International Food Research Journal 23(3): 1280-1288 (2016)
Journal homepage: *http://www.ifrj.upm.edu.my*

Optimization of raw-starch-digesting amylase (RSDA) production medium for *Enterococcus faecium* DMF78

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Article history

Received: 11 June 2015
Received in revised form: 22 August 2015
Accepted: 27 August 2015

Keywords

Enterococcus faecium
Raw-Starch-Digesting Amylase
Plackett-Burman
Box-Behnken

Abstract

In an effort to lower the cost of raw-starch-digesting amylase (RSDA) production for *E. faecium* DMF78, statistical tools were used to optimize production parameters. Plackett-Burman and Box-Behnken has been carried out to evaluate the effects of 17 different parameters and conditions. Proteose peptone, beef extract, and MRS salts were found to be positive significant effectors for amylase production. On the other hand, yeast extract, sodium dodecyl sulfate (SDS), and corn steep liquor (CSL) were negative effectors. The optimized medium resulted to a 466.67% increase in amylase activity and 41.99% decrease in cost compared to the modified MRS medium with the elimination of yeast extract, trub, SDS, and CSL.

Introduction

Today, there is an on-going global campaign towards a greener environment that promotes the use of more environment-friendly products. This allows the maximization of natural resources needed for the conversion of biomass, such as starch, into high value products. One step taken was the use of microorganisms as potential source of industrially important enzymes such as amylases. These enzymes have the capability of hydrolyzing starch into smaller molecules of sugars or dextrins that is necessary to circumvent the use of high temperatures for long hours (gelatinization and liquefaction) in a traditional starch hydrolysis process.

Successful isolation of amylase producing (amylolytic) microorganisms has been well documented. Examples of these isolates are *Lactobacillus amylophilus* JCM 1125 (Nakamura and Crowell, 1979; Yumoto and Ikeda, 1995), *L. amylovorus* JCM 1126 (Nakamura, 1981), *L. plantarum* A6 (Giraud *et al*., 1994) and *L. manihotivorans* LMG 18010T (Guyot *et al*., 2000). Aside from the previously mentioned isolates, the utilization of *Bacillus* sp. (Kelly *et al*., 1995), *Bacillus* sp. (Goyal et al., 2005), *Cytophaga* sp. (Shiau *et al.*, 2003), *Lactobacillus* sp. (Aguilar *et al*., 2000), and *E. faecium* DMF78 (Tabanao, 2008) for the production of raw-starch-digesting amylases (RSDA) have been explored.

Raw starch digesting amylases (RSDAs) have received a great deal of attention because of their perceived technological significance and economic benefits. RSDAs have the capability of hydrolyzing the native form of starches that can be used for the production of simple constituent sugars, which can be processed for the production of high value products like high fructose syrup (Limbaga, 2007), ethanol (Bandaru *et al*., 2006), and lactic acid (Cheng *et al.*, 1991). RSDAs circumvent not only the gelatinization-liquefaction steps but also the saccharification step needed for the conversion of starch to simple sugars. In other words, it allows the entire process of starch hydrolysis to proceed at a faster rate and at a lower cost.

Another factor that contributes to microbial enzyme cost is the production medium. According to Babu and Satyanarayana (1993), enzyme production by microorganisms is significantly affected by various physical and chemical parameters. There are different kinds of widely available substrates, minerals, carbon, and nitrogen sources to consider in enzyme production medium formulation. To reduce the cost of enzyme production medium, one must

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determine the cheapest possible set of components for the medium. The traditional way of screening variables is the "one-variable-at-a time" concept that has shown to work well when applied to process designs, especially in cases where only a small number of variables are being studied. However, this may become a problem when applied to an experiment that needs to consider large number of variables. This traditional method is time consuming, expensive, and does not allow the understanding of the interactions between the factors considered (Weuster-Botz, 2000 and Silva and Roberto, 2001). Nowadays, the use of statistical designs such as Plackett-Burman and Box-Behnken has been carried out. These advance statistical approaches offer a design that allows the study of several factors that requires minimum number of runs, thus, saving time and resources. These also allow the screening of critical factors; provide information regarding optimized levels of each factor and knowledge of the interactions between factors and its effect on amylase activity. Successful optimization of amylase production medium, carried out using statistical designs, were reported by Sharma and Satyanarayana (2011) using *Bacillus acidicola,* Hashemi *et al.* (2010) using *Bacillus* sp., and Dey *et al.* (2001) using *Bacillus circulans* GRS 313.

