CHAPTER 2

LITERATURE REVIEWS

2.1 Microbial exopolysaccharides

Long-chain, high-molecular-mass polymers which dissolve or disperse in water to give thickening or gelling properties are indispensable tools in food product formulation. Such food polymers are also used for secondary effects including emulsification, stabilization, suspension of particulate, control of crystallization, inhibition of syneresis (the release of water from processed foods), encapsulation, and film formation (Roller and Dea, 1992). Most of the biothickeners in current used in food industries are polysaccharides from plants (e.g. starch, pectin, locust bean gum, guar gum) or seaweeds (carrageenan, alginate). The animal proteinaceous hydrocolloids gelatin and casein are also used (de Vuyst *et al.*, 2001). The functional properties of these polymers in foods are determined by quite subtle structural characteristics. However, these polysaccharides may not always

be readily available in the quality needed or their rheological properties may not exactly match those required (Roller and Dea, 1992; Tombs and Harding, 1998).

Most of plant carbohydrates used are chemically modified to improve their structure and rheological properties. Their use is hence strongly restricted (Tombs and Harding, 1998).

An alternative class of biothickeners is microbial exopolysaccharides (EPS). Microbial EPS are extracellular polysaccharides, which are either associated with the cell surface in the form of capsules or secreted into the extracellular environment in the form of slime. They are referred to as capsular or slime EPS, respectively (Sutherland, 1985; 1990). EPS occur widely among bacteria, microalgae, yeast and fungi (Table 2.1).

EPS in their natural environment are thought to play a role in the protection of the microbial cell against desiccation, phagocytosis and phage attack, antibiotics or toxic compounds (e.g. toxic metal ions, sulfur dioxide, ethanol), predation by protozoans, osmotic stress, adhetion to solid surfaces and biofilm formation, and also in cellular recognition (Cerning, 1990). It is not likely that EPS serve as a food reserve, since most slime-forming bacteria are not capable of catabolizing

the EPS they produce (Cerning, 1990). In pathogenic bacteria, such as *Streptococcus pneumoniae* and *Streptococcus agalactiae*, capsular EPS and O-antigen lipopolysaccharides are involved in the immune response (Cerning, 1990).

Table 2.1 EPS from microorganisms

Microorganism	**Strain**	EPS forming	**Reference**
Bacteria	*Lactobacillus plantarum* EP56	Trisaccharide	Tallon *et al.* (2003)
	Agrobacterium radiobacter	Xanthan	McKellar *et al.* (2002)
	Lactobacillus rhamnosus KL37C	Pentasaccharide	Lipinski *et al.* (2002)
	Lactococcus lactis SK110	Polygalactan	Ruas-Madiedo and Zoon (2002)
	Azetobacter vinelandii	Alginate	Saude *et al.* (2001)
	Burkholderia caribensis MWAP71	Tetrasaccharide	Vanharerbeke *et al.* (2001)
	Xanthomonas campestris	Xanthan	Stredansky and Conti (1999)
Microalgae	*Anabaena sp.* ATCC33047	Octasaccharide	Moreno *et al.* (2003)
	Cyanospira capsulata	Octasaccharide	Garozzo *et al.* (1998)
Yeast	*Rhodotorula bacarum*	Pullulan	Chi and Zhao (2003)
Fungi	*Botryosphaeria* sp.	Botryosphaeran	Aneli *et al.* (2003)
	Cryphonectria parasitica	Trisaccharide	Molinaro *et al.* (2002)
	Guignardia citricarpa	β-galactofuranan	Guilherme *et al.* (2002)

Examples of industrially important microbial EPS are dextrans, gellan, pullulan, yeast glucans and bacterial alginates (Sutherland, 1986; Roller and Dea, 1992; Crescenzi, 1995). Novel microbial biopolymers may fill a gap in the market-available polymers or may replace a traditional product in terms of improving rheological and stability characteristics. Currently, microbial polysaccharides represent only a small fraction of the biopolymer market (de Vuyst *et al.*, 2001). Factors limiting the use of microbial EPS are their production, which requires a thorough knowledge of their biosynthesis and an adapted bioprocess technology, and the high cost of their recovery. Xanthan is a microbial EPS mainly approved in the food industry, mainly because of its unique rheological properties in foods and the possibility of low-cost production. It is produced in high amount by *X. campestris*, a phytopathogenic bacterium which is not generally recognized as safe (GRAS) (de Vuyst *et al.*, 2001). Recently, gellan from the phytopathogen *Sphingomonas elodea* has been introduced in the market (de Vuyst *et al.*, 2001).

