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BATCH FERMENTATION OF BLACK TEA BY KOMBUCHA: A CONTRIBUTION TO SCALE- UP

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ABSTRACT

Local domestic Kombucha was used in fermentation of 1.5 g L^{-1} of black tea (Indian tea, "Vitamin", Horgoš, Serbia and Montenegro), sweetened with 71.14 g L^{-1} of sucrose. Inoculation was performed by both 10% and 15% of fermentation broth from previous process. The fermentation was conducted in geometrically similar vessels of 0.4 L, 0.8 L, 4 L and 8 L, at 22±1 ^{0}C for 28 days. The samples were analysed after 3, 4, 5, 6, 7, 10, 14 and 28 days, so that their pH values, content of total acids, sucrose, glucose and fructose contents as well as quantity of ethanol and vitamin C were determined. Based on the plan of experiments as well as on the adopted independent variables (time, volume and inoculum concentration), a response surface was defined as a polynomial of the second order. The pH of the product was adopted to be a leading dependent variable. The derived response surface was suggested as a leading formula for scaling- up the process.

KEY WORDS:

Kombucha, fermentation, metabolites, vitamin C

1. INTRODUCTION

A beverage, which possesses characteristics of functional food, appears during sugared tea fermentation caused by activity of a symbiotic association of bacteria and yeasts, well known as Kombucha [1, 2]. The yeast cells hydrolyze sucrose into glucose and fructose, producing ethanol [3, 4], while acetic acid bacteria convert glucose into gluconic acid and fructose into acetic acid. Apart from two main metabolites (ethanol and acetic acid), Kombucha beverage contains most of tea ingredients and other compounds that appear as a result of numerous reactions [5-7]. Fermentation process induces synthesis of the B complex of vitamins and folic acid as well as synthesis of ascorbic acid - vitamin C [8-10].

On the other hand, production of vitamin C and other valuable compounds is related to the working conditions. It has been reported [3, 11, 12] that optimal conditions are: i) 7-th day fermentation possesses, ii) 22-28^{0}C interval of working temperatures, iii) composition of fermented tea and concentration of inoculum adjusted to the particular Kombucha

association iv) 50-100 g L^{-1} interval of sucrose concentration and v) 3.8-3.7 pH value of the product. However, all the data were determined at lab- scale processes, performed in flasks, with less than 0.5 L of liquid. In order to determine impact of liquid volume to the characteristics of the obtained product as well as to define a response of the system to the increase of the volume, experiments in this research were conducted in vessels, whose volumes varied from 0.4 L to 8 L. Some of the reported scaling- up strategies [13-16] were applied on the acquired data, giving a solution for Kombucha beverage processing on a large scale.

2. MATERIALS AND METHODS

2.1. KOMBUCHA CULTURE AND FERMENTATION

Local domestic Kombucha determined by Markov et al. [17] was used for the fermentation.

Tap water was boiled, sweetened with 71.14 g L^{-1} of sucrose and mass of 1.5 g L^{-1} of black tea (Indian tea, "Vitamin", Horgoš, Serbia and Montenegro) was added and removed by filtration after 15 min. After cooling to room temperature, the tea was inoculated, alternatively, with 10% and 15% of fermentation broth from previous Kombucha fermentation. Geometrically similar vessels- bioreactors of 0.4 L, 0.8 L, 4 L and 8 L were covered with cheesecloth and incubated at constant room temperature of 22±1^{o}C for 28 days. The samples of products were taken after 3, 4, 5, 6, 7, 10, 14 and 28 days and following quantities were measured: pH values, sucrose-, glucose- and fructose- content, total acids, ethanol concentration and content of vitamin C.

2.2. METHODS OF ANALYSIS

pH values were measured with an electric pH meter.

Sucrose, glucose and fructose contents were determined using the test of Boehringer Mannheim (Cat. No. 716260).

Total acids content was determined by the volumetric method with sodium hydroxide and phenolphthalein as indicator.

Ethanol content was measured in accordance with the procedure of Boehringer Mannheim (Cat. No. 176290).

Vitamin C content was determined using the test of Boehringer Mannheim (Cat. No. 409677).

