Oct. 26, 1937. R. R. THOMPSON 2,096,866

METHOD AND APPARATUS FOR CULTURING BACTERIA ON SOLIDIFIED CULTURE MEDIA

Filed March 22, 1934 2 Sheets-Sheet 1

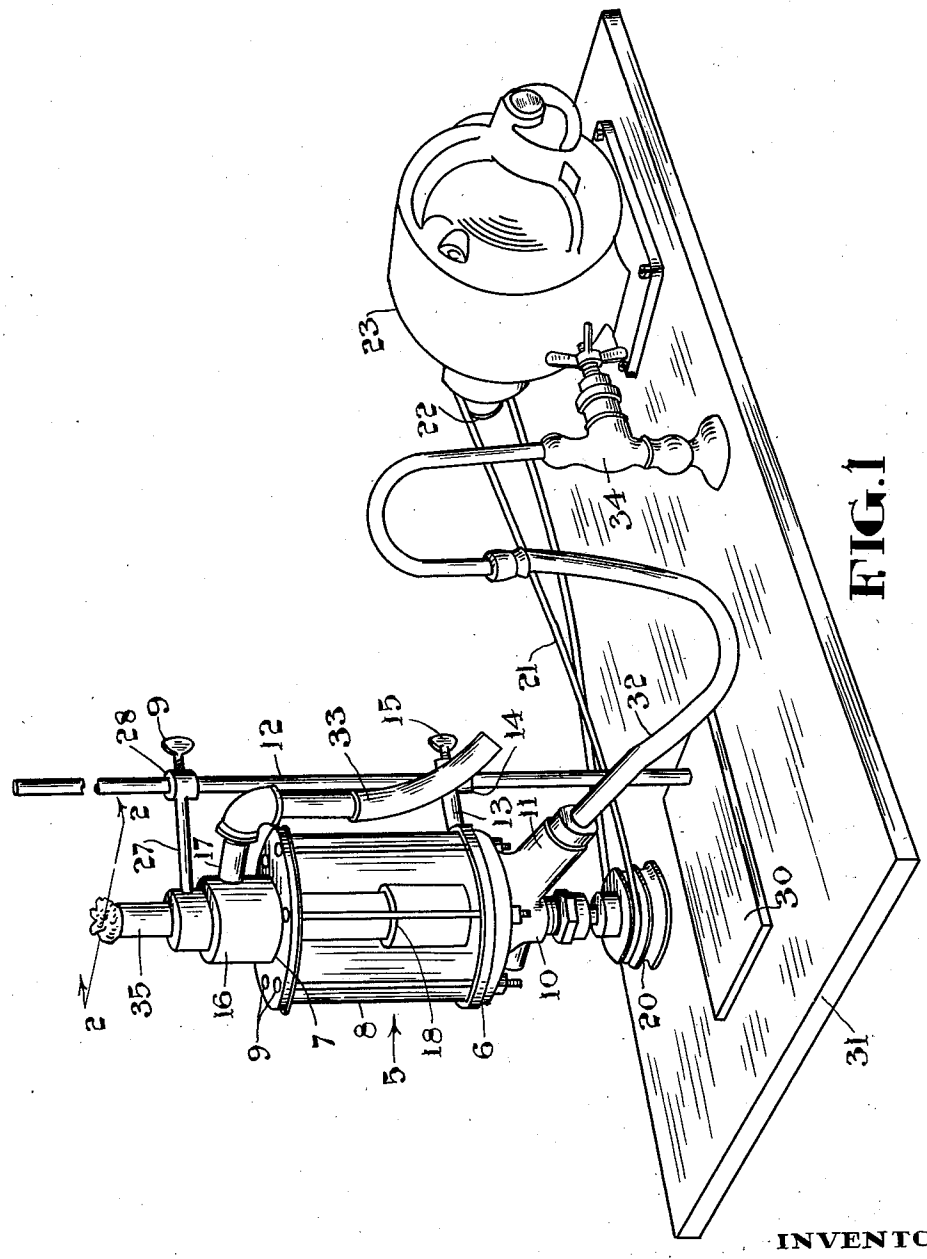


FIG. 1

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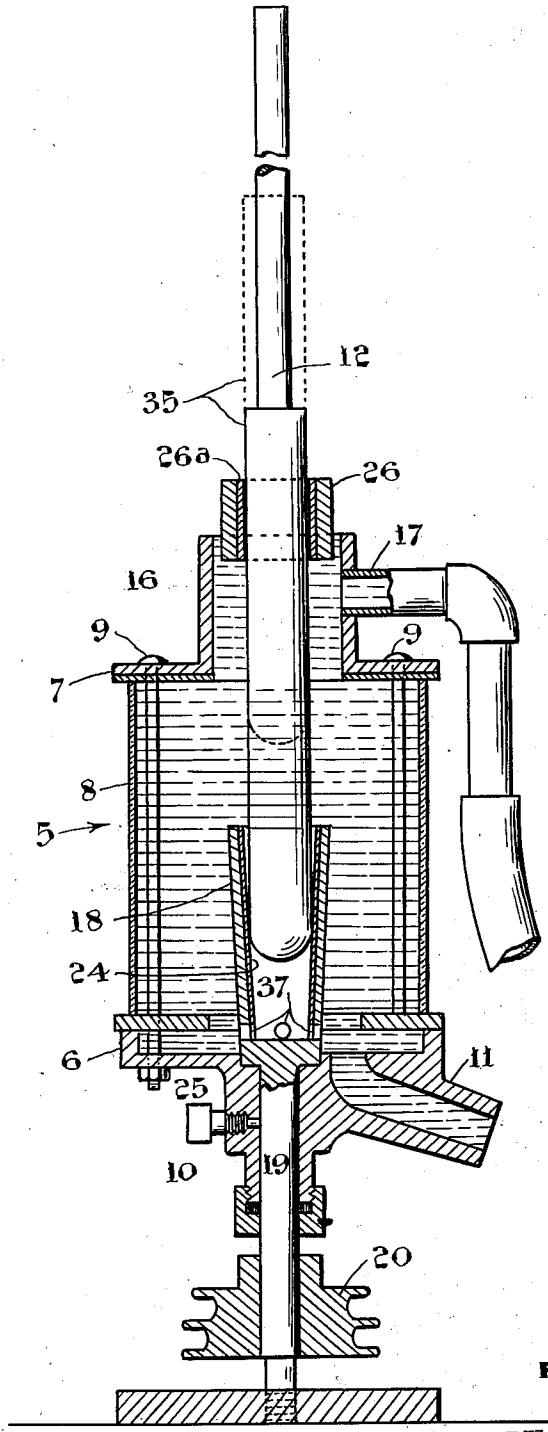


FIG. 2

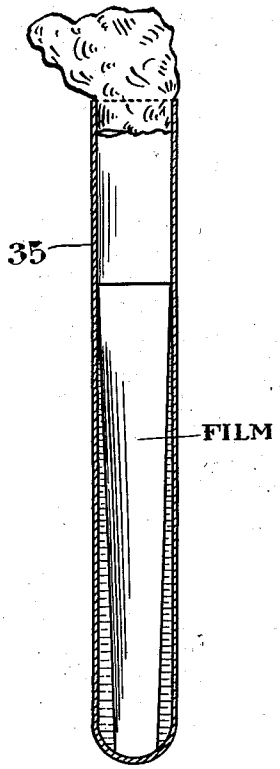


FIG. 3

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METHOD AND APPARATUS FOR CULTURING BACTERIA ON SOLIDIFIED CULTURE MEDIA

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4 Claims. (Cl. 23—259)

This invention relates to an improved method and apparatus for use in connection with the culturing of bacteria on the surface of solidified culture media.

