The MicroFermentation Cassette (MFC) Application: The continuous production of honey vinegar

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Abstract

The MicroFermentation Cassette system has been described . The performance of the MFCassette was evaluated in a double fermentation system. The first in order MFC was assigned for the production of an alcoholic beverage from honey and the second MFC connected to the first MFC was for the fermentation of ethanol to acetic acid.
The MicroFermentation Cassette has proved to be an efficient and flexible bioreactor in the system tried. The evaluation of cost for the construction of the MicroFermentation cassette has also been presented. The system ran under different conditions of dilution rates and initial honey concentrations. The vinegar produced was evaluated for its organoleptic properties.

Key words: Microfermentation Cassette, microencapsulation, yeast, mead, vinegar, fermentation

Introduction

The need for new, versatile and low cost bioreactors for teaching in schools and colleges as well as for research purposes are the reasons for designing the Microfermentation Cassette (Nerantzis, 2006).
The MFCassette is a bioreactor combining several properties among of these are: Versatility on the size and media to be used and on the microbial species as well as flexibility in the mode of their cultivation.
The MFC is appropriate for the production of metabolites when microbial immobilized systems or microencapsulated microbial systems are to be used.
The MFCassette developed as a continuation of the WITY system designed by Nerantzis *et al*. (1995).
The WITY system was a cascade system of tower fermentors filled with microencapsulated yeast cells. The only limitation of the WITY system was the minimum size of the towers should be above 1.0 litre for its optimum performance. The MFCassette has no limitations as far as the minimum size for its optimum performance. The other advantage is the possibility to scaling up.
The MFCassette can be scaled up in a pilot or even in an industrial size.
A large version of the cassette could have applications in industry, as the amount of biomass included in the cassette is multiple of that of the free cells reactors of the same volumetric capacity. In this way there is a better control of the fermentation and the cost of the initial investment is lower.
The microbial cells immobilization as method for the production of several metabolites has been used for the production of vinegar using various immobilization materials. Examples for these immobilization techniques and methods can be

mentioned such as : ceramics by Ghommidh et al., (1981), wood chips by Namba et al., (1985), and more recently calcium alginate by Levitsky et al., (1998) and siran, wood chips and polyurethane foam by Ory et al., (2004).
The microencapsulation technique has been reported for the production of ethanol (Kierstan & Bucke 1977, Williams & Munnecke 1981).
The fermentation of honey has been already studied and the parameters affecting the production of the alcoholic beverage, as is mead. In fact has been studied the effect of temperature and the duration of the fermentation in the aroma of the resulted mead by Rose (1977).
In the present work the performance of the MFCassette is evaluated using a defined medium based on honey wine as the sugary raw material to produce vinegar using two MFCassettes. The first was dedicated to the alcohol production from honey and the second to the acetic acid production.
The performance of the MFCassettes has been evaluated during batch and continuous operation.

Methods and Materials

Description of the Microfermentation Cassette:

The MFCassette consists mainly of a rectangular vessel with a metallic body, closed with a lid at the top. All-metallic parts were stainless steel 316 food grade. The lid carries 4 ports. The first port is used for the temperature control. This is achieved with the use of serpentine tubing connected to a temperature controller. The other ports are used for air exit, the pH electrode and the thermistor. There are also 2 ports build at the sidewalls of the main body. These are used for the introduction of both, air and medium. The inlet for the medium and air introduction consists of perforated tubing along side to the bottom of the main vessel. 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. The whole system is integrated by two vessels one for the feeding medium and the other at the end of the line for the disposal of the fermented medium.

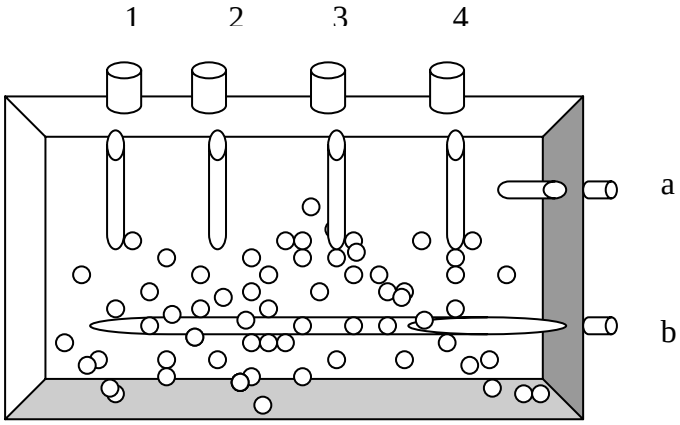

Figure 1. The Microfermentation Cassette. Ports (2,3), for the positions of different sensors, the air exit (4) and the temperature control (1). Ports (a) and (b) are the feed exit and entrance respectively.

