Figure 1

hCAII_NK0000 1 mshhwgygkhngpehwhkdfpiakgerqspvdidthtakydpslkplsvsydqatslrilnngharnvefddsqdkavlkggpldgtyrl
His94
His119
hCAII_NK0000 271 iqfhfhwgsldggqgschtvdkkkyaaelhlvhwntkygdfgkavqqpdglavigifikvgsakpglqkvvdvldsiktkgksadfcnfdp
His96
hCAII_NK0000 541 rglppesldywtypgsltppllecvtwivlkepisvssequlkfrklnfngegepeelmvdnwrpaqplknrqikasfk

Figure 2

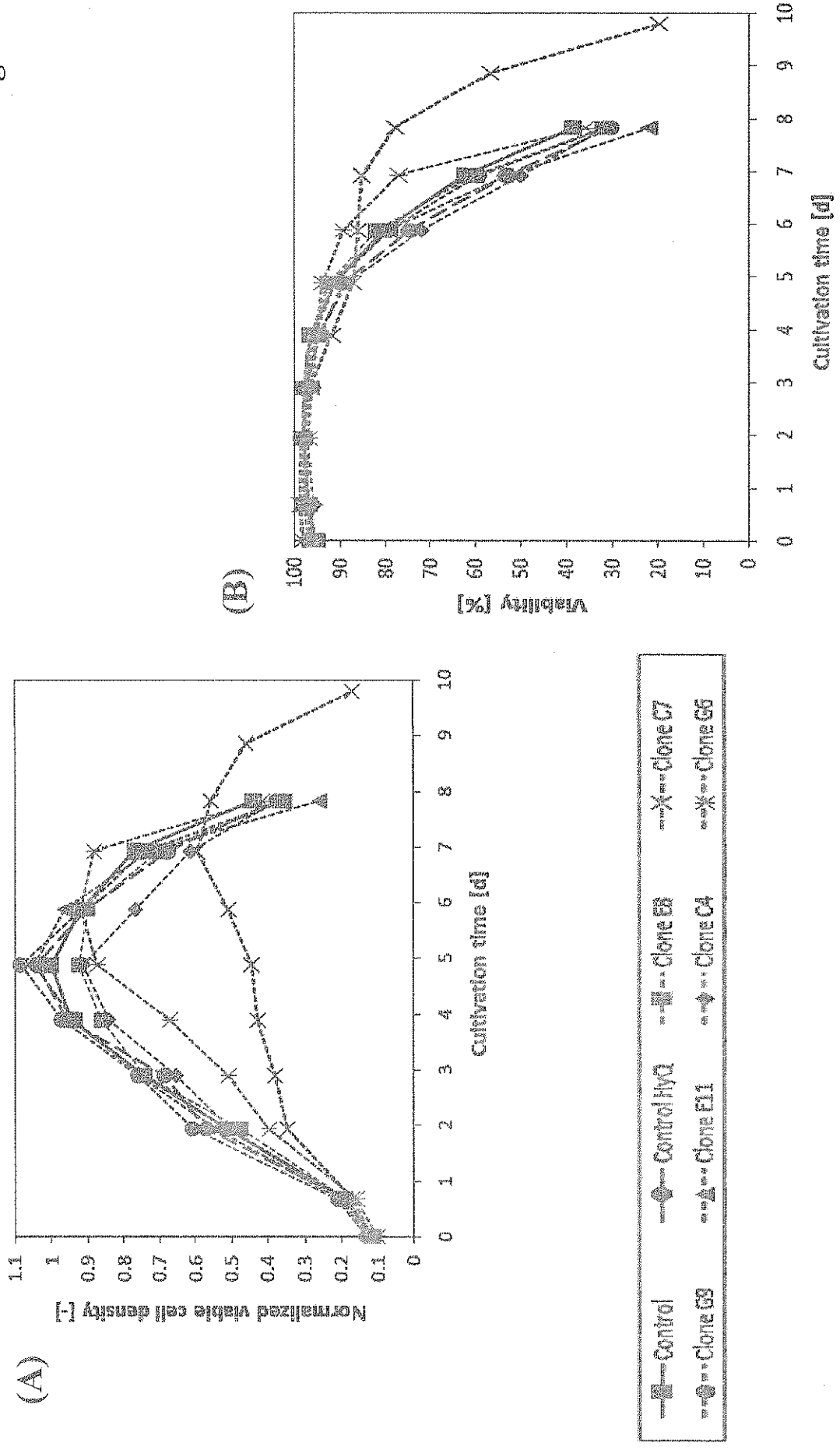
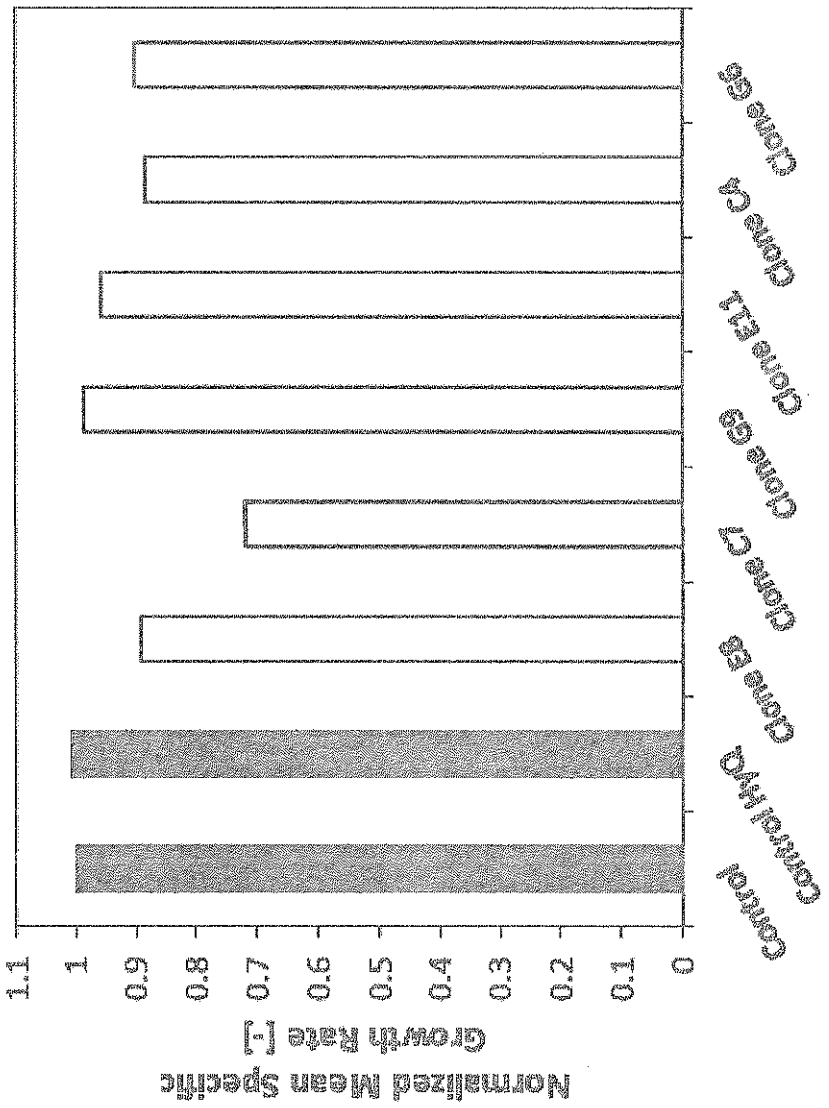

(A)
Normalized viable cell density [-]
Cultivation time [d]
(B)
Viability [%]
Cultivation time [d]
Control
Control Hyq
Clone E8
Clone C7
Clone G9
Clone E11
Clone C4
Clone G5

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 B3
clone C7
clone G9
clone E1
clone A
clone G8

(C)

Figure 3

(A)
Glucose concentration [mM]
Lactate concentration [mM]
Cultivation time [d]
c(glc)-Control
c(glc)-Clone C7
c(glc)-Clone C4
c(glc)-Control HyQ
c(glc)-Clone G3
c(glc)-Clone G5
c(lac)-Control HyQ
c(lac)-Clone E8
c(lac)-Clone G3
c(lac)-Clone E11
c(glc)-Clone E8
c(glc)-Clone E11
c(lac)-Control
c(lac)-Clone C7
c(lac)-Clone C4
(B)
Specific glucose consumption rate [pmol/(cell.day)]
Specific lactate production rate [pmol/(cell.day)]
Cultivation time [d]
4/26

Figure 4

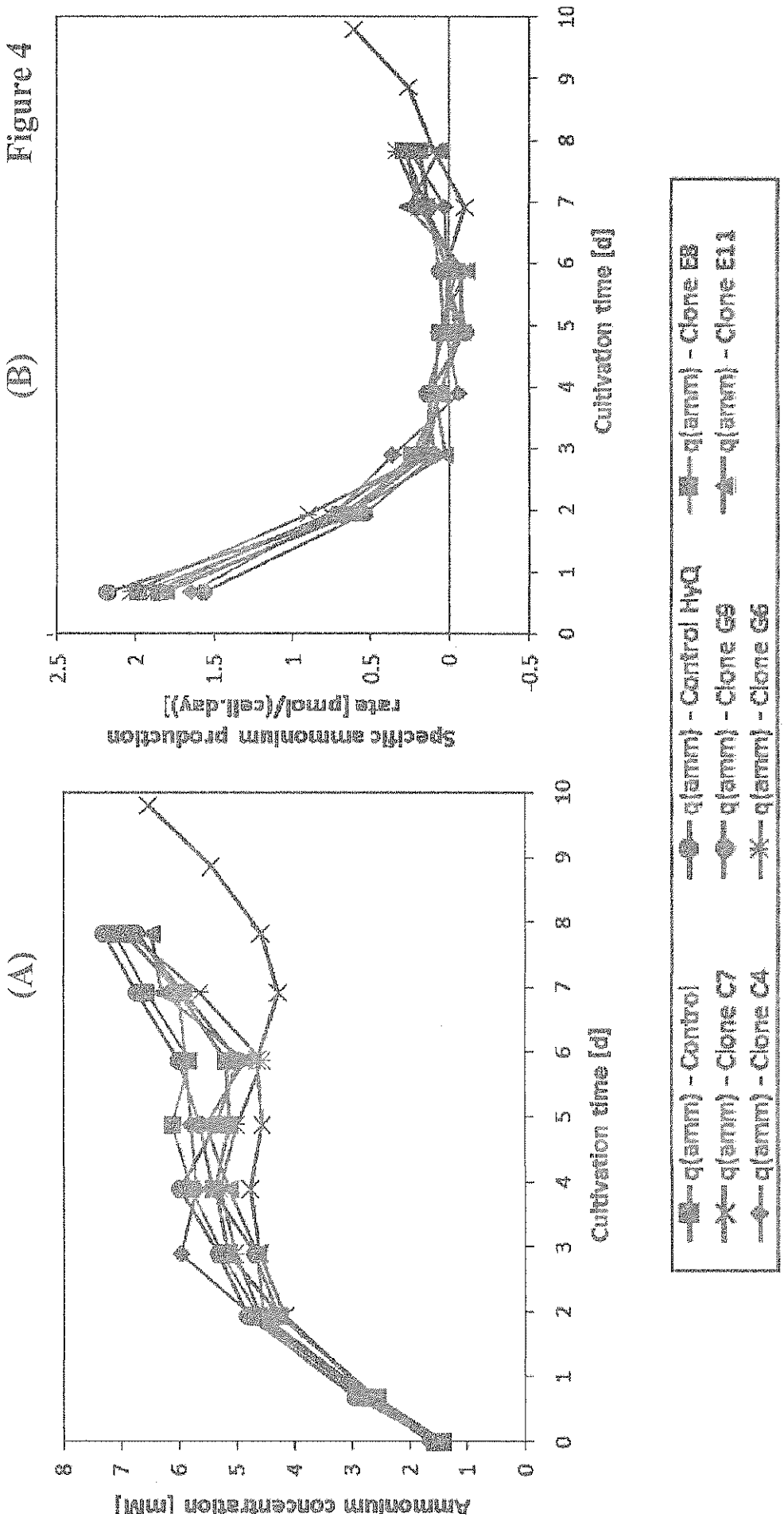

(A)
Ammonium concentration [mM]
Cultivation time [d]
(B)
Specific ammonium production rate [pmol/(cell.day)]
Cultivation time [d]
q(amm) - Control
q(amm) - Clone C7
q(amm) - Clone C4
q(amm) - Control HyQ
q(amm) - Clone G9
q(amm) - Clone G6
q(amm) - Clone E8
q(amm) - Clone E11

