Aug. 27, 1963 J. H. BREWER 3,102,082

APPARATUS AND METHOD FOR CULTURING MICRO-ORGANISMS

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FIG. 1

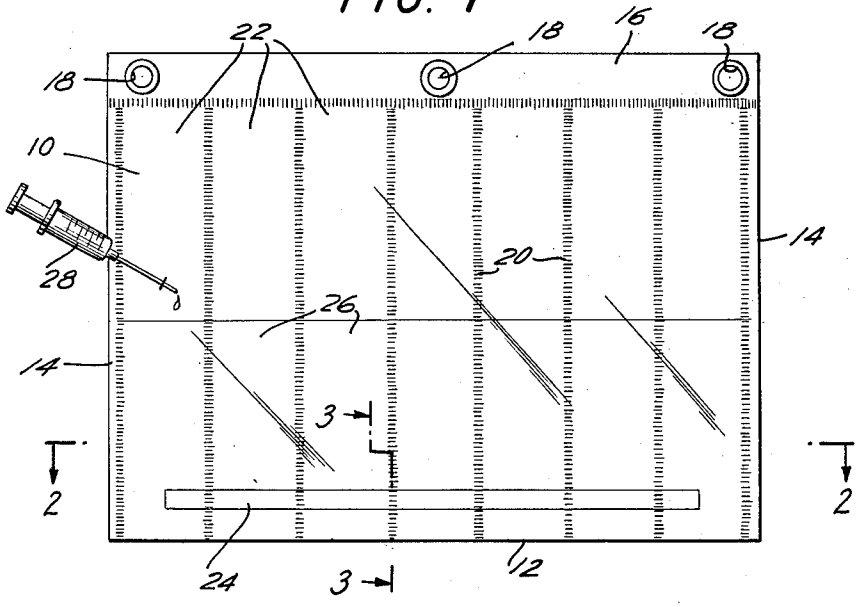
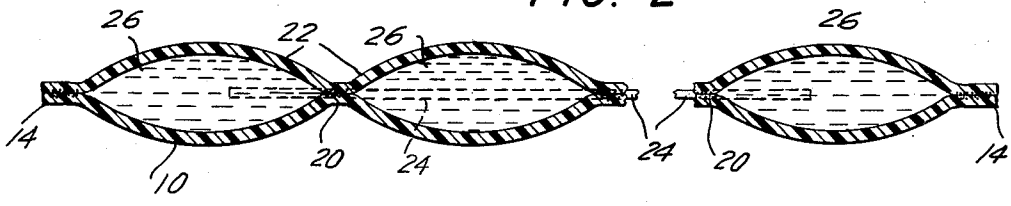


