Research Article

Mannanase and Cellulase Enzyme Production from the Agricultural Wastes by the *Bacillus subtilis* P2-5 Strain

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Abstract
The objective of this research was to use agricultural wastes as substrates in enzyme production processes, instead of using a commercial substrate named locust bean gum (LBG), in order to determine an alternative way of managing agricultural wastes, to reduce waste disposal costs, and to reduce enzyme production costs. There are four potential factors generally considered in enzyme production, which are designated as a substrate, an inoculum, temperature, and an incubator shaker speed. In this research, three agricultural wastes, namely tea waste, coffee grounds, and copra meal were considered as substrates. A new bacterium that was isolated from the soil named *Bacillus subtilis* P2-5 was considered as the inoculum, and it was tested at 1 and 5% (v/v). A factorial experiment was then conducted, in order to study the effects of these factors and their interactions on mannanase and cellulase activities. The results showed that the three wastes can be used as substrates to produce mannanase and cellulase. Tea waste obtained the highest enzyme activities when compared to coffee grounds and copra meal. The optimal condition to maximize both of the mannanase and cellulase activities was to use tea waste, 1% (v/v) of inoculum, 37°C, and 150 rpm. Although the enzyme activities obtained from the tea waste were lower than those obtained from LBG, the proposed idea is still recommended, since benefits from these agricultural wastes can be obtained, rather than merely casting them aside.

Keywords: Agricultural wastes, Mannanase, Cellulase, Submerged fermentation, Enzyme production

1 Introduction

The agricultural industry is one of the main economic sectors of developing countries, especially in Thailand. This sector produces many different kinds of agricultural wastes, which are defined as the residues from growing, and afterward, the processing of the raw agricultural products, such as fruits, vegetables, meat, poultry, dairy products, and crops. They are non-product outputs that contain useful materials, but whose economic values are less than their managerial costs, which are mostly reflected by their transportation and disposal costs. Hence, many research papers have tried to propose various approaches, in order to gain benefits from these wastes, rather than by simply disposing of them. One of the popular approaches, as reported in the literature, is to use them as substrates for producing enzymes. Table 1 shows the research

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papers, which are related to this approach that have been published in the past two decades. For example, sugarcane bagasse, textile waste, corn husk, and cellulosic are used to produce an enzyme known as cellulase [1]–[4]. The residues from agro-industry are used to produce amylase [5]. Wheat husk, sugarcane bagasse, and soy residues are used to produce xylanase [6]–[9]. Sweet sorghum bagasse is used to produce lignocellulolytic [10]. Konjac-mannan (glucomannan), sugar beet, soy residues, and konjac powder are used to produce a useful enzyme named mannanase [11]–[13], [9]. The waste that is obtained from the bakery industry is used as a substrate for producing protease and glucoamylase [14]. Orange peel is used to produce pectinase [15]. Sugarcane baggase is used to produce glucose and xylooligosaccharides [16].

It can be concluded from Table 1 that these agricultural wastes can be used as substrates for enzyme production. However, only specific enzymes can be produced. This is dependent on what materials are in the wastes, what microorganisms are being used, and what mediums are being applied.

Based on the survey, agricultural wastes, such as coffee grounds, tea waste, and copra meal can easily be found in Thailand. From a chemical perspective, these wastes are constituent groups of lignocellulose, which are the major component of plant cell wall. It consists of three major components, namely hemicellulose, cellulose, and lignin, which are high potential to produce mannanase and cellulase enzymes [17]–[19].

These enzymes are pertinently useful for the chemical industries. For example, mannanase and cellulase are the most important enzymes for the bio-bleaching of pulp and paper [20]. For the food and feed industries, mannanase can be hydrolyzed into mannose and mannooligosaccharides (MOS), which are very useful in a prebiotic process [20]. For the paper recycling industry, cellulase is used to improve deinking efficiency. In the coffee industry, mannanase is used to reduce viscosity when extracting polysaccharides [20]. Finally, for the detergent industry, mannanase and cellulase are used to remove stains from the soil, the grass, eggs, blood, and fat [21].

