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Microbiology under Pressure

Soft-matter mechanisms, experimental strategy and high-pressure processing applications

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CONTEXT

Why pressure microbiology matters now

> Pressure is a physical variable that reorganizes hydration, packing and weak interactions before it becomes a microbiological phenotype

Deep biosphere

Most ocean volume is cold, dark and pressurized; pressure-adapted microbes are not rare exceptions

High-pressure processing

HPP links pressure microbiology with food safety, shelf-life extension and process validation

Soft-matter bridge

Cells are hydrated, crowded, deformable systems; pressure exposes this physics clearly

Take-home framing: pressure is not simply “cold heat”; it is a distinct way to perturb biological matter

CONTEXT

Learning goals and roadmap

- Read pressure scales without over-extrapolating between ecology and processing.
- Explain why membranes, proteins, hydration and crowding are pressure-sensitive.
- Choose experimental platforms while controlling decompression artefacts.
- Interpret HPP survival curves and limits in a mechanistic way.

Scales — Soft matter — Cells — Methods — Applications — Open questions

For early-stage researchers: the goal is a reusable mental model, not a catalogue.

CONTEXT

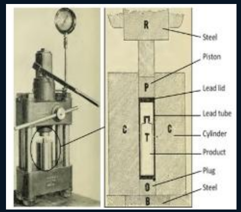
A short timeline of high-pressure microbiology

1870s

Challenger expedition challenges the azoic view

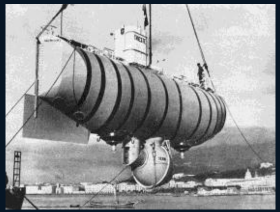
1899

Hite demonstrates pressure preservation of milk



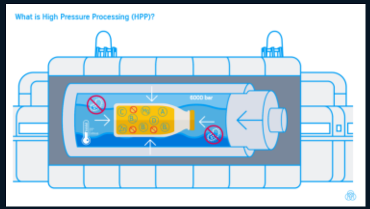
1960

Trieste reaches Challenger Deep



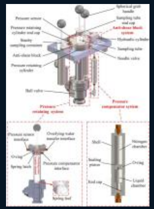
1980s–90s

Commercial HPP and deep-sea microbiology expand



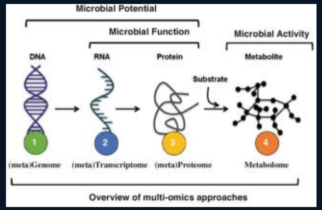
2000s

Pressure-retaining tools and high-pressure microscopy



2020s

Lipidomics, systems biology and refined validation



What changed?

The field moved from “can anything live there?” and “can pressure preserve food?” to mechanism-rich questions about interfaces, assemblies and pressure histories.

What should remain?

Historical anchors are useful, but the updated lecture should prioritize mechanisms and experimental reasoning for new researchers.

CONTEXT

Pressure scales from cell physiology to industrial processing

Same unit, different biological meaning

Ecological pressure ≠ industrial pressure

but the same soft-matter principles help to interpret both

PRIMER

Soft-matter primer

01

The minimum physics needed to understand microbial pressure response.

PRIMER

pressure selects volume-sensitive states

Thermodynamic intuition for non-specialists

Constant temperature

Pressure shifts equilibria toward states with lower partial molar volume

$$dG = VdP - SdT$$

Simplified lesson: ask where volume, cavities, hydration and interfaces change

Do not teach it as “temperature backwards”

- Temperature changes kinetic energy and heat flow
- Pressure changes volume, packing, hydration and equilibria
- P–T combinations can be synergistic or antagonistic

Pressure-sensitive systems are often those with voids, interfaces or weak assemblies.

Balny et al. 2002; Oger & Jebbar 2010

PRIMER

what matters biologically

Soft-matter essentials relevant to microbial cells

Interfaces

membranes, S-layers, biofilm matrices: small energetic changes can alter function.

Hydration

water structures and solvation volumes help determine pressure response.

Crowding

cytoplasm is not dilute; diffusion and assembly are context-dependent.

Assemblies

ribosomes, divisomes and respiratory complexes are volume-sensitive networks.

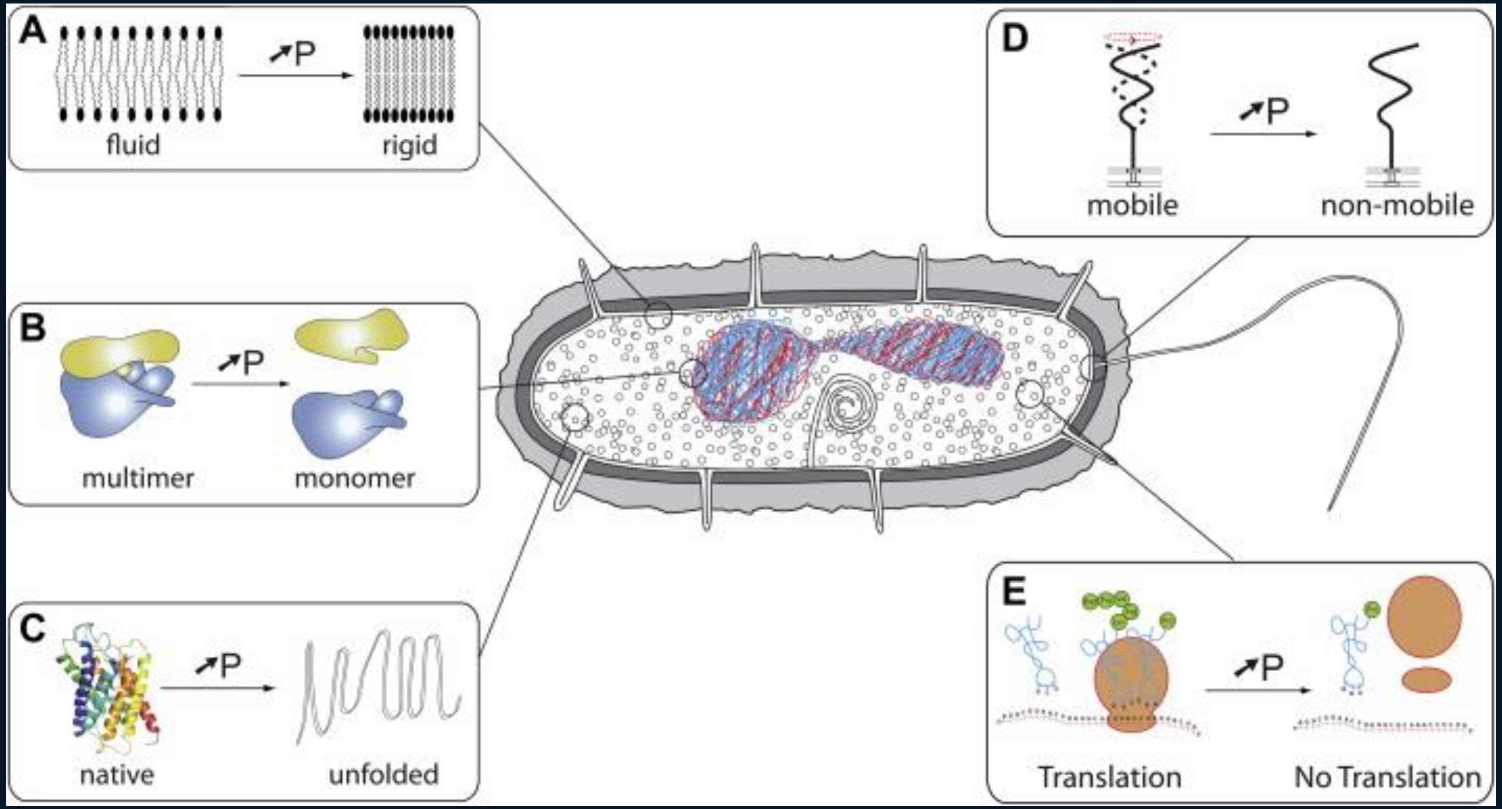
lipids — proteins — nucleic acids — cell wall — cytoplasm

