United States Patent Office 2,769,750 Patented Nov. 6, 1956

2,769,750

PROCESSES EMPLOYING HOMOGENOUS MIXTURE OF INERT ADSORBENT AND SUBSTRATE

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No Drawing. Application March 20, 1953, Serial No. 343,807

18 Claims. (Cl. 195—116)

This invention relates to improvements in processes involving the action of microorganisms on organic substances. More particularly, the invention relates to improvements in processing wherein hydrocarbons are subjected to the action of microorganisms.

The subject application is a continuation-in-part of Serial No. 12,896 which was filed March 3, 1948, now abandoned. The parent application disclosed the catalytic effect of a finely divided adsorbent on the action of microorganisms on organic compounds, particularly petroleum hydrocarbons. This present continuation-in-part discloses that the action of microorganisms on oxygenated hydrocarbons such as alcohols, aldehydes, acids, ketones and esters is also accelerated by the presence of a finely divided adsorbent, and describes a large number of hydrocarbon-consuming microorganisms whose utilization of hydrocarbons and oxygenated hydrocarbons is catalyzed by the presence of a finely divided adsorbent.

In the conversion of hydrocarbons to various products by the action of microorganisms, the reactions have been notoriously slow and replete with undesired secondary reactions leading to loss of the desired products and the production of undesirable products. Such reactions, even on a small scale, have been matters of hours and even days. It is an object of the present invention to increase the speed of utilization of organic substances by microorganisms with the consequent development of a minimum of side reactions and maximum production of the desired products.

Another object of the invention is to provide an improved process for effecting the interaction of microorganisms with organic substances wherein the culture concentration of the microorganisms is maintained at a high level, the reaction being correspondingly accelerated in the desired direction.

Briefly, the present invention is concerned with the improvement in and acceleration of processes involving interactions between microorganisms and organic substances by effecting reaction therebetween in the presence of finely divided and relatively inert adsorbent material. More specifically, the present invention discloses that the metabolism of hydrocarbons and oxygenated hydrocarbons by microorganisms is significantly catalyzed by the presence of a finely divided adsorbent. The contact of organic compound, adsorbent and microorganisms is effected in the presence of a nutrient particularly suited for the microorganism or microorganisms.

Organic materials whose utilization by microorganisms is accelerated by the presence of a finely divided adsorbent are broadly defined as hydrocarbons and oxygenated hydrocarbons comprising alcohols, aldehydes, ketones, esters and acids. The invention is particularly useful in the processing of hydrocarbons and oxygenated hydrocarbons containing more than 12 carbon atoms which are difficult to disperse in the nutrient-microorganism mixture and which are utilized only very slowly by microorganisms in the absence of the finely divided inert adsorbent. The

processing of hydrocarbon oil fractions boiling above 400° F. such as gas oil, lube oil fractions and residual fractions is particularly enhanced by the addition of a finely divided adsorbent. The addition of a finely divided adsorbent particularly increases the action of microorganisms on solids such as petroleum wax, petroleum resins, asphalt, solid alcohols and solid esters such as beeswax and Chinese wax and on high boiling liquid hydrocarbon fractions such as gas oil, lube oil and residual fractions. Although the catalytic action of a finely divided adsorbent on high boiling hydrocarbons and oxygenated hydrocarbons is particularly noteworthy, the processing of lighter hydrocarbon fractions and oxygenated hydrocarbons is also improved by the presence of an adsorbent.

Examples of oxygenated hydrocarbons whose processing by microorganisms is materially enhanced by the presence of an adsorbent are high boiling alcohols such as dodecyl alcohol and eicosyl alcohol, organic acids such as linoleic, oleic and stearic acids, esters such as lauryl stearate, beeswax, ketones such as lauryl stearyl ketone and aldehydes such as stearyl aldehyde.

Since the process of the invention is of particular value in the processing of hydrocarbons, the major portion of the subsequent description will concern the use of a finely divided adsorbent to accelerate the action of microorganisms on hydrocarbons by microorganisms.

A naturally occurring earth or clay is the preferred inert adsorbent. Kaolinic and montmorillonitic clays have been found very satisfactory. Fuller's earth and Ione clay are particularly preferred natural clays.

