Figure 1

hCAII_NK0000 1 mshhwgygkhngpehwhkdfpiakgerqspvdidthtakydpslkplsvsydqatslrilnnghafnvefddsqdkavlkggpldgtyrl
His94
His119
hCAII_NK0000 271 iqfhfhwgsldgqgsehtvdkkkyaaelhlvhwntkygdfgkavqqpdglavlgiflkvgsakpglqkvvdvldsiktkgksadfcnfdp
His96
hCAII_NK0000 541 rgllpesldywtypgslttppllecvtwivlkepisvsseqvlkfrklnfngegepee1mvdnwrpaqplknrqikasfk

Figure 2

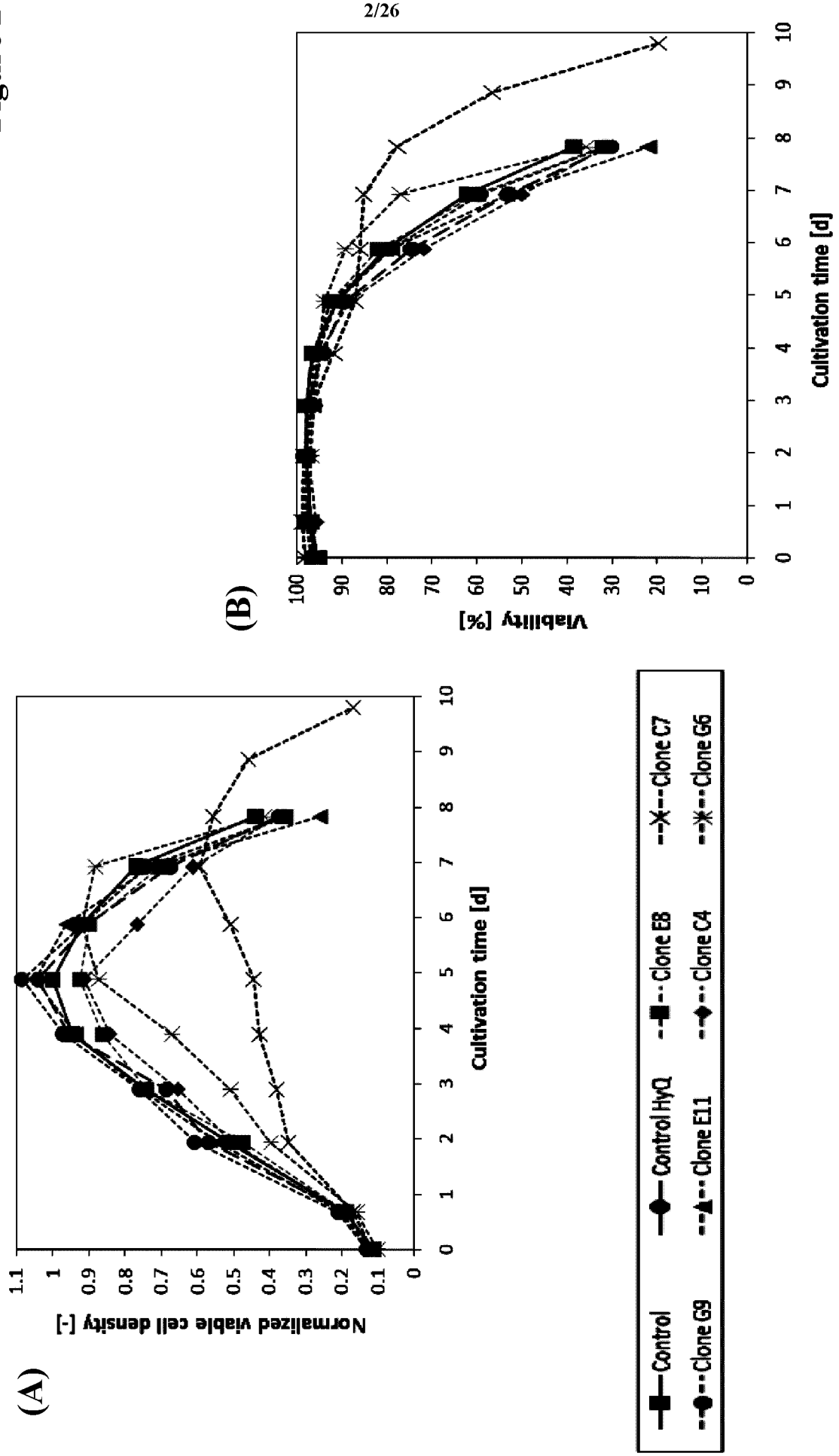
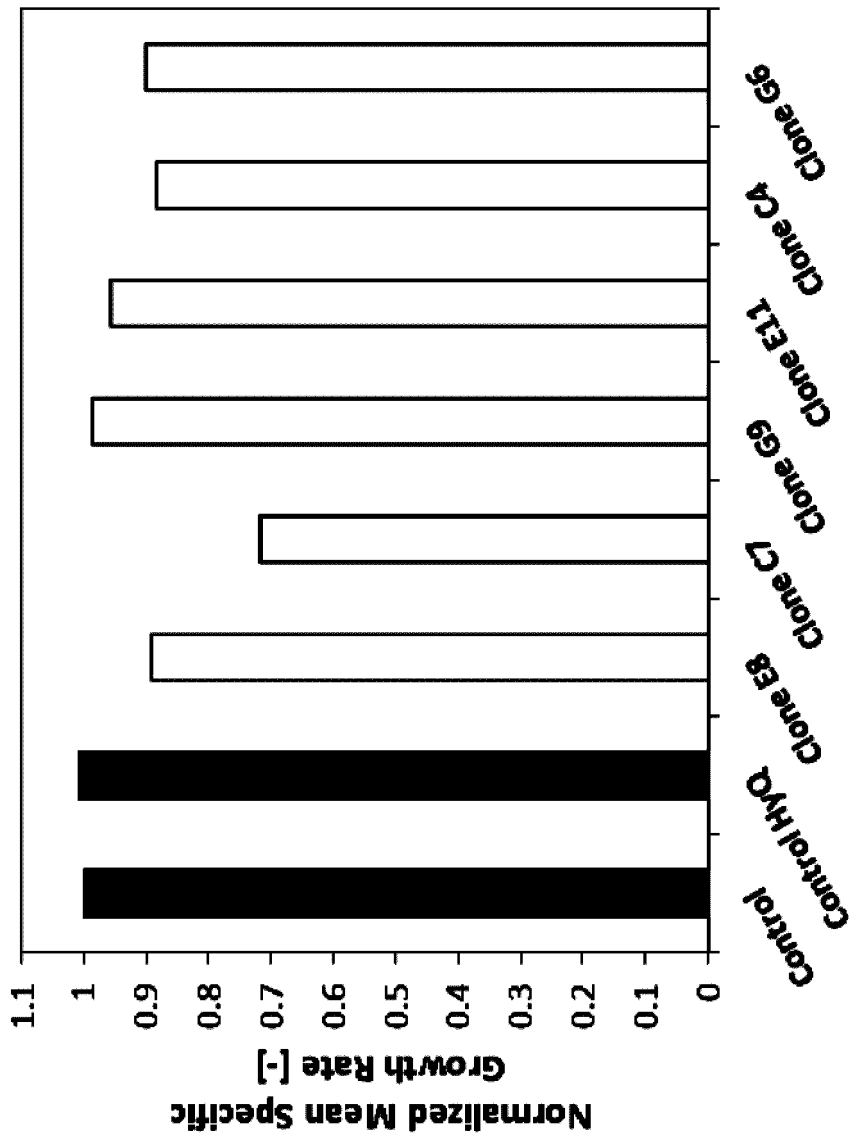

(A)
Normalized viable cell density [-]
Cultivation time [d]
(B)
Viability [%]
Cultivation time [d]
Control
Control HyQ
Clone E8
Clone C7
Clone G9
Clone E11
Clone C4
Clone G6

Figure 2


Normalized Mean Specific Growth Rate [-]
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Control
Control HyQ
Clone E8
Clone C7
Clone G9
Clone E11
Clone C4
Clone G6

(C)

Figure 3

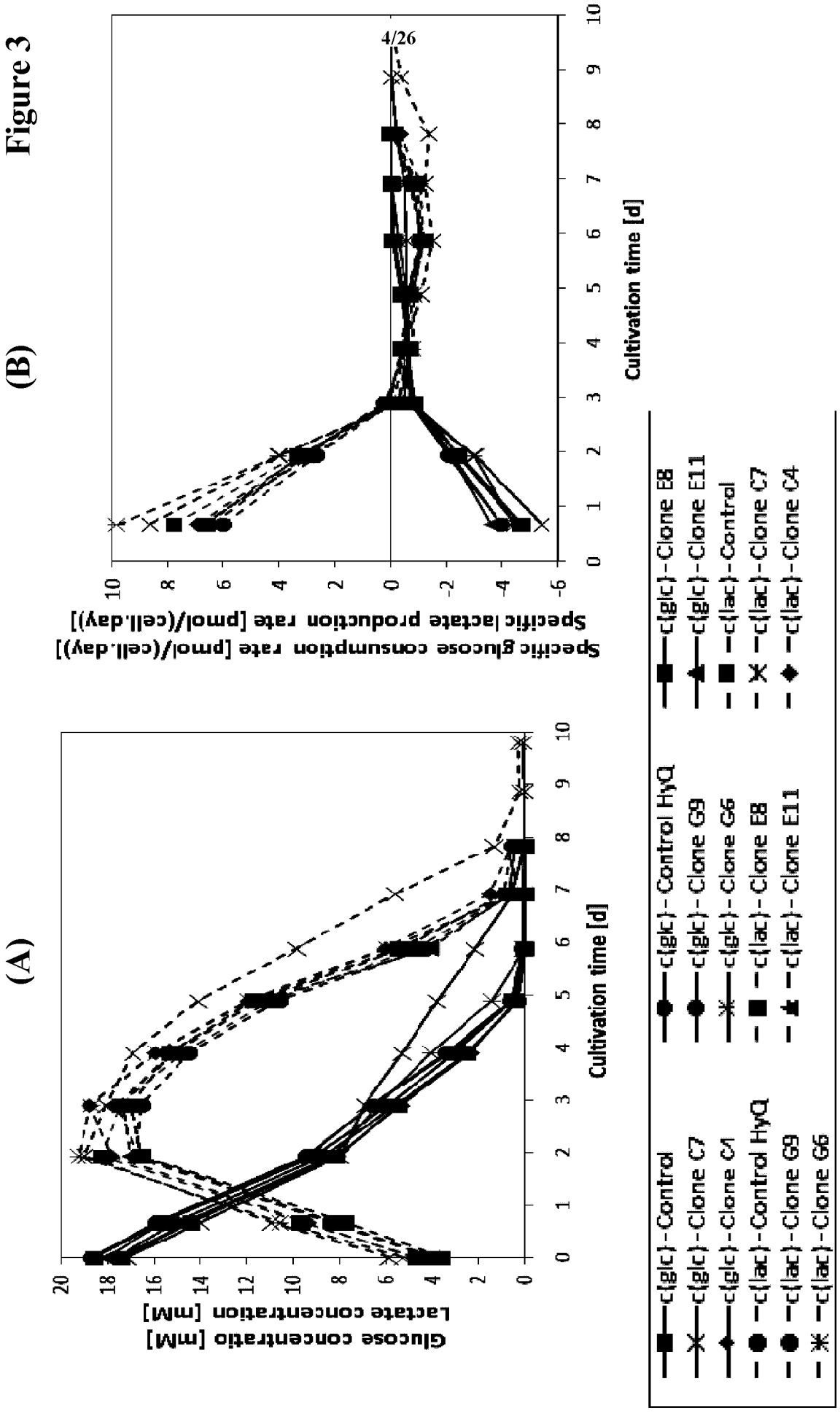

(A)
Glucose concentratio [mM]
lactate concentration [mM]
Cultivation time [d]
c(glc)-Control
c(glc)-Clone C7
c(glc)-Clone C4
c(lac)-Control HyQ
c(lac)-Clone G9
c(lac)-Clone G6
c(glc)-Control HyQ
c(glc)-Clone G9
c(glc)-Clone G6
c(lac)-Control
c(lac)-Clone E8
c(lac)-Clone E11
(B)
Specific glucose consumption rate [pmol/(cell.day)]
Specific lactate production rate [pmol/(cell.day)]
Cultivation time [d]
c(glc)-Clone E8
c(glc)-Clone E11
c(lac)-Control
c(lac)-Clone C7
c(lac)-Clone C4

