Spring 5-23-2012

Microbial fermentation: New tools to speed-up vaccine antigen development and increase process knowledge

Catherine Jourdat
Sanofi Pasteur

Microbial fermentation: New tools to speed-up vaccine antigen development and increase process knowledge

Catherine Jourdat

Sanofi Pasteur

Bioprocess R&D Upstream platform

Vaccine technology IV May 23rd 2012,

Albufeira, Portugal

SANOFI PASTEUR

SANOFI PASTEUR :
The vaccines division of SANOFI group

- **World leader in vaccines**
  - 20 diseases
  - More than 1 billion doses/year
  - More than 500 million people vaccinated/year
  - 13 vaccines in development(1)

- **Nearly 13,000 employees(2)**

- **12 production/R&D sites in the world**
  - France (Marcy l'Etoile and Val de Reuil)
  - US (Swiftwater, Cambridge, Canton, Orlando and Rockville)
  - Canada (Toronto)
  - Argentina (Pilar)
  - China (Shenzhen)
  - India (Hyderabad)
  - Thailand (Chachoengsao)

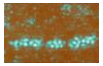
And 3 new facilities under construction: Mexico (Ocoyoacac), France (Neuville) and China (Shenzhen)

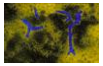
1. As of July 28, 2011, from phase I to "submitted"
2. FTEs as of December 2010 – Vaccines activities

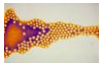
MARCY L'ETOILE, OUR VACCINES

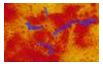
- The site manufactures vaccines against 11 of the 20 diseases that Sanofi Pasteur's vaccines protect against throughout the world

