

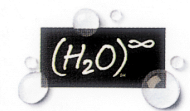
Millipore Corporation Laboratory Water Division

純水原理及用水探討
美商密理博公司純水部

純水技術應用專員：劉正儀

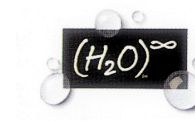


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討論內容

- 水質定義.
- 水中污染物種類及說明.
- 各種污染物之檢測單位及其定義.
- 各種純化方法之說明.
- 純水系統管理及菌膜(Biofilm)的形成
- 用水觀念之探討.



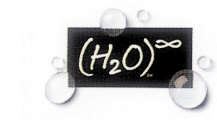
為何要使用純水/超純水？

■ 水中污染物降低可使化學分析儀器之背景降低並可得到較佳的儀器偵測極限. 在配製Standard、Rinse容器及稀釋樣品時使用超純水可減少污染的來源

■ 通常水中污染物會與樣本或標準溶液有複雜的反應, 而水中污染物之種類及濃度又無法確認及穩定供給, 所以再現性(Reproducibility)會有很大的麻煩.

■ 超純水的重要性在於能提供----

1. 低污染物背景值. 提高S/N ratio
2. 一致性. 穩定性之實驗品質.



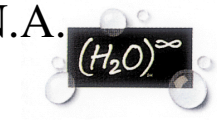
實驗用水之一般性定義

	無機鹽類	有機物	微生物	懸浮固體
超純水	* 0.055 us/cm * 18.2Mohm.cm at 25 °C * Ions = ppt (10-50 ng/L range)	* 3-20ug/L	* < 1cfu/ml	* 大於 0.22um 之顆粒數應為零
純水	* 0.1-1.0 us/cm * 1-10Mohm.cm * Ions = ppb (ug/L range)	*50-200ug/L	*10-100cfu/ml	*需有合理的過濾處理
自來水	*100-2000us/cm	*500 -7000ug/L * 0.5 - 7 mg/L	* <100cfu/ml	N.A. (H ₂ O) [∞]

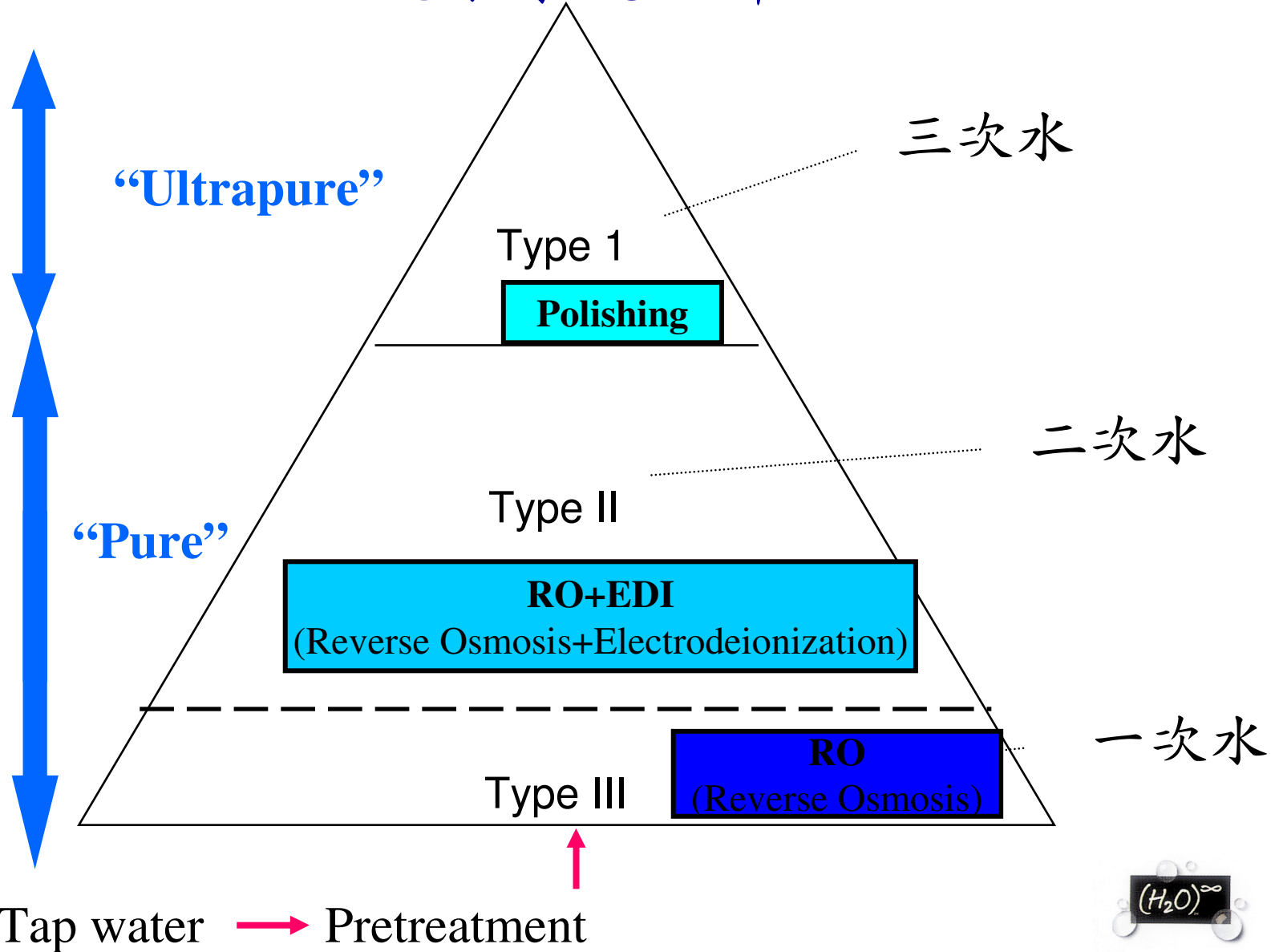
ASTM Water Specifications

	Type I	Type II	Type III
Resistivity 比阻抗值 (Mohm-cm to 25oC)	18	4	1
Total Organic Carbon 總有機碳 (ppb)	50	100	200
■ Sodium , max (ppb)	1	5	10
■ Chlorides , max (ppb)	1	5	10
■ Total Silica , max (ppb)	3	3	500
■ Max. Bacteria Count (CFU)	10/1000 ml	10/100 ml	100/10 ml
■ Endotoxin (Eu/ml)	<0.03	<0.25	N.A.

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純水系統設計



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Purity Requirements for Different Applications

Quality Norms					Quality Norms				
Applications	Type III	Type II	Type I	Purified Water	Applications	Type III	Type II	Type I	Purified Water
Analytical chemistry			Milli-Q		General laboratory applications	RiOs	Elix		Elix
Atomic Absorption			Milli-Q		Glassware rinsing	RiOs	Elix		Elix
Autoclave (steam)	RiOs				HPLC			Milli-Q	
Biochemistry automates		Elix			Humidity chambers	RiOs			
Blank preparation			Milli-Q		ICP-MS			Milli-Q	
Cell Culture			Milli-Q		Ion Chromatography			Milli-Q	
Cellular biology			Milli-Q		Microbiological culture media		Elix		
Clinical analyzers		Elix	AFS		Molecular biology			Milli-Q	
Dilution of samples			Milli-Q		Production				Elix
Dissolution testing				Elix	Rinsing: wafers			Milli-Q	
Electrophoresis			Milli-Q		Stability chambers		Elix		Elix
Fog generation		Elix			Washing machines	RiOs	Elix		
					Weatherometers		Elix		

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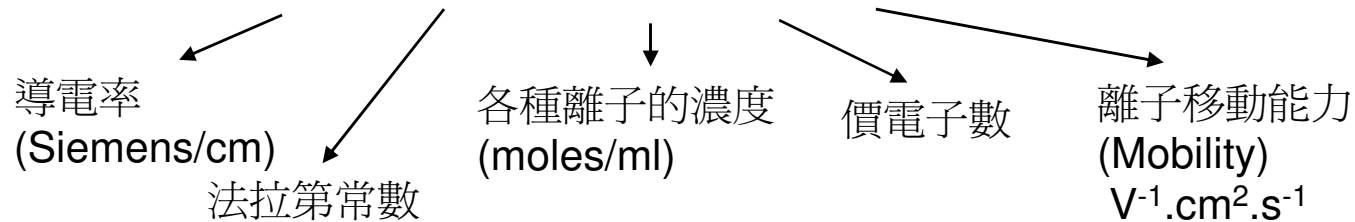
But they need different water qualities...

Water Type	Application
Type 1 (Ultrapure)	CRITICAL Water for HPLC,GC, HPLC ,AA , ICP-MS, for buffers and culture media for mammalian cell culture & IVF, reagents for molecular biology...
Type 2 (Pure)	REGULAR Buffers, pH solutions,culture media preparation ,clinical analysers and weatherometers feed.
Type 3 (Pure)	BASIC Glassware rinsing, heating baths and autoclaves filling,