FIG. 2



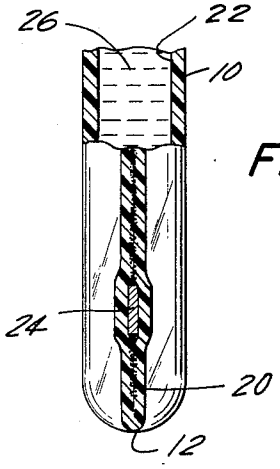


FIG. 3

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FIG. 4

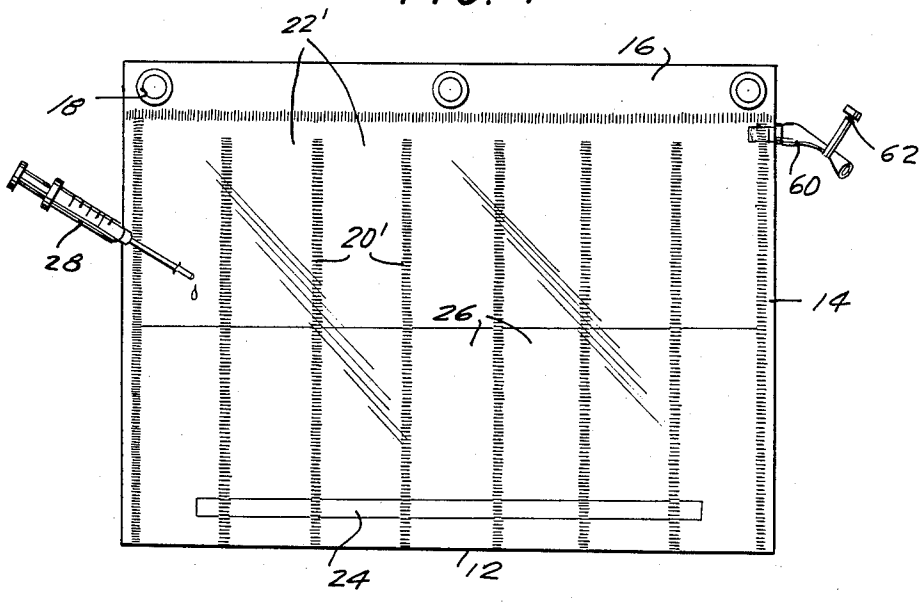
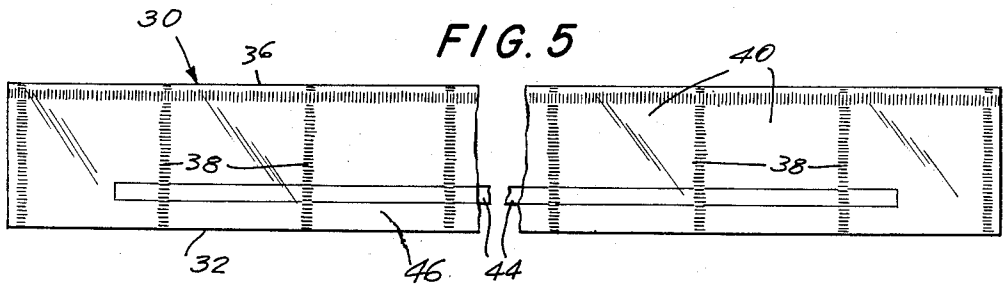


FIG. 5



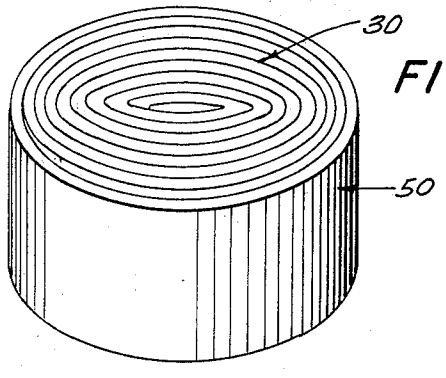


FIG. 6

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3,102,082
APPARATUS AND METHOD FOR CULTURING MICRO-ORGANISMS

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12 Claims. (Cl. 195—139)

This invention relates to an improved apparatus and method for culturing microorganisms such as bacteria, yeasts and molds.

My invention has particular application to an apparatus and method for automatically inoculating successive units of culture media and for continuously culturing the microorganisms without the necessity for successive manual inoculations.

The presently used apparatus and methods for culturing microorganisms such as bacteria, yeasts and molds present difficulties and disadvantages. Thus, in order to maintain stock cultures it is necessary to inoculate fresh culture media units at regular and repeated time intervals. Failure to do this will result in the loss of certain stock cultures. Because of this, laboratories require the attendance of skilled technicians to maintain the stock cultures during weekends and holiday periods if twenty-four hour cultures are to be maintained.

In addition, the apparatus heretofore used for culturing microorganisms of this type has been cumbersome, difficult to handle and store and has occupied a great deal of space. In addition, the inoculation requires the attention and attendance of highly skilled technicians, the use of elaborate sterilization apparatus and elaborate care at each transfer of the culture in order to prevent contamination.

It is an object of the present invention to overcome the difficulties and disadvantages heretofore encountered and to provide an improved method and apparatus in which the culturing process is a continuous one with the inoculation of successive culture media units being automatically made.

A further object is the provision of a method and apparatus of the above character in which the apparatus is flexible and relatively compact and light so that it is easy to handle and occupies a minimum amount of space; which is inexpensive and, therefore, is disposable; which does not require the close attention and attendance of a skilled technician; and which eliminates or minimizes the danger of contamination.