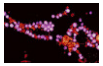
Viral diseases

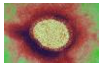

Yellow fever

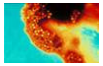

Mumps


Poliomyelitis


Measles


Rubella

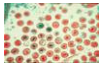

Influenza


Hepatitis A


Hepatitis B


Rabies


Japanese Encephalitis


Chickenpox

Bacterial diseases


Pertussis


Diphtheria


Haemophilus influenzae type b Infections


Meningococcal meningitis


Pneumococcal infections


Tetanus


Tuberculosis


Typhoid Fever

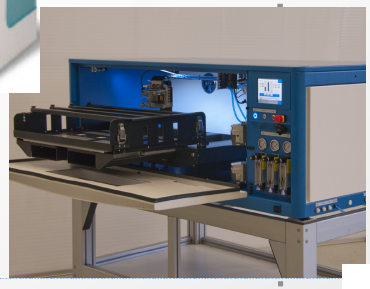

Cholera


Marcy l'Etoile also purifies immunoglobulins

SANOFI PASTEUR

Microbial fermentation

Innovative tools for high density aerobic and pathogenic strains



Standard stainless steel bioreactors 20-2000L

In-Process Analytic

High cell density aerobic strains & anaerobic strains

Anaerobic or low cell density aerobic strains

Small scale production

Process Development

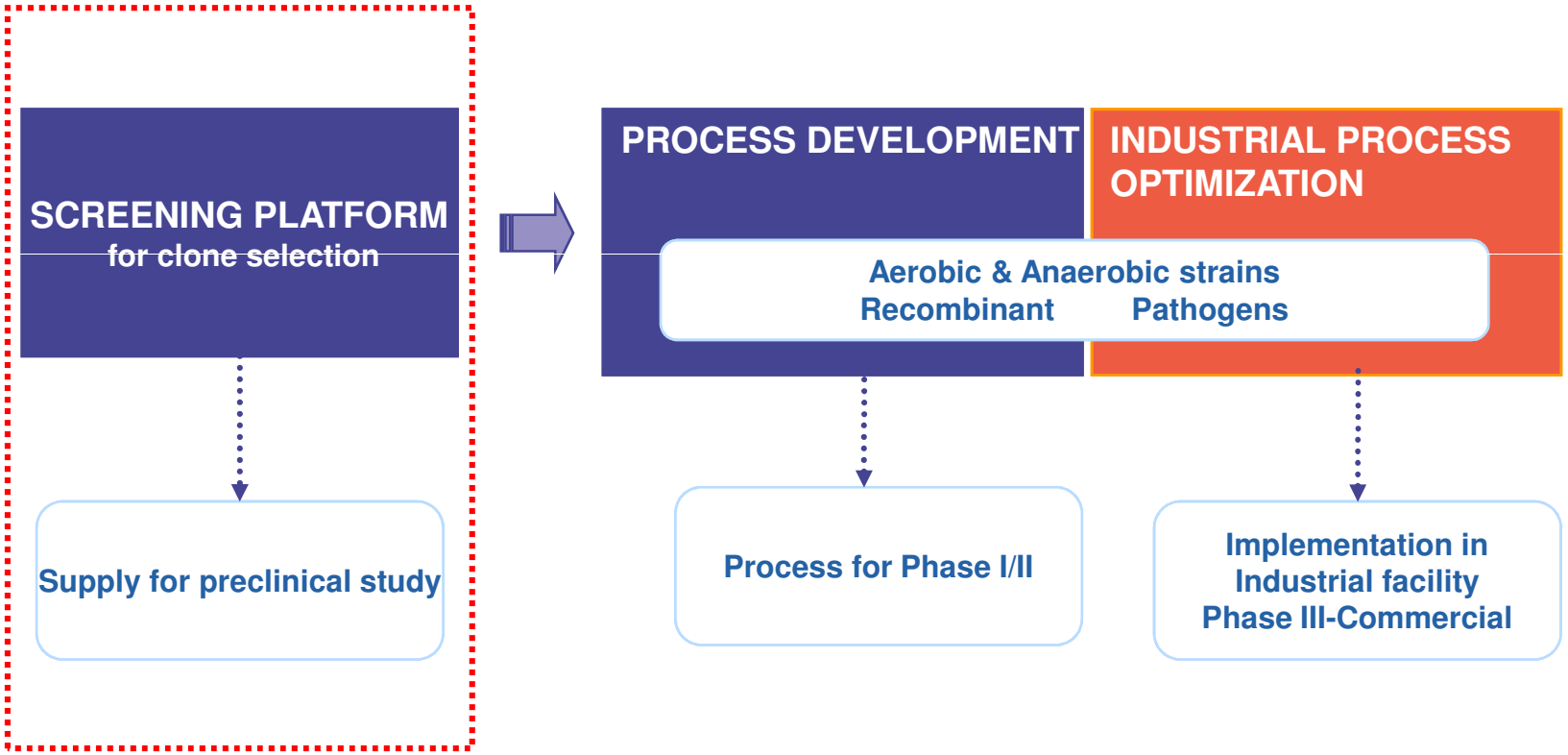
Scale-up

SANOFI PASTEUR

Microbial fermentation

Different sequential process screening/development platforms

- Tools architecture organized in each platform to speed-up strain selection, process development and optimization of industrial processes



Screening platform

µscale and disposable bioreactors for fast material supply

µscale bioreactors for clone selection

- ***Autoinducible media***
- ***Overnight culture***
- ***Criteria of clone selection:***
  - ***Final OD***
  - ***Antigen production***
- ***Seed lot production***

Expected protein quality & quantity

Efficient disposable system for microbial production

- ***Production at 1L or 10L scale***

2 weeks

Screening platform

Set-up of predictive µscale tools for difficult to express protein

Preliminary Strains Selection → **Culture condition selection** → **Preclinical material supply**

Commercial or in house customized media:
- Composition to favor soluble protein production
- Defined or complexe media
- Different final biomass
- Autoinduction
- Fed batch

-Shake flask in optimum conditions*
-µscale bioreactors for microbial system*

➢ ***Criteria of clone selection:***
Final OD
Antigen production
Soluble protein

→ **Strain selection based on its potential in different conditions**

→ **Set of data on strain behaviour**

****Tools optimized to be representative of 20-200L scale bioreactor performances - Based on kLa measurement in our culture conditions (media, temperature)***

