Journal of Applied Sciences

ISSN 1812-5654

Journal of Applied Sciences 12 (11): 1166-1170, 2012
ISSN 1812-5654 / DOI: 10.3923/jas.2012.1166.1170

Effect of Operational Parameters on Solid State Fermentation of Cassava Peel to an Enriched Animal Feed

Parveen Jamal, Ruqayyah I.D. Tijani, Md. Zahangir Alam and Md. Elwathig S. Mirghani
Bioenvironmental Engineering Research Unit (BERU),
Department of Biotechnology Engineering, Faculty of Engineering,
International Islamic University Malaysia (IIUM), P.O. Box 10, 50728 Kuala Lumpur, Malaysia

Abstract: Response surface methodology based on the Face-Centered Central Composite Design (FCCCD) was employed to determine the effects of process conditions on the production of an enriched animal feed from cassava peel by a locally isolated white rot fungus *Panus tigrinus* (M609RQY). Seventeen experimental runs based on three parameters (pH, inoculum size and moisture content) as designated by FCCD were carried out under solid state fermentation. The effect of these parameters on lignin degradation in cassava peel was evaluated. Statistical analysis of the results showed that, only moisture content exerted a highly significant effect ($p<0.01$) on lignin degradation. The optimum parameter combination was found at 70% v/w of moisture content, 6% v/w inoculum size and pH of 5.30. Under this optimum, 50.62% lignin loss was obtained. This study presents a viable option to the management of cassava peel for production of value-added-product animal feed.

Key words: Face-centered central composite design, cassava peel, animal feed, *Panus tigrinus*, solid state fermentation

INTRODUCTION

Cassava is the third most important source of calorie after wheat and rice in the developing countries (FAO, 2008), with an estimated world production of 233 million tonnes in year 2008. Cassava peel is a by-product of cassava processing either to food or other industrial products. This peel could make up to 10-20% of the wet weight of the roots (Obadina *et al*., 2006), thus indicating its enormous potential for biotechnological processes. However, these peels are regarded as wastes and are openly dumped causing serious environmental problem associated with their decomposition. Cassava peel represents a potential animal feed however, very small quantity is used for animal feeding, due to its low protein content, high level of hydrocyanide cum high crude fibre and poor digestibility.

Several studies have been reported using solid state fermentation to enhance the nutritional value of cassava peel as animal feed (Aderemi and Nworgu, 2007; Oboh, 2006). However, this had been centred on protein increment alone, whereas degradation of cell wall content especially lignin that hinders the digestibility of cassava peel have not been given priority. Lignin has been described as a key factor limiting the quality of lignocellulosic residue as animal feed and bioconversion using white rot fungi has been proposed to increase the nutritive value of such materials (Sharma and Arora, 2010; Villas-Boas *et al*., 2002).

The white rot fungi *P. tigrinus* is one of the promising locally isolated fungi that is capable of producing the three lignin modifying enzymes (lignin peroxidase, manganese peroxidase and laccases) (Tijani *et al*., 2011a). In addition, reports have shown that this fungus also produce cellulases, pectinases and xylanases (Lechner and Papinutti, 2006). All these enzymes help in the degradation of cell wall content of cassava peel. The secretion of degradative enzymes by white rot fungi depends mainly on strain, substrate composition and cultivation condition such as pH, temperature, carbon and nitrogen sources, moisture content, aeration, inoculum size, etc. (Revankar and Lele, 2006; Stajic *et al*., 2006).

Single factor optimization have been mainly used for the optimization of process conditions for production of animal feed, with a major disadvantage that it does not consider interaction (Antai and Mbongo, 1994). There is scarcely report about the use of RSM for optimization of process parameters for production of animal feed from cassava peel during solid state fermentation. However,

Corresponding Author: Parveen Jamal, Bioenvironmental Engineering Research Unit (BERU),
Department of Biotechnology Engineering, Faculty of Engineering,
International Islamic University Malaysia (IIUM), P.O. Box 10, 50728 Kuala Lumpur,
Malaysia Tel: +6-03-65964558 Fax: +6-03-61964442

Sharma and Arora (2010) used RSM to optimize moisture level, concentration of NH_4Cl and malt extract during solid state fermentation of wheat straw by *P. floridensis* using lignin degradation profile and production of different lignocellulolytic enzymes as response. Optimization of operating parameters is a vital tool to an efficient cell growth and improved secretion of various enzymes that help in degradation.

Hence, the aim of this study was to determine the effects of operational parameters and identify their optimum levels using response surface methodology for the production of animal feed from cassava peel by a locally isolated white rot fungus *Panus tigrinus*.