導電率或比阻抗

- 水中之陰陽離子含量可由
導電率(Conductivity)或比阻抗(resistivity) 表示
兩者互為倒數運算關係。

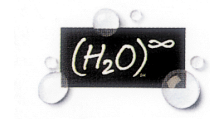
$$\chi = F \cdot \Sigma C_i Z_i u_i$$



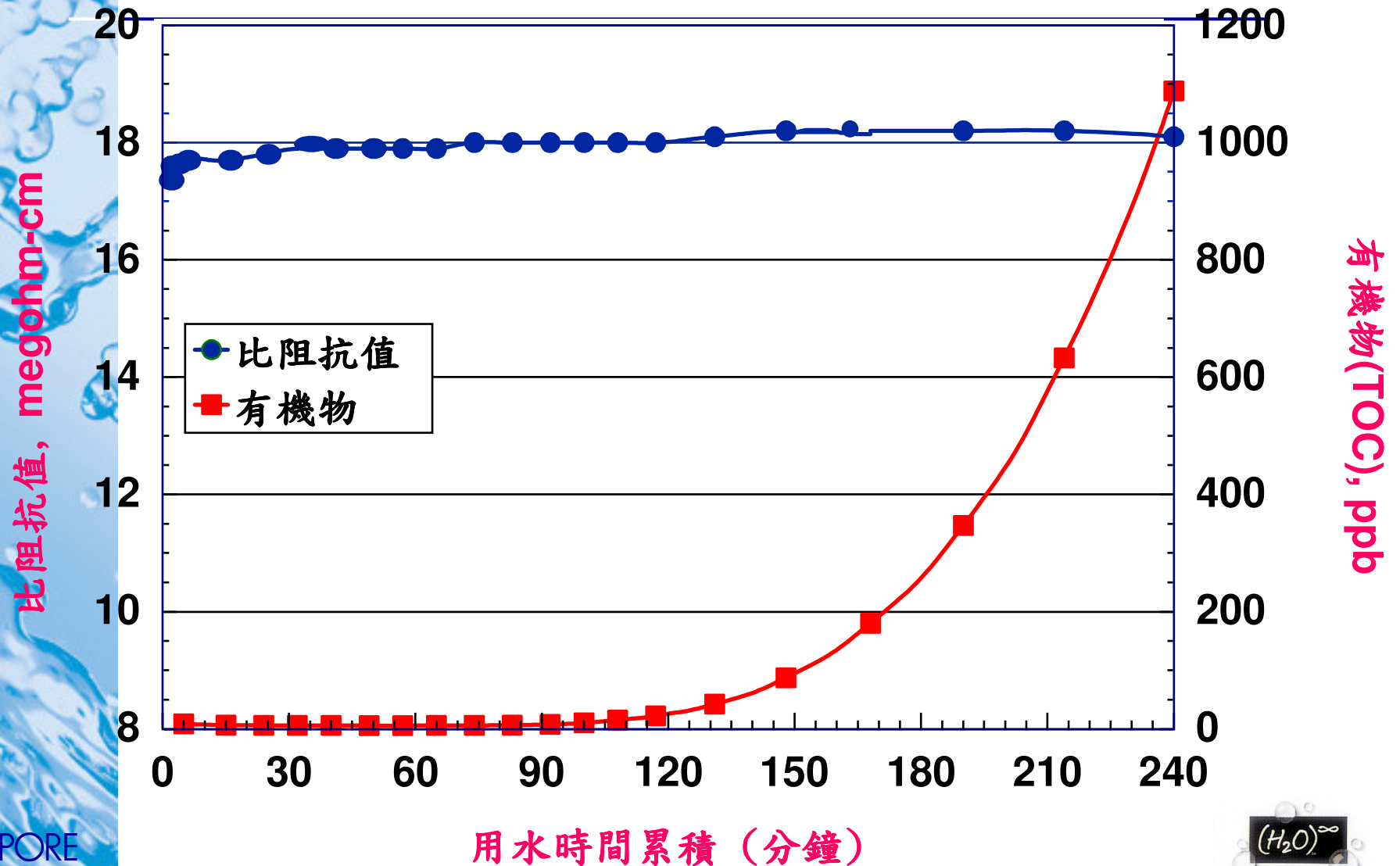
$$\text{Resistivity} \quad R = 1/\chi \quad \text{Conductivity}$$

$$0.1 \text{ M}\Omega \cdot \text{cm} = 10 \mu \text{ S/cm}$$

$$18.2 \text{ M}\Omega \cdot \text{cm} = 0.055 \mu \text{ S/cm} \quad \text{超純水 (25}^\circ\text{C)}$$



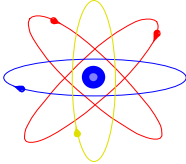
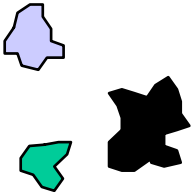
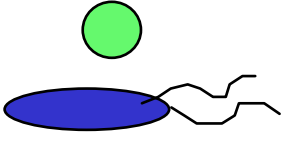
以刻意添加有機物(蔗糖)於純水中,做為
Milli-Q之進水後,其所產製的超純水之水質變化



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(H₂O)[∞]

水中各種污染物之分類

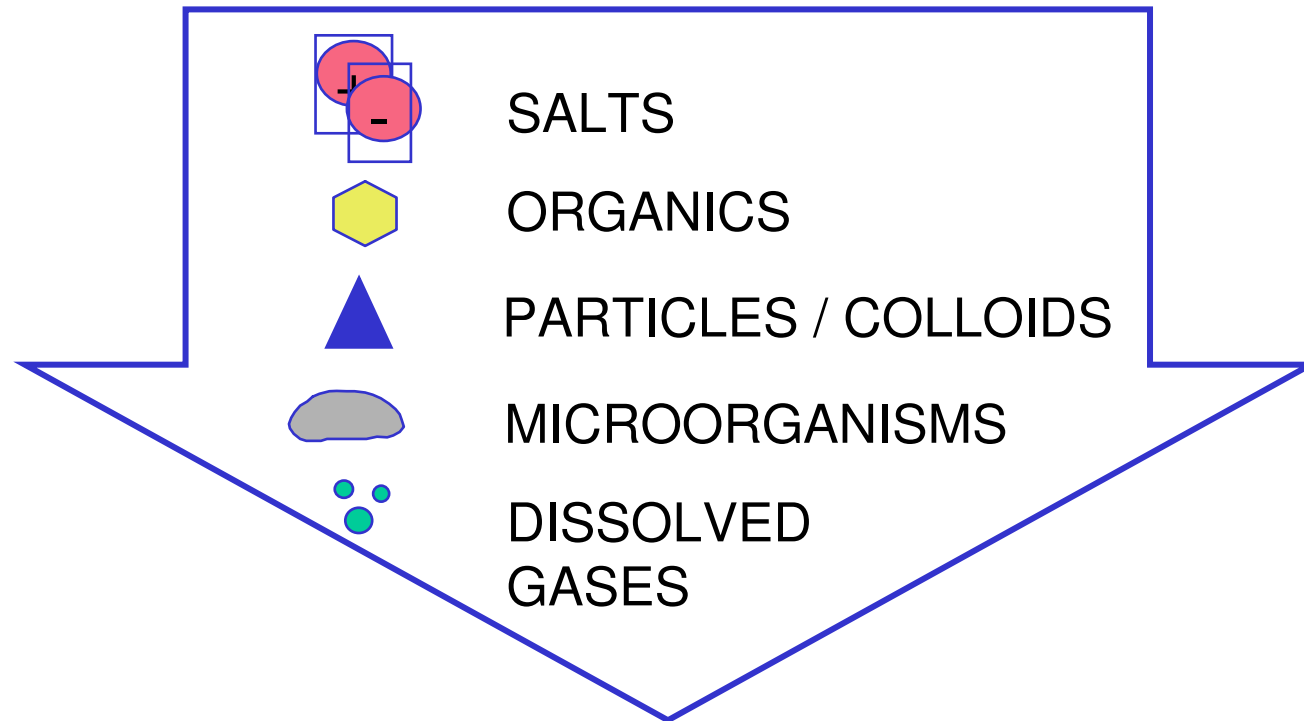
無機鹽類 	陽離子 Na^+ Ca^{2+}	陰離子 Cl^- HCO_3^-
有機化合物 $\begin{array}{c} \text{H} \quad \text{H} \\ \quad \\ \text{H}-\text{C}-\text{C}-\text{OH} \\ \quad \\ \text{H} \quad \text{H} \end{array}$	天然來源 單寧酸/腐植酸	環境污染物 殺蟲劑/除草劑
懸浮顆粒 (膠體) 	非可溶性顆粒性物質	
微生物 (內毒素) 	細菌／藻類／真菌 (polyliposaccharide of Gram negative bacterial cell wall)	

水中各種污染物之量測單位

污 染 物	檢 測 名 稱	使 用 單 位
無機鹽類	比電導度 (比阻抗值)	μ S/cm (Megohm.cm)
有機物	總有機碳 (Total Organic Carbon)	p.p.b. (ug/L)
懸浮顆粒	Silt Density Index / Fouling Index	Rate of pluggage of 0.45 um membrane.
微生物	生長在 0.45 微米濾 膜上之菌落數.	CFU/ml (Colony Forming Unit/ml)
內毒素	- Rabbit Inoculation test - LAL Test	Endotoxin units/ml EU/ml

(H₂O)[∞]

Water Purification Techniques

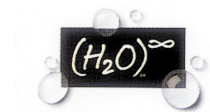


Millipore Purification Techniques



Pure and Ultrapure water

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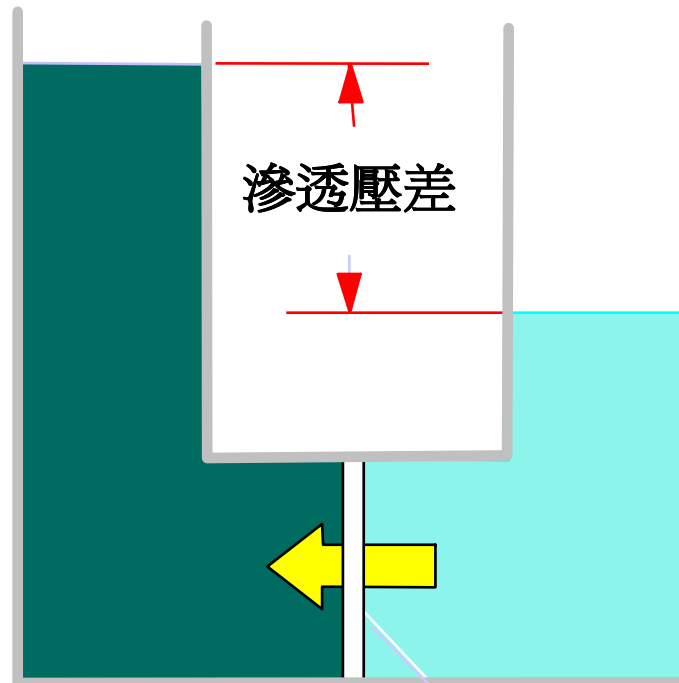


水質純化方法介紹及說明

- 過濾法
- 活性碳過濾法
- 逆滲透法
- 離子交換樹脂法(混床式. 電子式)
- 結垢控制
- 超過濾法
- UV (185nm) 光氧化有機物法
- UV (254nm) 紫外光殺菌法

自然狀態下之滲透現象

含有各種污染物之
自來水
(滲透壓相對較高)



純水
(滲透壓相對較低)

(具有選擇性過濾之半通透膜)

水流流動方向

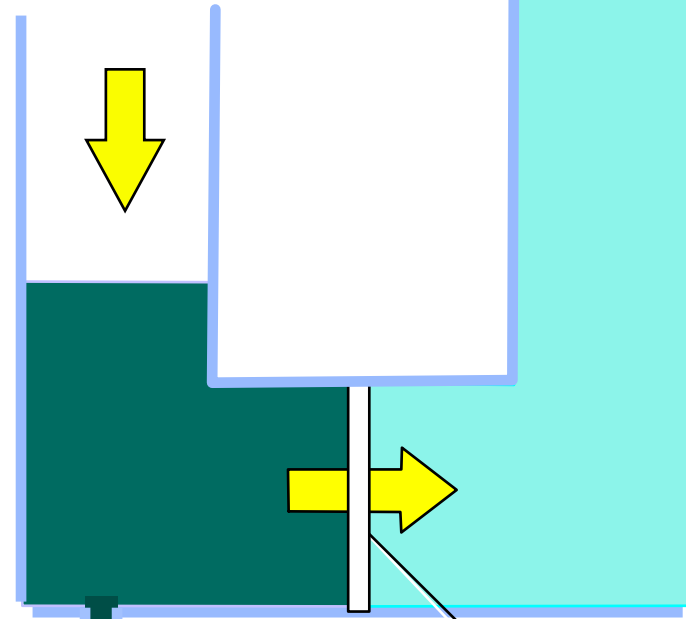
水流自低滲透壓自然流向高
滲透壓,此乃正常滲透現象

逆-滲透現象

Reverse - Osmosis

較高滲透壓
較高雜質之
自來水

外力加壓



逆滲透純水
(低滲透壓)

