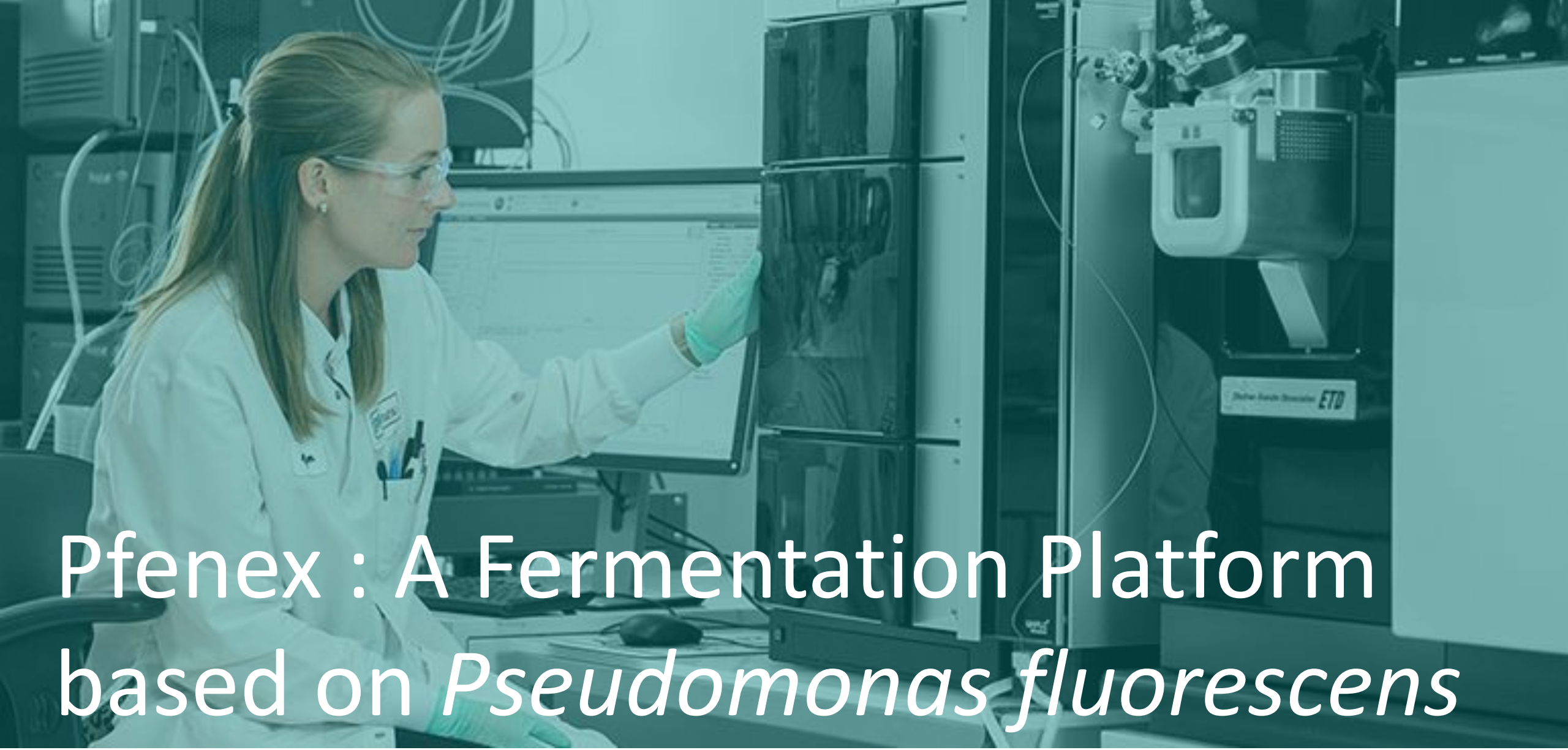

Pfenex : A Fermentation Platform based on *Pseudomonas fluorescens*

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Innovative Solutions for Global Health

Deisy Corredor, PhD. Upstream Group Leader
Global Bio-Production Summit – Feb 6th - 2018

Outline

- Fermentation Process Development
- Scale-Up / Down Pfenex Approach
- The Future in Upstream Development

