EFFECT OF BIOLOGICAL TREATMENTS BY CELLULOLYTIC BACTERIA ON CHEMICAL COMPOSITION AND CELL WALL CONSTITUENTS OF SOME ROUGHAGES

H.M. Gado; H.M. Metwally; H.S. Soliman and Etab R.I. Abd El-Galil

Animal Production Department, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Cairo, Egypt.

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SUMMARY

This paper considers the separation of five strains of cellulolytic bacteria (*Cellulomonas sp., Acetobacter sp., Thermonospora sp., Ruminococcus sp.* and *Bacillus sp.*) from rumen liquor of Baladi goats and evaluation of these bacteria by electrophoresis method. Then these bacteria were used as biological treatments of silage to study the changes that occur on chemical composition and cell wall constituents of three roughages. Silages were made from three roughages (corn stalks, bagasse and rice straw) and incubation for two months. The additive (additive/ DM,w/w)contained water 200%, urea 3%,molasses 4%, formic acid 0.03%, acetic acid 0. 5% and one of the cellulolytic bacteria at the rate of 2 liters/ ton. Results indicated that using cellulolytic bacteria caused marked increase in crude pro tein from average 1.98%to 15.16% and decrease crude fiber from average 52.6% to 38.1% in all roughages compared with the untreated roughages. All treatments significantly decreased NDF, ADF and ADL. It was concluded that the five strains of bacteria were different significant and all strains secretes cellulase enzymes according to the electrophoresis method. In addition, the biological treatments by *Cellulomonas sp.* and *Ruminococcus sp.* were succeeded more with bagasse and corn stalks while *Thermonospora sp.* and *Bacillus sp.* succeeded more with rice straw. However, the biological treatments of corn stalks, bagasse and rice straw silage improved its chemical composition and cell wall structure.

Keywords: biological treatments, cellulolytic bacteria, roughages, cell wall constituents,chemical composition.

INTRODUCTION

Analysis of roughages by detergent procedures (Goering and Van Soest, 1970) showed that they are high in lignin, cellulose and hemicellulose. Also, most cereal straw is characterized by low crude protein, low available energy and deficient in certain minerals. These low quality roughages are inefficiently utilized by

ruminants. This is due to low digestibility and poor nutritive value associated particularly with cereal straws. Their utilization is also limited because of low voluntary intake of the animals and their huge bulk, which makes transportation more costly (Batch 1976 and El-Shimmawy 1990). Agricultural residues, such as wheat straw, contain considerable quantities of cellulose and hemicellulose that can be excellent energy sources in ruminants feed (Detroy *et al.*, 1980). The availability of these components is controlled by lignin – carbohydrate complexes, which limit the digestion of cellulose and hemicellulose. Most of the linkage types are not hydrolysable (El-Moyson and Verachtert, 1991). The carbohydrate components of cereal straws represent a potential source of dietary energy for ruminants; the structural polysaccharides, which comprise the carbohydrate fraction are, however, only partially degraded by rumen microorganisms severely limiting the value of modified straw as a feed component (Chesson *et al.*, 1983). Cereal straw is composed primarily of cellulose and hemicellulose, which could form part of the feed as roughage for ruminants. Unfortunately, its low digestibility and protein contents, poor palatability and bulkiness, discourages its use as the sole source of feed. Straw, the major roughage of ruminants, contains very little protein and mineral and very high amount of lignin and silica. The lignin component creates the barrier to efficient utilization, conversion or degradation of the polysaccharides in lignocelluloses to useful products. Lignocellulosic residues are not high value feeds, they are classified as low quality roughage, i.e. high in fiber, low in protein, vitamins and minerals and high lignifications make them less utilization than the green fodder. Physical, chemical and microbiological treatments of the cellulosic materials have been tried for improving the nutrient availability from such materials to the animals (Lyo and Antai, 1988; Singh *et al.*, 1993;McHan, 1986 and Hunt *et al.*, 1992). Intake and utilization of low quality roughages may be increased by supplementary feeding and by various pretreatment (Devendra, 1985). The plant cell wall, or fiber, is not a discrete entity: its composition depends on a wide number of variables, the species of plant, the botanical of the plant, the environmental conditions and the states of health of the plant, all have an effect. Previous ideas that fiber contained cellulose, hemicellulose (pectin) and lignin which were separate species have had to be modified (Bailey, 1973). Other components, such as acetyl groups and phenolic acids are found in low concentrations in fiber, but their function has a large influence on the structure and bioavailability of the fiber (Bacon, 1980). From all the available evidence, it is only cellulose that has not been shown to be covalently bound to any other component of the fiber. The acetate groups are ester- linked to xylose residues in the hemicellulose and phenolic acids are ester – linked to arabinose in the hemicellulose as well as being reported linked to lignin (Mueller-Harvey *et al.*, 1986 and Higuchi *et al.*, 1987). Lignin and hemicellulose are linked together by bonds that are more stable than ester bonds. A hypothesis has been proposed that secondary cell walls are two –

component systems comprising cellulose microfibrils embedded in a ligno-hemicellulosic matrix which also contains the other minor components as integral parts (Morrison 1974 and Morrison 1983). The degradability of particular components of the ligno – hemicellulosic matrix will have specific effects not only the ligno – hemicellulosic matrix but also on the degradability of the cellulose and hemicellulose components.

The first objective in silage making is to produce a palatable and preserved feed; this can be achieved by ensuring the success of the lactic fermentation using various biological additives or by making the process less dependent on fermentation by acidifying the crop with organic or inorganic acids. Although both approaches can achieve satisfactory preservation, the composition of the resulting silages differs in that those prepared with biological additives tend to be extensively fermented whereas the addition of acids, especially formic, restricts fermentation. The same authors in a previous study found that biological treatment by *Cellulomonas sp.* of bagasse improved its nutritive value (TDN = 69%, approximately nutritive value of corn) by increasing the crude protein from 1.7% to 15.5%. In addition, these bacteria were very active to secrete the cellulase enzymes causing degradation of cell wall constituents of bagasse and decrease the crude fiber from 44.9 % to 30.6% (Abd- El- Galil, 2000).

The current investigation was carried out to study the effect of biological treatments (cellulolytic bacteria) on poor quality roughages (bagasse, corn stalks and rice straw) to improve its chemical composition and cell wall structure.

MATERIALS AND METHODS

The present study was divided into two Experiments:

The First Experiment:

A-Preparation of bacterial cultures:-

In the laboratory of the Rumen Ecology Center, Animal Production Department, Faculty of Agricultural, Ain-Shams University . Five strains of cellulolytic bacteria were prepared to be examined by electrophoreses method. The separated strains are:

1- *Cellulomonas cellulasea* (T2).
2- *Acetobacter xylinum* (T3).
3- *Thermonospora fusca* (T4).
4- *Ruminococcus albus* (T5).
5- *Bacillus sp.* (T6).
6- control or marker protein (T1).

B- Pure culture media:

Isolation of species using the streak-plate or the pour-plate method is easily accomplished when the organism is the dominant species in a sample (Hungate, 1975).

