PROCESS FOR THE PRODUCTION OF MICROBIAL BIOMASS AND THE TREATMENT OF SERUM FROM MILK OR OTHER WASTE OF THE MILK INDUSTRY

DESCRIPTION

Technical field of the invention

The present invention relates to a process for the production of microbial biomass, in particular yeasts and milk bacteria, starting from whey and other waste of dairy industry. The process according to the present invention represents a new approach for managing dairy waste limiting waste, reducing disposal costs by guaranteeing a low environmental impact. At the same time, it provides an alternative method for the production of microbial biomass.

State of art

In the state of art it is known that the whey can be used as carbon source for the microbial growth. In fact, in literature several examples of cultivation of microorganisms (yeasts or bacteria) are described, which however nowadays not always can be practically applied due to the still low yields and to the extreme variability in the input raw material which make difficult to manage them.

Currently the yeast production is performed by using molasses, sugar residues from processing of beet and sugar cane, with a supplement of nitrogen source. The standard process is a fermentation in *fed-batch* mode, during which molasses is added to the bioreactor gradually soon after the addition of cells. The profile therewith the *feed* of molasses is provided in the industrial processes is not wholly known. Several attempts are currently performed with the purpose of further improving this productive process by inserting new raw materials as fermentation substrates or variations in the process type (Patent EP0821057B1, Baker's yeast production from molasses/cheese whey mixtures; Ferrari et al. 2001). However, it is known that the feed profile is adapted to the growth rate of cells until a maximum rate which depends upon the transfer rate of oxygen of bioreactor and upon the critical condition under which the cells start to produce ethanol, by decreasing the biomass yield and the quality of the final product (Patent EP0821057B1).

1) The whey and the other milk waste of dairy industry are characterized by a considerable variability in terms of chemical-physical composition which depends upon the type of milk therefrom they derive and above all upon the processing procedure thereto the milk is subjected which can be considerably different depending upon the dairy industry of origin. In fact, currently, all data available in literature are focused on the use of one single type of waste (Ex. The acid whey, Patent CA1133753A) which is pre-treated thermally (Patent: US07890513; Improved *Saccharomyces Cerevisiae* Growth on Cheese Whey by Controlling Enzymatic Lactose Hydrolysis.; Pisano et al. 2015) or by ultrafiltration (UF) to remove the protein fraction (Patent CA1133753A). The membrane treatment process today is widely used for concentrating serum proteins, sugars or the serum itself, however nowadays these concentrated fractions are usually dried up and put into the market as such.

2) One of the main problems associated to the use of serum as source of sugars for the growth is the inability of several microorganisms to use directly lactose as carbon source. This is the case of some species of *Saccharomyces*, as those usually used for the production of leavened baked products, beers and wines. Such problem does not exist for the so-called lactic yeasts (ex. *K. lactis*) or lactic bacteria which are capable of hydrolysing lactose in the glucose or galactose monomers. In order to make up for this lack several alternatives were proposed, from genetic manipulation to induce the expression of genes allowing lactose consumption (Lactose utilization by *Saccharomyces cerevisiae* strains expressing *Kluyveromyces Lactis* LAC genes; Rubio Texeira et al. 2000), to the conversion of lactose, obtained from ultrafiltered, lyophilizated and re-hydrated serum, by means of *S. termophilus* in lactic acid subsequently used by *S. cerevisiae* as carbon source for the growth (Production of Bakers' Yeast in Cheese Whey Ultrafiltrate; Champagne et al.1989). The most recent work of Pisano et al. demonstrates that the controlled and repeated addition of the beta-galactosidase enzyme increases the yields of produced biomass and reduces the inhibitory effect from substrate accumulation. However, this procedure results to be hardly scalable at industrial level as it would require a controlled and refrigerated line for the enzymatic feed with consequent energy consumption and costs.

3) An additional critical issue found upon using the serum is the lack in source of nitrogen (N) which can invalidate the microbial growth; even for this problem several variants were proposed, such as the addition of corn streap liquor (Champagne et al.1989), inorganic sources of N or yeast extract with a considerable increase in process costs. Usually sources of nitrogen are added with a percentage variable between 25% and 50% (EP0821057B1).

4) Several variants in the operating modes of the whey fermentative process, in particular batch and continuous process, were described (Champagne et al.1989; Pisano et al. 2015; Patent CA1133753A; J.P., Production of yeast extract from whey using *K. marxianus*. Braz. Arch. Biol. Technol; 2003 de Palma Revillon et al.; Conversion of cheese whey to single-cell protein. Bio-technology Bioengineering; 1983 Sandhu et al.). The patent CA1133753A of 1978 describes a process wherein, after the batch fermentation step, a continuous step is performed during which serum and yeast are inserted constantly (constant time and volumes; volumes of feed equal to the volumes of product which is removed), for a total duration of about 18 hours of batch process and 4 hours of continuous process.

