Original research article

ISSN 2321-0125

Journal of Pharmaceutical and BioSciences
JPBS
An International Journal

www.jpbs-online.com

Optimization of Solid State Fermentation Conditions for the production of Pectinases by Aspergillus niger

Barkavi Durairajan*and P. Siva Sankari

Department Of Botany and P. G. Department of Microbiology Lady Doak College
(An Autonomous Institution of Madurai Kamaraj University), Madurai, India.

Received on 27th March 2014, Accepted on 04th May 2014, Available online from 02nd June 2014

Abstract

The objective of this study was optimization of Solid State Fermentation conditions for the production of Pectinases by using fungus Aspergillus niger. The effect of basic fermentation parameters (pH, Temperature, Moisture Contents, Moistening agent, Carbon Sources and Nitrogen Sources) on enzyme production was studied. Maximum pectinases production was in PME (5.58 mg/ml) using Banana peel as substrate. The optimum conditions for pectinases production for Banana peel were found to be: initial Moisture Content - 70%, initial medium pH - 6 and 7, temperature - 35ºC, concentration of carbon sources - 2% glucose, concentration of nitrogen sources – 3% Urea. The optimal incubation time for production was six days. Results indicate the scope for further optimization of the production conditions to obtain higher Pectinases using the strain under SSF.

Keywords: Solid State Fermentation, Aspergillus niger, Pectinolytic enzyme assay, Orange, banana and pineapple peels

INTRODUCTION

Solid state fermentation (SSF) holds tremendous potential for the production of enzymes. It can be of special interest in those processes where the crude fermented product may be used directly as the enzyme source (Collmer et al., 1986). Pectinolytic enzymes catalyzing the degradation of pectic substances are of great industrial importance (Spanga et al., 1995). Application of pectinases included fruit juice extraction and clarification; wine processing, oil extraction, coffee, tea fermentation, retting and degumming of fibres (Kashyap et al., 2000). Fungal Polygalactouronase used in industrial processes for juice clarification, mainly obtained from Mesophilic, Aspergilli and penicillia, the range of enzyme source were extended through new recombinant fungal strains (Maheswari et al., 2000). Selection of a particular strain however remains a tedious task, especially when commercially competent enzyme yields are to be achieved. It has been reported that a strain of Aspergillus niger produced 19 types of enzymes that was being produced by as many as 28 microbial cultures (Panday et al., 1992). Thus, the selection of a suitable strain for the required purpose depends upon a number of factors in particular upon the nature of the substrate and environmental conditions.

Harris and Dennis, 1992 reported that pectin enzymes possessed high temperature stability for different species of Rhizopus and Mucor Sp. Leuchtenberger and Mayer, 1992 reported 2-3 fold increase in Polygalactouronase and pectin lyase production was observed in mutants of Aspergillus niger. Brumano et al., 1993 found that in Pencillium Griseoroseum the optimum level of activity for

*Corresponding author *E - mail: barkavi84@gmail.com*

pectinolytic enzyme occurred at neutral pH and at high temperature, which is ideal for natural processing of material. The effect of different carbon source on pectinase synthesis by fungi in submerged and solid state fermentation has been studied. It is generally agreed that the optimum medium for the enhanced production of extracellular pectinase is that containing pectin materials as an inducer (Panayotou et al., 1993). Contrastly, Studies have been conducted on the fruit extracts of Cordia dichotoma act as a good source of antimicrobial agent against Escherichia coli, Klebsiella pneumoniae and Proteus vulgaris. Although Cordia dichotoma is used ethno medicinally in Urinary tract infection, there is no specific study on antibacterial activity against the pathogens causing urinary tract infection (S.R. Deore et al., 2013).