The pour-plate technique for isolation of pure cultures was conducted according to A.T.C.C. (1992). A liquid suspension of the microbe was diluted serially in melted nutrient agars that were support the growth of the microbe. Agar at 45°C remains liquid but does not kill microbes. Each dilution in the series contains a lower concentration of the microbes per milliliter, so individual bacteria are spatially separated from one another in the liquid. The agar for

each dilution is poured into Petri dishes, it is allowed to solidify, and the plates are incubated, Bacteria suspended in the milted agar are trapped in the agar as it solidifies. During incubation, the isolated bacteria develop into visible colonies wherever the bacteria had become localized in the agar or on the agar surface. At the higher dilutions, in which bacteria are physically separated from each other, discrete colonies are observed.

C- Preparation of bacterial culture to electrophoresis:-

1- SDS-Polyacrylamide gel electrophoresis:

According to Laemmlia (1970), 100 ml of the gel was prepared:- 40.9 ml of stock acrylamide solution was diluted with 25 ml by separating gel buffer stock. One hundred µl of N, N, N, N-tetramethyl ethylene diamine (TEMED) were added and made up to 99 ml with bidistilled water. Degassed, then 1.0 ml of freshly prepared 10% ammonium persulphate solution was added shacked and immediately cast the gel. In order to prepare 50 ml of the stocking gel:- 6.5 ml of the acrylamide solution stock were diluted by 12.5 ml of gel buffer stock. Fifty µL of TEMED were added and the volume was brought up to 49.5 ml with distilled water. The mixture was immediately added to the precasted separating gel and the comb was placed at the same moment. The slab was left till polymerization of the gel completed.

2- Cell protein preparation:

Ten ml of bacteria were inoculated with freshly prepared cultured bacteria slants (16 hours old) of each standard strain *Cellulomonas cellulasea, Bacillus sp., Acetobacter xylinum, Thermonospora fusca* and *Ruminococcus albus*, their mutants and the obtained fusants. Cultures were incubated at 30°C on a shaking water bath (100cycle /minute) for 16 hrs. For each strain, 10 ml aliquots were centrifuged at 1000 rpm for 30 minutes. The supernatant was discarded and one ml of lysis buffer was added to each pellet, followed by addition of 30 mg of lysozyme. The suspensions were thoroughly mixed with vortex and the tubes were incubated at 30°C on a shaking water bath for 30 minutes. The protoplasted cells were centrifuged at 1000 rpm for 5 minutes. The supernatants were discarded and the pellets were washed twice with sterile bidistilled water, then the protoplasted cells were resuspended in 1.0 ml sterile distilled water. The suspensions were shacked vigorously by Vortex, thus the protoplasts were ruptured and the cell proteins were readily available for electrophoresis. In clean dry Eppendorff tubes, equal volumes of cell protein and 2x sample buffer were mixed and one drop of bromophenol blue was added. The tubes were immersed for 30 seconds in a boiling bath in order to be ready for injection. Ninty µl from each sample (strains) were injected in each well of Pharmacia slab (160 x 180 x 1.5 mm). 15 µl wide range molecular weight calibration kit proteins were injected in the protein marker well. Electrophoresis was carried out at 50 volts per the double slab till the bromophenol blue marker dye reached the bottom of the stacking gel and began to enter the separating gel. The voltage was then raised to 100 till the marker dye reached the bottom of the separating gel.

The separated protein bands were fixed in the gel by 50% TCA for 30 min., stained overnight with freshly prepared 1.0% Coomassia brilliant blue dye. The gel was then repeatedly distained by freshly prepared distaining solution.

The Second Experiment:

A-Ensiling:

1 - The samples of roughages (bagasse, corn stalks and rice straw) were sun dried to 90% DM and chopped to an approximate 1-3 cm.

2 - The samples of roughages were mixed with 2 water : 1 air dry roughages (McHan *et al.*, 1974) and mixed with 5% w/w molasses (Le Dividich *et al.*, 1978), 3% w/w Urea (Jakhmola *et al.*, 1993), and 0.3% w/w formic acid (Narasimhalu *et al.*, 1992)and 0.5% w/w acetic acid (Magara *et al.*, 1989).

3 -The samples were treated with one of the following treatments by 2 liters (6×10^5 viable anaerobes/kg of wet silage) /ton:

T1:- Untreated Roughage.
T2:- Treatment by *Cellulomonas cellulasea.*
T3:- Treatment by *Acetobacter xylinum.*
T4:- Treatment by *Thermonospora fusca.*
T5:- Treatment by *Ruminococcus albus.*
T6:- Treatment by *Bacillus sp.*

4- Treated samples were pressed in jars (2 liters) for laboratory use or barrels (200 liters) for farm use and incubation for two months.

B-Proximate chemical analysis:-

The Proximate chemical analysis of treated and untreated roughages (bagasse, corn stalks and rice straw) was determined according to A.O.A.C. (1990). The proximate chemical analyses were used to determine dry matter (DM), crude protein (CP), crude fiber (CF), Ether Extract (EE) and ash. The nitrogen free extract (NFE) was obtained by difference.

C- Cell wall constituents analysis:-

Raw rice straw, corn stalk and bagasse, compost treated rations were analyzed according to Van Soest and Breston (1979) to determine neutral detergent fiber (NDF), Acid detergent fiber (ADF) and acid detergent lignin (ADL). Hemicellulose, Cellulose and Lignin were determined by difference.

D- Statistical analysis:

The data of chemical analysis and fiber fraction were statistically analyzed according to statistical analysis system User's Guide, S.A.S. (1998). Separation among means was carried out by using Duncan Multiple test. The following model was used:

$Y_{ij} = \mu + T_i + \alpha_{ij}$ Where:

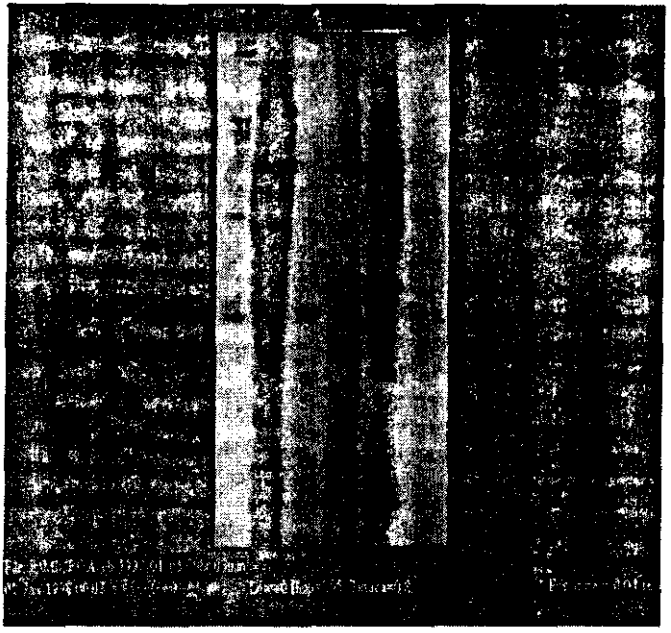
Y ij = The observation of the model.
μ = General mean common element to all observation.
Ti = The effect of the treatment (i = 1... 6), α ij = The effect of error.

