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(71) Applicant: **BIOMAR GROUP A/S** [DK/DK]; Værkmestergade 25, 8000 Aarhus C (DK).

(72) Inventors: **DETHLEFSEN, Markus Wied**; Violinvej 32, 2730 Herlev (DK). **HJERMITSLEV, Niels**; Holger Danskes Vej 8, 8700 Horsens (DK).

(74) Agent: **PLOUGMANN VINGTOFT A/S**; Rued Langgaards Vej 8, 2300 Copenhagen S (DK).

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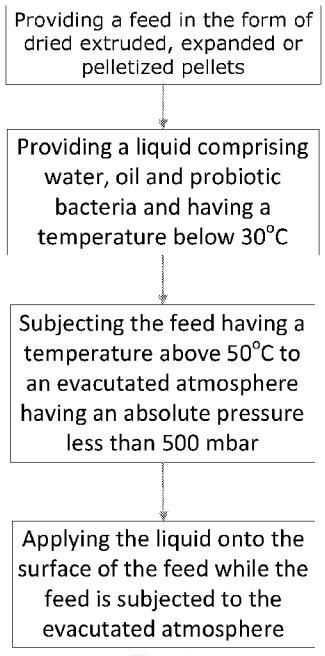

Fig. 1

(57) Abstract: The present invention relates to a method for producing a feed containing probiotic bacteria, the method comprising providing a feed granular having a temperature above 50 °C; providing a probiotic liquid comprising water, oil and probiotic bacteria, and having a temperature below 30 °C; subjecting said feed having a temperatures above 50 °C to an evacuated atmosphere having an absolute pressure less than 500 mbar, and while the feed is subjected to said evacuated atmosphere, applying said probiotic liquid onto the surface of the granular feed.

METHOD AND DEVICE FOR PRODUCING A FISH FEED CONTAINING PROBIOTIC BACTERIA

FIELD OF THE INVENTION

The present invention relates to a method for producing a feed containing probiotic bacteria, the method comprising providing a feed granular having a temperature above 50 °C; providing a probiotic liquid comprising water, oil and probiotic bacteria, and having a temperature below 30 °C; subjecting said feed having a temperatures above 50 °C to an evacuated atmosphere having an absolute pressure less than 500 mbar, and while the feed is subjected to said evacuated atmosphere, applying said probiotic liquid onto the surface of the granular feed.

The invention also relates to a device for applying bacteria to feed granulates, the device comprising an application chamber being adapted to provide and maintain an evacuated atmosphere having an absolute pressure less than 500 mbar; a fluid applicator adapted to sprinkle or spray a liquid into the interior of the application chamber; a feed granulate inlet and a feed granulate outlet.

BACKGROUND OF THE INVENTION

Fish feed containing probiotic bacteria is found to be useful in connection with fish farming e.g. in order to increase and/or maintain a desired health condition of the fish.

Fish feed is often produced in manners where the fish feed and/or the raw materials experience(s) a temperature that high that it has a lethal impact on the bacteria. Thus, it has been found that the most efficient way of producing a fish feed containing e.g. probiotic bacteria is to apply the probiotic bacteria as a subsequent step after the fish feed has otherwise been made ready for consumption by the fish. This process involves that the ready for consumption fish feed is cooled to a temperature below the temperature above which the bacteria would be inactivated. Such a cooling process is typically carried out by use of cooling devices which extract heat from the fish feed and such cooling device represents substantial costs both with respect to installation and energy

consumption during use. Additionally, the available space in existing process environments can be a limitation as well. Thus, a cooling process increases the costs for producing such fish feed substantially.

Hence, an improved method and apparatus of providing a fish feed with probiotic bacteria would be advantageous, and in particular a more efficient and/or reliable method and apparatus for providing such a fish feed would be advantageous.

OBJECT OF THE INVENTION

An object of the present invention is to provide an alternative to the prior art.

In particular, it may be seen as a further object of the present invention to provide a method and apparatus that solves the above mentioned problems of the prior art with high losses of the probiotic bacteria.

SUMMARY OF THE INVENTION

Thus, the above described object and several other objects are intended to be obtained in a first aspect of the invention by providing a method for producing a feed, preferably a fish feed, containing probiotic bacteria, the method comprising providing a feed granular having a temperature above 50 °C; providing a probiotic liquid comprising water and/or oil and probiotic bacteria, and having a temperature below 30 °C; subjecting said feed having a temperatures above 50 °C to an evacuated atmosphere having an absolute pressure less than 500 mbar, and while the feed is subjected to said evacuated atmosphere, applying said probiotic liquid onto the surface of the granular feed. While the liquid is applied to the surface of the feed, feed often contains pores with opening in the surface and the liquid may enter into the pores and end up inside the feed.

By the formulation comprising water and/or oil and probiotic bacteria is preferably meant a liquid comprising one of the following combinations:

i) water and probiotic bacteria
ii) oil and probiotic bacteria
iii) water and oil and probiotic bacteria

Each of these combinations may comprise further components, or may consist, such as consist essentially (accounting for impurities, a fluid in which bacteria is in suspension or the like), of the mentioned components (water, oil, probiotic bacteria).

In the present context terms are used as ordinary to a skilled person. However, some of the used terms are elaborated below:

Atmosphere is preferably used to reference gas surrounding the feed granulates or pellets.

Absolute pressure is preferably used to mean the total vapour pressure.

Temperature of feed granulates or pellets is preferably used to reference an average temperature of the pellets, the average temperature may be calculated as:

$$T = \frac{1}{Vol} \int T dvol$$

that is a volumetric average, where $dvol$ is an infinitesimal volume element of the feed and T is the temperature of $dvol$ and Vol is the volume of the granulate or pellet.

