Research in Biotechnology, 4(6): 21-29, 2013 ISSN: 2229-791X
www.researchinbiotechnology.com

Regular Article

Effects of medium and culture conditions on folate production by *Streptococcus thermophilus* BAA-250

Seyed Sadegh Mousavi[1], Hassan Moeini[2], Rosfarizan Mohamad[1,2*], Mojdeh Dinarvand[1], Arbakariya Ariff[1,2], Foo Hooi Ling[1,2] and Abdul Rahim Raha[2, 3]

[1]Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malatsiay, Selangor, Malaysia
[2]Institute of Bioscience, Universiti Putra Malaysia, Selangor, Malaysia
[3]Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, Selangor, Malaysia
*Corresponding author Email: farizan@biotech.upm.edu.my

The present study was conducted to investigate the effects of culture conditions on folate production by *Streptococcus thermophilus* BAA-250. Lactose (3g/L) and yeast extract (20g/L) were found to be the more suitable carbon and nitrogen sources for folate production by *S. thermophilus* BAA-250. *para*-aminobenzoic acid (*p*ABA) higher than 1 µM had no significant effect on folate biosynthesis. The optimum pH for folate production was shown to be 7.0 with a folate yield and productivity of 54.53 (µg/L) and 2.27 (µg/L.h), respectively. Optimum folate production obtained in the presence of lactose and yeast extract in a controlled pH of 7 during batch fermentation in bioreactor. Kinetic studies indicated that folate production by *S. thermophilus* is growth-associated process.

Keywords: Folate, Lactic acid bacteria, Kinetic model, *Streptococcus thermophilus*.

Folate is a water soluble group of B-vitamins with related biological activity which is synthesized from guanosine-5'-triphosphate (GTP), *p*-aminobenzoate (PABA), and glutamate (Basset et al., 2005; Wegkamp et al., 2007). Various green plants and some microorganisms produce folate. Numerous studies have shown that lactic acid bacteria (LAB) such as *Lactococcus lactis, Lactobacillus bulgaricus* and *Streptococcus thermophilus* have the ability to produce folate in fermented foods (Crittenden et al., 2003; LeBlanc et al., 2007; Lin & Young, 2000; Papastoyiannidis et al., 2006). There is limited information about the parameters that influence folate biosynthesis by LAB used for the production of dairy products (Crittenden et al., 2003; Lin & Young, 2000; Sybesma et al., 2003b). Therefore, the present study was conducted to investigate the effect of culture conditions, such as carbon and nitrogen sources, *p*-aminobenzoic (PABA) concentration, and pH on influence folate production by *S. thermophilus* BAA-250. In this work, kinetics models for folate production and the growth of the strain were also described.

Materials and Methods

Microorganism, Media and Cultivation Condition

Streptococcus thermophilus ATCC BAA 250/LMG 18311 and *Lactobacillus casei* subsp. *rhamnosus* (ATCC 7469) were respectively used as folate-producing and reference strains (Arcot & Shrestha, 2005). The bacterial strains were cultivated in a modified M17 medium containing yeast extract (2.5 g/L), peptone from meat (5 g/l), peptone from casein (5 g/l), $MgSO_4$ (0.25 g/l), K_2HPO_4 (0.6 g/l), *p*ABA (0.1 mg/l), lactose (5 g/l) and ascorbic acid (0.5 g/L) (Galia et al., 2009; Sybesma et al., 2003b; Zisu & Shah, 2003). The medium were sterilized at 121°C for 15 min. Ascorbic acid and *p*ABA were filter sterilized using 0.22-µm sterile syringe filter.

Shake-flask Fermentation

To investigate the effect of different carbon sources on folate production by *S. thermophilus* BAA 250, the strain was cultured in the modified M17 medium containing 5 g/l of glucose, maltose or sucrose, or 1-10 g/l of lactose. Folate production was also evaluated in the in presence of lactose (3 g/l) as carbon source and different nitrogen sources. The nitrogen compounds tested were organic [meat extract (3 %), yeast extract (0.5-3%), pentone/casein (3%) or pepton/meat (3%)] and inorganic [ammonium nitrate (3%) or ammonium sulfate (3%)]. To rule of exogenous folate addition, the folate content of the organic nitrogen sources was determined before the experiments (data not shown). In the medium with the optimized amount lactose and yeast extract, the effect of *p*ABA (1-150 µM) was also tested.

