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Research Article

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Effect of complexation conditions on microcapsulation of *B. bifidum BB01* in xanthan–chitosan polyelectrolyte complex gels

[1]He Chen, [1]Yajuan Song, [1]Nina Liu, [2]Hongchang Wan, [1]Na Liao and [1]Guowei Shu*

[1]College of Life Science & Engineering, Shaanxi University of Science & Technology, Xi'an, China
[2]Shaanxi Yatai Dairy Co., Ltd., Xianyang, China

ABSTRACT

There are many reports on the study of microencapsulated bifidobacteria, a variety of embedding materials using to embed probiotics have been applied in those reports. This study investigated that Bifidobacterium bifidum BB01 was immobilized with xanthan/chitosan gel using extrusion method, and the viable counts and encapsulation yield (EY) of B. bifidum BB01 encapsulated in different chitosan solution pH (5, 5.3, 5.6 and 5.9) ,in different chitosan concentration (0.4%, 0.7%, 1.0% and 1.3%), in different xanthan concentration (0.5%, 0.7%, 0.9% and 1.1%), in different cell suspension-xanthan ratios (1:3, 1:5, 1:7 and 1:9), in different mixed bacteria glue liquid-chitosan ratios (1:3,1:4,1:5 and 1:6), have been reported. It was studied by single factor experiment method, the results showed that this several factors impacted the viable counts and encapsulation yield (EY) of B. bifidum BB01 significantly, and the optimum chitosan solution pH for B. bifidum BB01 was 5; the optimum chitosan concentration was 1.3%; the optimum xanthan concentration was 0.5%; the optimum cell suspension-xanthan ratio was 1:3; the optimum mixed bacteria glue liquid-chitosan ratio was 1:6.

Keywords: xanthan; chitosan; *Bifido bacterium bifidum*; microencapsulation; extrusion

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INTRODUCTION

Considering the increasing demand for functional foods, probiotics became one of the most important healths promoting food enhancement in recent years [1]. Probiotics are viable microorganisms which are beneficial to the host when administered in adequate amounts [2].

Bifidobacterium bifidum is the most common genera of bacteria used as probiotics for the production of dairy products In order to exert beneficial effects for probiotics, they must be able to tolerate the acidic conditions of the stomach environment and the bile in the small intestine [3,4]. The acidic environment of the stomach and the bile salts secreted into the duodenum are the main obstacles for the survival of the ingested bacteria. However, the tolerance of bifidobacteria to the pH values of the gastric juice is generally considered low [5-8] and the bifidobacteria are vulnerable to oxygen. As a result, the viable counts of bifidobacteria in probiotic dairy products often have a exponential curve downward trend, which lead to those products have a difficult to achieve the healthy effect [9] .

Microencapsulated form has received reasonable attention, since it can protect probiotic organisms against an unfavorable environment, and to allow their release in a viable and metabolically active state in the intestine [10, 11]. In the process of microencapsulation of Probiotics, coatings and mixtures of suitable biopolymers, such as alginate, k-carrageenan, gellan-gum are applied [12,13]. However, those embedding materials have some defects in the process of microencapsulated Probiotics, e.g. alginate is not stable in acidic conditions.

Chitosan is a natural biological macromolecules, it has excellent biological properties such as biocompatibility,

biodegradability, lack of toxicity, and so on [14, 15]. Xanthan gum is stable over a wide range of temperatures and pH, which finds many applications in food [16,17]. The hydrogel net-work formed through the ionic interactions between the amino groups of chitosan and carboxyl groups of xanthan shows pH-sensitive swelling characteristics, which enable the controlled release of entrapped materials such as therapeutic agents, enzymes and bacteria [18, 19]. Therefore, xanthan–chitosan hydrogels are recognized as promising candidates for targeted delivery and controlled release of encapsulated products for oral administration.

