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Lalit Pandey
Pankaj Tiwari *Editors*

Microbial Enhanced Oil Recovery

Principles and Potential

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Lalit Pandey · Pankaj Tiwari
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Editors
Lalit Pandey
Department of Biosciences
and Bioengineering
Indian Institute of Technology Guwahati
Guwahati, Assam, India

Pankaj Tiwari
Department of Chemical Engineering
Indian Institute of Technology Guwahati
Guwahati, Assam, India

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Editors and Contributors

About the Editors

Prof. Lalit Pandey is an Associate Professor in the Department of Biosciences and Bioengineering, IIT Guwahati. He has completed his Ph.D. in Chemical Engineering from the Indian Institute of Technology, Delhi. He was awarded Erasmus Mundus India4EU Fellowship to pursue his research at Laboratoire des Matériaux et du Génie Physique (LMGP), Grenoble-INP, France, for 18 months during 2010 to 2012. Before joining the IIT Guwahati, he worked as a Scientist with the Central Pollution Control Board, Ministry of Environment & Forests, Government of India, from 2009 to 2014 and was researched on water and air pollution in agro-based industries. He has received several awards, including the Shastri Covid-19 Pandemic Response Grant (SCPRG) 2020, the DST-UKIERI Award 2018, the IEI (The Institution of Engineers [India]) Young Engineers Award 2017, Innovation in Science Pursuit for Inspired Research (INSPIRE) Faculty Award 2014, and Early Career Research Award from Science and Engineering Research Board (SERB) by Department of Science & Technology, Government of India. His research area includes biointerfaces and biomaterials, biochemical engineering and environmental biotechnology. He has published over seventy five scientific articles in international journals and serves on the editorial board of five international journals. He is serving as Associate Editor in Frontiers in Bioengineering and Biotechnology and Frontiers in Materials. He has co-edited one book titled "Biointerface Engineering: Prospects in Medical Diagnostics and Drug Delivery" published by Springer Nature in 2020. He is a life member of the International Association of Engineers (IAENG), the Indian Institute of Chemical Engineers (IIChE), the Biotech Research Society, India (BRSI), and Nano and Molecular Society (India), and serves as a reviewer for several peer-reviewed journals.

Prof. Pankaj Tiwari is an Associate Professor in the Department of Chemical Engineering, IIT Guwahati. He has received Ph.D. in Chemical Engineering from the University of Utah, USA. He is actively working on various enhanced oil recovery schemes, such as chemical EOR, gas-chemical EOR. He has successfully executed five sponsored projects from various funding agencies. He has an active collaboration in the area of EOR and also working on a project with Oil India Limited toward implementation of polymer and CO2 flooding in oil fields of Oil India Limited. He has published 40+ papers in reputed international journals including Journal of Petroleum Science and Engineering, Energy & Fuels, 2019, Colloids and Surfaces A and 6+ book chapters. He is a life member of the Indian Institute of Chemical Engineers (IIChE) and a regular member of the Institute of Engineers (India), the Society for Polymer Science, India, American Institute of Chemical Engineers (AIChE), and Society of Petroleum Engineers (SPE) and serves as a reviewer for several peer-reviewed journals. His research group is also actively working in the area of Pyrolysis, Biofuels, Fluidized bed, Multiphase flow and waste to wealth.

Contributors

Bhargav Baruah is a PhD research scholar at the Department of Chemical Engineering, Indian Institute of Technology Guwahati, India. He acquired his bachelors in Mechanical Engineering from Gauhati University (2015) and MTech from the Department of Energy, Tezpur University (2017). His topic of research is Investigating the thermal maturation mechanism of oil shales. His research interest includes thermochemical conversion, reaction kinetics, heat transfer in solids and fluids, engineering thermodynamics, reservoir engineering, and engineering design. He has multiple international publications and conference presentations in the field of pyrolysis. He is skilled in sophisticated analytical instruments such as HP-TGA, TGA, GC, FTIR, BET, NMR, and XRD. He is also a teaching assistant for Natural Gas Engineering under NPTEL.

Prof. Lalit M. Pandey is an Associate Professor at the Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati. He is also faculty associated with the Centre for the Environment of Indian Institute of Technology Guwahati. He received his PhD in Chemical Engineering from the Indian Institute of Technology Delhi. Before joining the IIT Guwahati, he worked as a Scientist with the Central Pollution Control Board, Ministry of Environment & Forests, Government of India. He has published over seventy research articles in international journals and serving as Associate Editor in Frontiers in Bioengineering and Biotechnology and Frontiers in Materials. His main research interests include Biointerfaces and Biomaterials, Biochemical Engineering, and Environmental Biotechnology.

Prof. Pankaj Tiwari is an Associate Professor in the Department of Chemical Engineering at the Indian Institute of Technology Guwahati. He is also faculty associated with the Centre for the Environment of Indian Institute of Technology Guwahati. He has completed his Bachelor of Engineering and Master of Technology, both in chemical engineering from MITS Gwalior and IIT Kanpur respectively. He has received his PhD degree from the University of Utah, USA in 2012. His research area focuses on Biofuel Production, Pyrolysis, Enhanced Oil Recovery Schemes, Reservoir Engineering, Multiphase Flow Phenomena and Fluidized bed.

Poulami Datta is a PhD scholar at the Centre for the Environment of Indian Institute of Technology Guwahati, India. She acquired her B.Tech and M.Tech in Biotechnology from West Bengal University of Technology, Kolkata in 2013 and 2015, respectively. She has been working in the field of "Indigenous Biosurfactant Producing Strains for Potential Applications in Enhanced Oil Recovery" since 2015 for her PhD research work. Her research interest includes Microbiology, Bioprocess Engineering and Environmental Biotechnology. She has published two papers in Bioresource Technology and Journal of Petroleum Science and Engineering as the first author and is a co-author of three other papers. She has also attended many national and international conferences in the field of Environmental Biotechnology. She has expertise in several sophisticated analytical instruments such as FTIR, HPLC, LC-MS, NMR and TGA.

Puja Kalita is currently pursuing PhD at Indian Institute of Technology Guwahati in the Department of Chemical Engineering. Prior to this, she has worked as an Assistant Professor at Uttaranchal University, Dehradun (2017-2019). She completed her bachelor's degree in Petroleum Engineering from Dibrugarh University (2015) and master's degree from Indian Institute of Technology (Indian School of Mines) Dhanbad (2017). Her topic of research is investigating the feasibility of using nanofluid in Enhanced Oil Recovery through modeling and simulation. Her research interest includes reservoir engineering, enhanced oil recovery, colloids and interfaces and reservoir modelling and simulation.

Dr. Rahul Saha has joined the post of Assistant Professor in the Department of Chemical Engineering at National Institute of Technology Hamirpur, Himachal Pradesh, India in November 2019. He was also a former Assistant Professor in School of Petroleum Technology at Pandit Deendayal Petroleum University, Gandhinagar, Gujarat, India. He received his PhD and Masters (M Tech) degree in Chemical Engineering from Indian Institute of Technology Guwahati, India in 2019 and 2013 respectively. He completed his Bachelors in Chemical Engineering from Pune University, India in 2010. His research area is focused on chemical (alkali, surfactant Polymer) induced enhanced oil recovery, colloids & interface science, adsorption kinetics, rheology, flow through porous media, nanotechnology in oil recovery and biodiesel.

Dr. Ranjan Phukan is currently working as an Assistant Professor in the Department of Petroleum Technology, Dibrugarh University, Assam. He obtained his PhD degree from IIT, Guwahati, M.Tech. degree from Dibrugarh University, and B.E.degree from Gauhati University. He has more than 12 years of experience in academia/research, and 2 years of professional experience in the oil industry. His research interests include reservoir engineering, improved oil recovery – both conventional and hybrid methods. He has a number of international publications and conference presentations to his credit. At Dibrugarh University, he is teaching some of the industry oriented courses in Petroleum fluids, Reservoir Engineering and Production Engineering. He is also the in-charge of the state of the art Core Analysis and Core Flood Laboratories.

Swati Sharma is a PhD research scholar at the Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, India. She acquired her bachelors in Biotechnology from RV College of Engineering, Bangalore (2014) and MTech from the Department of Biotechnology, NIT Durgapur (2016). Her topic of research is enhanced crude oil bioremediation using surface modified nanoparticle coated on immobilized inherent microbial consortia. Her research interest includes petroleum Microbiology, Bioremediation, Adsorption, and Hydrophobic surface modification. She has multiple international publications and conference presentations in the field of microbial biodegradation. She is skilled in sophisticated analytical instruments such as GC, FTIR, HPLC, and AES.

Sombir Pannu completed his B.Tech from Kurukshetra University in Chemical Engineering and M.tech from Indian Institute of Technology Guwahati, in Chemical Engineering (Petroleum Science and Technology). His master's project involved working on the Microbial and Chemical Enhanced oil recovery methods: Both experimental and simulation studies. He joined PhD in Indian Institute of Technology Delhi, in July 2020. His area of research focuses on the sequestration of Carbon dioxide in deep sedimentary geological formation.

Vikas Kumar Sharma is a PhD research scholar at chemical engineering department, Indian Institute of Technology Guwahati, India. He received his master's degree in Petroleum Engineering from Rajiv Gandhi Institute of Petroleum Technology, Jais (2017) and Bachelor's degree in Applied Petroleum Engineering from University of Petroleum & Energy Studies, Dehradun (2013). He is currently working on Two phase flow studies in microfluidic porous medium for Enhanced oil recovery applications. His research area is flow through porous media, enhanced oil recovery, Microfluidic using Micro particle image velocimetry.

Abbreviations/Nomenclature

ABC	ATP-binding cassette
AD	Anaerobic digestion
AMPS	2-Acrylamido-2-methyl propane sulfonate
ANN	Artificial neural network
ANOVA	Analysis of variance
AOR	Additional oil recovery
APG	Alkyl polyglucoside
API	American Petroleum Institute
ASAG	Alkaline-surfactant-alternated-gas/CO_2
ASP	Alkali surfactant polymer
ATCC	American type culture collection
ATP	Adenosine triphosphate
BBD	Box-Behnken design
BET	Brunauer–Emmett–Teller
BOAST	Black oil applied simulation
BPR	Back pressure regulator
BS	Biosurfactant
C/N	Carbon to nitrogen ratio
CCD	Central composite design
CCR	Carbon catabolite repression
CCS	Carbon dioxide capture and storage
CEOR	Chemical-enhanced oil recovery
CEWAG	Chemically enhanced water alternated gas
CMC	Critical micelle concentration
CMR	Cyclic microbial recovery
CNPC	China National Petroleum Corporation
COD	Chemical oxygen demand
CS	Chemical surfactant
CT	Computed tomography
CTAB	Hexadecyltrimethylammonium bromide
CWOW	Cycle of washing wells
DCT	Drop collapse test

DGGE	Denaturing gradient gel electrophoresis
DNA	Deoxy ribonucleic acid
DNB	Denitrifying bacteria
DOE	Design of experiment
EDX	Energy-dispersive X-ray analysis
EEOR	Enzyme-enhanced oil recovery
EI	Emulsification index
EOR	Enhanced oil recovery
EPS	Exopolysaccharides
FDM	Finite difference method
FEM	Finite element method
FE-SEM	Field emission scanning electron microscope
FMB	Fermentation bacteria
FTIR	Fourier-transform infrared spectroscopy
GA	Genetic algorithm
GC-MS	Gas chromatography–mass spectroscopy
GMEOR	Genetically engineered MEOR
GOR	Gas–oil ratio
HAAs	3-(3-Hydroxyalkanoyloxy) alkanoates
HBL	Hydrophilic–lipophilic balance
HCPV	Hydrocarbon pore volume
HDB	Hydrocarbon degrading bacteria
HPAM	Hydrolysed polyacrylamide
HPLC	High-performance liquid chromatography
IB	Iron bacteria
IFT	Interfacial tension
IMEOR	Indigenous microbial enhanced oil recovery
IMPES	Implicit pressure, explicit saturation
IOP	Initial oil in place
IRS	Institute of Reservoir Studies
LC-MS	Liquid chromatography–mass spectroscopy
LPG	Liquefied petroleum gas
LSWI	Low salinity water injection
M	Mobility ratio
MALDI-TOF-MS	Matrix-assisted laser desorption ionization time-of-flight tandem mass spectrometry
MBE	Material balance equation
MEL	Mannosylerythritol lipids
MEOR	Microbial-enhanced oil recovery
MFR	Microbial flooding recovery
MMP	Minimum miscibility pressure
MPN	Most probable number
MRST	MATLAB reservoir simulation toolbox
MSM	Minimal salt medium
MSPR	Microbial selective plugging recovery

MWR	Microbial wax removal
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NGS	Next-generation sequencing
NMR	Nuclear magnetic resonance
NP	Nanoparticle
NRB	Nitrate reducing bacteria
NRPS	Non-ribosomal peptide synthetase
NVP	N-Vinylpyrrolidone
O/W	Oil in water
ODT	Oil-displacement test
OFAT	One-factor-at-a-time
ONGC	Oil and Natural Gas Corporation Limited
OOIP	Original oil in place
OPEC	Organization of the Petroleum Exporting Countries
OPM	Open porous media
PAH	Polyaromatic hydrocarbon
PAM	Polyacrylamide
PBD	Plackett Burman design
PCR	Polymerase chain reaction
PG	Pressure gauge
PHAs	Polyhydroxyalkanoates
PHB	Polyhydroxybutyrate
PIMP	*Pseudomonas* and its metabolic products
ppm	Parts per million
PT	Pressure transducer
PV	Pore volume
R/A	Resin to asphaltene ratio
R/P	Reserve to production ratio
rDNA	Recombinant deoxyribonucleic acid
RDPI	Reservoir drive performance index
rRNA	Ribosomal ribonucleic acid/recombinant ribonucleic acid
RSM	Response surface methodology
SAG	Surfactant alternated gas
SAGD	Steam-assisted gravity drainage
SB	Sulfur bacteria
Scf/bbl	Standard cubic foot per barrel
Scf/Stb	Standard cubic foot per stock tank barrel/barrel
SDS	Sodium dodecyl sulphate
S_{oi}	Initial oil saturation
S_{or}	Residual oil saturation
S_{orbf}	Oil collected over residual oil saturation after biosurfactant flooding
S_{orwf}	Residual oil saturation after water flooding
SP	Surfactant polymer

SRB	Sulphate reducing bacteria
ST	Surface tension
S_{wi}	Initial water saturation
TCA	Tricarboxylic acid
TERI	The Energy and Resources Institute
TGB	Total growth bacteria
T-RFLP	Terminal restriction fragment length polymorphism
UDP	Uridine diphosphate
US-DOE	US Department of Energy
VFA	Volatile fatty acid
VSM	Vibrating sample magnetometer
W/O	Water in oil
WAG	Water alternating gas
X_i	OOIP − Volume of oil collected after water flooding
XPS	X-ray photoelectron spectroscopy
XRD	X-ray diffraction

Symbols

μ	Fluid viscosity (Nsm^{-1})
μ_{inj}	Viscosity of injected fluid (cp)
μ_o	Viscosity of oil (cp)
^{13}C	Carbon-13
^{1}H	Proton
A	Cross-sectional area (m^{2})
AccA	Acetyl-CoA carboxylase
acdH	Alcohol dehydrogenase
acS	Acyl-CoA synthetase
addH	Aldehyde dehydrogenase
$A_i X_i$	Linear expression as the effect of each variable on response factor
$A_{ii} X_i^2$	Square terms interactions
$A_{ij} X_i X_j$	Interaction between independent variables and their role in response output
Al_2O_3	Aluminium oxide
AlgC	Phosphomannomutase
AlkB-Rub	Alkane hydroxylase
bbl	Barrel of oil
B_g	Gas formation volume factor
B_o	Oil formation volume factor
C	Carbon
C/N	Carbon to nitrogen ratio
C=O	Carbonyl

Ca^{++}	Calcium ion
c_f	Formation compressibility of rock
CH	Methine
CH_2	Methylene
CH_3	Methyl
CH_4	Methane
C_N	Total number of replica tests performed for the centric point
CO_2	Carbon dioxide
CoA	Coenzyme A
C-O-C	Ether
CO-NH	Amide
CuO	Copper oxide
c_w	Water compressibility
cyp153	Alkane monooxygenase
dTDP	Deoxythymidine diphosphate
dTDPL-rhamnose	Deoxythymidine diphosphate L-rhamnose
E_g	Expansion of gas cap
E_o	Expansion of oil and total dissolved gases
$E_{w,f}$	Expansion of connate water and rock
E_X	Effect of variable X
F	Total fluid withdrawn
f_{inj}	Fraction of injected fluid
Fr	Mole fraction of intermediates
G_{inj}	Cumulative gas injected
G_p	Cumulative gas produced
H	Hydrogen
H_2	Hydrogen gas
Int	Oil intermediates (C_2-C_4, H_2S and CO_2)
K	Effective permeability (mD)
k	Biodegradation rate constant
k_La	Volumetric mass transfer coefficient
k_o	Effective permeability of oil (mD)
L	Length (m)
L	Total number of experiments in Taguchi model
M	Mobility ratio
m/z	Mass to charge
mg/L	Milligrams per liter
Mg^{++}	Magnesium ion
mN/m	Milli Newton per meter
mN/m	Milli Newton per meter
MW_{C5+}	Molecular weight of pentance plus fractions
MW_{C7+}	Molecular weight of heptance plus fractions
N	Nitrogen
N	Total number of trials performed in PBD/ Number of independent variables in CCD

N_2	Nitrogen gas
Na_2CO_3	Sodium carbonate
$NaHCO_3$	Sodium bicarbonate
NaOH	Sodium hydroxide
N-H	Amine
NO_3^-	Nitrate ions
O	Oxygen
Ø	Porosity
O_2	Oxygen gas
p	Pressure (psi)
P	Polymer
p	Statistical significance determinant P-value
p_c	Reservoir pressure (psi)
P_c	Critical pressure
PhbA	β-ketothiolase
PhbB	Acetoacetyl-CoA reductase
PhbC	PHB synthase
PO_4^{2-}	Phosphate ions
ppm	Parts per millions
Q	Flow rate (m^3s^{-1})
q_t	Total flow rate (bbl/day)
RhlA	3-(3-Hydroxyalkanoyloxy) alkanoate synthetase
RhlB	Rhamnosyl transferase
RhlC	dTDP-L-rhamnose using enzyme rhamnosyl transferase
RmlA	Glucose-1-phosphate thymidylyltransferase
RmlB	dTDP-D-glucose 4,6-dehydratase
RmlC	dTDP-4-dehydrorhamnose 3,5-epimerase
RmlD	dTDP-4-dehydrorhamnose reductase
R_p	Cumulative gas–oil ratio
rpm	Rotations per minute
R_s	Gas solubility
S	Sulphur
sg	Specific gravity (kgm^{-3})
SiO_2	Silicon dioxide
SO_4^{2-}	Sulphate ion
sp.	Unspecified species
spp.	Several (plural) species
T_c	Reservoir temperature (°R)
T_c	Critical temperature
TiO_2	Titanium dioxide
T_{res}	Reservoir temperature ^oR,
U/mL	Units of activity per millilitre
v/v	Volume by volume
Vol	Oil volatiles (C_1 and N_2)
vvm	Volume of liquid per minute

w/v	Weight by volume
W_e	Cumulative water influx
Wi	Mass of *i*th fraction
W_{inj}	Cumulative water injected
W_p	Cumulative water produced
X	Factor X
Y(X)	Predicted response in RSM
Yi	Mole fraction of *i*th fraction
Y_{XH}	Yield of variable X at a high level
Y_{XL}	Yield of variable X at a low level
α-D	Alpha-dextro
$\Delta\rho$	Density difference between injected fluid and oil (gm/cm^3)

Petroleum Reservoirs and Oil Production Mechanisms

Bhargav Baruah, Puja Kalita, Lalit Pandey, and Pankaj Tiwari

1 Introduction

A petroleum reservoir is a subsurface distribution of pore networks formed between strata of sedimentary rock formations, consisting of two or more hydrocarbon fluids and water. The hydrocarbons are formed by degradation of organic matter, both marine and terrestrial origins by the influence of high pressure and temperature over a long period. This phenomenon occurs in the source rock (Ardakani et al. 2017; Baruah and Tiwari 2020). The produced hydrocarbons then migrate into the empty pores and voids present in the rock formations and form a pool (reserve) of hydrocarbon fluids (Phukan et al. 2019a; Saha et al. 2018a). The reservoir rock requires to be capped to prevent migration (seepage) of these fluids to the surface under influence of buoyancy, capillarity, and other forces (Aplin and Macquaker 2011). The most important properties of a reservoir are the volume of oil and gas, recovery factory of the oil and gases in the reservoir, compositional and physical properties of the reservoir rock, and types of hydrocarbons present in the reservoir (Aplin and Macquaker 2011; McCain Jr 1973).

The reservoir fluids (oil, water, and gas) which are originally present within the pore spaces at the time of discovery contribute to the energy responsible for inducing flow and production of these fluids from the reservoir. These fluids are initially contained in the reservoir under very high pressure until drilling and production operations are carried out to release the trapped energy within the reservoir. The reservoir pressure starts declining steadily as fluids are produced from the reservoir to the surface. Therefore, when the pressure of the reservoir is reduced, the fluids are subjected to changes due to the expansion of the fluids and compressibility of the fluids and rocks (Dusseault 2011). The natural flow of hydrocarbons from

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B. Baruah · P. Kalita · L. Pandey · P. Tiwari (✉)
Department of Chemical Engineering, Indian Institute of Technology Guwahati, Guwahati 781039, Assam, India
e-mail: pankaj.tiwari@iitg.ac.in

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the reservoir rock occurs due to the expansion of reservoir rock, expansion of an aquifer underlying the oil zone, expansion of the fluids, and gravitational energy that segregates the fluids in the reservoir (Amit 1986; Dusseault 2011). The performance of a reservoir is hence dependent on the type of energy capable of drawing fluids from the reservoir to the wellbore and then to the surface. This energy governs the producing mechanism of a crude oil system and is commonly known as the drive mechanism for a reservoir.

The recovery of petroleum from its reserve is achieved by three methods: (i) primary recovery, (ii) secondary recovery, and (iii) tertiary recovery (Vishnyakov et al. 2020). In primary recovery, the hydrocarbons present in a particular reservoir are extracted to the surface by natural reservoir drive mechanisms such as, aquifer water moving the crude oil downwards from the reservoir pores into the production well, expansion of reservoir caps, expansion of dissolved gases in the crude oil, and gravity drainage as a result of the movement of crude oil from higher to lower saturation in the reservoir. The extraction of hydrocarbons by primary recovery has been recorded to be about 5–15% of the original oil in place (OOIP) (Vishnyakov et al. 2020; Viswanathan 2017). Over the lifetime of a production well, as the production of hydrocarbons from the reservoir increases, the reservoir pressure decreases. After a certain period, the prevailing reservoir pressure is inadequate to drive the hydrocarbons from the reservoir to the surface. In this situation, secondary recovery methods are utilized. The secondary recovery techniques provide supplementary energy to the reservoir by injecting different fluids such as water and gas (gas produced from the reservoir or carbon dioxide (CO_2)) (Srivastava and Huang 1997; Talebian et al. 2014) to increase the reservoir pressure, thus increasing or substituting the natural reservoir drive and therefore improve the mobility of the in situ hydrocarbons. Secondary recovery techniques have shown an average recovery of 35 and 45% of OOIP (Tzimas et al. 2005). However, with the application of primary and secondary recovery methods about 60% of OOIP remains in the reservoir (Gbadamosi et al. 2019a, b). Therefore, to increase the production of hydrocarbons tertiary recovery or enhanced oil recovery (EOR) methods are used (Datta et al. 2018; Datta et al. 2020; Phukan et al. 2019b; Saha et al. 2018b). In this chapter, the characterization and classification of petroleum reservoirs and production of reservoir fluids through various possible drive mechanisms are discussed.

2 Reservoir Potential

2.1 Geological Setting

Petroleum reservoirs are primarily found in the sedimentary rocks of the earth's crust (Selley 2003; Selley and Sonnenberg 2015b). Sedimentary rocks are formed by sedimentation, compaction of eroded rock particles into denser mass, and cementing with minerals or chemical precipitates (Zhang et al. 2019). The sediments are compacted and cemented after burial under additional layers of sediment, thereby leading to the formation of multiple strata or layers. Over a long duration of time, numerous organic beings such as dead animals, plants, planktons, etc. are trapped in these strata during compression, and under the influence of temperature and pressure, the trapped organic matter undergoes different biological, biochemical, and thermochemical alterations leading to the formation of hydrocarbons (Hunt 1995). The process of conversion of organic matter into kerogen is called diagenesis, and subsequent decomposition of kerogen into oil and gas is called catagenesis (Hunt 1995; Tissot and Welte 1984). The sedimentary rocks in which hydrocarbons originate are called source rocks (McCarthy et al. 2011). These rocks are broadly classified into two types: (i) *clastic sedimentary rocks*—formed due to weathering and depositions of rock particles of different grain sizes (e.g., sandstones, mudstones, and shales) (Fralick and Kronberg 1997), and (ii) *chemical or biochemical sedimentary rocks*—formed by chemical processes (e.g., carbonate and carbonate precipitates like calcite, limestone, dolomite, halite, and gypsum) (Boggs Jr and Boggs 2009). The geological setting of a petroleum system significantly affects the diagenesis process and reservoir quality (Ehrenberg and Nadeau 2005). Around 60% of the total world crude oil reserves originate in carbonate reservoirs. The Gulf countries contain 62% of the total oil reserve, out of which 70% reservoirs are carbonate reservoirs (Joshi and Singh 2020b).

The produced hydrocarbons in the source rock migrate into the adjacent porous rocks or reservoir rocks due to an increase in pressure. This movement of produced hydrocarbons from the source rock into the voids of the reservoir rock is termed primary migration (Chapman 1972; Eseme et al. 2007). The accumulated hydrocarbons travel and settle in the inter-connected pore networks of the strata of adjacent reservoir rocks. This movement of hydrocarbons in the reservoir rocks is known as secondary migrations. The movements of the hydrocarbons accumulated in the pore networks of the reservoir rocks are restricted by certain rock formations known as traps (Harding and Lowell 1979; Mitra 1990). The accumulated hydrocarbons (crude oil and natural gas) are followed by water and other inorganic gases (carbon dioxide (CO_2), carbon monoxide (CO), etc.). The natural gases along with the trapped inorganic gases occupy the top section of the trap and the water occupies the bottom section. An impermeable rock known as the caprock (trap) prevents any movement of the hydrocarbons out of the reservoir rock. The traps are generally classified into three types: structural, stratigraphic, and hydrodynamic. Structural traps are formed as a result of geological and tectonic activities (faulting, folding, etc.) in the subsurface which leads to the occurrence of structural changes such as folds, anticlines, and domes in different strata (Allen and Allen 2005). Stratigraphic traps are created by variations in the porosity, thickness, and texture of the reservoir rocks, and by lateral and vertical differences in its lithology (Allen and Allen 2013). Hydrodynamic traps are formed due to the variance in water pressure and the flow of underground aquifer water, leading to the formation of a tilt in the water–hydrocarbon interface in the subsurface (Allen and Allen 2013). The accumulated hydrocarbons are prevented migrating from the reservoir rock to the surface by a geological structure known as a seal. A seal is formed when the capillary pressure across the pore throats of the reservoir rock is greater or equal to the buoyancy pressure of the moving hydrocarbons (Bradley and Powley 1994; Watts 1987). The capillary seals are of two types; (i) hydraulic and (ii) membrane seal, which keep the fluids in the reservoir domain (Bradley and Powley 1994).

2.2 Petroleum Reserves

Petroleum reserves are categorized into three types by the analysis of different geological and engineering survey records: (i) *proven reserves*—petroleum reservoirs which can be projected with realistic assurance to be commercially recoverable, (ii) *probable reserves*—unproved petroleum reserves which are more likely than not to be recoverable, and (iii) *possible reserves*—unproved reservoirs whose hydrocarbon potential is less likely to be recoverable by existing operation methodologies (Flåm and Moxnes 1987; Garb 1985; SPE 1997). Hydrocarbon reservoirs are considered probable reserves if the probability of hydrocarbon recovery is at least 50% of the sum of estimated proven and probable reserves. These reservoirs are predicted to be proven by normal drilling methods where subsurface control systems are insufficient to categorize them as proven reservoirs (SPE 1997). Possible reserves may include reservoirs that could feasibly occur outside of areas categorized as probable reserves and seem to contain hydrocarbons based on well log and core analyses but may not sustain commercial production due to technical and geological limitations (SPE 1987, 1997).

Global-proven oil reserves have been recorded to be around 1734 billion barrels in 2019 (BP 2020; CIA 2020; OPEC 2019). Among the oil reserves around the world, South and Central American oil reserves have the highest estimated reserve to production (R/P) ratio of 144 years, whereas Europe has the lowest of 12 years (BP 2020). The distribution of the total proven crude oil reserves around the world is presented in Fig. 1. The countries which possess the highest oil reserves are Venezuela (17.5%), Saudi Arabia (17.2%), and Canada (9.8%) (CIA 2020; Joshi and Singh 2020b). As of November 2020, the top 10 proven reserves in the world are Venezuela (304 billion barrels), Saudi Arabia (298 billion barrels), Canada (170 billion barrels), Iran (156 billion barrels), Iraq (145 billion barrels), Russia (105 billion barrels), Kuwait (102 billion barrels), UAE (98 billion barrels), United Nations (69 billion barrels), and Libya (48 billion barrels) (BP 2020). As per the annual statistical bulletin of 2019 by Organization of Petroleum Exporting Countries (OPEC) (OPEC 2019), 79.4% of the total proven oil reserves are located in OPEC member countries.

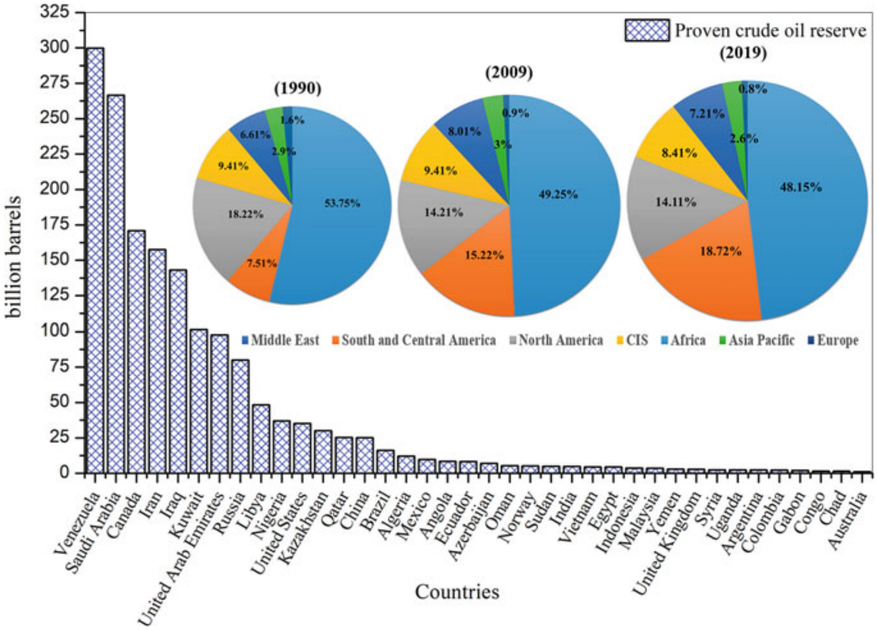

Fig. 1 Proved crude oil reserves for different countries [data are extracted from CIA Energy Outlook, August 2020 (CIA 2020)]

3 Physicochemical Characterization of a Petroleum Reservoir

The primary objective of any petroleum industry is the recovery or extraction of hydrocarbons from the discovered petroleum reservoirs. The composition of a petroleum reservoir is critical to petroleum recovery specifically to the implementation of enhanced oil recovery techniques. The mineralogical composition of the reservoir, surface morphology, and pore structure and distribution are of critical importance to the petroleum industry, both from the scientific and industrial point of view.

3.1 Composition and Mineralogy of Petroleum Reservoir

Petroleum reservoirs are predominantly composed of sandstone or carbonate (Bjørlykke and Jahren 2010). Sandstone reserves possess a high percentage of quartz and sand grains, along with the presence of feldspar, and clay minerals such as Illite and Kaolnite (Baruah et al. 2019; Saha et al. 2017). Indian reserves are mostly sandstone reservoirs. Sandstone reserves exhibit high porosity and permeability compared to carbonate reservoirs. Carbonate reservoirs are found abundantly in Gulf and Russia with recent discoveries in Brazil, Egypt, Kazakistan, and Libya (Joshi and Singh 2020a). Carbonate reserves are formed by the deposition of calcareous minerals and compounds. The compositional, morphological, and petrographic properties of petroleum reservoirs are widely studied by using different analytical techniques such as X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), field emission scanning electron microscope and electron dispersive X-ray spectroscopy (FE-SEM/EDX), Brauner–Emmet–Teller (BET), computed tomography (CT) scan, Fourier-transform infrared spectroscopy (FTIR), vibrating sample magnetometer (VSM), porosimeter, and permeameter (Al-Jaroudi et al. 2007; Ehrenberg and Nadeau 2005; McCarthy et al. 2011; Phukan et al. 2019a; Qiao et al. 2020; Saha et al. 2017).

The composition of a reservoir rock is studied by identifying the minerals present in the sample by using XRD and FE-SEM/EDX. These instruments provide a quantitative analysis of the minerals present in the reservoir rock sample. The XRD analysis also shows the crystallinity index of the rock sample. Identification of the mineral composition of reservoir rock demonstrates the charge (cationic or anionic) present in the reservoir rock. The information about the charge of the reservoir rock along with the ionic behavior of oil present in the reservoir helps in the identification and selection of surfactants to be used for enhanced oil recovery (EOR) in the particular reservoir. Sandstone or silica reserves are negatively charged, whereas carbonate rocks are positively charged with some carbonate reserves showing a neutral charge. FTIR analysis is performed to recognize various functional groups present in the reservoir rock. Along with the minerals, the presence of certain elements in the reservoir rock may affect the charge of the reservoir rock as well as the interactions of chemicals (surfactant) with the reservoir rock during the EOR process. XPS analysis can identify different elements and their chemical and overall electronic structures. FE-SEM, CT scan, and BET analyses help in the identification of surface morphology of the reservoir rock and pore structure, fracture size, and pore volume. SEM analysis is extensively used to provide qualitative information about the pore geometry of rocks by both direct and indirect methods (Phukan et al. 2019a; Saha et al. 2017, 2019). The porosity of the reservoir rock can be identified using a mercury or helium porosimeter, and the permeability is measured by using a permeameter (steady-state or transient state). The magnetic properties of a reservoir rock (ferromagnetic or paramagnetic) can be identified using VSM analysis. The effect of magnetic properties of the reservoir is generally observed during well logging.

3.2 Characterization of Pore Distribution

Sedimentary rocks are formed by continuous weathering activities such as erosion, transportation, and deposition. The surface morphology and the compositions of the sedimentary rocks depend on the mineralogy of the parent rock and the effect of chemical and physical alterations on the weathered rocks of different grain sizes and shapes during deposition and transportation. This leads to the formation of pore networks in the reservoir rocks. The distribution of oil and gas in a petroleum reservoir depends primarily on the porosity and permeability of the reservoir rock.

Porosity is a measure of the void or empty space in reservoir rocks (ratio of pore volume to bulk volume). Porosity is generally expressed as (i) total porosity—the void space present inside the reservoir irrespective of the voids being interlinked or isolated and (ii) effective porosity—the total void of the interconnected pore network. Total porosity is further classified into primary porosity, secondary porosity, and fracture porosity (Ganat 2020). The porosity ($\emptyset$) of a reservoir rock is governed by the pore volume (V_P) and the bulk volume (V_B). Mathematically porosity can be expressed as

$$\phi = \frac{V_B - V_G}{V_B} = \frac{V_P}{V_B} \tag{1}$$

where V_G is the grain volume of the reservoir rock.

Porosity can be measured by (i) core analysis, which is a direct method in which a core sample of the reservoir rock is taken and the pore distribution is studied by using a porosimeter, and (ii) well logging, which measures the porosity as a function of the electrical properties of the rock and termed as indirect method (Hu and Huang 2017). Sandstone reserves generally exhibit porosity in the range of 10–40%, and carbonate reservoirs possess porosity in the range of 5–25% (Morton-Thompson and Woods 1993).

Permeability is defined as the ability of a fluid to flow in the pores of a reservoir rock. Permeability is classified as (i) absolute permeability—measures of the permeability of a single fluid through a pore network and (ii) effective permeability—the ability of a reservoir rock to favor the flow or of a particular fluid through the rock in the presence of different immiscible fluids which are accumulated in the reservoir rock (Fanchi 2010). Permeability (unit is Darcy (D) or millidarcy (mD) is measured by using Darcy law (Eq. 2)

$$Q = \frac{KA(P_i - P_o)}{\mu L} \tag{2}$$

where K is the effective permeability, P_o is the outlet fluid pressure, P_i is the inlet fluid pressure, Q is the flow rate, μ is the fluid viscosity, L is the tube length, and A is the cross-sectional area.

The permeability of petroleum reservoirs ranges from 0.1 to more than 1000 mD. A petroleum reservoir is graded to be poor, fair, moderate, good, and very good for permeability values (mD) of $k < 1$, $1 < k < 10$, $10 < k < 50$, $50 < k < 250$, and $k > 250$, respectively. The permeability of a petroleum reservoir is affected by geological factors such as, the shape and size of sand grains, lamination, cementing, fracturing, and solutions (Tiab and Donaldson 2016).

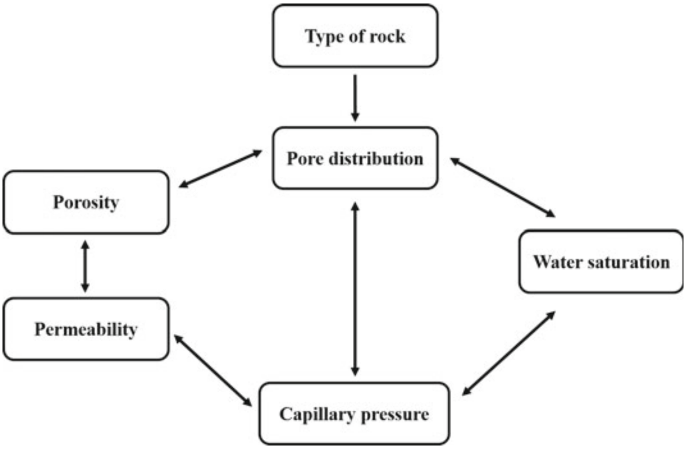

Fig. 2 Relation between basic rock pore properties [data extracted from Archie (1950)]

The correlations between porosity and permeability have been extensively studied in reservoir characterization and petroleum geology. Permeability of void space is always expressed as a function of porosity; however, different factors such as grain size, packing, and compaction of grain particles affect the relationship between porosity and permeability. Though the porosity of a rock is not influenced by the grain size, the permeability of a rock is inversely proportional to the particle size (Nelson 1994; Tiab and Donaldson 2016). Porosity and permeability generally decrease with an increase in depth (Bloch et al. 2002). Ehrenberg and Nadeau (Ehrenberg and Nadeau 2005) reported a comprehensive study on porosity and permeability of sandstone and carbonate reservoir distribution around the world. With an increase in depth of dolomite or limestone (calcite) reservoirs, the porosity appeared to be much less for limestone reservoirs as compared to dolomite reserves. The permeability has been recorded to be the same for both the reservoirs. In sandstone reservoirs, the porosity and permeability were found to increase dramatically after a depth of 4 km (Ehrenberg and Nadeau 2005). The rock properties such as capillary pressure and water saturation are directly reliant on the pore distribution of a reservoir rock (Fig. 2) and are directly influenced by the inherent porosity and permeability present in the reservoir rock (Archie 1950).

3.3 *Reservoir Fluid Properties*

The hydrocarbons present in the reservoir are classified based on their compositions, API gravity, formation volume factor, liquid and gas specific density, solution gas–oil ratio, bubble point, saturation and dew point pressure, and critical point. Crude oils are graded based on their physicochemical properties such as specific gravity (sg), sulfur content, and viscosity. Natural gas is classified into two types, viz., wet gas, and dry gas based on the solution gas-oil ratio (GOR). The market value of crude oil is governed by its API gravity and compositions, especially sulfur content. Based on API gravity crude oil is classified as light component oil (API > 31.1°, sg < 870 kg/m^3), medium quality oil (22.3° < API < 31.1°, sg 870–920 kg/m^3), heavy crude oil (10° < API < 22.3°, sg 920–1000 kg/m^3), and the extra-heavy crude (black) oil (API < 10°, sg > 1000 kg/m^3). The average composition of crude oil is 79.5–87.1% carbon (C), 11.5–14.8% hydrogen (H), 0.1–3.5% sulfur (S), and 0.1–0.5% nitrogen (N) and oxygen (O) (Demirbas et al. 2015; Sharma and Pandey 2020; Sharma et al. 2019).

The hydrocarbon mixtures or reservoir fluids after production are analyzed using different laboratory techniques. The analytical techniques are (i) *primary testing*—specific gravity, viscosity, and GOR, (ii) *routine or secondary testing*—compositional analysis of the samples, expansion test, differential analysis test, fluid separation test, and depletion test, and (iii) *specialized laboratory tests*—slim tube test for MMP measurement and fluid swelling test. The detailed explanation of the testing methods has been discussed extensively by Ahmed and coworkers (Ahmed 2019).

4 Classification of Petroleum Reservoir

Hydrocarbon reservoirs are classified as (i) *oil reservoir*—reservoir temperature is lower than critical temperature of the hydrocarbon mixture and (ii) *gas reservoir*—reservoir temperature is greater than the critical temperature of the hydrocarbon mixture. Oil reservoirs contain high molecular weight hydrocarbons or crude oil with small fractions of natural gas saturated in the oil. Gas reservoirs contain a high concentration of natural gas with a small percentage of lower molecular weight oil. The oil and gas reservoirs are further classified into several sub-divisions based on the following four criteria: (i) Composition of hydrocarbon mixture, (ii) types of the reservoir drive mechanism, (iii) prevailing pressure and temperature of the reservoir, and (iv) pressure and temperature at surface facilities (eg. separator). The phase diagrams for different types of reservoir fluids are presented in Fig. 3.

Based on composition, fluid properties, and pressure–temperature relation, oil reservoirs are classified into four categories (Fig. 3).

Black oil reservoirs contain a high concentration of higher molecular weight hydrocarbons, with a very small percentage of intermediate and lower molecular weight fractions. Black oil reservoirs have an initial GOR between 200 and 700 scf/STB, and a gravity of 15–40°API. Black oil reservoirs are classified into (i) *under saturated*—single-phase liquid system with reservoir temperature below the critical temperature, and (ii) *saturated*—entirely saturated by natural gas, and the reservoir temperature and pressure are in the two-phase region.

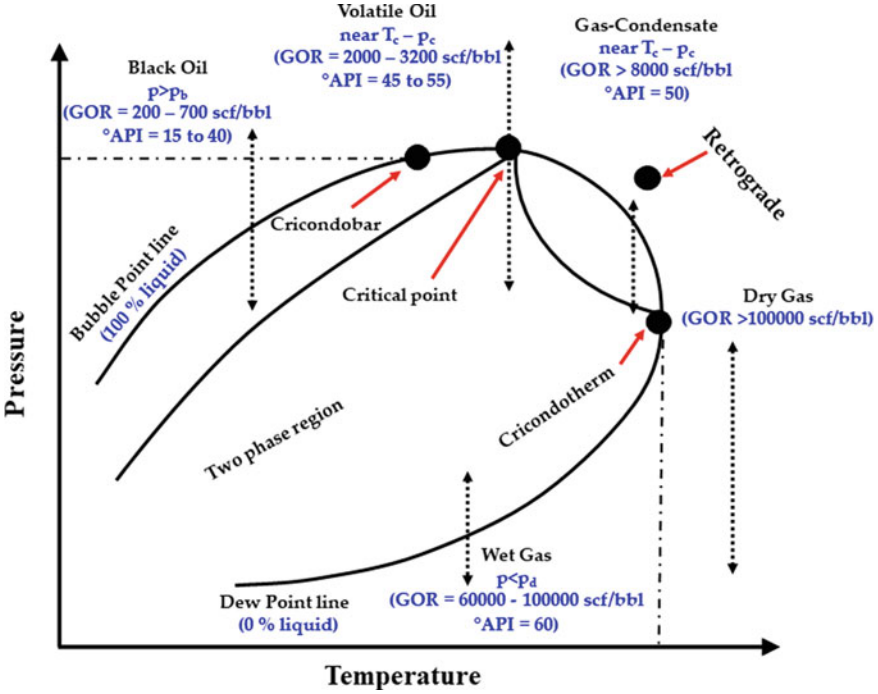

Fig. 3 Phase diagram of different types of petroleum reservoirs. The phase changes with pressure during the isothermal process are shown with broken line-arrow for different fluids

Low shrinkage oil reservoirs are composed of intermediate to higher molecular weight hydrocarbons with formation volume factor (Bo) < 1.2 bbl/STB (Stock tank barrel), GOR < 200 scf/STB, and gravity of <35°API.

Near critical crude oil reservoir approaches the critical temperature of the hydrocarbon mixture with GOR > 3000 scf/bbl and oil formation volume factor of 2 bbl/STB, and contains a lower concentration of methane and a high concentration of ethane through hexane.

Volatile oil reservoirs contain a high percentage of lower and intermediate molecular weight hydrocarbons with GOR in the range of 2000–3200 scf/bbl, formation volume factor of 2 bbl/STB, and gravity of 45–55° API. Volatile oil or high shrinkage oil reservoirs are found at higher depths with a high reservoir pressure and the reservoir temperature is lower than the critical temperature. The oil converts to gases as reservoir temperature approaches the critical point and produces high gas and low liquid yields.

Gas reservoirs are also classified into four categories based on the temperature and pressure of the formation and the surface facilities (Fig. 3).

Dry gas reservoirs: Hydrocarbon mixtures are composed of methane and light hydrocarbon gases and remain in the gas phase both in the reservoir and at the surface. The gases do not undergo a phase change, and no liquid formation occurs as pressure decreases during production. Dry gas reservoirs generally possess GOR greater than 100000 scf/STB.

Wet gas reservoirs: Hydrocarbon mixtures inside the reservoir are in the vapor phase and remain in the same phase during the production when the pressure depleted isothermally. The gas enters a two-phase region during the production (the temperature and pressure decreases) and liquid begins to form (condensation on the surface/separator) with GOR between 60000 and 100000 scf/STB gravity of 60° AP.

Retrograde gas condensate reservoirs: Reservoir temperature appears to be between the critical temperature and the cricondotherm temperature of the hydrocarbon system, and the fluid production is controlled by the thermodynamics. The GOR lies between 8000 and 70000 scf/STB with condensate API gravity of 50° API.

Near critical gas condensate reservoirs: Reservoir temperature occurs in the vicinity of near critical temperature. The GOR and API gravity are similar to retrograde gas condensate reservoirs.

5 Reservoir Drive Mechanisms

Recovery of the oil depends on the drive mechanism active in the reservoir. To optimize maximum recovery from a reservoir, the type of drive present should be identified (Clark 1960). The primary recovery technique utilizes natural energy (drives) existing in the reservoir to produce the crude oil to the surface. Figure 4 shows different drive mechanisms (combined) that contribute toward the production in a typical petroleum reservoir. The primary recovery consists of six driving mechanisms which are characterized primarily in terms of reservoir pressure, GOR, and water-cut (Fig. 5).

Rock and Liquid Expansion Drive: With the production of reservoir fluids, the reservoir pressure diminishes. The liquids and the rock expand due to their compressibility (Ahmed 2006). The expansion of the grain particles in the rock and the compaction of the formation decrease the pore volume and push the liquid out of the pores to the production well. The efficiency of this type of recovery is the least and helps to recover only a small amount of the fluid from the reservoir with a constant value of GOR.

Depletion Drive: The natural gas dissolved in the crude oil provides the energy for the production, hence also known as solution gas drive. The reservoir pressure reaches the bubble point pressure and the natural gas dissolved in oil evolves as bubbles. These bubbles expand as the fluid pressure drops further (Ahmed 2006). The reservoir pressure is maintained as long as these bubbles keep expanding to

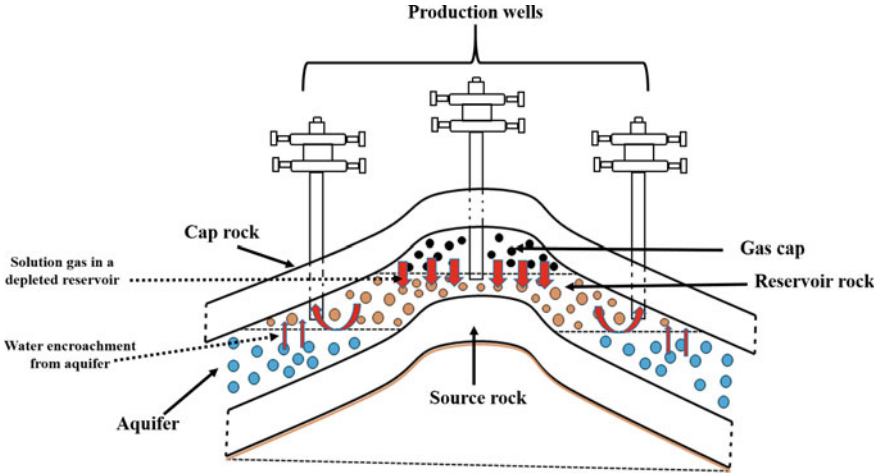

Fig. 4 Different drive mechanisms in a typical petroleum reservoir

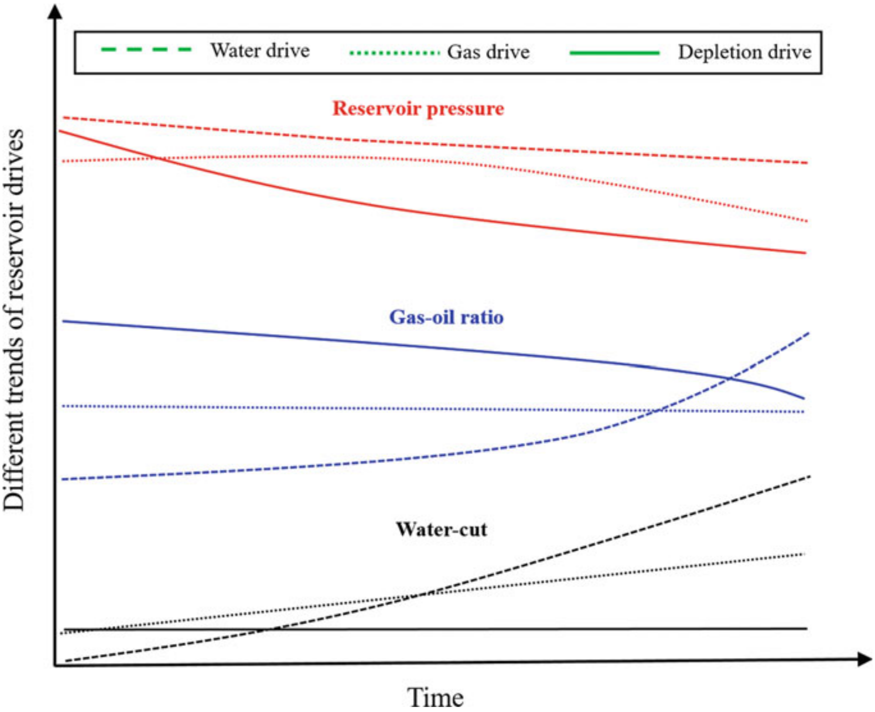

Fig. 5 History of different reservoir drive mechanisms over time

aid in production. The value of GOR increases with production and the reservoir pressure needs to be maintained higher than that of the critical gas saturation (Selley and Sonnenberg 2015a).

Gas cap Drive: As oil is produced from the oil zone, the gas cap expands and maintains the pressure of the reservoir. The gas pushes the oil to the production well (Pope and Nelson 1978). A gas cap is present in the reservoir below the bubble point pressure and it produces very little or no water (Ahmed 2006).

Water Drive: The natural source of energy in this drive is water. The water influx from an aquifer maintains the pressure of the reservoir by occupying the pore spaces created due to oil production. Water drives are used to produce about one-third of the world's reservoirs. Oil production holds steady initially because the pressure is maintained by the water encroached into the oil zone (Selley and Sonnenberg 2015a). The producing GOR is always constant (Glover 2000).

Gravity Drainage Drive: Gravity acts as the drive mechanism for the production of hydrocarbons. It is the natural tendency of oil, gas, water to segregate during production due to their density differences (Alamooti and Malekabadi 2018). This segregation does not directly result in expelling fluid from the reservoir toward the production well. The oil settles to the bottom and the gas migrates to the top portion of the reservoir. An important prerequisite for efficient recovery from gravity drainage is the oil viscosity. Fluid displacement increases as the viscosity of oil decreases. Hence, the recovery rate increases as the viscosity of crude oil decreases (Druetta and Picchioni 2020).

Combination Drive: This type of drive mechanism is usually an association between a gas cap and an active aquifer. The energy available in water and free gas aids in displacing the oil from the reservoirs (Ahmed 2006). The recovery of this drive is also dependent on several factors such as the size of the gas cap, capacity of the aquifer, and the position of the wells (Glover 2000). An oil rim reservoir is another example of a combined drive mechanism in which the accumulation of a small to medium column of oil is in communication with a large gas cap over it and an active aquifer (Lawal et al. 2020).

6 Material Balance Equation (MBE)

The material balance equation has been the most reliable interpretation and prediction method for reservoir engineers to define the initial oil-in-place based on production from the reservoir and static reservoir conditions. Mathematically, the balanced equation depicts the performance of the reservoir (Tank model) by relating liquid and rock expansion to liquid withdrawal and facilitates to (i) estimate the original fluids in place, (ii) determine the producing mechanism, and (iii) predict the prospect reservoir performance (Ahmed 2006).

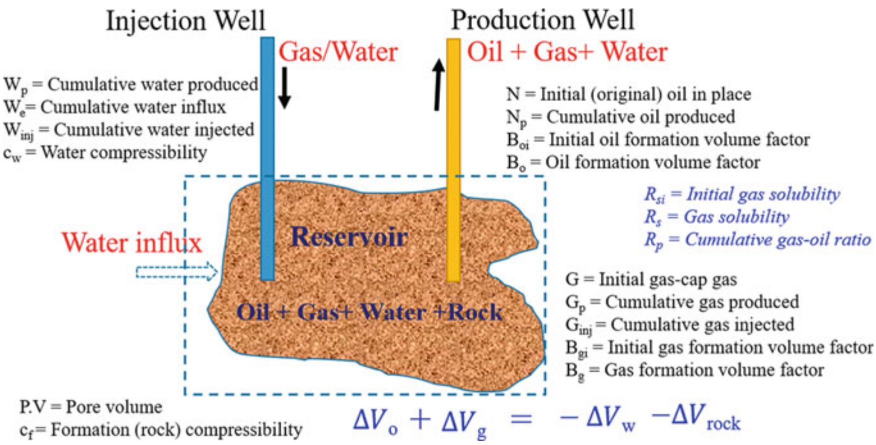

Fig. 6 A schematic of a tank model for material balance

The MBE is a volumetric balance of the reservoir (tank) with the assumption that the reservoir is at constant values of volume and temperature and equilibrium pressure (Fig. 6). The general form of MBE accounts for four phenomena: (i) the reservoir fluid volume withdrawn (cumulative oil and gas production): $N_p\left[B_o + \left(R_p - R_s\right)B_g\right]$, (ii) the net water influx that remains inside the reservoir: $W_e - W_p B_w$, (iii) the net expansion of gas cap that occurs with the production N_p: $mB_{oi}\left(B_g/B_{gi} - 1\right)$, and (iv) the compressible nature of fluids.

The material balance equation considering the external gas injection in the reservoir can be arranged as (Havlena and Odeh 1963)

$$F = N\left[E_O + mE_g + E_{f,w}\right] + \left[W_e + W_{inj}B_w + G_{inj}B_{ginj}\right] \tag{3}$$

where

$F =$ Total fluid (oil, gas, and water) withdrawal, $N_p\left[B_o + \left(R_p - R_s\right)B_g\right] + W_pB_w$
$E_o =$ Expansion of oil and its originally dissolved gas $(B_o - B_{oi}) + (R_{si} - R_s)B_g$
$E_g =$ Expansion of the gas cap, $B_{oi}\left[\left(B_g/B_{gi} - 1\right)\right]$
$E_{f,w} =$ Expansion of connate water and rock, $B_{oi}\left[\frac{C_wS_{wc} + C_f}{1 - S_{wc}}\right]\Delta p$
$m =$ Ratio of initial gas-cap-gas reservoir volume to initial reservoir oil volume
$R_{si} =$ Initial gas solubility
$R_s =$ Gas solubility
$R_p =$ Cumulative gas–oil ratio
$N =$ Initial (original) oil in place
$N_p =$ Cumulative oil produced
$B_{oi} =$ Initial oil formation volume factor
$B_o =$ Oil formation volume factor
$W_p =$ Cumulative water produced
$W_e =$ Cumulative water influx

W_{inj} = Cumulative water injected
G_p = Cumulative gas produced
G_{inj} = Cumulative gas injected
B_{gi} = Initial gas formation volume factor
B_g = Gas formation volume factor
c_f = Formation (rock) compressibility
c_w = Water compressibility.

The three important aspects of the developed MBE are: (i) The value of OOIP, N, (ii) the water encroached, W_e, and (iii) the volume of the gas and the oil, m, can be determined considering the special cases exist in the reservoir domain. For a reservoir with no initial gas cap ($m = 0$) and no water influx ($W_e = 0$), the MBE reduces to

$$F = NE_O \quad \text{where} \quad F = N\left[E_o + mE_g + E_{f,w}\right] + W_e \tag{4}$$

Here, a plot of F versus E_o yields a straight line passing through the origin with N as the slope.

The MBE can be simplified for different cases present in a reservoir production system and the important aspects can be evaluated by obtaining an equation of a straight line. Plots for total withdrawal versus total expansion are shown in Fig. 7 for different cases.

Case 1: Volumetric and Undersaturated Reservoir: For a reservoir with no gas injection or water influx, the linear form of MBE can be expressed as

$$F = N\left[E_o + E_{f,w}\right] \tag{5}$$

$$N = \frac{F}{E_o + E_{f,w}} \tag{6}$$

A plot of F versus $E_o + E_{f,w}$ gives a straight line passing through the origin with N as the slope. The deviation for the linearity represents the presence of a water drive reservoir.

Case 2: Volumetric saturated oil reservoir (without water influx): The MBE equation simply reduces to $F = NE_o$.

Case 3: Gas cap drive reservoir: When the reservoir has only a gas cap drive mechanism for the oil production and the size of the gas cap is known, the MBE equation takes the following form:

$$F = N\left[E_o + mE_g\right] \tag{7}$$

The F versus $E_o + mE_g$ relationship gives a straight line that passes through the origin. For a case when the gas cap is not known, the plot of $\frac{F}{E_o}$ versus $\frac{E_g}{E_o}$ gives N as intercept.

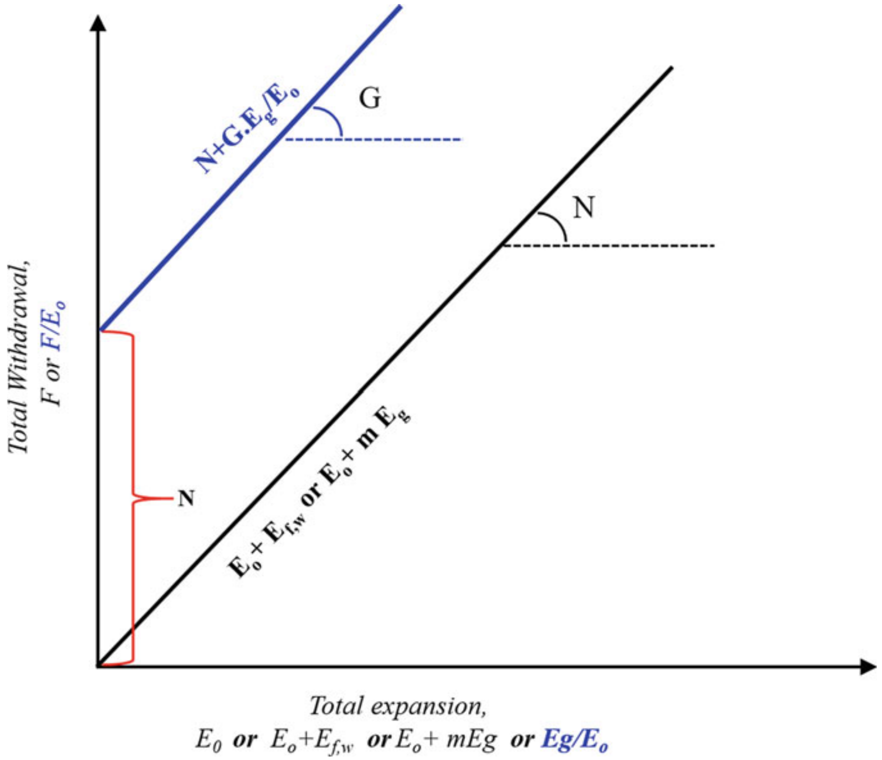

Fig. 7 Havlena and Odeh plots for total withdrawal versus total expansion for different drives

7 Reservoir Drive Performance Indexes (RDPI)

During the formation of oil and gas reservoirs, the volume of the reservoir reduces. This can be attributed to the compaction of the formation and invasion of water into the reservoir due to the pressure drop while producing from the reservoir. Both porosity and water influx into the reservoir are known to compensate for the decrease in pressure. Corresponding to the productivity of the aquifer, the reservoir pressure becomes high and reservoir pressure drops significantly when the productivity of the aquifer is low. Therefore, the ratio of decrease in volume of the reservoir to drop in pressure can evaluate the efficiency of the aquifer and thus can determine the reservoir drive mechanism. To study the changes in the relationship between a decrease in the pore volume of the reservoir and reservoir pressure in different drive mechanisms and to propose a different technique for characterizing the drive mechanism, Jamalbayov and Veliyev (Jamalbayov and Veliyev 2017) proposed a hypothetical gas condensate reservoir model as

$$\phi = \phi_0 e^{c_m(p-p_0)} \tag{8}$$

Table 1 Relation between RDPI and drive mechanisms (Jamalbayov and Veliyev 2017)

$\overline{\Omega_m}$	$\overline{\Omega_p}$	Reservoir drive mechanism
>1	1	Gas drive
>1	<1	Water expansion drive
<1	<1	Strong-water drive

$$\overline{\Omega_p} = \frac{\overline{\Omega}}{\overline{p}} = \text{pore volume at current reservoir pressure} \tag{9}$$

$$\overline{\Omega_m} = \frac{\overline{\Omega}}{\overline{\varphi}} = \text{pore volume after compaction} \tag{10}$$

$$\overline{\phi_p} = \frac{\phi(p)}{\phi_0}, \text{ the ratio of current formation porosity to its initial porosity} \tag{11}$$

$$\overline{p} = p/p_0 \text{ refers to the ratio of current reservoir pressure to original reservoir pressure} \tag{12}$$

where

p_o = initial reservoir pressure
ϕ_0 = initial reservoir porosity
p = reservoir pressure
ϕ = reservoir porosity
c_m = formation compaction factor

There is a relation between the parameters $\overline{\Omega_p}$, $\overline{\Omega_m}$ and the actual reservoir drive. It is reported that under the influence of water drive and gas drives, $\overline{\Omega_p}$ is always greater than unity, whereas in strong-water drive it is less than unity. $\overline{\Omega_m}$ is equal to unity in the gas drive, while it is always less than unity in water drive reservoirs. It is understood that when there is no water influx into the reservoir, the pore volume reduction occurs due to the overburden pressure and is always equal to $\overline{\phi}$.