Materials and Methods

Microorganism

Pure culture of *E. faecium* DMF78 was obtained from the Department of Food Science and Chemistry, University of the Philippines Mindanao. Stock cultures were prepared using a modified MRS medium (sago starch as substrate).

Wort preparation

Germination of sorghum was done according to Igyor *et al.* (2001). Worting was done according to Agu and Palmer (1999) with modifications. Fifty grams of sorghum malt was added with 360 mL of distilled water pre-equilibrated at 65°C, and stirred at 250 rpm for 1 hour at 65°C.

Enzyme assay

Amylase activity was determined using the starch reduction analysis procedure according to Yoo *et al.* (1987) with modifications. Pre-equilibrated substrate solution at 30°C was added with 100 µL enzyme and incubated for 10 minutes. The reaction was stopped with the addition of 1 mL of 0.1 M HCl. Five hundred micro liters of the sample was obtained as aliquot and was added with 5 mL working iodine reagent. The solution was allowed to stand for 5 minutes and was read at 660 nm. One unit of amylase activity is defined as the amount of enzyme required to hydrolyze 1 mg of soluble starch for 10 minutes at specified assay conditions.

Optimization of amylase production media

Screening of factors by Plackett-Burman design

A randomized design utilizing the Plackett-Burman design (PBD) was generated using Minitab® 16.1.0 Statistical Software. Seventeen factors were considered (effects of initial pH, substrate pre-treatment, agitation (rpm), % inoculum size, % substrate, Ca^{2+} concentration, % MRS salts, % sodium dodecyl sulfate, % Tween 80, % Triton® X-100, % yeast extract, % proteose peptone, % beef extract, % corn steep liquor, % wort, % trub, and % urea). Low and high values were considered based on literature. Table 1 shows the different factors considered in uncoded levels.

The various media, based in the experimental design, were prepared in flasks. Right amounts of cell suspension were introduced. The flasks were incubated at room temperature for 6 hours (0 rpm, 50 rpm, and 100 rpm). Enzymes were harvested from the media by centrifugation at 10,000 rpm, 4°C for 10 minutes. Enzymatic activity was checked.

Washed cells, mixed in 70 mL sterile ultrapure water, served as the starter inoculum for the optimization studies of amylase production.

Optimization of factors by Box-Behnken design

Based on the analysis using PBD, Box-Behnken design (BBD) was employed to establish the optimal values for the identified factors to study. Five percent cell suspension was inoculated onto various prepared media and controls and incubated at 30°C for 6 hours without agitation. The extracellular enzymes from the media were harvested by centrifugation and enzyme activity was checked.

Verification of the optimized medium: shake flask fermentation and time course analysis

Amylase production using the optimized medium was verified 5 times to confirm the optimization results. Time-course analysis was done using the 2-L Fermentor (Labo Controller MDL-8C, BNRN-2L B.E Marubishi). The optimized enzyme production medium was incubated at 30°C for 24 hours, at pH 6.5 and agitated at 48 rpm. Samples were collected at 0, 15^{th}, 30^{th} and 45^{th} minute and 1^{st}, 2^{nd}, 4^{th}, 6^{th}, 12^{th}, 18^{th}, and 24^{th} hour of fermentation. Crude enzyme was harvested by centrifugation and enzyme activity was checked. Microbial count (CFU/mL) was determined

Table 1.Factors considered and its corresponding uncoded levels

Code	Factor	Low	High	Bibliographic Reference
A	Initial pH	5.5	6.5	Sajedi *et al.* 2005; Asgher *et al.* 2007; Tabanao 2008
B	Substrate Pretreatment	Raw	Gelatinized	Tabanao 2008
C	% Inoculum Size	5	10	Sharma and Satyanarayama 2011
D	% Substrate	1	3	Original Media Formulation
E	Ca^{2+} Concentration, mM	0	1	Asgher *et al.* 2007
F	% MRS Salts	50	100	Original Media Formulation
G	% Sodium Dodecyl Sulfate	0	0.5	Reddy *et al.* 1999
H	% Tween 80	0	0.1	Reddy *et al.* 1999
J	% Triton® X-100	0	0.1	Reddy *et al.* 1999
K	Agitation, rpm	0	100	Tabanao 2008
L	% Yeast Extract	0	0.05	Sharma and Satyanarayama 2011; Dey *et al.* 2001
M	% Proteose Peptone	0	0.1	Sharma and Satyanarayama 2011;
N	% Beef Extract	0	0.1	Goyal *et al.* 2005
O	% Corn Steep Liquor	0	25	Hamilton *et al.* 1999; Goyal *et al.* 2005
P	% Trub	0	5	Tan 2010
Q	% Wort	0	25	Tan 2010; Mesta 2005
R	% Urea	0	3	Metin *et al.* 2010; Kunamneni 2005

by pour-plating methods. The colonies were counted after 24 hours and reported as CFU/mL.

