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PAPER A CHEMISTRY

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CLIENT'S LETTER

ClearWater Revival plc



Working Towards a Scumless Future

17 March 2000

Rumpole & Mortimer
European Patent Attorneys
9 Morse Road
Oxford

Dear Sirs,

Re: Filter Device and method for its preparation

We would like you to file a European patent application for us. As you know, we are active in the field of water treatment technology.

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.

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 days.

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. 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.

In one preferred form of the filter device, 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.

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.

If the filter device is to be freeze-dried, then 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 m²) 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 the 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.

Example 1

The Maintenance Medium is the Medium B solidified with 1.5 per cent (w/v) agar. For long-term storage, bacteria grown on Maintenance Medium at 30 °C for 48 hours are suspended in Medium B containing glycerol (20% w/v) and stored at - 70 °C in screw-capped bottles. In all cases the glucose, sterilised separately by autoclaving at 121 °C for 15 min, is added after the medium has been sterilised in the same way.

(a) Growth of inoculum.

The slime-producing bacterium is inoculated from a maintenance plate into 50 ml Medium A in a 250 ml capacity conical flask and incubated in a shaker-incubator, at 30 °C for 16 hours. This culture is used to inoculate (2% vol/vol) 50ml of the same medium, and the culture is incubated as above for 6 hours.

(b) Inoculation of water-permeable material and growth of bacteria.

Sterile 5 cm diameter discs of a cellulosic sponge material that has been washed at 121 °C in distilled water under pressure in an autoclave, are incubated in the above inoculum, under the same conditions, for 3 hours. The inoculated filter discs are transferred aseptically to 50 ml of Medium B in a 250 ml capacity conical flask, and incubated at 30 °C, in an orbital incubator until sufficient slimy biofilm has been established to provide a resistance to water flow such that a linear flow rate of approximately 0.2 m/hour is obtained through the filter under a hydrostatic head of 10 cm. A typical time of incubation taken to achieve this amount of biofilm will be between 8 and 16 hours depending on the initial pore size of the support material. A larger pore size predicates a longer incubation period.

Example 2

The procedure described in Example 1 is used to establish biofilms in 5 cm diameter discs of 1 to 2 mm thick layers of the non-woven fabric sold under the trade mark "Vilene". A suitable biofilm was formed after 8 hours of incubation.

Example 3

By the same procedure as used in Examples 1 and 2, a biofilm was formed in 5 cm diameter discs comprising a 10 mm thick layer of coconut fibre matting strengthened with plastic net. The final incubation time was 16 hours.

Example 4: Laboratory measurement of filter performance

Performance is assessed using 5 cm diameter biofilm-impregnated discs as prepared in Examples 1 to 3 in standard laboratory filter holders with plastic mesh support screens, under a hydrostatic head of 10 cm. Two types of test water are used (a) faecal coliform-contaminated natural water, typically incoming water to a municipal water treatment works; (b) phosphate-buffered saline containing a laboratory strain of *Escherichia coli* that carries a nalidixic acid-resistance gene (between 10,000 and 20,000 bacteria / 100 ml for both types of test water). The coliform bacterial count (used as a measure of water quality) is measured in both waters by the standard international procedures (principally the use of a selective medium - McConkey's medium - in multiple tube and filter assays, and standard confirmatory tests for *E. coli*). Nalidixic acid-resistant bacteria are counted by plating 0.1 ml samples of the contaminated water on to Nutrient Agar plates containing nalidixic acid at 10 µg/ml. The effluent water from the filters is assayed for coliform contamination as described above in successive 200 ml batches of filtrate. Typically the coliform count is reduced to less than 10 bacteria per 100 ml in the second 200 ml of filtrate and remains below this level in subsequent 200 ml batches (at least 10).