All the experiments were carried out in triplicates in 500 ml Erlenmeyer flasks containing 250 ml of the medium inoculated with 5% (v/v) of the inoculums, followed by 24 h incubation at 37°C and 150 rpm in a shaker incubator (Stuart, USA). Cell concentration, folate production and substrate consumption were determined at each 24 h intervals. Substrate utilization was determined using Di-nitro salicylic acid (DNS) method as previously described by Rosfarizan and Ariff (Rosfarizan & Ariff, 2006). Cell concentration was assessed at OD_{610} using spectrophotometer (Thermo, USA). Biomass concentration was determined by dry weight measurement (Rosfarizan & Arrif, 2007).

Batch culture fermentation

For batch fermentation, a 2-liter stirred tank bioreactor (Biostat, B. Braun, Melsungen, Germany), filled with one liter of the growth medium inoculated with 5% (v/v) of the inoculums. Temperature was maintained at 37°C, and pH and dissolved oxygen were controlled using controller hardware and software systems. Agitation speed was set at the constant rate of 150 rpm using 2 six-bladed turbine impellers. Fermentation was carried out at 37°C at the constant pH of 6.0, 7.0 or 8.0. After 0, 3, 6, 9, 12, 18 and 24 h of incubation, folate content, cell concentration and sugar consumption were assessed.

Microbiological assay for intra- and extracellular folate concentrations

Intra- and extracellular folate was quantified using *Lactobacillus casei* microbiological assay according to the method described by Horne and Patterson (Horne & Patterson, 1988). Briefly, 5 ml of the bacterial culture was precipitated at 8,000 ×g for 5 min. To determine extracellular folate, the supernatant was diluted (1:1) with 0.1 M sodium acetate buffer (pH 4.8) containing 1% (v/v) of ascorbic acid. Ascorbic acid is used to prevent oxidation reactions of folate (Jones & Nixon, 2002; Scott et al., 2000). To measure intracellular folate, the cell pellets were washed and resuspended in sodium acetate buffer (0.1 M, pH 4.8) containing 1% (v/v) ascorbic acid. Folate was released from folate binding protein by heating at 100°C for 5 min (Sybesma et al., 2003a). Total folate, including

polyglytamyl folate, was determined after enzymatic deconjugation of the folate samples at 37°C and pH 4.8 for about 5 h using clarified human plasma (Sigma-Aldrich, Germany) as the source of γ-glutamyl hydrolase (Sybesma et al., 2003a).

Kinetic methods

The following kinetic models were used for cell growth, substrate consumption and folate formation in culture fermentation based on Logistic, Gompertz, Monod and Luedeking-Piret equations(Lobry et al., 1992; Mu et al., 2006; Omar et al., 2006; Rosfarizan & Ariff, 2006).

Cell growth:

$$dX/dt = [\mu_{max}(1-x/x_{max})]x \quad [1]$$

Substrate consumption:

$$dS/dt = \alpha (dX/dt) + \beta x \quad [2]$$

Product formation:

-Growth associated

$$P/dt=\alpha(dX/dt) \quad [3]$$

-Mixed growth

$$dP/dt = \alpha (dX/dt) + \beta X \quad [4]$$

-Non-growth associated

$$dP/dX= \beta X \quad [5]$$

Where α and β are equation coefficients corresponded to growth-associated and non-growth-associated product formation, respectively. Accordingly, products accumulated in a fermentation process were classified into three categories according to the values of α and β as follows: A>0 and β ¼0, growth-associated product accumulated as the result of cell growth only; α ¼0 and β >0, non-growth-associated product accumulated as a result of cell maintenance only; and α >0 and β >0, mixed-growth-associated product accumulated as the results of the both cell growth and cell maintenance. The kinetic models (equations 1 to 5) were fitted to the experimental data by non-linear regression with a Marquadt algorithm using SIGMAPLOT computer software. The model parameter values were firstly evaluated by solving equations 1 to 5 and then the computer program was used as a search method to minimize the sum of squares of the differences between the predicted and measured values. The predicted values were then used to simulate the profiles of cell, substrate and product concentrations during the cultivation.

RESULTS

Effect of carbon Source

As shown in Table 1, higher level of folate (41.23 µg/L) and cell concentration (2.82 g/l) was obtained in the presence of 3 g/l lactose, whereas a dramatic decrease was observed in the presence of sucrose (12.19 µg/l). The highest level of folate per biomass or cell efficiency to produce folate (48.27 µg/g) was found in the lactose concentration of 1 g/l. The shortest lag phase was shown to be at the low lactose concentration of 3 g/l. Cell growth rate was almost the same at the different concentrations of lactose except for 1 g/l where a decrease was observed (0.81 g/l).

