doi:10.2533/chimia.2009.327 Chimia 63 (2009) 327–330

Lonza: Biotechnology – A Key Ingredient for Success in the Future

Karen T. Robins*, Antonio Osorio-Lozada, Manuela Avi, and Hans-Peter Meyer

Abstract: Lonza began as a small Swiss electricity company and it has successfully adapted to change throughout its history to become a global custom manufacturing company serving the needs of the life-science industry. One of the crucial decisions and changes in its development was the implementation of biotechnology. This article outlines briefly the history of change during Lonza's development, some of the problems that confronted the chemical industry approximately a decade ago and how they affected the area of biotransformation. There are still many chemical reactions that are difficult, inefficient and environmentally unfriendly. Lonza believes that some of these problems can be solved by biotechnology if the biocatalytic platform can be widened and improved so that the biocatalysts can be easily integrated into a chemical process.

Keywords: Biocatalytic platform · Biotechnology · Lonza

Lonza – A History of Changes

Since ancient times, human beings used wood, charcoal and later anthracite and gases to catalyze and fire the transformation of matter at temperatures of up to 1300 °C. With the appearance of electricity and the development of the electric arc furnace by the French chemist Henri Moissan, reaction temperatures of up to 3500 °C were now within reach for chemical transformation. Lonza started in 1887 as a producer of calcium carbide and acetylene using the electricity from turbines powered by the steep waters of the Lonza river to run its ovens (Fig. 1).

$$CaO + 3C \rightarrow CaC_2 + CO$$

Calcium carbide reacts with water to give the gas acetylene used for illumination purposes. The calcium carbide market collapsed as acetylene was increasingly replaced by electricity for lighting. However, innovative minds found other outlets for calcium carbide such as calcium cyanamide (fertilizer) and as a basis for the production of *e.g.* acetic acid anhydride and many other chemicals resulting in a carbide boom during the First World War and lasting well into the 1950s.

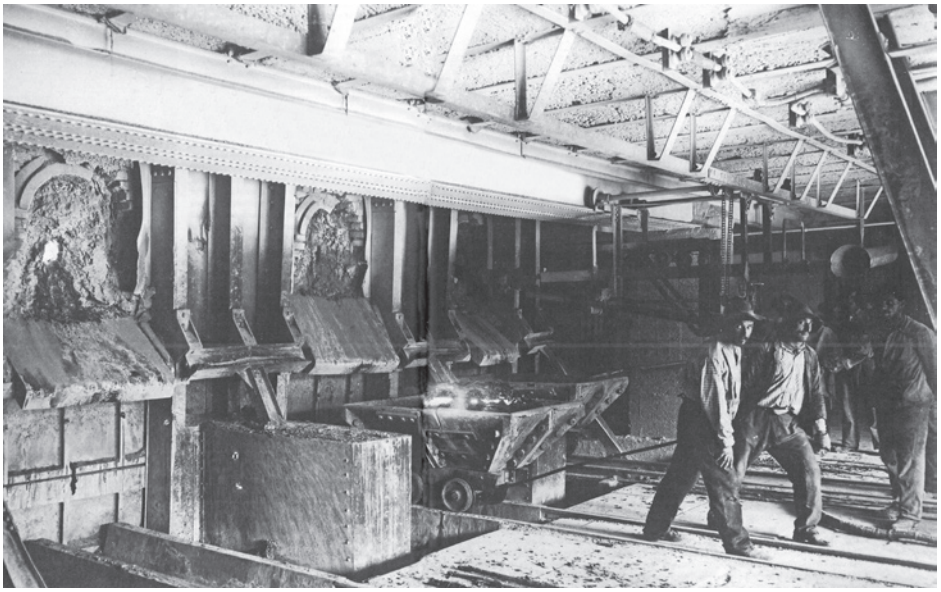


Fig. 1. Lonza's calcium carbide oven at the turn of the last century (Copyright Lonza Group Ltd, Basel).

