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Review Article

IMMOBILIZATION OF MICROBIAL PECTINASES: A REVIEW

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ABSTRACT
Immobilization technology creates exciting new opportunities for commercial development in a wide range of industries. There are several reasons for using an enzyme in an immobilized form. In addition to more convenient handling of the enzyme, it provides for its facile separation from the product, thereby minimizing or eliminating protein contamination of the product. To date, pectinases have been immobilized by various techniques (adsorption, cross linked enzyme aggregates (CLEAs), covalent attachment etc.). In this review, we have focused on pectinase immobilization techniques and some applications of immobilized pectinases. Microbial pectinases have tremendous potential to offer mankind which can be efficiently used by applying immobilization principles on them.

Key Words: *Chitosan, CLEAs, Immobilization, Nylon-6, Pectinases*

INTRODUCTION
Pectin is structurally and functionally the most complex polysaccharide in plant cell wall (Mohnen, 2008). The primary chain of pectin is composed of α -1,4- linked residues of D- galacturonic acid (Jayani *et al.,* 2005). The enzymes depolymerising pectin i.e. pectinases can be divided into hydrolases and lyases (Sakai *et al.,* 1993). Pectinases are distributed in many higher plants and microorganisms. They play a very important role in plants since they help in cell wall extension and softening of some plant tissues (Jayani *et al.,* 2005). Pectinases are produced by a large number of organisms such as bacteria (Magro *et al.,* 1994), fungi (Servili *et al.,* 1992) and yeasts (Fontana and da Silveira, 2012). Certain *Aspergillus* species can be characterized by the types of pectinolytic enzymes they are able to produce (Alimardani-Theuil *et al.,* 2011; Maciel *et al.,* 2011; Fontana and Da Silveira, 2012). The most widely occurring enzymes are polygalacturonase (PGs), pectin methylesterase (PMEs) and pectate lyase (PLs) produced during the infection process and during culturing (Jia *et al.,* 2009). The fixed bed reactor with orange peel support and using *Aspergillus niger* URM5162 is a promising process for polygalacturonase production at the industrial level (Maciel *et al.,* 2013). Alkaline pectinases find application in degumming and retting of plant material, plant protoplast formation and treatment of fruit-processing waste streams. Acidic pectinases are widely used for extraction and clarification of fruit juice. Alkaline pectinases are predominately produced by alkalophilic bacteria like *Bacillus* sp. (Kashyap *et al.,* 2001), whereas acidic pectinases are excreted by fungal sources, mainly *Aspergillus* sp. (Tuttobello and Mill, 1961). Some biochemical properties of PGs produced by different fungi are shown in Table 1.
The term immobilized enzymes refers to enzymes physically confined or localized in a certain defined region of space with retention of their catalytic activities, and which can be used repeatedly and continuously. Immobilization means associating the biocatalysts with an insoluble matrix, so that it can be retained in proper reactor geometry for its economic reuse under stabilized conditions. Immobilization thus allows, by essence, to decouple the enzyme location from the flow of the liquid carrying the reagents and products. Since in food industry it is preferable to avoid the presence of extraneous compounds in the final products the possibility to remove the enzyme is a significant advantage. In literature there is data about immobilization of pectinolytic enzymes on different supports by various methods (Vaillant *et al.,* 2000; Rao *et al.,* 2000; Sarioglu *et al.,* 2001; Demirel *et al.,* 2004; Sardar and Gupta, 2005; Brena and Batista-Viero, 2006). Immobilization can be performed by several methods, namely, entrapment/microencapsulation, binding to a solid carrier, and cross-linking of enzyme aggregates, resulting in carrier-free macromolecules. The latter presents an alternative to carrier-bound enzymes, since these introduce large portion of noncatalytic material. This can account to about 90% to more than

99% of the total mass of the biocatalysts, resulting in low space-time yields and productivities, and often leads to the loss of more than 50% native activity, which is particularly noticeable at high enzyme loadings (Sheldon, 2007). A broad, generalized overview of the advantages and drawbacks of the different immobilization approaches is given in Table 2.