Figure 5

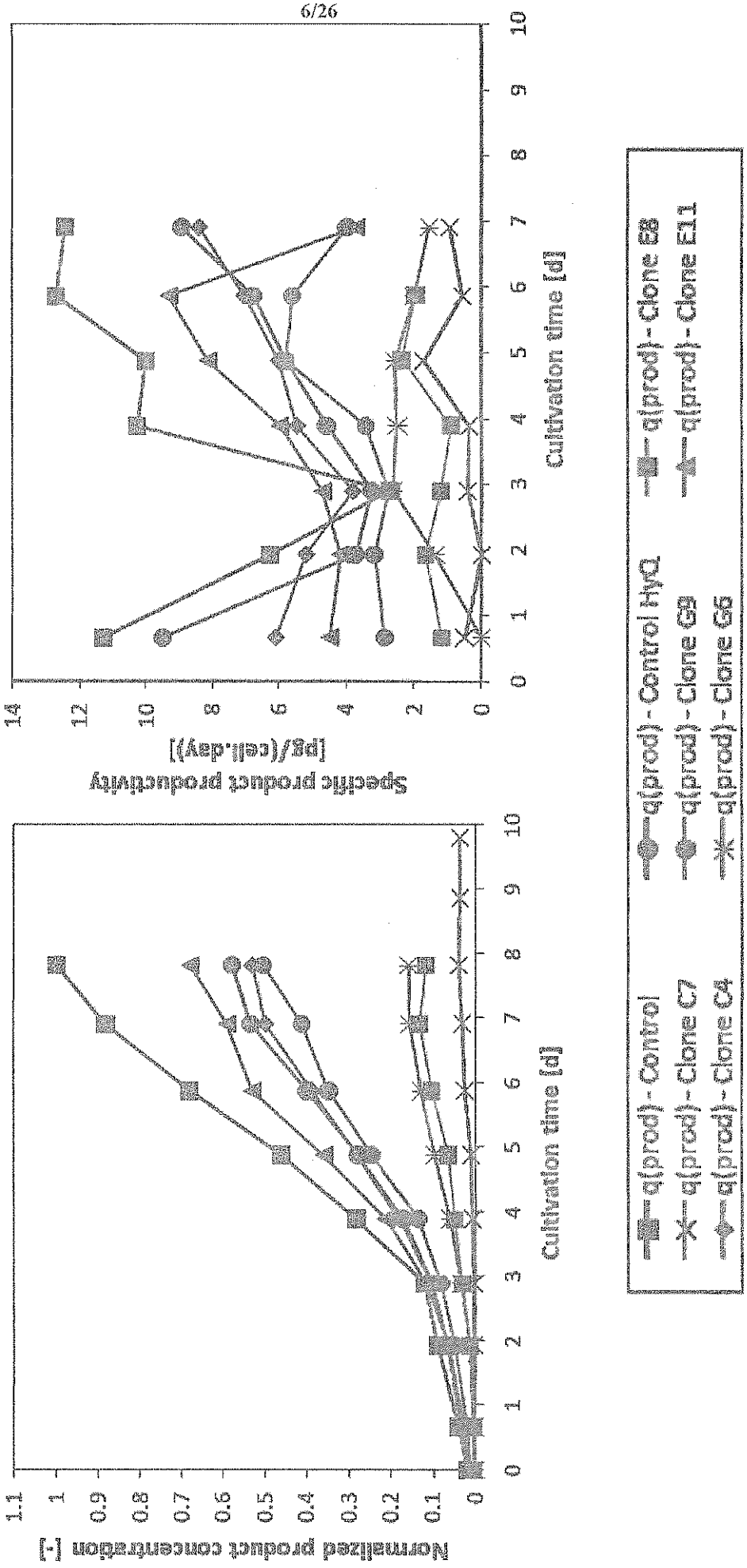

(A)
Normalized product concentration [-]
Cultivation time [d]
(B)
Specific product productivity
[pg/(cell.day)]
Cultivation time [d]
q(prod) - Control
q(prod) - Control HyQ
q(prod) - Clone E8
q(prod) - Clone C7
q(prod) - Clone G9
q(prod) - Clone E11
q(prod) - Clone C4
q(prod) - Clone G5

Figure 6

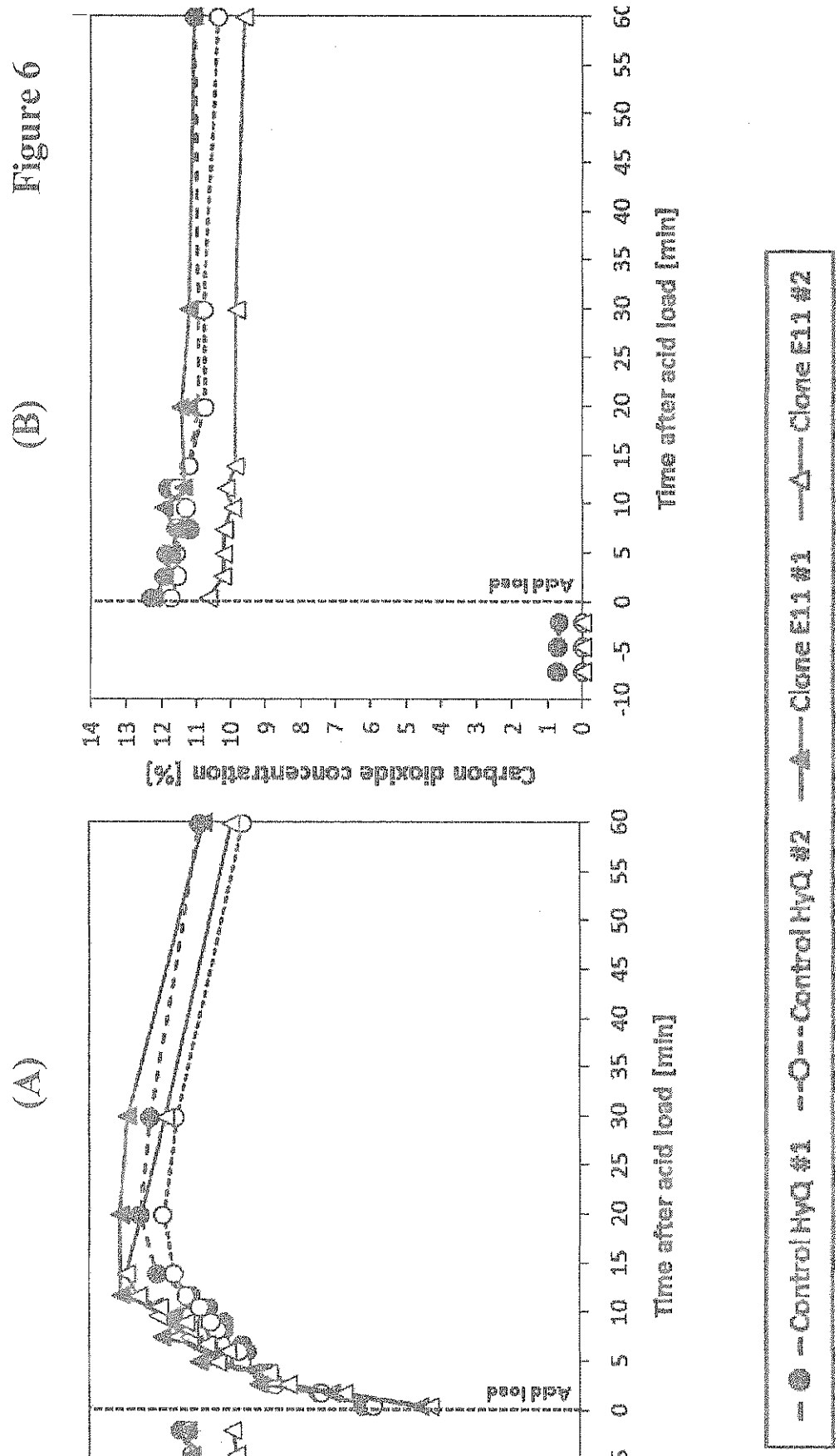

(A)
Intracellular pH value [-]
Time after acid load [min]
Acid load
(B)
Carbon dioxide concentration [%]
Time after acid load [min]
Acid load
Control HyQ #1
Control HyQ #2
Clone E11 #1
Clone E11 #2

Figure 7

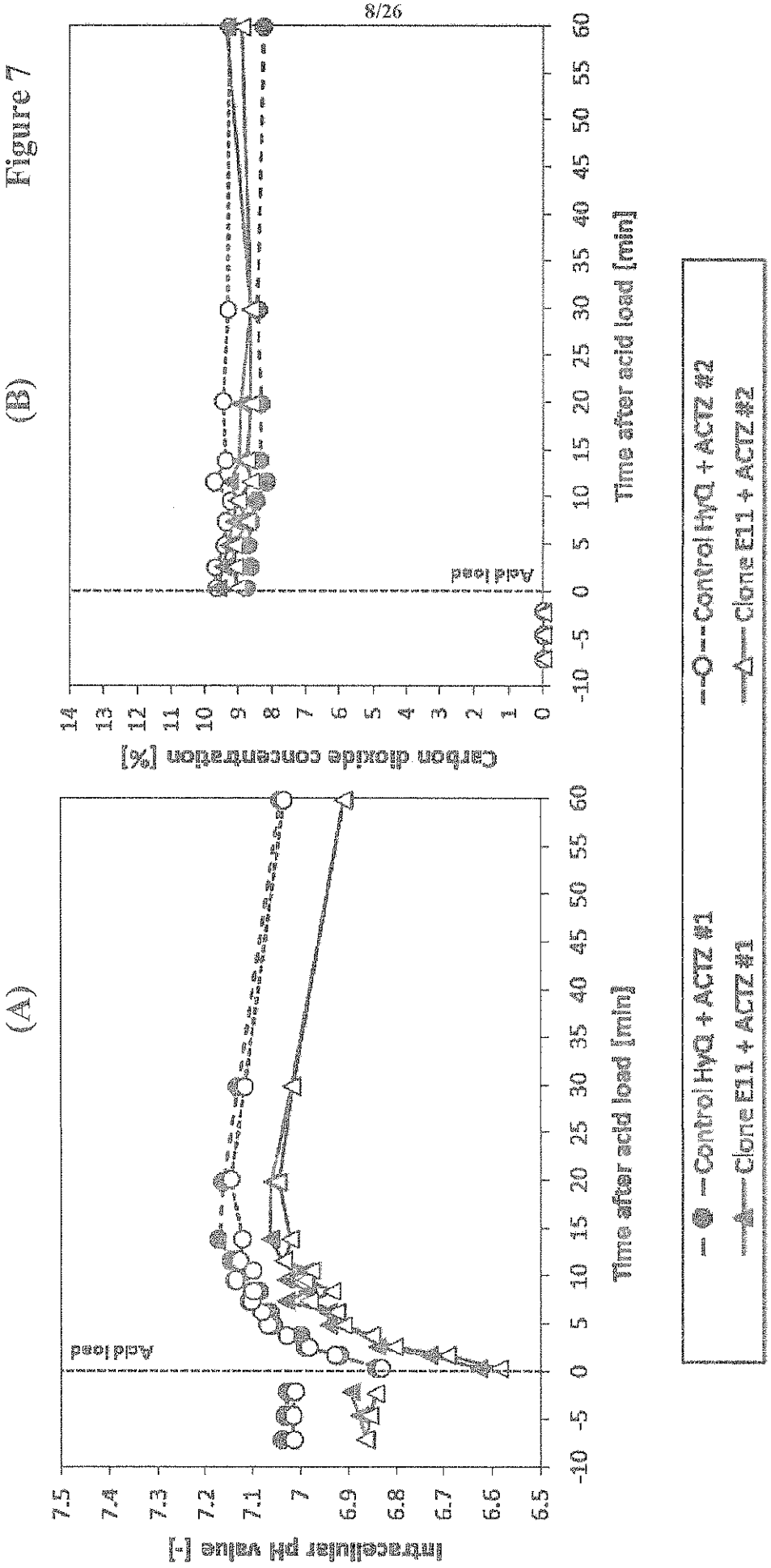

(A)
(B)
Acid load
Acid load
Time after acid load [min]
Time after acid load [min]
Carbon dioxide concentration [%]
Control HyQ + ACTZ #1
Control HyQ + ACTZ #2
Clone E11 + ACTZ #1
Clone E11 + ACTZ #2