Figure 4

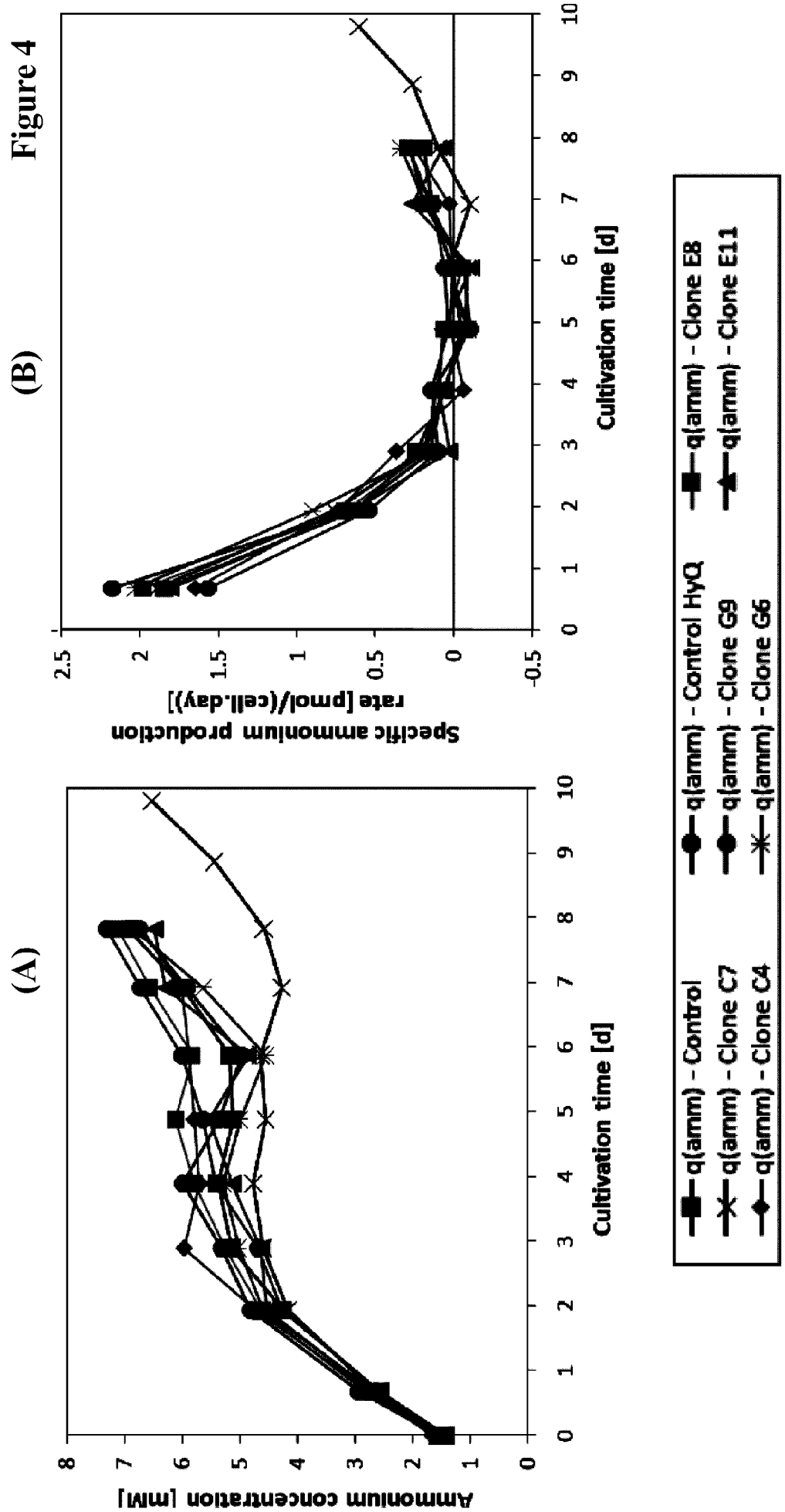

(A)
Ammonium concentration [mM]
Cultivation time [d]
(B)
Specific ammonium production rate [pmol/(cell.day)]
Cultivation time [d]
q(amm) - Control
q(amm) - Clone C7
q(amm) - Clone C4
q(amm) - Control HyQ
q(amm) - Clone G9
q(amm) - Clone G6
q(amm) - Clone E8
q(amm) - Clone E11

Figure 5

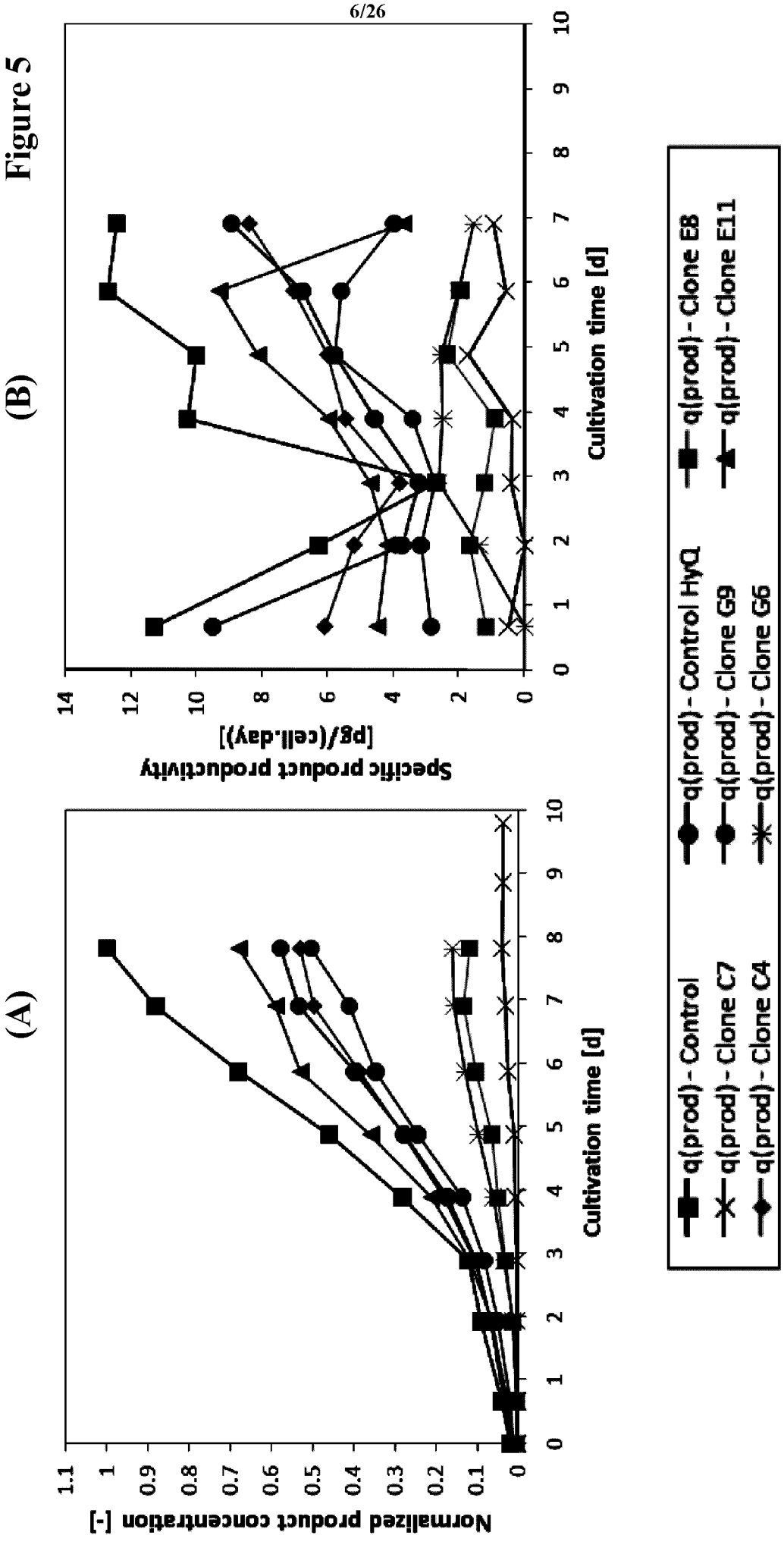

(A)
Normalized product concentration [-]
Cultivation time [d]
(B)
Specific product productivity [pg/(cell.day)]
Cultivation time [d]
q(prod) - Control
q(prod) - Control HyQ
q(prod) - Clone E8
q(prod) - Clone C7
q(prod) - Clone G9
q(prod) - Clone E11
q(prod) - Clone C4
q(prod) - Clone G6

Figure 6

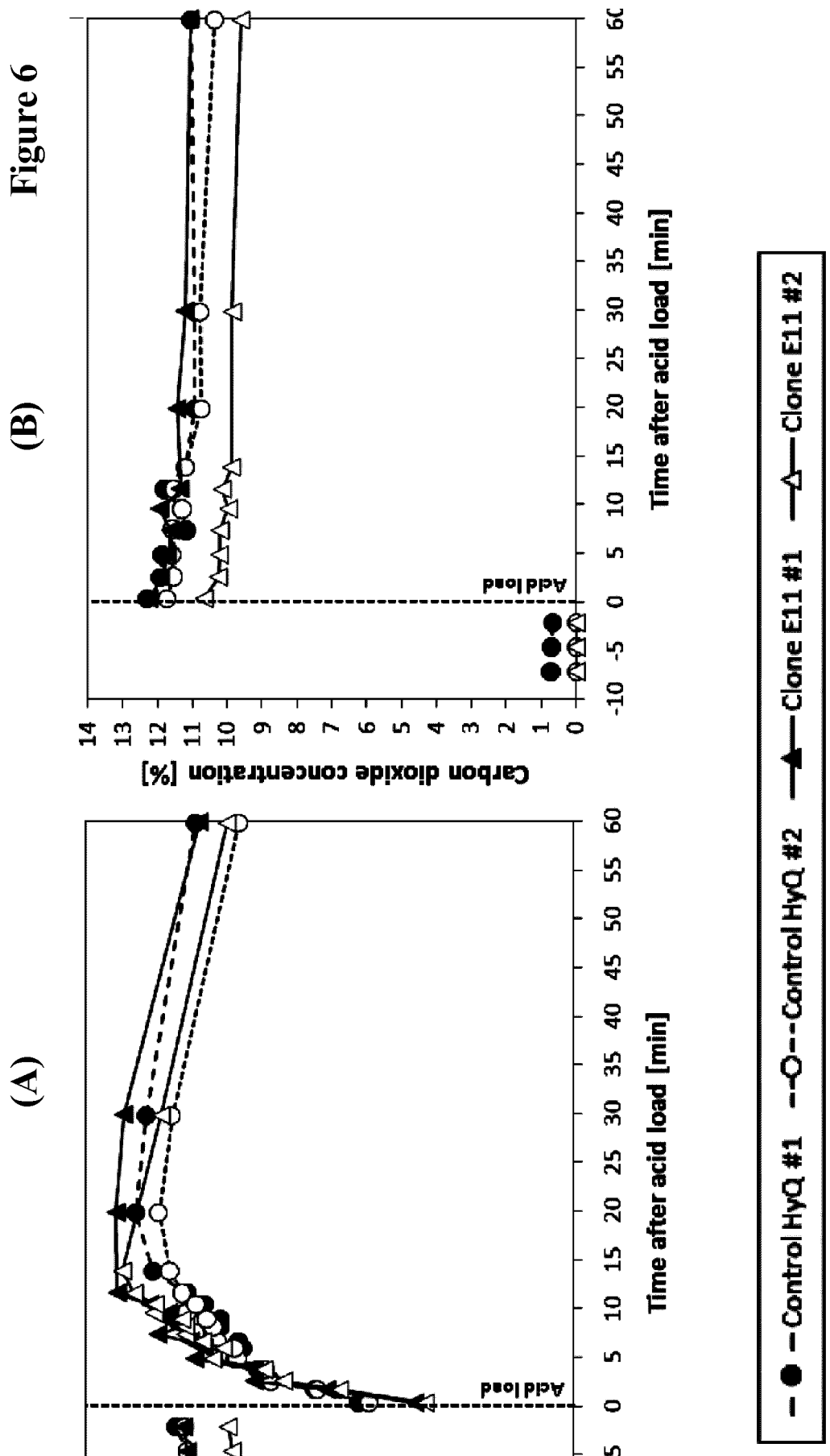

(A)
Intracellular pH value [-]
7.5
7.4
7.3
7.2
7.1
7
6.9
6.8
6.7
6.6
6.5
-10 -5 0 5 10 15 20 25 30 35 40 45 50 55 60
Time after acid load [min]
Add load
(B)
Carbon dioxide concentration [%]
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0
-10 -5 0 5 10 15 20 25 30 35 40 45 50 55 60
Time after acid load [min]
Add load
Control HyQ #1
Control HyQ #2
Clone E11 #1
Clone E11 #2

