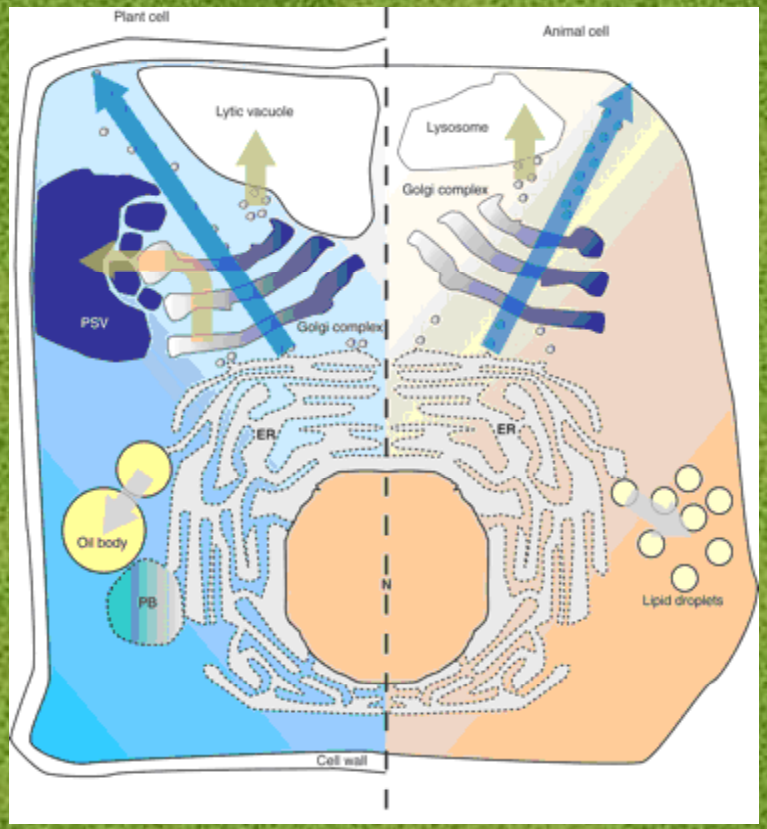
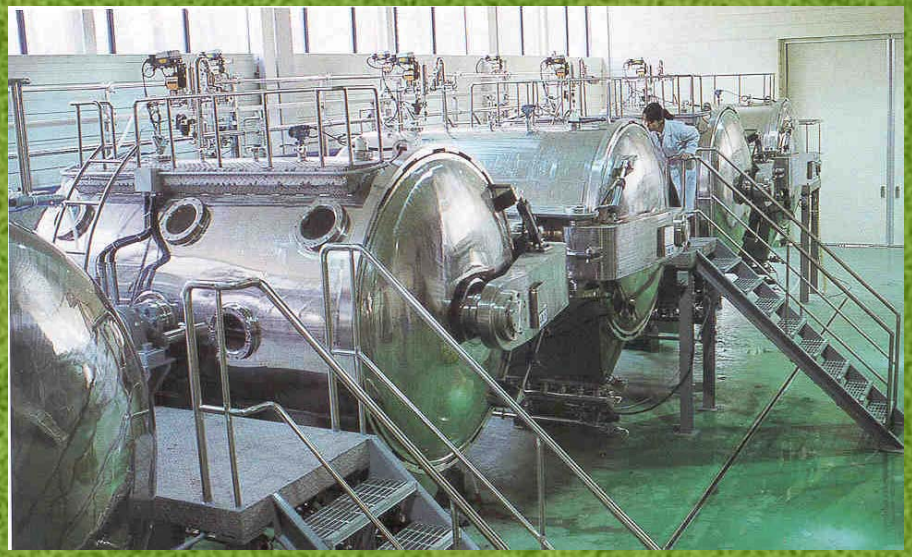
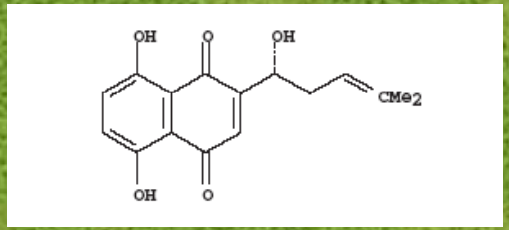
BIORREACTORES

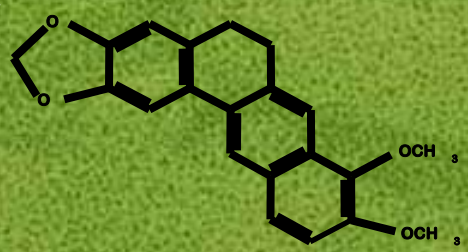




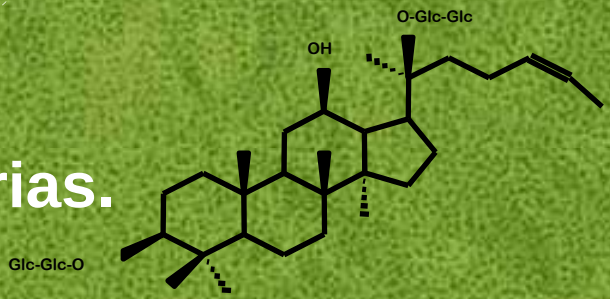
En la actualidad se encuentran desarrollados a escala industrial de producción los procesos para obtención de shikonina por cultivo de células de *Lithospermun erythrohizon*, puesto a punto por la Industrias Petroquímicas Mitsui,



así como la producción de berberina



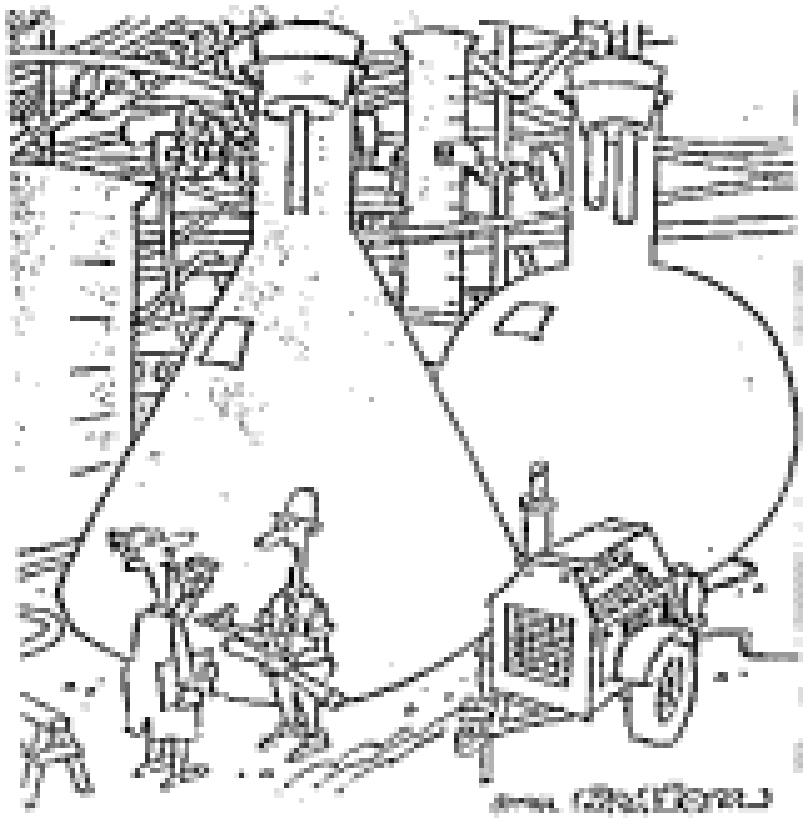
y biomasa de ginseng en otras industrias.



In order to cultivate plant cells in a large-scale, fermentors with different sizes are useful.

