Figure 1a

Wild type ptr (protease III) 5'.

```
  *  M  P  R  S  T  W  F  K  A  L  L  L  L  V
TGA ATG CCC CGC AGC ACC TGG TTC AAA GCA TTA TTG TTG TTA GTT

  A  L  W  A  P  L  S
GCC CTT TGG GCA CCC TTA AGT
```

Mutated Δ ptr (protease III) 5'.

```
    EcoR I
    ~~~~~~~~
  *  I  P  R  S  T  W  F  K  A  L  L  L  L  V
TGA ATT CCC CGC AGC ACC TGG TTC AAA GCA TTA TTG TTG TTA GTT

                      Ase I
                      ~~~~~~~~
  A  L  W  A  H  *  C
GCC CTT TGG GCA CAT TAA TGT
```

Figure 1b

Wild type Tsp 5'.

```
  M  N  M  F  F  R  L  T  A  L  A  G  L  L  A
ATG AAC ATG TTT TTT AGG CTT ACC GCG TTA GCT GGC CTG CTT GCA

  I  A  G  Q  T  F  A
ATA GCA GGC CAG ACC TTC GCT
```

Mutated Δ Tsp 5'.

```
    EcoR I
    ~~~~~~~~
  M  N  S  F  L  G  L  P  R  *  L  A  C  L  Q
ATG AAT TCG TTT TTA GGC TTA CCG CGT TAG CTG GCC TGC TTG CAA

                      Ase I
                      ~~~~~~~~
  *  Q  A  R  H  *  L
TAG CAG GCC AGA CAT TAA TTG
```

Figure 1c

Wild type DegP

202	D	A	A	I	N	R	G	N	**S**	G	G
949	GAT	GCA	GCG	ATC	AAC	CGT	GGT	AAC	***TCC***	GGT	GGT

Mutated DegP S210A

Ase I

202	D	A	A	I	N	R	G	N	**A**	G	G
949	GAT	GCA	GCG	***ATT***	***AAT***	CGT	GGT	AAC	***GCC***	GGT	GGT

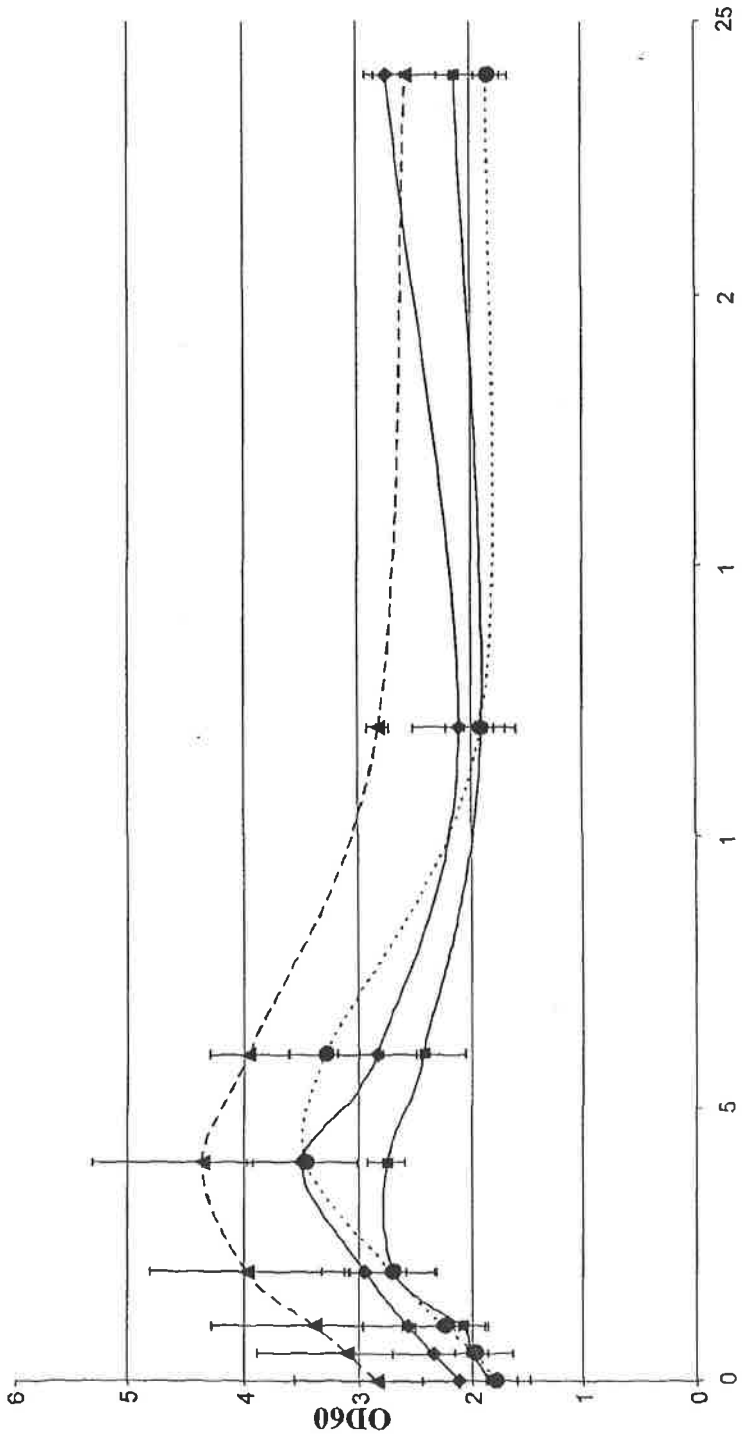

OD60
Time hrs
W3110 pMXE117
MXE005 (DegP S210A, Δ Tsp) pMXE117
W3110 pMXE117
MXE001(Δ Tsp)