水流方向: 自高滲透壓流向
低滲透壓, 此乃逆滲透現象

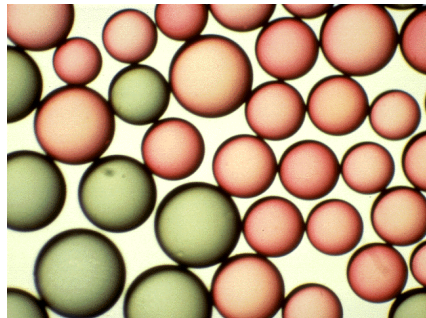
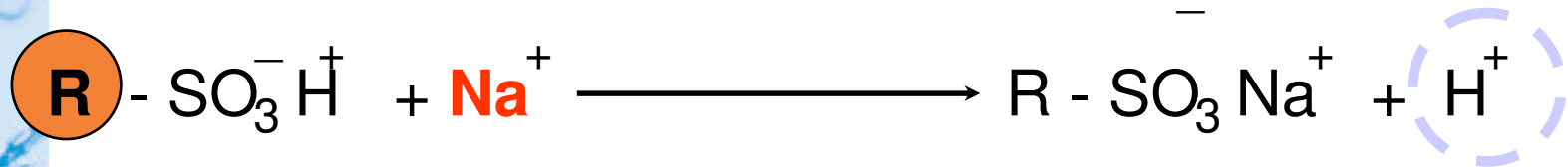
含有高濃度污染物之
排放水

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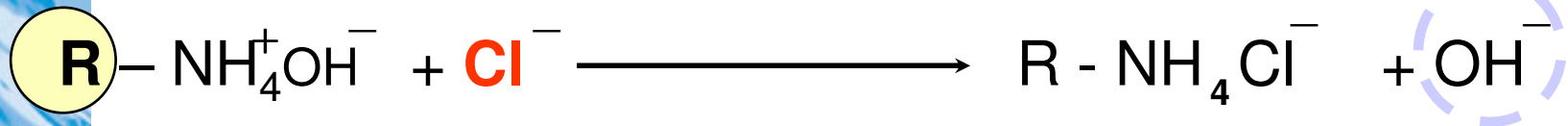
95 - 99%陰陽離子 去除率(Rejection)
99%有機物 去除率(Rejection)
99%顆粒及微生物 去除率(Rejection)

離子交換樹脂(De-Ionization)

陽離子交換樹脂



H_2O



陰離子交換樹脂

$(\text{H}_2\text{O})^\infty$

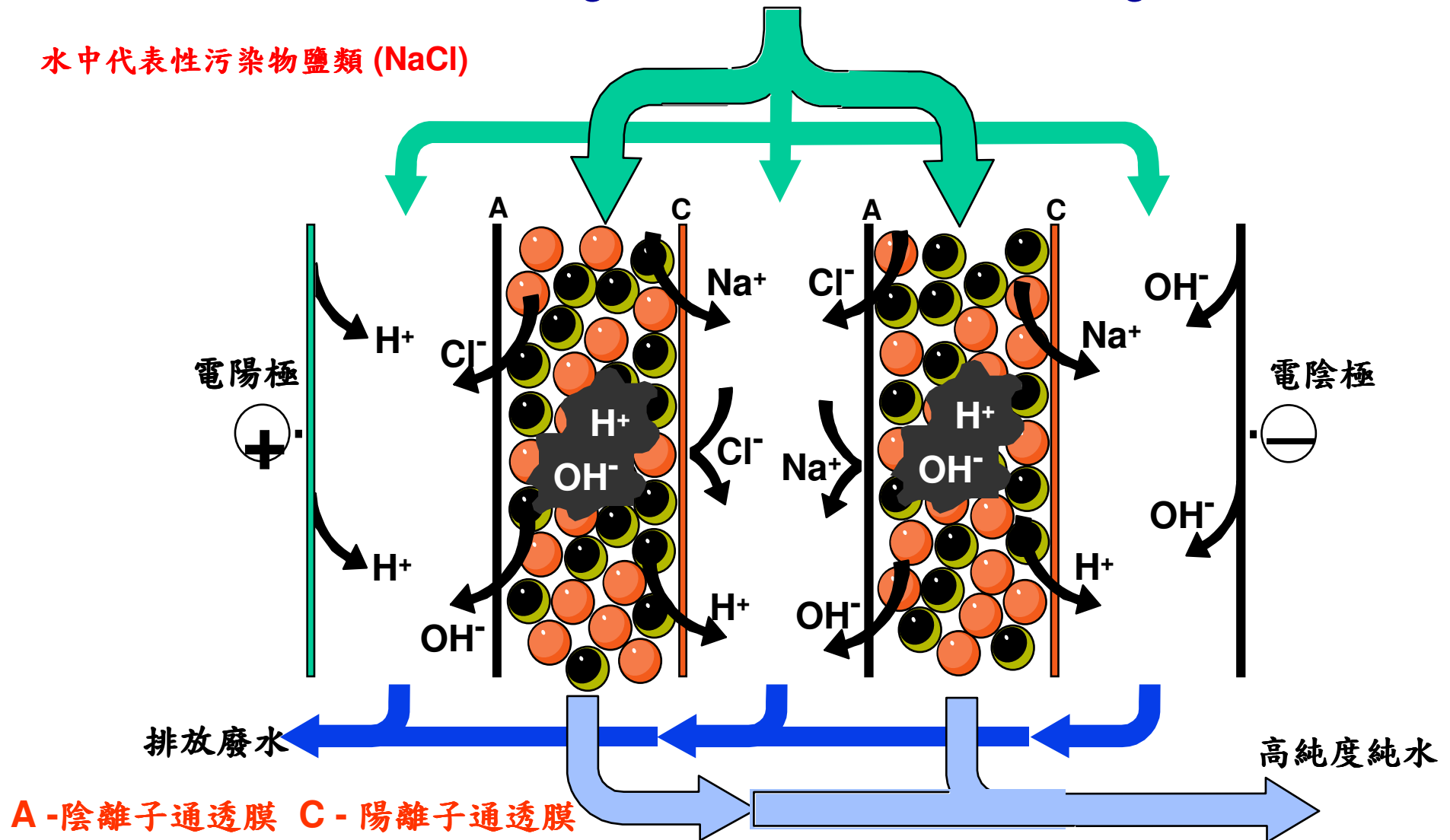
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電子連續式去離子純化原理(EDI)

Electric De-Ionization

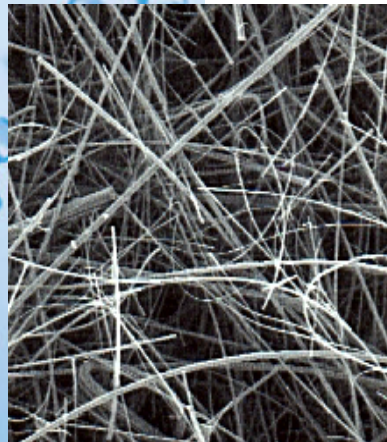
Electrochemical Regeneration of Ion Exchange Resins

水中代表性污染物鹽類 (NaCl)



微過濾 (Micro Filtration) - Depth filter/ Screen filter

Glass Fiber SEM



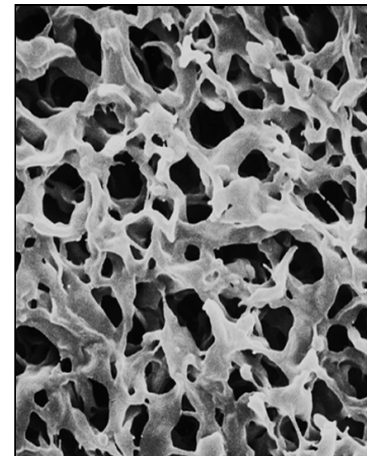
Depth filters

深層過濾

結構較凌亂
孔隙度一致性低
用來攔截顆粒性污染
可過濾大量顆粒性雜質
工作量大

Nominal

Durapore Membrane - SEM



Screen filters

微孔濾膜過濾

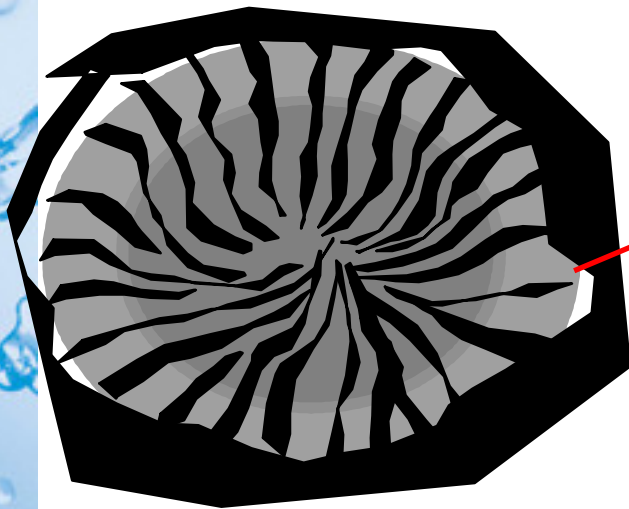
結構一致性高
孔隙度準確
濾膜表面阻隔顆粒為
低顆粒性雜質處理能力

Absolute

- Depth filters : for RO protection from fouling (high particle capacity).
- Membrane filters : for retention of small amounts (quick clogging) of small *particulates* like bacteria.
- Additional treatment:
 - Depth filters : for heavily loaded feed water with particles (SDI over specification).
 - Screen filters : for a final low pore size filtration downstream the system.
 - Charge filters : for pyrogen removal, in-line distribution loop

(H₂O)[∞]

活性炭 (Active Carbon)