This research attempted to gain benefits from the agricultural wastes, namely tea waste, coffee grounds, and copra meal, by using them as substrates. These wastes were fermented with a new bacteria named *Bacillus subtilis* P2-5, in order to produce mannanase and cellulase. The reasons for selecting these particular wastes were that they are costless and the materials that are contained in these wastes have a high potential to produce the mentioned enzymes, as stated earlier. The enzyme activities obtained from the proposed agricultural wastes were quantitatively compared with

Table 1: Literature regarding the use of agricultural and non-agricultural wastes to produce enzymes

Substrate	Microorganism	Medium	Enzyme	Ref.
Sugarcane bagasse	*Penicillium* sp. and *Rhizomucor* sp.	SSF	Cellulase	[1]
Textile waste	*Aspergillus niger* CKB	SSF	Cellulase	[2]
Corn husk	*Bacillus cereus*	SmF	Cellulase	[3]
Cellulosic	*Bacillus subtilis* AS3	SmF	Cellulase	[4]
Agro residues	*Bacillus cereus* amy3	SmF	Amylase	[5]
Wheat husk	*Aspergillus fumigatus* R1	SmF	Xylanase	[6]
Sugarcane bagasse	*Bacillus megaterium* BM07	SmF	Xylanase	[7]
Sugarcane bagasse	*Clostridium thermocellum*	SSF	Xylanase	[8]
Soy residue	*Bacillus circulans* BL53	SSF	Xylanase and mannanase	[9]
Sweet sorghum bagasse	Fungi	SSF	Lignocellulolytic	[10]
Konjac glucomannan	*Bacillus subtilis* BE-91	SmF	Mannanase	[11]
Sugar beet	*Aspergillus sojae*	SSF	Mannanase	[12]
Konjac powder	*Bacillus licheniformis* NK-27	SmF	Mannanase	[13]
Bakery waste	*Aspergillus awamori* and *Aspergillus oryzae*	SSF, SmF	Glucoamylase and protease	[14]
Orange peel waste	*Aspergillus niger*	SmF	Pectinase	[15]
Sugarcane bagasse	*Penicillium oxalicum*	SSF	Glucose and xylooligosaccharides	[16]

SmF: Submerged Fermentation, SSF: Solid State Fermentation

those obtained from locust bean gum (LBG), in order to evaluate the efficiency of the enzyme production proposed in this research. Note that LBG was selected since it is constitution groups of galactomannan consisted of mannose and galactose [22]. LBG is a commercial substrate that is normally used in enzyme production. It was used as a benchmark substrate for the efficiency comparison purposes.

The main contributions of this research were not only to determine an alternative way to manage agricultural wastes but to also reduce LBG usage, which would then result in reducing the enzyme production costs.

The rest of this research is organized as follows: Section 2 presents materials and methods. Results and discussion are provided in Section 3. Finally, conclusion and recommendations for further study are given in Section 4.

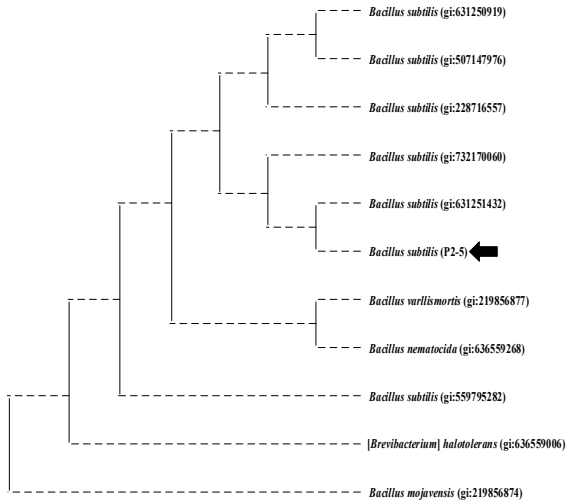

Figure 1: Phylogenetic analysis of the P2-5 strain that was based on the 16S rDNA gene sequences.

2 Materials and Methods

2.1 *Material preparation*

There were four substrates, which were locust bean gum (LBG) and three agricultural wastes, namely tea waste, coffee grounds, and copra meal. They were dried at 60°C by a hot air oven for 48 h. Afterward, they were blended and milled to 0.5 mm in size. LBG was purchased from Sigma Chemical, USA. All of the other chemicals used were of an analytical grade.

2.2 *Microorganisms and Inoculum preparation*

2.2.1 Microorganisms

Many researchers have tried to isolate bacteria from the soil [23], [24]. They have found that one of the bacteria species that is isolable from the soil is *Bacillus subtilis* [25]–[27]. *Bacillus subtilis* is mainly microorganisms for producing mannanase and cellulase, which have been widely used in the chemical industries [4], [11]. In this research, *Bacillus subtilis* P2-5 was used as an inoculum. It was isolated from the soil in Pathum Thani province in Thailand, and analyzed by a Korea company named Macrogen (www.macrogen.com). The DNA of P2-5 was analyzed by 16S rDNA sequences using the forward primer 785F (5'-GGA TTA GAT ACC CTG GTA-3') and the reverse primer 907R (5'-CCG TCA ATT CCT TTR AGT TT-3'). The obtained sequences were compared with other DNA sequences in the GenBank database of the BLAST program.

