

Candidate's Answer Paper A (Chemistry)

Claims

1. A method for preparing a freeze-dried filter device comprising exo-polysaccharide producing, gram-negative bacteria supported on a water-permeable material which is non-toxic to micro-organisms and to human beings, is resistant to temperatures within the range from $-15\text{ }^{\circ}\text{C}$ to $+65\text{ }^{\circ}\text{C}$ and is not readily biodegradable and is of low solubility or insoluble, in aqueous liquids, wherein the water-permeable material is first impregnated with the bacteria and the freeze-dried.
2. A method according to claim 1, wherein microbial nutrients are added to the impregnated material before freeze-drying.
3. Freeze-dried filter device obtainable by the method according to claim 1 or 2.
4. Freeze-dried filter device according to claim 3, which is vacuum-packed in a material impermeable to water vapour.
5. A method for water treating wherein the water is added to a freeze-dried filter device according to claim 3 to reactivate the bacteria, the filter device is placed in contact with a filter support medium and water to be treated is passed through the filter device and the filter support medium in turn.
6. A method according to claim 5, wherein the filter support medium is a bed of sand and the filter device is laid horizontally upon the bed of sand.
7. A method according to claim 5, wherein the filter support medium is a rigid porous support medium and the filter device is attached in a vertical position to one or more blocks of the rigid porous support medium.
8. A method according to any of claims 5 to 7, wherein microbial nutrients are added to the water used for reactivation.

Note

- I would file a separate application for a filter unit as illustrated in Figs. 3 and 4.

Filter unit comprising a filter device comprising exo-polysaccharide producing, gram-negative bacteria supported on a water-permeable support material, wherein the filter device is attached in a vertical position to one or more blocks of a rigid porous support medium.

This filter unit is novel (not disclosed in the prior art) and has the advantage that the filter device 23 and support block 24 can be replaced without the need to take the unit out of service.

Non-unity if in the same application.

- Non freeze-dried filter devices comprising bacteria on a water-permeable material are not novel:
 - DII discloses support material (any kind of permeable or non-permeable support material) coated with biomass.
 - In DI, bacteria on a support material are formed during operating the sand bed.
- Filter unit comprising filter device comprising bacteria on a water-permeable material upon sand bed not novel:
 - in DI: sand bed used and a layer of bacteria is formed during operating the sand bed for water treating → results in a filter unit comprising filter device comprising bacteria on a water-permeable support.

The present invention relates to a method for preparing a freeze-dried filter device comprising exo-polysaccharide producing, gram-negative bacteria supported on a water-permeable material, to freeze-dried filter devices obtainable by such a method and to a method for water treating using such freeze-dried filter devices.

Our recent researches have been directed to filter devices for the treatment of water to render it potable, and to methods of making the filter devices. The present invention is of particular value in emergency situations where microbiologically safe water is required with the minimum of delay and where disinfecting agents are not readily or continuously available.

Nearly all water-treatment plants include as an essential feature the filtration of the available water through sand. The two main types of sand filtration process are described respectively as slow and rapid sand filtration, which names reflect the relative rates of flow of the aqueous liquid through the filter medium. However, these two types of sand filtration process are also distinguished by fundamental differences of operating procedure.

In the case of rapid sand filters, the water is treated to coagulate the finely-divided and suspended impurities (including many of the harmful micro-organisms present) in flocculating tanks, after which the large particles formed by coagulation are removed in settlement tanks. Pretreatment of the water in this way makes it possible to carry out the filtration through sand at a faster flow-rate. No such pretreatment is carried out in the case of slow sand filters.

In slow sand filters, removal of impurities, and in particular harmful microorganisms, is effected not only by physical straining through the upper layers of the sand grains, but also by the entrapment of the impurities by microorganisms which develop in the upper layer of the sand. This is commonly known as the "Schmutzdecke" layer, which initially may take several weeks to develop and in the slow sand filter plays a key role in producing a high quality water.

In an emergency situation where supplies of safe potable water need to be established quickly and where sources of chemical treatment agents such as disinfectants may not

be readily available, it would in principle be desirable to use a slow sand filter. However, the creation of the necessary Schmutzdecke layer takes such a long time that slow sand filters have until now been wholly unsuitable for use in an emergency.

