

Bridging the Gap

How Process Technology Entered Research & Development



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Today, industrial production of biopharmaceutical goods without reproducible processes in preceding research and development are out of the question. This article gives a glance at the rise of process development in R&D scale with selected showcase applications.

The Early Years of Process Technology

Since the beginning of fermentation in human history, the improvement of product quantity and quality was the driving force. Production of food and especially beverages such as alcohol led to empiric secret knowledge such as the brewing of sake or soy sauce in Japan. But with the beginning industrialisation more and more process relevant parameters were identified and therefore the different techniques of

process development established. The first large scale vessels appeared prior to 1900 in brewing industry. Already in the nineteenth century, fed-batch operations and aeration were used for growing yeast cell mass.

Over the following two world wars, shortages of food, petrol and ammunition led to deeper insights in processes due to the production of yeast, glycerol and (the nowadays more and more required) bioethanol and, subsequently, to the follow-

ing process development techniques: e.g. media-development, sterility and cooling, tray fermentation and aerated, suspended cells with biomass recycling.

The growing demand for the first fermented medicine penicillin (Fleming, 1928) started the first real strain screening and optimisation task in history. As the first strain – still the wild type discovered by Fleming – was grown in flasks such as milk bottles, several hundreds of them were necessary to produce enough

1. Need: Tighter Time Frames

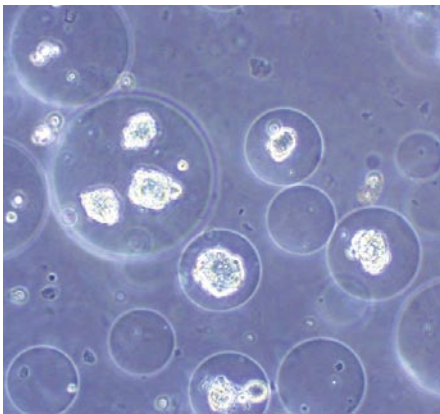
The pressure of faster time to market leads to the need for smaller and easier to handle cultivation scales in parallel as the number of experiments and therefore handling time and media consumption rises to impossible numbers and costs. Thus, even during the first screening processes there is a high need for valuable information from the reaction to set the right parameters in the later scale-up.

However, few suppliers, e.g. Applikon and Dasgip, have come up with intelligent high throughput fermentations systems yet.

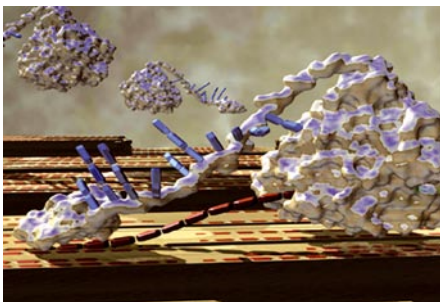


A cantaloupe delivered the strain of interest for Flemings penicillin.

penicillin for one single patient. A world wide search for new strains was started – ironically successful on a moldy cantaloupe in Peoria in direct neighbourhood to the laboratories involved in the fungal research. After a short period of surface cultures the researchers fell back on the reactors developed so far and designed large vessels of up to 25.000 gallons working volume for the submerge production of the required amount of the so called “wonder drug”: while initially in 1943, 29 pounds were produced, by the end of the war the production was sufficient to treat 7 million patients per year. This was the first time engineers and biologists worked together joining forces in



Stem Cells: Encapsulated mice ES cells in suspension culture (Source: Kelly Purpura, University Toronto)



Biofuels: Illustration of an enzymatic interaction with cellulose (Source: Laboratory for Industrial Microbiology and Biocatalysis, Faculty of Bioscience Engineering, Ghent University)

strain development and chemical engineering (influence of power input, shear, aeration rates and the necessary supply of sterile air).

Consequently research created the new branch of study. In 1947 the first courses of biochemical engineering were set up and in 50s the Journal of Biotechnology and Bioengineering was founded.

The design of the first reactors for antibiotics (stirred tank reactors) set the benchmark for fermenters until today. In the 60s the steamable dissolved oxygen probe was invented allowing the control of oxygen in solution, the first cell cultivation steps for the production of vaccines such as pox, mumps and polio were developed and the largest fermenter so far was put into operation with a stunning working volume of 1,5 million litre.

The high capacity fermenters on the one side needed a match on the small side for efficient and cost effective process development. The idea of “scale down” and “scale up” was born. Already in the 50s the big antibiotic producing companies such as Merck, Sharp and Dohme (MSD) set up R&D labs aiming at cost efficient production for antibiotics as the first commercial biologicals. Merck had already a dozen 5L fermenters for the detailed research of pH, OD, temperature and later DO. It was the time of the characterisation of vessels due to chemical engineering aspects of mixing times, power inputs while at the same time the first reaction kinetic research such by Monod appeared.

The necessary steps for the development of an efficient process led to the design of equipment in effect even today: mass screening, small-scale fermentation towards the scale up to the later production scale.

