Compulsion ventilation screening system

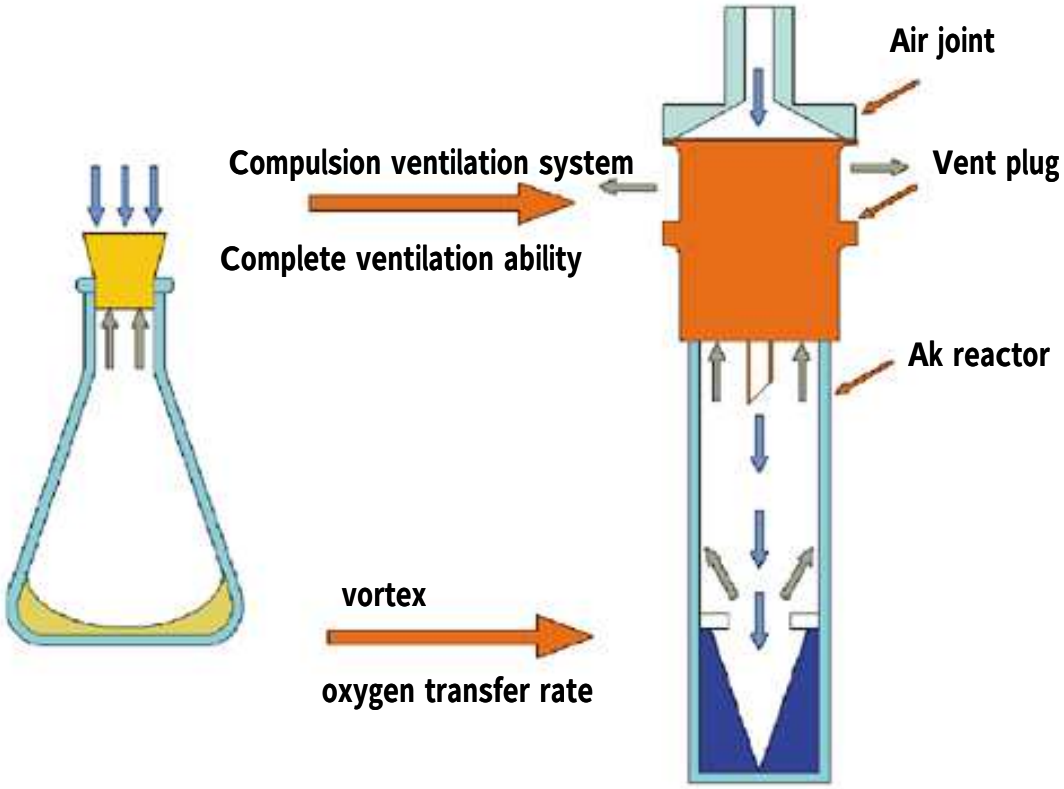
AK reactor

Patent No.4751255

Microorganisms need oxygen, nutrients and optimum temperature for growth and product formation.
Microorganisms cannot metabolize substances that have not been dissolved in liquid solvent.
The solubility of oxygen in 100 mL flask at 30°C are 0.76 mg while that of glucose is several tens of g.
In other words, oxygen (and other gas) solubility is very low and often limiting in many bio-processes.
During cultivation, oxygen demand in many bio-processes is 2-6 times higher than that of glucose.
Oxygen is usually limiting and needs to be supplied continuously during the cultivation.
It is therefore necessary consider oxygen supply as a rate controlling factor during growth and product formation by many microorganisms.

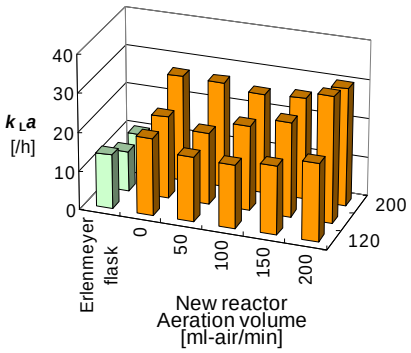
The problems of ventilation and oxygen transfer in flask cultures can be solved by using the AK reactor.
It is a ventilated screening system developed to process a large number of small samples.