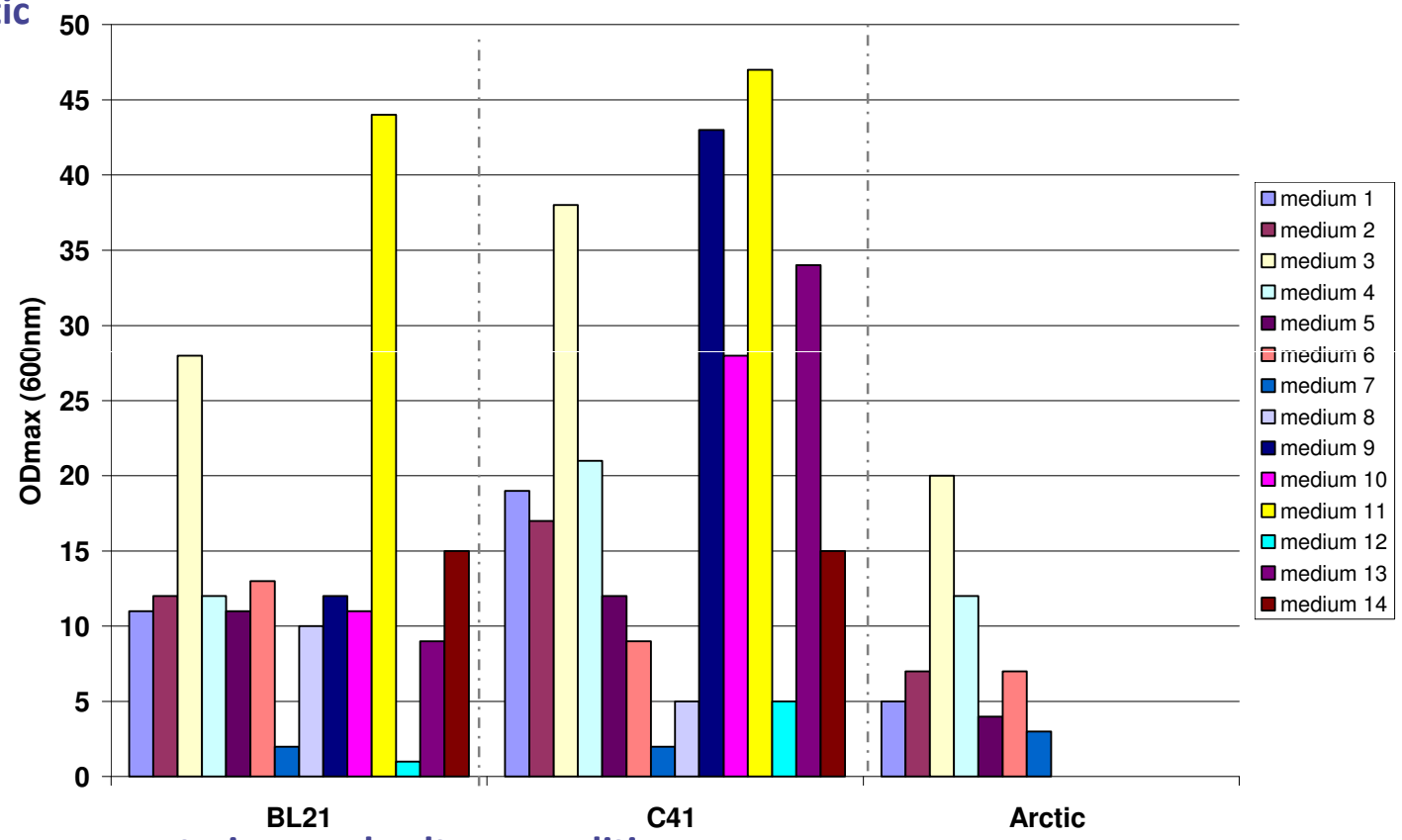
~8 weeks

Screening platform

Culture condition selection

Case study: *screening of different hosts/media in µ24 bioreactors*

- **Difficult to solubilize protein for vaccine antigen subunit**
- **Evaluation of different culture conditions, under induction, for pre-selected functional clones: *E. coli* BL21, C41 and Artic**



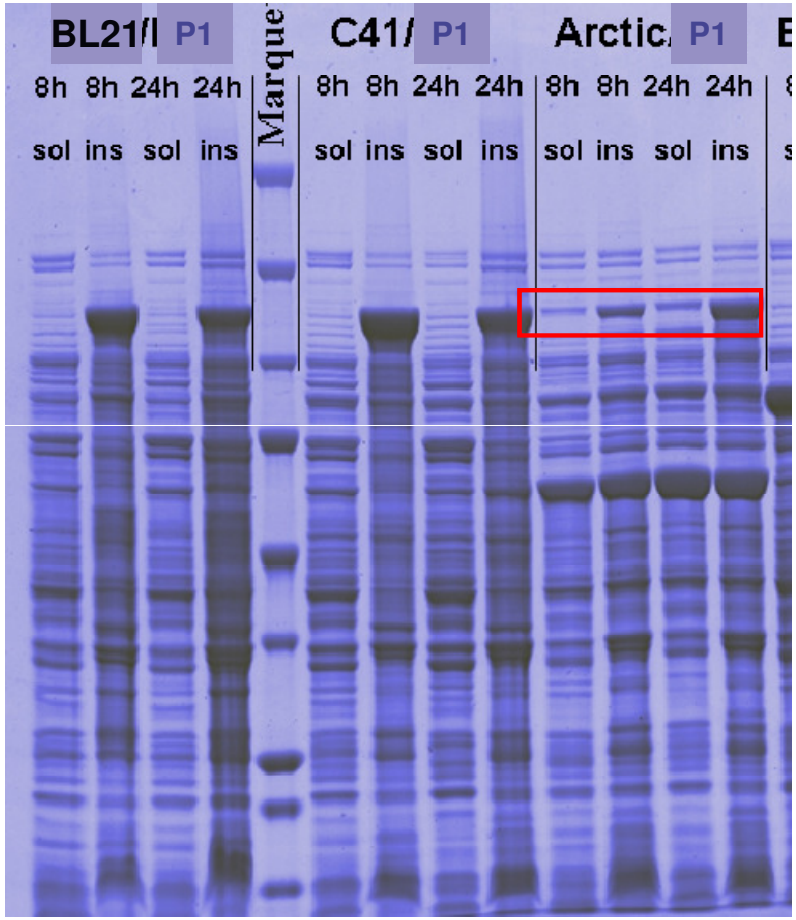
- **Differential behaviour among strains, and culture conditions**