The parameters $\overline{\Omega_m}$ $(\overline{p})$ and $\overline{\Omega_p}$ $(\overline{p})$ are the indicators of the aquifer activity. So, the drive mechanism can be identified and the productivity of the aquifer is evaluated. These two parameters $\overline{\Omega_m}$ $(\overline{p})$ and $\overline{\Omega_p}$ $(\overline{p})$ are identified as Reservoir Drive Performance Indexes (RDPI) (Jamalbayov and Veliyev 2017). The relation between RDPI and drive mechanisms is summarized in Table 1.

8 Conclusion

Physicochemical study of a petroleum reservoir rock and fluid is performed to investigate the petrographic origin, rock morphology, pore network and distribution, the type of hydrocarbon present in the reservoir, the reservoir fluid properties, and flow characterization. The analysis of reservoir mineralogy helps in identifying the origin of the reservoir, and its porosity and permeability values. The study of the pressure–temperature profiles and drive mechanisms of a petroleum reservoir helps in identifying the type of hydrocarbon present in the reservoir and its flow characteristics, and in the estimation of the total recoverable hydrocarbons from a reservoir. The combination of the natural forces that act on the hydrocarbons present in the reservoir enables the primary production at the surface. The knowledge of the drive mechanisms further opens the pathway into the adoption of different advanced recovery techniques for the efficient production of hydrocarbons from petroleum reservoirs.

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Secondary and Tertiary Oil Recovery Processes

Puja Kalita, Vikas Sharma, Lalit Pandey, and Pankaj Tiwari

1 Introduction

Pressure is the main driving force for the production of oil naturally from the reservoir, and the process is known as primary recovery. Early in the producing life of a reservoir, the natural pressure energy depletes and the dominant driving forces in the reservoir cannot push the crude oil to the surface. For a longer producing life of the reservoir, it becomes necessary to re-pressurize the reservoir. Pressure energy is usually achieved by the injection of water and/or gas. This arrangement of maintaining the reservoir at high pressure is known as the secondary recovery of oil. The supplementary energy is provided by injecting water (waterflooding) or injecting gas (gas flooding). The secondary recovery method continues to produce from the reservoir until a substantial amount of the displacing fluid is produced from the production well (Vishnyakov et al. 2020). The first and second stages of oil recovery extract only 20–30% of the original oil in place (OOIP) and the remaining crude oil still remains trapped in reservoir rocks (Mokheimer et al. 2019). The OOIP that remained in pores is subjected to the tertiary stage, also known as enhanced oil recovery (EOR).

EOR methods are broadly classified as thermal, chemical, gas and microbial (Alvarado and Manrique 2010; Datta et al. 2018, 2020). In this process, the additional recovery of oil is obtained by altering the properties of the crude oil (i.e., viscosity), rock (i.e., wettability, permeability) and interfacial properties to mobilize the trapped oil (Saha et al. 2018a, c; Sharma and Pandey 2020). Hence, the selection of these methods depend on the fluid properties (density, viscosity), reservoir rock properties (porosity, permeability, oil saturation, pay zone thickness, formation

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P. Kalita · V. Sharma · L. Pandey · P. Tiwari (✉)
Department of Chemical Engineering, Indian Institute of Technology Guwahati, Guwahati
781039, Assam, India
e-mail: pankaj.tiwari@iitg.ac.in

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depth) and environmental conditions (temperature, pressure and salinity). The implementations of EOR strategies have successful outcomes of recovering an additional oil of up to 30% depending on the method applied and reservoir properties.

The most important terms to describe the mechanisms responsible for recovering oil are the interfacial tension, emulsification, wettability alteration, sweep efficiency, and mobility ratio. The formation of emulsion types depends on the hydrophilic-lipophilic balance (HLB) of the chosen chemical (surfactants). The emulsion can be either water in oil (w/o, 3–6 HLB) (Pei et al. 2013; Saha et al. 2018c; Wang et al. 2010) or oil in water (o/w, 8–16 HLB) (Saha et al. 2019a; Saha et al. 2018b). A more detailed understanding on the emulsion behaviour can be achieved by analysing and understanding the phase behaviour studies (Bera et al. 2011; Green and Willhite 1998a). The phase behaviour represents Winsor type microemulsion (type I, II and III) and identifies the optimum condition (detection of lowest IFT) that can assist in selecting the optimum chemical EOR system. Interfacial tension (in chemical/microbial EOR) indicates the tension that exist between oleous phase oil and aqueous chemical phase. The reduction in interfacial tension promotes the movement of oil through the porous media by releasing the residual/trapped oil. This reduction in interfacial tension is dependent on the pH, salinity and temperature of the formation. The reduced interfacial tension value and the ultra-low interfacial tension value (~10^{-6} mN/m) can be detected by using spinning drop tensiometer. Wettability can be estimated by contact angle measurement. Alteration of wettability measures the potential of the reservoir rock to change the wettability from oil wet to favourable intermediate or water–wet (Saha et al. 2018b, c, 2019a). This alteration induces the flow of trapped residual oil from the pore formation towards the surface production platform. The injected chemicals cause an alteration in wettability of the reservoir by penetrating the water phase inside the solid rock induced by ion pairs formed by the reaction between crude oil-surfactant (Saha et al. 2018a) and ion-binding mechanisms (Liu et al. 2010). The value of mobility ratio, $M \leq 1$, is desirable as under this scenario, the viscosity of the injected fluid is more than the oil viscosity which reduces the viscosity fingering effect and enhances the oil recovery factor. If the value of M reaches more than 1, the injected fluid will bypass the oil phase and enhances the viscous fingering effect ultimately resulting in poor recovery efficiency. The displacement efficiency is considered to be one of the most important and essential parameters which directly hinders the overall recovery factor of crude oil. The displacement efficiency includes the overall area covered by the injected fluid in order to displace the residual crude oil. This chapter provides an overview of secondary and tertiary recovery processes, the various oil recovery mechanisms, screening criteria and some recent studies on oil recovery using lab-scale core flooding and computational domain.

2 Important Parameters and Mechanisms of EOR

Factors affecting enhanced oil recovery performance

Interfacial tension and surface tension—Interfacial tension is the force acting at the interface separating two immiscible liquids per unit length of the interface. Surface tension is the force acting per unit length of an interface between air–liquid. It has a unit of dynes/cm and denoted by 'σ'. The value of surface tension for water is 73 dynes/cm at room temperature. Surface tension decreases with an increase in temperature. Surface or interfacial tension can be calculated using the following formula:

$$\sigma_{ow} = \frac{rgh(\rho_w - \rho_o)}{2\cos\theta} \tag{1}$$

If the two phases are water and oil, then σ_{ow} is the interfacial tension of the oil–water system, ρ_w, ρ_o are the density of water and oil, respectively, and r, h are the radius and height of the capillary tube, respectively. θ and g are represented as contact angle and gravitational acceleration, respectively.

Wettability—Wettability is a property which shows the ability of a fluid to spread on or adhere to a solid surface in the presence of other immiscible fluids. Wettability of rock influences the type of fluid saturations within the porous medium. Contact angle (θ) is used to quantify the wettability of the rock. The rock is water-wet if $\theta < 90°$ and oil-wet if $\theta > 90°$. The rock is strongly water-wet when θ tends to 0° and strongly oil-wet when θ tends to 180°. If rocks show affinity for both water and oil, the value of 0° is near to 90° and it is a state of intermediate wettability. Wettability alteration from oil-wet to water-wet improves crude oil recovery.

Mobility Ratio and Mobility—Mobility ratio (M) is the ratio of the mobility of the displacing fluid (water or any chemicals) to the mobility of the fluid to be displaced. The mobility of any fluid, λ is the ratio of its effective permeability to viscosity of the phases present in the reservoir. Mobility ratio depends on the viscosity of the injected fluid and the fluid which is trapped in the pores. It is considered favourable when $M < 1$. A high mobility ratio ($M > 1$, *unfavourable*) creates poor sweep efficiency.

$$\text{Mobility for Oil} = \lambda_o = \frac{k_o}{\mu_o} = \frac{kk_{ro}}{\mu_o}, \quad \text{Water} = \lambda_w = \frac{k_w}{\mu_w} = \frac{kk_{rw}}{\mu_w},$$

$$\text{Gas} = \lambda_g = \frac{k_g}{\mu_g} = \frac{kk_{rg}}{\mu_g}$$

$$\text{Mobility ratio} = M = \frac{\lambda_w}{\lambda_o} = \frac{k_{rw}}{k_{ro}}\frac{\mu_o}{\mu_w}, \tag{2}$$

where λ_o, λ_w, λ_g are the mobility of the oil, water, and gas, respectively; k_o, k_w, k_g are effective permeability to oil, water, and gas, respectively; k_{ro}, k_{rw}, k_{rg} are relative permeability to oil, water, and gas, respectively; μ_w and μ_o are viscosity of oil and water, respectively; w and o represent water and oil phase, respectively.

Capillary Pressure—The pressure difference which exists across the interface between the non-wetting phase (crude oil) and the wetting phase (water) is defined as capillary pressure. Capillary pressure (P_c) is related to fluid–fluid IFT, wettability of the fluids, and the capillary size.

$$P_c = P_{nw} - P_w = gh(\rho_w - \rho_o) = \frac{2\sigma_{ow}\cos\theta}{r}, \tag{3}$$

where P_c is the capillary pressure; σ_{ow} is the oil–water interfacial tension; r is capillary radius; h is the fluid height, and θ is the contact angle. P_{nw} and P_w are the pressure of the non-wetting and wetting phase, respectively. ρ_w and ρ_o are the density of water and oil, respectively. g is the gravitational acceleration, and h being the height of the liquid in the capillary tube.

Viscous Forces (Poiseuille's Law)—Viscous forces, for fluid flow through porous media are measured by the pressure drop in a bundle of parallel capillary tubes (approximation). The pressure drop for laminar flow through a single tube is given by Poiseuille's law. Viscous force in a porous medium can be expressed in terms of Darcy's law,

$$\Delta p = -\frac{\mu L \bar{v} \varphi}{k}, \tag{4}$$

where k is the permeability of the bundle of capillary tubes (Darcy, cm^2, mm^2), d is the diameter of the capillary tubes, and φ is the effective porosity of the bundle of capillaries. For a bundle of equally sized capillaries, the permeability (k) is given by $k = 20 \times 10^6\, d^2\varphi$.

Capillary Number—Capillary number (N_{ca}) is the ratio of viscous forces to the capillary forces. It is denoted by N_{ca}

$$N_{ca} = \frac{F_V}{F_C} = \frac{v\mu_w}{\sigma_{ow}\cos\theta}, \tag{5}$$

where F_V and F_C are viscous and capillary forces, respectively. v is the interstitial velocity, μ_w is the viscosity of the wetting (displacing) phase, and σ_{ow} is the interfacial tension. Waterflood typically operates at $N_{ca} < 10^{-6}$.

Relative Permeability—The absolute permeability (k) is a property of the porous medium and is a measure of its capacity to flow fluids through the medium. *Effective* permeability is the ability to transmit fluids, when two or more fluids flow at the same time, and the relative permeability of each phase at a specific saturation is the ratio of the effective permeability of the phase to the absolute permeability

$$k_{ro} = \frac{k_o}{k};\, k_{rw} = \frac{k_w}{k};\, k_{rg} = \frac{k_g}{k}, \tag{6}$$

where k_{ro}, k_{rw}, k_{rg} are the relative permeability to oil, water, and gas, respectively? k_o, k_w, k_g Are effective permeability to oil, water, and gas, respectively? k is called the absolute permeability.

Overall Recovery Factor—The overall recovery factor (R.F.) is the product of (i) displacement efficiency,E_D (ii) areal sweep efficiency,E_A and (iii) vertical sweep efficiency, E_v.

$$RF = E_D \times E_A \times E_v \tag{7}$$

Displacement Efficiency—The displacement efficiency of crude oil is equal to the product of microscopic and macroscopic displacement efficiency. The microscopic displacement account for driving or mobilization of crude oil at pore level of the formation. The macroscopic efficiency represents the displacement or mobilization of the crude oil in areal and vertical direction of the reservoir. Microscopic displacement efficiency (E_D) is expressed as

$$E_D = \frac{1 - S_{orw}}{S_{oi}}, \tag{8}$$

where S_{oi} is the initial oil saturation, and S_{orw} is the residual oil saturation.

Volumetric Sweep Efficiency—Volumetric sweep or macroscopic displacement efficiency is defined as the fraction of the reservoir volume that is contacted by the injected fluid in situ. It is conceptually the product of vertical sweep efficiency (E_v) and areal sweep efficiency (E_A).

$$E_{vol} = E_A \times E_v \tag{9}$$

Vertical sweep efficiency can be defined as the height of the vertical fraction of the reservoir swept by injection fluid and is function of mobility ratio, gravity and permeability.

Areal sweep efficiency—The fraction of the total flood area from which reservoir fluid is displaced by the injected fluid. The pattern is such that only part of the contacted area is swept at breakthrough.

Emulsification—Emulsification is the process of misciblizing two immiscible liquid phases by the application of foreign substances like surfactants. Surfactants facilitates the dispersion of one immiscible phase over another immiscible phase resulting in droplet formation of different size.

Emulsification and Entrainment—The reduction of interfacial tension (due to interaction of crude oil and chemicals) leads to the formation of an oil/water emulsion. Heavy oil is dispersed (entrained) as tiny droplets within the aqueous phase (water). With time aqueous phase is enriched by oil droplets and form an elongated emulsion string.

Emulsification and Entrapment—The emulsion formed due to the interaction of crude oil and the chemical (alkaline) solution generates some small and large droplets of oil in water. This emulsion passes through the pore throat zones or small constrictions and traps in the reservoir.

Viscous Fingering—In waterflooding operation, water is injected into the reservoir through the injector well to displace the oil towards the producers. This injected fluid displaces oil from the pore spaces in a relatively simple manner. If the injected fluid travels at a less velocity then the injected fluid does not get past the one to be displaced and the horizontal interface between the two fluids is undisturbed. However, if the displacing fluid flows more easily it creates undue tongues at the interface because of viscosity differences.

3 Secondary Oil Recovery Methods

3.1 Waterflooding

Waterflooding is a viable alternative to bring back the wells to production by the maintenance of pressure (Civan 2007; Phade and Gupta 2008). The injected water controls the pressure of the reservoir and governs the displacement of oil to the producing wells (Speight 2015). Water injection offers certain cost-effective benefits; abundance of water resources and desired properties such as viscosity, density and wetting characteristics. The amount of producible oil reserves and the nature of drive mechanisms of the reservoir determine the time and schemes to be implemented for the waterflooding. Solution gas-drive reservoirs are considered the potential candidates for waterflooding. To maintain the reservoir pressure and improve oil production, water injection is recommended in undersaturated reservoirs as dissolved gas expands and supports the production.

Waterflooding was used all over the world in the 1960s in most of the fields in an attempt to recover more and more oil. The success of a waterflooding project depends on the properties of the crude oil and the rock formation such as the residual oil saturation, initial gas saturation rate of injection and arrangement of the injectors and producer (Alfarge et al. 2020). The design of waterflooding depends on the proper characterisation of the reservoir parameters (Ahmed 2019; Witte et al. 2008). The important factors which help in assessing the economic and technical feasibility of waterflooding are listed in Table 1. The reservoir geometry determines the location and number of wells to be drilled and the flooding pattern to be chosen (Ogbeiwi et al. 2017). Effective porosity is required to conduct water injection operations. Crude oil viscosity affects the displacement mechanism and controls the mobility ratio (An 2019).

Waterflooding in a carbonate reservoir does not displace all the oil from the pore spaces because of the capillary pressure difference and nature of wettability of the reservoir rock (Jia 2012; Shehata et al. 2014). Carbonate reservoirs are naturally fractured reservoirs characterized by vugs, multiple porosity and extremely less homogeneity which lead to poor sweep efficiency of oil (Jiang et al. 2019). Carbonate

Table 1 Important parameters to be considered for waterflooding reservoir model

Fluid properties	Rock properties	Reservoir properties
API oil gravity	Porosity	Depth of oil water contact
Solution gas oil ratio	Permeability	Initial reservoir pressure
Water density	Vertical and horizontal permeability	Bubble point pressure
Water compressibility	–	Reservoir temperature
Water viscosity	–	–
Oil formation volume factor	–	–
Oil viscosity	–	–

rocks contain a huge amount of oil in place and are preferentially of oil-wet nature; thus, the recovery of oil by waterflood is relatively low (Mohan et al. 2011; Ruidiaz et al. 2018). Low salinity water injection (LSWI) shifts the wettability of the rock towards more water-wet nature and releases the oil trapped in the pores (Al-Harrasi et al. 2012; Al-Shalabi et al. 2014). Several studies have been conducted on the impact of salinity of brine and ionic composition on oil recovery by waterflooding in carbonate reservoirs (Mohan et al. 2011). The main mechanism responsible is altering the wettability from oil-wet to water-wet for improving the oil recovery (Yousef et al. 2012). Low salinity water injection is an emerging and improved oil recovery technique, and several aspects of design to identify its suitability have been analysed (Dang et al. 2015; Derkani et al. 2018). Table 2 shows the summary of different screening parameters for low salinity water injection in carbonate rocks.

Table 2 Screening parameters for low salinity waterflooding in carbonate reservoirs

Formation	Carbonates	References
API gravity	36.5	Park et al. (2018)
Acid number	High	Sari et al. (2019), Witte et al. (2008)
Clay minerals	Low	Chavan et al. (2019)
Salinity of brine	5000 ppm	Bartels et al. (2019)
Reservoir minerals	Calcite, dolomite	Saw and Mandal (2020)
Wetting characteristics	Oil-wet or mixed wet	Chavan et al. (2019)
Injection water	Ca^{++}/Mg^{++}, SO_4^{2-}	Dang et al. (2015)
Reservoir temperature	~90 °C	Zahid et al. (2012)

It can be seen that low salinity water injection is applied in light crude oil reservoir. There should be some amount of clay, and clay content must not be high. High temperature, low acid number and the presence of Ca^{++} and Mg^{++} or SO_4^{2-} have proved to be favourable for low salinity injection in carbonate reservoirs.

3.2 Gas Injection

Gas injection started in 1864 and is one of the oldest methods used for fluid injection process to maintain the pressure of the reservoir in a cost-effective manner. Gas injection can be implemented, prior to production or when the pressure declines significantly, in two schemes (i) ***Crestal gas injection***—the gas is injected into gas cap of the pay zone and (ii) ***Pattern gas injection***—it involves distributing the gas injection wells throughout the oil reservoir in a particular pattern and the injection wells are deployed into the oil column (Donaldson et al. 1989). Typical gases used for injection are methane, nitrogen, carbon dioxide, associated petroleum gases, flue gases and air (Johns 2004). The gas is injected below the minimum miscibility pressure (MMP) (Selamat et al. 2008). The following factors are to be considered in conducting a gas injection pilot test (Elkins and Cooke 1948); (i) The gas injection rate and the time period should be sufficient enough; (ii) Continuous monitoring and measurement are essential; (iii) Sufficient wait time before and after gas injection and (iv) Gas injection pilot test to be conducted early in the life of the developed field. A successful oil recovery project is dependent on the volume of the reservoir contacted by the injected fluid and the mechanism by which the injected fluid displaces the remaining oil. However, injected gases have an adverse mobility and hence have a tendency to overrun the residual oil trapped in the reservoir. The injected gas must contact much of the volume of the reservoir to get maximum recovery.

Using material balance equation and instantaneous gas-oil ratio (GOR), Tarner and co-worker developed a mathematical solution to predict reservoir performance (Turner et al. 1969). The assumptions made to simplify the computation were (i) constant values for the rock and fluid properties, (ii) gravity forces are not significant and non-existence of pressure gradient and (iii) constant values for pore volume and water saturation, and no water encroachment. The factors which contribute to deciding candidates for gas injection in oil reservoirs are, net pay thickness of the reservoir, size and relative thickness of the gas cap, pressure and temperature of the reservoir, and dissolved gas and shrinkage factor (Barton and Dykes 1958; Leibrock et al. 1951). A high API gravity oil and reservoir temperature favour higher sweep and displacement efficiencies (Cook et al. 1967; Mohammadi et al. 2014). The screening criteria related to reservoir characteristics and crude oil properties for the injection of different gases are shown in Table 3.

Table 3 Important parameters and their ranges for the screening of gas injection schemes (Taber 1983)

Gases injected	API gravity	Viscosity (cp)	Oil saturation (PV) (%)	Depth (ft)
Hydrocarbon gases	>35	<10	>30	>1000 LPG–>5000 (HP gas)
Nitrogen or flue	>24	<10	>30	>4500
CO_2	>26	<15	>20	>2000

3.3 Buckley Leverett Model to Oil Recovery

Buckley and Leverett developed a onedimensional mathematical linear system to describe the immiscible displacement of two-phase (Buckley and Leverett 1942). It consists of semi-analytical solution saturation equations for (a) fractional flow (b) frontal advance which predominantly characterizes the phenomena of oil displacement throughout the reservoir and used for validation of numerical modelling.

The fractional flow equation developed by Leverett is expressed as

$$f_{inj} = \frac{1 + \left(\frac{0.001127 k_o A}{\mu_o q_t}\right)\left[\frac{\partial P_c}{\partial x} - 0.433 \Delta\rho \sin\theta\right]}{1 + \frac{k_o}{k_{inj}} \cdot \frac{\mu_{inj}}{\mu_o}}, \tag{10}$$

where f_{inj} = fraction of the injected fluid, k_o = effective permeability of oil, md; k_{inj} = effective permeability of injected fluid, md; $\Delta\rho$ = density difference between injected fluid and oil, g/cm^3, q_t = total flow rate, bbl/day; μ_o = viscosity of oil, cp; μ_{inj} = viscosity of the injected fluid.

This is general expression as the capillary forces always increase the movement of the injected phase irrespective of the direction of flow or the displacing phase. If the gravity and capillary forces are not taken into account, the fractional flow of the injected fluid varies only with pressure and saturation (Eq. 2)

$$f_{inj} = \frac{1}{1 + \frac{k_o}{\mu_o} \frac{\mu_{inj}}{k_{inj}}} \tag{11}$$

Buckley and Leverett modified the equation further to relate the fractional flow at a given location and saturation with time (Buckley and Leverett 1942) which is the frontal advance approach given by

$$\left(\frac{dx}{dt}\right)_{sinj} = (\vartheta)_{sw} = \frac{5.615 q_t}{\emptyset A} \left(\frac{df_{inj}}{dS_{inj}}\right)_{sinj} \tag{12}$$

This equation is basically derived from the material balance equation of injected phase within the porous media and describes the velocity with which the injected phase with a constant saturation moves through the medium. In case of displacement by gas, the gravitational force and the force exerted by the injected gas affect the displacement of oil. Here, capillary forces are ignored (Dardaganian 2005), and the equation is simplified to

$$f_g = \frac{1 + 0.001127 \frac{k_o}{\mu_o} \frac{A}{q_t} (-0.433 \Delta\gamma \sin\theta)}{1 + \frac{k_o}{\mu_o} \frac{k_{inj}}{\mu_{inj}}} \tag{13}$$

At any point of time, the position of gas-oil contact can be estimated by the following equations:

$$x = \frac{5.61 q_t t}{\emptyset A} \frac{df_g}{dS_g} \quad \text{or} \quad t = \frac{\emptyset A}{5.61 q_t \left(\frac{df_g}{dS_g} \right)} \tag{14}$$

4 Tertiary Oil Recovery Methods

The crude oil in the reservoir rock, after secondary recovery, can be recovered by overcoming the capillary force which holds the crude oil in the reservoir rock. A suitable technique that can reduce capillary force, lower interfacial tension between the phases and increase the capillary number can result in additional crude oil recovery. Enhanced oil recovery or tertiary recovery methods are such processes that alter the properties and interactions of reservoir formation; crude oil and rock. EOR methods are broadly categorised as thermal EOR, chemical EOR, gas EOR and microbial EOR methods (Alvarado and Manrique 2010; Datta et al. 2020; Sharma et al. 2019b) as shown in Fig. 1. These methods are selected based on the reservoir rock properties (porosity, permeability, crude oil saturation, pay zone thickness, formation depth) and fluid properties (crude oil density, viscosity).

4.1 Thermal EOR Methods

Thermal EOR is the process in which heat is transferred to the formation in the form of steam or hot air to alter reservoir rock and fluid properties. The thermal EOR methods are suitable for heavy oil reservoirs; viscosity and density (<22 °API) are very high (Santos et al. 2014). The thermal energy escalates reservoir temperature which reduces the viscosity of crude oil and displaces it towards the production well

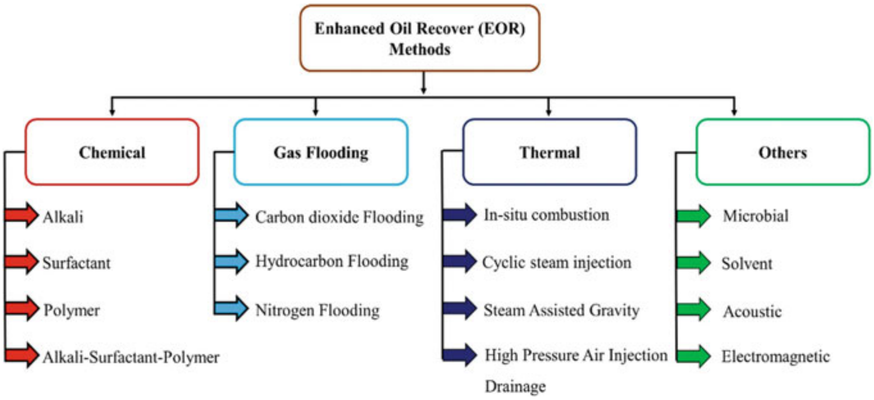

Fig. 1 A schematic representation of different enhanced oil recovery (EOR) methods (Adapted from Saha 2018)

(Green and Willhite 1998b). Steam flooding, hot water injection, cyclic steam injection, in situ combustion, and steam-assisted gravity drainage (SAGD) are commonly used thermal EOR methods (Fig. 2). Electric heating and electromagnetic heating are also used as non-aqueous thermal EOR processes to recover crude oil (Mokheimer et al. 2019). Thermal EOR methods are more successful in high porosity sandstone heterogeneous reservoir (Carcoana 1992). These EOR methods are most widely used all over the world (Mokheimer et al. 2019) and the projects are currently in progress in the United States of America, Canada, Brazil, China, and Venezuela (Alvarado and Manrique 2010).

Continuous steam injection—Also termed as steam drive, it is the process in which steam is injected continuously from the injection well into the reservoir. The crude oil properties such as thermal expansion, viscosity reduction, and thermal cracking change in this process. This causes wettability alteration of the reservoir rock and initiates dissolved gas drive (Carcoana 1992). The steam changes to hot water on

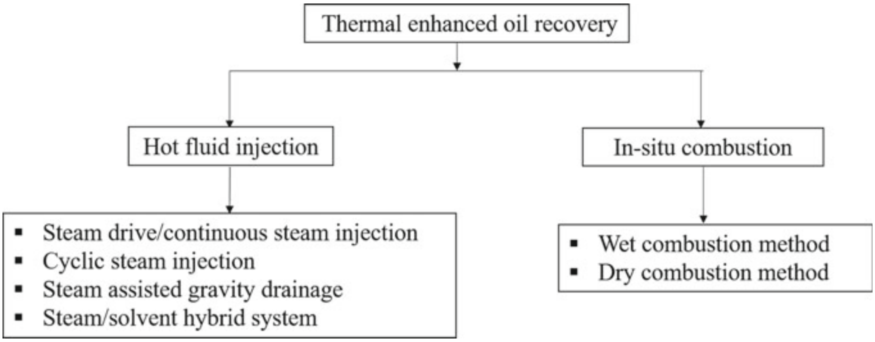

Fig. 2 Typical classification of thermal EOR method (Carcoana 1992; Mokheimer et al. 2019)

temperature reduction. The water pushes the crude oil to the production well due to pressure gradient and up to 50% of oil recovery can be achieved (Ghoodjani et al. 2012). One of the disadvantages of the steam drive is that there is a difference between crude oil and steam density and results in steam override.

Cyclic steam injection: This method was discovered by Shell Oil Company in 1959 in Venezuela (Alvarez and Han 2013) and then widely used by other countries such as Brazil, Canada, and Venezuela to harness their heavy oil resources (Ghoodjani et al. 2012). In this process single well is utilized for both injection and production and steam is injected at regular time intervals through the injection well into the reservoir. Each cycle consists of three principal phases: steam injection, soaking period (few weeks), and oil production. The crude oil recovery is relatively less (10–40%) in comparison to other thermal EOR methods and the heat loss to the surrounding formations limits its implementation.

Steam assisted gravity drainage (SAGD): This is one of the most famous thermal EOR methods to recover viscous and highly viscous crude oil (Thomas 2008). This method utilizes two parallel horizontal wells separated by some distance, at the same plane. The top well serves as a steam injector, whereas the well at the bottom serves as a producer. The steam after injection rises to the top and form a steam chamber. Further, the steam condenses into hot water and heats the oil which reduces the viscosity of oil and improves mobility. Gravity drainage is the main driving force for the movement of crude oil from the reservoir towards the production wells (Ghoodjani et al. 2012; Mokheimer et al. 2019). Crude oil recovery is very high (up to 60–80%) compared to other thermal EOR methods (Pang et al. 2015). However, the steam generation cost is significantly higher in this method (Hosseini et al. 2017) and large quantities of natural gas and water are required.

In situ *combustion*—this method is also called ‘fire flooding’ and was started in the 1950s in Pennsylvania, USA (Ghoodjani et al. 2012). Oxygen-enriched air is injected into the reservoir where it interacts with the crude oil causing the combustion of some portion of crude oil (~10% OOIP). The combustion process heats the rock and the fluid up to 450–600 °C which results in reducing crude oil viscosity. The sweep efficiency improves significantly, and the trapped residual oil saturation decreases (Mokheimer et al. 2019; Thomas 2008). In situ combustion process is classified as (i) *wet combustion*—air and water are introduced alternatively into the reservoir. The generated steam has high heat carry capacity compared to the oil and assists more heat transfer in the formation. (ii) *dry combustion*—only oxygen-enriched air is injected into the formation. As air heat carrying capacity is not good, wet combustion is the preferred process (Carcoana 1992).

4.2 Chemical EOR Methods

Chemical enhanced oil recovery (a non-thermal EOR) is the most prominent techniques that started in the 1980s during a rapid increase in the crude oil price (Gbadamosi et al. 2019). Heavy oil reservoirs with thin pay zone are produced using chemical EOR methods (Chen et al. 2015). It uses a combination of chemicals (alkali, surfactant, polymer) to alter physicochemical properties of reservoir rock and fluids such as interfacial tension, wettability, and relative permeability to recover residual oil within the capillaries of the reservoir rocks (Mandal 2015). Most widely used chemical EOR methods include alkaline flooding, polymer flooding, surfactant flooding, alkaline polymer flooding, alkaline surfactant flooding, surfactant polymer flooding, and alkaline surfactant polymer flooding.

Alkaline flooding is one of the simplest chemical EOR methods. The primary mechanisms (Fig. 3) of oil recovery using alkaline flooding are emulsification, entrainment, wettability alteration (oil-wet to water-wet), emulsification, and entrapment (Gong et al. 2016; Pei et al. 2013; Sheng 2010). Alkaline solution (such as NaOH, Na_2CO_3, and $NaHCO_3$) is injected into the reservoir where it reacts with acidic components present in crude oil and forms an in situ surfactant at the interface of the two phases, crude oil and alkaline solution (Fig. 4). Emulsification of crude oil, interfacial tension reduction, reduction in water mobility, reduction of severity of viscous fingering, and

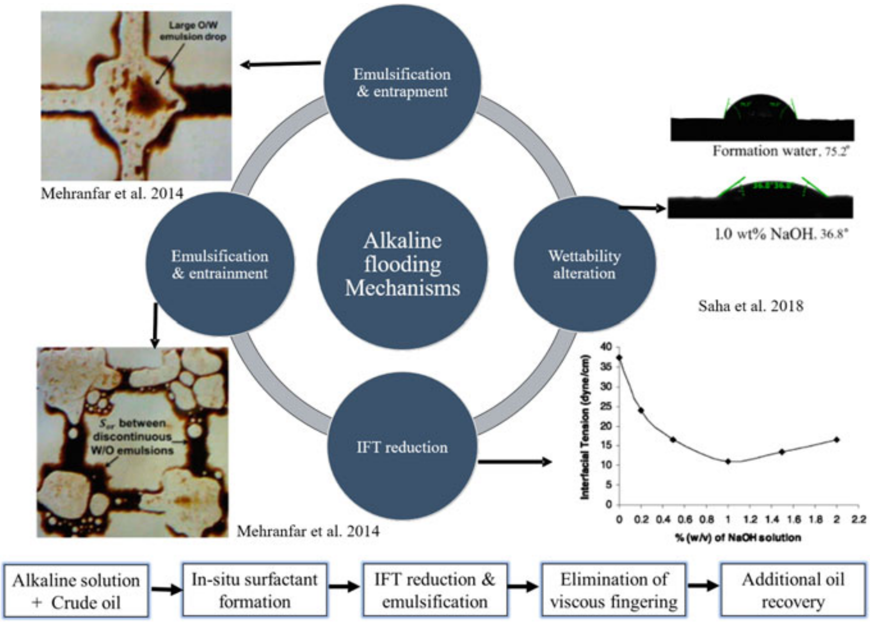

Fig. 3 Mechanisms of alkaline flooding and Interaction of alkaline solution with crude oil in the reservoir (Adapted with permission from Mehranfar and Ghazanfari 2014 and Saha 2018)

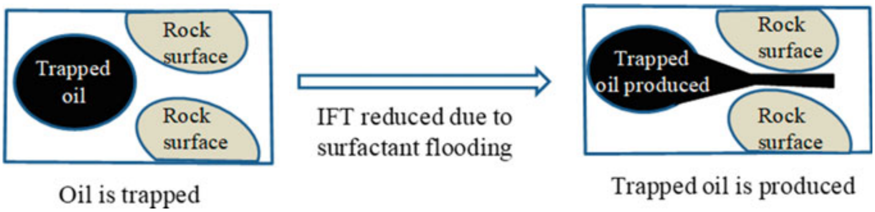

Fig. 4 Mechanism of oil recovery by surfactant flooding illustrating the effect of low IFT on residual oil (Adapted with permission from Kumar and Mandal 2017)

improvement in sweep efficiency by emulsification are the mechanisms of crude oil recovery using alkaline flooding within the porous medium (Jennings Jr et al. 1974; Pei et al. 2011, 2013). The crucial parameters that affect alkaline flooding are the viscosity of crude oil, the concentration of alkali, rheology of emulsion, and presence of other chemicals like polymer (Bahmanabadi et al. 2016; Cheraghian 2015; Du et al. 2013; Elyaderani and Jafari 2019; Pei et al. 2014).

Surfactant flooding—It is one of the well-known methods of chemical EOR. A specific concentration of chosen surfactant lowers the interfacial tension between the resident crude oil and water to a very low value, alters the wettability of rock surface and causes emulsification of the crude oil to achieve the additional recovery of trapped crude oil from the capillaries of the reservoir rocks (Gbadamosi et al. 2019). An increase in the capillary number results in a decrease in the residual oil saturation and an increase in crude oil recovery. The main mechanisms responsible for crude oil recovery using surfactant flooding are interfacial tension reduction and wettability alteration (Fig. 4). A surfactant molecule consists of two functional groups: (i) *hydrophilic group* (water-soluble) and (ii) hydrophobic group (oil soluble). The hydrophilic part of the surfactant (head) reacts with the aqueous phase, and the hydrophobic part (tail) reacts with the oleous phase to lower the interfacial tension and change the wettability. This weakens the capillary forces and leads to an increase in crude oil recovery (Sheng 2010). Different types of surfactants such as anionic, cationic, non-ionic, and zwitterionic are used in the EOR process. Anionic surfactants are most widely used in chemical EOR because of the low adsorption on sandstones. Non-ionic surfactants are used as co-surfactant and increase the tolerance to high salinity. Zwitterionic surfactants can withstand high temperature and high salinity. Cationic surfactants have a high adsorption rate on sandstones (Gurgel et al. 2008).

Polymer flooding—It is an enhanced oil recovery process in which a water-soluble polymer is added to increase the viscosity of the injected water and reduce its mobility (<1). This results in a uniform displacement of crude oil. It eliminates the problem of viscous fingering and improves the sweep efficiency (Gbadamosi et al. 2019; Sheng 2010). Two main types of polymers are used for field applications in chemical EOR. These polymers are biopolymer (i.e., Xanthan gum) and synthetic polymer (i.e., hydrolyzed polyacrylamide (HPAM)). Xanthan gum can withstand up to a temperature of less than 200°F. Whereas HPAM degrades at high temperature and high shear (Carcoana 1992; Gbadamosi et al. 2019; Green and Willhite 1998b). Polymer flooding is considered a right candidate for enhanced oil recovery when there is high water coning, high permeability areas, and highly viscous crude oil.

Alkaline surfactant polymer (ASP) flooding—a combination of chemicals such as alkali, surfactant, and polymer formulates an ASP slug. Alkali (sodium carbonate, sodium hydroxide, potassium hydroxide) components of the slug interact with the acidic part of the crude oil and form an in situ surfactant and termed as a co-surfactant in the ASP slug (Pei et al. 2011, 2013; Saha et al. 2018c). Surfactant (sodium dodecyl sulfate, petroleum sulphonates, and triton X100) within the ASP slug is surface-active agents with an affinity for organic solvent (crude oil) and water. The surfactants lower the interfacial tension between the crude oil and water to an ultra-low value so that the capillary pressure within the trapping zones is reduced to a significant level (Gbadamosi et al. 2019). Water-soluble polymers within the ASP slug helps in increasing the viscosity of ASP solution. Improvement in sweep efficiency and control of viscous fingering phenomena are the outcomes of adding polymers in ASP slug (Carcoana 1992; Saha et al. 2018b). Interactions of alkali and surfactant with the crude oil result in a microemulsion, which has a high viscosity. This emulsion controls the mobility of the water, channelling, and early breakthrough of water. In ASP flooding, all of the three chemicals have their different roles in altering the reservoir rock and fluid properties and increasing sweep and displacement efficiencies (Gong et al. 2017; Mandal 2015; Sheng 2010).

Nanotechnology for recovering the trapped oil is being exploited to formulate the chemical slug with desired properties (Cheraghian et al. 2020). Several studies have been conducted in recent decades on the applications of nanoparticles in water-wet rocks (Ju et al. 2002). Roustaei et al. experimentally proved the efficiency of hydrophobic and lipophilic polysilicon with the polysilicon nanoparticles in recovering additional oil by 32% and 28%, respectively, without causing damage to the formation (Roustaei et al. 2012). Hu and coworkers reported a maximum recovery of 33% at the breakthrough by the use of TiO_2 nanoparticles (Hu et al. 2016). Nanoparticles enhance the recovery of oil by altering wettability, reducing capillary forces and thereby improving the sweep efficiency (Dehaghani and Daneshfar 2019).

4.3 Gas EOR Methods

The gas based EOR methods are widely used to recover light-volatile oil trapped within the capillaries of the rocks. Crude oil swelling and viscosity reduction are the main mechanisms used in gas EOR methods. The gas based EOR methods include nitrogen injection, LPG injection, CO_2 injection, and flue gas injection to alter reservoir rock and fluid properties.

Carbon dioxide flooding—CO_2 EOR is the most feasible and environmentally friendly practice. When CO_2 capture and storage (CCS) is combined with CO_2

flooding, it reduces the greenhouse effect and increases oil recovery up to 15–20%. CO_2 enhanced oil recovery projects have been implemented in Brazil, Canada, Croatia, Hungary and USA (Alvarado and Manrique 2010). CO_2 is soluble in crude oil but not in water. When injected into the reservoir, CO_2 swells the crude oil, reduces its viscosity, and increases its density. CO_2 then interacts with the resident water to form carbonic acid which results in the dissolution of calcium and magnesium carbonate. This EOR method can be classified as immiscible flooding and miscible flooding depending on reservoir depth and crude oil compositions (Heidary et al. 2016). It is challenging for CO_2 to achieve miscibility with heavy oil (Nguyen et al. 2014). A significant number of challenges such as viscous fingering, gravity override, asphalting deposition, and precipitation are the problems related to CO_2 flooding (Irawan et al. 2012; Motealleh et al. 2013; Wang et al. 2017). Viscous fingering can be eliminated using carbonated water injection, water alternating gas injection, and hybrid water alternating gas injection (Nasir and Demiral 2012). Supercritical CO_2 injection and polymer thickened CO_2 injection are considered for reducing viscous fingering and eliminating the gravity override (Khosravi et al. 2014). CO_2 foam flooding can also be employed as an enhanced oil recovery method. Surfactants such as CTAB and SDS and/or various nanoparticles such as aluminium oxide (Al_2O_3), copper oxide (CuO), silicon dioxide (SiO_2) and titanium dioxide (TiO_2) are utilized to prepare stable CO_2 foam for a considerable time (Manan et al. 2015; Nguyen et al. 2014). LPG injected with CO_2 foam reduces the minimum miscibility pressure of the crude oil and also eliminates the gravity override and increases oil recovery (Cho and Lee 2016).

Advancements in enhanced oil recovery techniques resulted in development of such a technique that can eliminate the problems of viscous fingering and low volumetric sweep efficiency to a greater extent. This technique is alkaline-surfactant-alternated-gas/CO_2 flooding. The application of this technique results in stable displacement and high volumetric sweep efficiency using a combination of chemical flooding techniques (including alkaline and surfactant) and immiscible CO_2 flooding (Phukan et al. 2019a, b).

4.4 Microbial EOR

The microbial enhanced oil recovery (MEOR) process uses microorganisms to extract the residual oil trapped in the capillaries of reservoir rocks. It is usually employed when the thermal and chemical EOR methods have been implemented and a significant amount of crude oil still remains to be recovered from the reservoir rocks. The main mechanisms of enhanced oil recovery in MEOR are modification in the distributed porosity and permeability of the reservoir, alteration in the wettability of the rock, oil solubilization, interfacial tension reduction, emulsification and lowering mobility ratio (Datta et al. 2018; Lazar et al. 2007; Sharma et al. 2019b). The microorganisms (bacteria) react with the crude oil in the reservoir to form gases (H_2, N_2, CH_4, CO_2), acids, and solvents. The gases (H_2, N_2, CH_4, CO_2) reduce the oil viscosity or swell the oil and displace the oil in place. The low molecular weight fatty acids dissolve the carbonate precipitates, long-chain hydrocarbons (asphaltenes, resins, and wax) in pore throats, thereby improving the permeability as well as porosity (Lazar et al. 2007). Solvents produced are biosurfactants, biopolymers and other acids (Sharma et al. 2019a; Sharma and Pandey 2020; She et al. 2019; Verma et al. 2020). Biosurfactants are injected into the reservoir to recover crude oil from unswept zones. Biosurfactants lower the interfacial tension, promote emulsification, and alter the wettability and in turn improve the oil displacement efficiency. While biopolymers are used to improve the viscosity of the aqueous phase (water) and improve the mobility ratio. Microbial EOR has the advantage; microorganisms are inexpensive, products (biosurfactants, biopolymers), environment friendly, biodegradable and suitable for carbonate oil reservoirs.

5 Screening Criteria for EOR Methods

The production of hydrocarbons from a petroleum reservoir is governed by different properties such as porosity, permeability, compressibility, pay zone thickness, reservoir fluid properties, and reservoir drive mechanism. IFT reduction, wettability alteration and emulsification mechanisms are achieved using different EOR schemes. The selection of these EOR methods is a complex process that mainly includes reservoir rock and resident fluid properties. The screening criteria reported in the literature for various EOR schemes are summarized in Table 4. For the surfactant to be effective in lowering the interfacial tension of the crude oil in the reservoir, the physicochemical and petrophysical properties of the reservoir should be suitable (Saha et al. 2017, 2018a, 2019a). CO_2 EOR is highly effective in reservoirs with low porosity and permeability and is also influenced by the composition of reservoir rock (Feng et al. 2016). While, steam EOR works effectively in heavy oil reservoirs (Dong et al. 2019).

6 Core Flooding Experiments for EOR

Core flooding apparatus is utilized for flooding experiments. A schematic of laboratory scale core flooding experimental setup (for chemical-EOR) is shown in Fig. 5. Different parts of the experimental setup are high pressure pumps, transfer vessels, core holder, overburden pressure pump, back pressure regulators, separators, and gauge pressure. A core holder reactor that accumulates core or sand pack of size specific, length and diameter. Pressure transducers are attached at both the inlet and outlet of the reactor to calculate the pressure difference across the core reactor, and a back pressure regulator is installed at the outlet to create reservoir pressure in upstream. The pumps are required for injection of fluid (crude oil, water and chemical slug) at a constant rate to the core and an additional pump for maintaining overburden pressure to mimic reservoir conditions. The core assembly is kept at a constant

Table 4 Screening criteria for thermal enhanced oil recovery processes

Parameters	EOR schemes[f]								
	Steam flooding	In-situ combustion	ASP flooding	Polymer flooding	Alkaline flooding	Surfactant flooding	CO_2 miscible flooding	CO_2 immiscible flooding	Microbial flooding
Oil gravity (°API)	10–34	NA[c]	23–34	13–42.5	<35	>20	22–45	22.6	>20
Oil viscosity, (cp)	≤15000	<5000	875.8	>123.2	<150	<150	2.08–35	65.5	30–150
Oil saturation (S_o)[a]	≥10	>30	73.7	64	>35	>30	15–89	42–78	>25
Reservoir depth (ft.)	≤3000	300–11500	2984.5	700–9460	500–9000	500–9000	1500–13365	1150–8500	<10000
Pay zone thickness	≥20	NA[c]	NC[d]	NC[d]	NC[d]	NC[d]	NC[d]	NC[d]	NC[d]
Rock type	Both[e]	Both[e]	Sandstone	Sandstone	Sandstone	Sandstone	Both[e]	Both[e]	NA[c]
Porosity (%)	≥20	≥20	26–32	10.4–33	NA[d]	NA[c]	~15	17–32	≥20
Permeability(m D), average	250	>100[b]	–	–	>20	>20	1.5–4500	30–1000	≥150
Reservoir pressure (Psi)	≤1500	NA[c]	NA[c]	NC[d]	NC[d]	NC[d]	NA[c]	NA[c]	<4267
Reservoir temperature (°F)	NA[c]	NA[c]	121.6	74–237	<200	<200	82–257	82–198	<194

[a] At the start of flooding

[b] For shallow reservoirs

[c] NA—Not Available

[d] NC—Not Critical

[e] Both—Sandstone and Carbonate

[f] Data taken from various sources (Aladasani 2012; Brashear and Kuuskraa 1978; Bryant and Burchfield 1989; Carcoana 1992; Carcoana 1982; Dickson et al. 2010; Green and Willhite 1998b; Guo et al. 2015; Lake and Walsh 2008; Mokheimer et al. 2019; Patel et al. 2015; Safdel et al. 2017; Sheng 2015a, b; Taber et al. 1997a, b)

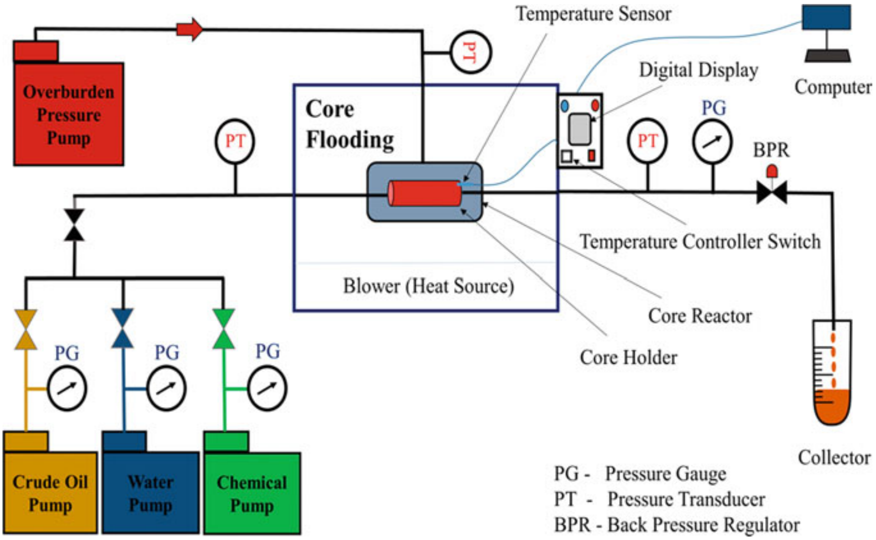

Fig. 5 The experimental set-up of core flooding apparatus (Adapted from Saha 2018)

temperature using a heat source (blower/heating element) with a temperature sensor to achieve and monitor the desired temperature across the core samples. The system is also facilitated with digital control to monitor the pressure and temperature reading. The setup can be used to perform CO_2 EOR, Chemical-EOR and Microbial-EOR with slight modifications.

Core flooding experiments are conducted using these four steps: experiment design, preparation of core and its characterization, saturation of core with brine and core flooding with various compositions of slugs followed by chase water. The experimental procure to conduct the core flooding studies (Chemical-EOR) is illustrated in a schematic diagram (Fig. 6). The core is first fitted in the reactor (core holder), and then reservoir formation water is injected inside the reactor using a hydraulic pump. After saturating the core with formation water, crude oil is then injected at the desired pressure (around 800–1000 psi) until the brine-cut at the effluent reaches <1%. The system at such pressurized condition is left for more than 24 h to maintain uniformity. The porosity, permeability and original oil in place (OOIP) can be calculated through mass balance. The waterflooding is then conducted to estimate the secondary oil recovery percentage. Finally, chemical flooding followed by chase waterflooding is executed to estimate the residual oil recovery.

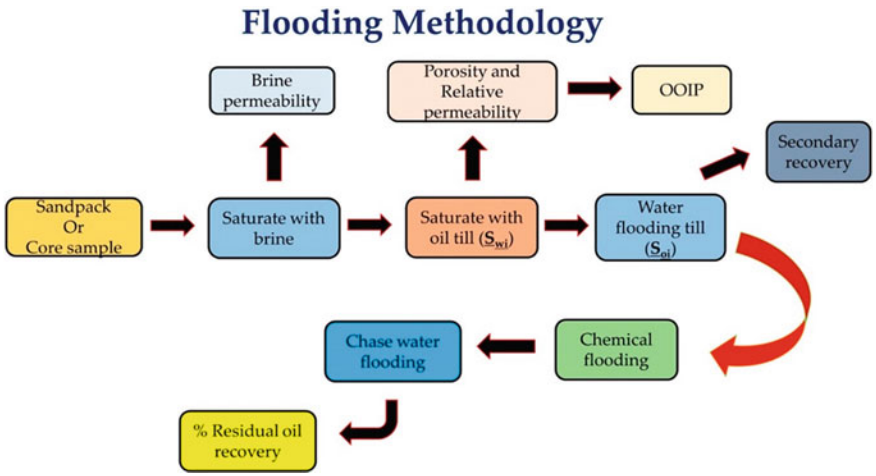

Fig. 6 A schematic diagram to represent the working of core flooding apparatus (Adapted from Saha 2018)

7 Modeling and Simulation of EOR Process

The computer simulation in enhanced oil recovery has been encouraged by the experimental investigations which proposed various mechanisms for oil recovery. The complex mechanisms that take place in the porous media of the reservoir are not feasible to monitor in lab-scale experiments. For example, in chemical based enhanced oil recovery process, the important uncertainties for full field production forecasts are adsorption of chemical on the rock surface, interfacial tension and residual oil saturation, which are critical to be quantified in core flood experiments. For such cases, the computer modeling approach is often to analyse the process more efficiently and simulating the reservoir process for scaling up to field operations.

Reservoir simulators are needed to account for the chemical and physical phenomenon present in EOR processes. The simulators validate the model against the data obtained from the well-controlled experiments conducted at the lab scale. All aspects of reservoir engineering can be addressed with a reservoir simulator such as to predict reservoir performance (Hemmat Esfe and Esfandeh 2020). Several reservoir simulation software such as Computer Modelling Group (IMEX, GEM, STARS), Schlumberger's ECLIPSE, Petrel and INTERSECT, UTCHEM, Crunch-Flow, Black Oil Applied Simulation Tool (BOAST), MATLAB Reservoir Simulation Toolbox (MRST) and Open Porous Media (OPM) have been developed to simulate complex EOR processes. Table 5 summarizes the studies carried out for nano- assisted EOR process in the recent decade. Several authors have reported that for the accurate mathematical representation of a system, physical phenomenon like adsorption, dispersion, interfacial tension reduction, modeling in chemical EOR still needs to be developed.

Table 5 Recent simulation studies conducted using nanofluids in porous media

Type of Model	Method/ Software	Investigating parameters	Purpose	References
3D numerical simulation	COMSOL/FEM	Effect of injection flow rate, nanoparticle volume fraction, porosity of the medium on oil recovery	To simulate nanofluid flooding over time	Hemmat Esfe and Esfandeh (2020)
2D heterogeneous model	Ansys fluent	Mixture of different nanofluids on oil recovery	To investigate the efficacy of ethanol to improve nanofluid oil recovery	Ejeh et al. (2020)
Three-dimensional, three-phase	CMG STARS	Effect of the physical and chemical mechanisms	To study the co-injection of polymer and nanoparticles	Loaiza et al. (2020)
Two-phase	CMG STARS	Efficiency of gemini surfactant/polymer/nanoparticle flooding	To simulate and match the coreflood experiments	Pal and Mandal (2020)
Two-phase, two-dimensional model	MATLAB, FDM	Aggregation, retention, modifications in porosity and permeability on oil recovery	To investigate the feasibility of polymer flooding model enhanced by nanoparticles	Druetta and Picchioni (2019)
Three-phase	GEM	CO_2 with CO_2 nanofluid on sweep efficiency	To test the feasibility of nanofluid alternated with gas in EOR performance	Erdmann and Gallo (2017)
Two-phase	CMG STARS	Model IL (Ionic Liquid) flooding on a field scale	To simulate IL solution flooding	Bin Dahbag et al. (2016)
1D, two-phase	SENDRA	To obtain relative permeability curve by history matching with coreflooding experiments,	To determine the efficiency of silica nanofluid on oil recovery	Safari et al. (2014)
Two-phase flow	IMPES	Variation of water saturation, concentration of nanoparticles n and porosity ratio	To describe nanoparticle transport in porous media	El-Amin et al. (2013)
Two phase water–oil flow	IMPES	Capillary pressure and mixed relative permeabilities to observe the variation in wettability	To describe nanoparticle transport in porous media	El-Amin et al. (2012)

Two phase or multiphase flow simulation model is governed by the conservation of the mass, the energy and the momentum. The material balance equations for all the fluids and Darcy's law for the motion of the fluids along with the correlations for relative permeability, capillary pressure equations, and phase equilibrium equations in the porous media are solved simultaneously. There exist a variety of approaches for solving these equations, such as the IMPES, SS, sequential, and adaptive implicit methods. IMPES is very popularly used in the petroleum industry to handle multiphase flow equations. CMG-STARS is a well-known commercial software based on finite difference method that describes mathematical equations for fluid flow in a petroleum reservoir. CMG-STARS is capable of modelling oil displacement results and handling complex behaviour of multiphase-porous media systems at both laboratory-scale and field-scale (Lashgari et al. 2015; Pal and Mandal 2020). CMG simulation model can be useful for understanding the displacement behaviour depending on the type of injection fluid (Yin and Pu 2008). STARS is recognized for its capability to represent both experimental and field results (Hatzignatiou et al. 2013). Goudarzi et al. collected data like phase behaviour, rheological studies, pressure drop and oil recovery data from secondary and tertiary core-floods and evaluated the performance of different reservoir simulators with different characteristics and developed an EOR benchmark to improve the chemical design (Goudarzi et al. 2016). Kumar et al. developed a model using CMG STARS to simulate the core-flooding experiments using Zwitterionic surfactants and nanoparticles.

8 Conclusion

The oil that remained in the reservoir after primary production is first subjected to secondary and tertiary oil recovery processes. Physicochemical and petrophysical properties of the reservoir and reservoir fluids should be suitable for the implementation of EOR schemes. Recent years have witnessed considerable development in areas of improved oil recovery (IOR) methods which consider EOR methods at any stage of the production to design a cost effective recovery process. The advancements in technology for the uses on combined EOR schemes (chemical alternate water and gas injections, integration of microbial and chemical EOR), chemical slug with nanoparticles, smart (low salinity) water injection, application of ultrasonic and electromagnetic waves and plasma pulse may be effective and environmental friendly to achieve the maximum possible recovery of crude oil from complex reservoirs.

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CO_2-Based Enhanced Oil Recovery

Ranjan Phukan, Rahul Saha, Lalit Pandey, and Pankaj Tiwari

1 Introduction

Gas injection is the second most commonly used enhanced oil recovery (EOR) method, next only to thermal EOR processes in heavy oil fields. Figure 1 illustrates the worldwide project share of the major EOR method. Among the subcategories of EOR methods, miscible gas injection is chosen after steam flooding (Al Adasani and Bai 2011). Gas injection involves the displacement of oil by lean hydrocarbon gases or pressurized non-hydrocarbon gases. The oil displacement mechanisms, either miscible or immiscible, depends on the prevailing conditions in the reservoir, pressure and temperature of the formation, and the compositions of the crude oil. An essential factor in these gas injection processes is the mutual exchange of mass between the displacing and the displaced phases through vaporizing and condensing gas drives (Archer and Wall 1986). In practice, several fluids like light reservoir oil, hydrocarbon gases, liquefied petroleum gas (LPG), CO_2, and N_2, have been employed for displacing the oil during gas injection.

Field and research experience do not recommend LPG injection for oil recovery due to higher expenses and risk involved in practical applications. Hydrocarbon gases are sometimes used, but require relatively high minimum miscibility pressure (MMP). Flue gas, which is mainly N_2 gas along with CO_2, SO, and O_2, has been employed in miscible processes but impurities increase the MMP value. Experimentally, CO_2 has been proven to be superior and is a preferred choice due to its low cost, higher density, and ability to provide an additional benefit of CO_2 sequestration in the reservoirs (Lake et al. 2019). CO_2 flooding for EOR was first employed as early as 1930, but most of its development happened only after the 1970s (Srivastava et al. 2000). CO_2 flooding is considered to be the most efficient EOR method for

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R. Phukan · R. Saha · L. Pandey · P. Tiwari (✉)
Department of Chemical Engineering, Indian Institute of Technology Guwahati, Guwahati 781039, Assam, India
e-mail: pankaj.tiwari@iitg.ac.in

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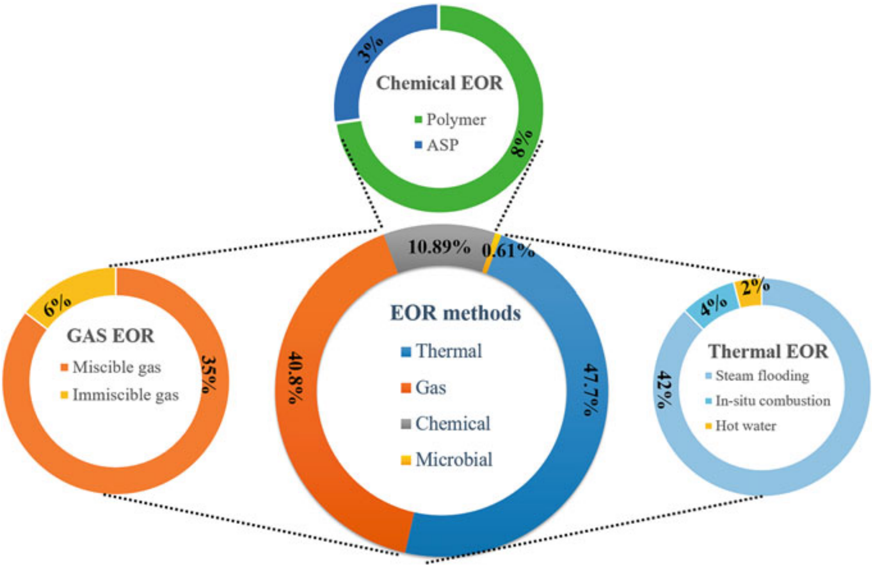

Fig. 1 Worldwide (1959–2010) EOR project [Data taken from (Al Adasani and Bai 2011)]

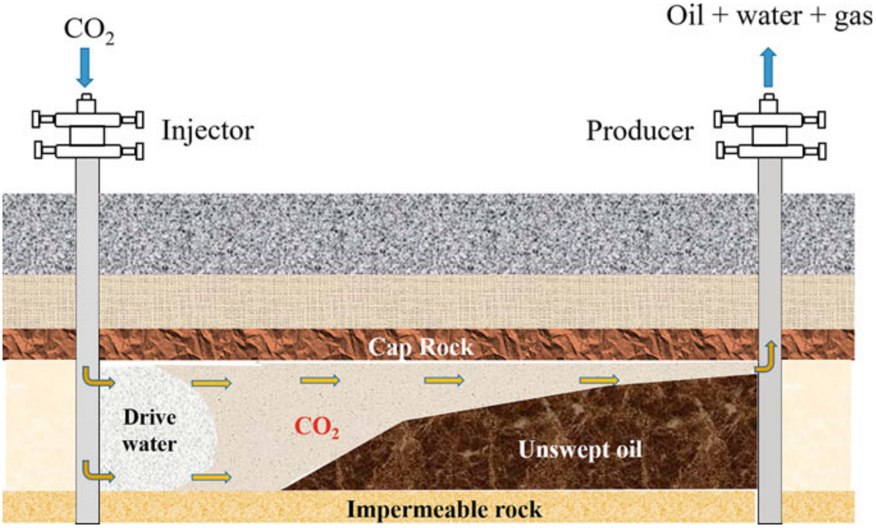

Fig. 2 Schematic representation of continuous CO_2 flooding showing early injection gas breakthrough [Adapted from Phukan (2020a)]

crude oils of light to medium gravity. Implementing the CO_2 flooding in a particular reservoir can lead to an additional oil recovery of 15–25% of original oil in place (OOIP), thereby extending the production life of the reservoir by another 15–20 years (Yongmao et al. 2004). Injection of CO_2 gas into the reservoir leads to its sequestration in the subsurface rocks, thus reducing its emission in the environment (Gogoi and Kakoty 2017; Karmakar 2016; Stocker et al. 2018). With the injection of anthropogenic CO_2 gas into an oil reservoir, two-fold benefits can be obtained: increase in oil recovery and reduce the emission of greenhouse gas.

According to the International Energy Agency (IEA), the total numbers of worldwide CO_2-EOR projects were 166 in 2017, with more number of miscible CO_2-EOR projects compared to the immiscible projects. The largest miscible CO_2 flooding in the world was developed in 1972 in Texas, USA by Chevon. CO_2 was recovered from flue gas of four gas plants, and after dehydration was transported 220 miles for injection (Qin et al. 2015). The Bati Raman project in Turkey has been acknowledged as the world's largest application of the immiscible CO_2-EOR project (Spivak et al. 1989). Started in 1986 after successful lab tests with the availability of a large amount of CO_2 gas in a neighboring field about 55 miles away, the project produced 7,000 barrels of oil per day (bpd) (Sahin et al. 2007). In the USA, the principal source of CO_2 comes from natural CO_2 reservoirs which are mainly injected in the Permian Basin CO_2-EOR projects (Godec 2011). Weyburn project in Canada has been reported to be one of the biggest global anthropogenic CO_2-EOR projects (Perera et al. 2016). The source of the CO_2 gas was the large gasification plant situated in North Dakota from where the gas is transported. About $1{,}600 \times 10^3$ ton/year of CO_2 is sequestered which was equivalent to 67% of the injected gas amount. As far as India is concerned, Oil and Natural Gas Corporation (ONGC) has been mainly involved in studying the feasibility of CO_2-EOR application in Indian oilfields. Experimental and modeling studies provided encouraging results for the CO_2-EOR pilot project for the Ankleshwar oil field of Western India. These studies indicated that CO_2-EOR is technically feasible in the fields, and recovery could be expected to improve by approximately 4% in the project life of 35 years. The anthropogenic CO_2 for injection was supplied from an adjacent gas processing plant in Hazira for injecting into the oilfield. It is expected to sequester 5 to 10 million tons of CO_2 by this process (Kumar and Mani 2007; Viebahn 2012). In this chapter, the various aspects of CO_2-based EOR schemes such as recovery mechanisms involved, process selection criteria, gas-alternate options along with CO_2 availability are elaborated (Fig. 2).

