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Biochemical Aspects of Microbial Product Synthesis: a Relook

G. Gallastegui[1]*, A. Larrañaga*[2]*, Antonio Avalos Ramirez*[3]*,*
and Thi Than Ha Pham[3,4]

[1] *Department of Chemical and Environmental Engineering, Faculty of Engineering Vitoria-Gasteiz, University of the Basque Country (UPV/EHU), Spain*
[2] *Department of Mining-Metallurgy Engineering and Materials Science & POLYMAT, Faculty of Engineering of Bilbao, University of the Basque Country (UPV/EHU), Spain*
[3] *Centre National en Électrochimie et en Technologies Environnementales, Shawinigan, Québec, Canada*
[4] *Université de Sherbrooke, Sherbrooke, Québec, Canada*

1.1 Introduction

Microbes are living unicellular or multicellular organisms (bacteria, archaea, most protozoa, and some fungi and algae) that must be greatly magnified to be seen. Despite their tiny size, they play an indispensable role for humanity and the health of ecosystems. For instance, until the discovery of an artificial nitrogen fixation process by the German chemists Fritz Haber and Carl Bosch in the first half of the 20th century, some soil microbes on the roots of peas, beans, and a few other plants were the solely responsible for the nitrogen release necessary for plants growth (Hager, 2008). This invention allowed to feed billions more people than the earth could support otherwise.

Besides, humanity has exploited some of the vast microbial diversity like miniature chemical factories for thousands of years in the production of fermented foods and drinks, such as wine, beer, yogurt, cheese and bread. In fact, the use of yeast as the biocatalyst in foodstuffs making is thought to have begun around the Neolithic period (ca. 10 000-4000 BCE), when early humans transitioned from hunter-gatherers to living in permanent farming communities (Rasmussen, 2015). Vinegar, the first bio-based chemical (not intended as a beverage) produced at a commercial scale was known, used and traded internationally before the time of the Roman Empire (Licht, 2014).

The staggering transformation undergone by biotechnology from serendipity and black-box concepts to rational science and increasing understanding of biological systems has led to not only a direct influence of microbes on human lives, but the emergence of new industries that take advantage of these organisms in

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large-scale processes devoted to the manufacture of high value-added compounds, energy production and environmental protection. Nevertheless, scientists and engineers are still discovering the broad array of complex signalling that microorganisms have developed to ensure their survival in a wide range of environmental conditions, and making their utmost effort to direct them towards our own ends (Manzoni et al., 2016). In this chapter, a brief summary regarding the historical production of microbial products, their niche in the current global market and the importance of microbial sensing (and other new disciplines) to convert biological systems in industrially relevant actors is presented.

1.2 History of Industrial Production of Microbial Products

In the 1800s, Louis Pasteur (and later Eduard Buchner) proved that fermentation was the result of microbial activity and, consequently, the different types of fermentations were associated with different types of microorganisms. In more recent times (1928), Alexander Fleming understood that the *Penicillium* mould produces an antibacterial bio-chemical (antibiotics discovery), which was extracted, isolated and named penicillin. Subsequent periods of conflicts (e.g., World Wars I and II) intensified the needs of the population and, at the same time, the creativity and inventiveness of scientists and engineers, who developed large-scale fermentation techniques to make industrial quantities of drugs, such as penicillin, and biofuels, such as biobutanol and glycerol, giving rise to industrial biotechnology. In 1952, Austrian chemists at Biochemie (now Sandoz) developed the first acid-stable form of penicillin (Penicillin V) suitable for oral-administration and achieved an extraordinary success in the treatment of infections during World War II (Williams, 2013).