Screening platform

Case study: screening of different hosts/media

Culture condition selection

- **Evaluation of production in the soluble and insoluble fractions for each condition**
- **Main results**
  - Soluble fraction for the protein P1 expressed in *E. coli* Arctic host in a specific medium
  - No soluble fraction with *E. coli* BL21 whatever the culture conditions
- **Next step: optimize best clone culture conditions to increase the protein production in the soluble fraction**

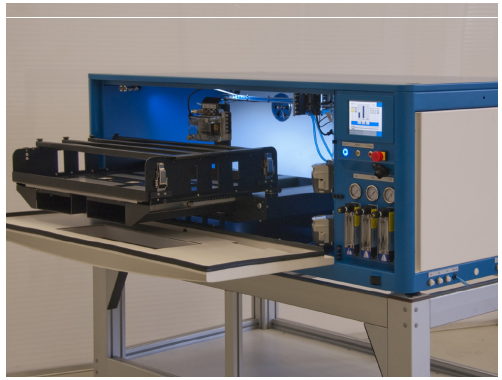


Screening platform

Preclinical material supply

Case study: Cell tainer system® evaluation

- **Initial process in stainless steel fermentor (30L scale)**
  - ***E.coli*** **strain, protein production induced with IPTG**
  - **Regulations:**
    - **pH: 7.00, 37 °C, DO: 30% (cascade: stirrer , air flow then oxygen enrichment)**
- **Evaluated system for fast preclinical production turn around: Cell tainer system**
  - **Flexibility (1 to 15L, batch or fed batch mode, optional on line glucose measurement (TRACE system)**
  - **Atypical agitation system allowing to reach high Kla values**



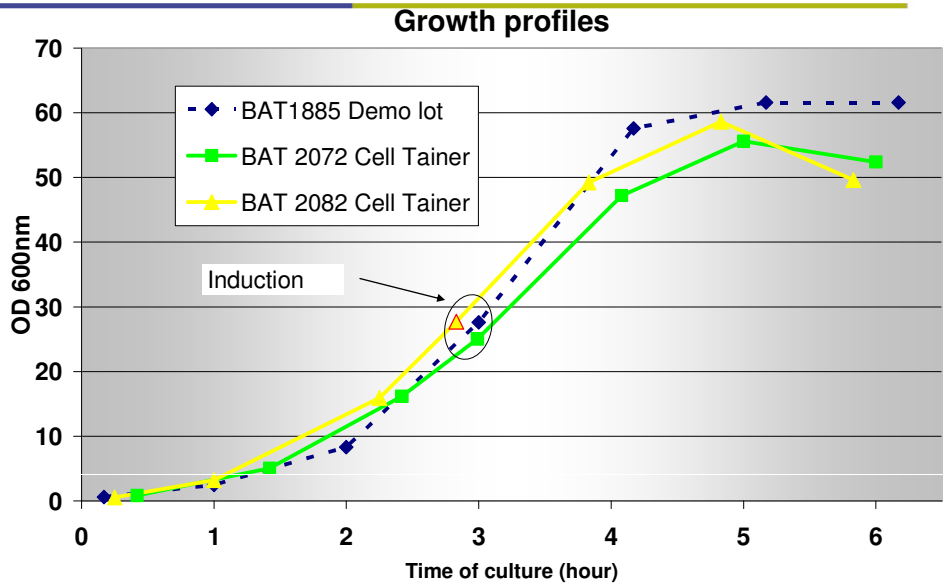
- **Preliminary run performed with working volume at 11 liters :**
  - **Foam in out air filter, Oxygen limitation**
- **Optimal settings identified via kLa measurement :**
  - **Working volume (7L), stirrer (45 rpm)**
  - **Addition of anti-foam before inoculation**
  - **Angle of agitation fixed at the maximum**

⇒ **Max kLa value: 300h^{-1}**

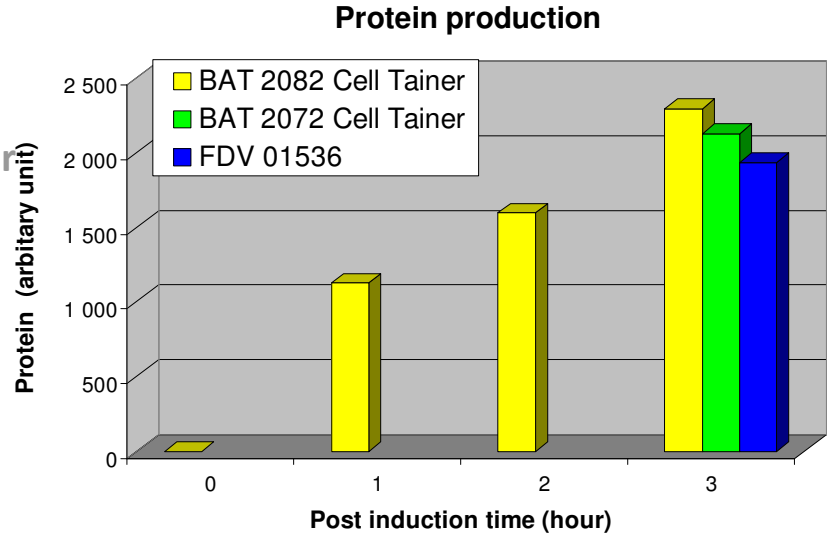
Batch mode process performance

Preclinical material supply

- **Growth kinetics with optimum settings**
  - similar growth and biomass between stainless steel reactor and cell tainer system