Since the system is ventilated, the culture conditions (environment) similar to those of a mini jar fermenter can be maintained.
An existing shaking machine can start the ventilation type screening as a drive unit.
It is the best culture device for enrichment culture s and for gene recombination.



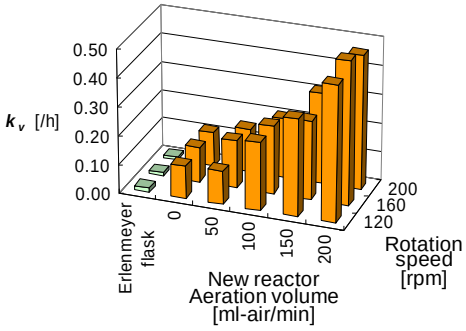
Comparison between AK reactor and Erlenmeyer flask

○ k_La



Comparison of k_La between Erlenmeyer flask and new reactor

○ k_v



Comparison of k_v between Erlenmeyer flask and new reactor

K L a

K L a x axis is the airflow rate, y axis is the rotation speed, and z axis is kLa.

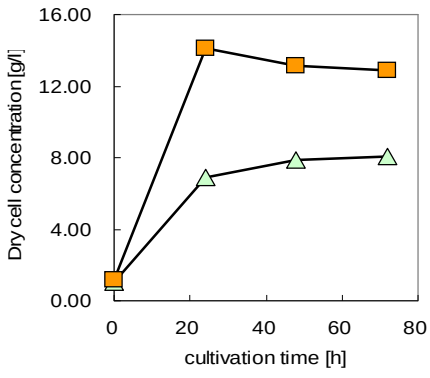
With this system, a kLa of 30.2 can be obtained, which is about three times higher than the 10.3 obtained in Erlenmeyer flask

K v

K v x axis is a airflow rate, y axis is a rotation speed, and z axis is the kv.

With the Ak reactor, the kv was 0.46 which is about 40 times higher than the 0.01 obtained in Erlenmeyer flask.

Phaffia rhodozyma



：Erlenmeyer flask
：New reactor

Effect of aeration on the growth of *Phaffia rhodozyma*

Condition

24℃ ：200 rpm

200 ml-air/min(new reactor.)

Number of bacteria (72 h)

$Y_{X/S\_\text{Erlenmeyer flask}} = 0.40$

$Y_{X/S\_\text{New reactor}} = 0.64$

1.59times

Astaxanthin (72 h)

O. D. $_{480\ nm\ \text{Erlenmeyer flask}} = 0.11$

O. D. $_{480\ nm\ \text{New reactor}} = 0.48$

4.41times

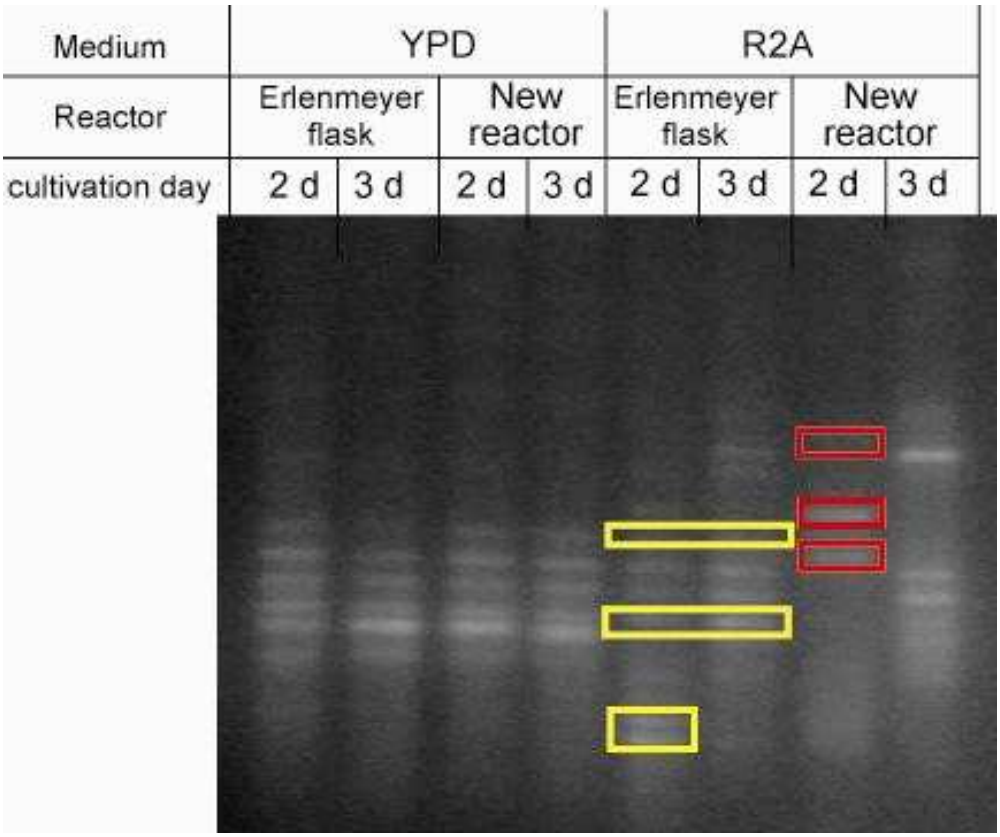
Both the biomass yield and astaxanthin production obtained in the AK reactor were higher than those obtained in Erlenmeyerflask.