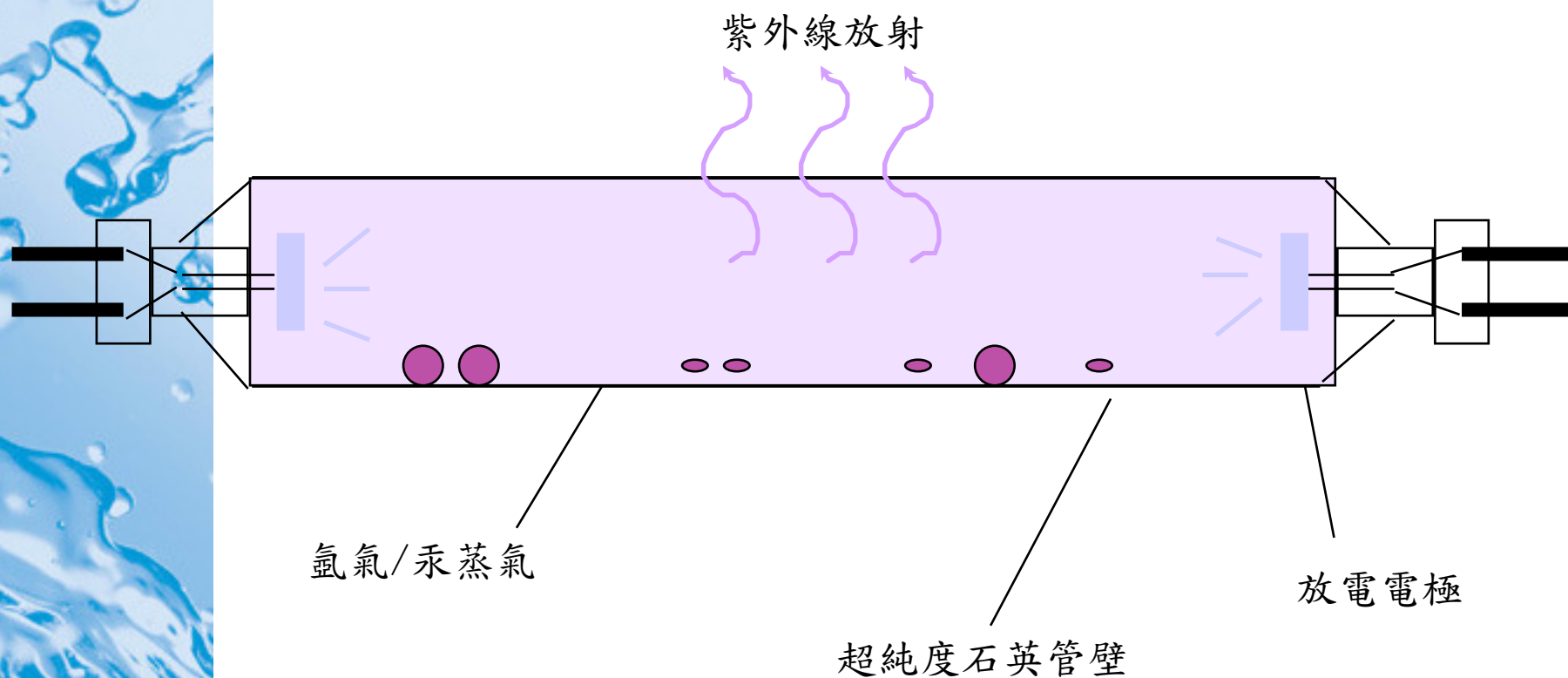
經活化處理過之活性炭產生大量之大/小孔隙,使整體表面積大增,使表面吸附能力大增.

**活性炭可吸附之表面積
1000 m² /gram**

- 可有效中和漂白水等強氧化劑
- 可大量吸附水中有機物質

紫外線光氧化原理

使用低汞蒸氣壓燈



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$(H_2O)^\infty$

紫外線/光氧化之輻射波長

相對輻射強度%

100%

20%

殺菌用波長 254 n.m.

造成光氧化作用之波長

185 n.m.

波長 (n.m.)

(H₂O)[∞]

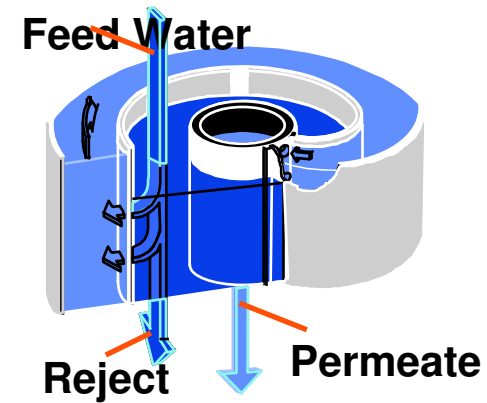
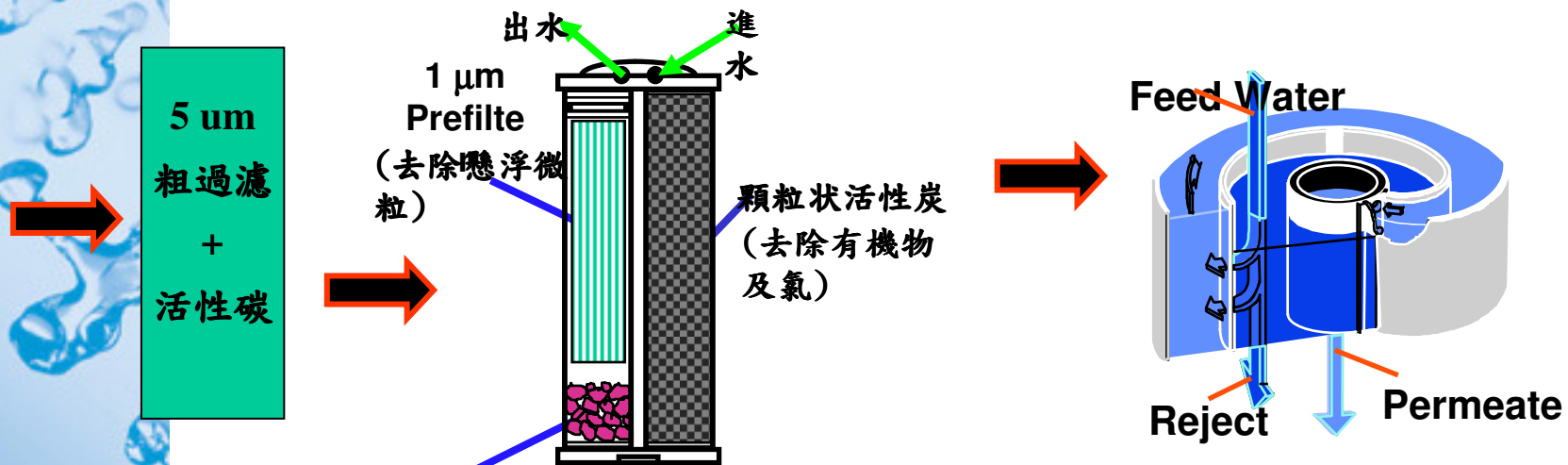
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水質純化方式比較

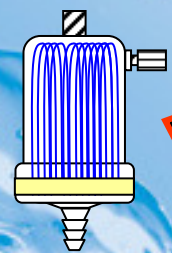
	蒸餾	離子 交換樹脂	逆滲透	超濾膜	微濾膜	活性碳
無機鹽						
有機物						
懸浮物. 膠體						
微生物						

系統內菌膜(Biofilm)防治

Purification Process



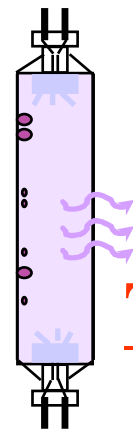
Type 3



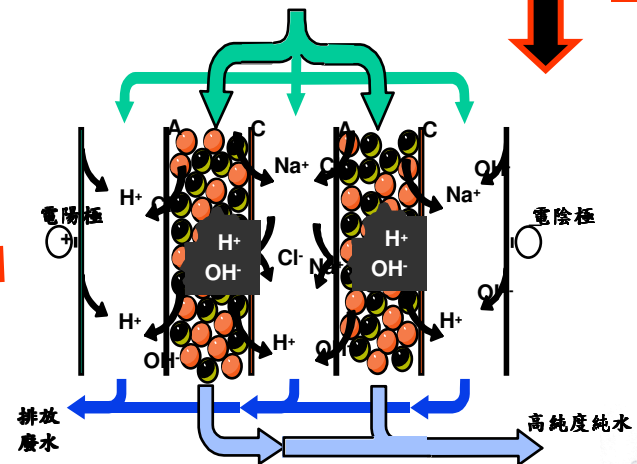
Type 1

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混床式
離子
交換樹脂
+
活性炭

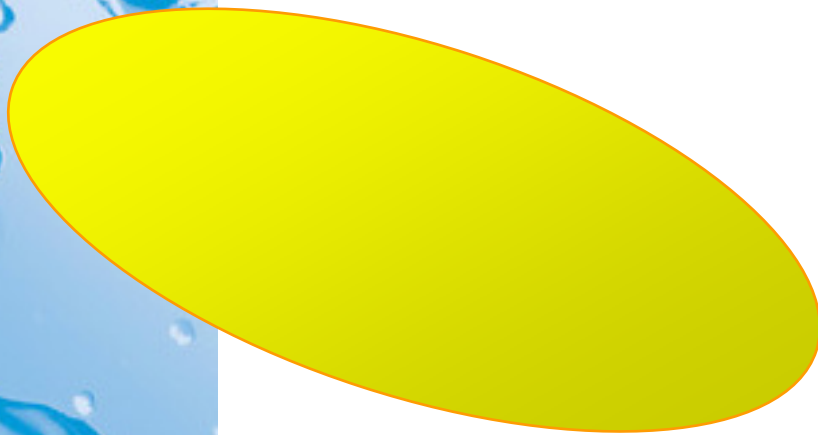


Type 2



菌膜(Biofilm)之形成過程

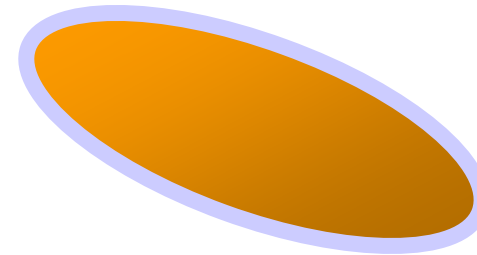
在純水中微生物適應之道



10 um



“具黏性”
多醣類物質黏膜
(Glycocalyx)