Microbial pressure response is the biology of soft, hydrated molecular assemblies under compression.

PRIMER

Cell mechanics under hydrostatic pressure

A microbial cell as hydrated soft matter with load bearing interfaces



Pressure is isotropic,
but biological response is not

Oger & Jebba (2010) Research in Microbiology. doi:10.1016/j.resmic.2010.09.017

PRIMER

Pressure effects on membranes and proteins

Packing, hydration and conformational landscapes

Membrane soft matter

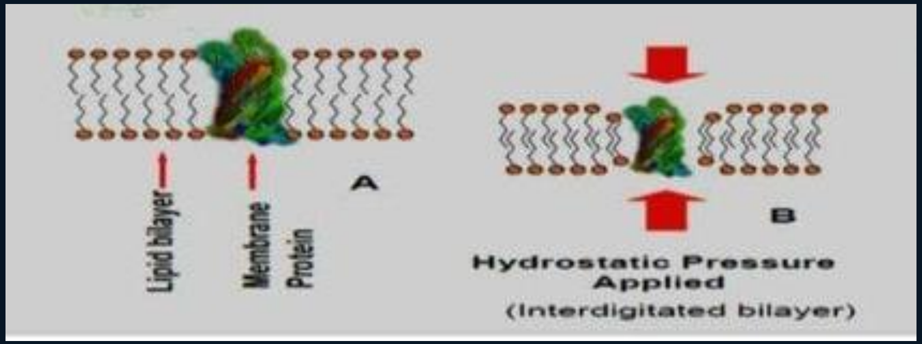
Lower pressure: larger free volume
Higher pressure: lighter packaging, lower fluidity

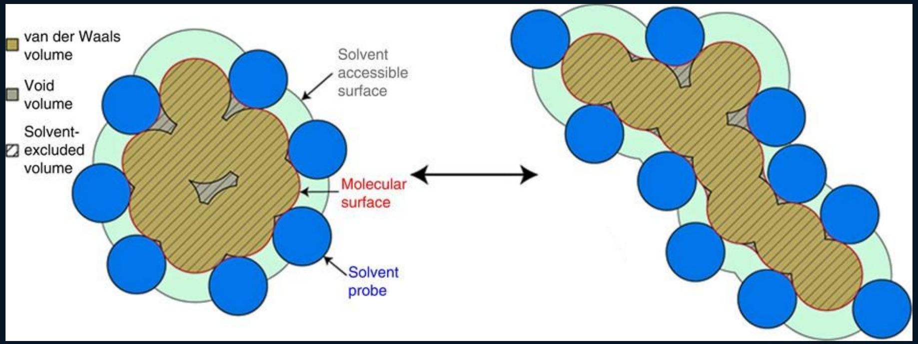
- **Permeability**
- **Transport**
- **Embedded enzymes**

Protein / enzyme landscape

- **Oligomeric state**
- **Active-site geometry**
- **Catalytic rate**

Pressure favour lower-volume states but can disrupt cavities, hydration and assemblies



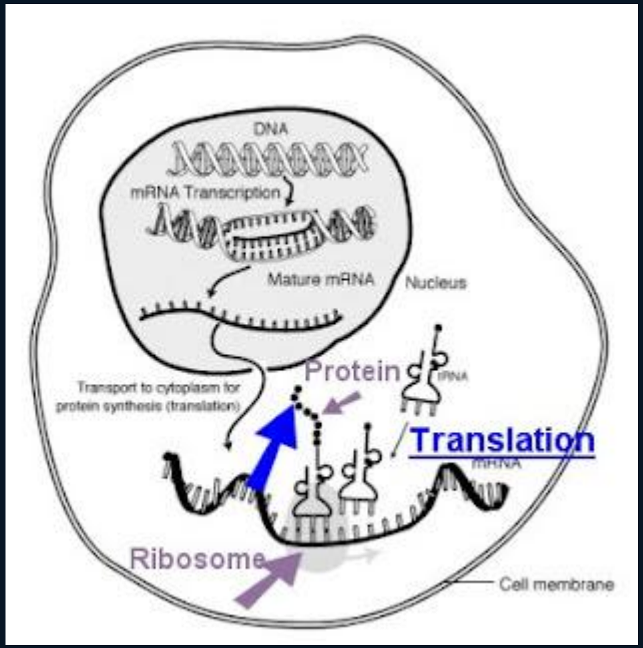


Winter & Jeworrek 2009; Tamby et al. 2023; Ichiye 2018; Chen & Makhatadze 2017

PRIMER

beyond membrane-only explanations

Cytoplasm, hydration and crowding



Diffusion is not guaranteed

Molecular mobility can change as pressure alters hydration and local viscosity

Assemblies have pressure histories

Ribosomes, divisomes and stress granules can respond before the whole cell fails

Recovery is biological

Post-pressure repair and resuscitation affect what the experiment appears to show