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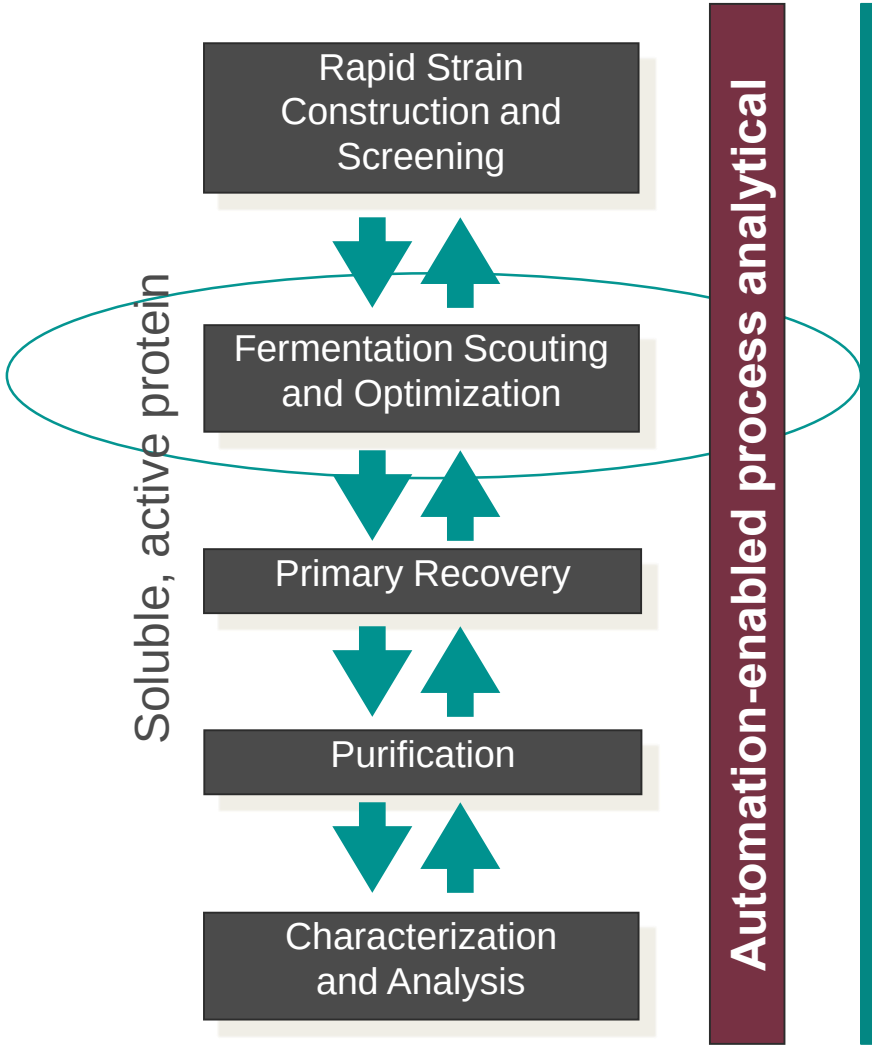
Pfenex – *Pseudomonas fluorescens* Production Platform

Extensive experience in protein expression including :

Novel enzymes, vaccines , therapeutic enzymes, engineered proteins (antibodies and fragments) .

120 protein expression programs for novel biologics , 80% expression success .



Strain Engineering
Thousands of strains- rapid cloning, periplasmic expression, 96-well screening

Process Analytical
Robotic sample processing, microchip SDS-CGE analysis and biolayer interferometry binding assays

Fermentation Development
Multiple strains each evaluated in multiple scalable fermentation processes

Protein Purification
Primary recovery and chromatography options evaluated in parallel microtiter plate format

Product Quality Analysis
Detailed characterization (QTOF MS, RP-HPLC, SEC, fluorescence); impurity analysis (HCP, DNA, LPS)

Fermentation Process Development

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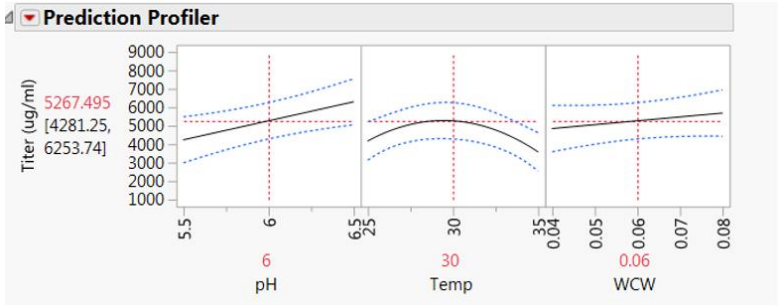
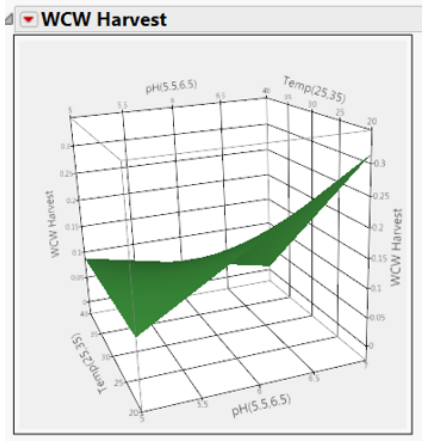
Upstream Process Development