Figure 7

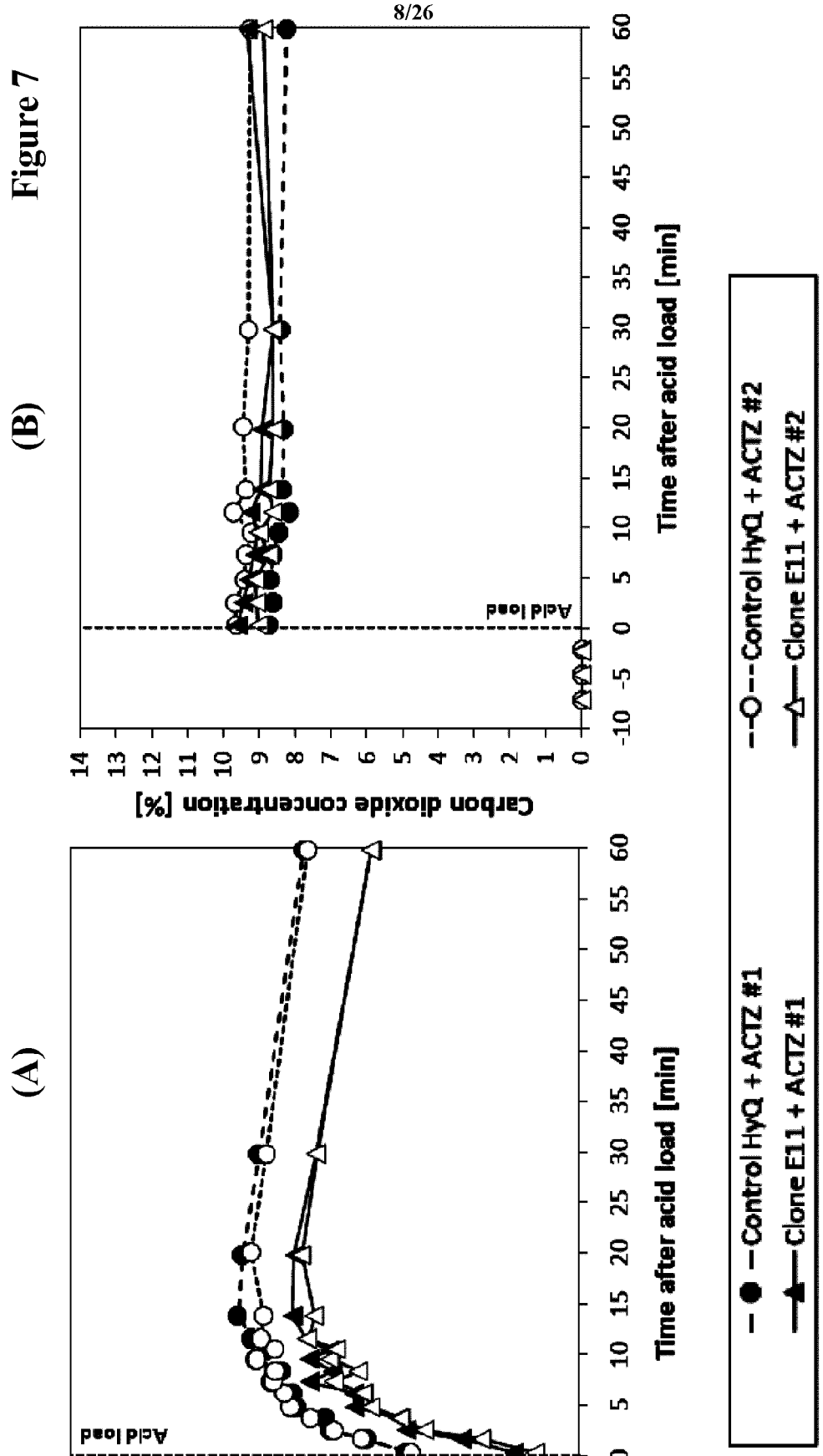

(A)
(B)
Intracellular pH value [-]
Carbon dioxide concentration [%]
Time after acid load [min]
Time after acid load [min]
Acid load
Acid load
Control HyQ + ACTZ #1
Control HyQ + ACTZ #2
Clone E11 + ACTZ #1
Clone E11 + ACTZ #2

Figure 8

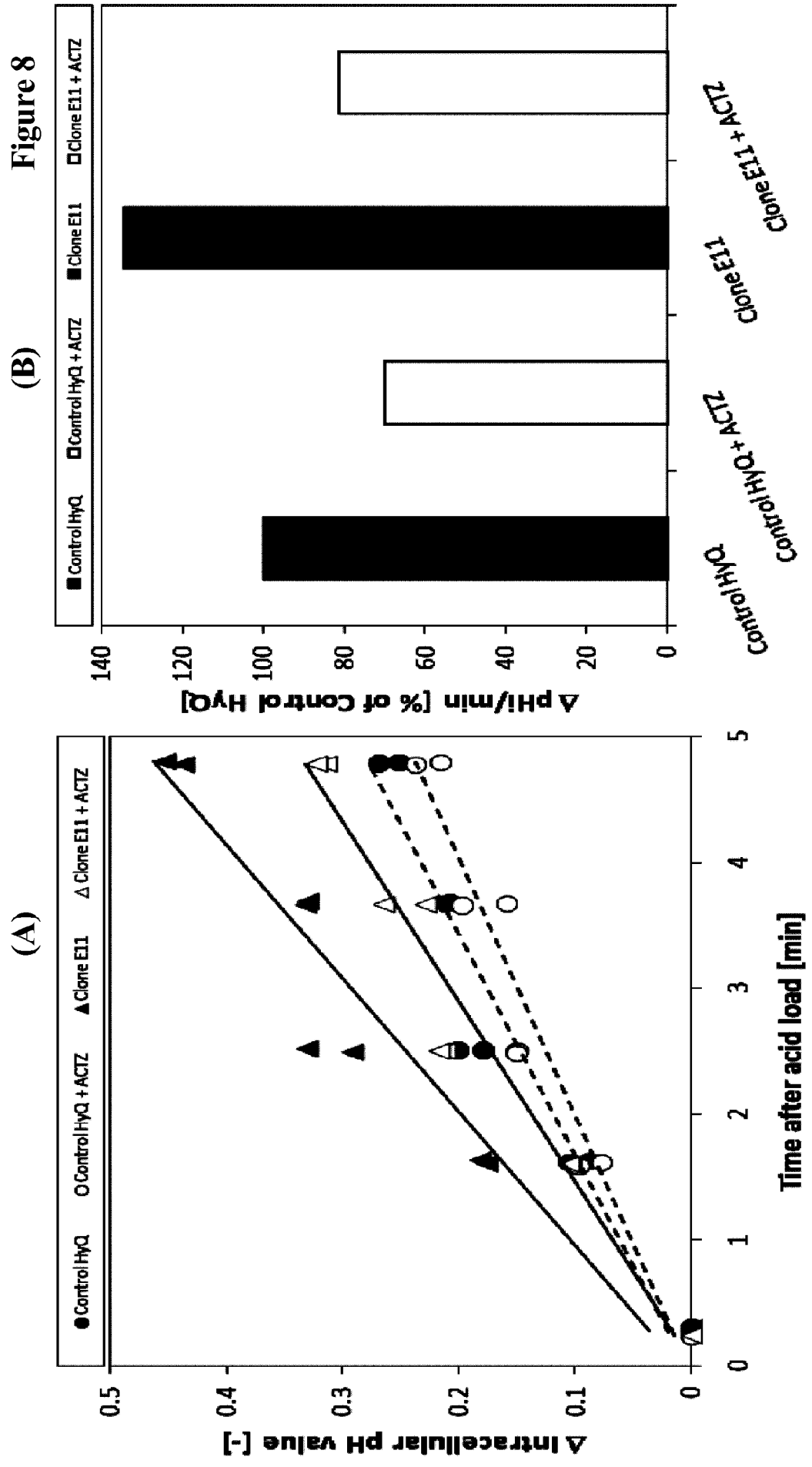

(A)
Control HyQ
Control HyQ + ACTZ
Clone E11
Clone E11 + ACTZ
Δ intracellular pH value [-]
Time after acid load [min]
(B)
Control HyQ
Control HyQ + ACTZ
Clone E11
Clone E11 + ACTZ
Δ pHi/min [% of control HyQ]

Figure 9

(A)

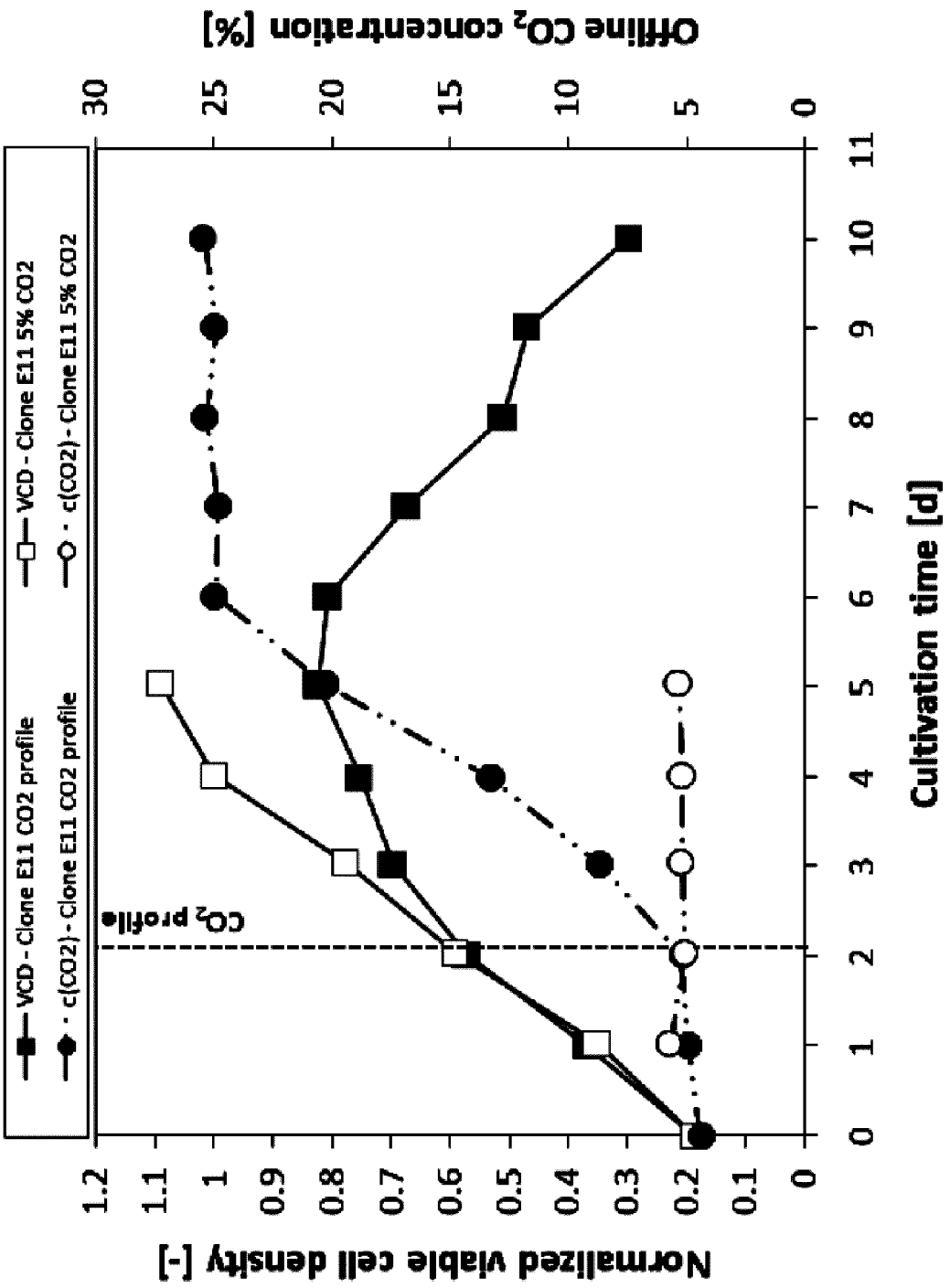

VCD - Clone E11 CO2 profile
c(CO2) - Clone E11 CO2 profile
VCD - Clone E11 5% CO2
c(CO2) - Clone E11 5% CO2
CO2 profile
Normalized viable cell density [-]
Offline CO2 concentration [%]
Cultivation time [d]
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
1.1
1.2
0
5
10
15
20
25
30
0
1
2
3
4
5
6
7
8
9
10
11