In this study, *Bifidobacterium bifidum BB01* was immobilized with xanthan/chitosan gel using extrusion method. Some factors, such as chitosan solution pH and concentration, xanthan concentration, cell suspension- xanthan ratio, mixed bacteria glue liquid-chitosan ratio have been investigated. The optimum conditions of microencapsulated *Bifidobacterium bifidum BB0I* will be observed. The results will be helpful to further optimize the process of *Bifidobacterium bifidum* microencapsulation, and provide reference for obtaining higher viable counts and entrapped yield of *Bifidobacterium bifidum* microcapsules.

EXPERIMENTAL SECTION

Preparation of chitosan and xanthan solutions

Chitosan with a minimum of 80~95% deacetylation and a molecular weight of 370000 was purchased (Nantong Xingcheng Biological Co., Ltd., Jiangsu). A known amount of chitosan was dissolved in 1N HCl by agitating. The desired solution pH was adjusted by 1M NaOH and DI water was added to bring it to the final volume. Xanthan gum with a molecular weight of 1.02 million was kindly supplied by Zibo Zhongxuan biochemistry .A predetermined amount of xanthan gum was dissolved in DI water under heating and agitation. Xanthan solution was autoclaved(110℃,15min) before use.

Microorganism

Bifidobacterium bifidum BB01 was obtained from College of Life Science & Engineering, Shaanxi University of Science & Technology, it was cultured in MRS medium at 37℃ for 24h. The cells were harvested by centrifugation at 4000g for 10 min at 4℃and washed twice before resuspending them in 5mL normal saline. The final cell concentration was adjusted to 2.0×10^9 CFU/mL.

Microencapsulation

In this study the extrusion method was used. The xanthan solutions and 1mL of cell suspension were mixed and the content was vortexed to homogeneity. Capsules were formed by dropwise addition of mixture into a solution of chitosan using a manually operated syringe with a 0.45-mm cannula. The chitosan solution was agitated continuously for 40 min to allow crosslinking and avoid coalescence of capsules. The capsules were filtered through a 160 mm Milliporenylon filter, washed twice with DI water.

Viable count

The sample to be tested with sterile saline solution into the bacterial suspension, then it was diluted at 10 times, and taking the dilution of 10^{-7} to 10^{-8} of the suspension inoculation of 1mL to the top agar medium. After the bacterias were cultured for 48h at 37 ℃, we can observe and count the average values, and investigate the various factors on the microencapsulation of Bifidobacterium viable counts. The viable counts of microcapsules were weight through a formula according to Eq. (1):

$$VC=N\times T\times 10 \quad (1)$$

Where VC is viable counts of the original suspension on a per milliliter (CFU/mL).N is average colony number of 3 repeat solid culture in the same dilution (CFU) .T is times of dilution.

Encapsulation yield (EY)

Encapsulation yield (EY), which is a combined measurement of the efficiency of entrapment and survival of viable cells during the microencapsulation procedure, was calculated according to Eq. (2):

$$EY=N\ /\ N_0\times 100\% \quad (2)$$

Where N is the number of viable entrapped cells released from the microspheres, and N_0 is the number of free cells added to the biopolymer mix during the production of the microspheres.

RESULTS AND DISCUSSION

Effect of chitosan solution pH on encapsulation of *B. bifidum BB01*

According to the initial preparation conditions of microcapsulation, chitosan solution pH was adjusted to 5, 5.3, 5.6and 5.9, the results as shown in Figure1.

