A method and apparatus for recovering cells and their analysis.

Technical Field

The present invention relates to the field of separating and/or recovering and analysing cells. In particular, but not exclusively, the present invention relates to the field of recovering and analysing viable cells from fluid or gas.

Background

Filtration is used to separate and/or recover cells from fluid or gas. With a conventional filter apparatus, the fluid or gas sample typically enters through an inlet, passes through a membrane filter comprising a large number of pores and exits through an outlet. After filtration, the trapped cells may be recovered for further analysis. In one method to recover the cells, any water remaining in the apparatus is removed and the membrane filter is also removed and placed in a liquid bath with agitation to enable the liquid to dislodge the trapped cells from the filter to be suspended in the liquid (termed "lateral shaking" method). The liquid comprising cells may be collected for further analysis.

For capturing cells from gas (e.g. airborne cells), the gas sample (e.g. air sample) similarly enters the inlet, passes through the membrane filter and out through the outlet. The same process as described above may be used to recover the trapped cells.

Another method to recover the trapped cells, termed "back-flushing", involves allowing an eluent to enter through the outlet and to flow through the membrane to flush out the trapped cells before exiting through the fluid inlet, followed by collecting the eluent comprising cells for further analysis.

The above processes may not recover cells from the membrane filter effectively nor efficiently. Both lateral shaking and back-flushing may not effectively dislodge cells trapped in the pores and some cells may still be left trapped in the membrane. The membrane filter also traps particles other than cells which may further reduce the efficiency of recovering cells from the membrane filter. For example, the recovery rate may only be 30-70% and this affects the overall detection sensitivity, in particular for cells present in low proportions.

It is therefore desirable to develop methods for improving analysis of recovered cells.

Summary

According to a first exemplary aspect there is provided a method of separating and/or recovering and analysing cells comprising:

separating cells from a fluid or gas with a filter apparatus comprising a filter;

substantially lysing the cells to release nucleic acid molecules on the filter; and

eluting nucleic acid molecules from the filter for analysis, detection and/or identification.

The method may be used for separating and/or recovering and analysing cells of any organism. For example, the method may be used for bacteria and/or protozoa cells and in particular, cells of pathogenic organisms.

In order that the invention may be fully understood and readily put into practical effect there shall now be described by way of non-limitative example only exemplary embodiments, the description being with reference to the accompanying illustrative drawings.

Brief description of the figures

In the drawings:

Figure 1 shows an example of a filter apparatus for the exemplary method.

Figure 2 illustrates an example of the exemplary method with the filter apparatus of Figure 1. The top view of the membrane filter after each step of the method is also illustrated in Figures 2(A) to (E). Figure 2(F) also shows an illustration depicting the eluent comprising unbound DNA derived from viable and/or membrane-intact cells and covalently bound DNA from dead or membrane-compromised cells.

Figure 3 shows the (A) cross-sectional view and (B) top view of an example of a fluidic apparatus for the exemplary method.

Detailed description of the exemplary embodiments

The present exemplary embodiments relate to a method of separating and/or recovering and analysing cells, for example, from fluid or gas. The fluid or gas may be filtered through a filter apparatus comprising a filter which separates the cells from the fluid or gas, trapping them on the filter. After filtration, the trapped cells on the filter may be lysed to release nucleic acid molecules. Any suitable method for lysing the cells may be used. For example, various methods may be used for lysing the cells, including but not limited to using heat, chemicals, ultrasound and freeze-thawing. The nucleic acid molecules may then be eluted from the filter apparatus for further analysis, detection and/or identification.

The method may also further include steps for differentiating viable cells.

The following further steps may be performed:

contacting the cells before lysing with a phenanthridium compound capable of preferentially penetrating dead or membrane-compromised cells over viable and/or substantially intact cells to intercalate with at least one nucleic acid molecule; and

exposing cells on the filter to a light source to covalently bind the phenanthridium compound to at least one nucleic acid molecule of penetrated cell(s).