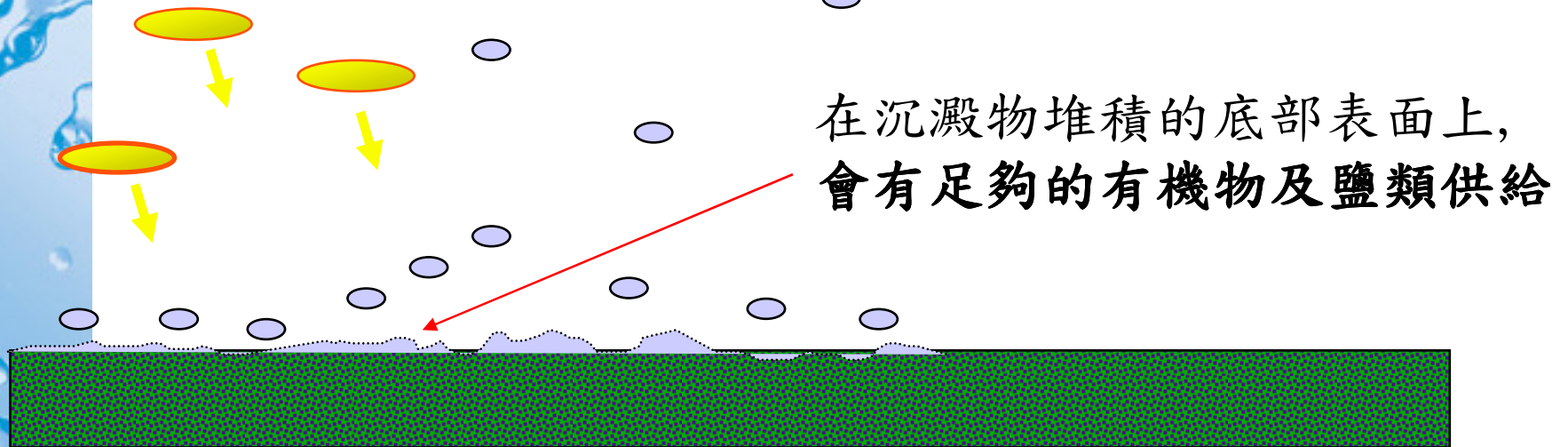


3 um

<微生物在純水系統中, 受營養跟環境壓力限制, 以菌膜方式存在>

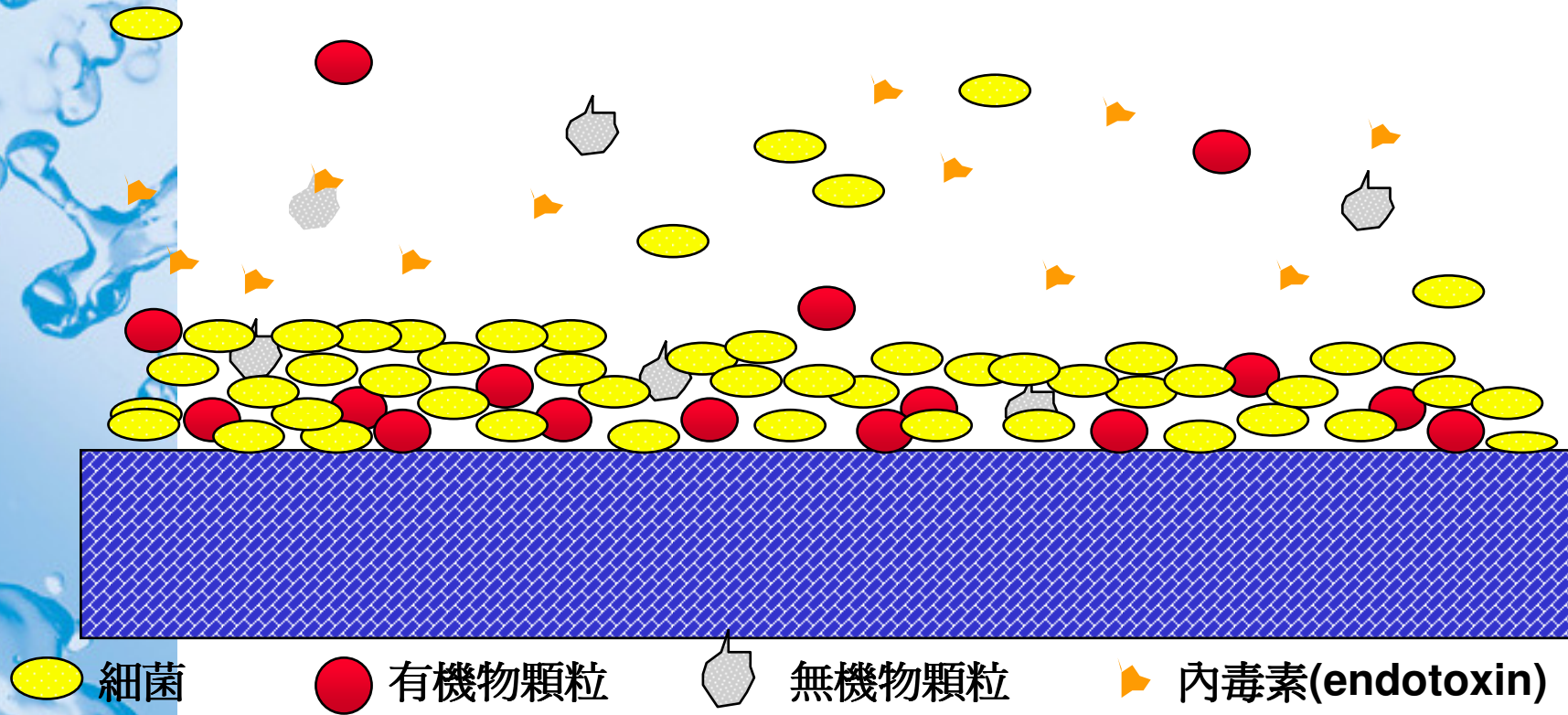
菌膜(Biofilm)之形成過程

細菌會黏在各種沾濕材料(wetting material)上，以主動方式分解及吸收所需養份。



<細菌體積變小更有利於吸收養份，因體表面積變大>

菌膜(Biofilm)生態體系之形成

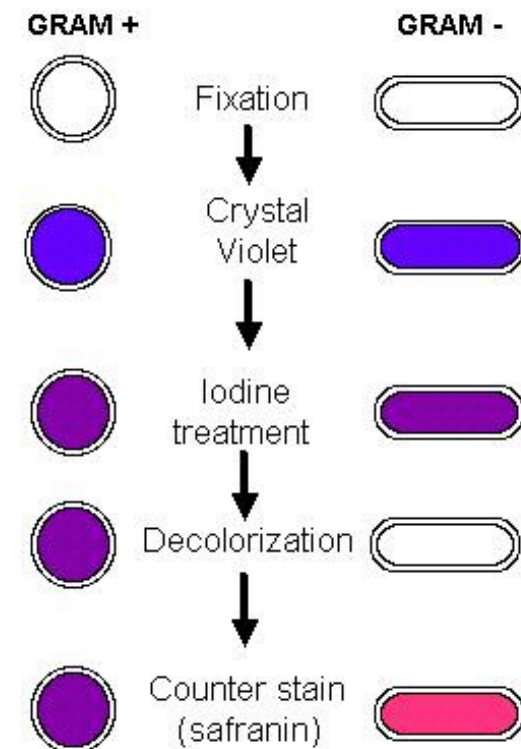


菌膜 → 內毒素, 酵素及高分子有機分解物

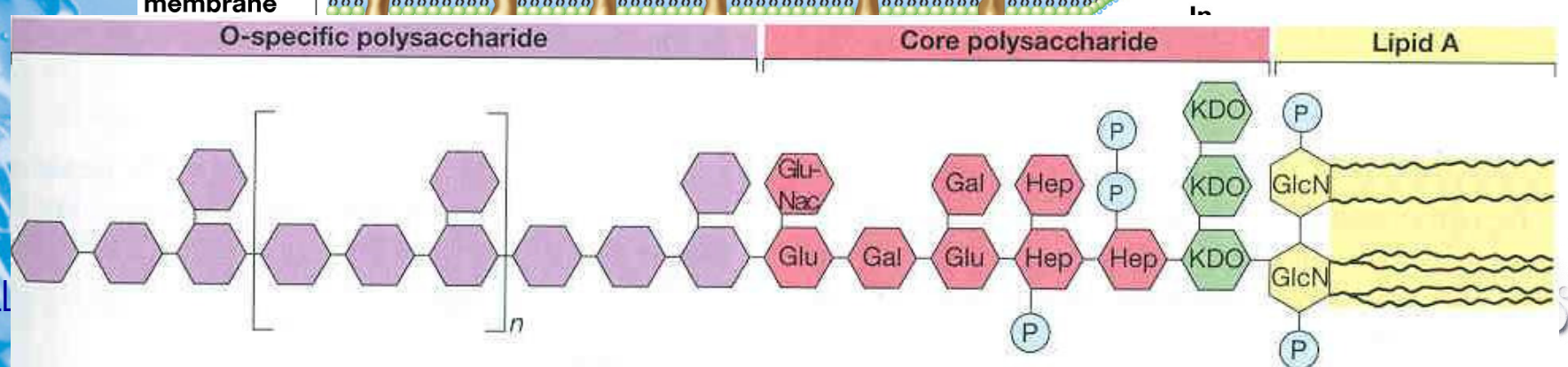
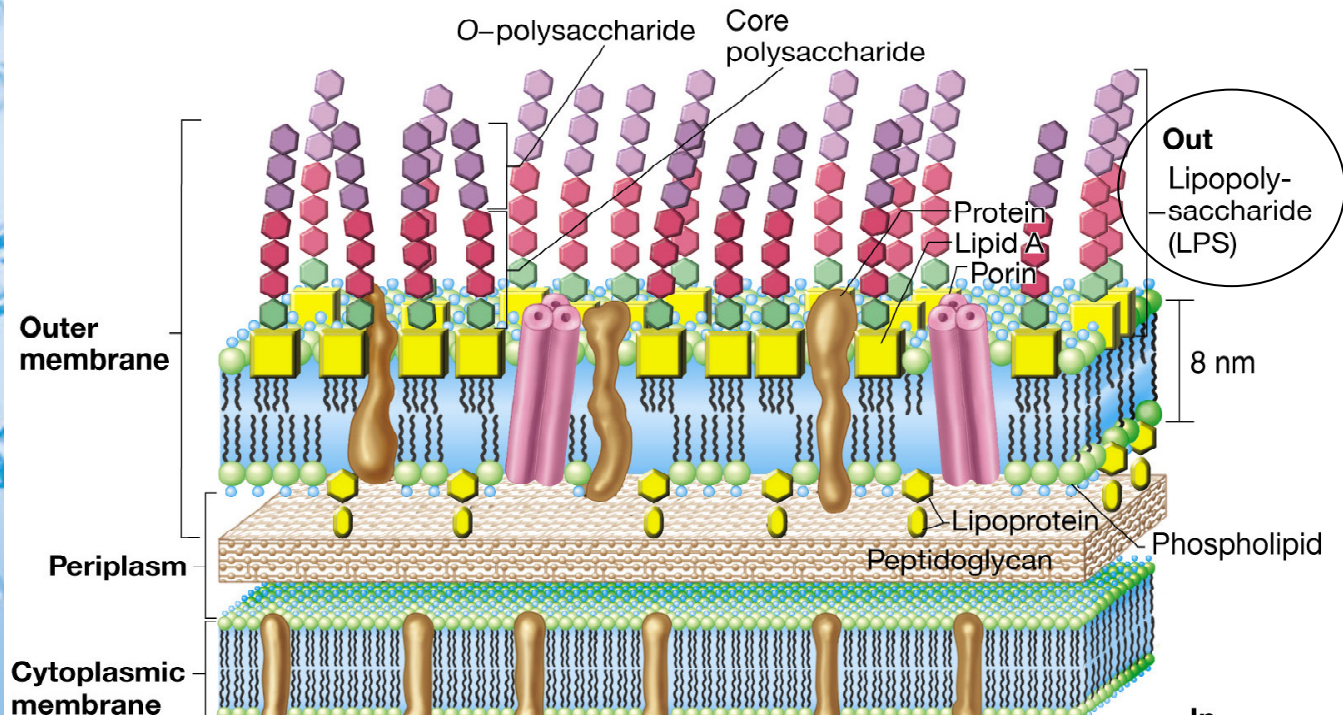
菌膜在純水系統亦會阻塞RO膜, 影響水質