Figure 9

(B)

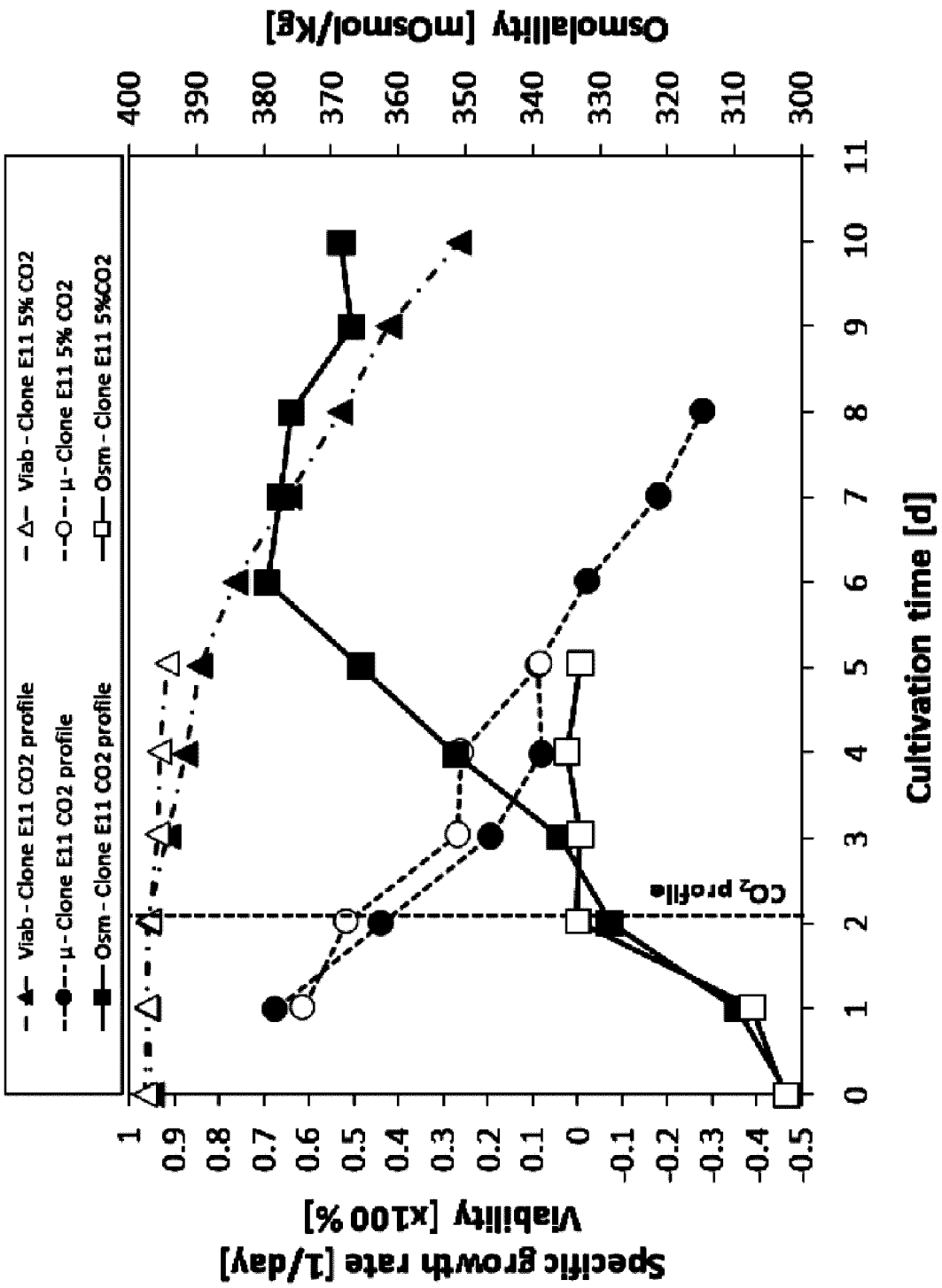

Viab - Clone E11 CO2 profile
Viab - Clone E11 5% CO2
µ - Clone E11 CO2 profile
µ - Clone E11 5% CO2
Osm - Clone E11 CO2 profile
Osm - Clone E11 5%CO2
Specific growth rate [1/day]
Viability [x100 %]
Osmolality [mOsmol/kg]
Cultivation time [d]
CO2 profile

Figure 10

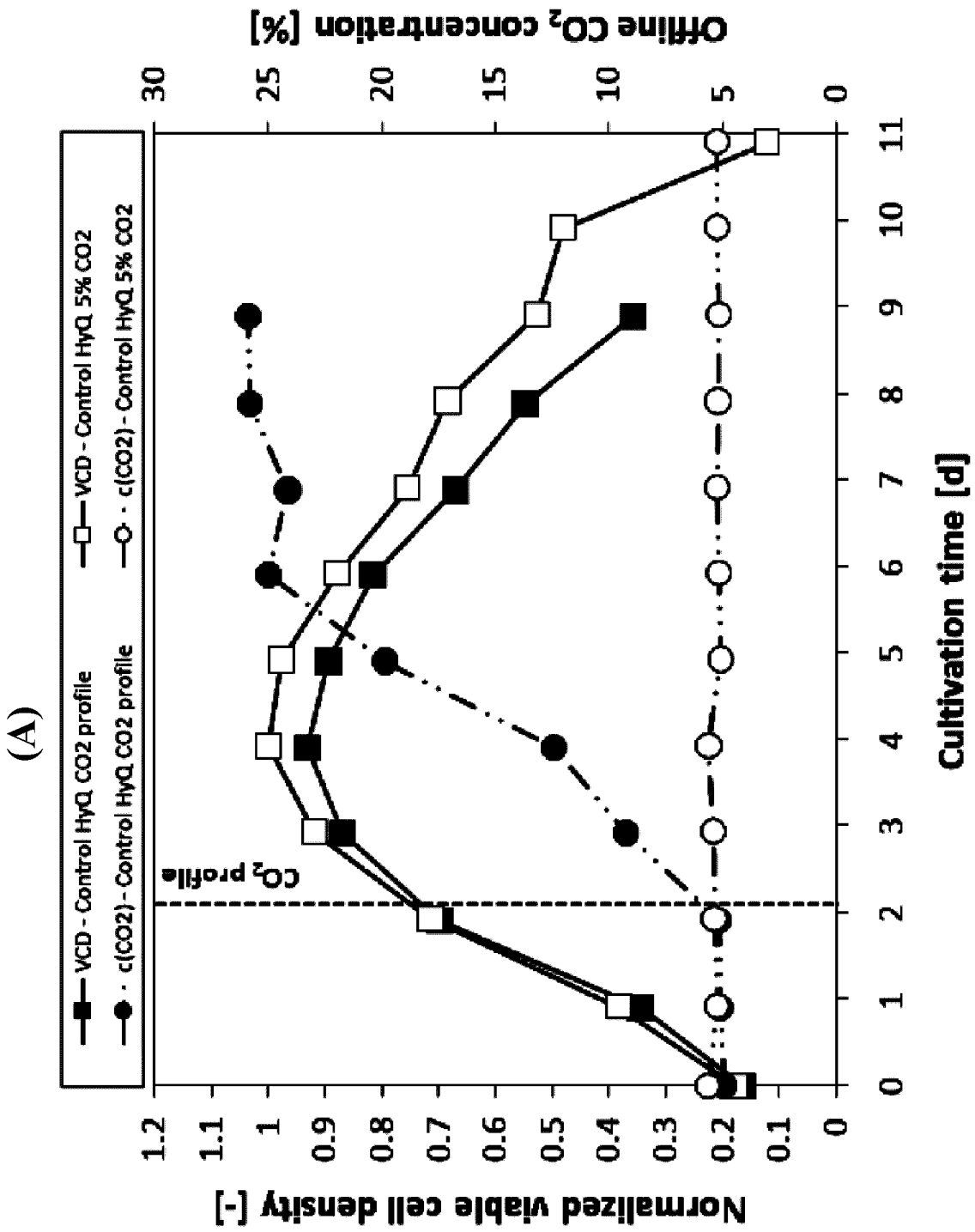

(A)
VCD - Control HyQ CO2 profile
c(CO2) - Control HyQ CO2 profile
VCD - Control HyQ 5% CO2
c(CO2) - Control HyQ 5% CO2
CO2 profile
Normalized viable cell density [-]
Offline CO2 concentration [%]
Cultivation time [d]
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
1.1
1.2
0
5
10
15
20
25
30
0
1
2
3
4
5
6
7
8
9
10
11

Figure 10

(B)

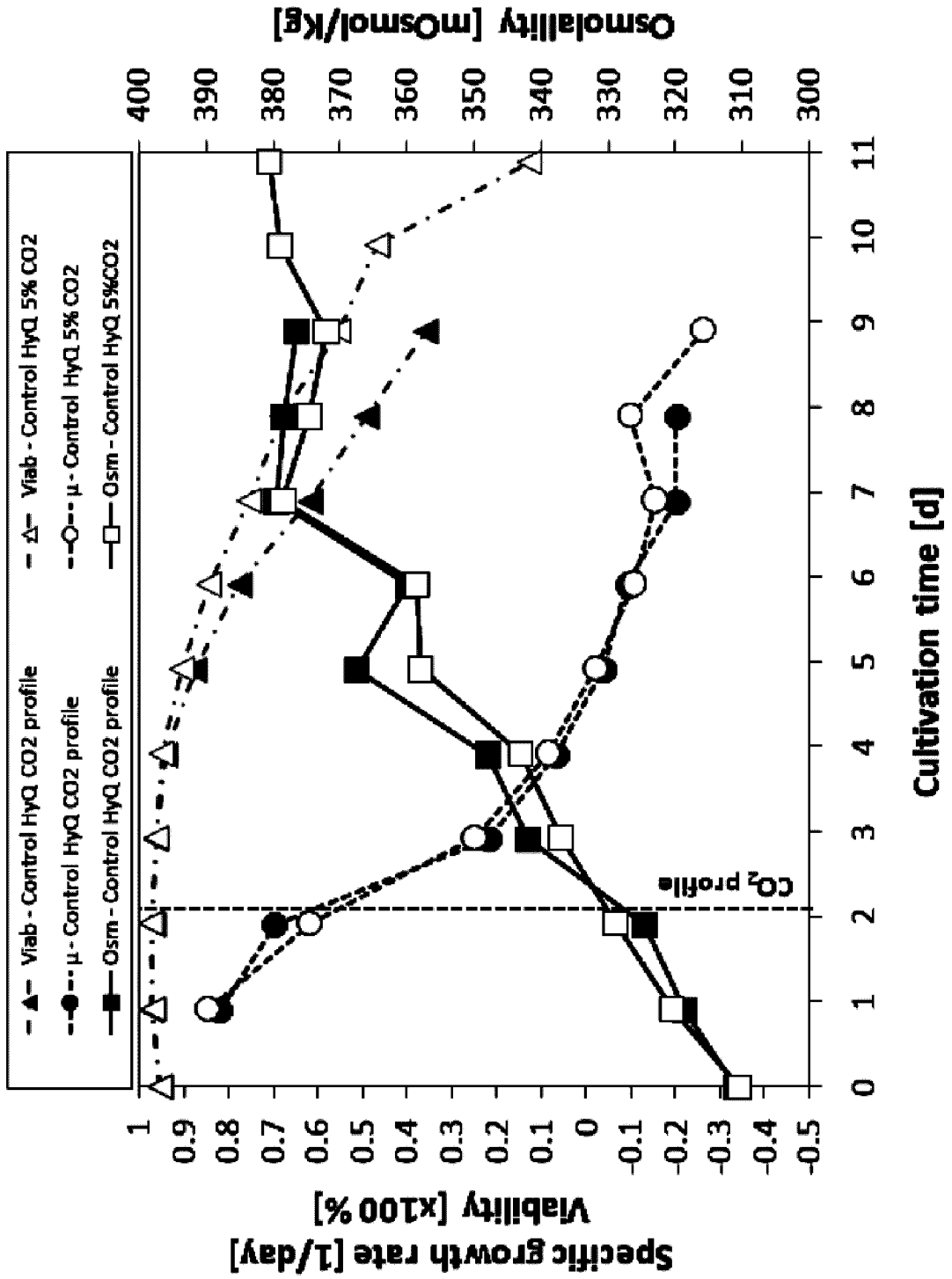

Viab - Control HyQ CO2 profile
Viab - Control HyQ 5% CO2
µ - Control HyQ CO2 profile
µ - Control HyQ 5% CO2
Osm - Control HyQ CO2 profile
Osm - Control HyQ 5%CO2
CO2 profile
Specific growth rate [1/day]
Viability [x100 %]
Osmolality [mOsmol/kg]
Cultivation time [d]

