Fed-batch fermentation at various constant rate feed without induction

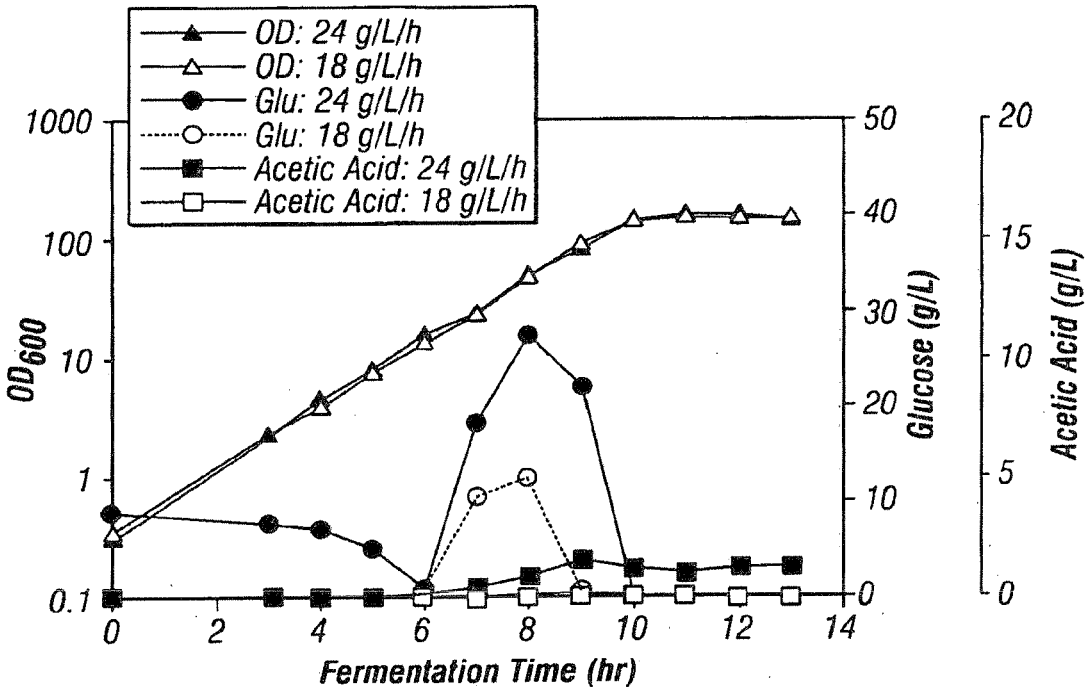


FIG. 1

Fed-batch fermentation at various constant rate feed without induction

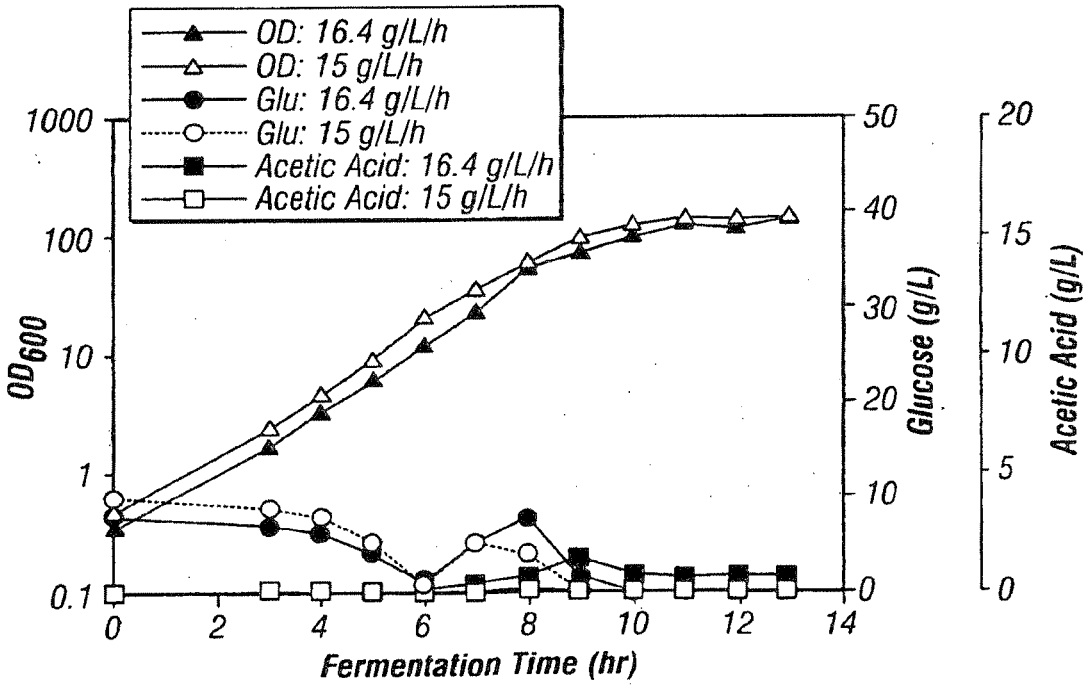


FIG. 2

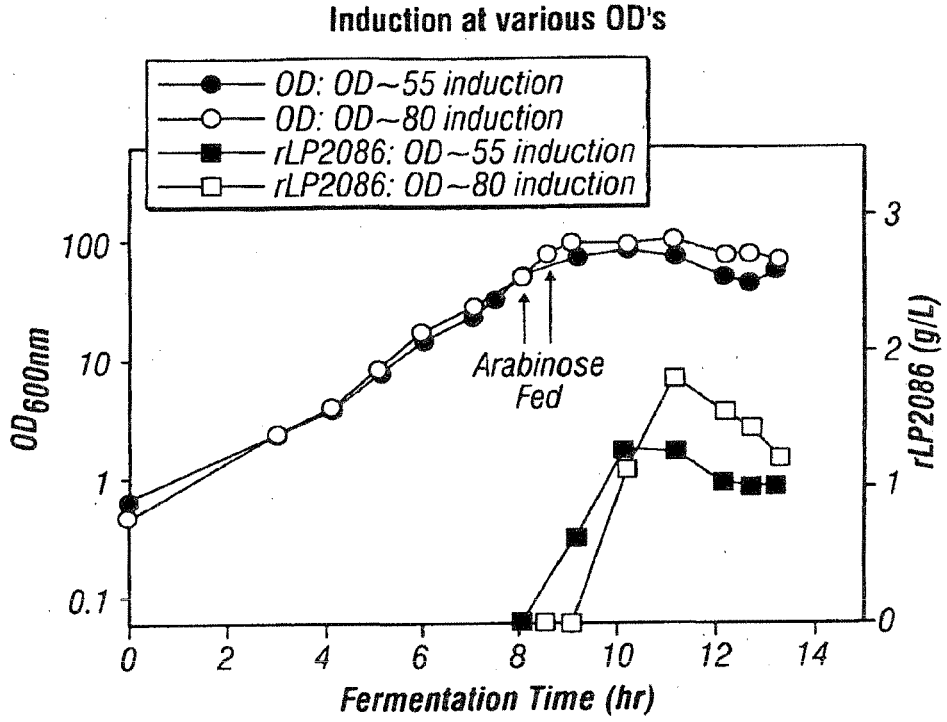

Induction at various OD's
OD: OD~55 induction
OD: OD~80 induction
rLP2086: OD~55 induction
rLP2086: OD~80 induction
Arabinose Fed
OD600nm
rLP2086 (g/L)
Fermentation Time (hr)