However, the strains of GRAS, food grade micro-organisms, in particular lactic acid bacteria (LAB), that are able to produce EPS in large enough quantities could be the solution to many of the above-mentioned disadvantages (de Vuyst *et al.*, 2001).

2.2 Exopolysaccharides from lactic acid bacteria

Lactic acid bacteria (LAB) are food grade microorganisms, which can produce EPS (Cerning, 1995). Most of the EPS-producing LAB strains studied in more detail were isolated from dairy products (Table 2.2).

Table 2.2 EPS-producing LAB

Microorganism strains	Source	EPS forming	EPS yield (g/L)	Reference
Lactobacillus reuteri LB121	Naterland's fermented food	Levan	9.8	van Geel-Schutten *et al.* (1999)
L. lactis subsp. *cremoris* NIZO B40	Naterland's fermented food	Pentasaccharide	0.0085	Petronella *et al.* (1999)
Leuconostoc mesenteroides	Sugar beets	Dextran	8.7	Tallgren *et al.* (1998)

Table 2.2 (continue)

Microorganism strains	**Source**	EPS forming	EPS yield (g/L)	Reference
Lactobacillus delbrueckii subsp. *bulgaricus*	Homemade fermented milk	Heptasaccharide	0.17	Petry *et al.* (2000)
Lb. delbrueckii subsp. *bulgaricus* RR	Yoghurt	Heptasaccharide	0.354	Kimmel *et al.* (1997)
Lb. delbrueckii subsp. *bulgaricus* NCFB 2772	Yoghurt	Heptasaccharide	0.055	Grobben *et al.* (1998)
Streptococcus thermophilus LY03	Yoghurt starter culture	Heptasaccharide	0.356	Degeest and de Vuyst (1999)
S. thermophilus Sfi12	Nestle's yoghurt	Hexasaccharide	0.105	Lemoine *et al.* (1997)
Lactobacillus sp. LM17	Kefir grains	Kiferan	2.00	Micheli *et al.* (1999)

2.2.1 Classification of EPS from LAB (de Vuyst and Degeest, 1999)

EPS from LAB can be subdivided into two groups :

(1) Homopolysaccharides

Homopolysaccharides, which are produced from LAB, contain only one type of monosaccharide such as fructose or glucose. In fact, most of these homopolysaccharides share the feature of being synthesized by extracellular glycansucrases using sucrose as the glycosyl (fructose or glucose) donor (Fig 2.1). The homopolysaccharide consists of three subgroups including α-D-glucans, β-D-glucans and fructans (Table 2.3) (Monsan *et al.*, 2001).

(a) α-D-glucans, it mainly composes of α-1,6-linked glucose residues with variable (strain specific) degrees of branching at position 3, and less frequently at positions 2 and 4, such as alternan from *Leuc. mesenteroides* and mutans from *Streptococcus mutans* and *Streptococcus sobrinus* both are composed of α-1,3- and α-1,6-linkages.

(b) β-D-glucans, it composes of β-1,3-linked glucose molecules with β-1,2-branches, produced by *Pediococcus* sp. and *Streptococcus* sp.

(c) Fructans, it mainly composes of β-2,6-linked D-fructose molecules, such as levan with some β-2,1-branching through the O1 site (*Streptococcus salivarius*)

