Effects of modified atmospheres on lab-grown fungi cultures

MCQ Gas Blender 100 Application

Introduction

Fungi is a term that refers to a large family of organisms (mushroom, yeasts, moulds etc..), which counts more then 1.5 million species on Earth. Fungi have a strong link with most terrestrial (and some aquatic) ecosystems. Fungi act as a major decomposer, degrading organic matter to inorganic molecules which are re-integrated in the biochemical cycles through assimilation by plants or other organisms. The human use of fungi is practice rooted in the past which is currently extremely extensive. Food-related applications are one of the major example, for mushroom farming and gathering represent a large industries in many countries. New, interesting application fields have been discovered through the years, all of them connected with the capacity of fungi to produce a wide range of products with important biological properties. Nowadays a large share industrial production of antibiotics, vitamins and anti-cancer cholesterol-lowering drugs relies on cultured fungi. Productivity and quality of fungi cultures strongly depend on growing conditions.

A micrograph of Penicillium and its spores.

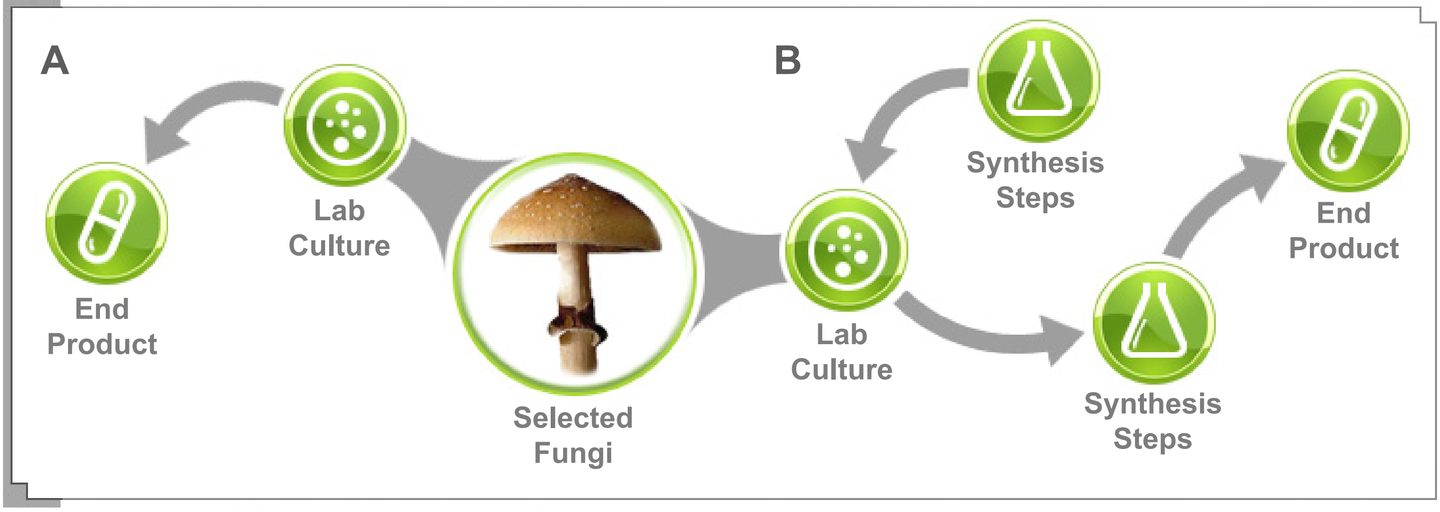
Among all the parameters that affect a culture, the surrounding atmosphere composition is one of the most crucial. Fungi are cultured in aerobic or anaerobic conditions, and most recently the use of modified atmosphere (greatly different from the standard air composition) have been implemented. For all these reasons, R&D demands professional instruments in order to perform accurate studies on fungi culture, and the MCQ Instruments faces these needs with its Gas Blender 100 Series. Highly accurate, reliable and easy to use, the MCQ Gas Blender 100 is the ideal instrument to create 3 component gas mixtures and to manage them dynamically.

