Proteins Purification Solutions

From Innovation to Industrial Implementation

Conference Adebiotech – 29/10/13

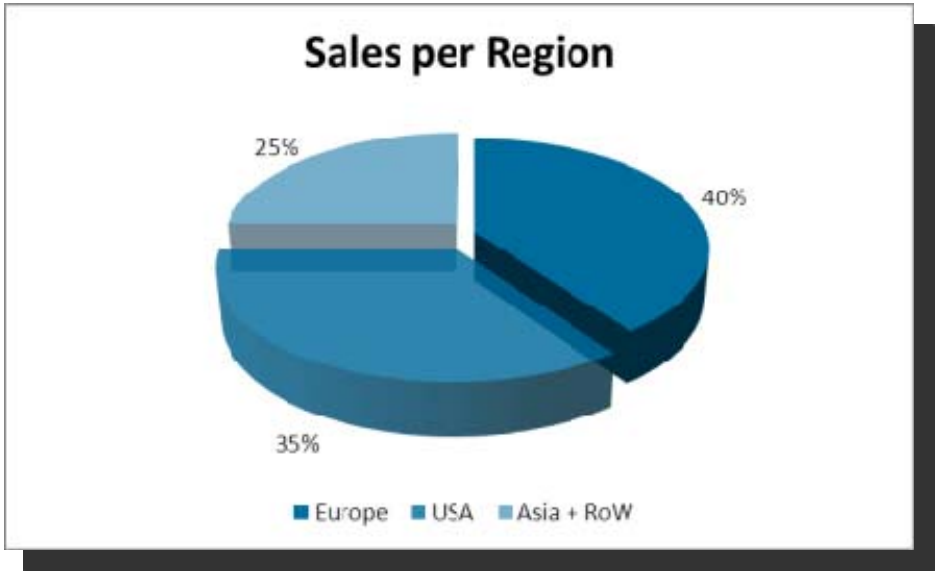
Frédéric Schab
R&D Project Manager

Novasep Group Today

300 M€ turnover

1200 people, 200 in R&D

13 sites



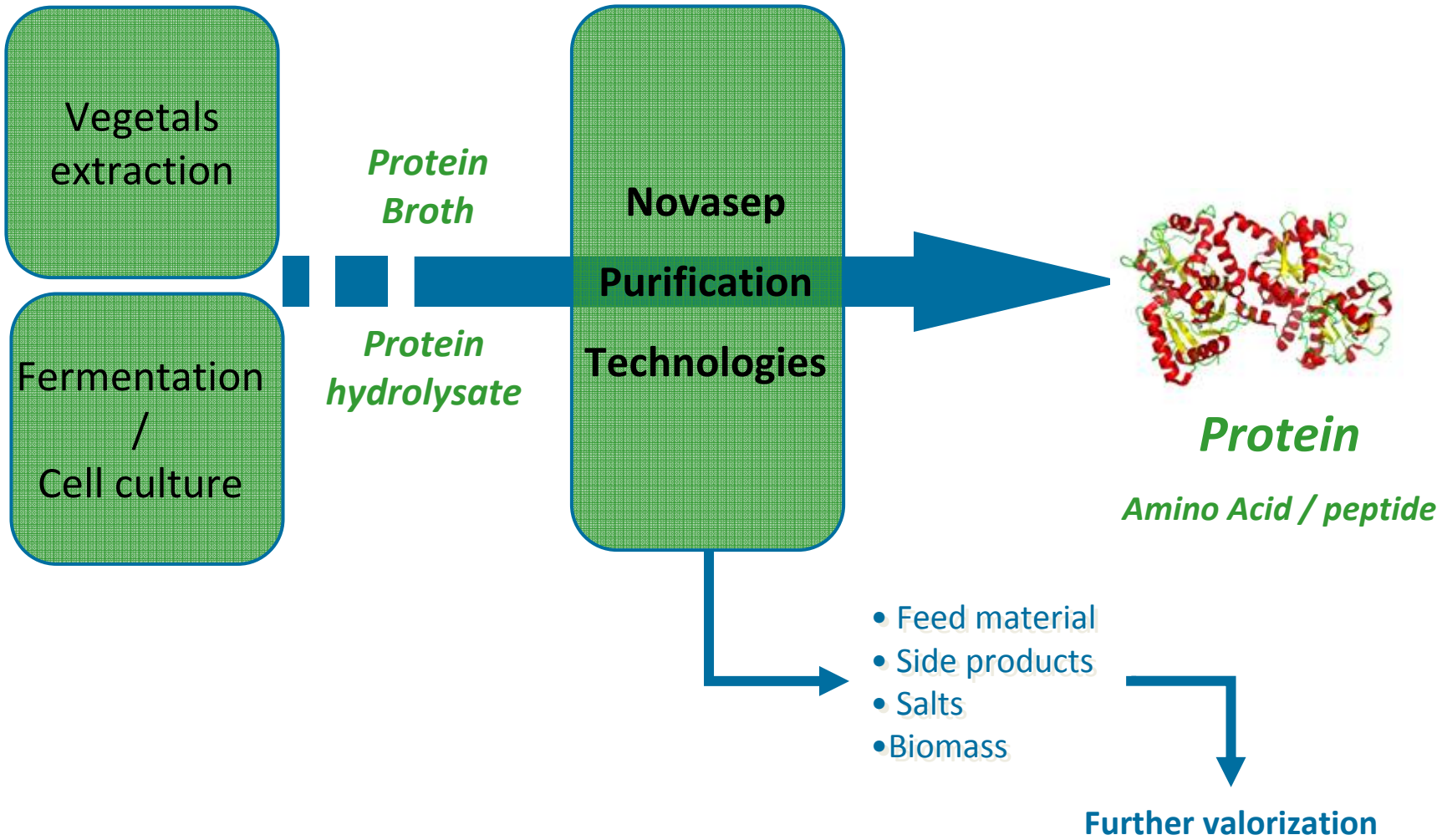
Over **650 customers** served worldwide

Over **100 R&D projects** per year

Over **100 different active molecules** produced per year

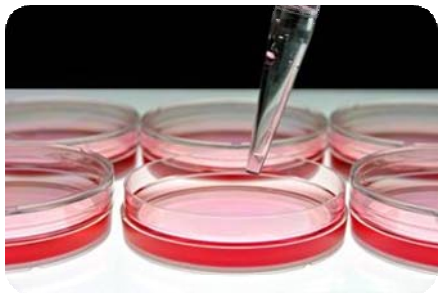
Over **2,000 purification systems** installed worldwide

Pure Proteins from Complex Mixtures



Industrial Biotech at Novasep

Biopharma



Blood Fractions
mAbs
r-Proteins
Vaccines
Cell Therapy

Food & Functional Ingredients

Sucrose
Starch and derivatives
Milk
Polyphenols
Anthocyanes
Proteins extract

Bio-Industries

Bio Based Chemicals
Organic Acids
Aminoacids/proteins
Antibiotics
Vitamins

Industrial Biotech at Novasep

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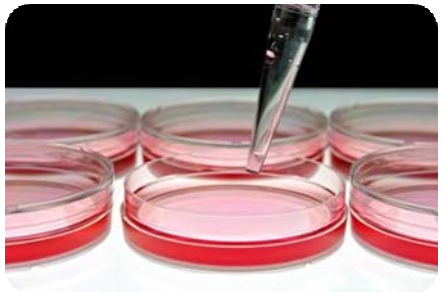


