THE REMOVAL OF LINSEED OIL VAPORS BY BIODEGRADATION

THESIS

Presented to the Graduate Council of the

University of North Texas

Fullfillment of the Requirements

For the Degree of

MASTER OF SCIENCE

By

Patamaporn Sukplang B. S. (Med. Tech.)

Denton, Texas

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Linseed oil is very important in industry but its use is limited due to noxious vapors produced by oxidation on exposure to air. Since some of the products are toxic, release of linseed oil vapors to the environment is normally prohibited. In order to remove the odorous compounds, a biofilter system based on bacterial metabolism was designed and the major premises of bioremediation were studied.

A total of five bacterial strains capable of using linseed oil vapors as their sources of carbon and energy were isolated from soil. The individual organisms were also mixed to form a bacterial consortium. The mixed population was able to degrade linseed oil vapors with more than 99 per cent efficiency.

According to this research, a successful biodegradation system was designed and, theoretically, this system could be applied to the removal of linseed oil vapors in any industrial plant air stream.

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CHAPTER I

INTRODUCTION

1.1. Linseed oil

1.1.1. Historical background

Linseed oil is a product made from the seeds of the flax plant, *Linum usitatissimum*, grown in many temperate areas of the world including the United States, Argentina, India and Russia. The flax that is grown to produce fiber for making linen and the flax grown for its oil belong to the same species. The seeds of this plant are produced in globular pods containing about 10 long, flat, elliptical seeds that vary substantially in size. The typical seeds are 3 to 4 mm long, 2 to 3 mm wide, approximately 0.5 mm thick, and weigh 3 to 9 mg (11, 13). Crude linseed oil from flaxseed is obtained by expression or solvent extraction. Various refining and bleaching methods are used to produce a clear almost colorless product. The amount of oil in the seed is variable and depends on several factors including the variety of the plant, maturity of the seed, soil type the plant is grown in, and ambient temperature, rainfall, and other climatic conditions in which the plants are grown (13).

Crude linseed oil has a dark amber color and a strong characteristic odor. The odor of linseed oil and of other linolenic acid oils, seems to be associated to a large extent with the unsaturated fatty acid content of the oil. Oils with strong, objectionable odors can be deodorized by steaming, but upon sitting the odors return. Linseed oil is classified as a drying oil because of its tendency to form films on drying. The hard, water impermeable film is formed by oxidative polymerization. The industrial quality of linseed oil for use as a drying agent depends largely on the degree of unsaturation and on the iodine number. Since this characteristic is extremely variable, oils of different types are blended to obtain a product of consistent qualities. Because of its significance of the iodine number in this regard, the iodine number is used as sole criterion of the quality of the oil.

1.1.2. Unsaturation

Unsaturation refers to the number of carbon atoms that share more than one valence bond. These appear in chemical notation as C=C. Unsaturated fats, which generally come from plant sources, have at least one hydrogen missing from one of their fatty acids and the carbon-carbon double bond occurs instead. If there is only one double bond, the fatty acid is called monounsaturated; fatty acids with two or more double bonds are said to be polyunsaturated.

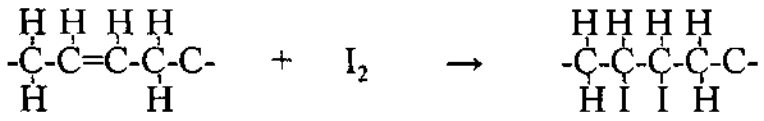
The drying properties of fats and oils are indicated by the number of unsaturated carbons in the fatty acids that make up the fat or oil. The amount is conventionally expressed as the iodine value. The measurement of unsaturation is an alternative to the determination of the individual fatty acids for the identification of natural oils, since natural oils have their own range of unsaturation values. The iodine value is a satisfactory measure of the relative drying time and speed of polymerization among a group of oils of the same type. Determination of the unsaturation of natural drying oils is described in the reference manual, Annual Book of ASTM Standards published by the American Society for Testing and Materials (2).

The reaction with oxygen is the most important characteristic of drying oils. Oxidation gives rise to *trans*-isomers, to polymers, and also to cleavage of the carbon chain with the formation of volatile products (11). A high degree of unsaturation makes desirable drying oils because of the high degree of autoxidation that leads to film formation and faster drying time. Oils which have low proportions of unsaturated fatty acids and low iodine values are not employed in the paint and coating industry. Polyunsaturated fatty acids react with oxygen at a far faster rate than monounsaturated ones. This fact explains why fats containing linoleic or linolenic acids

in large proportion are preferred for making drying oils, while those with oleic oils are less desirable. The avidity of fatty acids to cross link upon oxidation leads to the "drying" of the film. The amount and type of fatty acids determine the hardness and durability of the films produced.

1.1.3. Iodine value

The iodine value is a measurement of the unsaturation of oils and fatty acids and is expressed in terms of the number of centigrams of iodine per gram of sample (weight per cent of absorbed iodine). The determination of the iodine value of oils containing double bonds is based on the absorption of halogen under constant conditions (3).



The amount of iodine absorbed is determined by back titration of the excess reagent with sodium thiosulfate (NaS_2O_3) and comparing this to a blank determination. The iodine value (I) can be calculated as follows:

$$I = \frac{[(B-V)N \times 12.69]}{S}$$

V = NaS_2O_3 solution required for titration of specimen(ml)

B = NaS_2O_3 solution required for titration of blank (ml)

N = Normality of the NaS_2O_3 solution

S = Sample used (g)

The iodine number is a convenient test for expressing the degree of unsaturation of any fatty acid. According to such iodine values, oils are generally divided into three classes, drying, semidrying, and nondrying. Rheineck and Austin (36) classify oils with iodine values higher than 140 as drying oils, those between 125 and 140 are semidrying oils, and those below 125 as nondrying oils.

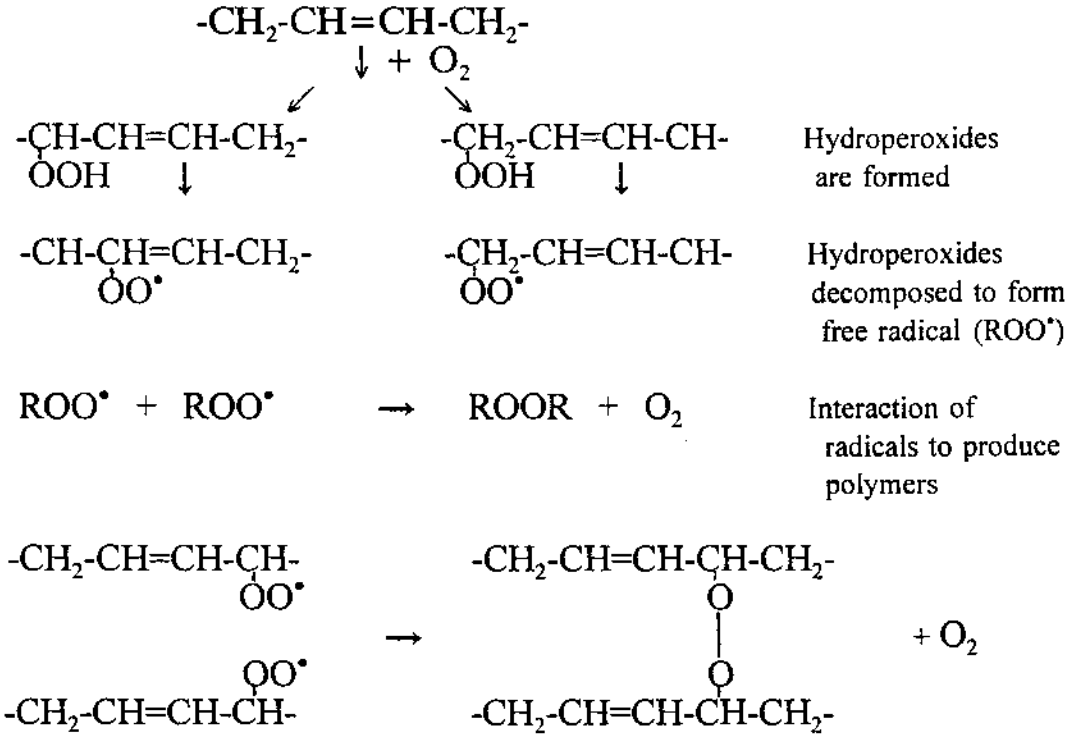
1.1.4. Characteristics and properties

Linseed oil is slighty less viscous than most vegetable oils. It contains both saponifiable and unsaponifiable fatty acids. The major fatty acid components are linolenic acid and linoleic acid which are found at levels of 50 to 55% and 20 to 25% of the total weight, respectively. The remainder are fatty acids of various kinds as shown in table 1.1 (13, 28, 32). The drying capacity is due primarily to the

Table 1.1 Fatty acid composition of linseed oil produced commercially in two different parts of America.

Fatty acids	Source	
	Argentina (per cent)	U.S.A (per cent)
Myristic	3.5	1.9
Palmitic	5.4	3.2
Stearic	2.8	2.9
Arachidic	1.8	1.1
Oleic	15.4	24.1
Linoleic	18.2	17.4
Linolenic	52.9	49.4

quantity of linolenic and linoleic acids. These are normally present in the form of glycerides. Such glycerides polymerize spontaneously on exposure to air since they fix oxygen and thereby are polymerized (16). The steps in film formation with linseed oil can be summarized as follows. First, an induction period in which little visible change in chemical or physical properties occurs but during which oxygen is absorbed. This follows a substantial increase in oxygen uptake with the appearance of hydroperoxides and conjugated dienes. Next, hydroperoxides form free radicals and the reaction becomes autocatalytic. Finally, the onset of chain cleavage and polymerization occurs. The reactions are shown as follows:



High molecular weight polymers are formed by cross-linking while low molecular weight products are formed by chain cleavage (11). The properties of the products formed as a result of these reactions make linseed oil highly useful in the manufacture of paints and related products.

Steamed linseed oil normally has no flavor or odor, but when these are present they are seen as slighly pleasant. This quality has led to the consideration of this oil as a potential food product. However, the oxidized products of linseed oil invariably possess objectionable odors and tastes. Also, the polymerization products of linseed oil make it totally unsuited as a food substance. The fatty acids in linseed oil become rancid when they oxidize resulting in disagreeable flavors and odors (14). Polymerizations occur when oxygen from the air reacts with the unsaturated fatty acids at or adjacent to the double bonds to form hydroperoxides that then decompose to yield other, noxious, products. Although these products are found only in trace amounts, they are thought to cause the strong odors associated with aged or rancid linseed oil (11, 21, 28).
Itoh et al. (26, 27) separated unsaponifiable substances (0.7% of the oil) from crude Canadian linseed oil by thin layer chromatography. They found hydrocarbons, aliphatic alcohols, triterpene alcohols,

methylsterols and sterols in all oils tested. Fedeli *et al.* (15) reported that linseed oil contains a triterpene alcohol component, possibly of the cuphane series.

1.1.5. Commercial products

Linseed oil is produced in different grades and various viscosities. Raw linseed oil is the unmodified oil obtained by pressing the flax seeds. When raw linseed oil is used as the vehicle in paints, the paint requires four to six days to dry to a hard film. Boiled linseed oil was developed in order to shorten the drying time. Boiled oils contain small amouts of such added driers as manganese or cobalt in the form of metallic salts which catalyze the drying process. These can be introduced in several ways: by blending oil and drier in the cold (although the product is still designated boiled oil), by heating oil and metal salts at 95 to 120°C while in the presence of air, or by heating oil and metal salts at 230 to 270°C in the absence of air (22). The boiled oil becomes thicker and darker, qualities considered very desirable in the paint industry. An even more desirable product can be obtained if the oil is doubled-boiled. This increases the viscosity and makes the oil dry much faster than plain boiled oil. Blown linseed oil is modified by bubbling air through it at 93°C to increase its viscosity, and the resulting product dries to a harder film than

unmodified oils.

1.1.6. Use of extracted seeds

Linseed oil meal is produced by crushing and extracting residual oil from flaxseed and cooking it with steam. The resulting product is sold for its high protein content and is generally used as an animal food. Linseed meal is available as fine-ground meal and is compressed into cubes and pellets for sale to feed-lot operators and ranchers for range feeding. The fine-ground linseed meal is sold largely to formula feed manufacturers for use as a high-protein ingredient of formula feed (12, 31).

1.1.7. Uses of linseed oil

Linseed oil is used in both food and nonfood products, depending upon the manner in which it is treated and refined. However, its use in edible products is not as common as in nonedible products because of its high rate of autoxidation and thermal polymerization. Also, it becomes rancid through oxidation resulting in disagreeable flavors and persistent odors which lessen desirability in the food industry. Because of its high linolenic acid content and its high iodine value, linseed oil is extensively used in the manufacture of paints, varnishes, linolenum, oilcloth, and printing inks, as well as in

other industrial products. The most common uses are as the vehicle for protective coatings and as a component of oil paints and varnishes. Other miscellaneous uses include manufacture of synthetic resins, caulking, and soap (12, 43). The use of linseed oil has declined since paint and coating manufacturers started using petroleum based solvent oils in coating and painting. The disadvantage of using these products is the evaporation of petroleum based solvents which yield toxic volatile compounds. Another disadvantage is that as the solvent evaporates, the film shrinks. To date, it appears that linseed oil products are still superior to synthetic products used by coating and paint manufactures. Linseed oil coatings do not shrink, because instead of evaporating as solvents do, the linseed oil becomes part of the coating film, creating a better covering than conventional coatings (20).

1.1.8. Odorous compounds in oil products

Non-volatile compounds such as fatty acids can play three important roles in producing odors and flavors in the products derived from them. First, they modify the odor and flavor of other components by reacting chemically with them. Second, they are precursors of odor and flavor compounds. And third, many of them have odors and flavors themselves (17). The characteristics of volatile

molecules that cause odor may develop in the processing of linseed oil products. Chemical changes and autoxidation are involved in the development of many undesirable odors. It was pointed out that the volatile odoriferous substances formed by autoxidation of highly unsaturated glycerides can be attributed to volatile free fatty acids, carbonyl compounds, and possibly aldehyde acids (45).

Little is known about linseed oil odors. On the other hand, the volatile substances in other vegetable oils that have components similar to those of linseed oils have been investigated and characterized chemically. Tables 1.2 and 1.3 show the fatty acid composition of vegetable oils and the volatile substances given by some of these oils (41).

Picuric-Jovanovic and Milovanovic (35) analyzed volatile compounds in almond and plum kernel oils. The most important volatile compounds from both oils were derivatives of n-alkanes, aliphatic acids, cycloalkanes, aromatics and furan compounds. These compounds are products formed by the decomposition of unsaturated fatty acids during the oxidation reaction. Frankel (18) found pentane, propanal and hexanal in soybean oils while Snyder and Mounts (41) reported that propanal, pentane, pentanal, hexanal, 2,3-hexenal, 2-heptenal, octen-3-ol, nonanal,and 2,4-decadienal were found in

Table 1.2 Fatty acid composition of various oils with characteristic odors

Acid	Per cent of fatty acids in the oil named							
	Peanut	Cottonseed	Soybean	Corn	Sunflower	Olive	Palm	Linseed
Myristic	-	0.5	0.3	-	-	1.2	1.5	0.2
Palmitic	10.3	21.9	9.5	11.4	6.8	15.6	42.9	5.4
Stearic	3.2	1.9	1.9	1.9	3.9	2.0	4.7	3.5
Arachidic	2.5	0.1	0.8	0.3	0.1	-	-	0.6
Oleic	56.0	30.7	22.9	24.6	14.1	65.6	39.8	17.0
Linoleic	26.0	44.9	50.5	56.8	74.5	15.6	11.3	24.0
Linolenic	-	-	7.1	-	-	-	-	47.0

Table 1.3 Volatile compounds of vegetable oils (41)

Vegetable oils	Volatile compounds
Corn	propanal, pentane, pentanal, hexanal, 2,3-hexenal, 2-heptenal, octen-3-ol, nonanal, 2,4-decadienal
Low erucic rapeseed	propanal, pentane, pentanal, pentanol, hexanal, 2-pentenol, 2,3-hexenal, 2-heptenal, octen-3-ol, 2,4-heptadienal, nonanal, 2,4-decadienal
Soybean	propanal, pentane, pentanal, hexanal, 2,3-hexenal, 2-heptenal, octen-3-ol, 2,4-heptadienal, nonanal, 2,4-decadienal
Sunflower	propanal, pentane, pentanal, pentanol, hexanal, 2,3-hexenal, 2-heptenal, octen-3-ol, nonanal, 2,4-decadienal
High oleic sunflower	propanal, pentane, pentanal, pentanol, hexanal, 2,3-hexenal, 2-heptenal, 2,4-heptadienal, nonanal, 2,4-decadienal

sunflower and soybean oils. Two compounds, 2-pentenol and 2,4-heptadienal, were also found in soybean oils.

Similar studies reveal that the odor of linseed oil is due to its high content of unsaturated fatty acids which lead to the release of volatile compounds by autoxidation and chain cleavage. The cause of the oxidation is now believed to be a chain reaction and it can be assumed that free radicals are intermediates. The compounds isolated are relatively short chain molecules and include aldehyde acids, hydroxy acids, ketones, and keto acids (23, 25, 33). Aldehydes are to be expected as main products resulting from the autoxidation of oleic acid, linoleic acid and linolenic acid (Table 1.4). The mechanism of autoxidation of linolenic acid is given in Figure 1.1.

1.2. Biodegradation

Biodegradation can be described as the decomposition of a substance through the action of biological agents, specially microorganisms. In a stricter perception, biodegradation has come to represent the complete microbial break down, or mineralization of complex constituents into simple inorganic compounds such as carbon dioxide, water, and mineral substances. Biodegradation is essential for the recycling of elements such as carbon, hydrogen, oxygen, nitrogen, and sulfur in nature. Without the resultant cycles of matter, these

Figure 1.1. Mechanism of the autoxidation of linolenic acid (38). The initial formation of a methylene radicals (I) is then mesomerically stabilized by contributions of forms (Ia) and (Ib). Subsequently, three isomeric linolenic acid monohydroperoxides are formed and these give rise, through chain cleavage at the hydroperoxy groups at C-13, C-11, and C-9, to saturated as well as singly and doubly conjugated unsaturated aldehydes (II-VII).

