DESCRIPTION

Title of Invention

CULTURE INFORMATION-GENERATING DEVICE, CULTURE DEVICE AND CULTURE METHOD

Technical Field

[0001]

The present invention relates to a culture information-generating device, a culture device, and a culture method.

Priority is claimed on Japanese Patent Application No. 2010-15844, filed January 27, 2010, the content of which is incorporated herein by reference.

Background Art

[0002]

When a target protein is produced using a virus, a virus into which a gene for generating a specific protein is introduced is seeded and cultured in an animal cell or the like, and the protein is separated from the cultured virus. Further, when a vaccine is produced using a virus, the virus itself sometimes becomes the target to be produced, and is seeded and proliferated in a properly cultured animal cell. In such a virus culture, as a technique of evaluating culture conditions, a method of sampling a virus-containing culture medium to count the number of the virus (titer measurement), or a method of checking a state of cells in a sample using a microscope is generally performed. An example of such a virus culture technology is disclosed in Patent Document 1 and Non-Patent Documents 1 to 3 below.

Citation List

Patent Document

[0003]

[Patent Document 1] Japanese Unexamined Patent Application, First Publication No. 2002-148258

Non-Patent Document

[0004]

[Non-Patent Document 1] *Mass production of protein using baculovirus-insect cell development system,* Protein Science Society Archives No. 022

[Non-Patent Document 2] *Proliferation of* Bombyx mori *nuclear polyhedrosis virus*, Report on Sericultural Insect Research No. 7; 9-29 (1993)

[Non-Patent Document 3] *Expression and purification of an influenza hemagglutinin-one step closer to a recombinant protein-based influenza vaccine*, Vaccine 24P2176 (2006)

Summary of Invention

Technical Problem

[0005]

In the conventional evaluation technology of culture conditions, since the culture target is the virus, the sampling must be operated in a sterilized and closed system, and thus is complicated. In connection with this sampling, the culture tank may be contaminated by bacteria. Further, since the titer measurement requires several days and is difficult, a measurement error may be occurred depending on a measurer, and thus it is difficult to secure timely measurement and measurement accuracy. Furthermore, the evaluation using the microscope cannot warrant objectivity because a difference between individual observers easily takes place.

[0006]

The present invention has been made in view of the above circumstances, and has the following objects.

(I) To timely evaluate culture conditions of a virus.

(II) To provide a technique of evaluating the culture conditions of the virus while securing objectivity compared to the related art.

(III) To prevent a culture tank from being contaminated by bacteria.

Solution to Problem

[0007]

To accomplish the objects of the present invention, the present invention employs the following techniques:

(1) The present invention provides a culture information-generating device that inputs a measurement signal indicating a change in concentration of a target component of a gas exhausted from a culture tank holding a virus-containing culture medium, and performs predetermined information processing on the measurement signal, thereby generating culture information indicating culture conditions of a virus.

[0008]

(2) In the culture information-generating device set forth in (1), when the virus is seeded and cultured in a predetermined cell that serves as nutrition for the virus in the culture tank, the generated culture information may indicate that a maximum value of the concentration of the generated gas is generated a predetermined number of times.

[0009]

(3) In the culture information-generating device set forth in (1) or (2), the gas may be carbon dioxide (CO_2).

[0010]

(4) The present invention provides a culture device, which includes: a culture

tank in which a virus is cultured; an exhaust gas analyzer that analyzes and measures an exhaust gas exhausted from the culture tank and outputs a measurement signal indicating the measured result; and the culture information-generating device set forth in any one of (1) to (3).

[0011]

(5) The culture tank of the culture device set forth in (4) may terminate culture of the virus based on the culture information.

[0012]

(6) The present invention also provides a culture method in which culture of a virus is terminated based on a change in concentration of a target component of a gas generated by culturing the virus.

[0013]

(7) In the culture method set forth in (6), when the virus is seeded and cultured in a predetermined cell that serves as nutrition for the virus, culture of the virus may be terminated when a maximum value of the concentration of the generated gas is generated a predetermined number of times.

[0014]

(8) In the culture method set forth in (6) or (7), the gas may be carbon dioxide (CO_2).

Advantageous Effects of Invention

[0015]

According to the present invention, it is possible to automatically detect the culture conditions of the culture tank on the basis of the exhaust gas exhausted from the culture tank in an on-line state. As such, it is possible to timely evaluate the culture conditions of the virus, and to provide a technique which evaluates the culture conditions

of the virus and the objectivity of which is improved compared to that of the related art. Further, it is possible to prevent the culture tank from being contaminated with bacteria.

