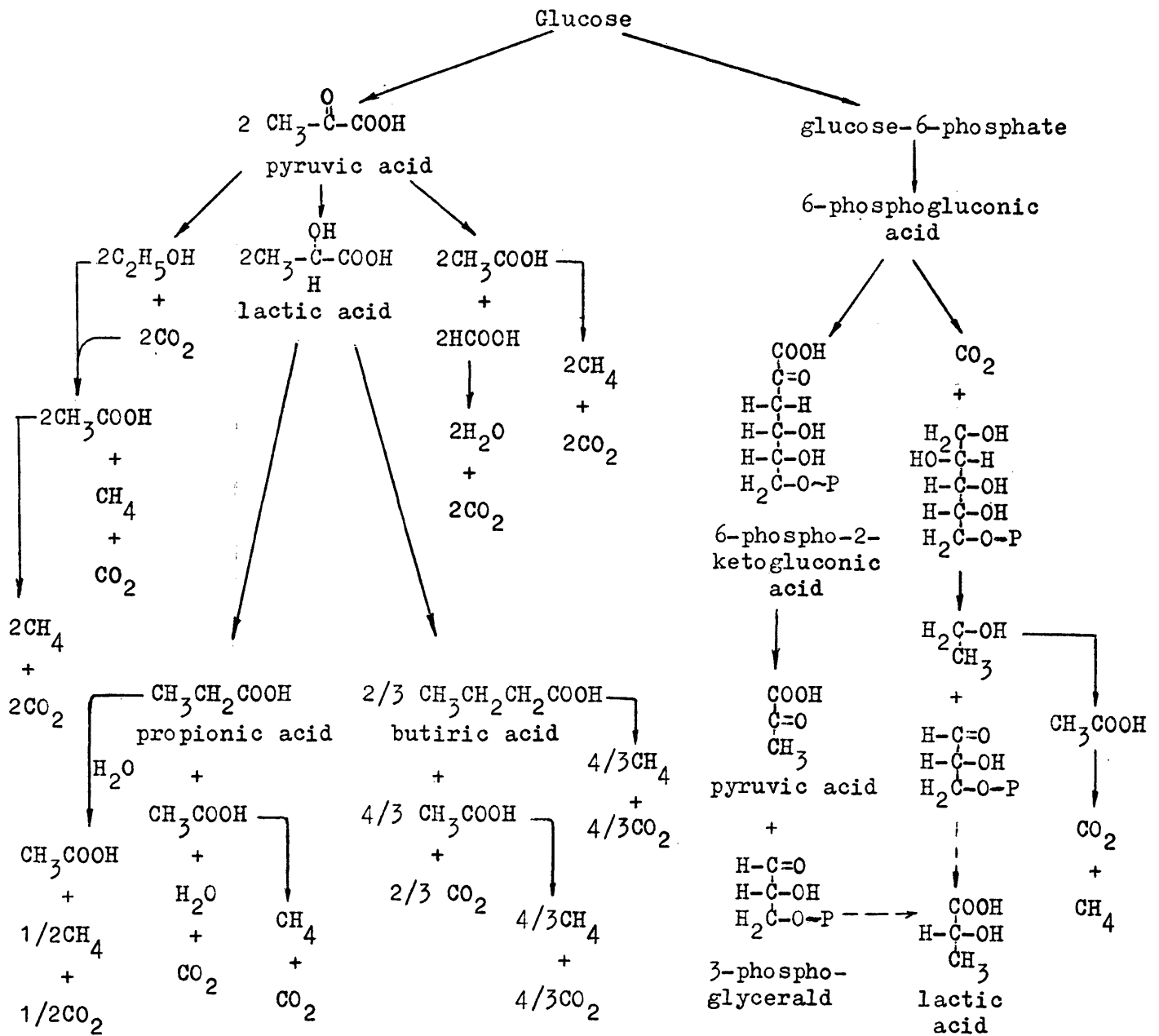
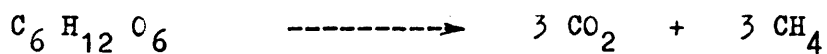


Figure 12g. Summary of the fermentation of glucose to CO_2 and CH_4 .

A complete methane fermentation of one mole of glucose will produce 3 moles of CO_2 and 3 moles of CH_4 .



The break down of fatty acids in microorganisms generally follows the β -oxidation mechanism (58,59). The fatty acid is converted to its coenzyme A derivative (an energy rich thioester) and then oxidized in a series of stages as shown in Figure 12h.