Pressure biology is often recovery biology

Balny et al. 2002; Oger & Jebbar 2010

CELLS

Microbial responses

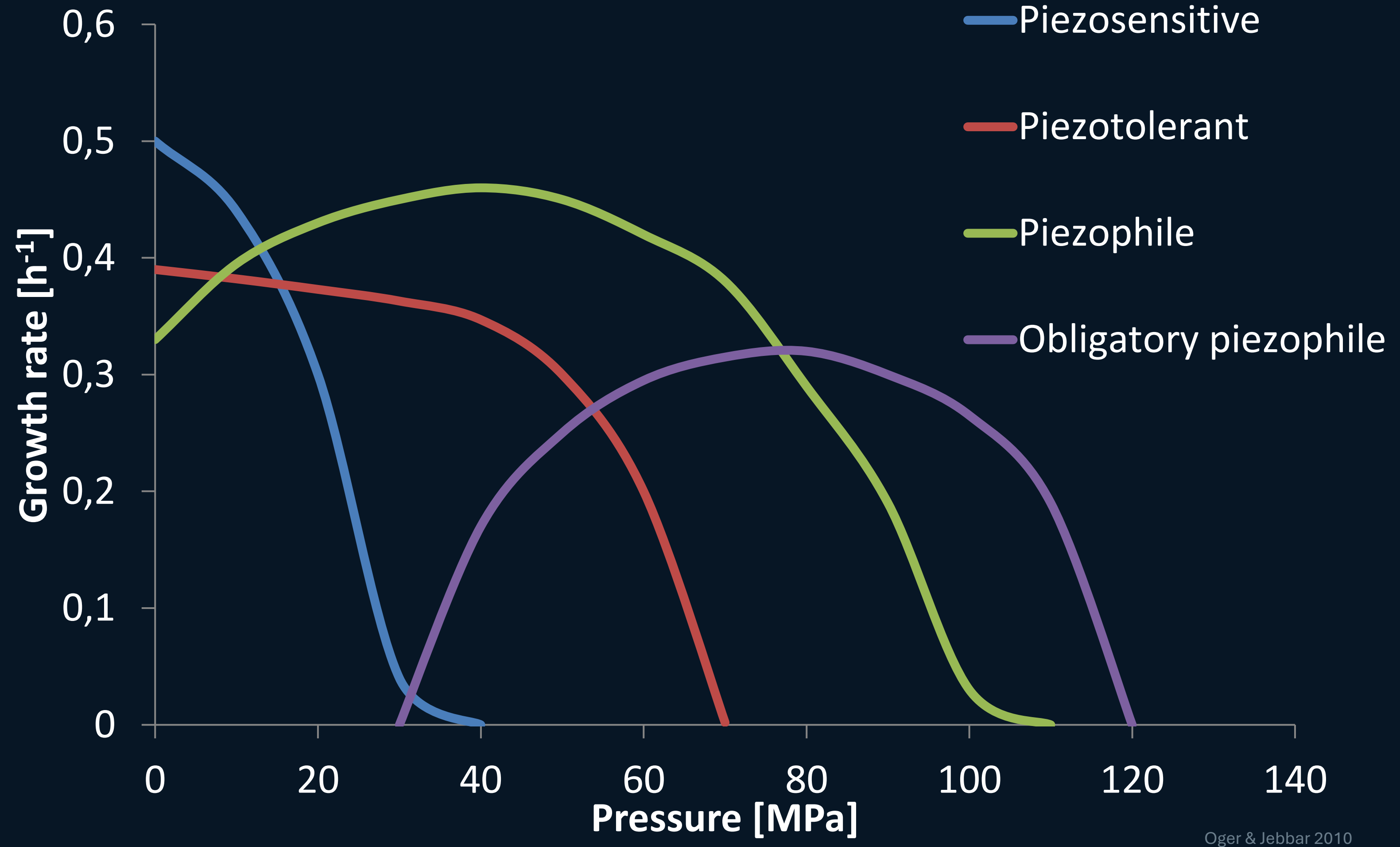
02

How physical perturbations become growth, adaptation, injury or death.

