

## Gas Detection as a Means to Control Microbial Metabolism in Biorefineries and Reduction of Environmental Emissions

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Microbiological cultures can be monitored on the basis of their gas emissions. We have used them to characterize metabolic activities, interactions and microflora developments in biorefineries. Gas flow also can be used for controlling bioreactions. Besides numerous individual industrial projects, this approach was applied in the European Union Baltic Sea region ABOWE project (Advanced Concepts for the Biological Utilization of Waste), in its Pilot A mobile unit (Fig. 1a). See also [www.abowe.eu](http://www.abowe.eu). Planning of the Pilot A was supervised by the author and his company, Finnoflag Oy, as the key technology providers. Microbiological process design was based on earlier research using the PMEU device (Portable Microbe Enrichment Unit, Finnoflag Oy, Finland; Fig. 1) (Hakalehto 2011; Hakalehto et al. 2009).

One fundamental feature of the PMEU is the adjustable gas flow. This gas flow can be aerobic, microaerobic or anaerobic. The volatile emissions into this flow can be effectively used for detecting and characterizing microbial cultures and communities in the enrichment containers in the PMEU Scentrion<sup>®</sup>. These principles were transferred into a larger scale (200-300 liters of effective liquid volume) in the ABOWE experimental pilot station. There the incoming gas flow could be controlled and directed onto two levels as two different mixtures, if necessary (Fig. 1).



Figure 1. The ABOWE Pilot A (a movable biorefinery system) designed by E. Hakalehto. A: the interior; B: a patented bioreactor of Pilot A.

Among the emitted gases, particular interest has been directed to hydrogen, which could be used as an energy reserve in the future. Practically all environmental microbiological suspensions and biomasses emit biohydrogen at some point. Its liberation also can be detected from the human intestines, and during biotechnical runs. Figure 2a presents the volatile emissions from Polish tests with Pilot A.

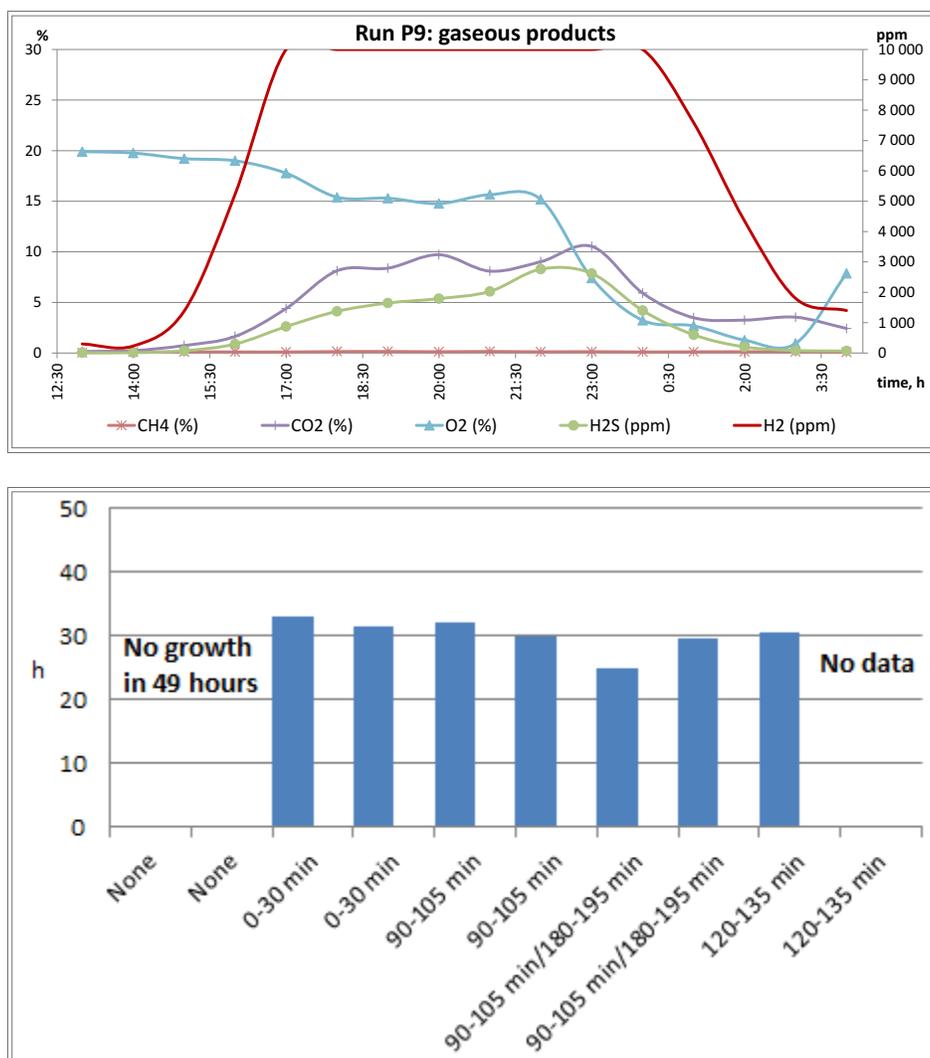


Figure 2. Two examples of volatile emissions in the biorefining. (a): Biohydrogen production (ppm) and other gases liberated (%) in ABOWE Pilot A from potato industry waste mixed with sorted restaurant biowastes. This pattern of gas formation was measured from a mixed culture of natural microbes in waste and some inoculation with *Klebsiella mobilis* (HAMBI 1271). Graph by Prof. Emilia den Boer, Wrocław University of Technology, Poland. Hydrogen in red and oxygen in turquoise color. (b): Effect of CO<sub>2</sub> on the onset of *Clostridium acetobutylicum* strain ATCC 185 growth by carbon dioxide in the PMEUSpectrion® cultures (see also Hakalehto 2015). The time required for observable bacterial growth is illustrated by the blue bars. The duration of the impulses is indicated in x-axis as minutes from the inoculation. Then a constant nitrogen (100%) flow was interrupted with one or two pulses of 45% CO<sub>2</sub> with duration of 15-30 min soon after the beginning of the cultivation. The triggering CO<sub>2</sub> impulses produced bacterial growth. One of the two parallel cultivations with CO<sub>2</sub> gas impulse starting at the 120 min time point was not producing data due to blocked tubes. No growth occurred in 49 hours in the absence of CO<sub>2</sub>.

Carbon dioxide has been used in our experiments to increase microbial growth. It shortened remarkably the onset of bacterial growth in pure *Clostridium butyricum* cultures when the outgoing gas from the PMEUSpectrion® cultivation syringe was passed through sterile filtration and directed into the next syringe (Hakalehto and Hänninen, 2012). This effect was also demonstrated with *Clostridium acetobutylicum*, *Escherichia coli* and *Klebsiella mobilis* (Hakalehto 2011; Hakalehto 2015). The growth stimulation of *C. acetobutylicum*

cultures by bubbled CO<sub>2</sub> flow periods is illustrated in Figure 2b. In fact, bacterial cultures have also a potential to assimilate the CO<sub>2</sub>, and in mixed cultures the carbon dioxide from lactobacilli increased clostridial growth (Hakalehto and Hänninen, 2012). This recycling of carbon could be exploited for biotechnical purposes, and also for preventing climatologically harmful emissions as the exhaust gases from combustion processes also can be reused biotechnologically. Nitrogen emissions from the bioreactors also can be studied, and the gaseous N<sub>2</sub> is potentially reutilized in the bacterial growth cycles.

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