Constant Systems Ltd

since 1989

Continuous High Pressure Cell Disrupter

連續式高壓細胞破碎機

尚偉(股)公司 客服部

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大綱

- 連續式高壓細胞破碎簡介
- 連續式高壓細胞破碎原理
- 操作流程
- 注意事項

連續式高壓細胞破碎簡介

型號：TS 0.75kW(桌上型連續式)

- 破菌壓力：40 kpsi (2700 Bar)
- 處理量：40~115 ml/min
- 樣品需要最小體積：20 mL
  (每次抽取10ml)
- 注意不要產生氣泡

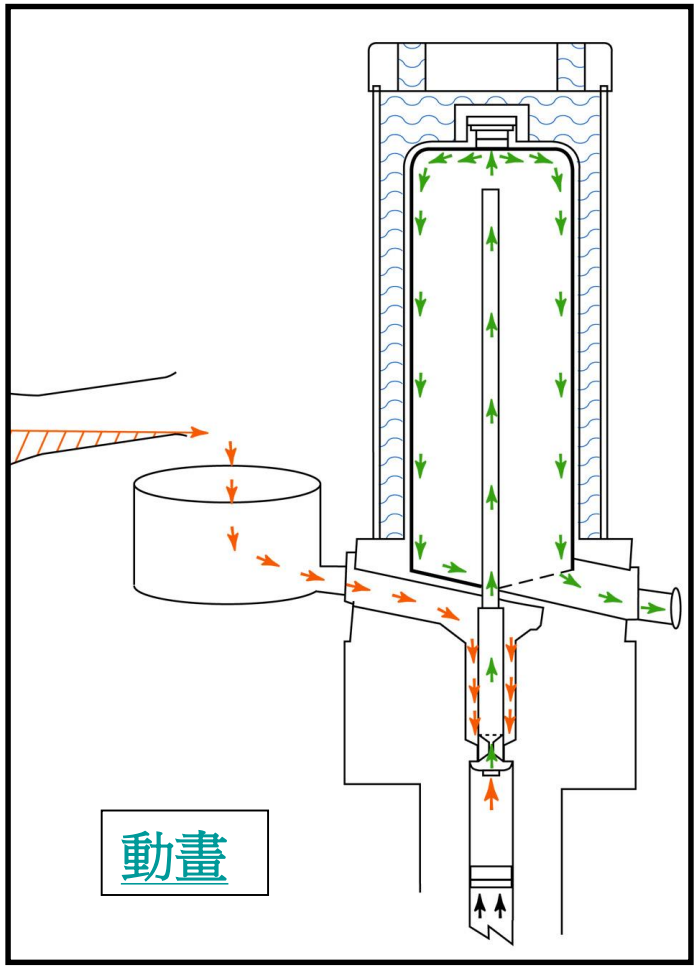
細胞破碎簡介

Algae, animal cells, bacteria, environmental samples, fungi, yeast, mammalian cells and tissue, parasites, plant cells and tissues and viruses.

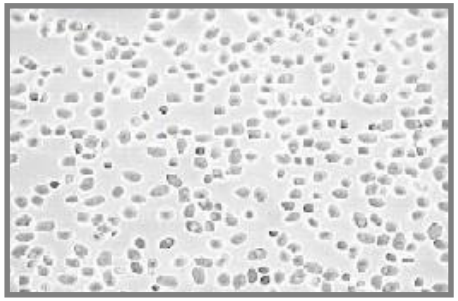
- **Protein & DNA Extraction**
- **DNA Manipulation**
- **Enzyme Release**
- **Single Cell Isolation**
- **Selective Breakage**
- **Tissue Disruption**