FIG. 3

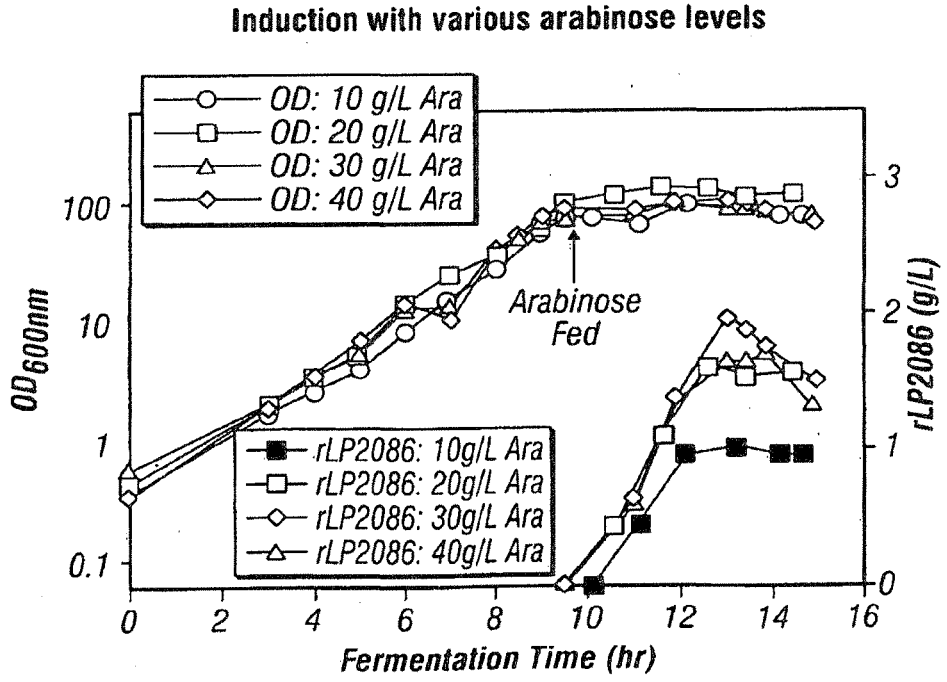

Induction with various arabinose levels
OD: 10 g/L Ara
OD: 20 g/L Ara
OD: 30 g/L Ara
OD: 40 g/L Ara
Arabinose Fed
rLP2086: 10g/L Ara
rLP2086: 20g/L Ara
rLP2086: 30g/L Ara
rLP2086: 40g/L Ara
OD600nm
rLP2086 (g/L)
Fermentation Time (hr)

