Bioengineering Challenges of Solid-State Cultivation

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Outline

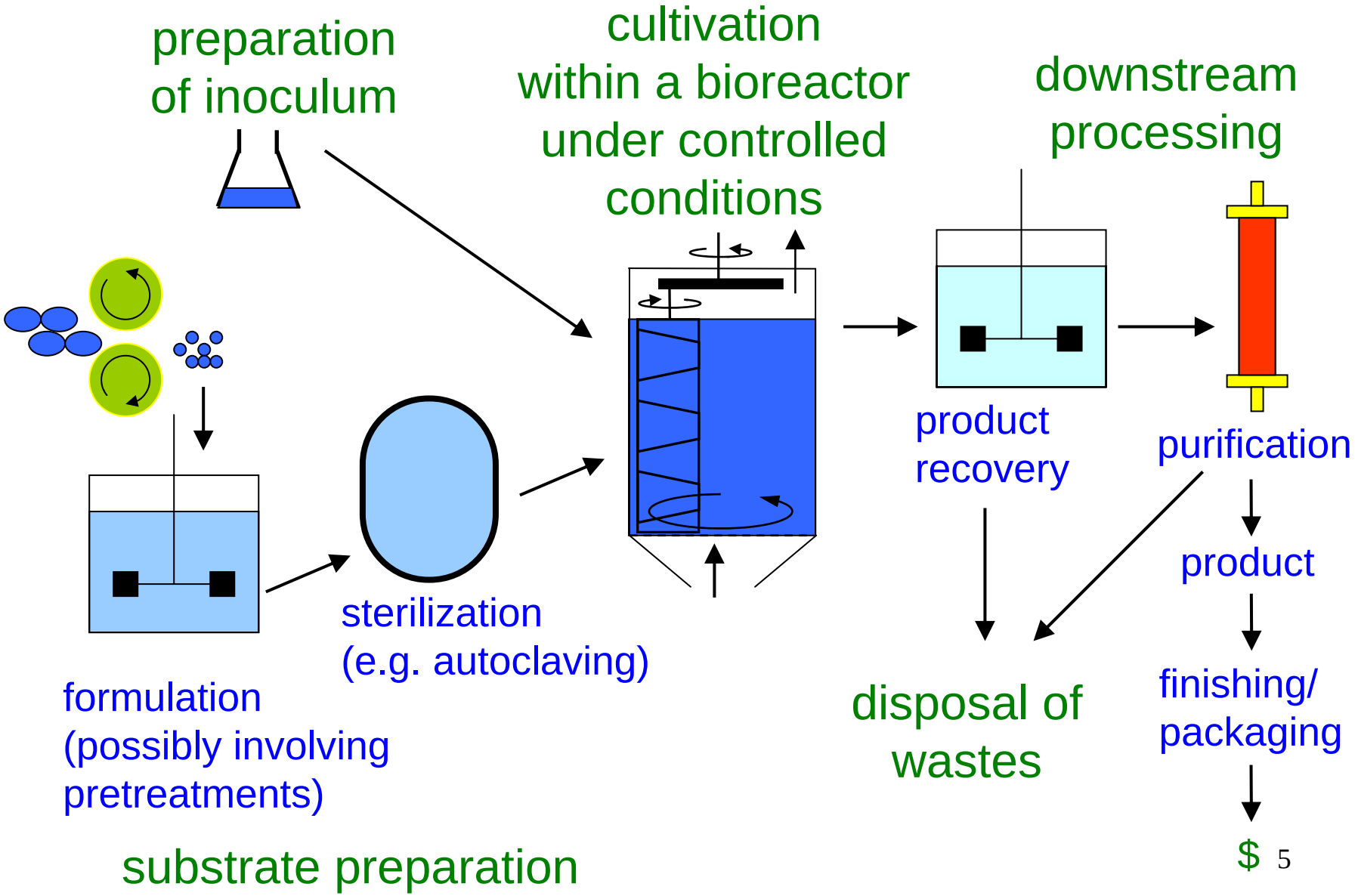
- What is solid-state cultivation?
- Why should we be interested in it?
- Why isn't it used as much as it might be?
- What types of bioreactors are used for SSC?
- What heat and mass transfer phenomena occur within SSC bioreactors?
- What do we know and what don't we know about...
  - how "microscale phenomena" affect the performance of the system?
  - how "macroscale phenomena" affect the performance of the system?
  - control of SSC bioreactors?

What is solid-state cultivation?

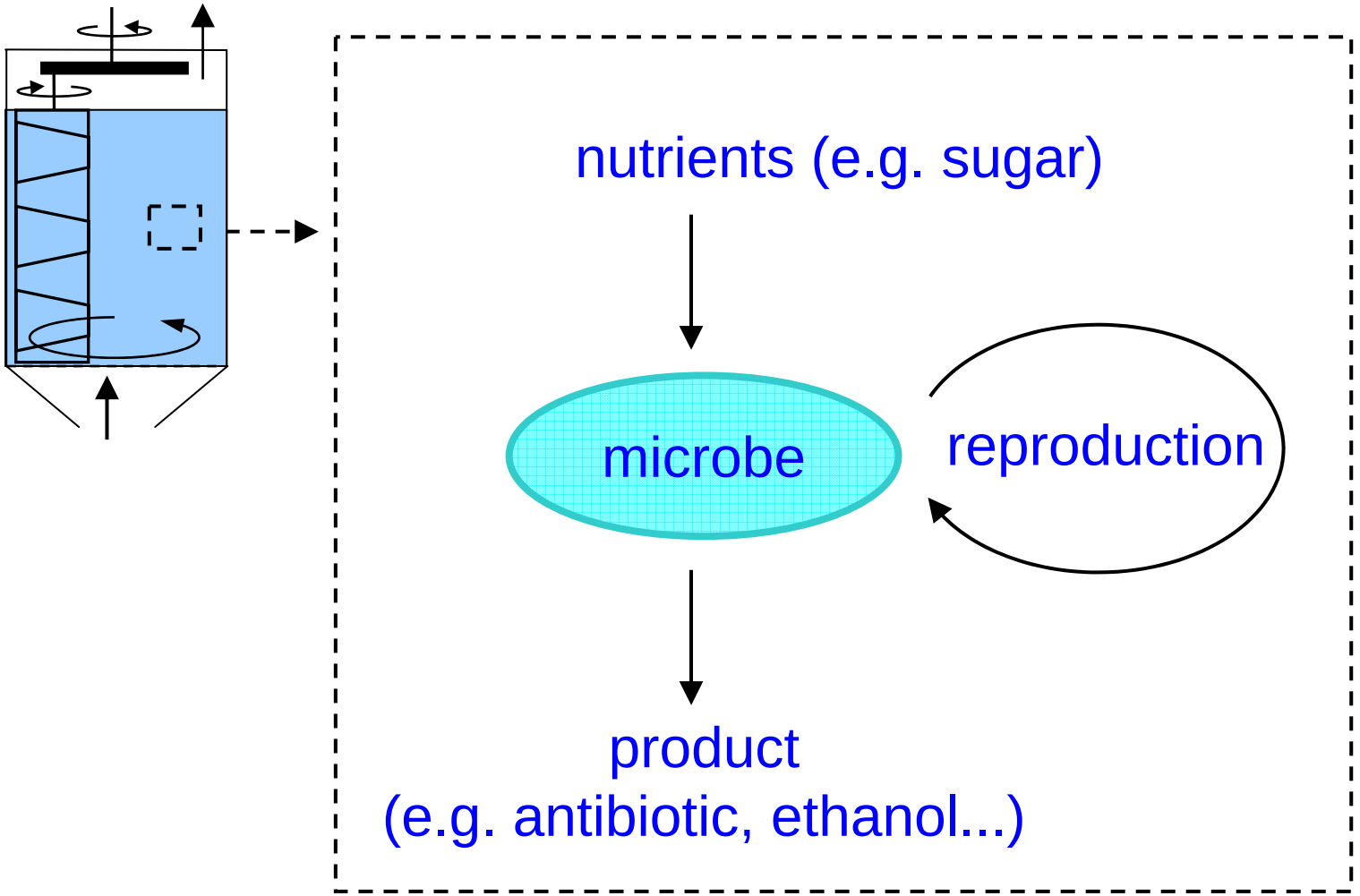
First of all, what is "cultivation" of a microorganism?

- we use the word "cultivation" to describe the growth of microbes (esp. bacteria, fungi, yeasts) under controlled conditions, using them as biocatalysts to produce valuable products
- in some senses these catalysts are just like catalysts in other chemical engineering processes
- in other senses, they are different
  - they are more complex
  - they grow
  - they tend to be quite sensitive to extreme conditions

A typical process for cultivation of a microorganism

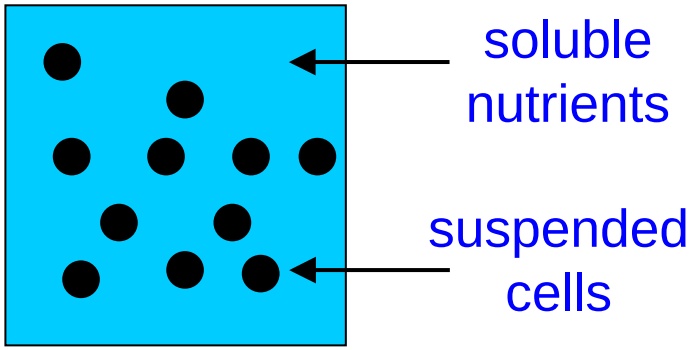


Our interest is in the cultivation step itself – where the biotransformation step takes place

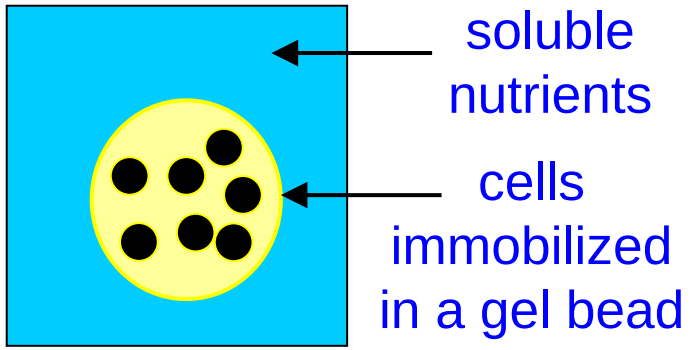


What cultivation methods are there?

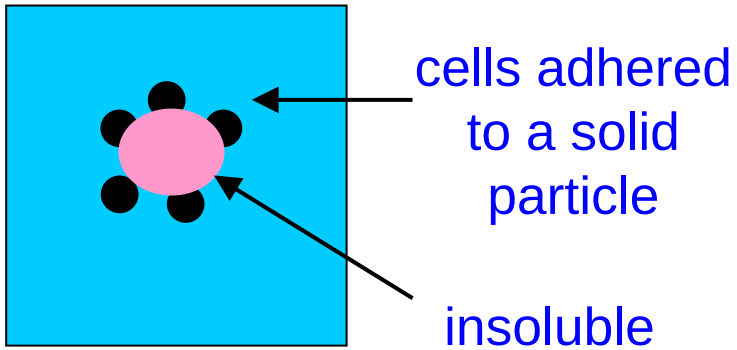
- classical submerged liquid culture (SLC)



- immobilized cells



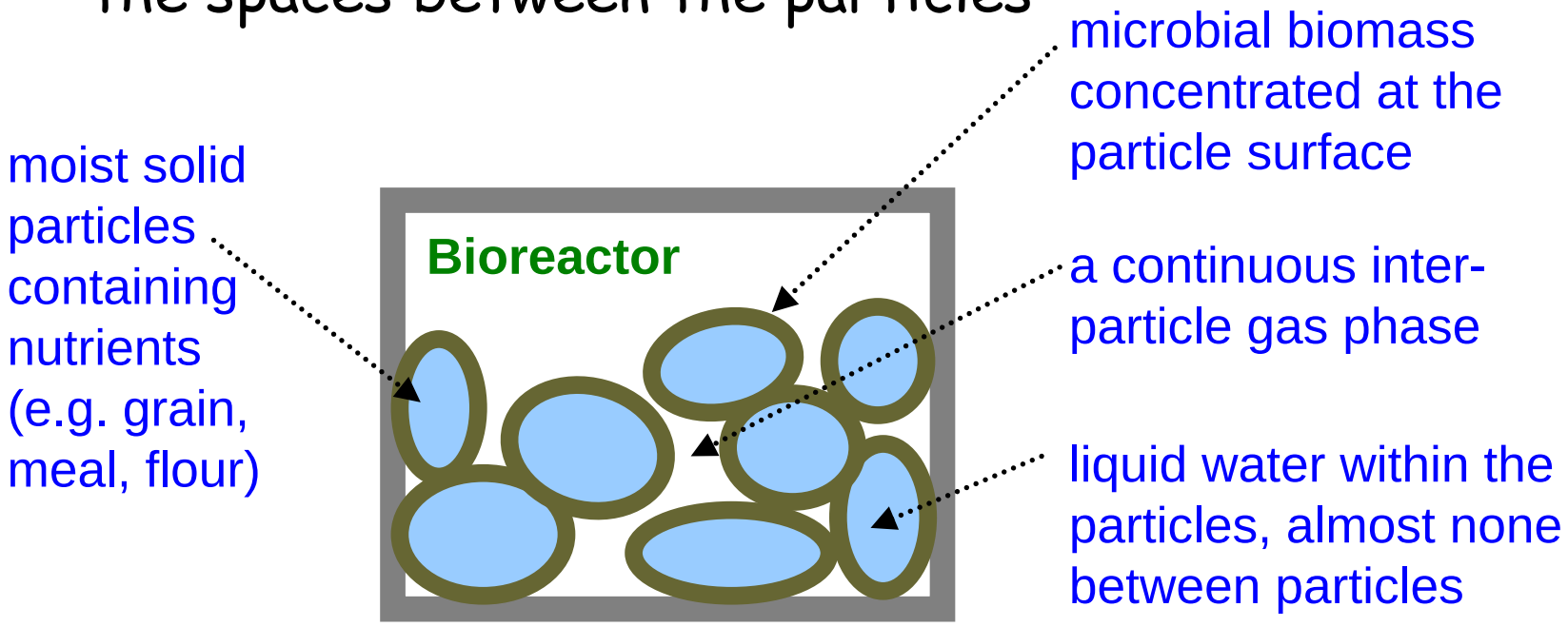
- suspension of a solid substrate



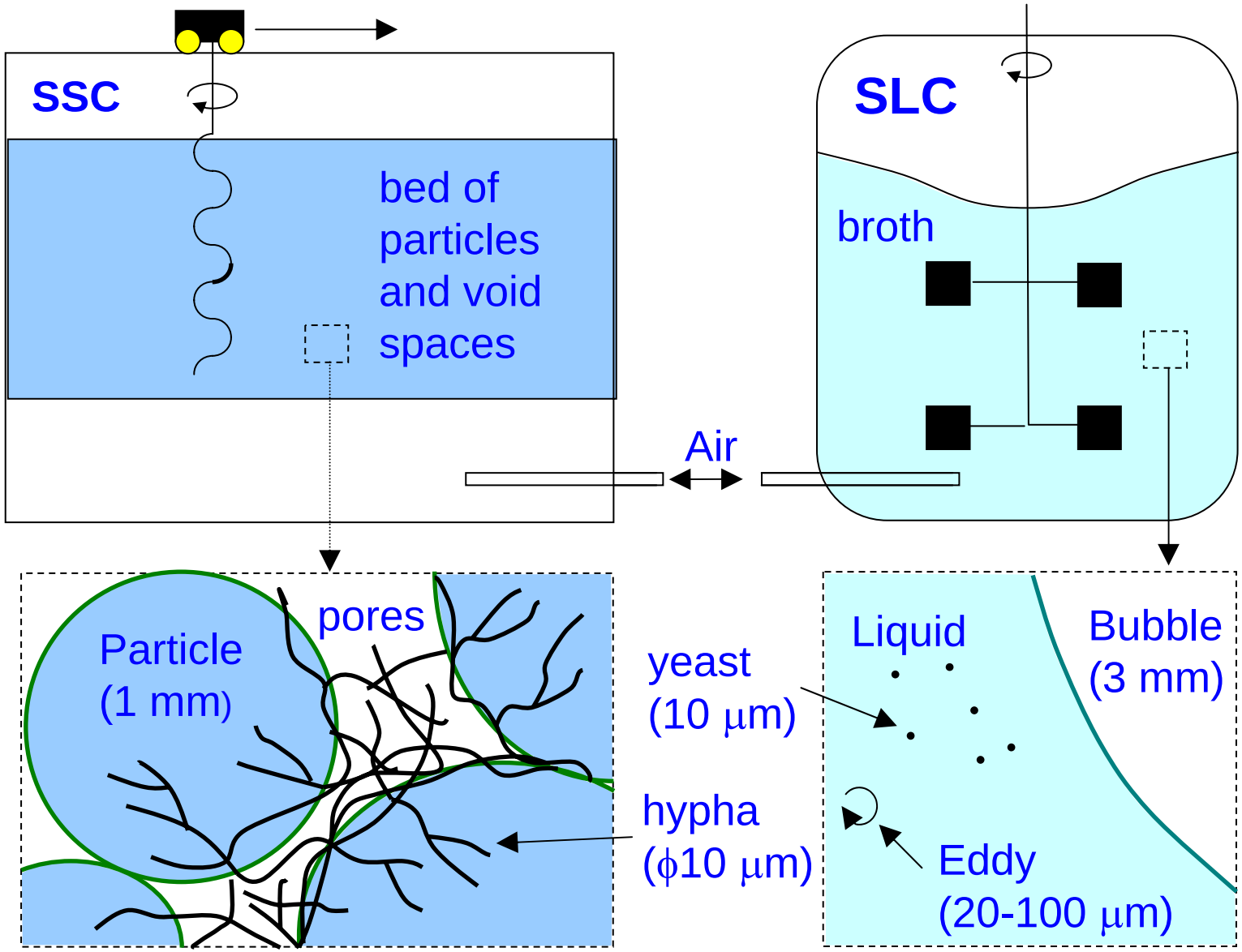
In all these cases the cells are totally surrounded by a continuous liquid phase