Figure 11

(A)

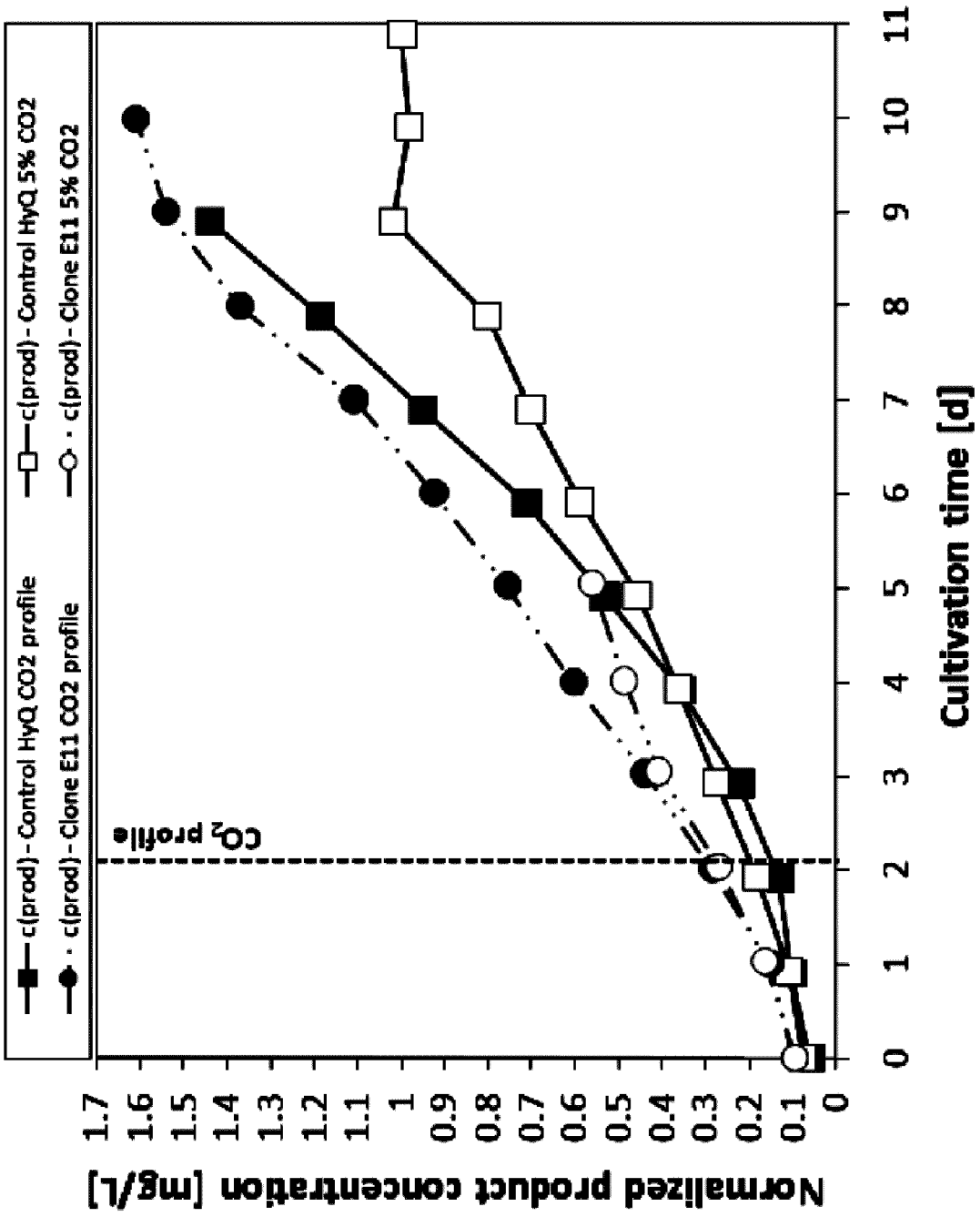

c(prod) - Control HyQ CO2 profile
c(prod) - Clone E11 CO2 profile
c(prod) - Control HyQ 5% CO2
c(prod) - Clone E11 5% CO2
CO2 profile
Normalized product concentration [mg/L]
Cultivation time [d]
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
1.1
1.2
1.3
1.4
1.5
1.6
1.7
0
1
2
3
4
5
6
7
8
9
10
11

Figure 11

(B)

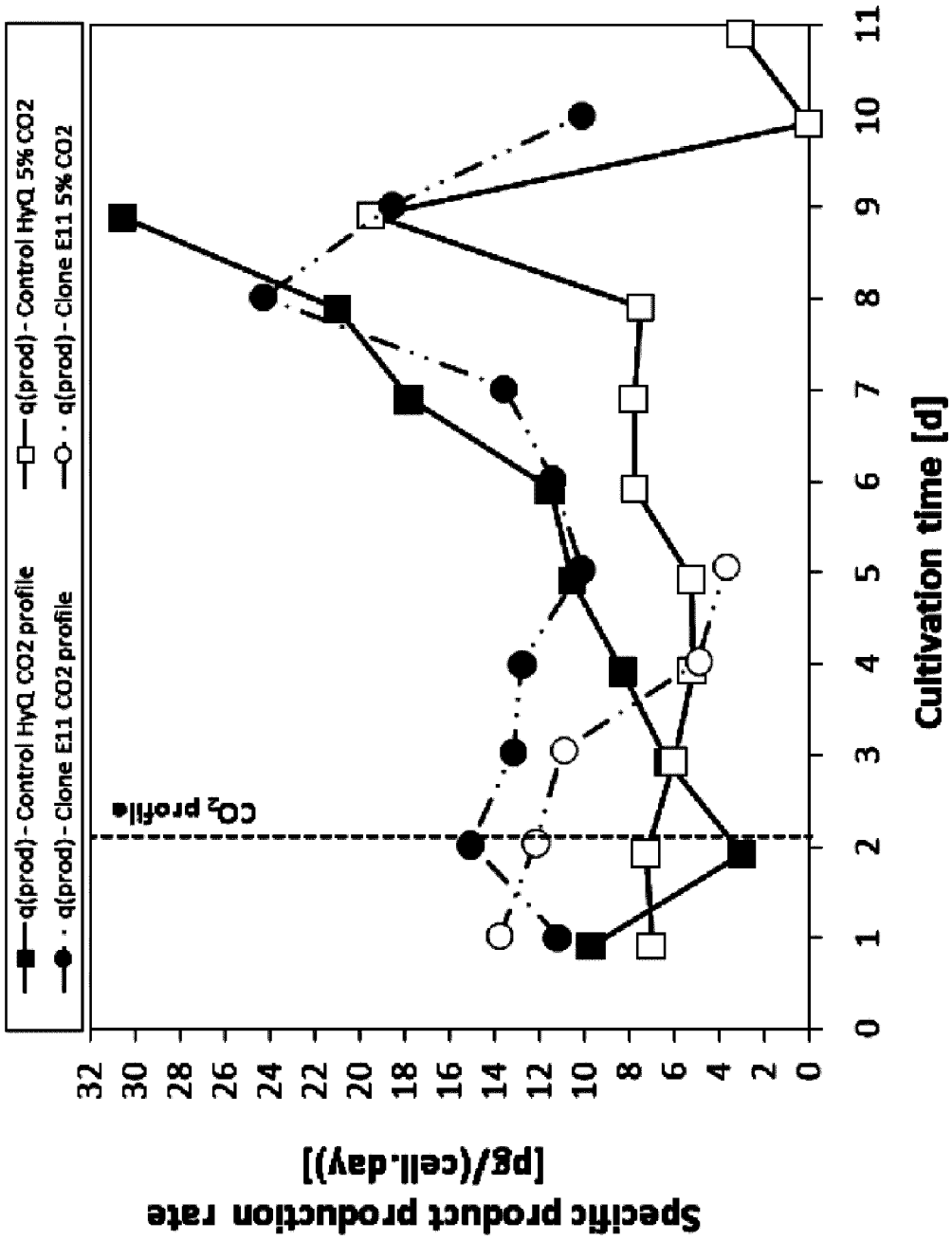

q(prod) - Control HyQ CO2 profile
q(prod) - Clone E11 CO2 profile
q(prod) - Control HyQ 5% CO2
q(prod) - Clone E11 5% CO2
CO2 profile
Specific product production rate
[pg/(cell.day)]
Cultivation time [d]
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
0
1
2
3
4
5
6
7
8
9
10
11

Figure 12

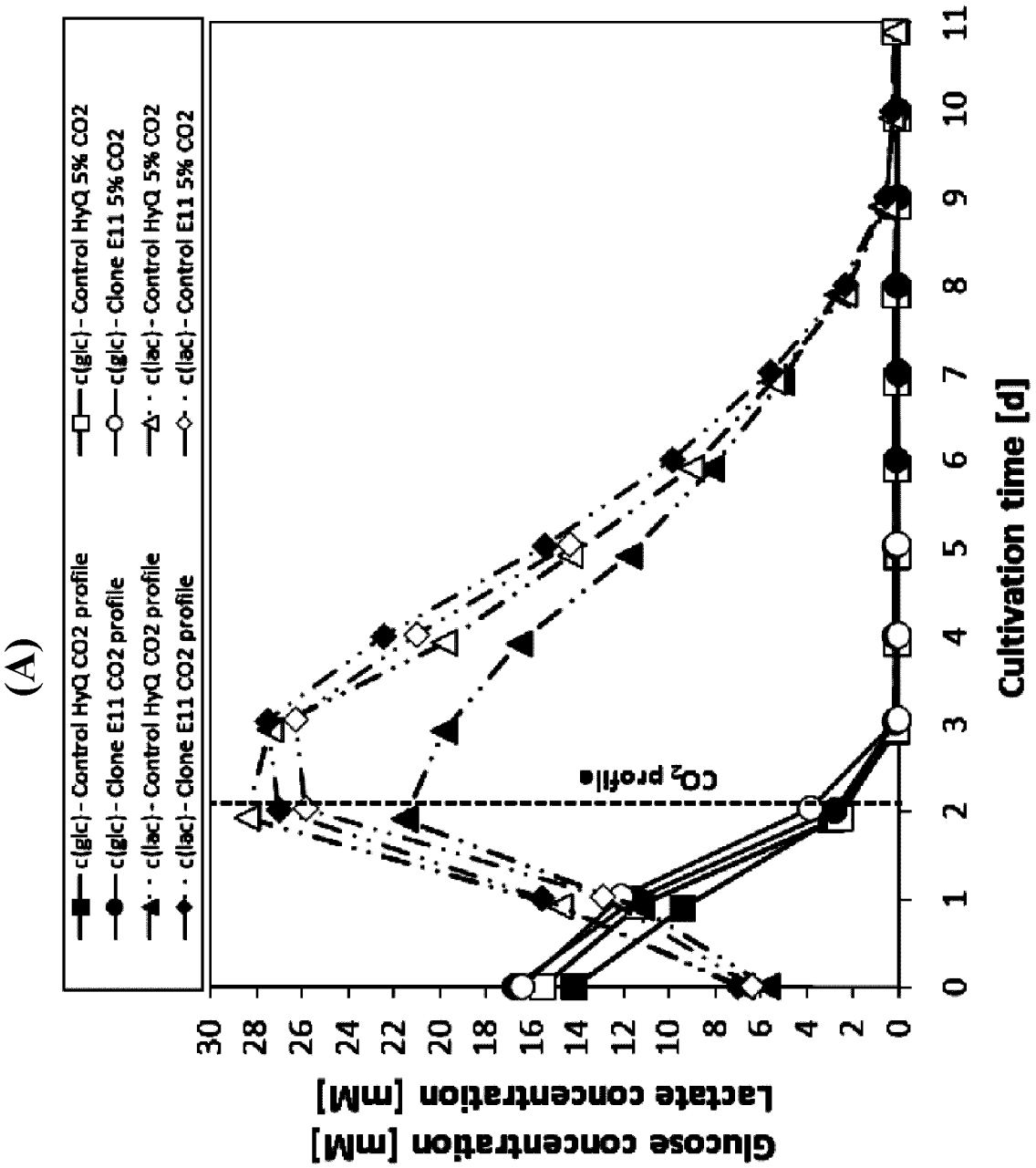

(A)
c(glc) - Control HyQ CO2 profile
c(glc) - Clone E11 CO2 profile
c(lac) - Control HyQ CO2 profile
c(lac) - Clone E11 CO2 profile
c(glc) - Control HyQ 5% CO2
c(glc) - Clone E11 5% CO2
c(lac) - Control HyQ 5% CO2
c(lac) - Control E11 5% CO2
CO2 profile
Glucose concentration [mM]
Lactate concentration [mM]
Cultivation time [d]
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
0
1
2
3
4
5
6
7
8
9
10
11