Figure 2

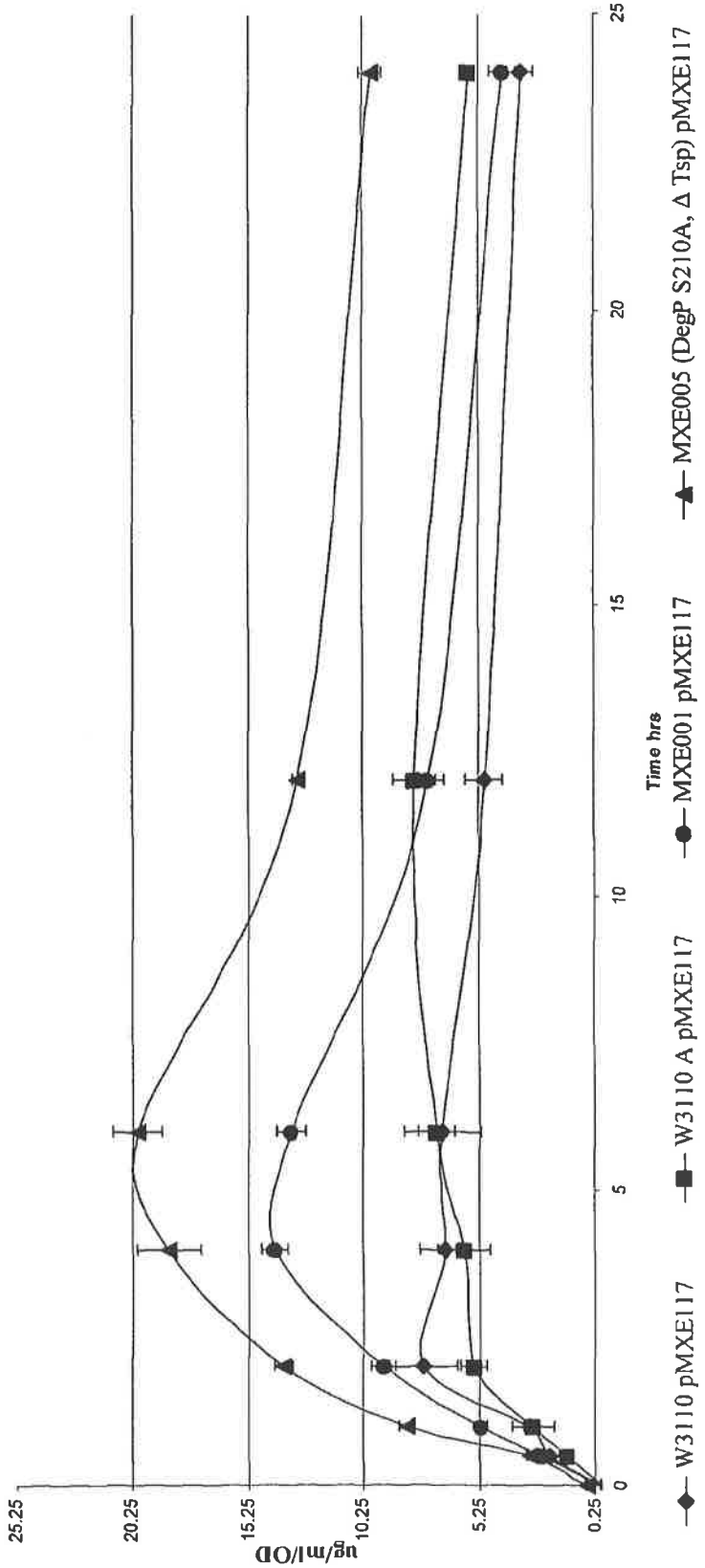

25.25
20.25
15.25
10.25
5.25
0.25
ug/ml/OD
0
5
10
15
20
25
Time hrs
W3110 pMXE117
W3110 A pMXE117
MXE001 pMXE117
MXE005 (DegP S210A, Δ Tsp) pMXE117