- **Protein production analysis by SDS – PAGE / Scan**
  - Comparable protein expression between Cell Tainer and stainless steel reference

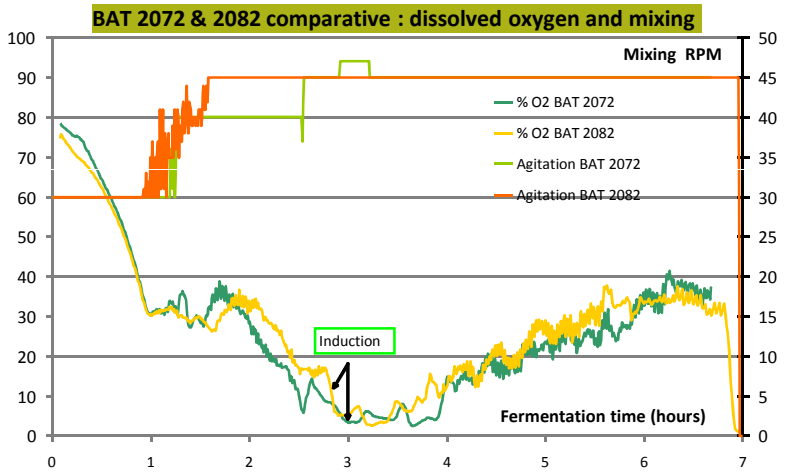


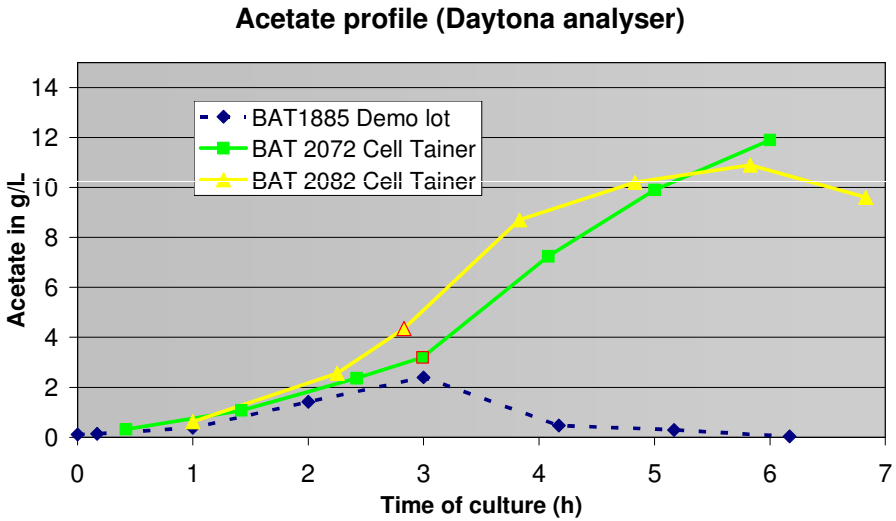
SANOFI PASTEUR

Batch mode process performance

Preclinical material supply

- **Process parameters and metabolites follow-up:**
  - Kinetic of glucose consumption identical to our standard process
  - No oxygen limitation but set point not maintained during the culture
  - High level of acetate production at the end of the culture





- **Fed batch process:**
  - Preliminary work shows than we can maintain DO above 20%
  - Acetate level is low (below 2.5 g/L)