Example 5: Production on a large laboratory scale

The water-permeable support material used is a 1 m² sheet of cellulosic sponge material which is 15 to 20 mm thick. This sheet is washed in distilled water by autoclaving at 121 °C for 2 hours and is subsequently squeezed dry. The sheet is fully immersed in Medium A in a laboratory fermenter and sterilised with the medium, in-situ. The glucose is sterilised separately as in Example 1, and added aseptically. The fermenter temperature is equilibrated at 30 °C and a 5% (by volume) inoculum of bacterial culture, prepared as for the inoculum in Example 1, is added. The fermenter is aerated at 1 litre air/min/litre of culture medium, and stirred at 200 rpm. After 8 hours, sterile glucose (40% w/v) is added to give a final concentration of 10 g/litre and incubation is continued under the same conditions for a further period of between 16 and 24 hours. Dissolved oxygen levels are not controlled.

The impregnated sheet obtained is suitable for use in a filter unit such as one of the two types illustrated in the accompanying drawings.

Example 6

Biofilm containing non-woven fabric prepared in accordance with example 2 was freeze dried under standard conditions and then vacuum packed. It was established that the bacteria could be reactivated after a storage period of 3 months without a loss of bacterial activity.

We would like to point out that the bacteria mentioned in this letter are well known in the art. These bacteria and the use thereof in forming a Schmutzdecke was the subject of a scientific article published last year.

We hope that the foregoing provides you with enough information to file a European application. We are including two documents, Document I and Document II, which represent the prior art.