Until now, pectinase has been immobilized on various supports including nylon (Lozano *et al.,* 1987), ionexchange resin (Kminkova and Kucero, 1983), silk (Zhu *et al.,* 1998), and chitin (Iwasaki *et al.,* 1998). Recently, pectinase was immobilized in alginate by simple inclusion (Ipsita *et al.,* 2003; Busto *et al.,* 2006), however, its residual activity was slightly lower or had a lower stability making it difficult to use on an industrial scale, pectinase was covalently immobilized onto the macroporous polyacrylamide microspheres (Lie and Jiang, 2006) and immobilized on an activated agar-gel support by multipoint attachment (Li *et al.,* 2008). The endo-polygalacturonase from *Aspergillus niger* has been immobilized by adsorption on porous polyethylene terephthalate (Rexova-Bankova *et al.,* 1982). Omelkova *et al.,* (1985) (Omelkova *et al.,* 1985) immobilized endopolygalacturonase on to porous poly (6-caprolactam) activated by glutaraldehyde with a relative activity of 24%. (The relative activity is the ratio of the activities of the bound and free enzyme expressed in percentage). Other matrices which have been used for immobilization of polygalacturonases are poly (2, 6- dimethyl-p-phenylene oxide) (Rexova-Benkova *et al.,* 1983), granular poultry bones (Findlay *et al.,* 1986), porous glass (Romero *et al.,* 1987) and nylon (Lozano *et al.,* 1987). Pectinase was immobilized on Fe_3O_4/SiO_2-g-poly (PSStNa) nanocomposite microspheres by covalent attachment (Lie *et al.,* 2009). Szaniawski and Spencer (Szaniawski and Spencer, 1997) examined the effect of immobilized pectinase on the microfiltration of dilute pectin solutions by macroporous titanium membranes and immobilized enzyme was found to be very effective for the degradation of the pectin solution. Endopectinlyase immobilization onto tailor-made core-shell microspheres is another research on the immobilization of the pectolytic enzymes (Dinella *et al.,* 1996).

In this review, various studies carried out on immobilization of pectinases on different matrices are discussed.

Pectinase Immobilization on Polyacrylonitrile Copolymer Membrane

In the present study commercial pectinase from *Aspergillus niger* was immobilized on polyacrylonitrile copolymer membrane via adsorption on the membrane or covalently after activation of the support with glutaraldehyde. The methods used for immobilization are simple and effective. The chosen support has suitable pore size that allows easy penetration of the enzyme; it has high mechanical, temperature and chemical stability and can be separated from the reaction mixture without contaminating the final product (Delcheva *et al.,* 2007).

Pectinase Immobilization on ion Exchange Resins

The ion exchange resin, Dowex Marathon WBA was used to obtain strong electrostatic interaction for the immobilization of commercial pectinase without using any other chemicals like glutaraldehyde, carbodiimide or cyanogens bromide. Pectolytic enzyme preparation was immobilized on to anion exchange St-DVB macroporous base resins, and the kinetics of immobilized commercial pectinase was studied (Demir *et al.,* 2001).

Bone as solid support for immobilization of pectinase

Poultry bone residue was found to serve as a solid support matrix to which catalase, pepsin, pectinase, lactase and invertase could be insolubilized by covalent attachment and adsorption. Bone has great potential for enzyme immobilization since it is inexpensive, abundant, chemically functional, porous, non-toxic and mechanically strong (Findlay *et al.,* 1986).

Pectinase Immobilization on Aminated Silica gel

Aminated silica gel was used as a support for the covalent immobilization of the enzyme. Endo-polygalacturonase from *Aspergillus ustus* when immobilized on to modified silica gel retained 28% of its original activity. The immobilized enzyme could be re-used through 10 cycles of reaction with almost 90% retention of its original activity. It had increased thermostability over its soluble form: the half-life of the soluble enzyme at 40°C was less than 10 h whereas the immobilized enzyme retained 82% of its

activity after 10 h at 40°C. Similarly, at 50°C the half-life of the soluble enzyme was 30 min whereas that of the immobilized enzyme was 5 h. When the enzyme was treated with trinitrobenzene sulfonate, a reagent which binds specifically to lysine residues in proteins, the enzyme failed to bind to the matrix, indicating that binding takes place through the "-amino groups of lysine residues on the surface of the enzyme. Previous studies on chemical modification of the active site residues of the endo-polygalacturonase had shown that lysine is not essential for catalytic activity (Narsimha *et al.*, 1996).