Studies have been conducted on comparative production of pectinases in systems of SmF and SSF (Solis –Pereira et al., 1993, Minjares – Carranco et al., 1997). When the fermentation medium was supplemented with different carbon sources, like glucose, sucrose and galactouronic acid, Polygalactouronase (PG, EC-3.2.1.15) production by Aspergillus niger CH4 increased in SSF system but decreased in SmF system. Overall productivity by SSF was 18.8 and 4.9-fold higher for endo-PG and exo-PG, respectively, than those obtained by SmF. Media acidity plays a significant role on pectinases production by SSF processes. Cavalitto et al., 1996 studied growth and pectinases production by Aspergillus foetidus and Aspergillus awamori respectively in SSF systems at different media acidities. Thus the objective of the present study was to optimize various factors like moistening agents, Moisture contents, pH, Carbon source, nitrogen source and temperature for maximum production of pectinases in SSF using cheaper renewable orange, banana peel, pineapple peels as a substrate by fungal strain Aspergillus niger.

MATERIALS AND METHODS

I. Maintenance of Culture

Aspergillus niger used in this study was obtained from pure culture under laboratory condition. It was grown and maintained at 28ºC and 4ºC on potato dextrose agar slants. Inoculums (Seed culture) was prepared by transferring cells from a fresh agar slant into 250ml Erlenmeyer shake flask, containing 50ml of the culture medium. Culture was maintained on agar slants at 4ºC and sub cultured twice a month. The pH of the medium was kept at 5.0 and sterilized at 121ºC (15 psi) for 20 min, incubated at 28ºC for at least 6 days depending upon the growth of the culture.

II. Solid state fermentation (SSF) and optimization of parameters for enzyme production:

The Medium used for inoculum preparation contained for 1 liter potato 200g; Dextrose 20g; streptomycin 50g; pH 5.5; Distilled H2O – 1 liter. This medium was sterilized by autoclaving 121ºC pressure of 15 psi for 15 min. The culture was incubated and shaken at 30ºC for 48 hrs in an orbital shaking incubator at 150 rpm before transferring to the production medium 20g of orange, banana and pineapple peels were moisture with 50 ml of distilled water solution pH (5 to 5.2) was taken in 500 ml Erlenmeyer Flask bottle and sterilized at 121ºC and 15 psi for 15 min by autoclaving. The fermentation was continued for 3 to 6 days and flasks withdrawn at regular intervals of 1 day for further analysis. The enzyme was extracted from fermented peels (Orange(S1), Banana (S2) and Pineapple (S3)) by using the

Moistening agents such as distilled water, Pectin 5.0%, Citrate Buffer (Solution A: 0.1m citric acid (2.1014g in 100ml of dis H2O), Solution B: 0.2 Na2 Hpo4 (5.6784g in 200ml of dis H2O), Tris HCL buffer Stock Solution (0.2 ml solution of this 24.2g in 1000 ml, 0.2 ml HCL, 50ml of A + xml of B, diluted to then total of 200 ml), Acid (0.02 M of Thiobarbutaric Acid – 5 ml, In HCL - 1.25 ml), pH 4.8.

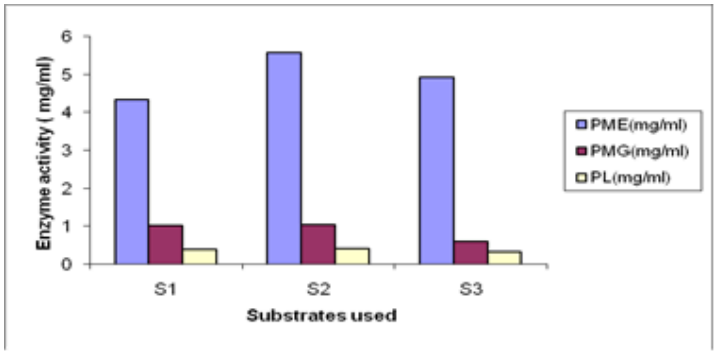
The pooled extract was filtered with muslin cloth, centrifuged at 3000-3500 rpm for further clarification, and then used for enzyme assay (Singhania et al., 2006). The parameters were studied in various moisture levels (50%, 60% and 70 %), pH (5, 6, 7 and 8), incubation temperature (25ºC, 35ºC, 45ºC and 55ºC), Carbon sources such as Glucose, Galactose and starch with varied level of concentration (0.5%, 1% and 2%) and Nitrogen sources such as Urea, yeast extract and ammonium sulphate with varied level of concentration (1%, 2% and 3% for maximum yield of pectinases in SSF using cheaper renewable orange, banana peel, pineapple peels as a substrate by fungal strain Aspergillus niger (Barkavi Durairajan, et al., 2014).

