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Specific interfacial area as a key variable in scaling-up Kombucha fermentation

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Abstract

A mathematical model based on the specific interfacial area as a key independent variable and titratable acidity content as a final goal was proposed for scaling-up Kombucha fermentation. The model was derived by processing data obtained from the experiments performed using the local tea fungus in sweetened black tea inoculated with 10% of fermentation broth from the previous process. The fermentation was conducted at 28 ± 1 °C in six reactors of the different liquid phase volumes, i.e. having various specific interfacial areas. The fermentation broth samples were collected during the process to measure their pH values and titratable acidity. Validity of the scale-up model was verified by comparing the fermentation duration calculated from the model and duration of the real process in a large reactor, and a high agreement was found. Also, the model was successfully applied to extremely small volumes, proving its wide-span applicability.

Keywords: Scale-up; Specific interfacial area; Kombucha; Fermentation

1. Introduction

Kombucha is a traditional beverage prepared by fermenting sweetened black tea with tea fungus. The product is a slightly sweet, carbonated, acidic tea beverage which is consumed worldwide, but historically in China, Russia, and Germany. The Kombucha has been claimed to be a prophylactic and therapeutic agent beneficial to human health – from weight loss to curing cancer and AIDS (Dufresne & Farnworth, 2000; Greenwalt, Steinkraus, & Ledford, 2000).

The tea fungus is a symbiotic culture of acetic acid bacteria (*Acetobacter aceti*, *Acetobacter pasteurianus*, *Gluconobacter oxydans*) (Greenwalt et al., 2000; Janković & Stojanović, 1994) and yeasts (*Saccharomyces* sp., *Zygosaccharomyces kombuchaensis.*, *Torulopsis* sp., *Pichia* sp., *Brettanomyces* sp.) (Greenwalt et al., 2000; Kurtzman, Robnett, & Basehoar-Powers, 2001; Liu, Hsu, Lee, & Liao, 1996). Exact microbial composition of tea fungus and its autochthonous characteristics are determined by geographic and climatic conditions of cultivation, as well as by the local species of wild yeasts and bacteria (Mayser, Fromme, Leitzmann, & Gruender, 1995). Sucrose as carbon source in the cultivation medium is hydrolyzed by the enzyme invertase from tea fungus yeasts. Acetic acid bacteria take up the monosaccharides (glucose and fructose) resulting from sucrose hydrolysis, but cannot use sucrose as such because they lack hydrolases and kinases. The yeasts ferment glucose and fructose to ethanol, which is then oxidized by acetic acid bacteria to acetic acid. This is the main metabolic path of Kombucha fermentation, and acetic acid is a dominant organic compound in the

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beverage (Sievers, Lanini, Weber, Schuler-Schmid, & Teuber, 1995). To obtain a pleasantly sour product, the fermentation should be terminated when titratable acidity content reaches the desired value, commonly accepted at a level of 4 g L^{-1} (Greenwalt et al., 2000; Reiss, 1994).

Kombucha is typically prepared by fermenting black tea (1.5–5 g L^{-1}), sweetened with sucrose (50–100 g L^{-1}) and inoculated with previously fermentated liquid tea broth at a level of 10–20% or tea fungus pellicle. The substrate is incubated statically under aerobic conditions for 10–12 days at 20–28 °C (Blanc, 1996; Reiss, 1994; Sievers et al., 1995). The conditions are usually determined in the lab-scale processes performed in flasks with less than 0.5 L (Steinkraus, Shapiro, Hotchkiss, & Mortlock, 1996; Teoh, Heard, & Cox, 2004) or up to 1 L of liquid (Chen & Liu, 2000; Sreeramulu, Zhu, & Knol, 2000). However, they can be applied onto larger systems under certain circumstances, i.e., if an adequate level of similarity between small and large systems is ensured. The need for scaling-up appears when considering production of fermented tea at a pilot or industrial scale. However, very limited amount of information on this subject can be found in the literature (Malbaša et al., 2006).