Blood Fractions
mAbs
r-Proteins
Vaccines
Cell Therapy

Low capacity / high added value

Environment : Single-use TFF, cGMP production, low to high pressure chromatography…

Food & Functional Ingredients

Sucrose
Starch and derivatives
Milk
Polyphenols
Anthocyanes
Proteins extract

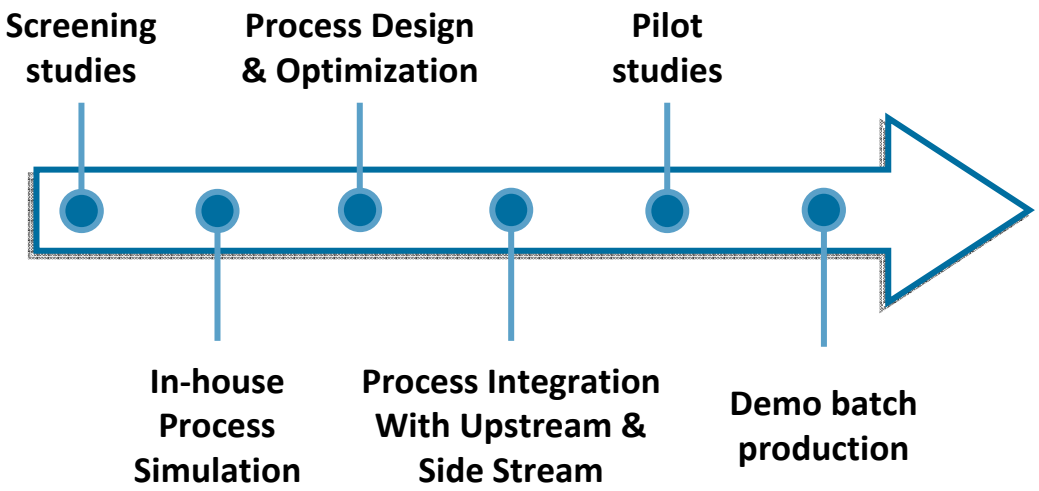
Bio-Industries

Bio Based Chemicals
Organic Acids
Aminoacids/proteins
Antibiotics
Vitamins

High capacity / low added value

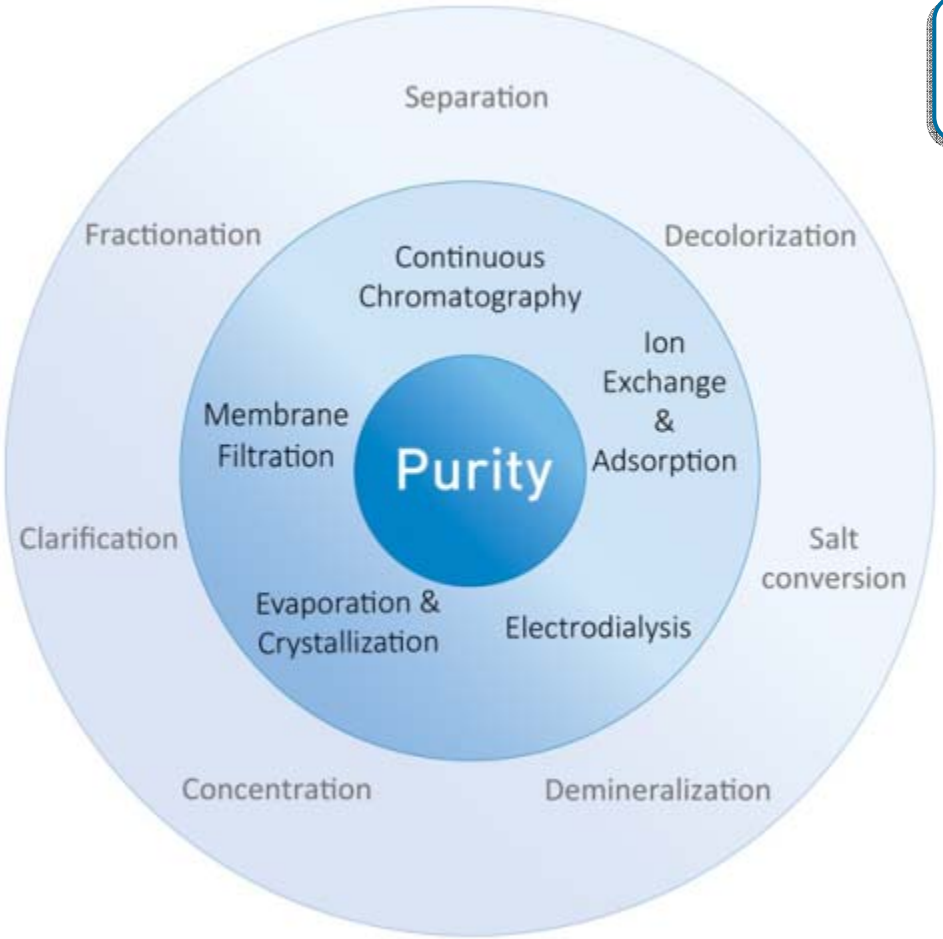
Environment : Re-usable units, low pressure chromatography…

A Methodology from Lab to Commercial



Supply of Equipment with Process Guaranteed

Cost-efficient Processes for Production Plants



Marketed products prices typically **< 2€/kg**
From **20 – 250 kT/year**

Demineralization units

Amino-acids & Proteins Production

- **From various feedstocks:**
  - Plant extracts : potato, colza…
  - Animal proteins :
    - Milk proteins:
      - casein, lactoferrin, alpha-lactalbumin, beta-lactoglobuline
      - whey protein concentrate (WPC)
      - milk protein concentrate (MPC)
    - Egg (lyzozyme)
  - Fermentation broths:
    - Amino-acids : lysin, threonin...

Low added value proteins

Need for cost-efficient process

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When purity is key.