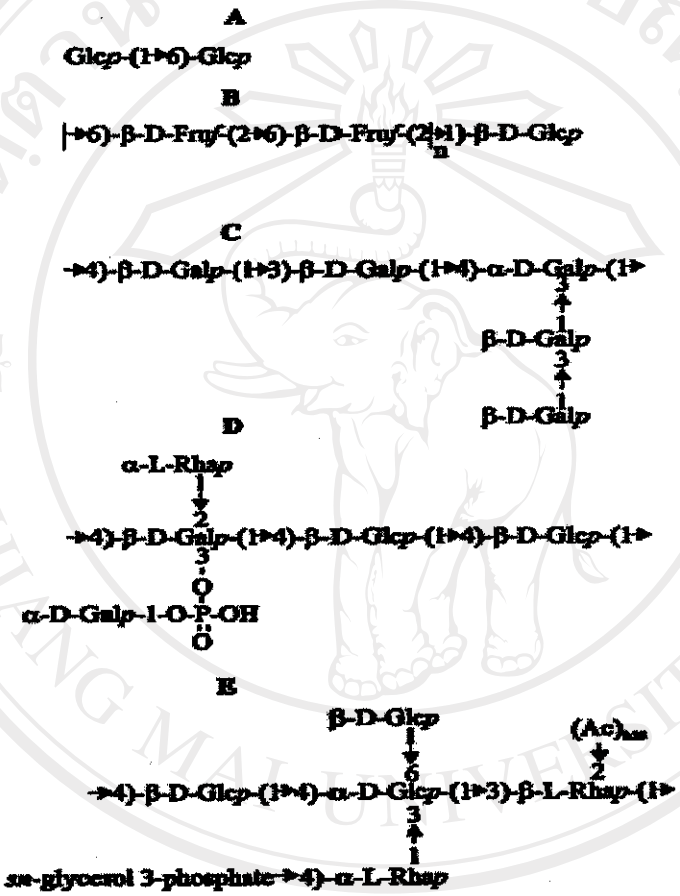


Fig. 2.1 Primary structure of EPS produced by LAB. 1) Homopolysaccharides : A, dextran ; B, levan ; C, polygalactan from *L. lactis* subsp. *cremoris* H414. 2) Heteropolysaccharides from mesophilic LAB : D, from *L. lactis* subsp. *cremoris* SBT 0495 ; E, from *Lb. sake* 0-1 (de Vuyst and Degeest, 1999).

Table 2.3 Classification of EPS from LAB

Subdivision of EPS	Subgroup of EPS	Strain	Reference
Homopolysaccharide	α-D-glucan	*Leuc. mesenteroides* B-512F	Soetaert *et al.* (1995)
		Streptococcus downei Mfe28	Mooser (1992)
		S. mutans GS5	Mooser (1992)
	β-D-glucan	*Lactobacillus* subsp. G-77	Duenas-Chasco *et al.* (1997)
		Pediococcus damosus 2.6	Duenas-Chasco *et al.* (1997)
		Streptococcus sobrinus	Abo *et al.* (1991)
	Fructans	Bacillus subtilis	Lebrun and van Rapenbush (1980)
		Streptococcus salivarius	Giffard *et al.* (1988)
		L. reuteri LB21	Van Geel-Schutten *et al.* (1999)
Heteropolysaccharide	Trisaccharide	*Lactobacillus* subsp. G-77	Duenas-Chasco *et al.* (1998)

Table 2.3 (continue)

Subdivision of EPS	Subgroup of EPS	Strain	Reference
Heteropolysaccharide	Tetrasaccharide	*S. thermophilus* Sfi39	Lemoine *et al.* (1997)
		Lactobacillus paracasei 34-1	Robijn *et al.* (1996)
		S. thermophilus Sfi 20	Navarini *et al.* (2001)
	Pentasaccharide	*Lb. rhamnosus* C83	Vanhaverbaka *et al.* (1998)
		Lb. lactis subsp. *cremoris* NIZO B891	Van Casteren *et al.* (2000)
		Lactobacillus acidophilus LMG 9433	Robijn *et al.* (1996)
	Hexasaccharide	*Lactobacillus helveticus* Lh59	Stingele *et al.* (1997)
		Streptococcus macedonicus Sc136	Vincent *et al.* (2001)
		S. thermophilus Sfi12	Lemoine *et al.* (1997)

Table 2.3 (continue)

Subdivision of EPS	Subgroup of EPS	Strain	Reference
Heteropolysaccharide	Heptasaccharide	*Lb. delbrueckii* subsp. *bulgaricus* LY03	Marshall *et al.* (2001)
		S. thermophilus NCFB 2393	Almiron-Roig *et al.* (2000)
		L. lactis subsp. *cremoris* NIZO B39	Van Casteren *et al.* (2000)
	Octasaccharide	*S. thermophilus* MR-1C	Low *et al.* (1998)

(2) Heteropolysaccharides

Heteropolysaccharides are produced by mesophilic and thermophilic LAB strains (Table 2.3). They are composed of a backbone of repeated subunits, which are branched (at positions C2, C3, C4, or C6) or unbranched, and that consisted of three to eight monosaccharides, derivatives of monosaccharides or substituted monosaccharides (Fig 2.1). The monosaccharides are present as the

α- or β-anomer in the pyranose or furanose form. The structure of the repeating unit of a LAB heteropolysaccharide produced by *S. thermophilus* was firstly determined by Doco *et al.* (1990).