2 Recovery Mechanisms for CO_2 Flooding

Depending upon the operating pressure, the injected CO_2 can develop miscible or immiscible conditions with oil in the reservoir. However, miscible or near-miscible flooding is the preferred method due to its potential to improve oil recovery. In miscible flooding, the increase in oil recovery occurs through the mobilization of lighter oil components, swelling of oil, reduction of oil viscosity, and reduction of interfacial tension (IFT) (Gogoi 2013; Verma 2015). This process occurs when the operating pressure of the reservoir is above the minimum miscibility pressures (MMP) of the crude oil, and CO_2 becomes miscible with oil through multi-contact or dynamic miscibility. Multi-contact miscibility, in general, can be achieved through two techniques: vaporizing gas drive and condensing gas drive; a lean gas is injected in the vaporizing drive which first vaporizes the lighter components (up to C6) present in the reservoir fluid. The mixture progressively mixes with heavier components until the injection gas is enriched enough to achieve miscibility with the original reservoir fluid. In a condensing gas drive, injected gas is an enriched C_{2+} hydrocarbons. The oil accomplishes sufficient enrichment and becomes miscible with the injected gas. In CO_2 flooding, the injected CO_2 gas vaporizes the intermediate components of crude oil (vaporizing gas drive method) and develops complete miscibility due to the mutual mass transfer between the two phases (Satter et al. 2007). Miscibility causes the lowering of IFT thereby eliminating the capillary pressure, and the result is near zero residual oil saturation (Jarrell 2002). Although the miscible process is more efficient for EOR, achieving miscibility is not always possible under various reservoir conditions and fluids properties due to technical and safety considerations.

Conversely, in immiscible displacement, the operating pressure is below the MMP, so less mutual interchange of components takes place between the crude oil and the injected gases. The main driving mechanisms of immiscible CO_2 flooding are oil swelling, oil viscosity reduction, solution gas drive, and reduction of IFT or a combination of these mechanisms which assists in mobilizing a part of the residual oil to improve oil recovery (Fath and Abdol-RasoulPouranfard 2014). In the immiscible flooding method, CO_2 gas is injected at subcritical pressures which not only provides energy to the reservoir to assist the flow of oil but also produces additional oil (Bagci 2007). Injected CO_2 dissolves in the crude oil to reduce oil viscosity which successively improves the mobility ratio resulting in better volumetric efficiency (E_V). Moreover, CO_2 gas upon contact with crude oil causes oil swelling by a process of dissolution. Swelling causes the discontinuous residual oil droplets to combine with the flowing oil phase, and the result is a lower residual oil saturation (Mangalsingh and Jagai 1996). Figure 3 illustrates the saturation pressure, oil viscosity, and swelling factor dependence on CO_2 solubility in crude oil. Saturation pressure increases as CO_2 solubility increases (Fig. 3a), and higher pressure is

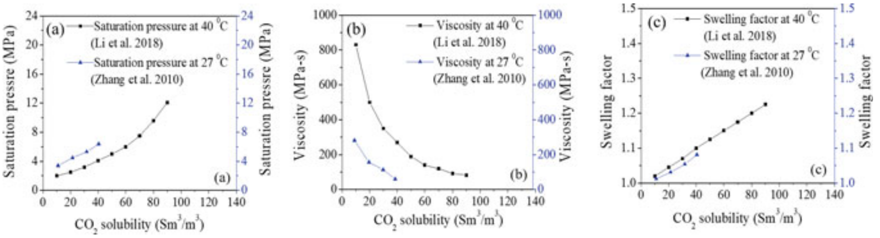

Fig. 3 CO_2 solubility in crude oils with respect to (**a**) saturation pressure, (**b**) viscosity, and (c) swelling factor [adapted from (Phukan, 2020b)]

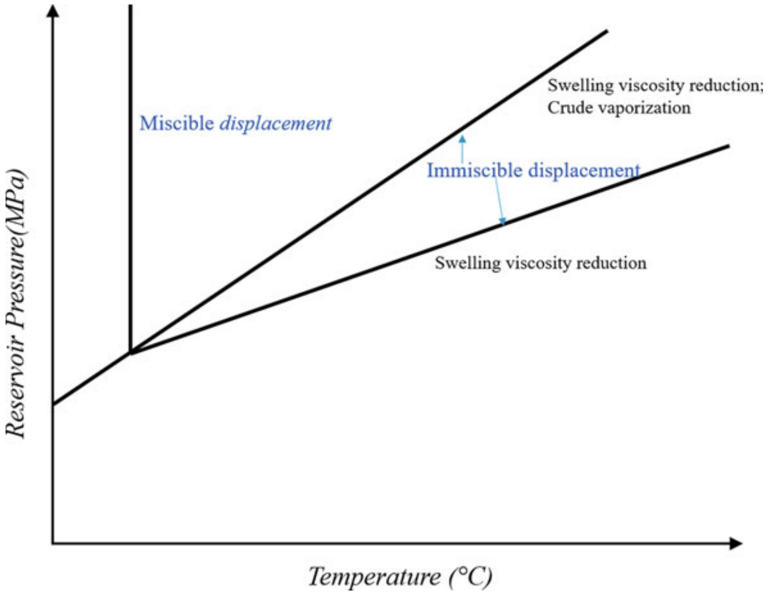

Fig. 4 A schematic representation of mechanisms of CO_2-based EOR depending on the reservoir pressure and temperature [adapted from Perera et al. (2016)]

required to gasify oil with a higher fraction of dissolved CO_2 gas (Li et al. 2018). The viscosity of crude oil decreases with an increase in CO_2 solubility in crude oil (Fig. 3b). This is the primary mechanism of heavy oil recovery by CO_2 flooding as the viscosity of heavy oil can be reduced considerably by CO_2 dissolution at a lower pressure (Song et al. 2018). The swelling factor is also observed to increase with CO_2 dissolution (Fig. 3c). Although miscibility between crude oil and CO_2 is not significant in immiscible flooding, CO_2 dissolves in the oil phase to cause a reduction of IFT and improve the oil relative permeability, and the oil is displaced towards the production well by the injected CO_2 gas. Oil swelling and viscosity reduction are the prominent effects of the immiscible CO_2-EOR process and additional recovery of 5 to 12% of OOIP may be achieved (Zhang et al. 2018). In addition, the choice of the mechanism of CO_2-based EOR relies on the reservoir pressure and temperature as shown in Fig. 4 (;).

3 Screening Criteria and Challenges Associated with CO_2 Flooding

The technical screening criteria for various EOR methods have been developed as a result of intensive studies in the laboratory, field pilot, and full-scale commercial operations (Al Adasani and Bai 2011; Taber et al. 1997a, 1997b). Taber et al. (1997a) first published the screening criteria for 12 EOR methods based on nine reservoir properties (Taber et al. 1997a), which was later updated based on 652 reported EOR projects (Al Adasani and Bai 2011). The updated screening criteria included new EOR categories and subcategories like microbial EOR, hot water flooding, miscible and immiscible WAG. Although these criteria cannot be considered exclusive, but provide sufficient guidance while assessing the viability of an EOR project in a new field. The technical screening guidelines established for miscible and immiscible CO_2 and WAG flooding are summarized in Table 1.

Although CO_2 flooding is considered a successful EOR technique, a large part (35–65%) of the OOIP remains un-retrieved even after injection of a sufficient volume of CO_2. Miscible CO_2 flooding typically recovers 10–20% OOIP using dense CO_2 injection corresponding to nearly 80% of the hydrocarbon pore volume (HCPV), while immiscible CO_2 flooding can recover only 5–10% of OOIP because of the non-zero IFT between the CO_2 and crude oil (Enick and Olsen 2012). The primary reason for the lower oil recovery of CO_2 flooding is related to its density and viscosity. The low density relative to oil leads to gravity overriding/segregation, a situation where CO_2 migrates toward the upper section of the pay zone. As a result, the lower portions of the formation remain unswept by CO_2 and lower E_{vo}. The mobility ratio is unfavorable ($M > 1$) due to the considerably lower viscosity of the injected CO_2 gas. The high mobility ratio promotes the flow in high permeability zones and viscous fingering. The resultant effect is an early gas breakthrough, reduced E_{vo}, and high residual oil saturation. The other problems associated with CO_2 flooding that reduce the efficiency of the process are corrosion and asphaltene precipitation. When CO_2 reacts with the formation water, carbonic acid is formed, making the formation water acidic. The acidic environment may corrode the downhole tubular and production equipment and increases the risk of leaks. During CO_2 flooding, the interaction of the CO_2 and crude oil may cause the asphaltene-to-resin ratio (R/A) of the crude oil to be reformed, leading to precipitation and subsequent deposition of the asphaltene (Srivastava and Huang 1997). Asphaltenes may also become unstable (Ghedan 2009).

Table 1 Screening criteria for miscible CO_2, immiscible CO_2, and WAG flooding [data adopted from references (Adasani and Bai 2011; Taber et al. 1997b)]

Parameters/GAS EOR method	Miscible CO_2	Immiscible CO_2	Miscible WAG
Oil gravity (0API)	22–45	11–35	33–39
Oil viscosity (cP)	35	0.6–592	0.3–0.9
Formation permeability (mD)	1.5–4500	30–1000	17–32
Rock porosity (%)	3–37	17–32	11–24
Oil saturation (%PV)	15–89	42–78	>42
Depth	1500–13,365	1150–8500	7545–8887
Temp. (^{o}F)	82–257	82–198	194–253
Rock type	Sandstone and carbonate	Sandstone and carbonate	Sandstone

The precipitation may obscure the movement of CO_2 into the portions of the reservoir containing residual oil and thereby lowering E_{vo}. Resins tend to retain asphaltenes in solution. A high R/A value indicates that asphaltenes are unlikely to separate (Bon and Sarma 2004). The conditions for the stability of asphaltene are: $R/A > 3.0$ as steady-state, $2.0 < R/A < 3.0$ as meta-steady state, and $R/A < 2.0$ as unsteady state Leontaritis and Mansoori (1987).

4 Water-Alternating-Gas/CO_2 (WAG) Flooding

Water-alternating-gas/CO_2 (WAG) flooding is the technological choice for CO_2 mobility control, where instead of continuous injection, CO_2 is alternately injected with water into the reservoir as short slugs to provide better E_{vo} and reduce CO_2 consumption. This technique lowers the relative permeability to CO_2 through increased water saturation and lowers CO_2 gas saturation in the pore spaces of the reservoir rock. The mobility of gas is controlled, and early gas breakthrough is alleviated through WAG injection which improves the displacement efficiency of the process (Bahadori 2018; Elwy et al. 2012). The first reported WAG field application was a pilot study in the North Pembina oil field, Alberta, Canada in the year of 1957 (Christensen et al. 2001; Rahimi et al. 2017). The obvious advantage of WAG lies in the fact that both the injected fluids are available in large volumes and so less costly. The schematic representation of the CO_2-WAG process is presented in Fig. 5.

Although WAG is the most widely used technique for mobility control in CO_2 flooding, the process still leaves behind significant oil unrecovered. The improvement of oil recovery by WAG injection is around 10% of OOIP (Christensen et al. 2001; Kulkarni and Rao 2005; Skauge and Stensen 2003). This low recovery is primarily due to the water blocking effect. Water blocking occurs when the injected water separates the residual oil from coming into contact with the CO_2 gas. Water itself cannot remove the capillary-held residual oil due to the high oil–water IFT, and consequently, the microscopic displacement efficiency is low. Moreover, in the case of viscous oil reservoirs, due to the adverse mobility ratio, viscous fingering of injected water and early injection gas breakthrough occurs. Thus, major areas of the reservoir with residual oil remain unswept by the injected fluids resulting in low oil recovery (Majidaie et al. 2015). Other concerns associated with CO_2-WAG injection are difficulty in controlling gas/CO_2 breakthrough as the WAG process matures, huge volumes of water injection delay the project duration, induce corrosion and water injectivity loss. Numerous studies have been conducted to enhance the performance of CO_2-WAG, which, have led to the chemically enhanced water-alternated gas injection (CEWAG) method. This method combines the benefits of both gas and chemical EOR methods. Different types of chemicals like surfactants, alkalis, co-surfactants, salts, polymers, co-solvents, and nanoparticles are used based on a specific application (Kumar and Mandal 2017; Talebian et al. 2013).

This mechanism is referred to as surfactant-alternated-gas (SAG) flooding in which surfactants are added to water during the WAG injection process resulting

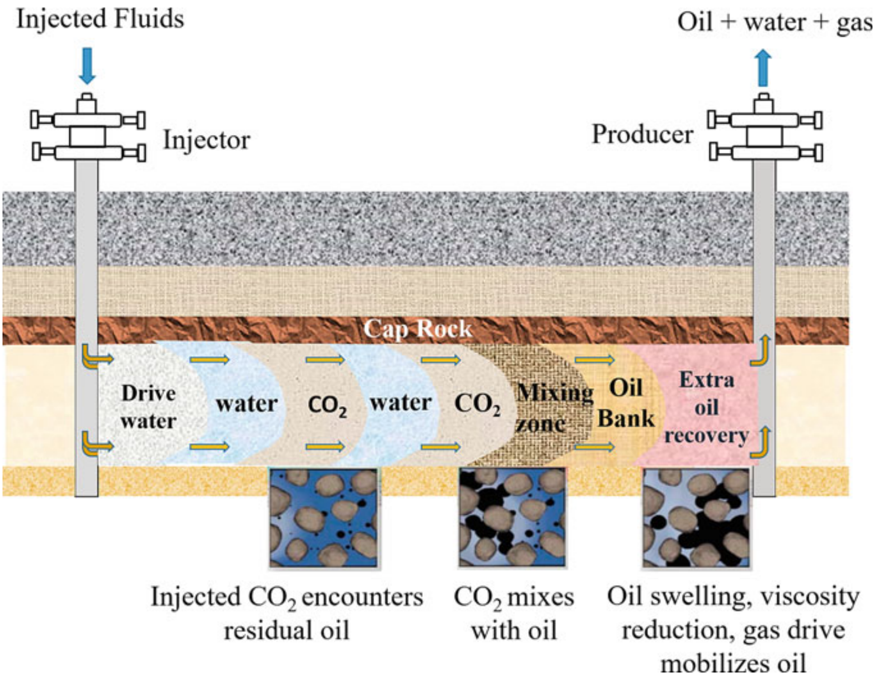

Fig. 5 Schematic representation of CO_2-WAG flooding showing the alternated CO_2 and water injection cycles [adapted from Phukan (2020a)]

in foam formation in the pore spaces of the reservoir rock (Kibodeaux and Rossen 1997; Memon et al. 2017). Previous studies have reported improvement in E_{vo} and a significant increase in oil recovery by SAG flooding compared to continuous CO_2 injection and CO_2-WAG injection (Aghdam et al., 2013; Gandomkar et al., 2016; Salehi et al., 2014; Yaghoobi et al., 1998). The higher oil recovery obtained by SAG injection can be attributed to several factors; IFT reduction of oil–water due to the presence of surfactants, favorable mobility due to foam formation, and effective mass transfer between the fluids. Foam increases the apparent viscosity of CO_2 gas, thereby reducing its mobility. Thus, channeling and viscous fingering problems are alleviated significantly. Additionally, foam decreases the permeability to water due to the higher trapped gas saturation in pore spaces of the reservoir rock (Bernard and Jacobs 1965). Although foam may form by the co-injection or alternate gas and surfactant solution injection in the reservoir. The alternate method is preferred over co-injection due to its characteristic advantages (Gandomkar et al. 2012; Salehi et al. 2014; Telmadarreie and Trivedi 2018). SAG minimizes contact between the water and gas/CO_2 in the surface facilities and pipelines reducing corrosion. SAG injection also increases gas injectivity due to changing saturation near the well-bore (Leeftink et al. 2015). Additionally, SAG injection can reduce gravity override problems without increasing injection well pressures which the continuous co-injection

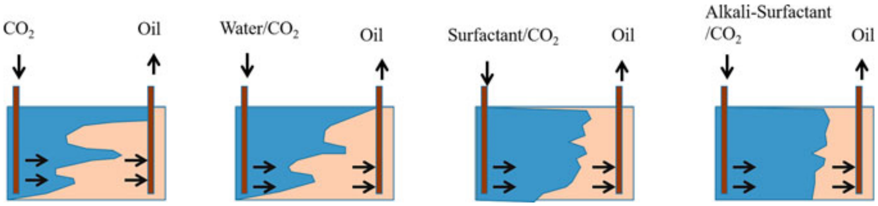

Fig. 6 Schematic representation of the displacement process in CGI, WAG, SAG, and ASAG injection scheme [adapted from Phukan (2020b)]

method cannot do (Shi and Rossen 1988). However, surfactants injected with CO_2 during the SAG process are prone to adsorption by the clay minerals in the rock matrix, which reduces the foaming process efficiency (Samanta et al. 2012). Traditionally, alkali has been used to decrease the adsorption of anionic surfactants onto the reservoir rock. For anionic surfactants, alkali plays the role of a sacrificial agent by fixing a negative charge to the rock surfaces, which creates an electrostatic repulsive force between the surface of the rock and the anionic surfactant, resulting in a significant decrease in surfactant adsorption (ShamsiJazeyi et al. 2013). Adding alkali also assists production of in-situ surfactants. The combination of injected surfactants and in-situ soaps generated helps in the formation of the microemulsion, which exhibits ultra-low oil–water IFT (<0.01 mN/m), thereby mobilizing residual oil for increasing oil recovery held by capillary pressure. In association with the CO_2 gas, the alkali-surfactant (AS) combination in the chemical slug results in a strong/stable in-situ foam in the reservoir (Phukan et al. 2020). These processes of alternate injection of gas/CO_2 and AS slug are referred to as alkaline-surfactant-foam (ASF) flooding, low tension gas (LTG) process, alkali-surfactant-gas (ASG) injection, and alkaline-surfactant-alternated-gas/CO_2 (ASAG) floodings (Cottin et al. 2012; Guo et al. 2012; Hosseini-Nasab and Zitha 2017; Lashgari et al. 2015; Srivastava et al. 2009; Phukan et al. 2019a, 2019b; Srivastava et al. 2011). Figure 6 shows a schematic representation of the displacement process in continuous gas injection (CGI), WAG, SAG, and ASAG floodings.

5 Sources of CO_2, Capture, and Storage

The CO_2 sources in the Permian Basin of the USA and Hungary are examples of natural sources. The anthropogenic sources mainly include the CO_2 emissions as a result of human activities. The combustion of fossil fuels is used for transportation, industrial applications, power generation, residential-commercial buildings. Some industrial applications that also contribute to CO_2 emission include manufacturing of cement, hydrogen production, and oil shale exploration. As large natural CO_2 resources are scarce, anthropogenic sources have become favorable options for CO_2-EOR applications. Capturing and geologically storing CO_2 is a process that involves the separation and recovery of CO_2 from the process or exhaust gases of large industrial installations, transportation to the site, and then injecting into a suitable subsurface formation for storing the gas. The process involves the following primary steps: capturing, transportation, and storage (Gabrielli et al. 2020; Rosenbauer and Thomas 2010).

Capturing techniques of CO_2: Based on the installation type, the capturing of CO_2 may take place at three stages; (a) *Post-combustion*—suitable for existing installation and is considered as the most mature technique. However, the capital expenditure and operating cost is high. Liquid solvents such as mono ethanolamine (MEA) is used for separating the CO_2 gas from the flue gases. (b) *Pre-combustion* involves treating the fuel by steam reforming (with steam and air) or partial oxidation (with oxygen) producing concentrated streams of CO and H_2, which facilitates CO_2 capture. The next step involves converting the CO in the presence of water and then separating the CO_2 for storage and capture. (c) *Oxyfuel combustion technique* produces a combustion gas rich in CO_2 (80–90% by volume) which can be used in existing installations. Instead of air, the process uses oxygen (of high purity) for combustion, thereby avoiding the primary difficulty of extracting oxygen from the air.

Transportation of CO_2: The transport of CO_2 can be done through pipelines by pressurizing up to 73 bar such that it reaches a supercritical state with high density, having liquid-like properties. For distances, more than 500 to 1,000 km, transportation of CO_2 in the liquid form has been done by ship.

Geological storage of CO_2 There are various ways for storing CO_2 at different maturity stages; (a) *Storage in depleted hydrocarbon reservoirs*—crude oil and natural gas reservoirs are advantageous for storing CO_2 due to the presence of a cap rock which provides the correct type of sealing to prevent the gas escape. Additionally, the existing infrastructure like the wells and pipelines used for hydrocarbon production can also be used for operations involving CO_2 storage, thereby providing a cost-effective approach. (b) *Storage in coal seams*—coal beds with impermeable cap rock can be used for CO_2 storage due to the absorption of the gas by the coal seams; this method can also be employed for enhanced coal bed methane recovery. (c) *Storage in saline aquifers*—another potential storage sites where a considerable amount of CO_2 can be stored. These are the porous and permeable saline aquifers found in different sedimentary basins of the world, both in onshore and offshore. In all cases, storage of CO_2 must be at a sufficient depth (greater than 800 m) such that the gas is in the supercritical state, thereby occupying the least volume.

6 Evaluation of CO_2-EOR Flooding

To investigate the suitability of CO_2 flooding for a particular reservoir and to provide sufficient information for oil field development, laboratory experiments are required to perform including (i) Calculation of minimum miscibility pressure (MMP), (ii) Measurement of PVT properties for reservoir fluid-CO_2 mixtures, and (iii) Evaluation of oil retrieval potential through core flooding tests (Srivastava et al. 2000).

6.1 *MMP Determination*

MMP is the lowermost pressure at which the crude oil develops multi-contact miscibility with injected CO_2. It is considered one of the most critical parameters for designing the CO_2 flood project and screening reservoirs for miscible or immiscible flooding (Chen et al. 2013). The MMP is determined prior to field implementation as it is essential for the operator to design the required surface facilities and develop injection conditions (Al-Hinai et al. 2014). MMP can be reliably determined by standard laboratory methods including slim tube test, rising-bubble apparatus (RBA), vanishing interfacial tension (VIT) method, and supercritical extractor method (Mansour et al. 2018). Among these experimental methods, the slim tube test is the most commonly used method. The rising-bubble apparatus (RBA) is considered a quicker and better visual alternative to the slim tube test. VIT method is a recently developed technique to evaluate the miscibility of crude oil-CO_2 systems built on the theory that IFT of the crude oil and CO_2 approaches zero for the two phases to develop miscibility (Cao and Gu 2013).

The standard experiment to evaluate the operating pressure for a potential CO_2 flood is done through the displacements of recombined reservoir fluid samples from a slim tube packed with sand or glass beads. The slim tube is a 1-D model of the reservoir and consists of a narrow tube (5–40 m in length) packed with sand or glass beads. A schematic diagram of the slim tube apparatus is shown in Fig. 7a. The slim tube is first saturated with oil at reservoir temperature and a pressure, above

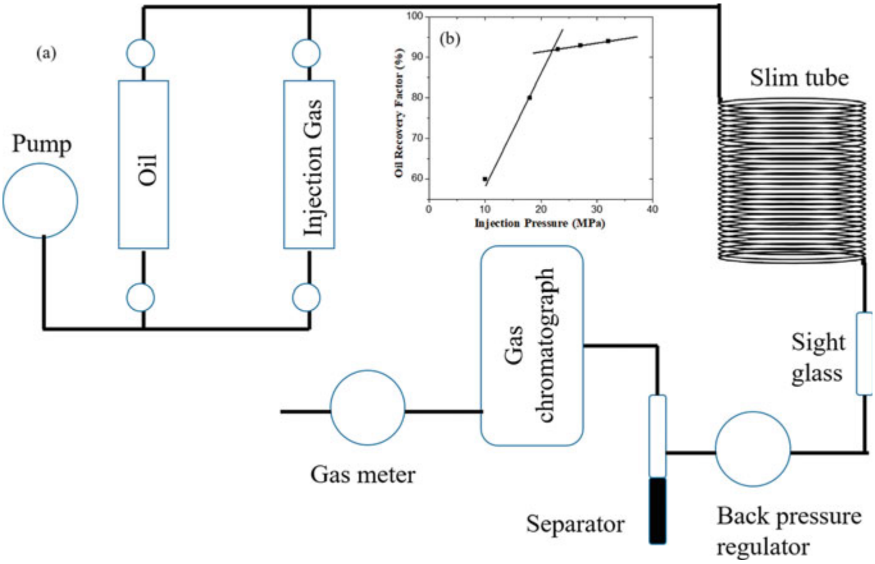

Fig. 7 **a** Schematic diagram representing the slim tube apparatus and **b** determination of the MMP of the crude oil-CO_2 system [adapted from Phukan (2020b)]

Table 2 Different correlations to determine the MMP value of CO_2-crude oil system

Correlation	References
$\text{MMP} = 15.988\, T_{\text{res}}^{\left(0.744206+0.0011038\,\text{MW}_{\text{C5}+}+0.0015279\text{M}_{\text{C1}}\right)}$ where $23.7° < \text{API} < 44°$ and $77 < \text{T}_{\text{res}} < 248$ °F	Cronquist (1978)
$\text{MMP} =$ $1833.7217 + 2.2518055T_{\text{res}} + 0.01800674T_{\text{res}}^2 - 103949.93/T_{\text{res}}$	Yellig and Metcalfe (1980)
$\text{MMP} =$ $0.000878\text{T}^{1.06}(\text{MW}_{\text{C5}+})^{1.78}\left(\text{Vol}/\text{Int}\right)^{0.136}\left(87.8/T_{\text{cm}}\right)^{\left(169.89/T_{\text{cm}}\right)}$ $\text{Tcm} = \sum_{i=1}^{n} \text{WiTci}$	Alston et al. (1985)
$\text{MMP} =$ $810.0-3.404\text{MW}_{\text{C7}+} + \left(1.70010^{-9}\text{MW}_{C_{7+}}^{3.730}e^{786.8\text{MW}_{C_{7+}}^{-1.08}}\right)T_{\text{res}}$ If mole fraction of intermediates $(F_{\text{r}}) < 18\%$: $\text{MMP} = 2947.9-3.404\text{MW}_{C_{7+}} +$ $\left(1.7 \times 10^{-9}\text{MW}_{C_{7+}}^{3.370}e^{786.8\times MW_{C_{7+}}^{-1.058}}\right)T_{\text{res}} - 121.2 \times F_{\text{r}}$	Glaso (1985)
For oil with bubble point pressure less than 0.345 MPa: $\text{MMP}_{\text{pure (MPa)}} = 7.43497 \times 10^{-5}(T_{\text{res}})^{1.1669}\text{MW}_{\text{C}_{5+}}^{1.201}(\text{Vol}/\text{Int})^{0.109}$	Emera and Sarma (2007)
$\frac{\text{MMP}_{\text{impure}}}{\text{MMP}} = 1.0 - 2.13 \times 10^{-2}(T_{\text{cm}} - 304.2) + 2.51 \times$ $10^{-4}(T_{\text{cm}} - 304.2)^2 - 2.35 \times 10^{-7}(T_{\text{cm}} - 304.2)^3$ $T_{\text{CM}} = \sum_{i=1}^{N} y_i T_{ci}$	Sebastian et al. (2013)

Where
T_{res} = Reservoir temperature, R, F_{r} = Mole fraction of intermediates
MWC_{5+} = Molecular weight of pentance plus fractions, Y_i = Mole fraction of ith fraction
$\text{MW}_{\text{C7}+}$ = Molecular weight of heptance plus fractions, Wi = Mass of ith fraction
P_{c} = Critical pressure, T_{c} = Critical temperature
Int = Oil intermediates (C_2–C_4, H_2S, and CO_2), Vol = Oil volatiles (C_1 and N_2)

the bubble point pressure of the oil. The oil is then displaced by CO_2 gas into the slim tube at a constant inlet/outlet pressure controlled by the backpressure regulator. The effluent coming out of the slim tube is flashed at atmospheric conditions, and the recovery rate is measured along with the density and composition of the fluids produced. The breakthrough of the gas is also monitored by continuously analyzing the effluent gas composition and the gas–oil ratio. The displacement experiments are conducted at numerous pressure conditions, and the oil recovery is monitored simultaneously to determine the miscibility conditions. MMP is a strong function of the crude oil composition, reservoir temperature, and injected gas composition. Oil recovery increases rapidly with an increase in injection pressure below the MMP.

Although CO_2 injection at higher pressures would result in greater oil recovery, injection above MMP reduce the rate of increase in oil recovery. The trend of oil recovery as a function of injection pressure is close to linear on both sides of MMP (Fig. 7b). Near MMP, the ultimate oil recovery reaches 90–95% or 80% of gas breakthrough, and after reaching the MMP, additional oil recovery is minimal. Thus, the MMP of the reservoir fluid-CO_2 mixture is the break-over point of oil recovery versus injection pressure. The slim tube experiment is an appropriate technique that can provide direct information about potential operating pressures, and it should be performed as early as possible to evaluate the field prospects for CO_2 flooding. However, the test apparatus is expensive and takes sufficient time to complete miscibility measurement (4–5 weeks).

The laboratory techniques for determining MMP are usually very expensive and time-consuming. Various empirical correlations for estimating MMP are developed as listed in Table 2. Most of these correlations are based on the composition of the crude oil, the composition of the injection gas, and reservoir temperature (Ahmed 1997; Zhang et al. 2016); hence, to be selected carefully for a particular reservoir. As the miscibility of CO_2 is higher with the intermediate components of crude oil than with the light and the heavy components, MMP is lower for crude oils with a larger fraction of the intermediate components. However, CO_2 can achieve miscibility with heavy components of crude oil through multiple/dynamic contact miscibility. Typically, CO_2 has higher miscibility with crude oil under reservoir conditions than methane and nitrogen, so they are generally considered as the impure components of the injection gas. Thus, injection gas with more of these gases increases the MMP. Further, CO_2 MMP increases with increasing temperature, and so deeper/hotter reservoirs have higher MMP values.

6.2 Fluid Sampling

Fluid sampling or collection of fluid samples is an essential component of reservoir fluid analysis and can be done in basically two ways: (i) direct subsurface sampling and (ii) surface recombination of the samples (Nagarajan et al. 2006). In both techniques, it is to be ensured that the sample collected represents the fluid in the reservoir at sampling. The sampling method to be employed is influenced by the volume of sample required, reservoir fluid type to be sampled, bottom hole pressure, and surface facilities available. In conventional subsurface sampling, a bottom hole sampler is lowered into the hole to the reservoir depth. The sample is collected from the subsurface well stream. The reservoir fluid sample is trapped in the pressure-tight sampler and brought to the surface where it is repressurized into a single-phase fluid before being transferred to the laboratory. The surface recombination sampling consists of collecting separate samples of oil and gas at separator conditions. A representative reservoir fluid sample is formed in the laboratory by recombining the samples, oil, and gas, in the correct proportion based on the producing gas–oil ratio. This method can easily obtain large volumes of reservoir fluid samples. Another method to get a representative reservoir fluid sample is by mixing the oil and gas samples from the separator in a ratio to maintain the bubble point pressure (Dake 1978).

6.3 Measuring PVT Properties for Reservoir fluid-CO_2 Mixtures

The mechanism of oil recovery by CO_2 flooding is associated with the mutual interaction between the CO_2 and reservoir fluid phases. So, it is indispensable to measure the phase-behavior and fluid-property at different reservoir conditions to gain a better insight into the underlying mechanisms and improve performance prediction. The physical properties such as oil swelling, viscosity reduction, and density alteration with CO_2 solubility in oil are required to be ascertained for a rational design of the process and to better simulate the oil recovery mechanism. These properties can be determined either experimentally and/or by prediction using empirical correlations. Accurate and reliable measurements of CO_2 solubility in oil, change in the swelling factor, and viscosity reduction of oil can be obtained through PVT tests in a high pressure-high temperature PVT apparatus. Additionally, the mutual interchange of oil components with the injected CO_2 gas can be quantitatively studied by measuring the composition of the remaining oil in a PVT apparatus. However, due to the large number of slow calculations involved, certain software like ECLIPSE is sometimes used to compute the oil viscosity reduction and swelling factors. Further, different correlations have been developed and reported in the literature to calculate the effects of CO_2 injection on the physical properties of oil that include CO_2 solubility, oil swelling factor, CO_2-oil viscosity, etc. The correlations developed by Emera and Lu (2005), Mehrotra and Svrcek (1982), Simon and Graue (1965), and Chung et al. (1988) are commonly used for predicting the fluid properties of CO_2-crude oil systems. The physical properties of the oil-CO_2 system by the model of Emera and Lu (2005) were found to be a better prediction with greater accuracy. In addition, this model covers the oil properties; gravity, viscosity (12,000 mPa.s), pressures up to 34.5 MPa, mPa.s, temperatures up to 140 °C, and oil molecular weight greater than 490 lb/mol.

6.4 Evaluation of Oil Recovery Potential by CO_2 Flooding

Core flood experiments are normally carried out to assess if an appreciable amount of additional oil could be recovered under reservoir conditions through the injection of CO_2 under different injection strategies at the laboratory scale. These experiments are performed under prescribed conditions using actual reservoir rock samples and crude oil samples to simulate the oil recovery by CO_2 injection under the reservoir environment. The key parameters measured are; (i) oil recoveries as a function of the volume of fluid injected, (ii) initial and final reservoir fluid saturations, and (iii) absolute and effective permeability, and pressure drop data. By calculating the pressure difference across the core with respect to flow rate, any change in rock permeability due to fine migration and asphaltene precipitation can be monitored. The core flooding works of Cao and Gu (2013) on sandstone reservoir core samples of Pembina Cardium oilfield with light crude oil showed that the oil recovery factor increased as the injection pressure of CO_2 flooding was increased up to the MMP. Above the MMP, the oil recovery increased marginally, and ultimately reached a nearly constant maximum value in the miscible CO_2 flooding. Additionally, during the core flooding experiments, the asphaltene content of the produced oil reduced with the pore volume of fluid injected due to asphaltene precipitation as a result of CO_2 injection. Wang et al. (2019) performed core flooding experiments on Chang Qing oilfield sandstone cores to examine the alteration of petrophysical properties during CO_2 and WAG flooding. Their experimental findings showed that the decrease in permeability was more significant in WAG compared to CO_2 flooding due to higher fine migration during WAG flooding. Nobakht et al. (2007) also conducted 13 high-pressure core flooding experiments to study the ramifications of injection pressure, injection rate, and volume of fluid injected on oil recovery by CO_2 flooding. The oil recovery data as a function of the injected fluid volume at various rates and injection pressures showed that oil recovery increased with CO_2 injection volume and achieved the maximum after 1.5 pore volume of CO_2 was injected. The oil recovery obtained was found to increase almost linearly with the injection pressure in the intermediate injection pressure range, whereas oil recovery was also increased with the rate of CO_2 injection and the injection pressure is maintained. However, in the lower or higher injection pressure ranges, the oil recovery remained practically unchanged irrespective of the injection rate (Nobakht et al. 2007).

6.5 Modeling and Simulation Study of CO_2 Flooding

Numerous empirical correlations and simulators have been developed to model the flow of fluids to estimate oil recovery and understand the underlying flow mechanism during CO_2 injection. Abass et al. (2018) used an entirely compositional simulation model representing a sandstone oil reservoir of Western Desert oil fields, Egypt to estimate CO_2 miscible flooding on the recovery of oil and optimize the injection mode such as straight CO_2 injection or WAG injection for pure and impure CO_2 injections. A comparison between different injection modes showed that better results are obtained with WAG application than with straight CO_2 injection. Reducing the cycle periods of the WAG processes could increase the oil recovery factor. Karimaie et al. (2017) carried out a simulation study using a realistic model of the North Sea oil reservoir to evaluate the oil recovery performance of CO_2 flooding. The various CO_2 injection schemes, and well locations and configurations have been studied to define the optimal injection scheme and well arrangement. The simulation results showed that a 3–8% increase in oil recovery factor could be obtained by CO_2 flooding which, to a significant magnitude, was dependent on the well configuration and injection scheme. CO_2 Simultaneous-Water-and-Gas (CO_2 SWAG) flooding showed favorable results as an event of an increase in mobility control. Regarding the storage potential, it was observed that for the marginal field under study, around 30% of the injected CO_2 volume could be sequestered in the reservoir. The experimental and numerical simulation studies of Kamali et al. (2015) to co-optimize the CO_2 injection process suggested that the near-miscible CO_2 injection provides the best option for coupling CO_2-EOR and storage. Mohammad et al. (2017) used a compositional reservoir simulator (CMG-GEM) to study the various design parameters of cyclic CO_2 injection that have maximum effect on the oil recovery and to understand the behavior of cyclic CO_2 injection in tight oil reservoirs. The results of the study specified that the most suitable time for CO_2 injection was after 18 months of primary depletion. Higher oil recovery could be achieved with a longer injection time in the cyclic CO_2 injection process, and extending the soaking period did not produce an increment in oil recovery. CO_2 dissolution in aquifer brine increased with increasing pressure, decreasing temperature, and decreasing brine salinity (Mohammad et al. 2017).

7 Conclusion

CO_2 flooding is considered as the most operational EOR method for the oil reservoirs of light and medium grade. In addition to oil recovery, the injection of CO_2 into the oil reservoir also helps in CO_2 sequestration. Experimental and modeling studies have confirmed that the mechanisms responsible for higher oil recovery by CO_2 flooding include the mutual mass transfer of components, oil swelling, viscosity reduction, and reduction of IFT value. Miscible displacement is the preferred mode of operation due to its higher oil recovery potential. Despite its ability to improve oil recovery, CO_2 flooding suffers the drawback of lower oil recovery (5–10% of OOIP), due to unfavorable mobility ratio and conformance issues. Efforts to improve CO_2 flooding performance have resulted in the development of the WAG and CEWAG methods, which are proven to enhance process efficiency. Simulation studies and techno-economic analysis would be required to forecast and estimate the performance of various CO_2 flooding schemes under various reservoir and operating conditions for a successful pilot and field-scale implementation.

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Optimum Formulation of Chemical Slug and Core Flooding Studies

Rahul Saha, Ranjan Phukan, Lalit Pandey, and Pankaj Tiwari

1 Introduction

The initial stage of oil production from reservoirs is encountered by the natural pressure energy that exists within the reservoir. This natural energy declines with time as oil production progresses and can be overcome by injecting water or gas. The term easy oil signifies the quantity of crude oil that can be recovered through primary and conventional water flooding method. The injected water pushes the oil to the production stage while maintaining the required pressure. Moreover, the conventional water flooding techniques are not effective towards higher oil recovery as water and oil are immiscible and water bypasses the crude oil due to viscous fingering. The primary and secondary oil recovery methods together cannot recover more than 25–30% of the original oil in place. The global hike in demand and consumption of energy along with the depletion of easy oil has put a tremendous burden towards recovering the residual oil from the mature reservoirs. Thus, the concept of tertiary or enhanced oil recovery (EOR) is introduced, which has the potential to recover a large amount of residual oil.

Chemical-enhanced oil recovery (CEOR) is one of the tertiary techniques, which is employed in the oil reservoirs to attain higher oil recovery. In this process, aqueous soluble chemicals like alkali, surfactants and polymers are injected into the reservoir. These chemicals either separately or in combination when injected in oil reservoirs have the ability to recover the left-out oil through several mechanisms. These mechanisms include reduction of interfacial tension (IFT), emulsification of crude oil, wettability alteration of the reservoir and overall displacement (Datta et al. 2018; Datta et al. 2020; Saha et al. 2021; Saha et al. 2018e; Sharma et al. 2019a; Sharma and Pandey 2020; Sharma et al. 2019b). Alkali–surfactant–polymer (ASP) flooding

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R. Saha · R. Phukan · L. Pandey · P. Tiwari (✉)
Department of Chemical Engineering, Indian Institute of Technology Guwahati, Guwahati 781039, Assam, India
e-mail: pankaj.tiwari@iitg.ac.in

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is a branch of chemical EOR, which is employed to recover the trapped residual oil from a reservoir. The ASP flooding combines the interaction of alkali, surfactant and polymer to reduce the IFT between oil and water, emulsify the crude oil and maintain a desirable mobility ratio between displaced fluid (oil) and displacing fluid (chemical aqueous phase) towards the maximum displacement efficiency (Olajire 2014; Sheng 2014). The overall impact of all these phenomena including wettability alteration of rock can severely enhance the oil recovery factor, which cannot be overcome by conventional water flooding schemes (Guo et al. 2017a, b). The consumption of alkalis in the reservoirs occurs through ion exchange mechanism, precipitation of hydroxide in the system and dissolution of the minerals (Dang et al. 2018; Mohammadi 2008). The presence of alkali in the ASP slug additionally blocks the active interaction sites present in the rock surfaces, which ultimately brings down the adsorption of surfactants and polymers. Moreover, a race between polymer and surfactant exists towards their adsorption on the active sites of the solid surfaces (Sheng 2013a). The ASP slug has been successfully implemented in several parts of the world like China, USA, Canada, South America, India, Kuwait and Venezuela, which accounts for over 25% additional oil recovery since decades (Charest 2013; Guo et al. 2017a, b; Jain et al. 2012; Li et al. 2003; Olajire 2014; Sheng 2013b2013b). The successful recovery of crude oil by ASP flooding depends on the formulation of a chemical slug for which several parameters are considered.

In this chapter, the various mechanisms responsible for additional oil recovery during the chemical injection, individual and combined slug are discussed along with the laboratory and field data.

2 Mechanisms Involved in Chemical EOR

The important mechanisms responsible for chemical induced enhanced oil recovery are: (i) ***Interfacial tension:*** the force/tension that exists between crude oil and the aqueous chemical phase. The reduction in IFT promotes the flow of oil through the porous media and depends on the pH, salinity and temperature of the formation. (ii) ***Emulsification:*** is the process of mixing two immiscible liquid phases by the application of foreign substances like surfactants. Surfactants facilitate the dispersion of one immiscible phase over another immiscible phase resulting in droplet formation of different sizes (Datta et al. 2020; Saha et al. 2018a; Saha et al. 2018b; Verma et al. 2020). (iii) ***Displacement Efficiency:*** measures the overall area covered by the injected fluid in order to displace the residual crude oil and accounts for both microscopic (mobilization of crude oil at pore level of the formation) and macroscopic (mobilization of the crude oil in the areal and vertical direction of the reservoir) displacement efficiencies. (iv) ***Mobility Ratio (M)***: ratio of the mobility (ability to flow) of displacing fluid (injected fluid) to the mobility of displaced fluid (crude oil). The value of $M \leq 1$ is desirable as the viscosity of the injected fluid is much higher than the viscosity of the oil. This reduces the viscosity fingering effect and enhances the oil recovery factor. If the value of M exceeds 1, the injected fluid bypasses the oil and enhances the viscous fingering effect, which ultimately results in poor displacement efficiency. ***(v) Wettability Alteration:*** is the ability of the reservoir rock to change the wettability (the ability of one fluid to adhere to the solid surface) from oil—wet to favourable intermediate or water—wet (Saha et al. 2019b; Saha et al. 2018b; Saha et al. 2018e; Sharma and Pandey 2020). This alteration eases the flow of trapped residual oil from the pore spaces towards the wellbore. The injected chemicals can lead to alteration in wettability of the reservoir by forcing the water phase inside the solid rock induced by ion pairs formed by the interaction between crude oil-surfactant (Saha et al. 2018a) and ion-binding mechanisms (Liu et al. 2010).

Conventional alkalis like sodium hydroxide (NaOH), sodium carbonate (Na_2CO_3) and potassium hydroxide (KOH) are commonly used for EOR process. Alkali component reacts with the carboxylic or naphthenic acid part of the crude oil and produces in-situ soap at the oil–water interface, which assists in reducing the IFT (Cooke et al. 1974; DeZabala et al. 1982; Jennings et al. 1974; Saha et al. 2018e; Sheng 2015a). This reduction in IFT helps in enhancing the emulsifying capacity of the crude oil. The IFT (and emulsification) is a function of pH, salinity and temperature of the reservoir (Cooke et al. 1974; Saha et al. 2018a; Saha et al. 2018b; Yuan et al. 2015). The literature reported several other mechanisms like emulsification (entrainment (Subkow 1942), entrapment (Jennings et al. 1974) and coalescence (Castor et al. 1981)), wettability alteration (Gong et al. 2016; Saha et al. 2018e) and displacement efficiency (Pei et al. 2012a) for successful alkali flooding (Sheng 2015a). Moreover, alkali flooding is sensitive to divalent ions (Mg^{2+}, Ca^{2+}, etc.), which can lead to severe scaling or corrosion. (Denney 2008; Guo et al. 2017a, b; Jiecheng et al. 2011). Hence, in order to predict the success of the process, proper selection and formulation of alkali (organic or inorganic) are essential considering the properties of the reservoirs (Fu et al. 2016; Tang et al. 2013).

Surfactant molecules possess a hydrophilic head and hydrophobic tail, which reduces the interfacial imbalance between the immiscible phases and aids in emulsification (Datta et al. 2018; Datta et al. 2020; Saha et al. 2021; Sharma and Pandey 2020). The surfactant introduces ultra-low IFT, which encourages emulsification of crude oil and creates a positive impact towards oil recovery by enhancing the oil displacement efficiency (Saha et al. 2019a; Saha et al. 2018a; Yuan et al. 20152015). The produced emulsion blocks the water channelling by diverting the aqueous chemical media to the unswept parts of the reservoir and ultimately improving the sweep efficiency.

Polymer helps in maintaining the desired mobility ratio between the displacing fluid (chemical phase) and the displaced phase (oil phase). It is expected that if the emulsion formed is too strong indicating unfavourable displacement efficiency then polymer can be implemented to displace the oil drop/emulsion through the porous media resulting in an enhanced recovery factor (Pei et al. 2014; Samanta et al. 2011).

3 Chemicals Selection Criteria

The commercial application of chemical EOR is feasible only if it is economical. The economic viability depends on several parameters like crude oil price, chemical cost, separation cost, operation cost, labour cost, etc. The important criteria are the cost of chemicals and crude oil price per barrel that extensively decides the economy of the system. The price of crude oil is administered by the organization of the petroleum exporting countries (OPEC), however, the cost of chemicals and its formulation for different reservoir conditions can significantly impact the process economics. Thus, based on the overall conditions, screening criteria of chemicals (alkalis/surfactants/polymers) have been formulated and discussed.

3.1 Alkali Screening Based on Crude Oil and Reservoir Properties

The process of alkali flooding depends on several mechanisms like reduction in IFT between oil–water phase, emulsification of crude oil, stability of the emulsion, displacement efficiency and wettability alteration (Almalik et al. 1997; Cooke et al. 1974; DeZabala et al. 1982; Dong et al. 2012; Ehrlich et al. 1974; Gong et al. 2016; Jennings 1975; Jennings et al. 1974; Johnson 1976; Pei et al. 2011; Pei et al. 2013a; Pei et al. 2012a; Rudin and Wasan 1992a; Rudin and Wasan 1992b; Saha et al. 2018e; Wang et al. 2010). Apart from the mechanistic investigation, the criterion for screening of alkali is also important to validate the process with respect to the economy of the system. The major factors are rock and oil properties, formation water characteristics, temperature of the reservoir, gas cap, and aquifer support.

Alkali–acid groups (of crude oil) reactions result in the formation of in-situ soap at the oil–water interface as shown in Fig. 1. The in-situ soap is the main component that controls the IFT reduction phenomena (Jennings 1975; Jennings et al. 1974; Saha et al. 2018e). The role of acid value is accountable for the generation of in-situ soap, thereby enhancing the oil recovery factor. Studies reported that the acid value of $\geq$ 1.5 mg KOH/gm of a sample can be effective in recovering residual crude oil (Cooke et al. 1974). The oil recovery can be enhanced with an increase in the acid value of the crude oil (Ge et al. 2012). Though the acid groups of crude oil are the main factor, a general value or lower acid number is not available to predict the success of alkali flooding. This is because, the acid groups determined by the titration method are not in a state to identify such groups, which form in-situ soap and consume alkali but do not produce soap (Jouenne 2020). Additionally, no direct correlation is available that can relate acid value with IFT and residual oil recovery factor. Crude oil with zero acid value can also reduce the IFT with alkali concentration.

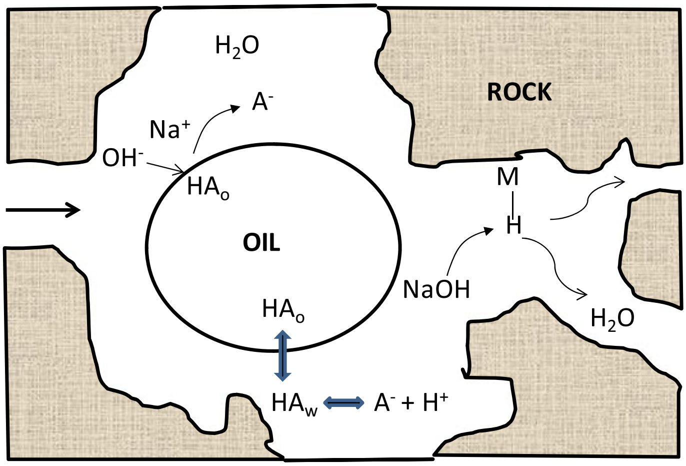

Fig. 1 A schematic of soap formation by reaction between acid groups of oil and injected alkali. Schematic representation of crude oil—alkali reaction mechanism [Adapted from (Phukan 2020)]

Inorganic alkali reacts with the divalent ions (like Ca^{2+}, Mg^{2+}, etc.) present in the formation water and induces water-insoluble soap that can severely hamper the drilling platform due to scaling or corrosion in addition to crude oil recovery (Pei et al. 2012a). Rock properties like porosity and permeability are important for flow through porous media but the damage in formation can hinder the oil recovery Alkali flooding can dissolve clay or other rock minerals and the migration of these fine particles along with the scaling due to divalent ions can severely block the pore throat and alter the permeability of the reservoir rock (Gbadamosi et al. 2019; Levitt and Pope 2008). Therefore, to avoid such issues, chelating agents as Na_4EDTA and Na_3NTA (Sorbie 2013), scale inhibitors (Gbadamosi et al. 2019) or organic alkali compatible with divalent ions have been developed and deployed (Fu et al. 2016; Pei et al. 2011; Sun et al. 2020; Yin and Zhao 2017). Alkali flooding is mostly carried out in sandstone reservoirs, as carbonate rock possesses anhydrite, which undergoes precipitation in addition to higher consumption of alkali (Sheng 2015b). Additionally, minimum clay content and reservoir temperature in the range of 40–60 °C are favourable for flooding the reservoir with alkali (Gaillard et al. 2015). However, in some scenarios, a temperature of around 93 °C has been found to be suitable for alkali flooding (Goodlett et al. 1986; Sivakumar et al. 2021; Taber et al. 1997a; Taber et al. 1997b). Moreover, the gas cap/aquifer support should be weak as the injected alkali acts as additional pressure support for the system (Sheng 2015b).

3.2 Surfactant Selection and Flooding in Reservoirs

Surfactant flooding can be implemented when alkali flooding is no longer effective in recovering the residual crude oil from oil reservoirs. Surfactants employed in reservoirs for enhancing oil recovery belong to the category of anionic (Kumar et al. 2017; Saha et al. 2019a; Yuan et al. 2015), cationic (Saha et al. 2018a), non-ionic (Saha et al. 2018a; Yuan et al. 2015), zwitterionic surfactant (Holt et al. 2009), gemini surfactants (Kamal 2016; Yuan et al. 2015) and natural surfactants (Al-Amodi et al. 2016; Saha et al. 2019b). Surfactant has the capacity to reduce the IFT between oil and aqueous phase to ultra-low value, thereby emulsifying the crude oil towards improved displacement efficiency and resulting in higher cumulative oil recovery (Chen et al. 2013b; Pu et al. 2016; Yuan et al. 2015). However, the selection of surfactant for different reservoirs needs to be identified by conducting phase behaviour studies (Bera et al. 2011; Green and Willhite 1998; Kamal et al. 2017). In phase behaviour studies, oil and aqueous surfactant phases are mixed, which results in the formation of thermodynamic stable microemulsions (Green and Willhite 1998; Sheng 2013a). These microemulsions induce the lowest IFT, which assist in recovering residual oil from both carbonate and sandstone oilfields (Adasania and Bai 2011; Lake et al. 1992; Santanna et al. 2009). The microemulsions are divided into, Winsor Type- I, Winsor Type II & Winsor Type III. In Type I (lower phase) microemulsion, oil in water emulsion is formed in which surfactant micelles are in the aqueous phase and solubilized oil at the cores. Similarly, in Type II (upper phase), the process is reversed and water in oil emulsions are formed (surfactant micelles in the oil phase and aqueous phase at the cores). In Type III (middle phase), the emulsions formed are bi-continuous in which the surfactant is equally partitioned in both excess oil and water phases. The lowest IFT is detected in Type III bi-continuous microemulsions (Dang et al. 2018; Firozjaii and Saghafi 2020) and the alteration from type I to Type II can be encountered by varying the salinity of the system at constant temperature (Fink 2003; Wang et al. 1979). However, the emulsification mechanisms may undergo a negative impact in which the displacement efficiency of oil can be severely affected due to poor mobility or flow of oil/emulsion and thereby reduces the recovery of residual oil (Pei et al. 2012a; Pei et al. 2012b; Saha et al. 2018a). The type of emulsion formed depends on the hydrophilic–lipophilic balance (HLB) of the selected surfactants. An emulsion can be either water in oil (w/o) (Pei et al. 2013a; Saha et al. 2018e; Wang et al. 2010) or oil in water (o/w) emulsion (Saha et al. 2019b; Saha et al. 2018b). The w/o emulsion is mostly formed with surfactants possessing HLB of 3–6 and o/w emulsion for surfactants with 8–16 HLB. The parameters that affect the phase behaviour studies include surfactant type, oil type, oil–water ratio, co-solvent, salinity and temperature (Green and Willhite 1998; Kamal et al. 2017).

The application of surfactants depends on the surface charge of the reservoir and has been successfully implemented in both sandstone (Chen et al. 2013b; Ko et al. 2014; Saha et al. 2019b; Samanta et al. 2011) and carbonate reservoirs (Aoudia et al. 2010; Ge and Wang 2015; Lu et al. 2014; Saha et al. 2018a; Sheng 2013c; Zendehboudi et al. 2013). The success of surfactant flooding in an oilfield is critical to several parameters like surfactant concentration, temperature, salinity and pH value (F et al. 2020; Saha et al. 2018a). Moreover, while executing surfactant flooding, adsorption of surfactants on rock mineralogy is inevitable and is also a major concern to the optimum formulation of chemical slug in addition to the economic constraints. Thus, to overcome these complexities, the adsorption behaviour of several synthetic surfactants (Bera et al. 2013; Curbelo et al. 2007; Muherei et al. 2009; Saha et al. 2017) and natural surfactants (Ahmadi and Shadizadeh 2015; Ahmadi and Shadizadeh 2013a; Ahmadi et al. 2012; Barati et al. 2016) that possess the potential to recover additional oil have been investigated. Surfactant adsorption phenomena on the reservoir rock surface depend on the type of surfactants (Bera et al. 2013; Park et al. 2015), surfactant concentration (Bera et al. 2013; F et al. 2020; Saha et al. 2017), rock mineralogy (Muherei et al. 2009; Saha et al. 2017), pH (Bera et al. 2013; F et al. 2020), salinity (Bera et al. 2013; F et al. 2020; Saha et al. 2017) and temperature (Barati et al. 2016; Bera et al. 2013; F et al. 2020; Saha et al. 2017) of the system. The mechanism through which this adsorption occurs involves ion association, ion exchange, hydrophobic bonding, dispersion forces and the polarization of π electrons (F et al. 2020; Gupta 2004; Kudaibergenov et al. 2015; Zhang and Somasundaran 2006; Zhapbasbayev et al. 2018). Though the surfactant adsorption process is natural, several studies are available on the successful reduction of the surfactant losses considering sacrificial agents and altering the mechanisms. Polyacrylate (molecular weight and concentration), polyacrylate-to-anhydrite molar ratio (ShamsiJazeyi et al. 2014) and different types of nanoparticles and their concentrations (Ahmadi and Shadizadeh 2013b; Wu et al. 2017; Zargartalebi et al. 2014) can significantly control the surfactant adsorption mechanisms on the rock surfaces.

3.3 Polymer Selection for Heavy Crude Oil

The implementation of polymer flooding has been initiated more than 50 years ago (Jewett and Schurz 1970; Pye 1964; Sandiford 1964) and has been a great success in countries like the USA (Chang 1978; Jewett and Schurz 1970) and China (Corlay et al. 1992; Wang et al. 2002; Wang et al. 2011) due to its lower capital cost (Saha and Tiwari 2019). Polymer flooding has been implemented in heavy crude oil reservoirs where oil recovery by conventional water flooding is extremely difficult (Fu et al. 2016; Pei et al. 2014; Saboorian-Jooybari et al. 2016; Saha et al. 2019b; Saha et al. 2018b). Polymer when injected in oil reservoirs enhances the overall displacement efficiency by achieving the favourable oil–water mobility ratio (Standnes and Skjevrak 2014). The important factors required for effective polymer flooding depend on polymer size and thermal stability, as the injected fluid must propagate through the rock pores without sacrificing the effective viscosity (Jouenne 2020).

The state-of-the-art of polymer flooding shows that synthetic polymer and biopolymer are two different categories of polymers that have been extensively used for oil recovery based on the reservoir characteristics. Synthetic water-soluble polymer like hydrolyzed polyacrylamide (HPAM) has the ability to reduce the mobility ratio significantly (Sorbie 2013; Thomas 2019). However, the application of synthetic polymer has its own limitations due to harsh reservoir conditions (i.e. temperature and salinity of the system) (Gaillard et al. 2015; Levitt and Pope 2008). The acrylate unit in polymer solution degrades at high temperature and undergoes precipitation in the presence of divalent cations (Ca^{2+} and Mg^{2+}) thus ultimately reducing the viscosity. The HPAM solutions have a tolerance limit up to 100 °C in the absence of divalent ions.

The success of polymer injection in Middle East oil reservoirs, usually under harsh reservoir conditions (temperature above 100 °C and salinity till 280 g/L) can unlock an enormous quantity of crude oil (Jouenne 2020). Therefore, to overcome such temperature limitations, researchers have developed a polymer using monomers of N-Vinylpyrrolidone (NVP) or 2-Acrylamido-2-Methyl Propane Sulfonate (AMPS) (Jouenne 2020). Despite several drawbacks of biopolymers like high propensity to biodegrade, industrial availability, high cost, complex logistic and handling, plugging tendency and reduced permeability (Jouenne 2020), they have extensively been investigated for EOR applications due to their tolerance to salinity, high viscosifying power and compatibility towards shear resistance.

4 Optimum Slug Formation for Chemical EOR

The formulation of optimum chemical slug either alkali, surfactant, polymer or combined chemicals to maximize oil recovery depends on the reservoir characteristics like crude oil properties, porosity and permeability of the rock, temperature and salinity of reservoir, interaction of chemicals when injected into the reservoirs, and participation of active mechanisms like IFT reduction, emulsification, rheology, displacement efficiency and wettability alteration. The economy of the process is also a critical factor in predicting the successful implementation of the chemical EOR schemes. Several studies have been conducted to explain the scope and limitations and develop a detailed understanding of the use of individual (alkali, surfactant and polymer) chemicals and combined chemical flooding schemes.

4.1 Individual Chemical Flooding

The individual chemical flooding to enhance recovery of residual oil from reservoirs indicates the application of alkali, surfactant or polymers. Alkali flooding has been implemented in different parts of the countries like China, USA, Canada and Saudi Arabia (Almalik et al. 1997; Johnson 1976; Tang et al. 2013; Wang et al. 2010; Xie et al. 2016). Alkali like sodium hydroxide, sodium carbonate, sodium metaborate, sodium tripolyphosphate, sodium silicate, potassium hydroxide, ethylenediamine ammonium hydroxide and ammonium carbonate are employed in oil reservoirs to improve the recovery of crude oil (Chen et al. 2013a; Chen et al. 2015; Kumar et al. 1989; Pei et al. 2011; Pei et al. 2013a; Pei et al. 2013b; Saha et al. 2019a).

The concept of alkali (NaOH) flooding for oil recovery has been introduced in 1927 for which a U.S. patent was assigned. Cooke et al. reported that alkali flooding could successfully recover residual oil from reservoirs when the acid value of the crude oil was in the range of 1.5 (Cooke et al. 1974). A formation of viscous emulsion bank by the crude-alkali reaction that assists in displacing the residual oil from rock pores to the production surface has been observed. It was also observed that other factors apart from lower IFT are responsible for the formation of an emulsion bank. However, the presence of divalent ions in the system can severely impact the oil recovery factor. The idea of rock reactivity was investigated by Jennings et al. (Jennings et al. 1974) and it was reported that moderate rock reactivity (caustic consumption) did not fail the caustic flooding however, an upper limit of reactivity has to be considered to avoid system failure. Ge et al. investigated several factors such as acid value, type and concentration of alkali, salinity and temperature which can impact the residual oil recovery of heavy crude oil (Ge et al. 2012). The deviation of these factors from the optimum condition can severely suppress the displacement efficiency and lower the residual oil recovery. The latest study by Saha et al. emphasized the activity and showed all possible mechanisms responsible for residual oil recovery (Saha et al. 2018e). The participation of mechanisms like lower equilibrium IFT, neutralization and saponification of acid group, emulsification, emulsion droplet distribution, wettability alteration and displacement efficiency as predicted by core flooding experiments were responsible towards a higher oil recovery of 25.5% of initial oil in place (IOP).

Surfactant flooding when deployed in an oil reservoir enhances the reduction in IFT to ultra-low value and simultaneously emulsifies the crude oil to improve the displacement efficiency. The enhancement in displacement efficiency of oil through the rock porous media portrays the improvement in residual oil recovery (Kamal et al. 2017; Pu et al. 2016; Saha et al. 2018a; Yu et al. 2011; Yuan et al. 2015). In addition, surfactant flooding further assists the oil recovery by alteration in wettability of the reservoir rock from oil-wet to favourable water/intermediate wet (Jarrahian et al. 2012; Saha et al. 2018a; Standnes and Austad 2000). Whatsoever, surfactants are costly and their stability at harsh reservoir conditions (high temperature and high salinity) greatly controls the oil recovery factor. The stability test of surfactant (Saha et al. 2018a) and additional adsorption of surfactant on rock surfaces (Saha et al. 2017) need to be examined.