Figure 12

(B)

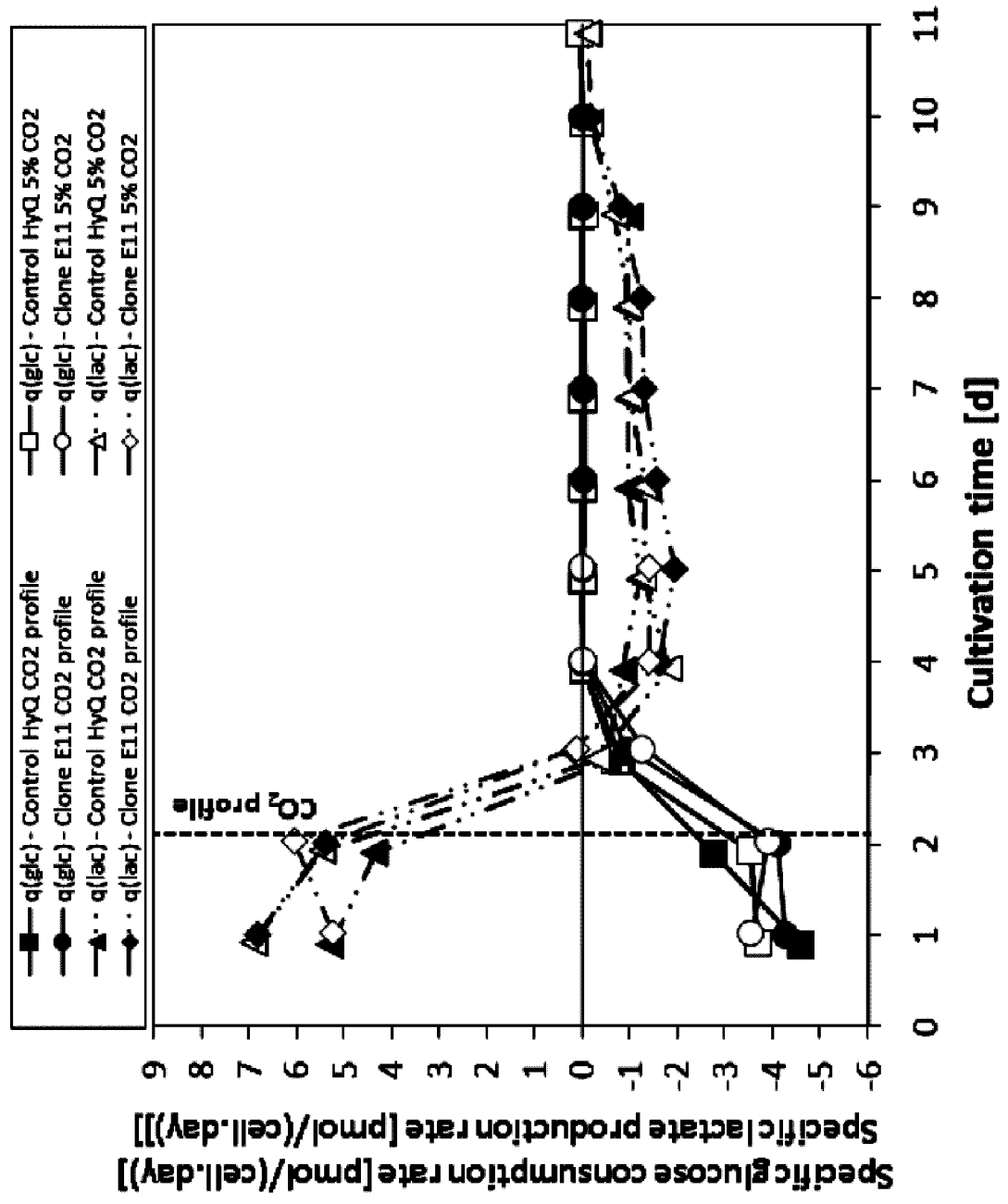

q(glc) - Control HyQ CO2 profile
q(glc) - Clone E11 CO2 profile
q(lac) - Control HyQ CO2 profile
q(lac) - Clone E11 CO2 profile
q(glc) - Control HyQ 5% CO2
q(glc) - Clone E11 5% CO2
q(lac) - Control HyQ 5% CO2
q(lac) - Clone E11 5% CO2
CO2 profile
Specific glucose consumption rate [pmol/(cell.day)]
Specific lactate production rate [pmol/(cell.day)]
Cultivation time [d]
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
0
1
2
3
4
5
6
7
8
9
10
11

Figure 12

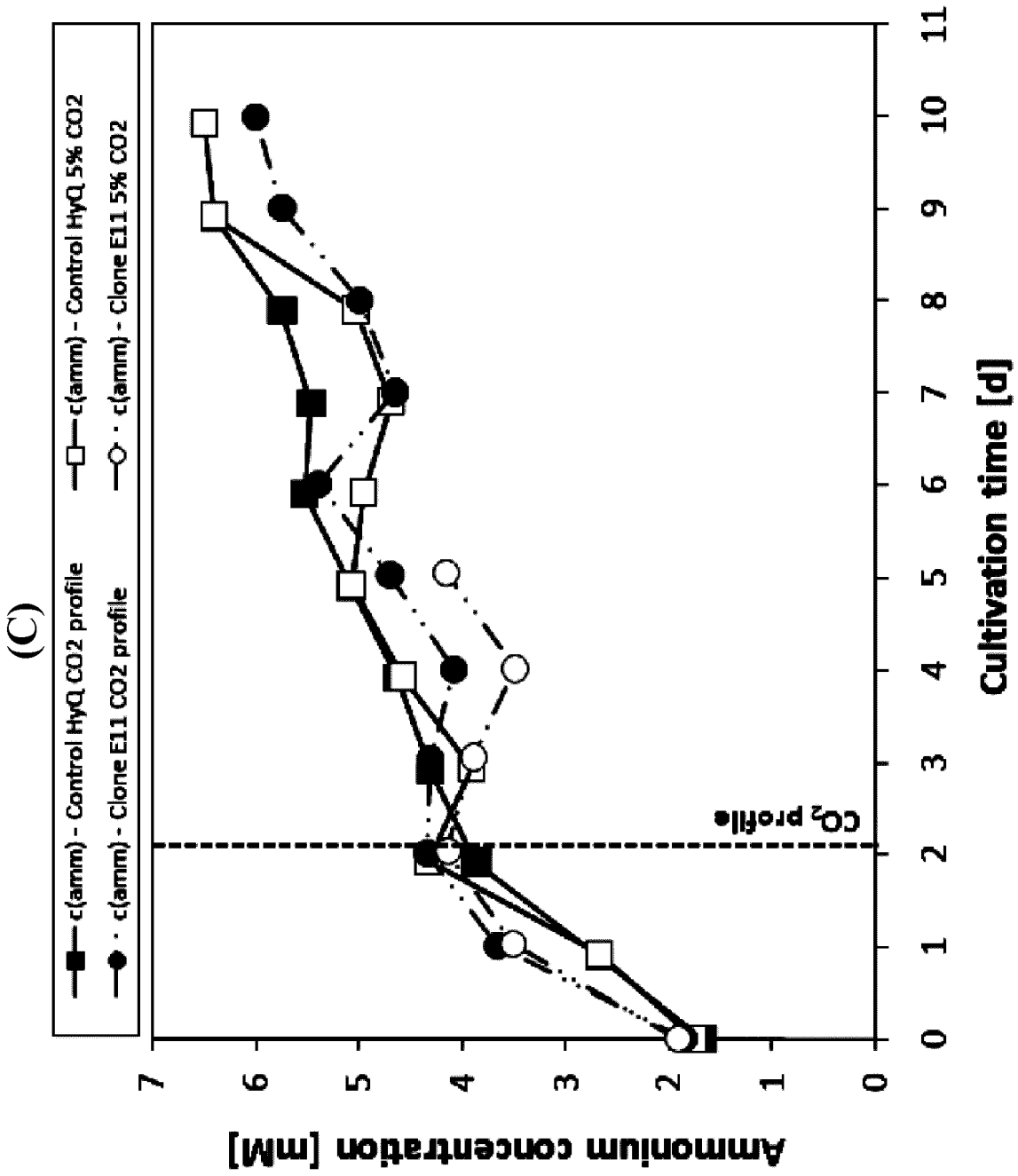

(C)
c(amm) - Control HyQ CO2 profile
c(amm) - Control HyQ 5% CO2
c(amm) - Clone E11 CO2 profile
c(amm) - Clone E11 5% CO2
CO2 profile
Ammonium concentration [mM]
Cultivation time [d]
0
1
2
3
4
5
6
7
8
9
10
11

Figure 12

(D)

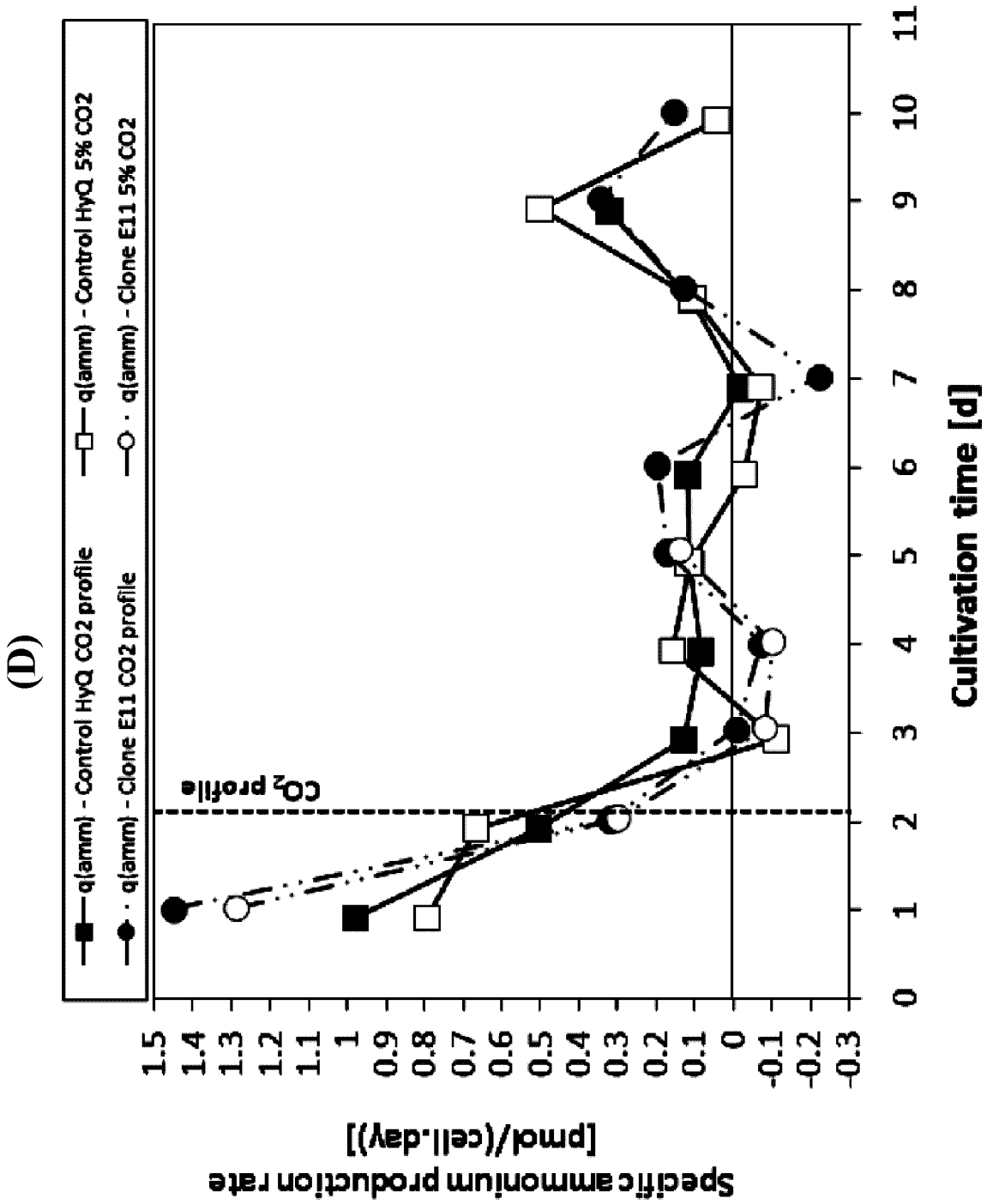

q(amm) - Control HyQ CO2 profile
q(amm) - Control HyQ 5% CO2
q(amm) - Clone E11 CO2 profile
q(amm) - Clone E11 5% CO2
CO2 profile
Specific ammonium production rate
[pmol/(cell.day)]
Cultivation time [d]
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
0
1
2
3
4
5
6
7
8
9
10
11

