Figure 1 Effect of media pH on cell growth and culture pH

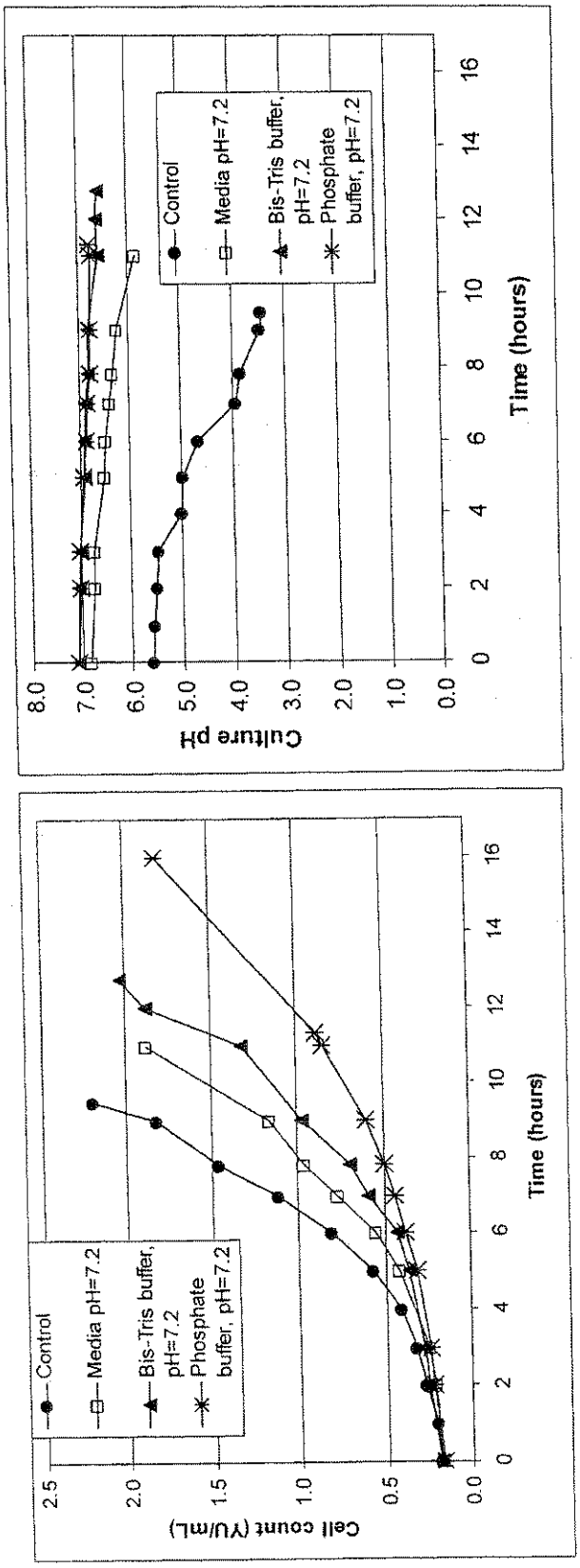
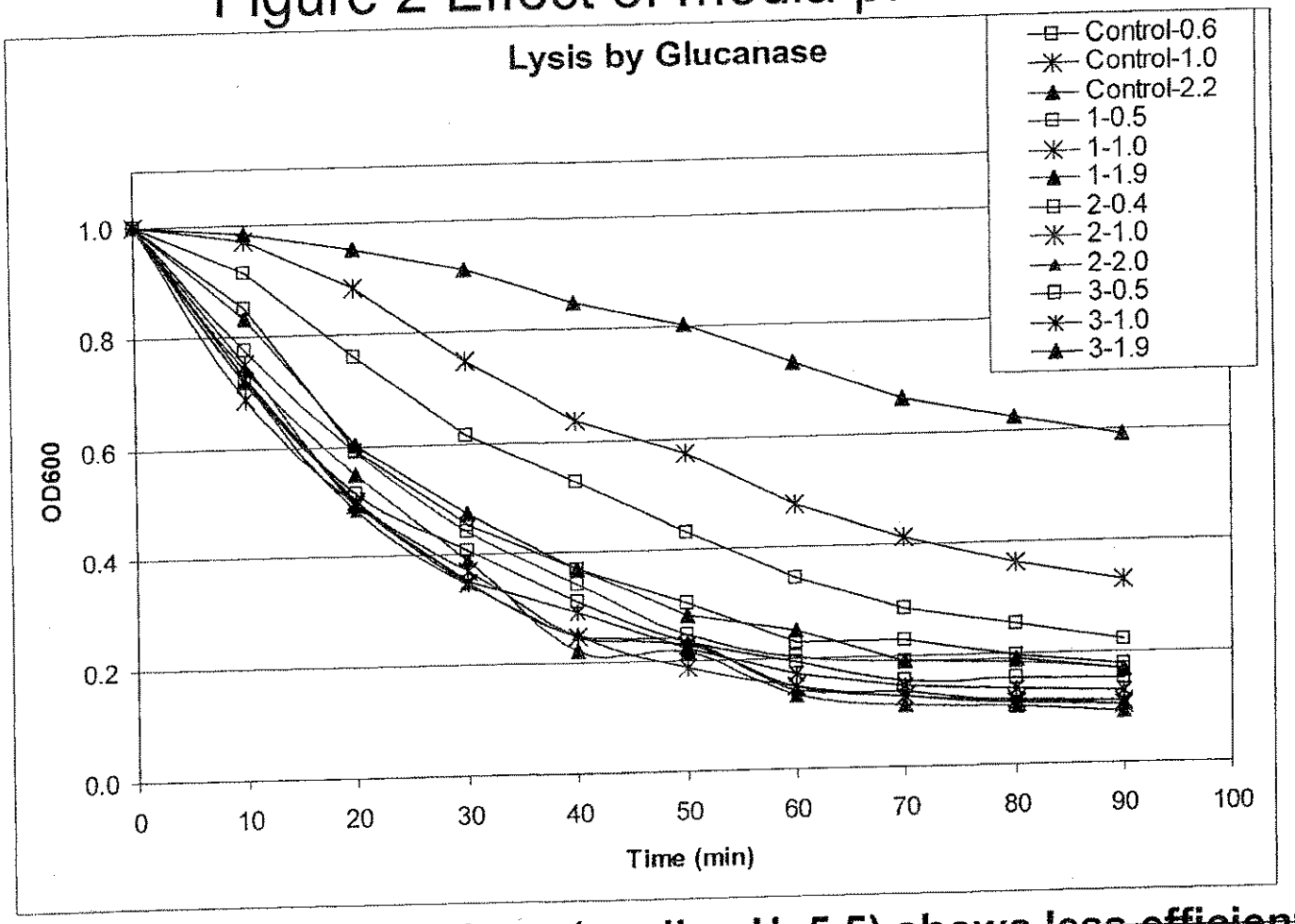


