DETERMINATION OF THE OPTIMUM CONCENTRATION OF MICROELEMENTS AND VITAMINES AND THEIR EFFECTS ON THE PROPAGATION OF *Candida utilis* [+]

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Abstract

The study was conducted to know the effect of five Microelements (Copper, Iron, Zinc, Calcium and Magnesium) and two vitamins (Biotin and Thiamine) on the activity and propagation *of Candida utilis* in data extract and molasses medium.

The results showed that the absence of these minerals and vitamins caused a significant decrease of growth and the addition of both $Fe^{2+}$and Mg^{2+} ions increased the yield of biomass 6 times , while the other ions (Ca^{2+} , Cu^{2+} and Zn^{2+}) caused a little effect on the growth which ganl a dry weight nearly (0.43 , 0.45 and 0.48) g/10 0 m l.

The results indicated also that biotin in the concentration of 0.1 Mg/L gave the best dry weight, whereas thiamine in 10 Mg/L was necessary for good growth.

المستخلص

تم دراسة تاثير العناصر المعدنية(النحاس،الحديد ،الزنك،الكالسيوم والمغنسيوم)والفيتامينات(البايوتين والثيامين)في تكاثر وفعالية خميرة *Candid utilis* عند تنميتها على عصير التمر والمولاس كوسط غذائي.

وقد اوضحت النتائج المستحصل عليها ضعف نمو الخميرة عند عدم احتواء وسط النمو على العناصر المعدنية والفيتامينات. حيث لوحظ بان كلا من ايونات Fe^{+2} و Mg^{+2} كان لها تاثير موجب قوي على نمو هذه الخميرة بحيث زاد من معدل نموها حوالي مايقارب ٦ مرات في حين اظهرت ايونات Cu^{+2} وCa^{+2} تاثير قليل على نموها وقد لوحظ تاثير مشابه لايون النحاس التي اعطت كتلة حيوية مقدارها (0.48,0.45,0.34 g/100ml).

Introduction :

The growth of any microbial organism can be considered as a continuous process of interaction between the characteristics of the organism and the environmental conditions in which it is being grown.

Yeast generally grow very well when the culture medium contain some microelements , e.g. Carbon , Oxygen, Nitrogen , Phosphorus and Sulphur to build up the cell constituents , Microelements such as Iron , Zinc and Magnesium in minute quantities as activators of enzymes are also essential for the growth of the yeast (1).

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A very et al., (2) reported that both Copper and Iron are essential nutrients for yeasts which act as cofactors in several enzymes , including the redox of the respiratory chain .youatt (3) reported also the physiological roles of $Ca^{"+}$ Ions in yeast growth and division . While Zhao and Eide (4) demonstrate the roles of Zn^{2+} in the growth of *Saccharromyces cervisiae* growth.

In addition to the nutrient element requirements detailed above, growth factors are also required either as an absolute requirement or in promotion of growth and optimal Cell yield, such as Biotin and Thiamin (l).

From these it produces a single cell protein, which is use in cattle food.

Materials and Methods :

Yeast strain

C. *utilis,* local strain (Biotechnology Department, college of science, University of Baghdad). Was used for all of the experimental work. A stock culture of the organism was maintained on Sabouraud Dextrose Agar (SDA) at 4°C.

Growth and preparation of Organism

The inoculum was prepared by transferring yeast cells from slant to 200ml sterilized synthetic medium, the flasks were subjected to the Orbital previously adjusted at 37°C and 180 rpm and incubated for 28 hrs.

To investigate the effect of metal salts and vitamins on the growth and activity of yeast cells , the following synthetic medium was used :

NH_4Cl	4g/L
KH_2PO_4	lg/L
K_2HPO_4	Ig/L
$MgSO_4.7H_2O$	0.5 g/L
Biotin	1Mg/L
Thiamine-HCl	50 Mg/L
$ZnCl_2$	0.5 mg/L
$FeSo_4.7H_2O$	0.5 mg/L
* Sugar	
$CaCl_2.2H_2O$	0.5 mg/L
$CuSO_4.5H_2O$	0.5 mg/L

* As date extract and molasses at the rate of (2:1) at the concentration (2.5%) (5).

Triplicate flasks containing sterilized media of different treatments were inoculated aseptically with 12 $x10^8$ cell/ml of already prepared inoculum. At the end of the incubation period (28 hrs) culture was harvested by centrifugation at 6000 Xg for 10 minutes to determine the yeast dry weight, and ATP.