The Ak reactor is suitable for cultivation of aerobic microorgan

Enrichment cultures

The media used for the culture were YPD and R2A media.
There was no significant change in DNA bands when YPD medium was used.
The DNA bands enclosed with red and yellow rectangles were observed when R2A medium was used.
The DNA band enclosed in red were not observed with Erlenmeyer flask but observed in the Ak reactor.
On the other hand, some DNA bands observed in Erlenmeyer flask (part that had been enclosed with yellow) were not observed in Ak reactor.
This has demonstrated that when enrichment culture is done in Ak reactor, there is a possibility that microorganisms different from those usually obtained in flask enrichment cultures will be isolated.
This is due the differences in the culture conditions, especially ventilation.

Thus, with the Ak reactor, it is possible to isolate novel microorganisms whose isolation has not been possible with flask enrichment cultures.

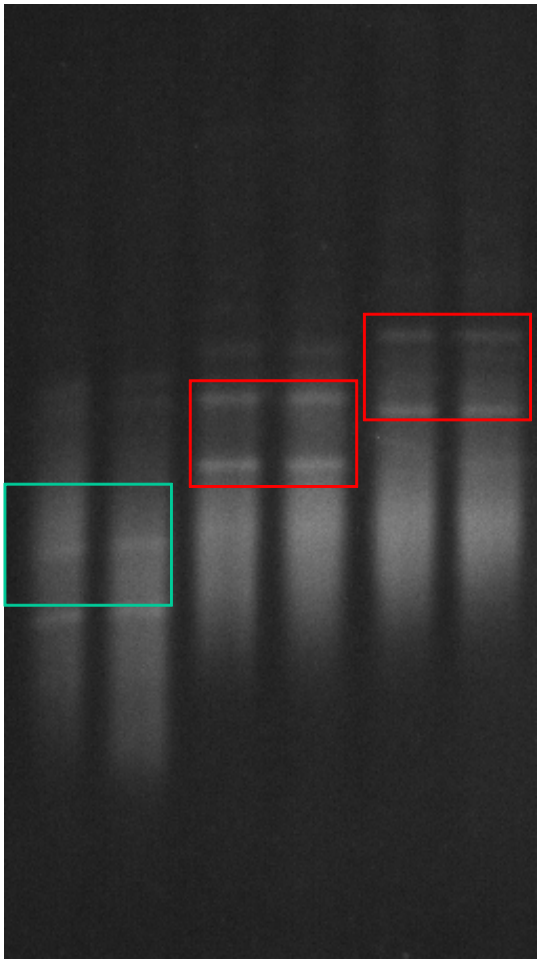


Influence of high temperature （50℃）

The enrichment cultures were done in both Erlenmeyer flask and the AK reactor.
There was no ventilation in the flask cultures (b2 and b4) but there was ventilation in the Ak reactor (c2 and c4).
There was no ventilation in the flask cultures (b2 and b4) but there was ventilation in the Ak reactor (c2 and c4).
Due to ventilation in the AK reactor, protein bands which were not observed in Erlenmeyer flask cultures were observed in Ak reactor.