微生物污染

- Bacteria
- Endotoxin : G(-)bacteria
outer membrane
- Lipopolysaccharide (LPS)



G(-)bacteria outer membrane



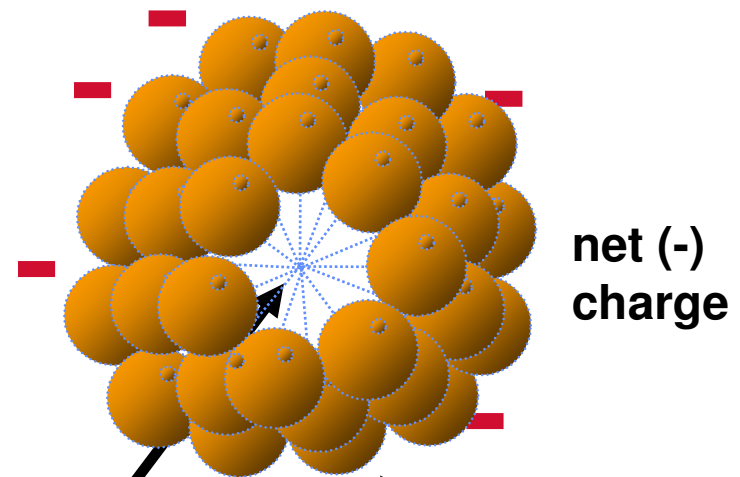
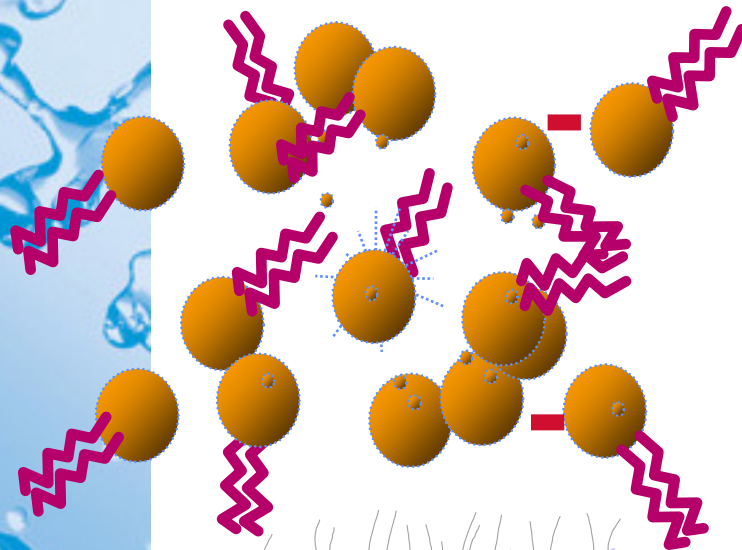
LPS Dispersion in Water

ions present

pure water
environment

subunits 10-20 KDaltons

micelle 300,000-10⁶ daltons



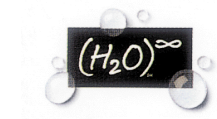
hydrophobic
core

hydrophilic
exterior

net (-)
charge

(Lipid A+O antigen)

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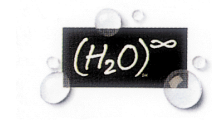
Endotoxin effects

■ Effects in vivo:

- ❖⇒ Production of factors involved in blood clotting, hypotension and fever.
- ❖⇒ Immunological reactions

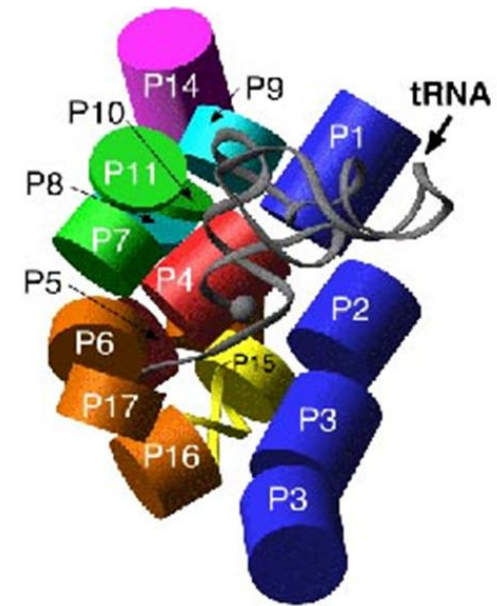
■ Effects in vitro:

- ❖Interference with membrane structure and other functions depending on the cell type or line (e.g. membrane and morphological effects, secretion / production of various substances)
- ❖Effect on IVF experimentations.
- ❖Impact on iso-focusing gel polymerization and molecular biology techniques such as PCR.

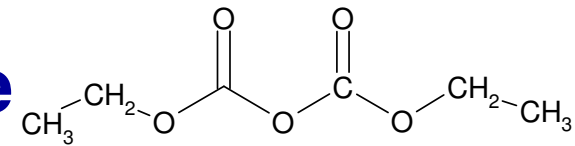


Ribonucleases (RNase)

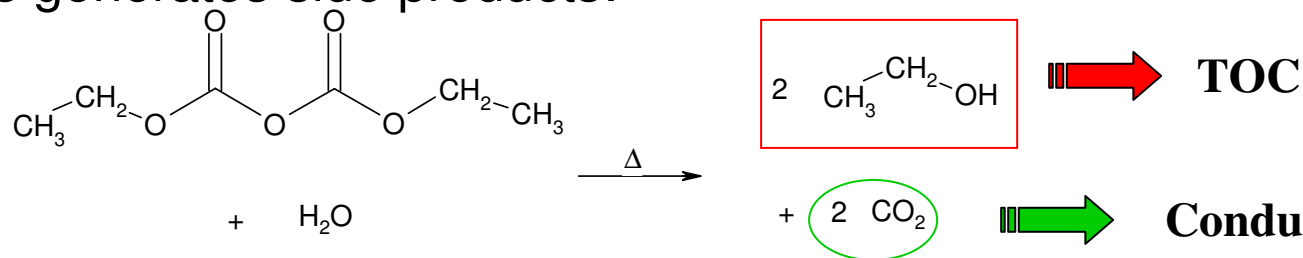
- RNases = enzymes ($\rightarrow$ = proteins) for ribonucleic acid (RNA) degradation.
 - RNA site specificity,
 - Picogram quantities are enough,
 - MW = 10,000 to 30,000 Da.
- RNases are:
 - omnipresent (hands, airborne...),
 - resistant to high temperature (up to 100°C), extreme pH,
 - fully active for weeks.
- Get rid of RNases = a challenge to molecular biologists
 - RNase present everywhere (glassware, researcher's hands...).
 - RNase active even in tiny quantities.
- RNase free water required for molecular biology applications such as:
 - gene sequencing, oligonucleotides synthesis, genetic engineering...
 - For the preparation of :
 - electrophoresis buffers
 - blotting test buffers
 - PCR solutions
 - sample dilutions,....



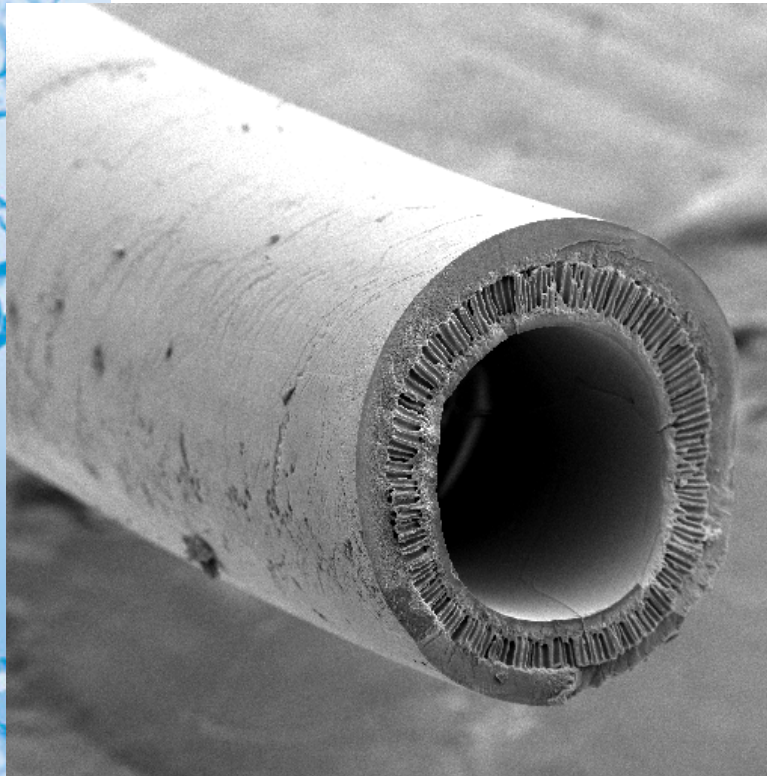
DEPC: diethylpyrocarbonate



- Chemical (suspected carcinogen) that inactivates the enzyme
- DEPC reacts with RNases and inactivates them, but they are not removed
- Excess DEPC is eliminated by autoclaving (15 min, 121 °C) procedure (would react with any biological material in further experiments if not removed)
- Cannot be used with buffers containing amine functions (Tris, HEPES)
- Process generates side products:



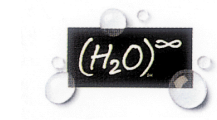
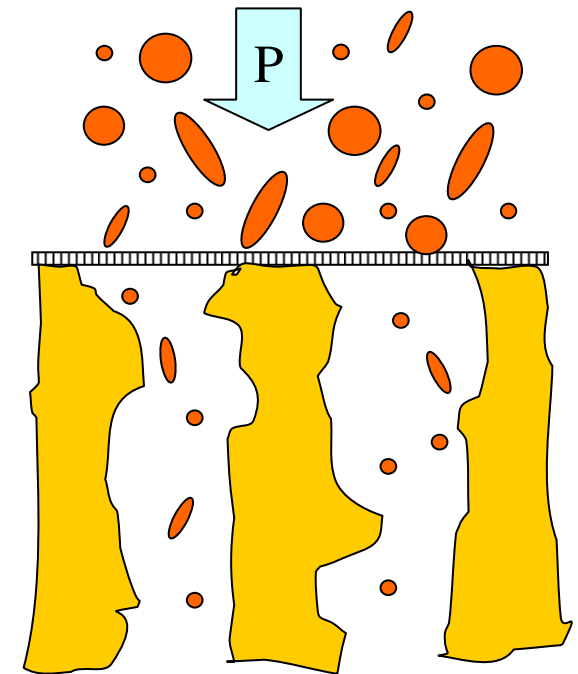
Ultrafiltration (超過濾)



