

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO	
RAD: 13-133429- -4	FECHA: 2014-07-03 15:20:43
DEP: 2020 DIRECCION DE NUEVAS CREACIONES	EVE: 378 FASE NACIONAL INCLUIDO CAPITULO I
TRA: 11 PCT-PATENTE DE INVENCION CAPITULOS I Y II	FOLIOS: 7
ACT: 519 PRESENTACION	

DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado(a)/Señor(a)
ALVARO CORREA ORDOÑEZ

Asunto: Radicación: 13-133429- -4
Trámite: 11
Evento: 378
Actuación: 519
Oficio: 8001
Folios: 7

Patente de Invención	X			Patente de Modelo de Utilidad	
Vía Nacional					
Vía PCT (Fase Nacional)	X	Capítulo I	X	Capítulo II	
No. Solicitud Internacional	PCT/US2011/058754				
Fecha de Presentación Internacional	01/11/2011				

Como resultado del Examen de Fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Rad. N° 13-133429-00000-0000	31/Mayo/2013
Reivindicaciones:	Rad. N° 13-133429-00000-0000	31/Mayo/2013
Dibujos:	Rad. N° 13-133429-00000-0000	31/Mayo/2013
Prioridades	US 61/408,731	01/Noviembre/2010

2. Análisis de la Prioridad

Se acepta la reivindicación de Prioridad porque esta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

De acuerdo con lo establecido en el numeral 1.2.2.2 del Capítulo Primero del Título X de la Circular Única para efectos del examen de patentabilidad, se tendrá en cuenta la tasa pagada por el solicitante al momento de comenzar el examen para las reivindicaciones adicionales; esto es que cuando la solicitud contenga más de diez (10) reivindicaciones y no se haya pagado la tasa complementaria, sólo se estudiarán las cubiertas por la tasa pagada. Por lo tanto se estudiaran las reivindicaciones 1 a 17.

Título de la solicitud: “Prevención de la agregación de biomasa en pozos de inyección” (Pág. 1, radicado N° 13-133429-00000-0000).

El problema planteado en esta solicitud consiste en la necesidad de obtener hidrocarburos de reservas subterráneas mediante la generación de biopelículas.

Para resolver este problema técnico, la solicitud en estudio proporciona un método de extracción de hidrocarburos de reservas subterráneas empleando un suministro de nutrientes y bacterias que generan biopelículas.

El objeto de la presente Solicitud se relaciona con la Clasificación Internacional de Patentes (CIP) E21B 43/22, C09K 8/58.

4. De la Solicitud de Patente

Reivindicaciones (Art 30 D 486, Art 22 PCT)

Las reivindicaciones 1, 12, 14, 16 no son claras porque las expresiones “*aproximadamente*” y “*al menos*” son imprecisas, ya que en este caso el alcance de la protección de las reivindicaciones deja de ser precisa y no permite distinguir la invención del estado de la técnica. Se sugiere sustituirlas por un término preciso o un rango concreto.

Las reivindicaciones 1, 4 y 6 no son claras porque las expresiones “*microorganismos*”, “*fluidos*”, “*primera formulación de solución nutriente*”, “*segunda formulación de solución nutriente*”, “*periodo de cierre*” y “*periódicamente*” son generales, por lo cual no permite distinguir la invención del estado de la técnica. Se sugiere sustituirlas por un término preciso o un rango concreto.

Las reivindicaciones 1, 2 y 17 no son claras porque las expresiones “*donde la primera solución sustenta el crecimiento de uno o más microorganismo de (a) en el lugar subterráneo objetivo pero no fomenta la agregación de biomasa ni la producción de biopelículas, en donde la segunda formulación de solución nutriente fomenta el biotaponamiento de poros y canales en el lugar subterráneo objetivo*”, “*ayuda a mover la suspensión de uno o más microorganismo de (a) al sitio subterráneo objetivo*”, “*el lactato fomenta el crecimiento de la Pseudomonas stutzeri y mantiene la P. stutzeri en suspensión*” y “*que fomenta la formación de biotaponos mediante la cepa P stutzeri de (a)*” son características funcionales que no le pueden brindar novedad ni nivel inventivo a las reivindicaciones de las cuales dependen. Se sugiere remplazarlas por las características esenciales estructurales que contribuyen a la obtención de ese efecto.

Descripción (Art 28 D 486, Art. 29 D486)

Dado que la invención se refiere a un procedimiento relativo a un material biológico, se debe allegar el depósito de material biológico de los microorganismos nombrados en la descripción y el capítulo reivindicatorio (reivindicación 9 y 17) diferentes a las cepas de los siguientes microorganismos, a los cuales si se allego el certificado de depósito: *Shewanella putrefaciens* LH4:18, *Pseudomonas stutzeri* LH4:15, *Arcobacter sp.* 97AE3-12, *Arcobacter sp.* 97AE3-3, *Pseudomonas stutzeri* BR5311, *Pseudomonas stutzeri* 89AC1-3

5. Determinación del Estado de la Técnica

El estado de la técnica se determinó con base en la fecha de presentación de la solicitud de prioridad invocada, es decir, el 01 de noviembre de 2010.

Consultadas las fuentes de información con que se cuenta, se encontraron los siguientes documentos que anticipan el objeto de esta solicitud.

Item	Documento	Título	Fecha de Publicación
D1	US 2009/0263887	"Identification, characterization, and application of pseudomonas stutzeri (lh4:15), useful in microbially enhanced oil release"	22/Octubre/2009
D2	Art 1 ¹	"In Situ Biosurfactant Production by Bacillus Strains Injected into a Limestone Petroleum Reservoir"	Febrero/2007

¹Youssef et al. Applied and environmental microbiology. 2007. Vol. 73(4). Págs.1239 a 1247.

El documento D1¹ divulga bacterias que producen biopelículas y un proceso para recuperar hidrocarburos basados en la generación de biopelículas (Pág. 15, reivindicaciones 1 a 10).

El documento D2² divulga la inyección de bacterias en una solución de nutrientes con una posterior adición de agua para mover dicha suspensión en la formación subterránea (Pág. 1240, Párr. 3).

6. Examen de Patentabilidad (Art 14 D486)

Nivel inventivo (Art 18 D486)

La reivindicación 1 consiste en *"Un método para el tratamiento de un lugar subterráneo..."* (Ver Pág. 119 del Rad. N° 13-133429-00000-0000).

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Características esenciales	D1	D2
Método para el tratamiento de un lugar subterráneo objetivo, el método comprende las etapas de, en orden	Método para analizar la acción de bacterias en la liberación de hidrocarburos (Pág. 10, Párr. 88 a 90).	Método para la recuperación de hidrocarburos en un pozo de producción (Pág. 1240, Párr. 3).
a)Preparar una suspensión de biomasa que comprende una o más especies de microorganismos	Se prepara una mezcla de microorganismos con agua de inyección empleada en un pozo y enriquecida con nitrato de sodio (Pág. 10, Párr. 90).	Se prepara una suspensión de bacterias en una solución de nutrientes (Pág. 1240, Párr. 2 y Párr. 3).
b)Introducir la suspensión (a) a un pozo de inyección del lugar subterráneo objetivo	Introducir una composición de microorganismos a una reserva de hidrocarburos (Pág. 15, reivindicaciones 7 y 8).	Se inyecta la suspensión de bacterias en el pozo de producción (Pág. 1240, Párr. 3).
c)Opcionalmente, introducir uno o más fluidos al pozo de inyección	No se menciona	Se adiciona agua después de la adición de la preparación de nutrientes y bacterias para mover una cierta distancia la preparación en la preparación subterránea (Pág. 1240, Párr. 3).
d)Introducir una primera formulación de solución nutriente al pozo de inyección, donde la primera solución sustenta el crecimiento de uno o más microorganismo de (a) en el lugar subterráneo objetivo pero no fomenta la agregación de biomasa ni la producción de biopelículas	Se adiciona una solución de nitrato en agua de inyección, esta solución permite la viabilidad y crecimiento de ciertas bacterias con o sin consumo de hidrocarburos* (Pág. 9, Párr. 87, Tabla 6).	No se menciona
e)Introducir una segunda formulación de solución nutriente al pozo de inyección, en donde la	Se tiene que una fuente de carbono escogida entre acetato y lactato favorece la	No se menciona

segunda formulación de solución nutriente fomenta el biotaponamiento de poros y canales en el lugar subterráneo objetivo	formación de biofilm, por lo que es cuestión de escoger la fuente carbono para producir el efecto deseado* (Pág. 9, Párr. 83).	
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*Adicionalmente la reivindicación 1 se encuentra afectada con el conocimiento del técnico, *“el experto medio deberá poseer experiencia y contar con un saber general en la materia y con una serie de conocimientos en el campo específico de la invención”*³, al conocer que se adicionan secuencialmente nutrientes para el crecimiento y taponamiento⁴.

³<http://www.google.com/patents/US20090263887>

Los documentos D1 y D2 se consideran el estado de la técnica más cercano a la invención definida en la reivindicación 1, porque divulgan métodos de recuperación de hidrocarburos con bacterias.

La diferencia entre la invención, definida en la reivindicación 1 y el método del documento D1 consiste en que D1 no menciona la adición opcional de fluidos después de la inyección de la suspensión de bacterias.

La diferencia entre la invención, definida en la reivindicación 1 y el método del documento D2 consiste en que en D2 no se menciona la adición de diversas formulaciones de nutrientes después de la inyección de la suspensión de bacterias.

El efecto que logra el incluir la la adición opcional de fluidos después de la inyección de la suspensión de bacterias es que permiten la obtención de hidrocarburos de forma más eficiente al trasladar la suspensión de bacterias al sitio deseado en la formación subterránea.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así *“¿cómo modificar el método de D1 para trasladar la suspensión de bacterias al sitio deseado en la formación subterránea y obtener hidrocarburos de forma más eficiente?”*.

Sin embargo, la inyección de agua posterior a la inyección de las bacterias, ya está divulgado en el documento D2 que se refiere a la producción de hidrocarburos de un pozo de producción (Pág. 1240, Párr. 3).

En consecuencia, una persona normalmente versada en la materia incluiría la adición de agua posterior a la inyección de las bacterias de D2 como etapa de recuperación de hidrocarburos en el procedimiento de la anterioridad D1, esperando con certeza lograr el método, como el de la reivindicación 1 de la solicitud, lo cual es considerado obvio y, por lo tanto, sin altura inventiva.

Asimismo, el documento D1 divulga las características de las reivindicaciones dependientes así:

Reivindicaciones 7 y 8: la suspensión de la biomasa se prepara en la solución nutriente que favorece el crecimiento, por lo que la etapa del suministro de esa solución se omite (Pág. 15, reivindicación 7).

Reivindicaciones 9 y 10: se emplea *Pseudomonas stutzeri* LH4:15 para el tratamiento (Pág. 15, reivindicación 7).

Reivindicaciones 11 a 16: se produce la biopelícula con acetato y no lactato, por lo que si se desea crecimiento se adiciona lactato, y en caso de desear biopelícula se adiciona acetato, el acetato y lactato están en una concentración de 0,6%, es decir 6000ppm. Adicionalmente el nitrato de sodio se usa en ambas soluciones a una concentración de

1600ppm (Pág. 9, Párr. 83 a Párr. 85, Pág. 9, Párr. 87 a Pág. 10, Párr. 90).

Asimismo, el documento D2 divulga las características de las reivindicaciones dependientes así:

Reivindicaciones 2, 3, y 5: se adiciona agua para mover dentro de la formación subterránea la preparación anteriormente inyectada, por lo que cuando se desee esta agua se puede aplicar (Pág. 1240, Párr. 3).

³Proceso 43-IP-2002, publicado en la G.O.A.C. N° 870, de 9 de diciembre de 2002, caso: formulaciones de péptidos hidrosolubles de liberación retardada.

⁴Springham Dereck. Microbiological methods for the enhancement of oil recovery. Biotechnological and genetic engineering reviews. Vol. 1 1984. Pág. 201, Párr. 2.

Reivindicación 4: aplicar un periodo de cierre cuando se desea el crecimiento de los microorganismos (Pág. 1240, Párr. 3).

Reivindicaciones 7 y 8: la suspensión de la biomasa se prepara en la solución nutriente que favorece el crecimiento, por lo que la etapa del suministro de esa solución se omite (Pág. 1240, Párr. 3).

Reivindicación 9: se emplean bacterias del género *Bacillus* (Pág. 1240, Párr. 3).

Asimismo, la reivindicación 6 se encuentra afectada con el conocimiento del técnico, “*el experto medio deberá poseer experiencia y contar con un saber general en la materia y con una serie de conocimientos en el campo específico de la invención*”³, al conocer que se adicionan los nutrientes en diferentes etapas dependiendo de los requerimientos nutricionales de las bacterias⁵.

Puesto que la reivindicaciones dependientes 2 a 16 no mencionan características adicionales que hagan inventiva la solicitud, se considera que tal reivindicaciones tampoco tienen nivel inventivo.

La reivindicación 17 consiste en “*un método para el tratamiento de un lugar subterráneo...*” (Ver Pág. 122 del Rad. N° 13-133429-00000-0000).

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Características esenciales	D1	D2
Método para el tratamiento de un lugar subterráneo objetivo, el método comprende las etapas de, en orden	Método para analizar la acción de bacterias en la liberación de hidrocarburos (Pág. 10, Párr. 88 a 90).	Método para la recuperación de hidrocarburos en un pozo de producción (Pág. 1240, Párr. 3).
a)Preparar una suspensión de biomasa que comprende una o más cepas de <i>Pseudomonas stutzeri</i>	Se prepara una suspensión de <i>Pseudomonas stutzeri</i> (Pág. 15, reivindicación 7).	No se menciona
b)Introducir la suspensión de (a) a un pozo de inyección del lugar subterráneo	Se inyecta la suspensión de <i>Pseudomonas stutzeri</i> en la reserva de hidrocarburo (Pág. 15, reivindicación 7).	Se inyecta la suspensión de bacterias en el pozo de producción (Pág. 1240, Párr. 3).
c)Opcionalmente, introducir uno o más fluidos al pozo de inyección	No se menciona	Se adiciona agua después de la adición de la preparación de nutrientes y bacterias para mover una cierta distancia la preparación en la preparación subterránea (Pág. 1240, Párr. 3).
d)Introducir una primera formulación de solución nutriente al pozo de inyección, en donde la primera solución nutriente comprende lactato y no acetato, y en donde el lactato fomenta el	Se adiciona una solución de nitrato en agua de inyección, esta solución permite la viabilidad y crecimiento de ciertas bacterias con o sin consumo de hidrocarburos*	No se menciona

crecimiento de la <i>Pseudomonas stutzeri</i> y mantiene la <i>P. stutzeri</i> en suspensión	(Pág. 9, Párr. 87, Tabla 6).	
e)Introducir una segunda formulación de solución nutriente al pozo de inyección en donde la segunda formulación comprende acetato que fomenta la formación de biotaponos mediante la cepa <i>P. stutzeri</i> de (a)	Se tiene que una fuente de carbono escogida entre acetato y lactato favorece la formación de biofilm, por lo que es cuestión de escoger la fuente carbono para producir el efecto deseado* (Pág. 9, Párr. 83).	No se menciona

⁵Jinfeng et al. The field pilot of microbial enhanced oil recovery in a high temperature petroleum reservoir. Journal of Petroleum Science and Engineering. 2005. Pág. 268, Col. 2, Párr. 2 y 3.

*Adicionalmente la reivindicación 17 se encuentra afectada con el conocimiento del técnico, *“el experto medio deberá poseer experiencia y contar con un saber general en la materia y con una serie de conocimientos en el campo específico de la invención”*³, al conocer que se adicionan secuencialmente nutrientes para el crecimiento y taponamiento⁴.

Los documentos D1 y D2 se consideran el estado de la técnica más cercano a la invención definida en la reivindicación 17, porque divulgan métodos de recuperación de hidrocarburos con bacterias.

La diferencia entre la invención, definida en la reivindicación 17 y el método del documento D1 consiste en que D1 no menciona la adición opcional de fluidos después de la inyección de la suspensión de bacterias.

La diferencia entre la invención, definida en la reivindicación 17 y el método del documento D2 consiste en que en D2 no se menciona la adición de diversas formulaciones de nutrientes después de la inyección de la suspensión de bacterias ni la que las bacterias corresponden a la especie *Pseudomonas stutzeri*.

El efecto que logra el incluir la la adición opcional de fluidos después de la inyección de la suspensión de bacterias es que permiten la obtención de hidrocarburos de forma más eficiente al trasladar la suspensión de bacterias al sitio deseado en la formación subterránea.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así *“¿cómo modificar el método de D1 para trasladar la suspensión de bacterias al sitio deseado en la formación subterránea y obtener hidrocarburos de forma más eficiente?”*.

Sin embargo, la inyección de agua posterior a la inyección de las bacterias, ya está divulgado en el documento D2 que se refiere a la producción de hidrocarburos de un pozo de producción (Pág. 1240, Párr. 3).

En consecuencia, una persona normalmente versada en la materia incluiría la adición de agua posterior a la inyección de las bacterias de D2 como etapa de recuperación de hidrocarburos en el procedimiento de la anterioridad D1, esperando con certeza lograr el método, como el de la reivindicación 17 de la solicitud, lo cual es considerado obvio y, por lo tanto, sin altura inventiva.

7. Conclusión

Los requisitos de Patentabilidad de la presente Solicitud están afectados así:

Item	Documento	Reivindicaciones afectadas	Requisito que afecta
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D1	US 2009/0263887	1 y 6 a 17	Nivel inventivo
D2	Art 1	1 a 9 y 17	Nivel inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir del día siguiente de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

Finalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones, no pagará la tasa de modificación voluntaria pero sí la correspondiente al pago por reivindicación adicional.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.



JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (c)

El anterior requerimiento fue notificado por fijación en lista, de fecha 2014-07-08

Elaboró: Jorge Eduardo Cortazar Gomez
Revisó: Rosa Patricia Tellez Chavarro

In Situ Biosurfactant Production by *Bacillus* Strains Injected into a Limestone Petroleum Reservoir

N. Youssef, D. R. Simpson, K. E. Duncan, M. J. McInerney,
M. Folmsbee, T. Fincher and R. M. Knapp
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In Situ Biosurfactant Production by *Bacillus* Strains Injected into a Limestone Petroleum Reservoir[∇]

N. Youssef,¹ D. R. Simpson,¹ K. E. Duncan,¹ M. J. McInerney,^{1*} M. Folmsbee,¹
T. Fincher,¹ and R. M. Knapp²

Department of Botany and Microbiology, University of Oklahoma, Norman, Oklahoma,¹ and School of Petroleum and Geological Engineering, University of Oklahoma, Norman, Oklahoma²

Received 25 September 2006/Accepted 6 December 2006

Biosurfactant-mediated oil recovery may be an economic approach for recovery of significant amounts of oil entrapped in reservoirs, but evidence that biosurfactants can be produced in situ at concentrations needed to mobilize oil is lacking. We tested whether two *Bacillus* strains that produce lipopeptide biosurfactants can metabolize and produce their biosurfactants in an oil reservoir. Five wells that produce from the same Viola limestone formation were used. Two wells received an inoculum (a mixture of *Bacillus* strain RS-1 and *Bacillus subtilis* subsp. *spizizenii* NRRL B-23049) and nutrients (glucose, sodium nitrate, and trace metals), two wells received just nutrients, and one well received only formation water. Results showed in situ metabolism and biosurfactant production. The average concentration of lipopeptide biosurfactant in the produced fluids of the inoculated wells was about 90 mg/liter. This concentration is approximately nine times the minimum concentration required to mobilize entrapped oil from sandstone cores. Carbon dioxide, acetate, lactate, ethanol, and 2,3-butanediol were detected in the produced fluids of the inoculated wells. Only CO₂ and ethanol were detected in the produced fluids of the nutrient-only-treated wells. Microbiological and molecular data showed that the microorganisms injected into the formation were retrieved in the produced fluids of the inoculated wells. We provide essential data for modeling microbial oil recovery processes in situ, including growth rates (0.06 ± 0.01 h⁻¹), carbon balances ($107\% \pm 34\%$), biosurfactant production rates (0.02 ± 0.001 h⁻¹), and biosurfactant yields (0.015 ± 0.001 mol biosurfactant/mol glucose). The data demonstrate the technical feasibility of microbial processes for oil recovery.