Many different types of heteropolysaccharide are secreted with respect to sugar composition and molecular mass, varying from 1.0×10^4 to 6.0×10^6 (Cerning, 1995 ; de Vuyst and Degeest, 1999). For instance, the constituting monosaccharides seem to be remarkably similar: D- galactose, D-glucose, and L-rhamnose are almost always present in different ratios. Tables 2.3 shows the variability in heteropolysaccharide produced by LAB with known structure.

2.2.2 Biosynthesis of EPS by LAB

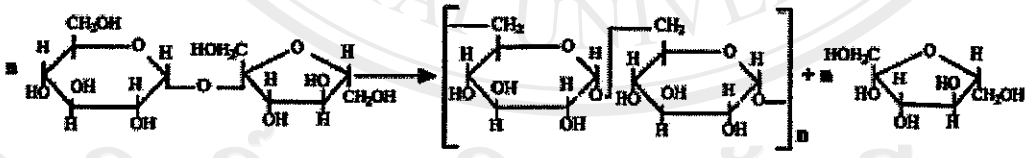
EPS-producing LAB, including the genera Streptococcus, Lactobacillus, Leuconostoc, and Lactococcus, produce a wide variety of structurally different polymers (de Vuyst and Degeest, 1999). The polymerization reaction in these cases proceeds via extracellular glycosyltransferases, which transfer a monosaccharide from a disaccharide to a growing polysaccharide chain. The other categories comprise homo- and hetero-polysaccharides with irregular repeating units which are

The EPS monomeric composition may not only be dependent on the sugar nucleotide level inside the cell, but probably also on the assembly of the EPS repeating unit. Polymerization of some hundreds to several thousands of the repeating unit takes place through sequential addition of sugar residues by specific glycosyl transferases from nucleotide sugar to a growing repeating unit which is coupled to the undecaprenyl phosphate carrier yielding the final EPS (Fig. 2.3). This isoprenoid glycosyl lipid carrier located in the cell membrane would act as the recipient molecule for the first sugar residue. In contrast with Gram-negative bacteria, there is only preliminary evidence for the existence of this lipid carrier in LAB (van Kranenburg *et al.*, 1998).

As the last step of EPS biosynthesis, the synthesized polysaccharide is translocated across the membrane to the exterior of the cell and is excreted in the environment (i.e. slime EPS) or remains attached to the cell (i.e. capsular EPS). Both polymerization and transportation may affect the amount or the sugar composition of EPS (de Vuyst and Degeest, 1999).

synthesized from intracellular sugar nucleotide precursors. Some of these sugar nucleotides serve as precursors for EPS biosynthesis (de Vuyst and Degeest, 1999).

Small numbers of homopolysaccharides were dextrans, mutans, alternan and levans. The biosynthesis process is extracellular and requires the specific substrate sucrose (Fig. 2.2). A highly specific glycosyl transferase enzyme (e.g. dextran sucrase or levan sucrase for dextran and levan biosynthesis, respectively) is involved in the polymerization reaction. The energy needed for polymerization comes from the hydrolysis of sucrose. The polysaccharide can be produced using either whole bacterial cell cultures or enzyme preparations (de Vuyst and Degeest, 1999).



n sucrose dextran *n* fructose

Fig. 2.2 Biosynthesis of the homopolysaccharide dextran (de Vuyst and Degeest, 1999).