Based on the Clustal Omega program, a phylogenetic tree of the 16S rDNA sequences was constructed and shown in Figure 1. The P2-5 isolate was found to be a 99% similar to *Bacillus subtilis* NCBI accession No. CP010052.1, and it was identified as the *Bacillus subtilis* P2-5 strain by Pangsri [27]. The efficiency of *Bacillus subtilis* P2-5 was observed by preliminary experiments. It was used to hydrolyze LBG on nutrient agar (NA) and carboxymethyl cellulose agar (CMC), in order to produce mannanase and cellulase, respectively. It was obvious that the ratio of the clear zone diameter to the colony diameter was high, which indicated that *Bacillus subtilis* P2-5 was an efficient bacterium to produce mannanase and cellulase enzymes.

2.2.2 Inoculum preparation

Bacillus subtilis P2-5 was stored at –20ºC on nutrient broth and was then transferred into a nutrient broth medium. The inoculated culture was incubated by using an incubator shaker speed of 150 rpm at 37°C for 18 h. The growth of P2-5 was measured by spectrophotometry when using an optical density of 600 nm (OD600). The value of the growth was 0.5, which is considered good for enzyme production [28]–[30].

2.3 *Submerged fermentation for enzyme production*

A submerged fermentation (SmF) method was selected for the enzyme production in this research. It was selected since an inoculum is a small amount of material containing bacteria that is used to start a culture. While using the SmF method, a medium was prepared in 250 Erlenmeyer flasks. It contained 100 mL of medium % (w/v), 0.4 bacto-peptone, 0.3 KH_2PO_4, 0.2 K_2HPO_4, 0.05 $MgSO_4.7H_2O$, 0.0002 $CoCl_2.6H_2O$, 0.0005 $FeSO_4.7H_2O$, 0.0002 $MnSO_4.H_2O$, 0.0016 $ZnCl_2$, and 1% (w/v) of a substrate (LBG, tea waste, coffee grounds, or copra meal). Finally, four mediums were obtained and they were sterilized by an autoclave at 121°C for 15 min. In order to produce the enzymes, each medium was fermented by using 1 and 5% (v/v) of *Bacillus subtilis* P2-5, with 150 and 200 rpm at 37 and 45°C, respectively, for 24 h. At the end of the submerged fermentation, they all were centrifuged at 4°C and 7,000 rpm for 20 min. The supernatant (crude enzyme) from each sample was then collected and measured for its enzyme activity.

2.4 *Determination of enzyme activity*

The mannanase activity was measured at 50°C for 30 min when using a reaction mixture containing 0.5 mL of crude enzyme and 0.5 mL of 0.1 M phosphate buffer (pH 7.0), with 1% (w/v) LBG. The amount of reducing sugar was determined by the dinitrosalicylic acid (DNS) method [31]–[33]. One unit of mannanase activity was defined as the amount of enzyme that produced 1 μmol of mannose per minute under each experimental condition. The cellulase activity was determined by estimating the reducing sugar that was produced during the enzymatic reaction by the DNS method [31], [34]–[37]. The reaction mixture was composed of 0.5 mL of crude enzyme, 0.5 mL of 0.05 M citrate phosphate buffer (pH 7.0), with 1% (w/v) CMC (Sigma-Aldrich). One unit of cellulase activity was defined as the amount of enzyme that was required for releasing 1 μmol of glucose per minute under each experimental condition.

2.5 *Experimental design*

There were four independent variables (factors) that were directly concerned with the enzyme production. These were a substrate, an inoculum, temperature, and shaker speed. In this research, LBG and three agricultural wastes, namely tea waste, coffee grounds, and copra meal were considered as substrates. *Bacillus subtilis* P2-5 was considered as the inoculum since it is one of the microorganisms that obtain high enzyme activities.

The levels (values) of each factor in this experiment were set by preliminary experiments that were conducted beforehand. The inoculum was set at values of 1 and 5% (v/v), while the temperature and the shaker speed were set, based on the equipment specifications in the laboratory. They are summarized in Table 2. By a factorial experiment with a single replicate design, there was a total of 32 runs required for the analyses. The reason for selecting a single replicate design was that 30 runs were reserved for the confirmatory experiments.