Polymer flooding on the other aspect is employed to maintain a favourable mobility ratio ($M < 1$) such that viscous fingering effects are minimized as shown in Fig. 2. Water-soluble polymers have been injected in numerous oil field located in different parts of the world like USA, China, Canada, India, Indonesia, Argentina, Russia, Germany and Oman (Kamal et al. 2015; Saboorian-Jooybari et al. 2016; Standnes and Skjevrak 2014). Standnes and Skjevrak compiled the details of polymer field projects both in the onshore and offshore oil fields. For most of the cases, i.e. around 92%, the injection of HPAM resulted in the success of the field projects and the remaining 8% was induced with biopolymers. The importance of several

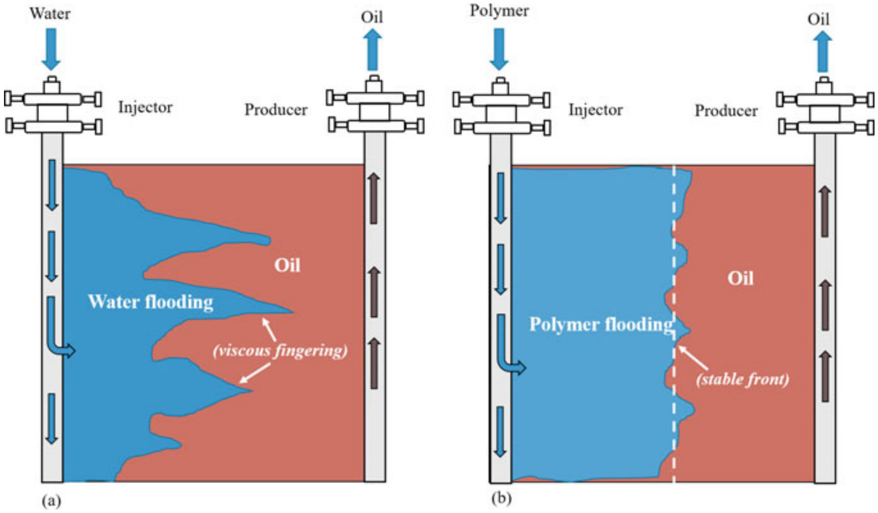

Fig. 2 A schematic representation of the typical mobility ratio in the case of (**a**) water flooding and (**b**) polymer flooding. The phenomena of viscous fingering are minimized in the case of the polymer flooding process. [Adapted from (Gbadamosi et al. 2019)]

parameters like reservoir permeability, polymer concentration, viscosity reduction, unacceptable infectivity and high polymer retention was highlighted (Standnes and Skjevrak 2014). Kamal et al. investigated and reviewed the role of different variables like polymer rheology, its thermal stability and adsorption, and application of various polymers such as HPAM, a copolymer of PAM, hydrophobically modified polyacrylamides, thermo-viscosifying polymers, biopolymers and cationic polymers. The other process parameters that impact the polymer flooding are the crude oil properties, rock charge, reservoir salinity and temperature (Kamal et al. 2015).

4.2 Combined Effects of Chemical Flooding

The combined chemical flooding emphasizes the applications of alkali–surfactant, surfactant–polymer, polymer–alkali and alkali–surfactant–polymer floodings in oil field reservoirs. The use of alkali flooding can be sufficient enough to recover the crude oil from the economical aspect as alkalis are cheaper than other chemicals. However, there are several consequences while running alkaline flooding, which includes high consumption of alkali, scaling of the production facilities and separation or demulsification of produced oil. Thus, to overcome these issues, a surfactant is added to the system through which the synergistic effect can be achieved. Additionally, the active sites on the reservoir rock through which adsorption occurs can be saturated with alkali thus reducing the surfactant loss. Chen et al. inspected the synergistic effects between alkali and surfactant and observed a higher oil recovery

(Chen et al. 2013b). The synergy between alkali and surfactant shows ultra-low IFT of the system in the range of 10^{-4} mN/m, thereby enhancing the oil displacement efficiency, with an additional oil recovery of around 19.4%. However, a higher concentration of surfactant in the alkali-surfactant system further increases the emulsification, which affects the displacement efficiency and reduces the recovery of highly viscous oil (Pei et al. 2012a). The combination of alkali–surfactant could not produce maximum oil recovery compared with alkali flooding even after generating ultra-low IFT values between oil and aqueous phase, because the W/O emulsion formed with alkaline is converted to O/W emulsion on the addition of surfactant, which increases the viscosity of oil and lowers the sweep efficiency. Thus, optimum formulation of the combined alkali–surfactant system is essential for a higher oil recovery, which depends on the crude oil properties, surfactant category, emulsion type, reservoir temperature and salinity (Liu et al. 2006; Pei et al. 2012a; Pei et al. 2012b; Saha et al. 2018a).

Alkali-polymer flooding, on the other hand, is further investigated for a higher oil recovery based on the emulsification formed by adding alkaline and its displacement by polymer flooding. Pie et al. studied the impact of polymer and alkaline flooding alone in addition to combined alkaline-polymer flooding for heavy crude oil in an oilfield of China as shown in Fig. 3 (Pei et al. 2014). There was no oil recovery by polymer flooding as the viscosity of the polymer solution was lower than the viscosity of crude oil and thus the sweep efficiency decreased. Alkaline flooding showed better results as compared with polymer flooding due to the formation of W/O emulsion by the infiltration of alkali solution in the heavy oil. This emulsion mechanism further enhances the reservoir overall sweep efficiency by diverting the aqueous phase towards the area of the reservoir untouched by the injected fluids, hence resulting in improved oil recovery. Moreover, the synergistic effect of alkali and polymer flooding maximized the oil recovery to 43.4% OOIP (Fig. 3) and such high recovery is dependent on the formation of W/O emulsion droplet incorporated

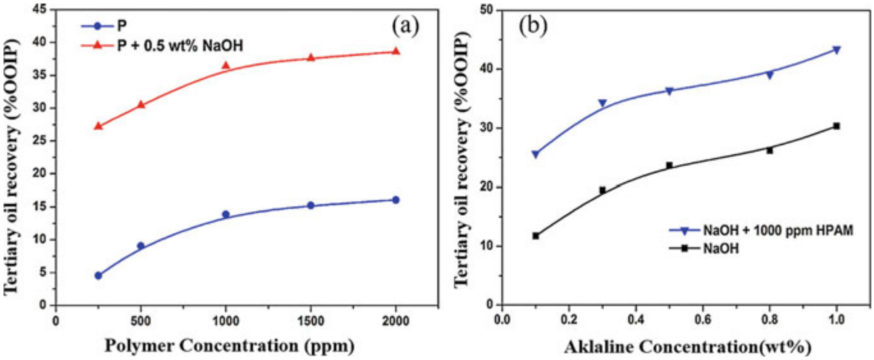

Fig. 3 **a** Effect of polymer (HPAM) concentrations and **b** effect of alkaline (NaOH) concentrations on the enhanced oil recovery in the case of AP flooding. [Adapted with permission from (Pei et al. 2014)]

by the addition of alkali on optimum polymer concentration. However, the addition of polymer in alkali solution can hamper the formation of emulsion droplets and in such a case, the oil recovery cannot be maximized.

For the case of surfactant–polymer (SP) flooding, the oil recovery depends on the ultra-low IFT achieved by using surfactants and favourable mobility ratio induced by polymer to enhanced sweep efficiency (Yin and Zhao 2017). The SP floodings do not show any scaling issues due to the absence of alkali and have been implemented in several oil fields of China and the field test recovered an enhanced oil recovery of 20% in a high permeability reservoir of Liaohe oilfield flooding (Sun et al. 2020). A higher concentration of surfactant is often used to counter the adsorption effect in strata. The SP flooding is not promising for low permeability reservoirs due to higher surfactant losses by adsorption and in such cases, the operating costs affect the profitability of the implementation of alkali–surfactant–polymer (ASP) flooding (Sun et al., 2020). Several other limitations of SP flooding such as surfactant solubilization in the oil phase, surfactant adsorption on the rock surface, polymer and surfactant interaction and chromatographic separation of chemicals can be detrimental for the overall process. Hence, polymeric surfactants are being explored for EOR applications (Raffa et al. 2016).

4.3 Potential and Progress of Alkali–Surfactant–Polymer Flooding

The ASP flooding showcases the combined effects of alkali, surfactant and polymer, which are injected as a single slug. The ASP flooding has been implemented in China, USA, Canada, India and Venezuela (Cao et al. 2002; Charest 2013; Jain et al. 2012; Li et al. 1999; Olajire 2014; Sheng 2014; Wang et al. 1999). In China, ASP flooding showed significant improvement in oil recovery when applied in Daqing oilfield. The ASP flood resulted in 3.5 million tons of additional oil, which is almost 9% of the total oil production in all wells of Daqing oil field. The ASP slug when injected in Henan oil field reservoirs which were under a high temperature of around 80 °C produced an incremental oil recovery of 7.7% (Guo et al. 2017a, b). Oilfields in the USA produced an additional oil recovery of 17% (Tanner), 26% (West Kiehl, Wyoming) and 28% (Cambridge Minnelusa) with ASP flooding. In Canadian oilfields (Taber South), the oil cut was improved from 1.7 to 7.3% with an injection of ASP slug, which accounted for oil production of 1502 bbl/day from 300 bbl/day (Olajire 2014). ASP flooding has also been investigated for carbonate reservoirs to recover viscous oil (Panthi et al. 2016). Two different alkalis, i.e. sodium metaborate (to tolerate the hard water formation) and sodium carbonate in the ASP slug were examined. Sodium metaborate was equally effective in reducing surfactant adsorption as compared with sodium carbonate. Sodium metaborate when used in ASP slug resulted in lowering of IFT, formation of type III emulsion and also found effective in reducing the adsorption of surfactants on carbonate cores with cumulative oil recovery of around

92.7% reducing oil saturation to about 6.1%. Therefore, from the literature, it can be observed that chemical EOR has huge potential to recover residual oil from reservoirs and many successful pilot and commercial oil field projects have been completed using different chemical EOR schemes with several limitations.

The screening criteria for ASP flooding take into account the crude oil properties like viscosity, API gravity, oil saturation, the salinity of reservoir, concentration of divalent ions, rock type (lithology), depth of the reservoir, clay content, the permeability of reservoir and reservoir temperature (Adasania and Bai 2011; Al-Bahar et al. 2004; Dickson et al. 2010; Lake et al. 1992; Taber et al. 1997a; Taber et al. 1997b). The preferable conditions for ASP flooding are; oil viscosity ≤12.9 cP, API gravity >20, oil saturation ≥0.3, salinity <200 000 TDS, divalent ions up to 1000 ppm, rock type of sandstone in nature, depth <9000 ft, low clay content, permeability of reservoir >10 mD and reservoir temperature <95 °C (Sheng 2014). The ASP flooding has been implemented in Chinese oilfields with crude oil having a viscosity of 10 cP with a maximum limit of 70 cP (Sheng 2014). However, as per literature data, an oil viscosity of more than 200 cP is also acceptable for ASP flooding (Firozjaii and Saghafi 2020). The limits on screening parameters as summarized in Table 1, which is based on the data available in the literature and may be used as guidelines for successful ASP floodings (Al-Bahar et al. 2004; Dickson et al. 2010; Lake et al. 1992; Sheng 2014; Taber et al. 1997a; Taber et al. 1997b).

5 Core Flooding of Alkali–Surfactant–Polymer in Laboratories

The ultimate recovery of crude oil can be estimated by performing core-flooding experiments using core samples gathered from oilfields or sand pack or Berea cores of desired size (Chap. 2, Sect. 2.6). Numerous successful ASP flooding experiments have been conducted on a laboratory scale in sandstone and carbonate reservoirs to estimate the cumulative oil recovery (Table 2). The laboratory ASP flooding using viscous crude oil from Karazhanbas oilfield showed promising results in terms of residual oil recovery (31–37%) at varying concentrations of chemical slugs (Kudaibergenov et al. 2015). The oil recovery with ASP flooding on cores saturated with oil samples from Eastern Moldabek and Karazhanbas showed additional oil displacement of 19 and 37%, respectively (Kudaibergenov et al. 2015; Zhapbasbayev et al. 2018). A laboratory study on ASP flooding experiments using Indian crude oil and sand pack core was successful to achieve an additional oil recovery between 23 and 33% of OOIP by varying the concentration of alkali, surfactant and polymer (Samanta et al., 2012). Another ASP flooding study using diesel oil as core saturation (cores collected from Sanand Mesana oil field, Cambay basin of India) resulted in an impressive oil recovery of around 18–20.5% of OOIP (Khan et al. 2009). A comparative study between alkali and alkali–surfactant–polymer flooding for carbonate reservoirs (in Italy) showed promising outcomes with ASP flooding,

Table 1 Range of different properties/parameters for ASP flooding

Properties	Viscosity of oil (cP)	Oil saturation (%)	Permeability (mD)	Reservoir temperature (°C)	Salinity (ppm)	Divalent ions (ppm)	API gravity	Depth (ft)
Range	<200	≥0.3	≥473	<95	<200,000	≤1000	>20	≤9000

Table 2 ASP flooding conducted in laboratory scale in different laboratories

Oil field	Oil viscosity (mPa S)	Crude category	Oil saturation (%)	Reservoir category	Porosity (%)	Permeability (D)	Residual oil recovery (% OOIP)
Karazhanbas	300	Light crude	0.68–0.77	Sandstone	40.4–48.4	5.8–6.5	31–37
Eastern Moldabek	407	Viscous	0.62–0.63	NA	36	1.3–1.4	19
Karazhanbas	300	Viscous	0.72–0.73	NA	18–19	1.3–1.4	37
Ahmedabad	50.12	NA	0.81–0.85	Sandpack	36–39	0.2–1.2	14–24
Diesel Oil	4.2	NA	0.68–0.69	Sandstone	21	0.001–0.0615	23–33
Oelic acid + Soltrol 170	NA	NA	NA	Carbonate	30	0.004–0.005	86.3%[a]
Stock tank oil	105	Viscous	0.70–0.84	Carbonate	18.8	0.081	93–95%[a]

[a] Cumulative oil recovery

and cumulative oil recovery for this case was improved up to 86.3% (Bortolotti et al. 2009). Similar laboratory experiments using carbonate (dolomite) rock and viscous stock tank oil were conducted in the USA with a successful cumulative oil yield of around 93–95% with less surfactant retention on the dolomite rock as desirable (Panthi et al. 2016). The studies conducted at laboratory scales to predict the success of ASP flooding provide a pathway for commercialization of the ASP flooding processes.

6 Field Application of Alkali–Surfactant–Polymer Slug

Apart from laboratory-scale experiments, alkali–surfactant–polymer flooding has been successfully implemented to a large extent in numerous pilot-scale floodings and oil fields around the world (Table 3). The majority of the ASP floodings have been implemented in China's oil fields; Daqing oil field, Shengli (Gudong) and Karamay. The additional oil recovery (AOR) from various Daqing oil reservoirs, possessing average porosity of 26% and permeability between 400 to 3800 mD, started in 1994 and the AOR varied in the range of 18%–25% of OOIP (Dang et al., 2018; Olajire, 2014). The flooding in the Gudong oil field using ASP slug started in 1992 in which the oil recovery was found around 26% (Wang et al. 1979) and in another project AOR of 13.4% has been reported (Qu et al. 1998). The oil recovery by ASP flooding in Gudao (western part) reservoir, which started in 1997 enhanced the oil recovery by 15.5% (Olajire 2014). In the Karamay oil field that is a heterogeneous conglomerate

Table 3 ASP flooding conducted in pilot and oil field worldwide

Country	Oil Field	Crude Oil Category	Reservoir type	Increase oil recovery (% OOIP)	References
China	Daqing	Light	Sandstone	18–25%	(Dang et al. 2018; Guo et al. 2017a, b; Li et al. 2003; Olajire 2014; Wang et al. 1999)
	Gudong	Heavy	Sandstone	13.4%	(Dang et al. 2018; Guo et al. 2017a, b; Qu et al. 1998)
	Karamay	Medium	Conglomerate	15–25%	(Gu et al. 1998; Guo et al. 2017a, b; Olajire 2014; Qiao et al. 2000)
	Gudao	Heavy	Sandstone	16%	(Cao et al. 2002; Dang et al. 2018; Olajire 2014; Yang et al. 2002)
Canada	Taber	Heavy	Sandstone	16%	(Dang et al. 2018; Olajire 2014)
	Upper Mannville	Heavy	Sandstone	10%	(Dang et al. 2018)
	Mooney	Heavy	Sandstone	25%	(Dang et al. 2018)
	Etzikorn	Heavy	Sandstone	12%	(Dang et al. 2018)
	Warner Mannville B	Light	Sandstone	11.1%	(McInnis et al. 2013)
India	Viraj	Heavy	Sandstone	18%	(Dang et al. 2018; Pratap and Gauma 2004)
	Mangala	Medium	Sandstone	20%	(Dang et al. 2018)
	Jhalora	Light	Sandstone	NA	(Jain et al. 2012)
	Kalol	Light	Sandstone	5%	(Hanotia et al. 2015)
USA	Tanner	Heavy	Sandstone	18%	(Dang et al., 2018; Olajire, 2014)
	West Kiehl	Medium	Sandstone	21%	(Dang et al. 2018; Olajire 2014)
	West Moorcroft	Heavy	Sandstone	15%	(Dang et al. 2018)
	Isenhour	Light	Sandstone	12%	(Dang et al. 2018)
	Sho-Vel-Tum	Light	Sandstone	5 times	(French; Olajire 2014)
	White Castle	Medium	Sandstone	10%	(Dang et al. 2018)
Kuwait	Sabiriyah Mauddud	Medium	Carbonate	NA	(Abdullah et al. 2015; Dang et al. 2018)
Venezuela	Lake Maracaibo	Light	Sandstone	At least 10%	(Hernandez et al. 2002)

reservoir, the ASP flooding started in 1995 and was successful to recover around 15–25% of OOIP (Gu et al. 1998; Olajire 2014; Qiao et al. 2000).

Similarly, ASP flooding showed significant results when implemented in Canadian Oil fields (10–25% of OOIP). Indian oil fields possess three types of light, moderate and heavy crude oil, the recovery with ASP flooding varies between 5 and 20% of OOIP (reference). In the USA, ASP flooding at West Kiehl was initiated in 1987, which resulted in additional oil recovery of 26% produced in an economical way (Clark et al. 1993; Meyers et al. 1992; Olajire 2014). The Cambridge Minnelusa field that is in actual contact to Kiehl field showed an impressive oil recovery of 28% of OOIP. The Tanner field ASP flooding successful produced around 17–18% of OOIP (Dang et al. 2018; Olajire 2014; Pitts et al. 2006). In Sho-Vel-Tum field located in Oklahoma, the ASP slug enhances the oil recovery from initial oil production of 4 bbl/day to 20 bbl/day (almost 5 times) (French; Olajire 2014). The other oil fields in the USA such as West Moorcroft, Isenhour, White Castle and Lawrence field Illinois (Cypress and Bridgeport) when exposed to ASP flooding revealed an improved oil recovery factor of 15%, 12%, 10% and 21–24% of OOIP (21% Cypress and 24% Bridgeport), respectively (Seyler et al. 2012). The other countries where pilot-scale ASP floodings have been executed involve North Kuwait (Abdullah et al. 2015; Dang et al. 2018) and Venezuela (Hernandez et al. 2002). North Kuwait showed an encouraging result in terms of oil recovery and in Venezuela, the recovery by ASP flooding was at least improved by 10% of OOIP.

7 Technical Issues and Their Solutions of ASP Flooding

The process of ASP flooding for enhanced oil recovery has been successfully executed in several pilot-scale and oil fields. However, the mechanisms/processes involved are highly complex and hence laboratory data cannot be directly implemented in a real field or for commercialization purposes. The understanding of ASP flooding physics is very important to sort out the complexities that are usually encountered during the process. The importance of complexities lies in the impact of geology; heterogeneity of the reservoirs such as porosity, pore throat opening, permeability, minerals, clays, etc. and these point out the opportunities and threats of the system (Dang et al. 2018). The complexities because of the type of crude oil, the salinity of formation water, rock charge and temperature of reservoirs play a dominant role in identifying the active mechanisms responsible for the higher oil recovery. Additionally, the formulation of optimum ASP slug is among the most imperative parameters towards the successful execution/implementation of ASP flooding either in laboratory, pilot or commercial scale processes. The impact of chemical dilution due to adsorption or retention, chemical degradation due to reservoir heterogeneity, injection pattern, injectivity, well pattern design, temperature, salinity and other parameters must be considered while designing an economical ASP flooding scheme. Thus, numerical modelling considering all the complexities is necessary to correlate laboratory data to reduce the complexities for commercialization. Moreover, several operational difficulties can also be faced while performing ASP chemical flooding. The chromatography separation of alkali, surfactant and polymer results in a chemical break, which can hinder the chemical flow in porous media thereby impacting significantly the recovery of residual oil. The production stream consists of emulsions and demulsification of the stream is required, which is a challenging task. In addition, the produced water is contaminated due to dissolved oil, chemicals and suspended solids and thus treatment of the effluent is mandatory before disposing to the environment. Furthermore, scaling and precipitation are also some of the major issues encountered during ASP flooding, which can damage the wellbore equipment and formation of the oil reservoir (Olajire 2014; Sheng 2014).

The detrimental effect of chemical dilution/degradation due to reservoir heterogeneity can be overcome by developing a more realistic and economical model for successful chemical EOR applications. The remedies such as injecting pre-slug prior to chemical flooding to neutralize/normalize extreme reservoir salinity, injection of cheap alkalis to reduce the active adsorption sites on reservoir rock surfaces and choosing or screening of chemicals that are stable under extreme reservoir temperature or conditions. Other operational difficulties like scaling and precipitation can be overcome by the use of scale and precipitation inhibitors. The use of hydrophilic scale inhibitors when added to scaling water reduces the formation of scale by minimizing the growth of scale crystals and their attachment on the solid surfaces. The scale treatments are useful especially in areas where mechanical treatment is not possible. The efficacy of this treatment depends on the surface area to mass/volume ratio (Bezemer and Bauer 1969; Chen et al. 2004; Conne 1983; Crabtree et al. 1999; Fink 2003; Olajire 2014). The conventional scale inhibitors are toxic, hazardous, bioaccumulation and non-biodegradable, which limits their applications. Hence, green scale inhibitors (phosphorous free and eco-friendly) are on-trend, which have the potential to overcome the above limitations (Gupta 2004; Holt et al. 2009; Kumar et al. 2010; Taj et al. 2006). The amount of divalent ions in the aqueous phase can be reduced by strong/weak acid cation resins or selection sequestration treatment.

8 Conclusion

The formulation of the chemical slug for the enhanced oil recovery depends on the (i) *reservoir properties* (i.e. rock lithology, permeability, divalent ions, salinity, depth, and temperature), (ii) *types of crude oil (*i.e. density, viscosity, acid value) and (iii) *chemistry and compatibility of chemicals* (i.e. scaling, corrosion, and emulsification). Alkali–surfactant–polymer (ASP) flooding due to its synergistic effect has the advantages of improving displacement efficiency, favour mobility of the oil, and lower the value of IFT which results in enhanced oil recovery. Combined ASP floodings have been found to be efficient for heavy oil reservoirs. ASP flooding has been recommended considering the amount of oil recovered and competitive cost. Recent trends indicated that nanoparticle-assisted polymer flooding has a significant impact on the recovery factor. However, the synergy between the crude oil–polymer nanoparticle system needs to be evaluated in the context of associated trade-offs; a higher concentration of nanoparticles may clog the porous structure of the reservoirs and is a detriment to oil recovery. Also, the fate and impact of nanoparticles on the environment need to take into considerations. In addition, a detailed investigation of the chemical nanoparticles solution is required for punitive reservoir conditions to accomplish the optimum chemical slug.

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Screening of Extremophiles for Microbial Enhanced Oil Recovery Based on Surface Active Properties

Poulami Datta, Pankaj Tiwari, and Lalit Pandey

1 Introduction

Over the past decades, researchers and scientists have been fascinated by the enthralling microorganisms which reside in severe environmental conditions. Such microbes, identified as extremophiles, flourish in surroundings that are unbearably hostile or even fatal for other terrestrial life-beings. They can prosper in extremely hot niches, snow, and halophilic conditions, as well as acidic and alkaline situations. Some of them may also grow in toxic waste products, heavy metals, organic solvents, or in numerous other territories which were formerly assumed to be inappropriate for life (Magot et al. 2000; Shen et al. 2018; Van Hamme et al. 2003). Few extremophilic microbes can even endure more than one extreme condition, for example, tremendous heat with high salinity, low temperature with high acidity, high-pressure levels with high alkalinity in which normal microorganisms would have been destroyed. For specific extreme environmental conditions, a group of microbes have exhibited their endurance in such extreme conditions, however, in some cases, those conditions are essential for their existence.

The capability of microbes to thrive under severe environmental conditions has encouraged scientists to investigate those microorganisms for a better understanding of their features and subsequently employ them for different purposes such as various biotechnological and commercial applications (Datta et al. 2018, 2020; Fopase et al. 2020; Sharma et al. 2019a, b; Sharma and Pandey 2020; Tango and Islam 2002; Verma et al. 2020). Extremophiles also possess some other characteristics such as limited nutrition requirements, high mass transfer rate, better solubilization of substrates, lower risk of pollution during the course of several industrial applications (Chen and

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P. Datta · P. Tiwari · L. Pandey (✉)
Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati,
Guwahati 781039, Assam, India
e-mail: lalitpandey@iitg.ac.in

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Jiang 2018). Extremophiles along with some extremozymes have been reported to be used as pre-treatment technologies for various biorefinery approaches (Raddadi et al. 2015). Biorefinery industries usually operate in very hostile conditions. Employing the extremophiles having special traits also reduces the overall expense of the process and ensures the smooth occurrence of the entire procedure (Zhu et al. 2020).

The major focus of this chapter mainly includes the concept of oil-retrieval-assisting extremophiles, their classifications and abundance in various environments. Depending on their survival environment, they have been named accordingly such as thermophiles, psychrophiles, acidophiles, alkaliphiles, halophiles and so on (Farias et al. 2018). The parameters for screening extremophiles are described in detail with their endurance limitations. Their role in *in-situ* microbial enhanced oil recovery (MEOR) from the oil reservoir fields has also been discussed. Additionally, along with the naturally occurring extremophiles, the concept of genetically engineered MEOR (GMEOR) has been elaborated which is primarily based on rDNA technology. A comprehensive table is included that lists the classification of extremophiles depending on their endurance parameter range and their probable contribution to EOR. The sustainability of the microbial species in extremely harsh conditions as well as their potentials for successful oil recuperation globally in *in-situ* conditions have also been elaborated.

2 Screening of Extremophiles

2.1 Concept of Extremophiles in MEOR and Their Classifications

It was assumed for a long time that the extreme subsurface ecosystems are difficult for microbial endurance but now there is much substantiation that proves the presence of extensive microbial diversity inside the oil reservoir. These extremophiles also participate in the internal biochemical processes at the adverse conditions (high temperature, salinity and pressure) of the oil reservoir. Extremophiles are broadly classified into two broad classes: extremophilic microorganisms, which need one or more harsh surroundings for growing and extremotolerant microorganisms, which can endure the extreme level of one or more physicochemical conditions although their optimum growth occurs at normal conditions. Extremophiles can be further divided depending upon the environment they grow and survive; such as thermophiles and hyperthermophiles (microbes that prefer higher temperatures above 50 ºC), psychrophiles (microbes that prefer comparatively lower temperature in the range of 0–15 ºC), acidophiles and alkaliphiles (microbes growing optimally at acidic or basic pH conditions, respectively), barophiles (microbes which adapt themselves under high pressure) and halophilic (microbes that need NaCl for growth and development). The bacterial strains which could thrive in a high concentration of carbon dioxide are termed as capnophiles (Rampelotto 2013). The reservoir microflora which could only utilize hydrocarbons as their substrate are termed as hydrocarbonclastic (Handaruni et al. 2020). Both Gram-positive and Gram-negative thermophilic bacteria have been isolated from thermal and normal atmospheres which can consume hydrocarbon as their only source of carbon and energy (Phillips and Perry 1976). One of the major proficient approaches in MEOR is the biosurfactant production by intrinsic microorganisms that can thrive in the existing harsh surroundings of oil reservoir systems, possessing high thermal tolerance, salinity and depleted oxygen level are termed as allochtonous (Farias et al. 2018). Other major operational reservoir extremophiles include acetoclastic strains (converts acetic acid to methane) and hydrogenotrophic methanogens (utilize molecular hydrogen as their energy source) such as *Methanobacterium*, *Methanosaetaceae* and *Methanoculleus* along with thermophilic, thermotolerant, and spore-forming bacteria from *Clostridiaceae* and *Thermotogaceae* families (Kim et al. 2018).

2.2 Strategies of Extremophiles to Perform MEOR

MEOR is the utilization of microbes and/or their produced metabolic products to improve the efficiency of oil recovery from oil reserves. Mainly, two general strategies are employed for MEOR: indigenous microorganisms' stimulation by supplementing with suitable nutrients, or seeding reservoirs with particular microbes (plus amendments) to launch new microflora with the anticipated functionalities. Extremophiles (thermophiles, halophiles and barophiles) are usually considered to be in the biostimulation category whereas non-extremophiles are generally based on bioaugmentation (Dourado et al. 2015; Jones 2006). Previously, the incorporation of biosurfactant-synthesizing bacteria and the necessary supplements into the reservoir has been mainly examined MEOR method. Although, the injected strains need to survive, nurture and be metabolically dynamic in the extreme internal reservoir environment of elevated temperature and pressure. Inherent bacteria isolated from reservoir soil, formation water or crude oil sample are assumed to be the model strains over the extremophiles from other sites (Miyazaki et al. 2012). Though there are several reports on biosurfactant or bioemulsifier producing mesophilic microorganisms, information about surface-active compounds secreting thermophilic microbes are comparatively rare (Banat 1993). The primary benefit of using these thermophiles for biosurfactant synthesis is because of comparatively faster reaction rates, which decreased the chances of contamination, declined viscosity of growth, improved solubility of bio-molecules in the growth medium and exclusion of pathogenic microbes for elevated incubation temperature (Wiegel et al. 1985). Numerous biosurfactant-producing bacterial isolates have been designated suitable for the *in-situ* MEOR purposes, for example, *Bacillus* sp. and *Pseudomonas* sp. due to their thermal and halotolerance capacity (Karlapudi et al. 2018).

Another strategy was implemented by combining the suitable isolated strains with the native endogenous strains and proceeds for MEOR. Two heavy oil viscosity-reducing bacteria, *Bacillus licheniformis* SH-2 along with *Bacillus subtilis* SH-3, were screened from the formation fluid of a high-temperature petroleum reservoir of Central China by enrichment culture technique (Sun et al. 2017). They produced biosurfactants as well as biogases that could degrade heavy crude oil constituents. Further, these strains were combined with endogenous bacteria (*Pseudomonas*, *Acinetobacter*, *Anoxybacillus*, *Arcobacter*, *Symbiobacterium*, *Clostridium*, *Caloramator* and *Geobacillus*) and applied for the pilot-scale trial of microbial huff and puff. Monitoring the functional microflora before and after bio-slug flooding was done to recognize the structural dissimilarity of the activated native and injected microflora as well as their effectiveness in introducing successful MEOR practices. The stimulation of hydrocarbon-degrading bacteria (HDB), fermentation bacteria (FMB) and denitrifying/nitrate-reducing bacteria (NRB) were performed efficiently, while the sulfate-reducing bacteria (SRB) *Desulfovirgula* were found to be repressed during microbial huff and puff which were previously studied to be unfavourable for MEOR (Sun et al. 2017).

In another study, for analysing the variability of the microflora of a crude oil reservoir during MEOR, injection water and formation water from several wells of Shengli oil field; China, were scrutinized via molecular microbial techniques for almost 2 years (Song et al. 2017). In the vastly permeable (1.5–2.5 μm^2) and high-temperature (65 °C) oil-rich reservoir, microbial diversity mainly consisted of thermophilic and anaerobic phyla such as *Euryarchaeota*, *Deferribacteres*, *Proteobacteria* and *Firmicutes*. These isolates improved oil output to 5×10^3 kg per day. The methanogens in the formation fluid were constituted by diverse genera, *Methanothermobacter, Methanococcus* and *Methanosaeta.* Thermophilic and barophilic sulfur metabolizing genus *Thermococcus* was also found in the formation water. Another non-methanogenetic archaea, *Archaeoglobus*, was also present and grew at exceptionally high temperatures via sulfate reduction. Later on, due to increased acetate concentration, a community shift occurred with the dominance of *Enterobacter* and a considerable decline in oil output was observed. The long-term dynamics of reservoir microflora revealed the necessity for distinguishing efficient species for establishing an *in-situ* MEOR (Song et al. 2017).

2.3 Screening of Extremophiles from Various Environments

The isolation of extremophiles from various environmental niches is represented in Fig. 1. The sustainability of the microbes in reservoir conditions depends on whether they can survive in the anoxic conditions because it is a challenge to perform the metabolic activities in limited oxygen availability. Typical petroleum reservoirs exhibit very harsh conditions, possibly provide long-term selection criteria for hydrocarbon-degrading extremophiles along with associated microflora. Extremophiles sometimes impose hostile economic effects because of their harmful activities within oil reservoirs, and on the contrary advantageous environmental effects during their use for oil spills mitigation and industrial applications (Foght and McFarlane 1999). To obtain extremophile bacteria, oil and aqueous samples of

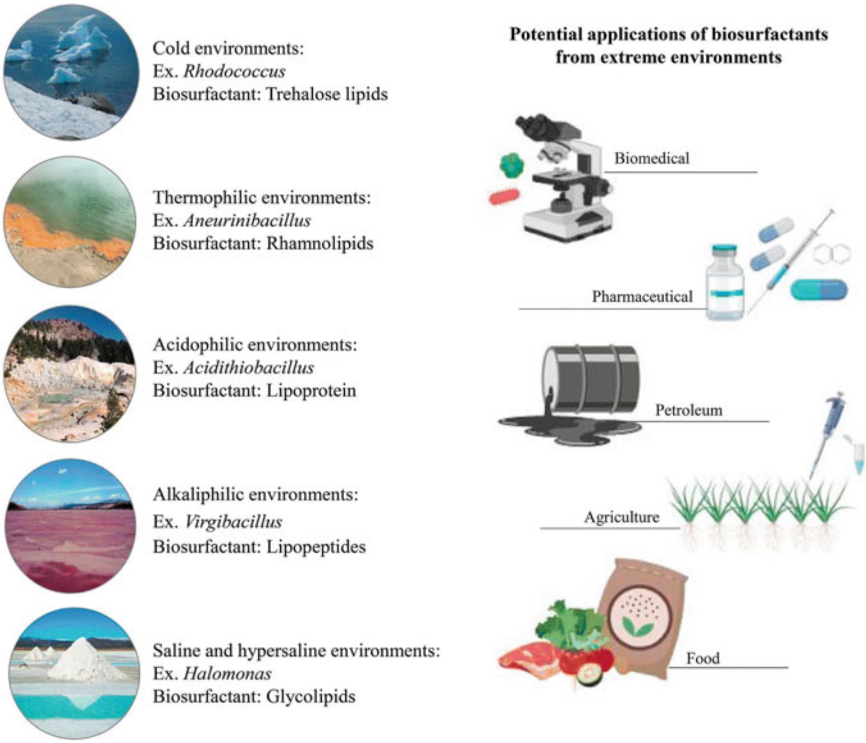

Fig. 1 Isolation of extremophiles from different extreme environments along with potential applications of produced biosurfactants from isolated producers. [Adapted with permission from (Schultz and Rosado 2020)]

a Mexican oilfield were grown in anaerobic conditions for hydrocarbon utilization capability. Their metabolic analysis resulted in the isolation of eight methanogenic, two fermentative, three nitrate-reducing, five thiosulphate-reducing, five sulphate-reducing and two iron-reducing strains. These isolates were able to grow at 60, 70 and 80 ºC and produced metabolites (bio-surfactants, gases (CO_2 and methane), acids and solvents) with potential application in MEOR (Muñoz et al. 2007).

Methanopyrus kandleri strain 116 was isolated from hydrothermal deep-sea sediment from the Gulf of California, which could grow at as high as 122 °C (252 °F, the maximum documented temperature) (Su et al. 2013). Whereas another genus *Picrophilus* (e.g. *Picrophilus torridus*) was first isolated from a dry solfataric field in northern Japan was reported as the most acidophilic microorganism among the other identified microbes, which could grow even at a pH of 0.06 (Fütterer et al. 2004; Rampelotto 2013). The sediment samples were collected to isolate hydrocarbonoclastic and biosurfactant-producing strains, which could utilize the crude oil as their sole substrate and members of *Curtobacterium, Brevundimonas*, *Marinobacter, Psychrobacter*, *Ochrobactrum* and *Staphylococcus* genera were mentioned as a potential anaerobic synthesizer of biosurfactants. The microbial populations from the deep sea, hydrothermal vents, mud volcano are mainly the geological diverse ecosystems to search for such bacterial diversity which can also sustain in the harsh reservoir conditions (Domingues et al. 2020). In another study, the microbial assortment of the Tang and Pirgal mud volcano of Iran and the isolation of capnophiles were investigated. *Enterobacter*, *Arthrobacter*, *Bacillus* and *Pseudomonas* were reported to be the dominant genera depending upon the biochemical tests. Their bio-emulsifying and bio-demulsifying properties revealed that *Bacillus thuringiensis* and *Bacillus anthracis* with superior surface activities and indicated their further utilization in the MEOR process (Parsia and Sorooshian 2017).

2.4 Screening Parameters for Extremophiles

The screening parameters for the biosurfactant-producing extremophiles isolated from various harsh surroundings are shown in Fig. 2. The biosurfactant production by the extremophiles may be screened via a number of techniques, which include oil-displacement test (ODT), measurements of interfacial tension (IFT), drop collapse test (DCT) and emulsification index (EI) (Al-Bahry et al. 2013). The biotechnological importance of the special enzymes present in the extremophiles (extremozymes) is not well documented due to information deficiency about the production of surface-active compounds. Further development could be accomplished when more innovative approaches would be established. Molecular biology practices and specifically gene expression monitoring are expected to meaningfully contribute to the identification and functional regulations involved in real-time *in-situ* processes. Yet, the present knowledge of genes for biosurfactant production is still inadequate and needs to be further explored for better controlling of the production strategies and improving products yields (Khire 2010; Sen 2010). EOR trials employing sand-pack columns or core-flooding apparatus under simulated reservoir conditions are conducted as screening procedures (Geetha et al. 2018).

Various thermophilic halophiles have been isolated for MEOR purposes. A novel strain of *Bacillus licheniformis* JF-2, isolated from petroleum-field injection fluid, could grow with up to 10% NaCl, 50 ºC temperature and in the pH range of 4.6–9 and produced Lichenysin anaerobically (Jenneman et al. 1983). Yakimov et al. examined the prospect of several *Bacillus licheniformis* isolates for EOR and testified that it produced noteworthy surfactant comparable to Surfactin at 55 °C temperature and up to 12% salinity (Yakimov et al. 1997). Another halo-thermotolerant strain, *B. licheniformis* ACO1 demonstrated high bioemulsifier production capacity up to 60 °C temperature and 180 g/L NaCl concentrations. The optimal salinity, temperature and pH for the production of bioemulsifier were reported to be 4% (w/v), 45 °C and 8, respectively (Dastgheib et al. 2008).

Among the thermophilic halophiles, bioemulsifier was extracted from *Methanobacterium thermoautotrophicum* that could grow up to 80 °C and metabolically functional over a broad pH range of 5–10, at a very high salt concentration.

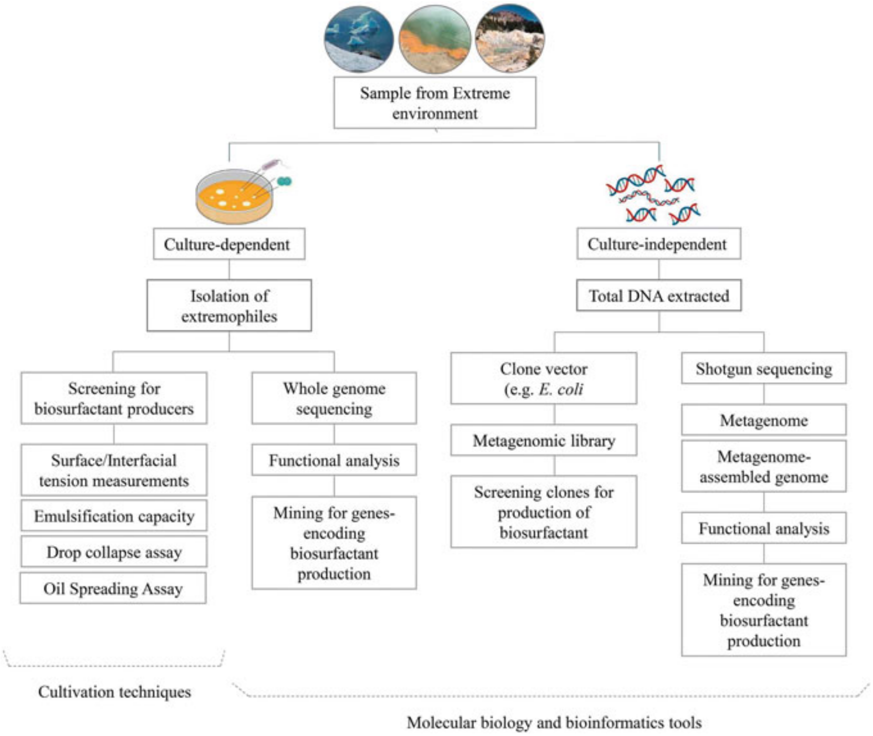

Fig. 2 Schematic representation of screening possibilities of biosurfactant-producing extremophiles by culture-dependent and culture-independent methods. [Adapted with permission from (Schultz and Rosado 2020)]

Bioemulsifier was also extracted from another thermophilic halophile, *Methanobacterium thermoautotrophicum* which could grow up to 80 °C and metabolically active over a broad pH range of 5–10 along with a very high salt concentration up to 200 g/L (Trebbau and Mclnerney 1996). Methanogenic *Methanocalculus halotolerans* also from an oil well showed maximum growth up to 20% salinity and 45 °C temperature (Ollivier et al. 1998). *Bacillus subtilis* MG495086 was isolated from the formation water of an oil reservoir and found to be stable in a pH range of 3–11, temperature range of 25–65 °C and salinity up to 30 g/L (Datta et al. 2018). The isolated bacterium was metabolically active up to a higher temperature of 78 °C.

Approximately, 30 diverse bacterial strains were screened from hot water springs to access their competence for synthesizing biosurfactants in very high thermal conditions. The novel biosurfactant production by *Bacillus stearothermophilus VR-8* was reported (Gurjar et al. 1995). It was not affected when heated for 30 min at 80 °C while at 90–100 °C, 60% emulsification index was determined and 5% NaCl resulted in only 8% loss of surface activity (Gurjar et al. 1995). *B. subtilis* and *B. licheniformis* could survive in 5% salinity and up to 50 °C temperature (Daryasafar et al. 2016). Makkar and Cameotra selected two *Bacillus subtilis* strains which showed very high biosurfactant production at thermophilic conditions (Makkar and Cameotra 1998). McInerney et al. studied more than 200 strains of *Bacillus subtilis*, *B. licheniformis*, *B. mojavensis* and *B. sonorensis* for their biosurfactant synthesis capability and compared their surface property anaerobically at 5% salinity which resulted in IFT reduction (McInerney et al. 2005).

There are very limited studies available regarding biosurfactant synthesis from psychrophiles. A novel biosurfactant producing strain *Arthrobacter protophormiae* was isolated from Antarctica which was further cultivated at 10 °C and in high salinity (10.0–100.0 g/L) by supplementing n-hexadecane as the main carbon substrate. The produced biosurfactant reduced the surface tension (ST) of the medium from 68 to 31 mN/m and was stable up to a temperature of 100 °C and a pH value of 12. The recovery of up to 90% of residual oil was observed from an oil-saturated sand pack column (Pruthi and Cameotra 1997; Sen 2010).

2.5 Technological Advancement in MEOR by Employing rDNA Technology and Genetically Engineered Microbes

Apart from the isolation of extremophiles from different sources, the advancements in genetic engineering have enabled construction of engineered strains suitable for MEOR or other applications. The major challenge for *in-situ* MEOR microbes remains to tolerate as well as synthesize suitable metabolic products in extreme reservoir conditions. Due to low metabolic activities, the indigenous microorganisms could not be employed at the commercial scale but such limitations can be addressed by genetic engineering approaches. Hence, genetically engineered microbial enhanced oil recovery (GMEOR) and enzyme-enhanced oil recovery (EEOR) came into the picture and constitute advanced MEOR methods (Zhang et al. 2020).

The genetic manipulation is mainly performed in two manners; site-specific mutation of the target enzyme and changing the characteristics of the protein structurally, chemically and functionally. With the help of genetic engineering strategies, the DNA sequences of microbes are injected into a host via protoplast fusion or the competent cells are incorporated by recombinant plasmid DNA to form hybrid strains. Secondly, the construction of mutants via random mutagenesis and high throughput screening with anticipated characteristics in the structural and functional properties of proteins are not involved.

These techniques enlarge the opportunity of MEOR by presenting innovative perspectives of metabolic engineering, which assist in the synthesis and utilization of more promising biochemical products along with bacterial biomass in oil reservoirs. The advancements in genetic engineering tools and practices offer advantages of manipulating, engineering and constructing novel strains that can endure hostile environmental settings and also produce a considerable amount of metabolites (Patel et al. 2015). The ultimate target is to attain microorganisms with the innate capability of prevailing and withstanding severe environmental surroundings for example very high pH, temperature, pressure and salt concentration. Engineering of bacteria can lead to degradation of long-chain fractions to short-chain ones and heavy crude oil is converted to light oil and improve the synthesis of related metabolic products that result in the application for heavy oil recovery (Zhang et al. 2020). The regulation of the long-chain alkane degrading key enzymes, alkane monooxygenase along with the co-encoded *alkB* and *ladA* genes, is a possible way to make them more adaptable for extreme environmental conditions (Lin et al. 2019).

Initially, the genomic analysis helped to scrutinize the potential functional microbial population in the petroleum reservoir which were monitored at the starting and ending of MEOR treatment employing the method of 16S rRNA gene clone libraries. *Anoxybacillus*, *Tepidiphilus*, *Bacillus* were present in the highest abundance in the reservoir and *Enterococcus*, *Ochrobactrum* and *Pseudomonas* were present in the samples after MEOR treatment. After the completion of MEOR process, the exogenous surfactant-producing bacteria (ESPB) along with the nitrate-reducing bacteria (NRB) including *Acinetobacter junnii*, *Lysinibacillus*, *Sulfuri hydrogenibium* have been plentifully stimulated in the operative wells (Wang et al. 2019).

The surfactin-producing engineered strains possess some advantages like high specific growth rate, low nutrient requirements and efficient generation of inoculum. Surfactin is synthesized by large multifunctional non-ribosomal peptide synthetase (NRPS) containing 3 modules, *SrfAA*, *SrfAB* and *SrfAC*. The key genetic engineering approaches to enhance the yield and creating novel Surfactin structures include: (a) promoter engineering by replacing the natural promoters P_{srf} of *srfA* components, responsible for Surfactin production; (b) establishing the efflux system of Surfactin by over-expression of associated proteins and surfactin transporters; (c) adjusting the transcriptional regulatory genes of surfactin synthase (*srfA*) and (d) the genomics and transcriptomics scrutiny of NRPS domain and combinatorial biosynthesis (Hu et al. 2019).

EEOR is among the latest *ex-situ* research trends in which the enzymes are employed to improve oil recovery is another novel conception. Enzymes are mainly proteins that catalyze several biochemical reactions. They could be used in consortium with other enzymes or biosurfactants (via enhancing active site mechanisms for enzymes) and subsequently applied for EOR (Nasiri 2011). These enzyme mixtures specially hydrolases break down crude oil components, modify the oil–rock–water interface dynamic by means of wettability and capillary action and the adsorption ability of enzyme proteins enhance easy recovery of oil (Nasiri 2011; Sun et al. 2011). Despite its higher expenditure, it predicts promising prospects for industrial applications. Some industrial sectors have launched products, such as Greenzyme, which have experienced both lab-scale as well as restricted field-scale testing. The incorporation of EEOR to present MEOR practices can finally lead to more effective functions for the petroleum sector.

To achieve the goal, BioSurfDB database was planned which highlights primary perceptions in the sector of biosurfactant formation along with the degradation, collecting information on 3736 genes, 3430 proteins, 1077 organisms and 58 pathways and a compilation of 96 cured biosurfactants assembled via synthesizing microbes and biosurfactant type (Oliveira et al. 2015). The significance of such cured databases for biosurfactant producers and their analyzing tool is a solution and prerequisite for functionality examination of the extremophilic microbial population and the selection of biosurfactant-producing microbes. The concept of multi-omics to search new biosurfactants is still very recent, and specifically the exploration of extremophiles is still emerging that requires widespread future study for (a) accumulating and recovering information of genes that are involved for biosurfactant generation from extremophiles, and (b) formulate strategies to evaluate and explore extracted information (Schultz and Rosado 2020). Summarily, GMEOR and EEOR are still being largely examined at the lab scale however more research is required to implement it at a large field scale (Patel et al. 2015).

2.6 In-Situ MEOR by Screened Extremophiles

For successful *in-situ* MEOR, bacteria need to grow in hostile circumstances which sustain in petroleum reserves for example very high temperature, pressure and salt concentration with limited oxygen content. Numerous aerobic and anaerobic thermophilic strains which are also pressure-tolerant as well as moderately halotolerant, were observed to increase the mobility of crude oil in lab-scale. Though extremophilic microorganisms could be isolated from several habitats, inherent microbes from petroleum reserves are considered to be the perfect contenders for further oil recovery purposes. There are certain disadvantages of using the exogenous strains in comparison to the indigenous bacteria. It is assumed that *in-situ* MEOR prefers the indigenous microbial communities existing in the oil reservoirs and are able to grow or survive in extreme environments (Khire 2010; Sen 2010). The detailed categorization of extremophiles based on their survival parameter range and their probable contribution in EOR is described in Table 1.

In most of the matured oil reservoirs, the temperature is expected to be as high as 70 °C and sometimes it can reach up to 100 °C. Although most of the researchers have reported the maximum reservoir temperature to be 93.3 °C (Kargarpour 2017). Anaerobic microbes are more prevailing species in oil reservoirs (Fardeau et al. 2004). *Thermoanaerobacter* and *Thermoanaerobacterium* were also revealed to grow well in low salty reservoir conditions (Fardeau et al. 2004; Nazina et al. 2006). *Methanothermobacter thermoautotrophicus* and *Gelria glutamica* were found in the highly thermal environment of the oil wells (Guan et al. 2013; Plugge et al. 2002). To endure high thermal conditions, the microbes are mostly spore-forming and contain some thermally stabilizing enzymes (Gomes et al. 2016). Most commonly observed thermophile hydrocarbon biodegraders include mainly *Bacillus*, *Thermoanaerobacter*, *Thermus*, *Thermococcus*, *Thermotoga* and *Thermodesulfobacterium,* which were isolated from very high thermal Chinese oil reservoirs possessing 90 °C (Lin et al. 2014); California at 80–90 °C (Orphan et al. 2000); and the North Sea at 70 °C (Dahle et al. 2008) are considered to be perfect genus to perform MEOR (Kaster et al. 2009).

Table 1 Classification of extremophiles depending on their endurance parameter range and their probable contribution to EOR

Parameter	Class of microbes	Microorganisms	Contribution in EOR	References
	Aerobic	*Pseudomonas*, *Bacillus*, *Corynebacterium*, *Streptococcus, Xanthomonas*	Produces glycolipid, lipopeptide and glyco-lipopeptide biosurfactants	Safdel et al. (2017); Saravanan et al. (2020)
Respiration Type	Anaerobic	*Clostridium*, *Desulfovibrio*, *Leuconostoc mesenteroides*, *B. licheniformis*	Produces glycoprotein at 96 °C and 37% oil recovery; utilizes sole hydrocarbon as substrate; produces exopolymer dextran	Al-Sayegh et al. (2015); Arora et al. (2019); Nikolova and Gutierrez (2020); Saravanan et al. (2020); Soudmand-asli et al. (2007)
	Facultative	*Arthobacter protophormie*; *Enterobacter cloacae* and *Enterobacter Hormaechei*	85–90% oil repossession from a sand pack column; efficient in IFT reduction and wettability alteration by biosurfactant production	Hosseini and Tahmasebi (2020); Pruthi and Cameotra (1997); Safdel et al. (2017); Sarafzadeh et al. (2013); Saravanan et al. (2020)
	Piezotolerant (1–40)	*Rhodococcus ruber* Z25 and *Rhodococcus erythropolis*; Consortia of *Arthrobacter* sp; *Pseudomonas* sp, *Bacillus* sp; *Rhodococcus qingshengi*i TUHH-12	Degrades long-chain hydrocarbons which reduce the crude oil viscosity and results in 8% oil recovery Alkane degradation	Bachmann et al. (2014); Jinfeng et al. (2005); Ke et al. (2018); Schedler et al. (2014); Xia et al. (2011); Zheng et al. (2012)
Pressure (MPa)	Barophilic (40–50)	*Thermococcus barophilus*	Hydrocarbon biodegradation	Marteinsson et al. (1999); Sakthipriya et al. (2017)
	Piezophiles (50–65)	*Thermococcus piezophilus*, *Shewanella benthica*	Biogas solubility, oil viscosity and IFT is influenced by increased pressure	Halim et al. (2009); Mamo and Mattiasson (2020)
	Extreme piezophiles (65–100)	*Shewanella* and *Moritella*,	High pressure affects the fluidity, permeability and carbonate dissolution	Kato et al. (1998); Marshall (2008); Sakthipriya et al. (2017)
	Extreme Psychrophile (−20 to 10)	*Rhodotorula psychrophenolica*, *Rhodotorula psychrophila*;	Produce sophorolipids	Perfumo et al. (2018)

(continued)

In a field study, *Bacillus* strains were injected to scrutinize *in-situ* biosurfactant production in the Viola limestone petroleum reservoir for mobilizing the residual oil (Youssef et al. 2007). To achieve this, five wells were selected among them two wells were inoculated with a combination of *Bacillus* RS-1 along with *Bacillus spizizenii* NRRL B-23049 with essential nutrient supplements. Two oil wells were provided

Table 1 (continued)

Parameter	Class of microbes	Microorganisms	Contribution in EOR	References
	Psychrophile or cryophile (<20)	*Pseudoalteromonas*; *Rhodococcus* sp., *Burkholderia*, *Flavobacterium*; *Halomonas neptunia* ANT-3b	Produces biosurfactant using hydrophilic substrates; produces emulsifying glycolipid using n-hexadecane	Pepi et al. (2005); Perfumo et al. (2018)
Temperature (°C)	Mesophile (20–50 °C)	*Pseudomonas*, *Halomonas*, *Lysinibacillus*, *Achromobacter*; *Acinetobacter*, *Sphingomonas*, *Brevundimonas*, *Methylobacterium*, *Acidaminococcus*, *Methanoplanus petrolearius*	Degrades alkane fraction of crude oil	Dahle et al. (2008); Mnif et al. (2011); Nikolova and Gutierrez (2020)
	Thermophiles (50–70 °C)	*Bacillus licheniformis*, *Geobacillus pallidus*, *Brevibacillus thermoruber*; *Clostridium*, *Thiobacillus*, *Desulfotomaculum*; *Pseudoxanthomonas* sp. G3; *Clostridium* sp. TERIK	ST and IFT decline with increasing temperature, oil mobilization, reaction rate and bioavailability are improved, production of rhamnolipid	Astuti et al. (2019); Dahle et al. (2008); Kazak et al. (2010); Lin et al. (2019); Mnif et al. (2011); OSMAN et al. (2019); Sharma et al. (2020); Tao et al. (2020)
	Extreme thermophile or hyperthermophile (70–100 °C)	*Thermotogales, Thermococcus; Thermoanaerobacter, Thermodesulfobacterium; Thermofilum, Thermus, Methanobacterium thermaggregans, Thermincola, Thermanaeromona, Bacillus atrophaeus*	Degrades aliphatic hydrocarbon and produces endospore to survive in the extreme reservoir conditions	Dahle et al. (2008); Lin et al. (2014); Nikolova and Gutierrez (2020); Orphan et al. (2003); Orphan et al. (2000); Zhang et al. (2016)
	Extreme Acidophiles (0–2)	*Sulfolobus acidocaldarius, Acidianus brierleyi, Ferroplasma acidarmanus*	Produces extremozymes such as oxidases, proteases, cellulases, amylases, glucoamylases	Eswari et al. (2019); Mamo and Mattiasson (2020)
	Acidophiles (2–5)	*Desulfovibrio desulfuicans, Acidophilium cryptum, Bacillus acidocaldarius, Acidothermus cellulolyticus, Thermoplasma acidophilum*	Produces thermostable enzymes for surviving in the extreme reservoir surroundings	Debnath et al. (2019); Margesin and Schinner (2001); Sakthipriya et al. (2017)
pH	Neutrophiles (5–8)	*Thermococcus celer, Pyrobaculum*; *Rhodococcus; Smithella, Syntrophobacter, Thauera, Thermodesulfobacterium; Gallionella, Sideroxydans*	Biosurfactant (trehalolipid) production	Chen et al. (2020); Eswari et al. (2019); Gupta et al. (2019); Kuyukina and Ivshina (2019)

(continued)

Table 1 (continued)

Parameter	Class of microbes	Microorganisms	Contribution in EOR	References
	Alkalophiles (8.5–11.5)	*Marinobacter*, *Micrococcus*, *Oceanobacillus* and *Bacillus*; *Alcalibacter saccharofermentans,* *Alkalibacterium psychrotolerans,* *Bacillus halodurans,* *Alkaliphilus transvaalensis*	Synthesizes alkaline active enzymes, for example, pectinases, Xylanases, Proteases, cellulases, lipases	Al-Awadhi et al. (2007); Dahle et al. (2008); Mamo and Mattiasson (2020)
	Extreme Alkalophiles (11.5–13.5)	*Ochrobactrum intermedium*	Potential candidates for ASP flooding	Mamo and Mattiasson (2020); Zarinviarsagh et al. (2017)
Salinity (M)	Halophiles or halotolerants (2–5)	*Halobacterium salinarium,* *Haloferax volcanii* and *Halobacterium distributum,* *Halomonas eurihalina,* *Gordonia amicalis*; *Planococcus maritimus* SAMP MCC 3013; *Halobacterium halobium*; *Halococcus*; *Dunaliella salina,* *Wallemia ichthyophaga,* *Bacillus halodurans,* *Haloarcula,* *Halorubrum*	Salinity affected the growth and metabolism of microbes along with the biosurfactant yield	Belyaev (1992); Debnath et al. (2019); Hao et al. (2008); Hosseini and Tahmasebi (2020); Kazak et al. (2010); Mamo and Mattiasson (2020); Nikolova and Gutierrez (2020); Schultz and Rosado (2020); Waghmode et al. (2019)
Chemicals	Metallophile	*Geobacter* (Fe^{3+}), *Alkaliphilus metalliredigens* (Co^{2+} and Fe^{3+}), *Amphibacillus* (Cr VI); *Gallionella,* *Sideroxydans* (iron oxidizer);	Appropriate for heavy-metal-containing oil reservoirs	Gupta et al. (2019); Ibrahim et al. (2011); Kim et al. (2018); Mamo and Mattiasson (2020)
	Toxitolerant	*Aeribacillus pallidus* strain SL-1	Tolerates 1000 mg/L naphthalene and 200 mg/L phenanthrene, degrades alkanes and aromatic hydrocarbon and improves bioavailability	Tao et al. (2020)

with only nutrients and the rest one well was given only formation fluid. Only CO_2 and ethanol were detected in the wells provided with the nutrient only, however, wells with inoculated microorganisms also generated 90 mg/L of lipopeptide biosurfactant, which was about ninefold higher than the amount required to mobilize entrapped oil from sandstone cores. Microbial growth rates, biosurfactant production rates, and biosurfactant yields were reported to be $0.06 \pm 0.01\ h^{-1}$, $0.02 \pm 0.001\ h^{-1}$ and 0.015 ± 0.001 mol biosurfactant/mol glucose, respectively. This demonstrated the procedural practicability of microbial processes for oil recuperation *in-situ* MEOR (Youssef et al. 2007).

The MEOR in high temperature (73 ºC) and salinity (16.79 g/L) oil reservoir has been reported in the Dagang oilfield in China (Jinfeng et al. 2005). Three bacterial isolates such as *Arthrobacter* sp. (A02), *Bacillus* sp. (B24) and *Pseudomonas* sp. (P15) were isolated from petroleum reservoir sample among which A02 and P15 strains displayed good oil biodegradation capability whereas B24 was found to be more competent in IFT reduction between oil and formation water because of the biosurfactant-production capability. When these microbes were injected along with nutrient amendments into seven wells, the oil recuperation was gradually augmented. Following six months of the incubation period (March–July 2001), approximately 8700 tonnes of extra oil was attained by July 2004 which was quite a huge amount when compared with the anticipated oil recovery via solely water-flooding (Jinfeng et al. 2005).

The stratal microflora was successfully utilized for EOR in the high-thermal Kongdian bed (60 ºC) of the Chinese Dagang oil field. The biostimulation strategy was adapted by injecting water–air mixtures along with nitrogen and phosphorus mineral salts into the oil wells so as to induce the stratal microfloral function for generating oil-releasing metabolic products. In addition to that, the cell density of thermophilic, fermentative, hydrocarbon-oxidizing, methanogenic and sulfate-reducing microbes were upsurged to 10–10,000-fold. Consequently, the water proportion was decreased in the formation fluid and the oil content improved and resulted in additional 14,000 tons of recovery over a period of 3.5 years (Nazina et al. 2017).

In India, the Oil and Natural Gas Corporation (ONGC) Limited, in association with The Energy and Resources Institute (TERI, New Delhi) along with the Institute of Reservoir Studies (IRS), Ahmedabad, performed several field-scale experiments utilizing the Huff and Puff method. The indigenously developed consortium of halophilic, barophilic, thermophilic (up to 90 °C) and anaerobic extremophiles (*Clostridium* type *Thermoanaerobacterium* sp. and *Thermococcus* sp.) were employed, which were previously isolated from the candidate reservoirs. The field trials were carried out in nearly 109 oil wells belonging to 9 dissimilar fields of ONGC and 8 oil wells of Naharkatia oilfield of OIL, Duliajan, Assam. Cumulative oil retrieval of 61,000 m^3 was achieved after 6–8 months of operation (Patel et al. 2015; Woodward 2006).

3 Conclusion

The presence of extremophiles proficient in growing on petroleum constituents has been acquainted for ages. Some literature reported regarding the *in-situ* modification of petroleum in hostile environments due to the activity of indigenous microflora, a which improvises the oil recovery. Contemporarily, the expansion of novel metagenomics strategies has provided prospects for characterizing the inherent microbial population, both phylogenetically and functionally in petroleum reservoirs to an unparalleled depth of resolution. However, that is not adequate to reveal the sustainability mechanism of the indigenous reservoir microflora as there are still various unexplored crude-oil-degrading extremophiles. The increased focus on the biotechnological perspective of these microbes would stimulate inclusive microbiological investigations of possible habitats and result in dynamic culturing procedures for isolating these intriguing microbes. Moreover, the next challenge is the exploration of crude-oil-degrading extremophiles possessing combined tolerances, such as thermophilic-halophiles, or psychrophilic-oligotrophs. The pioneering metagenomics approaches would assist further to comprehend the oil reservoirs microbiology to reveal probable microbial isolates which are expected to be scientifically innovative and open newer chances to harness their MEOR potential. The development of genetically engineered strains explores a new horizon for the sustainability of extremophiles inside the harsh reservoir conditions as well as the expansion of vigorous industrial processes for biometabolite production.

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Effect of Reservoir Environmental Conditions and Inherent Microorganisms

Poulami Datta, Pankaj Tiwari, and Lalit Pandey

1 Introduction

The implementation of the *in-situ* MEOR procedure is dependent on the various intrinsic and extrinsic factors including the type of the reservoirs, appropriate screening of prospective microbial community, the viable functioning of the microbes at the internal reservoir environment, amount of metabolites produced and mobilizing residual oil along with cost-effective aspects (Jang et al. 1983; Zhang et al. 2020b). Worldwide oil reservoirs are full of diversity in terms of temperature, salinity, traits of crude oil as well as the oil–water proportion of the reservoir. Because of the reservoir heterogeneity, the occurrence of microbes variation is obvious compared to the native population to the individual oil wells (Phukan et al. 2019; Rabiei et al. 2013; Rathi et al. 2018; Saha et al. 2018a, b).