MILLIPORE

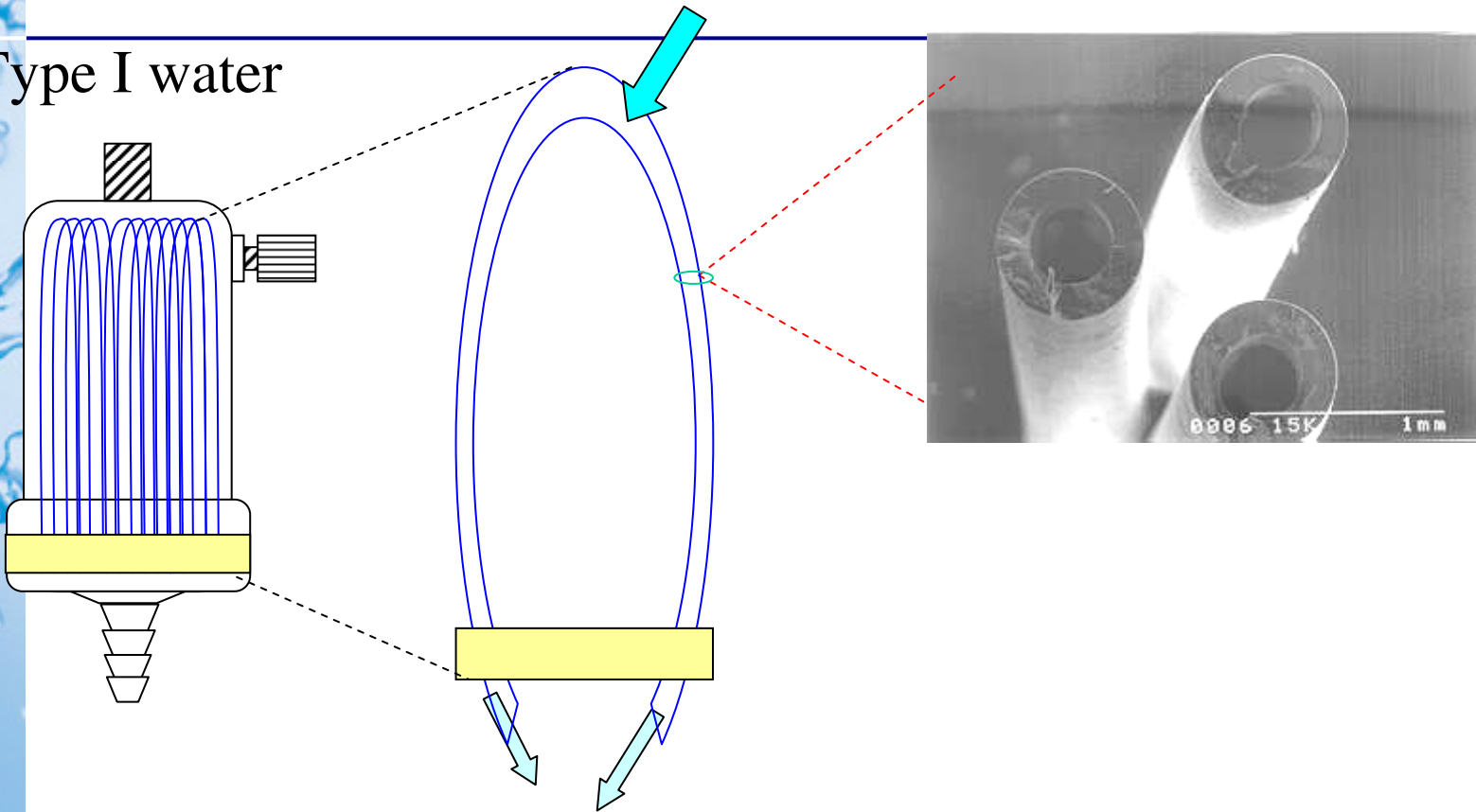
Active layer ($1\mu\text{m}$)

Support ($100\mu\text{m}$)



Ultrafiltration (超過濾)

Type I water



使用超濾膜可有效去除水中之 **內毒素, Nuclease**.

一般皆使用分子量攔截 **MWCO** (Molecular Weight Cut-Off)

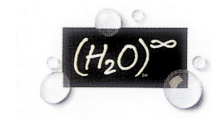
或 **LRV** (Log Reduction value) 做為有效之污染物去除指標. $(H_2O)^\infty$

重要用水觀念

- 儘量避免純水系統中有菌膜(Biofilm)的形成,因為一旦菌膜形成,沒有任何方法可以有效根除.

影響Biofilm發展的因素

- 1.微生物養分限制
- 2.表面材質
- 3.液體流速,靜量避免靜止狀態的死水



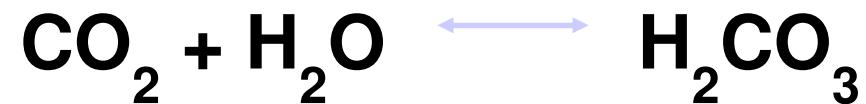
重要用水觀念 -續-

■ 防治菌膜的方法

- ❖ 準時消毒 RO 膜.
- ❖ 定期清洗儲水槽.(可加裝紫外燈)
- ❖ 準時更換耗材,不管用水量大小,因為凡是浸泡在水中之耗材,皆會無可避免的會形成菌膜.
- ❖ 合理的用水量,可以確保純水系統的穩定性.

空氣中二氧化碳與純水所產生之 化學反應公式

空氣中之二氧化碳 + 純水 = 碳酸



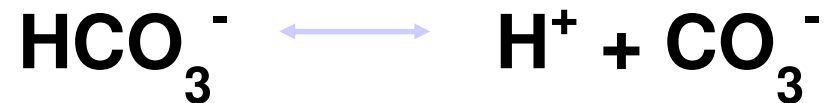
碳酸

氫離子 + 重碳酸根離子



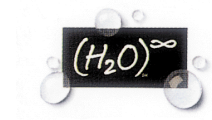
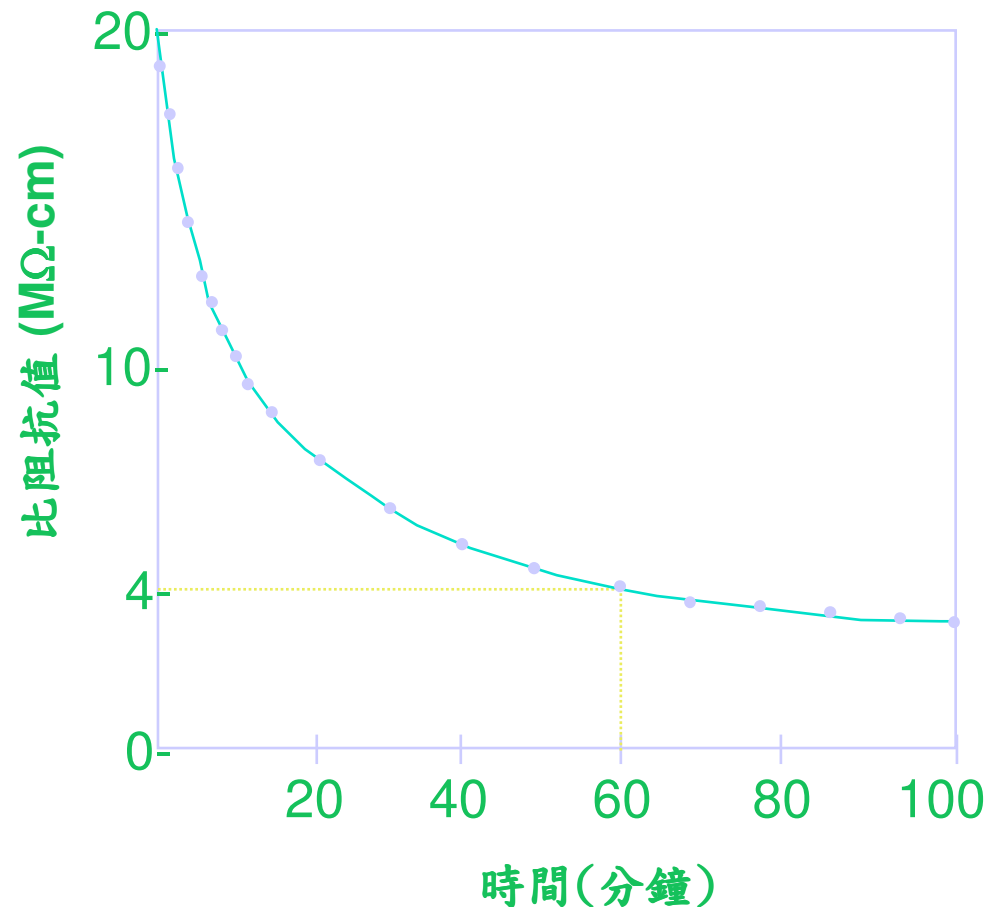
重碳酸根離子

氫離子 + 碳酸根離子



因空氣中二氧化碳溶於超純水而造成水質之劣化

1. 從碳酸中解離出之氫離子濃度 $[H^+] = 2 \times 10^{-6}$ 或 $pH = 5.7$
2. 因 氫離子 及 碳酸根離子 增加, 造成比阻抗值下降至 4 左右.



重要用水觀念 -續-

- 愈純的實驗室用水, 愈不宜久放. 因為對18.2 Megohm.cm 之超純水來說, 放置一小時之後, 阻抗會下降至4 Mohm.cm, pH 則降至5.7左右, 更遑論用水環境及容器所造成的污染 (如空氣中灰塵, 接觸容器所產生之溶出物以及儲水桶之微生物污染).
- 所以現取現用是非常重要的一个觀念.

重要用水觀念 -續-

- 取用超純水之環境良窳及容器的好壞對超純水之最終水質表現具有關鍵性影響。
- 室內灰塵缺乏過濾？空氣對流強烈？
 - 一開始前段水有無排放？
 - 容器 Rinse 夠不夠徹底？
 - 容器密封性足夠否？
 - 容器易有溶出物等問題？

重要用水觀念 -續-

- 純水系統濾材的更換原則, 有“條件說”及“時間說”兩種, 兩者並重:

- **條件說:**

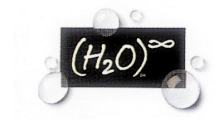
1. 儀表顯示值劣於標準值.
2. 壓力損失過大, 表示明顯有了阻塞現象.
3. 水質檢測結果顯示水質有問題時.

