FIGURE 1

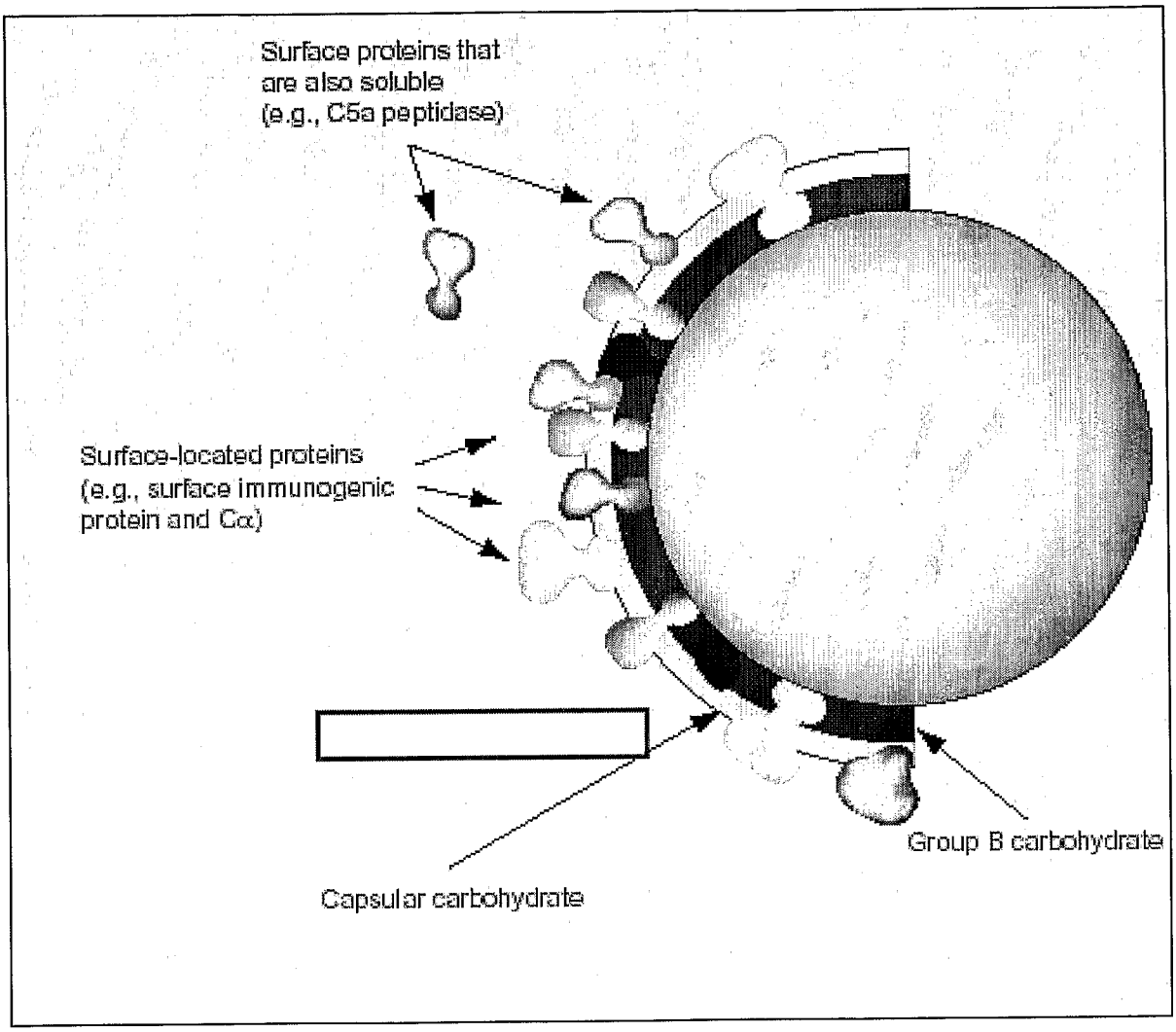

Surface proteins that
are also soluble
(e.g., C5a peptidase)
Surface-located proteins
(e.g., surface immunogenic
protein and Cα)
Group B carbohydrate
Capsular carbohydrate

FIGURE 2

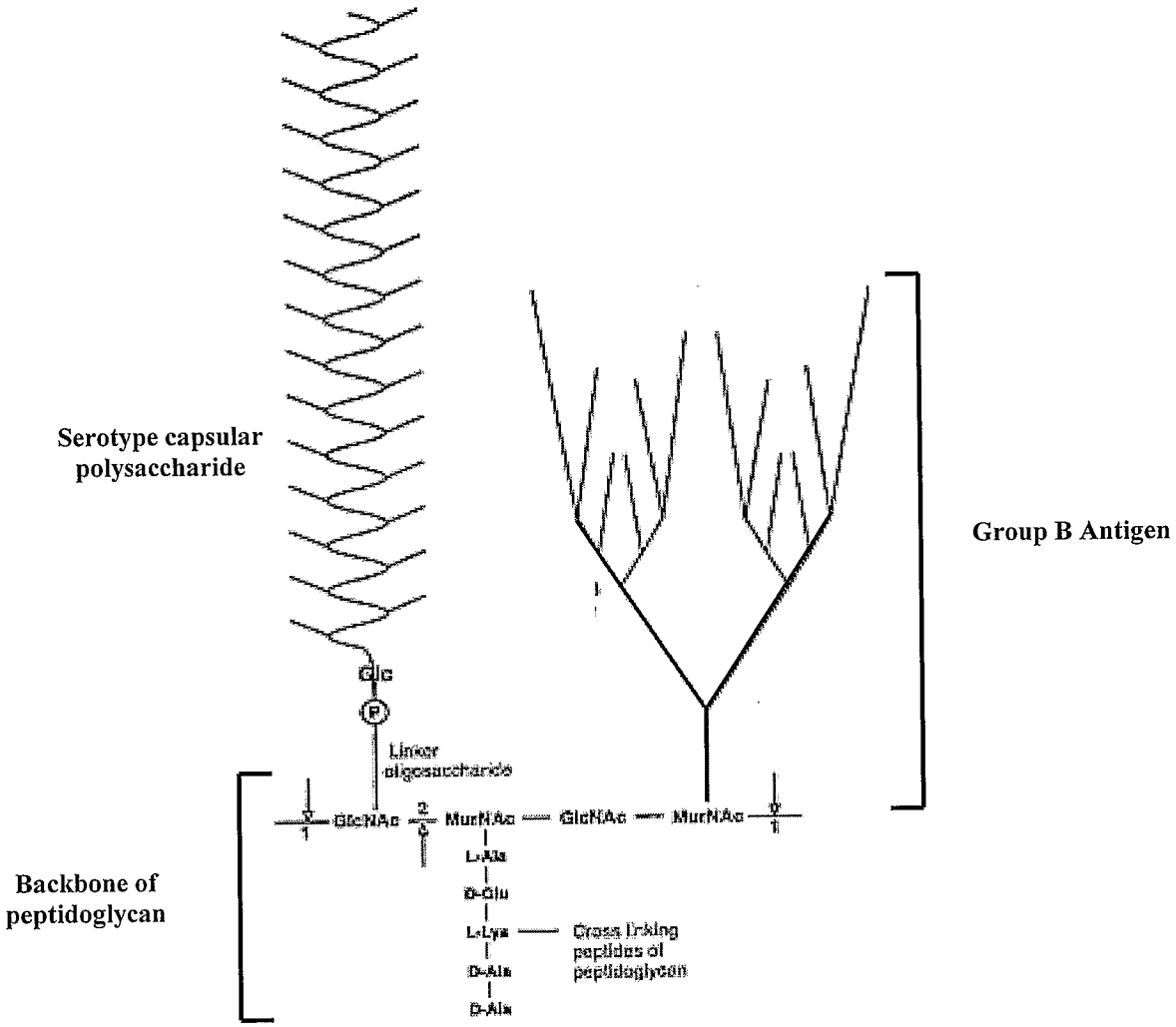
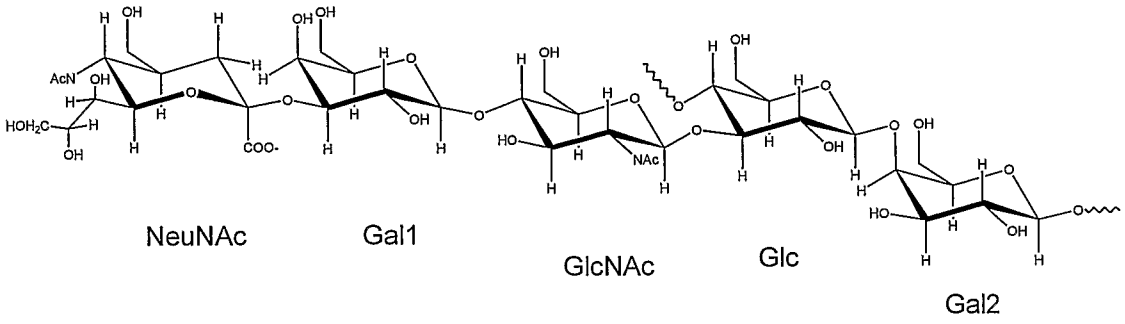

Serotype capsular polysaccharide
Group B Antigen
Backbone of peptidoglycan
Glc
P
Linker oligosaccharide
GlcNAc
MurNAc
GlcNAc
MurNAc
L-Ala
D-Glu
L-Lys
D-Ala
D-Ala
Cross linking peptides of peptidoglycan

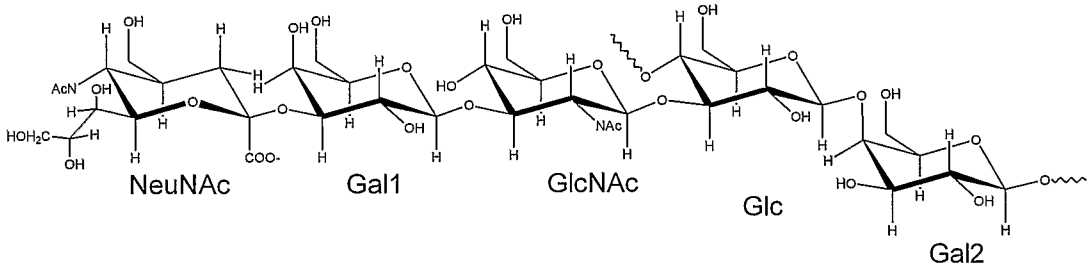
FIGURE 3A

Type Ia (090)



→4)-β-D-Glcp-(1→4)-β-D-Galp-(1→
3
↑
1
β-D-GlcpNAc
4
↑
1
β-D-Galp
3
↑
2
α-D-NeupNAc

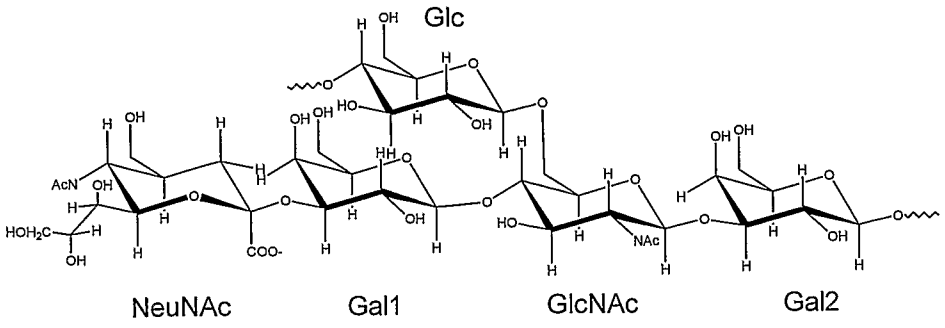
Type Ib (H36b)



→4)-β-D-Glcp-(1→4)-β-D-Galp-(1→
3
↑
1
β-D-GlcpNAc
3
↑
1
β-D-Galp
3
↑
2
α-D-NeupNAc

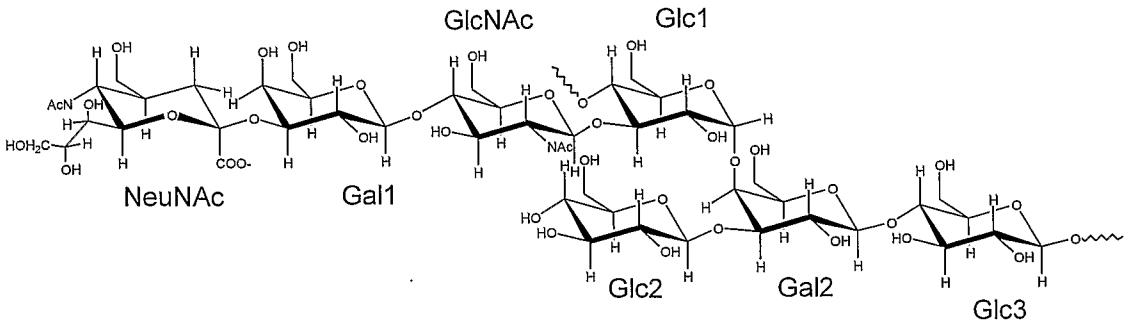
FIGURE 3B

Type III (M781)



→4)-β-D-Glcp-(1→6)-β-D-GlcNpAc-(1→3)-β-D-Galp-(1→
3
↑
1
β-D-Galp
3
↑
2
α-D-NeupNAc

Type V (CJB111)



→4)-α-D-Glcp-(1→4)-β-D-Galp-(1→4)-β-D-Glcp-(1→
6 3
↑ ↑
1 1
β-D-GlcpNAc β-D-Glcp
4
↑
1
β-D-Galp
3
↑
2
α-D-NeupNAc