Oil is an essential source of energy and one of the main factors that drive the economic development of the world (16). Current oil production technologies recover only about one-third to one-half of the oil originally present in an oil reservoir (16, 26, 37). The exploitation of oil resources in existing mature reservoirs is essential for meeting future energy demands as the number of oil-exporting countries dwindles. A key to exploiting this untapped resource is to overcome the capillary forces that entrap oil in small pores within the reservoir. Enhanced oil recovery (EOR), i.e., the use of heat, chemicals such as surfactants, microbial processes, and miscible gas injection (15), has the potential to recover a significant portion of this entrapped oil. However, oil recovered by EOR constitutes less than 10% of the total amount of oil produced in the United States (<http://www.fe.doe.gov/programs/oilgas/eor/>). Interfacial tension between the hydrocarbon and aqueous phases is largely responsible for trapping the hydrocarbon in the porous matrix, and a reduction of several orders of magnitude in interfacial tension is needed for hydrocarbon mobilization (1, 15, 47). To achieve large reductions in interfacial tension, surfactant concentrations significantly above that needed to form micelles (i.e., the critical micelle concentration) are required (7, 41, 42). The high chemical costs have prevented the widespread use of surfactants for enhanced oil recovery.

Microbially enhanced oil recovery (MEOR) processes employ the use of microbial metabolites, such as biosurfactants, to lower interfacial tension between brine and oil and hence mobilize entrapped oil (2, 8, 24, 32). Several biosurfactants, in particular the lipopeptides made by *Bacillus* species, generate the low interfacial tensions between the hydrocarbon and the aqueous phases required to mobilize residual hydrocarbon (25, 30). MEOR has several advantages compared to other EOR processes in that it does not consume large amounts of energy, as do thermal processes, nor does it depend on the price of crude oil, as do many chemical processes (2, 31, 32). MEOR can also be cost-effective, since microbial products can be produced from inexpensive, renewable resources, and several MEOR processes have been shown to produce incremental oil for about \$19 per m³ (\$3 per barrel) (6, 9, 31, 32). Nevertheless, microbial processes have always been viewed with considerable skepticism for a number of reasons. First, the lack of quantitative information regarding the reaction rates, stoichiometries, product concentrations, and yields needed to simulate the performance of microbial processes makes it difficult to extrapolate the results for a given field test to those for other reservoirs (31, 32). Second, it is not clear whether microbial processes can generate the necessary metabolites in sufficient quantities and at rates needed to mobilize entrapped oil in oil reservoirs (10, 31, 32). Third, technical performances in many field trials have been inconsistent (14, 23). Last, it is unclear whether the microbial strains used as inocula actually grow and metabolize in the reservoir (31, 32).

Here, we show in a well-controlled field experiment that biosurfactants are produced in situ in amounts sufficient to

* Corresponding author. Mailing address: Department of Botany and Microbiology, University of Oklahoma, 770 Van Vleet Oval, Norman, OK 73019. Phone: (405) 325-6050. Fax: (405) 325-7619. E-mail: mcinerney@ou.edu.

[∇] Published ahead of print on 15 December 2006.

mobilize substantial amounts of entrapped oil. We provide, for the first time, data on in situ product concentrations and yields, rates of growth, substrate utilization, and metabolite formation and an excellent carbon mass balance. This quantitative information shows that biosurfactant-mediated oil recovery is technically feasible and will facilitate the use of computer simulations to determine the efficacy of MEOR in different reservoirs.

MATERIALS AND METHODS

Preparation of the inoculum. Two halotolerant biosurfactant producers, *Bacillus* strain RS-1 and *Bacillus subtilis* subsp. *spizizenii* strain NRRL B-23049, were used as the inoculum. Both strains grow in medium with 5% NaCl. *Bacillus* strain RS-1 and *Bacillus subtilis* subsp. *spizizenii* strain NRRL B-23049 were each grown in 200 ml of medium E (48, 49). When the culture reached the late exponential phase of growth, it was used to inoculate a 10-liter carboy of the same medium, which was incubated at 37°C for 48 h. Agitation and aeration were maintained by using glass gas dispersion tubes with fritted cylinders (Fisher Scientific, Inc.). The cells were concentrated by using a tangential membrane flow system (0.45- μ m-pore-size filter) (Millipore, Bedford, MA) to yield on average 2 liters of cell concentrate from each 10-liter carboy. The concentrated cells were stored at 4°C. One liter of cell concentrate was used to inoculate a 132-liter tank with the following components (in grams per liter of tap water): dibasic potassium phosphate (1.2 g/liter), monobasic potassium phosphate (0.23 g/liter), sucrose (8.6 g/liter), sodium chloride (8.6 g/liter), sodium nitrate (0.86 g/liter), and *Saccharomyces cerevisiae* extract (0.86 g/liter). The medium was prepared septically due to the lack of facilities on site, and tanks were incubated for approximately 48 h at an ambient temperature.

Field experiment. A push-pull test was conducted on five oil production wells in the Bebee field (Section 19, T5N, R5E; Pontotoc City, OK) that produce from the same formation (a Viola limestone). Two wells received an inoculum of the *Bacillus* strains and nutrients, two wells received just nutrients, and one well received an equivalent volume of formation water and served as the negative control. Each well that was inoculated received 396 liters of *Bacillus* strain RS-1 and 264 liters of *B. subtilis* subsp. *spizizenii* NRRL B-23049. The nutrient package consisted of 79.5 kg of glucose; 7.9 kg of sodium nitrate; 19.9 g of magnesium sulfate; 2.0 g each of manganese sulfate, zinc sulfate, and iron sulfate; 0.2 g each of copper sulfate, aluminum potassium sulfate, boric acid, and sodium molybdate; 0.1 g of sodium selenate; and 0.6 g of nickel chloride per well. Each well that received nutrients also received fluorescein (125 g) and sodium bromide (2 kg). Fluorescein was used to detect visually (by a green signal) the production of the slug and to determine when to start and stop sampling. Bromide served as the conservative tracer to account for dilution in the reservoir. The nutrients, inoculum, and tracers were mixed with formation water (~8,000 liters) by circulation supplied by the pump truck. The formation water was obtained from a storage tank located near the production wells. Each well received an initial injection (preflush) of ~1,600 liters (10 barrels) of formation water and an injection of ~8,000 liters (50 barrels) of the treatment (nutrients and cells, nutrients only, or formation water), followed by an injection of ~8,000 liters (50 barrels) of formation water as a postflush treatment, to make a total of ~17,500 liters (110 barrels) of fluids injected per well. The postflush treatment was used to move the nutrient package 1.2 to 2.4 m into the formation. After injection of the treatment package, production from each well was stopped for 108 h to allow time for growth and metabolism to occur in the formation. After this incubation period, the pump was started and samples from each of these wells were collected with time.

Flow meters were attached to the tubing of three of the five wells to measure the volume of fluids produced. The total volume produced was recorded when each sample was collected. Since all the wells were set to pump at almost the same rate, the volume of fluid produced during a given time interval in the two wells that did not have flow meters was estimated from the average volume produced in the three wells that had the flow meters attached.

Sampling. Samples for chemical and microbiological analyses were collected on several occasions prior to treatment and for a 32-hour period after production recommenced after the 108-hour incubation period. Each sample was collected in a 2-liter glass bottle that was allowed to overflow to minimize contact with air. The temperature of the sample was immediately recorded with a handheld probe. A sample for chemical analyses was filtered through a 0.45- μ m membrane filter to remove particulate material and oil. The remainder of the unfiltered sample was used for measurement of oil-spreading activity and for microbiolog-

ical enumerations. All samples were stored on ice until analyzed. The analyses for pH, conductivity, nitrate, nitrite, ammonium, alkalinity, oil spreading, and surface tension were completed on site within 2 hours after sample collection. For the other analyses, the samples were transported back to the laboratory and stored at 4°C until analyzed. All measurements for each sample were done in duplicate unless otherwise indicated.

Detection of biosurfactant production. The biosurfactant activities of the unfiltered samples were measured by using the oil-spreading technique (33, 49). The diameter of the clear zone on the oil surface was measured in triplicate for each sample. Biosurfactant activity was defined as the diameter of the clearing on the oil surface in centimeters. The surface tensions of the filtered samples were measured with a Du Nuoy ring tensiometer (Fisher Scientific Inc., Hampton, NH) calibrated with water as the high-surface-tension standard and isopropanol as the low-surface-tension standard (25, 30). The lipopeptide biosurfactant was quantified by using high-performance liquid chromatography (HPLC) with a reversed-phase C₁₈ column (250-mm length by 1.5-mm inside diameter [ID]) and 60% acetonitrile in water as the mobile phase (48). Twenty microliters of 1:4 and 1:2 dilutions of filtered samples was injected onto the column. The retention times for the biosurfactant were 2, 2.3, and 3.1 min, corresponding to three different fatty acid tails of the lipopeptide. The peak areas of the three peaks were added together, and the concentration was calculated from standard curves prepared in a similar manner with surfactin (Sigma Chemical Co., St. Louis, MO) and the highly purified lipopeptides (48) produced by each of the two microorganisms, *Bacillus* strain RS-1 and *B. subtilis* subsp. *spizizenii* NRRL B-23049. The standard curve was linear up to 500 mg/liter of the lipopeptide. The lipopeptides detected in the samples had the same retention times as the highly purified biosurfactant obtained from cultures of each inoculum strain.

Fermentation analyses. A modified orcinol-H₂SO₄ method was used to determine the amount of glucose in each sample (45). Acetate, ethanol, and 2,3-butanediol were measured by using gas chromatography with an 80/120 Carbowax B-DA*4% Carbowax 20 M (2-m length by 2-mm ID) glass column (Sigma Chemical Co., St. Louis, MO). Helium was used as the carrier gas at a flow rate of 24 ml/min (18, 22). The injector temperature was 200°C; the flame ionization detector was set at 180°C. The column temperature was kept constant at 155°C for 3.5 min and then increased to 180°C at 30°C/min. The temperature was then held at 180°C for 10 min. One microliter of the sample diluted in 30 mM oxalic acid was injected onto the column. Oxalic acid was added to the samples in order to condition the column according to the manufacturer's recommendation. Lactate was measured by using HPLC with an Alltech Prevail organic acid column (250-mm length by 1.5-mm ID) (Alltech Associates, Deerfield, IL) and 25 mM KH₂PO₄ (pH 2.5) as the mobile phase according to the manufacturer's instructions. A 50- μ l aliquot of a 1:10 dilution of the sample was used. The above-mentioned metabolites were identified and quantified by comparison of their retention times and peak areas, respectively, with those of known standards.

To test whether the two *Bacillus* strains produced the same fermentation products from glucose as those detected in the produced fluids of the inoculated wells, *Bacillus* strain RS-1 and *B. subtilis* subsp. *spizizenii* NRRL B-23049 were each grown anaerobically in duplicate serum bottles containing 75 ml formation water supplemented with glucose, sodium nitrate, and metals at the same concentration as that used for the field experiment. The cultures were incubated at 37°C without shaking for 48 h. A three-series most-probable-number (MPN) technique (see below) was used to enumerate the number of viable cells immediately after inoculation and after 48 h of incubation.

Other chemical analyses. The pHs were measured for the filtered samples by using a handheld pH/conductivity meter (EXTECH Instruments, Waltham, MA). Nitrate, nitrite, ammonium, and alkalinity (in mg/liter) were measured colorimetrically by using Hach kits (Hach Chemical Co., Loveland, CO) according to the manufacturer's instructions. The alkalinity values and pHs were used to calculate the CO₂ concentrations in the samples by using the Henderson-Hasselbach equation, $\text{pH} = \text{pK}_a + \log (\text{value for ionized species/value for unionized species})$. Bromide in filtered samples was analyzed by using liquid chromatography with an analytical anion exchange column (IonPac AS4A-SC, 4 by 250 mm; Dionex Corporation, CA) (39). The concentrations of bromide in the samples were calculated from the standard curve of NaBr.

Microbiological analysis. A three-series MPN technique was used to enumerate total heterotrophic bacteria, spore-forming bacteria, and halotolerant bacteria in unfiltered samples from both the injected and the produced fluids. The procedure was modified for use of 96-well plates. Three columns of wells were used for each sample, which was serially 10-fold diluted from 10⁻¹ to 10⁻⁵.

The physiological properties of *Bacillus* species, e.g., biofilm formation (17), sporulation (17), and halotolerance (32), allowed the use of specific media and/or manipulations for the MPN analysis as follows. *Bacillus* biofilm growth medium (BBGM) (17) was used to enumerate heterotrophic bacteria and to promote

TABLE 1. Preinjection chemical and microbial analyses for all the wells^a

Well group	Temp (°C)	pH	Glucose (μg/liter)	Nitrate (mg/liter)	Ammonium (mg/liter)	Bromide (μM)	Alkalinity (mg/liter)
Inoculated ^b	27.9 ± 1.2	7.54 ± 0.55	4.22 ± 5.7	11.3 ± 2.4	8.12 ± 3.75	1.62 ± 0.02	745 ± 34
Nutrient only treated ^b	27.8 ± 3	7.4 ± 0.38	4.2 ± 4.3	11.8 ± 2.4	6.25 ± 1.4	1.64 ± 0.03	710 ± 66
Negative control ^c	28.8 ± 0.3	7.6 ± 0.43	1.97 ± 1.34	9.5 ± 3	7.5 ± 5	1.62 ± 0.01	720 ± 280

^a Acetate, lactate, ethanol, 2,3-butanediol, nitrite, the biosurfactant, total heterotrophic bacteria, and biosurfactant producers were not detected in any of the samples before injection. The detection limits for acetate, ethanol, and butanediol were 0.1 mM. The detection limit for lactate was 0.2 mM. The biosurfactant detection limit was 10 mg/liter.

^b Numbers are averages ± standard deviations for four replicates (two samples collected for each of two wells within a 1-week interval).

^c Numbers are averages ± ranges for two samples collected within a 1-week interval.

biofilm formation since biosurfactant production has been associated with biofilm formation in *Bacillus* species (5, 19, 44). Since *Bacillus* species are known to sporulate (17), a portion of each sample was heat treated at 85°C for 20 min and then diluted in BBGM to estimate the number of spore-forming bacteria. Finally, to estimate the number of halotolerant bacteria and to avoid underestimation of *Bacillus* species due to heat treatment, plate count broth (Difco Laboratories Inc., Detroit, MI) with 5% NaCl was used. To estimate the number of biosurfactant producers, 5 μl of sterile crude oil was added to the surface of the medium in all wells where growth was observed and the dissipation of the oil drop was noted. The MPNs of biosurfactant producers in the samples were estimated from those wells where the oil dissipated on the surface. Published tables (www.fs.usda.gov/Ophs/Microlab/Appendix2.02.pdf) were used to calculate the MPNs.

Samples (50 μl) from the injected and produced fluids of each well were patch inoculated onto blood agar plates and incubated overnight at 37°C. Multiple blood agar plates were subsequently streaked from the growth patch. Resulting beta-hemolytic (12) clearing zones were picked and streak purified until pure isolates were obtained. The beta-hemolytic isolates with the same colony morphologies as those of *Bacillus* strain RS-1 or *Bacillus subtilis* subsp. *spizizenii* NRRL B-23049 were used for further culture-dependent molecular analyses (see below).

Molecular analysis. Formation water samples (2 liters) before and after treatment were vacuum filtered on a polyethersulfonate membrane with a 90-mm diameter and a 0.2-μm pore size (VWR, West Chester, PA) after oil separation. Membranes containing the microorganisms were cut and used for culture-independent DNA extraction. Cells were lysed by rapid thawing and bead beating to shear the membrane into small pieces (typically less than 5 mm), followed by mixing with stool lysis buffer (QIAGEN, Valencia, CA), vortexing for 2 min, and incubating at 95°C for 10 min in a water bath. Using a modified QIAamp DNA stool mini kit protocol (QIAGEN, Valencia, CA), DNA was extracted from lysed cells and amplified in a *Taq* DNA PCR, using degenerate primers designed to hybridize with the *gyrA* gene sequences of a variety of *Bacillus* strains (13, 40).

PCR products were gel extracted (QIAGEN), pGEM cloned (Promega), and plasmid prepped (QIAGEN). Four clones from each *gyrA* amplicon were sequenced by the Oklahoma Medical Research Foundation (OMRF; Oklahoma City, OK). Sequences were analyzed by DNAMAN multiple sequence alignment (Lynnon Biosoft) with the sequences obtained for *Bacillus* strain RS-1 (GenBank accession number DQ995270) and *Bacillus subtilis* subsp. *spizizenii* strain NRRL B-23049 (GenBank accession numbers DQ995271 and AF272020).

Cell templates from isolates obtained on blood agar plates (see above) were utilized as a source of culture-dependent DNA for 16S rRNA gene (34, 35) and *gyrA* analyses as well as repetitive extragenic palindromic (REP)-PCR (21, 46). Three clones of each gene from each isolate were prepared and analyzed as described above. Consensus sequences were obtained by multiple sequence alignment and compared with those from other isolates and the strains used as the inoculum. The GenBank accession numbers for the 16S rRNA gene (551 bp) of *Bacillus* strain RS-1 and *Bacillus subtilis* subsp. *spizizenii* strain NRRL B-23049 are DQ995269 and AF074970, respectively. REP-PCRs were resolved first on a 1% agarose gel and subsequently on a 5% polyacrylamide gel with 0% denaturant at 75 V for 3.5 h at 60°C.

Calculation of total recoveries. The total amount of glucose utilized (C_{ST}) in a well was calculated from the equation $C_{ST} = N - \sum C_{si} \cdot V_n$, and the total amount of each metabolite produced (C_{PT}) was calculated from the equation $C_{PT} = \sum C_{pi} \cdot V_n$, where N is the number of moles of glucose injected in the well, C_{si} or C_{pi} is the molar concentration of glucose or metabolites, respectively, in the produced fluid of the n th sample collected from the well, and V_n is the volume of fluid produced during the time interval between the collection of samples $n - 1$ and n from the well. The total MPN of cells (C_{XT}) was calculated from the equation $C_{XT} = \sum C_{xi} \cdot V_n$, where C_{xi} is the MPN per liter of cells in the produced fluid of the n th sample, and V_n is the volume of fluid produced during the time interval between the collection of samples $n - 1$ and n .

Bromide was used as a conservative tracer to estimate the amount of dilution of the nutrient package by dispersion in the formation (4, 39). The bromide recovery factor was calculated for each of the three wells that had total volu-

TABLE 2. MPN analysis of total and spore-forming heterotrophic bacteria and biosurfactant producers in the injected and produced fluids^a

Well group	Log MPN (C_{XT}) of indicated organism in indicated fluid ^b			
	Injected		Produced	
	Heterotrophic bacteria	Biosurfactant producers	Heterotrophic bacteria	Biosurfactant producers
Inoculated				
Total	15 ± 0.01	10.3 ± 0.6	14.6 ± 0.4	— ^c
Spore formers	11.2 ± 0.6	11.2 ± 0.6	10.3 ± 0.2	10.3 ± 0.2
Nutrient only treated				
Total	11.5 ± 2.3	9.6 ± 0.6	12.1 ± 0.8	—
Spore formers	—	—	—	—
Negative control				
Total	9.15 ± 2.3	—	9.7 ± 0.11	—
Spore formers	—	—	—	—

^a BBGM was used for the MPN analysis.

^b Numbers are average log₁₀ total MPNs ± standard deviations for four independent determinations except for the negative control, where numbers are averages ± ranges for two independent determinations.

^c —, no growth was detected in the MPN medium.

TABLE 3. MPN analysis of halotolerant heterotrophic bacteria and biosurfactant producers in the injected and produced fluids^a

Well group	Log MPN (C_{XT}) of indicated organism in indicated fluid ^b			
	Injected		Produced	
	Heterotrophic bacteria	Biosurfactant producers	Heterotrophic bacteria	Biosurfactant producers
Inoculated	12 ± 0.3	11.9 ± 0.3	15 ± 0.5	12.2 ± 0.05
Nutrient only treated	12 ± 0.45	10.6 ± 1.4	13.7 ± 0.3	9.8 ± 0.3
Negative control	12.5 ± 0.4	9.4 ± 0.4	11.1 ± 0.04	— ^c

^a Plate count broth supplemented with 5% NaCl was used for the MPN analysis.

^b Numbers are average $\log_{10}$ MPNs ± standard deviations for four independent determinations except for the negative control, where numbers are averages ± ranges for two independent determinations.

