Figure 1

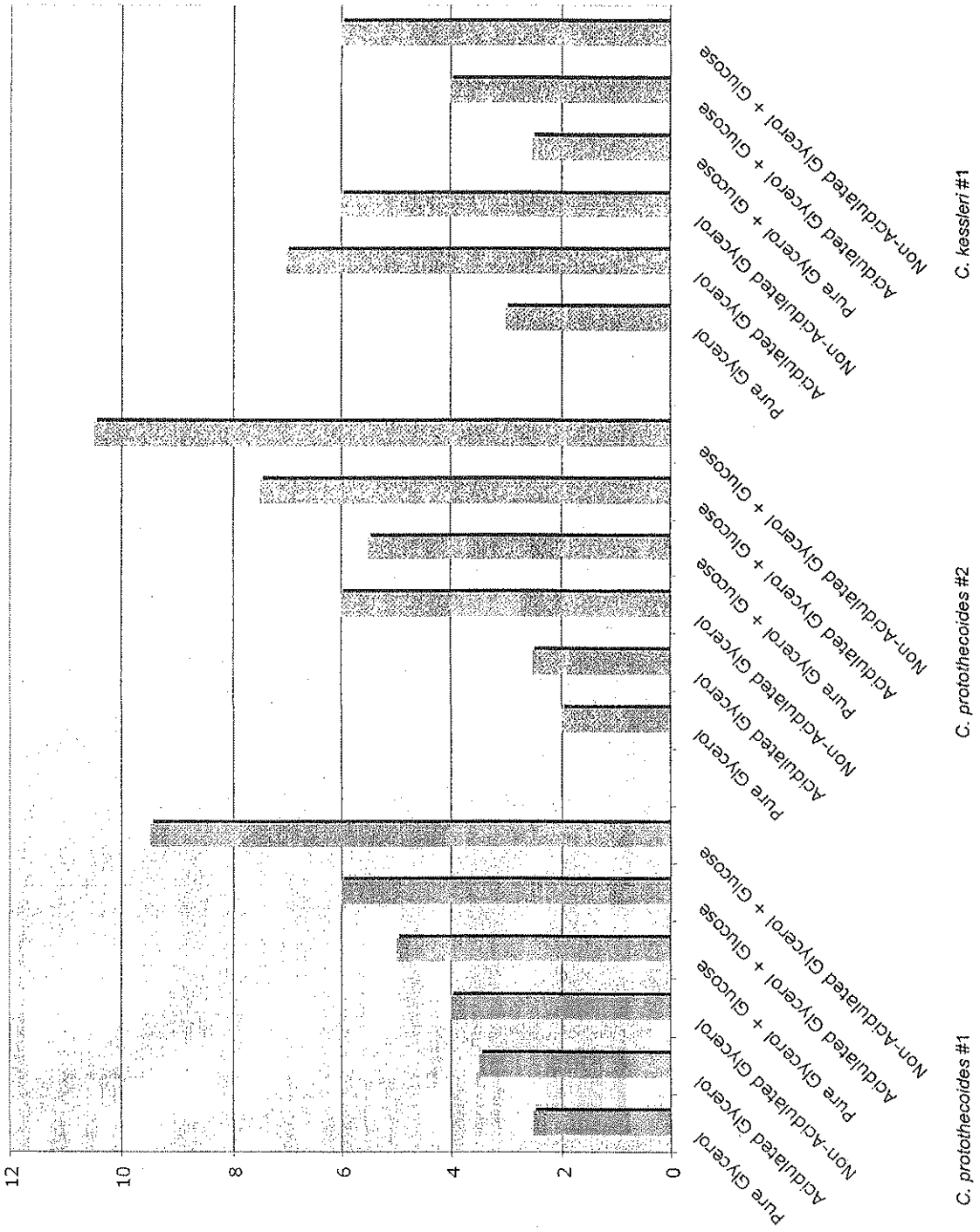

Dry Cell Weight (grams/Liter)
12
10
8
6
4
2
0
Pure Glycerol
Acidulated Glycerol
Non-Acidulated Glycerol
Pure Glycerol + Glucose
Acidulated Glycerol + Glucose
Non-Acidulated Glycerol + Glucose
C. protothecoides #1
C. protothecoides #2
C. kessleri #1

Figure 2

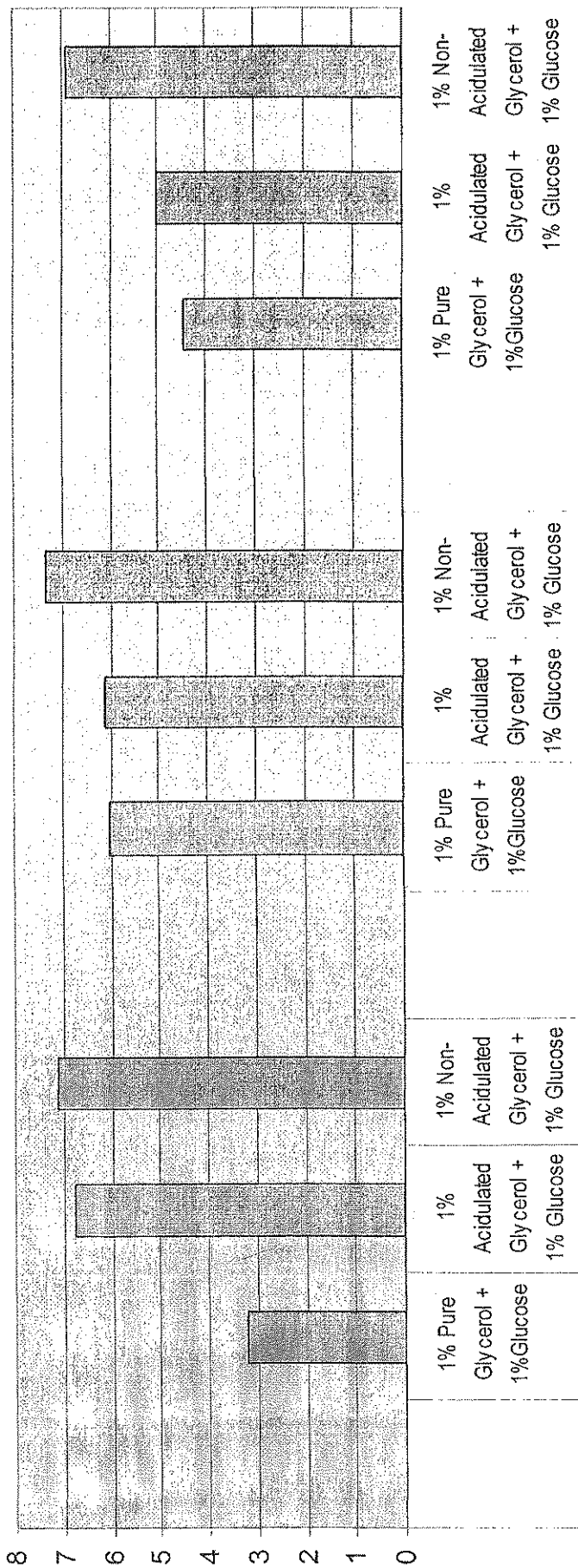

Dry Cell Weight (grams/Liter)
8
7
6
5
4
3
2
1
0
1% Pure Glycerol + 1%Glucose
1% Acidulated Glycerol + 1% Glucose
1% Non-Acidulated Glycerol + 1% Glucose
C. protothecoides #3
C. protothecoides #1
C. kessleri #2

Figure 3

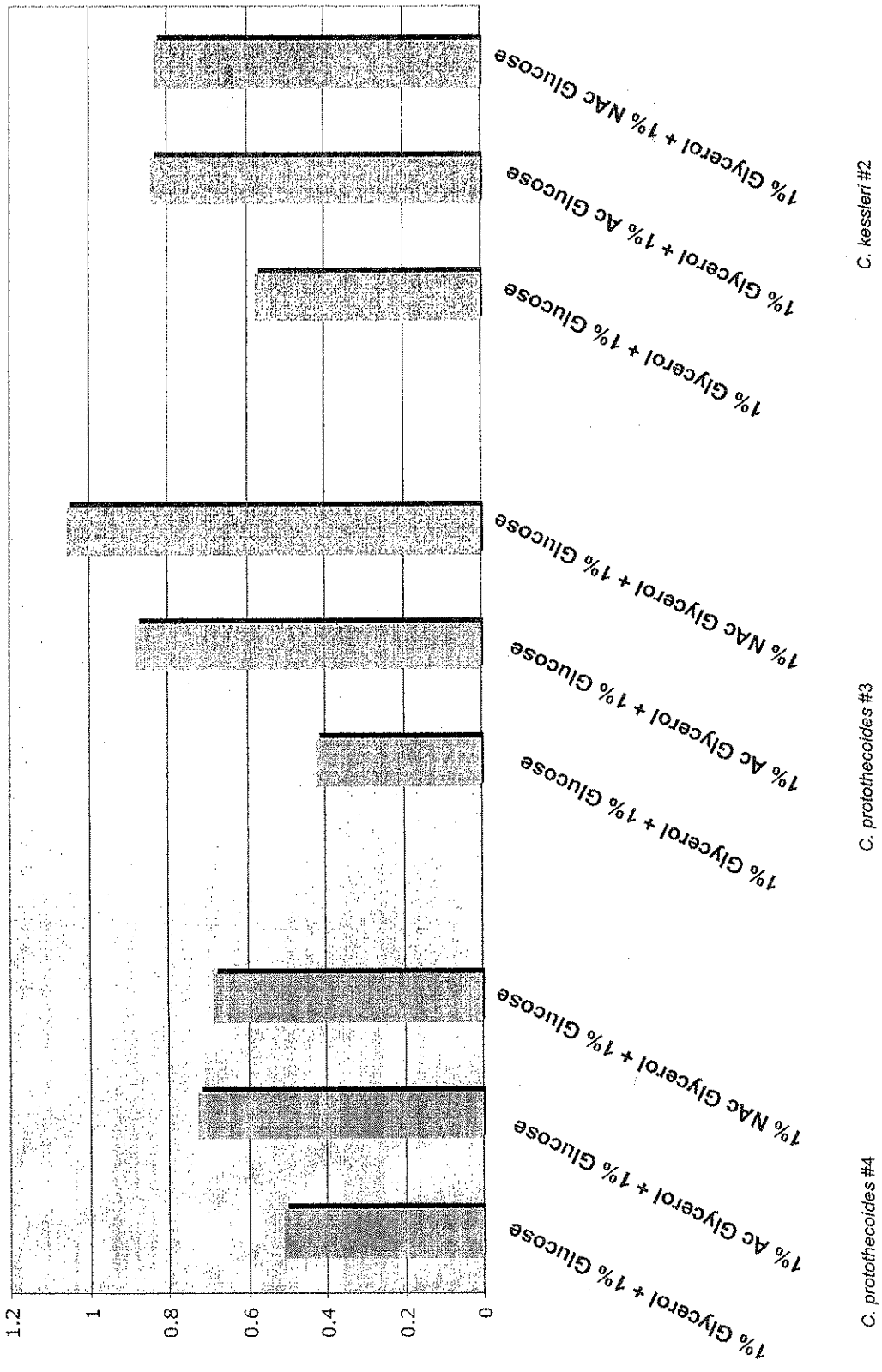

Lipid OD 530nm
1.2
1
0.8
0.6
0.4
0.2
0
1% Glycerol + 1% Glucose
1% Ac Glycerol + 1% Glucose
1% NAc Glycerol + 1% Glucose
1% Glycerol + 1% Glucose
1% Glycerol + 1% Ac Glucose
1% Glycerol + 1% NAc Glucose
C. protothecoides #4
C. protothecoides #3
C. kessleri #2