What cultivation methods are there? (cont'd)

- solid-state cultivation (SSC)
- SSC involves the growth of microorganisms (usually filamentous fungi) on a bed of particles of a moist solid substrate, with the minimum of free water in the spaces between the particles

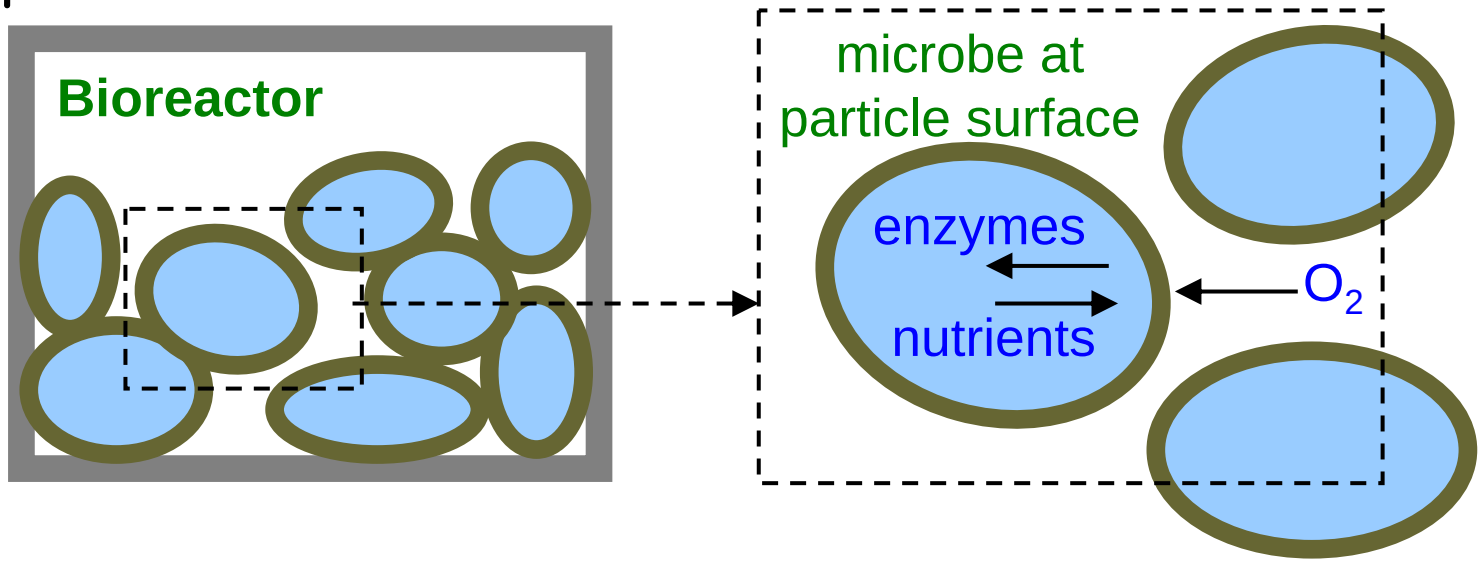


How does SSC differ from SLC?



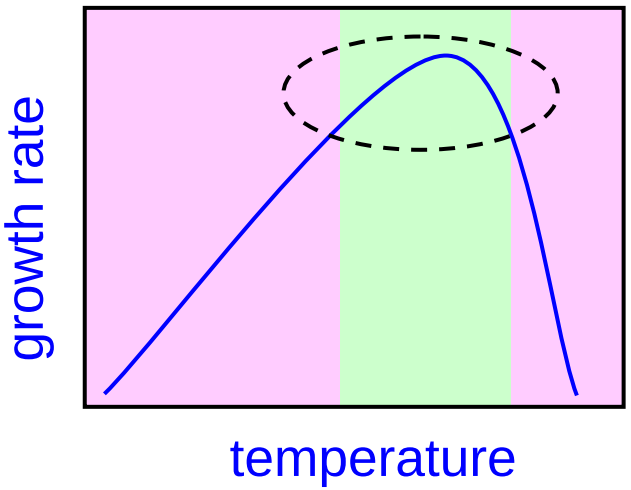
Growth of the microbe in SSC depends on what?

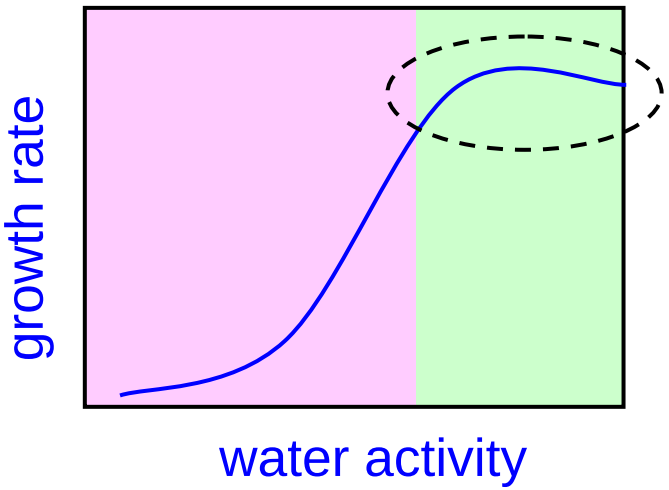
- the microorganism needs nutrients – note that the carbon source is typically present within in the substrate in the form of non-diffusible polymers, needing to be liberated by hydrolytic enzymes secreted by the microorganism
- the microorganism needs O_2 – which must diffuse in from the continuous gas phase between the particles



Growth of the microbe in SSC depends on what? (cont'd)

- the temperature should be near the optimum temperature for growth of the microorganism
- the water activity of the substrate should be near the optimum water activity for growth of the microorganism





Why should we be interested in solid-state cultivation?

Why should we be interested in SSC?

- Note that for the majority of processes, submerged culture (SLC) will give better yields than SSC and is much easier to operate
- However, in specific cases there may be reasons for strongly considering the SSC route:
  - When the product can only be produced by SSC

    solid fermented foods
  - When the product is produced both in SSC and SLC but the product yield is significantly higher in SSC

    this is often the case with fungal enzymes

Reasons for strongly considering the SSF route (cont'd)

- When the product is produced both in SSC and SLC, but the product produced in SSC has desirable properties due to the conditions that this cultivation method imposes on the organism

  fungal spores for use as biopesticides are more robust when produced in SSC than when produced in SLC

- When there is a desire to use a particular solid waste
- When you are thinking about biorefineries....

Biorefineries

- as petroleum resources dwindle, we will be forced to find alternative routes to many products that are currently based on the petroleum industry – fuels, plastics etc....
- the idea of a biorefinery is to use biological routes, including cultivation of microbes, in an integrated manner, to produce a range of organic products
- SSC will have an important role to play in any future biorefineries – in two ways
  - as a central processing step that minimizes water consumption (when compared to SLC processes)
  - as a means of taking advantage of solid by-products to produce value-added final products

Some examples of SSC products

- "Traditional"
  - koji step of soy sauce production

    involves the growth of the filamentous fungus *Aspergillus oryzae* on soybeans
  - tempe

    an Indonesian meat substitute that involves the growth of the filamentous fungus *Rhizopus oligosporus* on soybeans

- "Modern" (either under research or already commercial)
  - microbial enzymes

    for use in food processing, effluent treatment, or for use as biocatalysts (e.g. in biodiesel production)
  - antibiotics
  - biopesticides

    especially those based on fungal spores
  - organic acids
  - etc...

Why isn't solid-state cultivation used as much as it might be?

Why isn't SSC used as much as it might be?

...because it presents bioengineering challenges that have only been partially solved

- we don't know enough about how to design and operate SSC bioreactors
- we don't understand enough about how the various phenomena that occur in the system control the performance of the system
- our knowledge-base is lacking – there are relatively few examples of large scale processes and there is relatively little literature about the bioengineering principles of SSC

Why isn't SSC used as much as it might be? (cont'd)

- as a result, most companies consider it as a "risky technology"
  - in the West, SSC is underutilized in comparison to its potential (most companies choose SLC because it is a "proven" technology, even when SSC has the potential to perform better)
  - note that in Asia, where fermented foods based on SSC technology are common, this is not the case

So, what is our challenge?

To make SSC technology a "viable choice" for microbial cultivation processes!

What does it mean "to make SSC technology a viable choice"? Ideally, if you have a particular product that you want to produce by microbial cultivation, it should be possible to:

(1) evaluate both SLC and SSC for that product

(2) select the cultivation method that will work best

In order for this to be possible...

So, what is our challenge? (cont'd)

...we must have strategies for selecting, designing and operating bioreactors that are based on bioengineering principles and which will therefore ensure successful operation at large scale

In other words, we need to understand more about SSC bioreactors

- we need to understand the mass and heat transfer phenomena that control how the bioreactor performs
- we must understand the kinetics of the growth of the microorganism in the bioreactor

So, what kinds of bioreactors are used in SSC?...

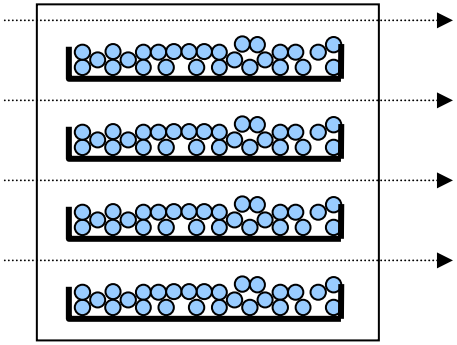
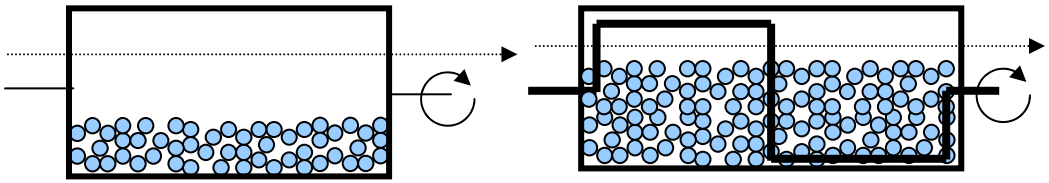
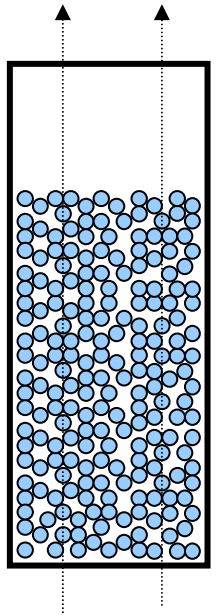
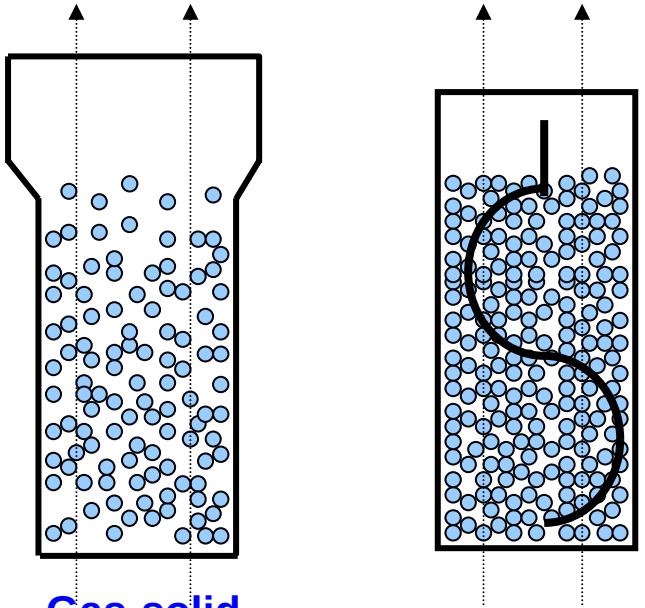
What types of bioreactors are used in solid-state cultivation?

What types of bioreactors are used in SSC?

The main functions of an SSC bioreactor are to

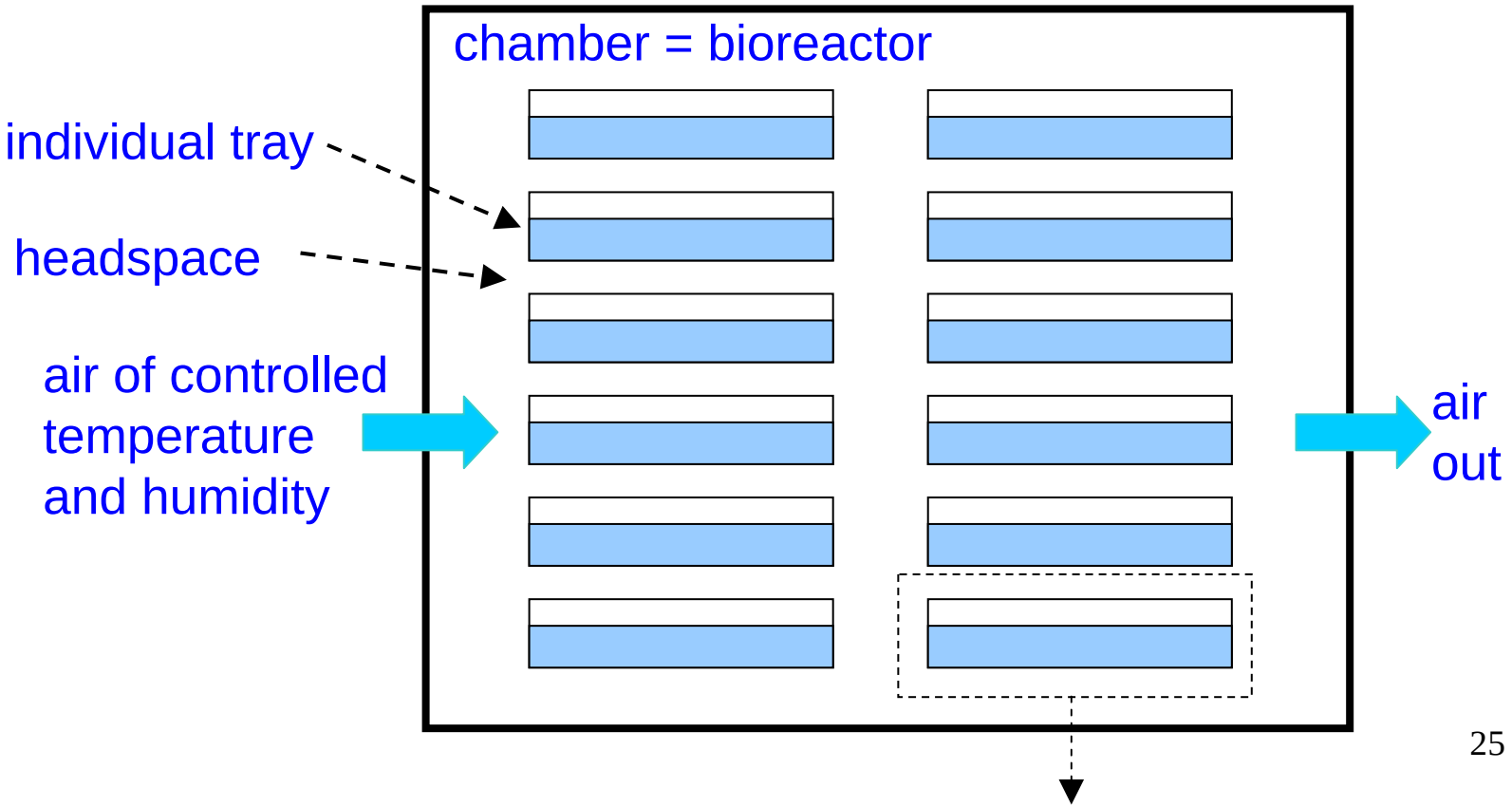
- bring O_2 to the particle surface
- allow control of the temperature of the bed
- allow control of the water activity of the bed

Although many details might be different, it is useful to classify SSC bioreactors based on mixing and aeration strategies...