Various types of fermentors have been designed by many researchers since the end of 1950's.

Pero no siempre ha sido fácil……



"Got a few problems going from lab scale up to full-scale commercial."

Los procesos industriales requieren de tres puntos fundamentales:

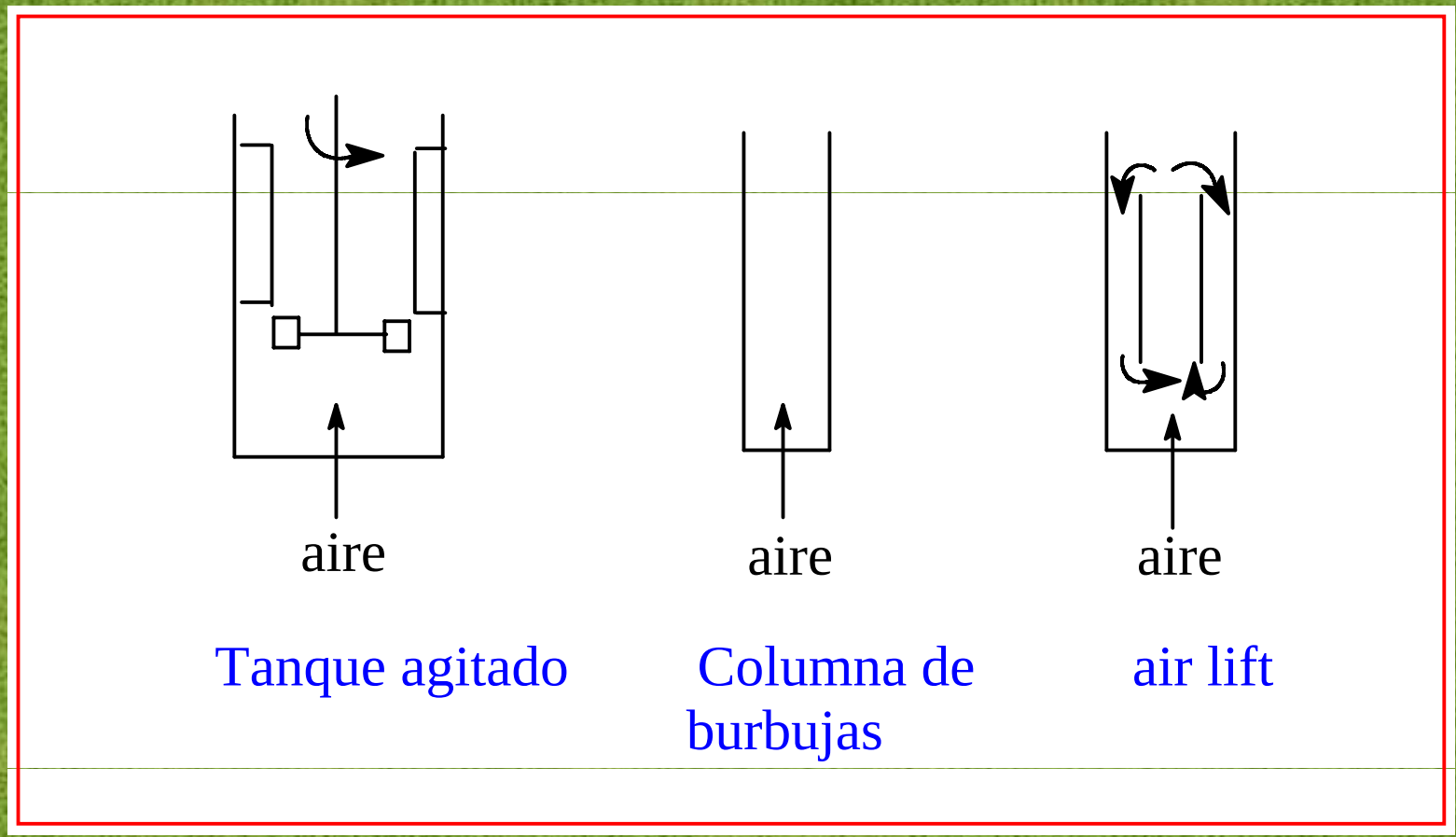
- **Crecimiento eficiente en grandes volúmenes**
- **Acumulación de metabolitos secundarios en el cultivo**
- **Si el proceso implica bioconversión o producción de enzimas, que esto ocurra en las condiciones de operación del proceso**

Es necesario ajustar algunos parámetros :

- tensión de oxígeno
- dióxido de carbono
- pH
- Agitación
- Mezclado
- densidad del cultivo

Características	células microbianas	células vegetales dediferenciadas	consecuencias en el biorreactor
Tamaño	2-10 μm	10-200 μm	Rápida sedimentación, mayor sensibilidad al corte
Células individuales	pueden obtenerse	forman agregados	Rápida sedimentación
Velocidad de crecimiento	Alta t_d 1-2 horas	Baja t_d 2-5 días	Largos procesos, problemas para mantener esterilidad
Densidad del inóculo	pequeño	5-20 %	Problemas de manipuleo. Dificulta la posibilidad de escalado
Sensibilidad al esfuerzo de corte	no sensitivo	sensitivo/tolerante	Disminución de la velocidad de agitación
Aireación	alta	baja	Baja demanda de oxígeno, bajo $K_L a$

Diseños básicos de biorreactores para múltiples propósitos



Factores fundamentales que afectan el diseño:

transferencia de oxígeno

- bajos requerimientos de oxígeno (1-10 mmol.h-1l-1)
  (microorganismos 5-200 mmol.h-1l-1)
- baja solubilidad de O_2 (sustrato limitante)
- La medida de transferencia de O_2 es el $\mathbf{K_L a}$ (coeficiente volumétrico de transferencia de oxígeno)

$K_L a$ (coeficiente volumétrico de transferencia de oxígeno)

$$dCL/dt = K_L a\ (C^* - CL)$$

$$\ln (C^* - CL\ ) = K_L a\ .\ t$$

$K_L a$ es afectado por:

- la velocidad del flujo de aire
- el grado de agitación
- las propiedades reológicas del cultivo y los antiespumantes

agitación  **transferencia de oxígeno**

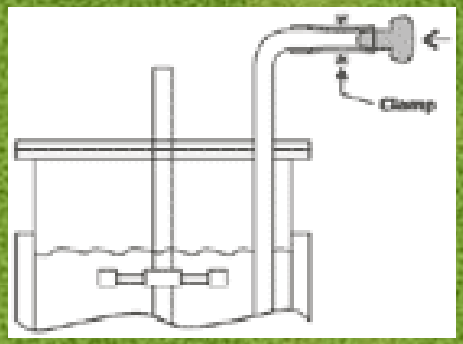
- Aumenta el área de transferencia de oxígeno por la formación de pequeñas burbujas
- Retarda el escape de burbujas desde el líquido
- Previene la coalescencia de las burbujas de aire
- Disminuye el grosor de la interface gas/líquido al crear un flujo turbulento

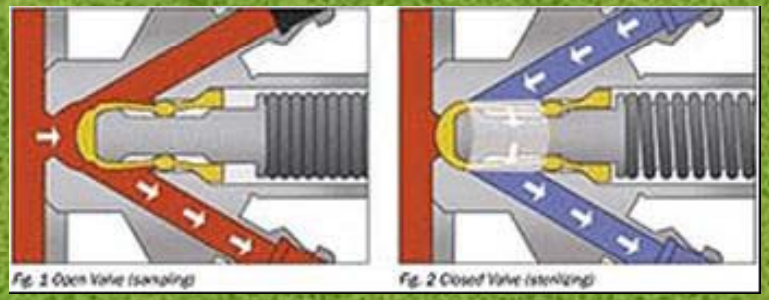
Efecto de corte

MIXING/AERATION
AS AN ESSENTIAL MASS TRANSFER FACTOR

- Supply of the nutrient components to cells (more precisely, cell agglomerates)
- Prevention of sedimentation
- Securing of heat transfer
- Solubility of the nutrient's components which are less soluble.