Figure 13

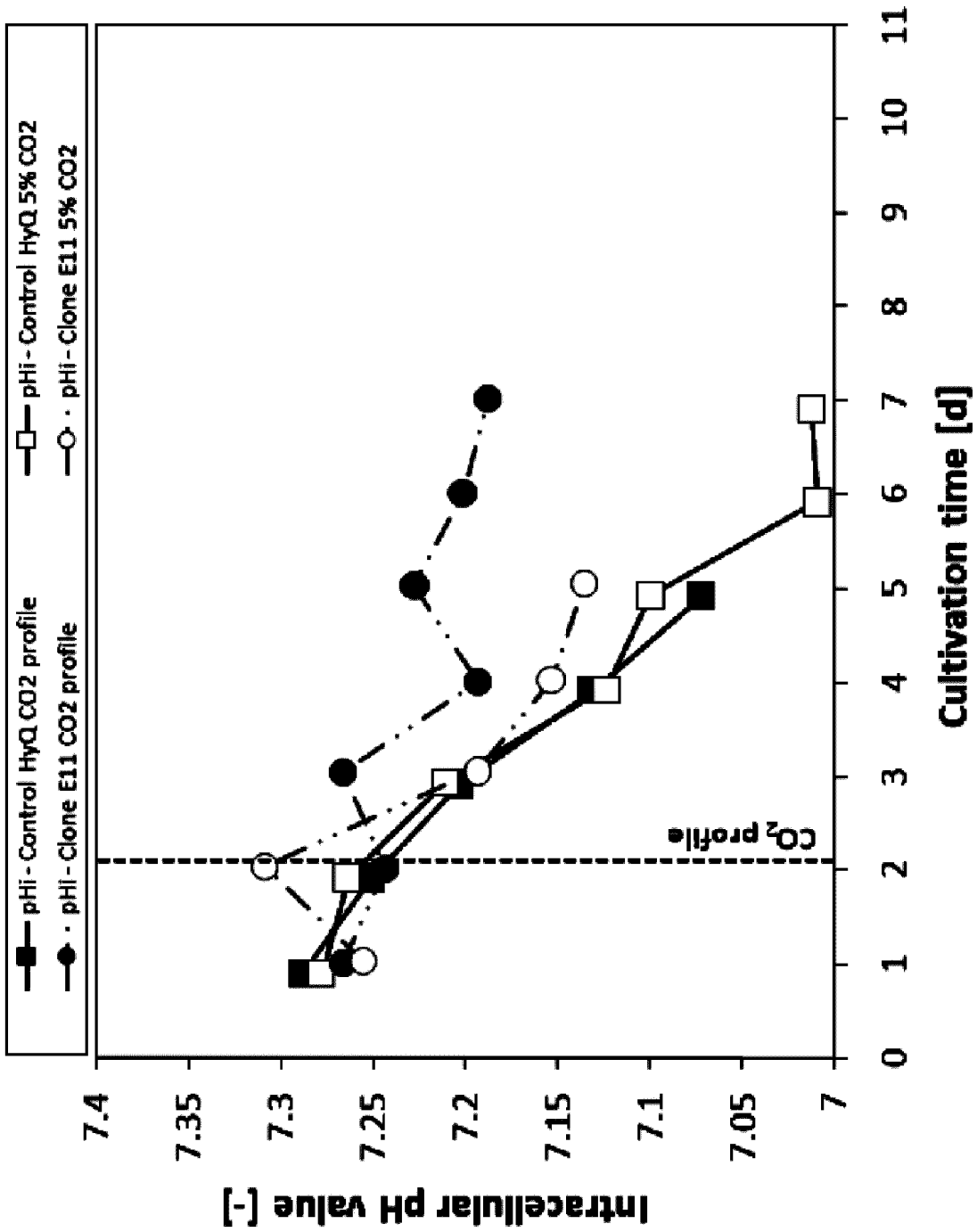

pHi - Control HyQ CO2 profile
pHi - Control HyQ 5% CO2
pHi - Clone E11 CO2 profile
pHi - Clone E11 5% CO2
CO2 profile
Intracellular pH value [-]
Cultivation time [d]
7.4
7.35
7.3
7.25
7.2
7.15
7.1
7.05
7
0
1
2
3
4
5
6
7
8
9
10
11

Figure 14

(A)

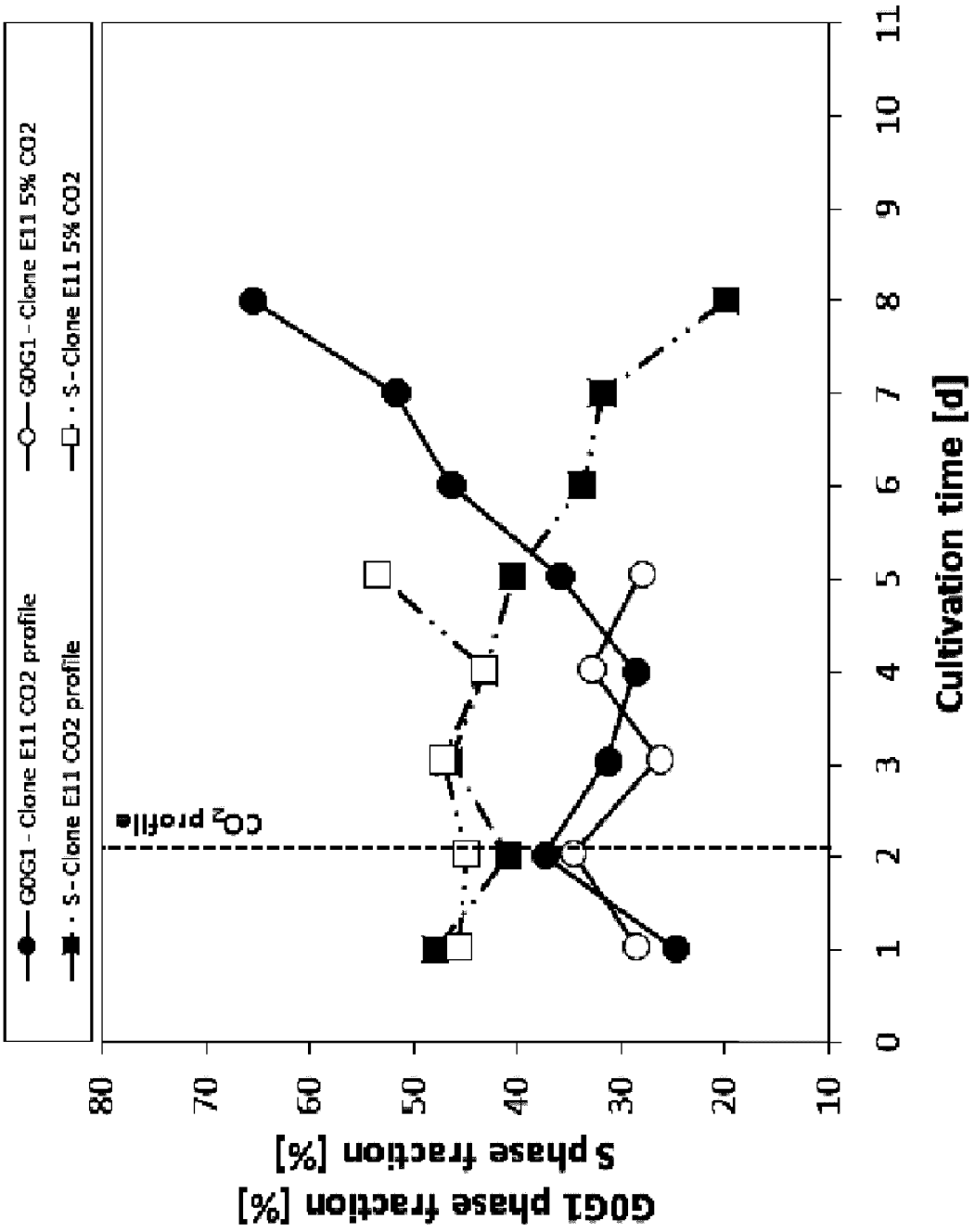

G0G1 - Clone E11 CO2 profile
S - Clone E11 CO2 profile
G0G1 - Clone E11 5% CO2
S - Clone E11 5% CO2
CO2 profile
G0G1 phase fraction [%]
S phase fraction [%]
Cultivation time [d]
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11

Figure 14

(B)

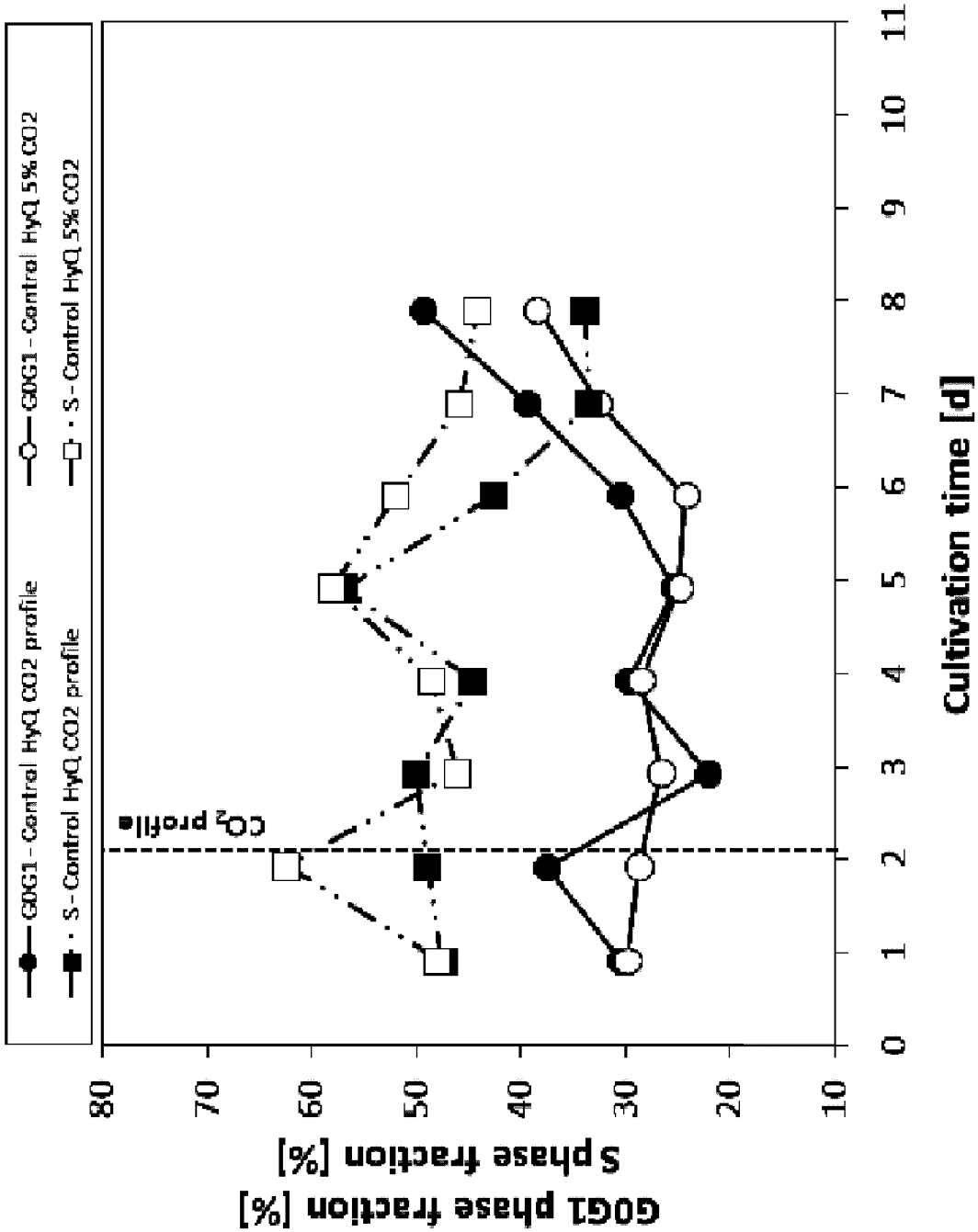

G0G1 - Control HyQ CO2 profile
S - Control HyQ CO2 profile
G0G1 - Control HyQ 5% CO2
S - Control HyQ 5% CO2
CO2 profile
G0G1 phase fraction [%]
S phase fraction [%]
Cultivation time [d]
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11