^c —, no growth was detected in the MPN medium.

metric flow meters attached. The average bromide recovery factor from the three wells was 1.09 (24% variation), which indicated that little dispersion or adsorption of the tracer occurred. The values for C_{ST} , C_{PT} , and C_{XT} from these three wells were divided by the corresponding bromide recovery factors obtained for those wells. For the other two wells, the average bromide recovery factors were used. The corrected numbers of moles of glucose utilized and moles of end products were used for percent carbon recovery calculations.

RESULTS

Preinjection data. Samples were collected from all the wells 1 and 2 weeks prior to the treatment. The biosurfactant and common bacterial fermentation end products, such as acids and alcohols, were not detected in any of the wells prior to treatment (Table 1). In addition, we did not detect any viable bacteria at any dilution used for the MPN enumerations (Table 1).

Evidence that the injected strains were maintained in the inoculated wells. MPN analysis using the BBGM showed biosurfactant producers in the injected fluids of both the inoculated wells and the nutrient-only-treated wells (Table 2). The total number of biosurfactant producers (C_{XT}) in the injected fluids was 1 order of magnitude higher for the inoculated wells than for the nutrient-only-treated wells (4.2×10^{10} compared to 3.7×10^9) (Table 2). No spore-forming microorganisms were detected in either the injected or the produced fluids of the nutrient-only-treated wells or the negative control. Since

Bacillus species are known to sporulate (17), the absence of spore-forming microorganisms in the nutrient-only-treated wells indicates that the biosurfactant producers that were introduced into these wells were probably not *Bacillus* species but microorganisms present in the storage tank. On the other hand, in the inoculated wells, spore-forming, biosurfactant-producing microorganisms were both introduced in and retrieved from the injected and produced fluids, respectively (Table 2). The C_{XT} values for spore-forming biosurfactant producers in the injected and produced fluids of the inoculated wells were not significantly different (Table 2). These data indicate that biosurfactant producers in the inoculum maintained viability but did not grow during the incubation period.

Although data in Table 2 suggest that biosurfactant-producing *Bacillus* species were present only in the produced fluids of the inoculated wells and not in those of the nutrient-only-treated wells, it was essential to eliminate any underestimation of *Bacillus* MPN due to the heat treatment. Plate count broth medium modified to contain 5% salt was used for the MPN analysis to select for the halotolerant *Bacillus* species (Table 3). Although halotolerant biosurfactant producers were detected in the produced fluids of the nutrient-only-treated wells, the total MPN (C_{XT}) of these microorganisms was 53-fold lower than that in the injected fluids of the same wells. On the

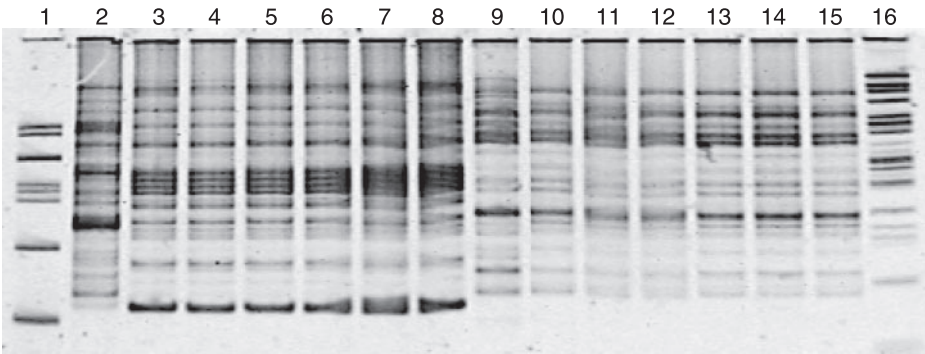


FIG. 1. Polyacrylamide gel of the REP-PCR for identification of *Bacillus* strains in the injected and produced fluids of the inoculated wells. DNA was extracted from isolates that had colonies with the same morphologies as those of the two strains used as the inoculum (*Bacillus* strain RS-1 and *Bacillus subtilis* subsp. *spizizenii* NRRL B-23049) and used for the PCRs. Lanes 2 and 9 are DNA from *B. licheniformis* and *B. subtilis* subsp. *spizizenii* type strains, respectively. Lanes 3 and 10 are DNA from laboratory-grown *Bacillus* strain RS-1 and *B. subtilis* subsp. *spizizenii* NRRL B-23049, respectively. Lanes 4 to 8 are *Bacillus* strain RS-1-like isolates, while lanes 11 to 15 are *B. subtilis* subsp. *spizizenii* NRRL B-23049-like isolates. Lanes 4 and 11 are DNA from isolates obtained from the tanks used for the inoculation. Lanes 5, 6, 12, and 13 are DNA from isolates obtained from the injected fluids. Lanes 7, 8, 14, and 15 are DNA from isolates obtained from the produced fluids. Lanes 1 and 16 are the DNA ladder.

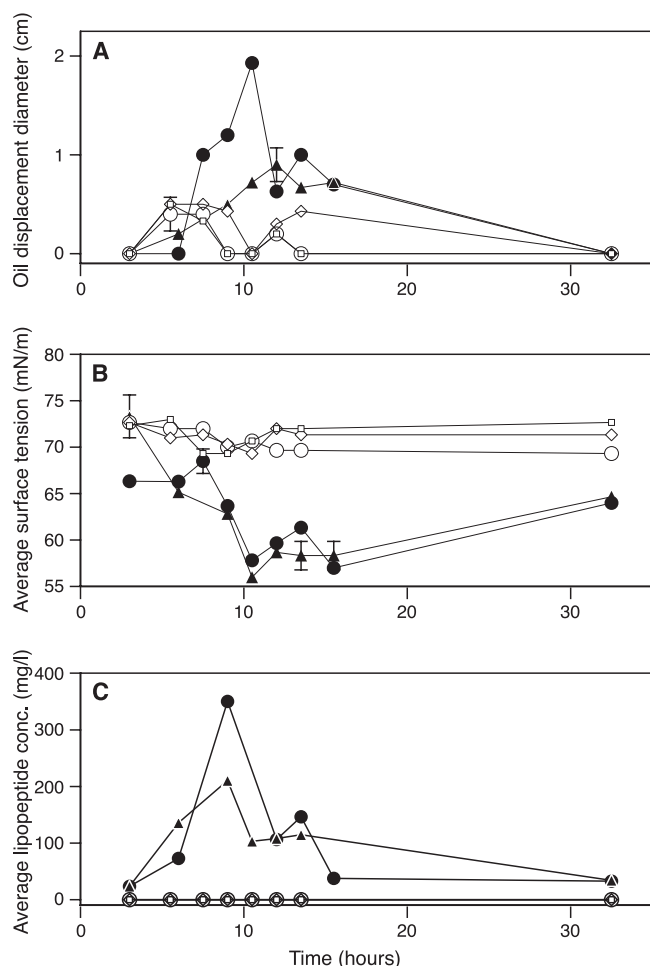


FIG. 2. Evidence for in situ biosurfactant production. The x axis represents the time in hours after the production started from the wells. Closed circles and triangles represent data from the two inoculated wells, open squares and open diamonds represent data from the two nutrient-treated wells, and open circles represent data from the negative-control well. (A) By the oil-spreading technique. Error bars represent standard deviations for three measurements. (B) By surface tension measurement. Error bars represent standard deviations for three measurements. (C) By determination of biosurfactant concentration (conc.) by high-performance liquid chromatography. Error bars represent ranges for duplicate measurements.

other hand, MPN data showed that the total MPN (C_{XT}) of halotolerant biosurfactant producers in the produced fluids of the inoculated wells was about 1.5 times that present in the injected fluids of the same wells.

The MPN data suggest the absence of *Bacillus* species (no spore-forming, biosurfactant-producing microorganisms) and the presence of non-spore-forming, halotolerant biosurfactant producers in the nutrient-only-treated wells. The latter were probably introduced into the wells with the treatment (e.g., from the storage tank formation water) but were not maintained. On the other hand, MPN data from the inoculated wells indicate the survival of *Bacillus* species (spore-forming, halotolerant, biosurfactant-producing microorganisms) in these wells.

Colonies with the same morphologies as those of the two

strains used as the inoculum, *Bacillus* strain RS-1 and *B. subtilis* subsp. *spizizenii* NRRL B-23049, were retrieved on blood agar plates from both the injected and the produced fluids of the inoculated wells but not from the injected and produced fluids of the wells that received only nutrients or the negative-control well. The 16S rRNA and *gyrA* gene sequences of these clones were 100% similar to those of *Bacillus* strain RS-1 and *B. subtilis* subsp. *spizizenii* NRRL B-23049.

Culture-independent *gyrA* amplicons were obtained with DNA extracted from produced fluids of the inoculated wells and not from DNA extracted from produced fluids of the wells that received only nutrients or the control well that received brine. The *gyrA* amplicons were not detected with DNA extracted from produced fluids of the inoculated wells collected prior to treatment. The resulting sequences of the *gyrA* clones obtained were 99.72% similar to those of *Bacillus* strain RS-1 and *B. subtilis* subsp. *spizizenii* NRRL B-23049.

REP-PCR reactions (21, 46) on blood agar plates containing isolates from the injected and produced fluids of the inoculated wells showed patterns similar to those of the inoculum strains, *Bacillus* strain RS-1 and *B. subtilis* subsp. *spizizenii* NRRL B-23049 (Fig. 1).

Collectively, microbiological and molecular data show that the microorganisms injected into the formation were retrieved in the produced fluids of the inoculated wells.

Lipopeptide biosurfactant production. The presence of the biosurfactant in produced fluids was followed over the time of sampling by using three different methods. The oil-spreading technique (33, 49) and surface tension measurement (25) were used to detect the presence of surface-active compounds in the produced fluids of all the wells. Surface activity was observed only in production fluids from the inoculated wells as evidenced by an increase in oil-spreading activity and a decrease in surface tension (Fig. 2A and B). No evidence for surface activity was detected in the production fluids from the nutrient-only-treated wells or the negative-control well (Fig. 2A and B). To determine the natures and the concentrations of the biosurfactant produced in the inoculated wells, HPLC was used (48). The HPLC profile of the samples from the produced fluids of the inoculated wells matched those of the lipopeptide biosurfactants purified from laboratory cultures of the two strains used as the inoculum, *Bacillus* strain RS-1 and *B. subtilis* subsp. *spizizenii* NRRL B-23049. The average concentration of the lipopeptide biosurfactant in the produced fluids of the inoculated wells was about 90 mg/liter (a total amount of 7 mol in ~80,000 liters of produced water) (Table 4 and Fig. 2C). The maximum concentration was as high as 350 mg/liter in the produced fluids of the inoculated wells (Fig. 2C). These maximum concentrations are more than 20 times higher than the critical micelle concentration reported for lipopeptide biosurfactants (ranging from 10 to 20 mg/liter) (33). HPLC analysis of the produced fluids of the nutrient-only-treated wells and the negative-control well showed no lipopeptide biosurfactant production.

Glucose utilization and product formation. Glucose was the carbon and energy source in our treatments. While complete glucose utilization did not occur, it was clear that large amounts of glucose were used since only 20 to 40% of the glucose added to the nutrient-treated wells (both inoculated and uninoculated) was recovered in the produced fluids and

TABLE 4. In situ mass balance and product formation rates and yields compared to those obtained in laboratory culture

Product	Value for indicated treatment group ^a								
	Inoculated wells			Nutrient-only-treated wells			Laboratory culture		
	No. of moles ^b	Rate (h ⁻¹) ^c	Yield ^d	No. of moles ^b	Rate (h ⁻¹) ^c	Yield ^d	No. of moles ^b	Rate (h ⁻¹) ^c	Yield ^d
Acetate	17 ± 7	0.04 ± 0.004	0.03 ± 0.01	ND	NA	NA	0.57 ± 0.03	0.04 ± 0.001	0.12 ± 0.007
Butanediol	68 ± 44	0.06 ± 0.007	0.13 ± 0.08	ND	NA	NA	0.54 ± 0.014	0.04 ± 0.0005	0.11 ± 0.003
Lactate	40 ± 0.9	0.06 ± 0.0002	0.08 ± 0.002	ND	NA	NA	2.1 ± 0.19	0.06 ± 0.001	0.42 ± 0.04
Ethanol	900 ± 50	0.08 ± 0.001	1.8 ± 0.28	130 ± 48	0.07 ± 0.003	0.36 ± 0.14	0.39 ± 0.12	0.03 ± 0.007	0.08 ± 0.03
CO ₂	640 ± 113	0.02 ± 0.001	1.3 ± 0.22	200 ± 76	0.01 ± 0.003	0.6 ± 0.21	11 ± 0.56	0.07 ± 0.001	2.3 ± 0.11
Biosurfactant	6.8 ± 0.67	0.02 ± 0.001	0.02 ± 0.001	ND	NA	NA	0.11 ± 0.05	0.05 ± 0.009	0.02 ± 0.009
Cells ^e	6.7 ± 7.7	0.06 ± 0.01	0.01 ± 0.02	0.24 ± 0.14	0.18 ± 0.006	0.001 ± 0.0004	0.12 ± 0.17	0.18 ± 0.06	0.03 ± 0.03

^a The numbers of moles of glucose used were 506 ± 118 mol, 360 ± 34 mol, and 4.88 ± 0.0004 mol for the inoculated wells, nutrient-only-treated wells, and laboratory cultures, respectively. Percent carbon recoveries were 106.7 ± 34, 20.7 ± 9, and 94.5 ± 12.8 for the inoculated wells, nutrient-only-treated wells, and laboratory cultures, respectively. ND, not detected; NA, not applicable.

^b The numbers are averages ± standard deviations for four independent determinations. Values were corrected for adsorption using the bromide recovery factor.

^c The rates were calculated from the equation $\ln(C_t/C_0) = k \cdot t$, where C_t is the concentration at time t , C_0 is the initial concentration, k is the rate of production, and t (time) was 108 h. The numbers are averages ± standard deviations for four independent determinations.

^d Yields are shown in mol · mol glu⁻¹ and were calculated from the equation $Y_{p/s} = \text{number of moles of product} \cdot \text{mol}^{-1}$ of substrate used. The numbers are averages ± standard deviations for four independent determinations.

^e The numbers of moles of cells were calculated from the following equation: number of moles of cells = $C_{XT} \cdot \text{mass of one cell/molecular weight of the cell}$, where the mass of a single cell in grams was assumed to be 1E-13 g, and the molecular weight of the cell was calculated from the cell's empirical formula, CH_{1.8}O_{0.5}N_{0.2}.

products of microbial metabolism were detected in the produced fluids. The produced fluids of all the nutrient-treated wells (both inoculated and uninoculated) showed increases in alkalinity and carbon dioxide concentrations compared to pretreatment levels, most likely resulting from microbial growth and activity. The produced fluids of the inoculated wells had much higher CO₂ concentrations following treatment than those of the nutrient-only-treated wells (Table 4 and Fig. 3E). In addition to CO₂, acetate, lactate, ethanol, and 2,3-butanediol were detected in the produced fluids of the inoculated wells (Table 4 and Fig. 3A to D). The percent carbon recovery was 107% ± 34% for the inoculated wells (Table 4). Similar fermentation products from glucose metabolism were obtained by the pure cultures of the strains used as the inoculum, *Bacillus* strain RS-1 and *B. subtilis* subsp. *spizizenii* NRRL B-23049, when the strains were individually grown in formation water supplemented with the nutrients used for well treatments (Table 4). These data argue that the products detected in the produced fluids of the inoculated wells were products of anaerobic glucose metabolism by the inoculum. Although glucose was partially utilized in the wells that received only nutrients, none of the above-mentioned fermentation products were detected, with the exception of CO₂ and some ethanol (Fig. 3). We do not know the fate of the remaining glucose carbon in these wells.

The detection of 2,3-butanediol, a product often produced by *Bacillus* species during fermentation (32, 43), and the biosurfactant indicates that we stimulated the microorganisms responsible for biosurfactant production.

DISCUSSION

A major concern with MEOR is whether exogenous microorganisms will be metabolically active in the presence of diverse, natural populations of microorganisms that inhabit oil reservoirs (2, 8, 32). Biosurfactant-mediated oil recovery is even more problematic in that a metabolite, e.g., the biosurfactant, not related to the main energy metabolism of the cell

must be produced. The data presented in Fig. 2 and 3 and Table 4 clearly show that the appropriate metabolism was stimulated in the formation and resulted in the production of the biosurfactant and products indicative of a *Bacillus* fermentation (45). The MPN data indicate that microorganisms physiologically similar to those used as the inoculum (halotolerant, spore-forming biosurfactant producers) were present in high numbers in the produced fluids of the inoculated wells after the incubation period. Molecular characterization of the isolates obtained from the produced fluids of the inoculated wells clearly showed that the same strains used in the inoculum were retrieved from the produced fluids of the inoculated wells. These data provide clear evidence that biosurfactant-mediated oil recovery is technically feasible.

Even though microorganisms exist in the reservoir (as evidenced by growth and glucose utilization in the wells that received only nutrients), the indigenous microorganisms did not prevent the strains used as the inoculum from establishing and metabolizing in the reservoir. The reason for the success of the inoculation procedure might be that the type and amount of nutrients used were more favorable for *Bacillus* species than for indigenous microorganisms. Pretreatment sampling did not detect the presence of microorganisms in the produced fluids of the wells that were capable of growing in the various media used for enumeration (Table 1). So, it is possible that the injection of nutrients created a niche that allowed the injected strains to be metabolically active. The MPN analysis did not indicate that the biosurfactant producers in the inoculated wells grew, since there was not a significant difference between the total MPN of the spore-forming biosurfactant producers (Table 2) or the halotolerant biosurfactant producers (Table 3) present in the injected fluids and that in the produced fluids. The lack of growth may have been due to a nutrient limitation. Nitrate was used as the nitrogen source, and limiting amounts of nitrate were added to the treated wells to shift the carbon flow from cell mass production to a secondary metabolite production. Previous studies show that nitrogen limitation is associated with an increase in biosurfactant production (11).

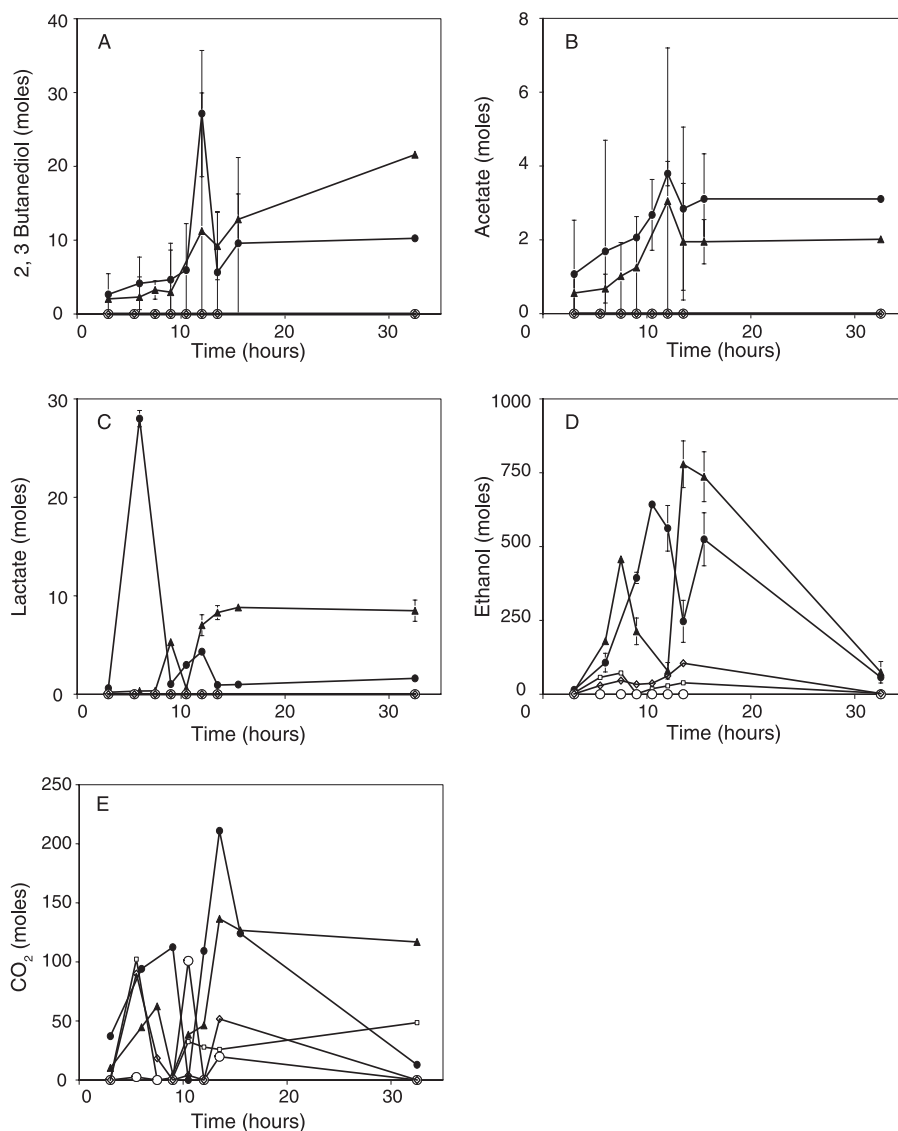


FIG. 3. Total numbers of moles of glucose fermentation products in the produced fluids of the wells. The x axis represents the time in hours after the production started from the wells. Closed circles and triangles represent data from the two inoculated wells, open squares and open diamonds represent data from the two nutrient-treated wells, and open circles represent data from the negative-control well. (A) 2, 3-Butanediol. (B) Acetate. (C) Lactate. (D) Ethanol. (E) CO_2 .

