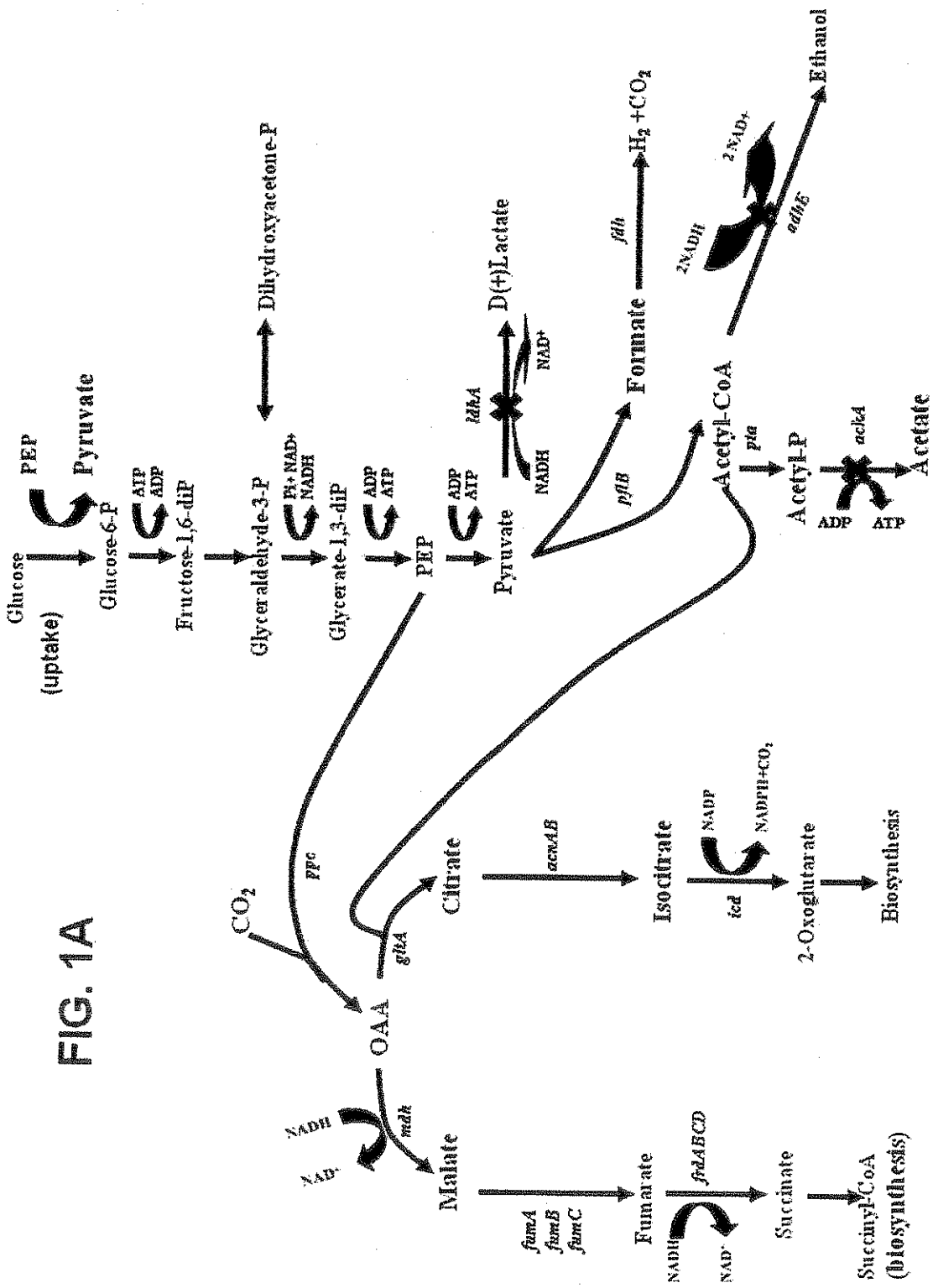
FIG. 1A


Glucose
(uptake)
PEP
Pyruvate
Glucose-6-P
ATP
ADP
Fructose-1,6-diP
Glyceraldehyde-3-P
Dihydroxyacetone-P
Pi+ NAD+
NADH
Glycerate-1,3-diP
ADP
ATP
PEP
ADP
ATP
Pyruvate
ldhA
NADH
NAD+
D(+)Lactate
pflB
Formate
fdh
H2 +CO2
2NADH
2NAD+
adhE
Ethanol
Acetyl-CoA
pta
Acetyl-P
ADP
ATP
ack4
Acetate
CO2
ppc
OAA
gltA
Citrate
acnAB
Isocitrate
NADP
icd
NADPH+CO2
2-Oxoglutarate
Biosynthesis
NADH
NAD+
mdh
Malate
fumA
fumB
fumC
Fumarate
NADH
NAD+
frdABCD
Succinate
Succinyl-CoA
(biosynthesis)

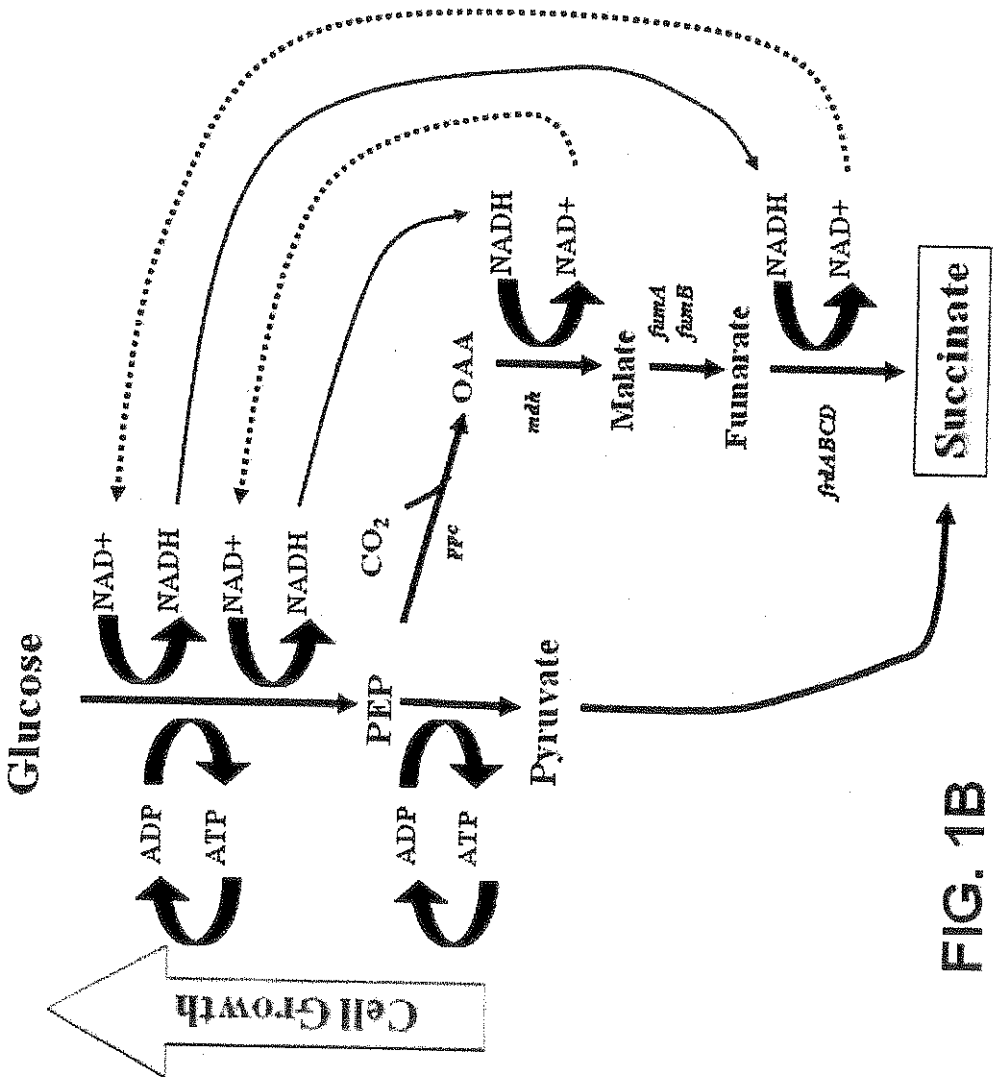

Glucose
NAD+
NADH
NAD+
NADH
ADP
ATP
ADP
ATP
PEP
CO_2
ppc
OAA
Pyruvate
NADH
NAD+
mdh
Malate
fumA
fumB
Fumarate
NADH
NAD+
frdABCD
Succinate
Cell Growth

FIG. 1B

FIG. 2A

FIG. 2B

FIG. 2C

FIG. 2D

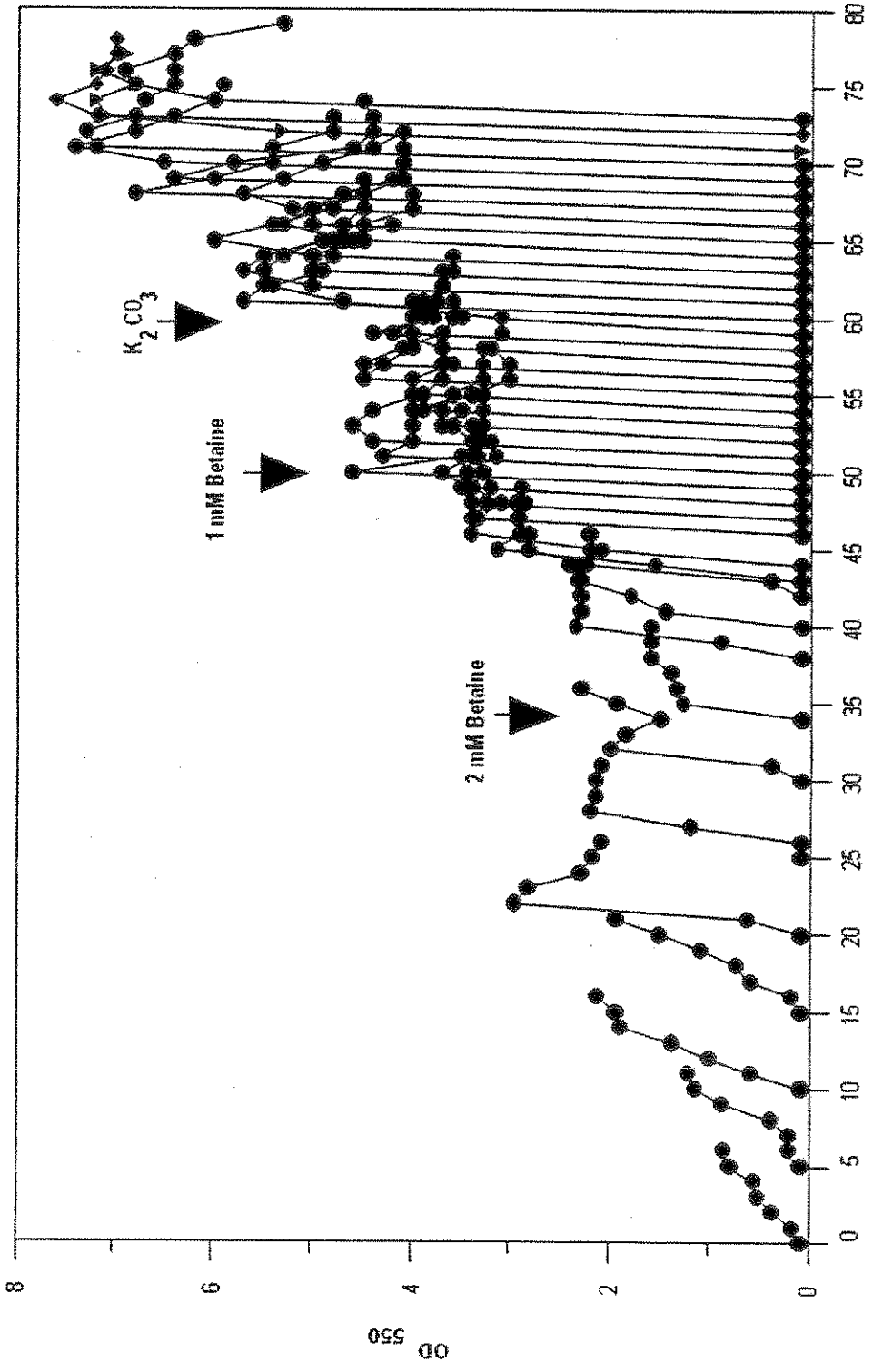

K_2CO_3
1 mM Betaine
2 mM Betaine
OD 550
Time (days)
0
2
4
6
8
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80