FIG. 4

Effect of arabinose addition method on rLP2086 yield

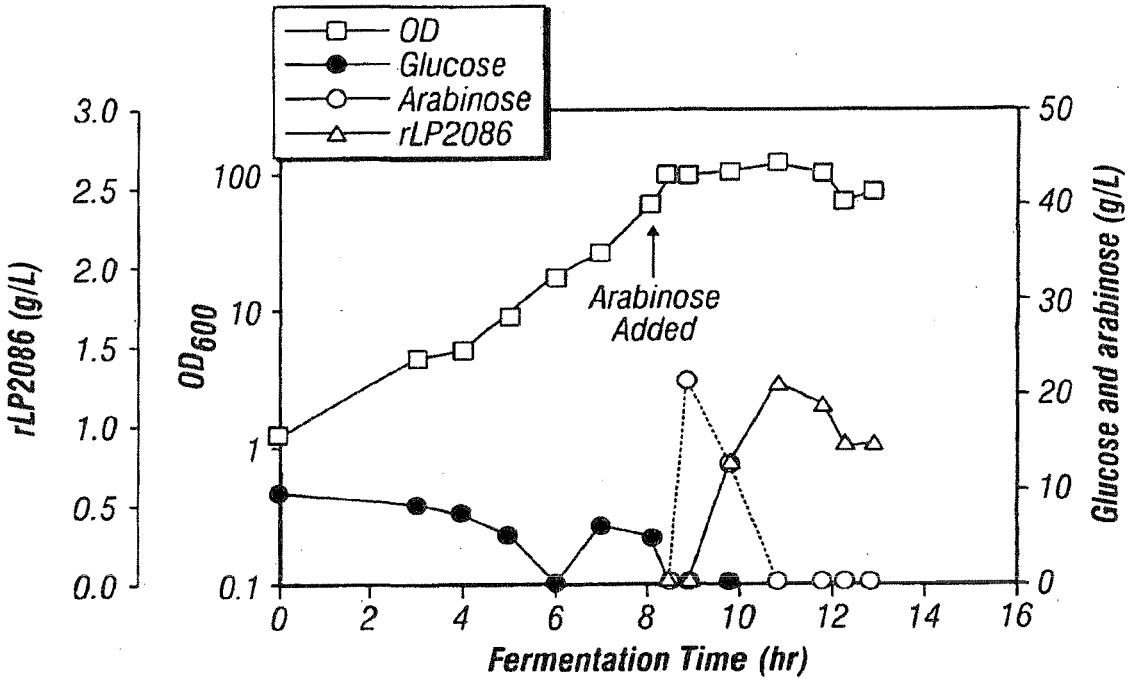


FIG. 5

Effect of arabinose feed rate on rLP2086 production

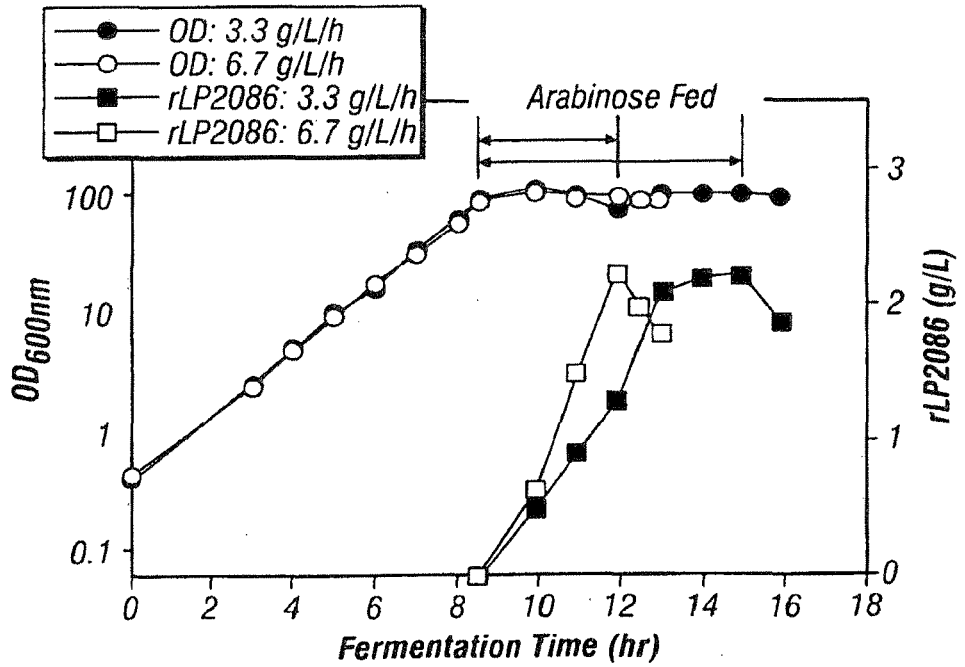


FIG. 6

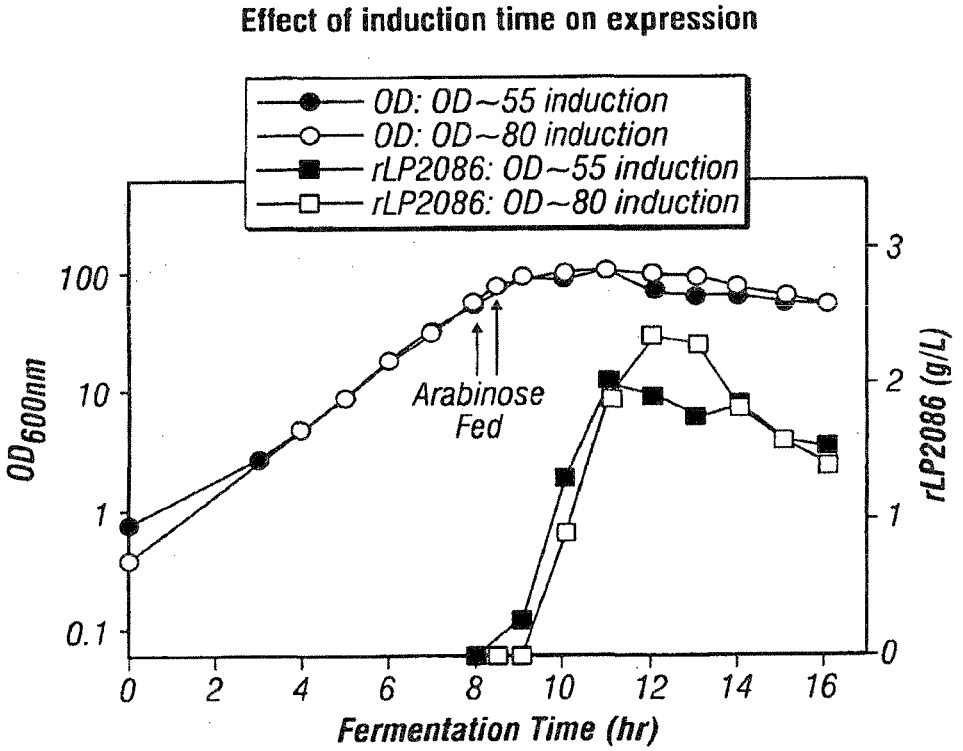

Effect of induction time on expression
OD: OD~55 induction
OD: OD~80 induction
rLP2086: OD~55 induction
rLP2086: OD~80 induction
100
10
1
0.1
OD600nm
3
2
1
0
rLP2086 (g/L)
Arabinose Fed
0
2
4
6
8
10
12
14
16
Fermentation Time (hr)

FIG. 7

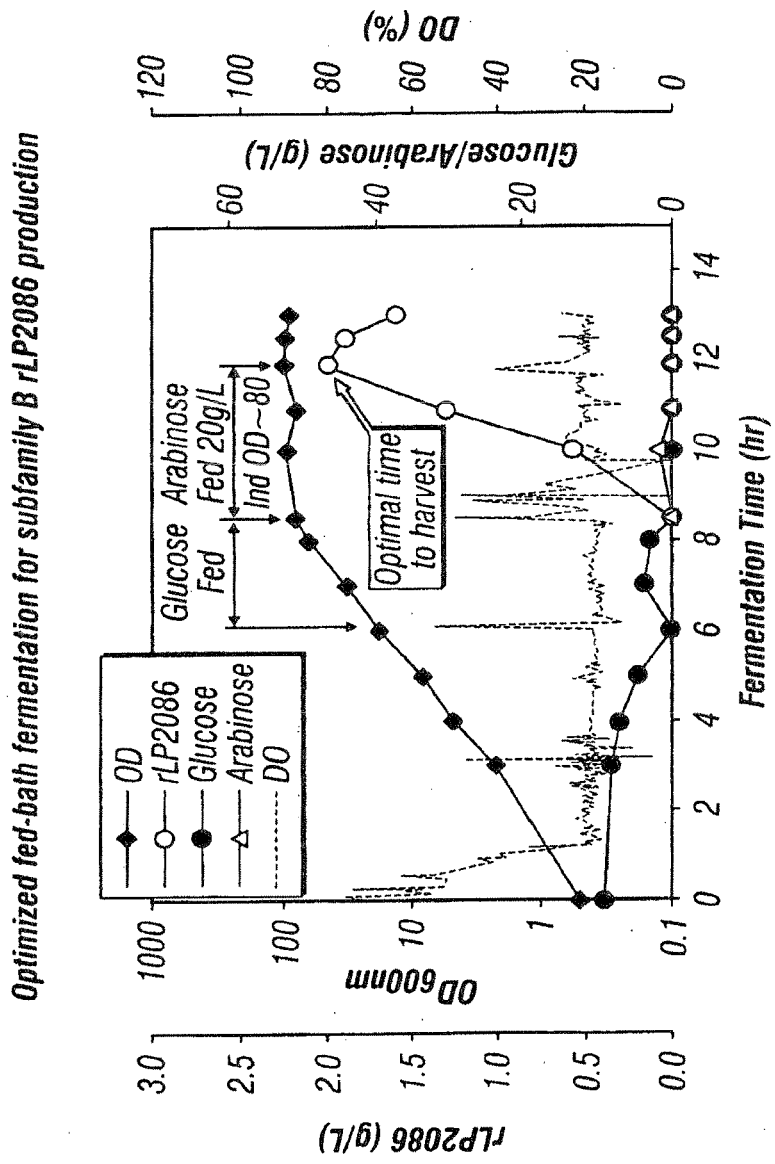

Optimized fed-bath fermentation for subfamily B rLP2086 production
rLP2086 (g/L)
OD600nm
Glucose/Arabinose (g/L)
DO (%)
Fermentation Time (hr)
OD
rLP2086
Glucose
Arabinose
DO
Glucose Fed
Arabinose Fed 20g/L
Ind OD~80
Optimal time to harvest

