APPENDIX

1. G. *oxydans* cell dry weight calibration

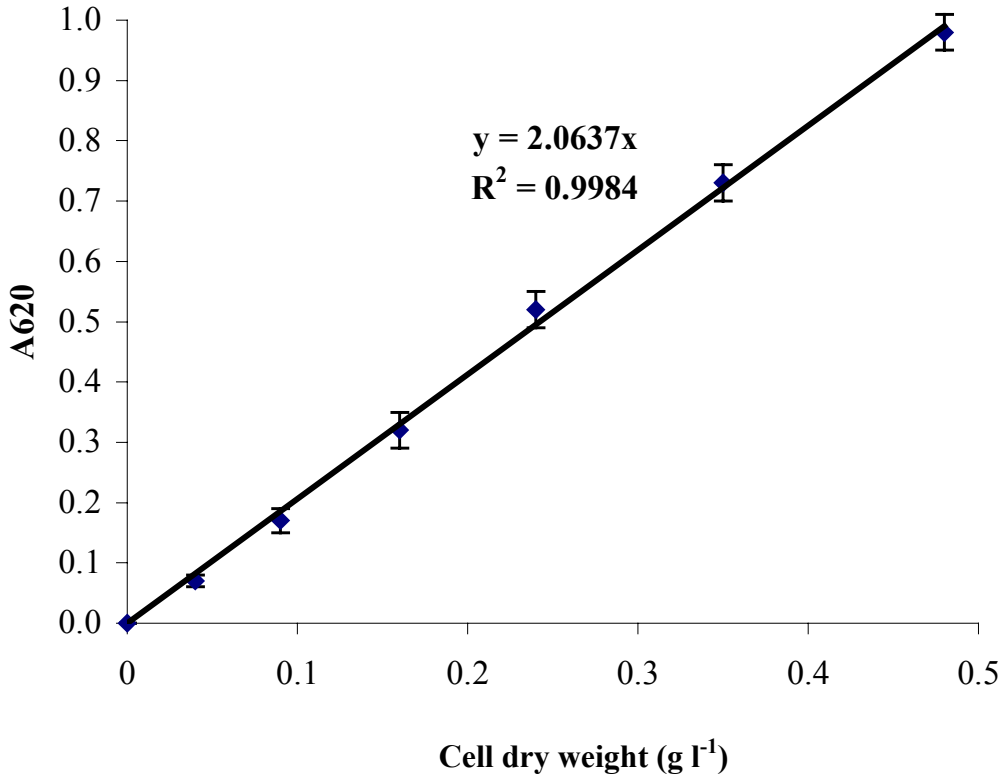


Figure 1.1 Calibration curve for the determination of *G. oxydans* cell dry weight by absorbance measurements at 620 nm

2. Total sugar concentration by the phenol-sulphuric acid assay

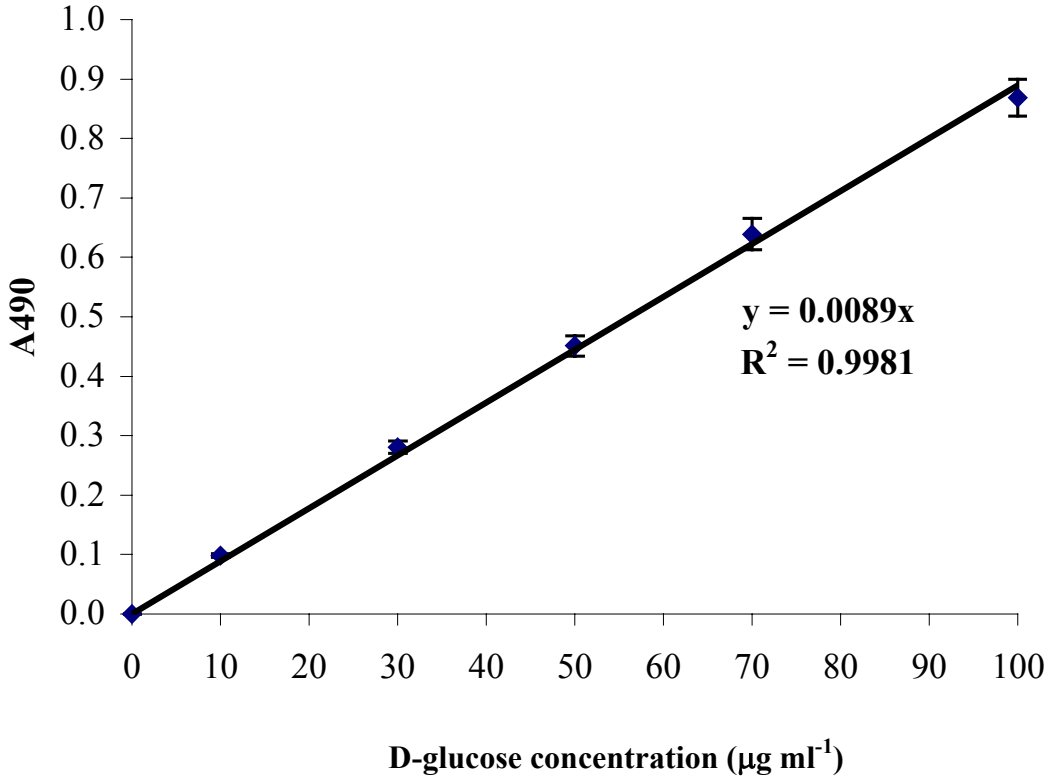


Figure 2.1 Calibration curve for the phenol-sulphuric acid assay (Dubois *et al*., 1956) for the determination of total sugar concentration expressed in glucose equivalents (µg ml^{-1}). Points are means ± s.d. of assays done in triplicate