The sampling construction should be such so that measures for preventing non-sterility before and after the sampling be avoided.





The most simple vessel is a carboy system described by **Tulecke and Nickell in 1959**

A rubber-stoppered 20 L carboy

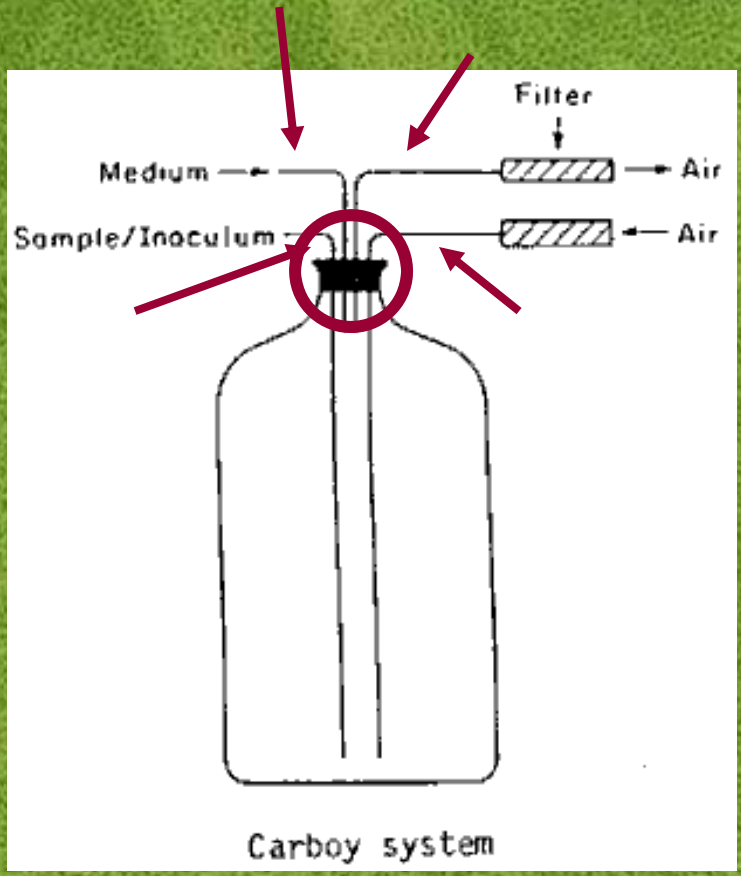
fitted with four tubes:

air-in

air-out

medium-in

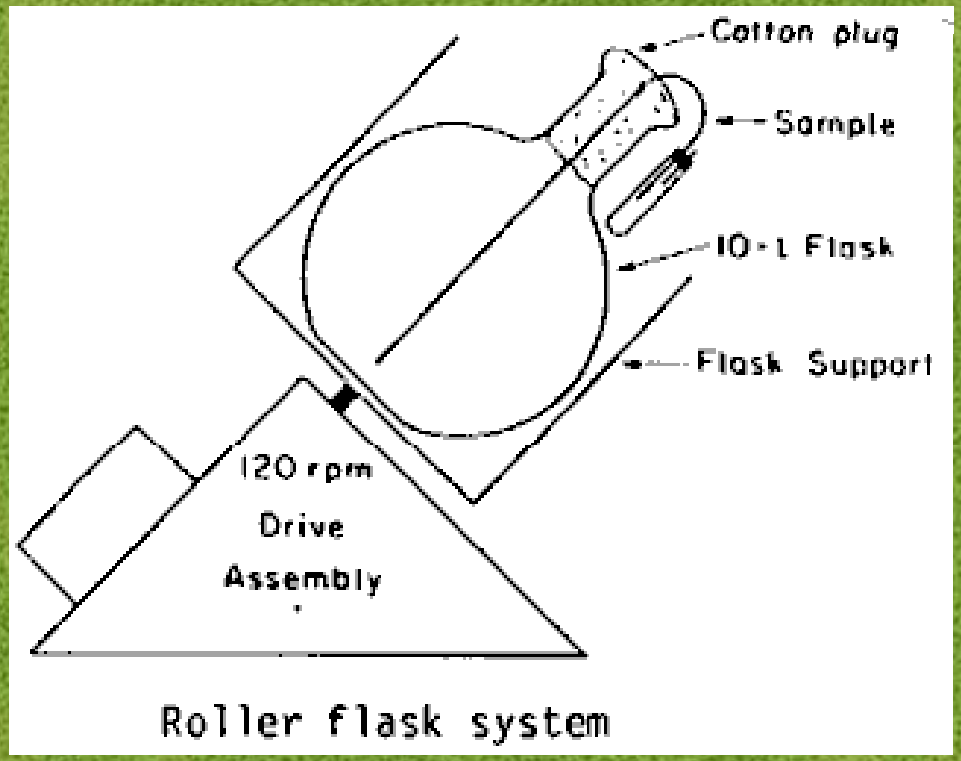
sample-out/Inocula in



Carboy system

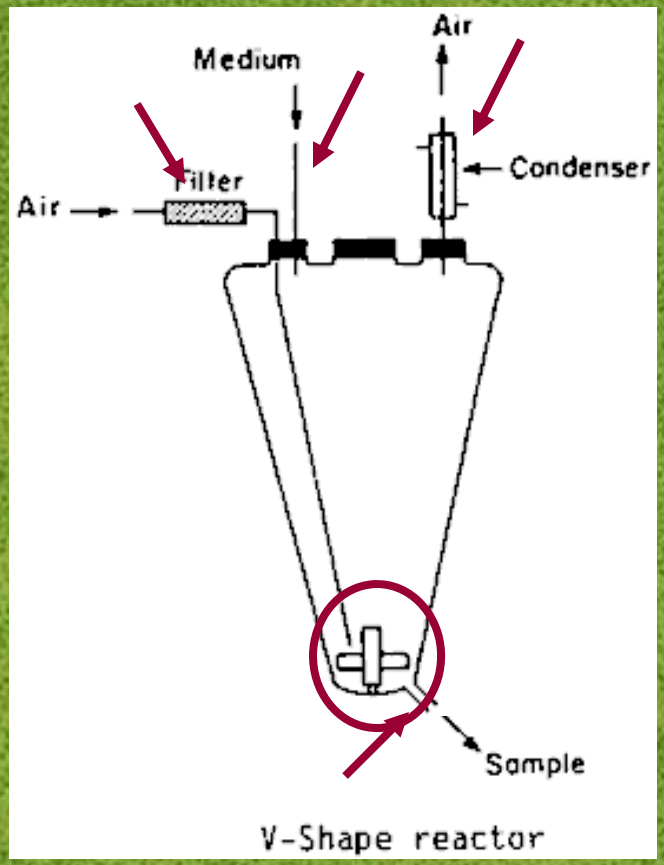
Filtered compressed air is employed for oxygen supply, aeration and agitation of the medium.