- 能獲得大量產物
- 避免在破碎過程中變性
- 避免有害物質產生而影響後續實驗

連續式高壓細胞破碎原理

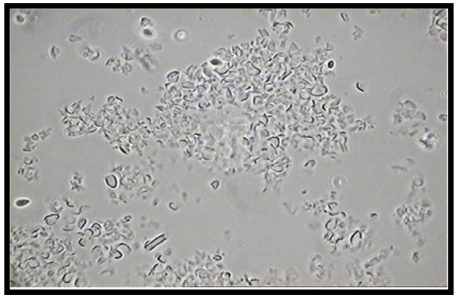


1. 撞擊力
2. 壓力
3. 應力



Candida Albicans Yeast

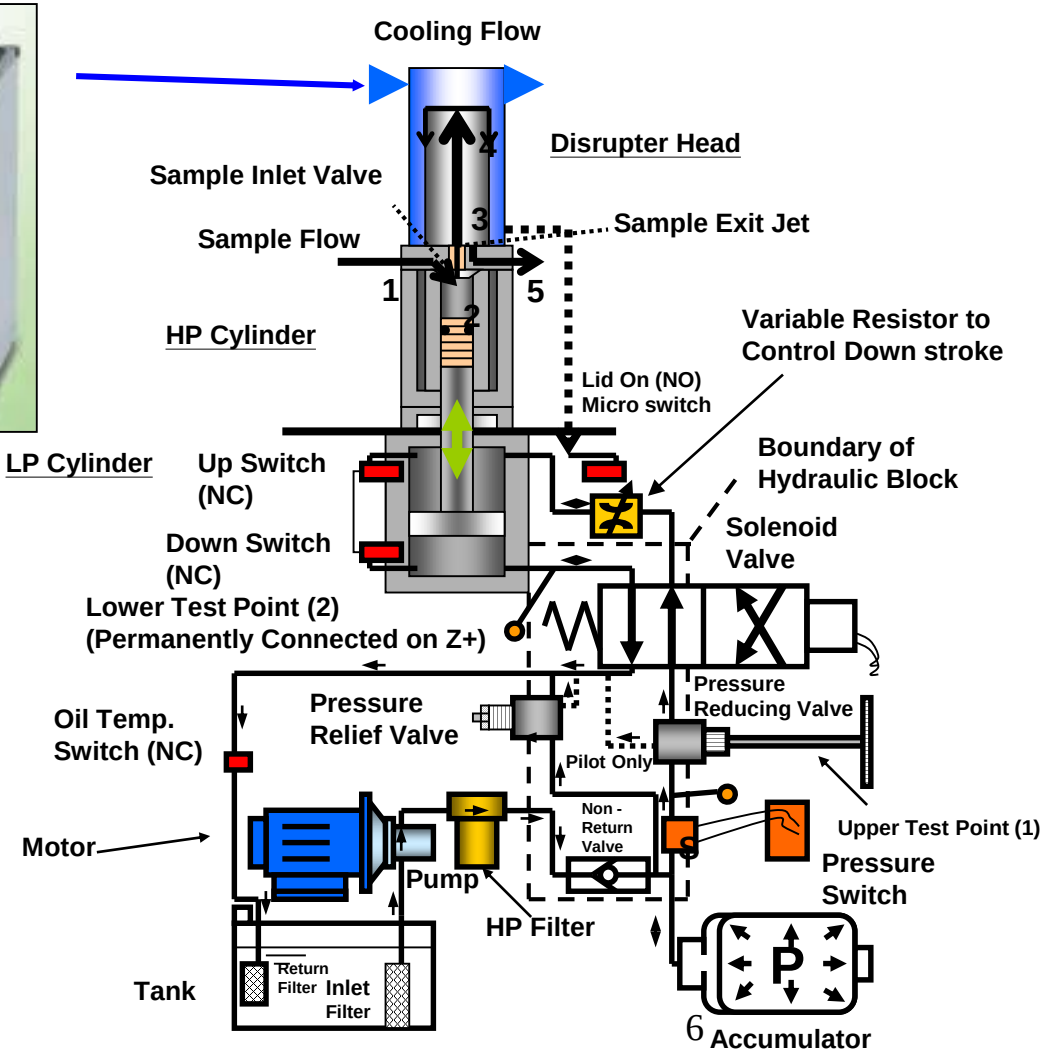
Form Unbroken



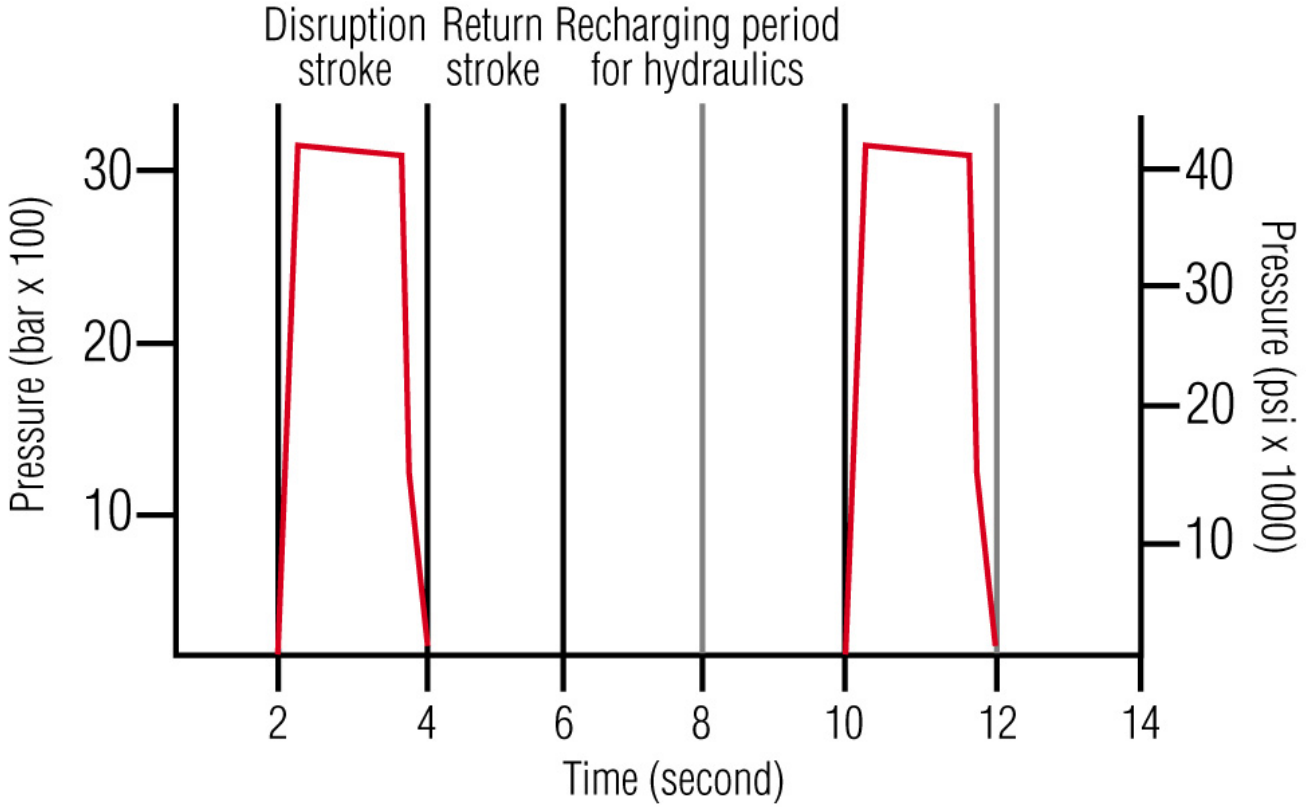
Candida Albicans Yeast

Form Broken

TS 0.75KW 系統


Cooling Flow
Disrupter Head
Sample Inlet Valve
Sample Flow
Sample Exit Jet
3
1
5
2
HP Cylinder
Variable Resistor to
Control Down stroke
Lid On (NO)
Micro switch
LP Cylinder
Up Switch
(NC)
Boundary of
Hydraulic Block
Down Switch
(NC)
Solenoid
Valve
Lower Test Point (2)
(Permanently Connected on Z+)
Pressure
Reducing Valve
Oil Temp.
Switch (NC)
Pressure
Relief Valve
Pilot Only
Non -
Return
Valve
Upper Test Point (1)
Motor
Pump
Pressure
Switch
HP Filter
Tank
Return
Filter
Inlet
Filter
P
Accumulator