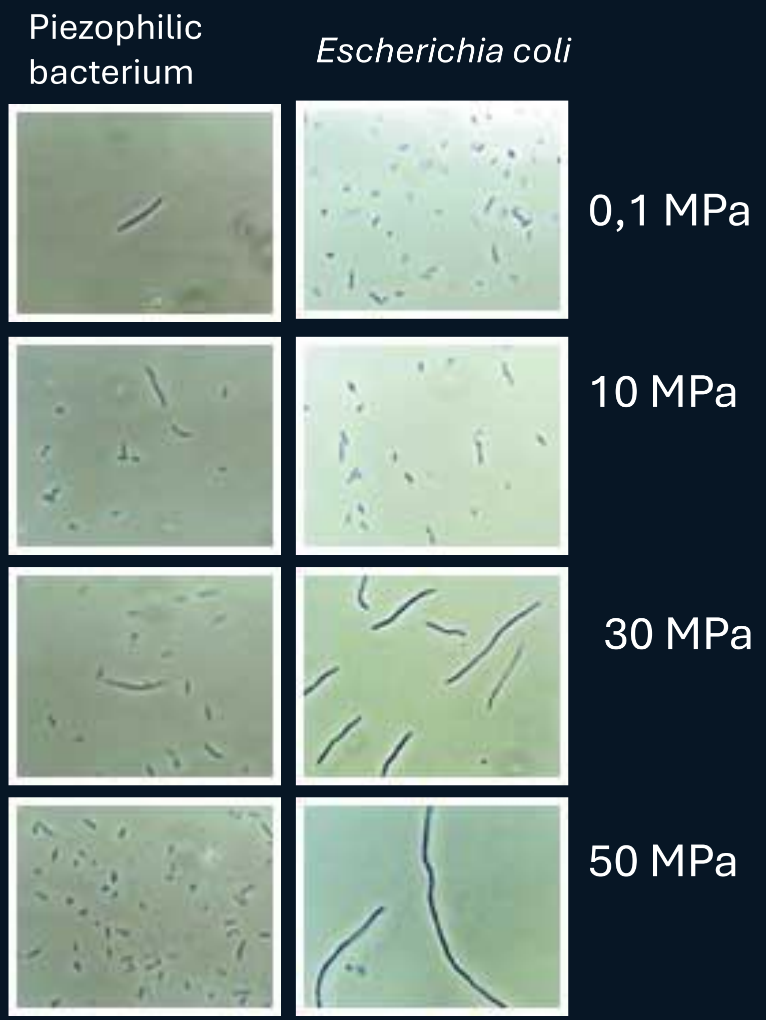
Terminology and pressure phenotypes

Growth phenotypes under pressure

A vocabulary for pressure-growth response curve



Oger & Jebbar 2010



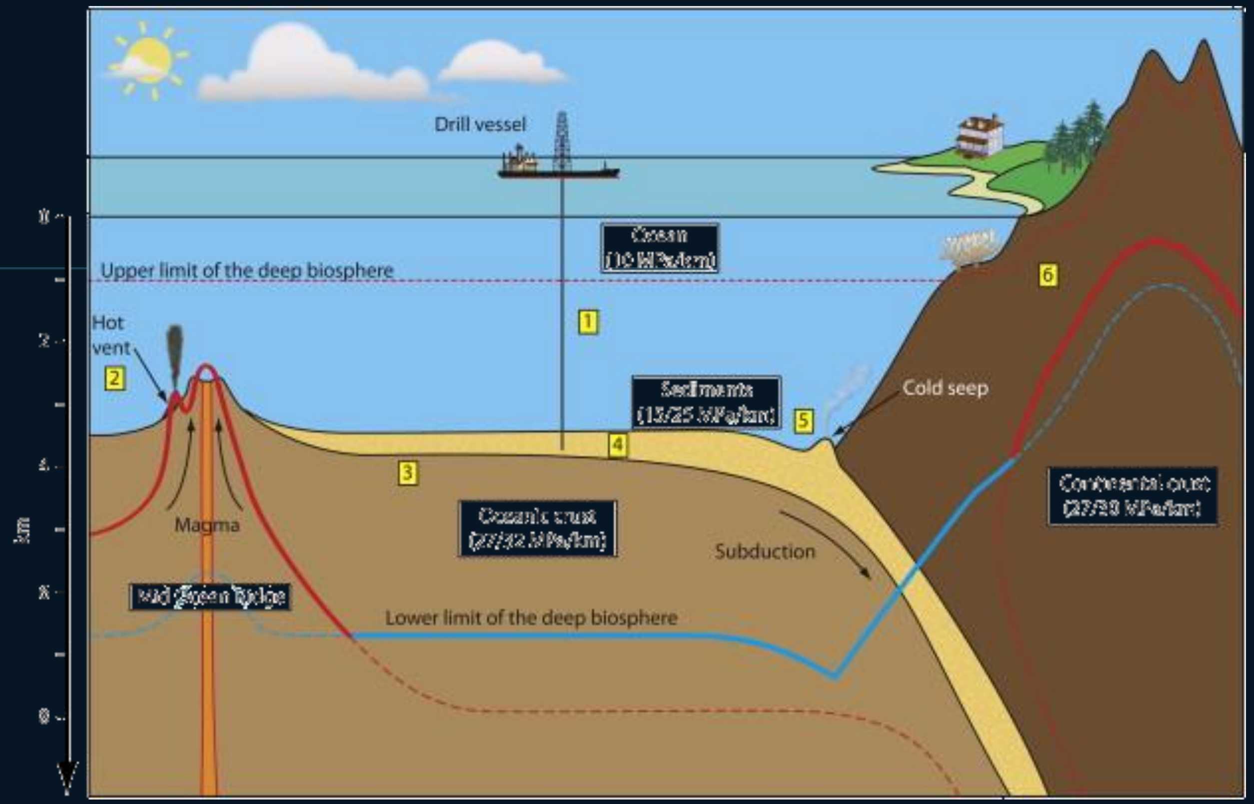
http://www.jamstec.go.jp

CELLS

habitats and gradients

Where pressure-adapted microbes are found

Deep ocean, trenches, hydrothermal vents and subsurface pores



- **Epipelagic** 0-200 m
- **Mesopelagic** 200-1000 m
- **Bathypelagic** 1000-4000 m
- **Abyssal** 4000-6000 m
- **Hadal trenches** 6000-11000 m

Pressure gradient

~0,1 MPa per 10 m in sea water

Biological consequence

Low temperature + high pressure + low organic carbon select for psycropiezophilic and oligotrophic strategies

Hot deep biosphere

Vent and crustal habitats add strong temperature gradients


Organisms found at the bottom of a coal mine
CARGA
AUTORRESCATADORES

Representative model organisms and what they teach us

Model	Pressure style	Useful for teaching
Photobacterium profundum SS9	psychropiezophile	membrane and gene regulation under pressure
Shewanella violacea DSS12	facultative piezophile	respiration and cytochrome regulation
Thermococcus / Pyrococcus spp.	thermopiezophilic archaea	proteins, lipids and high P–T limits
Colwellia marinimaniae	hyperpiezophile	hadal adaptation and growth at very high pressure

Teaching move: choose one model per mechanism rather than one mechanism per species.

Kato & Nogi; Jebbar group; Kusube et al. 2017

Membrane adaptation is the first soft-matter story

Bacteria

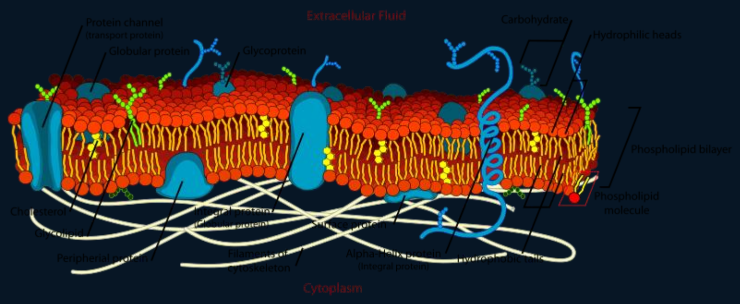
change fatty-acid length, branching, saturation and headgroup composition to tune packing.

Archaea

ether lipids and tetraether monolayers can stabilize membranes under combined extremes.

Universal problem

keep the membrane functional without making it leaky or rigid.



Membrane adaptation is a materials-engineering solution evolved by cells.

Tamby et al. 2023; Winter & Jeworrek 2009

Proteins, respiration and energy metabolism

protein cavities — hydration shell — conformation — complex assembly — electron transfer — ATP yield

Enzyme activity

Piezophilic enzymes may balance flexibility and stability differently from mesophilic homologues.

Respiratory chains

Pressure can alter membrane proteins and redox-complex organisation.

Energy stress

A small decline in energy supply can become a cell-division phenotype.

Mechanistic caution: pressure response is not one target, but a network of volume-sensitive steps.

Ichiye 2018; Oger & Jebbar 2010

Nucleic acids, ribosomes and cell division

DNA / RNA

base pairing and stacking can be pressure-sensitive; local opening matters.