Mixing → / ↓ Aeration	No mixing (or very infrequent)	Continuous mixing (or frequent intermittent mixing)
No forced aeration (passes around the bed)	I  Tray chamber	III  Rotating drum Stirred drum
Forced aeration (air forced through the bed)	II  Packed bed	IV  Gas-solid fluidized bed Stirred bed

Group I – Bioreactors without agitation (or with very infrequent agitation) and without forced aeration: tray bioreactors

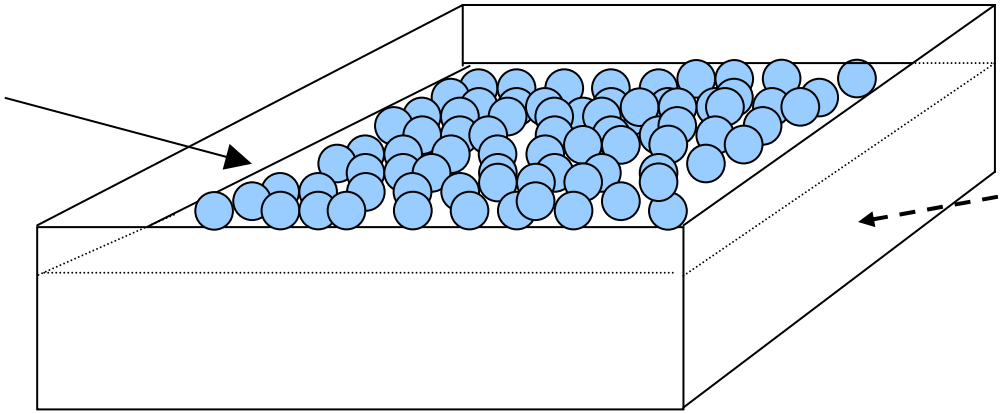
A tray bioreactor consists of many trays in a chamber with control of the temperature and humidity of the atmosphere



An individual tray

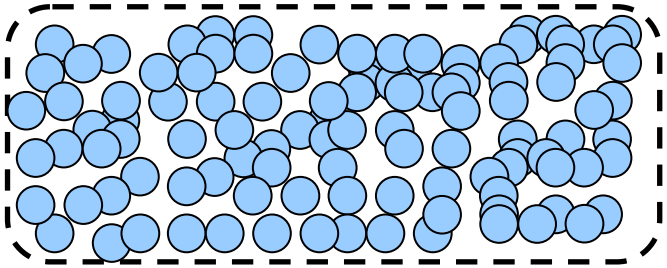
Air circulated around the tray

a thin layer of substrate, either completely static or agitated by hand one or two times per day

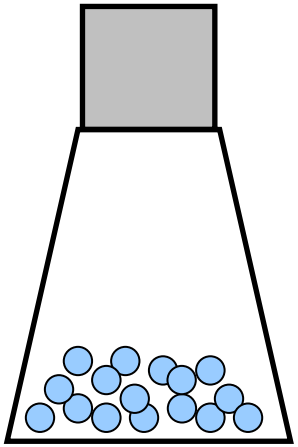


the sides and bottoms are typically perforated to aid in gas exchange

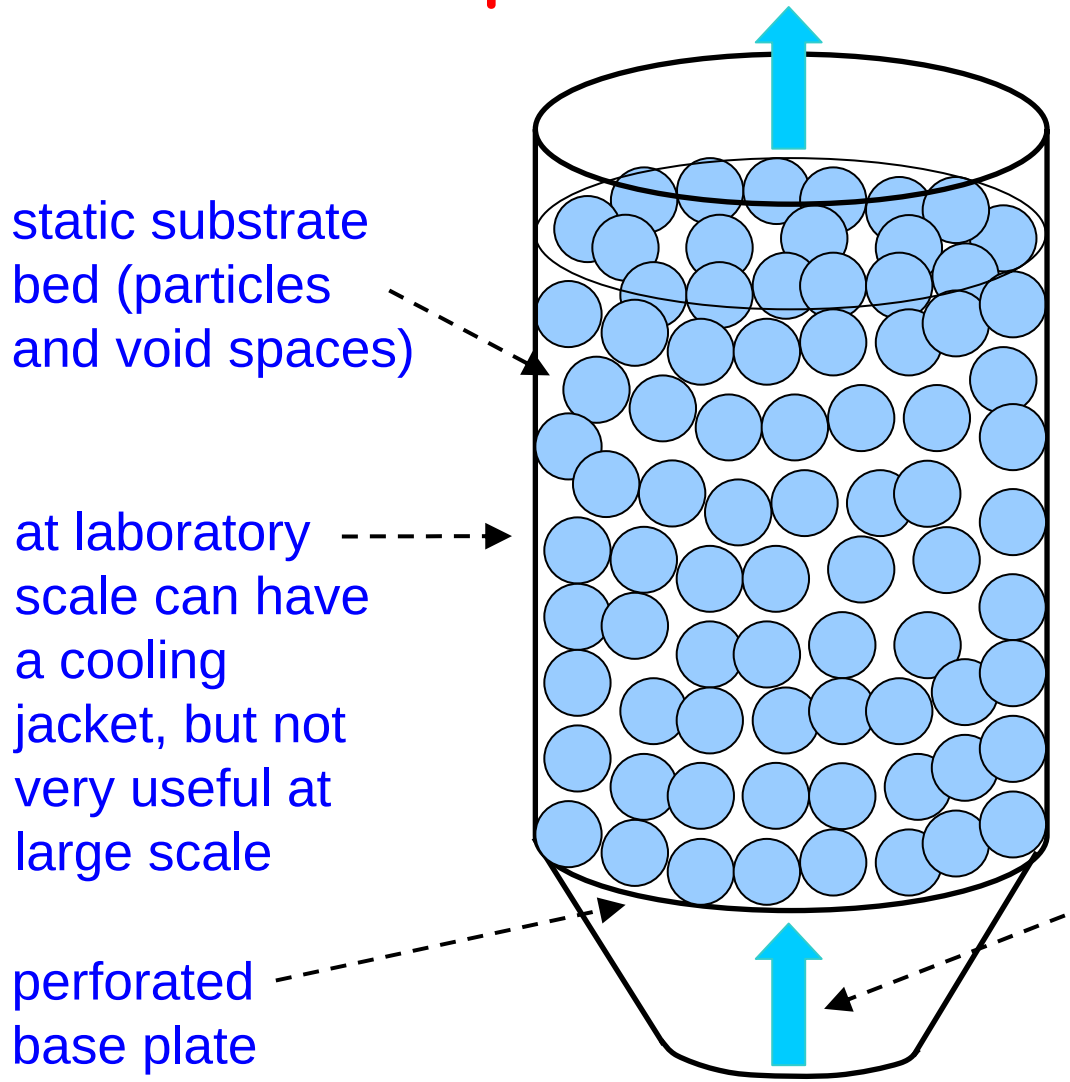
Microporous plastic bags have also been used



Erlenmeyer flasks represent this type of system



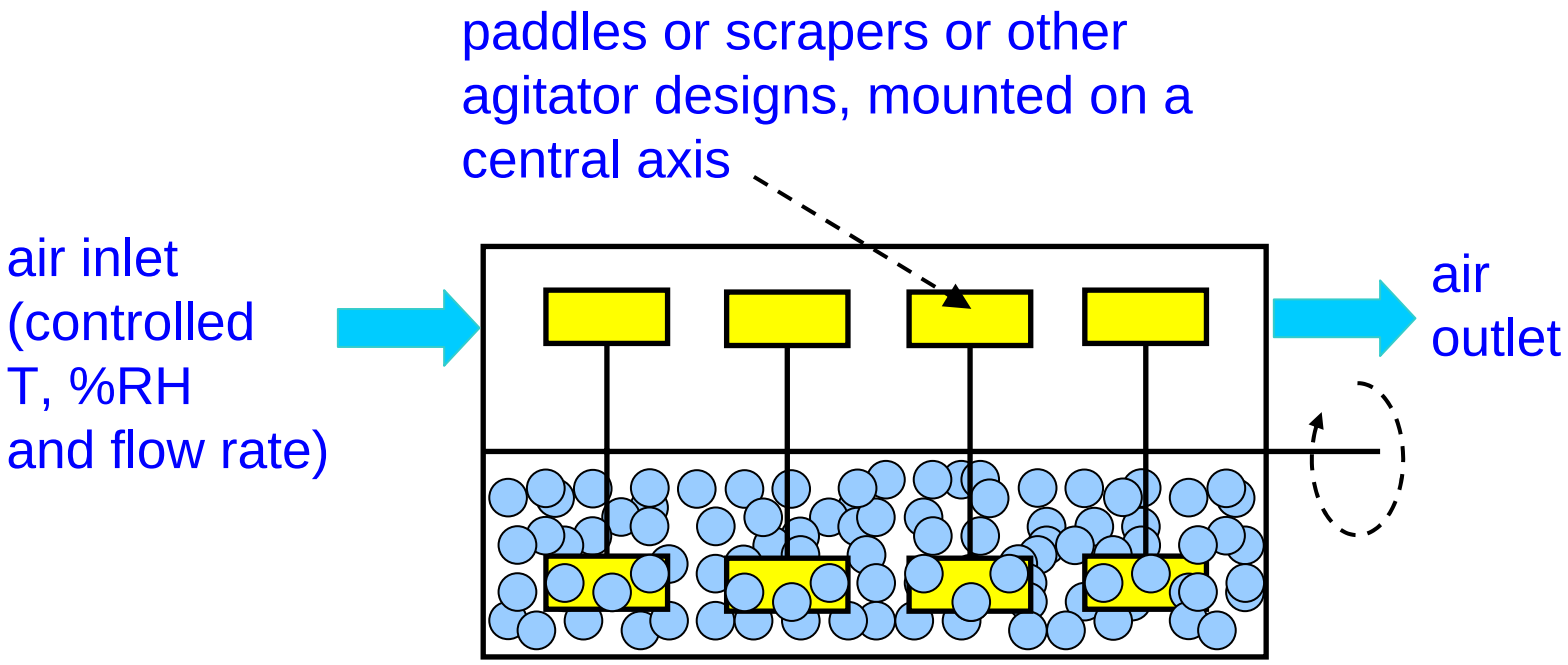
Group II – Bioreactors without agitation but with forced aeration: packed beds



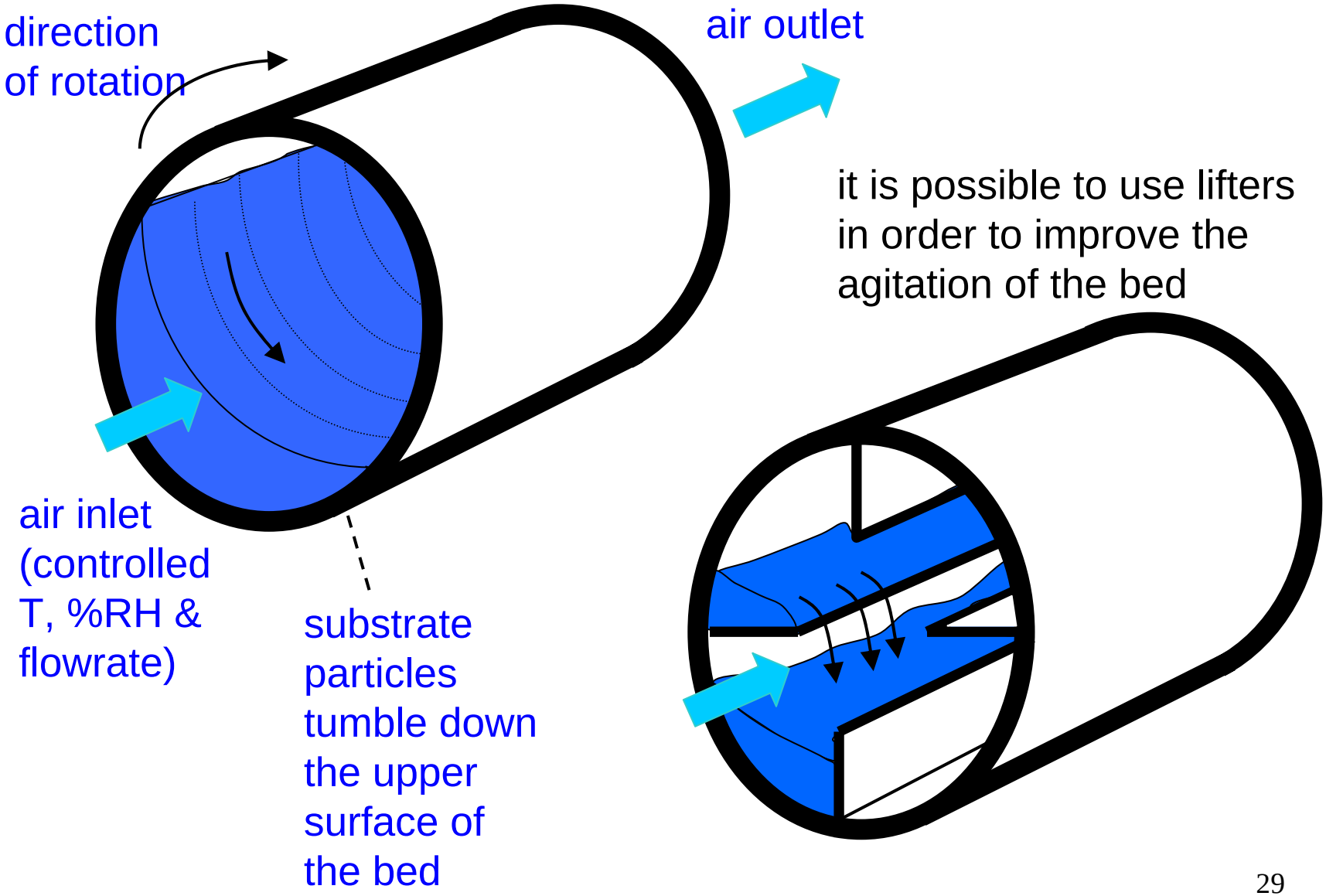
- forced aeration
- controlled T
- typically the air is saturated

Group III – Agitated, but air blown through the headspace and not forcefully through the bed: rotating drums and stirred drums

Stirred drum

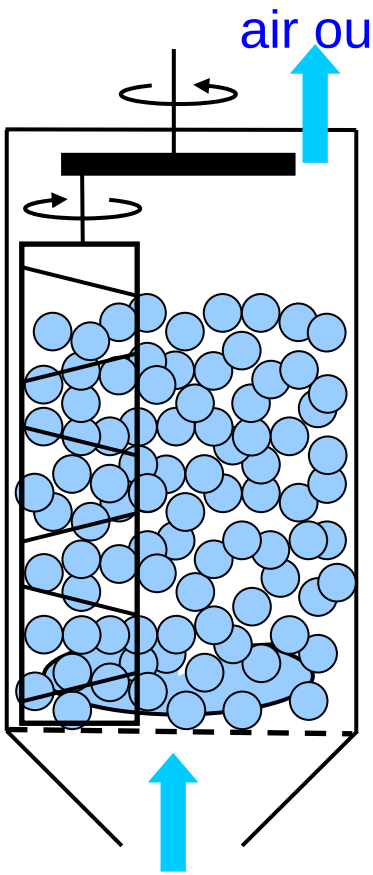


Rotating drum

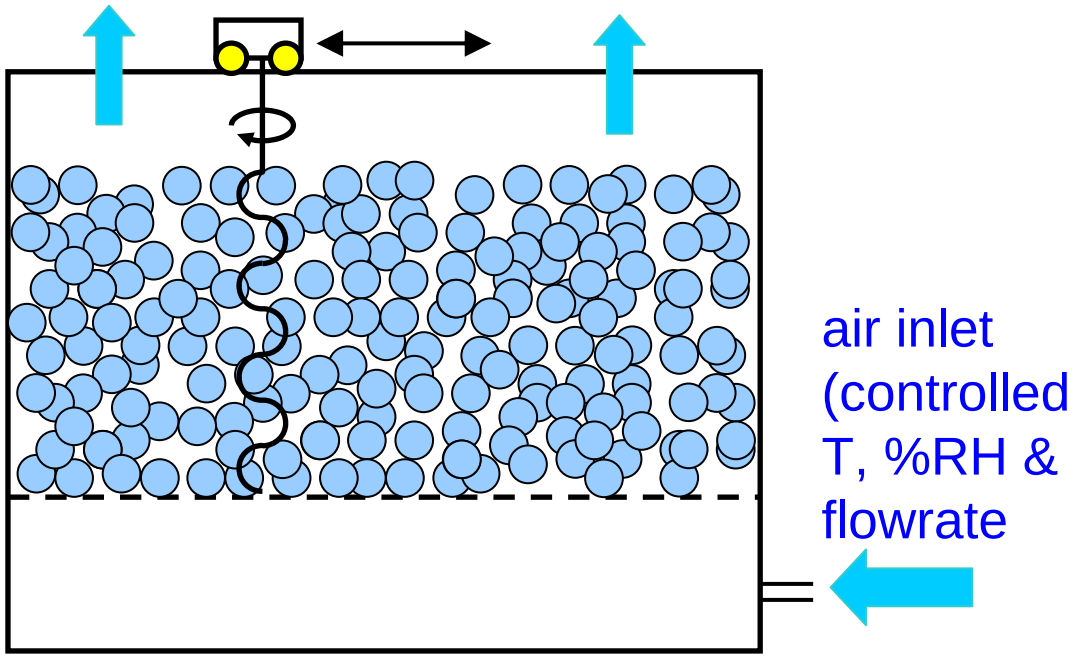


Group IV – agitated, with forced aeration: Stirred bed, fluidized bed

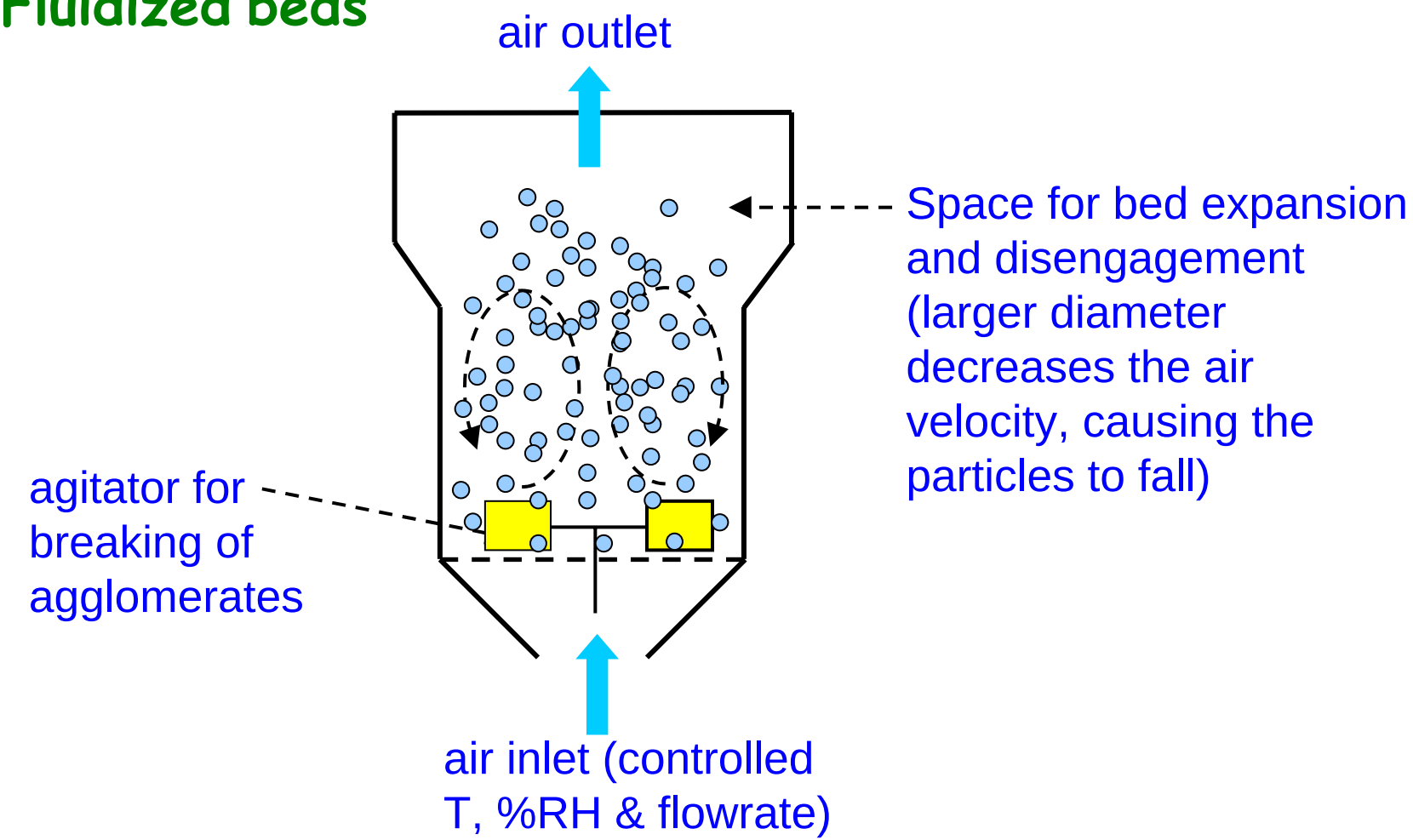
Stirred beds



agitation can be continuous or intermittent (with relatively frequent mixing events)