Oil reservoirs host numerous indigenous microorganisms which can endure high pressure, salinity and temperature conditions. *In-situ* MEOR encompasses stimulating inherent reservoir microorganisms or inoculating specifically screened microbes into the oil reserve to induce certain metabolic activities. This promotes the biosynthesis of different metabolites including biosurfactants to improve oil retrieval. MEOR utilizes microbes or their metabolites for residual oil repossession with low permeability or high viscosity. Microbes utilize two types of nutrients: subsurface crude oil or additives to the injected fluids (Safdel et al. 2017; Wood 2019). However, the incorporation of biosurfactant-synthesizing bacteria and the necessary supplements into the reservoir have been mainly examined in the MEOR method. The injected strains need to survive, nurture and be metabolically dynamic in the extreme internal reservoir environment of elevated temperature and pressure. In this regard, indigenous bacteria isolated from reservoir soil, formation water or

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P. Datta · P. Tiwari · L. Pandey (✉)
Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Guwahati 781039, Assam, India
e-mail: lalitpandey@iitg.ac.in

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crude oil sample are assumed to be the ideal candidates over the extremophiles from other sites (Miyazaki et al. 2012).

The sustainability and success of the reservoir ecosystems are controlled by complex and cooperative microbial activity. Microbes are the most diverse group of living organisms on the planet; hence it is very important to understand both diversity and metabolic proficiencies in a specified range of environmental factors for illustrating their potential for recovery of residual oils. Even though microbes have been reported to prefer the oxygen-rich environment theoretically, advanced investigations have revealed some exclusive and effective biodegradation pathways are followed by microbes under sub-oxic (i.e., extremely low dissolved oxygen; occasionally coexisting with sulfides) environments inside the reservoirs.

The holistic procedure involves the selection of strains that would thrive under the reservoir conditions and mobilize the crude oil by synthesizing biosurfactants or by biotransformation. This requires many efforts for the identification, incubation, and potential assessment of the strains at the lab scale. The next step involves properly formulation of injection bio-slug, which facilitates the exogenous bacteria to effectively build a colony inside the reservoir. Whenever nutrients are introduced, they should be adequate to enhance the long-term metabolic activity of the microbes. Finally, the oil wells, relevant accessories and other amenities should be cautiously equipped before MEOR operations. These additional facilities need to be treated with steam for removing debris and undesirable microbes to ensure the injection of only the desired microbial species into the reservoir (Gao 2018).

This chapter lists key reservoir environmental conditions including lithology, porosity, permeability, crude oil composition, temperature, pressure, pH and salinity and their influence on the microbial community. Different culture-dependent and independent microbiological approaches for detecting inherent microorganisms are summarized. Various microbial populations isolated from different locations worldwide oil reservoirs and explored for their suitability for MEOR applications are detailed. Important reservoir environmental conditions for the screening and conducting MEOR trials are outlined.

2 Influence of Reservoir Environmental Conditions

Oil reservoirs are one of the main modules of the extensive biosphere, where intrinsic microbial inhabitants have survived over a prolonged period (Gao et al. 2018; Li et al. 2017). The activity of the microbes is influenced by environmental as well as geological parameters of the reservoirs like lithological composition, reservoir type, internal temperature, porosity, permeability, crude oil gravity and fluid attributes. These factors directly or indirectly control microbial growth, movement and metabolism (Dhanarajan et al. 2017; Hong et al. 2019; Sharma et al. 2019a, b; Verma et al. 2020). Temperature is one of the prime significant parameters, which governs the bacterial growth and the functionalities of the strains (Aditama et al. 2017). Porosity and permeability mainly control the migration of the microbes or internally produced metabolites and very small pores leave a negative influence on this progression (Gabitto and Barrufet 2005). Salinity is one of the most imperative influencer assuming saline soil and fluid samples. At elevated salinity, biological activity is reduced due to less cell functioning and plasmolysis of microbes, which affected the survival of most microorganisms (Hadibarata et al. 2017). Besides these, pH, pressure and crude oil viscosity furthermore influence the microbes for its application in MEOR practice drastically (Bachmann et al. 2014; Kamari et al. 2014; Sari et al. 2019). The environmental parameters as well as the reservoir conditions are pictorially represented in Fig. 1.

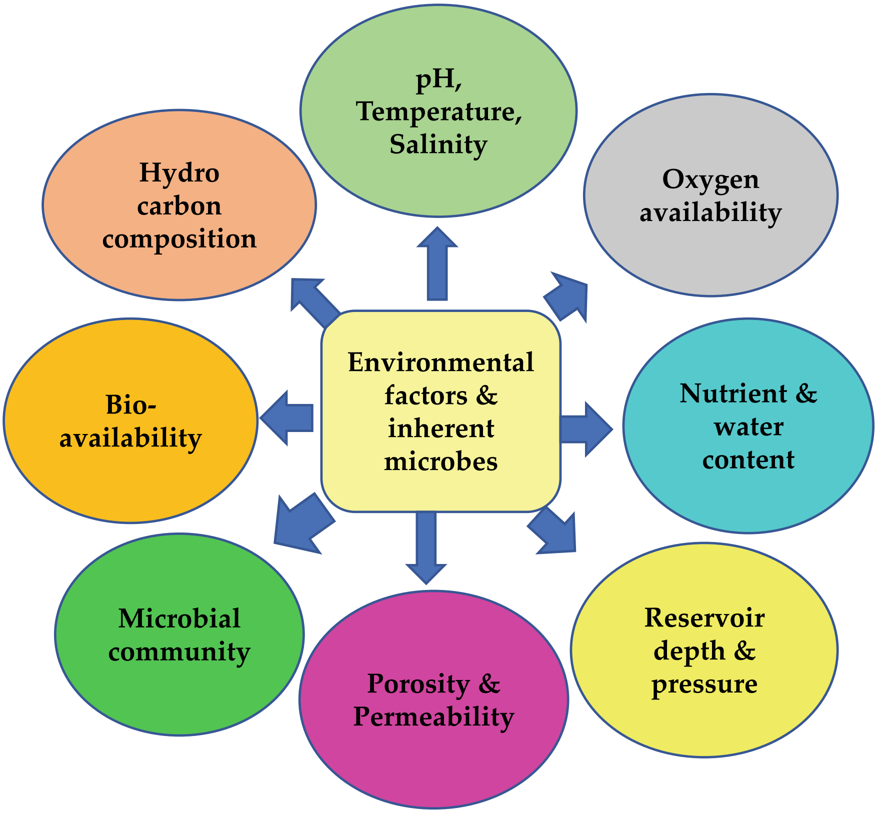

Fig. 1 Environmental factors and the associated microbial population influencing MEOR

2.1 Properties of Crude Oil

Petroleum is a naturally occurring, complex organic mixture, comprising numerous components which can be mainly classified into the following types: alkanes (paraffins), cycloalkanes (naphthenes), aromatics, and more complex asphaltenes and resins. Crude oil is usually categorized into bitumens, heavy oils, medium oil and light oils based on American Petroleum Institute (API) gravity (Santos et al. 2014). Heavy crude oils are well-defined as liquid petroleum possessing 10–22.3° API gravity or >100 mPas viscosity in normal reservoir environments. Medium crude oil possesses API gravity of 22.3–31.1° and 33.4–100 mPas viscosity; light oil has an API gravity of >31° and viscosity <33.4 mPas (Zhang et al. 2020a). The sequential degradation usually occurs following the trend of hydrocarbon susceptibility to bacterial degradation from light to heavy chain contents. The degradation rates of aliphatic hydrocarbons were established to be higher than aromatic hydrocarbons, which can be expressed in this order: aliphatic (linear > branched) > light aromatic (mono aromatics) > cycloalkane > heavy aromatic (substituted or poly aromatics) > asphaltenes and resins (Sharma and Pandey 2020; Van Hamme et al. 2003).

MEOR technology was implemented mostly in the light conventional oil reserves largely in the USA, Russia and China from 1980 to 2010 (Belyaev et al. 2004; Youssef et al. 2009). But relatively less MEOR work has been done in heavy crude oil (density of 920–1000 kg/m^3) reservoirs. The difference between heavy crude oil and traditional conventional oil are mainly high density, more complex composition possessing increased asphaltenes, resins, sulfur, nitrogen, and metal-containing compounds as well as low gas content and low hydrogen/carbon ratio. Heavy oil reservoirs can store about seven times more than conventional oil reservoirs (Leon and Kumar 2005). The largest heavy oil reservoir is located at the Orinoco oil belt of Venezuela. There are a number of heavy oil fields in Oman, where crude oil recovery is complex and expensive because of its high viscosity (Leon and Kumar 2005). The viscosity reduction of heavy oil facilitates improving oil retrieval from a reservoir due to improving the flow behaviour and minimizing the pressure drop (Santos et al. 2014). Different physical methods like heating and dilution are employed for this purpose (Santos et al. 2014).

The reduction in viscosity is also achieved majorly by two microbial mechanisms: either bioconversion of heavy into light oil fractions or by the production of microbial metabolites (e.g., biosurfactants) that modify the physical attributes of the oil, such as lowering its IFT. Several microbial isolates and produced enzymes contribute to the degradation of saturate and aromatic aerobically or anaerobically (Mbadinga et al. 2011; Nie et al. 2014; Rojo 2009; Widdel and Rabus 2001). Indigenous microflora can improve the fluidity of heavy crude oil by altering its high viscosity and subsequently forming lighter oil components (Pineda-Flores and Mesta-Howard 2001). Oil viscosity can also be reduced by produced CO_2 gas (a byproduct of microbial metabolic activity), which creates pressure within the reservoir and push the crude oil upwards by fractional degradation of the large molecular components of crude oil. The produced biomass gathers between the oil and the well-rock surface and displaces the oil, improving the mobility for easier recovery from the well (Marchant and Banat 2012; Safdel et al. 2017). However, there are very few reported literature that addressed the microbial systems with the capability of biological degradation or biotransforming heavy oil fractions (asphaltenes) and in turn improve the oil recovery by reducing the oil viscosity.

2.2 Rock Lithology

The physical characteristics of rocks regulate the occurrence of indigenous microbes and the adsorption behaviour of metabolites, i.e., surfactants. Microorganisms are classified depending on their occurrence in the reservoir rock and their role in the biogenic weathering of the reservoirs. Endolithic microbes are alive inside the rock or in the pores between mineral grains, while epiliths survive firmly on the rock surfaces. Chasmoendoliths accumulate in rock fractures or excavated formations. Euendoliths accumulate on carbonate surfaces by penetrating rock strata via dissolution and form the borings through active digging (Hoppert et al. 2004). The biosurfactant quantity essential for recovering oil is dependent on the adsorption capacity on the rock surface. Various rock formations have dissimilar absorption values. Generally, sandstone can adsorb 0.1–1 mg surfactant/g of rock, however, this also depends on the initial concentration of the surfactant. In the sandstone reservoirs, the anionic surfactant flooding is more competent than the carbonate reservoirs due to the high adsorption capacity of carbonates (Nikolova and Gutierrez 2020). However, in the carbonate reservoirs, the wettability alteration (from oil-wet state to water-wet state) plays a more prominent role in oil recovery. Water-wet state is comparatively favourable for improved oil recovery as oils in carbonate rocks are concentrated in bigger pores, which can be easily accessed during flooding (Kowalewski et al. 2006).

2.3 Reservoir Temperature and Pressure

The highest reservoir temperature has been projected to be about 137 °C at a depth of 4 km by the researchers and the surfactants need to be functional at such a high temperature (Kargarpour 2017). The reservoir depth is not a restrictive parameter for microbial flooding as long as it is complying with the reservoir temperature limit (Sheng 2013). There is an optimal temperature for microbial growth, which is adversely affected when the optimal temperature limit is surpassed (Chen et al. 2001). *Arthrobacter paraffineus*, thermophilic *Bacillus* and *Pseudomonas* strain DSM-2874 are comparatively temperature-sensitive. The composition of the produced biosurfactant is also controlled by temperature (Roy 2017). Temperature increase results in better solubility of hydrophobic components, decline viscosity, enhances long-chain n-alkanes diffusion and transfer from solid phase to fluid phase. The pressure in oil reservoirs varies in the range of 10–100 MPa. The effects of pressure on microbes are also systematically connected with temperature as elevated pressures in natural environments are related to temperature differences (Marshall 2008). Thus, pressure also affects microbial survival and metabolite production. The microbial decay rate was related to the pressure and exposure time (Jeong et al. 2021).

2.4 Environmental pH

The growth profile of the microbes along with their produced biometabolites is controlled by the pH of the environment. Biosurfactants production from different strains like *Bacillus* sp., *Pseudomonas* sp., *T. bombicola*, and *N. corynebacteroides* was found to be dependent on pH (Datta et al. 2020; Sharma and Pandey 2020). pH value influences the interfacial adsorption properties as well as CMC, ST, emulsifying activity and coefficient of elasticity of rhamnolipids (Özdemir et al. 2004). The carboxyl group that is responsible for the anionic nature of rhamnolipid molecules is largely influenced by the pH. The dissociation of the Rhamnosyl structure takes place at higher pH values ($pH > 11$) and leads to different behaviours. The alkaline conditions enhance rhamnolipid solubilization, and therefore cell permeability is increased, which significantly escalates the levels of extracellular carbohydrates, proteins and metabolites (Özdemir et al. 2004). In fact, the emulsifying property is greatly influenced by the pH of surfactin and its emulsions are stabilized quite well above a pH value of 7.4. Approximately, 98% oil emulsification ratio was achieved at pH 11; whereas this emulsification property got quickly and entirely vanished when pH was decreased below 3. This revealed the significance of pH in selecting the suitable reservoir to conduct biosurfactant-mediated EOR (Long et al. 2017).

2.5 Fluid Salinity

The salinity of the injected fluid must be close to the reservoir water salinity, so that the lower most IFT between oil and fluid could be obtained at the optimal salt concentration, which is dependent on several factors including types of crude oil and surfactants. For most of the surfactants, the optimal salinity is not too high (Sheng 2015). In a study, the significance of salt concentration was explained where it was stated that 41% crude oil degradation could be achieved after 4 months of incubation in soil samples without the addition of salt, while only 12% crude oil biodegradation was observed after 4 months incubation in the same soil samples when excess salt (50 g/L NaCl) was supplemented (Minai-Tehrani et al. 2006). However, the effect of salinity is reported to decrease the oil/water IFT values (Rostami et al. 2019).

2.6 *Permeability*

Low-permeability reservoirs are projected to possess decreasing production in comparison with high-permeability reservoirs. Thus permeability improvement is expected to result in improved oil reclamation. Organic acids (acetate, butyrate) production by bacteria (e.g., *Clostridium*, *Enterobacter aerogenes*) in *in-situ* mode can dissolve formation rocks and expose additional pore volume, specifically in carbonate reserves, and consequently, the permeability and fluids flow are improved (Van Hamme et al. 2003). Imbibition of surfactant, brine and surfactant was explored as an important means for the better recovery of oil from low permeability reservoirs (Xu et al. 2019).

3 Microbiological Approaches for Detecting Inherent Microorganisms

Microbial activity is a universal and significant part of the petroleum reserve systems; nevertheless, present information about the composition, assortment of active microflora in offshore petroleum reservoirs remains inadequate, which is vital to understand metabolic activities to employ suitable exploration strategies in the oil reservoirs. The variation of the microbial community of production wells is very diverse and at the same time complex. There are an extensive collection of microbes explored in MEOR, which can be classified broadly into two distinct classes (Youssef et al. 2009). The first one is autochthonous or indigenous microbes that already exist in oil reserves, and the second one is allochthonous or exogenous microbes, which are developed purposely by injecting into reservoirs. Exogenous microbes imply suitable microflora screened by employing reservoir-like conditions to increase oil reclamation by its proliferation and production of metabolites (Cheng et al. 2006; She et al. 2019).

Conventionally, culture-dependent techniques have been utilized to enrich and isolate microbial species from petroleum reserves (Singh et al. 2014). The cultivation-based approach assists in the improvement of the physiological potentials of some indigenous microbes that perform MEOR processes. However, this is not the only approach that reflects the actual environmental microbial diversity. As huge proportions of environmental bacteria cannot be cultured, the culture-independent approach is preferred during analyzing microbial population in reservoirs (Bordoloi and Konwar 2008; Brown and Vadie 1997; Wang et al. 2014).

As only a small portion of microbes could be grown using culture-dependent techniques so it is quite difficult to evaluate the inclusive reservoir microflora composition. In order to get rid of these cultivation restrictions, culture-independent methods have been established for characterizing the complex microbial population. Advanced microbiological and molecular strategies for detecting inherent microbial populations are pictorially described in Fig. 2. Particularly, some molecular tech-

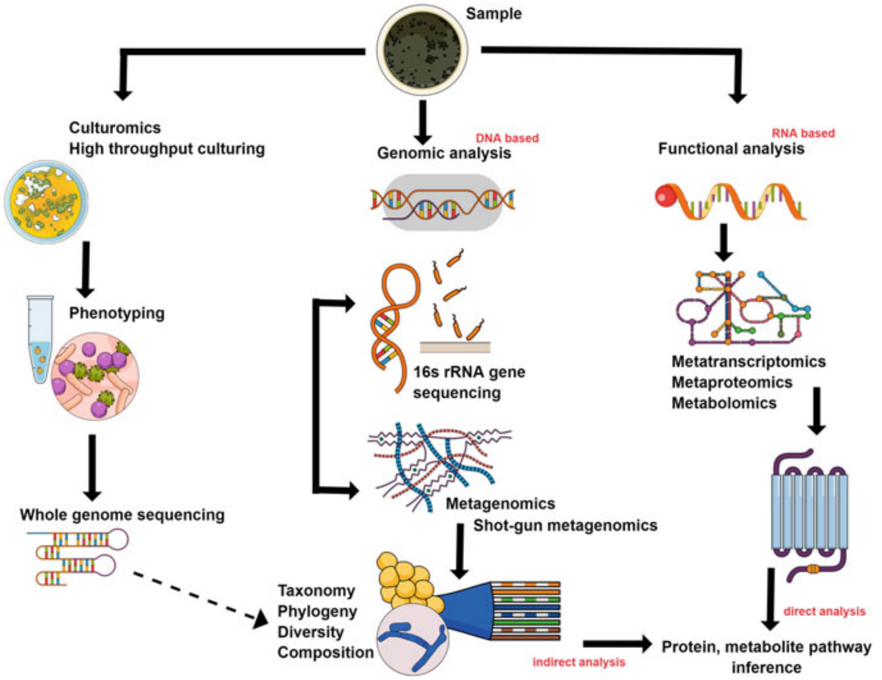

Fig. 2 Advanced microbiological approaches for detecting indigenous microbial communities [Adapted from (Philips et al. 2020)]

niques such as DNA extraction, polymerase chain reaction (PCR)-based approaches, 16S rRNA and functional genes sequencing and next-generation sequencing (NGS) have improved overall proficiency for identifying microbes in petroleum reserves. RNA-based sequencing methods could also be another substitution for analyzing active and inactive species. The internal microbial community could be identified by complex high-tech biotechnological culture-independent techniques (She et al. 2019) including terminal restriction fragment length polymorphism (T-RFLP), denaturing gradient gel electrophoresis (DGGE) (Xingbiao et al. 2015), gene bank (Liu et al. 2010), clone library and most probable number (MPN) (Cheng et al. 2006). Thus, metagenomics or microbial community investigation using whole metagenome-based strategy provides suitable insight into the inherent microbiome structurally and functionally. Therefore, expansion from culture-based strategy to metagenomics, metatranscriptomics or single-cell genomics have commenced a paradigm shift in perceptive of microbial assortment and genomic collection of intrinsic microbes (Gupta et al. 2019). Advanced metagenomics strategies in combination with metaproteomics, metabolomics, stable isotope probing and single-cell sequencing apprise innovative viewpoint in the microbial ecophysiology of the subsurface ecosystems (Ismail et al. 2017).

4 Microbial Diversity in Worldwide Oil Reservoirs

Hydrocarbon degrading microorganisms are termed as hydrocarbonoclastic. The indigenous hydrocarbonoclastic community of a reservoir is anticipated to possess specific benefits over other microorganisms or exogenous microbial consortiums due to better adaptability of the former in the internal reservoir conditions. The primary adaptations include their utilization of hydrocarbons, extreme endurance in high temperature, salinity and pressure, as well as their metabolic activity anaerobically.

The common hydrocarbon-degrading microbial genera are: *Bacillus*, *Pseudomonas*, *Acinetobacter*, *Micrococcus luteus*, *Nocardia*, *Rhodococcus*, *Streptomyces*, *Vibrio*, *Xanthomonas maltophilia* (Mariano et al. 2007). *Acinetobacter, Burkholderia, Paraburkholderia* and *Luteibacter* mainly contributed to petroleum hydrocarbons degradation under optimal conditions (Cui et al. 2020). However, the proficient oil-byproduct degrading bacterial genera are: *Acidovorans*, *Acinetobacter*, *Agrobacterium*, *Alcaligenes*, *Aeromonas*, *Arthrobacter*, *Beijemickia*, *Burkholderia*, *Comomonas*, *Corynebacterium*, *Cycloclasticus*, *Flavobacterium*, *Gordonia*, *Microbacterium*, *Moraxella*, *Mycobacterium*, *Neptunomonas*, *Paracoccus*, *Pasteurella*, *Polaromonas*, *Ralstonia*, *Sphingomonas* and *Stenotrophomonas* (Tonini et al. 2010). Marine microbes, *Alcanivorax*, *Oleiphilus*, *Oleispira*, *Thalassolituus*, *Planomicrobium alkanoclasticum* MAE2 uses a variety of saturated hydrocarbons, however, *Cycloclasticus* could utilize a range of polycyclic aromatic hydrocarbons (Head et al. 2006). Some microbes from the marine environment such as *Pseudomonas*, *Pseudoalteromonas*, *Marinomonas*, *Oceanobacillus, Halomonas*, *Rhodococcus*, *Sphingomonas* and *Cobetia* have been designated as biosurfactant synthesizers (Schultz and Rosado 2020).

Anaerobic microbes are more widespread species in oil reservoirs (Fardeau et al. 2004). *Bacillus*, *Pseudomonas*, *Mycobacterium*, *Micrococcus* and *Rhodococcus* can oxidize hydrocarbon present in oil reserves (Saravanan et al. 2020). Methanogenesis is an obligatory anaerobic biological mechanism prevalent in oil reservoirs (Zhou et al. 2020). Methanogenic pathways could be categorized into hydrogenotrophic, acetoclastic and methylotrophic methanogenesis based on the utilizing substrate. Methane could be produced by hydrogenotrophic (*Methanocalculus*, *Methanoplanus*, *Methanothermobacter* and *Methanolinea*), acetoclastic (*Methanosaeta*), methylotrophic (Candidatus *Methanomethylicus* and *Methanomassiliicoccaceae*, genera *Methanosarcina*, *Methanhalophiluus* and *Methermicoccus*) and other (Candidatus *Methanofastidiosum*) methanogens (Meslé et al. 2013; Youssef et al. 2009; Zhou et al. 2020). The phyla, namely *Acidobacteria*, *Atribacteria*, *Chloroflexi*, *Fusobacteria, Nitrospira*, *Planctomycetes*, *Spirochaetes*, *Synergistetes* and *Thermodesulfobacteria* were found in much less amount in oil reserves. Other less commonly identified archaeal diversity comprised of *Desulfurococcales*, *Fervidicoccales, Haloferacales*, *Methanocellales*, *Methanomassiliicoccales*, *Thermoproteales*, *Thermoplasmatales* and *Sulfolobales* (Li et al. 2017).

Three thermotolerant hydrocarbonoclastic strains of *Bacillus*, *Geobacillus*, and *Petrobacter* were isolated from high-temperature oilfield and described to endure

55 °C anaerobically and degrade hydrocarbon (Shibulal et al. 2014; Wang et al. 2008), signifying their appropriateness to participate MEOR process. Mesophilic genera such as *Arcobacter, Clostridium*, *Desulfuromonas*, *Geobacter*, *Marinobacter* and *Pseudomonas* could also be traced in large propensity in comparatively lower temperature oil fields (Hubert et al. 2012; Okpala et al. 2017; Zhang et al. 2012).

The primary bacterial and archaeal lineages detected in oil reservoirs to carry out *in-situ* MEOR are shown in Fig. 3. Different phylum, class and family of bacterial and archaeal lineages can be found in detail elsewhere (Rosenberg et al. 2014). *Proteobacteria* are a major phylum of Gram-negative bacteria and are distributed into nine different classes including *alpha* to *zeta-proteobacteria.* The phylum *Deferribacteres* are Gram-negative anaerobic bacteria. The phylum *Bacteroidetes* are non-spore-forming Gram-negative and facultative bacteria commonly spread in the environment. The family Firmicutes are a phylum of Gram-positive bacteria. *Actinobacteria* are a group of unicellular filamentous Gram-positive bacteria and occur in terrestrial or aquatic environments. The phylum *Thermotogae* include Gram-negative anaerobic and thermophilic/hyperthermophilic bacteria. Archaea are also unicellular organisms and the most ancient. Many archaea are extremophiles, but a few mesophiles.

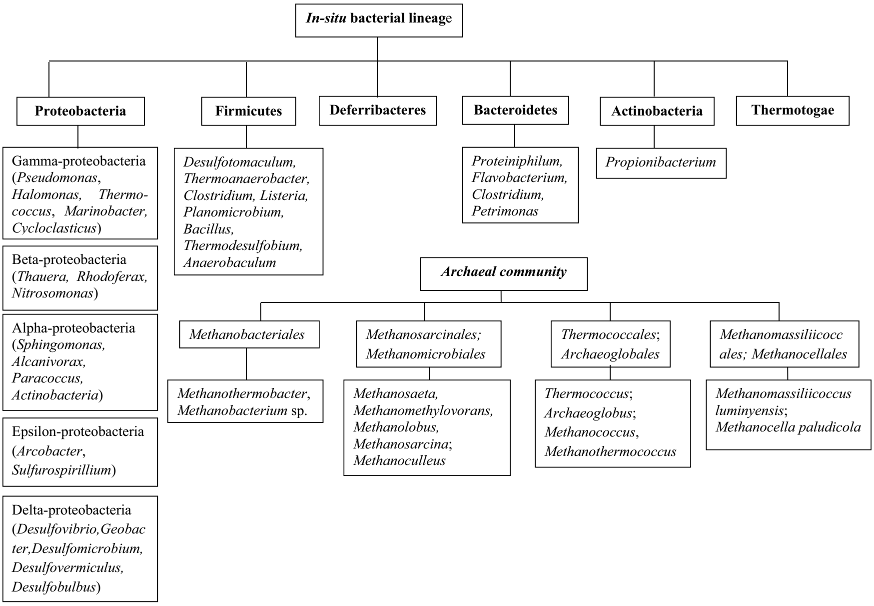

Fig. 3 Reservoir inherent hydrocarbon utilizing microbial population. Prepared based on the information from the reference (Youssef et al. 2009)

4.1 Various Microbial Populations in Worldwide Oil Reservoirs

Oil reserves are distributed across the globe and mainly concentrated in Saudi Arabia, Venezuela, Russia and Iran (Tong et al. 2018). Similarly, various microbes have been isolated from different locations worldwide and explored for their suitability for MEOR applications. A few of the potential isolates from oil reservoirs are discussed here.

Molecular approaches analysis of formation water of a high-temperature oil reservoir in Russia (Samotlor) showed the presence of certain genus such as *Thermoanaerobacter*, *Thermotoga*, *Geobacillus*, *Petrotoga*, *Thermococcus* and *Thermosipho* including some previously unidentified taxa *Desulfurobacterium. Thermovibrio* cluster of *Desulfurococcus* and *Thermus* genera were also identified via targeted oligonucleotide microchip analysis (Bonch-Osmolovskaya et al. 2003). Physico-chemical and biotechnological properties of formation waters of low-temperature heavy oil reservoirs of Russia (particularly Chernoozerskoe, Severo- Bogemskoe and Yuzhno-Suncheleevskoe oilfields) harboring limited microbial species, as well as the other (Vostochno-Anzirskoe and Cheremukhovskoe) oilfields comprising comparatively diverse microbial community were studied (Nazina et al. 2017). Sulphate reduction and methanogenesis were not observed in the samples from water-flooded sections. Metagenomics study and 16S rRNA sequencing of microbial gene fragments in the population of injection fluid displayed the presence in the order of descending quantity consisted of *Proteobacteria*, *Betaproteobacteria*, *Alphaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*, *Epsilonproteobacteria*, as well as some Firmicutes, Bacteroidetes, and Archaea. DGGE examination of *mcrA* genes in the microbial population of injection fluid showed the occurrence of methanogens genera including *Methanobacterium*, *Methanothrix*, *Methanospirillum*, *Methanoregula*, *Methanosarcina*, and *Methanoculleus*, along with undetected *Thermoplasmata.* Pure strains of *Cellulomonas, Gordonia*, *Pseudomonas*, *Rhodococcus* genera accomplished biosurfactant synthesis by supplementing with heavy oil. Fermentative bacteria supplemented with enrichment media containing sacchariferous substrates produced noteworthy amounts of volatile organic acids (VFA) (acetic, butyric and propionic), which were dissolved in the carbonates of oil-bearing rock proficiently (Nazina et al. 2017).

In the Tatariya carbonate oil field (Russia), *Clostridium* increased the oil production by 28–46% (Sakthipriya et al. 2017). The low-temperature heavy oil fields (Russia) accommodated a microbial community that was competent enough to produce oil-displacing metabolic compounds. Aerobic strains *Rhodococcus erythropolis* HO-KS22 and *Gordonia amicalis* 6–1 isolated from Russian oil reserves reported to oxidize heavy crude oil to produce biosurfactants which considerably reduced ST and IFT as well as exhibited prospects for paraffin degradation, MEOR and the hydrocarbon bioremediation (Nazina et al. 2020).

B. subtilis, *P. aeruginosa*, and *Bacillus cereus* isolated from oil-contaminated sites of Iran could withstand extreme reservoir surroundings (120 °C, pH 4, 25 g/L salinity) and ST was decreased from 72 to about 26 mN/m because of the production of biosurfactants (Amani et al. 2010; Bachmann et al. 2014). *Bacillus mojavensis* were isolated in Masjed-I Soleyman carbonate field (Iran) having a pressure of 3–4 MPa. The produced biosurfactant reduced the ST to 26.7 mN/m and a temperature of 42 °C (Ghojavand et al. 2012). *Bacillus licheniformis* was isolated from the Zilaei oil reservoir in southwest Iran, which grew optimally and produce biosurfactant at 50 °C which reduced the ST from 72 to 23.8 mN/m along with the IFT from 36.8 to 0.93 mN/m (Daryasafar et al. 2016). This same species was further isolated from the Niage field of the Egyptian Western Desert, which exhibited considerably good surface properties including a highest emulsifying index of 96% and ST reduction to 36 mN/m when incubated for 72 h at 45 °C (El-Sheshtawy et al. 2015).

Among several isolated spore-forming bacteria from soil samples of Oman oil fields, an autochthonous strain, *Paenibacillus ehimensis* BS1 was reported to improve heavy oil recovery due to a great endurance in stressful conditions with long dormancy period, in high thermal condition, drying and presence of acid. The isolate exhibited utmost growth when supplemented with elevated heavy oil and incubated for four days. Biotransformation of heavy crude oil (API 4.57°) to light aliphatic and aromatic constituents along with its prospects in EOR was accessed aerobically and anaerobically (Shibulal et al. 2017). Five dominant identified genera of Suwaihat oilfield, Oman were *Halomonas*, *Cenothrix*, *Methylobacter*, *Burkholderia* and *Crenarchaeote*. The most commonly detected bacterial genera were *Rhodococcus* followed by *Petrotoga*, *Diaphorobacter*, *Thermotoga* and *Actinobacterium* in Wafra Oil Wells, Kuwait, identified by gene sequencer (culture-independent technique) (Al-Wahaibi et al. 2013).

Marinobacter hydrocarbonoclasticus was isolated from the upper segment of a Vietnamese oil well, which grew on high salinity of 5% NaCl. It could biodegrade n-hexadecane, pristane along with some other crude oil constituents (Huu et al. 1999). The microbiological aspects along with functional gene analysis were predicted from metagenomics 16S rRNA gene data of the microbial population from onshore Mae Soon Reservoir, Thailand, which signified the prospects of the native microbes to facilitate the MEOR processes by synthesizing biosurfactants. The biosurfactant-synthesizing bacteria from the oil-bearing sandstone reservoir was identified as *Bacillus licheniformis* MS5-16 and the produced biosurfactant decreased ST from 72 to 32 mN/m. Promising gene sequences accountable for biosurfactant production were (*licA3* 0.26 kb), lipase generation (*lipP1* 0.63 kb) and catechol 2,3-dioxygenase for hydrocarbon assimilation (*C23O* 1.27 kb), which also indicated their applicability to oil recovery. The genes encrypting MEOR-associated functional proteins for alkane degradation were enoyl-CoA hydratase, alcohol dehydrogenase and alkane 1-monooxygenase (Phetcharat et al. 2019).

The whole genome of hydrogenotrophic thermophilic methanogen *Methanococcus maripaludis* strain X1, isolated from an offshore Malaysian oilfield subsurface production fluid, was reconstructed from high-quality metagenomics datasets (Wang et al. 2011). The microflora was targeted by certain genes such as dissimilatory sulphate reductase (*dsrA/B*) and dissimilatory nitrate reductase (*narG/napA*). The subsequent biodegradation process includes the methanogens, that convert hydrogen and CO_2 (hydrogenotrophic) or acetate to methane (CH_4) (acetogenic), maintaining equilibrium during the course of syntrophic biodegradation of hydrocarbons to methane, by eliminating the intermediates (hydrogen and CO_2). This population is detected by the methyl coenzyme-A reductase (*mcr*) gene (Gupta et al. 2019). The isolated and characterized native strains of a heavy oil reservoir of South Sumatra could effectively degrade heavy crude oil constituents and subsequently reduced oil viscosity. Three candidates were chosen as G3, G7, and N6 which reduced oil viscosity up to 22.67%, 23.14% and 24.36%, respectively. Isolate G3 which was identified as *Pseudoxhantomonas taiwanensis* degraded 38% aromatics along with 29% resin, isolate G7-*Brevibacillus agri* degraded 61% aromatic fraction while N6, identified as *Bacillus subtilis* degraded 52% asphaltenes fraction. This implied the suitability of these isolates for MEOR technology (Purwasena et al. 2018).

Institute of Reservoir Studies (IRS), India, indigenously formulated consortia, namely IRSM-1 and IRSM-2 were anaerobic thermophilic and halophilic (3% salt concentration) bacterial mixture comprising small cocci and short rod size (1.5–2 μm), with pH endurance of 6–8.5 and thermal tolerant up to 65 °C. This microbial system could produce suitable metabolites like fatty acids, biosurfactants and biogases in the oil fields. The MEOR field trials via huff and puff were conducted in Badarpur (3 wells), Kosamba (1well) and Padra (1well) of Mehsana asset of Cambay basin. IRS also prepared two more anaerobic consortia, NJS7-91 and NJS4-96 developed from the microbes of formation waters of Nandej and Sobhasan oil wells of Ahmedabad and Mehsana oil fields. These consortia were hyperthermophilic (grew at 91 and 96 °C) and halophilic (grew in 7% and 4% salt concentration) (Patel et al. 2015).

A bacterial isolate (*Garciaella petrolearia*) from Mumbai, India, could grow well-exploiting asphaltenes substrate and favorably degraded asphaltenes as well as aromatics present in crude oil (Lavania et al. 2012). The different strains were isolated from the formation water of the Assam oil reservoir field based on crude oil degradation and biosurfactant production. These strains were identified using 16S rRNA sequencing and found to be *Stenotrophomonas* sp. MG520349, *Bacillus subtilis* MG520348 and *Bacillus subtilis* MG495086. Among isolates, *Bacillus subtilis* MG495086 under optimal conditions i.e. 3.8% (v/v) of light-paraffin oil as a sole carbon source at 62.4 °C and pH 7.7 produced 6.3 ± 0.1 g/L of lipopeptide biosurfactant in 96 h and reduced ST to 29.85 mN/m (Datta et al. 2018). In another study, *Bacillus tequilensis* MK 729017 and *Bacillus subtilis* MK729018 were isolated from the reservoir soil of the Assam oil reservoir field. Among isolated strains, *Bacillus tequilensis* MK 729017 was chosen based on the better surface-active properties. The produced lipopeptide, surfactin was found to be thermal and colloidal stable and reduced ST to 30 ± 2 mN/m. It also decreased the wettability of hydrophobic rock surface from $90 \pm 1°$ to 26 ± 1 (Datta et al. 2020).

Similarly, the microbial population of other reservoirs production fluid was also scrutinized and many of them were found to be biosurfactants producers. Initially, the dominant genera present in the production well were found to be *Arcobacter*, *Flexistipes*, *Hyphomonas*, *Parvibaculum*, *Pseudomonas*, *Syntrophus*, *Treponema* and

Wolinella. During the bioaugmentation process, dominant genera somehow changed to *Pseudomonas*, *Arcobacter*, *Acinetobacter*, *Shewanella*, *Enterococcus*, *Flavobacterium.* However, *Pseudomonas aeruginosa* DQ3 strain remained the most dominant in Daqing oil reservoirs and could produce maximum biosurfactant of 228 mg/L anaerobically at reservoir temperature (42 °C), which was sufficient because the minimum biosurfactant concentration necessary for mobilizing the entrapped oil from the sandstone reservoir was already known to be only 10 mg/L (Youssef et al. 2007, 2013). *Brevibacillus brevis* and *Bacillus cereus* were also employed at Daqing low permeability high-temperature oil field at 65 °C which could reduce 40% of oil viscosity. *Enterobacter*, *Bacillus licheniformis* utilized at Fuyu oil field of China (1.95–2.95 MPa, 28 °C) and increased oil recovery by twofold (Sakthipriya et al. 2017).

G. amicalis LH3, isolated from oil-contaminated water samples of the Chinese Jidong oilfield, could degrade 18% (2% w/v) paraffin anaerobically at a rate of 4.4 mg/d after 10 days of cultivation at 40 °C with 5% NaCl. The strain could also reduce 45% of oil viscosity and degraded 10.5% (w/w) oil aerobically after 7 days of incubation (Hao et al. 2008). Two *Pseudomonas aeruginosa* strains (Gx and Fx) were isolated from oil-saturated soils of Yanchang oilfield, China, and produced biosurfactants using crude oil heavy components as their substrate. The oil displacement prospects of Gx and Fx were examined by the degradation capability of pure asphalt and crude oil asphaltenes where almost 10% of pure asphalt and 59–72% of asphaltenes were biodegraded utilizing bacterial supernatants showing the oil-displacing diameter of 15–17 cm. The lighter fractions (saturates and aromatics, maximum 11%) content were augmented while the heavier fractions (resins and others, maximum 75%) contents were bio-transformed with approximately 50% reduction of the oil viscosity (Gao et al. 2017).

Several microbes were detected and isolated from the production water samples of Jilin oilfields of China for the formulation of crude oil and asphaltenes-degrading microcosm based on dissimilar bacterial types. After two or three weeks of enrichment period, Gas chromatography-mass spectrometry (GC–MS) and Fourier transform infrared spectroscopy (FTIR) results proved the biodegradation of crude oil and asphaltene. The leading genera which formed crude oil-degrading microcosm were *Alcanivorax*, *Devosia*, *Hydrogenophaga,* *Parvibaculum*, *Pseudomonas* and *Dietzia* and similarly asphaltenes-degrading microcosm was prepared by combining *Alcanivorax*, *Flavobacterium,* *Hyphomonas*, *Parvibaculum* and *Reyranella.* This imparted an innovative dimension to understand the microbial miscellany in reservoir production fluid and its prospective function for oil degradation (Song et al. 2018). The microbial diverse community and their relative abundance were scrutinized in consecutive indigenous MEOR (IMEOR) metabolic phases. *Pseudomonas*, *Citrobacter* and *Burkholderia* were displayed to be dominant genera in the aerobic, facultative and early anaerobic stages whereas *Bacillus*, *Achromobacter*, *Rhizobiales*, *Alcaligenes* and *Clostridium* became prevailing in the later anaerobic phase which demonstrated the unique characteristics of microbial succession stimulated by wheat bran supplementation in the Chinese oilfields (Zhan et al. 2017).

Culture-independent DGGE and clone-library-based examination of production and formation water from high temperature (85 °C) offshore petroleum reserve in the Norwegian Sea focused on the microbial miscellany and recognized a common bacteriological assembly including *Arcobacter*, *Halomonas* and *Pseudomonas* (Brakstad et al. 2008). A pyrosequencing-based entire metagenomic investigation of Norwegian shelf oil fields samples (2.5 km deep, 85 °C, 250 bar) designated the microflora composition by mainly sulfate-reducing bacteria (SRB) from δ or ε *Proteobacteria* (*Campylobacterales, Desulfovibrionales* and *Desulfuromonadales*) along with *Thermotogales* and methanogenic archaeal *Methanococcus* (Kotlar et al. 2011). *Kosmotoga olearia*, an anaerobic, thermophilic, heterotrophic strain from the Troll B oil platform in the North Sea could grow in the temperature range of 20 to 80 °C, pH of 5.5 to 8.0 and 10–60 g/L of salinity (DiPippo et al. 2009). Moderately thermophilic and halophilic SRB, *Petrotoga halophila*, was isolated from an offshore oil well of Congo, West Africa, capable of hydrocarbon degradation (Miranda-Tello et al. 2007).

A culture-independent metagenomics analysis in formation water from the North Alaska slope oil field exhibited the dominance of thermophilic *Thermoanaerobacter*, *Desulfonauticus* and *Archeaoglobus* at a high temperature of 80 to 83 °C and 2.5 km depth site Ivishak (Hu et al. 2016). It was reported from a DGGE-based investigation on heavy oil carbonate reservoir in Cordoba platform, Veracruz, Mexico, that the consortia consisting thermophilic anaerobic, acetogenic *Thermoanaerobacter* were functional at 60–80 °C temperature and 535 g/L salinity, respectively (Castorena-Cortés et al. 2012a; Castorena-Cortés et al. 2012b). A bacterial consortium (*Bacillus* sp., *Corynebacterium* sp., *Brevibacillus* sp. and *Staphylococcus* sp.) was formulated which consumed asphaltenes of Mexico's Maya crude oil as their only carbon substrate (Pineda-Flores et al. 2004). The indigenous microflora in the Gulf of Mexico beach sands denoted the predominance of *Gammaproteobacteria* and *Alphaproteobacteria* as the chief contributor in oil biodegradation (Kostka et al. 2011).

A dual culturable and 16S rRNA gene clone-library-based study of the microbial assortment of biodegraded and non-biodegraded terrestrial oil from Potiguar basin, Brazil, identified the occurrence of *Actinobacteria*, *Firmicutes*, *Proteobacteria* (culturable representatives) and *Thermotogae* in both samples (Silva et al. 2013). Additionally, the prominent candidates were *Bacteroidetes*, *Deferribacteres*, *Spirochaetes*, and *Synergistetes* in the biodegraded sample and *Chloroflexi* and *Thermotoga* in the non-biodegraded sample along with archaeal family Methanomicrobiaceae. Similar investigation on three other oil fields of Brazil implied a core microbiome comprising of three bacterial (γ*-proteobacteria*, *Clostridia* and *Bacteroidia*) and one archaeal (*Methanomicrobia*) classes (Sierra-Garcia et al. 2017). Nine bacterial groups have been reported from Brazilian oil reservoirs samples, such as: *Acinetobacter*, *Arcobacter*, *Bacillus*, *Halanaerobium*, *Leuconostoc*, *Marinobacter*, *Propionibacterium, Streptomyces* and *Streptococcus* (Sette et al. 2007; Souza et al. 2014). A fungal strain, *Neosartorya fischeri*, isolated from Venezuela exploited asphaltenes substrate and efficiently degraded asphaltenes as well as aromatics present in crude oil (Uribe-Alvarez et al. 2011).

Comparative analysis to develop a better understanding of the microbial population was conducted by collecting the production fluid from four deep subsurface high-temperature oil reservoirs which revealed surprising resemblance among geographically distant oil reservoirs. The similarity was observed in oil wells situated in the Segno oilfield near Houston, USA (80–85 °C) and Crossfield oilfield, Canada (75 °C), as a preliminary investigation (Lewin et al. 2014). A similar study was carried out in four oil secluded reservoirs (China, Lousiana, Norwegian Sea, Danish North Sea). The pH range of the four production fluids was 5.53 to 8. The major species consisted of acetoclastic and hydrogenotrophic methanogens (*Methanosaetaceae*, *Methanoculleus and Methanobacterium*), along with Clostridiaceae and Thermotogaceae families, which were also found to be thermotolerant and/or spore-forming. The major microbial taxa were *Pseudomonadales*, *Clostridiales*, *Burkholderiales*, *Methanococcales*, *Rhizobiales* and *Synergistales*. A strong concurrence association was noticed among the orders *Bacteroidales, Sphaerochaetales*, *Desulforomonadales* and *Oceanospirillales*, and all were obligatory anaerobic hydrocarbon-utilizing organisms (Kim et al. 2018).

5 Reservoir Environmental Screening Parameters to Conduct MEOR Trials

The efficiency of the MEOR procedure is dependent upon the following constraints; reservoir temperature, crude oil viscosity and API gravity, brine salinity, porosity, permeability, water cut, pressure, residual oil saturation, reservoir depth, wax content along with indigenous microbial content and diversity of the reservoir systems (Sen 2008). The constructive outcome or the success rate of the MEOR process significantly depends upon the microbial consortia present in reservoirs along with the reservoir category. The MEOR progression involves primarily hydrocarbon-consuming microbes (Saravanan et al. 2020). The suitable reservoir conditions to implement the MEOR process have been described in various literature, which could be generalized as the temperature below 93 °C, salinity 100,000 ppm and permeability 75 mD (Yernazarova et al. 2016). The inoculum size and oxygen availability were also found to affect the MEOR process through cellular activities. MEOR has been attempted in more challenging Chinese reservoirs possessing high temperature, high salt concentration, low permeability and heavy crude oil. In some of the cases, reservoir temperature was reported to be as high as 80 °C, salt tolerance up to 46,000 ppm, oil viscosity of 43,000 cP, and reservoir permeability as low as 25 mD. Field-scale assessments proved that MEOR attained accomplishments even under such adverse environments (Gao 2018). Due to the variation in the reservoir environmental conditions, different organizations such as the US Department of Energy (US-DOE) (Bryant and Douglas

1988), China National Petroleum Corporation (CNPC) (Guo et al. 2015; She et al. 2019) and Institute of Reservoir Studies (IRS), India, (Patel et al. 2015) proposed standard parameter ranges for wells selection to carry out MEOR process. The detailed information of the screening parameters of the reservoirs for an individual country to perform the MEOR process is represented in Table 1.

6 Conclusion

The MEOR process performance is tough to anticipate due to the inducing environmental situations inside the oil reservoir which influence the microbial growth and metabolic product formation. As the environmental factors fluctuate in different seasons in a reservoir and are dissimilar among various reservoirs, the MEOR procedure needs to be tailored for precise settings in each of the reservoirs to increase its success rate. At present, oil-producing companies consider MEOR as a high-risk technology for achieving competent and predictable oil retrieval. While modelling strategies for foreseeing consistent oil repossession under replicated reservoir environments shed a ray of assurance and advancement. Though it had been revealed to be a relatively slow development. The probable progression of a “universal” formulation, comprising a combination of nutrients, particular microbes and biosurfactants is an optimistic elucidation subjected to future research in the field scales.

Table 1 Detailed characterizations of oil reservoir fields worldwide to carry out MEOR trials

Countries	Parameters											
	Reservoir lithology	pH	Temperature (ºC)	Permeability (mD)	Porosity, %	Depth (m)	Pressure, (kg/cm^2)	Water cut, %	Salinity, (g/L)	Viscosity, cP	ºAPI gravity	Reference
IRS	Sandstone	6–9	<90	>50		<2400	<300	30–90	<10	<20	>20	Patel et al. (2015), Safdel et al. (2017)
US DOE	Sandstone		<71	>100		<3048			<10		18–40	Bryant and Douglas (1988), Niu et al. (2020)
CNPC	Sandstone	6–8	30–60	≥ 150	17–25	2500		60–85	≥100	30–150		Gao (2018), Guo et al. (2015), He et al. (2018), She et al. (2019)
Iran	Carbonate		60	50	19.5	1450	1200	20		2000	14	Kamari et al. (2014), Sari et al. (2019)

(continued)

Table 1 (continued)

Countries	Parameters											
	Reservoir lithology	pH	Temperature (ºC)	Permeability (mD)	Porosity, %	Depth (m)	Pressure, (kg/cm^2)	Water cut, %	Salinity, (g/L)	Viscosity, cP	ºAPI gravity	Reference
Argentina (Tupungato Refugio)	Sandstone		71	300	18	1800	100	63.5	42	9	28	Gao and Zekri (2011), Nnaemeka et al. (2018)
Norwegian petroleum Directorate	Carbonate		61–155	1–20,000	11–35	1300–4208			14–273	0.1–4.83		Awan et al. (2008), Safdel et al. (2017)
Romanian Academy (Bragadiru field)				150–300		780			60–300	9		Sen (2008)

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Optimization of Culture Conditions for the Production of Biosurfactants

Swati Sharma, Pankaj Tiwari, and Lalit Pandey

1 Introduction

Metabolism of crude oil is a complex process with many underlying pathways, which work simultaneously to achieve maximum biodegradation. One such pathway involves the production of biosurfactant in order to break the hydrocarbons into smaller units for easy accessibility and bioavailability to the microbes. Biosurfactants are amphiphilic surface-active compounds released by microbes as primary/secondary metabolites (Akbari et al. 2018). It is typically composed of a polar head group with a non-polar fatty acid tail. Such structural characteristics aid the formation of micelles, emulsification and foaming activity in a non-polar and polar liquid mixture. These chemical molecules possess silent features such as decreasing the interfacial tension (IFT) of the oil–water interface, improving the solubility and hence bioavailability of non-polar compounds such as hydrocarbons into water and soil environment (Patowary et al. 2018). In addition, they are proven to be highly stable with no loss in the activity, in a broad range of pH, temperature, and salinity, thereby restricting the reliance on non-biodegradable chemical surfactants (Akbari et al. 2020; Saenge Chooklin and Saimmai 2020).

There have been ample debates on the behavior of biosurfactant production to be growth-associated or non-growth-associated. Various studies support that biosurfactants production is a characteristic feature of hydrocarbon-degrading microbes, however, some studies suggest that not all hydrocarbon-degrading strains are biosurfactant producers (Patowary et al. 2017; Wu et al. 2019). Even after decades of research in this field, the exact biosurfactant production pathway is not clearly understood till date as the process varies from microbial strain to strain. Few studies

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S. Sharma · P. Tiwari · L. Pandey (✉)
Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati,
Guwahati 781039, Assam, India
e-mail: lalitpandey@iitg.ac.in

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hypothesize the biosurfactant production process to be induced during stress conditions, however, there are evidences where contradictory results have been reported (Chakraborty et al. 2012; Santos et al. 2016). However, every literature supports the role of biosurfactants in hydrocarbon biodegradation and reduction of IFT (Abdulsalam et al. 2016; Akbari et al. 2020; Datta et al. 2018; Jimoh and Lin 2020; Parthipan et al. 2017; Patowary et al. 2017; Sharma et al. 2019b). Hence it's a vital part of microbial enhanced oil recovery (MEOR), bioremediation and other environmental cleanup processes. Yet, the challenge lies in the limited availability of such a proficient surface-active agent at the site of action owing to its low microbial productivity. Recently, various researchers are emphasizing on optimization for the maximum biosurfactant production in order to successfully achieve the intended applications (Datta et al. 2018; Ghazala et al. 2019; Haloi and Medhi 2019; Jimoh and Lin 2020, 2019; Ohadi et al. 2017; Verma et al. 2020; Vigneshwaran et al. 2018).

Understanding the basic underlying principle behind the biosurfactant production pathway by the biodegrading microbes is the key to design a successful production model. Presuming the biosurfactant to be a growth-associated product, the aim is to optimize the growth of microbe of interest in order to achieve higher productivity. For effective culture growth, the associated culture conditions and physiological parameters play a vital role. In this chapter, major factors controlling biosurfactant production are discussed. Selection of proficient microbes, the role of physiological parameters and media composition towards biosurfactant production are outlined. Based on these major factors, different associated optimization techniques are elaborated with their associated merits and demerits.

2 Optimizing Parameters

A high yield of biosurfactants is a must for competing with the commercial surfactant and be eligible for industrial production (He et al. 2020). Maximizing biosurfactant production is majorly controlled by two factors: (a) improving the activity of strain which is classified as strain engineering, and (b) optimizing the culture conditions which is classified as process optimization. Various studies put forward that maximum biosurfactant production occurs at optimized microbial growth conditions, signifying the metabolite to be growth associated (Devaraj et al. 2019; Sharma and Pandey 2020; Yaraguppi et al. 2020). Understanding the effect of each media component and their impact on process conditions are crucial before designing an optimization model. In this regard, various researchers have restricted the role of the following major parameters as the deciding factors governing the overall microbial production of biosurfactants: Carbon source, nitrogen source, their respective concentrations, culture pH, temperature and other salt concentration.

2.1 pH

The pH factor is regarded as one of the major factors controlling microbial growth and thereby biosurfactant production. Most of the biosurfactant production studies are conducted in the pH range of 4–10, where pH 7 is the most preferred choice as most of the microbial enzymes are effective at this pH and hence the best-known biosurfactant producers are reported to be neutrophils (Datta et al. 2018; Jimoh and Lin 2020; Schultz and Rosado 2020; Suganthi et al. 2018). Microbes that prefer acidic, neutral and basic environments for their growth are termed as acidophiles (pH < 7), neutrophiles (pH 7) and alkaliphiles (pH > 7), respectively. Culture pH immensely controls the production and bioavailability of biosurfactants in the culture media. Durval et al. observed an acidic change in culture pH from 7.2 to 6.5, with increased release in biosurfactant in the culture media after 48 h of incubation (Durval et al. 2019). On the contrary, Dikit et al. observed an increase in pH from 7 to 7.5 with an increase in the biosurfactant production ability of the bacterium *Marinobacter hydrocarbonoclasticus* ST1 (Dikit et al. 2019). Corresponding to these studies, it was concluded that the biosurfactant production was significantly higher in the pH range of 6–8, and was found to be drastically reduced outside this range (Jimoh and Lin 2019). However, the exception exists in the case of acidophiles and alkaliphiles, where the microbes are well equipped with suitable aids for surviving in such extreme conditions (Datta et al. 2018; Sharma and Pandey 2020). Furthermore, Lingquing et al. suggested that pH aided microbial cell growth of *P. aeruginosa* during the early incubation stage and played a critical role in biosurfactant production in the later stages of growth. It was reported that bacteria tend to internalize the biosurfactant within the cell or on the surface in mild basic or neutral pH, hence in the first 24 h the pH was maintained at 7–7.5, however, post 24 h, rhamnolipid production initiated and pH value was changed to 6–6.5 for the extracellular release of biosurfactant (Zhu et al. 2012). Hence, pH plays an influential role in microbial growth, biosurfactant production as well as its release in the given cultural conditions.

2.2 Temperature

Temperature is another critical parameter that decides the fate of microbial growth and metabolism. Based on the preference of microbial growth over their surrounding temperature, they are classified as psychrophilic (capable of growth at low temperature <20 °C), mesophilic (suitable growth in the range of 25–45 °C), and thermophilic (prefers growth at extreme temperature range >45 °C). Since, biosurfactant is a growth-associated metabolic product, the most preferred temperature for the maximum biosurfactant production is highly driven by the fact that the growth of microbe should be the highest in the range of temperature provided. Most of the biosurfactant producers (*Pseudomonas* sp., *Bacillus* sp., *Paenibacillus* sp.) are found to be mesophilic in nature (Jiang et al. 2019; Jimoh and Lin 2020; Yaraguppi et al.

2020). However, certain thermophilic bacterium such as *Bacillus subtilis* MG495086 was found to be a highly efficient biosurfactant producer in the thermophilic condition, i.e. 62.5 °C temperature and reduced the surface tension to 29.85 mN/m endorsing their suitability in enhanced oil recovery (EOR) applications (Datta et al. 2018). Another *Bacillus* species, *B. licheniformis* also exhibited excellent biosurfactant production and emulsification activity and decreased the surface tension from 72 to 23.8 mN/m at a high temperature of 50 °C (Daryasafar et al. 2016). Similarly, few of the prevailing biosurfactant producers in cold climate includes species of genus *Rhodococcus, Bacillus* and *Pseudomonas* (Cai et al. 2014; Malavenda et al. 2015). These observations endorse temperature also as a crucial factor for the growth of bacterium and thus their ability of biosurfactant production.

2.3 Carbon (C) Source

Carbon act as the basic source of energy for microbial growth and metabolite production. Apart from its vital role in the growth of biomass, C acts as the building block for the synthesis of biosurfactants. Microbes produce biosurfactants by utilizing a broad range of substrates varying from simple sugars, hydrocarbons and few fatty acids as found in used/waste frying oil (Datta et al. 2018,2020; Sharma et al. 2019a, b; Sharma and Pandey 2020). The biosynthesis of biosurfactants requires the production of polar and non-polar moieties for the formation of polar head groups and lipidic non-polar tails (George and Jayachandran 2013). Most of the bacteria utilize simple sugars such as glucose, sucrose as C sources for maximum biosurfactant production owing to their easy metabolism. However, these C sources are not cost-effective for bulk production and are often disregarded for scale-up studies. Hydrocarbons are complex C sources and often cause the production of biosurfactants in the process of their catabolic assimilation. Hydrocarbon-rich waste crude oil and fatty-acid-rich used vegetable oils have gained immense attention for their inherent waste-to-wealth concept (Chen et al. 2018; Jimoh and Lin 2020; Lan et al. 2015; Sharma et al. 2019a). Thus, in contrast to easily assimilating glucose and sucrose as C sources, hydrocarbons and used oils have been classified as slowly assimilating C sources.

Various studies support the suitability of these slowly assimilating C sources for the production of metabolites such as biosurfactant (Cai et al. 2014; Dikit et al. 2019; Nur Asshifa et al. 2017; Omarova et al. 2019; Ramos et al. 1995; Sharma et al. 2018; Sharma et al. 2019b; Xu et al. 2018). The carbon catabolite repression (CCR) phenomenon improvises the ability of microbes to prefer one C source over the other. CCR is a mechanism where less preferred substrate assimilation is restricted due to inhibition of the genes required for its expression in the presence of a more preferred substrate (Magnus et al. 2017). This phenomenon is evident in the case of hydrocarbon-degrading microbes where no biosurfactant production is observed in the presence of polar substrates such as glucose. These polar substrates are more involved in the promotion of biomass growth and were found to be interfering with biosurfactant production (Klekner and Kosaric 1993). On the contrary, few studies support the presence of simple sugars along with such complex assimilating C sources to maintain the balance of required cofactors for driving the biosurfactant production pathway. During a crude oil biodegradation study, it was reported that the presence of assimilating sugar in the form of glucose was found to enhance the overall *A. fabrum* SLAJ731 biodegradation of non-assimilating crude oil by replenishing the nicotinamide adenine dinucleotide (NADH) cofactors required for the catabolism of oil (Sharma et al. 2019b). Hence there is an ambiguity in the choice of simple sugars or complex hydrocarbons as suitable nutrients for the production of biosurfactants.

While looking for a suitable C source for the bulk production of metabolite another factor that needs to be kept in mind is the availability and cost. For instance, the use of waste or spent crude oil or discharged sludge could be suitable low-cost C sources for the biosurfactant yet the challenges such as toxicity and poor microbial assimilation rate diminish its uses as the sole C source. Hence, apart from the choice of C source, their concentration also plays a vital role in their suitability for microbial utilization. Various studies suggest the inhibitory impact of excess concentration of C sources and propose the need to optimize the concentration of substrate prior to use (Datta et al. 2018; Ma et al. 2016; Ohadi et al. 2017; Ravi 2019; Vigneshwaran et al. 2018). Hence, it is appropriate to use a low-cost C source that has a fast assimilation rate and is non-interfering in the biosurfactant production pathway.

2.4 Nitrogen (N) Source

Opting for a suitable N source is a significant factor for biosurfactant production as microbes use N sources as their building units for the synthesis of biosurfactants (particularly peptide fragments of lipopeptides) similar to C sources. Different studies revealed that microbes are capable of utilizing both organic and inorganic nitrogen sources for their metabolism (Sharma et al. 2019a, b; Sharma and Pandey 2020). Major organic N source preferred by microbes for biosurfactant production includes peptone, beef extract, urea and yeast extract (Dikit et al. 2019, 259). Microbes are also found to show a significant N preference towards nitrates over the ammonium ion. This could be due to the complex reduction of nitrate salts to their respective nitrites form and further reduction to ammonia, which later integrates into the glutamine–glutamate pathway (Sharma et al. 2019a). Thus, in comparison to easily assimilated form of ammonium ion as N source, nitrates are preferred as it creates a nitrogen limiting environment due to its slow assimilation rate, leading to the improved biosynthesis of biosurfactant (Ma et al. 2016). Davis and group also revealed the importance of ammonium ion as N source in the cell growth and nitrate form of N source in biosurfactant production (Davis et al. 1999). Additionally, few amino acids are also found to enhance the biosurfactant production ability of microbes. Apart from the source, the concentration of N also plays an important part in biosurfactant production. Lower availability of N source might lead to pre-cell death leading to accumulation of biosurfactant production. On the other hand, excess of N in the media is found to deviate the overall metabolic flux towards cellular growth and division causing an exponential growth of cells and diminishing the production of biosurfactants (Lan et al. 2015). Overall, not only N source plays important role in the production but its concentration also controls the fate of microbial biosurfactant biosynthesis.

2.5 C/N Ratio

Optimum concentrations of carbon to nitrogen in the media aid the overall biosurfactant production. Most studies revealed that a high C to N ratio supports the better biosurfactant yield (Jimoh and Lin 2019; Saimmai et al. 2013; Sharma et al. 2019a). It is suggested that a high C/N ratio leads to restriction in cell growth and promotion of cell metabolism for metabolites production. Joy et al. also observed a similar effect of C/N ratio on biosurfactant production by *Achromobacter* sp. (PS1) using lignocellulosic biomass as a C source and a mixture of sodium nitrate and beef extract as N source. The C/N ratios were varied from 6.2 to 12.5. It was reported that the C/N ratio in the range of 6.2–8.3 increased the rhamnolipid production emphasizing the importance of N limitation on the biosurfactant synthesis. However, when the ratio was further enhanced in the range of 8.3–12.5, a decline in biosurfactant production was observed (Joy et al. 2019a). This was due to poor assimilation of C source in the N limitation (Santos et al. 2016). It indicates that the concentration of N in the media should be lesser than the C, however too low N source also limits the metabolism of C sources and thereby decreases the biosurfactant production. Summarily, the C/N ratio is a highly sensitive parameter, which varies from strain to strain as well as the chemical composition of C and N sources used.

2.6 Other Factors

Even though the key role in biosurfactant production is played by the aforementioned factors, studies also suggest the importance of optimizing other non-essential reaction conditions based on a particular species and/or application. Few such factors are discussed in the sections below.

2.6.1 Salinity Concentration

Salinity plays a vital role in the structural stability of *Halobacterium* and various other marine bacteria that require a high salt concentration of 100–150 g/L for their survival (Oren 2008). The average salinity of seawater despite the geographical region is 3.5% (35 g/L), hence exploration of such halotolerant microbes is required for the oil-spill remediation and MEOR application (Darvishi et al. 2011). Since most of the marine bacteria are well adapted to such salinity, studies have revealed the synergistic effect of salinity on the biosurfactant-based emulsification activity

(Vecino Bello et al. 2012). Yet, beyond the optimized condition, the emulsification activity is compromised with an increase in salt concentration. Hence, the tolerance of bacteria to salinity levels needs to be tested for better results. Deng et al. studied the effect of salt concentration on the biosurfactant production ability of *Achromobacter* sp. A-8 and reported a decrement in emulsification activity for the salt concentration beyond 10 g/L (Deng et al. 2020). Likewise, a moderately halophilic bacterial strain *Bacillus subtilis* MG 495,086 was found to exhibit maximum growth at 6 g/L salinity, where further increase in salt concentration decreased the reduction in surface tension (Datta et al. 2018). Though most of the freshwater and soil bacteria are not tolerant to high salinity concentration, yet the crude biosurfactants extracted from these bacteria are well stable at extreme pH, temperature and salinity and hold a potential role in the in situ remediation and oil recovery studies (Chen et al. 2018; Jiang et al. 2019; Jimoh and Lin 2020; Joy et al. 2019a).