Cross Linked Enzyme Crystals and Cross Linked Enzyme Aggregates

Methods to immobilize enzymes without the use of supports are gaining in importance, however, because they offer the advantages of high volumetric productivity and lower production costs and also because they are composed only of protein and a small amount of cross-linking agent. Examples of such carrier-free preparations include crosslinked enzyme crystals (CLECs) (Quiocho and Richards, 1964; Alter *et al.*, 1967; Clair and Navia, 1992) cross-linked enzymes (Habeeb, 1997; Jansen and Olson, 1969), and the recently developed methodology of cross-linked enzyme aggregates (CLEAs) (Cao *et al.*, 2000). In this procedure, the enzyme is precipitated from an aqueous solution by adding a salt or a water-miscible organic solvent or polymer, such as poly (ethylene glycol). In a subsequent step, the physical aggregates of enzyme molecules are cross-linked with a bifunctional agent (Cao *et al.*, 2000; Cao *et al.*, 2001; Lopez *et al.*, 2002). Polyfunctional polymers with a high molecular weight (e.g., 100 to 200 kDa), containing numerous reactive aldehyde groups, are known to be effective cross-linkers of proteins or subunits (Kazan *et al.*, 1997; Fernandez *et al.*, 1999a; Fernandez *et al.*, 1999b). An interesting feature of the CLEAs is that these preparations do not require extensive purification of the enzyme activities. In this respect, CLEAs differ from the CLEC™, another form of enzyme aggregates prepared by chemical cross-linking of enzyme crystals (Presichelli *et al.*, 1995). Thus, a CLEA may catalyze a sequence of reactions. Such CLEAs have been called Combi-CLEAs (Sheldon *et al.*, 2005; Dalal *et al.*, 2006). The general protocol for the preparation of CLEAs consists of precipitating the enzyme activity by adding salt or an organic solvent (Schoevaart *et al.*, 2004; Shah *et al.*, 2006). This is followed by addition of cross-linking reagent, which is generally glutaraldehyde. A multipurpose CLEA with substantial activities of pectinase, xylanase and cellulase was prepared and characterized. The other two activites, xylanase and cellulase, also have well known and extensively documented applications in biotechnology (White and Brown, 1981; Subramaniyam and Prema, 2002). Table 3 shows the remarkable thermostabilization of the enzymes present in the preparation. In all three of the cases, half-lives have increased upon CLEA formation. Cellulase activity was most thermostable and its thermoinactivation was measured at 70°C. The largest increase in stability was in the case of pectinase in which the half-life increased from 17 to 180 minutes (Dalal *et al.*, 2007).

Pectinase Immobilization on Macroporus Polyacrylamide

The essential requirement for any carrier is the need to have a large surface area. In this respect, porous polymeric materials, which have obvious advantage of high internal surface areas, have been increasingly employed as the solid supports (Blanco *et al.*, 2004; Li *et al.*, 2010). It has been found that the pore sizes and specific surface area play an important role in the enzyme loading and activity expression (Keeling and Brennan, 2001; Tsai and Doong, 2007; Das *et al.*, 2010). However, a very high loading may produce diffusion constraint, which is not favorable for enzyme immobilization. It is convenient to use supports with a very large specific surface, such as macroporous polyacrylamide (PAM), which provide substrate and product transport with the least diffusional restriction. Macroporous PAM microspheres, a kind of macroporous amino resin (Liu and Guo, 2006) were chosen as immobilization supports because of their prominent advantages, such as availability of plentiful surface amino groups, perfect mechanical strength, large surface area (Tang *et al.*, 2001) amenable to chemical modifications, adjustable particle size, easy regeneration, low operational cost, high performance of antipollution, good selectivity, and favorable chemical stability. The advantages above may provide the pectinase immobilization: (i) a certain number of available binding sites and a very simple, mild, and time-saving process, (ii) the reuse support (Pessela *et al.*, 2003) (iii) the reduction of immobilization costs. Pectinase was immobilized onto the macroporous

PAM. The immobilized pectinase exhibited higher relative activity and stability than the free enzyme in the solution.