- **時間說:** 定期更換

以避免菌膜(Biofilm)的發生以及維持純水系統之最佳狀態, 以避免實驗上所發生的不確定風險.

總結

- 水質定義.
- 水中污染物種類及說明.
- 各種污染物之檢測單位及其定義.
- 各種純化方法之說明.
- 純水系統管理及菌膜(Biofilm)的形成
- 用水觀念之探討.



Thank you for your attention!

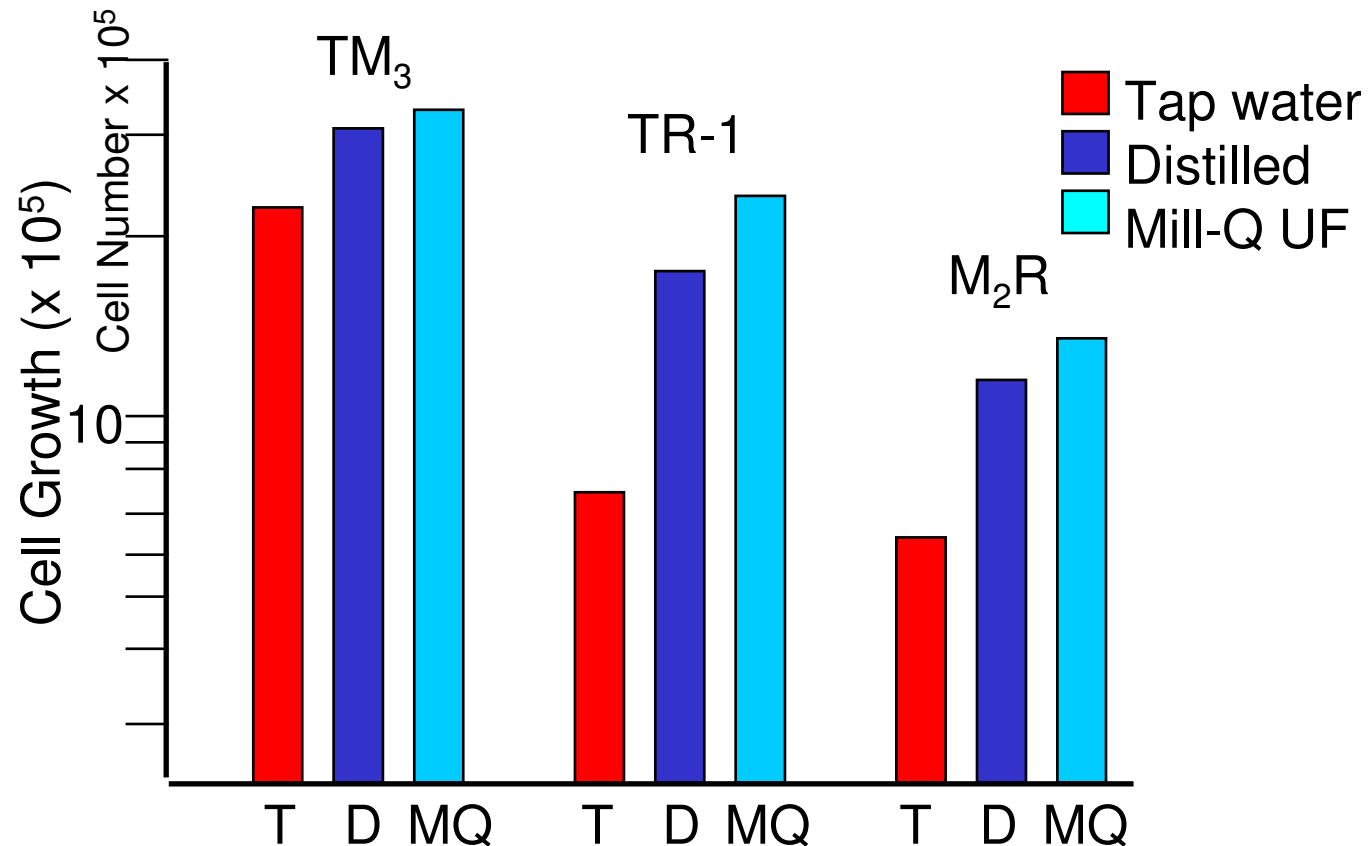
Q&A



$(H_2O)^\infty$

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Water Quality Effect on Cell Growth



Cell Line

MILLIPORE

J.Mather et al. Biotechniques, Vol 4, n° 1 (1986).

(H₂O)[∞]

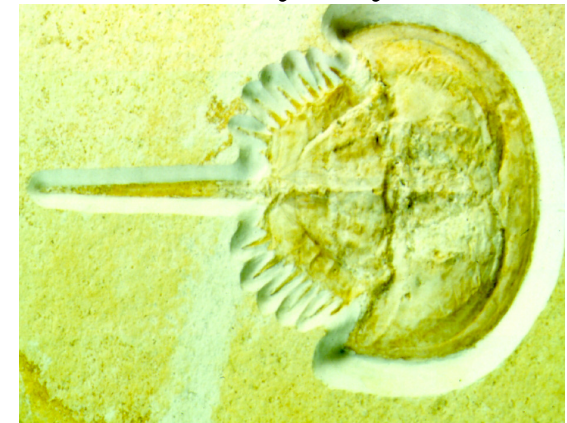
Pyrogen Detection

LAL (Limulus Amebocyte Lysate) test
Dr. Bang's 1955
horseshoe crab blood clots

Limulus
Polyphemus

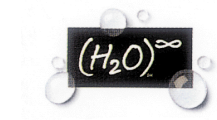


Amebocyte lysate

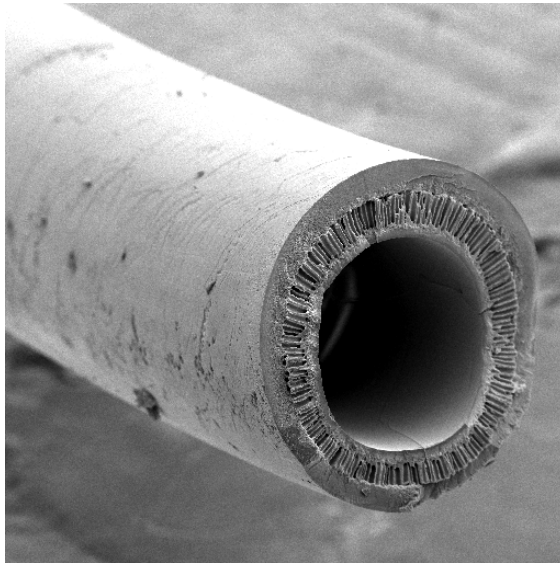


Endotoxin Units per ml (EU/ml)
1 EU/ml $\cong$ 0.1 ng/ml
= 0.1 ppb Endotoxin

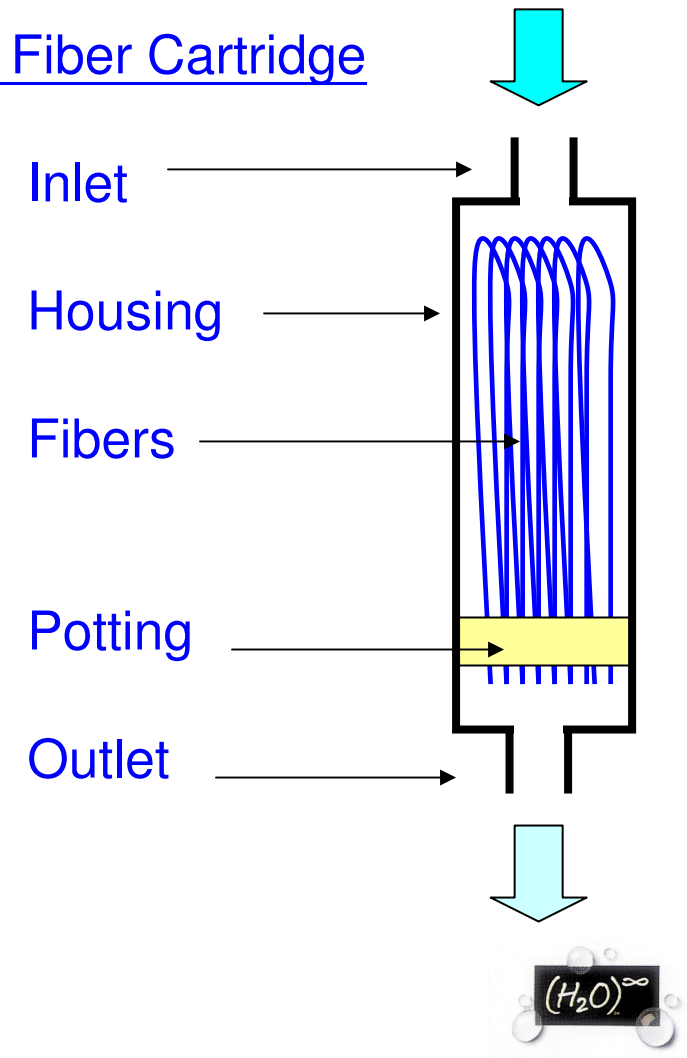
MILLIPORE



Ultrafiltration cartridge



Hollow Fiber Cartridge



MILLIPORE

Pyrogen level in Milli-Q Water with BioPak

The detection of pyrogens with the high sensitivity test was performed in « worst conditions »: after 142 days of operation and demonstrated that the pyrogen level was still < 0.001 Eu/mL

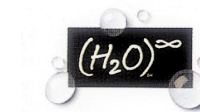
Serial number	Acceptation criteria	Endotoxin measurement after 1 L flush, EU/ml			Result
		Day 18*	Day 32*	Day 142**	
B0400072	< 0.01 EU/ml	Not detected	Not detected	0.00024	Compliance
B0400060		Not detected	Not detected	0.00026	Compliance
B0400048		Not detected	Not detected	0.00025	Compliance
Millipak 40	NA	Not detected	Not detected	0.00042	

*Detection limit by chromogenic LAL QCL-1000: 0.01 EU/ml

**Detection limit by Kinetic QCL: 0.00025 EU/ml

MILLIPORE

Typical < 0.001 Eu/mL



Type of Cell Culture

■ Mammalian

- ❖ Epithelial (in contact with outside world)
- ❖ Endothelial
- ❖ Skeletal
- ❖ Neural
- ❖ Stem cell
- ❖ Hybridomas
- ❖ CHO (Chinese Hamster Ovaries)
- ❖ Embryo/blastocyst

■ Insect cell

■ Plant, callus

■ Bacterial, microbial