The MFCassette system is a cascade of cassettes (vessels). Each cassette is filled with microencapsulate microbial cells of one species.
In this work the MFCassette system consisted of two vessels, which were connected to each other with the manner described above. 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 2).
The yeast microencapsulation technique has been described elsewhere.

A

B

Photos A and B.: Photos of the actual MicroFermentation cassettes. The photo A shows the connections of four cassettes in series and photo B shows the level of microencapsulated yeast cells in the cassettes

The principle of its design is based on the use of small amounts of media with in an easy to control environment. The total volumetric capacity of the MFCassette is 520ml. Its active volume was approx. 500ml.

The medium. 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 microorganisms. The microorganism in the first vessel was Saccharomyces cerevisiae N45 and in the second was a mixed culture from an active acetifier operating in a batch mode. (The yeast strains were kindly offered by Anchor biotechnologies South Africa).

The yeast Microencapsulation technique. The yeast cells were encapsulated in a double Ca-alginate layer with the apparatus (Yokotsuka *et al.* 1977) provided kindly by Professor Koki Yokotsuka.
The method of microencapsulation has been described by many research workers (kierstan and Bucke , 1977, Williams and Munnecke, 1981, Park J.K. and Chan H.N.2000)

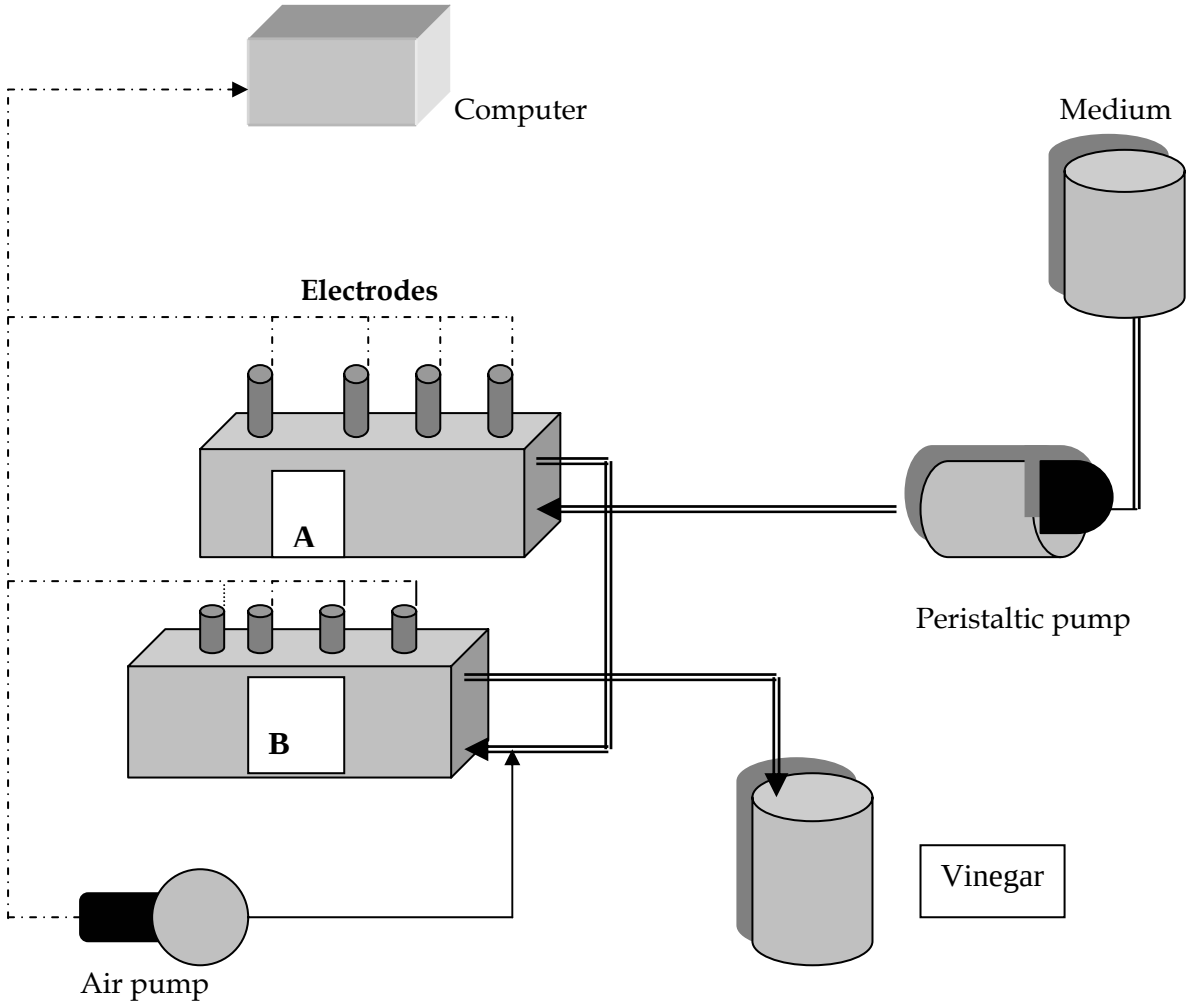

Figure 2 Double fermentation system using the Microfermentation Cassettes. The cassette A contains yeast in microencapsulated state. The microfermentation Cassette B contains acetic acid bacteria in immobilized state in bacterial cellulose