The SEM (Scanning electron microscope) images of the resulting macroporous PAM microspheres are shown in Figure 1. It can be seen in Figure 1 that the macroporous PAM microspheres, after being washed with methanol, are perfect microspheres, with a diameter of less than 50 μm (Figure 1a), and that their surfaces are smooth. The surface morphologies of the macroporous PAM microspheres exhibit porous structures (Figure 1b). Their porous structures did not change much after being washed by methanol and the diameter of their porous is about 25 nm. Fig 1c showed that the internal morphologies of the macroporous PAM microspheres exhibit porous structures (Lie and Jiangi, 2006).

Immobilization of Polygalacturonase on Activated Polyethylene

Polyethylene is a convenient matrix for enzyme immobilization. It is easily removed from fruit juice, is inexpensive, inert, non-toxic and readily available. The use of synthetic polymers for enzyme immobilization has several advantages viz. inertness to microorganisms, higher chemical resistance and option to use complex buffer system mostly required in biosensor systems (Lei and Bi, 2007). *Aspergillus niger* Van Tieghem (MTCC 3323) produced polygalacturonase when grown in modified Riviere's medium containing pectin as single carbon source by fed-batch culture. The enzyme was precipitated with ethanol and purified by gel filtration chromatography (Sephacryl S-100) and immobilized onto glutaraldehyde-activated polyethylene. The method is very simple and time saving for enzyme immobilization. Various characteristics of immobilized enzyme such as optimum reaction temperature and pH, temperature and pH stability, binding kinetics, efficiency of binding, reusability and metal ion effect on immobilized enzymes were evaluated in comparison to the free enzyme. Both the free and immobilized enzyme showed maximum activity at a temperature of 45 °C and pH 4.8. Maximum binding efficiency was 38%. The immobilized enzyme was reusable for 3 cycles with 50% loss of activity after the third cycle (Saxena *et al.,* 2008).

Immobilization of Pectinase on Polymer Nanocomposite Microspheres

Polymer nanocomposite microspheres (PNCMs) represent an attractive family of composite materials in which the nanometer sized reinforcing fillers are uniformly dispersed in the polymer on a nanometer scale compared to conventional phase-separated macrocomposites (Dyal *et al.,* 2003; Weng and Wei, 2003; Kahraman *et al.,* 2007). Polymer nanocomposite microspheres (PNCMs) as solid supports can improve the efficiency of immobilized enzymes by reducing diffusional limitation as well as by increasing the surface area per mass unit. The PSStNa support presents a very simple, mild, and time-saving process for enzyme immobilization, and this strategy of immobilizing pectinase also makes use of expensive enzymes economically viable, strengthening repeated use of them as catalysts following their rapid and easy separation with a magnet. In this work, to build more stable assembly, the polyelectrolyte brush PSStNa was grafted onto the surface of Fe_3O_4/SiO_2 composite particles by surface-initiated atom transfer radical polymerization (SI-ATRP) using modified magnetic silica as initiator. Subsequently, introducing a layer-by-layer (LbL) method, deposition occurs by electrostatic interactions between the adsorbed PSStNa and chitosan layer with opposite charges. It was further found that Fe_3O_4/SiO_2-g-PSStNa nanocomposite microspheres with modified multishells enhanced the stability of both nanopaticles (compared to adsorption) in solution and the immobilized pectinase. This strategy of pectinase immobilization opens new avenues for the application of bioparticles and represents a promising route for the creation of complex catalytic particles (Lie *et al.,* 2009).

Pectinase Immobilization on Silica Coated Chitosan

As a matter of fact, chitosan has been shown to be a superior supporter for the enzyme immobilization, compared to polysaccharides such as alginate (Centinus and Oztop, 2000; Ibrahim *et al.,* 2002). Furthermore, chitosan has been found to exhibit a considerable protein-binding capacity and a high recovery of enzyme activity, allowing that the enzyme immobilized thereon remains considerably active (Gallifuoco *et al.,* 1998). However, severe shrinkage and deformation could not be easily avoided upon drying the chitosan carriers into the corresponding gels (Michael and Arlon, 2001). This can be improved