提供穩定的壓力



Graph showing a typical controlled pressure cycle in the cell disrupter

操作流程

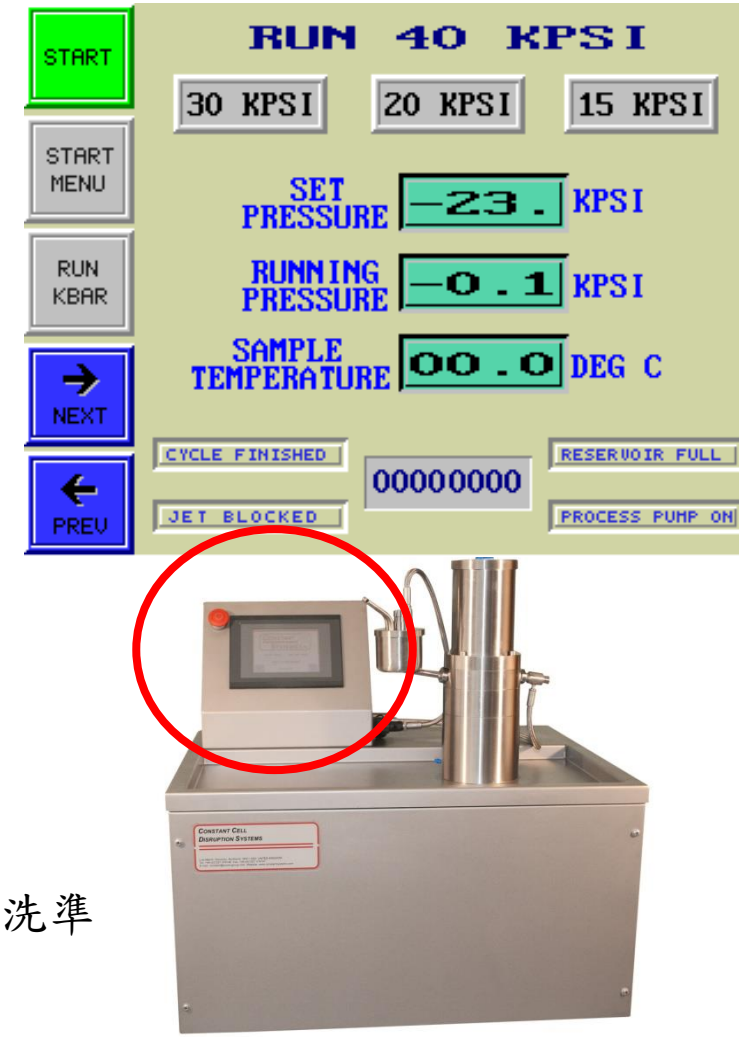
準備

1. 打開冰水檢查冰水機水位。
2. 開電源，按SET設定溫度，按COOLING鍵啟動壓縮機，按PUMP鍵啟動循環幫浦。
3. 接上破碎機電源，按START MANU鍵進入操作選單，按RUN MODE(PSI)鍵進入操作畫面。
4. 戴上橡膠手套。

操作流程

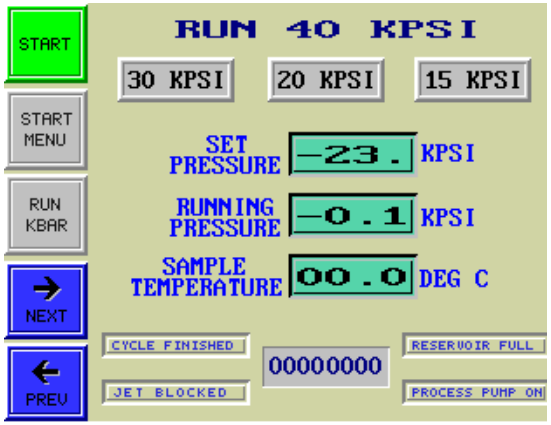
破菌前清洗

1. 於破碎出口接上一段可滅菌的矽膠管。
2. 置一廢水容器於破碎出口。
3. 倒入50mL BUFFER於左方金屬樣品杯。
4. 先選擇15KPSI範圍，用手旋轉機器前下方ADJUST PRESSURE鈕，SET PRESSURE為10KPSI，
5. 按綠色START鍵啟動清洗。
6. 待BUFFER 打完，按紅色停止鍵，即完成清洗準備。



操作流程

破菌中



1. 倒入SAMPLE於左方金屬樣品杯。(最大量約200mL，可連續添加)
2. 置SAMPLE容器於破碎出口。
3. 先選擇KPSI的範圍，再用手旋轉機器前下方ADJUST PRESSURE鈕，設定PRESSURE值，按綠色START鍵啟動破菌。(機器每打一次固定10mL)
4. 待SAMPLE 打完，按紅色停止鍵即完成。
5. 避免第一次打出空氣，可於SERVICE MODE，按住UP鍵按直到停止，再按住DOWN鍵直到停止，手動導入SAMPLE。

操作流程

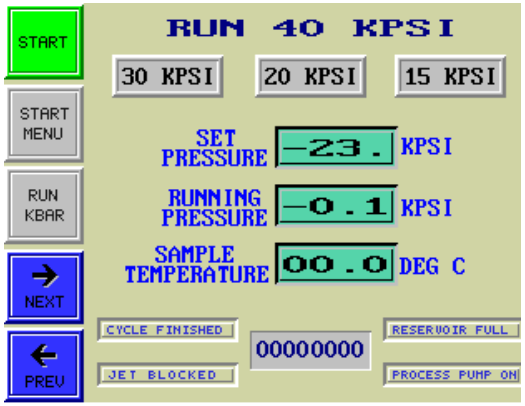
破菌後清洗

1. 於破碎出口與水龍頭接上逆洗水管，並置一廢水容器於樣品杯下方。
2. 開啟小量自來水，讓水緩慢由樣品杯溢出，流入廢水容器。
3. 並以擦洗樣品杯內部，目視水色由白轉澄清。
4. 關閉水龍頭。
5. 樣品杯旋轉向下倒光液體，再旋轉回原位。
6. 置一廢水容器於破碎出口。
7. 倒入50mL 75%酒精於左方金屬樣品杯。