MATERIALS AND METHODS

Sample collection and preparation: Cassava peels were collected from a small scale Kerepek (local snack) industry in Kuala Langat, Selangor, Malaysia. They were immediately washed to remove sand, tuber head and dried at 60°C in an air forced oven for 48 h to avoid deterioration and growth of unwanted microbes. The dried cassava peel was milled to pass through 1 mm sieve and stored in air tight container.

Microorganism and inoculum preparation: The white rot fungus *Panus tigrinus* M609RQY was locally isolated and maintained on malt extract agar (MEA) (Merck) plate at 4°C and subcultured forth nightly. Inoculum suspension was prepared by washing 4 MEA plates cultured for 7 days at 30°C with 60 mL of sterilized distilled water to yield a concentration of 0.865 g L^{-1} of biomass.

Solid state fermentation: For this study, optimized cassava peel medium based on previous study was employed (Tijani *et al*., 2011b). The optimized cassava peel medium consists of milled dried cassava peel of 1 mm particle size at 25.7% (w/w), co-substrate 4.3% (w/w), mineral solution 5% (v/w) and 59% (v/w) distilled water. The fermentation media were prepared in 250 mL Erlenmeyer flasks, autoclaved at 121°C for 20 min; cool to room temperature before inoculation and incubated at 30°C for 15 days. Samples were carried out in triplicates.

Response surface methodology (face-centered central composite design): Response surface methodology is a powerful mathematical technique use in evaluating the relationship that exists between variables and their response. It involves three major steps: (i) Carrying out statistically design of experimental (ii) Calculating the coefficient in a mathematical model (iii) Predicting the response and validating the adequacy of the model (Myers and Montgomery, 2002). Face-Centered Central Composite Design (FCCCD) under the response surface methodology was used to evaluate the effect of process parameters on the production of enriched cassava peel as animal feed. The boundary levels for each parameter were as follows: pH (3 and 7), inoculum (4 and 8%) and moisture content (60 and 80%) with lignin content (%) as response to the design. Seventeen experimental runs with three centre points were generated by the design using statistical software package Design-Expert_6.0.8 (Stat Ease Inc., Minneapolis, USA). The three parameters were chosen as the crucial variables and were denoted as A, B and C as shown in Table 1. In order to determine the relationship that exists between the dependent and independent variables, a second order polynomial Eq. 1 was fitted to the data by multiple regression procedure:

$$Y = \beta_0+\beta_1A+\beta_2B+\beta_3C+\beta_{11}A^2+\beta_{22}B^2+\beta_{33}C^2+ \beta_{12}AB +\beta_{13}AC+ \beta_{23}BC \qquad (1)$$

where, Y is the predicted response (Protein (mg g^{-1}) or lignin content (%), β_0 is a constant; β_1, β_2 and β_3 and are the linear coefficients; β_{11}, β_{22} and β_{33} are the quadratic coefficients and β_{12}, β_{23} and β_{23} are the interaction coefficients. Data were analyzed by using the software Design-Expert_6.0.8 (Stat Ease Inc., Minneapolis, USA). Multiple regression equation was developed and was evaluated by analysis of regression coefficient, ANOVA (analysis of variance), P-values and t-test. The quality of fit of this model was expressed by the coefficient of determination (R^2).

Analytical methods: The bioconverted substrate was milled in order to have a homogeneous sample. This was used for analysis of lignin. The method of Goering and van Soest (1970) was used in the estimation of acid detergent lignin. Acid detergent fiber was first determined by using Cetyl Trimethylammonium Bromide (CTAB). Acid detergent cellulose was determined by solubilization of acid detergent fiber residue with 72% sulphuric acid. Acid detergent lignin was calculated as the organic matter loss during ashing of residue left in estimation of acid detergent cellulose at 500°C for 3 h.

Validation of the experimental model: Different combinations predicted by the point prediction feature of the statistical software package Design-Expert_6.0.8 were used to validate the FCCCD model. Four sets of experiments were performed and the observed results were compared with the predicted results.

