

CHAPTER 3

RESULTS AND DISCUSSION

1. Optimization for production of oligodextran

1.1 Cell growth characteristics

1.1.1 Cell growth by shake-flask cultivation

The cell concentration of *Gluconobacter oxydans* NCIMB 4943 in GY medium in shake-flasks (175 rev min^{-1}) at 30°C increased rapidly for 18 h and subsequently remained constant for 24 h. The maximum dry cell weight (0.32 g l^{-1}) was obtained at 24 h. Culture pH rapidly declined (from 6.8 to around 4.0) within the first 15 h of cultivation and was nearly constant thereafter (Figure 3.1).

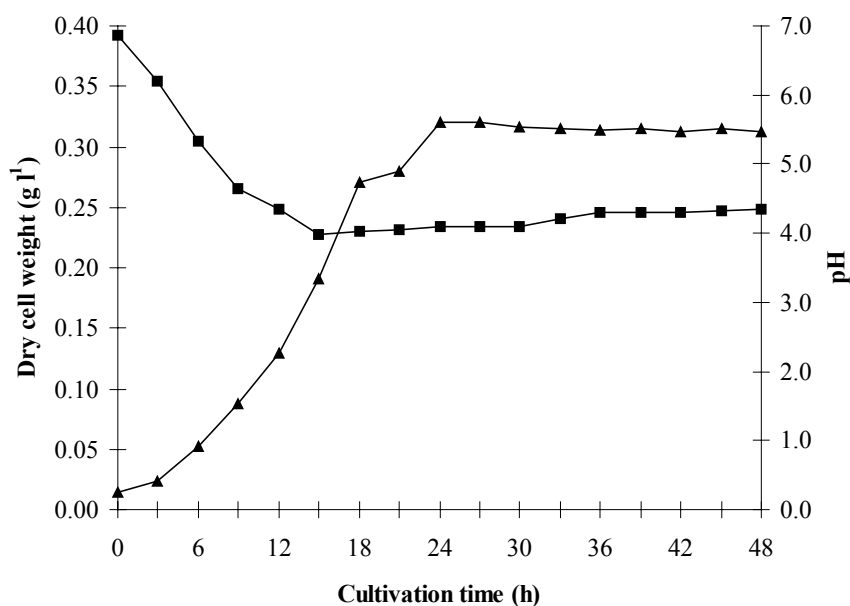


Figure 3.1 Growth characteristics of *G. oxydans* NCIMB 4943 cultivated in GY medium in shake-flask culture (175 rev min^{-1} , 30°C)

▲ dry cell weight; ■ culture pH

1.1.2 Cell growth by batch fermenter cultivation

Production of *G. oxydans* cells in GY medium in non-pH controlled and pH-controlled fermenters was compared. Growth under controlled pH (at 6.8) conditions was found to be much better than that under uncontrolled pH conditions when the culture pH dropped rapidly from 6.8 to around 3.8 within the first 18 h of cultivation. The maximum dry cell weights obtained were 0.51 and 0.36 g l⁻¹ at 24 h of cultivation, respectively (Figure 3.2). This clearly indicated that culture pH affected directly the growth of *G. oxydans* and the low pH resulted in lower growth of the bacterium.

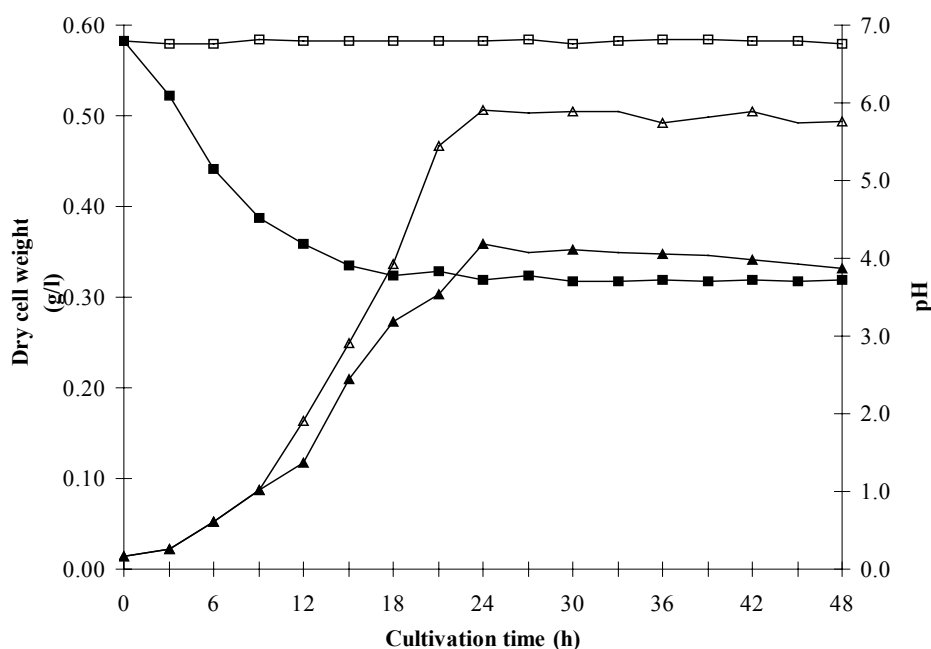


Figure 3.2 Growth and culture pH during cultivation of *G. oxydans* NCIMB 4943 in GY medium using 10 l batch fermenter, under uncontrolled and controlled culture pH (6.8), agitation speed of 150 rpm, 30°C for 48 h
 ■ pH (uncontrolled); □ pH (controlled at 6.8); ▲ dry cell weight (uncontrolled pH); △ dry cell weight (controlled pH)

1.2 Comparison of oligodextran production by the culture method and the cell suspension method

Oligodextran production was affected by the production method. The cell suspension method had significantly lower ($P < 0.05$) oligodextran yields compared to

the culture medium method despite a similar production rate during the first 16 h of cultivation. In the cell suspension method, the synthesis reaction was carried out using 0.5% (w/v) cell suspension of *G. oxydans* and 1% (w/v) of G19 maltodextrin in acetate buffer (pH4.5). The culture medium method was carried out by cultivation of *G. oxydans* in maltodextrin complex medium containing 1% G19 maltodextrin as carbon and energy source. Oligodextran yields (mean \pm SD) obtained from the maltodextrin complex medium and using cell suspensions at 48 h cultivation were 30.35 \pm 1.56% and 25.30 \pm 1.76%, respectively (Figure 3.3). The cell suspension method was chosen for the next study, although it gave lower yields, because the cell suspension method gave a higher purity product, facilitating separation and recovery.

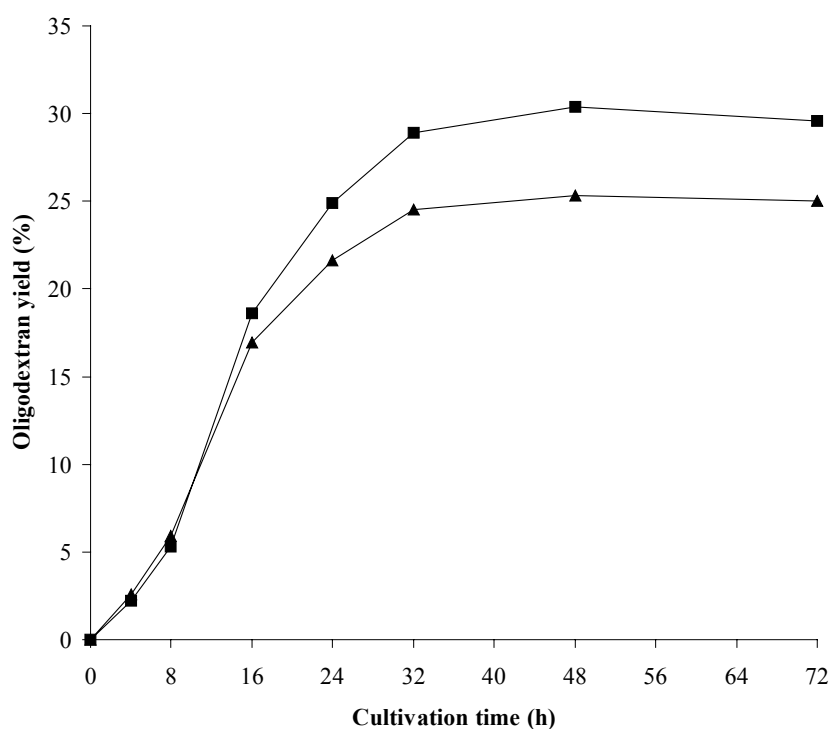


Figure 3.3 Comparison of oligodextran yields obtained by the culture method and the cell suspension method on 10 g l⁻¹ G19 maltodextrin by 5 g l⁻¹ *G. oxydans* cells

■ culture medium method; ▲ cell suspension method

Oligodextran production reached higher yields when *G. oxydans* NCIMB 4943 was grown in maltodextrin complex medium suggesting that dextrin dextranase (DDase) activity correlated to growth of cell biomass. However, DDase expression was found to be non-growth associated in a previous study (Mountzouris *et al.*, 1999). Using purified DDase, dextran yields on maltotriose, maltotritol, maltotetraose, maltotetratol, maltopentaose, maltohexaose, short chain amylose and soluble starch were 11.0, 22.4, 13.4, 36.8, 25.0, 30.2, 57.6 and 21.4%, respectively (Yamamoto *et al.*, 1992; 1993b). This showed that dextran yield was largely dependant on chain length of the substrate. In addition, low DE (high DP) maltodextrin had lower conversion rates to oligodextran compared to low DE maltodextrin. This result is consistent with the mode of action of DDase which catalyses the transglucosylation of non-reducing terminal residue of a donor substrate to an acceptor (Yamamoto *et al.*, 1993a). Long glucose chains have lower concentrations of non-reducing terminal residues than an equivalent mass of short glucose chains.

1.3 Effect of cell concentration

Oligodextran was produced rapidly in the first 24 h and increased only slightly by 48 h. No statistically significant further increase was seen at 72 h using the cell suspension method. Oligodextran yields (mean \pm SD) obtained at the cell concentrations (g l⁻¹) of 5, 10, 15 and 30 at 72 h were 26.70 \pm 1.35%, 27.00 \pm 1.59%, 26.40 \pm 1.60% and 25.80 \pm 1.87%, respectively. The maximum oligodextran yield (27.00%) at 72 h using 10 g l⁻¹ cell concentration (Figure 3.4). Thus the optimal cell concentration of *G. oxydans* for oligodextran production was 5 g l⁻¹ using cell suspension method was chosen for next study.

1.4 Effect of buffer pH

The pH of the reaction mixtures affected oligodextran production from *G. oxydans* NCIMB 4943. The maximum oligodextran yields (mean \pm SD) were 24.00 \pm 1.39%, 24.60 \pm 1.22% and 22.90 \pm 1.48%, at pH 3.5, 4.5, 5.5, respectively and these were obtained at 72 h (Figure 3.5). However, no significant differences were seen between 48 h and 72 h at all buffer pHs tested. The optimal buffer pH (sodium acetate) for oligodextran production using cell suspension method was 4.5.

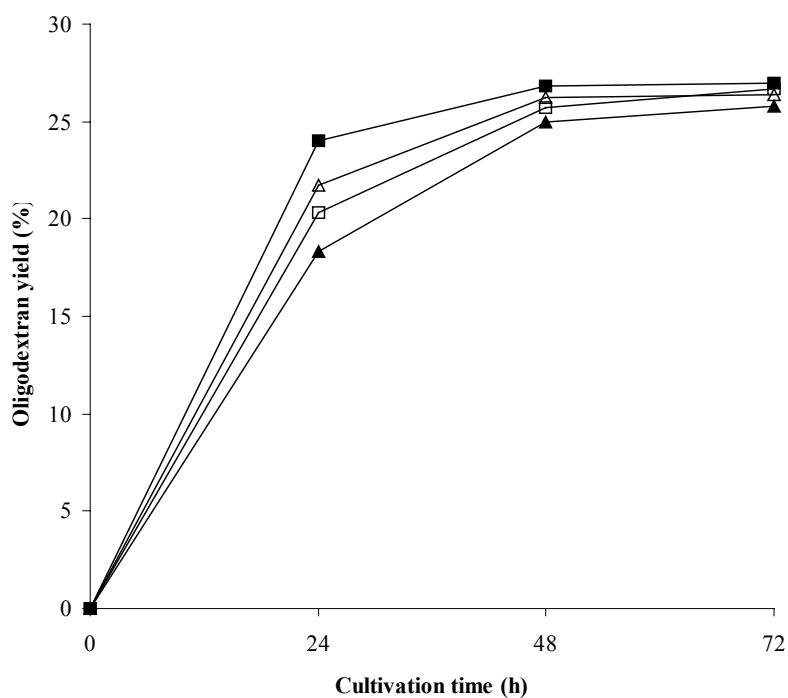


Figure 3.4 Effect of concentrations of *G. oxydans* NCIMB 4943 cells performed in shake-flask culture (200 rev min⁻¹, 30°C) using the cell suspension method containing 10 g l⁻¹ G19 maltodextrin dissolved in acetate buffer (pH 4.5)
 □ 5 g l⁻¹; ■ 10 g l⁻¹; △ 15 g l⁻¹; ▲ 30 g l⁻¹

Buffer pH in the range tested was found to have no effect on the oligodextran yield which agreed with a previous report (Mountzouris, 1999). The optimum conditions of purified DDase from *A. capsulatus* were 37-45°C, pH 4.0-4.2 with the enzyme being stable at temperatures below 45°C and at pH 3.5-5.2 for 30 min (Yamamoto *et al.*, 1992). The cell suspensions used in this study had slightly different optimum conditions of pH 4.5 and 30°C.

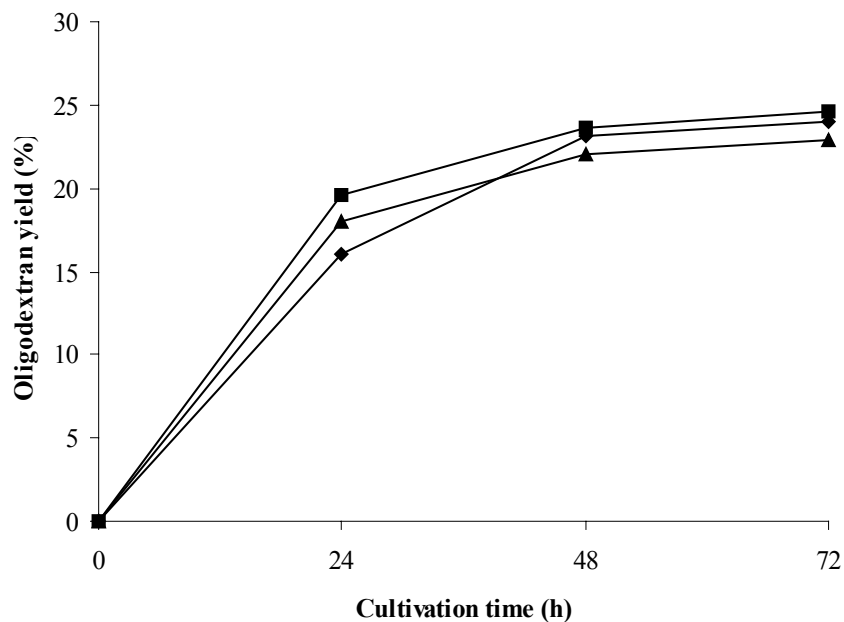


Figure 3.5 Effect of sodium acetate buffer pH on oligodextran yields carried out in shake-flask culture (200 rev min^{-1} , 30°C) using the cell suspension method containing 5 g l^{-1} *G. oxydans* cells, 10 g l^{-1} G19 maltodextrin dissolved in sodium acetate buffer at various pH values

◆ pH 3.5; ■ pH 4.5; ▲ pH 5.5

1.5 Effect of temperature

The maximum oligodextran yield (25.94%) was obtained at a reaction temperature of 30°C at 72 h reaction time whereas the values were 25.30% and 23.40% at 25°C and 35°C , respectively (Figure 3.6). The optimal temperature for oligodextran synthesis was between 25°C and 30°C . A reaction temperature of 30°C was chosen for further study.

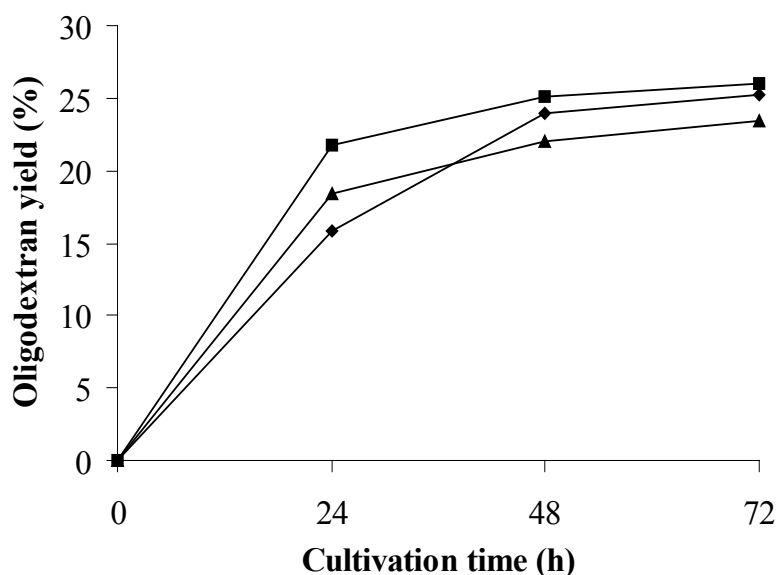


Figure 3.6 Effect of reaction temperature on oligodextran yield carried out in shake-flask culture (200 rev min^{-1}) at various temperatures using the cell suspension method containing 5 g l^{-1} *G. oxydans* cells, 10 g l^{-1} G19 maltodextrin dissolved in sodium acetate buffer (pH 4.5)

◆ 25°C; ■ 30°C; ▲ 35°C

1.6 Effect of substrate type

1.6.1 Culture medium method

Growth of *G. oxydans* on various maltodextrin substrates is shown in Figure 3.7. Higher DE (dextrose equivalent) substrates such as Glucidex29 (G29), Goldex30 (G30) and Goldex37 (G37) maltodextrins gave better growth during the first 16 h compared to lower DE substrates. This may be due to the higher DE substrate containing a higher proportion of low molecular weight components, particularly glucose, which is preferred for growth of the bacteria. Since Glucidex19 (G19) maltodextrin gave the highest growth of *G. oxydans* NCIMB 4943 it was chosen for further study.

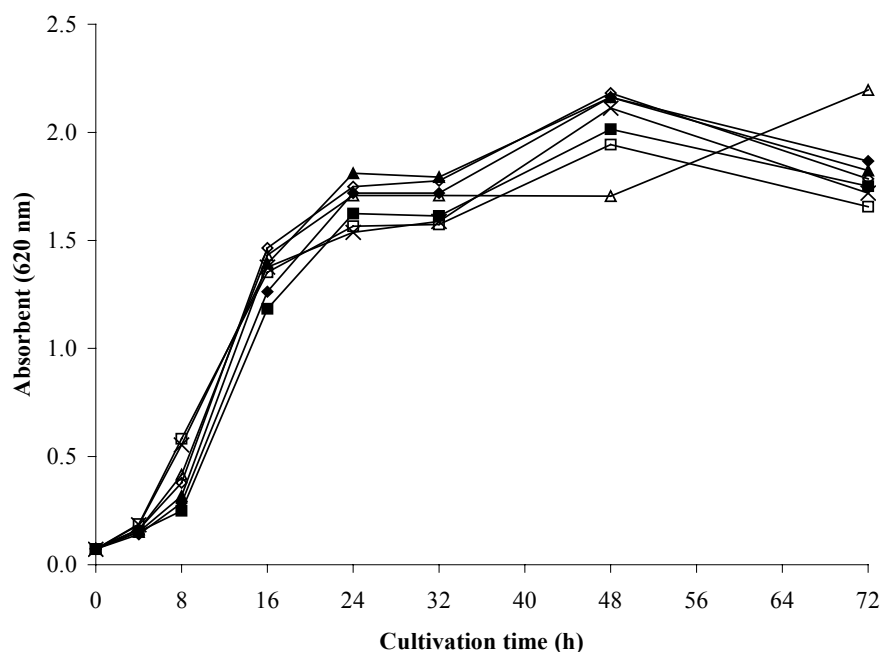


Figure 3.7 Effect of various commercial maltodextrins on growth of *G. oxydans* NCIMB 4943 cultivated in maltodextrin complex medium in shake-flask culture (200 rev min⁻¹, 30°C)

◆ G12; ■ G15; ▲ G19; ◇ G20; □ G29; △ G30; × G37

Culture pH rapidly declined on all maltodextrins tested (from 5.5 to around 3.0) within the first 24 h of cultivation (Figure 3.8). Typically, higher DE (low DP) maltodextrins gave a greater reduction in culture pH, particularly maltodextrins with DE higher than 20 such as Glucidex29 (G29), Goldex30 (G30) and Goldex37 (G37).

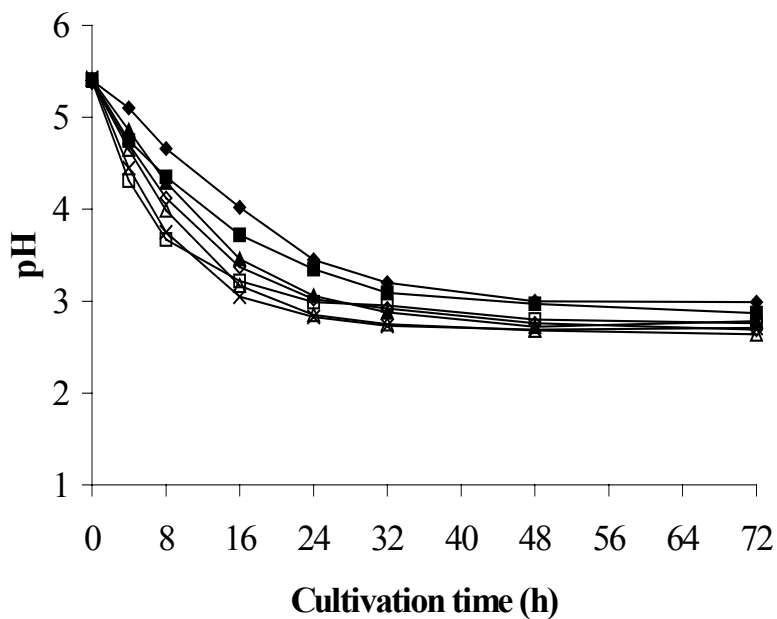


Figure 3.8 Effect of various commercial maltodextrins on culture pH of *G. oxydans* NCIMB 4943 cultivated on maltodextrin complex medium in shake-flask culture (200 rev min⁻¹, 30°C)
 ◆ G12; ■ G15; ▲ G19; ◇ G20; □ G29; △ G30; × G37

Oligodextran yield depended on the type of maltodextrins used with a maximum oligodextran yield of 30.02% on G30 in maltodextrin complex medium. In addition, G29 and G37 maltodextrins also gave high oligodextran yields whereas low DE substrates such as G12 and G15 gave the lowest yields. Oligodextran yields were 18.53, 15.31, 17.66, 18.94, 26.84, 30.02 and 22.25% obtained from G12, G15, G19, G20, G29, G30 and G37, respectively at 72 h (Figure 3.9).

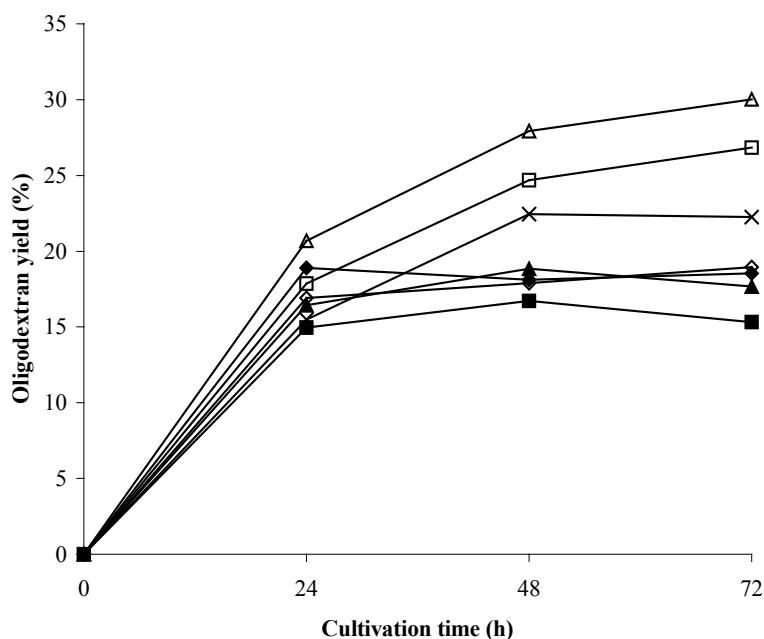


Figure 3.9 Effect of substrate types on oligodextran yields from *G. oxydans* NCIMB 4943 cultivated on maltodextrin complex medium with 10 g l⁻¹ maltodextrin by shake-flask culture (200 rev min⁻¹, 30°C) using the culture medium method

◆ G12; ■ G15; ▲ G19; ◇ G20; □ G29; △ G30; ×, G37

1.6.2 Cell suspension method

Each substrate type significantly ($P < 0.05$) affected the oligodextran yield. All of the maltodextrins tested with the cell suspension method gave significantly lower ($P < 0.05$) oligodextran yields than with the maltodextrin complex medium. Oligodextran yields (mean±SD) obtained at 72 h were 10.71±1.45, 14.31±2.10, 13.91±1.09, 14.58±2.11, 15.90±1.89, 18.70±2.32 and 11.96±1.56% from G12, G15, G19, G20, G29, G30 and G37 maltodextrins, respectively (Figure 3.10). The maximum oligodextran yield (28.77±2.40%) was obtained at 48 h using G30. These results suggested higher DE maltodextrins gave higher oligodextran yield.