Effect of nitrogen Source

The highest folate production (46.31 µg/l) and productivity (1.93 µg/l/h) were detected in the growth medium supplemented with yeast extract as nitrogen source (Table 1). Compared to the organic nitrogen compounds, inorganic nitrogen supplements were found to be less effective for folate production. No significant increase in biomass and folate production was detected in the low concentration of yeast extract (5 g/l), however, a significant increase in folate production (46.32 µg/l) was measured in the presence of 20 g/l yeast extract (Table 1).

Effect of *ρ*-Aminobenzoic acid

The highest folate production was obtained at 1 µM *p*ABA. However, as shown in Table 1, *ρ*-Aminobenzoic acid had no significant effect on folate yield and cell growth.

Table 1: Effect of culture condition on the production parameters.

Kinetic Parameter Values*		X_{max} (g/L)	P_{max} (µg/L)	$S_{consume}$ (g/L)	μ (h^{-1})	$Y_{x/s}$ (g/g)	$Y_{p/s}$ (µg/g)	$Y_{p/x}$ (µg/g	*Productivity* (µg/L.h)
Carbon Sources (g/L)	Glucose 5 g/L	2.34±0.03	17.52^{b} ± 0.38	0.98±0.05	0.07	0.24	1.79	07.50	0.73
	Lactose (g/L)								
	1	0.81±0.03	39.15^{c} ± 0.75	0.55±0.03	0.12	0.68	71.62	48.27	1.63
	3	1.65±0.05	41.23^{a} ± 0.35	1.27±0.04	0.18	0.59	32.40	24.95	1.72
	5	1.79±0.02	40.79^{b} ± 0.97	1.46±0.07	0.14	1.22	27.93	22.86	1.70
	10	2.03±0.05	40.56^{b}± 1.27	1.31±0.05	0.16	1.55	30.96	19.97	1.68
	Maltose 5 g/L	1.16±0.04	14.24^{c} ± 0.49	1.37±0.06	0.09	0.16	1.93	12.22	4.53
	Sucrose 5 g/L	2.69±0.09	12.19^{d} ± 0.15	0.71±0.03	0.10	0.37	1.68	04.53	0.51
Nitrogen sources	Meat extract 3.0%	1.57±0.02	44.11^{a}±0.99	1.34±0.07	0.08	1.17	32.97	28.07	1.58
	Yeast extract								
	0.5 %	0.78±0.03	37.72 ^{c}±0.72	0.35±0.04	0.12	2.23	108.17	48.48	1.57
	1.0 %	1.08±0.05	43.15 ^{b}±1.15	0.88±0.05	0.10	1.22	49.24	40.23	1.80
	2.0 %	1.50±0.08	46.31 ^{a}±0.79	1.14±0.06	0.15	1.31	40.62	30.95	1.93
	3.0 %	1.76±0.07	44.86^{b}±0.86	1.30±0.04	0.10	1.35	34.49	25.61	1.87
	Peptone/casein 3.0%	0.57±0.04	32.84^{c}±0.32	0.27±0.03	0.10	2.11	121.63	57.61	1.37
	Peptone/meat 3.0%	1.67±0.07	41.72^{b}±0.34	0.99±0.05	0.10	1.69	42.14	24.98	1.74
	$(NH_4)_2SO_4$ 3%	0.57±0.03	36.09^{d}±0.54	0.62±0.03	0.05	0.92	58.02	63.31	1.50
	NH_4NO_3 3%	0.42±0.04	35.93^{d}±0.12	0.60±0.04	0.06	0.70	59.57	85.60	1.50
***p*ABA (µM)**	0	1.45±0.06	42.61^{b}±0.38	1.05±0.05	0.11	1.39	40.73	29.33	1.78
	1	1.63±0.08	46.33^{a}±0.25	1.26±0.07	0.12	1.29	36.67	28.39	1.93
	3	1.65±0.07	43.98^{b}±0.76	1.06±0.06	0.12	1.55	41.29	26.61	1.83
	5	1.53±0.05	44.32^{b}±0.37	1.00±0.05	0.11	1.53	44.36	28.97	1.85
	10	1.53±0.06	44.19^{b}±0.17	1.25±0.07	0.11	1.22	35.26	28.89	1.84
	50	1.47±0.04	44.47^{b}±0.31	1.12±0.06	0.11	1.31	39.65	30.29	1.85
	100	1.53±0.06	44.50^{b}±0.13	1.34±0.07	0.11	1.14	33.26	29.09	1.85
	150	1.40±0.04	44.47^{b}±0.27	0.91±0.06	0.11	1.53	48.65	31.82	1.85
pH In shake-flask	6	1.68±0.04	42.00^{c}±0.4	0.73±0.07	0.11	2.31	57.88	25.05	1.75
	7	1.63±0.06	46.91^{a}±0.2	1.29±0.06	0.12	1.26	36.33	28.88	1.95
	8	1.77±0.05	45.68^{b}±0.4	1.26±0.09	0.10	1.40	36.17	25.82	1.90
In Bioreactor	6	1.77±0.09	50.04 ^{b}±0.2	1.61±0.08	0.09	1.10	31.05	28.35	2.08
	7	1.75±0.07	54.53 ^{a}±1.0	2.44±0.11	0.12	0.72	22.34	31.13	2.27
	8	1.27±0.06	51.14 ^{b}±0.7	1.57±0.09	0.11	0.80	32.49	40.47	2.13