Figure 4

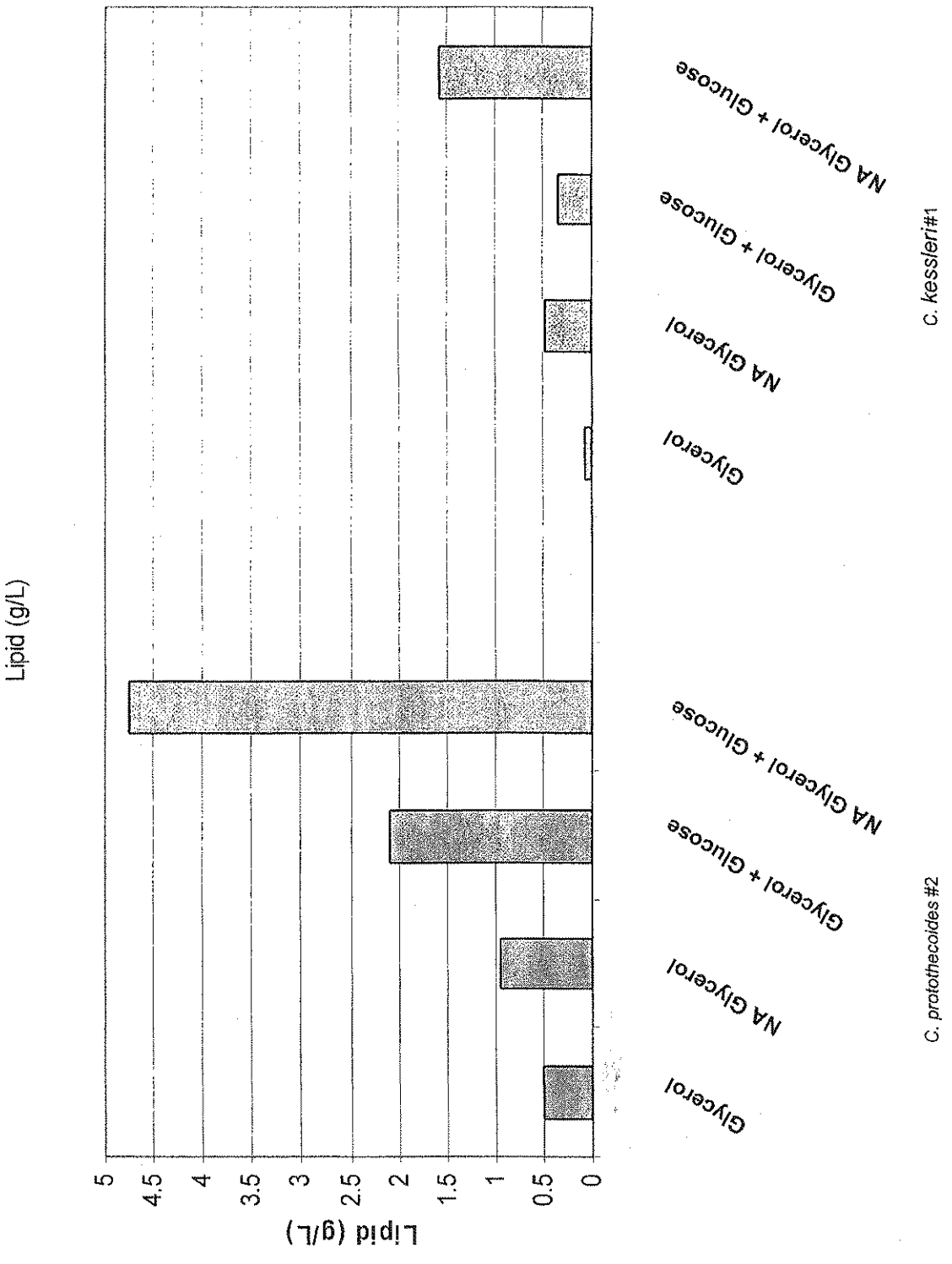

Lipid (g/L)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Lipid (g/L)
Glycerol
NA Glycerol
Glycerol + Glucose
NA Glycerol + Glucose
Glycerol
NA Glycerol
Glycerol + Glucose
NA Glycerol + Glucose
C. protothecoides #2
C. kessleri#1

Figure 5

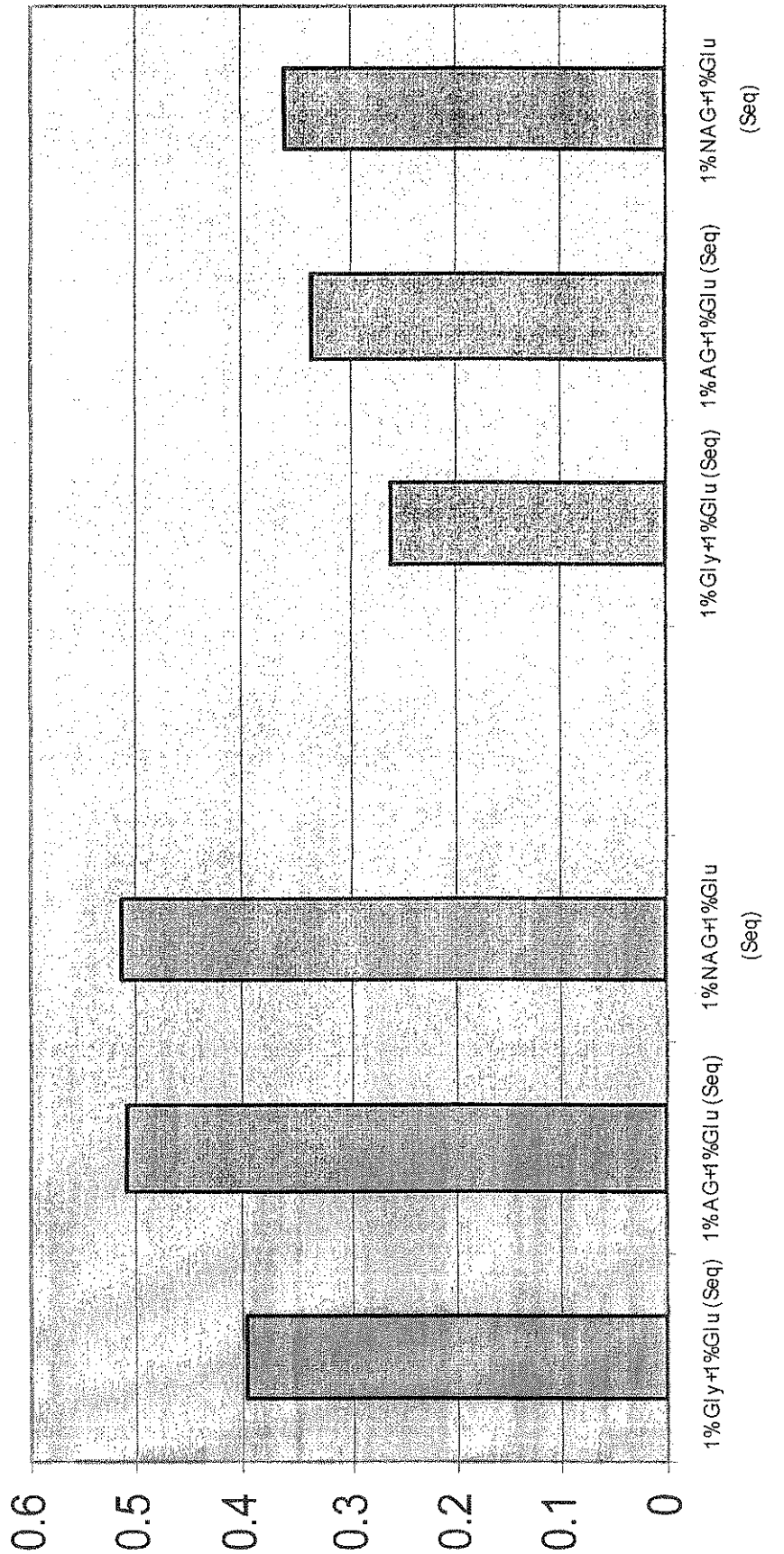

% Lipid
0.6
0.5
0.4
0.3
0.2
0.1
0
1% Gly+1%Glu (Seq)
1% AG+1%Glu (Seq)
1% NAG+1%Glu (Seq)
1% Gly+1%Glu (Seq)
1% AG+1%Glu (Seq)
1% NAG+1%Glu (Seq)
C. prototheocoides #3
C. kessleri #2

Figure 6

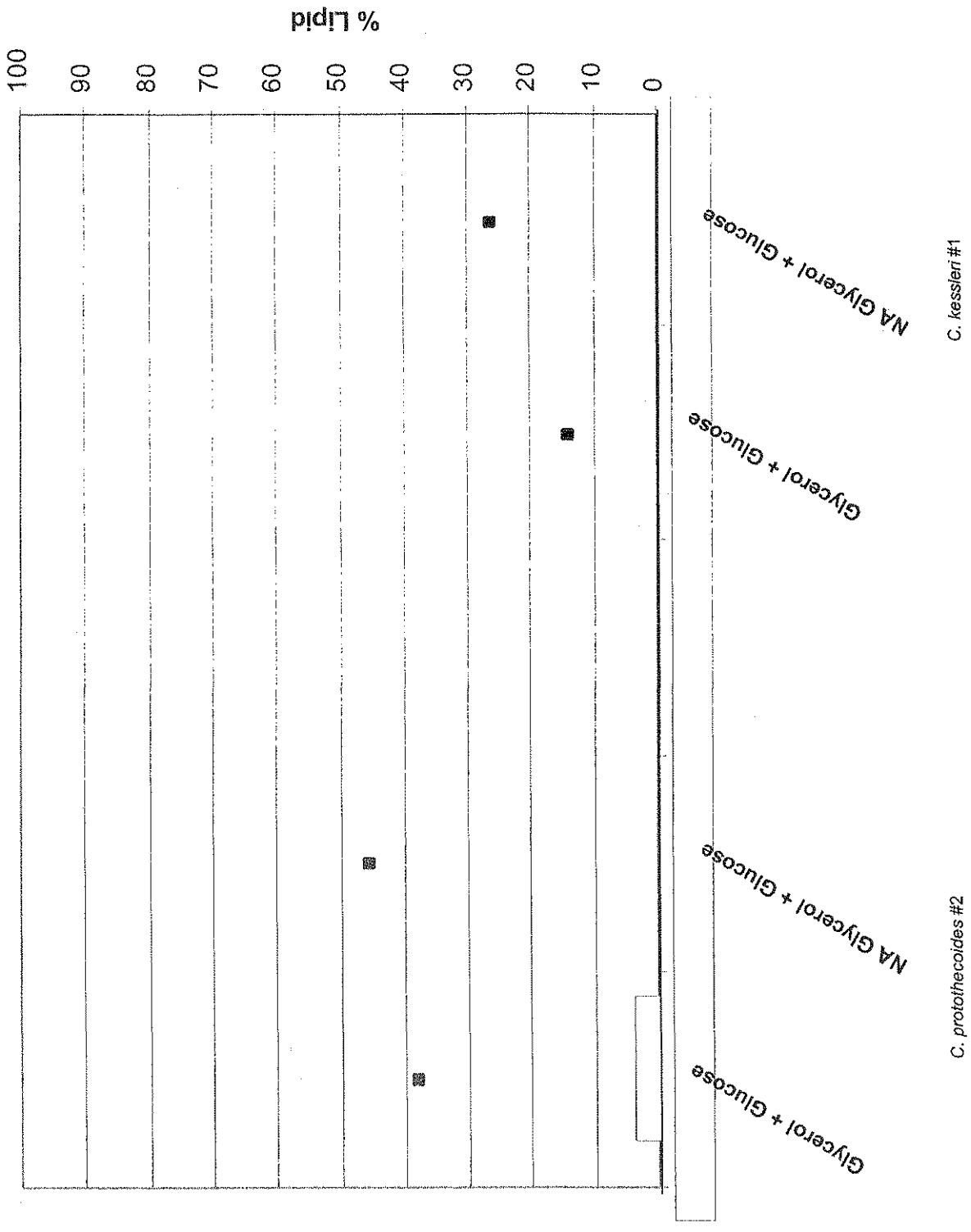

% Lipid
100
90
80
70
60
50
40
30
20
10
0
Glycerol + Glucose
NA Glycerol + Glucose
Glycerol + Glucose
NA Glycerol + Glucose
C. prototheocoides #2
C. kessleri #1

Figure 7

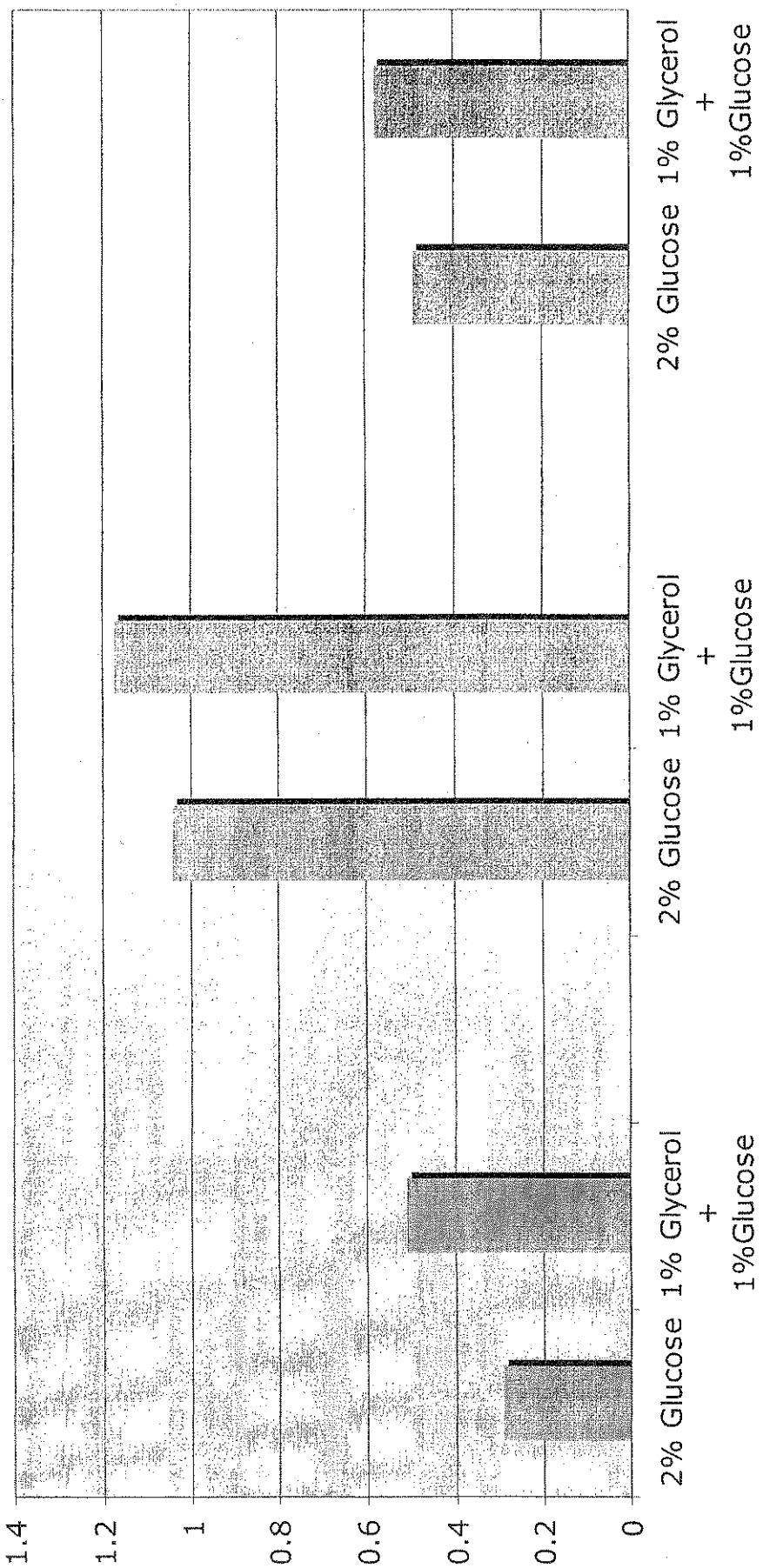

Lipid OD 530nm
1.4
1.2
1
0.8
0.6
0.4
0.2
0
2% Glucose 1% Glycerol + 1%Glucose
2% Glucose 1% Glycerol + 1%Glucose
2% Glucose 1% Glycerol + 1%Glucose
C. protothecoides #4
C. protothecoides #1
C. kessleri #2

