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(54) Title: DETECTION OF MICROORGANISMS WITH A FLUORESCENCE-BASED DEVICE

(57) Abstract: A device and method for detecting by fluorescence microbial growth from sample substances is disclosed. The device includes a container which is at least partially transparent to electromagnetic radiation in the visual and/or the ultraviolet wavelength ranges, and fluid disposed in the container for cultivating microorganisms therein. A fluorescing indicator substance is disposed in the fluid layer for undergoing transformation in the presence of microorganism growth. A second layer, composed of a semi-fluid substance, indicators and other substances, such as growth media, is disposed in the container. The substances within the semi-fluid phase are in equilibrium with the substances in the fluid layer and provide a barrier to solid substances introduced into the fluid layer while providing a zone within which fluorescence changes in the indicator substance due to microbial growth can be detected.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT APPLICATION
DETECTION OF MICROORGANISMS WITH A FLUORESCENCE-BASED DEVICE

Technical Field.

The present invention relates to fluorescence-based devices for detecting microbial growth from test samples.

Background of the Invention.

It is necessary to test various industrial substances, such as food, pharmaceuticals, cosmetics and water, for microbial contamination. One area of biological testing of food, dairy, pharmaceutical, cosmetic and related types of products involves the estimation of total numbers of bacteria, yeasts and molds, as well as concentrations of specific groups of organisms within the material. One widely used method is known as the "Standard Plate Count" method and involves culturing a diluted sample of the product in an agar growth medium. The plates containing the sample and the growth medium are incubated (e.g., 32°C - 40°C) for 24 hours to 5 days, depending upon the assay. After incubation, colonies of microorganisms which have grown in the agar are counted.

Optical methods have been successfully used to classify microorganisms in clinical samples (e.g., PASCO by Difco, Detroit, Michigan). Although it would be desirable to utilize a colorimetric method, or any other optical method, for detecting microbial growth in industrial samples, the solid substances of the test samples disposed in an aqueous media usually cause optical interference for a detection system. More specifically, when solid substances are disposed in a media to allow for culturing microorganisms, the colorimetric detection system must pass light either through or reflect light from the media containing the solid substance. In most of the cases, the solid substances interfere with the spectral characteristics of the media, yielding a poor signal-to-noise ratio of the detection system.

A device for continuously monitoring the biological activity in a specimen is described by Eden in U.S. Pat. No. 5,366,873. It describes a device and method for detecting microbial growth from a sample substance. The device includes a

container which is at least partially transparent and fluid disposed in the container for cultivating microorganisms therein. An indicator substance is disposed in the fluid layer for undergoing transformation in the presence of microorganism growth. A second layer, composed of semi-fluid substance, indicators and other substances, such as growth media, is disposed in the container. The substances within the semi-fluid phase are in equilibrium with the substances in the fluid layer and provide a barrier to solid substances introduced into the fluid layer while providing a zone within which changes in the indicator substance, due to microbial growth, can be detected. In practice, the indicator substance has been dyes that are affected by the PH variations in the fluid layer.

Summary of the Invention.

The present invention extends the scope of the above patent by employing fluorescing indicator substances to enhance the measurement sensitivity and the group of detected microorganisms. In accordance with the present invention, there is provided a device and method for detecting microbial growth from a sample substance. The device includes a container which is at least partially transparent to electromagnetic radiation in the visual and/or the ultraviolet wavelength ranges and fluid disposed in the container for cultivating microorganisms therein. A fluorescing indicator substance is disposed in the fluid layer for undergoing transformation in the presence of microorganism growth. A second layer, composed of at least one semi-fluid substance, indicators and other substances, such as growth media, is disposed in the container. The substances within the semi-fluid phase are in equilibrium with the substances in the fluid layer and provide a barrier to solid substances introduced into the fluid layer while providing a zone within which fluorescence changes in the indicator substance due to microbial growth can be detected.

Brief Description of the Drawings.

Other advantages of the present invention will be readily appreciated as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying drawings wherein:

FIG. 1 shows one embodiment of the present invention; and

FIG. 2 shows an alternative embodiment.

Description of the Preferred Embodiments.

Generally, the present invention provides a device for detecting microbial growth from a sample substance wherein the device includes a container which is at least partially transparent to visual and/or ultraviolet (UV) radiation. A fluid layer is disposed in the container for cultivating microorganisms therein. A fluorescing indicator substance is disposed in the fluid layer for undergoing transformation in the presence of microorganism growth. A barrier layer is disposed in the container which is a semi-fluid substance, the fluid portion of which is the same composition as the fluid layer in which the microorganisms are cultivated. Therefore, the fluid in the semi-fluid layer is in equilibrium with the fluid layer. The semi-fluid substance provides a barrier to solid substances introduced into the fluid layer while providing a zone within which changes in the fluorescing indicator substance, due to microbial growth, can be detected.