In parallel to these chemical innovations and starting at the turn of the 19th century the principles of fermentation and biotransformation were established in laboratories in the rest of the world. These innovations would be used by Lonza some 75 years later. Kühne used the name 'enzyme' for the first time and Berzelius described the catalytic reaction of starch hydrolysis by the enzyme diastase. Christian Hansen was the first to start an enzyme laboratory and company followed by J. Takamine and Röhm. Emil Fischer elaborated further the essentials of enzyme catalysis and Eduard Buchner published a description of fermentation with cell free extracts from yeasts.

Back to chemistry: Lonza, who had used coal for the last 50 years, was suddenly coerced to yet another change, as oil started to replace coal. A cracker (one of the smallest worldwide) was constructed in the 1960s. But small is relative as the cracker uses 12,500 liters of naphtha per hour – the same amount as a Boeing 747 at full throttle.

Through all these changes and years Lonza had become a fine chemical company producing even more complex molecules, including chiral intermediates and active ingredients. This lead naturally to the use of biotechnology in the early 1980s and yet another crucial change occurred after 80 years of pure chemistry. Today

*Correspondence: Ms K. T. Robins
Lonza AG
CH-3930 Visp
Tel.: +41 27 948 6932
Fax: +41 27 947 6932
E-mail: karen.robins@lonza.com

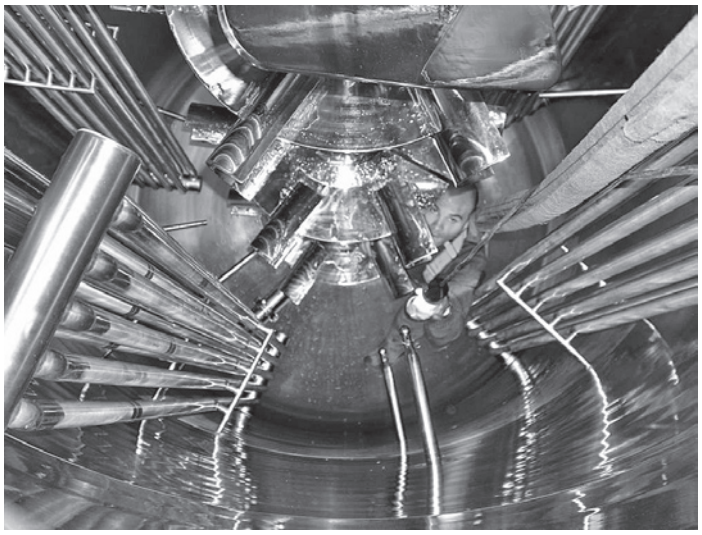


Fig. 2. Interior of a Lonza 15 m^3 high performance bioreactor used for high cell density fermentations (HCDF). The vessel is also explosion proof for the HCDF and production of enzymes with methylotrophic yeasts such as *Pichia pastoris*. Well visible are the cooling baffles which allow a cooling capacity of 650 kW. The stirring power input is 75 kW.[1] (Copyright Lonza Group Ltd, Basel)

biotechnology has not only conquered all divisions within our company (Fig. 2), but we are now also one of the world's leading manufacturers of monoclonal antibodies, and cell therapy services are part of our custom manufacturing business.