5) The process for the production of yeasts is a typically aerobic process, performed at a temperature of about 30°C under pH control conditions. Many fermentation processes provide a pH continuous control to avoid a stress on the growing cells (Champagne et al.1989; Patent CA1133753A). However, even this control requires considerable cost and energy consumption in an industrial optics.

6) The fermentative process is preceded by a step of pre-cultivation of cells which can be performed in synthetic growth medium or directly in serum.

The article of Ferrari et al. Biotechnology letters vol. 16 pages 205-210 describes a process for the production of bioethanol wherein the whey is firstly subjected to an atomisation process by spray-drying to obtain lyophilised lactose which is added to a second amount of whey as such in order to obtain a higher concentration of lactose in serum.

The patent application WO20141164 describes a mode for recovering lactose starting from a liquid source containing lactose through crystallization and

optional filtration steps for the removal of cells, proteins, polypeptides, polysaccharides, lipids, ions and salts.

However, the state of art as above reconstructed highlights several disadvantages, in particular in terms of effectiveness and high costs linked to the production of yeasts by means of processes using as growth medium the whey or other milk waste of dairy industry.

Summary of the invention

The technical problem placed and solved by the present invention is then to provide a process for preparing yeasts and lactic bacteria by using as growth medium the whey or other milk waste of dairy industry allowing to obviate the drawbacks mentioned above and allowing at the same time to reduce the polluting organic load of the whey or of other milk waste of dairy industry. The present invention then relates to a process for the treatment and re-qualification of dairy waste aimed at producing microbial biomass.

Such problem is solved by a process according to claim 1.

Preferred features of the present invention are set forth in the depending claims.

In the process according to the present invention the whole milk waste of dairy industry is subjected to a pre-treatment (microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), optionally reverse osmosis (OI)) and afterwards to a fermentation step. The first three steps make homogeneous the raw material which is then used for fermentation. In particular, MF allows to remove coarse particulate and fats, UF removes the protein fraction which is then lyophilised and used as food supplement, NF allows to concentrate lactose by removing part of salts; this fraction together with the liquid fraction of UF is then used in the fermentation step. On the contrary, the step of reverse osmosis allows to produce demineralized water which is inserted as process water in the steps for keeping the fermentative process or washing temperature.

The fermentation process provides two steps, a first batch step performed with the deproteinized serum deriving from UF and a second fed-batch step wherein a feed controlled in serum (or other waste) deriving from UF or NF is provided. The used raw material, both in batch and fed-batch, is hydrolysed enzymatically,

by adding beta-galactosidase, contemporary to the fermentative process: in this case one speaks about SSF (*simultaneous saccharification and fermentation*) process. This combination of pre-treatment and fermentative process has resulted to be very effective for the production of microbial biomass of different type, in particular yeasts for different applications (*Saccharomyces sp.; Kluyveromyces sp.; etc.*) and lactic bacteria (*Lactobacillus sp.*).

The process according to the present invention then provides a valid alternative to the classical expensive disposal, having a high environmental impact, of dairy waste. At the same time, it provides an alternative method for the production of microbial biomass. The pre-treatment and developed fermentative process together allow to reduce the presence of proteins, sugars and other substance constituting the polluting organic load of the whey, by allowing on one side to obtain market products of interest, on the other side to solve the problem of disposing this waste.

Other advantages, features and use modes of the present invention will result evident form the following detailed description of some embodiments, shown by way of example and not for limitative purposes.

Brief description of the figures

- Figure 1 describes the flow diagram of a preferred embodiment of the process according to the present invention;
- Figure 2 describes the flow diagram of a preferred embodiment only of the fermentation step of the process according to the present invention;
- Figure 3 summarizes the main parameters used in a preferred embodiment of the fermentation step of the process according to the present invention;
- Figures 4A and 4B show the course of the feed volumes with respect to the biomass growth (expressed in Optical Density, OD) during the fed-batch step. In particular Figure 2A describes the course of a feed with a UF Permeate, Figure 2B with a NF Concentrate.
- Figure 5 describes the consumption of sugars existing in whey during the fermentative process.

