Biocatalysis and Agricultural Biotechnology 19 (2019) 101119

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Intestinal adsorption of glucose, cholesterol and bile salt by simultaneous incorporation of edible microbiosorbent and intestinal bacteria

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ARTICLE INFO

Keywords:
Microbiosorbent
Adsorption
Cholesterol
Probiotic
Microalgae
Lactobacillus plantarum.

ABSTRACT

Natural edible adsorbents can play an important role in human healthcare via lowering the harmful materials from gut and regulating the microflora composition. In this work, several microalgae and plant sources, including *Chlorella vulgaris* (Ch), zucchini (Zu), ginger (Gi), rice bran (RB) and Zahedi date (ZD), were studied on the production of edible and bacterial biosorbents for the adsorption of glucose, cholesterol and bile salt *in vitro*. By screening the sources, Ch, Zu and Gi were selected for maximizing the production of *Lactobacillus plantarum.* Afterward, the optimized mixture of zucchini-ginger-*C. vulgaris* (ZuGiCh), obtained from the combination of the microparticles of zucchini (48.80 g/l), ginger (35.00 g/l) and *C. vulgaris* (39.30 g/l), maximized *L. plantarum* cells to $2.37 \pm 1.86 \times 10^9$ CFU/ml and minimized *Escherichia coli* and *Staphylococcus aureus* cells to $4.53 \pm 0.41 \times 10^8$ and $8.10 \pm 0.61 \times 10^8$ CFU/ml, respectively. The simultaneous incorporation of *S. aureus* and ZuGiCh as an edible microbiosorbent resulted in the highest adsorption of glucose (57.65%) and cholesterol (83.65%) at initial concentration of 40 g/l adsorbate while the incorporation of *L. plantarum* and ZuGiCh showed the highest adsorption of bile salt (42.59%) at initial concentration of 80 g/l bile salt. The ZuGiCh microparticles can be used as a potentially intestinal adsorbent for improving microflora and lowering of health hazards from gut.

1. Introduction

Diabetes and cardiovascular diseases develop due to the potent effects of high levels of glucose and cholesterol or several cholesterol oxides, respectively (Kim et al., 2002). Focusing on the natural prevention methods has a high priority to the drug therapy of diseases. Two natural methods of the adsorption by microparticles, dietary fibers and gut flora, and the biodegradation by enzymes and bacteria for removal of glucose, cholesterol and bile salts were mostly studied (Arun et al., 2017; Chen et al., 2015; Miremadi et al., 2014).

Various soluble dietary fibers, extracted from plant sources such as oats, barley, psyllium husk, wheat bran, guar and *Musa paradisiaca,* reduced the glucose level by *in vitro* adsorption (Ahmed et al., 2011; Arun et al., 2017). The removal of bile salts and other components synthesized from cholesterol, leads to increase the demand of cholesterol for synthesis of them, and thus lowering the cholesterol level in blood. An interesting approach for the removal of cholesterol is a suitable combination of probiotics and prebiotics. It was demonstrated that prebiotics improved the viability of probiotics for the cholesterol-lowering activity in animal models (Liong and Shah, 2005). Another useful approach was the encapsulation of probiotic cells that either polymeric matrices comprised of dietary fibers adsorbed cholesterol or probiotic bound it to the cell walls (Fareez et al., 2017). Thirty-four strains of bifidobacteria were reported as promising probiotics able to efficiently adsorb cholesterol (Bordoni et al., 2013). On the other hand, the cholesterol biosorption methods were suggested for the removal of bile salts (Miremadi et al., 2014). To reduce the level of cholesterol, bile salts and glucose *in vitro*, various natural sources such as apple peels, wheat bran, foxtail millet bran, soybean-seed hull, tomato peel, onion by-products and cocoa bean shells exhibited sufficient biosorption capacities (Benítez et al., 2017; Niu et al., 2018; Nsor-Atindana et al., 2012; Zhang et al., 2011; Zhu et al., 2018).

It was hypothesized that (1) the intestinal bacteria involving probiotics and pathogens could play adsorbents role in the adsorption of glucose, cholesterol and bile salt, (2) the bacteria could ferment natural edible materials for their growth, (3) the fermented materials could

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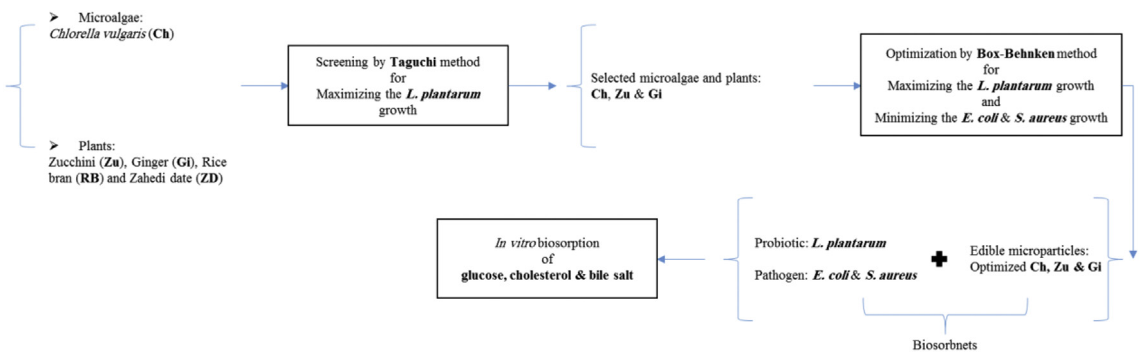
https://doi.org/10.1016/j.bcab.2019.101119
Received 28 February 2019; Accepted 31 March 2019
Available online 04 April 2019
1878-8181/



Scheme 1. Selection and optimization of edible microparticles for regulating the bacteria growth to adsorb glucose, cholesterol and bile salt.

adsorb glucose, cholesterol and bile salt in intestine and (4) the incorporation of the bacteria with the fermented materials showed the synergistic adsorption of glucose, cholesterol and bile salt. Therefore, in this work the several microalgae and plants were screened for increasing the production of *Lactobacillus plantarum*. Afterward, the combination of selected microalgae and plants was optimized as a culture medium for regulating the growth of *L. plantarum*, *Escherichia coli* and *Staphylococcus aureus* as model bacteria in the intestinal bacteria community. Then, it focused on the adsorption capabilities of the optimized plant-microalgae, regulated intestinal bacteria and fermented plant-microalgae for the adsorption of glucose, cholesterol and bile salt (Scheme 1).

2. Materials and methods

2.1. Materials

Probiotic strain of *Lactobacillus plantarum* (IBRC-M 10817) and pathogen strains of *Escherichia coli* (IBRC-M 11074) and *Staphylococcus aureus* (IBRC-M 10917) were supplied by Iranian Biological Resource Center (IBRC, Tehran, Iran). De Man Rogosa Sharpe (MRS) and nutrient broth (NB) media were purchased from Merck (Germany). The plant sources of zucchini (Zu), ginger (Gi), rice bran (RB) and Zahedi date (ZD) were purchased from local market. The microalgae source of *Chlorella vulgaris* (Ch) was obtained from Biotechnology Laboratory, Faculty of Chemical Engineering, Tarbiat Modares University, Tehran, Iran. Glucose, cholesterol and bile salt were supplied by Merck (Germany). The total cholesterol (CHOD-PAP) and glucose (GOD-PAP) assay kits were obtained from Pars Azmun Co. (Tehran, Iran). The other chemicals, used as received without any further purification, were purchased from Merck (Germany).