Milk protein purification

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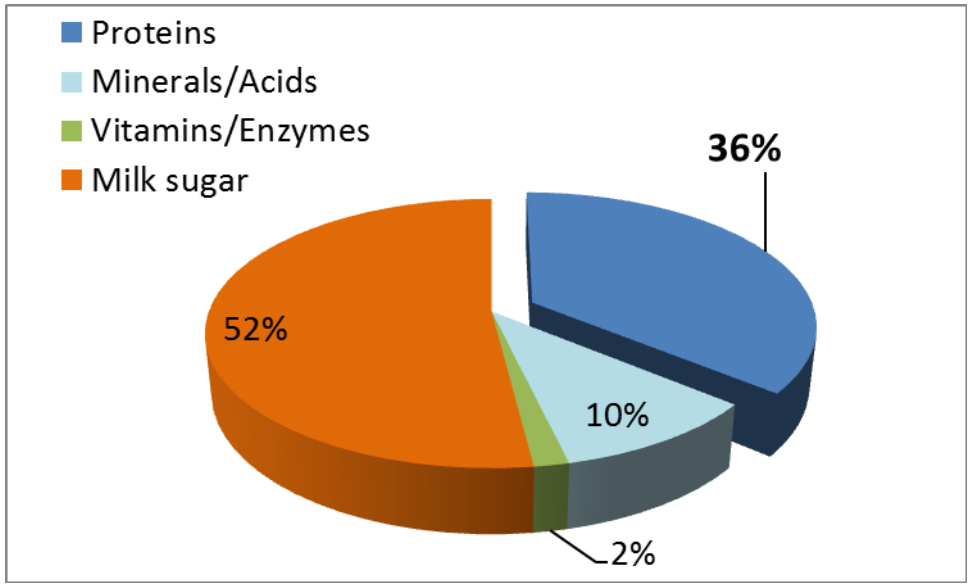
Lactoferrin case study

Milk proteins

- **Milk proteins**
  - Casein
  - Lactoferrin
  - Alpha-lactalbumin
  - Beta-lactoglobulin
  - Immunoglobulin

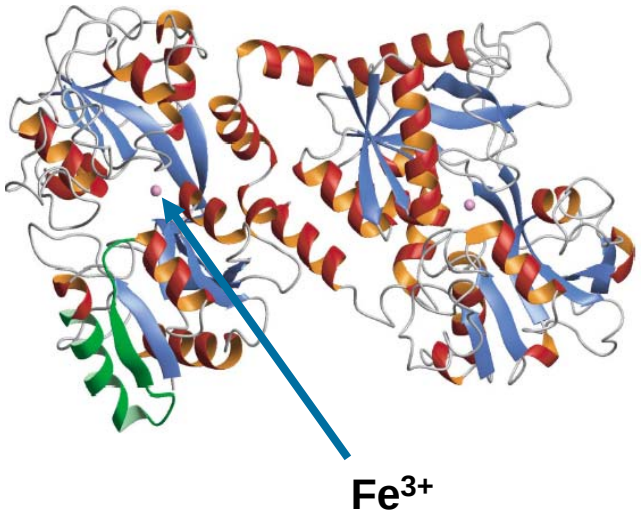
- **Other species :**
  - Lactose
  - Glucose, galactose
  - Vitamins / enzymes
  - Fat
  - Minerals



Composition of non-fat milk solids

- **Complex mixture presenting various impurity families**

Principle of Separation of the Lactoferrin



In whey, **lactoferrin** is generally complexed with serum albumine or β-lactoglobuline

Protein	Approx.% of skim milk protein	Isoelectric point	Molecular weight
alpha-Casein	45-55	4.1	23,000
kappa-Casein	8-15	4.1	19,000
beta-Casein	25-35	4.5	24,000
gamma-Casein	3-7	5.8-6.0	--
alpha-Lactalbumin	2-5	5.1	14,437
beta-Lactoglobulin	7-12	5.3	18,000
Blood serum albumin	0.7-1.3	4.7	68,000
Lactoferrin	**0.2 - 0.8**	**8,7**	**87,000**
IgG1	1-2	--	160,000
IgG2	0.2-0.5	--	160,000
IgM	0.1-0.2	--	~1,000,000
IgA	0.05-0.10	--	~400,000
Proteose peptone fraction	2-6	3.3-3.7	4,100 to 200,000

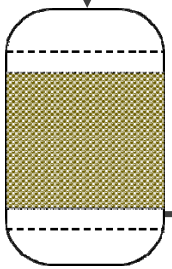
Separation: adsorb the lactoferrin selectively on a cationic resin

pKa(resin) < pH < isoelectric point (8,7)

Lactoferrin production process

Whey
(lactoferrin + impurities)

Cationic resin →



Selective elution with salts
(lactoferrin + salts)

→ **NanoFiltration** →

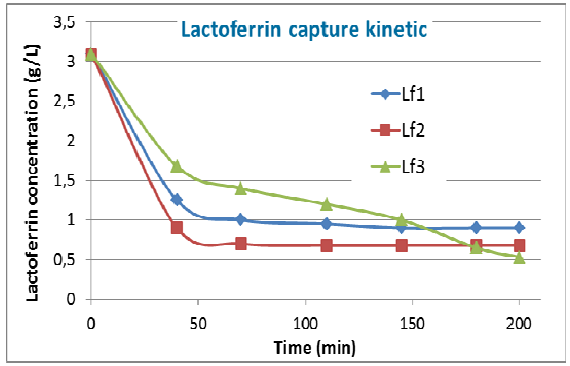
Concentrated lactoferrine
(retentate)

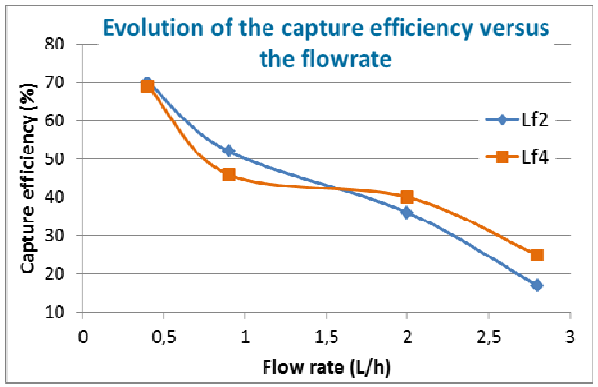
Salts
(permeate)

Fixation yield : 80-90 %
Elution yield > 80 %
Purity : > 60-85 % after cation resin
Sanitary design

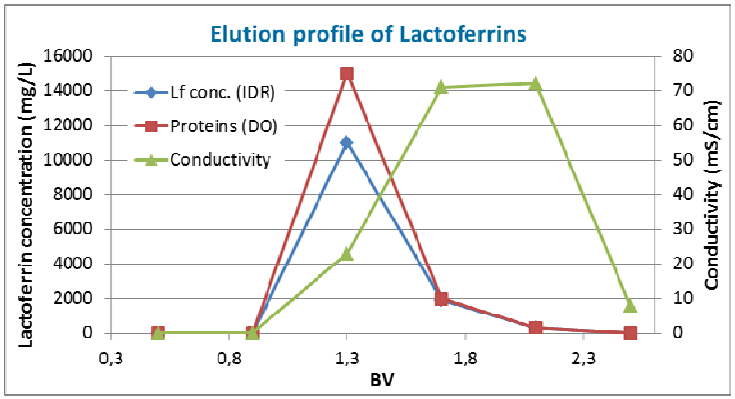
Process Optimization Studies

- **Resins comparison**
  - Adsorption capacity
  - Kinetic effects study
  - Resin form study
  - Selectivity