Detailed description of preferred embodiments

The present invention relates to a process for the production of microbial biomass by means of whey and/or other milk waste of dairy industry. The whey, deriving from the dairy processing procedures, is a substrate rich in organic matter which then can be used in the fermentation processes wherein microorganisms use the existing carbon sources, in particular sugars. One of the advantages of the process according to the present invention is the possibility of using both whey and other dairy waste, such as ricotta whey and buttermilk. Under the term whey in particular one refers to the liquid fraction deriving from the formation of curd during cheese making; under the term ricotta whey one refers to the liquid fraction which is obtained after coagulation of ricotta, whereas under the term buttermilk the remaining waste water is designated deriving from the stretching process and from washing the stretched curd.

The process provides a step i) of microfiltration (MF) of whey and/or of other milk waste of dairy industry.

According to an embodiment MF is performed by means of membranes with pores having diameter ranging from 0.1 to 1 μm capable of keeping the coarser particulate and the fats which would invalidate the subsequent growth step and the protein composition. Preferably membranes made of polyethylene sulfone (PES) could be used.

The process provides a step ii) of ultrafiltration (UF) of the permeate obtained from MF.

According to an embodiment the membranes of UF have a porosity ranging from 5 to 50 KDa, preferably 30 KDa, which do not allow the passage of the protein fraction ("UF concentrate"), but they let sugars, mineral salts and water ("UF permeate") to go through. During this step the protein fraction concentrates and it can be recovered for lyophilization, drying or hydrolysis of serum proteins. The US permeate is partially sent to fermenter.

In the subsequent step iii) the UF permeate is nanofiltrated (NF), for example with membranes ranging from 150 to 300 Da allowing to keep the lactose by

concentrating it ("NF concentrate"), whereas it lets a portion of salts and water ("NF permeate") to go through.

Whereas NF concentrate is used in fermentation, NF permeate can be subjected advantageously to reverse osmosis (OI) or it can be used as such as process water for example in keeping fermentation temperature. OI process for example could be implemented by means of spiral-wound membranes made of PES with a flow mainly depending upon the used pumping system and its rate of flow.

The process according to the present invention provides a step iv) for fermenting a culture of microorganisms. Such step is herein described in details.

According to an embodiment the fermentation step is performed under aerobic conditions with controlled flow of sterile air, for example between 0.5 and 5 L/min, at a temperature between 25 and 40°C and a stirring kept constant between 600 and 1000 rpm.

The fermentation could, or could not, provide the *pH* control, but it was observed that this does not induce significant variations on the yields of the produced biomass.

The cells are pre-cultivated preferably in synthetic growth medium, then transferred in a small volume of UF permeate and subsequently transferred in fermenter. UF permeate and NF concentrate which are used as growth medium could have not to be sterilized thermally if the used membranes comply with current sterilization and sanitization criteria; otherwise they are subjected to suitable thermal sterilization.

During fermentation it is necessary to add a nitrogen source to the substrate used for the fermentative process; it is possible to add anyone of the nitrogen sources commonly used in the process for producing yeast (for example yeast extract, bactopeptone, ammonium sulphate, urea etc.). The nitrogen concentration is selected so as not to be limiting but it is kept at a minimum level which does not compromise the process cost-effectiveness.

In the process several nitrogen sources, in particular urea, bactopeptone, yeast extract and ammonium inorganic sources, were tested by evaluating the effect on the growth of microorganisms. These sources were tested in variable

percentages, from 0.5 to 40% w/v. The highest concentrations guarantee higher biomass yields, but with greater costs. In fact, the minimum percentages, comprised between 0.05 and 5% w/v, were detected which guarantee a good microbial growth without invalidating the process yields significantly, but allowing considerable cost savings due to the reduction in the concentration of added nitrogen source. According to a preferred embodiment in fermentation a nitrogen source in a concentration between 0.05 and 5% w/v then will be used.

In case microorganisms are used which are not capable of consuming lactose (for example *S. cerevisiae*) beta-galactosidase is added during fermentation, in particular at the beginning of the batch step in concentration proportional to the sugars existing in the culture medium. In SSF processes the enzymatic hydrolysis is performed directly in the reactor wherein fermentation and growth of microorganism take place at the same time. In particular, in our case, during the first batch step the enzyme is added at the beginning of the process, without impacting on process and plant cost-effectiveness, contemporary to the inoculum of cells. The used enzyme amount is selected so as to avoid the accumulation of product (glucose and galactose) inhibiting the growth. Variable amounts of enzyme were tested, from 5 to 5000 enzymatic units per litre (UE/L) to detect a range allowing the complete hydrolysis without inhibiting the process and with the minimum economic impact on the process. The most suitable amounts resulted to be comprised between 800 and 2500 UE/L.

The illustrated processes were tested even by evaluating the effect on the growth of microorganisms with different concentrations of enzyme to detect the suitable concentrations which could not inhibit the growth by allowing even an economical advantage. Concentrations comprised between 5 and 5000 enzymatic units (UE) per litre were evaluated.