RESULTS AND DISCUSSION

The First Experiment:-

-PAGE electrophoresis protein banding pattern of cellulolytic bacteria and their fusants cell following SDS-PAGE:

The results obtained are presented in Figure (1). The marker proteins



LANE 1 2 3 4 5 6

Figure (1): Electrophoretic separation of cell protein of the protein marker and strains using SDS PAGE

Lane (1): The protein marker (control)
Lane (2): *Ruminococcus albus*
Lane (3): *Thermonospora fusca*
Lane (4): *Acetobacter xylinum*
Lane (5): *Bacillus sp.*
Lane (6): *Cellulomonas cellulasea*

Table (1): The electrophoresis standard of protein (Lane 1)

Band number	Relative front	Mol.Wt (K.Da)	Optical Dinesity O. D
1	0.015	116.000	0.483
2	0.038	97.000	0.797
3	0.161	89.000	0.525
4	0.194	66.000	0.391
5	0.230	55.000	0.324
6	0.344	45.000	0.336
7	0.539	36.000	0.409

Table (2): The electrophoresis of *Ruminococcus albus* (Lane 2).

Band Number	Relative front	Mol. Wt. (K. Dalton)	Optical Dinesity
1	0.000	131.474	0.511
2	0.033	100.533	0.514
3	0.084	93.919	0.784
4	0.113	92.061	0.747
5	0.132	90.796	0.850
6	0.148	89.825	1.117
7	0.179	75.882	1.220
8	0.210	60.940	1.077
9	0.236	54.367	1.109
10	0.258	52.309	1.214
11	0.283	50.135	1.095
12	0.309	47.866	1.075
13	0.366	43.873	1.007
14	0.402	42.129	0.889
15	0.430	40.763	0.828
16	0.453	39.742	0.891
17	0.483	38.356	0.850
18	0.545	35.727	0.765
19	0.576	34.481	0.660
20	0.514	37.018	0.982
21	0.609	33.194	0.556
22	0.649	31.713	0.586
23	0.722	29.167	0.595
24	0.775	27.445	0.419
25	0.799	26.690	0.479
26	0.823	25.959	0.588
27	0.857	24.987	0.694
28	0.892	23.994	0.744

(wide range calibration protein kit)had the following molecular weights *B*-glycosidase (116.000), phosphorylase (97.000), fructose-6-phosphate kinas (84.000), albumin(66.000), glutamic dehydrogenase (55.000), ovalbumin (45.000), glyceraldahyde-3-phosphate (36.000). Figure(1) and Table(1) represented the scanning pattern of the marker protein .The marker protein was lane (1) in Figure (1) and Table (1) showed the variation in number, optical density (OD) and relative front of the protein bands among the protein marker. Table (2) and lane (2) represented the scanning pattern of *Ruminococcus albus*. Also, Table (2) showed that the band which has Mol.Wt of approximately between 60.9 to 40.7 were present in most strains which it was the molecular weights of cellulase enzymes, but in the strain named *Ruminococcus albus* present another enzymes carbonic anhydrase (29.000) and trypsinogen (24.000). Figure (1) in lane (3) and Table (3) showed the scanning pattern of the marker protein and *Thermonospora fusca*. Also, Table (3) showed that the band which has Mol.Wt. present in most strains between 60-40 approximately, the bands were Mol.Wt. of cellulase enzymes. Table (4) and Figure (1) in lane (4) represented the scanning pattern of the marker protein and *Acetobacter xylinum*. Also, Table (4) showed the variations in number, O.D, R.F. and Mol.Wt. of the protein bands among the *Acetobacter xylinum*, the table indicated that Mol.Wt. of the *Acetobacter xylinum* has 56.000to41.000was present in most strains. Table (5) and Figure (1) in lane (5) represented the scanning pattern of the protein of strain *Bacillus sp.* Also, Table (5) cleared that the differences in R.F, O.D, and Mol.Wt. of the protein bands of *Bacillus sp.* which has Mol.Wt 63.000to 40.000was present in most strains. Table (6) and Figure (1) in lane (6) cleared the scanning pattern of the protein of strain *Cellulomonas cellulasea*. Also, Table (6) showed the differences in R.F., O.D. and Mol.Wt. of the protein bands. The results indicated that the bands of *Cellulomonas cellulasea* Mol.Wt. was from 57.000 to 40.000 present in most strains. The results indicated that the relationships between the genes and their products (i.e. proteins) are parallel. This is reflected by changes in the electrophoretic protein banding pattern either appearance of new bands by variation in Mol.Wt. of bands. Polyacrylamide gel electrophoresis (PAGE) of cellular protein has been used as a good tool for classifying and identifying microorganisms (Ibrahim *et al.*, 1990 and Ibrahim and Abu-Seada, 1991). As computer comparisons of electrophoretic protein patterns can be a faster, easy and powerful tool for classification and identification of bacteria (Kersters and Deley, 1975).

The data showed that all strains secret cellulase enzymes. Crawford *et al.*, (2004) noticed those cellulases are endogenous enzymes required for degradation of cellulose in plants, but microbes are producing the necessary enzymes. Protein purification from gastric fluid of animal via fast performance liquid chromatography (F P L C) indicated the presence of two endoglucanase enzymes. The molecular weights of these components were determined by matrix-assisted laser desorption /ionization-time of flight (MALDI-TOF) to be 47.887 K. Da.(GEN L 1) and 50.295 K. Da.(GEN L 2). In general, it can be concluded

Table (3): The electrophoresis of *Thermonospora fusca* (Lane 3)

Band No.	Relative front	Mol. Wt. (K. Dalton)	Optical Dinesity
1	0.086	93.775	0.154
2	0.194	66.000	0.148
3	0.212	60.249	0.176
4	0.241	53.949	0.216
5	0.258	52.309	0.259
6	0.278	50.523	0.245
7	0.291	49.367	0.253
8	0.364	43.985	0.226
9	0.444	40.147	0.201
10	0.481	38.453	0.200
11	0.534	36.183	0.278
12	0.561	35.099	0.199
13	0.618	32.859	0.185
14	0.658	31.393	0.167
15	0.759	27.937	0.201
16	0.892	23.994	0.297

Table (4): The electrophoresis of *Acetobacter xylinum* (Lane 4)

Band No.	Relative front	Mol. Wt. (K. Dalton)	Optical Dinesity
1	0.000	131.474	0.525
2	0.029	104.195	0.286
3	0.082	94.063	0.263
4	0.097	93.057	0.236
5	0.143	90.151	0.289
6	0.168	83.834	0.311
7	0.185	71.478	0.407
8	0.225	56.268	0.657
9	0.249	53.128	1.014
10	0.280	50.329	0.732
11	0.325	46.590	0.655
12	0.347	44.866	0.780
13	0.358	44.381	0.637
14	0.364	43.985	0.594
15	0.373	43.541	0.591
16	0.395	42.451	0.535
17	0.417	41.388	0.486
18	0.450	39.843	0.510
19	0.481	38.453	0.729
20	0.481	38.453	0.729
21	0.501	37.586	0.610
22	0.539	36.000	0.526
23	0.558	35.188	0.660
24	0.572	34.656	0.531
25	0.592	33.874	0.496
26	0.618	32.859	0.415
27	0.671	30.919	0.398
28	0.671	30.919	0.398
29	0.702	29.841	0.363
30	0.724	29.094	0.358
31	0.784	27.168	0.382
32	0.819	26.088	0.461
33	0.874	24.486	0.870