Figure 8

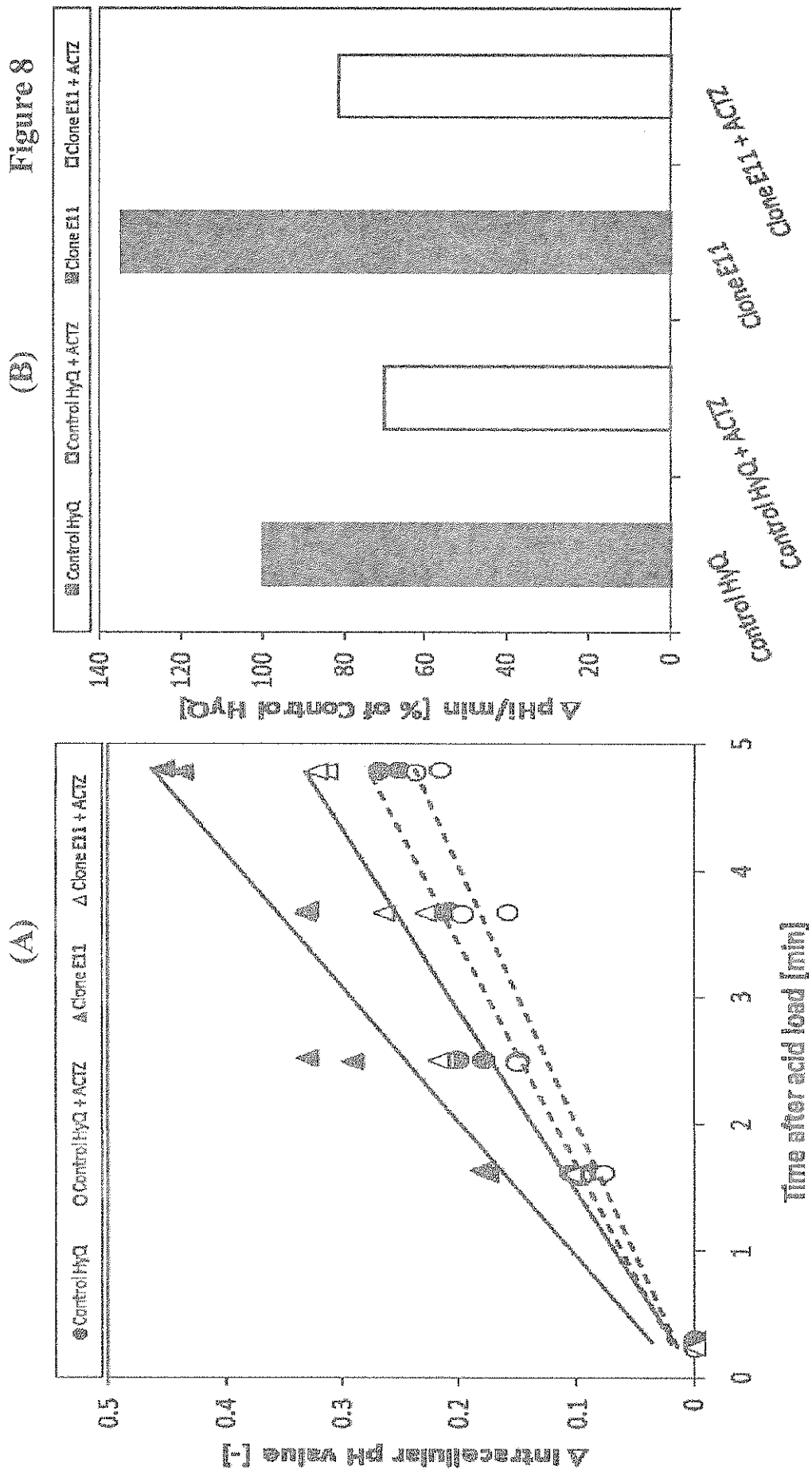

(A)
Control HyQ
Control HyQ + ACTZ
Clone E11
Clone E11 + ACTZ
Δ intracellular pH value [-]
Time after acid load [min]
(B)
Control HyQ
Control HyQ + ACTZ
Clone E11
Clone E11 + ACTZ
Δ pH/min [% of control HyQ]
Control HyQ
Control HyQ + ACTZ
Clone E11
Clone E11 + ACTZ

Figure 9

(A)

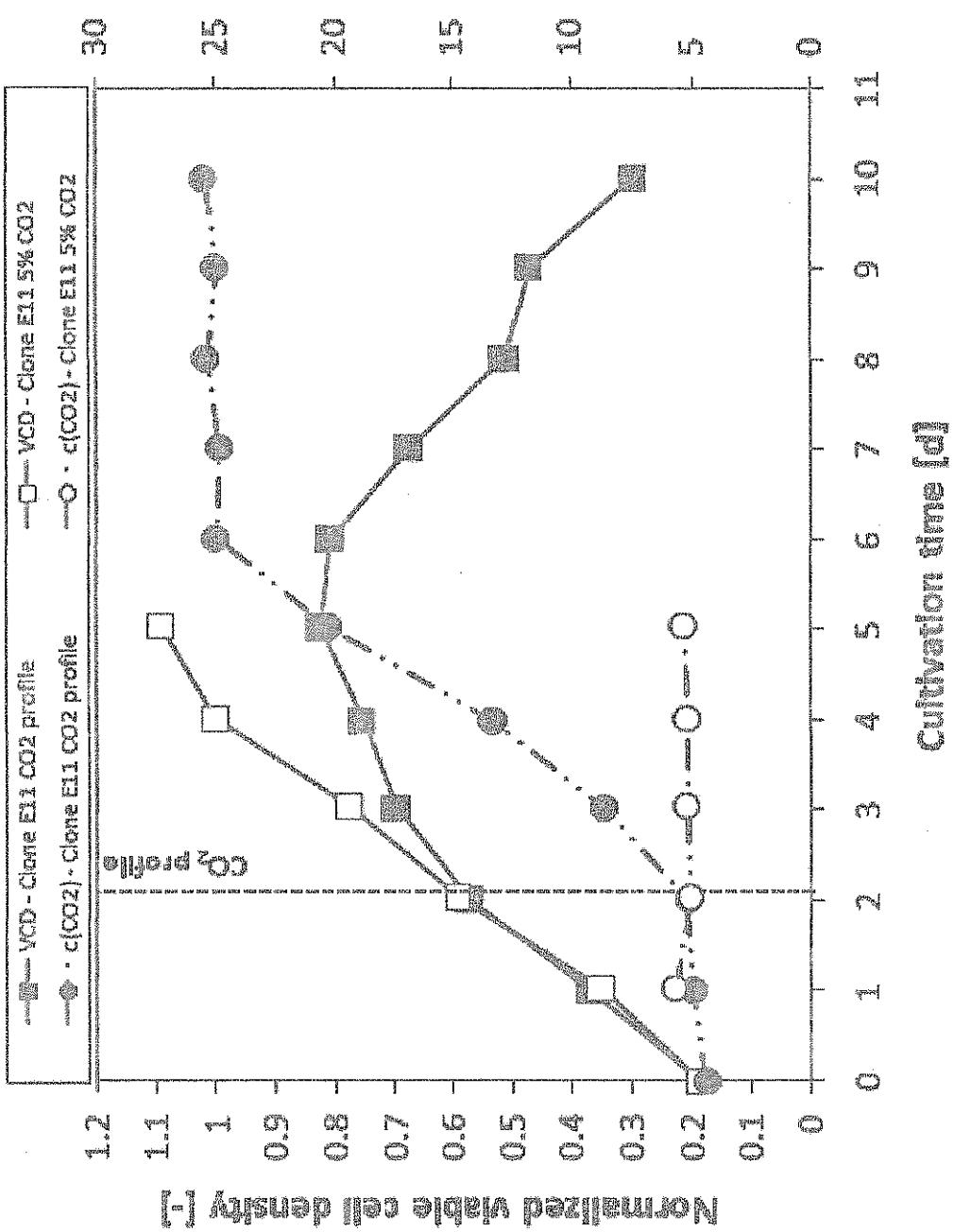

VCD - Clone E11 CO2 profile
c(CO2) - Clone E11 CO2 profile
VCD - Clone E11 5% CO2
c(CO2) - Clone E11 5% CO2
CO2 profile
Normalized viable cell density [-]
Offline CO2 concentration [%]
Cultivation time [d]
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
30
25
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9 10 11

Figure 9

(B)

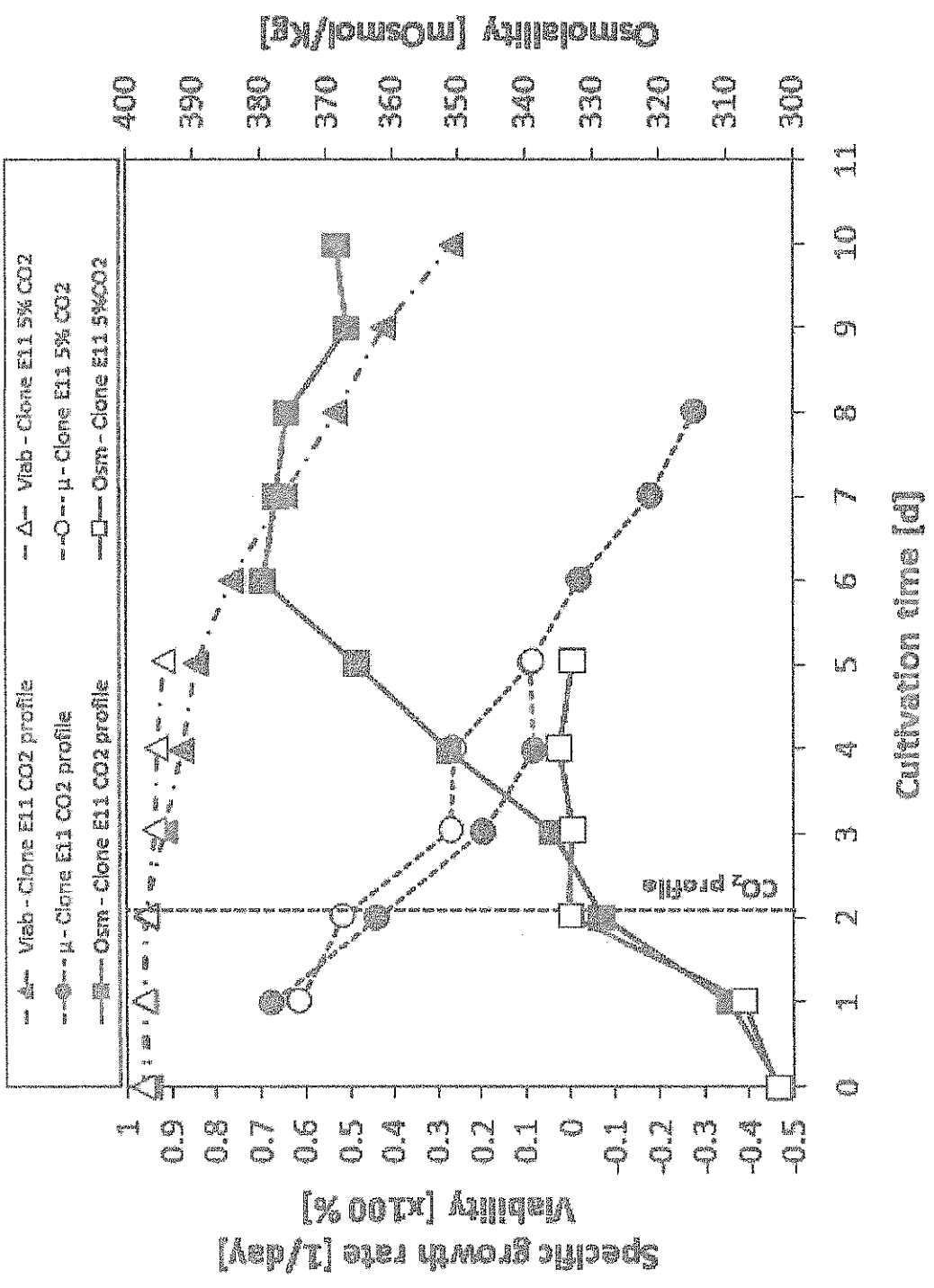

Specific growth rate [1/day]
Viability [x100 %]
Osmolality [mOsmol/Kg]
Cultivation time [d]
CO2 profile
Viab - Clone E11 CO2 profile
μ - Clone E11 CO2 profile
Osm - Clone E11 CO2 profile
Viab - Clone E11 5% CO2
μ - Clone E11 5% CO2
Osm - Clone E11 5%CO2

