Manufacturing and GMOs

GMO large scale

Martin Kuster, MD MOH

USDA ARS Feb 3, 2015

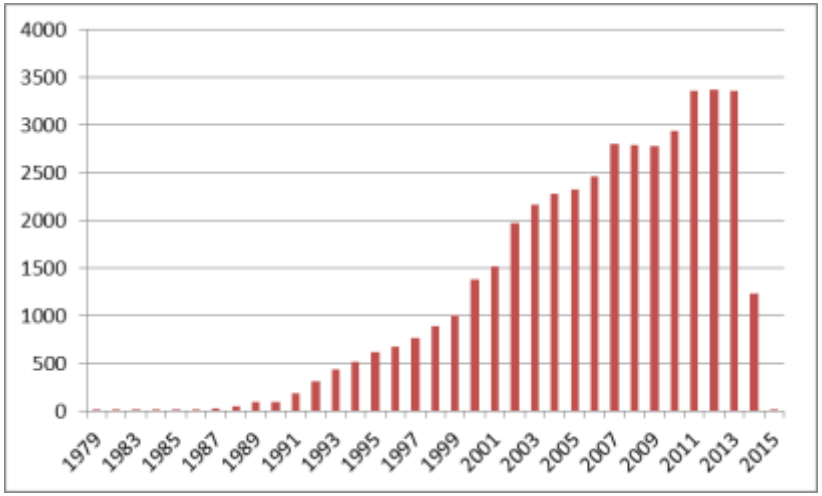
NOVARTIS

What is a Genetically Modified Organism (GMO)

Definition as per Wikipedia

- A **genetically modified organism** (**GMO**) is a bacterium, yeast, insect, plant, fish, or mammal whose genetic material has been altered using genetic engineering techniques.

- If you search «medline» for GMO: you get 42342 articles over 35 years (Nov 26, 14)

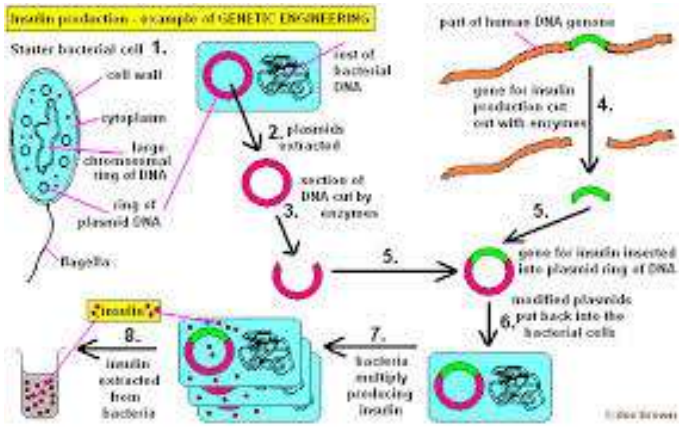


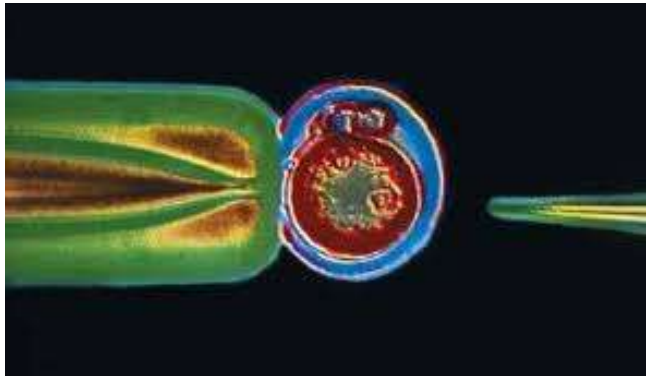
NOVARTIS

How are GMOs produced?