Sand-packed-column studies have shown that oil recoveries up to 95% occur when the columns are treated with lipopeptide biosurfactants (3, 27, 36, 38). Recent studies have shown that at least 11 mg/liter of a lipopeptide biosurfactant is required to mobilize oil from sandstone cores, and recoveries as high as 40% of entrapped oil were obtained with as little as 38 mg/liter of the lipopeptide biosurfactant (20, 28). In the current field test, the average concentration of lipopeptide biosurfactant in the produced fluids of the inoculated wells was about 90 mg/liter. This concentration is approximately nine times the minimum concentration required to mobilize entrapped oil from sandstone cores (28). These results showed that in situ lipopeptide biosurfactant production indeed meets this important engineering criterion.

Previous laboratory studies that used both sandstone cores

and sand-packed columns suggested that 2.2 ml of oil could be recovered per mg of a lipopeptide biosurfactant (29). Based on this information, the 7 mol of lipopeptide biosurfactant recovered from the produced fluids of the two inoculated wells could recover approximately 16 m³ (100 barrels) of oil. The total material expenses for producing 7 mol of biosurfactant were about \$164 (\$82 per well). The cost of the in situ biosurfactant production process could be as low as \$10 per m³ (\$1.6 per barrel). Since the main goal of the study was to test whether in situ biosurfactant production is possible, the volume of the reservoir that was contacted was small, and thus, significant amounts of additional oil were not expected. However, the company followed oil production before and after treatment and these data can be used to judge the effectiveness of the process. The lease that had the two inoculated wells, one of the

nutrient-only-treated wells, and several other wells showed an average increase in oil production of one barrel of oil per day compared to the oil production rate before treatment. This increase in oil production was maintained for a period of 7 weeks following treatment. On the other hand, the oil production rate of the other well that received only nutrients slightly decreased during the 7 weeks after treatment compared to pretreatment oil production rates. Although the oil production data suggest that the in situ process was not as efficient and cost-effective as predicted from laboratory data, the data still argue for the cost-effectiveness of MEOR compared to the current price of oil (>\$65/barrel). However, more definitive results can be obtained if the size of the treatment is scaled up.

Our data show that in situ biosurfactant production is possible and occurred in amounts exceeding the engineering criterion for mobilizing oil from sandstone cores. This work also provides essential data for modeling MEOR processes in situ (Table 4), including growth rates ($0.06 \pm 0.01 \text{ h}^{-1}$), carbon balances ($107\% \pm 34\%$), biosurfactant production rates ($0.02 \pm 0.001 \text{ h}^{-1}$), and biosurfactant yields ($0.015 \pm 0.001 \text{ mol biosurfactant/mol glucose}$). These data can be used to study computationally microbial activity in subsurface environments, including petroleum reservoirs. We should note that this is the first time that an in situ carbon balance has been obtained for any MEOR process. Overall, the work emphasizes the technical feasibility and cost-effectiveness of MEOR.

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Microbiological Methods for the Enhancement of Oil Recovery

DEREK G. SPRINGHAM

*School of Biological Sciences, Queen Mary College, University of London,
Mile End Road, London E1 4NS, UK*

Introduction

Conventional processes for extraction of oil from reservoir rocks are surprisingly inefficient. The proportion of the original oil in place which is extracted can vary from about 5% to about 90% but the average recovery is not much more than 30%.

Oil is a vital ingredient in the economy of most nations, and its availability and price have profound and widespread economic and political impact. The total quantity available in the earth's crust, although not exactly known, is finite, and certainly insufficient to meet the future needs and aspirations of either consuming or producing nations in the next 50–100 years.

Some idea of the potential significance of methods aimed at the extraction of extra oil (Enhanced Oil Recovery, EOR) can be gained from figures presented by Geffen (1976). The known oil deposits in the United States, at the beginning of 1975 totalled 440×10^9 barrels. Of this total, some 106×10^9 barrels (24%) had already been produced. The reserves which could be produced by conventional methods amounted to 39×10^9 barrels (9%), equivalent to about seven times the US consumption in 1982. Forty per cent (176×10^9 barrels) were considered to be irrecoverable by any present or envisaged technologies, leaving 120×10^9 barrels (27%) as the target for enhanced oil recovery techniques. On a world-wide basis the target for EOR is correspondingly greater, and it has been calculated that the world's oil fields contain roughly 1.2×10^{12} barrels of oil with a current value of about US $\$3.5 \times 10^{13}$ which is not recoverable by conventional techniques (V. Moses, personal communication). Engineers have been working for some years on chemical and physical methods of EOR. So far, applications have been limited to specially favourable circumstances and results have often been disappointing in terms of the extra oil recovered and of the economics of the process. An alternative approach is to make use of micro-organisms, deliberately injected into the oil-bearing rock, to release oil. Such processes, known as Microbial Enhancement of Oil Recovery (MEOR), are less well established than the chemical and physical methods, but may have

special advantages such as low operating costs and, perhaps, improved efficiency, which would make them especially attractive in the long run (Moses and Springham, 1982). They form the subject matter of this review.

Conventional procedures for oil recovery

To understand the potential uses of micro-organisms an outline knowledge of the conventional processes for oil recovery is necessary. A simplified account, aimed at the microbiologist, has been presented by Moses and Springham (1982) and a broader account of this and other aspects of the oil industry is that by Stockil (1977).

Crude oil is an extremely complex mixture consisting mainly of alkanes, cycloalkanes and aromatics. Unsaturated compounds are rare. Sulphur compounds are present, usually in small amounts and there are traces of oxygen, nitrogen and metal-containing compounds. The specific gravities of crude oils range from 0.78 to 1.0. Viscosities are usually higher than that of water, values ranging over seven orders of magnitude. Gases, mainly methane, are found in solution. Oil is believed to have been formed from plant or animal remains deposited in marine sediments. It is found in porous sandstone or carbonate rocks having been forced upwards by displacement with water. It is found in commercially useful quantities only where a layer of impermeable clay or shale, the cap rock, prevents further upward movement. Water is always present in the oil-bearing layer (connate water). It often has a high salt content and is referred to as brine. Frequently a layer of brine underlies the oil.

The reservoir rock can be characterized in terms of two very important properties: porosity and permeability. The porosity is the proportion of the rock volume not occupied by rock particles; it defines the maximum quantity of oil which a given volume of rock can contain. Permeability, measured in darcies ($d = 1 \mu\text{m}^2$), is a measure of the ease with which a fluid can be forced through the rock. Permeabilities of reservoir rocks vary from fractions of a millidarcy up to several darcies. In the former case oil will flow only with great difficulty.

If a well is drilled into the correct region of a reservoir, oil will be forced into the well bore by one or more so-called 'primary mechanisms'. The gas in the cap (usually under considerable pressure) will expand, gas may come out of solution from the oil, and water may flow in from surrounding rocks. The pressure may be sufficient to force oil to the surface as a 'gusher' or alternatively a pump may be used to raise it. Sooner or later the flow of oil due to primary mechanisms will decrease and secondary recovery will usually be undertaken. This involves drilling one or more injection wells and pumping in fluid to maintain pressure or to displace oil directly. Waterflooding is the most common method of secondary recovery. The extra wells (which may be in a five-spot pattern usually with four injection wells surrounding one production well, or may form a more extensive array in a larger field) are drilled down into the brine layer beneath the oil. Water quality is critical for injection. If the salinity is unsuitable, clay particles in the reservoir rock may swell and reduce the permeability. Careful filtration is usually practised to remove organic and

inorganic materials which might block ('plug') the formation, together with de-aeration to prevent corrosion, and the addition of scale inhibitors, corrosion inhibitors, and biocides as considered appropriate.

As primary and secondary production proceeds, the oil produced will be mixed with increasing proportions of water. Production will normally cease, not because there is no more oil, but because the proportion of oil is too low to justify continued operation. We have seen that at this point some two-thirds of the oil, on average, remains in place: it is now appropriate to consider why this is so.

In particular fields some oil will be cut off from the wells by impermeable layers; this can be released only by engineering procedures such as drilling more wells, and will not be considered further. Some oil will not be displaced because of the nature of flow patterns of fluids between injection wells and production wells. *Figure 1a* shows a portion of a reservoir in plan view with injection wells and production wells. To achieve 100% recovery of the oil, the area enclosed by the rectangle must be swept by water from the injection well. *Figure 1b* shows a more realistic flooding pattern. Water is almost always less viscous

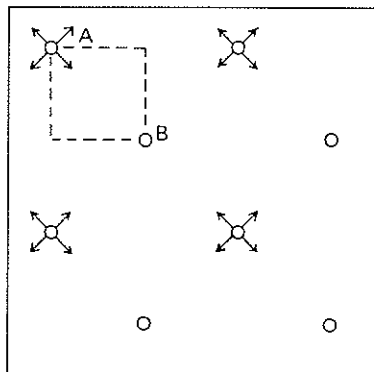


Figure 1(a). Part of a reservoir in plan view showing injection wells (X) and production wells (○). The dotted rectangle shows the area which, ideally, should be swept by flow of water from A to B.

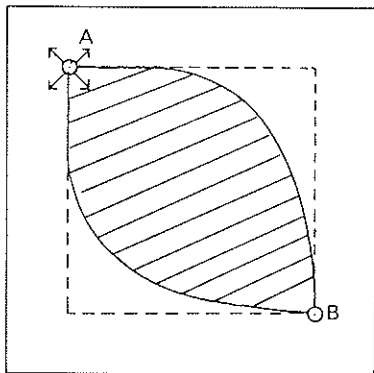


Figure 1(b). Enlarged view of part of the same area. The shaded area shows a more likely flow pattern of water between A and B. Waterflooding will not displace oil from outside this zone.

than the oil it is displacing and takes the easiest path between the two routes so the swept area is only a proportion of the whole. In thick oil-bearing layers, water tends to slide under the oil instead of displacing it evenly, thus reducing the sweep efficiency still further. A consideration of *Figure 1* suggests that it should be possible to improve the sweep efficiency by drilling more wells; this is indeed the case. More oil could be obtained by drilling wells at shorter intervals but the high cost of drilling largely determines the drilling pattern in the first instance.

Another departure from the optimum flow pattern is attributable to a process known as fingering. When a fluid such as water displaces a more viscous fluid such as oil in a porous matrix, the displacement fronts are highly uneven, with irregular fingers of water extending into the oil region. Once water flows in a particular zone, the resistance to flow is decreased because of its lower viscosity and the uneven flow is enhanced. In this way water can reach the production well at a comparatively early stage. As fingering becomes more severe the oil/water ratio in the produced fluid will decrease. If the structure of the reservoir rock is uneven (as it usually is), fingering will be promoted by preferential flow in regions of high permeability. Such regions, which may be due to high permeability strata such as the Brent Sand layers in many of the North Sea reservoirs or to rock fractures, are known as thief zones. In extreme cases preferential flow along thief zones may be so severe that only a small percentage of the oil in place is recoverable.

A further mechanism by which oil is cut off occurs on a microscale and involves the stranding of globules (ganglia) of oil in capillaries by the waterflood. *Figure 1c* shows an oil ganglion which has been cut off by water. The pressure differential across the ganglion tends to move it in the direction of the waterflood but to pass through the narrow throat of the capillary work must be done to distort the shape, because the surface area has to increase. With the range of normal parameters applying during a waterflood, a substantial proportion of the oil will be permanently cut off in this fashion.

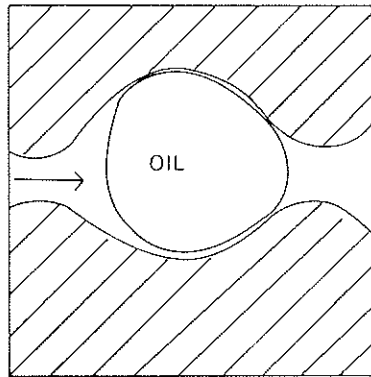


Figure 1(c). Oil globule trapped in a rock pore. The arrow shows the direction of the waterflood tending to displace the oil globule. In order to escape from the pore the globule must be deformed. This would increase the oil:water interfacial area and would require work to be done.

Chemical and physical methods for enhanced oil recovery

Only a brief consideration of non-biological methods is appropriate here. Detailed accounts are available (Groszek, 1977; Stewart, 1977; Stockil, 1977; Brown, 1979; Van Poollen, 1980; Shah, 1981). The methods fall into four classes: thermal methods, miscible displacement, chemical flooding and selective plugging. Thermal methods raise the temperature of regions of the reservoir by injection of hot water, steam or other gas or by conducting a combustion *in situ* of oil or gas. The principal aim is to reduce oil viscosity.

Miscible displacement methods involve the injection of fluids which are fully miscible with the oil, thus avoiding many of the difficulties of waterflooding. Carbon dioxide is a useful displacing fluid. As it dissolves in oil, the oil swells and its viscosity is reduced; at high pressures the fluids are fully miscible. Carbon dioxide flooding is an attractive method but large quantities of carbon dioxide are required: estimates range from 112 to 280 m³ per barrel of oil (Meyer, 1977; Shah and Wittmeyer, 1978; Doscher and Kuuskraa, 1979).

In a chemical flood, chemicals are injected with the waterflood to improve the displacement efficiency. In polymer flooding, a viscous solution of polyacrylamide or polysaccharide is introduced as a discrete slug in the waterflood. By raising the viscosity of the displacing fluid to equal or exceed that of the oil, the mobility ratio and efficiency of displacement can be greatly improved. Another way of making displacement more efficient is to lower the interfacial tension between the oil and the displacing fluid by the use of surfactant solutions. The preferred method is to produce a microemulsion from a mixture of surfactants (often sulphonated petroleum fractions) together with other components such as alcohols, brine and sometimes chelating agents. The aim is to push an oil-miscible micellar slug into the oil-bearing region to flush out residual oil. The displaced oil droplets coalesce to form a continuous layer or oil 'bank' which is itself very effective at dislodging more oil. The micellar slug follows behind the oil bank preventing oil ganglia being left behind. Usually a polymer will be used in conjunction, to optimize the sweep pattern.

Micellar flooding and polymer flooding work well on the laboratory scale but tend to perform less well in the reservoir. Success undoubtedly depends on choosing the right reservoir and treating it in the optimum manner: the formulation of the surfactant mix is especially critical. Several difficulties are peculiar to large-scale operations. Because of the high cost of the chemicals used they are not injected continuously but as a discrete slug. Correct function depends on the integrity of the slug so that piston-like displacement is achieved and optimum concentration of surfactant is maintained. The slug, however, tends to disintegrate as it proceeds through the reservoir, due to adsorption, degradation, precipitation or backmixing of components.

The last method, selective plugging, aims to inject viscous polymers or time-setting gels into thief zones. Since these zones accept the bulk of the injected aqueous flow the expectation is that they will be effectively plugged: floodwater will then be forced into the less permeable regions which will have received only minor amounts of polymer.

Enhanced oil recovery procedures are undoubtedly becoming more effective

as they become better understood but they are expensive to operate and their cost-effectiveness can be assessed from the large subsidies paid by the US Department of Energy to companies using them. Dafter (1980) has reviewed the prospects for the chemical and physical methods. It should be noted that in some cases EOR methods are best applied at an early stage.

Microbial enhancement of oil recovery (MEOR)

EARLY EXPERIMENTS ON MEOR

The first suggestion that bacteria might have a useful role in enhanced oil recovery came from Beckman in 1926. Claude ZoBell followed up the suggestion with an extensive series of laboratory experiments (*see*, for example, ZoBell, 1946, 1947a, b, 1953). He used cultures of sulphate-reducing bacteria designated as *Desulfovibrio hydrocarbonoclasticus* and *D. halohydrocarbonoclasticus*, claiming that treatment of oil-bearing formations with bacteria could potentially release oil by a number of mechanisms. Crude oil hydrocarbons were utilized by bacteria, leading to the production of carbon dioxide and acids. The acids reacted with rock carbonates, increasing permeability and producing more carbon dioxide.

Carbon dioxide, and other gases which might be produced, increased reservoir pressure and dissolved in the oil, forcing it to swell and reducing its viscosity, thus aiding displacement. It was also reported that the growth of bacteria on the surface of oil-bearing rocks in contact with nutrient media led to films of oil forming on the (aqueous) liquid surface, and it was suggested that displacement of oil from surfaces by bacterial growth was occurring. Some degree of oil utilization was reported and it was suggested that oil viscosity fell as a result of a reduction in the average molecular weight of oil components. The bacteria were also said to produce surface-active agents, reducing surface tensions from about 70 to about 50 mN/m, thus potentially aiding oil release. MacKenzie (1952) supported many of ZoBell's claims.

Beck (1947), using similar bacteria, found that utilization of oil was slow, and was unable to demonstrate enhanced oil release attributable to bacteria in model systems. Updegraff and Wren (1954), working in consultation with petroleum engineers, were unable to demonstrate consistent enhancement of oil release by sulphate-reducing bacteria alone under conditions relevant to reservoir operations. La Rivière (1955) expressed some reservations about ZoBell's conclusions but was able to demonstrate the reduction of surface tension by a number of bacteria. He pointed out that peptone, used by ZoBell, would itself reduce surface tension. On the other hand, Updegraff and Wren (1953) and Updegraff (1957) patented techniques in which a variety of other organisms including *Escherichia coli*, *E. freundii*, *Aerobacter aerogenes* and members of the genera *Clostridium* and *Bacillus* or sulphate-reducing bacteria in conjunction with other types, were claimed to produce significant enhancements of oil release when supplied with carbohydrates and mineral nutrients.

Possible mechanisms of oil release

The early publications of ZoBell proposed a series of possible mechanisms by which bacteria might release oil: several more have been added since. Not all are equally plausible.

Modification of reservoir permeability by acids. Many bacteria certainly produce acids under anaerobic conditions. Many reservoir rocks consist partly or entirely of carbonate. Bacterial action may result in dissolution of reservoir rock carbonate (see for example Yarbrough and Coty, 1983) and changes visible to the naked eye can be produced in calcite and dolomite cores by bacterial action (Bubela, 1983a), but it is not obvious that effects on permeability will necessarily prove favourable. There is a danger that fine particles, released by acid, might be swept into pore throats resulting in plugging. Extensive dissolution of a pure carbonate rock would require massive amounts of acid and hence of substrate. Acid might well preferentially attack the largest channels and there appear to be signs of this in the photographs presented by Bubela (1983a). This would increase permeability but would result in bypassing most of the oil. On the other hand, production of acid resulting in pore enlargement might well be favourable where the vital region immediately round the injection well has become partly plugged, and Johnson (1979) appears to have exploited this possibility.

2. *Repressurization by production of carbon dioxide, hydrogen, methane, nitrogen.* This seems a plausible mechanism and many field trials report pressure increases on bacterial treatment, sometimes of substantial magnitude and duration. Yarbrough and Coty (1983) reported that *Clostridium acetobutylicum* could generate pressures up to 8.8 MPa in laboratory culture in the absence of a gas phase, mainly due to production of hydrogen which has a very low solubility in water. Carbon dioxide generated from rock carbonate could contribute to this mechanism.