8. 用手旋轉機器前下方ADJUST PRESSURE鈕，SET PRESSURE為10KPSI，
9. 按綠色START鍵啟動清洗。
10. 待50mL 75%酒精打完，按紅色停止鍵，即完成清洗滅菌。

操作流程

復原歸位



1. 關閉所有機器電源。
2. 樣品杯以乾淨的橡膠手套覆蓋。
3. 其他東西復原歸位。

Set pressure and Start , that's all !

Constant Systems 應用

- **Algae** : 30kpsi
- **Bacteria** : 30 – 40kpsi
- **Fungi** : 20kpsi
- **Mammalian** : 100psi – 15kpsi
- **Yeast** : 40kpsi

Common Cell Disruption Models

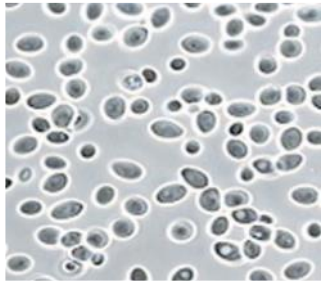
Yeast - *Saccharomyces Cerevisiae*

Protein Release **- more than 99% after one pass at 40 Kpsi**

Membrane Breakage at ATPase activity **- best at 30 Kpsi**

Protein Purification **- best at 20 Kpsi**

Recombinant Protein **- best at 13.5 Kpsi**



Common Cell Disruption Models

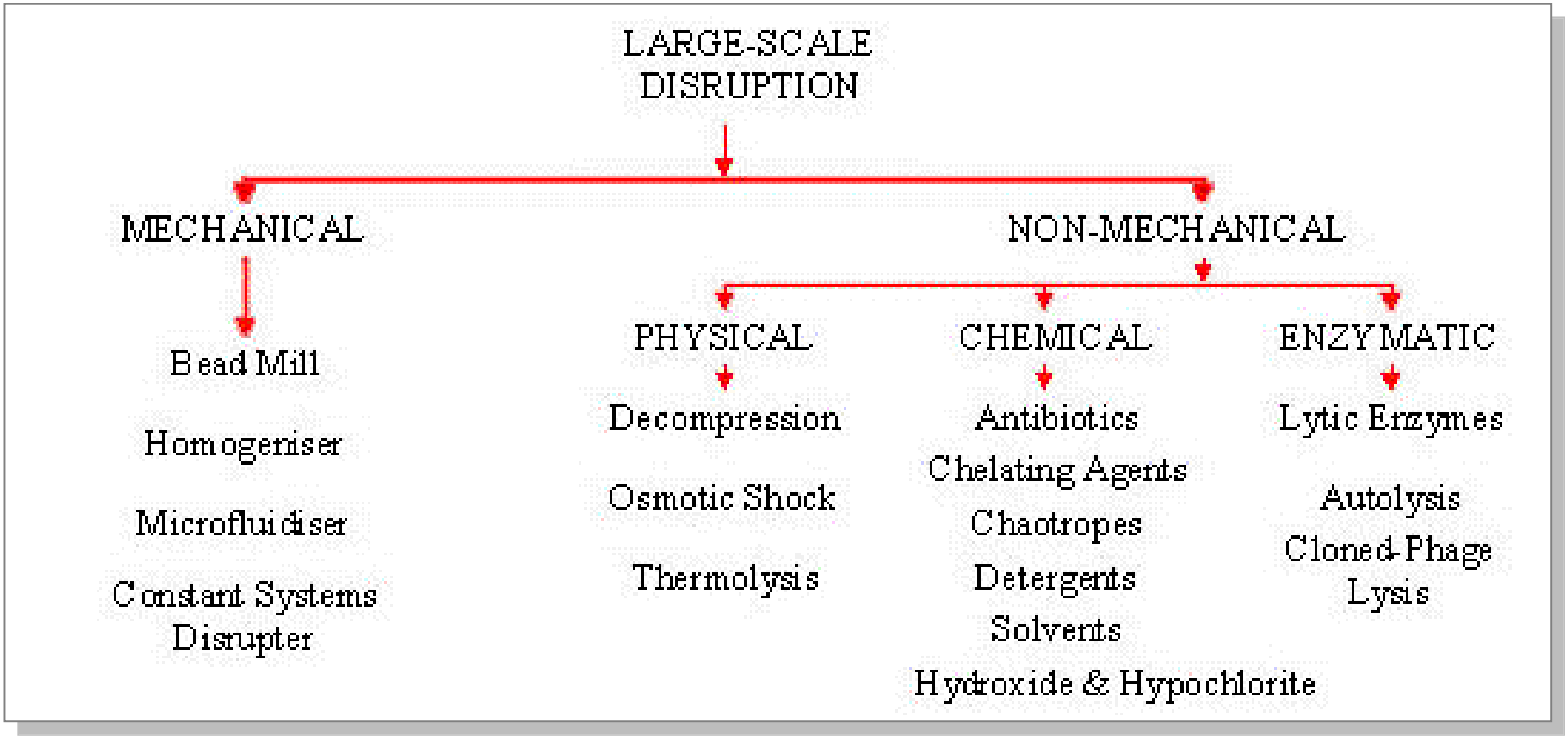
Bacteria - *Escherichia Coli (E-Coli)*