*(X_{max}): Maximum cell concentration; (P_{max}): Maximum folic acid concentration; ($S_{consume}$): Substrate Consumed; (μ): Specific growth rate; ($Y_{x/s}$): Growth yield coefficient; ($Y_{p/s}$): Product yield coefficient; ($Y_{p/x}$): Cell efficiency coefficient.
^{a} ,^{b,c,d} One way ANOVA of Folate (µg /L) means at different hours between culture inoculation variables. Means in the same column and title with different superscript are significantly different ($p< 0.05$)

Effect of initial pH

The effect of the initial pH on folate production in shake-flask was also studied over a pH range of 6.0 to 8.0. As shown in Table 1, the total amount of folate and productivity were found to be significantly higher at pH 7 (46.91 µg/L) than those of the initial pH of 6 and 8. Maximum specific growth rate and yield per biomass were also observed at pH 7.

Batch fermentation in Bioreactor

Compared to the shake-flask culture (19.29 µg/L), batch fermentation in bioreactor at pH 7 led to about two-fold increase in folate yield (54.53 µg/L) (Table 1). The highest specific growth rate (0.12 h^{-1}) and productivity (2.27

µg /l/h) were also detected at this pH. However, it was found that pH had no significant effect on the intra- and extracellular distribution of folate and the ratio of intracellular and extracellular folate excretion. Cell growth was significantly inhibited at pH lower than 6 and higher than 8.

Kinetic model and parameter estimation

The kinetic parameter values of the culture in shake-flask and bioreactor are summarized in Table 2. Monod model was fitted to the empirical approach and the data of the biomass, while Logistic model was not (Figure 1). Gompertz model was also not completely fitted with the experimental data in the both shake-flask and bioreactor. According to the data, the behavior of cell growth was in line with the model equations in shake-flask and bioreactor. As shown in Figure 2, the performance of substrate consumption in bioreactor was approximately fitted with the Luedeking-Piret substrate model (Figure 2). The simulation curves were fitted well, with more than 93% confidence, to the experimental data in shake-flask and bioreactor. Statistical analysis of the t-test revealed no significant deviations between the calculated and the experimental data ($p< 0.05$), suggesting that the proposed models based on the Monod and Luedeking-Piret equations are sufficient to describe the cell growth, lactose consumption and folate production. Although, cell concentration was similar for the processes, maximum specific growth rate and growth associated rate constant for folate formation in the bioreactor were about two times lower than the values calculated for the shake-flask. For the both cultivations, the values of β were zero, suggesting that the production of folate is growth associated (Figure 2).

Table 2: Comparison of the performance and the kinetic parameter values of folate production in batch culture of S. thermophilus BAA-250 using shake-flask and the bioreactor.