Yours,

T. Credence
ClearWater Revival plc

CLIENT'S DRAWINGS

1/1

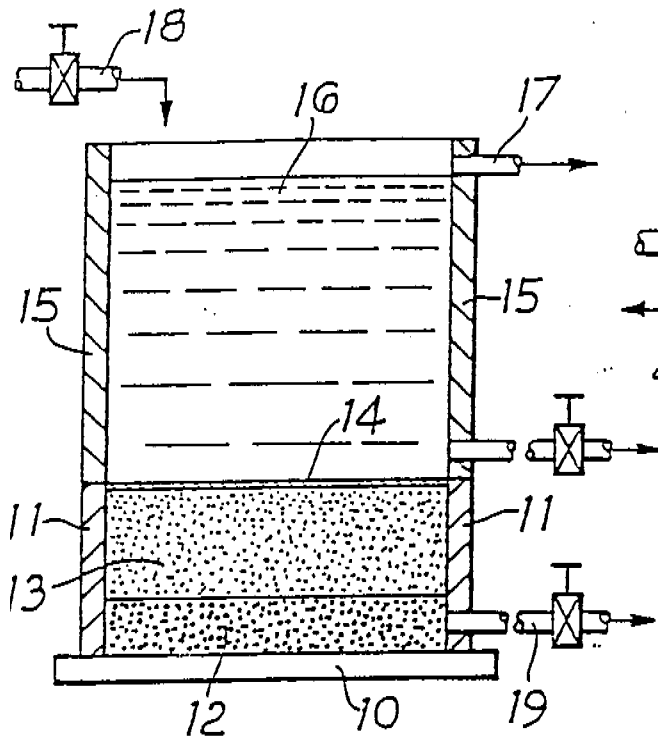


Fig. 1

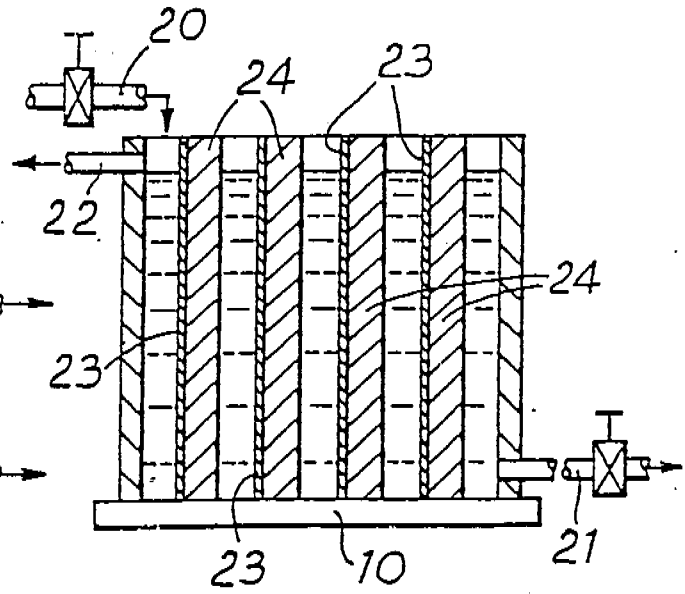


Fig. 3

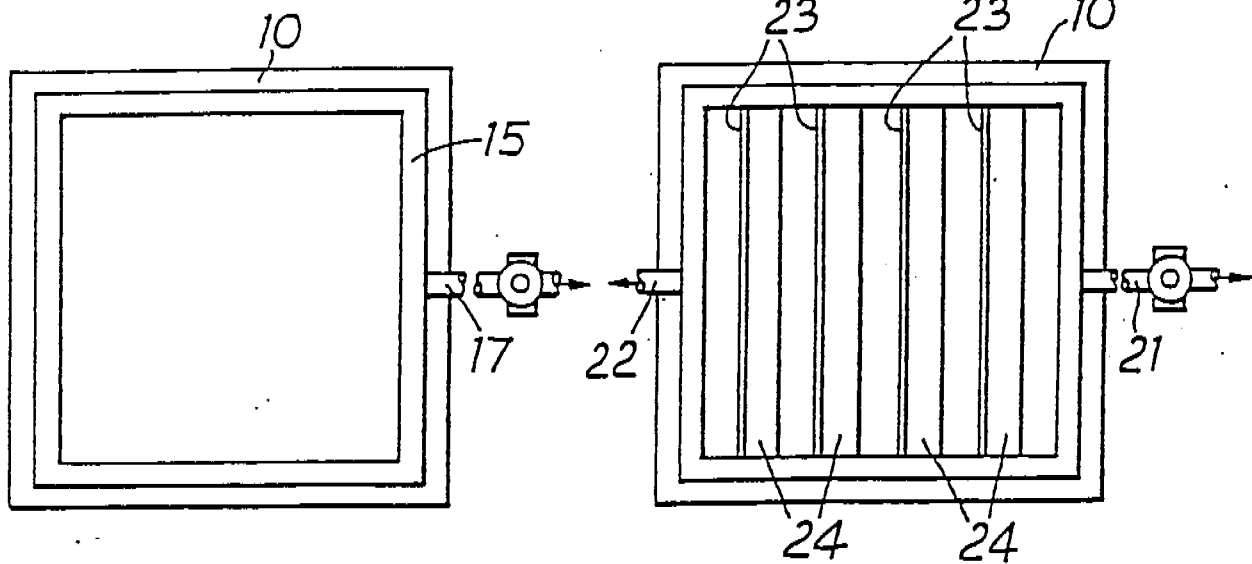


Fig. 2

Fig. 4

DOCUMENT DI (State of the Art)

Improvements Relating to Clean Water Treatment

In clean water treatment, water from a reservoir is subjected to a primary filtration by a coarse filter and then to a secondary filtration by a large scale slow sand filter.

5 The slow sand filter comprises a much larger flat bed of sand (e.g. in the region of 3,500 m²). The water, from a head maintained above the bed by the supply of filtered water from the coarse filter through this sand filter, filters relatively slowly. In the course of time, silt collects and algae and bacteria begin to grow on the surface of the sand bed, forming what is known as "Schmutzdecke". This layer contains exo-polysaccharide producing, gram-negative bacteria, which help to filter the water. As the thickness of the Schmutzdecke increases, the filtration rate of water through the sand bed decreases, and eventually the filter must be drained in order to remove the Schmutzdecke and restore the bed to operational efficiency. The Schmutzdecke is removed by mechanically skimming the top 2 to 5 cm of sand (and with it the Schmutzdecke) from the bed, and usually this has to be done every few months. This is a relatively laborious and time consuming operation, and although the removed sand may be cleaned for re-use, the cleaning operation is fairly expensive. The filter bed may be initially about 75 cm thick, and when this has been reduced by successive skimming operations to below about 30 cm it is usual to remove the remainder of the bed completely and to replace it with a thick bed of fresh sand, again a time consuming and expensive operation having regard to the amount of sand involved.

