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THE ROLE OF SOME ADDITIVES ON THE PRODUCTION OF VITAMIN B_{12} AND FOLATE USING *KLEBSIELLA PNEUMONIAE*

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ABSTRACT

The effect of different additives (cobalt , zinc, magnesium, iron and amino acids L-glycine, glutamic) on the production of B_{12} using *Klebsiella pneumoniae* were investigated. It was grown on mixture of agriculture wastes (wheat straw and rice bran). The results showed that the best B_{12} out puts (104.41, and 67.20 ug/l) were obtained at 2mg/L Fe^{+2} for both vitamin B_{12} and folate, respectively. On the other hand, the lowest induction effect was noticed by the addition of magnesium since, it gave 41.40ug/l of vitamin B_{12}compared to the control. The statistical analysis of the obtained results had been carried involving analysis and variance (ANOVA) and least significant difference (LSD) .The data showed that addition of Fe^{+2}, Zn^{+2} , Co^{+2} and glycine were highly significant compared to the other sources under investigation.

KEYWARDS: Production, B_{12}, folate, *Klebsiella pneumonia.*

INTRODUCTION

VitaminB_{12} like other B vitamins is important for the metabolism. It helps in the formation of red blood cells and in the maintenance of the central nervous system.[1] The deficiency of B_{12} is associated with hematologic, neurologic and psychiatric manifestation it is a common cause of megaloblastic anemia.[2] Also the deficiency may exert indirect cardiovascular effects in addition to the hematologic and neuropychatric manifestation.[3,4]

On the other hand, folic acid is necessary for fertility in both men and women .In men it contributes to spermatogenesis but in women enhance oocycte maturation placenta ion.[5] For the above mentioned factors and many other this work intended to investigate the effect of some additives on the production of B_{12} and folic acid under using some raw materials involving agriculture wastes wheat straw and rice bran for the magnification of the production process.

2-MATERIALS AND METHODS

2.1- Microorganisms

The microorganism used in the current work *Klebsiella pneumoniae* was isolated from fecal bacterial strains and identified in previously discussed work.[6] The fungus *Rhizopus nigricans* was provided from Natural and Microbial products Department, National research Center(NRC).

2.2-Substrate preparation

The agriculture wastes used (wheat straw, rice bran) in a mixture of 1:2 (w/w), respectively. These wastes were pretreated by cutting, grinding and alkaline hydrolysis with sodium hydroxide to separate the lignin components according to.[7,8,9]

2.3-Cultivation

Erlenmeyer flasks (250 ml) each containing 200 gm. of the solid agriculture substrate and 100ml sterile distilled water. Which previously inoculated with 1 X 10^6 spores of the fungus /ml and $4.5X10^8$ bacterial cell/ml , the initial pH was adjusted at 8.The flasks were incubated statistically at $30C^0$ for three days.[10,2,8] The flasks contents filtered to separate the culture growth and substrate from the culture media the filtrate was centrifuged at 4000 rpm for 10 min. The substrate consumed was also determined after each experiment by calculating the percentage of the difference of weight before and after the experiment. The contents of vit.B_{12} and folic acid were determined.

2.4-Estimation of vitamin $B_{12}$and folic acid

Radio-immunoassay (RIA) and HPLC analysis were used for the simultaneous quantitative determination of vitamin B_{12} and folic acid in comparison with standard according to the methods described by.[11,12]

2.5-Statistical analysis

The obtained results were statistically analyzed using the analysis of variance (ANOVA) to determine the degree of significance for the variation of both vit.B_{12} and folic acid yields. F test and the least significance different (LSD) at 0.05 level was also calculated for the mean values .All the statistical methods were applied according to the method described by.[13]

3-RESULTS AND DISCUSSION

3.1-Cobalt concentration

Cobalt plays important role in microbial metabolism. Different cobalt concentrations (0.5,1.0, 2.0, and 3.0 mg/l) were added as cobalt chloride to the fermentation medium. The results presented in table (1) showed that at cobalt conc. (0.5mg/l) produce the maximum out puts (42.37 and 49.29 ug/l)of B_{12} and folate, respectively . At the higher concentrations (1, 2, 3 mg/l) the yields of the both products were considerably decreased[14] The statistical analysis showed that the addition of different cobalt concentrations were highly significant ($P < 0.001$) for vitamins B12 and folate the medium constituents showed moderately significant ($p<0.001$).