Probiotic liquid and probiotic fluid is a live probiotic bacteria mixed into water and/or preferably oil.

Granulate is preferably used to reference a feed material composed of spatial distinguishable pieces such as pellets, granules or other.

Pellet is preferably used to reference a coherent element, which typically is made by a pelletizing process. *Pellets* are typically granular which typically are created by compressing, expanding or extruding a feed material.

Applying, as used e.g. in applying said probiotic liquid onto the surface of the granular feed, is preferably used to reference a process in which the probiotic fluid is broad into contact such as deposited on the surface of the feed granulate.

Sprinkling is typically used to reference a process in which the probiotic liquid is fed through a nozzle to form droplets which are directed towards the surface feed granulate. *Spraying* is typically used to reference a process in which the probiotic liquid is fed through a nozzle to form aerosols which are directed towards the surface of the feed granulate.

Agitate is preferably used to reference a process in which the granulates or pellets are moved around inside a void.

In some preferred embodiments, the feed granulate may be pellets which may be provided by an extrusion, an expansion or a pelletizing process.

In some preferred embodiments, the method may further comprise agitating the feed while the liquid is applied onto the surface of the feed.

In some preferred embodiments, the probiotic liquid applied to the surface of the granulates may be applied by sprinkling or spraying.

In some preferred embodiments, the temperature of said probiotic liquid while being applied onto the surface of the feed may be below 25 °C, such as below 20 °C, preferably below 15 °C, such as below 10 °C, preferably below 5 °C.

In some preferred embodiment, the probiotic liquid contains at least 0.4 vol %, such as at least 0.8 vol %, preferably at least 1.2 vol % water, and at least 2 vol %, such as at least 3 vol %, preferably at least 4 vol %, such as at least 10 vol %, preferably at least 20 vol %, such as at least 30 vol %, and even at least 35 vol % oil.

In some preferred embodiments, the amount of water in a probiotic liquid containing oil is less than 5 vol %, such as less 4 vol % preferably less than 3 vol %, such as less than 2.5 vol %, preferably less than 2 vol %, such as less than

1.5 vol %. In further embodiments, the amount of water in a probiotic liquid containing oil is between 0.25 vol % and 5 vol %, such as between 0.5 vol % and 3 vol %.

In some preferred embodiments, the probiotic liquid may contain water in an amount between 0.5 to 3 vol %, probiotic bacteria in an amount between 0.2 to 3 vol %, or between 0.1 to 2 vol % and the remaining amount of the probiotic liquid being oil.

In some preferred embodiments, the probiotic liquid consists, such as consists essentially (e.g. includes impurities), of water, oil and probiotic bacteria and preferably, the amount of water is at least 0.4 vol % water, such as at least 0.8 vol % water, preferably at least 1.2 vol % water, such as at least 2 vol % water, preferably at least 3 vol %, the amount of probiotic bacteria being less than 3 vol %, such as less than 2.5 vol %, such as less than 2 vol %, preferably less than 1.5 vol %, and the remaining fraction of the probiotic liquid being oil.

In some preferred embodiments, the oil may preferably be a vegetable oil preferably selected from the group consisting of rape seed oil, soya oil, palm oil, olive oil, sunflower oil, or an animal oil, such as a fish oil or a combination of one or more of said oils.

In some preferred embodiments, the probiotic liquid may preferably contain less than 25 grams, preferably less than 18 grams, such as less than 12 grams, preferably less than 1 grams of bacteria per kg granulates.

In some preferred embodiments, the bacteria (probiotic bacteria) may be selected from the group consisting of *Pediococcus acidilactici* or other live probiotic, such as live probiotic as used in aquaculture feed, such as *Bacillus* species.

In some preferred embodiments, the pressure of the evacuated atmosphere may be less than 400 mbar, such as less than 300 mbar, preferably less than 200 mbar, such as less than 100 mbar, preferably less than 50 mbar.

In some preferred embodiments, the steps of subjecting said feed to an evacuated atmosphere, and applying the liquid onto the surface of the feed may be carried out on a batch of feed granulates. In such, and other embodiments, the method may comprise introducing a batch of feed granulates into an application chamber configured for providing the evacuated atmosphere inside the chamber and sprinkling and/or spraying said probiotic liquid onto the batch of feed granulates while the feed granulates are agitated.

In some preferred embodiments, the feed granulates may have a water content of less than 25 vol %, such as less than 20 vol %, preferably less than 15 vol % as determined immediately prior to being subjected to application of the probiotic liquid.

In some preferred embodiments, the step of providing a probiotic liquid may comprise:

- mixing water and/or oil and probiotic bacteria, preferably in a mixing chamber, preferably at atmospheric pressure, such as in region of 1 bar, so as to provide said probiotic liquid prior to applying said probiotic liquid onto the surface of the granulate feed,

wherein the temperature of the mixed water and/or oil and probiotic bacteria preferably is below 30 °C.

In some preferred embodiments, the step of providing a probiotic liquid comprising water and/or oil and probiotic bacteria, and preferably having a temperature below 30 °C may comprise:

- mixing a liquid comprising water and/or oil having a temperature less than 30 °C, such as less 25 °C, preferably below 20 °C, such as below 15 °C, preferably below 10 °C, such as below 5 °C with a probiotic bacteria in a mixing chamber at atmospheric pressure, such as in region of 1 bar, so as to provide said probiotic liquid prior to applying said probiotic liquid onto the surface of the granulate feed.

In some preferred embodiments, the feed granulates after said probiotic liquid has been applied to the surface thereof may be cooled, preferably to a temperature less than 45 °C.

