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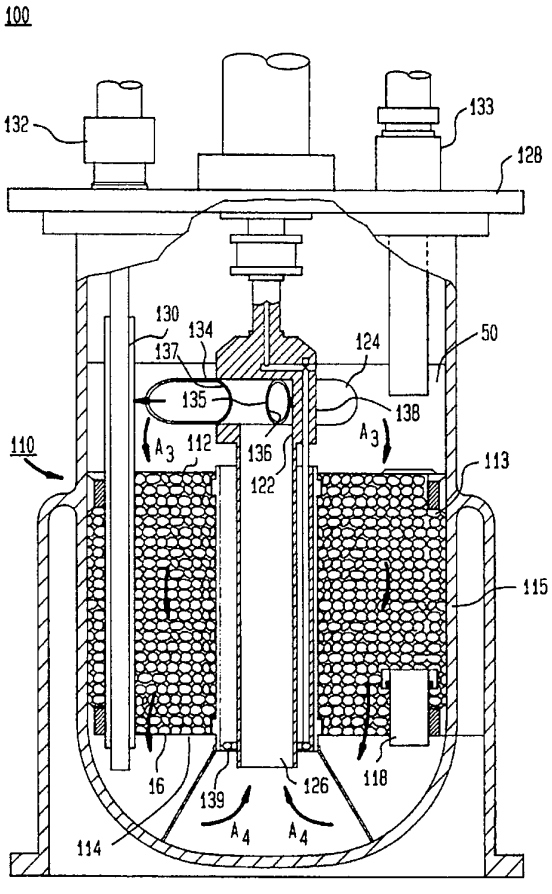
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(54) Title: METHOD AND APPARATUS FOR ANCHORAGE AND SUSPENSION CELL CULTURE

(57) Abstract

A method and apparatus for cultivating anchorage and non-anchorage cells in a reactor (100). A reactor (100) includes a basket type packed bed (113) and an internal recirculation device (124). The basket (113) includes a top (112) and bottom (114) portion for allowing medium to flow uniformly through the basket (113). Medium flows downwardly from the top (112) of the basket (113) through the basket (113) for providing gentle flow and preventing removal of cells from the carrier (16). Preferably, the carrier (16) is formed of a fiber matrix.



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TITLE: METHOD AND APPARATUS FOR ANCHORAGE AND SUSPENSION CELL CULTURE

INVENTORS: DAVID FREEDMAN, GUOZHENG WANG, AND AVINOAM KADOURI

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to a method and apparatus for cultivating anchorage and nonanchorage dependent cells.

2. Description of the Related Art

The cultivating of cells is desired for a variety of purposes such as for the production of hormones, enzymes, antibodies, vaccines and the like. Certain cell lines are anchorage dependent as is well known in the art. This means that the cells must adhere and grow as monolayers and multilayers anchored to a glass or plastic substrate.

Culture of anchorage dependent cells has conventionally been performed by attaching the cells to stacked petri dishes or by growing the cells in roller bottles. These approaches have the drawbacks of being unable to achieve high volumetric cell density or of having inadequate circulation necessary to the growth of the cells. In addition, cells tend to detach from their anchorage surface under conditions such as high or low serum concentrations, monolayer saturation or viscous

sheer caused by stirring or perfusion of the culture medium.

Alternative methods have been suggested for increasing the surface area for attachment to increase proliferation of cells. Microcarrier systems have been described in which minute beads are formed of a specific gravity such that the beads float in culture fluid under gentle agitation. Once suspended, cells attach, spread and grow on the external surface of the microcarriers.

In particular, U.S. Patent No. 5,114,855 describes using a microcarrier formed of microspheres. The microspheres have a diameter of less than about 60μm. Microcarrier systems have the disadvantage in that the necessity for suspension and mixing creates handling problems. Also, cells are subjected to mechanical stress which might result in cell rupture.

It is also known that certain types of cells can be grown in suspension. Suspension cell lines conventionally have been grown using a stirred-tank reactor. For certain applications, these suspension cells can be conveniently cross-linked to a solid matrix or encapsulated in gelatin, alginate, agarose, etc. to protect the cells from mechanical stress.

Conventional immurement methods have been used for facilitating the harvesting of cells in suspension culture. Examples of immurement techniques are: hollow

fiber (Hopkinson, J. "Hollow Fiber Cell Culture Systems For Economical Cell-Product Manufacturing", Bio/Technology 3:225-230 (1985)); and membrane reactors (Scheirer, W., "High Density Growth of Animal Cells Within Cell Retention Fermentors Equipped with Membranes", In: Spier, R.E. and Griffiths, J.B. (Eds.) Animal Cell Biotechnology, Vol. 3, pp. 263-281 (1988)). These techniques have the disadvantage that scale-up of these methodologies is difficult.

In addition, entrapment methods can be used for entrapping non-anchorage dependent cells within a matrix. A matrix used for entrapping cells is described in UK Patent No. GB 2178447. This patent describes a matrix of a nonwoven fabric having a pore diameter of from 10μm to 100μm for providing high internal volume with pores that are 1 to 20 times the volume of individual cells. A porous support sheet can be bonded to the matrix for providing dimensional stability and physical strength. The matrix sheets are used as liners for the bottom of petri dishes or are wound into a spiral and immersed in a container of medium. The matrix can also be used for growing anchorage dependent cells.

Packed bed reactors for the cultivation of anchorage dependent cells have been in use for many years. A packed bed bioreactor for growing either anchorage or nonanchorage dependent cells has also been

described in U.S. Patent No. 4,833,083. In this patent, a vessel contains a packed bed of a support or matrix material. Cells or cell components attach or are entrapped to the solid support material or matrix material of the packed bed. The solid support material is formed of diatomaceous earth, silica, alumina, ceramic beads, charcoal, polymeric beads or glass beads. Medium is pumped horizontally inwardly across the packed bed for radial flow of the medium through the packed bed.

Of possible general relevance are U.S. Patent Nos. 5,081,036, 4,888,294, 5,100,799 and 5,019,512 related to impellers for generating flow of the medium within the cell culture. Other patents of possible general relevance to the invention are U.S. Patent Nos. 4,087,327, 4,906,577, 5,079,161 and 5,126,269, 4,727,040 and 4,634,675 related to attachment surfaces and bioreactors.

A need exists for a device in which cells are protected against mechanical stress and the cells are provided with optimum levels of nutrients and oxygen. The highest possible cell densities or concentrations are needed to achieve economical and large quantity production of the cells of interest.