The present invention relates primarily to this secondary filtration process, and aims to reduce the time, effort, and expense involved in periodically removing the Schmutzdecke in order to restore the slow sand filter bed to operational efficiency.

25 According to the invention therefore, we propose a method of operating a large scale slow sand filter whereby the surface of the sand bed is covered by one or more thin, flexible, water permeable sheets to form a protective filter layer. In this way, the Schmutzdecke forms on the protective layer instead of on the surface of the sand, and the Schmutzdecke is removed either from the protective layer in situ or by removing the protective layer, with the Schmutzdecke adhered to it, from the filter.

In this way, the removal of the Schmutzdecke leaves the sand bed substantially clean and intact.

Although it is expected that the period between successive Schmutzdecke removals will be much the same as at present, the time taken for each removal will be much less. The method in accordance with the invention should therefore be very much quicker, easier, and more economical than the present
5 skimming method, and should prove to be of considerable benefit to Water Authorities operating large slow sand filter beds.

Each sheet forming the protective filter layer comprises a fabric, which should be inert in water. The fabric is preferably denser than water so that it will rest naturally on the surface of the sand bed, but if
10 it has a tendency to float the sheets may be held down on the surface of the bed in any suitable manner, for example, by metal mesh laid on top thereof. Preferably also, the fabric has a permeability to water which is substantially the same as the sand bed, and is similarly resistant to silt penetration. Suitable fabrics include woven, non-woven and sponge like materials or a fibre mat. Fabrics that have successfully been employed include a coconut fibre matting, a cellulosic sponge, a cotton cloth and a non-woven fabric sold under the trade mark "Vilene".

These fabrics typically exhibit porosities of greater than 75 percent, and pore diameters of 40-60 μm . Such fabrics, once the Schmutzdecke is formed, will have pore diameters of about 10-30 μm , this ensures an optimal flow-rate of the water through the filter layer.

If the Schmutzdecke is removed from the protective layer in situ, this may be done by suction dredging using a conventional suction dredging apparatus floating on the water above the filter bed, and in this case it may be necessary to prevent the sheet or sheets lifting from the surface of the bed, such as by metal mesh laid on top of the sheets as mentioned above.

If the Schmutzdecke is removed by removing the protective layer from the filter, this may be done by manually or mechanically rolling or folding up the or each sheet after first draining the water from the filter. One or more clean replacement sheets are then placed on the surface of the sand to form a fresh protective filter layer before refilling the filter with water and recommencing operation of the filter.

- 5 Dirty sheets removed from the filter may be washed for subsequent re-use, which should prove a much less time and energy consuming process than the washing of the skimmed sand as carried out at present.

Alternatively, if economically viable, the dirty sheets may even be discarded.

Claims

1. A method of operating a large scale slow sand filter in which the Schmutzdecke which forms on the surface of the sand bed is periodically removed, characterized in that the surface of the sand bed is covered by one or more thin, flexible, water permeable sheets to form a protective filter layer so that the Schmutzdecke forms on the protective layer instead of on the sand, and the Schmutzdecke is removed either from the protective layer in situ or by removing the protective layer, with the Schmutzdecke thereon, from the filter.
2. A method according to Claim 1, in which the sheet or sheets forming the protective filter layer also cover the side walls of the filter.
3. A method according to Claim 1 or Claim 2, in which the or each sheet is a woven or non-woven synthetic fabric which is inert in water.
4. A method according to any one of the preceding Claims, in which the or each sheet has a permeability to water which is substantially the same as that of the sand bed.

5. A method according to any one of the preceding Claims, in which the or each sheet is denser than water so that the protective filter layer will rest naturally on the surface of the sand bed.
6. A method according to any one of Claims 1 to 4, in which metal mesh panels are laid on top of the protective filter layer to hold it on the surface of the sand bed, and the metal mesh panels are removed prior to removal of the protective filter layer.
7. A method according to any one of the preceding Claims, in which the protective layer, and with it the Schmutzdecke, is removed from the filter by rolling or folding up the or each sheet after first draining the water from the filter.
8. A method according to any one of the preceding Claims, in which the or each sheet is scraped and washed to remove the Schmutzdecke from the sheet.

