Bioprocess Engineering 19 (1998) 355–361 © Springer-Verlag 1998

Production of pectolytic enzymes – a review

G.S.N. Naidu, T. Panda

Abstract Pectolytic enzymes play an important role in food processing industries and alcoholic beverage industries. These enzymes degrade pectin and reduce the viscosity of the solution so that it can be handled easily. These enzymes are mainly synthesized by plants and microorganisms. *Aspergillus niger* is used for industrial production of pectolytic enzymes. This fungus produces polygalacturonase, polymethylgalacturonase and pectinlyase. This review mainly concerns with the production of pectolytic enzymes using different carbon sources. It also deals with the effect of operating parameters such as temperature, aeration rate, agitation and type of fermentation on the production of these enzymes.

1
Introduction

Pectin and other pectic substances are complex plant polysaccharides. They contribute structure to plant tissue as a part of primary cell wall and as middle lamella component. The principal constituent present in pectin is a D-galacturonic acid in the form of macromolecule based on α-1,4 glycosidic linkages. The uronide carboxyl groups are 60–90% esterified by methanol. Rhamnose units can be inserted into the main uronide chain and often side chains of arabinan, galactan or arabinogalactan are linked to rhamnose [1].

Pectic enzymes may be divided into two main groups, viz., the depolymerizing pectic enzymes and saponifying enzymes or pectic esterases [2]. The enzymes are described in groups which is shown in Table 1, within each group the enzymes are listed according to the origin.

Elimination of pectic substances is an essential step in many food processing industries and wine industries. The principal objective is to reduce the viscosity of the solution so that it can be handled and processed easily. Pectic substances are degraded by pectolytic enzymes which are multiple and various due to complex nature of their substrates. These enzymes are mainly synthesized by plants and microorganisms. *Aspergillus niger* is used for the industrial production of pectolytic enzymes. This fungus synthesizes polygalacturonases, polymethyl galacturonases, pectin lyases and pectin esterases. The advantage of *Aspergillus niger* is the possession of GRAS (Generally Regarded As Safe) status which permits the use of its metabolites in the food industry [3] and also the distribution of these enzymes is in appreciable amount:

This review article mainly concerns with:

(i) The production of pectolytic enzymes using different carbon sources
(ii) Effects of physical parameters such as temperature, aeration rate and type of fermentation on the production of pectolytic enzymes

Received: 28 October 1997

G.S.N. Naidu, T. Panda
Biotechnology Research Centre,
Indian Institute of Technology,
Madras, Chennai - 600 036, India

Correspondence to: T. Panda

2
Production of pectolytic enzymes

Studies on the selection of *Aspergillus niger* strains for the production of pecolytic enzymes was made by different workers. 109 different strains of pure cultures belonging to *Aspergillus niger* species from the collection of the Industrial Microbiology Laboratory of Galati University were subjected to qualitative and quantitative screening for the selection of those strains which were the best producers for pectic enzymes. The results shows that 4 strains were choosen and they were tested for their exopolygalacturonase, pectinesterase and pectinlyase activities [4].

A process was developed for the production of polygalacturonase by *Aspergillus niger* VTT-D-77050 and its mutant VTT-D-86267. High levels of production of these enzymes were obtained in laboratory fermentations on a beet extraction waste medium. The beet waste was formulated to a concentration of 30 kg/m^3 and added to the medium containing other nutrients. Culture was performed at between pH 4.6 and 5.0 with agitation of 200 rpm and aeration of 10 l/min. Pilot plant studies were also performed in 200 l fermenter with an aeration rate of 75 l/min and the yields were low [5]. Studies on *Candida boidinii* and *Aspergillus niger* for the production of multiple forms of polygalacturonase was made. Pectic acid, the carbon source in the culture medium, induced the production of pectic enzymes. Both exopolygalacturonase are induced by the addition of galactopyranuronic acid to pectin. Production can be enhanced by altering the pH of the maintenance and culture media [6]. Schmidt et al. [7] studied *Aspergillus niger* growth and pectolytic enzyme formation in a 30 l stirred and aerated glass fermenter. The spore concentration in the fermentor was adjusted to 1000 spores/cm^3 at the beginning of the experiment and fermentation was carried out at 30 °C with 700 rpm and 60–100% air saturation. Biomass formation showed a lag phase. The stationary period is observed when cell lysis

Table 1. Classification of deploymerizing pectic enzymes [2]

Type of Enzymes	Nomenclature
Pectic enzymes acting on pectin	Polymethyl galacturonases(PMG)
	1. Endo PMG (3.2.1.41)[a]
	2. Exo PMG
	Pectin lyases[b] (PL)
	1. Endo PL (4.2.2.3)
	2. Exo PL
Pectic enzymes acting on pectic acid	Polygalacturonases (PG)
	1. Endo PG (3.2.1.15)
	2. Exo PG (3.2.1.40)
	Pectatelyases[b] (PAL)
	1. Endo PAL (4.2.2.1)
	2. Exo PAL (4.2.2.2)

[a] Numbers based on the recommendations on enzyme nomenclature of the International Union of Biochemistry [49]
[b] The term 'lyase' is preferred to 'transellminase' by International Union of Biochemistry [49]

occurred. The enzyme synthesis took place in two steps. The first was growth dependent and completed with the end of the log phase of biomass growth. The second occured at the end of catabolite repression and was not growth dependent. A mathematical model was also developed for this purpose [7].