The acetic acid bacteria immobilization technique . The acetic acid bacteria were immobilized in a perlite matrix. An active culture of Acetobacter was taken from an active acetifier and added to 15 grams of perlite in the second cassette. The acetic acid bacteria after a 12-hour recycling with the use of a peristaltic pump were considered as immobilized within the pores of the perlite. During the recycling of the acetic acid culture the cassette was continuously aerated using an air pump. The temperature of the culture was 29^0 C.

Analytical methods

The reducing sugars. Reducing sugars were measured with the method of DNS by Miller G.L. (1959).

The total acidity. Total acidity was determined with titration. In fact 6 ml of sample was titrated against 0.1N NaOH, The amount of NaOH used for the neutralization of the existing acid was expressed in concentration of acetic acid percent by weight.

The dilution rate. 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 active volume for the ethanol cassette was considered as the volume occupied by the beads. The active volume of the acetic acid producing cassette was considered as the volume occupied by the bacterial cellulose filaments.

Alcohol content. The alcohol content was measured using standard oenological methods.

The acetic acid conversion rate. The conversion rate was calculated using the formula:

Conversion rate (%) = [Acetic acid] (0.77)/ [initial alcohol] x100

Operation of the MFCassettes.

Alcoholic fermentation.
The first stage for the production of vinegar was the production of alcohol by converting the sugar content of honey in to ethanol. For this purpose microencapsulated yeast cells filled the first cassette. In order to synchronize the production of the alcoholic substrate procured by he 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 temperature of the fermentation was 29^0 C.
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 $0.3h^{-1}$. The performance of the fermentation was observed and corrected. At the beginning of the fermentation started during the batch operation the culture was foaming but it was not detrimental to the fermentation performance. The foaming reduced to minimum when the fermentation reached the stationary phase. During the continuous mode no foaming was observed. The beats with the encapsulated yeast were fermenting with not major problems. The fluidization phenomena in the cassette (A) prevented by the yeast cell separator. The yeast cell separator was a devise made out of Plexiglas. On its body were holes to allow the circulation of air and medium. The yeast cell separator kept the beads under the surface of the liquid during the whole duration of the culture. The mixing of the liquid phase of the system was facilitated by the evolution of CO_2 as well as from the medium introduction method (Figure 1, a).
No contamination affected the first MFCassette because of the high dilution rate 0.3 h^{-1}, which is higher than the specific growth rate of most anaerobic bacteria cultured in a suppressing medium with high content of ethanol too.

Acetic acid fermentation
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.
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.

Results and discussion

For the production of vinegar from mead is not necessary the stage of maturation that mead should pass through before it will be consumed. Maturation of mead would not affect significantly the fermentation performance of the acetic acid production and its quality.
Therefore the mead was immediate inserted the cassette for the acetic acid fermentation and the consequent production of vinegar.
The fermentation started with a batch mode and after the 18-hour period followed by the continuous mode when the culture was fed continuously with fresh medium. 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. 2) 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.

	MFCassette A Yeast	MFCassette B Acetic acid bacteria	Double system
Total reducing Sugars	48g/l%	3 g/l	3g/l
Total Acidity	6g/l	63g/l	63g/l
Foaming	At the beginning of the batch mode	Not at all	Not at all
Turbidity in the cassette	Traces	Weak	Weak
Dilution rate	$0.3h^{-1}$	$0.3h^{-1}$	$0,15\ h^{-1}$

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

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 was taken in consideration the amount of ethanol lost due to evaporation.
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.

Conclusion

The fermentation of honey for the production of vinegar using the microfermentation cassette system was tried in a double stage fermentation system using the Microfermentation Cassette system.
The fermentation of diluted honey by the encapsulated yeast cells was performed in a constant rate and did not presented any operational problems such as fluidization phenomena and foaming to be detrimental to the final product.
The production of acetic acid was performed using bacteria of acetic acid immobilized on the perlite matrix. The biomass concentration immobilized on the perlite matrix was not accurately evaluated. Therefore the actual productivity of the second stage was not accurately evaluated.
It is therefore suggested that further investigation of the system for the production of homey vinegar is needed. In fact, several dilutions of honey should be fermented and then fermented in the second cassette for the production of acetic acid. More experimental work also is needed for the evaluation of the bacterial biomass immobilized on the perlite.

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