FIG. 8

SDS-PAGE and Western Blot of rLP2086 subfamily B induction

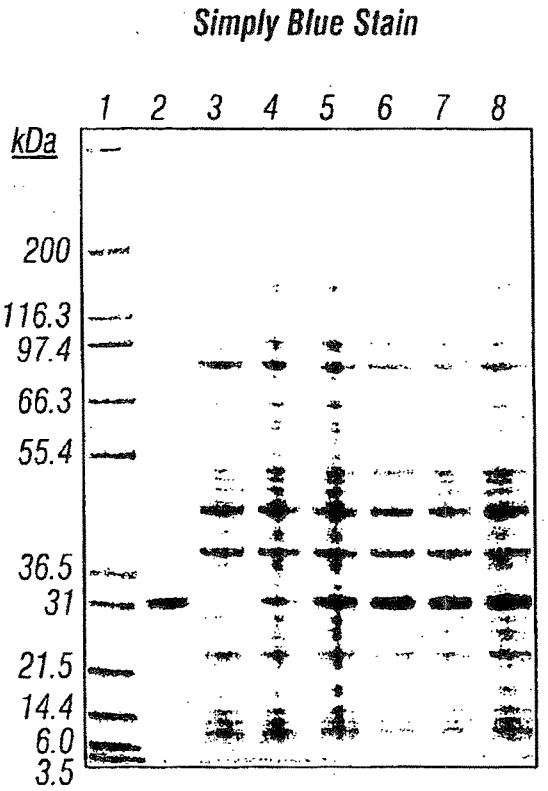


FIG. 9A

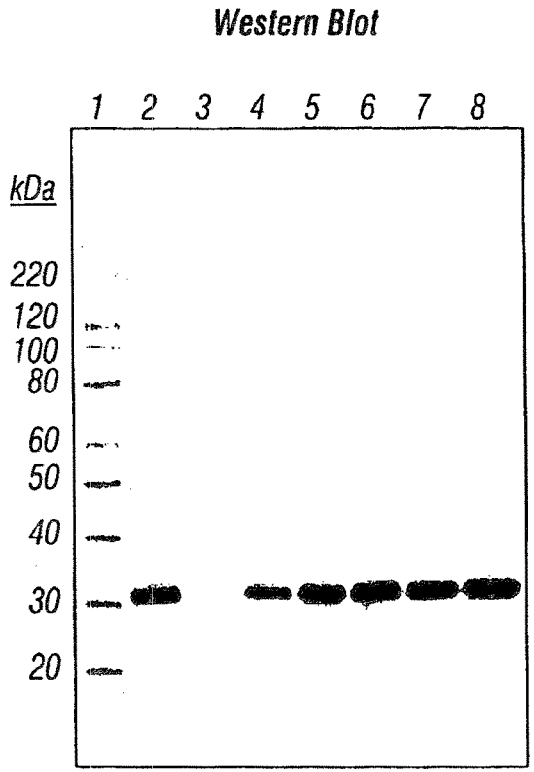


FIG. 9B

Lane 1. MWt Standard; Lane 2. rLP2086; Lane 3. Pre-induction; Lane 4. Induction 1.1 hr; Lane 5. Induction 2.0 hr; Lane 6. Induction 3.0 hr; Lane 7. Induction 3.6 hr; Lane 8. Induction 4.0 hr