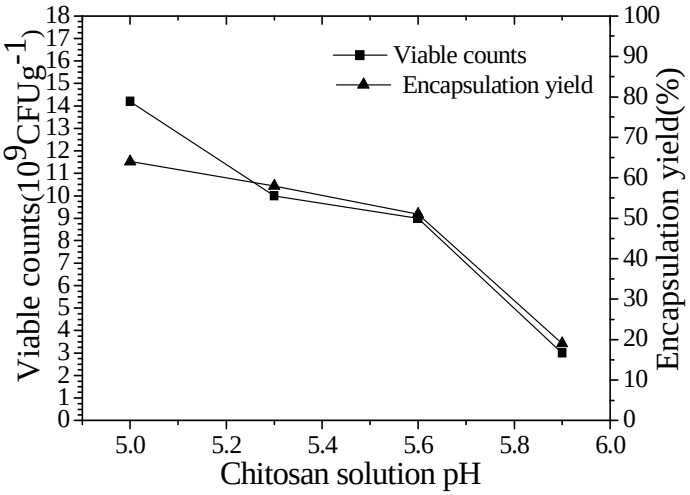

Fig.1 Effect of chitosan solution pH on viable counts and entrapped yield of *B. bifidum BB01* of microcapsules

According to the Figure1，with increasing of the chitosan solution pH, the viable counts and entrapped yield of *B. bifidum BB01* microcapsules continually decreased. This phenomenon may be due to the change of the chitosan solution physic chitosan solution pH increased, the amino groups became less charged, which lead to fewer ionic linkages would occur between the two polymers. As a result, the crosslinking densities of xanthan–chitosan hydrogel capsules decreased, diffusion coefficient became higher for chitosan chains, so amounts of bacteria spread out from xanthan–chitosan hydrogel capsules, viable counts of encapsulation and encapsulation yield of *B. bifidum BB01* microcapsules will be reduced, correspondingly.

As a result, there is a preliminary determination about the chitosan solution pH for *B. bifidum BB01* microencapsulated.The optimum chitosan solution pHwas 5, which corresponds to viable counts and entrapped yield were 1.4×10^{10}CFU/g and 64%, respectively.

Effect of chitosan concentration on encapsulation of *B. bifidum BB01*

According to the initial preparation conditions of microcapsulation, chitosan concentration was adjusted to 0.4%, 0.7%, 1.0% and 1.3%, the results as shown in Figure2.

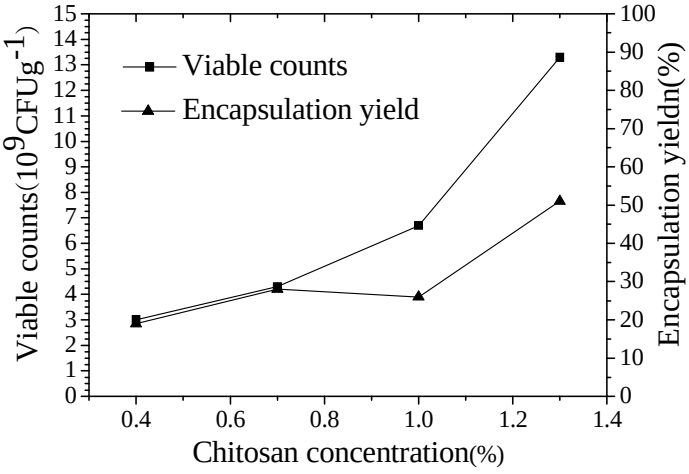

Fig.2 Effect of chitosan concentration on viable counts and entrapped yield of *B. bifidum BB01* of microcapsules

According to the Figure2，with increasing of the chitosan concentration, the viable counts and entrapped yield of *B.*

bifidum BB01 microcapsules continually increased. Since chitosan concentration increased, the amino groups become more charged, which lead to more ionic linkages would occur between the two polymers. As a result, the crosslinking densities of xanthan–chitosan hydrogel capsules increased, amounts of bacteria will be embedded completely, viable counts of encapsulation and encapsulation yield of *B. bifidum BB01* microcapsules will be increased, correspondingly.

As a result, there is a preliminary determination about the chitosan concentration for *B. bifidum BB01* microencapsulated. The optimum chitosan concentration was 1.3%, which corresponds to viable counts and entrapped yield were 1.3×10^{10}CFU/g and 51%, respectively.