A roller-bottled system using a round flask was used by **Lamport in 1964.**



Roller flask system

A **V-shape fermentor** was proposed by Veliky and Martin

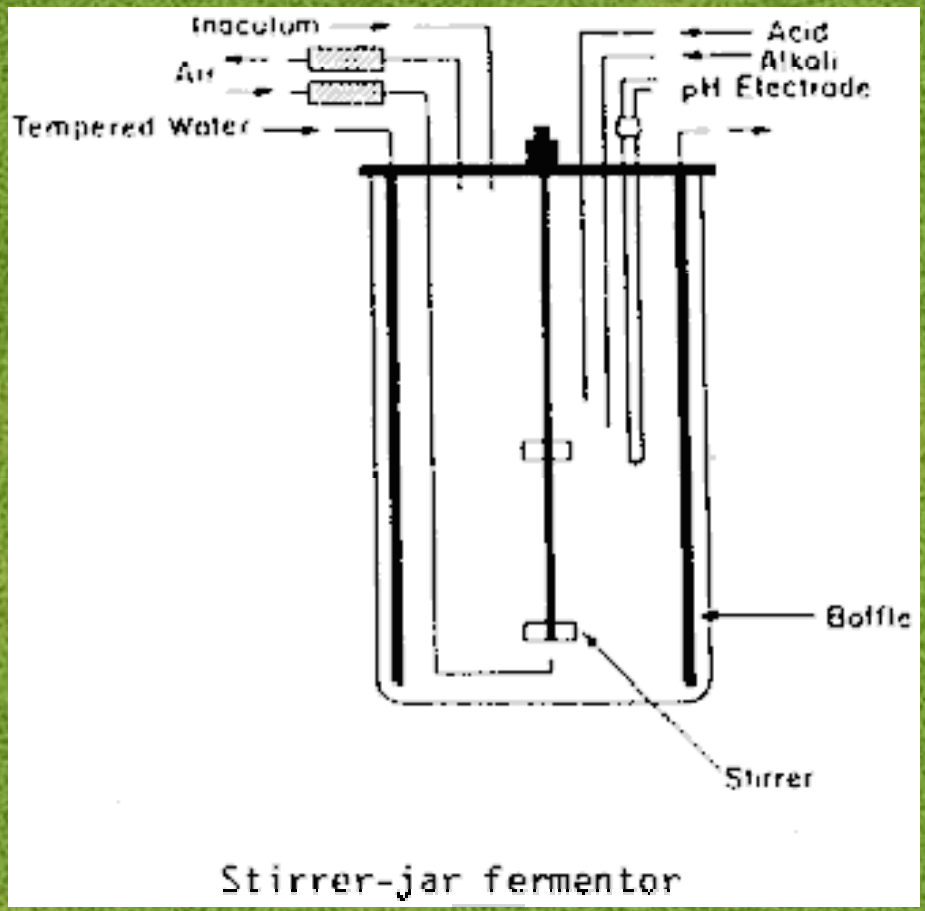


It is an inverted flask carrying two teflon-coated stirring bars on a glass pin situated at the bottom of the flask.

A drain/sample port is also located at the bottom.

The top of the flask is fitted with three tubes:
air-in
air-out
medium-in

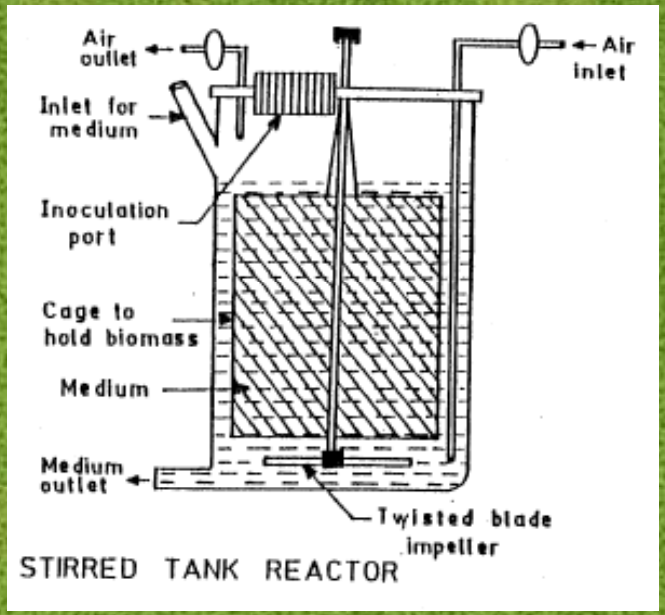
the most common types of system is a stirred-jar fermentor

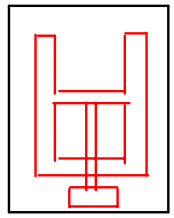


Stirrer-jar fermentor

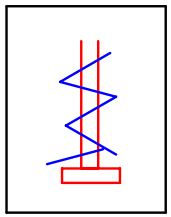
Kato et al. suggested that an agitation speed of **50 to 100 r.p.m.** was most appropriate for the growth of tobacco cells in stirred-jar fermentors.

It is true that cultured plant cells are more fragile than microbial cells, however, Martin noted: "it seems obvious that cell lines differ in their resistance to shear effects and that a single optimum agitation speed cannot be designed for all lines"

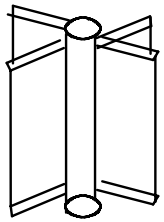




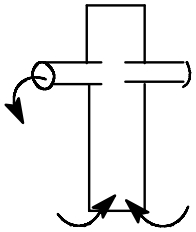
ancla



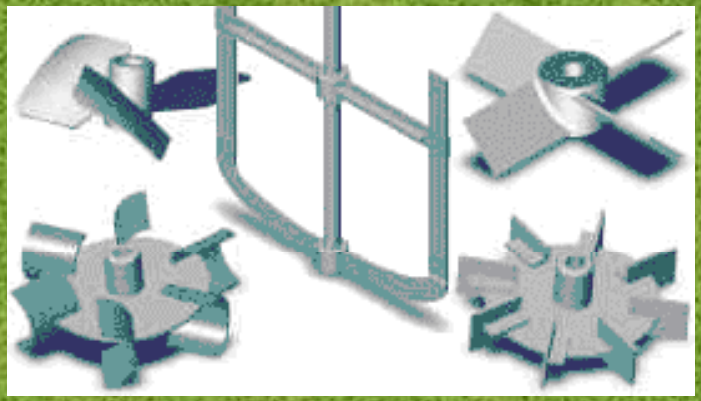
espiral



paletas planas



celulas ascendent

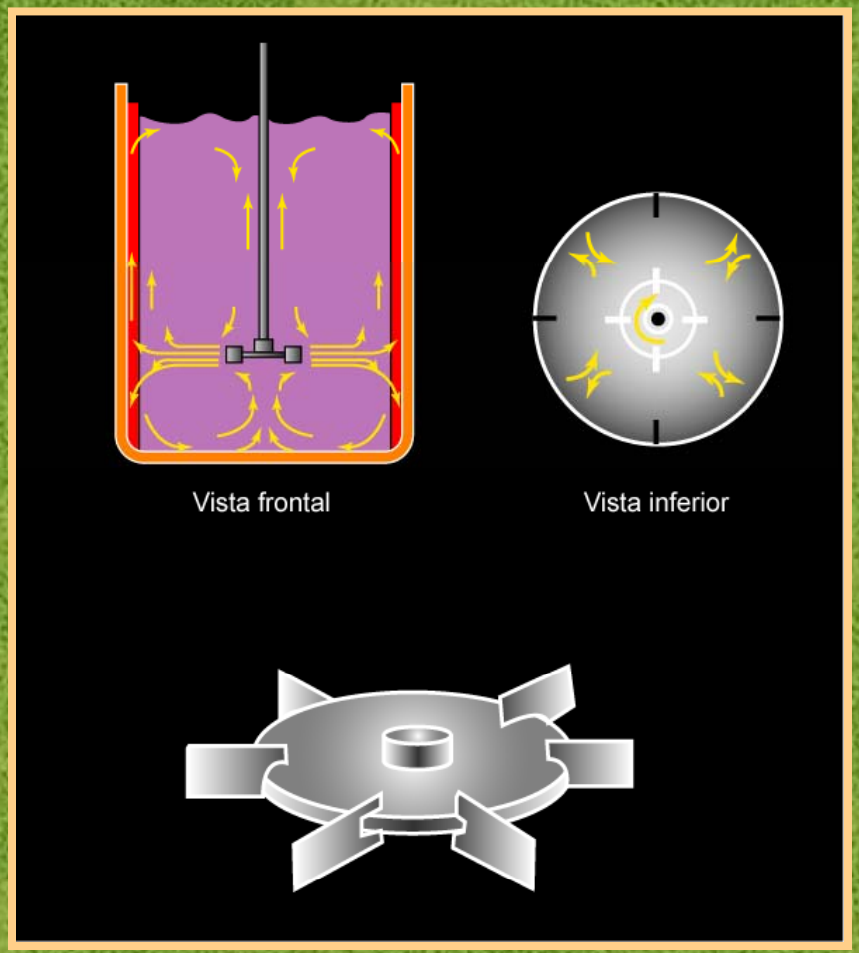


The mixer's diameter is normally 1/3 - 1/2 from the diameter of the reactor vessel