Lonza – Our World Today

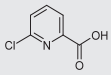
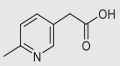
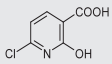
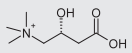
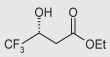
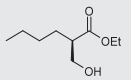
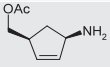
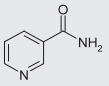
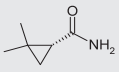
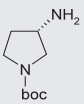
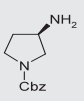
About 10 years ago while biopharmaceuticals were experiencing a growing market, the area of biotransformation suffered a set back. The chemical industry was seriously affected by over-capacity and extreme time pressure along with a decrease in the number of new chemical entities (NCEs) and increased failure rate in clinical trials. Lonza adapted to these changes by increasing the throughput of projects in the R&D. The biotransformation group could no longer compete with the pure chemical synthesis within the short time frames given for the projects. This forced Lonza to re-think its strategy by working on second generation processes to alleviate the time pressure. The benefit from Lonza's extensive strain collection that had been built up over the last 20 years was extended by mining our collection of over 700 wild type organisms for known and novel enzyme activities. Libraries of strains covering different enzyme classes and geni known for their versatile metabolism like *Rhodococcus*, *Pseudomonas* and *Streptomyces* were set up in deep well microtitre plates to enable high throughput screening protocols (see Table 1).[2] In the same manner large libraries of commercially available proteases, lipase and esterases were also established. External collaborations became even more important because Lonza could profit from the expertise of specialists in each respective field as required.

In the last few years the biotransformation group has also profited from Lonza's proprietary expression system XS Technologies™[30] that have been developed in the biopharmaceutical division for the production of microbial peptides and proteins. These systems[30] have been developed for *Escherichia coli*, *Pichia pastoris*, *Bacillus subtilis* and *Pseudomonas* species. The advantages of these expression systems are that they have high expression levels, and cheap, non-toxic inducers, extremely low background and high cell density fermentation protocols for *E. coli* and *P. pastoris* can be used.

Perspective

Today there is a growing academic, public and private interest in industrial (or

Table 1. Lonza's current biocatalytic toolbox.

Reaction class	Reaction type	Substrate class	Example of products	Ref.[a]
Oxidation	Methyl group oxidation	heteroaromatic compounds		[**3**]
	Ethyl group oxidation	heteroaromatic compounds		[**4**]
	Selective oxidation	polyols		[**5**,6]
	Hydroxylation	heteroaromatic carboxylic acids		[7,8,**9**,10]
		stereoselective hydroxylation of unactivated carbons		[**11**,12]
Reduction	Carbonyl reduction	β-oxo esters		[13,**14**]
	Double bond reduction	activated enols		[15,**16**]
		alkenes		b
Hydrolysis	Acetyl hydrolysis	α-aminoacids Penicillin		[17,**18**]
	Ester hydrolysis	α-hydroxy carboxylic acids esters		[**19**]
	Nitrile partial hydrolysis	*N*-containing heterocyclic cyanides		[20–23,**24**]
	Amide hydrolysis	*N*-containing heterocylic 2-carboxamides		[21,**25**,26,27]
Amination	Transamination	*N*-containing-3-amino heterocycles (asymmetric synthesis)		[**28**]
		N-containing-3-oxo heterocycles (racemic resolution)		[**29**]

[a]References in bold refer to the product examples. [b]R: Custom synthesis (undisclosed information)

Table 2. Reactions identified to be inefficient and/or environmentally unfriendly by ACS GCI[32] and Lonza and their biocatalytic alternative.

Reaction	Problems	Biocatalytic alternative	Disadvantages of biocatalysis
amide formation	poor atom economy reagents	peptidases, acylases, amidase, nitrile hydratases	narrow substrate range, solubility problems
reduction of amides	hydride reagents	–	only one organism known, research in its infancy
oxidation, hydroxylation, epoxidation	chlorinated solvents, heavy metals in catalysts, allylic oxidation, selectivity (regio and chemo)	cytochrome P450s, monooxygenases, dioxygenases, Baeyer Villiger monooxygenases, laccases, *etc.*	stability of enzymes, substrate range, low enzyme expression
asymmetric synthesis of amines	reductive amination methods limited to specific cases and asymmetric version difficult to realize	transaminases	reaction equilibrium on the wrong side, very few (*R*)-selective enzymes known
asymmetric hydrogenation	unfunctionalized olefins/ enamines/imines	enoate reductase	only activated double bonds
fluorination	harsh conditions, corrosive and hazardous reagents	fluorinase	narrow substrate range, stability of enzyme

Table 3. A selection of interesting technologies for Lonza.