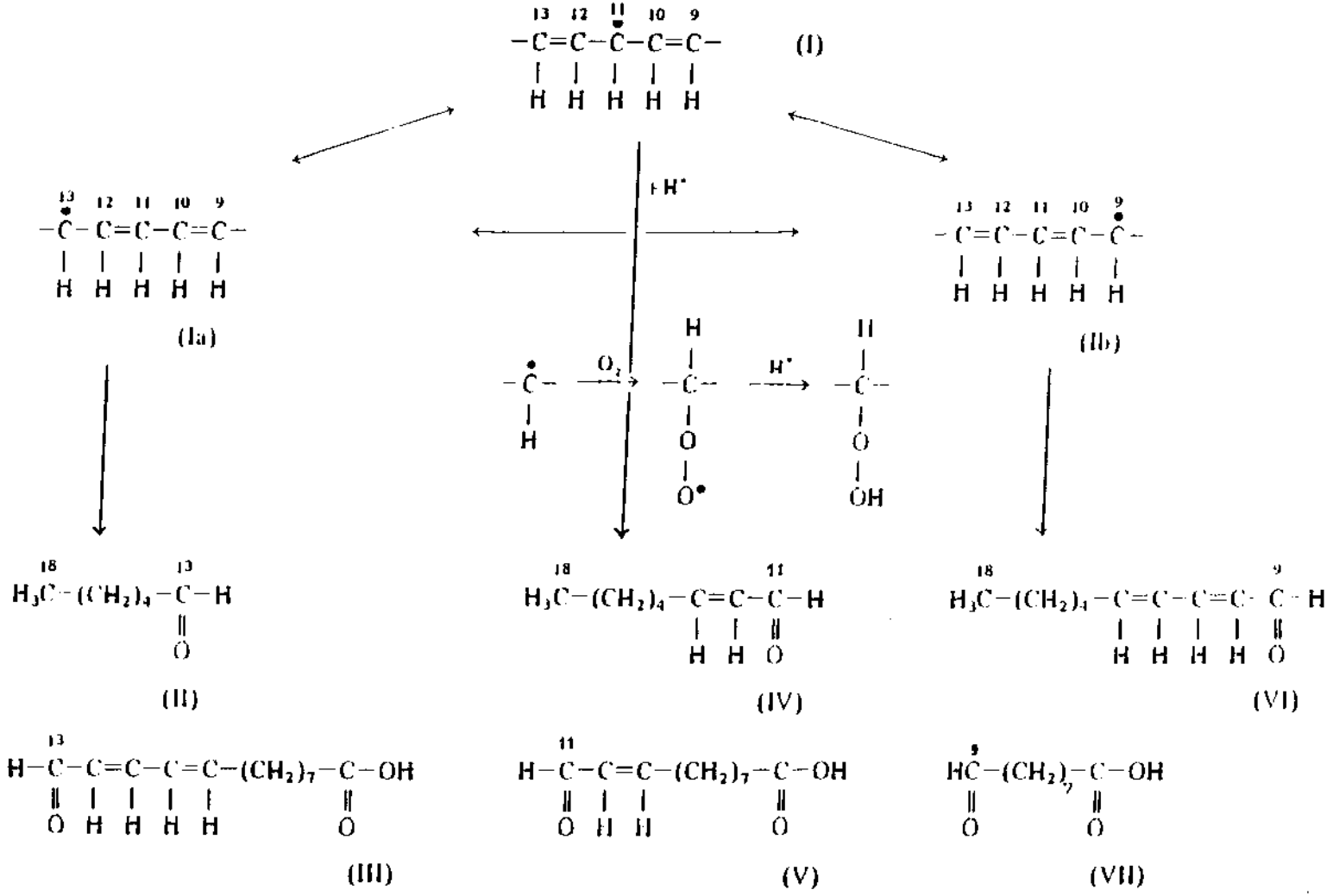

(I)
+H•
(Ia)
(Ib)
O2
H•
(II)
(IV)
(VI)
(III)
(V)
(VII)

Table 1.4. Aldehylic autoxidation products of oleic, linoleic, and linolenic acids (38)

Fatty acid	End product
Oleic	heptanal, octanal, nonanal, decanal, 2-*trans*-decenal, 2-*trans*-undecenal
Linoleic	pentanal, hexanal, heptanal, octanal, 2-*trans*-heptenal, 2-*trans*-octenal, 2-*cis*-octenal, 2-*trans*-nonenal, 2-*cis*-decenal, 3-*trans*-nonenal, 3-*cis*-nonenal, 2,4-*trans*-nonadienal, 2,4-decadienal, 2-*trans*,4-*cis*-decadienal
Linolenic	acetaldehyde, propanal, hexanal, 2-*trans*-pentenal, 2-*cis*-pentenal, 2-*trans*-crotonaldehyde, 2-*trans*-hexenal, 3-*trans*-hexenal, 3-*cis*-hexenal, 2-*trans*-heptenal, 2-*trans*,4-*trans*-heptadienal, 2-*trans*,4-*cis*-heptadienal, 2-*cis*,5-*cis*-octadienal, 2-*trans*,6-*cis*-nonadienal, 2-*trans*,4-*cis*,7-*cis*-decatrienal, 2-*trans*,4-*trans*,7-*cis*-decacatrienal

atoms might be tied up in complex substances and, therefore, unavailable to re-enter the natural cycles of living things in nature. The activity of microorganisms on certain substances can also, in many cases, result in their removal from the environment or in reduction of their toxicity. Worldwide, bacteria are the most important degradative entities of all natural agencies. Bacteria or other microorganisms can degrade a great variety of organic compounds under appropriate conditions. Generally, they oxidize organic substances to generate energy and nutrients for their growth. Microorganisms that degrade a given substrate are able to proliferate utilizing both the energy derived from it and the atoms of which it is composed. Thus, a common consequence of biodegradation of a compound is an increase in the number of microorganisms degrading that substance. Alternatively, although microorganisms can degrade the substance, they may not be able to employ the products for their own use. In such cases, the organism responsible for the catabolic reactions depends on other, alternate sources of energy and nutrients. This phenomenon, known as cometabolism, appears to result from a lack of specificity of certain microbial enzymes. Such enzymes can modify the compound and generate products that other microorganisms can employ or further degrade. Nevertheless, in a natural ecosystem, biodegradation is

frequently equated with the catabolism of particular organic compounds by a single microbial strain. However, it is becoming increasingly apparent that biodegradation in the natural environment is carried out by mixed microbial communities, and even under laboratory conditons it has been shown that a mixed group of microorganisms, a consortium, may be more effective than any of the component organisms acting alone (20).

The major advantage of biodegradation is that compounds degraded are finally converted into carbon dioxide and water, instead of being transferred from one place to another in environment (5).

1.3. Biodegradation of certain compounds in fats and oils

Many organisms have the ability to utilize fats and oils for both cell carbon and as a source of energy. Besides fatty acids which are the major component of fats and oils, the compounds isolated from oxidation products of fats and oils include alkanes, aldehydes, ketones and some short chain organic acids.

Aerobic bacteria, such as Pseudomonads, Acinetobacter, Bacillus, and Escherichia usually degrade fatty acids by the catabolic pathway called β-oxidation. In this process, the fatty acids undergo oxidative removal of successive two carbon units in the form of acetyl coenzyme A (CoA), starting from the carboxyl end of the fatty acyl

chain. First, the fatty acid is converted to the fatty acyl CoA. This reaction is catalyzed by the enzyme fatty-acyl-CoA-synthetase. Then, fatty acyl CoA is reduced to yield *trans* Δ^2 enoyl CoA. The enzyme responsible for this first step is acetyl CoA dehydrogenase. In the second step, water is added to the double bond of *tran* Δ^2 enoyle CoA to form β-hydroxyacyl CoA. This reaction is catalyzed by enoyl CoA hydratase. In the third step, β-hydroxyacyl CoA is dehydrogenated to form β-ketoacyl CoA by the action of β-hydroxyacyl CoA dehydrogenase. The last step of fatty acid oxidation is catalyzed by acyl CoA acetyl transferase, which promotes the reaction of the β-ketoacyl CoA to split off the carboxyl terminal two carbon fragment of the original fatty acid as acetyl CoA (19). The fatty acid β-oxidation pathway is shown in Figure 1.2 which is taken from Stryer (42).

Gaseous, liquid, and solid hydrocarbons in the aliphatic, olefinic and naphthenic series are also susceptible to microbial decomposition. Hydrocarbons are attacked by microorganisms growing under both aerobic and anaerobic conditions. Some bacterial species are also able to grow on such volatile substances as ethane, propane, butane, and hydrocarbons up to C_8 (19). Shennan and Levi (39) reported that aliphatic hydrocarbons were assimilated by many microorganisms but that aromatics are assimilated less efficiently. Long chain n-alkanes

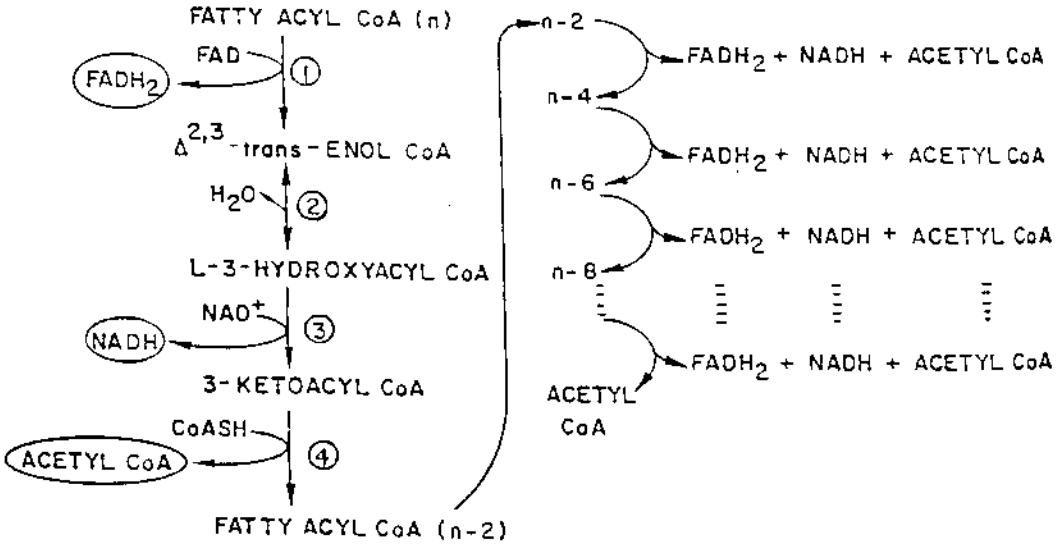


Figure 1.2. The oxidation of fatty acids by the β-oxidation pathway as shown in Stryer (42).

1 = fatty acyl CoA dehydrogenase

2 = enolhydratase

3 = L-3-hydroxyl CoA dehydrogenase

4 = 3-ketothiolase

are assimilated more readily than short chain n-alkanes. Saturated aliphatic hydrocarbons are degraded more readily than unsaturated ones, while branched chain compounds are degraded less readily than straight chain ones (39).

A simple system for aerobic microbial degradation was described for volatile substances. The results revealed that the odor disappeared completely after contact with selected microorganisms (37). Hydrocarbons differ in their susceptibility to microbial attack and have generally been ranked in the following order of decreasing susceptibility: n-alkanes > branched alkanes > low molecular weight aromatics > cyclic alkanes (29).

1.4. Techniques for removing odorous compounds

Many technologies are available for the treatment of volatile substances. A number of methods can be chosen including the following: 1) biofiltration 2) chemical wet scrubbers 3) thermal regenerative oxidation and 4) activated sludge scrubbing (46).

1.4.1. Biofiltration

Biofiltration techniques have been used to treat odorous compounds which may cause air pollution from waste processing plants. Biofilters are currently one of the most commonly used odor

control techniques because they achieve high removal rates with low energy requirements, material costs or maintenance. This technique can be used in pollution prevention applications, and their acceptance is expanding over traditional, as well as other novel methods to remove volatile organic compounds. Odorous organic compounds, inorganic compounds, and volatile organic compounds have been removed successfully by biofiltration (4, 48). This method depends on the degradation of the volatile organic compounds into water and carbon dioxide. The biofiltration system consists of a filter bed, water saturated medium bearing active bacterial agents. The beds contain a mixture of substances that make up a substrate that can support the growth of microorganisms which are allowed to grow on the solid support. Streams of gas containing odorous molecules are passed over or through these beds to bring them into direct contact with the bacteria therein. Microbial metabolism leads to destruction of the contaminants and the release of nonodorous waste products. Volatile compounds that can be degraded in biofilters include benzene, ethyl benzene, toluene, xylene, ethyl alcohol, naphthalene, acetone, propionaldehyde, such volatile sulfur compounds as hydrogen sulfide and mercaptans, esters, and ketones (1, 24). To operate an efficient biofilter, the medium must provide a suitable environment for

microorganisms. The most common medium currently used in biofilters appears to be soil and compost (48, 49). There is a report that over 110 soil biofilters are being operated in Japan for the purpose of removing odorous substances from effluent air (44). Composts such as sewage sludge, yard waste, and solid wastes of various kinds have a greater number and diversity of microbial populations than soils, making them a preferred medium for biofilter applications (48, 49). A biofilter system, designed by Ambient Engineering Inc., Matawan, N.J. has been created using styrofoam balls as the bed medium for supporting bacterial growth. The medium can last several years when operated under industrial plant conditions (6). The simple operating theory of biofilters states that when odorous or contaminated gases are passed through the bed medium, two basic removal mechanisms, absorption/adsorption and biooxidation, occur simultaneously. Odorous gases passing through a biofilter will be adsorbed on the surface layer of medium particles. Microorganisms, principally bacteria including actinomycetes, and fungi, attached to the filtering medium will utilize the biodegradable organic constituents of odorous substances in industrial plant air streams. The medium particles serve as a nutrient supply and substrate for the microorganisms, thereby supplementing those

nutrients which may not be contributed in the gas stream being treated. When a biofiltration system performs appropriately, carbon dioxide, water, and mineral salts are formed as final products of the degradation. As odorous compounds are degraded, adsorptive sites on the biofilter medium become available for additional compounds in the gas stream, thus self-regenerating the filter odor removal capacity. In order to maximize odor removal rates, the rate of microbial degradation of sorbed odorous compounds must equal or exceed the absorption/adsorption rates. If absorption sites are filled faster than they are regenerated by bioxidation, the filters will be overloaded, and odorous elements in the gas stream will flow into the atmosphere (48,49).

Actinomycetes have been found to be especially useful for removal of dimethyl sulfide, dimethyl disulfide and volatile fatty acids. The removal of toluene, butanol, ethyl acetate and butyl acetate from waste gas streams using a peat compost biofilter was described by Ottengraf and Van Den Oever (34). Beerli and Rotman (7) demonstrated the removal of volatile organic compounds through a biofilter system using a pear/sphagnum peat moss as medium particles. Removal efficiencies ranged from 65 to 92 percent of the total volatile organic compounds present in the gas stream. William (46) reported

that the removal of volatile amine compounds using a sludge compost biofilter was greater than 95% on a volume to volume basis. Volatile organics such as methane, propane, and isobutane have also been removed successfully with biofilters (8, 34). Generally speaking, to determine removal capacities, continuous biofilter operation of more than 10 days allows the establishment of optimal removal rates of odorous compounds. In addition, a high degree of microbial activity in the biofilter must be maintained whether or not organic materials are present inthe air stream. Performance depends on numerous factors, including the type of odorous compound to be removed, the characteristics of the filter medium, the moisture content of the bed, environmental temperature and humidity and the time the biofilter has been in operation. It is essential that the biofilter environment be suitable for the microorganisms to survive and metabolize waste gases to accomplish maximum odor treatment because the odor removal mechanism is based on metabolic bioxidation reactions (40, 48, 49).

1.4.2. Chemical wet scrubbers

Chemical wet scrubbers remove odorous compounds from an air stream according to the absorptive capacity of the chemicals in the absorbent. Normally, sodium hypochloride and sodium hydroxide are used as scrubbant solutions. A typical wet scrubbing system

consists of centrifugal dust collectors followed by scrubbers, forming parallel treatment trains. Treated air from the wet scrubbers is discharged through a dispersion rack. Wet scrubbers are not as effective as are biofilters at removing many types of odorous substances from industrial plant air streams (46). It has been reported that the odor removal efficiency of chemical scrubbers is not in the 75 to 95% effectiveness range as are biofilters (50).

1.4.3. Thermal regenerative oxidation

Thermal regenerative oxidation consists of exposing the malodorous air stream to temperatures of approximately 1,400°F for about one second. The removal efficiencies of this method have been reported to be similar to, but not better than, multi-stage wet scrubbing. However, the cost for this system is significantly higher than for wet scrubbing and biofiltration (46).

1.4.4. Activated sludge scrubbing

Activated sludge scrubbing consists of diffusing the odorous air stream through a mixed liquor in the aeration basin of treatment plants. Typically, exhaust air from the composting facility is introduced into the inlet side of the aeration blowers. The effectiveness of this odor control technology depends on the nature

and concentration of the odor compounds, the volume of air treated, and the area and depth of the aeration basins (46).

1.4.5. Other odor removal techniques

Bioscrubbing and activated carbon are also used as odor removal techniques. Bioscrubbing uses the same principle as biofiltration, but in a gas/liquid system rather than a gas/solid system. Odorous air is bubbled through bacteria-laden liquid such as that found in the activated sludge tank at a sewage plant. Odors in the air are removed by microbial action and broken down biologically.

Activated carbon has been utilized but with little success, primarily because dust and moisture in industrail air streams fill active adsorption sites in the activated carbon thereby reducing effectiveness. Chemical counteractants have shown some success in odor removal but are not as widely used because of cost and technical complexity. They generally are not as effective as wet chemical scrubbers and much less effective than biofilters.

Based on case studies, multi-stage chemical scrubbers and biofilters are the two odor treatment technologies most commonly used because they offer the most promising results (50). Richard Ziminski of Ambient Engineering Inc. predicts that ten years from now biofilter techniques will be the major control technology for both

odors and volatile organic compound emissions (6).

1.5. Purpose of this project

Research was performed to develop a biodegradation technique to remove linseed oil odors from industrial plant air streams. In this work, it is assumed that the drying ability of linseed oil is due to the oxidation and chain cleavage of unsaturated fatty acids. It is also assumed that these processes lead to volatile products that have strong noxious odors that may be toxic for human beings. Since linseed oil is commonly used in many industrial processes including the paint, coatings, plastics, and other industries, its vapors represent a hazard for production personnel in these industries. This makes it imperative that techniques be found to remove linseed oil vapors from plant environments and before the air is released to the outside air. The working hypothesis is that bacteria present in nature are capable of removing such vapors from the air by oxidative metabolic reactions. The research proposed here centers on finding these bacteria and of using them to degrade linseed oil vapors. The bacteria should be capable of using linseed oil vapors as the carbon source and degrading them to carbon dioxide and water or to nonodorous and nonnoxious substances. The system sought should remove more than 99% of volatile substances from linseed oil in a single-pass and at a rate of

one volume of air per volume of biofiltration medium per minute. The working objective also includes development of a method for monitoring the presence of linseed oil vapors in effluent industrial plant emissions.

CHAPTER II

MATERIALS AND METHODS

2.1. Biofiltration system

A glass column, 60 cm in length and 4 cm in diameter was packed with styrofoam balls, 3 to 5 mm in diameter, and filled with soil compost obtained from an industrial plant that uses linseed oil in its operations. The M9 solution, without a carbon source, was added to the column to provide an aqueous environment and a source of minerals for the bacteria. The composition of the M9 solution in one liter of distilled water was as follows: dibasic sodium phosphate (Na_2HPO_4) 6 g; monobasic potassium phosphate (KH_2PO_4) 3 g; ammonium chloride (NH_4Cl) 1 g; and sodium chloride (NaCl) 0.5 g. After autoclaving, 2 ml of a 1 M sterile solution of magnesium sulfate ($MgSO_4.7H_2O$) and 0.1 ml of a 1 M sterile solution of calcium chloride ($CaCl_2$) were added. The column is pictured in Figure 2.1.

2.2. Linseed oil vapors

Linseed oil was placed in an amber flask in order to protect it from exposure to light. A glass tube, 3 mm in diameter, was used to connect the flask to the glass column as shown in Figure 2.2.