Table (5):The electrophoresis of *Bacillus sp.* (Lane 5)

Band No.	Relative front	Mol. Wt. (K. Dalton)	Optical Dinesity
1	0.035	98.735	0.285
2	0.091	93.483	0.412
3	0.091	93.483	0.412
4	0.115	91.914	0.318
5	0.139	90.371	0.481
6	0.161	88.825	0.526
7	0.179	75.554	0.546
8	0.201	63.459	0.638
9	0.263	51.567	0.775
10	0.311	47.195	0.833
11	0.322	46.231	0.779
12	0.338	44.886	0.763
13	0.373	43.366	0.841
14	0.406	41.811	0.672
15	0.430	40.688	0.614
16	0.486	38.226	0.783
17	0.512	37.093	0.608
18	0.541	35.898	0.584
19	0.567	34.833	0.584
20	0.589	33.970	0.508
21	0.614	33.046	0.418
22	0.642	31.984	0.462
23	0.695	30.123	0.274
24	0.735	28.827	0.275
25	0.779	27.437	0.276
26	0.830	25.954	0.346
27	0.883	24.486	0.584

Table (6): The electrophoresis of *Cellulomonas cellulasea* (Lane 6)

Band No.	Relative front	Mol. Wt. (K. Dalton)	Optical Dinesity
1	0.161	88.826	0.138
2	0.168	83.571	0.138
3	0.188	69.655	0.131
4	0.199	64.198	0.131
5	0.219	57.847	0.150
6	0.274	50.537	0.173
7	0.329	45.636	0.295
8	0.344	44.603	0.251
9	0.430	40.688	0.288
10	0.461	39.292	0.229
11	0.510	37.188	0.232
12	0.528	36.447	0.232
13	0.576	34.495	0.203
14	0.623	32.745	0.173
15	0.894	24.486	0.223

that *Cellulomonas cellulasea, Acetobacter xylinum, Thermonospora fusca, Ruminococcus albus,* and *Bacillus sp.* were originally isolated from the rumen, which can grow under anaerobic conditions. There are major cellulolytic bacteria within the rumen. One of the reasons for its predominance isolation is its ability to readily degrade various forms of crystalline cellulose and lignocelluloses materials. These strains were different which in lane (2) the percent of cellulase enzymes was 28.57% , in lane (3) 43.75% , in lane (4) 30.30% , in lane (5) 25.92% , and in lane (6) 33.33% . These results showed that differences between all strains due to different of effect on cell wall constituents.

The Second Experiment:-

- ***Effect of biological treatments on roughages by cellulolytic bacteria:***

1-Effect of biological treatments on chemical composition of bagasse:

Results of chemical composition of treated and untreated bagasse are presented in Table (7). However, the effects were mainly due to the effect of biological treatments, which the increased of DM in T2, T3, T4, T5 and T6 due to the additives. In regard to crude protein (CP) values of untreated (T1)and treated bagasse T2,T3, T4 , T5 ,andT6 were 1.91 , 17.26,19.18 ,15.47,12.44 and 12.10% ,respectively. It was noticed that greater difference ($p<0.05$) was cleared between untreated and treated bagasse, but the highest values were recorded for T2 and T3 (17.26 and 19.18) while T5andT6 (12.44 and 12.10) were the lowest values.

These effects were mainly due to nitrogen content of added urea (about 3% w/w), microbial protein from bacteria treatments and nitrogen content of growing cellulolytic bacteria in silage of bagasse. Crude fiber decreased from 67.64% in untreated bagasse to 32.42 and 32.97 %in treated bagasse by T2 and T5, respectively. The decline of crude fiber values in the experimental could be results of the cellulase enzymes secreted by cellulolytic bacteria. Treatment with different strains of cellulolytic bacteria resulted in an increase ($p< 0.05$) in ether extract from 2.24 % in untreated bagasse (T1) to 4.91 ,4.34 and 5.88% in treated bagasse (T2, T3 and T4) . The increase of ether extract due to synthesis fatty acids through growth of bacteria. There was a decrease ($p<0.05$) in the NFE contents of treatments bagasse from 23.76% in T1 to 18.45 and 16.23% in treated bagasse T3 and T4. But treated bagasse (T2, T5 and T6) were increased ($p<0.05$) to 26.88, 40.66 and 35.82 % in comparison of the untreated bagasse (T1). It is clear that ash values increased from 4.44 and 3.82 %in untreated (T1) and treated bagasse (T3) to 18.53, 19.42, 11.25, and 8.63 % followed the treatments T2, T4 , T5 and T6, respectively. These effects were due to added media of growing bacteria, degradation organic matter to ash and inorganic matter ,molasses and strains of bacteria to bagasse .In general , it can be concluded that all strains of cellulolytic bacteria as biological treatments, had a great effect on degrading crude fiber (from 67.64 to 32.42 %) and increasing crude protein content (from 1.91 to 19.18 %) of bagasse.

2-Effect of biological treatments on chemical composition of rice straw:

Table (7): Effect of biological treatments on chemical composition of bagasse (On DM basis)

Item	T1	T2	T3	T4	T5	T6	S.E
DM	89.00^{c}	87.59^{d}	84.00^{e}	85.00^{e}	93.57^{b}	95.02^{a}	± 0.201
OM	95.56^{b}	81.47^{e}	96.18^{a}	80.58^{e}	88.74^{d}	91.37^{c}	± 0.197
CP	1.91^{e}	17.26^{b}	19.18^{a}	15.47^{c}	12.44^{d}	12.10^{d}	± 0.123
CF	67.64^{a}	32.42^{e}	54.21^{b}	43.00^{c}	32.97^{e}	41.46^{d}	± 0.112
EE	2.24^{c}	4.91^{b}	4.34^{b}	5.88^{a}	2.67^{c}	1.99^{d}	± 0.076
NFE	23.76^{d}	26.88^{c}	18.45^{e}	16.23^{f}	40.66^{a}	35.82^{b}	± 0.296
Ash	4.44^{d}	18.53^{a}	3.82^{d}	19.42^{a}	11.25^{b}	8.63^{c}	± 0.055

a,b,c,d,e&f means within the same row with different superscripts differ significantly(P<0.05)
Where: -
T1: - bagasse untreated. T2: - bagasse treated with *Cellulomonas cellulasea.*
T3: - bagasse treated with *Acetobacter xylinum.* T4: - bagasse treated with *Thermonospora fusca.*
T5: bagasse treated with *Ruminococcus albus.* T6: bagasse treated with *Bacillus sp.*

