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Taguchi's - A Recent Statistical Approach to Optimize the Microbial Pectinases Screened from Fruit Waste Yard

R. Gopinath* and Suneetha V.

School of Bio Sciences and Technology, VIT University, Vellore-632 014, Tamil Nadu, India.

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Pectinase posses potent industrial application and hence pectinolytic bacteria *Bacillus cereus* (KC571175) that posses ability to produce pectinase enzyme are of prime importance. The main theme of work concentrates around isolation of potent pectinolytic bacteria from fruit processed industrial waste to aid in wide commercial application and to recycle the waste to produce a clean and sustainable environment. Series of processes in a calculated way to carry out careful fermentation has led to production of enzymes that show high activity. The Taguchi method is a simple statistical tool to design the experiments involving a system. The orthogonal array is an unbiased. The experimental runs were carried based on the factors and levels. To predict the significant contribution of the each factors signal-to-noise ratio was used. It shows a signification different in various levels of the factors. At Temperature 39°C, pH 5, RPM 75, Pectin 7% and Incubation (in hours) 120 the maximum activity of polygalacturonase and pectin lyase were observed.

Key words: Pectin lyase, Polygalacturonase, Taguchi, SN ratio, *Bacillus cereus.*

Pectin is the useful carbohydrate polymer which is available in the natural sources. It presents the cell wall of the plant and enriched in citrus fruits[1]. Pectins are polysaccharides enriched in galacturonicacid and galacturonic acid methyl ester units[2] Combined with proteins and other polysaccharides, pectins form skeletal tissues of plants, which are chemically stable and physically strong.Pectic substances are polysaccharides consisting of mostly the chains of galacturonan molecules interspersed with a much smaller number of rhamnose molecules and side chains of galacturonan and some other five carbon sugars[3]. The enzymes that degrade pectic substances are known as pectinases or pectinolytic enzymes[4]. Some chain splitting pectinases, called polygalacturonases (PG: EC 3.2.1.15), split the pectin chain by adding a molecule of water and breaking (hydrolyzing) the linkage between two galacturonan molecules; pectin lyases (PL: EC 4.2.2.2)[5], split the chain by removing a molecule of water from the linkage,there by breaking it and releasing products with an unsaturated double bond. Both the enzymes break the pectin chain at random sites or terminal linkage (exo-pectinases) of the chain and release single unit of galacturonan[6].

* To whom all correspondence should be addressed.
Tel.: +91 9445450004.
E-mail: gopinath877@gmail.com

MATERIALS AND METHODS

Collection of soil samples

Soil samples were collected from fruit industrial waste yard in Chittoor, Andhra Pradesh, India. Soil samples are collected in sterile container and stored in deep freeze for further analysis.

Isolation of Microorganisms

The soil samples were taken for isolation of pectinolytic enzymes. One gram of soil was taken

and serial dilutions were made for the screening of bacteria. Then 0.1 ml of the diluted sample were taken and add in the Isolation medium of following composition, g/L (Pectin, 10; tryptone, 3; yeast extract, 2; KCl, 0.5; $MgSO_4.7H_2O$, 0.5; $MnSO_4.5H_2O$, 0.01; $(NH_4)2SO_4$, 2) supplemented with mineral salt solution of composition g/100 mL ($CuSO_4.5H_2O$, 0.04; $FeSO_4$, 0.08; Na_2MoO_4, 0.08; $ZnSO_4$, 0.8; $Na_2B_4O_7$, 0.004, $MnSO_4$, 0.008), pH 5.5-6.0 was used[7,8]. All the chemicals were analytical grade and had been purchased from Hi Media Chemicals, Mumbai. From the isolation medium one ml were taken and pour plats method was procedure. Pure culture was sub cultured onto slant media and maintained for identification and enzyme studies.

Identification of potential isolated bacteria

The isolated bacteria was identified using 16S rRNA sequencing. The sequence was submitted to GENBANK with the Accession No-KC571175 and the stain was identified as *Bacillus cereus*.

Taguchi's orthogonal array

Taguchi's method has used to estimate the optimal condition of various parameter used in the production process with minimum number of trials with the MINI TAB 15. In this method all the parameter at different level and each combination will appear an equal number of times[8] The effects of the other factors will be balanced. There is a relative value representing all the factors. The experimentation size is given in symbolic designation of the arrays[9 10], L25, has 25 trails. The degree of freedom is calculated by no of trials minus one[11].The factors are given in Table 1. All the factors are given equal no of levels such as 1,2,3,4 and 5 respectively. Table 2 shows the experimental condition designed using L25 orthogonal array system. All the parameters were conducted using mineral medium supplemented with pectin containing 1 g KH_2PO_4, 2 g $NaNO_3$, 0.50 g $MgSO_4.7H_2O$, 0.05 g KCl, 0.01 g $FeSO_4$, in 1 L.dH_2O[7] and incubated at appropriate temperatures with agitation. The protein concentration was assayed using Lowry's method.