Ribosomes

large macromolecular assemblies translate pressure into growth-rate changes.

Divisome

cell division can fail before viability is lost; filamentation is a clue.

Look for “growth without division”, “metabolism without colony formation”, and “injury without immediate death”.

These distinctions are especially important when using post-pressure plating as the main readout.

Oger & Jebbar 2010; Kumar & Libchaber 2012

CELLS

visible phenotypes are mechanistic clues

Morphology, motility, biofilms and recovery

compression → membrane stress → motility changes → filamentation → injury / VBNC → recovery or death

Morphology

elongation and division defects can appear before complete inactivation.

Motility

flagella and chemotaxis depend on membranes and energy supply.

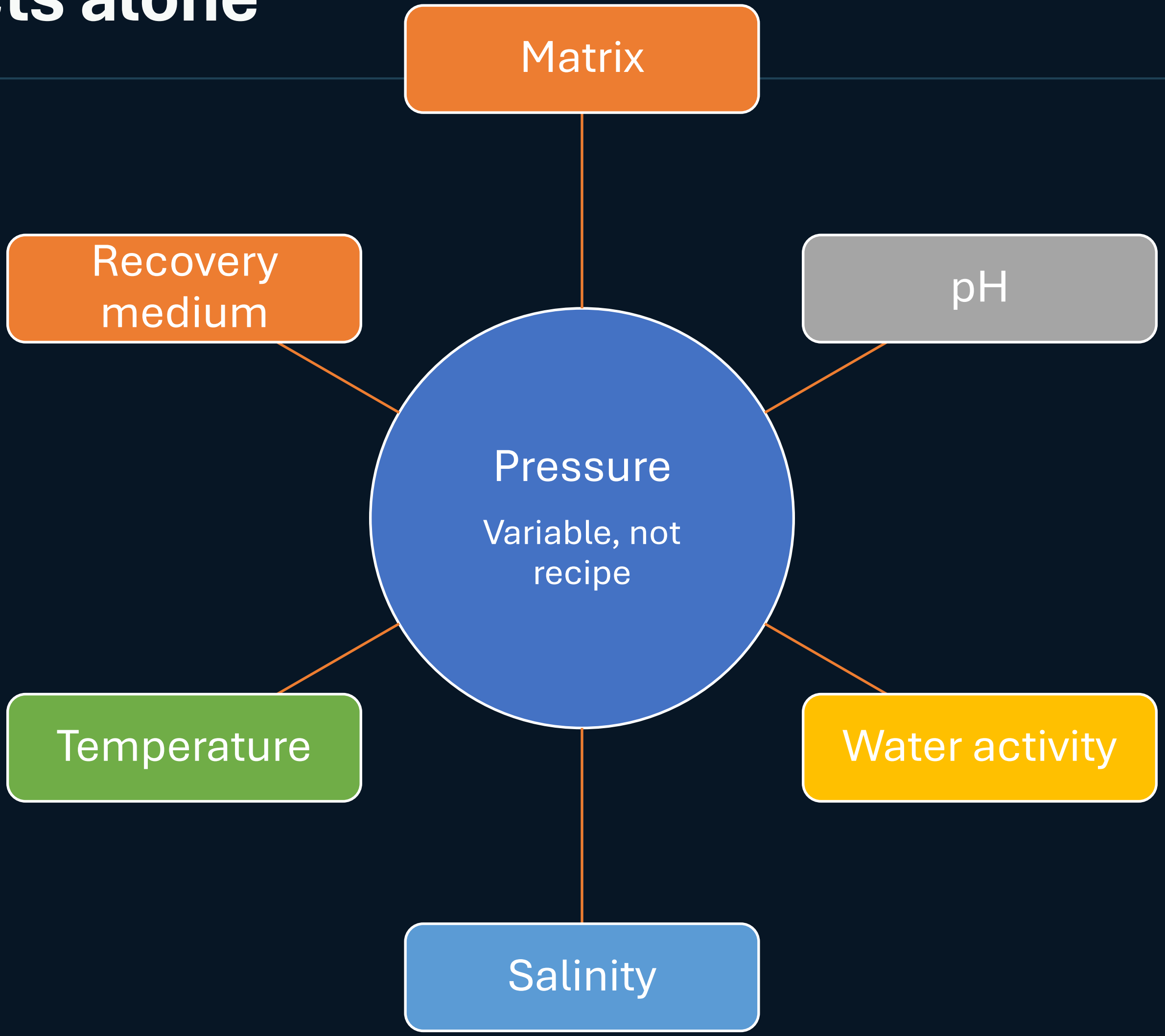
Biofilms

EPS and multicellular organisation can change pressure tolerance.

Plate counts are not the whole phenotype

Kumar & Libchaber 2012; HPP recovery literature

Pressure never acts alone

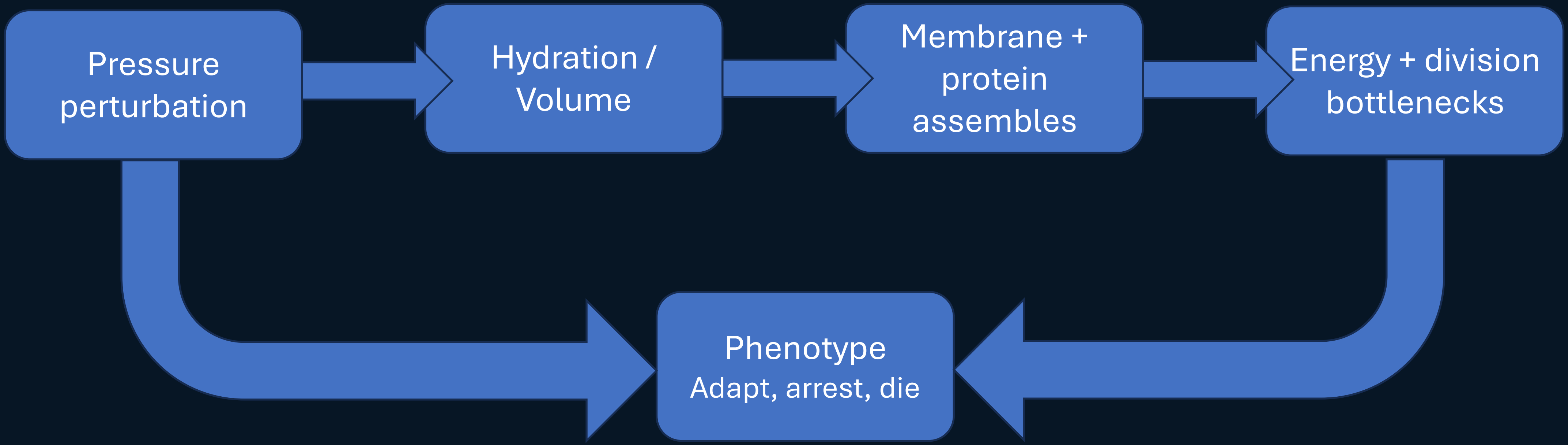


Experimental design should report the full pressure ecosystem, not only MPa and minutes

CELLS

From physical perturbation to microbiological phenotype

A compact map for interpreting pressure experiments



The same physical driver can yield growth, dormancy, injury or death depending on the context

METHODS

Experimental strategy

03

How to measure pressure response without changing the answer during sampling.