Table (8): Effect of biological treatments on chemical composition of rice straw (On DM basis)

Item	T1	T2	T3	T4	T5	T6	S.E
DM	90.50^{a}	84.25^{c}	90.50^{a}	85.92^{b}	89.89^{a}	85.53^{b}	± 0.139
OM	85.91^{a}	82.43^{b}	82.41^{b}	78.61^{c}	86.32^{a}	78.79^{c}	± 0.269
CP	1.44^{d}	12.66^{c}	15.88^{a}	13.91^{b}	16.36^{a}	14.37^{b}	± 0.114
CF	44.08^{a}	39.39^{c}	39.33^{c}	28.14^{e}	41.12^{b}	35.31^{d}	± 0.131
EE	2.21^{b}	3.67^{a}	2.87^{b}	4.07^{a}	2.33^{b}	3.74^{a}	± 0.049
NFE	38.18^{a}	26.71^{c}	24.35^{d}	32.49^{b}	26.49^{c}	25.37^{d}	± 0.213
Ash	14.09^{c}	17.57^{b}	17.59^{b}	21.39^{a}	13.68^{c}	21.21^{a}	± 0.065

a,b,c,d&e means within the same row with different superscripts differ significantly(P<0.05)
Where: -
T1: - rice straw untreated. T2: - rice straw treated with *Cellulomonas cellulasea.*
T3: - rice straw treated with *Acetobacter xylinum.* T4: - rice straw treated with *Thermonospora fusca.*
T5: - rice straw treated with *Ruminococcus albus.* T6: - rice straw treated with *Bacillus sp.*

Results of chemical composition of treated and untreated rice straw are presented in Table (8). .In regard to crude protein (CP) values of untreated (T1), treated rice straw T2, T3,T4, T5 and T6 were 1.44 , 12.66,15.88, 13.91,16.36 and 14.37 %,respectively .

It was noticed that greater difference ($p<0.05$) was clear between untreated rice straw (T1) and treated rice straw (T2, T3, T4, T5 andT6), but the highest values were for treated rice straw T3and T5 (15.88 and 16.36%), while treated rice straw T2and T4 (12.66 and 13.91%) were the lowest values. These effects were mainly due to nitrogen content of added urea (about 3% w/w), microbial protein from bacteria treatments and nitrogen content of growing cellulolytic bacteria of rice straw silage .Crude fiber decreased from 44.08% in untreated rice straw to 35.31 and 28.31% in treated rice straw (T6 and T4, respectively). The decline of crude fiber values in the experimental treatments could be as results of the cellulase enzymes secreted by cellulolytic bacteria (Abd El-Galil, 2000). Treatment with different strains of cellulolytic bacteria resulted in an increase of ether extract from 2.21% in untreated rice straw (T1) to 4.07% in treated rice straw (T4). There was a decrease ($p<0.05$) in the NFE contents of treatments rice straw from 38.18% in untreated rice straw (T1) to 26.71,24.35 ,32.49,26.49 and 25.37 % in treated rice straw(T2, T3,T4,T5 andT6),respectively . But treated rice straw (T3) was the lowest value of NFE in comparison of the untreated rice straw (T1). It is clear that ash content increased from 14.09 in untreated rice straw (T1) to 17.57 and 21.39 %when it was treated (T2) and (T4), while it decreased to 13.68 in T5. There effects were due to added media of growing bacteria, degradation organic matter to ash and inorganic matter, molasses and strains of cellulolytic bacteria as biological treatments to rice straw. There had a great effect on degrading crude fiber content (from 44.08 to 28.14 %) and increasing crude protein content (from 1.44 to 16.36 %) of rice straw .

3-Effect of biological treatments on chemical composition of corn stalks:

Results of chemical composition of treated and untreated corn stalks are presented in Table (9). The differences observed in dry matter contents could be due to the effect of biological treatments. On the other hand, the values of crude protein of untreated corn stalks (T1) and treated corn stalks (T2, T3, T4, T5 and T6) were 2.59, 14.42, 16.62, 12.51, 17.12 and 15.19 %, respectively. The present data indicated that the highest values were obtained for treated corn stalks T3 and T5 (16.62 and 17.12 %), while for T2 and T4 it were the lowest values (14.42 and 12.51%). These effects were due to nitrogen content of added urea (about 3% w/w), microbial protein from bacteria treatments and nitrogen content of growing cellulolytic bacteria in silage of corn stalks. No explanation for this phenomena which was repeated in different studies by Abd El-Galil, (2000); Zhao, *et al.* (2002) and Shoukry *et al.* (1985). However, it can be suggested that N content increased due to contamination by water, soil or plastic container. Crude fiber decreased from 46.10% in untreated to 27.65 and 34.04 %in treated T 2 and T5, respectively. The decreasing of crude fiber values in the experimental

Table (9): Effect of biological treatments on chemical composition of corn stalks (On DM basis)

Item	T1	T2	T3	T4	T5	T6	S.E
DM	88.50^{c}	85.86^{d}	85.50^{d}	89.50^{b}	89.97^{b}	90.50^{a}	± 0.182
OM	92.37^{a}	77.42^{d}	74.03^{e}	82.23^{c}	88.98^{b}	88.07^{b}	± 0.175
CP	2.59^{d}	16.42^{b}	16.62^{a}	12.51^{c}	17.12^{a}	15.19^{b}	± 0.114
CF	46.10^{a}	27.65^{f}	37.61^{d}	39.55^{c}	34.04^{e}	42.09^{b}	± 0.179
EE	4.52^{b}	5.17^{b}	6.90^{a}	3.50^{c}	3.11^{c}	2.33^{d}	± 0.043
NFE	39.15^{a}	30.68^{c}	12.90^{f}	26.67^{e}	34.66^{b}	28.45^{d}	± 0.450
Ash	7.63^{f}	22.08^{b}	25.97^{a}	17.77^{c}	10.87^{e}	11.93^{d}	± 0.060

a,b,c,d,e&f means within the same row with different superscripts differ significantly (P<0.05)
Where: -
T1: - corn stalks untreated. T2: - corn stalks treated with *Cellulomonas cellulasea.*
T3: - corn stalks treated with *Acetobacter xylinum.* T4: - corn stalks treated with *Thermonospora fusca.*
T5: corn stalks treated with *Ruminococcus albus.* T6: corn stalks treated with *Bacillus sp.*

Table (10): Effect of biological treatments on cell wall constituents of bagasse (On DM basis)