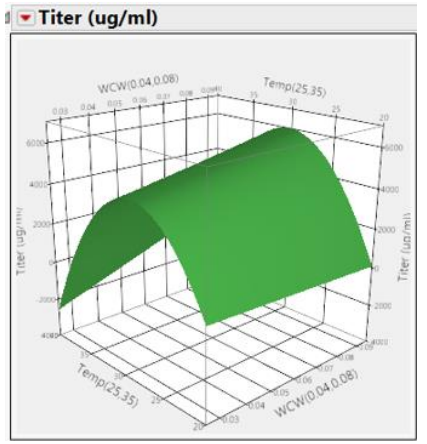
~ 200 fermentations

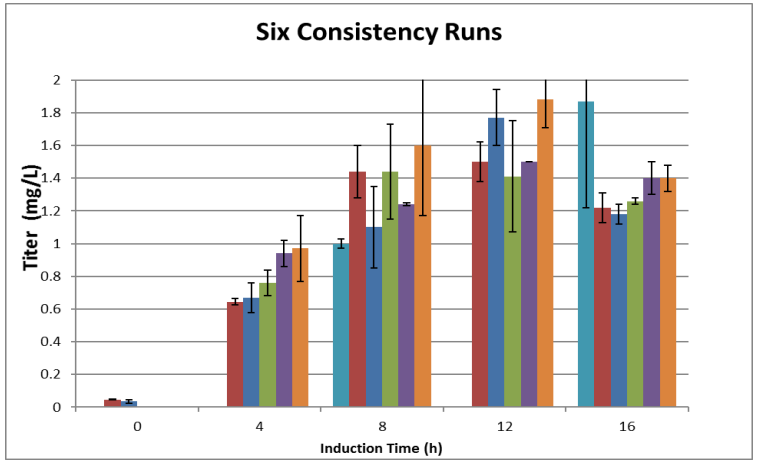
- Sets of experiments in 2L reactors aiming to screen several strains, find optimum fermentation conditions Temp , pH, WCW, Inducer Concentration.
- Pre-process characterization RSM design to model trends in 2 L reactors. Useful for further RA, PC and PPQ.
- Sets of experiments in 20L reactors , aiming to Scale-Up and find optimum Process Parameters: Pressure , Airflow and RPM.
- Consistency Runs in 20L or 75 L reactors to demonstrate reproducibility and / or deliver material. Batch Records from runs form the basis of TT to GMP.

6 – 8 months

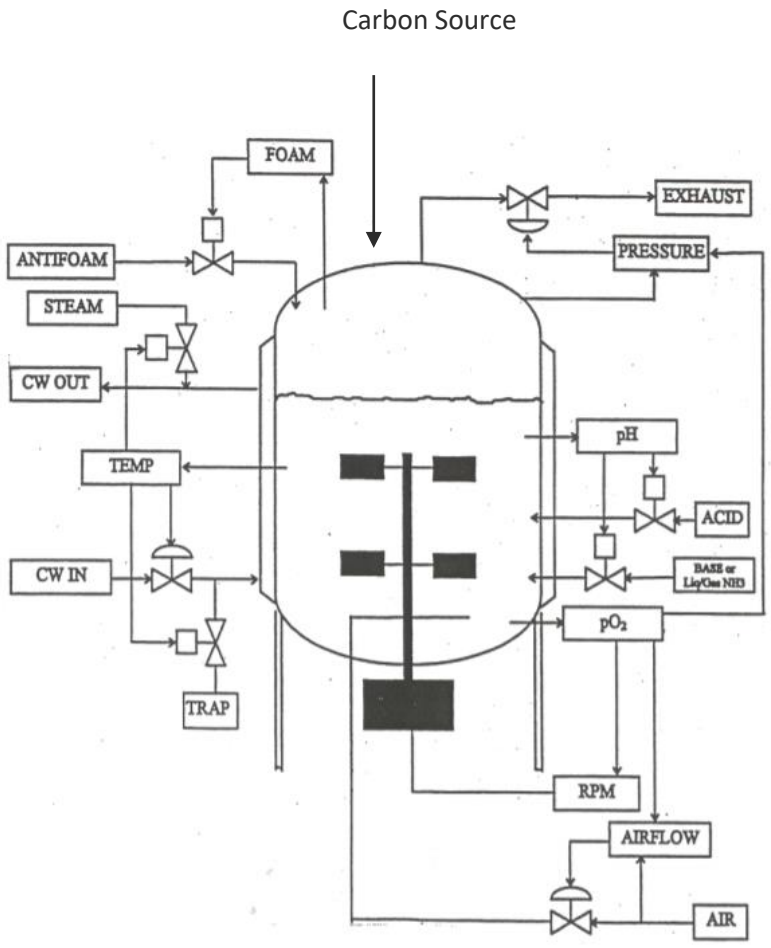








Pfenex - Fed-Batch / DO–Strategy Fermentation



- Low Nutrient Batch Medium present at inoculation of fermentor
- Concentrated limiting nutrient feed added during fermentation
- Culture harvested at termination of fermentation
- 10-50 X biomass / L increase over batch process is common. Cell densities increase by regulation of nutrients feed.
- Feed can be used to regulate growth rate , prevent accumulation of toxic products

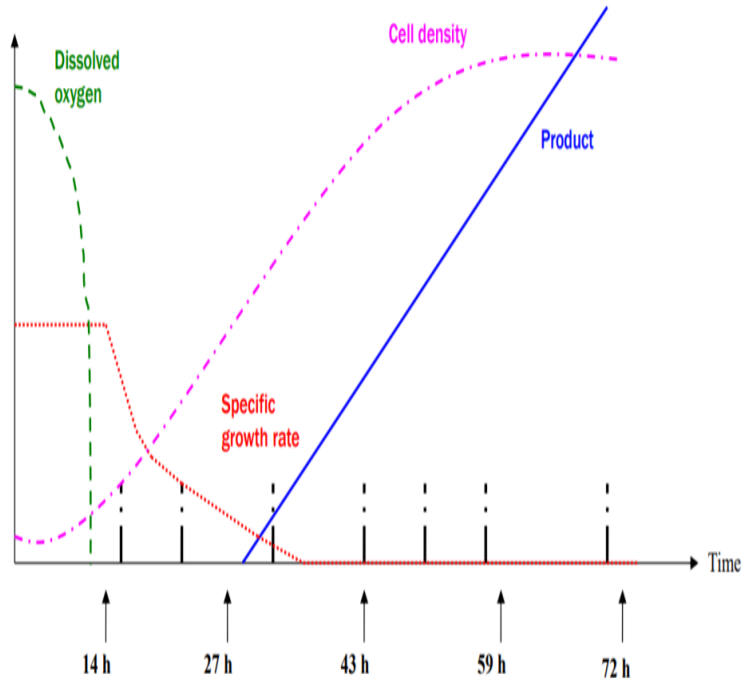
Advantages : DO-Stat Feeding vs Continuous Feeding