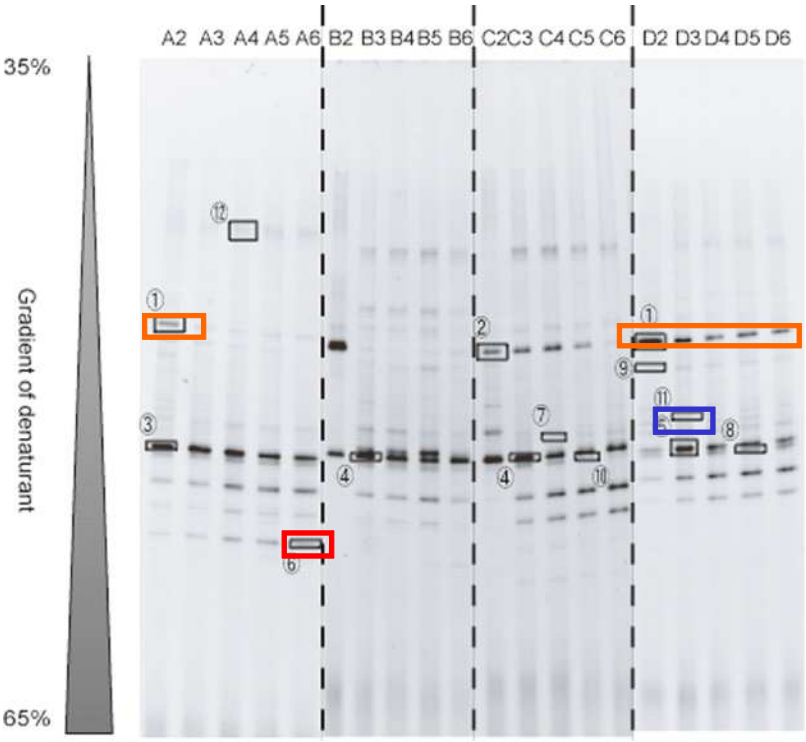
Enrichment culture in the AK reactor (ventilation) can be used to obtain bacteria that cannot be isolated by Erlenmeyer flask enrichment culture.

a2,a4, b2,b4, c2,c4



		Ventilation	Flow
a	Erlenmeyer flask	×	×
b	AK reactor	△	0 v.v.m.
c	AK reactor	○	1 v.v.m.

Effects of oxygen transfer rate and ventilation



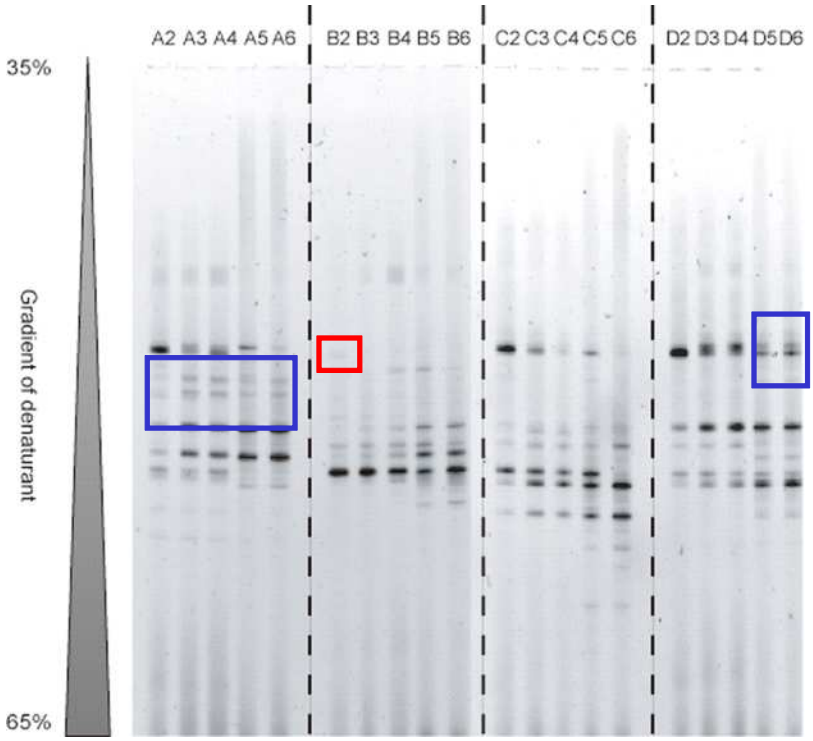
	k_La	k_v
A	Low	Low
B	Low	High
C	High	Low
D	High	High