Biobutanol production is recognized as one of the oldest industrial-scale fermentation processes. It was generated by anaerobic ABE (acetone–butanol–ethanol) fermentation of sugar extract using solventogenic clostridia strains, with a typical butanol:acetone:ethanol mass fraction ratio around 6:3:1. Until the 1920s, acetone was the most sought-after bioproduct of commercial interest. An emerging automotive paint industry and the need of quick-drying lacquers, such as butyl acetate, changed the economic landscape and by 1927 butanol displaced acetone as the target product (Rangaswamy et al., 2012). From 1945 to 1960, about two thirds of the butanol production in North America was based on the conventional ABE fermentation. Nevertheless, butanol yield by anaerobic fermentation remained sub-optimal, and this biobased product was progressively replaced by low cost petrochemical production (Maiti et al., 2016).

When Watson and Crick (with the valuable help from Wilkins and Franklin) worked out the structure of DNA in 1953, they barely imagined that this latter discovery supposed a milestone in the development of modern industrial biotechnology. Thus, in the following decades traditional industrial biotechnology merged with molecular biology to yield more than 40 biopharmaceutical products, such as erythropoietin, human growth hormone and interferons (Demain, 2000). Since then, biotechnology has steadily developed and now plays a key role in several industrial sectors, such as industrial applications, food and beverages, nutritional and pharmaceuticals or plastics and fibers, providing both high value products and commodity products (Heux et al., 2015).

Although, as shown in the previous paragraphs, the use of microorganisms and enzymes for the production of essential items has a long history, the recent linguistic term “white biotechnology” has been assigned to the application of biotechnology for the processing and production of chemicals, materials and energy. It is based on microbial fermentation processes and it works with nature in order to maximize and optimize existing biochemical pathways that can be used in manufacturing. The development of cost effective fermentation processes has allowed industry to target previously abandoned fermentation products and new ones which used to be of small interest for the naphtha-relying chemical industry, such as succinic acid or lactic acid. In the latter case, and although the chemical synthesis of lactic acid from petrochemical feedstock is more familiar to chemists, approximately 90% of its production is accomplished by microbial fermentation (Wang et al., 2015). Nowadays, this platform molecule is used as a building block for the synthesis of chemicals such as acrylic acid and esters (by catalytic dehydration), propylene glycol (by hydrogenolysis) and lactic acid esters (by esterification) (Figure 1.1).

1.2.1 Advances of Biochemical Engineering and Their Effects on Global Market of Microbial Products

Economic viability of bio-derived products, especially in the case of biofuels, has been traditionally limited to a large extent by the selection of cheap carbon-rich raw materials as feedstock, applied production mode, downstream processing and the scarcity of naturally occurring microorganisms that are able to deliver the desired compounds at a high production-rate. Conventional bio-based products ultimately turned out so expensive to compete with petroleum-derived chemicals that they were hardly worth producing.

Despite these drawbacks, advances in biotechnology in recent years have enabled the reengineering of the bioprocesses incorporating several transformation or purification steps into only one, reducing time and operating costs. This has involved the increase of bioprocesses yield, boosting production of biobased materials. Currently, biotechnology advances (microbial, enzymatic and biology engineering) can be considered among the new technological revolutions, having huge impacts in industry, society and economy, as nanotechnology-materials, informatics and artificial intelligence.

Therefore, a resurgence in the production of fermentation chemicals including biofuels, chemical building blocks, such as organic acids, amino acids, alcohols (diols, thiols) and specialty chemicals, such as surfactants, thickeners, enzymes, antibiotics and fine chemicals (pigments, fragrances, etc.) is expected in the years to come. The global fermentation chemicals market was $51.83 \cdot 10^6$ tons in 2013 and is expected to reach $70.76 \cdot 10^6$ tons by 2020, growing at a Compound Annual Growth Rate (CAGR) of 4.5% from 2014 to 2020, with North America emerging as the leading regional market and accounting for 33.8% of total market volume (Grand View Research, 2014).