2.3. METHOD OF SCALING- UP

The method for scaling- up, whose algorithm is given in Fig. 1, is based on a series of particular processes in a small- scale reactor to obtain the information for scale- up [15]. The effect of process variables and their variation to a chosen, controlled characteristic of product (here pH value of the beverage) is determined and decision is made concerning the scale- up of the process.

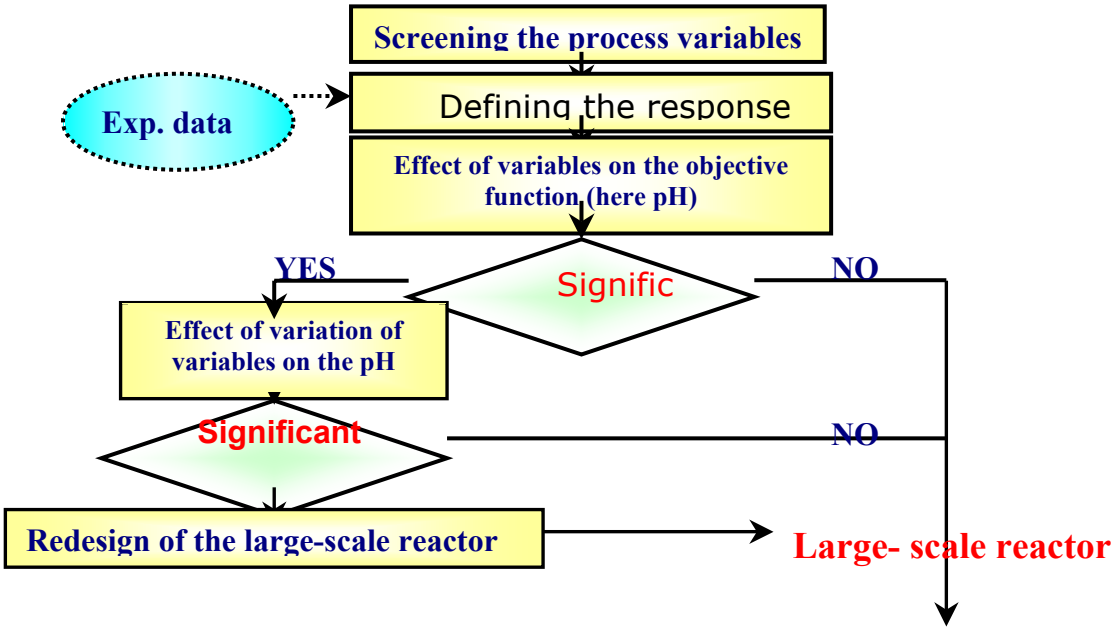

FIG. 1. ALGORITHM OF THE SCALING-UP METHOD [15]

3. RESULTS

3.1. SCREENING THE PROCESS VARIABLES

In order to investigate influence of various working conditions upon Kombucha lead fermentation process, experiments were performed and the results presented in Figs. 2-8 were obtained.