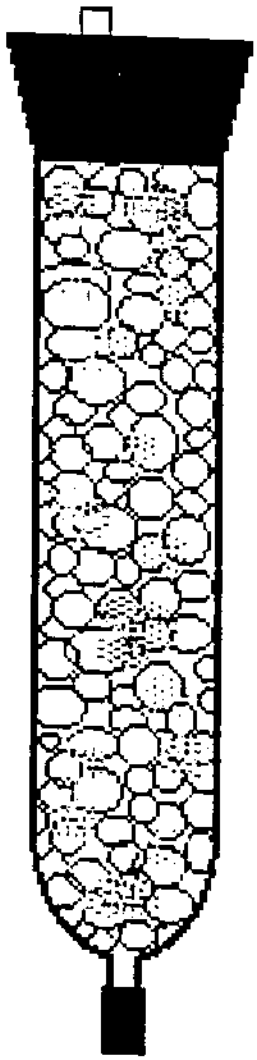


Figure 2.1. Glass column packed with styrofoam balls and filled with soil compost suspended in M9 salts solution.

Air from the lab supply pump was blown through the linseed oil in the flask, saturating it with linseed oil odor. Liquid droplets were removed by a cotton filter in the flask and another in the exit tube. The air containing the volatile substances from linseed oil in the flask passed through the glass tube and bubbled through the glass column at a rate of 50 ml per minute. These linseed oil volatiles were the sole source of carbon, other than that in the compost, for the bacteria attached to the surface of the styrofoam balls. The experimental system is illustrated in Figure 2.2.

2.3. Bacterial cultures

After allowing the linseed oil odor to bubble through the column for two weeks without adding any other sources of carbon, some of the styrofoam balls were removed from the column and streaked on the surfaces of plates containing M9 solution solidified with purified agar (Difco, Detroit, MI). The culture plates were inverted and one drop of linseed oil was added to a 5.5 cm in diameter of filter paper in the lid of the plate. This arrangement insured a supply of linseed oil volatiles were then supplied to the inoculum. The plates were incubated at 26°C in an aerobic condition for two days. A control was inoculated and incubated on the same kind of medium but using distilled water instead of linseed oil.

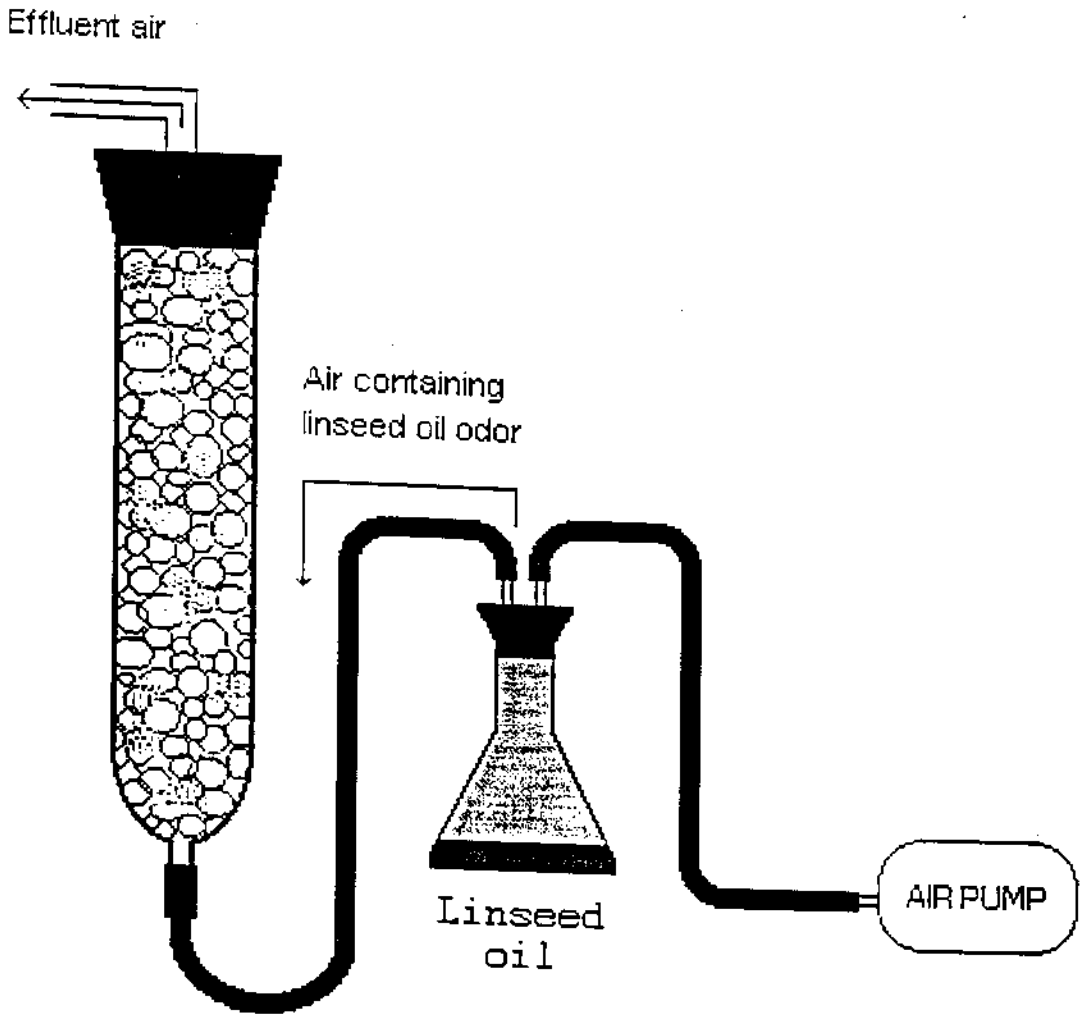


Figure 2.2. Experimental biofilter system using styrofoam balls and soil compost as a solid phase with linseed oil vapor source.

The bacteria that grew on plates with linseed oil were subcultured, isolated, and purified on Nutrient Agar plates (Difco, Detroit, MI). Organisms that grew on plates supplied with distilled water were discarded. Gram stains were made to determine the morphology of the isolated bacteria. Biochemical tests such as Triple Sugar Iron agar (TSI), motility, glucose fermentation, catalase, and oxidase tests were also carried out. Different bacterial strains were obtained and maintained as stock cultures on Nutrient Agar slants for further studies.

Confirmation of growth: Each isolated organism was transferred to 5 ml of Nutrient Broth and incubated at room temperature overnight. After incubation, the bacterial cells were separated by centrifugation, washed three times with sterile normal saline solution (0.85% NaCl), and resuspended in 5 ml of M9 salts solution with no carbon source.

The five cultures were mixed in equal proportions in terms of the number of cells. The mixed culture, a bacterial consortium, was used as a uniform suspension and was diluted with M9 salts solution to a final concentration of 1×10^4 cells per ml. Cell density was determined by culture turbidity from a reference curve constructed for this purpose. The total number of cells in the suspension was

adjusted by adding either bacterial cells from the original mixture or sterile M9 salts solution. Ten ml of the mixed suspension of bacterial cells were transferred to sterile screw-top side arm flasks containing 20 ml of M9 salts solution. One ml of linseed oil was placed in the side arm of each flask and pleated filter paper immersed in the linseed oil to augment volatilization (see Figure 2.3). Controls consisted of flasks with 1 ml of distilled water in the side arm fitted with the filter paper evaporator. The culture flasks were incubated at room temperature for 10 days, and the growth of the bacterial consortium was monitered daily by removing 1 ml of culture and making a ten fold dilution series for plate counts by the spread plate method. Plate count agar (Difco, Detroit, MI) was used to determine the total number of cells and total growth rates on both the distilled water control and the linseed oil volatiles.

2.4. Characterization of linseed oil by gas chromatograph

Chromatographic equipment: Gas liquid chromatography was performed with a Hewlett Packard 5710 Gas Chromatograph equipped with a flame ionization detector. A glass column (2 meters long and 4 mm i.d.) packed with 15% diethylene glycol succinate (DEGS) on chromosorb WHP, 100/120 mesh was used. Nitrogen was used as as the carrier gas, and hydrogen and air were used to operate the flame

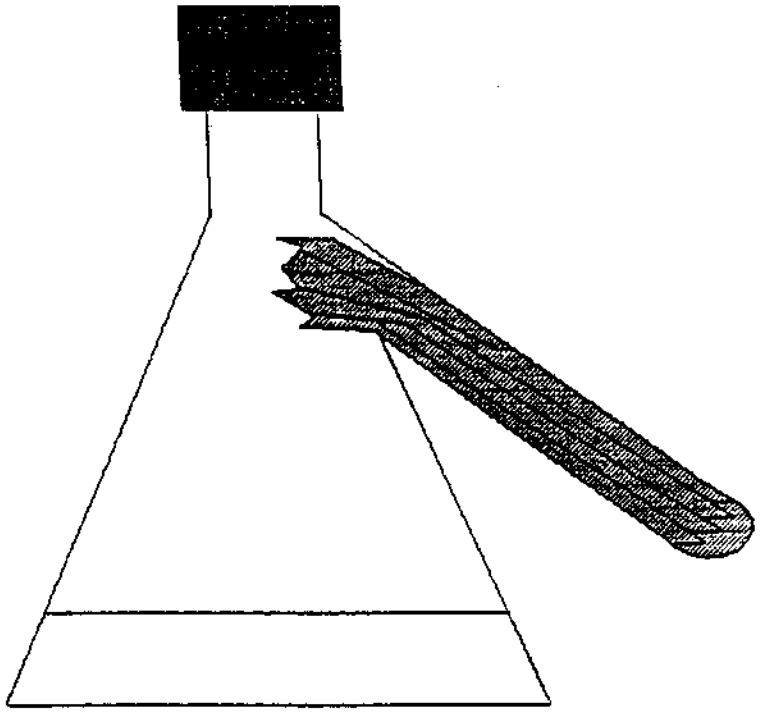


Figure 2.3. Flask containing linseed oil in the side arm fitted with pleated filter paper evaporator.

ionization detector. The samples were analyzed in two different forms, liquid and vapor. Flow rates for each gas and temperature program for sample analysis are described below.

Linseed oil composition analysis: Linseed oil was converted to the fatty acid methyl esters for analysis by gas liquid chromatograph. The method for the preparation of the methyl esters is decribed in Figure 2.4 (9). Fatty acid methyl ester standards (Sigma Chemical Company, St. Louis, MO), namely methyl palmitate, methyl oleate, methyl linoleate, and methyl linolenate were used as references. Two μl of the prepared samples for analysis and the standards that accompanied each analysis were injected into the gas chromatograph. The syringe was washed with hexane between injections. The carrier gas flow rate was maintained at 75 ml per min. Hydrogen and air were supplied to the detector at 60 and 240 ml per min, respectively. The oven temperature was isothermally held at 180°C, while the detector and injection port temperatures were held at 250°C.

Linseed oil vapor analysis: 5 ml of the vapor were injected directly into the gas chromatograph with a gas-tight syringe. The carrier gas flow rate was maintained at 40 ml per min. Hydrogen and air were supplied at the same rate as in the linseed oil methyl ester samples described previously. Temperature program sequences were

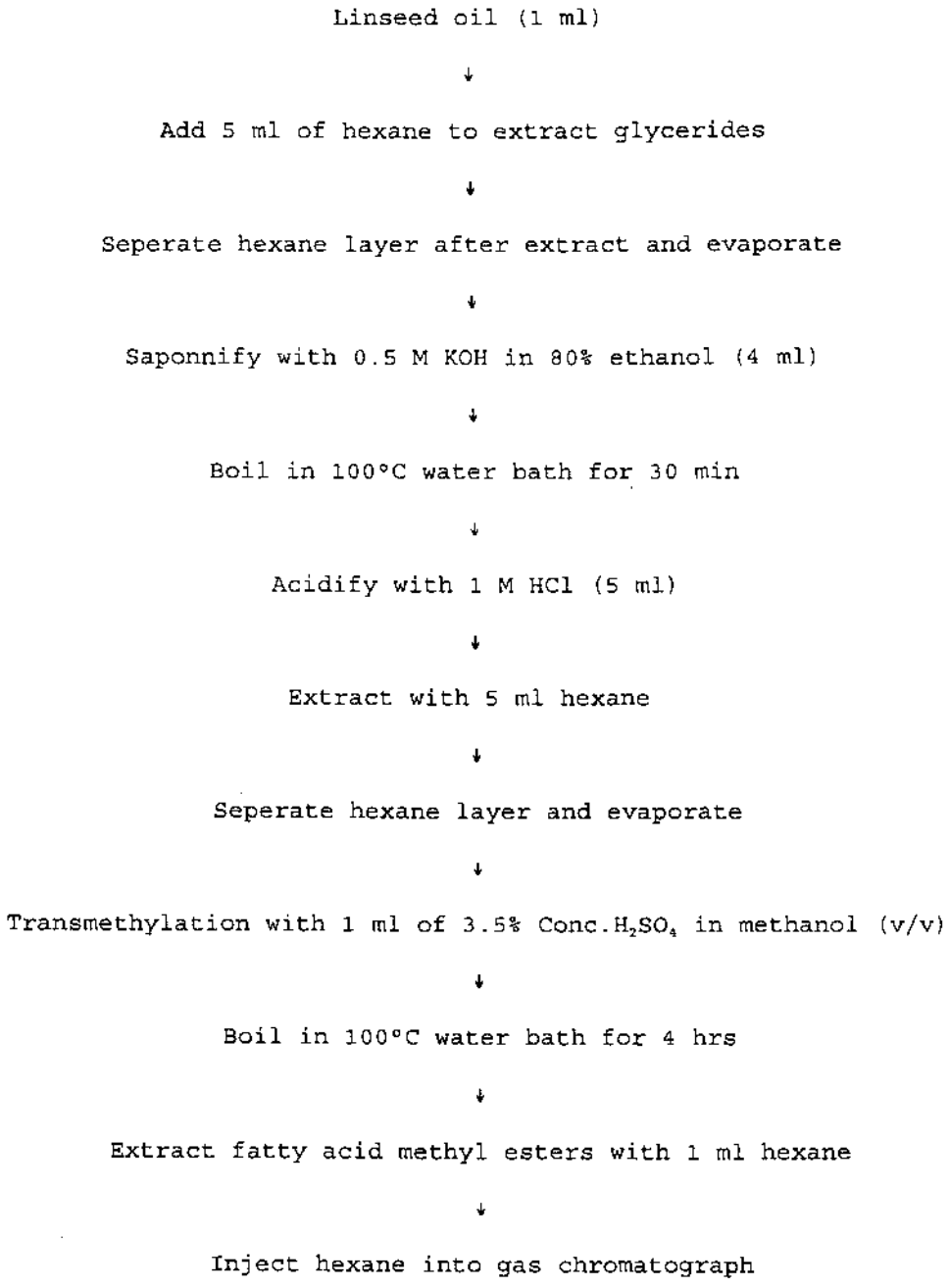


Figure 2.4. Preparation of methyl esters of linseed oil fatty acids for gas chromatography.

as follows: 55°C isothermal for 4 min, increased at 8°C per min to 140°C and held at the final temperature for 2 min. Detector and injection port temperatures were held at 250°C and 200°C, respectively.

2.5. Degradation of volatile compounds of linseed oil by bacteria

Narrow mouth culture bottles were prepared and used as follows: M9 agar was sterilized in the culture bottle and solidified in a horizontal position. The bacterial isolates were cultured individually in the bottles and all the strains mixed to represent equal numbers as previously described except that the total number of cells was adjusted to 1 x 10^8 cells per ml of the final mixture. The total number was determined using the McFarland nephelometer series. This mixture was then cultured on M9 salts solution in the same culture bottles. The bottles were inverted, and a piece of filter paper containing 0.5 ml of linseed oil was placed on the bottom of each bottle. The linseed oil volatiles in these bottles were the sole source of carbon for bacterial growth. This operating technique was based on the same principle as that described previously for the culture of bacterial isolates (Section 2.2). The culture bottle with linseed oil is illustrated in Figure 2.5. All of the culture bottles were incubated at room temperature. To determine the degradation of linseed oil odor by the

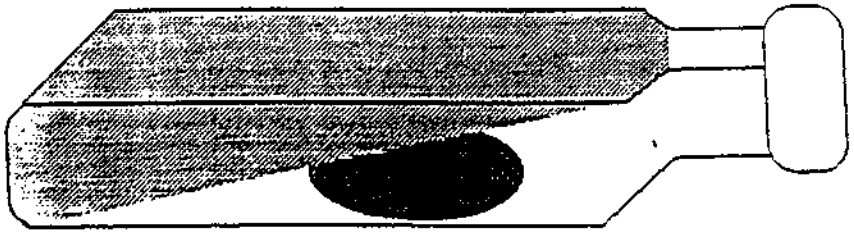


Figure 2.5. Culture bottle with linseed oil for determination of degradation of linseed oil odor by bacteria.

bacteria, the linseed oil vapor in the culture bottle was analyzed by gaschromatography on the first, third, fifth, seventh, fifteenth, and thirtieth days of incubation. Linseed oil volatile controls were determined in the same manner.

2.5. Organoleptic test

An organoleptic test was performed by having 10 people smell the linseed oil odor both before and after degradation by the bacteria. These people were randomly selected and had not received previous information on linseed oil odors or the difference between test samples and controls. They were asked to smell two culture bottles containing linseed oil, one incubated with a mixed culture of bacteria, and the other a control without bacteria. They were asked to described their impression of the odor in the bottles. The data collected are shown in the Results section.

CHAPTER III

RESULTS

3.1. Bacterial cultures

A total of 5 bacterial strains collected from the experimental biofilter were able to grow well on the M9 medium using linseed oil vapor as the source of carbon. The bacteria were isolated in pure culture and are maintained as stock cultures in the laboratory. Three of the 5 isolates were Gram negative aerobic rods and two were Gram positive aerobic rods. These were designated UNTA, UNTB, UNTC, UNTD, and UNTE. The criteria used to distinguish one from the other are given in Table 3.1.

3.2. Confirmation of growth

The total number of mixed bacterial cells cultured in M9 broth with and without linseed oil vapor are shown in Table 3.2. These cultures were in 250 ml side arm flasks with 1.0 ml of linseed oil in the side arm fitted with folded filter paper to increase vaporization. These data show that the total number of bacteria in cultures exposed to the vapors of linseed oil increases markedly while the controls did not. This experiment was performed at least three times using the

Table 3.1. The characteristic of isolated bacteria capable of degrading linseed oil odors.

Organisms	Tests					
	Morphology	Oxidase	Catalase	TSI*	Motility	Glucose fermentation
UNTA	Gram positive large rod	+	+	NA	+	+
UNTB	Gram negative rod	+	+	K/N	+	-
UNTC	Gram positive rod	+	+	NA	+	+
UNTD	Gram negative rod	-	+	K/N	+	-
UNTE	Gram negative rod	+	+	K/N	+	-

* Triple Sugar Iron: K/N = Alkaline slant/No change in bottom, NA = Not Applicable

Table 3.2. Growth of bacteria with and without linseed oil vapors.

