Title	Analytical methods for soluble microbial products (SMP) and extracellular polymers (ECP) in wastewater treatment systems : a review(Tables)
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Table 1. Pretreatments and analytical methodologies for the identification of SMPs and ECPs

Substrate	Type of Biological treatment	Aerobic	Anaerobic	Parameters	Pretreatment	Analysis	References
sludge	activated sludge, acidogenic sludge (fermentor), methanogenic sludge (UASB)	✓	✓	ECP	Five extraction methods: EDTA, cation exchange resin, formaldehyde, formaldehyde plus NaOH, and formaldehyde plus ultrasound After extraction: centrifugation 20,000G for 20 min, filter through 0.2 µm and dialysis membrane, lyophilization at -50°C	Anthrone method [carbohydrate] Lowry Folin method [protein, humic substance] m-hydroxydiphenyl sulfuric acid method [uronic acid] diphenylamine colorimetric method [DNA]	Liu and Fang (2002)
mixed liquor, effluent	CSTR, SAMBR		✓	SMP, ECP, cell lysis	SMP: centrifugation 13,000 rpm (10 min), filtration by 0.2µm Ultrafiltration: MWCO 1kDa, 10kDa LLE (hexane, monochloro benzene, dichloro methane, ethyl acetate), for GC-MS SPE (sorbent ENV+), for GC-MS ECP extraction: steaming, formaldehyde Cell lysis: autoclave 120°C (20min)	SEC, pH, COD, VSS HPLC-UV (C-8 and C-18 columns) (210nm) [VFA], (254nm) [other compunds] UV-Vis spectrophotometer: (595nm) [total protein], (490nm) [total carbohydrate], (600nm) [total DNA] GC-MS (electron ionization mode (EI) [identification of m/z 40 to 600] Matrix assisted laser desorption ionization time-of-flight mass spectrometry MALDI-TOF-MS [identification of high MW]	Aquino (2004)
Sludge	activated sludge	✓		ECP	ECP extraction: glutaraldehyde, EDTA, formaldehyde + NaOH, cation exchange resin, sonication, cation exchange resin associated to sonication, heating, centrifugation	TOC-meter, colorimetric method [protein, humic acids, polysaccharide, uronic acids, lipid, nucleic acid], Infra-red spectrometry [functional group]	Comte *et al.* (2006)
Effluent	activated sludge	✓		SMP	Prefiltered by 0.45 µm filters	DOC, UVA (254nm), TDS, SEC	Jarusutthirak and Amy (2006)
Sludge	activated sludge	✓	✓	SMP, ECP	SMP: centrifugation at 6,000G (10min), separated, added acetone to supernatant, precipitatation (4°C, 24hrs), precipitate is SMP ECP extraction: ultrasonication Filtration: 0.45 µm filter (EEM sample)	COD, SS Fourier-transform infrared (FTIR) spectrophotometer [compositions] Zetasizer [surface change, floc size] EEM- fluorescence spectroscopy Jar test with PACl [chemical coagulation test]	Ramesh *et al.* (2006)