A further object of the invention is the provision of an improved method and apparatus of the above character which may be used in the generation and collecting of gas such as hydrogen, methane and carbon dioxide.

In carrying out my invention, I contemplate the provision of a plurality of separate units of culture media communicating with each other only through interstices and pores of a size and arrangement so that the microorganisms to be cultured will slowly diffuse or grow therethrough whereby the units can be arranged in sequence and after inoculation of the first unit of culture media the microorganisms will multiply and will grow or diffuse successively from unit to unit thereby providing automatic inoculation and continuous culturing. The apparatus and method can be controlled so that fixed predetermined time intervals are measured by the inoculation and culturing of the successive units. Thus, each unit may represent a separate day of the year so as to permit the study of the culturing of bacteria over any desired calendar period, such as a year, without the necessity of manual inoculations.

My invention also contemplates that by selection of the culture media, i.e. by utilizing carbohydrate media such as dextrose or glucose, sufficient hydrogen, methane

or carbon dioxide gas can be generated on a programmed schedule and collected from the successive units.

In the accompanying drawings:

FIG. 1 is an elevational view of one form of apparatus embodying my invention;

FIGS. 2 and 3 are sectional views in the direction of the arrows on the lines 2—2 and 3—3 of FIG. 1;

FIG. 4 is an elevational view similar to FIG. 1 of a modified apparatus which may be used in generating gas;

FIG. 5 is a fragmentary view of a further modified type of apparatus in the form of a relatively elongated envelope; and

FIG. 6 is a perspective view of the apparatus shown in FIG. 5 wound in helical form and disposed in a cylindrical container.

In the several forms of my invention I provide a casing which is inert and impermeable to the culture media and the microorganisms to be cultured and I separate the interior of the casing by a plurality of partition means into a plurality of separate compartments each having culture media therein. The partitions are provided with portions, preferably wicks extending through and sealed therein, having interstices and pores of a size and arrangement so that the microorganisms to be cultured will slowly grow or diffuse therethrough.

In the form of my invention shown in FIGS. 1, 2 and 3, the casing is an envelope **10** made of a flexible, plastic material inert to and impermeable to the culture media and the microorganisms to be cultured. Suitable plastic materials for this purpose are polyethylene, polyvinyl chloride and the co-polymers thereof, polypropylene, tetrafluoroethylene and trifluorochloroethylene. Where the microorganisms or bacteria are anaerobic the plastic material should be impermeable to air. Where the microorganisms or bacteria being cultured are aerobic, then the plastic material should be permeable to air while at the same time being impermeable to the culture media. Thin sheets of polyethylene and polypropylene in the order of .002" thick will transmit oxygen and are suitable for use with aerobic microorganisms. Thicker sheets of such material in the order of .006" or more in thickness and also laminated thinner sheets generally do not transmit oxygen. Where aerobic microorganisms are to be cultured, either the entire envelope or only one side thereof may be made of plastic material which transmits oxygen.

For certain purposes it is preferred that the plastic material be transparent so that the bacteriologist or technician can observe the media and culture inside the casing.

From the standpoint of ease and simplicity of manufacture I prefer to employ a thermoplastic material so that heat sealing can be employed for sealing the casing and for forming the partitions therein.

The envelope illustrated in FIGS. 1 to 3 is formed of a single sheet of plastic material folded upon itself along its lower edge **12** and then heat sealed along the lateral edges **14** and across the top as at **16** so as to provide a completely sealed envelope. The top of the envelope as shown may be provided with apertures **18**, preferably fitted with grommets for supporting or suspending the envelope in vertical or upright position.

The interior of the envelope is provided with a plurality of vertical horizontally spaced partitions formed by heat sealing the two plies of the envelope together from top to bottom as shown. The interior of the envelope is thereby divided into a plurality of successive compartments **22**. The partitions **20** are provided with portions having interstices or pores communicating with the culture media in the compartments on the two sides thereof and the interstices or pores should be of a size

and arrangement so that the microorganism being cultured will slowly grow or diffuse therethrough.