FIGURE 4

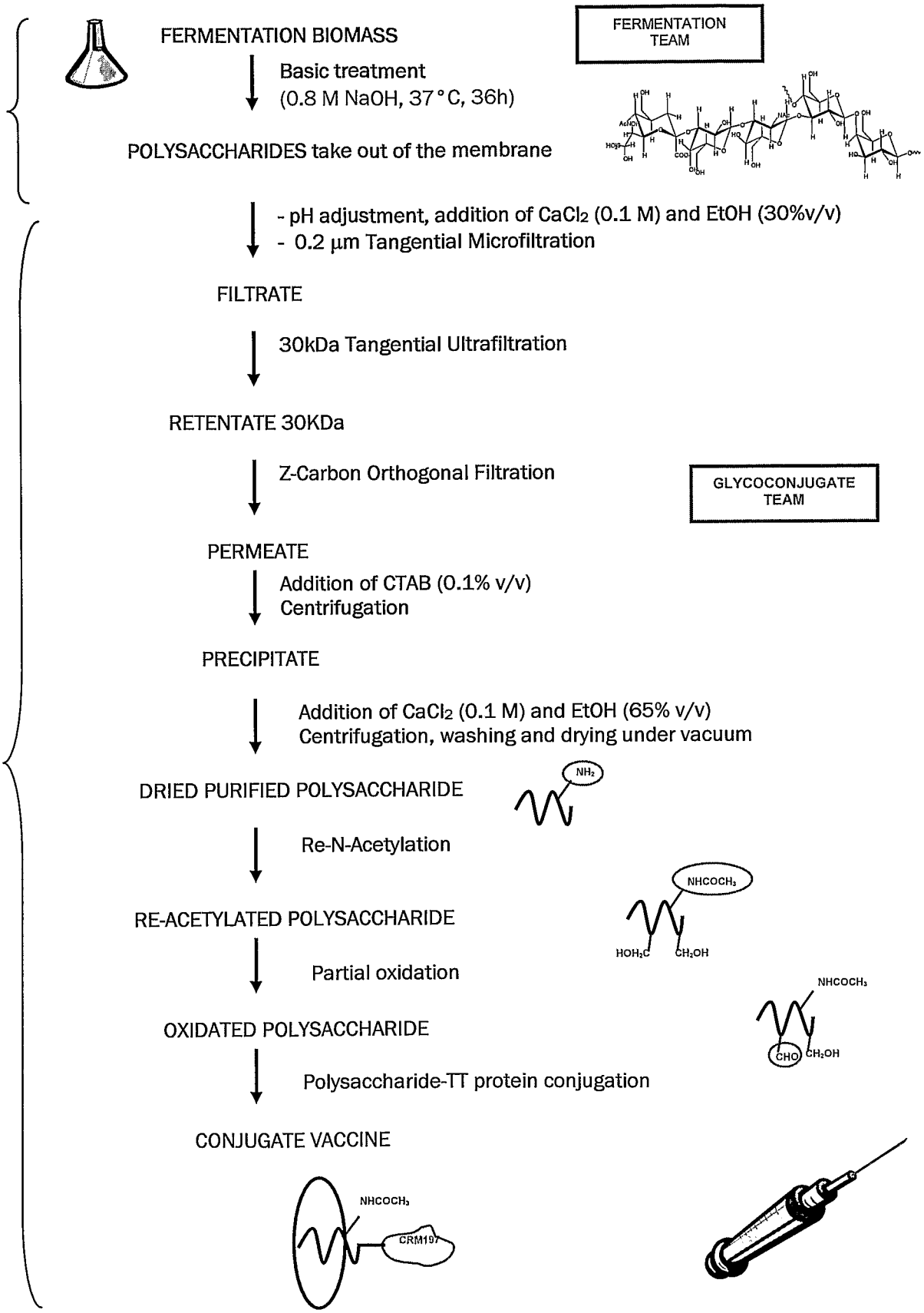
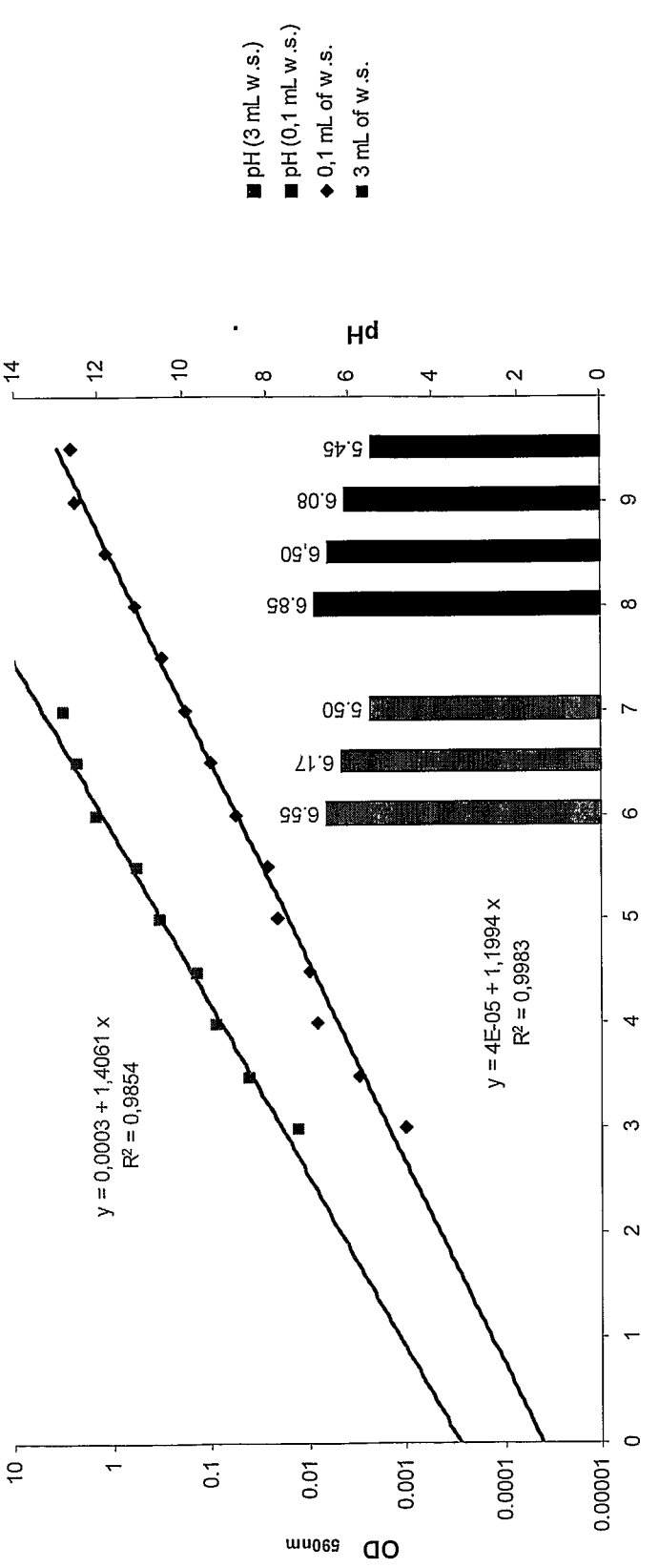
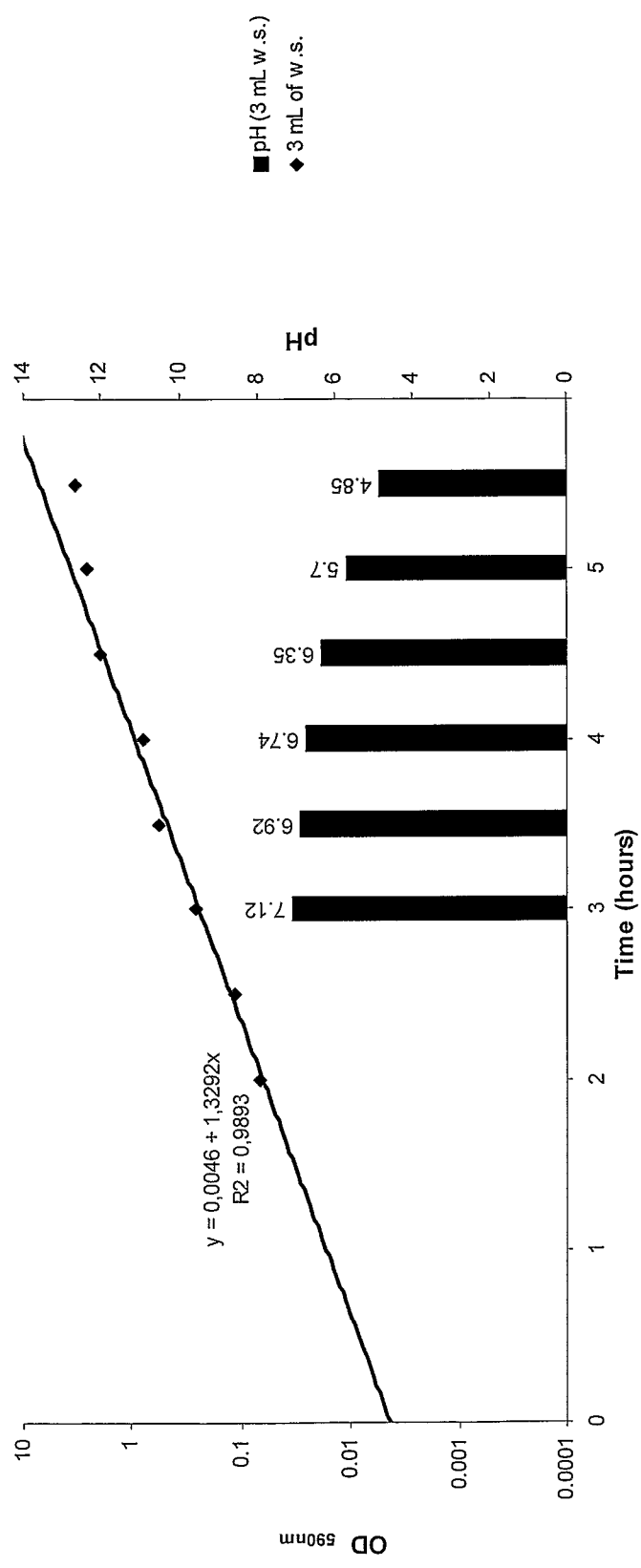

FERMENTATION BIOMASS
FERMENTATION TEAM
Basic treatment
(0.8 M NaOH, 37 °C, 36h)
POLYSACCHARIDES take out of the membrane
- pH adjustment, addition of CaCl2 (0.1 M) and EtOH (30%v/v)
- 0.2 µm Tangential Microfiltration
FILTRATE
30kDa Tangential Ultrafiltration
RETENTATE 30KDa
Z-Carbon Orthogonal Filtration
GLYCOCONJUGATE TEAM
PERMEATE
Addition of CTAB (0.1% v/v)
Centrifugation
PRECIPITATE
Addition of CaCl2 (0.1 M) and EtOH (65% v/v)
Centrifugation, washing and drying under vacuum
DRIED PURIFIED POLYSACCHARIDE
NH2
Re-N-Acetylation
NHCOCH3
HOH2C
CH2OH
RE-ACETYLATED POLYSACCHARIDE
Partial oxidation
NHCOCH3
CHO
CH2OH
OXIDATED POLYSACCHARIDE
Polysaccharide-TT protein conjugation
CONJUGATE VACCINE
NHCOCH3
CRM197

FIGURE 5A



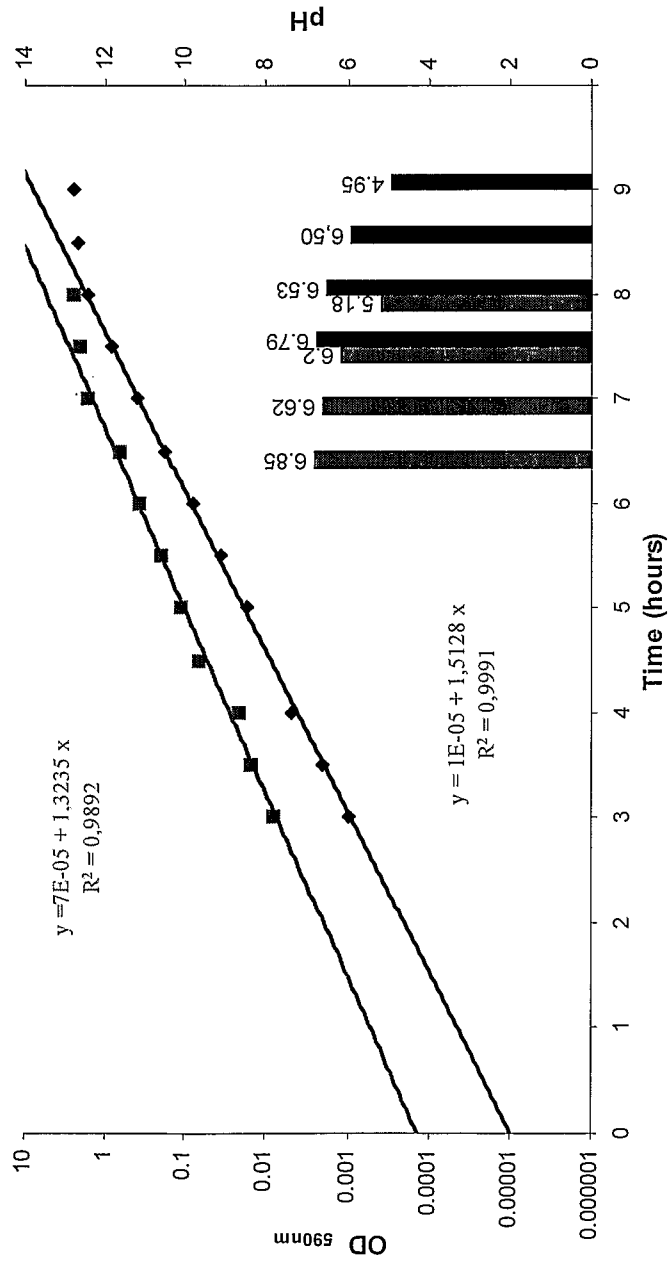
Growth curve and pH of medium for the 090 strain
(μ was calculated with OD values in a range 0.1-2.4)