Figure 3

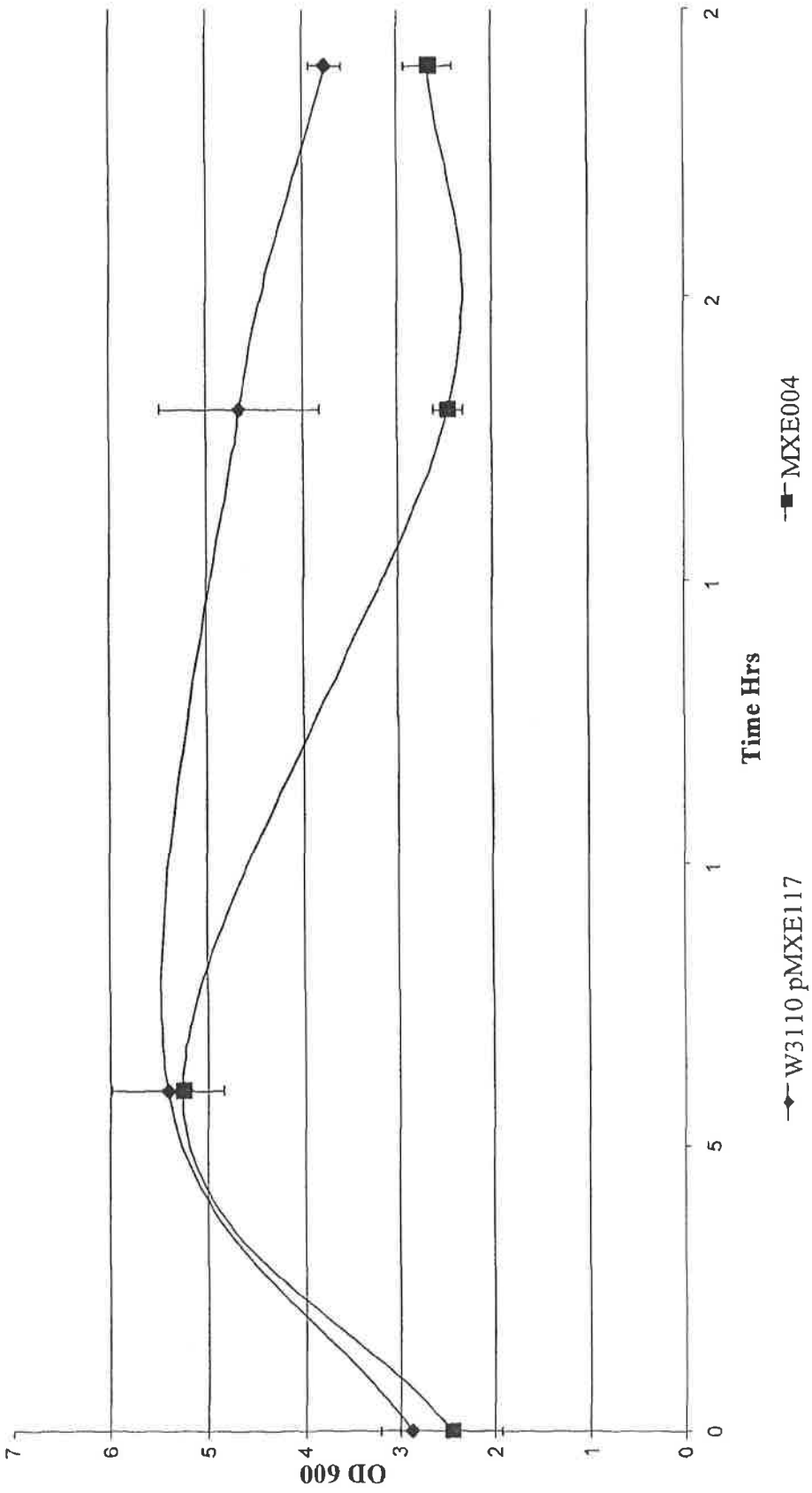


Figure 4

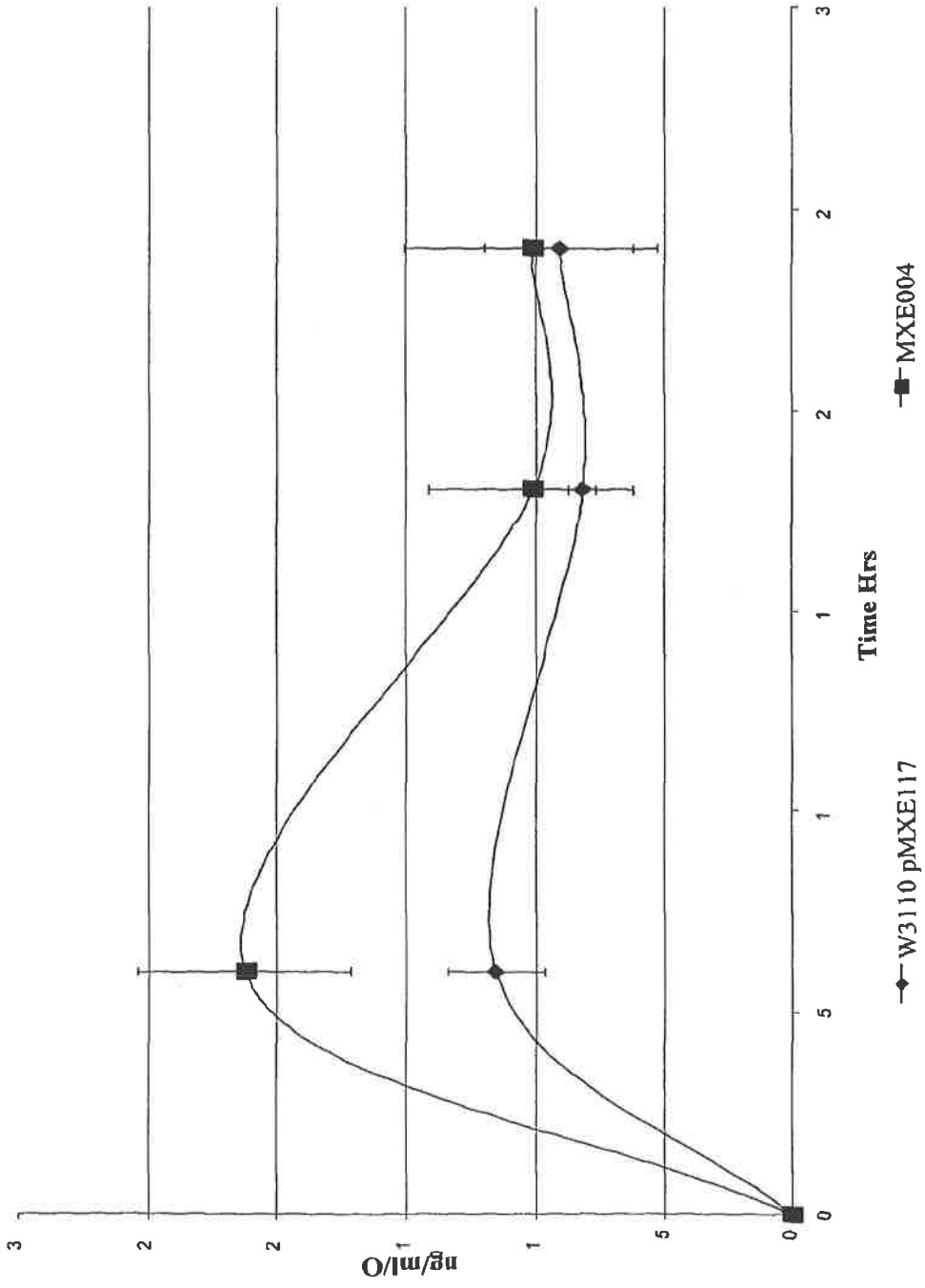


Figure 5

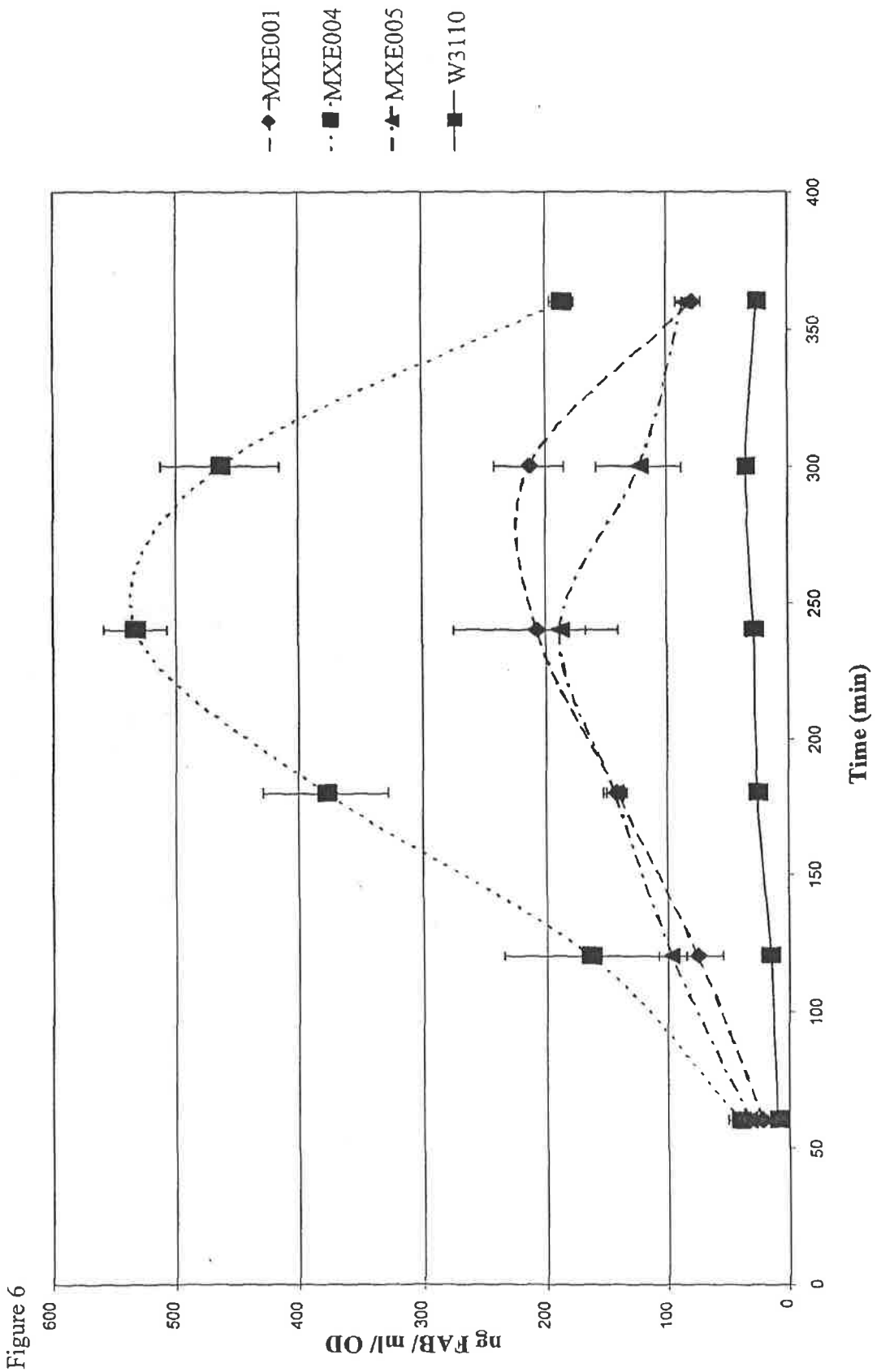

MXE001
MXE004
MXE005
W3110
ng FAB/ ml/ OD
Time (min)
0
100
200
300
400
500
600
0
50
100
150
200
250
300
350
400