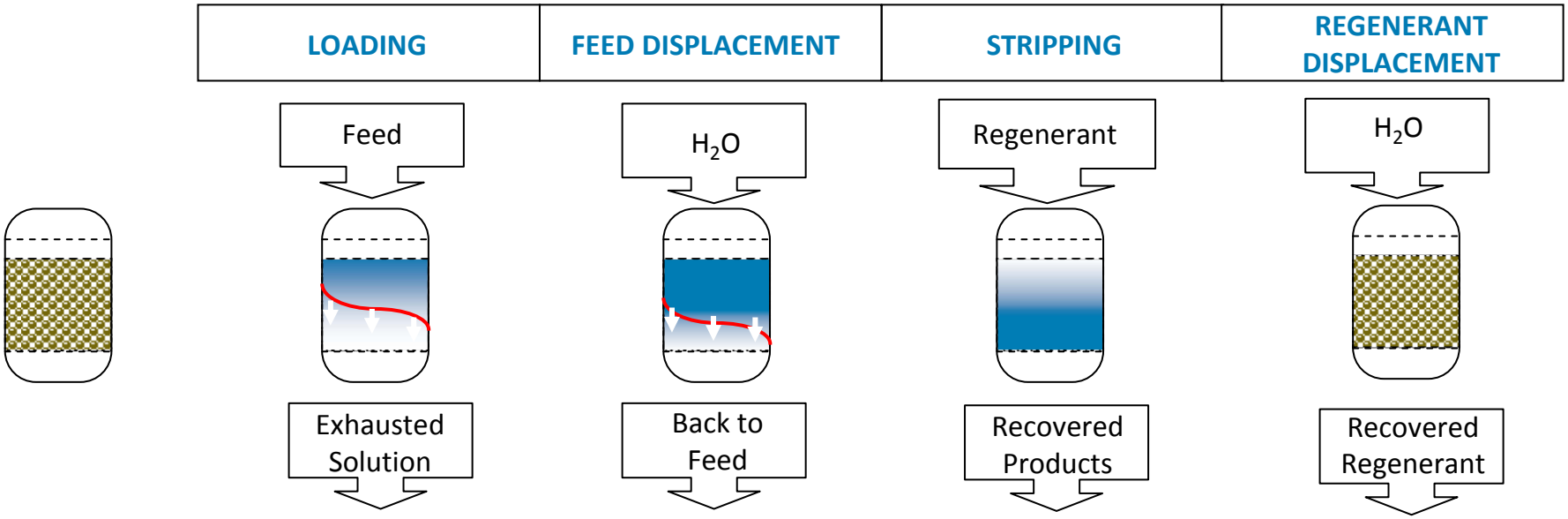


- **Operating mode optimization**
  - Feed's pH adjustment
  - Flow rate optimization
  - Elution conditions optimization
  - Column regeneration conditions
  - Purity study



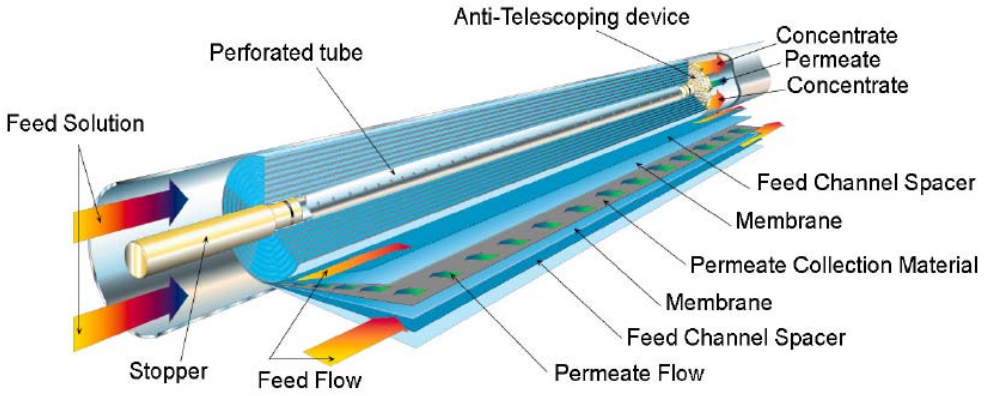
Novasep's solutions for Ion Exchange

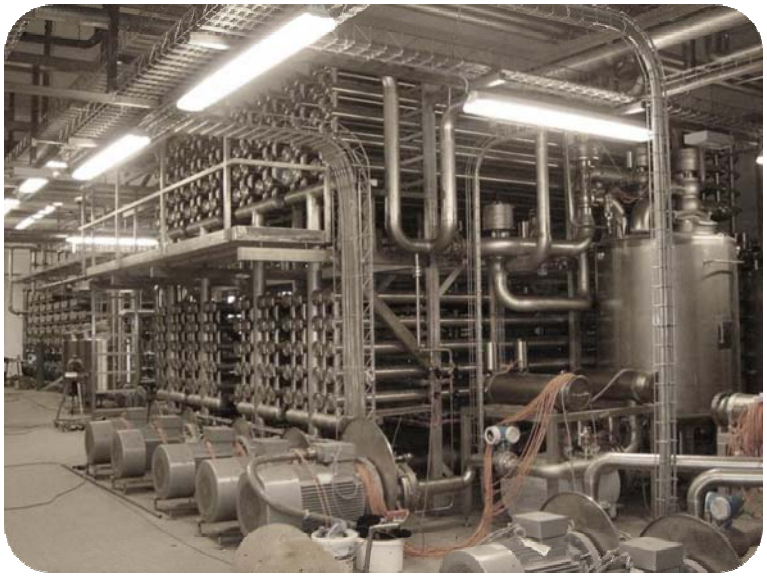
- Batch and continuous ion exchange (> 150 units designed by Novasep)
- Adsorption on resins and activated carbon
- Applications
  - Decolorization, desalting, salt conversion, purification
  - Proteins isolation, polyphenols extraction



Organic membranes

- Ultrafiltration
  - Proteins recovery
  - Proteins / enzymes elimination
- Nanofiltration
  - Concentration
  - Whey demineralization
  - Fractionnation
- Reverse osmosis
  - Concentration
  - Process water polishing





novasep
When purity is key.