SUMMARY OF THE INVENTION

Briefly described, the present invention comprises an improved reactor for fermentation and tissue

culturing for both anchorage and non-anchorage dependent cells. The reactor is designed as a packed bed with a means which provides internal recirculation. Preferably, a fiber matrix carrier is placed in a basket within a reactor vessel. A top and bottom portion of the basket has holes therethrough for allowing medium to flow uniformly through the basket.

A recirculation means provides flow of medium through the space occupied by the fiber matrix for assuring a uniform supply of nutrients and the removal of wastes while simultaneously assuming that a negligible amount of the total cell mass is suspended in the medium. The combination of the basket and the recirculation means also provides a bubble free flow of oxygenated medium through the fiber matrix.

In comparison to other culturing systems, the present invention offers several significant advantages. With a fiber matrix carrier, the cells are protected against mechanical stresses such as agitation and foaming. The free medium flow through the basket provides the cells with optimum regulated levels of oxygen, pH and nutrients. The product can be continuously removed from the culture. The harvested product is free of cells and produced in low-protein medium which makes subsequent purification steps easier. Also, the unique design of this invention offers an

easier way to scale-up the reactor.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a front elevational view of a first embodiment of the reactor of the present invention.

Fig. 2 is a front elevational view of a second embodiment of the reactor of the present invention.

Fig. 3 is a front elevational view of a third embodiment of the reactor of the present invention.

Fig. 4A is a front elevational view of a carrier of the present invention.

Fig. 4B is a side elevational view of the carrier shown in Fig. 4.

Fig. 5 is a top plan view of a triangular shaped carrier.

Fig. 6 is a top plan view of a square shaped carrier.

Fig. 7 is a top plan view of a hexagonal shaped carrier.

Fig. 8A is a top plan view of a carrier having a plurality of contoured surfaces.

Fig. 8B is a side elevational view of the carrier shown in Fig. 8A.

Fig. 9 is a graph of results for oxygen uptake in the reactor of the present invention.

Fig. 10 is a graph of results for Mab production and harvested Mab.

Fig. 11 is a graph of impeller speed versus flow rate for the reactor of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

During the course of this description like numbers will be used to identify like elements according to the different figures which illustrate the invention.

Fig. 1 illustrates reactor 10 of a first embodiment of the present invention. Reactor 10 includes vessel 12 and basket 14 disposed within the vessel. Carrier material 16 is positioned within basket 14. Screens 18 and 20 form the top and bottom portions of basket 14 and allow flow of medium through basket 14. Screens 18 and 20 are preferably formed of stainless steel. Preferably, permeable silicone tubing forms sides 22 of basket 14 for enabling spent medium 50 to be dispensed from the basket.

Cover 24 is attached to the lower surface of screen 20 for directing medium 50 which has passed through basket 14 to impeller 28. Impeller 28 is rotated by shaft 40 for circulating medium 50 which has passed through basket 14. Impeller 28 includes downwardly facing blade 29 pushing medium away from basket 14 in direction A_1. Medium 50 flows upwards between vessel 12 and sides 22 of basket 14. Thereafter, medium 50 flows in a downwardly direction A_2 through screen 20 and through basket 14. O-ring 26 seals basket 14 to immersed

housing 41.

Additionally, impeller 28 can rotate at varying speeds depending on the needs of the system. In particular, the preferred rotation speed is between about 20 to about 225 rpm. The preferred flow rate is between about 0.2 to 2.5 cm/s. A working volume of 1.0 to 1000 liters can be used with the reactor. The preferred working volume is about 2.5 to about 5.0 liters.

Head plate 36 can be removed for introduction of the carrier and cells into vessel 12. Plugs 38, 39 extend from the upper surface of head plate 36 and receive medium during cell cultivation. Top guiding tube 30 is attached with O-ring 34 to plug 38 for directing medium to the top of basket 14. Bottom guiding tube is attached with O-ring 35 to plug 39 for directing medium to the bottom of basket 14.

Vessel 12 is provided with probe 42 attached to head plate 36 for measuring dissolved oxygen. Tip 43 of probe 42 extends below the surface of medium 50 above basket 14. A second dissolved oxygen probe 44 is positioned below basket 14. A pH probe 46 is attached to head plate 36. It will be appreciated that other sensors and control devices can be used with the present invention.

Fig. 2 illustrates reactor 100 of a second embodiment of the present invention. Vessel 110 houses

packed bed 113 of carrier material 116. Top screen 112 and bottom screen 114 surround packed bed 112. Packed bed 113 extends on either side of hollow shaft 126 to inside wall 115 of vessel 110. Port 132 aspirates PBS and medium through aspirating tube 130. Spent medium is removed from vessel 110 by tube 133. Aspirating tube 130 extends through packed bed 112 to the bottom of vessel 110.

Agitator 124 is attached to end 122 of hollow shaft 126. Agitator 124 includes tubulations 134 which project laterally from hollow shaft 126. Tubulations 134 are hollow and include a first end 135 defining an exit opening 136 and a second end 137 connected to an opening 138 in the hollow shaft 126. Fluid flows through hollow shaft 126 and exits through tubulations 134 out exit openings 136. Agitator 124 operates in a manner consistent with the agitator described in U.S. Patent No. 4,634,675 (the '675 patent) for creating a suction effect, the '675 patent is incorporated herein by reference. Agitator 124 directs fluid flow from exit openings 136 in downward direction A_3 through packed bed 113. Agitator 124 also sucks medium through hollow shaft 126 for directing flow of medium in direction A_4 upwardly through hollow shaft 126. The above-described reactors provide for flow and aeration of cell structures attached to a carrier positioned in the basket while minimizing

detachment of the cells from the carriers. Air is introduced from sparger 139 at the bottom hollow shaft 126 for providing an oxygenated medium and disengaging the bubbles at the upper fluid surface. Agitator 124 provides a recirculation means for providing bubble-free medium to the packed bed basket. The basket protects cells from turbulence created at higher stirring rates thereby providing greater oxygen exchange to increase cell growth.

In this embodiment, carrier 16 is a non-woven material. However, other materials such as polyester, nylon and inorganic materials can also be used.

In the alternative, carrier material 16 can be formed of a plurality of disks, as illustrated in Fig. 4A. Carrier 16 has a contoured upper surface 60, as shown in Fig. 4B. Upper surface 60 is raised a height of about .005 inch to about .015 inches above lower surface 62. Carrier 16 has a disk shape with a diameter of about 3/16 to about 3/8 of an inch.