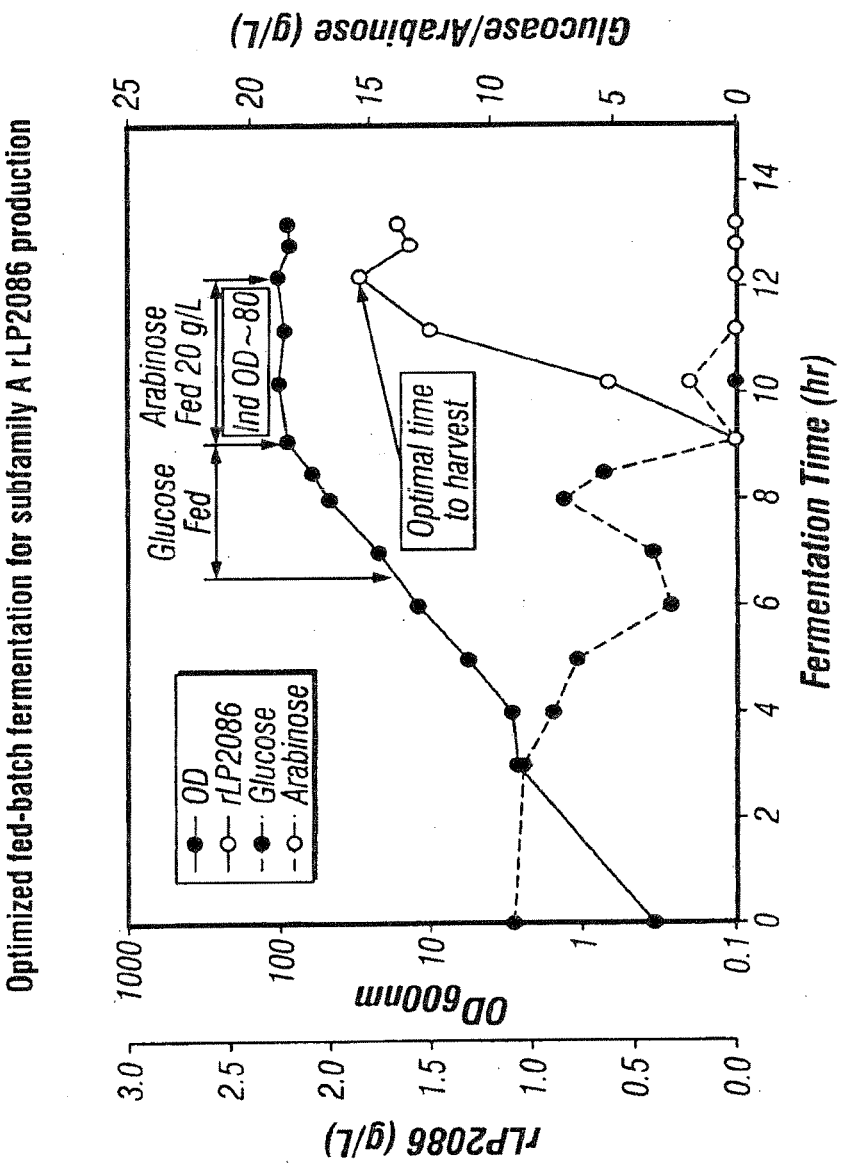

Optimized fed-batch fermentation for subfamily A rLP2086 production
rLP2086 (g/L)
OD600nm
Glucose/Arabinose (g/L)
Fermentation Time (hr)
OD
rLP2086
Glucose
Arabinose
Glucose Fed
Arabinose Fed 20 g/L
Ind OD~80
Optimal time to harvest

FIG. 10

SDS-PAGE and Western Blot of rLP2086 subfamily A induction

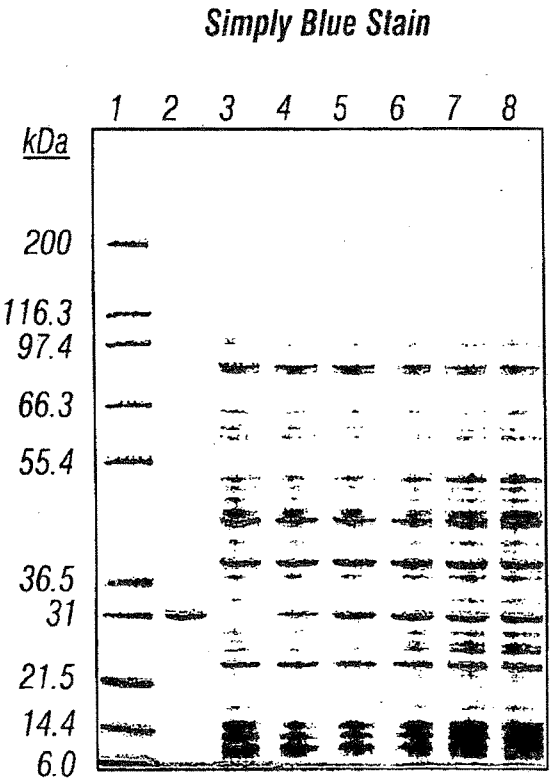


FIG. 11A

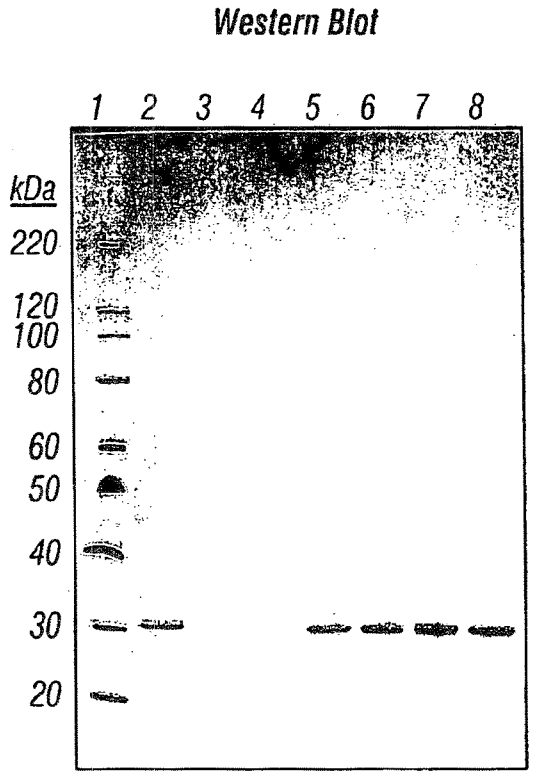
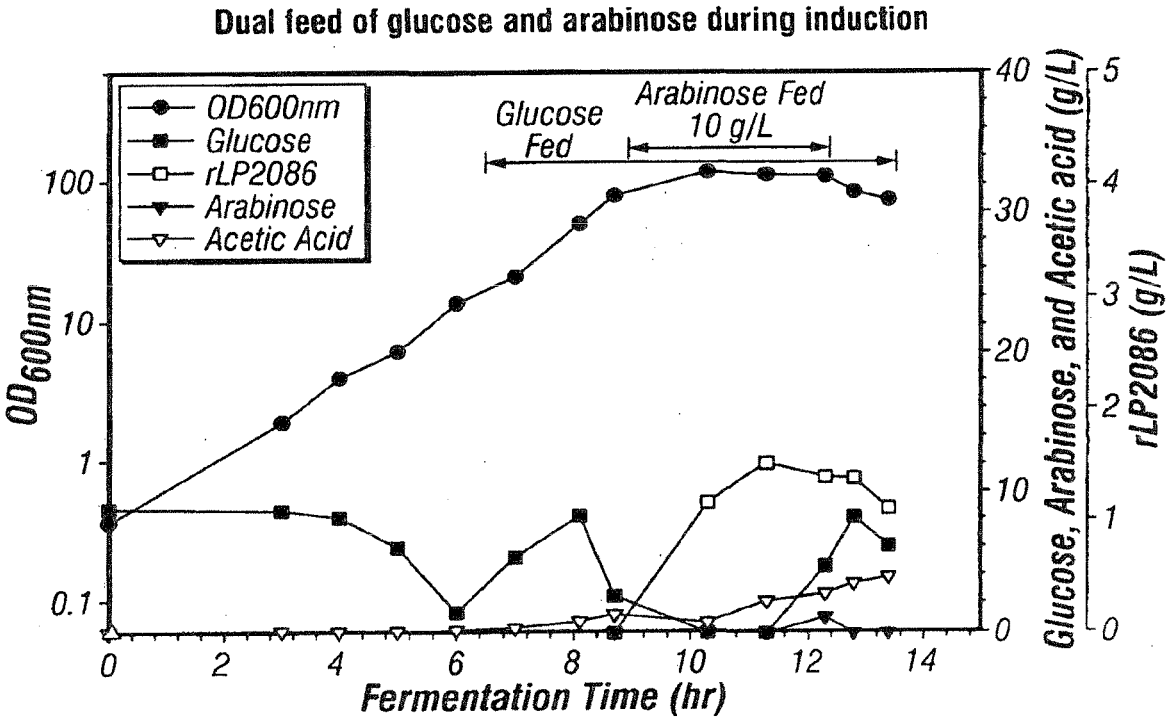