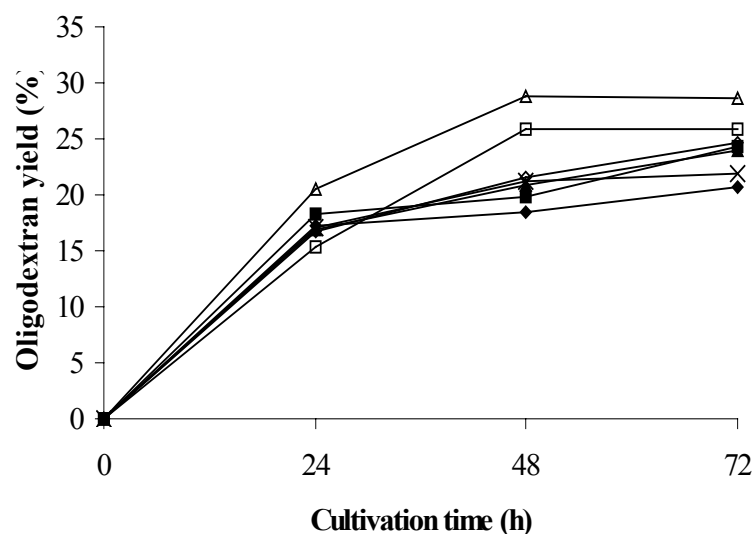


Fig. 3.10 Effect of substrate type on oligodextran yields performed in shake-flask culture (200 rev min^{-1} , 30°C) using the cell suspension method containing 5 g l^{-1} *G. oxydans* cells, 10 g l^{-1} maltodextrin dissolved in sodium acetate buffer (pH 4.5)

◆ G12; ■ G15; ▲ G19; ◇ G20; □ G29; △ G30; × G37

1.7 Effect of substrate concentration

Although, G30 maltodextrin have the highest oligodextran yield however due to the shortage of G29 and G30 maltodextrins for awhile so that G19 maltodextrin was chosen for next study. In addition, next study confirmed that G19 maltodextrin was suitable substrate due to it gave the highest ratio of 1,6 linkages contained in oligodextran product.

Substrate concentration had a significant effect ($P < 0.05$) on oligodextran formation except at 10 g l^{-1} and 25 g l^{-1} . The highest oligodextran yield (24.80%) was obtained at 72 h using 10 g l^{-1} of G19 maltodextrin and 0.5 g l^{-1} *G. oxydans* cells in sodium acetate buffer (pH 4.5) at 30°C in shake-flask culture at 200 rev min^{-1} (Figure 3.11). The optimal concentration of maltodextrin to give the maximum oligodextran yield was 10 g l^{-1} . High concentrations ($50, 100 \text{ g l}^{-1}$) of maltodextrin gave lower oligodextran yields. Increasing the concentration of maltodextrin did not result in

higher oligodextran yields, rather it resulted in lower yields. This may be due to substrate inhibition affecting the DDase enzyme.

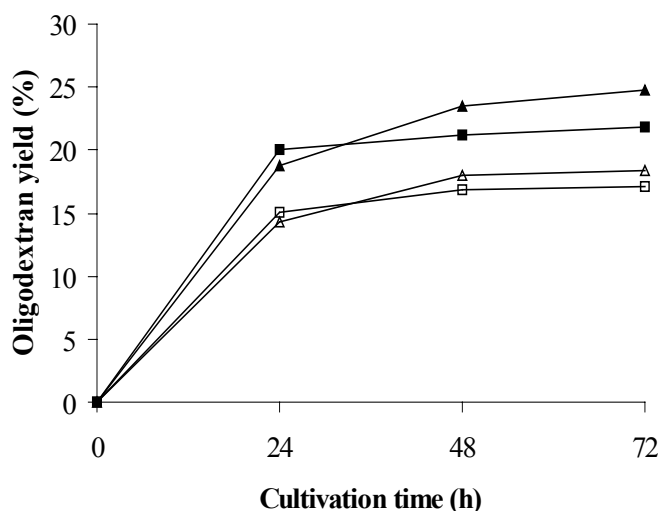


Figure 3.11 Effect of substrate concentration on oligodextran yields carried out in shake-flask culture (200 rev min⁻¹, 30°C) using the cell suspension method containing 5 g l⁻¹ *G. oxydans* cells and various amounts of G19 maltodextrin dissolved in sodium acetate buffer (pH 4.5)
G19 maltodextrin; ▲ 10 g l⁻¹; ■ 25 g l⁻¹; △ 50 g l⁻¹; □ 100 g l⁻¹

2. Studies on the influence of reaction parameters on the molecular weight distribution and chemical structure of oligodextran

2.1 Molecular weight distribution of oligodextran

2.1.1 Culture medium method

The profiles of molecular weight distribution varied as cultivation time increased. The G19 maltodextrin substrate contained large amounts of low molecular weight material (at 0 h cultivation) as eluted from the HPSEC column at about 50 min. This low molecular weight fraction reduced as cultivation time increased with a concomitant increase of the high molecular weight fraction (Figure 3.12).

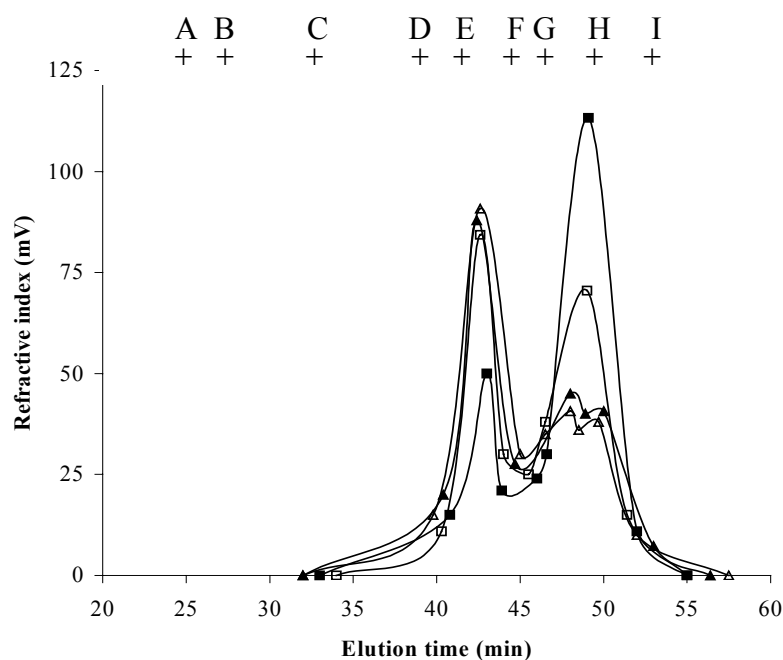


Figure 3.12 Molecular weight distributions of maltodextrin substrate (at 0 h) and products formed by *G. oxydans* NCIMB 4943 cultivated in maltodextrin complex medium with 10 g l⁻¹ G19 maltodextrin in shake-flask culture (200 rev min⁻¹, 30°C) using the culture medium method 0 (■), 24 (□), 48 (▲), 72 (△) h cultivation
Crosses (+) labeled with the letter A, B, ..., I; represent the elution time at peak height of the standards used to calibrate the chromatographic system. A: dextran 38000 kDa for V₀; B: dextran 2000 kDa; C: dextran 464 kDa; D: dextran 68.4 kDa; E: dextran 42 kDa; F: dextran 19.5 kDa; G: dextran 9.5 kDa; H: maltoheptaose 1.15 kDa; I: glucose 0.18 kDa

2.1.2 Cell suspension method

The molecular weight distribution of products from G19 and the G19 maltodextrin substrate displayed a similar pattern to those obtained using the maltodextrin complex medium although the oligodextran yields were lower in the cell suspension method (Figure 3.13). The reaction mixtures had two fractions; the low molecular weight fraction remaining from unconvertible maltodextrin substrate (≈1 kDa) and the high molecular weight oligodextran-containing fraction (7.8-65.6 kDa).

Oligodextrans produced by either method had exactly the same molecular weight (7.8-65.6 kDa).

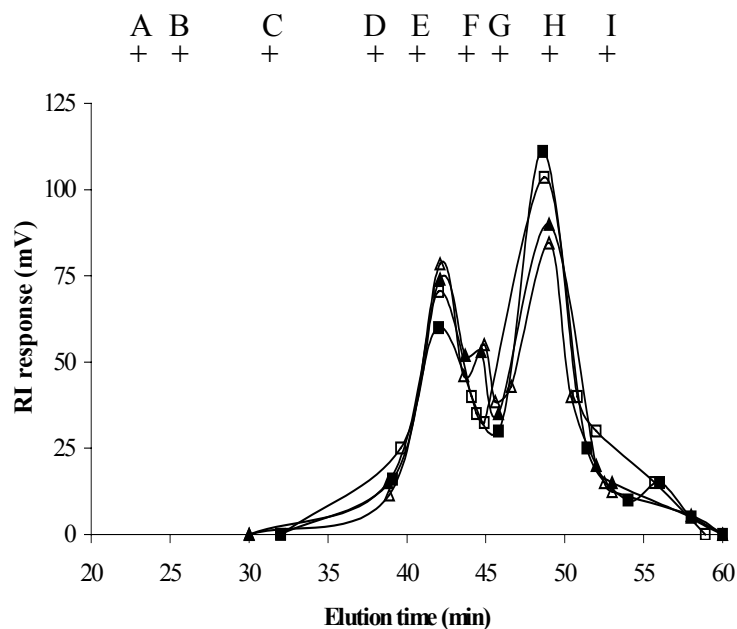


Figure 3.13 Molecular weight distributions of maltodextrin substrate (0 h) and products formed by *G. oxydans* NCIMB 4943 cultivated in shake-flask culture (200 rev min^{-1} , 30°C) using the cell suspension method (containing 5 g l^{-1} *G. oxydans* cells, 10 g l^{-1} G19 maltodextrin dissolved in sodium acetate buffer (pH 4.5)

0 (■), 24 (□), 48 (▲), 72 (△) h cultivation

Crosses (+) labeled with the letter A, B, ..., I; represent the elution time at peak height of the standards used to calibrate the chromatographic system.

A: dextran 38000 kDa for V_0 ; B: dextran 2000 kDa; C: dextran 464 kDa; D: dextran 68.4 kDa; E: dextran 42 kDa; F: dextran 19.5 kDa; G: dextran 9.5 kDa; H: maltoheptaose 1.15 kDa; I: glucose 0.18 kDa

2.2 Structural analysis

2.2.1 Linkage analysis by methylation analysis

2.2.1.1 Products of the culture medium method

Oligodextran products from several maltodextrans made using the culture medium method consisted of α -1,4, α -1,6 and α -1,4,6-D-glucosidic linkages by

methylation analysis (Table 3.1). The ratio of these linkages was calculated relative to the terminal glucose (t-Glc). Use of G19 maltodextrin as a substrate resulted in a product which contained α -1,6-D-glucosidic linkages at 3.99 mol mol⁻¹ of terminal-D-glucose. Low DE substrate such as G12, 15 maltodextrins gave higher ratio of α -1,4 in their products with the values of 4.27 and 3.82, respectively. Maltodextrin substrate having a medium level of DE (DE 19-20) gave the highest ratio of α -1,6 contained in the product, particular G19 and G20 maltodextrins.

Table 3.1 Effect of maltodextrin type on the molar ratio of linkages in oligodextrans made using the culture medium method, as determined by methylation analysis

Linkage type	Maltodextrin (DE)						
	G12	G15	G19	G20	G29	G30	G37
t-Glc	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1,6	2.94	3.29	3.99	3.77	2.51	2.60	1.43
1,4	4.27	3.82	3.40	3.35	2.17	2.20	1.48
1,4,6	0.73	0.68	0.93	0.82	0.59	0.52	0.31

t-Glc: terminal glucose

2.2.1.2 Products of the cell suspension method

G19 maltodextrin was found to be suitable for oligodextran production using the cell suspension method, although the ratio of α -1,6-D-glucosidic linkage was lower than the culture medium method (Table 3.2). The product produced from G19 maltodextrin gave the highest ratio (3.76) of α -1,6 linked glucose. Reduction of α -1,6 linked glucose was found using maltodextrin having DE higher than 20 (G29, G30, G37 maltodextrin) and DE lower than 19 (G15, G12 maltodextrin). The ratio of α -1,4,6- increased as the ratio of α -1,6- increased. Products obtained by the cell suspension method had lower yields than those obtained by the culture medium method (Figure 3.3), resulting in a higher ratio of α -1,4 linked residues and less α -1,6 linked residues. Thus G19 maltodextrin was the best substrate giving the highest content of α -1,6-D-glucosyl residues in the oligodextran product. Thus the culture

medium method is a good method giving high oligodextran yield with high content of α -1,6- whereas the cell suspension method seems to be a good alternative method for retrieving high purity oligodextran products.

Table 3.2 Effect of maltodextrin type on the molar ratio of linkages in oligodextrans made using the cell suspension method, as determined by methylation analysis

Linkage type	Maltodextrin (DE)						
	G12	G15	G19	G20	G29	G30	G37
t-Glc	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1,6	2.73	3.26	3.76	3.69	2.45	2.58	1.37
1,4	4.36	3.87	3.49	3.40	2.23	2.25	1.53
1,4,6	0.653	0.65	0.77	0.73	0.53	0.50	0.31

t-Glc: terminal glucose

2.2.2 ^1H -NMR analysis

Since the G19 and G20 maltodextrins gave the highest ratios of α -1,6-linked residues relative to terminal glucose by methylation analysis, they were studied by ^1H -NMR analysis. G19 and G20 maltodextrins were found to contain mostly α -1,4-D-glucosidic linkages and had an average degree of polymerization (DP) of 6-7 glucose residues. Oligodextrans obtained from both maltodextrins had similar linkage ratios, however, the oligodextran derived from G19 maltodextrin had slightly higher content of α -1,6-D-glucosidic residues (Table 3.3). The ratios of α -1,4- and α -1,6- were similar to those obtained by methylation analysis. The chemical structure of G19 oligodextran elucidated by NMR was proposed to contain 44 α -1,4-D-glucosidic residues and 49 α -1,6-D-glucosidic residues whereas G20 oligodextran contained 41 α -1,4-D-glucosidic residues and 45 α -1,6-D-glucosidic residues. This suggests that the oligodextran product had at least 86 glucose residues in total.

Table 3.3 Effect of maltodextrin type on the molar ratio of linkages in oligodextrans made using the culture medium method as determined by $^1\text{H-NMR}$

Linkage type	Goldex20		Glucidex19	
	t=0	t=48	t=0	t=48
t-Glc	1.00	1.00	1.00	1.00
1,6	0	44.91	0	48.60
1,4	6.73	40.57	7.39	43.83

t-Glc: terminal glucose

The linkage composition of the dextran was, however, largely independent of substrate chain length and contained an average of 83 mol% α -1,6-, 8 mol% α -1,4-, 6 mol% terminal-glucose and 3 mol% α -1,4,6-D-glucose linkages (Yamamoto *et al.*, 1994). Oligodextran produced in this study contained 48% α -1,6-, 40% α -1,4- and 11% α -1,4,6-D-glucose linkages, consistent with the use of maltodextrin substrate with a DP higher than 28 (Sims *et al.*, 2001). NMR analysis confirmed the linkage composition in the oligodextran products and showed that the oligodextran had a DP of at least 86 glucose residues, almost half of which were linked by α -1,4-linkages and half by α -1,6-D- linkages.

3. Pilot-scale production and recovery of oligodextran

3.1 Production of oligodextran

Production of oligodextran by the cell suspension method required two steps, cell production and product synthesis.

3.1.1 Cell production

The inoculum was prepared by cultivation of *G. oxydans* NCIMB 4943 in a batch fermenter (10-l) under controlled pH (6.8) conditions and this was used as inoculum for cell production in a pilot-scale fermenter (150-l). Growth of *G. oxydans* NCIMB 4943 reached a maximum at 24 h giving a dry cell weight yield of 0.52 g l⁻¹ (Figure 3.14).

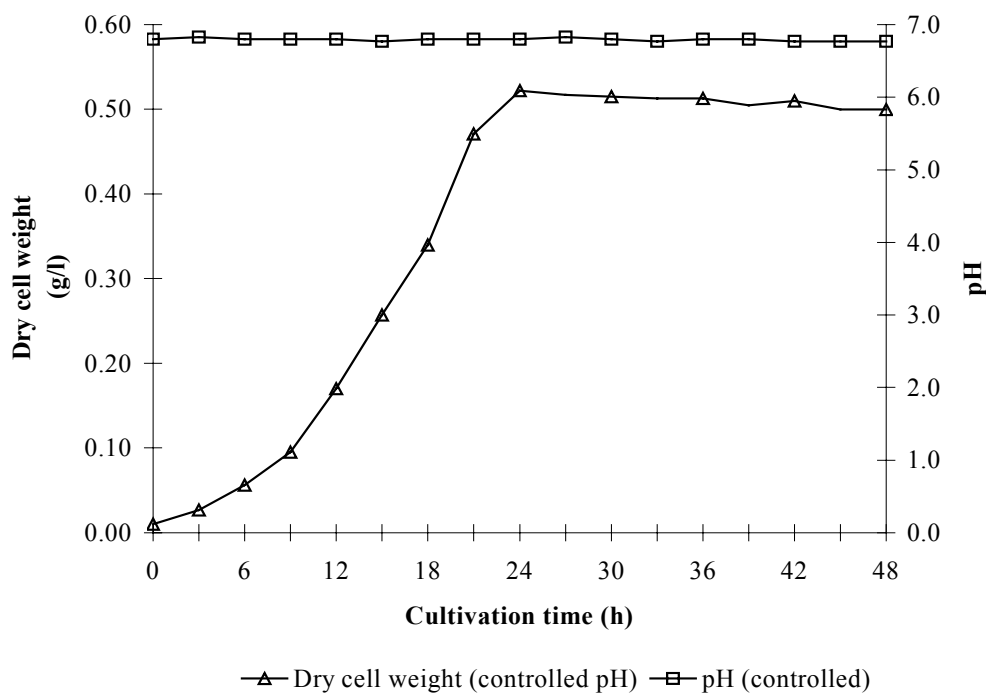


Figure 3.14 Growth of *G. oxydans* NCIMB 4943 in GY medium under controlled pH (6.8) conditions in a pilot-scale fermenter (150-l, agitation speed of 150 rpm, 30°C)

3.1.2 Product synthesis

G. oxydans cells were used in the pilot-scale (150-l) fermenter for oligodextran synthesis. The oligodextran product yield was similar to that obtained in shake-flask culture, giving a maximum value of 22.21% at 48 h cultivation (Figure 3.15). In addition, the molecular weight distribution of the product was also exactly the same as that produced in shake-flask batch cultures (7.8-65.6 kDa, Figure 3.16). This indicated that production of oligodextran was possible in pilot-scale however difficulties were experienced with the filter sterilization and cell recovery steps. These problems could be overcome by using the cell suspension method that requires only one step and steam sterilization could replace filter sterilization.

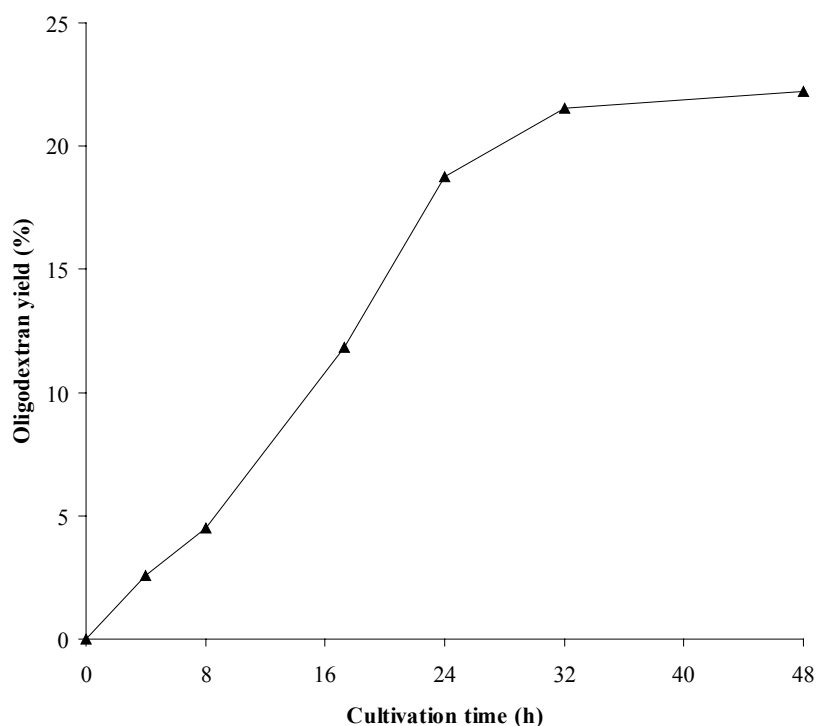


Figure 3.15 Production of oligodextran by *G. oxydans* NCIMB 4943 in a pilot-scale fermenter (150-l, agitation speed of 150 rpm, 30°C) using the cell suspension method containing 5 g l⁻¹ cells and 10 g l⁻¹ G19 maltodextrin dissolved in sodium acetate buffer (pH 4.5)

3.2 Recovery of oligodextrans

Recovery of oligodextrans produced from the pilot-scale fermenter was performed using a pilot-scale ultrafiltration system. The first trial was performed using a membrane with a nominal molecular weight cut off of 10 kDa, and the molecular weight distribution of the retentate and permeate fractions were determined by HPSEC. The retentate fraction contained both oligodextran product and a large proportion of low molecular weight maltodextrin residue. Consequently a membrane with a nominal molecular weight cut off of 25 kDa was tested. This membrane successfully separated low molecular weight maltodextrin residues from the higher molecular weight oligodextran products. Cell-free viscous culture supernatant obtained from different types of maltodextrin substrate had an influence on the

efficiency of separation. Highly viscous samples, particularly those produced from G20 maltodextrin using the culture method, gave the lowest permeate flux ($13.2 \text{ l m}^{-2} \text{ h}^{-1}$) whereas the highest permeate flux ($30.6 \text{ l m}^{-2} \text{ h}^{-1}$) was obtained with samples produced by the cell suspension method using the same maltodextrin type (Table 3.4).

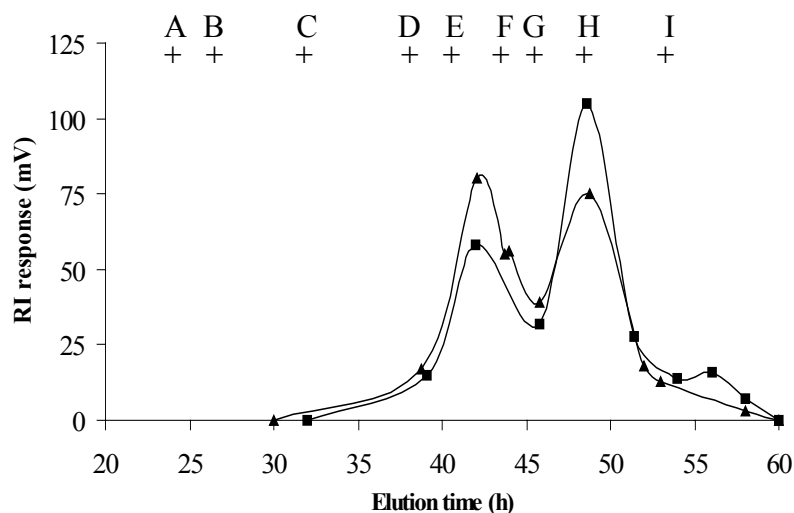


Fig. 3.16 Molecular weight distributions of maltodextrin substrate and oligodextran formed by *G. oxydans* NCIMB 4943 cultivated for 48 h in a pilot-scale fermenter (150-l, 150 rpm, 30°C) using the cell suspension method containing 5 g l^{-1} *G. oxydans* cells and 10 g l^{-1} G19 maltodextrin dissolved in sodium acetate buffer (pH 4.5)

■ G19 maltodextrin substrate; ▲ Oligodextran product

Crosses (+) labeled with the letter A, B, ..., I, represent the elution time at peak height of the standards used to calibrate the chromatographic system. A: dextran 38000 kDa for V_0 ; B: dextran 2000 kDa; C: dextran 464 kDa; D: dextran 68.4 kDa; E: dextran 42 kDa; F: dextran 19.5 kDa; G: dextran 9.5 kDa; H: maltoheptaose 1.15 kDa; I: glucose 0.18 kDa

Table 3.4 Permeate flux of cell-free culture sample produced from various maltodextrin types obtained by pilot-scale ultrafiltration

Sample	Permeate flux ($\text{l m}^{-2} \text{h}^{-1}$)
G19 maltodextrin*	23.4
G20 maltodextrin*	30.6
G20 maltodextrin**	13.2
G29 maltodextrin*	28.8
G37 maltodextrin*	27.6

* produced by cell suspension method

** produced by culture medium method

4. Studies on the prebiotic properties of oligodextran

4.1 Stability of oligodextran and maltodextrin to acid

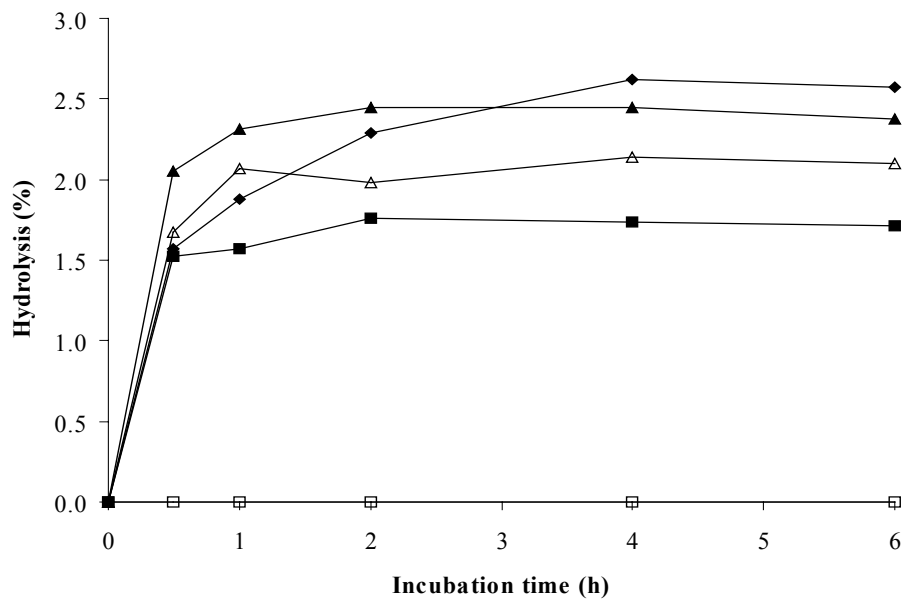
4.1.1 HCl

4.1.1.1 G19 and G20 oligodextran

The aim to use HCl was to mimic the strong acid condition in human stomach in case stomach was empty. Hydrolysis of G19 oligodextran at pH 1-5 reached equilibrium at 2 h incubation except pH 1, which reached equilibrium at 4 h and gave the maximum hydrolysis of 2.62%. No hydrolysis occurred at pH 5 while less than 3.0% of G19 oligodextran was hydrolyzed by HCl at all pH tested. Percentages of hydrolysis at pH 1, 2, 3, and 4 were 2.62, 2.45, 2.14 and 1.74%, respectively incubated for 4 h (Figure 3.17a).

