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PREPARATION OF LIQUIDS OF GRADED DENSITY

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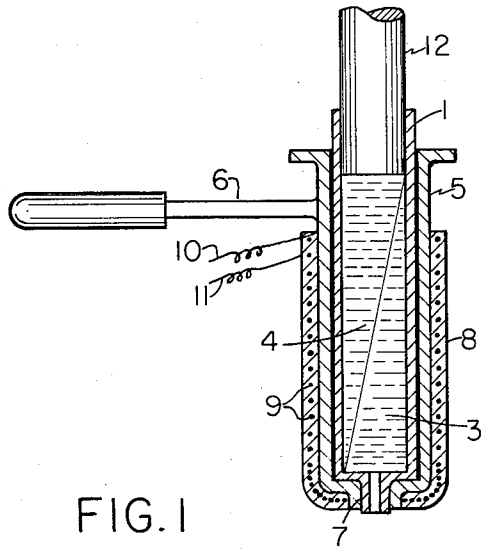


FIG. 1

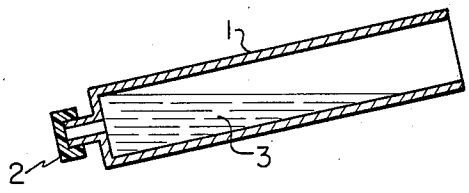


FIG. 2

L.E. SACKS
INVENTOR
BY
ATTORNEY

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PREPARATION OF LIQUIDS OF GRADED DENSITY

Lawrence E. Sacks, Berkeley, Calif., assignor to the United States of America as represented by the Secretary of Agriculture

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A non-exclusive, irrevocable, royalty-free license in the invention herein described, throughout the world for all purposes of the United States Government, with the power to grant sublicenses for such purposes, is hereby granted to the Government of the United States of America.

This invention relates to and has among its objects the provision of novel means and methods for preparing graded density liquids. Further objects and advantages of the invention will be evident from the following description taken in connection with the annexed drawing.

In the drawing:

FIGURE 1 is a side view, partly in cross-section, of an embodiment of apparatus useful in carrying out the process of the invention.

FIGURE 2 is a side view in cross-section illustrating formation of a wedge of solidified liquid of selected density.

In carrying out certain microbiological investigations it is often desirable to provide a body of liquid having a graded density, that is, a density which varies from top to bottom of the body of liquid. Such materials are useful, for example, in isolating different microbial species from a mixture thereof. To this end, within a centrifuge tube is deposited a liquid of increasing density, considered in the direction from top to bottom. A specimen of the microbial mixture is placed at the top of the liquid and the tube then centrifuged. After this operation, the tube is examined and it will be seen that microbial species of different intrinsic densities are located at different levels of the liquid. These separate deposits of microbial material can then be isolated from one another by careful decanting, pipetting, or the like.

In the prior art the usual way of establishing the liquid of varying density involves the following steps: A series of solutions are prepared, each having a different density as determined by the concentration of selected soluble salts therein. The individual solutions are then poured one after another into the centrifuge tube starting with the solution of greatest density. This technique has several disadvantages. One is that extreme caution must be observed during the pouring operation lest the individual solutions be mixed. This would, of course, destroy the density gradient pattern. Another point is that the finished product has a density pattern which varies step-wise rather than continuously. For this reason it is difficult to get clean-cut separations of samples differing by a small degree of density.

In accordance with the present invention the above disadvantages are eliminated. Liquids having continuously-varying density gradients may readily be prepared and the problem of careful pouring is eliminated. Other advantages of the invention will be apparent from the following detailed description of a preferred embodiment of the invention.

In applying the process of the invention, a pair of solutions of different density is first prepared. The particular densities selected will depend on the microbial species or other materials to be separated. Adjustment of density of each solution may be carried out in known manner, for example, by adding to water the required proportion of

a solute, such as a mineral salt, sucrose, glycerine, etc. The solutions are warmed and into them is incorporated an amount of a gelling agent sufficient to cause them to gel when cooled. The type of gelling agent is not a critical item and any of the materials known to have such properties may be used. Typically, one may employ gelatine, agar, algin, gum arabic, or similar heat-reversible gelling agents, that is, materials that form gels that may be re-liquefied by heating.

In the next operation, the two solutions are formed into a composite structure having two complementary wedge shaped blocks of the solidified solutions. To this end, there is provided a conventional hypodermic syringe with the needle removed. The stem end of the syringe is capped and the syringe is about half-filled with the denser solution. The syringe is then tipped on its side and held in such position until the solution is cooled and solidified. FIGURE 2 illustrates this step in the process. In this figure syringe **1**, provided with cap **2**, is shown in the tipped position which is retained while the solution is cooled and solidified, forming wedge shaped block **3** of solidified solution. Having prepared the syringe with solidified block **3** in place, the syringe is then turned upright and an equal amount of the other solution added. The syringe is held in upright position until the second solution is cooled and solidified forming wedge shaped block **4** (FIG. 1).