Advantages / Perspectives

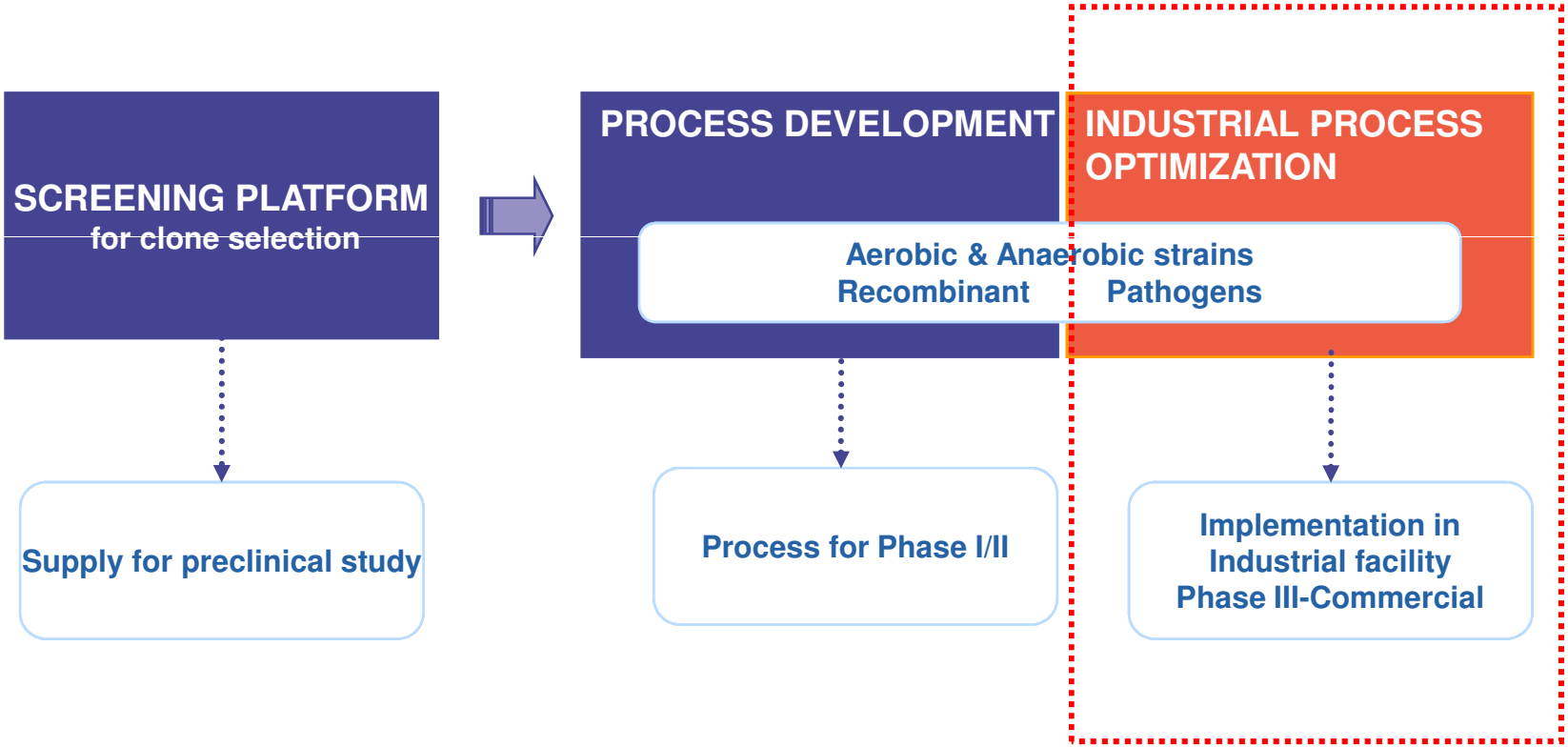
Preclinical material supply

- **First efficient *E.coli* batch process in a single use bioreactor**
  - Similar biomass (around 20g/L cdw) and protein production versus standard fermentor
  - Batch and fed batch processes capabilities
  - Good reproducibility
- **Advantages thanks to the high turn-around for process development and small scale batches**
- **However, system scalability limited for purposes beyond preclinical supplies, seed train, phase I GMP production**

Microbial fermentation

Different sequential process screening/development platforms

- Tools architecture organized in each platform to speed-up strain selection, process development and optimization of industrial processes

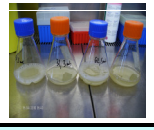


Industrial process optimization

Case study: µscale to industrial scale

- Aerobic pathogenic strain – Industrial process
- Aim of the study:
  - To model the impact of 3 process parameters (pH, DO, T) on biomass and antigen production and impurity level
  - To identify optimal process productivity conditions

Implementation of micro scale-down and DOE combination for process modelization and improvement of culture parameters for an industrial process