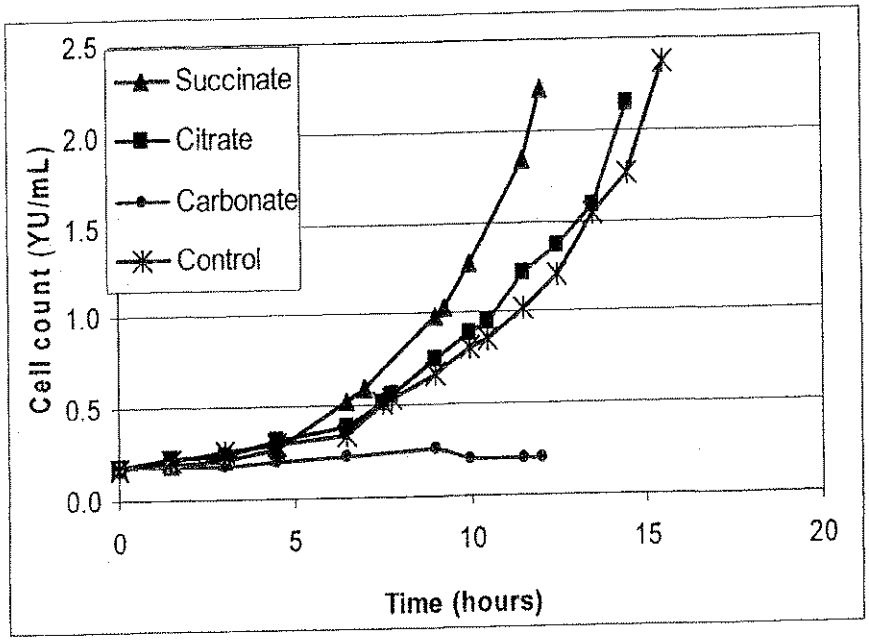
Figure 2 ~~Effect of media pH on cell wall thickness~~