FIG. 3A

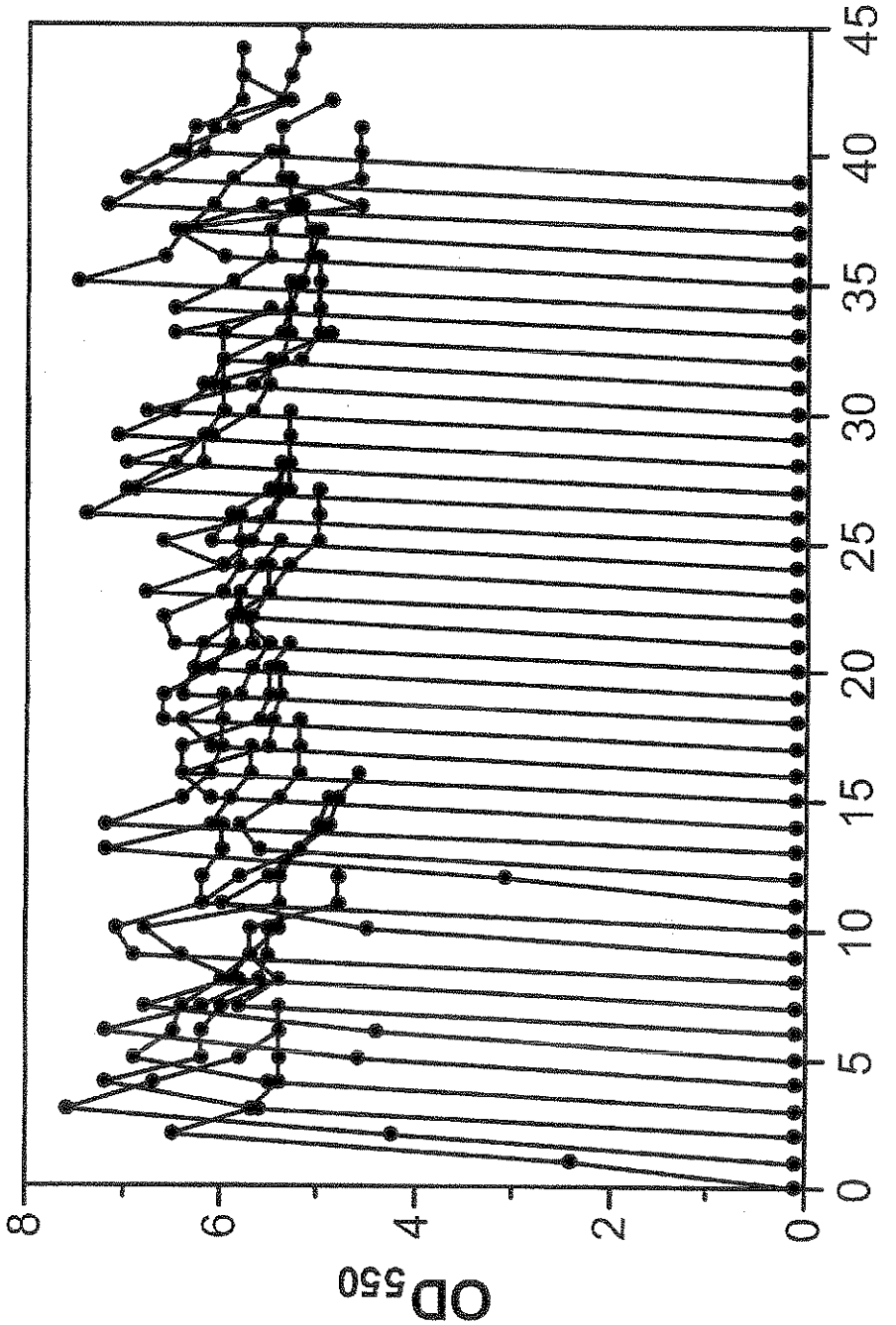


FIG. 3B

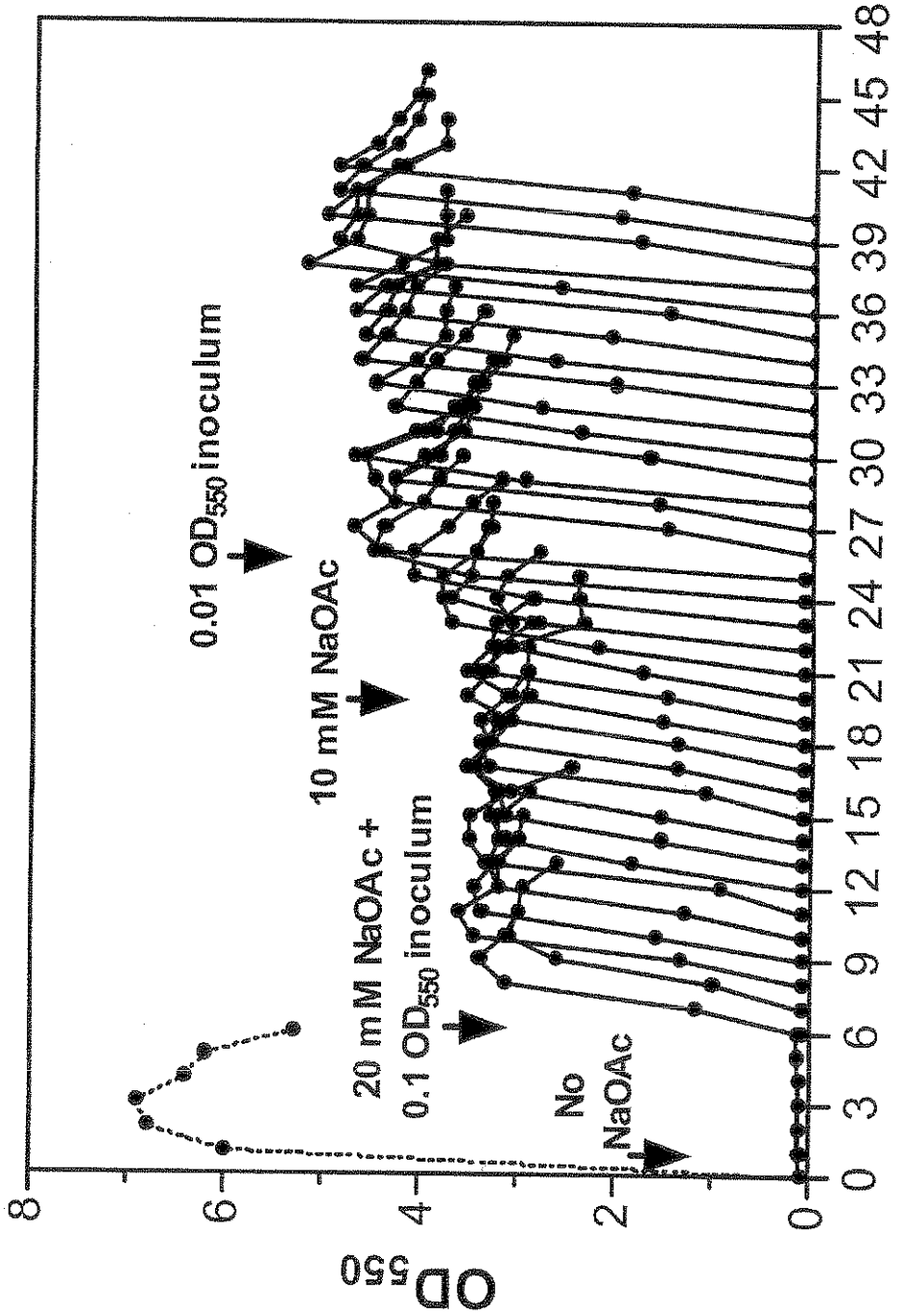

OD550
Time (days)
0.01 OD550 inoculum
10 mM NaOAc
20 mM NaOAc + 0.1 OD550 inoculum
No NaOAc
0
2
4
6
8
0 3 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48

FIG. 3C

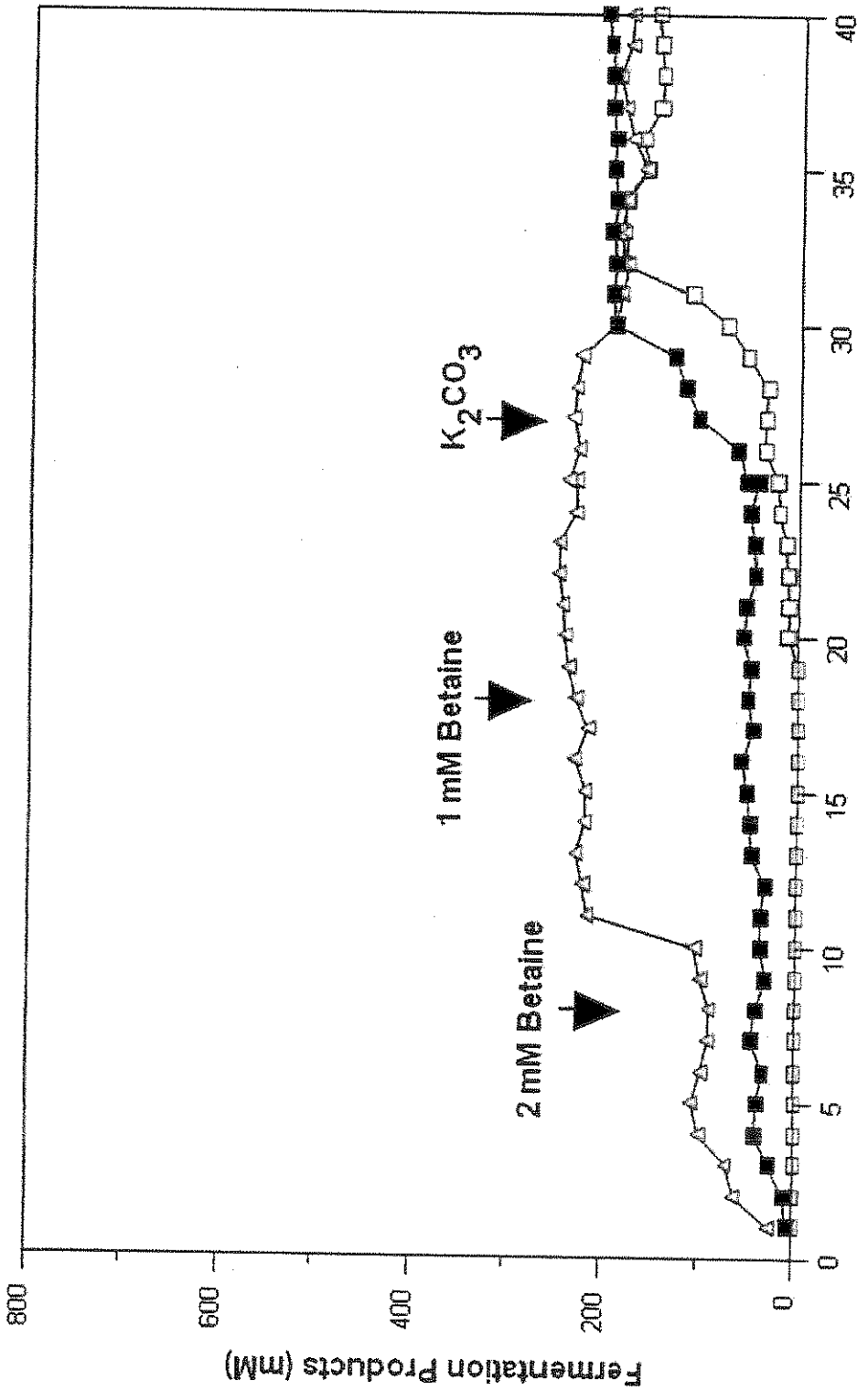

Fermentation Products (mM)
800
600
400
200
0
0
5
10
15
20
25
30
35
40
Number of Transfers
K_2CO_3
1 mM Betaine
2 mM Betaine

FIG. 4A

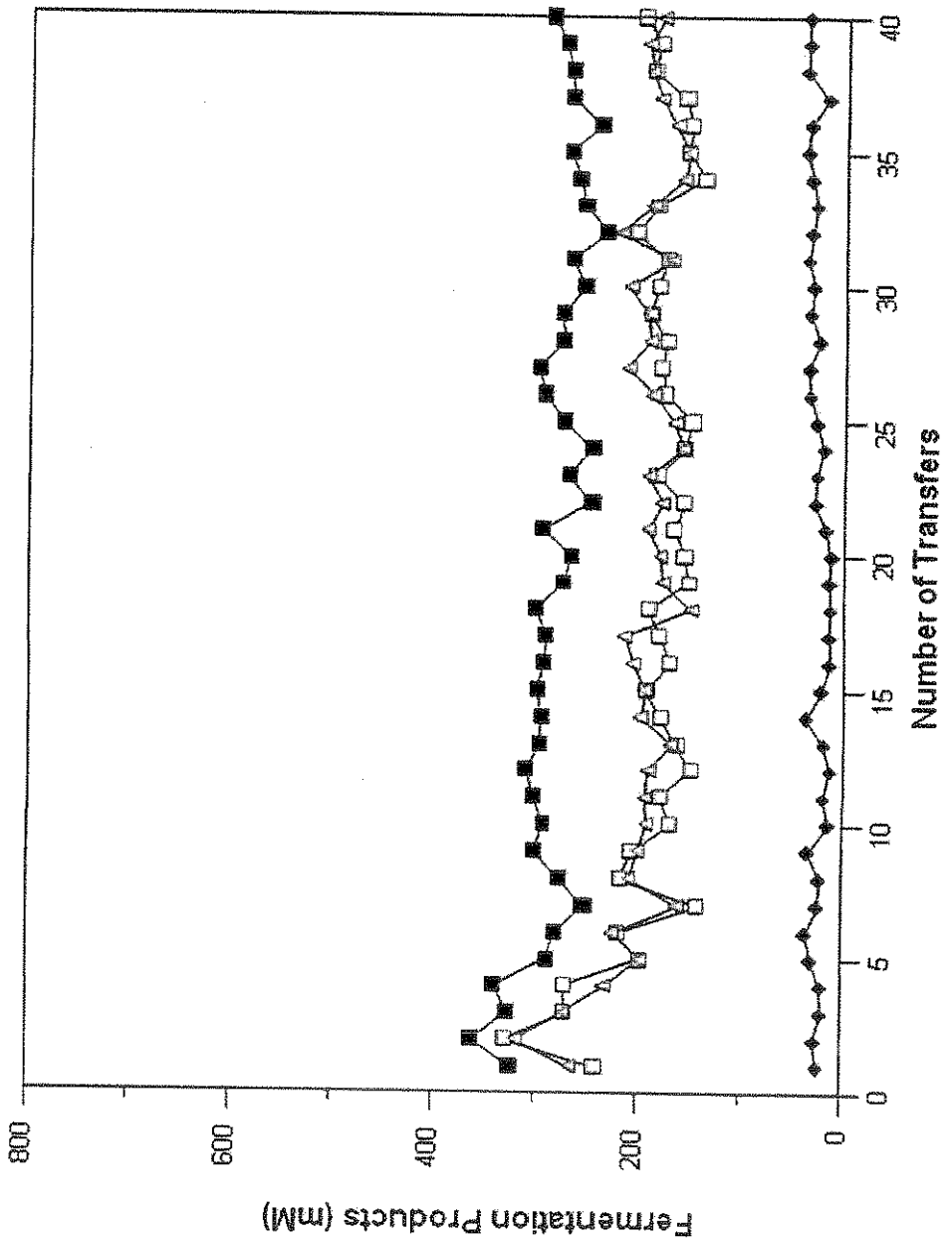

Fermentation Products (mM)
800
600
400
200
0
0
5
10
15
20
25
30
35
40
Number of Transfers

