Bubble bioreactor

65999.00

Operating instructions

Fig. 1

1 PURPOSE AND DESCRIPTION

Bioreactors (fermenters) are used for the production of biotechnological products by means of bacteria, yeasts, cell cultures, etc. Magnitudes such as the pH value, the temperature, thorough mixing, gassing, etc. must be very carefully adjusted in large bioreactors. The bubble bioreactor 65999.00 (fig. 1) is a very simple type of bioreactor. It consists of a long glass tube, the temperature of which can be controlled, fitted with an insert which goes in up to the tip. The lower orifice is used to introduce gas into the bioreactor, the two upper orifices are used for the addition of nutrient solution or to remove final products. In order to assure temperature regulation, the bioreactor can be integrated into a water circulation circuit over the two lateral hose couplings.

2 HANDLING

To begin with, the interior tube is introduced through the gasket ring of the screw-on cap GL 32 up to the stop and fastened by screwing the cap tight. The now ready bioreactor is fastened to a support with two clamps (fig. 2).

To vent the bioreactor, a small pointed glass tube is set in at the lower end so the end of the point will just stand above the lateral orifices in the interior tube. The small glass tube is connected to an aquarium pump by means of a flexible silicone pipe. The pump must be installed higher than the bioreactor, so the investigated suspension will not flow back into the pump when the latter is switched off, thus contaminating it.

To adjust a constant temperature in the bioreactor, the two hose couplings of the exterior glass mantle are connected to a recirculating thermostat (fig. 2). If the room temperature is sufficient for the production process, no exterior temperature regulation is required.

The bioreactor is filled with the suspension to be investigated up to approximately 1 cm above the upper end of the interior tube. A thermometer is fixed to the central upper orifice, the lateral upper orifice is closed with a cotton wad.

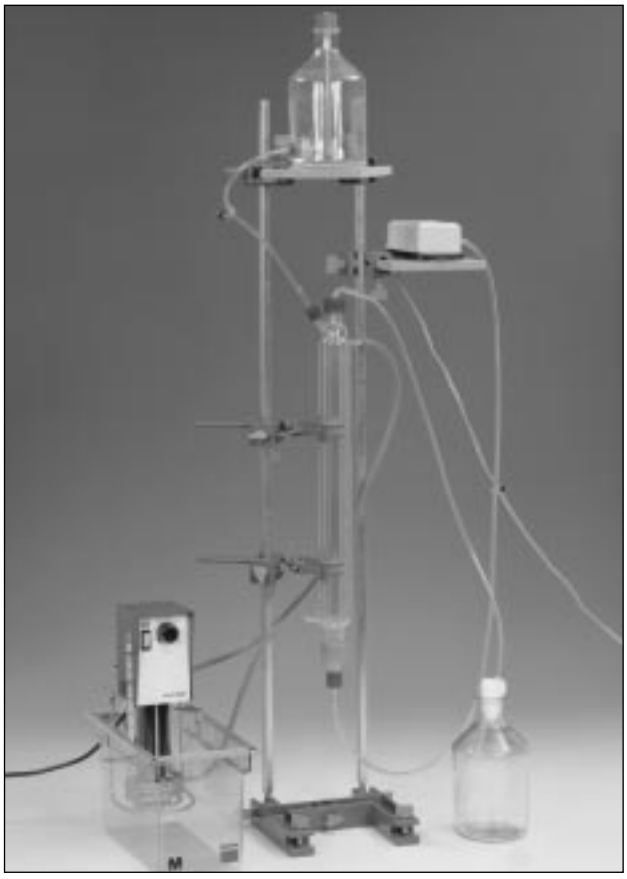

Fig. 2

After switching on the aquarium pump, air bubbles rise inside the tube, thus initiating an upwards movement of the suspension within the interior tube. When the suspension has reached the upper part of the tube, it falls again in the central tube, returning to the interior tube through the orifi-

ces specially foreseen for this purpose. The air bubbles thus assure a continuous thorough mixing of the suspension, thus avoiding the use of an electric stirring unit. The air inlet, and thus the degree of mixing, can be adjusted precisely by means of a hose clamp.
The above mentioned so-called batch process is a **discontinuous** process, that is, after a certain time, production is interrupted and the product can be collected. The bioreactor is then started again with a new filling load (batch). The **continuous** process, however, is being used more and more, because the required working time per kg of final product is much shorter. This method, for which the initial products are continuously fed into the reactor and the final products are continuously removed from the reactor, can also be carried out using a bubble bioreactor. The bottle containing the initial suspension is placed above the bioreactor (fig. 2). The suspension drips through a small pointed glass tube through the lateral upper orifice into the gap between the central and the interior tube. The drip rate is adjusted and kept constant by means of a hose clamp and of a venting tube which nearly reaches down to the bottom of the bottle. The final products run through a small pointed bend tube fastened in the central upper orifice of the bioreactor, and from there through a flexible silicone pipe into the lower bottle. The orifice of this bottle is closed with a cotton wad.