	Traditional Technology	Developing Technology	Advantage
Upstream	Fermentation	Microbioreactors	Modular systems Flexibility Cost savings Space saving Speed Process simplification
		Electro-Kinetic-Bioreactors[37]	
	Whole cells or commercial enzymes (some immobilized)	Immobilization lamination of microorganisms[38] CLEA®s[39] other matrices	
	Aqueous and organic systems	Unconventional media ionic liquids expanded liquids supercritical solvents	
Downstream	Extraction Crystallization Distillation	ISPR 2-Phase systems membrane based resin based precipitation	Remove inhibition factors Increase productivity Simplify work-up Environmentally friendly

white) biotechnology. The potential global market for industrial biotechnology is much larger than the market for red (pharmaceutical biotechnology). The number of industrial biocatalytic processes has increased over the last years and more biocatalytic reactions can easily be integrated in large scale chemical plants. This is assisted by several factors:

– the increased availability of different classes of commercial enzymes from companies like Novozymes, Amano, Libragen, EnzySource and Codexis that allows for fast screening and scale-up. The best example is the large number of commercially available ketoreductases acting on a large substrate spectrum and allowing for fast screening and scale-up which competes with the chemical equivalent both on efficiency and development time.
– the increasing availability of enzymes immobilized on hydrophilic or hydrophobic carriers assists the tuning of enzyme to the appropriate reaction media and leads to cost reduction through the possibility of recycling the enzyme. They are offered by several companies like Sprin, Resindion, Chiral Vision, Viazyme and CLEA Technologies.
– the combination of a deeper understanding of enzyme structure, maturing technologies like directed evolution, metagenomics, modeling and quantitative structure-activity relationship (3D-QSAR)[31] and increasing advances in instrumentation which allows extremely high throughput screening protocols to be executed.
– the increasing public and government awareness of global warming and the necessity to implement environmentally friendly processes. With this awareness injection of funds into the biotechnology sector, which is pushing advances in research, is increasing.

There are still improvements to be made on the available toolbox of biocatalysts. Unnatural substrates, low activity, low stability, product and substrate inhibition and their suitability for industrial processes often pose great challenges. In 2005 the ACS Green Chemistry Institute (GCI) together with global pharmaceutical corporations identified some key research areas,[32] where neither chemistry nor biotechnology offer reasonable solutions for efficient and environmentally friendly processes.

Lonza is also repeatedly confronted with a number of reactions that are difficult and inefficient to perform in a classical chemical manner. Lonza has an interest in saving resources and reducing the emission of green house gases, the use of organic solvents and the production of waste. The reactions identified to be critical from a chemical and environmental point of view overlap to some extent with the research areas identified by GCI (Table 2). Therefore Lonza is expanding the strategy followed in the last years and developing new approaches that will overcome existing shortcomings of biotechnology for broad industrial application.

One of the major goals is to expand the biocatalytic platform broadening the substrate spectrum of the existing collection and making new reactions accessible. In addition to the classical methods of enrichment and engineering metagenomic approaches[33,34] are also being considered since 99% of the microorganisms are not culturable.[35] Strain development for resistance of microorganisms to organic solvents,[36] high temperature and pH are also important aspects of biocatalytic processes development. The importance given to these areas is reflected in the fact that the biocatalytic toolbox project will be addressed within the Lonza Innovation for Future Technologies (LIFT) program.

Other challenges that Lonza is addressing are the introduction of new large scale technologies into a generally conservative industry where fermenters are the stalwart of biotechnology (see Table 3).

In the same manner innovation in product isolation and *in situ* product recovery (ISPR) need to be introduced to make the

biotechnological processes more efficient, more environmentally friendly and more economical. Lonza is already using novel, environmentally friendly technologies such as micro-, nano- and ultrafiltration, reversed osmosis and electrodialysis and is attempting to implement technologies still in development (for examples see Table 3) in order to achieve these goals.