2.6.2 Inoculum Size

Optimized inoculum size also plays an important role in the overall production of biosurfactant. Various studies revealed that with the increase in inoculum size there is an increase in the overall biosurfactant production. However, beyond certain optimum concentration, further increase in microbial concentration can lead to depletion in dissolved oxygen. This occurs due to the overgrowth of microbes which results in increased cellular respiration, leading to a negative effect on biomass growth and metabolite production (Suganthi et al. 2018). Jimoh et al. stated a similar decrease in the biosurfactant production ability of *Paenibacillus* sp. D9 when the inoculum size was increased above 1.5% (v/v) (Jimoh and Lin 2019). Sharma et al. also supported the importance of using a low inoculum size of 2% (v/v) for higher biosurfactant production (Sharma et al. 2018). Overall the inoculum size should be appropriate in a way that it doesn’t cause either substrate feedback inhibition (i.e. higher substrate availability than required), or substrate limitation while incubation due to excessive growth.

2.6.3 Agitation Speed

Agitation speed ensures the proper availability of oxygen and substrate throughout the culture volume to prevent any dead zones. It plays an important part in the case of the immiscible or poorly miscible substrate such as oils and sludge samples as a C source. Since these substrates limit the overall dissolved oxygen transfer rate, optimized agitation is required to make oxygen available for the microbes present in the culture. Usually, fast dividing aerobic cultures require a higher agitation rate than facultative and anaerobic cultures. Studies revealed that a high agitation rate favoured biosurfactant production (Bertrand et al. 2018; Brumano et al. 2017; Fonseca et al. 2007). Yet, where low agitation limits microbial growth due to poor availability of dissolved oxygen, high agitation leads to cell membrane disruption due to high shear force. As observed by Asshifa et al. the oxygen transfer rate in a diesel enriched culture medium was highest in the case of 500 rpm agitation rate as compared to 400 and 600 rpm, leading to the highest biosurfactant production by *P. aeruginosa* USM-AR2. It was stated that volumetric mass transfer coefficient 'k_La', was found to increase from 48.21 (at 400 rpm) to 70.38 h^{-1} (at 500 rpm), however, it further decreased to 65.31 h^{-1} at 600 rpm, leading to a decrease in biomass as well as biosurfactant production. This is due to restriction in the increase of interfacial area 'a', suggesting the limitation in the role of agitation rate in overall oxygen transfer (Nur Asshifa et al. 2017). At a high agitation rate >500 rpm, few *Bacillus* sp. were found to form endospores that also resulted in decreased surfactin productivity (Ha et al. 2018). Another issue with biosurfactant production is the foam formation with increasing agitation, which limits the overall biosurfactant productivity (Shaligram and Singhal 2010). Hence, it is important to analyse the best-suited agitation rate for maximum microbial growth and biosurfactant production.

2.6.4 Incubation Time

Incubation time plays a crucial role in cases of secondary metabolite production. Since the time of the release of biosurfactant varies from early exponential phase to stationary phase, it is always important to understand the relation between the bacterial growth profile and its biosurfactant release profile. As biosurfactant metabolite production is growth associated in a few species and non-growth associated in some, it is not easy to predict its time of extracellular release. Thus, the incubation period plays important role in biosurfactant production as during the late stationary phase, microbes tend to internalize the biosurfactant within the cell, leading to a drop in the biosurfactant production and hence the overall productivity (Durval et al. 2019). Abdulsalam et al. reported a similar trend of increase in biosurfactant production till 144 h which then decreased after 168 h (Abdulsalam et al. 2016). Similarly, a decrease in biosurfactant concentration (up to 0.5 g/L) was observed after 72 h of incubation by *B. subtilis* MG495086, stating that bacterial cells tend to consume the biosurfactant as a secondary carbon source during the late stationary phase (Datta et al. 2018). Such loss of metabolite productivity makes the entire production system forfeited. Hence, it is essential to perform the extraction of the released metabolite from the culture medium at an optimized time interval, i.e. before cells enter the late stationary phase.

Although a number of studies have reported the essential factors that play a crucial role in maximizing biosurfactant production, yet it is also clear that these factors vary from strain to strain. Any suitable combination of these parameters cannot be concluded as best suitable, and hence comes the need of optimization model particular for each strain making the entire production of biosurfactant as time and cost-effective.

3 Optimization Designs

Optimization is a scientific approach to regulate each culture parameter in a way that their integration provides the best output response. Various researchers have critically investigated the importance of media composition and culture reaction conditions for the bulk production of microbial biosurfactant. The most conventional technique employed for optimization of culture condition was “one factor at a time approach” (OFAT). However, this technique involves numerous experiments diluting its overall accuracy as well as making the entire model time-consuming and expensive. Later, with the advent of computational tools in the field of mathematical and statistical analysis, various optimization models have been developed. These models are more accurate, robust and efficient in predicting response and hence are gaining more attention by present research communities. The aim of optimization designing is to understand the impact of each experimental variable on the final output response (Bertrand et al. 2018). Figure 1 depicts the hierarchy of development of optimization model designing, where the pioneer optimization model is OFAT. Later, with a consistent emphasis on the role of the interdisciplinary approach, various mathematical and statistical models have been designed for successful optimization. In the section below, a detailed understanding of the most widely employed model designs targeted for the maximum biosurfactant production has been discussed along with an attempt in understanding the basics of each model stating their pros and cons.

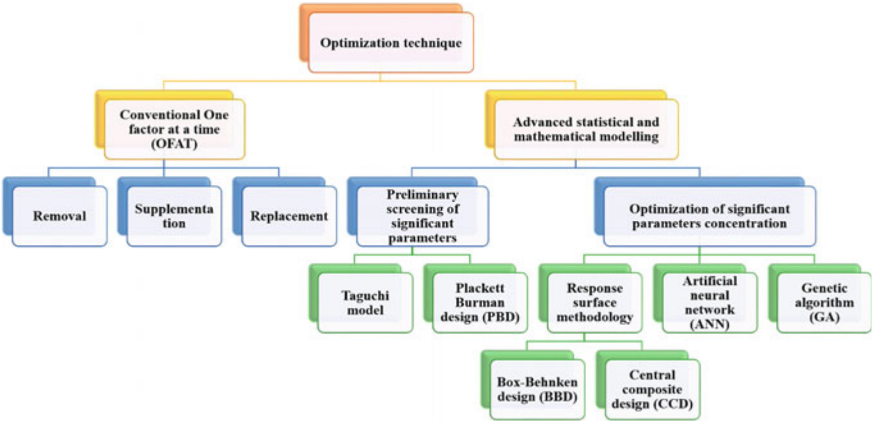

Fig. 1 The schematic representation of various optimization techniques [Adapted with permission from Singh et al. (2017)]

3.1 One-Factor-At-A-Time (OFAT)

OFAT is the most classical optimization technique practiced by researchers for the preliminary optimization of factors, owing to its ease of operation. In the OFAT technique, only one factor is varied in each experimental trial keeping the other factors constant. Since only one variable is explored at a time, there is a need to optimize each factor in a sequential manner. For example, in most cases, initially, pH is optimized followed by temperature, further based on the optimized pH and temperature, the C source and N source are optimized. Various studies support the use of the OFAT model for the optimization of biosurfactant production (Parthipan et al. 2017; Sharma et al. 2019a, b).

The OFAT technique is further performed in three sequential steps: (1) Removal, (2) Supplementation and (3) Replacement technique (Singh et al. 2017). The fundamental of the removal technique is to analyse the effect of each parameter when it is absent from the culture medium. This technique is mostly used to design the composition of a culture medium. Initially, the component of interest is removed from the system and the change is analysed with respect to the control model. Based on this, the essential components are segregated for further optimization (Singh et al. 2008).

In the supplementation technique, the importance of any component (e.g. carbon or nitrogen source) is analysed when it is supplemented to the minimal salt medium (MSM). To date, this technique is widely utilized in optimizing the suitable C and N sources during the media designing experiments. For example, in the basic biosurfactant optimization technique, researchers have confined the major nutrient supplementation beneficial for the biosurfactant production as C and N. Hence, all the techniques optimize these nutrients separately in comparison to other macronutrients (Sharma et al. 2019a). Finally, upon selecting and shortlisting the major factors playing important role in governing the overall product outcome, these factors are varied in a defined range. Like a selected range of pH and temperature, or replacement with low-cost nutrients in the case of C and N sources in optimization studies. Hence this technique is termed ‘replacement’.

OFAT technique has been used as a preliminary optimization technique in order to sort major controlling parameters (Hema et al. 2019; Joy et al. 2019a). The overall experimental trials to be performed are expressed in Eq. 1:

$$\begin{aligned}\text{Total number of trials} = [&\text{Number of factors to be studied} \\ &\times (\text{Number of levels} - 1) + 1]\end{aligned} \tag{1}$$

Overall such expression causes an increase in the number of experimental trials making the process time-consuming, laborious and non-economic. Discouragingly, this technique lacks the study of the interaction between two independent parameters and doesn’t minimize the noise caused due to random experimental errors, hence making the outcomes highly compromised. Another drawback associated with OFAT includes the Domino effect, which concludes that error in one experimental parameter can cause inconclusive optimized results.

In this regard, a statistical approach was proposed by various researchers in the late 1960s as a suitable solution to lacunas of the classical OFAT model. Design of experiment (DOE) is a statistical technique exploring a set of variables at a time and gaining information about their cumulative effects on the response. This technique was more acceptable as it could take into account various controllable and noise factors. Also, based on an advanced mathematical and statistical model, it could gain more insights into the possible relation between various independent parameters in limited experimental trials. When a DOE technique utilizes all the factors and their possible combinations it is termed as 'full factorial designing', on the other hand using only few factors and choosing only significant combinations is termed as 'partial factorial designing'. Due to the interventions of mathematical model fittings, the suggested solutions are more precise and accurate in comparison to OFAT. In l991, Silveria et al. supported the accuracy of the DOE optimization model as it resulted in 1.3 times improved response output than the conventional OFAT model (Silveira et al. 1991).

3.2 Plackett–Burman Design (PBD)

This technique was pioneered in 1946 by two scientists, namely R. L. Plackett and J. P. Burman. PBD model involves 2-level factorial designing where each variable is varied at two levels (+1, −1). In a typical PBD design, only the essential parameters are considered, ignoring the non-essential ones. There are two categories of variables—real and dummy. The real variable is varied to its highest and lowest levels, whereas dummy variables are kept constant throughout the process design. The overall statistical variances of dummy variables are used to estimate the experimental error. The total number of experimental trials in a typical PBD with "n" essential variables is obtained as "n + 1". The contribution of each variable is given by Eq. 2:

$$E_X = 2 \times \frac{\sum Y_{XH} - \sum Y_{XL}}{N} \tag{2}$$

where, $E_{X,}$ $Y_{XH,}$ Y_{XL} and N represent the effect of variable X, the yield of variable X at a high level, the yield of variable X at a low level and the total number of trials performed, respectively. Sunkar et al. utilized the PBD technique to design the culture media for *Bacillus cereu*s HM998898 using gingley oil as a C source. Assuming no interaction within variables, 7 media components (KNO_3, Oil, K_2HPO_4, KH_2PO_4, $MgSO_4 \cdot 5H_2O$, $FeSO_4 \cdot 7H_2O$ and NaCl) were optimized using an 8-run PBD design. The significance of each factor was obtained as expressed in Eq. 2 where the average difference between the high and low levels was considered. In the order of their importance from Pareto plot analysis, 3 variables were obtained, namely (1) KNO_3, (2) K_2HPO_4 and (3) gingley oil, which under optimized condition yielded 11.32 g/L of biosurfactant (Sunkar et al. 2019). Similarly, Yaraguppi et al. also screened the significant culture media components involved in the biosurfactant production by *Bacillus aryabhattai* ZDY2 using crude oil as substrate. Among the 8 variables (yeast extract, $NaNO_3$, KCl, glucose, NaCl, KH_2PO_4, $MgSO_4{\cdot}7H_2O$, and Crude oil), 3 factors were obtained as significant ($p < 0.05$) based on regression analysis namely $NaNO_3$, yeast extract and crude oil. It was reported that among the 3 significant variables, crude oil was most significant with $E_X = 1.485$, followed by yeast extract ($E_X = 1.4$) and $NaNO_3$ ($E_X = 1.01$). It was concluded that *B. aryabhattai* ZDY2 positively used crude oil as the sole C source and both organic and inorganic N source for the maximum biosurfactant production, yielding 5.88 g/L of biosurfactant (Yaraguppi et al. 2020).

Overall this technique immensely decreases the total experimental trials making the design implementation simple, easy, time and cost-effective and also statistically efficient. However, due to the lack of incorporating the interaction within the essential factors, this technique is restricted to the study of parameters that are mutually exclusive and doesn't mask the effect of one another (Biniarz et al. 2018). The technique is mostly used for the screening of essential variables, which can further be optimized using more statistically robust techniques (Câmara et al. 2019).

3.3 *Taguchi Model*

Taguchi model, developed in the late 1950s by Genichi Taguchi, is a robust optimization model that is preferred when the optimizing factors are too many and the involved noise quotient is high. It's a 3-level (+1, 0, −1) statistical modelling, where the interaction between essential variables is explored, unlike PBD. In addition, the noise factors are also incorporated in the design of the experiment. However, the Taguchi model is only efficient in analyzing the main factors, with no interactions. It is used when the target is to obtain the best optimal design that not only is in the range of specification provided but is more centric to the mean of the provided parameters. The advantage includes its ability to incorporate the noise factors while designing the most suitable optimized condition. The noise factor here means the factor which is not predefined or controllable during the culture conditions. In general, the number of experimental trials to be done by Taguchi design can be expressed in Eq. 3:

$$\text{Total experimental trial} = 1 + (\text{Parameters studied}) \times (\text{Levels} - 1) \tag{3}$$

In a typical Taguchi model, an orthogonal array is selected in order to find the minimum number of experiments required to analyse the optimal conditions. The total experiments to be conducted is determined as the suffix to 'L'. For instance, for three parameters with three corresponding levels, L9 orthogonal matrix is chosen.

In a recent study by Raza and co-workers, rhamnolipid biosurfactant production by *Pseudomonas putida* was optimized using waste frying oil as a C source, using Taguchi model. L9 orthogonal matrix was chosen with 9 experimental trials were suggested for the optimization purpose. The 3 major factors studied include C source, fermentation setup and the incubation time. The optimized model enhanced the biosurfactant yield from 3.4 to 4.1 g/L (Raza et al. 2020). Similarly, Haloi and coworkers reduced the effect of uncontrollable parameters on the glycolipid production by *Achromobacter* sp. TMB1 by optimizing 3 parameters with 4 levels each. These parameters included: C source, N source, and inducer concentration using an L16 orthogonal matrix. Taguchi model summarizes the contribution of each parameter studied making it suitable to understand the role of each parameter in the overall biosurfactant contribution. For example in the above study, yeast extract in the form of an inducer played a vital role in maximum biosurfactant production (Haloi and Medhi 2019). With the presence of large experimental factors to be studied, this technique endeavors advantages such as (1) lesser number of experiments, (2) time and cost-effective and (3) highly precise. The only disadvantage with this model is the number of parameters to be studied needs to be significantly higher than the noise factors.

3.4 Response Surface Methodology (RSM)

RSM is an optimization technique that uses mathematical regression analysis such as ANOVA along with statistical experimental design for the formulation of optimized conditions in the presence of a set of inter-dependable parameters/factors. This design focuses on obtaining the best outcome using finite experimental runs reducing noise factors. The unique feature of RSM includes its surface-plot-based representation of output results. The response obtained is expressed in terms of a simple, mathematical expression with associated regression analysis. One such general quadratic response expression is mentioned as Eq. 4:

$$Y(X) = A_0 + \sum_{i=0}^{N} A_i X_i + \sum_{i<j}^{N} A_{ij} X_i X_j + \sum_{i=0}^{N} A_{ii} X_i^2 \tag{4}$$

Here, Y(X) stands for the predicted response, A_i X_i represents the linear expression as the effect of each variable on response factor, A_{ij} X_i X_j denotes the interaction between independent variables and their role in response output and A_{ii} $X_i{}^2$ represents the square terms.

RSM provides a quantitative analysis of each factor on the response output. In RSM, we can study the effect of 2 or more process parameters within a range and predict the optimized yield which doesn't necessarily be one of the experimental trial conditions performed. In the case of biosurfactant production, RSM uses optimization designs such as Central Composite Design (CCD) or Box–Behnken Design (BBD) for the formulation of a statistically valid optimization model (Fig. 2).

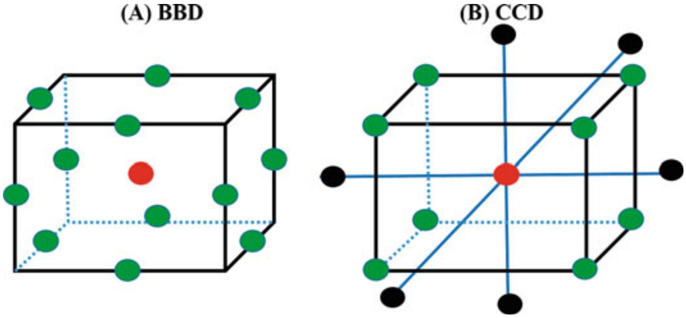

Fig. 2 RSM optimization designs **a** BBD and **b** CCD

3.4.1 Box–Behnken Design (BBD)

In this model, each factor is studied at 3 levels, two factorial points and one centric point. Centric point is often replicated for error evaluation. In this regard, El-Housseiny *et al.* explored the effect of pH, temperature and inoculum size on rhamnolipid production. Each factor was studied at 3 levels. Upon using the RSM-BBD model, the optimized conditions were obtained as pH 8, 30 °C with the inoculum size of 1% reporting a remarkable improvement of rhamnolipid yield (El-Housseiny et al. 2019). RSM techniques are unique for their ability to deduce the correlation within the variables and their integrated effects on the overall output response. In this regard, Ghazala et al. explored 4 variables (glucose, glutamic acid, temperature and salinity) for the evaluation of their impact on the biosurfactant synthesis by *Bacillus mojavensis* I4. A set of 29 runs were performed to analyse the possible reaction among the 4 variables each varied at 3 levels (+1, 0, −1). RSM-BBD analysis provided a second-order polynomial equation expressing the biosurfactant yield in terms of all 4 variables and their interaction. It was observed that apart from salinity, all the other three factors positively affected the biosurfactant yield. Among them, the coefficient of glucose and glutamic acid were 0.49, whereas the coefficient for temperature was 0.16, describing the importance of C and N sources on the overall production of biosurfactants. However, salinity inversely affected the overall productivity. The model proposed the optimal biosurfactant yield of 4.12 g/L at 3% of glucose concentration, 0.6% of glutamic acid concentration, 35 °C of temperature and 10 g/L of salinity (Ghazala et al. 2019).

Similarly, Jimoh et al. observed the interrelation between C source, N source and trace elements on the overall biosurfactant production ability of *Paenibacillus* sp. D9. Waste canola oil was used as a C source and KNO_3 as an N source. BBD design expressed the significant integrated effect of N source and metal supplementation on the overall biosurfactant yield. The optimized concentrations for all three variables were obtained as 5, 2 and 1% for C, N and metal supplementation, respectively, yielding 5.31 g/L of biosurfactant concentration. The model suggested the constitutive effect of high canola oil concentration along with N-trace element interaction, positively driving the overall production of biosurfactants. On the other hand, C-trace elements concentration and C-N interaction negatively repressed the overall yield of biosurfactants (Jimoh and Lin 2020).

Hence, the BBD technique statistically expresses the importance of the significant role played by individual factors along with their inhibitory or inducing effect on other variables. Such insights are helpful in examining the media composition and other physical parameters, in a finite number of runs, making the entire design statistically relevant and time and cost-effective. However, the BBD model is highly sensitive to mismeasure of experimental runs, or missing data points as the number of runs are highly restricted. Hence, the entire model is compromised with loss or wrong data points.

3.4.2 Central Composite Design (CCD)

Among all the RSM designs, CCD is the most employed design. It utilizes second-order model fitting equation for the analysis of each variable on output and on each other. RSM-CCD factorial design is a more advanced tool for optimization study, where each variable is explored at 5-levels. There are two factorial design points, two axial points and one centric point as shown in Fig. 3. Studying each variable at 5 levels ensures that losing data or mismeasurement won't affect the entire model design, unlike BBD, making the entire design highly robust. A typical experiment runs in the CCD model can be expressed as Eq. 5:

$$\text{Number of experimental runs} = 2^{\mathrm{N}} + 2\mathrm{N} + \mathrm{C_N} \tag{5}$$

where, N is the number of independent variables, and $\mathrm{C_N}$ represents the total number of replica tests performed for the centric point (Cornell and Khuri 1987; Sahoo and Barman 2012). In the case of the experimental design for the CCD and BBD model for three independent variables. The number of runs in the BBD model is 15, which increases to 20 in the case of CCD optimization. This is due to the analysis of points beyond the maxima and minima ranges.

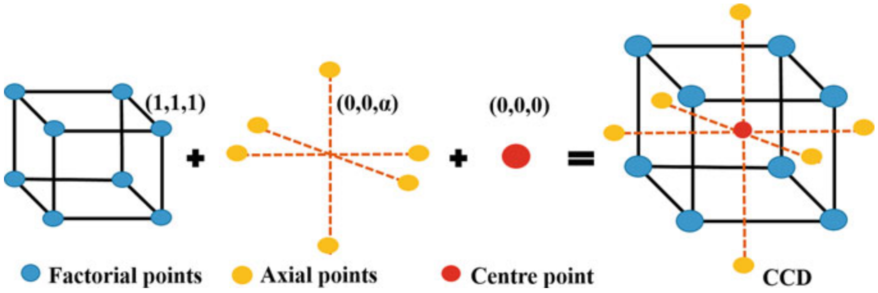

Fig. 3 5-level factorial design in CCD optimization technique

In a biosurfactant production study, 3 variables (pH, Temperature and C source) were optimized using the RSM-CCD technique using 20 sets of equations. A quadratic equation was obtained determining the constitutive effects of each variable and their interactions on the response factor. The optimal biosurfactant concentration was obtained as 6.3 g/L in the presence of 3.89% paraffin oil as a C source, pH 7.73 and 62.5 °C incubation temperature (Datta et al. 2018). Similarly, Khademolhosseini et al. analysed the biosurfactant production ability of *Pseudomonas aeruginosa* HAK01 using 5-level factorial designing considering C source concentration, inoculum size and salinity as 3 variables. The optimization experiments revealed the sensitivity of strain to high salt concentration, yielding 2.07 g/L of biosurfactant in the presence of 22.9 g/L sunflower oil as C source, 2.77% (v/v) inoculum size and a very low salt concentration of 0.19% (w/v) (Khademolhosseini et al. 2019).

Another study utilized the 5-level factorial designing for the optimization of biosurfactant production using *Pseudomonas aeruginosa* UCP 0992 in the presence of corn steep liquor and vegetable oil residue, as low-cost C and N substrate, keeping four reaction parameters in consideration: agitation speed, incubation time, aeration, and inoculum size. It was reported that using optimal conditions of 1 vvm aeration rate, 225 rpm agitation rate, 3% inoculum size and 120 h of incubation a remarkably high biosurfactant yield of 26 g/L was achieved (Silva et al. 2018). Even though CCD optimization techniques yield the most suitable optimized condition, yet its numerous experimental runs make the entire process time-consuming and laborious.

Apart from all the mentioned advantages, there exist associated disadvantages of RSM. To list a few, RSM uses second-order polynomial order for response analyses which loses its precision with the increase in variable counts and their levels and hence is restricted to the prediction of low levels of parameters. In a biological system, like bioreactors, a lot of complex reaction with unknown kinetics decreases the reproducibility of predicted response. In such cases, an integration of two or more models is performed to screen out significant variables from the rest, decreasing the strain on further optimization models. For instance, Biniarz *et al.* exploited the PBD optimization technique for screening of parameters essential for biosurfactant production followed by further optimization using the CCD technique. Primarily, the media formulation was done by studying 11 different media constituents. This was followed by the analysis of media additives, which was performed using CCD. The three significant variables, namely glycerol, tryptone and leucine were chosen as major elements in media formulation for the maximum biosurfactant by *Pseudomonas fluorescens* BD5, yielding 610.4 ± 5.9 mg/L of biosurfactant. These 3 factors were further optimized using the CCD technique resulting in a twofold increase in biosurfactant yield of 1187 ± 13.0 mg/L. Hence, there was an enhanced biosurfactant production upon using an integrated optimization model (Biniarz et al. 2018).

Joy et al. also explored the advantage of CCD after the OFAT optimization study for biosurfactant production using *Achromobacter* sp. (PS1). Initially, the lignocellulose hydrolysate streams comprising C_5 and C_6 residues were screened using the OFAT technique as C source, and the optimal biosurfactant concentration was obtained as 3.3 g/L, however, the authors further optimized the concentration of C source (C_6 hydrolysate) and other 5 factors ($NaNO_3$, yeast extract, $FeSO_4$, phosphate and agitation rate) using the CCD technique. The CCD optimization further improved the overall rhamnolipid yield to 5.46 g/L (Joy et al. 2019b). Summarily, the RSM model has been extensively explored for maximizing metabolite production and optimization studies. Integration of optimization techniques has been proven to be capable of improving the biosurfactant yield by many folds and should be further explored for more robust model development. Yet, its inability to unwind complex biological interactions which are not linearly dependent has caused a lacuna in its suitability for living systems and has opened doors for further research in this domain.

3.5 Artificial Neural Network and Genetic Algorithm

To combat with optimization of the complex biological system, a more precise model has been recently introduced known as Artificial Neural Network (ANN). ANN represents a highly adaptable model which is trained for making changes to internal factors with change in the external environment. It's a computational model, which is modelled as a black box, where no prior information or knowledge is required to be incorporated by the user. In each ANN model, there are 3 levels, (1) input layer; (2) hidden layer; and (3) output layer. The ANN programming has two main domain, (a) supervised, where the user train or calibrate the program by providing asset of known inputs and its expected outputs; (b) unsupervised, where the system studies the pattern in clusters of inputs and decides the possible output (Singh et al. 2017).

Similar to ANN, Genetic Algorithm (GA) works on the principle of Darwin's "survival of the fittest" model. It is programmed with a biological phenomenon such as crossing over and mutation. There are 3 steps in which a typical GA works: (1) selection, where the GA studies the set of inputs provided and decides the best parents for next-generation prediction based on the selection process; (2) Cross over, where the possible combination of parents is explored for next-generation children predictions; (3) Mutations, here random changes are made to individual parents and thus changes in the next generation children is analysed. Taking care of all such natural biological phenomenon, GA is capable of analyzing a large set of biological data with precision (Singh et al. 2017). Pal et al. explored the effectivity of ANN-GA-based optimization technique over statistical optimization models, for the biosurfactant production ability of *Rhodococcus erythropolis* MTCC 2794. The authors explored the importance of yeast extract and meat peptone as N sources and toluene and sucrose as C sources in biosurfactant production. ANN-GA technique showed a remarkably low average error of 3% with a correlation coefficient of 0.99%, whereas ~ 6% error was reported by a statistical optimization technique. In this regard, ANN-GA also reported 3.5 folds enhanced biosurfactant yield (7.2 g/L) in comparison to RSM-based optimization (2.05 g/L). The incorporation of sensitivity analysis in the ANN-GA model has proved to be the key component in providing a more accurate prediction capacity owing to higher yield (Pal et al. 2009).

Similarly, Sivapathasekaran et al. also showed a 70% enhancement in biosurfactant production by *Bacillus circulans* MTCC 8281, by optimizing 4 independent parameters (glucose, urea, $SrCl_2$ and $MgSO_4$). In comparison to statistical CCD optimization where the optimal concentration of biosurfactant was found to be 2.95 g/L, ANN-GA reported an enhanced yield of 4.35 g/L (Sivapathasekaran et al. 2010). The same group of researchers also explored the applicability of ANN-GA over CCD in a bioreactor study using the bacterium *Bacillus circulans* MTCC 8281. In this study, the influences of pH, temperature, agitation and aeration rate were explored for biosurfactant productivity. A 52% enhanced productivity was reported after the ANN-GA optimization model (6.98 g/L) than CCD-based optimization (4.61 g/L) (Sivapathasekaran et al. 2010).

The major advantage of this computational technique is its efficacy in handling a large volume of information, complex pathways and data with no prior mechanistic information provided. In addition, ANN-GA works effectively with parameters that are not linearly linked with each other and failure of any unit doesn't affect the overall output of the process due to in-built parallel programming. Yet, the system needs highly skilled user and quality data for effective analyses (Bertrand et al. 2018).

4 Bottlenecks of Optimization

Table 1 reports various optimization models explored for maximizing the overall biosurfactant production ability of different bacterial species. In contrast to tremendous improvement in biosurfactant productivity using various optimization techniques, yet individual optimization techniques possess certain limitations. The optimization techniques involve a lot of experimental trials, which are time-consuming, labour-intensive and expensive. It is also evident that the results of every optimization reported in the literature are uniquely restricted to the strain, media and physiological parameters used, and hence cannot be generalized. Further, in most cases, the shake flask results don't fit in bioreactor-based experiments. It is due to a number of uncontrollable parameters in shake flask studies that if controlled in reactor studies can provide exceptionally different results. Hence, the optimization results vary at different scale studies.

Since biological species consist of complex metabolic pathways which may undergo mutation based on the external change in the environment leading to underexpression or overexpression of output yield. It is suggested to look into mutagenic species as well as control to understand the insights of external optimization on the internal metabolic activity of biological species. Another factor is the restriction of optimization techniques to liquid-based medium, there is scarcity in the availability of information regarding the optimization model for solid-state or semi solid-state models. Furthermore, since most of the optimization models are aimed at increasing the overall productivity, which deviates each time with the change in any media component.

Table 1 List of optimization models used for maximizing biosurfactant production by various bacterial species, in the presence of mentioned reaction parameters

S. no.	Optimization model used	Substrate	Bacteria studied	Reaction parameters	Biosurfactant concentration	References
1	OFAT	Oily sludge	*Shewanalla chilikensis*, *Bacillus firmus* and *Halomonas hamiltonii*	pH 7.0, 35 °C, 1% oily sludge as substrate 15% inoculum size, 7 days incubation time	152 mg/g	Suganthi et al. (2018)
2	OFAT	Kitchen waste oil	*Pseudomonas aeruginosa*	pH 8.0, 35 °C, 2.0 g/L yeast extract concentration,10% (v/v) inoculum size	NA	Chen et al. (2018)
3	OFAT	*Parthenium hysterophorus* biomass	*Pseudomonas mosselii* F 01	pH 6.0, 35 °C, 3% (w/v) biomass substrate, 96 h incubation time glucose and yeast extract as C and N source	1.94 g/L	Devaraj et al. (2019)
4	OFAT	Molasses	*Marinobacter hydrocarbonoclasticus* ST1	pH 7.0, 30 °C, 5% (v/v) inoculum size, 2.5% (w/v) molasses and 0.3%(w/v) sodium nitrate, 72 h incubation time	6.25 g/L	Dikit et al. (2019)
5	OFAT	Waste frying oil	*Bacillus cereus* BCS0	2% (w/v) frying oil and 0.12% (w/v) peptone, 250 rpm and 48 h	3.5 g/L	Durval et al. (2019)

(continued)

Table 1 (continued)

S. no.	Optimization model used	Substrate	Bacteria studied	Reaction parameters	Biosurfactant concentration	References
6	OFAT	Rapeseed oil	*Pseudomonas aeruginosa* KT1115	pH 7.5, 37 °C, 1% (v/v) inoculum size, 180 rpm and 8 days incubation time, 60 g/L rapeseed oil, 6 g/L $NaNO_3$, 3 g/L yeast extract, 1 g/L KH_2PO_4, 1 g/L Na_2HPO_4, 0.1 g/L $CaCl_2 \cdot 2H_2O$, 0.1 g/L $MgSO_4$	44.39 g/L	Jiang et al. (2019)
7	OFAT	Diesel fuel	*Paenibacillus* sp. D9	pH 7.0, 30 °C, C/N (ammonium sulfate) ratio of 3:1, 4.0 mM $MgSO_4$, and 1.5% (v/v) inoculum size	4.11 g/L	Jimoh and Lin (2019)
8	OFAT	Dextrose	*Achromobacter* sp. (PS1)	pH 7.0, 30 °C, 120 rpm, C/N ratio 8.3 using sodium nitrate and beef extract	4.13 g/L	De Meester et al. (2016)
9	PBD	Glycerol	*Pseudomonas aeruginosa*	pH 7.37, 30.17 °C, C/N ratio of 32.35, 9.36% (v/v) Glycerol concentration of and 10.26 days incubation time	0.88 mg/L	Câmara et al. (2019)

(continued)

Table 1 (continued)

S. no.	Optimization model used	Substrate	Bacteria studied	Reaction parameters	Biosurfactant concentration	References
10	PBD	Gingley oil	*Bacillus cereus* HM998898	KNO_3 (1 g/L), Gingley oil (2 mL), K_2HPO_4 (2.5 g/L), KH_2PO_4 (0.75 g/L), $MgSO_4 \cdot 5H_2O$ (0.5 g/L),$FeSO_4.7H_2O$ (0.005 g/L) and NaCl (0.025 g/L)	11.32 g/L	Sunkar et al. (2019)
11	RSM-BBD	Waste canola oil	*Paenibacillus* sp. D9	pH 7.0, 30 °C, waste canola oil (2% v/v), 2 g/L KNO_3, and 48 h incubation time	5.31 g/L	Jimoh and Lin (2020)
12	RSM-BBD	Glucose	*Bacillus subtilis* MJ01	1.49 g/L Yeast extract; 7.62 g/L KH_2 PO_4; 33.68 g/L K_2HPO_4; 11.9 g/L Glucose	1.14 g/L	Veshareh et al. (2019)
13	RSM-BBD	Glucose	*Bacillus mojavensis* I4	35 °C, 3% (w/v) glucose, 0.6% (w/v) of glutamic acid, 10 g/L Salinity	4.12 g/L	Ghazala et al. (2019)
14	RSM-CCD	Whey and vinasse	*Lactococcus lactis* CECT-4434	15% (w/v) whey, 3% (w/v) vinasse, 1% (w/v) sucrose, 1.5% (w/v) yeast extract	0.11 g/L	Vera et al. (2018)
15	RSM-CCD	Glucose	*Bacillus brevis*	pH 8.0, 33 °C, 10 days incubation time and 8.5 g/L glucose	NA	Mouafi et al. (2016)

(continued)

Table 1 (continued)

S. no.	Optimization model used	Substrate	Bacteria studied	Reaction parameters	Biosurfactant concentration	References
16	RSM-CCRD	Vegetable oil	*Pseudomonas aeruginosa* UCP 0992	0.5% (w/v) corn steep liquor, 4.0% (w/v) vegetable oil, 1.0 vvm aeration rate, 3.0% (v/v) inoculum size, 225 rpm and 120 h incubation time	26 g/L	Silva et al. (2018)
17	RSM-CCD	Light Paraffin oil	*Bacillus subtilis* MG495086	pH 7.7, 62.4 °C, 96 h and 3.8% (v/v) of light-paraffin oil	6.3 g/L	Datta et al. (2018)
18	RSM-CCRD	Glucose	*Bacillus subtilis* BR-15	pH 7.0, 37.5 °C and 72 h incubation time, 2.17% (w/v) glucose, 0.5% (w/v) yeast extract and 4% (v/v) inoculum	1.72 g/L	Sharma et al. (2020)
19	RSM-CCRD	Glycerol	*Bacillus amyloliquefaciens* SAS-1	pH 7.0, 37.5 °C, 4% (v/v) inoculum size, 72 h incubation time, 5% (v/v) glycerol, and 0.5% (w/v) Yeast extract	2.40 g/L	Sharma et al. (2020)
20	RSM-CCD	Poultry slaughter greasy effluent	*Pseudomonas aeruginosa* ATCC 10145	30 °C, 48 h incubation time, 1.2 vvm aeration, 600 rpm, and 1.0 g/L inoculum concentration	5.37 g/L	Borges et al. (2015)

(continued)

Table 1 (continued)

S. no.	Optimization model used	Substrate	Bacteria studied	Reaction parameters	Biosurfactant concentration	References
21	RSM-CCD	Sunflower oil	*Pseudomonas aeruginosa* HAK01	144 h incubation time, 25 g/L of C, 4% (v/v) inoculum size, 2.5% (w/v) salinity	2.07 g/L	Khademolhosseini et al. (2019)
22	RSM-CCD	Glucose	*Bacillus amyloliquefaciens* IT-45	pH 6.8, 30 °C, 48 h incubation time, agitation rate 200 rpm, 25 g/L glucose syrup, 15 g/L yeast extract, and 2 g/L calcium chloride	5.5 g/L	Lima et al. (2020)
23	RSM-CCD	Brewery waste	*Bacillus subtilis* N3-1P	pH 6.41, 27 °C, 7% (v/v) brewery waste, 6.22 mg/L ammonium nitrate, agitation 150 rpm	0.66 g/L	Moshtagh et al. (2019)
24	OFAT -RSM-CCD	Industrial rice-straw hydrolysate	*Achromobacter* sp. (PS1)	30 °C, 8 days incubation time, Total sugars 40 g/L, sodium nitrate 6.0 (g/L), yeast extract 2.0 (g/L), ferrous sulphate 0.2 (mg/L), phosphate 1000 mM, and agitation 100 rpm	5.46 g/L	Joy et al. (2019b)

(continued)

Table 1 (continued)

S. no.	Optimization model used	Substrate	Bacteria studied	Reaction parameters	Biosurfactant concentration	References
25	OFAT-RSM	Glucose	*Planococcus* sp. MMD26	pH 7.0, 48 h incubation time,2.5% (w/v) salt concentration, 4% (w/v) glucose, and 1% (w/v) ammonium nitrate	NA	Hema et al. (2019)
26	PBD- RSM-CCD	Glycerol	*Pseudomonas fluorescens* BD5	20–100 g/L glycerol, 15 g/L tryptone, 10 g/L Leu/Val, 0.5 g/L K_2HPO_4, 0.1 g/L $MgSO_4$,50 mg/L $Fe_2(SO_4)_3$, 100 mM MOPS	1.19 g/L	Biniarz et al. (2018)
27	PBD-RSM-CCD	Crude oil	*Bacillus aryabhattai* ZDY2	pH 7.0, 35 °C, 4 days incubation time, 200 rpm, 4.0% (v/v) crude oil, 0.7% (w/v) yeast extract and 3% (w/v) $NaNO_3$	8.86 g/L	Yaraguppi et al. (2020)

5 Conclusion

Media optimization is the best way to improve the yield of the overall system. Since, in the output, the major role is played by the media composition and culture conditions, most of the studies are inclined to optimizing these parameters for better results. In this chapter, we have taken a significant insight into each of these parameters and their role in overall output production. We have explored various optimization designs, their advantages and shortcomings, and explored their stability using regression and statistical analyses. However, there are a few information that are still not explored and need to be looked up. Overall this chapter provides a rationale regarding the importance of various factors involved, their optimization designs and techniques in order to improve the bulk production of metabolites.

One of the major factors that are less estimated during the overall optimization process is cost prediction. Since the once optimized condition cannot be compromised, what could be regulated is the substrate replacement with low cost or waste materials. The use of waste material will not only improve the economy but also the eco-friendliness of the overall process. Another aspect that is scarcely discussed is the role of strain improvement in the overall media optimization studies. Though various mutagenic strains are available in the market for the mass production of interested metabolite yet they are highly sensitive to their media conditions and formulation and hence demand high maintenance cost, dipping down the overall profit quotient of the system. In spite of this, one can explore and isolate new microbes already chosen by nature for this job, which not only have high productivity for the desired metabolite but also natural immunity to ecological fluctuations.

In addition to these, few other factors that could be looked up include new interventions such as the use of immobilized microbes for mass production, as it saves the overall cost. Likewise, the use of a fed-batch or chemostat model could limit the wastage of unused substrate that otherwise can repress the overall metabolic productivity of the microbe due to feedback inhibitions. Moreover, for metabolites such as biosurfactants whose complete metabolic pathway is yet unknown, it is suggested to explore deeper insights into the possible pathways while designing the media. This will ensure that there is no limitation to the availability of cofactors and substrates that restrict the pathway of its production.

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Design of Consortium for the Production of Desired Metabolites

Swati Sharma, Pankaj Tiwari, and Lalit Pandey

1 Introduction

Microbes ubiquitously exist in nature and are continuously involved in various ecological interactions and aid each other in prevailing bio-geochemical cycles. The diversity in their functional and metabolic capabilities is responsible for the continuous cycling of various complex nutrients preventing their hazardous accumulation (Che and Men 2019). There is no place in nature, where a single microbial species could exist. The occurrence of multiple species of various genus deciphers the co-inhabitance phenomenon, chosen by nature for their successful survival. Such co-inhabiting also leads to a division of labor and harbor resilience and resistance against environmental changes. The role of a special class of microbes in the degradation of hydrocarbons and remediation of hazardous wastes are well foreseen in nature (Datta et al. 2018; Liu et al. 2016; Sharma and Pandey 2020). In fact, isolation of potential bio-degraders of various petroleum and heavy metal-based hazardous wastes has occurred primarily from the sites where the contamination occurred, deciphering the role of nature in selectively employing specific species as biological degraders in the extreme/contaminated niches (Akbari et al. 2020; Datta et al. 2018, 2020; Miyazawa et al. 2020; Ohadi et al. 2017; Ramos et al. 1995; Tuleva et al. 2009; Verma et al. 2020; Veshareh et al. 2019).

Various microbes have been actively participating as bio-remediating agents due to their native exposure and hence acclimatization to such persistent and recalcitrant compounds. Microbes such as various fungi, yeast and algae have shown promising biodegradability towards various precarious compounds, however, bacteria act as the most active primary bio-remediating agent (Speight 2018; Ummalyma et al. 2018).

S. Sharma · P. Tiwari · L. Pandey (✉)
Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati,
Guwahati 781039, Assam, India
e-mail: lalitpandey@iitg.ac.in

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These bacteria are rich in catalytic and degrading enzymes responsible for the degradation of complex hydrocarbons or other toxic contaminants. Apart from biodegradation ability, another class of microbes is known for producing biosurfactant, an effective tool for improving the bioavailability of hazardous wastes. Few studies also suggest the significance of non-biosurfactant producers and biodegraders in overall bioremediation. Hence, every contaminated site is found to be crammed with microbes with a diverse mode of operations, working synchronously in achieving the maximum biodegradation. This chapter discusses the importance of such diverse bacteria involved in the bioremediation of various persistent compounds focusing on their activity in consortium versus pure forms.

1.1 Biosurfactant-Producing Microbes

Various poly-aromatic and aliphatic hydrocarbons contribute to a large part of existing crude oil in extreme reservoir conditions or recalcitrant contaminants (Sharma et al. 2019b). They possess limited solubility and dissolution rate, which is the major reason for their persistency in the ecosystem. This is due to their non-polar nature and inherent hydrophobicity that makes them poorly bioavailable for the microbes to act on them. Biosurfactants are amphipathic molecules released by microbes to pseudo-solubilize oils and hydrocarbons (Datta et al. 2020; Santos et al. 2016). These chemically active agents either directly modify the hydrocarbons by emulsifying them, or solubilize them using micellar structure formation. Unlike chemical surfactants, biosurfactants are efficient in lowering the surface tension of the hydrocarbon–water interface at a very low concentration known as critical micelle concentration (CMC). In addition, biosurfactants are non-toxic, biodegradable and comparatively more stable in a wide range of environmental conditions (Santos et al. 2016).

Biosurfactants are primarily classified as low molecular weight (glycolipids, and phospholipids) and high molecular weight (lipopeptides and complex polymeric compounds) based on their structural composition. Glycolipids can be further categorized as Rhamnolipid, Sophorolipids, Trehalose lipids and Mannosylerythritol lipids (MEL). These types of biosurfactants are majorly produced by *P. aeruginosa, P. alcaligenes, P. desmolyticum, R. erythropolis, Arthrobacter, Mycobacterium, Nocardia, R. ruber, R. opacus* and *Micrococcus luteus* (Christova et al. 2013; Gein et al. 2011; Jadhav et al. 2011; Muthusamy et al. 2008; Niescher et al. 2006; Oliveira et al. 2009; Tuleva et al. 2009; Zaragoza et al. 2013). These glycolipids have proven to improve the bioremediation of hydrocarbons by mobilization and solubilization of otherwise persistent hydrocarbons. Similarly, lipopeptides have also been subdivided into Surfactins, Iturins, Amphisin, Fengycins, Viscosin, Subtilisin, Polymyxins and Putisolvin. The major producers of these lipopeptide biosurfactants are *Bacillus* spp., *Serratia* spp., *Acinetobacter* spp., *Agrobacterium* spp. *Streptomyces* spp., *Halomonas* spp., *Marinobacter* spp. and a few *Pseudomonas* spp. (Chang et al. 2011; Coutte et al. 2017; Datta et al. 2018; Sharma et al. 2019a; Sharma and

Pandey 2020; Sharma et al. 2019b; Verma et al. 2020). Among various lipopeptides, Surfactin has been found to be highly potent in the emulsification of hydrocarbons.

Biosurfactants act on poorly bioavailable contaminants by either mobilizing, solubilizing or emulsifying them based on their chemical composition (Fig. 1). In a typical mobilization phenomenon, biosurfactants present in concentration below their CMC, reduces the capillary force between oil and soil, reducing the interfacial tension between soil-oil improving its mobility for bacterial action (Lin et al. 2017; Ma et al. 2018). Gradually, when the concentration of biosurfactant raises above CMC, there is the formation of micellar structures entrapping the non-polar entity within the core, thus solubilizing it. A similar observation was made by Li et al. while exploring the solubility of pyrene, naphthalene and phenanthrene in the presence of rhamnolipid biosurfactant released by *Bacillus* Lz-2. With the increase in the biosurfactant concentration above CMC, a linear increase in the solubility of these compounds was reported (Li et al. 2015). High molecular weight biosurfactants are more advanced in forming a highly stable emulsion of non-polar and polar compounds, e.g. oil-in-water emulsion. This characteristic feature is essential in oil washing and oil recovery operation in the petroleum industries. Datta et al. reported the applicability of surfactin biosurfactant produced by *Bacillus* spp. in microbial enhanced oil recovery (MEOR) operation and oil washing, owing to its remarkable emulsification activity of >65% (Datta et al. 2018, 2020). Apart from this, biosurfactants are also involved in modifying the cell surface hydrophobicity of microbial cell membrane, enhancing its affinity to non-polar substrates (e.g. complex hydrocarbons) (Kaczorek et al. 2018; Zhong et al. 2015).

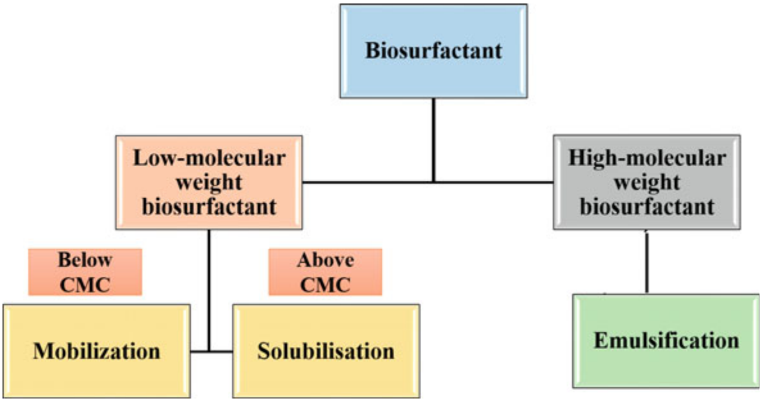


Fig. 1 Biosurfactant classification and their mode of action

1.2 Hydrocarbon-Degrading Microbes

Apart from biosurfactant production, different non-biosurfactant-producing bacterial strains have also been explored for their remarkable role in the biodegradation of hydrocarbons. Various studies have explored and identified the presence of hydrocarbon-degrading microbes in the formation water, core and oil-spilled surroundings (Allamin et al. 2020; Peng et al. 2008; Ramos et al. 1995; Sierra-Garcia and de Oliveira 2013; Suyama et al. 1996; Varjani 2017). A rare-to-dominant change in the bacterial community is often evidenced at the site of contamination. Yakimov et al. reported a similar phenomenon in the sudden rise in the count of few obligate hydrocarbonoclastic bacteria, namely (1) *Oleispira* sp., (2) *Alcanivorax* sp., (3) *Cycloclasticus* sp., (4) *Marinobacter* sp. and (5) *Thallassolituus* sp., from undetectable, explaining their significance in the biodegradation and bioremediation (Yakimov et al. 2007). The abundance of such microbes varies from the kind of sites and other physiological factors. These microbes are enriched with degradative enzymes involved in the uptake and catabolism of the complex hydrocarbons and other hazardous substrates as their source of C and energy demands. The most employed degradation mechanism is an oxidation reaction. Few classes of oxidoreductases like alkane hydroxylases, cytochrome P450, alkane monooxygenases, methane monooxygenases and alcohol dehydrogenases play a crucial role in the biodegradation of such hydrocarbons.

These microbial enzymes are substrate-specific and capable of catabolizing a specific class of hydrocarbons. Aliphatic hydrocarbons are saturated hydrocarbon units that are preferably oxidized by alkane hydroxylases and oxygenases, and found to be actively biodegraded by *Dietzia* sp., *Pseudomonas* sp., *Oleispira* sp., *Rhodococcus* sp, *Geobacillus* sp., *Alcanivorax* sp. and *Gordonia* sp. (Waikhom et al. 2020). The aliphatic hydrocarbon of chain length C1-C4 are easily oxidized by methane mono-oxygenases, C5-C16 are oxidized by cytochrome P450, further higher chain length aliphatic hydrocarbons are catalyzed by alkane hydroxylase catabolic enzymes. Among these *Rhodococcus* sp., *Geobacillus* sp., *Alcanivorax* sp. and *Gordonia* sp. were also reported to be involved in cyclic aliphatic biodegradation (Delacuvellerie et al. 2019; Kim et al. 2019; Laczi et al. 2015; Yusoff et al. 2020).

Likewise, the aromatic hydrocarbons are effectively catabolized by microbial enzymes such as catechol dioxygenases, and benzoyl-CoA reductase. Microbes such as *Achromobacter* sp., *Aeribacillus* sp., *Mycobacterium* sp., *Pseudomonas* sp., *Cycloclasticus* sp., *Bacillus* sp., *Sphingomonas* sp., *Novosphingobium* sp. are reported to be enriched with polyaromatic hydrocarbon (PAH) catabolizing enzymes for effective biodegradation (Fida et al. 2017; Kwak et al. 2016; Messina et al. 2016; Nzila et al. 2018; Tao et al. 2020). Enzymes such as catalases and lipases also have been reported to be involved in the catabolism of various hydrocarbons. Lipases are known to catalyze the hydrolysis of non-polar oils and other hydrocarbons and catalases are involved in the alleviation of heavy metals induced oxidative stress by scavenging hydrogen peroxidase (Suganthi et al. 2018). Furthermore, in order to catabolize comparatively more complex hydrocarbons such as resins and asphaltenes, few microbiological studies have revealed the presence of specific enzymes in *Pseudomonas* sp., *Bacillus* sp., *Citrobacter* sp., *Enterobacter* sp., *Staphylococcus* sp., *Lysinibacillus* sp. and *Micrococcus* sp. (Ali et al. 2012). The main aim of these hydrocarbon-degrading strains is to bio-transform toxic hydrocarbons into the easily accessible substrate to be used by microbes. To date, decades of research have put forward millions of microbial species involved in the bioremediation of different contaminants, however, the problem still persists. No single microbial species can biodegrade a diverse set of hazardous contaminants that exist in the nature and so it's mandatory to rely on diverse taxonomies for more efficiency.

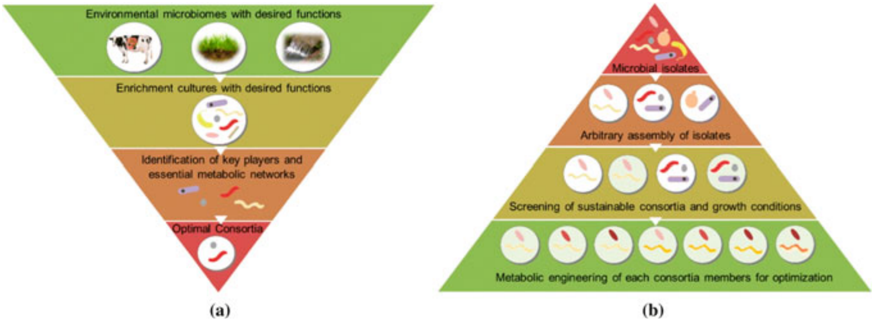
2 Design of Microbial Consortium

Micro-consortium is an associative term referred to the symbiotically or cooperatively inter-linked populations of two or more microbes of different genera or taxa, co-inhibiting under the same physiological condition. Often the term is misunderstood with mixed culture, however, the key concept is the cooperative interaction which is mandatory for the consortium, unlike mixed cultures. Nature is abundant with effective microbial communities yet their contribution remains un-highlighted due to the presence of interfering species along with symbiotic ones. In a study, microbial consortia were screened from Omani oil wells (Wafra oil wells and Suwaihat Gathering Station) and overall 33 genera and 58 species were identified using 16S rRNA gene sequences. However, the identified microbial consortia from Wafra oil wells were dissimilar from that of Suwaihat Gathering Station. These isolated were found to be anaerobic, thermophilic and halophilic indicating their suitability for MEOR applications. The isolated microorganisms produced different metabolites including biogases, bio-solvents and biosurfactants (Al-Bahry et al. 2013). Hence, comes the need to look into designing a stable micro-consortium. In agreement with this, various researchers have also suggested the upper hand of the consortium over single bacterial bio-degradative activity (Birolli et al. 2020; Jannat et al. 2020; Krainara et al. 2020).

Co-cultivation of a set of microbes with diverse metabolic enzymatic activity and adaptability towards physiological factors ensures improved stability and expands the possibility of secondary metabolites production (Hoshino et al. 2019). The co-cultivation of biosurfactant producing and hydrocarbon-degrading bacterial cultures confers a mutualistic effect on the overall biodegradation activity of individual bacteria. The biosurfactant producers improved the bioavailability of hydrocarbons by increasing their desorption from the sites and further catalytic activity of hydrocarbon degraders aided for the effective degradation (Sharma et al. 2019b). Yet, it's not the sole mutual activity of hydrocarbon degraders and biosurfactant producers that leads to complete hydrocarbon remediation. Studies have revealed the presence of non-hydrocarbon degrading species at the site of contamination along with the aforementioned major ruling microbes (Alves et al. 2019; Ebadi et al. 2017; Wanapaisan et al. 2018). These non-degraders thrive on the intermediates released during the primary hydrocarbon catabolism.

In order to design a micro-consortium, two approaches are commonly exploited, namely “top-down” and “bottom-up” approaches. The first case focuses on exploring a diverse and complex microbial populations, followed by screening and shortlisting of selective species of interest which act as key contributors in the micro-consortium. For example, sorting only hydrocarbon-degrading microbes from diverse environmental sites. The top-down approach puts forward the naturally selected, interlinked and inherent microbial communities. Yet, the presence of non-cultivable complex microbial communities in top-down prevents researchers from understanding the complete metabolic activities and pathways involved in the process. In contrast, the second approach focuses on “bottom-up” designing, where each microbe in the consortium (need not be of the same place of origin) is selected with required traits and later enriched in the microbial consortium (Ibrar and Zhang 2020). The advantage of the bottom-up approach lies in its inclusivity with the use of engineered microbes as a part of the consortium making it the most adapted technique in the formulation of the synthetic consortium (Tuleva et al. 2009, Zaragoza et al. 2013). Figure 2 depicts the two conventional approaches explored in the designing of the micro-consortium.

Overall the challenging part in consortium design exists in the unavailability of accurate information regarding the genomics, metabolic fluxes and catabolic enzymes involved in the biosynthesis of the product of interest limiting the designing of synthetic consortium. Advancement in metabolic engineering, system biology, metagenomics and single-cell techniques is required for the successful designing of a robust and stable microbial consortium.



(a) (b)

Fig. 2 Top-down (**a**) and bottom-up (**b**) approaches for synthetic consortia construction. [Adapted with permission from (Che and Men 2019)]

3 Criteria for Designing Microbial Consortium

A microbial consortium involves complex underlying inter-microbial interactions which are dependent on the molecular and metabolic pathways involved. Hence a thorough knowledge of these aspects is vital in the rational designing of the microbial consortium. While designing a petroleum degrading consortium, choosing a potential biosurfactant producer and hydrocarbon degrader is a very crucial step. Though a consortium rich in numerous potential degraders with the aforementioned capabilities are promising candidates yet often the results in pure cultures are contradictory to expectations. Rizzo et al. investigated one such incongruity in consortium designing. Three biosurfactant-producing strains (*Joostella* sp. A8, *Pseudomonas* sp. A6 and *Alcanivorax* sp. A53) were explored for their hydrocarbon degradation ability in pure culture as well as in consortium. While performing the study on pure culture, it was indicated that each strain grown significantly in mineral medium with diesel oil supplementation exhibiting biodegradation efficiency of 26.8%, 38.2% and 52.7% by *Joostella* sp. *Pseudomonas* sp. and *Alcanivorax* sp., respectively. However, during the co-culture study of *Joostella* sp. with *Pseudomonas* sp. (J-P) and *Alcanivorax* sp. (J-A), the biodegradation activity of 99.4% in the case of the J-A consortium and 99.2% by the J-P consortium was reported. Their study also witnessed strong competitiveness in the J-P consortium, where an abundance of *Joostella* sp. decreased with an increase in the growth of *Pseudomonas* sp. (Rizzo et al. 2018). Hence, it's important to analyse the growth behavior of each strain with their co-inhabiting species before using them in a consortium.

Interaction within co-inhabiting species is the major governing factor in the functioning of consortium. These interactions can be majorly classified as positive (+, beneficial), negative (−, detrimental) and neutral (0, no effect). Table 1 summarizes the various modes of microbial interactions within two species in a consortium.

Various researches report the suitability of mutualism (+, +) and commensalism (+,0) as the major interactions involved in cumulative growth and robustness of microbial consortium. Mutualism is the interaction within two microbes where both are benefitted from one another. The best example is cross-feeding, where there is an exchange of metabolic products between the two species. Commensalism is a one-way interaction, where one species is benefited whereas the other species is neither benefitted nor negatively affected. For instance, non-hydrocarbon degraders

Table 1 Various modes of microbial interaction within co-inhabiting species [Symbols: Beneficial (+); Detrimental (-) and No effect (0)]

Mode of interaction	Microbe A	Microbe B
Mutualism	+	+
Commensalism	0	+
Parasitism/Predation	-	+
Competition	-	-
Amensalism	-	0
Neutralism	0	0

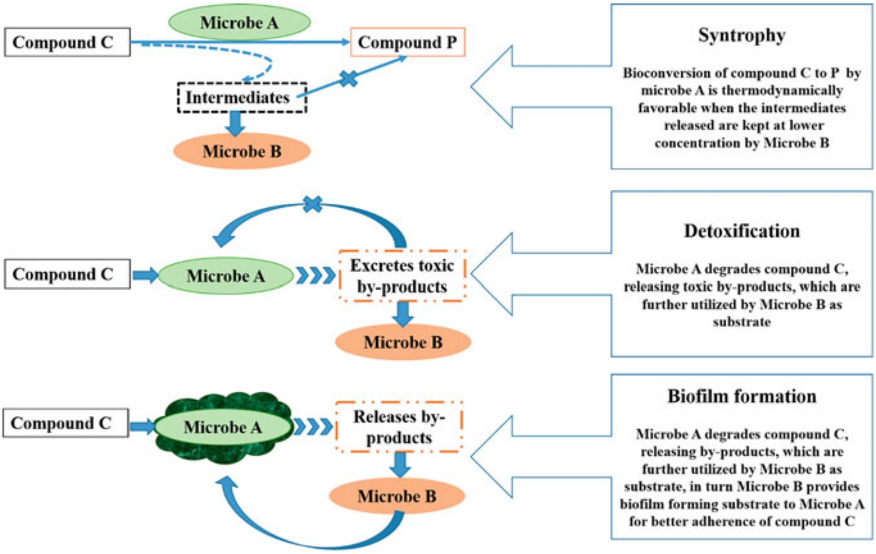


Fig. 3 Various modes of positive microbial interaction

surviving on the intermediate metabolites produced by the key species. The benefits involved in every co-species interaction are intended for (1) cell growth, (2) substrate utilization and (3) balancing of redox factors such as Nicotinamide adenine dinucleotide (NADH), Nicotinamide adenine dinucleotide phosphate (NADPH). Major microbial interaction reported in biodegradation/bioremediation involves syntrophy, bio-film formation and detoxification, as shown in Fig. 3.

Similar to pure bacterial culture, the biodegradability and secondary metabolite productivity of consortia is dependent on the physiological culture conditions. Hence, a designed bacterial consortium needs to be optimized for obtaining the appropriate culture growth condition in order to achieve desired optimal results. Suganthi et al. isolated 3 hydrocarbon degraders (*Shewanalla chilikensis* MG452729, *Halomonas hamiltonii* MG452731 and *Bacillus firmus* MG452730) from oily sludge and used these isolates as a consortium for further bioremediation studies. Culture conditions such as pH, incubation time, temperature, biomass concentration and oily sludge concentration were optimized using the one-factor-at-time (OFAT) technique. The authors explored bacterial growth, selective enzyme activity and biosurfactant yield as response factors for the analyses. The optimized culture condition obtained were: pH value of 7, temperature of 35 °C, 1% (w/v) of oily sludge concentration, 15% (v/v) of biomass concentration and 7 days of incubation time. Under these optimized conditions, the authors reported 96% biodegradation of total petroleum hydrocarbon concentration along with remarkably improved enzyme activity of hydrocarbon degradative i.e. 68 U/mL oxidoreductase activity, 80 U/mL lipase activity and 46 U/mL catalase activity (Suganthi et al. 2018). Summarily, the use of syntrophic microbes which share either mutualistic- or commensalism-based interactions is the preliminary screening criteria of microbes prior to be considered as consortia members. Later, the growth conditions of microbes are optimized to achieve cumulative maximum growth and metabolite production.

4 Advantages of Microbial Consortium Over Pure Isolates

Crude oil and other petroleum products are chemically complex hydrocarbons with diverse aliphatic and aromatic structural compositions. Various researchers have debated on the inefficiency of a single microbial system (axenic) to utilize such complex substrates as their energy source. In this regard, Kumari et al. used a diverse mixture of microbes namely, *Ochrobactrum anthropic* IITR07*, Pseudomonas mendocina* IITR46, *Pseudomonas aeruginosa* IITR48, *Microbacterium esteraromaticum* IITR47 and *Stenotrophomonas maltophili*a IITR87. The ability of crude oil bioremediation was analysed in three modes (1) Axenic culture, (2) Microconsortium and (3) Consortium supplemented with rhamnolipid JBR- 425. Focusing on major contributors of crude oil i.e. PAHs; the axenic culture showed the highest naphthalene degradation by IITR47 strain (80.4%), which increased to 97.3% when used in the consortium. Similarly, phenanthrene was the highest biodegraded by 67.1% by strain IITR48, which raised to 96.5% when performed by micro-consortium. Other PAH such as benzo(b)fluoranthene and fluorene also showed 1.4 folds and 1.6 folds higher biodegradability in the consortium than axenic culture. The authors highlighted the role of coordinated metabolic activity in the consortium that led to better results (Kumari et al. 2018). Table 2 summarizes the comparative changes in biodegradation ability of compounds by few bacterial species when grown in micro-consortium over axenic culture.