Photograph of DGGE for 16S rDNA of bacteria after cultivation at 30℃. Alphabet represents culture condition. Number represents cultivation time (day).

A is Erlenmeyer flask while B, C, and D are AK reactors.
The type of microorganism isolated is greatly influenced by both the kLa and kv values (blue frame).
Thus some microorganisms (bands) observed in B, C, and D are different from those in A.
The band that is enclosed in orange was observed in A on the second day while it appeared in D on the 6th day.
This implies that the growth of the microorganism that produced this band is highly influenced by ventilation(kv).

Thus Ak reactor is very effective in isolating such ventilation-sensitive micro-organisms.

Influence of CO2 ventilation



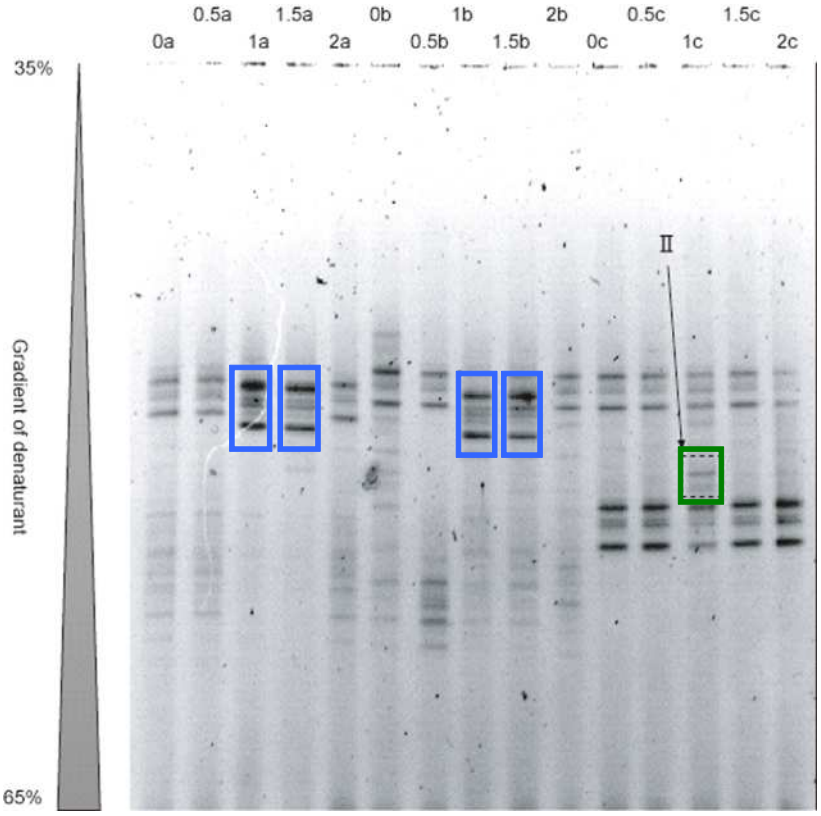
	k_La	k_v
A	Low	Low
B	Low	High
C	High	Low
D	High	High

Photograph of DGGE for 16S rDNA of bacteria after cultivation at 30℃.
Alphabet represents culture condition. Number represents cultivation time (day).

The ventilation gas was changed from air to 5%CO2.
Comparison between cultures aerated with air and 5%CO2
There was no significant change in the type of microorganisms under condition (C) but there were major differences in culture condition (A) and (B) and (D).
The blue bands are those that were observed when 5%CO2 was used for ventilation while the red bands are those that disappeared when 5%CO2 was used for ventilation.