Figure 8

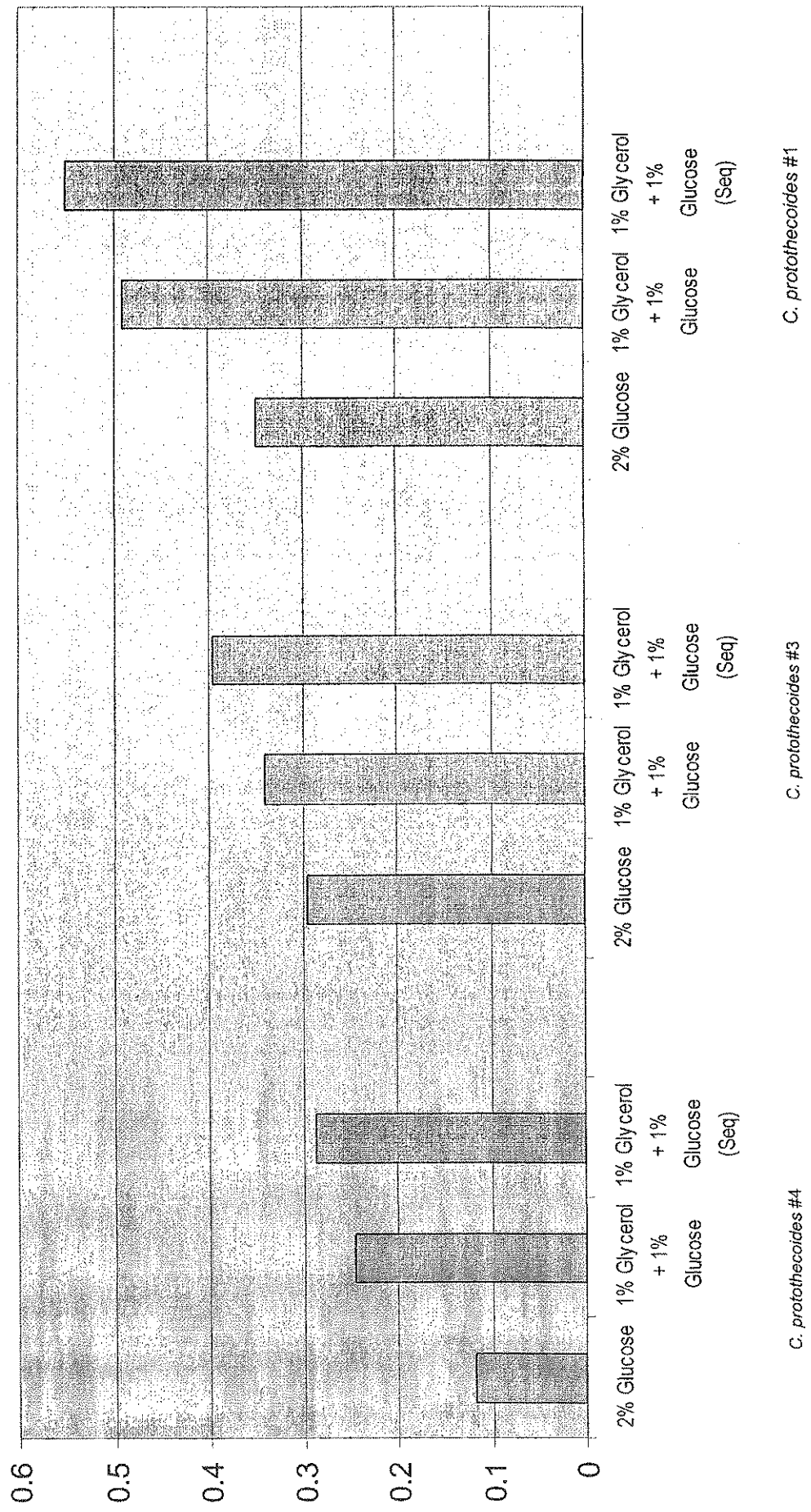

% Lipid
0.6
0.5
0.4
0.3
0.2
0.1
0
2% Glucose
1% Glycerol + 1% Glucose
1% Glycerol + 1% Glucose (Seq)
C. protothecoides #4
C. protothecoides #3
C. protothecoides #1

Figure 9

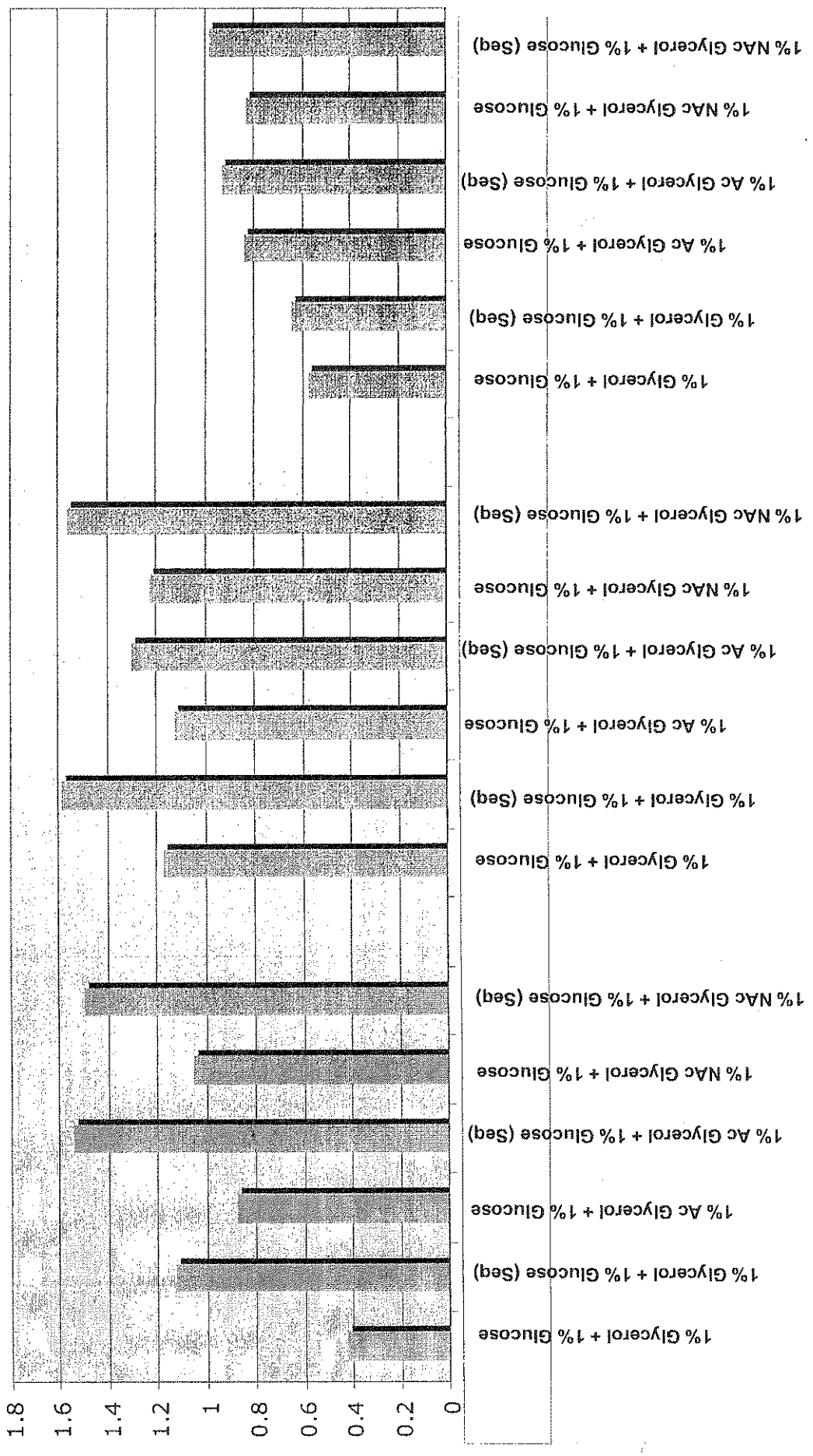

Lipid OD 530nm
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1% Glycerol + 1% Glucose
1% Glycerol + 1% Glucose (Sed)
1% Ac Glycerol + 1% Glucose
1% Ac Glycerol + 1% Glucose (Sed)
1% NAc Glycerol + 1% Glucose
1% NAc Glycerol + 1% Glucose (Sed)
C. prototheocoides #3
1% Glycerol + 1% Glucose
1% Glycerol + 1% Glucose (Sed)
1% Ac Glycerol + 1% Glucose
1% Ac Glycerol + 1% Glucose (Sed)
1% NAc Glycerol + 1% Glucose
1% NAc Glycerol + 1% Glucose (Sed)
C. prototheocoides #1
1% Glycerol + 1% Glucose
1% Glycerol + 1% Glucose (Sed)
1% Ac Glycerol + 1% Glucose
1% Ac Glycerol + 1% Glucose (Sed)
1% NAc Glycerol + 1% Glucose
1% NAc Glycerol + 1% Glucose (Sed)
C. kessleri #2