The invention relates in a second aspect to a device for applying bacteria to feed granulates, preferably fish feed granulates, the device preferably comprising an application chamber being adapted to provide and maintain an evacuated atmosphere having an absolute pressure less than 500 mbar; a fluid applicator adapted to sprinkle or spray a liquid into the interior of the application chamber; a feed granulate inlet and a feed granulate outlet.

In some preferred embodiments, the device may comprise a pump in fluid connection with the interior of the application chamber for sucking out gas from the application so as to reduce to and/or maintain the pressure preferably at less than 500 mbar in the application chamber.

In some preferred embodiments, the device may comprise an agitator arranged inside the application chamber adapted to agitate the feed granulate while inside the application chamber.

In some preferred embodiments, the device may comprise a mixing chamber adapted to mix oil, water and probiotic bacteria so as to provide a probiotic liquid.

In some preferred embodiments, the feed granulate inlet and the feed granulate outlet may both be closeable, preferably by comprising a shut-off valve, so as to seal the feed granulate inlet and feed granulate outlet.

In a third aspect, the invention relates to a fish comprising granulates being coated with a probiotic liquid containing probiotic bacteria, said fish feed preferably contains less than 25 grams, preferably less than 18 grams, such as less than 12 grams, preferably less than 1 grams of bacteria per kg granulates.

In some preferred embodiments, the probiotic bacteria is selected from the group consisting of *Pediococcus acidilactici* or other live probiotic, such as live probiotic as used in aquaculture feed, such as *Bacillus* species.

In a fourth aspect, the invention relates to a fish feed obtained by or obtainable by a method according to the first aspect of the invention.

The first, second, third and fourth aspect of the present invention may each be combined with each other. These and other aspects of the invention will be apparent from and elucidated with reference to the embodiments described hereinafter as well as in the patent claims.

BRIEF DESCRIPTION OF THE FIGURES

The present invention and in particular preferred embodiments thereof will now be disclosed in greater details with reference to the accompanying figures. The figures disclose ways of implementing the present invention and are not to be construed as being limiting to other possible embodiments falling within the scope of the attached claim set.

Figure 1 is a flow chart illustrating steps preferably carried out for applying bacteria to feed granulates/pellets according to the invention,

Figure 2 is a flow chart illustrating steps preferably carried out in connection with the invention in connection with providing feed granulates/pellets and applying bacteria to feed granulates/pellets according to a preferred embodiment of the invention,

Figure 3 is a schematically illustration of a device for applying bacteria to feed granulates/pellets according to a preferred embodiment of the invention,

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Reference is made to fig. 1 schematically illustrating a method for producing a feed containing probiotic bacteria in a flow chart. As illustrated, the method comprising the steps of:

- providing a feed granular having a temperature above 50 °C
- providing a probiotic liquid comprising water, oil and probiotic bacteria, and having a temperature below 30 °C,
- subjecting said feed having a temperatures above 50 °C to an evacuated atmosphere having an absolute pressure less than 500 mbar, and

- while the feed is subjected to said evacuated atmosphere, applying said probiotic liquid onto the surface of the granular feed.

It is noted that the above sequence does not necessarily have to be followed as for instance the probiotic liquid and the feed granulate may be provided timewise parallel or the probiotic liquid may be provided before the feed granulates are produced. However, once the feed is subjected to the evacuated atmosphere, it is in general preferred to apply the probiotic liquid to the granulates within or keep the granulates at the reduced atmosphere for no longer than 45 minutes, such as maximum 30 minutes, preferably 20 minutes, such as maximum 15 minutes, preferably 10 minutes. This is typically preferred to avoid e.g. leakages of substances from the granulates as a result of a prolonged exposure to an evacuated atmosphere.

Without being bound by theory, it is believed that although the granulates have a surface temperature being well above the temperature at which most bacteria are inactivated, the presence of water in the probiotic liquid in combination with the evacuated atmosphere provides an evaporation of water on the surface granulates which keeps the surface temperature at a sufficient low level to avoid massive decimation of bacteria.

While it is believed that the water will play a role in cooling the surface of granulates, the oil – which also has a low temperature – has a sufficient heat capacity to avoid the surface temperature to reach temperatures being critical to the bacteria's survival after the water has at least substantially evaporated and the feed granulates is left for further cooling at e.g. room temperature (20 °C) prior to be packed into feed containers.

Further, it is generally preferred to avoid subjecting the feed granulates to pressure fluctuations. For instance, the pressure of the evacuated atmosphere should not fluctuate more than 10%.

Often, the feed granulate is pellets which is provided by an extrusion, an expansion or a pelletizing process. Such methods are known to produce a feed having an elevated temperature (above 50 °C) and a feed produced in these

manner may be treated directly by the method according to the present invention – thus, in general there is no need for cooling of the produced feed prior to application of the probiotic liquid.

Fish feed in the form of granulates or pellets has a three dimensional shape and it is preferred to apply the probiotic liquid to all of the outer surface of granulates or pellets (it is noted that a granulate or pellet may comprise pores opening into the outer surface whereby some of the probiotic liquid may go into the pores). To accomplish this, it is preferred that a method according to the invention comprising agitation of the feed while the liquid is applied onto the surface of the feed. The agitation and the amount of probiotic liquid applied are aligned so as to assure that all granulates or pellets receive a substantial equal amount of probiotic liquid. Although it is possible to calculate the amount of probiotic liquid sprayed e.g. per second per m^2 and comparing this with the exposed surface area of granulates, it has been found that it is more efficient to set the operating parameters of an agitator 11 and a liquid applicator 7 (see fig. 3) according to experiments.

The probiotic liquid applied to the surface of the granulates or pellets is applied by sprinkling or spraying.

