



APPLICATION OF BIOFILM REACTOR TECHNOLOGY FOR BIOPRODUCTION: A CLOSER LOOK

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Abstract

Applications of customized biofilm reactors have markedly enhanced the productivity of different bioproducts. Implementation of novel concepts in designing of cost effective, durable and commercially scalable substrata has proven their positive impacts on the product features. To make such approaches more generalized in biofilm reactor technology, it is important to highlight the factors that decisively act on the compatibility between microorganisms and solid supports used for different bioproducts. The contents of the review have been strongly oriented towards the broader application of substrata for many bioproducts. Correlations between the variations in the product features and biofilm associated factors have been highlighted. Plastic composite support has been given a special attention. Some of the thermodynamic and interface properties of microorganisms and substrata have been considered. Role of extended Derjaguin, Landau, Verwey, Overbeek theory in assigning the parameters for substrate selection has been discussed. The influence of water structure on the formation of biofilm, and quantitative analysis of physical factors namely adhesion energy, contact angles and primary/secondary minima in selection of substrata have been well addressed. The key issues taken into the consideration and suggestions made in context of the present review can further aid in the customization of biofilm reactor technology.

Keywords

Biofilm reactors, plastic composite support, customization, XDLVO theory, adhesion energy, water structure.

INTRODUCTION

Biofilm reactor (BR) technology has found wider applications in the production of different bioproducts (fine and bulk chemicals, biofuels, organic acids and biomolecules) and wastewater (industrial and municipal origin) treatment processes (Cheng et al., 2010; Qureshi et al., 2005; Wang and Sang, 2009; Straathof et al., 2002; Lazarova and Manem, 1995, 2000; Martin and Nerenberg, 2012). The biological means of wastewater management dates back to 1940, when trickling filters were first introduced at industrial scale in UK (Mishra and Sutton, 1991). In the practice of wastewater engineering, design and operation of trickling filters are now well established (Metcalf and Eddy, 1991). Both fixed- medium system and moving-medium system based BRs have been successfully applied in wastewater treatment for various purposes. In particular, rotating biological contactors (RBC) are the most widely and effectively used moving-medium system type BRs in

wastewater treatment (reduction in the level of chemical oxygen demand (COD)/biological oxygen demand (BOD) and nitrification/denitrification) (Kargi and Dincer, 1999; Kargi and Ekar, 2001; Gonce and Harremoes, 1985). Since the first commercial-scale application of Biofilm Fluidized Bed (BFB) in the mid-1970s in USA, particulate BRs of different configurations (biofilm upflow sludge blanket, biofilm fluidized bed, expanded granular sludge blanket, biofilm airlift suspension, and internal circulation reactors) have been designed and experimented for lab and large scale wastewater treatment processes in the last two decades (Metcalf and Eddy, 1991). Metabolic efficiencies of different microbial strains has facilitated the use of wide spectrum of cheap and renewable carbohydrate sources in the production of biofuels employing BR technology. Effluents from dairy industries have been utilized as alternative substrates for butanol and acetone-butanol-ethanol production (Jones and Wood, 1986). The newly emerged plastic composite support

(PCS) technology has successfully designed substrata for biofilm development utilizing agro based waste products (Kunduru and Pometto, 1996). Moreover, industrial waste gases (organic and inorganic) can also be utilized as sources of energy or carbon for microbial metabolism (Ottengraf, 1987).

Novel concepts are being introduced to meet the challenges of limiting parameters of biofilm reactor based production and this has led to the custom designing of BRs (Wood et al., 2001). In addition, extensive molecular study on biofilms of different microbial origin has technically revolutionized the designing of BRs. Concept of designing an air-membrane surface (AMS) bioreactor for the production of secondary metabolites (bacitracin and a red pigment) by *Bacillus licheniformis* strain EI-34-6 is an ideal example of BRs configuration based on molecular aspects of biofilms. The bacitracin molecule played a role of biofilm-specific inducer causing the formation of biofilm at air-membrane interface (Yan et al., 2003). Customization of rotating disc biofilm reactor (RDBR) is another such approach made for *Streptomyces* sp. MS1/7 for the production of antimicrobial compounds (Sarkar and Mukherjee, 2010). The basic outcome of the newly emerged BR technology is the exploitation of microbes for quality enhancement of different products.

However, designing of biofilm reactor and optimization of process parameters seems to be suffering from narrowing down its application. The extent to which the advantages/disadvantages can be generalized for a particular biofilm reactor must be addressed properly. For biofilm forming microbes, change in external stimuli causes drastic physiological, spatial and structural changes of the concerned biofilm. As any alternation in the biofilm directly influences the overall performance of a biofilm reactor, the pros and cons can be predicted for an application of different BRs for a particular product provided that the quality controlling factors are kept optimum. Present review encompasses most of important BR technology based bioproducts for analyzing pros and cons of the contemporary production processes and emphasizes on the need of further customizations and generalizations of the techniques.

2. Biofuel

2.1. Ethanol

Production of ethanol either with *S. cerevisiae* or *Z. mobilis* in BRs of different configurations has been very productive (Table 2). However, at production and compatibility levels, *Z. mobilis* has proven to be more efficient than *S. cerevisiae*.

2.1.1. Ethanol production and *S. cerevisiae*

The ability to form biofilm by *S. cerevisiae* and its responses under different culture conditions affects productivity of ethanol. In broader sense, adaptability of the yeast to technically different BRs is controlled by all those factors that can cause functional or structural changes of the biofilm. Reynold and Fink (2001) in their investigation on molecular

aspects of biofilm formation by *S. cerevisiae*, traced out the key factors influencing the establishment of biofilm on a substratum. These factors and their role in favouring or disfavoring the biofilm formation have been tabulated in Table 3. Glucose concentration vs. adherence ability, controlling over expression level of the yeast gene FLO11, role of nitrogen concentration in determining the phenotype (multicellular pseudohyphae or mat type) of biofilm, importance of sliding motility, Ploidy condition and possible exploitation of the mutant strain flo 11 Δ have been well elaborated in the article. This information is important to justify and explain the adoption of a new biofilm reactor technology of ethanol production employing *S. cerevisiae*. Emphasis should be given to sticking to the basic principles of biofilm formation for a microorganism used in fermentative biology while designing a model biofilm reactor.