- ~~Control culture (media pH~5.5) shows less efficient lysis~~ as cell density increases.
- Flask1 = media pH 7.2, no buffer
- Flask 2 = media pH 7.2, Bis-Tris buffer
- ~~Flask 3= media pH 7.2, phosphate buffer~~

Figure 3 ~~Testing different buffers (pH 6.5 media)~~

~~Effects on cell growth and culture pH~~



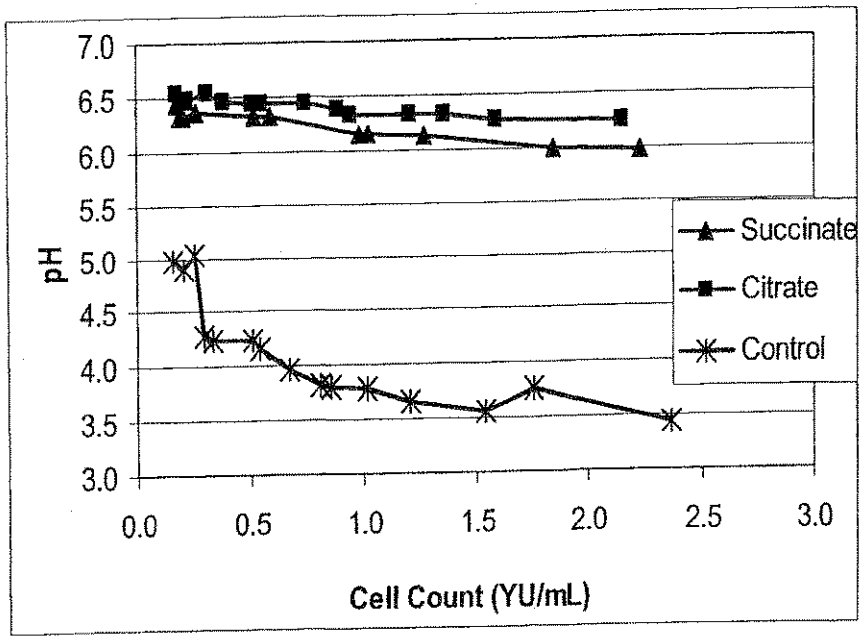


Figure 4 Testing new buffers (pH 6.5 media)