Particle size distribution of the adsorbent and the method by which the substance to be treated is mixed therewith are important in increasing the efficiency of the process. Best results are obtained when the adsorbent is formed of particles 75 to 90 percent of which have diameters of less than 1.0 micron. The following example is cited to illustrate a preferred particle size distribution for a good adsorbent:

Particle Diameter in Microns	Percent Distribution in Samples
Less than 0.1	5
0.1 to 1.0	80
1.0 to 10.0	10
10 and above	5

The solid adsorbent is premixed with the hydrocarbon or oxygenated hydrocarbon substrate. The total amount of adsorbent added is in the range of 1 to 10 volumes of adsorbent per volume of hydrocarbon or oxygenated hydrocarbon being treated. The proportions of adsorbent to hydrocarbon depend on the nature of the substance to be treated; the polarity and ease of adsorption of the treated material are important in determining how much adsorbent is to be used. Normally, 1 to 3 parts of adsorbent to 1 part of liquid or solid hydrocarbon are employed.

Sufficient substantially anhydrous adsorbent is mixed with a substance to insure a thoroughly homogeneous mixture. It is preferred to add sufficient adsorbent to convert the hydrocarbon-adsorbent mixture to a crumbly, powdery phase. This dry mixture is termed an adsorbent complex. In this modification, the adsorbent complex is introduced into a nutrient solution that has been inoculated with the desired microorganism.

The addition of the finely divided adsorbent exerts a catalytic action on the rate at which a wide variety of microorganisms utilize hydrocarbons and oxygenated hydrocarbons. In general, the action of all hydrocarbon-consuming microorganisms in processing hydrocarbons

and oxygenated hydrocarbons is accelerated by the presence of a finely divided adsorbent. Hydrocarbon-utilizing bacteria, actinomyces, molds, yeasts and fungi may be employed in the process of this invention. The wide application of the process of the invention is illustrated in the following partial enumeration of microorganisms whose activity in processing hydrocarbons and oxygenated hydrocarbons is catalyzed by the presence of an inert adsorbent solid:

Bacteria—*Bacterium aliphaticum, Micrococcus paraffinae.*

Bacteria like forms—*Mycobacterium phlei, Mycobacterium laticola, Mycobacterium smegmatis.*

Actinomyces—*Actinomyces chromogenes albus, Actinomyces asteroides, Actinomyces salmonicolor, Proactinomyces paraffinae.*

Molds—*Aspergillus versicolor, Aspergillus flavus, Penicillium glaucum.*

Yeasts—*Monilia murmanica.*

Nutrient solution is employed in the reaction mixture in an amount such that the hydrocarbon or oxygenated hydrocarbon substrate is present in an amount approximately 1 to 10 percent by weight of the entire reaction mixture including adsorbent and nutrient solution. Preferably, the portion of substrate present in the total reaction mixture is in the lower portion of the 1 to 10 percent range.

The conditions as to temperature, pH and other factors are selected so as to provide optimum conditions for the particular processing reaction being effected. As is well known, the optimum conditions vary with the substrate being treated, the microorganism used and the reaction product that is the goal of the processing reaction. The reaction mixture is usually subjected to continuous agitation during the processing, although the catalytic effect of the adsorbent on the reaction is also realized when the processing is effected under quiescent conditions. When anaerobic microorganisms are employed in the processing, care must be taken during the agitation to keep the reaction mixture out of contact with oxygen. It is also advisable to sterilize the adsorbent and the hydrocarbon substrate either separately or jointly in complex form prior to contact with the selected microorganism in order to prevent any undesirable contamination or interaction.

The nature of the nutrient medium or solution (a mixture of basic mineral salts required for microbial metabolism) will vary with the microorganism to be handled in accordance with well-known bacteriological practice. For example, a nutrient solution suitable for Pseudomonas would comprise 1,000 ml. of distilled water having substantially the following mineral content:

	Grams
$NaH_2PO_4H_2O$	1.0
K_2HPO_4	2.0
NH_4NO_3	2.0
$MgCl_26H_2O$	0.25
$MnCl_24H_2O$	0.01
$CaCl_22H_2O$	0.01
$FeCl_2$	0.01

The addition of certain ions such as copper, mercury, zinc, bismuth and iodine to the medium in trace amounts will stimulate the activity of the microorganisms. Care must be exercised in the addition of such ions. When present in more than trace amounts, they tend to poison the microorganisms. Preferably, the pH of the medium is adjusted so that it is about 7 after sterilization.

With the present process, the concentration of the culture can be kept at a high level, so that the reaction is carried out at a much faster rate, and is relatively unaffected, particularly in a continuous process, by the addition of new reactants or by the removal of any portion of the aqueous phase. This is insured by the presence of the relatively large proportion of nutrient solution. Secondary reactions leading to the production of such undesirable end products as carbon dioxide and water are minimized.