Pharmaceutical applications

- Natural compounds expression

Fungi produce a wide array of natural compounds with useful biological activities that are of great interest for pharmaceutical applications. Fungi expression capacity is used for industrial production of metabolites that are the major source of pharmacologically active drugs. The major example of fungi culture application is the production of antibiotics, especially the penicillins. Although natural occurring penicillins are known to be good antibiotic, modern penicillins are semisynthetic compounds, structurally altered, through chemical modifications, to obtain products with improved biological activities and specific desirable properties. Currently there are many drugs produced by fungi, like the immunosuppressant ciclisporin (commonly used during transplant surgery), the fusidic acid, whose main application is the bacterial infection control and the statins, used to inhibit cholesterol synthesis. The use of fungi-derived compounds is a promising application field, and in the next few years, many new products with cardiovascular, anticancer, antiviral,

A) Products naturally expressed by fungi are extracted and refined to produce drugs.
B) Fungi cultures as new syrthetic route, used to replace complicated and expensive steps.



antibacterial, antiparasitic, anti-inflammatory, and anti-diabetic properties are supposed to be refined to become globally available. Aside from the production of antibiotics and drugs, fungi are also used for the expression of various enzymes particularly useful for industrial processes. Moreover fungi act efficiently as biological pesticides to control weeds, plants diseases and insect pests.

- Future base for R&D

The use of natural compounds expressed by mushrooms, yeasts and moulds is not the only promising application of fungi R&D. For the pharmaceutical industrial production fungi don't have to produce compounds to be useful. Fungi are organisms capable to bring about a variety of chemical transformations in a reliable and reproducible way, and therefore can be used in the intermediate steps of compounds synthesis. The production of steroids is the major example of this application. During their manufacturing process, base steroids are transferred in a fungi culture in order to be opportunely transformed. About twenty to forty hours later the new processed steroids can be extracted. This relatively simple procedure avoids up to thirty steps of pure chemistry, with a direct positive impact on process productivity and production costs. Success with steroid transformation has led to a relevant consideration: fungi can be the base for the future pharmaceutical R&D. Similar approaches can be applied to a wide range of active compounds, to reduce natural toxicity or to enhance biological activity. Using fungi in this way gives the possibility to easily manufacture specific compounds which would be otherwise very difficult and expensive to produce by direct chemical synthesis.

Shelf life enhancement

Fungi R&D is not only focused on the growth of these organisms, but also on their suppression. Acting as decomposers, mushrooms and moulds are the main factor (along with bacteria) that contributes to shorten the shelf-life of stored foods. Mushrooms and moulds grow from spores, which are extremely diffused and virtually present (even in small amount) on the surface of every food. In stored products, the fungi growth control is then achieved through the inhibition of spores proliferation. Inhibition is obtained through storing products in a modified atmosphere, usually poor of oxygen. This technique, called Modified Atmosphere Packaging, is currently the most widespread method used to enhance the shelf-life of general goods (more information regarding the Modified Atmosphere Packaging and the related MCQ Gas Blender 100 Series applications can be found here).

Fungi culture atmosphere

The composition of the atmosphere surrounding the culture strongly affects the growth rate of fungi. For all the fungi applications previously described, a proper optimization of growth/inhibition process parameters is definitely required and represents a research field which modern science is currently focused on. Fungi are usually grown in air (standard atmosphere) but, especially in the last 10 years, many studies have proved the crucial influence of modified atmospheres on cultures. Depending on the type of fungi in study, both high [1] and low [2,3] oxygen concentrations in gas phase can be used as a growth-promoting factor.

Increasing the CO_2 concentration in the culture atmosphere usually involves a growth-suppression effect, detrimental for drugs expression [4,5] but useful for shelf-life enhancement [6]. The combination of low oxygen (>0,5%) and high CO_2 (>15%) is a condition used both to suppress [7,8] and to enhance [9] fungi proliferation, while high oxygen high CO_2 atmospheres are less common but still promising [10].