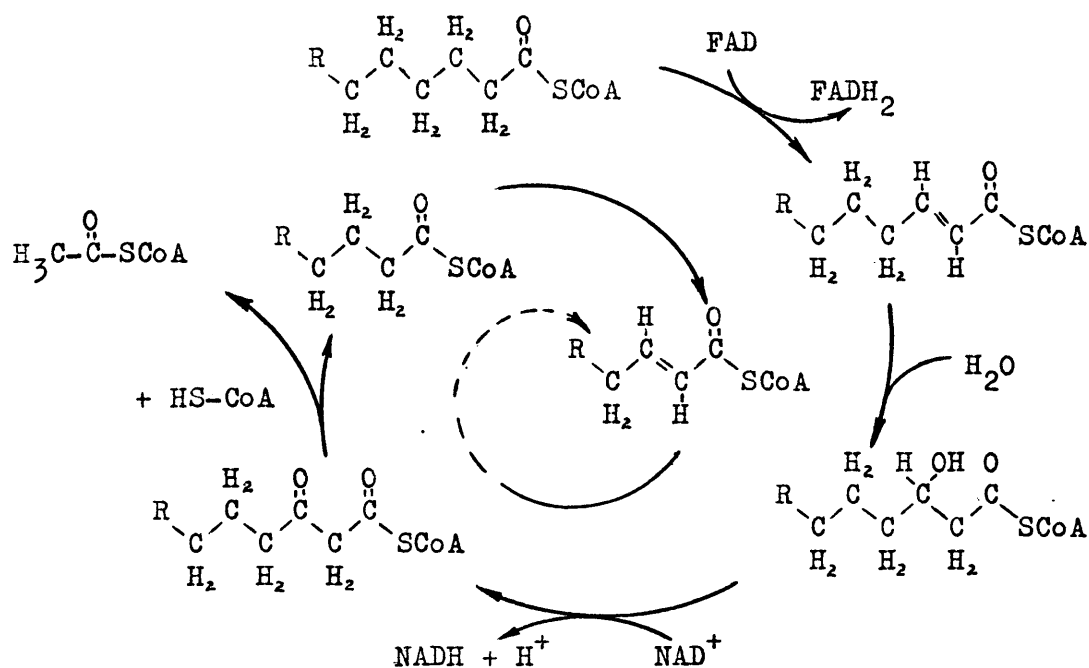


Figure 12h. β -oxidation of fatty acids (59).

Two carbon atoms are split off at a time in the form of acetyl-CoA. Under aerobic conditions the acetyl-CoA then enters the tricarboxylic acid cycle in which it is oxidized to CO₂. The H (bound to the coenzyme) will react with the O₂ in the respiratory chain. In an anaerobic system the oxidation of fatty acids also proceeds according to the β -oxidation mechanism (56); the liberated acetate is oxidized to CO₂ and CH₄ by methane bacteria. The H acceptor in this case might be a carbon compound such as CO₂ (8H + CO₂ → CH₄ + 2H₂O), or a sulfur compound such as inorganic sulfate (which would be reduced to H₂S). Since our anaerobic study employed anaerobic sludge, the presence of a H acceptor and the methane bacteria seems plausible.

C O N C L U S I O N

The Test Method: The CO₂ generation method used in the above biodegradation experiments shows a reasonably good reproducibility, particularly of the maximum biodegradation rates. The lag phase and the die-away curves, sometimes show variations; this is so even with the tests employing C¹⁴-sucrose ester, which has a small analytical error and no uncertainty due to endogenous respiration (see Figures 2b, p.30; 5a-b, p.39-40; 5g-h, p.43). This might be due to some uncontrolled variables that affect the viability of the bacteria.

The Sucrose Esters: Studies on rates of ultimate biodegradation of pure sucrose monolaurate, sucrose monolaurate (C¹⁴UL sucrose), and sucrose monolaurate (1-C¹⁴on laurate) have shown that the sugar ester is readily biodegradable in synthetic media with river bacteria, river water, and in sludge under aerobic and anaerobic conditions. Studies in synthetic media have shown that the biodegradability of the sugar ester is almost comparable to that of glucose, sucrose, and lauric acid, and is superior to that of the surfactants used in domestic detergent, C₁₂LAS and tergitol 15-S-9.

The aerobic batch sludge die-away test shows that the rate of CO₂ production from sucrose monolaurate is slower than that of glucose and sucrose only at the very early period of incubation; then

the ultimate degradation proceeds as fast as glucose and sucrose. Therefore, in a sewage treatment plant, if sucrose monolaurate is fed continually to the system, the ester would be removed and converted to CO_2 and H_2O in about the same rate as glucose and sucrose.

Complete ultimate biodegradation of the ester is readily achieved in river water and in sludge under aerobic and anaerobic conditions at 27°C .

Studies of the temperature effect using synthetic media have shown that within the range of 15° to 27°C , temperature has little effect on rate. At 5°C , the rate decreases substantially. However, at that temperature the rate of conversion of 1-C on laurate to CO_2 is still quite rapid, though much slower than at 15° and 27°C . This indicates that if the sugar ester is present in natural water, degradation will occur even in winter.

Studies on various kinds of sucrose esters with fatty acid chain length ranging from C_{12} to C_{22} show that the ester with C_{12} fatty acid (sucrose monolaurate) displays the highest biodegradation rate. The ester with C_{14} fatty acid (sucrose monomyristate) shows a slightly slower rate. A mixed ester with C_{16} and C_{18} fatty acids (Nitto ester) shows a rate of about the same as the C_{14} fatty acid ester. A mixed ester of C_{18} , C_{20} , and C_{22} fatty acids (sucrose monostearate, arachidate, behenate) shows a very slow rate of biodegradation. Sucrose monolaurate would be the best detergent among its higher series in the curtailment of water pollution caused by detergent residues.

Biodegradation of sucrose esters proceeds essentially via hydrolysis of the ester linkage. This behavior offers a great advantage:

After hydrolysis takes place, the products will be sucrose and fatty acid, two natural products whose ultimate biodegradabilities under aerobic and anaerobic conditions are well known.

The ability of sucrose monoesters to undergo degradation under anaerobic condition (as shown by sucrose monolaurate) indicates that these surfactants would be degraded even in septic tanks, cesspools, and in underground water, where sufficient oxygen is not available. Under those conditions LAS are not readily degraded.

The results of these studies lead to the conclusion that if sucrose mono-fatty acid esters (with fatty acids not higher than C₁₈) are used instead of LAS in detergent formulations, any water pollution problem due to detergent residues might be largely solved even in areas where sewage treatment is non-existent or inadequate.