Figure 10

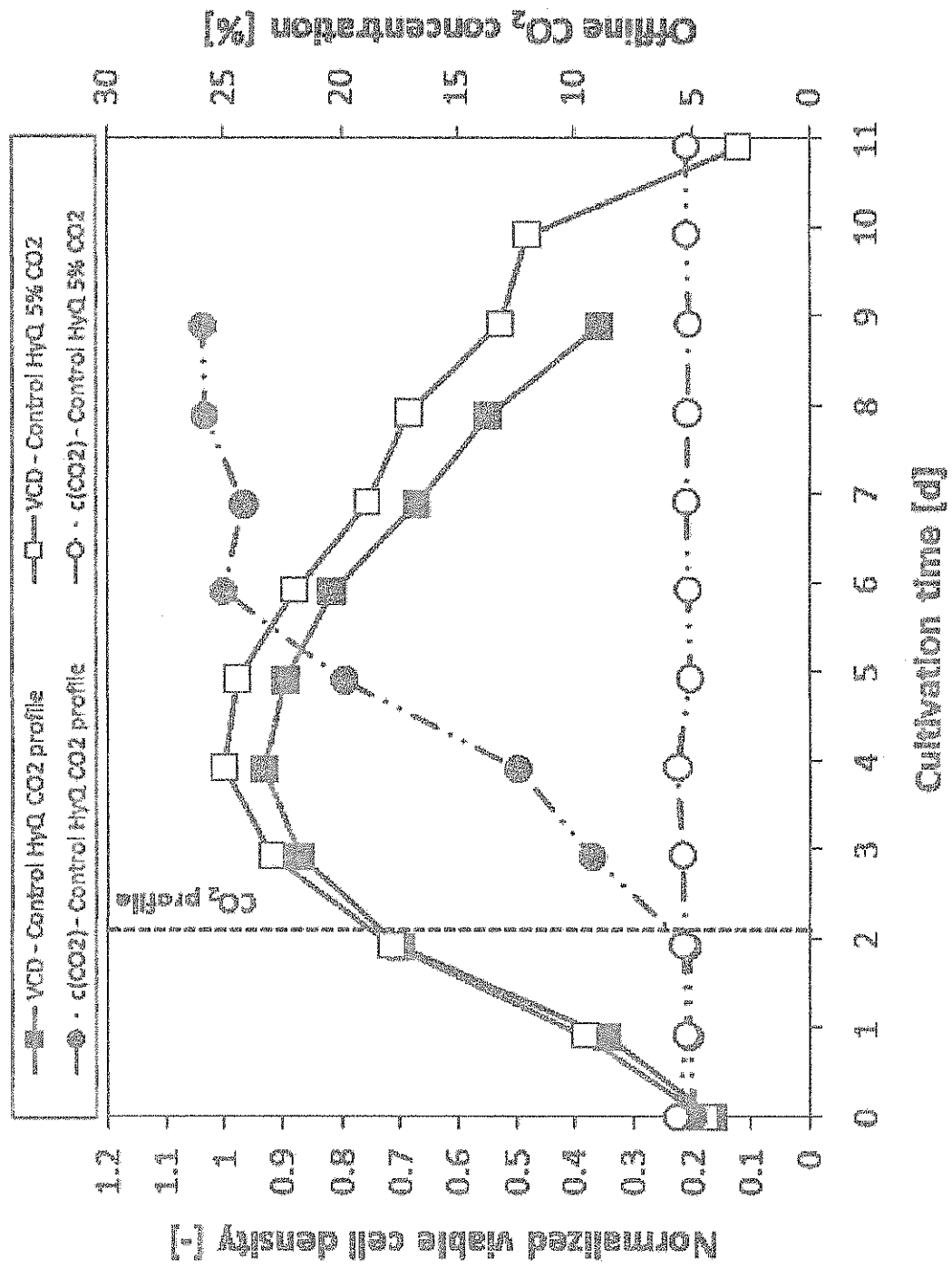

(A)
VCD - Control HyQ CO2 profile
c(CO2) - Control HyQ CO2 profile
VCD - Control HyQ 5% CO2
c(CO2) - Control HyQ 5% CO2
CO2 profile
Normalized viable cell density [-]
Offline CO2 concentration [%]
Cultivation time [d]

Figure 10

(B)

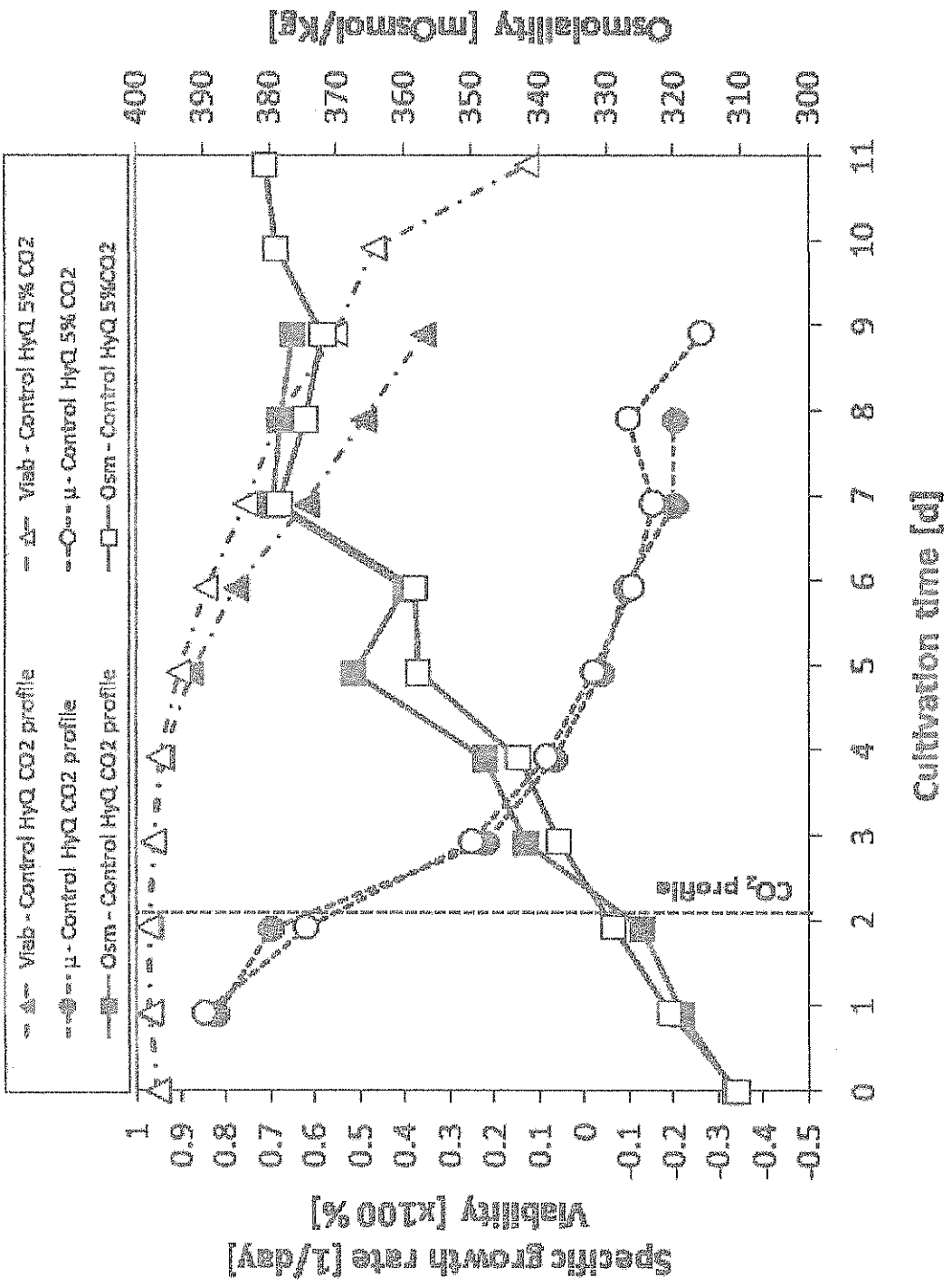

Viab - Control HyQ CO2 profile
μ - Control HyQ CO2 profile
Osm - Control HyQ CO2 profile
Viab - Control HyQ 5% CO2
μ - Control HyQ 5% CO2
Osm - Control HyQ 5%CO2
CO2 profile
Specific growth rate [1/day]
Viability [x100 %]
Osmolality [mOsmol/kg]
Cultivation time [d]

Figure 11

(A)