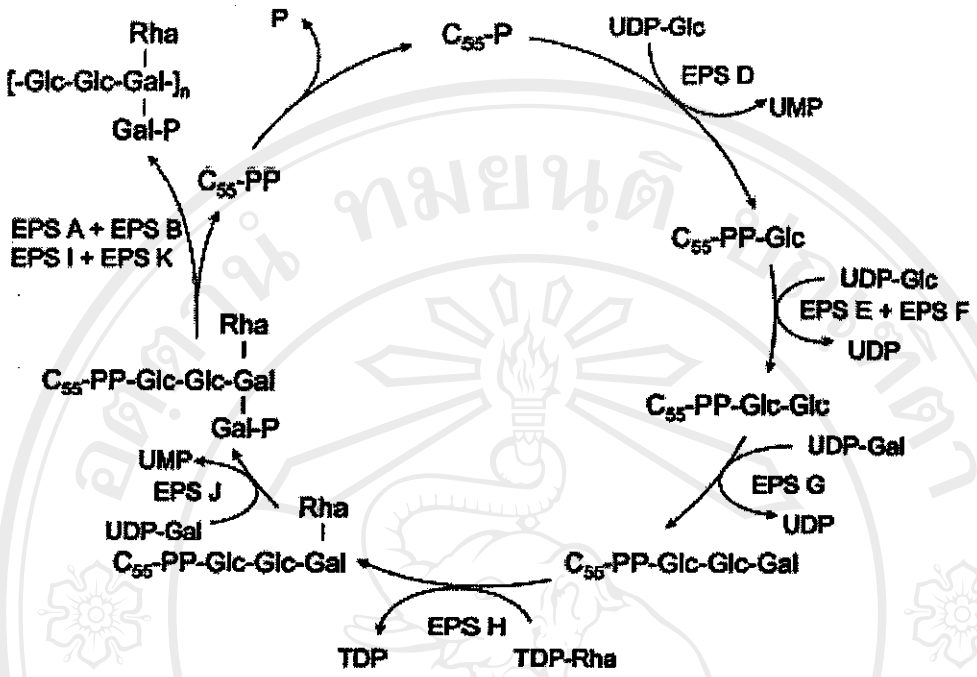


Fig. 2.3 Model for EPS biosynthesis in *L. lactis* subsp. *lactis* NIZO B40. C55-P, lipid carrier; Glc, glucose; Gal, galactose; Rha, rhamnose; UDP-Glc, UDP-Gal and TDP-Rha are nucleotide sugars (van Kranenburg *et al.*, 1998).

2.3 Factors affecting EPS production

Most EPS producing bacteria produce their products under suitable conditions but the quantity and the composition of EPS are affected by fermentation conditions and medium composition (Sutherland, 1972). Some factors affecting EPS production are summarized in Table 2.4.

Table 2.4 Factors affecting EPS production

Strain	Product	Factor	Optimal value	Yield	Reference
Rhodotorula bacarum	Pullulan	Glucose concentration	20 g/L	7.5 g/L	Chi and Zhao (2003)
		Soybean concentration	5 g/L		
		Incubation time	60 h		
Gyrodinium impudicum KG 03	Sulfated polysaccharide	Vitamin B_{12}	0.75 μg /L	0.134 g/L	Yim *et al.* (2003)
		Plant growth hormone	7.5 μg /L		
		Incubation temperature	22.5 °C		

Table 2.4 (Continue)

Strain	Product	Factor	Optimal value	Yield	Reference
L. lactis subsp. *cremoris* NIZO B40	EPS	Incubation temperature	25 °C	0.52 g/L	Looijesteijn and Hugenholtz (1999)
		Initial pH	5.8		
Agrobacterium radiobacter	EPS	Sucrose concentration	26.2 g/L	2.26 g/L	Triveni *et al.* (2001)
		Inoculum concentration	10 g/L		
		Initial pH	6.24		
Azotobacter vinelandii MTCC2460	Alginate	Sucrose concentration	20 g/L	3.2 g/L	Meenakshi *et al.* (1995)
		Initial pH	5.0		
		Inoculum concentration	20 %		
		Incubation temperature	34 °C		

2.4 Applications of EPS from LAB

The discovery of the EPS-producer *Leuc. mesenteroides* in 1878, responsible for the thickening and gelling of sugar beet and sugar cane syrup, can be considered as the start of the possible use of EPS from LAB in food products (de Vuyst and Degeest, 1999). The use of xanthan from *X. campestris*, the firth microbial polysaccharide that was allowed in food products, was approved by the US Food and Drug Administration in 1969 (de Vuyst and Degeest, 1999). Recently, gellan from *S. elodea* has also been commercialized (de Vuyst and Degeest, 1999). EPS from LAB, which are GRAS bacteria, may form a new generation of biothickeners (de Vuyst and Degeest, 1999).

