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[54]	Title:	A PROCESS TO REDUCE OR ELIMINATE BACTERIA IN LIVE BIVALVE MOLLUSK	
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[57]	Abstract:	This invention provides a less complicated way of eliminating bacteria in live mussels, whereby decontamination is achieved by placing live mussels into tanks with treated filtered seawater and applying recirculating depuration system. This is done by having a shorter time of 18 to 24 hours per batch/cycle. The same seawater may be used for two cycles. Through this invention, small scale aquaculture farming of mussels can be done consistent with industry safety standards.	

A PROCESS TO REDUCE OR ELIMINATE BACTERIA IN MUSSELS

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TECHNICAL FIELD OF THE INVENTION

This invention relates to a method of treating and packaging live bivalve mollusk
5 and more particularly to depuration protocol.

BACKGROUND OF THE INVENTION

There are various ways of reducing or eliminating bacteria in live mussels. One
such technology is taught by EP3231282A1 ('282), which provides a method of
10 treating and packaging live bivalve mollusk (LBM), in particular mussels,
characterized by reducing, preferably before packaging, the number of
microorganisms inside the live bivalve mollusk to less than 1000 colony forming
units (CFU) per gram. Specifically, '282 discloses one or more of these steps -
depuration, cleaning, cooling, buffering, and/or packaging - in at least
15 substantially sterile water was found to be an effective way of reducing the
number of micro-organisms in and on the live bivalve mollusk.

In an embodiment the water is purified by means of at least one of UV radiation,
preferably pulsed UV radiation, ozonation, (electrochemical) chlorination, (cold)
plasma treatment or (hydrogen) peroxide treatment.

20 In a further embodiment of '282, the UV radiation has a power of at least 20
mJ/cm², preferably at least 30 mJ/cm², preferably at least 35 mJ/cm², preferably
a power in a range from 20 mJ/cm² to 100 mJ/cm².

In another embodiment of '282, before UV purification, particles, such as silt, are
removed from the water. In a refinement, the particles are removed by means of
25 a filter, for instance a sieve, preferably a bow screen sieve.

In another embodiment of '282, before UV purification, the water is subjected to skimming, in particular protein skimming, e.g. using an Emyg skimmer or similar skimming apparatus.

5 In another embodiment of '282, before UV purification, the UV transmittance of the water is increased to at least 75%, preferably at least 80%.

Lowering and maintaining the number of micro-organisms below the CFU values specified above requires efficacious and consistent measures, preferably a cascade of such measures, preferred examples of which are mentioned above. E.g., filtering and increasing the UV transmittance of the water followed by
10 (pulsed) high power UV treatment appeared an effective way of reaching a sufficiently low CFU value.

In an embodiment of '282, the water is purified to at least a 5 log, preferably at least a 6 log reduction of bacteria and/or at least a 3 log, preferably at least a 4 log reduction of viruses.

15 In another embodiment of '282, to preserve the obtained increase of shelf life, the live bivalve mollusk are cooled to and packaged at a temperature in a range from -1 to 5 °C, preferably from 0 to 4 °C, preferably from 0,5 to 3 °C.

In another embodiment of '282, the method comprises the step of transferring the live bivalve mollusk to a package, e.g. a tray of a thermoplastic material, creating
20 a subatmospheric pressure in the package, preferably a subatmospheric of more than 200 mbar, preferably more than 300, preferably more than 400 mbar below atmospheric pressure, and sealing the package.

The invention also relates to a package, e.g. a sealed tray or a hessian bag, containing live bivalve mollusk, in particular mussels, characterized in that the

number of microorganisms inside the live bivalve mollusk is less than 1000 colony forming units (CFU) per gram.

In an embodiment '282, the number of microorganisms inside the live bivalve mollusk bivalves in the package is less than 800, preferably less than 600, preferably less than 500, preferably less than 400, preferably less than 300, preferably less than 200 colony forming units (CFU) per gram.