3. Reducing sugar concentration by the dinitro-salicylic acid assay (DNS)

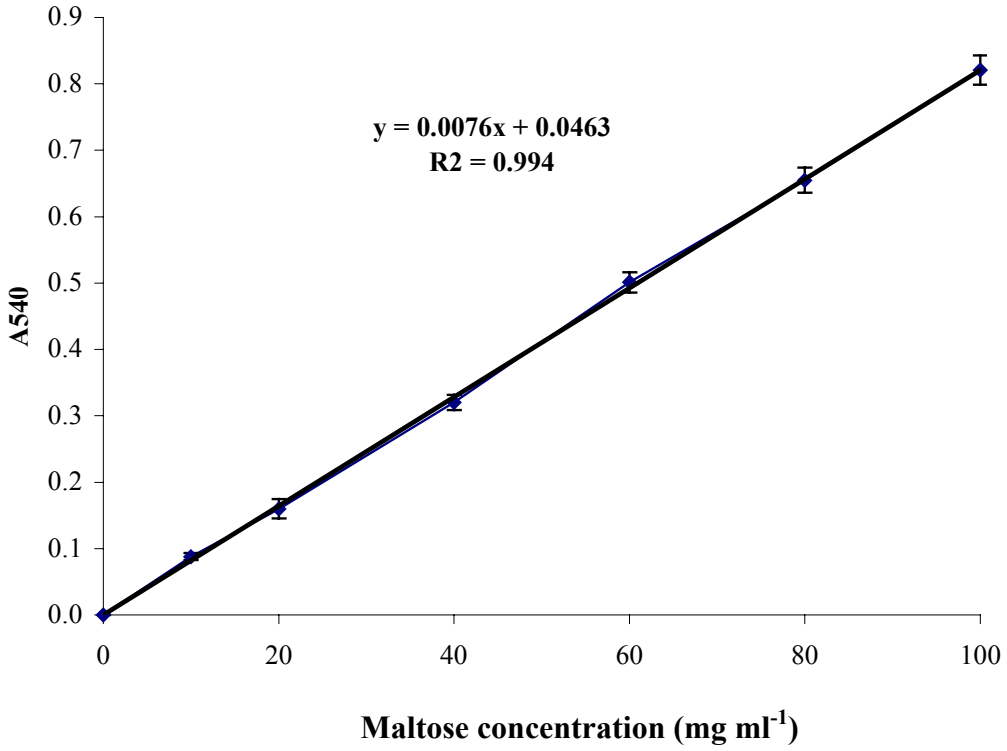


Figure 3.1 Calibration curve for the DNS assay for the determination of reducing sugar concentration expressed in maltose equivalents (mg ml^{-1}). Points are means ± s.d. of assays done in triplicate

4. Calibration of the high performance size exclusion chromatographic system (HPSEC)

Table 4.1 Elution characteristics of the dextran standards used to calibrate the HPSEC system and shown as molecular weight markers on all the HPSEC chromatograms in this work

Labels used in graphs	Dextran standard MW (kDa)[+]	Elution time (min)[*]	Elution volume V_e (ml)[**]	K_{av}[#]	Log_{10} MW
A (V_0)	38000	28.67	17.20	0	7.58
B	2000	29.70	17.82	0.039	6.30
C	464	36.21	21.72	0.285	5.67
D	260	36.80	22.08	0.308	5.41
	68.4	40.22	24.13	0.438	4.84
E	42	42.29	25.37	0.516	4.62
F	19.5	45.02	27.01	0.620	4.29
G	9.5	46.43	27.86	0.673	3.98
H	1.15	49.69	29.81	0.796	3.06
I	0.18	52.08	31.25	0.887	2.26

[+]: Molecular weight supplied by the manufacturer (Sigma Chemicals, Poole, UK).

[++]: Dextran 260 kDa used in the MW calibration of the HPSEC system but it is not shown in the HPSEC graphs in this work

[*]: Determined chromatographically. Values presented are means of determinations done in duplicate

[**]: Calculated by multiplying the appropriate elution time (min) by the eluent flowrate (0.6 ml min^{-1})

[#]: Calculated by the following formula (Nilsson and Nilsson, 1974): $\mathbf{K_{av} = V_e - V_0}$ where $\mathbf{K_{av}}$ is the partition coefficient, $\mathbf{V_e}$ is the elution volume of the sample at peak height, $\mathbf{V_0}$ is the void volume of the system taken at peak height of dextran 38000 kDa and $\mathbf{V_t}$ is the total volume of the system taken at the end of glucose elution (i.e. elution time 52.08 min corresponding to elution volume 31.25 ml)