Membrane Protein	**- best between 27 and 35 Kpsi**
Cytoplasmic Protein	**- best between 20 and 27 Kpsi**
Inclusion Bodies	**- best between 15 and 20 Kpsi**
Release of DNA	**- best below 15 Kpsi**

注意事項

- 樣品請勿小於 20mL
- 操作完後的清洗，人必須在現場等清洗，結束後執行復原歸位步驟才能離開。

細胞破碎的方式


LARGE-SCALE DISRUPTION
MECHANICAL
NON-MECHANICAL
Bead Mill
Homogeniser
Microfluidiser
Constant Systems Disrupter
PHYSICAL
CHEMICAL
ENZYMATIC
Decompression
Osmotic Shock
Thermolysis
Antibiotics
Chelating Agents
Chaotropes
Detergents
Solvents
Hydroxide & Hypochlorite
Lytic Enzymes
Autolysis
Cloned-Phage Lysis

細胞破碎的方式之比較

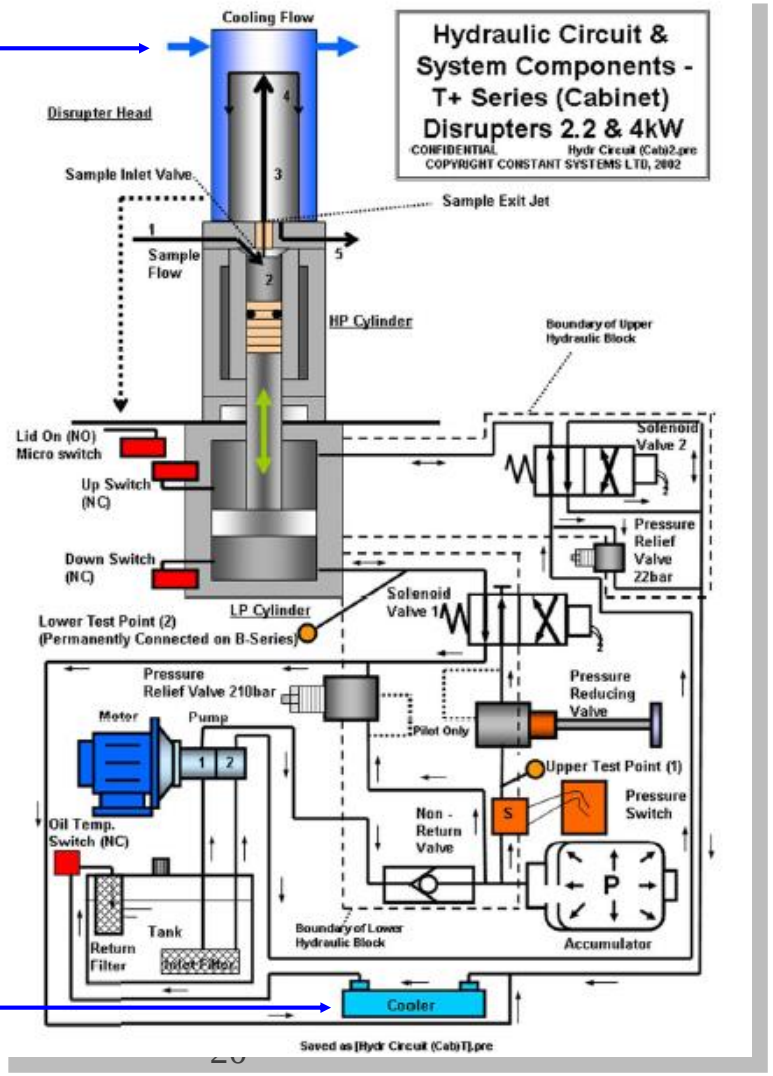
原理	優點	缺點	應用實例
溫度	操作簡單便宜	蛋白質會失活	動物細胞
酵素	具專一性	成本貴	細菌
化學物質	具專一性	蛋白質會失活,後續處理麻煩	細菌/酵母菌
超音波	方便	量產不易	假單細胞菌
剪力破碎	破碎效果佳	操作時間長	酵母菌/孢子/藻類
高壓破碎	破碎效果佳/操作時間短/易放大	熱轉換不易	E.coli