Lower surface 62 of carrier 16 is formed of a non-woven screen material of a network of fibers. Examples of a screen material useful for practicing the invention include polyethylene, polypropylene and Teflon®. Teflon® is a registered trademark of Dupont, Delaware. It will be appreciated that other non-woven materials for the carrier and screen can be used within

the teachings of the present invention. Lower surface 62 can be laminated or bolted to upper surface 60.

Fig. 3 illustrates an alternate reactor 300 of a third embodiment of the present invention. Vessel 310 houses a packed bed 320 of carrier material 16. Top cap 314 is attached with gasket 312 to vessel 310. A pair of side ports 316 are positioned on either side of vessel 310. Side ports 316 can be used for sampling and changing media. Side cap 318 covers membrane 319. Side cap 318 provides a sterile seal of vessel 310.

O-ring 322 attaches screen cover 320 to base 315. Grooves 323 and 325 are formed in base 315. Screen cover 321 can be attached with O ring 322 either to groove 323 or groove 325 for altering the volume of packed bed 320. Preferably, the basket volume is about 100 ml for holding 10 grams of carrier 16 when screen 321 is attached to groove 323 and the basket volume is about 50 ml for holding 5 grams of carrier 16 when screen 321 is attached to groove 325. Preferably, the working volume of vessel 310 is about 500 mls with a total volume of about 1000 mls.

An impeller 324 is driven by a magnet for providing flow in the direction of arrows A_5 and A_6 from the top of packed reactor bed 320 towards impeller 324.

Attached metabolites can be easily removed with carrier material 16 without filtering from the system.

The reactor of the present invention has the advantage that separation of cells from secreted proteins and waste products is simplified because they remain trapped in carrier material 16 during perfusion. The reactor provides a high surface to volume ratio for cell growth and avoids exposure of cells to gas liquid interfacial forces and shear from the impeller. Anchorage dependent cells attach to the surface of carrier material 16. Cells grown in suspension are retained within the packed bed of carrier material 16.

The reactor of the present invention has the advantage that separation of cells from secreted proteins and waste products is simplified since both anchorage-dependent and suspension cells remain trapped in carrier material 16 during the culture process.

Many anchorage dependent cell lines have been attached to carrier material 16. These include: CHO, rCHO, C127, BHK and VERO. Surprisingly, many suspension lines have also been attached to the carrier material 16 such as: hydridomas e.g.: DA 4.4; γ69B, 123A and 127A and insect lines e.g.: TN-368 and Sf -9. Those skilled in the art would recognize that other types of cells can be propagated with the reactors of the present invention.

Figs. 5-8 illustrate alternate shapes of carrier 16. Upper surface 70 of carrier 16 can have a triangular shape as shown in Fig. 5, or upper surface 80

can have a square shape, as shown in Fig. 6. Alternatively, upper surface 90 of carrier 16 can have a hexagonal shape, as shown in Fig. 7. In the above carriers, lower surface 65 is correspondingly shaped to the shape of the upper surface. It will be appreciated that other shapes could be used for carrier 16.

Figs. 8A and 8B illustrate an alternative embodiment of the carrier shown as carrier 200. Concentric contoured upper surface 202, 204, 206 are raised above respective lower surfaces 208 and 210. This embodiment has the advantage of channelling of medium in grooves 212 and 214.

The reactor design of the present invention has the advantage of reducing channelling through the packed bed and providing uniformity of flow in the reactor. The use of the carrier in a packed bed has the advantage of supporting propagation of hybridomas and promoting cell growth at a high cell density per unit of the reactor. Hybridomas can be retained within the packed bed of carrier material in a productive state under perfusion with low levels of serum or serum free medium. The reactor is capable of being easily scaled-up because of the low pressure drop across the basket.

EXAMPLE 1

To illustrate the use of the reactor of the present invention for growth of cells or cell cultures,

the reactor is packed with polyester non-woven disk carrier. 110 grams of polyester disks were packed into the reactor basket. Cells are mouse-mouse hybridoma, DA4.4 (ATCC HB-57) producing an IgG1 against human IgM. Dulbecco's modified Eagle medium (DMEM) was used for growth of cells in the reactor. Additional supplements were added:

Primatone RL (Sheffield)	2.5 g/L
Oxalacetic Acid	150 mg/L
Mercaptoethanol	3.92 mg/L
Insulin	75.5 µg/L
Sodium Pyruvate	110 mg/L
Streptomycin	100 mg/L
Penicillin	100 Units/L
Bovine Calf Serum (Hyclone)	5% by Volume

PBS (without Ca^{+2}, Mg^{+2}) was added as media to the vessel and the cover is attached to the vessel. Media was added and incubated overnight. The reactor was seeded.

The culture conditions were as follows:

Temperature	37°C
Agitation	60-140 rpm
Top Dissolved Oxygen (DO)	50%
Bottom DO	>25%
pH	7.2
Medium flowrate	45-100 ml/sec

During cultivation, samples were taken and analyzed off-line. Glucose, Lactate and Ammonia concentrations of the medium were determined by enzymatic assays (Sigma Chemical Co.). Monoclonal antibody concentration was determined by radial immunodiffusion assay. Trypan blue cell counts and citric acid/crystal violet nucleus counts by hemocytometer were used to determine the cell numbers in the media and attached to the carrier respectively.

EXAMPLE 2

Cell Attachment

The cell attachment rate in the reactor was determined. After inoculation, cells were maintained in the medium without perfusion to allow the cells to attach to the carrier material 16 inside the basket. The following table illustrates the DA 4.4 cell attachment in the reactor.

TABLE

Innoculation Cells (cells/ml)	After 5 hours cells attached to material 16 (% of inoculum)	After 1 day cells attached to material 16 (% of inoculum)	After 30 days cells attached to material 16 (% of inoculum)
1.7×10^5	97.1	99.5	99.9

These results indicate that the hybridoma cells were easily trapped in the disk carrier, even if continuous stirring was used during the attachment period.

EXAMPLE 3

Cell cultivation was operated for 36 days for hybridoma 123A producing an IgG type antibody against human gamma interferon. Nuclear counting was performed after the reactor was disassembled. The calculated values indicated an inoculum concentration of 1.8×10^6 cells/cm^3 in the packed bed of material 16 and 9.4×10^7 cells/cm^3 at the end of the experiment. In addition, 5×10^4 cells/ml were measured in the supernatant which

represents less than 0.1% of the cells in the reactor.