Plant proteins purification

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Plant proteins case study

Proteins from Plant Extracts

- **Various processes for plant extracts:**
  - Pressing
  - Diffusion
  - Infusion

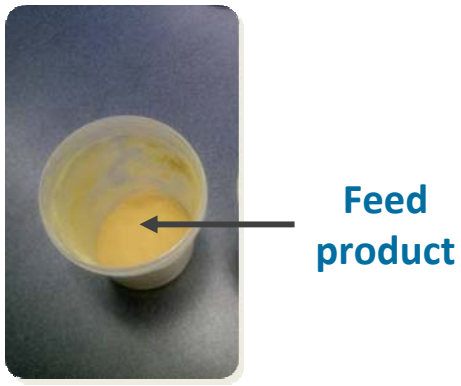
- **With the following typical composition:**
  - Insolubles: cellulose, lignin, starch
  - Soluble macro-molecules: lignin, dextrans, pectins
  - Mono- and Poly-saccharides, **proteins**
  - Dyes: anthocyans, polyphenols
  - Minerals: monovalents, divalents
  - Water

- **Purification techniques have to be chosen according to the target fraction or molecule**

Plant extract : project development

- **Protein extract composition:**

% Dry Substance	pH	Colour OD_{420nm}	Protein content (%/DS)
3.4	7.2	5.95	41

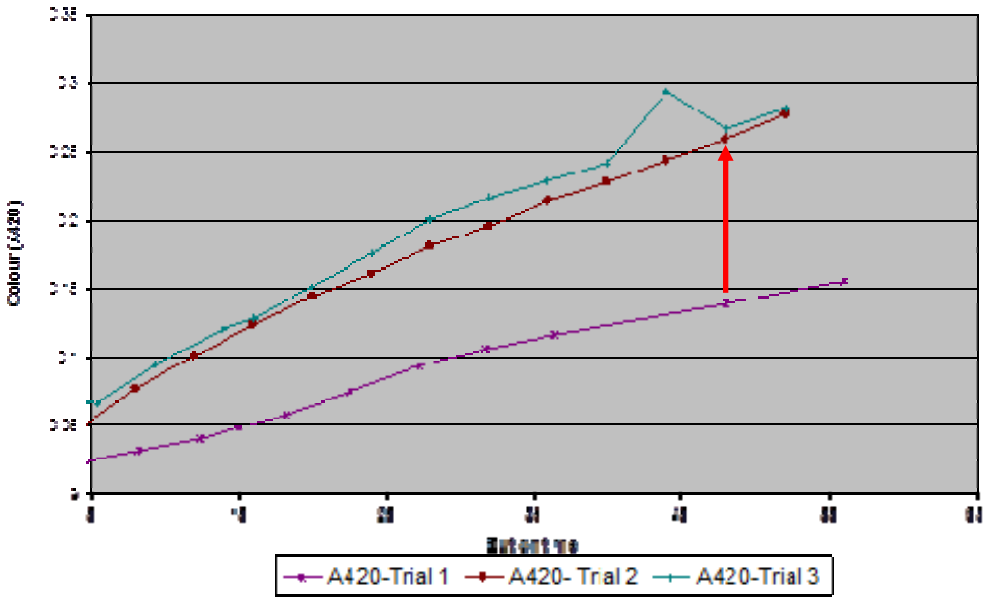


- **Target:**
  - Remove color
  - Remove flavor

- **Technical solution : adsorption on resin**
  - Color reduction (polyphenols)
  - Taste and flavor reduction

Plant extract Proteins: Lab Scale Results

- **Lab results**
  - Reduction of colour by 80%
  - Reduction of taste (organoleptic tests)
  - Adapted operating conditions (pH, T°C, flowrate) to avoid protein precipitation and denaturation



Feed product

Before/After decolorization

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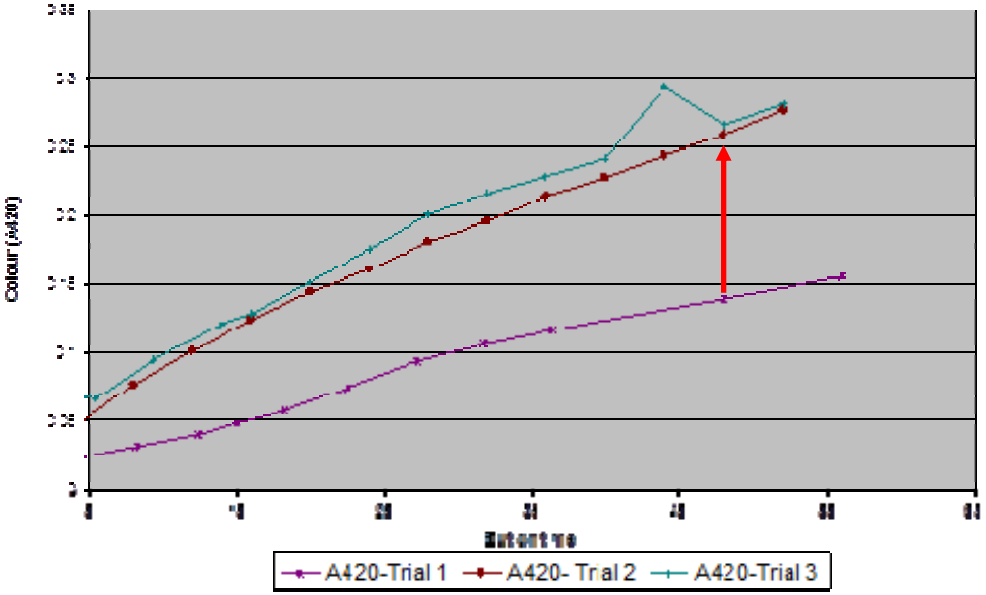


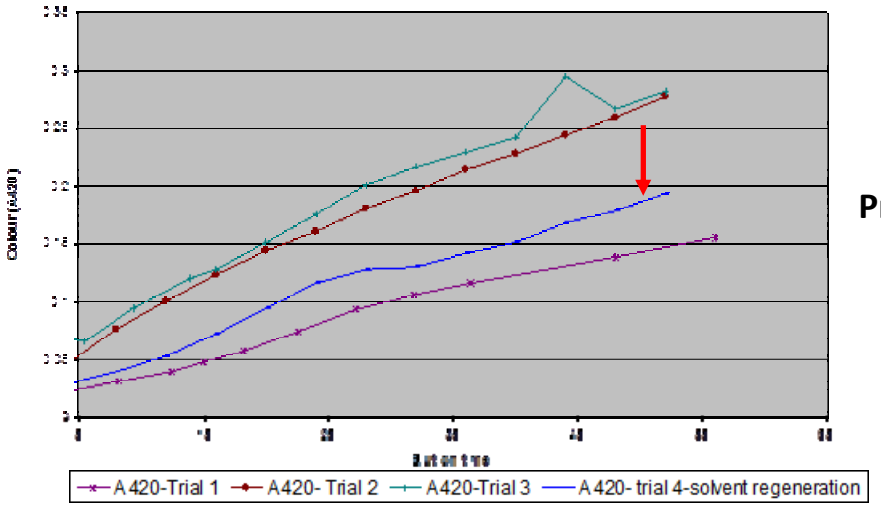
Feed product
Before/After decolorization