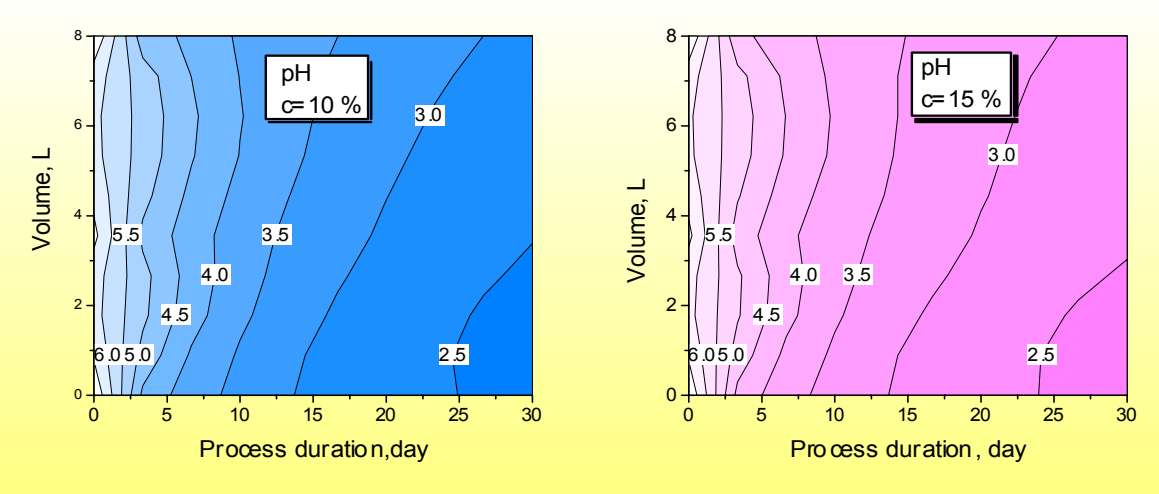

FIG. 2. pH AS A FUNCTION OF PROCESS DURATION, VESSEL VOLUME AND INOCULUM CONCENTRATION

Dependence of pH value of the beverage (Fig. 2) on process duration is very significant. Volume affects pH values almost negligible at the beginning of the process; in late phase of the process this influence slightly increases.

Almost the same conclusion follows from the Fig. 3, where changes of sucrose content are presented.