Figure 6

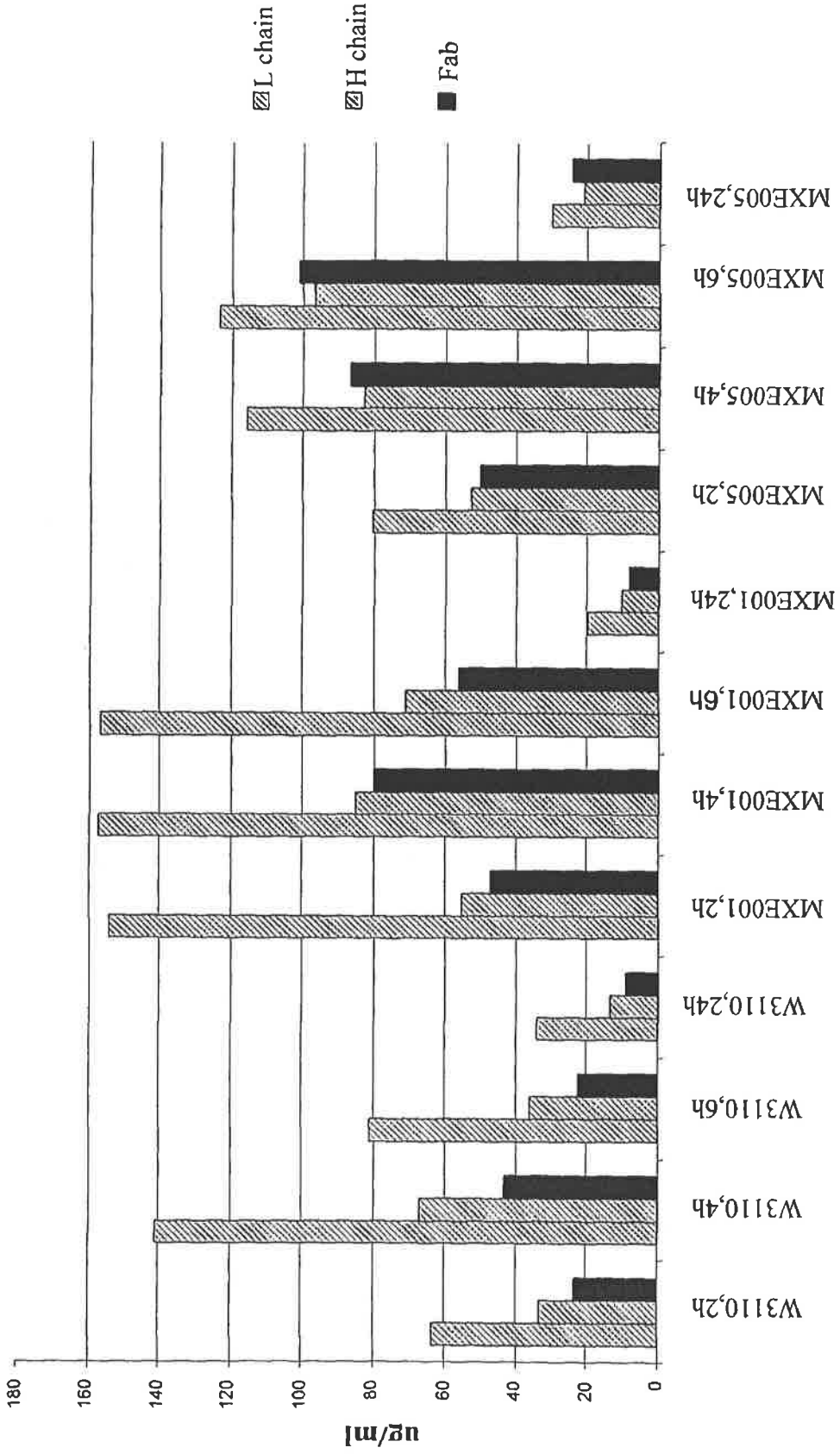

L chain
H chain
Fab
µg/ml
0
20
40
60
80
100
120
140
160
180
W3110,2h
W3110,4h
W3110,6h
W3110,24h
MXE001,2h
MXE001,4h
MXE001,6h
MXE001,24h
MXE005,2h
MXE005,4h
MXE005,6h
MXE005,24h

Figure 7

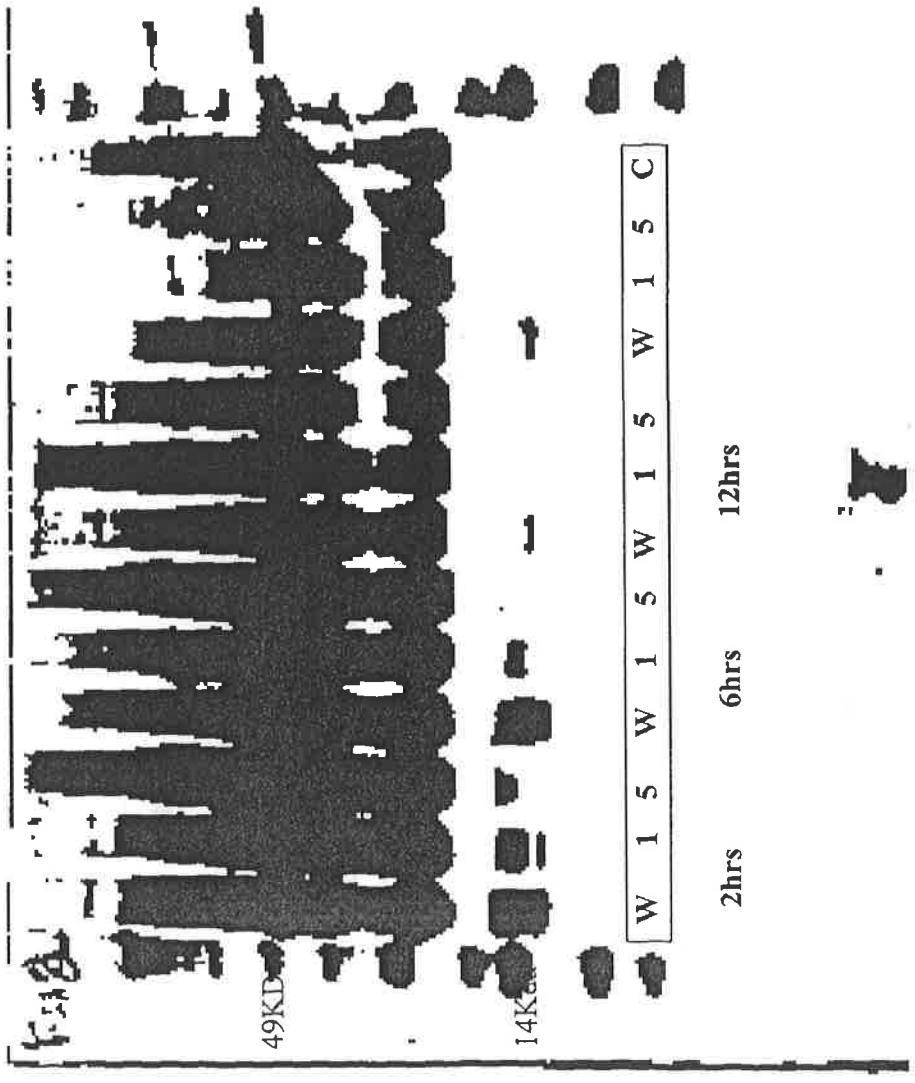


Figure 8

W = W3110
1 = MXE001 (ΔTsp)
5 = MXE005 (ΔTsp, DegP S210A)