連續式高壓細胞破碎機型

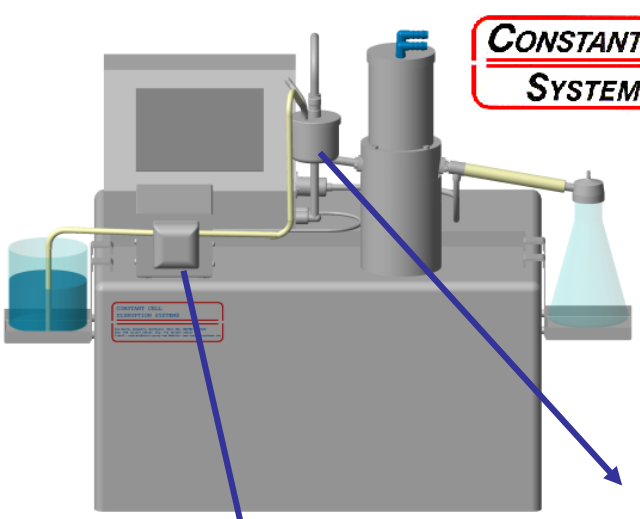
	Flow	Pressure	Flow Rate	Features
One Shot Model 0.75KW	One Shot	Up to 40,000psi	0.5ml - 20ml per shot	Research
TS 0.75kW	Continuous Flowing	Up to 40,000psi	40ml - 115ml /min	Research
TS 1.1KW	Continuous Flowing	Up to 40,000psi	100ml - 255ml /min	Pilot Scale
TS 2.2KW	Continuous Flowing	Up to 40,000psi	190ml - 310ml /min	Pilot/Production Scale
TS 4KW	Continuous Flowing	Up to 40,000psi	405ml - 565ml /min	Production Scale