Table (1): Effect of different cobalt concentrations

Cobalt conc.(mg/l)	Vitamin B_{12}content(ug/l)	Folate content(ug/l)	Substrate consumption (%)
Control	19.37±0.3	36.32±0.2	10±1
0.1	22.14±0.8	36.45±0.3	11±1.5
0.5	42.37±0.9	49.29±0.6	11±1
1	25.11±0.3	40.78±0.7	9±0.0
2	22.54±0.4	36.16±0.5	10±0.1
3	17.89±0.6	29.44±0.4	10±0.2
P value	***	***	NS
Ftest	196	101	2.3
LSD (at 0.05)	1.10	1.32	1.73

Initial pH 8, incubation period 3 days, temp. 30 C^o, substrate W.S + R.B) ratio 1:2 w/w) substrate weight 200 g/l

*** highly significant ($p< 0.001$) NS non significant ($p > 0.05$)

Control without additions

3.2-Zinc concentrations

In the present experiments different concentrations of zinc (0.5, 1, 2, 3 mg /l) were added to the fermentation medium . The data in table (2) showed that at the lowest concentrations of

Zinc sulphate (0.5mg/l) the maximum yields of (58.78 and 44.67ug/l) were obtained for Vitamin B_{12} and folate, respectively.[9] on the other hand, at the higher concentration (1,2,3mg/l) the out put of the products were considerably reduced out puts.[15,16]

The statistical analysis of the obtained data revealed that the addition of zinc showed to be highly significant ($p<0.001$) for both vitamin B_{12} and folate productivity and was moderately significant ($p<0.01$) for substrate consumption.

Table (2) Effect of different concentrations of zinc

Zinc conc.(mg/l)	Vitamin B_{12}content(ug/l)	Folate content(ug/l)	Substrate consumption (%)
Control	19.37±0.3	36.32±0.2	10±1
0.1	42.14±0.8	42.45±0.7	11±1.5
0.5	58.78±0.4	44.67±0.5	11+1
1	47.41±0.3	40.78±0.8	9±0.0
2	45.34±0.2	36.16±0.6	10±0.1
3	41.8±0.8	29.44±0.4	10±0.2
P value	***	***	NS
F test	471	89	7.2
LSD (at 0.05)	1.13	1.31	1.29

Initial pH 8, incubation period 3 days , temp . 30 C^o , substrate W.S + R.B) ratio 1:2 w/w) substrate weight 200 g/l .*** highly significant ($p< 0.001$)NS non significant ($p > 0.05$) Control without additions.

3.3- Magnesium concentration

Different concentration of magnesium sulphate (25, 50, 75,and 100mg/l) were added as magnesium sulphate to the fermentation medium. The results presented in table (3) showed that the best vitamin B_{12} and folate (43.41 and 49.78ug/l) were obtained at 75mg/l Magnesium sulphate concentration.[17] on the other hand , the concentrations below and above 75mg/l of magnesium sulphate showed reduced concentrations of B_{12} and folate. The statistical analysis of the obtained data revealed that the Addition of magnesium showed to be highly significant ($p<0.001$) for both vitamin B_{12} and folate productivity and was moderately significant ($p<0.01$) for substrate consumption.

Table (3): Effect of different concentrations of magnesium

Magnesium conc.(mg/l)	Vitamin B_{12}content(ug/l)	Folate content(ug/l)	Substrate consumption (%)
Control	19.36+0.3	36.32+0.2	10+1
25	26.14+0.8	35.45+0.7	11+0.0
50	28.78+0.4	42.65+0.5	11+1.0
75	43.41+0.3	49.78+0.8	11+1.0
100	35.34+0.2	36.16+0.6	11+0.1
P value	***	***	NS
F test	471	89	7.2
LSD (at 0.05)	1.13	1.31	1.29

Initial pH 8, incubation period 3 days, temp . 30 C^o, substrate W.S + R.B) ratio 1:2 w/w) substrate weight 200 g/l

*** highly significant (p< 0.001)NS non significant (p > 0.05)

Control without additions

3.4-Iron concentrations

In the present experiment the effect of different iron concentrations (0.5, 1, 2 and 3 mg/l) were added as ferrous sulphate to the fermentation medium. The data presented in table (4) revealed a remarkable variations in the vitamins productivity, however the additions of 2 mg/l of iron produce noticeable increase in the B_{12} out put (104.41ug/l.