DOCUMENT II (State of the Art)

Process and apparatus for the preparation of a biomass reaction surface on a support material

The present invention concerns a process and an apparatus for the preparation of a biomass reaction surface on a support. The present invention is particularly useful in a water treatment plant for the treatment of waste water by passage through a solid granular support, such as sand.

- 5 Industrial waste water effluents produced by industry often contain organic or mineral contaminants. These must be removed before the water can be discharged into the environment. The treatments of such effluents may be physico-chemical, for example by involving addition of materials which provoke a precipitation of the pollutants, or biological, whereby the contaminants are removed by contacting the waste water with micro-organisms to eliminate for example carbon and nitrogen containing impurities.

The biological reaction is generally carried out by contacting the waste water with biomass in a biomass reactor. Biomass is a biological mass of materials which form an ecosystem comprising a number of discrete bacteriological entities. The bacteria in the biomass consume the polluting substances. These bacteria are in turn consumed by other bacteria, which are consumed by other bacteria, and so on. Effectively, the biomass acts as a food chain. In many cases, a useful biomass layer develops quite naturally within the oxygenated bioreactors used in water treatment plants. These reactors contain a support material upon which the biomass grows. Typically, the bacteria in these reactors are exo-polysaccharide producing, gram-negative bacteria. Typical bioreactors in this sense include the sort of slow sand filters used in large scale water treatment processing.

- It has been shown that the rate of coating of these support materials with the biomass depends on the state of the surface of the support. With a non-porous support, such as sand, the rate is very slow, and generally takes from two to three weeks. On the other hand, it has been shown that on microporous
25 support materials, the rate of coating can be greatly accelerated. The problem with the microporous supports is, however, that they are very expensive.

The present inventors have found that effective pre-treatment of the granular support so as to coat the grains of granular material with micro-organisms provide very significant advantages with respect to supports which have not been pre-treated. Specifically, the "pre-seeding" of the granular support material allows for greatly increased rates of establishment of the necessary concentrations of the
5 biomass microorganisms.

The present invention relates to a process for the pre-treating of a support material for use in a biological reactor, whereby the support material is coated with biomass and then frozen and kept at temperatures below freezing so as to preserve the biomass layer on the support materials up until the
10 time they are introduced into the reactor.

This provides a film on the surface of the granular support which favours the rapid establishment of the necessary ecosystem in the reactor. The rate of establishment of the necessary biomass within the reactor using the frozen coated granular support materials of the invention compares favourably with results obtained with the very much more expensive (uncoated) microporous supports. Thus, whereas reactors containing normal granular supports require two to three weeks before reaching a steady effective state, the coated granular materials of the present invention are operational within a day or so.

The granular supports are brought into contact with the biomass prior to freezing by immersing the granular support in a biomass broth. The granular support may then be frozen.

The frozen support materials may be used to start processing in a new reactor, or to re-start processing in an existing reactor following a period of hibernation or a period off-line for
25 maintenance. To do this, the reactor is filled with waste water, possibly diluted with clean water, and the recycling circuit is started up. The frozen support materials are then simply introduced into the reactor and allowed to come up to operating temperature. Start-up time (i.e. the time needed for a steady state to be achieved so that water may be effectively treated) is reduced from two to three weeks to about a day.

The invention is of course not limited to the embodiments specifically described relating to the treatment of waste water, but is applicable generally to areas where biomass is to be fixed on support materials.

- 5 In addition, although the invention has been more particularly described having regard to the use of sand as support material, it is to be understood that the invention is not so limited, and may be applied to any kind of permeable or non permeable support material, including both granular support material, and the woven or non-woven textile materials commonly employed in the art in such reactors.

Claims:

1. Process for the start-up of a bioreactor comprising biomass fixed on support materials, characterised in that it comprises introducing into the reactor a support comprising one or more elements which have been previously coated in biomass material, then frozen and stored in the frozen state.
2. Support element for bioreactors, characterised in that it comprises a solid support coated in frozen biomass.
3. Support element according to claim 2, characterised in that the solid support is a granular material.
4. Support element according to claim 3, characterised in that the granular material is a grain of sand.