Just to summarize

- The foreign genetic information is either cut from the «donor» or more often synthesized
- The information is either used straight or mounted on:
  - Plasmids, Viral vectors, Beads etc
- The information is transported into the cells by:
  - Salt gradient plus heat
  - Electroporation/»gene guns»
  - Transfection (viral vectors)
  - Direct injection
- Depending on method, the information
  - Inserts into genome of cell
  - Uses the metabolism of the cell as extrachromosomal elements





NOVARTIS

What makes GMOs for production attractive

Source Nature Reviews Genetics October 2003

- Single cell organisms are easy to manipulate
  - You have to treat them well, so they produce at large scale!
- The table below summarizes the position
  - Is applicable for all large scale production, not only proteins

Table 1 | **Comparison of production systems for recombinant human pharmaceutical proteins**

System	Overall cost	Production timescale	Scale-up capacity	Product quality	Glycosylation	Contamination risks	Storage cost
Bacteria	Low	Short	High	Low	None	Endotoxins	Moderate
Yeast	Medium	Medium	High	Medium	Incorrect	Low risk	Moderate
Mammalian cell culture	High	Long	Very low	Very high	Correct	Viruses, prions and oncogenic DNA	Expensive
Transgenic animals	High	Very long	Low	Very high	Correct	Viruses, prions and oncogenic DNA	Expensive
Plant cell cultures	Medium	Medium	Medium	High	Minor differences	Low risk	Moderate
Transgenic plants	Very low	Long	Very high	High	Minor differences	Low risk	Inexpensive

NOVARTIS

Production in plants tried..and now ZMAPP!

Issue: Purification!! *Source Nature Genetics October 2003*

Table 2 | **Important pharmaceutical proteins that have been produced in plants**

Protein	Host plant system	Comments	References
Human biopharmaceuticals			
Growth hormone	Tobacco, sunflower	First human protein expressed in plants; initially expressed as fusion protein with *nos* gene in transgenic tobacco; later the first human protein expressed in chloroplasts, with expression levels ~7% of total leaf protein	6,14
Human serum albumin	Tobacco, potato	First full size native human protein expressed in plants; low expression levels in transgenics (0.1% of total soluble protein) but high levels (11% of total leaf protein) in transformed chloroplasts	15,98
α-interferon	Rice, turnip	First human pharmaceutical protein produced in rice	99
Erythropoietin	Tobacco	First human protein produced in tobacco suspension cells	100
Human-secreted alkaline phosphatase	Tobacco	Produced by secretion from roots and leaves	59,60
Aprotinin	Maize	Production of a human pharmaceutical protein in maize	101
Collagen	Tobacco	First production of human structural-protein polymer; correct modification achieved by co-transformation with modification enzyme	13,26
α1-antitrypsin	Rice	First use of rice suspension cells for molecular farming (see REF. 102 for discussion of antibody production in rice cell culture)	103
Recombinant antibodies			
IgG1 (phosphonate ester)	Tobacco	First antibody expressed in plants; full length serum IgG produced by crossing plants that expressed heavy and light chains	7
IgM (neuropeptide hapten)	Tobacco	First IgM expressed in plants and protein targeted to chloroplast for accumulation	104
SIgA/G (*Streptococcus mutans* adhesin)	Tobacco	First secretory antibody expressed in plants; achieved by sequential crossing of four lines carrying individual components; at present the most advanced plant-derived pharmaceutical protein	89,90, 105
scFv-bryodin 1 immunotoxin (CD 40)	Tobacco	First pharmaceutical scFv produced in plants; first antibody produced in cell-suspension culture	106
IgG (HSV)	Soybean	First pharmaceutical protein produced in soybean	72
LSC (HSV)	*Chlamydomonas reinhardtii*	First example of molecular farming in algae	107
Recombinant subunit vaccines			
Hepatitis B virus envelope protein	Tobacco	First vaccine candidate expressed in plants; third plant-derived vaccine to reach clinical trials stage	8,19,20
Rabies virus glycoprotein	Tomato	First example of an 'edible vaccine' expressed in edible plant tissue	77
Escherichia coli heat-labile enterotoxin	Tobacco, potato	First plant vaccine to reach clinical trials stage	21,108
Norwalk virus capsid protein	Potato	Second plant vaccine to reach clinical trials stage	22
Diabetes autoantigen	Tobacco, potato	First plant-derived vaccine for an autoimmune disease	109
Cholera toxin B subunit	Tobacco, potato	First vaccine candidate expressed in chloroplasts	65
Cholera toxin B and A2 subunits, rotavirus enterotoxin and enterotoxigenic *E. coli* fimbrial antigen fusions	Potato	First plant-derived multivalent recombinant antigen designed for protection against several enteric diseases	110
Porcine transmissible gastroenteritis virus glycoprotein S	Tobacco, maize	First example of oral feeding inducing protection in an animal	111