RESULTS AND DISCUSSION

Optimization of operational parameters by response surface methodology: Three operational parameters based on their vital roles played in SSF were selected to optimize the production of an enriched animal feed. Face Centered Central Composite Design (FCCCD) based on seventeen runs for three variables was carried out with three centre points. The replication of the centre point allows for estimation of the pure error of the process. In order to identify the optimum conditions, data was best fitted by polynomial second order regression models that shows the relationship between response "Y_L (Lignin content) Eq. 2 and the operating parameters, A: pH, B: inoculum size and C: moisture content. This was obtained by multiple regression analysis of the experimental data. Linear, quadratic and interaction terms of variables that contributed to the model are retained in the reduced equation. Eq. 2 is a reduced equation for lignin degradation as this is the best selection for the model.

$$Y_L\,(\%) = +7.11+0.19A-0.20B+1.06C-0.14A^2+0.95B^2 +1.16C^2 - 0.14AB - 0.022BC \qquad (2)$$

The predicted and the experimental values obtained for the response (Lignin content) is presented in Table 1. The adequacy of the model was tested by coefficient of determination (R^2) and analysis of variance based on fisher's statistical test. The closer the value of (R^2) to 1, the better is the correlation between the observed and predicted value. The R^2 for the model was 0.8590 indicating that 85.90% variations in the response data can be accounted for by the two fitted model equations (Table 2). This demonstrates a satisfactory representation of the process by the model. The model F-value of 6.09 ($p<0.0097$) indicates model terms are highly significant for lignin content. To ascertain the model further, the non significant lack of fit calculated by the ratio between mean square of model error and replicate error indicates that the probability is having a non significant lack of fit suggesting that the observed experimental response sufficiently fit the model.

Table 1: Actual values of FCCCD experiment with three parameters showing the experimental and predicted response for enrichment of cassava peel

Run	A: pH	B: Inoculum size (%)	C: Moisture content (%)	Lignin (Exp.) (%)	Lignin (Predict.) (%)
1	5.3	6	70	6.91	7.11
2	5.3	6	70	6.49	7.11
3	3.3	8	60	7.96	7.93
4	7.3	8	80	10.69	9.98
5	7.3	6	70	7.01	7.16
6	5.3	6	60	8.21	7.21
7	3.3	8	80	9.58	9.89
8	5.3	8	70	7.68	7.86
9	5.5	6	80	8.90	9.33
10	3.3	4	60	7.22	7.90
11	3.3	6	70	7.49	6.78
12	7.3	8	60	7.46	7.87
13	7.3	4	60	8.46	8.55
14	3.3	4	80	10.10	10.01
15	7.3	4	80	10.60	10.44
16	5.3	4	70	9.01	8.27
17	5.3	6	70	6.82	7.11

Table 2: Analysis of variance of reduce quadratic model for lignin content

Source	Sum of squares	F- value	p-value
Model	24.81	6.09	0.0097**
A	0.35	0.69	0.4317
B	0.41	0.80	0.3967
C	11.16	21.92	0.0016**
A^2	0.05	0.11	0.7517
B^2	2.43	4.76	0.0606
C^2	3.59	7.06	0.029*
AB	0.16	0.31	0.5925
BC	0.00	0.01	0.9337
Lack of fit	3.97	13.32	0.0715

$R^2 = 0.8590$, Adjusted $R^2 = 0.7179$, **Significant at $p< 0.01$, *Significant at $p< 0.05$

Table 3: Validation of experimental model

No. of experiments	pH	Inoculum size (%)	Moisture content (%)	Lignin experimental	Lignin predicted
1	5.3	6	70	7.19	7.16
2	5.3	5	70	8.42	7.52
3	5.3	7	70	7.65	7.27
4	5.3	7	75	8.01	8.03

Interaction of different operating parameters: The reduced quadratic model ANOVA results in Table 2 shows the strength of interaction between the independent variables and their individual effects as indicated by the coefficients which is determined by t-values and p- values. The lower the p- value the higher the significant of the corresponding coefficient. According to the ANOVA result (Table 2), only moisture content in the linear and square term showed significant effect, all other terms are insignificant. This means that moisture content could act as a limiting parameter and little variation in its concentration would affect lignin degradation. In solid state fermentation, moisture plays a vital role in ligninolytic enzyme production, since the availability of water, either in low or high concentration affects substrate utilization and microbial activity (Bhattacharya *et al*., 2011). Moreover, lignin degradation is an oxidative process that requires oxygen supply which is easily achieved in SSF. Similar result was obtained during solid state fermentation of wheat straw by *Phlebia floridensis* where by lignin degradation was favoured at lower moisture level (Sharma and Arora, 2010). Inoculum level is another important factor that affects enzyme and biomass production in SSF. In this study, Inoculum level at the centre point gave maximum lignin degradation, though the linear effect of this parameter is not significant. This indicates that, a lower inoculum level may cause an insufficient biomass production resulting in decreased ligninolytic enzyme production whereas a higher inoculum may produce too much biomass and result in poor secretion of ligninolytic enzyme (Zhang *et al*., 2006).

