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Complex of technical tools for fish cells cultivation

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Abstract. The solution of problems of viral diseases of fishes which are quite often arising in an aquaculture requires holding diagnostic actions with use of cell cultures, at the same time creation of new cell lines will allow to expand researches as much as possible. Technological means for simplification and mechanization of preparation of cellular suspension and also for large-scale cultivation of the continuous cell cultures in a monolayer are presented in this article. The offered devices are intended for laboratory or for industrial use as a part of technology park for the solution of tasks in the field of bionanotechnology.

1. Introduction

Aquaculture is recognized in the world practice as the most powerful economic factor affecting the state of the world's economies, ensuring food independence and saturation of the domestic market, as well as increasing employment and increasing export earnings [1]. Successful development of aquaculture is largely determined by effective scientific support of the entire complex of breeding, growing and processing of hydrobionts. The problem of infectious diseases of hydrobionts arising during cultivation in connection with the inevitable intensification of the breeding processes can be solved by timely diagnostic, prophylactic, anti-epizootic measures and vaccination of planting material, with appropriate financial support for this process [2].

Currently, in diagnostic studies and in the isolation of viruses, the main laboratory cell models are transplantable cell lines due to the undoubted convenience of their use, stability, characterized properties and some other advantages [3, 4, 5]. The availability of modern equipment makes it possible to obtain various cell lines and use them for diagnosis, as well as for solving a number of other problems [6].

2. Technical solutions to obtain monolayer cell cultures

To mechanize and simplify the fish tissue trypsinization, a device representing a trypsinization vessel placed into a water bath with an electrical motor-driven mixer connected with the vessel for trypsin solution via tubing and with a flask for disaggregated tissue placed into ice where underpressure is created by means of a vacuum pump.

This technology ensures fast cell outflow irrespective of the number of suction done and weight of the loaded tissues, which contributes to a significant increase in the output of trypsinized tissues and the entire process becomes much more mechanized and simplified.

Figure 1a schematically shows a device consisting of a cylinder with automatic temperature control placed into a water bath with a cotton filter and a mixer driven from an electric motor and a vessel for trypsin solution, a flask for disaggregated tissues placed into a vessel containing ice and a vacuum pump with an electric drive.



Figure 1b shows a cylinder being the main part of the device whose lower part contains glass beads used as a filter; the cylinder throat is closed with a plug through which a mixer rod and a filter tube are passed. The cylinder's upper branch is connected with a rubber tube through a siphon with a vessel for trypsinized tissue, the second siphon tube is connected with a rubber cylinder to inject the trypsin solution. The cylinder's lower branch is connected with a rubber tube with a flask for trypsinized tissue placed in ice, the flask is also connected with the vacuum pump.