FIGURE 5B



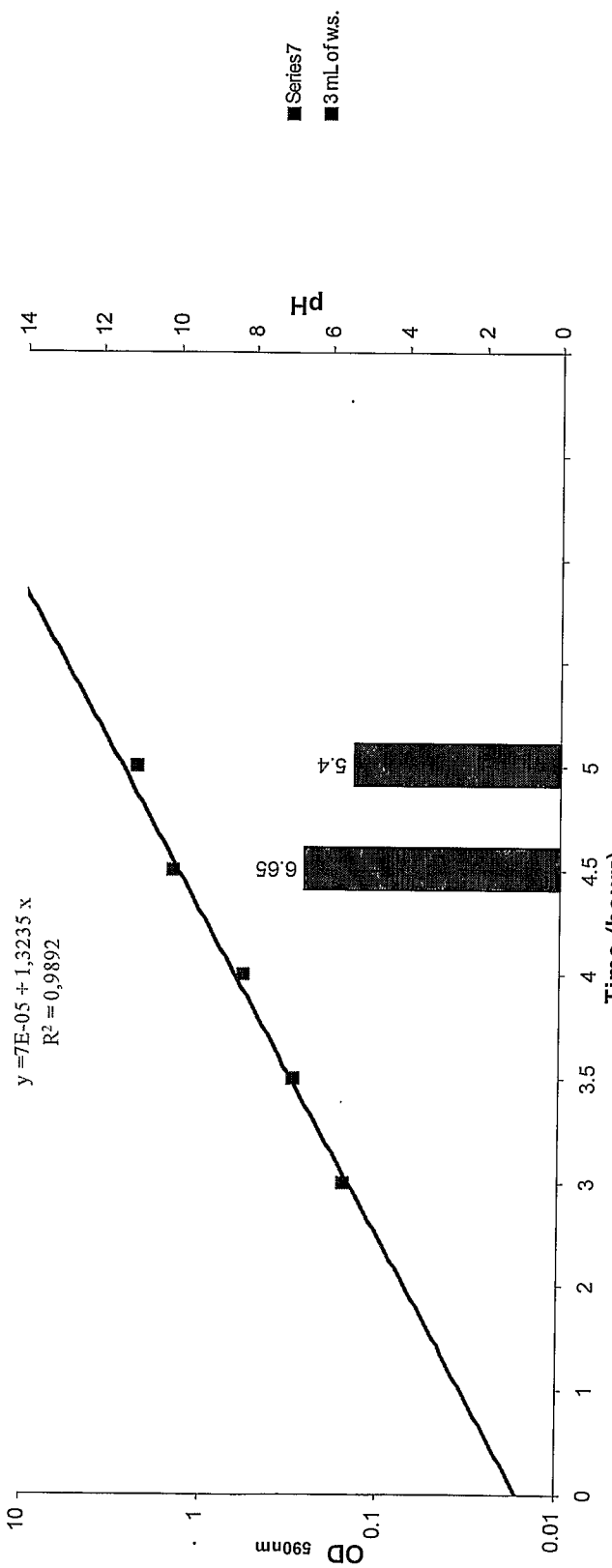
Growth curve and pH of medium for the H36b strain
(μ was calculated with OD values in a range 0.1-2.4)

FIGURE 5C



Growth curve and pH of medium for the M781strain
(μ was calculated with OD values in a range 0.1-2.4)

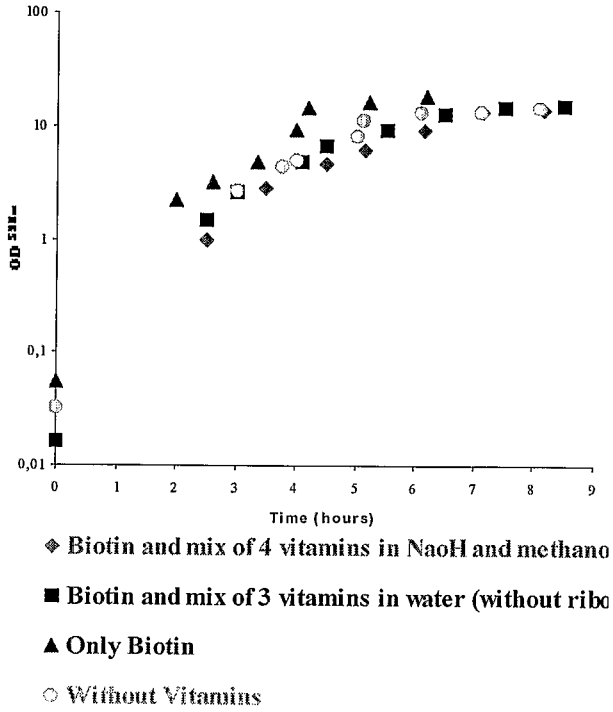
FIGURE 5D



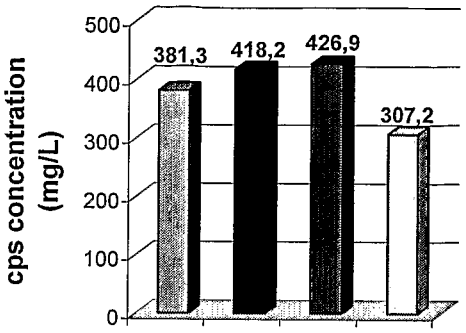
Growth curve and pH of medium for the CJB111strain
(μ was calculated with OD values in a range 0.1-2.4)

FIGURE 6

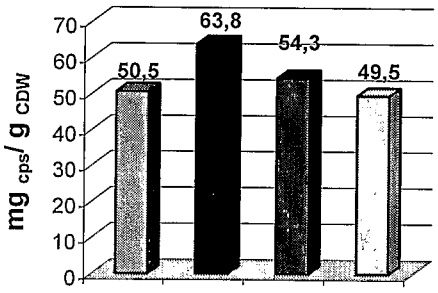
090 Strain:



(A) 090 Growth curve



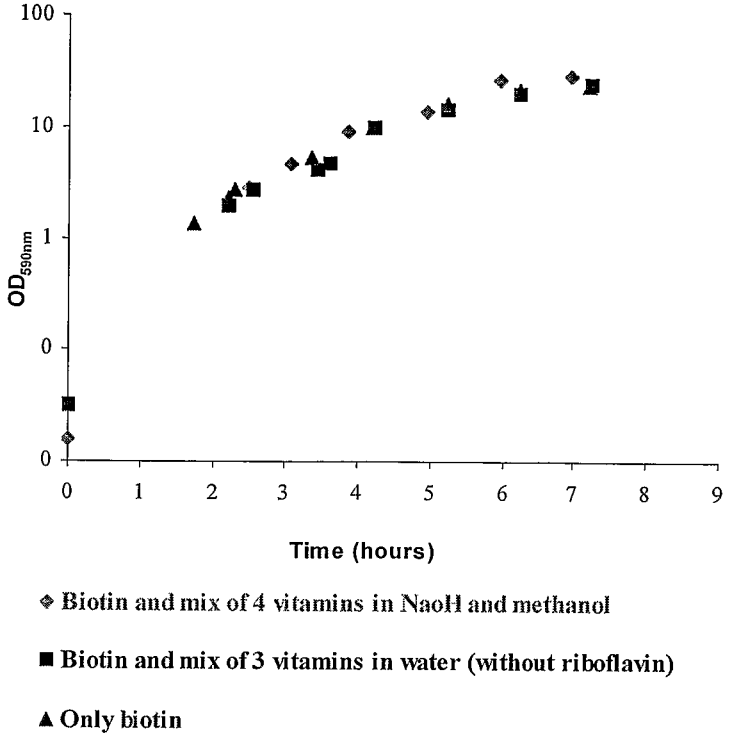
(B) Cps concentration by 090 strain



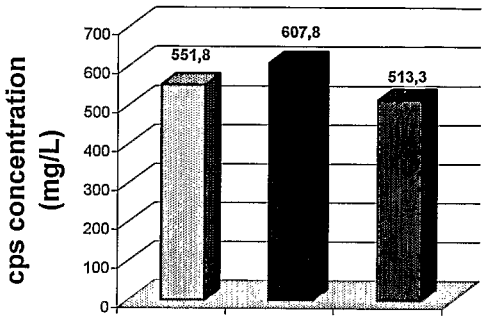
(C) Cps production by gram CDW for 090

FIGURE 7

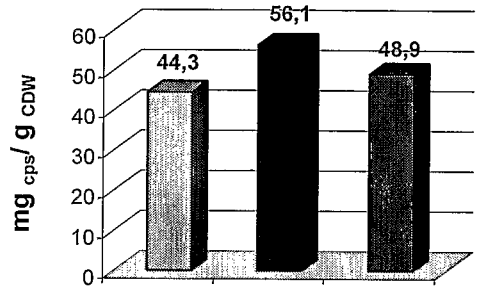
H36b Strain:



(A) H36b growth curve



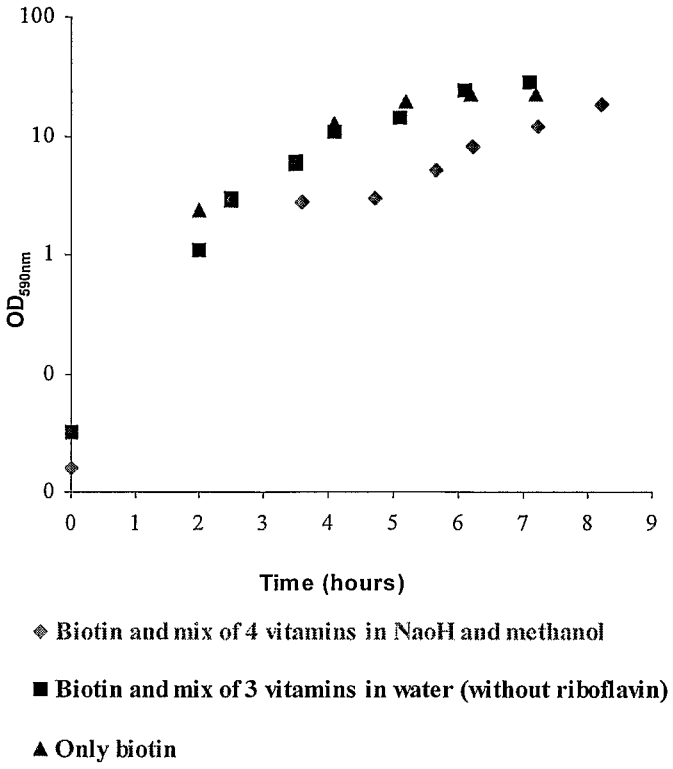
(B) Cps concentration by H36b strain



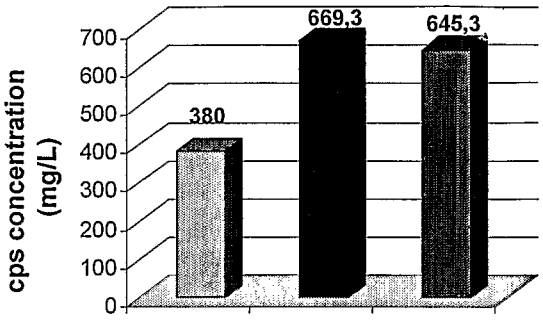
(C) Cps production by gram CDW for H36B

FIGURE 8

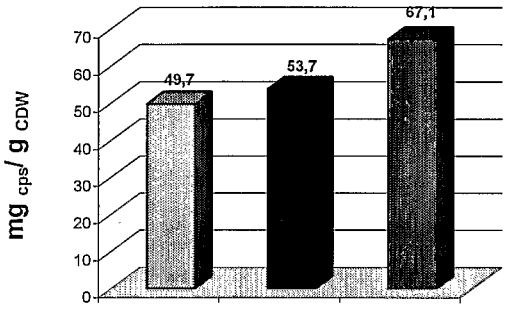
M781 Strain:



(A) M781 growth curve



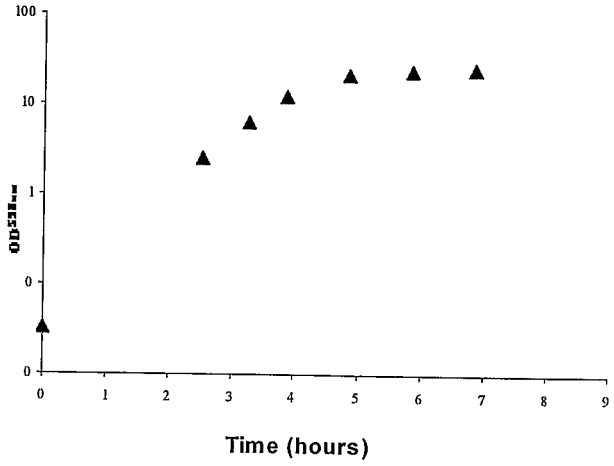
(B) Cps concentration by M781 strain



(C) Cps production by gram CDW for M781 strain

FIGURE 9

CJB111 Strain:



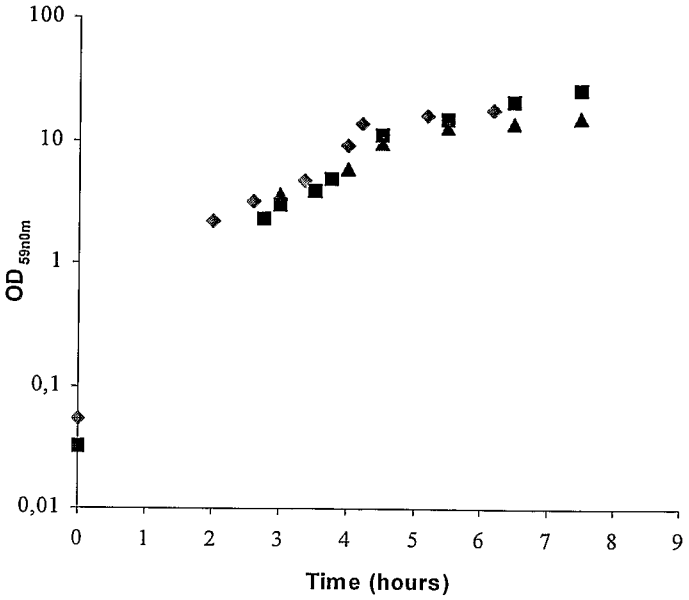
▲ Only biotin

mg/L	550
mg_{cps}/g_{cdw}	57,8

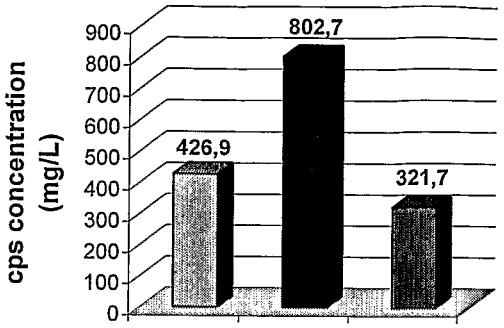
Cps production by CJB111 and
Cps production by gram CDW for CJB111 strain

FIGURE 10

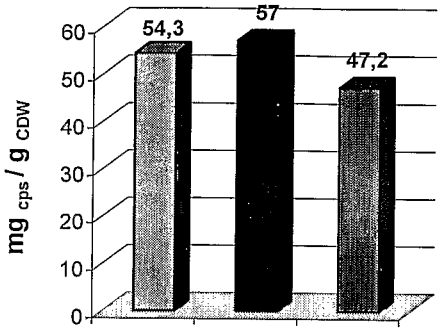
090 Strain:



(A) 090 Growth curve



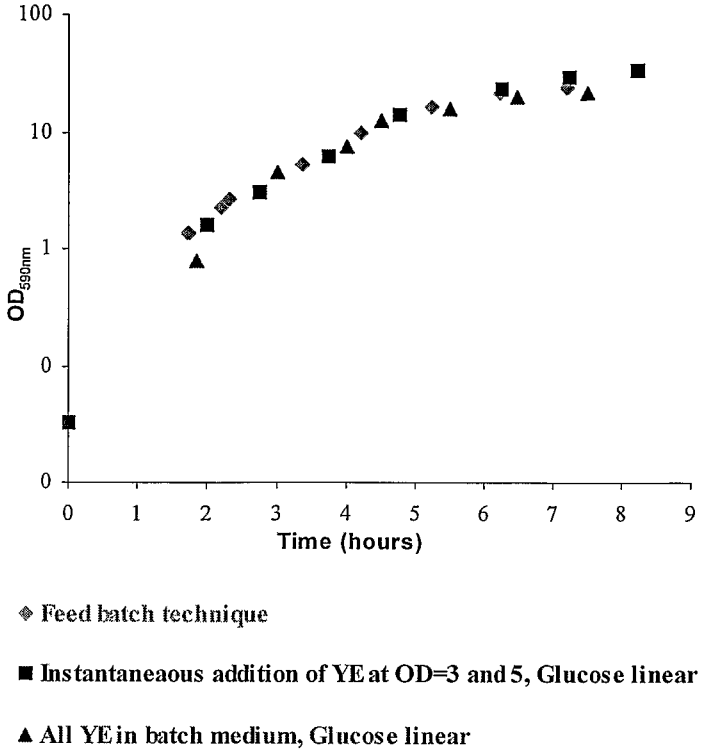
(B) Cps concentration by 090 strain



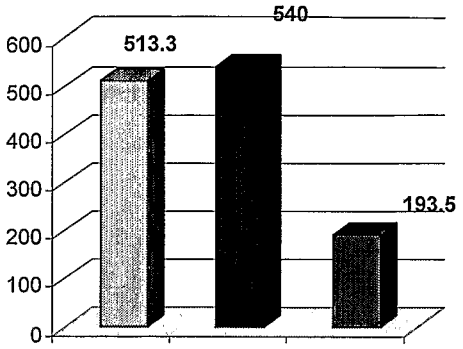
(C) Cps production by gram CDW for 090 strain

FIGURE 11

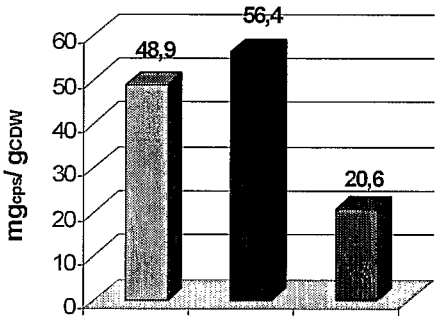
H36b Strain:



(A) H36b growth curve



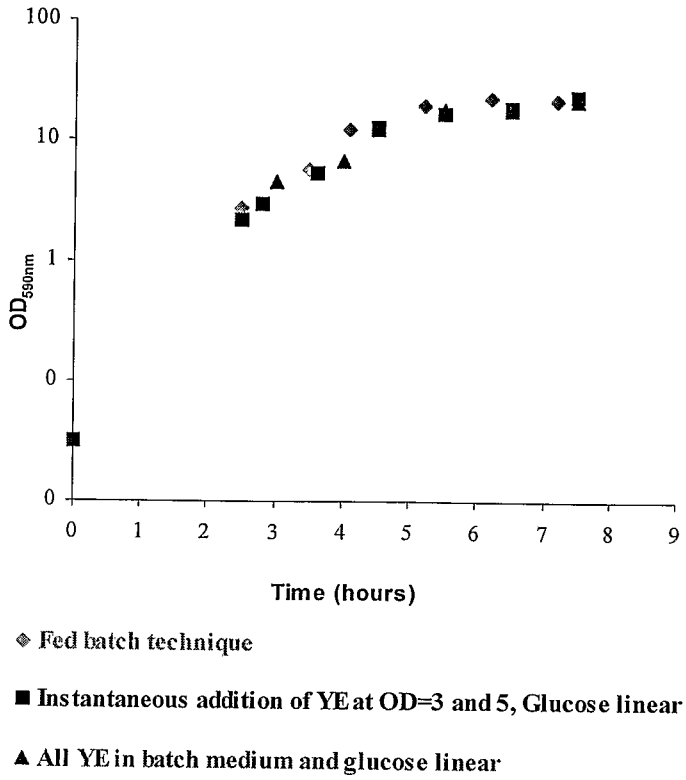
(B) Cps concentration by H36b strain



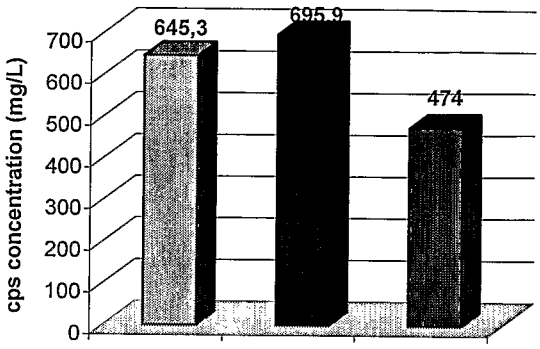
(B) Cps production by gram CDW for H36b

FIGURE 12

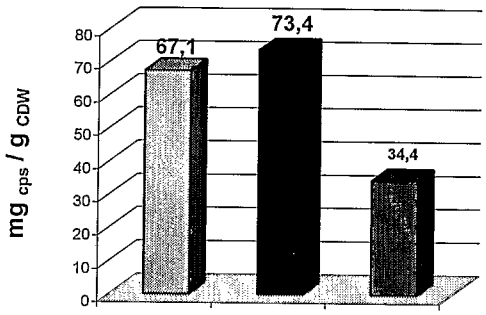
M781 Strain:



(A) M781 growth curve



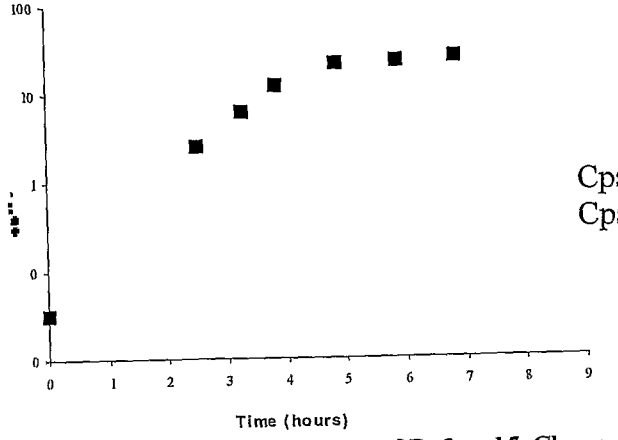
(B) Cps concentration by M781 strain



(C) Cps production by gram CDW for M781 strain

FIGURE 13

CJB111 strain:



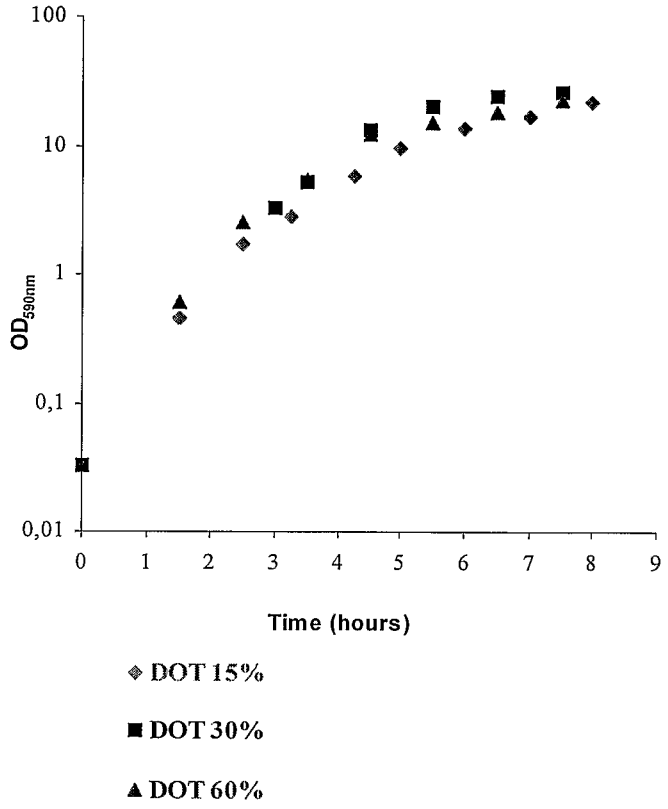
■ Instantaneous addition of YE at OD=3 and 5, Glucos

mg/L	550
mg_{cps}/g_{cdw}	57,8

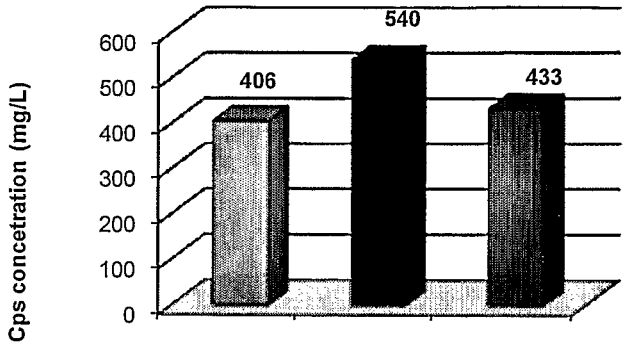
Cps production by CJB111 and
Cps production by gram CDW for CJB111 strain

FIGURE 14

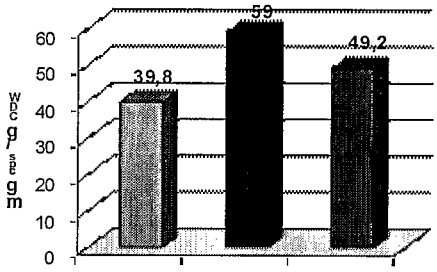
DOT study:



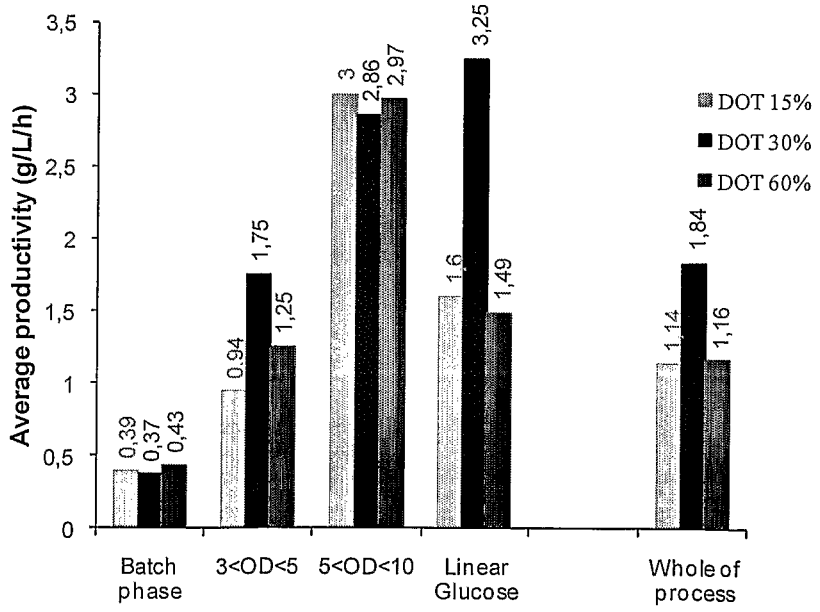
(A) H36b growth curve



(B) Cps concentration by H36b strain



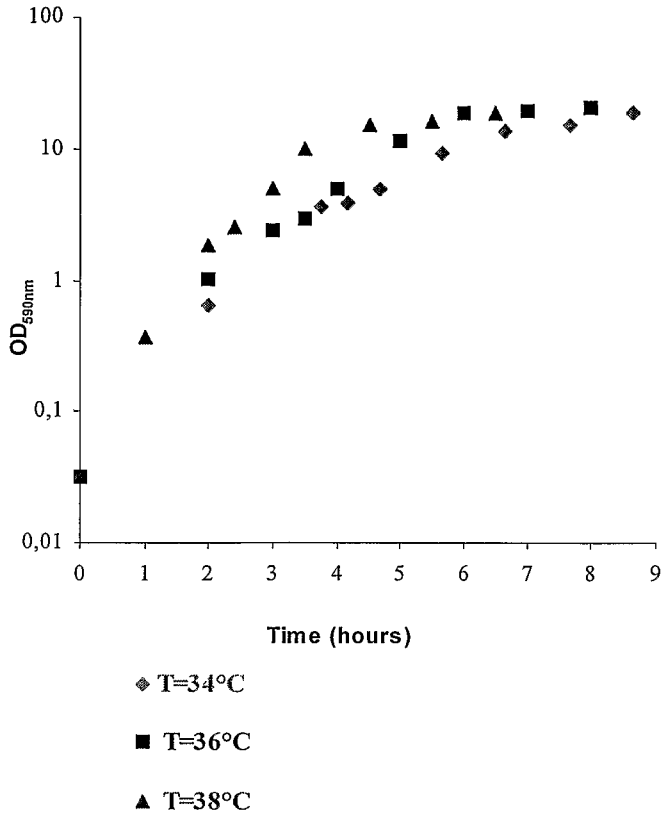
(C) Cps production by gram CDW for H36b strain