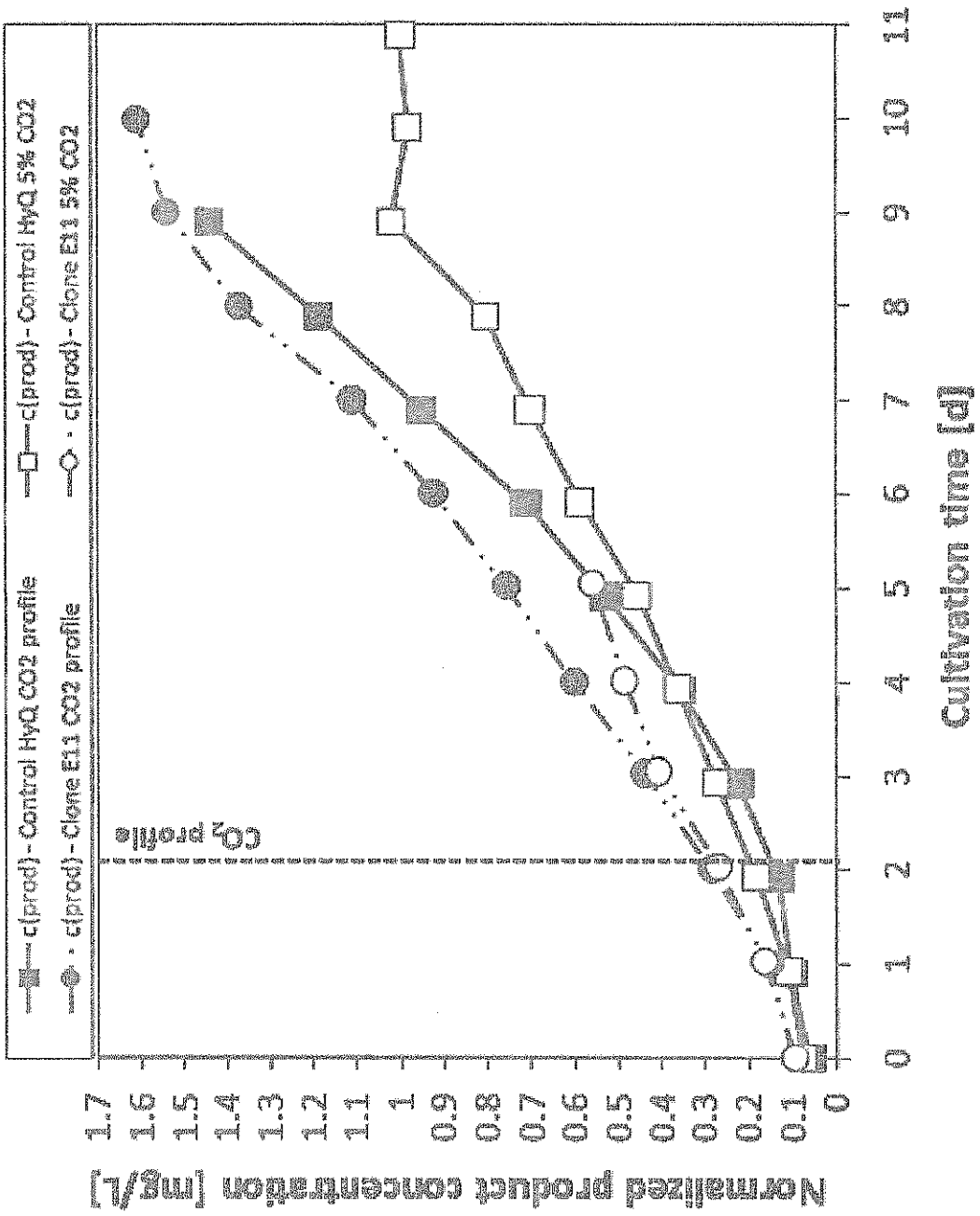

c(prod) - Control HyQ CO2 profile
c(prod) - Control HyQ 5% CO2
c(prod) - Clone E11 CO2 profile
c(prod) - Clone E11 5% CO2
CO2 profile
Normalized product concentration [mg/L]
Cultivation time [d]
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
1.1
1.2
1.3
1.4
1.5
1.6
1.7
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14

Figure 11

(B)

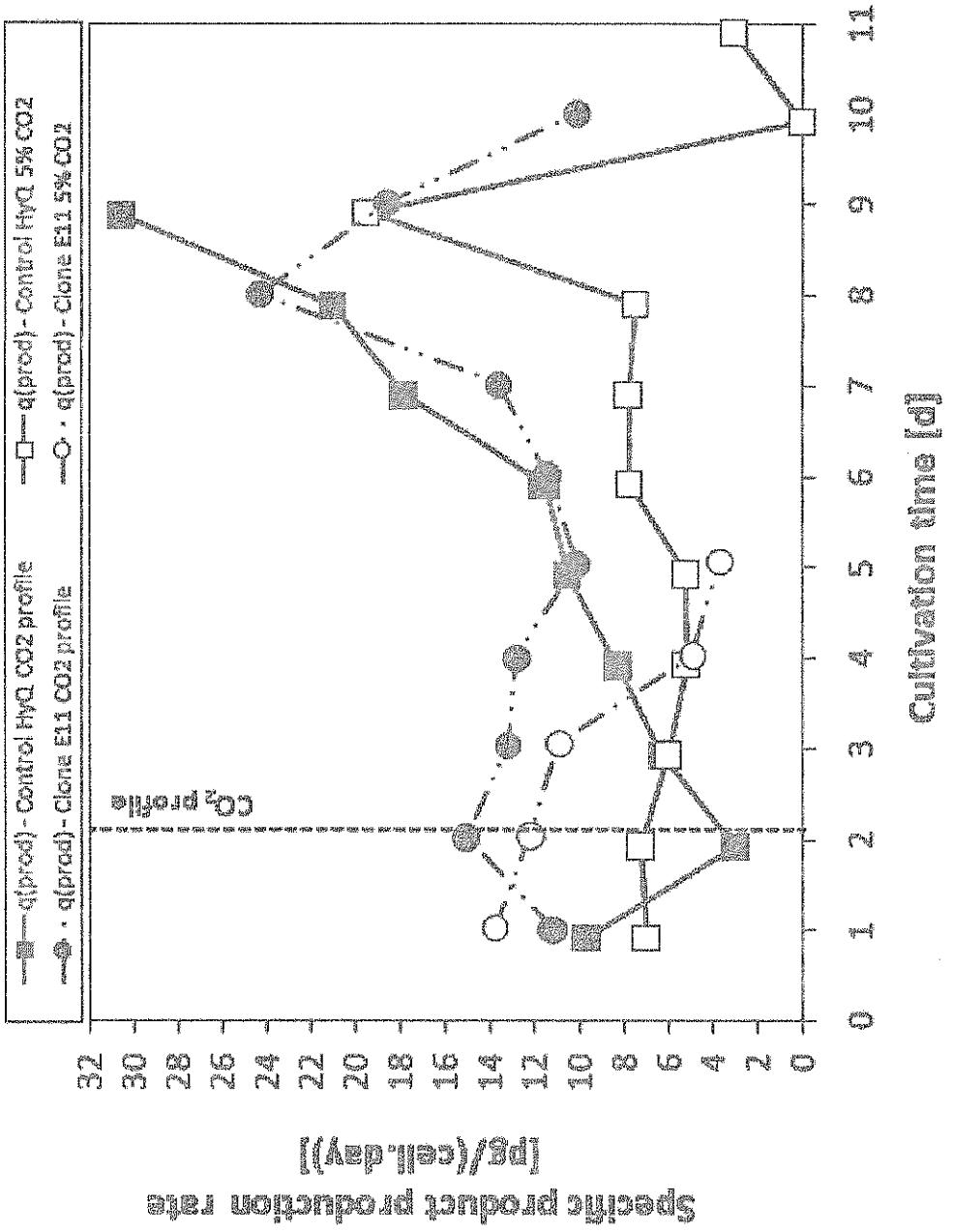

q(prod) - Control HyQ CO2 profile
q(prod) - Control HyQ 5% CO2
q(prod) - Clone E11 CO2 profile
q(prod) - Clone E11 5% CO2
CO2 profile
Specific product production rate
[pg/(cell.day)]
Cultivation time [d]
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
1
3
5
7
9
11

Figure 12

(A)

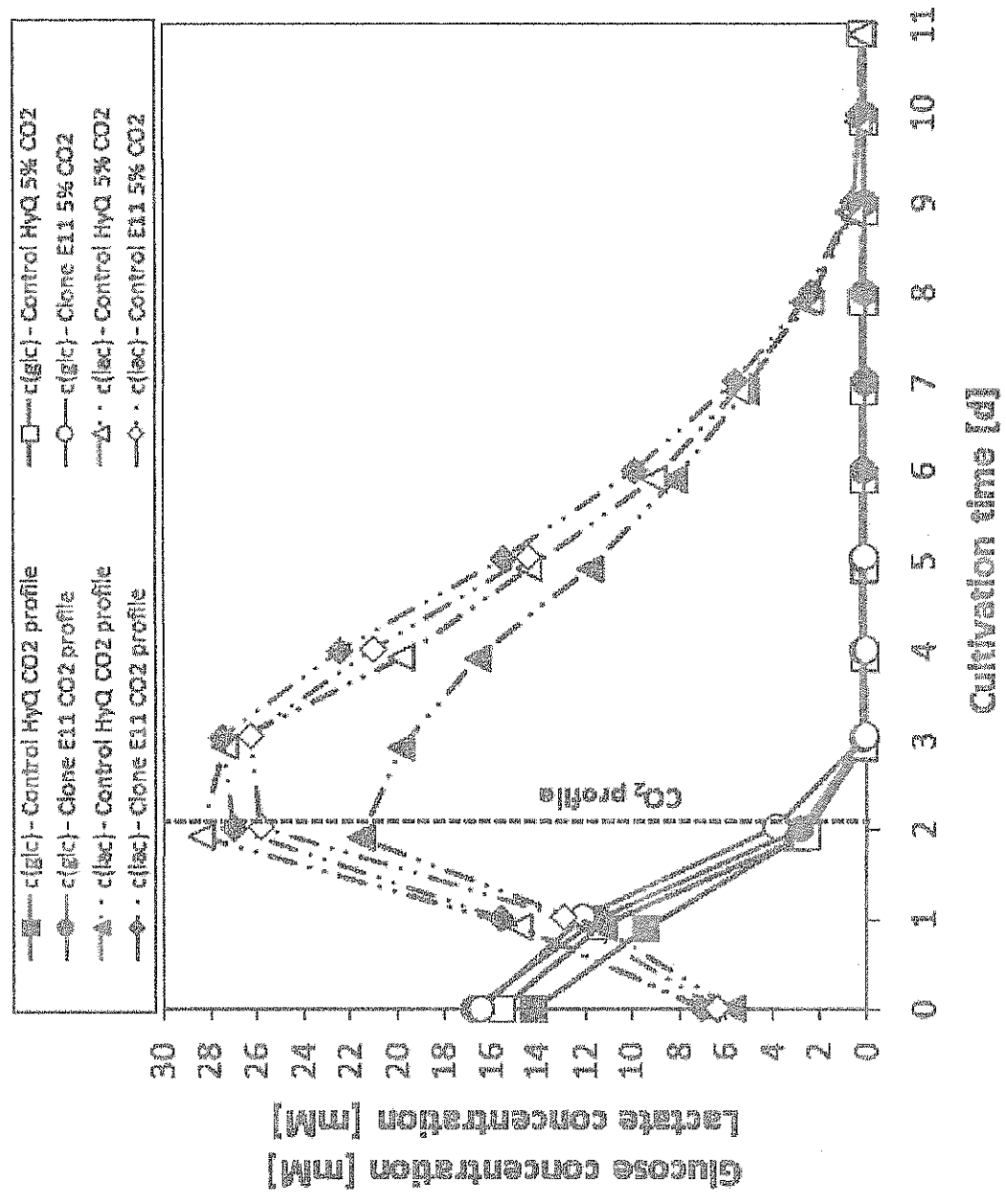

c(glc) - Control HyQ CO2 profile
c(glc) - Clone E11 CO2 profile
c(lac) - Control HyQ CO2 profile
c(lac) - Clone E11 CO2 profile
c(glc) - Control HyQ 5% CO2
c(glc) - Clone E11 5% CO2
c(lac) - Control HyQ 5% CO2
c(lac) - Clone E11 5% CO2
CO2 profile
Glucose concentration [mM]
Lactate concentration [mM]
Cultivation time [d]
0
2
4
6
8
10
12
14
15
16
18
20
22
24
25
26
28
30
1
3
5
7
9
11

Figure 12

(B)