2.1.2. Ethanol production and *Z. mobilis*

Z. mobilis is an ethanologenic and biofilm forming bacterium. Kunduru and Pometto (1996) demonstrated that *Z. mobilis* biofilms could be used in ethanol fermentation [Table 2]. However, formation and morphology of the *Z. mobilis* biofilms were not characterized in this study. The major findings on the different aspects of the biofilm of this bacterium were revealed by Li et al. (2006). They found that *Z. mobilis* cells are capable of forming a biofilm comprised of microcolonies with an average thickness of 20 μ m embedded in extracellular polysaccharide (EPS) and interspersed with open water channels. The experiment was carried out in a hydrophobically treated 3-mm glass beads packed biofilm reactor. Although the study was undertaken to examine the potential of surface-associated biofilms for biotransformation of chemicals into value-added products and benzyldehyde tolerance taking *Z. mobilis* as model organism, the findings can be implemented for different biofilm reactor based ethanol production. The authors mentioned about the possible role of alternation in gene expression resulting in physiological and/or structural changes during biofilm formation making the biofilm more resistant to benzyldehyde. For ethanol production, *Z. mobilis* has been well exploited using packed bed reactor (PBR), fluidized bed reactor (FBR) and expanded bed BRs [Table 2]. The different substrates used favored the establishment of *Z. mobilis* biofilms. The high ethanol tolerance level enhanced productivity of ethanol in plastic composite support (PCS) and high amenability to genetic manipulation of *Z. mobilis* becomes more understandable if considered as biofilm regulated processes.

2.2. Butanol

Compared to ethanol, application of different BRs for the production of butanol using either *Clostridium acetobutylicum* or *Clostridium beijerinckii* is more confined. PBR in particular, has been explored with different substrata resulting in marked variation in the productivities of butanol (Table 4). Bonechar has been found to be the best adsorbent for *C. acetobutylicum*. Due to the high compatibility between the innate properties of the organism and the substratum,

Table 1: Application and common advantages of conventional and customized BRs in the production of different bioproducts.

Biofilm reactor type	Conventional or Customized BR type	Products	Common advantages	Ref
Trickling bed reactor (TBR)	Conventional type	Acetic acid, Hydrogen	High cell concentration and productivity.	Kargi and Eker, 1999; Hekmat et al., 2007; Li et al., 2006; Qureshi et al., 2004; Krug and Daugulis, 1983; Qureshi and Maddox, 1987, 1998; Welsh et al., 1987; Zhang et al., 2004; Ho et al., 1997; Urbance et al. 2003, 2004; 33.Weusterbotz et al., Sanroman et al., 1996; Srivastava and Kundu, 1999; Cao et al., 1996; Wang, 2007; Tay and Yang, 2002
Packed bed reactor (PBR)		Dihydroxyacetone, Benzylalcohol, Ethanol, Butanol, Acetone+butanol, Ethanol+Butanol+Aetone, Poly(3-hydroxubutyrate), Lactic acid, Succinic acid		
Fluidized bed reactor (FBR)		Ethanol, Citric acid		
Airlift reactor (ALR)		Cephalosporin C,		
Membrane bed reactor (MBR)		Cellulose		
Rotating disc contactor (RDC)		Fumaric acid, Citric acid, Lactic acid,		
Membrane aerated biofilm reactor (MABR)	Customized type	Styrene-oxide	Substrate/product solubility and toxicity can be overcome.	Halan et al., 2010; Gross et al., 2010; Cotton et al., 2001; Cheng et al., 2010; Yang et al., 2003; Sarkar and Mukherjee, 2010; Jeremiasse et al., 2010; Logan et al., 2008; Rabaey et al., 2010; Cheng et al., 2009; 49.Steinbusch, et al., 2010; Nevin et al., 2010.
Segmented flow biofilm reactor (SFBR)	[(S)-styrene oxide]	Direct oxygen transfer, no mass transfer barrier and no excessive biofilm growth.		
Solid support membrane-aerated biofilm reactor (SMABR)	Enantiopure (S)-styrene oxide	High oxygen transfer rate		
Plastic composite support biofilm reactor (PCSBR)	Lactic acid, Bacterial cellulose, Pullulan	Shortened the product formation lag phase		
Rotating disc biofilm reactor (RDBR)	Bacitracin, Other anti-microbial compounds	Highly mimicking of the natural environmental growth conditions		
Electro-active biofilm reactor (EABR)	Biohydrogen, H ₂ O ₂ , Methane, Ethanol, Caustic soda, multi-carbon organic compounds	Can be combined to a microbial fuel cell (MFC)		

Table 2: Performances of different BRs in the production of ethanol.

BR type	Year of Reporting	Maximum productivity obtained (g l ⁻¹ h ⁻¹)	Organism used	Substratum	Ref
Expanded bed	1982	105	<i>Zymomonas mobilis</i>	Vermiculite	Bland et al., 1982
PBR	1982	28.6	<i>Saccharomyces cerevisiae</i>	Sugarcane bagasse	Tyagi and Ghose, 1982
PBR	1983	27.5		Ceramic rods	
PBR	1983	135.8	<i>Zymomonas mobilis</i>	Resin	Krug and Daugulis, 1983
FBR	1990	100		Coke-particles	
PBR	1996	374	<i>Zymomonas mobilis</i>	PCS + 25% various agricultural materials and nutrients	Demirci et al., 1997; Kunduru and Pometto, 1996a, 1996b
		148	<i>Zymomonas mobilis</i> + <i>Streptomyces viridosporus</i> (T7A)		
		190	<i>Saccharomyces cerevisiae</i> + <i>Streptomyces viridosporus</i> (T7A)		
		40	<i>Saccharomyces cerevisiae</i>		
PBR	1997	30	<i>Saccharomyces cerevisiae</i>	PCS + 50% various agricultural materials and nutrients	
FBR	2004	2.21(continuous) 0.28-0.90 (batch)	<i>Escherichia coli</i> FBR-5	Clay brick particles	Qureshi et al., 2004
EABR	2010		Mixed cultures		[50]

Table 3: *Saccharomyces cerevisiae* associated molecular factors influencing the production of ethanol and also showing the probabilities on the application of different BR types.