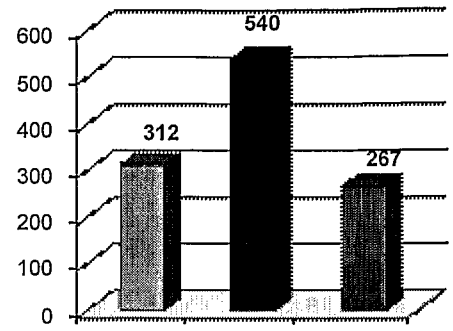
(D) Average productivity of H36b strain

FIGURE 15

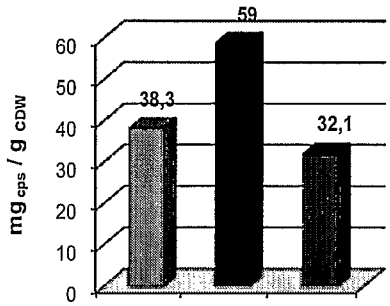
Temperature study:



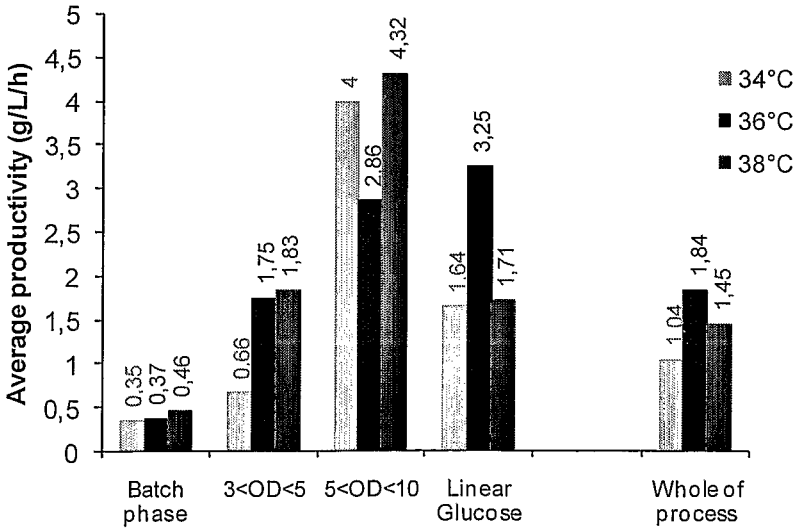
(A) H36b growth curve



(B) Cps concentration by H36b strain



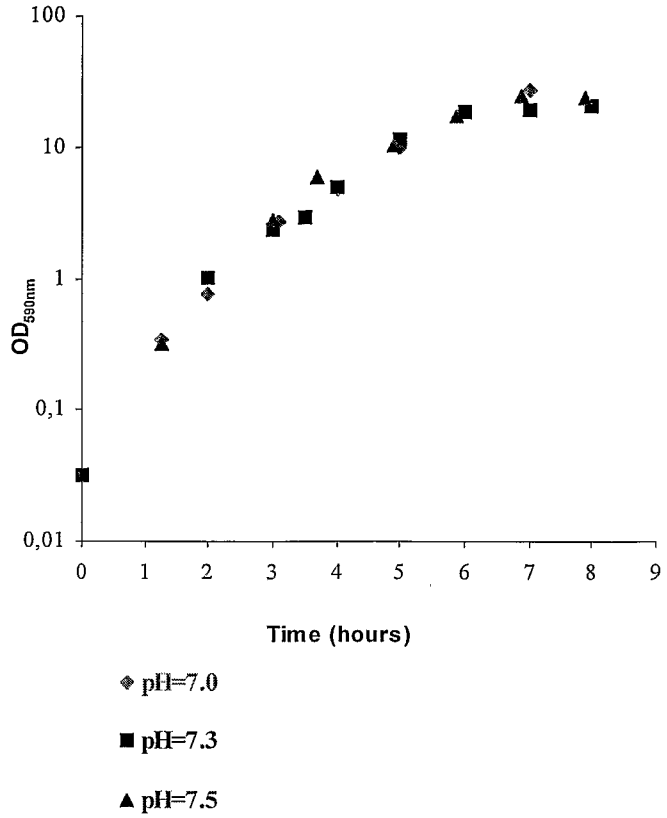
(C) Cps production by gram CDW for H36b strain



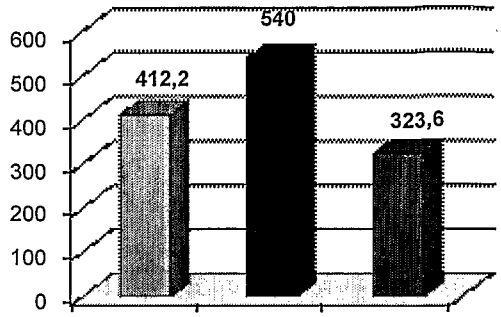
(D) Average productivity of H36bstrain

FIGURE 16

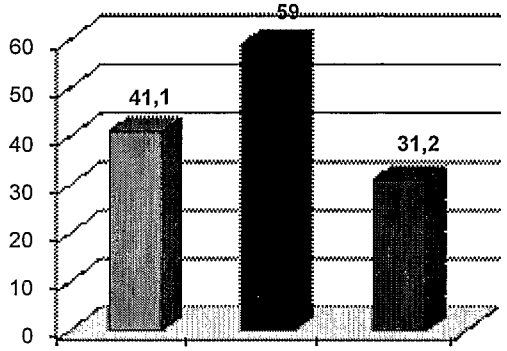
pH study :



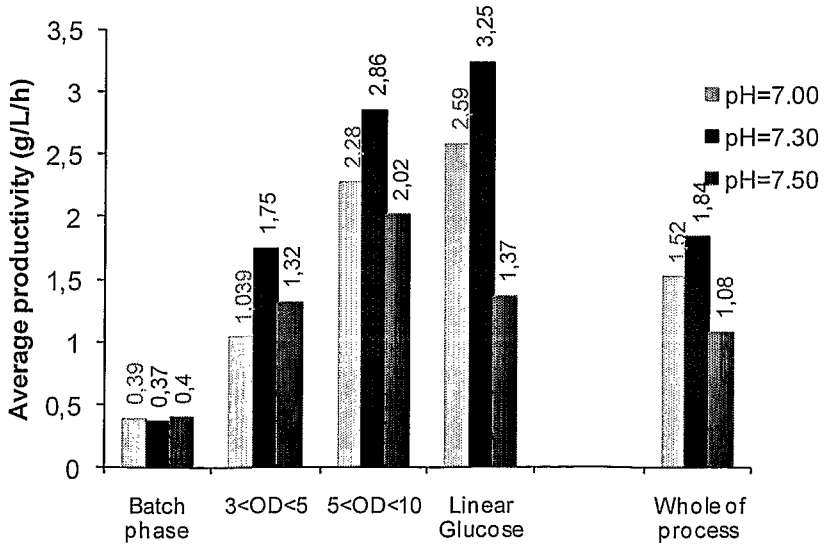
(A) H36b growth curve



(B) Cps concentration by H36b strain



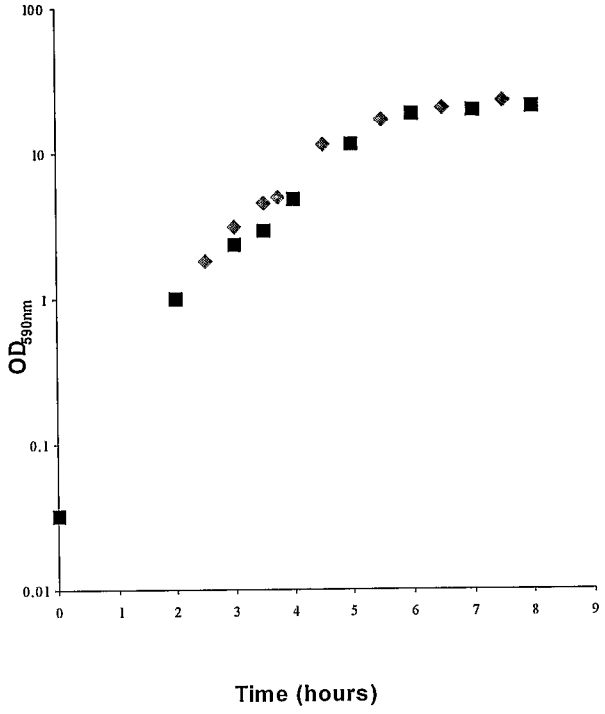
(C) Cps production by gram CDW for H36b strain



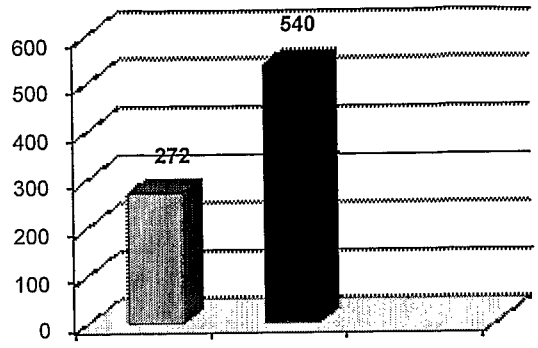
(D) Average productivity of H36bstrain

FIGURE 17

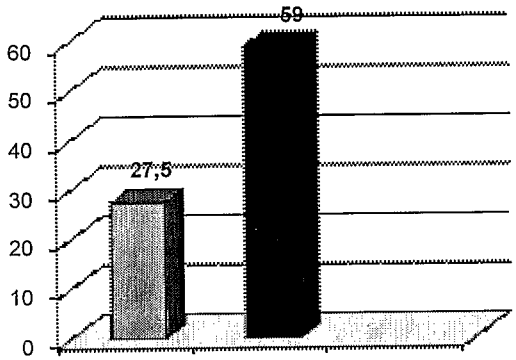
pressure study :