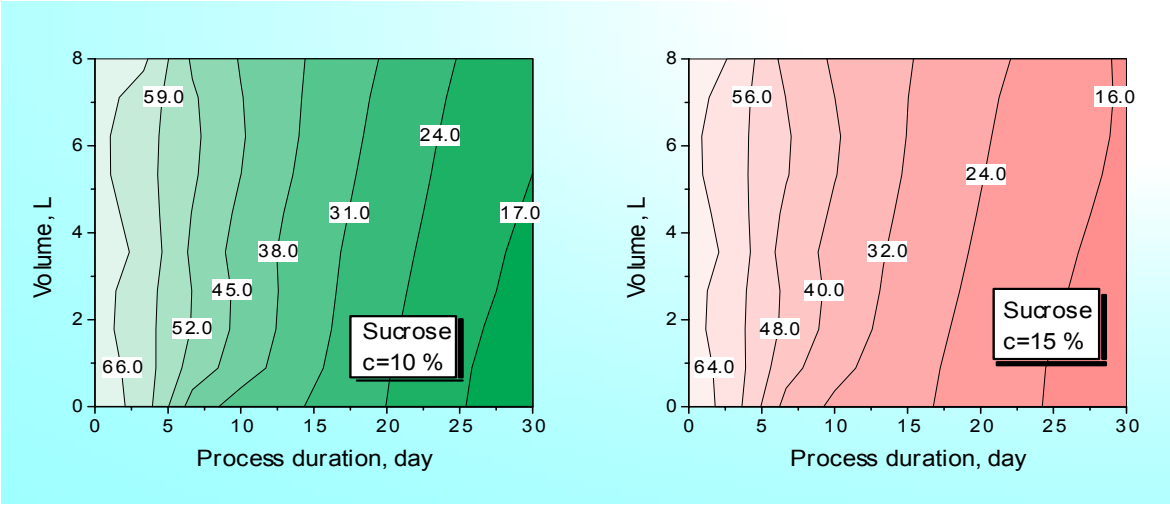

FIG. 3. SUCROSE CONTENT AS A FUNCTION OF PROCESS DURATION, VESSEL VOLUME AND INOCULUM CONCENTRATION

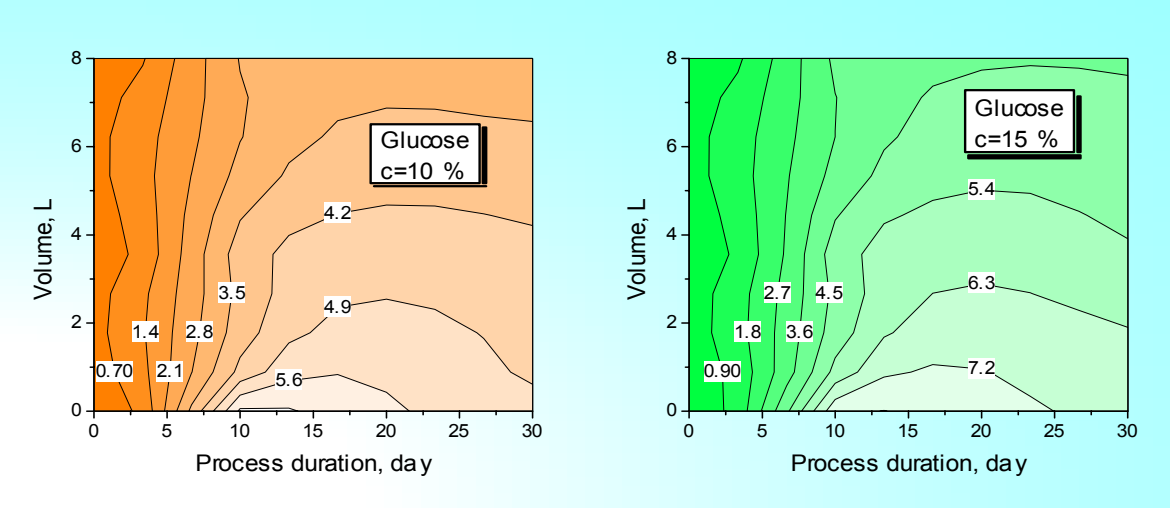

FIG. 4. GLUCOSE CONTENT AS A FUNCTION OF PROCESS DURATION, VESSEL VOLUME AND INOCULUM CONCENTRATION

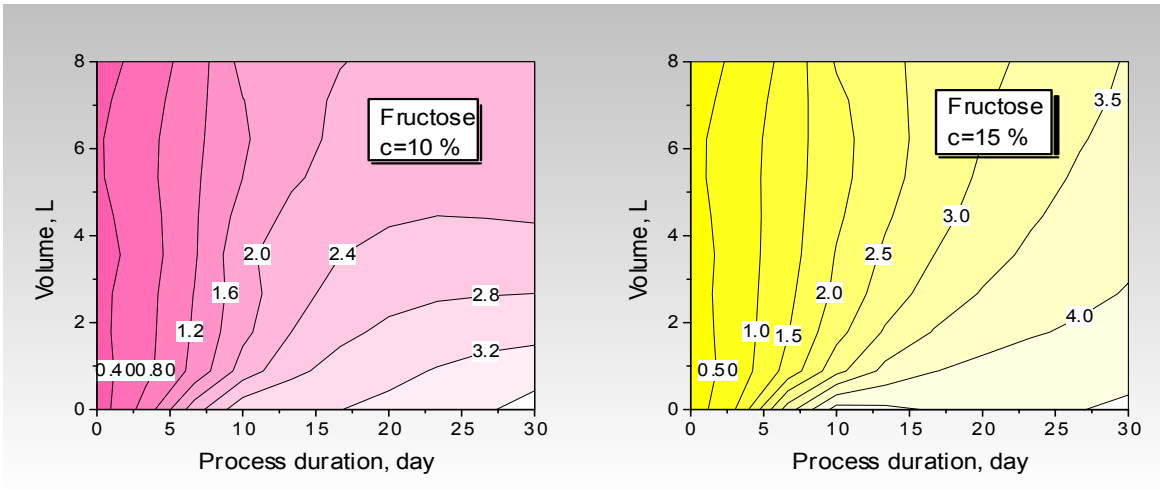

FIG. 5. FRUCTOSE CONTENT AS A FUNCTION OF PROCESS DURATION, VESSEL VOLUME AND INOCULUM CONCENTRATION

Change of glucose- and fructose- content (Figs. 4-5), dependent on the change of process duration and volume, follows more complicated mathematical model. However, this will not be taken in account while scaling- up the fermentation process.