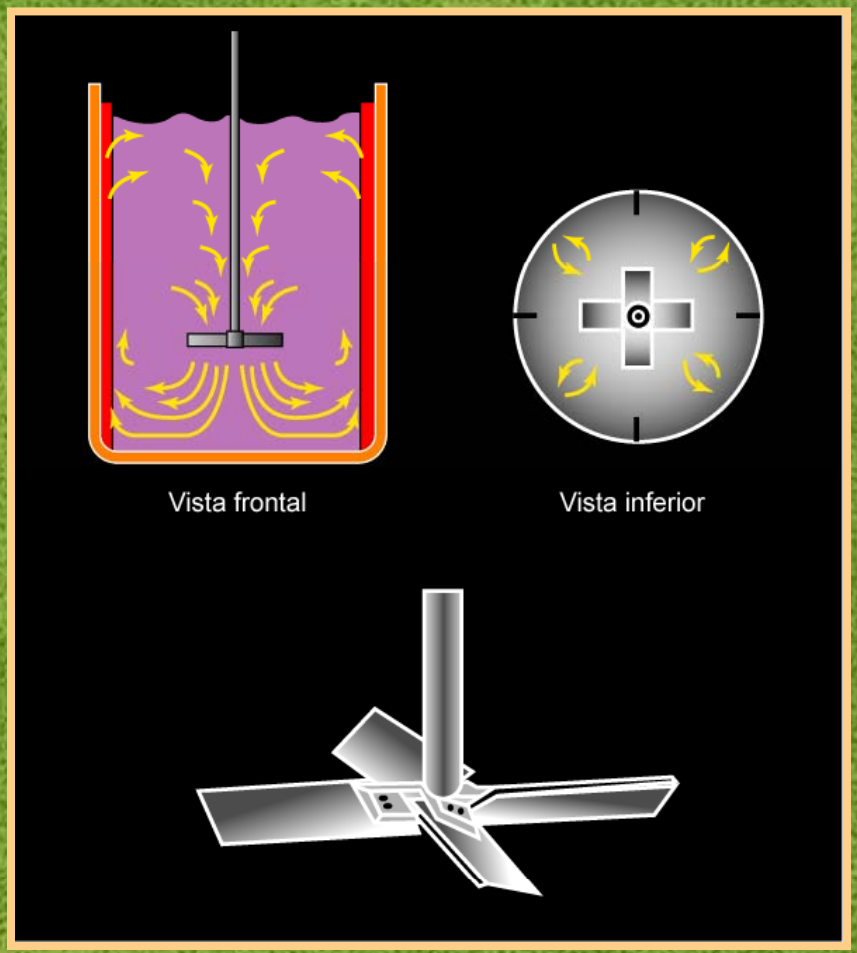
The location of the mixer's axis depends on the fact whether the bioreactor has the upper or lower drive

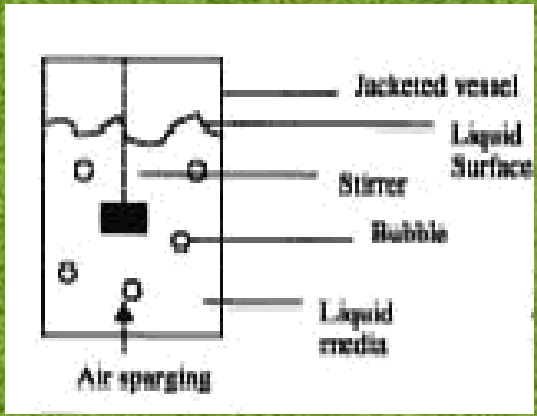
La turbina de Rushton es el modelo mas difundido
Su diámetro es de1/3 del diámetro del reactor
Genera un típico flujo radial

Agitador de paletas planas o Rushton

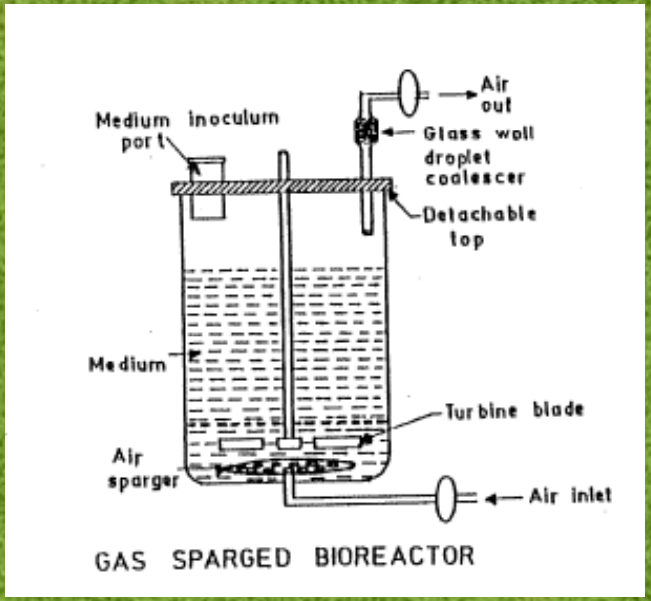


Agitador de paletas inclinadas





CSTR
mechanical mixing and air-sparged



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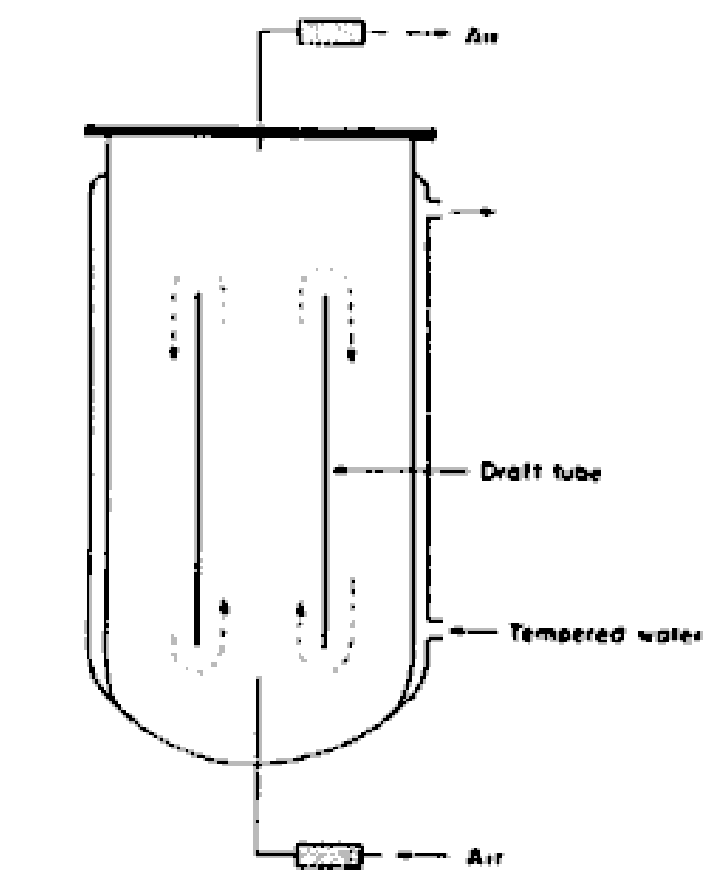
Biotechnology Advances 20 (2002) 101–153