Bridging the Gap between R&D and Production by Parallel and Controlled Small Scale Cultivation

Even with the first interdisciplinary teams formed in the 50s the gap between natural sciences and engineering still exists today. Scientists aimed at discovering if a special protein would be produced by a specific cell line or strain, need to work with high throughput methods due to the vast numbers of mutants and media compositions to be tested. The most appropriate choice of technology is since the 40s the shaking flask still being rampant for strain and media development.

The requirements in favour for these vessels in terms of fermentation conditions was to work in small volumes to save resources such as media, gasses and cell material leading on the other hand to

2. Need: Replacement of Crude Oil by Alternate Energy Sources

Since the crude oil prices have increased nearly 150% from 2001 to 2006, biologist and bioprocess engineers have been working with scientists from the chemical and energy fields to seek more cost-efficient and environmentally-sound solutions for the newest application of biotechnology – Biofuel development. Scientists seek and utilise tools to screen and select the desired host organisms and enzymes for first and second generation biofuels. They benefit from parallel operation of multiple vessels, which is ideal for running factorial designed experiments to optimise bioconversion processes and establish fermentation conditions. Furthermore, tight monitoring and accurate control of crucial values such as temperature, pH, DO or, in the case of anaerobic processes, oxidation reduction (redox) potential are required.

3. Need: Process Development for Highly Regulated Therapies

The process development in the growth and differentiation of stem cells is in the very beginning as there is no process so far gaining therapeutic amounts of clinical material. At the moment lots of manual work and empiric knowledge leads to the need of automation and consequently the set up of the first production scale process. The first steps are now undertaken to gain insight in the importance and relevance of the process parameters and variables: even small changes of the dissolved oxygen level, the pH or the medium composition can result in significant changes in the differentiation pattern and the proliferative potential. Efforts in process development to untangle the effects of acid (such as lactate) production, pH, medium utilisation and differences in oxygen tension on e.g. hematopoietic cell cultures have proven that each of the parameters seems to influence stem and progenitor cell responses. Only a set-up providing utterly accurate data and treatment of the highly sensitive cells can keep up with the requirements of stem cell research.

problems during the scale-up. It is not known how many promising clones could not be identified due to unfavourable process conditions in the simple bioreactors in comparison to the controlled fermenters (e.g. shaking vs. stirred, batch vs. fed-batch and buffers vs. pH-control).

So it was necessary to find a tool to bridge the gap between high throughput and the reproduction of later process conditions. Of course it was possible to set up multiple fermenters such as the lab suite at Merck but the acquisition as well as the running costs drove engineers and researchers to design new set-ups to cope with the increasing numbers of experiments under process conditions. Therefore, 1964 Holmström and Hedén introduced already a highly flexible 6 fold set-up with a small working volume of 80–800mL for anaerobic as well as aerobic fermentations. The design showed clearly the chemical background of the researches but the reactors were individually controlled for pH, temperature and foam.

In the following years the trend tended, nevertheless, to single benchtop fermenters and got a major boost with

the introduction of computer aided process control in the 70s when New Brunswick Scientific – well known so far for shakers – brought the first PC-controlled fermenter with supervisory software and data logging to the market. With this background it was possible to automate process control, lowering the necessity of sampling and devise processes able to be monitored, both saving manpower and minimising process variations.

As the research field and consequently production aspects focussed more and more on details like metabolic fluxes and cellular pathways the need grew for reliable reproducible and – of course scalable fermentation conditions. It was and still is very important to distinguish between the dynamics of the cell reaction and those of the environment, hence the vessel itself. These requirements led to the so-called high performance bioreactors. Flexible software control systems are implemented rather than classical single purpose controllers. Equipment suppliers were Applikon, B.Braun and Infors starting their development of small-scale bioreactors with 1–2 L working volume in the early 80s.

As many of these first generation small-scale bioreactors allowed single-vessel operation only, a logical consequence was the revival of parallel set-ups to ease the handling and increase the experimental throughput even in high performance bioreactors. The first parallel systems in the market were the Biostat Quattro by B.Braun (now Sartorius) and the Sixfors by Infors beginning of the 90s allowing the parallel operation of 4 respectively 6 vessels of working volumes of appr. 800 mL. In the mid 90s Dasgip addressed researches still relying on shaking flasks with a 16fold pH-controlled shaking flask system for batch and chemostat processes. End of the 90's Dasgip followed with its 16fold stirred vessels system and unto now implemented all control algorithms known from the fully equipped fermenters in a modular approach allowing combining its functions on request.

Today, and even more in the future, the bioreactors' design will depend on the process requirements and the industries they are utilised in. Fermentation and cultivation nowadays takes place in industries from feed, food, cosmetics, pharma, chemistry and medicine and have therefore manifold tasks and demands. The trend shifts from "one-size-fits-all" fermenters to highly versatile and adjustable tools leading to highly customised solutions.

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