For this purpose, I prefer to employ a wick **24** extending through and sealed in each of the partitions **20** and immersed in the culture media in each of the compartments. This can be simply accomplished by providing a wick extending transversely from the first compartment through each of the compartments to the last compartment. The wick is sealed in place in the partitions at the time that the heat sealed partitions **20** are formed.

The interstices or pores should be of a size so that when the microorganisms multiply in one compartment they will grow or diffuse through the wick to the next successive compartment. At the same time the wick pore size should not be so great as to permit the free flow of the culture media or of the metabolic products. The size of the interstices or pores should be varied with the speed of the transfer from compartment to compartment which is desired and also with the microorganisms being cultured. For speedier transfer, larger pores or a larger cross-sectional wick should be employed. The speed of the transfer may be decreased by using a wick with smaller pores or of smaller cross-sectional area or by adding agar or gelatin to the culture media.

For this purpose, various types of filter media may be employed such as filter paper or filter cloth, wet strength paper towelling, fibre glass tape, cotton ribbon, and cotton twill or cross grain ribbon. Commercial cotton twill tape .250" by .007" serves very satisfactorily.

Inside the envelope or casing in each of the compartments I place the culture media **26**. Any of the well known culture media may be employed such as dilute solutions of peptones and dextrose, sucrose, glucose, maltose and other sugars, gelatin, agar-agar, serum, blood and the like. The culture meria is placed in the compartments before the final sealing of the upper end of the casing or envelope. As previously indicated, the speed of the transfer of the microorganisms from compartment to compartment can be in part controlled by the culture media. By increasing the amount of agar or gelatin, the speed of transfer can be decreased.

After the envelope has been completed and the culture media inserted in the compartments, the assembly is sterilized in suitable manner, in the case of plastic materials which will withstand high temperatures such as polypropylene autoclaving at 15 pounds' steam pressure at 121° C. for 20 minutes or in the case of all of the plastic materials by radiation or by immersing the apparatus in an atmosphere of ethylene oxide.

In using my improved apparatus, I inoculate the first compartment as shown in FIG. 1, as for instance by inserting a hypodermic needle therein and injecting a small quantity of the microorganism or bacteria to be cultured. Upon withdrawal of the needle, the envelope or casing may be resealed with a strip of pressure sensitive tape. The envelope is then maintained in the desired thermal environment by being suspended on hooks from the apertures **18** in an incubator or similar apparatus. For most microorganisms, the temperature range will be between 20° and 45° C. In the case of psychrophiles the preferred temperature is between 0° and 20° C., in the case of mesophiles the preferred temperature is between 20° and 45° C. and in the case of thermophiles the preferred temperature is between 45° and 70° C. While thus maintained in the desired thermal environment, the microorganisms will multiply in the first compartment and will finally diffuse through the wick in the second compartment where the microorganisms will further multiply and diffuse through the wick into the third compartment. This process of continuous culturing and automatic inoculating of successive compartments continues sequentially through the envelope to the last compartment.

Where a uniform wick having uniform interstices and pores throughout its length is employed, the time sequence of progress from compartment to compartment is uniform. By controlling the size and arrangement of the interstices and pores and/or the width of the wick and/or the character of the culture media, the time sequence can be at any desired interval. Thus, it can be arranged on an hourly or daily basis so that successive compartments are inoculated and cultured on successive hours or days. By using all the compartments as shown in FIG. 1, a period of one week can be covered with separate cultures for each day. It will be appreciated that this can be accomplished on a monthly, yearly, or any other desired time or calendar basis.

Where it is desired to carry on the continuous culturing over a long period of time, as for instance 365 successive days, the form of the envelope can be changed to that shown in FIGS. 5 and 6. Thus, in FIGS. 5 and 6 I have illustrated a relatively elongated envelope **30** made of a similar plastic material as that described in connection with FIGS. 1 to 3. The plastic sheet material is folded upon itself at **32** and heat sealed along its upper edge **36** and at its two ends. The elongated strip is divided by transverse partitions or heat seals **38** into separate compartments **40** and a wick **44** preferably in unitary form extends longitudinally for the entire length of the envelope and extends through each seal or partition and is sealed therein. Culture media is placed in each compartment as in the first form of my invention and after sealing the assembly is suitably sterilized.