Figure 10

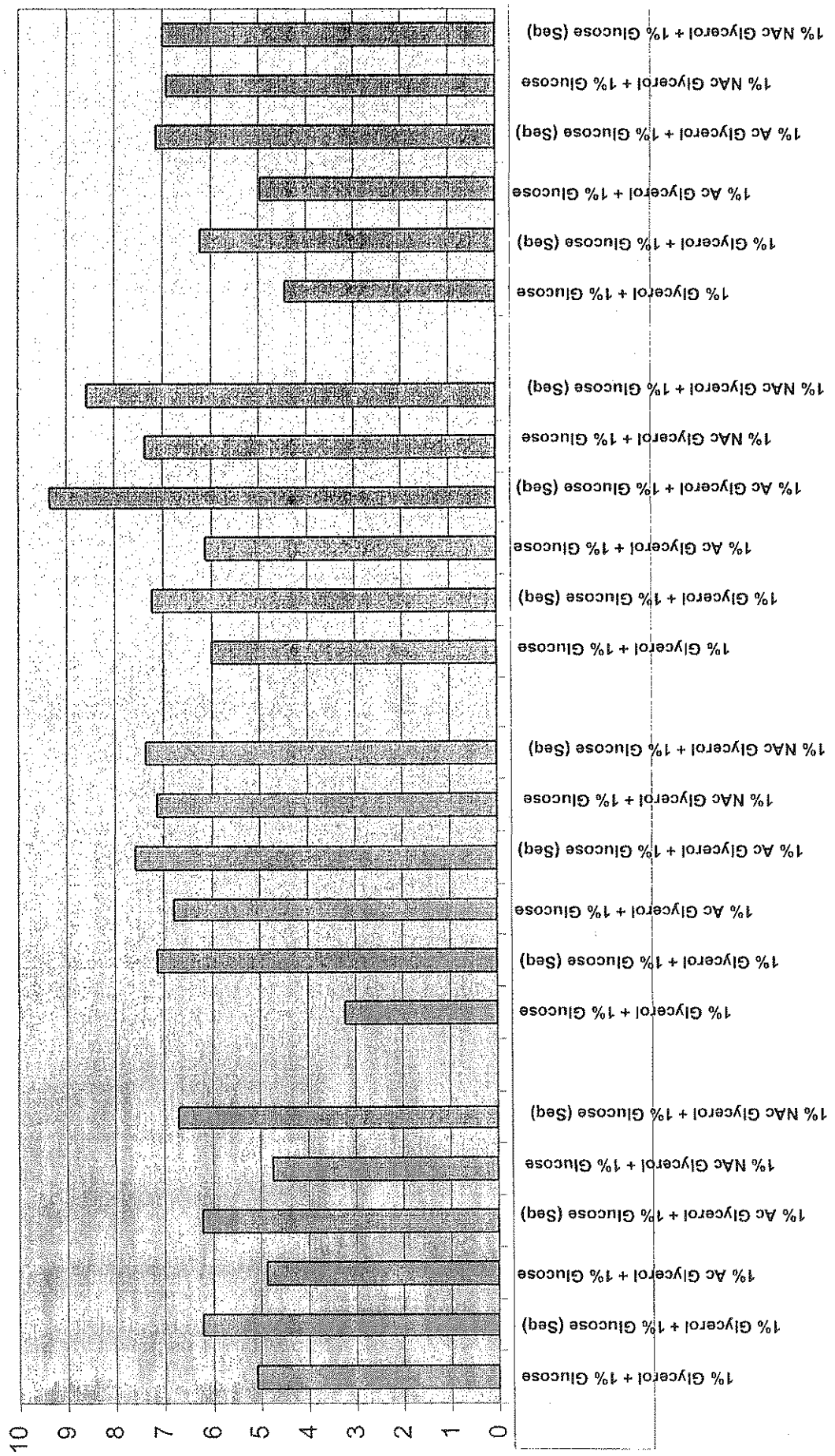
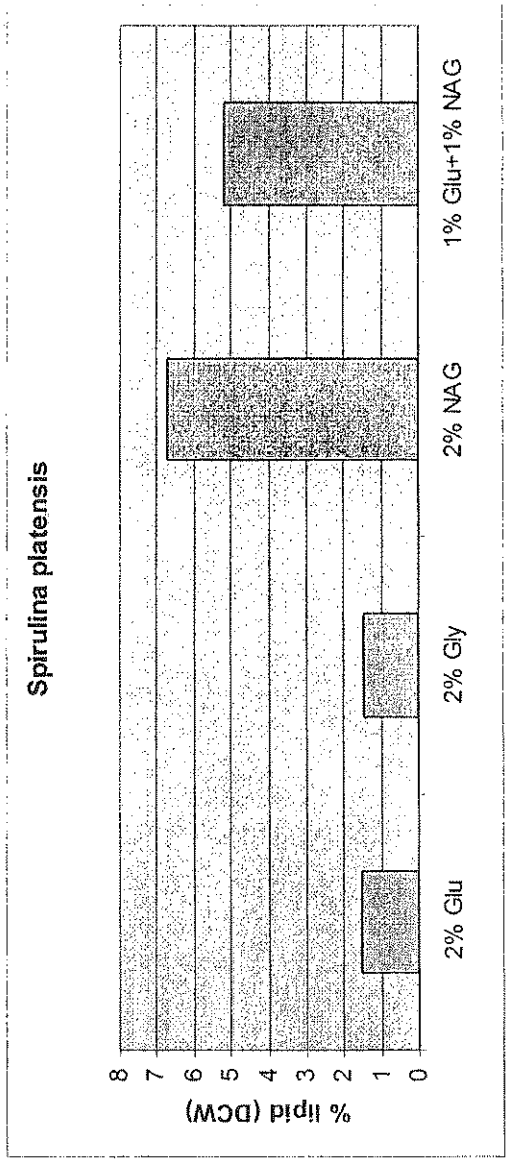
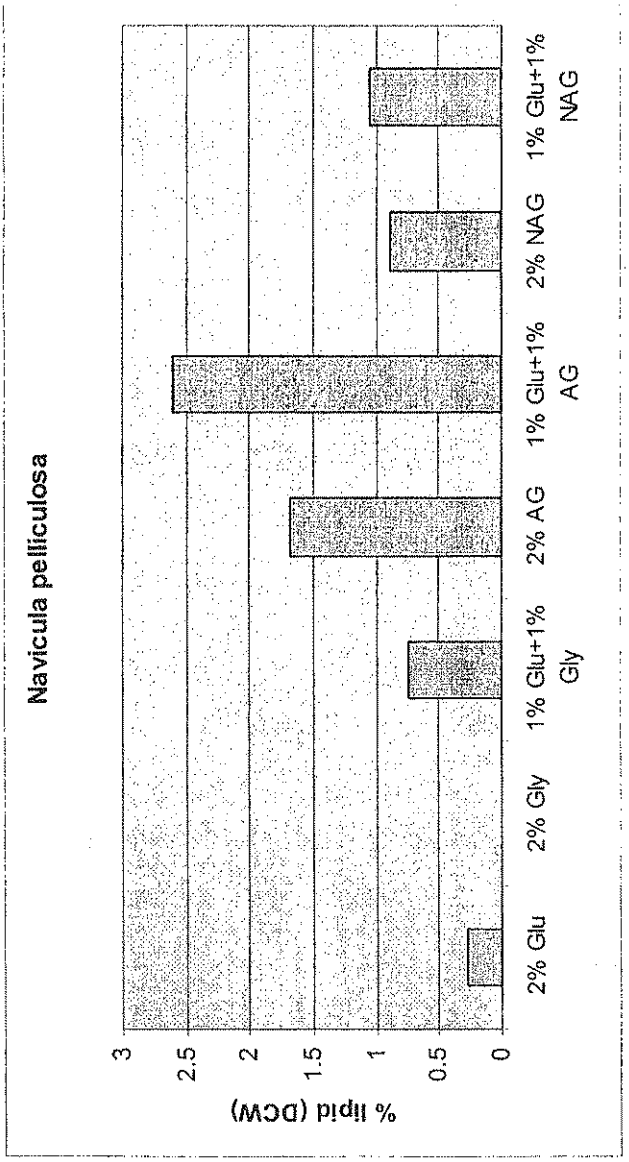

Dry Cell Weight (grams/Liter)
10
9
8
7
6
5
4
3
2
1
0
1% Glycerol + 1% Glucose
1% Glycerol + 1% Glucose (Seq)
1% Ac Glycerol + 1% Glucose
1% Ac Glycerol + 1% Glucose (Seq)
1% NAc Glycerol + 1% Glucose
1% NAc Glycerol + 1% Glucose (Seq)
C. prototheccoides #4
1% Glycerol + 1% Glucose
1% Glycerol + 1% Glucose (Seq)
1% Ac Glycerol + 1% Glucose
1% Ac Glycerol + 1% Glucose (Seq)
1% NAc Glycerol + 1% Glucose
1% NAc Glycerol + 1% Glucose (Seq)
C. prototheccoides #3
1% Glycerol + 1% Glucose
1% Glycerol + 1% Glucose (Seq)
1% Ac Glycerol + 1% Glucose
1% Ac Glycerol + 1% Glucose (Seq)
1% NAc Glycerol + 1% Glucose
1% NAc Glycerol + 1% Glucose (Seq)
C. prototheccoides #1
1% Glycerol + 1% Glucose
1% Glycerol + 1% Glucose (Seq)
1% Ac Glycerol + 1% Glucose
1% Ac Glycerol + 1% Glucose (Seq)
1% NAc Glycerol + 1% Glucose
1% NAc Glycerol + 1% Glucose (Seq)
C. kessleri #2

Figure 11

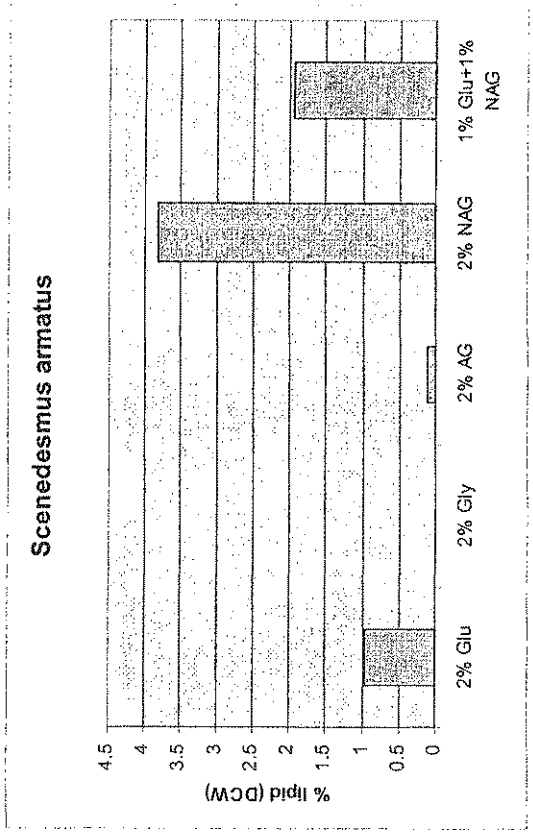

Spirulina platensis
% lipid (DCW)
0
1
2
3
4
5
6
7
8
2% Glu
2% Gly
2% NAG
1% Glu+1% NAG

(a)

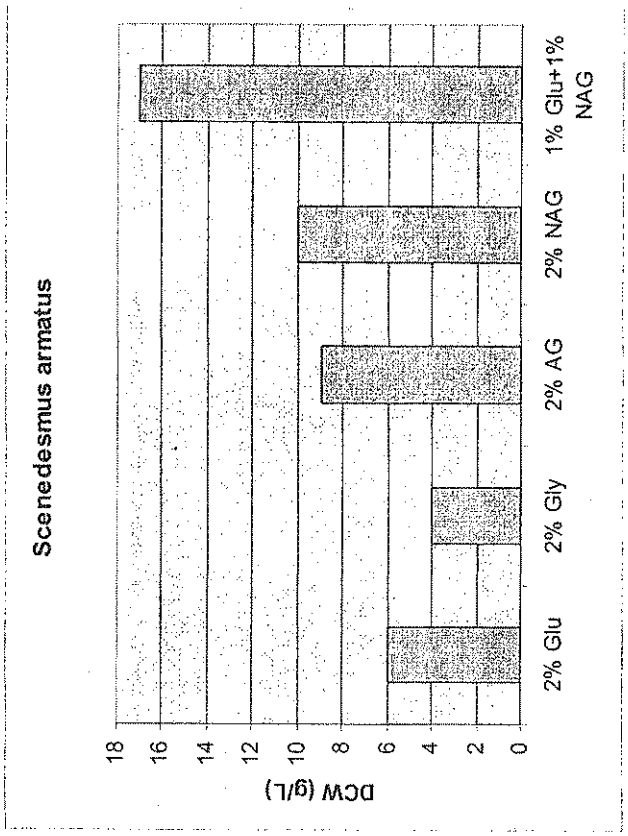

Navicula pelliculosa
% lipid (DCW)
0
0.5
1
1.5
2
2.5
3
2% Glu
2% Gly
1% Glu+1% Gly
2% AG
1% Glu+1% AG
2% NAG
1% Glu+1% NAG

(b)

Figure 12


Scenedesmus armatus
% lipid (DCW)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
2% Glu
2% Gly
2% AG
2% NAG
1% Glu+1% NAG

(a)


Scenedesmus armatus
DCW (g/L)
18
16
14
12
10
8
6
4
2
0
2% Glu
2% Gly
2% AG
2% NAG
1% Glu+1% NAG

(b)

Figure 13

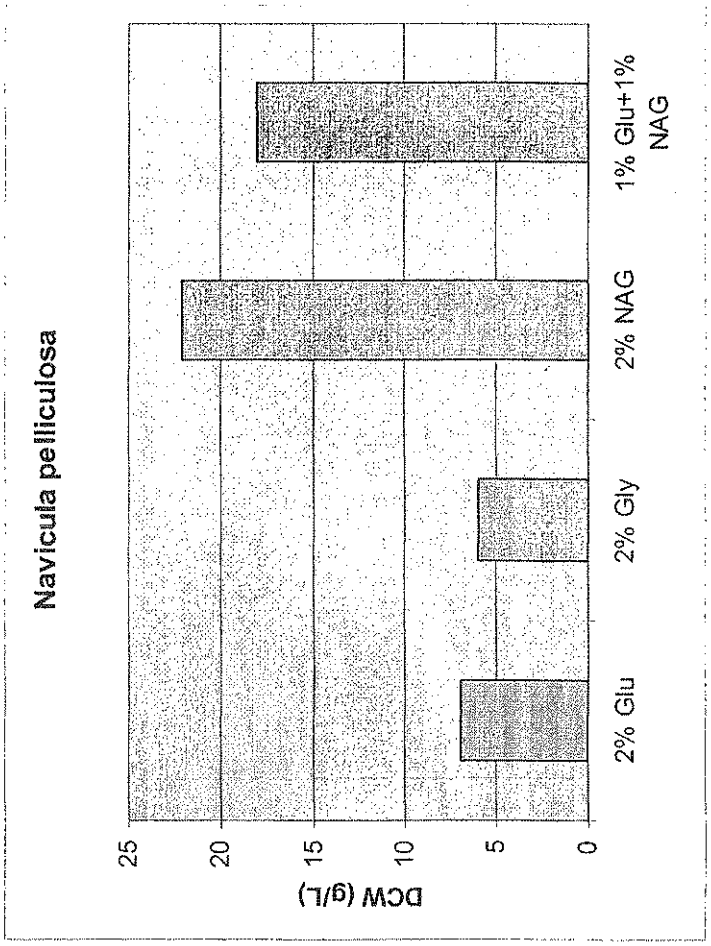

Navicula pelliculosa
DCW (g/L)
25
20
15
10
5
0
2% Glu
2% Gly
2% NAG
1% Glu+1% NAG