Statistical analyses

All experiments were carried out with three replicates unless otherwise stated. The data collected was tested for Normality using Kolomogorov-Smirrnov test for normality and normal set of data were analyzed using one-way Analysis of Variance (ANOVA) at 95% level of confidence. Tukey's test was used to determine if treatments were significantly different from each other using Minitab® 16.1.0 Statistical Software.

Results and Discussion

E. faecium DMF78 is a novel lactic acid bacterium that has been previously reported to have the ability to produce amylase at room temperature in a short period of time (Shibata *et al.* 2007). To further improve the amylase activity and reduce cost of production, optimization of amylase production medium was studied using various low-cost carbon and nitrogen sources. Different statistical approaches were used in order to generate designs of experiment that will allow a number of factors to be studied using only a few runs.

Screening of factors affecting amylase production by Plackett-Burman Design of experiment

Plackett-Burman design of experiment is a two factorial design that can be used to screen important independent variables (Plackett and Burman, 1946). Seventeen factors were investigated and an upper limit of 0.1% for proteose peptone and beef extract, 0.05% for yeast extract, and 100% (based from the original formulation) MRS salts were previously set. That is, this will be the maximum concentrations of these factors regardless of the results of statistical analysis because of the high cost that they contribute to the over-all production.

The response variable is amylase activity. Results were analyzed statistically in coded units to avoid any spurious statistical results due to different measurement scales of the factors (% vs. rpm) and to eliminate co-linearity among the terms in the models that increases the variability in the coefficient estimates making it difficult to interpret. The model has an r^2 value of 0.9554 and an adjusted r^2 value of 0.7948. The r^2 value of 0.9554 indicates that 95.54% of the proportion of the variability in the response can be explained by the model. However, it is recommended to assess model fit using the adjusted r^2 value because it modifies the r^2 value by taking into account the number of covariates or predictors included in the model. High value of adjusted r^2 (values ≥ 0.70) indicates that the model is fitting the data very well. The standard error or standard error of the estimate (S) value is a measure of prediction accuracy. The model has an S-value of 0.8698, which is very low, indicating the capability of the model to have accurate prediction results.

Based from the estimated effects and coefficients of the 17 factors that were screened, it was observed that the p-values of MRS salts, proteose peptone, and