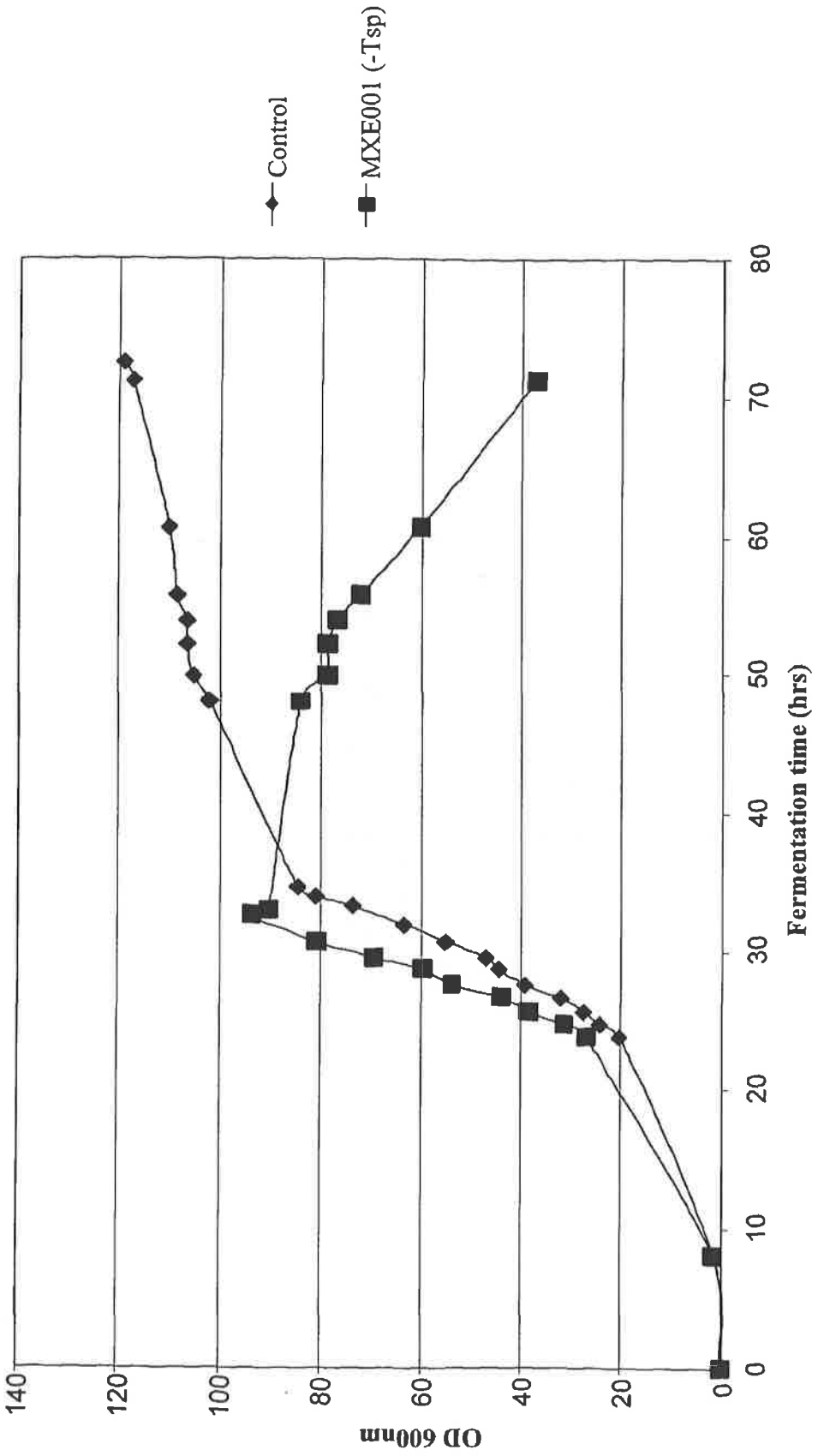

Control
MXE001 (-Tsp)
OD 600nm
0
20
40
60
80
100
120
140
Fermentation time (hrs)
0
10
20
30
40
50
60
70
80

Figure 9

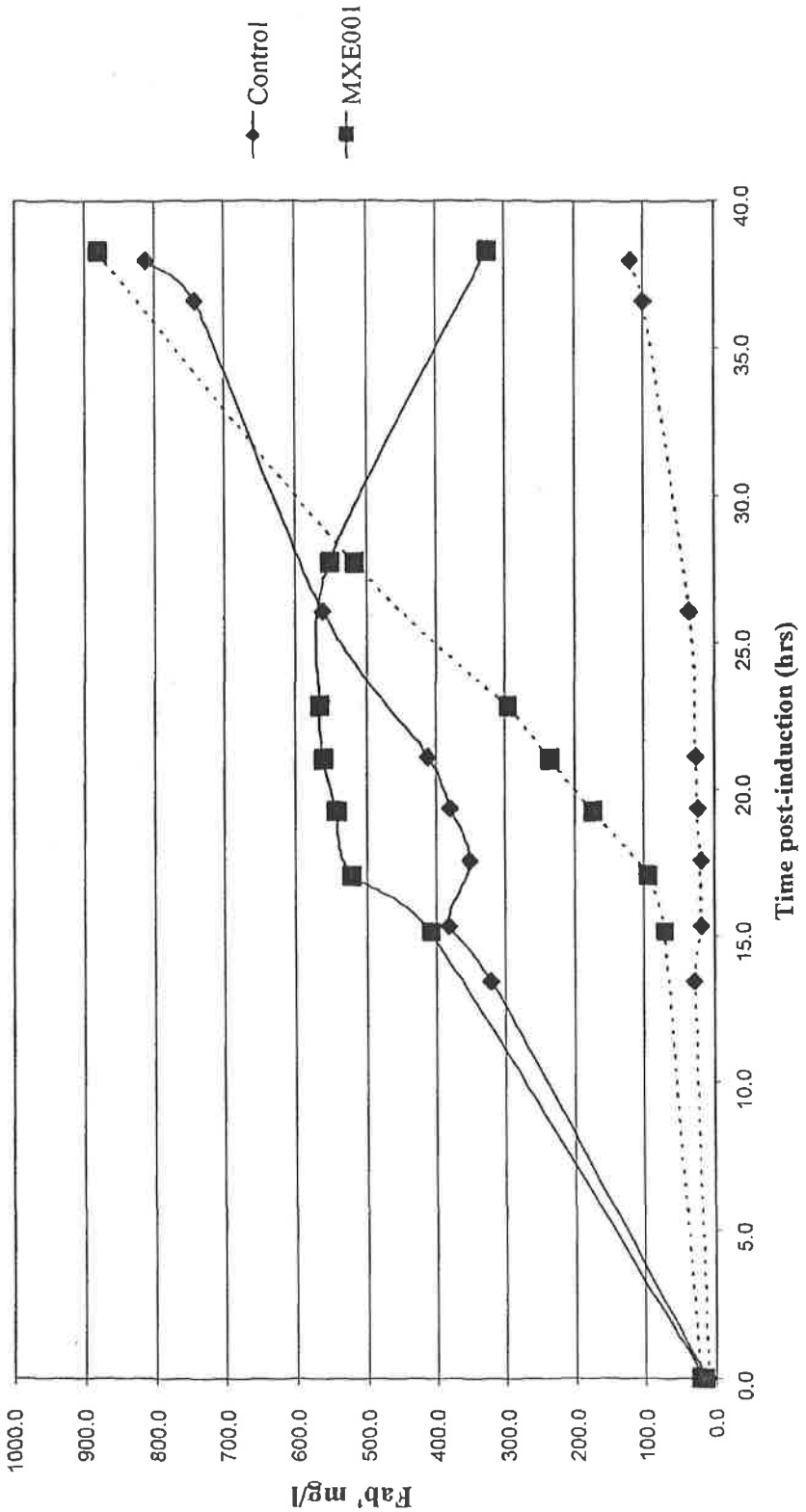

Control
MXE001
Fab' mg/l
1000.0
900.0
800.0
700.0
600.0
500.0
400.0
300.0
200.0
100.0
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
Time post-induction (hrs)