Sparger

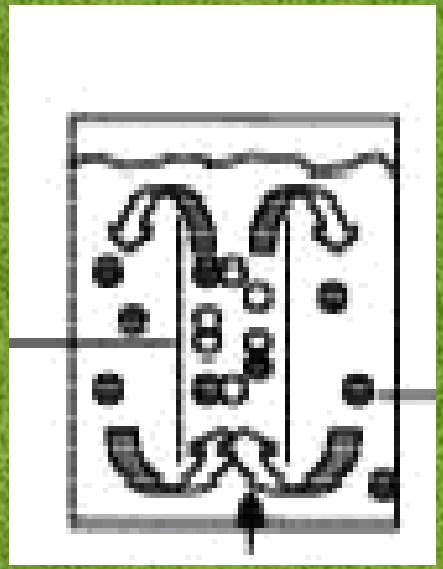
- **The supply of compressed air is realized**
- **The most widespread constructive is a loop pipe with small holes in the lower part (d = 0.05 - 0.15 mm).**

Dalton en 1978 diseñó un reactor de bajo esfuerzo de corte denominado **“air lift”**



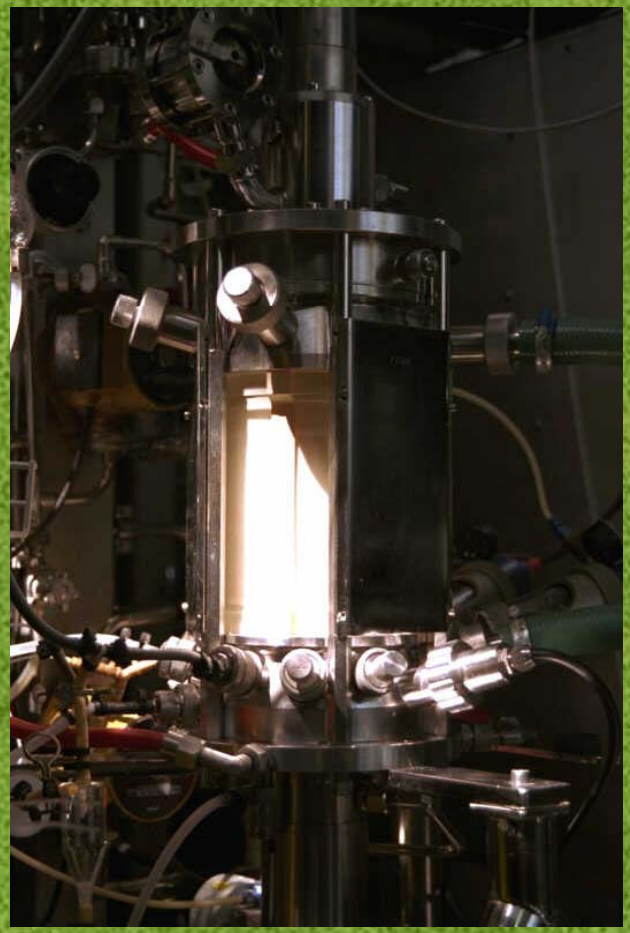
Airlift fermentor

Draft tube

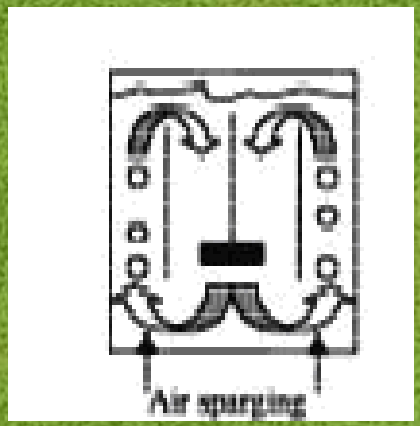


Bubble

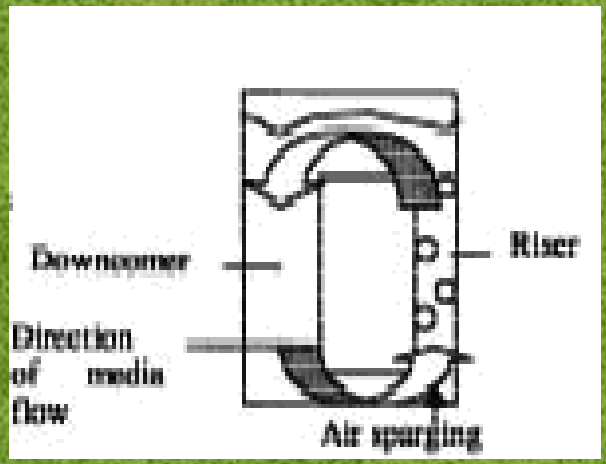
Aire



Airlift - uses air sparging to pneumatically mix the media

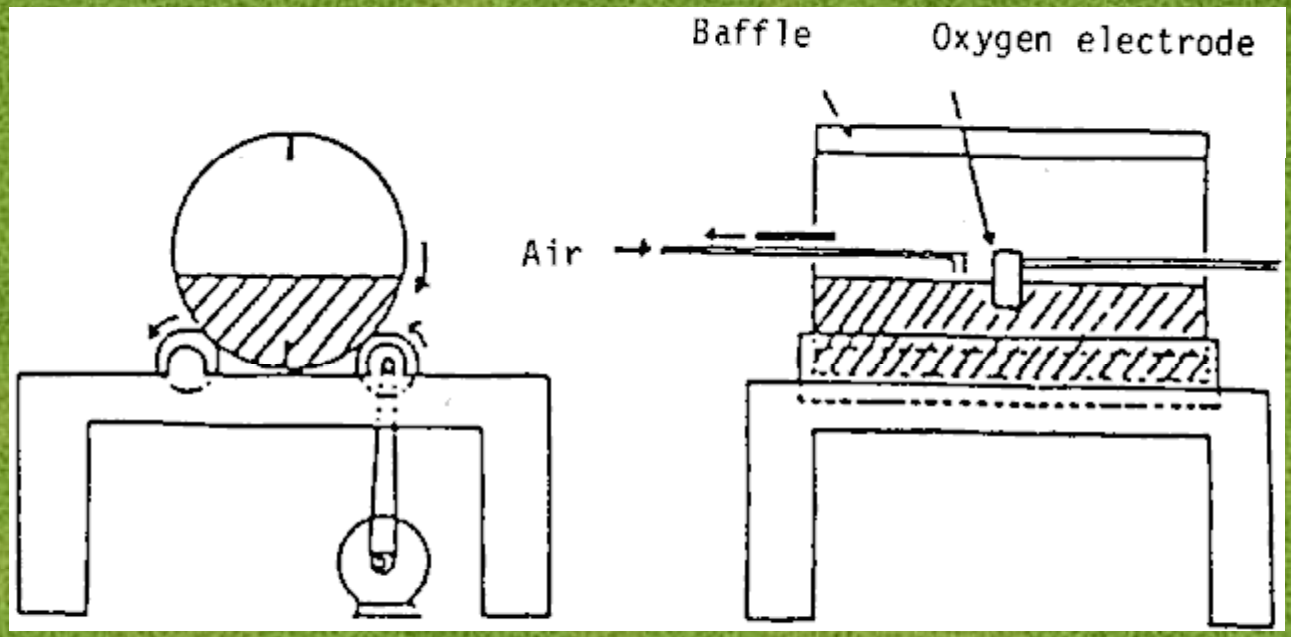