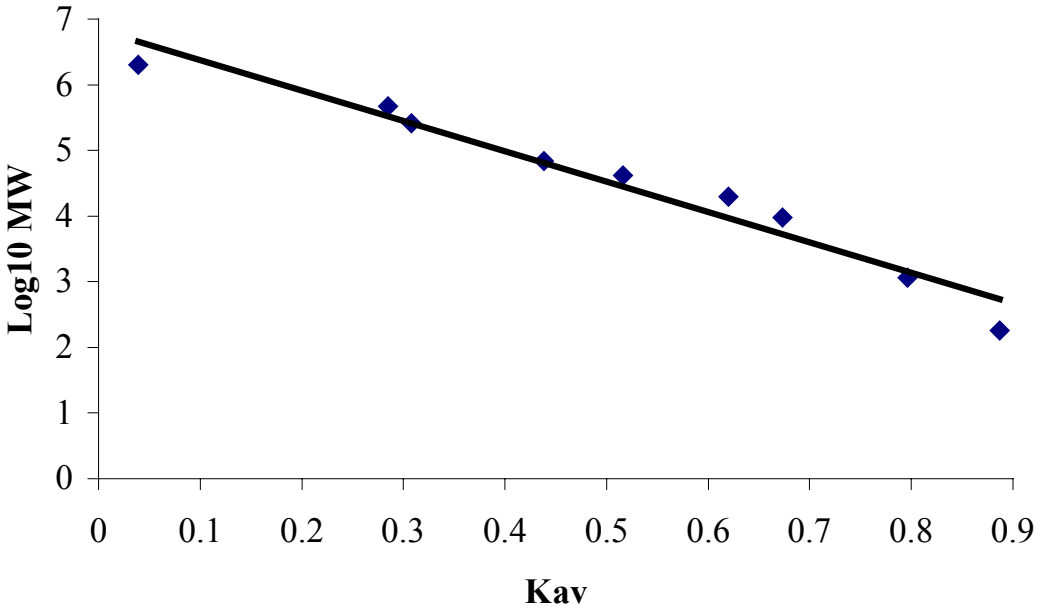
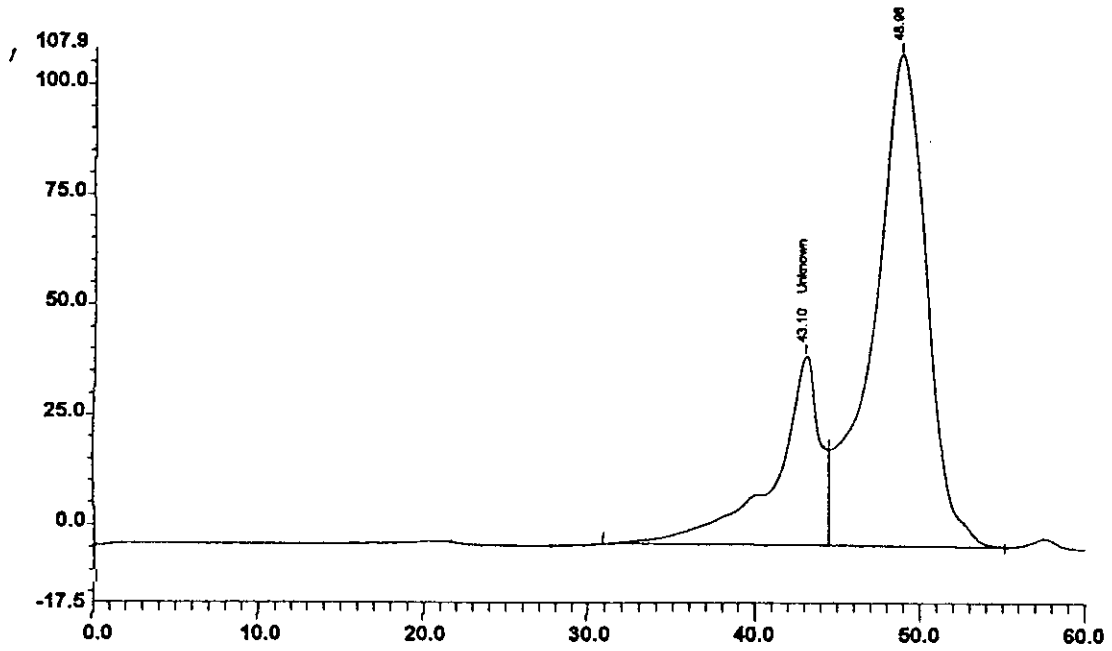


Figure 4.2 Molecular weight calibration curve for the HPSEC system

5. Calculation of the oligodextran yield

a)



b)

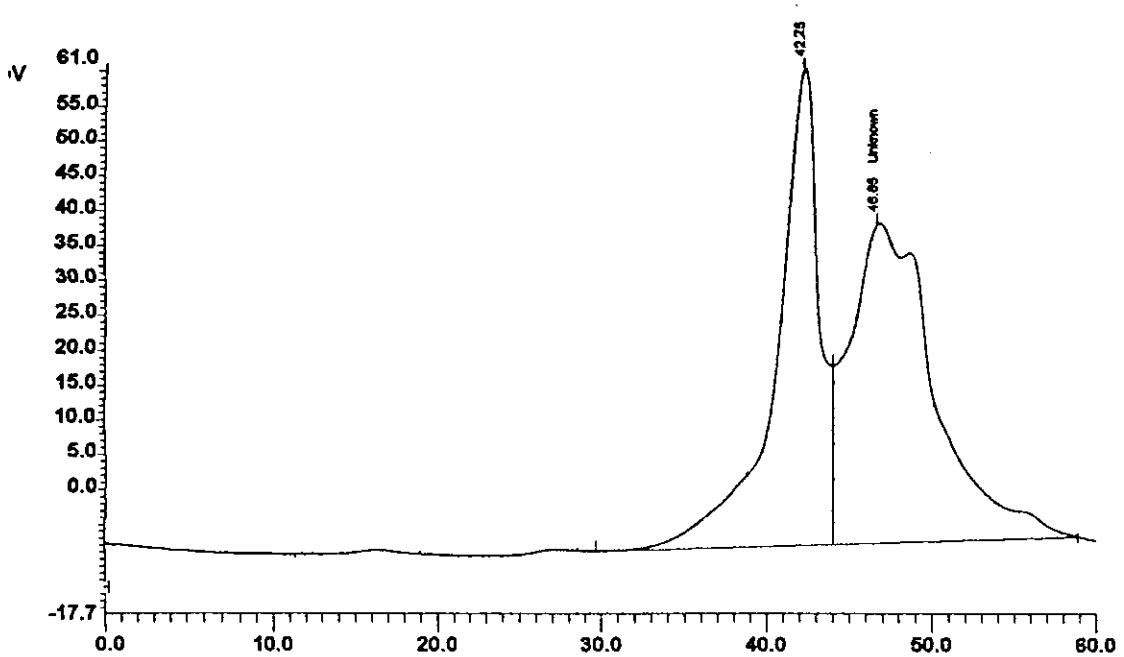


Figure 5.1 Chromatograph of maltodextrin substrate (a) and oligodextran product (b) obtained by HPSEC

Calculation:

Oligodextran yield (%) = $(X_t - X_0) / (X_t + Y_t) \times 100$

X_t = area of oligodextran peak at each cultivation time point

X_0 = area of maltodextrin substrate peak (0 h cultivation) eluted at about 42 min

Y_t = area of maltodextrin substrate peak at each cultivation time point eluted at about 50 min

Example. Chromatography of oligodextran produced from Goldex 20 maltodextrin was calculated as followed.