Thus, the type of microorganism that grow is highly depended on the gas that is used for ventilation.

Influence of CO2 ventilation, kv, and medium



	Broth
a	R2A medium
b	1/2 LB medium
c	YPD medium

Photograph of DGGE for 16S rDNA of bacteria after cultivation at 30℃.
Number represents volume per volume per minute. Alphabet represents culture broth.

The airflow of 5%CO2 was adjusted to 0, 0.5, 1.0, 1.5, or 2.0VVM to investigate the effect of gas flow rate on the type of microorganism.
The enrichment culture was done with three media.
In the Low nourishment medium, bands 1a, 1.5a, 1b, and 1.5b (in blue) appeared when ventilation was done at 1.15VVM.
The bands observed in High nourishment medium (YPD) at 1 vvm are in green.
It was confirmed that this band is peculiar.

By using CO2 for ventilation, it has become possible to cultivate microorganism (s) that has not been possible to culture under convention culture condition.

All these experiments were conducted in Prof. H. Aoyagi's Laboratory, Tsukuba University, Japan

Influence of oxygen movement speed and ventilation

There are differences in the light and shade of bands in A2 and D2.
The oxygen transfer rate in Erlenmeyer flask is not sufficient but AK reactor has sufficient oxygen transfer rate.
The bands in A3-A6 disappeared in D3-D6 because there is no ventilation in Erlenmeyer flask and the generated gas (CO_2)is not substituted by air fresh.
On the other hand, there is enough ventilation in the AK reactor and the generated gas is continuously replaced by air fresh.
Thus, the culture is only limited by depletion of the nutrients.
In other words, the limitations of Erlenmeyer flasks can be overcome by the Ak reactor with good ventilation system.

Possibility of AK reactor and ventilation screening system

From the result of these DGGE some microorganisms which could not be obtained with the conventional enrichment culture in Erlenmeyer flask can be obtained by changing the kLa, kv, the temperature, and the ventilation (aeration) gas.
These experiments were performed with microorganism bacterium flora.
If you replace the enrichment culture with the axenic culture.
Culture and change the culture conditions, you can change the metabolism and obtain new useful metabolites.
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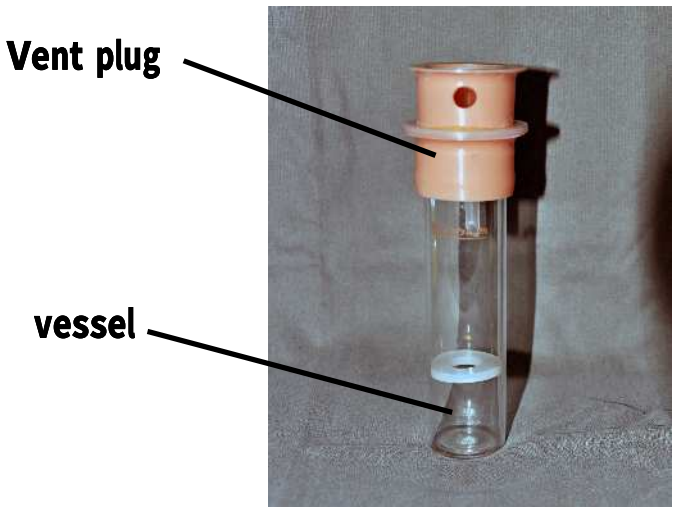
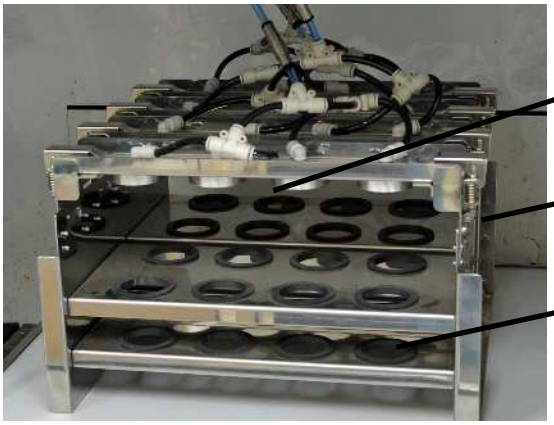
Ventilation screening system AK reactor



Vent plug that is newly developed for ventilation screening system.
It is a filter ventilation plug with inlet and outlet.
Contamination is prevented by using a double filter unit.
It is easily installed with a special installation tool, and because the installation position is free, high oxygen transfer rate can be obtained by adjusting liquid volume.
It is operated in the same way as Erlenmeyer flask.