It is generally preferred in methods according to the present invention that the temperature of said probiotic liquid while being applied onto the surface of the feed is below 25 °C, such as below 20 °C, preferably below 15 °C, such as below 10 °C, preferably below 5 °C. While being applied onto the surface typically refers to the temperature of the liquid as it leaves a liquid applicator, it may as well include that the probiotic liquid is stored at other temperatures prior to being applied, which also means that the probiotic fluid may be heated or cooled prior to being applied.

As presented herein, the probiotic liquid preferably contains a water and/or oil. The amount of water in the probiotic liquids is preferably at least 0.4 vol %, such as at least 0.8 vol %, preferably at least 1.2 vol %, and at least 2 vol %, such as at least 3 vol %, preferably at least 4 vol %, such as at least 10 vol % oil,

preferably at least 20 vol %, such as at least 30 vol %, and even at least 35 vol % oil.

While different types of oils and also mixtures of oils can be used in the connection with the present invention, it is preferred that the oil is a vegetable oil preferably selected from the group consisting of rape seed oil, soya oil, palm oil, olive oil, sunflower oil, or an animal oil, such as a fish oil or a combination of one or more of said oils.

The amount of probiotic bacteria in the probiotic liquid is typically selected so as to obtain a certain and predefined lower amount of probiotic bacteria deposited in/on the feed. This received amount depends on how much of the probiotic liquid that is received by the feed, taking into account that if the probiotic liquid contains a low amount of probiotic bacteria, larger amounts of probiotic liquid may be applied to the feed (and vice versa). However, in preferred embodiments of the invention, the following amounts of probiotic bacteria have proven to produce a useful content of probiotic bacteria in the feed, that is the probiotic liquid contains less than 25 grams, preferably less than 18 grams, such as less than 12 grams, preferably less than 1 grams of bacteria per kg granulates. Within these ranges, the amount of probiotic bacteria applied to the feed is counted determined at the end of the application process.

While the bacteria may in general be selected from live probiotics such as those used in aquaculture feed, the bacteria is preferably selected from the group consisting of *Pediococcus acidilactici* or other live probiotic, such as *Bacillus* species.

The present invention makes use of evacuated atmosphere during application of the probiotic liquid. As presented herein, the reduced pressure alters the boiling point of the water in the probiotic liquid, e.g. to provide an evaporative cooling of the feed granulates or pellets. While it is preferred that the pressure of the evacuated atmosphere may be selected so as to be less than 400 mbar, such as less than 300 mbar, preferably less than 200 mbar, such as less than 100 mbar, preferably less than 50 mbar, it could preferably be selected in due respect to the temperature of the probiotic liquid as if the pressure provides a boiling point being

lower than the temperature of the probiotic liquid, the water may evaporate before the liquid reaches the surface of the feed granulates or pellets, which in general is not preferred.

It is noted that the boiling point of water as presented herein may be influenced by the water being in a mixture with other fluids. Such changes in boiling point may either be determined theoretically or experimentally and the results of such considerations may be taken into account when selecting the pressure of the evacuated atmosphere and/or the temperature of the probiotic liquid.

While a method according to present invention can be implemented in a continuous process where the feed is fed in a continuous manner into an application chamber, it is preferred to implement the steps of subjecting said feed to an evacuated atmosphere, and applying the liquid onto the surface of the feed are carried out on a batch of feed granulates. Thereby, the evacuated atmosphere may be provided in an efficient and controllable manner as it may be carried out in an easy sealable chamber. It is noted that although the probiotic liquid is applied to the surface of the feed, the feed may contain open pores and that some of the probiotic liquid may enter into the pores.

Accordingly a method according to present invention, may preferably comprise the steps of introducing a batch of feed granulates into an application chamber 6 (see fig. 3) configured for providing the evacuated atmosphere inside the chamber and sprinkling and/or spraying said probiotic liquid onto the batch of feed granulates while the feed granulates are agitated. The agitation may be important in order to assure that all the feed granulates or pellets receive probiotic liquid and/or the probiotic liquid is evenly – or at least substantially evenly – distributed on the surface of the feed so that the amount of probiotic bacteria contained on/in different feed granulates or pellets is substantially the same.

The feed granulates or pellets after being produced and prior to be subjected to the application of the probiotic liquid often contains water. Such water may start to boil off when subjected to the evacuated atmosphere (which may be enhanced by the feed having a temperature of above 50 °C). This is in general not preferred as such boiling off may destroy the structure of granulates or pellets. It is in

accordance with this and other objectives preferred that the feed granulates are dried to have – or in general have – a water content of less than 25 vol %, such as less than 20 vol %, preferably less than 15 vol % as determined immediately prior to being subjected to application of the probiotic liquid.

As presented herein, the invention makes use of a probiotic liquid and methods according to the invention may preferably include a process of producing such a probiotic liquid. The probiotic bacteria used in connection with the invention is typically commercially available for purchase. Preferably, the process of producing the probiotic liquid comprises mixing water and/or oil and probiotic bacteria.

The mixing may preferably be carried out in a mixing chamber 1 (see e.g. fig. 3) preferably being at atmospheric pressure, such as in region of 1 bar, so as to provide said probiotic liquid prior to applying said probiotic liquid onto the surface of the granulate feed and – if pores exist – also into the pores of the feed. The mixing is carried out so that the temperature of the mixed water and/or oil and probiotic bacteria is below 30 °C. This often involves that the mixing process is designed so that the bacteria is added to a liquid having a temperature below 30 °C which may be provided by considering the heat capacity and amounts of the liquids mixed and securing the temperatures of the mixed liquid so that the temperature of the mixed liquid is below 30 °C. Alternatively, if e.g. only a single liquid is used for probiotic liquid, the temperature of that liquid is below 30 °C.