FIG. 4B

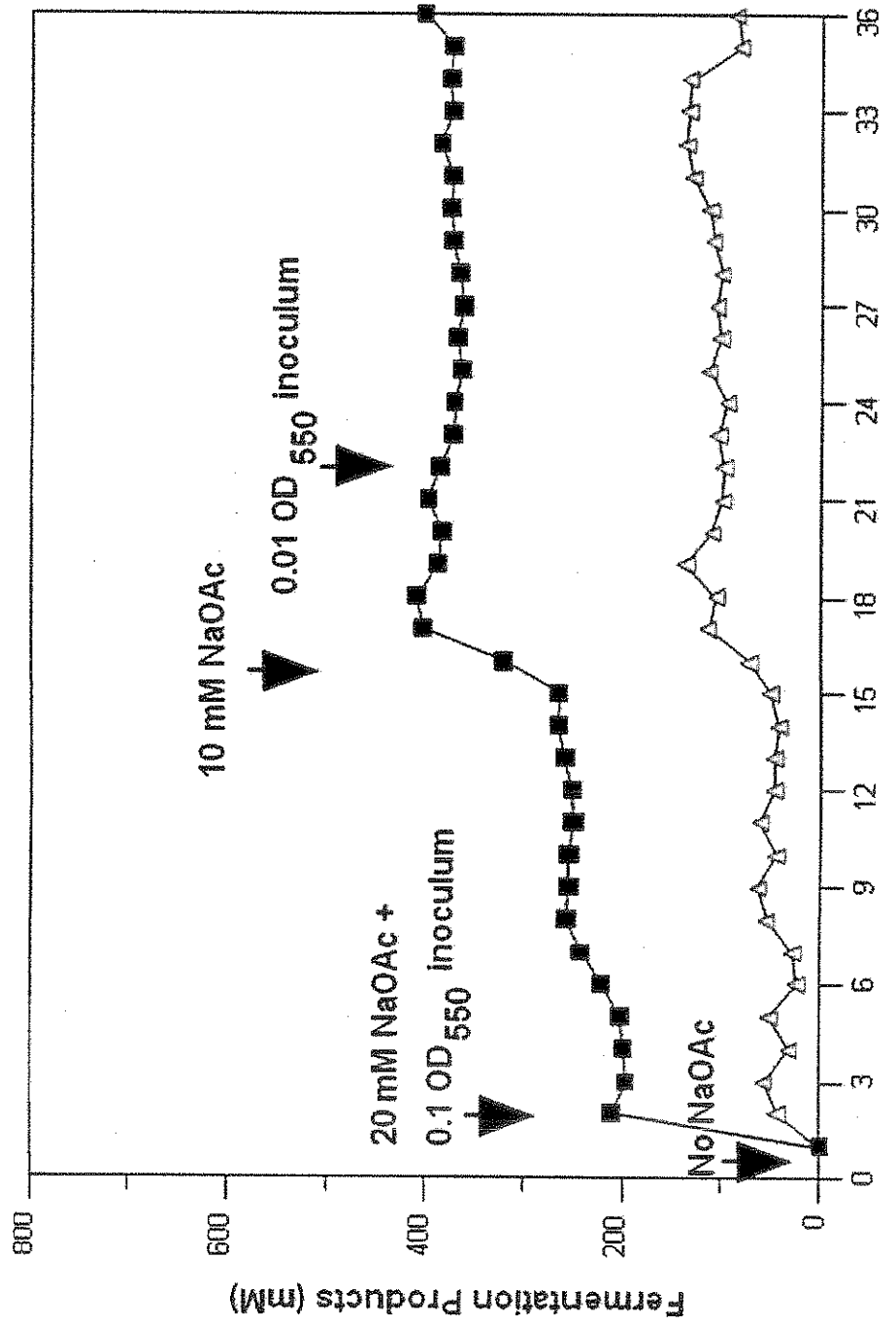

Fermentation Products (mM)
800
600
400
200
0
0
3
6
9
12
15
18
21
24
27
30
33
36
Number of Transfers
10 mM NaOAc
0.01 OD550 inoculum
20 mM NaOAc +
0.1 OD550 inoculum
No NaOAc

FIG. 4C

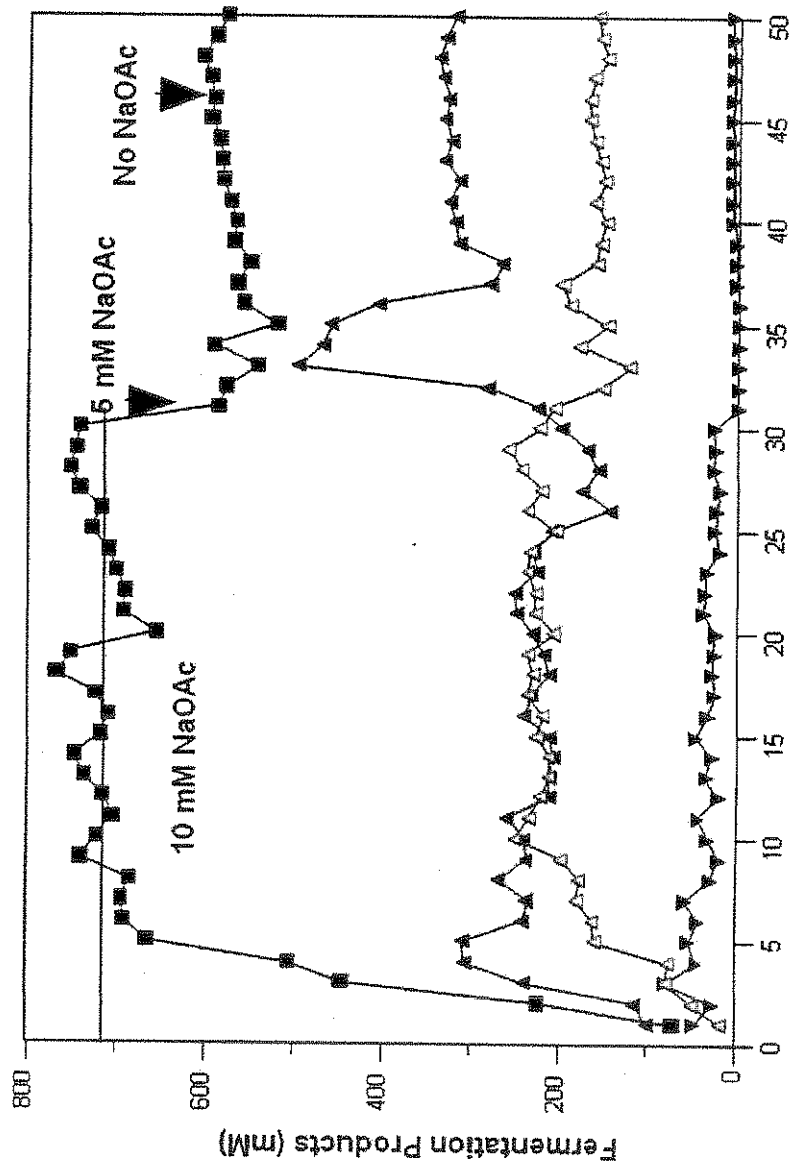

No NaOAc
6 mM NaOAc
10 mM NaOAc
Fermentation Products (mM)
Number of Transfers
0
200
400
600
800
0
5
10
15
20
25
30
35
40
45
50

FIG. 4D

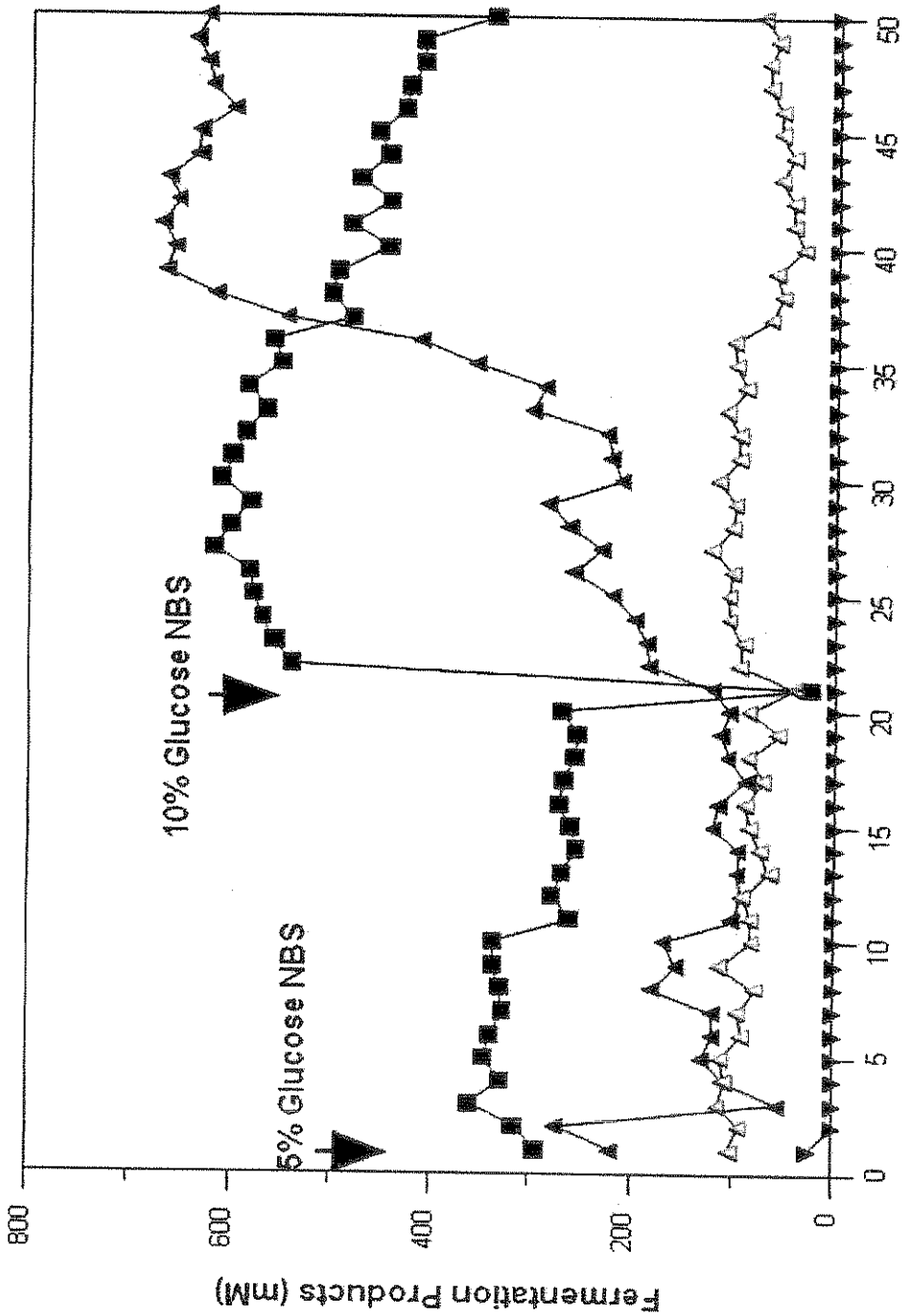

Fermentation Products (mM)
800
600
400
200
0
0
5
10
15
20
25
30
35
40
45
50
Number of Transfers
10% Glucose NBS
5% Glucose NBS

FIG. 4E

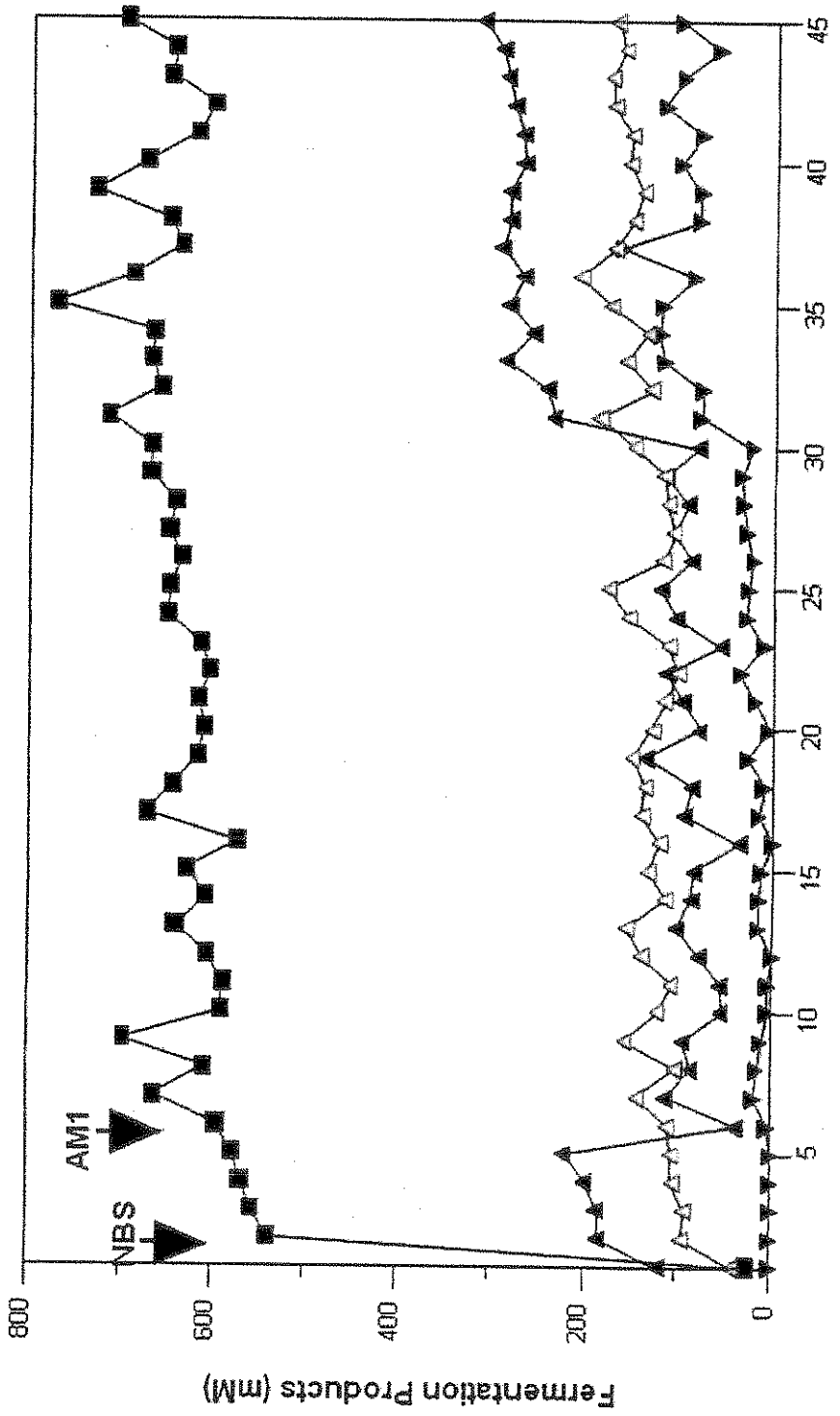

Fermentation Products (mM)
800
600
400
200
0
NBS
AM1
5
10
15
20
25
30
35
40
45
Number of Transfers