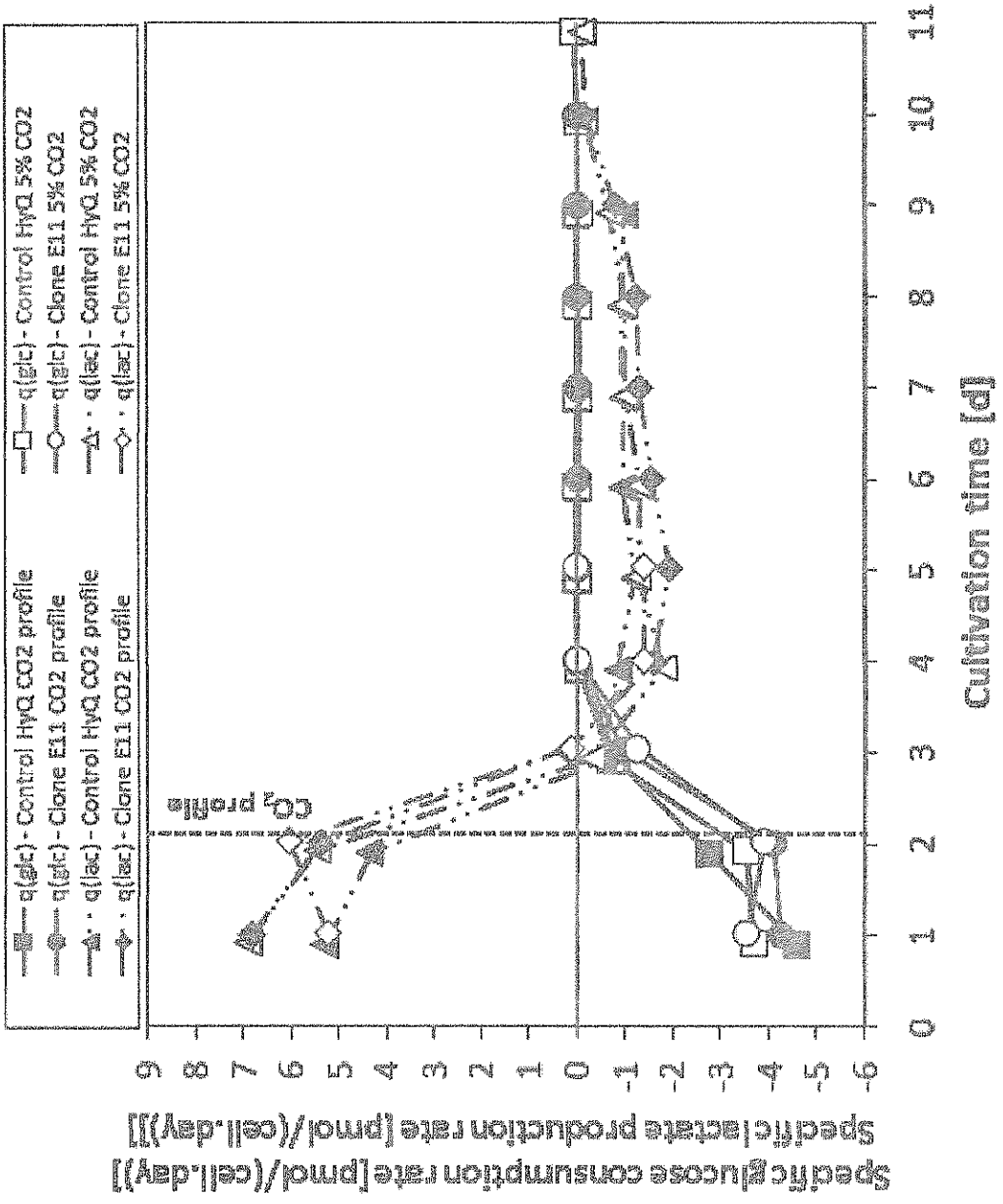

q(glc) - Control HyQ CO2 profile
q(glc) - Clone E11 CO2 profile
q(lac) - Control HyQ CO2 profile
q(lac) - Clone E11 CO2 profile
q(glc) - Control HyQ 5% CO2
q(glc) - Clone E11 5% CO2
q(lac) - Control HyQ 5% CO2
q(lac) - Clone E11 5% CO2
CQ profile
Specific glucose consumption rate [pmol/(cell.day)]
Specific lactate production rate [pmol/(cell.day)]
Cultivation time [d]

Figure 12

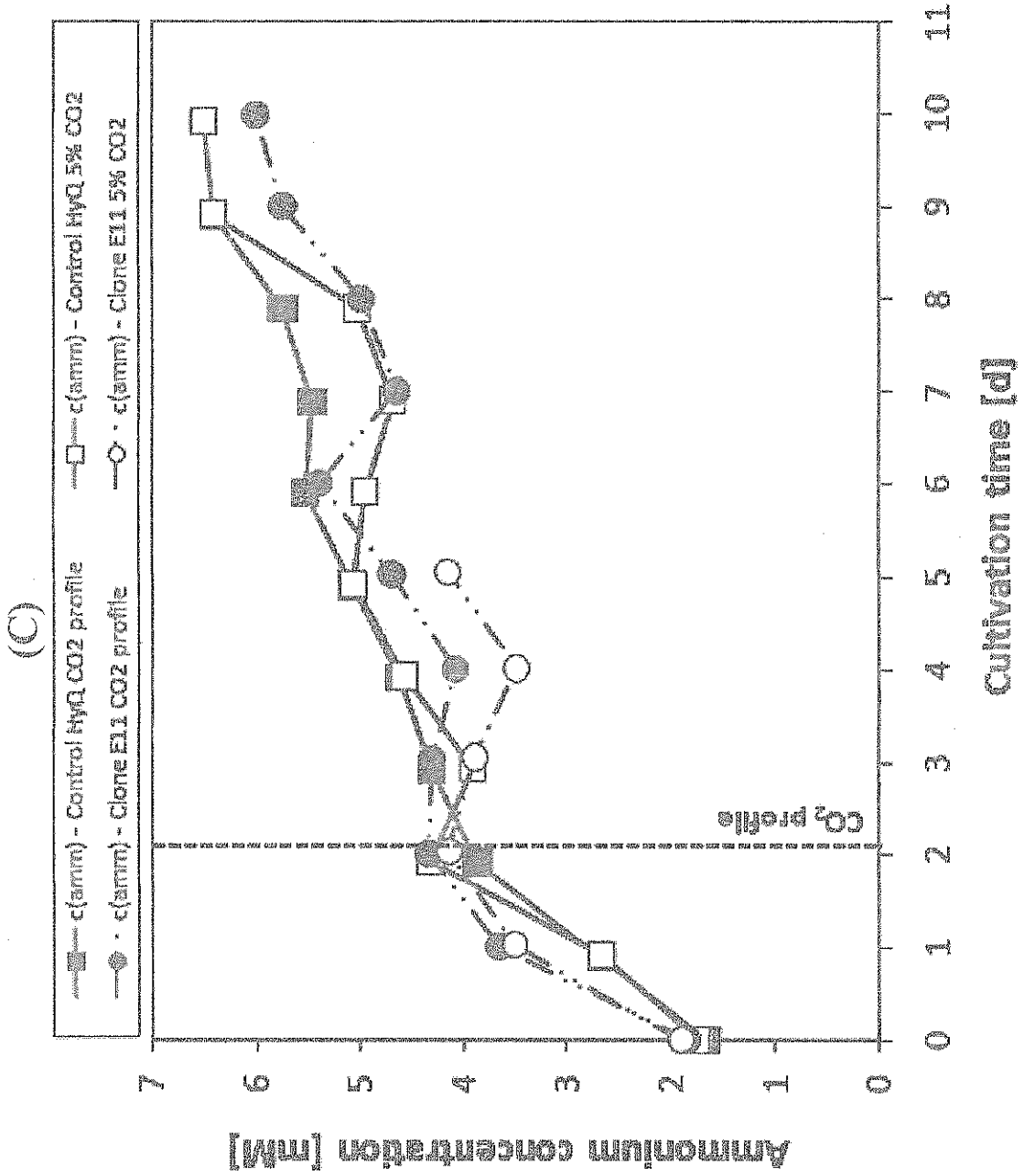

(C)
c(amm) - Control HyCl CO2 profile
c(amm) - Control HyCl 5% CO2
c(amm) - Clone E11 CO2 profile
c(amm) - Clone E11 5% CO2
CO2 profile
Ammonium concentration [mM]
Cultivation time [d]
0
1
2
3
4
5
6
7
8
9
10
11

Figure 12

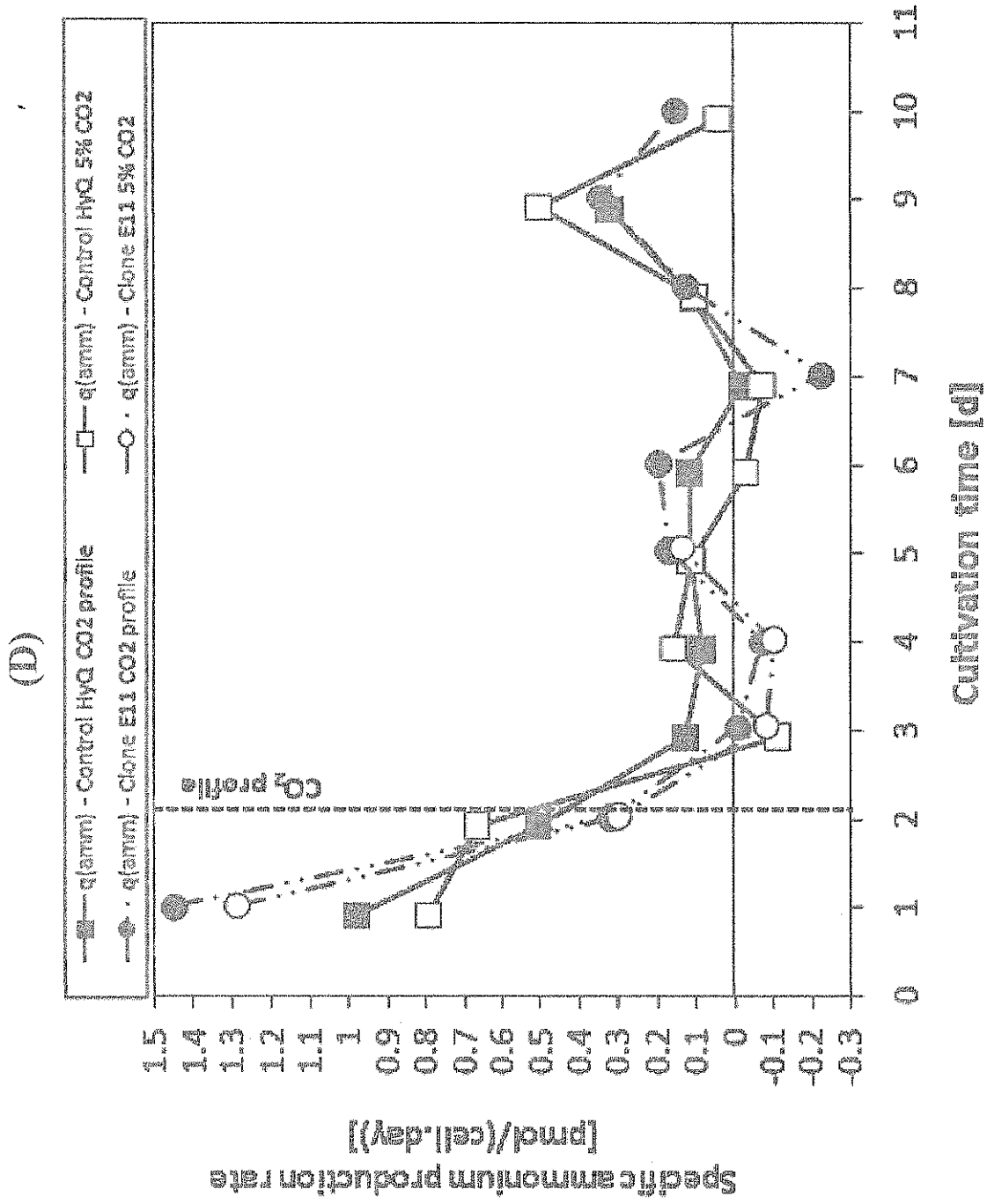

(D)
q(amm) - Control HyQ CO2 profile
q(amm) - Control HyQ 5% CO2
q(amm) - Clone E11 CO2 profile
q(amm) - Clone E11 5% CO2
CO2 profile
Specific ammonium production rate
[pmol/(cell.day)]
Cultivation time [d]