Days of incubation	Total number of viable cells	
	Cultured with linseed oil	Cultured without linseed oil
0	1.0×10^4	1.0×10^4
1	4.7×10^5	4.5×10^4
2	1.8×10^6	5.2×10^4
3	2.9×10^7	6.4×10^4
4	4.1×10^7	3.8×10^4
5	5.3×10^7	2.1×10^4
6	1.1×10^8	1.9×10^4
7	2.1×10^8	8.0×10^3
8	3.3×10^8	4.9×10^3
9	4.2×10^8	2.1×10^3
10	5.4×10^8	6.0×10^2

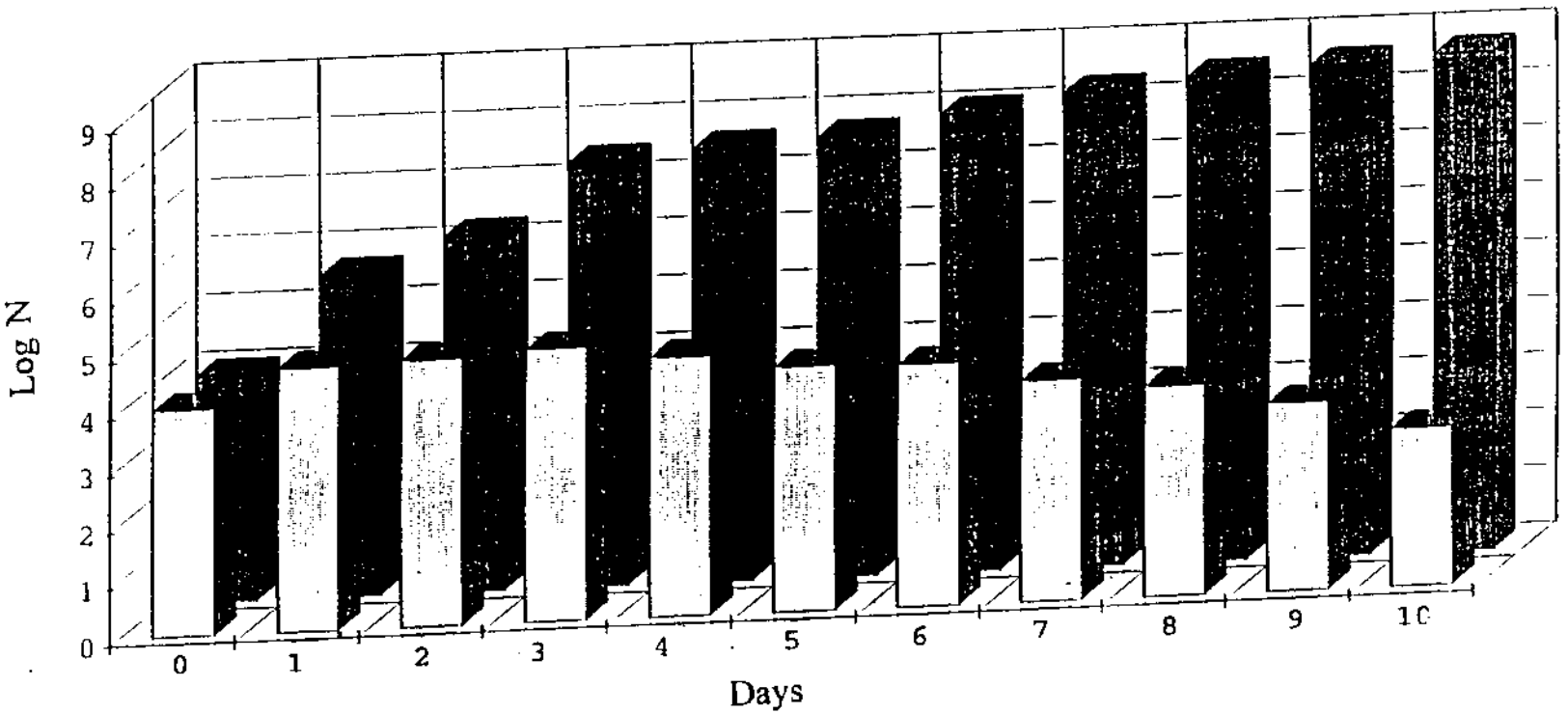


Figure 3.1. Comparison of the total number of bacteria cultured with and without linseed oil vapors. ■ With linseed oil □ Without linseed oil

biofilter culture as inoculum.

All the data comfirmed the observation that the bacteria grew at the expense of linseed oil vapors. This is interpreted as preliminary evidence that there are bacteria in nature capable of degrading linseed oil vapors. From this preliminary observation, it is assumed that the hypothesis developed for this research can be supported.

3.3. Chromatographic characteristic of linseed oil

Chromatographic resolution of fatty acid methyl esters in linseed oil is shown in Figure 3.2. These data agree completely with previously published results (12, 13, 32). The results show that linseed oil is composed of linolenic acid, the major component, linoleic acid, oleic acid, stearic acid and palmitic acid.

3.4. Chromatographic characteristic of linseed oil vapor

While linseed oil yielded six components on esterification of fatty acids, the vapor yielded 11 volatile substances. These are shown in Figure 3.3. Ten replicates of this analysis were performed to confirm the presence of these volatile materials. This was done to insure that linseed oil sampled at different temperatures and other ambient conditions yielded the same results.

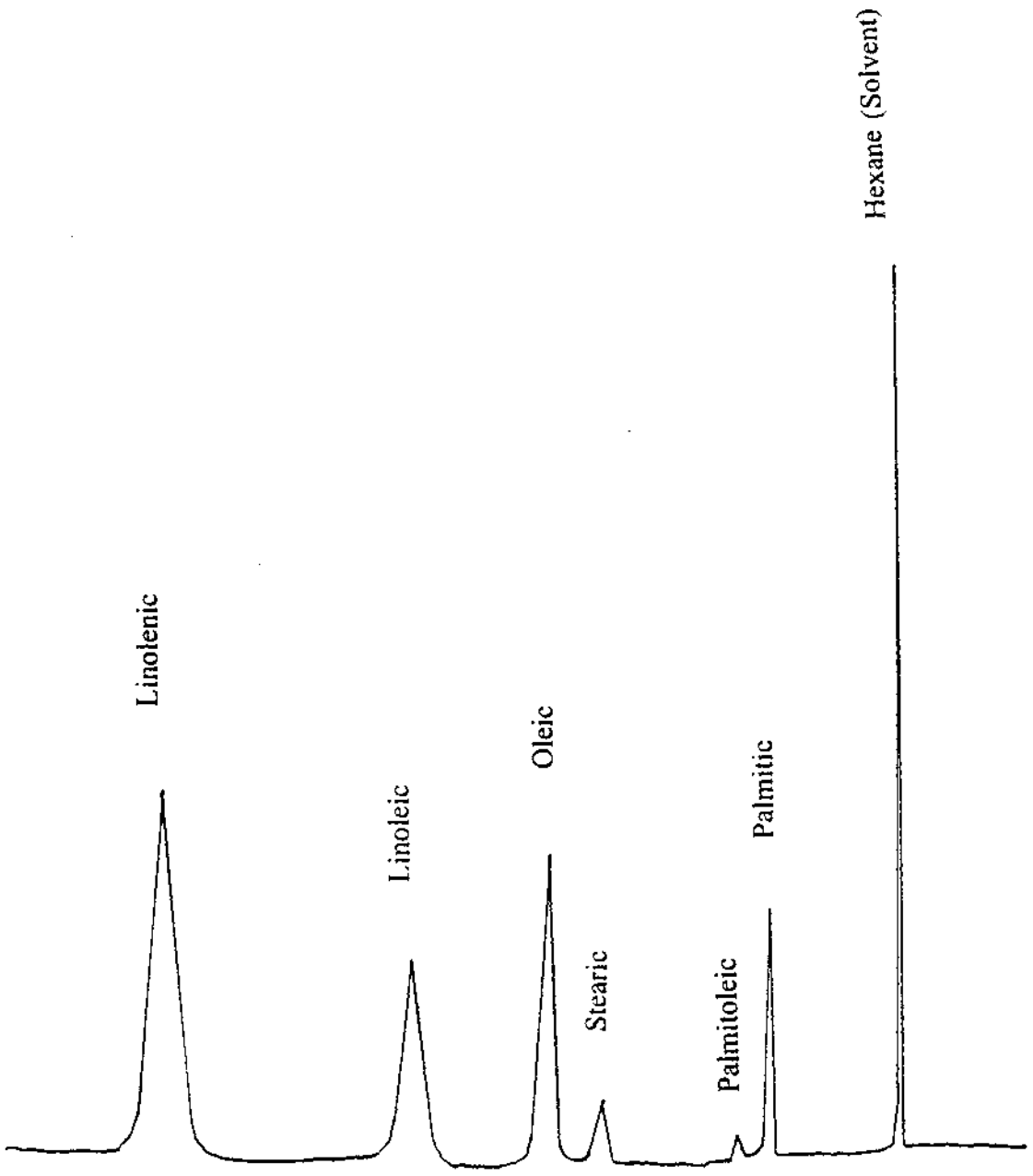


Figure 3.2. Gas chromatography of fatty acid methyl esters obtained from linseed oil.

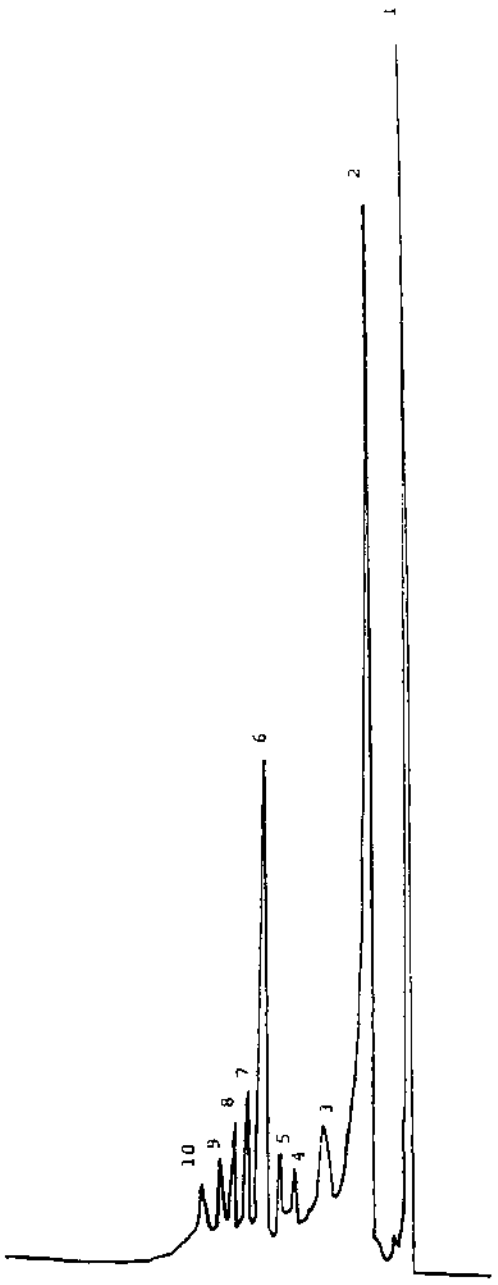


Figure 3.3. Gas chromatography of linseed oil vapors. The peak between #1 and #2 probably represents a separate compound but it may be a heat degradation product of #1 and, therefore, is not treated separately.

3.5. Biodegradation of linseed oil vapors

For each of the five bacteria in the collection and for the mixed culture, a profile of linseed oil odor degradation was generated by determining the decrease in the number of chromatographic peaks present after 1, 3, 5, 7, 15 and 30 days of incubation. The decrease in the number and type of peak in a given culture when compared to pertinent controls was assumed to be due to degradation of specific vapor components by the organism tested. All tests and controls were handled simultaneously and identically to avoid confusion which may be caused by spontaneous changes.

All of the organisms studied were able to degrade each compound at different periods of time. The decrease of each compound degraded by different bacteria was measured. The results are shown in Figure 3.4 to 3.26.

As shown in Figures, some bacteria did not degrade vapor components during brief periods of incubation, but were able to degrade them after longer incubation. It is noticable that the mixed culture was able to degrade linseed oil vapor compounds faster than did the individual organisms.

3.6. Organoleptic test

All of the ten people in the organoleptic test panel indicated

that the bottle containing linseed oil and the mixed bacterial culture did not have the smell of linseed oil while the other bottle (control) not gave off a strong smell of linseed oil. Table 3.3 lists the names of the ten people and the results obtained from each.

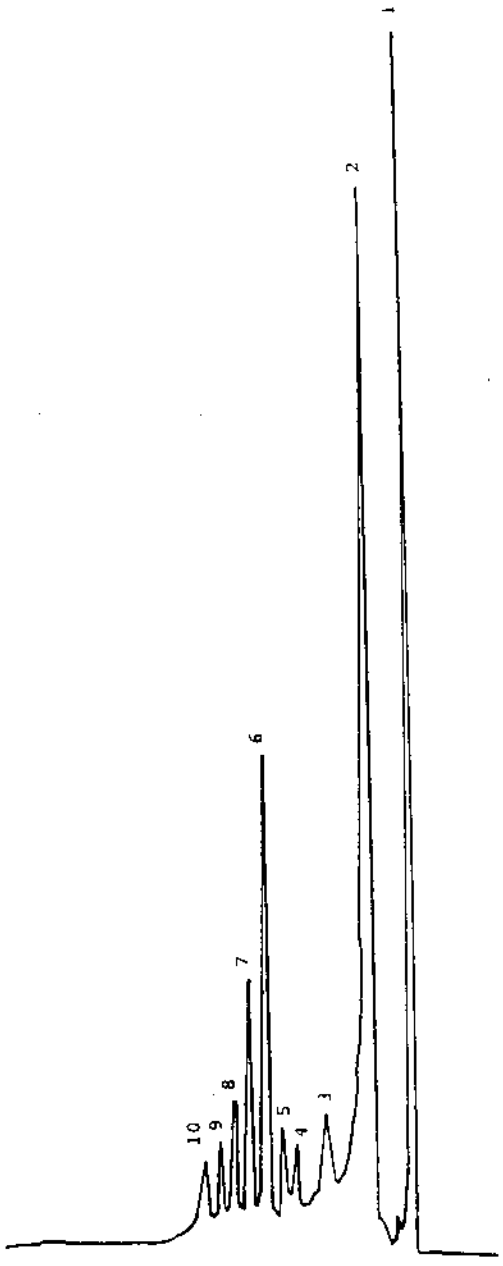
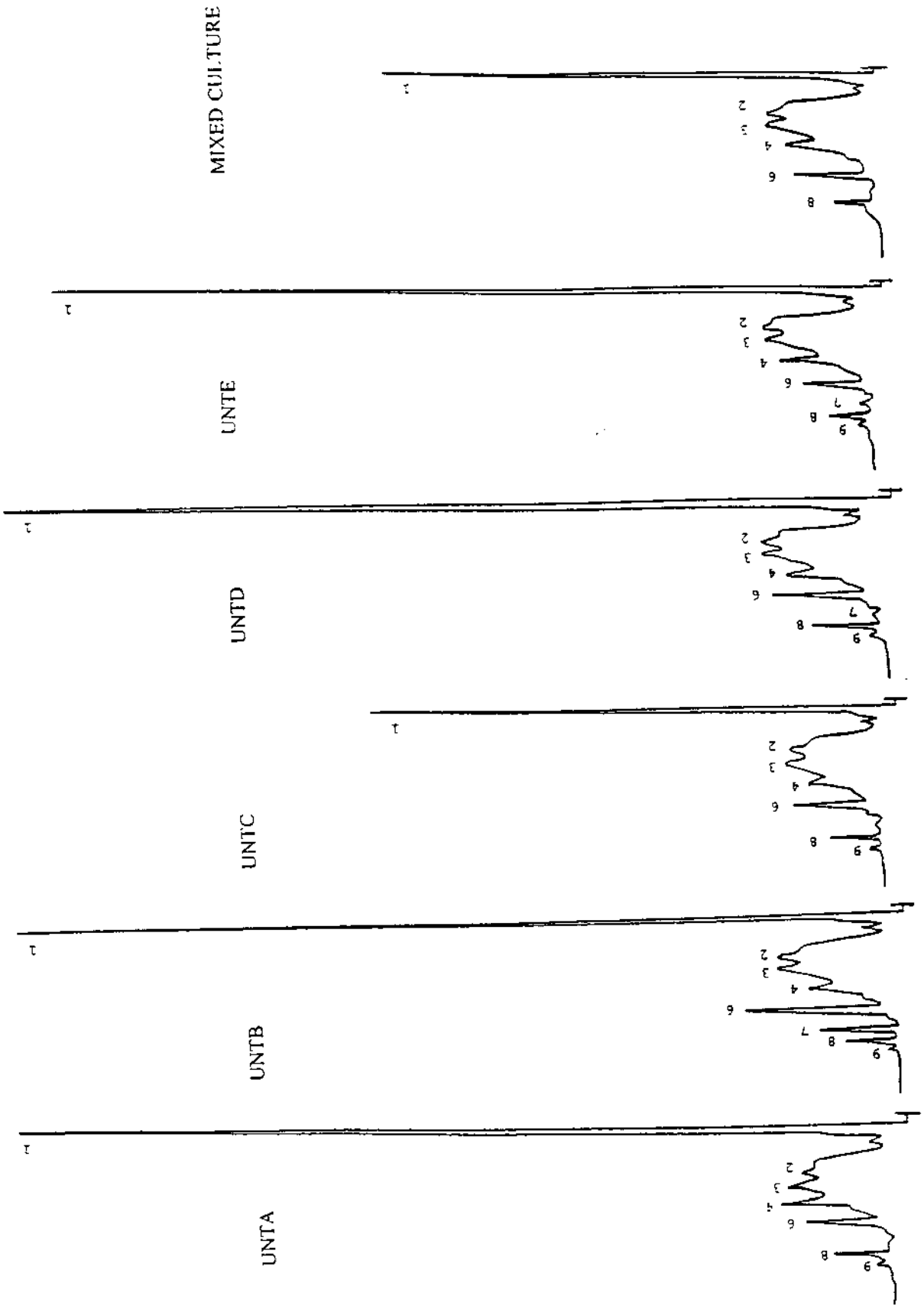


Figure 3.4. Control analysis of linseed oil volatiles without bacterial cultures by gas chromatography after one day of incubation.

Figure 3.5. Analysis of degradation of linseed oil volatiles by bacteria UNTA, UNTB, UNTC, UNTD, UNTE, and the mixed culture by gas chromatography after one day of incubation.


