Using Paper Waste and Local Bacterial Isolates to Produce Biomass and Cellulase Enzyme- A Study Waste Recycling

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Abstract

The study aims to isolate the cellulose-degradation bacteria that produce the cellulase enzyme as well as the production of SCP protein from Ramadi city soils and to obtain efficient local bacterial isolates in production and efficient isolation was selected in the production of cellulase enzyme and single cell protein which is *Bacillus subtillis* bacteria tested the efficiency of the isolated isolation to produce the enzyme and single cell protein. The cell using local media contains the paper waste available in the environment and contaminated with it. Initial transactions were made for this paper waste and these factors included (heating to boiling point, dilute sulfuric acid and dilute sodium hydroxide). The paper waste used included the white cardboard, yellow cardboard and white paper Which was used as a single source of carbon and energy, after testing the waste paper used, it was found that the white cardboard treated with heating gave the best production of the cellulase enzyme and single-cell protein. The bacterial isolation used showed the best growth and gave the highest production of the enzyme and the single cell protein at pH 7, while the best temperature for the production of the enzyme was 45 ° C. The optimum temperature for the production of the single cell protein was 40 ° C. As for the carbon concentration, which is the white cardboard treated with heating, it was better to produce than Concentration is 2%. As for the production of single cell protein, the highest output at concentration was 4%. The best bacterial vaccine size for enzyme production was 3 ml / 100 ml medium. As for the production of single cell protein, the size of the inoculum gave 1.5 ml / 100 ml the best production. The addition of the organic nitrogen source (urea) gave a clear increase in enzyme production and single cell protein, and when adding 0.5 g urea / 100 mL medium. Finally, the results showed that the best period duration to obtain the highest cellulase enzyme production was after 96 hours, while it was found that the best production of single-cell protein was after 24 hours incubation.

Key words: *cellulose, cellulase enzyme, single cell protein (SCP)*

Introduction

Paper, industrial and agricultural wastes and a lot of wastes containing carbohydrates cause accumulating a great pollution in nature. These wastes are an essential source of cellulose[1]

In order to get rid of these residues and for the continuity of the carbon cycle in nature, there is a need for the cellulose-analyzing neighborhoods to be by producing these neighborhoods for the enzyme cellulose that stimulates the process of cellulose decomposition into short chains and to the final product which is glucose, and we can get from them an important final result that is economically beneficial [2] .

The cellulase enzyme (EC 3.2.1.4) is one of the enzymes that produce microbes, which stimulates the process of cellulose decomposition into cellulose and then glucose. In the first two steps, it converts long cellulose chains into short chains, then the second step is by splitting the cycloid bonds (β, 1- 4) and converting the chains Short cellulosic to melted sugars[3]

Paper waste and industrial waste were exploited in the field of biotechnology, as Biomass was produced using the Submerged Cultures technology and the production of single cell Protein with high nutritional value, according

to the Food and Agriculture Organization (FAO), the percentage of single cell protein (SCP) Single Cell Protein ranges between 60-90% of the dry weight of the microbial cell [4].

Materials and Methods

ؤCulture media : An isolate of *Bacillus subtilis* was used, isolated from the soil of Al-Jazeera in Ramadi and planted with okra crop. The local symbol was given E7. The bacterial isolation was grown on the production medium consisting of (2 g ammonium nitrate, 0.5 g potassium dihydrogen phosphate, 0.2 g aqueous magnesium sulfate, 20 mg aqueous calcium chloride, 20 mg aqueous manganese sulfate, 20 mg aqueous ferrous sulfate, 0.2 g yeast extract, 0.2 g peptone [5]and used three carbon sources of paper waste including (yellow cardboard, white cardboard and white paper) after initial treatment For paper waste, the farm was sterilized and incubated at a temperature of 30 m and a pH 7-7.4 for a period of three days using a 1% inoculum and a CFU is 5 x 10^7 colony formation units / ml. conical flasks technique was used using 250 ml and put in each flask 100 ml of the farm .

Cellulase enzyme assay : The enzyme activity was measured according to a method[6], and the reducing glucose (glucose) was estimated according to the method[7] and using the spectrophotometer at a wavelength of 540 nm**.**

Single cell protein production: was obtained using a liquid medium containing paper waste as a single carbon source, distribute the medium in 250-ml flasks, sterilize the farm and put the bacterial vaccine and incubated for three days, after which the bacterial cultures were filtered using filter papers to separate the portion The steel (precipitate) from the filtrate of the farm in which the enzyme is present. The precipitate was washed with distilled water and dried in the oven at 60 ° C for 24 hours, then the dry biomass was weighed by the sensitive scale to estimate the weight of the dry biomass which represents the final product of the single cell protein [8]

Optimization environmental conditions for the growth of selective and degraded bacterial isolates of cellulose and single cell protein production:

The liquid medium containing the paper waste was used as the only source of carbon and energy in determining the factors affecting the production of the enzyme and the single cell protein, as it used conical flasks with a capacity of 250 milliliters and put in each of them 100 milliliters of the liquid medium and sterilized the decanters in the receptacle. And incubated in a rocker incubator at a speed of 120 shake / minute using various criteria including the pH, adjust the pH of the production medium (5,6,7,8,9,10) to determine the optimal pH for the production of the enzyme and single-cell protein for the bacterial isolation used. Different temperatures 20,25,30,35,40,45,50 C were selected to determine the optimum temperature for the production of the enzyme and protein. For the purpose of determining the appropriate carbon source for growth, three carbon sources containing cellulose were used: yellow and white cardboards, and white office paper as they were ground after Carrying out initial transactions for it, and the white carton was chosen in the light of the result obtained from this test, as the white cardboard gave the highest production value of the enzyme and single cell protein. The cell also used different concentrations from the carbon source. The white cardboard included 0.5,1,1.5,2,2.5,3,4 g Also, different vaccine volumes were used for the purpose of vaccinating the liquid medium from the selected bacterial isolation 0.5,1,1.5,2,2.5,3g Also, a nitrogen source such as urea and sodium nitrate was added in different concentrations, which are 0.5,1,1.5,2 g for the purpose of improving growth and increasing the production of enzyme and single cell protein. In light of the results obtained from the previous steps, I used different lap time periods that included from one day lap to Six days, the enzyme activity was estimated and the amount of biomass measured to determine the best bosom duration.

Statistical Analysis

All experiments were designed according to Complete Randomize Design (CRD) and using the system of global experiments. Statistical analyzes were carried out using Genestat multi-factor program and using the value of the least significant difference for LSD (Least signification difference) at the 5% probability level.

Results and Discussion

Optimal conditions for the production of cellulase and single cell protein:

The results shown in Table (1) showed that the enzyme productivity using *Bacillus subtilis* at pH 7 was higher than the other pH numbers after 3 days of lap, as the enzymatic efficacy reached (0.827 units / ml). As for the single-cell protein, it was higher Its value is at pH 7 and the amount of dry weight is (1.102 g / 100 ml). The effect of the pH on the production of the enzyme comes through its effect on the characteristics of the nutritional medium and the solubility of nutrients and their readiness for the organism. This in turn is reflected in the growth of the organism and the production of the enzyme as the pH affects In the direction of the course of metabolism, synthesis and enzyme production [9] . .

Table (1) shows the effect of pH on cellulase enzyme and single cell protein production

PH	Dry weight gm / 100ml	Enzymatic activity Unit / ml
5	0.572	0.588
6	0.987	0.797
7	1.102	0.827
8	0.959	0.157
9	0.919	0.155
10	0.843	0.239
	L.S.D=0.035	L.S.D=0.025

As for the effect of temperature on the productivity of bacteria for the enzyme and single cell protein The highest productivity of the enzyme was at a temperature of 45 ° C and using the bacterial isolate *Bacillus subtilis* at pH 7 and the carbon source was the white cardboard, as the enzyme activity reached (0.398) units / ml .

As for the single cell protein, its best yield at the temperature was 40 º C. As shown in Table (2), as the production of the single cell protein at this temperature reached (1.438 g / 100 ml)

The reason for the increase in the enzyme productivity due to the increase in temperature may be attributed to the fact that the high temperature affects the speed of enzymatic reactions within the cell or some of the factors that help the growth of isolation, such as the decrease in the dissolved oxygen ratio[10]

Table (2) shows the effect of temperature on the production of cellulase enzyme and single cell protein

Temperature C	Dry weight gm / 100ml	Enzymatic activity Unit / ml
20	1.126	0.239
25	1.146	0.252
30	1.152	0.380
35	1.244	0.368
40	1.438	0.380
45	1.021	0.398
50	1.011	0.129
	L.S.D=0.045	L.S.D=0.069

As for the concentration of the carbon source, the results showed as indicated in Table No. (3), as it was found that the highest enzyme yield was obtained at the concentration of 2 g / 100 mL and the enzyme efficacy reached (0.538) units / mL after a 3-day bosom duration at pH 7 and temperature 45 M . As for the single cell protein, the concentration of the carbon source of 4 g / 100 mL gave the highest production of the unicellular protein and reached 4.609 g / 100 mL, while the remaining concentrations of less than 4 g recorded the lowest biomass and were the least productive for the unicellular protein (1.232 g / mL) In terms of dry weight, the production of single cell protein depends on the type of raw material used in production and the importance of choosing the base material that is a source of carbon and that will result in high biomass production in a less time[11]

Table (3) shows the effect of the concentration of the carbon source on the production of cellulase enzyme and single cell protein