In embodiments of the invention, the step of providing a probiotic liquid comprising water and/or oil and probiotic bacteria, and having a temperature below 30 °C comprising mixing a liquid comprising water and/or oil having a temperature less than 30 °C, such as less 25 °C, preferably below 20 °C, such as below 15 °C, preferably below 10 °C, such below 5 °C with a probiotic bacteria in a mixing chamber (1) at atmospheric pressure, such as in region of 1 bar, so as to provide said probiotic liquid prior to applying said probiotic liquid onto the surface of the granulate feed and – if pores exist – also into the pores of the feed.

It is noted, that if the probiotic liquid is provided at pressure conditions being higher – or in general – different from the pressure in the application chamber 6, regulation between the pressure in the mixing chamber 1 and the application

chamber is typically applied. Such a pressure regulation may be accomplished by a valve, such as a shut-off valve, throttle valve, arranged in or in connection with the outlet 5 from mixing chamber 1. Alternatively or in combination thereto, the liquid applicator 7 may be configured to regulate the pressure, e.g. by suitable designing the openings in the liquid applicator 7. Still alternatively or in combination thereto, the probiotic liquid may be provided at a pressure equal to or in the range of the pressure in the application chamber 6.

After the application of the probiotic liquid, the feed granulates may still have a temperature which requires or makes it desirable to cool the feed e.g. prior to being packed into containers. Thus, in some embodiments, the feed granulates after said probiotic liquid has been applied to the surface thereof are cooled, preferably to a temperature less than 45 °C. Such a cooling may be active cooling where the feed is subjected to a cooling facility wherein the temperature of the atmosphere is kept lower than the temperature of the feed, e.g. below 10 °C, by use of cooling elements. Alternatively, the feed is subjected to passive cooling where the feed is cooled by being exposed to normal indoor temperature, e.g. 20 °C, in a production facility.

Reference is made to fig. 3 illustrating schematically a preferred embodiment of a device for applying bacteria to feed granulates, preferably fish feed granulates. The device is illustrated in a cross sectional like view so as to illustrate schematically the interior of the elements of the device.

As illustrated, the device comprising an application chamber 6 being adapted to provide and maintain an evacuated atmosphere having an absolute pressure less than 500 mbar. The application chamber 6 may be in the form of an encapsulation providing an enclosed void. The encapsulation is made so as to withstand the pressure difference between the pressure inside the chamber (less than 500 mbar) and the pressure outside the chamber, which outside pressure typically is atmospheric pressure.

The device further comprising a fluid applicator 7 adapted to sprinkle or spray liquid into the interior of the application chamber 6. As illustrated in fig. 3, the fluid applicator 7 is typically arranged at an upper extremity of the application

chamber 6, and the fluid applicator 7 directs the probiotic liquid towards a lower extremity of the application chamber 6.

Feed to have a probiotic liquid applied enters into the application chamber 6 through a feed granulate inlet 16 and leaves the application chamber 6 through a feed granulate outlet 9. Transport of the feed into, through and out of the application chamber is typically at least assisted by conveyer elements (not shown), such as screw transporters, conveyer belt or the like.

The evacuated atmosphere inside the application chamber 6 is preferably provided by a pump 8 in fluid connection with the interior of the application chamber 6 for sucking out gas from the application so as to reduce to and/or maintain the pressure at less than 500 mbar in the application chamber 6. While the pump in fig. 3 is shown as being located outside the application chamber 6, the pump may be located inside the application chamber 6. The pump 6 may be in the form of a piston pump or any other pump type suitable for reducing the pressure inside the application chamber 6 by sucking out gas from the chamber 6.

It is, as presented above, generally preferred that all feed granulates or pellets receive an equal amount (or substantial equal amount) of probiotic liquid and as the feed when introduced into the application chamber 6 often forms a layer and the liquid applicator 7 is arranged above and supply liquid to the surface of the layer, it may be necessary to agitate the feed to expose substantially all of the feed to the flow of liquid. Accordingly, a device according to the present invention preferably comprises an agitator 11 arranged inside the application chamber 6 which agitator is adapted to agitate the feed granulate or pellets while inside the application chamber 6.

Such an agitator may be in the form of moveable paddles, mixers or other types of mechanical devices suitable for providing a stirring of the feed; it should be noted that a too vigorous stirring could destroy the mechanical integrity of the feed and the agitation should therefore be implemented in a manner seeking to avoid mechanical damaging of the feed.

A device according to the present invention may comprise a mixing chamber 1 adapted to mix water and/or oil and probiotic bacteria so as to provide a probiotic liquid. In the embodiment shown in fig. 3, the mixing chamber has a separate liquid inlet 2 and a separate inlet for bacteria, so as to avoid contact between the liquid and the bacteria before entering into the mixing chamber 1 (the bacteria may be in dry form). The mixing chamber is equipped with an agitator 4 to provide a mixing of the liquid and the bacteria in the mixing chamber 1. The mixing chamber 4 has an outlet 5 connected to the liquid applicator 7. A valve (not shown) may be arranged to control the amount of liquid going into the liquid applicator 7 and – if needed – the liquid may be pressurised (not illustrated) to force the liquid through the liquid applicator 7.

The feed granulate inlet 16 and the feed granulate outlet 9 may both be closeable, preferably by comprising a shut-off valve 15, 13, so as to seal the feed granulate inlet 16 and feed granulate outlet 13.

Experimental results obtained by the present invention

In the following some experimental results obtained by a method and device according to the invention are provided.

The device used is as disclosed in fig. 3 is designed to be used in small scale experiment and can handle up to 25 kg of feed per batch.

The probiotic liquid is provided by mixing bacteria with oil in some trials and by mixing bacteria with oil and water in other trials. The thermodynamic state during the trials as well as details as to composition of probiotic liquid are presented in the below tables.