FIG. 4F

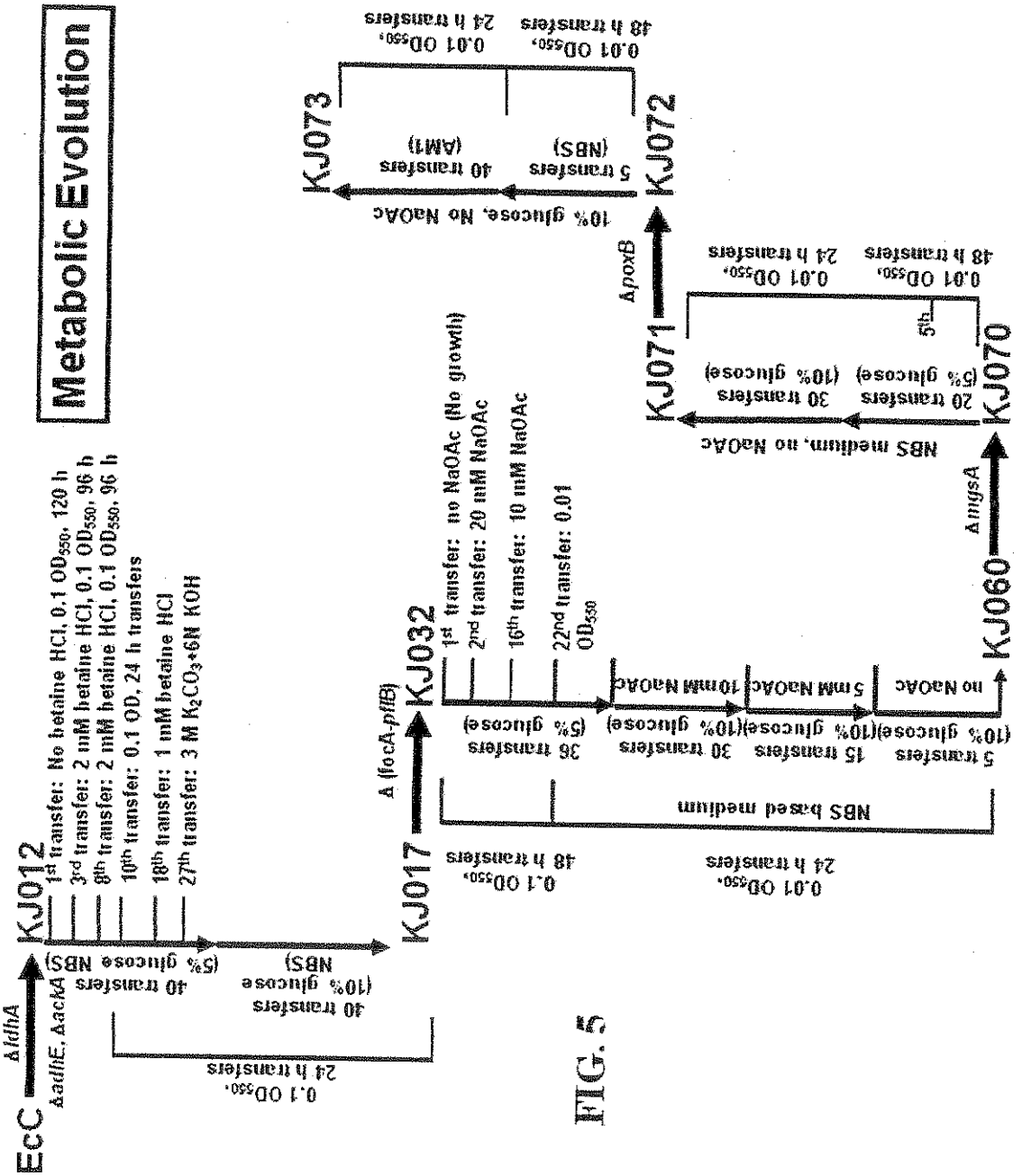
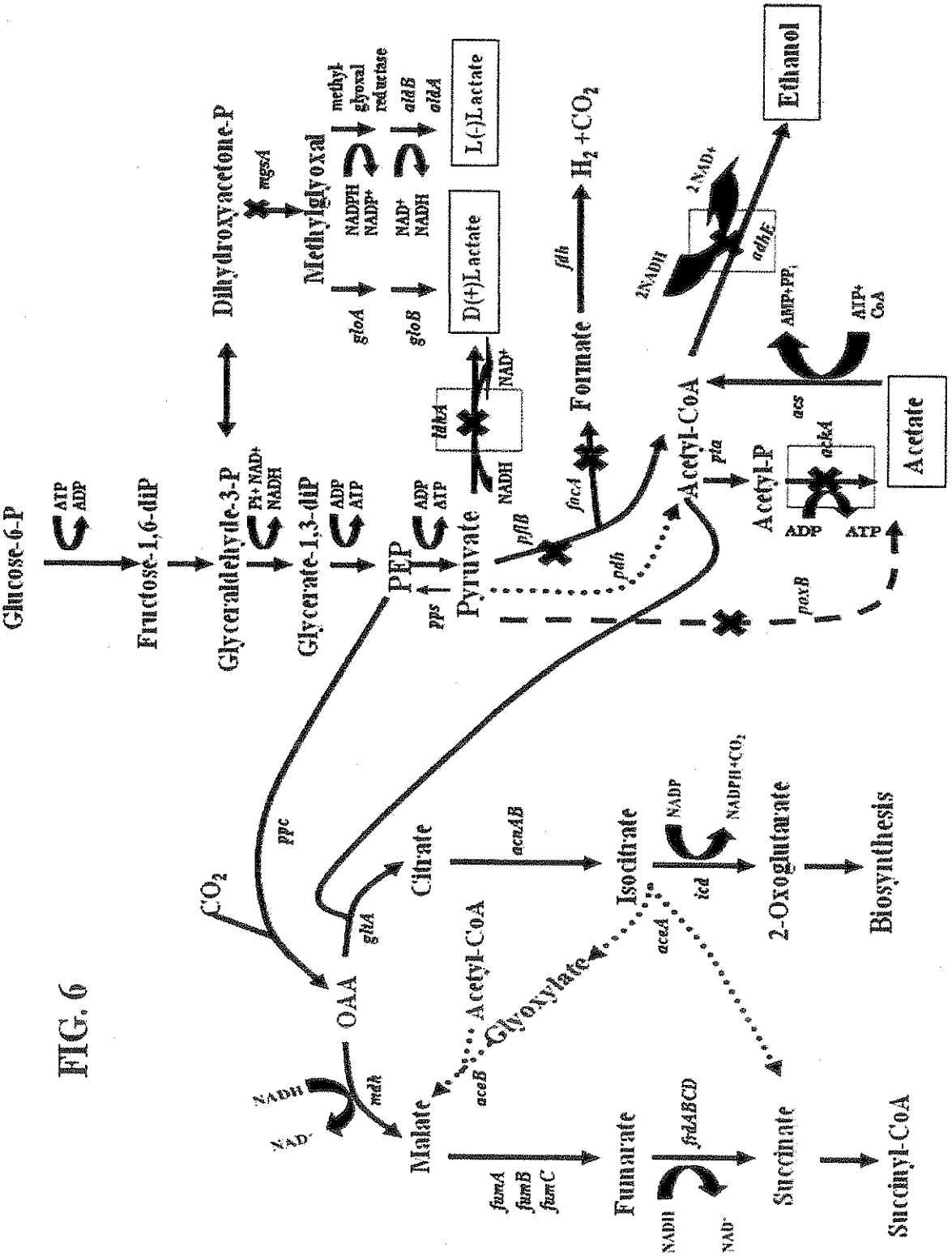

Metabolic Evolution
EcC
ΔadhE, ΔackA
ΔldhA
KJ012
1st transfer: No betaine HCl, 0.1 OD550, 120 h
3rd transfer: 2 mM betaine HCl, 0.1 OD550, 96 h
8th transfer: 2 mM betaine HCl, 0.1 OD550, 96 h
10th transfer: 0.1 OD, 24 h transfers
18th transfer: 1 mM betaine HCl
27th transfer: 3 M K2CO3•6N KOH
40 transfers (5% glucose NBS)
40 transfers (10% glucose NBS)
24 h transfers 0.1 OD550,
KJ017
Δ (focA-pflB)
KJ032
1st transfer: no NaOAc (No growth)
2nd transfer: 20 mM NaOAc
16th transfer: 10 mM NaOAc
22nd transfer: 0.01 OD550
36 transfers (5% glucose)
30 transfers (10% glucose)
15 transfers (10% glucose)
5 transfers (10% glucose)
10 mM NaOAc
5 mM NaOAc
no NaOAc
NBS based medium
18 h transfers 0.1 OD550,
24 h transfers 0.01 OD550,
KJ060
ΔmysA
NBS medium, no NaOAc
20 transfers (5% glucose)
30 transfers (10% glucose)
KJ070
5th
KJ071
ΔpoxB
18 h transfers 0.01 OD550,
24 h transfers 0.01 OD550,
10% glucose, No NaOAc
5 transfers (NBS)
40 transfers (AM1)
KJ072
KJ073
18 h transfers 0.01 OD550,
24 h transfers 0.01 OD550,

FIG. 6

FIG. 6


Glucose-6-P
ATP
ADP
Fructose-1,6-diP
Glyceraldehyde-3-P
Pi+ NAD+
NADH
Glycerate-1,3-diP
ADP
ATP
PEP
ADP
ATP
Pyruvate
NADH
Dihydroxyacetone-P
mgsA
Methylglyoxal
NADPH
NADP+
NAD+
NADH
methyl glyoxal reductase
aldB
aldA
L(-)Lactate
D(+)Lactate
NAD+
tdhA
gloA
gloB
pflB
fdh
Formate
H2 +CO2
fdhE
2NADH
2NAD+
Ethanol
AMP-PPi
ATP
CoA
acs
Acetyl-CoA
pta
Acetyl-P
ADP
ATP
ackA
Acetate
poxB
pdh
fdcA
ppc
ppsA
CO2
OAA
gltA
Citrate
acnAB
Isocitrate
NADP
NADPH+CO2
icd
aceA
aceB
Glyoxylate
2-Oxoglutarate
Biosynthesis
NADH
NAD+
mdh
Malate
fumA
fumB
fumC
Fumarate
frdABCD
NADH
NAD+
Succinate
Succinyl-CoA