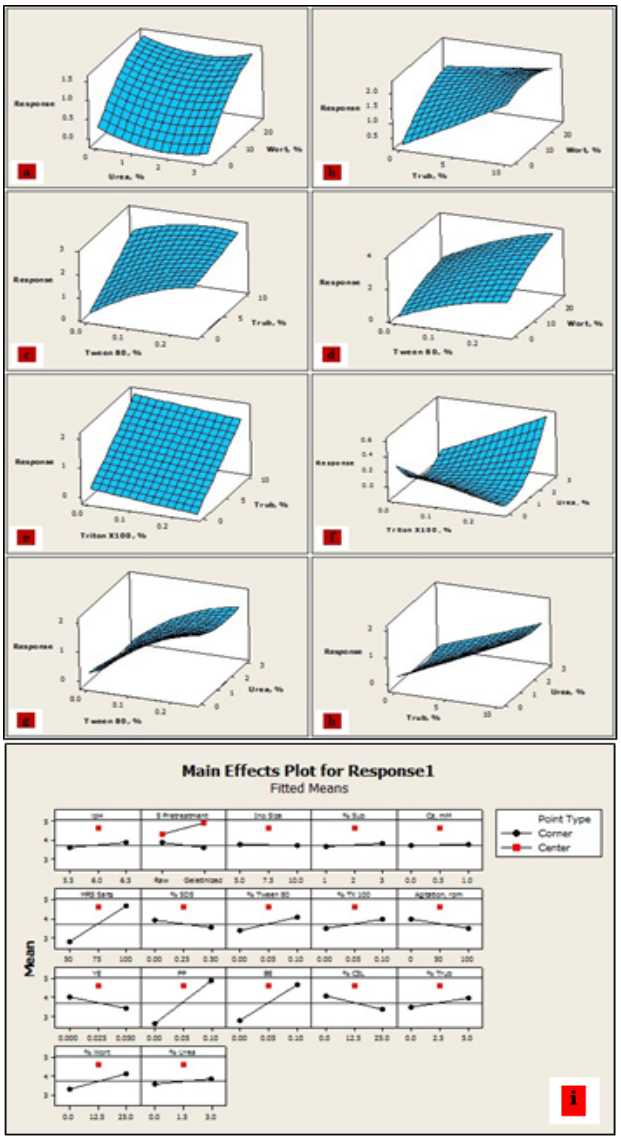

Figure 1. Surface plots of the interaction effects of (a) urea and wort, (b) trub and wort, (c) Tween 80 and trub, (d) Tween 80 and wort, (e) Triton® X-100 and trub, (f) Triton® X-100 and urea, (g) Tween 80 and urea, and (h) trub and urea, on amylase activity (response). (i) Main effects plot of 17 screened factors.

beef extract were less than 0.05 making these factors significant contributors to the increase in amylase activity.

Figure 1i shows the main effects plot that presents the effect to the response of the various levels of the factors being studied. The main effects plot is used to compare the relative strength of the effects. It can be observed in Figure 1i that initial pH, inoculum size, % substrate, and Ca^{2+} concentration had almost no effect on the amylase activity even at its highest concentrations. Thus, Ca^{2+} can be eliminated for future formulations of the amylase production media. In the case of substrate concentration (1%-3%), it did not show any significant effect on amylase activity, even though literatures have reported that it almost always affects fermentation processes. Typically, as the substrate concentration is increased, there is also a corresponding increase in amylase activity. However, this was not the case for this study. The result can be due to substrate saturation wherein subsequent addition of substrate poses no effect on amylase activity because all active sites in the enzyme were already occupied. On the other hand, % SDS, % yeast extract, and agitation showed a possible negative effect. It was observed that as the concentration and level increased, there was a decrease in the enzyme activity. Thus, it is better to set these factors at the lowest possible concentrations and level. % MRS salts, % Tween 80, % Triton® X-100, % proteose peptone, % beef extract, % wort, % trub, and % urea showed a possible significant effect as shown by the slope of the line generated by these factors. It can also be observed that as the concentration of these factors were increased; there was a corresponding increase in the amylase activity. Thus, it is recommended to further study the effects of these factors at increased concentrations.

These results also concurred with the results from previous study on amylase production using *E. faecium* DMF78. Based on the study by Tabanao (2008), *E. faecium* DMF78 preferred a pH of 6.5, 0 rpm agitation, and the use of raw sago starch as substrate. *E. faecium* DMF78 is a facultative anaerobe and hence can grow well with or without the presence of oxygen. Zero agitation preference for increased enzyme activity may indicate that the microorganism may prefer low levels of oxygen for its enzyme production.

In the study conducted by Dey *et al.* (2001) using a statistical tool to enhance amylase production from *Bacillus circulans* GRS 313 by optimization of nutritional constituents of the media, results showed that yeast extract was found to be less significant for the enhancement of amylase yield. Tanyildizi *et al.* (2005) also reported yeast extract to exhibit no effect on amylase activity.

It is important to note that only MRS salts, proteose peptone, and beef extract are the positive significant factors that were identified. The maximum levels of MRS salts, proteose peptone and beef extract was previously set in such a way that these will not contribute to the biggest percentage in the cost of the medium. Thus, it was decided that even though these three factors are candidates for further optimization studies using increased concentration, it shall be overlooked due to its contribution to high production costs. So the remaining factors that exhibit increase in amylase activity with increased concentration (% Tween 80, % Triton® X-100, % wort, % trub, and % urea) were considered for further optimization, with

MRS salts, % proteose peptone, and % beef extract at its constant levels.

Optimization of the factors by Box-Behnken design

The Box-Behnken design of experiment generated a randomized design of experiment based on the five factors that were considered, producing 46 base runs with 6 center points. Amylase activity was determined using starch reduction assay method and response surface regression analysis showed an r^2 of 0.9115, which means that 91.15% of the proportion of the variability in the response can be explained by the model, an adjusted r^2 value of 0.8184, and an S-value of 0.2831. Furthermore, under the Analysis of Variance for Response table, the lack of fit (the variation due to model inadequacy) had a p-value of 0.074, making it insignificant. Thus, there is no evidence that the model does not adequately explain the variation in the responses.