DII describes support materials for use in a bioreactor, which includes slow sand filter units for water treatment, which are coated with biomass materials, then frozen and stored in the frozen state. Upon introducing the frozen support in a bioreactor, the start-up time is about a day.

However, storage and transport of support material in a frozen state is complicated and thawing of the material during storage or transport may inactivate the bacteria. Moreover, start-up of a bioreactor with the frozen material still takes about a day.

Against this background, we have developed a filter device which is of value in emergencies and other situations and which may be used to establish supplies of safe water in a matter of hours rather than a day and which can be very easily be transported. Accordingly, the present invention relates to a freeze-dried filter device obtainable by the method according to claim 1.

Our new filter device comprises exo-polysaccharide producing, gram-negative bacteria supported upon a water-permeable material which is non-toxic to microorganisms and to human beings, is resistant to temperatures within the range from - 15 °C to + 65 °C, and is not readily biodegradable. The device is freeze-dried after the bacteria have been applied to the water-permeable material. The freeze-dried product may then be vacuum packed so as to exclude moisture and stored until required for use. When an emergency arises in which potable water is required urgently, the product may be reactivated within a few hours by the addition of water and then be used, supported on sand or other support, for the purification of available water in the manner of a slow sand filter. This novel filter device, which may be used as a replacement in a slow sand filter for the conventional Schmutzdecke layer, is available for producing potable water in a fraction of the time which would be required for the creation of a usual Schmutzdecke layer.

DI describes the formation of a "Schmutzdecke" on a water-permeable support. The "Schmutzdecke" is formed when the filter unit is in operation and takes the same amount of time as the formation of a "Schmutzdecke" on the sand bed itself.

*The exo-polysaccharide producing, gram-negative bacteria employed in the filter device we have developed are of a type which is found in Schmutzdecke layers. These bacteria occur naturally in the biofilm layer of a standard slow-sand water filter, especially in the region within 5 cm, more especially 2.5 cm, of the surface of the filter medium. Such naturally-occurring bacteria are characteristically producers of copious amounts of polysaccharides in the form of a viscous or gelatinous material, under conditions of low nutrient concentrations. The bacteria used may be a mixture of bacteria obtained directly from a Schmutzdecke layer. Alternatively, pure cultures of single strains of a bacterium may be used singly or in mixtures. Among suitable bacteria may be mentioned strains of *Pseudomonas vesicularis*, for example NCIB40121; *Zoogloea ramigera*, for example ATCC 25935 or NCIB 10340; *Pseudomonas sp.*, for example NCIB 11264;*

Achromobacter georgiopolitanum, for example ATCC 23203; and non-pathogenic alginate-producing pseudomonads such as *Pseudomonas mendocina*, for example NCIB 10541.

The particularly preferred bacterium for use in our filter device is one which is part of the dominant microbial flora in the surface biofilm of an established conventional slow sand filter and which is deposited as NCIB 40121. It has the following properties, namely unpigmented rapid growth on Medium A (see below), copious polysaccharide slime production on Medium B (see below) both in liquid medium and on medium solidified with 1.5 per cent agar, no or very poor growth on full strength standard bacteriological Nutrient Agar media, and no growth on McConkey Agar. In the foregoing and hereinafter, Medium A and Medium B are proprietary products well known in the trade.

The selected bacterium or mixture of bacteria is supported upon a water-permeable material of the characteristics specified above. The material employed should be not readily biodegradable. Material which biodegrades relatively slowly, for example over the period of use of the device, which may typically be say from 3 to 6 months, is suitable for this purpose. Preferably the material is resistant to ultraviolet radiation, to enable it to be used in conditions of prolonged strong sunlight. In order to permit the colonisation of microorganisms on its surface, it is desirable that the surface of the material should be not highly polished nor smooth. Of course the selected water-permeable material should be of low solubility, or insoluble, in aqueous liquids.

The water-permeable material may take various forms. Thus, for example, it may be a rigid or compressible porous material such as an expanded polymeric material, or a mat such as a coconut fibre mat, or a non-woven fabric such as a paper-like product, or a woven product such as cotton or a cellulosic material. A suitable expanded material is cellulosic sponge. When a flexible material of this type is used, it may be stored and/or conveyed in rolled and/or compressed form. A suitable non-woven material is the product sold under the trade mark "Vilene", which is offered for sale as a tailor's interfacing material. These sheet materials such as "Vilene" may be used in single or multiple layers, or sandwiched with other materials for support.