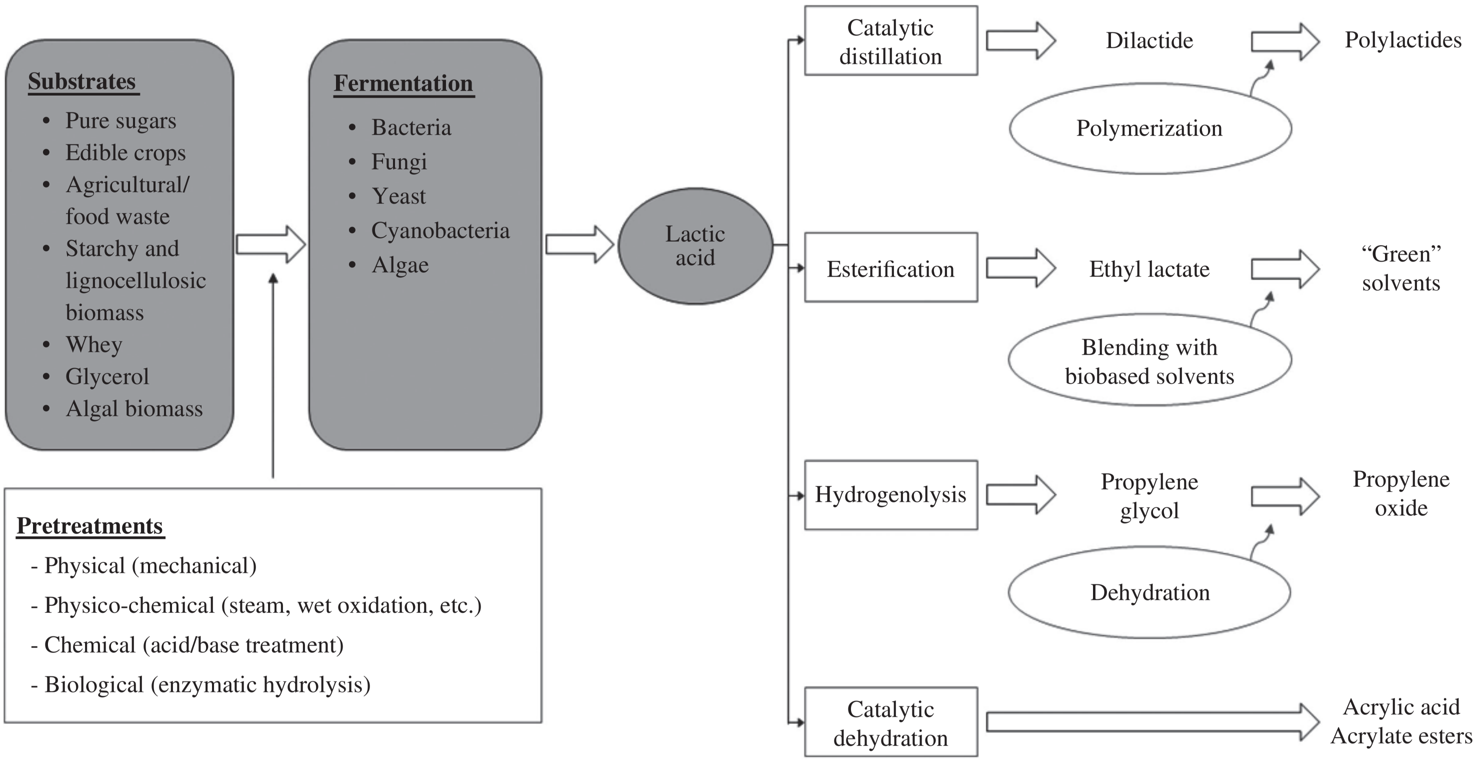

Figure 1.1 Production of lactic acid by microbial fermentation and its derivatives.

Among all the possible products and value streams obtained from biomass in the biorefineries, the chemical market (both commodity and fine chemicals) is expected to grow at a rate almost double to that of biofuels, since chemicals are on average priced 15 times higher than energy (Deloitte, 2014), which will entail that by 2025 at least a 45% share of chemicals will be accounted by biorenewable chemicals in the USA (Bardhan et al., 2015). In Europe, biobased chemicals account at present time for 5.5% of total turnover for chemicals produced in the EU, and they are expected to grow up by over 5% per year, until reaching a total proceeding of sales of about $44 billion in 2020 (Schneider et al., 2016).