- DO-Stat strategy was initially developed back in the 70's *, to elaborately control feed addition as a pulse-bolus, using the abrupt increase of DO as an indicator for carbon depletion and trigger carbon source feeding.

- Principles of DO-Stat can be used / adapted and **applied to similar strains, independent of the product , empirical models of cell growth and cell growth rate, being versatile enough for low or high cell densities fermentations.**

- DO-Stat strategy is comparable across different scales since it is easier to implement without online monitoring of rate of carbon source depletion. Strategy is more representative when **"Scale Down" studies are necessary to facilitate and / or troubleshoot "Scale Up".**

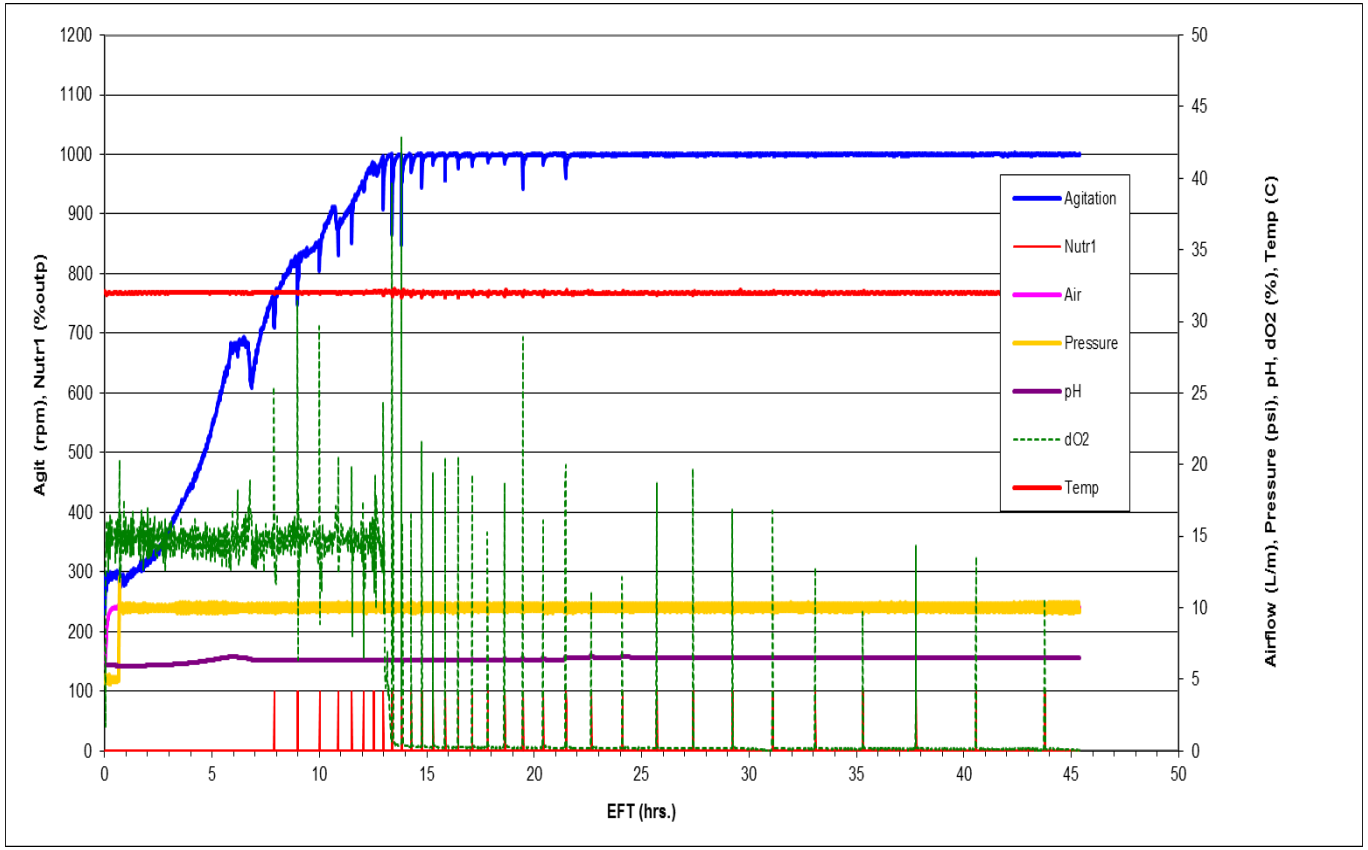
- Continuous feeding or exponential feeding needs **constant monitoring of carbon source consumption and / or PID tuning for linear or exponential variable feed . Sometimes requires online sensor monitoring.**



Applied Mechanics and Materials , 2014 , Vol 541 pP1198

*Mori, H., Yano., T., Kobayashi, T. and Shimizu, S. (1979). High density cultivation of biomass in fed-batch system with DO-stat. *Journal of Chemical Engineering of Japan,* 12(4), 313-319.