While a batch process has been described, it is to be understood that the invention is applicable as well to a continuous process wherein the substance to be treated is continuously mixed with a stream of the adsorbent, and the inoculated nutrient is continually mixed with the resultant adsorbent complex under suitable conditions. Products of the interaction may be recovered by precipitation from the aqueous phase, distillation of the aqueous phase, processing of the adsorbent, and other well known chemical methods. After the process has been completed, the adsorbent may usually be regenerated by the application of heat or the use of solvents.

The process of the invention is illustrated in the following example which demonstrates the catalytic effect of a finely divided adsorbent on the conversion of a large quantity of paraffin wax to degradation products in the process of the invention.

100 g. of paraffin wax was mixed with 200 cc. of nutrient solution and contacted with a wax-destroying species of Pseudomonas; after three months, the wax sample was found to have undergone no substantial decomposition. Another 100 g. sample of the same paraffin wax stock was mixed with 150 g. of adsorbent clay, and 200 cc. of nutrient solution of the type described above; this mixture of wax, adsorbent and nutrient was inoculated with the same wax-destroying Pseudomonas species with the result that the wax was entirely converted within 72 hours to oxygen-containing decomposition products.

The catalytic effect of a finely divided adsorbent on the utilization of hydrocarbons by hydrocarbon-consuming microorganisms has also been demonstrated on a microscale employing small quantities of hydrocarbons. In this test, the results of which are shown in the accompanying table, the increased conversion of high molecular weight hydrocarbons by microorganisms in the presence of a finely divided adsorbent is shown by the increased oxygen utilization obtained when the hydrocarbon is subjected to microbial decomposition in the presence of a finely divided adsorbent. In this test, homogeneous powdery hydrocarbon-clay complexes were prepared by heating approximately 100 g. of the hydrocarbon substrate with 150 g. of clay at a temperature of approximately 120° C. Sufficient of this complex was then added to 50 ml. of nutrient solution in an 8 ounce bottle to make a 2.5 percent hydrocarbon concentration in the total culture medium. A parallel series of experiments was set up to demonstrate the normal rate of hydrocarbon conversion by microorganisms in the absence of the catalytic adsorbent material; in this series, sufficient hydrocarbon was added to 50 ml. of nutrient solution to impart to the resulting culture mixture a hydrocarbon concentration of 2.5 percent. Both series of reaction mixtures were then inoculated with a hydrocarbon-clay culture of the test microorganisms; each reactor bottle was seeded with 2.5 percent of the appropriate seed culture.

The nutrient medium employed in this evaluation had the following composition:

NH_4NO_3		2.0
K_2HPO_4		2.0
KH_2PO_4		1.0
$CaCl_2 \cdot 2H_2O$		0.05
$FeSO_4 \cdot 7H_2O$		0.01
KI		trace
$MgSO_4 \cdot 7H_2O$		0.5
$MnCl_2 \cdot 4H_2O$		0.2
$CuSO_4 \cdot 5H_2O$		trace
Distilled H_2O	ml.	1,000
pH 7.0		[1] 7.2

[1] After autoclaving.

The air-filled reactor bottles were then placed in a shaking apparatus and were maintained during shaking at a temperature of 28 to 30° C. When growth was observable in the reactor bottles, shaking was stopped, nitrogen gas introduced to bring the pressure back to atmospheric and the volume of oxygen utilized calculated from the percentage of residual oxygen in each bottle which was determined by a Beckman oxygen analyzer. The following table clearly demonstrates that the presence of finely divided adsorbent effects a two- to seven-fold increase in oxygen utilization micro evaluation of the process of the invention:

Organism	Hydrocarbon	Hours in Shaker	Ml. O_2 utilized	
			Hydrocarbon	Hydrocarbon clay complex
Achromobacter sp. TDC (B–148).	Paraffin wax	26	10.6	40.0
Alcaligenes sp. TDC (A–47).	do	22	14.5	36.1
Aspergillus sp. TDC (946).	do	154	4.0	29.4
Aspergillus sp. TDC (946).	Arabian crude oil.	154	37.2	55.6

The results in the foregoing table clearly demonstrate the catalytic effect exerted on the action of microorganisms by the use of a finely divided adsorbent.

The presence of clay caused a four-fold increase in the utilization of paraffin wax by a species of Achromobacter, approximately a two and one half-fold increase in the utilization of paraffin wax by a species of Alcaligenes, a seven-fold increase in the utilization of paraffin wax by a species of Aspergillus and almost a two-fold increase in the utilization of Arabian crude oil by the same Aspergillus species.

The Aspergillus species (TDC 946) mentioned in the previous table has been described as follows:

Source of isolation.—A mixed sample composed of soils taken from areas in which oils were undergoing decomposition and water taken from sumps, tank bottoms, and waters which had been in contact with refinery wastes; decomposing asphalt, wax and used motor oil were included in the sample.