RESULTS & DISCUSSION

Initial screening of the various substrates such as Orange (S1), Banana (S2) and pineapple (S3) peels for their potential to support pectinases production indicated that among the tested substrates, banana peel (S2) was found to be the best Pectin content was high in S2 (5.58 mg/ml) than the S1 and S3. The enzyme activity of the selected strains Aspergillus niger used for the enzyme production. Among the three enzymes assayed, polymethylesterase (PME) was found to be higher in all the treatments than in Polymethyl galactouronase (PMG) and in pectin lyase (PL).

The Orange, Banana and Pineapple peels were supplemented with suitable moistening agent to meet the water requirement and additional nutrients to the growing cultures using SSF. The Moistening agent such as distilled water, citrate buffer, pectin 5.0% was used. The Mineral salt solution such as Citrate buffer, Tris HCL buffer, Stock Solution, Thiobarbutaric Acid and orange, banana, pineapple peels medium were used for this enzyme production using pectinolytic enzyme assay shown in Figure 1.

Figure 1: Estimation of Pectinase assay using Aspergillus niger in the selected substrates



S1 - Orange peels treated with Aspergillus niger, S2 - Banana peels treated with Aspergillus niger and S3 - Pineapples peels treated with Aspergillus niger

The Maximum yield of the enzyme 5.58 mg/ml in S2 banana peel was obtained at 70% of moisture level. Mekala et al., 2008 showed that at high moisture (70%) the substrate prevents oxygen penetration and facilitates the contamination, whereas low moisture level inhibits the growth enzyme activity and accessibility to nutrients. it was noticed that 70% of the moisture content responded well in all the substrates using Aspergillus niger and produced high enzyme activity (Table 1), (Figure 2). Contrast, 50% and 60% exhibited only moderate enzyme production in Aspergillus niger treatments.

Figure 2: Effect of 70% moisture content on Pectinase production in all the selected substrates treated with Aspergillus niger

Table 1: Effect of Moisture content in the production of Pectinolytic enzymes in the selected substrates

% Moisture Content	Substrate	Enzyme Activity		
		PME	PMG	PL
		mg/ml	mg/ml	mg/ml
50%	S1	3.1	0.0135	0.0125
	S2	2.48	0.0099	0.0153
	S3	3.1	0.009	0.0135
60%	S1	4.34	0.009	0.018
	S2	4.34	0.0972	0.018
	S3	3.72	0.0477	0.0117
70%	S1	4.96	0.0324	0.0126
	S2	5.58	0.0549	0.0198
	S3	4.34	0.0279	0.0135

The effect of pH on enzyme production was studied by adjusting the pH of the moistening agent (Mineral salt solution) from pH 5 to pH 8 as shown in figure 3. It indicates that the enzyme production was favoured in range of pH6 and pH7 of maximum enzyme yield was 5.58 mg/ml and 5.56 mg/ml in PME. Experiments performed to find out the optimum pH for better enzyme production showed that S2 responded well at pH 6 & 7 and considerable enzyme production was observed in S2 than in other treatments (Table 2). PME and PMG activity was higher in all the treatments tested compared to PL activity. The pH 5 and pH 8 showed a lower production of enzymes. Studies showed that only low enzyme productions (PMG) were observed at pH 5 that was isolated by gel filtration in Czapeckdox Medium.