Figure 14

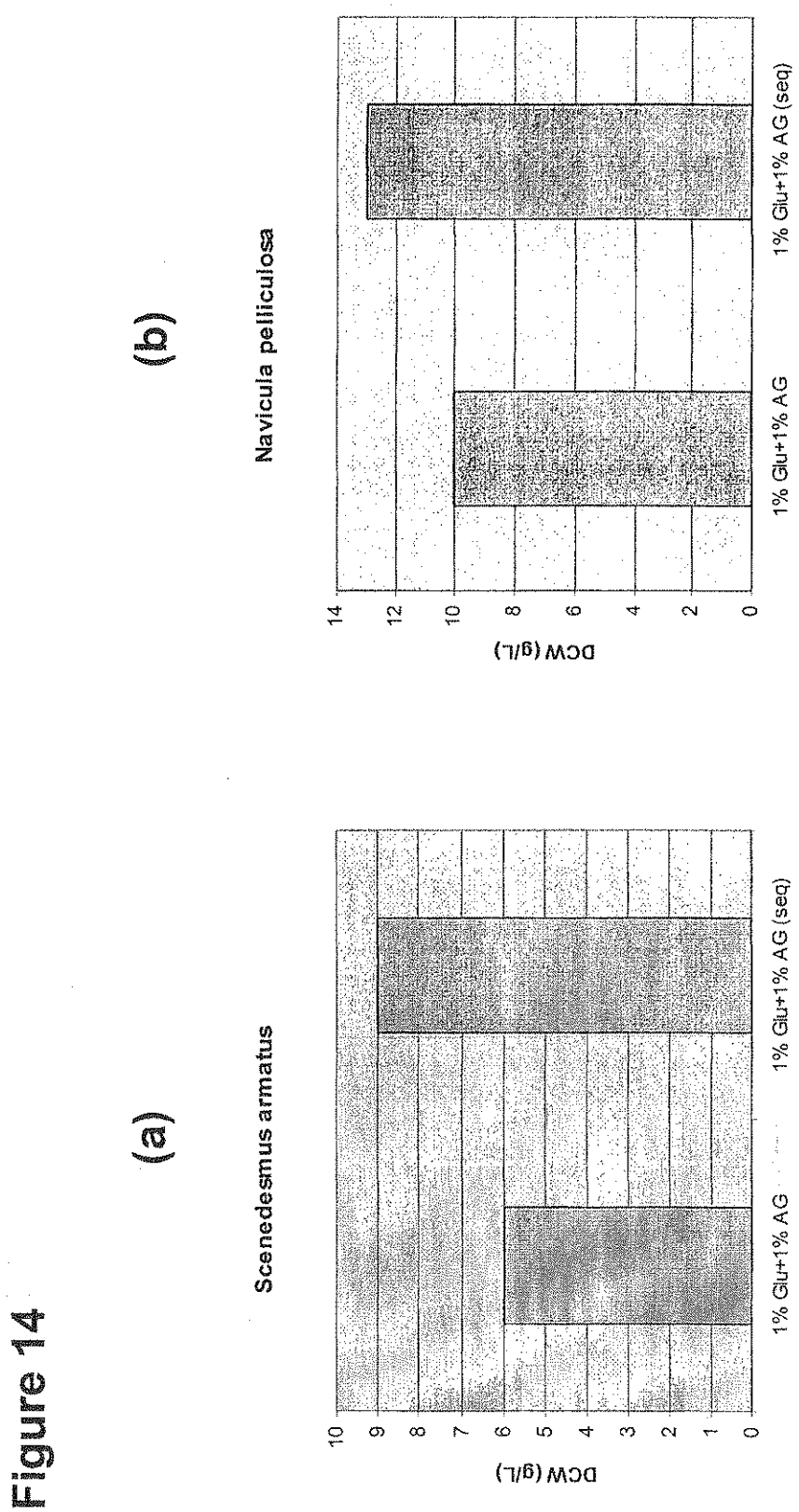

(a)
Scenedesmus armatus
DCW (g/L)
0
1
2
3
4
5
6
7
8
9
10
1% Glu+1% AG
1% Glu+1% AG (seq)
(b)
Navicula pelliculosa
DCW (g/L)
0
2
4
6
8
10
12
14
1% Glu+1% AG
1% Glu+1% AG (seq)

Figure 15

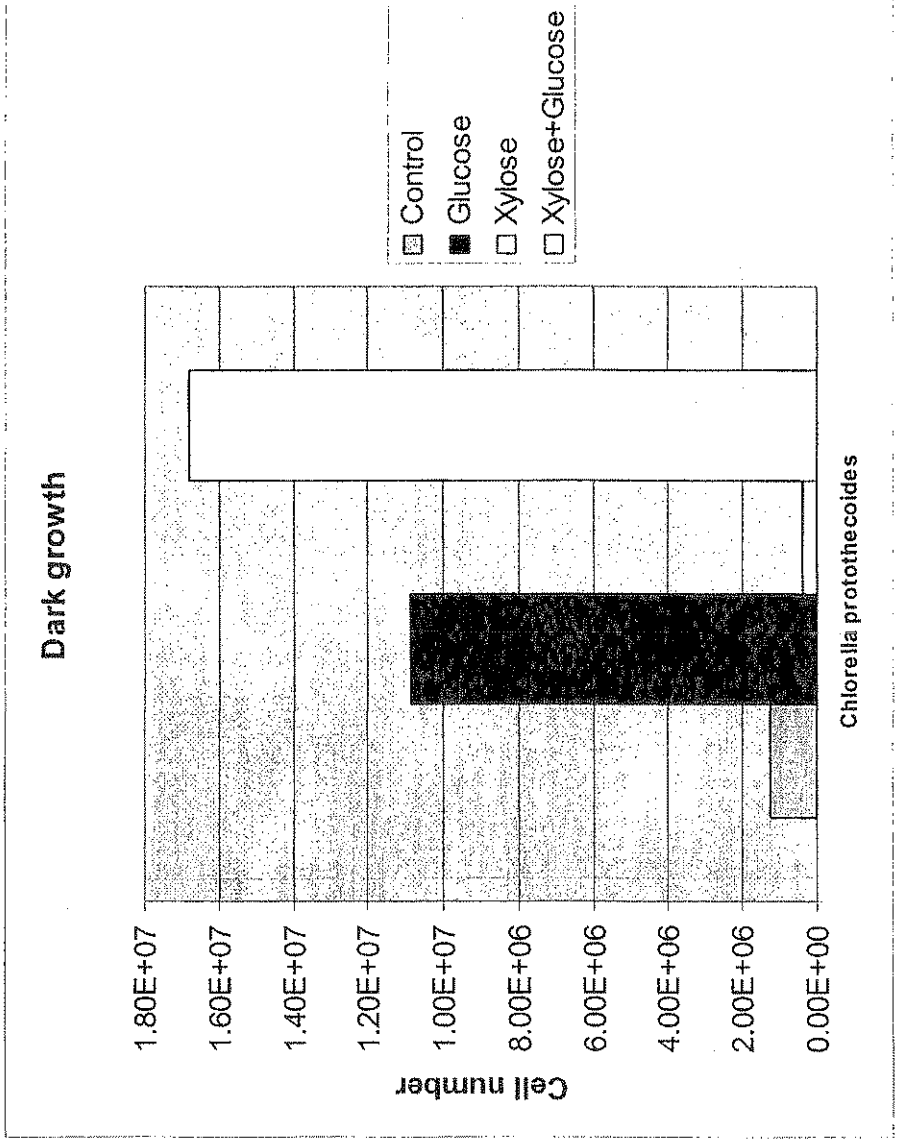

Dark growth
Cell number
1.80E+07
1.60E+07
1.40E+07
1.20E+07
1.00E+07
8.00E+06
6.00E+06
4.00E+06
2.00E+06
0.00E+00
Chlorella protothecoides
Control
Glucose
Xylose
Xylose+Glucose

Figure 16

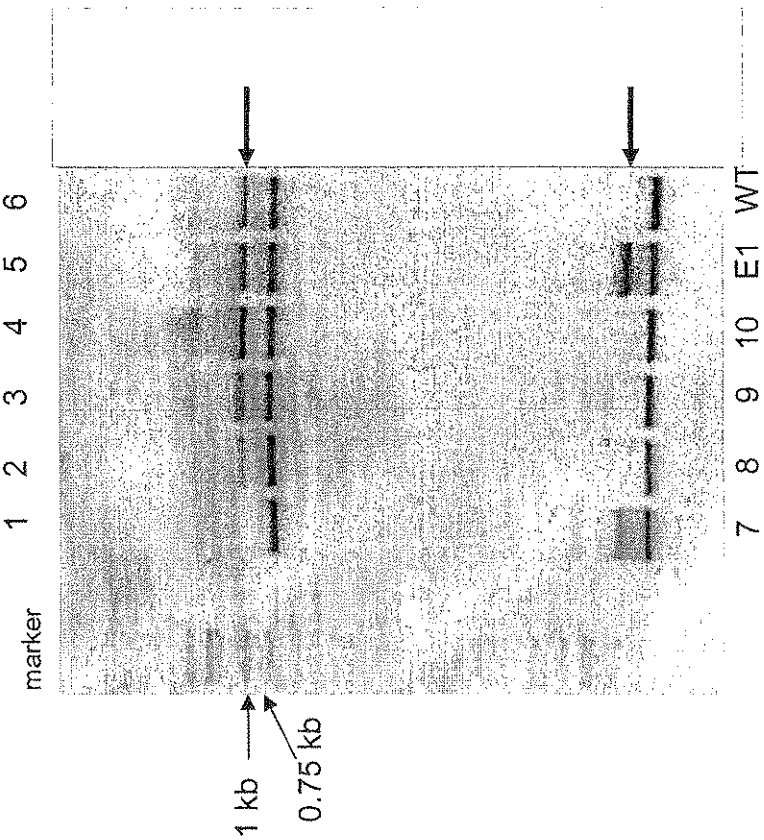

Lanes 1-10: biolistic transformants
Lane E1: electroporation transformant
Lane: WT genomic DNA
marker
1 2 3 4 5 6
7 8 9 10 E1 WT
1 kb
0.75 kb

Figure 17

Chlorella protothecoides codon usage table

Phe	UUU	39	(0.82)	Ser	UCU	50	(1.04)
	UUC	56	(1.18)		UCC	60	(1.25)
Leu	UUA	10	(0.20)		UCA	46	(0.96)
	UUG	46	(0.91)		UCG	43	(0.89)
Tyr	UAU	15	(0.59)	Cys	UGU	46	(0.77)
	UAC	36	(1.41)		UGC	73	(1.23)
ter	UAA	9	(0.00)	ter	UGA	43	(0.00)
ter	UAG	15	(0.00)	Trp	UGG	69	(1.00)
Leu	CUU	49	(0.97)	Pro	CCU	80	(0.98)
	CUC	73	(1.45)		CCC	88	(1.08)
	CUA	22	(0.44)		CCA	93	(1.14)
	CUG	103	(2.04)		CCG	65	(0.80)
His	CAU	50	(0.88)	Arg	CGU	39	(0.76)
	CAC	63	(1.12)		CGC	63	(1.23)
Gln	CAA	59	(0.84)		CGA	46	(0.90)
	CAG	82	(1.16)		CGG	47	(0.92)
Ile	AUU	24	(0.69)	Thr	ACU	32	(0.67)
	AUC	61	(1.76)		ACC	76	(1.60)
	AUA	19	(0.55)		ACA	41	(0.86)
Met	AUG	42	(1.00)		ACG	41	(0.86)
Asn	AAU	26	(0.75)	Ser	AGU	23	(0.48)
	AAC	43	(1.25)		AGC	67	(1.39)
Lys	AAA	32	(0.54)	Arg	AGA	51	(1.00)
	AAG	86	(1.46)		AGG	61	(1.19)
Val	GUU	36	(0.75)	Ala	GCU	57	(0.79)
	GUC	54	(1.13)		GCC	97	(1.34)
	GUA	30	(0.63)		GCA	89	(1.23)
	GUG	71	(1.49)		GCG	47	(0.65)
Asp	GAU	60	(0.95)	Gly	GGU	35	(0.60)
	GAC	66	(1.05)		GGC	78	(1.33)
Glu	GAA	41	(0.68)		GGA	54	(0.92)
	GAG	80	(1.32)		GGG	67	(1.15)

Figure 18

A. Dunaliella salina most preferred codons
TTC (Phe)
TAC (Tyr)
TGC (Cys)
TAA (Stop)
TGG (Trp)
CCC (Pro)
CAC (His)
CGC (Arg)
CTG (Leu)
CAG (Gln)
ATC (Ile)
ACC (Thr)
AAC (Asn)
AGC (Ser)
ATG (Met)
AAG (Lys)
GCC (Ala)
GAC (Asp)
GGC (Gly)
GTG (Val)
GAG (Glu)

B. Chlorella pyrenoidosa most preferred codons
TTC (Phe)
TAC (Tyr)
TGC (Cys)
TGA (Stop)
TGG (Trp)
CCC (Pro)
CAC (His)
CGC (Arg)
CTG (Leu)
CAG (Gln)
ATC (Ile)
ACC (Thr)
GAC (Asp)
TCC (Ser)
ATG (Met)
AAG (Lys)
GCC (Ala)
AAC (Asn)
GGC (Gly)
GTG (Val)
GAG (Glu)