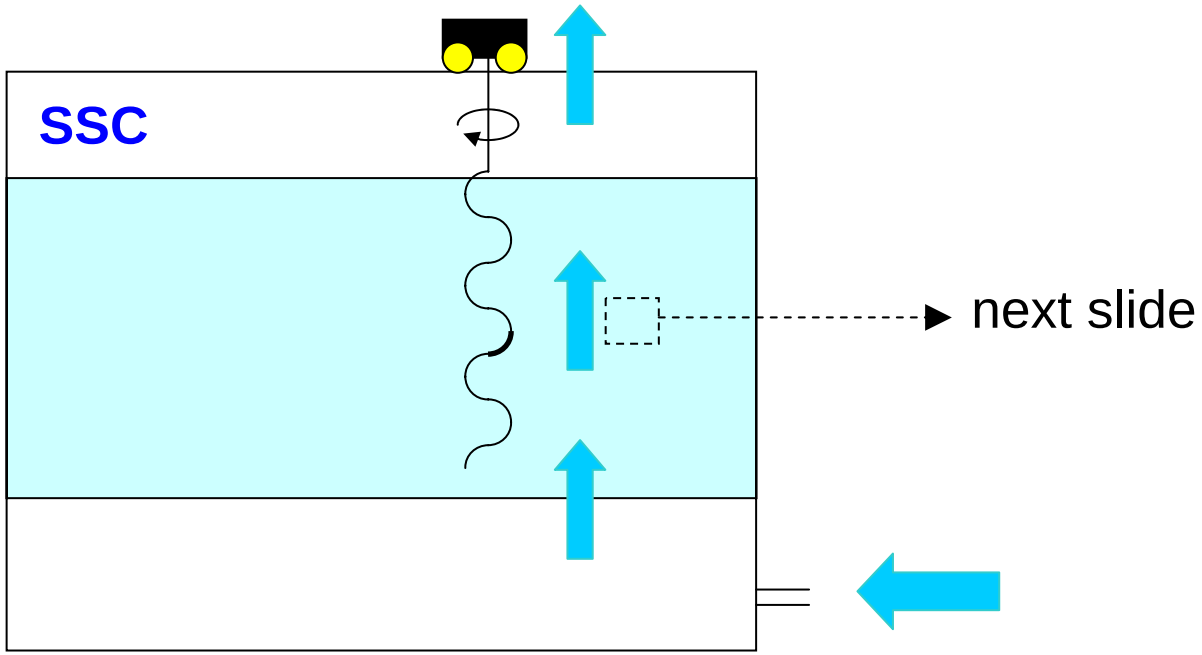
Fluidized beds



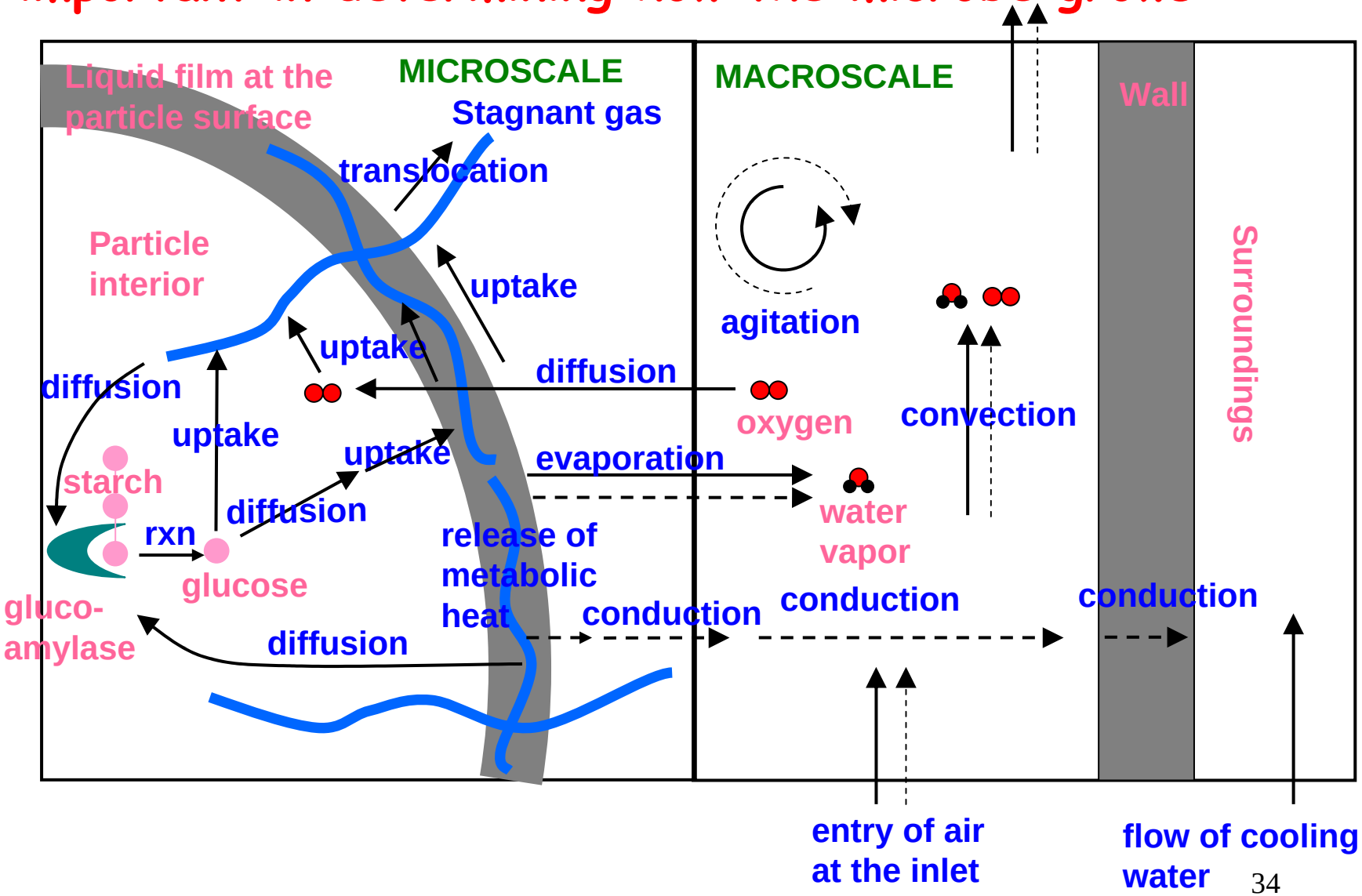
The air is blown at sufficient velocity to fluidize the substrate particles

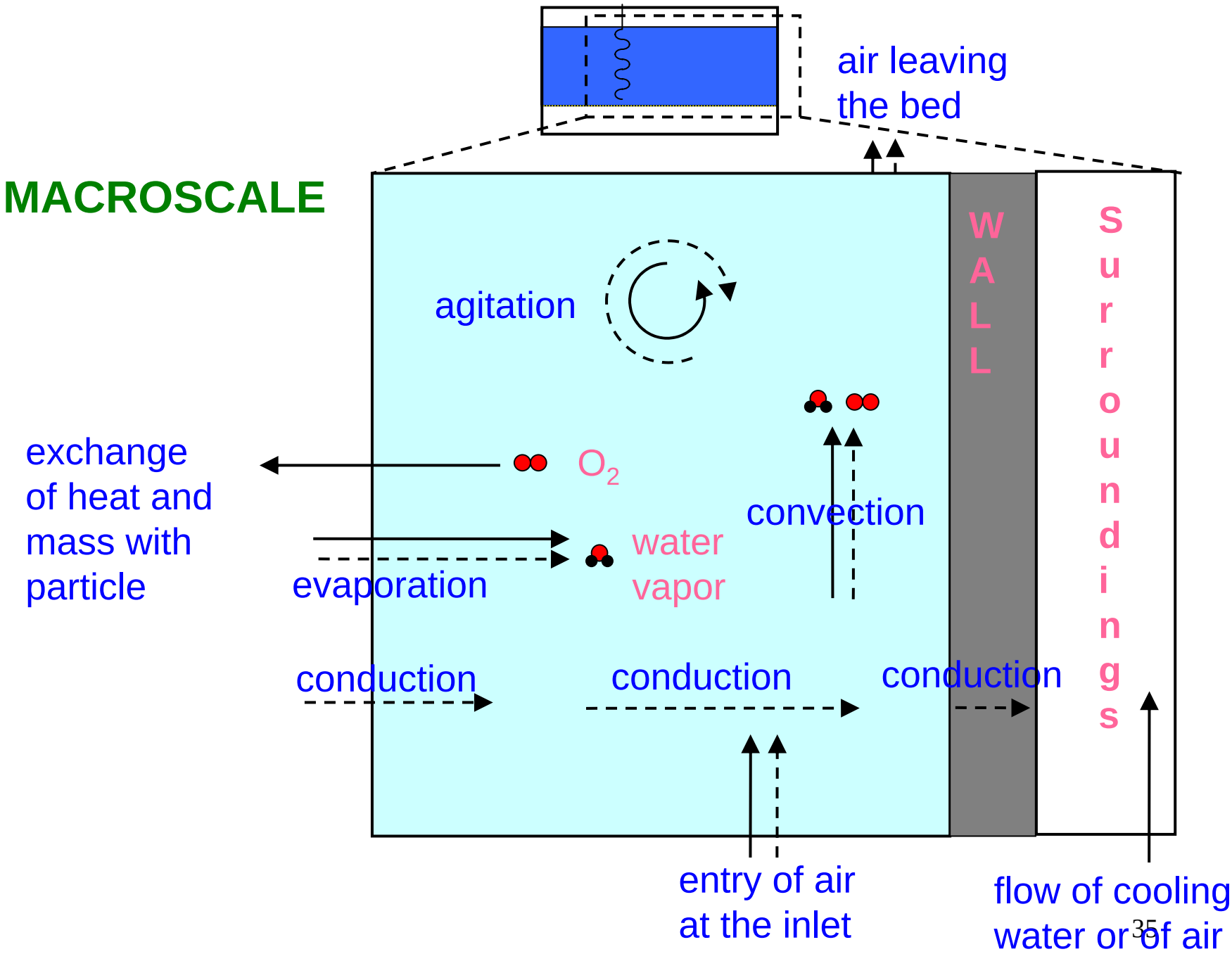
What heat and mass transfer phenomena occur within solid-state cultivation bioreactors?

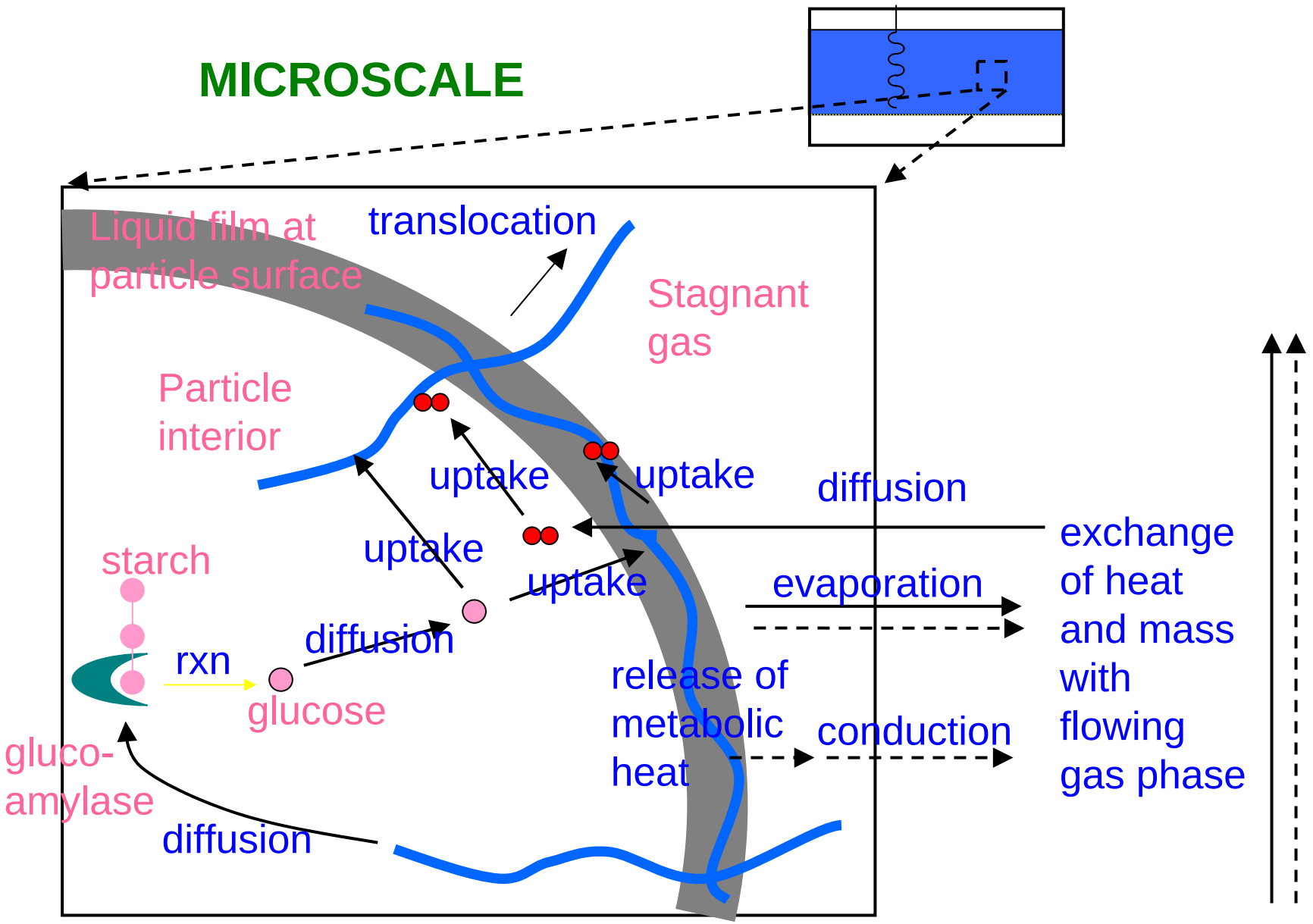
The performance of an SSC bioreactor depends on the various microscale and macroscale processes that occur and how they interact



Both "microscale" and "macroscale" phenomena are important in determining how the microbe grows




MACROSCALE
air leaving
the bed
agitation
W
A
L
L
S
u
r
r
o
u
n
d
i
n
g
s
exchange
of heat and
mass with
particle
O_2
convection
water
vapor
evaporation
conduction
conduction
conduction
entry of air
at the inlet
flow of cooling
water or of air


MICROSCALE
Liquid film at particle surface
translocation
Stagnant gas
Particle interior
uptake
uptake
uptake
uptake
diffusion
exchange of heat and mass with flowing gas phase
starch
evaporation
rxn
diffusion
glucose
release of metabolic heat
conduction
gluco-amylase
diffusion

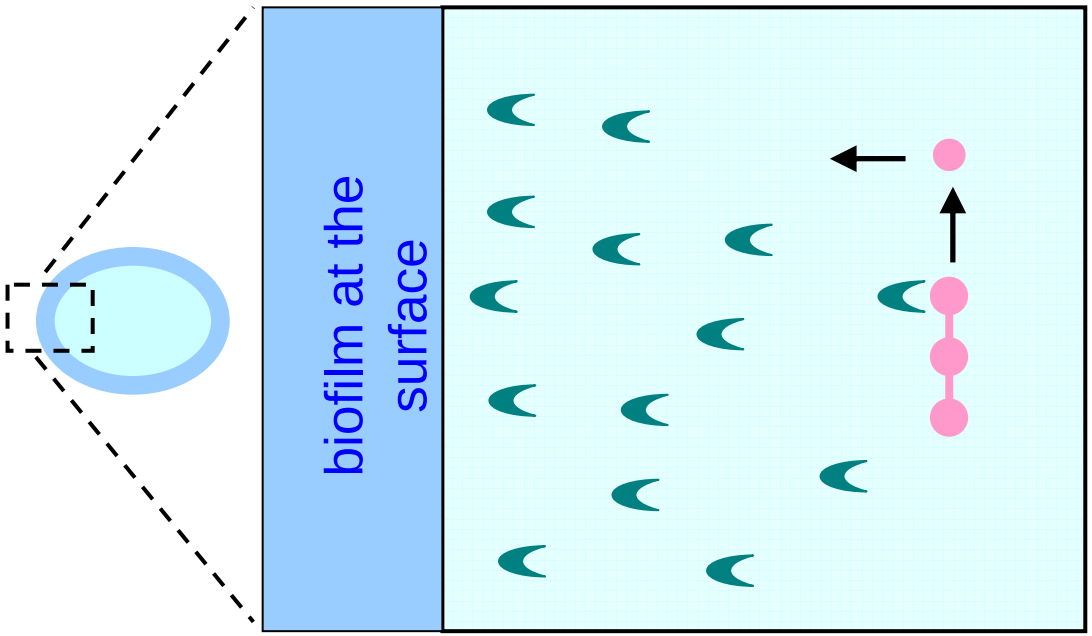
What do we know and what don't we know about how "microscale phenomena" affect the performance of the system?