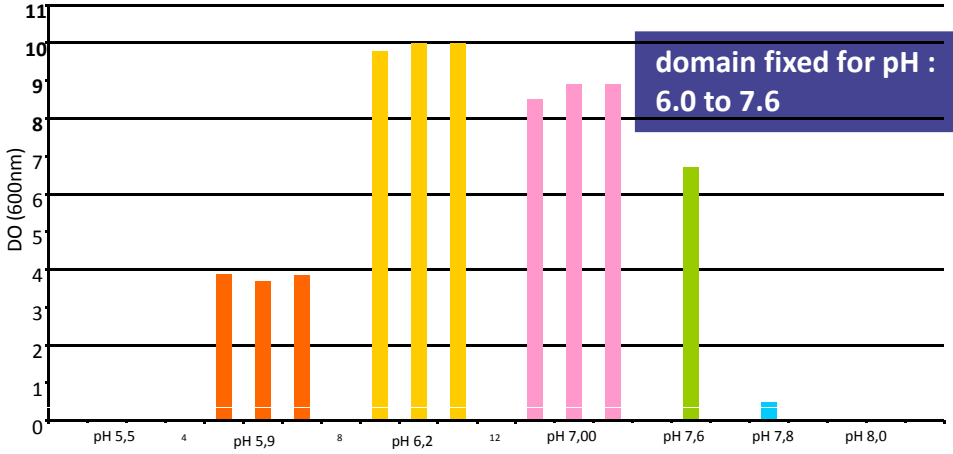

Scale down model tested	flask	Micro - fermentor	Fermentor 1L	Fermentor 30L	Reference Industrial Fermentor 1000L
Working volume	150 ml	3 ml	0.5L	30L	1000L
Capacity of fermentors available in the lab	No limit	24	4	2	/
Results Biomass (OD_{600nm})	7 9	9 - 10	9 -10	8 - 9	8 - 9
Results Product yield (g/L)	0.4	0.75	0.8	0.71	0.62

Experimental domain definition :

identification of limit values for each parameter investigated

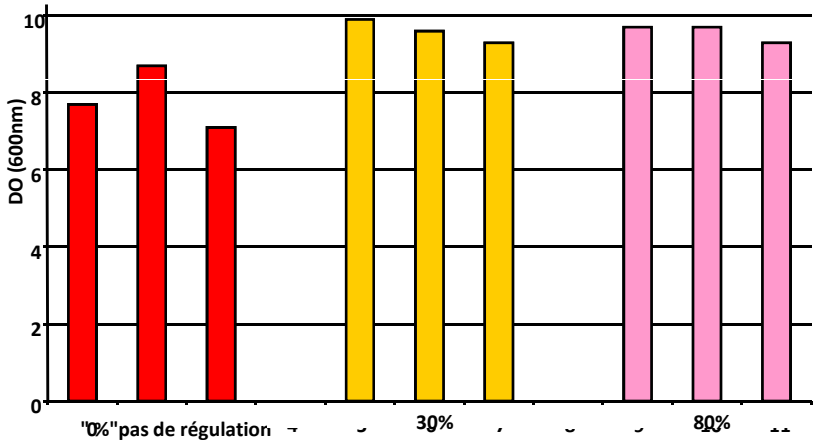
INDUSTRIAL PROCESS OPTIMIZATION

- **Effect of pH on biomass (T at 37°C , DO_2 at 30%)**

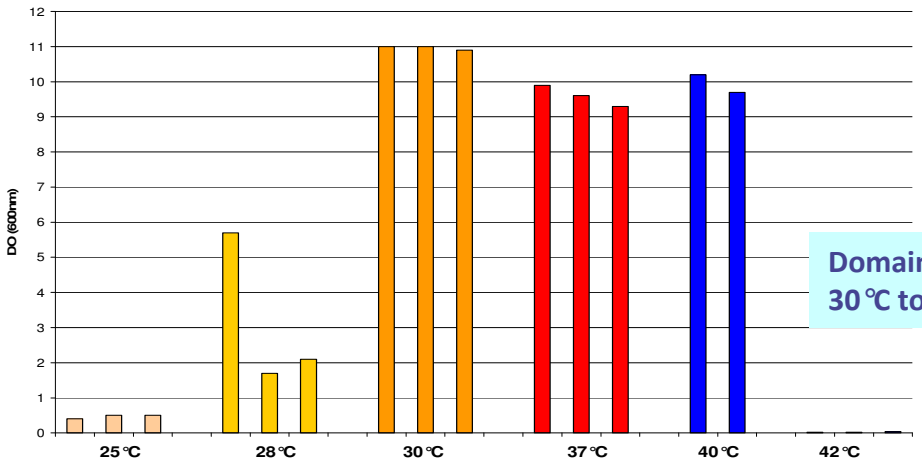


domain fixed for pH : 6.0 to 7.6

- **Effect of DO_2 on biomass (no pH regulation and T at 37°C)**



- **Effect of Temperature on biomass (no pH regulation, DO_2 at 30%)**



Domain fixed for T° : 30°C to 40°C

Domain fixed for DO_2 : 5% to 70%

SANOFI PASTEUR

DOE matrix selection for the study

N°Exp	température	pH	pO2
	°C	Unité	%
1	30.0	5.90	6.0
2	40.0	5.90	6.0
3	30.0	7.60	6.0
4	40.0	7.60	6.0
5	30.0	5.90	70.0
6	40.0	5.90	70.0
7	30.0	7.60	70.0
8	40.0	7.60	70.0
9	30.0	6.75	38.0
10	40.0	6.75	38.0
11	35.0	5.90	38.0
12	35.0	7.60	38.0
13	35.0	6.75	6.0
14	35.0	6.75	70.0
15	35.0	6.75	38.0
16	35.0	6.75	38.0
17	35.0	6.75	38.0
18	33.0	6.55	32.7
19	37.0	6.55	32.7
20	35.0	7.15	32.7
21	35.0	6.75	54.0

Response surface model (central composite matrix)

	Facteur	Unité	Centre	Pas de variation
U1	température	°C	35.0	5.0
U2	pH	Unité	6.75	0.85
U3	pO2	%	38.0	32.0