Within the framework of the present invention, Colony Forming Units or CFU inside the live bivalve mollusk is measured in accordance with NEN-EN-ISO 4833-1:2013. The number of microorganisms in the water surrounding the live bivalve mollusk (used e.g. for depuration, cleaning, cooling, buffering, and/or packaging) is measured in accordance with NEN-EN-ISO 6222:1999. In practice, CFU is also referred to as colony count or culturable micro-organisms.

These embodiments of '282, however, teach complicated processes in removing the bacteria from LBM. Thus, there is a need to provide a more economically feasible processes and apparatuses for cleaning LBMs that can be used by small scale aqua farmers, particularly in developing countries.

SUMMARY OF THE INVENTION

This invention provides a less complicated way of eliminating bacteria in live mussels, whereby decontamination is achieved by placing live mussels into tanks with treated filtered seawater and applying recirculating depuration system. This is done by having a shorter time of 18 to 24 hours per batch/cycle. The same seawater may be used for two cycles.

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a schematic diagram of the invention.

Figures 2a and 2b show photographs of the tanks used in the process disclosed by this invention.

5 **DETAILED DESCRIPTION OF THE INVENTION**

Harvesting of mussel has to be done during high tide and not after the heavy rain when effluents from the coastal areas containing contaminants may be high.

10 ***Pre-depuration protocol***

Mussels intended for depuration are kept alive during harvest and transported to the depuration facility. Live mussel samples are harvested and cleaned on-site by removing sediments and other foreign materials attached to the shell valves and washed with clean seawater. During cleaning, byssus are kept intact to ensure
15 that mussels are transported live. Samples are placed in clean large plastic container (LDPE) and stored inside the styropore boxes layered with ice at the bottom observing the 1:10 ratio (ice to mussel ratio), keeping the temperature of 18-21°C and not exceeding the optimum temperature level of their natural habitat. Care should be taken that mussels do not directly come in contact with
20 ice during transport to keep them alive. A layer of plastic bag is placed between the ice and live mussel. During transport, mussels are sprayed with chilled seawater (the top portion) to prevent stress to the organism which may induce spawning and/or lead to high mortality rate.

25 Upon arrival in the depuration facility, cleaning of mussels is done by lightly scrubbing the outer shells, if needed, to remove the mud, attached barnacles and other foreign materials. Filtered seawater is used in washing the mussels. Tap water should never be used for washing as it may result to mortality of the mussels. It is recommended that mussels have to be acclimatized in filtered

seawater for 30 minutes before depuration. Any floating or dead mussels have to be removed.

Depuration Protocol

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Depuration makes use of fiberglass tanks with large recirculating filtration system. Recirculating depuration facility. Figure 1 provides the system that requires installation of fiberglass depuration tanks with desired capacity, filtered seawater reservoir, filtration system (sand and carbon), UV sterilizer, pumps and housing facility. This system could reduce or eliminate bacterial load at a shorter time of 10 18-24 hours per batch/cycle. The same seawater may be used for two cycles. Figure 2a and 2b shows the fabricated recirculating system having a maximum capacity of 120 kg of live mussel per tank which requires approximately 900-1000L of UV-treated filtered seawater can depurate live mussel for 18-24 hours.

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It is important to maintain the seawater parameters like dissolved oxygen of at least 5mg/L and turbidity of less than or equal to 15 NTU during the process to keep the mussels alive and continue its metabolic activity.

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The preferred embodiment of this invention is described in the above-mentioned detailed description. It is understood that those skilled in the art may conceive modifications and/or variations to the embodiment shown and described therein. Any such modifications or variations that fall within the purview of this description are intended to be included therein as well. Unless specifically noted, it is the 25 intention of the inventors that the words and phrases in the specification and claims be given the ordinary and accustomed meanings to those of ordinary skill in the applicable art. The foregoing description of a preferred embodiment and best mode of the invention known to the applicant at the time of filing the application has been presented and is intended for the purposes of illustration

and description. It is not intended to be exhaustive or to limit the present invention to the precise form disclosed, and many modifications and variations are possible in the light of the above teachings.