The wick is immersed in the culture media **46** in each of the compartments **40**. The interstices and the pores of wick **44**, the cross-sectional area of the wick and the character of the culture media, are such that the microorganisms in the envelope will grow or diffuse from one compartment to another so as to culture the media therein on a daily basis. An envelope long enough to have 365 separate compartments is required to cover a yearly period. For convenience and compactness, this elongated envelope may be wound in helical form as shown in FIG. 6 and packed in a cylindrical casing or cassette **50** during storage, shipment and use.

In using the apparatus shown in FIGS. 5 and 6 the media in the first compartment is inoculated in the manner shown in FIG. 1. The envelope in helical form is packed in its casing and the assembly may be kept in the desired thermal environment as previously indicated by resting on a shelf in an incubator. This permits the study of cultures as for instance mutations of bacteria on a daily basis over an entire year period.

In the apparatus shown in FIGS. 1 to 3 and 5 to 6 the compartments are shown as being of uniform size. It will be appreciated that the compartments may be of varying size. Thus, the first compartment may be relatively larger to thereby develop starter cultures for use in various fermentation processes and dairy products production.

My improved apparatus and method may also be used in the generation and collection of various gases such as hydrogen, methane, CO_2 and the like for various purposes as for instance so as to permit the bacteriologist to check or confirm the nature of the microorganisms by an analysis of the gases.

In FIG. 4 I have shown apparatus suitable for this purpose. This apparatus is generally the same as shown in FIGS. 1 to 3 with the exception that the heat sealed partitions **20'** extend upwardly from the lower end of the casing to a point spaced from the upper end thereof so that the compartments **22'** intercommunicate at the upper end thereof. In this form of my apparatus I provide an outlet **60** adjacent the upper end of the casing above the partitions **20'** so that the gas generated in each compartment as a result of the culturing may be collected through the outlet. A suitable shut-off valve **62** may be provided for the outlet as shown. Where my apparatus is used in generating gas I prefer to employ a carbohydrate culture

media such as a dilute solution of glucose, dextrose, sucrose or other sugars and peptone, since this results in the generation of a greater quantity of gas.

In each form of my invention, suitable indicators may be added to any of these culture media, such as brom cresol purple, and after growth of the organism a change in color would result, as well as turbidity, to indicate which compartment the organism had reached at any given time.

Specific examples of the culturing or microorganisms pursuant to my invention are as follows:

Example I

An envelope of the type shown in FIGS. 1 to 3 is made from polypropylene plastic material (.006" th.), and has the following dimensions: Unit length is 14½"; unit width 7½"; compartment length 13"; compartment width 2". Before final heat sealing, 20 ml. of culture medium having the following composition per liter is inserted in each compartment: Trypticase—17 gms.; phytone—3 gms; NaCl—5 gms.; K_2HPO_4—2.5 gms.; and dextrose—5 gms; pH—7.2. The wick material employed was cotton twill tape .007" thick by ½" wide and it was in the form of a unitary wick extending transversely from the first compartment through each of the partitions to the last compartment. The bag is then sterilized by an appropriate method. The culture medium in the first compartment was inoculated by a hypodermic needle with *Clostridium welchii,* an anaerobe, and the aperture left by the needle after inoculation was sealed with a suitable sealing tape having a pressure sensitive adhesive. The envelope was suspended in an incubator in a thermal atmosphere of 35° C. The bacteria in the first compartment multiply and after 24 hours diffuse or grow through the wick and inoculate the medium in compartment #2 where the bacteria again multiple and inoculate the medium in succeeding compartments. It was observed that the bacteria proceeded sequentially in uniform time intervals from compartment to compartment and that the culture in each compartment was free from contamination.