Studies on the production of pectolytic enzymes using *Aspergillus niger* was carried out in a pilot plant 10 l fermentor and relative activity was around 210 U/cm^3 [8]. Some modification in the production medium for production of pectolytic enzymes using submerged culture of *Aspergillus niger* was made without sucrose and increasing the concentration of pectin to 5%. This favours the production of exopolygalacturonase at the expense of endopolygalacturonases. The enzyme displays its full activity in the presence of Hg^{2+} ions [9].

Pectinases were produced by *Aspergillus* species using various pretreated lemon peel as the carbon source instead of pectin. It was found that the production of polygalacturonase was about the same and that of pectinesterase substantially higher when unwashed fresh lemon peel was used instead of pectin [10]. A strain of *Aspergillus niger* was used for the synthesis of pectolytic enzymes. During the production of the enzyme methanol is liberated into the medium due to cleavage of pectin molecule which is the carbon source. It was reported that methanol is consumed by the organism but neither the synthesis nor the activity of pectinesterase and polygalacturonase was affected by methanol. It was proposed that the mechanism involved in the utilization of methanol is similar to that for methylotrophic yeast [11].

Endopolygalacturonase production was investigated by *Aspergillus sp.* CH-Y-1043 using untreated lemon peel as the sole carbon source. Results show that the activity of endopolygalacturonase was more at 39 °C than at 29 °C. Untreated lemon peel proved to be a better carbon source than citrus pectin for endopolygalacturonase production. A 10 fold increase in endopolygalacturonase was observed by modifying the medium and decreasing the medium pH to 2.8 [12].

Determination of pectinase production by *Aspergillus flavus* was made by measuring the clear zones formed around colonies stained with ruthenium red. Several isolates produced red zones instead of clear zones. Red zones were reproduced with pectinesterase and correlated with the absence of specific polygalacturonase. Out of 87 isolates tested, 15 produced red zones [13].

Polygalacturonase and pectinesterase were synthesised by a strain of *Aspergillus niger* which was isolated from the rotten lemon. The production of these enzymes were repressed by glucose even in the presence of pectin which acts as an inducer. It was observed that there exists negative effect of the sugar on the synthesis of these enzymes. When the level of the sugar decreases to low concentration due to growth, the enzymes synthesis increased. The mycelium was harvested during stationary phase and washed free of glucose and incubated in a glucose free medium containing pectin. The production of these enzymes were observed in this case. This proved the reversability of the repression mechanism. The results suggested that the catabolite repression occurs at the translational level [14]. The synthesis of pectinase was investigated using six species of *Aspergillus* with five media differing either in their carbon sources. The six species used are *Aspergillus niger* NCIM 548; *Aspergillus niger* NCIM 616; *Aspergillus foetidus* NCIM 505; *Aspergillus foetidus* NCIM 510; *Aspergillus foetidus* NCIM 1027 and *Aspergillus awamori* NCIM 885. Five among six synthesized pectinase in appreciable amount in media containing sugars.

Pectinase synthesis was highest for *Aspergillus niger* NCIM 548 with all sugar containing media. It synthesised about 1.3 units of pectinase. *Aspergillus foetidus* NCIM 510 was the only one among the organisms which synthesised pectinase in appreciable amount in medium containing pectin in the absence of additional sugars supplied in medium [15].

Using various carbon sources and nitrogen sources as well as natural products were investigated as inducers for the production of amylases and pectinases using *Aspergillus niger* C. Wheat bran extract was best for the production of both amylases and pectinases. High pectinase activities were also observed when polygalacturonic acid, fructose, mannose, saccharose and cellobiose were used as stimulators. Optimum pH for the production of pectinase was 6.0 and temperature was 35 °C [16].

Friedrich et al. [17] studied the effect of different sugars as carbon source on *Aspergillus niger* to synthesis pectolytic enzymes. It was found that pectinesterase and pectinlyase activities were found similar to those obtained in the medium containing pectin. By increasing sugar concentration from 1.5 to 15%, the activities are increased as follows:

polygalacturonase – 1.8 to 20 U/cm^3
pectinlyase – 0.14 to 0.65 U/cm^3 and
pectinesterase – no effect.

A study on the different synthesis of polygalacturonase and pectinesterase using aggregated mycelium of the strain *Aspergillus niger* ZIMET 43962 was made. The synthesis of the enzymes was regulated to an extent by the kind and concentration of carbon source and fermentation

time. It was reported that higher amount of polygalacturonase was synthesised with higher sugar concentration and pectinesterase activity is small except in the case of lactose [18]. An endopolygalacturonase produced by *Rhizopus stolonifer* using citrus pectin as the sole carbon source. Maximum activity of 4 U/cm^3 was observed during 48–52 h of fermentation. This strain does not produce pectinesterase and pectinlyase [19].