Hibrido CSTR- air lift

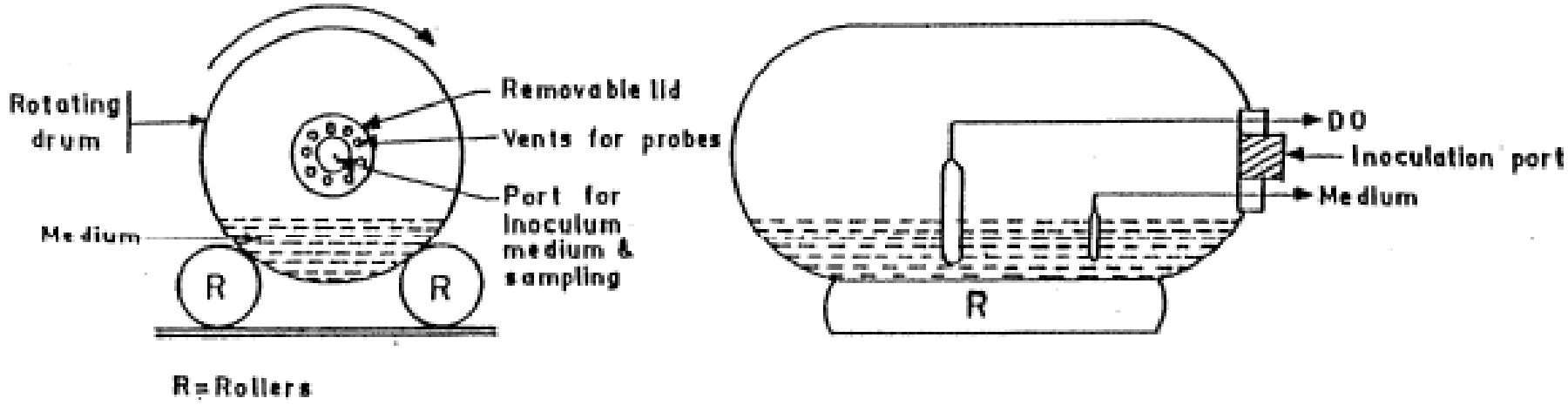


Air lift de loop externo

Tanaka et al. designed a **rotary-drum type fermentor** having an in-let and an out-let at the side of the fermentor



Tanaka, H., et al., Biotechnol. Bioeng., 24 2359 (1983)



ROLLER DRUM REACTOR

The fermentor itself rotates slowly like as a rotary bottle.

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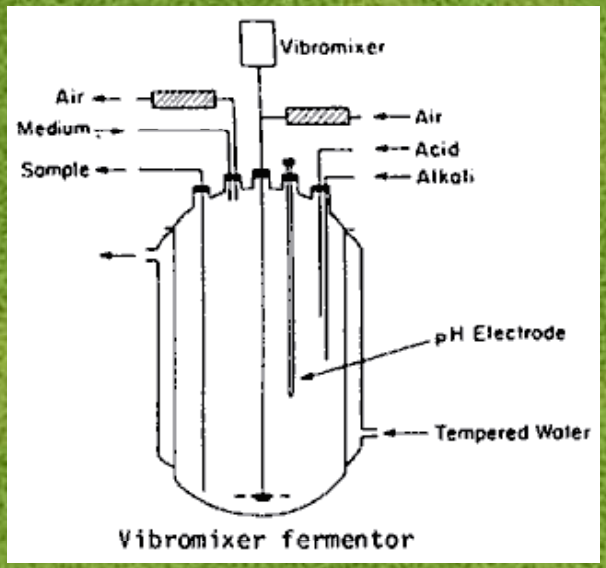
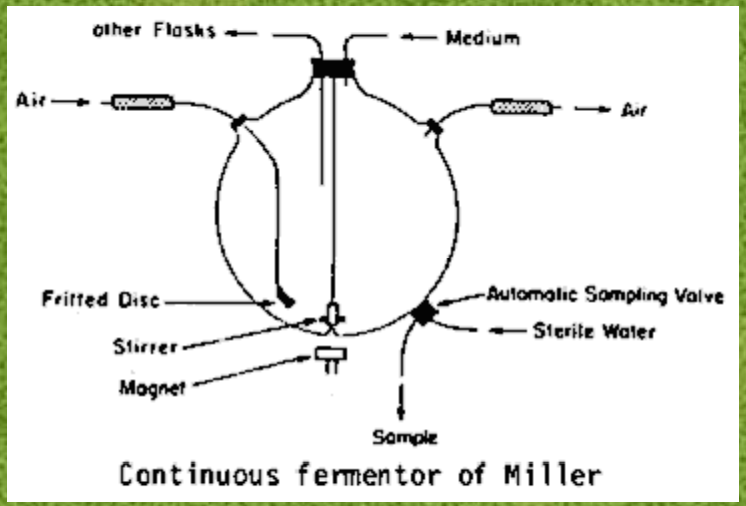
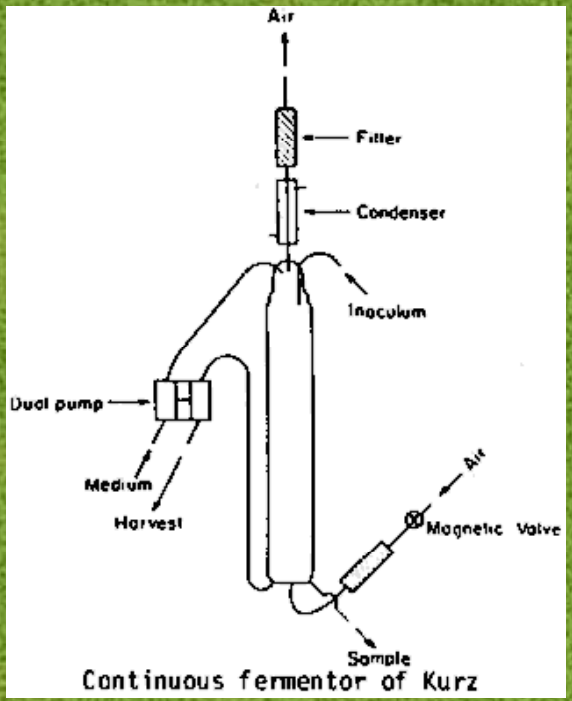
Otros modelos para células indiferenciadas