G20 oligodextran was found to resist HCl acid hydrolysis at various pH (1-5) and less than 2.5% was hydrolyzed within 6 h. The lower pH of HCl gave higher hydrolysis and no hydrolysis occurred at pH 5. Acid hydrolysis by HCl occurred rapidly within 1 h and reached equilibrium after 2 h at pH 4. Percentages of hydrolysis at pH 1, 2, 3 and 4 were 1.86, 1.90, 1.82 and 1.44%, respectively at 2 h (Figure 3.17b).

a)



b)

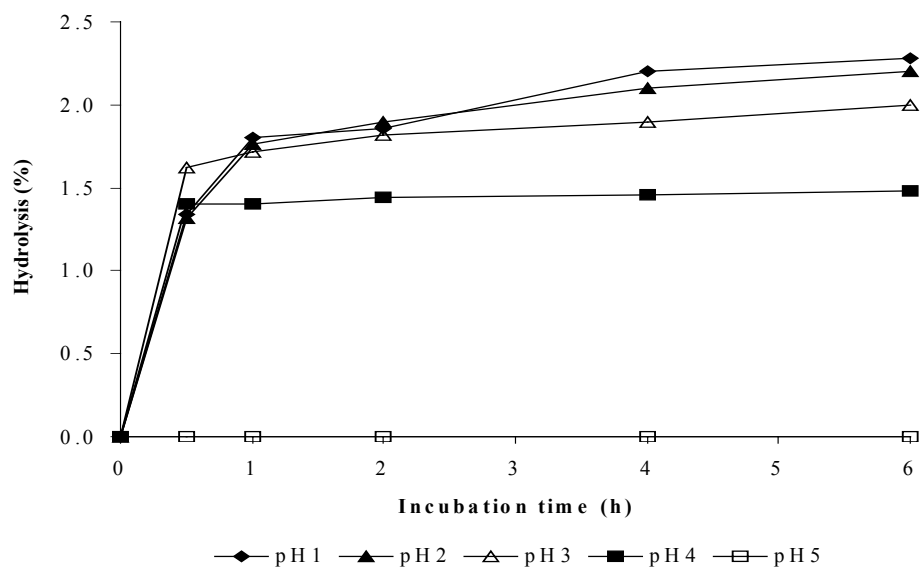


Fig. 3.17 Susceptibility of G19 oligodextran (a) and G20 oligodextran (b) on HCl (pH1-5) incubated for 6 h, at 37°C

◆ pH 1; ▲ pH 2; △ pH 3; ■ pH 4; □ pH 5

4.1.1.2 G19 and G20 maltodextrins

Rapid hydrolysis of G19 maltodextrin by HCl occurred within 30 min incubation and reached equilibrium at 2 h. The hydrolysis rate at pH 3 and 4 dramatically reduced after 30 min and was constant thereafter at approximately 3% hydrolysis. The maximum hydrolysis (4.20%) was obtained at pH 2 with incubation time of 4 h. Percentages hydrolysis at pH 1, 2, 3, and 4 were 4.12, 3.92, 3.04 and 2.92%, respectively at 2 h (Figure 3.18a).

G20 maltodextrin showed higher hydrolysis, as expected, compared to G20 oligodextran at the same pH tested. This indicated the higher acid stability of 1→6 linkages relative to 1→4. The maximum hydrolysis was 5.12% obtained at pH 1 incubated for 2 h. Equilibrium was obtained after 2 h incubation for all pH tested. This confirmed that maltodextrins, particularly G19 and G20 maltodextrin had lower acid resistance compared to the oligodextrans produced from them. Percentages of hydrolysis at pH 1, 2, 3 and 4 were 5.12, 4.48, 3.72 and 2.64%, respectively incubated for 2 h (Figure 3.18b).

4.1.2 HCl buffer

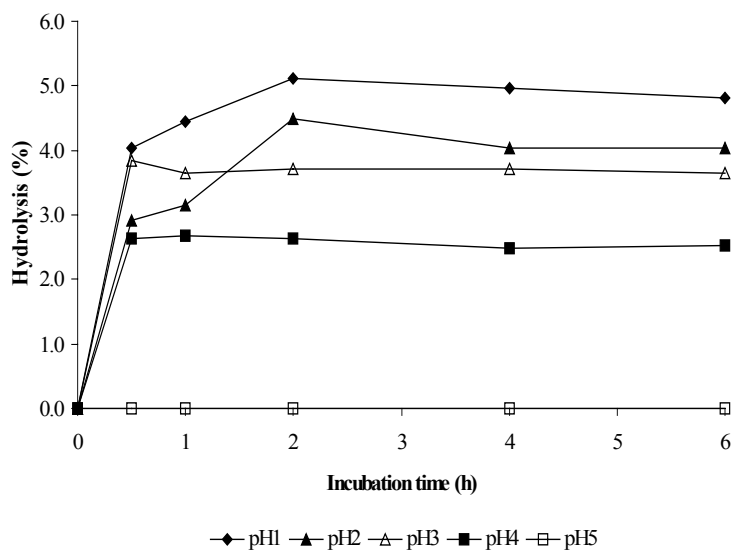
4.1.2.1 G19 and G20 oligodextrans

HCl buffer was used to stimulate acidic condition in human stomach in normal situation. No hydrolysis of either G19 or G20 oligodextran was seen in HCl buffer. HCl buffer contained similar components to the acidic contents of the stomach of a healthy human with a typical pH of 2-4. Hence, this result confirmed that these carbohydrate samples would not be hydrolyzed by the acidic condition in the stomach.

4.1.2.2 G19 and G20 maltodextrins

Hydrolysis of G19 and G20 maltodextrins by HCl buffer increased rapidly after 30 min incubation and reached equilibrium after 1 h incubation. Both samples gave similar hydrolysis values of less than 2.0% (Figure 3.19).

a)



b)

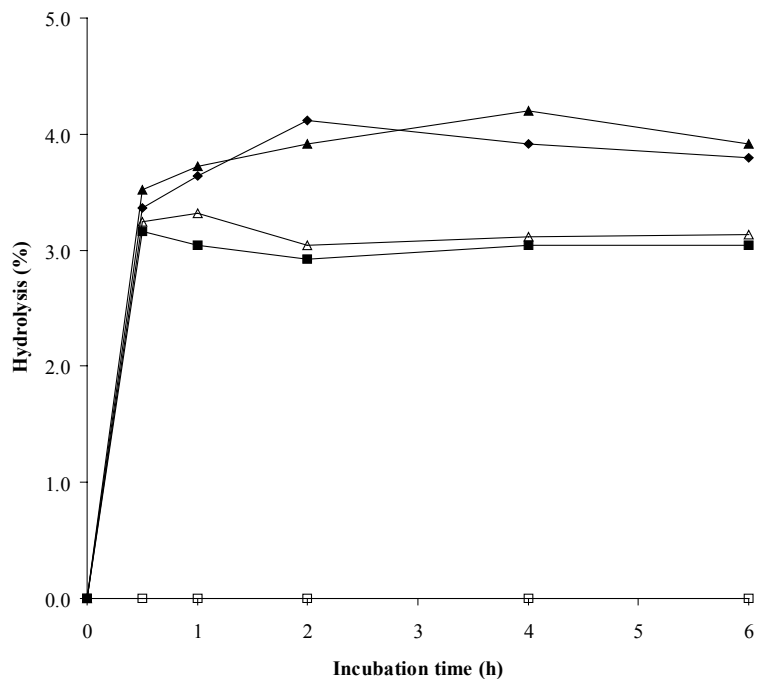


Figure 3.18 Susceptibility of G19 maltodextrin (a) and G20 maltodextrin (b) to HCl (pH1-5) incubated for 6 h, at 37°C

◆ pH 1; ▲ pH 2; △ pH 3; ■ pH 4; □ pH 5

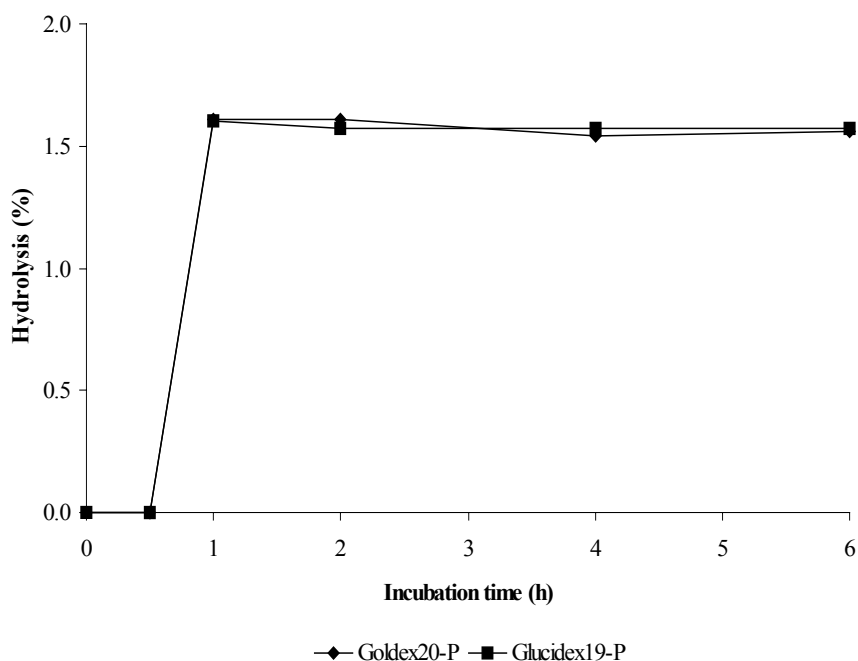


Figure 3.19 Susceptibility of G19 oligodextran to HCl buffer (pH 1) incubated for 6 h, at 37°C

▲ G19 maltodextrin, ■ G20 maltodextrin

4.2 Susceptibility of oligodextran to enzymatic digestion

4.2.1 Effect of sources of amylase

4.2.1.1 α -amylase from human saliva

A. G19 oligodextran

The susceptibility of G19 oligodextran to human salivary amylase at pH 4-8 and an enzyme concentration of 1-2 U ml⁻¹ was studied. It was found that higher pH and higher enzyme concentration gave higher degree of hydrolysis. The maximum hydrolysis was obtained at pH 8 and 4 h incubation. A high rate of hydrolysis was observed at 2 h, reaching equilibrium at 4 h. No significance difference in hydrolysis was seen between enzyme concentrations of 1 and 2 U ml⁻¹. Percentages of hydrolysis using human salivary amylase of 2 U ml⁻¹ at pH 4, 5, 6, 7 and 8 were 7.94, 10.36, 12.44, 13.60 and 14.23%, respectively at 4 h (Figure 3.20). The maximum hydrolysis

(14.23%) of G19 oligodextran by human salivary amylase was achieved using 2 U ml⁻¹, pH 8 and 4 h incubation.

A contour plot of the degree of digestion of G19 oligodextran by human salivary amylase is shown in Figure 3.21. It was found that the optimal conditions for the highest hydrolysis were at pH of 7.86 and incubation time of 4.33 h. The predicted maximum hydrolysis (18.26%) was obtained as shown in three dimension response surface.

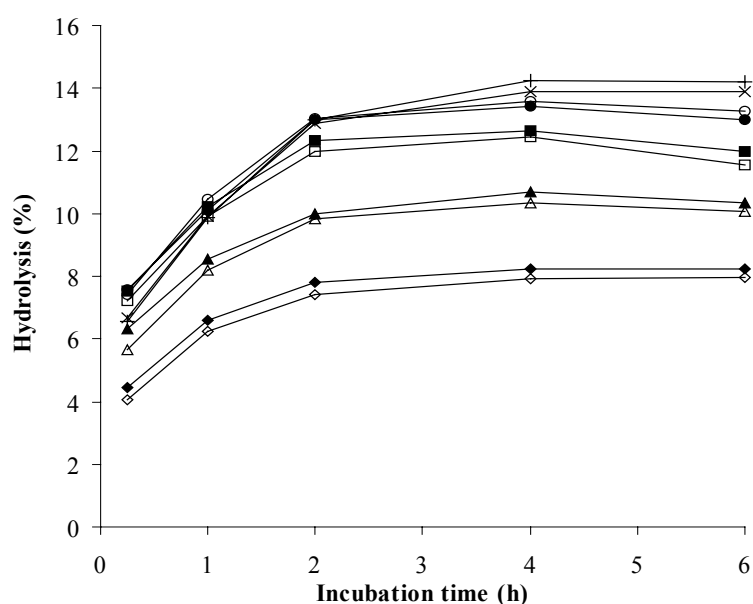


Figure 3.20 Susceptibility of G19 oligodextran to human salivary amylase at pH 4-8,

1-2 U ml⁻¹ enzyme, incubated at 37°C for 6 h

◆, pH 4, 1 U ml⁻¹; ◇, pH 4, 2 U ml⁻¹; ▲, pH 5, 1 U ml⁻¹; △, pH 5, 2 U ml⁻¹; ■, pH 6, 1 U ml⁻¹; □, pH 6, 2 U ml⁻¹; ○, pH 7, 1 U ml⁻¹; ●, pH 7, 2 U ml⁻¹; ×, pH 8, 1 U ml⁻¹; +, pH 8, 2 U ml⁻¹

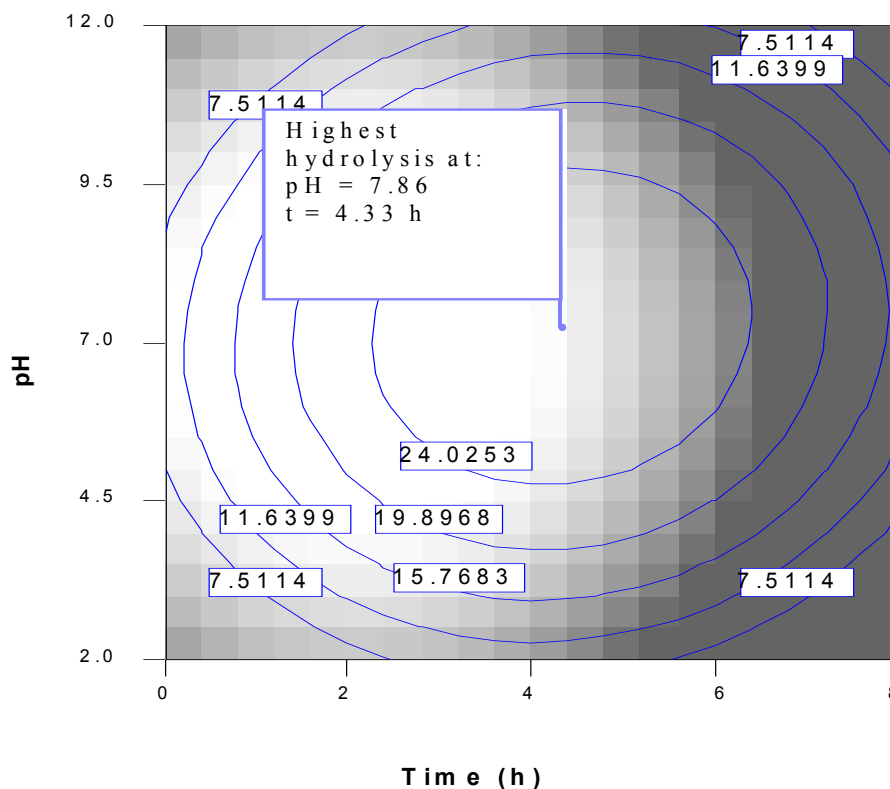


Figure 3.21 Contour plot of susceptibility of G19 oligodextran to human salivary amylase as a function of pH and time, 1-2 U ml⁻¹ enzyme, incubated at 37°C

B. G20 oligodextran

The percentage of hydrolysis of G20 oligodextran by human salivary amylase (1-2 U ml⁻¹) at pH 4-8 was less than 14% (Figure 3.22). Equilibrium was reached after 2 h incubation for all pH values tested except pH 8, which reached equilibrium at 4 h. Percentages of hydrolysis of G20 oligodextran using 2 U ml⁻¹ enzyme, incubated for 4 h at pH 4, 5, 6, 7 and 8 were 6.44, 9.41, 11.29, 13.28 and 14.13%, respectively. Percentages of hydrolysis of G20 oligodextran was similar to G19 oligodextran. This means G20 and G19 oligodextrans had similar susceptibility to human salivary amylase.

A contour plot of degree of digestion of G20 oligodextran by human salivary amylase is shown in Figure 3.23. It was found that the optimal conditions for the highest hydrolysis were at pH 7.93 and incubation time of 3.34 h.

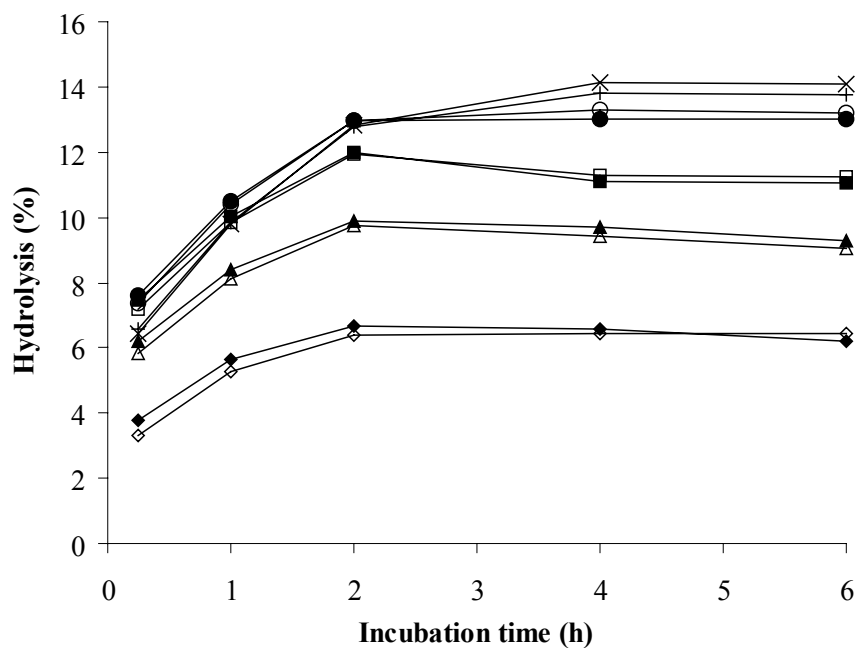


Figure 3.22 Susceptibility of G20 oligodextran to human salivary amylase at pH 4-8, 1-2 U ml⁻¹ of enzyme, incubated at 37°C for 6 h
 ◆, pH 4, 1 U ml⁻¹; ◇, pH 4, 2 U ml⁻¹; ▲, pH 5, 1 U ml⁻¹; △, pH 5, 2 U ml⁻¹; ■, pH 6, 1 U ml⁻¹; □, pH 6, 2 U ml⁻¹; ○, pH 7, 1 U ml⁻¹; ●, pH 7, 2 U ml⁻¹; ×, pH 8, 1 U ml⁻¹; +, pH 8, 2 U ml⁻¹

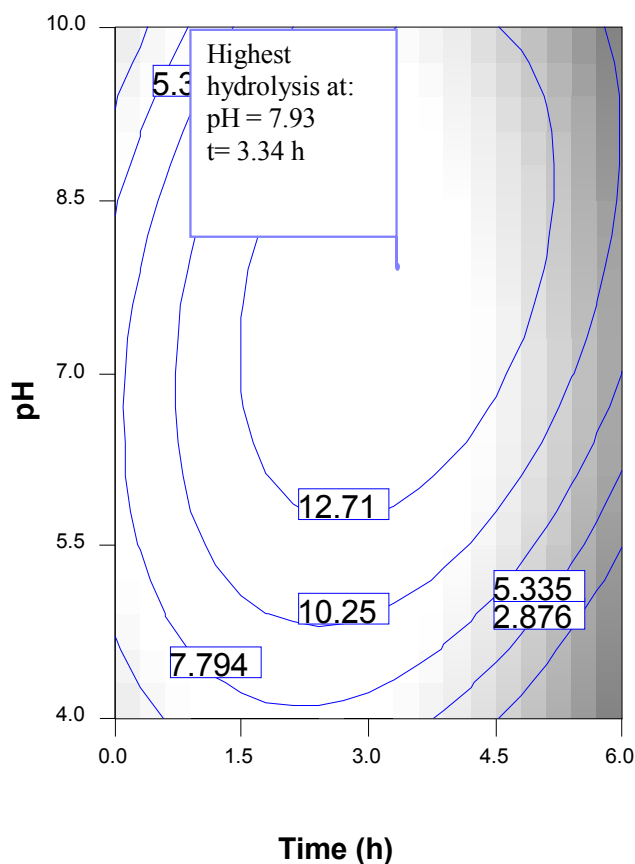


Figure 3.23 Contour plot of susceptibility of G20 oligodextran to human salivary amylase as a function of pH and time using 1-2 U ml⁻¹ enzyme and incubated at 37°C

C. G20 maltodextrin

Hydrolysis equilibrium was reached after 1 h for all pH values tested except for pH 8 which reached equilibrium at 2 h incubation. Percentages of hydrolysis of G20 maltodextrin using 2 U ml⁻¹ enzyme and 2 h incubation at pH 4, 5, 6, 7 and 8 were 14.70, 26.2, 32.25, 35.35 and 33.06%, respectively (Figure 3.24). The percentage of hydrolysis of G20 maltodextrin by human salivary amylase at pH 4-8 and 1-2 U ml⁻¹ enzyme was higher than those of G19 and G20 oligodextran and the maximum hydrolysis was 35%.

A contour plot of digestion of G20 maltodextrin by human salivary amylase is shown in Figure 3.25. Optimal conditions for the highest hydrolysis were found to be

pH of 6.88 and incubation time of 1.63 h. This confirmed that oligodextran were more resistant to amylase digestion than the maltodextrin which they were made from.

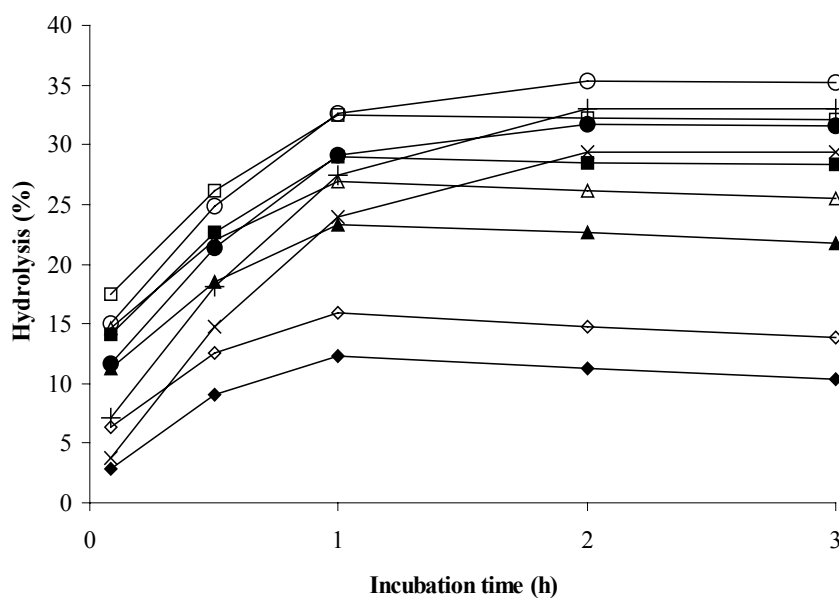


Figure 3.24 Susceptibility of G20 maltodextrin to human salivary amylase at pH 4-8, 1-2 U ml⁻¹ of enzyme, incubated at 37°C for 3 h

◆, pH 4, 1 U ml⁻¹; ◇, pH 4, 2 U ml⁻¹; ▲, pH 5, 1 U ml⁻¹; △, pH 5, 2 U ml⁻¹; ■, pH 6, 1 U ml⁻¹; □, pH 6, 2 U ml⁻¹; ○, pH 7, 1 U ml⁻¹; ●, pH 7, 2 U ml⁻¹; ×, pH 8, 1 U ml⁻¹; +, pH 8, 2 U ml⁻¹

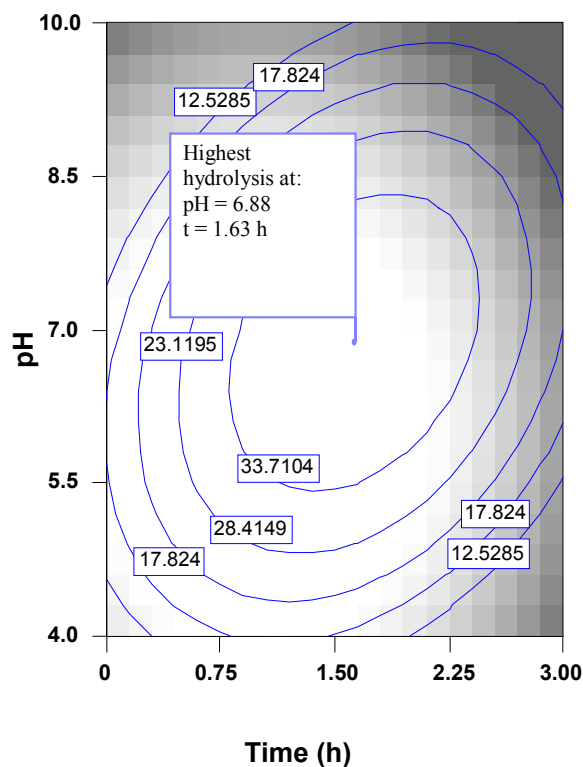


Figure 3.25 Contour plot of susceptibility of G20 maltodextrin to human salivary amylase at various pH and times using 1-2 U ml⁻¹ enzyme and incubation at 37°C

4.2.1.2 α -amylase from human pancreas

Digestion of G19 oligodextran by human pancreatic amylase at pH 4-8 and 1-2 U ml⁻¹ enzyme gave the maximum hydrolysis of 23.6% at pH 7 and 1 U ml⁻¹ enzyme. Rapid hydrolysis was found at 1 h and reached equilibrium after 2 h except pH 7 and 8 which reached equilibrium after 4 h. Percentages of hydrolysis of G19 oligodextran by human pancreatic amylase using 2 U ml⁻¹ enzyme at pH 4, 5, 6, 7 and 8 were 6.8, 10.6, 15.8, 22.4 and 23.0%, respectively (Figure 3.26).