Brief Description of Drawings

[0016]

FIG. 1 is a block diagram showing the configuration of a virus culture device according to an embodiment of the present invention.

FIG. 2 is a flowchart showing the operation of essential parts of the virus culture device according to the embodiment of the present invention.

FIG. 3 shows measured results showing a change in concentration of carbon dioxide (CO_2) and oxygen (O_2) exhausted from a culture tank in the embodiment of the present invention.

FIG. 4 is a characteristic diagram showing a change in product concentration ratio over culture time in the embodiment of the present invention.

Description of Embodiments

[0017]

Hereinafter, an embodiment of the present invention will be described with reference to the drawings.

As shown in FIG. 1, a virus culture device A according to the embodiment includes a culture tank 1, a virus feeder 2, an exhaust gas analyzer 3, and a culture information-generating device (culture condition information generating device) 4. This virus culture device A seeds a virus incorporating a gene for generating a predetermined target protein in an insect cell, and cultures the virus, thereby producing the target protein, or proliferating the virus itself.

[0018]

The culture tank 1 is equipment for culturing the insect cell and the virus, both of

which are culture targets, and is an installation that cultures the culture targets while keeping an environment in a tank body (culture environment) optimal for the culture. That is, the culture tank 1 includes an agitator that agitates a culture medium in the tank body, an oxygen feeder that feeds oxygen required for the culture to the tank body, and a temperature controller that maintains the tank body at an optimum temperature for the culture. A certain type of culture tank, for instance, that carries out agitation using aeration without an agitator, is generally known as a type of the culture tank. In the present embodiment, any type may be employed as the culture tank 1.

[0019]

The virus feeder 2 feeds the virus to the culture tank 1 at a predetermined timing after culture initiation. A gene for generating a specific protein is incorporated into the virus. The protein is a final product of the virus culture device A. The culture tank 1 cultures the insect cell at its initial stage. However, when the virus is fed from the virus feeder 2 at the predetermined timing after culture initiation, the virus seeded in the insect cell is thereafter cultured in the tank body.

[0020]

The exhaust gas analyzer 3 is a device that analyzes and measures components and concentration of an exhaust gas exhausted from the culture tank 1 in the culture process in an on-line state.

This exhaust gas analyzer 3 analyzes and measures concentration of, for instance, carbon dioxide (CO_2) among the components included in the exhaust gas, and outputs the result as a measurement signal to the culture information-generating device 4. As this exhaust gas analyzer 3, for instance, an optical on-line gas detector using a difference in optical absorption wavelength based on a difference in gas component may be used.

[0021]

The culture information-generating device 4 is a most characteristic component in the virus culture device A.

As shown in FIG. 1, the culture information-generating device 4 is a sort of computer that is essentially made up of an information processing unit 4a and a storage unit 4b. The information processing unit 4a processes the measurement signal on the basis of a certain culture information-generating program, generates culture information showing the culture conditions of the culture target in the culture tank 1, and outputs the generated culture information to a built-in or separately provided display device (not shown) and/or the culture tank 1. The storage unit 4b is a semiconductor memory made up of a non-volatile memory region that stores the culture information-generating program and a volatile memory region that temporarily stores the calculated results.

[0022]

Next, an operation of the virus culture device A configured in this way will be described in detail with reference to FIGS. 2 to 4.

[0023]

The virus culture device A initiates culture with only insect cells held in the culture tank 1. Then, when a predetermined time has lapsed from the culture initiation, the virus feeder 2 is operated to feed viruses to the culture tank 1. In the culture tank 1, the insect cells and the viruses are agitated and mixed by the agitator, and the insect cells are further cultured. When a constant time has lapsed from the culture initiation, the culture of the insect cells is terminated.

[0024]

Changes in concentration of carbon dioxide (CO_2) and oxygen (O_2) exhausted from the culture tank 1 between the culture initiation and termination of the insect cells,

which are measured results of the exhaust gas analyzer 3 between the culture initiation and termination of the insect cells, are as shown in FIG. 3. When attention is focused on the change in the concentration of the carbon dioxide (CO_2), the virus feeder 2 is operated at a point of time when 45 hours have lapsed from the culture initiation. Thereby, the viruses are fed to the culture tank 1, and thus begin to be seeded in the insect cells. Each insect cell is smoothly cultured from the culture initiation, and thereby the concentration of the carbon dioxide (CO_2) is gradually raised. However, slight turbulence occurs on a rising curve of the concentration of the carbon dioxide (CO_2) at the time of seeding.