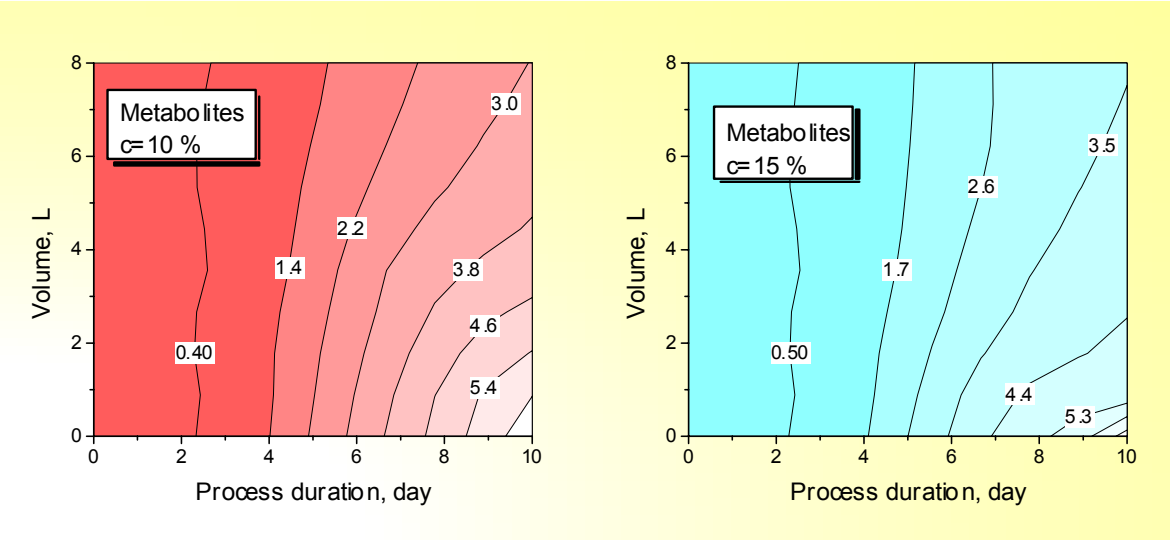

FIG. 6. METABOLITES QUANTITY AS A FUNCTION OF PROCESS DURATION, VESSEL VOLUME AND INOCULUM CONCENTRATION

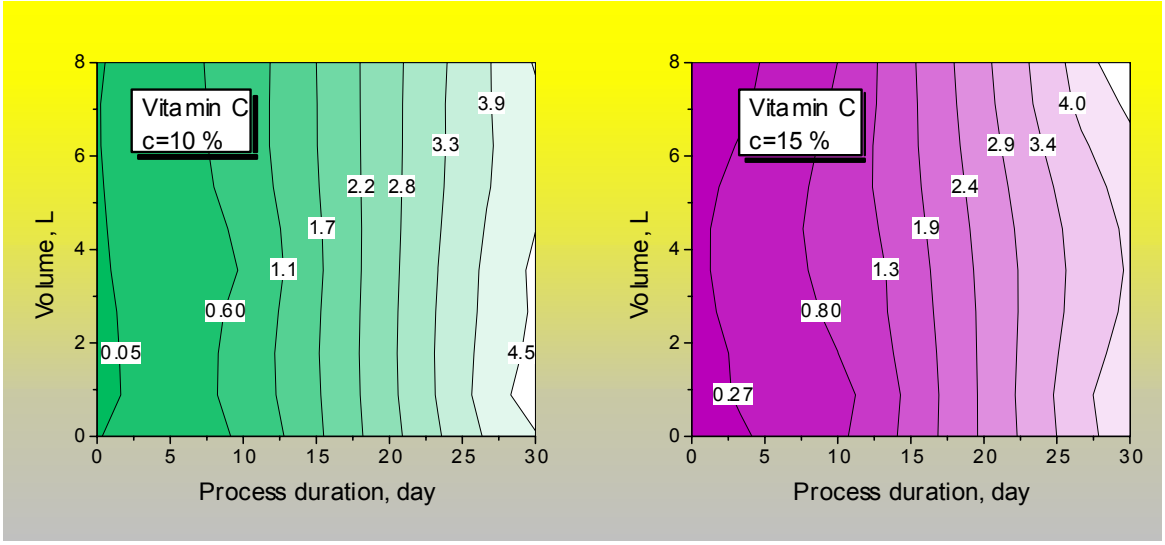

FIG. 7. VITAMIN C CONTENT AS A FUNCTION OF PROCESS DURATION, VESSEL VOLUME AND INOCULUM CONCENTRATION

During fermentation of sucrose, two main metabolites (acetic acid and ethanol) appear. However, other acids (that gives total acidity) are also components of the obtained product and should be taken into account. As for metabolites quantity (Fig. 6), it increases significantly with the increase of time, but decreases slightly with the increase of vessel volume.