Along with improved metabolic activity, augmented biosurfactant production was also reported by Alves et al. during co-culturing of biofilm-forming bacterial strains *Pseudomonas aeruginosa* ATCC 27853 with model biosurfactant producing *Pseudomonas* sp. During the axenic study, the overall rhamnolipid production was reported to be 53.5 mg/L, which enhanced by 2.4 times (i.e. 129 mg/L) in the case of co-culturing with biofilm-forming *Pseudomonas aeruginosa,* indicating its role as inducer and stimulator in the consortium (Alves et al. 2019). Hence, in a consortium, microbes complement one another by acting as an inducer or stimulator of essential metabolic pathways.

Interestingly, apart from improved biosurfactant production, different isoforms of biosurfactant have also been reported during consortia study over axenic growth. In this approach, Ibrar et al. constructed a microbial consortium with intentions to enhance the overall biodegradation activity and biosurfactant yield. More than 60% biodegradation of glyceryl tributyrate (GT) was obtained by using a micro-consortium comprising 4 strains of *Lysinibacillus* spp. (HC_B, HC_C, HC_4, and

Table 2 Comparison of biodegradation ability toxic compounds by consortium over pure culture

S. no	Compounds	Microbes used	Reaction conditions	Efficiency	Reference
1	Natural rubber	*Rhodococcus pyridinivorans* Consortium (indigenous soil-inhabiting microbes including *Rhodococcus pyridinivorans*)	Incubated in MSM supplemented with dried latex glove pieces (0.6%, w/v) as a sole carbon source at 30 °C, 150 rpm for 4 weeks	9.36% 18.38% .38	Nawong et al. (2018)
2	Saturated fractions of oily sludge	*S. acidaminiphila* *B. megaterium* *B. cibi* *P. aeruginosa* *B. cereus* Consortium (all the above-mentioned strains)	Incubated with 1% of oily sludge as the sole carbon source, and kept at 100 rpm, and 30 °C for 40 days	91.7% 89.0% 89.7% 86.7% 88.4% 90.7%	Cerqueira et al. (2011)
	Aromatic fractions of oily sludge	*S. acidaminiphila* *B. megaterium* *B. cibi* *P. aeruginosa* *B. cereus* Consortium (all the above mentioned strains)		33.2% 39.6% 64.3% 39.5% 40.3% 51.8%	
3	Phenanthrene	*Bacillus* sp. ASP1 *Pseudomonas* sp. ASP2 *Stenotrophomonas maltophilia* ASP3 *Staphylococcus* sp. ASP4 *Geobacillus* sp. ASP5 *Alcaligenes* sp. ASP6 Consortium (all the above-mentioned strains)	Incubated with Phenanthrene (300 ppm) using 4% (v/v) inoculum at 37 °C, and 150 rpm for 120 h	29% 38.66% 52% 38% 43% 43% 76%	Patel et al. (2013)

(continued)

Table 2 (continued)

S. no	Compounds	Microbes used	Reaction conditions	Efficiency	Reference
4	Crude oil	*Raoultella ornithinolytica* PS *Bacillus subtilis* BJ11 *Acinetobacter lwoffii* BJ10 *Acinetobacter pittii* BJ6 *Serratia marcescens* PL Consortium (all the above-mentioned strains)	Incubated in 10% (v/v) inoculum, 0.4% crude oil (w/v), 30 °C,180 rpm for 10 days	83.5% 81.1% 75.80% 74.90% 70.00% 94.00%	Abena et al. (2019)
5	Bisphenol A (BPA)	*Pseudomonas knackmussii* Consortium (Inherent microbes from contaminated river sediment predominantly *Pseudomonas knackmussii*)	Basal salt medium (BSM) supplemented with 10 ppm of BPA, 150 rpm at 30 °C	100% in 7 days 100% in 28 h	Peng et al. (2015)
6	Octachlorodibenzo-p-dioxin (OCDD)	*P. mendocina* NSYSU Consortium (Inherent microbes from soil with bioaugmentation of *P. mendocina* NSYSU)	NB broth, room temperature (20 °C) for 65 days incubation	68% 62% (due to competition)	Tu et al. (2014)

HC_4L), *Paenibacillus* sp. (HC_A), *Gordonia* spp. (HC_8A) and *Cupriavidus* sp. (HC_D). The axenic growth of *Cupriavidus* sp. exhibited a poor biodegradative activity of 30–45% and the other bacterial species (*Lysinibacillus, Paenibacillus* and *Gordonia*) exhibited 45–60% of biodegradation. The authors suggested that the production of new isoforms of heptapeptide-based lipopeptide by the mutualistic growth of consortium, caused the increased emulsification activity and thus improved biodegradation (Ibrar and Zhang 2020).

Similarly, Kanaly et al. also reported the induction of new metabolic activity in *Rhodanobacter* sp. which otherwise was unable to grow on benzo[*a*]pyrene as a sole substrate. On the other hand, as a part of a consortium, it could utilize intermediates of catabolism formed by the action of mineralization and solubilization of benzo[*a*]pyrene by other members of the consortium. Thus *Rhodanobacter* sp. actively participated in the overall degradation, exhibiting twofold higher biodegradative activity (Kanaly et al. 2002). Such induction of new metabolite production elucidates the assertive effects of micro-consortium over pure cultures.

Consortium also reveals commensalism within members, where one species initiates the degradation so that the rest of the co-surviving species can thrive on catabolic metabolites released and thus degrade more effectively. Such synergistic effect of the microbial consortium over pure culture was also suggested by Wanapaisan and group. In the study, pyrene hydrocarbon was used as a model high molecular weight contaminant to analyse the bioremediation activity of consortium obtained from mangrove sediments. The study stated that the inherent sediment micro-consortium was primarily enriched with *Mycobacterium* spp. strains (PO1 and PO2), capable of utilizing pyrene as C source, however, the other components of consortium, i.e. *Novosphingobium pentaromativorans* PY1, *Ochrobactrum* sp. PW1 and *Bacillus* sp. FW1 were not able to grow in pyrene enriched agar. Such different growth patterns of various components of the consortium revealed the co-existence of non-pyrene degraders. Further bioinformatics study explained the occurrence of genes in strains PY1 and PW1 responsible for the catabolism of intermediates of the pyrene degradation pathway. Yet, strain FW1 lacked genes involved in the biodegradation of pyrene or its intermediates. Interestingly, FW1 was responsible for the assimilation of pyrene improving its bioavailability for other bacteria, due to its ability to produce biosurfactants. Hence, the presence of such diverse bacteria in the consortium (PO1, PO2, PY1, PW1 and FW1) led to > 80% degradation of pyrene within 72 h of incubation, which was more than twofold higher than the biodegradation activity of axenic *Mycobacterium* spp. (PO1, PO2) (Wanapaisan et al. 2018).

Indigenous microbes definitely are major biodegrading/bioremediating agents at the site, however, due to low bioavailability and biotoxicity at higher concentrations of hazardous contaminants, the inherent biodegradability of such micro-consortium is severely compromised. Exogenously augmented microbes add the genetic and metabolic diversity to such sites and hence significant tolerance is attained by the consortium and their degradation capability is also broadened. Thus in few cases, it's vital to bioaugment the sites with new microbes for improving the overall survivability and thus bioremediation/metabolic activities (Yuan et al. 2018). Ebadi et al. explored the bioremediation activity in a harsh salt-rich contaminated site. For this study, consortia enriched with oil-degraders and biosurfactant producers, *Pseudomonas aeruginosa* strains (T4, T27, T30, and E1) were prepared. The biodegradation rate constant, k (day^{-1}) of inherent consortium varied from 0.002 to 0.0009 with an increase in salinity from 0 to 300 mM in the presence of 30 g/kg of initial crude oil concentration. However, the k values improved to 0.0049 to 0.0035 along with *Pseudomonas* strains, expressing the positive role of bio-augmenting. Accordingly, the authors also investigated the effect of catabolic enzyme dehydrogenase in the given experimental conditions. The results reported an approximate twofold boost in the enzyme activity in the case of bio-augmented (6.35 ± 0.62 μg g^{-1} h^{-1}) than inherent consortium (3.41 ± 0.59 μg g^{-1} h^{-1}) (Ebadi et al. 2017). Various researchers have also reported the enhancement in the catabolic and degradative enzyme activities in the presence of metabolically enriched consortia (Loureiro et al. 2020; Suganthi et al. 2018).

Summarily, a microbial consortium has been found to be highly significant in the overall biodegradation of complex hydrocarbons or other toxic contaminants over pure cultures due to the cooperative and mutualistic effects among members that leads to an increase in metabolic activity and sometimes induces the novel pathways for the degradation and metabolite production. The development of synthetic microbial consortium has been successfully exploited in various environmental remediation and industrial metabolite production applications. Yet, potential insights in this domain need to be explored by applying smart system biology, bioinformatics and metagenomics tools.

5 Conclusion

Biodegradation of complex hydrocarbons like crude oil or other toxic contaminants by various biological agents has been very promising due to their cost effectiveness, no secondary pollutant generation and complete mineralization making it the most eco-friendly solution. Despite various researches and remarkable lab-scale successes, the technique has been a massive failure in the real site of application. Imitating the experimental results at the real site is difficult due to limited knowledge about the existing microbial communities and their interactions with one another. The underlining issue is the alteration in microbial behaviour from axenic growth to consortium growth. However, there are new advances made in the field of synthetic biology, bringing forward advanced tools to amend the knowledge gaps among complex microbial communities. These tools will elucidate the interaction within microbial communities understanding the metabolic pathways involved. These advances will help in the rational designing of stable synthetic microbial consortiums for real site applications. The cooperative and mutualistic effects among members lead to an increase in metabolic activity and sometimes induce novel pathways for degradation and metabolite production.

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Identification of Various Metabolites like Gases, Biopolymers and Biosurfactants

Swati Sharma, Pankaj Tiwari, and Lalit Pandey

1 Introduction

Microbes are the most abundant living being on this planet as they are exquisitely capable of surviving in extremely harsh environmental conditions. One of the survival technique exploited by microbes is the secretion of metabolites for adjusting to their surroundings. These metabolites could be either growth associated or non-growth associated based on their time of production by the microbes. Other than guarding microbes for their survival, these metabolites hold remarkable potential in various pharmaceutical, cosmetics, remediation, oil recovery and other industrial activities (Fopase et al. 2020; Madhumitha et al. 2019; Pham et al. 2019). These metabolites epitomize a completely new class of chemistry comprising chemically active entities with varied compositions like surface-active amphiphilic molecules, biodegradable polymeric compounds, solvents and often gaseous products. More than 43,000 microbial metabolites have been identified till date, yet merely 2% of these are accessible for research due to limitations of extraction and purification techniques (Berdy 2005). Poor extraction leads to a limited yield of these compounds and that slows down the entire exploration and research activities. Among the various metabolites, very few have been examined by researchers however, their potential roles in different sectors are still been investigated.

In order to explore these metabolites, the understanding of their chemistry is the foremost clue to their activity and hence, identification of these metabolites is the primary step to be focused on. The use of mathematically and statistically designed techniques and highly automated equipment are the prime requisites. Techniques such as infrared spectroscopy, nuclear magnetic resonance spectroscopy and liquid/gas chromatography-mass spectroscopy have been extensively explored in

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S. Sharma · P. Tiwari · L. Pandey (✉)
Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Guwahati 781039, Assam, India
e-mail: lalitpandey@iitg.ac.in

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this regard. Further, there is a requirement to improve the yield of these products, which mandates an in-depth knowledge of the associated metabolic pathways for the synthesis of these compounds. Metabolic engineering, metagenomics and computational biology techniques have paved the route to look into the microbial pathways associated with the synthesis of different metabolites (Kodzius and Gojobori 2015; Liu et al. 2019). In this chapter, we have emphasized the major microbial metabolites, their synthesis route and their identification techniques to gain knowledge of their chemical nature and potential applications.

2 Production of Microbial Metabolites

2.1 *Biosurfactants*

Biosurfactants are the major metabolite released by microbes during the biodegradation of hydrocarbons. These molecules are amphiphilic in nature with a hydrophobic tail and polar head. Alike to synthetic surfactants, these molecules are capable of drastically reducing the interfacial tension of the polar and non-polar (oil–water) interface and possess a high surface activity and emulsification index (Akbari et al. 2018). Poor availability of nutrients (during nitrogen limitation) and complex C sources such as hydrocarbons (Sharma et al. 2019a, b) leads to the production of such biological agents by the microbes as a response. Apart from hydrocarbon, various other substrates have also shown promising results in the production of biosurfactants. Complex substrates such as molasses and vegetable oils have shown promising biosurfactant production abilities (Al-Bahry et al. 2013; De Vrieze et al. 2015; Saimmai et al. 2011; Sharma et al. 2019a; Verma et al. 2020). They emulsify the poorly bioavailable non-polar substrate into small entities by the action of emulsification and solubilization, making them suitable for microbial uptake (Sharma et al. 2019a; b; Sharma and Pandey 2020; Yaraguppi et al. 2020).

Having biological origin, these molecules are biodegradable, biocompatible and non-toxic along with various economic and eco-friendly benefits. The biosurfactants are categorized based on their microbial origin, chemical type and structure. Major classes of biosurfactant include (a) Lipopeptide, produced by *Bacillus* sp., *Streptomyces* sp., and *Thiobacillus* sp.; (b) Glycolipids, produced by *Pseudomonas* sp., *Alcanivorax* sp., *Arthrobacter* sp. and *Acinetobacter* sp.; (c) Phospholipids, produced by *Aspergillus* sp., and *Corynebacterium* sp. and (d) Polymeric biosurfactants, produced by *Halomonas eurihalina, Mycobacterium thermoautotrophium*, and *Sphingomonas paucimobilis* (Datta et al. 2020; Joy et al. 2019; Khademolhosseini et al. 2019; Lima et al. 2020; Santos et al. 2016; Suganthi et al. 2018; Thavasi et al. 2011). These surface-active molecules are involved in the reduction of surface tension and own very low critical micelle concentration (CMC) values ranging from 0.5 to 500 mg/L (Kot and Krawiec 2015; Patowary et al. 2017; Sharma et al. 2019a). When administered below CMC these biosurfactants are involved in the mobilization of non-polar entities, on the other hand, when used above CMC, it leads to complete solubilization, demonstrating its vital role in the Microbial enhanced oil recovery (MEOR)-based applications (Datta et al. 2018, 2020; Pacwa-Płociniczak et al. 2011). In addition, biosurfactants are extensively used in various pharmaceuticals, food, cosmetics and remediation sectors and are on the verge of getting the commercial market replacing existing chemical surfactants (Santos et al. 2016).

2.2 Biopolymers

Biopolymers are the result of a set of microbial enzymatic reaction involved in the process of conjugating simple sugars, amino acids or fatty acid units into high molecular weight compounds such as polysaccharides, polyamides, polyesters and polyphosphates (Moradali and Rehm 2020). These high molecular weight entities are innocuous and biocompatible with characteristics of high strength, stability and tensile activity (Chang et al. 2016). These compounds are released as secondary metabolites by microbes in stress conditions to combat the threats. Few studies also suggest the production of biopolymer being associated with microbial defense or storage needs (Sukan et al. 2015). These polymers impart better physical strength, durability and eco-friendly characteristics than existing synthetic polymers.

Exopolysaccharides (EPS) constitute the major class of biopolysaccharide released during cell starvation or harsh physiological conditions of microbial growth. Due to this, these compounds endure exemplary features which have been of keen interest by various industrial sectors (Fontana et al. 2015; Gonzalez-Gil et al. 2015; Gupta et al. 2019; Gupta and Diwan 2017). Most studies support the presence of EPS as essential exo-polymers released by microbes to strengthen microbial cell membranes against predation, desiccation and biofilm formation for the improved adhesion to substrates. These EPS are primarily rich in long-chain organic molecules, protein, lipid and uronic acid units (Gupta and Diwan 2017). The organic monomers of the saccharide unit could be homopolymeric such as inulin, dextran, alternan, levan, or it could also be comprised of alginate, xanthan, hyaluronan, categorized as heteropolymeric saccharides. Such compositions impart a negative charge to the EPS, which further have been exploited by various researchers in the remediation of heavy metals and other polar contaminants (Gupta and Diwan 2017).

Biopolyesters are another major class of microbial biopolymers that functions as storage units and energy reserves. They also act as an electron sink during biofilm formation under anaerobic conditions (Moradali and Rehm 2020). Polyhydroxyalkanoates (PHAs) constitute a major type of biopolyesters. PHA are bioplastics that are equally efficient in physiochemical strength as commercial plastics yet biodegradable. Various studies have revealed its induced synthesis by different microbes during stress caused by a limitation of O_2, PO_4^{2-} and NO_3^-. Strains like *Alcaligenes eutrophus*, *Cupriavidus necator*, *Protomonas extorquens*, *Pseudomonas* spp. and *Protomonas oleovorans* are known to possess stress-driven PHA production as an energy reserve in cytoplasmic granular entities (Aljuraifani et al. 2018; Muneer et al.). These compounds are kept as a reserve for energy and nutrients in the presence of excess carbon in the media. However, few engineered *E. coli* sp., *Bacillus* sp., *Alcaligenes* spp. and *Azotobacter vinelandii* are known for constitutive PHA production (Khanna and Srivastava 2005). Polyhydroxybutyrate (PHB) was the first bioplastic produced by bacteria *Bacillus megaterium.* Its remarkable biocompatibility and biodegradability have been explored for the replacement of petroleum-originated plastics, however, due to poor thermal stability, its industrial application is still at the infant stage. Likewise, various other bio polyesters have shown promising potential in the field of tissue engineering, implant designing and food packaging applications (Aljuraifani et al. 2018). The aforementioned unique features and ongoing experimental results have paved the way for biopolymers to be exploited as adsorbents, drug delivery vehicles, tensile, conditioner, lubricants and adhesive in various pharmaceutical, food and remediation sectors (Kaur et al. 2014).

2.3 BioGases

Biogas has gained attention in recent decades due to its inherent characteristic of renewable energy resource where non-renewable resources are rapidly depleting. The biological production of gases such as hydrogen, carbon dioxide and methane is a result of microbial fermentation. The fermentation is an anaerobic process driven by a set of biochemical reactions where complex organic matter such as carbohydrates, lipids and proteins are consecutively digested into biogases. It is a 4-staged process, each being catabolized by a special set of microbes, i.e. (a) Hydrolysis, (b) Acidogenesis, (c) Acetogenesis and (d) Methanogenesis (Fig. 1) (Ersahin et al. 2011). This process leads to the biological production of valuable biogases such as H_2, CO_2 and CH_4. Each of these processes is catalyzed by a special class of microbes equipped with necessary catalytic enzymes, however, the nutrient availability, pH, temperature and chemical oxygen demand (COD) are few essential factors deciding the overall yield of anaerobic digestion (AD). For instance, while the initial steps of AD are more favored under a mild acidic environment (pH 5.5 to 6.5), yet the methanogenesis is more evident at basic pH 6.5–8.2 (Lee et al. 2009). Temperature also plays a vital role in controlling the efficacy of the AD process. Chae et al. reported a 17.4% decrease in biogas yield with a shift in temperature from 30 to 25 °C. However, a mere 3% loss in yield was observed at 35 °C (Chae et al. 2008). Reports also suggest the C/N ratio of 12–30 as optimal for AD (Cho et al. 1995). In addition, the high COD content of the substrate (Mathew et al. 2015) and the presence of metal ions such as Cu, Fe, Ni, Zn, Co also have been reported as essential factors for improving the overall biogas yield by microbial AD (Goswami et al. 2016; Qiang et al. 2012).

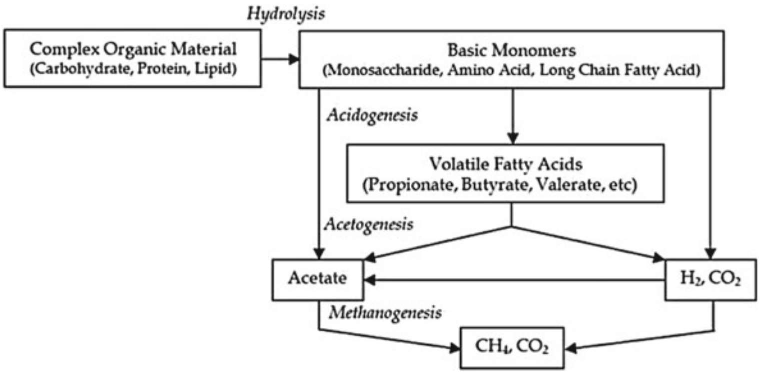


Fig. 1 Schematic representation of anaerobic digestion pathway. [Adapted from (Ersahin et al. 2011)]

Hydrogen gas has been extensively explored as a clean, green and renewable fuel to meet energy demands. "Hydrogenogens" are the class of microbes involved in the hydrolysis of organic matters for the production of hydrogen gas (Mohr et al. 2018). These microbes are rich in [Fe–Fe] hydrogenase enzyme responsible for the reduction of hydrogen, as shown in reaction stated in Eq. (1);

$$2H^{+} + 2Ferredoxin_{reduced} \rightarrow 6H_2 + 2Ferredoxin_{oxidized} \tag{1}$$

During the reduction of hydrogen to hydrogen gas, the reduced Ferredoxin is fetched from another photochemical reaction where water molecules act as an electron donor. The reduction of ferredoxin is a result of the oxidation reaction catalyzed by pyruvate-ferredoxin oxidoreductase. These enzymes are mostly evinced in algae and cyanobacteria. However, few bacterial species such as *Clostridium* spp., *Enterobacter* spp., *Thermotoga* spp. and *Bacillus* spp. are also reported to be involved in the production of hydrogen gas using non-photo driven dark fermentation reaction (Korres and Norsworthy 2017).

Similarly, methanogenic bacteria utilize hydrocarbons for the production of methane gas. Clavijo et al. have explored the applicability of using methanogenic bacteria with hydrocarbon-degrading micro-consortium. The syntrophic microbes effectively utilized hydrocarbons to produce metabolites such as acetate, H_2 and CO_2, which are further utilized by methanogens for the production of methane (Berdugo-Clavijo and Gieg 2014). The basic chemical reactions involved are mentioned in Eqs. 2 and 3:

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \tag{2}$$

$$CH_3COOH \rightarrow CH_4 + CO_2 \tag{3}$$

Apart from acetate fermenters such as *Methanosarcina* and *Methanosaeta*, a few microbial families are also involved in the methanogenesis of formate which includes *Methanopyrales, Methanobacteriales*, *Methanococcales* and *Methanomicrobiales* (Pan et al. 2016). Overall these potential biogases are very promising in refinery-based oil recovery application, where these gases are essential in decreasing the viscosity of crude oil for its easy recovery, and sometimes are essential in causing pressure-based oil extraction (Al-Sulaimani et al. 2011).

2.4 Other Metabolites

Apart from the production of biogases, biosurfactant and other polymers, microbes are also associated with the production of certain solvents such as acids and alcohols. Various known bio-acids such as lactic acid, succinic acid, acetic acid, formic acid and butyric acid are released by microbes as a result of anaerobic fermentation (Özcelik et al. 2016). Lactic acid fermenting bacteria are the major producers of organic acids, however, the kind of acid produced and its quantity depend on the type of strain, culture conditions and substrate used. *Lactococcus* sp., *Leuconostoc* sp., *Pediococcus* sp., *Weisella* spp. and *Aerococcus* spp. are the major bacteria explored for the synthesis of bio-acids (Desniar 2013; Özcelik et al. 2016). The acidification step in anaerobic fermentation is reported to play a key role in the biosynthesis of organic acids. Luo et al. revealed a 1.8-fold improved production of acetic acid during the biodegradation of phenanthrene by waste-activated sludge. The authors reported an induction in the acidification step in the anaerobic degradation of phenanthrene leading to overexpression of enzymes responsible for acetic acid production (Luo et al. 2016). Similar improved production of propionic acid and acetic acid as metabolites during fermentation of liquor effluent by activated sludge was also reported. The study also revealed the increase in the population of *Firmicute* sp., *Bacteroidetes* sp. and *Proteobacteria* sp., which are exclusively responsible for the conversion of acetic acid production using complex organic macromolecules (Luo et al. 2018). Overall the acidification step in the anaerobic fermentation plays a major role in the production of organic acids as metabolites. These metabolites are separately used as feedstock and energy reserves for various bioprocess and microbiological applications.

Likewise, another key metabolite produced by microbes as a result of fermentation involves bio-solvents and primarily alcohols. Various researchers have explored the microbial alcohol formation as a product of fermentation of lignocellulose biomass, vegetable and animal oils as well as whey protein solutions (Ariyanti and Hadiyanto 2013; Kamoldeen et al. 2017; Ko et al. 2016; Staniszewski et al. 2007). Various strains like *Saccharomyces cerevisiae*, *Scheffersomyces stipites* and *Kluyveromyces marxianus* have been utilized for ethanol production. Alcohols are also sometimes involved as surfactant ingredient to decrease the interfacial tension of oil–water during extraction. Table 1 summarizes the essential microbial metabolites produced by microbes and their major role in oil remediation, extraction and refinery applications.

Table 1 List of microbial metabolites and their important roles in oil-based applications

S. No	Metabolites	Microbes	Essential roles
1.	Surface-active agent	*Acinetobacter* sp., *Arthrobacter* sp., *Bacillus* sp., *Enterobacter* sp., *Clostridium* sp., *Agrobacterium* sp., *Pseudomonas* sp.	• Lowering interfacial tension, • Reduce surface tension, • Enhance emulsification activity, • Alteration of reservoir rocks wettability • Solubilization and mobilization of heavy crude oil
2.	Bio-polymers	*Bacillus polymyxa*, *Brevibacterium viscogenes*, *Ralstonia eutropha*, *Lactobacillus plantarum*, *Leuconostoc mesenteroides*, *Micrococcus* sp., *Xanthomonas campestris, Microbacterium* sp., *Enterobacter* sp.	• Regulate oil mobility, viscosity and injectivity profiles
3.	Biogases (CO_2, CH_4, H_2)	*Acetobacterium woodii, Clostridium* sp.*, Enterobacter aerogens, Methanobacterium* sp.*, Moorella thermoacetica,*	• Increase in oil permeability via solubilization of carbonate salts, • Enhancement in pressurization activity in the reservoir, • Reduction in heavy oil viscosity, • Reduces interfacial tension, • Causing swelling of oil
4.	Others (Bio-acids, Biomass, Bio-solvents)	*Clostridium* sp.*, Enterobacter aerogens, Bacillus* sp.*, Leuconostoc mesenteroides, Xanthomonas campestris, Zymomonas mobilis*	• Surface modification causing alteration reservoir rocks wettability • Enhance porosity and permeability, • Emulsification, • Oil biodegradation, • Selective/nonselective plugging, • Aid in crude oil desulfurization, • Decrease viscosity of heavy crude oil and Enhanced oil dissolution

3 Metabolic Pathways Involved in the Production of the Above Metabolites

As discussed in the above section these metabolites are crucially important for various applications such as feedstock resource, cosmetics and drug synthesis, oil recovery and extraction and various other oil remediation sectors. In this section, the major metabolic pathways responsible for the synthesis of these microbial metabolites are elucidated.

3.1 *Biosurfactant Synthesis*

Among the various biosurfactants, two major classes of biosurfactants extensively explored for their surface activity and interfacial activities are rhamnolipid and surfactin. The biosynthesis pathways of these two biosurfactants are discussed in the subsections below.

3.2 *Rhamnolipid Biosynthesis*

Rhamnolipid biosurfactant comprises a hydrophilic head (rhamnose unit) with hydrophobic tails (hydroxyl fatty acid), making the entire molecule amphiphilic in nature. The synthesis of these polar and non-polar moieties takes place separately in the microbes. The substrate for sugar synthesis is glucose whereas acetyl CoA act as a precursor for the synthesis of lipid group (Fig. 2a). A typical rhamnolipid biosynthesis comprises four stages: (1) Synthesis of 3-(3-Hydroxyalkanoyloxy) alkanoates (HAAs), which is initiated using acetyl CoA as

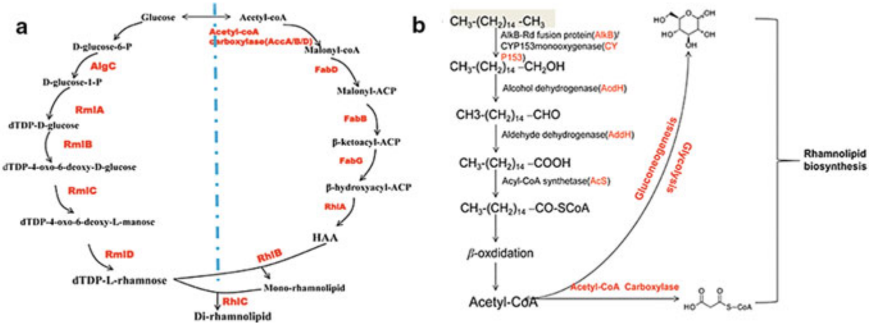


Fig. 2 General pathway of **a** Rhamnolipid biosurfactant synthesis; **b** Hexadecane catabolism as a substrate to obtain precursor Acetyl CoA. [Adapted from (Wang et al. 2014)]

mentioned above. The HAAs are obtained through de-novo fatty acid synthesis reaction catalyzed by a set of enzymes such as Acetyl-CoA carboxylase (AccA/B/D), FabD/B/G and 3-(3-hydroxyalkanoyloxy) alkanoate synthetase (RhlA). (2) Rhamnose synthesis, which takes place using glucose as a precursor. Phosphomannomutase (AlgC), glucose-1-phosphate thymidylyltransferase (RmlA), dTDP-D-glucose 4,6-dehydratase (RmlB), dTDP-4-dehydrorhamnose 3,5-epimerase (RmlC) and dTDP-4-dehydrorhamnose reductase (RmlD) are key enzymes involved in this step. (3) Production of mono-rhamnolipid using deoxy thymidine diphosphate L-rhamnose (dTDPL-rhamnose) and HAA catalyzed by rhamnosyl transferase (RhlB). (4) Production of di-rhamnolipid using mono rhamnolipid as a precursor and dTDP-L-rhamnose using enzyme rhamnosyl transferase (RhlC) (Li 2017; Varjani and Upasani 2017; Wang et al. 2014).

However, in the case of substrates other than sugars, an additional oxidation pathway is exploited by microbes for the synthesis of Acetyl CoA as the important preliminary precursor for biosurfactant synthesis (Fig. 2b). Wang et al. explored the rhamnolipid synthesis pathway by bacteria *Dietzia maris* As-13–3 using n-hexadecane as the sole carbon source. The study revealed the involvement of enzymes such as alkane hydroxylase (AlkB-Rub), alkane monooxygenase (cyp153), alcohol dehydrogenase (acdH), aldehyde dehydrogenase (addH), acyl-CoA synthetase gene (acS) along with tricarboxylic acid (TCA) cycle and gluconeogenesis related enzymes (Wang et al. 2014).

3.2.1 Surfactin Biosynthesis

Surfactin is a cyclic lipopeptide that is synthesized in four parts: (1) Fatty acid biosynthesis; (2) Formation of fatty Acyl-CoA; (3) Biosynthesis of amino acids; and (4) Surfactin assembly. Importantly, the synthesis of surfactin is majorly catalyzed by non-ribosomal peptide synthetases (NRPS). This NRPS primarily comprises 3 modes: SrfAA, SrfAB and SrfAC, each comprising 7 modules. Figure 3 depicts a detailed view of the synthesis of surfactin biosurfactant using glycerine as substrate. A 5-staged process is responsible for the synthesis of surfactin: (1) Glycolysis of substrate for the biosynthesis of Acetyl CoA; (2) Amino acid biosynthesis; (3) TCA cycle; (4) Biosynthesis of fatty acids and (5) Amino acid–lipid bio assembly.

3.3 Biopolymer Synthesis

3.3.1 Exopolysaccharide (EPS) Synthesis

EPS synthesis involves the cooperation among several sets of enzymes and regulatory molecules catalyzing a set of microbial pathways. During the synthesis of homo-polysaccharides such as dextran, monomeric units (α-D-glucose or sucrose) are linked in a polymeric chain using enzymes such as dextran-sucrase. On the

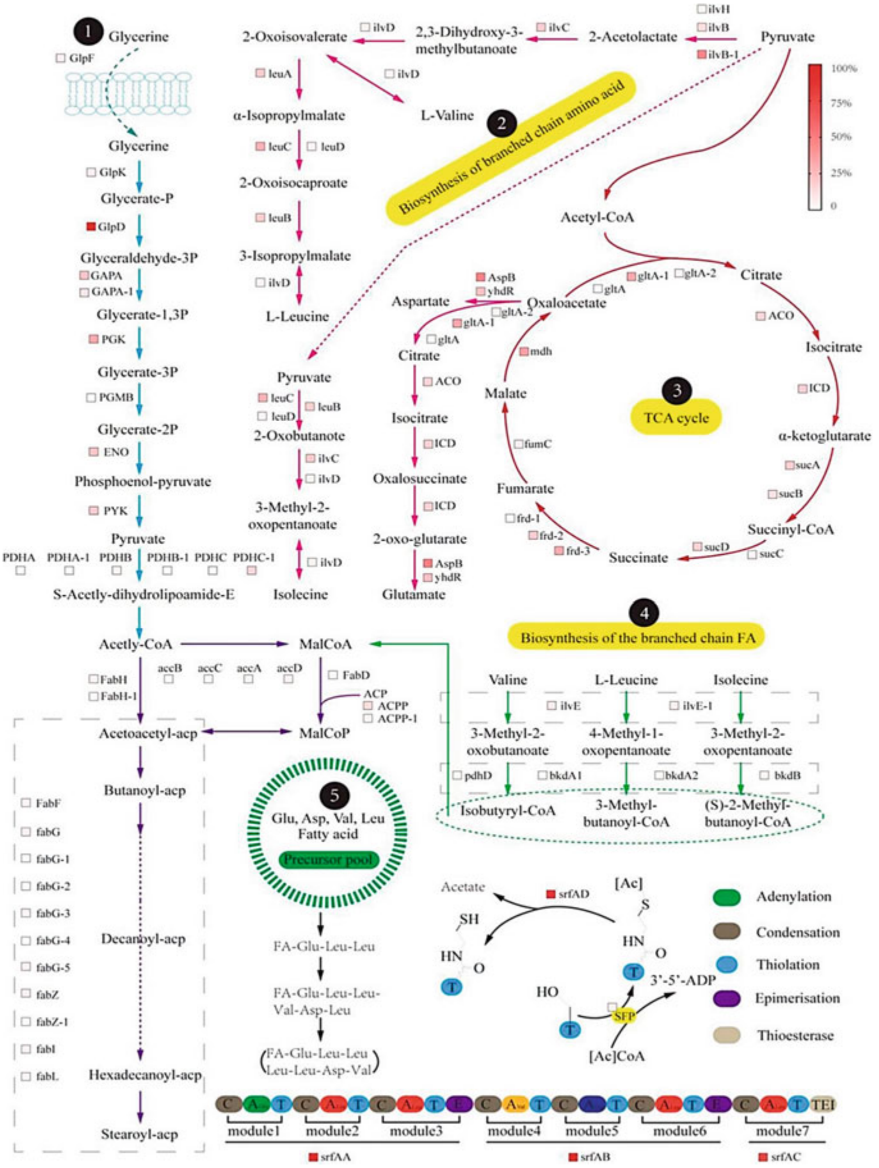


Fig. 3 Metabolic pathways for surfactin synthesis using Glycerin as initial substrate. [Adapted from (Zhou et al. 2019)]

other hand, for the synthesis of hetero-polysaccharides, a more complex metabolic pathway is followed. Most hetero-polysaccharides are prepared intracellularly which commences with the intake of monomeric substrate units within the cell. Further, within the cytoplasm, the substrate undergoes an oxidation or phosphorylation reaction in order to provide sufficient energy to drive the polymerization reaction, called as activation step. Independent addition of nucleotides monophosphates or diphosphates unit to precursor's monomeric substrates occurs in the periplasmic space itself. Few such intermediates involved in EPS synthesis include dTDP-rhamnose and Uridine diphosphate glucose (UDP-glucose). These activated units are further transferred by glycosyltransferase to a 55-C isoprenoid lipid moiety present in the cytoplasmic membrane. This lipid carrier ensures the accurate structural arrangement of monomeric units for the synthesis of polysaccharides. Upon the rearrangement of monomeric units on the lipid carrier backbone, an elongation step is performed. This step generally takes place upon translocation of carrier chain to the cell membrane in case of gram-positive bacteria. However, in the case of gram-negative bacteria, this step is performed by the ABC transporter-dependent pathway. In this case, the assembly and elongation are carried out in the periplasmic space. Overall, upon the completion of polymerization, the biopolymer either stays attached to the cell membrane as a capsule or is released into extracellular space as a slime. Figure 4 represents the major steps and catabolizing enzymes involved in the initiation, activation, coupling, elongation and transportation of polysaccharides biopolymer in bacteria (Gupta and Diwan 2017).

3.3.2 PHB Synthesis

The process of bioplastic synthesis occurs in three steps as shown in Fig. 5. Preliminarily, the substrate is oxidized in the glycolysis cycle yielding acetyl CoA as a precursor for the synthesis of PHB. The major steps include: (1) Formation of acetoacetyl-CoA via condensation reaction catalyzed by β-ketothiolase (PhbA), (2) Reduction of acetoacetyl-CoA to (R)-3-hydroxybutyryl-CoA catalyzed by acetoacetyl-CoA reductase (PhbB) and (3) Polymerization, where 3-hydroxybutyryl-CoA to PHB is catalyzed by PHB synthase (PhbC) (Osman et al. 2016).

3.4 Biogases Synthesis

The major biogases production is a result of an anaerobic fermentation reaction. Figure 6 represents the major reaction involved in the biosynthesis of biogases: CO_2, H_2, CH_4 using various class of substrates such as carbohydrates, protein and lipids. The primary substrate for biogases production is pyruvate. A set of specialized bacteria are involved in the synthesis of these gases. Initially, hydrolytic bacteria are involved in the hydrolysis of polymeric substrates into their monomeric units. These monomeric sugars, fatty acids and amino acids thereafter are oxidized to pyruvate

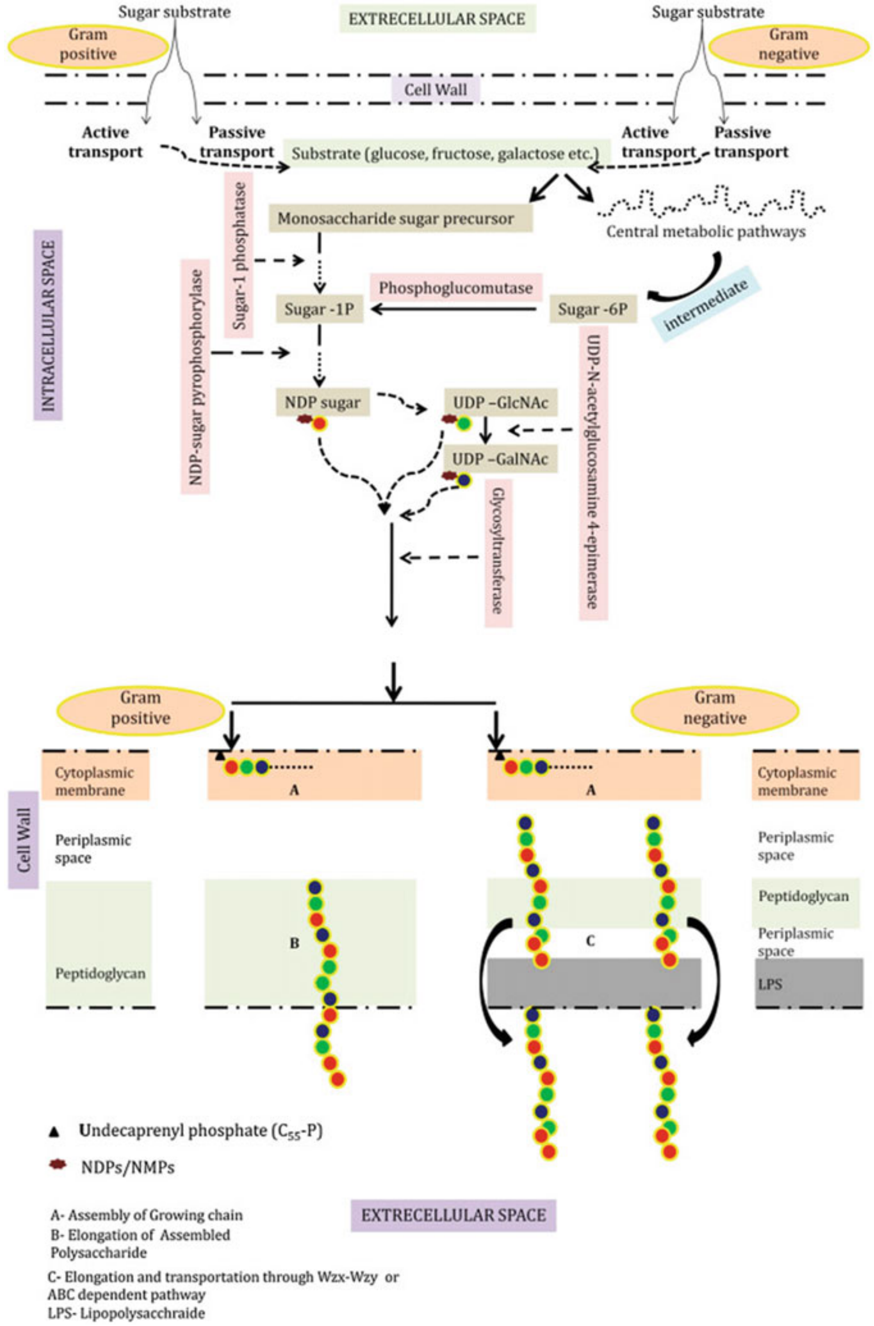
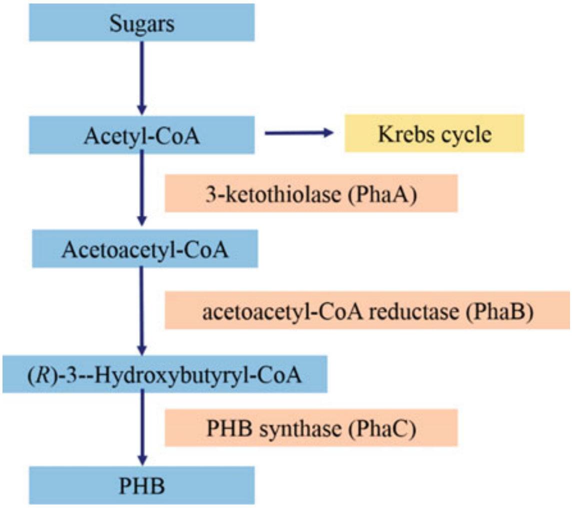


Fig. 4 General pathway for the synthesis of EPS by gram-positive and gram-negative bacteria. [Adapred from (Gupta and Diwan 2017)]

Fig. 5 Schematic representation of PHB production using sugars as a primary substrate



using another category of bacteria namely acidogenic bacteria. The pathways associated with this breakdown involve, glycolysis, amino acid oxidation and fatty acid oxidation (α, β, ω oxidation) reactions (Nelson et al. 2008). During the acidogenesis reaction, the conversion of pyruvate into short-chain acids such as formic acid, lactic acid and propionic acid along with the formation of H_2, and CO_2 takes place. Hydrogenase enzymes with two metallic prosthetic groups: [Fe–Fe] and [FeNi] play major enzyme in these conversions (Sikora et al. 2017). In the acetogenesis reaction, the prior acidic residues act as substrates for the formation of CO_2, H_2 and acetate as final products using acetogens as a specialized class of microbes. The final step of anaerobic fermentation is methanogenesis, wherein thus formed acetate is broken down into CO_2 and methane using methanogens (Du Shin et al. 2017).

4 Identification Techniques Involved in the Synthesis of Metabolites

Identification of metabolites produced by microbes is the preliminary affirmation of obtaining the desired compounds both qualitatively and quantitatively. The basic identification techniques explored for the identification of these metabolites are discussed in the following subsections.

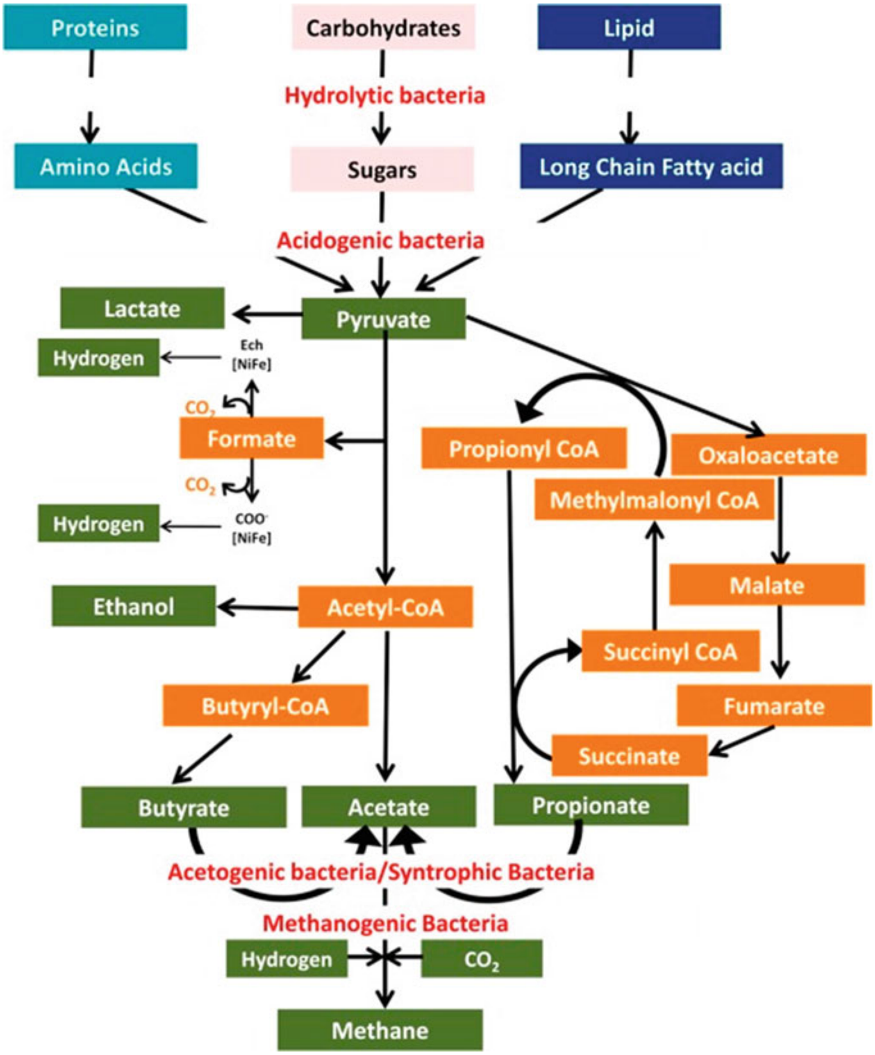


Fig. 6 Schematic pathways of anaerobic fermentation for biogas synthesis. [Adapted with permission from (Dahiya et al. 2018)]

4.1 *Fourier Transform Infrared Spectroscopy (FTIR)*

This technique is applied for identifying the chemical groups and functional groups present in the molecule of interest. FTIR spectroscopy uses infra-red radiation to vibrate and rotate bonds within the compound at a specific frequency which is recorded by the detector as a peak intensity. The nature of the peak (stretching/bending) and its intensity describe the chemical groups present in the chemical structure of the test compounds (Mohamed et al. 2017). FTIR has been a widely accepted identification and characterization technique employed for characterizing biosurfactants and biopolymers produced by microbes. A typical FTIR spectrum of lipopeptide surfactin shows the presence of specific peaks in regions associated with its chemical groups. The recorded spectrum showed peaks for N–H stretching in the 3300–3400 cm^{-1} region, CO–NH peptide bond specific to lipopeptide class biosurfactant in the region 1650–1700 cm^{-1}, and peaks at 1200–1400 cm^{-1} associated to aliphatic $–CH_3$ and $–CH_2$-groups in the lipid chain (Al-Wahaibi et al. 2014; Joshi et al. 2016, 2015). In the case of rhamnolipid biosurfactant, certain peak positions associated with rhamnose sugar and lipid chain are used to identify the compound. These peaks are in the region of 1170 cm^{-1} and 1632 cm^{-1}, which represent C–O–C group and $C = O$, respectively. Also, $(–CH_2)_n$ group fatty acid chain was represented by a peak at 718 cm^{-1}. Various other peaks in the region 2900–2800 cm^{-1} represent C–H, $–CH_2$ and CH_3 groups (Sharma et al. 2019a).

Similar to biosurfactant, various researchers have used FTIR spectroscopy for the identification of biopolymers released by bacteria during growth. One such biopolymer extensively explored using FTIR is PHB. The FTIR spectrum of pure PHB showed peaks at 1728 cm^{-1} and 1282 cm^{-1} standing for $C = O$ and –CH groups in the biopolymer, respectively (Sindhu et al. 2011). Similar results have been reported in literature along with small peaks at 1452 cm^{-1}, 1380 cm^{-1} and 1230 cm^{-1} for the $–CH_2$ and $–CH_3$ groups (Florez et al. 2019; López et al. 2012; Özgören et al. 2018). Likewise, exopolysaccharides (EPS) are another biopolymer extensively studied using FTIR spectroscopy. Peaks at 3274, 1577, 1454, 1236 and 1122 cm^{-1} represent $C = C\text{-}H$, $\text{-}C = C\text{-}$, CH_2, C-H and C-O chemical groups, respectively, as major components of EPS (Borah et al. 2019; Osińska-Jaroszuk et al. 2014).

4.2 Nuclear Magnetic Resonance (NMR)

NMR technique is an analytical technique used for determining the structure of any compound based on its magnetic spin orientation under an external magnetic field. Different biosurfactants have been characterized by ^1H and ^{13}C NMR spectroscopy. ^1H NMR characterizations of biosurfactants, PHB and EPS are concisely outlined. In a study, rhamnolipid ^1H atoms were characterized exhibiting rhamnose sugar peaks at 5.3–5.1 ppm for –CH-O-C-, and 4.9 ppm and 3.3 ppm for –CH-OH. Peaks for β-hydroxy fatty acids stand in the region 0.8 ppm for $–CH_3$, 1.2 ppm for $–(CH_2)_5$-, and 1.5, 2.5 and 3.5 ppm for CH_2-COO chains (Moussa et al. 2014; Singh et al. 2013; Varjani and Upasani 2016). Surfactin biosurfactant has also been explored using NMR spectroscopy. The $(-CH_3CH_2\text{-})$ region was demonstrated as peaks at 0.6–1.02 ppm signifying the protons from myristic fatty acid. Similarly, a chemical shift in the range 1.3–1.8 ppm stands for palmitoleic fatty acid (Ramalingam et al. 2019). The presence of various protonated amino acids was also reported by Lee et al. in the region 8.5–7.5 ppm signifying –NH group, 3.6–4.3 ppm for CH_2 in amino acids and 2.5–3.0 ppm for fatty acid chains. CH_3 in fatty acids in the range 5.2–5.5 ppm and 2.3–3.0 ppm for amino acids were observed (Jakinala et al. 2019; Lee et al. 2012).

The ^1H NMR characterization of PHB, produced by *Bacillus cereus* PS 10, showed spectral chemical shifts at 1.2, 2.3 and 2.7 ppm corresponding to chemical groups CH_3, CH_2 and CH, respectively, while 4.6 and 5.7 ppm corresponded to alkene H atoms (Sharma and Bajaj 2015). Pradhan et al. also characterized the PHB produced by *P. hysterophorus*, exhibiting peaks at 5.2, 3.5, 2.6–2.4 and 1.3 ppm representing -CH, RCOOR, -CH_2 and CH_3 chemical groups, respectively. Similar peaks were obtained by *E. crassipes* and *Bacillus* sp. PHB (Hassan et al. 2016; Pradhan et al. 2017). EPS has also been successfully characterized by various researchers using ^1H NMR spectroscopy. A typical polymeric organic saccharide structure comprises sugar ring protons in the region 3.2–4.8 ppm. For deoxy sugars peak at 2.3 ppm was observed. Peaks before 3.2 ppm stand for the presence of lipid or protein substitute within the saccharide molecules (Fontana et al. 2015; Gonzalez-Gil et al. 2015; Gupta et al. 2019). Overall this technique has been successfully used for the characterization of various biosurfactants and biopolymers.

4.3 Mass Spectroscopy

Mass spectroscopy is another technique to identify the organic macromolecules such as biosurfactant and biopolymers based on their mass to charge (m/z) ratio. In this regard the compounds are initially ionized, accelerated, deflected and finally detected. Based on the obtained m/z, the results are matched with the existing library to obtain the exact molecular weight of a metabolite. This technique is often used with chromatographic tools such as liquid chromatography–mass spectroscopy (LC–MS), Gas chromatography–mass spectroscopy (GC–MS) and Matrix-assisted laser desorption ionization time-of-flight tandem mass spectrometry (MALDI-TOF–MS) for the characterization of various compounds. Table 2 lists the m/z values of a number of metabolites including biosurfactants, PHB and EPS produced by different microbes.

5 Conclusion

Microbial metabolites are eco-friendly chemicals with remarkable chemical, physical and biological properties. Biodegradable plastics, renewable fuel source in the form of biogases and highly surface-active biosurfactant are few such microbial metabolites produced by microbes during starvation, stress and nutrient limitation conditions. With the advancement in the system and computational biology tools, it has been possible to get an insight into the pathways involved in the synthesis of these compounds. Their chemical compositional analysis and characterization

Table 2 Identification of microbial metabolites using mass spectroscopic techniques

S. No	Metabolites	m/z			Microbes	Ref
		LC–MS	MALDI-TOF–MS	GC–MS		
1	Surfactin	980.6, 994.6, 1008.7, 1022.7, 1036.7, 1038.7, 1050.7, 1054.7, 1064.7 and 1078.7	1064	NA	*Bacillus amyloliquefaciens* BPD1	(Liao et al. 2016)
2	Surfactin	1030, 1044, 1058 and 1072	1043, 1065 and 1081	NA	*Bacillus subtilis*	(Bernat et al. 2016)
3	Surfactin	1036.8, 1036.8, 1050.8 1050.8 and 1064.8	NA	NA	*Bacillus atrophaeus* strain AKLSR1	(Routhu et al. 2019)
4	Surfactin	NA	861, 882, 900, 912, 934, 950, 956, 1016, 1024, 1042, 1048, 1064, 1070, 1102, 1123, 1145, 1174, 1196, 1508 and 1527	NA	*Bacillus amyloliquefaciens*	(Dimkić et al. 2017)
5	Surfactin	NA	1051, 1067, 1083 and 1100	NA	*Bacillus subtilis* MG495086	(Datta et al. 2018)
6	Di-rhamnolipid	673.40	673.33	NA	*Pseudomonas fluorescens* (VCRC B426)	(Prabakaran et al. 2015)
7	Di-rhamnolipid	649.4, 675.5 and 677.5	NA	NA	*Pseudomonas putida* KT2440 pVLT33_rhlABC	(Behrens et al. 2016)
8	Mono-rhamnolipid	503.3, 529.3 and 531.4				
9	Mono- and Di-rhamnolipid	502 and 647	527 and 673.5	NA	*Pseudomonas aeruginosa* MTCC7815	(Sharma et al. 2019a)
10	PHB	NA	128	NA	*Methylopila oligotropha*	(Karolski et al. 2018)
11	PHB	NA	86 and 100	NA	*Clostridium*, *Pseudomonas* and *Rhodopseudomonas*	(Guerra-Blanco et al. 2018)

(continued)

Table 2 (continued)

S. No	Metabolites	m/z			Microbes	Ref
		LC–MS	MALDI-TOF–MS	GC–MS		
12	EPS	NA	4093 and 5228	NA	*Thalassiosira pseudonana*	(Ai et al. 2015)
13	EPS	NA	1970	434 and 390	*Neopestalotiopsis* sp. SKE15	(Fooladi et al. 2019)
14	EPS	NA	1123.9	658.3	*Bifidobacterium longum* subsp. *Longum* 35624TM	(Altmann et al. 2016)

have elucidated their outstanding activity over existing chemically synthesized alternates. These compounds are economic, non-hazardous with biocompatibility and biodegradability aspects. However, their poor yield still obstructs their commercialization and is a major drawback that needs to be studied extensively. Understanding the pathways involved in their synthesis is a crucial step and researchers should look for syntrophic microbial consortium designing in order to improve the product yield. Another aspect to be looked into is low-cost substrates or waste products as the energy source to make the entire system cost-effective and eco-friendly.

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Core Flooding Studies Using Microbial Systems

Poulami Datta, Sombir Pannu, Pankaj Tiwari, and Lalit Pandey

1 Introduction

Microbial enhanced oil recovery (MEOR) is a green substitute and feasible strategy using biological molecules for the tertiary retrieval of crude oil from the reservoirs. One of the major potential bio-products for MEOR application is the biosurfactants, which are a class of amphiphilic or amphipathic biomolecules containing both hydrophobic or non-polar and hydrophilic or polar moieties in the same biomolecule. Hence biosurfactants possess the capability of accumulating at interfaces of a system and notably alter the free-energy of these interfaces (Osman et al. 2019; Putra and Hakiki 2019; Rosen 1989; Wood 2019; Zhang et al. 2020). In comparison to the synthetic chemical surfactant counterpart, biosurfactants are primary/secondary metabolic products, synthesized by the living organisms and possess certain key properties, for example, no eco-toxicity, higher biodegradability, greater stability in an extensive range of pH, salinity and temperature, milder production environment, biocompatibility, more eco-friendly and diverse structure with properties. Further, the production of biosurfactants using economical renewable raw materials makes the process cost-effective. These advantages allow their employment and probable replacement of chemical surfactants in numerous industrial processes including oil recovery applications (Fenibo et al. 2019; Sáenz-Marta et al. 2015).

The petroleum industry sector has been a significant customer of surfactants as they could increase the solubility of the petroleum constituents and subsequently enhance the biodegradation process (Falatko and Novak 1992). Likewise, microbial biosurfactant production is known to be a vital microbial strategy to influence the

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P. Datta · S. Pannu · P. Tiwari · L. Pandey (✉)
Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Guwahati 781039, Assam, India
e-mail: lalitpandey@iitg.ac.in

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bioavailability of hydrophobic compounds (Xia et al. 2014). In the crude oil biodegradation approach, biosurfactants mobilize and retrieve the crude oil via mainly four different mechanisms; lowering oil viscosity along with oil–water and oil-brine interfacial tension (IFT), altering wettability or by emulsifying crude oil in the pH range of 5 to 6.5 and porosity and permeability alteration of the rock surfaces (Gao 2018; Gao and Zekri 2011; Karlapudi et al. 2018; Nikolova and Gutierrez 2020; Saxena et al. 2017; Varjani and Upasani 2017; Wood 2019). The complex constituents are transformed into simpler ones which change the traits of the crude oil and the viscosity is reduced which improves fluidity and recovery of crude oil. In addition, even biosurfactants in the crude form can be used in oil industries as they do not require a very high level of purity unlike food and health applications (Aitken et al. 2004). Moreover, biosurfactants are also quite compatible with the reservoir brine and the adsorption rate of biosurfactants on the reservoir formation rock is very low which makes the microbial surfactants ideal candidate to be employed for the EOR (Pal et al. 2018). Thus, introducing crude biosurfactants into the reservoir (ex-situ MEOR) or reservoir-simulated systems with the biosurfactant-producing microbes (in-situ MEOR) upsurge the recovery performance proficiency because biosurfactants are well-tolerant to a broad range of physicochemical and environmental alterations, such as high salt concentration ($\leq 20\%$), pH (2–12), and temperature (30–100 °C) (Jahan et al. 2020; Udoh and Vinogradov 2019).

The common bacterial producers of biosurfactants are as follows: *Bacillus* produces Surfactin (Pereira et al. 2013), Iturins and Lichenysin (Bonmatin et al. 2003; Halim et al. 2017), *Pseudomonas* produces Rhamnolipid (Bhardwaj et al. 2013; Bordoloi and Konwar 2008; Dobler et al. 2016; Makkar et al. 2011; Sharma et al. 2019a; Souza et al. 2014; Varjani and Upasani 2017), *Acinetobacter* produces Emulsan (Suthar et al. 2008) and Alasan (Mujumdar et al. 2019; Navon-Venezia et al. 1995), *Rhodococcus* produces Viscosin and Trehalose lipids (Silva et al. 2014). Many yeast strains such as *Candida tropicalis*, *Geotrichum candidum, Galactomyces pseudocandidum, Aureobasidium pullulans* and *Galactomyces geotrichum* are reported to produce glycolipids, form stable emulsions and reduce ST (Brumano et al. 2017; Eldin et al. 2019; Mulligan 2005; Sáenz-Marta et al. 2015; Yalçın et al. 2018). *Candida* produces Sophorolipid (Saborimanesh and Mulligan 2015). In MEOR, glycolipids along with lipopeptide biosurfactants are recognized to offer huge commercial and industrial prospectives towards extensive applications (Bachmann et al. 2014).

Core flooding studies are one of the laboratory scale methods to examine the residual oil mobilization caused by microbial activity or their metabolites. Core flooding experimental trials are performed to simulate and assess reservoir conditions and typically comprise of a sample holder with rock samples through which different solutions are injected for the oil recovery. Core flooding studies for MEOR are performed either using microbes (in-situ MEOR) or their metabolites (ex-situ MEOR). Among the wide diversity of biosurfactants, surfactin (lipopeptide) and rhamnolipid (glycolipid) have been commonly considered for core flooding studies (Kubicki et al. 2019; Niu et al. 2020; Osman et al. 2019; Patel et al. 2015; Zhang et al. 2020). In this chapter, the fundamentals of core flooding experiments for MEOR are described. Core flooding investigations utilizing biosurfactant and mixed biosurfactant systems and key findings are discussed. A comprehensive table listing various isolated microbes and their metabolites explored for the MEOR studies in core flooding is provided for a better demonstration of potential applications. Different mathematical models developed to simulate oil recovery from reservoirs by applying MEOR have been briefly discussed.

2 Basics of Core Flooding Experiments Using Biosurfactant Systems

Core flooding experiments are systematically conducted to simulate and assess reservoir conditions for the validation of EOR practices (Phukan et al. 2019, 2020; Saha et al. 2018a, b). Four types of core setups are commonly used for oil recovery purposes including natural reservoir cores, artificial cores, micro-models and sand-packed columns (Sun et al. 2011). Different types of oil-field simulating bioreactors are used like glass bioreactors (Callbeck et al. 2011), syringe bioreactors (Bordoloi and Konwar 2008; Gudina et al. 2013), acrylic bioreactors (Rellegadla et al. 2020; Suthar et al. 2008) and many more. The overall efficiency of core flooding is dependent upon several factors such as the reservoir properties (i.e. lithology, nature of the porous materials, dimension of the simulated system, porosity, permeability), reservoir temperature, incubation period, crude oil API gravity and composition/constituents of flooding fluids (Dhanarajan et al. 2017). In the case of MEOR, core-flooding studies investigate the ability of the microbes and their metabolites especially biosurfactants to improve oil recovery. A schematic representation of the sand pack column flooding process for biosurfactant mediated MEOR is shown in Fig. 1. Sequential steps involved to perform core flooding experiments using biosurfactants are briefly discussed here (El-Sheshtawy et al. 2015; Suthar et al. 2008).