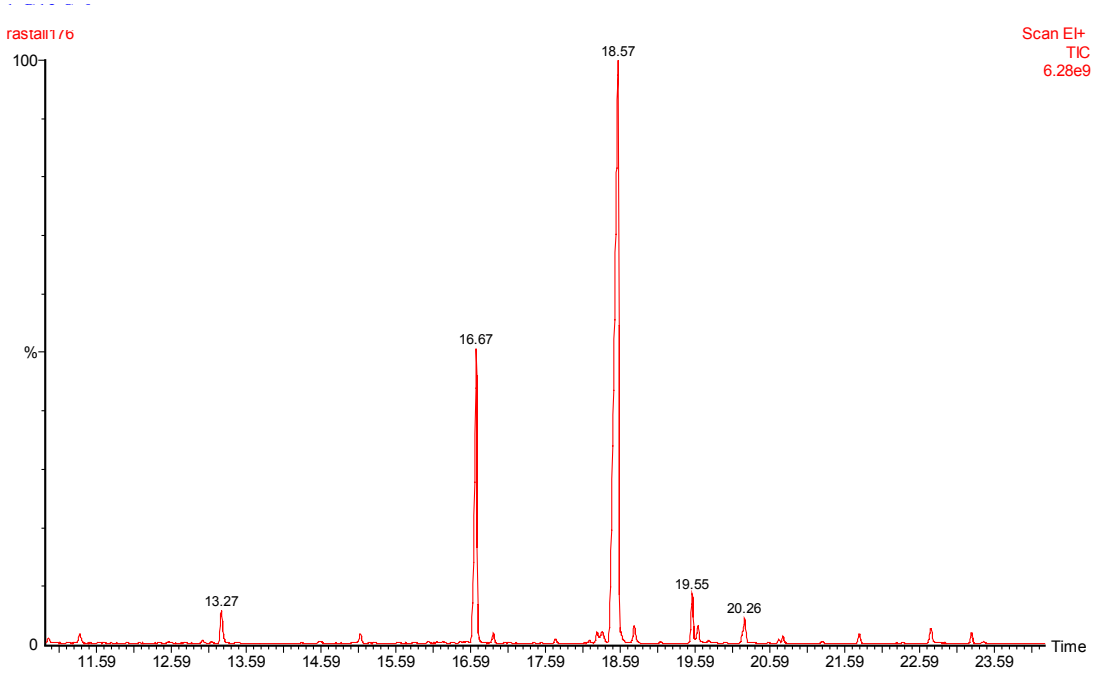
G20 oligodextran yield (%) = [(128761005 - 86214791)/(128761005 + 171575642)] × 100

= (42546214 / 300336647) × 100

= 14.17

6. Calculation of the ratio of linkages obtained by methylation

a)



b)

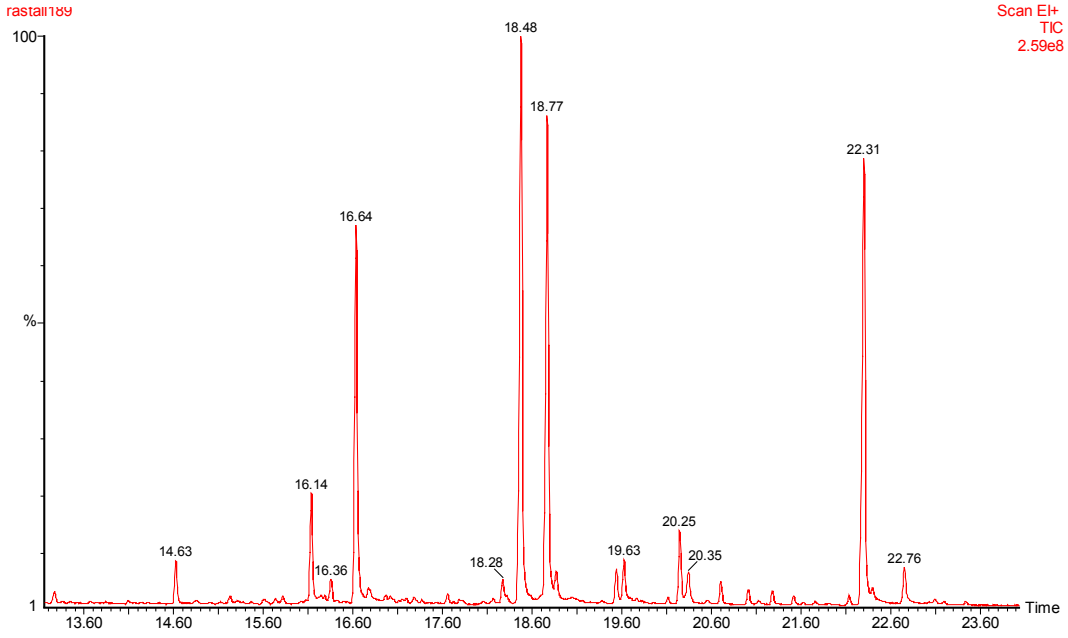


Figure 6.1 Chromatograph of G19 maltodextrin substrate (a) and G19 oligodextran product (b) obtained by GC-MS

Calculation:

Ratio of α-1,4 = A/t

A = area peak of 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylhexitol (indicated 1,4 linkage) eluted at 18.57 min

t = area peak of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylhexitol (indicated terminal residue) eluted at 16.67 min

Ratio of α-1,6 = B/t

B = area peak of 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylhexitol (indicated 1,6 linkage) eluted at 18.77 min

Ratio of α-1,4,6 = C/t

C = area peak of 1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methylhexitol (indicated 1,4,6 linkage) eluted at 20.25 min

7. Calculation of degree of polymerization (DP) by ^{1}H-NMR

a)

b)