MIXED CULTURE
UNTE
UNTD
UNTC
UNTB
UNTA

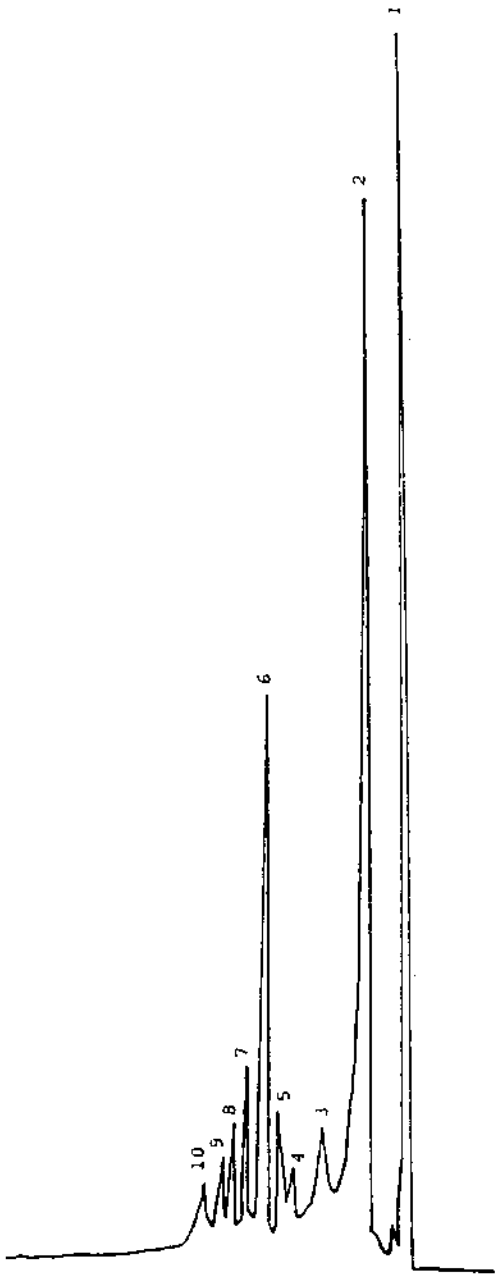
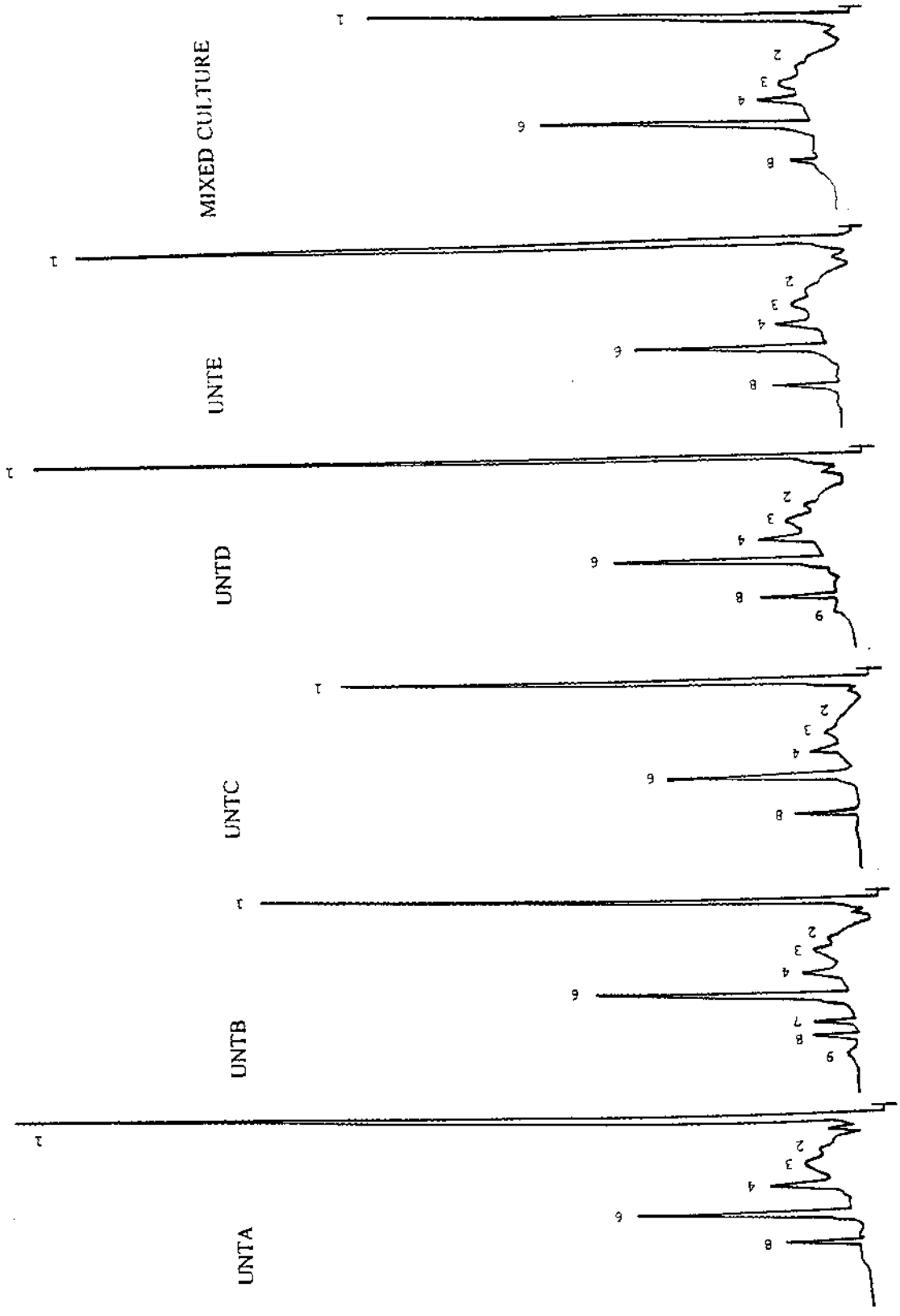


Figure 3.6. Control analysis of linseed oil volatiles without bacterial cultures by gas chromatography after three days of incubation.

Figure 3.7. Analysis of degradation of linseed oil volatiles by bacteria UNTA, UNTB, UNTC, UNTD, UNTE, and the mixed culture by gas chromatography after three days of incubation.


UNTA
UNTB
UNTC
UNTD
UNTE
MIXED CULTURE

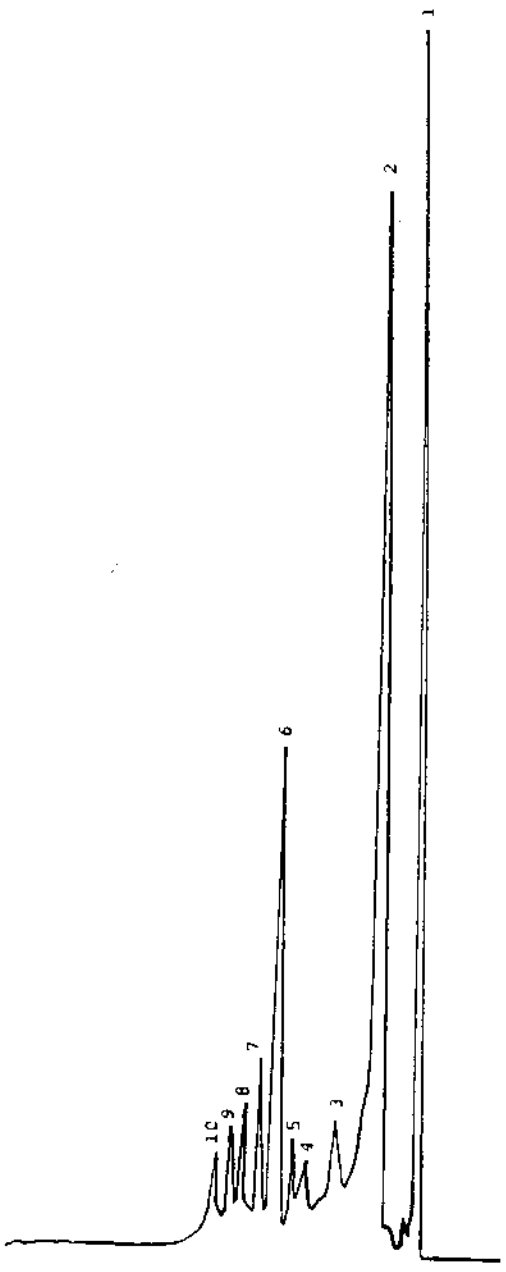
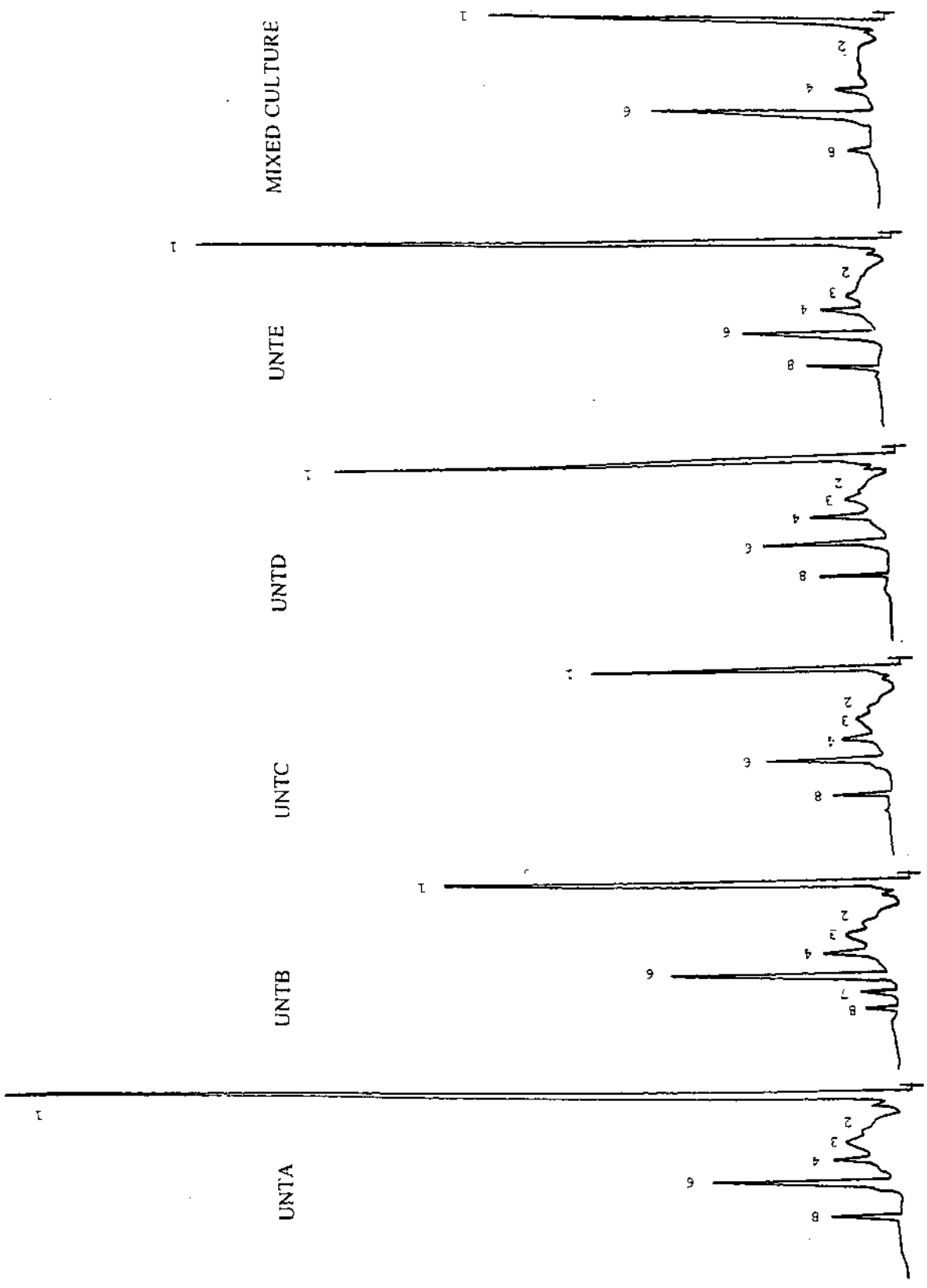


Figure 3.8. Control analysis of linseed oil volatiles without bacterial cultures by gas chromatography after five days of incubation.

Figure 3.9. Analysis of degradation of linseed oil volatiles by bacteria UNTA, UNTB, UNTC, UNTD, UNTE, and the mixed culture by gas chromatography after five days of incubation.


MIXED CULTURE
UNTE
UNTD
UNTC
UNTB
UNTA

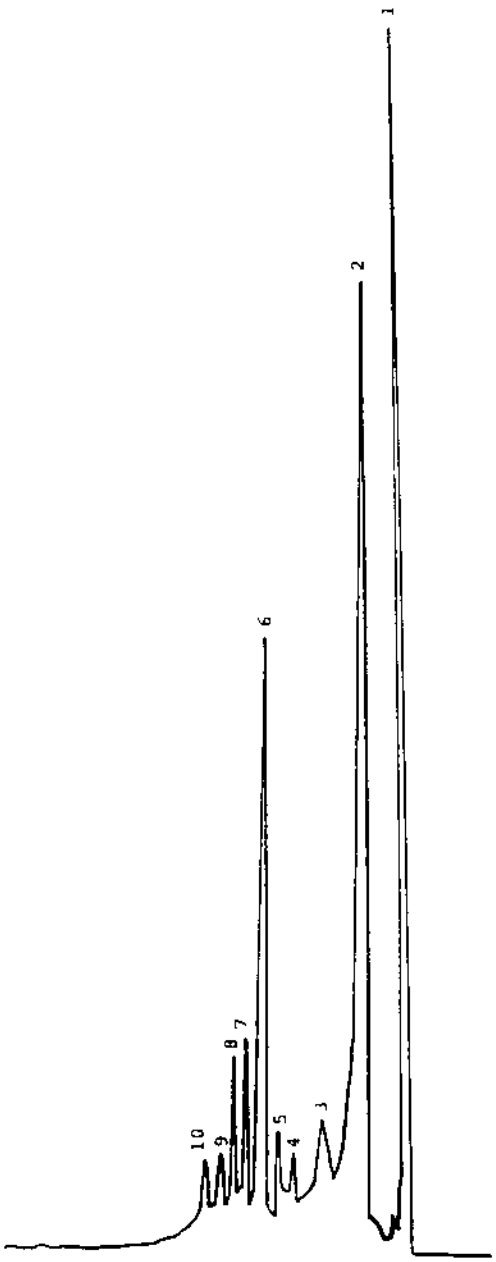
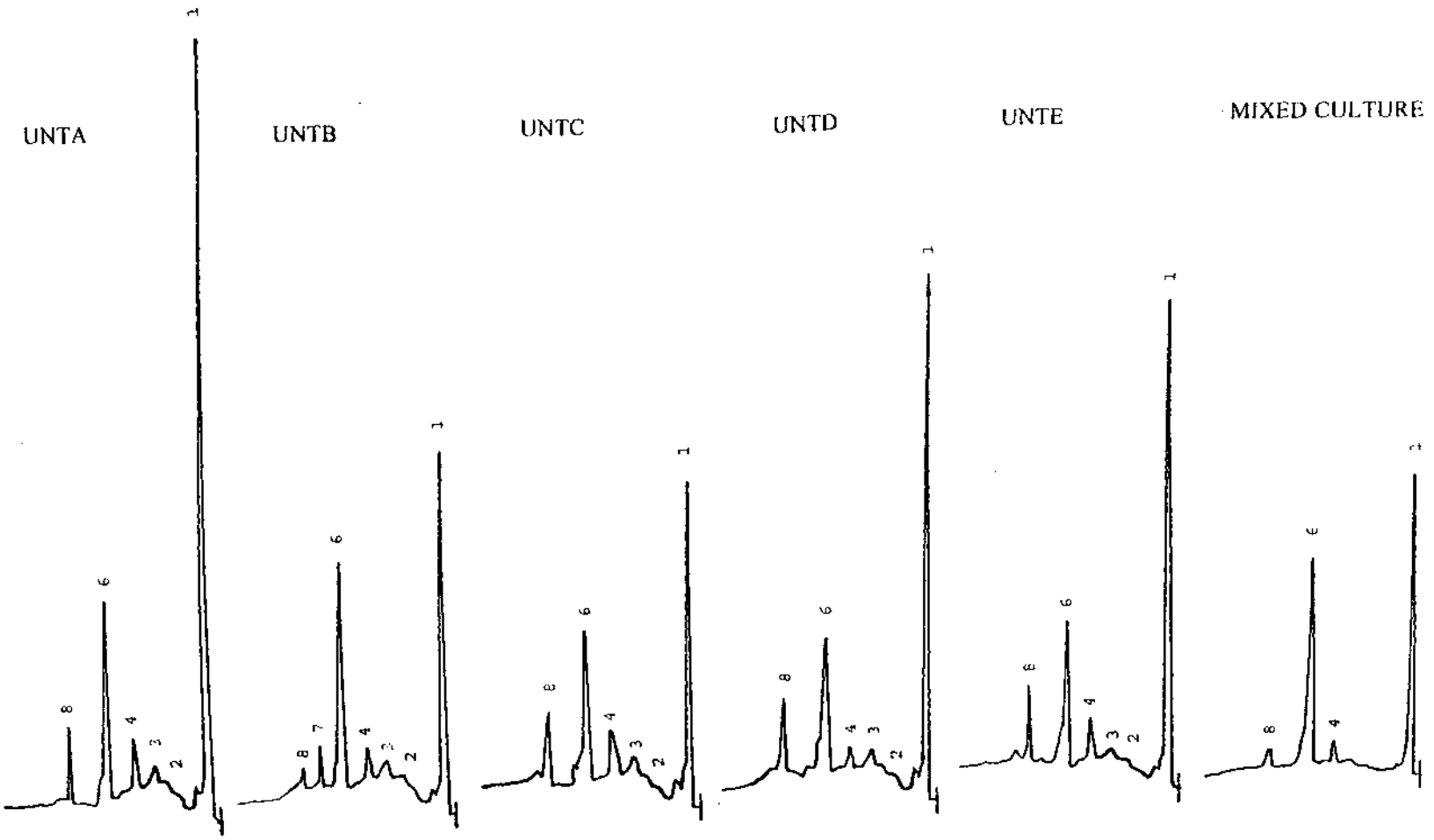


Figure 3.10. Control analysis of linseed oil volatiles without bacterial cultures by gas chromatography after seven days of incubation.

Figure 3.11. Analysis of degradation of linseed oil volatiles by bacteria UNTA, UNTB, UNTC, UNTD, UNTE, and the mixed culture by gas chromatography after seven days of incubation.


