Microfermentation Cassette : A low budget bioreactor for schools and industry

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Introduction

Bioreactors in teaching and research in microbial biotechnology comprise a necessary equipment vital to carry microbial fermentations. The use of a bioreactor usually comprises the main equipment that has to be available in different volumetric capacities in research and development projects. There are many models in bioreactor design. The main vessel usually is cylindrical. These models are used mainly with free microbial cells. The vessels have moving parts and strong aeration devices to aerate and mix the medium in the fermentation vessel. The cost of the vessels and the accessories is normally very high. The idea of the Microfermentation Cassette (MFC) comes to alleviate the cost of the fermentation system so the whole process to become easily available to the majority of laboratories in both, in industry and in academia.

Material and methods

The MicroFermentation Cassette (MFC) combines mainly two advantages: The compact form of its geometry and the low budget of its construction. The MFC came as the consequence of the necessity to run fermentations in a continuous and semi-continuous modes using immobilised microbial cells as well as in running in a cascade system. The predecessor of the MFC was the WITY system (Nerantzis *et al*., 1995), which consisted of a series of tower fermentors running in a cascade system. The MFCassette has no limitations as far as the minimum size for its optimum performance. The other advantage is the possibility to scaling up and the flexibility to adapt to any existing fermentation process, cell or microbial.

The MFC in a cascade system has been used in the production of wine and vinegar (Nerantzis, 2006). Mainly it consists of a rectangular vessel in with dimensions of 10x 20x 3 cm. The skeleton is made out of stainless steel (316 food grade) and the walls of glass. The top is removable and it consists of stainless steel containing of 4 ports where are located the air exit as well as the ports for different electrodes or temperature control. On the side walls there are two ports: on bottom there is the port for the input of the substrate and at the top there is the port of the product exit. The exit also consists of short metallic tubing perforated at the tip in order to prevent the alginate beats to passing through. A peristaltic pump and a thermo circulator for the temperature control support the MFCassette. Several MFC's can be connected in series creating a cascade system (photo 1, figure 1).

The MFC can find applications in the teaching of fermentation in schools and university colleges. The simplicity of its construction and its low budget of its construction can reach laboratories in the majority of the laboratories aiming in teaching, research and even development of products. There are several experiments which can be used for teaching purposes. Among these are: Alcoholic fermentation of sugary raw materials, Continuous Wine and vinegar production (immobilized yeast, and lactic acid bacteria), Malolactic fermentations for wines reach in malic acid, Beer production (free and microencapsulated yeast), Lactic acid fermentations for the production of processed yoghurt, Citric acid production, Microalgae production (*Spirulina*, *Chlorella*, etc), Fine chemicals from microalgae, Fungal spore production (*Aspergillus niger*, *Trichoderma viridae*), Production of *Bacillus thurigiensis*, Production of Cyclosporin.

Photos 1. Photos of the actual MicroFermentation Cassettes, .1st photo (connections of four cassettes in series), 2nd photo (microencapsulated yeast cells in the cassettes).

Applications in the continuous and semicontinuous wine and vinegar production

Various number of Cassettes (cascade) can be used and each one to contain a particular yeast. Consequently the product of the first is the substrate of the next and so on. The end product is the result of the consecutive fermentations of the yeast immobilised in the Cassettes. In this experiment for mead vinegar production the MFCassette system consisted of two vessels. The first in order vessel contained microencapsulated yeast cells and the second contained *Acetobacter xylinum* cells immobilized in perlite. The first cassette was fed with a help of the peristaltic pump and the other was fed from the first cassette with overflowing (Figure 1).

The total volumetric capacity of the MFCassette is 520ml. Its active volume was approx. 500ml. The medium used made out of honey. 1 kg of honey was diluted in 6 parts of tap water. The initial pH was adjusted for the alcohol fermentation to 4.5 with acetic acid. The microorganism in the first vessel was *Saccharomyces cerevisiae* N45 and in the second was a mixed culture from an active acetifier (*Acetobacter xylinum*) operating in a batch mode at 29°C. (The yeast strains were kindly offered by Anchor biotechnologies South Africa). The yeast cells were encapsulated in a double Ca-alginate layer with the apparatus (Yokotsuka *et al*. 1997) provided kindly by Professor Koki Yokotsuka. The method of microencapsulation has been described by many research workers (Kierstan and Bucke, 1977; Levitsky *et al.,* 1998; Park and Chan, 2000, Tataridis *et al.*, 2006). In order to synchronize the production of the alcoholic substrate procured by the first cassette with the second cassette, which produced acetic acid in a lower production rate, a 30% of the cassette was filled with microencapsulated yeast in alginate beads. The dilution rate of the system was measured by dividing the total output F by the active volume V_{active} of the fermentor, which was 480ml. The conversion rate was calculated using the formula: Conversion rate (%) = [Acetic acid] (0.77)/ [initial alcohol] x100.