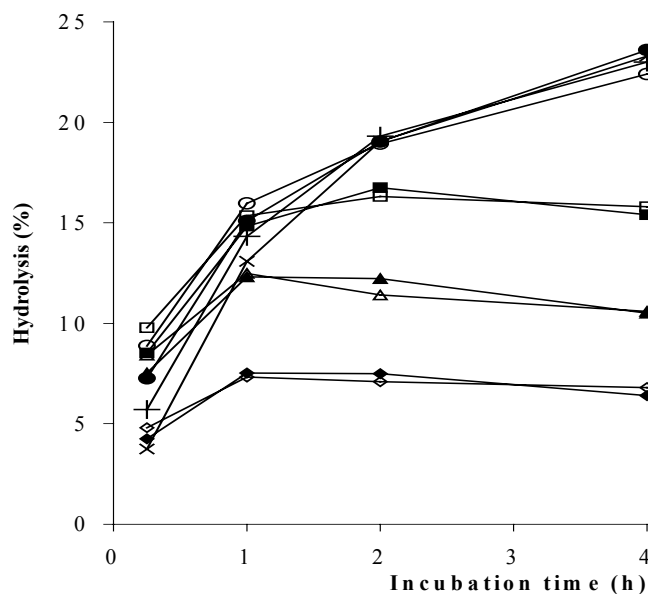


Figure 3.26 Susceptibility of G19 oligodextrans to human pancreatic amylase at pH 4-8 using 1-2 U ml⁻¹ enzyme and incubation at 37°C for 6 h
 ◆, pH 4, 1 U ml⁻¹; ◇, pH 4, 2 U ml⁻¹; ▲, pH 5, 1 U ml⁻¹; △, pH 5, 2 U ml⁻¹; ■, pH 6, 1 U ml⁻¹; □, pH 6, 2 U ml⁻¹; ○, pH 7, 1 U ml⁻¹; ●, pH 7, 2 U ml⁻¹; ×, pH 8, 1 U ml⁻¹; +, pH 8, 2 U ml⁻¹

A contour plot of digestion of G19 oligodextrans by human pancreatic amylase is shown in Figure 3.27. The maximum hydrolysis was anticipated to be at pH 7.73 and incubation time of 2.17 h. α -amylase from human pancreas resulted in a higher degree of hydrolysis of G19 oligodextrans compared to α -amylase from human saliva.

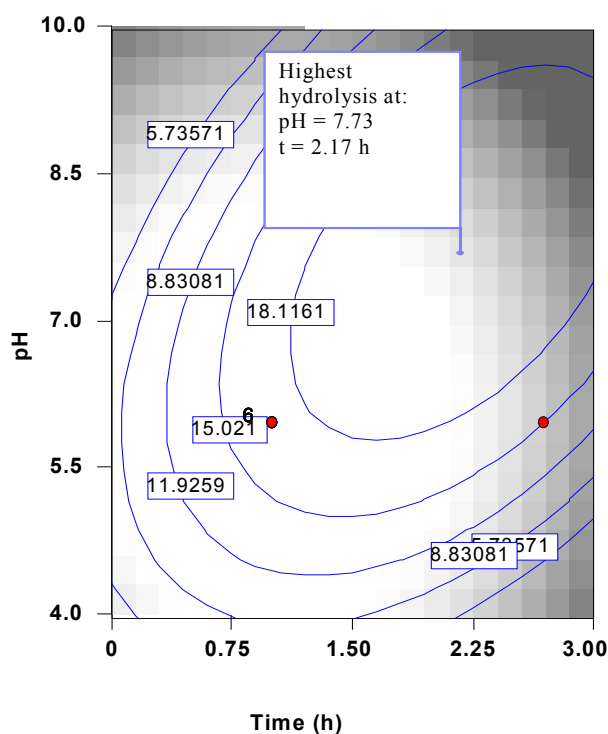


Figure 3.27 Contour plot of susceptibility of G19 oligodextran to human pancreatic amylase as a function of pH and time using 1-2 U ml⁻¹ enzyme and incubation at 37°C

4.2.1.3 α -amylase from *Aspergillus oryzae*

A. G20 oligodextran

The aim of using α -amylase from other sources beside human origin (saliva and pancreas) is to evaluate their resistance to enzymes which might be present in food applications of these oligodextrans such as baking processing. The digestion rate of G20 oligodextran by α -amylase from *A. oryzae* was lower than by enzymes of human origin. The maximum hydrolysis of G20 oligodextran by α -amylase from *A. oryzae* was 16.03% using 2 U ml⁻¹ enzyme at pH 6 and incubation time of 4 h. The hydrolysis was constant after 4 h incubation. Percentages of hydrolysis at 2 U ml⁻¹ enzyme, incubated for 4 h at pH 4, 5, 6, 7 and 8 were 13.84, 15.39, 16.03, 15.77 and 14.60%, respectively (Figure 3.28).

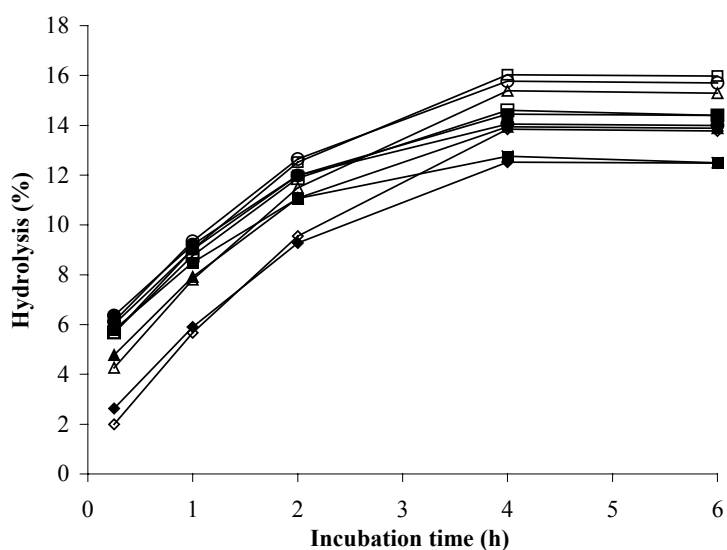


Figure 3.28 Susceptibility of G20 oligodextrans to α -amylase from *A. oryzae* at pH 4-8 using 1-2 U ml⁻¹ enzyme and incubation at 37°C for 6 h

◆, pH 4, 1 U ml⁻¹; ◇, pH 4, 2 U ml⁻¹; ▲, pH 5, 1 U ml⁻¹; △, pH 5, 2 U ml⁻¹; ■, pH 6, 1 U ml⁻¹; □, pH 6, 2 U ml⁻¹; ○, pH 7, 1 U ml⁻¹; ●, pH 7, 2 U ml⁻¹; ×, pH 8, 1 U ml⁻¹; +, pH 8, 2 U ml⁻¹

A contour plot of digestibility of G20 oligodextrans by α -amylase from *A. oryzae* is shown in Figure 3.29. The maximum hydrolysis was predicted to be at pH 6.10 and incubation time of 4.47 h giving the maximum value of 16.20%. The maximum hydrolysis of 16.20% from using α -amylase of *A. oryzae* was lower than the digestibility of G20 oligodextrans using the enzyme from human origin.

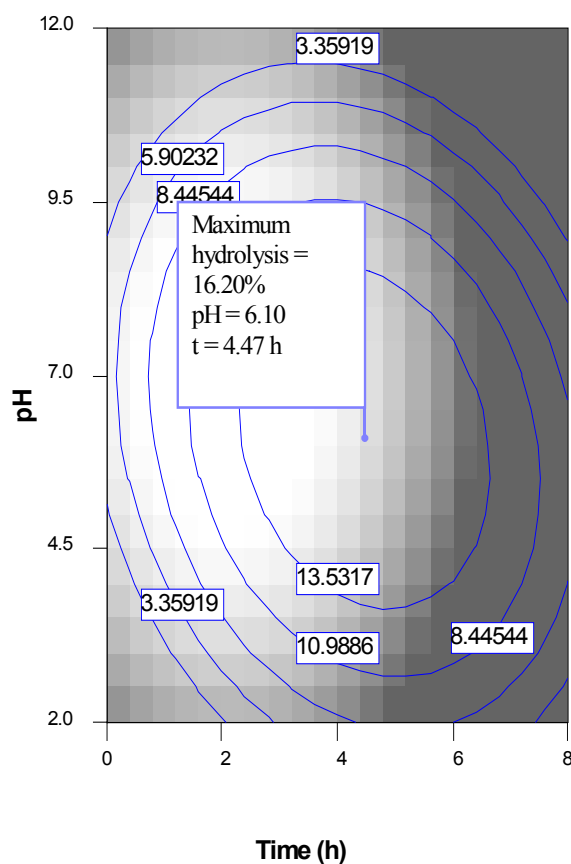


Figure 3.29 Contour plot of susceptibility of G20 oligodextran to α -amylase from *A. oryzae* as a function of pH and time using 1-2 U ml⁻¹ enzyme and incubation at 37°C

B. G20 maltodextrin

The susceptibility of G20 maltodextrin to α -amylase from *A. oryzae* was studied. It was found that the hydrolysis equilibrium was reached at a shorter incubation time (2 h) compared to G20 oligodextran (4 h). So that G20 oligodextran was more susceptible than G20 maltodextrin. The percentage of hydrolysis was also higher than those obtained from G20 oligodextran. The maximum hydrolysis was found at pH of 6, 2 U ml⁻¹ enzyme giving the value of 29.05%. Percentages of hydrolysis at 2 U ml⁻¹ enzyme, incubated for 2 h at pH 4, 5, 6, 7 and 8 were 25.24, 28.02, 29.05, 28.33 and 25.85%, respectively (Figure 3.30).

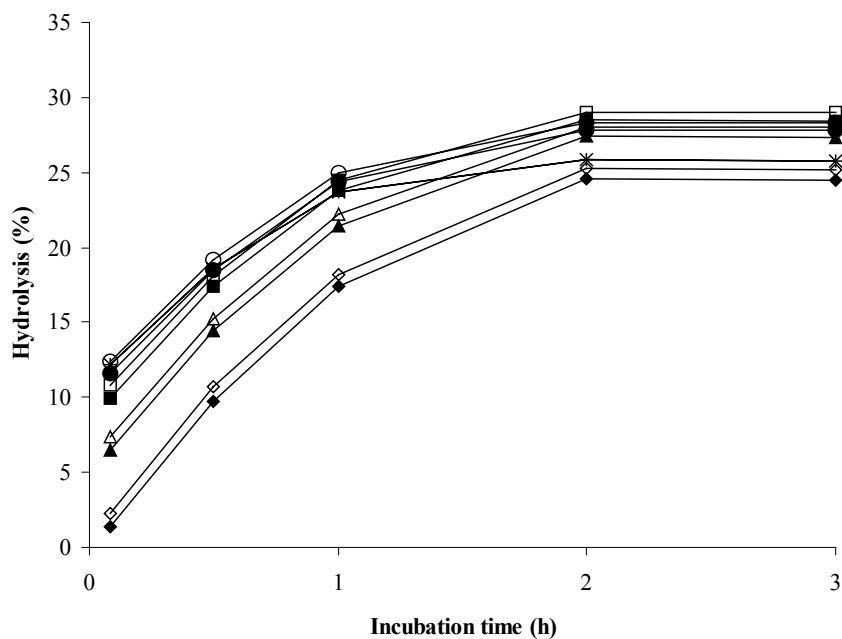


Figure 3.30 Susceptibility of G20 maltodextrin to α -amylase from *A. oryzae* at pH 4-8 using 1-2 U ml⁻¹ of enzyme and incubation at 37°C for 3 h
 ◆, pH 4, 1 U ml⁻¹; ◇, pH 4, 2 U ml⁻¹; ▲, pH 5, 1 U ml⁻¹; △, pH 5, 2 U ml⁻¹; ■, pH 6, 1 U ml⁻¹; □, pH 6, 2 U ml⁻¹; ○, pH 7, 1 U ml⁻¹; ●, pH 7, 2 U ml⁻¹; ×, pH 8, 1 U ml⁻¹; +, pH 8, 2 U ml⁻¹

A contour plot of digestibility of G20 maltodextrin by α -amylase from *A. oryzae* is shown in Figure 3.31. The maximum hydrolysis was anticipated to be 29.44% at pH 6.16 and incubated for 1.91 h. Optimal pH for α -amylase from *A. oryzae* on hydrolysis of either G20 oligodextran or G20 maltodextrin was in slightly acidic condition (pH 6.1).

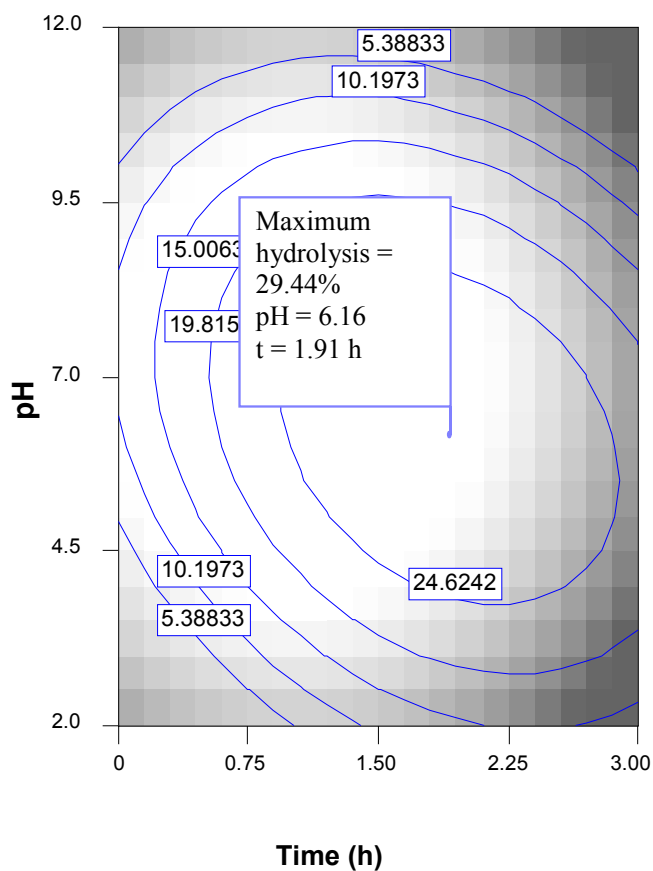


Figure 3.31 Contour plot of susceptibility of G20 maltodextrin to α -amylase from *A. oryzae* as a function of pH and time using 1-2 U ml⁻¹ enzyme and incubation at 37°C

4.2.1.4 α -amylase from *Bacillus licheniformis*

A. G20 oligodextran

The susceptibility of G20 oligodextran to α -amylase from *B. licheniformis* is shown in Figure 3.32. It was found that a high rate of hydrolysis was obtained within the first 2 h and equilibrium was reached after 4 h. The maximum hydrolysis was found at pH 7, 2 U ml⁻¹ enzyme, giving the value of 16.3%. Percentages of hydrolysis at 2 U ml⁻¹ enzyme, incubated for 4 h at pH 4, 5, 6, 7 and 8 were 9.86, 13.15, 15.30, 16.30 and 16.16%, respectively. The percentage of hydrolysis was lower than those obtained with enzymes of human origin.

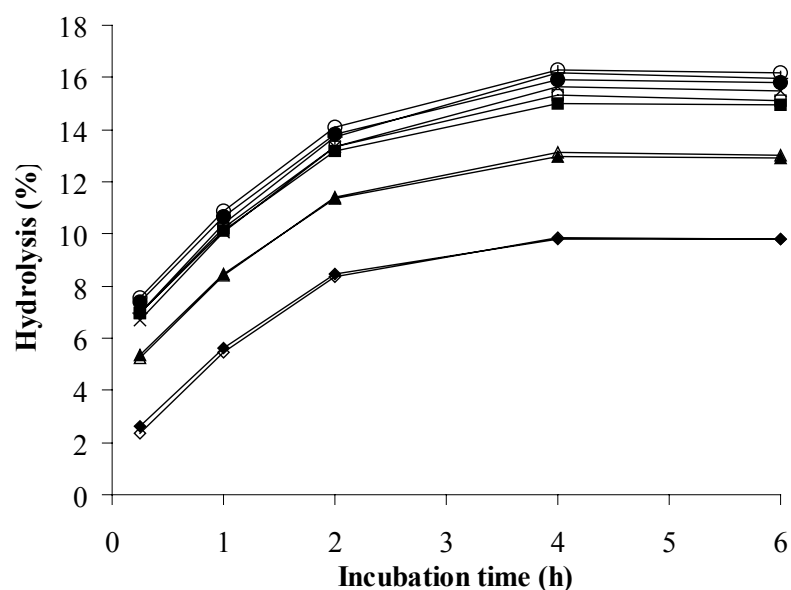


Fig. 3.32 Susceptibility of G20 oligodextran to α -amylase from *B. licheniformis* at pH 4-8 using 1-2 U ml⁻¹ enzyme and incubation at 37 °C for 6 h
 ◆ , pH 4, 1 U ml⁻¹; ◇ , pH 4, 2 U ml⁻¹; ▲ , pH 5, 1 U ml⁻¹; △ , pH 5, 2 U ml⁻¹; ■ , pH 6, 1 U ml⁻¹; □ , pH 6, 2 U ml⁻¹; ○ , pH 7, 1 U ml⁻¹; ● , pH 7, 2 U ml⁻¹; × , pH 8, 1 U ml⁻¹; + , pH 8, 2 U ml⁻¹

A contour plot of digestion of G20 oligodextran by α -amylase from *B. licheniformis* is shown in Figure 3.33. It was found that the conditions giving the maximum hydrolysis was predicted to be at pH 7.33 and incubation time of 3.80 h giving the value of 16.46%. Optimal pH for α -amylase from *B. licheniformis* on hydrolysis of G20 oligodextran was pH 7.33, similar to the enzymes of human origin.

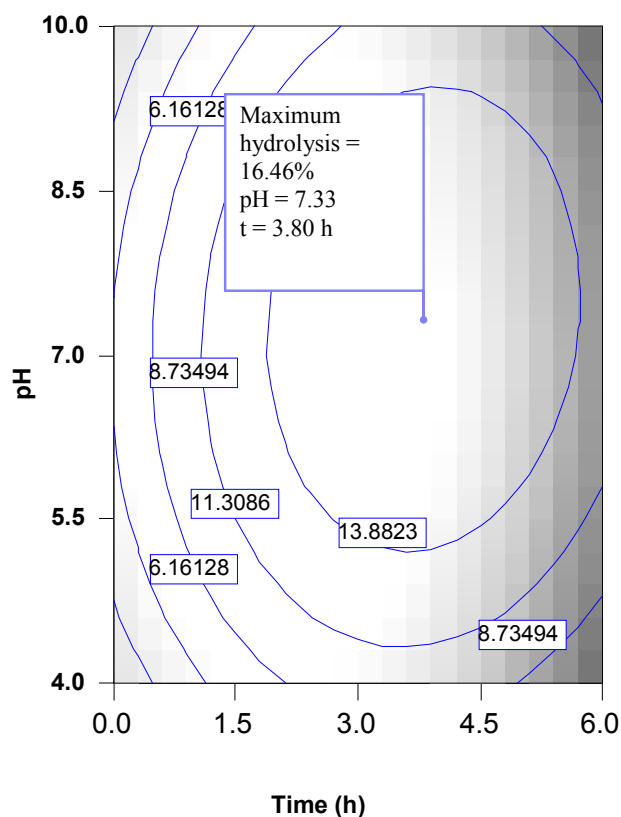


Figure 3.33 Contour plot of susceptibility of G20 oligodextran to α -amylase from *B. licheniformis* as a function of pH and time using 1-2 U ml⁻¹ enzyme and incubation at 37°C

B. G20 maltodextrin

The susceptibility of G20 maltodextrin to α -amylase from *B. licheniformis* is shown in Figure 3.34. It was found that a high rate of hydrolysis was found within the first 1 h and reached equilibrium after 2 h. The maximum hydrolysis was found at pH 7 using 2 U ml⁻¹ enzyme, giving the value of 26.47%. Percentages of hydrolysis at 2 U ml⁻¹ enzyme, incubated for 2 h at pH 4, 5, 6, 7 and 8 were 15.85, 22.03, 25.56, 26.47 and 24.74%, respectively. The percentage of hydrolysis was higher than that obtained with G20 oligodextran.

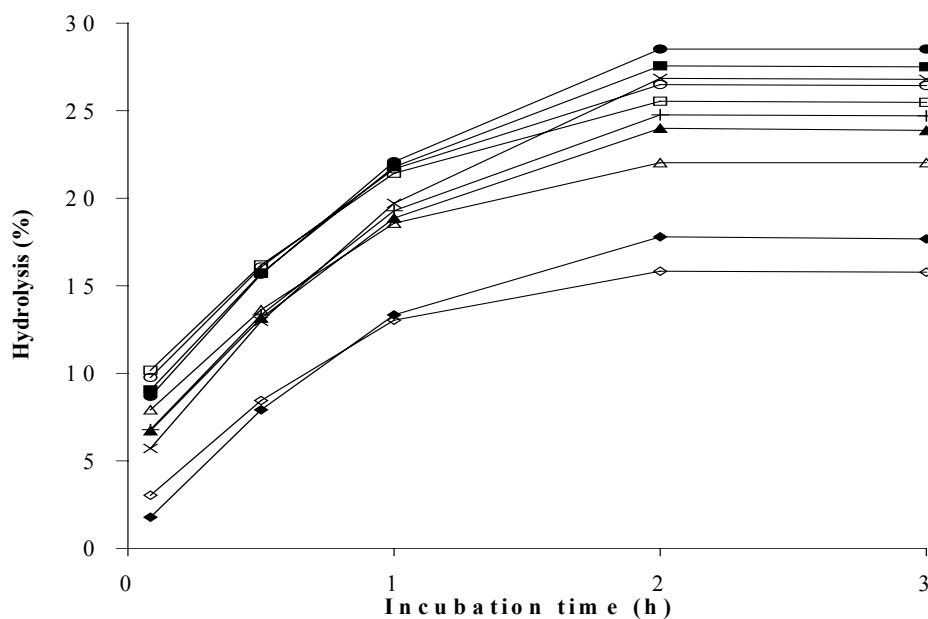


Figure 3.34 Susceptibility of G20 maltodextrin to α -amylase from *B. licheniformis* at pH 4-8 using 1-2 U ml⁻¹ enzyme and incubation at 37°C for 3 h
 ◆, pH 4, 1 U ml⁻¹; ◇, pH 4, 2 U ml⁻¹; ▲, pH 5, 1 U ml⁻¹; △, pH 5, 2 U ml⁻¹; ■, pH 6, 1 U ml⁻¹; □, pH 6, 2 U ml⁻¹; ○, pH 7, 1 U ml⁻¹; ●, pH 7, 2 U ml⁻¹; ×, pH 8, 1 U ml⁻¹; +, pH 8, 2 U ml⁻¹

A contour plot of digestibility of G20 maltodextrin by α -amylase from *B. licheniformis* is shown in Figure 3.35. It was found that the condition to give the maximum hydrolysis was predicted to be at pH 6.92 and incubation time of 2.26 h giving value of 28.82%. Optimal pH for α -amylase from *B. licheniformis* on hydrolysis of G20 maltodextrin was pH 6.92.