An additional new aspect of the process consists in the type of fed-batch which was developed: firstly, in the batch step, the cells are inoculated in a fixed volume of UF permeate, this step lasts from 2 to 8 hours. At the end of this step a *feed* step starts which can be performed with UF permeate or NF concentrate. The use of NF concentrate in a fermentative process is an important new aspect in this context. In fact, even the use of UF permeate allows the microbial growth,

but, by performing the *feed* with NF concentrate the working volumes are reduced considerably with a significant increase in the yields.

Even in this case should microorganisms not capable to consume lactose be used, an addition of the beta-galactosidase enzyme in the *feed* volume is performed. The flow of *feed* is increased gradually to provide an amount of sugars instantaneously proportional to the number of cells existing in the reactor. This step can have a duration of 10-20 hours and if performed in this way, it allows the complete consumption of the sugars existing in the medium. The variability in feed time depends upon the composition of the input waste; this allows a certain flexibility in using the substrates with different amounts of sugars, as it allows to customize the fermentation profile (time and feed volumes) based upon the substrate features.

The rate and amount of feed which are input in the reactor are calculated based upon: amounts of sugars in the feed, amounts of cells existing in the reactor when the feed starts and theoretical maximum yield (defined in advance and known). By considering these parameters it is possible to calculate a feed profile wherein the administration rate increases exponentially parallelly to the exponential increase in microbial biomass in the reactor. In this way in each instant the cells will be capable of consuming all sugars which are provided.

According to an embodiment the feed volume is defined according to the following equation:

$$F = \frac{\mu * X * V_0 * \exp(\mu * t)}{Y_{x/s} * C}$$

wherein:

F= litres of feed per hour

μ= specific growth rate (h^{-1})

X= concentration of cells in the reactor at the end of the batch (g/L)

V_0= growth medium volume contained in the reactor at the end of the batch (L)

t= time

$Y_{x/s}$= maximum theoretical biomass yield on substrate

C= concentration of sugars in the growth medium expressed in glucose equivalents (g/L).

In the fermentation step different types of microorganisms could be used; in particular, good yields were obtained for the different species of the genus *Saccharomyces* which are not capable of using lactose; the process results to be also applicable for the different species of the genus *Kluyveromyces*.

At the end of the process the microbial biomass is recovered by centrifugation or filtration. The obtained microbial biomass is separated by centrifuge and it can be lyophilized or dried to obtain a powder product.

Example

EXAMPLE 1.

20 litres of whey, provided by a local dairy company, were subjected to an initial filtration process by means of a small-scale membrane system having a maximum yield of 21 L/min; with spiral-wound membranes made of PES. The first passage of MF, performed with membranes having a porosity of 1μm, allows to remove the coarse particulate. During this passage a significant volume loss is not observed. MF permeate is subjected to UF with 30-KDa membranes. In this step MF permeate is made to circulate several times inside the membrane by allowing the formation of UF concentrate rich in the protein fraction (about a tenth of the initial volume) and deproteinized UF permeate which is then partially subjected to NF. NF passage performed with 300-Da membranes allows to concentrate the sugar fraction; even in this case UF permeate is made to circulate inside the filtration system until obtaining a NF concentration equal to one fourth of the initial volume (5L). For each liquid fraction obtained from this process, the concentrations of sugars (lactose, glucose and galactose) are measured by HPLC. One litre of UF permeate is autoclaved and inserted in a 2-L bioreactor. To such volume b-galactosidase enzyme and the nitrogen source are added. An inoculum of cells of *S. cerevisiae* is inserted in the fermenter so as to obtain a

final optical density (OD) comprised between 0.5 and 5. Such cells are pre-cultivated previously for about ten hours in 50 ml of synthetic growth medium and then transferred into 100 ml of sterile UF permeate added with b-galactosidase for further ten hours. During this batch step the system is subjected to continuous and controlled stirring of 800 rpm and the temperature is kept between 28 and 32° C; the air flow is kept at 1.5 L/min. The growth is monitored by measuring OD every two hours. With the same time interval the course of sugars in the medium is evaluated by means of HPLC analysis. The batch step lasts 10 hours; at the end of this step the fed-batch step is started during which a feed of UF permeate is added for about 7 hours. Several combinations in volumes of batch and fed-batch and even in the duration of the different steps were evaluated. This feed is increased proportionally to the growth of the biomass in the reactor; in particular the feed volume which is added in a certain time unit is proportional to the amount of cells existing in the reactor; this guarantees a supply of sugars suitable to favour the growth of cells with the maximum performances by following an exponential course. At the end of the 7 hours of feed the fermentation proceeds for about further two hours by favouring the consumption of all sugars existing in the reactor. Even during these steps the cell growth is monitored by measuring OD and the course of sugars in the medium is monitored by means of HPLC analysis. At the end of the process the medium is collected and centrifuged for separating and recovering the microbial biomass.