Figure 10

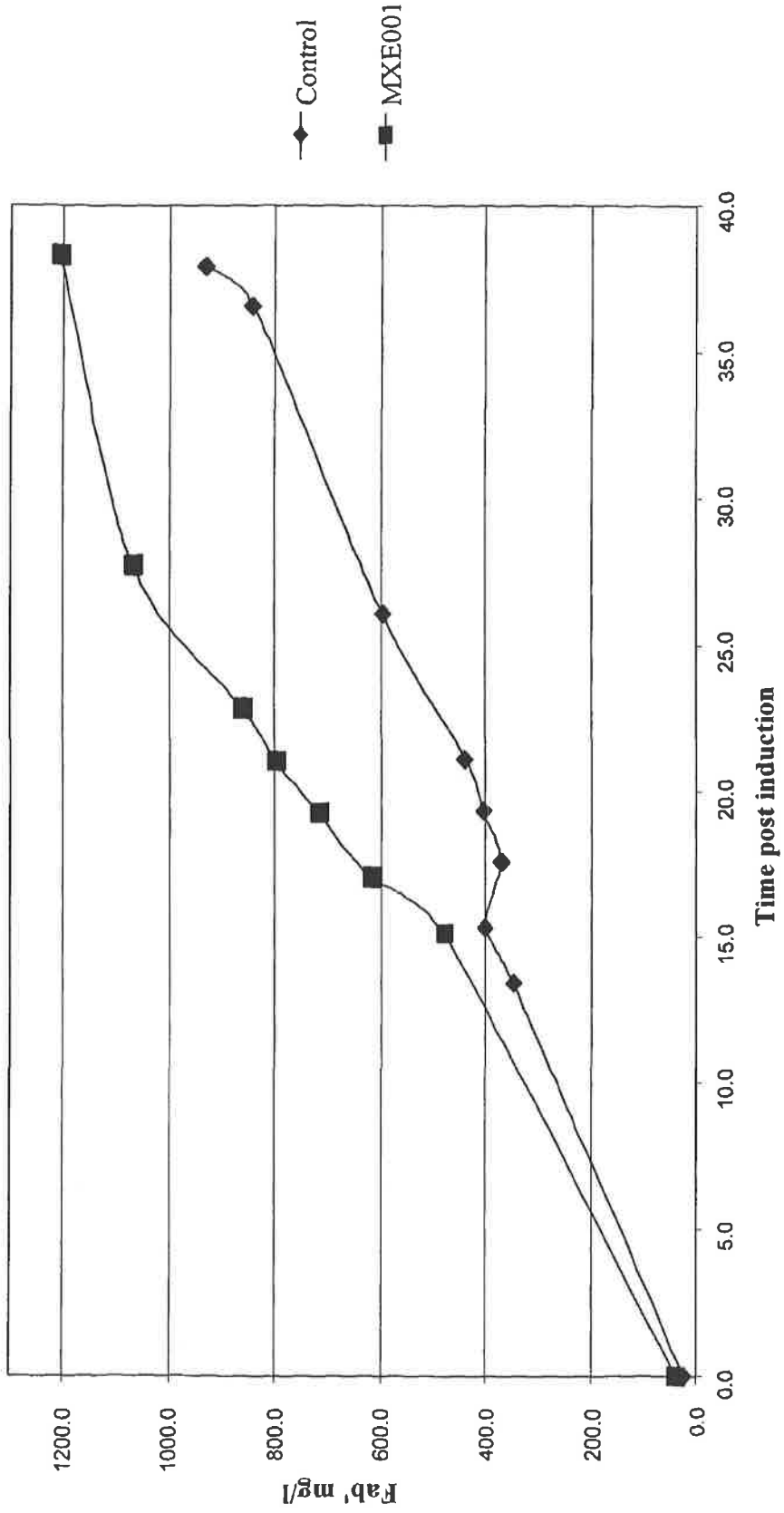

Control
MXE001
Fab' mg/l
1200.0
1000.0
800.0
600.0
400.0
200.0
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
Time post induction