Measurement of yeast activity

The activity of yeast cells was measured according to ATP production by sampling the yeast suspension in a final concentration 5% (v/v) $HclO_4$ on cooling, the mixture was left for 30 minutes in ice cold water bath, the supernatants were collected by centrifuging at 6000 Xg for 10 minutes and ATP was determined

spectrophotometrically by following the increase in absorbance at 260 nm, which converted to ATP concentration by using standard curve (5).

Results and Discussion:

• Construction of achemically 0 defined medium for production yeast Biomass.

When *C. utilis* is inoculated into an aerobic culture the growth and ATP- Producing activity of yeast cells varied with different cultivations with synthetic medium. Results in Fig. (l) Indicate that when the vitamin mixture or metal salt mixture was removed from the medium, the yeast showed no or only low growth, which interfere with ATP production (7). This may be due to the ability of *C. utilis* to grow under effects of different environmental factors and their interaction in *C. utilis* production and growth (1).

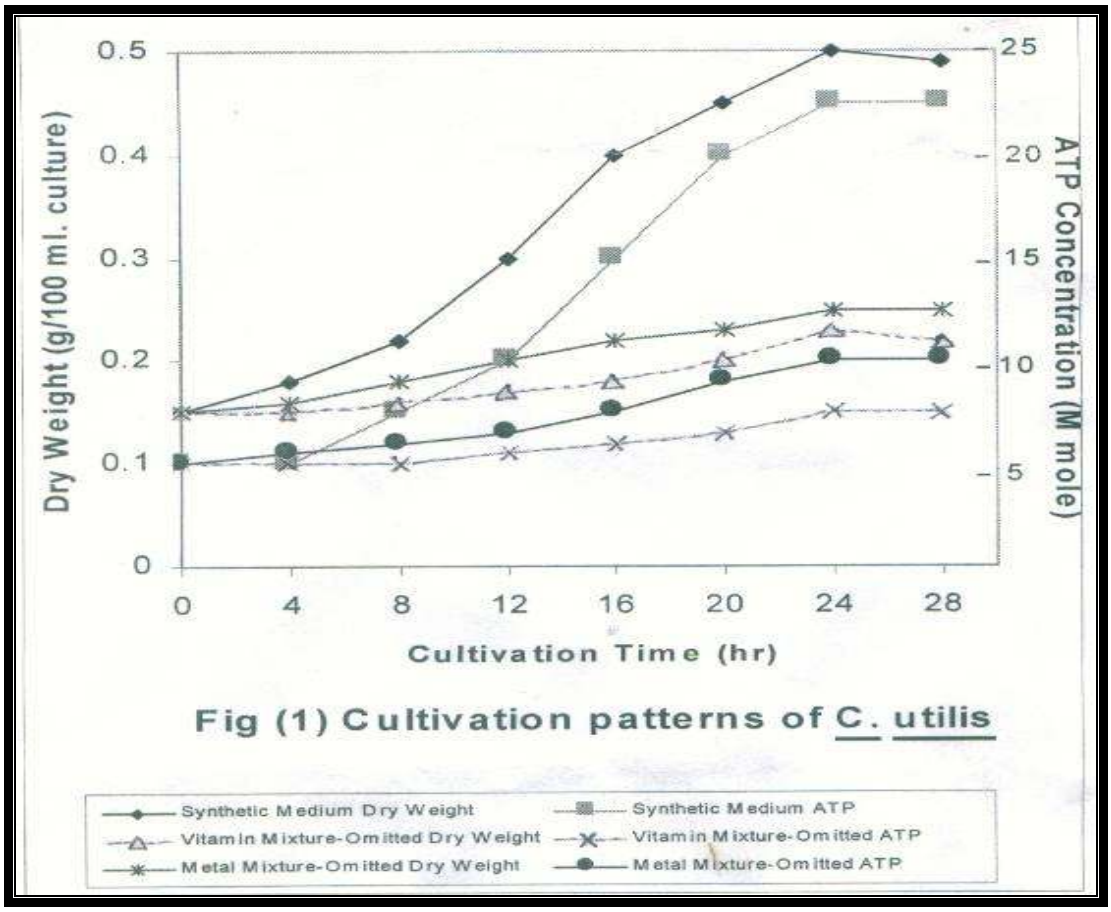

Fig (1) Cultivation patterns of C. utilis

• **Effect of Biotin**

Results in Fig. (2) Indicate that Biotin is essential for the growth and activity of yeast, it can be seen that the yeast required biotin at a concentration of 0. 1Mg/L for full growth.

This could be due to the fact that C. *utilis* was characterized as a biotin- required yeast (1), because it was involved in several areas of yeast metabolism including (6):

Synthesis of protein, nucleic acid, formation of poly saccharides and fatty acids. Therefore, Biotin is commonly added to promote growth and from their experiments the low quantity of Biotin added to synthetic media does not reflect the major important of this vitamin (6) which is essential to yeast growth.