in conjunction with other solid powders to increase its density and strengthen its physical properties, and thus to expand its applications (Bai *et al.,* 2002). The layer-by-layer (LbL) technique provides an easy, low cost, and versatile method for the fabrication of the silica coated chitosan support. By virtue of the attraction of oppositely charged molecules, chitosan, owing to its cationic polyelectrolyte nature, spontaneously forms water insoluble complexes with anionic polyelectrolyte (Dumitriu and Chornet, 1998; Kubota and Kikuchi, 1998; Singla *et al.,* 2001). Pectinase was immobilized onto a new type of silica-coated chitosan support from layer by layer approach and the properties of immobilized enzyme were compared with those of free pectinase. The immobilized pectinase revealed acceptable pH stability over a broad experimental range. This simple strategy seems to permit very good results in terms of immobilization rate and stability, offering some advantages when compared to the immobilization on glutaraldehyde pre-activated supports (Lie and Bi, 2007).

Pectinase Immobilization on Nylon-6

Unlike most other nylons, Nylon-6 is not a condensation polymer, but instead it is formed by ring–opening polymerization. During polymerization, the peptide bond within each caprolactam molecule is broken; with the active groups on each side reforming two new bonds as the monomer becomes part of the polymer backbone. The activation of nylon involved partial acid hydrolysis of the Nylon-6 surface to generate amino groups (and carboxyl groups), which could be coupled to proteins with glutaraldehyde (Sundaram and Hornby, 1970). Pectin lyase [PNL, poly (methoxygalacturonide) lyase; E.C. 4.2.2.10] from *Penicillium italicum* was immobilized by covalent binding to Nylon 6 in order to compare physico-chemical and kinetic properties of the soluble and immobilized counterpart. The immobilization caused a marked increase in the thermal stability of the enzyme. The immobilized PNL was extraordinarily stable during storage at 4°C. No loss of activity was observed when the immobilized enzyme was used for 12 consecutive cycles of operation (Alkorta *et al.,* 1996). Polygalacturonase from *Aspergillus niger* Van Tieghem was immobilized by covalent binding method on glutraldehyde activated Nylon-6 and used for apple juice clarification (Shukla *et al.,* 2010).

Immobilization of Aspergillus niger Pectinase on Magnetic Particles

A commercial preparation of pectinase, pectinex was purified with the help of alginate-magnetite beads. The purified pectinase was immobilized on magnetic latex beads via carbodiimide coupling. The pH optimum (pH 4.5 for both free as well as immobilized enzyme) and K_m (0.7 mg/ml for free enzyme; 1 mg/ml for immobilized enzyme) did not vary significantly upon immobilization. While the half life of free enzyme was calculated as 9 min., the immobilized preparation remained stable upto 3 h at 60°C (Tyagi and Gupta, 1995). In a previous study pectinase from *Leucoagaricus gongylophorus* immobilized on magnetic particles (Adalberto *et al.,* 2012).

Applications

Immobilized Pectinase in Hollow Fibre Ultrafiltration (HFUF) of Apple Juice

Commercial pectic enzymes or pectinases are used in apple juice manufacturing to depectinize pressed juices in order to remove turbidity and prevent cloud-forming (Grampp, 1976). The available commercial pectinase preparations used in apple processing generally contain a mixture of pectinesterase (PE), polygalacturonase (PG) and pectinlyase (PL) enzymes (Dietrich *et al.,* 1991). Endo-polygalacturonase and pectinlyase among others have been immobilized on different organic and inorganic supports, with uneven results (Pifferi and Preziuso, 1987; Spagna *et al.,* 1995). Enzyme immobilization by physical adsorption is a simple and well established technique (Gekas, 1986; Szaniawski, 1996). However, immobilized pectinase enzymes are not currently available commercially. In view of the high molecular weight and viscosity of pectin, the use of immobilized pectinase in most fruit processing applications may be rather limited (Kulp, 1975). Despite the different types of supports and reactor configurations proposed for a continuous performance of enzymatic reaction, immobilization of enzymes on micro, or ultrafiltration membranes, appear as interesting alternatives for treating cloudy fruit juices (Alkorta *et al.,* 1995).