A strain of *Aspergillus niger* isolated from onion used to produce large amount of polygalacturonase. Sodium polypectate was used as the main substrate in medium in addition to onion, salt and glucose. Results showed the presence of two exo-polygalacturonase forms (polygalacturonase I and polygalacturonase II) and one endo-polygalacturonase form (polygalacturonase III). All three polygalacturonase forms are optically active at pH 5.0 and preferred sodium pectate as substrate over pectin [20].

Endopolygalacturonase lyase was produced from *Streptomyces thermovulgaris* CR 42. It is a thermophile isolated from forest soil and produces only endopolygalacturonase lyase within 48 h of fermentation. Complete degradation of pectin was observed and optimum temperature for growth and enzyme production was 55 °C. The optimum pH of the medium was 7.6 and there is no product of enzyme if the initial pH of the medium is less than 6.7 [21]. Optimization of polygalacturonase synthesis was made for *Aspergillus niger* strains R1/214, J36 and J41. *Aspergillus niger* was cultivated in three different production media. The pH of the medium was 4.5 and cultivation was done either by a single or by a two step process. The single step process involves the growth in any one of the medium at 25 °C for 96 h on a shaker. A two step process involves a preculture medium containing 4% sugarbeet slices prior to the growth in a production medium at 30 °C for 168 h in a shake culture. It was concluded that enzyme overproduction was defined as a pathological effect due to limitation of oxygen and nutrient supply [22].

The effect of sucrose on the hydrolysis of pectin was studied was found that the medium containing sucrose as the sole carbon source has tendency to produce limited hydrolysis while medium containing pectin shows complete hydrolysis. The use of mixture of sucrose and pectin as the carbohydrate source favours higher activities. The effect of different carbohydrate other than sucrose has some difference in shake flask but they virtually disappeared in stirred fermenters. It was also reported that the addition of lucerene powder to media increased the yield of pectic enzymes from *Pencillium notatum* and similar effect was observed in *Aspergillus niger*. The great effect was found with boiled extract of groundnut meal [23].

The microbial flora of coffee beans collected in the regions of Sao Paulo contained *Cladosporium*, *Fusarium* and *Aspergillus* species. The pectolytic enzymes liberated from the fungi are isolated from culture media. These enzymes are capable to break down pectic acid and galactoarabinan [24]. Three forms of polygalacturonase were present in orange peel infected by *Pencillium italicum*. The three polygalacturonase forms are designated as polygalacturonase I, polygalacturonase II and polygalacturonase III. Polygalacturonase is characterized as an exoenzyme while polygalacturonase II and III were characterized as an endoenzyme [25]. Sachin et al. [26] attempted to synthesis pectinase from agricultural waste using fungi. They used sunflower torus as energy source with glucose. The effect of pH, composition of medium were also studied. *Aspergillus niger* strains produce 3.91–5.12 kg enzyme/100 kg raw material. Pectolytic enzymes were produced by cultivation of *Aspergillus niger* on a support that develops concentrated mycelial structure. *Aspergillus niger* was cultivated from spores for 3 days in medium containing pectin and mycelium was filtered on a piece of textile of 6.5 m^2 area and the textile was placed in fresh medium and incubated for 6 days. Polygalacturonase produced was 660 U/cm^3 culture compared to 20 U/cm^3 by diffusive mycelium.

Aureobasidium pullulans LV10 produced extracellular pectolytic enzymes when grown on medium containing apple pectin as a carbon source. Maximum enzyme production was 22 U/cm^3 for polygalacturonase and 9 U/cm^3 for pectin lyase was obtained after 4 d of fermentation [27]. Dried sweet whey was used as a complete medium for production of polygalacturonase by *Kluveromyces fragilis*. The optimum concentration of whey for enzyme production was 0.5% (w/v), two days of fermentation at 25 °C. Supplementation of whey with sodium polypectate does not increase enzyme production [28].

Exopolygalacturonase, endopolygalacturonase and pectinesterase are produced from *Trichoderma reesei QM 9414* in a 30 l fermenter at 30 °C with an air flow rate of 0.3 m^3/(m^3) (min). Citrus pectin with a degree of esterification of 65% was used as a carbon source. After 10 d of fermentation, pectinolytic enzymes attain maximum activity. The maximum enzyme activity obtained were 130 nkat/cm^3 for pectinesterase and 2.06 nkat/cm^3 for polygalacturonase [29]. A study was made to compare the production of Pectinase by *Aspergillus niger* CH4 in solid state fermentation and submerged fermentation. Production of pectinase by solid state fermentation was not reduced when glucose or sucrose were added to culture medium containing pectin. Moreover, the activity was increased when the concentration of carbon source was also increased. In this submerged fermentations, the activity were decreased. This shows that the regulatory phenomena such as induction-repression or activation-inhibition related to pectinase synthesis by *Aspergillus niger CH4* are different in two types of fermentation [30]. Studies were made in both shake flask and bioreactors for the production of pectolytic enzymes from *Aspergillus ochraceus*. The specific pectolytic activity obtained in shake flask (380 rpm) are 500–600 spa/cm^3. Pilot plant run of 1000 l capacity fermenter yielded 100–140 spa/cm^3 and in 10,000 l bioreactor yielded 200–250 spa/cm^3 [8].