EXAMPLE 4

Comparison of Cell Growth and Productivity

Fig. 9 illustrates oxygen uptake rate during hybridoma 123A cell cultivation with a packed bed reactor of the present invention. The dissolved oxygen concentrated at the top of the basket was controlled at 50% by gas flow rate and the dissolved oxygen content at the bottom of the basket was greater than 25% by liquid flow via the impeller.

The following table illustrates MAb productivity for various modes of production for the culture of 123A cells.

MODE OF CELL CULTIVATION		PRODUCT YIELD (mg/day/L)
Repeated batch Suspension	T-Flask Stirred Tank	23 20
Perfusion Immobilization	Packed-bed Reactor	277

The results indicate a 14 fold increase for the packed bed reactor of the present invention as compared to a stirred-tank reactor.

Fig. 10 illustrates MAb production from hybridoma 123A in a packed bed reactor system. MAb produced was about 0.5 grams per day during the production phase and total yield was about 11.5 grams.

EXAMPLE 5

Anchorage - Dependent Cell Culture

rCHO cell line was cultivated in the reactor for 24 days. DMEM/F12 culture medium was used with supplements of 1-5% calf serum. The total volume of the reactor was 2.2 liters, packed by 65 grams carrier material 16. Culture condition was: temperature 37°C, pH 7.2, dissolved oxygen concentration 50%, perfusion rate was maintained at 3.0-3.4 liter per day since day 7. The continuous operation led to a 60 fold increase in cell density reaching 1.2 x 10^8 cells per cm^3 of bed volume at the end of a 24 day run.

EXAMPLE 6

The average shear stress (τavg) on the surface of the cells in the packed-bed can also be estimated by the following equation.

$$\tau\ avg = \frac{\Delta p}{L_b(S/V)}$$

where Δp is the pressure drop in the bed; L_b the height of the bed; and S/V the fiber surface-to-volume ratio of the bed reactor. The calculated τ avg. at liquid velocity of 1.0 cm/s was found to be 0.6 dyn/cm^2. The shear stress is significantly lower than what is needed to cause shear damage to the cells.

A pitot tubing method was used to determine

liquid flow rate for the reactor shown in Fig. 2. A tracer (acid and base) method was also used to measure liquid flow rate.

A 2.2 liter reactor as shown in Fig. 2 was used. The cross section surface of the packed bed was $100cm^2$. The superficial velocity was measured to be 0.65cm/s to 1.76 cm/s at impeller speeds of 80 - 200 rpm as shown in Fig. 11. These results indicate that the liquid flow rate provided by the impeller is sufficient and meets the oxygen and nutrient demands of the immobilized cells.

Pressure drop of the disk bed, Δp, was measured with a glass tube manometer at different liquid flow rates and was calculated by the equation:

$$\Delta p = \Delta h \ (\sigma_L - \sigma_o) \ g = k\mu Lu$$

where Δh is manometer reading (cm); σ_L is density of liquid (g/cm^3); σ_o is density of air (g/cm^3); g is gravity acceleration (cm/s^2); μ superficial viscosity (poise). The resistance coefficient of the disk bed, k, was calculated from pressure drop measurements to be 2×10^4 cm^2.

Low pressure drops provide for scale-up of the reactor. A volume of 1 to 1000 L can be used in the reactor while maintaining cell densities.

EXAMPLE 7

It is known that insect cells are shear

sensitive and can be damaged by gas sparging. It is also known that insect cells have a high oxygen demand in comparison to mammalian cells.

To illustrate the use of the reactor of the present invention for growth of insect cell lines, the reactor shown in Fig. 2 was used for a TN-368 cell line. A serum free medium Ex-cell 400 manufactured by JRH Bioscience, Kansas was used with the reactor. The growth media was added to the reactor and the reactor contents were incubated overnight. The following is a table of the volume of the reactor.

Equipment:	2.2 liter basket reactor
Working volume:	1.3 liter
Total volume:	2.2 liter
Basket volume:	0.65 liter
Carrier:	Material 16
Weight:	65 grams

Inoculation conditions were pH 6.2, temperature 27°C, dissolved oxygen 50%. After one hour of inoculation all cells were attached to the carrier in the packed bed in the basket. Glucose concentration decreased from 2.0 g/liter to 0.83 g/liter during the first two days. Perfusion was initiated on day 3 at 1.05 liters per day. From day 9 to day 13 the perfusion rate was maintained at 1.53 liters per day.

Trypan blue cell counting was performed after 13 days. The total cells in the 0.65 liter packed bed was 5.6×10^{10} and the cell density was 8.6×10^{7}. This results in a cell density that is 20 times greater as

compared to conventional suspension batch culture systems. The cells were distributed uniformly in the carrier of the packed bed.

The present invention has the advantage of promoting cell growth on a carrier while producing gentle flow of growth medium over the carrier. Both anchorage and suspension cells are entrapped within a carrier. The carrier provides a high surface to volume ratio for cell growth which results in high cell densities and a carrier having a contoured surface promotes attachment and continuous growth of hybridoma cells, thereby having high Mab production rates. The reactor provides gentle flow of medium downwardly through a basket containing the carrier for reducing removal of cells from the carrier. Scale up is possible due to the low pressure drop across the basket and column, and oxygen uptake rate can be calculated and used to optimize perfusion. The reactor can be used with a variety of anchorage dependent cells and suspension cells.

While the invention has been described with reference to the preferred embodiment thereof, it will be appreciated by those of ordinary skill in the art that modifications can be made to the structure and form of the invention without departing from the spirit and scope thereof.

WE CLAIM:

1. An apparatus for use in cell cultivation comprising:

a vessel having a closed bottom portion and side walls extending from said closed bottom portion;

basket means including a top and bottom portion with holes therethrough and side portions positioned between said top and bottom portion, said basket positioned in said vessel; and

an impeller positioned below said basket for circulating fluid,

wherein fluid flows from said bottom of said vessel between said side walls of said vessel and said sides of said basket to said top of said vessel and returns to said bottom of said vessel through said basket.

2. The apparatus of claim 1 wherein said basket includes an upper and lower screen forming said top and bottom portions of said basket.

3. The apparatus of claim 2 further comprising a removable cover attached to a top portion of said vessel.

4. The apparatus of claim 3 further

comprising a reaction medium inlet positioned through said cover.