The result from the ANOVA was also confirmed by the response surface plot which is a graphical representation of the regression equation used to identify the optimum levels and the interaction among variables that were investigated in the production of delignified animal feed from cassava peel. A perfect interaction between two independent variables give an elliptical or saddle shape (Muralidhar *et al*., 2001). The plots presented in Fig. 1-2, showed the interaction between two variables while the other variable was fixed at its optimum level for minimum lignin. Although the interaction between moisture content and inoculum size and interaction between inoculum size and pH were not significant (Fig. 1 and 2) in the optimization process however, the goal of the response which is minimum lignin content was obtained. In Fig. 1, lower and higher level of inoculum does not favour lignin degradation while varying pH level does not show any effect on lignin content. In the case of Fig. 2, increasing and decreasing both parameters did not support lignin loss.

To validate the applicability of the model developed; four sets of experiments replicated three times, were performed (Table 3), pH was fixed at the centre point based on the ANOVA result and the 3D response curve which shows that pH has a non contributory effect to lignin degradation within the range (3.3 to 7.3) experimented in this study. *P tigrinus* has been widely cultured within the pH range of 5-7 mostly in liquid cultures (Nazareth and Sampy, 2003; Quaratino *et al*., 2008) and pH 5 in solid state of wheat straw (Lechner and Papinutti, 2006). Nazareth and Sampy (2003) observed that increasing the pH of *P. tigrinus* medium from 5.6 to 7.0 did not show any effect on xylanase production but at higher pH, laccases production was reduced. This present analysis has not only allowed us to see the optimum conditions but also shows the effects of the combinations of the three variables. From the validation result, it was observed that, the predicted results from three out of the four sets of experiments favour the goal of the response (lignin loss) than the experimental results. Hence, the optimum conditions obtained for animal feed production from cassava peel was pH, 5.30; inoculum size, 6% (v/w) and moisture content, 70% (v/w). At this optimum, 50.62% loss in lignin was obtained.

P. tigrinus causes a 56% loss in lignin during a 4 months degradation of hard wood sawdust (Nazareth and Sampy, 2003). Lechner and Papinutti (2006) reported 21.49% delignification in wheat straw in 110 days fermentation with *P. tigrinus*. Sharma and Arora (2010) observed a loss of 27.6% in lignin during optimization of moisture content, ammonium chloride and malt extract for production of lignocellulolytic enzymes in a 20 days solid state fermentation of wheat straw by *Phlebia floridensis*.

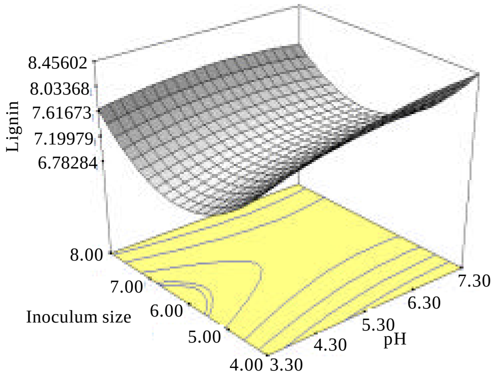

Fig. 1: The 3D response surface curves showing the interaction effects between inoculum size and pH on production of enriched animal feed from cassava peel with lignin content as response

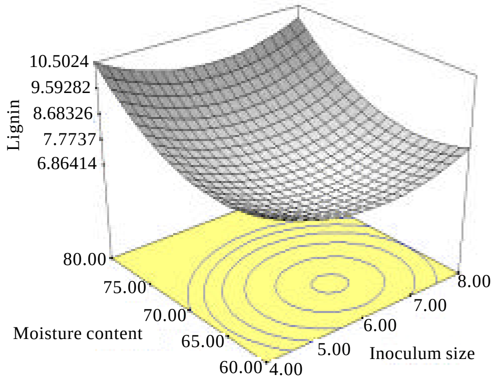

Fig. 2: The 3D response surface curves showing the interaction effects between moisture content and inoculum size on production of enriched animal feed from cassava peel with lignin content as response

CONCLUSION

This study showed that the delignification of cassava peel as animal feed by 50.62% was achieved within 15 days of solid state fermentation as compared to other studies on enrichment of agro-industrial wastes by white rot fungi. The optimum parameter combination that produce this result was found at 70% v/w of moisture content, 6% v/w inoculum size and a pH of 5.30. This bio product from cassava peel will serve as a promising inexpensive alternative to maize and invariably reduce the environmental pollution caused by their disposal.