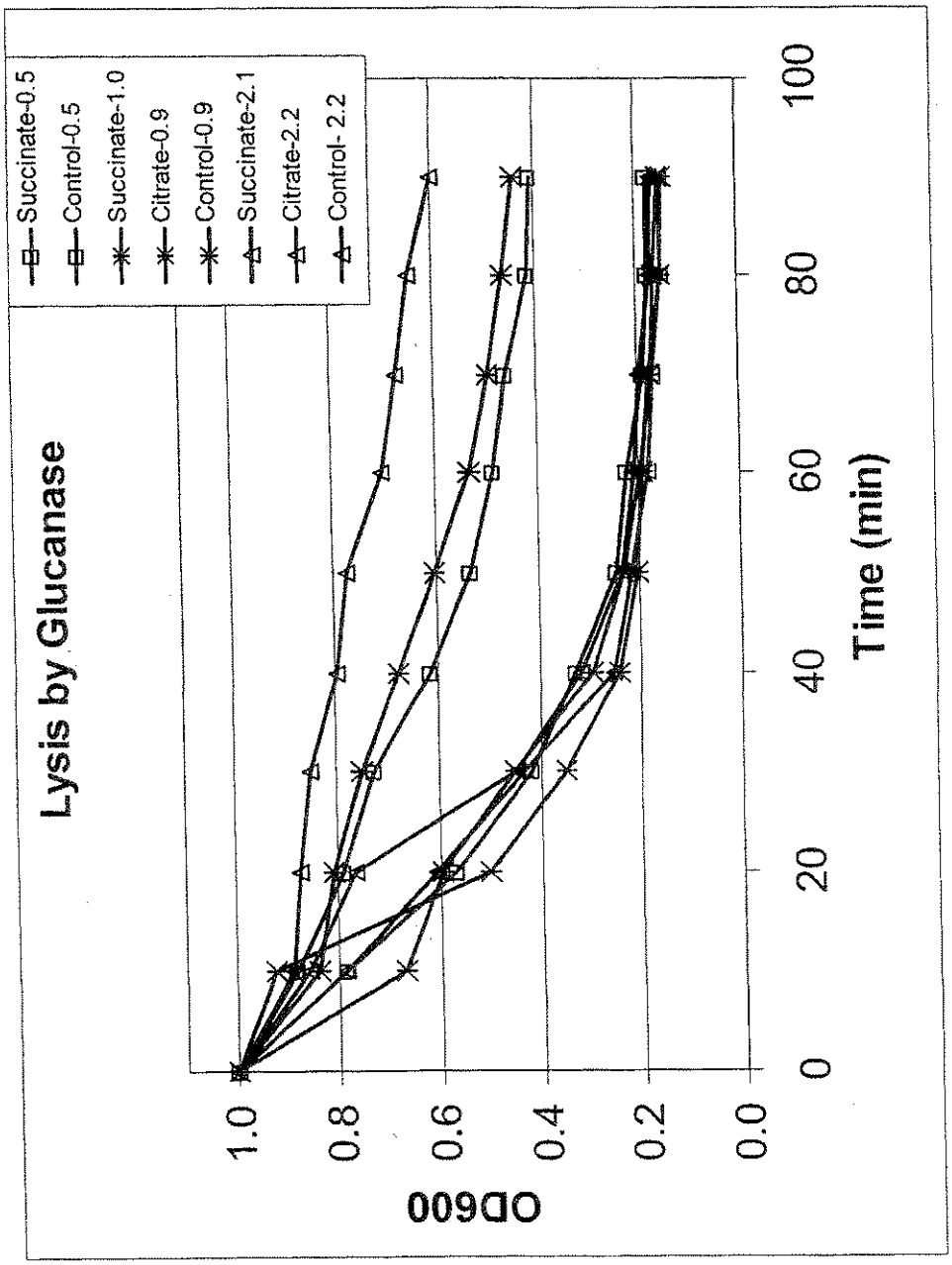
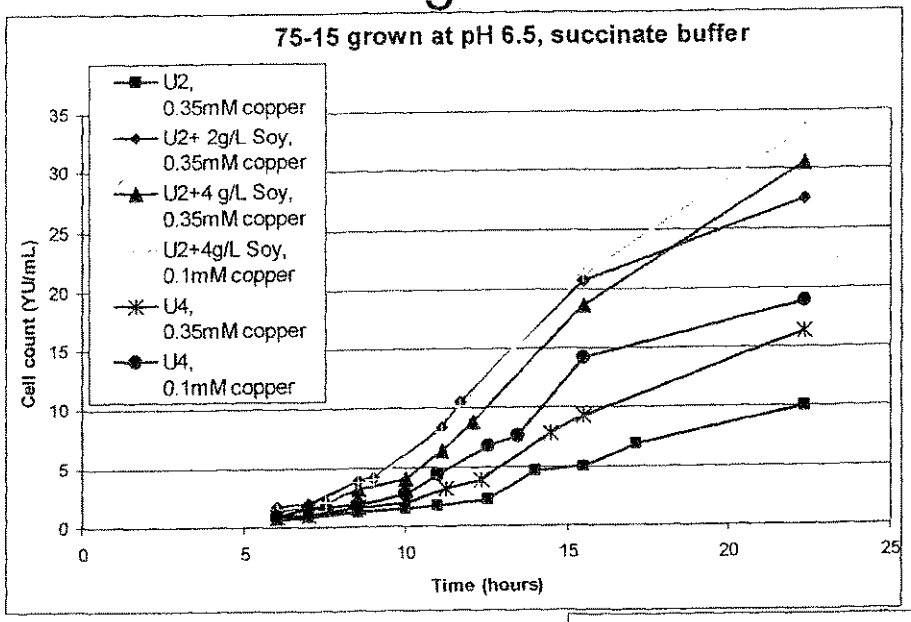