3 Influence of physical parameters and nature of fermentation on pectolytic enzymes production

Fermenter cultivations were carried out in a Chemap CF 2000 bioreactor of capacity of 10 l for the production of polygalacturonase by *Aspergillus niger* mutant strain VTT-D-86267. It was found that the production was efficient at 18 °C and insufficient at 30 °C. β-glucosidase production

by the same organism was almost unaffected by temperature between 18 °C and 30 °C. A technical medium was developed containing less readily available nutrients, production of both enzymes was considerably more efficient at 30 °C than 18 °C [31].

Three strains of fungi, i.e., *Trichoderma viride TS*, *Aspergillus niger* ATCC 22343 and *Aspergillus niger* SS have been cultivated in solid state media with different carbon dioxide partial pressures in gaseous environments. The production of pectinolytic enzymes was studied on a sugar beet pulp. Only *Aspergillus niger* SS produced three enzymes, viz. polygalacturonase, pectinesterase and pectin lyase. Enzyme activities of 230, 30 and 0.5 U/(g of dry matter) of polygalacturonase, pectinesterase and pectin lyases were obtained. Pectinesterase production was unaffected by pCO_2. Upto 2% pCO_2 no significant effect occurs on polygalacturonase and pectinlyase. For higher pCO_2 significant differences between these two enzymes were observed. At 10% pCO_2, polygalacturonase increases by 22% and drop of about 8% occurs for pectinlyase production. At 20% pCO_2, there is no significant influence on polygalacturonase but there is a decrease of 40% in pectinlyase [32].

Pectolytic enzymes were synthesized during the cultivation of *Aspergillus niger* in a medium with a glucose concentration of 140 kg/m^3. Experiments were performed in a 10 l Bioengineering stainless steel bioreactor at 30 °C. During the fermentation dissolved oxygen consumption, aeration and agitation had to be increased upto 1 m^3/(m^3)(min) and 600 rpm after 60 h. Maximum value of pectinolytic enzymes were obtained within 140–180 h. The maximum activities were 3 U/cm^3 for polygalacturonase, 13 U/cm^3 for pectinesterase and 0.5 U/cm^3 for pectin lyase. Maximum biomass concentration was 13 kg/m^3 and maximum amount of citric acid formed was 60 kg/m^3 [33]. A study on the production of pectinases in fedbatch culture using *Aspergillus niger* was done by Aguilar et al. [34].

Pectolytic enzymes produced in a 10 l bioreactor was studied using *Aspergillus niger* mutant A138. Maximum activity was achieved after 95 h of fermentation and thereby the activity remained constant. The specific morphology of fungus with long peripheral hyphae, resulted in very dense and viscous broth which resulted in problems for heat and mass transfer. To overcome these difficulties an attempt was made by different agitation and aeration regimes. These parameters does not affect morphology but had a marked influence on enzyme synthesis. At the time of maximum growth rate aeration was increased from 0.5 (m^3/m^3)(min) to 1.2 (m^3/m^3)(min) and agitation from 300 rpm to 500 rpm. The depectinizing activity was doubled in comparison with the results obtained when 0.5 vvm and 300 rpm throughout the fermentation [35].

The effect of inoculum size and potassium hexacyanoferrate II hydrate on pectinase synthesis by *Aspergillus niger* in submerged cultivation were studied. Higher spore concentration lead to 25% or even upto 50% decrease in activity. Polygalacturonase showed decrease in activity with the increase in spore concentration while pectinlyase showed the opposite relationship and pectinesterase does not show any relationship. A pronounced effect of potassium hexacyanoferrate II hydrate on fungal morphology as well as on the enzymatic activity was observed. Increasing the concentration of potassium hexacyanoferrate II hydrate the morphology gradually changed from loose pellets to smaller compact ones and the enzymatic activity was markedly improved. In bioreactor the biomass was reduced about to 10 to 8 kg/m^3. The activities were improved in comparison to fermentation without potassium hexacyanoferrate II hydrate. The improved activities were as follows:

0.8 to 3.3 U/cm^3 for polymethylgalacturonase,
32 to 49 U/cm^3 for pectin esterase and
0.05 to 0.12 U/cm^3 for pectinlyase.

The fermentation time was reduced from 96 h to 68 h [36].

The influence of inoculation and agitation techniques on the production of *Rhizopus arrhizus* polygalacturonase were studied in 14 l fermenter. The maximum activity was achieved after 56–64 and 24–32 h of fermentation when inoculation was carried out using spore suspensions and vegetative cells, respectively. Agitation made by using a perforated ring tube showed a positive effect on polygalacturonase activity compared to mechanical stirring. The highest activity was obtained when date extract medium containing 4% reducing sugars, mineral salts and 1% pectin at pH 4.0 was inoculated with 24 h old vegetative cells [37].