Figure 3. Effect of pH- 6.0 on pectinase production in the selected substrates

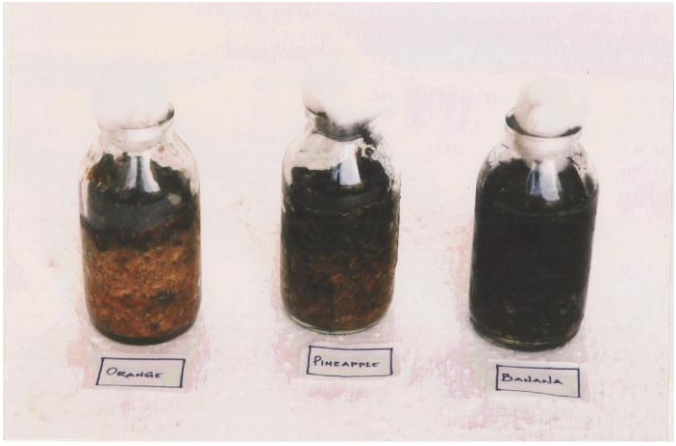
Table 2: Effect of pH in the production of Pectinolytic enzymes in the selected substrates

pH	Substrate	Enzyme Activity		
		PME	PMG	PL
		mg/ml	mg/ml	mg/ml
5	S1	3.72	0.4797	0.0153
	S2	4.34	0.7254	0.0234
	S3	4.96	0.1602	0.0423
6	S1	4.96	0.3654	0.0162
	S2	5.58	0.8757	0.1359
	S3	4.34	0.2214	0.0603
7	S1	4.96	0.4023	0.0132
	S2	5.56	0.7308	0.0315
	S3	4.93	0.1674	0.0203
8	S1	5.56	0.3627	0.0123
	S2	4.96	0.7497	0.0234
	S3	4.37	0.0126	0.0558

The Overall effects of pH in the production of Pectinolytic enzymes using selected substrates were observed, more enzyme activity in pH 6 as shown in the Table 2.

The effect of temperature on different treatments was analyzed at 25ºC, 35ºC, 45ºC and 55ºC as shown in the figure 4.

Figure 4. Effect of temperature (55 °C) on pectinase production in the selected substrates



The maximum enzyme yield at 35ºC in banana peel (S2) 5.56 mg/ml whereas the enzyme yield was reduced in Orange peel (S1) 4.34 mg/ml and Pineapple peel (S3) 4.36 mg/ml in PME. In PMG and PL was showed a lower production of enzyme in S2 (0.2493 mg/ml and 0.2493 mg/ml). It was noticed that the treatments exposed to 35ºC showed high enzyme activity on 4th day of incubation. It was also found that S2 responded well at 35ºC and considerable enzyme production was observed (Table 3). But at 25ºC temperature, PME activity was high in S3, PMG production was more in S1 and high production of Pectin Lyase was observed in all treatments. Reports also showed that Pectinase from Aspergillus strains have been described as susceptible to deviation in temperature above 50ºC (Bailey et al., 1990).

With reference to the effect of various carbon sources on Pectinolytic activity by solid state fermentation using Aspergillus niger, carbon sources such as Glucose, Galactose and starch with varied level of concentration (0.5%, 1% and 2%) are used to enhance the enzyme production. It was clearly noted that the addition of 2% Glucose in S2 (5.58 mg/ml) with 70% moisture content showed the maximum enzyme production in PME than 1% (4.34 mg/ml) and 0.5% (4.96 mg/ml) of Glucose (Table 4), (Figure 5).