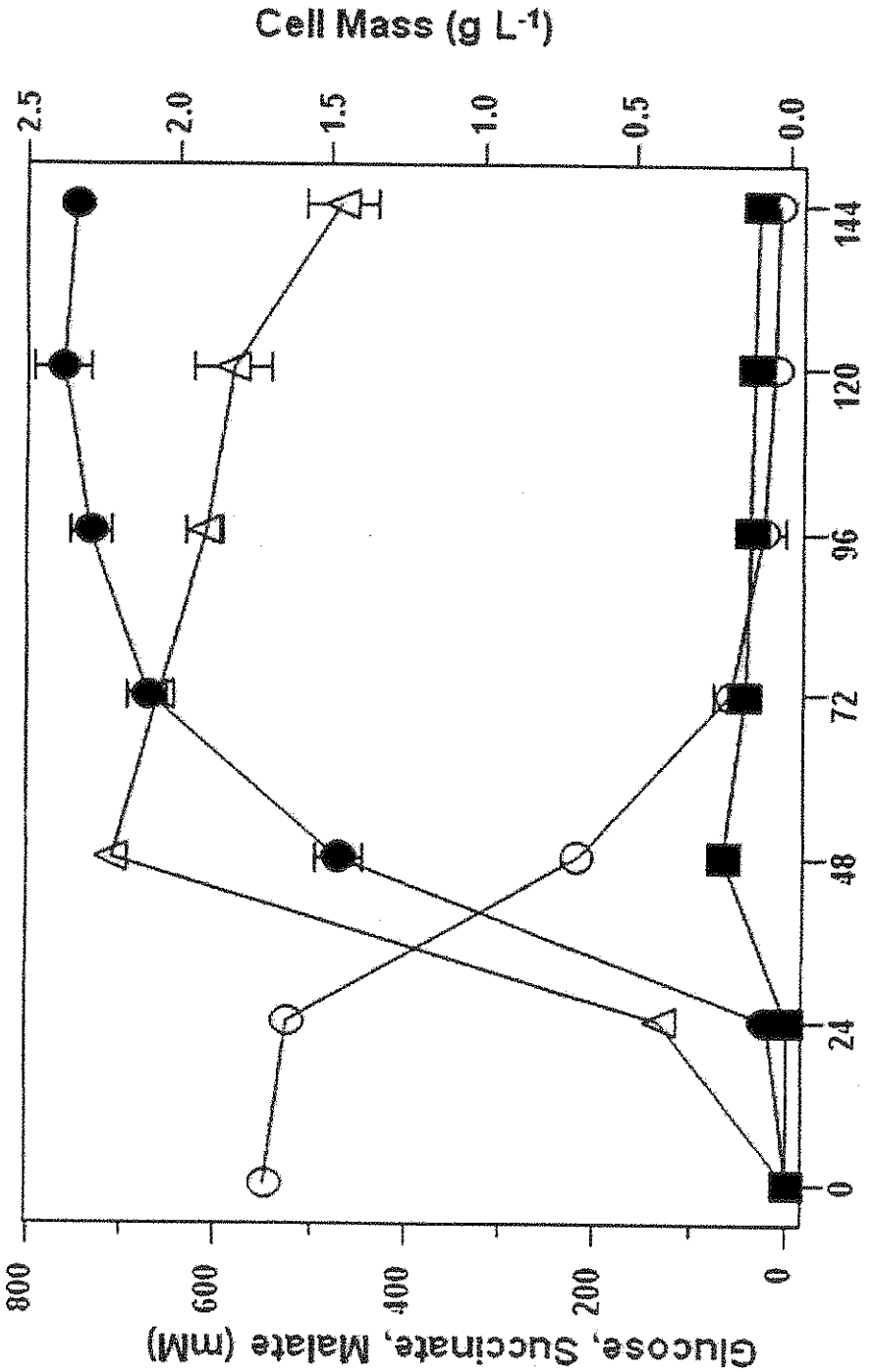

Cell Mass (g L-1)
2.5
2.0
1.5
1.0
0.5
0.0
144
120
96
72
48
24
0
Time (h)
800
600
400
200
0
Glucose, Succinate, Malate (mM)

FIG. 7A

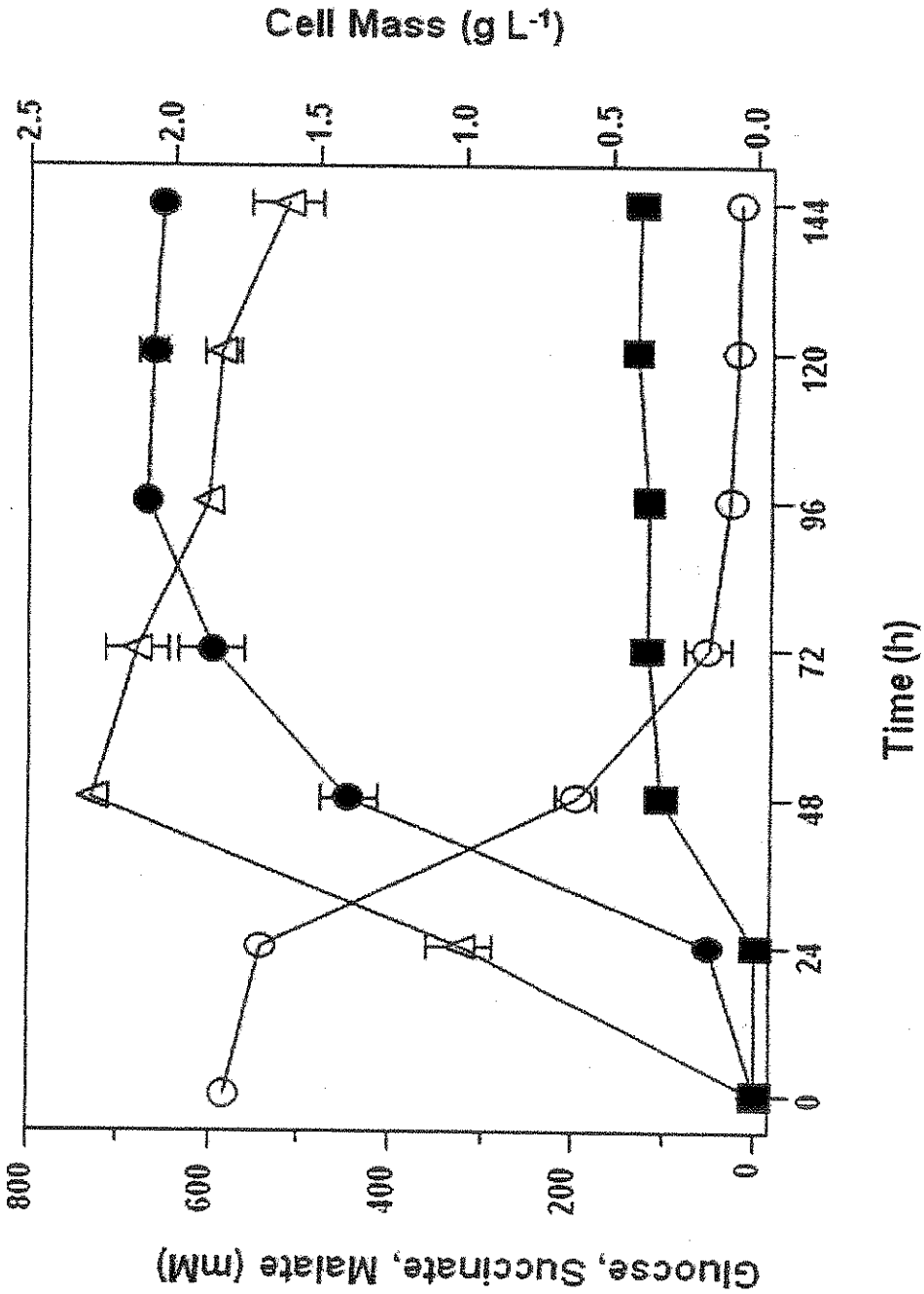

Cell Mass (g L-1)
2.5
2.0
1.5
1.0
0.5
0.0
144
120
96
72
48
24
0
Time (h)
800
600
400
200
0
Glucose, Succinate, Malate (mM)

FIG. 7B

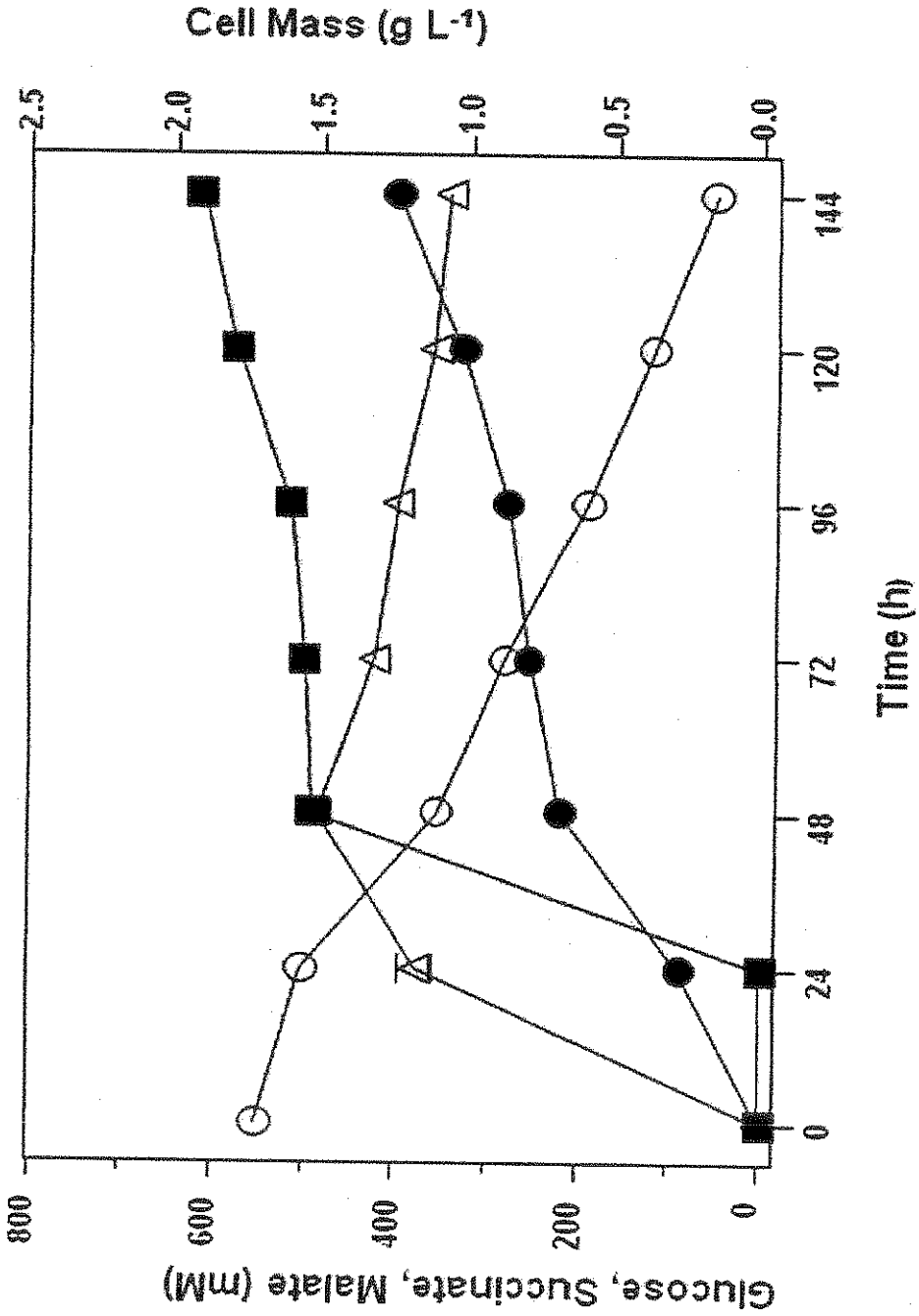

Cell Mass (g L-1)
2.5
2.0
1.5
1.0
0.5
0.0
144
120
96
72
48
24
0
Time (h)
800
600
400
200
0
Glucose, Succinate, Malate (mM)