Pfenex - Representative Fermentation Profile



Literature Typical Fermentation Trend*

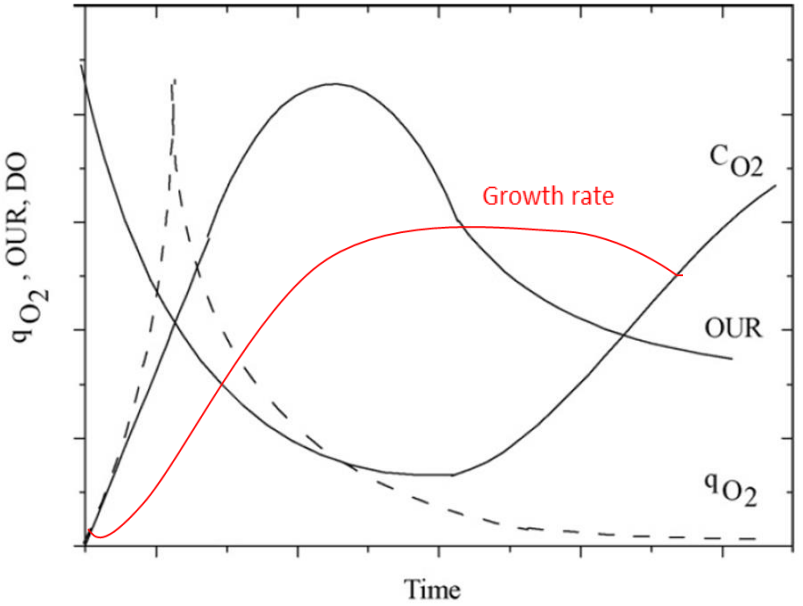


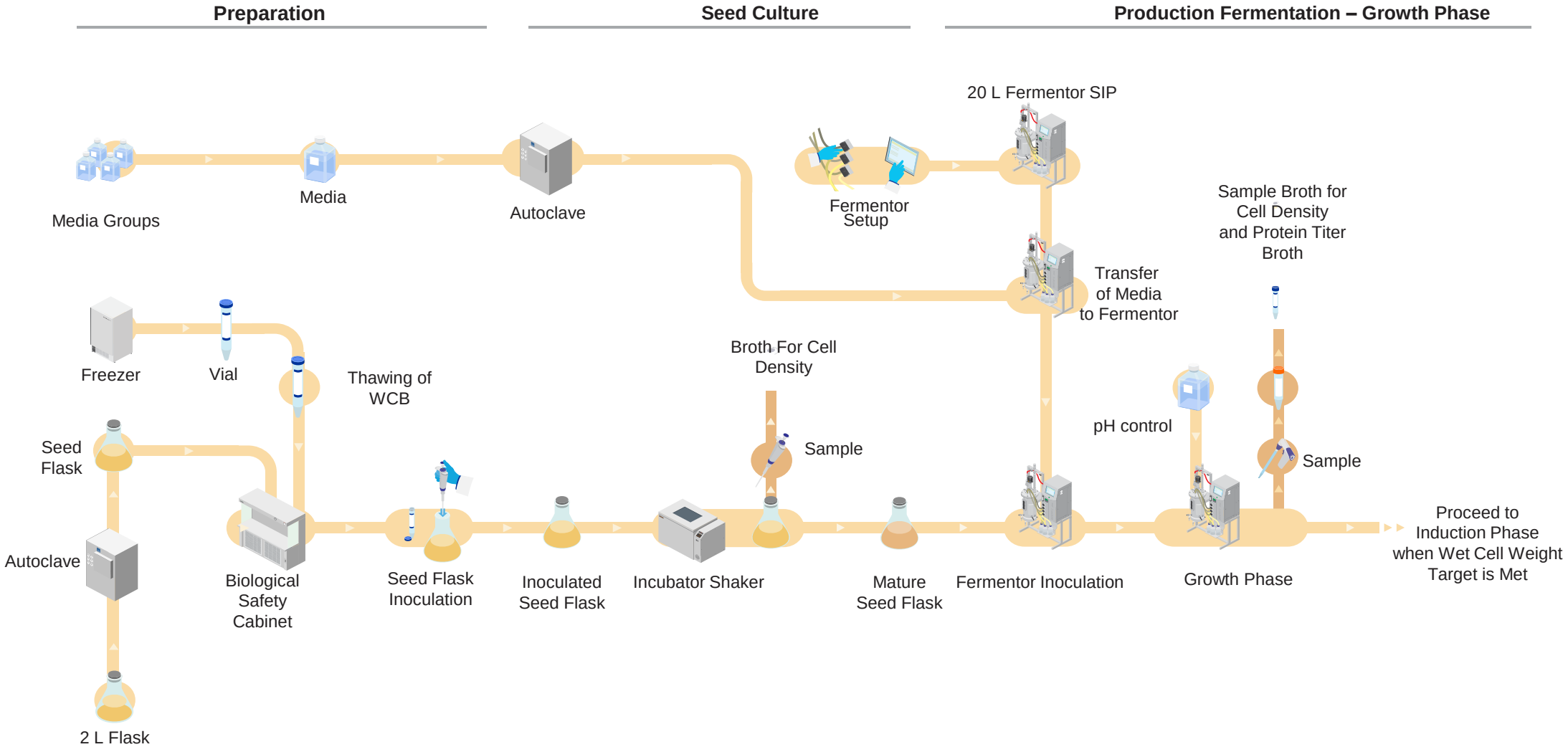
Fig. 4. Typical evolution of oxygen uptake rate (OUR), specific oxygen uptake rate (q_{O_2}) and DO concentration (C_{O_2}) in time course of fermentation.