The polysaccharide is incorporated into foods to alter the rheological properties of the water present, and has found applications, which take advantage of many of its physical properties (Table 2.5). EPS have many other novel properties to offer and the discovery of immune modulation and tumouristasis by β–D-glucans and the use of bacterial cellulose in audio membranes. Semisynthetic polymers and polysaccharides are the source of oligosaccharides (Sutherland, 1998).

Table 2.5 Application of microbial EPS

Properties	Use	Polymer
Biological properties	Antitumour agents	β-D-glucan
	Eye and joint surgery	Streptococcus EPS
	Heparin analogues	*E. coli* K5 EPS
	Wound dressings	Bacterial cellulose
Chemical properties	Enzyme substrates	*E. coli* K4 and K5 EPS
	Oligosaccharide preparation	Curdlan, Pullulan, Scleroglucan
Physical properties :		
Emulsion stabilization	Foods, thinxotropic paints	Xanthan
Fibre strength	Acoustic membranes	Bacterial cellulose
Film formation	Food coatings	Pullulan
Floculant	Water clarification	Various
Foam stabilization	Beer, Fire-fighting fluids	Xanthan
Gelling agents	Cell and enzyme technology	Gellan
	Foods	Curdlan, Gellan
	Oil recovery	Curdlan, Xanthan
Hydrating agent	Cosmetics, Pharmaceuticals	Streptococcus EPS
Inhibitor of crystal formation	Frozen foods, Sugar syrups	Xanthan

Table 2.5 (continue)

Properties	Use	Polymer
Shear thinning and viscosity control	Oil-drilling "muds"	Xanthan
Suspending agent	Food	Xanthan
	Paper coatings	Various
	Agrochemical pesticides and sprays	Xanthan
Viscosity control	Jet printing	Xanthan

From : Sutherland, 1998

2.5 *Pediococcus urinae-equi*

Pediococci are lactic acid bacteria (LAB) which divide alternately in two perpendicular directions to form tetrads. They are invariably spherical and produce lactic acid without gas from glucose. The genus is heterogeneous and includes organisms, which is able to grow in beer and those active during soya sauce manufacturing (Sneath, 1989).

P. urinae-equi produces less lactic acid than other pediococci. The use of this strain has no report.

Cells of *P. urinae-equi* are 0.8 – 1.0 μm in diameter (Fig. 2.4), occurring singly, in pairs, tetrads or irregular clusters. On agar medium, they form circular colonies, 1 – 2 mm in diameter (Fig. 2.5), which are greyish – white in colour and raised (Sneath, 1989).

Growth of *P. urinae-equi* is generally improved if the initial pH of the medium is alkaline. Optimal pH is between 8.5 and 9 although the growth will take place in media with an initial pH of 6.5 – 7.0 and the final pH is about 5.0. Optimum temperature are found to be 25 – 30 °C. Growth can occur in media which does not contain added carbohydrate (Sneath, 1989).

The mol% G+C of the DNA is 39.5 % (T_m). The species does not appear to be widely distributed and the number of isolations are few (Sneath, 1989).

Stiles and Holzapfel (1996) reported that *P. urinae-equi* had not associated with food. Whereas, Wongroung *et al.* (2002) found that *P. urinae-equi* which was isolated from Thai traditional fermented Pork Sausage can produce EPS from Man, Rogosa and Sharpe (MRS) medium.

Fig. 2.4 The characteristic of *P. urinae-equi* TISTR 1499 *X*1000

Fig. 2.5 *P. urinae-equi* TISTR 1499 colonies on MRS agar medium

2.6 Solid state fermentation by using solid supports

Solid state fermentation (SSF) has shown much promise in the development of several bioprocesses and products. SSF is defined as any fermentation process occurring in the absence or near-absence of free water, employing a natural substrate, or an inert material as solid support (Pandey *et al.*, 1992).