Example II

An envelope of the type shown in FIGS. 1 to 3 is made from very thin polypropylene (.002") or polyethylene which will transmit oxygen and has the following dimensions: Unit width is 7½"; unit length 14½"; compartment length 13"; compartment width 2". Before final heat sealing, 20 ml. of culture medium is inserted in each compartment. The culture medium is whole cow's milk. The wick material employed was cotton twill tape .007" thick x ½" wide and it was in the form of a unitary wick extending transversely from the first compartment through each of the partitions to the last compartment. The bag is then sterilized by an appropriate method. The culture medium in the first compartment was inoculated by a hypodermic needle with *Lactobacillus acidophilus,* an aerobe, and the aperture left by the needle after inoculation was sealed with a suitable sealing tape having a pressure sensitive adhesive. The envelope was suspended in an incubator in a thermal atmosphere of 45° C. The bacteria in the first compartment multiply and after 24 hours diffuse or grow through the wick and inoculate the medium in compartment #2 where the bacteria again multiply and inoculate the medium in succeeding compartments. It was observed that the bacteria proceeded sequentially in uniform time intervals from compartment to compartment and that the culture in each compartment was free from contamination.

Example III

The elongated envelope similar to that shown in FIGS. 5 and 6 is made from similar materials to that described in connection with Example I. Each compartment has a dimension of approximately 1" wide x 2" high. The

same twill tape is used as a wick. In this case, however, 365 compartments are made and the whole is placed in a metal can or cassette. This particular arrangement is of value in carrying cultures for a very long period of time, as for instance for a period of one year with transfers from compartment to compartment being made on a daily basis. Suitable indicators may be added to any of these culture media, such as brom cresol purple, and after growth of the organism a change in color would result, as well as turbidity, to indicate which compartment the organism had reached at any given time.

It will be appreciated that modifications may be made in the illustrative embodiments and examples of my invention within the scope of the appended claims. Thus, the specific size, shape and configuration of the envelopes may be changed and the envelopes may be made of any suitable materials having the indicated characteristics.

I claim:

1. Apparatus for use in culturing microorganisms comprising means providing a casing impermeable to culture media and the microorganisms to be cultured, partition means separating the interior of said casing into separate culturing compartments, and culture media disposed inside said casing in said separate compartments, said partition means including a portion having interstices and pores in communication with the culture media in both compartments of a size and arrangement so that the microorganisms to be cultured will slowly grow and diffuse therethrough but so as to prevent the free flow of the culture media therethrough.

2. Apparatus for use in culturing microorganisms and for generating gas comprising means providing a casing impermeable to culture media and the microorganisms to be cultured, partition means extending upwardly from the lower end of the casing and terminating short of the upper end thereof to divide the interior of the lower portion of the casing into separate culturing compartments which intercommunicate at the upper portion of the casing and culture media disposed in said casing in said compartments at a level lower than the upper end of the partition means, said partition means including a portion having intersttices and pores of a size and arrangement so that the microorganisms to be cultured will slowly grow and diffuse therethrough but so as to prevent the free flow of the culture media therethrough and an outlet for gas generated in said compartments formed in said casing near the upper portion thereof.

3. Apparatus for use in culturing microorganisms comprising means providing a casing impermeable to bacteria and the microorganisms to be cultured, partition means separating the interior of said casing into separate culturing compartments for holding culture media and wick means extending through and sealed in said partion means so as to communicate with both the adjacent compartments on opposite sides of the partition means, said wick means having interstices and pores of a size and arrangement so that the microorganisms to be cultured will slowly grow and diffuse therethrough but so as to prevent the free flow of the culture media therethrough.

4. Apparatus for use in culturing microorganisms comprising means providing a casing impermeable to culture media and the microorganisms to be cultured, partition means separating the interior of said casing into separate culturing compartments, culture media disposed inside said casing in said compartments and wick means extending through and sealed in said partition means so as to communicate with the culture media in both compartments on opposite sides of the partition means, said wick means having interstices and pores of a size and arrangement so that the microorganisms to be cultured will slowly grow and diffuse therethrough but so as to prevent the free flow of the culture media therethrough.