The cells may be contacted with the phenanthridium compound before or after filtration. For example, the cells may be mixed with the phenanthridium compound and the mixture is then filtered. Alternatively, the cells may be filtered first and the phenanthridium compound may then be loaded through the filter, contacting the cells on the filter while flowing through the filter.

After being exposed to the light source, the covalently bound nucleic acid molecules of the dead or membrane-compromised cells are unsuitable or unable to take part in further reactions, for example, the polymerase chain reaction (PCR).

The filter may also be washed to substantially remove any excess, residual and/or unbound phenanthridium compound. Either exposing the filter to a light

source may be performed before washing the filter or alternatively, washing the filter may be performed before exposing the filter to a light source.

Removal of excess, residual and/or unbound phenanthridium compound by washing before lysing the cells is optional and may be performed so that when the nucleic acid molecules from viable and/or substantially intact cells are released after lysis, they do not covalently bind to phenanthridium compound. In addition, nucleic acid molecules released from viable and/or substantially intact cells should not covalently bind to any excess, residual and/or unbound phenanthridium compound unless exposed to a light source after cell lysis. Nucleic acid molecules from substantially intact cells which are not covalently bound with the phenanthridium compound would be able to take part in further analytical, detection and/or identification reactions, for example PCR and in particular, real-time PCR. While real-time PCR involves exposure to a light source, the excess, residual and/or unbound phenanthridium compound does not substantially inhibit PCR, in particular, if present at low concentrations.

Any suitable phenanthridium compound may be used. In particular, the compound propidium monoazide may be used. Propidium monoazide is able to preferentially penetrate dead or membrane-compromised cells over viable and/or substantially intact cells to intercalate with DNA molecules and on exposure to a blue light source, covalently binds with the DNA molecules.

Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention.

EXAMPLES

Example 1

In one embodiment, the filter apparatus may comprise a filter cartridge comprising a membrane filter 3 as shown in Figure 1. The filter cartridge comprises a lid 1 with an inlet 2, a membrane filter 3 on a filter support 4 and an outlet 5. The method of the invention including the optional step differentiation of viable cells will now be described, with reference to Figure 2.

As illustrated in Figure 2(A), a fluid sample comprising *Escherichia coli* for example, was allowed to enter the inlet, pass through the membrane filter 3 and out through the outlet 5, as indicated by the arrows. The fluid sample may comprise both viable and dead *E. coli*, for example, by mixing a sample of *E. coli* heated at 72 °C for 10 minutes with a sample of live *E. coli* before using for the filtration. The membrane filter 3 traps particles including both viable and/or membrane intact *E. coli* 6 and dead or membrane-compromised *E. coli* 7 and other particles 8 (e.g. dirt) from the fluid.

In the next step as illustrated in Figure 2(B), 4 ml of 50 micromolar propidium monoazide was added via the inlet of the filter apparatus and the filter apparatus was then incubated in the dark for 10 minutes. The flow of propidium monoazide through and out of the filter apparatus is indicated by the arrows. Any suitable volume and concentration of phenanthridium or propidium monoazide may be added for any suitable incubation time. The volume and concentration of the phenanthridium compound or propidium monoazide used may be altered as appropriate, depending on target organisms to be analysed, detected and/or identified. The propidium monoazide molecules, represented by black dots 9 in Figure 2(B) preferentially penetrate the dead or membrane-compromised cells 7 over viable or membrane-intact cells 6.

In the next step as shown in Figure 2(C), which is an optional step, a washing fluid (e.g. 4 ml of water) may be added to wash away any excess propidium monoazide. The propidium monoazide molecules represented by the black dots 8 which had penetrated the dead or membrane-compromised cells were not washed away.

In the next step as illustrated in Figure 2(D), the lid of the filter cartridge may be removed and the membrane filter 3 may then be exposed to a light source 10, for example, a blue light from a light emitting diode (LED). On exposure to the blue light for a suitable timeframe (e.g. 20 minutes), the propidium monoazide covalently binds to the DNA 11 of the penetrated cells. Alternatively, the lid of the filter cartridge may be made of a substantially optically transparent material

(or substantially optically transparent to the blue light used) and in this case, the lid need not be removed when exposing the membrane filter 3 to the light source.