Figure 13

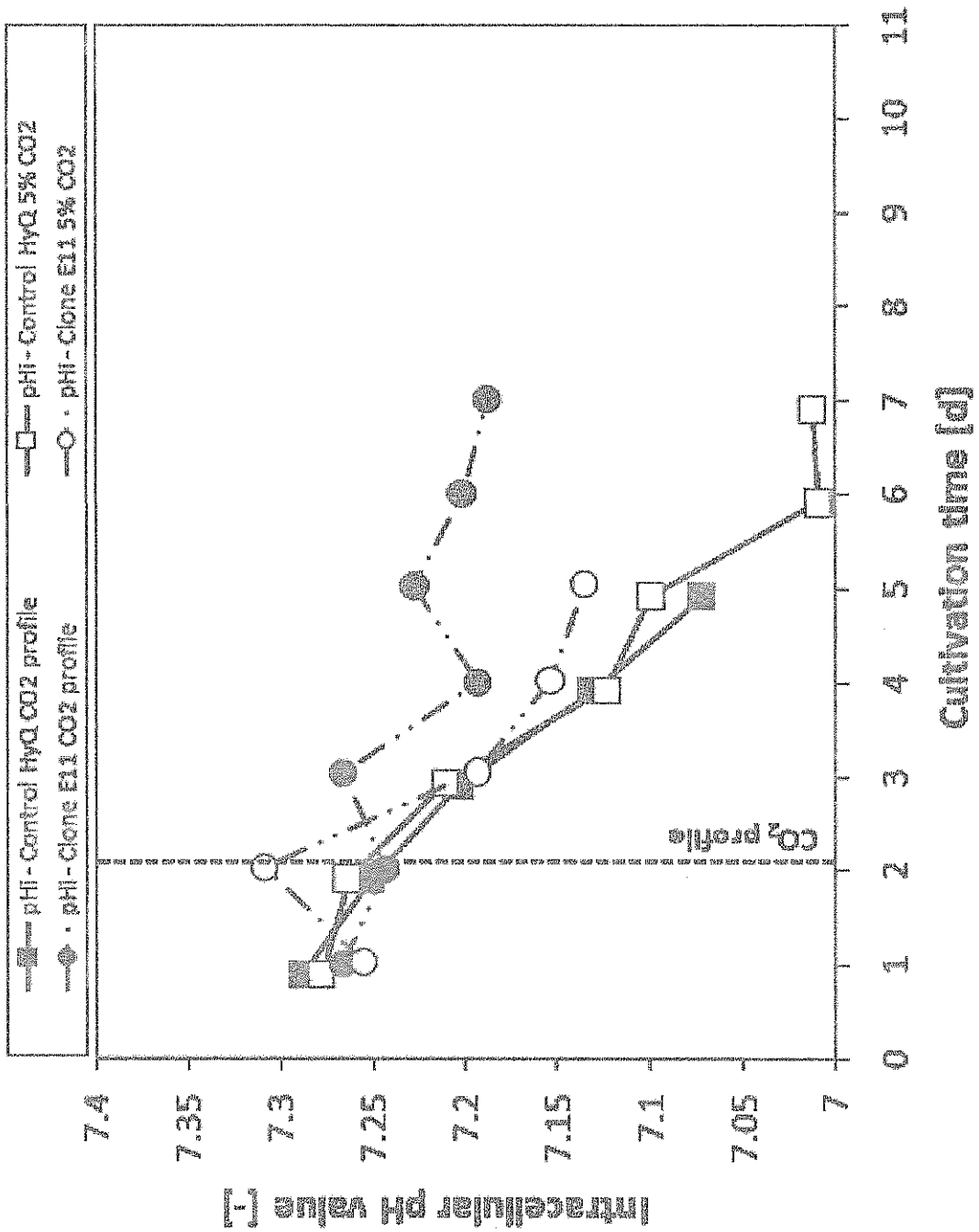

pHi - Control HyQ CO2 profile
pHi - Control HyQ 5% CO2
pHi - Clone E11 CO2 profile
pHi - Clone E11 5% CO2
CO2 profile
Intracellular pH value [-]
Cultivation time [d]
7.4
7.35
7.3
7.25
7.2
7.15
7.1
7.05
7
0
1
2
3
4
5
6
7
8
9
10
11

Figure 14

(A)

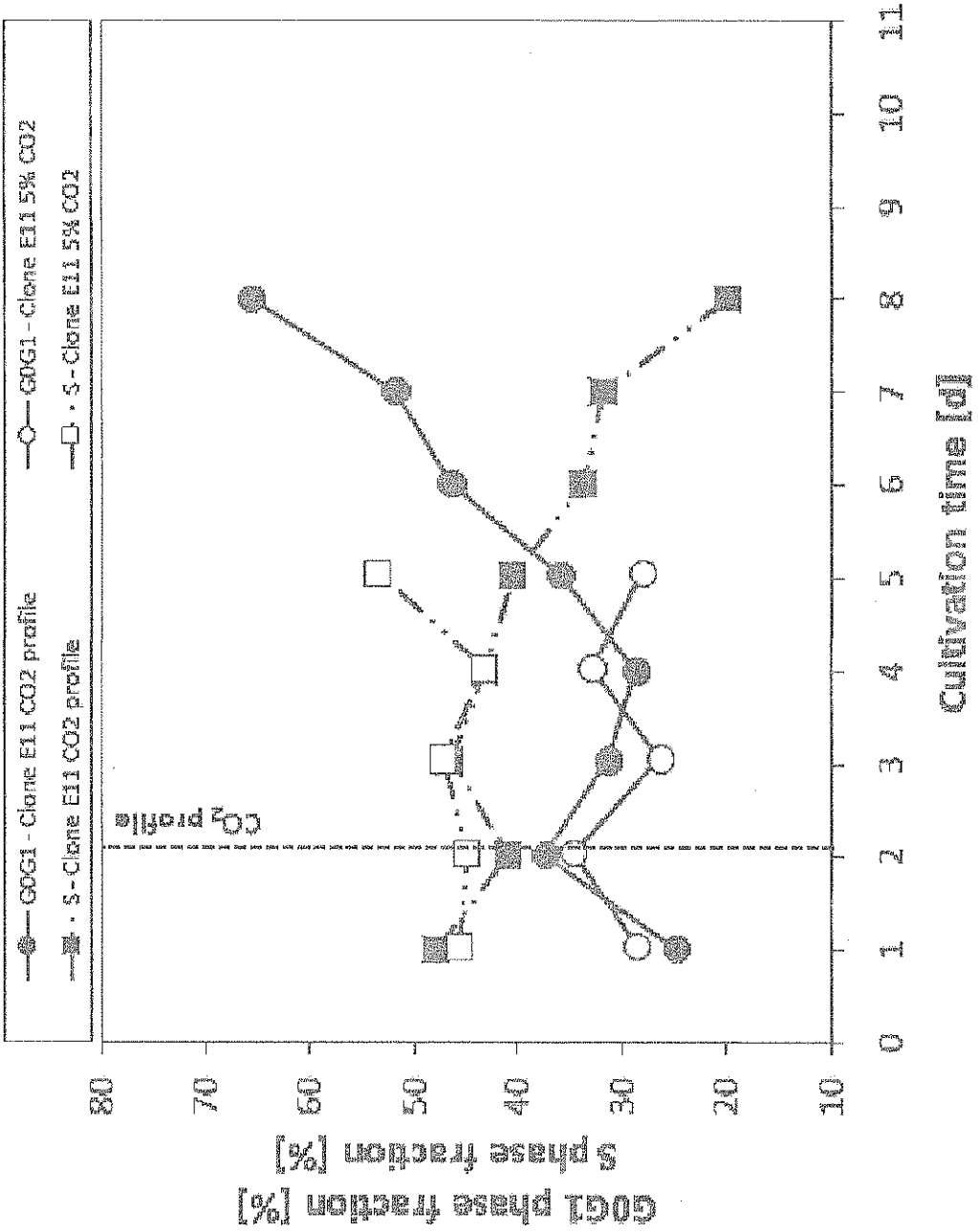

G0G1 - Clone E11 CO2 profile
S - Clone E11 CO2 profile
G0G1 - Clone E11 5% CO2
S - Clone E11 5% CO2
CO2 profile
G0G1 phase fraction [%]
S phase fraction [%]
Cultivation time [d]
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11

Figure 14

(B)

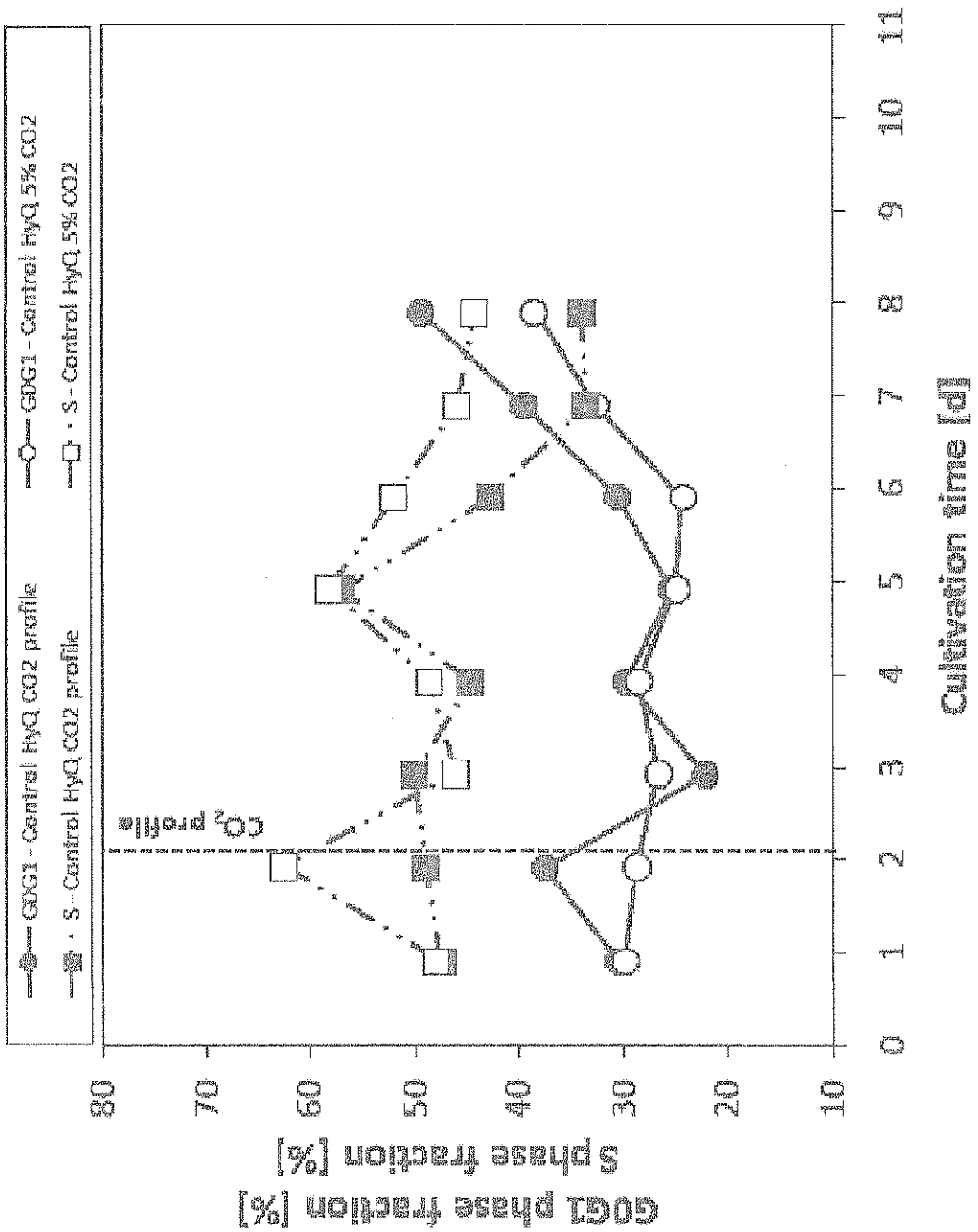

G0G1 - Control HyQ CO2 profile
S - Control HyQ CO2 profile
G0G1 - Control HyQ 5% CO2
S - Control HyQ 5% CO2
CO2 profile
G0G1 phase fraction [%]
S phase fraction [%]
Cultivation time [d]
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11