Gas Blending: common solution

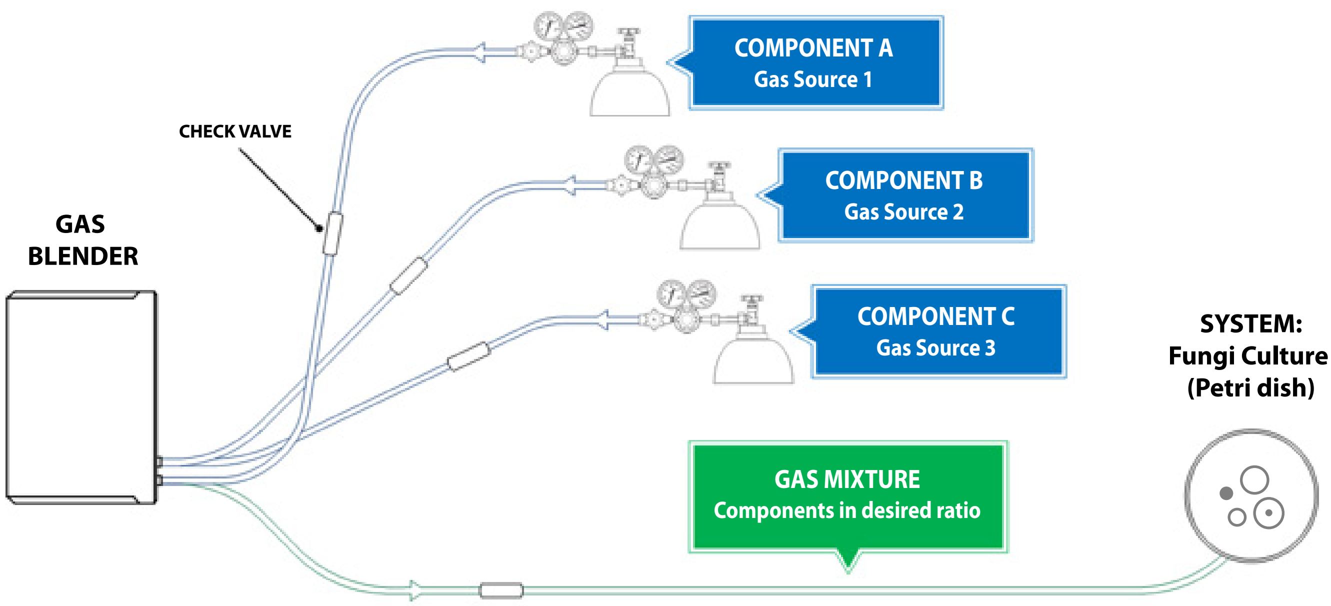
The standard gas blending solution used for culture application consists in 3 single channel mass flow controllers (usually calibrated for nitrogen, oxygen and carbon dioxide), which work independently from each other. All mass flow controllers are connected to an external control unit, used to blend the mixture and to manage the mixture settings (i.e. the relative amount of each compound in the gaseous phase). This standard hardware configuration demands a considerable amount of laboratory space, resulting inappropriate in case of installation under fume hoods, and the mixture parameters changes are often laborious, making it almost impossible to work with culture conditions that requires dynamic mixture adjustments.

• MCQ solution

The MCQ solution for gas mixing applications is the MCQ Gas Blender 100 Series. The Gas Blender 100 Series is a professional instrument designed for high precision 3 components mixture blending. The MCQ ensures a precision of 1% of the setpoint, a repeatability of 0,16% of the reading value and a 50 ms response time for setpoint changes (currently the fastest on the market). The Gas Blender 100 Series requires no external control unit, for all the mixture parameters and other gas settings can be managed by the user with the MCQ Gas Mixer Manager, the software specifically created to access all the Gas Blender features. The software only requires a desktop or a laptop computers compatible with any Windows operating systems starting from Windows XP.