A P P E N D I X I

Table 1. Rate of CO₂ production from the degradation of glucose (C¹⁴ UL) in synthetic media with S₆, at 23 ppm, 27°C, initial pH 7.0, final pH 6.9

Time (hours)	blank		glucose (C ¹⁴ UL)			
	mg CO ₂	mg CO ₂	% CO ₂ **	mg CO ₂ -blank	% CO ₂ -blank	% C ¹⁴ O ₂ ***
14	--	0.06				
24.5	0.10*	0.13	0.31	0.03	0.07	0.17
37.5	0.35	0.46	1.09	0.11	0.26	1.11
46	0.52	7.61	18.0	7.09	16.7	16.0
52	0.66*	14.7	34.7	14.0	33.1	31.9
61	0.71	18.7	44.4	18.0	42.6	41.5
74.5	0.78	20.8	49.3	20.0	47.3	47.0
115	0.78*	22.1	52.2	21.3	50.4	49.8

activity remained in solution****						
(% initial)						42.3

activity recovered						92.1%

* These values were determined experimentally, while the others were taken from graph of cum. mg CO₂ produced in blank vs time.

** Cum. CO₂ production (% theoretical) without subtracting the amount of CO₂ produced by blank.

*** Counting error = ± 0.2 to 0.3 %

**** The bacteria were not removed from the solution.

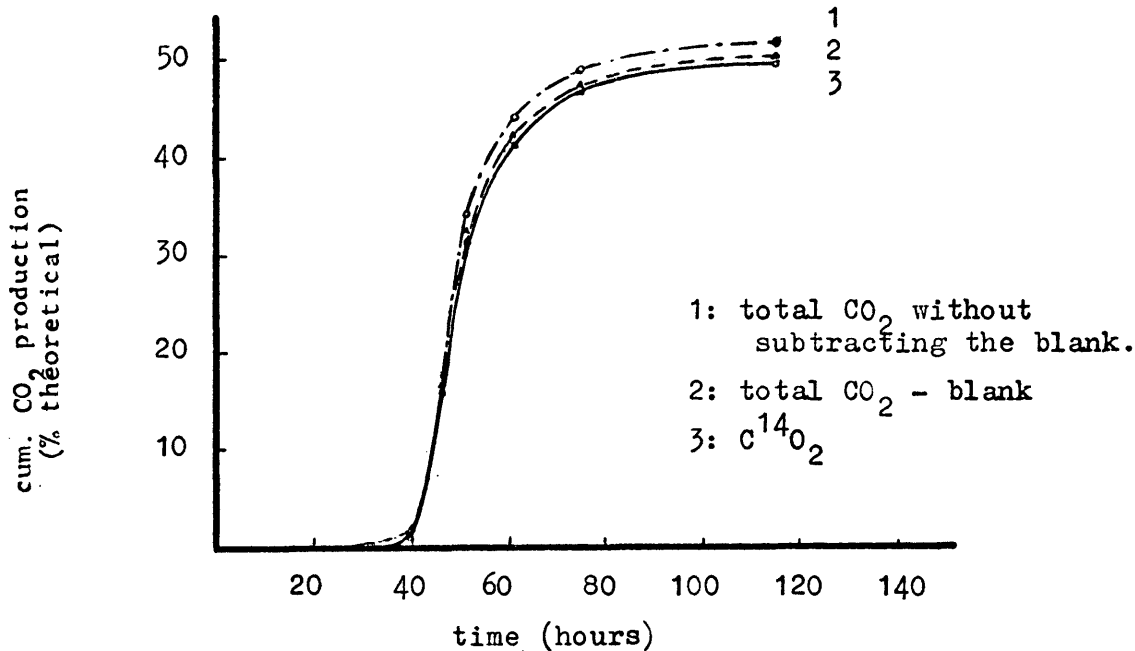
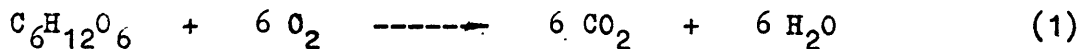


Figure 13. Rate of CO₂ production from the degradation of glucose ²(C¹⁴UL) diluted with reagent grade glucose in synthetic media with S₆, at 23 ppm, 27°C, pH 7.0

Figure 13 shows the rate of cumulative CO₂ production in % theoretical calculated from the equation:



Thus, for complete oxidation, 23 ppm glucose (28.9 mg in 1250 ml solution) will produce:

$$\frac{28.9 \text{ mg}}{180 \text{ g/mole}} \times 6 \times 44.0 \text{ g/mole} = 42.4 \text{ mg CO}_2$$

in which 180 g/mole = mole wt of glucose
44.0 g/mole = mole wt of CO₂

Curve 1, rate of total CO₂ production in which endogenous respiration was neglected:

$$\frac{\text{cum. CO}_2 \text{ production}}{\text{at time } t} \left(\frac{\% \text{ theoretical}}{\% \text{ theoretical}} \right) = \frac{\text{cum. mg CO}_2 \text{ production in time } t}{42.4 \text{ mg}} \times 100\%$$

For example:

$$\begin{aligned} \text{cum. CO}_2 \text{ production} &= 14.7 \text{ mg} \\ (\% \text{ theoretical}) &= \frac{\quad}{42.4 \text{ mg}} \times 100\% \quad (\text{see table 1}) \\ \text{in time} = 52 \text{ hrs} & \\ &= 34.7\% \end{aligned}$$

Curve 2, the amount of CO₂ produced was subtracted by the CO₂ produced in the blank at the corresponding time:

$$\begin{aligned} \text{cum. CO}_2 \text{ production} &= \frac{(\text{cum. mg CO}_2 \text{ produced}) - (\text{cum. mg CO}_2 \text{ produced})}{42.4 \text{ mg}} \times 100\% \\ (\% \text{ theoretical}) &= \frac{\text{in time } t \quad \text{by blank in time } t}{} \end{aligned}$$

For example:

$$\begin{aligned} \text{cum. CO}_2 \text{ production} &= \frac{14.7 \text{ mg} - 0.66 \text{ mg}}{42.4 \text{ mg}} \times 100\% \quad (\text{see table 1}) \\ (\% \text{ theoretical}) & \\ \text{in time} = 52 \text{ hrs} & \\ &= 33.1\% \end{aligned}$$

Curve 3, rate of C¹⁴O₂ production: According to equation (1), all of the carbon from glucose could be converted to CO₂. Therefore, if the oxidation is complete, all of the C¹⁴ in glucose would be converted to C¹⁴O₂, and all of the C¹⁴ activity in glucose would be found as C¹⁴O₂. Hence, cumulative C¹⁴O₂ production (% theoretical) could be calculated from the cumulative activity found in the CO₂ absorber divided by the initial activity of the substrate.