The model contains 5 linear effects (% Tween 80, % Triton® X-100, % wort, % trub, and % urea) and the p-values of Tween 80, trub, and wort were less than 0.05. Therefore, there is a significant linear effect for Tween 80, trub and wort. That is, the yield differed depending on the Tween 80, trub and wort. On the other hand, the p-values of urea and Triton® X-100 were greater than 0.05 and thus, the yield did not change with changes in urea and Triton® X-100.

As for the squared effects, it can be used to evaluate whether or not there is curvature (quadratic) in the response surface. The p-values of Tween 80 and wort were less than 0.05 and thus implied significant quadratic effects. That is, the individual relationship of Tween 80 and wort to amylase activity follow a curved line, rather than a straight line.

The model contains ten two-way interaction that allows the determination of which among the factors had a significant interaction with each other. Interactions between Tween 80 and trub, and trub and wort had p-values less than 0.05 thus, there was a significant interaction effect. That is, the effect of Tween 80 on the amylase activity depended on trub and the effect of trub on the amylase activity depends on wort.

Results showed the estimated regression coefficients for the response (amylase activity) which can be used to construct an equation representing the relationship between the response and the factors.

Amylase activity = 0.2584 + 11.8678 (%Tween 80) - 1.4940 (Triton® X-100) + 0.1721 (Trub) - 0.4147 (Urea) + 0.0949 (Wort) - 18.5878 (Tween 80)2 + 0.4300 (Triton® X-100)^2 + 0.0011 (Trub)^2 + 0.1088 (Urea)^2 - 0.0017 (Wort)^2 + 9.4603 (Tween 80*Triton® X-100) - 0.4862 (Tween 80*Trub) - 0.2346 (Tween 80*Urea) + 0.0912 (Tween 80*Wort) + 0.0034 (Triton® X-100*Trub) + 1.2841 (Triton® X-100*Urea) - 0.0339 (Triton® X-100*Wort) - 0.0162 (Trub*Urea) - 0.0057 (Trub*Wort) + 0.0025 (Urea*Wort)

The Box-Behnken design of experiment is used essentially to find out the optimum values of the considered variables for which the response is maximized. Statistical analysis showed that amylase activity was highest when Tween 80 was at 0.25%, Triton® X-100 at 0.25%, trub at 0%, urea at 3%, and wort at 25%. The effect of interaction of various parameters on the amylase activity was studied using 3-dimensional response surface curves against any two independent variables and is shown in Figure 1a-h. For example, Figure 1a shows effect of the interaction of urea and wort on amylase activity. It can be observed that amylase activity is low when the concentrations of urea and wort are low. On the other hand, the amylase activity is high when the concentrations of these factors were also high.

Verification of optimized media: shake flasks

The verification process involves performing experiments applying the predicted "best" processing conditions. Results of response optimization gave a global solution ("best" combination of factor settings for achieving the desired responses). A predicted amylase activity of 4.69 U/mL can be achieved with 0.25% Tween 80, 0.25% Triton-X100, 0% trub, 3% urea, and 25% wort. However, the amylase activity of 4.69 U/mL was not achieved but rather, only 2.5 U/mL (mean of all verification trials). But on the positive note, the amylase activity of the optimized (DTF) medium offers a 400% increase in enzyme activity compared to the control, or modified MRS medium. Figure 2 shows the amylase activity of the DTF medium in comparison with the control. This showed that the optimized medium offers higher amylase activity or higher amylase production at a lower cost than the original enzyme production medium.