Table 1: Some biochemical properties of fungal polygalacturonases

Fungal source	PG form	pH optimum	Mw (kDa)	pI	Reference
Aspergillus niger	II	3.8 – 4.3	61	-	(Tuttobello and Mill, 1961)
	IV	3 – 4.6	38	-	
Aspergillus niger	Exo I	-	82	-	(Kester and Visser, 1990)Rao *et*
	Exo II	-	56	-	*al.*, 1996)
Aspergillus awamori	I	-	41	6.1	(Nagai *et al.*, 2000Gainvors *et al.*, 2000)
Aspergillus carbonarius	I	4.0	61	-	(Devi and AppuRao, 1996)
	II	4.1	42	-	
	III	4.3	47	-	
Aspergillus tubingensis	Exo PG	4.2	78	3.7 - 4.7	(Kester *et al.*, 1996)Singh and AppuRao, 2002)
Aspergillus kawachii	I	2 - 3	60	3.55	(Contreras Esquivel *et al.*, 2004) (Sakamoto *et al.*, 2002)
Aspergillus ustus	I	5.0	36	8.2	(Rao and Kembhavi, 2000)Kester and Visser, 1990)
Botrytis cinerea	Exo I	5	65	8.0	(Cabanne and Doneche, 2002)
	II	5.2	52	7.8	(Nagai *et al.*, 2000)
Fusarium oxysporum f. sp. *lycopersici*	I & II	5	37	-	(Semenova *et al.*, 2003)
Fusarium oxysporum	I	-	35	8.3	(Garcia-Maceira *et al.*, 2001) (Kester *et al.*, 1996)
Fusarium oxysporum f. sp. *lycopersici*	Exo PG2	5	74	4.5	(Di Pietro and Roncero, 1996) (Contreras Esquivel *et al.*, 2004)
Fusarium moniliforme	I	5	36	8.1	(Niture *et al.*, 2001) (Cabanne and Doneche, 2002)
Kluyveromyces marxianus	I	4	41.7	-	(Serrat *et al.*, 2002) (Strand *et al.*, 1976)
Mucor circinelloides	-	5.5	65	-	(Pahwa *et al.*, 2010) (Garcia-Maceira *et al.*, 2001)
Penicillium frequentans	I	3.9	74	4.2	(dos Santos *et al.*, 2002)
Penicillium frequentans	I	4.0 - 4.7	20	5.6	(De Fatima Borin *et al.*, 1996)
Postia placenta	I	3.2 - 3.9	34	3.3	(Clausen and Green, 1996)
Phytophthora parasitica	I	-	39.2	5.2	(Yan and Liou, 2005)
Rhizoctonia fragariae	I	-	36	6.76	(Cervone *et al.*, 1977)
	II	-	36	7.08	
Rhizopus oryzae	I	4.5	31		(Saito *et al.*, 2002)
Sclerotinia borealis	I	4.5	40	7.5	(Takasawa *et al.*, 1997)
Sclerotinia sclerotiorum	I	-	42	4.8	(Martel *et al.*, 1998)
	II	-	41.5	4.8	
Sclerotinia sclerotiorum	Exo I	5	60	-	(Riou *et al.*, 1992)
Saccharomyces cerevisiae	I	3 - 4.5	42	-	(Gainvors *et al.*, 2000)
Saccharomyces cerevisiae	I	5.5	65	-	(Blanco *et al.*, 1994)
Thermomyces lanuginosus	I	5.5	59	-	(Kumar and Palanivelu, 1999)
Thermoasus aurantiacus	I	5.5	30	-	(Martins, 2007)
Trichoderma harzianum	II	5.0	31.0	4.5	(Mohamed *et al.*, 2006)

Table 2: A generalized characterization of immobilization methods

Parameter	**Immobilization method**				
	Carrier binding				
	Covalent	Ionic	Adsorption	CLEAs, CLECs	Entrapment
Activity	High	High	Low	Intermediate/ High	High
Range of application	Low	Intermediate	Intermediate	Low	Intermediate/High
Immobilization efficiency	Low	Intermediate	High	Intermediate	Intermediate
Cost	Low	Low	High	Intermediate	Low
Preparation	Easy	Easy	Difficult	Intermediate	Intermediate/ Difficult
Substrate specificity	Can not be changed	Can not be changed	Can be changed	Can not be changed	Can be changed
Regeneration	Possible	Possible	Impossible	Impossible	Impossible