Date of isolation.—October 1, 1947.

Location of isolation.—Signal Hill Laboratory, The Texas Company, Long Beach, California.

Media used for isolation.—A hydrocarbon-mineral salts medium composed of 5 per cent Arabian crude oil (topped) and the following medium at a pH of 4.5; this medium is generally designated Turfitt's Yeast and Mold Medium.

Compound:	Grams per liter
$NaNO_3$	2.0
KH_2PO_4	1.0
$MgSO_4 \cdot 7H_2O$	0.5
KCl	0.5
$FeSO_4 \cdot 7H_2O$	0.01

Sea water, 10.0 ml.
Distilled water, 990.0 ml.

Conditions of isolation.—Aerobic, at 40° C.

Microscopic examination and a comparison of the essential morphological features with those of molds described in the literature indicate that the mold belongs to the *fumigatus* group of the Aspergilli, the type species of which is *Aspergillus fumigatus*. A complete description thereof can be found in "A Manual of the Aspergilli" by Charles Thom, The William and Wilkins Company, Chapter X, pp. 148–151.

The Achromobacter species (TDC B–148) has been described as follows:

Source of isolation.—Soils and waters taken from areas in which oils are undergoing decomposition.

Date of isolation.—1946.

Location of isolation.—Signal Hill Laboratory, The Texas Company, Long Beach, California.

Media used for isolation.—A hydrocarbon-mineral salts medium composed of 5 per cent Arabian crude oil (topped) and the following medium at approximate pH of 6.5:

Compound:	Grams per liter
$(NH_4)_2SO_4$	2.0
$NaH_2PO_4 \cdot H_2O$	1.0
K_2HPO_4	2.0
$MgSO_4 \cdot 7H_2O$	0.25
$MnCl_2 \cdot 4H_2O$	0.01
$CaCl_2 \cdot 2H_2O$	0.01
$FeSO_4 \cdot 7H_2O$	0.01
KCl	0.00001
$CuSO_4 \cdot 5H_2O$	0.00001

Distilled water to 1,000 ml.

Conditions of isolation.—Aerobic at 30° C.

Culture 148 is a gram negative rod which gives the following biochemical reactions: negative on arabinose, lactose, dextrin, maltose, dextrose and iron peptone. It gave a slightly acid test on mannite, sucrose and glycerol. It peptonized and reduced litmus milk, liquefied gelatin and reduced nitrate.

The Alcaligenes species (TDC A–47) has been described as follows:

Source of isolation.—Soils and waters taken from areas in which oils are undergoing decomposition.

Date of isolation.—1946.

Location of isolation.—Signal Hill Laboratory, The Texas Company, Long Beach, California.

Media used for isolation.—A hydrocarbon-mineral salts medium composed of 5 per cent Arabian crude oil (topped) and the following medium at an approximate pH of 6.5:

Compound:	Grams per liter
NH_4NO_3	2.0
$NaH_2PO_4 \cdot H_2O$	1.0
K_2HPO_4	2.0
$MgCl_2 \cdot 6H_2O$	0.25
$MnCl_2 \cdot 4H_2O$	0.01
$CaCl_2 \cdot 2H_2O$	0.01
$FeCl_2$	0.01
KCl	0.00001
$CuCl_2 \cdot 2H_2O$	0.00001

Distilled water to 1,000 ml.

Conditions of isolation.—Aerobic at 30° C.

Culture 47 is a gram negative rod which gives the following biochemical reactions: negative on arabinose, mannite, sucrose, glycerol, lactose, dextrin, maltose, dextrose, gelatin and iron peptone. Litmus milk gave a reduced and alkaline reaction. Nitrate was reduced.

The process of this invention is applicable to many different types of reactions such as the desulfurization of petroleum fractions and the production of chemicals such as antibiotics, acids, alcohols, esters, ketones, aldehydes and other organic compounds from hydrocarbons and carbonaceous material.

The process of this invention also finds application in the treatment of hydrocarbon-containing waste liquids from petroleum refineries. Conversion of hydrocarbon-containing aqueous waste from petroleum refineries into innocuous products by the action of microorganisms has not been commercially feasible prior to this invention because of the extremely slow rate at which the hydrocarbon pollutants are attacked by microorganisms. Since the rate at which hydrocarbon pollutants present in aqueous refinery waste are decomposed is increased two- to seven-fold by the presence of the finely divided adsorbent during the microbial utilization, conversion of refinery waste into innocuous products by microbial treatment has become a commercial reality. In using the

process of the invention in microbial conversion of refinery waste into innocuous products which can be disposed into rivers or streams without causing their pollution, the previously described conditions of adsorbent concentration, particle size and use of nutrient medium are observed.