Because the glucose was uniformly labeled, therefore, theoretically, the curve representing the rate of total CO₂ production should coincide with that of C¹⁴O₂. The C¹⁴O₂ curve might be expected to be slightly lower, because C¹⁴ has a higher mass than C¹²; hence the reaction rate might be slightly slower. Curve 2, in which the CO₂ produced was subtracted by the CO₂ produced by blank at the corresponding time, shows good agreement with the prediction.

On the basis of this result a blank was always run in conjunction with the test samples, and the CO_2 production was subtracted. Endogenous respiration was assumed to be the same in fed samples and in the blank, though it was not necessarily true for different types of substrate, seed, and conditions. However, for most of the experiments only information regarding relative rates were required; thus, uncertainty due to endogenous respiration might compensate to some extent from one experiment to another.

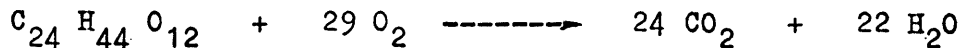
In attempting to calculate the radioactive material balance, % activity found as C^{14}O_2 + % activity in the culture at the end of the test, which in this experiment = 49.8% + 42.3% (see table 1), indicated that 92.1% of the initial activity was recovered. Failure to achieve 100% recovery may be due to adsorption of the radioactive intermediates onto the wall of the incubation flask and its content, particularly on the fritted glass tubing, which has a very large surface area.

A P P E N D I X II

Table 2a. Rate of CO₂ production from the degradation of sucrose monolaurate in synthetic media with S₆, S₁₃ and S₀, at 20 ppm, 27°C, pH 7.1 - 7.0

S ₆		S ₁₃		S ₀	
time (hours)	% CO ₂	time (hours)	% CO ₂	time (hours)	% CO ₂
10	0.97	36	--	12	0.25
48	18.7	106	0.96	17	1.82
72	29.8	128	1.54	25	10.4
96	37.2	154	5.96	36	17.2
120	46.1	161	8.66	61	31.8
144	48.6	177	16.0	87	44.2
		184	18.2	113	51.7
		204	27.2	161	58.5
		228	38.5	216	62.4
		252	42.9	316	66.8

Note: %CO₂ = cumulative CO₂ production (% theoretical) which was determined as described in Appendix I. The equation for complete oxidation is



Theoretically, 20 ppm of sucrose monolaurate (25.0 mg per 1250 ml solution) would produce

$$\frac{25 \text{ mg}}{524 \text{ g/mole}} \times 24 \times 44.0 \text{ g/mole} = 50.4 \text{ mg CO}_2$$

Table 2b. Rate of CO₂ production from the degradation of sucrose monolaurate and glucose in synthetic media with S₆, at 20 ppm and 23 ppm respectively, 27°C, pH 7.1

sucrose monolaurate			glucose					
	I	II	I	II	III			
time (hours)	% CO ₂	% CO ₂	time (hours)	% CO ₂	time (hours)	% CO ₂	time (hours)	% CO ₂
10	0.97	--	24.5	0.07	24	0.41	12	--
48	18.7	12.1	37.5	0.26	35	2.50	25	10.2
72	29.8	22.8	46	16.7	48	31.7	30	20.1
96	37.2	31.1	52	33.1	52	36.2	35	29.2
120	46.1	38.5	61	42.6	58	41.2	42	36.9
144	48.6	40.6	74.5	47.3	70	46.0	62	42.6
168		44.5	115	50.4	96	49.4	160	47.8
					145	51.6		

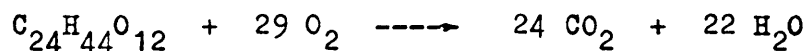
Table 2c. Rate of $C^{14}O_2$ production from the degradation of sucrose monolaurate (C^{14} UL sucrose) and sucrose (C^{14} UL) in synthetic media with S_6 , at 20 ppm, 27°C, pH 7.0 - 6.9

sucrose monolaurate(C^{14} UL sucrose)		sucrose(C^{14} UL)	
time (hours)	% $C^{14}O_2$	time (hours)	% $C^{14}O_2$
13	0.18	24	0.10
20.5	1.78	48	0.56
26	5.73	58	3.00
33	13.3	70	21.0
38	18.8	78	30.2
45	25.4	83	34.5
51	29.9	96	38.5
76	37.5	121	41.7
99	40.6	147	43.4
147	44.6	198	45.1
223	49.1	267	46.6
----- $C^{14}O_2$ obtained after acidifying the soln. at the end of the test (% theoretical)		0.22	
----- activity remained in solution (% initial)		38.9	45.6
----- activity recovered		88.2%	92.2%