SSF are those processes in which all or parts of the substrate are in a solid state (Hesseltine, 1972). They are more natural than liquid fermentations because they approach the conditions under which most microorganisms grow in the wild. While some groups of microorganisms, such as some bacteria and yeasts, grow in liquid, a large number of other microorganisms grow attached to solid substrates (Pandey *et al.*, 1992).

2.6.1 Type of solid support

In relation to SSF processes using inert substrates, two approaches have been adopted; one in which, natural materials such as sugarcane bagasse are used as the inert solid support and other which synthetic materials such as amberlite or polyurethane are used (Pandey *et al.*, 2000).

2.6.1.1 Agricultural waste as natural solid support

Researches on the selection of a suitable substrate has mainly centered around tropical agro-industrial crops and residues (Table 2.6).

Biotechnological potential of several agro-industrial residues for value addition in SSF has been reviewed recently (Pandey *et al.*, 1992; 2003). It was reported that processes were developed to utilize these as raw materials for the production of bulk chemicals and value-added fine products such as ethanol, single-cell protein (SCP), mushrooms, enzymes, organic acids, biologically active secondary metabolite, including exopolysaccharide (Pandey *et al.*, 1992). It was highlighted that advances in industrial biotechnology offer potential opportunities for economic utilization of agro-industrial residues such as cassava bagasse, sugarcane bagasse, coffee pulp and coffee husk. Due to their rich in organic substance, agro-industrial residues can serve as ideal substrates for microbial processes for the production of value added products. SSF has been mostly employed for bioconversion processes. Application of agro-industrial residues in bioprocesses, on the one hand, provides alternative substrates, and on other, helps in solving pollution problems, which may cause their disposal (Pandey *et al.*, 2000).

Table 2.6 Products by using agricultural waste for SSF

Product	Micro-organism	Substrate
L-glutamic acid	*Brevibacterium* sp.	Sugarcane bagasse
Pigments	*Monascus purfireus*	Sugarcane bagasse
Carotenoid	*Penicillium* sp.	Corn meal
Xanthan gum	*X. campestris*	Apple pomace, Grape pomace, Spent malt grains[a]
Succinoglycan	*Agrobacterium tumefaciens*	Spent malt grains
Ethanol	*Saccharomyces cerevisiae*	Sorghum carob pods, Sugar beet
Aroma compounds	*Rhizopus oryzae, B. subtilis*	Coffee husk, Soy bean
Vitamins B_{12}, B_6, riboflavin	*Citrobacter freundii*	Soy bean
Gamma-linoleic acid	*Cunninghamella japonica*	Cereals
Biosurfactants	*B. subtilis*	Agro-industry residues[a]
Biopesticides / Bioherbicide	Entomopathogenic and mycoparatisitic fungi	Rice flour

[a] As inert solid support

From : Pandey *et al.*, 2000

2.6.1.2 Synthesized material

The inert materials, on impregnation with a suitable medium, provide a homogenous aerobic condition throughout the fermentor, and do not contribute impurities to the fermentation product (Aidoo *et al.*, 1982). It maximal recovery of the productivity with low viscosity and high specificity for the target product (Prabhu and Chandrasekaran, 1995). The polystyrene, a commercially available insulating and packaging material, could be used as an inert solid support for production of enzymes (Sabu *et al.*, 2000). While ion exchange resins (Auria and Benet, 1990), polyurethane foam (Zhu *et al.*, 1994) and computer cards (Madamwar *et al.*, 1989) were also been used as inert carriers for SSF with fungi (Table 2.7). However, the use of polystyrene for EPS production under SSF with bacteria has not been reported.

Table 2.7 Products by using synthesized solid support for SSF

Product	Strain	Solid support	Reference
Extra-cellular phytase	*Aspergillus ficauum* TUBF-1165	Polystyrene beads	Gautam *et al.* (2000)
Caffeine inhibitor	*Aspergillus tamarii* V12A25	Polystyrene foam	Hakil *et al.* (1999)
Mycelial fungi	*Aspergillus niger*	Ion exchange resin	Mariano *et al.* (1998)
Nuclease P1	*Penicillium citrinum*	Polyurethane foam	Yang *et al.* (1996)
Fungal tannase	*Penicillium glabrum*	Polyurethane foam	van de Lagemaat and Pyle (2001)
Cellulase	*A. niger*	Computer card	Madamwar *et al.* (1989)

2.6.2 Factors affecting EPS production under SSF condition

Factors affecting the secretion of second metabolites by microorganism under SSF include selection of solid substrate, level of salt solution, initial moisture content, inoculum level, incubation temperature, incubation time, initial pH, various carbon and nitrogen additives, etc. These factors must be optimized by adopting a search technique (Banerjee and Bhattacharyya, 2003) and varying parameters one at a time.