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- j. cleaning of mussels is done by lightly scrubbing the outer shells upon arrival in the depuration facility to remove the mud, attached barnacles and other foreign materials using filtered seawater;
 - k. acclimatizing mussels in filtered seawater for 30 minutes before depuration; and
 - l. removing of any floating or dead mussels.
3. The method according to Claim 1, wherein the depuration protocol comprises the steps of:
- a. making use of fiberglass tanks with large recirculating filtration system;
 - b. making use of the same seawater for two cycles;
 - c. using 120 kg of live mussel per tank with approximately 900-1000L of UV-treated filtered seawater for 18-24 hours; and
 - d. maintaining the seawater parameters like dissolved oxygen of at least 5mg/L and turbidity of less than or equal to 15 NTU during the process to keep the mussels alive and continue its metabolic activity.

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CLAIMS

- 5 1. A method of removing or eliminating bacteria from live bivalve mollusks comprising the steps of:
- a. conducting a pre-depuration protocol; and
 - b. conducting a depuration protocol.
- 10 2. The method according to Claim 1, wherein the pre-depuration protocol comprises the steps of:
- a. harvesting of mussels during high tide;
 - b. keeping alive the mussels during harvest and transported to the depuration facility;
 - c. cleaning the mussels on-site by removing sediments and other foreign materials attached to the shell valves and washing with clean seawater;
 - 15 d. keeping intact the byssus to ensure that mussels are transported live;
 - e. placing the live mussels in clean large plastic container (LDPE) and storing inside the styropore boxes layered with ice at the bottom observing the 1:10 ratio (ice to mussel ratio);
 - 20 f. keeping the temperature of 18-21°C;
 - g. maintaining the optimum temperature level of their natural habitat of mussels;
 - 25 h. keeping mussels away from direct contact with ice during transport by providing a layer of plastic bag between the ice and live mussel;
 - i. spraying mussels with chilled seawater (the top portion) during transport to prevent stress to the organism which may induce spawning and/or lead to high mortality rate;