UNTA
UNTB
UNTC
UNTD
UNTE
MIXED CULTURE

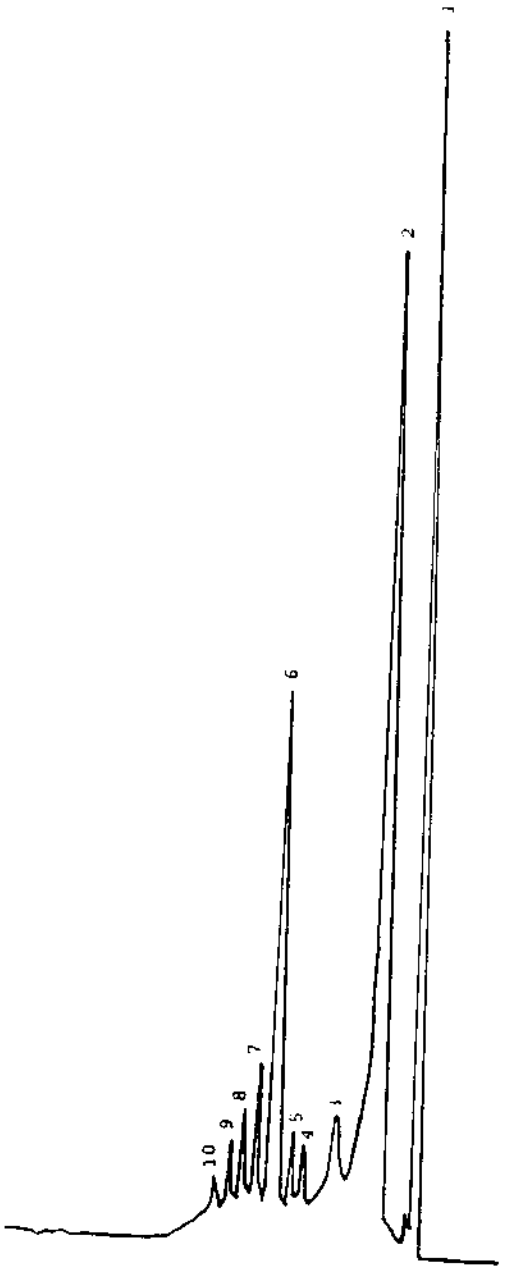
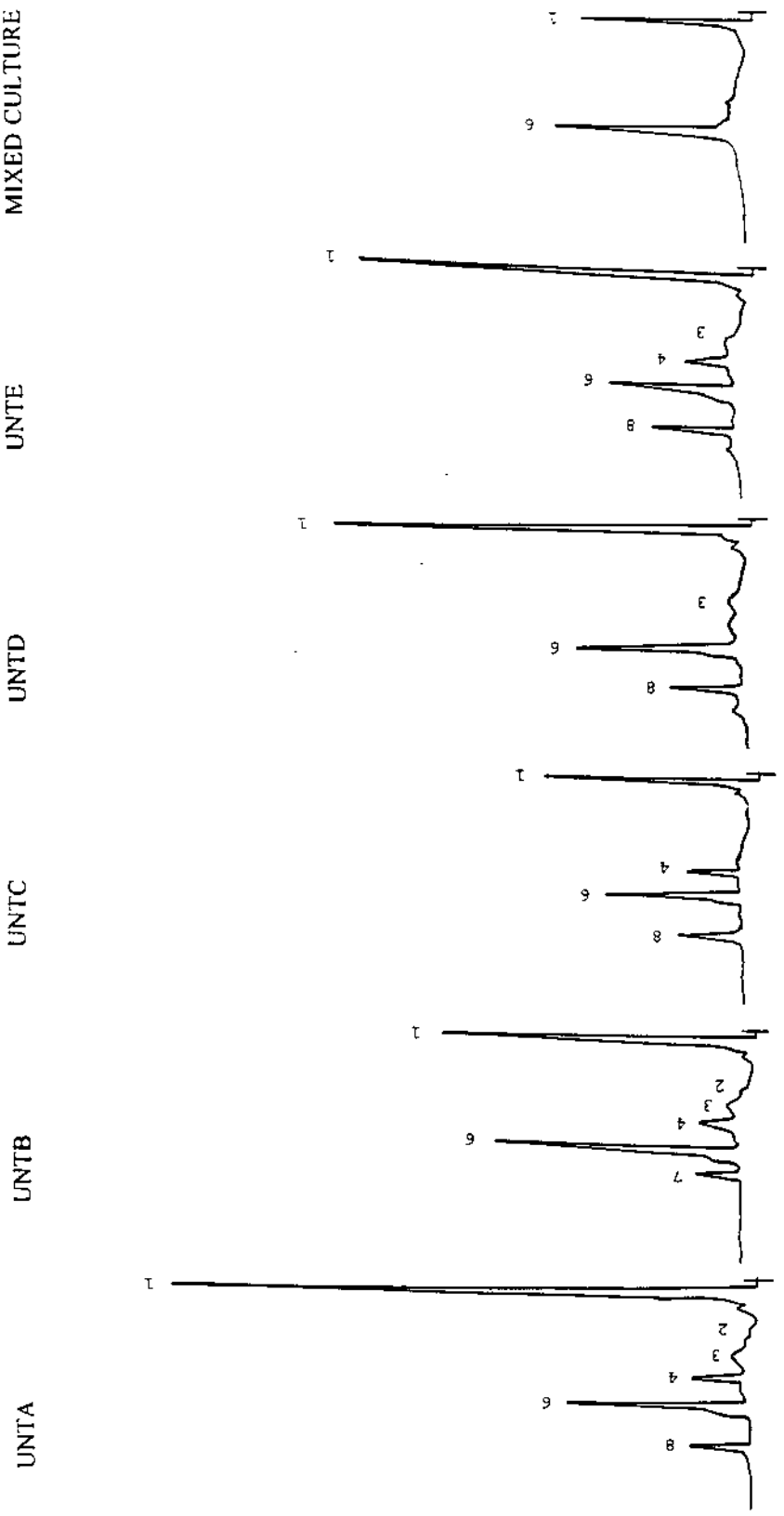


Figure 3.12. Control analysis of linseed oil volatiles without bacterial cultures by gas chromatography after fifteen days of incubation.

Figure 3.13. Analysis of degradation of linseed oil volatiles by bacteria UNTA, UNTB, UNTC, UNTD, UNTE, and the mixed culture by gas chromatography after fifteen days of incubation.


UNTA
UNTB
UNTC
UNTD
UNTE
MIXED CULTURE

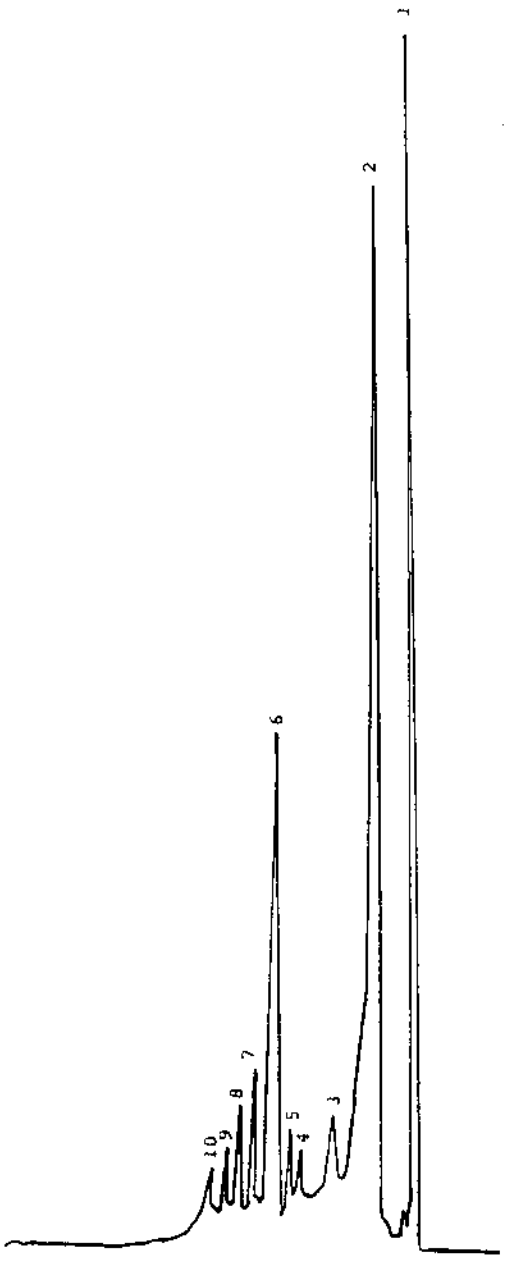


Figure 3.14. Control analysis of linseed oil volatiles without bacterial cultures by gas chromatography after thirty days of incubation.

Figure 3.15. Analysis of degradation of linseed oil volatiles by bacteria UNTA, UNTB, UNTC, UNTD, UNTE, and the mixed culture by gas chromatography after thirty days of incubation.

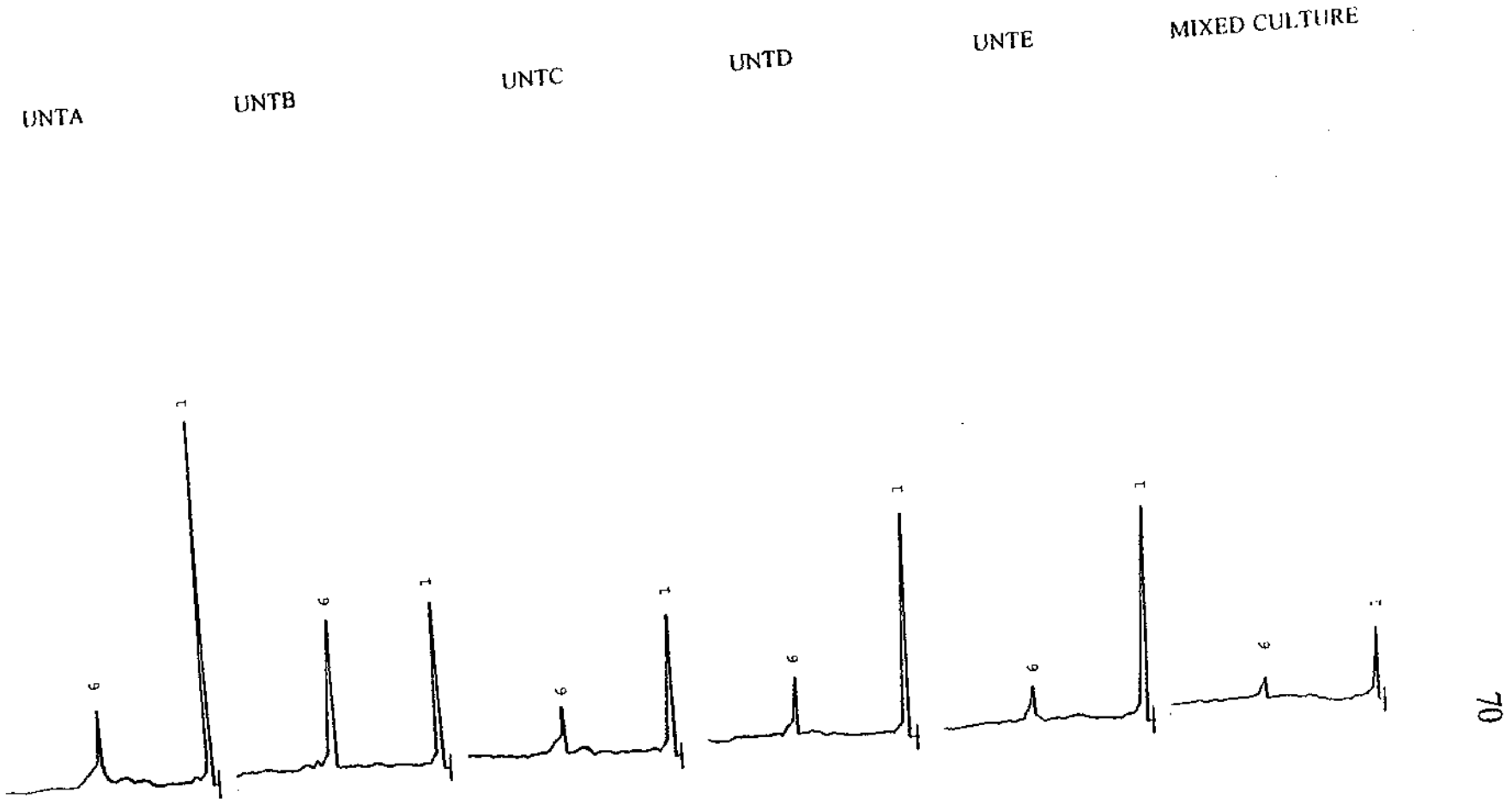
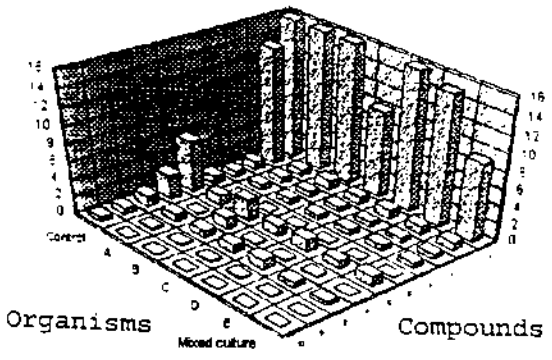
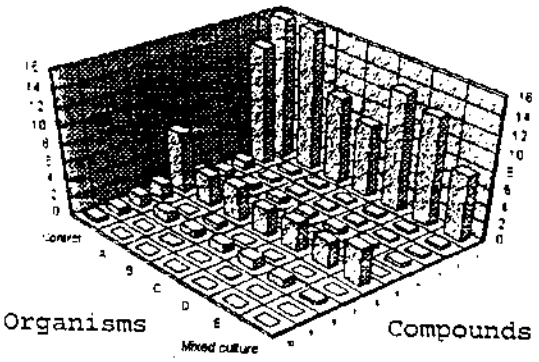

UNTA
UNTB
UNTC
UNTD
UNTE
MIXED CULTURE

Figure 3.16. Comparison of linseed oil volatiles degradation by isolated bacteria and by the mixed culture at different incubation periods.

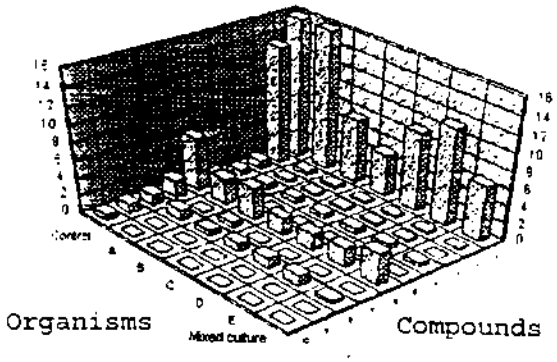
Day 1



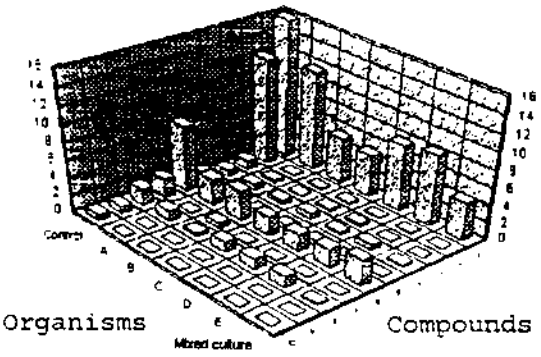
Day 3



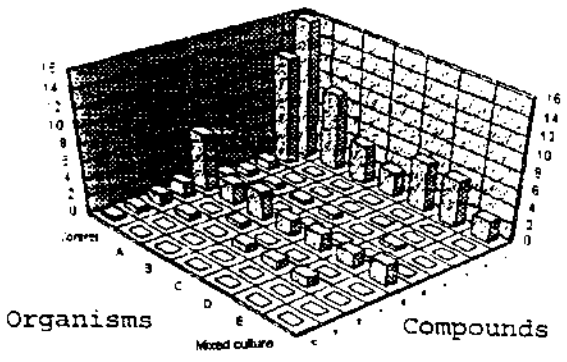
Day 5



Day 7



Day 15



Day 30

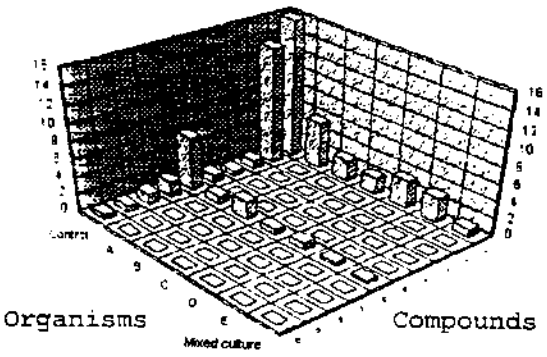


Figure 3.17. Comparison of degradation of compound 1 of linseed oil volatiles by different bacteria at various incubation periods.

Compound 1

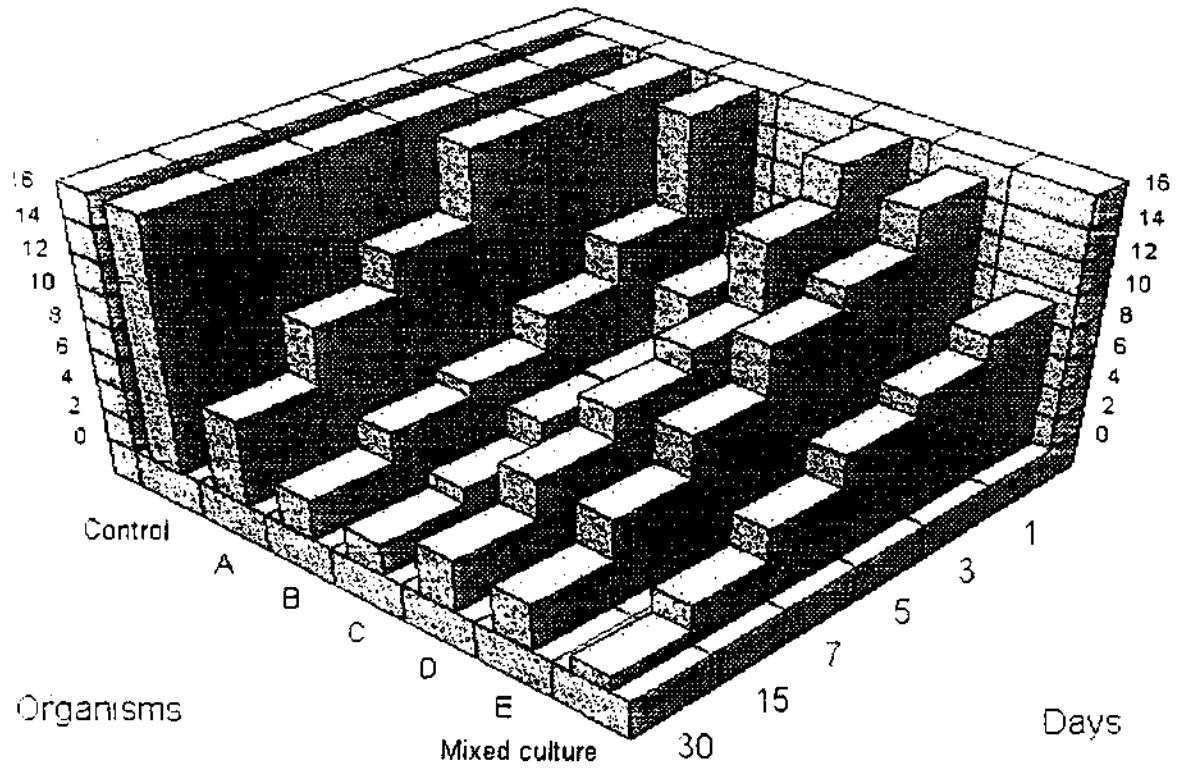

16
14
12
10
8
6
4
2
0
Control
A
B
C
D
E
Mixed culture
Organisms
1
3
5
7
15
30
Days

Figure 3.18. Comparison of degradation of compound 2 of linseed oil volatiles by different bacteria at various incubation periods.