AK holder

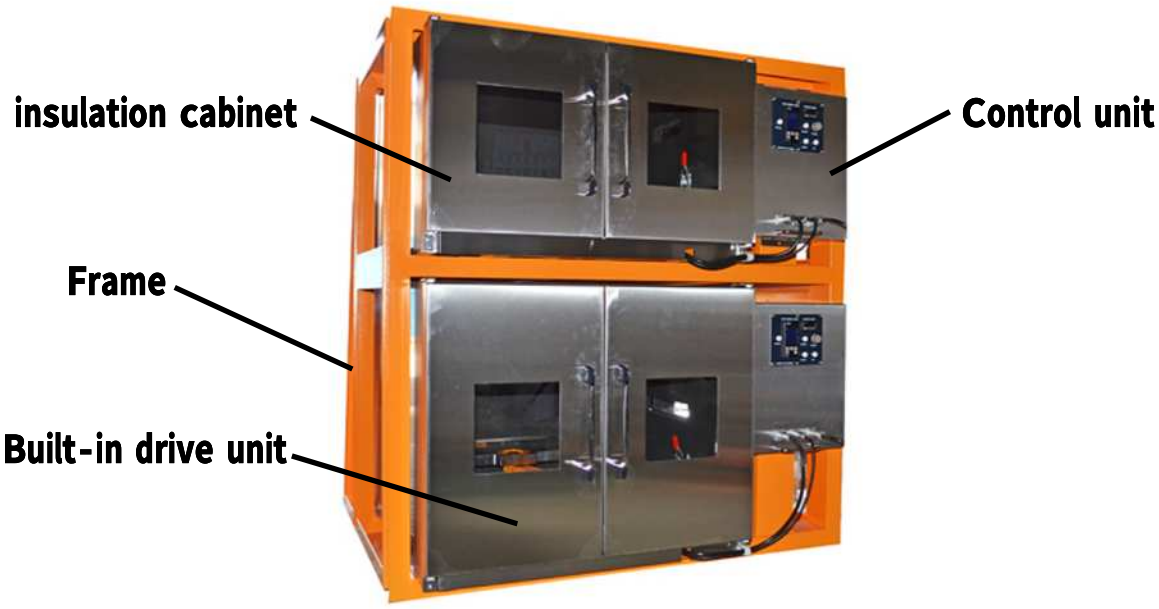


It was set.

Drive unit

A shaking culture machine is used as the drive unit to make the vortex..
The amplitude is fixed at 70mm.
To obtain best kLa, the rotation speed is adjusted.(usually 200 rpm)

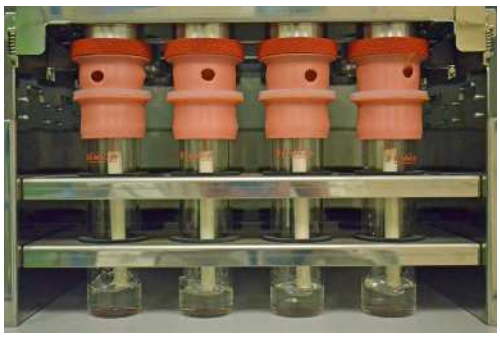
The entire system



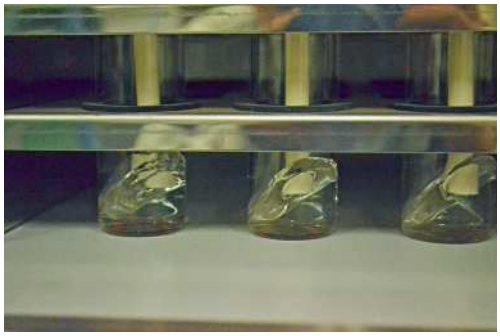
insulation cabinet	It was lined by the heat insulator.
Control unit	A refrigerator, heater, a circulation fan, a drive unit, a controller, and an operation equipment, are built in.
Frame	It is composed of an insulation cabinet, a controller, and the drive unit.