The resulting composite structure of abutting blocks (**3**, **4**) of solidified solutions is then subjected to progressive melting to yield the solution of graded density. This step may conveniently be carried out in the device shown in FIG. 1. The illustrated device includes a cylinder **5**, proportioned to hold syringe **1** snugly. The cylinder is provided with a handle **6** and an aperture **7**, the latter serving to allow projection of the stem of the syringe. About cylinder **5** is a jacket **8** of insulating material in which is embedded heating coil **9**. The individual turns of coil **9** are arranged so that they are more concentrated at the lower end of the device, thus to furnish most of the heat output near the base of syringe **1**. Terminals **10** and **11** are provided for connection of coil **9** to a suitable source of electrical energy.

In operating the melting device, syringe **1** containing the solidified blocks **3**, **4** is placed within cylinder **5** such that, when viewed in horizontal planes, the cross section of block **3** increases and that of block **4** decreases progressing from top to bottom, as shown in FIG. 1. The heating coil **9** is then energized. As the system heats up, the portion of solidified material near the base of syringe **1** melts and the solution runs out of the stem of the syringe. The first increments of effluent thus removed will obviously be of high density because they contain a high proportion of liquid derived from the denser liquid **3**. In succeeding increments, the density of the effluent will progressively decrease. The effluent, as it is removed from the syringe, is received in a conventional centrifuge tube. During the operation a slight pressure may be maintained on the composite block of solidified material by applying a finger or a weight on piston **12**, thus to keep the solidified block seated against the base of the syringe and hence ensure that the melting will take place mainly at the base of the block of material. It is evident that as the process continues with melting taking place mainly at the base of the syringe, the effluent solution will gradually change in density. Initially, it will contain a maximum proportion of solution of block **3** with a minimum proportion of solution of block **4**. Later, these proportions will approach equality. Then the situation will be reversed and solution **4** will become the major constituent until the final drop when the effluent contains a maximum of solution of block **4** and a minimum of

solution of block 3. It is thus apparent that the solution received in the centrifuge tube held below the syringe will be in prime condition for isolating particles of different density as it consists of a liquid, the density of which varies continuously from top to bottom of the tube. During the progressive melting, the amount of heat applied to the system is preferably controlled so that the effluent emerges at a slow rate so that it may be received in a centrifuge tube with minimum mixing of one portion of the effluent with successive portions.

Having prepared a centrifuge tube containing the graded density solution, the material to be separated is placed on top of this solution. The tube is then centrifuged at a temperature high enough that the solution remains liquid. After centrifugation the tube may be recooled to solidify the contents. A pinhole is then formed in the base of the tube and the solid block of solution blown out by air pressure. The block of solution may be cut into sections for isolation of the particles located in different areas of the solidified block. This method of isolating the different particles constitutes a more selective and accurate technique than methods which involve pipetting of different portions from a mass of the material in liquid form.

It is obvious that it is within the ambit of the invention to carry out the various steps of the invention in alternate ways. For example, in preparing the solidified blocks 3, 4, these solidified blocks of liquid may be cast separately or jointly in molds designed for the purpose, then placed in a syringe or other apertured vessel for progressive melting. Also, instead of being cast into the desired shape, the wedge shaped blocks or blocks of other suitable shapes of varying cross-section may be cut from large masses of the solidified solutions.

In the preferred modification the blocks of solidified liquid are subjected to heating directed mainly at the bottom of the composite structure of the abutting blocks as this structure moves down so that its base is kept in the region where most of the heat is applied. In this way the melting may be considered as progressing from the bottom of the structure upwardly to the top of the structure. However, it is entirely within the purview of the invention to hold stationary the structure of the complementary wedges while moving a heat-source, such as an electrically heated coil, progressively upward from bottom to top of the structure, or even in the reverse direction. Moreover, if the abutting blocks are laid on their sides the source of heat may be moved horizontally from one end of the structure to the other.

Having thus defined the invention, what is claimed is:

1. The method which comprises providing a first solution containing a gelling agent, providing a second solution containing a gelling agent and having a density different from the first solution, solidifying and forming the solutions into a composite structure containing a block of solidified first solution and an abutting block of solidified second solution, the cross section of one of said blocks increasing as it is viewed in horizontal planes progressing from top to bottom of the structure, the cross-section of the other block decreasing as it is viewed in horizontal planes progressing from top to bottom of the structure, applying heat mainly to the bottom of said structure to cause progressive upward melting thereof, and removing the increments of liquid formed by the melting.

2. The method which comprises providing a first gellable liquid and a second gellable liquid of density different from that of the first liquid, gelling and forming the solutions into a composite structure of a pair of abutting complementary blocks each formed from one of said liquids, the cross-section of one of said blocks increasing as it is viewed in horizontal planes progressing from top to bottom of the structure, the cross-section of the other block decreasing as it is viewed in horizontal planes progressing from top to bottom of the structure, progressively melting the said structure starting from the bottom thereof and continuing upwardly and removing the increments of liquid formed by the melting.

3. The method which comprises providing a first gellable liquid, forming and gelling said liquid into a first solid block of such dimensions that the cross-section thereof increases as it is viewed in horizontal planes progressing from top to bottom thereof, providing a second gellable liquid of density different from that of the first liquid, forming and gelling said second liquid into a second solid block abutting said first block, the cross-section of the second block decreasing as it is viewed in horizontal planes progressing from top to bottom thereof, applying heat mainly to the bottom of said blocks to cause progressive upward melting thereof, and removing the increments of liquid formed by the melting.

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