ACKNOWLEDGMENT

The research was supported by a research grant approved by the Research Management Centre (RMC), International Islamic University Malaysia (IIUM). The authors are grateful to RMC and Department of Biotechnology Engineering, IIUM for supporting and providing the laboratories facilities.

REFERENCES

Aderemi, F.A. and F.C. Nworgu, 2007. Nutritional status of cassava peels and root sieviate biodegraded with *Aspergillus niger*. Am. Eurasian J. Agric. Environ. Sci., 2: 308-311.

Antai, S.P. and P.M. Mbongo, 1994. Utilization of cassava peels as substrate for crude protein formation. Plant Foods Human Nutr., 46: 345-351.

Bhattacharya, S.S., V.K. Garlapati and R. Banerjee, 2011. Optimization of laccase production using response surface methodology coupled with differential evolution. New Biotechnol., 28: 31-39.

FAO, 2008. Cassava for food and energy security. FAO Newsroom: News Stories. http://www.fao.org/newsroom/en/news/2008/1000899/index.html

Goering, H.K. and P.J. van Soest, 1970. Forage Fiber Analysis: Apparatus, Reagents, Procedures and Some Applications. U.S.D.A. Agricultural Research Service, USA., Pages: 20.

Lechner, B.E. and V.L. Papinutti, 2006. Production of lignocellulosic enzymes during growth and fruiting of the edible fungus *Lentinus tigrinus* on wheat straw. Process Biochem., 41: 594-598.

Muralidhar, R.V., R.R. Chirumamila, R . Marchant and P. Nigam, 2001. A response surface approach for the comparison of lipase production by Candida cylindracea using two different carbon sources. Biochem. Eng. J., 9: 17-23.

Myers, R.H. and D.C. Montgomery, 2002. Response Surface Methodology. John Wiley and Sons, New York.

Nazareth, S.W. and J.D. Sampy, 2003. Production and characterisation of lignocellulases of *Panus tigrinus* and their application. Int. Biodeterior. Biodegrad., 52: 207-214.

Obadina, A., O. Oyewole, S. Abiola and L. Sanni, 2006. Short communication-fungal enrichment of cassava peels proteins. Afr. J. Biotechnol., 5: 302-304.

Oboh, G., 2006. Nutrient enrichment of cassava peels using a mixed culture of *Saccharomyces cerevisae* and *Lactobacillus* sp. solid media fermentation techniques. Electr. J. Biotechnol., 9: 46-49.

Quaratino, D., M. Ciaffi, E. Federici and A. D'Annibale, 2008. Response surface methodology study of laccase production in *Panus tigrinus* liquid cultures. Biochem. Eng. J., 39: 236-245.

Revankar, M.S. and S.S. Lele, 2006. Enhanced production of laccase using a new isolate of white rot fungus WR-1. Process Biochem., 41: 581-588.

Sharma, R.K. and D.S. Arora, 2010. Production of lignocellulolytic enzymes and enhancement of *In vitro* digestibility during solid state fermentation of wheat straw by *Phlebia floridensis*. Bioresour. Technol., 101: 9248-9253.

Stajic, M., L. Persky, D. Friesem, Y. Hadar, S.P. Wasser, N. Nevo and J. Vukojevic, 2006. Effect of different carbon and nitrogen sources on laccase and peroxidases production by selected *Pleurotus* species. Enzyme Microbiol. Technol., 38: 65-73.

Tijani, I.D.R., P. Jamal, Z. Alam and E. Mirghani, 2011a. Lignin modifying enzyme activities by some Malaysian white rot fungi. ACT-Biotechnol. Res. Comm., 1: 14-19.

Tijani, I.D.R., P. Jamal, Z. Alam and E. Mirghani, 2011b. Valorization of cassava Peels by the white rot fungus *Panus tigrinus* M609RQY. Aust. J. Basic. Applied Sci., 5: 808-816.

Villas-Boas, S.E., E. Esposito and D.A. Mitchel, 2002. Microbial conversion of lignocellulosic residues for production of animal feeds. Rev. Anim. Feed Sci. Technol., 98: 1-12.

Zhang, W.J., Z.R. Xu, S.H. Zhao, J.F. Jiang, Y. Wang and X.H. Yan, 2006. Optimization of process parameters for reduction of gossypol levels in cottonseed meal by Candida tropicalis ZD-3 during solid substrate fermentation. Toxicon, 48: 221-226.