Analysis of Taguchi's experiment

When the design was conformed the PG and PL assay was analysis for different trials. The assay data was analysis using taguchi's lager is better method. The SN ratio was plotted for PG and PL. A fermentation run was carried out in a batch fermenter (Lark Innovative Fine Teknowledge Company product) with 3L working volume to validate the results obtained during optimization of the process in shake flask.

Pectin lyase assay

The activity of pectin lyase is assayed by measuring the increase in optical density at 235 nm due to formation of 4, 5-unsaturated oligogalactouranotes[12] assayed using the procedure of Albersheim *et al.*, 1966[13] .

Polygalacturonase assay

Polygalacturonase activity was checked by quantifying end groups released during the reaction[14] and it was assayed using the procedure of Panda *et al.*, 1999[15].

Protein assay

Protein in culture filtrate was estimated using standard method bronsted lowry protein assay[16].

RESULTS AND DISCUSSION

Experimental analysis of taguchi's

The *Bacillus cereus* used in the process was culture under submerged fermentation and study with the experimental design as per taguchi's method. The protein was estimated for all the trials and plotted in (Fig. 1). The polygalacturonase activity for different trials were plotted in (Fig. 2).

Table 1. Factors and levels used in Taguchi's method

Factors	Levels				
	1	2	3	4	5
Temperature(°C)	36	37	38	39	40
pH	5	6	7	8	9
Incubation in hours	24	48	72	96	120
Pectin (%)	3	5	7	9	10
Rpm	75	100	125	150	175

Table 2. Experimental Condition Designed by Taguchi's method

Trial No	Temperature(°C)	pH	Incubation in hours	Pectin (%)	RPM
1	36	4	24	3	75
2	36	5	48	5	100
3	36	6	72	7	125
4	36	7	96	9	150
5	36	8	120	10	175
6	37	4	48	7	150
7	37	5	72	9	175
8	37	6	96	10	75
9	37	7	120	3	100
10	37	8	24	5	125
11	38	4	72	10	100
12	38	5	96	3	125
13	38	6	120	5	150
14	38	7	24	7	175
15	38	8	48	9	75
16	39	4	96	5	175
17	39	5	120	7	75
18	39	6	24	9	100
19	39	7	48	10	125
20	39	8	72	3	150
21	40	4	120	9	125
22	40	5	24	10	150
23	40	6	48	3	175
24	40	7	72	5	75
25	40	8	96	7	100

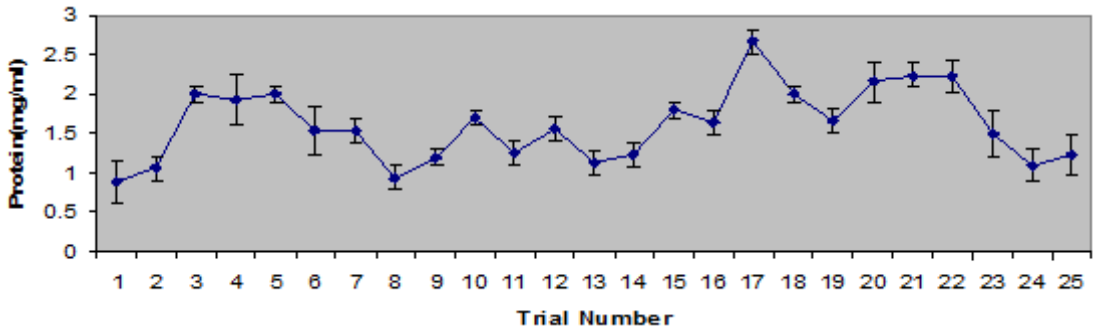

Fig. 1. Protein Estimation (Lowry's Method)