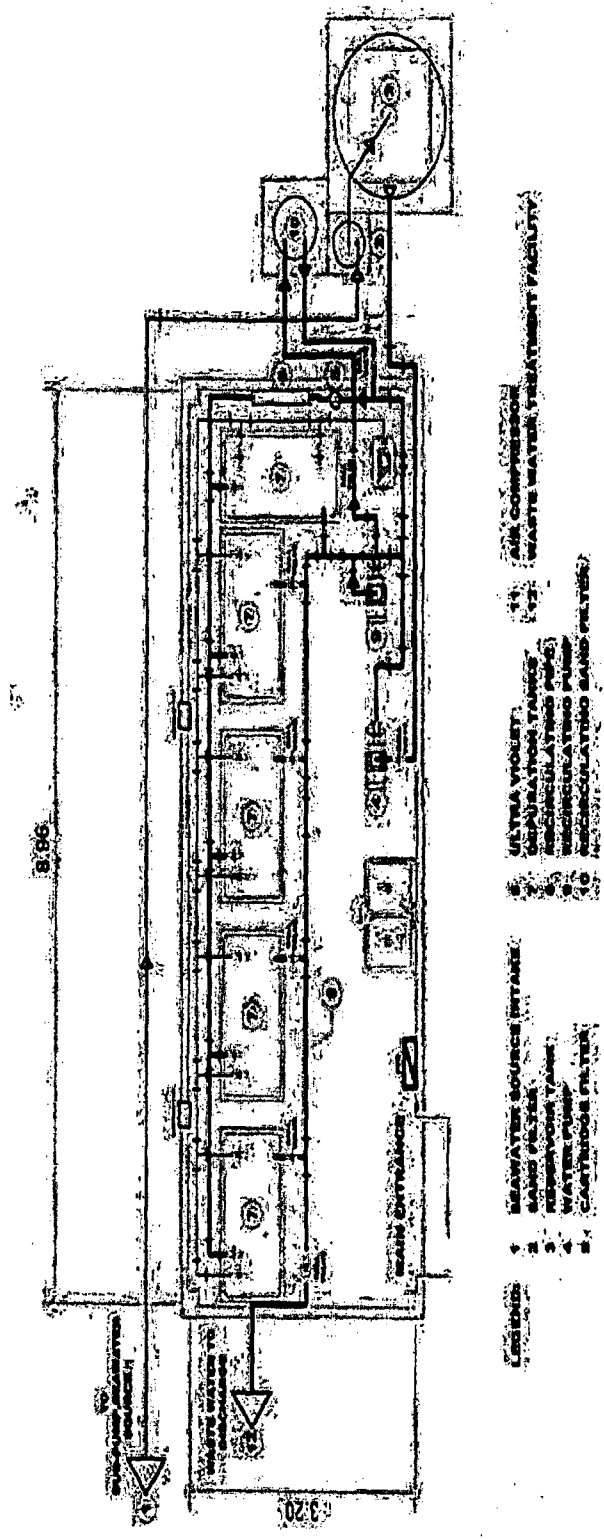


FIGURE 1

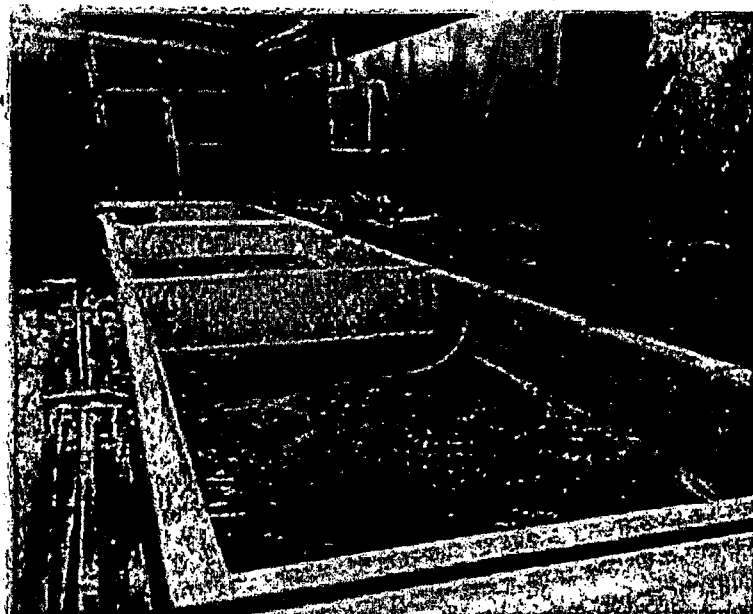


FIGURE 2A

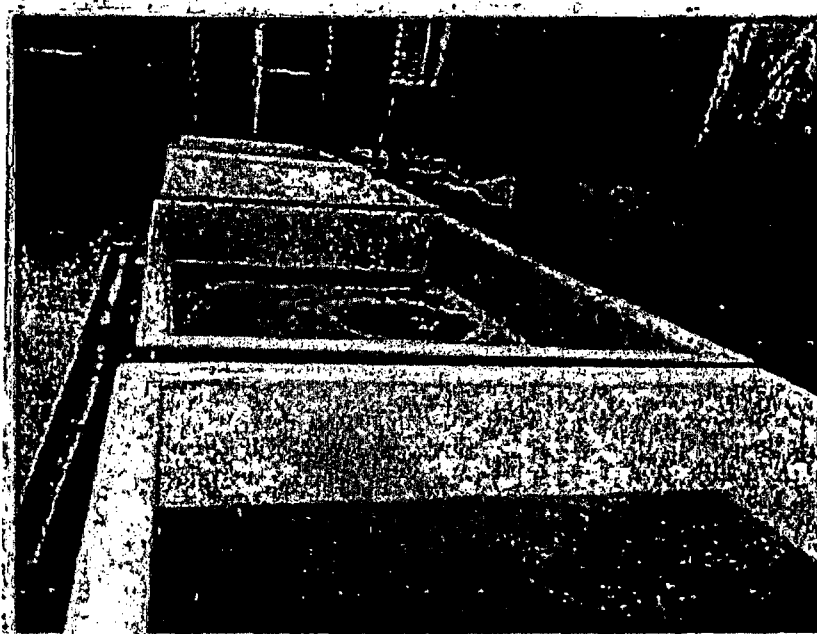


FIGURE 2B

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