More specifically, the barrier layer is comprised of gelling agents, such as agar. In carrying out the present invention, any type of gelling substance or agar, as defined in the Merck Index, can be utilized. There are several commercial gelling products available which are suitable, including gelatin, carrageenan and pectin.

The important property of such gelling agents used in the present invention is their ability to transfer ions, such as H+ and small molecules, while blocking out bacteria and larger debris particles. If the concentration of the small particles changes due to organism growth (e.g., pH or Redox reactions), the concentration of the identical particles in the barrier layer will track those changes as well. The diffusion coefficient of the barrier layer determines the rate in which variations in the liquid layer are tracked by identical changes in the barrier layer.

FIG. 1 illustrates a typical configuration of the various components of a system which can be utilized in accordance with this invention. The vial 10 is made of UV-transparent material (e.g., glass, UV-transparent plastics). The barrier layer 16 may be composed of any available agar (e.g., Muller Hinton Agar by Difco, Detroit, Michigan) and non-toxic fluorescing dye 14, such as Umbelliferon. This layer is manufactured by dispensing said mixture, thermally

sterilized, to the bottom of the vial 10 and letting it solidify at room temperature. A sterile mixture of the liquid media 12 and dye 14 is poured at room temperature on top of the barrier layer.

The test sample 28 is placed in the fluid layer. The vial 10 is then placed in an incubating device, at an appropriate temperature, to promote growth of organisms. The incubating device can be an air incubator, heating and cooling blocks or heat exchanger.

An ultraviolet light source 18 is positioned at the bottom part of the vial 10 such that the transmitted UV light is directed through the UV-transparent walls of the vial 10 and the barrier layer 16. The light source can be any long or short wave ultraviolet from various UV sources. In the preferred embodiment of the invention, a long wavelength ultraviolet Light Emitting Diode (LED) (e.g., 350 to 400 nanometers) can be utilized. A multiplicity of LEDs can be controlled by the controller 20, which provides electrical energy which is spatially uniform and stable.

In dealing with fluorescing essays, one should remember that the wavelength of the radiation emitted from the fluorescing dye is longer than that of the light source. For example, radiating Umbelliferon with a UV light source of 380 nanometers (invisible) generates a blue-green visible radiation. Consequently, care should be taken that the light sensor will not be influenced by stray light generated by the UV light source. If the UV source 18 is placed directly facing the light sensor 22, as shown in FIG. 1, an additional band pass optical filter 23 is required to block the influence of the UV radiation on the sensor. Alternatively, the UV light source 18 and the sensor 22 can be placed next to each other facing the UV-transparent section of the vial at specific angles, as shown in FIG. 2, so that the fluorescing radiation is reflected back to the light sensor. Since the fluorescing radiation is equally radiated in all directions, the specific angles can be set to minimize UV reflected light, thereby allowing the light sensor to measure only the fluorescing energy.

The dynamic changes of the fluorescing light, which is the indicator of bacterial activity, is converted to electrical energy utilizing a light sensor 22. Although a wide variety of sensors may be utilized (e.g., photo voltaics, photodiodes, phototransistors, photo multipliers, charged coupled devices (CCD) and multi-channel devices) low-cost solid state sensors can be employed due to

the high energy of light reaching the sensor. Therefore, each vial can have its own pair of light source and sensor, thus eliminating complex mechanical indexing devices utilized in optical readers and thereby increasing the reliability and the operating life of the instrument. The LED can provide either stationary (constant) or pulsated energy. If an additional LED operating in the visible range is employed, one of the LEDs can be driven at a constant level of energy while the other can be pulsated, allowing a single light sensor to detect both signals. In another embodiment, both UV LED and the visible-range LED can be combined in a single package forming dual-band UV and visible light sources that can be independently activated.

In the preferred embodiment, readings are taken every six minutes, and the analog data is converted by the converter 24 to digital form. The process data is transferred to a processor 26, where it can be displayed, stored and analyzed for real time detection.

The gelling agent or agar is positioned in the container such that it is in a transparent region of the container to facilitate measurement of changes in this phase of the system when in use. If the container is a vial or tube, typically the agar could be placed at the bottom of such receptacle, as illustrated in FIG. 1, and would be approximately 2 to 3 mm thick. The agar also could be in the form of a disc, attached to any wall of the container or other configuration as may be convenient in accomplishing the measurement which is the object of the present invention.