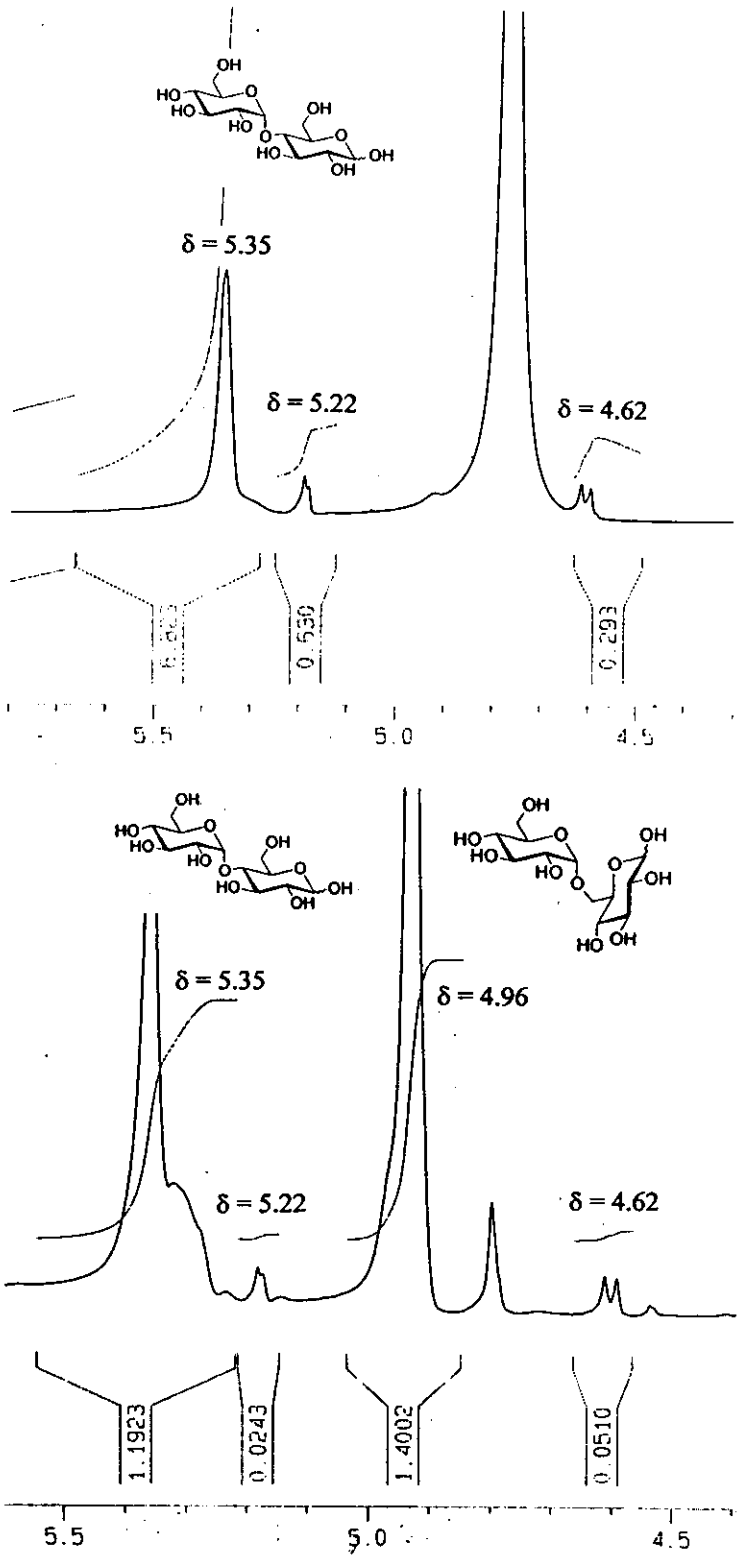


Figure 7.1 ^{1}H-NMR of C_1 of Glucidex 19 maltodextrin substrate (a) and G19 oligodextran (b)

Calculation:

DP of residue linked by 1,4 = A/t

A = area peak of 1,4 linked at 5.35 ppm

t = summary of area peak of α-terminal at 5.22 ppm and β-terminal at 4.64 ppm

DP of residue linked by 1,6 = B/t

B = area peak of 1,6 linked at 4.96 ppm

8. Values of the responses used for prediction on oligodextran hydrolyzed by human amylases

Table 8.1 Numeric values of the responses used in the persistence of the G19 oligodextran to human salivary amylase using response surface methodology

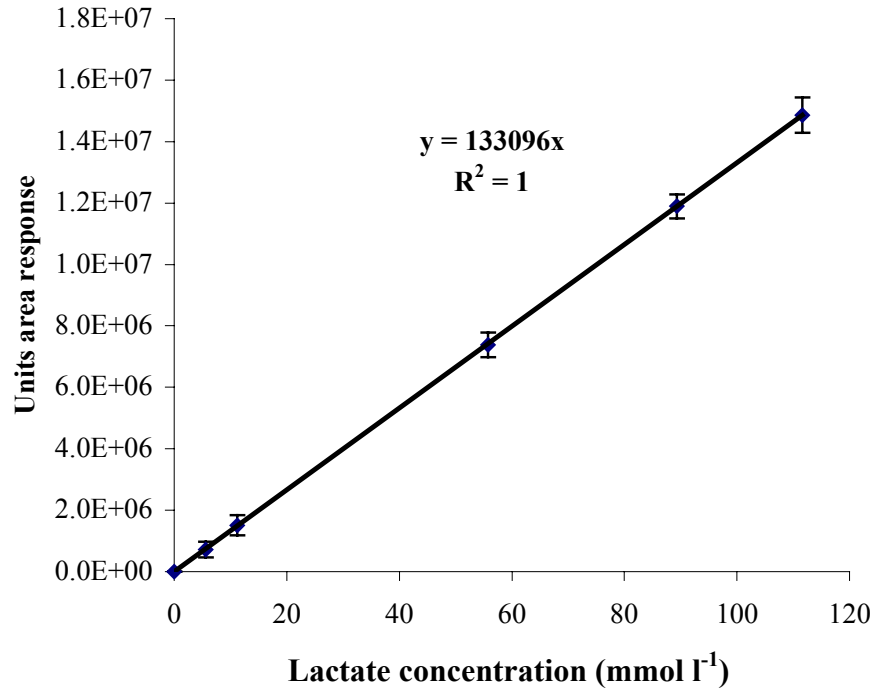
Run	**Reaction time (h)**	**Buffer pH**	**Enzyme conc. (U ml^{-1})**	**Hydrolysis (%)**
1	2	7	1.5	23.68
2	2	7	1.5	22.74
3	4	10	2	22.42
4	0	4	1	0
5	2	7	0.66	7.82
6	4	4	1	17.18
7	4	10	1	22.08
8	4	4	2	21.64
9	5.36	7	1.5	21.84
10	0	4	2	0
11	0	7	1.5	0
12	2	7	1.5	20.82
13	2	7	1.5	22.46
14	2	7	1.5	21.52
15	2	7	2.34	21.88
16	0	10	1	0
17	2	12.05	1.5	5.34
18	2	7	1.5	24.36
19	2	1.95	1.5	4.78
20	0	10	2	0