The optimized conditions and corresponding proportion of components for amylase production (DTF) medium using *E. faecium* DMF78 are as follows: proteose peptone (0.10%), beef extract (0.10%), Triton® X-100 (0.25%), Tween 80 (0.25%), urea (3%), sorghum wort (25%), raw sago starch (3%), and MRS salts (100%-based from the original). Proteose peptone that has been commonly used as nitrogen source in culture media because of its high amino acid content that contributes to the essential nitrogen requirement and beef extract complements

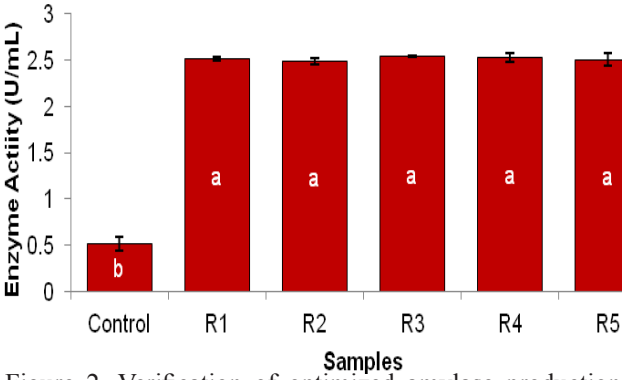

Figure 2. Verification of optimized amylase production (DTF) medium. (Bars of different letters mean statistical difference at 95% confidence level.)

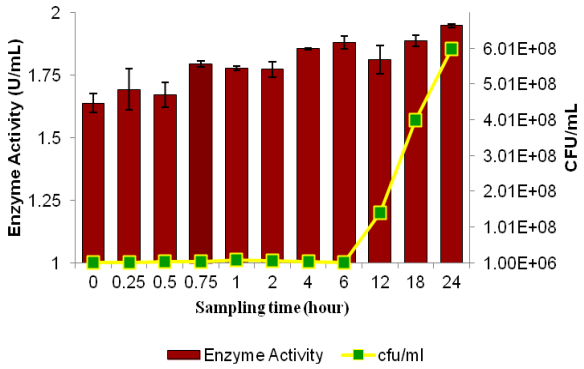

Figure 3. Time course analysis of *E. faecium* DMF78 enzyme activity and microbial count at different time intervals.

the nutritive properties of peptone by contributing minerals, phosphates, energy sources (BD Bionutrients™ Technical Manual). Peptone and beef extract were also part of the original MRS medium that is used for the cultivation of lactic acid bacteria and had been shown to be very essential in the growth and amylase production of *E. faecium* DMF78. Tanyildizi *et al*. (2005) reported that the use of peptone increased amylase activity. The medium also contains surfactants like Triton® X-100 and Tween 80 which are known to increase the permeability of the cell membrane thus increasing the secretion of proteins (Hashemi *et al.*, 2010). Wort has always been believed to be protein-rich and full of nutrients, making it suitable for growth and fermentation processes (Stewart, 2006). The use of sorghum wort was attempted due to unavailability of barley in the Philippines and Etokakpan (1992) reported that the free amino nitrogen levels of sorghum were higher compared to barley if mashed in a modified procedure. The presence of an organic nitrogen source like urea has been reported to enhance amylase production (Anto *et al.,* 2006).

Time course analysis

The DTF medium was validated using the 2-L fermentor (Labo Controller MDL-8C, BNRN-2L B.E Marubishi). Figure 3 shows the enzyme activity at various time intervals. Enzymes harvested after 24 hours of fermentation gave the highest enzyme activity of 1.95 U/mL. This was followed by enzymes harvested after 18 hours, 6 hours, and 4 hours with enzyme activities of 1.88 U/mL, 1.88 U/mL, and 1.85 U/mL respectively. Statistical analysis results showed that enzyme activities of the samples collected in these time intervals had no significant difference from each other. Thus, it is recommended that 4 hours fermentation for enzyme production of *E. faecium* DMF78 was enough. Previous study on amylase activity of E. faecium DMF78, conducted by Tabanao (2008), showed that highest enzyme activity can be acquired after 1 hour fermentation using the modified MRS medium. However, this medium contained expensive carbon and nitrogen sources such as proteose peptone, beef extract, and yeast extract. This study showed that there was a need for a slight increase in time for the enzyme to be produced. However, it offered the use of a much cheaper enzyme production medium which is very much advantageous for large scale production.

Samples were also collected at designated time intervals for microbial count determination. According to Goyal *et al.* (2005), enzyme production is associated with microbial growth and is induced by the presence of substrate in the medium. Figure 3 shows that the lag phase of *E. faecium* DMF78 extended up to the 6th hour after which log phase followed. Figure 3 shows that the highest enzyme activities were observed on the onset of the log phase that indicates a growth related association of microbial growth and enzyme production. However it was also observed in preliminary experiments (data not shown), wherein the sole carbon source of the microorganism is starch, that the association of microbial growth and amylase activity is not-growth related. This is because the microorganism needs to break down the starch first to obtain its glucose vitally needed for growth. And thus, *E. faecium* DMF78 shows a mixed (growth and not-growth) association of microbial growth and enzyme production.