5. The apparatus of claim 4 wherein said sides of said basket are formed from silicone tubing.

6. The apparatus of claim 5 wherein said impeller includes a downwardly facing blade for pushing said medium away from said basket.

7. The apparatus of claim 6 further comprising:

a carrier positioned in said basket, said carrier having a first surface formed of a non-woven screen material and a second surface formed of a non-woven material, said second surface being contoured and raised above said first surface wherein cells are immobilized on said carrier.

8. The apparatus of claim 7 further comprising magnetic driving means for rotating said impeller.

9. The apparatus of claim 8 wherein a base extends within said basket, said base including at least one groove wherein said upper screen attaches to said

groove.

10. The apparatus of claim 9 wherein said cells are selected from the group of: rCHO; C127; BHK; VERO; DA4.4; γ69B; 123A; 127A; TN-368 and Sf-9.

11. An apparatus for use in cell cultivation comprising:

a vessel having a closed bottom portion and side walls extending from said closed bottom portion;

a hollow tube displaceably positioned in said vessel having a lower open end;

a basket including a top and bottom portion with holes therethrough, said basket extending around said hollow tube to the side walls of said vessel; and

agitator means affixed to said hollow tube, said agitator including tubulation means having an input opening coupled to said hollow tube and an exit opening,

wherein fluid flows from said bottom of said vessel through said hollow tube to the top of said vessel and returns to the bottom of said vessel through said basket.

12. The apparatus of claim 11 wherein said basket includes an upper and lower screen forming said top and bottom of said basket.

13. The apparatus of claim 12 further comprising a reaction medium inlet positioned through said cover.

14. The apparatus of claim 13 further comprising a sparger positioned at the bottom end of said hollow tube.

15. The apparatus of claim 14 further comprising a carrier positioned in said basket, said carrier having a first surface formed of a non-woven screen material and a second surface formed of a non-woven material, said second surface being contoured and raised above said first surface wherein cells are immobilized on said carrier.

16. The apparatus of claim 15 wherein said cultivated cell are selected from the group of: rCHO; C127, BHK; VERO; DA4.4; γ69B; 23A; 127A; TN-368 and Sf-9.

17. A method for cell cultivation comprising the steps of:

placing a carrier within a basket, said basket positioned within a vessel;

adding cells to said basket, said cells being immobilized on said carrier; and

circulating medium through the basket, wherein said medium flows to the bottom of said vessel through said basket.

18. The method of claim 17 further comprising the steps of providing means for adding said medium to said vessel.

19. The method of claim 18 wherein the step of circulating medium through said basket is carried out by positioning an impeller below said basket.

20. The method of claim 19 wherein the step of circulating medium through said basket is carried out with an agitator means positioned above said basket, said basket including a hollow tube, said agitator means including tubulation means, said tubulation means having an inlet attached to said hollow tube and an outlet, wherein fluid flows from the bottom of said vessel through said hollow tube to said inlet and through said outlet of said tubulation means to the top of said vessel and returns to the bottom of said vessel through said basket.