3. *Solution of gases in oil resulting in swelling and decreased viscosity.* Gases, particularly carbon dioxide, dissolve in oil under pressure, reducing its viscosity and increasing its volume. An increase in volume of sufficient magnitude might free some oil from ganglia and the effect on oil viscosity might be useful.

4. *Reduction of oil viscosity by degradation of large molecules.* Singer *et al.* (1983) isolated an aerobic organism, designated H13, which reduced the viscosity of heavy crude by as much as 98%. Part of this reduction was due to formation of an oil-water emulsion under the influence of a glycolipid surfactant. The surfactant alone reduced viscosity by about half, the remaining effect being apparently due to changes in oil composition, as yet only partly characterized (Donaldson, 1982). Similar results have been reported by Zhang and Quin (1983). These reports serve to demonstrate that bacteria can reduce oil viscosity, apparently by selective degradation as well as by emulsion formation, but as yet there is no demonstration that this is possible under the anaerobic conditions

pertaining in the reservoir and the persistent emulsions generated would be disadvantageous.

5. *Reduction of oil–water interfacial tension by surfactants.* The introduction of chemical surfactants into the reservoir together with the waterflood forms the basis for a well-known EOR technique. The production of surfactants of different chemical types by a variety of bacteria is well documented. Many of the cultures used for MEOR operations are reported to reduce the surface tension of culture broths. However, where interfacial tension measurements have been reported for bacterial surfactants, the values with one exception fall far short of that required to effect oil mobilization under reservoir conditions (*see below*). The operation of this mechanism is thus hypothetical.

6. *Dissolution of oil by solvents e.g. alcohols and ketones.* It seems unlikely that appropriate substances would be produced in high enough concentrations to dissolve oil but they might act as co-surfactants, modifying the behaviour of the surfactant itself (*see below*).

7. *Sulphonation of oil by bacteria.* Sulphonated petroleum fractions are used as surfactants for EOR. No evidence exists of bacterial surfactant production in this way.

8. *Displacement of oil from rock surfaces by bacterial growth.* Most reservoir rocks are water-wet. The oil is trapped by pore throats rather than stuck to a surface. The mechanism might operate in oil-wet rocks.

9. *Increase in viscosity of the aqueous phase caused by bacterial polymers.* Polymers, mainly of bacterial origin, are already used to increase the viscosity of injected water. Polymers can be produced under anaerobic conditions (*see below*) so it is feasible to consider their production *in situ*.

10. *Selective plugging of regions of high permeability.* If permeability variations are a serious problem this is a highly plausible mechanism. Bacterial cells can block pores either directly or by producing extracellular slimes (*see below*).

CONDUCTING MEOR OPERATIONS

As a result of early laboratory experiments a picture emerged on the way in which an MEOR operation might be conducted in the field. The work of ZoBell (1946, 1947a, b, 1953) had placed emphasis on sulphate-reducing bacteria and on utilization of crude oil as a substrate for growth. Later workers had difficulty in demonstrating oil utilization under anaerobic conditions and sometimes found that sulphate-reducing bacteria were ineffective at removing oil (Beck, 1947; Updegraff and Wren, 1954) and were likely to cause a variety of problems (*see below*). Emphasis therefore shifted to the use of other bacteria, either strict or facultative anaerobes, and to the provision of a carbohydrate substrate, usually molasses, or an organic acid such as lactate, for growth together with

a range of mineral nutrients (Updegraff and Wren, 1953, 1954, 1957). Hitzman (1962) pointed out that certain problems might arise if vegetative cells, injected together with nutrients, were used as the inoculum. Growth would begin in the vicinity of the well where, for geometrical reasons, large volumes of fluid would pass through a very small cross-sectional area of rock. Plugging of the formation at this point by cells or metabolic products (*see below*) would be particularly detrimental (Crawford, 1983), whereas the beneficial effects of bacterial growth and metabolism would be most effective further into the reservoir. Vegetative cells are susceptible to environmental hazards, such as high shearing forces, especially in the absence of nutrients, and Hitzman proposed to overcome these problems by first injecting the inoculum in the form of spores, introducing nutrients only after the spores had penetrated deep into the formation. He pointed out that spores penetrated sandpacks more readily than vegetative cells, an observation confirmed by later reports (e.g. Jang *et al.*, 1983). Later, as an alternative way of avoiding plugging, he proposed injecting the inoculum into the brine layer below the oil-bearing formation, the injection well being shut off from the brine layer. The inoculum would, it was claimed, grow at the oil-water interface, repressurizing the reservoir by producing methane for a 10–30-year period, but causing no dangers of plugging because bacterial growth would be outside the main oil-bearing layer (Hitzman, 1965).

Generally, the use of aerobic organisms has not been seriously considered because of the low oxygen status of reservoirs, because of the difficulties of introducing large quantities of oxygen without interfering with the waterflood and because engineers normally go to much trouble and expense to exclude oxygen to avoid corrosion of metal pipework. However, Jones (1967) proposed the use of aerobic bacteria, injected along with oxygen or air, to metabolize oil hydrocarbons *in situ*. Fracturing the rock formation to facilitate bacterial penetration was also proposed.

In many cases MEOR operations have proceeded as part of the waterflooding operations with nutrients and inoculum injected at one point and oil produced elsewhere (water-drive method). Alternatively the 'huff-and-puff' method has been used in which nutrients and inoculum are injected into a well which is then sealed for a period of months. Oil is produced by opening the well and reversing the flow.

POTENTIAL ADVANTAGES OF MEOR

MEOR offers, in principle, three advantages over other EOR systems. It is cheap, because the main raw material is an inexpensive carbohydrate source such as molasses, with or without inorganic nutrients. It is further expected that MEOR might be more effective than some types of EOR operation because the active materials would be generated throughout the reservoir, and their effectiveness would not be reduced by adsorption during migration to the site of action. It is also thought that bacteria, by diffusing out of the main lines of fluid flow, could mobilize oil in regions not accessible to other methods. The latter two advantages have yet to be proved.

LABORATORY MODELS OF MEOR

The techniques used to study oil release on a laboratory scale are of critical importance. Most reservoir rock is water-wettable and the surface is covered with a layer of water even when oil fills most of the pore-space. Oil is held in place by surface forces (*see later*). Thus the displacement of oil from oil-wet surfaces is not likely to be of importance in most reservoirs.

In order to simulate the porous reservoir for displacement studies, two main devices—sandpacks and cylindrical rock cores—usually derived from outcrop rocks, have been used. Sandpacks consist of glass or plastic tubes containing fine sand or other material such as glass beads. They are easy to make in a fairly uniform fashion but the grains are not normally fused as with a rock core, and it can be difficult to obtain permeabilities in the lower range. Yarbrough (personal communication) has used lucite (perspex) tubes packed with sand and then heated to fuse the sand to the tube wall in order to prevent edge channelling. Rock cores have been used in a variety of shapes and sizes. Berea sandstone has been a favourite material because of its fairly uniform structure and easy availability in the US in a range of permeabilities. Cylindrical shapes are usually used with fluid flow induced across the ends. To prevent the escape of fluids from along the length, the surfaces can be coated with an epoxy resin or the core can be sealed in a tightly fitting rubber sleeve (Hassler cell). In the latter case it is comparatively easy to apply uniform pressure to the core independent of the pressure differential across the ends. Occasional use has been made of other materials to simulate porous rock. Thus Bubela (1983a) described the use of concrete, which can be cast in a wide range of permeabilities, and cores of sintered alundum have also been used.

In some instances visual monitoring of the progress of an experiment may be useful. For studies of oil displacement a variety of systems have been devised in which a thin layer of the porous matrix is sandwiched between sheets of plastic or glass so that flows of oil and water can be observed directly (Dawe, personal communication). Similar arrangements have been used for bacterial experiments by the group at Queen Mary College (Andrew McKay, personal communication) and by Bubela (1983a).

The preparation of cores or sandpacks for an experiment is critical. Several workers steam-clean outcrop rocks before use to remove humic acids (Hank Yarbrough, personal communication, Jenneman *et al.* 1983). Sterilization may be carried out at this stage but is difficult to achieve. Jenneman *et al.* (1983) used a commercial chlorine dioxide preparation. This, they said, reduced the counts of indigenous bacteria for 24–48 hours but the population became re-established after this time or upon introducing nutrients. Even after steaming cores for two weeks, autoclaving for 12 hours and drying at 121°C, variable numbers of indigenous bacteria remained, presumably due to failure of steam fully to penetrate the pore structure. Jang *et al.* (1983) and Jang and Yen (1983) reported the use of 70% ethanol for sterilization. This, too, is unlikely to have been fully effective. The use of mercuric chloride or of low temperature to inhibit bacterial action in controls is not acceptable as both treatments reduce oil mobility (Davis and Updegraff, 1954).

Cores or sandpacks are first flooded with water (brine). Vacuum or pressure infiltration is necessary to get good penetration. Crude oil is then pumped in until no more water is displaced. In this way the rock surfaces become water-wet while the capillaries are full of oil as in a reservoir. If water is now injected at a steady rate, oil will be expelled. It is essential to continue with this process until no more oil emerges. This represents waterflooding to exhaustion and can be used both as a control and as a preliminary to bacterial injection. The lack of an explicit mention that this operation has been properly performed, makes it difficult or impossible to evaluate some of the reports in the literature. It is of no practical use to demonstrate that a bacterial culture (plus the waterflood) will release a certain percentage of the retained oil if the waterflood alone will produce as much or more.

Oil released can be measured volumetrically. Jang and Yen (1983) extracted it into toluene, evaporated the solvent and determined the oil by weighing. This seems advantageous if oil is released as an emulsion, as it may be if bacteria or synthetic surfactants are used, or if the quantities released are small. Small cores (say 7.5 cm $\times$ 2.5 cm) are frequently used commercially to test the effects of surfactants or polymers, but larger cores may be useful to facilitate accurate measurements of released oil and to avoid the possibility of end effects. Fluctuations in back pressure are almost inevitable: these are easily monitored by solid-state differential-pressure transducers connected across the column. Peristaltic pumps are widely available and convenient, but flow rates are very susceptible to fluctuations in back pressure. Continuous-action motor-driven syringe pumps are more expensive but can be virtually pulse-free and are much less sensitive to back pressure. Electronic flow-measuring devices are available but expensive.

Microbial activities and MEOR

PLUGGING AND PENETRATION OF RESERVOIR ROCK BY BACTERIA

Many authors have described the presence of a wide variety of bacteria in oil samples, formation rock and produced water. With some justification a range of undesirable activities has been attributed to bacteria and these include degradation of oil in reservoirs (Kuznetsov, Ivanov and Lyalikova, 1963; Anonymous, 1972; Westlake, 1983) the promotion of corrosion (Allred, 1976; Miller, 1971), souring by the production of H_2S , and formation plugging. The sulphate-reducing bacteria are considered to be especially undesirable (for general discussions see Beerstecher, 1954; Davis and Updegraff, 1954; Kuznetsov, Ivanov and Lyalikova, 1963; Davis, 1967; Moses and Springham, 1982).

Pressure maintenance and waterflooding operations involve the injection of enormous volumes of water into reservoirs, and micro-organisms present in the water may cause serious plugging problems. Crawford (1983) has calculated the effects of different degrees of plugging on waterflooding operations. Reservoir engineers have devised methods of treatment to alleviate this and other problems, and water treatment prior to injection may involve the use of flocculating agents to remove solids, de-aeration, addition of biocide to prevent bacterial growth

on filters, passage through various filters and, finally, the addition of substances such as oxygen scavengers, corrosion inhibitors, scale inhibitors and biocides. Two dangers arise in respect of MEOR operations: the suspension of treatment to permit bacterial inoculation and growth may permit undesirable organisms to enter, and the inoculum itself may have a detrimental effect.

It is not easy to generalize on the dangers of plugging. Some of the early work demonstrated that serious plugging of cores could occur very rapidly (Plummer *et al.*, 1944) but the waters used for the study contained a variety of bacteria, fungi, algae, protozoa, as well as precipitates of calcium carbonate and metal sulphides. Beck (1947) studied injection waters which had given rise to plugging problems and noted the presence of a variety of bacteria together with ferrous sulphide and ferric hydroxide precipitates. He recommended pretreatment of the water by filtration and the addition of germicides. It is clearly important to distinguish between plugging caused by cells and that caused by metabolic products, and to ask to what extent different types of cells cause different degrees of plugging, so that bacteria intended for MEOR can be chosen to eliminate or minimize these problems. Particularly serious problems are likely to be caused by iron bacteria and sulphate-reducing bacteria producing ferric hydroxide and ferrous sulphide precipitates respectively, and by slime-forming organisms; these would normally be avoided in MEOR operations or used only for special purposes.

Many investigators have used killed or non-growing suspensions of bacteria, injecting them into a variety of rock cores and measuring the pressure drop as an index of plugging. In some cases pressure was measured at a series of points along the core. There is general agreement that the injection of large volumes of dense bacterial suspensions produces a progressive reduction in permeability (Hart, Fekete and Flock, 1960; Kalish *et al.*, 1964; Raleigh and Flock, 1965; Jenneman *et al.*, 1983). The most severe plugging occurs at, or close to, the injection face. The greater the concentration of cells injected, the greater the degree of plugging. There is a concentration effect: a given number of cells causes more plugging if injected at low concentration than at high concentration (Hart *et al.*, 1960; Kalish *et al.*, 1964). Kalish *et al.* (1964) found that permeability values tended to stabilize after the injection of large volumes of suspension: the proportional reductions in permeability at this point were greatest in the formations of highest permeability. They attributed this to the ability of cells to penetrate further into the more permeable formations and thus cause plugging in depth. By contrast Hart, Fekete and Flock (1960) found that in some cases permeability was reduced almost to zero and were unable to relate permeability reductions to the initial permeability values of their rock samples. Using different bacterial species, Kalish *et al.* (1964) concluded that large cells cause more plugging than small cells and clumps cause more plugging than single cells. Jack, Thompson and DiBlasio (1983) isolated an anaerobic rod-shaped isolate which grew as discrete cells on sucrose medium but as chains on glucose/fructose medium. The chains were much more effective at plugging than the discrete cells. Bubela (1983b) found that rod-shaped organisms caused greater plugging than did cocci and that the plugs are more difficult to shift by the application of pressure.

Kalish *et al.* (1964) found that the adverse effects of bacteria on core permeability could be overcome readily by increasing the applied pressure, which increased permeability apparently by dislodging cell plugs, or by acid treatment followed by reverse flooding. Chlorine treatment is an alternative (Crawford, 1983). Sharpley (1961) discussed the various problems of plugging in waterflood operations and specified safe limits for the numbers of bacterial cells in injection water. The general validity of these limits may be open to question. Allred (1976), also discussing field operations, suggested that no such generalizations could be made.

The risks of serious plugging by the inoculum itself do not seem very high. The cells are an inoculum, not a reagent, and very high numbers should not be necessary. According to Kalish *et al.* (1964) the injection of 10^{11} cells of *Pseudomonas aeruginosa* into a core of 323 md permeability, reduced the permeability by only about 15% and, even with a 32 md core, 5×10^{10} cells reduced the permeability only by about half. The most vulnerable part of the formation, for geometrical reasons, is the part nearest to the face of the injection well, but that is the part easiest to clean.

Cells actually growing in the formation are likely to present a more serious problem (Jenneman *et al.*, 1983). Hitzman (1962) recommended introducing the bacteria first in the form of spores, nutrient being pumped in later when the spores had moved into the formation. This suggestion does not seem to have been followed up in field trials; in most instances the cells and medium are injected together. Despite this, plugging does not seem to have caused any serious problems. Yarbrough (personal communication) found that injectivity decreased as a result of inoculation but the effect could be overcome by pumping instead of letting fluids flow in by gravity.

One would expect that a number of factors might influence the ability to penetrate and the rates of movement. These include characteristics of the bacteria such as size, shape, deformability, the nature of the cell surface, whether or not cells aggregate into chains, production of extracellular slimes, and motility; reservoir characteristics such as permeability, pore structure, the chemical nature of the rock, nutrient and salt content of the connate water or waterflood, and temperature and pressure (which may affect motility) and also the fluid flow rate and the concentration of cells. Effects of cell size and shape on plugging have already been mentioned and these factors presumably have equivalent effects on penetration. Penetration can be studied by total or viable counts on cells emerging from rock cores or sandpacks, by staining cells and examining the appearance of rock to detect penetration (Kalish *et al.*, 1964) or by labelling cells with radioactive isotopes (Myers and McReady, 1965). Springham *et al.* (1983) have pointed out the problems which may arise in using ^{32}P -labelled cells due to release of soluble label and to isotope-induced lysis.

Failure of cells to penetrate could be caused by settling of cells out of suspension, by adsorption to rock surfaces or by sieving. Some idea of the likely importance of the latter mechanism can be obtained from data on so-called pore size distribution. This is routinely obtained by forcing mercury into rock samples and measuring the relationship between applied pressure and the volume of mercury which penetrates into the rock pores (Ritter and Drake,

1945). Strictly speaking, it measures the distribution of the constrictions controlling entry, rather than the pores proper. Burdine, Gournay and Reichertz (1950), demonstrated a relationship between pore throat size distribution and the permeability of reservoir rocks. Davis and Updegraff (1954) found that pore throats must be at least twice the diameter of cocci or short bacilli for cells to pass through readily and concluded that this would require permeabilities in excess of 100 md. Several workers have suggested figures in this region as representing minimum values for MEOR. A summary is given by Moses and Springham (1982).

Quantitative studies of penetration rates have been made by Yarbrough (personal communication) who found negative semilogarithmic relationships between spore numbers and distance moved, using a series of sample points along the length of sandpacks. Jang *et al.* (1983), using *Pseudomonas putida*, *Clostridium* sp. and *Bacillus subtilis*, found that, in general, cells were detectable in the fluid emerging from their rock cores after one pore volume of fluid had passed through. For several more pore volumes of fluid, cell concentrations remained more or less constant at a fraction of the input concentration. Eventually cell concentrations rose to equal the input level. They interpreted their data in terms of a deep bed filtration model, calculating the filtration coefficient (K_o) for cells and spores as:

$$K_o = \frac{\ln \left(\frac{C_i}{C_L} \right)}{L}$$

where C_i = initial cell concentration, C_L = emerging cell concentration, L = length of core.

They studied the effects of ions and chelating agents and concluded that, at low cell concentrations, filtration was mainly due to adsorption on to rock surfaces. Spores had lower filtration coefficients than cells and the presence of residual oil lowered the filtration coefficient, suggesting that cells might penetrate waterflooded reservoirs more readily than most experiments with rock cores would suggest.

In contrast to the results of Jang *et al.* (1983), Jenneman *et al.* (1983), measuring the emergence of *Pseudomonas* cells from a rock core, found that the concentration of cells emerging fluctuated with time, with no discernible pattern. The cell concentration in the effluent never exceeded 1% of the input concentration. Unfortunately Jang *et al.* (1983) presented only a generalized curve, and not the results of individual experiments, so an exact comparison is not possible.

Linear rates of cell movement can be calculated from laboratory data and the values obtained are not wholly incompatible with MEOR operations, even where considerable distances are involved between wells (Moses and Springham, 1982). The reports of field trials generally indicate that bacteria can become distributed fairly rapidly (see the compilation of data presented by Hitzman, 1983) and, subject to the permeability limits mentioned above, there is no sign so far that penetration is a serious limitation to MEOR.

SELECTIVE PLUGGING BY BACTERIAL CELLS

Under some circumstances, plugging by bacterial cells may be positively advantageous. Von Heininger, Jan de Haan and Jensen (1958) injected cultures of slime-forming bacteria, together with carbohydrate media, with the deliberate intention of restricting flow in the more permeable regions of the formation, thus redistributing the waterflood into the less permeable regions. A similar rationale lay behind the trial described by Yarbrough and Coty (1983) who intended to achieve a similar end by the generation of gas bubbles. Crawford (1961, 1962) pointed out that the proportional reduction in permeability due to bacterial cells was greatest in rocks of highest permeability and suggested that this might result in redistributions of flow in favour of the less permeable zones.