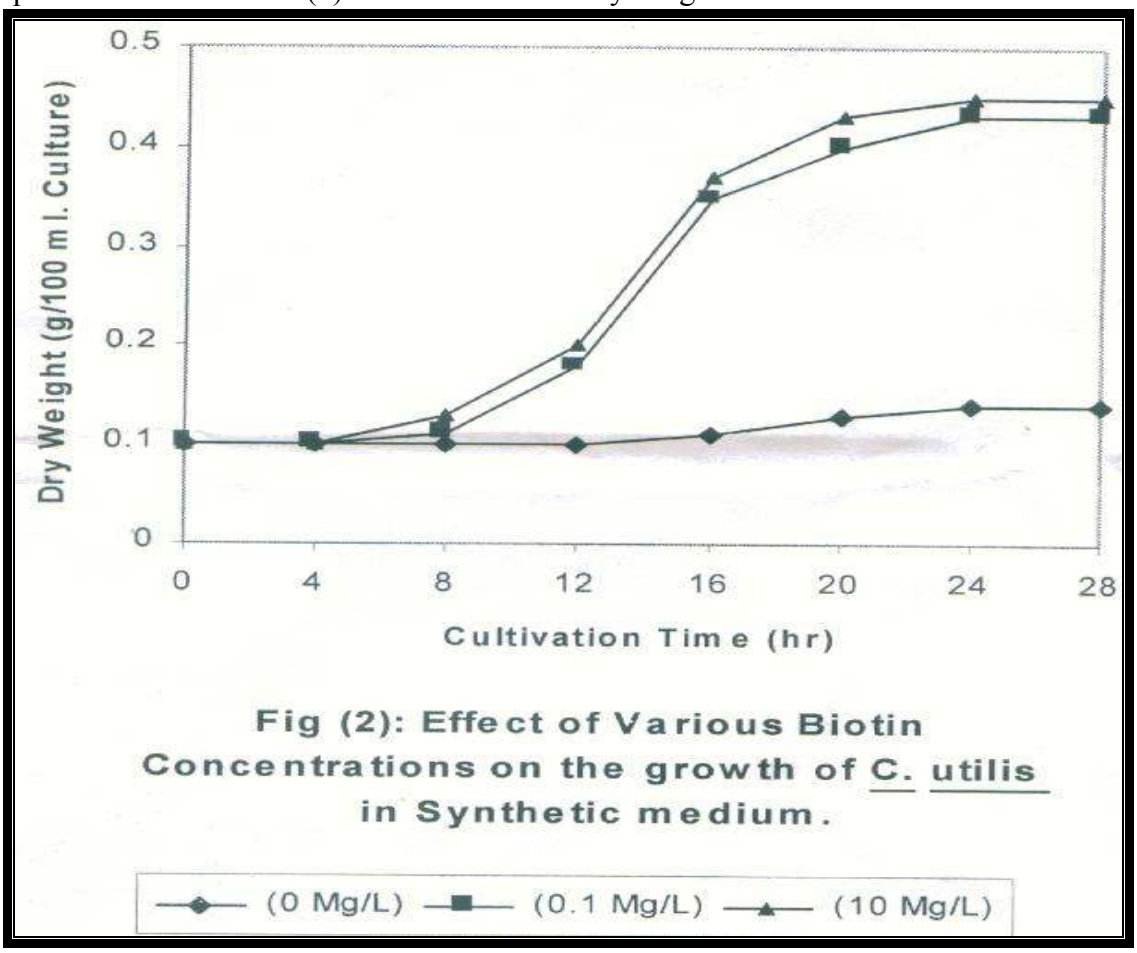

Fig (2): Effect of Various Biotin Concentrations on the growth of C. utilis in Synthetic medium.

•Effect of thiamine

The effect of thiamine or vitamin B_{12}eficirncy on yeast physiology has been studied and the results in Fig. (3) show that thiamine was not essential for growth but accelerated the growth rate of the yeast (8), therefore the minimal concentration of thiamine- HCL for good growth was 10 Mg/L .which is necessary to maintain optimal physiological activities of C. *utilis,* because it plays an important role in several yeast metabolism including: Glycolysis , fatty acid , sterol and lipid formation and also respiratory competence(9).