BR type	Use for ethanol production	Comments	Fundamental factors affecting the biofilm formation by <i>Saccharomyces cerevisiae</i> .	Ref
TBR	Not used.	May be used, as the nutrient deficient may induce phenotypic changes in the biofilm.	<ol style="list-style-type: none"> Glucose concentration: Low glucose concentration favors adherence to plastic surfaces like polystyrene, polypropylene and polyvinylchloride. Complete absence of glucose retards the adherence. Genetic factors: (a) FLO11, a gene required for the production of a cell surface glycoprotein (Flo11p) which helps in both filamentous growth (multicellular pseudohyphae) and mat formation. Flo11p favours hydrophobicity. (b) FLO8, a gene that encodes a regulatory protein required for FLO11 expression. Ploidy condition: Haploid and Diploid cells do not adhere to plastic supports, but round yeast-form cells do only. Nitrogen Concentration: <i>S. cerevisiae</i> switch from round yeast-form to filamentous growth under nitrogen starved condition. Sliding Motility: Required for reduced friction between the cells and the substrate thus increases hydrophobicity. Expression level of Flo11p controls the phenomenon. Mutant Strain: flo 11 Δ is an isogenic strain lacking FLO11 gene. It prefers hydrophilic surface for adherence. 	Renolds and Fink, 2001.
PBR	Mostly used.	Hydrophobicity and sliding motility favored the biofilm formation		
FBR	Used.	More surface area on the used particles helped in better adherence		
ALR	Not used.	Difficulties may arise in maintaining the sliding motility and hydrophobicity is not favoured		
MBR		Hydrophilic as well as hydrophobic membranes can support the growth and thus biofilm formation.		
RDC	Not used.	Hydrophilicity is favored so the mutant strain flo 11 Δ can be employed.		
MABR		Not preferred for anaerobic processes.		
SFBR				
SMABR				
PCsBR	Used.	Polypropylene supported the biofilm formation.		
RDBR	Not used.	Not preferred for anaerobic processes.		
EABR	Used	Electrode surfaces (anode/cathode) supported the biofilm formation.		

Table 4: Production summary of butanol obtained with different BRs, employing *Clostridium acetobutylicum* or *Clostridium beijerinckii* and justification for their respective performances.

Organism used	BR type	Substratum	Productivity (gL ⁻¹ h ⁻¹)	Comments	Ref
<i>C. acetobutylicum</i>	PBR	Beechwood shavings	1.53	Control over substratum roughness, hydrophobicity, porosity and expression level of the cell surface protein moieties rich in hydrophobic amino acids can further enhance the productivity.	Qureshi et al., 2005; Qureshi and Maddox, 1987; Welsh et al., 1987; Napoli et al., 2010; Forberg and Haggstrom, 1985
		Whey permeate	4.5		
		Coke	1.2		
		Bonechar	6.5		
		Glass beads	0.93		
		Glass wool	0.30		
		Polypropylene tow	0.58		
		Stainless steel wire balls	0.15		
	Tygon® rings	4.4			
Membrane cell reactors		6.5			
	FBR		1.65	Hydrophobicity and large surface area of the particles favored formation of the biofilm.	Qureshi et al., 2000
<i>C. beijerinckii</i>	PBR	Clay brick	15.8		

Table 5: Performances of different microorganisms, substrata and BRs used in the production of different organic acids and comments made against variations in the productivities.

Organic acid	BR type	Organism	Productivity (g ^l h ⁻¹)	Substratum used	Comments	Ref
Lactic acid	FBR	<i>Streptococcus thermophilus</i>	12	Activated carbon	PBR with PCS can be the best choice	Ho et al., 1997; Tay and Sang, 2002; Cotton et al. 2001; Demirci et al. 1993a, 1993b, 1995
	PBR	<i>Streptomyces viridosporus T7A + Lactobacillus casei</i>	13 (g ^l h ⁻¹)	Polypropylene + soy hulls-zein (25% w/w)		
		<i>Lactobacillus casei</i>	102	PCS		
	ALR	<i>Rhizopus oryzae</i>	104.6 (g ^l h ⁻¹)	Mineral support + 5 ppm poly(ethylene oxide)		
	ALR	<i>Rhizopus oryzae</i>		Polyurethane foam cubes		
	Rotating fibrous bed	<i>Rhizopus oryzae</i>	60	Fibrous matrix		
	PBR	<i>Lactobacillus Casei subsp. rhamnosus</i>	9	Grid-like orientation PCS biofilm reactor		
<i>Lactobacillus casei</i>		7.6	PCS			
Acetic acid	Multistage shallow flow biofilm reactor	<i>Acetobacter aceti M7</i>	4.3		MABR, SMABR, SFBR can be explored.	Park and Toda, 1992
	TBR	<i>Acetic acid bacteria</i>	1.67	Beechwood shavings		
Citric acid	FBR	<i>Aspergillus niger</i>	0.13	Polyurethane foam (PUF)	PCS can be a better choice to PUF.	Ricciardi et al., 1997; Sanroman et al., 1996; Wang, 2000.
			0.11	Polyurethane foam particles		
	RDC	<i>Aspergillus niger</i>	0.9	Plastic discs + PUF		
Fumaric acid	RDC	<i>Rhizopusoryzae</i>	4.25	Polysulfone Plastic discs	SMABR can be explored	Cao et al., 1996
	CSTR	<i>Rhizopus oryzae</i>	0.9			
Succinic acid	PBR	<i>Actinobacillus succinogenes</i>	2.08	PCS	No correlation between biofilm formation and succinic acid production was observed.	Urbance et al. 2003, 2004

Table 6: Different types of antibiotics and MABs produced employing BR technology and comments made against variations in the productivities.

Antibiotic/Antibody	BR type	Organism/Cells	Substratum	Productivity (g ⁻¹ h ⁻¹)	Comments	Ref
Penicillin	FBR (steady state analysis)			Theoretical development	Complete-mixed contacting pattern resulted in higher specific productivity.	Park and Wallis, 1984
	Inverse fluidized bed bioreactor (IFBBR)	<i>Penicillium chrysogenum</i>	Expanded polystyrene in the form of beads	5.79×10^{-4} g (biomass) ⁻¹ .h ⁻¹	IFBBR favored more production	Ramsay et al., 1991
	CSTR	<i>Penicillium chrysogenum</i>	Agar beads	0.026	2.5 times more stable product	Swarooparani et al., 2003
	ALR	<i>Mutant Penicillium chrysogenum P2</i>	Celite		Overcame the problem of the free cell mass	Keshavarz et al., 1990
Penicillin-G	FBR	<i>Penicillium chrysogenum</i>	Celite R-630	0.11 g Pen-G (K+)/g lactose		Jones et al., 1986; Deo and Gaucher, 1984
			K-carrageenan beads	1.2 mg/g cells/h		
Cephalosporin C	FBR	<i>Cephalosporin acremonium</i>	Celite particles	Production was improved by 1.9		Park and Seo, 1998
	ALR	<i>Cephalosporin acremonium</i>	Siran particles, Silk sachets, Pellets	Specific Productivity: 180% 150% 125% (as compared to 100% for free cells)	Immobilization modes exhibited enhanced volumetric oxygen transfer coefficient	Srivastava and Onodera, 1998
Nisin	PCSBR	<i>Lactococcus lactis</i>	PCS tubes attached on the agitator Shaft.	4,314 U/mL		Pongtharangkul and Demirci, 2006c
Monoclonal antibody (IgG2b)	Fibrous-bed bioreactor (FBBR)	Hybridoma HD-24 cells	Fibrous matrix	7 mg/h.l	Productivity was about 23 times higher to flask cultures	Zhu and Yang, 2004
MAB	FBBR	Hybridoma cells	Non-woven polyester fibrous matrix	6.5	Highly porous fibrous matrix was advantageous	Yang et al., 2004
Anti-digoxin MAB	PBR	Mouse hybridoma cell	Fibra-Cel	116-120 microg/day per ml	Continuous-feeding mode was more efficient for large-scale MAB production than a batch culture.	Golmakany et al., 2005