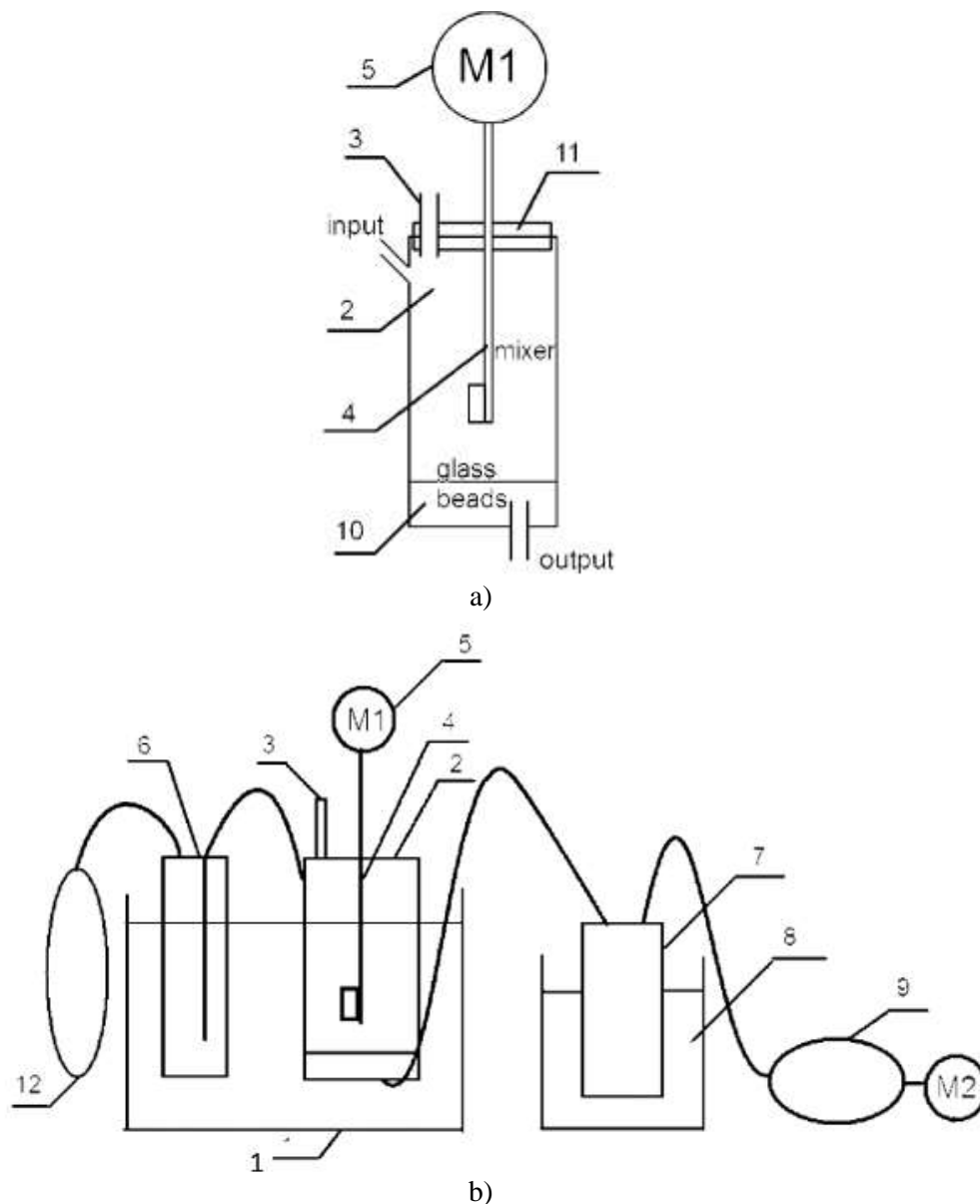


Figure 1. Components of the trypsinization device and cylinder being the main part of the device: 1 - water bath; 2 - cylinder; 3 - cotton filter; 4 - mixer; 5 - electrical motor; 6 - vessel for trypsin solution; 7 - flask for disaggregated tissue; 8 - ice vessel; 9 - vacuum pump with electrical drive; 10 - glass beads; 11 - plug; 12 - cylinder to inject trypsin solution.

During operation, the tissue to be disaggregated, after being weighed in a phosphate-buffer saline, is placed into the cylinder, then the trypsin solution is supplied using a rubber cylinder and the mixer is turned on, the resuspended tissue is sucked through the flask going through the filter made of glass

beads. Depending on the water bath size, several cylinders can be placed in it simultaneously, whose mixers are driven for rotation from a single electrical motor.

To achieve higher mechanization of the production process of tissue trypsinization during mass production of cell tissues, it is recommended to use a device with a friction-magnetic drive, a heater, a relay and a contact thermometer as well as a chamber with an evaporator. In this case, automatic temperature control in the water bath is done using tubular heaters, contact thermometers and relays. Instead of an ice vessel, a cooling chamber is used where temperature is maintained and automatically controlled using evaporators located on the sides of the chamber, contact thermometers and relays. Temperature in the water bath is controlled within 0 to 21°C, and 0 to 10°C in the cooling chamber. It is possible to place friction magnetic mixers under water baths, which are driven using an electrical motor through a friction drive on the horizontal shaft where rubber rollers move using levers, above which steel disks are located that are driven by rollers for rotation. Magnets connected with disks through vertical axes start rotating, which drives cylindrical magnets in a fluoroplastic shell placed into trypsinization flasks. The rotation rate of magnetic mixers is adjusted within 100-500 rpm.

3. Technical solutions for large-scale cultivation of single-layer cellular cultures

To cultivate cells in the production of anti-virus vaccines, special devices are used with an increased growth surface formed with various structural elements representing a tightly sealed vessel with a gas duct for aeration and multiple flat elements for culture growth located above each other. Cells in such devices grow on plates placed into a culture medium the vessel is filled with. For culture medium circulation and its saturation with oxygen, magnetic mixers, pumps and air lift are used.

In various versions, the device for cultivation of cellular cultures in a monolayer always has a tight vessel, since all cultural processes are done in aseptic and antiseptic conditions. The growth surface can be done as cuvettes having curvilinear coupling of walls with the bottom ensuring that the cultural medium gets to underlying cuvettes when supplied into upper cuvettes by overflowing the walls, or a rotor having the growth surface as a single-cut full-wall screw with a continuous surface.

The device for cultivation of cells in a monolayer (figure 2) contains a glass vessel fixed between two stainless steel discs using connection bolts and sealed with sealing gaskets; a rotor installed in the vessel and having the growth surface made as a single-cut full-wall screw with a continuous surface, and tubes to supply and remove the culture medium and gas. The rotor is installed on the shaft, the rotor drive comprises an electrical drive, a magnetic coupling whose semi-couplings are installed on rotor and electrical motor shafts using nuts. The rotor shaft is installed in self-lubricated bearings; the disks have legs to place the device in upright position.