When culturing certain organisms it is desirable to inoculate onto the surface of solidified culture medium. The present practice of using Petri plates for this work is quite satisfactory when the culturing is carried on in a well equipped laboratory and when it is possible to deliver the inoculum to the laboratory in a fresh condition. Cases arise, however, where these requirements cannot be met and where the use of Petri plates gives rise to various difficulties. For example, when bacteriological investigations are carried out at locations hundreds of miles distant from the base laboratory, it is frequently impossible to transport inoculum, such as blood or milk, to the laboratory in the condition necessary to ensure a satisfactory bacteriological analysis. In such cases poured Petri plates have been used for carrying out the bacteriological investigation at the place of infection but it was found difficult to prevent contamination of the poured plates during transportation and to obtain satisfactory inoculation and distribution of inoculum in available places such as private homes and poorly equipped laboratories. Contamination, subsequent to inoculation, is also a troublesome factor in connection with the use of Petri plates, since these plates have to be incubated for periods of two to three weeks when culturing the organisms from blood.

The present invention overcomes the above mentioned difficulties by the provision of a method and apparatus which makes feasible the employment of glass test-tubes (commonly called rolled-tubes), in place of Petri plates, for surface inoculation of solidified culture media. In addition to lessening the risk of contamination, during transportation and use, rolled tubes are more convenient to carry than Petri plates and are much cheaper.

The roll-tube method of culturing bacteria, as heretofore practiced, consists in adding the inoculum to liquefied culture medium contained in the tube and then rotating the tube to spread the inoculated culture medium in the form of a film. The tubes are usually rotated by hand while lying in a horizontal plane. More recently, there has been developed a mechanical tube spinning machine which spreads the inoculated culture medium over the walls of the tube by centrifugal force. This machine has the advantage of turning out uniform culture tubes free from wrinkles and is more rapid than the hand rolling. However, so far as I am aware, none of the previous workers in this field have developed any method or apparatus which makes feasible the employment of rolled tubes, instead of Petri plates, for culturing bacteria on the surface of a previously prepared film of culture medium.

According to the present invention, a tube, containing the culture medium in liquid form, is supported in an upright position in a body of cooling liquid with the lower end of the tube received in the cavity of a rotary tube spinning cup. When the cup is rotated at a sufficiently high rate of speed (usually above 1500 R. P. M.) the centrifugal force developed causes the culture medium to spread over the walls of the spinning tube to form a solidified film gradually diminishing in thickness toward the upper limit. The tube is then removed and stored away in a cold place until the inoculum is to be added and distributed over the surface of the prepared film. It is desirable that the spreading of the inoculum be conducive to the growing of well isolated colonies at some location on the prepared film. This is accomplished in the following manner. After addition of the inoculum the prepared tube is dropped into the cooling liquid and engaged with the spinning cup which is made to revolve at a speed of about 2640 R. P. M. Owing to the centrifugal force developed at this speed and to the sloping surface of the film the inoculum is caused to climb up the film to a point near its upper limit. With this procedure the density of the seeding diminishes toward the upper limit of the film and ensures the growing of well isolated colonies.

Another feature of this invention resides in a peculiar hydraulic clutching action resulting from the submergence of the tube spinning cup in a casing containing the cooling liquid into which the tube is dropped through a suitable opening or tube guide aligned with the cup. With this assembly the tube floats in the liquid out of contact with the cup when the latter is at rest or rotating at a slow speed but, in the case of the particular apparatus illustrated, is automatically drawn down into the cup by the action of the disturbance set up in the cooling liquid when the cup is rotated at relatively high rates of speed ranging upwards from about 1500 R. P. M. When the cup is slowed down or brought to rest the action of the liquid is such that the tube is then floated out of the cup to a released position remote therefrom. It will thus be seen that the cup and tube constitute driving and driven mem-

bers which are clutched together or released by the action of the cooling liquid in response to variations in the operating speed of the cup. Obviously this feature of the present invention is susceptible of wide application in the mechanical arts since the tube may be replaced by another form of driven member adapted to actuate some mechanical contrivance when moved to and from the driving cup by the action of the liquid in which the cup is submerged.

Proceeding now to a more detailed description of the different features of this invention reference will be had to the accompanying drawings wherein—

Figure 1 is a perspective view of a preferred embodiment of the apparatus by means of which the invention is reduced to practice.

Figure 2 is a view in vertical section taken substantially along the line 2—2 of Fig. 1.

Figure 3 is a view in longitudinal section of a rolled tube showing the manner in which the culture medium is distributed in accordance with this invention to form a film of varying thickness.