What do we know and what don't we know about how "microscale phenomena" affect the performance of the system?: (1) Reaction and diffusion

- A part of bioengineering is to understand the microscale phenomena that determine biocatalyst performance
- What we know:
  - that reaction/diffusion phenomena affect the growth of the microorganism because they affect the availability of nutrients and O_2

Microscale – effect of reaction and diffusion phenomena on growth kinetics

Representation of growth of a biofilm of unicellular microorganisms at the surface of a particle

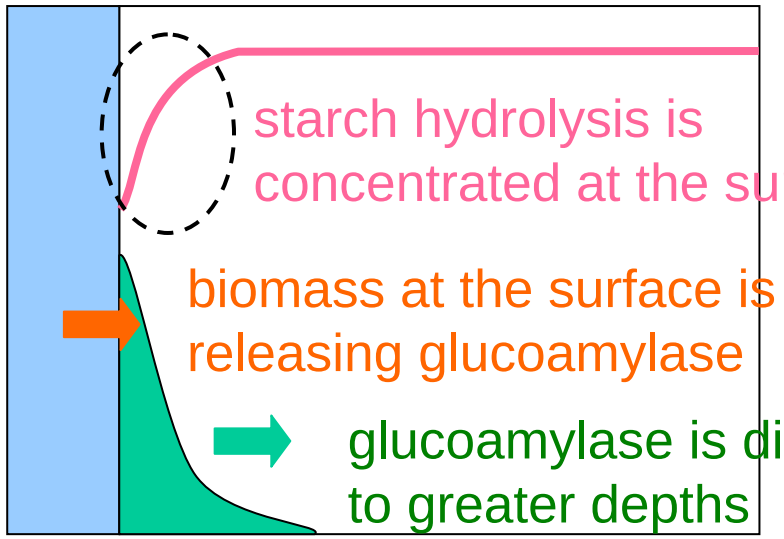


Experimental and modeling work have shown that:

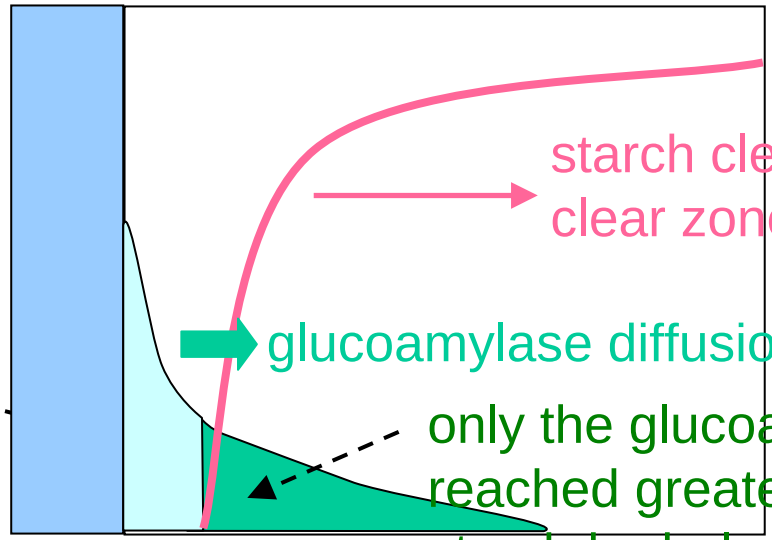
- Reaction/diffusion phenomena within the substrate are important
- O_2 or glucose can be limiting in the biofilm

Reaction/diffusion phenomena within the substrate

early on



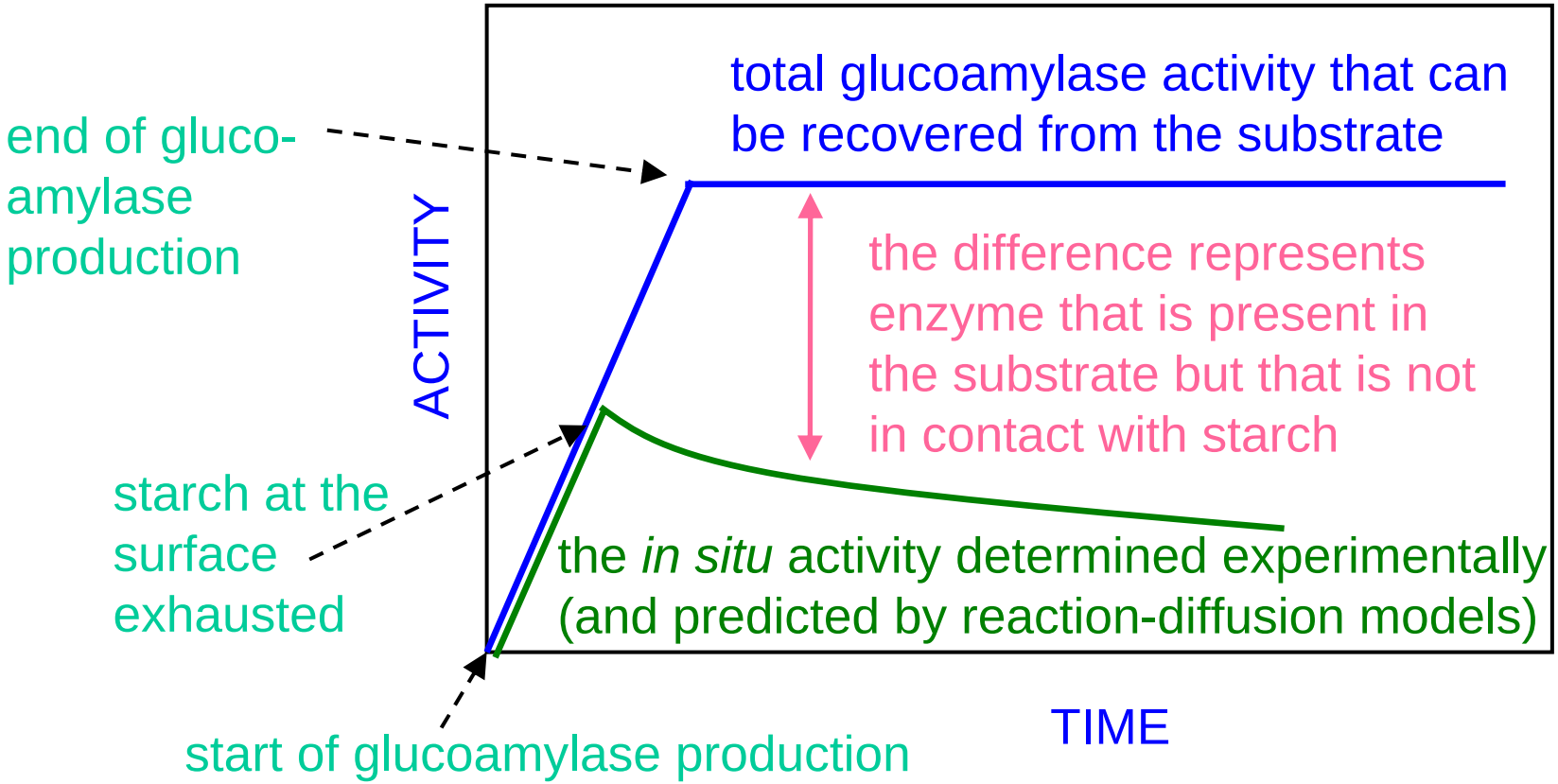
later



the enzyme here has no substrate!

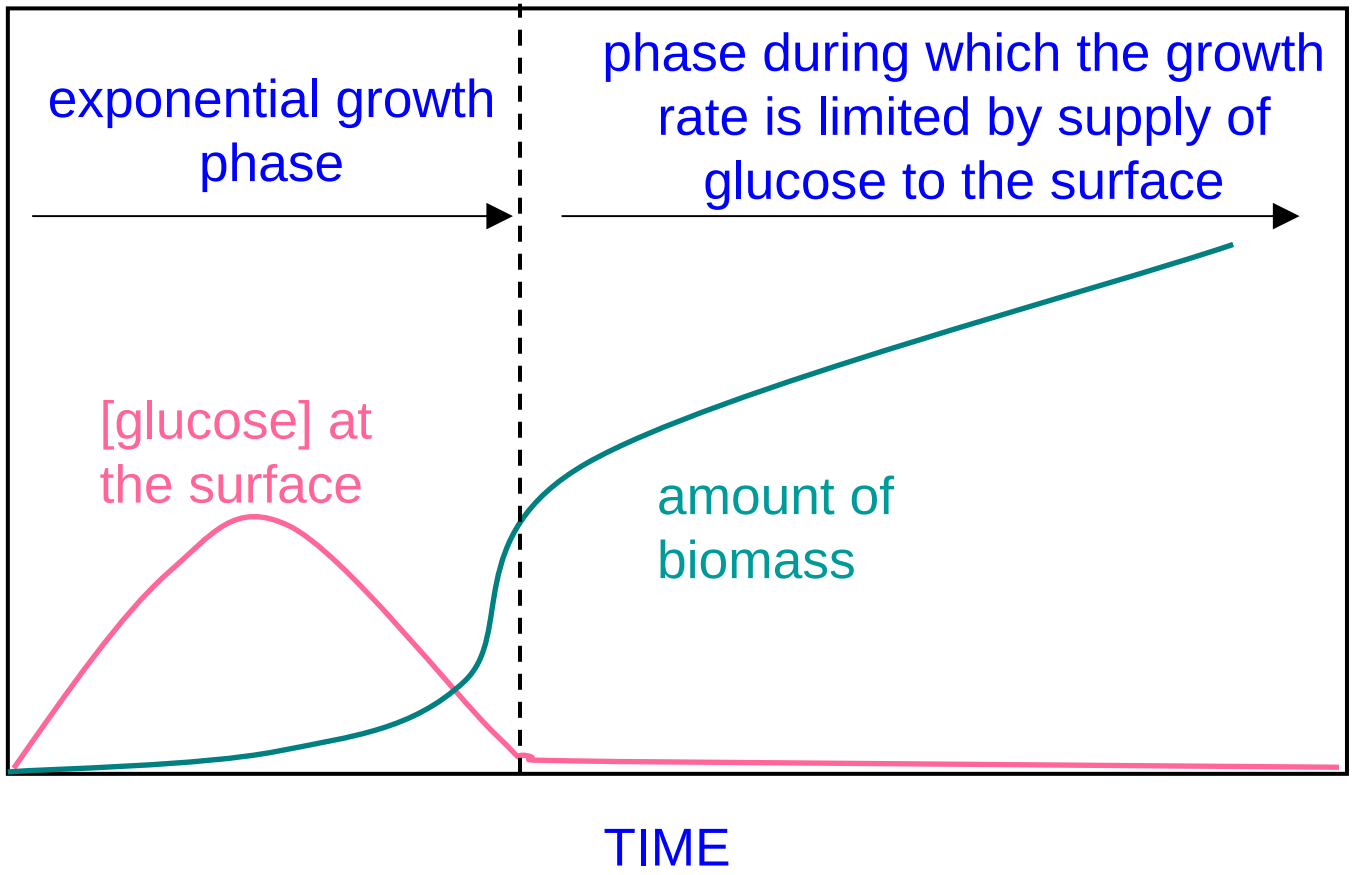
Reaction/diffusion phenomena within the substrate (cont'd)

As a result of these reaction/diffusion phenomena, after exhaustion of starch at the surface, the release of glucose within the substrate decreases with time



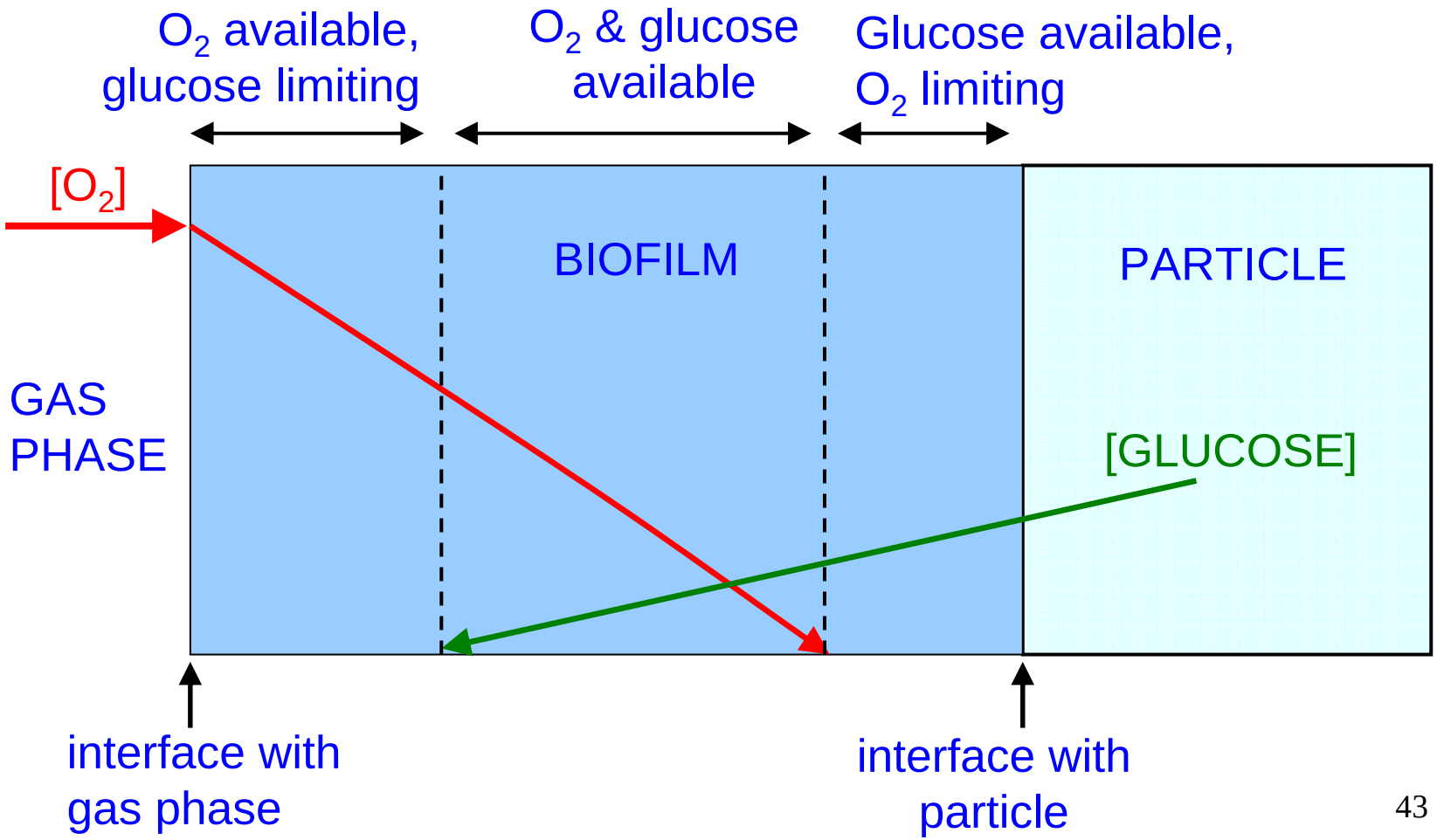
Reaction/diffusion phenomena within the substrate (cont'd)

Modeling work suggests that the supply of glucose to the microorganism at the surface can limit its growth rate



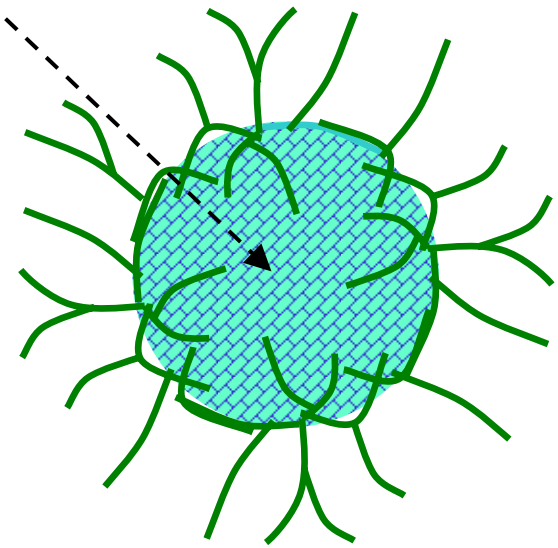
Both O_2 & glucose can limit growth within the biofilm