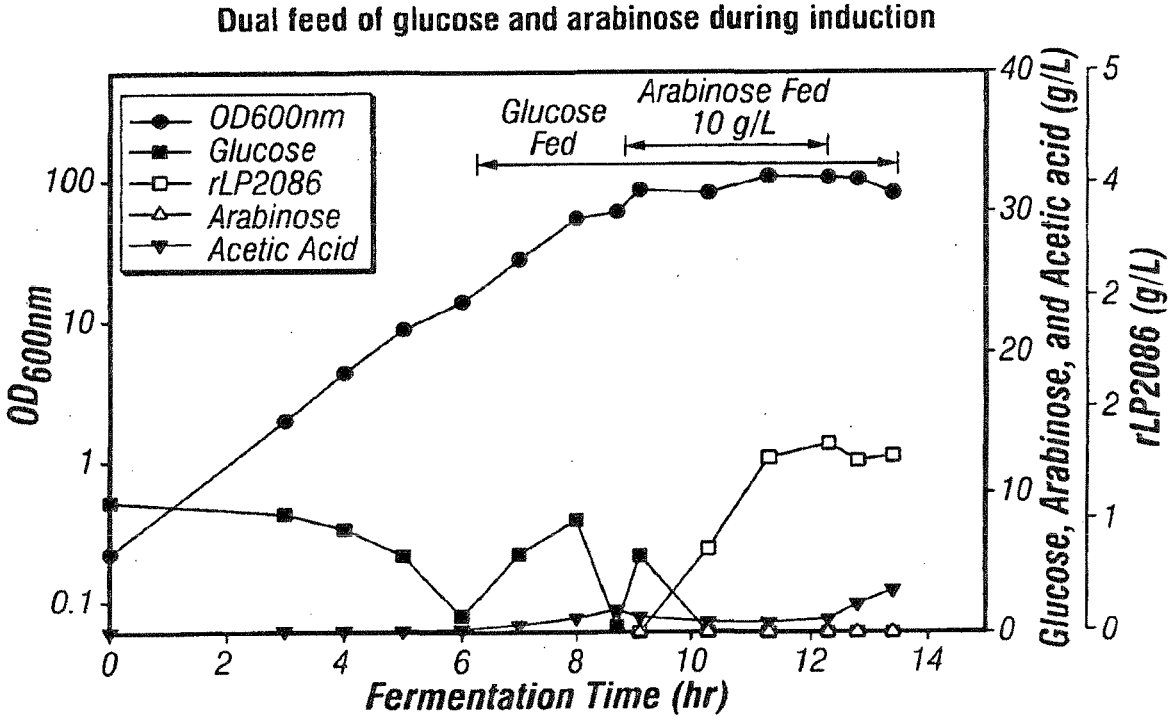
FIG. 11B

Lane 1. MWt Standard; Lane 2. rLP2086 (A); Lane 3. Pre-induction; Lane 4. Induction 1.1 hr; Lane 5. Induction 2.0 hr; Lane 6. Induction 3.1 hr; Lane 7. Induction 3.6 hr; Lane 8. Induction 4.0 hr