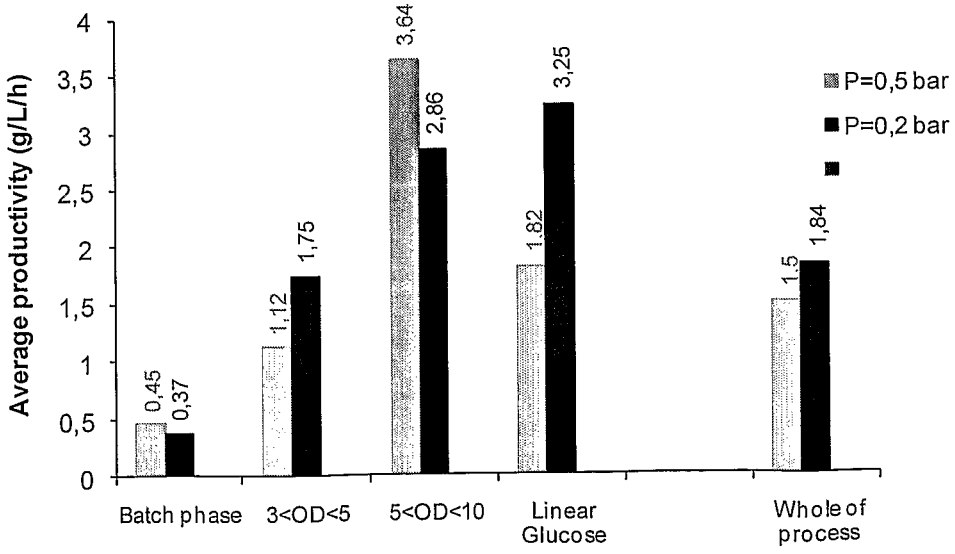
(A) H36b growth curve



(B) Cps concentration by H36b strain

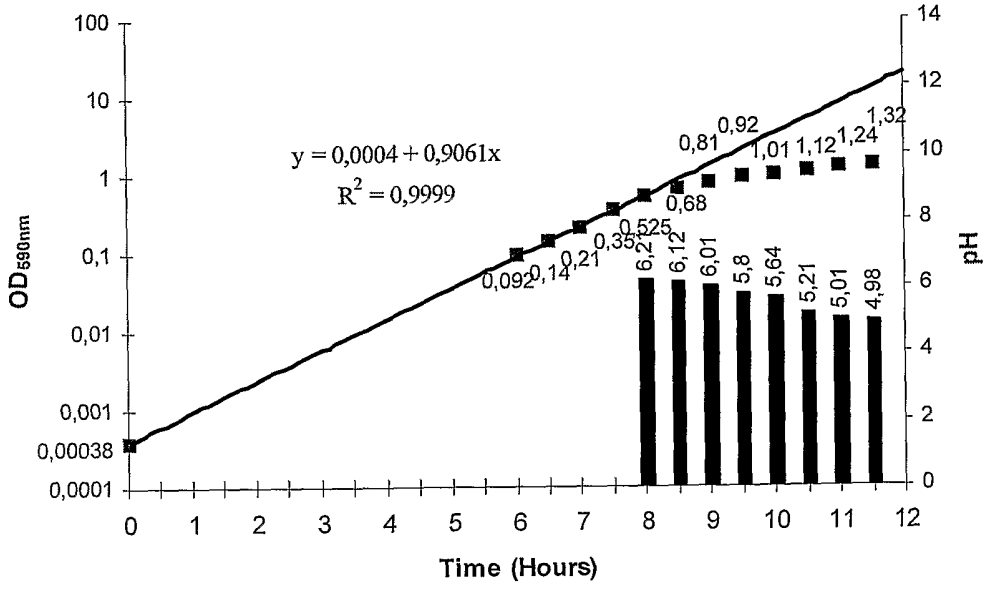


(C)Cps production by gram CDW for H36b strain

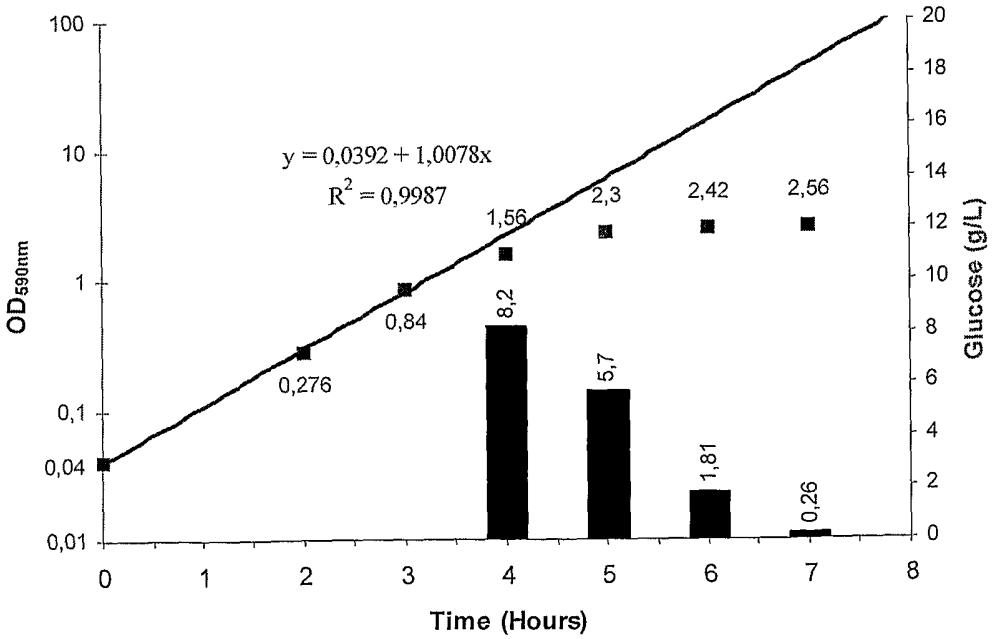


(D) Average productivity of H36bstrain

FIGURE 18

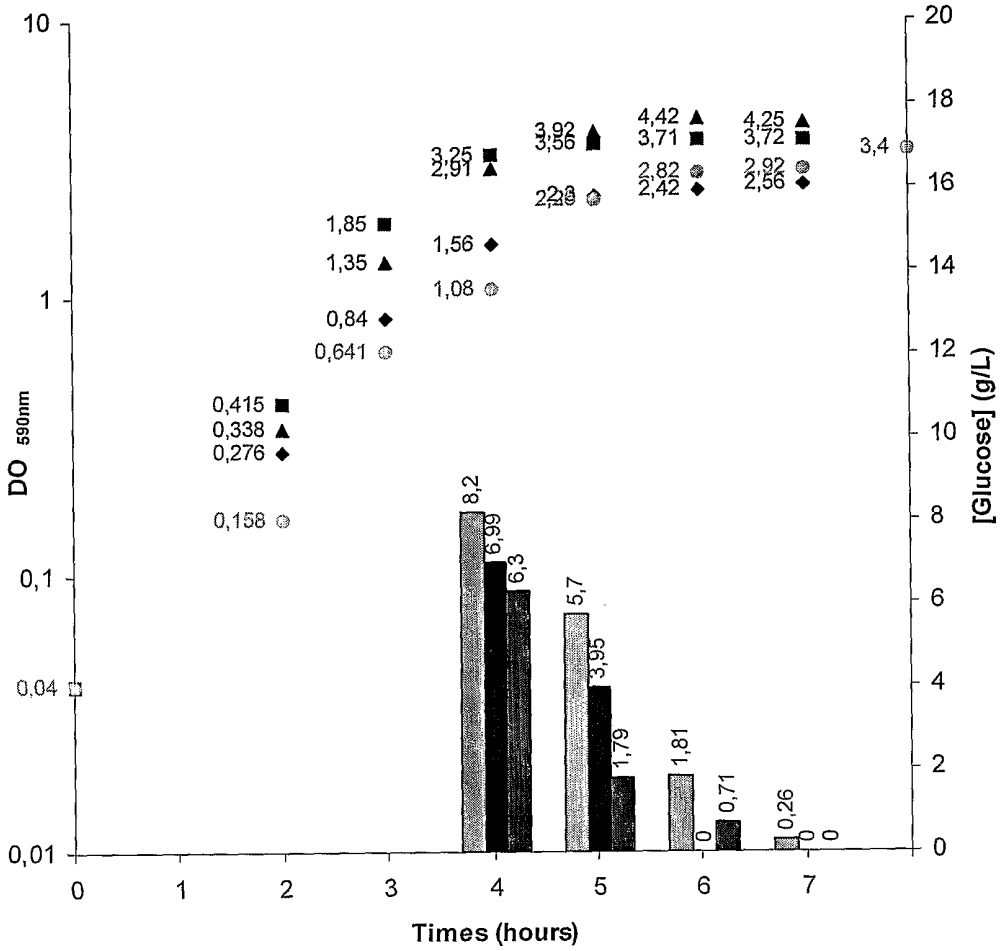


(A) M781Growth curve (0.1mL of w.s.) in a 500mL Erlenmeyer flasks containing 100mL of CDM
(μ was calculated with OD values in a range 0.1-0.7)



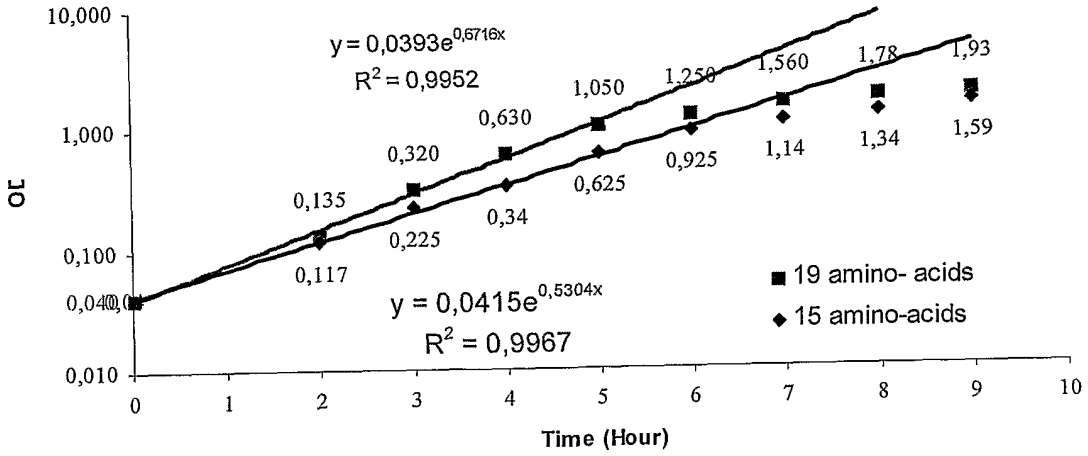
(B) M781Growth curve in a 2 liters fermentor
(μ was calculated with OD values in a range 0.1-1)

FIGURE 19



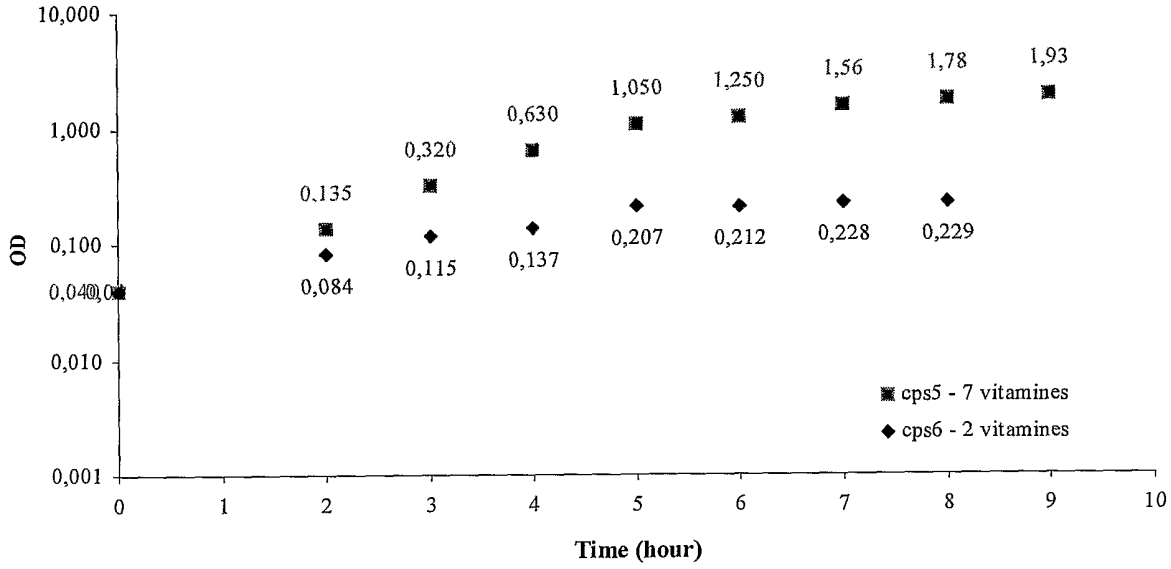
Growth curve and glucose consumption of M781strain

FIGURE 20



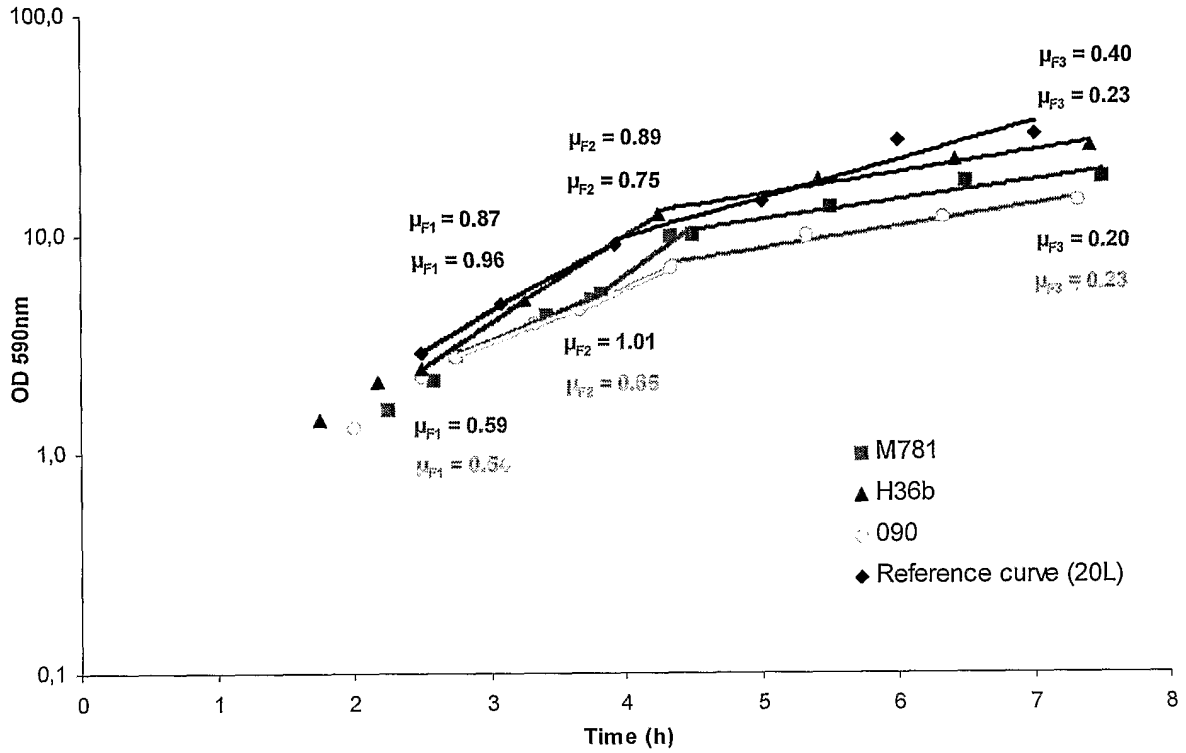
Effect of omission of alanine, aspartic acid, glutamine and proline on growth of strain M781 of GBS in a CDM