TS 2.2KW & TS 4KW 系統

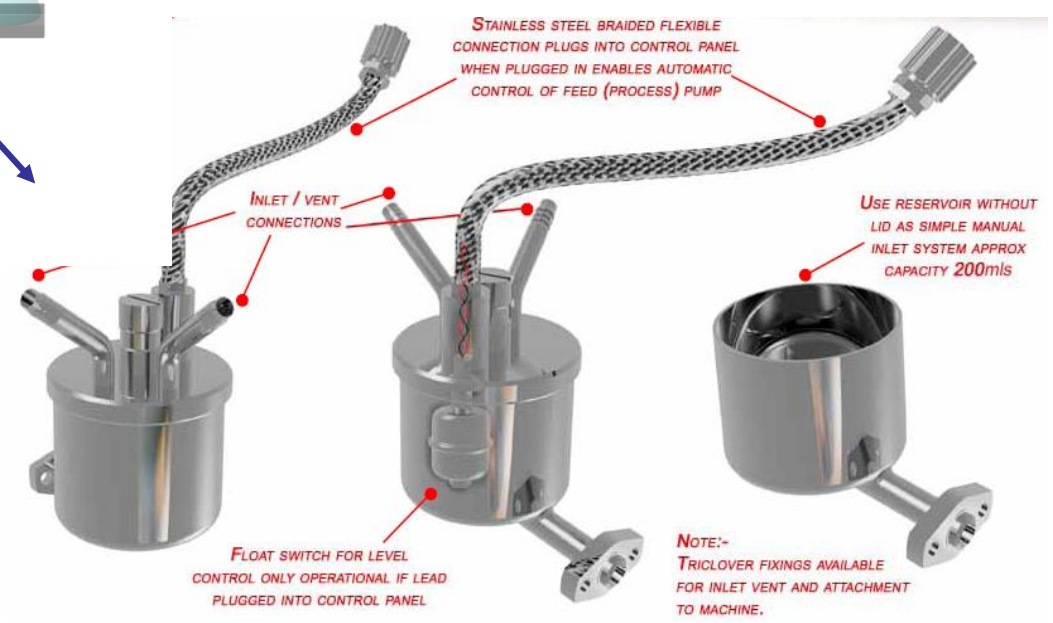
chiller



更改配件即可連續破碎



optional pump



Constant Systems 優勢

- 再現性佳
- 放大量產容易
- 破碎效果佳
- 操作時間短
- 可控制溫度
- 應用廣泛
- 進料/破碎/出料全自動化

Examples of High Pressure Disruption

2. The effect of pressure on the cell disruption of Saccharomyces Cerevisiae expressing protein X

Completed at an independent and confidential customer site, 2003.

Preparation:

Cell pellet was resuspended in chilled 20mM Tris/1 mM EDTA, pH 7.98. The required lysis buffer was calculated as follows: (46.82% w/v/20%w/v) x 233ml = 545ml. The resuspended cells were mixed for 30 minutes at 6°C. pH and conductivity of resuspended paste; 6.98 at 12.2°C and 1.448mS/cm at 13.8°C. OD_{600} of resuspended cells; 76.

Method 1:

40ML of the resuspended cells were passed through a Constant Systems disrupter once at the following pressures; 8, 10, 15, 20, 25 and 27kpsi.

Pressure	Protein mg/ml
Non Disrupted	0.88
8	2.20
10	2.89
15	4.16
20	4.34
25	4.41
27	4.61

Result after 1 pass

Sample	% Cell Disruption
Non Disrupted	0
8	52%
10	68%
15	73%
20	87%
25	96%
27	98.9%

Examples of High Pressure Disruption

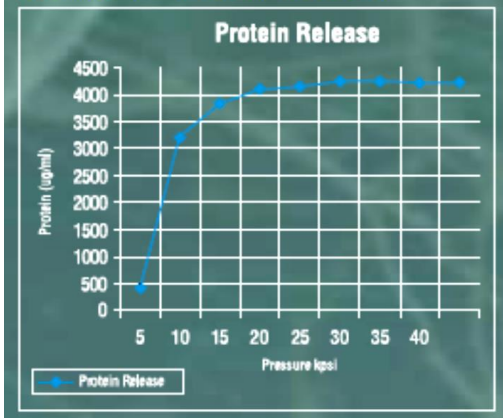
1. The effect of pressure on the cell disruption of Esherichia coli expressing soluble protein X

Completed at an independent and confidential customer site, 2003.

Preparation:

Cell paste (87g WCW) resuspended in 870ml 20mM TrisHCl/5 mM EDTA, pH 8.0. 40ml of the suspension was passed through Constant Cell Disruption Systems (Z Plus Series) once with the following disruption pressures: 10, 17, 20, 24, 27, 30, 33 and 35kPSI respectively. Samples were centrifuged at 8000 rpm for 20 minutes at 4°C. Determined protein released with coomassie assay. Thereafter repeat passes of the same sample were passed through the cell disrupter to determine if multiple passes improved efficiency in protein yield between passes.

Results 1 – Protein Release:



Pressure (kpsi)	Protein (ug/ml)
0	414
10	3221
17	3838
20	4106
24	4162
27	4261
30	4247
33	4240
35	4236

Number of Passes	Protein ug/ml
1	4261
2	4840
3	4884
4	4488

For this particular strain of e.coli 27kpsi was the optimal pressure.

Cell Disruption following 1 pass through the cell disrupter = 99.87%. 99.87% breakage would leave 0.13% intact = 7.2×10^7 cfu/ml

Cell Disruption following 2 pass through cell disrupter = 99.997%. 99.997% breakage would leave 0.003% intact = 1.7×10^5 cfu/ml.

Constant Systems使用客戶

- Pfizer -UK
- NIH -USA
- University of Cambridge -UK
- Tokyo University -Japan
- SmithKline Beecham -Belgium
- Academia Sinica -Taiwan
- NHRI-Taiwan
- And More…

Thanks for Your Attending.