Table 8.2 Numeric values of the responses used in the persistence of the G19 oligodextran to human pancreatic amylase using response surface methodology

Run	**Reaction time (h)**	**Buffer pH**	**Enzyme conc. (U ml^{-1})**	**Hydrolysis (%)**
1	0	8	1	0
2	2.68	6	1.5	18.48
3	2	4	2	0
4	1	6	1.5	16.6
5	1	2.64	1.5	3.8
6	0	8	2	0
7	1	9.36	1.5	10
8	1	6	1.5	17.26
9	1	6	1.5	16.44
10	2	4	1	3.4
11	1	6	2.34	16.76
12	1	6	1.5	15.46
13	2	8	2	17.62
14	0	4	1	0
15	1	6	1.5	17.38
16	1	6	0.66	12.3
17	0	6	1.5	0
18	0	4	2	0
19	1	6	1.5	17.46
20	2	8	1	18.14

9. Area response for the quantification of SCFA by HPLC

a)



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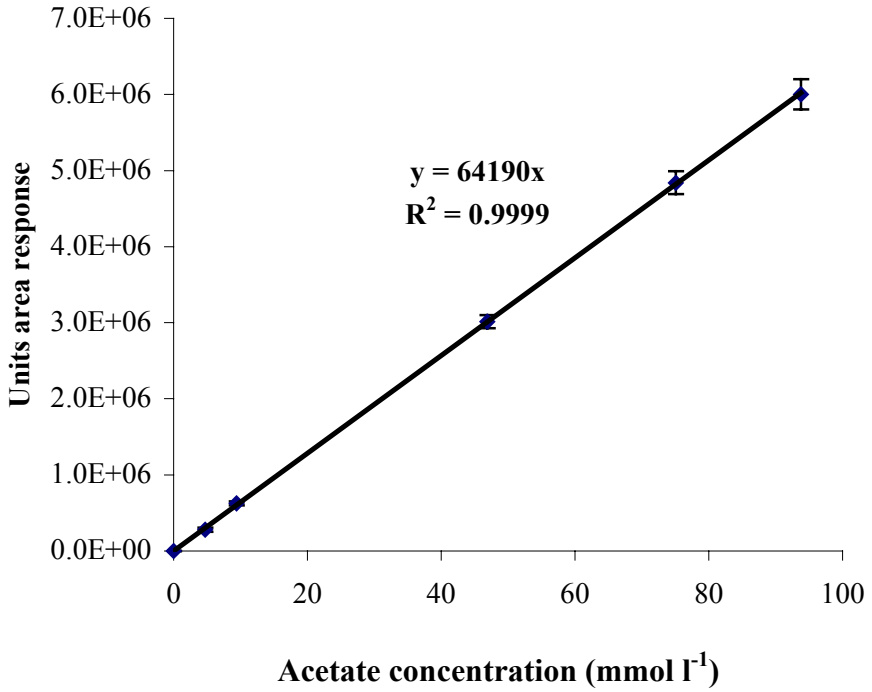

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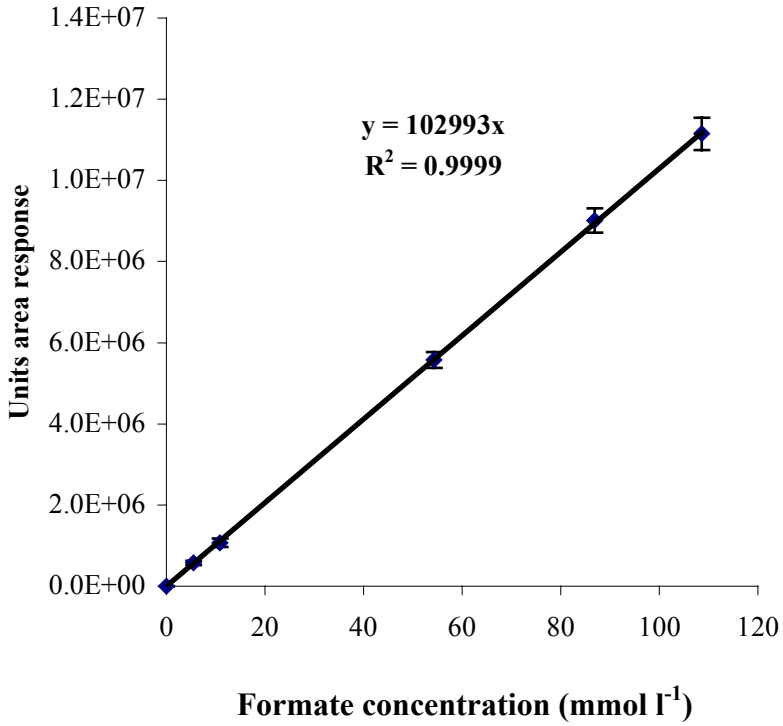
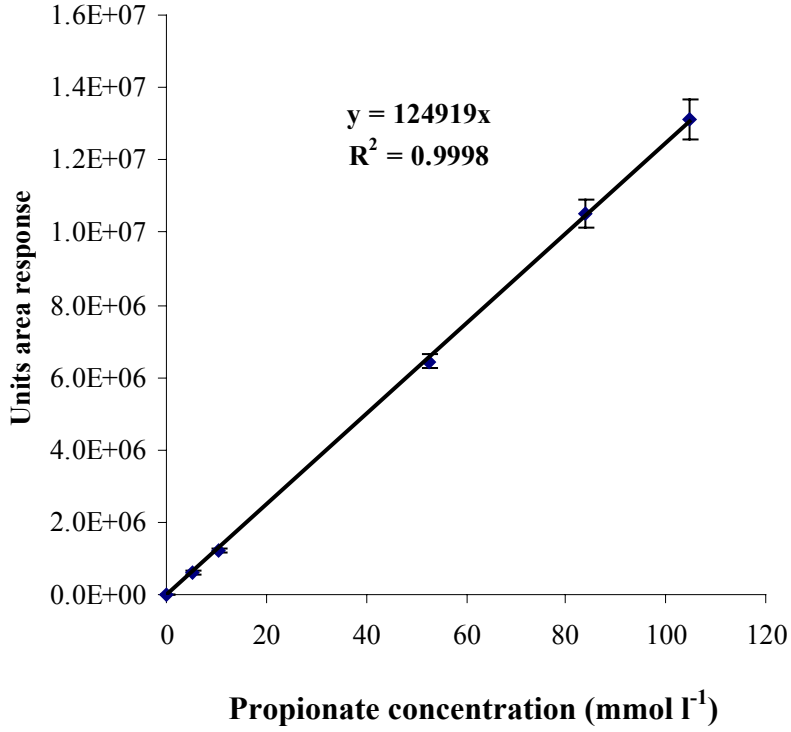