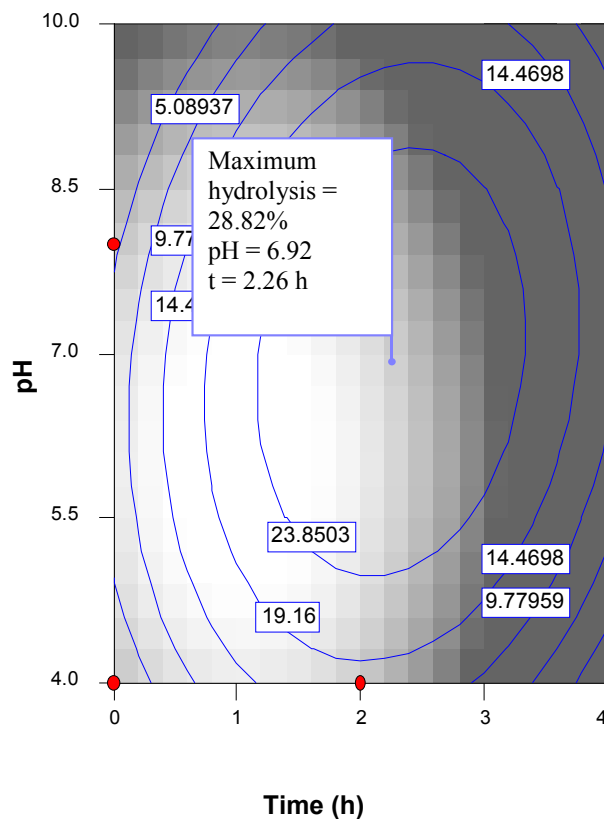


Figure 3.35 Contour plot of susceptibility of G20 maltodextrin to α -amylase from *B. licheniformis* as a function of pH and time using $1-2 \text{ U ml}^{-1}$ enzyme and incubation at 37°C

4.2.1.5 α -amylase from *Bacillus spp.*

A. G20 oligodextran

Susceptibility of G20 oligodextran to α -amylase from *Bacillus spp.* is shown in Figure 3.36. It was found that rapid hydrolysis was obtained in the first 2 h and reached equilibrium after 4 h. The maximum hydrolysis was found at pH 7 and 2 U ml^{-1} enzyme giving value of 10.49%. Percentages of hydrolysis using 2 U ml^{-1} enzyme, incubated for 4 h at pH 4, 5, 6, 7 and 8 were 7.16, 8.88, 9.99, 10.49 and 10.39%, respectively. The percentage of hydrolysis was lower than those obtained with enzymes from human sources.

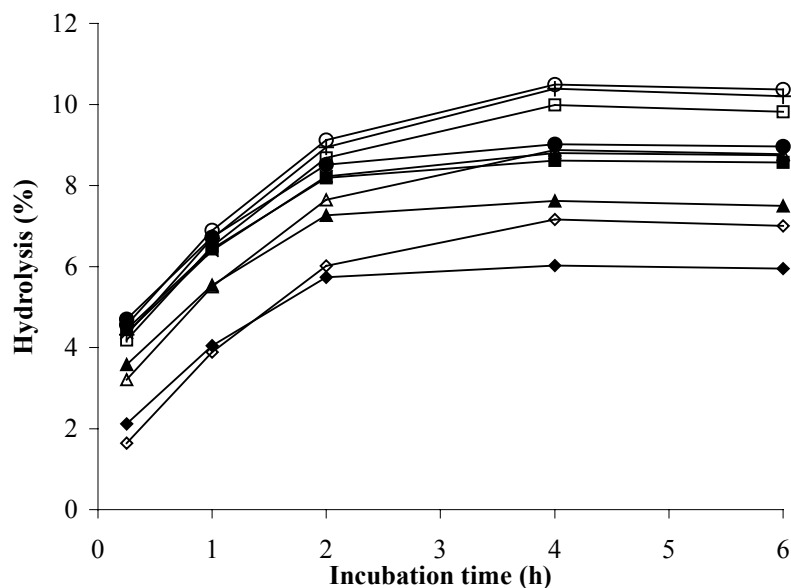


Figure 3.36 Susceptibility of G20 oligodextran to α -amylase from *Bacillus spp.* at pH 4-8 using 1-2 U ml⁻¹ enzyme and incubation at 37°C for 6 h
 ◆, pH 4, 1 U ml⁻¹; ◇, pH 4, 2 U ml⁻¹; ▲, pH 5, 1 U ml⁻¹; △, pH 5, 2 U ml⁻¹; ■, pH 6, 1 U ml⁻¹; □, pH 6, 2 U ml⁻¹; ○, pH 7, 1 U ml⁻¹; ●, pH 7, 2 U ml⁻¹; ×, pH 8, 1 U ml⁻¹; +, pH 8, 2 U ml⁻¹

A contour plot of digestibility of G20 oligodextran by α -amylase from *Bacillus spp.* is shown in Figure 3.37. The maximum hydrolysis was predicted to be at pH 7.28 and incubation of 3.60 h giving value of 10.65%. Optimal pH for hydrolysis of G20 oligodextran by α -amylase from *Bacillus spp.* was pH 7.28.

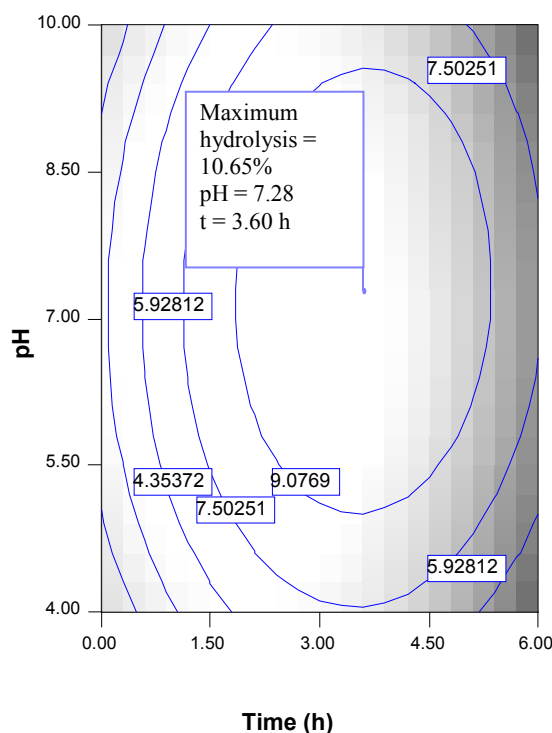


Figure 3.37 Contour plot of susceptibility of G20 oligodextran to α -amylase from *Bacillus spp.* as a function of pH and time using 1-2 U ml⁻¹ enzyme and incubation at 37°C

B. G20 maltodextrin

The susceptibility of G20 maltodextrin to α -amylase from *Bacillus spp.* is shown in Figure 3.38. It was found that rapid hydrolysis occurred within 1 h and reached the equilibrium after 2 h. The maximum hydrolysis was found at pH 6 and 2 U ml⁻¹ enzyme giving value of 17.13%. Percentages of hydrolysis at 2 U ml⁻¹ enzyme, incubated for 2 h at pH 4, 5, 6, 7 and 8 were 11.14, 15.14, 17.13, 17.11 and 15.08%, respectively. The percentage of hydrolysis was higher than those obtained from G20 oligodextran.

A contour plot of digestion of G20 maltodextrin by α -amylase from *Bacillus spp.* is shown in Figure 3.39. It was found that the condition to give the maximum hydrolysis was predicted to be at pH 6.40 and incubation time of 1.92 h giving value of 18.47%. Optimal pH for hydrolysis of G20 maltodextrin by α -amylase from *Bacillus spp.* was pH 6.40.

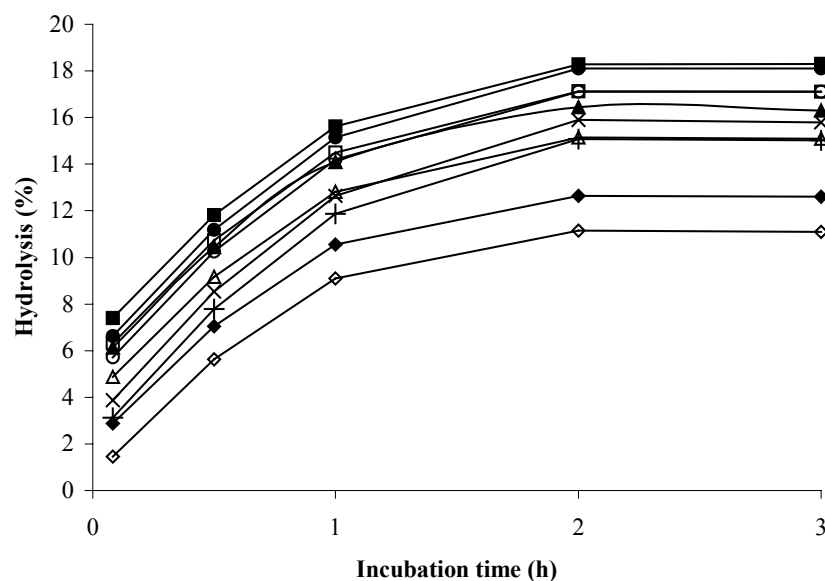


Figure 3.38 Susceptibility of G20 maltodextrin to α -amylase from *Bacillus spp.* at pH 4-8 using 1-2 U ml⁻¹ enzyme and incubation at 37°C for 3 h
 ◆, pH 4, 1 U ml⁻¹; ◇, pH 4, 2 U ml⁻¹; ▲, pH 5, 1 U ml⁻¹; △, pH 5, 2 U ml⁻¹; ■, pH 6, 1 U ml⁻¹; □, pH 6, 2 U ml⁻¹; ○, pH 7, 1 U ml⁻¹; ●, pH 7, 2 U ml⁻¹; ×, pH 8, 1 U ml⁻¹; +, pH 8, 2 U ml⁻¹

4.2.1.6 β -amylase from barley

A. G20 oligodextran

The aim of using β -amylase beside α -amylase is to determine the degree of resistance in case these materials are used in food processing with β -amylase although this enzyme would not be found in human saliva or the small intestine. Hydrolysis of G20 oligodextran had similar hydrolysis rate compared to α -amylases. The equilibrium was reached at 4 h incubation. However, the percentage of hydrolysis was lower than obtained with amylases from human saliva and pancreas. The maximum hydrolysis was obtained at pH 6 and 2 U ml⁻¹ enzyme, giving the value of 14.22%. Percentages of hydrolysis at 2 U ml⁻¹ enzyme and incubated for 4 h at pH 4, 5, 6, 7 and 8 were 11.46, 13.24, 14.22, 12.94 and 13.81%, respectively (Figure 3.40).

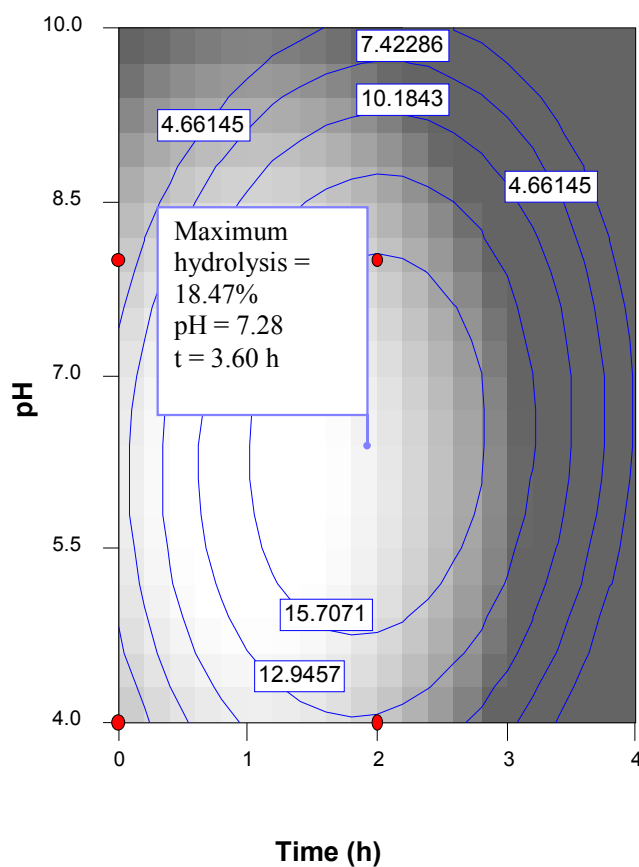


Figure 3.39 Contour plot of susceptibility of G20 maltodextrin to α -amylase from *Bacillus spp.* as a function of pH and time, 1-2 U ml⁻¹ enzyme, incubated at 37°C

A contour plot of digestibility of G20 oligodextran by β -amylase from barley was shown in Figure 3.41. It was found that the condition to give the maximum hydrolysis was predicted to be at pH 6.64 and incubation of 4.32 h giving value of 14.55%. Optimal pH for hydrolysis of G20 oligodextran by barley β -amylase was pH 6.64.

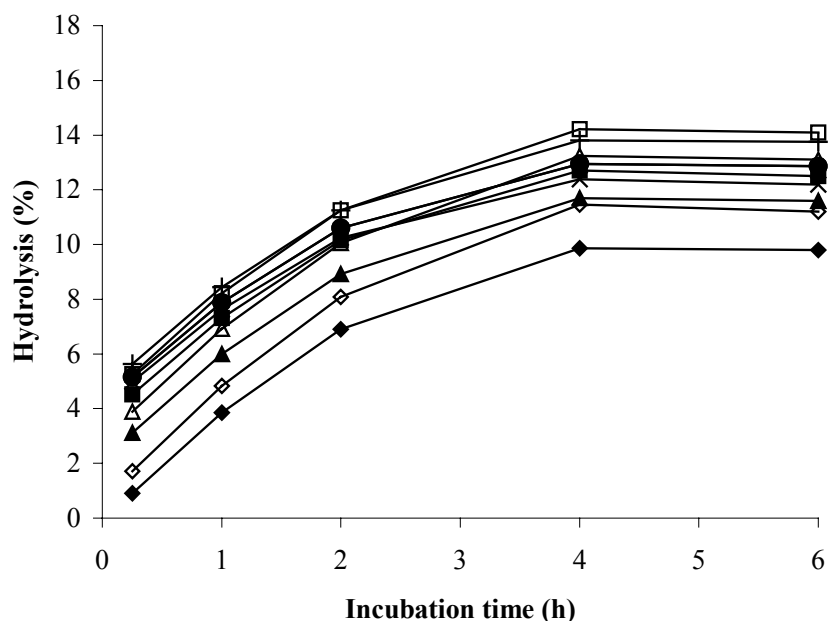


Figure 3.40 Susceptibility of G20 oligodextran to β -amylase from barley at pH 4-8 using 1-2 U ml⁻¹ enzyme and incubation at 37°C for 6 h
 ◆, pH 4, 1 U ml⁻¹; ◇, pH 4, 2 U ml⁻¹; ▲, pH 5, 1 U ml⁻¹; △, pH 5, 2 U ml⁻¹; ■, pH 6, 1 U ml⁻¹; □, pH 6, 2 U ml⁻¹; ○, pH 7, 1 U ml⁻¹; ●, pH 7, 2 U ml⁻¹; ×, pH 8, 1 U ml⁻¹; +, pH 8, 2 U ml⁻¹

B. G20 maltodextrin

G20 maltodextrin was more rapidly hydrolysed than G20 oligodextran and equilibrium was reached at 2 h. Maximum hydrolysis was found at pH 5 and 2 U ml⁻¹ enzyme, giving the value of 34.74%. Percentages of hydrolysis at 2 U ml⁻¹ enzyme and incubation of 2 h at pH 4, 5, 6, 7 and 8 were 34.20, 34.74, 32.27, 26.78 and 18.27%, respectively (Figure 3.42). However, percentage of hydrolysis was lower than with amylases from human saliva and pancreas.

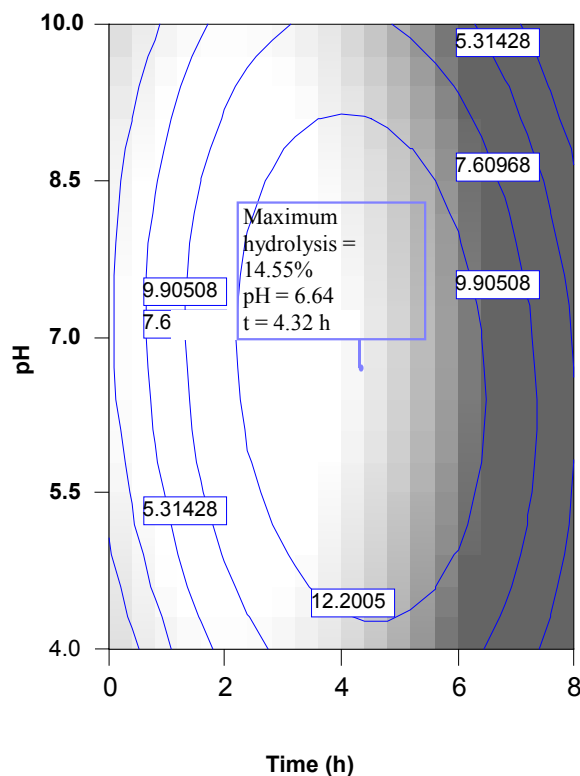


Figure 3.41 Contour plot of susceptibility of G20 oligodextran to β -amylase from barley as a function of pH and time using $1\text{-}2\text{ U ml}^{-1}$ enzyme and incubation at 37°C

A contour plot of digestion of G20 maltodextrin by β -amylase from barley is shown in Figure 3.43. The maximum hydrolysis predicted to be at pH 4.57 and incubation of 2.25 h giving value of 36.85%. Optimal pH for hydrolysis of G20 maltodextrin by barley β -amylase was pH 4.57.

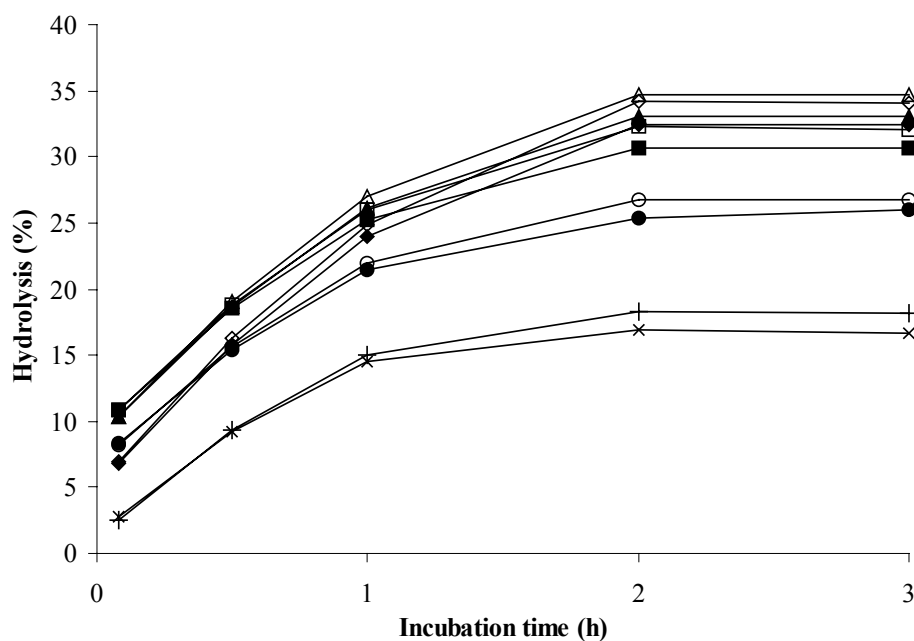


Figure 3.42 Susceptibility of G20 maltodextrin to β -amylase from barley at pH 4-8 using 1-2 U ml⁻¹ of enzyme and incubation at 37°C for 3 h
 ◆, pH 4, 1 U ml⁻¹; ◇, pH 4, 2 U ml⁻¹; ▲, pH 5, 1 U ml⁻¹; △, pH 5, 2 U ml⁻¹; ■, pH 6, 1 U ml⁻¹; □, pH 6, 2 U ml⁻¹; ○, pH 7, 1 U ml⁻¹; ●, pH 7, 2 U ml⁻¹; ×, pH 8, 1 U ml⁻¹; +, pH 8, 2 U ml⁻¹

4.2.2 Effect of temperature on digestion by amylases

The susceptibility of G19 oligodextran to various sources of enzyme (2 U ml⁻¹), pH 7 at 20, 30 and 37°C were tested. It was clearly seen that incubating temperature affected the hydrolysis and higher temperature had higher percentages of hydrolysis. Percentages of hydrolysis using amylases from human saliva, *Bacillus spp.*, *A. oryzae*, barley and *B. licheniformis* were 14.66, 14.44, 13.72, 12.39 and 11.68%, respectively at 37°C (Figure 3.44).

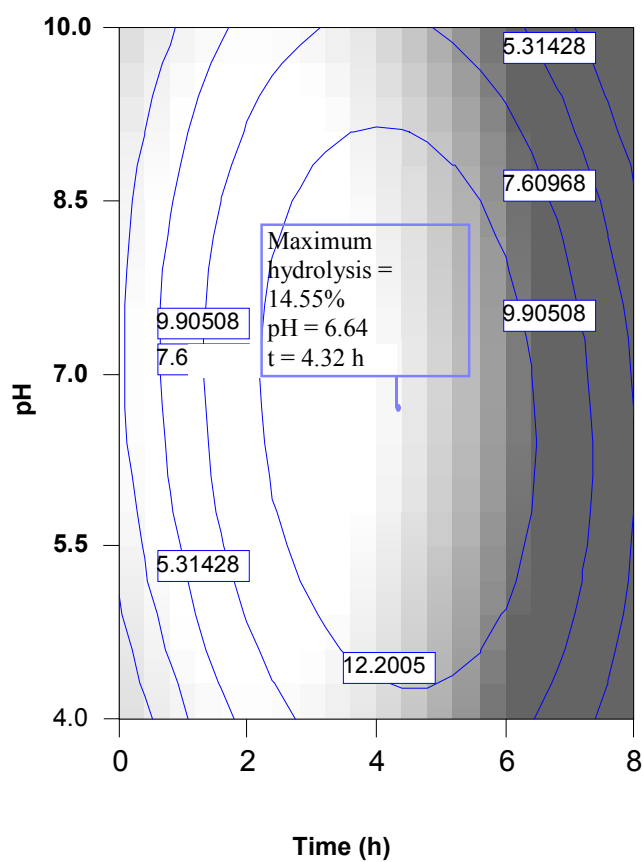


Figure 3.43 Contour plot of susceptibility of G20 maltodextrin to β -amylase from barley as a function of pH and time using 1-2 U ml⁻¹ enzyme and incubated at 37°C

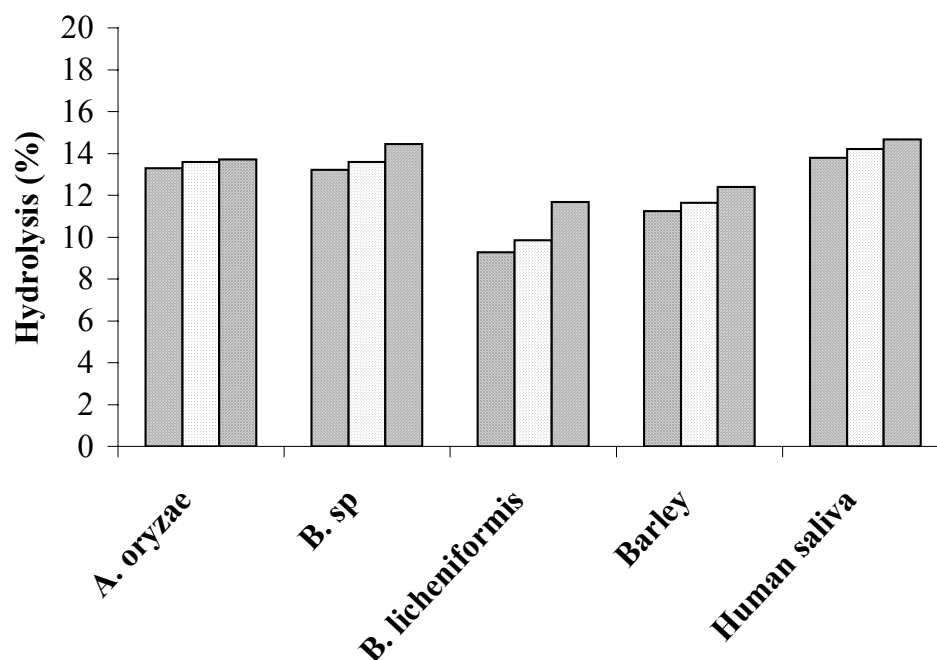


Figure 3.44 Effect of temperatures used for incubation of G19 oligodextran with various sources of α -amylases and β -amylase (2 U ml^{-1}) at pH 7
 ▨, 20°C; □, 30°C; ▩, 37°C

In this study, the maximum degree of hydrolysis of oligodextran product and maltodextrin substrate were 15% and 35%, respectively. This indicated that oligodextrans were over twice more resistant than maltodextrins. This result correlated with the linkages present in that the ratio of α -1,6 linkages was more than twice that of α -1,4 linkages. This study showed a similar degree of hydrolysis of maltodextrins (35%) either by α - or β -amylase.

The hydrolysis equilibrium of oligodextrans and maltodextrins by amylases in this study was reached at four and two h, respectively. This suggested that the maltodextrin was less complex than raw starch, which needed 24-48 h to reach equilibrium (Sarikaya *et al.*, 2000). The hydrolysis equilibrium of oligosaccharides (G6-G7) by α -amylases (isolated from rice) was reached at 200-400 min (Terashima *et al.*, 1996) which was in the same range as this study.

The degradation of oligodextrans by amylases was similar to that seen in previous work (Hreczuk-Hirst, *et al.*, 2001). They studied on degradation of maltodextrins (51.1 kDa) by porcine pancreatic α -amylase and reported that maltodextrin was completely hydrolyzed within 90 min whereas half of maltodextrin was hydrolyzed within 3 h using rat plasma α -amylase (Hreczuk-Hirst, *et al.*, 2001). Therefore, pancreatic α -amylase gave much higher degree of hydrolysis of maltodextrin and oligodextran than α -amylase from other sources.

4.3 Fermentability of oligodextran by human colonic microflora

4.3.1 Stirred pH-controlled batch culture fermentation

4.3.1.1 Microbial population change

A. G12 oligodextran and G12 maltodextrin

The fermentability of various carbohydrates by human colonic microflora in stirred pH-controlled batch cultures was tested. The changes of microbial populations were determined by fluorescent *in situ* hybridization accordance with the related probes used. Microbial change by fermentation of G12 oligodextran (Figure 3.45a) indicated that this substrate gave significant ($P < 0.05$) increase in total bacterial count and especially bifidobacteria and lactobacilli although they were slightly decreased at 48 h of fermentation. The numbers of bacteroides increased at 6 h and no significantly changed thereafter. The numbers of clostridia and eubacteria increased significantly ($P < 0.05$) within 24 h and decreased thereafter.