Item	T1	T2	T3	T4	T5	T6	S.E
NDF	80.33^{a}	70.20^{b}	79.10^{a}	64.90^{e}	68.29^{c}	62.42^{f}	± 0.176
ADF	$61..51^{b}$	40.30^{e}	63.40^{a}	40.10^{e}	54.84^{c}	46.21^{d}	± 0.210
ADL	19.60^{b}	21.13^{a}	22.80^{a}	20.90^{b}	22.50^{a}	22.07^{a}	± 0.123
Hemicell.	18.80^{c}	29.90^{a}	15.70e	24.80^{b}	13.45^{f}	16.21^{d}	± 0.41
Cellulose	41.90^{a}	19.17^{e}	40.60^{b}	19.20^{e}	32.34^{c}	24.14^{d}	± 0.291

a,b,c,d,e&f means within the same row with different superscripts differ significantly (P<0.05)
Where: -
T1: - bagasse untreated. T2: - bagasse treated with *Cellulomonas cellulasea.*
T3: - bagasse treated with *Acetobacter xylinum.* T4: - bagasse treated with *Thermonospora fusca.*
T5: - bagasse treated with *Ruminococcus albus.* T6: - bagasse treated with *Bacillus sp.*

treatments could be as results of the cellulase enzymes secreted by cellulolytic bacteria. In regard to ether extract (EE) value of untreated (T1) it was 4.52%, while the treated T2, T3, T4, T5 andT6 were 5.17, 6.9, 3.5, 3.11 and 2.33 %, respectively. It is clear that ash values increased from 7.63% in untreated (T1) to 25.97 % when treated by *Acetobacter* (T3). The values of NFE was 39.15 %in untreated (T1) but in the treated T2,T3,T4,T5 andT6 it were 30.68, 12.9, 26.67, 34.66 and 28.45% respectively. So, NFE values decreased from 39.15 to 12.9 % when corn stalks were treated by *Acetobacter* (T3). In general , it can be concluded that all strains of cellulolytic bacteria had great effect on increasing crude protein content (from 2.59 to 17.12%) and increasing degrading crude fiber content (from 46.10 to 27.65 %) of corn stalks. The present finding is in agreement with Bakshi and Langer (1991). They reported that crude fiber decreased from 42.92 to 17.87 % in the compost and spent with the treatment of cellulase enzymes .On the same trend, supportive results were reported by Streeter *et al.* (1982) ;Reader and Mc Queen (1983) ;Eduardo and Etienne (1985) and Larwence and Abada (1987) . On the other hand, Sheperd and Kung (1996) found that, when corn was ensiled in mini silos with enzyme (cellulase and hemicellulase), the results showed no differences in DM content between treatments during storage. However, some studies referred that the inoculation by bacteria or fungi produced little improvement in silage composition (Gordon, 1989), but in other studies, it produced a faster and more efficient fermentation (Seale, 1989) with reduction of DM losses. But Abd- EL-Galil (2000) found that , when bagasse treated by *Cellulomonas sp. bacteria* decreased of crude fiber from 44.9 % to30.21 % and increased crude protein from 1.75 % to 16.9% with reduction of DM losses . The present results confirmed the results obtained by Shoukry *et al.*, (1985) and Abdul- Aziz *et al.*, (1997). Gado (1999) fermented rice straw and bagasse with *Trichoderma ressei* and reported that the CP, CF, EE, NDF, ADF, cellulose and hemicellulose were lowered significantly ($p<0.05$) in both treated rice straw and bagasse. Bader (2001) found that the biological treatments by *P.florida* decreased CF content than that in raw, being 3.5%, while combined fungi and bacteria at level of 3% were more efficient in decreasing crude fiber content from 37.85% to 18.42% ,followed by incubation of corn stalks *by P.florida and E. carotovora* at level 2% (being 47.37% of control). The addition of urea only into maize silage decreased DM digestibility. It can be concluded that urea and molasses can be used as silage additive to increase crude protein and energy content of silage (Denek and Deniz, 2004). Jachmann *et al.*, (1998) showed that the pH range, supporting growth of *pleurtus sp* .was 1.5 to 12.0 units and good growth of *pleurtus sp.* with urea was 1.5 to 3.0%. Shoukry *et al.*, (1985) found an increase in CP, EE and ash content when treated sugarcane bagasse treated with 4 different microorganisms *(Trichoderma viride 253, Basidomycetes sp.,* ∏ *and Gliocadium sp. Q230).* Marwaha *et al.*, (1990) reported that the ammonuation (through urea) had resulted in a decrease 3.21% in crude fiber (CF), while fungal treatment has showed a high decrease in CF when

compared with that of untreated wheat straw. Alfalfa, corn, sorghum and wheat forages were chopped and inoculated with *Lactobacillus plantarum* (107 cells/kg silage). The effect of the inoculum was observed more on alfalfa and wheat silage. Treated silage had increasing in crude protein, decreasing in crude fiber for alfalfa and wheat, and had a positive effect on nutrients digestibility (Lane and Maxsudweeks, 1981). Zhao, *et al.* (2002) conducted an experiment, whereas, a mixture of Panicum and rice straw with no additive (control), acremonium cellulolytic and molasses as additives. They found that additives had resulted in lower pH and CF content, but higher CP content.

4-Effect of biological treatments on cell wall constituents of bagasse:-

It is clear from Table (10) that biological treatments with different cellulolytic bacteria had a significant effect on cell wall constituents of bagasse. Biological treatments decreased ($p<0.05$) NDF contents from 80.33 %in untreated (T1) to 70.2 ,79.1 ,64.9 ,68.29 and 62.42 % in treated bagasse (T2,T3,T4,T5 and T6, respectively) . The highest decrease in NDF content was recorded with treated by *Bacillus sp.* (T6), while the lowest decrease in NDF content was recorded with treated by *Acetobacter sp.* (T3). There were significant differences among treatments detected in ADF content from 61.50% in untreated (T1) to 40.3, 63.4, 40.1, 54.84 and 46.21 % in treated T2, T3, T4, T5 and T6, respectively. The lowest decrease in ADF content was recorded with bagasse treated by *Acetobacter sp.*(T3) , while the highest decrease in ADF content was found in T2 and T4. On the other side ,values of hemicellulose content were 18.8%, 29.9% ,15.7%,24.8%,13.45% and 16.21% in untreated(T1) ,treated T2,T3,T4,T5 and T6. respectively. It was noticed that hemicellulose content increased in T2 andT4, while it decreased in T3, T5 and T6. Cellulose content in untreated was 41.9% while it was 19.17, 40.6, 19.2, 32.34and 24.14% in T2, T3, T4, T5 and T6, respectively. It was noticed that the lowest decrease in cellulose content was recorded with treatment T3 with *Acetobacter sp.* In all biological treatments by cellulolytic bacteria lignin increased from 19.6% to more than 22 % with *Acetobacter sp.*, *Ruminococcus sp.* and *Bacillus sp.* In general, results indicated that biological treatments of bagasse by cellulolytic bacteria were significant by affect on cell wall constituents.