Experimental challenge: decompression can change the answer

in-situ state → sampling → pressure history → decompression → recovery medium → observed phenotype

What can be lost

labile transcripts, pressure-dependent assemblies, stressed subpopulations.

What can be created

decompression injury, oxygen exposure, temperature shocks.

What to report

compression rate, hold time, decompression rate, recovery conditions.

The end point is often a pressure-history phenotype, not a pure pressure phenotype

METHODS

new figure

Experimental platforms across the pressure toolbox

Choose the instrument after choosing the biological question

P

Batch vessel

Process validation

High throughput; post-pressure readout

Typical HPP 100-700 MPa

S

Pressure retaining sampler

In situ recovery

Keeps deep sample under pressure

Sampling / cultivation continuously

μ

High pressure microscopy

Live dynamics

Small volume; Optical access

Growth, morphology, fluorescence

◇

Diamond Anvil cell

Extreme limits

Tiny sample; high pressure range

Raman / X-ray; ice phases, survival

Vass et al. 2010; pressure-retaining sampler literature; DAC studies

METHODS

new figure

Experimental platforms across the pressure toolbox

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Diamond anvil cell

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METHODS

maintain the sample’s pressure history

Pressure-retaining sampling and cultivation strategies

Recover without decompression

captures cells that may not survive atmospheric handling

Culture under pressure

tests growth optima and obligate piezophily directly

Pair with molecular readouts

links activity, composition and viable cultivation

Design principle

If the scientific claim is about in-situ physiology, the pressure history must be preserved or explicitly modelled

Pair atmospheric-pressure controls with pressure-retaining workflows to separate decompression sensitivity from true in-situ adaptation

High-pressure microscopy and spectroscopy

Live morphology

growth, filamentation, motility and division under controlled P–T.

Fluorescence

membrane integrity, reporters and stress markers when optics allow.

Spectroscopy

metabolite or structural signatures in small high-pressure samples.

A small field of view can answer a question that a million colony counts cannot: when does the cell stop dividing?

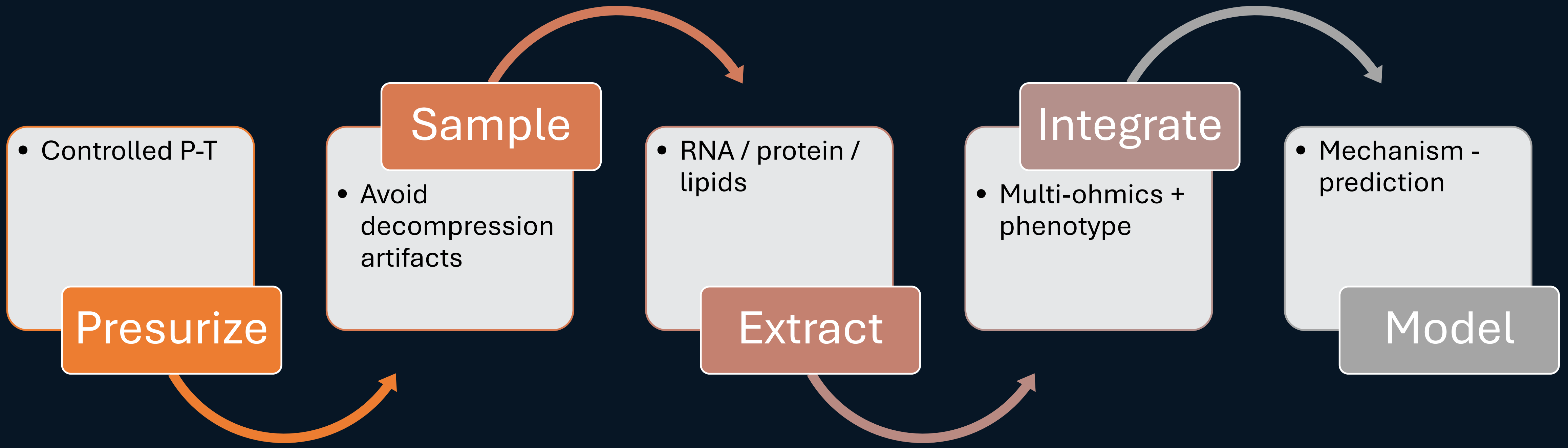
Limitations: small sample volume, optical constraints, calibration, and pressure/temperature gradients.

Vass et al. 2010; Sharma et al. 2002

METHODS

Omics and systems microbiology under pressure

Make pressure history explicit in every sample



Pressure is metadata, not just a treatment label

Comparative table of core experimental methods

Method selection matrix

The method defines the biological question you can answer

Method	Pressure window	Readout	Strength	Caution
Batch HPP vessel	100–700 MPa	survival, quality	validation	post-pressure readout
Pressure-retaining sampler	10–120 MPa	in-situ communities	ecological realism	low throughput
HP microscopy	0.1–700 MPa	live morphology	dynamics	small volumes
DAC / spectroscopy	0.1–GPa	metabolism, physics	extreme range	tiny sample

Pressure, decompression and recovery are part of the measurement chain.

Figure 08 — original teaching diagram

METHODS

general principles, not a protocol

Safety and operational good practice

Stored energy

treat pressure systems as energy-containing equipment

Vessel integrity

use rated components, maintenance logs and interlocks

Thermal control

compression heats; decompression cools; both affect biology

Biosafety

contain viable organisms before, during and after treatment

Good microbiology under pressure is reproducible engineering plus reproducible biology

Report compression rate, decompression rate, temperature profile, vessel loading, sample geometry and recovery conditions

APPLICATIONS

04

Applications and limits

From microbial inactivation to translation gaps in real matrices.