Fermentation of G12 maltodextrin (Figure 3.45b) gave significant ($P < 0.05$) increase in total bacteria after 6 h fermentation. The large increase in numbers of bifidobacteria (from 5×10^8 to 2×10^9 cell ml^{-1}) was found at 24 h, however, the number of lactobacilli (9×10^7 cell ml^{-1}) was much lower than that from G12 oligodextran fermentation (7×10^8 cell ml^{-1}) despite it showed slightly increased after 6 h. Bacteroides increased at 6 h and decreased thereafter whereas clostridia had the lowest bacterial numbers (5×10^7 cell ml^{-1}) with slightly increased after 6 h. Eubacteria had quite high numbers and slightly increased at 48 h.

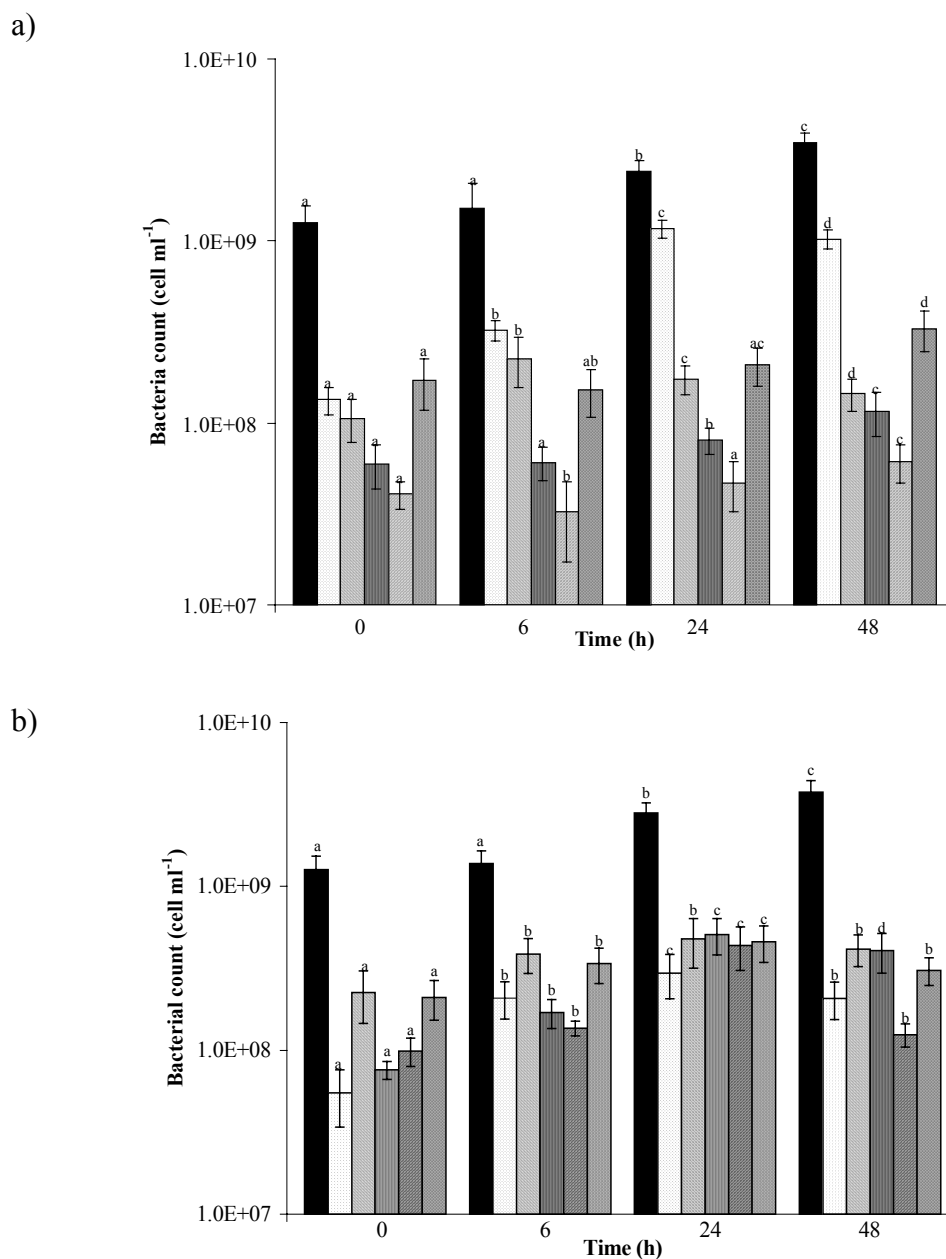


Figure 3.45 Changes in bacterial populations enumerated using fluorescent *in situ* hybridization in stirred pH-controlled batch culture fermentation with G12 oligodextran (a) and G12 maltodextrin (b)

■, total bacterial counts; □, bifidobacteria; □, bacteroides; □, lactobacilli/enterococci; ▨, clostridia (perfringens/histolyticum subgroup); ▩, Eubacterium rectale-Clostridium coccoides subgroup

Different letters (a, b, c, d) mean significant differences ($P < 0.05$) for the same bacterial species

B. G19 oligodextran and G19 maltodextrin

Fermentation of G19 oligodextran (Figure 3.46a) showed the increase of total bacteria numbers (from 2×10^9 at 0 h to 2×10^9 cell ml⁻¹ at 24 h), however it was slightly decreased at 48 h. Large increase in bifidobacteria (from 8×10^7 to 5×10^8 cell ml⁻¹) and lactobacilli (from 9×10^7 to 6×10^8 cell ml⁻¹) were observed within 24 h and no significant change thereafter. Bacteroides (from 3×10^8 to 7×10^8 cell ml⁻¹) and eubacteria (from 3×10^8 to 6×10^8 cell ml⁻¹) numbers increased within 24 h and significantly ($P < 0.05$) decreased thereafter.

For G19 maltodextrin fermentation (Figure 3.46b), the increase in total bacteria slightly decreased at 48 h. Significantly increases of bifidobacteria (from 7×10^7 to 3×10^8 cell ml⁻¹) and lactobacilli (from 8×10^7 to 2×10^8 cell ml⁻¹) were observed at 6 h and no significant change thereafter. Bacteroides did not change significantly between 0 and 48 h of fermentation while eubacteria started to change significantly ($P < 0.05$) at 24 h (3×10^8 cell ml⁻¹) and decreased at 48 h (2×10^8 cell ml⁻¹).

The fermentation rate of oligodextran is estimated to be between dextran and starch. Dextran has been reported to be metabolized by lactobacilli, bifidobacteria and *E. coli* with the growth rates of 0.10, 0.10 and 0.35 h⁻¹ whereas clostridia is not able to metabolize dextran. Growth rate of lactobacilli, bifidobacteria, *E. coli*, and clostridia on starch were 0.18, 0.22, 0.80 and 0.01 h⁻¹, respectively (Fooks and Gibson, 2002).

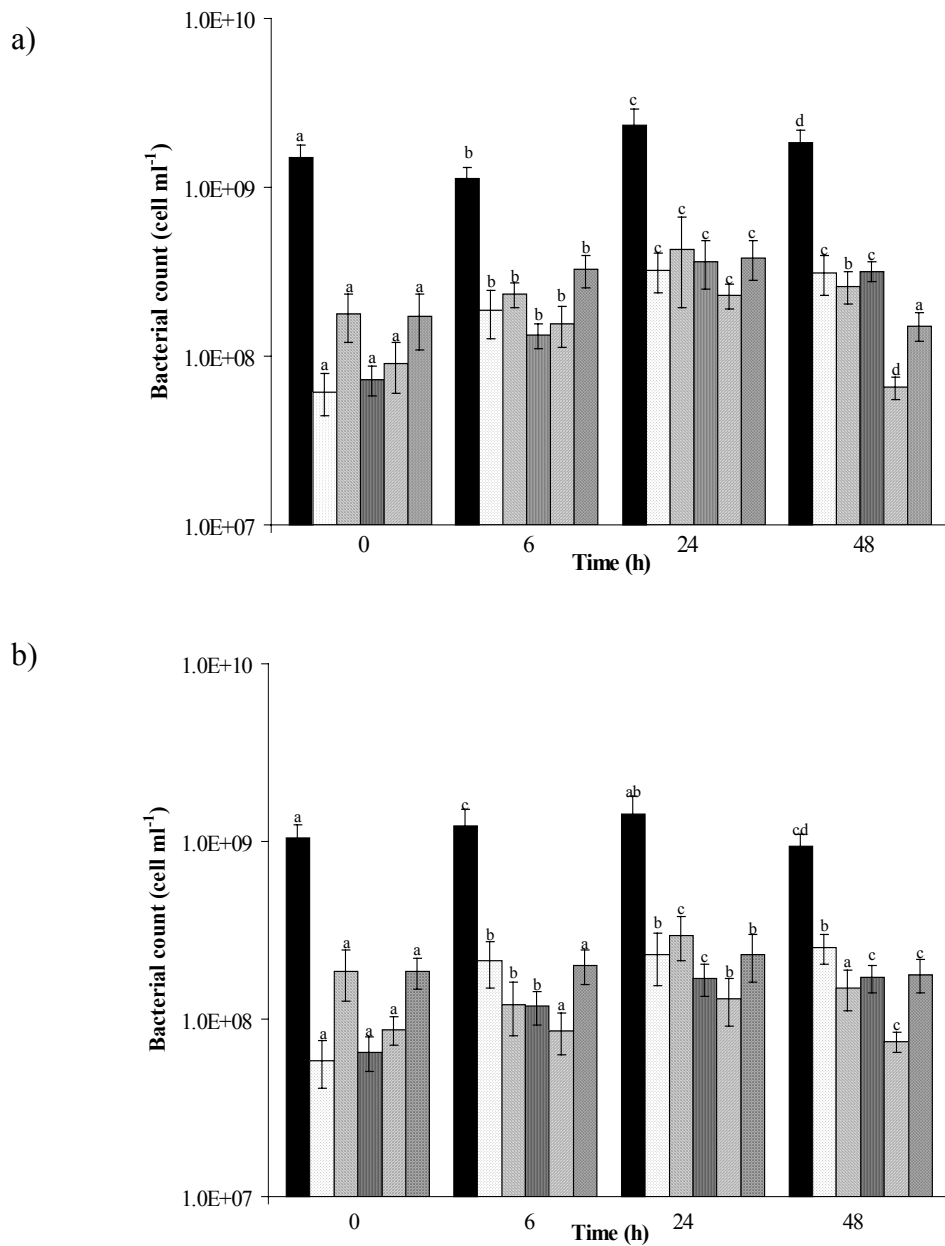


Figure 3.46 Changes in bacterial populations enumerated using fluorescent *in situ* hybridization in stirred pH-controlled batch culture fermentation with G19 oligodextran (a) and G19 maltodextrin (b)

■, total bacterial counts; □, bifidobacteria; □, bacteroides; □, lactobacilli/enterococci; ▨, clostridia (perfringens/histolyticum subgroup); ▩, Eubacterium rectale-Clostridium coccoides subgroup

Different letters (a, b, c, d) mean significant differences ($P < 0.05$) for the same bacterial species

C. G20 oligodextran and G20 maltodextrin

G20 oligodextran fermentation (Figure 3.47a), the increase in total bacteria appeared at 24 h (5×10^9 cell ml⁻¹) and not significant decreased at 48 h. Significantly increases of bifidobacteria (from 2×10^8 to 4×10^8 cell ml⁻¹) but not for lactobacilli were observed at 24 h and no significant change thereafter. Bacteroides decreased significantly between 0 h (3×10^8 cell ml⁻¹) and 6 h (1×10^8 cell ml⁻¹) however it increased again at 48 h (2×10^8 cell ml⁻¹) of fermentation while clostridium was slightly decreased at first 6 h (from 6×10^7 to 4×10^7 cell ml⁻¹) and it was increased significantly at 24 h (2×10^8 cell ml⁻¹) and 48 h (1×10^8 cell ml⁻¹). Eubacteria had decreased significantly ($P < 0.05$) after 6 h (from 4×10^8 to 2×10^8 cell ml⁻¹) and slightly increased thereafter.

Fermentation of G20 maltodextrin is shown in Figure 3.47b, total bacterial number increased significantly ($P < 0.05$) according to the fermentation time increased (from 1×10^9 at 6 h to 5×10^9 cell ml⁻¹ at 24 h) but no significant changed between 24 and 48 h fermentation. Slightly decrease in bifidobacteria (from 2×10^8 at 0 h to 1×10^8 cell ml⁻¹) was found at 24 h and increased to the original number (2×10^8 cell ml⁻¹) thereafter. Lactobacilli slightly decreased between 0 h (4×10^7 cell ml⁻¹) and 6 h (3×10^7 cell ml⁻¹) and it was slightly increased to 5×10^7 cell ml⁻¹ before decreased to less than 3×10^7 cell ml⁻¹ at 48 h. Bacteroides increased significantly at 24 h (3×10^8 cell ml⁻¹) and slightly decreased to the similar number to the original value (1×10^8 cell ml⁻¹) thereafter while clostridium slightly decreased at 6 h (3×10^7 cell ml⁻¹) and significant increase (1×10^8 cell ml⁻¹) was observed at 24 h. Eubacteria decreased but not significant from 2×10^8 cell ml⁻¹ at 0 h to 1.5×10^8 cell ml⁻¹ at 48 h.

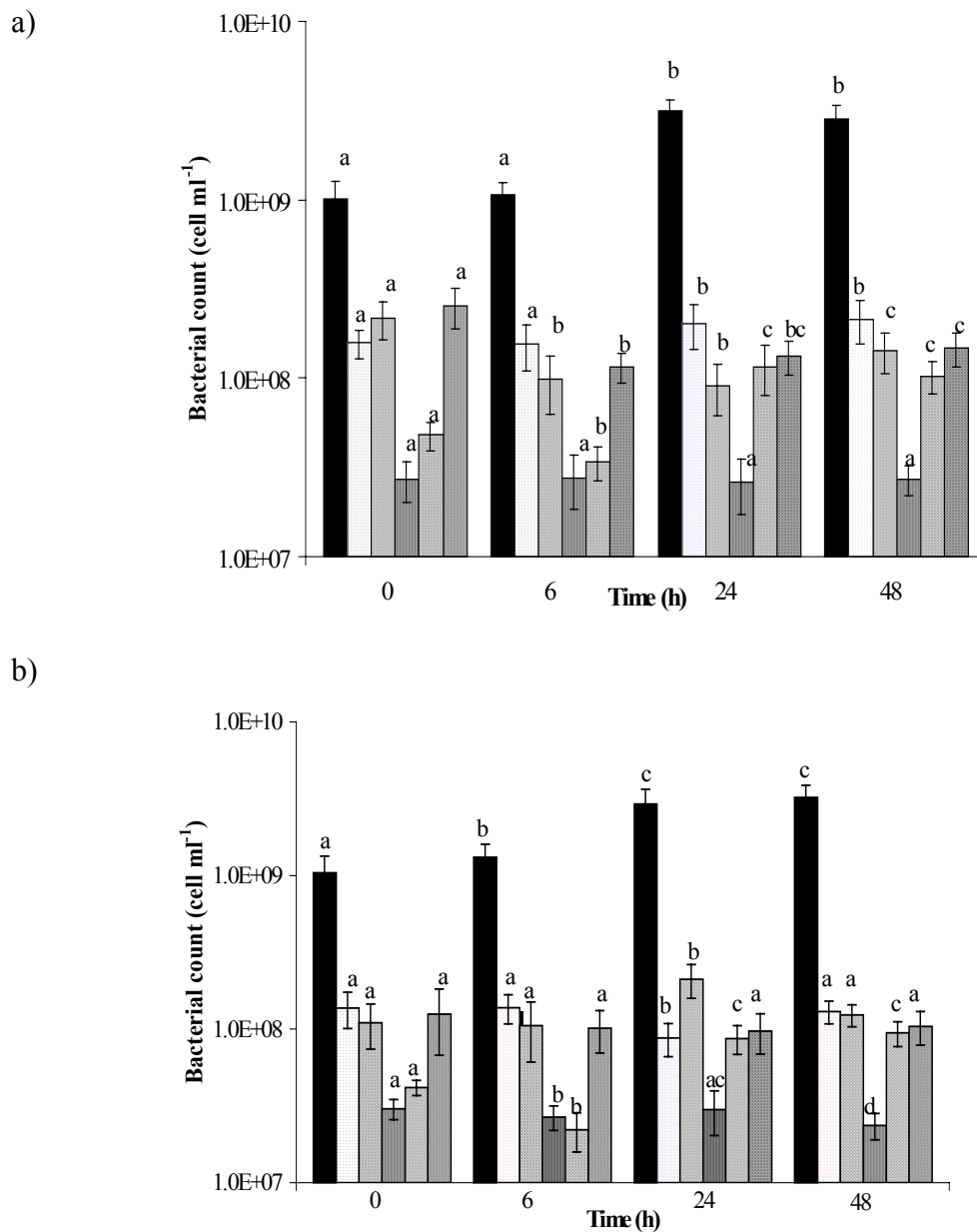


Figure 3.47 Changes in bacterial populations enumerated using fluorescent *in situ* hybridization in stirred pH-controlled batch culture fermentation with G20 oligodextran (a) and G20 maltodextrin (b)

■, total bacterial counts; □, bifidobacteria; ▒, bacteroides; ▓, lactobacilli/enterococci; ▒, clostridia (perfringens/histolyticum subgroup); ▒, Eubacterium rectale-Clostridium coccoides subgroup

Different letters (a, b, c, d) mean significant differences ($P < 0.05$) for the same bacterial species

D. G29 oligodextran and G29 maltodextrin

Fermentation of G29 oligodextran is shown in Figure 3.48a. The increase in total bacteria appeared at 24 h (5×10^9 cell ml⁻¹) and slightly decreased thereafter (4×10^9 cell ml⁻¹). The number of bifidobacteria significantly ($P < 0.05$) increased at 24 h (1×10^9 cell ml⁻¹) and slightly decreased thereafter (8×10^8 cell ml⁻¹). Lactobacilli significantly increased at 6 h (1×10^8 cell ml⁻¹) and 24 h (3×10^8 cell ml⁻¹) however slightly decreased was observed at 48 h (1×10^8 cell ml⁻¹). Bacteroides significantly increased till 24 h (8×10^8 cell ml⁻¹) and decreased thereafter (3×10^8 cell ml⁻¹) while clostridium significant increased at 6 h (from 5×10^7 cell ml⁻¹ at 0 h to 1×10^8 cell ml⁻¹) and kept slightly constant thereafter. Eubacteria increased at 6 h (from 3×10^8 cell ml⁻¹ to 7×10^8 cell ml⁻¹) and significantly decrease at 24 h (2×10^8 cell ml⁻¹), then increased thereafter (6×10^8 cell ml⁻¹).

Fermentation of G29 maltodextrin is shown in Figure 3.48b. The increase in total bacteria appeared till 24 h (6×10^9 cell ml⁻¹) and slightly decreased thereafter (5×10^9 cell ml⁻¹). The number of bifidobacteria significantly ($P < 0.05$) increased at 6 h (2×10^9 cell ml⁻¹) and 24 h (3×10^9 cell ml⁻¹) and slightly decreased thereafter (1×10^9 cell ml⁻¹). Lactobacilli significantly increased at 6 h (1×10^8 cell ml⁻¹) and decrease was observed thereafter (7×10^7 cell ml⁻¹). Bacteroides increased significantly till 24 h (from 1×10^8 at 0 h to 3×10^8 cell ml⁻¹) and significantly decreased thereafter (1×10^8 cell ml⁻¹) while number of clostridia significant increased at 24 h (1×10^8 cell ml⁻¹) and kept slightly constant thereafter. Eubacteria increased at 6 h (1×10^8 cell ml⁻¹) before significantly increased at 24 h (2×10^8 cell ml⁻¹) and decreased thereafter.

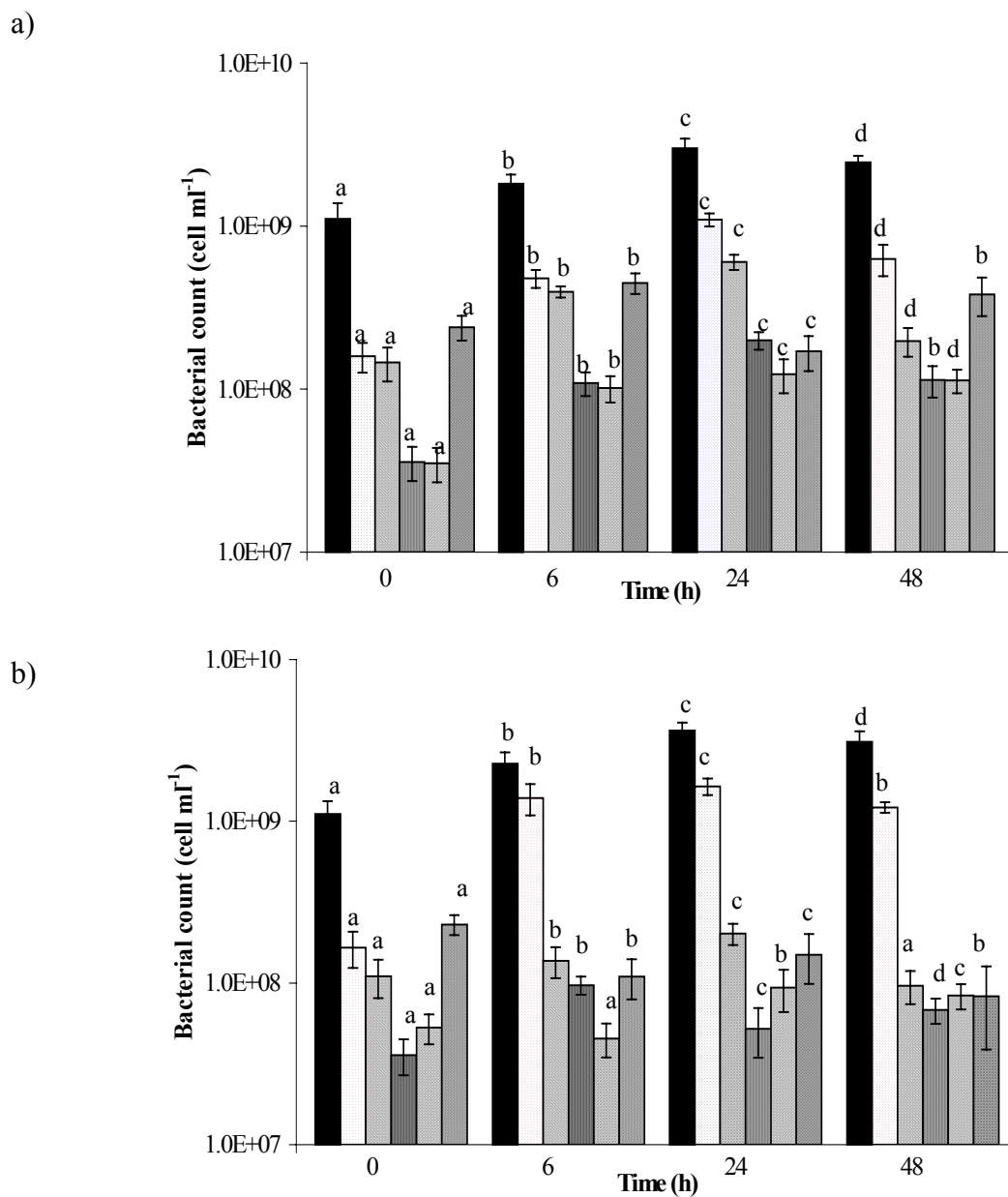


Figure 3.48 Changes in bacterial populations enumerated using fluorescent *in situ* hybridization in stirred pH-controlled batch culture fermentation with G29 oligodextran (a) and G29 maltodextrin (b)

■, total bacterial counts; □, bifidobacteria; ▒, bacteroides; ▓, lactobacilli/enterococci; ▨, clostridia (perfringens/histolyticum subgroup); ▩, Eubacterium rectale-Clostridium coccoides subgroup

Different letters (a, b, c, d) mean significant differences ($P < 0.05$) for the same bacterial species

E. G37 oligodextran and G37 maltodextrin

G37 oligodextran increased total bacteria within 24 h (5×10^9 cell ml⁻¹) and kept constant thereafter (Figure 3.49a). Large increase in bifidobacteria (2×10^9 cell ml⁻¹) was found within 24 h and slightly declined thereafter (1×10^9 cell ml⁻¹). Lactobacilli increased in 6 h (2×10^8 cell ml⁻¹) and declined at 24 h (8×10^7 cell ml⁻¹) with no change thereafter. Similar behavior was observed for bacteroides but the increase was obtained at 24 h (7×10^8 cell ml⁻¹) and no significant changed thereafter. Clostridia increased significantly from 6 h (6×10^7 cell ml⁻¹) to 24 h (3×10^8 cell ml⁻¹) and declined thereafter. Significant ($P < 0.05$) increase of eubacteria was found only at 48 h (5×10^9 cell ml⁻¹) fermentation.

For fermentation of G37 maltodextrin (Figure 3.49b), total bacteria increased within 24 h (5×10^9 cell ml⁻¹) and no change thereafter. Large increases of bifidobacteria (1×10^9 cell ml⁻¹) and lactobacilli (2×10^8 cell ml⁻¹) were found at 6 h but declined thereafter whist the reduction of bacteroides (1×10^8 cell ml⁻¹) were detected at 48 h except clostridium (7×10^7 cell ml⁻¹) and eubacteria increased (3×10^8 cell ml⁻¹).