Finally, quantity of vitamin C, which is one of the numerous valuable compounds, does not depend of the vessel volume, but increases with the increase of process duration (Fig. 7). This might be important conclusion when scaling- up the fermentation process.

3.2. DEFINING THE RESPONSE SURFACE

Based on the plan of experiments as well as on the adopted independent variables, following form of response surface was chosen:

$$y = b_1 + b_2 x_1 + b_3 x_2 + b_4 x_3 + b_5 x_1 x_2 + b_6 x_1 x_3 + b_7 x_2 x_3 + b_8 x_1^2 \quad (1)$$

where x_1 denotes time, x_2 is volume of beverage in a vessel and x_3 denotes inoculum concentration. Polynomial (1) proved the best function when compared with several alternative functions, based on the following criterion:

$$\delta = \frac{\sum_{i=1}^{N} \left(y_{\exp} - y_{cal} \right)_i^2}{N} \quad (2)$$

where y_{exp} denotes experimental value, y_{cal} represents calculated value while N corresponds to the total number of experiments. It is obvious that the function (1) consists of linear terms and non- linear terms, whereas non- linear terms represent interaction of two variables (as their product) as well as quadratic time- term.

For the purpose of scaling- up Kombucha fermentation process, the mathematical model (1) should be applied so as to define a response of pH of the beverage, as a leading variable, to the variation of time, volume and inoculum concentration. After statistical processing of the measured data by the regression analysis method, parameters (b) for the pH- response surface are obtained as follows:

$$y = 6.113 - 0.3814\, x_1 + 6.311E-02\, x_2 - 1.246E-02\, x_3 + 1.869E-03\, x_1 x_2 + 3.551E-04\, x_1 x_3 - 1.2E-03\, x_2 x_3 + 8.749E-03\, x_1^2 \quad (3)$$

When significances of all b- parameters are considered, it was concluded that process duration (time) is the most significant variable. On the other hand, inoculum concentration is the least significant variable, single and in interactions with other variables.

4. APPLICATION OF THE RESPONSE SURFACE FOR SCALE- UP

Due to the fact that the most important dependent variables (pH of the Kombucha beverage, sucrose content and quantity of vitamin C) do not depend significantly on the independent variables (such as volume of the reactor, etc.), the response surface can be suggested as the leading scale- up formula. The Equation (3) can be expressed in a form:

$$(x_1)_1^2 = \frac{-B \pm \sqrt{B^2 - 4AC}}{2A} \quad (4)$$

where:

A= $8.749E-03$

$$B = -0.3814 + 1.869E-03\,x_2 + 3.551E-04\,x_3$$

$$C = 6.113 + 6.311E-02\,x_2 - 1.246E-02\,x_3 - 1.2E-03\,x_2x_3 - y$$

$$y = pH$$

and can be used for estimation of process duration (x_1) in a specified (enlarged) vessel volume (x_2), when inoculum concentration is one of two (10 % or 15 %).

For example, if volume of vessel changes from 8 L to 20 L, the product will achieve optimal pH value (3.8) after times presented in Table 1. The estimated values of process duration increase with the increase of the beverage volume quite expectedly. As far as the inoculum concentration is concerned, it can be noticed that greater value of inoculum concentration makes shorter process duration, for the same volume of the beverage and the same required pH value.

TABLE 1. ESTIMATED PROCESS DURATION IN ENLARGED VESSELS

c=10%, pH=3.8		C=15 %, pH=3.8	
Volume, L	Process duration, day	Volume, L	Process duration, day
8	9.20	8	8.74
10	9.91	10	9.35
15	12.12	15	11.21
20	16.05	20	14

5. CONCLUSION

The method for scaling- up, based on a series of particular processes in a small- scale reactor as well as to an application of the response surface (defined for the obtaining experimental information), was successfully applied to a batch process with Kombucha fermentation. By using the method, process duration was estimated for a series of enlarged batch reactors satisfying the required pH value of the product.

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