Baysal *et al.* (2003) studied the initial moisture content, particle size of solid support and inoculum concentration for α-amylase production by *B. subtilis* on SSF. Narang *et al.* (2001) also studied the particle size of solid support, inoculum age, initial moisture content and harvest time for xylanase production by *Melanocarpus albomyces* IIS68 on SSF.

The critical importance of moisture level in SSF media and its influence on the biosynthesis and secretion of product can be attributed to the interference of moisture in the physical properties of the solid particles. Increasing in moisture aeration level is believed to reduce the porosity of the solid support, thus limiting oxygen transfer (Balakrishnan and Pandey, 1996).

On the other hand, the low moisture content causes reduction in the solubility of nutrients and low degree of swelling (Murthy *et al.*,1999).

Gowthanmen *et al.* (1993) reported that increasing the aeration flow rate could improve activity of microbial enzyme when cultured on SSF. Shojaosadati and Babaeipour (2002) reported that increasing in moist-air circulation could improve citric acid yield when cultured in the muti-layer packed bed solid-state bioreactor.

The factors affecting EPS production by microorganism, were substrate moisture, carbon source and nitrogen source (Stredansky *et al.*, 1999).

2.6.3 Advantages and disadvantages of SSF

2.6.3.1 Advantaged of SSF

(1) The support may be very simple, often using agricultural waste (Pandey *et al.*, 2000).

(2) Since less substrate and water are used to produce the product, pollution problems are greatly reduced. Because there is no liquid fraction to be disposed and the residual substrate can be dried easily or shipped wet as a feed (Hesseltine, 1981).

(3) When product such as enzymes or EPS has to be extracted from the fermentation, less solvent is required for recovery. The recovery may be carried out directly in the fermentation vessel (Hesseltine, 1981 and Pandey *et al.*, 2000).

(4) The SSF process can be scaled up to either a large batch or a continuous fermentation (Pandey *et al.*, 2000).

(5) When the solid material is not suspended in liquid, aeration is easier because of the large void spaces between the solid particles (Hesseltine, 1981).

2.6.3.2 Disadvantages of SSF

(1) Probably, the most important disadvantage is the generation of heat which occurs even in small fermentations of only 1 kg. Excess heat must be controlled, otherwise the temperature

becomes so high and destroys the desired microbial product or stops the microbial growth and fermentation completely (Hesseltine, 1987).

(2) If existing equipment does not suffice, it is necessary to develop devices to monitor temperature and to measure pH, O_2 and CO_2 levels, moisture content, and the concentration of desired product (Hesseltine, 1981).

(3) The substrate must be treated to make more easily infected by the inoculum and to increase the surface area (Hesseltine, 1987).

(4) With the concentrated medium, the initial amount of inoculum required may be quite high. It is even higher in a continuous fermentation because inoculum has to be added continuously to the fermentation (Hesseltine, 1981).

(5) For large fermentations requiring agitation, the energy costs may be high. Although the constant agitation of solid material would become quite expensive, it should be recognized that constant agitation might not be the best way to produce a given product (Hesseltine, 1987).

(6) Moisture is a critical and limiting factor in many fermentations because microorganisms may require different moisture levels in strictly solid media (Hesseltine, 1987).

2.7 Concluding remark

In summary, EPS are biothickeners that can be added to a wide variety of food productions, to be served as viscosifying, stabilizing, emulsifying and gelling agents. Where appropriate suggestion is made for production processes, so the SSF was the choice of production process that may contribute to the economic soundness of EPS production such as decreased solvent using during extraction process. Further advantage of SSF is cheap and available. Recently, the SSF for EPS production has been interested and the optimum conditions of SSF for EPS production were studied (Stredansky *et al.*, 1999). However, the moisture content, inoculum level, incubation temperature, incubation time and initial pH are the important factors to be optimized for bacterial growth and EPS productivity.