Outlook

The economic potential for industrial biotechnology is enormous in theory. The global chemistry market of 2,292 billion US$ in 2008 is expected to grow to 3,235 billion US$ by 2015 and to 4,000 billion US$ by 2020.[40] However only about 50 billion US$ (not including biofuels) of today's chemical market are generated by biotechnology. Of these 50 billion US$ biotechnology products, about 25% are fine chemicals. The share of these biotechnologically produced fine chemicals is expected to grow from 8% to 60% between 2001 and 2010.[41] It is also estimated that at least 20% of the global chemicals could be produced by biotechnological means by 2020!

However, to realize this potential which seems to be significantly larger than the (red) pharmaceutical biotechnology market, substantial technological gaps must be bridged. There is a need for radical thinking in both technical terms and the way we collaborate. To foster out of the box thinking leading to truly innovative ideas, products and processes, Lonza for example has created the corporate program LIFT (Lonza Innovation for Future Technologies). This program finances long term, high risk and high reward approaches and products in all business sectors. The program includes the search for radically new approaches to improve chemical manufacturing and the BIOTRANS symposium is an excellent platform to catalyze truly innovative considerations.

Received: February 2, 2009

[1] H. P. Meyer, K. T. Robins, *Monatsh.* **2005**, *136*, 1269.
[2] W. A. Duetz, L. Rüedi, R. Hermann, K. O'Conner, J. Büchs, B. Witholt, *Appl. Environ. Microbiol.* **2000**, *66*, 2641.
[3] A. Kiener, *Angew. Chem Int. Ed. Engl.* **1992**, *31*, 774.
[4] A. Kiener, *Chemtech* **1995**, *9*, 31.
[5] F. Molinari, R. Gandolfi, R. Villa, E. Urban, A. Kiener, *Tetrahedron: Asymmetry* **2003**, *14*, 2041.
[6] T. Locher, E. M. Urban, F. Molinari, F. Aragozzini, J.-P. Roduit, PCT Patent Appl. No. WO00/22153, **2000**.
[7] P. Lehky, H. Kulla, S. Mischler, Eur. Patent Appl. EP 0152948B1, **1991**.
[8] H. G. Kulla, *Chimia*, **1991**, *45*, 81.
[9] A. Tinschert, A. Tschech, K. Heinzmann, A. Kiener, *Appl. Microbiol. Biotechnol.* **2000**, *53*, 185.
[10] A. Kiener, J.-P. Roduit, A. Tschech, A. Tinschert, K. Heinzmann, *Synlett* **1994**, *10*, 814.
[11] T. P. Zimmermann, K. T. Robins, J. Werlen, F. W. J. M. M. Hoeks in 'Chirality in Industry II', Eds. A. N. Collins, G. N. Sheldrake, J. Crosby, John Wiley and Sons Ltd, Chichester, **1997**, pp. 287–305.
[12] S. I. de Azevedo Wäsch, J. R. van der Plog, T. Maire, A. Lebreton, A. Kiener, T. Leisinger, *Appl. Environ. Microbiol.* **2002**, *68*, 2368.
[13] M. Petersen, O. Birch, S. Shimizu, A. Kiener, M.-L. Hischier, S. Thoni, PCT Patent Appl. No. WO99/42590 A1, **1999**.
[14] N. M. Shaw, K. T. Robins, A. Kiener, *Adv. Synth. Catal.* **2003**, *345*, 425.
[15] J. E. Leresche, H. P. Meyer, *Org. Process Res. Dev.* **2006**, *10*, 572.