5. Apparatus for use in culturing microorganisms and for generating gas comprising means providing a casing

impermeable to culture media and the microorganisms to be cultured, partition means extending upwardly from the lower end of the casing and terminating short of the upper end thereof so as to separate the lower portion of the casing into separate compartments intercommunicating at the upper end thereof, culture media disposed inside said casing in said compartments, and wick means extending through and sealed in said partition means and communicating with the culture media in the compartments on both sides of said partition means, said wick means having interstices and pores of a size and arrangement so that the microorganisms to be cultured will slowly grow and diffuse therethrough but so as to prevent the free flow of the culture media therethrough, and an outlet for the gas generated in said compartments formed in said casing at the upper portion thereof.

6. Flexible, disposable and relatively compact apparatus for use in culturing microorganisms comprising an envelope made of flexible, plastic material inert and impermeable to culture media and the microorganisms to be cultured, partition means separating the interior of said envelope into separate culturing compartments for holding culture media and wick means extending through and sealed in said partition means and communicating with the compartments on both sides thereof, said wick means having interstices and pores of a size and arrangement so that the microorganisms to be cultured will slowly grow and diffuse therethrough but so as to prevent the free flow of the culture media therethrough.

7. Flexible, disposable and relatively compact apparatus for use in culturing aerobic microorganisms as set forth in claim 6 in which at least a portion of the envelope is made of a plastic material which is pervious to oxygen.

8. Flexible, disposable and relatively compact apparatus for use in culturing microorganisms as set forth in claim 6, in which said wick means comprises cotton twill tape.

9. Flexible, disposable and relatively compact apparatus for use in culturing microorganisms and generating gas comprising an envelope made of plastic material inert and impermeable to the culture media and the microorganisms to be cultured, partition means extending upwardly from the lower end thereof and terminating short of the upper end for separating the lower portion of the interior of the envelope into separate compartments, culture media disposed in said compartments at a level lower than the upper ends of the partitions when the casing is in upright position, and wick means extending through and sealed in said partition means and communicating with the culture media in the compartments on both sides thereof and an outlet for the gas generated in said compartments formed in said casing above the upper end of said partitions, said wick means having interstices and pores of a size and arrangement so that the microorganisms to be cultured will slowly grow and diffuse therethrough but so as to prevent the free flow of the culture media therethrough.

10. Apparatus for use in automatically inoculating and culturing microorganisms on a chronological basis comprising a relatively narrow elongated envelope made of flexible, plastic material having a plurality of spaced seals forming partitions extending across the envelope so as to divide the envelope into a plurality of separate culturing compartments each representing a predetermined time interval with respect to the adjacent compartment, culture media disposed inside said envelope in each of said compartments, and a continuous wick extending through and sealed in each of said partitions and disposed in the culture media in each compartment, said wick having interstices and pores of a size and arrangement so that the microorganisms to be cultured will slowly grow and diffuse through the portion sealed in each partition within the aforesaid predetermined time interval but so as to prevent the free flow of the culture media therethrough, said envelope being flexible and subject to being arranged in a tightly compacted helix, and a circular container for holding said envelope when disposed in helical form.

11. The method for automatically and sequentially inoculating and culturing microorganisms in successive separate units of culture media which comprises providing a plurality of separate culturing compartments in sequential arrangement with partitions beween the compartments provided wih a section having interstices and pores of a size and arrangement so that the microorganisms to be cultured will slowly grow and diffuse therethrough but so as to prevent the free flow of the culture media therethrough, inoculating the culture media in the first compartment with the microorganisms to be cultured and maintaining the entire assembly in a thermal environment to cause said microorganisms to multiply and to diffuse sequentially through the partition sections having interstices and pores from compartment to compartment.

12. The method for automatically and sequentially inoculating and culturing microorganisms in successive separate units of culture media which comprises providing an elongated flexible plastic envelope divided into a plurality of sequentially arranged compartments having culture media therein by partitions having wicks extending therethrough formed with interstices and pores of a size and arrangement so that the microorganisms to be cultured will slowly diffuse therethrough but so as to prevent the free flow of the culture media therethrough, inoculating the culture media in the first compartment with the microorganisms to be cultured and maintaining the assembly in the proper thermal environment to cause the microorganisms to multiply and diffuse sequentially through the wicks from compartment to compartment.

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