In some formations, permeability variations can be considerable. Fox (1983) mentions the problems caused by rapid water movement along the Brent Sands layers in many of the North Sea fields, Jack, Thompson and DiBlasio (1983) refer to the problems of the Lloydminster reservoir where bypassing is exacerbated by high oil viscosity, and renewed interest is being shown generally in selective plugging as a possible MEOR mechanism. Cells actively forming slime cause very severe plugging, whereas the same cells injected under nutrient conditions which prevent slime formation have little effect (Jack, Thompson and DiBlasio, 1983). Injections of cells can thus be made deep into the formation together with nutrients which do not support slime formation, and followed by modified nutrient solutions which promote slime formation and plugging. Jenneman *et al.* (1983) used two cores of different permeabilities connected in parallel, and by injecting cells of *Bacillus subtilis* followed by nutrients were able to achieve selective plugging of the more permeable core. Initially, the low-permeability core had accepted 24% of the liquid flow; after selective plugging it accepted 90%.

In my opinion, selective plugging is a mechanism of great potential importance. Its usefulness will depend on the degree to which plugging can be confined to the more permeable zones. It may be the case that it will not be limited to instances where there are obvious and major differences in rock permeability. It is likely that even rocks whose structure is apparently uniform have permeability variations on the micro scale. This is suggested by the data on pore size distribution in reservoir rocks (Burdine, Gournay and Reichertz, 1950) and by the results of Kalish *et al.* (1964) who examined cores which had been flushed with suspensions of stained bacteria and noted the very uneven extent to which cells had penetrated the matrix. It might also account for the rather surprising fact reported by Myers and McReady (1965) that bacteria appeared to penetrate cores of very low permeability (<0.1 md).

Jang and Yen (1983) examined the release of oil from sandpacks which had been saturated with oil and then waterflooded. A culture of *Bacillus subtilis* injected into the rock caused release of about 35% of the residual oil, whereas sterile medium released only 12%. If sterile medium was pumped into a column after a bacterial inoculum, more oil was released and this coincided with an increase in the pressure drop across the column. From the brief details published,

selective plugging seems the most likely reason for oil release in this instance.

PRODUCTION OF POLYMERS AND SURFACTANTS

We have seen that polymers and surfactants can play a part either in chemical or microbial EOR. Where such materials are produced by bacteria they could either be produced above ground and injected into the reservoir, or the organisms could be injected to produce them *in situ*.

The properties required of a polymer for EOR are fairly well understood. Water viscosity must be increased up to about the same value as that of the oil being displaced. The solution must be resistant to shear degradation as high shearing forces are normally experienced when water is injected into the formation. Shear thinning has the advantage that the viscosity of the aqueous phase drops (temporarily) on injection. Reservoir brines frequently contain high concentrations of salts so these must not seriously affect viscosity. The internal surface area of reservoir rocks is enormous: Hesselink and Teeuw (1981) mention 0.1–1.0 m² surface area per gram of sandstone. It is therefore important that adsorption is minimal. The two polymers most used (by injection from the surface) are partly hydrolysed polyacrylamides and a polysaccharide, xanthan. Polyacrylamides are good viscosifiers at low salt concentrations but lose most of their viscosity above 1 g/dm³ NaCl and are very sensitive to shear degradation. Xanthan, a bacterial product, is a good viscosifier and is relatively insensitive to shear degradation and salt content.

Little work has been published on the generation of polysaccharide *in situ* as opposed to its injection from the surface. Xanthan itself is synthesized under aerobic conditions but some polysaccharides such as dextrans and levans can be produced anaerobically. A valuable body of experience concerning the selection and strain movement of suitable organisms is available (Sutherland, 1983). Improvements include the selection of high-yield mutants, modification of polysaccharide properties, and mutation to eliminate unwanted products. Successful *in situ* polysaccharide generation will also require an appropriate nutrient balance. In general, for good polysaccharide production, carbohydrate must not be limiting and high carbohydrate:nitrogen ratios are generally favourable. There is some evidence that different limiting nutrients may affect the precise chemical nature of the product.

Rather less is known in general terms about the factors controlling surfactant production but it appears that these, too, would probably require control of the nutrient status in the reservoir to achieve high levels of production. The properties required of a microbial surfactant can also be predicted from a consideration of oil displacement.

Under normal waterflooding conditions a large proportion of the oil is trapped by capillary (surface) forces, even in regions which are fully swept by the water-flood. Oil globules which are surrounded by water are unable to move through constrictions because this would require an increase in the interfacial surface area as the globule is distorted. If the flow rate of water is made sufficiently high, the viscous (flow) force tending to displace oil will overcome the capillary forces resisting displacement. Taber (1968) and Melrose and Brandner (1974)

have stressed the utility of a single dimensionless parameter representing the balance between viscous and capillary forces. The one most frequently used is the capillary number:

$$N_c = \frac{\mu_w V_w}{\sigma}$$

where μ_w = water viscosity, V_w = water velocity and σ = oil:water interfacial tension.

After waterflooding, the local displacement efficiency of oil (in well-swept zones) is likely to be about 50% and the capillary number about 10^{-6} . To exceed significantly this displacement efficiency, capillary values above 10^{-3} are required, and to approach 100% displacement, the capillary number has to be increased to about 10^{-2} (Shah, 1981). The applied pressure gradient is limited by the operating equipment and by the need to avoid fracturing the reservoir rock and cannot be increased to the necessary values (Taber, 1968). Reduction of the interfacial tension from 30 mN/m to 10^{-2} or 10^{-3} mN/m can be achieved by the use of surfactants, thus increasing the capillary number to the required value.

The attainment of ultralow values of interfacial tension is by no means straightforward. Shah (1981) provides a useful survey of some of the complexities. Interfacial tension is affected by several factors, including the properties of both the surfactant and the oil, surfactant concentration, salt concentration, temperature, and the nature of the cosurfactant. Surfactant formulations for chemical EOR are thus designed for the specific oil type, salinity and temperature of a particular reservoir. The surfactant slug injected may contain one or more surfactants, salt, a cosurfactant such as a short-chain alcohol (which has a number of desirable effects), a sacrificial agent which will preferentially adsorb to rock surfaces and minimize loss of the surfactant, and perhaps a chelating agent to remove ions which may interfere with the surfactant.

The surfactant slug moves through the reservoir displacing trapped oil droplets which are pushed ahead and merge together to form the so-called 'oil bank'. Formation and maintenance of an oil bank is considered to be essential for efficient oil recovery. Displaced oil droplets must coalesce readily so the interfacial viscosity must be low. The surfactant slug must be of an appropriate viscosity to minimize fingering, the materials must not be unstable or insoluble under reservoir conditions, and loss by adsorption on to the enormous rock surface area must be minimized. Usually a polymer slug will follow the surfactant flood to optimize the flow pattern. It can be seen that, once ideal conditions have been achieved in the surfactant slug, there will be a tendency for the composition to change by adsorption, partitioning and degradation of components and for the slug itself to break up. These factors will tend to limit the efficiency of surfactant flooding operations over any but the shortest distances.

For micro-organisms to release oil by surfactant production requires more than just production of a surfactant. An effective product must have certain specific properties, especially the ability to lower oil:water interfacial tension to 10^{-2} or 10^{-3} mN/m, and it must be produced in sufficient concentration.

Cationic surfactants are unlikely to be useful because of adsorption on to negatively charged rock surfaces. Low values of interfacial viscosity are essential so that displaced oil ganglia can coalesce to form an oil bank. A wide variety of surface-active agents is known to be produced by micro-organisms (*see* Zajic and Panchal, 1976; Finnerty and Singer, 1983).

Where interfacial tension has been measured, the values have usually been too high to effect oil mobilization, although it should be remembered that even the best synthetic surfactants give ultralow interfacial tensions only in the presence of critical concentrations of salt. Singer *et al.* (1983) have isolated a bacterial strain (H13) which produces a glycolipid surfactant when grown on crude oil. The crude glycolipid solution gave an interfacial tension of 2×10^{-2} mN/m against hexadecane, and Finnerty and Singer (1983) mention a minimum interfacial tension of less than 10^{-4} mN/m with 0.5% pentanol as cosurfactant. The material has apparently not yet been tested for oil mobilization. As it is reported to stabilize oil-in-water emulsions it would presumably inhibit oil-bank formation and would therefore not mobilize oil effectively in a conventional recovery procedure.

However, there are recent indications from other sources that microbial surfactants can give low interfacial tensions and are very effective at mobilizing oil from sandpacks and rock cores without the addition of cosurfactants. The latter property would be particularly useful for *in situ* MEOR as it would simplify the attainment of critical conditions within the reservoir.

FIELD TRIALS

Over the past 30 years, field trials of MEOR processes have been conducted in Czechoslovakia, Hungary, Poland, the USSR, Romania, the Netherlands and the USA. Over 40 separate trials have been reported in varying degrees of detail. The reports are scattered and most are not in English. Moses and Springham (1982) have discussed the more important trials and Hitzman (1983) has published a tabulated summary. A single example discussed in detail, together with briefer consideration of others, will be used to illustrate the way in which such trials have been conducted and the difficulties of interpretation they pose.

In 1954, Coty, Updegraff and Yarbrough conducted one of the first-ever field trials in Union County, Arkansas. A detailed account has recently been presented (Yarbrough and Coty, 1983). The rationale was as follows: it was intended that the injected bacteria should produce free gas *in situ*, thereby blocking the most permeable channels and diverting flow into unswept regions of the reservoir. It was hoped that generating gas *in situ* would require much less energy than forcing in gas from the surface and that the bacteria, because they could migrate outside the main streamlines, would generate gas and release oil from areas not accessible to processes operated direct from the surface.

A strain of *Clostridium acetobutylicum* was chosen as the test organism because of its ability to generate hydrogen, carbon dioxide and organic acids under anaerobic conditions from media containing sugars and mineral salts. Hydrogen and carbon dioxide were produced in approximately a 1:1 ratio and, in liquid-

filled systems, pressures up to 8.8 MPa could be generated. The organism grew well at about 40°C and could tolerate high pressures of carbon dioxide as well as sodium chloride concentrations up to 30 g/dm³. It was not inhibited by crude oil and produced no detectable changes in oil properties. Laboratory trials with sandpacks and rock cores demonstrated its ability to release oil; the most important variables appeared to be the nature of the rock and the pressure generated.

The wells chosen were in the Lisbon field, producing from the Nacatoch sand, a layer about 7 m thick at a depth of about 650 m. The formation was loosely consolidated sand containing about 8% carbonate and with high porosity and permeability (30% and up to 5.7 d respectively). Down-hole temperature was about 34°C and the formation water contained 42 g/dm³ sodium chloride. The temperature was suitable for growth of the test organism, and the high permeability was expected to permit easy penetration, but the field was far from ideal in most other respects. Thus the salt content of the formation water was sufficient to inhibit bacterial growth and the amount of oil remaining in the formation was extremely low. The field had been discovered in 1925. After many years of primary production, waterflooding was started in 1949. Rock core samples, taken when extra wells were drilled for the waterflood operation, showed residual oil saturations of only 5–9% of the pore space. A single well, No. 30, was chosen as the injection well for the experiment and well 31, 123 metres to the south, which had been abandoned but was re-opened for the trial, was used as the production well.

Waterflooding operations in the field, resulting in a general flow of water from north to south, continued throughout the trial. It was considered that the predominant flow of water between the two wells was determined by the main waterflooding operation rather than by the injections into well 30.

Before the experiment started, water containing 20–25 g/dm³ NaCl had been injected into well 30 to lower the salt concentration in the reservoir. In May 1954, fresh water was injected for two months and in July 1954 the experiment was started by the injection of dense bacterial suspensions, 18 separate batches totalling 18 m³ being injected over a 4-month period. Simultaneously, injection of beet molasses was started, a concentration of 2% being maintained in the injection water for 5½ months. At first the substrate solution was allowed to flow in by gravity; later, the resistance to flow increased slightly and a pump was installed to force the solution into the formation. Samples of gas, water and oil were taken at intervals from all the production wells in the area.

Fresh water injected at well 30 appeared 70 days later at well 31. From 80 to 90 days after inoculation until the termination of sampling in May 1955, unused sugars, seven different short-chain fatty acids, carbon dioxide and methane, together with traces of ethanol, butanol and acetone appeared at well 31. The gas produced from well 31 contained 38–82% carbon dioxide: gas from wells not affected contained 0.3–8.8%. By measuring the ¹⁴C:¹²C ratio of the carbon dioxide it was estimated that 20% came from acid dissolution of rock carbonate. Carbon dioxide produced by fermentation was equivalent to 19% of the sugar injected. The remaining gas produced was mainly methane: from ¹⁴C:¹²C ratios it was calculated that 20% of this originated from sugars.

Virtually no hydrogen was detected. Total acid production was equivalent to 59% of the sugar injected.

Neither the spectrum nor the ratios of fermentation products were those expected from a pure *Clostridium acetobutylicum* fermentation, which would produce only formic, acetic and butyric acids equivalent to 35–40%, carbon dioxide equivalent to about 47%, and hydrogen equivalent to 2% of the sugar used, but no methane. This suggests that the bacteria active in the formation were not exclusively derived from those injected. Bacteria, (not *Clostridium acetobutylicum*) were detected at the production well, but counts were low and erratic and no correlation with the production of fermentation products could be made.

The production of oil from well 31 dropped rapidly from 3.5 barrels per day when it was reopened to less than 1 barrel per day at inoculation (Figure 2). The dotted line shows the field engineers' estimate of continued oil production had no disturbance occurred. Oil production rose sharply, if not steadily, after July. Water production varied between about 400–500 barrels per day, the water:oil ratio fluctuating wildly. Yarbrough and Coty (1983) estimated that oil production from November to May, when measurements terminated, was 250% more than that projected. No change could be detected in oil properties. Since the test wells were part of a larger pattern of wells subjected to a major water-flooding operation, it was vital to decide whether increases in oil production had occurred at other producing wells. Changes in oil production during the period of the experiment at the nearest three production wells, nos. 33 and 34 to the south-west and no. 25 to the north-east were monitored. No significant increases were observed, showing that the increased production at well 31 was not a result of any general change in that part of the field.

The monitoring of fermentation products showed that, despite the absence of increased bacterial activity at the production well, an underground fermentation had been induced, and all those connected with the trial, both engineers and microbiologists, seem to have been convinced that a genuine stimulation of oil production had been obtained.

Two Dutch trials reported by Von Heiningen, Jan de Haan and Jensen (1958) are of interest because of the explicit intention to enhance oil production by selective plugging. Few details are provided but *Betacoccus dextranicus* was used in one case and a mixture of *Leuconostoc mesenteroides*, *L. dextranicum*, *Bacillus polymyxa* and *Clostridium gelatinosum* was used in the other. Molasses was the nutrient. An increase in oil production was reported in the first case and an increase in the oil:water ratio in the second.

Dostalek's group in Czechoslovakia (Dostalek and Spurny, 1957) reported the isolation of a soil *Clostridium* which would grow on petroleum, or on carbohydrate and yeast extract, with the production of large quantities of gas. Laboratory trials indicated that gas production was the critical factor in releasing oil. In a series of field trials starting in 1954, cultures of *Desulfovibrio* and *Pseudomonas* were injected together with molasses. Bacterial counts in the formation water were increased in every case. In three of the seven trials, increased production of oil was reported. In one case individual wells showed increases of 12–36% and the whole formation showed an increase of 7% over

the 6-month trial. Trials in which either inoculum or molasses were omitted gave no increases in oil production. In the successful trials, permeabilities were in excess of 3 d.

A Soviet trial was described by Kusnetsov, Ivanov and Lyalikova (1963). A mixed inoculum, grown anaerobically on molasses, was injected together with 54 m³ of 4% molasses solution. The well was closed for 6 months. On reopening, oil production increased by about 3 tonnes, from 37 to 40 t/day. Wellhead pressure increased and the proportion of co-produced water decreased. Oil viscosity increased by about 20%. Four months later, oil production had fallen to 36.5 t/day. The brief increase in production cannot have compensated for the loss during the 6-month shutdown. This trial is chiefly remarkable for the high productivity of the well chosen. In another Russian trial the inoculum consisted of a complex mixture of aerobic and anaerobic bacteria (including sulphate-reducing, denitrifying, putrefactive, butyric acid-producing, and cellulose-digesting activities), was grown up in oil and formation water and contained nutrient substances derived from peat and silt but no molasses. In this instance substantial increases in oil production were recorded, together with a reduction in oil viscosity, an increase in gas production, changes in gas composition, and

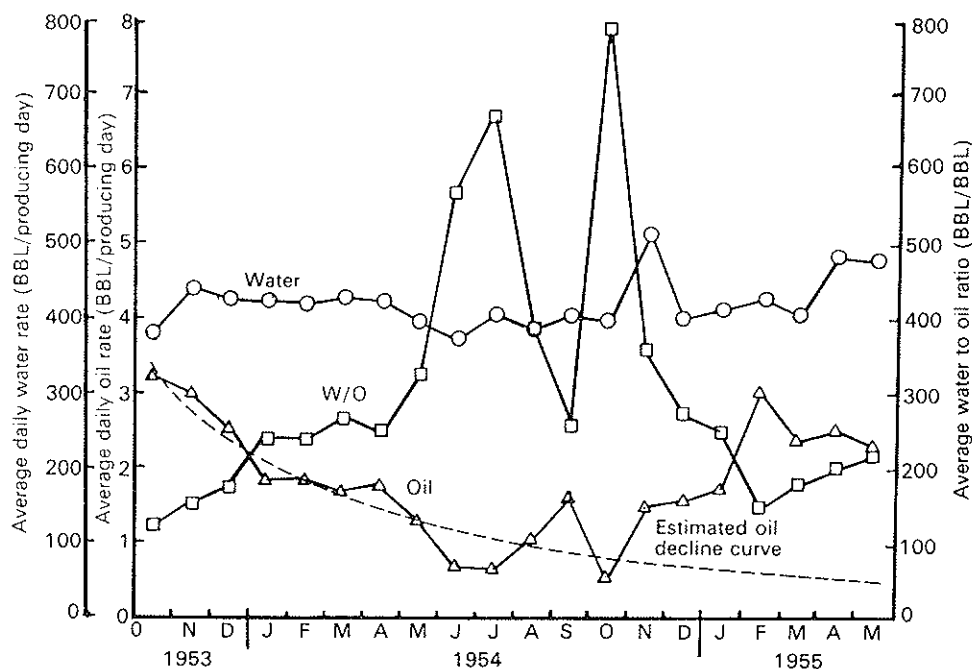


Figure 2. Production of oil and water from well 31 in the Arkansas field trial. The first bacterial inoculation was in July, 1954. Reproduced from Yarbrough and Coty (1983). (BBL = barrels).

an increase in bacterial counts in produced water. Russian workers have shown some interest in stimulating and controlling the natural population of the reservoir. This approach would appear to be useful only if the natural population has properties which can lead to oil mobilization. Particular attention has been paid to methanogens (Ivanov and Belyaev, 1983).

In a series of trials in Hungary (Jaranyi *et al.*, 1963; Dienes and Jaranyi, 1973) the inoculum consisted of mixed cultures of *Pseudomonas*, *Clostridium* and *Desulfovibrio* or of mixed sewage sludge cultures containing *Desulfovibrio desulfuricans*, and the complex injection protocols involved the addition of molasses, potassium nitrate, inorganic phosphate and sucrose. In some cases the injection well was closed for a period and operated on a huff-and-puff basis. In others, the injection well remained open, oil being produced from nearby wells. Sometimes supplementary charges of nutrient were made at a later date. Among the effects reported were reductions in oil viscosity, reductions in water pH, increases in gas production, increases in bacterial counts (in one case 60000 m³ of formation was said to be affected). Increases of oil production varying from 0 to 60% were reported over periods up to 18 months. In two trials, the failure to increase oil production was attributed to low permeability (stated to be 10–70 md and 'low' respectively) but stimulation of oil production occurred in one instance where permeabilities as low as 67–104 md were recorded. One trial is of particular interest: it was performed on a reservoir nearly 2500 m deep with a temperature of 97°C and pressure of 22.8 MPa. Well spacings (injection to production) were 300–1700 metres. Increases in the numbers of *Desulfovibrio desulfuricans* and of the indigenous population were recorded throughout the formation, with a decrease in water pH from 9 to 6, a halving of oil viscosity and a 60% increase in oil production. This remarkable result indicates the potential of MEOR techniques even in deep hot reservoirs with widely spaced wells.