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

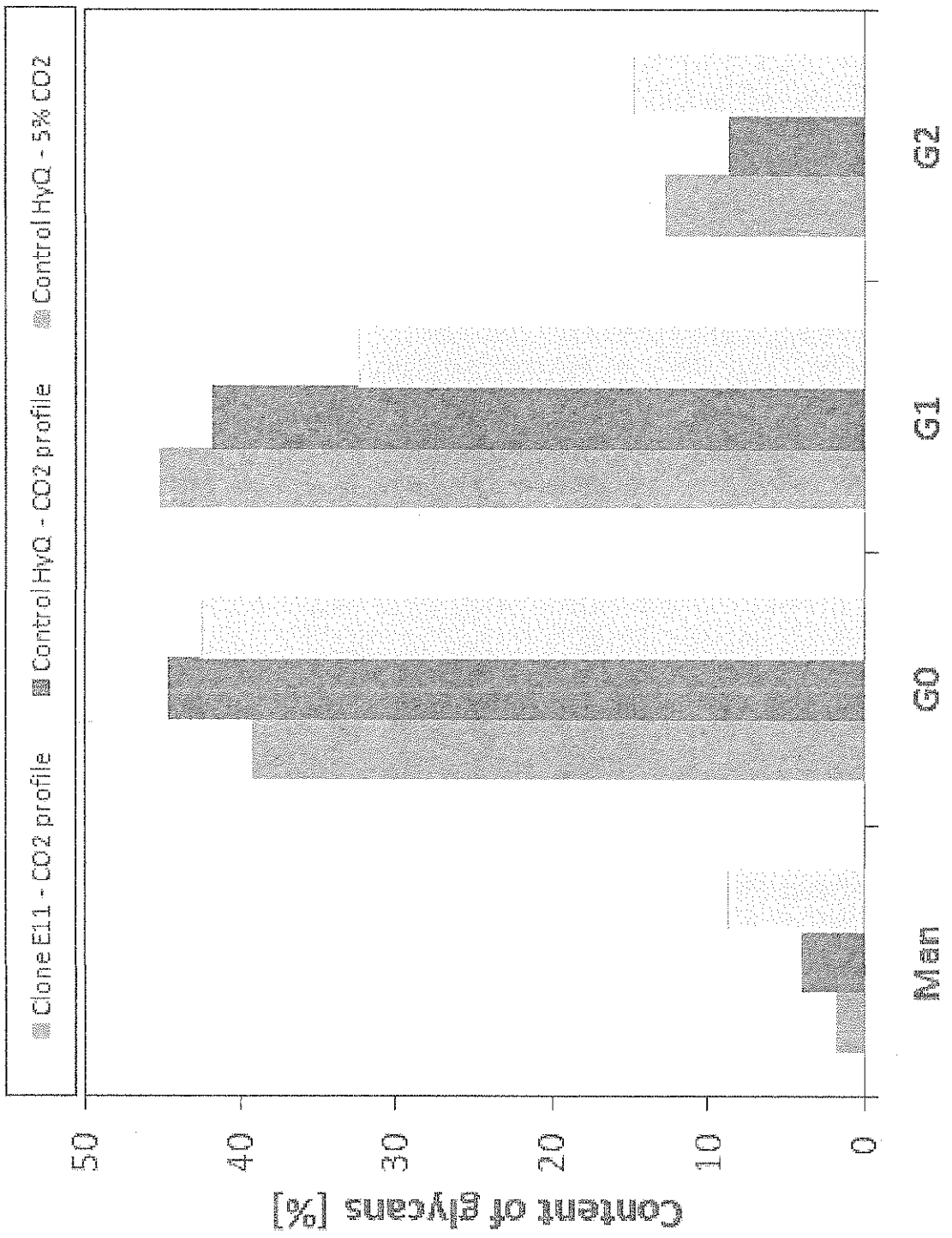

Clone E11 - CO2 profile
Control HyQ - CO2 profile
Control HyQ - 5% CO2
Content of glycans [%]
0
10
20
30
40
50
Man5
G0
G1
G2

Figure 17

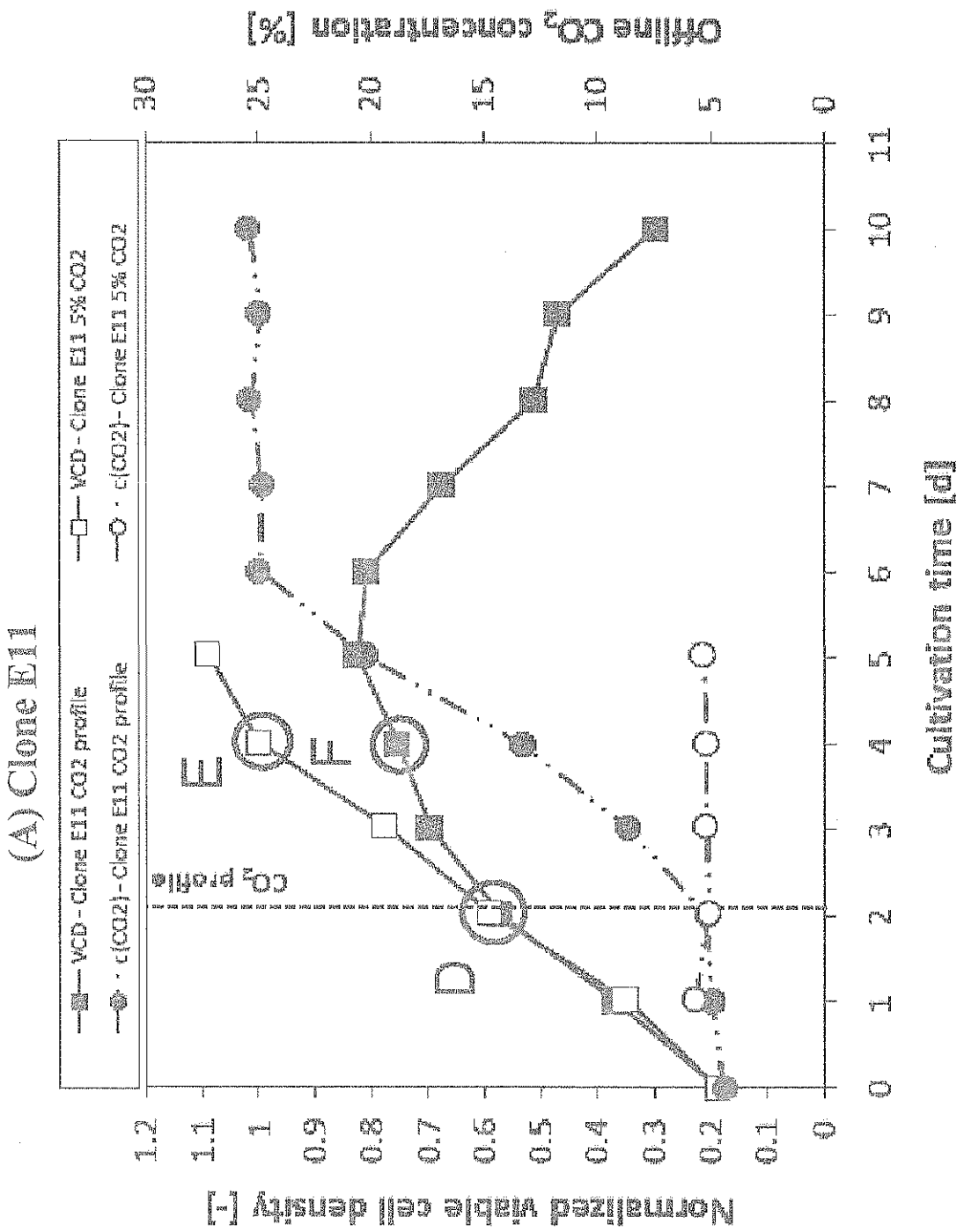
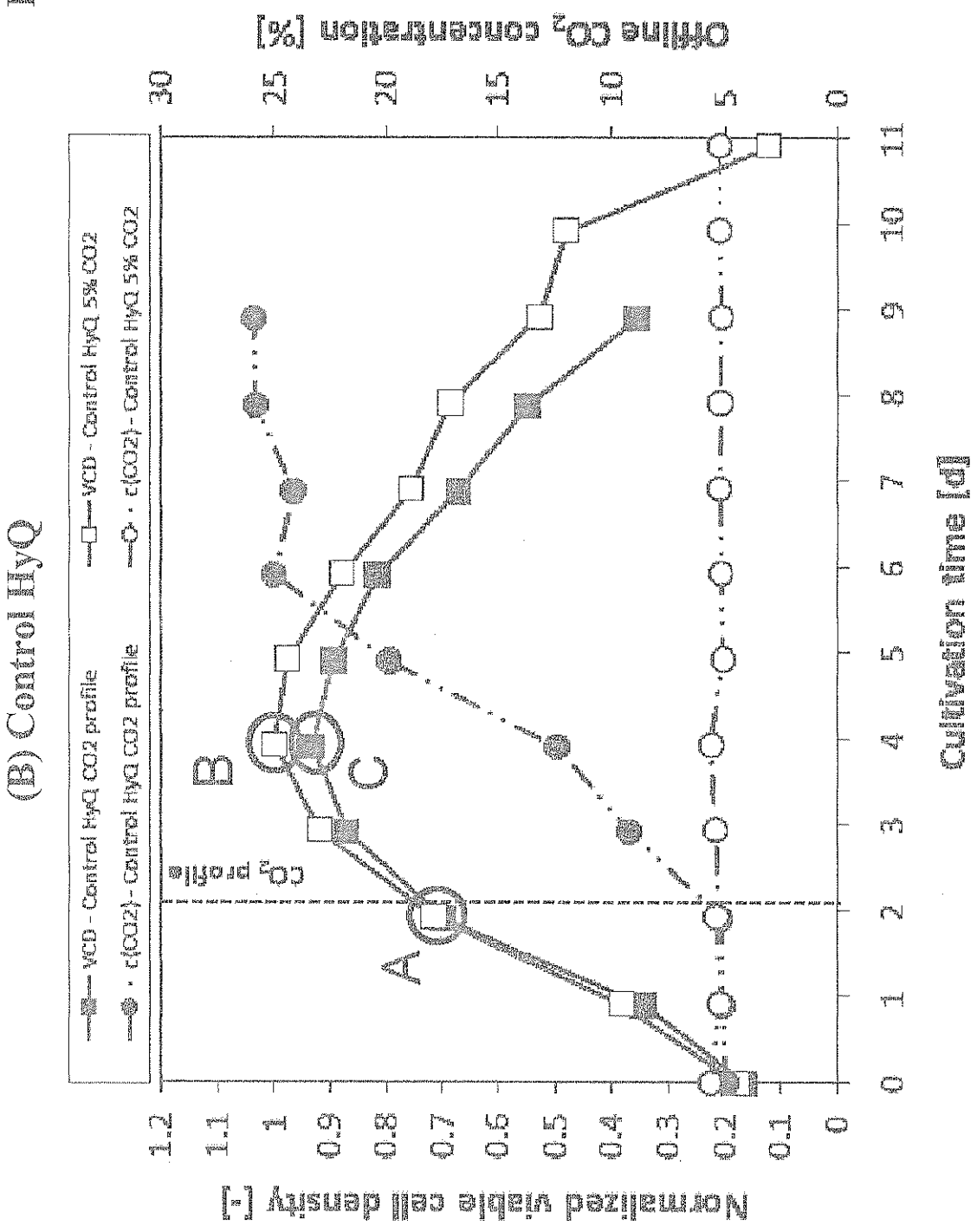

(A) Clone E11
WCD - Clone E11 CO2 profile
c(CO2)- Clone E11 CO2 profile
WCD - Clone E11 5% CO2
c(CO2)- Clone E11 5% CO2
CO2 profile
D
E
Normalized viable cell density [-]
Offline CO2 concentration [%]
Cultivation time [d]

Figure 17


(B) Control HyQ
Normalized viable cell density [-]
Offline CO_2 concentration [%]
Cultivation time [d]
VCD - Control HyQ CO2 profile
c(CO2) - Control HyQ CO2 profile
VCD - Control HyQ 5% CO2
c(CO2) - Control HyQ 5% CO2
CO_2 profile
A
B
C