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Thermotolerance Study of Bagasse-Alginate Encapsulant using Effective Thermal Conductivity Model for Sustainable Probiotic Feed

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A great potential in the synthesis of heat resistant microcapsules for probiotic was developed via the synthesis of heterogeneous material. In the study of heterogeneous material, effective thermal conductivity is widely used in measuring the thermal behaviour of the material and is regarded as a key thermophysical property. Production of heterogeneous material could be created via filler incorporation. Incorporation of filler in the microcapsule matrix could enhance the thermotolerance characteristic of the microcapsule. The encapsulation wall concentration also could contribute to the value of effective thermal conductivity of the heterogeneous material. This research is aimed to model fitting the series model of effective thermal conductivity using data collected from immobilised Lactobacillus rhamnosus NRRL 442 in alginate microcapsule (NaA) with incorporated sugarcane bagasse (SB). Different alginate concentration of 1 %, 2 % and 3 % and NaA : SB ratio of 1 : 0, 1 : 1 and 1 : 1.5 were used in synthesising the microcapsules. The microcapsules were produced using the extrusion method. The lowest effective thermal conductivity was obtained from the same microcapsule preparation using 3 % of alginate concentration and at a NaA : SB concentration ratio of 1 : 1.5. The microcapsules prepared using 1 % of alginate concentration and a NaA : SB concentration ratio of 1:0 showed the highest effective thermal conductivity. The regression values (R^2) for all alginate concentrations (1 %, 2 % and 3 %) were found to be 0.9997, 1.0000, and 0.9973. The regression models of the effective thermal conductivity in all alginate concentrations versus filler (SB) concentration were plotted. High coefficient of determination was obtained and showed strong correlation between effective thermal conductivity and SB concentration for different alginate concentration. This study could be beneficial in the production of products that necessitate heat treatment thermo tolerance especially in pelleted probiotic feed.

1. Introduction

Heterogenous solid materials have been broadly used in processes of heat transfer and thermal managements (Agrawal and Satapathy, 2013). In quantifying the thermal behaviour of heterogenous materials, effective thermal conductivity is widely measured as a key thermophysical property. The main factors of effective thermal conductivity value are the composition and structure of heterogenous material (Agrawal and Satapathy, 2013). There are two fundamental structural models that have been previously developed and applied; parallel model (Carson et al., 2005) and series models (Bujard et al., 1994). The physical structure of heterogenous materials influences the developing of a model in defining the effective thermal conductivity (Wang et al., 2008). The structure of heterogenous materials involved in series and parallel models was the laminate (layered) structure (Wang et al., 2008).
Various previous studies were performed in evaluating the modelling of heat conduction in microstructure (Cho et al., 2012). A procedural function-based constructive approach was used to model heterogenous materials (Pasko et al., 2009). Another approach known as the hybrid Voronoi-B-Spline was applied. This

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approach is based on the geometric representation method when irregular porous structures was used (Kou and Tan, 2010). The random morphology description functions (RMDF) was another alternative approach for modelling a heterogenous structure (Vel and Goupee, 2010).
The effective thermal conductivity of series model was referred as the heat flux model for the heterogenous-spherical structure in the study. This study is a continuity and complementation to the findings obtained in Shaharuddin and Muhamad (2015). The beneficial step of probiotic immobilisation before microencapsulation was significantly enhanced microencapsulation efficiency and cell survivability after heat exposure of 90 ºC for 30 s (Shaharuddin and Muhamad, 2015). A great potential in the synthesis of heat resistant microcapsules for probiotic was discovered.
The model fitting of this heterogeneous microcapsule in relation with effective thermal conductivity using double protection wall is scarce. The objective of this study was to fit the series model of effective thermal conductivity of immobilised Lactobacillus rhamnosus NRRL 442 (Lr) in bagasse-alginate microcapsule. The model fitting of microcapsules was analysed through the series model using element of alginate and sugarcane bagasse concentrations, and thermal conductivity. The regression models of the effective thermal conductivity in all alginate concentrations versus filler (sugarcane bagasse) concentration were plotted and coefficient of determination was analysed.

2. Materials and Methods

2.1 Materials

Sodium alginate (Q-ReC), calcium chloride (Q-ReC), peptone water (Sigma Aldrich), sodium citrate (Q-ReC), MRS broth (Merck), MRS agar (Merck) and potassium bromide (Merck) were of analytical grade. All chemicals were used without further purification. Lactobacillus rhamnosus NRRL 442 used as probiotic model was received from Agricultural Research Service (ARS, USA). Fresh sugarcane bagasse (SB) was collected from local supplier in Johor Bahru, Johor, Malaysia. Three replications were performed for each sample. Fresh SB was ground and sieved using 75 µm mesh. Then SB was dried using hot air oven at 40 °C for 48 h and kept in freezer (-20 °C).