HSV, herpes simplex virus; IgG, immunoglobulin G; IgM, immunoglobulin M; LSC, long single chain; *nos*, nopaline synthase; scFv, single-chain FV fragment; SIgA, secretory immunoglobulin A.

NOVARTIS

Uses of genetic modification for production

Many are in research stage, several are used since years

- Agriculture: Food!
  - Well known, not part of the disucssion here
    - There are big differences US/EU: GMO-legislation
- Medications: Advanced and in use since years
  - Production of «chemicals»: Antibiotics, Immunosuppresors
    - Bacteria, fungi as «workhorses»
  - Production of «biologicals»: Hormones, Proteins (Antibodies)
    - E. coli, Yeasts and mammalian cells as «workhorses»
  - Research or early development:
    - Genetic modification of human cells: CTL019 for Leukemia
    - Genetic modification of plants: ZMAPP for Ebola in Tobacco
    - Genetic modification of Goats: Hormones in Goat milk
- Material science: Research stage: Fuel, Polymers etc
- Env. Sciences: GMO indicator plants for heavy metals, Solvents etc

Issues of production

The scale adds a different dimension

- The quantities and processes in a research lab are small (few milliliters up to about 10 Liters)
- «Large Scale» has no clear definition
  - If the agent does not infect people or animals: >2000 l are considered large scale in industry
  - The «virus production» for retroviral vectors for CTL019 is about 70 Liters. Due to the high virus titer, this must be considered «large scale», as it will transfect cells of workers, so exposed.
- GMO-legislation applies to ALL uses the same:
  - Based on «Asilomar principles»
  - Industrial production uses often «safety plasmids» technolgoy in E. coli, Pichia; and other «GRAS» organisms
  - You have to perform extensive risk assessments
    - Infectivity to people, animals, plants; potential of transfer of genetic material (e.g. plasmids)
  - Public perception puts them all in the same bucket

Example Antibiotics Fermentation

Here you have the real large volumes

- The volumes are large: up to 250 m3
- You need well trained people
  - Prevention of superinfection
- You need mature HSE programs
  - Physical hazards
  - Chemical hazards
  - Environment: Full inactivation unless GRAS, no Antibiotic left: Resistance!
- You need to understand all facets of your material streams
  - Raw materials and Energy
  - Purification steps
  - Where is waste generated
  - What to do with your waste