1.4E+07
1.2E+07
1.0E+07
8.0E+06
6.0E+06
4.0E+06
2.0E+06
0.0E+00
Units area response
y = 102993x
R2 = 0.9999
0
20
40
60
80
100
120
Formate concentration (mmol l-1)

d)


1.6E+07
1.4E+07
1.2E+07
1.0E+07
8.0E+06
6.0E+06
4.0E+06
2.0E+06
0.0E+00
Units area response
y = 124919x
R2 = 0.9998
0
20
40
60
80
100
120
Propionate concentration (mmol l-1)

e)

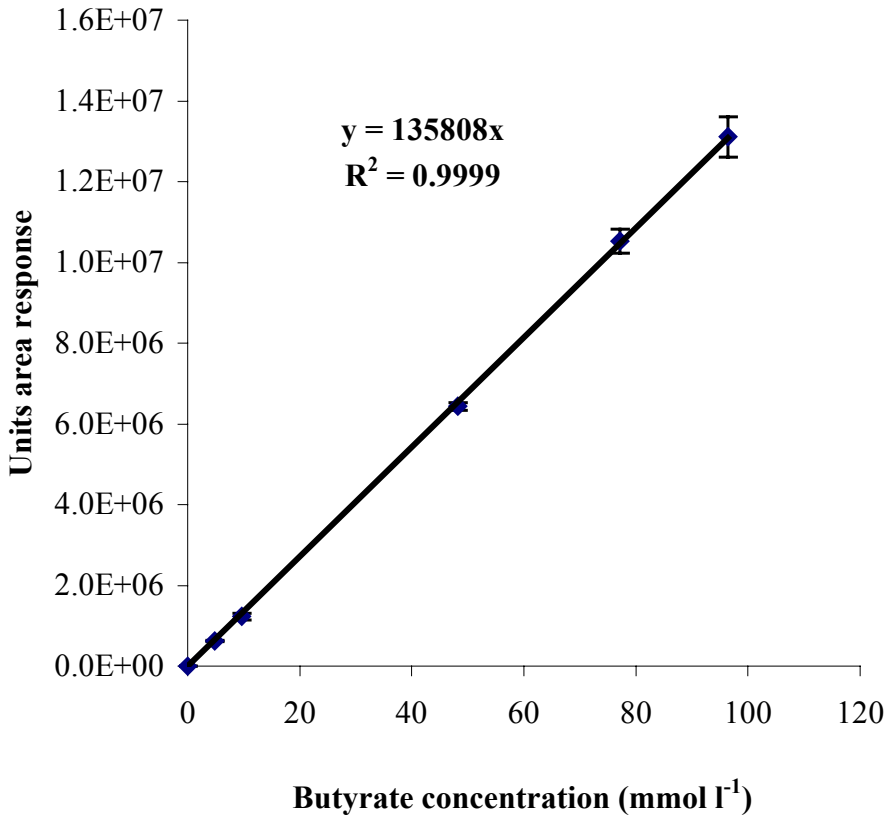


Figure 9.1 Calibration curves for the quantification of SCFA by HPLC with UV detection at 210 nm. The column was Aminex HPX-87H and the eluent was 0.005 M H_2SO_4 at 50°C. Points are means ± s.d. of determinations done in triplicate for lactate (a); acetate (b), formate (c); propionate (d) and butyrate (e) at each concentration.