Table 2: Factors and levels

Factor	Level	Detail
Substrate (*Sb*)	4	LBG, CG, TW, CM
Inoculum (*I*)	2	1 and 5% (v/v)
Temperature (*T*)	2	37 and 45°C
Incubator Shaker Speed (*S*)	2	150 and 200 rpm

TW: Tea waste, CG: Coffee grounds, CM: Copra meal

There were two dependent variables (responses) measured from each run. They were the mannanase and cellulase activities (U/mL). The experimental data of 32 runs along with the corresponding responses are shown in Table 3.

The results were analyzed based on a multi-response analysis tool in Minitab 17, in order to obtain a condition to maximize both of the enzyme activities. After that a set of 30 runs was conducted based on the obtained condition to ensure that it was reliable. Finally, the maximum enzyme activities that were obtained from all of the agricultural wastes, were quantitatively compared with those that were obtained from LBG, in order to evaluate the efficiency of the proposed enzyme production idea of this research.

3 Results and Discussion

The results and the discussion are separated into four parts. Firstly, the effects of the factors on the mannanase and cellulase activities are discussed.

Secondly, a regression model for predicting the responses, and a factor setting to maximize both of the enzyme activities are determined. Thirdly, a confirmatory run with the hypothesis test is performed and concluded. Finally, the efficiency of using the wastes and *Bacillus subtilis* P2-5 to produce mannanase and cellulase is discussed.

Table 3: Experimental data

Sb	*I*	*T*	*S*	M (U/mL)	C (U/mL)
LBG	1	37	150	1.7290	1.0319
LBG	1	37	200	2.0321	1.1397
LBG	1	45	150	1.4362	0.7876
LBG	1	45	200	1.4729	1.0919
LBG	5	37	150	1.3844	1.1479
LBG	5	37	200	1.5980	0.8858
LBG	5	45	150	1.1345	0.8490
LBG	5	45	200	0.9329	1.0141
TW	1	37	150	0.6533	0.5692
TW	1	37	200	0.8652	0.4777
TW	1	45	150	0.8240	0.5760
TW	1	45	200	0.6695	0.5405
TW	5	37	150	0.7755	0.5037
TW	5	37	200	0.7681	0.4313
TW	5	45	150	0.6386	0.5077
TW	5	45	200	0.6283	0.5132
CG	1	37	150	0.6445	0.3412
CG	1	37	200	0.6990	0.1406
CG	1	45	150	0.6592	0.2320
CG	1	45	200	0.6931	0.0123
CG	5	37	150	0.7534	0.3044
CG	5	37	200	0.5885	0.0532
CG	5	45	150	0.7652	0.2566
CG	5	45	200	0.5121	0.2006
CM	1	37	150	0.1192	0.0996
CM	1	37	200	0.0824	0.0546
CM	1	45	150	0.1015	0.0382
CM	1	45	200	0.0677	0.0218
CM	5	37	150	0.1207	0.0887
CM	5	37	200	0.0839	0.0273
CM	5	45	150	0.1001	0.0682
CM	5	45	200	0.1016	0.0382

M: Mannanase activity, C: Cellulase activity

3.1 *Effects of the factors on mannanase and cellulase activities*

The results obtained by the analysis of variance method (ANOVA), were shown in Figure 2.

Based on Figure 2, all of the factors and the interactions of *Sb*I*, *Sb*T*, and *T*S* significantly affected the mannanase activities, while only *Sb*, *S*, *T*, and the interactions of *Sb*S* and *T*S* significantly affected the cellulase activities. The associations between both of the enzyme activities and the factors as shown in Figure 2, were at a very good level since each R^2 (adjusted) was very high (> 95%). This meant that the single replicate design was adequate to estimate the final model.