Compound 2

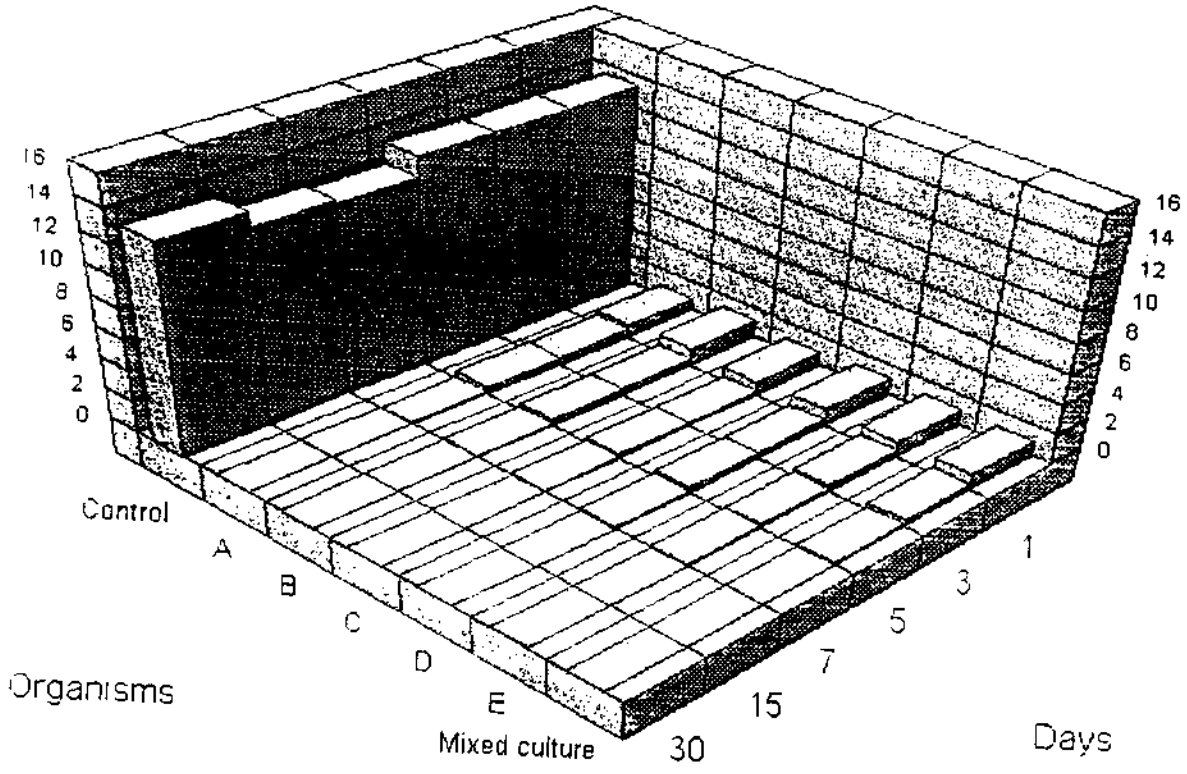

16
14
12
10
8
6
4
2
0
Control
A
B
C
D
E
Mixed culture
Organisms
1
3
5
7
15
30
Days

Figure 3.19. Comparison of degradation of compound 3 of linseed oil volatiles by different bacteria at various incubation periods.

Compound 3

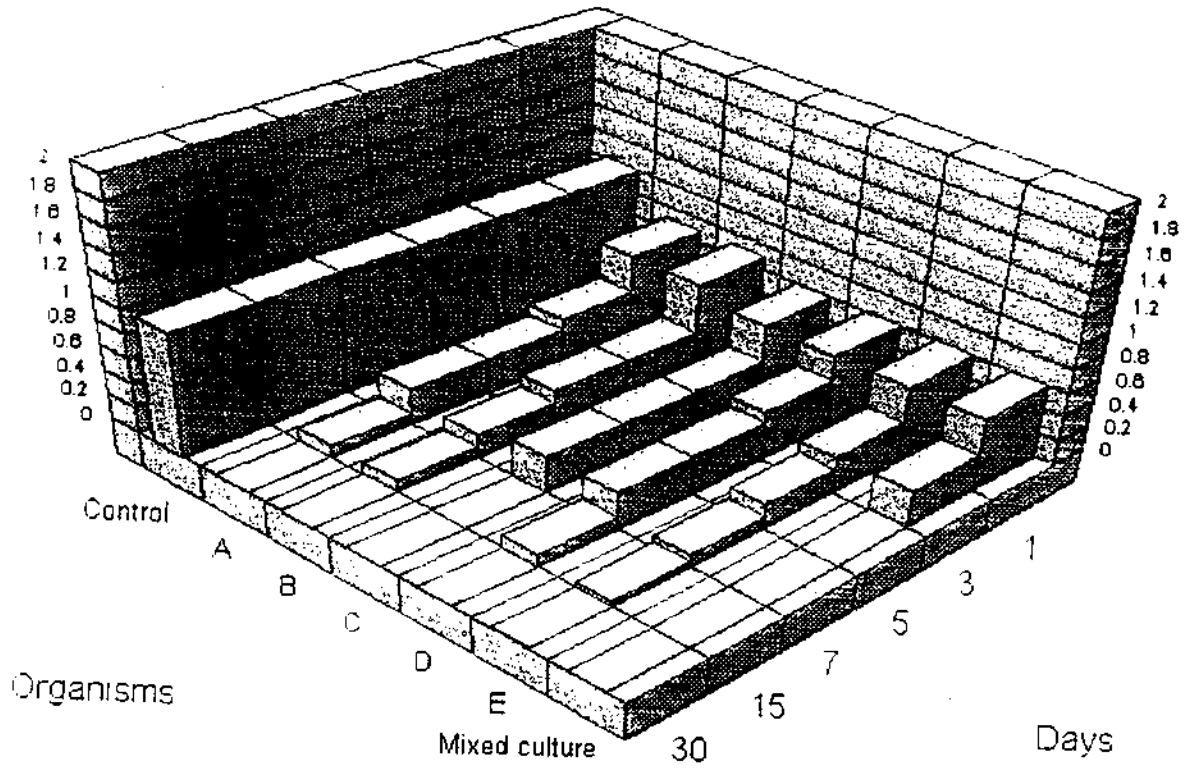

2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Control
A
B
C
D
E
Mixed culture
Organisms
1
3
5
7
15
30
Days

Figure 3.20. Comparison of degradation of compound 4 of linseed oil volatiles by different bacteria at various incubation periods.

Compound 4

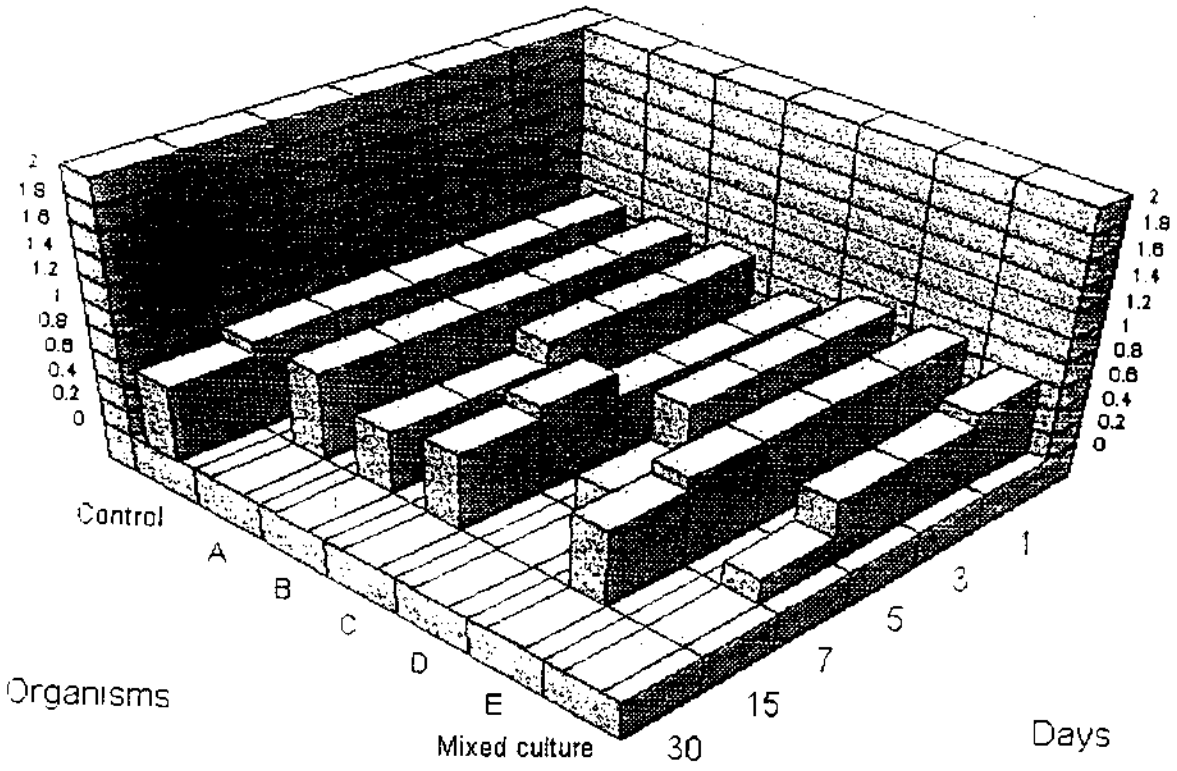

2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Control
A
B
C
D
E
Mixed culture
Organisms
1
3
5
7
15
30
Days

Figure 3.21. Comparison of degradation of compound 5 of linseed oil volatiles by different bacteria at various incubation periods.

Compound 5

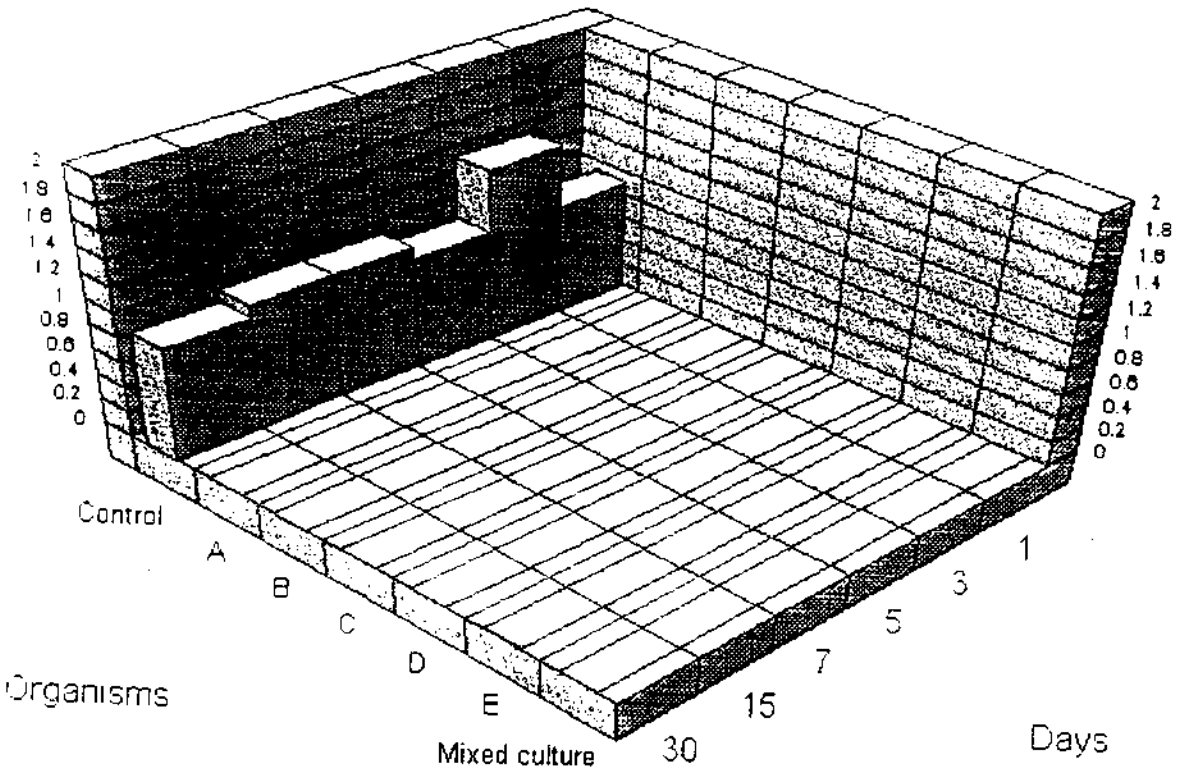

2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Control
A
B
C
D
E
Mixed culture
Organisms
1
3
5
7
15
30
Days

Figure 3.22. Comparison of degradation of compound 6 of linseed oil volatiles by different bacteria at various incubation periods.

Compound 6

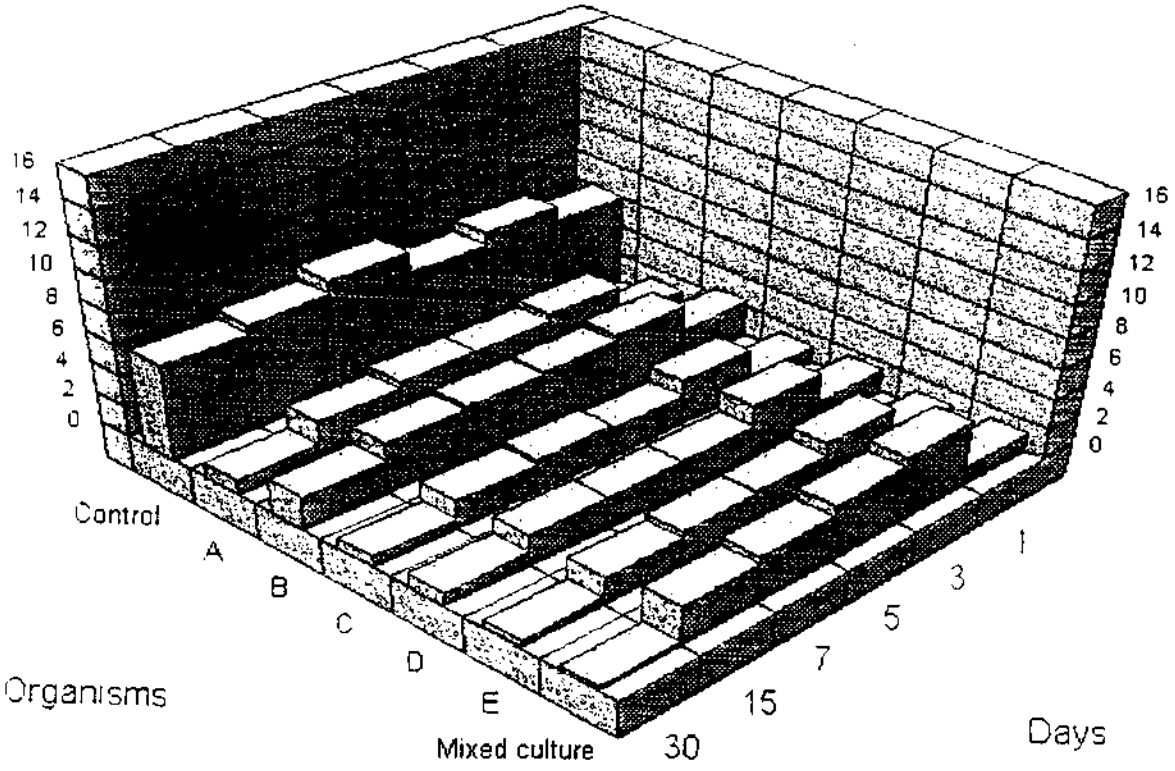

16
14
12
10
8
6
4
2
0
Control
A
B
C
D
E
Mixed culture
Organisms
1
3
5
7
15
30
Days

Figure 3.23. Comparison of degradation of compound 7 of linseed oil volatiles by different bacteria at various incubation periods.

Compound 7

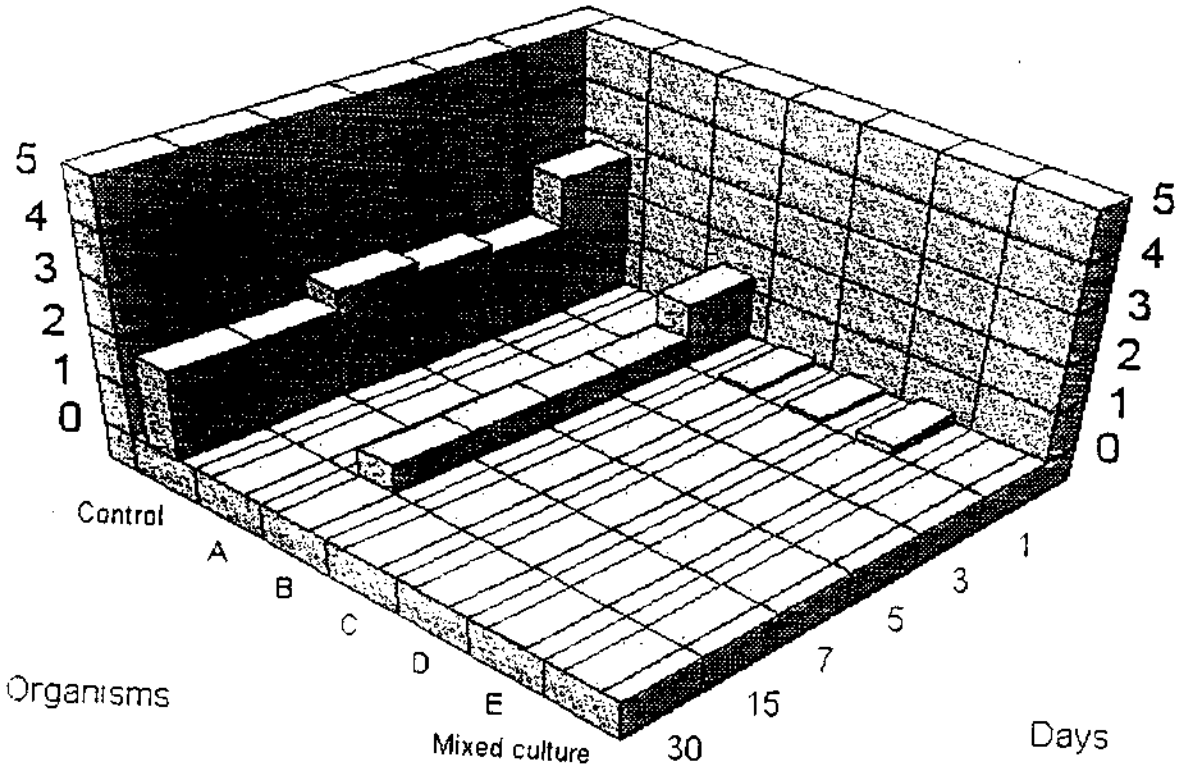

5
4
3
2
1
0
Control
A
B
C
D
E
Mixed culture
Organisms
1
3
5
7
15
30
Days

Figure 3.24. Comparison of degradation of compound 8 of linseed oil volatiles by different bacteria at various incubation periods.