Kinetic Parameter Values	Shake-flask			Bioreactor		
Models	**Logistic**	**Gompertz**	**Monod**	**Logistic**	**Gompertz**	**Monod**
t(h)	24	24	24	24	24	24
μ_{max} (h^{-1})	0.6467 ± 0.2640	1.0000 ±0.10200	0.6830 ± 0.2810	0.6105 ± 0.1310	1.0000 ± 0.1261	0.7451 ± 0.1310
X_0 (g/L)	0.1070 ± 0.0800	0.0715 ± 0.0318	0.1070 ± 0.0293	0.0110 ± 0.0060	0.0794 ± 0.0195	0.0110 ± 0.0465
X_m (g/L)	1.7100 ± 0.0480	1.8555 ± 0.1764	1.8350 ± 0.1461	1.5300 ± 0.0230	1.8633 ± 0.1116	1.5635 ± 0.0273
P_0 (µg/L)	33.3770 ± 0.7620	33.3770 ± 0.7620	33.3770 ± 0.7620	41.9220 ± 1.6850	41.9220 ± 1.6850	41.9220 ± 1.6850
P_m (µg/L)	47.9120 ± 0.0826	47.9120 ± 0.0826	47.9120 ± 0.0826	56.5320 ± 0.9620	56.5320 ± 0.9620	56.5320 ± 0.9620
a (g folate/g cell)	4.7380 ± 0.5190	4.7380 ± 0.5190	4.7380 ± 0.5190	7.1090 ± 1.3240	7.1090 ± 1.3240	7.1090 ± 1.3240
β (g folate/g cell.h)	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
S_0 (g/L)	3.5590 ± 0.0263	3.5590 ± 0.0263	3.5590 ± 0.0263	3.4510 ± 0.0540	3.4510 ± 0.0540	3.4510 ± 0.0540
m (g lactose/g cell)	0.7400 ± 0.0228	0.7400 ± 0.0228	0.7400 ± 0.0228	1.1460 ± 0.0560	1.1460 ± 0.0560	1.1460 ± 0.0560
n (g lactose/g cell.h)	0.0030 ± 0.0010	0.0030 ± 0.0010	0.0030 ± 0.0010	0.0230 ± 0.0030	0.0230 ± 0.0030	0.0230 ± 0.0030

DISCUSSION

The results of this study revealed that lactose (3 g/L) and yeast extract (20 g/L) were the most suitable carbon and nitrogen sources for folate production by *S. thermophilus* BAA-250. The adaptation of *S. thermophilus* to lactose is due to the fact that it only utilizes the glucose derived from lactose (van den Bogaard et al., 2004). The highest level of folate per biomass or cell efficiency to produce folate (48.27 µg/g) was obtained in the lactose concentration of 1 g/L due to the very low amount of biomass (Fu & Mathews, 1999). Matched up well with the previous reports by Foucaud and Poolman (1992) and Yuksekdag and Aslim (2008), our findings showed that sucrose was a poor carbon source for cell growth and folate production.

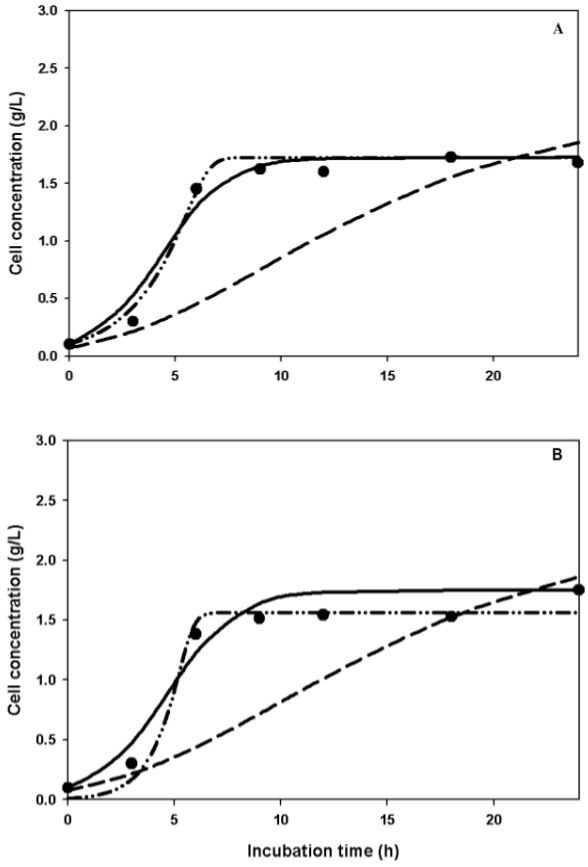

Figure 1: Modeling of cell growth of *S. thermophilus* BAA-250 in shake-flask (A) and bioreactor (B). Cell concentration (●), Logistic (▬), Gompertz (−−) and Monod (·−).