The semi-fluid layer (e.g., the agar or gelling phase) is situated in the liquid phase within the container such that the liquid substances within the agar are in equilibrium with the remaining liquid in the container. In the practice of the present invention, the liquid phase within the container is a liquid medium suitable for culturing microorganism growth. A sample of a substance which may harbor microorganisms is placed in the liquid phase in the container and incubated to promote growth of the microorganisms. When microorganisms are present, their growth will result in changes in the composition of the liquid phase throughout the container inasmuch as the liquid in the semi-fluid or agar phase is in equilibrium with the remainder of the liquid in the container. The contents of the liquid growth medium can be selected to result in a wide variety of changes in the liquid composition that can be detected and measured, as set forth in more detail below.

The change in the composition of the liquid growth medium can be detected and measured in the semi-fluid phase, which is free of the sample that is being tested and free of microorganisms. The sample being tested is usually too large molecularly to penetrate the agar phase, as are the microorganisms. Thus, the semi-fluid phase provides a zone within which changes in the liquid phase, brought on by microorganism growth, can be readily detected and measured without any interference from the test sample.

The liquid phase of the present invention is a medium suitable for the promotion of microorganism growth and for the maintenance of the viability of the microorganisms. Such growth media are well known in the art.

After a test sample has been placed in the liquid phase of the container, the container is incubated at an appropriate temperature (e.g., about 15°C to 65°C) for about 24 to 48 hours, or some other suitable time period, after which changes in the fluorescing substances can be measured. Changes in the fluorescing substance are detected and measured in the semi-fluid phase by analyzing the fluorescence changes related to microorganism growth. Changes in the indicator substance can be detected and measured in the semi-fluid phase since the liquid in this phase is in equilibrium with the remaining liquid in the container. Thus, any changes which occur in the fluorescence substance will be present throughout the container. Detection and measurement in the semi-fluid phase free of large molecules (e.g., the sample being tested) and microorganisms provides an accurate and consistent means of detecting microorganism growth with a high signal-to-noise ratio.

The container used in the present invention can be glass or long UV-transparent plastics, such as polystyrenes. The entire container need not be transparent, but the portion of the container surrounding the semi-fluid phase must be transparent to permit measurement of any change in the indicator substance in response to microorganism growth. Also, the container can be any shape or size, but typically will be a vial or a tube which can be closed once the agar phase and liquid phase are incorporated therein. Once the two phases are loaded in the container, they can be shipped to the site needed for performing analysis of test samples. No special temperature or storage requirements for the container exist.

The invention has been described in an illustrative manner, and it is to be understood that the terminology which has been used is intended to be in the nature of words of description rather than of limitation.

Obviously, many modifications and variations of the present invention are possible in light of the above teachings. It is, therefore, to be understood that within the scope of the appended claims, the invention may be practiced otherwise than as specifically described.

CLAIMS

1. A method for the generation and detection of visible-band fluorescence signals generated by at least one fluorescing compound excited by ultraviolet energy, comprising the steps of:

targeting and exciting the fluorescing compound with ultraviolet energy emitted from a light-emitting diode with spectral energy located substantially below 400 nanometers wavelength causing said ultraviolet energy to interact with the fluorescing compound to generate the visible fluorescence signal; and

detecting the visible-band fluorescence signal with a light detector sensitive to electromagnetic energy located substantially above 400 nanometers wavelength.

2. The method of claim 1 wherein said light detector is a photo-transistor with diminished sensitivity below 400 nanometers wavelength.

3. The method of claim 1 wherein said light-emitting diode generates a stationary level of energy.

4. The method of claim 1 wherein said light-emitting diode generates pulsated energy.

5. The method of claim 1 wherein said fluorescing compound is the dye Umbelliferon.

6. The method of claim 5 wherein said fluorescing compound is dissolved in liquid.

7. The method of claim 5 wherein said fluorescing compound is dissolved in agar.

8. The method of claim 5 wherein said fluorescing compound is impregnated in a matrix.

9. The method of claim 7 wherein said liquid is media capable of growing biological cells.

10. The method of claim 9 wherein said biological cells are microorganisms.

11. A device for generating and detecting visible-band fluorescence signals generated by at least one fluorescing compound excited by ultraviolet energy, comprising:

an ultraviolet light-emitting diode generating electromagnetic radiation residing substantially below 400 nanometers wavelength and targeted to the fluorescing compound; and

a light detector sensitive to electromagnetic energy residing substantially above 400 nanometers wavelength for the detection of visible-band fluorescence signals generated by the fluorescing compound.

12. The device of claim 11 wherein said light detector is a photo-transistor with diminished sensitivity below 400 nanometers wavelength.