FIG. 7C

FIG. 8

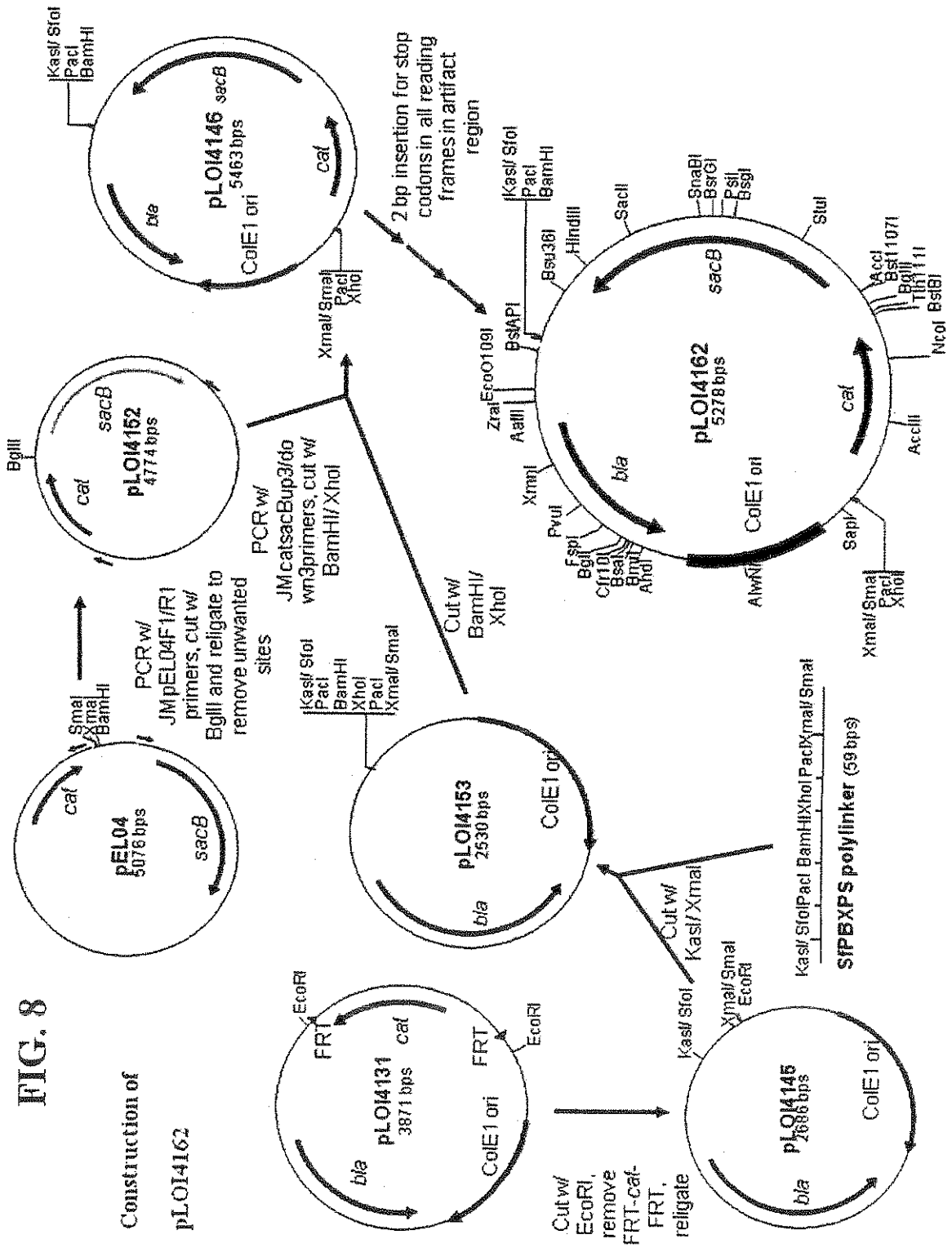
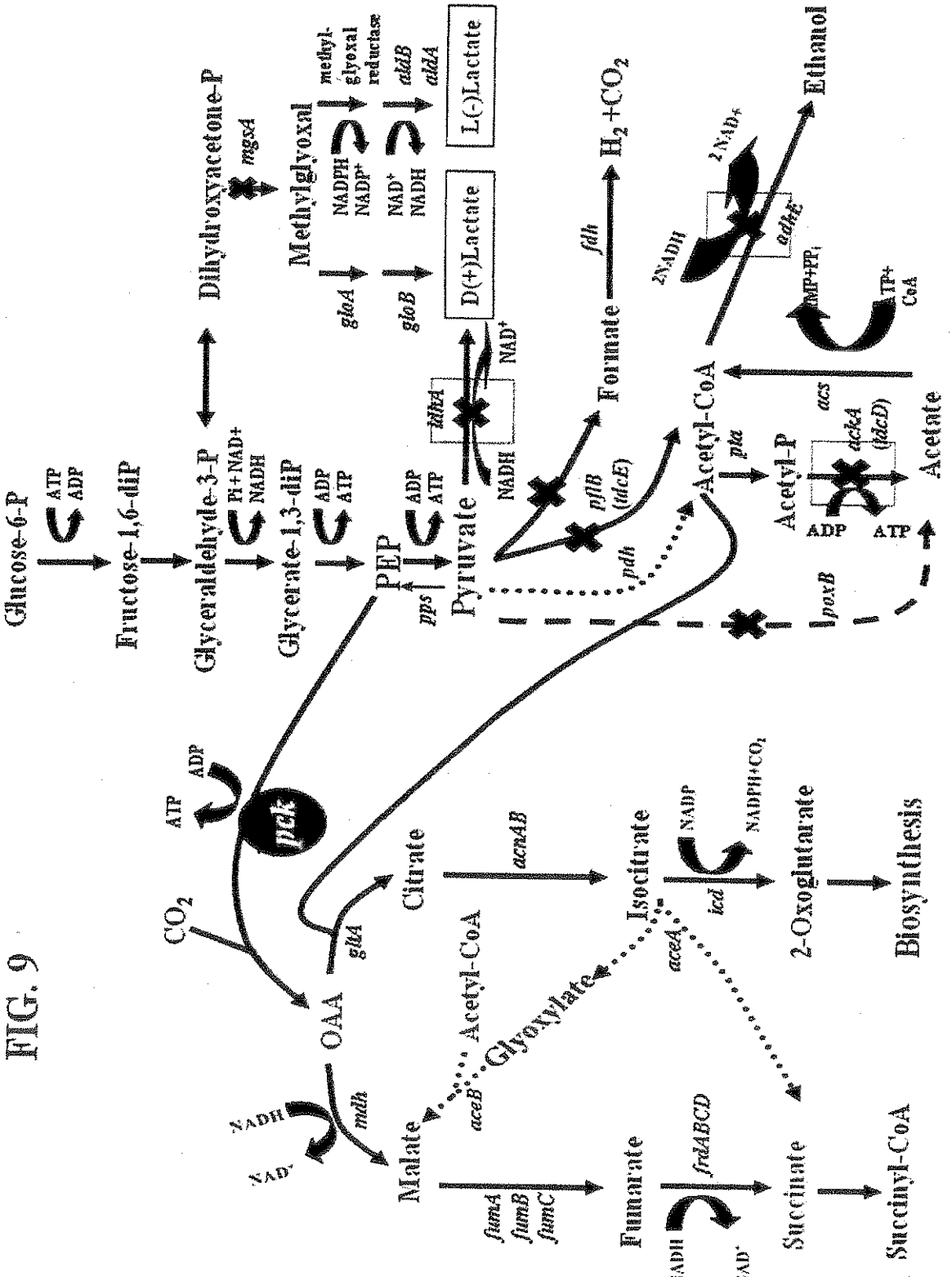

Construction of
pLOI4162
pEL04
5076 bps
caf
sacB
SmaI
XmaI
BamHI
PCR w/
JMpEL04F1/R1
primers, cut w/
BglII and religate to
remove unwanted
sites
BglII
pLOI4152
4774 bps
caf
sacB
PCR w/
JMcatsacBup3/do
wn3primers, cut w/
BamHI/XhoI
XmaI/SmaI
PacI
XhoI
pLOI4146
5463 bps
sacB
bla
caf
ColE1 ori
KasI/SfoI
PacI
BamHI
2 bp insertion for stop
codons in all reading
frames in artifact
region
Cut w/
BamHI/
XhoI
pLOI4153
2530 bps
bla
ColE1 ori
KasI/SfoI
PacI
BamHI
XhoI
PacI
XmaI/SmaI
pLOI4162
5278 bps
bla
sacB
caf
ColE1 ori
ZraI
EcoO109I
AatII
BstAPI
Bsu36I
HindIII
SacII
SnaBI
BsrGI
PsiI
BsgI
StuI
AccI
Bst1107I
BglII
TliI
NcoI
BstBI
AccIII
XmmI
PvuI
FspI
BglI
Cfr10I
BsaI
BmgI
AhdI
AlwNI
SapI
XmaI/SmaI
PacI
XhoI
Cut w/
KasI/XmaI
pLOI4131
3871 bps
bla
caf
ColE1 ori
FRT
EcoRI
FRT
EcoRI
Cut w/
EcoRI,
remove
FRT-caf-
FRT,
religate
pLOI4145
2686 bps
bla
ColE1 ori
KasI/SfoI
XmaI/SmaI
EcoRI
KasI/SfoIPacI BamHIXhoI PacIXmaI/SmaI
SfPBXPS polylinker (59 bps)

FIG. 9


Glucose-6-P
ATP
ADP
Fructose-1,6-diP
Glyceraldehyde-3-P
Pi+NAD+
NADH
Dihydroxyacetone-P
mgsA
Methylglyoxal
methyl-glyoxal reductase
NADPH
NADP+
aldB
aldA
NAD+
NADH
L(-)Lactate
gloA
gloB
Glycerate-1,3-diP
ADP
ATP
PEP
pps
Pyruvate
D(+)Lactate
ldhA
NAD+
NADH
Formate
fdh
H2+CO2
pflB
(ldcE)
pdh
Acetyl-CoA
pta
Acetyl-P
ADP
ATP
ackA
(ldcD)
acs
Acetate
poxB
2NADH
2NAD+
adhE
Ethanol
AMP+PPi
ATP+
CoA
CO2
ATP
ADP
ppc
OAA
gltA
Citrate
acnAB
Isocitrate
NADP
NADPH+CO2
icd
aceA
2-Oxoglutarate
Biosynthesis
NADH
NAD+
mdh
Malate
aceB
Glyoxylate
fumA
fumB
fumC
Fumarate
frdABCD
NADH
NAD+
Succinate
Succinyl-CoA

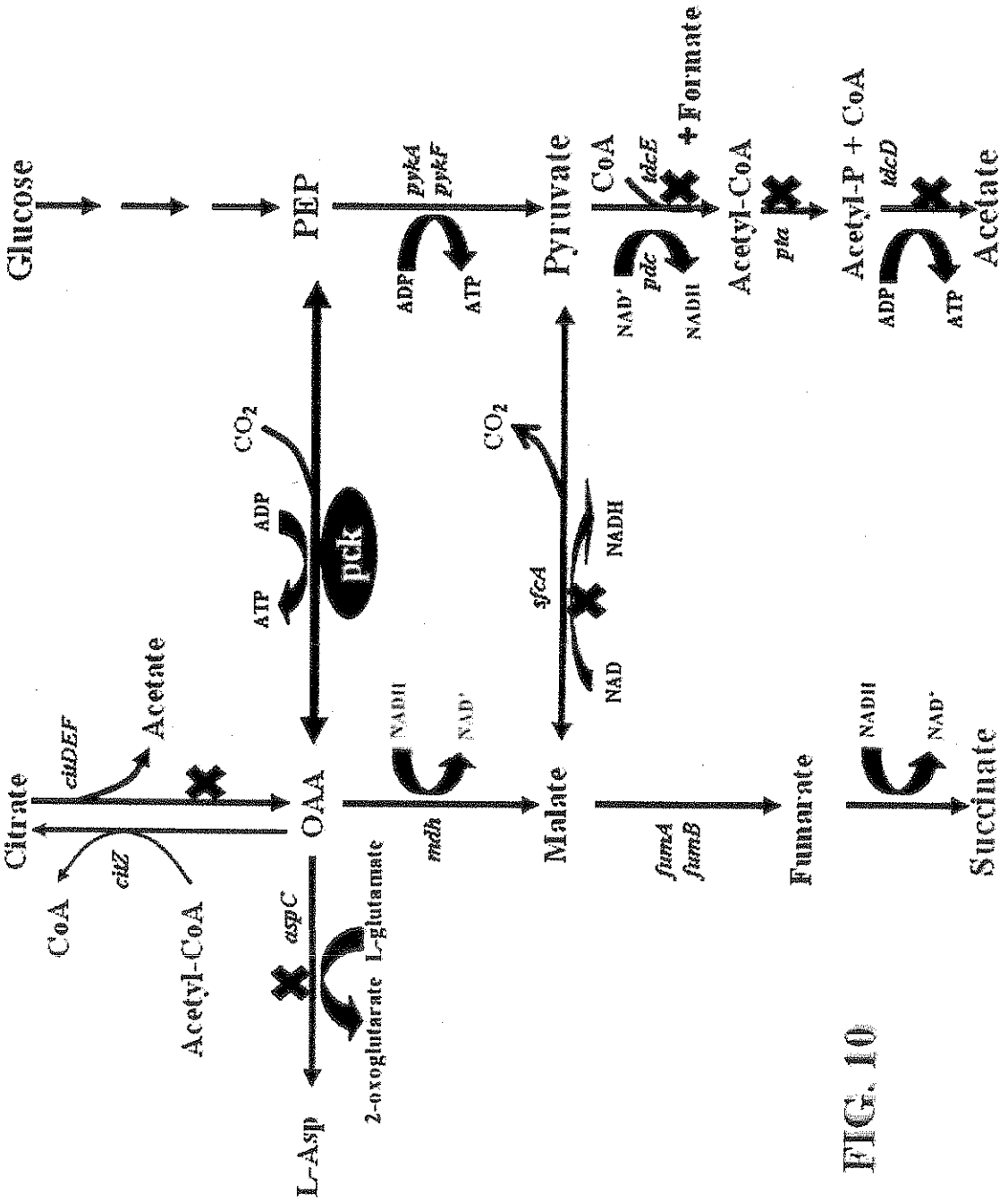

Glucose
PEP
pykA
pykF
ADP
ATP
Pyruvate
CoA
ldcE
+ Formate
Acetyl-CoA
pta
Acetyl-P + CoA
ldcD
ADP
ATP
Acetate
NAD'
pdc
NADH
CO2
ADP
ATP
pck
sfcA
NADH
NAD
Citrate
citDEF
Acetate
CoA
citZ
Acetyl-CoA
OAA
aspC
L-glutamate
2-oxoglutarate
L-Asp
NADH
NAD'
mdh
Malate
fumA
fumB
Fumarate
NADH
NAD'
Succinate

FIG. 10

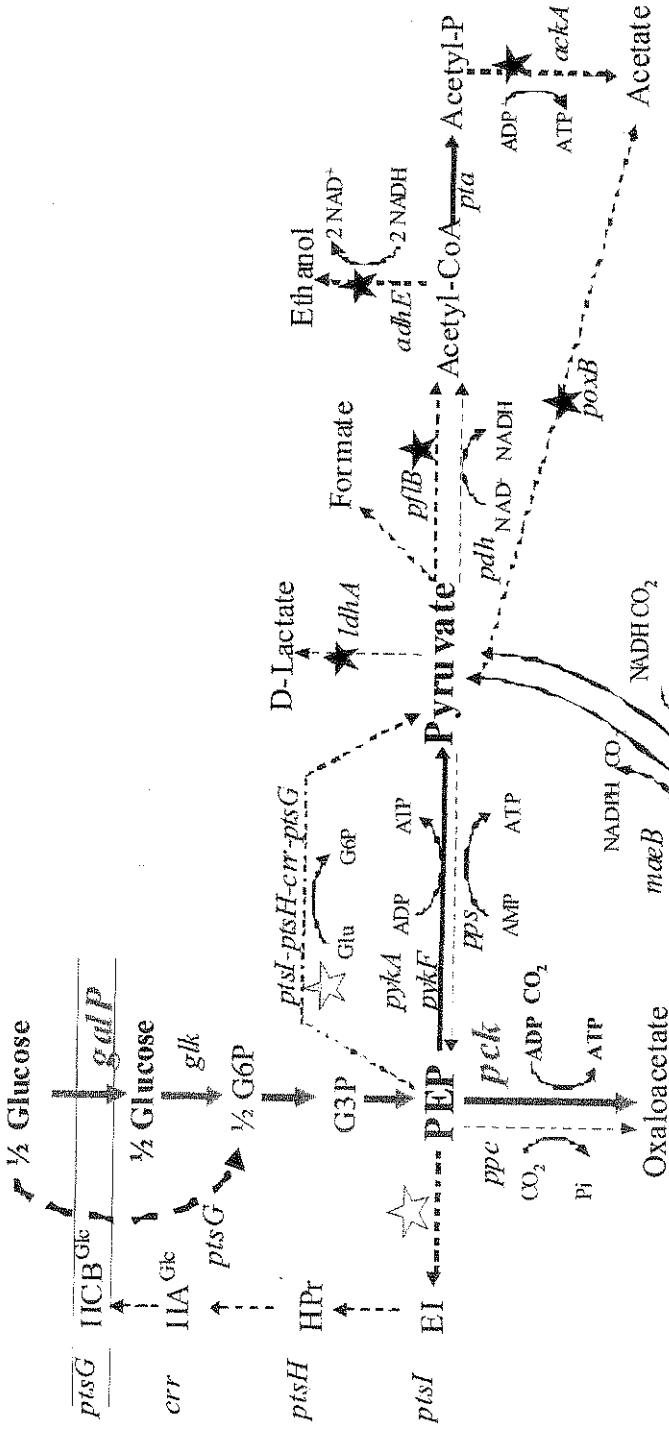

½ Glucose
ptsG
galP
½ Glucose
crr
IIA Glc
glk
ptsG
½ G6P
ptsH
HPr
ptsI-ptsH-crr-ptsG
G6P
Glu
G3P
ptsI
EI
PEP
pykA
pykF
ADP
ATP
Pyruvate
ppsA
AMP
ATP
ppc
CO2
Pi
pck
ADP CO2
ATP
Oxaloacetate
NADH
NAD+
mdh
Malate
fumABC
H2O
Fumarate
NADH
NAD+
frdABCD
Succinate
D-Lactate
ldhA
Formate
pflB
Ethanol
2 NAD+
2 NADH
adhE
Acetyl-CoA
pta
Acetyl-P
ADP
ATP
ackA
Acetate
poxB
pdh
NAD+
NADH
NADH
CO2
maeB
NADP+
maeA
NAD+