Dual feed: Glucose feed 15 g/L/h; Arabinose feed 3.3 g/L/h

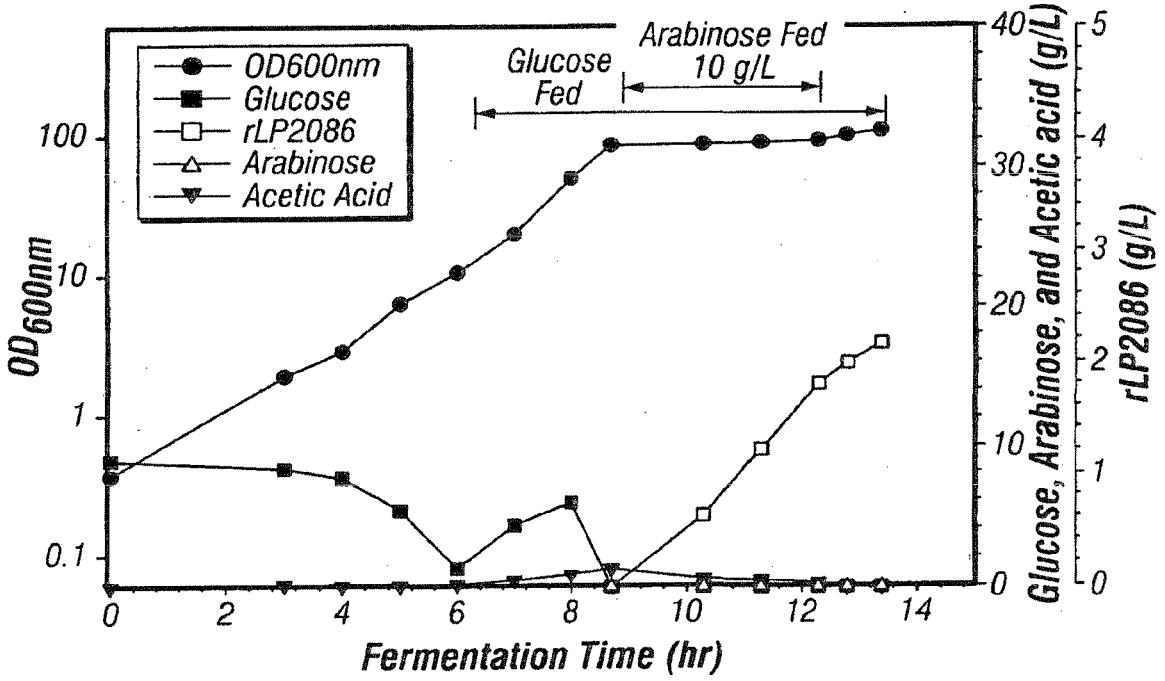
FIG. 12A



Dual feed: Glucose feed 15 g/L/h; Arabinose feed 3.3 g/L/h

FIG. 12B

Dual feed of glucose and arabinose during induction



Dual feed: glucose feed 3.75 g/L/h; Arabinose feed 3.3 g/L/h

FIG. 12C

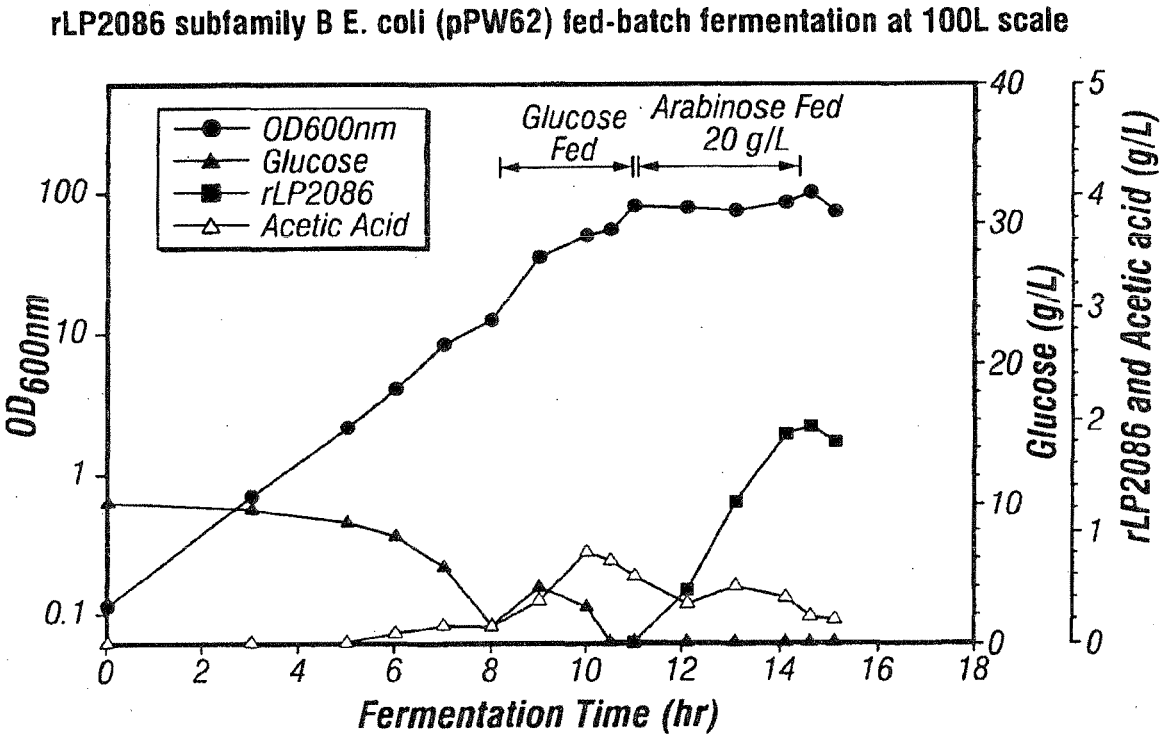

rLP2086 subfamily B E. coli (pPW62) fed-batch fermentation at 100L scale
OD600nm
Glucose
rLP2086
Acetic Acid
Glucose Fed
Arabinose Fed 20 g/L
OD600nm
Glucose (g/L)
rLP2086 and Acetic acid (g/L)
Fermentation Time (hr)

FIG. 13A

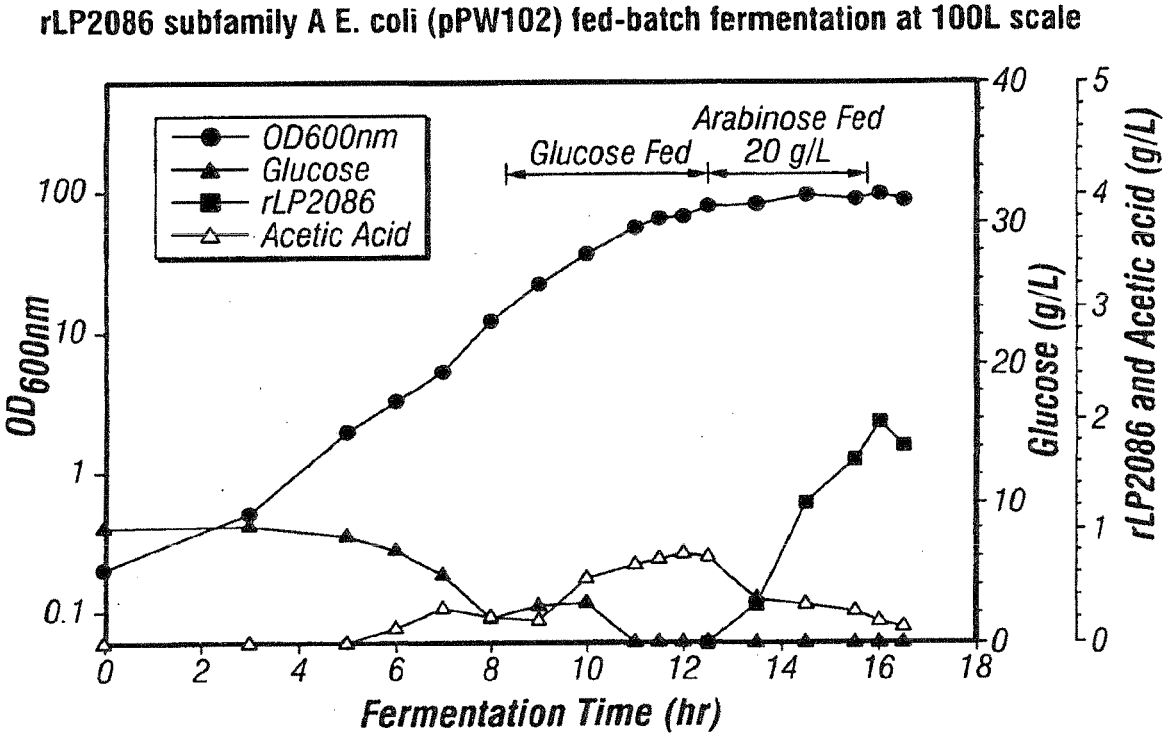

rLP2086 subfamily A E. coli (pPW102) fed-batch fermentation at 100L scale
OD600nm
Glucose
rLP2086
Acetic Acid
Glucose Fed
Arabinose Fed
20 g/L
OD600nm
Glucose (g/L)
rLP2086 and Acetic acid (g/L)
Fermentation Time (hr)
100
10
1
0.1
40
30
20
10
0
5
4
3
2
1
0
0
2
4
6
8
10
12
14
16
18