Table 7: Different types of enzymes and their productivity profiles obtained employing BRs..

Enzyme	BR/Substratum type used	Organism	Productivity	Comments	Ref
Cellulase	Woven nylon pads	<i>Aspergillus terreus</i>	453 U/ml	Designs of the biofilm reactors are highly innovative	Hui et al., 2010; Webb et al., 1986; Ahamed and Vermette, 2010;
	Spouted-bed reactor	<i>Trichoderma viride (QM9123)</i>	24.7–31.5 U		
	Draft-tube airlift bioreactor	<i>Trichoderma reesei RUT-C30</i>	200 U l ⁻¹ h ⁻¹		
Tagatose	PBR	<i>Escherichia coli cells containing Geobacillus stearothermophilus l-arabinose isomerase mutant (Gali 152)</i>	2.9 g/L.h		Jung et al., 2005
Lignin peroxidase (LiP)	Hollow fiber reactor and silicone membrane reactor	<i>Phanerochaete chrysosporium</i>			Venkatdari and Irvine, 1993; Linko, 1992
	Nylon web	<i>P. chrysosporium</i>	2430 U/L		
Lignin peroxidase/manganese peroxidase	PCS tubes attached on the agitator shaft	<i>P. chrysosporium</i>	50, 63 U/L		Khiyami et al., 2006
Manganese peroxidase (MnP)	FBR and fixed bed bioreactors with gas pulsation	<i>P. chrysosporium</i>			Moreira et al., 1998
Amylase	Silicone foam	<i>E.coli</i>	15-28 U		Oriel, 1988

Table 8: Different types of microbial polysaccharides obtained employing BRs.

Polysaccharide	BR/Substratum type used	Organism	Productivity	Ref
Pullulan	PCS tubes attached on the agitator shaft	<i>Aureobasidium pullulans</i>	32.9–60.7 g/l	Chen et al., 2010
	PCS	<i>A. pullulans</i>	1.33g/l/h	Cheng et al., 2011
	PCS (surface response methodology approach)	<i>A. pullulans</i>	60.7g/l	Cheng et al., 2010
Cellulose	PCS tubes attached on the agitator shaft	<i>Acetobacter xylinum</i>	7.1 g/l	Cheng et al., 2009
Xanthan	Centrifugal packed-bed Reactor (CPBR)	<i>Zymomonas campestris</i>	3 g ^l /h	Yang et al., 1996
	FBR (Celite particles)	<i>Z. campestris</i>		Robinson and Wang, 1985

formation of the biofilm was more favored compared to other used substrata. It has been claimed that bonechar has the shear force resistance due to its high porosity and roughness and it is hydrophobic in nature (Qureshi et al., 2005; Qureshi and Maddox, 1990). Microbial cells can escape from the detrimental effects of shear forces as shear forces are very low inside pores. By chemical composition, bone char is mainly calcium phosphate. Microbial cells grown in phosphate rich nutrient have a higher tendency to flocculate and adhere due to their increased hydrophobicity, while the cells depleted in phosphate are more hydrophilic and less likely to adhere (Bucks et al., 1998). The phosphate present in the structure might also aid in maintaining a high degree of hydrophobicity on the surfaces of bonechar and *C. acetobutylicum*. The inherent properties of bonechar make it a recognizable adsorbent for common application in biofilm reactor. However, it's not been in common practice for application in PBR reactors. For different products, role of bonechar in the variation of productivity can be evaluated in PBR, provided the reaction parameters are set at optimum conditions for each product. As production of butanol is manipulated at genetic level, efforts can be made to make *C. acetobutylicum* more adaptable to bonechar. Productivity of butanol was enhanced in FBR by more than two fold than in PBR with bonechar. This encouraged the researchers to scale up the FBR technology to pilot plant level for mass production of butanol (Qureshi et al., 2005). Introduction of bonechar into the FBR set up of butanol production after making necessary morphological changes can further enhance the productivity level.

3. Organic acids

Organic acids viz. lactic acid (LA), acetic acid (AA), citric acid (CA), fumaric acid (FA) and succinic acid (SA) have been produced using different BRs (Table 5). Conventional BRs such FBR, PBR, airlift reactor (ALR), rotating disc biofilm reactor (RDBR), stirred tank reactor (STR) and trickling bed reactor (TBR) have proven to be more productive over their respective suspension cell reactors. Based on the organism, substratum and the type of BRs being employed, productivity of a particular organic acid varied. In general, PCS exhibited better productivity along with technical feasibility for scaling up to pilot plant level. Customization of PCS in its texture or blending imparted better adaptability for application in PBR and FBR for LA production. The aqueous solution of ethanol in contact with air and under the influence of LA bacteria produces LA. The two phase (organic and aqueous) system and need of high oxygen rate transfer makes production of LA ideal for recently developed customized membrane biofilm reactor. Solid support membrane-aerated biofilm reactor (SMABR) and slug flow biofilm reactor (SFBR) are the modern BRs supporting production under aerobic conditions. In the production of CA, FBR and RDC have been explored using polyurethane foam (PUF) as supporting material for biofilm growth. As RDC is preferred for aerobic strains, it resulted in better productivity of CA over FBR. For FA, RDC increased the productivity by many folds over STR. This again

encouraged researchers to go for the aerobic process supporting modern BRs, such as SMABR. Productivity of SA was highly influenced when shifted from suspended cell fermentation to PBR with PCS. However, for comparative statement on performance, application of more BRs for SA production is required.