Figure 5. Effect of Glucose on pectinase production

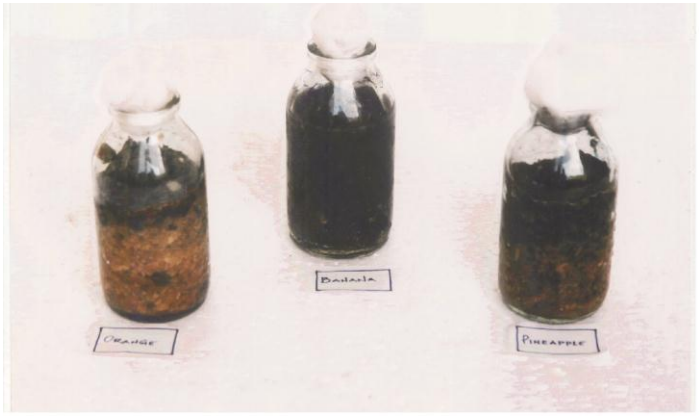


Table 3: Effect of temperature in the production of Pectinolytic enzymes in the selected substrates

Temperature	Substrate	Enzyme Activity		
		PME	PMG	PL
		mg/ml	mg/ml	mg/ml
25°C	S1	4.34	0.4734	0.4734
	S2	4.96	0.4347	0.4347
	S3	5.57	0.3294	0.3294
35°C	S1	4.34	0.2187	0.2187
	S2	5.56	0.2493	0.2493
	S3	4.96	0.0315	0.0315
45°C	S1	3.72	0.0117	0.0117
	S2	4.96	0.2178	0.2178
	S3	3.72	0.072	0.072
55°C	S1	5.58	0.053	1.053
	S2	4.96	0.0134	0.0134
	S3	4.37	0.0017	0.6017

The supplementations of Galactose in S2 were studied considerable production of pectinolytic activity. It was noted that the addition of 2% galactose was enhanced in PME production (5.58 mg/ml) and 2% galactose in PMG were showed maximum production (1.011 mg/ml)in S2 than the other concentrations used (Table 5),(Figure 6).

Table 4: Effect of Glucose in the production of Pectinolytic enzymes in the selected substrates

% Concentration of Glucose	Substrate	Enzyme Activity		
		PME	PMG	PL
		mg/ml	mg/ml	mg/ml
0.50%	S1	4.34	0.4491	0.0144
	S2	4.96	1.027	0.071
	S3	4.34	0.529	0.023
1.00%	S1	4.96	0.634	0.741
	S2	4.34	0.974	0.024
	S3	4.96	0.867	0.076
2.00%	S1	4.96	0.422	0.024
	S2	5.58	0.738	0.067
	S3	4.96	0.707	0.085

Figure 6. Effect of urea (3%) on pectinase production in the selected substrates

Table 5: Effect of Galactose in the production of Pectinolytic enzymes in the selected substrates

% Concentration of Galactose	Substrate	Enzyme Activity		
		PME	PMG	PL
		mg/ml	mg/ml	mg/ml
0.50%	S1	4.37	0.064	0.018
	S2	4.96	0.414	0.001
	S3	4.96	0.505	0.072
1.00%	S1	4.37	0.093	0.051
	S2	4.96	0.239	0.144
	S3	5.58	0.386	0.074
2.00%	S1	4.96	0.093	0.017
	S2	5.58	1.011	0.086
	S3	4.96	0.386	0.063

Augmentation of starch as carbon source revealed that 2% starch exhibited only moderate enzyme production in S2 (7.34 mg/ml) of PME than 1% (5.58 mg/ml) and 0.5% (5.58 mg/ml) (Table 6).The other treatments exhibited only low production of enzyme activity.

Table 6: Effect of Starch in the production of Pectinolytic enzymes in the selected substrates

% Concentration of Starch	Substrate	Enzyme Activity		
		PME	PMG	PL
		mg/ml	mg/ml	mg/ml
0.50%	S1	4.96	0.018	0.018
	S2	5.58	0.117	0.081
	S3	4.37	0.072	0.072
1.00%	S1	4.34	0.027	0.2898
	S2	5.58	0.0397	0.2466
	S3	4.96	0.027	0.253
2.00%	S1	5.58	0.0297	0.1755
	S2	7.34	0.378	0.3438
	S3	4.96	0.0324	0.1215