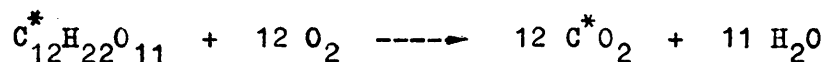
Table 2d. Rate of CO₂ production from the degradation of sucrose monolaurate (C¹⁴UL sucrose) and lauric acid in synthetic media with S₆ at 20 ppm, 27°C, pH 7.0 - 6.9

sucrose monolaurate (C ¹⁴ UL sucrose)				lauric acid	
time (hours)	% total CO ₂	% C ¹⁴ O ₂	% CO ₂ (laurate)	time (hours)	% CO ₂
10	--	--	--	17	0.79
48	12.1	10.0	14.2	30	7.50
72	22.8	22.5	23.1	48	25.9
96	31.1	32.6	29.6	65	33.6
120	38.5	42.4	34.6	80	37.1
144	40.6	45.8	35.4	100	39.3
168	43.2	47.4	38.9		

$$\% \text{ total CO}_2 = \frac{\text{weight of total CO}_2 \text{ (tagged and untagged)}}{\text{theoretical amount of CO}_2 \text{ obtained from complete oxidation of sucrose monolaurate according to the equation:}}$$

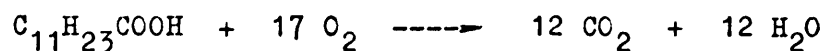


$$\% \text{ C}^{14}\text{O}_2 = \frac{\text{amount of C}^{14}\text{O}_2}{\text{theoretical amount of C}^{14}\text{O}_2 \text{ obtained from complete oxidation of the sucrose portion of the ester:}}$$



$$= \frac{\text{activity recovered as C}^{14}\text{O}_2}{\text{initial activity of the ester}}$$

$$\% \text{CO}_2(\text{laurate}) = \frac{\text{weight of CO}_2 \text{ originated from the laurate portion of the ester}}{\text{theoretical amount of CO}_2 \text{ obtained from complete oxidation of the laurate portion of the ester:}}$$



$$\% \text{CO}_2(\text{laurate}) = 2 \times \% \text{ total CO}_2 - \% \text{ C}^{14}\text{O}_2$$

Table 3. Rate of $C^{14}O_2$ production from the degradation of sucrose monolaurate ($C^{14}UL$ sucrose) in synthetic media with S_6 , at 20 ppm, 27°C, pH 7.0, using different flow rate of air stream.

flow rate:	32 ± 3 ml/min			9 ± 1 ml/min		
time (hours)	A	B	C	A	B	C
13	0.14	0.25	0.39	0.18		
20.5	0.31	0.33	0.64	1.78	0.77	2.55
26	0.78	1.25	2.03	5.73	7.40	13.1
33	4.06	5.11	9.17	13.3	9.65	23.0
38	9.45	6.51	16.0	18.8	9.28	28.1
45	17.0	7.40	24.4	25.4		
51	23.7			29.9	5.38	35.3
55		4.13				
60	29.7					
76	34.1	1.40	35.5	37.5	1.93	39.4
99	40.2			40.6		
147	44.8			44.6		
223	48.1	0.16	48.3	49.1	0.22	49.3
----- activity remained in solution (% initial)			42.1			38.9
----- activity recovered			90.4%			88.2%

A = % activity found as $C^{14}O_2$ trapped in ethanolamine.

B = % activity found as $C^{14}O_2$ and $HC^{14}O_3^-$ in solution.

C = A + B

For the experiment with the flow rate of 32 ml per min, an additional absorption vial was set in series. The activity found in the second vial did not exceed 0.3% of the first vial.

Table 4. Rate of CO₂ production from the degradation of sucrose mono-laurate (C¹⁴UL sucrose) in synthetic media with S₆, at 27°C, pH 7.0, with different ester concentration.

Run I							
time (hours)	10.0 ppm		20.0 ppm		41.5 ppm		blank
	total CO ₂ (mg)	C ¹⁴ O ₂ (mg)	total CO ₂ (mg)	C ¹⁴ O ₂ (mg)	total CO ₂ (mg)	C ¹⁴ O ₂ (mg)	CO ₂ (mg)
10	0.53	--	0.44	--	1.12	--	
48	9.24	2.72	8.88	2.52	17.5	5.24	2.83
56					22.1	7.55	
72	11.8	4.18	14.7	5.66	28.1	10.2	
96	13.5	5.07	20.5	8.21	37.9	15.1	5.06
120	14.6	5.71	25.2	10.7	45.9	19.8	
144	15.0	5.97	26.7	11.6	51.1	22.8	6.25

Run II							
time (hours)	10.0 ppm		20.0 ppm		41.8 ppm		blank
	total CO ₂ (mg)	C ¹⁴ O ₂ (mg)	total CO ₂ (mg)	C ¹⁴ O ₂ (mg)	total CO ₂ (mg)	C ¹⁴ O ₂ (mg)	CO ₂ (mg)
20.5	0.96	--	1.0	--	1.25	--	0.74
36.5	4.46	0.79	7.97	3.07	12.4	3.73	
48	6.01	1.45	10.6	4.66	20.8	7.07	2.65
60	7.78	2.22	14.2	6.70	26.1	9.70	
72	9.21	2.91	16.9	8.36	31.3	12.6	
96	12.0	3.94	19.9	10.1	40.4	16.3	5.91
120	13.5	4.50		11.1	43.0	18.7	6.45

Table 5a. Rate of CO₂ production from the degradation of sucrose mono-
laurate in synthetic media with S₆, at 20 ppm, pH 7.1 - 7.0