APPLICATIONS

one physics, multiple objectives

Application landscape

Food HPP

safe, fresh-like products; pathogen and spoilage control

Pharma / cosmetics

bioburden reduction, formulation stress tests, sensitive matrices

Biotechnology

pressure as perturbation tool for enzymes and cells

Ecology

deep life, subsurface, astrobiology analogues

The same mechanism can be useful or harmful depending on the application

In HPP we want irreversible injury; in deep-sea microbiology we try not to create it post-packaging pressure treatment

High-pressure processing in foods

packaged food → pressure vessel → water medium → 400–600 MPa → hold + release → chilled shelf life

Main advantage

inactivates many vegetative cells with limited covalent chemistry

Main caution

spores, matrix protection and recovery can dominate validation

Main design variable

pressure, temperature, pH, aw and product structure together

HPP is not just “MPa × minutes”: it is product-specific microbial ecology under compression.

Aganovic et al. 2021; HPP reviews

How pressure inactivates microbes

membrane permeability → ion gradients → energy stress → protein assemblies → division failure → injury / death

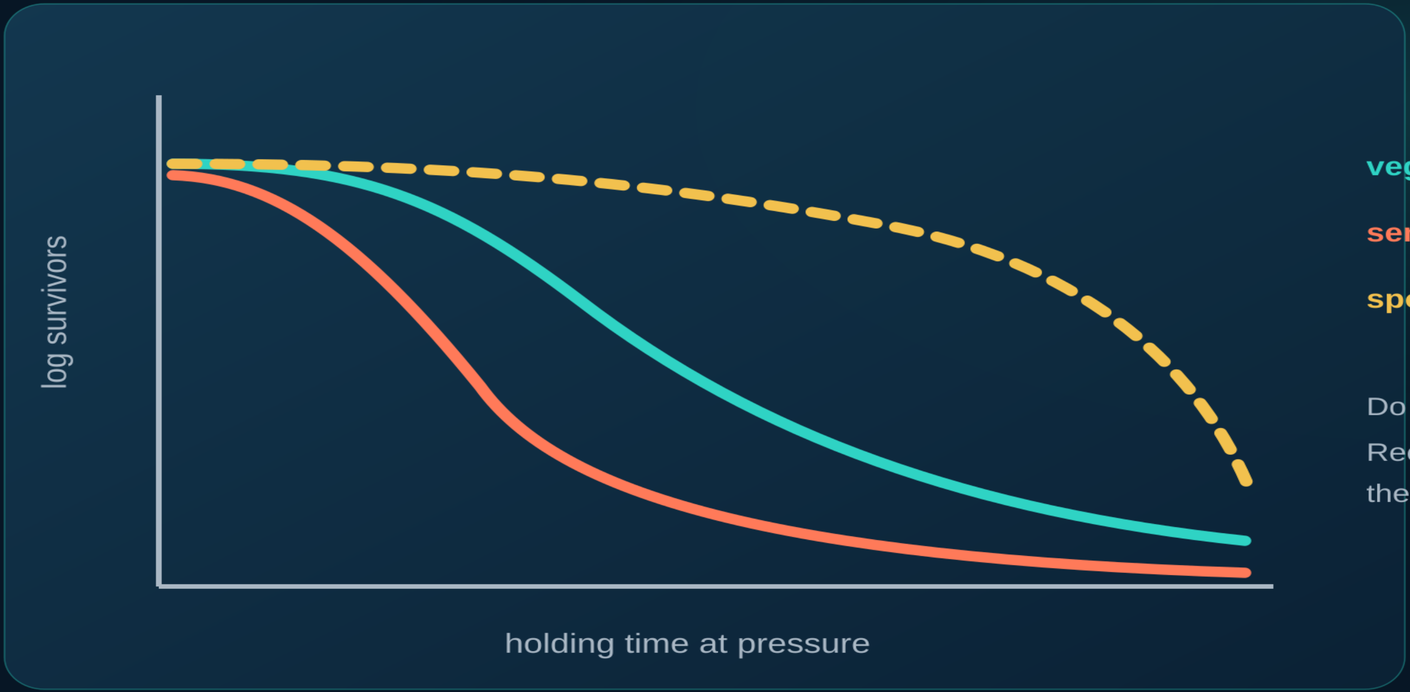
The lethal event may be a systems-level collapse, not a single broken molecule

This explains shoulders, tails and strong effects of recovery media in survival curves

APPLICATIONS

Pressure versus survival curves

Schematic HPP inactivation: shoulder, tailing and recovery



vegetative cells

sensitized cells + hurdles

spores / protected subpopulations

Do not fit a straight line too early.

Recovery medium and matrix often determine the apparent tail.

APPLICATIONS

why HPP is often pasteurization-like, not sterilization

Spores, persistence and recovery artefacts

Spores are not small vegetative cells

low water content, protective layers and dormancy change pressure response.

Germination can help

pressure-triggered germination may make spores more sensitive to follow-up hurdles.

Validation must be conservative

injured survivors and recovery conditions can change apparent log reductions.