<u>Mixing bacteria:</u>

Bacteria are mixed into oil, rape seed, soya, fish, palm, olive, sunflower.
Preferable vegetable oil.
Mixed into oil below 30 °C, preferable below 10 °C.

<u>Coating:</u>

Comparative experiments

Hot pellets -> Warm oil with bacteria (Bactocell) + Fish oil -> Coated on and into pellets during continues vacuum release.

Preferred:

Cold pellets -> Cold oil + water with bacteria (Bactocell) -> Coated on and into pellets during deep vacuum.

Results proving the effect:

Pellet temperature before coating: 53 degrees Celsius. 1.1*10^6 added bacteria. RO = Rape seed oil, BC = Bactocell, FO = Fish oil					
Experiment	Temp. of RO+BC	Temp. of FO	P [mbar]	Survived [%]	Added water
1	40	40	600	30.9	0%
2	40	40	400	43.9	0%
3	40	40	200	49.8	0%
4	40	40	100	60.7	0%
5	40	40	200	83.2	2%
6	5	40	200	89.5	0%

These results were used for a validating trial. The setup was identical to the trial covering experiment 1-6:

Experiment	Temp. of RO+BC	Temp. of FO	P [mbar]	Survived [%]	Added water
7	40	40	200	33	0%
8	5	20	50	72	2%
9	40	40	50	24	0%

Although the present invention has been described in connection with the specified embodiments, it should not be construed as being in any way limited to the presented examples. The scope of the present invention is set out by the accompanying claim set. In the context of the claims, the terms "comprising" or "comprises" do not exclude other possible elements or steps. Also, the mentioning of references such as "a" or "an" etc. should not be construed as excluding a plurality. The use of reference signs in the claims with respect to elements indicated in the figures shall also not be construed as limiting the scope of the invention. Furthermore, individual features mentioned in different claims, may possibly be advantageously combined, and the mentioning of these features in different claims does not exclude that a combination of features is not possible and advantageous.

List of reference symbols used

1	Mixing chamber
2	Liquid inlet of mixing chamber
3	Inlet for bacteria to mixing chamber
4	Agitator
5	Outlet from mixing chamber
6	Application chamber
7	Liquid applicator, such as a sprinkler or sprayer
8	Pump (vacuum pump)
9	Feed granulate outlet
11	Agitator
13	Outlet valve
14	Feed granulate reservoir
15	Feed valve
16	Feed granulate inlet
17	Feed granulate connection pipe

CLAIMS

1. A method for producing a feed, preferably a feed fish, containing probiotic bacteria, the method comprising

- providing a feed granulate having a temperature above 50 °C
- providing a probiotic liquid comprising water and/or oil and probiotic bacteria, and having a temperature below 30 °C,
- subjecting said feed having a temperature above 50 °C to an evacuated atmosphere having an absolute pressure less than 500 mbar, and
- while the feed is subjected to said evacuated atmosphere, applying said probiotic liquid onto the surface of the granular feed.

2. A method according to claim 1, wherein the feed granulate is pellets which are provided by an extrusion, an expansion or a pelletizing process.

3. A method according to claim 1 or 2, further comprising agitating the feed while the liquid is applied onto the surface of the feed.

4. A method according to any of the preceding claims, wherein said probiotic liquid applied to the surface of the granulates is applied by sprinkling or spraying.

5. A method according to any of the preceding claims, wherein the temperature of said probiotic liquid while being applied onto the surface of the feed is below 25 °C, such as below 20 °C, preferably below 15 °C, such as below 10 °C, preferably below 5 °C.

6. A method according to any of the preceding claims, wherein said probiotic liquid contains at least 0.4 vol %, such as at least 0.8 vol %, preferably at least 1.2 vol % water, and at least 2 vol %, such as at least 3 vol %, preferably at least 4 vol %, such as at least 10 vol % oil, preferably at least 20 vol %, such as at least 30 vol %, and even at least 35 vol % oil.

7. A method according to any of the preceding claims, wherein the probiotic liquid contains water in an amount between 0.5 to 3 vol %, probiotic bacteria in an

amount between 0.1 to 2 vol % or 0.2 to 3 vol % and where the remaining amount of the probiotic liquid being oil.

8. A method according to any of the preceding claims, wherein the oil is a vegetable oil preferably selected from the group consisting of rape seed oil, soya oil, palm oil, olive oil, sunflower oil, or an animal oil, such as a fish oil or a combination of one or more of said oils.

9. A method according to any of the preceding claims, wherein said probiotic liquid contains less than 25 grams, preferably less than 18 grams, such as less than 12 grams, preferably less than 1 grams of bacteria per kg granulates.

10. A method according to any of the preceding claims, wherein the bacteria is selected from the group consisting of *Pediococcus acidilactici* or other live probiotic, such as live probiotic as used in aquaculture feed, such as *Bacillus* species.

11. A method according to any of the preceding claims, wherein the pressure of the evacuated atmosphere is less than 400 mbar, such as less than 300 mbar, preferably less than 200 mbar, such as less than 100 mbar, preferably less than 50 mbar.

12. A method according to any of the preceding claims, wherein the steps of subjecting said feed to an evacuated atmosphere, and applying the liquid onto the surface of the feed are carried out on a batch of feed granulates.

13. A method according to claim 12, wherein the method comprises introducing a batch of feed granulates into an application chamber (6) configured for providing the evacuated atmosphere inside the chamber and sprinkling and/or spraying said probiotic liquid onto the batch of feed granulates while the feed granulates are agitated.

14. A method according to any of the preceding claims, wherein the feed granulates have a water content of less than 25 vol %, such as less than 20 vol

%, preferably less than 15 vol % as determined immediately prior to being subjected to application of the probiotic liquid.