4. Antibiotics & Monoclonal Antibodies

Application of different BRs in the production of antibiotics has been explored for a limited number of targeted molecules. Penicillin and its derivative Penicillin-G, new generation antibiotic Cephalosporin-C and the only FDA approved bacteriocin Nisin, have been the prime choice of researchers so far (Table 6). Conventional type BRs such as FBR, ALR and STR are in common practices for the production of these antibiotic molecules. Penicillin has also been produced in the new concept based inverse fluidized bed bioreactor (IFBBR). Production of Nisin was greatly enhanced by the introduction of PCS concept.

The production of single antigen specific monoclonal antibodies (MAbs) from hybridoma cells have also been carried out in BRs. Hybridoma cells are immobilized on different matrices to reach a highly viable and productive cell density. FBBR has been the common choice for MAbs production. Non-woven polyester matrix being highly porous is very efficient in mass transfer, supported the adhered cells for a long time and thus enhanced the productivity of MAbs compared to the entrapment method that employed Fibracel as supporting matrix.

5. Enzymes

The inherent enzyme producing property of many microbes has been positively manipulated employing a biofilm reactor set up. However, list of the targeted enzymes is very short (Table 7). Application of the innovative biofilm systems for long term growth of the fungus, *P. chryso sporium* resulted in more productivity of the two extracellular ligninolytic enzymes (LiP and MnP). *Trichoderma* species also exhibited good adaptation to different substrata for biofilm development and caused more productivity of cellulase compared to suspension cell cultures. Tagatose and amylase production were also enhanced under the biofilm reactor conditions. More studies are required to encompass all the basic elements supporting the optimum growth of biofilms and finally designing of a biofilm reactor with the scope of scaling up to pilot plant level for the enzyme of interest.

6. Microbial Polysaccharide

Application of BRs for the production of microbial polysaccharides has been sparsely experimented. Pullulan, cellulose and xanthan are the three microbial polysaccharides explored using BR technology (Table 8). A detailed description on the progress of pullulan production has been well reviewed (Cheng et al., 2011). In the production of cellulose, application of PCSBR enhanced the productivity. Production of xanthan was attempted in FBR using Celite particles. Centrifugal packed-bed reactor (CPBR) markedly enhanced production of xanthan.

7. Lacunae in the present knowledge / understanding of BR technology

7.1. Selection of solid support

The whole process of biofilm formation is an outcome of the complicated bio-physicochemical interactions between the microbial surfaces and the solid supports (Fig 2). However, the phenomenon of “biofilm formation” by microorganisms on a solid support follows the same basic principles in the form of some quantitative physical factors (contact angles, free energy of adhesion, total energy of interaction) originating from the close interactions between solid supports and microorganisms. These factors predict the suitability of a solid support for a particular microorganism. Irrespective of any biofilm forming microorganisms, type of BRs, customization of solid support and the product features of a bio-product, these physical factors can never be compromised. High precision in the calculation of these physical factors and their proper analysis would provide a better scientific backdrop support in the selection of a novel solid material for biofilm formation.

Van Oss et al. (1986) suggested that microbial adhesion to a solid support follows extended XDLVO (Derjaguin, Landau, Verwey, Overbeek) theory. This theory is based on the attractive Lifshitz van der Waals (LW), electrostatic double layer (EL) and short-range Lewis acid–base (AB) interactions between microorganisms and substrata. The polar AB component is the result of hydrogen bonding between two surfaces immersed in a polar solvent (e.g., water). XDLVO approach is more precise in quantifying the interaction energy in order to predict the adhesion. According to XDLVO theory, the total free energy of interaction is expressed as:

$$\Delta G^{TOT(XDLVO)} (\text{mJ} / \text{m}^2) = \Delta G^{LW} + \Delta G^{AB} + \Delta G^{EL} \quad (1)$$

The total interaction energy is evaluated as a function of the minimum equilibrium cut-off distance (y_0) between the interacting surfaces. At this distance physical contact is possible between two interacting flat surfaces and generally a value of 0.158 ± 0.009 nm is assigned (Speranza et al., 2004). The distance is also considered as the van der Waals boundaries between the non-covalently interacting molecules which signify the distance between the outer electron shells. Van Oss et al. (1986) mathematically expressed all the three components (ΔG^{LW} , ΔG^{AB} and ΔG^{EL}) contributing to the calculation of $\Delta G^{TOT(XDLVO)}$ per unit area in terms of y_0 . The equations are presented as follows:

$$\Delta G_{y_0}^{LW} = -2 \left(\sqrt{\gamma_s^{LW}} - \sqrt{\gamma_l^{LW}} \right) \left(\sqrt{\gamma_m^{LW}} - \sqrt{\gamma_l^{LW}} \right) \quad (2)$$

$$\Delta G_{y_0}^{AB} = 2 \left[\left(\sqrt{\gamma_m^-} - \sqrt{\gamma_s^+} \right) \left(\sqrt{\gamma_m^-} - \sqrt{\gamma_l^+} \right) - \left(\sqrt{\gamma_m^+} - \sqrt{\gamma_s^-} \right) \left(\sqrt{\gamma_m^+} - \sqrt{\gamma_l^-} \right) \right] \quad (3)$$

$$\Delta G_{y_0}^{EL} = \frac{\epsilon_0 \epsilon_r \kappa}{2} \left(\zeta_s^2 + \zeta_m^2 \right) \times \left(1 - \coth(\kappa y_0) + \frac{2\zeta_s \zeta_m}{\zeta_s^2 + \zeta_m^2} \text{csch}(\kappa y_0) \right) \quad (4)$$

where,

- (i) γ_s^{LW} , γ_l^{LW} and γ_m^{LW} represents the surface tension components of a solid surface (s), three probe liquid (l) and microorganism (m) respectively,
- (ii) γ^+ and γ^- represent the electron-accepting and electron-donating parameters of each surface tension component (γ_s^{LW} , γ_l^{LW} and γ_m^{LW}) and
- (iii) ϵ_0 ($=8.854 \times 10^{-12} \text{ CV}^{-1} \text{ m}^{-1}$) and ϵ_r ($=79$) are dielectric permittivities of a vacuum and water, respectively, κ ($=3.28 \times 10^9 \text{ I}^{1/2} \text{ m}^{-1}$, where I is the ionic strength of the electrolyte in terms of molarity) the inverse Debye screening length, and ζ_s and ζ_m the surface potentials of the solid surface and microorganism respectively.