Expanding a fermentation process from the lab-scale unit to a commercial one is a challenge because of the difficulty in assessing the factors that affect the scale-up process during the cultivation (Hsu & Wu, 2002). At the beginning of the process, significant amounts of ethanol and monosaccharides, needed by acetic acid bacteria, are provided by Kombucha yeasts. Therefore, the activity of acetic acid bacteria as strict aerobic organisms depends on the transfer of air oxygen in the fermentation broth. Under static condition, the amount of dissolved oxygen depends on the surface and volume of the medium. This relationship can be expressed by the specific interfacial area (a), which is a well-known kinetic factor, defined as the ratio of the reactor cross-section and volume of the reacting liquid phase. The specific interfacial area is related to other kinetic factors, such as liquid volume, reactor cross-section, mass transfer coefficient and concentration of dissolved oxygen (Nielsen, Villadsen, & Lidén, 2003). This means that the rate of Kombucha batch fermentation without agitation and without gas introduction depends on the specific interfacial area. It may be assumed that the two reactors having the same a value, although being different in size, provide similar mass transfer conditions. This paper introduces a method of scaling-up the Kombucha fermentation based on the specific interfacial area as a key variable.

2. Materials and methods

2.1. Tea fungus

Fermentation was performed using the local tea fungus culture, for which previous investigations (Lončar, Djurić, Malbaša, Kolarov, & Klašnja, 2006; Markov, Malbaša, Hauk, & Cvetković, 2001) showed that it contained at least five yeast strains (*Saccharomycodes ludwigii*, *Saccharomyces cerevisiae*, *Saccharomyces bisporus*, *Torulopsis* sp. and *Zygosaccharomyces* sp.) and two bacterial strains of the *Acetobacter* genera.

2.2. Fermentation conditions

All experiments were performed on a sweetened tea made by dissolving 70 g of sucrose in 1 L of tap water. To the boiled water, 3 g L^{-1} of black tea (“Fructus”, Bačka Palanka, Serbia) was added and removed by filtration after 15 min. After cooling to room temperature, the tea was inoculated with 10% of the fermentation broth from the previous fermentation obtained under the similar conditions. Six reactors of different volumes (three for deriving the model and three for model verification), whose characteristics are given in Table 1, were filled in with various volumes of inoculated liquid phase. In this way, specific interfacial area varied as presented in Table 1. The vessels were covered with cheesecloth, and the fermentation at

Table 1
Characteristics of the fermentation vessels and ranges of independent variables

Vessels	Characteristics of the vessels		Liquid volume, L	Ranges of independent variables	
	Vessel volume, L	Diameter, cm		Fermentation time, days	Specific interfacial area, cm^{-1}
Small flask	0.72	8	0.33	9	0.152
Common flask	5	16	4.18	9	0.0481
Large flask	10	19.5	5.5	9	0.0543
			6.8	9	0.0438
			8.08	12	0.0369
			8.25	12	0.0362
Cylinders	13	18	5.5	9	0.0462
			8.25	12	0.0308
			11	14	0.0231
Small reactor	25	30	11	9	0.0642
			13.8	9	0.051
			16.5	11	0.0428
			19.25	14	0.0367
Large reactor	110	63	90	13	0.0346

28 ± 1 °C was monitored for about two weeks. The fermentation broth samples were taken each other day to measure pH value and titratable acidity; in some cases, samples were taken in three-day intervals.

2.3. Methods of analysis

The pH values were measured using an electronic pH meter (HI 9321, HANNA Instruments) calibrated at pH 4.0 and 7.0.

The titratable acidity was determined according to OIV (1990). After removing CO_2 (according to OIV, 1990) from the fermentation broth, a 20-mL aliquot was taken and titrated with 0.1 mol L^{-1} of NaOH. The titratable acidity was expressed in grams of acetic acid per liter of the sample.