Substrate	Type of Biological treatment	Aerobic	Anaerobic	Parameters	Pretreatment	Analysis	References
mixed liquor, effluent	MBR	✓		SMP	Centrifugation 10,000 rpm (10 min at 4°C) then filtering through 0.45 µm membrane Ultrafiltration: Amicon (3, 10, 30 kDa)	COD, MLSS, MLVSS UV spectrophotometer 254nm Phenol-sulfuric method [carbohydrate] Lowry Folin method [protein] Column chromatographic method (borosilicate glass column) [hydrophobic/hydrophilic and charge]	Liang *et al.* (2007)
Sludge	activated sludge, aerobic granule	✓		ECP	ECP extraction: formamide plus NaOH	EEM, confocal laser scanning microscope technique	Adav and Lee (2007)
Influent, mixed liquor, Effluent	MBR	✓		SMP, ECP	ECP extraction: cation exchange resin SMP: centrifuged raw sludge for 15 min at 12,000G, take supernatant Ultrafiltration: MW separation (1 and 10kDa)	Colorimetric method by phenol-sulfuric acid reagent [carbohydrate] Relative hydrophobicity (mix with *n*-hexane) COD, MLSS, MLVSS	Jang *et al.* (2007)
Sludge	activated sludge	✓		SMP, LBECP, TBECP	ECP extraction: ultrasonication (LBECP) and ultrasonication plus heat (TBECP)	SEC, Tubidity, Zeta potential, pH, UV-Vis (230, 254, 280 nm), RID, EEM, DOC	Tsai *et al.* (2008)
effluent	UASB, SBR	✓	✓	protein, carbohydrate	Filtration: 0.45 µm filter cellulose membrane	Simultaneously scaning the excitation and emissiion monochromators form 200-650 nm [protein, carbohydrate]	Zhang *et al.* (2008)
Sludge	activated sludge	✓		ECP	ECP extraction: cation exchange resin	SEC, UV spectrophotometer 260nm [nucleic acid], COD MLVSS	Ni *et al.* (2009)
Effluent	UASB (full scale 3100m^3) activated sludge	✓	✓	SMP	Not mentioned	COD, VFA, MLSS, MLVSS Sulphuric acid-anthrone method [polysaccharide] Lowry Folin method [protein] GC-MS (electron ionization mode (EI)) [identification of m/z 33 to 500]	Zhou *et al.* (2009)
mixed liquor	MBR (pilot-scale)	✓		ECP	ECP extraction: heat LBECP, TBECP extraction: ultrasonication plus heat	SEC, Mean oxidation state (derive from TOC and COD), UV (254nm), phenol-sulfuric acid method [carbohydrate], Lowry method [protein] Fourier-transform infrared (FTIR) spectrophotometer [functinal group], EEM	Wang *et al.* (2009)
influent, effluent	UASB		✓	SMP	Ultrafiltration: Amicon (1, 10, 100 kDa)	COD (initial inert, soluble, total) [soluble COD produce by biomass or SMP]	Aquino *et al.* (2009)

Substrate	Type of Biological treatment	Aerobic	Anaerobic	Parameters	Pretreatment	Analysis	References
Sludge	activated sludge, anaerobic granular (UASB, EGSB), anaerobic digester	✓	✓	ECP	ECP extraction: cation-exchange resin coupled with ultracentrifugation/ultrasound and centrifugation	SEC, HPLC-diode array UV detector (280 nm)	Villian *et al.* (2010)
Effluent	activated sludge	✓	✓	SMP, ECP	ECP extraction: ion-exchage resin Cell lysis test: ultrasonication	COD, TSS, VSS, pH, Temperature, oxygen Phenol-sulfuric method [carbohydrate] High performance liquid chromatography -UV 210nm with ion exclusion column [VFA, glucose] Matrix assisted laser desorption ionization time-of-flight mass spectrometry MALDI-TOF-MS [high molecular weight identification] High resolution liquid chromatography ion trap time-of-flight mass spectrometry LC-IT-TOF-MS [identification of MW 100 - 4,000 Da]	Mesquita *et al.* (2010)
Effluent	activated sludge	✓		SMP	Prefiltered by 0.22 µm filters	SEC, HPLC (C18)-diode array UV detector (254 nm) EEM- fluorescence spectroscopy [tyrosine/tryptophan amino acid, tyrosine/tryptophan protein, polysaccharide, fulvic acid, polyaromatic type humic acid, polycarboxylate type humic acid]	Wang and Zhang (2010)
influent, effluent	SBR, Cyclic Activated Sludge Technology Anoxic/Oxic, Modified Carrousel Oxidation Ditch, Unifed-SBR, Anoxic/Oxic nitrogen removal process, Step-Feed Anoxic/Oxic nitrogen removal process, Anaerobic/anoxic and nitrifying nitrogen and phosphorus removal process	✓	✓	SMP	Centrifugation 2,810G for 10 min, filtration through 0.45 µm acetate fiber membrane filter	DOC, UVA SEC coupled with UV and fluorescence detectors	Guo *et al.* (2011)