5-Effect of biological treatments on cell wall constituents of rice straw:

Values of the effect of biological treatments by *Cellulomonas, Acetobacter, Thermonospora, Ruminococcus and Bacillus* on cell wall constituents of rice straw are shown in Table (11). There were significant ($p<0.05$) decrease in NDF content of rice straw treated by bacteria. The highest decrease was found in T3 andT6 (56.46 and 58.13%). The lowest decrease was found in T2and T4 (84.62 and 84.58 %). ADF content decreased from 49.25% in untreated (T1) to 30.94% in T3. However all bacterial treatment resulted in decreased ADF content. There was significant decrease hemicellulose content by cellulolytic bacteria. The highest reduction of

Table (11): Effect of biological treatments on cell wall constituents of rice straw (On DM basis)

Item	T1	T2	T3	T4	T5	T6	S.E
NDF	89.00^{a}	84.62^{b}	56.46^{e}	84.58^{b}	63.53^{c}	58.13^{d}	± 0.135
ADF	49.25^{a}	48.57^{a}	30.94^{e}	45.73^{b}	35.59^{d}	41.97^{c}	± 0.196
ADL	10.92^{d}	11.22^{c}	12.19^{b}	11.53^{c}	15.74^{a}	14.65^{a}	± 0.122
Hemicell.	39.75^{a}	36.05^{b}	25.52^{e}	38.85^{a}	27.94^{c}	16.16^{f}	± 0.393
Cellulose	39.33^{a}	37.35^{b}	18.75^{e}	34.20^{c}	19.85^{e}	27.32^{d}	± 0.280

a,b,c,d,e&f means within the same row with different superscripts differ significantly (P<0.05)
Where: -

T1: - rice straw untreated. T2: - rice straw treated with *Cellulomonas cellulasea.*
T3: - rice straw treated with *Acetobacter xylinum.* T4: - rice straw treated with *Thermonospora fusca.*
T5: rice straw treated with *Ruminococcus albus.* T6: rice straw treated with *Bacillus sp.*

Table (12): Effect of biological treatments on cell wall constituents of corn stalks (On DM basis)

Item	T1	T2	T3	T4	T5	T6	S.E
NDF	84.90^{a}	78.35^{b}	70.73^{d}	76.73^{c}	76.27^{d}	63.50^{e}	± 0.191
ADF	57.85^{d}	41.20^{c}	41.85^{c}	43.10^{b}	47.35^{a}	38.18^{e}	± 0.210
ADL	19.98^{a}	13.05^{c}	13.45^{c}	12.90^{c}	18.42^{a}	14.29^{b}	± 0.161
Hemicell.	27.05^{d}	21.15^{f}	28.80^{c}	33.63^{b}	22.92^{e}	35.32^{a}	± 0.220
Cellulose	37.87^{b}	44.15^{a}	28.40^{d}	30.20^{c}	28.93^{d}	23.39^{e}	± 0.245

a,b,c,d,e&f means within the same row with different superscripts differ significantly (P<0.05)
Where: -

T1: - corn stalks untreated. T2: - corn stalks treated with *Cellulomonas cellulasea.*
T3: - corn stalks treated with *Acetobacter xylinum.* T4: - corn stalks treated with *Thermonospora fusca.*
T5: corn stalks treated with *Ruminococcus albus.* T6: corn stalks treated with *Bacillus sp.*

hemicellulose content was in T6 (16.16%), which treated with bacteria.

On the other hand, a significant decrease in cellulose content was found in all treated rice straw, the highest decrease was recorded for T3,T5 and T6(18.75, 19.85 and 27.32 %, respectively). In addition there were significant increase in lignin content by cellulolytic bacteria; the untreated was 10.92 % while T2,T3,T4,T5 and T6 were 11.22, 12.19,11.53 ,15.74 ,14.65% ,respectively . Generally, it can be concluded that cellulolytic enzymes that secretions from bacteria affect on cell wall constituents of rice straw.

6-Effect of biological treatments on cell wall constituents of corn stalks:

Biological treatments decreased ($p<0.05$) in NDF contents from 84.8 %in untreated corn stalks (T1) to 78.35, 70.65, 76.73, 70.27 and 63.5 %in T2, T3, T4, T5 and T6, respectively(Table 12). The highest decrease in NDF content was recorded in T6, while the lowest decrease in NDF content was recorded in T2.

There were significant differences among treatments detected in ADF content from 57.85% in untreated (T1) to 41.2, 41.85, 43.1, 47.35 and 38.18 %in treated T2, T3, T4, T5 and T6, respectively. The lowest decrease in ADF content was recorded in T4; while the highest decrease in ADF content was found in T6. On the other side, values of hemicellulose content were 27.05, 21.15, 28.8, 33.63, 22.92 and 35.32 % in untreated (T1), treated T2, T3, T4, T5 and T6, respectively. It was noticed that hemicellulose content increased in T3, T4 andT6 while it decreased in T2 and T5. Cellulose content in untreated (T1) was 37.87 % while cellulose content in treated corn stalks were 44.15 ,28.4 ,30.2,28.93 and 23.39 % in T2,T3,T4,T5 and T6 , respectively. It was noticed that treatment T2 was increased in cellulose content which bacteria degraded the banding between cellulose and other components without degraded cellulose, but the other treatment there was decreased in cellulose content which bacteria degraded the banding between cellulose and other components with degraded cellulose. In all biologically treatment found that treated by cellulolytic bacteria decreased in lignin content, it reduced from 19.98 % to 12.90 % when it was treated by *Thermonospora* (T4).

In general, results indicated that biologically treatment by different cellulolytic strains with different poor quality roughage (i.e. bagasse, rice straw and corn stalks) had significant affect on cell wall constituents. Bader (2001) reported that biological treatments by fungi decreased ($p<0.05$) in NDF, ADF, ADL, cellulose and hemicellulose in rice straw and corn stalks. Autrey *et al.*(1975) reported a 13% decreased in the cellulose content of corn silage when it was treated with cellulase enzyme. Also, Spoelstra *et al.*(1992) and Stockes and Chen(1994)reported a reduction of NDF value after enzyme addition to corn silage . On the other hand, Sheperd and Kung (1996) found that enzyme treatment to whole plant corn silage (WPCS) reduced NDF and ADF contents. In addition, Leatherwood *et al.*(1983) showed that cellulase treatment reduced the cellulose content 10%more than control. Bakshi *et al.*(1985) reported that the spent wheat

straw *by Pleurotus sp.* lowered CF, NDF, ADF, cellulose and hemicellulose content than untreated wheat straw. Also, Eduardo *et al.*(1986) reported that a decrease in cell wall constituents from92.2 to 77.3% for untreated and treated wheat straw (by white rot fungi), respectively. Levanon *et al.*(1988) found that the content of lignin, cellulose and hemicellulose of wheat straw and cotton stalks decreased when fermented by *Pleurotus sp.*as effect of their enzymes. Filya (2002) confirmed that, using lactic acid bacteria +enzyme mixture inoculants as silage additives on maize silage, he found that both inoculants increased dry and organic matter insitu degradabilities but decreased NDF and ADF content. Wang *et al.*(2002) cited that the treatments of corn straw with microbial inoculants (209 gm *lactic acid bacteria or lactobacillus sp.)* and enzyme preparation 400 gm/100kg DM(100gm cellulase +200gm amylase +100gm glucoamylase). The results indicated that the treatments of corn straw increased degradable DM and NDF compared with control (untreated).