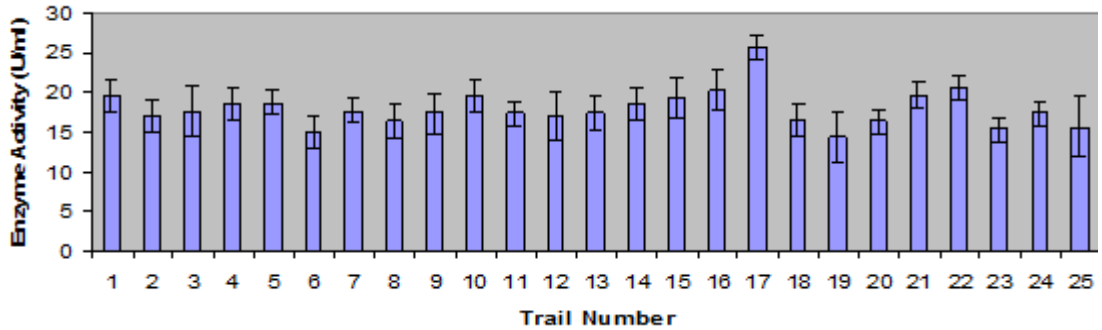

Fig. 2. Polygalacturonase Activity

Pectin lyase activity was determine for all trials and plotted in (fig 3). Trail 17 shows the maximum activity in protein, polygalacturonase and pectin lyase. Optimum condition was as follows Temperature 39°c, pH 5, RPM 75, Pectin 7% and Incubation (in hours) 120.

Signal to noise ratio

The SN ratio were analyzed using lager is better. The Signal to noise ratio for polygalacturonase was plotted in (figure 4) and for pectin lyase was plotted in (figure 5). In polygalacturonase the maximum effect of temperature is 36°c, pH is 5, rpm is 75, pectin (%) is 9 and incubation time is 120 hours. For pectin lyase had the maximum effect of temperature is 39°c, pH is 5, rpm is 75, pectin (%) is 9 and incubation time is 48 hours.