Among the organic and inorganic nitrogen sources, yeast extract was shown to be an effective growth factor and nitrogen source for folate production. It is encouraging to compare this result with the finding of Hujanen and Linko (1996) and Liew *et al*. (2005) that there is strong relationship between yeast extract and the growth of probiotic bacteria. Cell development and acids production are thought to be higher in present of organic nitrogen sources compared to inorganic nitrogen sources (Rosfarizan & Ariff, 2006; Sybesma et al., 2003b). Our finding were also consistent with those of Zisu and shah *et al*. (2003) who showed that compared to inorganic nitrogen sources, organic nitrogen sources improved EPS production by *S. thermophilus*. Free amino acids are thought to be required for the enhancement of the enzymes which are involved in folate biosynthesis (Wu et al., 2008).

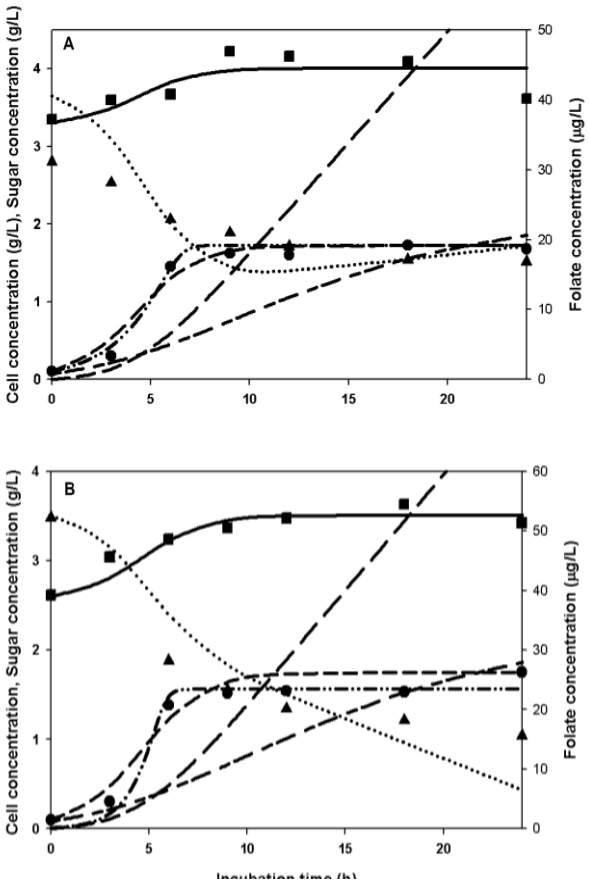

Figure 2: Experimental and simulated data for folate fermentation in the shake-flask (A) and bioreactor (B). Cell concentration (●), Substrate consumption (▲), Folate production (■), Luedeking-Piret growth associated (▬), Luedeking-Piret non-growth associated (— —), Luedeking-Piret product (····), Logistic (−−), Gompertz (— −), Monod-cell growth (−··).

Folate production is controlled by the biosynthesis of the folate precursor, *p*ABA, via the shikimate pathway (Wegkamp et al., 2007). The present work showed that *p*ABA higher than 1 µM had no significant effect on folate biosynthesis. This result was in contrast to the finding of Sybesma and

colleagues (2003b), who reported that high level of *p*ABA ranging from 1 µM to 100 µM increases folate production in *Lactococcus lactis* strains. It seems that the presence of *p*ABA is not necessary for folate production by *S. thermophilus*, because the strain is able to synthesis *p*ABA as previously reported from some other LAB (LeBlanc et al., 2012).

Under pH-controlled conditions in bioreactor, pH range of 6-8 was shown to be optimal for folate production by *S. thermophilus*. However, Sybesma et al. (2003b) reported pH between 7.3 and 9.3 as the optimum pH for folate production by *S. thermophilus* B119. In controlled batch fermentation, higher level of folate production was obtained at pH 7. In contrast, Sybesma and colleagues (Sybesma et al., 2003b) showed an increase in the excretion of extracellular folate, under controlled growth condition at lower pH. They also reported higher intracellular folate at pH higher than 7. However, in *S. thermophilus* BAA-250, the intra and extracellular distribution of folate and the ratio of intracellular and extracellular folate excretion was not a function of pH, as also reported by Pompei *et al.* (Pompei et al., 2007) for *B. adolescentis* MB 239 in chemostat experiment.