Maldonado et al. [38] showed that strain of *Aspergillus niger* producing pectinesterase and polygalacturonase exhibited diauxic growth when cultivated in a medium containing pectin as the carbon source at an initial concentration of 15 kg/m^3. The specific growth rate observed during the first growth phase is 0.14 h^{-1} while that of second phase was about ten times less than that of first one. Diauxic growth was suppressed by adding yeast extract to the medium. The specific production of polygalacturonase was decreased by increasing the initial concentration of pectin, while that of pectinesterase was increased upto 15 kg pectin/m^3. Yeast extract increased the production of biomass but decreased that of the enzymes. The temperature of incubation (25 to 40 °C) did not affect the production of biomass or that of polygalcturonase. But the production of pectinesterase was predominantly affected at 30 °C being the optimal temperature. Huerta et al. [39] used 130 l packed bed bioreactor for pectinase production by *Aspergillus niger* using absorbed substrate fermentation technique. Pectinase activity and relative CO_2 production were used as indicators of metabolic activity. It has been suggested that absorbed substrate fermentation is an efficient process for pectinase production and also an interesting model because the culture medium, water, nutrients and inducers can be designed at desired concentration. Studies on the production of exopolygalacturonase and endopolygalacturonase using *Aspergillus niger* CH4 at high initial concentrations of glucose in solid state fermentation. Cells were cultured in a packed column fermentor. The medium was supplemented with 100, 250, 350 and 450 kg/m^3 glucose. The results shows that exopolygalacturonase activity (35 U/g) was maximum at 72 and 20 h in medium containing 100 and 250 kg/m^3 glucose respectively. The

maximum endopolygalacturonase activity (9 U/g) was obtained at 72 h in medium with 250 kg/m^3 glucose. A decrease in polygalacturonase activity was observed at 350 and 450 kg/m^3 of glucose [40].

Studies on large scale production of pectolytic enzymes by solid state fermentation revealed that the production of enzyme by *Aspergillus carbanerius* CFTRI 1048 on wheat bran medium was maximum at 21 h of fermentation at 30 °C with an activity of 10.49 U/cm^3 [41]. An attempt was made to improve the mold strains for the production of pectinase by solid state fermentation of coffee pulp. 248 strains were isolated in Mexico's coffee growing areas, among which wild strains of *Aspergillus niger* attained a peak value of pectinase of 138 U/g dry pulp in 72 h. Pectinase producing mutant strain was isolated in a medium containing 2-deoxyglucose. This mutant strain produced 228 U/g of dry pulp [42].

An extracellular polygalacturonase was obtained from the extracts of solid-state cultures of *Aspergillus niger* 3T5B8. The enzyme was purified by salting-out fractionation, dialysis and gel filtration chromatography. The molecular weight determined for this polygalacturonase was 34700 daltons [43]. Solid-state cultures of *Aspergillus foetidus* NRRL341 were studied under different acidic conditions. Glass bottles containing 5 g of wheat bran and 7.5 cm^3 of 0.2, 0.3, 0.4 and 0.5 M HCl were autoclaved and inoculated with a spore suspension appropriately diluted and incubated at 30 °C. Total pectinase activity was expressed in terms of viscometric units per gram of wet substrate (VU/g) was affected by the initial acidity of the culture. It was shown that the pectinase activity increases with increase in initial acidity of the culture. Fungal growth was not observed at higher acidity (0.5 M HCl). Maximum activity (2535 VU/g) was reached after 36 h in 0.4 M HCl containing culture. With 0.2 and 0.3 M HCl, total pectinase activity was maximum at 30 h (1860VU/g). The composition of pectinase was also affected by the culture acidity. Culture acidity is inversely proportional to the pectinesterase activity and proportional for both polymethylgalacturonate lyase and polygalacturonase activity [44].

Protopectinase was produced from cultures of *Aspergillus awamori* IFO 4033 in solid state culture. 10g of wheat bran and 15 cm^3 of 0.2 M HCl were inoculated in Petridish and incubated for 48 h at 30 °C. Protopectinase activities were determined using lemon and apple as substrates. Protopectinase activity on lemon and apple was maximum at 24 h of culture (1490 and 610 U respectively) and then decreases. Pectinase activity on lemon and apple pectin and polygalacturonase activity were maximum at 48 h. It has been shown that the ratio of protopectinase lemon to protpectinase apple changed during culture which implies that there exists at least two protopectinases with different substrate specificity [45].

Studies on anaerobic fermentation of bacteria for the production of pectinase was studied. Samples from biogas digester, sewage ponds, animal house effluents and food processing wastes were used in enrichment systems seeking anaerobic pectinases. Among the 46 anaerobic consortia developed from various samples, four showed high pectinase activity under static anaerobic conditions. The optimum conditions for pectinase activity were pH 7.0, 45 °C and 72 h of growth with 0.5% pectin in the cultivation medium. Nearly 1.6 fold increase in pectinase activity was achieved under these conditions. The maximum yield of enzymes were: 62.72 U/cm^3 of polygalacturonase, 113.30 U/cm^3 of pectinlyase, 2.10 U/cm^3 of pectinesterase and 0.75 U/cm^3 of total cellulase was observed [46].