Humidifying device

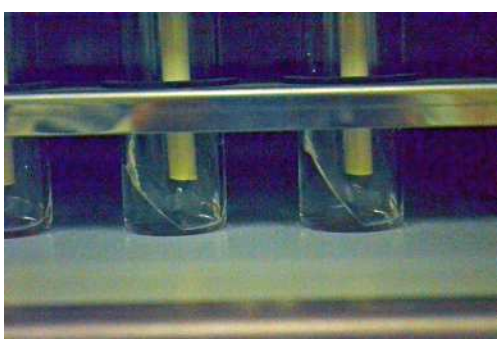
To prevent evaporation of the medium from the vessel, the air is first humidified by passing through a humidifying bottle before passing into the culture vessel.
It can be ventilated with gas of various composition.



Confirmation of ventilation



State of liquid (140rpm)



State of liquid (200rpm)

Decision of specification

Capacity of vessel
Number that is necessary
The size of the holder is decided from the number of vessels
Kind of control unit
R control unit(temperature range = 15-60 oC)
T control unit(temperature range = 15-80 oC)
It is possible to set it up in one insulation cabinet to eight insulation cabinets.
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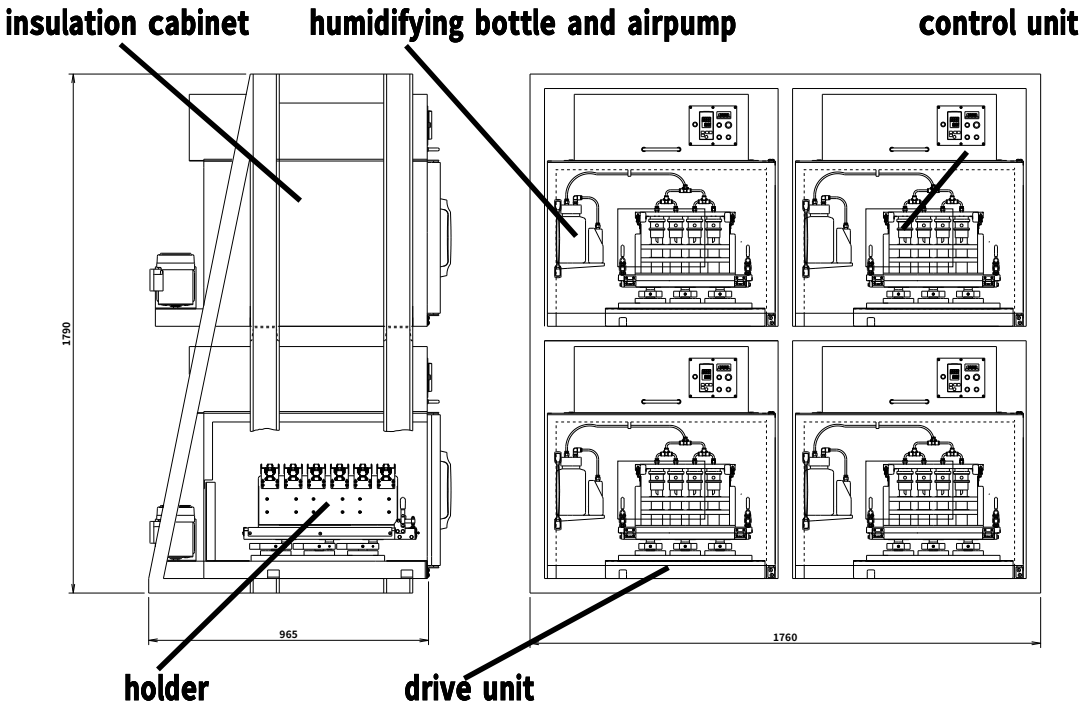
One example

The vessel is AK100.
The temperature control is four.
One each of.holder.
24 is stored in the holder.



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