Concentration of the carbon source	Dry weight gm / 100ml	Enzymatic activity Unit / ml
0.5	1.232	0.300
1	1.593	0.334
1.5	2.500	0.427
2	2.523	0.538
2.5	3.036	0.439
3	3.619	0.374
4	4.609	0.301
	L.S.D= 0.18	L.S.D=0.035

for the size of the vaccine in the production of the enzyme and the protein, Table (4) shows that the highest production of the enzyme from *Bacillus subtilis* when adding the size of the vaccine is 3 milliliters / 100 milliliters, and the enzymatic efficacy reached (0.558) units / ml after 3 days of incubation then the effectiveness decreased when increasing or the size of the vaccine decreased more than that, either when producing the single cell protein in terms of dry mass or the dry weight of the product, the size of the vaccine gave 1.5 ml / 100 ml the highest single cell production of 3.052 g / 100 ml medium while the sizes of the inoculum were recorded (0.5, 1, 2, 2.5, 3) (ml / 100ml) yields less than single cell protein. The decrease in enzyme production at the smallest sizes of the vaccine is due to the fact that the concentration of the vaccine is not sufficient to give a biomass from the isolation and thus reflects on the enzyme productivity. It may be due to the intense competition for nutrient exploitation in the middle [12]

Table (4) shows the effect of vaccine size on the production of cellulase and single cell protein

The size of the inoculum Ml	**Dry weight gm / 100ml**	**Enzymatic activity Unit / ml**
0.5	2.539	0.186
1	2.643	0.214
1.5	3.052	0.268
2	2.872	0.289
2.5	2.166	0.295
3	2.147	0.558
	L.S.D= 0.098	**L.S.D= 0.030**

As for the effect of the nitrogen source on enzyme and protein production, the results shown in Table (5) showed that the bacteria using *Bacillus subtilis* gave the highest efficacy of the enzyme cellulase using urea and reached (0.882) units / ml and was the highest efficacy of the enzyme when adding a concentration (0.5) g / 100 ml of urea, while the efficacy decreased slightly with the use of an inorganic source such as sodium nitrate at different concentrations such as (2,1,0.5 g / 100 ml medium) to reach (0.730) units / ml at a concentration of (0.5) g sodium nitrate / 100 ml

And single cell protein was the highest production when adding urea in different concentrations, as it reached its highest productivity when using a concentration of 0.5 g urea / 100 ml and the productivity was (3.683 g / 100 ml) and the productivity decreased when adding the inorganic nitrogen source (sodium nitrate) in different concentrations, reaching the highest output For single cell protein, when adding 0.5 g of sodium nitrate / 100 mL, the enzymatic activity is (2.522) units / ml .

Table (5) shows the type and effect of the nitrogen source in the production of cellulase enzyme and single cell protein

Concentration of nitrogen source gm/100ml	Enzymatic activity U / ml				Dry weight ml gm/100		
	Sodium nitrate	Urea			Sodium nitrate	Urea	
0			3.052				0.558
0.5	2.522	3.683			0.730	0.882	
1	2.037	2.207			0.638	0.778	
2	2.028	2.229			0.646	0.747	
	L.S.D=0.018	L.S.D=0.093			L.S.D=0.028	L.S.D=0.027	

Using the optimal production conditions that were shown by the previous steps, he studied the effect of the brood period in the production of the enzyme and protein. As shown in Table No. (6), the production of the enzyme increases with increasing the brooding period to reach a maximum after 4 days with an enzymatic efficacy of (0.996) units / ml of *Bacillus subtilis* bacteria. Then the effectiveness decreased with the increase of the bosom period to reach (0.550) units / ml after 6 days. This decrease in effectiveness with the increase in the bosom period may be attributed to the occurrence of environmental changes in the center of production as well as the possibility of self-dissolution of cells and the accompanying release of metabolic materials that affect Negatively in the enzyme productivity and this is confirmed by Aggelopoulos[13].

From the observation of Table (6), we find that the best production of the single cell protein was after a 24-hour bosom duration and the weight of the produced protein was (3.630) g / 100 ml medium, while the level of production decreased when the other brood period increased.

Table (6) shows the effect of brood duration on the production of cellulase enzyme and single cell protein

Incubate period	Dry weight gm/ 100ml	Enzymatic activity Unit / ml
1 day	3.630	0.798
2 day	3.520	0.816
3 day	3.062	0.819
4 day	1.883	0.996
5 day	1.703	0.822
6 day	1.454	0.550
	L.S.D=0.098	L.S.D=0.030

Implementation of optimal conditions for SCP production:

The optimum conditions were applied for the production of cellulase enzyme and single cell protein of pH, temperature and concentration of the carbon source, the size of the bacterial vaccine, the type of nitrogen source. After the end of the optimal brood period, the highest cellulase enzyme production was reached (0.996 u / ml) either As for the dry weight of the biomass, it reached (26.7 g / l) resulting from the decomposition of the paper waste used in the study, which is the white cardboard

Conclusion

The spread of cellulose-dissolving bacteria to varying degrees in agricultural soils in Ramadi city. The local isolate, *Bacillus subtilis*, possesses the highest capacity for analyzing cellulose from its various sources, carboxymethylcellulose, and paper waste (white and yellow carbord and white office paper), as well as having the highest activity of cellulase enzyme and the highest ability to produce a single cell protein. The possibility of using paper wastes available in the local environment as pollutants in many biological products through recycling them in microbial fermentation, and thus we achieve two goals to obtain certain cheap products and rid the environment of these pollutants .

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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