Compound 8

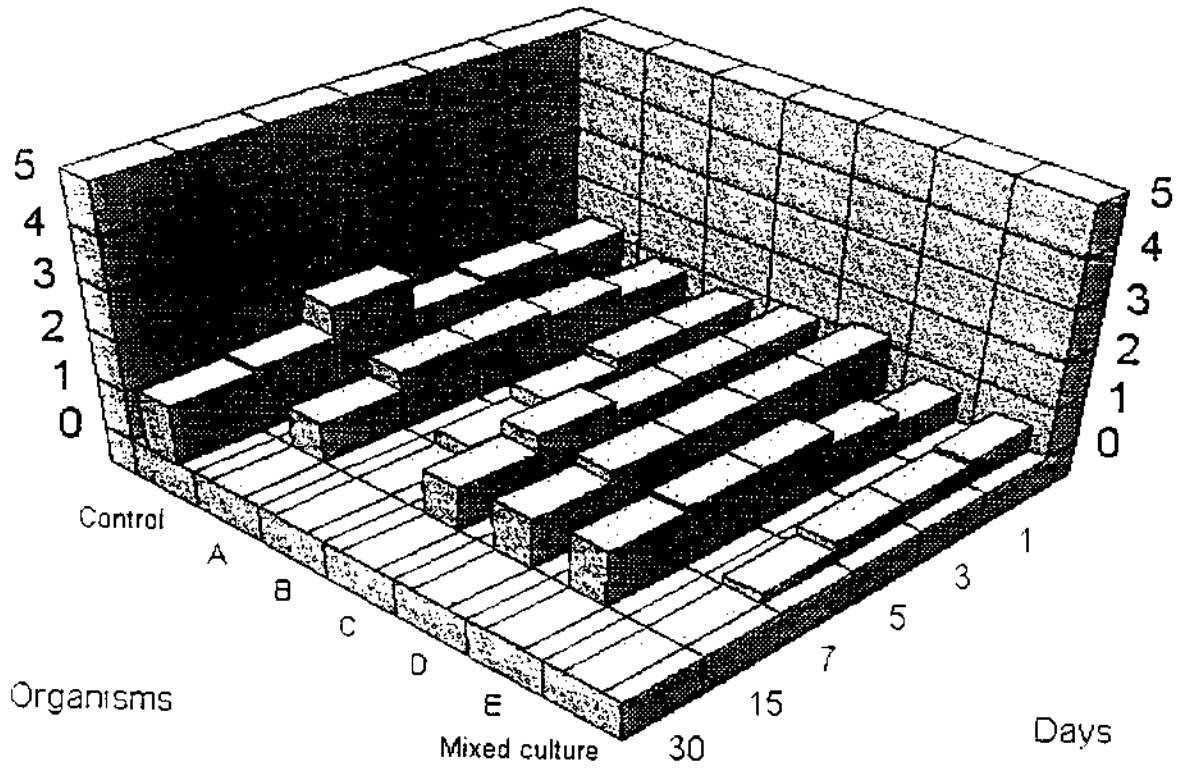

5
4
3
2
1
0
Control
A
B
C
D
E
Mixed culture
Organisms
1
3
5
7
15
30
Days

Figure 3.25. Comparison of degradation of compound 9 of linseed oil volatiles by different bacteria at various incubation periods.

Compound 9

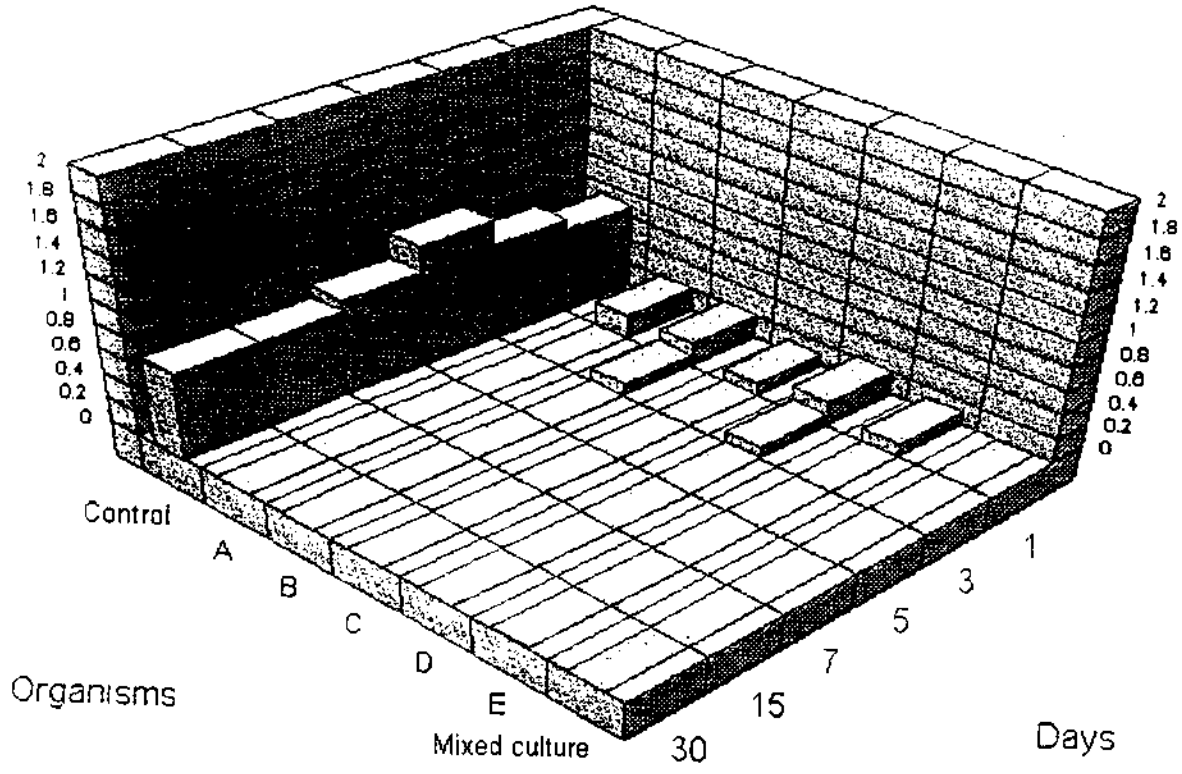

2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Control
A
B
C
D
E
Mixed culture
Organisms
1
3
5
7
15
30
Days

Figure 3.26. Comparison of degradation of compound 10 of linseed oil volatiles by different bacteria at various incubation periods.

Compound 10

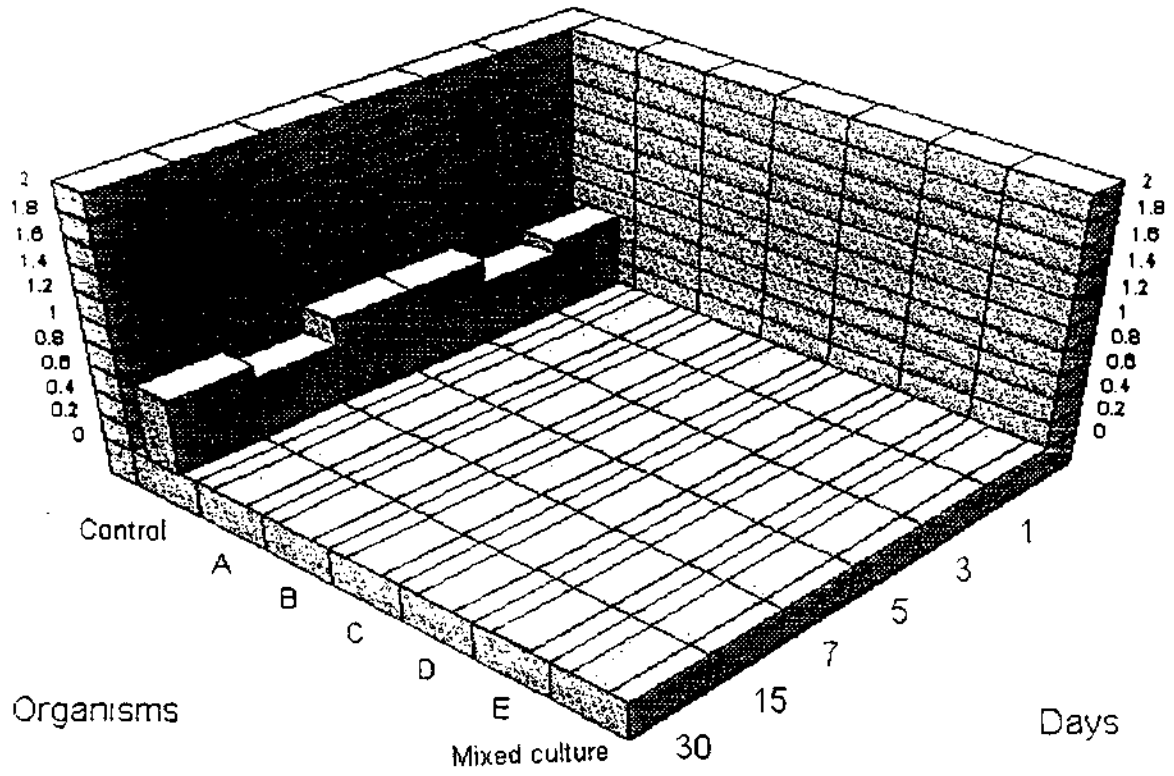

2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Control
A
B
C
D
E
Mixed culture
Organisms
1
3
5
7
15
30
Days

Table 3.3. Results of the organoleptic panel asked to sample linseed oil vapors with and without biodegradation by the mixed bacterial culture.

Panel member names	Smell of linseed oil	
	Without bacterial culture	With bacterial culture
Austin, Robin	Yes	No
Bejarano, Roxana	Yes	No
Brichta, Dayna	Yes	No
Greene, Brandon	Yes	No
Johnson, Zane	Yes	No
Kumar, Alan P.	Yes	No
Lee, Douglas	Yes	No
Meixner, Andy	Yes	No
Stewart, John E.B.	Yes	No
Thongmee, Acharawan	Yes	No

CHAPTER IV

DISCUSSION

Five organisms were isolated for the studies described here, but many more were present in the experimental biofilter that could have been tested for the ability to degrade the volatile substances given off by linseed oil. Two Gram positive and three Gram negative organisms were selected to emphasize the idea that degradation of linseed oil vapors is not restricted to one or a few classes of bacteria but rather widely scattered among soil organisms.

The bacteria selected grew well using linseed oil vapors as sole source of carbon and energy. Growth of the five isolates and also the consortium of the five in a single culture was rapid. The mixed culture showed a doubling time of approximately seven hours and maximal populations greater than 5×10^8 cell per milliliter. Growth was measured in M9 medium with linseed oil volatilized by a filter paper fan dipped in a reservoir of the liquid. Each of the components of the mixed culture, the bacterial consortium, was tested separately and each was found capable of growing independently of the others on the volatile products of linseed oil. In each case, growth was

measured against a control and the results of such comparisons were found to be unambiguous. The data in Figure 3.2 and 3.3 corroborate this assertion. The experiments described in these data were repeated 10 times or more and the results were similar in each case. This confirmed the finding that bacteria isolated from the soil can degrade linseed oil vapors.

This is a novel finding. A thorough search of the literature yielded no previous reports on such organisms or on the microbial oxidation of linseed oil vapor.

4.1. Chromatographic characteristics of linseed oil

Since linseed oil reacts with oxygen to produce various products such as aldehydes, ketones, short chain fatty acids, and esters (16, 17, 43), it is deemed necessary to see the effect of this phenomenon on microbial degradation. Chromatographic analysis of linseed oil after formation of the fatty acid methyl esters is shown in Figure 3.2. Six components were identified in this figure. The data shown in Figure 3.2 agree completely with data previously published (12, 13, 32).

4.2. Chromatographic characterization of linseed oil vapors

While linseed oil yielded six fatty acid esters on chromatographic analysis, the vapor yielded 10 volatile products (Figure 3.3). It is

assumed that the volatile compounds were produced when the linseed oil was exposed to air during a prolonged period of time. It was determined that the 10 components of the vapor are always present in linseed oil and therefore it is these substances that make up the vapor. The identity of these materials is not essential for testing the hypothesis under study. Because of this, no effort was made to identify these materials. It was shown that oleic, linoleic, and linolenic acids were not present in the vapor phase of linseed oil. Pure, authentic, samples of these three components of linseed oil were placed in sealed containers at ambient temperature and the liquid and vapor phases allowed to equilibrate. When the vapor phase was tested by the same method as that used for the data shown in Figure 3.3, no evidence of volatile substances was found in any of the three acids tested. This means that the volatile materials described chromatographically in Figure 3.3 are oxidation products of linseed oil and not the native compounds of the oil.

The chromatographic analysis of linseed oil vapors was not found in the readily available literature and is reported here for the first time. It must go without saying that it is these vapors that impinge on the use of linseed oil in the paint industry, the food industry, medicinal industry, and others. The vapors examined here

are noxious, pungent, irritating, and disagreeable. In addition, introducing them into the environment with exhaust air from industrial plants will undoubtedly lead to complaints from regulatory agencies. While linseed oil vapors are not classified as toxic agents by the National Institute for Occupational Safety and Health (NIOSH), they are responsible for the spontaneous production of formaldehyde, acetaldehyde, and hexanal which are classified as toxic. The latter are specifically produced from the autooxidation of linolenic acid under ambient climatic conditions.

4.3. Biodegradation of linseed oil vapors

The bacteria isolated from soil which were capable of growing in the vapors given off by linseed oil did so by degrading the various substances that make up the vapor. In each analysis performed, controls identical to the tests were included to assess the effect of autoxidation. Figure 3.4 shows that the vapors in uninoculated culture bottles were the same after 24 hours of incubation as those obtained from new samples (Figure 3.4). The same was true for incubation times of 3, 5, 7 ,15, and 30 days indicating that under the conditions of these studies, autooxidation has no discernable effect on the results obtained (Figures 3.6, 3.8, 3.10, and 3.14).

On the other hand, Figures 3.5, 3.7, 3.9, 3.11, 3.13, and 3.15 show that the individual organisms are capable of degrading the volatile compounds. These data show a progressive degradation going from day 1 to day 30. Degradation takes place by the individual organisms in pure culture and also by the consortium of the five organisms. The same data are presented in Figure 3.16 to show the fact that each organism utilizes the various components in a manner (rate) different from that of the others. The progressive oxidation of individual components as a function of time reveals different reaction mechanisms for each isolate. The consortium is, therefore, an overall average of these. The data in Figures 3.17 to 3.26 show the rate at which different compounds were removed by the different organisms and by the consortium.

An overview of these data shows that the bacteria studied grow at the expense of the linseed oil volatiles and that each of the compounds is degraded by one or more of the organisms employed. These data support the hypothesis examined and strongly assert that the hypothesis was not proven false. From this vantage point, my thesis affirms the idea that there are bacteria in nature that can degrade (remove) linseed oil vapors from the air and that they can do this rapidly and efficiently.

4.4. Organoleptic test

To test the use of the hypothesis in a setting similar to that of an industrial plant, a series of culture bottles was prepared and linseed oil added to all. Half of these bottles were inoculated with the mixed culture of bacteria while the other half, the controls, did not contain bacteria. After seven and 30 days of incubation, a panel of 10 individuals was asked to smell each bottle and comment on the odor detected. The results of this test are shown in Table 3.3.

4.5. Conclusion

All of the data strongly support the hypothesis since they show that linseed oil vapors were removed by the metabolic activities of the bacterial consortium. Even though bottles such as the ones used here are very different from the situation found in an industrial plant, the idea that bacteria remove linseed oil vapors from the air is tenable under any condition. The schematic diagram of a plant (Figure 4.1) employs the same basic principles investigated in this work. It is not radically different from applications to remove other plant effluent gases such as formaldehyde, ethanol, benzene, hexane, and acetoacetic acid (personal communication; Prof. G. R. Vela). The design in Figure 4.1 would be the most logical plan for the design of a bioremediation system for any plant producing linseed oil vapors. The

bacteria isolated for this work would be the organisms of choice for such a bioremediation system. The use of such a plan for the removal of linseed oil vapors has not been previously proposed and I offer it to the public as part of my masters work.

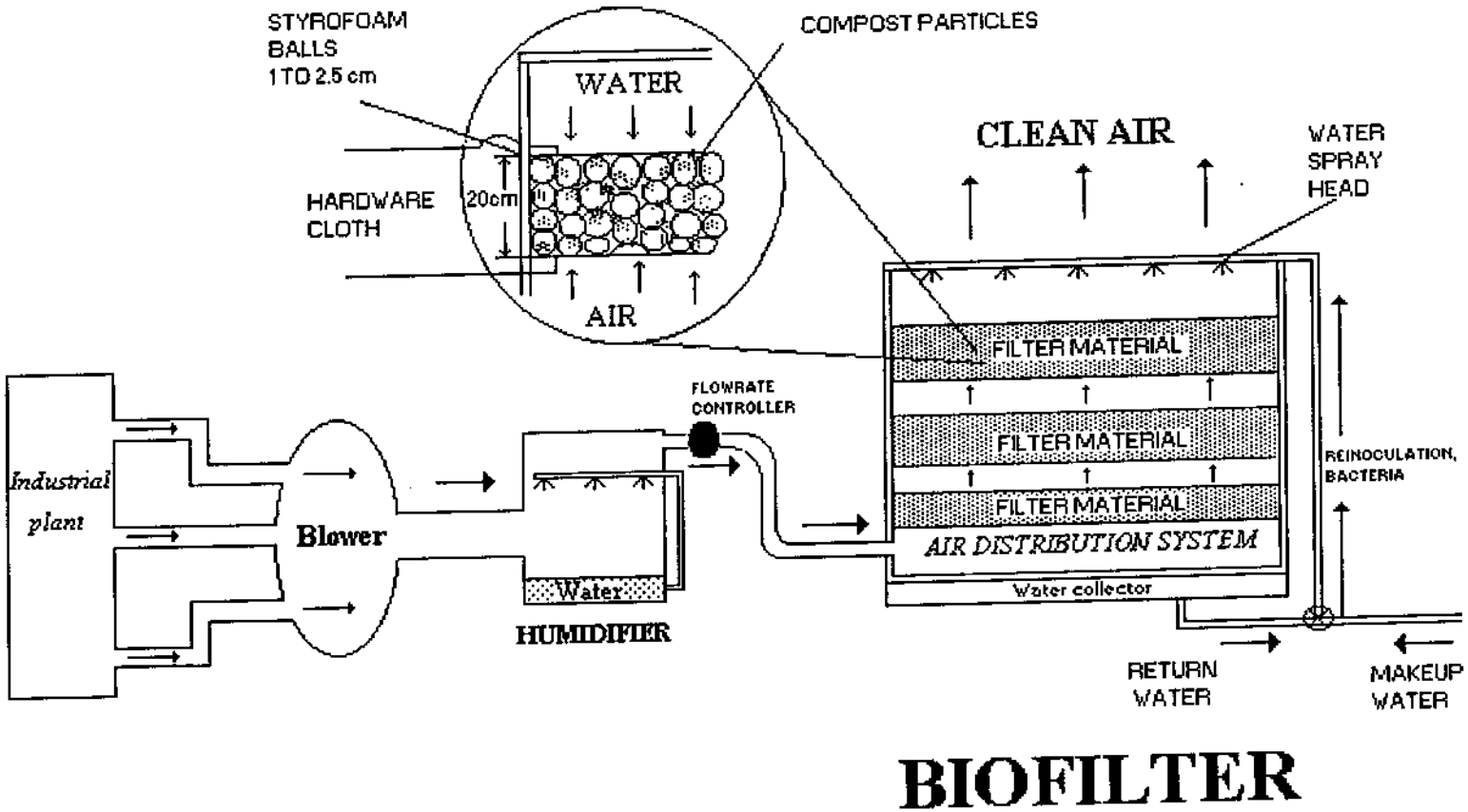


Figure 4.1. A multiple-bed biofilter system suggested by Prof. G.R.Vela (Personal communication).

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