Figure 19

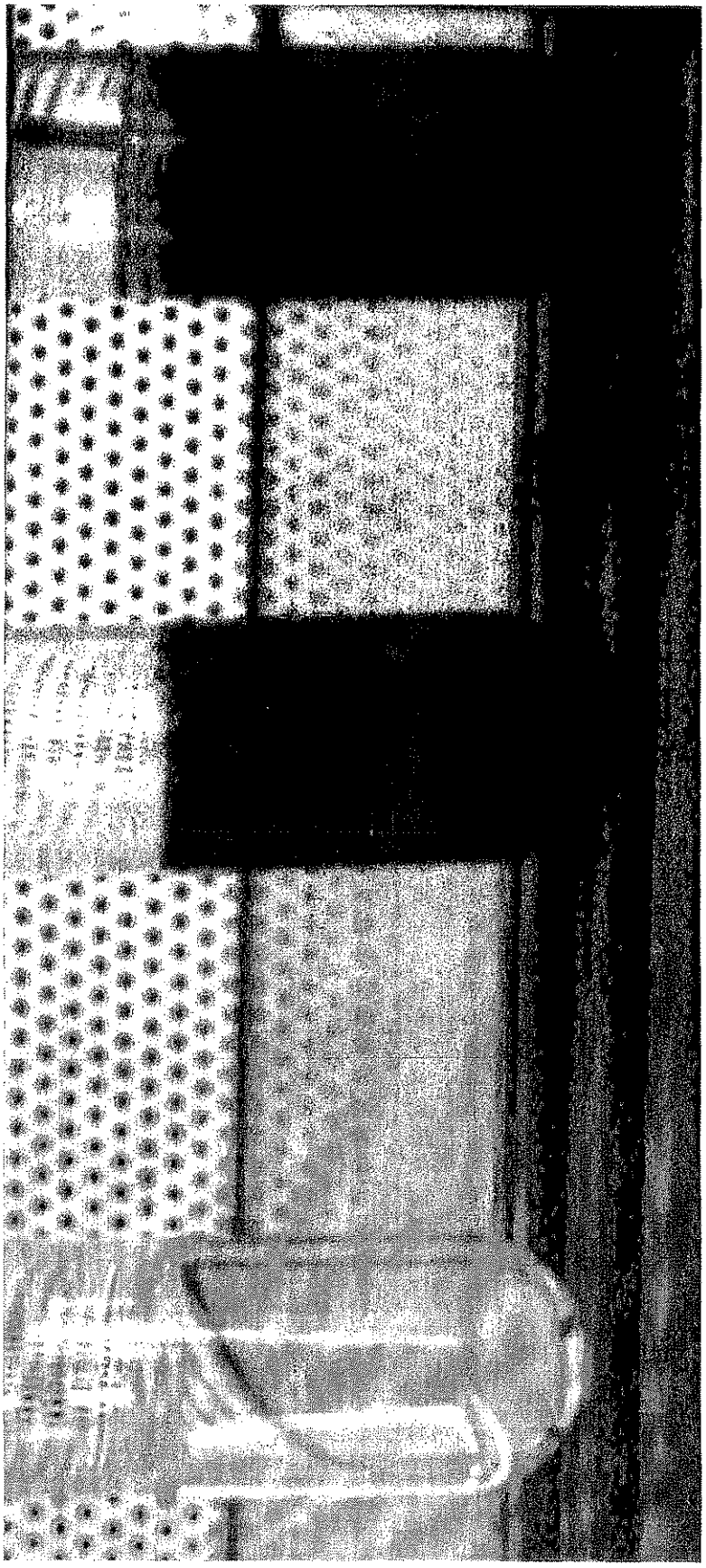


Figure 20

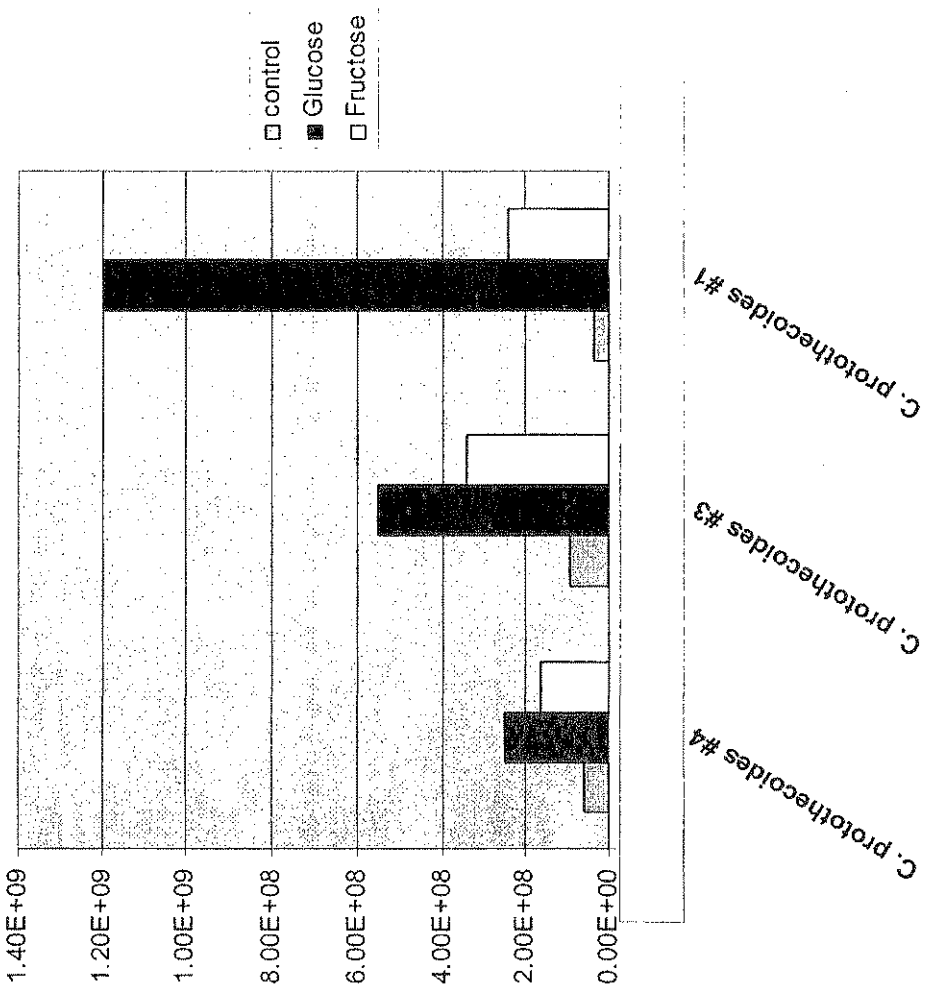
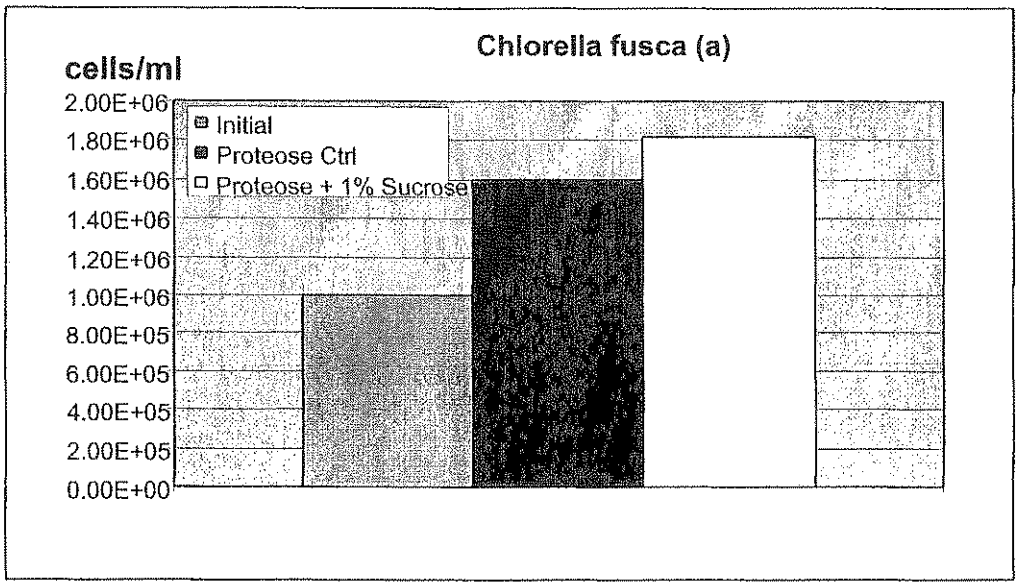
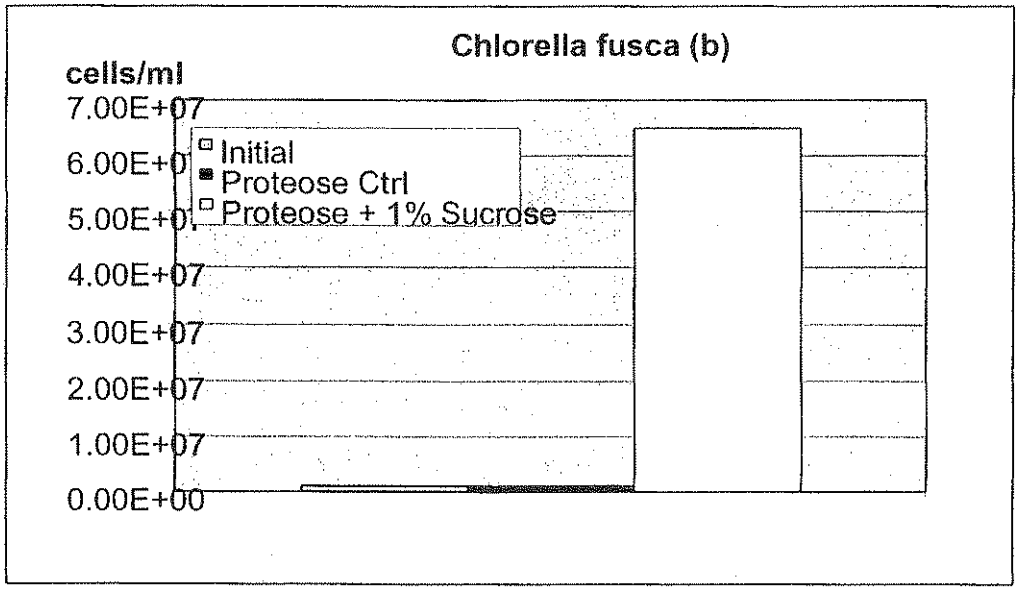
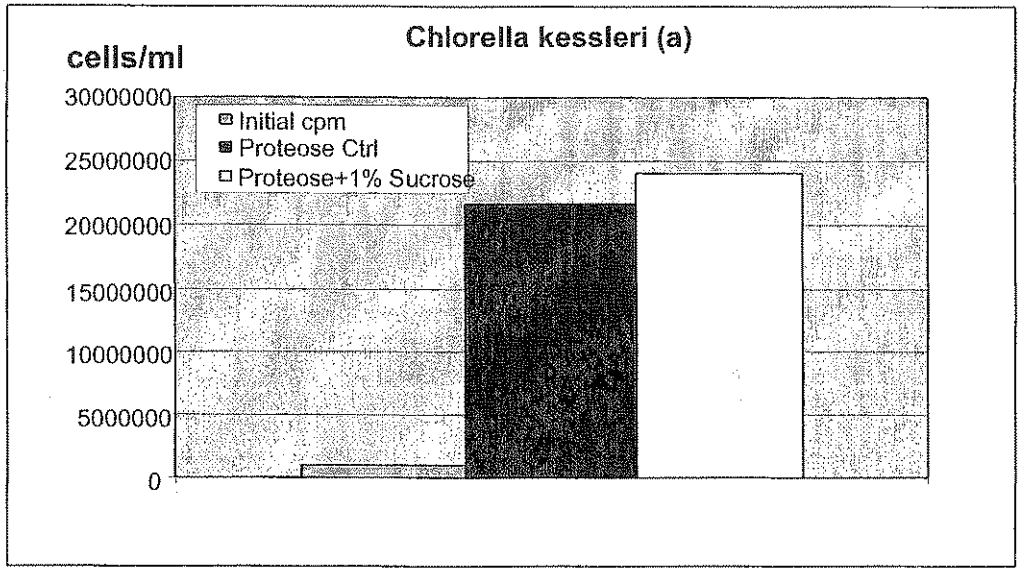

control
Glucose
Fructose
1.40E+09
1.20E+09
1.00E+09
8.00E+08
6.00E+08
4.00E+08
2.00E+08
0.00E+00
Cell density (cells/ml)
C. protothecoides #1
C. protothecoides #3
C. protothecoides #4

Figure 21


Chlorella fusca (a)
cells/ml
2.00E+06
1.80E+06
1.60E+06
1.40E+06
1.20E+06
1.00E+06
8.00E+05
6.00E+05
4.00E+05
2.00E+05
0.00E+00
Initial
Proteose Ctrl
Proteose + 1% Sucrose


Chlorella fusca (b)
cells/ml
7.00E+07
6.00E+07
5.00E+07
4.00E+07
3.00E+07
2.00E+07
1.00E+07
0.00E+00
Initial
Proteose Ctrl
Proteose + 1% Sucrose


Chlorella kessleri (a)
cells/ml
30000000
25000000
20000000
15000000
10000000
5000000
0
Initial cpm
Proteose Ctrl
Proteose+1% Sucrose