Total counts of cells of yeasts and acetic acid bacteria in the fermentation broth were determined by plate counting method. For yeasts, the medium was Sabouraud-4% Maltose Agar (Merck, Darmstadt, Germany) with addition of 50 mg L^{-1} of chloramfenicol (Sigma–Aldrich, St. Louis, USA), and the plates were incubated for 72 h at 28 °C. The medium for determining total count of acetic acid bacteria was Yeast Peptone Mannitol Agar (Difco, Detroit, USA), containing 500 mg L^{-1} cycloheximide (actidione; Sigma–Aldrich, St. Louis, USA) to inhibit yeasts growth. The incubation at 28 °C lasted 5–7 days.

All experiments were performed in duplicate, under the same conditions, while each quantity was measured three times. The obtained values used for further processing are the averages of all the measurements.

3. Results and discussion

3.1. Experimental results

3.1.1. Main characteristics of the applied inoculums

Kombucha beverages, i.e. the fermentation broths obtained in a traditional process of cultivation (3 L of sweetened tea in 5 L common flask inoculated with mentioned tea fungus pellicle) were used for inoculation. Average values of the chemical (pH and titratable acidity) and microbiological (number of yeast cells and acetic acid bacteria) parameters for all applied inoculums are given in Table 2. Basic chemical parameters of the inoculums (pH 3.0 and 4.11 g L^{-1} of acids after seven days of the process) are similar to the results obtained by other authors. Liu et al. (1996) obtained pH 3.5 and 4 g L^{-1} of acetic acid in the fermentation liquid after six days, while Sievers et al. (1995) the same acidity obtained after nine days of fermentation. The counts of viable yeasts and bacterial cells in the inoculums were 6.62 log cfu mL^{-1}, which corresponds to the same or higher exponential rate in similar fermentation media. For example, Sreeramulu et al. (2000) obtained 4.48 log cfu mL^{-1} yeast cells and 5.3 log cfu mL^{-1} bacterial cells in fermentation liquid after six days of the process, while corresponding counts reported by Chen and Liu (2000) were 7.55 and 4.5 log cfu mL^{-1}. Teoh et al. (2004) found the count of individual yeast species on the sixth day of the process between 5 and 7 log cfu mL^{-1}, depending on the species used. Some differences in chemical parameters, process duration and cell counts may be expected because Kombucha does not have standardized microbiological and chemical composition (Teoh et al., 2004). However, the results have shown certain similarity among different Kombucha cultures and the possibility to repeat fermentations under the same conditions on different locations.

Table 2
Main characteristics of the applied inoculums

pH value	Titratable acidity, g L^{-1}	Cell counts, log cfu mL^{-1}	
		Yeast	Acetic acid bacteria
3.04 ± 0.14	4.11 ± 0.76	6.62 ± 0.45	6.62 ± 0.39

3.1.2. pH values and titratable acidity

pH values and titratable acidity (TA) were measured in large flasks, cylinders and small reactors, whose characteristics are presented in Table 1. The average changes of pH and TA with time and in dependence of specific interfacial area are presented in Figs. 1–3. Each average value was obtained from six observations (in two parallel experiments, with three measurements). The values of TA serve thus as a database for deriving a mathematical model of the fermentation process. Besides, the same quantities were measured in large reactors (Table 1), to verify the possibility of applying the model in the process scaling-up. Finally, fermentation was performed in quite small vessels (small and common flasks, Table 1), to check the possibility of extrapolating the model to small volumes.

An analysis of the results presented in Figs. 1–3 shows that the pH values and TA depend significantly on the process duration, which is in accordance with previous

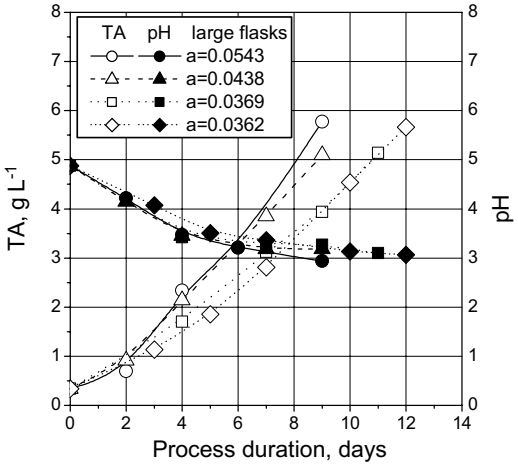

Fig. 1. Changes in pH values and titratable acidity in large flasks.