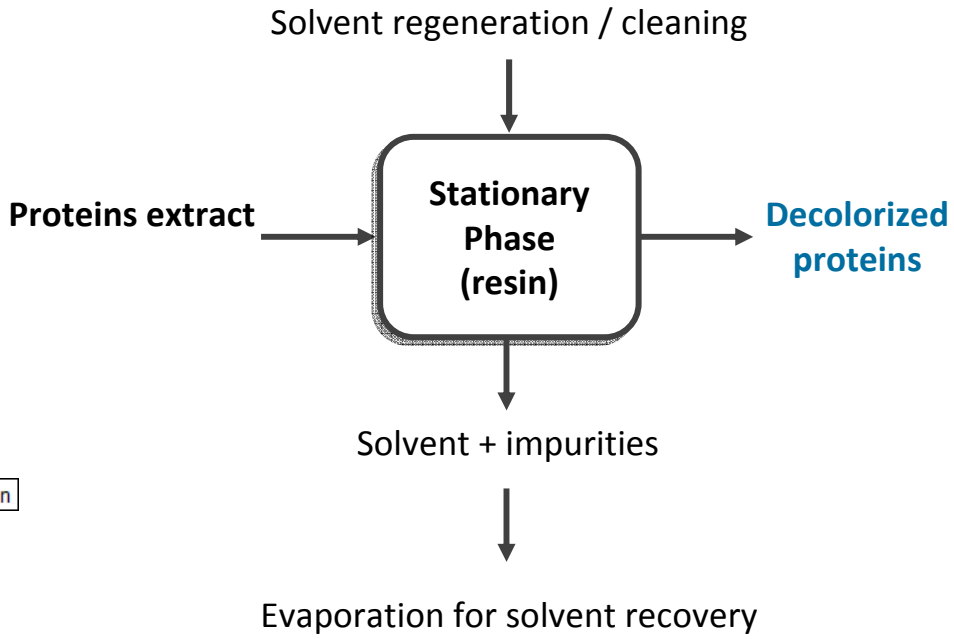
Decrease of decolorization performance cycle after cycle

Need to strengthen the robustness of the process by optimizing regeneration

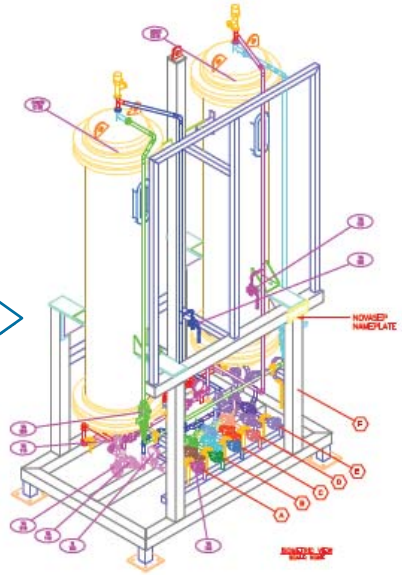
Plant extract Proteins : Process Optimization

- **Development of an enhanced regeneration procedure**
- **Combination of chemical and polar solvent regeneration**





Plant extract Proteins : from Lab to Scale-up



Build-up of an ATEX pilot unit comprising 1600 Liters of resin

Next scale

novasep
When purity is key.

Amino-acids from Fermentation

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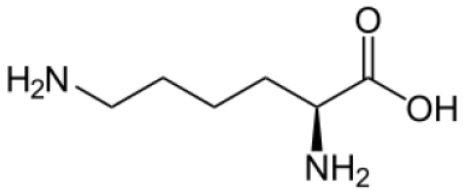
Lysine case study

Lysine : The Purification Challenge

What you think you will get!



What you actually get!

Lysine : Fermentation Broth

Broth composition		
Dry matter	*%DS*	20
pH		4
Lysine	*g/L*	130
Ashes *Mostly : Sulphate Chloride NH4, K*	*% / DS*	1,5
Turbidity *Suspended solids*	*%/ DS*	*5 - 10*

- **Lysine purity: 60 %**
- **Impurities of different nature**
- **Challenges**
  - Remove suspended materials
  - Remove colour, residual sugars, minerals…
- **Lysine has to be extracted from the broth**

Lysine : Process development

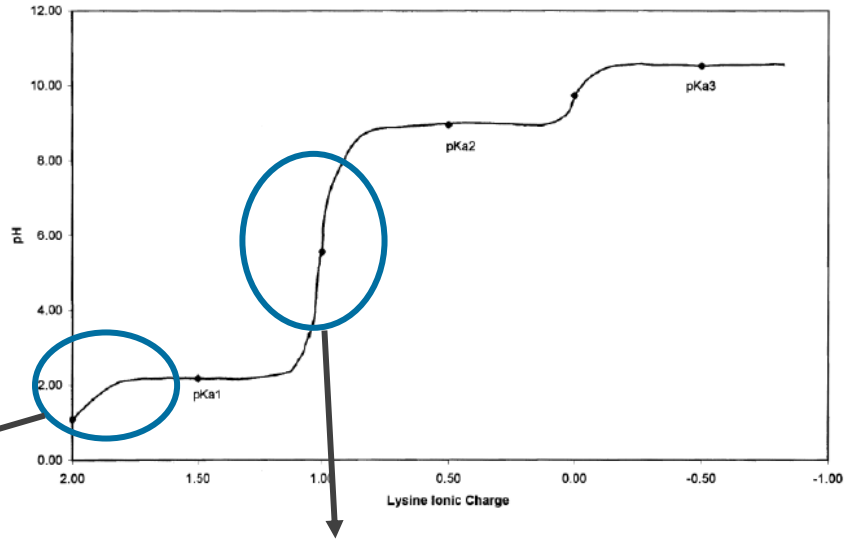
Solubility: 642 g/L in water in 20°C

Boiling Point: 312°C (760 mmHg)

Molar Mass: 146 g/mol

pKa = 2,2 ; 9,2 ; 10,7

pI = 9,7



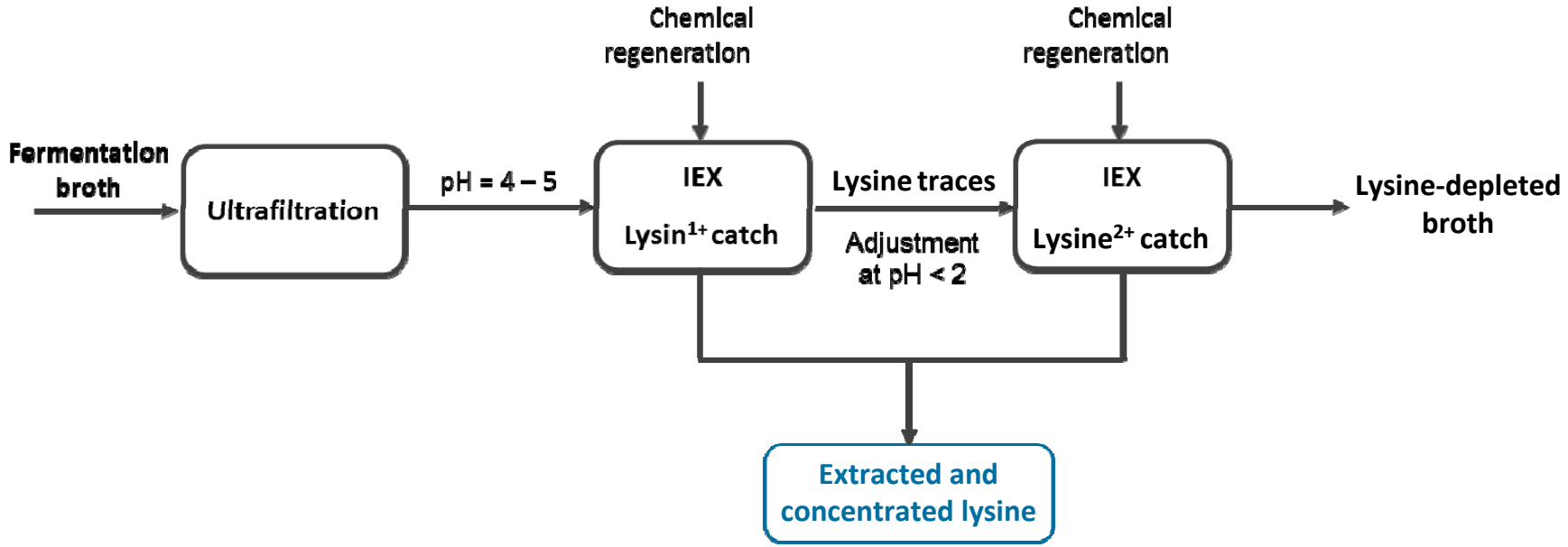
- Lysine can be caught under a cationic form by a cationic resin
- Lysin valence depends on the pH