NOVARTIS

Example Antibiotics Fermentation ctd

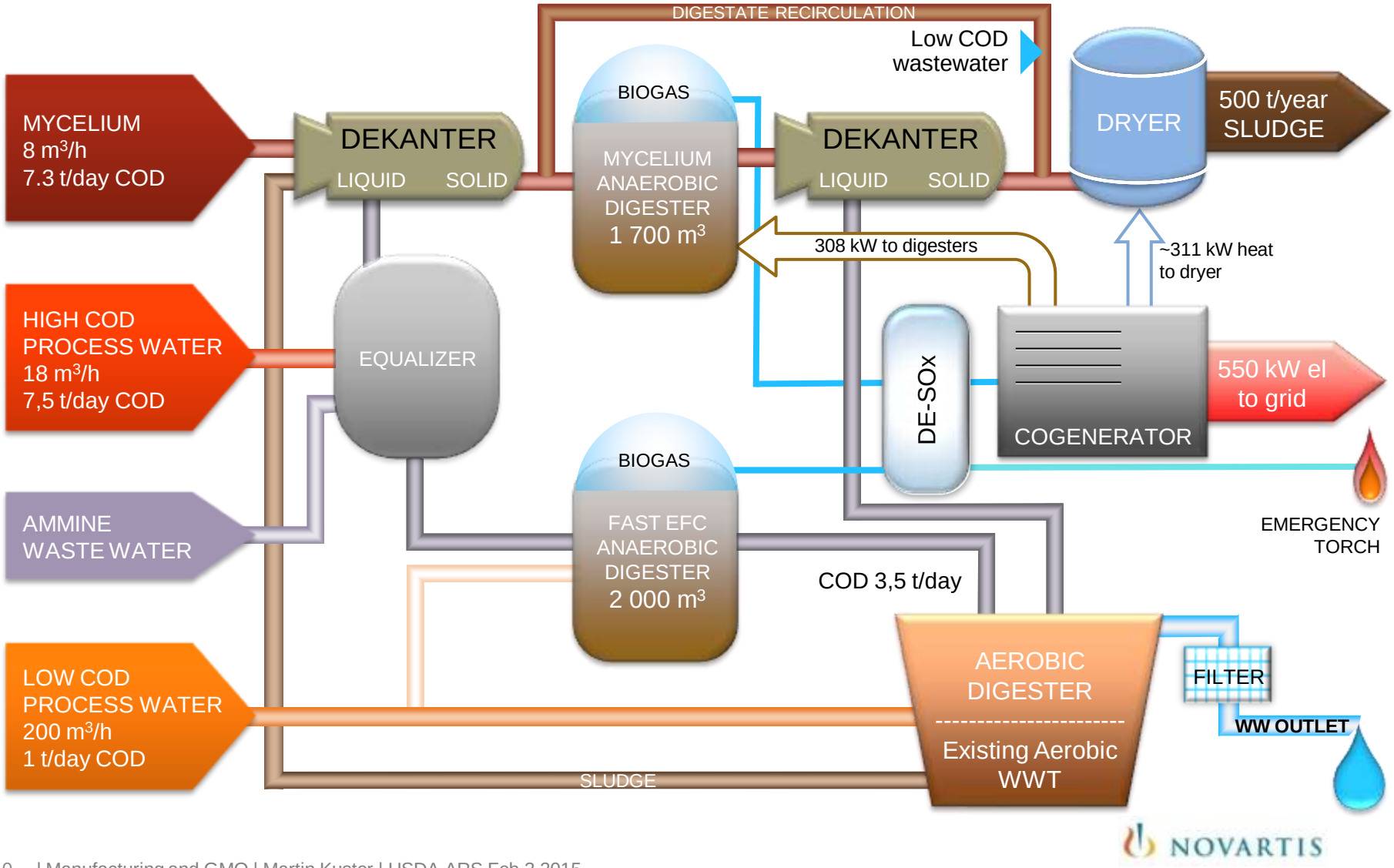
Think cradle to cradle

- You need a lot of engineering for protection of spills
- You need a waste water treatment plant
- You get fertilizer, too
  - So you understand your waste stream
  - «Biosol»: about 5000 t/year
    - Makes money, helps pay for the overall waste managemetn costs
- You might be able to produce Biogas
  - So making fertilizer is not an option
  - See following slides

NOVARTIS

Integrated Anaerobic-Aerobic WWTP

Designed new configuration: Live since 2012



For comparison: How big are 8 m3 (8000 Liters)