2.2. Preparation of microalgae and plant powders

In this study, culture media studied on the growth of *L. plantarum*, *E. coli* and *S. aureus*, were obtained from the microalgae and plant sources. The microalgae and plant sources washed, drained and sliced were then dried using oven (Wisd, Germany) at 60 °C. The dried samples were ground to obtain fine powders and stored in a cool dark until further use. Each medium consisted of the mixture of microalgae and plant powders. According to the experiments designed, the amount of each powder was weighted and added into water. The mixture of powders as a microalgae-plant culture medium was sterilized using autoclave at 121 °C for 20 min to investigate the growth of bacteria.

2.3. Selection of microalgae and plant powders using taguchi design

According to Taguchi design, a standard orthogonal array L_{16} (4^5) was designed to evaluate the effects of 4-level five variables on the growth of *L. plantarum*. The variables and their levels are listed in Table S1. 16 experiments were run according to the design given in Table S2. Each array consisted of a particular combination of levels and variables (microalgae and plant powders), which was a culture medium obtained as described earlier. Each medium aseptically inoculated with a 5% (v/v) inoculum of *L. plantarum* was then incubated at 37 °C for 24 h in an Erlenmeyer flask containing 50 ml of medium without shaking. To prepare the seed culture for inoculum, *L. plantarum* was grown in MRS medium at 37 °C for 24 h without shaking. The growth of *L. plantarum* was measured as the colony-forming unit of the cells (CFU/ml) and the dry cell weight of the biomass (g DCW/l). All experiments were carried out in triplicate.

2.4. Optimization of microalgae and plant powders using Box-Behnken design

Three important variables from the results of the Taguchi design were selected for the increase of *L. plantarum* and decrease of *E. coli* and *S. aureus* using a Box-Behnken design. These variables were Zu, Gi and Ch which were assessed at three coded levels (−1, 0 and + 1) given in Table 1. The number of experiments suggested by the three variables Box-Behnken design was 17. The experiments and responses for the growth of probiotic and pathogens are presented in Table 2. The experimental results of each response were fitted with a second-order polynomial model. Each experiment was a culture medium obtained as described earlier. To examine each experiment, each medium aseptically inoculated with a 5% (v/v) inoculum of bacteria (probiotic or pathogen) was then incubated at 37 °C for 24 h in an Erlenmeyer flask containing 50 ml of medium without shaking. To prepare the seed culture for inoculum, bacterial strain was grown in culture medium (MRS for probiotic and NB for pathogens) at 37 °C for 24 h without shaking. The growth of bacteria was measured as the colony-forming unit of the cells (CFU/ml) and the dry cell weight of the biomass (g/l). All experiments were carried out in triplicate.

2.5. Preparation and morphology characterization of biosorbents

A plant-microalgae based biosorbent was the optimized mixture of powders obtained from the optimization as described earlier. To prepare fermented and bacterial biosorbents together, the powder particles and bacteria cells incubated in the optimum mixture of powders (culture medium) were centrifuged at 4000 × *g* for 10 min, freeze-dried, and

Table 1
Selected variables and their levels for the Box-Behnken design.

Variable		Levels		
		−1	0	1
Zu (g/l)	x_1	20	35	50
Gi (g/l)	x_2	35	50	65
Ch (g/l)	x_3	20	35	50

Table 2
Box-Behnken design for the growth of *L. plantarum* and pathogen bacteria.

Exp. No.	Variable levels			Cell ($\times 10^8$ CFU/ml)			Biomass (g DCW/l)			L/E[a]	L/S[b]
	x_1	x_2	x_3	*L. plantarum*	*E. coli*	*S. aureus*	*L. plantarum*	*E. coli*	*S. aureus*		
1	−1	−1	0	14.72 ± 0.41	3.99 ± 0.10	9.18 ± 0.10	3.52 ± 0.10	0.95 ± 0.02	2.19 ± 0.02	3.68 ± 0.01	1.60 ± 0.01
2	1	−1	0	20.88 ± 0.81	5.90 ± 0.20	8.50 ± 0.41	4.99 ± 0.19	1.41 ± 0.05	2.03 ± 0.10	3.54 ± 0.02	2.46 ± 0.04
3	−1	1	0	14.00 ± 1.22	5.58 ± 0.31	8.39 ± 0.61	3.35 ± 0.29	1.33 ± 0.07	2.00 ± 0.15	2.51 ± 0.08	1.67 ± 0.12
4	1	1	0	22.43 ± 2.04	5.00 ± 0.41	10.19 ± 0.61	5.36 ± 0.49	1.20 ± 0.10	2.43 ± 0.15	4.48 ± 0.10	2.20 ± 0.12
5	−1	0	−1	12.56 ± 1.22	5.29 ± 0.31	8.03 ± 0.31	3.00 ± 0.29	1.26 ± 0.07	1.92 ± 0.07	2.37 ± 0.05	1.57 ± 0.05
6	1	0	−1	19.48 ± 0.00	3.64 ± 0.00	8.82 ± 0.00	4.65 ± 0.00	0.87 ± 0.00	2.11 ± 0.00	5.36 ± 0.00	2.21 ± 0.00
7	−1	0	1	18.54 ± 0.00	6.73 ± 0.00	11.34 ± 0.00	4.43 ± 0.00	1.61 ± 0.00	2.71 ± 0.00	2.75 ± 0.00	1.63 ± 0.00
8	1	0	1	22.18 ± 1.22	4.68 ± 0.31	8.86 ± 0.51	5.30 ± 0.29	1.12 ± 0.07	2.12 ± 0.12	4.74 ± 0.07	2.50 ± 0.09
9	0	−1	−1	20.48 ± 0.41	5.94 ± 0.00	7.38 ± 0.20	4.89 ± 0.10	1.42 ± 0.00	1.76 ± 0.05	3.45 ± 0.02	2.78 ± 0.04
10	0	1	−1	18.32 ± 2.44	5.36 ± 0.41	7.74 ± 0.71	4.38 ± 0.58	1.28 ± 0.10	1.85 ± 0.17	3.42 ± 0.14	2.37 ± 0.16
11	0	−1	1	23.98 ± 0.00	6.84 ± 0.00	8.86 ± 0.00	5.73 ± 0.00	1.63 ± 0.00	2.12 ± 0.00	3.51 ± 0.00	2.71 ± 0.00
12	0	1	1	13.32 ± 0.25	7.27 ± 0.10	9.00 ± 0.51	3.18 ± 0.06	1.74 ± 0.02	2.15 ± 0.12	1.83 ± 0.04	1.48 ± 0.10
13	0	0	0	22.32 ± 0.81	6.77 ± 0.20	8.78 ± 0.41	5.33 ± 0.19	1.62 ± 0.05	2.10 ± 0.10	3.30 ± 0.09	2.54 ± 0.13
14	0	0	0	23.47 ± 1.22	6.62 ± 0.20	8.86 ± 0.51	5.61 ± 0.29	1.58 ± 0.05	2.12 ± 0.12	3.54 ± 0.01	2.65 ± 0.02
15	0	0	0	22.75 ± 0.41	7.13 ± 0.10	8.71 ± 0.10	5.44 ± 0.10	1.70 ± 0.02	2.08 ± 0.02	3.19 ± 0.01	2.61 ± 0.01
16	0	0	0	22.54 ± 0.81	6.84 ± 0.20	8.71 ± 0.20	5.38 ± 0.19	1.63 ± 0.05	2.08 ± 0.05	3.29 ± 0.04	2.59 ± 0.04
17	0	0	0	21.82 ± 0.41	6.91 ± 0.10	8.64 ± 0.10	5.21 ± 0.10	1.65 ± 0.02	2.06 ± 0.02	3.16 ± 0.04	2.53 ± 0.04

[a] L/E: ratio of *L. plantarum* (cell or biomass) to *E. coli* (cell or biomass).
[b] L/S: ratio of *L. plantarum* (cell or biomass) to *S. aureus* (cell or biomass).

stored in a cool dark until further use. Followed by the preparation of fermented and bacterial biosorbents, the supernatant obtained after incubation was removed and remained particles were used for preparation of the fermented powders as fermented biosorbents as follows: LF (*L. plantarum*-fermented powder), EF (*E. coli*-fermented powder) and SF (*S. aureus*-fermented powder).