In the case of flat-spherical surfaces, interacting at minimum equilibrium cut-off distance (h), the total interaction energy (U^{TOT}) profile is calculated as per Derjaguin's approximation and expressed as:

$$U_h^{TOT} = U_h^{LW} + U_h^{AB} + U_h^{EL} \quad (5)$$

where,

U_h^{LW} = LW component of interaction energy

$$= 2\pi \Delta G_{\gamma_0}^{LW} \frac{\gamma_0^2 a_p}{h} \quad (6)$$

U_h^{AB} = AB component of interaction energy

$$= 2\pi a_p \lambda \Delta G_{\gamma_0}^{AB} e^{[\gamma_0 - h/\lambda]} \quad (7)$$

U_h^{EL} = EL component of interaction energy

$$= \pi \epsilon_r \epsilon_0 a_p \left[2\zeta_s \zeta_m \ln \left(\frac{1 + e^{-\kappa h}}{1 - e^{-\kappa h}} \right) + (\zeta_s^2 + \zeta_m^2) \ln (1 - e^{-2\kappa h}) \right] \quad (8)$$

a_p represents radius of the cell.

In general, calculation of total surface tension of a pure substance is expressed as the sum of a LW and AB components as suggested by van Oss *et al* (1986). The equation is given as:

$$\gamma^{TOT} = \gamma^{LW} + \gamma^{AB} \quad (9)$$

For each component, the expression is given as,

$$\gamma^{AB} \text{ or } \gamma^{LW} = 2\sqrt{\gamma^+ \gamma^-} \quad (10)$$

Again, for a solid surface or a microorganism under study, γ^{AB} or γ^{LW} can be calculated by putting the contact angle data of a three probe liquid (water, diiodomethane and ethylene glycol) in the extended Young equation which is expressed as:

$$(1 + \cos \theta) \gamma_l = 2 \left(\sqrt{\gamma^{LW} \gamma_l^{LW}} + \sqrt{\gamma^+ \gamma_l^-} + \sqrt{\gamma^- \gamma_l^+} \right) \quad (11)$$

Where, θ = Measured Contact angle.

For many microorganisms used in BR based production, predictive utility of XDLVO theory was found to be precise than the DLVO theory. Experimentation done on the adhesion behavior of bacteria and *S.cerevisiae* onto different treated surfaces confirmed the involvement of factors of XDLVO origin (Bayoudha et al., 2009; Kang and Choi,

2005). Although such studies are confined to specific microbial strains, the concept is also applicable to the unexplored ones.

PCS has been shown to be an excellent solid support material for many BR based bio-products. Compatibility of the used microbial strains to PCS resulted in manifold increase of productivity. Both quantitative (measurement of contact angle) and qualitative (scanning electron microscope based topological studies) data supporting the adhesion and biofilm formation respectively, have been included in those studies (Ho et al., 1997). However, mere consideration of surface hydrophobicity/hydrophilicity on the basis of contact angle measurement can only support DLVO theory. Surface manipulations (such as, blending, activation etc.) for better adhesion of microorganisms must be supported as all the criterions come under the XDLVO approach. Constant solution chemistry (culture media and other ingredients) will be a pre-requirement in obtaining precise data for XDLVO consideration. XDLVO approach combines the thermodynamic approach and DLVO theory to explain the experimental results of microbial adhesions (Katsikogianni and Missirlis, 2004). To overcome the limitation of broad application of a solid support, XDLVO can be exploited as a promising model for the prediction of physic-chemical interactions between solid support surface and microbes. In addition, chemical composition of a solid support is a deciding factor for the adhesions of microorganisms. Polymers of different molecular weights, lengths and molecular structures (isomers) might respond differently to a microorganism. If it is assumed that, agricultural waste products (AWP) and the plastic support present in PCS chemically inert to each other, the accessibility of AWP to a microorganism can still be sterically hindered by the orientation of the monomer chains constituting the plastic support. This concept is applicable to any novel solid support to be developed considering PCS as a model substratum. More investigation on the molecular interactions between a nutrient cum solid support material and microorganisms can reveal the governing factors for a better adaptability of microorganisms to be used for different bioproducts in BRs.

As opposed to the current tendency of random search, applications of biomaterials research findings and nanotechnology concepts in the direction of prospective design or selection of a novel solid support can give predictive outcome. Cellulose acetate (CLA), the photodegradable but not biodegradable and renewable biomaterial can be a good option as solid support and biofilm carrier (Hon, 1977). CLA has already found wider applications in biomaterials and tissue engineering (BMTE) field as it can mimic the topology of an extracellular matrix (Han and Gouma, 2006). Evaluation of CLA (sourced from cigarette waste filter rods) as a biofilm carrier in an integrated fixed film activated sludge (IFAS) process was very encouraging (Sabzali et al., 2011). Compared to the activated sludge (AS), the CLA integrated IFAS performed better in terms of the removal efficiencies of COD, ammonia and

phosphorus. Being a renewable (mainly sourced from wood pulp) and cheap material, CLA has the scope of more applications in BRs technology as solid support for biofilm formation and biofilm carrier. Application of nanotechnology in designing more efficient solid support for biofilm formation can also be vital. Electrospinning (a fabrication method used to form complex, porous, 3D structures with specific design in terms of geometry, morphology or topography in a single-step process) of solid support in its soluble form into nanosheets of desired porosity, thickness and surface area can give a better form of solid support for microbial adherence. Application of nanofibers (polyethylene + polyurethane) as a carrier of the biofilm of bacterial strain *Rhodococcus erythropolis* for wastewater treatment in a MBBR, found to be better than the commercially available AnoxKaldnes (type K3) carriers (Kriklavova and Lederer, 2010). Growth of the bacterial biofilm within the nanofibers not only facilitated more protection for the bacteria against the toxic effects of the surrounding environment of wastewater, but was also able to provide substrate and oxygen to the microorganisms in sufficient amount. Thus, it is obvious that application of biomaterials and nanotechnology concepts in the customization of solid supports can have serious impacts. However, in-depth studies are required to make these novel concepts fruitful and also an integral part of BRs technology.