CONCLUSION

It was concluded that all treatments had the most effects on chemical composition and cell wall constituents of silage. These results cleared that bagasse had the highest response when treated with *Cellulomonas* and *Ruminococcus*, rice straw showed the highest affect when treated by *Thermonospora* and *Bacillus*, while corn stalks showed the highest affect when treated with *Cellulomonas* and *Ruminococcus*. The different response due to different of cell wall structural of roughages and structural of cellulase enzymes that secreted from cellulolytic bacteria.

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تأثير المعاملات البيولوجية بالبكتيريا المحللة للسيليلولوز على التركيب الكيميائى وتركيب جدر الخلايا لبعض المخلفات المزرعية الخشنة.

هانى محمود جادو، حمدى موسى متولى، حسين سعد سليمان، عتاب رمضان ابراهيم عبد الجليل

قسم الانتاج الحيوانى، كلية الزراعة، جامعة عين شمس، شبرا الخيمة، القاهرة، مصر .

- اجريت هذه الدراسة بهدف تحسين استخدام بعض المخلفات المزرعية خاصة قش الارز ومصاصة القصب وحطب الذرة كعليقة للمجترات. وقد انقسمت مراحل البحث الى مرحلتين اساسيتين :

* فى المرحلة الأولى تم جمع سائل كرش من الماعز البلدى ثم تم عزل سلالات بكتيرية منها (محللات السيليلولوز) وتم عمل تنمية معملية لها ثم فحص هذه البكتيريا باستخدام طريقة الالكتروفيريسيس للتأكد من اختلاف هذه الأنواع عن بعضها وهذه السلالات هى :

Acetobacter xylinum & Cellulomonas cellulasea& Bacillus sp. & Ruminococcus albus & Thermonospora fusca.

* فى المرحلة الثانية من البحث استخدمت السلالات المحللة للسيليلولوز (بعد تنميتها بكميات تكفى للاستخدام) فى معاملات البيولوجية للسيلاج المصنع من الثلاثة مخلفات المستخدمة بالتجربة كلا على حدى ثم أجريت التحليلات المعملية الكاملة حيث قدر فيها الرطوبة الكلية والبروتين الخام والألياف الخام والمستخلص الخالى من الأزوت والمادة العضوية والرماد وكذلك الألياف الأساسية (السيليلولوز – الهيمسيليلوز – اللجنين) لدراسة تأثير المعاملات البيولوجية على التركيب الكيميائى وتركيب جدر الخلايا لهذه المخلفات .

*جاءت نتائج المرحلة الأولى من البحث: أن السلالات البكتيرية التى تم عزلها كمحللات للسيليلولوز هى سلالات مختلفة من حيث تركيب البروتينات الداخلة فى تكوينها وتشير النتائج بشكل عام الى اختلاف البكتريا فى الوزن الجزيئى والكثافة البصرية والمسافات التى تتحركها البروتينات من نوع لأخر وبالتالى تختلف كل سلالة بكتيرية فى الأنواع الانزيمية التى تفرزها لتحليل السيليلولوز ولكن وجد أنها تتشابه فيما بينها فى أنها طبيعيا كلها تعيش فى سائل الكرش معا واحتوائها جميعها على الوزن الجزيئى للانزيمات المحللة السيليلولوزوالتى يتراوح وزنها الجزيئى بين ٤٠-٦٠كيلودالتون.

*وجاءت نتائج المرحلة الثانية من البحث: أن الخمسة أنواع من البكتيريا المستخدمة فى عمل السيلاج و استخدمت معها المخلفات المزرعية الثلاثة وهى قش الأرز و مصاصة القصب وحطب الذرة كلا على حدى تم كمرها فى صورة سيلاج لمدة شهرين ثم فتحها بعد ذلك فوجد أن خواص السيلاج الناتج ذو رائحة جيدة وخالية من رائحة حمض البيوتيريك والا بثانول مما يدل على جودة طريقة الحفظ للسيلاج . و أوضحت نتائج التحليل للمخلفات بشكل عام وجود اختلاف فى الأستجابة للمعاملات من مخلف لأخرولكنها جميعها تتشابه فى أن معظمها بطبيعتها تكون مرتفعة فى محتواها من الألياف و منخفضة المحتوى من البروتين. وأثرت المعاملات البيولوجية على نسبة الألياف ونسبة البروتين ولكن بنسب مختلفة تبعا لنوع المخلف وأيضا تبعا لنوع البكتيريا المستخدمة فى المعاملة.

لوحظ فى مصاصة القصب ارتفاع نسبة البروتين الكلى بعد المعاملة فكانت تتراوح بين 12.10% الى 19.18% بالمقارنة بدون معاملة9.9%. وكان متوسط نسبة الألياف الخام بعد المعاملة يتراوح بين 32.42 % الى 54.21 % بالمقارنة بدون معاملة 67.64 %.و الهيمسيليلولوز أصبح بعد المعاملات البيولوجية يتراوح بين 13.45-29.90% بينما بدون معاملة كان 18.80%. السيليلولوز بعد المعاملات البيولوجية كان يتراوح بين 19.17-

32.34% بينما بدون معاملة كان 41.90% على أساس المادة الجافة. و بالنسبة لقش الأرز لوحظ ارتفاع نسبة البروتين الكلى بعد المعاملة فكانت تتراوح بين 12.66 % الى 16.36 % بالمقارنة بدون معاملة1.44 %. و كان متوسط نسبة الألياف الخام بعد المعاملة يتراوح بين 28.14 % الى 39.33 % بالمقارنة بدون معاملة كانت 44.08 %. الهيمسليلولوز أصبح بعد المعاملات البيولوجية يتراوح بين 16.16- 38.85% بينما بدون معاملة كان 39.33%. السليلولوز بعد المعاملات البيولوجية كان يتراوح بين 18.75-37.35% بينما بدون معاملة كان 39.33% . أما بالنسبة لحطب الذرة لوحظ ارتفاع نسبة البروتين الكلى بعد المعاملة فكانت تتراوح بين 12.51 % الى 16.62 % بالمقارنة بدون معاملة 2.59 % . وكان متوسط نسبة الألياف الخام بعد المعاملة تتراوح بين 27.65 % الى 42.09 % بالمقارنة بدون معاملة كانت 46.10 %. الهيمسليلولوز أصبح بعد المعاملات البيولوجية يتراوح بين 21.15- 35.32% بينما بدون معاملة كان 27.05%. السليلولوز بعد المعاملات البيولوجية كان يتراوح بين 23.39-44.15% بينما بدون معاملة كان37.87% على أساس المادة الجافة.

من النتائج السابقة يمكن استخلاص امكانية استخدام بكتيريا *Ruminococcus & Cellulomonas* لمعاملة المصاصة بينما بكتيريا *Bacillus & Thermonospora* لمعاملة قش الأرزوبكتيريا *Cellulomonas & Ruminococcus* لمعاملة حطب الذرة كمعاملات بيولوجية وهذا الاختلاف فى الاستجابة للمعاملة البيولوجية يرجع لاختلاف التركيب الكيميائى وتركيب جدر الخلايا للمخلفات بالاضافة لاختلاف تركيب الانزيمات المحللة للسيليلولوز التى تفرز من كل سلالة .