Note: COD = chemical oxygen demand, CSTR = continuous stirred tank reactor, DOM = dissolved organic matter, DOC = dissolved organic carbon, EEM =excitation and emission matrix, EGSB = expanded granular sludge bed, ECP = extracellular polymer, GPC = gel permeating chromatogrphy, LLE = liquid-liquid extraction, LOQ = limit of quantification, MBR = membrane bioreactor, MLSS = mixed liquor suspended solids, MLVSS = mixed liquor volatile suspended solids, RID = refractive index detector, SAMBR = submerged anaerobic membrane bioreactor, SBR = sequencing batch reactor, SEC = size exclusion chromatography, SMP = soluble microbial product, SPE = solid phase extraction, UASB = upflow anaerobic sludge blanket, VFA = volatile fatty acids

Table 2. ECP extraction procedures

Substrate	Type of Biological treatment	Extraction method used	Procedure of the selected method	References
Sludge	activated sludge, acidogenic sludge (fermentor), methanogenic sludge (UASB)	5 extraction methods: EDTA, cation exchange resin, formaldehyde, formaldehyde plus NaOH, and formaldehyde plus ultrasonication	Formaldehyde + NaOH: 10mL sludge, 0.06mL formaldehyde (36.5%, 4°C, 1h), 4mL 1N NaOH (4°C, 3h), centrifugation 20,000G (4°C, 20min), filtration by 0.2µm (25°C), purified with dialysis membrane (3500Da, 4°C, 24h), freeze-drying (-50°C, 48h)	Liu and Fang (2002)
Mixed liquor	CSTR (anaerobic), SAMBR	Steaming	Steaming extraction (ECP): centrifugation 13,000 rpm (10min), resuspended pellets by distilled water and steamed in autoclave (80°C, 1 bar, 10min), centrifugation, filtration by 0.2µm, ECP in solution	Aquino (2004)
		Formaldehyde solution	Formaldehyde extraction (ECP): centrifugation 13,000 rpm (10min), resuspended pellets by 0.5% v/v formaldehyde solution, mixed (5min), centrifugation, filtration by 0.2µm, ECP in solution	
Sludge	activated sludge	Centrifugation plus ultrasonication	Centrifugation at 6,000G (10min), added 0.85% w/w NaCl to dewatered cake, ultrasonication (20kHz, 2min), shaken 120rpm (10min), ultrasonication (20kHz, 2min), centrifugation at 8,000G (10min), added acetone to supernatant, precipitatation (4°C, 24h), precipitate is ECP	Ramesh *et al.* (2006)
Sludge	activated sludge	3 chemical procedures (Glutaraldehyde, EDTA, formaldehyde + NaOH)	Formaldehyde + NaOH: Centrifugation (4,300G, 10 min), resuspended in ultrapure water, add folmaldehyde 36.5% (4°C, 1 h) plus 1M NaOH (4°C, 3 h), 2 times ultracentrifugation 4°C (20,000G, 20 min plus 10,000G, 15 min), purification with a 3500D dialysis membrane (4°C, 2h)	Comte *et al.* (2006)