[16] K. T. Robins, 11th Swiss-Japanese Joint Meeting on Biotechnology and Bioprocess Development, Minuso, Switzerland, **2008**.
[17] M. Sauter, D. Venetz, F. Henzen, D. Schmidhalter, G. Pfaffen, O. Werbitzky, PCT Patent Appl. No. WO97/33987, **1997**.
[18] W. Brieden, J. Schroer, C. Bernegger-Egli, E. M. Urban, M. Petersen, J.-P. Roduit, K. Berchtold, H. Breitbach, US Patent Appl. No. US7358073, **2008**.
[19] W. Brieden, A. Naughton, K. Robins, N. Shaw, A. Tinschert, T. Zimmermann, Ger. Patent Appl. No. DE19725802, **1998**.
[20] A. Kiener, J.-P. Roduit, J. Kohr, N. Shaw, Eur. Patent Appl. No. EP0686698 B1, **1995**.
[21] E. Eichhorn, J.-P. Roduit, N. Shaw, K. Heinzmann, A. Kiener, *Tetrahedron: Asymmetry* **1997**, *8*, 2533.
[22] M. Petersen, M. Sauter, *Chimia* **1999**, *53*, 608.
[23] H. Yamada, T. Nagasawa, Eur. Patent Appl. No. EP0307926 B1, **1993**.
[24] K. T. Robins, T. Nagasawa, PCT Patent Appl. No. WO99/05306, **1999**.
[25] T. Zimmermann, K. T. Robins, O. M. Birch, E. Boehlen, Eur. Patent Appl. No. EP0524604 B1, **1998**.
[26] W. Brieden, A. Naughton, K. T. Robins, N. Shaw, A. Tinschert, T. Zimmermann, PCT Patent Appl. No. WO98/01568, **1998**.
[27] N. M. Shaw, A. Naughton, K. T. Robins, A. Tinschert, E. Schmid, M.-L. Hischier, V. Venetz, J. Werlen, T. Zimmermann, W. Brieden, P. de Riedmatten, J.-P. Roduit, B. Zimmermann, R. Neumüller, *Org. Process Res. Dev*. **2002**, *6*, 497.
[28] M. Höhne, S. Kühl, K. T. Robins, U. T. Bornscheuer, *ChemBioChem*. **2008**, *9*, 363.
[29] M. Höhne, K. T. Robins, U. T. Bornscheuer, *Adv. Synth. Catal*. **2008**, *350*, 807.
[30] H. P. Meyer, J. Brass, C. Jungo, J. Klein, J. Wenger, R. Mommers, *BioProcess Int.* **2008**, *6*, (Suppl 4), 10.
[31] P. Braiuca, L. Boscarol, C. Ebert, P. Linda, L. Gardossi, *Adv. Synth. Catal.* **2006**, *348*, 773.
[32] D. J. C. Constable, P. J. Dunn, J. D. Hayler, G. R. Humphrey, J. L. Leazer Jr., R. J. Linderman, K. Lorenz, J. Manley, B. A. Pearlman, A. Wells, A. Zaks, T. Y. Zhang, *Green Chem.* **2007**, *9*, 411.
[33] J. Handelsman, *Microbiol. Mol. Biol. Rev.* **2004**, *68*, 669.
[34] J. A. Eisen, *PLOS Biol.* **2007**, *5*, 384.
[35] R. I. Amann, W. Ludwig, K. H. Schleifer, *Microbiol. Rev.* **1995**, *59*, 143.
[36] J. A. M. de Bont, *Trends Biotechnol*. **1998**, *16*, 493.
[37] C. J. Knowles, S. A. Jackman, L. Hong, R. Mustacchi, J. G. Sunderland PCT Patent Appl. No. WO 2004/046351 A1, **2004**.
[38] M. C. Flickinger, J. L. Gosse, K. Jannek, M. Fidelao, S. Charaniya, C. W. Solheid, L. E. Scriven, *PMSE Preprints* **2006**, *95*, 185.
[39] R. Schoevaart, M. W. Wolbers, M. Golubovic, M. Ottens, A. P. G. Kieboom, F. van Rantwijk, L. A. M. van der Wielen, R. A. Sheldon, *Biotechnol. Bioeng.* **2004**, *87*, 754.
[40] U. Perlitz, *CHEManager* **2008**, *14*, 4.
[41] G. Festel, J. Knoell, H. Goetz, H. Zink, *Chem. Ing. Tech.* **2004**, *76*, 3007.