The series of trials conducted in Poland from 1961 (Karaskiewicz, 1974) include relatively detailed documentation. Some twenty wells in sandstone reservoirs between 500 and 1200 m deep were treated. Inocula consisted of complex mixtures derived from soil, crude oil, formation water, industrial wastes and sewage sludge and included strains of *Arthrobacter*, *Clostridium*, *Mycobacterium*, *Peptococcus* and *Pseudomonas*. Cultures were adapted and tested for the ability to mobilize oil by methods similar to those described by Lazar (1983a, b) and discussed below. Typically, 500 dm³ of culture, containing about 2×10^{12} cells, 2 tonnes of molasses, and 50 m³ of production water were injected followed by 150 dm³ of crude oil and the well was sealed for a period of months. In each case the injection well was reopened and oil produced from it by lift pump (i.e. huff-and-puff operation). Data for oil production appear to have been kept carefully, production being logged every 12 h, and detailed records were available for at least 12 months prior to injection, sometimes for much longer.

In most trials, increases in oil production were reported: often a peak occurred after reopening, which quickly compensated for the loss during closure. Enhanced production then continued for periods as long as 9 years at declining rates. Increases of up to 300% occurred in some cases after allowing for lost production, the figures depending very much on the period over which the

increase is calculated, and the projected oil-production curves used, which obviously became less reliable the longer the experiment continued. However, the projections appear to be conservative and the stimulations convincing. Some trials gave no increase in oil production. Sometimes oil viscosity decreased and fractional distillations revealed changes in oil composition. Increases in gas pressure were recorded in some cases. Water pH decreased in most, if not all, cases.

Bacterial counts increased in every case reported. In some trials, increased oil production was also observed in neighbouring wells. This was interpreted as a stimulation due to treatment and not to a general improvement due to other causes. Sometimes bacterial injection was combined with hydraulic fracturing to increase permeability and it is not always clear how much of a particular increase in oil production was due to bacterial action and how much to the fracturing. In one instance, a previous fracturing operation was performed without effect, suggesting a synergistic effect of the two treatments. An appreciation of the significance of these results demands a study of the oil-production graphs and the interested reader is strongly recommended to consult either the original monograph (Karasckiewicz, 1974) or the compilation presented by Hitzman (1983) which reproduces many of the original figures with English legends.

Lazar (1983a, b) has conducted trials on seven fields in Romania. Increases in bacterial counts were reported in all cases but only two fields showed overall increases in oil production, up to 200% increases being obtained for periods of 1–5 years. The successful trials appear to have given very convincing demonstrations of microbial stimulation. Good baselines are available for comparison as detailed records were kept for 2–3 years before inoculation. Some changes in oil viscosity, decreases in pH and alterations in composition of produced water were observed. Lazar regards the unsuccessful results as demonstrating the need for the correct choice of formation for treatment.

Detailed accounts have been given by Lazar (1983a) of the procedure for obtaining, adapting, testing and injecting bacterial cultures. His procedure is developed from those used by the Hungarian and Polish groups. Bacterial cultures were obtained from a wide variety of sources, the two which proved best in laboratory trials being formation water, which contained bacteria adapted to the reservoir conditions, and the solid sludge resulting from sugar refining, which contained vigorously fermenting populations. The organisms were cultured at reservoir temperature in formation water, supplemented with molasses and in contact with crude oil. The various 'adapted' populations which resulted were then tested in 'collectors': these are items of equipment of varying design in which the ability of growing bacteria to release oil from saturated sandpacks can be measured. Experience showed that strains isolated in pure culture released a maximum of 3.5% of the oil; mixtures of pure strains released up to 14.5%, whereas undefined but adapted mixed populations released from 18 to 25%. The best cultures contained predominantly strains of the following genera: *Pseudomonas*, *Escherichia*, *Arthrobacter*, *Mycobacterium*, *Micrococcus*, *Peptococcus*, *Bacillus* and *Clostridium*.

Recently Johnson (1979) carried out MEOR operations on over 150 wells in

the United States. His approach was highly empirical, taking the view that field trials carried out on stripper wells, (those producing less than 10 barrels per day), are cheaper and more conclusive than laboratory simulations. He used various *Bacillus* and *Clostridium* species. The cultures were mixed with a molasses solution containing unspecified mineral nutrients and immediately injected into the well. About 10–14 days were needed for growth.

Operations were conducted on either a huff-and-puff or a water-drive basis. Johnson (1979) claimed that, given an appropriate choice of oil type and reservoir conditions, 'an average increase of oil production in excess of 350% may be expected' at a cost roughly in the range of 15–30 cents per additional barrel (1979 prices). For comparison, at the time of writing (in 1983) the spot price of Saudi Arabian light crude on the Amsterdam market is US \$29.80 per barrel.

Johnson suggested that several mechanisms could be involved in oil release according to circumstances. The production of organic acids which react with reservoir carbonates (and incidentally thus produce more carbon dioxide) was reckoned to clean up formation damage, i.e. partial plugging in the region of the well bore. The copious production of gases, especially carbon dioxide and methane, could increase reservoir pressures and help to force out more oil. A variety of surfactants could help to reduce oil:water interfacial tension and thus aid oil release. No data were presented. It seems unlikely that Johnson was able to make very detailed collections of data either before or after well treatment. What makes his work particularly impressive is the number of trials carried out and the fact that he was apparently able to make an overall commercial success of his operations. This year (1983) Johnson has joined forces with other workers including Updegraff and the group at QMC Industrial Research Ltd in London, under the aegis of Petroleum Sciences Inc., to test a variety of newly isolated strains on twelve wells in Illinois (Anonymous 1983). In this instance detailed monitoring is being performed and the results are awaited with interest. It is reported in the same source that several other groups in the United States, including those at Oklahoma State University (E. Grula), the University of Texas, El Paso (J. Zajic), the University of Calgary, Alberta (T. Jack) and Phillips Petroleum (J. Norell), are also planning field trials.

In the earlier Romanian experiments, sources of inorganic nitrogen and phosphorus were injected together with molasses but Lazar (1983b) remarks that the mineral supplement was omitted in later trials since laboratory experiments showed that molasses and formation water provided adequate mineral nutrition.

About half of the trials reported throughout the world seem to have used mineral nutrients. Updegraff and Wren (1953) reported deficiencies in phosphate or nitrogen in many formation waters. Clark, Munnecke and Jenneman (1981), summarizing a survey in the top ten oil-producing states of the United States, reported general deficiencies in sulphur, nitrogen and phosphorus. Many successful trials have been conducted without mineral supplements, and Grula *et al.* (1983) reported that limestone or sandstone outcrop rock would support good growth of several clostridia supplied with molasses and ammonia. Analysis of formation water and molasses is clearly essential before a trial. The ability

of nutrients to move, in solution, through rocks has been examined by Jang *et al.* (1983) and by Jenneman *et al.* (1983).

GENERAL CONCLUSIONS FROM FIELD TRIALS

The data which are readily available are imperfect: essential facts on methods or conditions are frequently omitted; results are often given in vague terms; sometimes, errors appear to have crept in during translation. The most important result, namely the extent to which oil production has increased (if at all), is not easy to decide, even if a full set of data is available, because an adequate control in the normal sense is difficult or impossible. Although controls are frequently mentioned in connection with field trials, the term usually refers simply to a nearby well. The fact that this is not a control in the normal sense is demonstrated when an increase in 'control production' is accepted as further evidence of the effectiveness of the bacterial inoculation. One of the best controls explicitly mentioned is in the work of Coty, Updegraff and Yarbrough (Yarbrough and Coty, 1983). Here control wells were chosen aligned fairly closely with the test wells in the overall waterflood but positioned in such a way that the bacterial inoculation would not be likely to affect them during the period of the experiment. The absence of a response in these wells was good evidence against a significant change in that part of the field generally. Dostalek's group omitted either inoculum or molasses in some of their trials and reported no oil stimulation. The significance of these controls clearly depends on how comparable they were with the wells receiving full treatment, since negative results are not uncommon, even with normal injections.

Generally, stimulations in oil production have to be detected by extrapolating the oil-production curve forward into the experimental period. In many cases the results of this exercise show a fairly convincing stimulation of oil production, but production curves are not straight lines (nor even very smooth curves in many cases) and the hazards of interpretation must be appreciated. Where stimulations appear to continue for several years but at a slowly declining rate, calculation of the enhanced recovery depends critically on the slope of the projection curve and exact figures become meaningless. One particularly convincing demonstration of increased production, not yet described in a published report, arose where a series of injections was made. Oil production rose for a period of months after each injection and then decreased until the next injection was made.

Changes in flow patterns attributable to events in other parts of the formation, or even disturbances such as the temporary closing of a well, can affect oil production and reports of field trials should make it clear that this has not happened. Where disturbances are inevitable, the possible effects should be considered carefully. For instance, the author is not aware of any instance reported in the literature where a well has been capped for a period with no other treatment, and the results monitored as a prelude to a similar treatment coupled with bacterial inoculation, although personal conversations make it clear that this test has, in fact, been made in at least one instance. Hydraulic fracturing of formation rock has sometimes been practised in conjunction with

bacterial inoculation, to enhance overall permeability. In one of the Polish field trials it was reported that, previously, fracturing alone had been completely ineffective: more often, the results of the two treatments are indistinguishable.

Despite all these reservations, I am now convinced that genuine stimulations of oil production have occurred in a number of trials, if not in all cases where success has been claimed. Many trials were unsuccessful in that no stimulation of oil production resulted, but no disastrous effects of microbial inoculation were reported. In one of the Polish trials, slimy oil resulted, but no serious plugging or decline in oil production seems to have been experienced. The cynic might suspect that any such event would not quickly find its way into print.

In most cases it is difficult to draw reliable conclusions about the mechanism responsible for enhanced production, because cultures are usually chosen on the basis of empirical criteria. Most authors describe stimulation of bacterial counts in the reservoirs, encompassing both the strains inoculated and the indigenous flora. Two conclusions follow: (1) it is not always easy to be sure that a given effect results solely, or even partly, from the bacteria inoculated; (2) it may not be easy to produce a bacterial monoculture in the reservoir, and results therefore may be difficult to predict from laboratory-scale trials.

SPECIAL PROBLEMS OF MEOR OPERATIONS IN DEEP FIELDS

As the shallower oil fields on the earth's surface become insufficient to satisfy demand, increasing attention must be paid to deeper deposits. Temperature and pressure increase with depth and some of the fields now being exploited have ambient temperatures and pressures well beyond the limit tolerated by most forms of life. Thus, in the North Sea fields where formation depths exceed 2000m, pressures range from 20 to 50 MPa, temperatures from 80° to 110°C and formation water salinities exceed 30 g/dm³ dissolved solids (Fox, 1983). Additional difficulties in the North Sea are attributable to the offshore location, the depth of the sea (up to 200m) and the bad weather which makes supplying the platforms, and even access, difficult for much of the year. The North Sea fields represent the bulk of the known oil deposits in the United Kingdom and the ambient conditions are by no means unique. As a result of the cost of drilling, well spacings are in excess of 600m. It is thus of some interest to consider whether MEOR operations might be feasible in such fields.

The question of whether life was possible at all under these conditions was, until recently, open to doubt. Most bacteria will tolerate pressures up to 20 MPa but above this value an increasing proportion become inhibited by pressure, even when other conditions are optimal (for reviews *see* ZoBell and Kim, 1972; Marquis, 1976; Marquis and Matsumura, 1978; Marquis, 1983). Even if growth is possible, undesirable changes often occur, such as loss of motility (Meganathan and Marquis, 1973) and a tendency to filamentous growth. High temperature also prevents growth of most bacteria and, until recently, the highest temperatures reported for growth in culture were about 85°C, although ZoBell (1958) reported growth of an organism at 104°C and 100 MPa but was unable to

subculture it. There were signs that growth at up to 100°C occurred in certain natural environments (Brock, 1967; Bott and Brock, 1969; Brock and Darland, 1970; Brock *et al.*, 1971, 1972; Jackson, Ramaley and Meinschein, 1973; Tansey and Brock, 1978). Even if bacteria could grow at reservoir temperatures, the effect of combinations of different adverse conditions, especially temperature and pressure (Marquis, 1983), and the effects of heavy metals (Bubela, 1983b) are unknown.

Recently, the known limits for growth have been extended substantially. Thus Stetter *et al.* (1981) isolated a new species of methanogen from an Icelandic hot spring with an optimum growth temperature of 83°C and a maximum of 97°C. Shortly afterwards Stetter (1982) reported the isolation, from submarine volcanic areas, of an organism which required hydrogen, carbon dioxide and elemental sulphur for growth, generating hydrogen sulphide. This organism had an optimum growth temperature of 105°C. This year Baross and Deming (1983) have reported the growth under laboratory conditions of still more remarkable organisms which were cultured from submarine volcanic vents at a temperature of 306°C. These organisms grew at temperatures from 100°C at atmospheric pressure up to 250°C at a pressure of 26.5 MPa. Some care is necessary when attempting to demonstrate bacterial growth at very high temperatures as it is easy to obtain misleading results if parts of the apparatus are below the design temperature. In the three cases mentioned there is no reason to suspect the validity of the authors' conclusions.

Thus temperature and pressure alone do not appear to rule out the growth of bacteria even in the hottest reservoirs, although their effects in combination with salinity, heavy metals and other oil components are, as yet, unknown. Little is known about the newly discovered extreme thermophiles and no information is available to assess their suitabilities for MEOR operations. The difficulty of access to wells such as those of the North Sea, together with their wide spacing, give rise to further difficulties. A substrate-fed MEOR operation would require the provision of substrate at very high rates (because of the high injection rates) for very long periods. Estimates of the time taken for fluid to pass from one well to another vary from 5 to 12 years, posing enormous problems of supply, investment cost and system stability.

There are strong arguments that the only way in which a large-scale MEOR operation could be conducted would be for crude oil to provide the substrate. Molecular oxygen is not available in the reservoir, so some other electron acceptor would be necessary. Moses *et al.* (1983) described bacterial cultures which, under strictly anaerobic conditions, grew on crude oil as sole carbon source in the presence of inorganic nutrients. Growth was measured by increases in absorbance and protein concentrations: both methanogenic and nitrate-reducing cultures were described, the latter producing nitrite, molecular nitrogen and carbon dioxide. Injection of oxygen into the gas phase of nitrate-reducing cultures completely inhibited gas production, whereas injection of nitrogen had no detectable effect. Although anaerobic growth at the expense of crude oil has been demonstrated, the rates of growth were very low, the oil components being utilized were unknown, and no thermophilic activity has so

far been detected. Other workers have also reported signs of anaerobic growth on hydrocarbons (*see* Moses and Springham, 1982). When Bubela (1983a) incubated a bacterial culture with a crude oil sample for 3 months at 60°C under strictly anaerobic conditions, an increase was found in the asphaltene fraction. No change was detected in oil samples incubated at 30°C or at 60°C without bacteria, suggesting that the change was due to anaerobic metabolism of hydrocarbons.

Much remains to be done for a workable system to be developed and it may well be that gene manipulation will eventually have a role in transferring essential attributes into an extreme thermophile. Despite the recent spectacular advances in gene manipulation, the difficulties of doing this must not be underestimated since entire pathways rather than single enzymes may need to be transferred, and the problems of getting mesophilic enzymes to function at high temperatures (if that should be necessary) have only recently been addressed. However, one of the Hungarian field trials claimed success under reservoir conditions remarkably similar to those in the North Sea fields: perhaps the problem is not as difficult as it seems!

Summary and conclusions

The plethora of possible mechanisms for oil release has sometimes diverted attention from the vital necessity to determine which one is operating in a particular case. The temptation is strong to inject a mixture of organisms, offering between them the possibilities of several mechanisms, hoping that one or more will operate successfully. The author feels that this empirical 'eye of newt and toe of frog' approach must now give way to a determination to define the specific mechanism which is to function, and its critical parameters.

Gene manipulation may have a part to play both in increasing product yields and in producing desirable combinations of properties in a single strain. The problems will be much more difficult than those currently being tackled to produce pharmaceutical products, because the sort of property which may have to be transferred, e.g. polymer production, surfactant production, is likely to involve the products of a whole series of genes. Rational manipulation will require the use of single strains or, at most, of simple, well-defined mixtures, despite the present advantages which more complex cultures seem to enjoy (Lazar, 1983a).

For a number of reasons, an MEOR system which has been optimized in the laboratory may perform in a very different way in the reservoir. It is important to be aware of the problems, although in some cases effective solutions are not immediately obvious. Indigenous organisms may compete for substrate or, by supplying metabolic products or removing the products of the inoculum, may alter the expected metabolic balance. Thus the fermentation balance of *Ruminococcus albus* can be altered if the partial pressure of hydrogen is lowered by growing it together with *Vibrio succinogenes*, which removes the hydrogen by oxidation (Thauer, Jungermann and Decker, 1977; Tewes and Thauer, 1980). Interactions of this general sort could occur in the reservoir and their effects

would not be easy to predict. Evidence of metabolic interactions between inoculum and indigenous organisms was presented by Yarbrough and Coty (1983).

The bacterial population which develops in the reservoir may not be the one injected. It is certain that any attempt to eliminate indigenous organisms from the reservoir would be futile since it has proved extremely difficult to sterilize rock cores in the laboratory, but numbers might be reduced locally by a biocide injected prior to inoculation. Reservoir populations are likely to have been selected for their abilities to survive for long periods rather than for rapid growth, and the effects of competition could be minimized by using a large inoculum of organisms with a high maximum growth rate.

Mutation is unlikely to be a problem except where distances between wells are great and very long periods are involved. The growth of ineffective variants would be a particular danger if strong selection pressures had been applied to obtain high-yielding mutants (e.g. surfactant producers) and it will be necessary to check strains carefully for genetic stability. One favourable aspect arises from the highly directional flows within the reservoir. Since there is little back-mixing and cross-mixing, a mutant, unless it arises very early, could affect only a comparatively small sector of the reservoir.

The provision of nutrients by injection from the surface raises another type of problem. Before substrate can reach bacteria growing in distant parts of the formation it must pass through established populations in the vicinity of the injection well. There is a danger that the latter will grow sufficiently fast to utilize all the substrate, thus localizing the region of bacterial activity. I have constructed a simple computer model to predict how substrate concentration, bacterial concentration and growth rate would vary with distance from the injection well, using a deep-bed filtration equation to describe bacterial movement and the Monod equation to describe growth rate. The model is still being checked and refined, but preliminary results indicate that the outcome is highly dependent on the choice of parameters such as the concentration of substrate injected, flow rate and bacterial growth rate. According to circumstances, growth can occur deep in the formation or it can be confined to a region close to the injection well. It is, of course, only the concentration of the limiting nutrient component that is critical. Data from field trials should provide useful information but are not easy to interpret. Thus Yarbrough and Coty (1983) measured appreciable concentrations of carbohydrate at the production well, but this does not prove that bacterial growth was possible in this region because the limiting nutrient was not known. Similarly, where increased numbers of bacteria are found in parts of the reservoir remote from the injection well it does not follow that substrate is also present to permit their growth.

The weight of published information suggests that real increases in oil production can be achieved by MEOR under appropriate circumstances. With the wide dissemination of information and the considerable research efforts which have been exerted in recent years, the chances of success in the future should be greatly improved.

The experience of Johnson (1979) suggests that, with the easiest targets, a high percentage of success is already attainable, but full exploitation of the

potentialities requires considerable development work. A high degree of collaboration between disciplines is required for success: the properties of the reservoir are defined by geologists, geochemists and geophysicists; extraction of the oil requires the skills of engineers, chemists and mathematical modellers; microbiologists and geneticists must provide the organisms and the means of developing and controlling their activities. All parties involved must be fully aware of the requirements and limitations imposed by the other disciplines. Fortunately, the oil industry has a long experience of interdisciplinary collaboration.

The enormous potential benefits of MEOR were mentioned in the opening paragraphs of this review. MEOR is particularly attractive in comparison with other EOR techniques in that, despite the difficulties and uncertainties, the operating costs are low. Essentially all the cells and active substances are generated in the reservoir from relatively cheap raw materials and no expensive manufacturing facilities are required on the surface. The substrates are cheap sources of carbohydrate and, in some cases, mineral nutrients: if workable systems can be developed depending on anaerobic hydrocarbon degradation, no carbohydrate would be needed, although there would be a requirement for an electron-acceptor such as nitrate.