FIG. 1

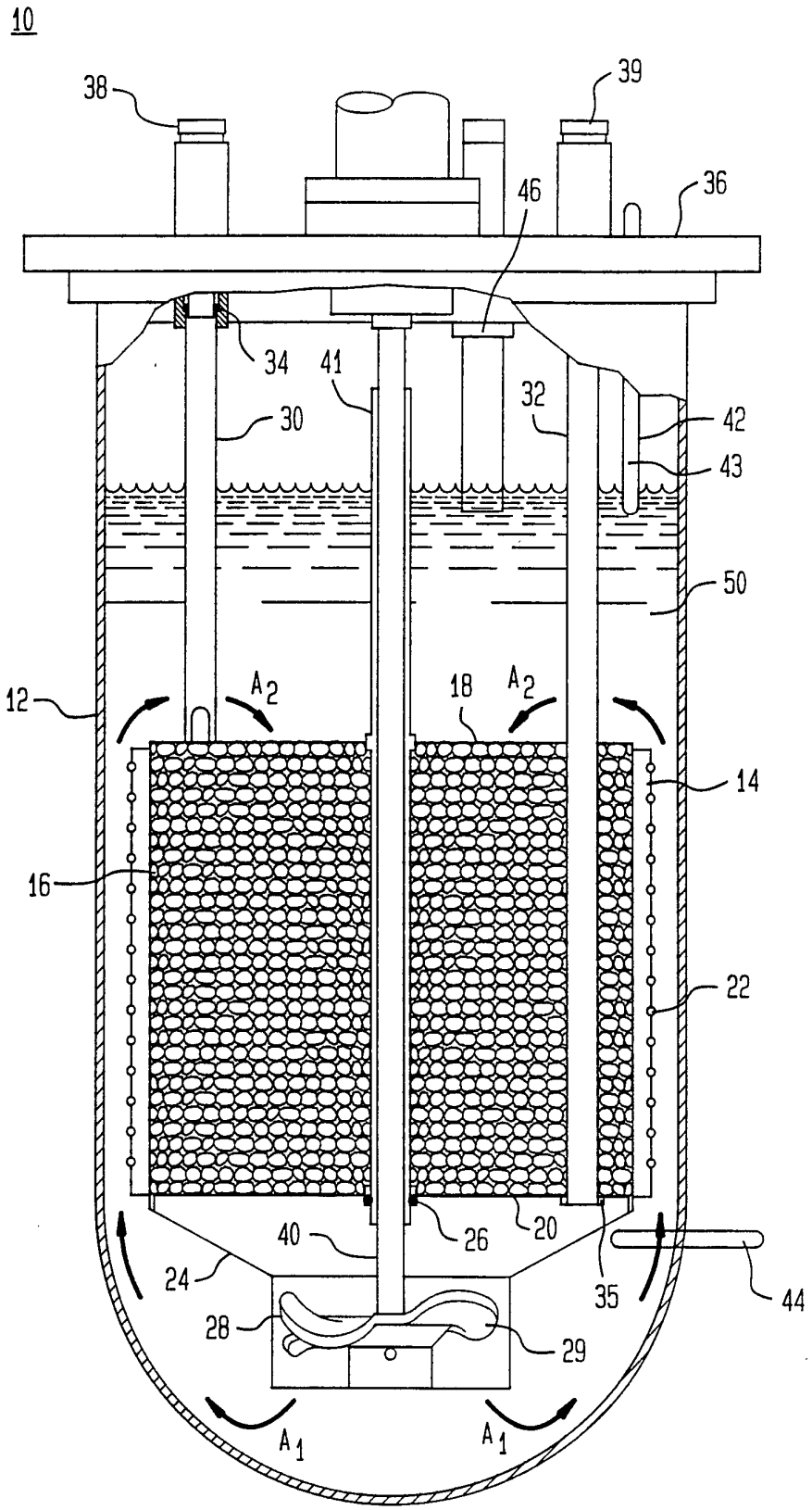

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A1

FIG. 2

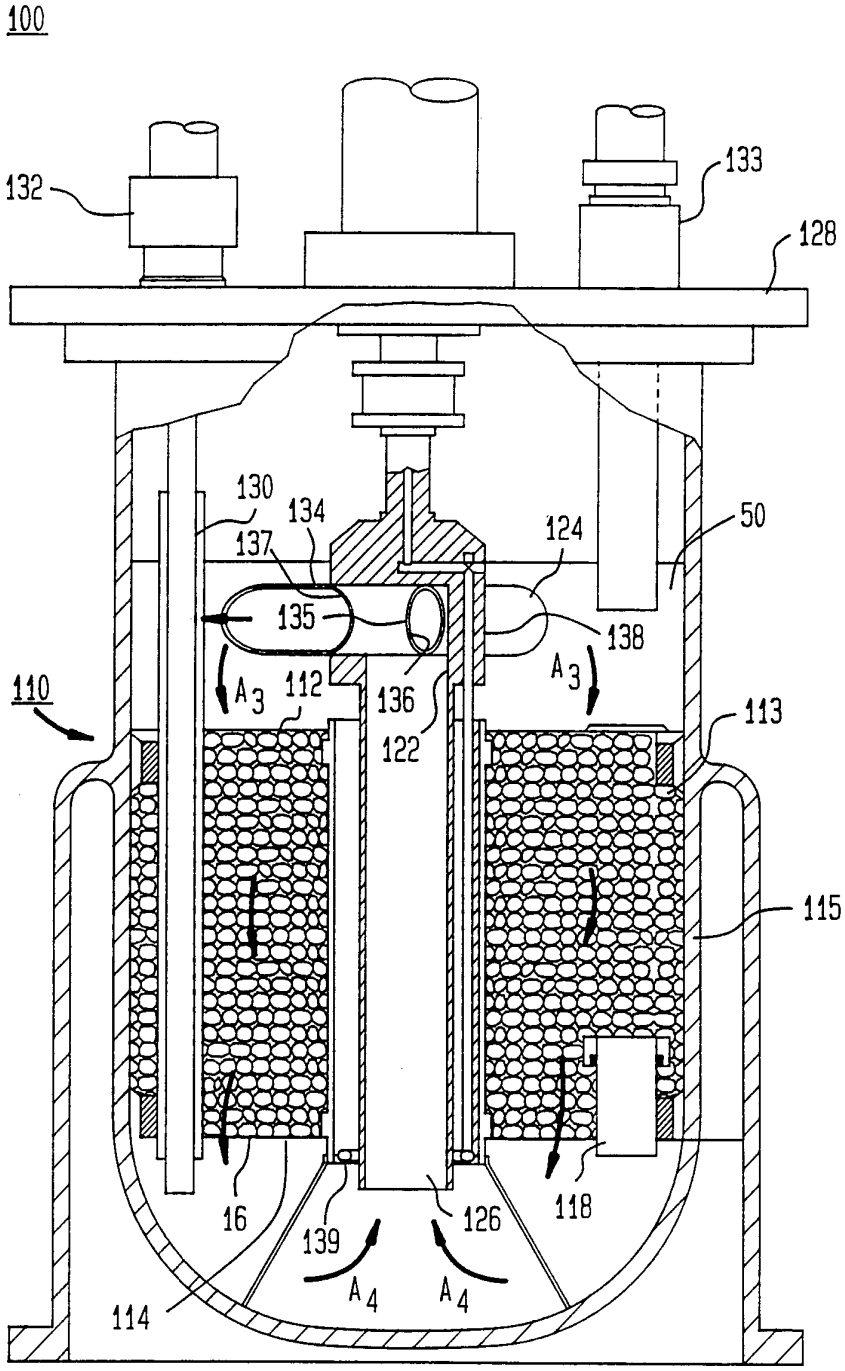

100
132
133
128
130
134
137
124
50
135
138
110
A3
112
136
122
A3
113
115
16
139
126
118
A4
A4
114

FIG. 3

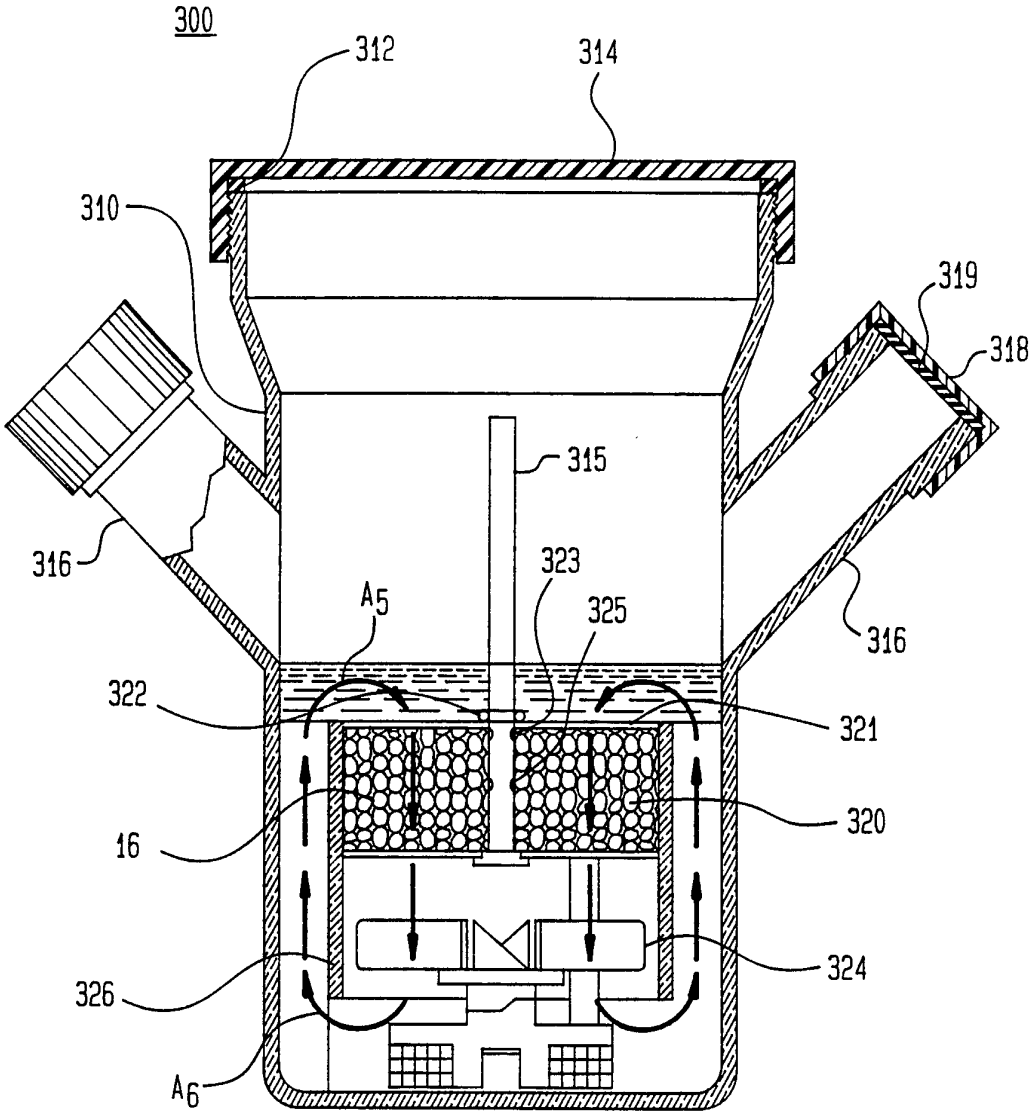

300
312
314
310
319
318
315
316
323
325
A5
316
322
321
16
320
324
326
A6

FIG. 4A

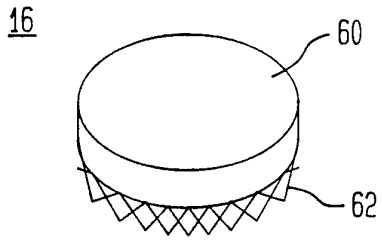