Biochemical Engineering Journal , 49, (2010) 289

Biotechnology and Bioengineering 112(4) · April 2015

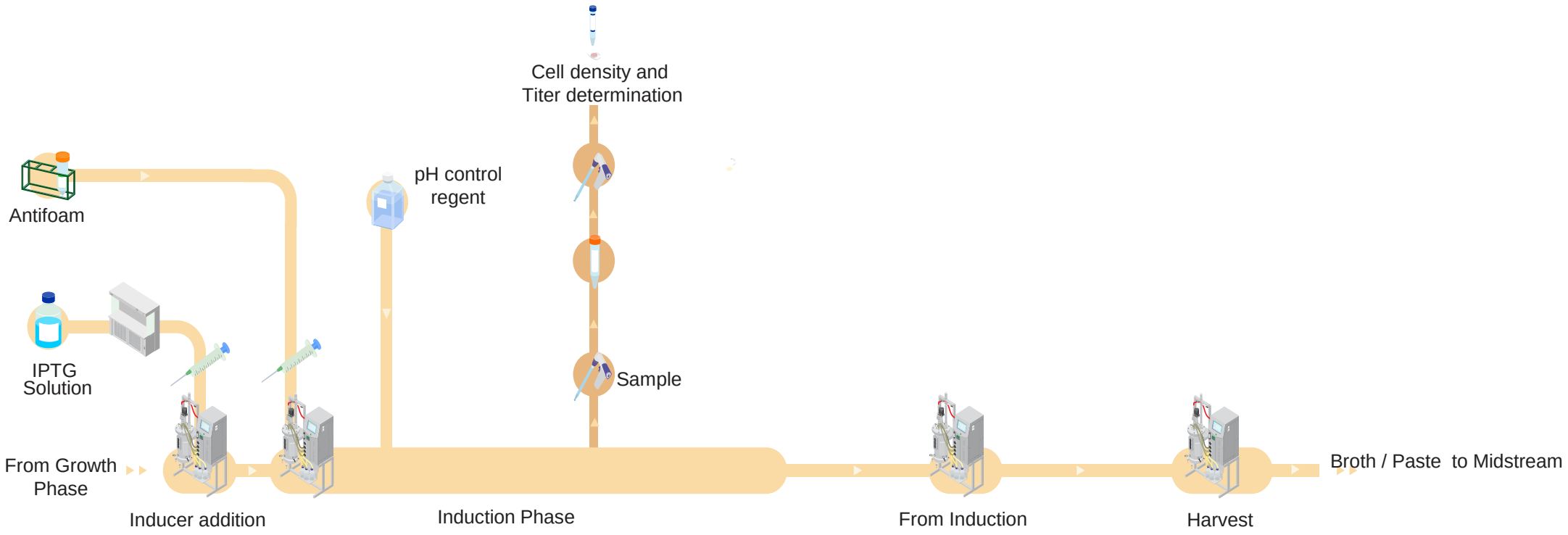
Pfenex fermentation strategy allows for sudden DO rise as an indicator for carbon depletion and exert greater control in a robust and reproducible fashion.

Pfenex – USP Process Flow Diagram



Process Flow Diagram…Cont.

Production Fermentation – Induction Phase | **Harvest**



Scale-Up / Scale-Down

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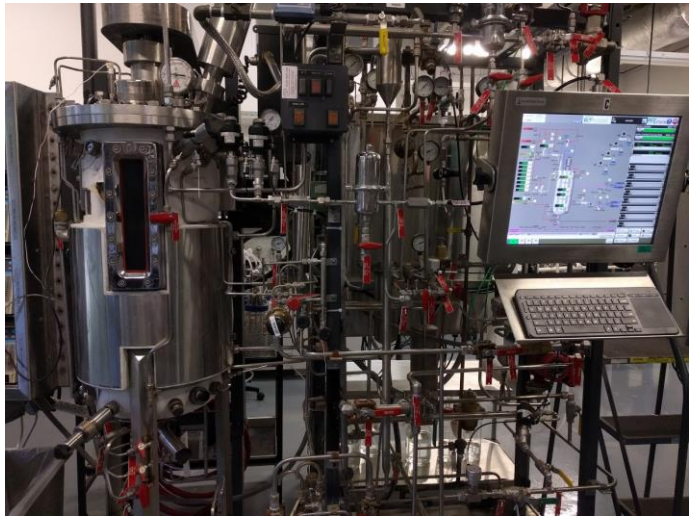
Bioreactor Scale-Up – Assessment