Effect of xanthan concentration on encapsulation of *B. bifidum BB01*

According to the initial preparation conditions of microcapsulation, xanthan concentration was adjusted to 0.5%, 0.7%, 0.9% and 1.1%, the results as shown in Figure3.

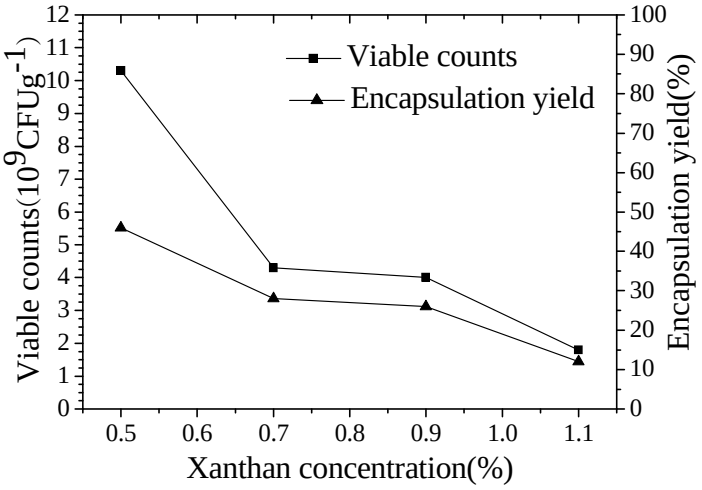

Fig.3 Effect of xanthan concentration on viable counts and entrapped yield of *B. bifidum BB01* of microcapsules

According to the Figure3，with increasing of the xanthan concentration, the viable counts and entrapped yield of *B. bifidum BB01* microcapsules continually decreased. This phenomenon may be due to the degree of swelling decreased when increasing

Xanthan solution from 0.5% to1.1%, this indicated the crosslink densities of xanthan–chitosan hydrogel capsules become increased. When we count the number of the bacteria in a certain period of time, increasing xanthan concentration resulted in significantly greater the crosslink density of xanthan–chitosan hydrogel capsules and lower the dissolution rate, As a result, it takes a lot of time to release probiotic cells under the degradation media, the viable counts and entrapped yield of *B. bifidum BB01* microcapsules will be decreased, correspondingly.

As a result, there is a preliminary determination about the xanthan concentration for *B. bifidum BB01* microencapsulated. The optimum xanthan concentration was 0.5%, which corresponds to viable counts and entrapped yield were 10×10^{9}CFU/g and 46%, respectively.

Effect of cell suspension-xanthan ratios on encapsulation of *B. bifidum BB01*

According to the initial preparation conditions of microcapsulation, the difference proportion of prepared bacteria suspension volume (mL) and xanthan solution volume (mL) were investigated, such as 1:3, 1:5, 1:7, and 1:9. The effect of various cell suspension- xanthan ratios on encapsulation of *B. bifidum BB01* was shown in Figure4.

According to Fig.4, with increasing of the proportion of xanthan and bacteria suspension, the viable counts and entrapped yield of *B. bifidum BB01* of microcapsules continually decreased, this phenomenon may be due to the high proportion of sodium alginate and bacteria suspension. Although the more volume of bacterial suspension leads to the more number of core material of the microcapsule contained and the viable counts and entrapped yield should be very high, the bacterial suspension volume increased, xanthan solution volume will be reduced. As a result, the phenomenon of incomplete embedded will emerge, and most of the cells were not embedded strongly.