Table (4): Effect of different concentration of iron

Iron conc.(mg/l)	Vitamin B_{12}content(ug/l)	Folate content(ug/l)	Substrate consumption (%)
Control	19.37+0.3	36.23+0.2	10+1
0.5	77.94+0.8	60.23+0.7	15+0.0
1	85.78+0.4	67.23+0.5	15+1.0
2	104.41+0.3	58.97+0.8	16+1.0
3	100.70+0.2	55.47+0.6	15+0.1
P value	***	***	NS
F test	3684	230	25
LSD (at 0.05)	1.35	1.67	1.22

Initial pH 8, incubation period 3 days , temp . 30 C^o , substrate W.S + R.B) ratio 1:2 w/w) substrate weight 200 g/l

*** highly significant (p< 0.001)NS non significant (p > 0.05)

Control without additions.

The best folate out puts (67.23ug/l) was obtained at 1 mg/l[8,9]. The concentrations above 2mg showed slight low yields. The statistical analysis of the results showed that iron concentrations were highly significant (p<0,001) for both vitamin B_{12} and folate concentrations.

3.5-Glycine concentrations

The effect of different amino acid concentrations was tested for the effect oh the production of vit.B_{12} and folate. These amino acids were added separately to the fermentation medium. In the present experiment different glycine concentration (1,2,3,4 and 5 g/l) were tested.

The results presented in table (5) revealed that the maximum yields of Vitamin B_{12} (89.81ug /l) was obtained at 4g/l glycine concentrations. On other hand the maximum folate (43. 93ug/l) was obtained at 3 g/l glycine concentrations[10,17] The results of the statistical analysis showed that the addition of glycine to the fermentation medium was highly significant (p<0.001) for both vitamin B_{12} and folate production, but non significant for the consumption of the substrate.

Table (5) Effect of different glycine concentration

Glycine conc.(mg/l)	Vitamin B_{12}content(ug/l)	Folate content(ug/l)	Substrate consumption (%)
Control	19.37±0.3	36.32±0.2	10±1
1	21.74±0.8	39.37±0.7	10±0.0
2	36.31±0.4	41.75±0.5	10±1.0
3	64.93±0.3	43.97±0.8	11±1.0
4	89.81±0.2	38.67±0.6	12±0.1
5	32.41±0.7	37.06±0.5	11±0.0
P value	***	***	NS
F test	`380	68	1.9
LSD (at 0.05)	1.26	1.07	1.82

Initial pH 8, incubation period 3 days, temp. 30 C^o, substrate W.S + R.B) ratio 1:2 w/w) substrate weight 200 g/l

*** highly significant (p< 0.001)NS non significant (p > 0.05)

Control without additions

3.6-glutamic acid concentration

Different concentrations of L- glutamic acid (1,2,3,4, and 5 g/l) were added to the fermentation medium. The results presented in table (6) showed that the addition of l glutamic acid showed greater effect on the production of vitamin B_{12} and folate. A slight

increase in the out puts (43.29 ug/l) was obtained at 2 g/L. The best out put (53.81ug/l) was obtained at 4g/l. Furthermore, increase in the amino acid concentration gave a remarkable decrease was noticed.[17,18]

Table (6) Effect of different L-glutamic concentration

L- Glutamic acid conc.(mg/l)	Vitamin B_{12}content(ug/l)	Folate content(ug/l)	Substrate consumption (%)
Control	19.37±0.3	36.32±0.2	10±1
1	40.94±0.8	52.37±0.7	12±0.0
2	43.31±0.4	53.75±0.5	13±1.0
3	45.93±0.3	58.97±0.8	13±1.0
4	53.81±0.2	59.67±0.6	14±0.1
5	32.41±0.7	54.06±0.5	14±0.5
P value	***	***	NS
F test	52	229	9.8
LSD (at 0.05)	1.61	1.14	1.15

Initial pH 8, incubation period 3 days, temp. 30 C^o, substrate W.S + R.B) ratio 1:2 w/w) substrate weight 200 g/l.

*** highly significant ($p < 0.001$)NS non significant ($p > 0.05$)

Control without additions

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