EXAMPLE 2.

An identical treatment and fermentation process was performed by replacing UF permeate with NF concentrate. The process parameters such as duration, temperature, stirring, aeration, were kept identical. The fermentative process in this variant allows to reduce considerably the feed volumes input in the reactor with a considerable saving in sizes of a plant on industrial scale and an increase in the yield of biomass obtained on the quantity of input raw material (see figures 2A and 2B).

EXAMPLE 3.

The illustrated processes were tested by evaluating the effect on the growth of microorganisms of several nitrogen sources, in particular urea, bactopeptone,

yeast extract and ammonium inorganic sources. These sources were tested in percentages variable from 0.05% to 40% w/v. The highest concentrations guarantee higher biomass yields but with higher costs. In fact, the minimum percentages were detected, comprised between 0.05 and 5%, which guarantee a good microbial growth without invalidating the process yields significantly, but allowing a considerable economic saving thanks to the decrease in the concentration of added nitrogen source. Among the suitable sources there are yeast extract and urea; the latter preferably is economically more advantageous, the performances being equal.

The illustrated processes were tested even by evaluating the effect on the growth of microorganisms of different concentrations of enzyme to detect the suitable concentrations which could not inhibit the growth by allowing even an economical advantage. Concentrations comprised between 50 and 5000 enzymatic units (UE) per litre were evaluated.

The present invention also relates to the microbial biomasses which can be obtained with the process according to anyone of the described embodiments. In particular it relates to biomasses of yeasts, for example of *Saccharomyces* in lyophilized or dried form.

* * *

The present invention has been sofar described with reference to some preferred embodiments. It is to be meant that other embodiments belonging to the same inventive core may exist, as defined by the protective scope of the herebelow reported claims.

CLAIMS

1. A process for the production of microbial biomass by whey and/or other milk waste of dairy industry comprising the following steps:

i) microfiltration of the whey and/or other milk waste of dairy industry;

ii) ultrafiltration of the microfiltration permeate obtained at step i);

iii) nanofiltration of the ultrafiltration permeate obtained at step ii);

iv) fermenting a culture of microorganisms, wherein said fermentation step is characterized by the use of the ultrafiltration permeate obtained at step ii) and of the nanofiltration concentrate obtained at step iii) as the growth medium of said culture.

2. The process according to claim 1 wherein said fermentation step comprises a step of pre-culture in synthetic growth medium and/or in the ultrafiltration permeate.

3. The process according to claim 1 or 2 wherein in said fermentation step there is provided:

- a first *batch* step wherein the cells of said culture of microorganisms are grown in a medium comprising a fixed volume of the ultrafiltration permeate obtained at step ii), in particular wherein said *batch* step lasts from 2 to 8 hours;

- a second *fed-batch* step wherein the ultrafiltration permeate obtained at step ii) and/or the nanofiltration concentrate of step iii) is added as growth medium to said cell culture.

4. The process according to the preceding claim wherein in said *fed-batch* step the rate and amount of *feed* are calculated based upon the amount of sugars in the *feed*, the amount of cells present in the reactor when one starts to administer the *feed* and the theoretical maximum yield, so that the *feed* administration rate increases exponentially parallelly to the exponential increase in microbial biomass in the reactor, wherein the *feed* volume is defined according to the following equation:

$$F = \frac{\mu * X * V_0 * \exp(\mu * t)}{Y_{x/s} * C}$$

wherein:

F= litres of *feed* per hour

μ= specific growth rate (h^{-1})

X= concentration of cells in the reactor at the end of the batch (g/L)

V_0= growth medium volume contained in the reactor at the end of the batch (L)

t= time

$Y_{x/s}$= maximum theoretical biomass yield on substrate

C= concentration of sugars in the growth medium expressed in glucose equivalents (g/L).

5. The process according to anyone of claims 1 to 4 wherein said culture of microorganisms is selected from species of the genus *Saccharomyces*, species of the genus *Kluyveromyces* and milk bacteria of genus *Lactobacillus*.

6. The process according to anyone of claims 1 to 5 wherein during said fermentation step a nitrogen source is added selected from yeast extract, bactopeptone, ammonium sulphate, ammonium salts or mixtures thereof in an amount comprised between 0.05% and 40% w/v, in particular between 0.05 and 5% w/v.