Studies on biosynthesis of pectolytic enzymes was made by immobilizing the mycelium. *Aspergillus niger* mycelium immobilized on these carriers and grown on the medium with wheat bran extract was partially released to the medium. Controlled growth and good stability of *Aspergillus niger* mycelium were obtained using pumice stones as carrier and a medium containing 1% pectin. Subsequently, thirty 24 h batches could be performed by using the same carrier at a small loss of polygalacturonase activity. Culture conditions such as pH, temperature, agitation speed and amount of pumice stones and pectin were optimized to improve the growth and enzyme biosynthesis by immobilized mycelium [47]. The biomass is grown in a fermentor on a support and the culture is continued till a compact layer of mycelium was formed on the support. The culture medium was withdrawn and fresh medium was charged in the fermentor several times. The harvested medium was concentrated for enzyme. It was concluded that various combinations of fresh feed and reused culture media can be performed in single fermentor or the reused media can be charged into another fermentor or series of fermentors. In this way, utilization of nutrients is improved. Maximum activity of polygalacturonase was 400 U/g.h. The production of pectolytic enzymes is effected by culturing *Aspergillus niger* on a solid support in a culture medium in a fermentor under growth and fermentation conditions. The initial pH during growth phase is 4.5. After 24 h of culture, the pH was reduced to 2.9 by adding an acid. This process gave high space time yields (800–2350 U/l.h). This process can also be operated as a cyclic batch process in which the enzyme containing culture medium was periodically removed and fresh medium was replaced. The pH was maintained at 2.6–3.5 during subsequent fermentation. The production of pectolytic enzymes is effected by culturing an immobilized fungus *Aspergillus niger*, under submerged conditions in a conventional medium. The ratio of polygalacturonase to pectinesterase is controlled by varying the fermentation time. This ratio was less than 20 when fermentation was stopped at between 72 and 120 h and greater than 100 when stopped after 120 h. Phosphate or citrate buffer of concentration 20–60 kg/m^3 was added to the medium which, in turn, enhanced the production of the enzymes especially polygalacturonase (Akad. Wiss. DDR).

4 Application of pectolytic enzymes

In the production of fruit juices, extracts and concentrates, these enzymes play an important role in maceration and solubalization of fruit pulps and in clarification [48].

By applying these enzymes on fruit pulps, it degrades pectin thereby reducing the viscosity and the fruit juices can be handled easily. These enzymes are used in clarification of wine, expression of fruit juices like banana,

mango, papaya, guava and apple. They are also used in the manufacture of hydrolyzed products of pectin. It is used in the retting of textiles and fibres. It is used in the manufacture of pectin free starch, in the refinement of vegetable fibres, in the curing of coffee, cocoa and tobacco. It is also used as analytical tool for the estimation of plant products [41].

5 Conclusions

Extensive study on the influence of carbon source on the production of enzymes was made. The effect of different carbon sources and concentration of carbon source was studied. The optimum medium constituents for the enhanced production of enzymes using pectin as an inducer was determined. Effect of initial pH, temperature, air flow rate on enzyme production were also studied. Batch reactor studies on the production of pectolytic enzymes were also carried out. Inadequate amount of work was done on novel reactors for the production of pectolytic enzymes except in packed bed reactors. Inadequate amount of work was done in anaerobic fermentation for production of pectolytic enzymes. Production of enzymes in immobilized cell reactors were also studied. Extensive work is yet to be carried out in fed batch reactors. Intensive work on kinetic studies in reactor was not done. Extensive work was carried out in solid state fermentation for the production of pectolytic enzymes. Solid state fermentation provides higher productivity due to larger enzyme yields and shorter fermentation times. In submerged cultures the enzyme activity decreases by increasing the supplementation of carbon source, but not in solid state fermentation. This shows that regulatory phenomena such as induction-repression or activation-inhibition was different in submerged culture and solid state fermentation.

6 Future work

The physical parameters such as pH, aeration and agitation should be optimized in a reactor for the production of pectolytic enzymes and their interaction effects are required to be addressed on this avenue. Extensive work on reactor kinetics can be done to analyze the whole process. Higher concentrations of carbon source inhibit enzyme synthesis. This conclusion strongly supports production of pectolytic enzymes by fed batch fermentation and extensive work should be carried out. Continuous mode of operation in reactor for production of these enzymes can be studied to develop the process. Literature on high cell density cultivation is not available. Further studies on design of level reactors for the production of pectolytic enzymes can be attempted. Studies on production of these enzymes using genetically modified strains for enhanced production can be done.