Figure 5 ~~Media formulation study~~



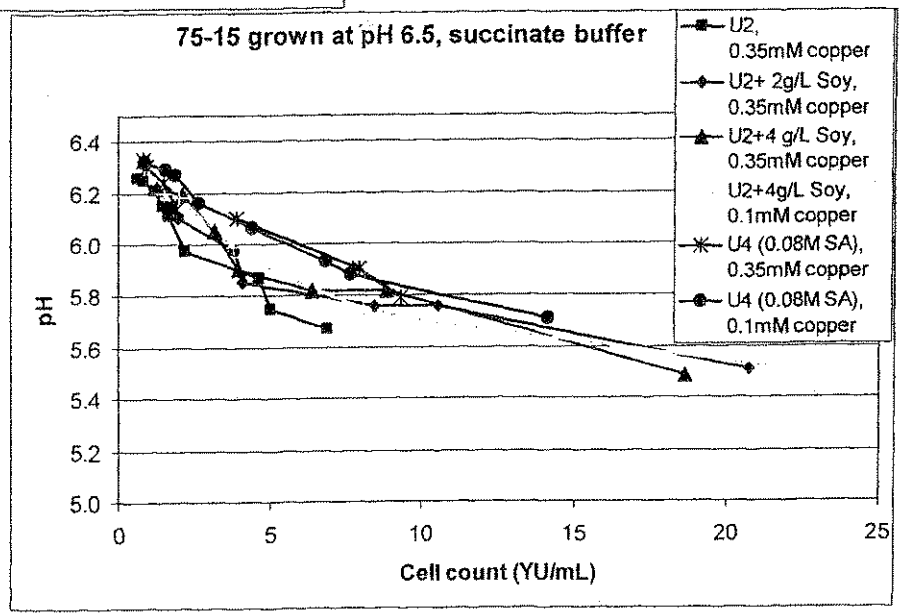


Figure 6 Detection of HA expression of yeast