Referring more particularly to the drawings, 5 designates a casing comprising a cast metal base 6, a cover member 7 and a glass cylinder 8, the latter being clamped between the base and cover by a series of clamping bolts 9. The base 6 is provided with a shaft bearing 10 and a water inlet passage 11 and is slidably mounted on a supporting standard 12 by means of the arm 13, sleeve 14 and clamping screw 15. The cover member 7 is provided with a neck extension 16 equipped with an overflow connection 17.

The casing 5 encloses a driving cup 18 provided with a depending shaft 19 extending downwardly through the bearing 10. A pulley 20 at the lower end of this shaft is driven, by a belt 21, from a pulley 22 on the armature shaft of an electric motor 23. As shown more particularly in Figure 2 the cup 18 is slightly conical and is provided with a friction lining 24 of rubber or other suitable material. The bottom of this cup rests in a recess 25 at the upper end of the shaft bearing 10.

A cylindrical tube guide 26 is fitted in the neck extension 16 of the cover member in axial alignment with the cup 18. This tube guide is provided with a liner or bushing 26a and is adjustably mounted on the standard 12 by means of the arm 27, sleeve 28 and clamping screw 29. The lower end of the standard 12 is screwed into a flat base 30 mounted on a main supporting plate 31 which also carries the motor 23.

A constant flow of water through the casing 5 is maintained by means of a water supply pipe 32 and an overflow pipe 33, the former being connected with the water passage 11 of the base 6 and the latter with the overflow connection 17 of the cover member. The flow through pipe 32 is controlled by a valve 34 mounted on the main supporting plate 31 and adapted for connection with any suitable water supply.

The manner in which the above mentioned apparatus is used in preparing rolled tubes for surface inoculation will now be described. A rolled tube 35, containing the culture medium in liquid form, is dropped through the tube guide 26 into the body of water contained in the casing 5. If the cup 18 is at rest or turning at a relatively slow speed, the tube 35 simply floats in the liquid in the vertical position indicated by dotted lines in Figure 2. If, now, the rotational speed of the cup is sufficiently accelerated a peculiar disturbance is set up in the water contained in the casing and cup and results in the tube 35 being automatically lowered into driving engagement with the friction lining 24, as illustrated by full lines in Figures 1 and 2. During the resulting rotation or spinning of the tube the centrifugal force developed causes the culture medium to climb the inner surface of the tube to form a solidified film gradually diminishing in thickness as it approaches its upper limit. This shaping of the film is clearly shown in Figure 3 and has certain advantages in connection with the subsequent distribution of inoculum as hereinafter explained. The time required to form the film is usually about one and one-half minutes after which the tube may be removed and placed in a cold room until it is to be used for culturing. It will thus be seen that a large number of tubes may be quickly prepared in accordance with this invention and stored away for future use with very little danger of contamination provided reasonable care is exercised by the operator.

The cup 18 is preferably slowed down or brought to rest before removing the prepared tube. When the turning speed of the cup drops below a certain value, the tube is automatically floated out of driving engagement with the cup and resumes the dotted line position shown in Figure 2. The operator then grasps the projecting part of the tube and removes it from the casing 5.

From the foregoing it will be seen that this invention presents, as one of its characteristic features, a tube spinning apparatus in which the liquid employed for cooling the rolled tube during the spinning operation co-operates with said tube and with the submerged spinning cup to provide a species of hydraulic clutch which causes the tube, representing the driven member of the clutch, to be automatically clutched to the cup or driving member when the latter attains a sufficiently high rate of speed and to be automatically released when the speed of the driving member or cup falls below that required for the spinning operation. It is also desired to point out that, in addition to its use in connection with the present invention, the aforesaid hydraulic clutch action is obviously susceptible of wide application in the mechanical arts since the rolled tube 35 may obviously be replaced by another suitable form of driven member intended to actuate some mechanical device when moved into and out of the driving cup in response to the action of the liquid disturbance caused by rotation of the cup at different speeds. While the exact nature of the liquid disturbance upon which the hydraulic clutching action in question is based is not entirely free from uncertainty, it is believed that the following is a correct description of what actually takes place.