2.2 Microencapsulation of immobilised Lactobacillus rhamnosus (Lr) NRRL 442

Culture medium of MRS broth (100 mL) was prepared as mentioned in Shaharuddin and Muhamad (2015). Immobilisation of probiotic started with the cell suspension of Lr was mixed homogenously with powdered SB at ratio of 8 : 1 (Guergoletto et al., 2010). The mixture was left for 1 h for adsorption of Lr in sterile condition following Saarela et al. (2006). Microencapsulation was performed by slight modification of the procedure described by Chen et al. (2007). The syringe was modified via adding a 10 µL tip into the syringe. This modification allows the decreasing of pore diameter and subsequently produced smaller size of microcapsule. The immobilised Lr was microencapsulated via extrusion technique. Coating material were prepared using 1, 2 or 3 % (w/v) of sodium alginate and autoclaved (121 ºC, 15 min). The coating material was mixed with immobilised Lr at different dry weight ratio of 1 : 0, 1 : 1 and 1 : 1.5 (w/w). The homogenised mixture of immobilised Lr and coating solution was extruded through modified syringe into sterile 0.1 M calcium chloride ($CaCl_2$). The formed microcapsules were allowed to stand for 1 h for solidification, then rinsed and subsequently kept in sterile 0.1 % peptone solution at 40 ºC for further analysis.

2.3 Model fitting of encapsulant on effective thermal conductivity (K_{eff})

The effective thermal conductivity was determined using equation of effective thermal conductivity (Eq(1)) developed by Bujard et al. (1994). The equation was shown as below:

$$K_{eff} = \frac{1}{\frac{v_1}{k_1} + \frac{v_2}{k_2}} \qquad (1)$$

Where K_{eff} is the effective thermal conductivity, v_1 is the volume fraction of the first layer of heterogenous material with thermal conductivity, k_1, and v_2 is the volume fraction of second layer of heterogenous material with thermal conductivity, k_2.
Then, the effective thermal conductivity versus SB concentration (filler) at different alginate concentrations was plotted. The model equation that relates both variables were obtained through curve fitting method (regression method). The established thermal conductivity of alginate and SB were obtained from previous studies where the values of alginate and SB are 0.6 W/m °C (Pawar and Sunnapwar, 2013) and 0.05 W/m ºC (Gertrude, 2011). The coefficients of determination (R^2) of the models were analysed to determine the suitability of the model in describing the effective thermal conductivity for heterogenous material.

3. Results and discussion

The series model of effective thermal conductivity for heterogenous material was fitted to each alginate concentration (Bujard et al., 1994). The microencapsulated probiotic was assumed as heterogenous material as it consisted of two different materials (alginate and SB) and form a heterogenous material or composite. Series model was previously used to explain relationship between heterogenous material and effective thermal conductivity (Carson et al., 2005).

Several reasons have been identified in the selection of the model such as both studies using heterogenous or composite material as sample, data from this study were compatible with this particular model compared to other models and similar conditions and assumption of heat flow direction were applied in both studies. The initial condition of series model is when two materials are arranged in a series where the lowest thermal conductivity material blocks the heat conduction most effectively (Bujard et al., 1994). The initial condition of heat exposure of microencapsulated probiotic was similar to the initial condition of this model. Heat movement of this model was in line with heat movement during heat exposure of microcapsule of immobilised Lr NRRL 442 which was illustrated from SEM result of alginate-bagasse microcapsule reported in Shaharuddin and Muhamad (2015).

For the three types of fillers (1 : 0, 1 : 1 and 1 : 1.5), the effective thermal conductivities were determined using Eq(1). Effective thermal conductivity in all alginate concentrations gradually decreased as the concentration of filler (SB) of the microcapsule was increased as shown in Table 1.

Table 1: The effective thermal conductivity in different alginate concentrations

Alginate concentration (%)	Alginate : SB ratio		
	1 : 0	1 : 1	1 : 1.5
	Effective thermal conductivity (W/m °C)		
1	1.67	1.54	1.47.
2	0.83	0.77	0.74
3	0.56	0.51	0.49

Table 1 shows that the highest effective thermal conductivity was obtained when an alginate concentration of 1 % and an NaA : SB concentration ratio of 1 : 0 was used. The results showed a generally high effective thermal conductivity can be obtained when an alginate concentration of 1 % was used, as compared to the low efeective thermal conductivity obtained at all three ratios when an alginate concentration of 3 % was used. The relationship between the filler (SB concentration) and the effective thermal conductivity are shown in the following figures. Figure 1 demonstrates the thermal conductivity obtained at all ratios when alginate concentration of 1 % was used.