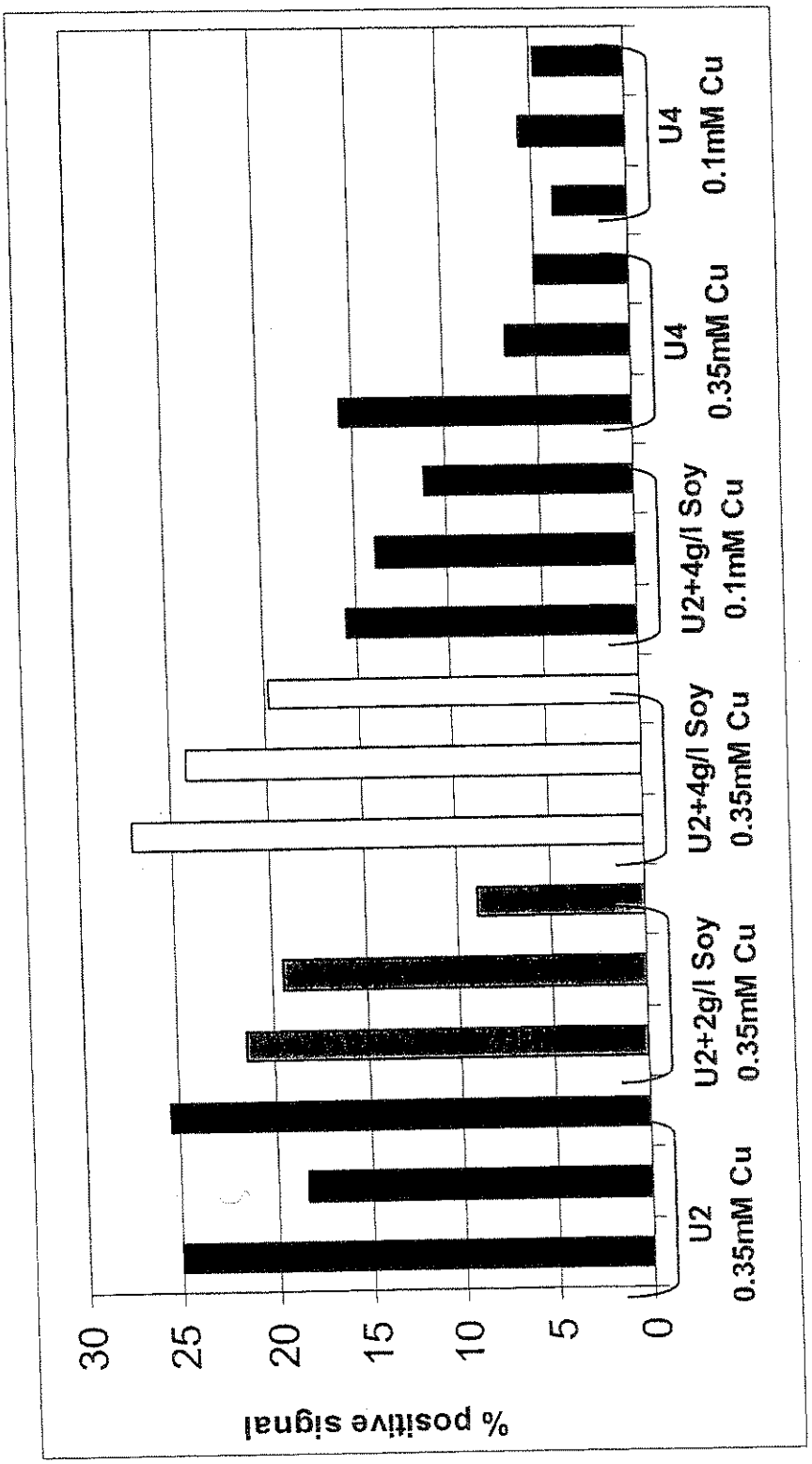
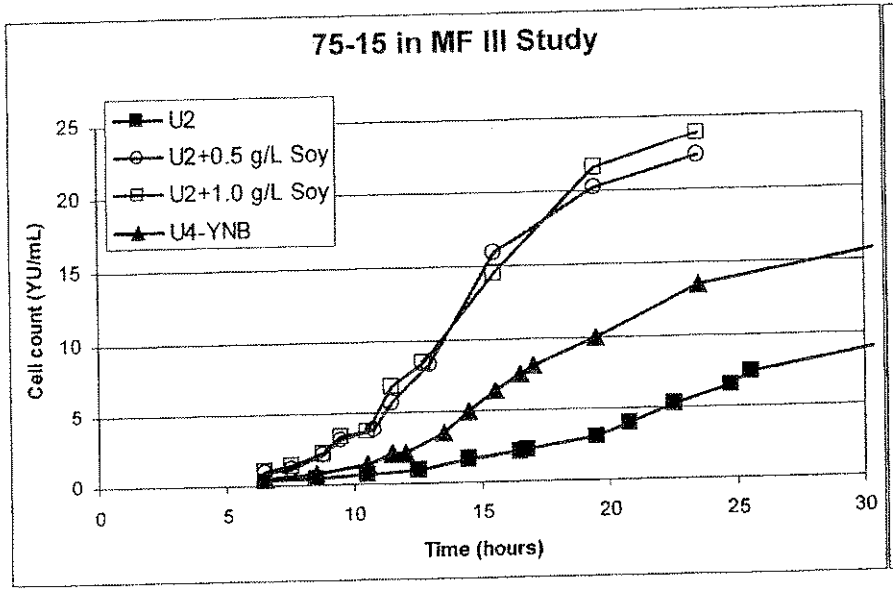
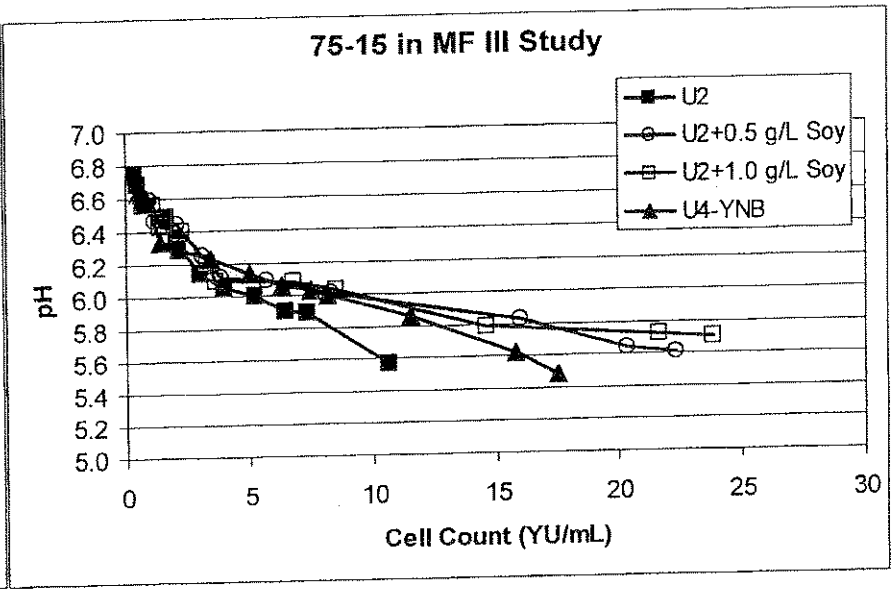