7. The process according to anyone of claims 1 to 6 wherein:

- in the microfiltration step a membrane is used with pores having a diameter between 0.1 and 1 μm; and/or

- in the ultrafiltration step a membrane is used with a cut-off between 5 and 50 KDa; and/or

- in the nanofiltration step a membrane is used with a cut-off between 150 and

300 Da.

8. The process according to anyone of claims 1 to 7 wherein during the fermentation step a concentration of b-galactosidase ranging from 800 to 2500 enzymatic units (UE) per litre is used.

9. The process according to anyone of claims 1 to 8 comprising a further step wherein the permeate of the nanofiltration is subjected to reverse osmosis (OI) or used as process fluid for maintaining the fermentation temperature.

10. The process according to anyone of claims 1 to 9 wherein said fermentation step is performed under aerobic conditions with controlled flow of sterile air between 0.5-5 L/min, at a temperature between 25 and 40°C and at a stirring between 600 and 1000 rpm.

11. The process for the treatment of whey and/or other milk waste of dairy industry comprising the following steps:

i) microfiltration of the whey and/or other milk waste of dairy industry;

ii) ultrafiltration of the microfiltration permeate obtained at step i);

iii) nanofiltration of the ultrafiltration permeate obtained at step ii);

iv) optionally subjecting the permeate of the nanofiltration to reverse osmosis, wherein:

-in the microfiltration step a membrane is used with pores having a diameter between 0.1 and 1 μm; and/or

-in the ultrafiltration step a membrane is used with a cut-off between 5 and 50 KDa; and/or

-in the nanofiltration step a membrane is used with a cut-off between 150 and 300 Da.

ABSTRACT

The present invention relates to a process for the production of microbial biomass, in particular yeasts and milk bacteria, starting from whey and other waste of dairy industry. The process according to the present invention represents a valid alternative to the classical expensive disposal, having a high environmental impact, of dairy waste. At the same time, it provides an alternative method for the production of microbial biomass.

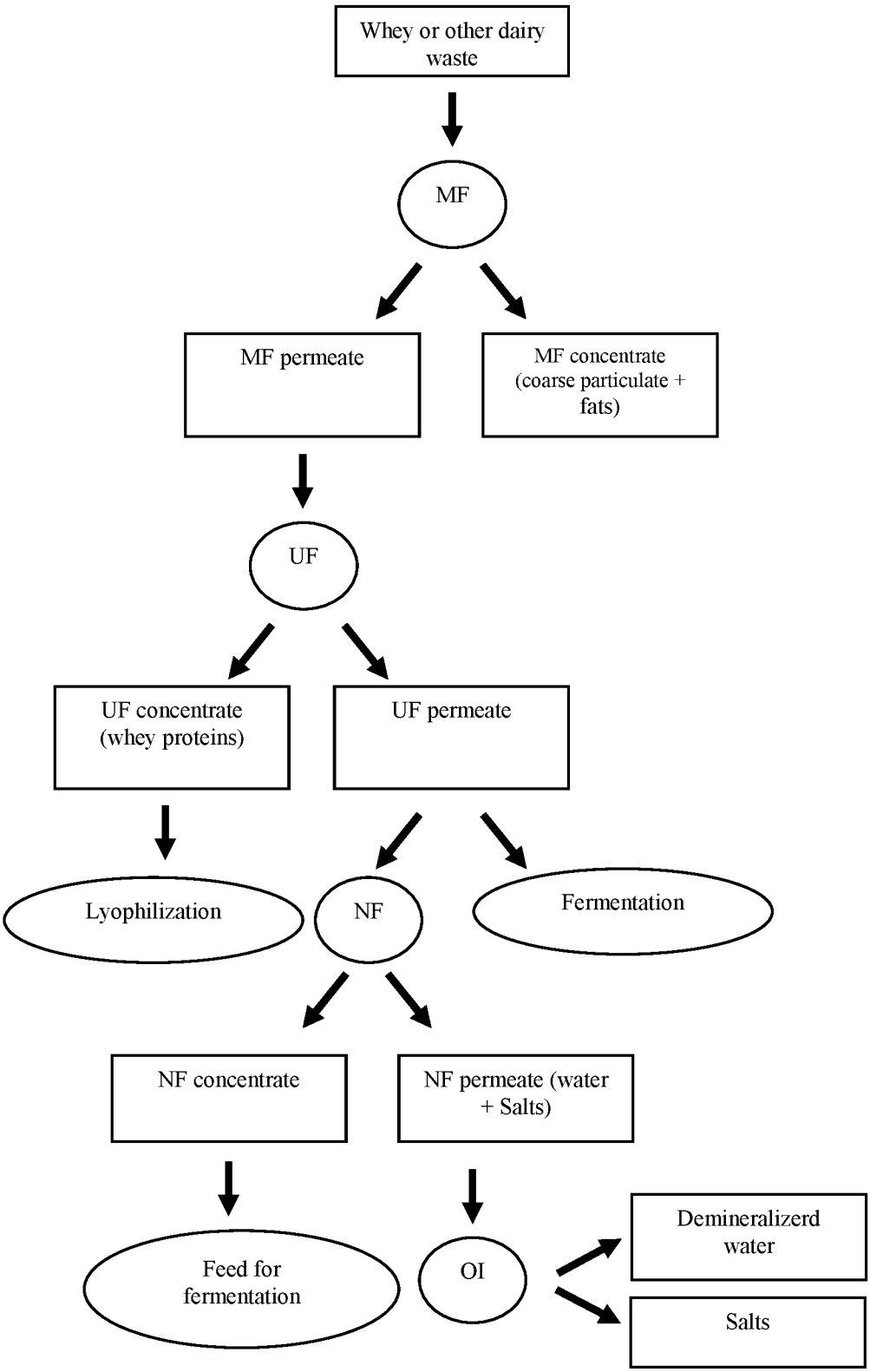
Whey or other dairy waste
MF
MF permeate
MF concentrate (coarse particulate + fats)
UF
UF concentrate (whey proteins)
UF permeate
Lyophilization
NF
Fermentation
NF concentrate
NF permeate (water + Salts)
Feed for fermentation
OI
Demineralizerd water
Salts