FIG. 4B

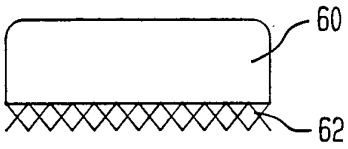


FIG. 5

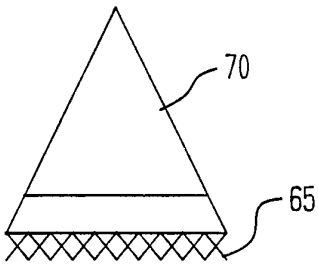


FIG. 6

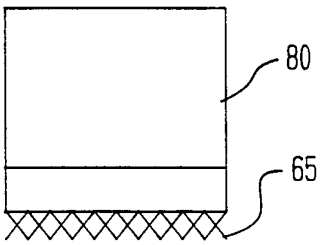


FIG. 7

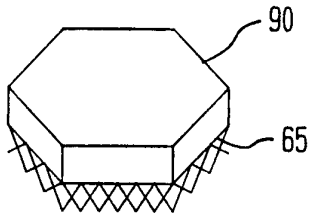


FIG. 8A

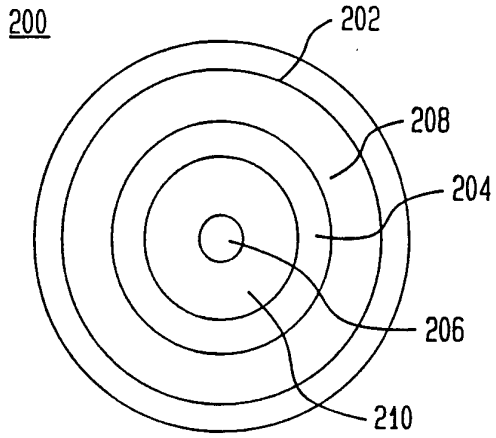


FIG. 8B

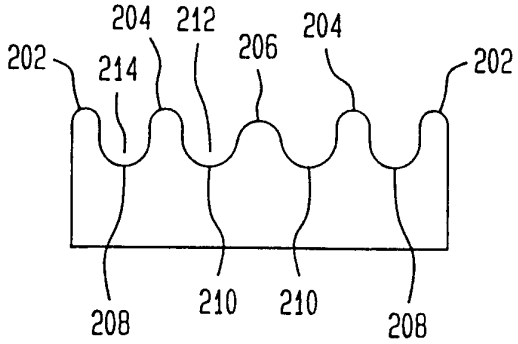


FIG. 9

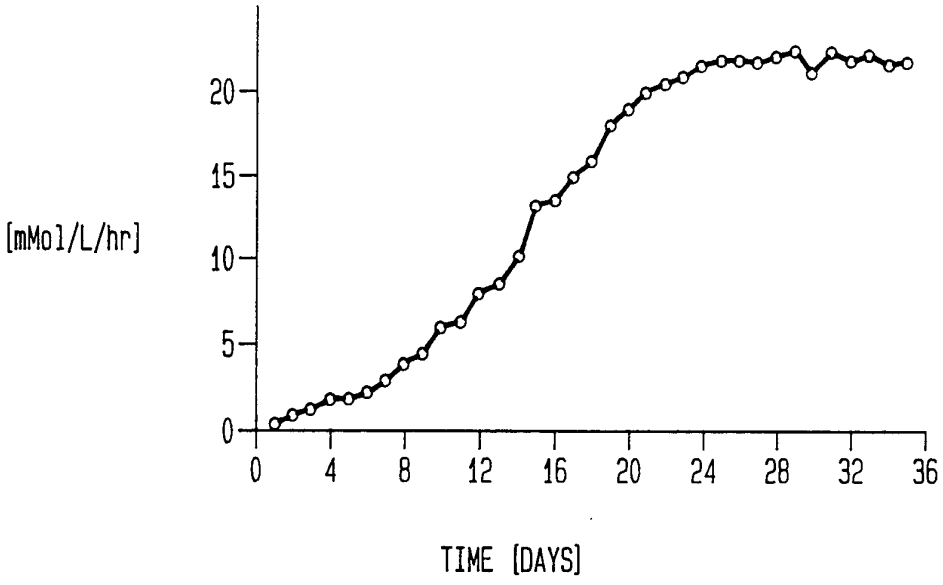

[mMol/L/hr]
20
15
10
5
0
0
4
8
12
16
20
24
28
32
36
TIME [DAYS]