600 ml

2 L

20 L



75 L

1500L

A Shake Flask IS NOT equivalent to a Bioreactor

Scale-Up – “Rules of Thumb”

- Main parameters to be maintained constant are :
  - Mass transfer and mixing
    - Impeller tip speed (focus on surrogate for agitation shear)
    - Mean Power / volume (Pg/V) (Power Input / Unit Volume)
      - Agitator motor size can be a constraint
    - Impeller Reynolds (mass transfer)
    - Volumetric gas flow rate per unit of liquid (vvm)

  - Process Specifications to maintain constant
    - Reactor geometry (H/D) (Di / Dt)
    - Volumetric oxygen transfer Kla (Oxygen transfer)
    - Superficial Gas Velocity (aeration rate)
    - Mixing time (Mass transfer)
    - Specific Growth Rate

It is known that during Scale-Up of Fermentation Processes it is difficult to maintain all parameters constant, Pfenex has been successful transferring to CMO’s by focusing on the most important parameters during Scale-Up (Highlighted)

Mean Power Input / Unit Volume

- Depends significantly on type of impeller and Power Number (power consumption of an impeller).
- Choosing the proper impeller and number of impellers is necessary for proper Scale-Up.

Marine Impeller

- Axial flow pumping impeller reduced shear for given P/V relative to a Rushton impeller. Traditional use on CHO fermentations. Power Number varies 0.3 – 1.3 .

CD-6 Impeller

- Gas and immiscible liquid dispersion impeller. The CD-6 can handle about 2.4 times the maximum gas capacity of Rushton impeller. Power numbers as high as 2.1 – 3.2.

Rushton Impeller

- Simple radial flow pattern that moves material from the center of the vessel outward. It is most commonly used in reactor tanks, two phase mixing (liquid/gas), and any application requiring high shear , high mass transfer. Traditional used in microbial fermentations. Power numbers varies 5.4 – 6.

Power Number , P/V ratio and Scale-Up - Example

		Impeller Dia (m)	min rpm	max rpm	max RPS	N^3*D^5	Theoretical Np	Motor Power required (kW)	Max in Place (kW)	total motor required (kW)	Design	P/V (Power / final volume)	Comments
75L	CD6	0.150	100	925	15.4	0.278	3.2	0.85	1.49	1.40	Current 75 L	26	
	HE3	0.200	100	925	15.4	1.173	0.25	0.28					
400L	Proposed CD6	0.269	59	700	11.7	2.237	3.1	6.41	7.5	**6.55**	**Option A**	**26**	Similar P/V values without exceeding motor capcity.
	Current Axial	0.180	59	700	11.7	0.300	0.25	0.07					
400L	Current CD6	0.220	59	700	11.7	0.818	3.1	2.35	7.5	2.49	Current 400 L	10	Lower P/V than expected
	Current Axial	0.180	59	700	11.7	0.300	0.25	0.07					
400L	Proposed CD6	0.269	59	700	11.7	2.237	3.1	6.41	7.5	**10.00**	Option B	40	Exceed motor capcity. Working outside safety operation.
	Proposed HE3	0.345	59	700	11.7	7.761	0.25	1.79					

$$N_p = \frac{P}{\rho N^3 D^5}$$

$$\rho = liquid\ density\ \left(\frac{kg}{m^3}\right)$$
$$P = PowerConsumption\ (kW)$$
$$N = rotational\ speed\ \left(\frac{rev}{s}\right)$$
$$D = Diameter\ impeller\ (m)$$

Oxygen Transfer – Scale-Up Bio-Reactor

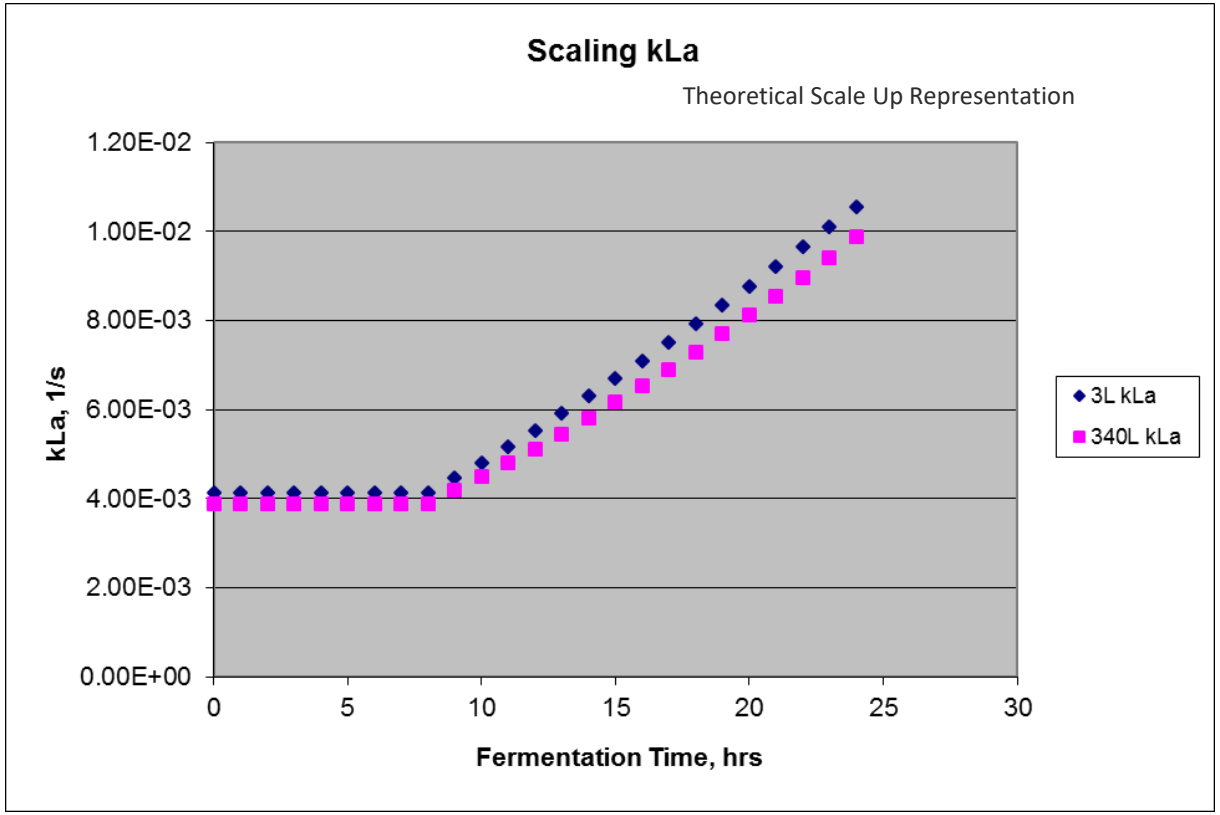
- $K_L a$ = Oxygen Transfer Rate / Mass Transfer Coefficient
  - A mass balance on DO gives:

$$\frac{dC_L}{dt} = OTR - OUR = K_L a(C_{sat} - C_L) - OUR$$

At steady state, the OUR can be estimated by:

$$OUR = K_L a\,(C_{sat} - C_L)$$

- *Csat* is saturated DO concentration (mg/l) (approx. 8 mg/l at 25 deg. C and 1 atm.)
- CL is the actual DO concentration in the liquid (mg/l)
- $K_L a$: Oxygen transfer rate coefficient



- Strategies to increase OTR :
  - Decrease growth rate , increase agitation rate, change impeller type, increase aeration rate, increase pressure, increase number and change placement of impellers.

The Future in Upstream Processing

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Bioreactors and Manufacture Discussion:

Bacterial Fermentation vs CHO Cell Culture & Single Use Bioreactors

	Bacteria (e.g *E.Coli – P. fluorescens*)	Mammalian Cells (e.g CHO)
Specific Growth Rate (1/h)	0.3 – 0.4	0.03
Duplication Time (h)	2-4 h	20- 24
Oxygen Mass Transfer (mM O2/L/h)	~ 200 - 300	~2 - 5
$K_L a$ Required (1/h)	>200	2 - 30
Bioreactors	SS – Stirred Tanks	Single Use - SS - Wave bags
Fermentation Strategy	Fed - Batch	Batch / Fed-Batch

Recent discussions in the industry to move towards single use reactors; however the type of reactor depends significantly on host organism requirements.

Is Continuous Manufacturing Processes an option?

Benefit	Upstream	Downstream	Scale Up and Regulatory
Smaller equipment and process foot print	**Sterility!!!**	**Hold times within Unit Operations**	**Requires flexibility in technology/facility**
Short process residence time	High productivity	**Require in-line and at-line sensors**	Real time assurance of product quality
Flexibility in Schedule (Rapid / Constant response to Demand)	Designed around a simplified process might require smaller equipment and/or single use technology	Designed around a simplified process might require smaller equipment and/or single use technology	**Lack of experience with new technologies**
Reduction in Capital Expense	**Controlling dilution rates to avoid washing**	*Functionally* closed systems	**Challenges to traditional regulation**
Increase Productivity (you may not need it that high)	**Challenging for non – growth associated products** $\frac{dP}{dt} = K_p X$ **Adding Complexity**	**Integration and process modeling for operation and control**	**Genetic instability – product degradation Continuous is sometime used for selecting mutants**

Recent discussions in the industry to move towards continuous processes; however technology is “not there yet” *.
Optionality in the process is key.

*Cooney, C et al; Biotechnology and Bioengineering , Vol 112, No. 4 April 2015

Conclusions

- Pfenex uses a systematic, science-based approach that utilizes unique aspects of our production platform. **Multiple strains , fermentation conditions and process parameters are evaluated in parallel and optimized at different scales** with robust and reliable models using Pfenex Process development.
- The DO-stat strategy works well for *P. fluorescens* defined media where nutrient depletion results in rapid DO rise. **DO stat strategy used in Pfenex fermentation development also allows process control in a robust and reproducible fashion**, and is a strong strategy for easy scale-up and transfer technology to different manufacturing capabilities.
- Key engineering considerations such as keeping P/V constant, impeller selection, engineering calculations and DOEs on key parameters are important for successful scale-up.
- Implementation of Carbon source feeding based on DO spikes leads to a robust/reproducible feed control across scales . Scale-Up/Down models are easily built based on the chosen feeding strategy and pre-process characterization models.
- New approaches (i.e continuous processes , single use technology) for microbial fermentation are still under discussion and need further understanding regarding new technologies and regulations.

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