10. Photography of the experiment

Figure 10.1 Growth of *G. oxydans* NCIMB 4943 on GYC medium

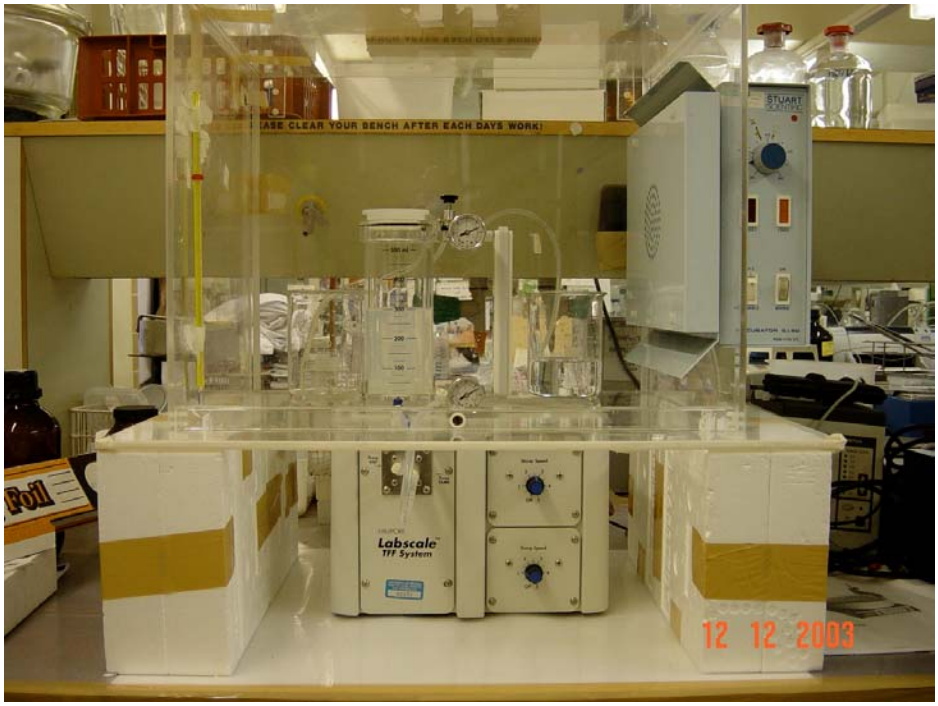


Figure 10.2 Assembly of lab-scale ultrafiltration system

Figure 10.3 Pilot-scale fermenter (150-l) for production of oligodextran

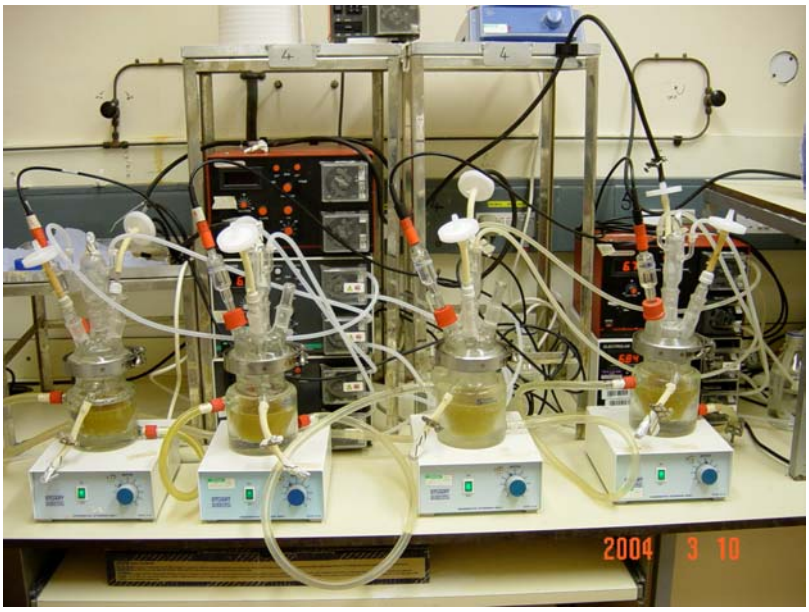


Figure 10.4 Stirred pH-controlled batch culture for fermentation studies

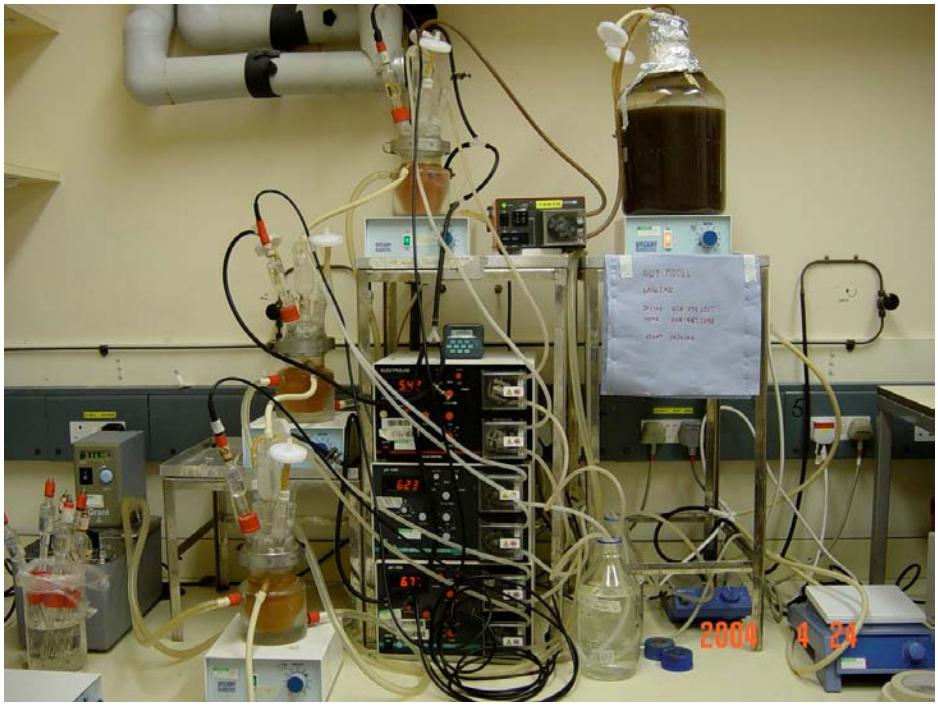


Figure 10.5 Three-stage continuous system (gut model) for fermentation of G19 oligodextran

PUBLICATION

1. S. Wichienchot, P. Prasertsan, T. Hongpattarakere and R.A. Rastall. 2005. Manufacture of oligodextrans by *Gluconobacter oxydans* NCIMB 4943. Enz. Microb. & Technol. (In press).

2. S. Wichienchot, P. Prasertsan, T. Hongpattarakere, R.A. Rastall and G.R. Gibson. 2005. *In vitro* Fermentation of Mixed Linkage Gluco-oligosaccharides Produced by *Gluconobacter oxydans* NCIMB 4943 on the Human Colonic Microflora. Curr. Issu. Intes. Microbiol. (In Press).

3. S. Wichienchot, P. Prasertsan, T. Hongpattarakere, R.A. Rastall and G.R. 2005. *In vitro* Fermentation of Gluco-oligosaccharides Produced by *Gluconobacter oxydans* NCIMB 4943 on the Human Colonic Microflora in Three-stage Continuous System. Curr. Issu. Intes. Microbiol. (In Press).