FIG. 10

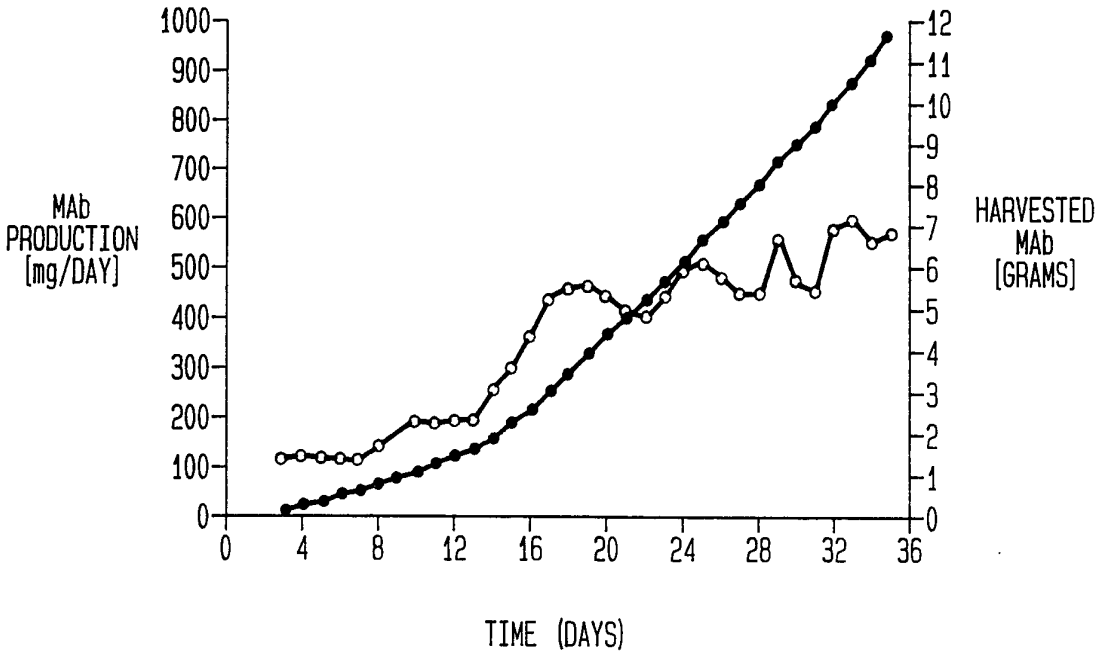
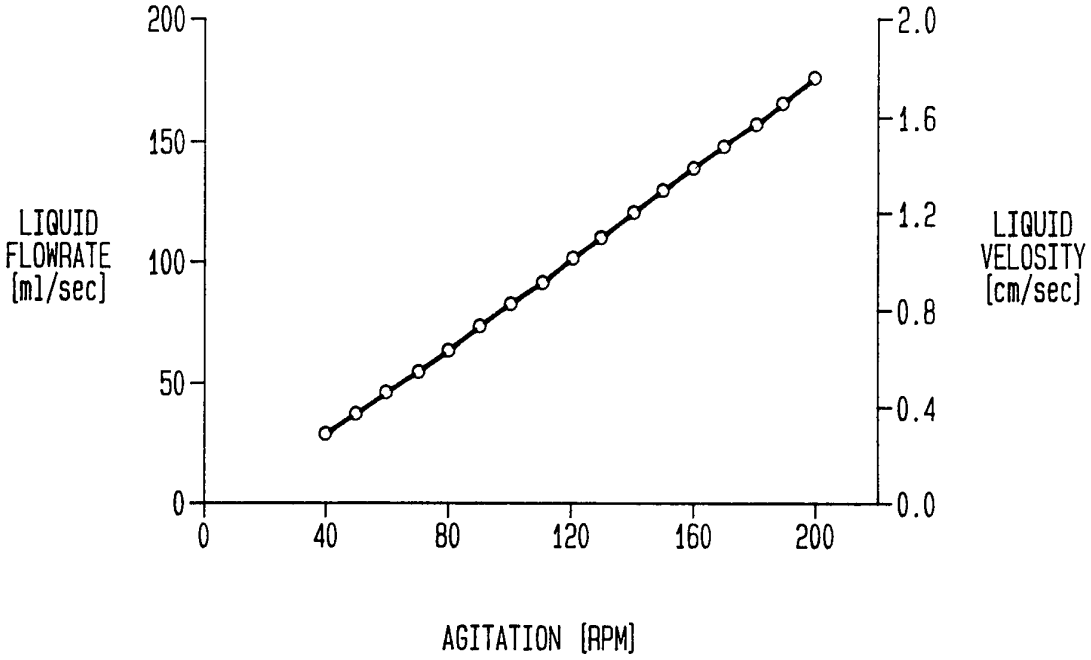

MAb PRODUCTION [mg/DAY]
1000
900
800
700
600
500
400
300
200
100
0
HARVESTED MAb [GRAMS]
12
11
10
9
8
7
6
5
4
3
2
1
0
0
4
8
12
16
20
24
28
32
36
TIME (DAYS)

FIG. 11


LIQUID FLOWRATE [ml/sec]
200
150
100
50
0
2.0
1.6
1.2
0.8
0.4
0.0
LIQUID VELOSITY [cm/sec]
0
40
80
120
160
200
AGITATION [RPM]

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/01089

A. CLASSIFICATION OF SUBJECT MATTER
IPC(5) :C12N 5/00; C12M 3/00
US CL :435/240.23, 313
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)

U.S. : 435/240.23, 204.24, 240.4, 284-286, 288, 299, 313-316, 813; 422/211, 218, 311; 210/615-617, 150, 151

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)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	US, A, 4,649,114 (MILTENBURGER ET AL) 10 March 1987, see entire document.	1-3 ------- 4-20
Y	GB, B, 2,178,447 (BOHAK ET AL) 28 December 1989, see pages 8-10.	7-20
Y	US, A, 4,727,040 (FREEDMAN ET AL) 23 February 1988, see entire document.	7-20
Y,P	US, A, 5,246,855 (KATINGER ET AL) 21 September 1993, column 3, lines 9-12.	11-20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"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
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"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
"E"	earlier document published on or after the international filing date	"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
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 20 APRIL 1994	Date of mailing of the international search report 05 MAY 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer WILLIAM H. BEISNER Telephone No. (703) 305-0196

Form PCT/ISA/210 (second sheet)(July 1992)*