The morphology of plant-microalgae biosorbent was identified using the scanning electron microscopy (SEM) (XL30, Philips, Netherlands). The samples were coated with gold using the sputtering technique to improve the conductivity of the samples. The dried samples were fractured and observed at beam energy of 20.0 kV.

2.6. In vitro biosorption of glucose, cholesterol and bile salt

In vitro biosorption of glucose (Qi et al., 2016), cholesterol (Arun et al., 2017) and bile salt (Xie et al., 2017) was carried out according to the methods reported with some modifications. Briefly, the adequate amount of freeze-dried biosorbent (ZuGiCh: 1 g, LF: 0.5 g, *L. plantarum*: 0.5 g, EF: 0.94 g, *E. coli*: 0.06 g, SF: 0.74 g, *S. aureus*: 0.26 g, LF + *L. plantarum*: 0.5 g + 0.5 g, EF + *E. coli*: 0.94 g + 0.06 g, SF + *S. aureus*: 0.74 g + 0.26 g) was mixed with 100 ml of glucose solutions (2–40 g/l), 100 ml of cholesterol solutions in ethanol (2–40 g/l) and 100 ml of bile salt solutions (4–80 g/l) at 37 °C for 1 h with shaking at 100 rpm. Then, the solution containing fermented and bacterial biosorbents were centrifuged at 4000 × *g* for 10 min. The final glucose, cholesterol and bile salt content in the supernatants were determined using the glucose assay kit (Pars Azmun Co., Tehran, Iran), cholesterol assay kit (Pars Azmun Co., Tehran, Iran) and the measurement of absorbance of the supernatant by spectrophotometer (Cary 100 UV–Vis, Agilent, USA) at 400 nm, respectively.

2.7. Statistical analysis

Software Design-Expert version 7 trial (Stat-Ease Inc., Minneapolis, USA) was used for the experimental design and statistical analysis of the experimental data. The analysis of variance (ANOVA) was used to estimate the statistical parameters. The statistical significance of the results was evaluated at the 95% confidence level ($p < 0.05$). The experimental results were expressed as mean ± SD (standard deviation) of triplicate measurements.

3. Results

3.1. Bacterial biosorbents production by plant-microalgae powders

Five variables were analyzed with regard to their effects on the growth of *L. plantarum* using Taguchi design. As shown in Table S2, the maximum experimental values recorded for the cells and biomass of *L. plantarum* were 1.69×10^9 CFU/ml and 4.04 g DCW/l, respectively. The variations observed in terms of the growth values at given experimental conditions revealed that the growth of *L. plantarum* was impacted by the concentrations of microalgae and plant sources (Fig. S1). The increase in the concentrations of Zu, Gi and Ch were noted to induce positive effects on the growth. As shown in Fig. S1, the Zu, Gi and Ch were noted to exhibit the maximum growth at concentrations of 35 g/l (level 3) (2.98 g DCW/l), 50 g/l (level 4) (2.95 g DCW/l) and 35 g/l (level 3) (2.73 g DCW/l), respectively. The findings also revealed that the change of RB and ZD concentration from 15 g/l (level 1) to 50 g/l (level 4) did not influence the growth as well as the other variables. The results indicated that the optimum levels for each variable in terms of achieving higher growth were as follows: 35 g/l Zu, 50 g/l Gi, 25 g/l RB, 25 g/l ZD and 35 g/l Ch. However, Zu, Gi and Ch were determined as the most significant variables and selected for further optimization by a Box-Behnken design.

To obtain the regulated intestinal bacterial community based on the most *L. plantarum* and the least pathogen (*E. coli* and *S. aureus*) cells, various culture media prepared with different combination of Zu, Gi and Ch powders were investigated. Taguchi design might neglect important variables, thus leading to loss of the supreme values of these variables and of the results. Therefore, Box-Behnken design was used to further optimize the three most important variables in the medium. 17 experiments, including the five center points, were performed (Table 2). Although the results showed that the cells and biomass of *L. plantarum* reached their highest (2.39×10^9 CFU/ml and 5.73 g DCW/l, respectively) during experiment 11, the aim of optimization was to reach the highest ratio of probiotic to pathogen. Hence, experiment 8 shows the optimal ratios (L/E = 4.74 and L/S = 2.5) whereas the difference between cells and biomass of *L. plantarum* in experiment 8 (2.22×10^9 CFU/ml and 5.30 g DCW/l, respectively) and 11 is negligible.

The experimental results were analyzed by ANOVA and fitted with second-order polynomial equations given in Table 3. As calculated *P*-values, the models were statistically significant at a probability level of

Table 3
Response surface models for the growth of *L. plantarum* and pathogen bacteria.

Response		Model	*P*-value	R^2
Cell	*L. plantarum*	$+22.58 + 3.14x_1 - 1.50x_2 + 0.90x_3 + 0.57 \times 1 \times {}_2 - 0.82 \times 1 \times {}_3 - 2.12 \times 2 \times {}_3 - 2.70{x_1}^2 - 1.87{x_2}^2 - 1.69{x_3}^2$	0.0493[a]	0.83
	E. coli	$+6.85–0.30x_1 + 0.07x_2 + 0.66x_3 - 0.62 \times 1 \times {}_2 - 0.10 \times 1 \times {}_3 + 0.25 \times 2 \times {}_3 - 1.50{x_1}^2 - 0.23{x_2}^2 - 0.27{x_3}^2$	0.0470[a]	0.83
	S. aureus	$+8.74–0.07x_1 + 0.18x_2 + 0.76x_3 + 0.62 \times 1 \times {}_2 - 0.82 \times 1 \times {}_3 - 0.05 \times 2 \times {}_3 + 0.67{x_1}^2 - 0.35{x_2}^2 - 0.15{x_3}^2$	0.0054[a]	0.91
Biomass	*L. plantarum*	$+5.39 + 0.75x_1 - 0.36x_2 + 0.21x_3 + 0.14 \times 1 \times {}_2 - 0.20 \times 1 \times {}_3 - 0.51 \times 2 \times {}_3 - 0.65{x_1}^2 - 0.45{x_2}^2 - 0.40{x_3}^2$	0.0493[a]	0.83
	E. coli	$+1.64–0.07x_1 + 0.02x_2 + 0.02x_3 - 0.15 \times 1 \times {}_2 - 0.02 \times 1 \times {}_3 + 0.06 \times 2 \times {}_3 - 0.36{x_1}^2 - 0.06{x_2}^2 - 0.06{x_3}^2$	0.0470[a]	0.83
	S. aureus	$+2.09–0.02x_1 + 0.04x_2 + 0.18x_3 + 0.15 \times 1 \times {}_2 - 0.20 \times 1 \times {}_3 - 0.01 \times 2 \times {}_3 + 0.16{x_1}^2 - 0.08{x_2}^2 - 0.04{x_3}^2$	0.0054[a]	0.91
L/E		$+3.30 + 0.85x_1 - 0.24x_2 - 0.22x_3 + 0.53 \times 1 \times {}_2 - 0.25 \times 1 \times {}_3 - 0.41 \times 2 \times {}_3 + 0.51{x_1}^2 - 0.25{x_2}^2 + 0.003{x_3}^2$	0.0340[a]	0.85
L/S		$+2.58 + 0.36x_1 - 0.23x_2 - 0.07x_3 - 0.08 \times 1 \times {}_2 + 0.06 \times 1 \times {}_3 - 0.20 \times 2 \times {}_3 - 0.48{x_1}^2 - 0.12{x_2}^2 - 0.13{x_3}^2$	0.0291[a]	0.85

[a] Significant at the 95% level.