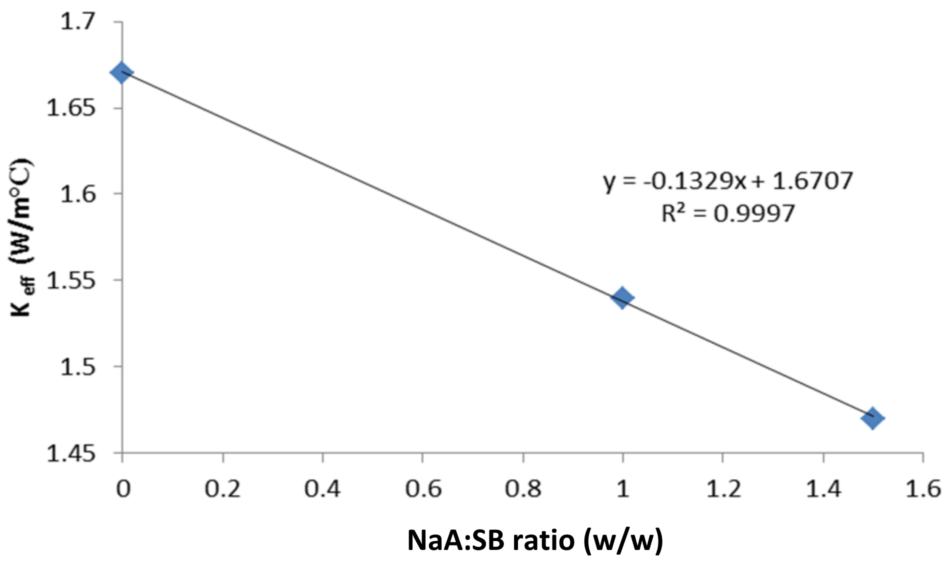

Figure 1: Series model fitting and linear mathematical equations representing for 1 % alginate concentration

The regression analysis from the series of model fitting and linear mathematical equations showed a good fit between the experimental data and calculated data, where R^2 is as high as 0.997. Similar results were obtained for the experimental and calculated effective thermal conductivity at all ratios when the alginate concentration was at 2 % and 3 %, as shown in Figure 2 and Figure 3.

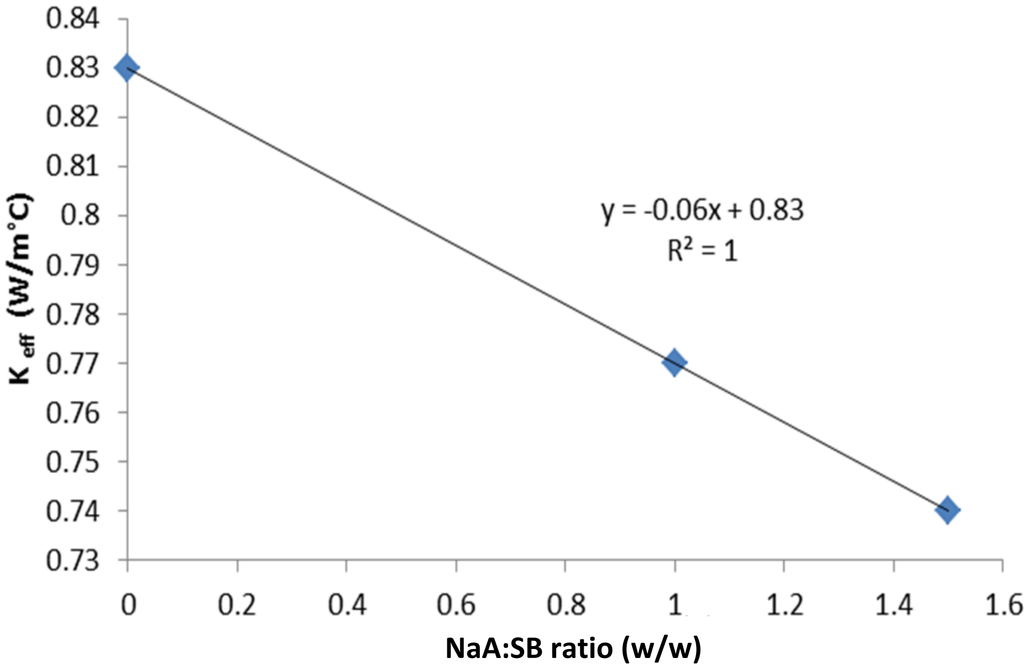

Figure 2: Series model fitting and linear mathematical equations representing for 2 % alginate concentration