FIG. 1

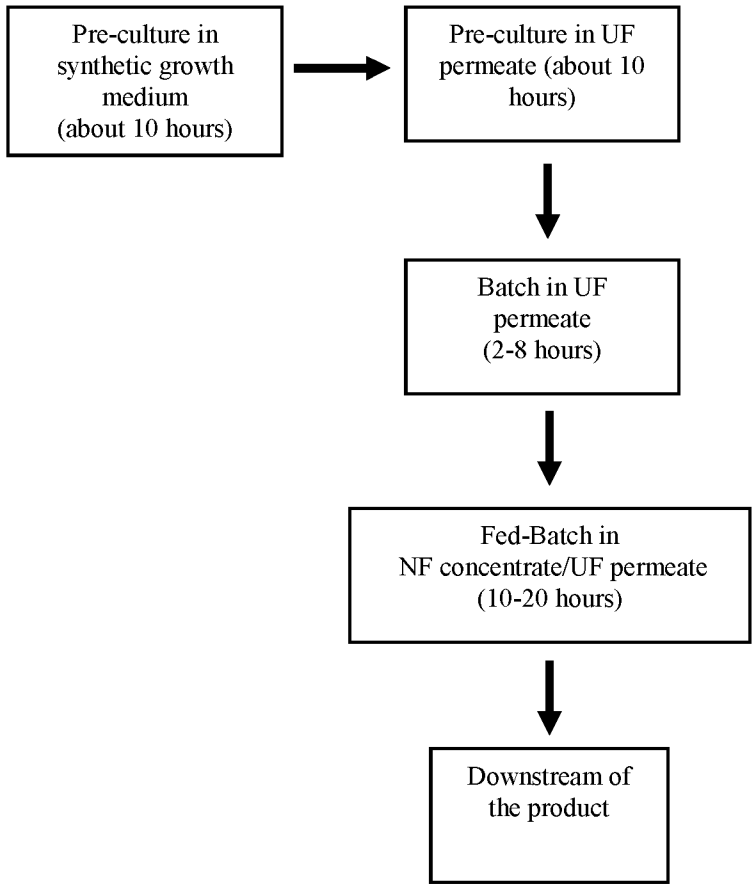
Pre-culture in synthetic growth medium (about 10 hours)
Pre-culture in UF permeate (about 10 hours)
Batch in UF permeate (2-8 hours)
Fed-Batch in NF concentrate/UF permeate (10-20 hours)
Downstream of the product

FIG. 2

Temperature (°C)	25-40
pH	Not controlled
Stirring (rpm)	600-1000
Aeration (L/min)	0.5-5
Nitrogen source (%w/v)	0.05-5
B-galactosidase (UE/L)	800-2500