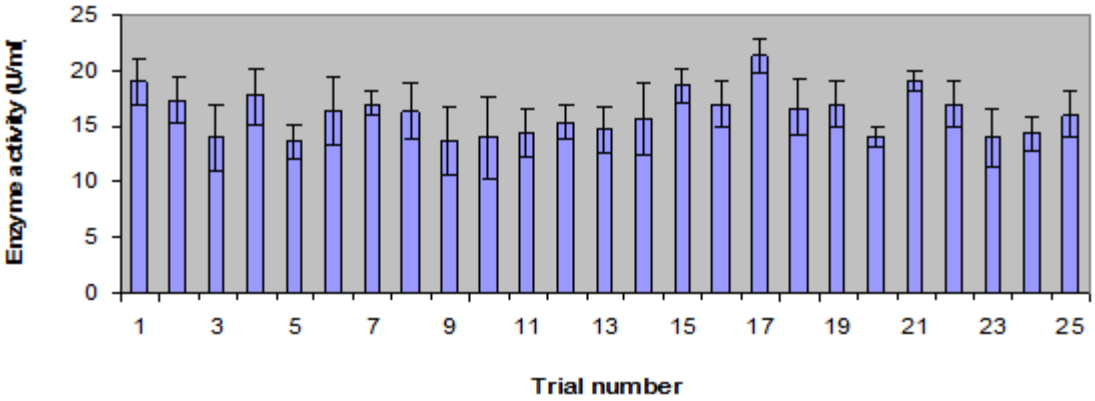

Fig. 3. Pectin lyase Activity

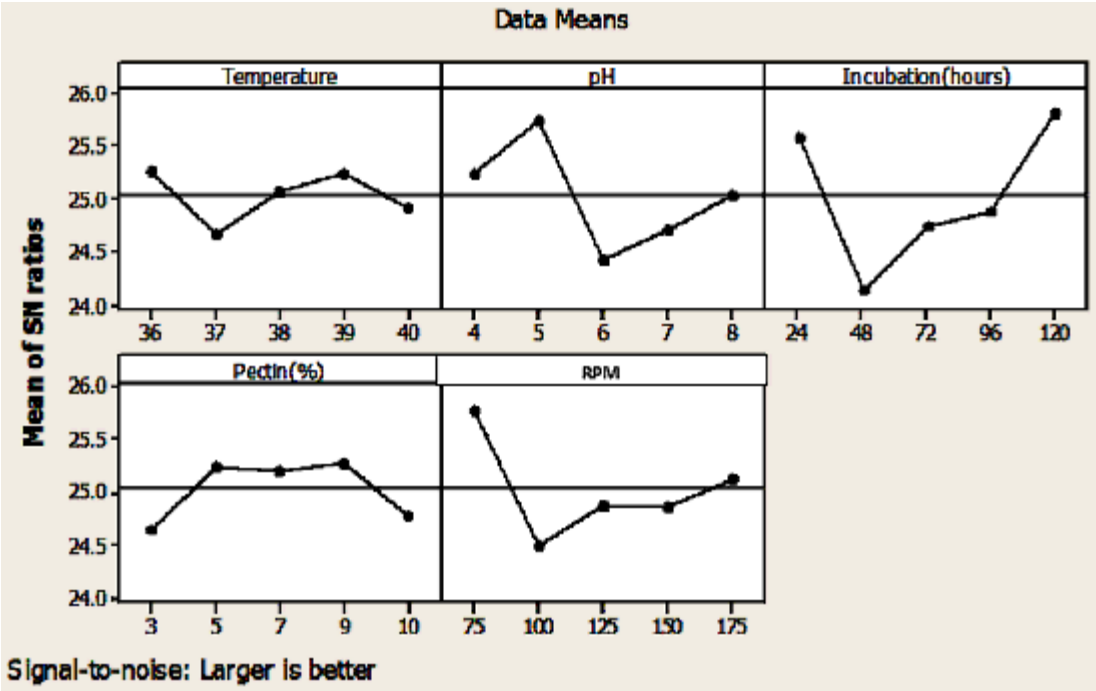

Fig. 4. Main Effects Plot for SN ratios

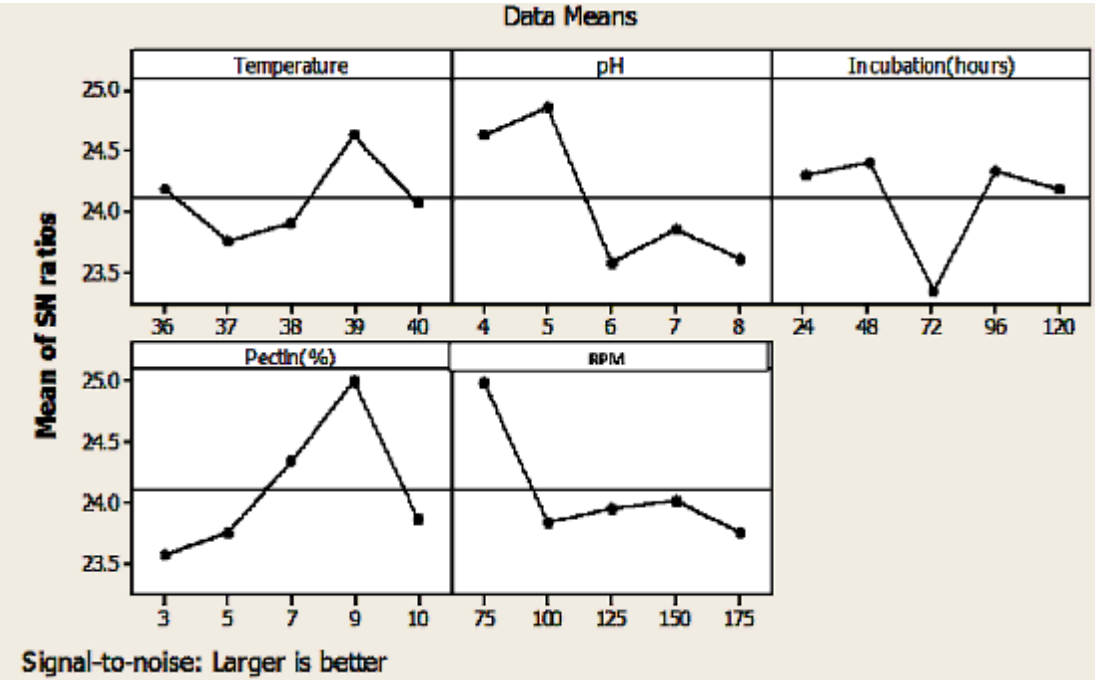

Fig. 5. Main Effects Plot for SN ratios

CONCLUSION

The optimum condition for the maximum polygalacturonase and pectin lyase activity is Temperature 39°C, pH 5, RPM 75, Pectin 7% and Incubation (in hours) 120. The enzyme activity is more at room temperature. The activity is more when the rpm is 75 it shows that the low rpm has maximum yield. Incubation time and pectin concentration has maximum yield when the concentration is more. Maximum polygalacturonase activity was found to be was (25.66 U/mL) and maximum pectin lyase activity was (21.33 U/mL).

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