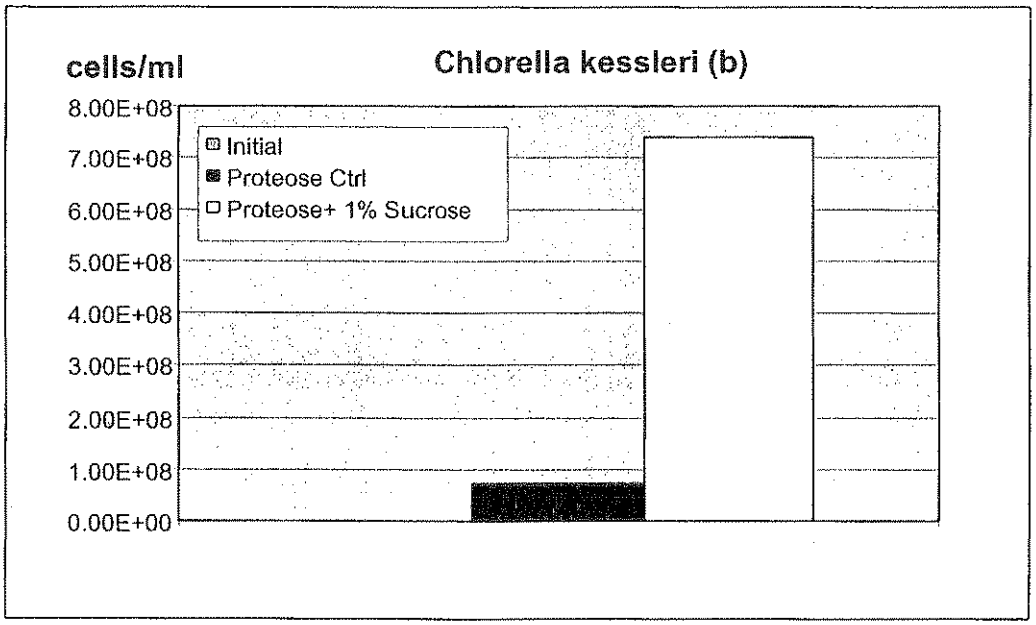

Chlorella kessleri (b)
cells/ml
8.00E+08
7.00E+08
6.00E+08
5.00E+08
4.00E+08
3.00E+08
2.00E+08
1.00E+08
0.00E+00
Initial
Proteose Ctrl
Proteose+ 1% Sucrose

Figure 22

Figure 23

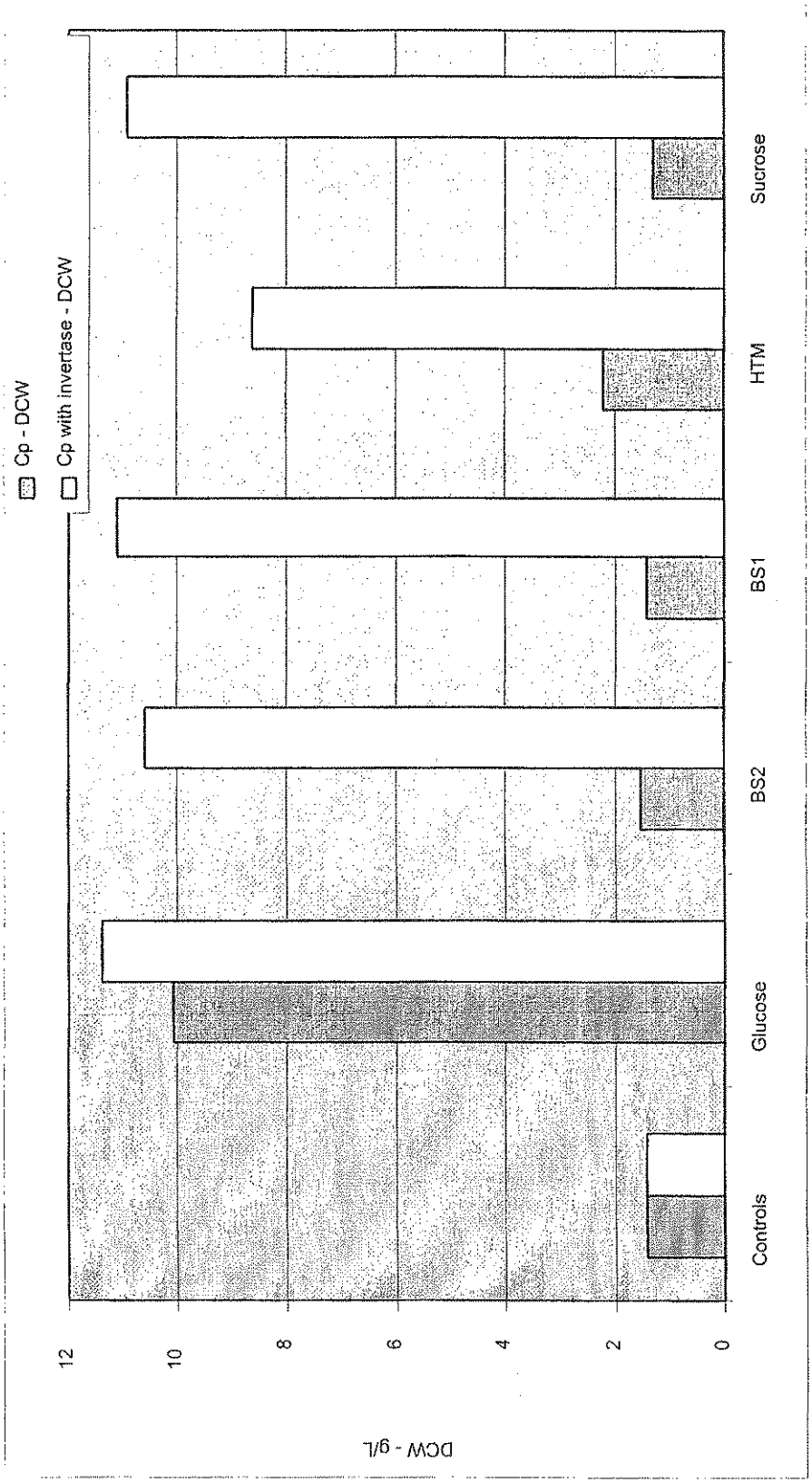

Cp - DCW
Cp with invertase - DCW
DCW - g/L
0
2
4
6
8
10
12
Controls
Glucose
BS2
BS1
HTM
Sucrose
Feedstock (Molasses/Sugar)

Figure 24

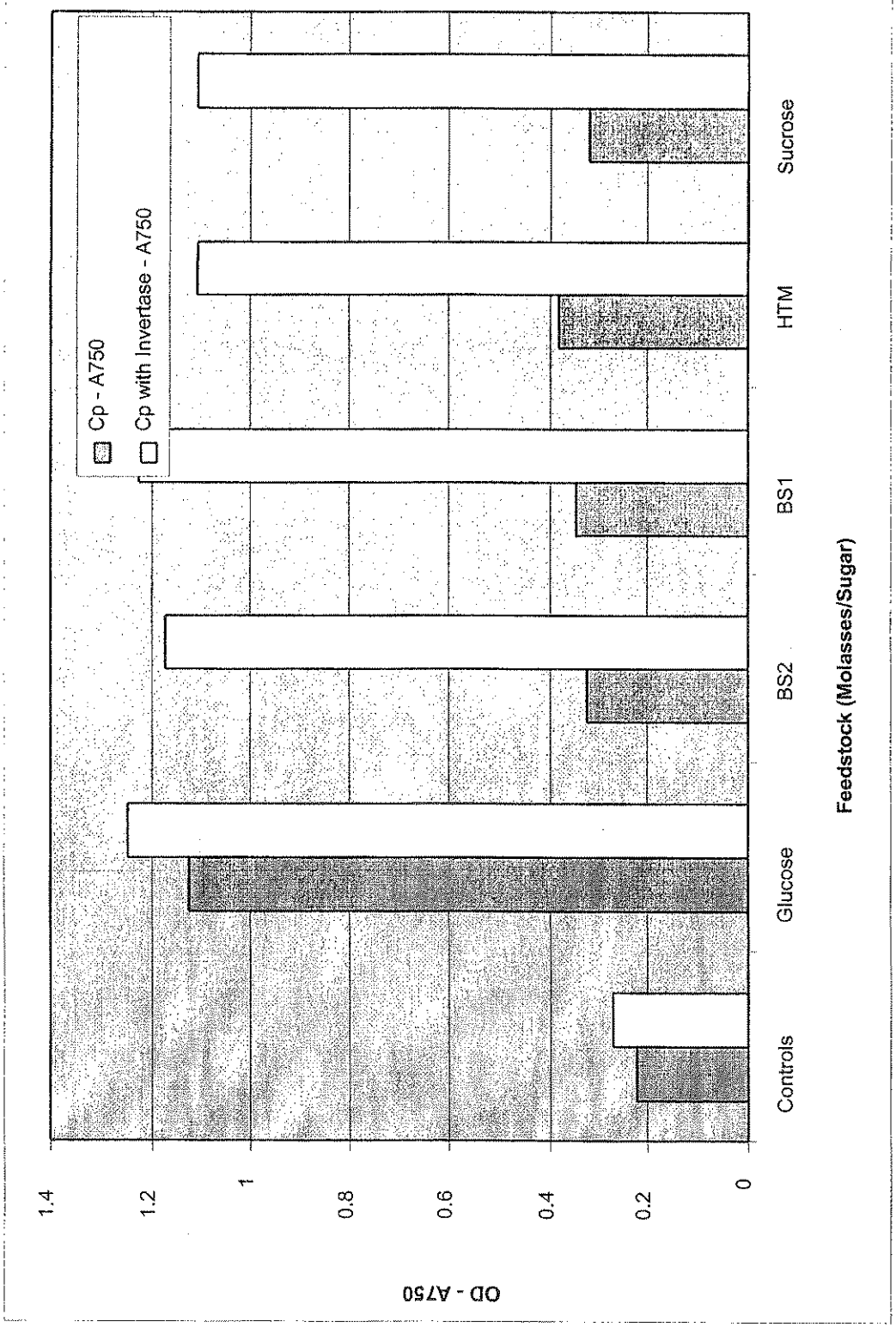

Cp - A750
Cp with Invertase - A750
OD - A750
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Controls
Glucose
BS2
BS1
HTM
Sucrose
Feedstock (Molasses/Sugar)