Figure 15

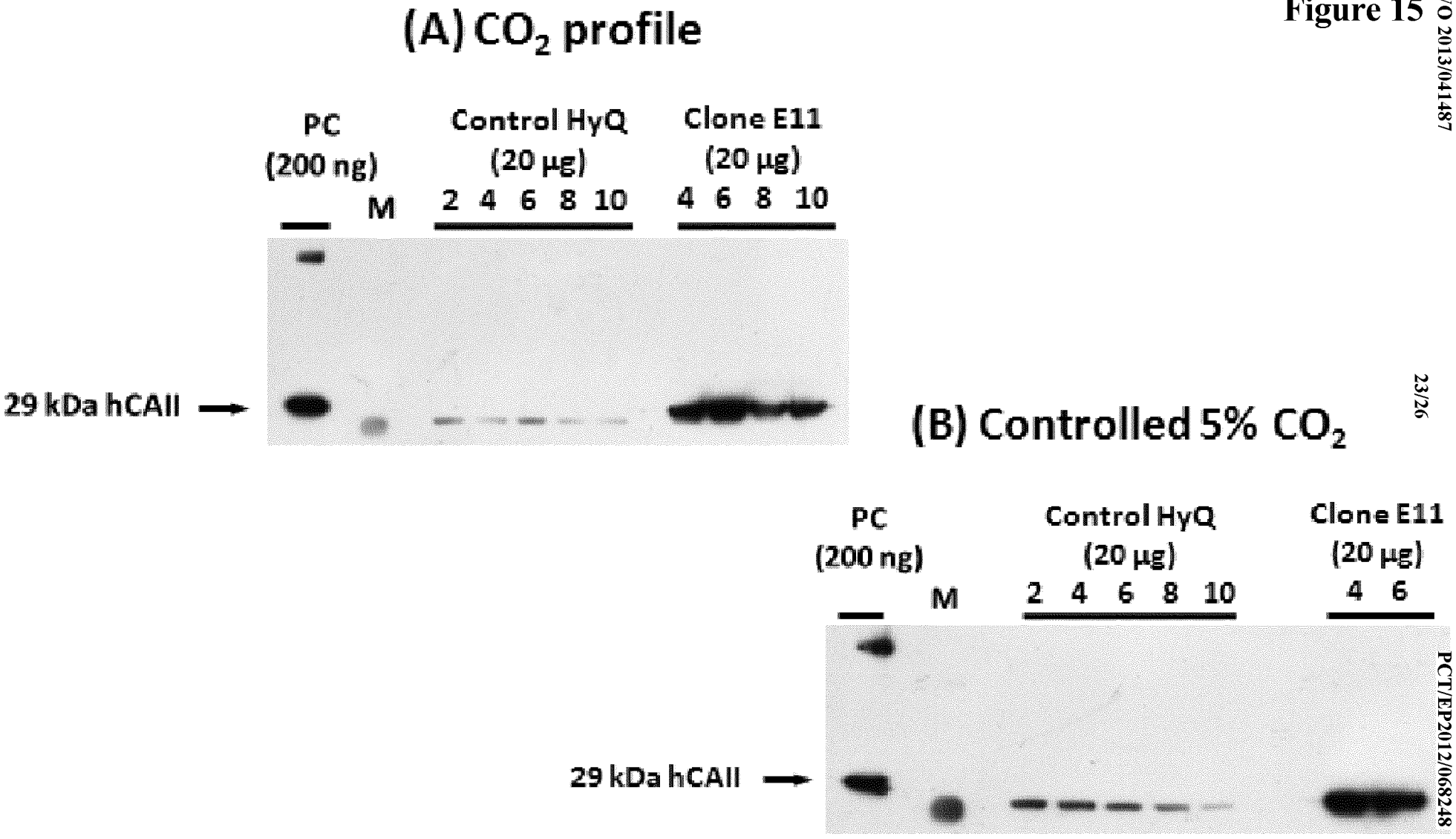

(A) CO_2 profile
PC
(200 ng)
M
Control HyQ
(20 µg)
2 4 6 8 10
Clone E11
(20 µg)
4 6 8 10
29 kDa hCAII
(B) Controlled 5% CO_2
PC
(200 ng)
M
Control HyQ
(20 µg)
2 4 6 8 10
Clone E11
(20 µg)
4 6
29 kDa hCAII

Figure 16

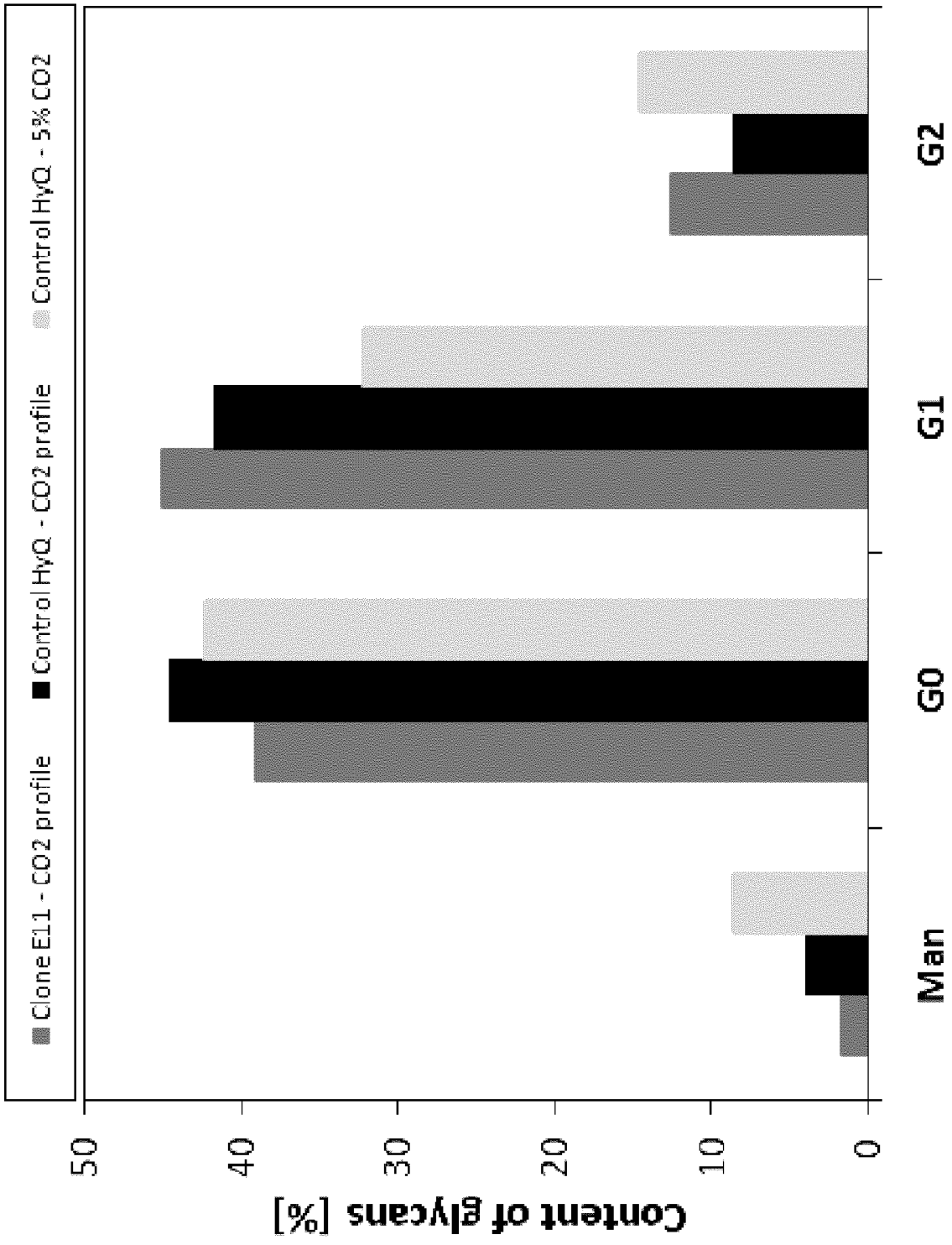

Clone E11 - CO2 profile
Control HyQ - CO2 profile
Control HyQ - 5% CO2
Content of glycans [%]
0
10
20
30
40
50
Man
G0
G1
G2

Figure 17

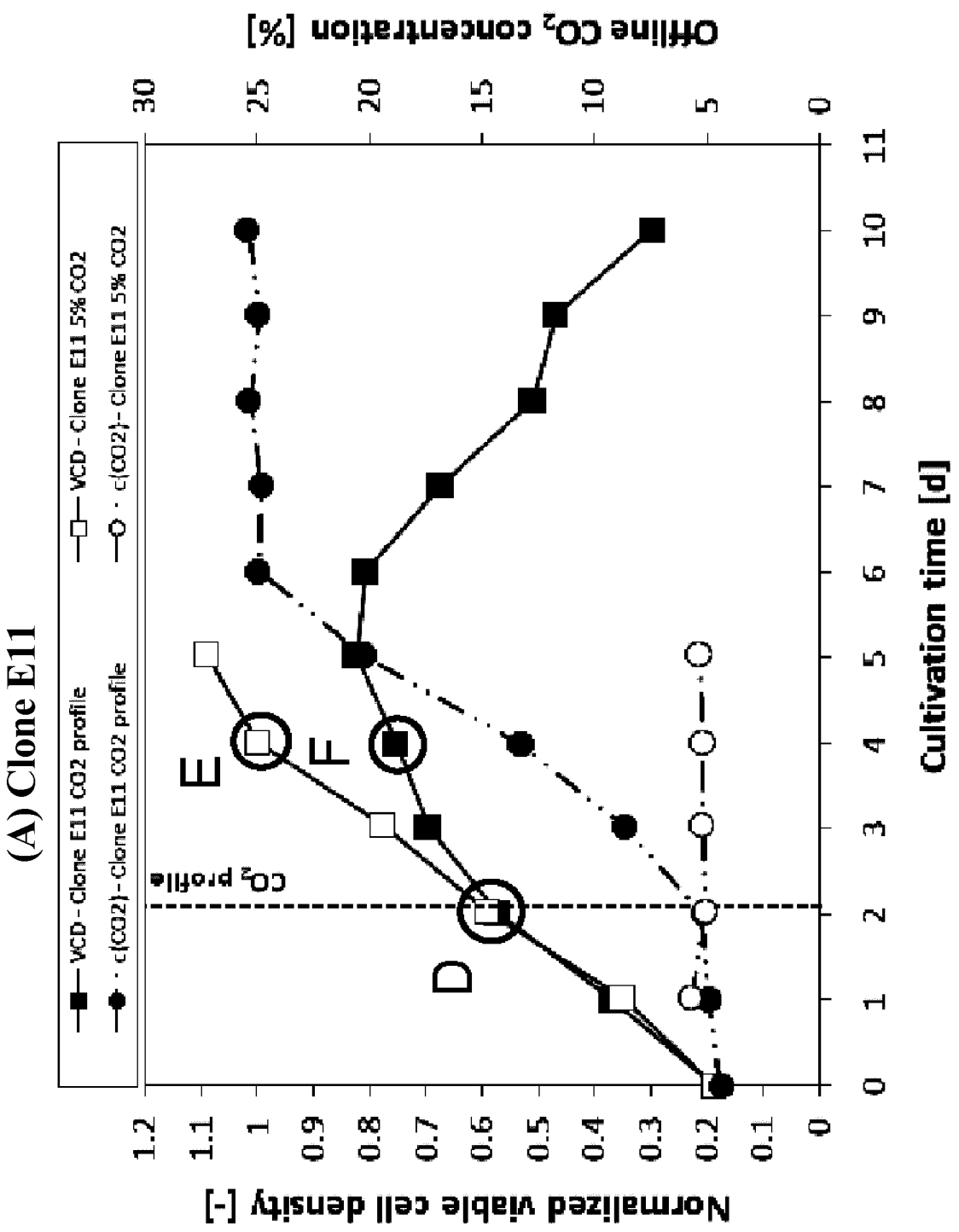

(A) Clone E11
VCD - Clone E11 CO2 profile
c(CO2) - Clone E11 CO2 profile
VCD - Clone E11 5% CO2
c(CO2) - Clone E11 5% CO2
CO2 profile
D
E
F
Normalized viable cell density [-]
Offline CO2 concentration [%]
Cultivation time [d]

Figure 17

(B) Control HyQ