		4 physical procedures (cation exchange resin, ultrasonication, cation exchange resin plus ultrasonication, heating)	Ultrasonication + resin: Centrifugation (4,300G, 10 min), resuspended in ultrapure water, ultrasonication 40W (2 min) + DOWEX RESIN (50X8) at 4°C (1 h, 600 rpm), 2 times ultracentrifugation 4°C (20,000G, 20 min plus 10,000G, 15 min), purification with a 3500D dialysis membrane (4°C, 2h)	
Sludge	activated sludge, aerobic granule	Ultrasonication plus formamide and NaOH	10mL of sludge or granule, ultrasonication, add 0.06 mL formamide 4°C, 1 h, 4mL 1 N NaOH 4°C, 3 h, centrifugation 10,000G 4°C (24h), filter by 0.2µm	Adav and Lee (2007)
Mixed liquor	MBR	Cation-exchange resin	Add cation exchange resin (75g of resin/g VSS) to 200mL sample, mix at 600 rpm (2h, 4°C), cetrifugation 15 min at 12,000G	Jang *et al.* (2007)
Sludge	activated sludge	Ultrasonication plus heat	1) Centrifugation 6,000G (10 min), add acetone (2 volumes) to supernatant and precipitate at 4°C, 24 h [SMP]. 2) Dewatered cake mix with 0.85% w/w NaCl with glass beads, ultrasonication at 20 kHz (2 min), shaken 120 rpm (10 min), ultrasonication (2 min), centrifugation 8,000G (10 min), add acetone (2 volumes) to supernatant and precipitate at 4°C, 24 h. [LBECP] 3) Residual solids mix with 0.85% NaCl, ultrasonication (2 min), heat (80°C, 30 min). supernatant mix with acetone (2 volumes) and precipitate at 4°C, 24 h. [TBECP]	Tsai *et al.* (2008)
Sludge	activated sludge	Cation-exchange resin	Centrifugation, washed by 100mM NaCl, add cation exchange resin, stirred, centrifugation, filtration by 0.45µm membrane	Ni *et al.* (2009)
Mixed liquor	MBR (pilot-scale)	Centrifugation plus heat	Centrifugation (3,200 rpm, 30min), discarded supernatant and resuspended with 0.9% NaCl, heat treatment (100°C, 1 h), centrifugation (3,200 rpm, 30min), supernatant is ECP Extraction [LBECP, TBECP]: centrifugation (3,200 rpm, 30min), discarded supernatant, resuspended with 0.9% NaCl, sonication (2min), centrifugation (3,200 rpm, 30min), supernatant is LBECP, sludge was resuspended, heated (100°C, 1h), centrifugation (3,200 rpm, 30min), supernatant is TBECP	Wang *et al.* (2009)
Sludge	activated sludge, anaerobic granular (UASB, EGSB), anaerobic digester	Cation-exchange resin coupled with ultrasonication and centrifugation	Centrifugation (4,300G, 10 min), resuspended in ultrapure water, ultrasonication 40W (2 min) + DOWEX RESIN (50X8) at 4°C (1 h, 600 rpm), 2 times ultracentrifugation 4°C (20,000G, 20 min plus 10,000G, 15 min), purification with a 3500D dialysis membrane (4°C, 2h)	Villian *et al.* (2010)