References

1. **Van Buren, J.P.:** The chemistry and technology of pectin. Academic Press, San Diego (1991) 1–22
2. **Rombouts, F.M.: Pilnik, W.:** Research on pectin depolymerases in the sixties - A Literature Review: CRC Critical Review in Food Technology (1972) 1–22
3. **Parzia, M.W.; Foster, E.M.:** Determining the safety of enzymes used in Food Processing. J. Food Protection 46 (1983) 453–468
4. **Dinu, D; Dan, V.:** Selection of Aspergillus niger strains producing pectic enzymes. Analele Universitatii Biologie 43 (1994) 33–37
5. **Bailey, M.J.; Pessa, E.:** Strain and process for production of polygalacturonase. Enzyme Microb. Technol. 12 (1990) 622–624
6. **Stratilova, E.; Breierova, E.; Vadkertiova, R.:** Factors influencing the production of multiple forms of polygalacturonase from the yeast Candida biodinni and Aspergillus niger. Folia Microb. 40 (1995) 565
7. **Schmidt, O.; Angermann, H.; Frommhold Treu, I.; Hoppe, K.:** Experimental and theoretical investigation of submerged fermentation and synthesis of pectinolytic enzymes of Aspergillus niger. Appl. Microb. Biotechnol. 43 (1995) 424–430
8. **Nyiri, L.:** Manufacture of Pectinases. Process Biochem. (1968) 27–30
9. **Mill, P.J.:** The pectic enzymes of Aspergillus niger, mercury activated exopolygalacturonase. Biochem. J. 99 (1966) 557–561
10. **Maldonado, M.C.; Antonia, N.; Danley Callieri, A.S.**: Production of pectinases by Aspergillus species using differently pretreated lemon peel as the carbon source. Biotechnol. Lett. 8 (1986) 501–504
11. **Maldonado, M.C.; Anna Maria Strasser de Saad.; Danley Callieri.:** Methanol utilization by a strain of Aspergillius niger: Influence on the synthesis and activity of pectinases. World J. Micro. Biotechnol. 9 (1993) 202–204
12. **Larios, G.; Gracia, G.M.; Huitron, C.:** Endopolygalacturonase production from untreated lemon peel by Aspergillus sp. CH-Y-1043. Biotechnol. Lett. 11 (1989) 729–734
13. **Cotty, P.J.; Medronho, R.A.; Leite S.G.F; Couri, S.**: Partial purification of a polygalacturonase produced by solid state cultures of Aspergillus niger 3T5B. Revista de Microbiologia. 26 (1995) 318–322
14. **Maldonado, M.C.; Anna Maria Strasser de Saad.; Danley Callieri.:** Catabolic repression of synthesis of inducible polygalacturonases and pectinesterase by Aspergillus niger species. Current Microb. 18 (1989) 303–306
15. **Nair, S.R.; Rakshit, S.K.; Panda, T.:** Effect of carbon sources on the synthesis of pectinase by Aspergilli. Bioprocess Eng. 13 (1995) 37–40
16. **Fiedurek, J.; Ilczuk, Z.; Lobarzewski, J.:** Influence of the mycelium growth conditions on the production of amylolytic, proteolytic and pectinolytic enzymes by Aspergillus niger C. Acta Biotechnol. 9 (1989) 355–361
17. **Friedrich, J.; Cimerman, A.; Steiner, W.**: Production of Pectolytic enzymes by Aspergillus niger on sucrose. Food Biotechnol. 6 (1992) 207–216
18. **Leuchtenberger, A.; Friese, E.; Ruttloff, H.:** Variation of polygalacturonase and pectinesterase synthesis by aggregated mycelium of Aspergillus niger in dependence of carbon source. Biotechnol. Lett. 11 (1989) 255–258
19. **Manachini, P.L.; Fortina, M.G.; Parini, C.:** Purification and properties of endopolygalacturonase produced by Rhizopus stolonifer. Biotechnol. Lett. 9 (1987) 219–224
20. **Behere, A.; Satyanarayan, V.; Padwal Desai, S.R.:** Separation and limited characterization of three polygalacturonase of Aspergillus niger. Enzyme Microb. Technol. 15 (1993) 158–161
21. **Niranjan, R.; Dhala, S.A.:** Endopolygalacturonase lyase of streptomyces thermovulgaris CR 42. J. Food Sci. Technol. 18 (1981) 171–175
22. **Hermersdoerfer, H.; Leutchenberger, A.; Wardsack, C.; Ruttloff, H.:** Influence of culture conditions on mycelial structure and polygalacturonase synthesis of Aspergillus niger. J. Basic Microb. 27 (1987) 309–315