FIGURE 21



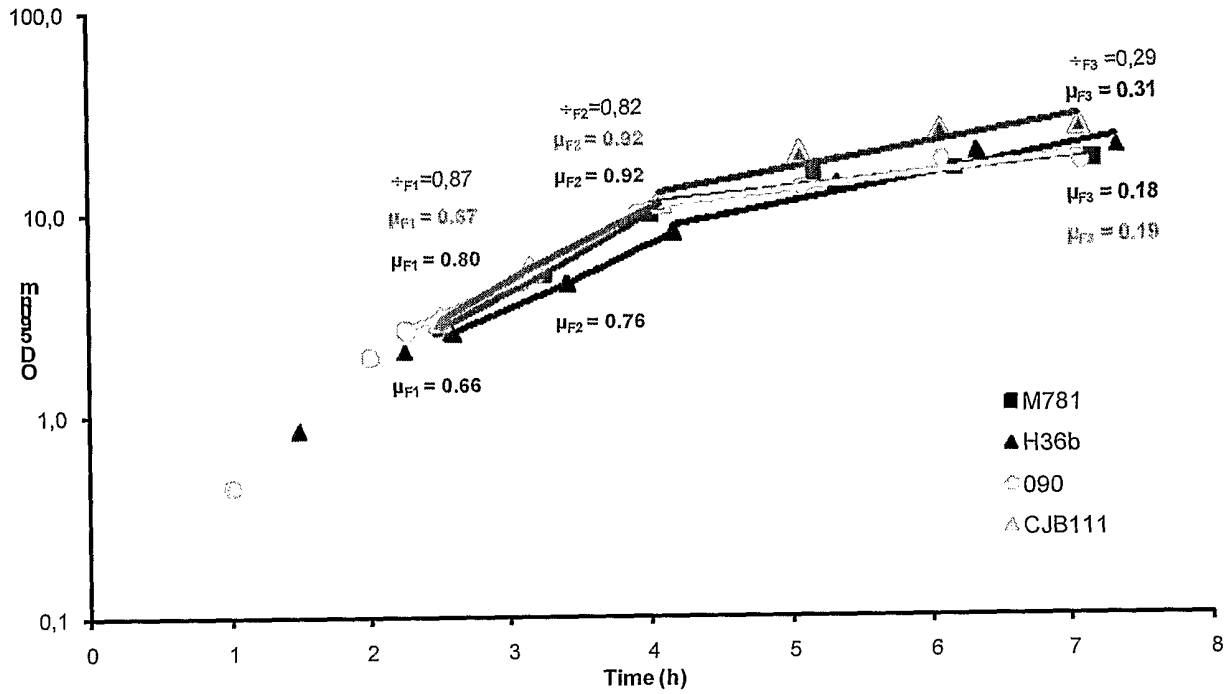
Effect of omission of biotin, folic acid, pyridoxine, riboflavin and thiamine on growth of strain M781 of group B streptococci in a chemically defined medium

FIGURE 22



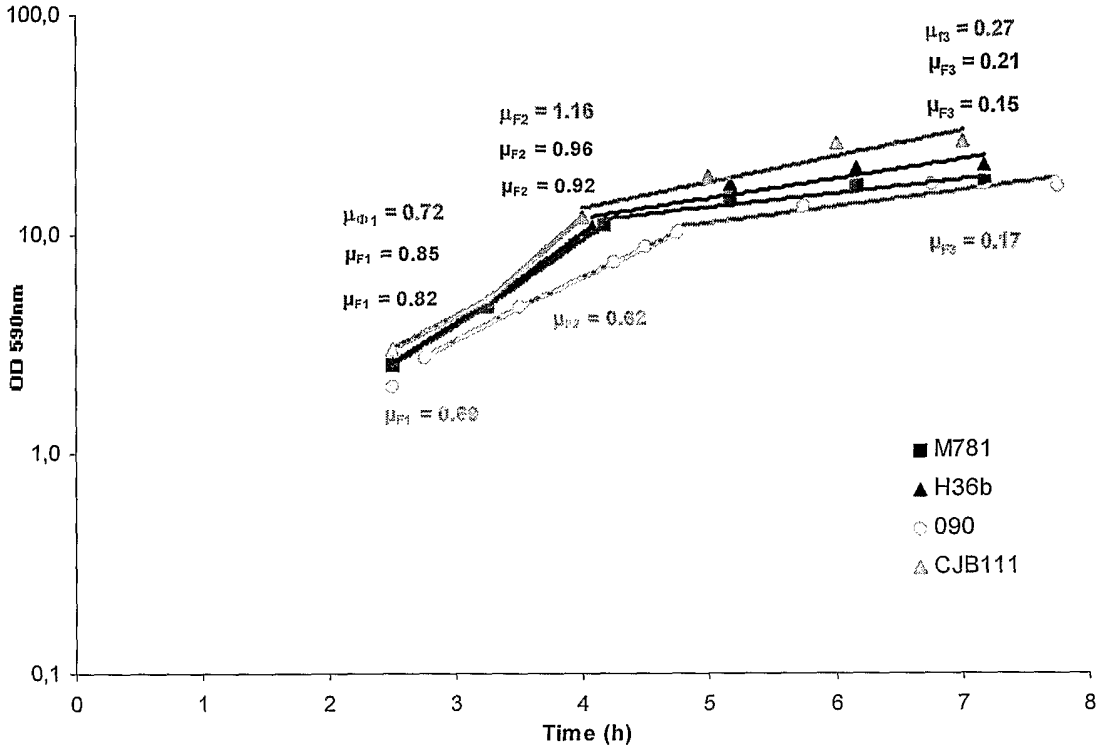
OD_{590nm} profiles of the first pre test runs fermentations

FIGURE 23



OD_{590nm} profiles of the second pre test runs fermentations

FIGURE 24



OD_{590nm} profiles of the test runs fermentations

FIGURE 25

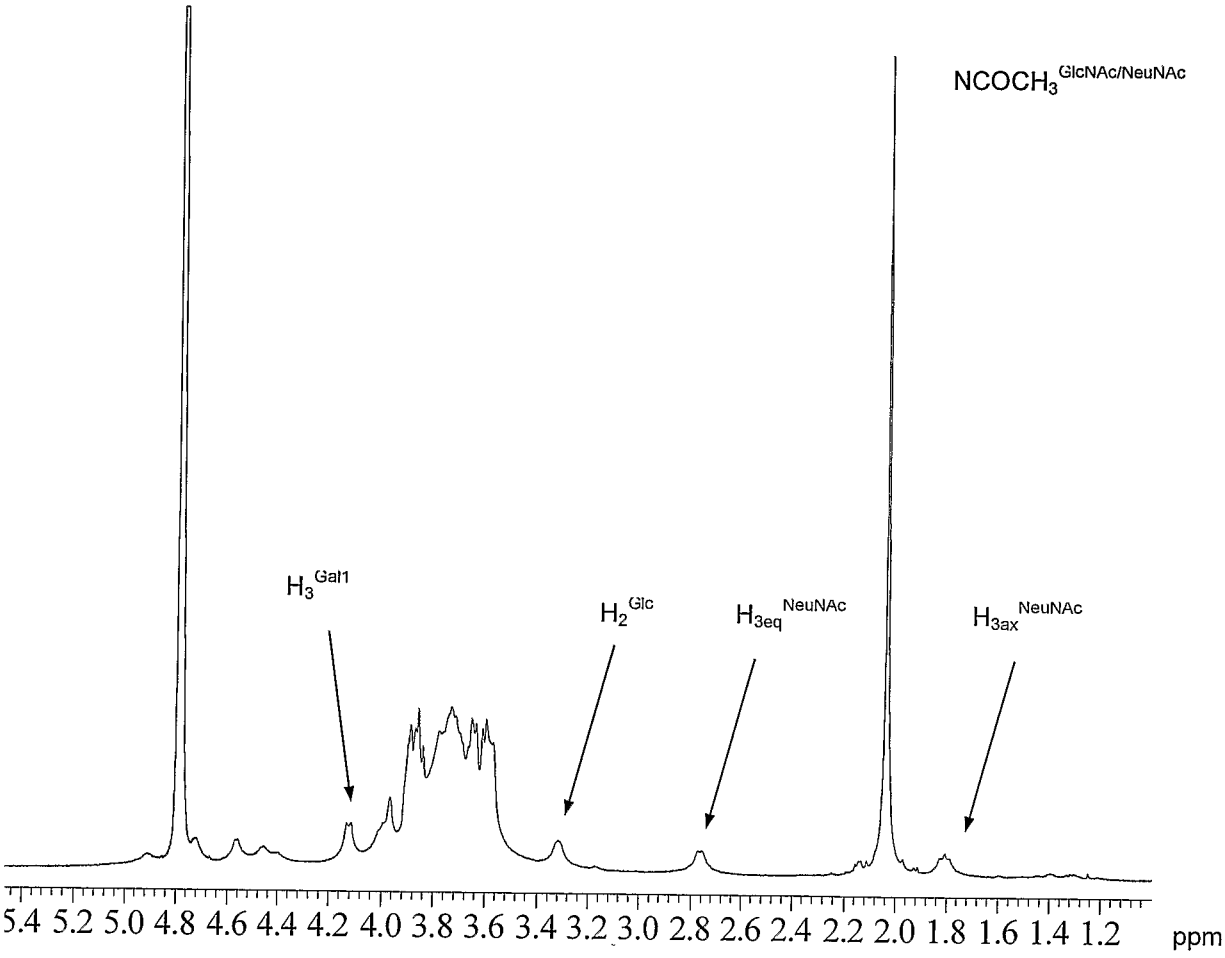

NCOCH3 GlcNAc/NeuNAc
H3 Gal1
H2 Glc
H3eq NeuNAc
H3ax NeuNAc
5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2
ppm

FIGURE 26

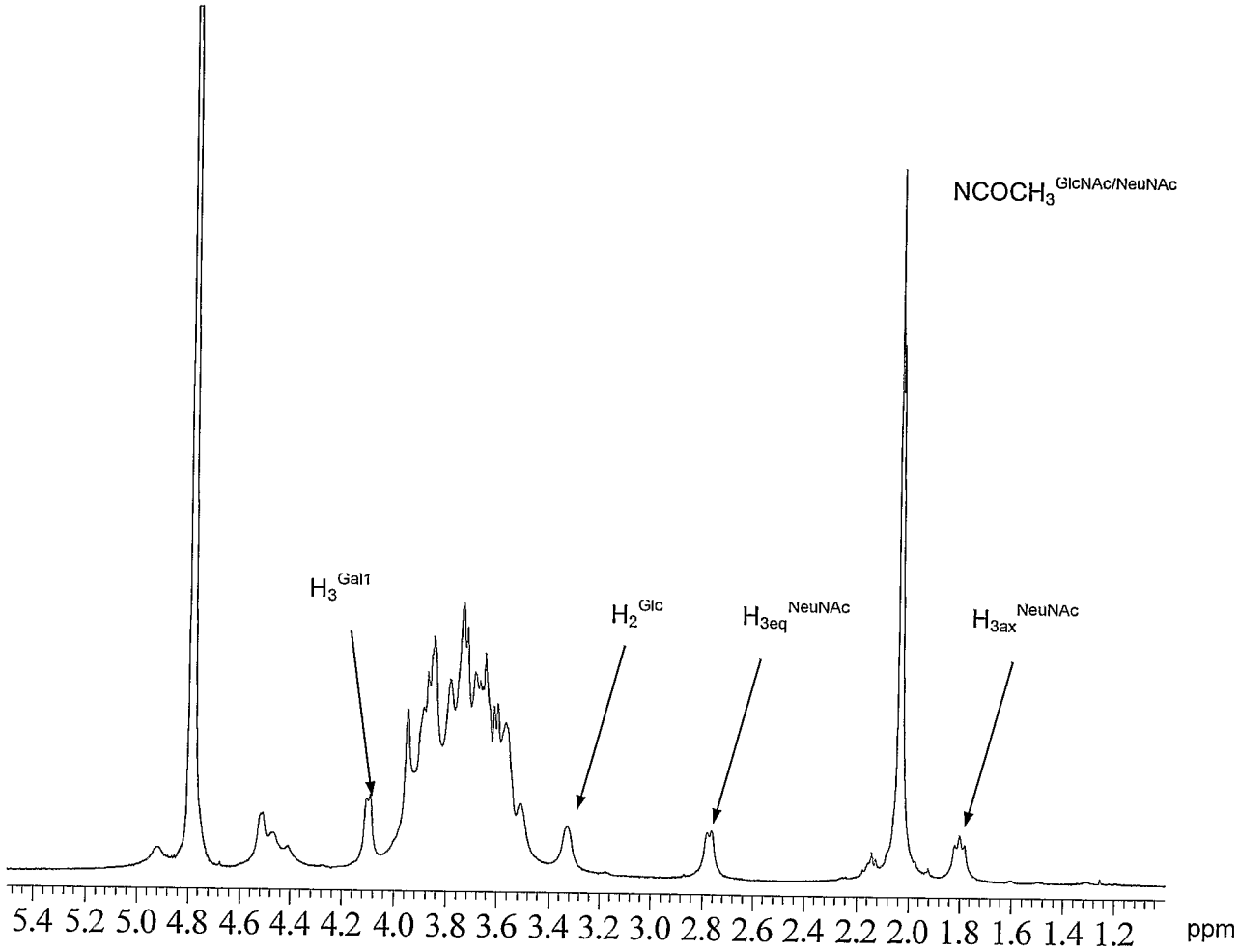

NCOCH3 GlcNAc/NeuNAc
H3 Gal1
H2 Glc
H3eq NeuNAc
H3ax NeuNAc
5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2
ppm