This is a 8000 Liter seawater aquarium
Or: 2x2x2 meters

NOVARTIS

Integrated Anaerobic-Aerobic WWTP

Digester and Co-gen engine

Integrated Anaerobic-Aerobic WWTP

Project delivers an excellent ROI

Investment

Digester	2 800 k€
Cogenerator	700 k€
Dryer	1 000 k€
TOTAL	**4 500 k€**

Savings

Sludge disposal	From 14 000 t/year to 500 t/year @ 100 €/t (75% abatement in anaerobic digester + dryer)	1 350 k€/year
Cogen electricity (RENEWABLE)	550 kW x 8 000 h/year x 0,28 €/kWh (incentive)	1 230 k€/year
Air compressor (Aerobic digester)	373 kW x 8 000 h/year x 0,10 €/kWh x 30%	90 k€/year
Dryer fuel Dryer electricity	15 m³/h x 0,30 €/Sm³ x 8 000 h 30 kW x 0,10 x 8 000 h	-36 k€/year -24 K€/year
TOTAL		**2 610 k€/year**

YEARS

2

PAY-BACK

Not considering…

Local authority incentives | Cogen heat recovery

NOVARTIS

Example Anti-body production

The scale is still sizable

- You need animal cells
  - Plants are not yet there
- Insert will be in genome predominantly
- You need well trained people
- You need mature HSE programs
  - Physical hazards
  - Chemical hazards
  - Environment: Full inactivation, due to GMO legislation
    - Energy savings possible by demonstrating that NaOH treatment and heat inactivated genes; Full sterilization of production effluent not necessary
- Scale still sizable
  - Less spill protection engineering

NOVARTIS

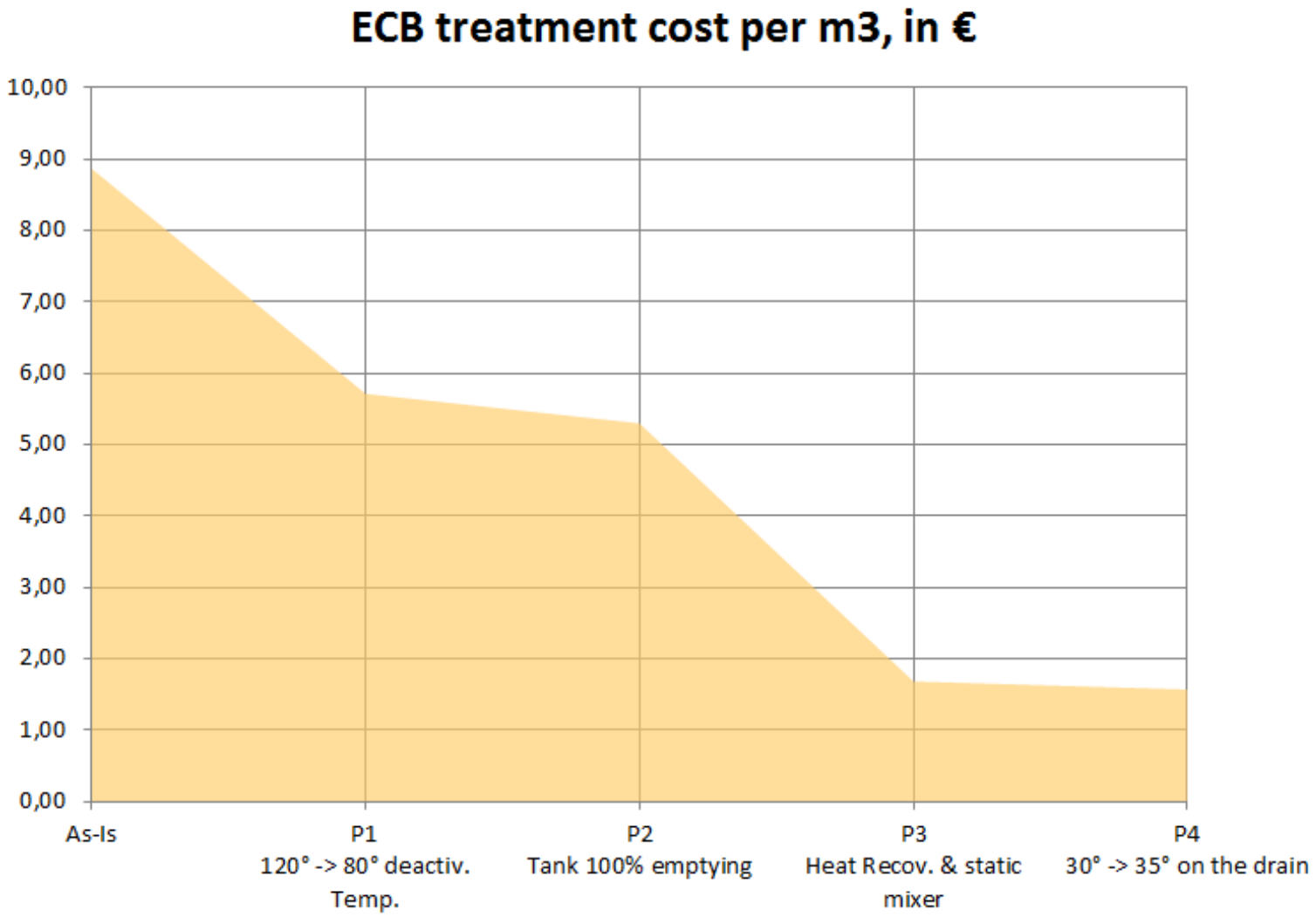
Full inactivation after change of process: Project summary