Thus the present study showed that the augmentation of Glucose enhanced the production of Pectinase enzyme compared to galactose and starch. In contrast, Denis Silva., 2002 reported that pectinlyase, Polygalactouronase production by a newly isolated Pencillium viridicatum strain Rfc3 was high by means of SSF using orange bagasse, and banana peels as carbon source. Similarly when nitrogen source was supplemented to the treatments, it was found that 3% urea showed the maximum production of pectinase activity compared to 1% and 2% of urea and the enzyme production was high in banana peel S2 (7.34 mg/ml) in PME than orange S1 (5.58 mg/ml) and pineapple S3 (4.96 mg/ml) peels were used (Table 7). Hence the PME shows maximum pectinases production than the PMG and PL activities. This may be due to the variation in nitrogen content of the raw substrate. Contrastingly, the addition of yeast

extract 2% showed the maximum production of pectinases activity compared to 1% and 3% of yeast extract. Hence the enzyme production was high in banana peel S2 (7.34 mg/ml) in PME than the orange peel S1 (4.34 mg/ml) and pineapple peel S3 (3.72 mg/ml) were observed (Table 8).

Table 7: Effect of Urea in the production of Pectinolytic enzymes in the selected substrates

% Concentration of urea	Substrate	Enzyme Activity		
		PME	PMG	PL
		Mg/ml	mg/ml	mg/ml
1.00%	S1	4.34	1.027	0.022
	S2	5.58	0.939	0.108
	S3	4.96	0.947	0.212
2.00%	S1	5.58	4.34	0.019
	S2	3.72	0.887	0.144
	S3	4.34	0.953	0.006
3.00%	S1	5.58	0.955	0.006
	S2	7.34	1.032	0.193
	S3	4.96	0.921	0.012

Table 8: Effect of Yeast Extract in the production of Pectinolytic enzymes in the selected substrates

% Concentration of yeast extract	Substrate	Enzyme Activity		
		PME	PMG	PL
		mg/ml	mg/ml	mg/ml
1.00%	S1	5.58	1.017	0.006
	S2	3.72	1.029	0.027
	S3	4.34	0.801	0.128
2.00%	S1	4.34	0.915	0.019
	S2	7.34	1.003	0.019
	S3	3.72	0.84	0.009
3.00%	S1	4.96	1.02	0.027
	S2	4.34	0.899	0.008
	S3	5.58	0.79	0.045

When ammonium nitrate used as the nitrogen source, 2% of ammonium nitrate was showed that the maximum production of pectinases activity compared to 1% and 3%. The enzyme production was high in banana peel S2 (5.58 mg/ml) in PME than the orange peel S1 (4.34 mg/ml) and pineapple peel S3 (3.72 mg/ml) were observed (Table 9). Hence, PME shows maximum pectinases production than the PMG and PL activities.

Table 9: Effect of Ammonium Nitrate in the production of Pectinolytic enzymes in the selected substrates

Concentration of ammonium Nitrate (%)	Substrate	Enzyme Activity		
		PME	PMG	PL
		mg/ml	mg/ml	mg/ml
1.00%	S1	4.96	0.936	0.021
	S2	4.34	0.951	0.027
	S3	5.58	0.815	0.074
2.00%	S1	4.34	1.018	0.023
	S2	5.58	0.905	0.052
	S3	3.72	0.967	0.092
3.00%	S1	5.58	0.944	0.018
	S2	4.34	0.951	0.216
	S3	5.58	0.861	0.067

The cost-effective technologies are needed for the production of enzyme and SSF is a suitable technology for economical production of pectinases using Orange, Banana and pineapple peel as a substrates. Major parameters affecting the fermentation process for enzyme production were studied and optimum levels were identified. It is concluded from the findings that the strategy to produce pectinases from banana peel (S2) was successful as it resulted in a considerably good amount this enzyme produced by newly strain Aspergillus niger under laboratory conditions. Furthermore, evolutionary operation factorial-design technique could be considerably effective in maximizing the yield of enzyme but all the parameters was optimized by one at a time method.