FIGURE 27

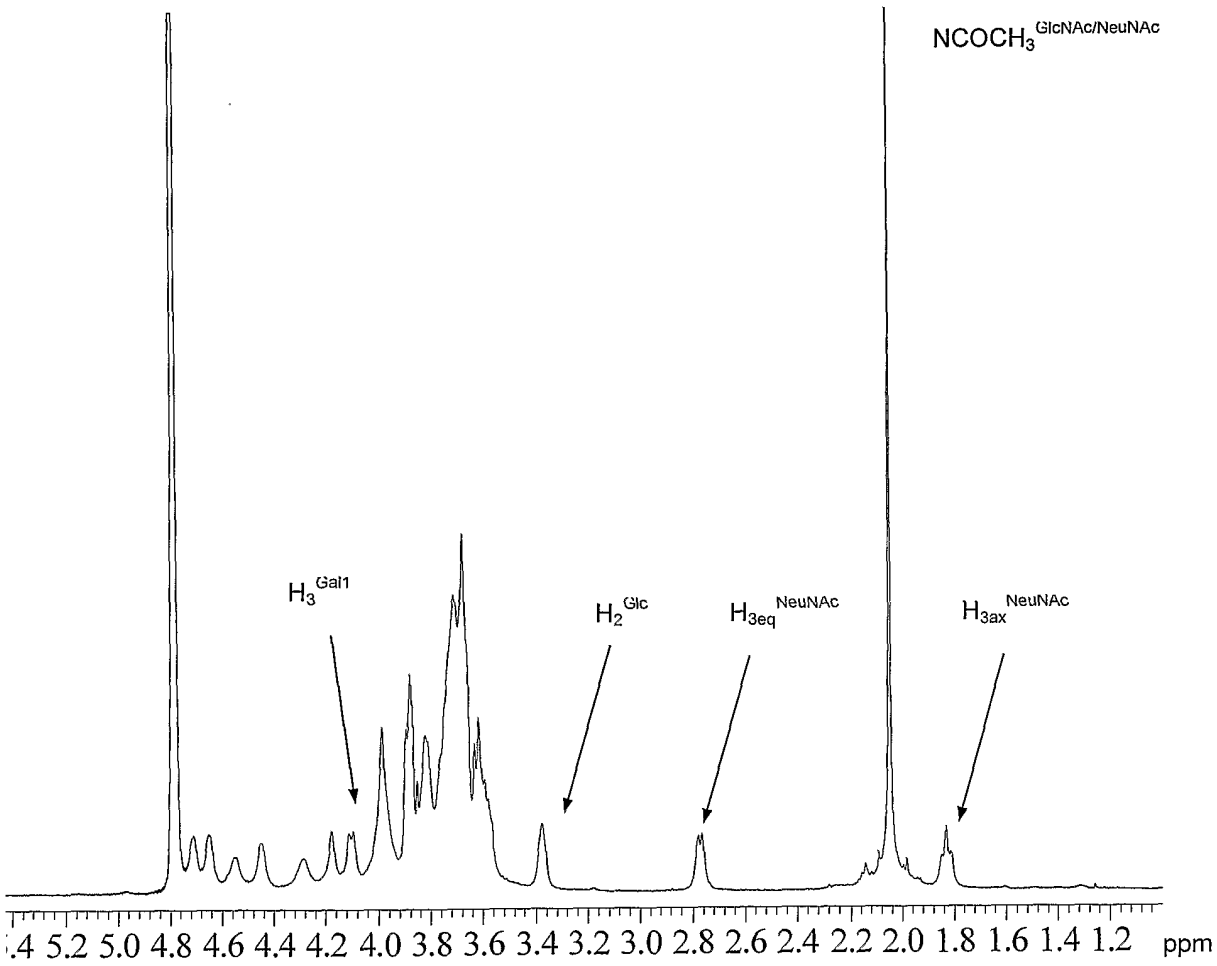

NCOCH3 GlcNAc/NeuNAc
H3 Gal1
H2 Glc
H3eq NeuNAc
H3ax NeuNAc
.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2
ppm

FIGURE 28

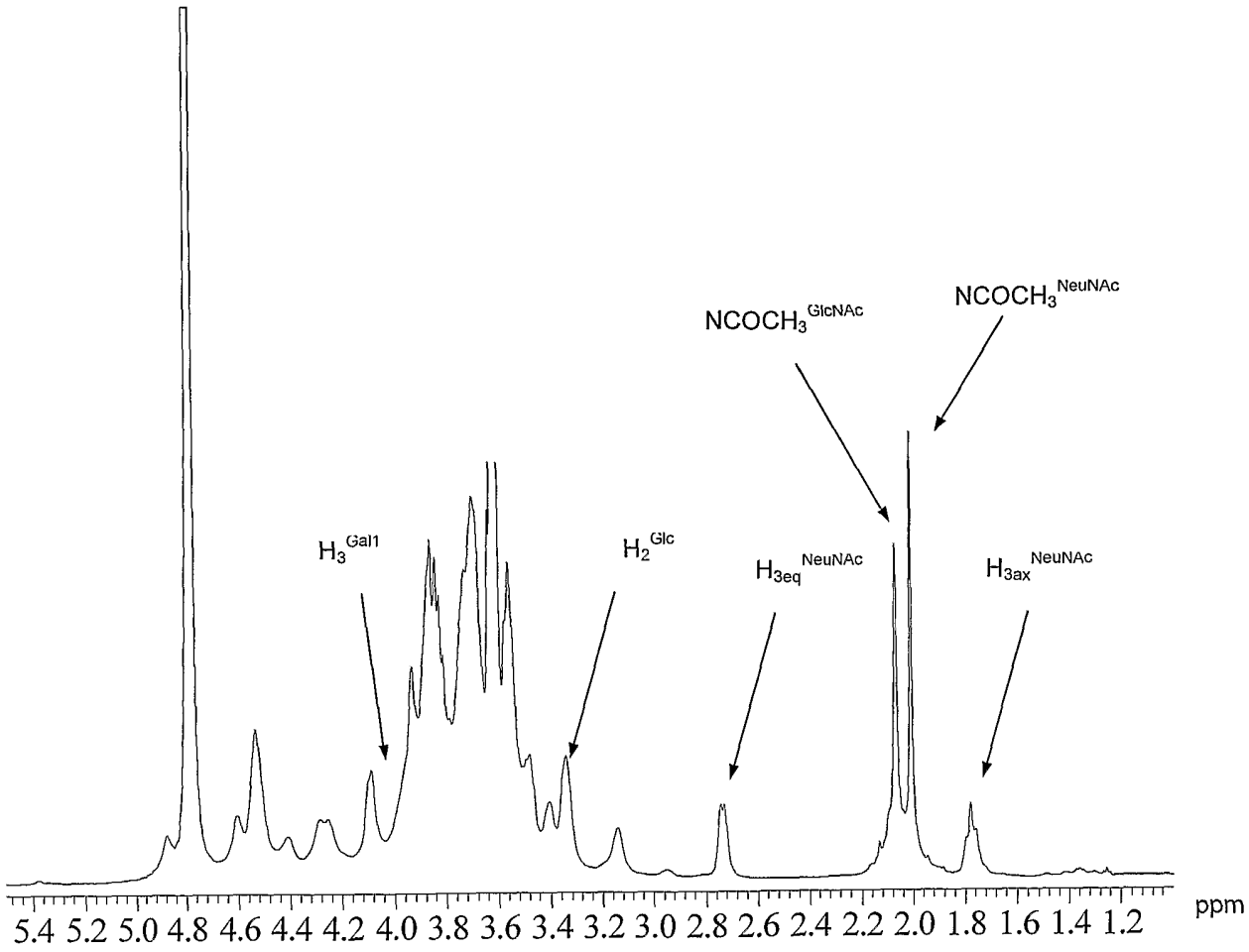

NCOCH3 GlcNAc
NCOCH3 NeuNAc
H3 Gal1
H2 Glc
H3eq NeuNAc
H3ax NeuNAc
5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2
ppm

FIGURE 29

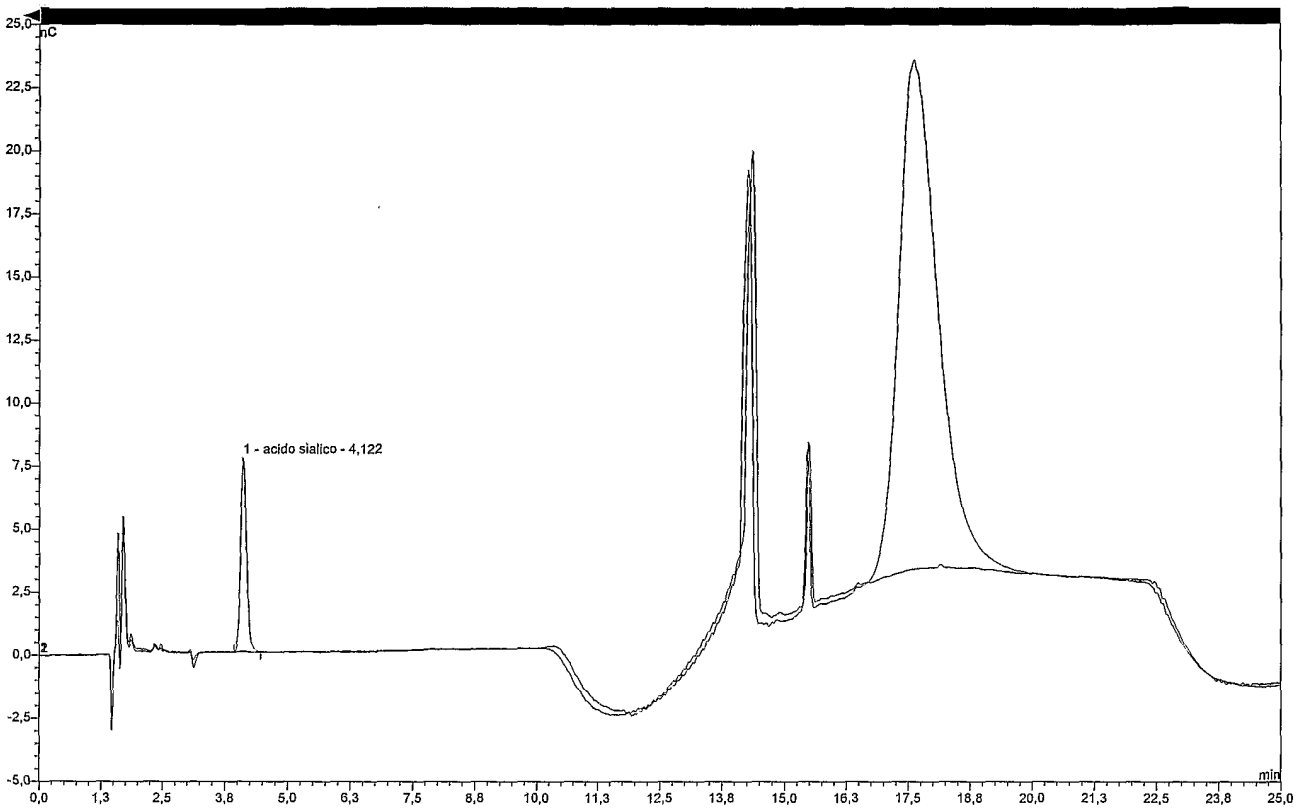
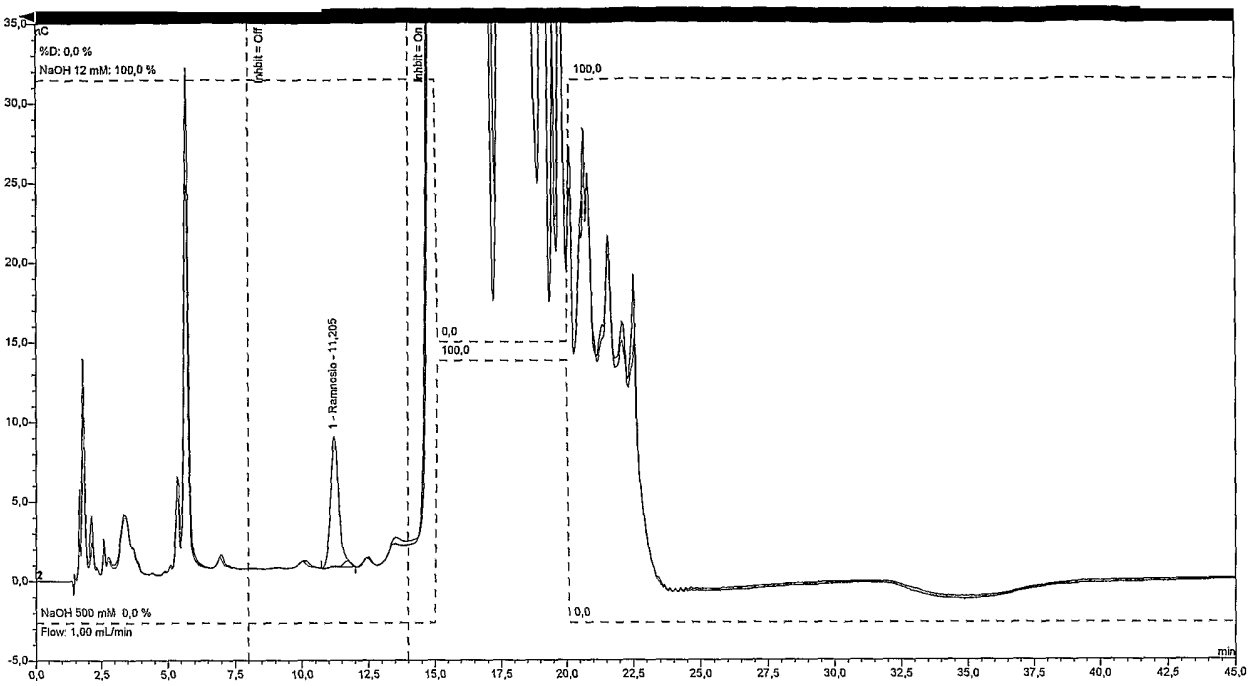

nC
25,0
22,5
20,0
17,5
15,0
12,5
10,0
7,5
5,0
2,5
0,0
-2,5
-5,0
1 - acido sialico - 4,122
2
min
0,0
1,3
2,5
3,8
5,0
6,3
7,5
8,8
10,0
11,3
12,5
13,8
15,0
16,3
17,5
18,8
20,0
21,3
22,5
23,8
25,0

FIGURE 30


nC
%D: 0,0 %
NaOH 12 mM: 100,0 %
Inhibit = Off
Inhibit = On
100,0
0,0
100,0
1 - Ramnosio - 11,205
0,0
NaOH 500 mM: 0,0 %
Flow: 1,00 mL/min
min