FIG. 13B

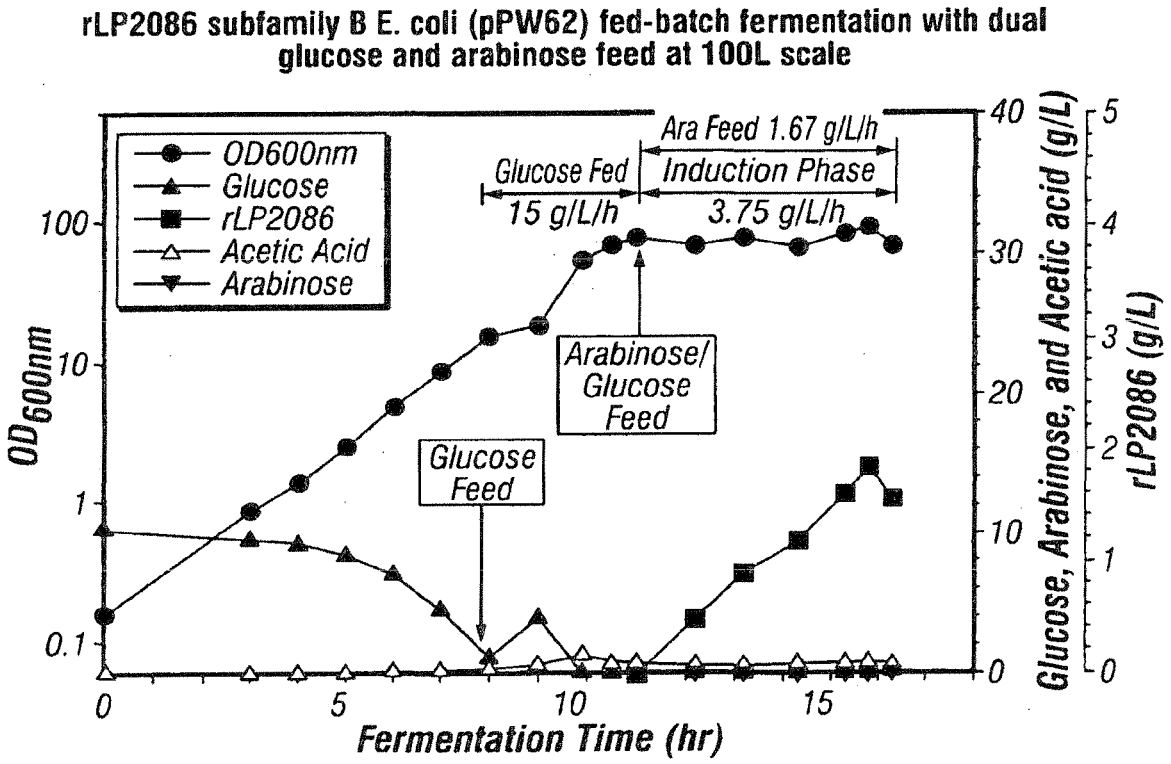

rLP2086 subfamily B E. coli (pPW62) fed-batch fermentation with dual glucose and arabinose feed at 100L scale
OD600nm
Glucose
rLP2086
Acetic Acid
Arabinose
Ara Feed 1.67 g/L/h
Glucose Fed
Induction Phase
15 g/L/h
3.75 g/L/h
Arabinose/ Glucose Feed
Glucose Feed
OD600nm
Glucose, Arabinose, and Acetic acid (g/L)
rLP2086 (g/L)
Fermentation Time (hr)

FIG. 14A

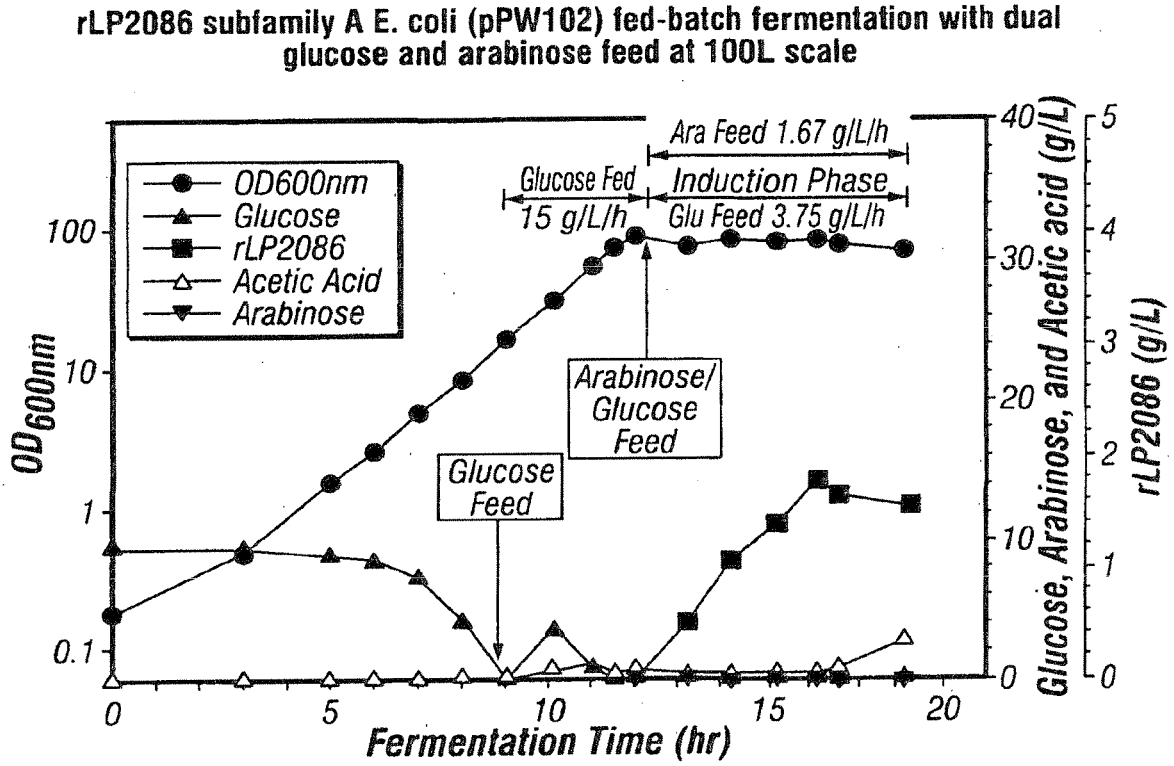

rLP2086 subfamily A E. coli (pPW102) fed-batch fermentation with dual glucose and arabinose feed at 100L scale
OD600nm
Glucose
rLP2086
Acetic Acid
Arabinose
Ara Feed 1.67 g/L/h
Glucose Fed 15 g/L/h
Induction Phase Glu Feed 3.75 g/L/h
Arabinose/ Glucose Feed
Glucose Feed
OD600nm
Glucose, Arabinose, and Acetic acid (g/L)
rLP2086 (g/L)
Fermentation Time (hr)
100
10
1
0.1
0
5
10
15
20
40
30
20
10
0
5
4
3
2
1
0

FIG. 14B