FIG. 11

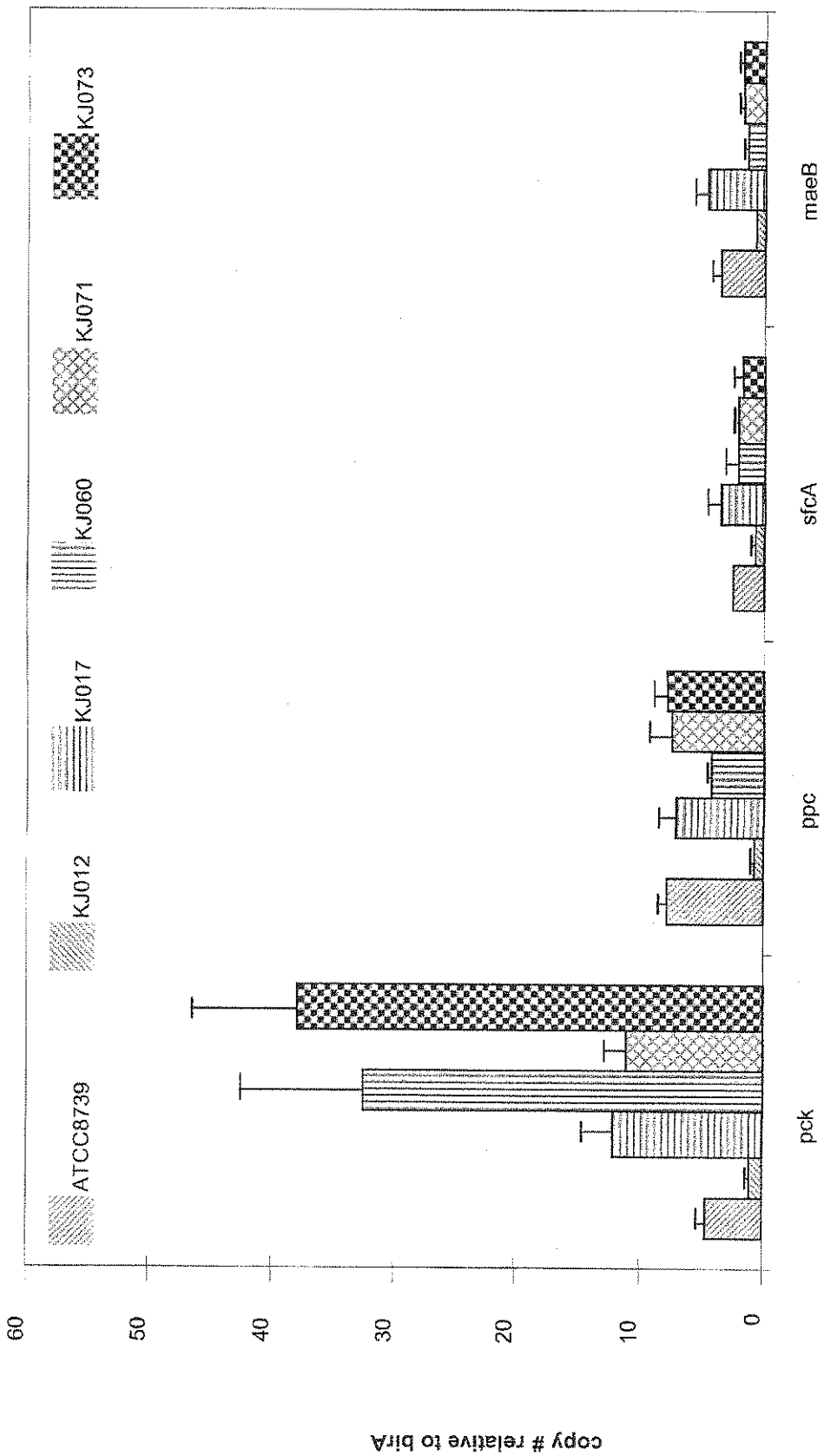

ATCC8739
KJ012
KJ017
KJ060
KJ071
KJ073
copy # relative to birA
0
10
20
30
40
50
60
pck
ppc
sfcA
maeB

FIG. 12A

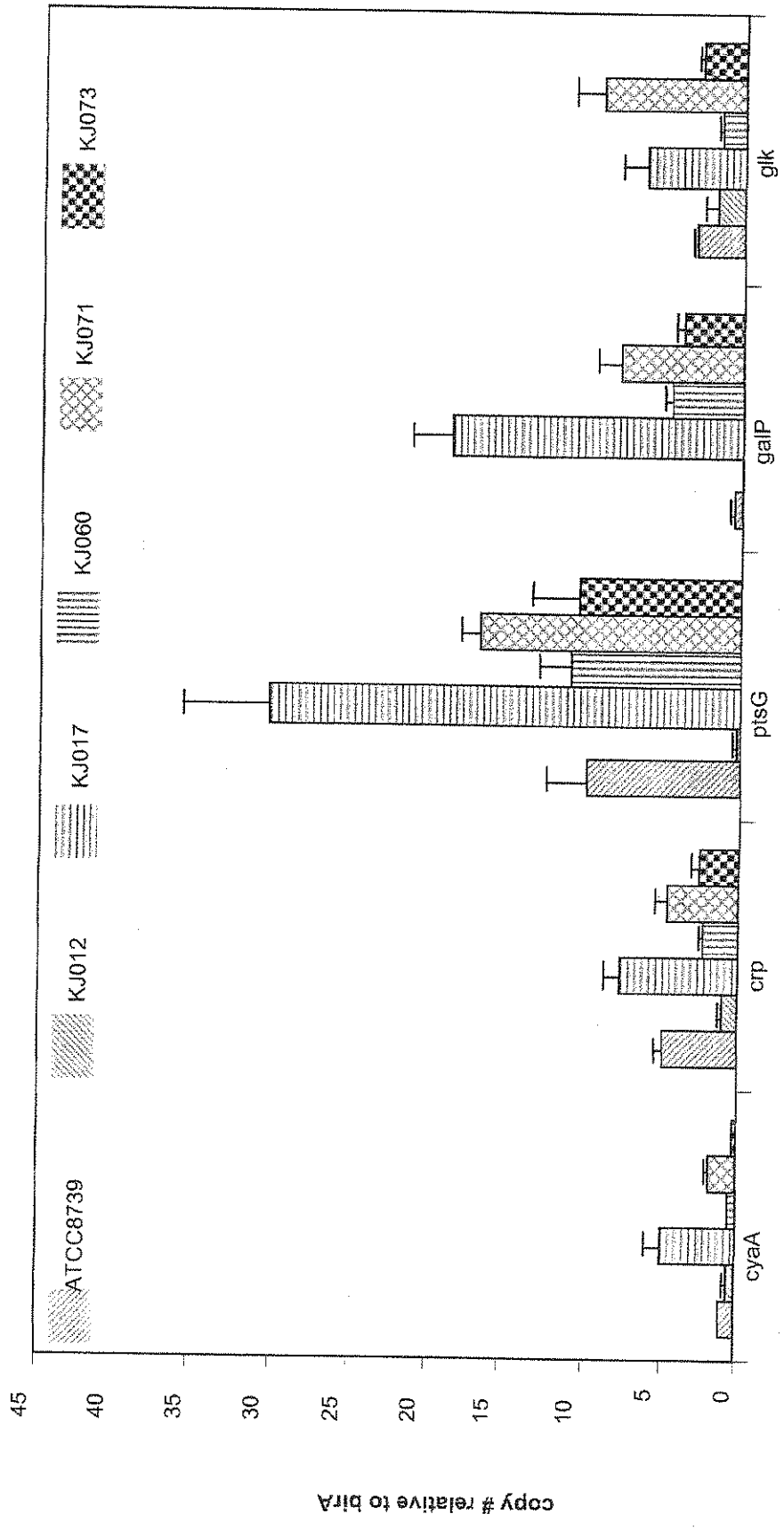

ATCC8739
KJ012
KJ017
KJ060
KJ071
KJ073
cyaA
crp
ptsG
galP
glk
copy # relative to birA
0
5
10
15
20
25
30
35
40
45

FIG. 12B

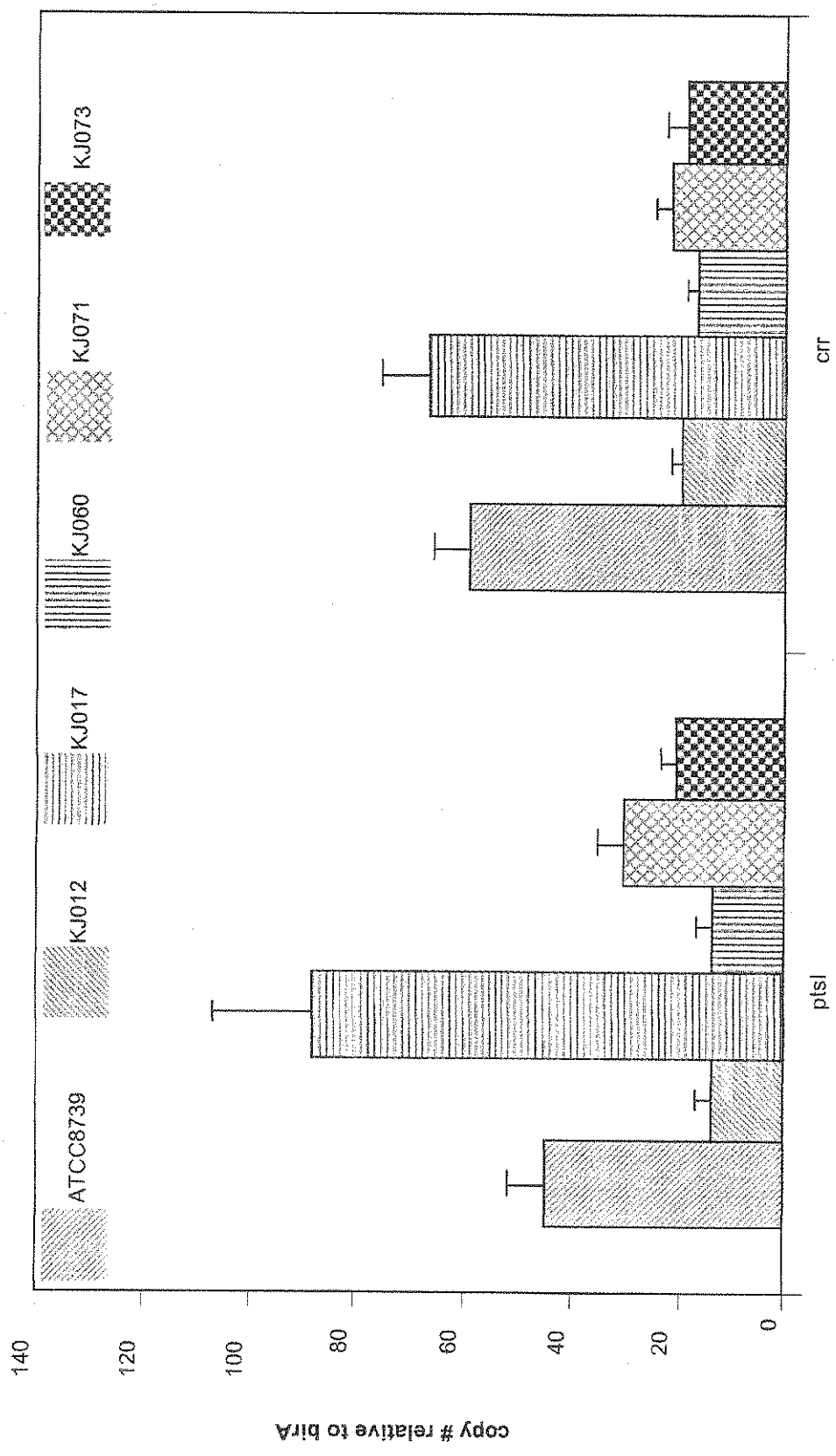

ATCC8739
KJ012
KJ017
KJ060
KJ071
KJ073
copy # relative to birA
0
20
40
60
80
100
120
140
ptsI
crr