95%. The high regression coefficient values ($R^2 > 0.80$) indicated that the experimental data were close to the predicted values from the models. To obtain optimum combination of medium and corresponding response surfaces, linear, quadratic, and linear interaction effects were calculated using the models obtained for the responses of L/E and L/S.

The interactions of variables (Zu, Gi and Ch) on the responses (L/E and L/S) are graphically illustrated by plotting the two-dimensional contour and the three-dimensional response surface plots (Figs. 1 and 2) in accordance with two variables, while the one other remains constant at its optimum level. Different combinations of two variables at once are represented by each response surface and contour plot for the responses. As shown in Fig. 1, L/E increased significantly at a higher concentration of Zu and lower concentrations of Gi and Ch. L/S increased significantly at higher concentrations of Zu and Ch, and a lower concentration of Gi (Fig. 2). Consequently, the increase of Zu and the decrease of Gi identified the optimum combination of the medium that maximized both responses of L/E and L/S. This optimum medium represented in Table 4 confirmed that the lowest level of Gi (35 g/l) was incorporated with the approximately highest level of Zu (48.80 g/l). On the contrary, the minimum responses were obtained at the lower level of Zu (35 g/l) and the highest level of Gi (65 g/l), demonstrated by experiment 12 (Table 4). The results of biomass (or cells) of *L. plantarum* and both responses (L/E and L/S) in the optimum medium were significantly higher compared with those in experiment 8 (Table 4). The results in Table 4 show that optimum zucchini-ginger-*Chlorella vulgaris* (ZuGiCh) is addressed as an alternative for the culture medium to increase the production of bacterial biosorbents. The use of ZuGiCh was an interesting and expected approach because it regulated simultaneously both of the bacterial biosorbents production, and then performed as a fermented biosorbent incorporated with the bacterial biosorbents.

3.2. Morphology of Zu, Gi, Ch and ZuGiCh biosorbent

The morphology of Zu, Gi, Ch and fermented ZuGiCh biosorbent are shown in Fig. 3. As shown in Fig. 3a-c, the morphology of Zu, Gi and Ch particles has disordered structures with the particle size of 100 μm in average. As compared to the morphology of Zu, Gi and Ch are about spherical. In Fig. 3d, the mixture of microparticles of Zu, Gi and Ch, fermented by intestinal bacteria, is shown as irregular 300 μm-in-average particles formed via sticking the particles together. Although some pores, holes and voids are not seen in the morphology of microparticles, the adsorption of glucose, cholesterol and bile salt on the ZuGiCh microparticles is sufficient because more adsorbing groups such as amine group, hydroxyl group, etc. belonged to plant and microalgae cell wall are present on the ZuGiCh surface.

3.3. ZuGiCh and bacterial biosorbents for adsorption of glucose, cholesterol and bile salt

As the aim of this research was to adsorb glucose, cholesterol and bile salt by the plant-microalgae and bacterial biosorbents, the ZuGiCh was employed as the plant-microalgae biosorbent. The effects of initial concentrations of the adsorbates (glucose, cholesterol and bile salt) on the performance of biosorbents are depicted in Figs. 4 and 5. As shown, all of the studied biosorbents were capable of removing glucose, cholesterol and bile salt with efficiencies varying significantly ($p < 0.05$) across the biosorbents and across the initial concentrations applied. It was as expected that the adsorbates concentrations relied considerably on their initial concentrations, in which the highest level of adsorbates concentrations was seen with the highest initial concentration of glucose (40 g/l), cholesterol (40 g/l) and bile salt (80 g/l) studied. The results in Figs. 4 and 5 revealed that the biosorbents of SF + *S. aureus* (10 g/l), ZuGiCh (10 g/l) and LF + *L. plantarum* (10 g/l) exhibited higher adsorbing capability of glucose, cholesterol and bile salt, respectively. It was observed that glucose was significantly ($p < 0.05$) adsorbed by SF + *S. aureus* with the maximum biosorption (73.98%) at an initial concentration of 6 g/l whereas it adsorbed only 57.65% of glucose with an initial concentration of 40 g/l (Fig. 5a). As shown in Fig. 5b, ZuGiCh exhibited a predictable trend in the biosorption of cholesterol and an adsorbing potential with the maximum efficiency of 88% at an initial concentration of 40 g/l. Since ZuGiCh was converted to the fermented ZuGiCh *in vitro*, SF + *S. aureus* was considered with the maximum efficiency of 83.65% at an initial concentration of 40 g/l. To adsorb the bile salt, behaviors of the biosorbents (except *L. plantarum* and LF + *L. plantarum*) were similar with the approximately constant biosorption efficiency (Fig. 5c). LF + *L. plantarum* biosorbent adsorbed constantly the bile salt with a maximum efficiency of 59% in the initial concentration range of 4–50 g/l whereas it was gradually inclined to the efficiency of 42.59% at an initial concentration of 80 g/l. In comparison between Figs. 4 and 5, it was demonstrated that the correlation between percentage of biosorption and adsorbate concentration was not mandatory. On the other hand, the increase in adsorbate concentration did not lead to the increase in the percentage of biosorption. The results indicated that the removal of cholesterol was higher than that of glucose and bile salt. Further, glucose was removed more efficiently as compared to bile salt. Consequently, the conversion of ZuGiCh biosorbent to the fermented ZuGiCh and bacterial biosorbents led to the increase in biosorption efficiency of glucose, cholesterol and bile salt. In contrast to an expectation that the consumption of ZuGiCh by gut flora leads to decrease the efficiency of biosorbents, this research revealed that the fermentation of ZuGiCh powder led to not only adsorb directly by fermented ZuGiCh powder but also increase adsorbing bacteria, thus improving the biosorption efficiency *in vitro*.

Biosorption capacities of the biosorbents for the removal of glucose, cholesterol and bile salt at equilibrium are presented in Fig. 6. The results in Fig. 6 indicate that the initial concentrations considerably influence the equilibrium biosorption as well as the adsorbate concentration in Fig. 4. It was noted that the adsorbed amount increased with the increase in the initial adsorbate concentration, due to the fact that this increase provided a driving force to overcome all mass transfer resistance, thus resulting in higher binding rates. *E. coli* as a particular