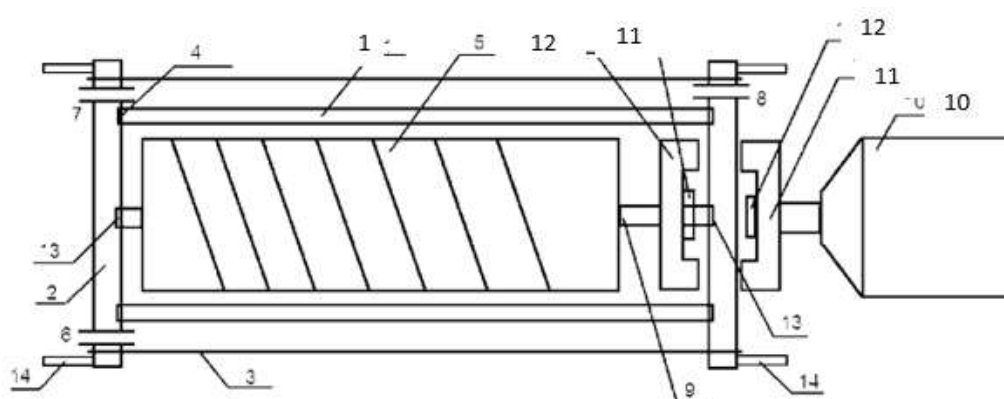


Figure 2. Device for cell cultivation in a monolayer: 1 - glass vessel; 2 - disks; 3 - connection bolts; 4 - sealing gaskets; 5 - rotor; 6,7,8 - tubes to supply and remove culture medium and gas; 9 - shaft; 10 - electrical motor; 11 - magnetic coupling; 12 - nuts; 13 - bearings; 14 - legs.

The device operates as follows: a 4L vessel is filled via tube 6 with a suspension of primary trypsinized cells in the IGLA culture medium saturated with 10% serum of cattle embryos. A horizontally position devices is rotated for 30 minutes at 4 rpm to evenly distribute the inoculating cell-rich fluid. The device is placed upright, by placing 35 onto legs 14, and after attaching cells to the surface of screw 5, the device is rotated by 180 degrees to attach cells to the opposite side of the screw surface, and then half of the culture medium is removed from the device through tube 6 to a backup bottle to collect the culture medium. The cultivation is done with the device in horizontal position in the thermostat at the required temperature depending on the type of fish, cell donors. The shaft speed is set to 96 rpm.

The culture is aerated through tube 7 with the mixture of 5% CO and 95% air. Every day, some part of the culture medium is replaced though tube 6 with a new one from the bottle collecting the culture medium. In 7-8 days, cells form a monolayer. 10X cell growth occurs in the device.

The device is used to grow monolayer cellular cultures and further cultivation of viruses. 100% use of the growth surface is used in the device, since, when placing, cells are evenly attached to it.

By changing the pitch of turns of screw 5, the growth surface can be increased or decreased in the same device and the mixing hydrodynamics can be changed in the device, which makes it more or less intensive. Screw rotation creates directional movement of both liquid and gas phases in the device, which promotes effective mixing and allows for the process to be carried out in homogeneous conditions in case of intensive mass exchange. During cultivation, the device is introduced with the setting cellular suspension that is evenly distributed along the entire device volume, the cells are not concentrated at the entrance, and mixing prevents sticking of individual cells into conglomerates. In these conditions, the cells evenly cover the growth surface when settled. Portions of liquid and gas introduced into the device are not localized at the entrance but distributed along the entire volume of the mixed liquid or gas, and all cells are supplied with the required components equally. Equal concentration of nutritional substances in the liquid and equal proportion of components in the gas phase along the entire volume of the device created by screw rotation allows using methods of automatic control and process adjustment. Similar devices are made in laboratory, semi-industrial or industrial versions.

4. Conclusion

A device developed to mechanize and simplify the fish tissue trypsinization is made as a trypsinization vessel placed into a water bath with an electrical motor-driven mixer connected with the vessel for trypsin solution via tubing and with a flask for disaggregated tissue placed into ice where underpressure is created by means of a vacuum pump. Using glass beads in the lower part of the vessel as a filter ensures a more intensive outflow of cells. A device for cell cultivation in the monolayer in industrial and semi-industrial conditions contains a sealed vessel and a rotor installed in it having an increased growth surface, which improves growth conditions and increases the yield of viable cells.

By means of the developed technical means perhaps further improvement of methods of cultivation, obtaining the new cell cultures having high sensitivity to viruses, creation of new cell systems, development and modernization of science and technology park for implementation of innovative projects in the field of nanotechnologies.

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