13. The device of claim 11 wherein said light-emitting diode generates a stationary level of energy.

14. The device of claim 11 wherein said light-emitting diode generates pulsated energy.

15. The device of claim 11 wherein said fluorescing compound is the dye Umbelliferon.

16. The device of claim 15 wherein said fluorescing compound is dissolved in liquid.

17. The device of claim 15 wherein said fluorescing compound is dissolved in agar.

18. The device of claim 15 wherein said fluorescing compound is impregnated in a matrix.

19. The device of claim 16 wherein said liquid is media capable of growing biological cells.

20. The device of claim 19 wherein said biological cells are microorganisms.

21. The device of claim 11 further including a band-pass filter located in the path of said electromagnetic radiation in front of the light sensitive area of said light detector.

22. An instrument for simultaneous measurements of a multiplicity of fluorescing compounds comprising multiple units each characterized by the device according to claim 11.

23. A device for generating and detecting visible-band fluorescence signals and visible-band secondary signals generated by a fluorescing compound excited by ultraviolet and visible-band energy, comprising:

an ultraviolet light-emitting diode generating electromagnetic radiation residing substantially below 400 nanometers wavelength, targeted to the fluorescing compound and interacting with the fluorescing compound, thereby generating the visible-band fluorescence signal;

a visible-band light-emitting diode generating electromagnetic radiation residing substantially above 400 nanometers wavelength, targeted to the fluorescing compound and interacting with the fluorescing compound, thereby generating the visible-band secondary signal; and

a light detector sensitive to electromagnetic energy residing substantially above 400 nanometers wavelength for detecting the visible-band fluorescence signal and the visible-band secondary signal, both generated by the fluorescing compound.

24. The device of claim 23 wherein one of said light-emitting diodes is generating stationary energy and the other light-emitting diode is pulsated, thereby generating a combination of constant energy and pulsated energy directed to said light detector and corresponding to the individual fluorescence signal and the secondary signal.

25. The device of claim 23 wherein one light-emitting diode is activated for a specific amount of time while the other light-emitting diode is deactivated, followed by activating said deactivated light-emitting diode and deactivating said activated light-emitting diode, thereby alternately generating the fluorescence signal and the secondary signal at consecutive periods of time.

26. The device of claim 23 wherein said ultraviolet and visible-band, light-emitting diodes are packaged in a single enclosure, forming a dual-band, light-emitting diode.

27. The device of claim 23 wherein the interaction of the visible-band secondary signal with the fluorescing compound characterizes the optical transmittance of the fluorescing compound.

28. The device of claim 23 wherein the interaction of the visible-band secondary signal with the fluorescing compound characterizes the optical reflectance of the fluorescing compound.

29. The device of claim 23 wherein the interaction of the visible-band secondary signal with the fluorescing compound characterizes the optical scattering property of the fluorescing compound.

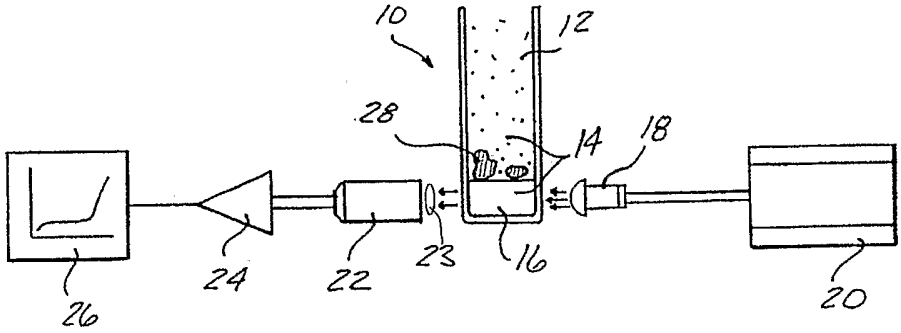

FIG-1

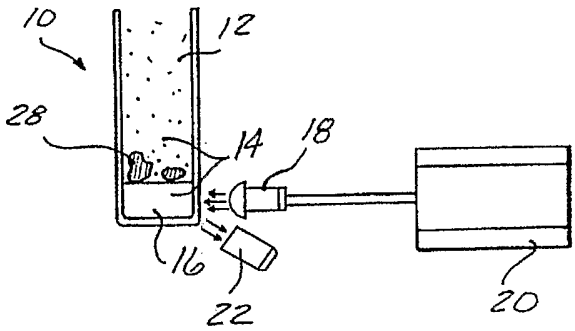

FIG-2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US05/04331

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C12Q 1/04
US CL : 435/34
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/34

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2002/0079454 A1 (YAMADA et al) 27 June 2002 (27.06.2002), Abstract.	1-29
Y	US 5,366,873 A (EDEN et al) 22 November 1994 (22.11.1994). col. 3, lines 1-30, col. 4, lines 5-60).	1-29
Y	US 5,164,301 A (THOMPSON et al) 17 November 1992 (17.11.1992), col. 4, lines 5-60.	1-29

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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