27°C ± 1°C				15°C ± 1°C			5°C ± 1°C		
I		II		I	II	I		II	
time (hours)	%CO ₂	time (hours)	%CO ₂	time (hours)	%CO ₂	%CO ₂	time (hours)	%CO ₂	%CO ₂
17	1.09	20.5	2.38	17	0.65	0.92	97	1.29	1.24
24	2.34	28	5.38	39.5	2.48	3.73	108		1.59
30	9.25	36.5	14.2	49	5.20	9.10	120	2.10	2.69
39.5	22.6	48	22.6	65	14.8	20.5	144	3.31	4.55
49	29.2	60	27.8	77	21.1	26.8	170	7.10	8.20
65	33.1	96	32.4	89	27.1		192	10.5	12.3
89	34.7	120	34.9	113	29.3	34.4	216	14.3	16.8
							264	20.0	23.1
							312	23.2	

Table 5b. Rate of $C^{14}O_2$ production from the degradation of sucrose monolaurate($C^{14}UL$ sucrose) and sucrose monolaurate($1-C^{14}on$ laurate) in synthetic media with S_6 , at 20 ppm, pH 7.1 - 7.0; at 27°C, 15°C, and 5°C.

sucrose monolaurate($C^{14}UL$ sucrose)														
27°C ± 1°C				15°C ± 1°C				5°C ± 1°C						
I		II		I		II		III		IV		I		
time (hr)	% $C^{14}O_2$	time (hr)	% $C^{14}O_2$	time (hr)	% $C^{14}O_2$	% $C^{14}O_2$	time (hr)	% $C^{14}O_2$	time (hr)	% $C^{14}O_2$	time (hr)	% $C^{14}O_2$	time (hr)	% $C^{14}O_2$
17	--	13	0.18	47	--	--	39.5	0.29	29	0.13	120	0.55		
24	0.47	20.5	1.78	58	1.02	1.18	49	1.48	44	0.41	144	1.45		
30	5.28	26	5.73	72	6.31	7.32	65	8.90	53	1.33	170	4.85		
39.5	24.7	33	13.3	82	13.1	13.3	77	15.1	60	3.29	192	8.61		
49	34.5	38	18.8	94	17.6	17.5	89	19.3	70	6.18	216	12.5		
65	41.0	45	15.4	106	19.7	20.0	113	25.1	83	12.7	264	18.7		
89	44.7	51	29.9						99	21.0	312	21.8		
		76	37.5						131	30.0	408	23.6		
		99	40.6						156	33.4	504	24.4		
		147	44.6						223	36.8				
		223	48.1											

sucrose monolaurate($1-C^{14}on$ laurate)							
27°C ± 1°C		15°C ± 1°C		5°C ± 1°C			
time (hr)	% $C^{14}O_2$	time (hr)	% $C^{14}O_2$	time (hr)	% $C^{14}O_2$	time (hr)	% $C^{14}O_2$
20.5	9.70	17	7.45	97	16.7		
28	16.7	39.5	25.6	108	21.8		
36.5	25.8	49	29.6	120	26.5		
48	32.7	65	34.3	144	32.3		
60	38.5	77	36.4	170	35.0		
96	42.6	113	39.4	192	36.5		
120	45.9			216	37.6		
				264	38.8		
				312	39.4		
				408	40.5		

Table 5c. Rate of $C^{14}O_2$ production from the degradation of sucrose monolaurate ($C^{14}UL$ sucrose) in synthetic media with S_6 , at 20 ppm, pH 7.0; 27° and $15^\circ C$.

time (hours)	$27^\circ C \pm 1^\circ C$			$15^\circ C \pm 1^\circ C$					
	A	B	C	A	B	C	A	B	C
20.5	1.78	0.77	2.55						
26	5.73	7.40	13.1						
29				0.13			0.11		
33	13.3	9.65	23.0						
38	18.8	9.28	28.1						
44				0.41	0.57	0.98	0.91	2.02	2.93
45	25.1								
51	29.9	5.38	35.3						
53				1.33	2.38	3.71	2.86	4.36	7.22
60				3.29	4.02	7.31	5.08	4.99	10.1
70				6.18	5.83	12.0	8.67	5.86	14.5
76	37.5	1.93	39.4						
83				12.7	5.21	17.9	14.8	5.86	20.7
99	40.6			21.0	4.77	25.8	22.2	4.95	27.2
131				30.0	2.10	32.1	31.6	2.59	34.2
147	44.6								
156				33.4			35.3		
223	49.1	0.22	49.3	36.8	0.38	37.2	39.2	0.63	39.8
----- activity remained in soln. (% initial) -----			38.9			50.7			49.5
activity recovered			88.2%			87.9%			89.3%

A = % activity found as $C^{14}O_2$ trapped in ethanolamine.

B = % activity found as $C^{14}O_2$ and $HC^{14}O_3^-$ in solution.

C = A + B

Table 6. Rate of CO₂ production from the degradation of pure sucrose monolaurate and sucrose monomyristate in synthetic media with S₆, at 20 ppm, 27°C, pH 6.9

time (days)	% CO ₂	
	sucrose monolaurate	sucrose monomyristate
0.5	0.24	--
2.5	4.60	4.48
3.5	8.01	8.45
4.5	14.4	10.52
5.5	22.8	17.6
6.5	27.8	23.0
7.5	34.7	27.4
10.5	44.6	
11.5		35.3
13.5	51.4	
14.5		41.5
15.2	54.4	
17.5		47.0
21.5		53.4

Note: The seed used in this experiment was not previously acclimatized. It was taken from agar slant and suspended in saline water.