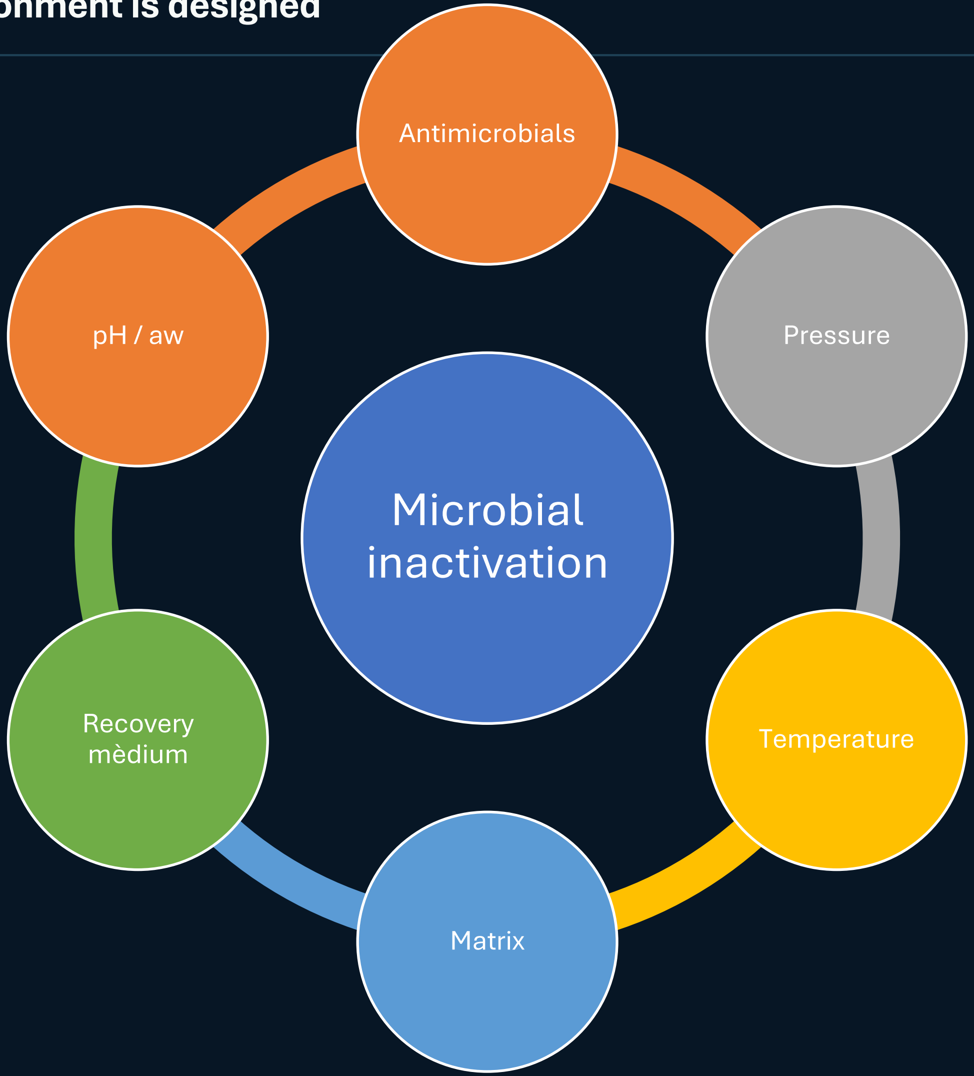
Practical message: HPP is strongest against vegetative cells; spore control usually needs a hurdle strategy.

Use non-selective and selective recovery, appropriate incubation windows and product-specific challenge tests.

APPLICATIONS

Combined hurdles and intensified processes

Pressure works best when the full environment is designed



Do not validate MPa in isolation. Validate the treatment ecosystem

Three concrete application examples

Ready-to-eat meats

Focus: Listeria risk reduction and shelf-life.
Key issue: product formulation and post-process contamination.

Juices and purées

Focus: fresh-like quality in acidic products.
Key issue: yeasts, moulds and enzyme residual activity.

Seafood

Focus: pathogen control and shellfish shucking.
Key issue: texture, enzymes and cold chain.

Each product is its own microbial ecosystem under pressure.

Biotech, pharma and cosmetics prospects

Enzyme engineering

pressure probes cavities, flexibility and catalytic robustness

Formulation stress tests

biologics, emulsions and cells can be screened for pressure sensitivity

Bioburden reduction

useful for selected matrices but not a universal sterilization replacement

Translational opportunity: use pressure as a controlled perturbation before using it as a process

This is especially useful for early-stage projects because mechanism and application can be linked in the same workflow

Key limitations and translation gaps

Heterogeneity

subpopulations, physiological state and stress history shape survival

Comparability

pressure, temperature and decompression protocols are often underreported

Transferability

deep-sea adaptation and HPP inactivation overlap mechanistically but not operationally

The biggest errors come from treating pressure as a scalar recipe rather than an experimental history

SYNTHESIS

Synthesis and discussion

05

How to turn pressure microbiology into good research questions

SYNTHESIS

questions that connect mechanism and application

Open research questions worth showing to ESRs

What lipid changes matter most?

composition vs material state

How much is decompression artefact?

real physiology vs handling

When does injury become death?

repair thresholds

Can we predict matrix protection?

food structure and solutes

Which assemblies are pressure bottlenecks?

ribosomes, divisome, respiratory complexes

How can data become comparable?

metadata and standards

Good questions define the pressure history before they define the measurement

SYNTHESIS

new figure

Good project ideas for early-stage researchers

Good early-stage research projects

Small enough to start, rich enough to matter

Membrane lipidomics
pressure × temperature

Single-cell recovery
VBNC vs injury

Model organisms
piezophiles + mesophiles

In-situ microscopy
morphology + division

Food matrix effects
pH, aw, fat, solutes

Data standards
protocol comparability

Each project links a measurable physical perturbation to a microbiological phenotype.

Figure 12 — original teaching diagram

SYNTHESIS

four-step heuristic

A practical framework for interpreting any pressure experiment

1. Define pressure history

2. Identify soft-matter targets

3. Choose readouts and controls

4. Interpret recovery explicitly

History

compression, hold, decompression, temperature

Targets

membrane, proteins, ribosomes, wall, cytoplasm

Readouts

growth, viability, morphology, omics, kinetics

Recovery

repair, VBNC, tailing, selective media

A pressure experiment is a story of perturbation, response and recovery.

SYNTHESIS

Take-home messages

- Pressure targets hydration, packing, interfaces and weak assemblies before covalent chemistry.
- Ecological pressure and industrial HPP overlap mechanistically, not operationally.
- Membranes, proteins, energy metabolism and division are central bottlenecks.
- Sampling, decompression and recovery conditions can change the answer.
- Good HPP microbiology designs the whole treatment ecosystem, not MPa alone.

The best pressure experiments are mechanistic, explicit and reproducible

Questions

Which bottleneck would you test first?

membrane state

decompression artefact

spore recovery

matrix protection

Thank you

BACKUP

optional deail

Backup: additional species and edge cases

Example	Reported context	Teaching angle
Pyrococcus yayanosii	obligate piezophilic hyperthermophilic archea	high P–T limits and archaeal adaptation
Marinitoga piezophila	thermo-piezophilic bacterium from hydrothermal vent	pressure + temperature + morphology
Colwellia marinimaniae	hyperpiezophile from Mariana Trench context	growth near / above hadal pressures
E. coli under pressure	mesophile model in microscopy studies	division, morphology and non-adapted response

Use edge cases to illuminate mechanisms, not to make universal claims

BACKUP

Backup: kinetic language and glossary

log reduction

decimal decrease in survivors; read with recovery conditions in mind

shoulder

initial region with little apparent inactivation

tailing

persistent survivor fraction or protected subpopulation

VBNC

viable but non-culturable state; method-dependent interpretation

PATP

pressure-assisted thermal processing

UHPH

ultra-high-pressure homogenization; continuous liquid treatment