General Factorial Regression: Mannanase activity

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	13	8.33377	0.64106	80.20	0.000
Linear	6	7.81256	1.30209	162.90	0.000
Sb	3	7.55826	2.51942	315.20	0.000
I	1	0.10848	0.10848	13.57	0.002
T	1	0.14576	0.14576	18.24	0.000
S	1	0.00006	0.00006	0.01	0.932
2-Way Interactions	7	0.52122	0.07446	9.32	0.000
Sb*I	3	0.22568	0.07523	9.41	0.001
Sb*T	3	0.25645	0.08548	10.69	0.000
T*S	1	0.03909	0.03909	4.89	0.040
Error	18	0.14387	0.00799		
Total	31	8.47765			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0894038	98.30%	97.08%	94.64%

General Factorial Regression: Cellulase activity

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	4.29997	0.47777	86.30	0.000
Linear	5	4.20091	0.84018	151.77	0.000
Sb	3	4.17351	1.39117	251.30	0.000
T	1	0.00941	0.00941	1.70	0.206
S	1	0.01800	0.01800	3.25	0.085
2-Way Interactions	4	0.09906	0.02476	4.47	0.009
Sb*S	3	0.06820	0.02273	4.11	0.019
T*S	1	0.03086	0.03086	5.57	0.028
Error	22	0.12179	0.00554		
Total	31	4.42176			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0744039	97.25%	96.12%	94.17%

Figure 2: ANOVA results for the mannanase and cellulase enzyme activities.

3.2 *Regression model and the recommended factor setting for each substrate*

Table 4 shows the regression coefficients of the factors in the final models for the mannanase and cellulase activities. Since each substrate was a categorical factor, a regression model was determined separately for each of the substrates. They are shown in Equations (1) to (8). Note that the interactions of *Sb*T* and *Sb*I* for mannanase, and *Sb*S* for cellulase, had already been included in the factors of *I*, *T*, and *S*.

Table 4: Coefficients of the factors in the final models

Cf		Cs	*I*	*T*	*S*	*T*S*
LBG	M	1.5301	–0.1013	0.0059	0.0143	–0.0004
	C	3.1211	0	–0.0586	–0.0112	0.0003
TW	M	–1.3502	–0.0126	0.0517	0.0143	–0.0004
	C	3.0881	0	–0.0586	–0.0137	0.0003
CG	M	–1.7501	–0.0048	0.0594	0.0143	–0.0004
	C	3.2231	0	–0.0586	–0.0164	0.0003
CM	M	–2.3601	0.0022	0.0601	0.0143	–0.0004
	C	2.5921	0	–0.0586	–0.0135	0.0003

Cf: Coefficient, Cs: Constant

Regression models for mannanase activity

LBG:

$$M = 1.5301 - 0.1013\,I + 0.0059\,T + 0.0143\,S - 0.0004\,T*S \quad (1)$$

Tea waste:

$$M = -1.3502 - 0.0126\,I + 0.0517\,T + 0.0143\,S - 0.0004\,T*S \quad (2)$$

Coffee ground:

$$M = -1.7501 - 0.0048\,I + 0.0594\,T + 0.0143\,S - 0.0004\,T*S \quad (3)$$

Copra meal:

$$M = -2.3601 + 0.0022\,I + 0.0601\,T + 0.0143\,S - 0.0004\,T*S \quad (4)$$

Regression models for cellulase activity

LBG:

$$C = 3.1211 - 0.0586\,T - 0.0112\,S + 0.0003\,T*S \quad (5)$$

Tea waste:

$$C = 3.0881 - 0.0586\,T - 0.0137\,S + 0.0003\,T*S \quad (6)$$

Coffee ground:

$$C = 3.2231 - 0.0586\,T - 0.0164\,S + 0.0003\,T*S \quad (7)$$

Copra meal:

$$C = 2.5921 - 0.0586\,T - 0.0135\,S + 0.0003\,T*S \quad (8)$$

For the mannanase activity, the inoculum had a negative effect, as shown in Equations (1) to (3). This meant that when LBG, tea waste, and coffee grounds were selected, by increasing the inoculum, a lower mannanase activity was obtained. However, this happened in the opposite direction when copra meal was selected, as shown in Equation (4). For all of the substrates, the temperature and the speed had positive effects, as shown in Equations (1) to (4). This meant that by increasing these factors, a higher mannanase activity was obtained. However, the temperature and the speed had to be carefully set since the interaction of these factors was significant.

For the cellulase activity, as shown in Equations (5) to (8), the temperature and the speed had negative effects. This meant that by increasing these factors, a lower cellulase activity was obtained. Similar to the mannanase activity, these factors had to be carefully set since their interaction was significant. This happened for all of the substrates. Note that the inoculums of 1 and 5% did not produce any significant differences in the means of the cellulase activity for all of the substrates. Therefore, this factor was excluded from the models. However, it may become a significant factor when it is tested by the other experimental ranges.