Additionally, compared to the production of first-generation biofuels, the production of more bio-based materials will not have a price enhancing effect on food products (van Haveren et al., 2008) since it would be based on the utilisation of the carbohydrate fraction of lignocellulosic biomass (i.e., cellulose and hemicellulose) and inedible oil seed crops or algal oil as feedstock. In the report edited by Deloitte, the authors estimated that replacing all petrochemicals would require just 5% of agricultural biomass production and global arable land, which is about 60 times less than what would be required to replace all fossil energy (Deloitte, 2014). Straathof (2014) reported in his extensive review about the biochemical formation of commodity chemicals from biomass that 21 of the compounds cited are already commercially produced (including carboxylic acids, alcohols and amino acids), and at least 9 others have been tested at pilot scale. Frost & Sullivan (2011) calculated that the global market for fermentation derived fine chemicals was $16 billion in 2009.

However, as with all the main human inventions, modern biotechnology presents contradictions and confronts the ethic principles of our societies. It is at the same time a tool to face the main human challenges (energy needs, environment conservation, human health, food supplying, etc.), but it also represents high risks to the environment and to human health if it is not properly used. Thus, even if the use of genetically modified microorganisms (GMM) has offered advantages over traditional methods of improving chemical selectivity and the supply of desired bioproducts thus reducing production cost (Bullis, 2013), their implementation has been controversial among the general public, especially when these microorganisms contain genes introduced from other species. Taking into account that newly isolated strains of microorganisms and GMMs can be patented, pressing questions arise regarding whether these organisms have any place in our ethical considerations and how they should be treated (Cockell, 2011).

Microbial sensing, microbial nanocontrol, smart fermentations, smart enzymatic systems, and the bioinformatics can be included amongst the main new developments which will revolute the biotechnology itself. The discovery in the 1970s of sophisticated cell-cell communication mechanisms (quorum sensing), became evident that microbial populations are synchronized at a certain cell density, by means of diverse signalling molecules that are synthesized and secreted by the microbes themselves (Bassler and Losick, 2006). Thus, the deep knowledge of the quorum sensing regulation on microbial metabolism and the control of microbial sensing will allow the complete redesign of all bioprocesses in terms of microbial signalization. We will be able to control better the bioprocesses (shortening residence times, controlling contamination, increasing production yields), to change the way to fight against microbial illnesses with new molecules (other than antibiotics) or antagonist bacteria, to improve life quality of livestock, to protect better the environment, etc.

As most of technological advances, several of these improvements obtained by microbial sensing appear so far or impossible to develop with time. But the biotechnological revolution is highly associated to the technological advances of other science branches, such as materials science, photonics, electronics, microscopy, and others. The development of new powerful and high-sensitive analytic equipment is essential to identify microbial signals and to construct mapping interactions (Moon et al., 2010).

In the near future, transformation of biomass into chemicals using enzymes or cells will be implemented with success only if the production process is more attractive than for alternative options (petrochemical route) to produce these chemicals based on their ecologic, social, and economic value. The present book tries to show a brief portrait of the state of the art of "four magic e" bioproducts (large-scale microbial fermentation products considering economic, ethical, environmental, and engineering aspects) and how microbial sensing has a main role in their present and future production.

1.2.2 Importance of Microbial Sensing in Product Formation

However, microbial sensing is so wide that it is necessary to delimit the goal of this work. This book presents a comparison among the different control concepts for the carbon transformation by microorganisms, analysing microbial, biochemical and molecular biology control concepts. The microbial sensing concept is emphasized showing the potentiality to use it for fermentation control and predict the scaling up.