Obviously many modifications and variations of the invention as above set forth may be made without departing from the spirit and scope thereof, and, therefore, only such limitations should be imposed as are indicated in the appended claims.

I claim:

1. A process for improving processes involving the action of hydrocarbon-consuming microorganisms on substrates selected from the group consisting of hydrocarbons, oxygenated hydrocarbons and mixtures thereof which comprises adding sufficient anhydrous inert adsorbent in solid particle form to said substrate to form a thoroughly homogenous mixture, mixing the resulting homogenous mixture with a nutrient medium and subjecting the resulting mixture to the action of said hydrocarbon-consuming microorganisms.

2. A process according to claim 1 in which sufficient of said adsorbent solid in substantially anhydrous form is added to said substrate to form a relatively dry, powdery adsorbent complex.

3. A process according to claim 1 in which said substrate is present in an amount of approximately 1 to 10 weight percent of the total mixture of adsorbent, substrate and nutrient medium.

4. A process according to claim 1 in which the substrate contains more than 12 carbon atoms.

5. A process according to claim 1 in which 75 to 95 percent of the particles of said adsorbent are of a diameter less than 1.0 micron.

6. A process according to claim 1 in which the mixture of substrate, nutrient and adsorbent is subjected to the action of Pseudomonas bacteria.

7. A process according to claim 1 in which the mixture of adsorbent, substrate and nutrient is subjected to the action of Achromobacter bacteria.

8. A process according to claim 1 in which the mixture of adsorbent, substrate and nutrient is subjected to the action of Alcaligenes bacteria.

9. A process according to claim 1 in which the mixture of adsorbent, substrate and nutrient is subjected to the action of Aspergillus fungi.

10. A process according to claim 1 in which fuller's earth is used as the inert adsorbent.

11. A process according to claim 1 in which Ione clay is used as the inert adsorbent.

12. A process for treating pollutant-containing aqueous waste from a petroleum refinery which comprises adding to said waste liquid sufficient anhydrous inert adsorbent in solid particle form to produce a thoroughly homogenous mixture, adding a nutrient medium to said homogenous mixture of waste liquid and adsorbent and contacting said resulting mixture to the action of the hydrocarbon-consuming microorganisms with the resulting acceleration of the conversion of said pollutants to a relatively innocuous residual liquid.

13. A method for improving processes involving the action of Pseudomonas on substrates selected from the group consisting of hydrocarbons, oxygenated hydrocarbons and mixtures thereof which comprises forming an adsorbent complex by mixing said substrate with an inert adsorbent solid in particle form and in sufficient amount to form a substantially homogeneous mixture, adding sufficient of said adsorbent solid in substantially anhydrous form to said mixture to insure said complex being in a relatively dry, powdery phase and subjecting the resultant adsorbent complex to the action of Pseudomonas bacteria.

14. A method for improving processes involving the action of Pseudomonas on substrates selected from the group consisting of hydrocarbons, oxygenated hydrocarbons and mixtures thereof which comprises forming an adsorbent complex by mixing said substrate with an inert adsorbent solid in particle form, mixing the resultant adsorbent complex with a nutrient medium inoculated with Pseudomonas bacteria and subjecting the resultant mixture to conditions favorable to the action of said bacteria.

15. The method according to claim 14 in which said substrate is present in an amount of approximately 1 to 10 weight percent of the total mixture of solid, substrate and nutrient medium.

16. A method for improving processes involving the action of Pseudomonas on substrates selected from the group consisting of hydrocarbons, oxygenated hydrocarbons and mixtures thereof which comprises forming an adsorbent complex by mixing said substrate with an inert adsorbent solid in particle form, the major portion of the particles having a diameter less than 10 microns and subjecting the resultant adsorbent complex to the action of pseudomonas bacteria.

17. The method of claim 16 wherein 75 to 95 per cent of the particles of said adsorbent solid are of a diameter less than 1.0 micron.

18. A method for improving processes involving the action of Pseudomonas on substrates selected from the group consisting of hydrocarbons, oxygenated hydrocarbons and mixtures thereof which comprises forming an adsorbent complex by mixing said substrate with an inert adsorbent clay in sufficient amount to form a relatively dry, powdery phase, mixing the resultant adsorbent complex with nutrient medium, said nutrient medium being added in sufficient amount to secure a portion of substrate of about 1 to 10 percent by weight in the total and subjecting the resulting mixture of adsorbent complex and nutrient medium to the action of Pseudomonas bacteria.

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