Note: CSTR = continuous stirred tank reactor, EGSB = expanded granular sludge bed, ECP = extracellular polymer, MBR = membrane bioreactor, SAMBR = submerged anaerobic membrane bioreactor, SBR = sequencing batch reactor, UASB = upflow anaerobic sludge blanket

Table 3. Size Exclusion Chromatography technique for identifying MW distribution in SMPs and extracted ECPs

Substrate	Type of Biological treatment	Parameters	Column	Mobile phase (flow rate)	Standard	Analysis	References
Mixed liquor, effluent	CSTR (anaerobic), SAMBR	SMP, ECP, cell lysis	Aquagel OH-30 single or in-line with Aquagel OH-40 (at ambient temperature)	Deionized water (1 mL/min)	Linear polyethylene oxide (PEO) and polyethylene glycol (PEG) from Polymer Labs	UV and refractive index (RID) detectors	Aquino (2004)
Sludge	activated sludge	SMP, loosely bound ECP (LBECP), tightly bound ECP (TBECP)	HW-50S column (TOYOPEARL resin with 20-40 µm particel size, TOSOH Bioscience LLC)	Phosphate mobile phase (0.0024M NaH_2PO_4 + 0.0016M Na_2HPO_4, pH 6.8) containing 0.025M Na_2SO_4 at ionic strength 0.1M (1 mL/min)	Polyethylene glycols (PEGs, 200, 1000, 4000, 8000 and 20000 g/mol)	UV-Vis (230, 254, 280 nm), refractive index detector, and CHF 100SA fraction collector (for EEM and DOC)	Tsai *et al.* (2008)
Mixed liquor	MBR (pilot-scale)	ECP	TSK G4000SW type gel column (TOSOH Corporation)	Not mentioned	Polyethylene glycols (PEGs) with molecular weight (MW) of 1215000 Da, 124700 Da, 11840Da, and 620Da	DOC, UV (254nm)	Wang *et al.* (2009)
Sludge	activated sludge	ECP	Series of ultrahydrogel 250, 500 and 200 column (40°C)	Deionized water (1 mL/min)	Standard polysaccharides of molecular mass 180, 738, 5900, 1180, 2280, 47300, 112000, 212000, 404000 and 788000 Da and standard proteins of molecular mass 13700, 45000, 67000, 200000 and 670000Da (ribonuclease A: R-4875, bamylase: A-7130, chicken egg albumin: A-5378 and bovine serum albumin: A-7906)	Diode array UV detector at 254 nm and refractive index detector	Ni *et al.* (2010)
Sludge	activated sludge, anaerobic granular (UASB, EGSB), anaerobic digester	ECP	Amersham Biosciences column, the Superdex 200 10/300 GL (10-600 kDa)	75 mM HEPES (pH 7) (0.4 mL/min)	Proteins standard: cytochrome C (124000Da), chicken albumin (443000 kDa), ovalbumin (66 000Da) and ferritin I (440000Da).	Diode array UV detector (280 nm)	Villian *et al.* (2010)
Influent, effluent	SBR, Cyclic Activated Sludge Technology Anoxic/Oxic, Modified Carrousel Oxidation Ditch, Unifed-SBR, Anoxic/Oxic nitrogen removal process, Step-Feed Anoxic/Oxic nitrogen removal process, Anaerobic/anoxic and nitrifying nitrogen and phosphorus removal process	SMP	Waters Protein-pak 125 column	Deionized water buffered with phosphate (0.0024 M NaH_2PO_4 + 0.0016 M Na_2HPO_4) to pH 6.8 and 0.025 M Na_2SO_4 was added to reach a total ionic strength of 0.1 M	Sodium polystyrene sulfonates with a molecular weight of 210, 1400, 3400, 13000, 32000 Da	A series-connected Ultraviolet (254 nm) and fluorescence (278 nm, 353 nm) detectors	Guo *et al.* (2011)

Note: CSTR = continuous stirred tank reactor, DOC = dissolved organic carbon, EEM =excitation and emission matrix, EGSB = expanded granular sludge bed, ECP = extracellular polymer, MBR = membrane bioreactor, RID = refractive index detector, SAMBR = submerged anaerobic membrane bioreactor, SBR = sequencing batch reactor, SMP = soluble microbial product, UASB = upflow anaerobic sludge blanket

Table 4. Sample pretreatment procedures and analytical methodologies of mass spectrometry for SMP and ECP analysis.

Substrate	Type of Biological treatment	Pretreatment	Analysis	References
Mixed liquor, Effluent	CSTR, SAMBR	LLE (hexane, monochloro benzene, dichloro methane, ethyl acetate), for GC-MS SPE (sorbent ENV+), for GC-MS	GC-MS [Column: SGE Phase BPX5, Identification of m/z 40 to 600] MALDI-TOF-MS [Identification of high MW]	Aquino (2004)
Effluent	UASB (full scale 3,100m^3) activated sludge	LLE (dichloromethane)	GC-MS [Column: DB 5MS, Identification of m/z 33 to 500, NIST98 and WILEY Registry 7.0]	Zhou *et al.* (2009)
Effluent	SAMBR	SPE (Oasis®HLB), Elution solvent: 10%methanol/90%MTBE	GC-MS [Column: SGE HT5, Identification of m/z 33 to 500, NIST05 library]	Trzcinski and Stuckey (2009)
Effluent	activated sludge	Lyophillized	MALDI-TOF-MS [high molecular weight identification (757.40 to 39,212.28 Da)] LC-IT-TOF-MS [Identification of MW 100 - 4,000 Da]	Mesquita *et al.* (2010)
Effluent	UASB	LLE (dichloromethane)	GC-MS [NIST98 and WILEY Registry 7.0]	Wu and Zhou (2010)

Note: CSTR = continuous stirred tank reactor, LC-IT-TOF-MS = High resolution liquid chromatography ion trap time-of-flight mass spectrometry, LLE = liquid-liquid extraction, MALDI-TOF-MS = Matrix assisted laser desorption ionization time-of-flight mass spectrometry, SAMBR = submerged anaerobic membrane bioreactor, SPE = solid phase extraction, UASB = upflow anaerobic sludge blanket