[0025]

The concentration of the carbon dioxide (CO_2) forms a rising curve because each insect cell continues to be proliferated after seeding. Thereafter, the rate at which the concentration of the carbon dioxide (CO_2) rises is gradually reduced, and forms a falling curve at a point of time when 75 hours have lapsed from the culture initiation. That is, the concentration of the carbon dioxide (CO_2) represents a first maximum value after seeding, as indicated by a circle. This change in the concentration of the carbon dioxide (CO_2) is caused by the fact that, after seeding, the viruses gradually infect the insect cells (primary infection) and interfere with the proliferation of the insect cells, or the fact that the destructed insect cells due to the proliferation of the viruses in the insect cells gradually increase.

[0026]

Then, the viruses proliferated to a predetermined number in the insect cells separate from the insect cells at a point of time when 100 hours have lapsed from the culture initiation, and then infect other insect cells (secondary infection). In the event of the secondary infection, the viruses proliferated in the insect cells go out from the insect

cells for a while. As such, as shown in FIG. 3 by a circle, the concentration of the carbon dioxide (CO_2) is abruptly increased and reduced. That is, the concentration of the carbon dioxide (CO_2) represents a second maximum value due to the secondary infection of the viruses.

[0027]

Among the proteins (products) generated in the viruses, certain proteins are gradually increased in accordance with the proliferation of the viruses in the culture tank 1 by the seeding after 45 hours have lapsed from the culture initiation. Thereafter, the proteins (products) are gradually increased with the lapse of time, and are remarkably increased in the event of the primary infection and the secondary infection. Then, when the secondary infection is terminated, the proteins (products) are extremely reduced. This reduction of the proteins (products) after the secondary infection is considered to be because the proteins (products) in the viruses are drastically decomposed by protease (proteolytic or peptidolytic enzyme) secreted from the destructed insect cells. Accordingly, to efficiently produce the proteins (products), it is very important to detect the generation of the secondary infection in an accurate and precise manner.

[0028]

The aforementioned change in the concentration of the carbon dioxide (CO_2) is the result analyzed and measured by the exhaust gas analyzer 3, and is supplied from the exhaust gas analyzer 3 to the culture information-generating device 4 as the measurement signal. The information processing unit 4a of the culture information-generating device 4 is operated on the basis of the culture information-generating program. Thereby, the information processing unit 4a sequentially stores the concentrations of the carbon dioxide (CO_2), which are indicated by the measurement signals input from the exhaust gas analyzer 3 at respective times, in the storage unit 4b as time-series data, and detects

the generation of the secondary infection on the basis of the time-series data, i.e. the concentrations of the carbon dioxide (CO_2) at current and past times, in accordance with the sequence shown in the flowchart of FIG. 2.

[0029]

The information processing unit 4a determines whether or not the seeding occurs after the culture initiation (step S1). If the determination is "Yes," the information processing unit 4a determines whether or not the primary infection occurs (step S2).

Here, the information processing unit 4a determines whether or not a lapsed time (clocking time of a timer) after the culture initiation matches a preset virus feed time by setting, for instance, an internal timer, thereby determining the occurrence of the seeding. Then, the information processing unit 4a checks on the basis of the time-series data whether or not the concentration of the carbon dioxide (CO_2) after seeding is changed from a rising curve into a falling curve, thereby determining the occurrence of the primary infection.

[0030]

Then, if the determination of step S2 is "Yes," the information processing unit 4a determines whether or not the secondary infection occurs (step S3). That is, the information processing unit 4a checks on the basis of the time-series data whether or not the concentration of the carbon dioxide (CO_2) after the primary infection has again risen and then fallen, thereby determining the occurrence of the secondary infection. If the occurrence of the secondary infection is detected in this way, the information processing unit 4a generates culture information indicating the occurrence of the secondary infection, and outputs the generated culture information to the outside and to the culture tank 1 (step S4). When the culture information is input from the culture information-generating device 4, the culture tank 1 rapidly terminates the culture to

prevent decomposition of proteins (products).

[0031]

In this manner, the information processing unit 4a of the culture information-generating device 4 is operated on the basis of the culture information-generating program, thereby automatically detecting the occurrence of the secondary infection on the basis of the time-series data associated with the concentration of the carbon dioxide (CO_2), which is one component of the exhaust gas exhausted from the culture tank 1, in an on-line state. Accordingly, according to the virus culture device A, it is possible to completely resolve the problems occurring in the related art, and to timely evaluate the culture conditions of the virus. Furthermore, it is possible to provide a technique which evaluates the culture conditions of the virus and the objectivity of which is improved compared to that of the related art.