Figure 7 Media formulation study on cell growth and pH profiles





Media	Doubling Time (hours)	Cell count at "peak"	Count when pH=6
U2	4.0	~7 YU/mL	5 YU/mL
U4-YNB	2.5	~10 YU/mL	8 YU/mL
U2 + 0.5 g/L Soytone	2.0	~15 YU/mL	8 YU/mL
U2 + 1.0 g/L Soytone	2.0	~15 YU/mL	8 YU/mL

Figure 8 ~~The effect of media pH levels on antigen~~

Grown in BT-UDM **(pH 6.7-6.8 at harvest)**	Grown in UDM (pH 4.0-4.3 at harvest)
1- YEX w/ Cu2+	2- YEX w/ Cu2+
3 – GI8103	4 – GI8103
5 – GI8103 w/ Cu2+	6 – GI8103 w/ Cu2+



➢Cells grown in pH 7, but not pH<5, have glycosylated surface HA that can be released by DTT

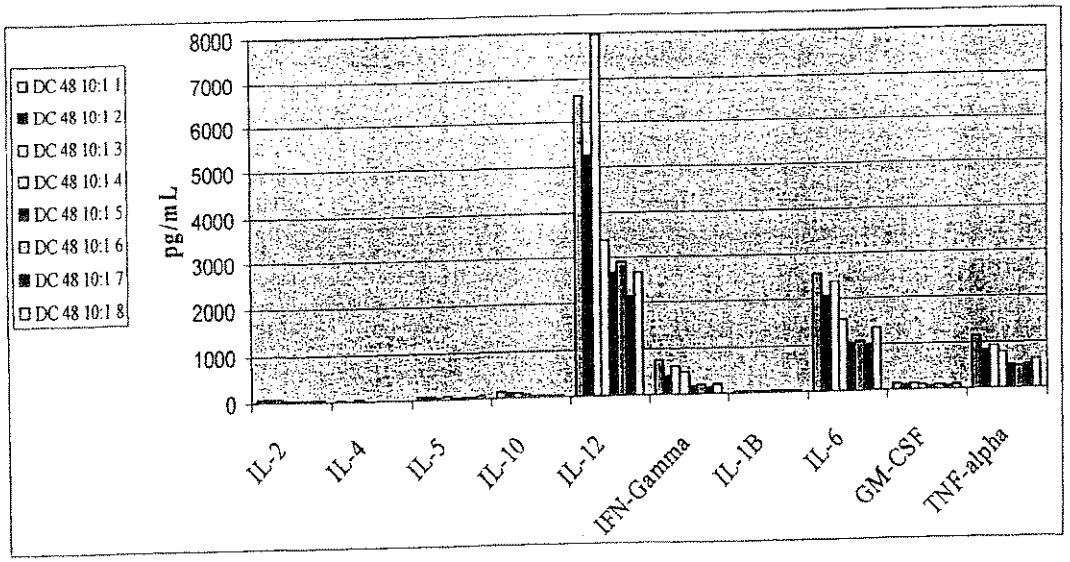
Novagen Anti-His MAb 1:1000

NB 126-51

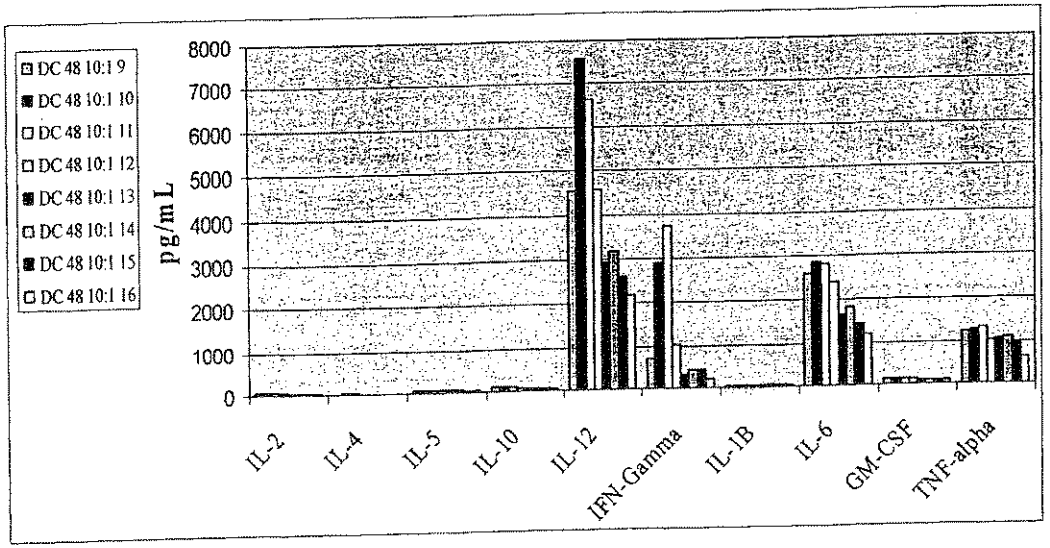
Figure 9 ~~The effect of neutral pH on cytokine production~~

Live vs. H-K
Low pH grown
DC 10:1 48hr

Sample #	Harvest density
9, 13	0.6 YU/mL
10, 14	2.6 YU/mL
11, 15	3.3 YU/mL
12, 16	6 YU/mL



Live vs. H-K
Neutral pH grown
DC 10:1 48 hr



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1. (WO2008097863) METHODS FOR PRODUCING YEAST-BASED VACCINES

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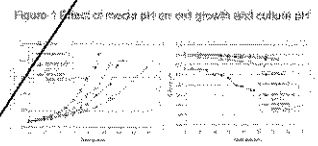
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Priority Data: 60/899,281 02.02.2007 US

Title
(EN) METHODS FOR PRODUCING YEAST-BASED VACCINES
(FR) PROCÉDÉS AMÉLIORÉS DE PRODUCTION DE VACCINS À BASE DE LEVURES

Abstract:
(EN) The invention provides methods for culturing yeast at a neutral pH level. Yeast cultured under neutral pH conditions exhibit desirable characteristics useful for biological purposes, such as the development of vaccines, prophylactics and therapeutics. The invention also provides for compositions and kits comprising yeast grown using the methodologies disclosed herein.



(FR)L'invention concerne des procédés de culture de levures à un niveau de pH neutre. Les levures cultivées dans des conditions de pH neutre présentent des caractéristiques intéressantes, utilisables à des fins biologiques, par exemple dans le cadre de la mise au point de vaccins tant prophylactiques que thérapeutiques. L'invention concerne également des compositions et des trousses comprenant des levures cultivées conformément aux méthodologies décrites dans les présentes.

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