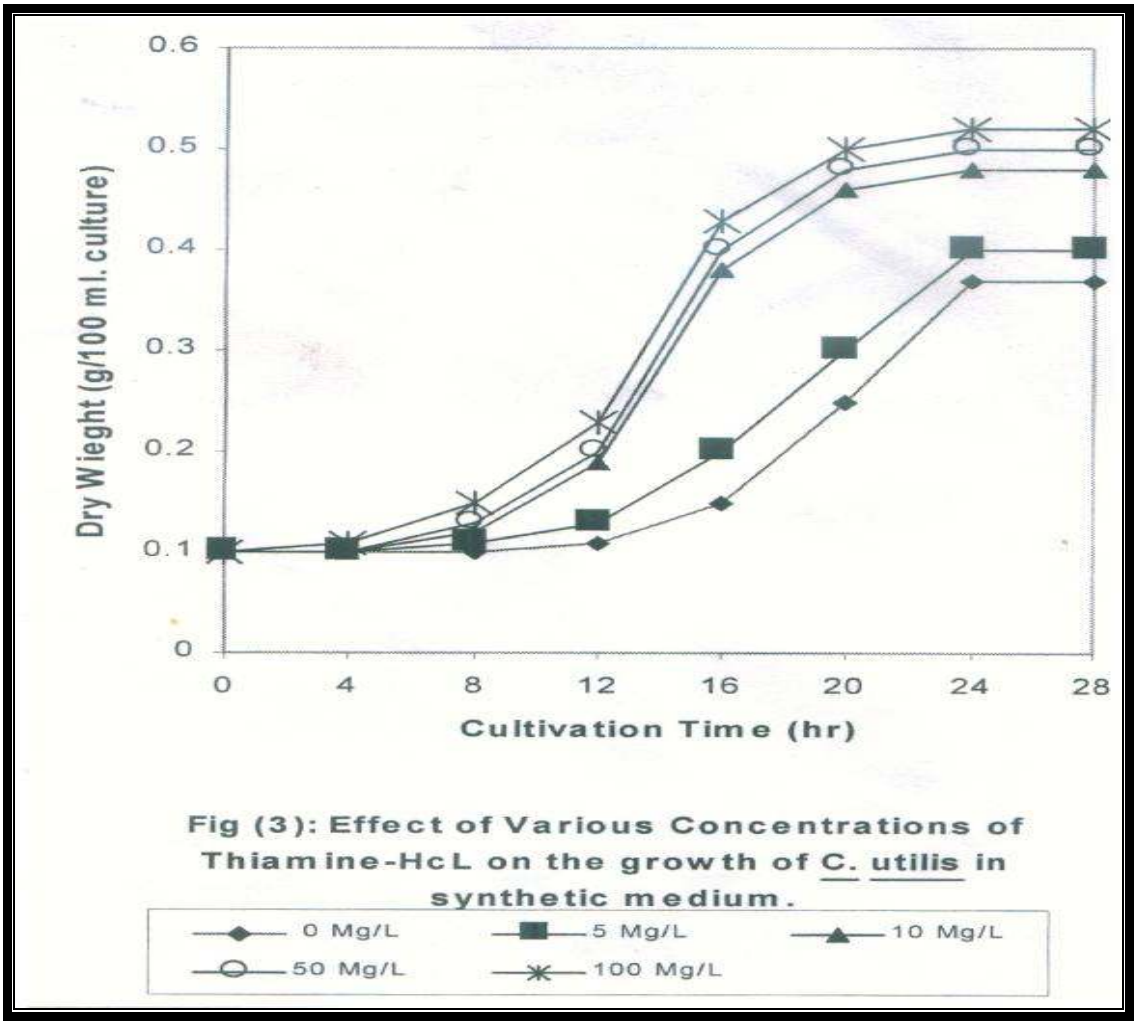

Fig (3): Effect of Various Concentrations of Thiamine-HcL on the growth of C. utilis in synthetic medium.

•Effect of metal salts

The metal sources in the synthetic medium consisted of Ca^{+2}, Cu^{+2}, Fe^{2+}, $Mg^{2+}$and Zn^{2+}as chloride or sulfate forms.

The result of growth experiments Fig.(4) with each metal salt Omitted showed that , Fe^{2+} and Mg^{2+} ions were effective for the growth of *C. utilis* which may be due to the fact that , Fe^{2+} and Mg^{2+} ions involved with many vital metabolic functions as components of coenzymes , for example they are known as a cofactor for an enzymes essential for ATP- production during energy transductions (10,11).

In an addition to this, the results showed that the omission of $Ca^{+2}$and Cu^{2+} ions hardly effected the cell growth.

This result was similar to results obtained by Walker (12) which found that the high levels of Ca^{2+} may be inhibitory to yeast due to antagonism of essential Mg^{2+} - dependent functions, therefore concentrations as low as 10-9 M have been reported by Youatt(3).