Figure 11

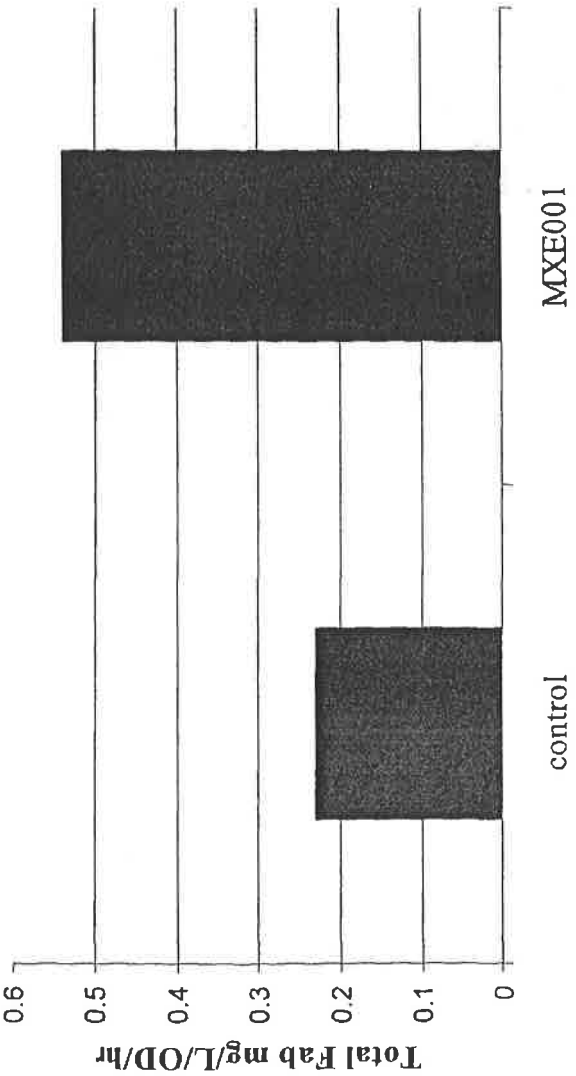


Figure 12

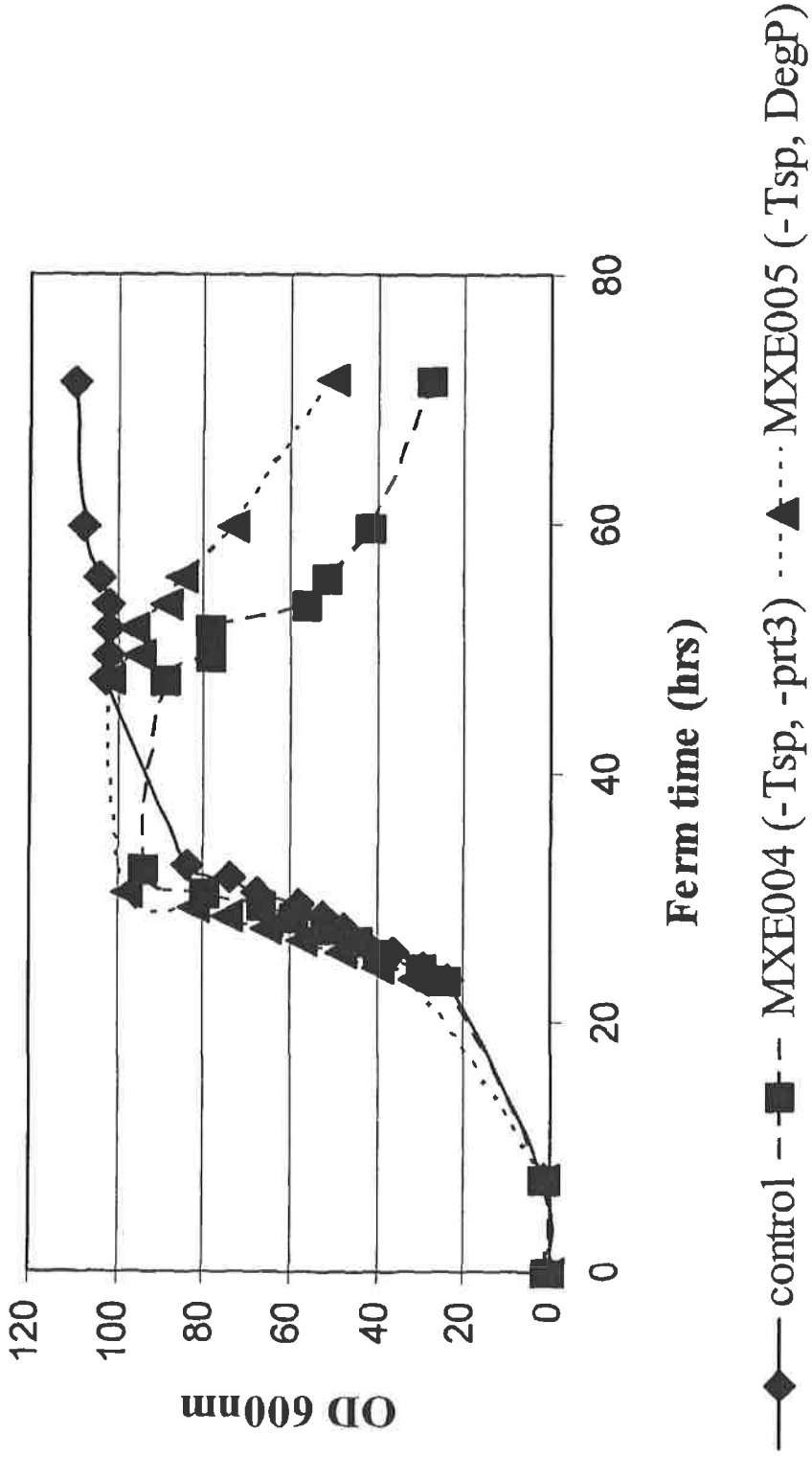

OD 600nm
120
100
80
60
40
20
0
0
20
40
60
80
Ferm time (hrs)
control
MXE004 (-Tsp, -prt3)
MXE005 (-Tsp, DegP)

Figure 13

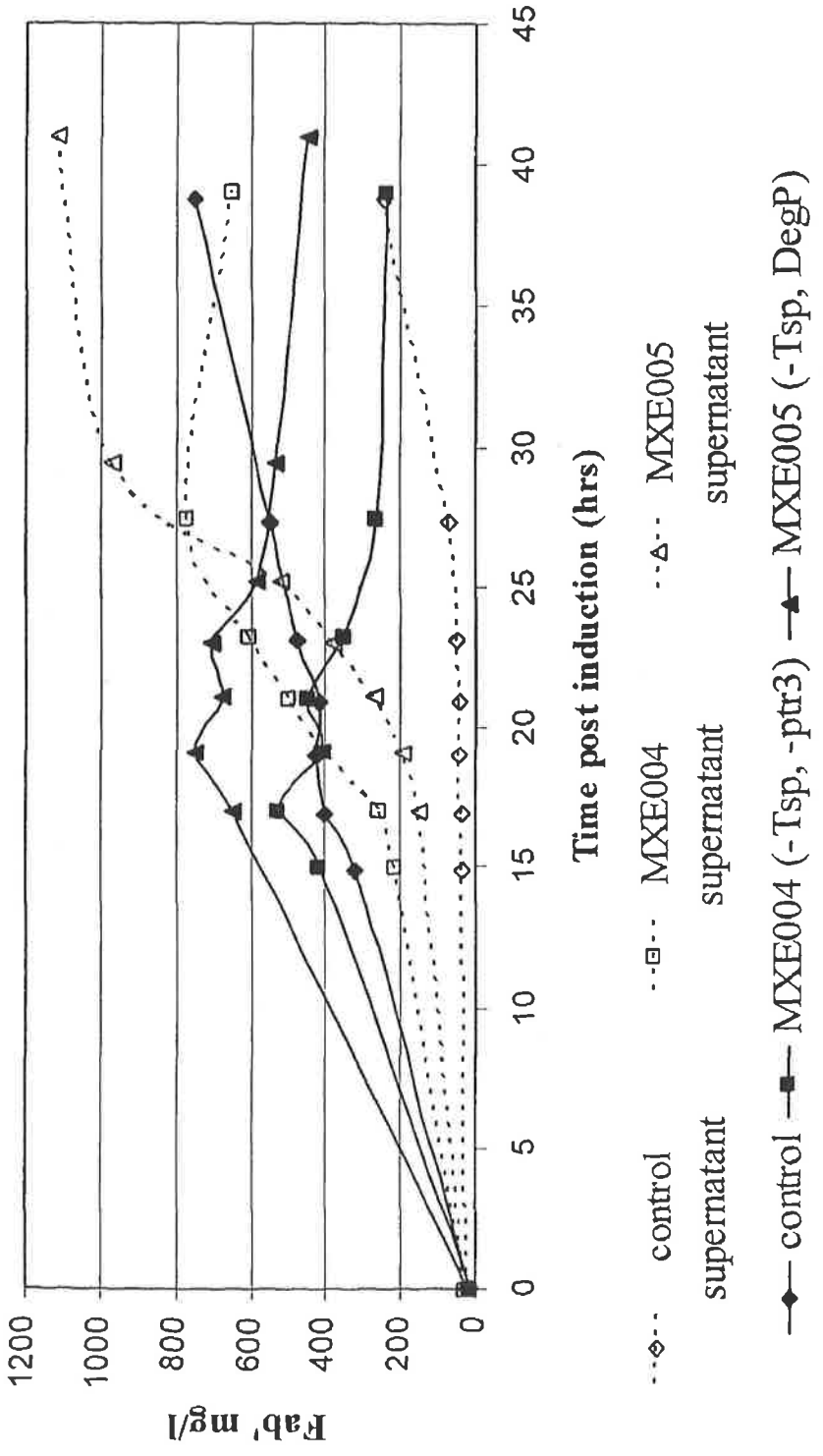

Fab' mg/l
1200
1000
800
600
400
200
0
0
5
10
15
20
25
30
35
40
45
Time post induction (hrs)
control supernatant
MXE004 supernatant
MXE005 supernatant
control
MXE004 (-Tsp, -ptr3)
MXE005 (-Tsp, DegP)