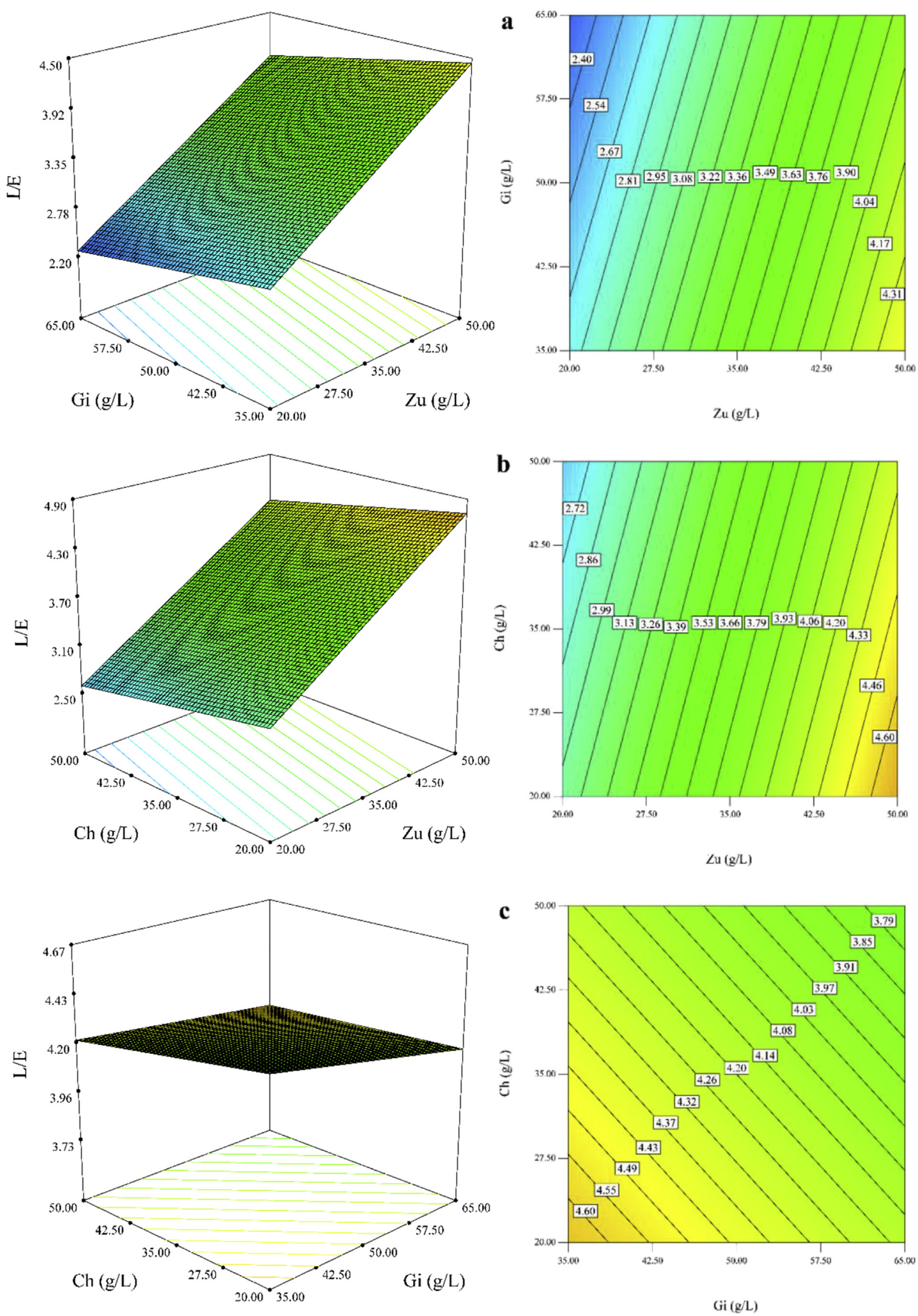


Fig. 1. Response surface and contour plots of (a) interaction of Zu (x_1) and Gi (x_2) at optimum level of Ch (39.30 g/l), (b) interaction of Zu (x_1) and Ch (x_3) at optimum level of Gi (35.00 g/l), and (c) interaction of Gi (x_2) and Ch (x_3) at optimum level of Zu (48.80 g/l) on L/E.

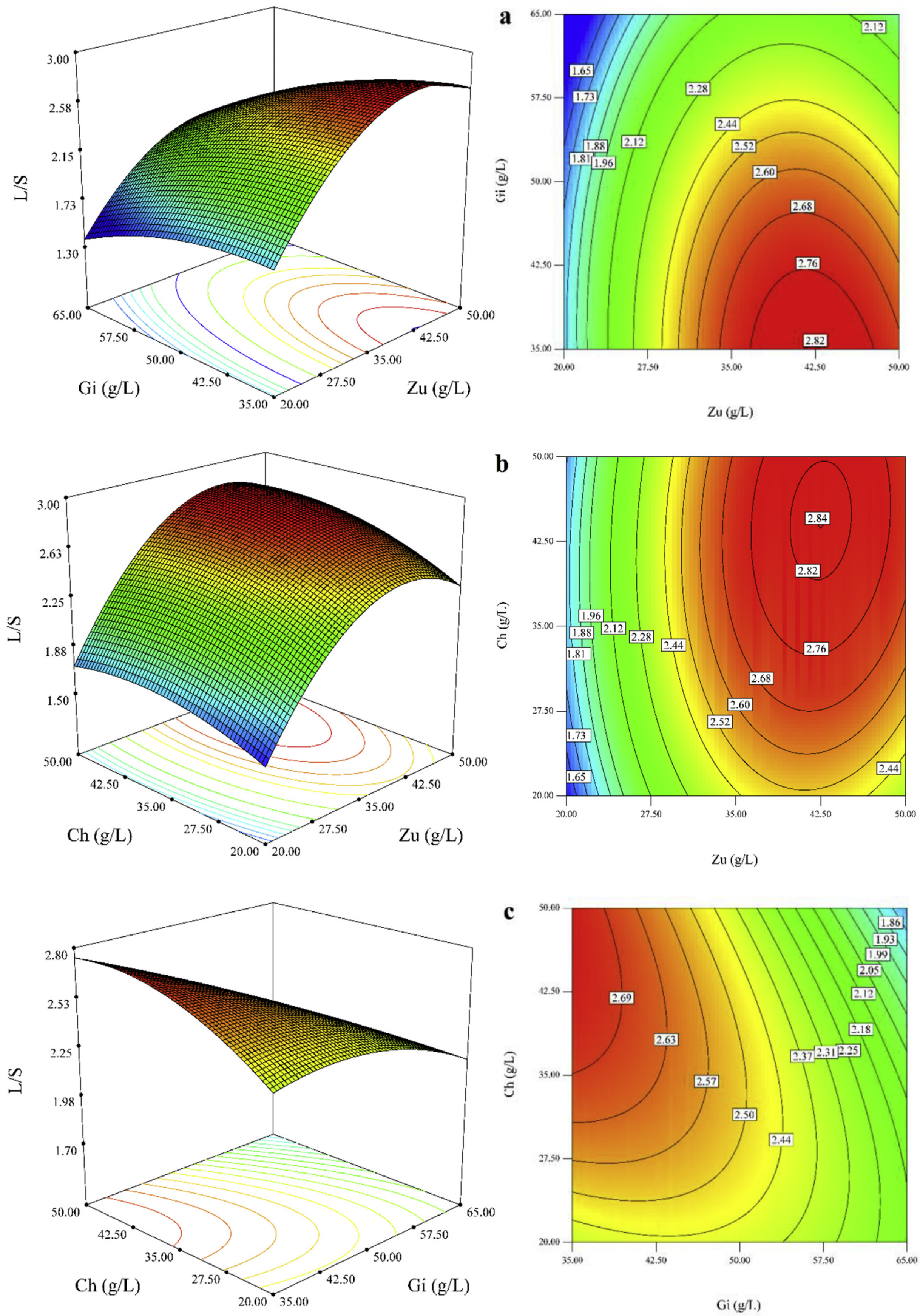


Fig. 2. Response surface and contour plots of (a) interaction of Zu (x_1) and Gi (x_2) at optimum level of Ch (39.30 g/l), (b) interaction of Zu (x_1) and Ch (x_3) at optimum level of Gi (35.00 g/l), and (c) interaction of Gi (x_2) and Ch (x_3) at optimum level of Zu (48.80 g/l) on L/S.

Table 4
ZuGiCh media for the growth of *L. plantarum* and pathogen bacteria.