According to the combination signals' origin-cell sensor, the microbial sensing defined as the identification of internal and external signals by microbial sensors can be classed in five main categories (Figure 1.2), as follows:

1) Internal signals. The molecules to be captured by microbial sensors are produced by the same cell in its cytoplasm. These signals are employed by the cell to control the production of functional cell structures (proteins, enzymes, organelles, etc.), as well as to control the cell aging.
2) Signals in a homogenous microbial community. They are produced by cells of the same species in a homogenous microbial community to control the interactions among them, for example the quorum sensing to conglomerate and begin the formation of homogenous biofilm.
3) Signals in a heterogeneous microbial community. They are produced by the cells of the different species present in a heterogeneous microbial community to develop synergistic or antagonistic interactions among them. For example, production of toxic molecules to inhibit the growth of competitive species.
4) Signals produced by the effect of environmental factors. They are caused by the effect of extracellular environmental factors such as light, humidity, ionic strength, pH or temperature.
5) Signals in host bodies. They are produced by both cells and infected bodies. The interactions among them can be both synergistic (e.g., probiotic microorganisms in human or animal gut) or antagonistic (e.g., pathogen infections).

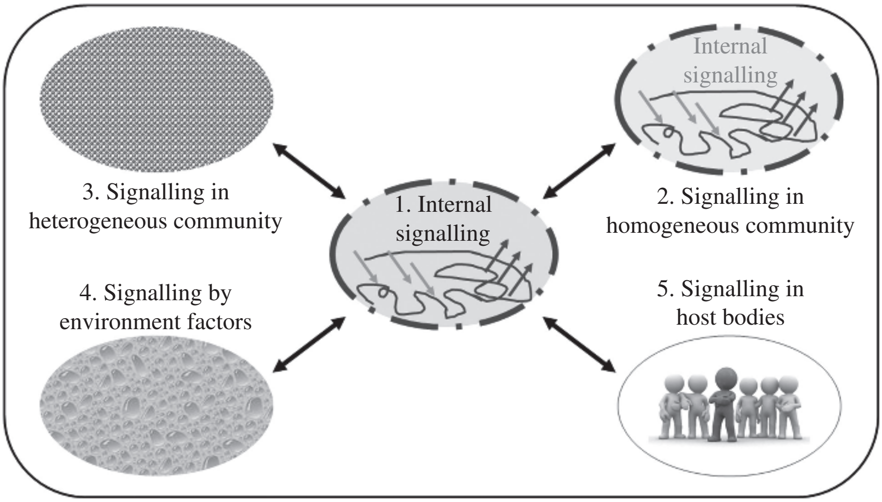

Figure 1.2 Microbial sensing classification according to signal origin. (*See insert for color representation of this figure.*)

The effect of environmental factors is the most known signalling mechanism of microbial sensing since their role has been clearly defined for several bioprocesses, such as alcoholic fermentations. Besides, the major efforts undertaken regarding the understanding of infections by pathogenic agents and host health and homeostasis, has contributed to gain appropriate and reliable information about the signals produced in host bodies (5th mechanism) (Kendall and Sperandio, 2016).

The remaining categories (1-3) became important at the end of last century, and it is precisely these categories which represent the state-of-the-art in this field. However, empiric and scientific data related to the first three cases is scarce, and there are still many gaps and uncertainties in the relevant scientific knowledge about signalling processing. In addition, the experimentation with animal or human models is a very sensitive subject constrained by ethic rules which must be respected, limiting the number and the quality of the scientific research. Therefore, even if all kind of microbial sensing is now studied around the world, there is a lack of updated reviews showing the most important advances done during the last 5-10 years.

1.3 Conclusion

The present book documents and critiques those aspects related to microbial production and performance, including the type of carbon source, cellular and biochemical control over the microbial products, etc. from the perspectives of molecular biology and biochemistry. Together with these aspects, the ways to quantitatively and qualitatively control the microbial products as well as approaches to scale-up and optimize these processes along with specific future market perspectives and policy initiatives are thoroughly reviewed in this book. Accordingly, it will be of particular interest for those researchers working in the field of microbial biotechnology but it will also cover those areas related to molecular biology, biochemistry and materials science, among others.

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