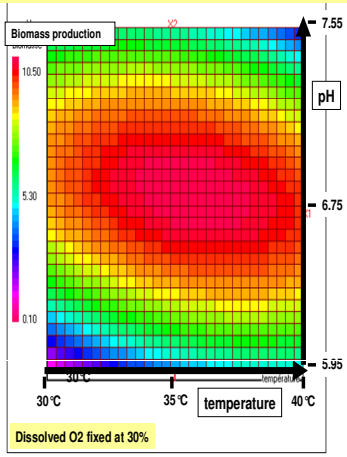
- Use of the software Nemrodw®
- Response surface model (central composite matrix)
- 21 cultures
  - 15 conditions for the DOE
  - 3 repetitions of central point
  - 4 test points to validate the model

DOE interpretation and modelization

INDUSTRIAL PROCESS OPTIMIZATION

First answer studied : biomass production

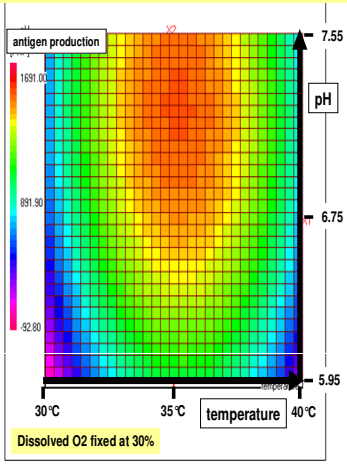
Modelling of the biomass in function of pH and temperature values



Significant effects identified :
- pH (+++)
- Interaction pH / DO_2 (++)
- Interaction temperature / pH (+)
- DO_2 (+)

Second answer studied : antigen production

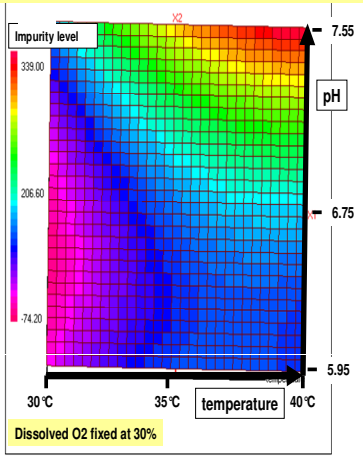
Modelling of the antigen production in function of pH and temperature values



Significant effects identified :
- temperature (+++)
- pH (++)
- Interaction DO_2 / pH (+)
- DO_2 (+)

Third answer studied : impurity production

Modelling of the impurity level in function of pH and temperature values



Significant effects identified :
- pH (+++)
- Interaction DO_2 / temperature (+)

- **Effect of the 3 parameters investigated on the 3 responses (single effect or interaction)**
- **But no condition allowing at the same time to maximize antigen production and minimize the impurity quantity**

SANOFI PASTEUR

DOE interpretation and modelisation

INDUSTRIAL PROCESS OPTIMIZATION

- **Modelisation of the phenomena investigated in the experimental domain**

- **Compromise :**

  Identification of conditions allowing at the same time the increase of the antigen production and the maintain/control impurity level

Implementation of results obtained through the DOE

INDUSTRIAL PROCESS OPTIMIZATION

- **Testing of new settings at intermediary scales (fermentors 1liter and 100 liters) then at industrial scale (fermentor 1000 liters)**

Confirmation of results obtained with DOE at micro scale :

increase of antigen production confirmed up to industrial scale

Settings	Initial	DOE predictions with new settings
Fermentor Scale	Fermentor 1L	
OD max (600nm	11	12
Product yield (g/L)	0.8	1.23
Impurity Level (UI/ml)	900 000	800 000

Conclusion

- **Technological evolution in the single use domain**
  - For microbial fermentation new single-use and µscale bioreactors have been recently developed than can deal with the high oxygen demand
- **Benefits of the single use and µscale bioreactors implementation**
  - Conduce fast expression system screening to select best host, clone, media, vector for antigen production at the expected quality and quantity
  - Generate predictible results at industrial scale thanks to parameters regulation
  - Increase process knowledge and robustness through DoE with limited workload
  - Reduced time of process development trough downscale, multiple parallel bioreactors
  - Ressource allocated to core expertise instead of non value tasks (cleaning, bioreactor decontamination)
  - Lead significant increase of the antigen production for two industrial processes
- **Next steps**
  - Implementation of highthrough-put sample treatment,
  - Automation of the different steps (culture, sampling, sample treatment, analytics),
  - Data management

Acknowledgement

Sandy DEMAY
Nicolas CHAUDET

Grégoire Exbrayat
Bénédicte Leroy
Nathaelle Luçon
Didier Roze
Nadine Venet

Jean Marc GUILLAUME –Head of Bioprocess R&D USP platform

KITE
Knowledge & Innovation for Technology Excellence

Thank you!
Questions ?

SANOFI PASTEUR