Figure 14

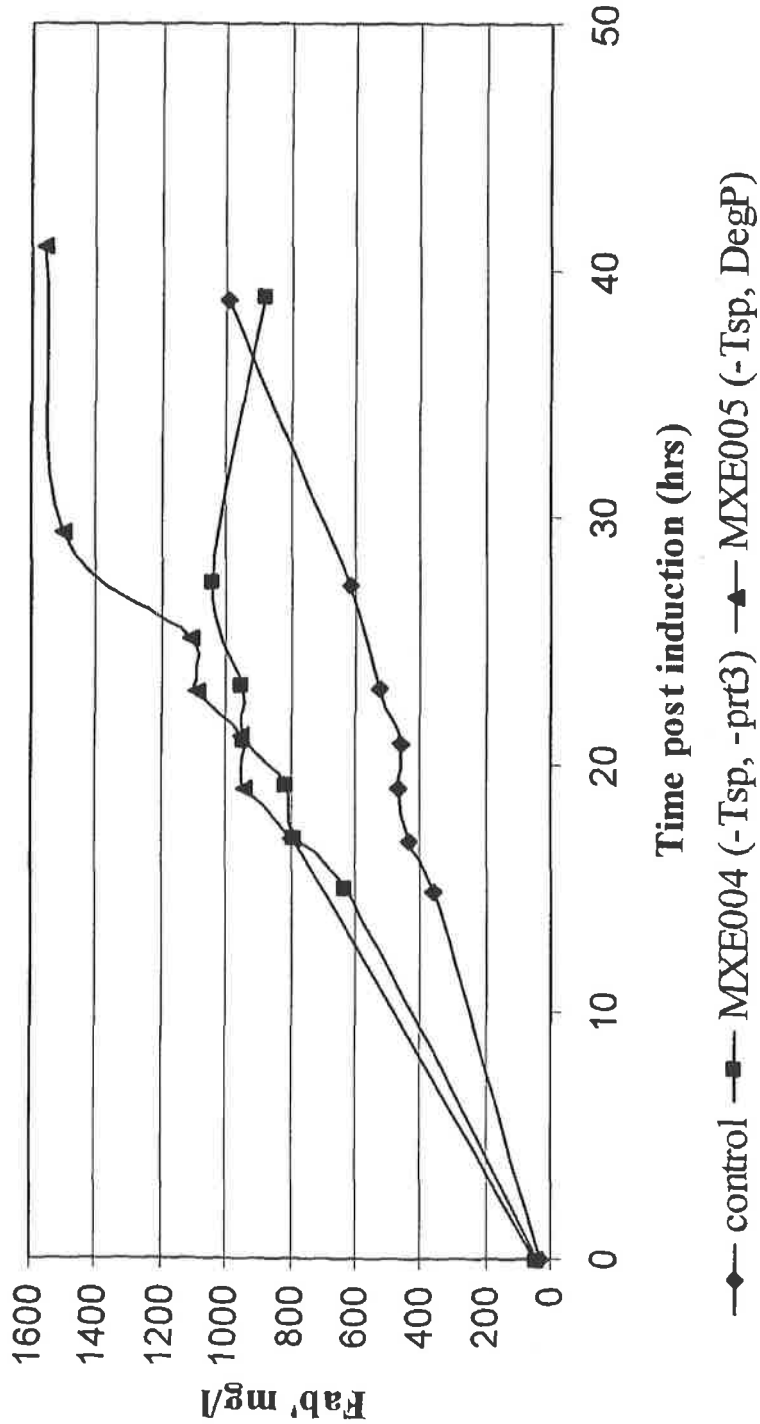

Fab' mg/l
1600
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
50
Time post induction (hrs)
control
MXE004 (-Tsp, -prt3)
MXE005 (-Tsp, DegP)

Figure 15

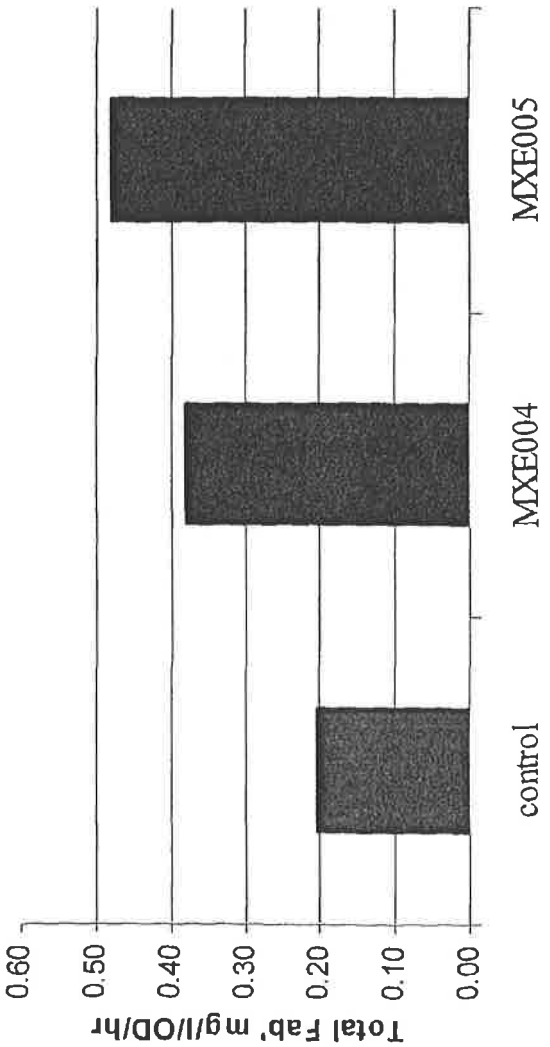

Total Fab' mg/l/OD/hr
0.60
0.50
0.40
0.30
0.20
0.10
0.00
control
MXE004
MXE005

Figure 16

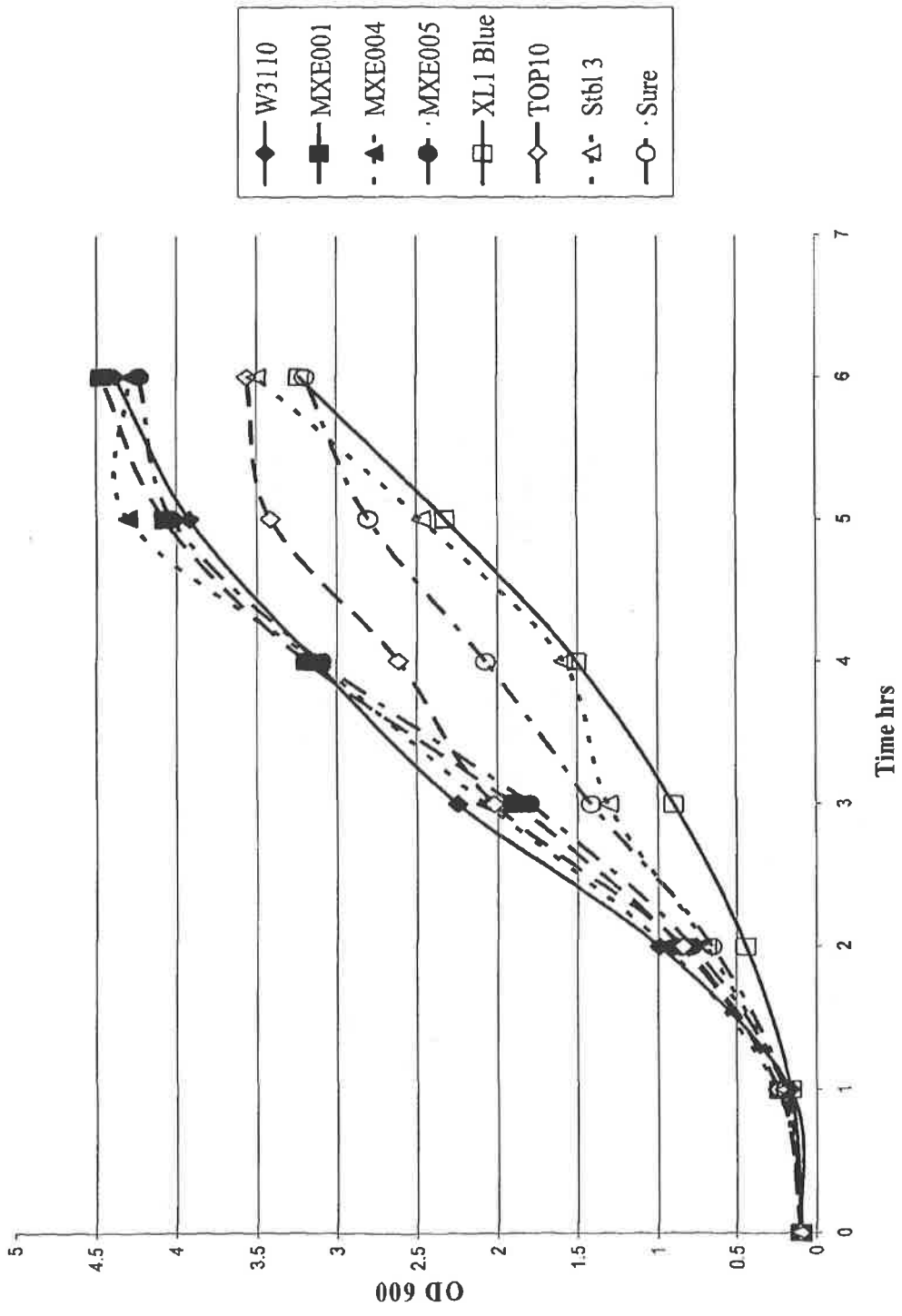

W3110
MXE001
MXE004
MXE005
XL1 Blue
TOP10
Stbl 3
Sure
OD 600
Time hrs
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
0
1
2
3
4
5
6
7

Figure 17