Kinetic models revealed that the growth was fitted with the Monod equations, while substrate consumption was fitted with the Luedeking-Piret equation. Luedeking-Piret model showed that folate production was growth associated process. On the other hand, Gompertz model can only be used to describe the experimental data up to 3 h of the growth phase. This models were also used by Song *et al*. (2008) to study succinic acid production by Mannheimia *succiniciproducens*. Monod model has been also used to described lactic acid production by *L. lactis* ATCC 19435 (Åkerberg et al., 1998). Overall, kinetic studies revealed the effect of specific growth rate on the biosynthesis of folate by *S. thermophilus*, where an increase in folate production was observed by decreasing specific growth rate. High specific growth rate may affect the metabolism of cell to produce more by-products rather than folate (Lin & Young, 2000; Sybesma et al., 2003a). However, in a recent study by Pompei *et al*. (2007), folate content in yeast cells showed highly correlation with the growth rate.

Taken together, lactose (3 g/L) and yeast extract (20 g/L) were shown to be the more suitable carbon and nitrogen sources for folate production by *S. thermophilus*. It was also shown that *p*ABA is necessary for folate biosynthesis in *S. thermophilus*. Furthermore, folate production was found to be significantly increase by maintaining the pH of the culture medium at 7 during batch fermentation in bioreactor. Kinetic models showed that folate production by *S. thermophilus* BAA-250 is growth associated process.

Acknowledgment:
The present study was supported by the Universiti Putra Malaysia.

References

Åkerberg, C., Hofvendahl, K., Zacchi, G., and Hahn-Hägerdal, B. 1998. Modelling the influence of pH, temperature, glucose and lactic acid concentrations on the kinetics of lactic acid production by Lactococcus lactis ssp. lactis ATCC 19435 in whole-wheat flour. Applied microbiology and biotechnology, 49(6): 682-690.

Arcot, J., and Shrestha, A. 2005. Folate: methods of analysis. Trends in Food Science & Technology, 16(6-7): 253-266.

Basset, G. J. C., Quinlivan, E. P., Gregory III, J. F., and Hanson, A. D. 2005. Folate Synthesis and Metabolism in Plants and Prospects For Biofortification. Crop Science, 45: 449-453.

Crittenden, R. G., Martinez, N. R., and Playne, M. J. 2003. Synthesis and utilisation of folate by yoghurt starter cultures and probiotic bacteria.

International Journal of Food Microbiology, 80(3): 217-222.

Foucaud, C., and Poolman, B. 1992. Lactose transport system of *Streptococcus thermophilus* Functional reconstitution of the protein and characterization of the kinetic mechanism of transport. Journal of Biological Chemistry, 267(31): 22087-22094.

Fu, W., and Mathews, A. P. 1999. Lactic acid production from lactose by *Lactobacillus plantarum*: kinetic model and effects of pH, substrate, and oxygen. Biochemical Engineering Journal, 3(3): 163-170.

Galia, W., Perrin, C., Genay, M., and Dary, A. 2009. Variability and molecular typing of *Streptococcus thermophilus* strains displaying different proteolytic and acidifying properties. International Dairy Journal, 19(2): 89-95.

Horne, D. W., and Patterson, D. 1988. *Lactobacillus casei* Microbiological Assay of Folic Acid Derivatives in 96-well Microtiter Plates Clinical Chemistry, 34(11): 2357-2359.

Hujanen, M., and Linko, Y. Y. 1996. Effect of temperature and various nitrogen sources on L (+)-lactic acid production by *Lactobacillus casei*. Applied microbiology and biotechnology, 45: 307-313.

Jones, M. L., and Nixon, P. F. 2002. Tetrahydrofolates Are Greatly Stabilized by Binding to Bovine Milk Folate-Binding Protein. The Journal of Nutrition, 132(9): 2690-2694.

LeBlanc, J. G., de Savoy, G. S., Smid, E. J., Hugenholtz, J., and Sesma, F. 2007. Folate production by lactic acid bacteria and other food-grade microorganisms. Communicating Current Research and Educational Topics and Trends in Applied Microbiology.

LeBlanc, J. G., Milani, C., de Giori, G. S., Sesma, F., van Sinderen, D., and Ventura, M. 2012. Bacteria as vitamin suppliers to their host: a gut microbiota perspective. Current Opinion in Biotechnology.

Liew, S. L., Ariff, A. B., Raha, A. R., and Ho, Y. W. 2005. Optimization of medium composition for the production of a probiotic microorganism, *Lactobacillus rhamnosus*, using response surface methodology. International Journal of Food Microbiology, 102(2): 137-142.