- The quantity of waste water that must be inactivated is about 22000 m^3 per year (average of years 2010, 2011 and 2012).
- Bio-inactivation is one of the major energy consumer of the site.
- It is considered as a “waste” activity because it does not add any value to our product. Thus, our objective is to reduce as much as possible the cost of that “waste”, to improve our productivity.
- Approach is to find all kind of opportunities to reduce the energy consumption and increase availability of the inactivation installation. 4 projects have been identified :
  - P1 – inactivation temperature reduction from 120° → 80°
  - P2 – inactivation tank 100% emptying
  - P3 – heat recovery & static mixing
  - P4 – reduction of drain water cooling from 65° to 35° instead of 30°C

Project P1 : inactivation temperature reduction from 120° to 80°C, and from 20 to 15 min

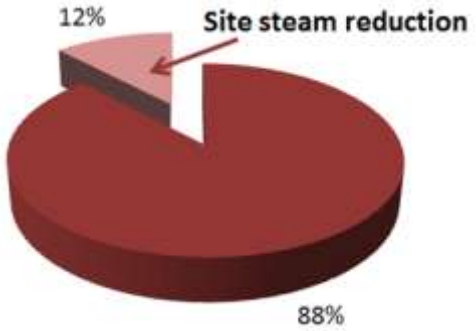
- A requirement was send to the authorities to change inactivation parameters from 120°C during 20 min, to 80°C during 15 min.
  - Change of parameters of the initial permit to produce
- We could demonstrate to the authorities that the required specifications for decontamination were also met at 80°C during 15 min, for all GMOs that are produced on site.
- With this set-up change, important benefits will be made in terms of energy and cost reduction, CO2 emissions and installation availability increase.
- Thus, authorities have accepted our change requirement in October 2012.

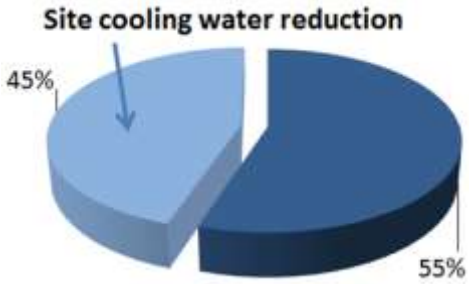
Inactivation and cooling cost evolution after project implementation



NOVARTIS

Benefits





- Saving of 2720 tons of steam representing 12% of site consumption !
- Saving of 206000 m^3 of cooling water representing 45% of site consumption !