- the organisms used are typically aerobic – they need O_2
- the sizes of limited zones varies during the process



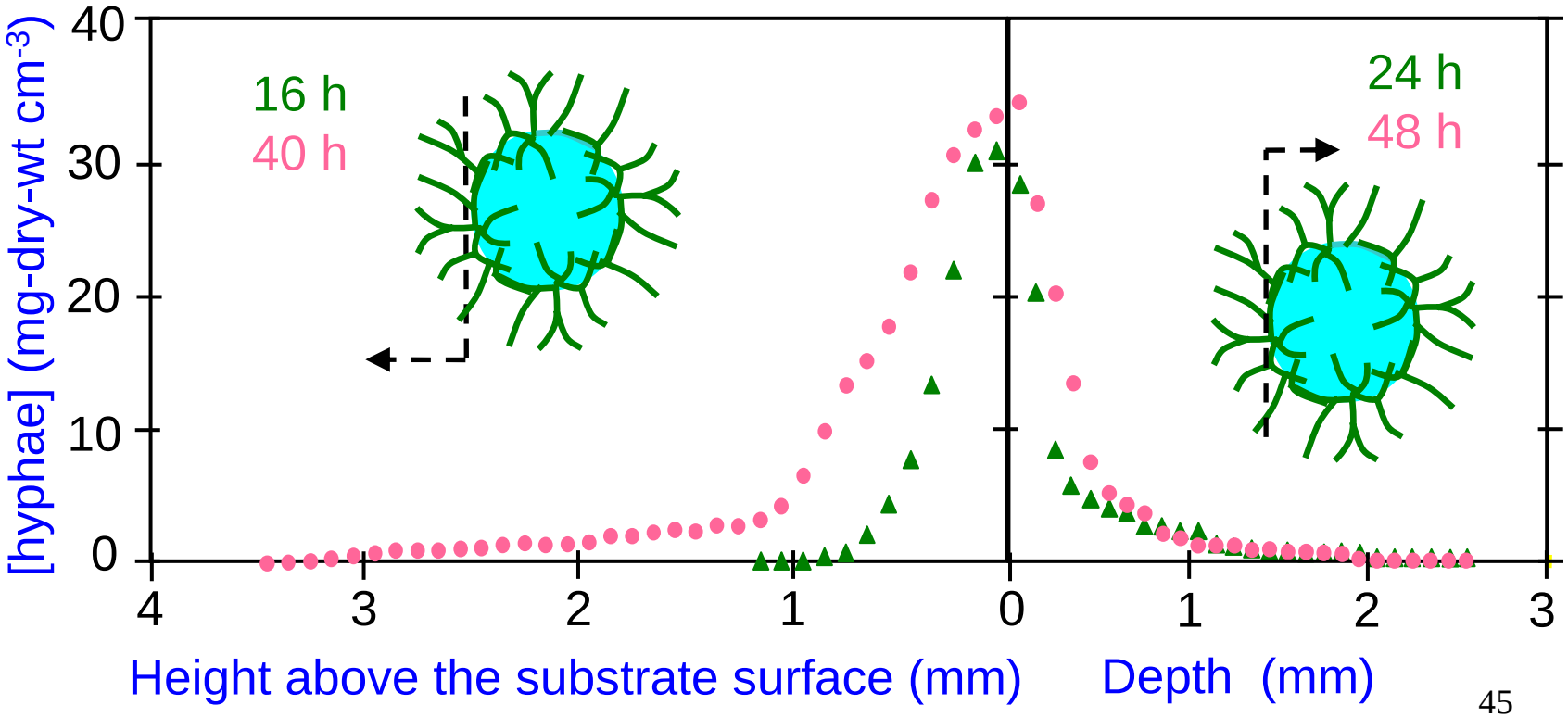
What we don't know about the microscale phenomena

- These studies have been done with artificial gels – real solid substrate particles may have more complex substructure. How does this affect the processes in the substrate?
- Many SSC processes involve filamentous fungi which grow into the air phase (and not as a wet biofilm). Note also that this can affect the pressure drop within aerated beds. What controls the growth of the "aerial hyphae"?



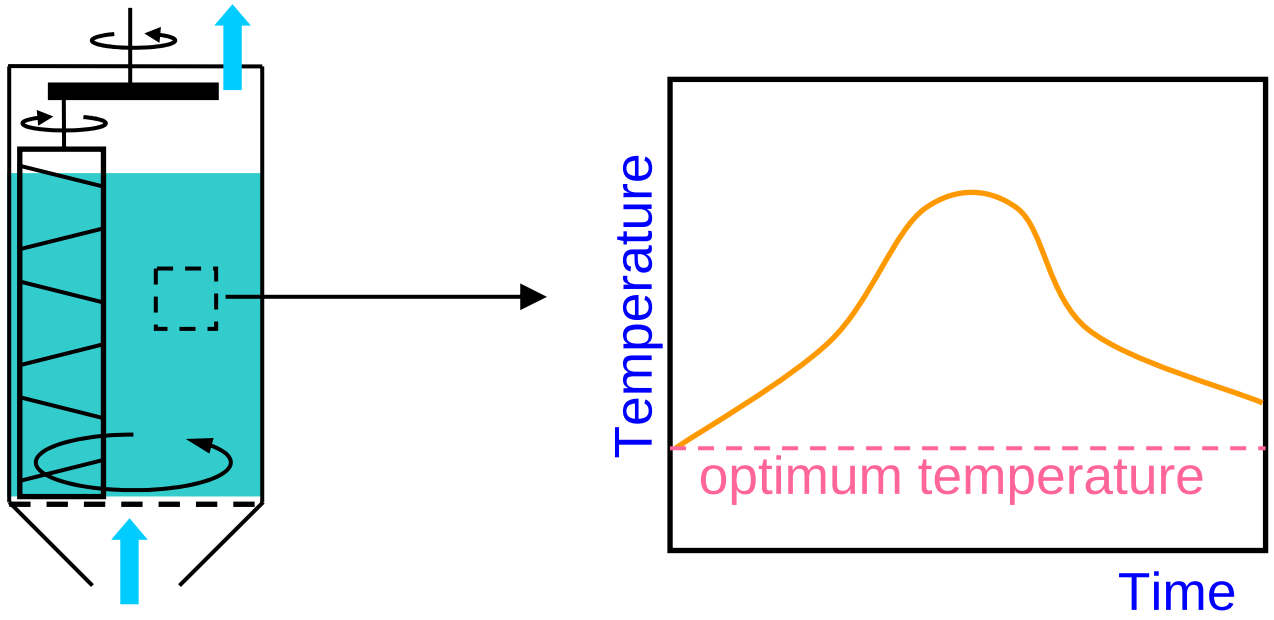
What we don't know about the microscale phenomena (cont'd)

- We have profiles for the density distribution of the fungal hyphae above and below the particle surface. Our challenge is to understand what controls these profiles.



What do we know and what don't we know about how "microscale phenomena" affect the performance of the system? (2) Effect of a varying temperature

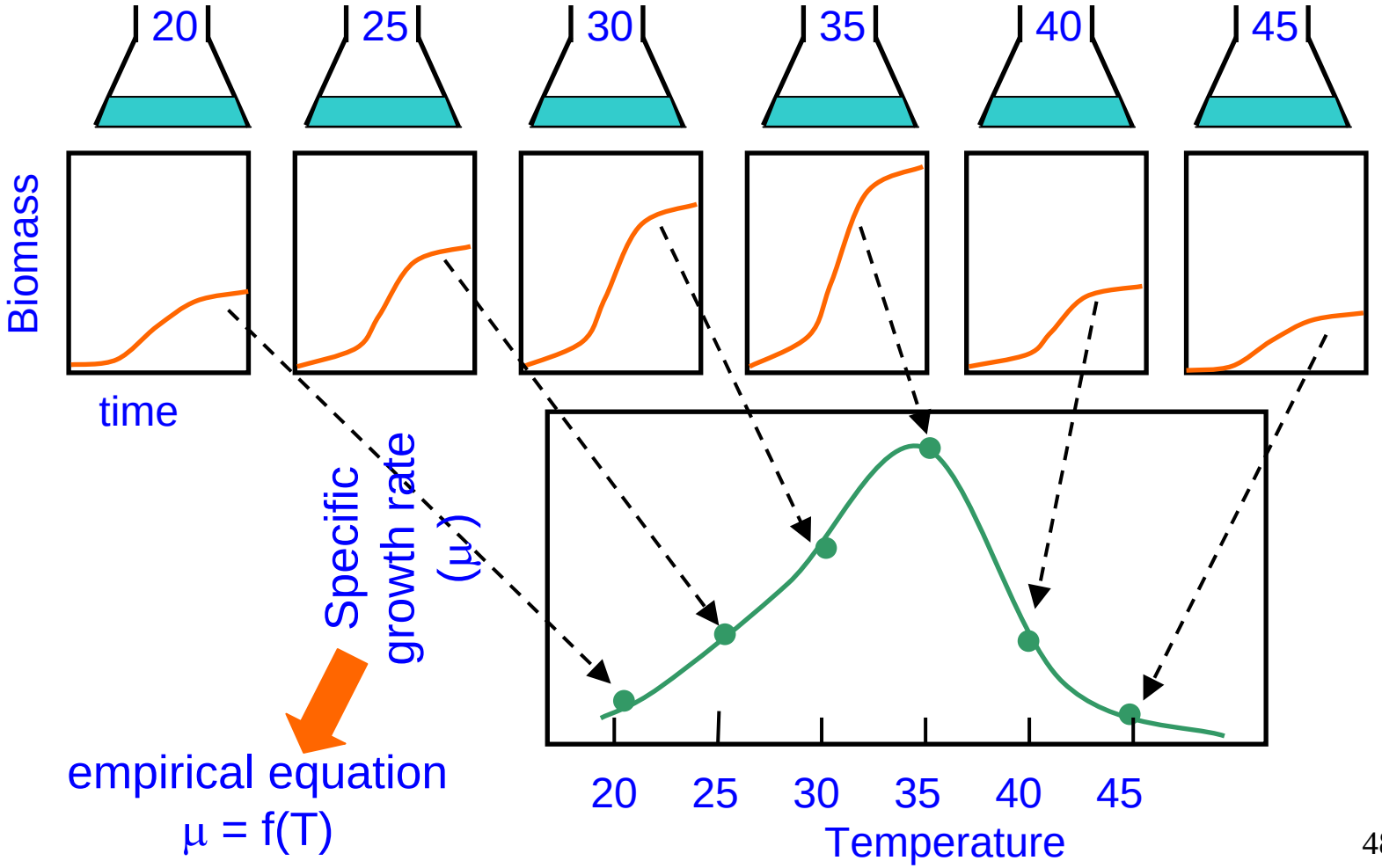
- Due to difficulties in heat removal, the temperature of the bed reaches values above the optimum temperature for growth during the culture

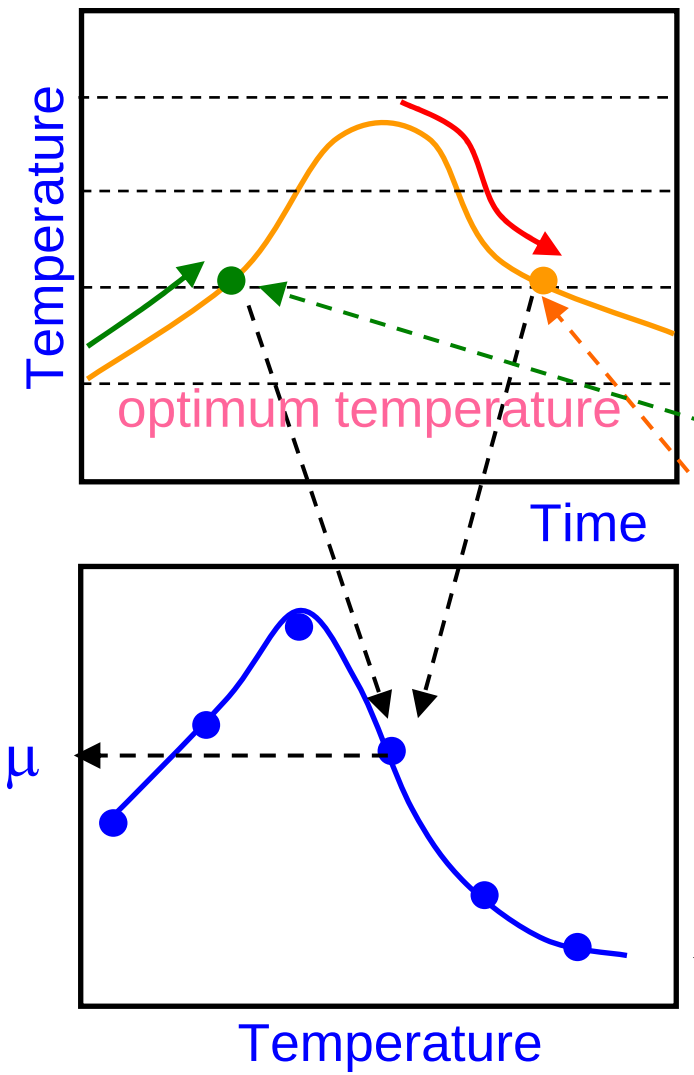


What do we know and what don't we know about how "microscale phenomena" affect the performance of the system? (2) Effect of a varying temperature (cont'd)

- We know that the growth kinetics of microorganisms depend on the temperature
- But just what is the effect of a varying temperature on the growth kinetics?
- The next slide shows the "classical" approach to determining the effect of temperature on growth kinetics – it was used traditionally in SLC and was later used in studies of growth in SSC

- in this traditional method, different cultures are incubated at different temperatures, with each culture being subjected to a constant temperature during its growth cycle





The dashed horizontal lines represent the temperature profiles experienced by the various cultures that were incubated according to the traditional method.

In reality, although the temperature is the same, the temperature history is different:

- initially, the organism has been recently exposed to near-optimal T
- later, the organism has been recently exposed to high T

The underlying specific growth rate constant would be expected to be different in the two situations...

...but according to the curve obtained by the "traditional method", for the same T, the value of μ will be the same!

What do we know and what don't we know about how "microscale phenomena" affect the performance of the system? (2) Effect of a varying temperature (cont'd)

- In order to have adequate models for the kinetics of growth of the microorganism in SSC:
  - We need to understand the effect of a history of temperature variations on the growth kinetics
  - In order to do this we need to collect good experimental data ! (this is currently not available)

What do we know and what don't we know about how "macroscale phenomena" affect the performance of the system?

What do we know and what don't we know about how "macroscale phenomena" affect the performance of the system?

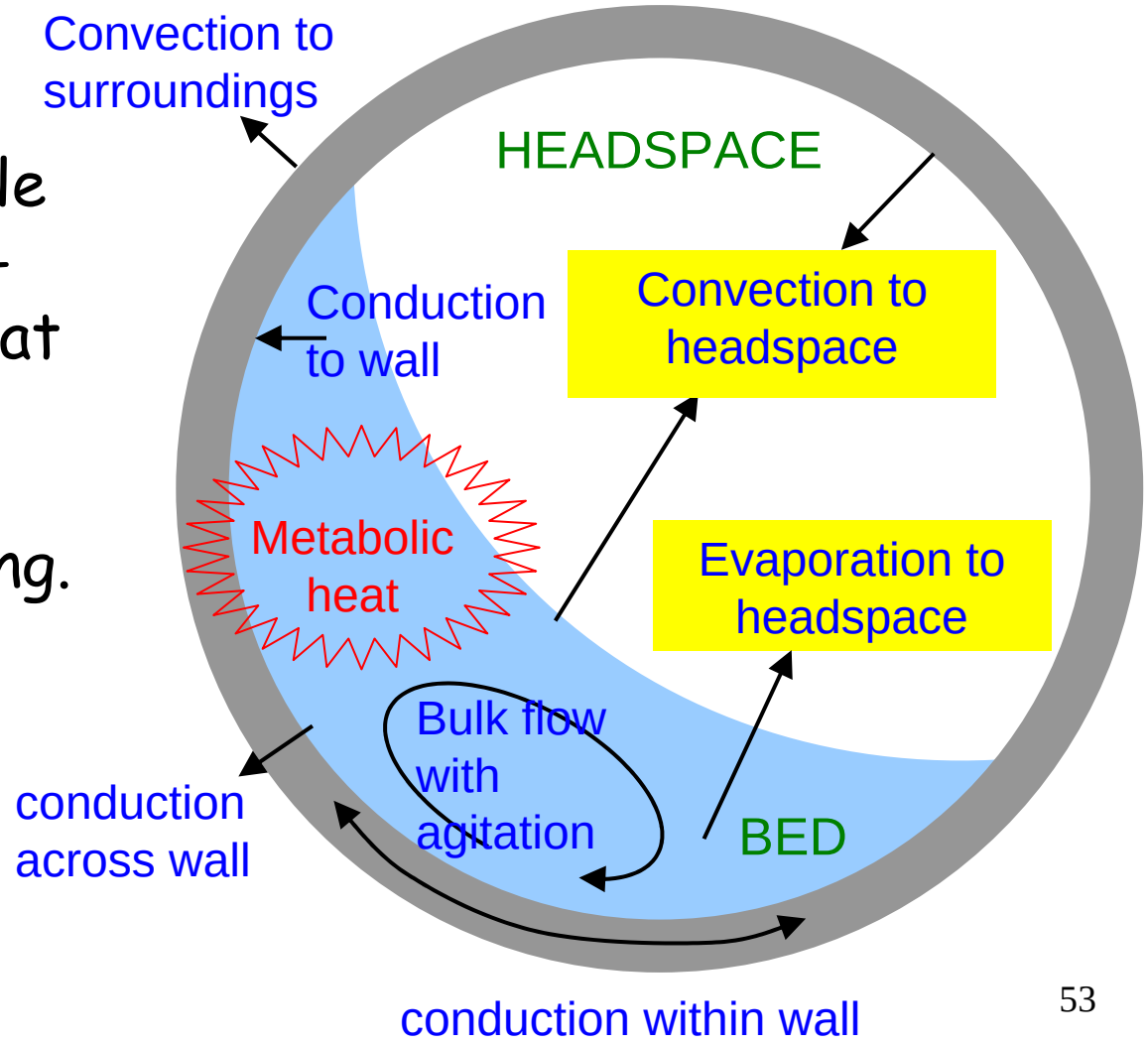
What we have:

- we have experimental work that characterizes various aspects of the performance of SSC bioreactors
- we have basic models of macroscale heat transfer and water transfer (evaporative cooling) within the beds of several different kinds of SSC bioreactors

In the following discussion we will consider "rotating drum", "packed bed " and "mixed and aerated " bioreactors

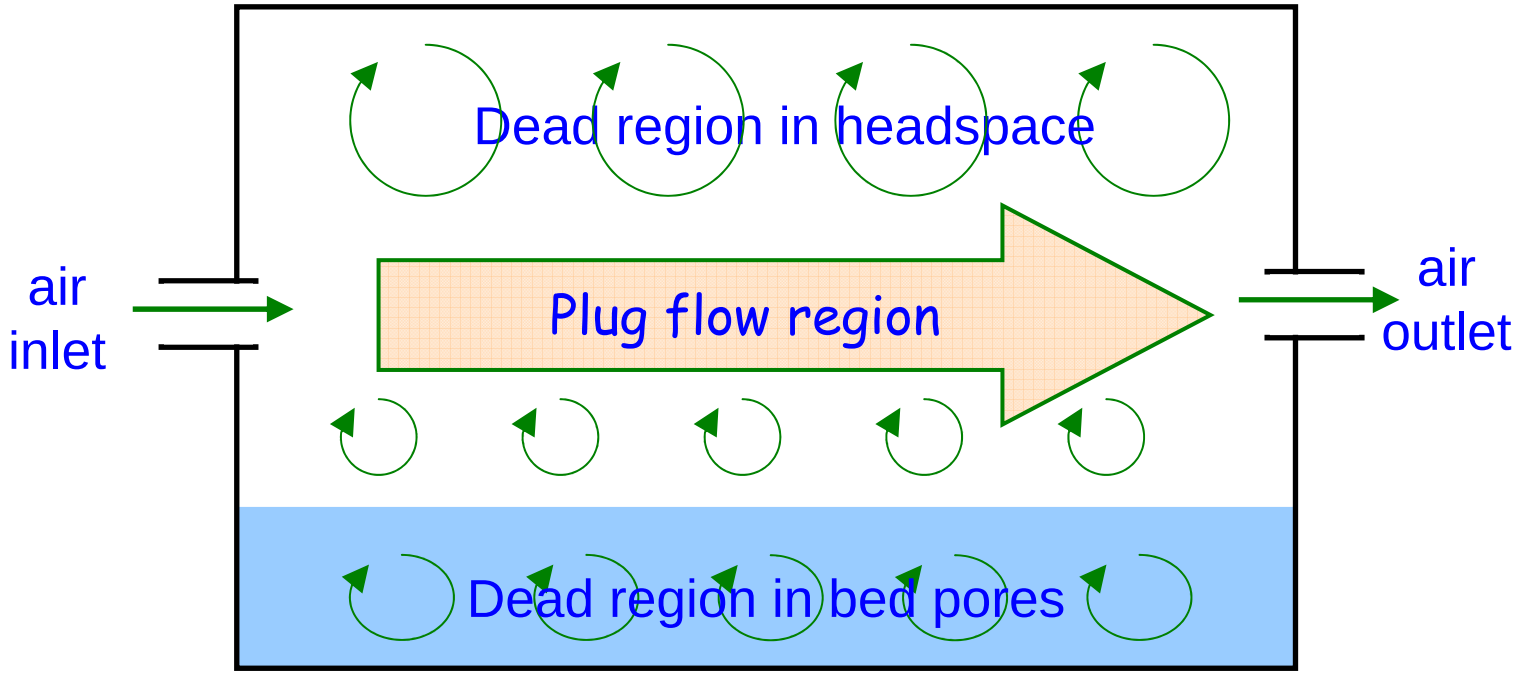
What do models and experimental results tell us about rotating drum type bioreactors...and what would we still like to know? (1) Cooling routes

- models suggest that in large scale bioreactors bed-to-headspace heat transfer will be the major route, but will be limiting. However, this is relatively poorly investigated experimentally



What do models and experimental results tell us about rotating drum type bioreactors...and what would we still like to know? (2) Gas flow patterns

- headspace gas flow patterns will affect bed to headspace heat and mass transfer
- some work has been done....residence time distribution patterns suggest that there are dead spaces



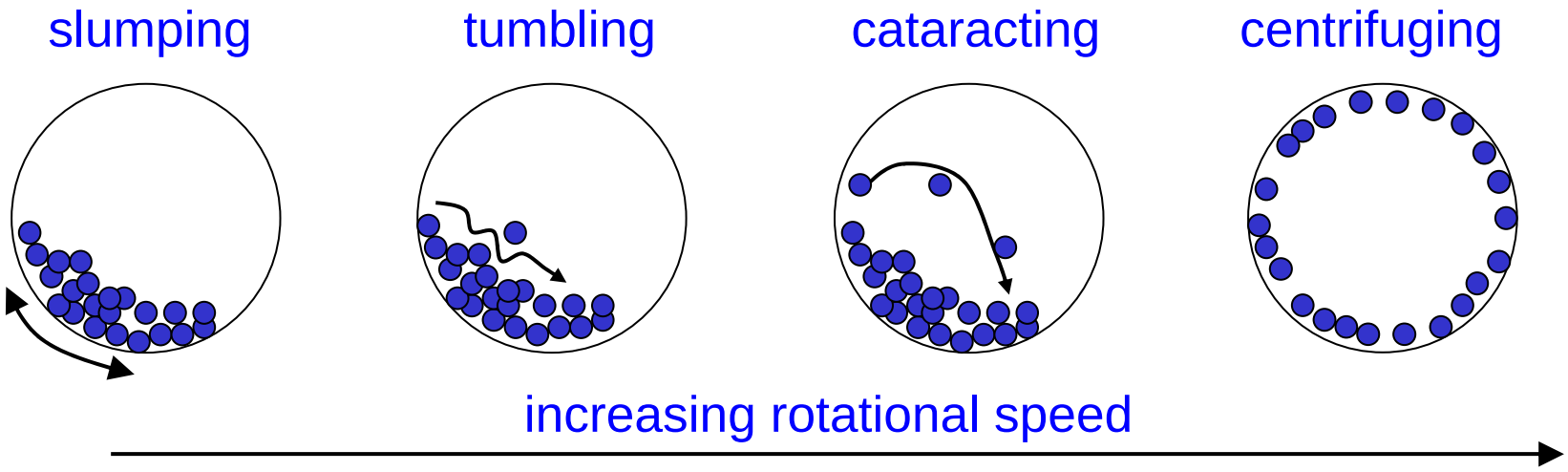
What do models and experimental results tell us about rotating drum type bioreactors...and what would we still like to know? (2) Gas flow patterns (cont'd)

...however, on the whole this has been poorly investigated...studies to date have been limited

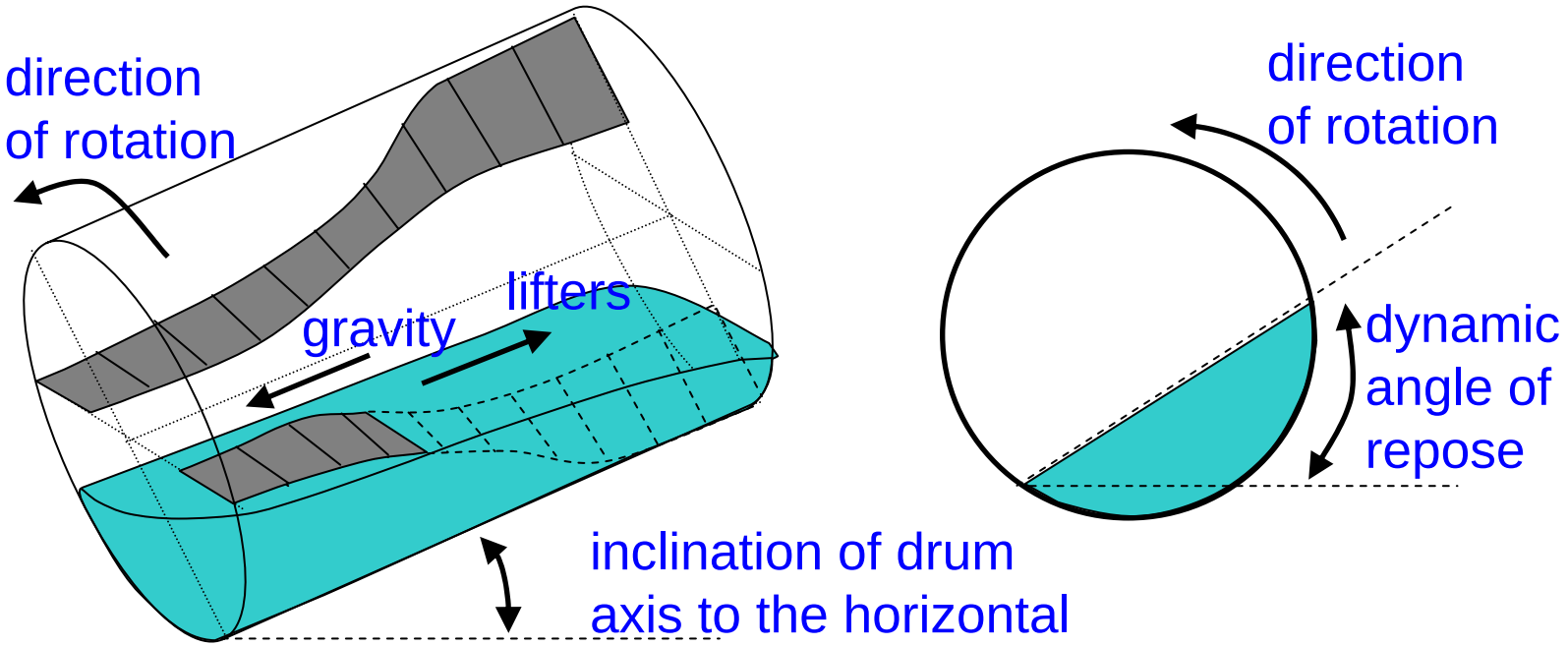
- There have not been any studies of how the design of the drum and of the air inlet and outlet will affect flow patterns
- The effect of drum design and operation on the resulting efficiency of bed-to-headspace heat and mass transfer has not been studied

What do models and experimental results tell us about rotating drum type bioreactors...and what would we still like to know? (3) Solids flow patterns

We know that we can expect different solids-flow regimes depending on the design and operation of the drum – for example, in the absence of baffles:



We know that we can improve mixing with lifters – discrete particle modeling has shown that best mixing will be obtained with an inclined axis (less than the dynamic angle of repose) and with angled lifters:

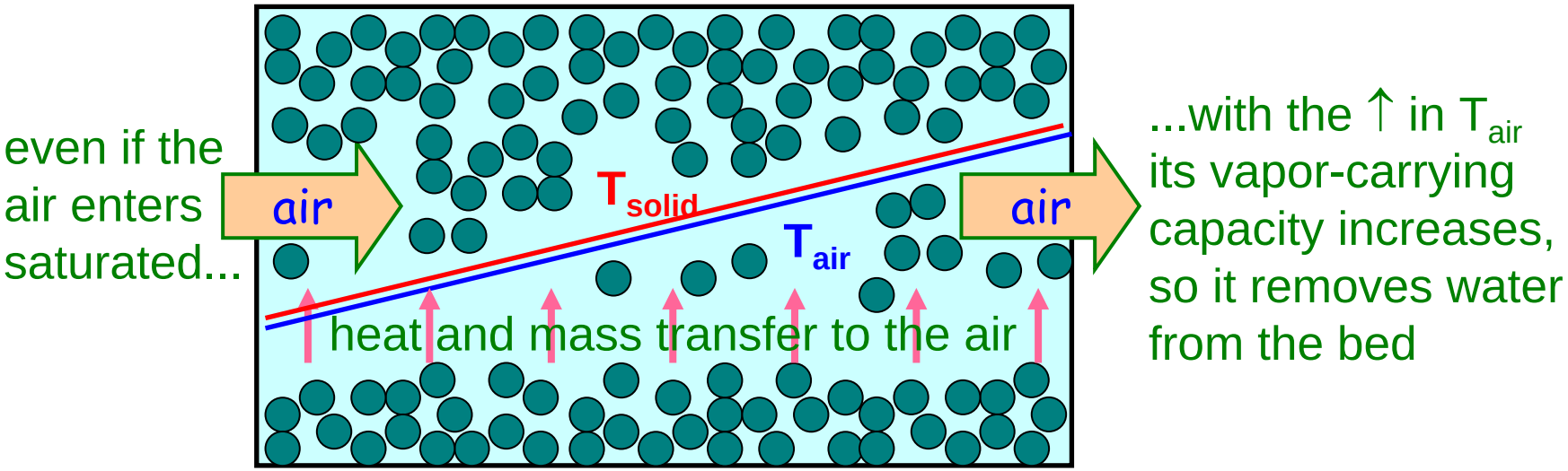


However, the bed volume decreases during growth and the properties of the particles change. We do not know how these changes influence solid mixing patterns.

What do models and experimental results tell us about packed-bed type bioreactors....and what would we still like to know?

We understand basic heat and mass transfer phenomena in packed beds reasonably well:

- temperature will increase with distance (this is normal for packed beds with exothermic reactions) and therefore the bed dries out

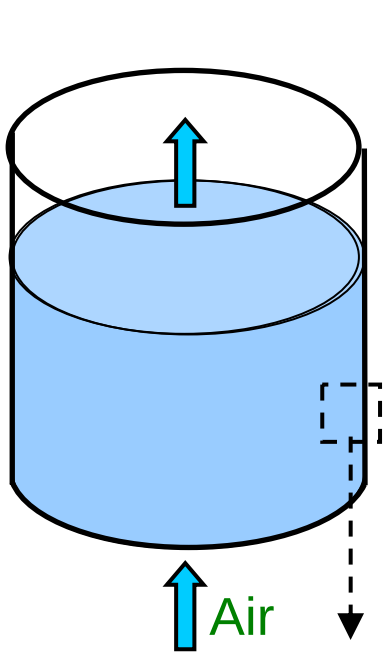


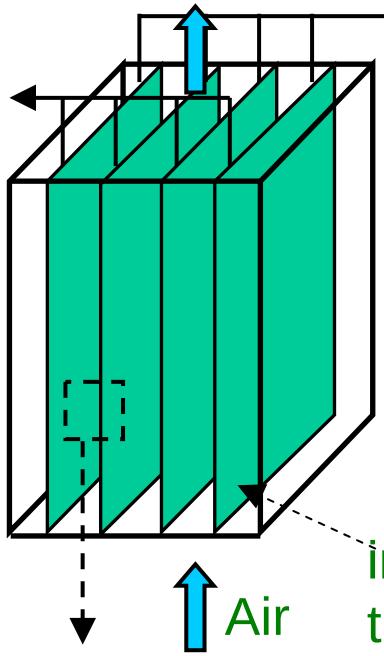
What do models and experimental results tell us about packed-bed type bioreactors....and what would we still like to know? (cont'd)

We know that internal heat transfer plates in the bed will help to minimize axial temperature gradients

traditional design (without internal heat transfer plates)

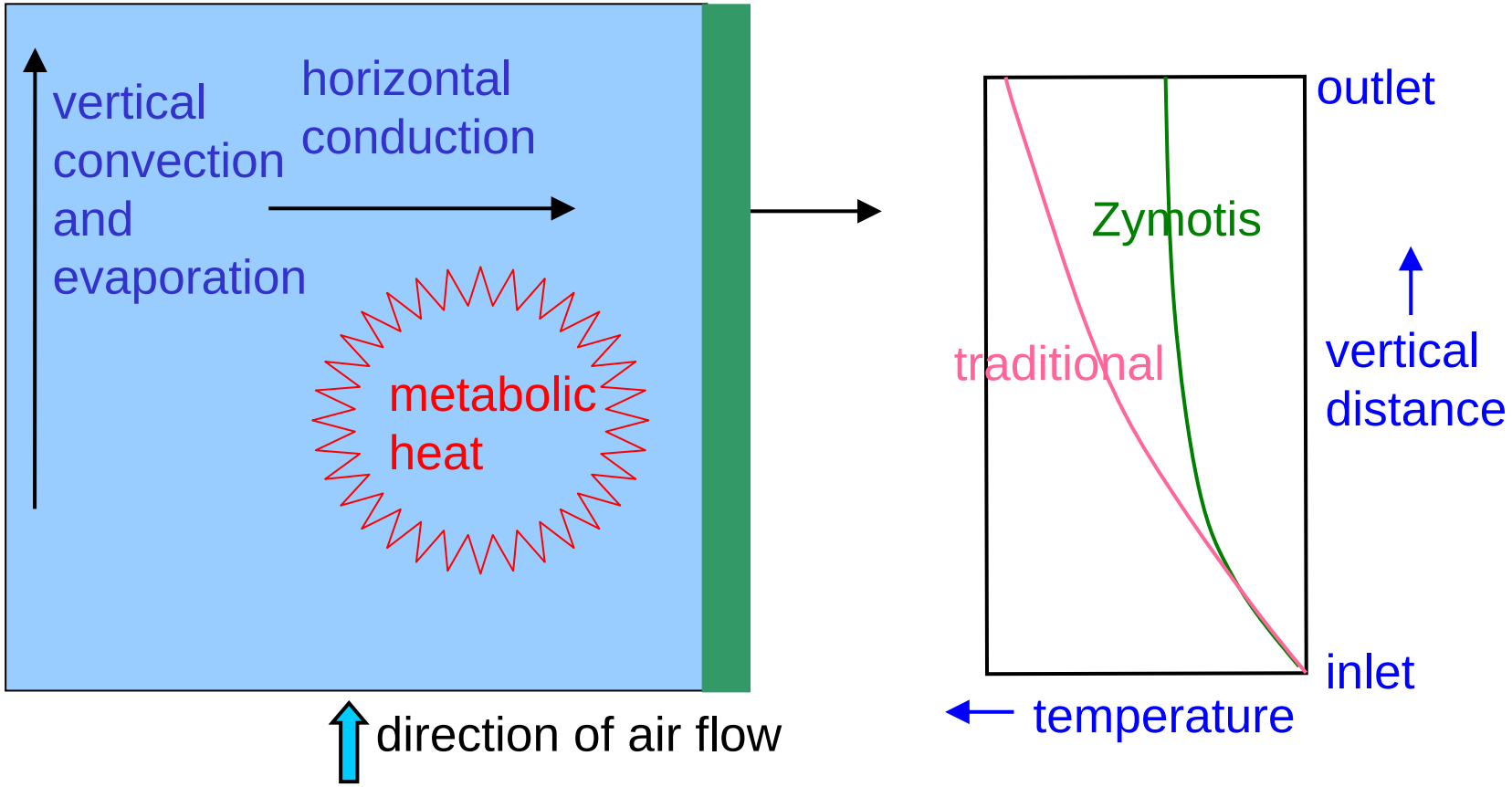
Zymotis type (with vertical internal heat transfer plates)





Conduction in the horizontal direction makes a relatively small contribution to heat removal

Conduction in the horizontal direction can contribute significantly to heat removal



The advantage of the Zymotis design is that it promotes horizontal heat transfer by conduction, reducing axial temperature gradients

What do models and experimental results tell us about packed-bed type bioreactors....and what would we still like to know? (cont'd)

However, we don't know much about the "dynamics" of particles in a static bed.