Also Avery et al., (2) demonstrated as the toxic effect of high level of copper ions towards yeast cells, therefore yeast require aminute amount of copper. Where as the results in the present study shows no effect on the dry weight was observed when molasses medium was used.

While Zn^{2+} ions showed a clear negative effect on the growth, which may be due to the role of Zn^{2+} ions as a potent all esoteric inhibitor and also an essential cofactor for yeast AMP deaminase to IMP which decreased the ATP yield in the yeast cell (4,13).

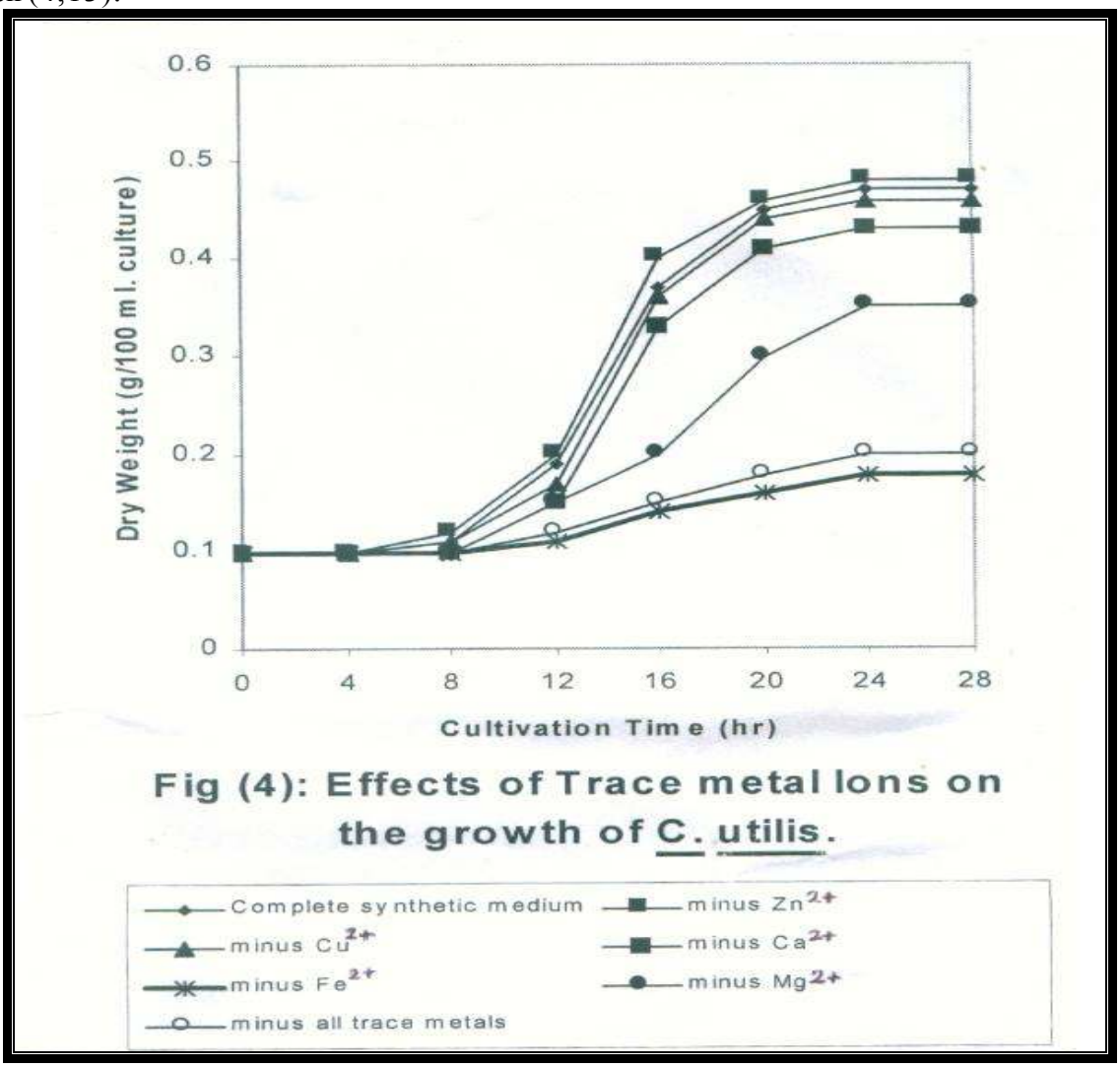

Fig (4): Effects of Trace metal Ions on the growth of C. utilis.

Recommendations :

More attempts are required to elucidate its genetic basis of local isolatel *C.utilis* to obtained amutant whose give the high activity of growth and used as "Food yeast".

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