FIG. 12C

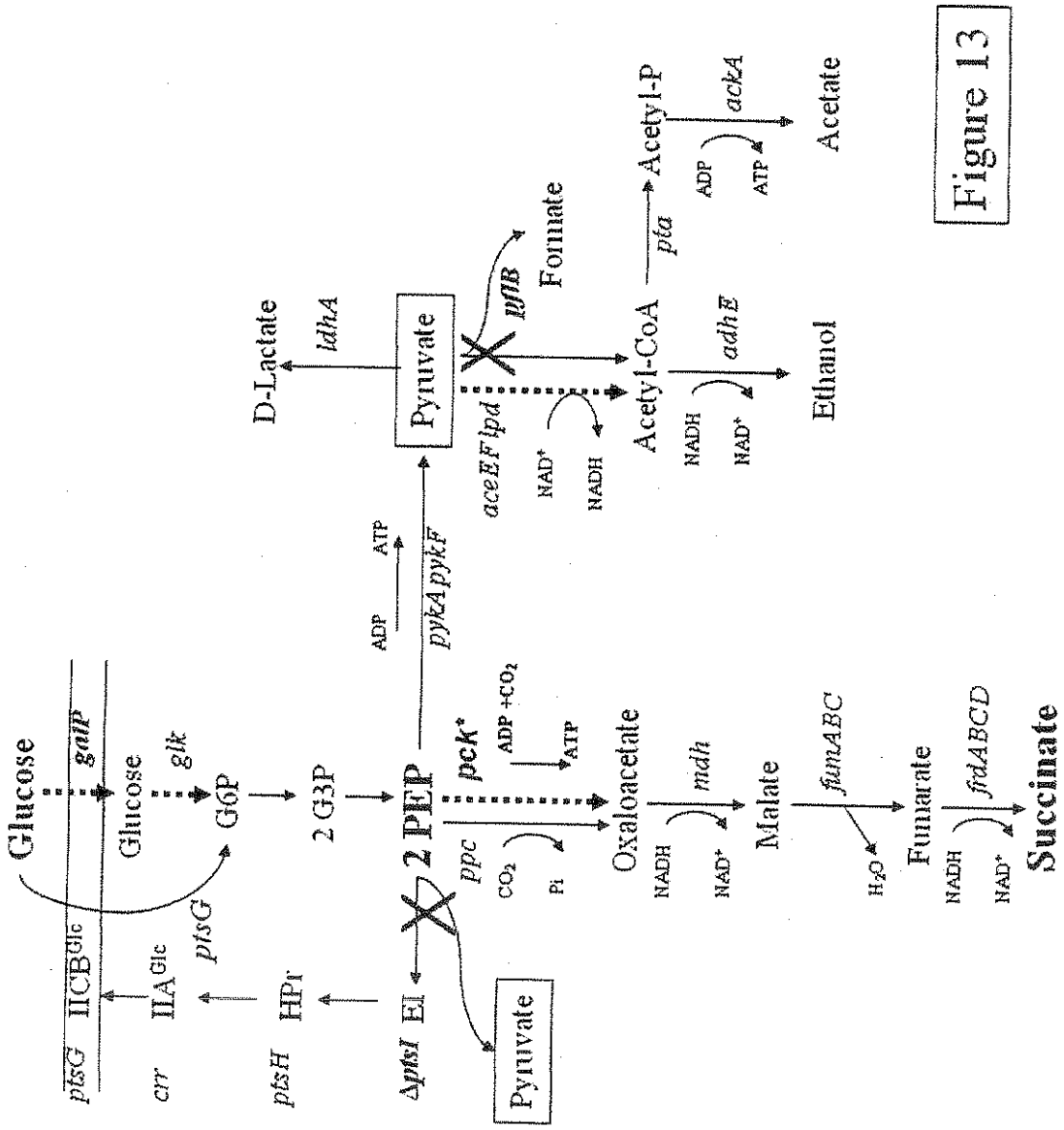

Glucose
ptsG
galP
Glucose
glk
G6P
ptsG
ptsH
HPr
crr
IIA Glc
IICB Glc
ΔptsI
EI
2 PEP
2 G3P
pykA pykF
ADP
ATP
pck*
ADP +CO2
ATP
ppc
CO2
Pi
Pyruvate
Pyruvate
ldhA
D-Lactate
aceEF lpd
NAD+
NADH
pflB
Formate
Acetyl-CoA
pta
Acetyl-P
ackA
ADP
ATP
Acetate
NADH
NAD+
adhE
Ethanol
Oxaloacetate
NADH
NAD+
mdh
Malate
fumABC
H2O
Fumarate
NADH
NAD+
frdABCD
Succinate

Figure 13

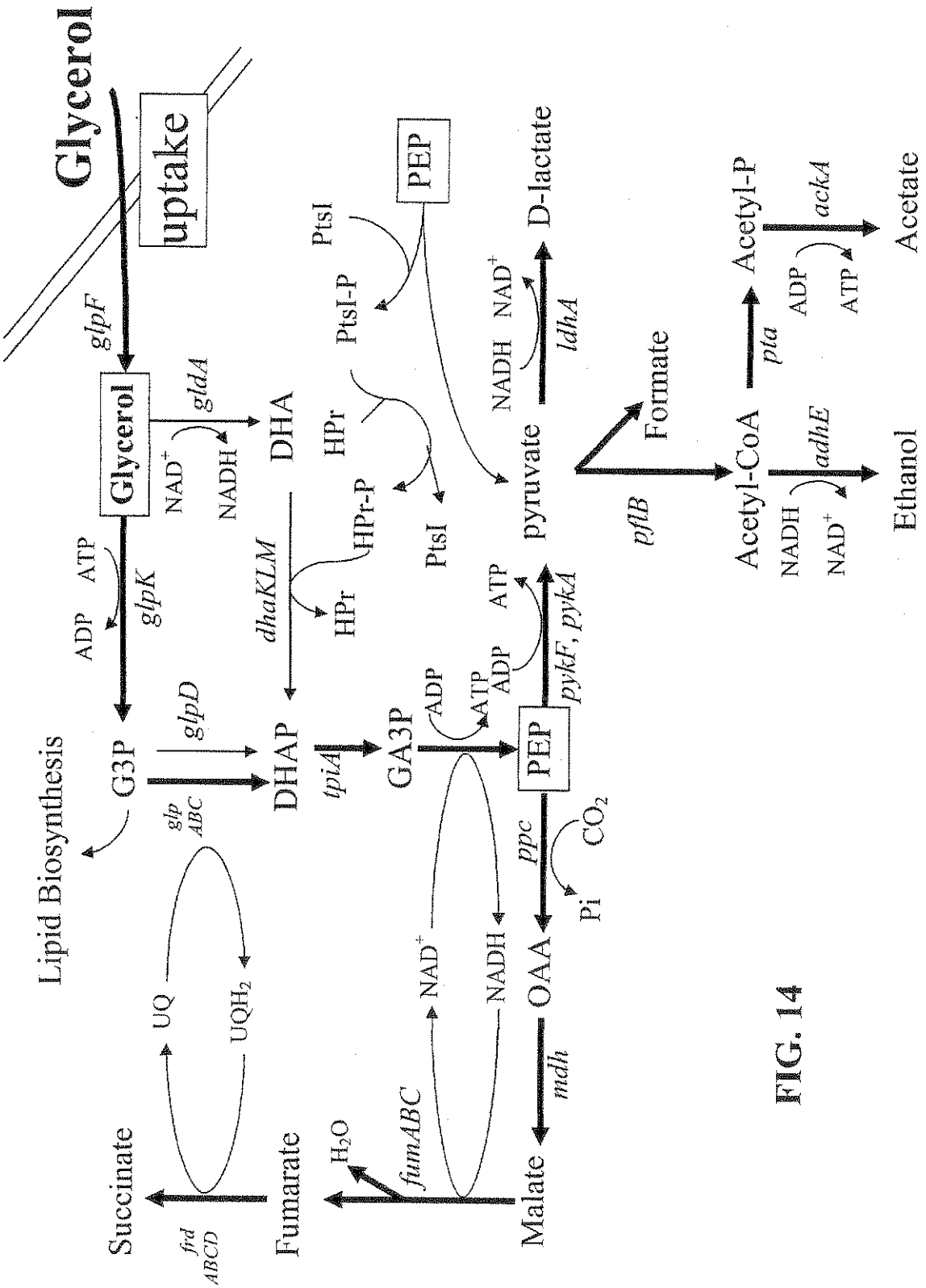

Glycerol
uptake
glpF
Glycerol
gldA
NAD+
NADH
DHA
ATP
ADP
glpK
dhaKLM
HPr
HPr-P
PtsI
PtsI-P
PEP
G3P
glpD
DHAP
tpiA
GA3P
ADP
ATP
ADP
ATP
pykF, pykA
pyruvate
NADH
NAD+
D-lactate
ldhA
Lipid Biosynthesis
glp ABC
UQ
UQH2
ppc
Pi
CO2
pflB
Formate
Acetyl-CoA
pta
ADP
ATP
ackA
Acetyl-P
Acetate
NADH
NAD+
adhE
Ethanol
NAD+
NADH
OAA
mdh
H2O
fumABC
Malate
Fumarate
frd ABCD
Succinate

FIG. 14

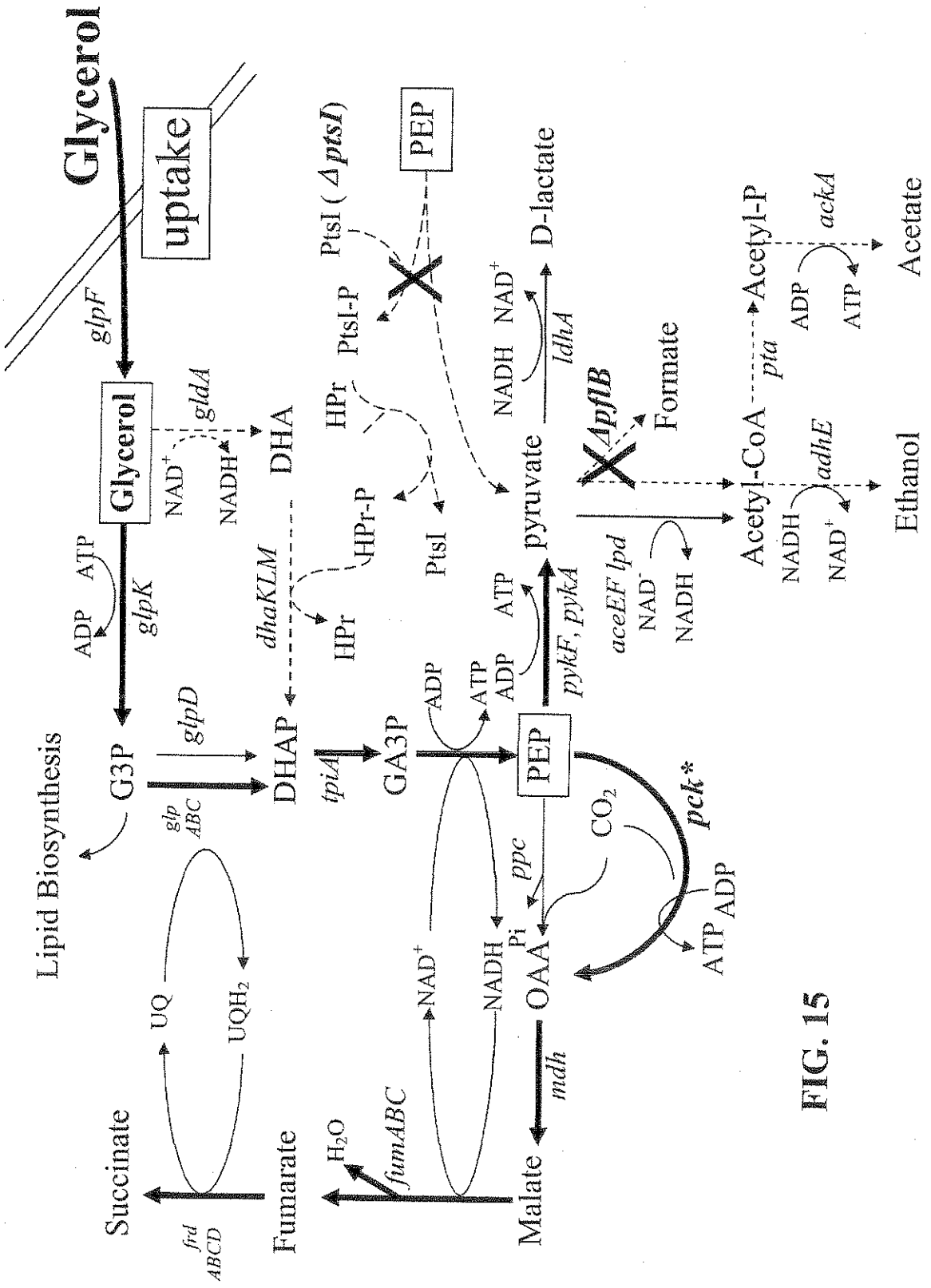

Glycerol
uptake
glpF
Glycerol
NAD+
gldA
NADH
DHA
ADP
ATP
glpK
dhaKLM
HPr
PtsI
PtsI-P
PEP
PtsI (ΔptsI)
HPr-P
PtsI
NADH
NAD+
D-lactate
ldhA
pyruvate
ΔpflB
Formate
Acetyl-CoA
pta
Acetyl-P
ackA
ADP
ATP
Acetate
NADH
NAD+
adhE
Ethanol
aceEF lpd
NAD+
NADH
Lipid Biosynthesis
G3P
glpD
DHAP
tpiA
GA3P
ADP
ATP
ADP
ATP
PEP
pykF, pykA
glp ABC
ppc
CO2
pck*
Pi
NAD+
NADH
OAA
ATP
ADP
mdh
Malate
fumABC
H2O
Fumarate
frd ABCD
Succinate
UQ
UQH2

FIG. 15