I. **Core saturation with brine**: Firstly, nitrogen gas is purged and passed through the entire system for deoxygenating followed by saturation of column with brine solution. The pore volume (PV) of the column is calculated by measuring the volume of brine essential to saturate the column.
II. **Saturation of core by oil**: Crude oil is passed through the column following the same protocol of brine flooding. During this process, the brine solution is discharged from the exit of the column. Initial oil saturation or Original Oil in Place (OOIP) is estimated by measuring the volume of brine solution displaced during the oil saturation.
III. **Water flooding**: The sand pack saturated with oil is flooded with water until no release of oil in the effluent. It could be assumed that residual oil saturation (S_{or}) is achieved. The volume of displaced oil is measured to estimate the oil recovery by water flooding and the quantity of crude oil retained in the column.
IV. **Microbial surfactant flooding**: This step is also performed similar to oil and brine flooding. A specific pore volume of biosurfactant solution is injected

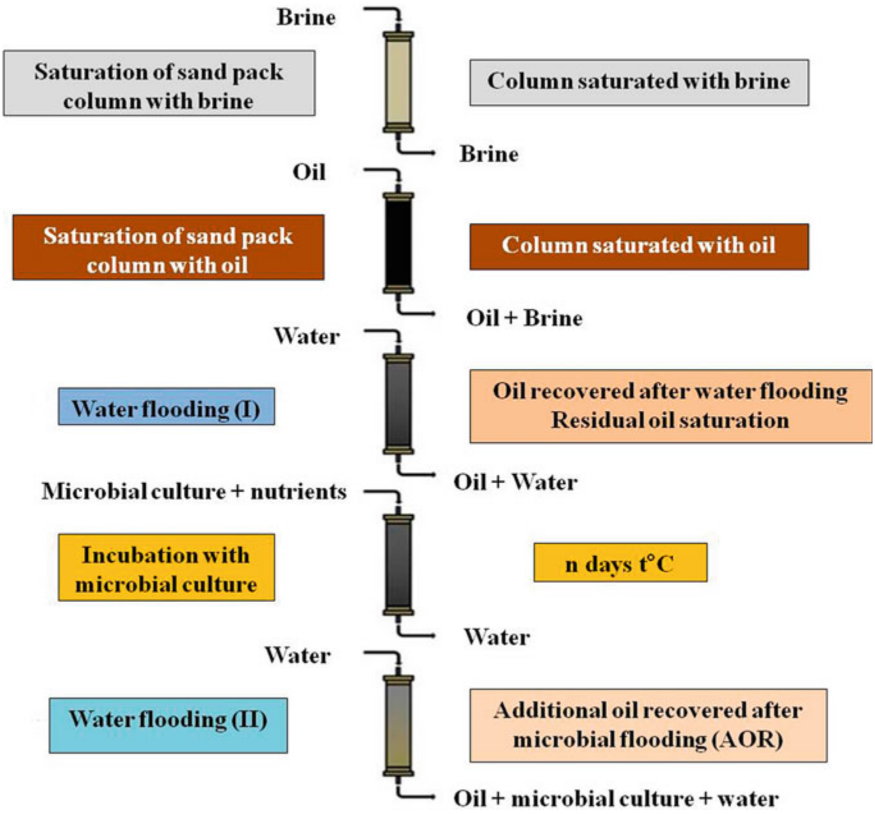

Fig. 1 Schematic diagram of sand pack column flooding process using biosurfactant mediated MEOR. [Adapted with permission from (Gudina et al. 2013)]

> into the column at a definite flow rate to maintain a particular duration of incubation. Then, once more the column is saturated with brine. Discharges from the column are collected to determine the amount of oil retrieved by biosurfactant solution.

The percentage of oil recovered is estimated using the followings expressions (Suthar et al. 2008):

Pore Volume (PV) (ml or cc) = Volume of brine essential to saturate the column.
Original Oil in Place (OOIP) (ml) = Volume of brine displaced by oil saturation.
S_{orwf} (ml) = Residual oil saturation after water flooding.
S_{orbf} (ml) = Oil collected over residual oil saturation after biosurfactant flooding

$$Initial\ Water\ Saturation\ (S_{wi})(\%) = (X/PV) \times 100 \tag{1}$$

where, X = Pore Volume − Volume of brine collected after injecting oil

$$Initial\ Oil\ Saturation\ (S_{oi})(\%) = (OOIP/PV) \times 100 \tag{2}$$

$$Residual\ Oil\ Saturation\ (S_{or})(\%) = (X_i/OOIP) \times 100 \tag{3}$$

where, X_i = OOIP − Volume of oil collected after water flooding

$$\begin{aligned} &Additional\ Oil\ Recovery\ Over\ S_{orwf},\ (AOR)(\%) \\ &= \frac{Oil\ recovered\ using\ biosurfactant}{Oil\ in\ column\ after\ water\ flooding} \times 100 \end{aligned} \tag{4}$$

3 Core Flooding Investigations Utilizing Biosurfactant Systems

Core flooding experiments are performed to examine the residual oil mobilization caused by microbial activity or their metabolites. A typical experimental setup for conducting core flooding trials is discussed in Sect. 2.6 of Chap. 2. The standard or natural cores (collected straight from the designated reservoirs) are coupled with a continuous flow system and termed as core flooding apparatus. The experimental procedures comprise the insertion of rock samples (i.e. Berea sandstone or carbonate core) inside a sample holder through which various fluids (formation brine solution followed by crude oil) are injected for simulating reservoir conditions. The temperature and pressure of the core are adjusted to imitate the targeted reservoir environment. The representative machineries of a core flooding system comprise of an oven having a temperature sensor for controlling temperature, a multichannel peristaltic pump (crude oil pump, water pump, chemical or microbial solution pump and overburden pressure pump), core holder and core reactor, injection cylinder, back pressure regulator (BPR), density meter, high-pressure regulator, fraction collector and an optional computerized tomography (CT) scanner (Rellegadla et al. 2020).

Core flooding trials usually facilitate in estimating the optimum options required for the maximum oil recovery. The number of crucial parameters is determined during the course of flooding for example relative permeability, oil saturation, wettability and additional oil recovery. Although the core flooding offers an accurate assessment of the prospects of microbes or their metabolites for the mobilization of residual oil, it has certain drawbacks. It requires a complex and expensive experimental setup. The availability and maintenance of native cores from reservoirs are another constraint. The screening of different cultures requires separate core setups (Rellegadla et al. 2020).

3.1 **In-Situ** *and* **Ex-Situ** *MEOR in Core Flooding Experiments with Biosurfactants*

Core flooding studies for MEOR are performed either using microbes or their metabolites. The incubation of microbes in core setup imitates in-situ MEOR, while injection of microbial metabolites, i.e. biosurfactants emulates ex-situ MEOR. Generally, inherent microbes isolated from the oil reservoir are injected with nutrients to the core sample and incubated for a certain period for the microbial growth and in-situ production of metabolites.

In a study, *B. licheniformis* was isolated from Zilaei oil reservoir of Southwest Iran which could utilize crude oil (API 37°) at 50 °C and subsequently synthesize glycolipid. The core flooding studies were performed in a sandstone core with 16% of porosity and 7.5 mD of permeability. The isolated bacteria with growth media (0.25 PV) was injected and incubated for 7 days at 50 °C. 18.1 mg/L of glycolipid was produced, which decreased ST and IFT to 23.8 and 0.93 mN/m, respectively. The oil viscosity was also decreased from 21.58 cP (41% reduction). This resulted in microbial tertiary oil recovery of 13.7% of OOIP at optimum conditions (Daryasafar et al. 2016). In a separate study, *Pseudomonas aeruginosa* L6-1, isolated from the formation brine of Xinjiang Oilfield China, was used for in-situ MEOR study in artificial cores with dimensions of 30.0 × 3.8 cm. 0.5 PV of nutrient solution with 2% (v/v) inoculum was injected into cores and incubated for 7 days at 35 °C under static culture conditions with and without aeration. The additional oil recovery (AOR) was 9.49% of OOIP in the case of anaerobic incubation, which enhanced to 12.58% of OOIP when 0.25 PV of air was pumped due to higher rhamnolipid production (Cui et al. 2017b). This study indicated the role of aeration in improving the production of biosurfactants (Datta et al. 2018). In another study, *Bacillus* sp., *Klebsiella* sp. and *Pseudomonas* sp. were screened for their biopolymer and biosurfactant producing ability among the 37 isolated hydrocarbon-degrading strains from hydrocarbon-contaminated soil samples from Gio, Ogoniland, Nigeria. Pure isolates, as well as consortia, were used for in-situ core flooding studies and 18.33–29.09% of AOR was achieved (Okotie et al. 2020).

The core flooding study was performed using thermo- and halotolerant rhamnolipid produced by *Pseudomonas aeruginosa* NCIM 5514, which was isolated from crude oil contaminated subsurface soil sample. The produced biosurfactant was stable under a broad temperature range of 30–100 °C, pH of 2–10 and salinity of 0–18%, w/v. The flooding experiments were carried out in Cerro-metal pack using Berea sandstone core (5.6 × 3.7 cm) at 70 °C. 0.5 PV of the biosurfactant solution was passed and 8.82% of AOR was achieved (Varjani and Upasani 2016). In another study, 0.6 PV of crude biosurfactant produced from isolated *Bacillus mojavensis* JF2, *Bacillus licheniformis* TT42 and *Bacillus licheniformis* K125 was injected in the column and incubated for 24 h. This was followed by flooding with brine. The AOR (%) was varied in the range of 31.7 to 46.8 (Suthar et al. 2008).

Comparative studies of in-situ and ex-situ MEOR in laboratory sand-pack columns were performed using *Pseudomonas aeruginosa* WJ-1, which was isolated from the production brine of Xinjiang Oilfield, China (Cui et al. 2017a). The core flooding experiments were carried out in stainless steel sand-pack columns with dimensions of 30.0 × 3.8 cm filled with oil sand at 35 °C. In the case of in-situ MEOR, 0.5 PV of microbes (1×10^7 CFU/mL) with media was injected and incubated for 10 days, while 0.5 PV of rhamnolipid solution (0.23 g/L) was injected and incubated for 10 days for ex-situ MEOR. The concentration of biosurfactant for in-situ MEOR was found to be 2.66 g/L. The in-situ and ex-situ MEOR altered the wettability of a hydrophobic plate (168.2°) to hydrophilic with a contact angle of 61.5° and 56.3°, respectively. This corresponded to the higher concentration of biosurfactant in the case of in-situ MEOR. Similarly, the oil–water IFT (mN/m) for in-situ was lower (1.71) than the ex-situ MEOR (5.49). The AOR for in-situ and ex-situ MEOR flooding studies were 7.46 and 4.64%, respectively (Cui et al. 2017a). This study highlighted the positive impact of biosurfactant concentration on the AOR.

4 Core-Flooding Studies Employing Mixed Biosurfactant Systems

The applicability of mixed biosurfactant systems (biosurfactant-chemical surfactant, biosurfactant-polymer, biosurfactant-bioalcohol system and biosurfactant-nanoparticles) have also been explored to maximize oil recovery. Table 1 summarizes the isolated biosurfactant-producing microorganisms from various terrestrial and aquatic environments worldwide, the maximum biosurfactant concentration and key surface properties, their influence in enhanced oil retrieval in different core flooding studies.

The effects of biosurfactant and chemical surfactant on EOR were explored in core-flooding experiments (5.17 × 3.75 cm) at 90 °C (Al-Wahaibi et al. 2016). Core plugs (average 23% of porosity, 13.7 mL of PV and permeability of 156 mD) from a Middle East heavy oil field containing $38 - 67\%$ of quartz and heavy crude oil (API of 13.5° and viscosity of 2450 cP) were used in core flooding studies. The chemical surfactant (ethoxylated sulfonate), biosurfactant (lipopeptide) and their mixture at a concentration of 0.25% (w/v) were applied for the EOR. The brine/oil IFT values reduced to 3.24, 3.97, 3.2, 3.11 and 4 mN/m in the presence of the chemical surfactant (CS), biosurfactant (BS), CS:BS; 75:25, CS:BS; 50:50 and CS:BS; 25:75, respectively. The injection of biosurfactant recovered more oil (6.8 to 18.5%) compared to chemical surfactant (1.4 to 11%). Further, the mixture of the biosurfactant and the chemical surfactant at different ratios resulted in the highest recovery of 27 to 34% (Fig. 2). It was hypothesized that the activity of the biosurfactant was enhanced in the mixture with the chemical surfactant presumably due to chemical interactions between the surface charges of the two surfactants. In turn, this synergetic effect enhanced the oil recovery by two folds. Thus the biosurfactant was structurally modified in the presence of the chemical surfactant and the mixture improved the recovery efficiency (Al-Wahaibi et al. 2016).

Table 1 Summary of core flooding experiments along with the contributing microbes and the biosurfactant system and their effect on interfacial properties and enhanced oil recovery

Microbes	Isolation site	Produced metabolites	Surface properties	Core flooding substrate	Core properties (L × D in cm), Porosity, Permeability	Flooding type (conditions), (Pore volume, mL)	Recovery	References
TERIK consortium of *Clostridium* sp.	High temperature oil reservoir of ONGC, Gujarat	Biomass (600 mg/L), bio-surfactant (325 mg/L), and VFA (250 mg/L) at 65°C	Reduction of ST to 34 mN/m	Crude oil from Kalol reservoir	(10.11 × 3.87), 17.8%, 2.14 to 1.39 mD	In-situ (65 °C, 1300 psi for 10 days), (21.1)	Total recovery of 75% with 19% incremental recovery	(Sharma et al., 2020)
Enterobacter aerogenes B19 and *Bacillus cereus* ISU-02	Formation water from Basra, South Iraq	Biosurfactant and bioemulsifier	ST reduction to 28.93 and 27.61 mN/m, respectively	Crude oil (API 29°)	Sandstone core plug, (16 and 24.8%),(197 and 843 mD), respectively	In-situ (40 °C for 14 days), (4 and 4.4)	34 and 66.9% oil recovery, respectively	(Hamzah et al., 2020)
Pseudomonas aeruginosa WJ-1 and *Enterobacter cloacae* FY-07	Menggulin and Jilin oilfield, China respectively	Rhamnolipid from WJ-1 and biopolymer from FY-07	ST reduction to 30 mN/m	Crude oil from Xinjiang Oilfield, China	Heterogeneous cemented cores (30 × 4.5 × 4.5), 28.4%, 1.092 μm^2	In-situ (34 °C for 14 days)	17% more oil recovery with combined FY-07 and WJ-1 flooding	(Bi et al., 2019)
Consortia TERIJ-188 of *Thermoanaerobacter brockii, T. italicus, T. mathranii, T. thermocopriae*	Formation fluid from oil-wells of Jotana, Ahmedabad, Gujarat	Biomass (850 mg/L), bio-surfactant (500 mg/L), and VFA (495 mg/L) at 70 °C	ST reduction to 32 mN/m	Crude oil from oil-head of Kalol and Jotana oilfields	(7.8 × 3.78), 22.5%, from 28.3 to 11.3 mD	In-situ (70 °C, 1874 psi for 10 days), (19.7)	19.2% incremental oil recovery	(Sharma et al., 2018)

(continued)

Table 1 (continued)

Microbes	Isolation site	Produced metabolites	Surface properties	Core flooding substrate	Core properties (L × D in cm), Porosity, Permeability	Flooding type (conditions), (Pore volume, mL)	Recovery	References
Consortium of *Enterobacter Cloacae* and *Enterobacter Hormaechei*	Formation water of Southwest Iranian oil reservoir	Biosurfactant (1.53 g/L)	ST and IFT reduction to 31 and 3.2 mN/m, respectively	MIS crude oil (API 35.93°), Gachsaran crude oil (24.2°)	Dolomite core (8 × 2.54), 14%, 11 mD	In-situ (40 and 60 and 80 °C, 7 days), (8.1, 7.9 and 7.78)	53.2, 56.2 and 61.33% of OOIP	(Hosseini & Tahmasebi, 2020)
Pseudomonas sp. TMB2	Oil contaminated soil of Tejpur, upper Assam, India	Rhamnolipid (2.8 g/L)	ST and IFT reduction to 33.4 to 0.8 mN/m, CMC of 120 mg/L, wettability change from 75° to 42°	Light crude oil from ONGC, Jorhat, Assam, India	Sandstone core plug (8.9 × 3.74), 19.7–22%,	Ex-situ flooding (70 °C), (19.25–21.5)	10.7–16.7% additional oil recovery, residual oil recovery of 39–55%	(Haloi et al., 2020)
Fungal strain *Trichoderma* MK116452	Seri Chermin filling station, Brunei Darussalam	Glycolipid (6.33 g/L)	EI of 17.82% and 7.3 cm oil displacement diameter	Crude oil	Sand pack column (50 g sand mixed with 15 mL crude oil in 100 mL Erlenmeyer flask)	Ex-situ biosurfactant flooding	63.33% oil recovery in which only 16% due to water-flood	(Shivanand et al., 2020)

(continued)

Table 1 (continued)

Microbes	Isolation site	Produced metabolites	Surface properties	Core flooding substrate	Core properties (L × D in cm), Porosity, Permeability	Flooding type (conditions), (Pore volume, mL)	Recovery	References
Bacillus subtilis 22.2	NA	Surfactin	IFT of 0.056 and 0.110 mN/m for Oil-1 and Oil-2, Wettability from 71° to 35°	Two stock tank crude oil (API 27.3 and 41.6°)	Sandstone core BS1(10.2 × 3.8), 21%, 292 mD, BS2 (10.2 × 3.8), 21.5%, 153mD	Ex-situ (25 °C)	1.3–5% additional oil recovery	(Hadia et al., 2019)
B. licheniformis	Hydrocarbon contaminated soil from Beijing, China	Lichenysin-A (1.1 g/L)	ST and IFT reduction to 26.21 and 0.26 mN/m, CMC 21 mg/L, EI 66.4%, wettability from 50° to 17°	Light crude oil from Xinjiang oilfield, China	Sand pack column (4.5 × 5 × 17.5)	Ex-situ flooding, (59–62 ml PV)	32% additional oil recovery	(Ali et al., 2019)

(continued)

Table 1 (continued)

Microbes	Isolation site	Produced metabolites	Surface properties	Core flooding substrate	Core properties (L × D in cm), Porosity, Permeability	Flooding type (conditions), (Pore volume, mL)	Recovery	References
Bacillus licheniformis W16	Soil sample near Omani oil well	Lichenysin-A (0.52 g/L)	Reduction of ST and IFT to 24.3 and 2.47 mN/m, wettability alteration from 56° to 19°	Light crude oil (API 36.51°) from Petroleum Development Oman (PDO)	Berea sandstone core (7.6 × 3.8) (18–22%), (250–260 mD)	Ex-situ (60 °C) (17–19 cc)	24–26% residual oil recovery	(Joshi et al., 2016)
Bacillus licheniformis DS1	Oil reservoir in Indonesia	Lichenysin A	CMC 157.5 mg/L, EI = 94.28%	Light crude oil from oil reservoir wellhead in Sumatra, Indonesia	Berea sandstone core (7.4 × 4.4), 12.84%	Ex-situ flooding, (50 °C for 12 h), (14.84 mL)	5.4% additional oil recovery	(Purwasena et al., 2019)
Biosurfactant from *Bacillus subtilis* W19, Biopolymer from *Schizophyllum commune* ATCC38548	NA	Biosurfactant-biopolymer *Schyzophyllan* and Na_2CO_3 (ASP flooding)	IFT reduction to 0.02 to 0.1 mN/m	Light crude oil (API 27.15°) from Omani oilfield	Reservoir core (5.8 × 3.6), 30–35% and Berea sandstone core plugs (7.2 × 3.7 cm), 20%, 500–1000 mD	Ex-situ (50 °C, 1000 psi) (22.18 for reservoir and 16.37 for Berea)	AOR values of 14 and 32% OOIP from reservoir core and Berea core, respectively	(Al-Ghailani et al., 2018)

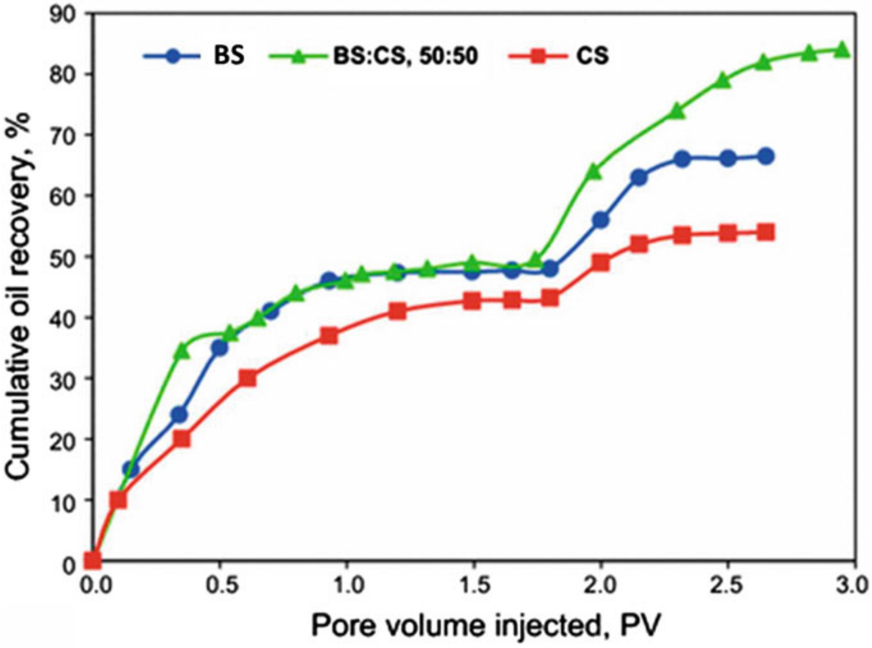

Fig. 2 Comparison of maximum cumulative oil recovery via hot water injection (continued until saturation) followed by ethoxylated sulfonate a chemical surfactant (CS), lipopeptide a biosurfactant (BS), or their binary mixtures. [Adapted from (Al-Wahaibi et al. 2016)]

The performances of biosurfactants, betaine (amphoteric chemical surfactant) and their mixtures were examined in core flooding experiments for high-temperature and high-saline reservoirs (Ding et al. 2017). Core flooding trials were carried out in artificial cores (20 × 2.5 cm) having a porosity of 28.1% and permeability of 697 mD. The biosurfactants were produced from isolated thermophilic strains from the Shengli Oilfield. The viscosity and density of the crude oil were 3456 cP (at 50 °C) and 0.9939 kg/m^3, respectively. 1 PV of biosurfactants, betaine or their mixture biosurfactants mixtures solution was injected and incubated for 7 days at 90 °C. The is followed by secondary water flooding. The lowest oil/water IFT value of biosurfactants, betaine and their mixture were 0.6, 0.1 and 5×10^{-3} mN/m, respectively. The AOR values were found to be betaine (5.9%) < biosurfactants (5.83 to 6.03%) < mixture of betaine and biosurfactants (10.2 to 11.8%) (Ding et al. 2017). This study also indicated that the interfacial behaviour and emulsifying ability of the biosurfactants mixtures were greatly improved due to the incorporation of chemical surfactants. In a similar core flooding study, experimentations were performed in Berea sandstone cores (7.2 × 4.5 cm) with the permeability of 50 mD, PV of 18 mL and 18% of porosity utilizing crude oil from the North sea (API 40°) and different surfactants. 13.6% of OOIP was recovered by injecting 0.3% surfactin solution as compared to 6.3% recovery using 0.3% SDS solution. The recovery was enhanced to

23.9% of OOIP by injecting their mixture (Saito et al. 2016). These findings highlight the better recovery efficacy of biosurfactants as compared to chemical surfactants, which can be further enhanced by adding chemicals to the biosurfactants.

The combinatorial efficiency of 0.3 wt % Na_2CO_3 and 2 wt % microbial cultures of *Bacillus subtilis* XT-1 and 0.2 wt % hydroxy-polyacrylamide (HAMP) was compared with the conventional ASP system using alkyl-benzene sulfonates as a chemical surfactant. The core flooding experiments were carried out in an artificial core (30 × 3.8 cm) from Xinjiang Oilfield. 0.5 PV of HAMP or ASP solutions were injected followed by incubation for 7 days at 45 °C. The AOR values were improved in the case of HAMP flooding (17.11–19.91%) as compared to ASP flooding (13.67–14.28%) (Wang et al. 2019b).

Apart from chemical surfactants, green surfactants were also amended with biosurfactants. The effect of green surfactant (Alkyl Polyglucoside or APG generated from coconut, palm oil, corn, potato or wheat residues) and bio-alcohol (butanol) along with biosurfactants (from anaerobic *Bacillus mojavensis* strain JF-2) has been investigated for the MEOR process. The core flooding experiments were carried out in Berea sandstone (8.15 × 3.85 cm) having PV of 18.95 mL, 20.45% of porosity and 470.86 mD of permeability with 0.5% APG, 45 mg/L of biosurfactant along with 0.5% butanol. Two preparations of surfactant and co-surfactant mixtures were chosen for core-flooding trials. In the first preparation, JF-2 biosurfactant was mixed with butanol which produced only 1.7% of AOR. Then the biosurfactant (anionic) was combined with non-ionic green surfactant APG and butanol; which resulted in about 24.7% of AOR (Haq et al. 2020, 2018).

The influence of nanomaterials such as silica nanoparticles (NP) along biosurfactants was investigated in instantaneous flooding. Micromodel flooding tests were conducted employing rhamnolipid and hydrophilic spherical silica NPs in which positive synergism was observed between them which altered the wettability to water-wet condition and decreased IFT (1.85 mN/m). The core flooding studies were performed using crude oil from the North Iranian Azadegan oilfield in a carbonate core (6.2 × 3.74 cm) with 14.1% of porosity and 1.45 mD of permeability at 80 °C. The BS-spherical NP solution yielded 26.1% of oil repossession which was slightly higher than that of the BS-sponge-like NP solution (25.1%). The spherical NPs were found to perform better due to the higher uniformity, which led to improved distribution and more effective interactions with crude oil constituents (Khademolhosseini et al. 2019). Another novel bio-nanofluid containing silica nanoparticles and rhamnolipid in brine was explored as a promising agent for core-flooding in low-permeability Berea sandstone (10 × 2.5 cm) using crude oil at 30 °C. 1 PV of the prepared bio-nanofluid was injected into the core followed by water flooding. The wettability alteration capability of bio-nanofluid from 135° to 55°, i.e. from oil-wet towards water-wet state resulted in AOR of 5.3–6.8% (Wang et al. 2019a).

5 Application of Mathematical Modelling for MEOR

Reservoir simulators (discussed in Chap. 2) hold the advantages of long flooding hours and can simulate the MEOR, with modification required to include the physical and chemical phenomena that occur during the process, to compare the lab-scale experimental results and predict the performances (Jeong et al. 2021) The mathematical models for MEOR include a set of continuity equations representing pressure, saturation, interfacial area, bacterial concentration, nutrient concentration, biosurfactant concentration, relative permeabilities and capillary pressure to predict the IFT, capillary number and residual oil saturation (Landa-Marbán et al. 2017). These models are based on certain assumption as MEOR is a complex process comprising mainly six components; reservoir core, oil, water, bacteria, substrate and metabolites/biosurfactant. Kowalewski and co-workers simulated MEOR in Eclipse for experimental data (Sunde et al. 1992) and compared the predicted oil production with the reported one (Kowalewski et al. 2006). The simulation was performed with a recursive set of relative permeabilities. Change in wettability was related to the set of relative permeabilities. The simulation data of oil production agreed well with the experimental observations (Fig. 3) (Kowalewski et al. 2006). Simulations of MEOR core flood depicted a gradual reduction in IFT and a change in wettability ass possible mechanisms of improved oil recovery. These predictions also correlated with various experimental observations (Datta et al. 2020; Sharma and Pandey 2020).

Nielsen *et al.* have developed a mathematical model of MEOR based on partitioning of metabolites between the oil and the water phases by combining reactive

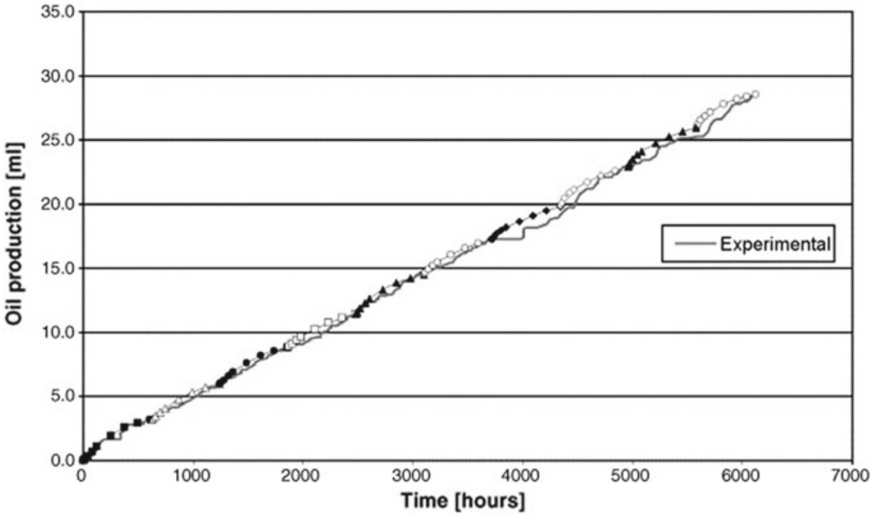

Fig. 3 The oil production curve from the simulations by applying MEOR. Symbols correspond to simulated data and line refers to experimental data. [Adapted with permission from (Kowalewski et al. 2006)]

transport with a simple compositional approach (Nielsen et al. 2010b). The modified model was based on the IMPEC (implicit pressure and explicit composition) to decouple flow and reactive transport. The chemical reaction includes the growth of microbes, the consumption of nutrients and the formation of metabolites or surfactants. The bacterial growth rate was described by the Monod model being independent of temperature, pressure, pH and salinity. Three methods namely the capillary number method, Coats' method and the Corey relative permeability interpolation method were implemented for determining the reductions of IFT and the final oil recovery was found to be similar for the three methods (recovery of 38 to 44%). The simulation results predicted an increment of 9% in oil recovery by implementing MEOR. The oil recovery was found to be affected by surfactant partitioning, bacterial growth rate and injection concentrations (Nielsen et al. 2010a; b).

Further, Hosseininoosheri and coworkers developed a new model incorporating first-order Monod kinetic equations as a function of temperature, salinity and pH (Hosseininoosheri et al. 2016). UTCHEM, a three-dimensional, multiphase and non-isothermal chemical compositional reservoir simulator, was used to implement the effect of environmental factors (i.e. temperature, salinity and pH) and predict the MEOR performances, i.e. oil recovery. The simulation predicted an incremental oil recovery of 10–15% by implementing MEOR. The effects of nutrients concentration, temperature, salinity and temperature on oil recovery were predicted to be significant, while pH had an insignificant impact (Hosseininoosheri et al. 2016). The results indicated that in-situ biosurfactant production can also be methodically modelled based on environmental factors (Hosseininoosheri et al. 2016), which agreed with various experimental observations (Datta et al. 2018; Sharma et al. 2019b; Verma et al. 2020). In a recent study, environmental factors (temperature, pressure and salinity) were considered in an integrated manner to accurately model MEOR performances (Jeong et al. 2021). Improvements in recovery efficiency by MEOR in field applications were related to increased recovery factor and decreased water–oil ratio. The actual reservoir model predicted a 7% increase in oil recovery by implementing MEOR at optimized conditions (Jeong et al. 2021).

6 Conclusion

MEOR is getting recognition with the fundamental establishment for a green substitute and feasible strategy for the tertiary recovery of crude oil from the reservoirs. The exploration of microbial communities from various environments allows identifying specific microbes and their probable further utilization in the oil recovery through the core flooding approach. Microbes along with nutrients or metabolites, i.e. biosurfactants alone or in combination with chemicals are applied for in-situ and ex-situ MEOR, respectively. The efficacies of these MEOR have been validated in various core-flooding studies. The positive impact of biosurfactant concentration on the AOR has been established. Biosurfactants are found to alter wettability, reduce oil–water IFT and emulsify crude oil. Further, the recovery efficiency is improved when biosurfactants are applied in combination with chemical surfactants. The biosurfactants are structurally modified in the presence of the chemical surfactant, which in turn improved the recovery efficiency for mixed systems. Further detailed investigations are needed to establish the optimum ratio of biosurfactants and chemicals for maximum oil recovery. Different reservoir simulators have been explored to predict the MEOR performances and compare them with lab-scale experimental results. More simulation studies are required in this front to model the actual reservoirs to comment on the applicability of MEOR and predict the oil recovery by MEOR. The new models should incorporate the maximum possible numbers of parameters influencing the efficiency of the MEOR process.

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Recent Case Studies of In-Situ and Ex-Situ Microbial Enhanced Oil Recovery

Poulami Datta, Pankaj Tiwari, and Lalit Pandey

1 Introduction

Microbial enhanced oil recovery (MEOR) is a kind of enhanced or tertiary oil recovery (EOR) explored when oil retrieval is no longer attainable employing the primary and secondary conventional methods (Singh et al. 2014). The MEOR strategy uses the microorganisms or their produced metabolites such as biopolymers (Couto et al. 2019; El-Hoshoudy and Desouky 2018; Gao et al. 2018; Hong et al. 2019b; Jia et al. 2018; Tianyuan et al. 2019; Wang et al. 2018c; Zhao et al. 2018), biogenic acids (Rathi et al. 2018), bio-solvents (Wu et al. 2006), bio-gas (Anderson et al. 2017; Ansah et al. 2018; Ma et al. 2018; Rathi et al. 2018; Shabani and Vilcáez 2017; Sugai et al. 2010; Vilcáez et al. 2018; Xu et al. 2019) and biosurfactants (Datta et al. 2018,2020; Sharma et al. 2019a, 2019b; Sharma and Pandey 2020; Verma et al. 2020) for mobilizing residual oil (Harner et al. 2011; Sen 2008). Each of the metabolites play a different role in the process of EOR. In some cases, two or even more types of biometabolites have been combined in order to increase the oil retrieval (Dhanarajan et al. 2017; Pedraza-de la Cuesta et al. 2018; Qi et al. 2018; Souza et al. 2018). The biological metabolites have also been combined with the chemical agents to maximize the oil recapture (Ding et al. 2017; Jia et al. 2017; Saxena et al. 2017). Even in some cases, the metabolites have been combined with the nanoparticles (Al_2O_3, TiO_2, and SiO_2) to formulate nanofluids, emphasizing the synergistic effects of surface area and interfacial properties for conducting the EOR process (Amani 2017; Wang et al. 2018a).

MEOR being a biological method is easy to operate, less energy-consuming, cost-effective, environment-friendly and does not require any major changes in the field infrastructure to implement this process (Lazar et al. 2007; She et al. 2019; Shibual

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P. Datta · P. Tiwari · L. Pandey (✉)
Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Guwahati 781039, Assam, India
e-mail: lalitpandey@iitg.ac.in

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et al. 2014) MEOR can be implemented mainly in two ways: (i) by injecting the externally produced metabolites into the reservoir (ex-situ) and (ii) by inoculating extremophilic isolated microbes or introducing suitable nutrients to stimulate the inherent microbes or stimulating them in some other way so that the native biosurfactant producing microorganism can function properly inside the oil reservoir (in-situ) (Bachmann et al. 2014; De Almeida et al. 2016; Jahan et al. 2020; Nikolova and Gutierrez 2020; Safdel et al. 2017; Singh et al. 2007). However, both approaches have their own characteristics with pros and cons. The in-situ process costs less than the ex-situ one but requires a longer incubation time and is affected by the internal environmental conditions (high salinity and temperature) of the reservoir. These are still some of the implications of the in-situ MEOR process. Whereas, in the case of ex-situ, the productivity is comparatively higher however, the production and purification costs are added. So from the economic point of view, ex-situ MEOR is not very preferred. However, the ex-situ process has a short operating cycle and has better reservoir condition adaptability as well as a higher success rate (Banat et al. 2010; Geetha et al. 2018; Patel et al. 2015; Youssef et al. 2009; Zhang et al. 2020).

In this chapter, contemporary in-situ and ex-situ case studies worldwide have been discussed. The role of internal reservoir microflora on in-situ MEOR and the other primary factors on which the processes are dependent have been elaborated. The consecutive phases of both the MEOR and the scale-up possibility from lab scale to pilot scale and finally to industrial scale have been explained with pictorial representations.

2 Case Studies of In-Situ MEOR

In-situ MEOR is carried out through (a) the activation of the endogenous reservoir microflora; and (b) incorporating stimulator to decrease oil viscosity and upgrade mobility of crude (Yue et al. 2017). The injection of microbes (indigenous or specifically screened) and/or nutrients into the reservoir is followed by a shut-in period for a few weeks to months. During this period, microbes synthesize a number of biometabolites that play an imperative task to extract petroleum (Youssef et al. 2007, 2013). The physiochemical properties of a reservoir play a significant role in the MEOR process. Hence this is vital to know about the suitable growth conditions for the isolates along with the ambience of the field (She et al. 2019). The accomplishment of the in-situ MEOR procedure is dependent on certain parameters such as the choice of the reservoir type, the appropriate screening of the prospective microbial community, the feasible activities of microbes in the internal reservoir environment, the quantity of metabolites produced, the mechanism of mobilizing oil and other cost-effective aspects (Jang et al. 1983). Oil reservoirs all over the world are full of diversity in terms of the interior environment and fluid characteristics. Because of the reservoir miscellany, variation in the native microbial population is also evident for individual oil wells, which contribute during the in-situ MEOR process (Rabiei et al. 2013; Rathi et al. 2018).

There are primarily three phases for the *in -situ* study to transfer the technology from “lab scale to field scale” as described in Fig. 1. In the initial phase, laboratory experiments are carried out with the indigenous or exogenous strains to analyze their ability for the desired metabolites, i.e. biosurfactant production in the reservoir conditions. This includes microbial isolation, screening and identification using modern molecular biology techniques, and testing interfacial behaviour. Then the suitable proportions of various nutrients are optimized under the environmental conditions of the oilfield of interest. In the second phase, bacterial growth kinetics, biosurfactant production kinetics, concerned oilfield mineralogy, previous flooding history and rheology of fluids are monitored. Thereafter, exogenous microbes and/or cheaper nutrients are inoculated into the oil wells (shut-in phase) to synthesize the desired metabolites, i.e. biosurfactants in an adequate concentration needed for the oil retrieval. After the shut-in phase, oil wells are flooded with formation brine, then the propagation of indigenous or injected bacteria along with additional oil recovery are tracked. The field trials are essential to scale up the process from a research laboratory scale towards an industrial level. So, in the final phase, depending on the pilot studies, it is decided whether to proceed with the field-scale trials or not (Geetha et al. 2018).

There are several techniques of MEOR field trials such as Microbial flooding recovery (MFR), Cyclic microbial recovery (CMR), Microbial selective plugging recovery (MSPR), microbial wax removal (MWR) and many more (Safdel et al. 2017). MFR is the most applied technique (33%) in the world as shown in Fig. 2. However, it varies in different countries. In China, MWR technique of MEOR is applied in more than 3000 wells (65%) out of 4600 wells (She et al. 2019). Likewise,

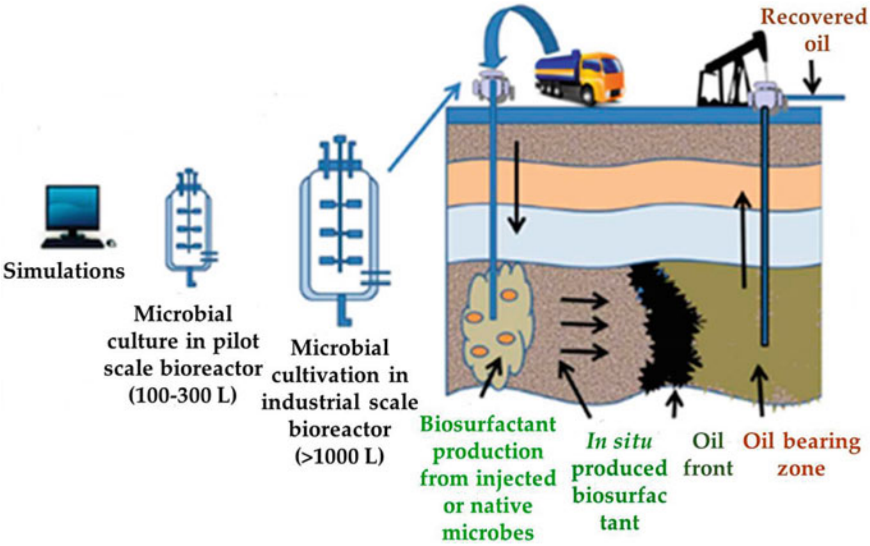

Fig. 1 Illustration of biosurfactant mediated in-situ MEOR. [Adapted from Geetha et al. (2018)]

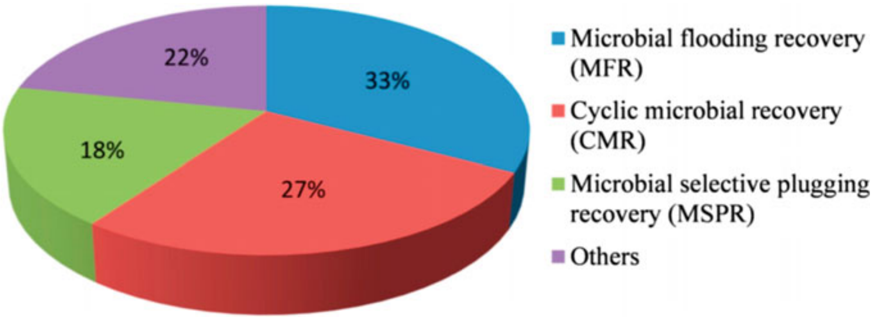

Fig. 2 Different technologies applied in MEOR trials worldwide. [Adapted with permission from Safdel et al. (2017)]

among various MEOR techniques, mainly CMR and MSPR have been employed in India utilizing thermophilic halophilic anaerobic bacterial consortia made up of *Clostridium* sp., *Thermoanaero* sp. and *Thermococcus* sp. where 3% of molasses was used as the nutrient (Patel et al. 2015). In India, the Oil and Natural Gas Corporation (ONGC) Limited in partnership with The Energy and Resources Institute (TERI, New Delhi) and with the Institute of Reservoir Studies (IRS, Ahmedabad) carried out a number of field trials using huff and puff technique utilizing the isolated anaerobic microbial community from the internal reserve. Thereafter, field tests of 12 wells in 4 fields exhibited three times augment in petroleum recovery indicating a major decline in water-cut (Sen 2008).

Safdel and co-workers compiled the 47 field trial cases from 21 countries as shown in Fig. 3 (Safdel et al. 2017). This specified the implementations of MEOR across the world and more efforts were made in the USA followed by China. In-situ MEOR was carried out in various Chinese oilfields including Shengli, Xinjiang Liuzhongqu, Dagangkongdia, Jilin, Huabe Baolige, Changing Jing'an Y9 and Daqing, and the

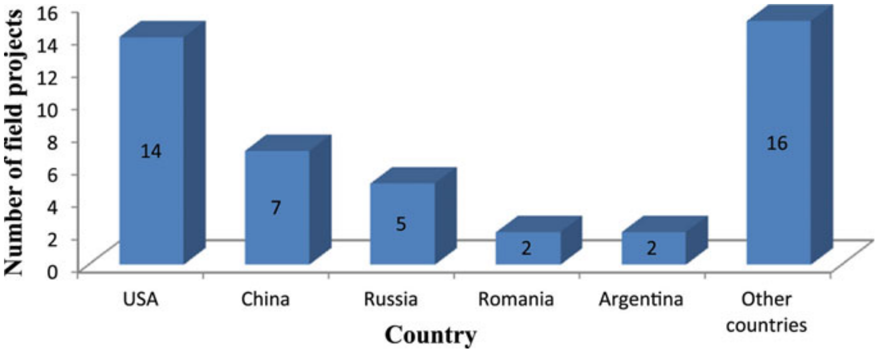

Fig. 3 Relative distribution of 47 field trial cases of MEOR in 21 countries. [Adapted with permission from Safdel et al. (2017)]

succeeding oil retrieval amount was very significant. The reservoir characteristics and the probable success rate were also analyzed and found to be more than 70% for MEOR which have been discussed elaborately elsewhere (Gao 2018; Gao and Zekri 2011; Safdel et al. 2017). In another study, among the San Andres project (Texas, USA), Tupungato-Refugio project (Medoza, Argentina), Xinjiang project (China), 2.5% and 13% oil recovery improvement were achieved for the first two projects, respectively (Nnaemeka et al. 2018). In a separate investigation, pilot-scale testing of MEOR was carried out in the Daqing oilfield of north China to investigate appropriate reservoir conditions and application necessities. By the end of 2012, cumulative oil recovery reached to 1.2×10^5 tons employing MEOR in the Daqing oilfield (Chaoyanggou, Lamadian, Songfangtun and Sabei block). Single-well microbial huff-and-puff in 518 wells produced 6.3×10^4 tons oil cumulatively, and 10 projects with 45 well patterns adopted microbial flooding and profile modification to obtain a collective incremental oil production of 5.7×10^4 tons (Le et al. 2015). A few in-situ MEOR case studies have been elaborately tabulated with the major microorganisms, produced metabolite, field details and oil properties along with the noteworthy oil recovery in Table 1. In recent reviews, cases of nineteen field trials in different types of reservoirs of nine countries were reported (Niu et al. 2020a). Another review summarized the MEOR field trial results of heavy oil recovery in China and six other countries (Zhang et al. 2020). These observations demonstrated the applicability of MEOR for the different kinds of reservoirs with light/heavy crude oil, low/high permeabilities and varying temperature, pressure and salinity, which proved the potential of MEOR for actual field applications.

2.1 Key Factors Affecting the In-Situ MEOR Process

The effects of various factors and their impacts on MEOR have been investigated by various researchers. A few of them are briefly discussed here.

Indigenous microflora

In a study, two heavy crude oil viscosity-declining bacteria, *Bacillus licheniformis* SH-2 and *Bacillus subtilis* SH-3, were screened from the formation fluid of elevated-temperature oilfields by enrichment culture method which produced biosurfactants and biogases by degrading heavy crude oil constituents (Sun et al. 2017). These two strains were combined with the endogenous bacterial population (*Pseudomonas*, *Acinetobacter*, *Anoxybacillus*, *Arcobacter*, *Clostridium*, *Caloramator*, *Geobacillus* and *Symbiobacterium*,), then applied for a pilot-scale trial of microbial huff and puff in an oilfield of Central China. The porosity, permeability and temperature of the reservoir were 14.32%, 22 mD and 50 °C, respectively. Suitable nutrients (molasses or corn steep powder) for activating reservoir-dwelling microflora were optimized to augment the retrieval by improving the mobility of heavy crude oil. After degassing treatment at 50 °C, the viscosity of the remaining oil was declined from 750 to 634 mPa·s after 54 days. Additionally, wax and resin-asphaltene fractions in the

Table 1 In-situ case studies in diverse field trials in different countries

Inoculation	Metabolites	Field details	Oil property, Shut-in period	Contribution in oil recovery	References
Indigenous microbe strain of *Bacillus* XJ2-1, *P. aeruginosa* XJ3-1, Dietz's bacteria Z4M8-2, inoculating microbes strain of *Bacillus* SLG5B10-17, nutrient solution, and activator	Mixed metabolites	Chunfeng Oilfield, Northwest China, temperature of 31 °C, depth of 590 m, sandstone reservoir with high porosity and permeability	Density of 0.956 7 g/cm^3, viscosity of 19 683 mPa s 166 days	Oil production of 3464 tonnes	Xuezhong et al. (2016)
Air, nutrients, *Pseudomonas*, and fermentative and other hydrocarbon-oxidizing bacteria	Biosurfactants	Low temperature (20.6 °C) heavy oil reservoir at Xinjiang oilfield, China Depth 480 m, porosity 19%	Oil viscosity 145 mPa s 7 days	Cumulative increment of 1872 tonnes of heavy oil	Chai et al. (2015)
Indigenous microbes	Biopolymer	Daqing field, North China	NA	518 wells in 10 blocks which lead to 63,386 tonnes of incremental oil production	Willenbacher et al. (2015)
Glucose-sodium nitrate-trace metal mixture and *Bacillus licheniformis* RS-1, *Bacillus spizizenii* NRRL B-23049	Lipopeptide	Viola limestone formation, Oklahoma	4.5 days	52.5 m^3 of additional oil was obtained in 60 days	Youssef et al. (2013)
Activation of indigenous microbes	NA	Mannville, Canada Reservoir depth 945–1024 m, temperature 31–35 °C, porosity 18–33%, permeability 400–1500 mD,	Oil API gravity 15–22.2°	Oil production increased from 1.25 m^3/d to 4.4 m^3/d	Govreau et al. (2013)

(continued)

Table 1 (continued)

Inoculation	Metabolites	Field details	Oil property, Shut-in period	Contribution in oil recovery	References
Activation of indigenous microbes	NA	Sparky reservoir, Canada Reservoir depth 600–661 m, porosity 14–30%, permeability 600–700 mD,	Oil API gravity 16–20°	Oil production increased from 1.4 $m^3\ d^{-1}$ oil to 9 $m^3\ d^{-1}$ oil	Govreau et al. (2013)
Nutrient and an electron acceptor to stimulate inherent microbes	Biomass and biopolymer	Brazilian onshore oilfield	NA	Pressure was increased and oil recovery was increased due to plugging of high permeability zone	Reksidler et al. (2010)
Inherent microbes and nutrient stimulation	Biomass	Saskatchewan, Canada Depth 1200 m, Temperature 47 °C, Porosity 15.2–21.5%, permeability 53–567mD	Oil API gravity 22–24° 7 days shut	Oil production was increased to 57 m^3/d to 164 m^3/d	Town et al. (2010)

(continued)

Table 1 (continued)

Inoculation	Metabolites	Field details	Oil property, Shut-in period	Contribution in oil recovery	References
Hydrocarbon degrading bacteria (HDB), denitrifying bacteria (DNB), methane-producing bacteria (MPB), sulfate-reducing bacteria (SRB), iron bacteria (IB), sulfur bacteria (SB) and total growth bacteria (TGB)	Biogas	S12 block in Shengli sandstone oilfield (surface area of 0.31 km^2), depth, temperature, average pressure, porosity and permeability of 1367–1450 m, 66 °C, 10.79 MPa, 20–23%, $89–263 \times 10^{-3}$ μm^2, respectively	Oil API gravity 28.2°, viscosity 54.3 mPa s at 50 °C	Oil production increment of 6.82%	Bao et al. (2009)
Brevibacillus brevis and *Bacillus cereus* along with other indigenous microbes	Organic acids	Daqing oilfield, China Low permeability $(0.1–200) \times 10^{-3}$ μm^2	Oil viscosity 8–100 mPa.s	Incremental oil of 11,000 tonnes in three years	Hou et al. (2008)
Inherent microorganisms and specific nutrients	Biosurfactants	Providencia and Lobitos fields, Northwest Peru	800 days	19,410 and 13,907 bbl of incremental oil, respectively	Bybee (2006)
150–300 m^3 of fluid containing 1% bacteria and 10% nutrient	CO_2 as biogas	Fuyu field, Northeast China, reservoir depth 300–500 m, 22–26% porosity, 100–300 mD permeability, temperature 28–33 °C	Viscosity 21–24 cP, 10–20 days	Among the 250 wells treated, 195 wells responded positively and produced 18,704 tonnes of additional oil	DI et al. (2005)

(continued)

Table 1 (continued)

Inoculation	Metabolites	Field details	Oil property, Shut-in period	Contribution in oil recovery	References
Hydrocarbon degrading anaerobic facultative microbes	Biosurfactant, biomass	Vizcacheras field (sandstone), Argentina Temperature 198 °F, porosity 25%, permeability 1000 mD,	Oil API gravity 29°, density 1.006 kg/m^3	Additional oil of 5887 m^3 in three years	Strappa et al. (2004)
Microbe thermophilic NG80, injection water, including NH_4Cl, K_2HPO_4	Biosurfactant, organic acid and biogas	G69 block, Northern China Temperature 73 °C	Dead oil density is 0.8801 g/cm^3, viscosity 6.8 mPa·s	13.8% additional oil recovery in 7 days	Feng et al. (2002)
Stimulation of indigenous bacteria	Gases, surfactants and acids	Trinidadian Oil Well, Trinidad and Tobago Reservoir porosity 19–31%, permeability 45–389 mD	Crude oil API gravity greater than 25°	Increase in oil recovery	Maharaj et al. (1993)

repossessed oil were also decreased by 12.3% and 17%, respectively. The average crude oil production was enhanced from 2.2 to 3.5 t/day followed by microbial exposure. The functional microbial population before and after microbial flooding was monitored to find out the structural dissimilarity of the activated native and injected microflora as well as their effectiveness in introducing successful MEOR practices. Hydrocarbon degrading bacteria (HDB), fermentation bacteria (FMB) and denitrifying bacteria (NRB) were stimulated efficiently, while the sulfate-reducing bacteria (SRB) of genus *Desulfovirgula* considered to be unfavorable for MEOR, were repressed during microbial huff and puff (Sun et al. 2017).

Low-temperature heavy oil resource

The reservoir geology of the Mengulin sandstone reservoir in Huabei oilfield was studied and reported to be a low-temperature heavy oil resource in which MEOR was implemented to improve oil displacement efficiency. Two microbial strains (HB1 and HB2) were screened in the laboratory, based on their growth pattern, compatibility and functional strategies with crude oil, and found to be appropriate for the reservoir setting. These screened isolates utilized petroleum hydrocarbon for their growth and metabolism, subsequently lowered oil viscosity and decreased oil–water IFT by producing biosurfactants with better surface activities. Nine oil wells were selected to perform pilot-scale microbial stimulus, among them 7 wells turn out to be more efficient with the improved experimental outcomes. The improved oil recovery was quantified to be 1,094 tons and continued up to 190 days with the calculated efficiency of 77.8% (Wang et al. 2018b).

Temperature, injection rate and nutrients

The efficiency of cold-water MEOR in an elevated-temperature reservoir was investigated by integrating temperature-dependent biokinetic and thermal multiphase model which was developed under non-isothermal conditions (Hong et al. 2019a). This model studied the growth pattern for *Bacillus subtilis* and Surfactin which were measured throughout the process at as high as 71 °C. The findings projected the effect of injection parameters which comprised nutrient concentration, injection rate and temperature (Fig. 4). Elevated sucrose concentration generated more surfactin which decreased the IFT and ultimately influenced the oil recovery. Higher injection rate developed further favorable thermal condition to stimulate growth, but the drawback was the microbes were being swept out before surfactin production. Injection temperature was associated with surfactin production along with oil viscosity change. Coldwater (< 21 ºC) was slowly heated up in a high-temperature reservoir to maintain an optimum temperature to support microbial growth and Surfactin production. The relationship between every injection parameter along with the oil recovery factor specified the effect of each parameter on oil recovery effectiveness. Additional 6% oil recovery was achieved by the optimum injection formulation (sucrose concentration of 100 g/L, injection rate of 7 m^3/d, and temperature of 19 °C) compared to control. Through the optimization process, 53% improved oil recovery was observed which was 8% higher than water-flooding (Hong et al. 2019a). This highlighted that the metabolic activities of the indigenous or exogenous microbes can be augmented

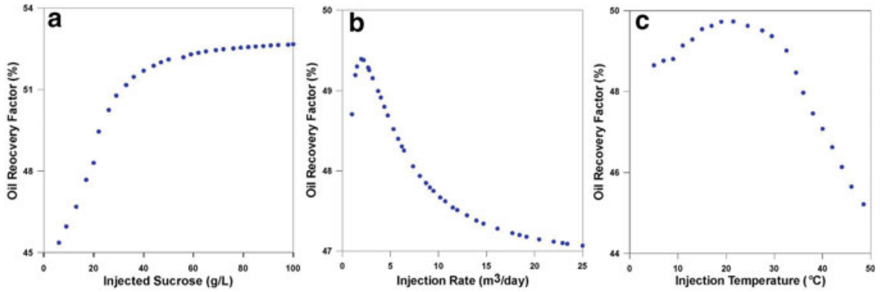

Fig. 4 Correlation of sucrose concentration, injection rate and injection temperature with the oil recovery factor. [Adapted from Hong et al. (2019a)]

by tuning the temperature of injection water to improve the synthesis of desired metabolites, which in turn positively impact the oil recovery.

Aeration

Microbiological and biogeochemical oil recuperation from the Dagang reservoir's high temperature (57–58.4 °C) and heavy oil (density 0.966–0.969 g/cm^3) field trials were reported (Nazina et al. 2017). The oil-dwelling microflorae were activated by supplying oxygen as an air–water mixture or H_2O_2 in combination with nutrients (supplementation of nitrogen and phosphorus mineral salts) throughout oil wells. As a result, aerobic, anaerobic microbes as well as methanogens (*Methanothermobacter* sp. and *Thermoanaerobacter*) were augmented in the formation water. The implementation of the strategy at the Kongdian bed led to 6331 tons of additional oil recovery with 11% of viscosity reduction. Entirely 46,152 tons of additional oil was retrieved from three investigation sites of the Dagang oilfield (North block and block no. 1 of the Kongdian sandstone bed and the Gangxi bed), indicating the in-situ MEOR as highly efficient expertise for activating the oil-well microbial community for heavy oil repossession from extremely thermal oil reservoirs (Nazina et al. 2017).

Pressure and Porosity

The oil -displacement mechanism was investigated by carrying out microbial flooding trials using endogenous mixed consortia under the reservoir situation of the Shengli Oilfield. A microscopic simulation visual model was employed for monitoring water flooding and microbial oil displacement under high thermal and high-pressure conditions for surveillance and scrutiny of flow distribution of the remaining oil. Results exhibited that the microbial metabolism was attenuated under high pressure conditions compared to atmospheric conditions, and the growth cycle was extended due to the presence of the porous medium for microbes which provided good adhesion. The microbial activities could effectively retrieve residual oil by boosting crude oil liquidity in the presence of metabolites and significantly progress oil displacement (Yue et al. 2017).

In summary, the reservoir internal environment differs for every system, accordingly the obviousness of the MEOR process changes depending on the multiphase flow of fluid, characteristics of reservoir rocks and crude oil properties (Shibulal et al. 2014). Various indigenous and exogenous extremophiles (i.e. thermophilic and halophilic) have been isolated and applied for in-situ MEOR. In fact, in-situ MEOR trials have been conducted in low-temperature heavy oil reservoirs (Wang et al. 2018b). Further, the injected microbes need to compete with indigenous microflora. Hydrocarbon degrading and denitrifying bacteria positively impact the in-situ MEOR, while sulfate-reducing bacteria negatively affect the process (Sun et al. 2017).

In addition, growth stimulators, i.e. nutrients, temperature, pressure and aeration along with reservoir properties like porosity contribute significantly to the success of in-situ MEOR. The key objective is to improve the microbial activities to produce the desired metabolites and mobilize the residual oil. Microbes require an optimum temperature, time and nutrients for the maximum production of desired metabolites (Hong et al. 2019a). The production of metabolites is also augmented by aeration, which can be achieved by injecting an air–water mixture or H_2O_2 along with nutrients (Nazina et al. 2017). Microbial metabolism is decreased under a high pressure condition as compared to atmospheric pressure. The presence of the porous medium offers good adhesion for microbes (Yue et al. 2017).

3 Case Studies of Ex-Situ MEOR

In the ex-situ MEOR, the metabolites are synthesized outside the reservoir and subsequently introduced in the oil reservoir during the flooding process. The ex-situ MEOR also comprises three phases for the technology transfer from laboratory to original oilfield as shown in Fig. 5. In the first phase, laboratory studies are carried out by isolating desired metabolites, i.e. biosurfactant producing microorganisms from relevant environmental sites. Then the surface activities of the produced metabolites like ST, IFT and emulsification index (EI) are measured along with wettability alteration and CMC value (Datta et al. 2018; Sharma et al. 2019a, b; Sharma and Pandey 2020; Verma et al. 2020). The stability and adsorption studies of the produced metabolites are examined at the extensive range of pH, temperature and salinity to confirm the suitability at the reservoir conditions (Datta et al. 2020). In the next phase of pilot-scale studies, computer simulations and mathematical models are framed after supervising the upstream scale production and downstream separation, product recovery and transportation of the metabolite to the oilfield. In this case, a longer shut-in period is not required unlike in-situ MEOR as there are no inoculation and incubations steps involved. Finally, the success rate of pilot studies is implemented and validated in the field applications (Geetha et al. 2018).

Pseudomonas aeruginosa (P-1) and its metabolic products (PIMP) were explored for oil recovery in Daqing Oilfield (Li et al. 2002). The strain was isolated from the crude oil-contaminated water. At the lab scale, the metabolites resulted in ST, IFT

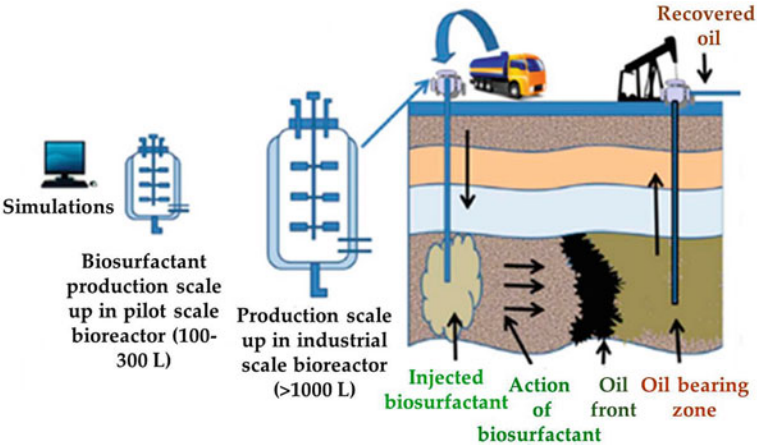

Fig. 5 Schematic of biosurfactant induced ex-situ MEOR. [Adapted from Geetha et al. (2018)]

and oil viscosity decrease of 27.3 mN/m, 0.65×10^{-2} mN/m and 38.5%, respectively. The PIMP were found to be stable at 70 °C for 16 h. More than 60 oil-producing wells were injected with PIMP and compared with six numbers of observation wells. The production of crude oil was increased to 12 tonnes after injection as compared to 3 tonnes before injection. PIMP also prolonged the cycle of washing wells (CWOW). The CWOW prolonged from 79 days before injection to 179 days because PIMP reduced the oil viscosity which in turn prevented wax deposition on the walls of wells. Thus, PIMP was found to enhance the oil recovery up to 11.2% and decrease the injection pressure up to 40.1%. The pilot studies indicated that PIMP positively impacted the total oil production by altering the interfacial properties, viscosity and prolonging the CWOW (Li et al. 2002). Although, biosurfactants are proven to be better surface-active agents and demonstrated their suitability for ex-situ MEOR. But the cost of production at a large scale is a main limiting factor for its actual applications. In this direction, various efforts are being made to use cheaper substrates, screen potential strains, improve productivity and alternate downstream processing in order to develop cost-effective bioprocessing of biosurfactants at a large scale.

Apart from MEOR, genetically engineered microorganisms for MEOR purposes (GMEOR) and enzyme enhanced oil recovery (EEOR) are also being explored (Niu et al. 2020a; Zhang et al. 2020). Genetic engineering is used to construct engineered strains with desired properties needed for EOR like extremophiles, halophiles, heavy oil degraders and better metabolites producers (Zhang et al. 2020). However, GMEOR is still being examined at the laboratory scale. Further, the stability of engineered microbes, operational cost and ethical requirements are key challenges for the implementation of GMEOR. In the case of EEOR, various enzymes like oxygenase, dehydrogenase, peroxidase and laccase can be applied for the purposes of EOR through enzymatic reactions (Zhang et al. 2020). Enzymes are found to be more specific and undertake biochemical reactions at a much fast rate as compared to whole cells. However, EEOR is still at the developing stage and the cost of enzyme production at the large scale poses economic constraints similar to that of ex-situ MEOR.

4 Conclusion

Since microbial activity in laboratory conditions cannot be projected to be the same in the field scale, it is impractical to anticipate the exact consequences of the MEOR process on a large scale. Therefore, pilot studies are conducing before performing actual field trials. Also, employing various modeling and simulations the prediction could be done to some extent. In-situ MEOR has been successfully tested across the world and positively impacted the enhanced oil recovery. However, the implementation of in-situ MEOR is dependent on the physical, chemical and biological properties of reservoirs. Novel strategies to monitor microbial activity in-situ are very essential in determining their usefulness in real oil recapture. Henceforth, research studies that emphasize comprehending various factors influencing MEOR accomplishment in diverse reservoir surroundings are recommended. For ex-situ MEOR advanced research is required to overcome the bottlenecks of yield and massive expense. GMEOR and EEOR have exhibited potential at the lab scale and further investigations need to be undertaken at pilot and field scales in the future.

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