Medium	Variables			Cell ($\times 10^8$ CFU/ml)			Biomass (g DCW/l)			L/E	L/S
	Zu (g/l)	Gi (g/l)	Ch (g/l)	*L. plantarum*	*E. coli*	*S. aureus*	*L. plantarum*	*E. coli*	*S. aureus*		
Exp. No. 12	35	65	50	13.32 ± 0.25	7.27 ± 0.10	9.00 ± 0.51	3.18 ± 0.06	1.74 ± 0.02	2.15 ± 0.12	1.83 ± 0.04	1.48 ± 0.10
Exp. No. 8	50	50	50	22.18 ± 1.22	4.68 ± 0.31	8.86 ± 0.51	5.30 ± 0.29	1.12 ± 0.07	2.12 ± 0.12	4.74 ± 0.07	2.50 ± 0.09
Optimum	48.80	35.00	39.30	23.68 ± 1.86	4.53 ± 0.41	8.10 ± 0.61	5.66 ± 0.71	1.08 ± 0.16	1.94 ± 0.23	5.23 ± 0.42	2.92 ± 0.01

biosorbent exhibited the highest equilibrium biosorption capacity for glucose (16.37 g/g at C_0 = 40 g/l), cholesterol (48.00 g/g at C_0 = 30 g/l) and bile salt (24.76 g/g at C_0 = 80 g/l). As the bacteria and ZuGiCh interacted with each other, their biosorption efficiency was higher when cooperated; however, their biosorption capacity could be lower. In this study, although *E. coli* had the highest biosorption capacity (Fig. 6), SF + *S. aureus* and LF + *L. plantarum* had the highest biosorption efficiency (Fig. 5).

4. Discussion

Although the amount of bacterial biosorbents is effective on *in vitro* biosorption, type of bacteria is important to healthcare. Since presence of pathogens is inevitable in gut flora, the best culture medium accessible for both probiotics and pathogens should be able to induce the growth of probiotics against pathogens. At the first step of this study, microalgae and plant sources were screened to increase the growth of *L. plantarum* not *E. coli* and *S. aureus*. Among the probiotic bacteria, *L. plantarum* traditionally used as the most common probiotic needs media containing expensive compounds such as amino acids, nucleic acids, peptides, and vitamins. As expected, Zu, Gi and Ch were determined as the best sources since they were rich in essential amino acids, protein, minerals, vitamins, dietary fiber, and a great range of antioxidants and bioactive substances with various health promoting effects, such as cholesterol-lowering, anti-diabetic effect, suppression of hypertension, protection against renal failure, regulation of intestinal microbiota and anticarcinogenic activity (Amaro et al., 2013; Andallu et al., 2003; Madkor et al., 2011; Pignolet et al., 2013; Simpson and Morris, 2014; Yi et al., 2009). Furthermore, the incorporation of soluble fibers extracted from Zu, Gi and Ch powders as prebiotics into *Lactobacillus* could exert growth stimulatory effect as compared to pathogens in the intestinal condition (Bartkiene et al., 2011; Chang et al., 2011; Leal et al., 2017; Lu et al., 2017; Palma et al., 2014) as well as the combination of these extracts and other herbal plants revealed synergistic effects on improving the intestinal microflora (Andallu et al., 2003; Kumoro et al., 2016; Lee et al., 2012; Madkor et al., 2011; Yi et al., 2009).

The biosorption capacity of ZuGiCh could be related to the viscous and gel forming properties of fibers extracted form ZuGiCh (Wu et al., 2014). In addition, more open internal structure (porosity) and functional groups on the surface area of ZuGiCh increased the glucose adsorption (Niu et al., 2018). Thus, the high biosorption capability of fermented ZuGiCh could be due to the adsorbing surface functionalized by fermentation. Since pectin as a soluble polysaccharide revealed gel formation in presence of cations and glucose, the soluble fibers extracted form ZuGiCh including pectic polysaccharides could be a stronger biosorbent for the glucose adsorption (Benítez et al., 2017). Larger particle size of ZuGiCh could increase the glucose biosorption since high hydration capacity of larger particles led to the reduction of water mobility on the particle surface and thus retaining glucose adsorbed on the surface (Gupta and Premavalli, 2011; Nsor-Atindana et al., 2012). Therefore, the ZuGiCh incorporated with the bacteria could lead to the formation of larger particles, which increased the biosorption capacity.

Generally, ZuGiCh especially containing non-starch polysaccharides such as pectin, glucans and gum showed a better binding capacity for cholesterol and bile salt as compared to insoluble fibers (Nsor-Atindana et al., 2012; Soh et al., 2003; Zhu et al., 2018). Soluble fibers extracted form ZuGiCh reduced cholesterol levels indirectly by adsorbing bile salts and components synthesized from cholesterol, or by directly adsorbing cholesterol (Arun et al., 2017). It was demonstrated that excessive blood cholesterol was bound by soluble fibers forming a gel in the intestines and eventually expelled from the body (Lv et al., 2017).

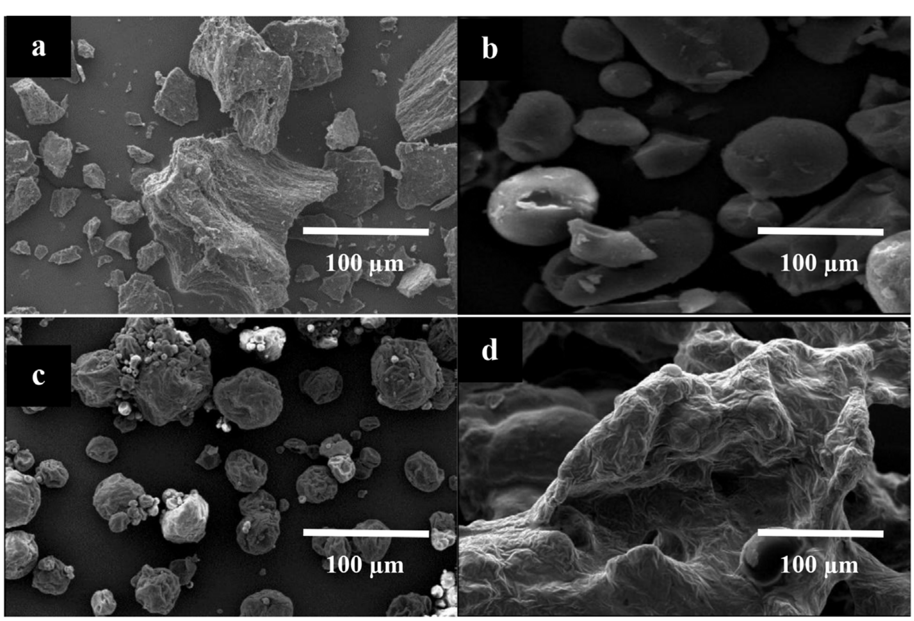


Fig. 3. SEM of (a) Zu, (b) Gi, (c) Ch and (d) ZuGiCh biosorbent.