Table 7. Rate of CO₂ production from the degradation of sucrose mono-laurate; sucrose monomyristate; sucrose mono-stearate, arachidate, behenate; and Nitto ester in synthetic media with S₆, at 12 ppm, 27°C, pH 7.1

	time (hours)	% CO ₂ *			
		laurate	myristate	stearate arachidate behenate	Nitto ester
Run I	6.8	0.33	0.51		0.33
	21	3.55	6.08	1.92	2.66
	36.5	15.0	13.8	3.38	10.9
	49.5	20.0	20.1	4.61	15.7
	65	22.7	23.4	6.35	18.7
	91	24.5	27.0	7.43	22.0
	121	27.1	31.0	8.30	25.0
	162			9.07	
	210			10.2	
Run II	12	0.93	0.51	0.47	0.36
	24	5.51	4.67	1.73	3.07
	36	16.0	11.9	3.56	10.6
	48	25.2	15.5	4.42	15.4
	60	30.4	17.9	5.09	18.3
	77	33.4	20.2	5.70	20.4
	146	40.7	27.6	8.38	26.2

* 100% CO₂ is based on the amount of CO₂ obtained from the dichromate-H₂SO₄ (HgSO₄) oxidation (50).

Table 8a. Rate of $C^{14}O_2$ production from the degradation of sucrose mono-laurate ($C^{14}UL$ sucrose) in river water from Clear Creek, at 12 ppm, 27° and $15^\circ C$, pH 8.4 - 8.3

time (hours)	$27^\circ C \pm 1^\circ C$						$15^\circ C \pm 1^\circ C$		
	A	B	C	A	B	C	A	B	C
38	0.53	4.63	5.16	0.43	5.30	5.73	0.10	2.14	2.24
85	3.30	14.0	17.3	2.76	12.3	15.1			
142	9.22	30.1	39.3	7.56	24.6	32.2	3.35	13.1	16.5
179	13.5	28.0	41.5	11.8	27.3	39.1			
229	18.9	27.4	46.3	16.8	27.1	43.9	6.58	22.3	28.9
285	24.3	26.1	50.4	22.3	29.9	52.2	9.68	26.1	35.8
333	27.8			25.8	29.9	55.7	12.0	26.7	38.7
381	30.7	27.3	58.0	28.9	28.6	57.5	14.2	28.3	42.5
----- activity remained in soln. (% initial)			30.8			29.7			44.5
----- activity recovered			88.8%			87.2%			87.0%

A = % activity found as $C^{14}O_2$ trapped in ethanolamine.

B = % activity found as $C^{14}O_2$ and $HC^{14}O_3^-$ in solution.

C = A + B

Table 8b. Apparent rate of $C^{14}O_2$ production from the degradation of sucrose monolaurate ($C^{14}UL$ sucrose) and sucrose monolaurate ($1-C^{14}$ on laurate) in river water from South Platte River, at 20 ppm, pH 8.4 - 8.3

time (hours)	% $C^{14}O_2$		
	sucrose monolaurate ($C^{14}UL$ sucrose) 27°C	sucrose monolaurate 27°C	($1-C^{14}$ on laurate) 15°C
21.5		5.86	0.95
23	4.53		
31		8.67	
32.5	7.20		
45		11.5	
46.5	9.76		4.60
68		14.8	7.25
69.5	13.3		
93		17.5	9.35
94.5	16.1		
116		20.8	10.9
118	18.9		
164		25.3	13.3
166	24.7		
213		28.6	15.4
215	28.8		
285		32.3	17.7
287	33.1		
381		35.2	21.0
383	36.8		
473		37.3	23.2
475	39.5		

$C^{14}O_2$ obtained after acidifying the river water at the end of the test (% theoretical)	53.4	49.0	35.9

total $C^{14}O_2$ production (% theoretical)	92.9	86.3	59.1

Table 9a. Rate of $C^{14}O_2$ production from the degradation of sucrose monolaurate ($C^{14}UL$ sucrose) and sucrose monolaurate (1- C^{14} on laurate) in activated sludge, pH 7.0 - 7.2

time (hours)	% $C^{14}O_2$					
	sucrose monolaurate ($C^{14}UL$ sucrose)			sucrose monolaurate (1- C^{14} on laurate)		
	10.5 ppm		40 ppm		40 ppm	
	27°C		27°C	15°C	27°C	15°C
	I	II				
2.5			1.50	0.65	7.48	3.96
3.5	2.7	1.8				
9	15.6	12.5				
11			11.4	4.90	23.8	12.5
12	21.4	18.0				
17			22.1	8.33	32.5	17.6
18	31.8	26.4				
21	37.6	31.4				
24	42.1	37.0	34.6	12.1	40.2	21.3
28	45.8	42.5				
35.5			43.3	16.6	47.0	25.3
38	49.9	45.2				
48	53.4	49.6				
49			52.4	20.1	50.8	28.7
61			57.2	22.1	53.2	30.0
74	62.6	53.5				
85			62.5	25.3	56.1	31.5
116	70.0	60.1				
134			70.1	31.2	61.6	33.6
207			78.8	41.3	67.1	36.3
263			84.6	48.9	75.6	38.1
----- $C^{14}O_2$ obtained after acidifying the sludge at time = 263 hours (% theoretical)			2.99	7.98	3.06	3.02
----- total $C^{14}O_2$ production (% theoretical)			87.6	56.9	78.7	41.1
----- activity remained in soln.			15.9	34.2	14.0	46.5
----- activity recovered			103.5%	91.1%	92.7%	87.6%

Table 9b. Rate of $C^{14}O_2$ production from the degradation of sucrose monolaurate($C^{14}UL$ sucrose), glucose($C^{14}UL$), and sucrose($C^{14}UL$) in activated sludge, pH 7.0 - 7.2

time (hours)	% $C^{14}O_2$		
	sucrose monolaurate ($C^{14}UL$ sucrose)	glucose($C^{14}UL$)	sucrose($C^{14}UL$)
2.5	1.50	8.15	4.68
6.5		22.5	17.2
11	11.4	31.2	27.0
17	22.1		
21		42.3	39.3
24	34.6		
33		50.6	48.2
35.5	43.3		
45.5		55.1	53.6
49	52.4		
61	57.2		
73.5			60.2
80		62.9	
85	62.5		
134	70.1		
176		78.2	75.2
207	78.8		
263	84.6		