Vibromixer fermentor

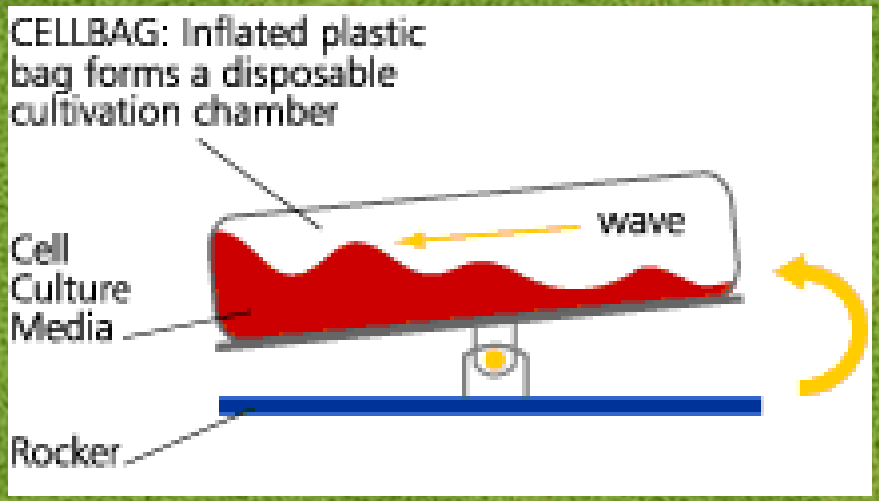


Continuous fermentor of Miller



Continuous fermentor of Kurz

Bolsas descartables



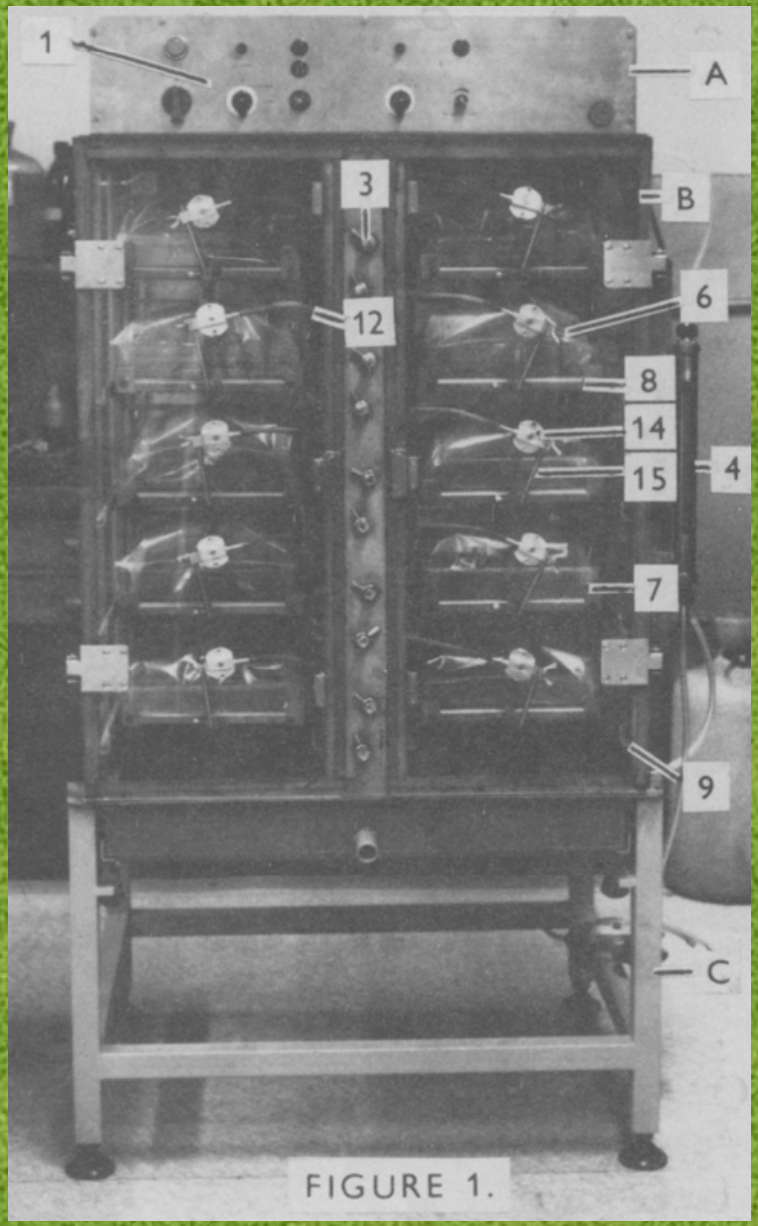


FIGURE 1.

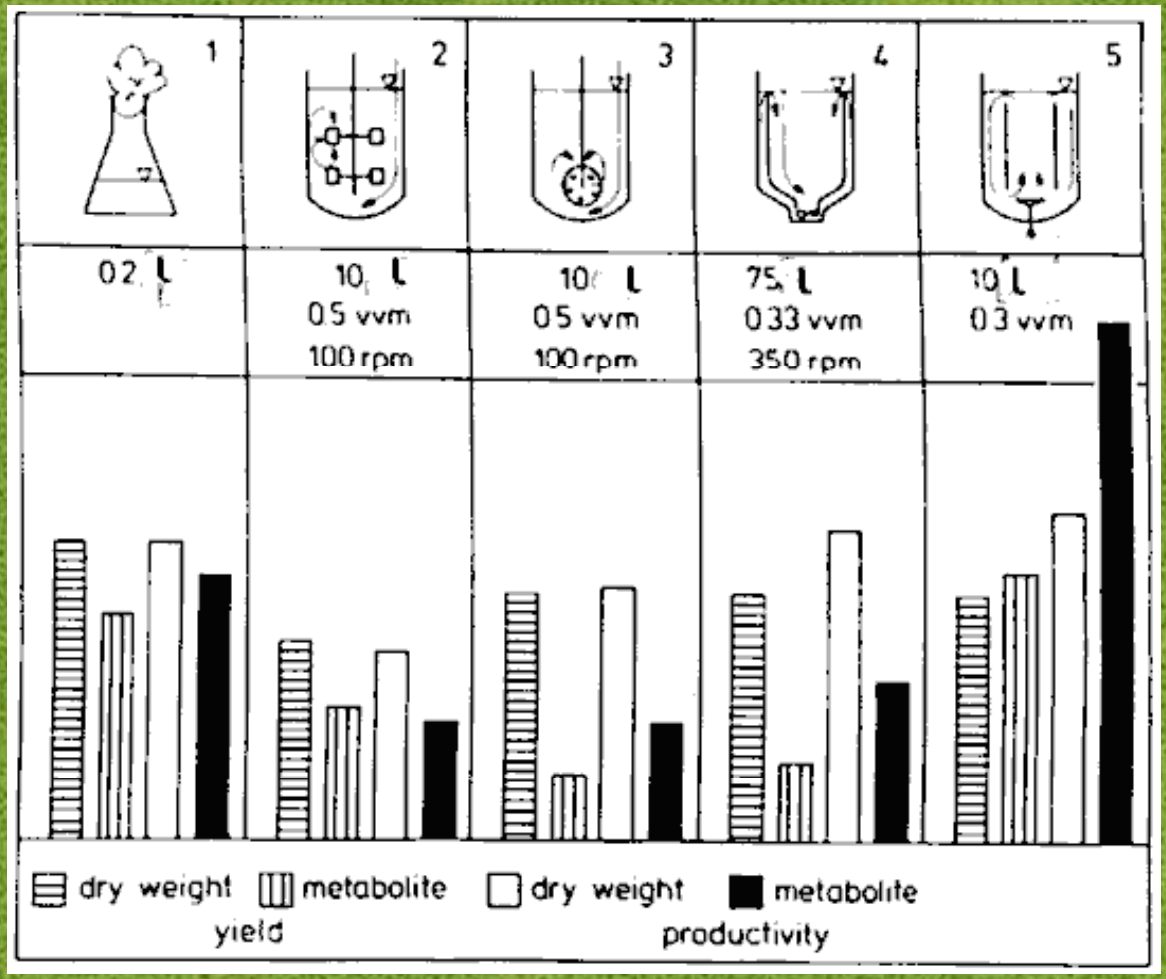
"A DEVICE FOR CULTIVATION OF PLANT AND ANIMAL CELLS"

Biotechnology Letters Vol 7 No 7 467-470 (1985)

Comparison of Yield and Productivity for Cell Mass and Anthraquinones in Varoius Reactor Systems

3. perforated disk impeller

1. Shake flask



5. air-lift reactor

2. flat blade turbine

4. draft tube reactor

Wagner, F. In "Plant Tissue Culture and Its Bio-technological application" Ed. Barz, W. et al., p. 250 (1977). Springer-Verlag, Berlin Heidelberg.t reactor