23. **Tuttobello, R.; Mill, P.J.:** The pectic enzymes of Asspergillus niger. Biochem. J. 79 (1961) 51–56
24. **Woriaki, G.; Zancan.; Galci, T.:** Enzymatic degradation polysacchharides in the pulp of coffee beans. Arq. Biol. Technol. 16 (1973) 129–134
25. **Hershonhom, J.; Manulis, S.; Barash, I.:** Polygalacturonase associated with infection of valencia orange by Pencillium italicum. Phytopathology. 80 (1990) 1374–1376
26. **Sachin, I.; Donmetz, S.; Kilic, O.:** Studies on pectolytic enzyme production from some agricultural wastes by some fungi. Chem. Microb. Technol. Lebensm. 8 (1983) 87–90
27. **Manachini, P.L.; Parini, C.; Fortina, M.G.:** Pectic enzymes from Aureobasidium pollulans LV 10. Enzyme Microb. Technol. 10 (1988) 682–685
28. **Donaghy, J.A.; McKay, A.M.:** Pectin extraction from citrus peel by polygalacturonase produced on whey. Bioresource Technol. 47 (1994) 25–28
29. **Markovic, O.; Slezarik, A; Labudova, I.:** Purification and characterization of pectinesterase and polygalacturonase from Trichoderma reesei. FEMS Microb. Lett. 27 (1985) 267–271
30. **Solis-Pereyra, S.; Favela-Torres, E.; Gustavo Viniegra-Gonzaka, G.; Rojas, G.:** Effects of different carbon sources on the synthesis of pectinase by Aspergillus niger in submerged and solid state fermentations. Appl. Microb. Technol. 39 (1993) 36–41
31. **Bailey, M.J.:** Effect of temperature on polygalacturonase production by Aspergillus niger. Enzyme Microb. Technol. 12 (1990) 622–624
32. **Desgranges, C.; Durand, A.:** Effect of pCO_2 on growth conditions and enzyme production in solid-state culture on Aspergillus niger and Trichoderma virde TS. Enzyme Microb. Technol. 12 (1990) 622–624
33. **Friedrich, J.; Cimerman, A.; Steiner, W.:** Concomitant biosynthesis of Aspergillus niger pectolytic enzymes and citric acid on sucrose. Enzyme Microb. Technol. 16 (1994) 703–707
34. **Aguilar, G.; Huitron, C.:** Application of fedbatch cultures in the production of extracellular pectinases by Aspergillus species. Enzymes Microb. Technol. 8 (1986) 541–545
35. **Friedrich, J.; Cimerman, A.; Steiner, W.:** Submerged production of pectolytic enzymes by Aspergillus niger: Effect of different aeration/agitation regimes. Appl. Microb. Technol. 31 (1989) 490–494
36. **Friedrich, J.; Cimerman, A.; Steiner, W.:** Production of pectolytic enzymes by Aspergillus niger: Effect of inoculum size and potassium hexacyanoferrate II hydrate. App. Microb. Technol. 33 (1990) 377–381
37. **Al Obaid, Z.S.; Aziz, G.M.; Al Bakir, A.Y.; Ali, M.A.:** Effect of fermenter conditions on the production of Rhizopus arrhizus polygalacturonase. J. Agri. Water Resource Res. Plant Production. 8 (1989) 65–76
38. **Maldonado, M.C.; Callieri, D.:** Influence of environmental conditions on the production of pectinesterase and polygalacturonase by Aspergillus niger. Mircen J. Appl. Microb. Biotechnol. 5 (1989) 327–334
39. **Huerta, S.; Favela, E.; Lopez-Vllibarri, R.; Ronseca, A.; Viniegra-Gonazalez, G, Rojas, M.:** Absorbed substrate fermentation for pectinase production with Aspergillus niger. Biotechnol. Lett. 8: 837–842
40. **Solis-Pereyra, S; Favela Torres, E.; Gutierrez Rojas, M.; Roussos, S.; Saucedo Castaneda, G.; Gunasekaran, P.; Viniegra Gonzalez, G.:** Production of pectinases by Aspergillus niger in a solid state fermentation at high initial glucose concentration. World J. Microb. Biotechnol. 12 (1996) 257–260
41. **Ghildyal, N.P.; Ramakrishna, S.V.; Nirmala Devi, P.; Lonsane, B.K.; Asthana, H.N.:** Large scale production of pectolytic enzymes by solid state fermentation. J. Food Sci. Technol. 18 (1981) 248–251
42. **Philip, A.; Alfredo, M.; Sevastianos, R.; Marice, R.; Viniegra-Gonzalez, G.:** Pectinase hyperproducing mutants of Aspergillus niger C28B25 for solid state fermentation of coffee pulp. Enzyme Microb. Technol. 15 (1993) 254–260
43. **Coelho, M.A.Z.; Medronho, R.A.; Leite, S.G.F.; Couri, S.:** Partial purification of a polygalacturonase produced by solid state cultures of Aspergillus niger 3T5B. Revista de Microbiologia. 26 (1995) 318–322
44. **Cavalitto, S.F.; Arcas, J.A.; Hours, R.A.:** Pectinase production profile of Aspergillus foetidus in a solid state cultures at different acidities. Biotechnol. Lett. 18 (1996) 251–256
45. **Hours, R.A.; Sakai, T.:** Protopectinase production in solid state culture of Aspergillus awamori. Biotechnol. Lett. 16 (1994) 721–726
46. **Shivakumar, P.D.; Nand, K.:** Anaerobic degradation of pectin by mixed consortia and optimization of fermentation parameters for higher pectinase activity. Lett. Appl. Microb. 20 (1995) 117–119
47. **Fiedurek, J.; Ilczuk, Z.; Lobarzewski, J.; Pleszcynska, M.:** Optimization of pectinolytic enzymes biosynthesis by immobilized mycelium of Aspergillus niger 71. Zentralbl. Mikrobiologie 147 (1992) 15–21
48. **Kilara, A.:** Enzymes and their uses in the processed apple industry: A Review. Process Biochem. 35 (1982) 35–41
49. **Florkin, M.; Stotz, E.H.:** Enzyme Nomenclature in Comprehensive Biochemistry. Elsevier, Amsterdam (1965)