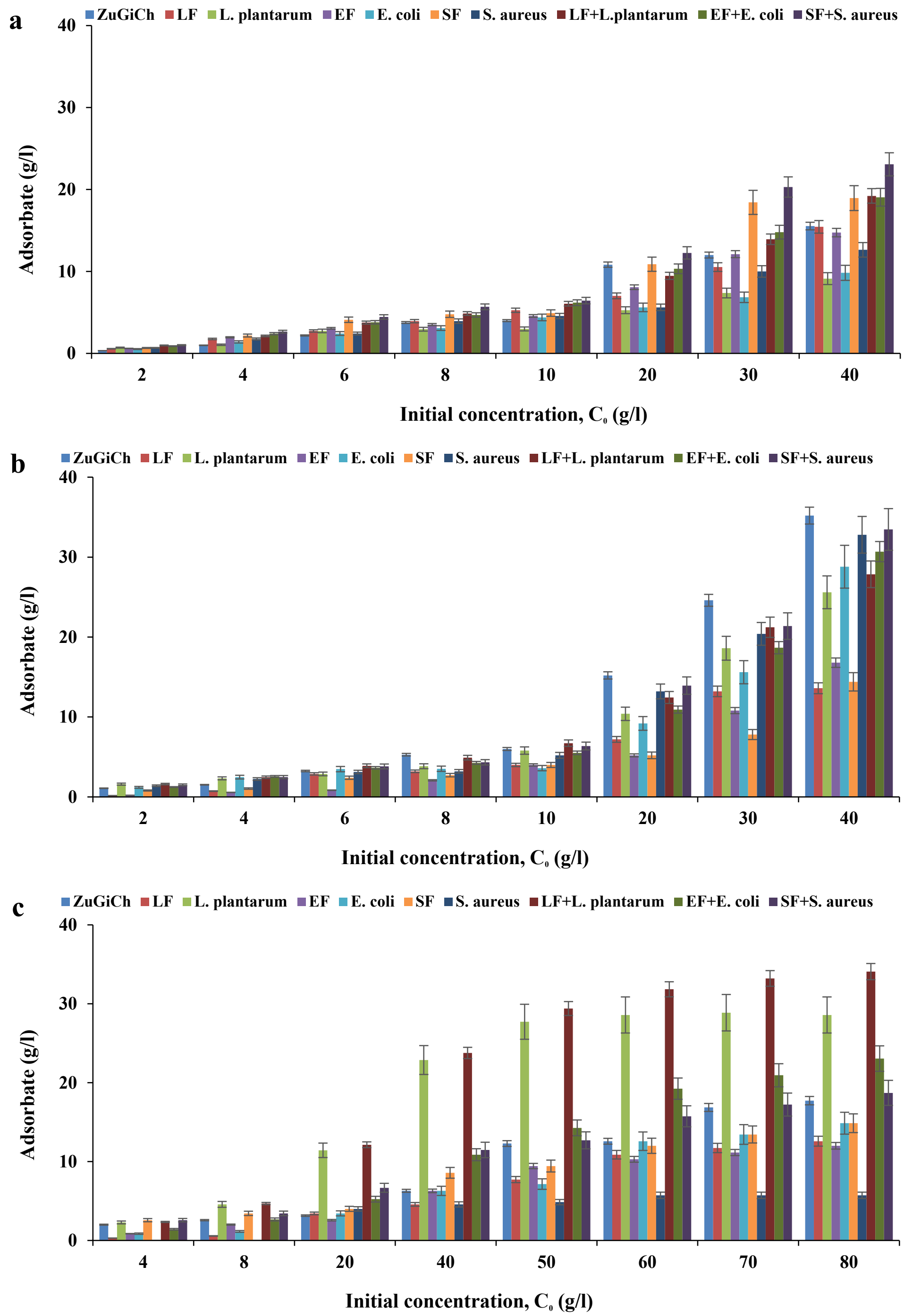


Fig. 4. Effect of initial concentrations of (a) glucose, (b) cholesterol and (c) bile salt on the adsorption using fermented and bacterial biosorbents.

Acidic polysaccharides such as pectin including uronic acids in the backbone structure and xanthan gum including galacturonic acid had higher cholesterol adsorption capacities (Soh et al., 2003). Further, some polyphenolic compounds reduced the solubility of cholesterol in micelles which resulted in delayed absorption (López-Marcos et al., 2015). The higher specific surface area is created through decreasing the particle size, which increases the adsorption capacity (Chen et al., 2015). The fermentation of ZuGiCh powder by the bacterial biosorbents led to the fine powder, and thus the fermented ZuGiCh biosorbents possessed the higher biosorption capacity than the ZuGiCh. Two possible mechanisms of the adhesion of cholesterol to the cell wall and the degradation of cholesterol by the cells were documented for the cholesterol lowering using bacteria (Tok and Aslim, 2010). *L. plantarum*, *E. coli* and *S. aureus* showed different capacities for the cholesterol biosorption on the cell walls because bacterial strains could be able to lower cholesterol with the different cholesterol-lowering potential (Fareez et al., 2017; Miremadi et al., 2014).

Bile salt biosorption capacity depends on the fractional composition of ZuGiCh, chemical structure of the bile salt, the osmotic concentration and the pH of the environment (Dziedzic et al., 2015). The high biosorption capacity of ZuGiCh could be due to pectins and β-glucans extracted as soluble polysaccharides which were the major fractions responsible for bile salt biosorbing. Fiber-bound bile salt without being digested passes through the intestinal tract and is excreted in the stool (López-Marcos et al., 2015). The fermented biosorbents enhanced the bile salt biosorption because of the disintegration and the degradation