- **Lysine^{2+} at low pH**
  - High capture efficiency on the resin
  - 2 equivalents occupied on the resin : more resin capacity needed

- **Lysine^{1+} at pH = 4 – 5**
  - Lower capture efficiency
  - 1 equivalent occupied on the resin : less resin capacity needed

Lysine : Process principle

- **Target: optimization of the resin usage by a combination of two operating conditions**



Lysine : Process principle

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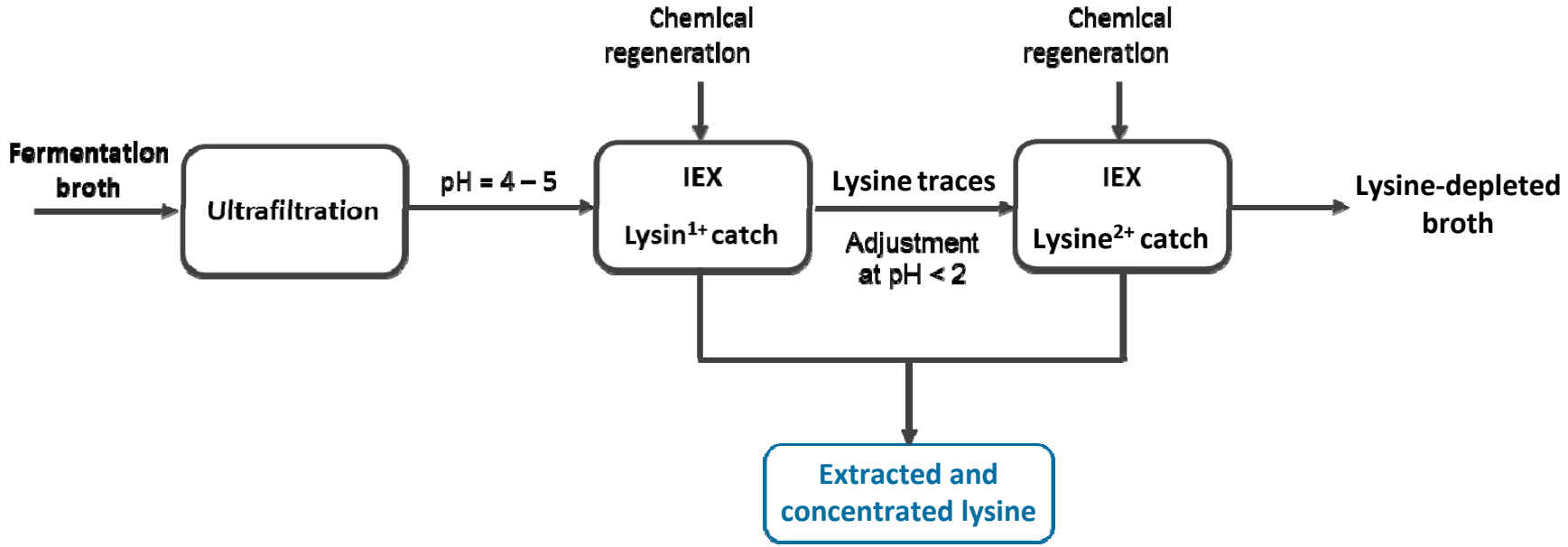


- Lysine: 0,9 eq/L in the feed product
- Cationic resin typical capacity: 1,8 – 2,1 eq/Lresin

High ionic load and fast saturation of resins

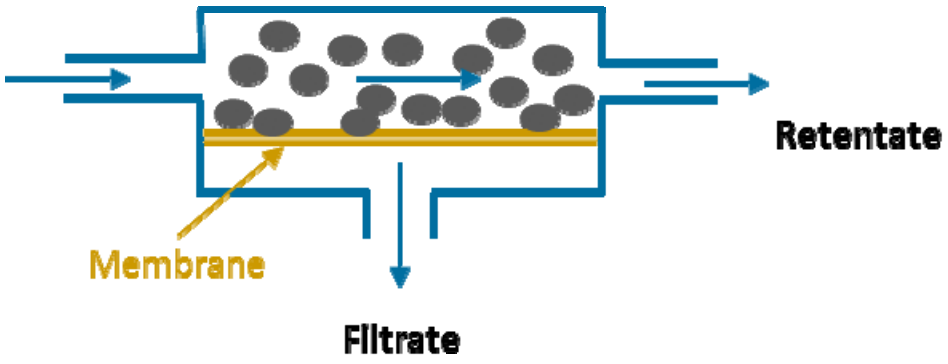
Non-neglectible regenerant amount

Need for a more cost-effective process:
Continuous Ion Exchange

Building the lysine extraction process

Ultrafiltration of Fermentation Broth

Obstacle to specifications	Related issue	Our technical solutions
Solids	Resin clogging → Pressure drop increase	Ceramic filtration



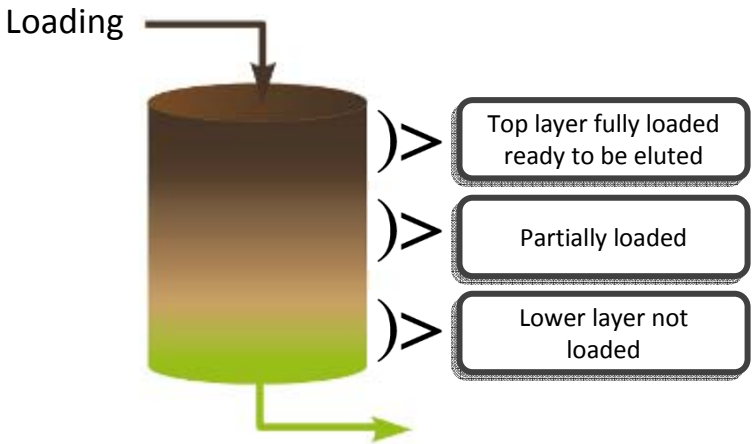
Removal of >99.5 % of solids in suspension
> 99,5 % yield