→Energy savings : 14100 GJ / year

→ GHG reduction : 460 t / year

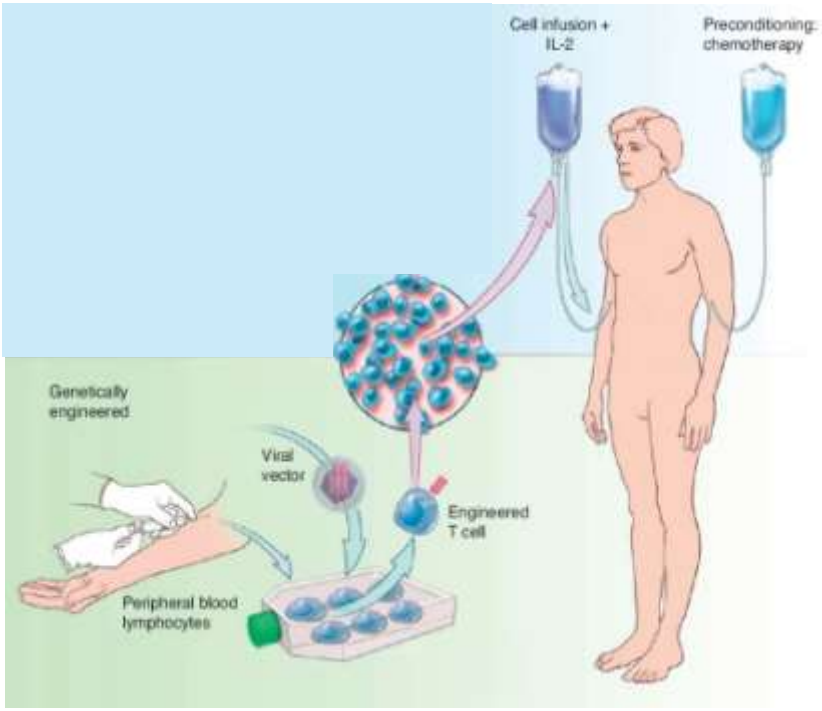
- Cost savings : 225 kUSD / year
- Payback : 2,5 years
- Increase in capacity: +37%
  - More volume, shorter cycle times

NOVARTIS

Example modified human cells

Viral vectors will be increasingly used in human therapeutics

- The first step is vector production
  - Well developed process
  - Vector able to transfect human cells
- The second step is to harvest correct cells from patient
  - Then you have to grow them in numbers
- The third step is to transfect your cloned cells and activate them
- The fourth step is to re-inject the cells



NOVARTIS

Example modified human cells ctd

Generating the viral vectors is most critical for Biosafety

- You need well trained people
  - HSE **and** GMP
- You need mature HSE programs
  - Physical hazards
  - Chemical hazards
  - Biosafety hazards
    - The insert might be the problem (research>>production)
    - Post exposure prophylaxis necessary where feasible e.g. Lentiviral vectors
  - Environment: Full inactivation of waste, due to GMO legislation
- Scale is about 100 l /»factory»
  - Virus titer at the end of cycle about 1 Mio/ml

Example genetically modified animals

Still predominantly for research

- Goats
  - Production of «bio-steel»
    - Company went bankrupt
    - Name used for energy drink now!
  - Very small production of hormones (HgH) or clotting factors (no products, though!)
- Pigs
  - Environmentally friendly meat (less phosphor in manure); abandoned
- Cattle
  - Production of human breast-milk
  - Research to early development in CN

Example modified animals ctd

The time is not ripe yet

- Research of a myriad of disease models based on genetically modified animals; typically mice and rats
- Basic research might profit from new GMO animals (fish, pigs, dogs) with fluorescent genes inserted
- Expansion into farm and/or companion animals difficult
  - Moral and ethical questions: especially EU/US
  - Very expensive

Summary

Genetic modification of cells for production is a reality

- Many of the major pharmaceuticals, vaccines or even enzymes in washing powders start with fermentation of cells
  - While several strains were produced by different methods in the past, today genetic engineering is the way to get a cell to produce something
  - Industry has developed and uses inherently biologically safe agents that will not transfer their «payload»
    - *E.Coli* K12; *Saccharomyces cerevisiae*; *Pischia pastoris*
- GMO has stirred a lot of discussion and legislation
  - Moral/ethical questions
  - Stringent handling of even the smallest quantities
- GMO will expand into other areas, with increased need of the human race for materials, medications and research