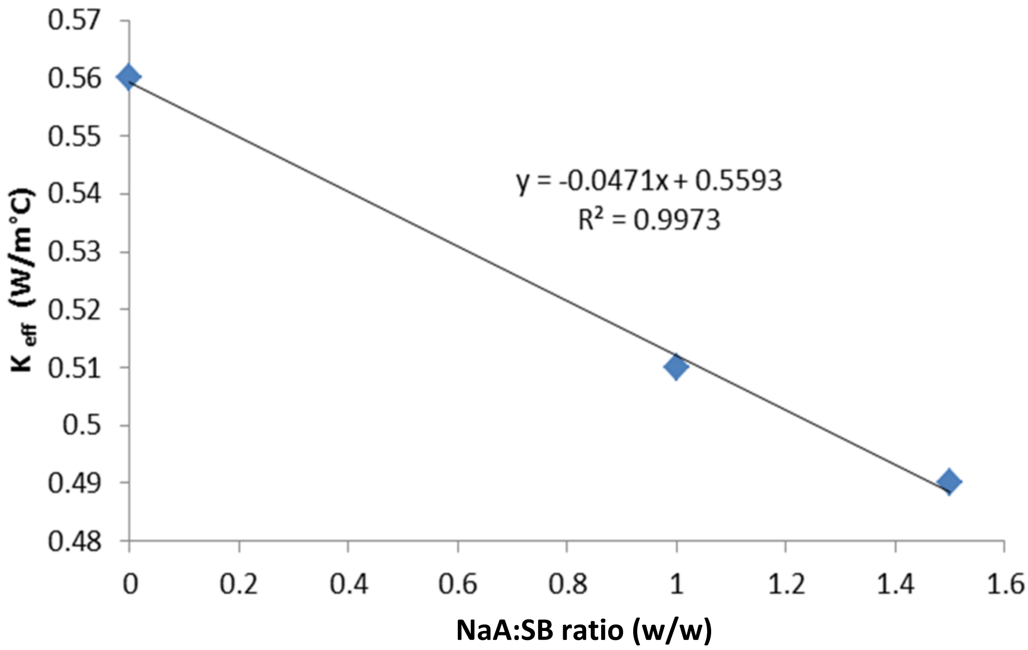

Figure 3: Series model fitting and linear mathematical equations representing for 3 % alginate concentration

The equation and the corresponding coefficients of determination (R^2) of the regression models for each alginate concentration were tabulated in Table 1. Hassan and Ramaswamy (2011) stated that the coefficient of determination, R^2, is the proportion of variation in the response attributed to the model rather than to random error. R^2 value was suggested to be higher than 0.8 is a well fitted model.

Higher values of R^2 demonstrate that the model is appropriate to explain the relation between variables. The R^2 values for all alginate concentrations (1 %, 2 % and 3 %) were found to be 0.9997, 1.0000, and 0.9973. This showed that the fitting model was suitable to explain the experimental variations in this study.

The models concluded that the effective thermal conductivity in all alginate concentrations gradually decreased with the increase of the filler concentration (SB) of the microcapsule as shown in Table 1. The value range (0.49 - 1.67 W/m °C) of the effective thermal conductivity in this study was within the value range (0.25 - 4.50 W/m °C) of the effective thermal conductivity in the series model demonstrated in Bujard et al. (1994). It showed the validity of the generated regression models in this study with the fitted series model of the effective thermal conductivity.

By using an alginate concentration of 3 %, low effective thermal conductivity was obtained for all ratios as compared to the effective thermal conductivity obtained using 1 % and 2 % alginate concentration. The lowest effective thermal conductivity (0.49 W/m °C) was obtained from the microcapsule preparation of 3 % of alginate concentration and 1 : 1.5 of alginate : SB concentration ratio. This finding correlated and is in agreement that the better survivability of microencapsulated probiotic after heat exposure obtained from the similar microcapsule preparation as described in Shaharuddin and Muhamad (2015). Evidently, the reduced effective thermal conductivity value enhances the chance of cell survivability during heat exposure and this was in agreement with the results that showed high percentage of cell viability for microencapsulated probiotic as compared to free cell as mentioned in Shaharuddin and Muhamad (2015). Kao et al. (2014) concluded that the increased blockage ratio in heat transfer system may decrease the transfer of heat and reduce the temperature obtained.

4. Conclusions

The regression models that relate the effective thermal conductivity in all alginate concentrations versus filler (SB) concentration were plotted and high coefficient of determination were obtained. The models showed strong correlation between the effective thermal conductivity and SB concentration for different alginate concentration. The regression models were well fitted and validated with the effective thermal conductivity results of the series model. The lowest effective thermal conductivity was obtained from the same microcapsule preparation using 3 % of alginate concentration and at a 1 : 1.5 of alginate : SB concentration ratio. These conclusions demonstrated the great potential in the synthesis of heat resistant alginate-bagasse microcapsules for probiotic and for inclusion as probiotic additive. This study could be useful in the production of pelleted feed or other products that necessitate thermo tolerance in heat treatment.

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