In what sense do we mean "dynamics" if we are talking about a static bed? The particle properties change drastically during the process!

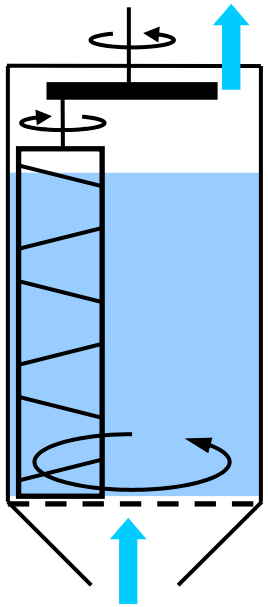
- the fungus knits particles together
- the bed dries and can shrink
- the pressure drop through the bed will increase

Although the presence of these phenomena has long been known, they are poorly characterized for beds in SSC bioreactors

What do models and experimental results tell us about "mixed and aerated" type bioreactors....and what would we still like to know? (1) Mixing of solids

We know something about mixing patterns in beds of solids and how these depend on agitator design - one group has used PEPT ("positron emission particle tracking) to track the motions of individual particles

If we are going to use intermittent mixing, then we have some idea about how frequently it will be necessary to mix the bed (basically the necessity of mixing is determined by the need to control the bed temperature)



What do models and experimental results tell us about "mixed and aerated" type bioreactors....and what would we still like to know? (cont'd)

However, studies to date about mixing have been limited – more needs to be done.

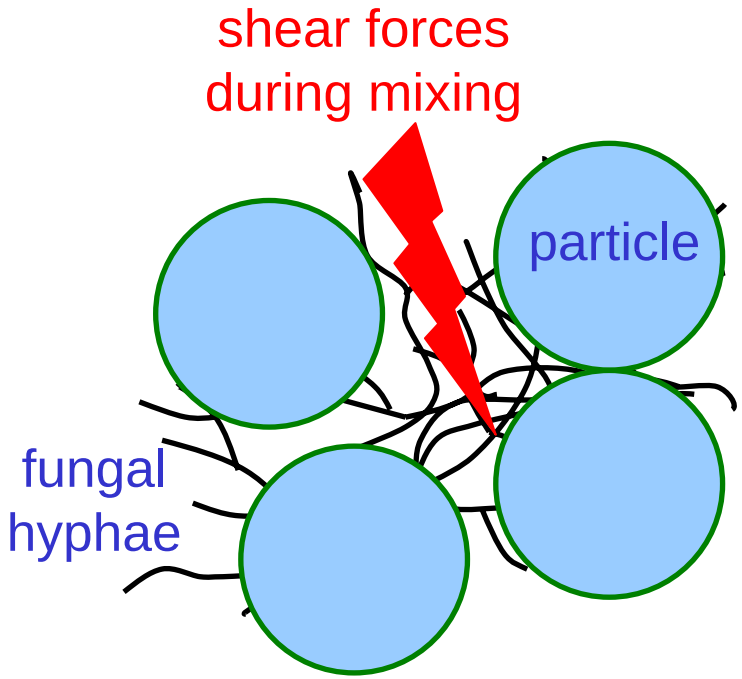
Also we do not know how the growth of the organism and the changes that it produces...

- knitting particles together (during static periods)
- particle shrinkage (due to consumption of polymers)
- change of surface properties (due to biomass)

...affect the effectiveness of mixing and of heat and mass transfer between the solid and gas phases in the bed

What do models and experimental results tell us about "mixed and aerated" type bioreactors....and what would we still like to know? (2) Effect of mixing on growth

We don't understand very well the effect of mixing on fungal hyphae – although it has been long recognized that mixing can break hyphae, these effect of this breakage on growth of the fungus is relatively poorly characterized.



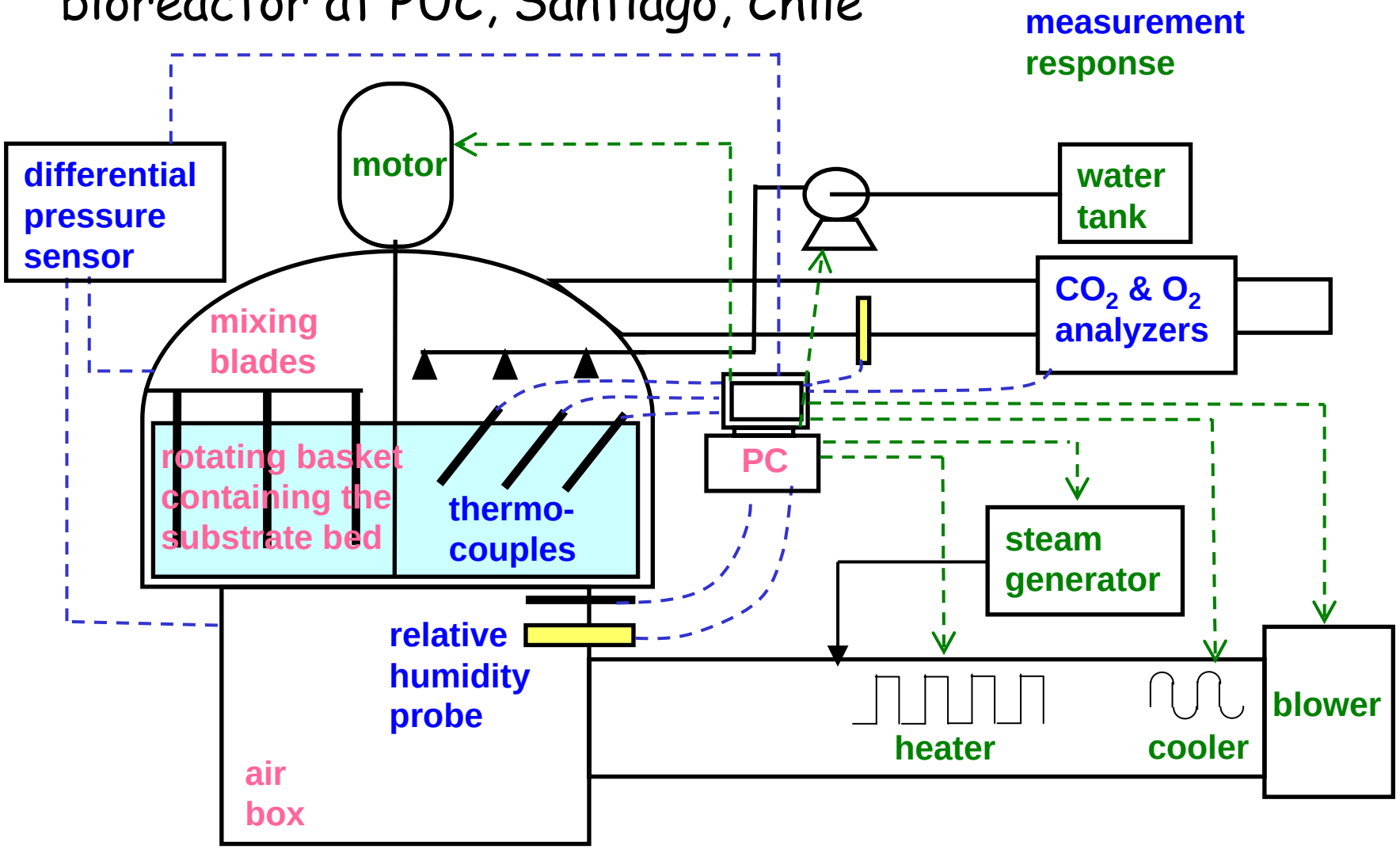
What do we know and what don't we know about how control of SSC bioreactors?

What do we know models and what don't we know about control of SSC bioreactors?

Firstly, remember that we need to supply O_2 to the microorganism and to try to control the temperature and water activity of the bed.

We know what we can measure and the operating responses that are feasible...

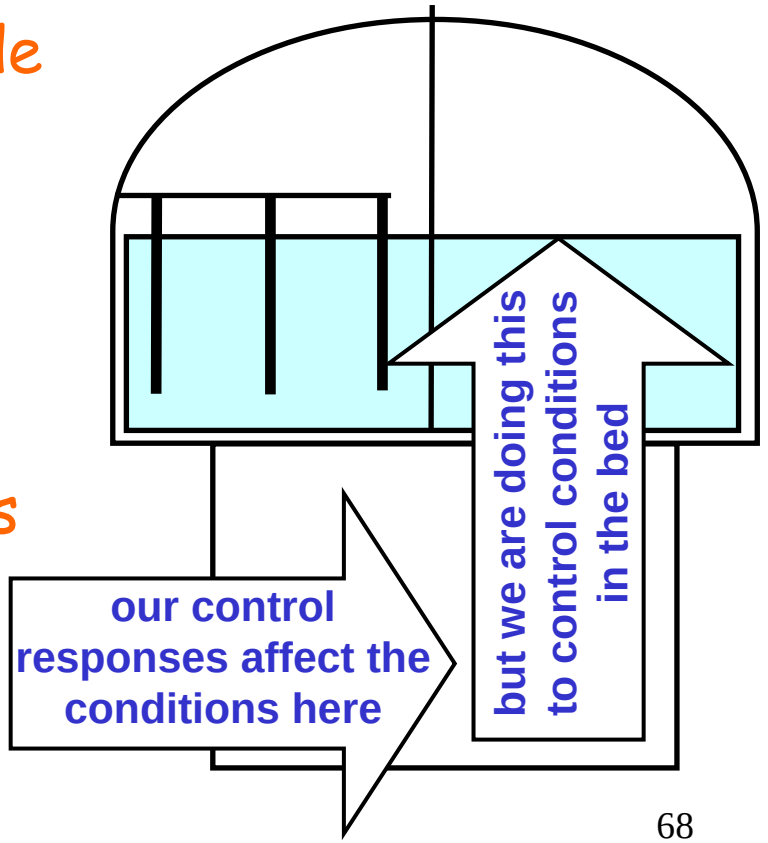
For example, the instrumented and controlled bioreactor at PUC, Santiago, Chile



What do we know models and what don't we know about control of SSC bioreactors? (cont'd)

However, control of SSF bioreactors brings various challenges to the application of control methods

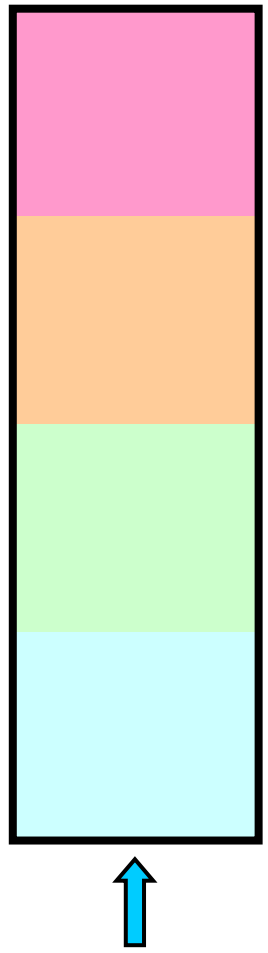
(1) we have a system of cascade control... we already have a control task in controlling the conditions of the inlet air (flow rate, T and RH)... but the control of the conditions of the inlet air is not an end in itself – our final aim is to control the conditions within the bed



Challenges to the application of control methods (cont'd)

(2) SSF bioreactor beds are inherently heterogeneous – this is a challenge because it raises questions of what is the best objective function if the value of the target variable varies across the bed

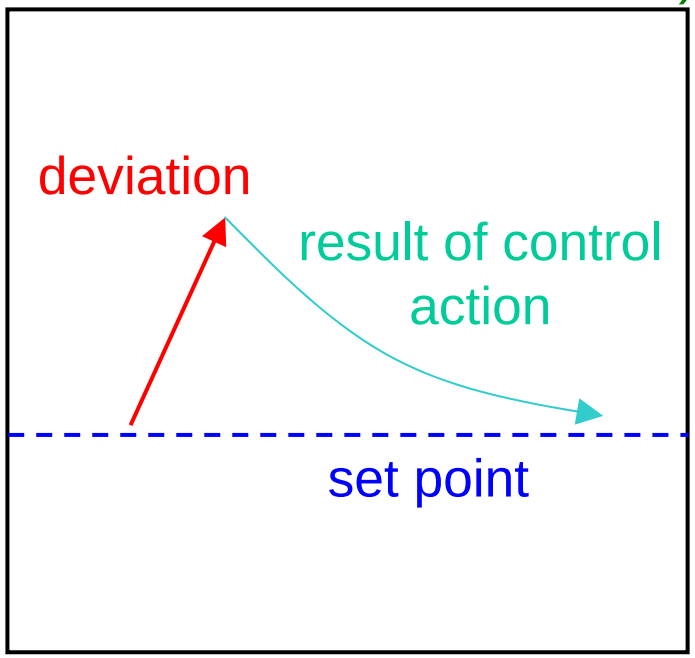
(the challenge is to minimize the AVERAGE deviations in TIME and in SPACE – in trying to avoid overly hot temperatures in one region, we must also avoid overly cold temperatures in other regions! – but this question has not been well investigated)



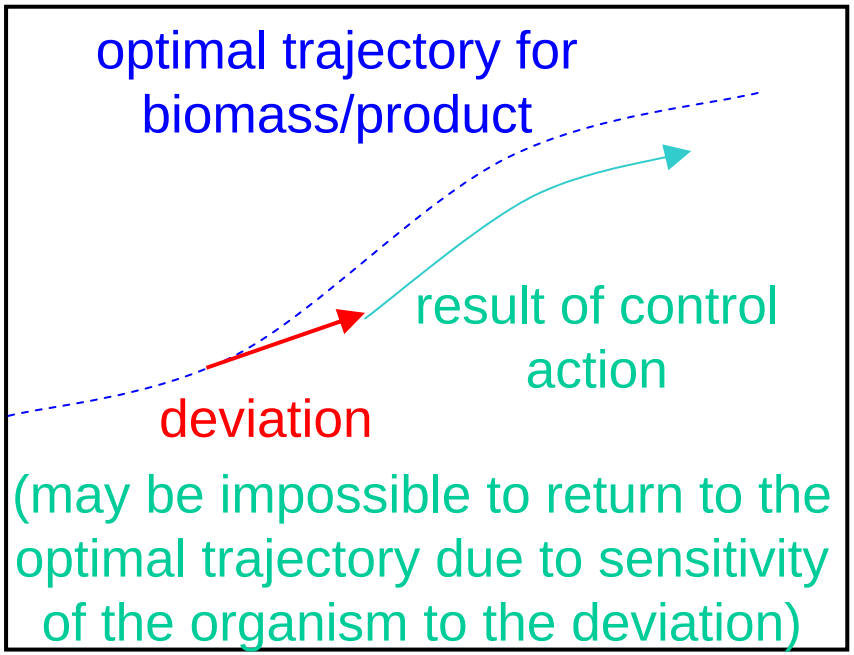
Challenges to the application of control methods (cont'd)

(3) We are not talking about set point control, but rather about optimal trajectories – this question has not been well explored in SSC bioreactors

Set point control (necessary for control of inlet air conditions)



Optimal trajectory control



Conclusions

- Solid-state cultivation should be considered as a cultivation method (in specific cases) for the production of microbial products
- However, there still remain many bioengineering challenges... I have mentioned only a few!