Table 3: Half-life of pectinase, xylanase and cellulase in CLEAs

Temperature(°C)	**Enzyme**	**Half life($t_{1/2}$) (minutes)**	
		Free	CLEAs
50	Pectinase	17	180
60	Xylanase	22	82
70	Cellulase	32	9

The use of pectinase immobilized on ultrafiltration membranes is expected to hydrolyze the pectin to lower molecular weight species (mainly anhydrogalacturonic acid, AGA) at the membrane-permeate interface, resulting in an increase of the permeate flux or at least an extension of the membrane operation without cleaning.

Mash Treatment

Enzymatic mash treatment is a well-known modern process for gaining more juice from fruits and vegetables. According to the technique, cell wall and middle-lamina pectin of the fruit are degraded by pectinase activities. Besides increasing press capacity and the yield of juice up to 20%, it has also a positive effect to achieve high carotene and dry matter content of the product. The aim of the research was to investigate the activity and reusability of immobilized commercial pectinase named as Pectinex Ultra SP-L on carrot puree. Immobilization process was carried out by using ion exchange resin particles. An average yield increment was 30.23% with respect to the yield obtained from non-enzymic processed carrot juice (Demir *et al.,* 2001).

Papermaking Industries

To find wide application of enzymes in lowering PGA concentration in papermaking industries, cross-linked chitosan beads were prepared. Results showed that the PGA-absorption capability of chitosan

beads was greatly affected by its cross-linking degree. The activity of immobilized pectinase on cross-linked chitosan beads were also investigated and the highest activity of binary immobilized pectinase on cross-linked chitosan was achieved using 1.00% of activating reagent or 0.005% of glutaraldehyde. Cationic demand of PGA solutions was obviously lowered by increasing the temperature of enzymatic treatment (Liu *et al.,* 2010).