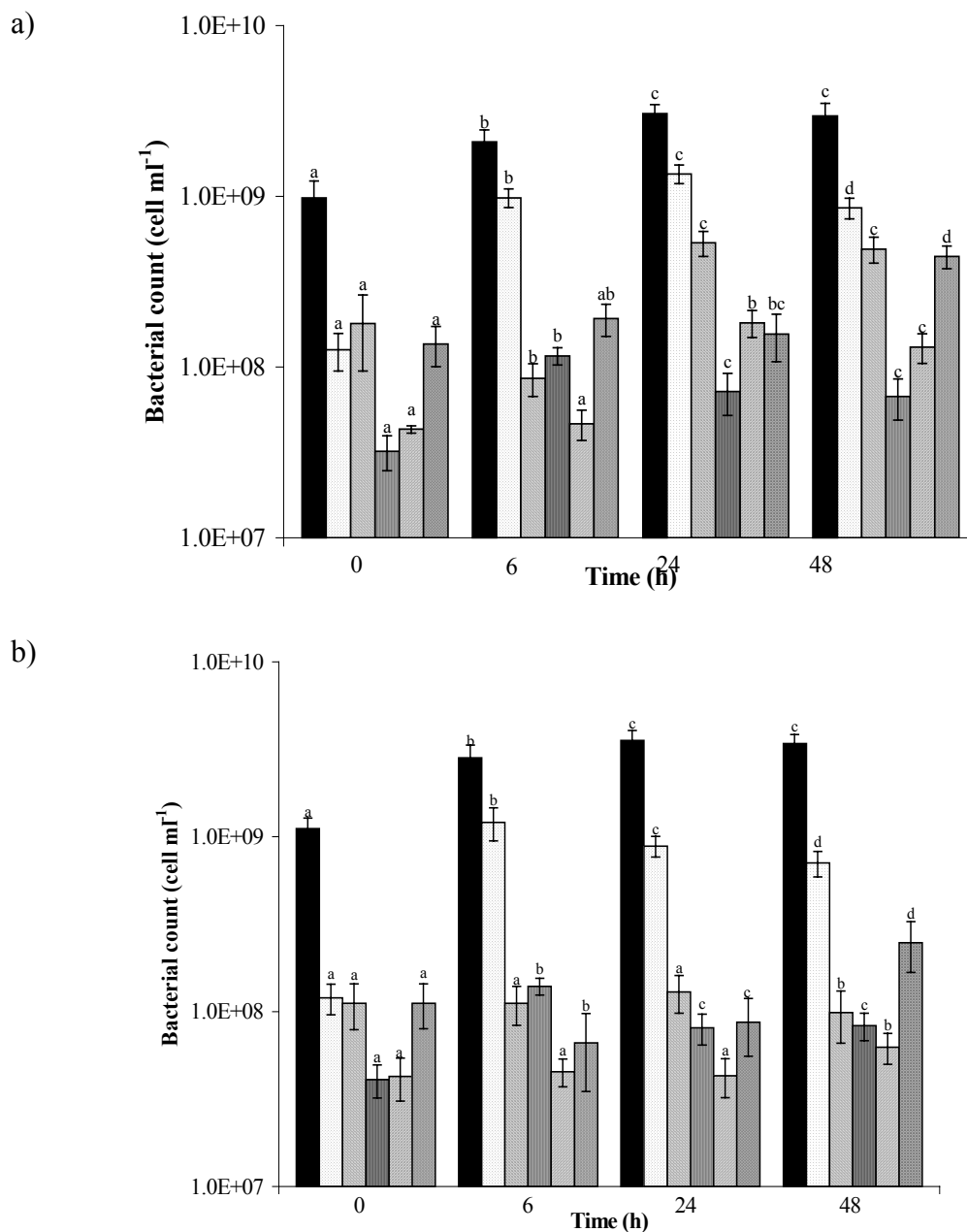


Figure 3.49 Changes in bacterial populations enumerated using fluorescent *in situ* hybridization in stirred pH-controlled batch culture fermentation with G37 oligodextran (a) and G37 maltodextrin (b)

■, total bacterial counts; □, bifidobacteria; ▨, bacteroides; ▩, lactobacilli/enterococci; ▤, clostridia (perfringens/histolyticum subgroup); ▥, Eubacterium rectale-Clostridium coccoides subgroup

Different letters (a, b, c, d) mean significant differences ($P < 0.05$) for the same bacterial species

F. Levan

Fermentation of commercial levan was shown in Figure 3.50, total bacteria was slightly increased at 6 h (2×10^9 cell ml^{-1}) and large increase was seen at 24 h (6×10^9 cell ml^{-1}) before slightly decreased thereafter. The number of bifidobacteria slightly increased at 6 h (2×10^8 cell ml^{-1}) and decreased thereafter. Levan did not stimulate the growth of lactobacilli thus no changes in number of lactobacilli were observed throughout the experiment. Reduction of bacteroides was seen at 6 h (9×10^7 cell ml^{-1}) and it was kept nearly constant until the end of fermentation. Clostridium significantly increased at 24 h (1×10^8 cell ml^{-1}) and large decrease was detected thereafter.

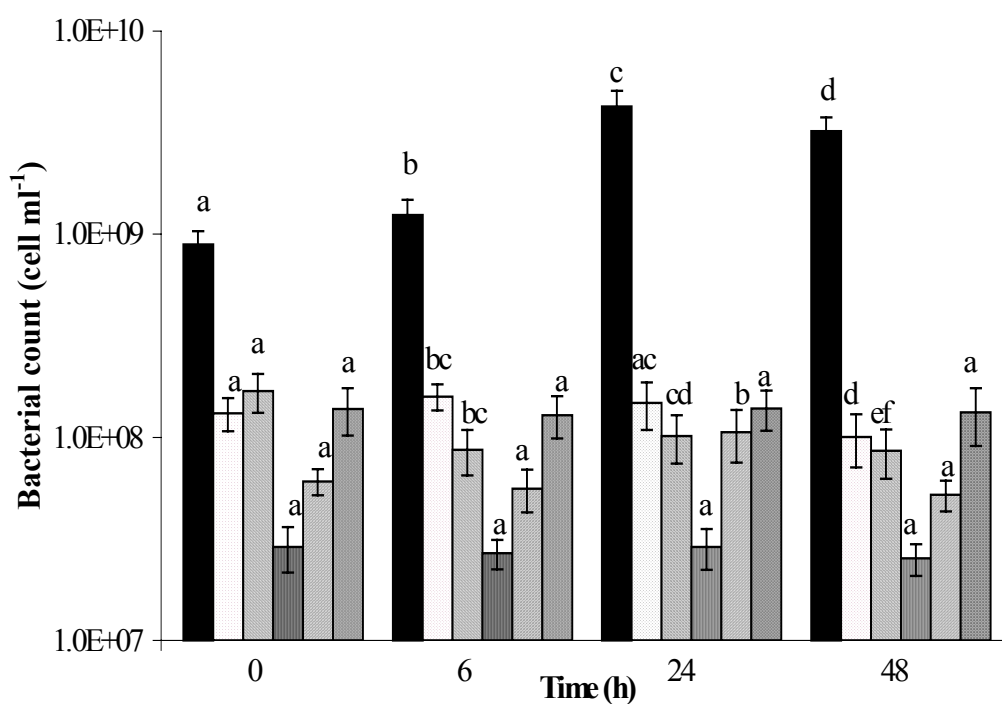


Figure 3.50 Changes in bacterial populations enumerated using fluorescent *in situ* hybridization in stirred pH-controlled batch culture fermentation with commercial levan

■, total bacterial counts; □, bifidobacteria; ▒, bacteroides; ▓, lactobacilli/enterococci; ▒, clostridia (perfringens/histolyticum subgroup); ▒, Eubacterium rectale-Clostridium coccoides subgroup

Different letters (a, b, c, d) mean significant differences ($P < 0.05$) for the same bacterial species

G. Inulin-ST

Fermentation of commercial prebiotic inulin-ST, standard inulin, was shown in Figure 3.51. The number of total bacteria increased throughout the fermentation. Large increase in number of bifidobacteria was observed within 24 h (1×10^9 cell ml^{-1}) and no change thereafter. Lactobacilli increased at 24 h (2×10^8 cell ml^{-1}) and slightly decreased thereafter. Bacteroides (7×10^8 cell ml^{-1}), clostridia (1×10^8 cell ml^{-1}) and eubacteria (7×10^8 cell ml^{-1}) increased at 24 h but declined slightly thereafter.

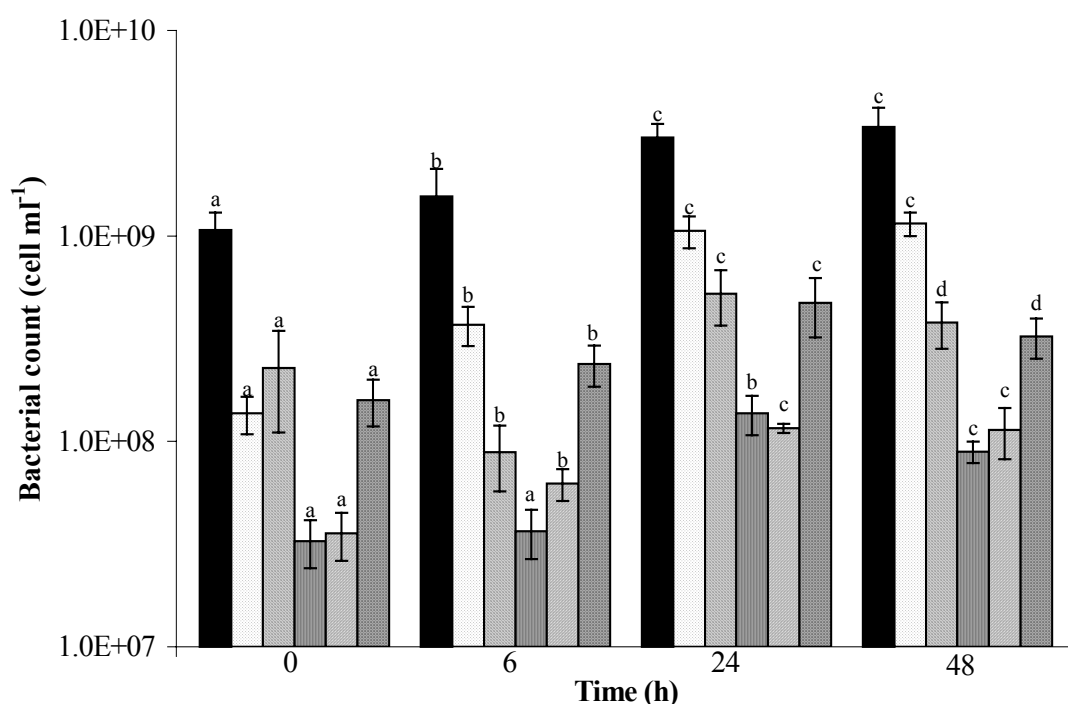


Figure 3.51 Changes in bacterial populations enumerated using fluorescent *in situ* hybridization in stirred pH-controlled batch culture fermentation with commercial inulin-ST

■ , total bacterial counts; □ , bifidobacteria; ▒ , bacteroides; ▒ , lactobacilli/enterococci; ▒ , clostridia (perfringens/histolyticum subgroup); ▒ , Eubacterium rectale-Clostridium coccoides subgroup

Different letters (a, b, c, d) mean significant differences ($P < 0.05$) for the same bacterial species

H. Inulin-HP

Fermentation of commercial prebiotic inulin-HP, high performance inulin, which consisted of high fraction of high molecular weight inulin was shown in Figure 3.52. The number of total bacteria increased throughout the fermentation. Inulin-HP did not support the growth of bifidobacter however slightly increase was observed at 24 h. Inulin-HP also did not support the growth of lactobacilli however slightly increase was found at 48 h (4×10^7 cell ml^{-1}). Bacteroides significantly increased at 24 h (3×10^8 cell ml^{-1}) and decreased thereafter. Clostridia and eubacteria decreased at 6 and 24 h but large increase of clostridium was observed at 48 h (1×10^8 cell ml^{-1}) whilst slightly increase in number of eubacteria was found at 48 h (2×10^8 cell ml^{-1}).

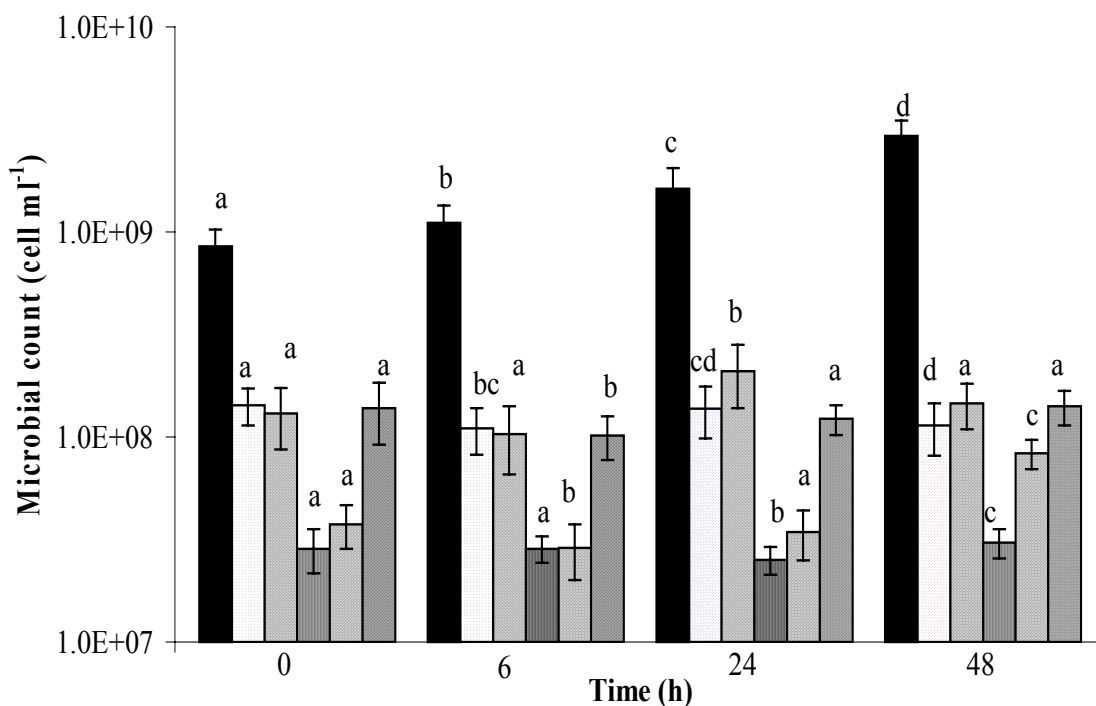


Figure 3.52 Changes in bacterial populations enumerated using fluorescent *in situ* hybridization in stirred pH-controlled batch culture fermentation with inulin-HP

■, total bacterial counts; □, bifidobacteria; ▒, bacteroides; ▓, lactobacilli/enterococci; ▤, clostridia (perfringens/histolyticum subgroup); ▥, Eubacterium rectale-Clostridium coccoides subgroup

Different letters (a, b, c, d) mean significant differences ($P < 0.05$) for the same bacterial species

4.3.1.2 Short-chain fatty acids (SCFA) production

Short-chain fatty acids produced by fermentation of G12, G19, G20 and G29 oligodextrans in batch culture were determined by HPLC (Table 3.5). Fermentation of G12 oligodextran produced low concentration of lactate within 6 h and disappeared thereafter whereas formate was not detected throughout the fermentation. High concentration of acetate was produced and increased with the increase in fermentation time. Propionate was generated in large quantity at 24 h and 48 h of fermentation. Butyrate increased as fermentation time increased and reached the maximum concentration ($13.44 \text{ mmol l}^{-1}$) at 48 h.

Fermentation of G19 oligodextran produced lactate within 6 h and disappeared thereafter whereas low concentration of formate was found after 24 h. Large quantity of acetate was found and kept constant after 24 h. Propionate was produced as fermentation time increased, however, the quantity was not much different at 24 and 48 h. The maximum butyrate concentration ($14.52 \text{ mmol l}^{-1}$) was found at 48 h of fermentation. Fermentation of G20 oligodextran also produced lactate within 6 h and slightly decreased thereafter whereas small amount of formate was detected at 24 h. The concentration of acetate increased as fermentation time increased and reached the maximum concentration ($43.25 \text{ mmol l}^{-1}$) at 48 h. Small increase in propionate was detected as fermentation time increased after 6 h, giving the highest value of $11.25 \text{ mmol l}^{-1}$ at 48 h. Butyrate was produced in small quantity according to the fermentation time increased and the maximum value (9.06 mmol l^{-1}) was obtained at 48 h of fermentation.

Fermentation of G29 oligodextran produced low concentration lactate production and formate only at 6 h and disappeared thereafter. Acetate, on the other hand, was increased with the increase in fermentation time between 6 h and 24 h giving the maximum concentration of $59.14 \text{ mmol l}^{-1}$ at 48 h. Propionate was generated in large quantity at 24 h and 48 h of fermentation, giving the maximum concentration of $25.51 \text{ mmol l}^{-1}$ at 48 h. Butyrate was detected after 24 h (4.23 mmol l^{-1}) and slightly increased thereafter (4.97 mmol l^{-1}).

Table 3.5 SCFA* production by G12, G19, G20 and G29 oligodextran fermentations in stirred pH-controlled batch culture

	G12 oligodextran				G19 oligodextran				G20 oligodextran				G29 oligodextran			
	0 h	6 h	24 h	48 h	0 h	6 h	24 h	48 h	0 h	6 h	24 h	48 h	0 h	6 h	24 h	48 h
Lactate	3.33	8.70	0.00	0.00	1.67	17.83	0.00	0.00	0.00	4.11	2.40	0.42	0.00	2.41	0.00	0.00
	±0.54	±1.87	±0.00	±0.00	±0.45	±2.49	±0.00	±0.00	±0.00	±0.53	±0.23	±0.11	±0.00	±0.25	±0.00	±0.00
Formate	0.00	0.00	0.00	0.00	0.00	0.00	0.77	0.37	0.00	0.00	1.99	0.73	0.00	1.52	0.00	0.00
	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.22	±0.12	±0.00	±0.00	±0.21	±0.11	±0.00	±0.77	±0.00	±0.00
Acetate	0.00	35.43	55.39	62.03	7.76	50.68	63.48	63.67	9.40	19.99	29.61	43.25	1.71	28.41	55.12	59.14
	±0.00	±9.65	±8.69	±11.45	±1.32	±9.97	±12.40	±13.45	±1.12	±2.85	±2.19	±3.65	±0.22	±0.64	±5.60	±5.91
Propionate	6.30	7.64	24.25	28.06	3.65	9.76	17.34	18.36	6.29	6.09	9.40	11.25	0.00	5.01	25.25	25.51
	±1.38	±2.10	±5.88	±2.54	±0.66	±1.64	±2.15	±2.37	±0.66	±0.50	±1.15	±0.88	±0.00	±0.83	±1.28	±3.37
Butyrate	0.00	5.67	10.03	13.44	0.00	6.56	12.96	14.52	0.00	1.32	6.00	9.06	0.00	0.00	4.23	4.97
	±0.00	±1.09	±1.25	±1.46	±0.00	±1.31	±2.00	±2.11	±0.00	±0.26	±0.54	±1.10	±0.00	±0.00	±0.73	±0.18

* Values were means ± SD from duplicate fermenters in mmol l⁻¹ concentrations

Short-chain fatty acids produced from fermentation of G37 oligodextran, commercial levan, commercial prebiotic inulin-ST and inulin-HP in stirred pH-controlled batch culture were summarized in Table 3.6. Acetate was generated in large amount and reached the maximum concentration ($79.42 \text{ mmol l}^{-1}$) at 48 h and quantitative concentration of propionate and butyrate were achieved from fermentation of G37 oligodextran.

Low concentration of lactate was produced only at 6 h fermentation of commercial levan and disappeared thereafter. Low concentration of formate was detected throughout the fermentation time. High concentration of acetate was produced as fermentation time increased and large increase was found at 24 h. The maximum concentration of acetate ($55.64 \text{ mmol l}^{-1}$) was found at 48 h. Concentration of propionate increased as fermentation time increased and reached the maximum concentration ($23.10 \text{ mmol l}^{-1}$) at 48 h. Butyrate was produced after 6 h, giving the maximum concentration of 3.99 mmol l^{-1} at 48 h fermentation.

Fermentation of commercial prebiotic inulin-ST produced low concentrations of lactate at 24 h whereas low concentration of formate was obtained at 24 and 48 h. Acetate produced in lower concentration compared to fermentation of other carbohydrates tested. Large increase was found at 24 h giving concentration of $24.52 \text{ mmol l}^{-1}$. Propionate was also produced in lower concentration compared to other carbohydrate samples tested and the maximum propionate ($17.69 \text{ mmol l}^{-1}$) was obtained at 48 h. Inulin-ST was a good source for butyrate production since high concentration of butyrate was produced at 24 h and 48 h, giving the values of 10.86 and $12.72 \text{ mmol l}^{-1}$, respectively.

Fermentation of commercial prebiotic inulin-HP had different profiles of SCFA production compared to fermentation of inulin-ST. For example fermentation of inulin-HP gave lower concentration of butyrate but higher concentrations of acetate and propionate. Thus inulin-HP seemed to be a good source for acetate and propionate but not for butyrate.

Table 3.6 SCFA* production by G37 oligodextrans, levan, inulin-ST and inulin-HP fermentations in stirred pH-controlled batch culture

	G37 oligodextrans				Levan				Inulin-ST				Inulin-HP			
	0 h	6 h	24 h	48 h	0 h	6 h	24 h	48 h	0 h	6 h	24 h	48 h	0 h	6 h	24 h	48 h
Lactate	0.00	2.02	0.00	0.00	0.00	5.02	0.00	0.00	0.00	0.00	5.52	0.00	0.00	3.28	2.50	0.00
	±0.00	±0.91	±0.00	±0.00	±0.00	±0.28	±0.00	±0.00	±0.00	±0.00	±0.55	±0.00	±0.00	±0.26	±0.16	±0.00
Formate	0.00	0.61	0.00	0.00	2.77	0.00	2.63	2.00	0.00	0.00	3.16	1.77	0.00	0.00	0.00	0.66
	±0.00	±0.12	±0.00	±0.00	±0.43	±0.00	±0.33	±0.20	±0.00	±0.00	±0.33	±0.15	±0.00	±0.00	±0.00	±0.10
Acetate	1.96	46.12	64.90	79.42	5.62	13.15	48.89	55.64	6.65	3.49	24.52	26.26	6.24	10.49	38.62	44.65
	±0.89	±8.87	±12.13	±15.35	±0.55	±1.24	±3.56	±5.54	±1.05	±0.54	±2.55	±2.56	±0.56	±1.23	±4.55	±2.89
Propionate	0.00	17.17	17.17	21.75	5.13	6.17	13.89	23.10	4.77	4.85	15.73	17.69	5.47	6.39	22.78	25.74
	±0.00	±5.56	±3.24	±5.59	±0.53	±0.45	±0.88	±2.67	±0.89	±0.65	±1.57	±1.88	±0.45	±0.88	±1.40	±2.21
Butyrate	0.00	7.05	7.05	7.40	0.00	1.78	2.62	3.99	0.00	0.77	10.86	12.72	0.00	0.39	6.81	6.50
	±0.00	±2.32	±1.99	±1.85	±0.00	±0.20	±0.55	±0.35	±0.00	±0.11	±1.26	±1.88	±0.00	±0.08	±0.55	±0.97

* Values were means ± SD from duplicate fermenters in mmol l⁻¹ concentrations

Fermentation of G12, G19, G20 and G29 maltodextrins in stirred pH-controlled batch culture was summarized in Table 3.7. G12 maltodextrin produced lactate within a short period of fermentation and disappeared after 6 h. No formate was detected throughout the fermentation. The maximum acetate concentration ($81.59 \text{ mmol l}^{-1}$) was obtained at 48 h. Propionate was detected after 6 h and slightly decreased at 48 h whilst low concentration of butyrate was found throughout fermentation.

G19 maltodextrin produced quantitatively concentration of lactate within 6 h and disappeared thereafter while no formate was detected throughout the fermentation. Acetate was produced constantly after 24 h and reached the maximum concentration ($60.98 \text{ mmol l}^{-1}$) at 48 h and not high concentration of propionate and butyrate were detected.

Fermentation of G20 maltodextrin produced lactate at 6 h for $12.19 \text{ mmol l}^{-1}$ and disappeared thereafter. Formate was produced at low concentration at 48 h but not produced at other fermentation times. Acetate increased as fermentation time increased and the maximum acetate ($46.05 \text{ mmol l}^{-1}$) was obtained at 48 h. Large propionate was produced at 24 and 48 h for 22.68 and $22.78 \text{ mmol l}^{-1}$, respectively. Butyrate was produced in low concentration and the maximum concentration (5.70 mmol l^{-1}) was obtained at 24 h.

G29 maltodextrin fermentation produced quite large lactate concentration ($17.78 \text{ mmol l}^{-1}$) at 6 h and disappeared thereafter. Formate was produced only at 6 h and disappeared thereafter. High concentration of acetate was produced since 6 h fermentation and slightly increased as fermentation time increased and the maximum concentration ($80.37 \text{ mmol l}^{-1}$) was detected at 48 h. Propionate was produced after 6 h till 24 h it had the highest concentration ($11.80 \text{ mmol l}^{-1}$) at 48 h. Butyrate was produced quantitatively at 24 and 48 h for 12.10 and $13.00 \text{ mmol l}^{-1}$, respectively.

Fermentation of G37 maltodextrin produced lactate only at 6 h with the detection of low concentration of formate at this time. High concentration ($93.83 \text{ mmol l}^{-1}$) of acetate was produced at 48 h and not high concentrations of propionate and butyrate were found throughout the fermentation (Table 3.8).