8.2. Water structure, solid surface and microbial response

Water structure (three-dimensional hydrogen-bonded network) associated with 'hydrophobic' and 'hydrophilic' solid surfaces are different as given in Fig 3. This property of water is attributed to the strong nature of self-association of its molecules. In a polar solvent system, such as water, molecular association is dependent on the acid-base interactions taking place between molecules in solution or between solution-phase molecules and a solid surface. Lewis acid and base is required for this polar environment which has a direct effect on the polar interactions among the molecules involved, thus influencing the interfacial phenomena. Another important aspect of water-solid surface interactions is the analytical measurement of hydrophobicity. Techniques that directly probe water structure rather than those that simply respond to water structure, such as contact angle and wettability, should be more preferred. Measurement of surface forces with surface force apparatus (SFA) and ancillary techniques are one such approach to quantifying hydrophobicity. Apart from the water structure and hydrophobicity that influence the water-solid support interactions, another major factor which contributes to the role of water in biological response (microbial surfaces) to materials (solid support) is the measurement of 'water wettability' in terms of 'adhesion tension' (denoted as τ^0), rather than surface energy (γ_s) or interfacial tension (γ_0) components that are found to be distantly related to water wettability (Vogler, 1998).

Water adhesion tension (τ^0) can be derived from the known value of water interfacial tension (γ^0) and measurement of contact angle (θ). The expression is given as:

Where,

$$\tau^0 = \gamma^0 \cos \theta \quad (12)$$

τ^0 = Water Adhesion Tension (dyne/cm)

γ^0 = Water Interfacial Tension (= 72.8 dyne/cm for pure water)

θ = Measured Contact Angle

8.3. Berg Limit

The concept of 'Berg Limit' can precisely be applied in the measurement of surface forces (attractive or hydrophobic/repulsive or hydration) acting on a solid surface immersed in water. Berg et al suggested for a 'threshold' value of contact angle ($\theta = 65^\circ$) for separating the zone of hydrophobicity and hydrophilicity of solid surfaces immersed in water (Berg et al., 1994). This contact angle value can be exploited to determine the 'threshold' value of τ^0 for predicting the water wettability properties of different solid surfaces.

From equation (12),

At 'Berg limit' $\theta = 65^\circ$

$$\begin{aligned} \tau^0 &= 72.8 \times \cos 65^\circ \\ &= 72.8 \times 0.4226 \\ &= 30.76 \text{ dyne/cm} \end{aligned}$$

Thus, according to 'Berg Limit' concept

- (1) For hydrophobic surfaces, $\tau^0 < 30 \text{ dyn/cm}$, $\theta < 65^\circ$ and
- (2) For hydrophilic surfaces, $\tau^0 > 30 \text{ dyn/cm}$, $\theta > 65^\circ$.

8.4. Primary & secondary minima of adhesion

Adhesion of microorganisms to different substrata under high flow velocity can either be reversible (partial or complete detachment) or irreversible (zero or negligible detachment). Due to the heterogeneity in the surface properties of different microorganisms and substrata, the adhesion energy profiles (U_n^{TOT}) of interactions at different contact points differ. The high adhesion energy profile causing the strong attraction at some contact points are called as "Primary Energy Minima", and those contact points where the adhesion energy profile is relatively weak are known as "Secondary Energy Minima" (Kang and Choi, 2005). Reversibility or irreversibility of microbial – substrata interactions is a net outcome of the relative abundances of primary or secondary energy minima, which in turn is highly susceptible to surface properties of microorganisms or substrata itself. Experiment on the detachment of microbial cells from a substratum surface is vital as the product features will be highly affected by too much reversibility nature of the adhesion energy profile. Thus, while adopting a novel solid support material for BR, test of reversibility seems to be mandatory for a proper scientific evaluation of applicability of a substratum at commercial scale. Favouring of hydrophobic or hydrophilic surfaces for biofilm development by different microorganisms and sustainability of irreversible condition (no detachment) in a long time running set up of a BR are the mere consequences of adhesion energy

minima profiles of the interactions. Reports on the successful application of a novel substratum for BR based productions are not inclusive of adhesion energy minima concept. Most of the findings are based on the measurement of contact angles and specifically designed for a particular strain of microorganism. This area of BR technology needs further exploration to make the application of a substratum even broader.

8.5. Entropy of Mixing

Mixing of two materials change the thermodynamic property called "entropy of mixing" even though they are chemically non-reacting. The entropy of mixing provides information about differences of intermolecular forces or specific molecular effects in the materials. Though not considered as a common factor in BR technology, analysis of entropy of mixing can be relevant to designing of composite solid supports like PCS. This thermodynamic property can have a predictive value in fixing the ratios of blending materials (organic or inorganic) in the development of a novel and better performing composite substratum to be applied in BRs. In a recently published report, it has been shown that even the method (ethylene oxide or gas plasma) adopted for surface sterilization of a substratum can have huge impact on the adherence level of bacteria (Kinnari et al., 2010). Analysis of entropy of mixing for different ratios of ingredients can help in knowing (a) any chemical interactions in-between the ingredients and (b) thermodynamic impact of each ingredient in the overall performance of a composite substratum. In the near future also, researcher might develop interest in designing a novel composite solid support, more efficient than PCS at the expense of even more cheaper waste materials from agricultural or other sources. In such an approach, it might be possible to select the ingredients in a more easy but accurate way by employing the concept of entropy of mixing.

Concluding remarks

The effort for utilizing the natural phenomenon of "biofilm formation" by microbes in the benefit of human has been well manifested in the form of BR technology. The systematic approach made towards the development of a novel BRs resulted in multiple impacts on the production level of different bioproducts. The key factors controlling the performance of biofilm can now be regulated at molecular level. However, when explored from the lab to commercial scale translation level, the present scenario of BR technology is not satisfactory except one or two bioproducts. The contemporary efforts made for enhanced productivity, utilization of waste materials as sources of carbon and energy, designing of composite solid supports and customization of BRs, in a collective manner has not been able to put the BR technology in an easily scalable platform by adopting common features. The divergences arises due to the application of bioproduct or microorganism or BR engineering aspect specific elements (solid support, culture condition, microbial strains and BR hydraulics) and they have restricted the scope from further scaling. Thus, a unified concept on the development of a substratum for a particular