Compared to other amylase producing bacteria, the time of harvest for this bacterium is desirable and advantageous because of the short time needed for enzyme production. According to the reports *B. flavothermus* needs 24 hours (Kelly *et al*. 1997), *B. subtilis* needs 32 hours (Konsula and Kyriakides, 2004), *Bacillus* sp. IMD 435 needs 41 hours (Hamilton *et al.* 1999), and *B. licheniformis* ATCC 9945a (Bozic *et al*. 2011) for optimum production. As

Table 2. Costing composition of modified MRS medium and DTF medium for amylase production by *E. faecium* DMF78

COMPONENT	MODIFIED MRS		DTF MEDIUM		
	1L	Lab Scale (PhP)*	1 L	Lab Scale (PhP)*	Industrial Scale (PhP)*
Proteose Peptone	10g	49.4	1g	4.943	0.43
Beef Extract	10g	74	1g	7.4	0.32
Yeast Extract	5g	21.55	0g	0	0
Triton® X-100	0g	0	2.5g	4.725	0.23
Tween 80	1g	12	2.5g	30	30
Urea	0g	0	30g	40.8	0.48
Wort	0mL	0	150mL	1.6	1.6
MRS Salts	50mL	2.85	50mL	2.85	2.85
Sago Starch	30g	0.9	30g	0.9	0.9
TOTAL		**160.7**		**93.218**	**36.81**

* Prices as of November 2014

of the moment, there are no other available literatures indicating a shorter time for enzyme production than that of *E. faecium* DMF78.

Cost comparison between modified MRS medium and DTF medium

The DTF medium formulation offered a higher amylase activity at a lower cost compared to that of the original medium (modified MRS) used. Table 2 shows the comparison of the cost of the different media formulations. It can be observed that the greater bulk of the cost of production was due to proteose peptone, beef extract, and yeast extract, thus an upper limit was imposed in the optimization procedures. The total cost of the modified MRS medium is PhP 160.70 per liter while the DTF medium costs PhP 93.218 per liter. Thus, the use of the DTF medium will offer 42% savings on the cost of enzyme production. Moreover, an increase of 466.67% (2.52 U/mL) in enzyme activity was noted in the use of DTF medium than modified MRS medium (0.54 U/mL). The use of the DTF medium therefore offers a high enzyme activity at a much lower cost.

Conclusion

This study primarily aimed to produce amylase from *E. faecium* DMF78 using a low-cost alternative medium. Statistical designs such as Plackett-Burman design of experiment was used to screen significant factors that may affect amylase activity and Box-Behnken design was used to determine the optimum levels of the factors being considered. Seventeen factors were considered as components and conditions for the alternative amylase production medium. Plackett-Burman results showed that 3 factors (SDS, yeast extract, and corn steep liquor) have a negative impact on amylase activity and thus can be eliminated. On the other hand, peptone, beef extract, and MRS salts were significant contributors to amylase activity. However, due to the ceiling values previously set, the next succeeding factors (% Tween 80, Triton® X-100, % wort, % trub, and % urea) that showed main effect (not necessarily significant) on amylase production were considered for Box-Behnken experiment.

Box-Behnken design of experiment gave a global solution that may be used to achieve highest amylase activity. The new optimized medium (DTF medium), with noted limitations, for amylase production of E. faecium DMF78 is composed of proteose peptone at 0.10%, beef extract at 0.10%, Triton® X-100 at 0.25%, Tween 80 at 0.25%, urea at 3%, sorghum wort at 25%, raw sago starch at 3%, and MRS salts (based from the original) at 100% with 5% inoculum. The medium must be maintained at pH 6.5 and at 0 rpm agitation.

Time course analysis showed that enzymes with high amylase activity can be harvested after just 4 hours of fermentation. Microbial tests showed that amylase activity is at its peak during the late lag phase to log phase of microbial growth.

The use of the DTF medium allows shorter enzyme production time (4 hours), 42% savings on enzyme production costs, and an almost 500% increase in enzyme activity in comparison to the modified MRS media. The use of the DTF medium therefore offers a high enzyme activity at a much lower cost.

Acknowledgement

This study was financially supported by the DOST-ASTHRD thesis grant and Sago- DOST Project of Dr. Dulce M. Flores in UP Mindanao.

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