Table 5 shows the maximum enzyme activities, along with their factor settings, as obtained from the multi-response optimizer. It was no surprise that LBG obtained the highest enzyme activities, when compared to the other substrates since it is a commercial substrate that is generally used in enzyme production. Among the agricultural substrates, tea waste obtained the highest enzyme activities, whereas copra meal obtained the lowest.

Table 5: Conditions to maximum enzyme activities

Sb	*I*	*T*	*S*	M (U/mL)	C (U/mL)
LBG	1%	37°C	200 rpm	1.9221	1.0192
TW	1%	37°C	150 rpm	0.7572	0.5873
CG	1%	37°C	150 rpm	0.6473	0.3318
CM	5%	41.2°C	150 rpm	0.1045	0.0713

Based on Table 5, when comparing tea waste and LBG, the mannanase and cellulase activities that were obtained from the tea waste were approximately 39.4 and 57.6% of those that were obtained from LBG, respectively.

3.3 *Confirmatory run and the hypothesis test*

A confirmatory run was performed by using only

tea waste since it obtained the highest enzyme activities. In order to accomplish this, 30 samples were 'confirmatory-run-performed' based on the tea waste setting, as shown in Table 5. The means and the standard deviations, along with their hypotheses, and the *p*-values that were obtained from the 30 samples, are shown in Table 6.

The hypotheses were performed in order to check whether the results in Table 5 were reliable. One-tailed t-tests (right side) were required for the tests, based on the question that the results obtained from the confirmatory run were better than those obtained from the optimizer. Since the *p*-values for both tests were 0.0000, the null hypotheses were rejected. This meant that the results can be implemented.

Table 6: Means and standard deviations of the confirmatory run

Statistics	M (U/mL)	C (U/mL)
Mean	0.7665	0.5992
Standard deviation	0.0064	0.0097
Hypothesis	H_0: μ = 0.7572 H_1: μ > 0.7572	H_0: μ = 0.5873 H_1: μ > 0.5873
p-value	0.0000	0.0000

3.4 *Efficiency of using tea waste and Bacillus subtilis P2-5 to produce mannanase and cellulase*

The efficiency of producing mannanase and cellulase by using the tea waste and *Bacillus subtilis* P2-5 was determined by comparing the enzyme activities, with the other related researches that produced the same enzymes, but used the different substrates and microorganisms. The efficiency comparisons were summarized in Table 7.

To evaluate the efficiency, the enzyme activities were directly compared to each other. The more the enzyme activity, the higher the efficiency. It clearly shows that the enzyme activities obtained from this research (see bottom row) were in a competitive level. Therefore, this can be a good reference for further studies.

4 Conclusions

In this research, three agricultural wastes, namely, tea waste, coffee grounds, and copra meal were selected and used as substrates. They were fermented with a new inoculum named *Bacillus subtilis* P2-5, in order to produce mannanase and cellulase enzymes. The results have shown that it was possible to use these agricultural wastes as substrates in an enzyme production process. Although the enzyme activities that were obtained from the tea waste were lower than those that were obtained from LBG, this idea is still well-worth implementing since the enzyme production costs and the agricultural waste disposal costs were both reduced when LBG was replaced by the tea waste. There are two main contributions resulting from this research. Firstly, an alternative way to manage agricultural waste is proposed. Secondly, when the proposed idea is implemented, the demand for LBG is also reduced, leading to lower enzyme production costs.

There are some limitations to this research. The experiments were conducted in a small-scale laboratory. Therefore, the levels of the factors were varied within narrow ranges. Other agricultural wastes, as well as microorganisms and mediums, should be investigated, in order to determine the potential ones for enzyme production. Further researches should be conducted to address these limitations.

Table 7: Efficiency comparison with the other researches in the literature

Substrate	Microorganism	M (U/mL)	C (U/mL)	Ref.
Coffee ground	*Bacillus subtilis* GA2(1)	0.26	-	[28]
Corn cobs	*Bacillus* sp. GA2(1) *Bacillus* sp. GA1(6)	0.53 0.25	0.33 0.15	[29]
CMC	*Bacillus* sp. P3-1 *Bacillus* sp. P4-6	- -	0.015 0.015	[38]
Sugarcane bagasse	*Bacillus* sp. SMIA-2	-	0.29	[39]
Mixed of Coconut and Tofu waste	*Bacillus amyloliquefaciens*	2.24	-	[40]
LBG		2.28	-	
Tea waste	***Bacillus subtilis* P2-5**	**0.7665**	**0.5992**	-

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