REFERENCES

1. Bailey, M.J., Pessa, E. Strain., 1990. Process for production of Polygalactouronase. Enzyme Microbiology Technology, 12: 266-271.
2. Barkavi, Durairajan, P. Siva Sankari, 2014. "Studies on the utilization of fruit peels for

pectinases production using different fungal species by solid state fermentation", Journal of Pharmaceutical and Biosciences, volume 2:36-43.

3. Brumano, M.H.N., Coleho, J.L.C., 1993. “Pectin lyase activity of pencillium grieseoroseum related to degumming of ramie”, Acta Microbiology Vol.24:175-178.
4. Cavalitto, S.F., Arcas, S.A., Hours, R.A., 1996. Pectinases production profile of Aspergillus foetidus in solid state cultures of different acidities. Biotechnol. lett., 18:251-256.
5. Collmer, A., N.T.Keen., 1986. The role of pectic enzymes in plant pathogenesis, Annu. Rev. Psychopathology Vol.24: 383-409.
6. D.R.Kashyap, P.K.Vohra, S.Chopra, K.Tewari., 2000. Application of pectinases in the commercial sector. A review, Bio resource technol. Vol.77:215-227.
7. Galiotou, Panayotou, M., Rodis, P., Kapantai, M., 1993. Enhanced Polygalactouronase production by Aspergillus niger NRRL – 364 grown on supplemented citrus pectin. Letters Appl. Microbial. 17:145-148.
8. Harris, S.E., Dennis, C., 1992. “Heat stability of fungal pectinolytic enzymes”, Journal of food science and Agriculture, 781-791.
9. Leuchtenberger, A., Mayer, G., 1992. “Changed pectinases synthesis by aggregated mycelium of some Aspergillus niger mutants”, Enzyme Microbiol. Tech., 14:18-22.
10. Maheswari, R., Bhardwaj, G., Bhat, M.K., 2000. Thermophilic fungi; their physiology and enzymes. Microbial. Mol Bio. Rev., 64: 461-488.
11. Mekala, N.K., R. R. Singhania, R. K. Sukumaran, A. Pandey, 2008. Cellulose production under solid state fermentation by Trichoderma reesei RUT C30; Statistical optimization of process parameters. Appl. Biochem. Biotechnology, 151:122-131.
12. Minjares, Cassanco, A., Trejor, Aguillas, B.A., Aguilar, G., 1997. Comparison between pectinases producing mutants of Aspergillus niger adopted solid state fermentation or submerged fermentation. Enzyme Microb. Technol. vol.21:26-27.
13. Reddy, S.M., Ram Reddy, S., 2000. Microbiology A laboratory manual BSL Publishers and Distributors. 136-138.
14. S.E.Solis, Pereiral, E.favella, Torres, G.Viniegra, Gonzalez, M.Gutierrez, Rojas, 1993. Effect of different carbon sources on the synthesis of pectinases by Aspergillus niger CH4 in submerged and solid-state fermentation, Appl. Microbiol. Biotechnol. Volume 39:34-38.
15. S.R.Deore, A.G.Namdeo, 2013. "In-vitro evaluation of anti-bacterial activity of Cordia dichotoma Forst on urinary tract pathogens", Journal of Pharmaceutical and biosciences, volume 1:110-113.
16. Silva, D., Martins, E.S., Silva, R., Gomes, E., 2002. Pectinase production by Penicillium viridicatum RFC3 by solid state fermentation using agricultural wastes and agroindustrial by-product. Brazilian Journal Microbiology Volume 33:318-324.
17. Singhania, R.R., R.K. Sukumaran, A. Pillai, P. Prema, G. Szakacs, A. Pandey, 2006. Solid state fermentation of lignocelluloses substrates for cellulose production by Trichoderma reesei NRRL 11460. Ind. J. Biotechnology, 5:332-336.
18. Spanga, G., Pefferi, P.G., Gillali, E., 1995. Immobilization of a pectin lyase from Aspergillus niger for Application in food technology. Enzyme Microb. Technol., 17:729-738.