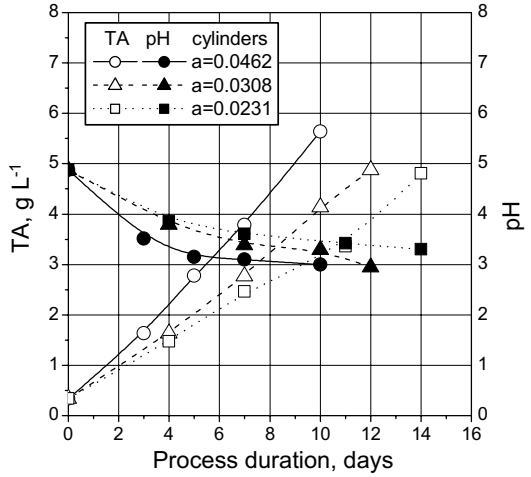

Fig. 2. Changes in pH values and titratable acidity in cylinders.

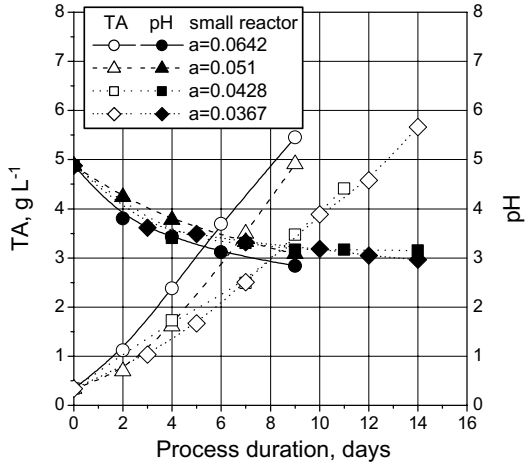

Fig. 3. Changes in pH values and titratable acidity in small reactors.

findings (Jayabalan, Marimuthu, & Swaminathan, 2007; Lončar et al., 2006). The pH value of the sweetened black tea was approximately 7.4, and it dropped to about 4.7 almost immediately after the inoculation with the fermentation broth. In first four days of cultivation, the pH value decreased by about 1.6 units as a consequence of the physiological activity of the tea fungus and synthesis of organic acids (primarily acetic acid). After this period, the pH value changed insignificantly, despite of the continued synthesis of organic acids. The same pH trend was observed by some other authors (Chen & Liu, 2000; Cvetković, 2003; Sreeramulu et al., 2000). Apparently, the fermentation broth exhibits a certain buffer capacity. Namely, during the fermentation, carbon dioxide is released, at first slowly and much faster after 2–3 days. The obtained water solution of CO_2 dissociates and produces the amphiprotic hydrocarbonate anion (HCO_3^-), which easily reacts with hydrogen ions (H^+) from the present organic acids, preventing further changes in pH, thus contributing to a buffer character of the system. As for the content of TA, it increased from the beginning till the end of the fermentation, in all investigated systems. Because of that, the pH value cannot be a critical parameter which determines the end of sweetened tea fermentation; this role is better played by TA of the sample.

As mentioned earlier, specific interfacial area (a) is related to the other kinetic factors in the following way:

$$V_L \frac{dc}{d\tau} = K_L(c^* - c)A \Rightarrow \frac{dc}{d\tau} = K_L a(c^* - c) \quad (1)$$

where V_L represents the liquid volume; A is the reactor cross-section; K_L stands for the coefficient of mass transfer; τ denotes time, while c and c^* are the actual and equilibrium concentration, respectively. The specific interfacial area is particularly related to the mass transfer, i.e. to the transfer of oxygen from the air to the liquid phase, which is especially important for the activity of acetic acid bacteria.