Figure 25

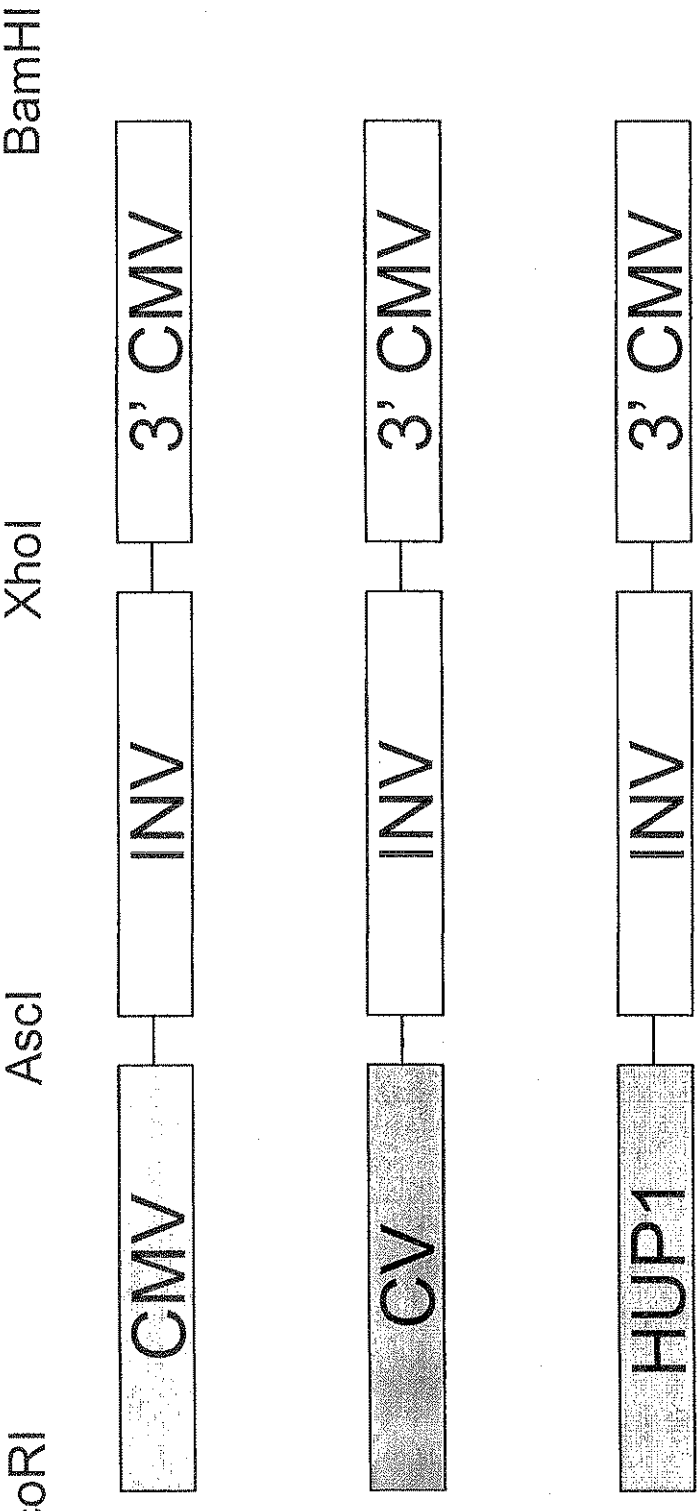


Figure 26

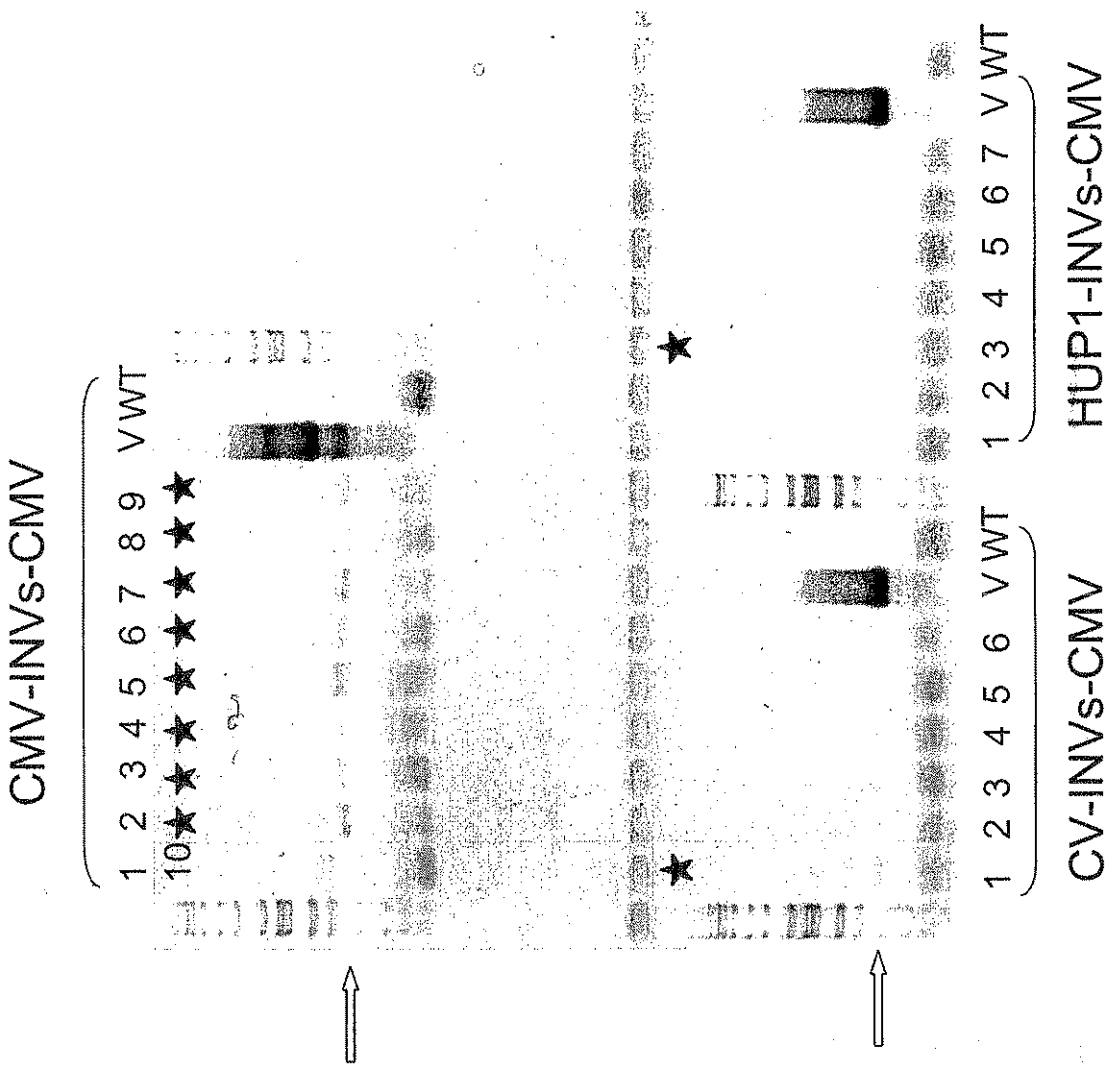
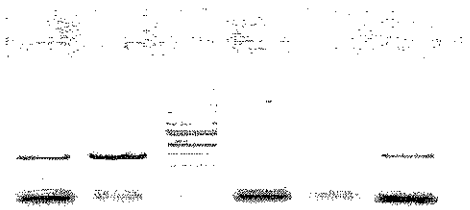

CMV-INVs-CMV
1 2 3 4 5 6 7 8 9 10 V WT
CV-INVs-CMV
1 2 3 4 5 6 V WT
HUP1-INVs-CMV
1 2 3 4 5 6 7 V WT

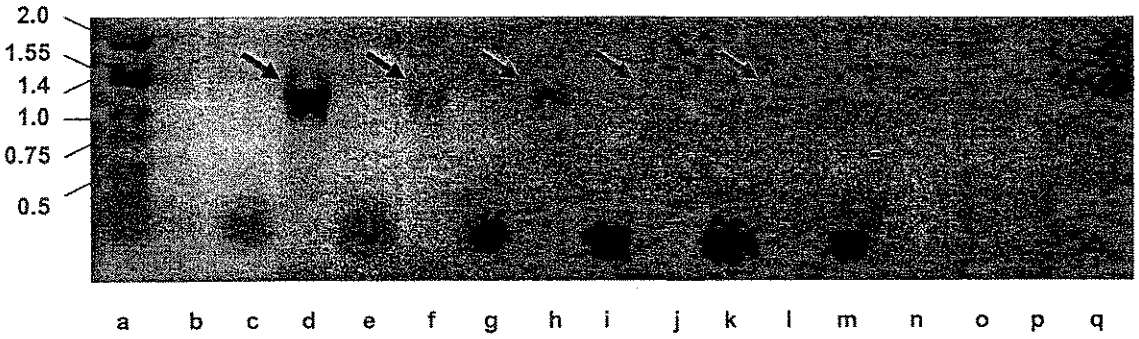
Figure 27



a b c d e

a -Plasmid (200 pg/ uL)
b -Plasmid (200 ng/ uL)
c –Wild type DNA
d -Transformant #1
e -Transformant #2

Figure 28



a. DNA size standards in kilo base pairs
b. Primer set 2 control (no template)
c. Primer set 4 control (no template)
d. 1 picogram of plasmid control, primer set 2
e. 1 picogram of plasmid control, primer set 4
f. Chlorella minutissima transformant #1, primer set 2
g. Chlorella minutissima transformant #1, primer set 4
h. Chlorella minutissima transformant #2, primer set 2
i. Chlorella minutissima transformant #2, primer set 4
j. Chlorella emersonii transformant #1, primer set 2
k. Chlorella emersonii transformant #1, primer set 4
l. Chlorella emersonii transformant #2, primer set 2
m. Chlorella emersonii transformant #2, primer set 4
n. wild type Chlorella minutissima, primer set 2
o. wild type Chlorella minutissima, primer set 4
p. wild type Chlorella emersonii, primer set 2
q. wild type Chlorella emersonii, primer set 4

Figure 29

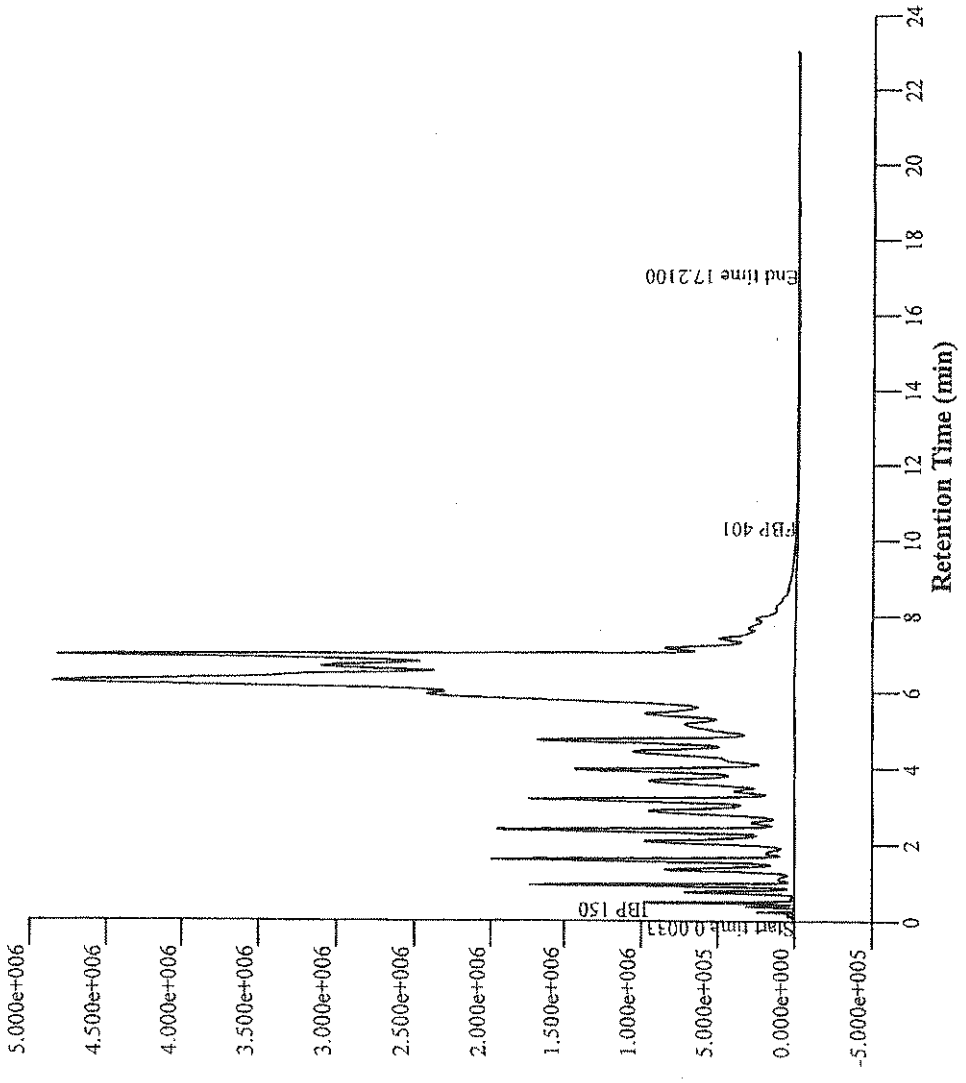
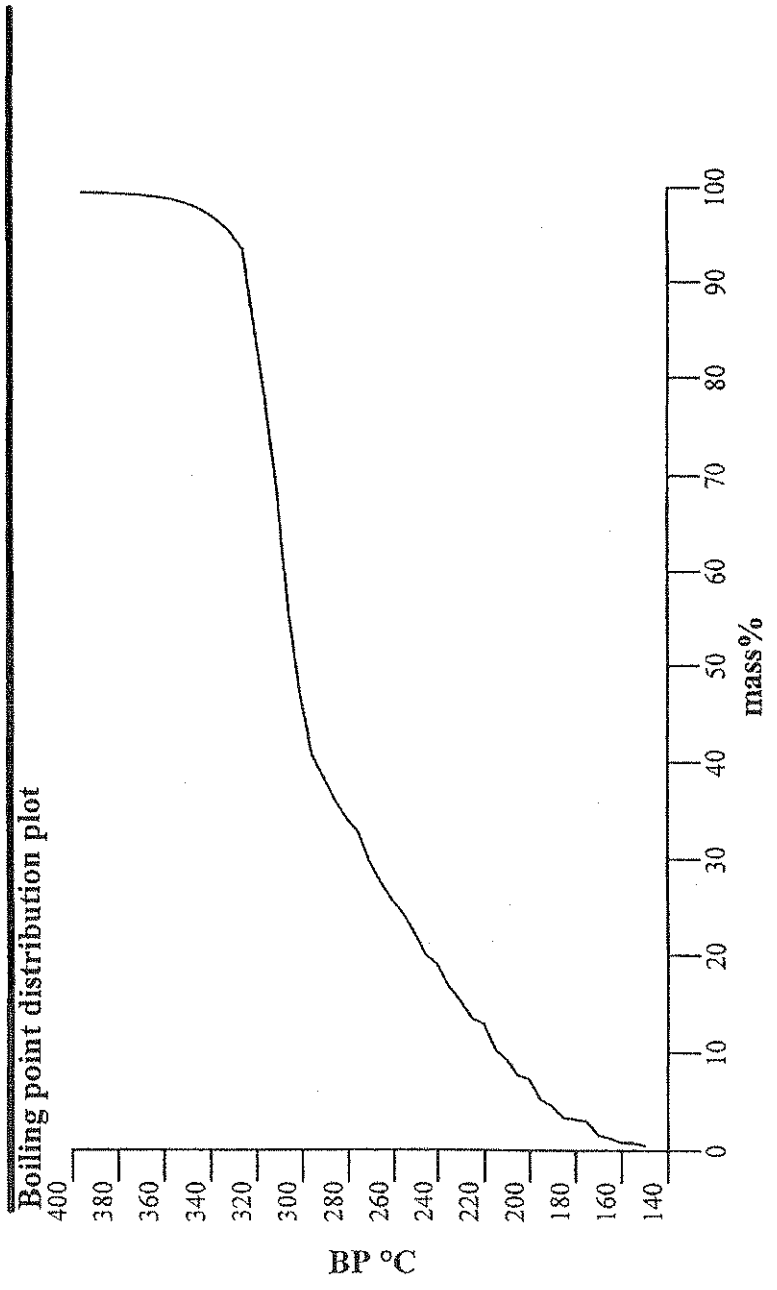

5.000e+006
4.500e+006
4.000e+006
3.500e+006
3.000e+006
2.500e+006
2.000e+006
1.500e+006
1.000e+006
5.000e+005
0.000e+000
-5.000e+005
0
2
4
6
8
10
12
14
16
18
20
22
24
Retention Time (min)
Start time 0.0033
JBP 150
FBP 401
End time 17.2100

Figure 30


Boiling point distribution plot
BP °C
400
380
360
340
320
300
280
260
240
220
200
180
160
140
0
10
20
30
40
50
60
70
80
90
100
mass%