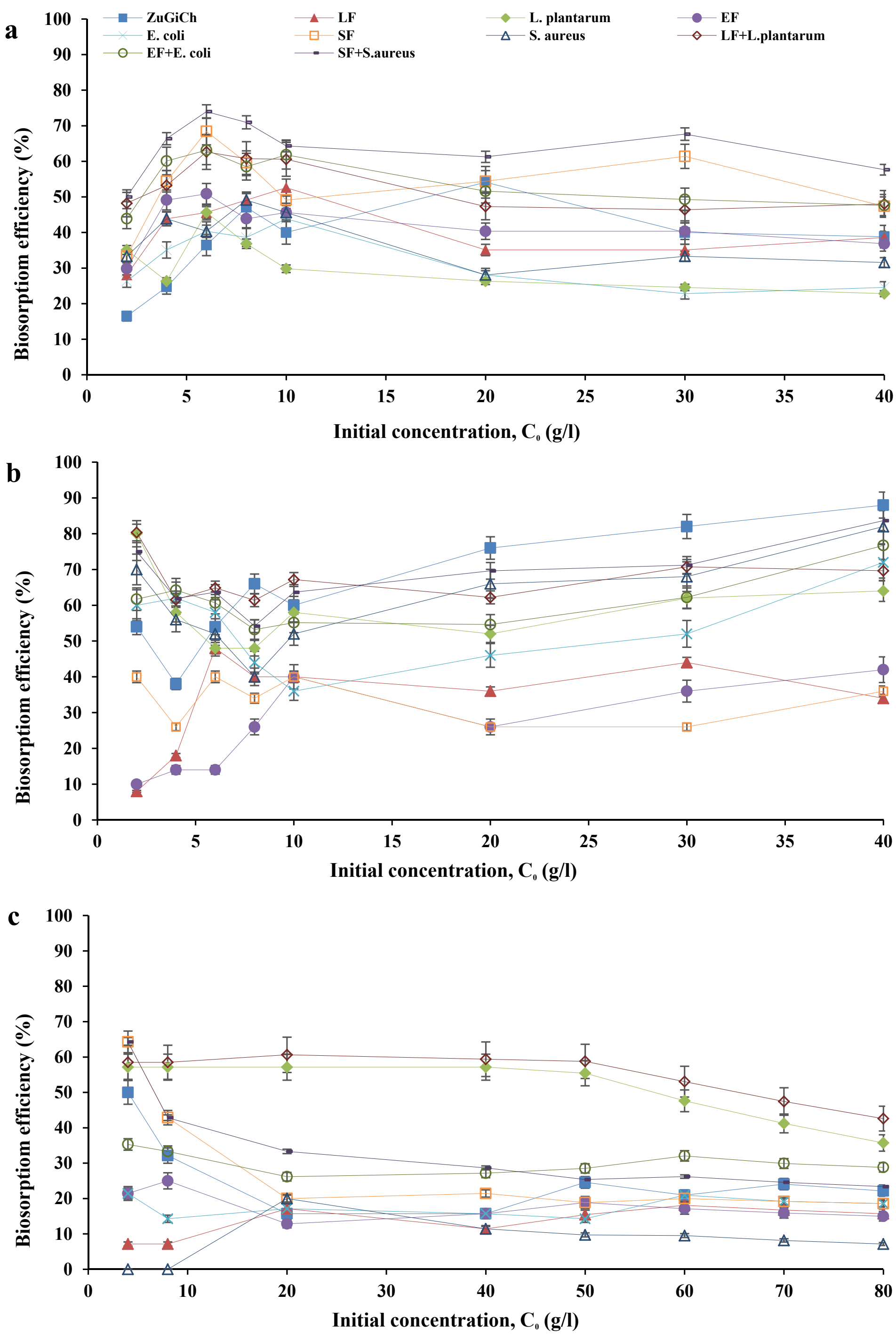


Fig. 5. Effect of initial concentrations of (a) glucose, (b) cholesterol and (c) bile salt on the biosorption efficiency.

of ZuGiCh particles that could be resulted by the activities of intestinal bacteria. As expected, the interaction of bacterial biosorbents and ZuGiCh resulted in a higher proportion of smaller fragments, which considerably increased the bile salt biosorption capacity of the fermented biosorbents (Cornfine et al., 2010).

In comparison with other studies which investigated the adsorption capacity of dietary fibers and bacteria for biosorbing glucose (Ahmed et al., 2011; Arun et al., 2017; Benítez et al., 2017; Gupta and Premavalli, 2011; Liu et al., 2017; Niu et al., 2018; Nsor-Atindana et al., 2012; Qi et al., 2016; Wu et al., 2014; Xie et al., 2017), cholesterol and bile salt (Araki et al., 2012; Arun et al., 2017; Belviso et al., 2009; Bordoni et al., 2013; Castorena-Alba et al., 2018; Chen et al., 2015; Cornfine et al., 2010; Daou and Zhang, 2014; Fareez et al., 2017; Funk et al., 2008; Huang et al., 2014; Kumar et al., 2013; Miremadi et al., 2014; Niu et al., 2018; Nsor-Atindana et al., 2012; Oakenfull and Fenwick, 1978; Soh et al., 2003; Sreenivas and Lele, 2013; Tok and Aslim, 2010; Xie et al., 2017, 2016; Zhang et al., 2011; Zhu et al., 2018), the results obtained by this study revealed the highest adsorption capacity for the biosorption of them, which have been reported yet.

5. Conclusions

Multifunctional ZuGiCh microparticles were evaluated to simultaneously enhance the bacterial adsorbent of probiotics against pathogens

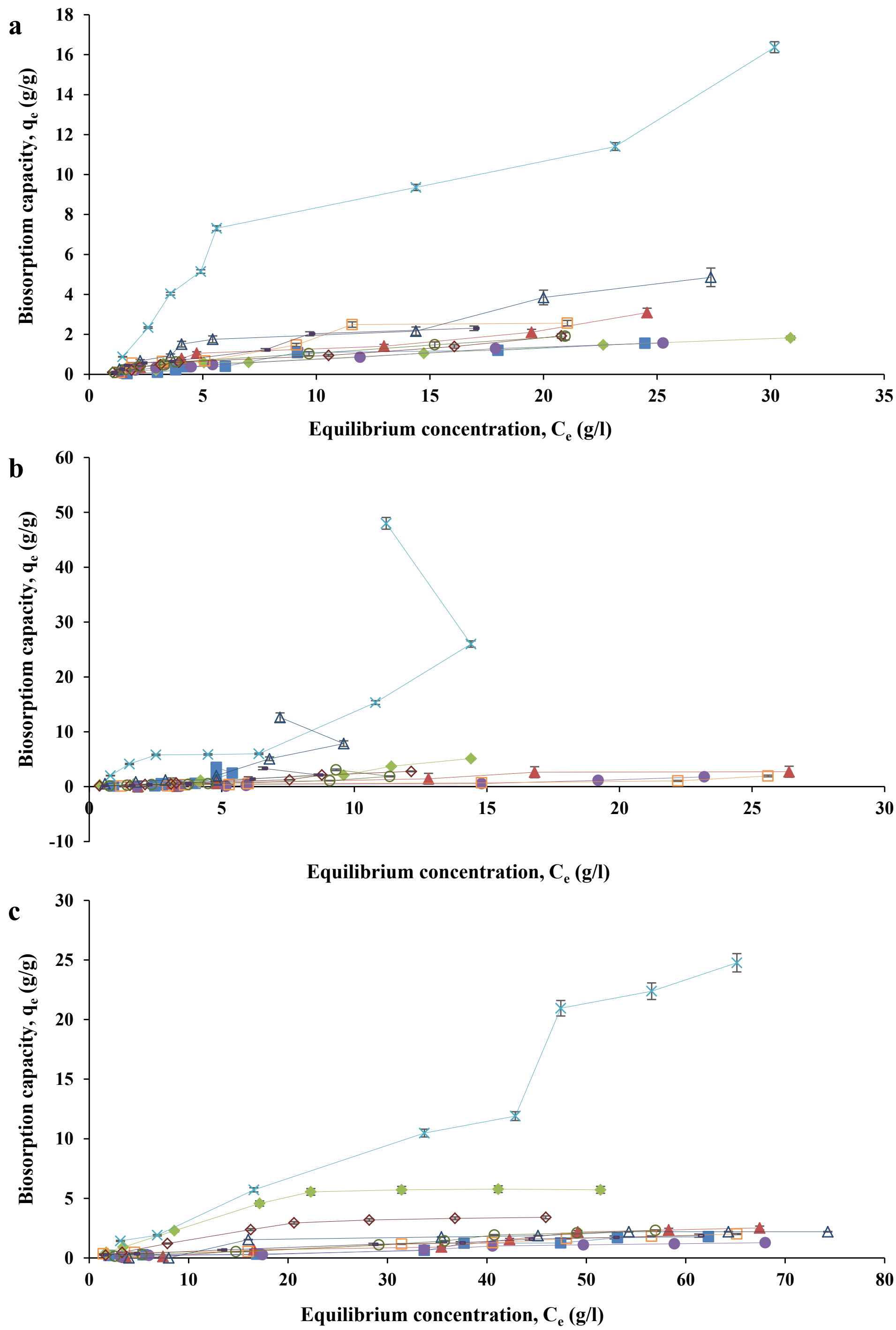


Fig. 6. Isotherms for the biosorption capacity of (a) glucose, (b) cholesterol and (c) bile salt onto the biosorbents.

and adsorb glucose, cholesterol and bile salt. The findings showed the importance of ZuGiCh for gastrointestinal health via the regulation of bacteria community and removal of glucose, cholesterol and bile salt. This study demonstrated that *E. coli* and *S. aureus* as well as *L. plantarum* could be potential benefits for human healthcare via adsorbing the unhealthy materials. Moreover, the simultaneous incorporation of bacteria and fermented ZuGiCh resulted in a synergistic effect on the adsorption of glucose, cholesterol and bile salt when compared with only ZuGiCh because the fermented ZuGiCh exhibited a higher adsorption capacity compared to ZuGiCh. Consequently, ZuGiCh micro-biosorbent can be consumed as a nutritional supplement to maintain gastrointestinal health.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

The authors gratefully acknowledge the financial support provided by Iran's National Elites Foundation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://

doi.org/10.1016/j.bcab.2019.101119.

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