An analysis of the dependence of TA on the specific interfacial area (Figs. 1–3) shows that a larger a value means a faster reaction, regardless of the size of the vessel. Also, equal a values guarantee equal process duration. For example, the required 4 g L^{-1} of TA were achieved after 9 days in large flasks ($a \cong 0.036$), after 10 days in cylinders ($a \cong 0.031$), and after 10 days in small reactors ($a \cong 0.037$). So, the equality of the a values of two reactors of different size will very probably guarantee similar mass transfer in the two systems. If so, the specific interfacial area may be used as a leading parameter in the scaling-up procedure, instead of the liquid volume as suggested Malbaša et al. (2006).

4. Mathematical model

4.1. Deriving the model

The regression analysis was applied onto the database consisting of 15 average values on the systems in cylinders, 21 average values on the systems in large flasks, and 23 average values on the systems in small reactors. In this way, a total of 59 average titratable acidity (TA) data were acquired and used to derive the function TA(τ,a):

$$\mathrm{TA} = b_1 a + b_2 \tau + b_3 a\tau + b_4 a\tau(a - \tau) + b_5 a\tau(a - \tau)^2 \quad (s^2 = 0.142) \quad (2)$$

Cylinders, large flasks and small reactors were filled with various volumes of the liquid phase (see Table 1), giving the systems with different interfacial areas and broadening the database.

The function (2) is a mathematical description of the relationship between the TA, on the one hand, and fermentation duration (τ) and specific interfacial area (a), on the other. Among several models, the model (2) turned out to be the best, according to the criterion:

$$s^2 = \frac{\sum_{i=1}^{n}(\mathrm{TA}_{i,\mathrm{exp}} - \mathrm{TA}_{i,\mathrm{calc}})^2}{n - k} \quad (3)$$

where $TA_{i,exp}$ denotes the average titratable acidity based on the experimental values, while $TA_{i,calc}$ represents the corresponding titratable acidity calculated by Eq. (2). Also, n stands for the number of average values (in our case 59), while k denotes the number of parameters in the model (in our case 5).

The problem of determining the parameters ($b_1 - b_5$) of the regression Eq. (2), according to the well-known method of least squares, reduces to finding the minimum of a function which expresses the sum of squared differences between the measured and calculated acidities. This function has as many first derivatives as there are unknown parameters; in mathematical statistics, they are known as a system of normal equations. A unique solution of this system is a set of b-values. The b-parameters estimated in our database are presented in Table 3, together with the corresponding t values relevant for the analysis of the significances of the parameters. Evidently, the largest t value is associated with the linear time term, proving the greatest effect of the fermentation duration on the TA value. Very significant are the parameters b_5 and b_4 related to the complex interaction of two variables (the fermentation duration and specific interfacial area). They introduce a considerable non-linearity into the model. Also significant in respect of the t value is the parameter b_1, which takes care of the influence of the specific interfacial area on the TA values. The least significant is the simple interaction between the two independent variables (parameter b_3).

4.2. Application of the model in scaling-up

The mathematical model (2) should be valid for every fermentation process conducted by adequately active Kombucha on a substrate of black tea sweetened with 70 g L^{-1} sucrose, if the content of synthesized acids does not exceed 5.78 g L^{-1}, if the process lasts not longer than 14 days, and if the specific interfacial area has a value in an interval of 0.0231– 0.0642 cm^{-1}. The function (2) was derived under these conditions, and it was used for scaling-up the fermentation process as follows.

When the requirement concerning TA($b = 4$ g L^{-1}) is substituted into Eq. (2), it transforms into:

$$7.28a + 0.202\tau - 0.61a\tau - 1.38a\tau(a - \tau) - 0.074a\tau(a - \tau)^2 - b = 0 \quad (4)$$

As this expression contains two independent variables, its numerical solving was performed in the following steps. As first, the process duration was specified and then the specific interfacial area was calculated by applying the Newton–Raphson procedure. Thus, the interfacial area needed for achieving 4 g L^{-1} TA after the specified period of time, was determined. When repeating these steps, a series of values of time-specific interfacial areas were obtained, as given in Table 4. It is obvious that a smaller a value required longer fermentation, which is a direct consequence of a slower mass transfer in the system. The calculated specific interfacial areas can be applied to estimate the reactor characteristics that will ensure satisfactory fermentation conditions, presented in Table 4 for several volumes of the product (20–100 L).