If the selected water-permeable material is porous it should, of course, be open-pored. The average pore diameter is preferably at least 10 μm both before and after impregnation with the bacteria. More preferably, the average pore diameter is at least 20 μm , especially of the order of 50 μm , before impregnation. Both the pore diameter and the pore density affect the rate at which the water to be purified can pass through the filter device and this should be borne in mind in selecting the water-permeable material to be used. With this in mind, porosities of 70 to 90 percent and higher are preferred.

Conditions typical for freeze-drying processes may be used for that purpose. Preferably the impregnated material is frozen at a temperature of the order of -70°C or lower. The subsequent removal of water by sublimation under vacuum is preferably carried out under a vacuum of 100 Pa or below that pressure. Following freeze-drying, the impregnated material is sealed in any suitable material which is impermeable to water-vapour, for example a sheet of synthetic polymeric material. This material can be folded down into a compact package. When so packed, our device is conveniently transportable in a form particularly suited to distribution to emergency situations. When the freeze-dried product is subsequently required for use, the vacuum seal is broken and water is added, with the result that within several hours (for example 6 to 8 hours) the microorganisms are reactivated and ready for use. To promote reactivation and growth of the freeze-dried microorganisms, microbial nutrients may be incorporated in the impregnated material before the freeze-drying step, or may be added to the water used for reactivation.

In order to use our filter device, it may be placed in contact with a bed of sand or another filter support medium and then the water to be purified is passed through the device and the filter support medium in turn. For example, the device may be laid horizontally upon a bed of sand or attached in a vertical position to one or more blocks of a rigid porous support medium. Suitable simple structures for this purpose are shown in the attached drawings, wherein:

- Fig. 1 is a vertical sectional elevation of a first form of filter unit;
- Fig. 2 is a plan view corresponding to Fig. 1;
- Fig. 3 is a vertical sectional elevation of a second form of filter unit; and
- Fig. 4 is a plan view corresponding to Fig. 3.

The filter unit illustrated in Figs. 1 and 2 is, as shown, square in plan (for example approximately 1 m^2) and somewhat taller than it is wide (say about 1.5 metres). It is formed of flanged flat tank sections made in glass-reinforced plastic, assembled in situ from a readily transportable pack, upon a support plinth 10. Within the lower part of the unit defined by side sections 11 are underdrains 12 of gravel or similar material and above the underdrains 12 is a support medium 13 of sand.

A filter device 14 according to the invention in the form of a bacterial layer on a flexible water-impermeable material is supported by the medium 13. The edges of the device 14 are held and sealed between the flanges of the side sections 11 and upper side sections 15. The level of water 16 in the unit is controlled by an overflow 17.

In using the unit, water for treatment is introduced to the upper part of the tank by an inlet pipe 18 and percolates through the filter device 14 and the support medium 13 to the underdrains 12, potable water being withdrawn via a valved outlet pipe 19. When, in use, the filter device 14 eventually becomes blocked, it is readily replaced by a new one.

The unit illustrated in Figs. 3 and 4 relies upon vertical filter panels, through which water flows in a generally horizontal direction from an inlet 20 to an outlet 21, the water level being controlled by an overflow 22. The filtering system consists of filter devices 23 according to the invention, attached at their edges to blocks 24 of a porous support medium, placed at spaced positions vertically in the water tank.

In the case of a unit of Figs. 3 and 4, when a filter device 23 eventually becomes blocked, it and the associated support block 24 may easily be replaced without the need to take the unit overall out of service. This offers obvious advantages over the unit illustrated in Figs. 1 and 2.

In experimental use of each of the illustrated units, high removals of pathogenic microorganisms have been achieved within hours of the initiation of the reactivation of the supported bacteria.

The following examples describe the preparation of two embodiments of our filter device, and the use of one of the resulting devices to purify contaminated water. In both cases, the bacterium used was the particularly preferred bacterium described above and identified by the Deposit No. NCIB 40121.