$C^{14}O_2$ obtained after acidifying the sludge at the end of the test (% theoretical)	2.99	4.60	5.10

total $C^{14}O_2$ production (% theoretical)	87.6	82.8	80.3

activity remained in the sludge (% initial)	15.9	14.3	17.8

activity recovered	103.5%	97.1	98.1

Table 10. Apparent rate of $C^{14}O_2$ production from the degradation of sucrose monolaurate ($C^{14}UL$ sucrose) and sucrose monolaurate ($1-C^{14}on$ laurate) in anaerobic sludge, at 40 ppm, pH about 8.

time (hours)	sucrose monolaurate ($C^{14}UL$ sucrose)				sucrose monolaurate ($1-C^{14}on$ laurate)	
	27°C		15°C		27°C	
	%activity recovered as $C^{14}O_2$	$C^{14}O_2$ % theoretical	%activity recovered as $C^{14}O_2$	$C^{14}O_2$ % theoretical	%activity recovered as $C^{14}O_2$	%activity recovered as $C^{14}O_2$ or %theoretical
10	4.02	8.04	1.68	3.36		7.80
22	9.35	18.7	3.37	6.74		17.0
37	14.1	28.2	5.15	10.3		23.9
61	18.5	37.0	7.12	14.2		30.8
85	21.8	43.6	9.02	18.0		35.2
133	26.4	52.8	12.1	24.2		41.2
165	28.9	57.8	13.9	27.8		43.9

$C^{14}O_2$ obtained after acidifying the sludge at the end of the test	19.0	38.0	16.1	32.2		22.7

total $C^{14}O_2$ production ²		95.8		60.0		66.6

Table 11. Rate of CO₂ production from the degradation of sucrose monolaurate, LAS, and tergitol 15-S-9 in synthetic media with S₀, at 20 ppm, 27°C, pH 7.0

time (hours)	sucrose monolaurate % CO ₂	LAS		time I (hours)	tergitol		
		% CO ₂	% CO ₂		%CO ₂	time II (hours)	%CO ₂
		I	II				
12	0.2	0.2	0.1	24	--	17.5	--
17	2.41			48	0.29	68	3.94
25	11.1			72	3.58	91	6.85
36	18.8	5.65	4.72	96	5.31	112	10.2
52		9.76	9.52	120	9.47	136	13.6
61	34.3	12.6	12.4	144	14.4	184	21.4
75.5		17.2	18.0	168	18.2	237	26.0
87	46.7			192	19.9		
96		21.6	23.4	288	28.7		
113	54.1			360	32.1		
121		25.7	27.9				
161	60.9	29.5	32.3				
216	64.9	36.0	38.2				
316	66.8	44.3	44.3				

CO ₂ obtained after acidifying the soln. at the end of the test (% theoretical)		1.9	1.7	1.4	0.54		0.77

total CO ₂ production (% theoretical)		68.7	46.0	45.7	32.6		26.8

Theoretical amount of CO₂ of LAS and tergitol were calculated from the following complete oxidation equation:

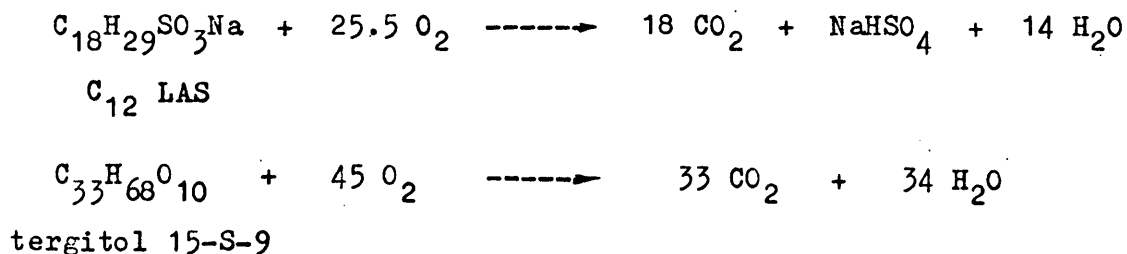


Table 12. Rate of CO₂ production from the degradation of sucrose monolaurate (1-C¹⁴ on laurate) in synthetic media with S₆, S₁₃, at 20 ppm, pH 7.1 - 7.0

			S ₆						S ₁₃		
			15°C			5°C			27°C		
27°C			15°C			5°C			27°C		
time (hr)	%C ¹⁴ O ₂	%CO ₂	time (hr)	%C ¹⁴ O ₂	%CO ₂	time (hr)	%C ¹⁴ O ₂	%CO ₂	time (hr)	%C ¹⁴ O ₂	%CO ₂
20.5	9.70	2.38	17	7.45	0.92	97	16.7	1.24	36	1.55	0.42
28	16.7	5.38	39.5	25.6	3.73	108	21.8	1.69	60	2.42	0.96
36.5	25.8	14.2	49	29.6	9.10	120	26.5	2.69	106	3.70	1.54
48	32.7	22.6	65	34.3	20.5	144	32.3	4.55	128	5.94	2.58
60	38.5	27.8	77	36.4	26.8	170	35.0	8.20	140	10.8	5.96
96	42.6	32.4	113	39.4	34.4	192	36.5	12.3	154	27.8	8.66
120	45.9	34.9				216	37.6	16.8	161	34.3	16.0
						264	38.8	23.1	177	42.1	18.2
						312	39.4		184	44.0	27.2
						408	40.5		204	47.1	38.5
									228	48.9	42.9
									252	50.5	

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