Lin, M. Y., and Young, C. M. 2000. Folate levels in cultures of lactic acid bacteria. International Dairy Journal, 10(5-6): 409-413.

Lobry, J. R., Flandrois, J. P., Carret, G., and Pave, A. 1992. Monod's bacterial growth model revisited. Bulletin of Mathematical Biology, 54(1): 117-122.

Mu, Y., Wang, G., and Yu, H. Q. 2006. Kinetic modeling of batch hydrogen production process by mixed anaerobic cultures. Bioresource Technology, 97(11): 1302-1307.

Omar, R., Abdullah, M. A., Hassan, M. A., Rosfarizan, M., and Marziah, M. 2006. Kinetics and modelling of cell growth and substrate uptake in *Centella asiatica* cell culture. Biotechnology and Bioprocess Engineering, 11: 223-229.

Papastoyiannidis, G., Polychroniadou, A., Michaelidou, A. M., and Alichanidis, E. 2006. Fermented milks fortified with B-group vitamins: vitamin stability and effect on resulting products. Food Science and Technology International, 12(6): 521-529.

Pompei, A., Cordisco, L., Amaretti, A., Zanoni, S., Matteuzzi, D., and Rossi, M. 2007. Folate Production by Bifidobacteria as a Potential Probiotic Property. Applied and Environmental Microbiology, 73(1): 179-185.

Rosfarizan, M., and Ariff, A. B. 2006. Kinetics of kojic acid production by *Aspergillus flavus* S44-1 using sucrose as a carbon source. Journal of Biotechnology and Bioprocess Engineering, 11: 72-79.

Rosfarizan, M., and Arrif, A. B. 2007. Biotransformation of various carbon sources to kojic acid by cell-bound

enzyme system of *A. flavus* Link 44-1. Biochemical Engineering Journal, 35(2): 203-209.

Scott, J., Re´beille, F., and Fletcher, J. 2000. Folic acid and folates: the feasibility for nutritional enhancement in plant foods. Journal of the Science of Food and Agriculture, 80(7): 795-824.

Song, H., Jang, S. H., Park, J. M., and Lee, S. Y. 2008. Modeling of batch fermentation kinetics for succinic acid production by *Mannheimia succiniciproducens*. Biochemical Engineering Journal, 40(1): 107-115.

Sybesma, W., Strrenburg, M., Kleerebenzem, M., Mierau, I., de Vos, W. M., and Hugenholtz, J. 2003a. Increased Production of Folate by Metabolic Engineering of *Lactococcus lactis*. Applied and Environmental Microbiology, 69(6): 3069-3076.

Sybesma, W., Strrenburg, M., Tijsseling, L., Hoefnagel, M. H., and Hugenholtz, J. 2003b. Effect of Cultivation Condition on Folate Production by Lactic Acid Bacteria. Applied and Environmental Microbiology, 69(8): 4542-4548.

van den Bogaard, P. T., Hols, P., Kuipers, O. P., Kleerebezem, M., and de Vos, W. M. 2004. Sugar utilisation and conservation of the gal-lac gene cluster in *Streptococcus thermophilus*. Systematic and Applied Microbiology 27: 10-17.

Wegkamp, A., van Oorschot, W., de Vos, W. M., and Smid, E. j. 2007. Characterization of the Role of *para*- Aminobenzoeic Acid Biosynthesis in Folate Production by *Lactococcus lactis*. Applied and Environmental Microbiology, 73(8): 2673-2681.

Wu, C. Y., Liang, Z. C., Lu, C. P., and Wu, S. H. 2008. Effect of Carbon and Nitrogen Sources on the Production and Carbohydrate composition of Exopolysaccharide by Submerged Culture of *Pleurotus citrinopileatus*. Journal of Food and Drug Analysis, 16(2): 61-67.

Yuksekdag, Z. N., and Aslim, B. 2008. Influence of Different Carbon Sources on Exopolysaccharide Production by *Lactobacillus delbrueckii* subsp. *bulgaricus* (B3, G12) and *Streptococcus thermophilus* (W22). Brazilien Archives of Biology and Technology, 51(3): 581-585.

Zisu, B., and Shah, N. P. 2003. Effects of pH, Temperature, Supplementation with Whey Protein Concentrate, and Adjunct Cultures on the Production of Exopolysaccharides by *Streptococcus thermophilus* 1275. Journal of Dairy Science, 86(11): 3405-3415.