Table 3.7 SCFA* production by G12, G19, G20 and G29 maltodextrin fermentations in stirred pH-controlled batch culture

	G12 maltodextrin				G19 maltodextrin				G20 maltodextrin				G29 maltodextrin			
	0 h	6 h	24 h	48 h	0 h	6 h	24 h	48 h	0 h	6 h	24 h	48 h	0 h	6 h	24 h	48 h
Lactate	0.00	7.28	0.00	0.00	2.64	27.83	0.00	0.00	0.00	12.19	0.00	0.00	0.00	17.78	0.00	0.00
	±0.00	±1.22	±0.00	±0.00	±0.70	±7.80	±0.00	±0.00	±0.00	±1.10	±0.00	±0.00	±0.00	±1.12	±0.00	±0.00
Formate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.48	0.00	0.00	0.00	0.46	0.00	4.39	0.00	0.00
	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.05	±0.00	±0.00	±0.00	±0.10	±0.00	±0.56	±0.00	±0.00
Acetate	0.00	15.46	80.96	81.59	0.00	46.44	58.41	60.98	4.40	14.01	42.64	46.05	0.00	75.74	77.47	80.37
	±0.00	±3.32	±15.83	±10.88	±0.00	±9.90	±12.20	±9.90	±0.88	±1.11	±4.55	±6.44	±0.00	±5.60	±8.50	±6.89
Propionate	0.00	0.00	20.88	19.74	5.34	3.81	15.61	16.68	3.67	4.18	22.68	22.78	0.00	7.08	11.46	11.80
	±0.00	±0.00	±4.56	±4.45	±1.00	±0.50	±5.12	±3.56	±0.60	±0.23	±1.78	±1.88	±0.00	±0.77	±1.00	±1.22
Butyrate	0.00	0.00	1.07	2.05	0.00	6.30	6.30	8.45	0.00	0.00	5.70	4.10	0.00	0.00	12.10	13.00
	±0.00	±0.00	±0.47	±0.66	±0.00	±1.10	±1.54	±2.25	±0.00	±0.00	±0.50	±0.45	±0.00	±0.00	±1.80	±2.01

* Values were means ± SD from duplicate fermenters in mmol l⁻¹ concentrations

Table 3.8 SCFA* production by G37 maltodextrin fermentations in stirred pH-controlled batch culture

	G37 maltodextrin			
	0 h	6 h	24 h	48 h
Lactate	0.00 ±0.00	14.24 ±3.20	0.00 ±0.00	0.00 ±0.00
Formate	0.00 ±0.00	5.17 ±1.10	0.00 ±0.00	0.00 ±0.00
Acetate	0.00 ±0.00	70.58 ±15.50	76.65 ±13.35	93.83 ±18.78
Propionate	0.00 ±0.00	7.05 ±1.04	10.09 ±2.50	10.74 ±2.50
Butyrate	0.00 ±0.00	0.00 ±0.00	7.77 ±1.10	5.22 ±1.00

* Values were means ± SD from duplicate fermenters in mmol l⁻¹ concentrations

SCFA produced by fermentation of inulin-HP in this study (mmol l⁻¹; lactate, formate, acetate, propionate, butyrate at 24 h were 2.50, 0, 38.62, 22.78 and 6.81 mmol l⁻¹, respectively) was not much different from other study (Corradini *et al.*, 2004) (mmol l⁻¹; lactate, formate, acetate, propionate, butyrate at 24 h were 15.44, 2.39, 36.84, 1.77 and 10.29 mmol l⁻¹, respectively). Although, the detection methods were different, i.e. the HPLC and the capillary zone electrophoresis (CZE) for this study and the study of Corradini and co-workers (2004), respectively. Fermentation of Fibregum AS[®] led to production of acetate, propionate and butyrate for 30.8, 9.9 and 6.2 mmol l⁻¹, respectively whereas standard-Fibregum led to production of acetate, propionate and butyrate for 25.0, 5.9 and 4.3 mmol l⁻¹, respectively (Michel *et al.*, 1998). This was clearly seen that acacia gums showed lower butyrogenic effect (lower butyrate concentration) than oligodextrans used in this study.

4.3.1.3 Prebiotic index calculation

Prebiotic index (PI) of various carbohydrates tested in batch culture were calculated and summarized in Figure 3.53. G12 oligodextran, as well as G12 maltodextrin, demonstrated prebiotic properties (PI higher than 2). G19 oligodextran exhibited the highest prebiotic properties (PI of 5.90 at 48 h) whilst G19 maltodextrin

had lower prebiotic properties. It should be noted that maltodextrin could reach colon less than oligodextran due to its higher resistant to human saliva and simulated gastric juice as shown in section 2.2.10 and 2.2.11. Neither G20 oligodextran nor G20 maltodextrin had low prebiotic property. G29 maltodextrin seemed to give higher prebiotic property compared to G29 oligodextran. G37 oligodextran had higher prebiotic property at 48 h compared to G37 maltodextrin. Particularly, inulin-ST showed prebiotic properties. However it gave lower PI value than G19 and G37 oligodextrans.

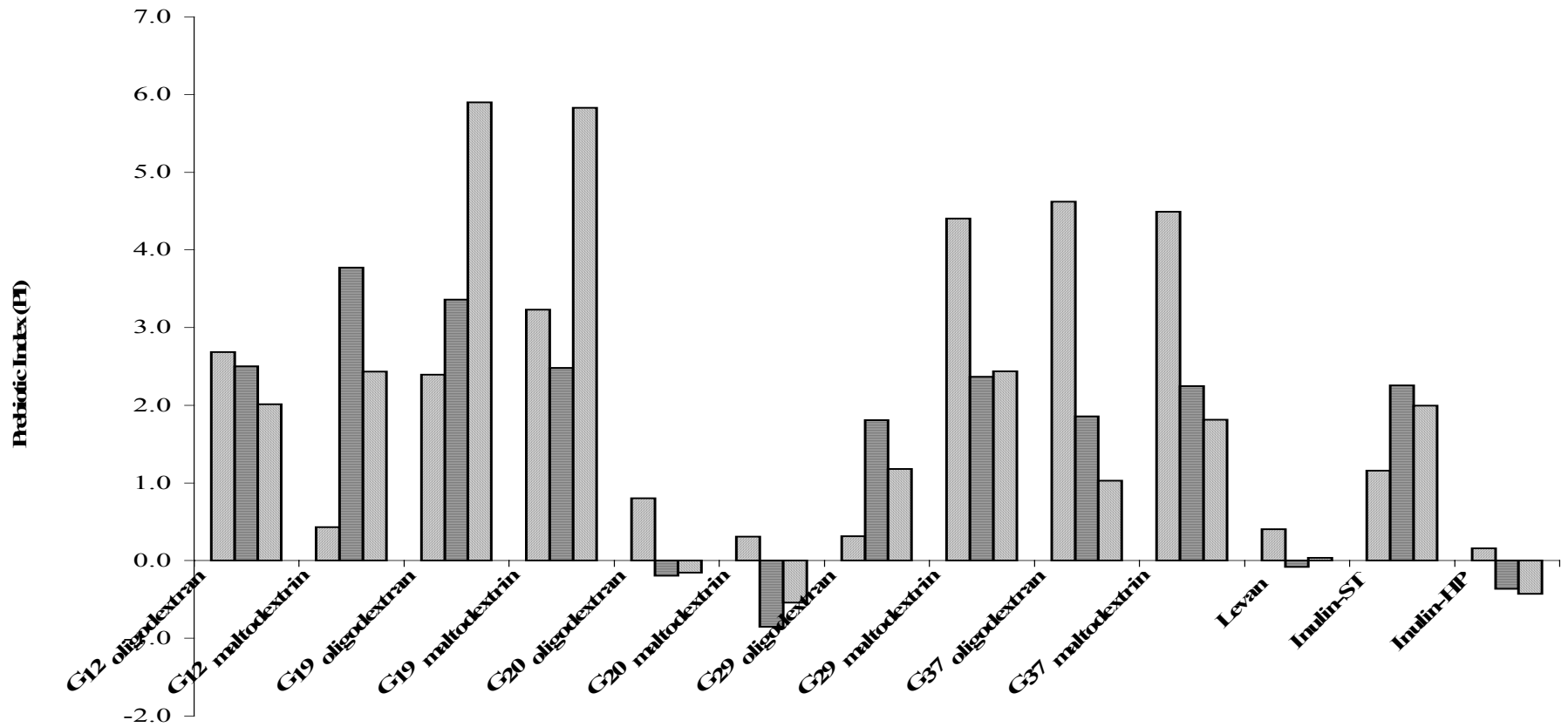


Figure 3.53 Prebiotic index (PI) scores from stirred pH-controlled batch culture fermentations of 1% (w/v) G12, G19, G20, G29, G37 oligodextrans, G12, G19, G20, G29, G37 maltodextrins, levan, inulin-ST and inulin-HP at 6 (□), 24 (▨) and 48 h (■) fermentations

4.3.2 Three-stage continuous system (gut model) fermentation

4.3.2.1 Microbial population change

A. Vessel 1

Microbial population changes in three-stage continuous system by G19 oligodextran in vessel 1 (V1, represented proximal colon) is given in Figure 3.54. Total bacterial number was small but significantly increased ($P<0.05$) after carbohydrate substrate was fed at day 10 and the numbers kept constant till the end of experiment. After feeding the carbohydrate tested, gradually increase of bifidobacteria was observed while lactobacilli appeared in large numbers as fermentation time increased and was constant till the end of experiment. G19 oligodextran did not support the growth of bacteroides throughout the fermentation. Clostridia significantly ($P<0.05$) decreased after carbohydrate tested was fermented.

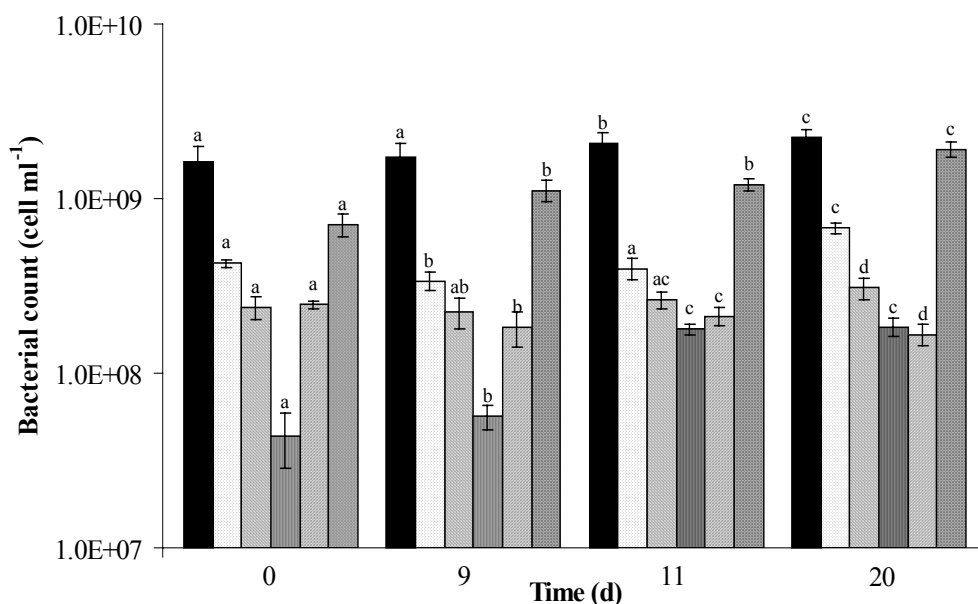


Figure 3.54 Changes in bacterial populations enumerated using FISH technique in V1 of three-stage continuous system and 1% (w/v) G19 oligodextran was supplemented during day 10 to 20

■, total bacterial counts; □, bifidobacteria; ▒, bacteroides; ▒, lactobacilli/enterococci; ▒, clostridia (perfringens/histolyticum subgroup); ▒, Eubacterium rectale-Clostridium coccoides subgroup

Different letters (a, b, c, d) mean significant differences ($P<0.05$) for the same bacterial species

B. Vessel 2

In vessel 2 (V2, represented transverse colon), slightly increase of total bacteria numbers with the increase of bifidobacteria was observed after day 11 (Figure 3.55). Significant ($P<0.05$) increase of lactobacilli appeared after supplementation of carbohydrate tested in the basal medium. Nevertheless, this had no influence on the numbers of bacteroides whereas clostridia numbers slightly decreased. The amount of eubacteria was high compared to other members and declined at the end of experiment.

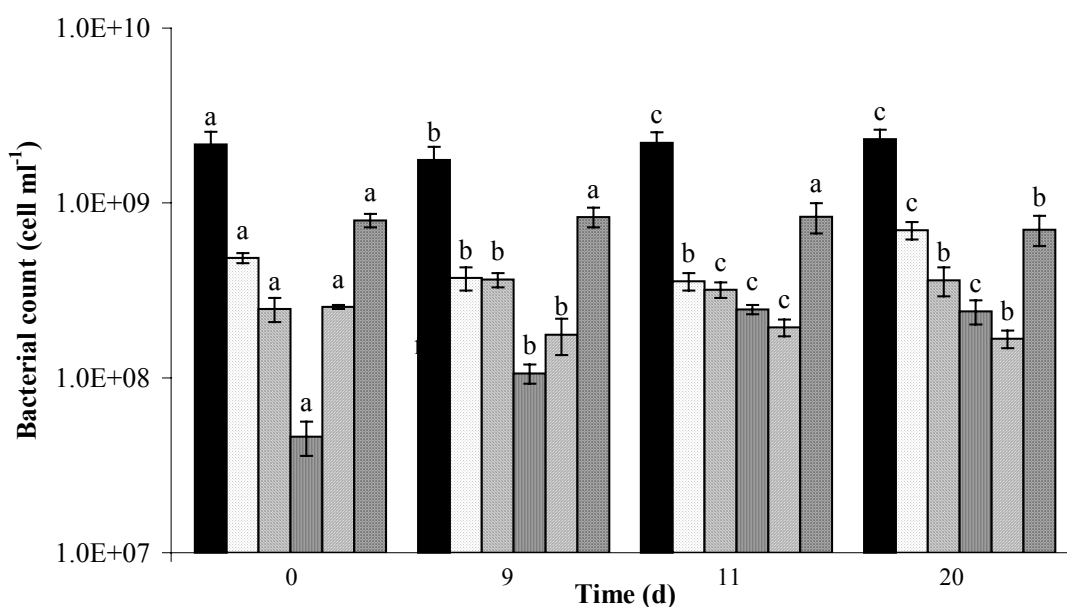


Figure 3.55 Changes in bacterial populations enumerated using FISH technique in V2 of three-stage continuous system and 1% (w/v) G19 oligodextran was supplemented during day 10 to 20

■, total bacterial counts; □, bifidobacteria; ▒, bacteroides; ▒, lactobacilli/enterococci; ▒, clostridia (perfringens/histolyticum subgroup); ▒, Eubacterium rectale-Clostridium coccoides subgroup

Different letters (a, b, c, d) mean significant differences ($P<0.05$) for the same bacterial species

C. Vessel 3

In vessel 3 (V3, represented distal colon), total bacteria significantly ($P<0.05$) increased (Figure 3.56). Bifidobacteria increased after day 11 while lactobacilli clearly increased. The numbers of clostridia decreased at day 9, no change at day 11 and slightly increase was observed at the end of fermentation. Eubacteria seemed to decrease after G19 oligodextran was fermented at day 11 but they increased again at the end of fermentation.

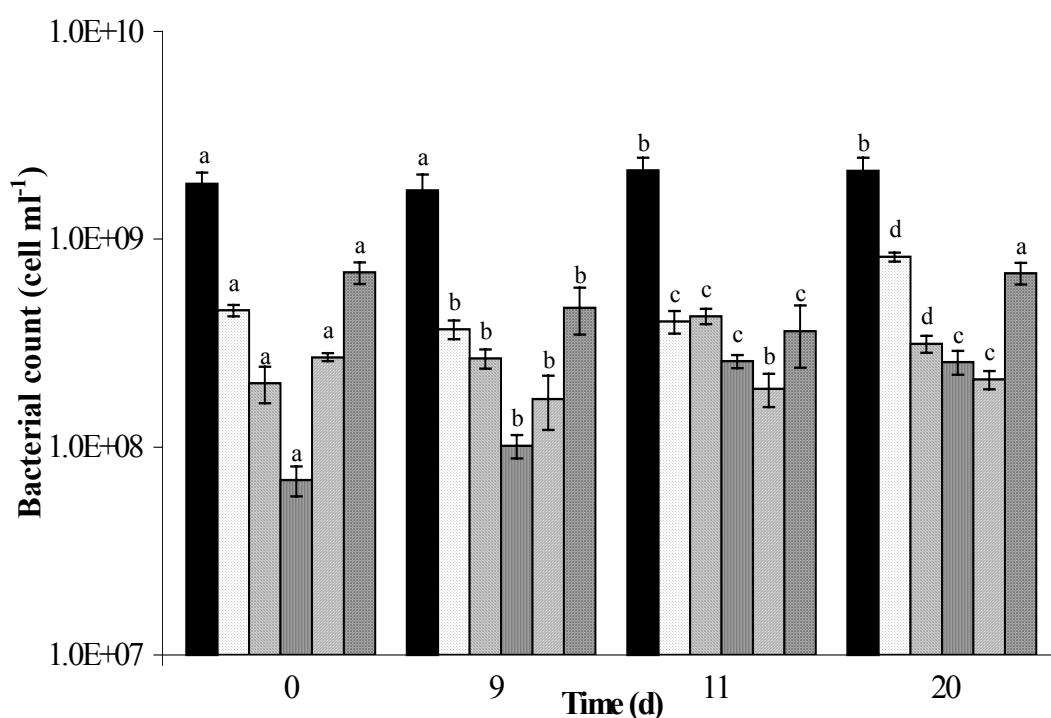


Figure 3.56 Changes in bacterial populations enumerated using FISH technique in V3 of three-stage continuous system and 1% (w/v) G19 oligodextran was supplemented during day 10 to 20

■ , total bacterial counts; □ , bifidobacteria; ▨ , bacteroides; ▩ , lactobacilli/enterococci; ▪ , clostridia (perfringens/histolyticum subgroup); ▩ , Eubacterium rectale-Clostridium coccoides subgroup
Different letters (a, b, c, d) mean significant differences ($P<0.05$) for the same bacterial species

4.3.2.2 Short-chain fatty acids (SCFA) production

Short-chain fatty acids obtained from fermentation of G19 oligodextran in three-stage continuous system are summarized in Table 3.9. In V1, the concentration of lactate was slightly increased (from 9.18 mmol l⁻¹ to 17.04 mmol l⁻¹ at day 11) after the substrate tested was utilized by human colonic microflora, however it was reduced thereafter. High concentration of formate was detected at day 11 and reduced thereafter. Very high concentration of acetate was found, especially after fermentation (after day 11) of the carbohydrate tested and kept constant thereafter. Propionate was generated after carbohydrate tested was not supplemented (day 0 to 9) and only small quantity of propionate produced from carbohydrate tested (day 11 to 20) whereas butyrate (23.42 mmol l⁻¹) was detected after fermentation of the carbohydrate tested at day 11 and decreased thereafter. In V2, concentrations of lactate and formate were lower than those obtained from V1. On the other hand, acetate concentration in V2 was higher concentration than those from V1 and it increased as fermentation time increased. Propionate did not much increase after fermentation of the carbohydrate tested. The concentration of butyrate was slightly higher than in V1 and reached the maximum concentration (24.98 mmol l⁻¹) at day 11. In V3, similar profiles and concentrations of SCFA were obtained. This confirmed that G19 oligodextran possesses butyrogenic properties since large quantity of butyrate was produced particularly in V2 and V3.

Table 3.9 SCFA* production by G19 oligodextrans** fermentations in V1, V2 and V3 of three stage continuous system

	V1				V2				V3			
	Day				Day				Day			
	0	9	11	20	0	9	11	20	0	9	11	20
Lactate	9.18	11.25	17.04	5.04	0.00	8.20	5.41	4.39	0.00	4.22	4.97	4.42
	±1.25	±4.50	±4.40	±1.10	±0.00	±1.20	±1.10	±1.12	±0.00	±1.10	±1.08	±0.88
Formate	7.51	13.65	21.07	7.67	9.53	7.19	2.20	1.22	8.22	1.27	1.41	1.14
	±2.20	±5.56	±3.45	±2.00	±1.56	±2.12	±0.86	±0.29	±2.00	±0.20	±0.89	±0.10
Acetate	48.10	49.62	81.73	73.92	57.43	65.56	71.90	96.09	60.60	66.61	69.48	101.02
	±9.00	±9.09	±14.40	±11.18	±8.80	±15.03	±9.86	±18.89	±9.90	±8.80	±9.00	±16.69
Propionate	23.01	29.91	31.99	33.45	35.40	50.92	56.37	55.54	35.78	52.01	57.62	53.82
	±7.69	8.05	±8.98	±8.90	±5.60	±11.05	±12.20	±13.80	±4.59	±6.60	±8.90	±9.78
Butyrate	0.00	0.00	23.42	19.47	0.00	2.72	24.98	24.28	0.00	0.00	19.65	27.53
	±0.00	±0.00	±3.88	±4.50	±0.00	±0.99	±5.50	±8.50	±0.00	±0.00	±5.20	±4.50

* Values were means±SD from duplicate samples in mmol l⁻¹ concentrations at day 0, 9, 11 and 20 of fermentation

** 1% (w/v) G19 oligodextrans was supplemented in basal medium during day 10 to 20

4.3.2.3 Prebiotic index calculation

Prebiotic index was highest value at day 20 except in vessel 1, giving prebiotic index of $V2 > V3 > V1$ whereas their prebiotic index was $V1 > V3$ at day 9 of fermentation. It clearly indicated that G19 oligodextran had the highest (PI of 4.23) prebiotic properties in V2 at day 20 of fermentation (Figure 3.57) in three-stage continuous system.

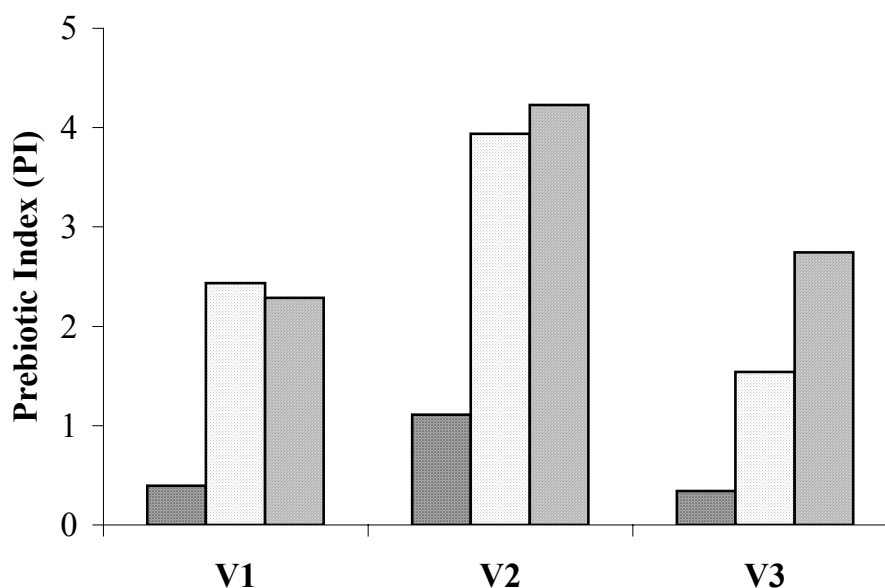


Figure 3.57 Prebiotic index (PI) scores in V1, V2 and V3 from three-stage continuous system with the supplementation of 1% (w/v) G19 oligodextran during day 10 to 20 of fermentation
 ■, day 9; □, day 11; ▨, day 20

Part of the α -1,4 linked glucose polymers contained in the oligodextran might reach the colon intact, to be metabolized by the gut microflora using the following route. Metabolism of α -1,4 linked glucose polymers (maltodextrins) of up to seven glucose units has been found in some microorganisms (*E. coli*, lactobacillus, fusobacterium) residing in the human colon using the maltose/maltodextrin transportation system (Boos and Shuman, 1998). Incoming maltose and maltodextrins of up to seven glucose moieties are metabolized to glucose and glucose-1-phosphate by the combined action of three cytoplasmic enzymes, amyloamylase, maltodextrin

phosphorylase, and maltodextrin glucosidase. Since maltodextrins larger than six glucose moieties are not very well transported, they are reduced in size by a periplasmic amylase (Boos and Shuman, 1998).

Oligodextran is thought to be fermented by enzymes produced from saccharolytic clostridia, bacteroides and bifidobacteria with similar to the fermentation of resistant starch (Cummings and Englyst, 1995; Topping and Clifton, 2001) whereas fermentation of starch gave rise of bacteroides, clostridia and *E. coli* (Roberfroid *et al.*, 1998). Lactobacillus strain such as *L. acidophilus* (cell envelop associated amylase), *L. fermentum* (extracellular amylase) possessed amylase activity that able to hydrolyze starch which escape digestive system (Lee *et al.*, 2001) which might be responsible to the metabolism of oligodextran. High amylose (linear α -1,4 D-glucose) starch was reported to give slightly higher stimulation on bifidobacteria compared to low amylose starch (Topping *et al.*, 2003) which according to this study that oligodextrans with different ratio of α -1,6- and α -1,4 linkages showed different degree of stimulation on bifidobacteria and gave lower bifidogenic effect compared to resistant starch.

The difference in fermentability of inulin and oligodextran was observed. SCFA produced by fermentation of inulin either in transverse and distal colon gave similar concentration suggested inulin was fermented completely at transverse colon whereas G19 oligodextran reached the distal. This means G19 oligodextran has longer chain-length thus needs longer fermentation time and shifts the butyrate-producing region to more distal where colon cancer is more often found. Thus G19 oligodextran is more targeted-site of colon cancer than inulin. Generally, the fermentation of oligodextrans in batch and continuous culture produced acetate > propionate > butyrate in accordance to the theoretical fermentation of the hexose sugars such as inulin, oligofructose, polydextrose, pectin, arabinogalactan except starch whose butyrate > propionate (Topping and Clifton, 2001; Cummings *et al.*, 2001). The molar ratio of acetate:lactate of 5:1 obtained from this study suggested bifidus pathway did not dominate the fermentation of oligodextrans by faecal flora because theoretically the bifidus pathway will ferment hexose sugars to give a molar ratio of 1.5:1 (Bezkorovainy, 1989).