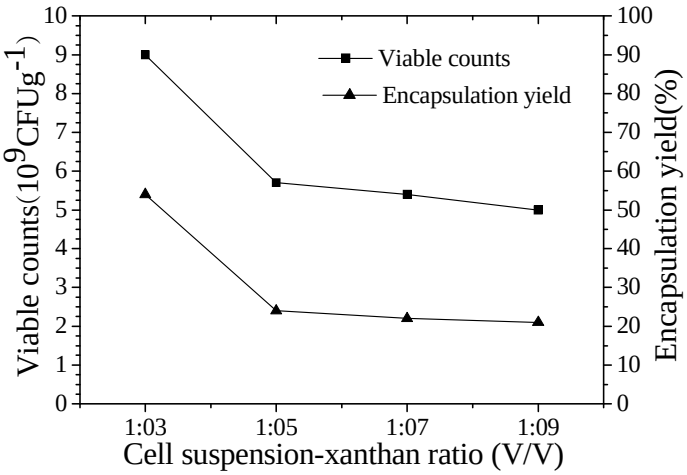

Fig.4 Effect of cell suspension- xanthan ratio on viable counts and entrapped yield of *B. bifidum BB01* of microcapsules

As a result, there is a preliminary determination about the cell suspension- xanthan ratio for *B. bifidum BB01* microencapsulated.The optimum cell suspension- xanthan ratio was 1:3, which corresponds to viable counts and entrapped yield were 9×10^9CFU/g and 54%, respectively.

Effect of chitosan-mixed bacteria glue ratios on encapsulation of *B. bifidum BB01*

According to the initial preparation conditions of microcapsulation, the different proportion of mixed bacteria glue liquid volume (mL) and chitosan volume (mL)were adjusted to 1:3,1:4,1:5,1:6, the results as shown in Figure 5.

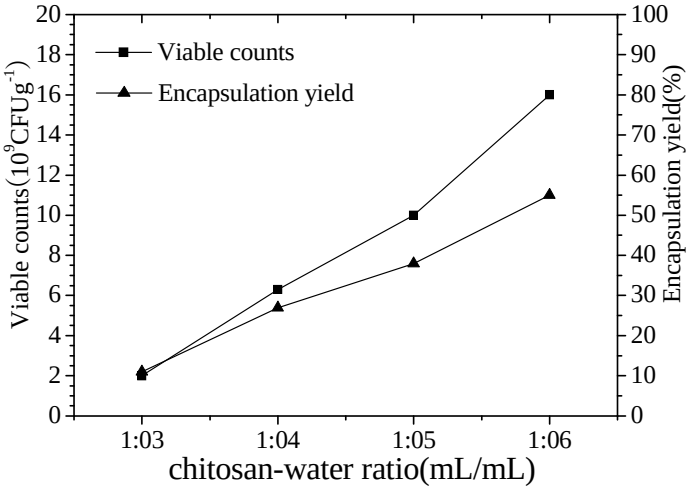

Fig.5 Effect of e chitosan-water ratio on viable counts and entrapped yield of *B. bifidum BB01* of microcapsules

According to Fig.5, with increasing of the chitosan-water ratio, the viable counts and entrapped yield of *B. bifidum BB01* microcapsules continually increased. The reason of this tendency on figure was that the high values about proportion of mixed bacteria glue liquid and chitosan. With increasing of chitosan solution, microcapsules will be crosslinked completely resulted in the large amounts of bacteria will be entrapped; the viable counts and entrapped yield of *B. bifidum BB01* microcapsules will be increased, correspondingly.

As a result, there is a preliminary determination about the cell suspension- xanthan ratio for *B. bifidum BB01* microencapsulated. The optimum mixed bacteria glue liquid and chitosan ratio was 1:6, which corresponds to viable counts and entrapped yield were 1.6×10^{10}CFU/g and 55%, respectively.

CONCLUSION

This present work showed that several factors, including chitosan solution pH and concentration, xanthan concentration, cell suspension-xanthan ratio, mixed bacteria glue liquid-chitosan ratio, have an important influence on microcapsulation of *B. bifidum BB01*. The optimum chitosan solution pH for *B. bifidum BB01* was 5; the optimum chitosan concentration was 1.3%; the optimum xanthan concentration was 0.5%; the optimum cell suspension-xanthan ratio was 1:3; the optimum mixed bacteria glue liquid-chitosan ratio was 1:6.

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