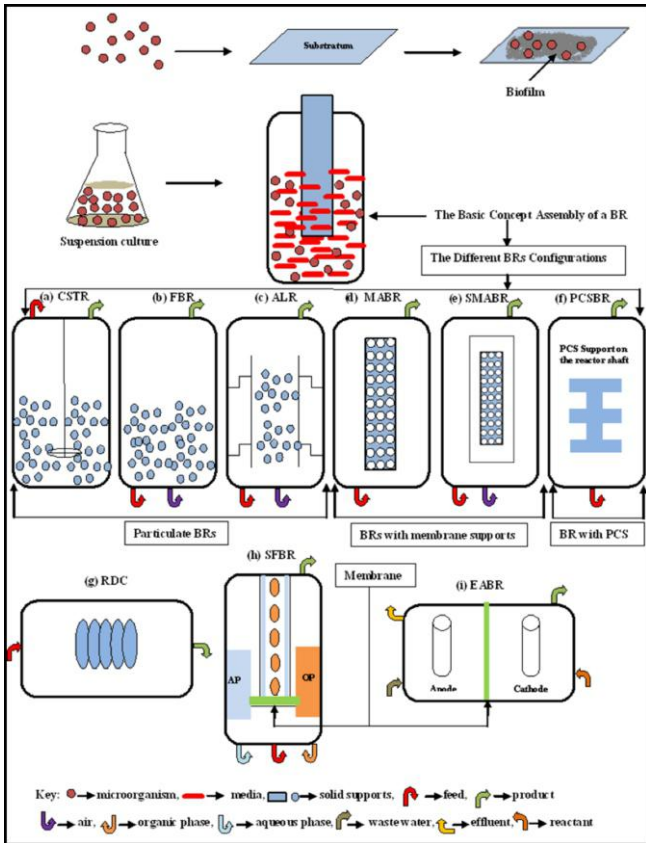


Fig 1: The concept of BR technology and its development.

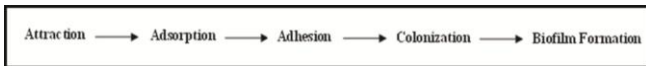


Fig 2: Schematic outline of different steps of biofilm formation mechanism.

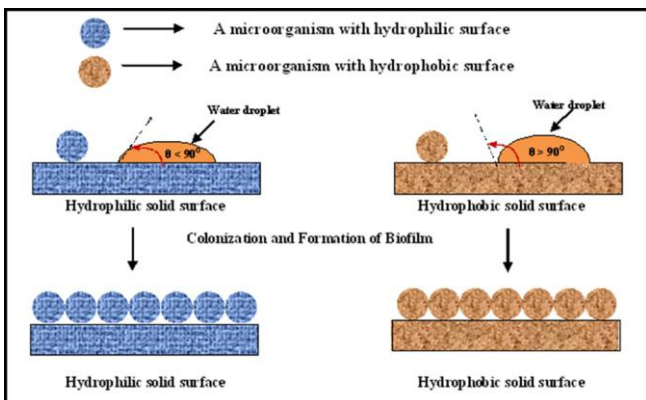


Fig 3: Summary of the surface properties of microorganism and water molecule involved during formation of a biofilm on a substratum.

bioproduct (single or multiple microorganisms specific) will be a preferred approach. The material chosen as a substratum alone or component for composite support designing must be subjected to the analysis for some quantified parameters (XDLVO analysis for adhesion energy, energy minima, Berg limit and entropy of mixing) before approval for common application in BR technology. In a positive sense, PCS can be foreseen as a substratum for broad applications, but more technical rectifications are required before approving it as a default choice in BR technology. Extensive molecular level

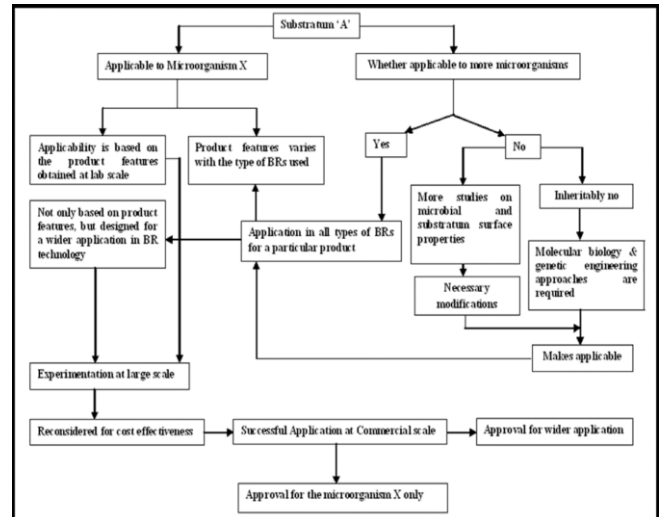


Fig 4: Schematic outline of the overall concept of the present review.

research in the direction of developing chimeras capable of adopting to the substratum of default use can make BR technology more converging in the aspect of prevailing great diversification due to the orthodox 'bioproduct-microorganism-substratum-BR type' working principles. The underexplored research areas of the substratum concerned, highlighted in this review article, in a straight forward way have practical impacts on the overall performance of BRs. Along with the customization of engineering aspects of BRs; proper exploration of the substratum associated issues mentioned in the present context can be fruitful in making BR studies technology more productive and uniform.

Declaration of competing interest

The author declares that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Abbreviations

BR, biofilm reactor; RBC, rotating biological contactors; COD, chemical oxygen demand; BOD, biological oxygen demand; BFB, biofilm fluidized bed; TBR, trickling bed reactor; PBR, packed bed reactor; FBR, fluidized bed reactor; ALR, airlift reactor, MBR, membrane bed reactor; RDC, rotating disc contactor; MABR, membrane aerated biofilm reactor; SFBR, slug flow biofilm reactor; AP, aqueous phase; OP, organic phase; SMABR, solid support membrane-aerated biofilm reactor; PCSBR, plastic composite support biofilm reactor; RDBR, rotating disc biofilm reactor; EABR, electro-active biofilm reactor; CSTR, continuous stirred tank reactor; PCS, plastic composite support; PUF, polyurethane foam; IFBBR, inverse fluidized bed bioreactor; CPBR centrifugal packed-bed reactor; OA, organic acids; LA, lactic acid; AA, acetic acid; CA, citric acid; FA, fumaric acid; SA, succinic acid; MABs, monoclonal antibodies; FDA, food and drug administration; DNA, deoxyribonucleic acid; Lip, lignin peroxidase; MnP, manganese peroxidase; XDLVO, Derjaguin, Landau, Verwey, Overbeek; LW, Lifshitz van der Waals; EL, electrostatic double layer; ZB, Lewis acid-base;

SFA, surface force apparatus; CLA, cellulose acetate; RSM, response surface methodology.

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