• Hardware Configuration

An example of MCQ Gas Blender 100 Series hardware configuration is represented in the image below. The instrument works with dry, non-aggressive gases. The gas sources can be both pure or mixtures (in our example pure gases have been chosen for simplicity). The gas cylinders are connected to the instrument through 6 mm diameter tubes and a check valve is installed along each line as backflow prevention device. Each gas media is connected and controlled by a dedicated channel of the Gas Blender 100. Another 6 mm tube finally connects the instrument to the working system in which the experiment takes place. A PC is connected to the Gas Blender through a simple USB connection. All the instruments features and the gas mixture properties can then be manage with the Gas Mixer Manager software.



MCQ Gas Blender hardware configuration

References

[1] **Wen-Xian Zhang and Jian-Jiang Zhong**, *Effect of oxygen concentration in gas phase on sporulation and individual ganoderic acids accumulation in liquid static culture of Ganoderma lucidum* – J Biosci Bioeng 109, 1 (2010) 37-40.

[2] **William R. Kenealy and Diane M. Dietrich**, *Growth and fermentation responses of Phanerochaete chrysosporium to O_2 limitation* – Enzyme Microb Technol 34, 2 (2004) 490-498.

[3] **S. Samapundo, B. De Meulenaer, A. Atukwase, J. Debevere and F. Devlieghere**, *The influence of modified atmospheres and their interaction with water activity on the radial growth and fumonisin B1 production of Fusarium verticillioides and F. proliferatum on corn. Part II: The effect of initial headspace oygen concentration* - Int J Food Microbiol 113, 3 (2007) 339-345.

[4] **M.H. Taniwaki, A.D. Hocking, J.I. Pitt and G.H. Fleet**, *Growth and mycotoxin production by fungi in atmospheres containing 80% carbon dioxide and 20% oxygen* – Int J Food Microbiol 143, 3 (2010) 218-225.

[5] **S. Samapundo, B. De Meulenaer, A. Atukwase, J. Debevere and F. Devlieghere**, *The influence of modified atmospheres and their interaction with water activity on the radial growth and fumonisin B1 production of Fusarium verticillioides and F. proliferatum on corn. Part I: The effect of initial headspace carbon dioxide concentration* – Int J Food Microbiol 114, 2 (2007) 160-167.

[6] **C.J. Downes, B.B.C. Page, C.W. van Epenhuijsen, P.C.M. Hoefakker and A. Carpenter**, *Response of the onion pests Thrips tabaci (Lind.) (Insecta: Thysanoptera: Thripidae) and Aspergillus niger (van Tieghem) (Fungi: Hyphomycetes) to controlled atmospheres* – Postharvest Biol Technol 48, 1 (2008) 139-145

[7] **M. H. Taniwaki, A. D. Hocking, J. I. Pitt and G. H. Fleet**, *Growth of fungi and mycotoxin production on cheese under modified atmospheres* – Int J Food Microbiol 68, 1-2 (2001) 125-133.

[8] **M.H. Taniwaki, A.D. Hocking, J.I. Pitt and G.H. Fleet**, *Growth and mycotoxin production by food spoilage fungi under high carbon dioxide and low oxygen atmospheres* - Int J Food Microbiol 132, 2-3 (2009) 100-108.

[9] **Wendy C. Schotsmans, Gordon Braun, John M. DeLong and Robert K. Prange**, *Temperature and controlled atmosphere effects on efficacy of Muscodor albus as a biofumigant* – Biol Control 44, 1 (2008) 101-110.

[10] **Yannick Masson, Paul Ainsworth, David Fuller, Hüseyin Bozkurt and Senolibanoglu** - *Growth of Pseudomonas fluorescens and Candida sake in homogenized mushrooms under modified atmosphere* – J Food Eng 54, 2 (2002) 125-131.