When rotated at a relatively high rate of speed, the driving cup apparently sets up a disturbance which causes the cooling liquid to flow out of the cup at its edges or periphery and in at the center, thus lowering the hydraulic pressure along the axis of the cup. This decrease in pressure along the axis of the cup apparently unbalances the forces holding the rolled tube in suspension so that the tube is automatically forced down into the cup and caused to rotate, by frictional contact, with the rubber lining. With a decrease in speed the liquid circulation in the cup is apparently reversed since the test tube is automatically floated out of the cup when the speed of the latter falls below a certain value. By

actual tests it has been ascertained that, at a speed of 1500 revolutions, there is a decrease in pressure of 2.54 centimeters of water at a point approximately one-half inch above the center of the driving cup. This would be equivalent to the rolled tube floating 2.54 centimeters lower in the water which, in the case of the construction illustrated in the drawings, is sufficient to bring the tube down into the cup. At higher speeds the decrease in pressure along the central axis of the cup is greater and would, of course, exert a greater downward pull on the rolled tube. I have also found that the hydraulic clutching effect due to the liquid circulation set up within the driving cup **18** may be enhanced by providing the cup with flow openings near its bottom wall, as indicated at **37** in Figure 2. When these flow openings are provided the downward pull on the rolled tube is stronger for a given speed of cup rotation than when they are omitted.

We come now to the mode of using the described apparatus in connection with the distribution of inoculum over the surface of the films contained in the previously prepared tubes. The inoculum is preferably added to a prepared tube by means of a pipette or hypodermic needle. If desired the inoculum may be thus added to a whole series of prepared tubes before resorting to the use of the spinning apparatus for distributing the inoculum over the prepared films. In the spreading of the inoculum it is desirable to have uniformity and to effect the spreading in a manner that will be conducive to the growing of well isolated colonies which permit of easy picking at some location on the film. These results are obtained by dropping the prepared tube, containing the inoculum, into the body of water in the casing **5** when the driving cup **18** is rotating at a speed of about 2640 R. P. M. The centrifugal force developed at this speed forces the inoculum to climb up the sloping exposed surface of the cone-shaped film to a point near the upper limit of the film. As the inoculum is forced upward the film is seeded with the organisms therefrom. This procedure results in the most dense seeding at the base of the film and a gradual lessening of the density of the seeding towards the upper limit of the film. The advantage of this is that well isolated colonies are obtained towards the upper limits of the film. After the inoculum has been thus distributed the tube is removed and placed in an incubator where it is held at the desired temperature.

Having thus described my invention, what I claim is:—

1. Apparatus of the character described comprising a stationary casing having an opening at the top for the insertion of a test-tube in an upright position, a cup shaped member rotatably mounted in the casing in line with said opening to receive the lower end of the test-tube, tube encircling means located above the opening in the casing adapted to assist in maintaining the upright position of the tube without interfering with rotation thereof, means for maintaining said casing filled with liquid to a level substantially above the top of the cup shaped member and means for rotating said cup.

2. Bacteriological apparatus comprising an upright stationary casing having an opening at the top to permit a test-tube to be inserted in the casing in an upright position, a test-tube supporting member in line with said opening adapted to encircle and support the upper portions of the test-tube without interfering with rotation thereof, a cup shaped member rotatably mounted in the lower portion of the casing in line with said opening and adapted to receive the lower end of the test-tube therein, means for maintaining said casing filled with liquid to a level high enough to completely submerge the cup and the lower portion of the inserted test-tube and means for rotating said cup.

3. Tube spinning apparatus of the character described comprising a casing containing a body of liquid, a rotatably mounted tube spinning cup submerged in said liquid, an upright test tube fitted in a top opening of the casing so that said tube floats in the liquid out of contact with the cup when the latter is at rest or is rotating at a slow speed but is automatically drawn down into the cup by the action of the disturbance set up in the liquid when the cup is rotated at relatively high rates of speed and means for rotating said cup.

4. Tube spinning apparatus as set forth in claim 3 in which the cup is provided, below the mouth portion thereof, with flow openings placing the cavity of the cup in communication with the interior of the casing.

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