FIG. 3

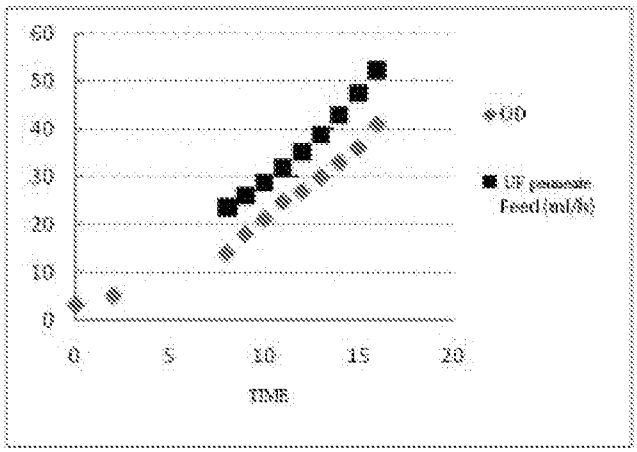

FIG. 4A

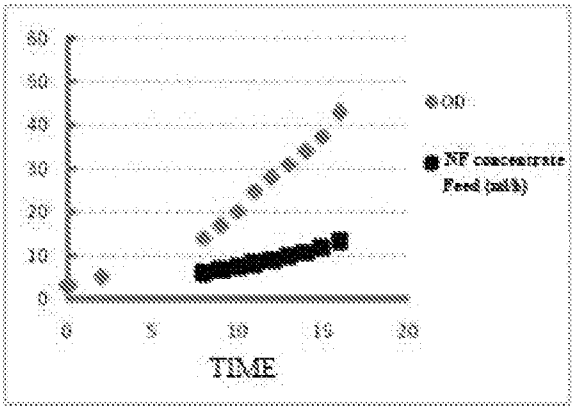

FIG. 4B

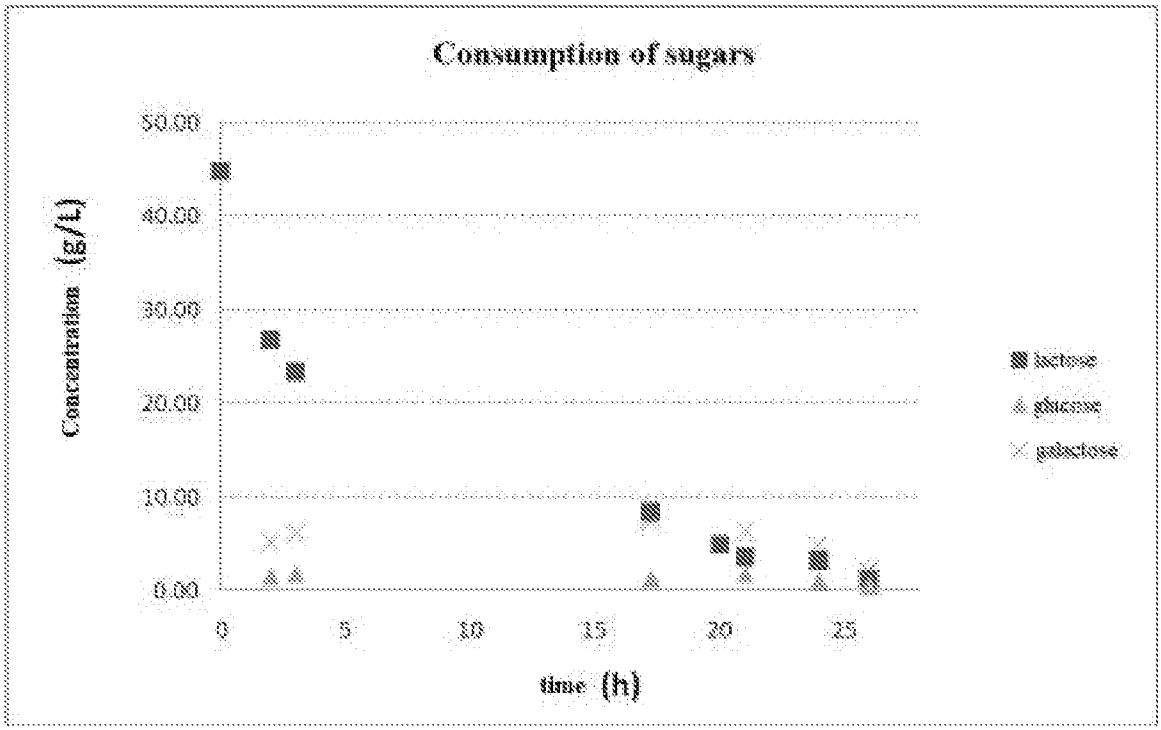
Consumption of sugars
Concentration (g/L)
50.00
40.00
30.00
20.00
10.00
0.00
0
5
10
15
20
25
time (h)
lactose
glucose
galactose

FIG. 5