[0032]

The present invention is not limited to the embodiment. For example, the following modifications may be considered.

(1) In the embodiment, the culture information-generating device 4 generates the information indicating the occurrence of the secondary infection as the culture information. However, in the present invention, the culture information is not limited to this information. For example, the culture information-generating device 4 may be configured to generate a trend graph representing the change in the concentration of the carbon dioxide (CO_2) as shown in FIG. 3, and to output the trend graph as the culture information to the built-in or separately provided display device and/or the culture tank 1. When this trend graph is generated as the culture information, a worker determines on the basis of this trend graph whether or not the secondary infection occurs, and takes predetermined procedure for terminating the culture in the culture tank 1.

[0033]

(2) As shown in FIG. 2, after the occurrence of the seeding is determined, it is determined whether or not the primary infection (generation of the first maximum value) and the secondary infection (generation of the second maximum value) occur. However, the determination associated with the occurrence of the seeding may be omitted. That is, the number of times the maximum value of concentration of generated gas, which is a parameter for deciding the termination of the culture, is not limited to two times as in the embodiment, and may be a predetermined number of times other than two.

[0034]

(3) The change in the concentration of the carbon dioxide (CO_2) as shown in FIG. 2 is measured for a specific insect cell and a specific virus. However, the primary infection and the secondary infection are similarly generated from a variety of viruses and animal cells other than an insect cell. As such, the first maximum value and the second maximum value are considered to be generated independently of a relation between a virus and a cell, such as an insect cell, that serves as nutrition for the virus. Accordingly, the present invention is not particularly limited to the relation between the virus and the cell that serves as nutrition for the virus.

[0035]

(4) In the embodiment, when the culture information indicating the occurrence of the secondary infection is input from the culture information-generating device 4, the culture in the culture tank 1 is terminated. The control of the culture conditions of the virus based on the culture information of the culture tank 1 is not limited to this termination control of the culture. A control other than the termination control of the culture may be carried out on the basis of a variety of culture conditions which are sequentially supplied from the culture information-generating device 4 to the culture tank

1. For example, the culture information-generating device 4 may generate the culture information indicating the primary infection (generation of the first maximum value) and provide the generated culture information to the culture tank 1, and the culture tank 1 may modify the culture conditions on the basis of the received culture information.

[0036]

(5) In the embodiment, the culture information is generated on the basis of the concentration of the carbon dioxide (CO_2) of the exhaust gas exhausted from the culture tank 1. The present invention is not limited to this configuration. The concentration of a component other than the carbon dioxide (CO_2) may be measured as long as the component is a gas component representing a special change in concentration in the event of the primary infection and/or the secondary infection.

Industrial Applicability

[0037]

According to the culture information-generating device, the culture device and the culture method of the present invention, it is possible to timely evaluate the culture conditions of the virus, and to provide a technique of evaluating the culture conditions of the virus whose objectivity is improved compared to that of the related art. Further, it is possible to prevent the culture tank from being contaminated with bacteria.

Reference Signs List

[0038]

A: virus culture device

1: culture tank

2: virus feeder

3: exhaust gas analyzer

4: culture information-generating device

4a: information processing unit

4b: storage unit

CLAIMS

1. A culture information-generating device that inputs a measurement signal indicating a change in concentration of a target component contained in carbon dioxide (CO2) exhausted from a culture tank holding a virus-containing culture medium, and performs predetermined information processing on the measurement signal, thereby generating culture information indicating culture conditions of a virus

wherein, when the virus is seeded and cultured in a predetermined cell that serves as nutrition for the virus in the culture tank, the generated culture information indicates that a maximum value of the concentration of the generated gas is generated a predetermined number of times.

2. A culture device comprising:

a culture tank in which a virus is cultured;

an exhaust gas analyzer that analyzes and measures an exhaust gas exhausted from the culture tank and outputs a measurement signal indicating the measured result; and

the culture information-generating device set forth in claim 1.

3. The culture device according to claim 2, wherein the culture tank terminates culture of the virus based on the culture information.

4. A culture method in which culture of a virus is terminated based on a change in concentration of a target component of carbon dioxide (CO2) generated by culturing the virus

wherein, when the virus is seeded and cultured in a predetermined cell that serves as nutrition for the virus, culture of the virus is terminated when a maximum value of the concentration of the generated gas is generated a predetermined number of times.