4.3. Model verification

In order to prove validity of the introduced model (4) as a tool for scaling-up the Kombucha fermentation, an experiment was performed in a large-scale reactor (90 L), whose diameter was 63 cm (Table 1). The resulting specific interfacial area was $a = 0.0346$, and the estimated duration of fermentation (according to the data in Table 4) was 9–10 days. The experiment showed that the required acidity of the product (4 g L^{-1}) was achieved in 10 days (Fig. 4). Thus, a high agreement between experimental and calculated TA during Kombucha fermentation was found. This may be taken as a proof that the scaling-up was achieved successfully, with the scaling-up ratio 1:5, suggested as the optimal one in the case of bioreactors (Junker, 2004). It may also be concluded that the method based on the specific interfacial area is precise and reliable, at least as the previously suggested method in which geometric similarity of small and large reactors was taken as the main criterion (Malbaša et al., 2006).

The robustness of the model (4) was checked by its application to extremely small vessels. For this purpose the experiment was performed in small flasks (0.33 L of

Table 3
Parameters in the mathematical model (3) and their t values

No.	Parameter		t-value
	Symbol	Value	
1	b_1	7.28	0.14
2	b_2	0.202	0.32
3	b_3	−0.61	0.015
4	b_4	−1.38	0.19
5	b_5	−0.074	0.20

Table 4
Characteristics of large reactors obtained by the scaling-up procedure

Process duration, days	Spec. inter. area, cm^{-1}	Reactors producing fermented tea with 4 g L^{-1} TA					
		V, L	d, cm	V, L	d, cm	V, L	d, cm
7	0.0573	20	38.2	50	60.4	100	85.4
8	0.0452	20	33.9	50	53.7	100	75.9
9	0.0366	20	30.5	50	48.3	100	68.3
10	0.0304	20	27.8	50	44	100	62.2

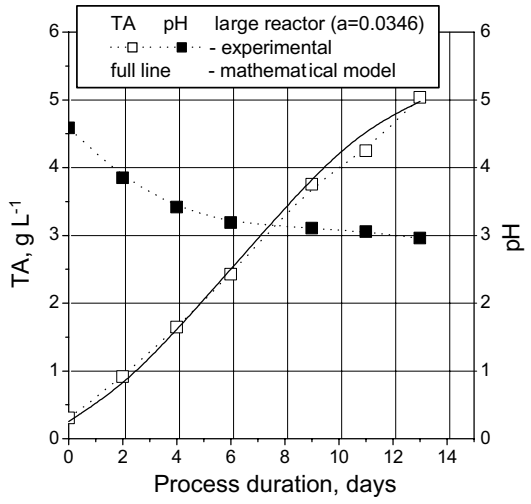

Fig. 4. Changes in pH values and titratable acidity in large reactor compared to the calculated acidity.

liquid volume), with 8 cm in diameter (Table 1). The resulting specific interfacial area was $a = 0.152$, which is approximately 2.5 times larger than the largest a value (0.0642) used for deriving the mathematical model. The fermentation time calculated from the model is 4 days, while the experiment to achieve the required TA value (4 g L^{-1}) lasted 4.5 days. The agreement is acceptable, especially having in mind that an extrapolation was done. At homes, Kombucha is usually prepared in common flasks (Table 1). The model (4) was applied to such systems as well. For example, in a 4.18 L of liquid volume, the TA of 4 g L^{-1} was achieved in about 6.5 days, while the calculated duration was approximately 7.5 days. A comparison of the experimental and calculated times showed that the former ones were longer than those estimated by using Eq. (4) when applied onto a system that was not included in the model derivation (small flasks and large reactors).

5. Conclusion

The reported investigation is a first step towards suggesting the specific interfacial area as a variable that controls the duration of Kombucha fermentation. Regardless of their size, the reactors having equal values of specific interfacial area will very probably ensure the production of a fermented tea of the given characteristics in the equal periods of time. This was concluded by analyzing the experimentally obtained results, as well as by applying a mathematical model derived for scaling-up a typical Kombucha fermentation system.

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