3 APPLICATION EXAMPLE

The fermentation of molasses to alcohol using yeast is shown as an example of the application of the bioreactor. Molasses is a by-product in the production of sugar and can be purchased at any sugar factory in Autumn of in Winter. The residual sugar contents of about 45 % are quite high. Molasses can also be purchased in health food shops.

To begin with, a suspension of molasses, nutrient salt, yeast, formaldehyde solution (against bacterial impurities) and oleic acid (antifoaming agent) is prepared as follows:

83.3 g of molasses
0.3 g of nutrient salt slurry
0.3 ml of formaldehyde dissolved in 250 ml of tap water
3.0 g of fresh yeast
2 drops of oleic acid

The nutrient salt slurry consists of:
50 g of $Ca(H_2PO_4)_2\ H_2O$
20 g of $(NH_4)_2\ SO_4$ dissolved in 75 ml of tap water
5 mg of $Mg\ SO_4$

The suspension, which must be fermented (also called “mash”), should have a pH value between 5 and 6. If the pH is not within these values, it is adjusted with sodium hydroxide solution or with sulphuric acid. The mash is filled into the bioreactor, and temperature is adjusted to 30 °C. The air inlet is adjusted so that aerobic conditions prevail within the interior tube (multiplication of the yeast) and anaerobic conditions prevail in the central tube (fermentation of the mash). 5 to 10 ml of air must be blown in per minute, in order to achieve this purpose.

After 24 hours, the fermented mash is distilled at 100 °C, the distillation product is weighed and the alcoholic content is determined from the density. If the density is for example 0.9887 g/ml, the distillate contains 6.5 $^{w}/_{o}$ or 65 g of ethanol according to the table. At the beginning of the fermentation process, a litre of mash contained 150 g of sugar. After total fermentation, and according to the reaction

$$C_{12}H_{22}O_{11} + H_2O \rightarrow 4\ C_2H_5OH + 4\ CO_2$$

this should yield 80.7 g of ethanol. The output thus is:
65/80.7 x 100 = 80.5 %

Table of densities for mixtures of ethanol and water

Density D^{20}_{20}	Weight % Ethanol	Density D^{20}_{20}	Weight % Ethanol
1,0000	0	0,9903	5,5
0,9991	0,5	0,9895	6,0
0,9981	1,0	0,9887	6,5
0,9972	1,5	0,9880	7,0
0,9962	2,0	0,9872	7,5
0,9953	2,5	0,9865	8,0
0,9945	3,0	0,9857	8,5
0,9936	3,5	0,9850	9,0
0,9927	4,0	0,9843	9,5
0,9919	4,5	0,9836	10,0
0,9911	5,0		

4. TECHNICAL SPECIFICATIONS

Volume:	280 ml
Exterior diameter:	50 mm
Length:	270 mm
Weight:	approx. 600 g

The glass components (DURAN), gaskets and screw-on caps can be sterilised (heat-proof up to 180 °C).

5. LIST OF ACCESSORIES

Bubble bioreactor		65999.00
Aquarium pump		64565.93
Recirculating thermostat		46994.93
Bath for thermostat		08487.02
PVC flexible pipe, *di* = 7 mm	(2x)	03985.00
Silicone flexible pipe, *di* = 5 mm	(3x)	39297.00
Small glass tube, bent to a right angle, 85x60 mm, package of 10		36701.52
Pointed small glass tube, 200 mm, package of 10		36701.63
PASS H - support base		02009.55
PASS support rod, *l* = 100 mm	(2x)	02028.55
PASS double clamp	(5x)	02040.55
Universal clamp	(2x)	37715.00
Blocks to place underneath the unit, 150 x 150 mm, package of 4	(2x)	02070.00
Flexible pipe clamp, 100 mm wide	(2x)	43631.10
Laboratory thermometer +15 ... +40 °C		38057.00

The following accessories are furthermore required for the **continuous process**:

Aspirator flask 1000 ml	34175.00
Narrow necked laboratory bottle, 1000 ml, colourless	41104.01
Rubber stopper with hole SB 19	39255.01
Rubber stopper with hole SB 29	39258.01

The following is required to perform distillation:

Heating hood 250 ml, 220 V WE	32255.93
Round bottom flask, 250 ml, GL 25/12	35812.15
Round bottom flask, 100 ml, GL 25/12	35841.15
Liebig condenser with additional top part GL 18/8	35817.15