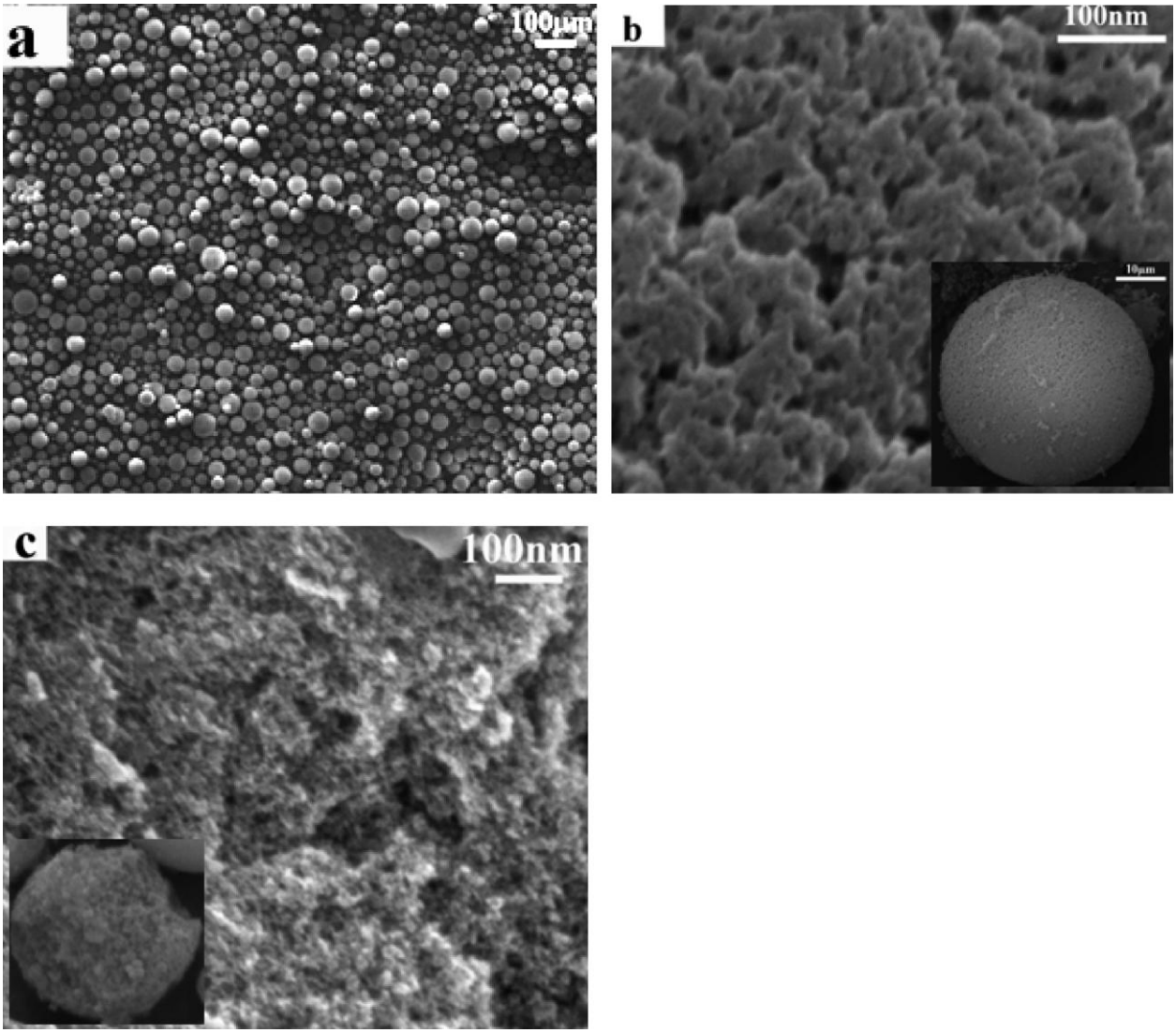

Figure 1: SEM (Scanning electron microscope) images of (a) the holistic morphologies of the PAM macroporous microspheres, (b) the surface morphologies of the PAM macroporous microspheres, and (c) the internal morphologies of the PAM macroporous microspheres

Production of Short Chain Fructooligosaccharides (FOS):

Functional foods as prebiotic fructooligosaccharides have become important and their significance has risen recently because of their favourable properties shown in human and animal nutrition as health foods and special feed additives (Yun, 1996). They have advantageous effects on the intestinal bacterial population and the general health conditions in the body (Bornet *et al.,* 2002; Tuony *et al.,* 2003). FOS are nondigestible oligosaccharides (NDO) and they are not decomposed in the small intestine by the

digestive enzymes, so they reach the colon where they are fermented by the microbial flora (e.g. *Bifidobacteria* sp., *Lactobacillus* sp.) to lactic acid and short chain fatty acids. Consequently, FOS stimulates the growth and fermentation of these microbes and decrease pH in the colon, inhibiting the growth of harmful pathogens (Losada and Olleras, 2002). In addition, they have low sweetness intensity, their caloric value is low, approximately 8–9 kJ g^{-1} (Durieux *et al.*, 2001) and because they avoid the digestion in the upper intestine, they cause no caries. These properties make them applicable as raw materials of diabetic products (Kaplan and Hutkins, 2000). FOS is natural components of many vegetables, for example onion, asparagus, rice, sugar beet, wheat, etc. The industrial scale recovery from these plants is not economical since their concentration is low. For this reason, FOS is produced commercially via biosynthetic as well as hydrolytic methods. The raw material of the biosynthetic way is sucrose; the process is catalyzed by fructosyl-transferase (Yun, 1996). The partial hydrolysis of inulin is also used practically to produce fructooligosaccharides (Kaur and Gupta, 2002). Pectinex Ultra SP-L, a commercial pectinase with fructosyl-transferase (FTF) activity, is able to catalyze the production of short chain fructooligosaccharides (FOS). It was immobilized onto an anion exchange resin by a combined method (Csanadi and Sisak, 2006).

Conclusion

Hopefully, it is clear from this review that the subject of pectinase immobilization continues to attract considerable attention from researchers in both industry and academia. Most of the studies performed so far on immobilization of pectinases resulted in applications of these immobilized enzymes in apple juice clarification, mash treatment and in paper making industries. These examples are just a few of the many ways enzymes touch our lives so more work is needed on this topic which is going on in many institutes and industries.

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