Novasep's Solutions for Filtration

- **Ceramic membranes**
  - Kerasep™ Modules
  - Large range of geometries and cut-offs
  - 99 membranes per modules at industrial scale
  - Compact, sanitary design

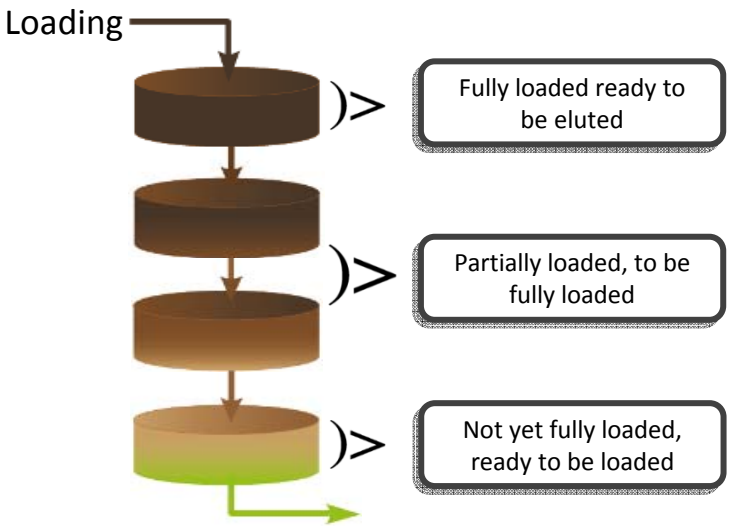
Building the lysine extraction process
From IEX to Continuous IEX

Batch IEX



Resin is **NOT** used at its maximum capacity

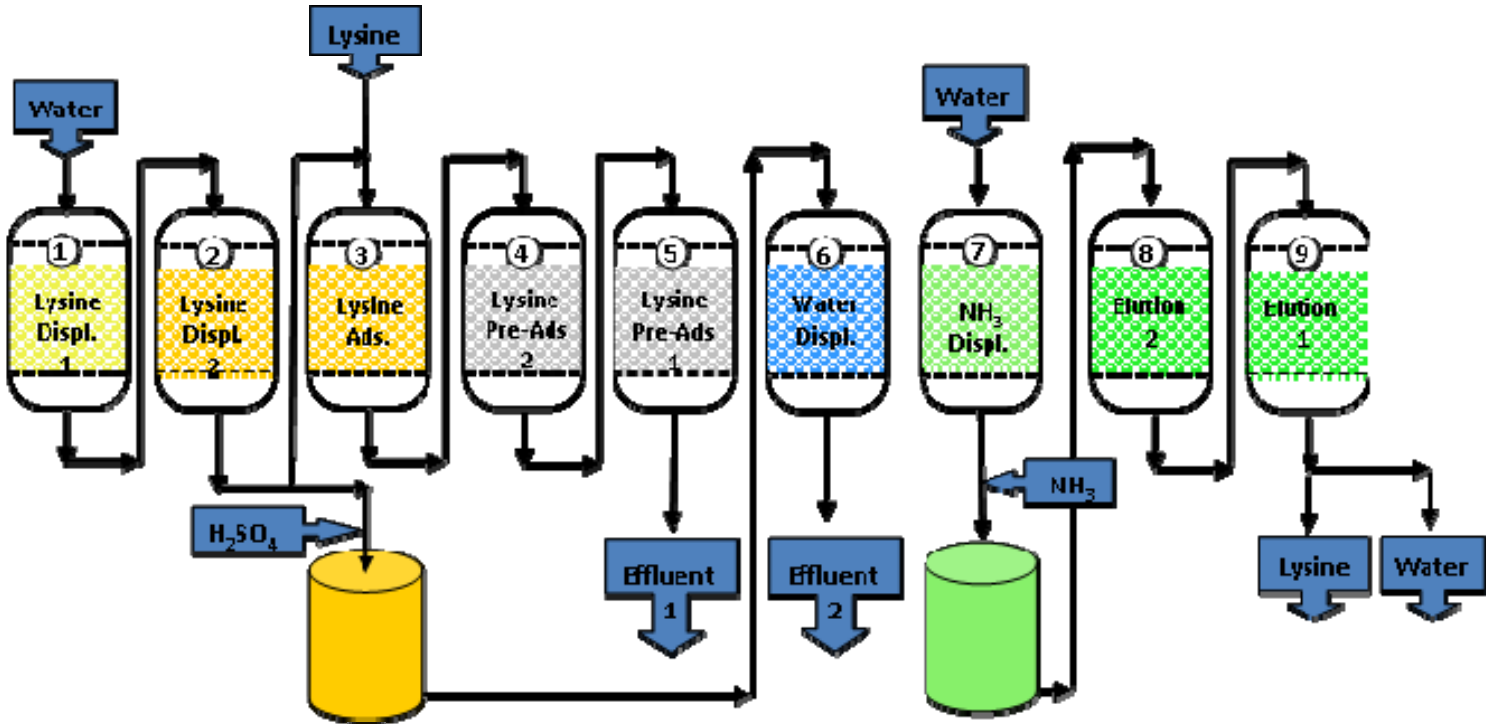
Continuous IEX



Resin used at max capacity

Optimization of resin capacity: lower OPEX due to resin volume reduction
High-recovery rate up to 99.8%
Strong decrease of water/chemicals consumption
Higher fractions concentrations
Recycling of water and regenerant

Lysine Processing with CIEX (1/3)



- **The system is divided into several process zones**
- Lysine cationic form adsorption (cells 1 – 6)
- Lysine desorption with dilute NH3 (cells 8 – 9)
- Resin regeneration and rinse (cells 7)
- Resin back-Wash (not represented) and resin backpulse (not represented)

Lysine Processing with CIEX (2/3)

Lab screening [mL-L]

Piloting [100L-m3]

Industrial Design

Build supply [100s m3]

Lysine Processing with CIEX (3/3)

Selective extraction from the fermentation broth by continuous ion-exchange
Operating temperature : 55°C
> 99,8 % capture rate achievable
Market price : 1,1 € / kg
Installed capacity by Novasep Process : approx 300 kT/y

- **Interest of Continuous IEX compared to IEX :**
  - Less lysine dilution and higher recovery
  - Approx 30 % less resin volume
  - Approx 20 % regenerant saving

Typical CIEX Design

- **Typically 10 cells + 1 spare for maintenance**
- **3 cells / columns for layout optimisation**

- **Simple design and equipment (inlets/outlets collectors and valves)**

Some CIEX Use Examples

- **For capture**
- Lysine and nucleic acids (GMP) : USA, Brazil, China
- Organic acids conversion
- Gluconic : Europe
- Succinic : France, USA, China (pilot)

- **For demineralization**
- Demin. of Biomass sugars, glycols desalting
- Can be adapted to proteins purification

CAPEX is higher but lead to a lower utilities consumption and wastes production

CIEX is recommended for

Removal of high ionic load (organic salt conversion)

Large-scale production (tons / year)

Compliance with strict regulations on waste streams, etc.

Thank you for your attention!

Frederic.schab@novasep.com
R&D Project Manager; PhD