The lid 1 may then be replaced on the filter apparatus. 200 μl of water 12 was then added to immerse the membrane fully within the filter apparatus and the cartridge may be immersed in ~ 95 °C hot liquid bath 13 to lyse the cells on the membrane filter 3. Heat causes the cell wall to break and the nucleic acid molecules are released into the water within the filter apparatus. Alternatively, a hot gas stream also of ~ 95 °C may be allowed to enter via the inlet 2 to pass through the filter apparatus to lyse the cells on the membrane filter. The entire filter apparatus may also be incubated at ~ 95 °C, for example in an oven or a hot gas stream of ~ 95 °C may also be directed to the exterior of the filter apparatus to lyse the cells. As another alternative example, a heater may be included as part of the filter apparatus for heating the membrane filter.

As a further example, a hot liquid of ~ 95 °C may also be introduced via the inlet to submerge the filter as indicated by the arrow and lyse the cells on the membrane filter 3. Both DNA from viable and/or membrane-intact cells 14 and covalently bound DNA from dead or membrane-compromised cells 11 are released with cell lysis. (See Figure 2(E)). Cell debris 15 may also be present. Other methods of cell lysis may be utilised, including but not limited to chemical lysis, freeze-thawing and ultrasonication.

In the next step as illustrated in Figure 2(F), the nucleic acid molecules may be eluted from the membrane filter 3 for example by adding 800 μl of TE buffer as the elution buffer via the inlet of the filter apparatus as indicated by the arrow. Any suitable eluent including but not limited to water or other solution or buffer may also be used. The eluted nucleic acid molecules may then be collected for further analysis, detection and/or identification. During elution, most of the cell debris 15 from lysis and other particles 8 remained on the membrane filter 3 while the nucleic acid molecules were eluted. Further analysis, detection and/or identification may be with PCR. For example, 1 μl of the eluent may be used for

PCR. During PCR, only DNA obtained from viable or membrane-intact cells 14 may be amplified. The covalently bound DNA from the dead or membrane-compromised cells 11 should not be amplified.

Example 2

In another embodiment, the filter apparatus may be in the form of a fluidic chip as illustrated in Figure 3. In this embodiment, the filter apparatus comprises three layers 16, 17 and 18 and a filter 3 bonded together to form a planar apparatus with an inlet 2, a channel 19 and an outlet 5. Layer 16 may be substantially optically transparent to light (or substantially optically transparent to the blue light used). The fluidic device is exposed to light from a LED (e.g. blue light), which may be positioned above layer 16. A heater and/or an ultrasonic device may be positioned below layer 18 for lysing the cells. As indicated by the arrows, the fluid enters via an inlet 2, passes through the membrane 3 which separates the particles including cells, passes through a channel 19 within the fluidic chip and out again via the outlet 5. The subsequent steps, including the optional step of differentiating viable cells with a phenanthrdium compound, as well as lysing the cells and eluting the nucleic acid molecules may also be carried out with the fluidic chip.

Claims

1. A method of separating and/or recovering and analysing cells comprising:

separating cells from a fluid or gas with a filter apparatus comprising a filter;

substantially lysing the cells to release nucleic acid molecules on the filter; and

eluting nucleic acid molecules from the filter for analysis, detection and/or identification.

2. The method according to claim 1 further comprising:

contacting the cells before lysing with a phenanthridium compound capable of preferentially penetrating dead or membrane-compromised cells over viable and/or substantially intact cells to intercalate with at least one nucleic acid molecule; and

exposing cells on the filter to a light source to covalently bind the phenanthridium compound to at least one nucleic acid molecule of penetrated cell(s);

3. The method according to claim 2, further comprising washing the filter before lysing to substantially remove excess, residual and/or unbound phenanthridium compound.

4. The method according to claim 2 or 3, wherein the phenanthridium compound comprises propidium monoazide.

5. The method according to any one of claims 2 to 4, wherein the light source comprises blue light.

6. The method according to any one of the preceding claims, further comprising analysing, detecting and/or identifying the nucleic acid molecules with PCR.