15. A method according to any of the preceding claims, wherein the step of providing a probiotic liquid comprises:

- mixing water and/or oil and probiotic bacteria, preferably in a mixing chamber (1), preferably at atmospheric pressure, such as in region of 1 bar, so as to provide said probiotic liquid prior to applying said probiotic liquid onto the surface of the granulate feed,

wherein the temperature of the mixed water and/or oil and probiotic bacteria is below 30 °C.

16. A method according to any of the preceding claims, wherein the step of providing a probiotic liquid comprising water and/or oil and probiotic bacteria, and having a temperature below 30 °C comprises

- mixing a liquid comprising water and/or oil having a temperature less than 30 °C, such as less 25 °C, preferably below 20 °C, such as below 15 °C, preferably below 10 °C, such as below 5 °C with a probiotic bacteria in a mixing chamber (1) at atmospheric pressure, such as in region of 1 bar, so as to provide said probiotic liquid prior to applying said probiotic liquid onto the surface of the granulate feed.

17. A method according to any of the preceding claims, wherein the feed granulates after said probiotic liquid has been applied to the surface thereof are cooled, preferably to a temperature less than 45 °C.

18. A device for applying bacteria to feed granulates, preferably fish feed granulates, the device comprising

- an application chamber (6) being adapted to provide and maintain an evacuated atmosphere having an absolute pressure less than 500 mbar
- a fluid applicator (7) adapted to sprinkle or spray liquid into the interior of the application chamber (6)
- a feed granulate inlet (16)

- a feed granulate outlet (9).

19. A device according to claim 18, wherein the device comprising a pump (8) in fluid connection with the interior of the application chamber for sucking out gas from the application so as to reduce to and/or maintain the pressure at less than 500 mbar in the application chamber (6).

20. A device according to claim 18 or 19 wherein the device comprising an agitator (11) arranged inside the application chamber adapted to agitate the feed granulate while inside the application chamber (6).

21. A device according to any of the preceding claims 18-20, the device comprising a mixing chamber (1) adapted to mix oil, water and probiotic bacteria so as to provide a probiotic liquid.

22. A device according to any of the preceding claims 18-21, wherein the feed granulate inlet (16) and the feed granulate outlet (9) both are closeable, preferably by comprising a shut-off valve (15, 13), so as to seal the feed granulate inlet (16) and feed granulate outlet (13).

23. A fish feed comprising granulates being coated with a probiotic liquid containing probiotic bacteria, said fish feed contains less than 25 grams, preferably less than 18 grams, such as less than 12 grams, preferably less than 1 grams of bacteria per kg granulates.

24. A fish feed according to claim 23, wherein the probiotic bacteria is selected from the group consisting of *Pediococcus acidilactici* or other live probiotic, such as live probiotic as used in aquaculture feed, such as *Bacillus* species.

25. A fish feed obtained or obtainable by a method according to any of the preceding claims 1-17.

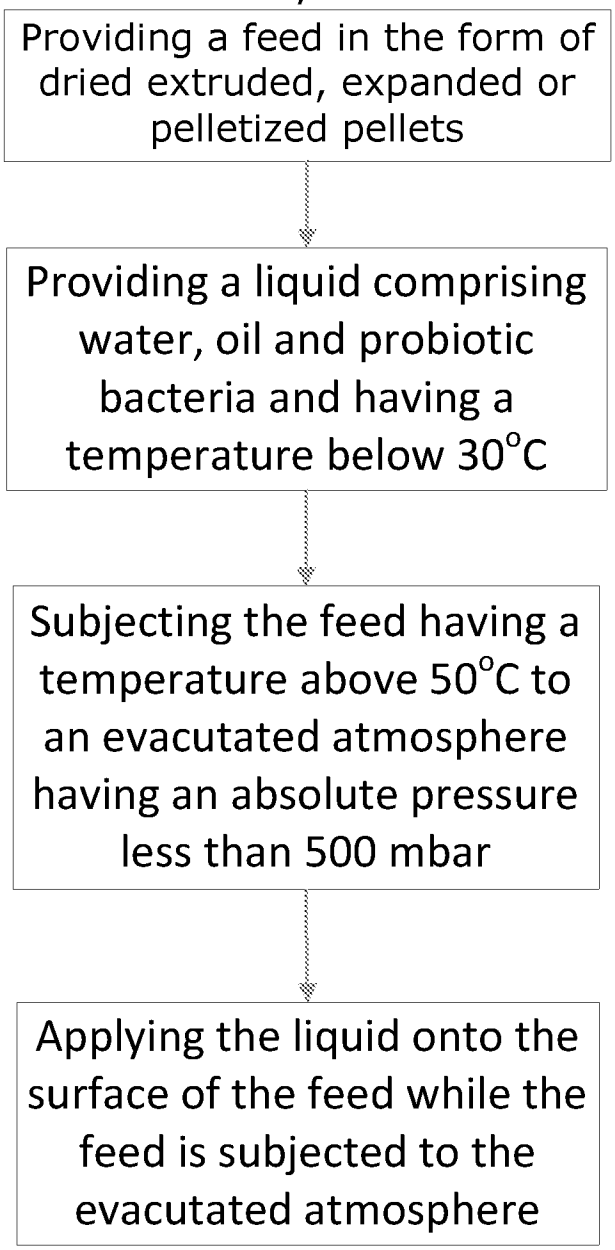
Providing a feed in the form of dried extruded, expanded or pelletized pellets
Providing a liquid comprising water, oil and probiotic bacteria and having a temperature below 30°C
Subjecting the feed having a temperature above 50°C to an evacutated atmosphere having an absolute pressure less than 500 mbar
Applying the liquid onto the surface of the feed while the feed is subjected to the evacutated atmosphere