The produced yeast cell alginate beats were left for 6 hours in a diluted honey 10% medium to be activated. After adding the alginate beats in the cassette the medium was added for the initial batch fermentation. After 12 hours the peristaltic pump started feeding with fresh medium with a dilution rate of $0.3h^{-1}$. After a period in which the culture of the first cassette (A) reached the stationary phase the pump was started and the alcoholic medium entered the second cassette B which was under continuous aeration.

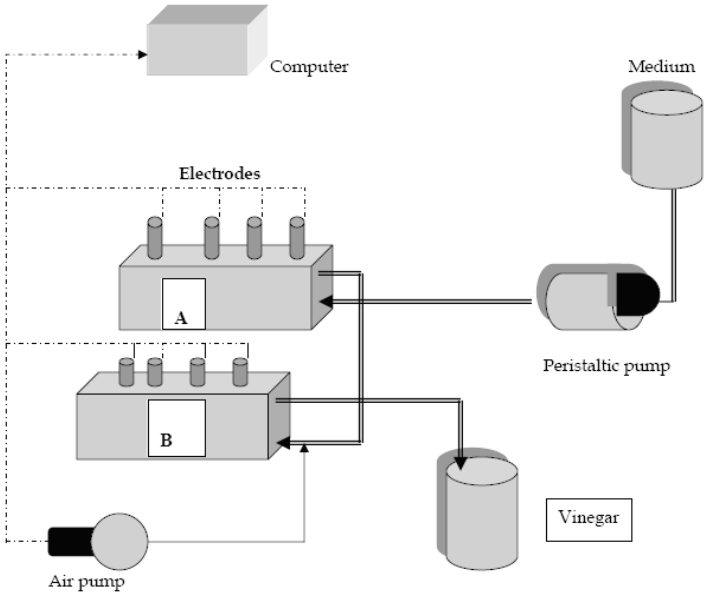

Figure 1 Double fermentation system using the MFC. Cassette A contains yeast in microencapsulated state. Cassette B contains immobilized acetic acid bacteria.

The second cassette B was filled with perlite where immobilized bacteria of acetic acid were immobilized as described above. There was cell separator preventing the fluidization of the perlite. The dilution rate was set to $0.3h^{-1}$, which is higher from the maximum specific growth rate of the yeast strain that is close to $0.25h^{-1}$. The performance of the cassette A (Fig. 1) was highly satisfactory. The double immobilization of the yeast cells helped to maintain a high active yeast biomass at all times during the fermentation of honey to mead. The high dilution rate applied to the system probably washed out all the free state bacteria and yeasts contaminants. Consequently the first fermentor was running with not obvious contamination. In the batch fermentation the results showed that the total reducing sugars concentration after 18 hours batch fermentation was near to exhaustion. The total acidity of the cassette A (Fig.1) was 0.6% and the alcohol concentration was 4.8% by weight. In the second cassette B (Fig. 1) the ethanol contents entered in a 4.8% (by weight) concentration and converted to acetic acid with conversion rate up to 98%. The concentration of the acetic acid in the final product was 6.3% (by weight). In the calculation of the conversion rate the amount of ethanol lost due to evaporation was also taken in consideration.

	MFCassette A Yeast	MFCassette B Acetic acid bacteria	Double System
Total reducing Sugars	48g/l%	3 g/l	3 g/l
Total Acidity	6g/l	63g/l	63g/l
Turbidity in the cassette	Traces	Weak	Weak
Dilution rate	$0.3h^{-1}$	$0.3h^{-1}$	$0.15h^{-1}$

Table 1. Analysis of the MFCassette fermentation system individually and in stages and in steady states.

Other applications of the MFC include production of microalgae. Where the glass walls permit the microalgae cells to photosynthesize and to produce fine chemicals. The MFC can be used for the production of Spirulina using in a cascade system the CO_2 produced in the alcoholic fermentation. MFC was also used in the production of bioethanol. The MFC can play a significant role in the production of bioethanol. The compact size of MFC gives the advantages of saving industrial space and have a better control of the active yeast biomass using it in high concentrations in a microencapsulated mode. The yield of ethanol in a continuous cascade system is much grater than that in a free cell system. This is because the concentration of yeast is higher in the fermentor during the whole duration of the fermentation. For the purpose of online monitoring of fermentation parameters MFC was connected via computer to a wide range of sensors from PASCO S.A. (USA) (Passport series sensors: temperature, pH, redox, conductivity, dissolved oxygen, dissolved CO_2, ion selective probes, etc).

Conclusions

In evaluating the system of the MFCassettes the overall production of the system was performing without any operational problems. The production of vinegar was predictable and in a steady rate. The immobilized biomass on the perlite as the immobilization is passive was not predictable as the biomass in the microencapsulated state.
The Microfermentation Cassette is a robust, versatile, low cost fermentation system, with high interconnectivity for a variety of sensors, ideal for laboratory experimentations in both, in industry and in academia

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