Fig. 1

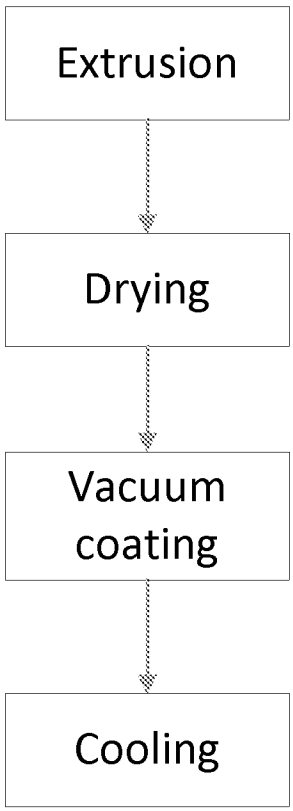

Fig. 2

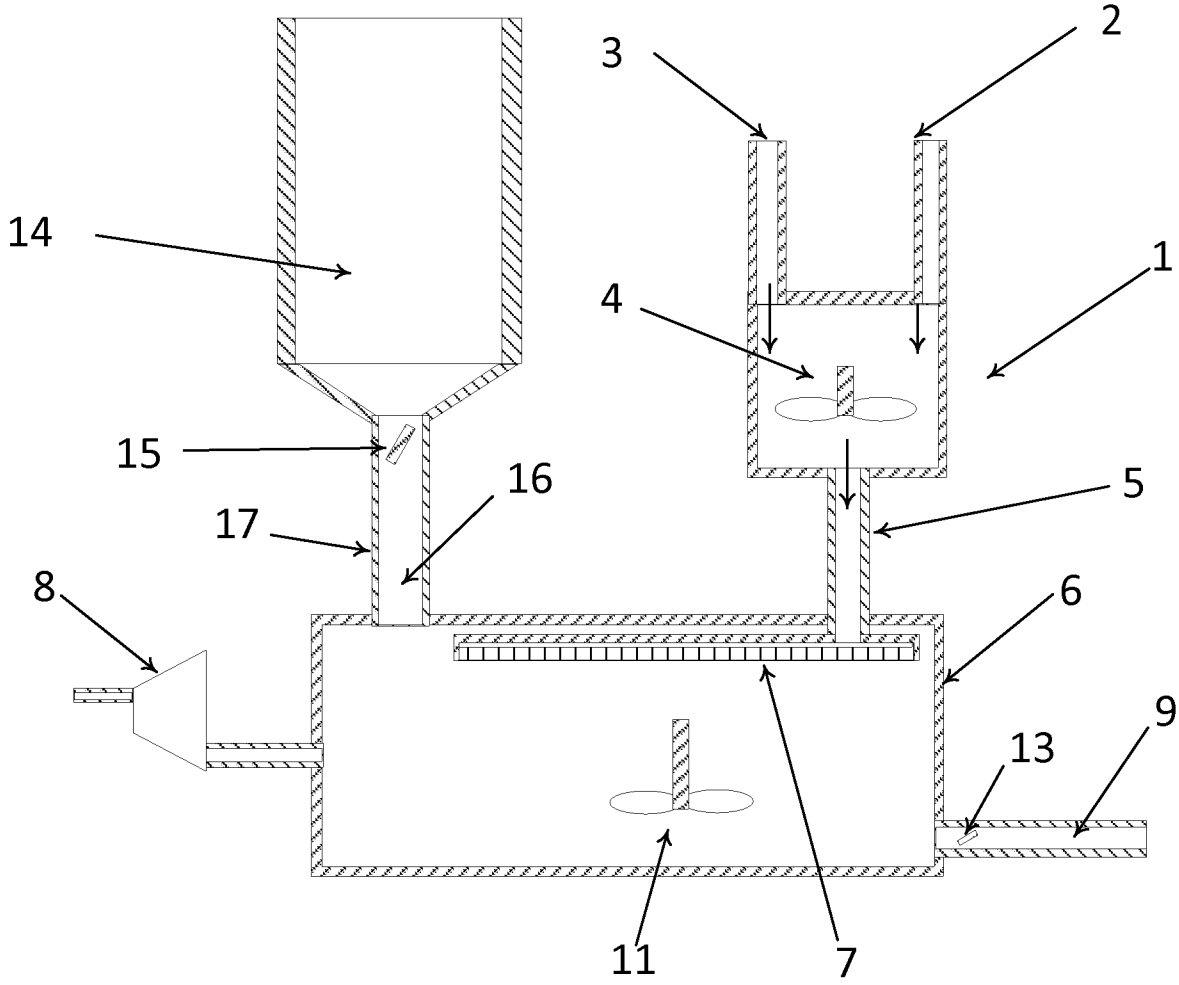

Fig. 3

INTERNATIONAL SEARCH REPORT

International application No
PCT/DK2017/050246

A. CLASSIFICATION OF SUBJECT MATTER
INV. A23K50/80 A23K10/18 A23K20/158 A23K30/00 A23K40/30 A23K40/25
ADD.
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)
A23K

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)
EPO-Internal, FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2012/076895 A1 (KIREJEVAS VYGANTAS [EE] ET AL) 29 March 2012 (2012-03-29) paragraph [0001] - paragraph [0046] paragraph [0074] - paragraph [0139] paragraph [0160] - paragraph [0206]; figure 4; examples 1-3,6-7	1-25
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A	US 2010/247712 A1 (RUDOLPH MARVIN J [US]) 30 September 2010 (2010-09-30) paragraph [0003] - paragraph [0012] paragraph [0060] - paragraph [0063]	1-25

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 August 2017	18/08/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Alevisopoulos, S

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/DK2017/050246

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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A	paragraph [0003] - paragraph [0017] paragraph [0049] - paragraph [0066] paragraph [0084] - paragraph [0092]; examples 1, 3	1-22
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