Almost all MEOR operations so far have been confined to stripper wells, so success in individual cases has resulted in only small quantities of extra oil. However, there are of the order of 400 000 stripper wells in the US alone. In 1981 these produced less than three barrels a day on average but accounted for about 13% of the US production, so the widespread and successful application of MEOR to these alone would be of some significance. The application of MEOR to more productive reservoirs has to face two sorts of hurdle: the first is that there may be technical difficulties not yet appreciated; the second is a matter of credibility and understanding. Many engineers still regard MEOR as unreliable and potentially harmful. Part of this mistrust will disappear if MEOR is widely used and regularly successful, but the technique will not take its place along other EOR techniques until the mechanisms are fully understood and the processes can, to some degree, be modelled successfully by computer.

The application to really difficult targets, as exemplified by the North Sea fields, will face the problems of extreme reservoir conditions, widely spaced wells and difficulty of access together with very high costs of maintaining equipment in working order. The high temperatures and pressures, although still serious problems, do not seem as formidable as they did a few years ago, but the economics and logistics of feeding organic substrates from the surface in such situations still look intractable. It remains to be seen if rapid anaerobic growth on crude oil components is possible. A considerable faith in the biological stability of MEOR systems working over long distances and extended times will be essential if depleted fields are to be kept operating for the long periods before results can be expected. It is indeed to be hoped that techniques, understanding and confidence develop to the necessary point before too many fields are finally abandoned.

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The field pilot of microbial enhanced oil recovery in a high temperature petroleum reservoir

Liu Jinfeng ^{a,b}, Ma Lijun ^b, Mu Bozhong ^{a,*}, Liu Rulin ^c, Ni Fangtian ^b, Zhou Jiaxi ^b

^aDepartment of Chemistry, East China University of Science and Technology, Shanghai 200237, China

^bDagang Oilfield Company, PetroChina, Tianjin, 300280, China

^cDepartment of Microbiology, Nankai University, Tianjin, 300070, China

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Abstract

To evaluate the technical feasibility and effectiveness of improving oil recovery by microbial enhanced water-flooding techniques in high temperature petroleum reservoirs, a field project was initiated with the nature-occurring microorganisms and nutrient injected into an integrated, close Unit with temperature of 73 °C and salinity of 16,790 mg/L in 2001 in Dagang Oilfield, PetroChina. This paper presents the field design and the results of the field pilot with a discussion on characteristics of the reservoir and the performance of the microorganisms injected. Field results show that microorganisms can thrive, proliferate and move in the high temperature reservoir matrix, the positive effect of the bio-treatment first and mainly occurs in those production wells which have good connectivity with injection wells, and the indigenous microorganisms in the petroleum reservoir may also contribute to improving oil recovery due to the injection of nutrient. Results from this project suggest that microbial enhanced water-flooding technique has significant potential for enhancement of oil recovery in high temperature petroleum reservoirs.

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Keywords: Enhanced oil recovery; Microbial water-flooding; High temperature reservoir; Field pilot

Extensive investigations on microbial enhanced oil recovery (MEOR) have been made in both the laboratories (Desouky et al., 1996; Stephens et al., 2000; Sui et al., 2001; Li et al., 2002; Wang, 2002) and field tests (Bryant and Burchfield, 1993; Zhang et al., 1996; Lazar, 1998; Feng and Chen, 1999; Nazina et al.,

1999; Wang et al., 2001) in the last decade. As to the investigation of field tests, although microbial enhanced water-flooding is believed to be one of the most promising technologies of enhancement of oil recovery, it is still applied under general reservoir conditions on a small scale. The major reason for this is that its technical feasibility and effectiveness in enhancement of oil recovery under difficult reservoir conditions (e.g. high temperature, etc.) have not been well documented by a little result from existing field tests.

* Corresponding author. Fax: +86 21 64252063.

E-mail address: bzmu@ecust.edu.cn (M. Bozhong).

A microbial enhanced water-flooding project was conducted in Guan69 Unit at Dagang Oilfield in China by injection of microbial formulation with nutrient through injection wells in an ongoing waterflood reservoir to evaluate its technical feasibility and effectiveness in enhancement of oil recovery in high temperature reservoirs. The temperature and brine salinity of the tested reservoir are as high as 73 °C and 16,790 mg/L, respectively. And more importantly, this reservoir is well integrated, and production water from every production well was monitored independently during the project period, which makes it a convincing way to evaluate changes in oil production and the effectiveness of the microbial treatment. This paper presents the reservoir properties, design of the project and the results of the field pilot.

1. Description of the oil reservoir

The Guan 69 Unit in Dagang Oilfield is located in Hebei Province, China, and the distribution of the production and injection wells is shown in Fig. 1. This Unit is relatively close with a surface area of 1.19 km², OOIP (Original Oil In Place) of 192 × 10⁴ t. The oil-bearing formation is the Sandstone consisting of two layers as SaI and SaIII with the temperature, porosity, and permeability of 70 and 73 °C, 27.6% and 24.9%, 468 × 10^{−3} μm² and 259 × 10^{−3} μm², respectively, as outlined in Table 1. The composition of the formation brine is shown in Table 2.

The Guan 69 Unit was operated in primary production in 1984. Water flooding of the reservoir began in May 1988, and from then on, it has been

Table 1
Characteristics of the testing reservoir

Items	Parameters	
	SaI	SaIII
Formation	Sandstone	Sandstone
Buried depth (m)	1801.3–1850.0	1906.6–1960.0
Oil bearing area (km ²)	1.05	1.19
Original Oil In Place (OOIP), 10 ⁴ t	81	111
Average net pay thickness, (m)	5.3	6.7
Average permeability, 10 ^{−3} μm ²	468.4	259.2
Porosity, %	27.6	24.9
Original pressure (MPa)	17.78	18.89
Saturated pressure (MPa)	6.66	ND
Temperature (°C)	70	73
Original oil–gas ratio, m ³ /t	32	ND
Oil viscosity (underground), mPa · s	6	ND
Oil viscosity (surface, 50 °C), mPa · s	75.8	42.5
Oil density (surface), g/cm ³	0.8787	0.8841
Solidification point (°C)	37.7	37.9
Paraffin content (%)	27.4	28.1
Sulfur content (%)	0.097	0.078
Asphaltene content (%)	23.4	25.8

ND, Not Determined.

in continuous water flooding to the present time by the use of recycled water from the production water from this reservoir. In February 2001, the Unit covered 5 injection wells with injection volume of 357 m³/d, and 7 production wells with total oil production of 34.6 t/day. The cumulative oil production from this reservoir was about 53.8 × 10⁴ t with the oil recovery of OOIP of 22.61%, average water cut of 94.5%, and the cumulative water injection of 184.88 × 10⁴ m³. It was estimated that the recoverable oil in this Unit was about 70.0 × 10⁴ t by water flooding, which implies that about 77% OOIP has been recovered from the beginning of this project.

Table 2
The property of the formation water

Item	Na ⁺ +K ⁺	Ca ²⁺	Mg ²⁺	Cl [−]	SO ₄ ^{2−}	CO ₃ ^{2−}	TDS
Value (mg/L)	6075	298	87	9874	37	419	16790

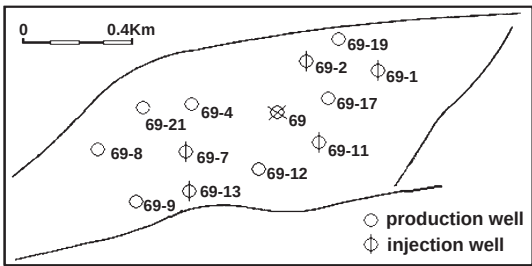


Fig. 1. The Map of Guan 69 Unit.

2. Laboratory experiments

2.1. Bacterial strains and the nutrients

Three microbial strains isolated from Dagang Oil-field were selected for the field project based on microbial compatibility tests of the strains with Guan 69 reservoir conditions. These strains were identified as *Arthrobacter* sp (A02), *Pseudomonas* sp (P15), and *Bacillus* sp (B24), respectively, according to their colony appearance, and physiology and cell morphology (Dong and Cai, 2001; Liang et al., 2004). A02 and P15 demonstrated a good capacity in degradation of oil, and B24 was more effective in reduction of the interfacial tension of oil and formation brine due to its production of biosurfactant from fermentation of crude oil.

A nutrient medium was developed based on growth behavior of the selected strains and the composition of the formation brine. The medium consists of crude oil (20 g/L), $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ (0.8 g/L), KH_2PO_4 (0.45 g/L), Yeast extract (0.25 g/L), Peptone (0.1 g/L), NH_4Cl (2 g/L), Na_2EDTA (0.25 g/L), where Na_2EDTA was designed to inhibit the deposition caused by interaction of phosphates in the medium and the divalent cations in the brine. All the three strains exhibited vigorous growth in the medium when incubated under the simulated reservoir conditions in laboratory. When the medium is used for the field trial, the crude oil is not added in it for the existence of crude oil in the tested layer.

2.2. Performance of bacteria

2.2.1. Biosurfactant production

The biosurfactants produced by bacteria in broths were obtained and identified by the following procedures. Initially, each mixture of 5 ml viable microbe broth of strain A02, P15, or B24 with 5 ml oil from Well 69-8 and 200 ml medium with nutrient composition mentioned above contained in flasks was incubated at 73 °C with a water rotary shaker at 150 rpm for about 7 days. The resulting culture broth was then centrifuged at $6000 \times g$ for 20 min to remove residual oil and filtered with $0.22 \mu\text{m}^2$ filters to remove cells. The interfacial tensions of oil and filtrates were measured at 73 °C as 22.9, 22.2 and 23.7 mN/m for strain P15, B24 and A02, respectively, which indicates the

reduction of 25.9%, 28.2% and 23.3% compared with that of the blank sample and implies the production of surface-active compounds in the broths. Following it, the primary sample of biosurfactants in the filtrate at pH=6 adjusted with 6 mol/L H_2SO_4 was extracted by chloroform–methanol (2:1, *V/V*) and concentrated by distillation. The extract was redissolved in chloroform and then applied to a silica column which was successively eluted by hexane, chloroform and the mixture of chloroform and methanol (8:1, *V/V*), and the biosurfactant sample was obtained by distillation of the eluent. Finally, the biosurfactants were identified by the method of Thin Layer Chromatography (TLC), which shows that the biosurfactants produced by the selected strains fall under a kind of Glycolipids. The biosurfactant concentrations in cell-free broths of strain P15, B24 and A02 were measured by the colorimetric method (Dubois et al., 1956), which shows the values of 950, 1170 and 800 mg/L, respectively.

2.2.2. Fatty acid production

The cell-free broths were obtained and treated by the procedures same as that motioned above. The pH in the broths were measured as 5–6, which implies the production of some acids in the broths. For qualitative determination, the Gas Chromatography (GS) was applied (Ma et al., 1995), and the acetic acids and other short-chain fatty acids were found in the broths of the three strains. The titration with NaOH at a concentration of 0.10466 mol/L and phenolphthalein as the indicator was adopted to determine the concentration of the fatty acids in the broth, which indicates the values of 149.8, 135.0, and 175.5 mg/L in the broths of P15, B24, and A02, respectively.

2.2.3. Crude oil alternation

For evaluation of the effect of the strains on the crude oil, the mixtures of viable microbe broth (50 ml), the medium (200 ml), and crude oil from Well 69-8 in the Unit (50 ml) in conical flasks were incubated at 73 °C in a rotary shaker at 150 rpm for 7 days. The blank experiment was run in the same conditions but without inoculation. After incubation, the crude oil sample in the flasks was collected and electrically dehydrated in a pressurized tank, and its viscosity was measured with DV-III Brookfield Viscosimeter at 73 °C. The solidification point and the content of paraffin and asphaltene were measured by

the silica gel column chromatograph method (Zhang et al., 2000). The results are shown in Table 3.

2.2.4. Core flooding

For evaluation of oil displacement of the selected strains, core-flooding experiments were performed at 73 °C. The heterogeneous man-made core models with the size of $20 \times 4.5 \times 1.1$ cm, the permeability of $0.2 \sim 0.6 \mu\text{m}^2$ and the porosity of 22–26% were used in experiments. The major procedures in the displacement are as follows: First, water-flooding of oil-bearing core with oil saturation of 65–70% was performed until no more oil was observed in the outlet of the core. Second, one pore volume of the bacterial suspension with a density of 1.5×10^7 cells/ml was injected into the water-flooded core, and followed by a 7-day shut-in period at 73 °C. Finally, water-flooding was again performed until no more oil was observed in the outlet of the core. The blank experiment was run in the same conditions but without injection of bacterial suspensions into cores. The results show that the incremental oil recoveries with bacterial suspensions of B24, A02 and P15 are 5.6%, 5.0% and 4.8% OOIP, respectively, compared with the blank core flooding results.

2.3. Detection of microorganisms in production water

For evaluation of the microorganisms in production water, the samples were collected at wellhead of the production wells in the tested reservoir in sterile glass bottles with rubber stoppers. These samples were serially diluted and viable counts were performed by the spread plate technique with LB (Luria–Bertani) medium. The incubation temperature was 73 °C. The

injected microorganisms were identified by the colony appearance developed on the LB medium and their physiology and cell morphology.

3. Field trial

3.1. Field-test design

The injection of the microbial suspension and the nutrient solution in the trial were initiated in March 2001 and completed in July 2001. The pattern of injection characterized by “nutrient-suspension-nutrient” was adopted. During the whole period of injection, the nutrient solution of about 350 m³/day was firstly injected for 3 days. The mixture of A02 and P15 suspensions of 210 m³ was then injected, and followed by a 10-day shut-in period of injection wells; the B24 suspension of 512 m³ was sequentially injected, and again followed by a 10-day shut-in period of injection wells. Following the injection of microbial suspension, the nutrient solution of about 350 m³/day was finally injected for 21 days. Before the injection, the microbial suspension was diluted by the nutrient solution to a microbial density of $1.8 \sim 2.4 \times 10^7$ cells/ml from its original density about 3.0×10^8 cells/ml. Finally, the water injection was restarted as usual.

The injection pattern “nutrient-suspension-nutrient” mentioned above was designed based on the knowledge of the target reservoir conditions and the mechanism of enhancement of oil recovery by the selected strains in the reservoir. The primary section of nutrient solution designed is with the purpose to establish a necessary nutrient environment for the following injected microorganisms, while the last one is to supply microorganisms with more nutrients to maintain and stimulate the activities of injected as well as indigenous microorganisms in the reservoir. As for the sections of microbial suspension, the volume and sequence of injection were designed according to the performance of the selected strains. Strain A02 and P15 were first injected in expectation of removing asphaltene and paraffin deposited in pore throats near wellbore region due to their good capacity of biodegradation of oil; the strain B24 as the body section was then injected in the expectation to improve microscopic oil displacement efficiency by biosurfactants, and more importantly, a 10-day shut-in of the injection

Table 3
Microbial performances in change in properties of oil from Well 69-8

Species	Viscosity reduction (%)	Solidification point reduction (°C)	Paraffin content reduction (%)	Asphaltene content reduction (%)
A02	15.8	2.6	2.6	3.3
P15	14.0	3.1	3.3	2.5
B24	15.4	2.0	1.8	1.6

The original values of viscosity, solidification point, paraffin and asphaltene content of the tested oil sample are 22.8 mPa·s, 42.4 °C, 26.1% and 23.1%, respectively.

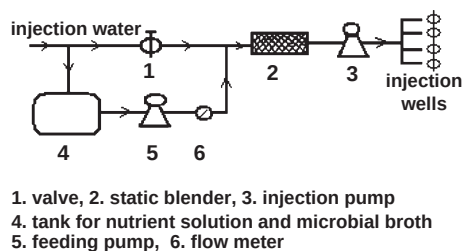


Fig. 2. Sketch of the bypass system for injection.

wells was followed with an aim to avoid microorganisms injected into the reservoir being excessively diluted by following injection and, therefore, to offer a time for their primarily proliferation in the reservoir.

The sketch of the bypass system for injection of microorganisms and nutrients is indicated in Fig. 2. As it shows in this figure that the microbial suspension or nutrient solution was first prepared in tank (4), and then mixed with water through a static blender (2) to achieve the designed concentration of microbial suspensions and nutrient solution, and finally injected by pump (3) into the target reservoir through injection wells.

3.2. Field results

Before the microbial water-flooding, the production performance and the related properties were monitored. During and after the microbial treatment, microorganisms in production water, the property of oil and production water, and the production performance in the Unit were also periodically monitored.

3.2.1. Growth and migration of microorganisms

According to the monitoring results of microorganisms in production water from all the 7 production wells in the Unit with a sampling interval of 15 days, no microorganisms same as the microbial species

Table 4

The surface tensions of the production water from the tested reservoir

Production wells	69-4	69-8	69-9	69-12	69-17	69-19	69-21
Baseline value (mN/m)	72.7	82.4	79.6	81.6	85.0	87.1	86.1
The minimum value (mN/m)	60.4	54.0	59.1	58.0	56.0	59.3	55.6
Reduction (mN/m)	12.3	28.4	20.5	23.6	29.0	27.8	30.5

Table 5

The oil property of Well 69-19

Item	Density (g/cm ³)	Viscosity (mPa·s)	Paraffin (%)	Asphaltene (%)
Before implement	0.8689	57.9	29.82	31.06
Aug, 2001	0.8671	54.1	27.84	30.54
March, 2002	0.8665	53.8	25.47	28.75

injected were detected in the samples collected before and in the beginning phase of the nutrient injection, but later, microorganisms in production water from Well 69-17 and Well 69-12 were first observed at a concentration of 2000–3000 cells/ml in two months; concentrations of microorganisms in production water from Well 69-9 and Well 69-8 were observed at a concentration of 6000–6800 cells/ml in five months. Subsequently, the microbial density was found in steady increase to its maximum about 1.6×10^5 cells/ml in about nine months. It is, therefore, reasonable to conclude that the injected microorganisms can proliferate in the target reservoir conditions. Furthermore, taking the well space (about 250 m) into consideration, the results suggest that the injected microorganisms may migrate through the reservoir matrix at a speed about 1.7–4.2 m/day.

In addition to microorganisms, the surface tensions of the production water were measured. It shows that the surface tension of the production water decreases due to biosurfactants produced by injected microorganisms and, probably, also by indigenous microorganisms in the reservoir. The maximum reduction in surface tension is 30.5 mN/m as shown in Table 4.

3.2.2. Change in properties of oil and gas

The change in properties of oil and gas from production wells were analyzed after bio-treatment,

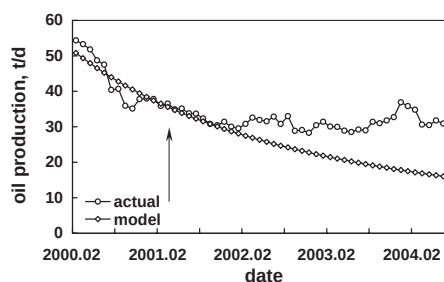


Fig. 3. Production curve of Guan69 Unit.

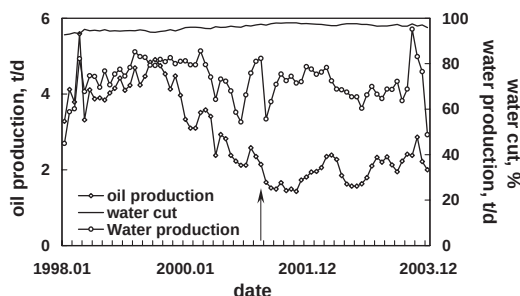


Fig. 4. Production curve of Well 69-9.

and a notable change in oil and connate gas was observed. The density, viscosity, paraffin and asphaltene content of crude oil from the Unit decreased as outlined in Table 5. The content of methane in the connate gas increased and that of carbon dioxide decreased. An example of change in the connate gas produced from Well 69-9 gives that the methane content increase from 85.4% to 90.2% and the carbon dioxide content decrease from 5.0% to 1.5%, respectively.

3.2.3. Increase in oil production

The oil production steadily increased after microbial water-flooding in the Unit. As it shows in Fig. 3 that, before and in the beginning phase of the injection, the oil production in the Unit decreased from 55 t/day in Jan. 2000 to 30.0 t/day in Sept. 2001, which implies a decline rate of 21%. However, this situation markedly changed six months later of the microbial treatment. By the end of July 2004, about 8700 t of additional oil was obtained compared with the predicted oil production by water flooding alone. All of the 7 production wells showed a positive response to the treatment, of which five Wells (69-9, 69-4, 69-17, 69-12 and 69-19) evidently increased in oil production. An example of the production performance of Well 69-9 is shown in Fig. 4.

4. Discussion

- (1) Microorganisms injected in reservoirs can thrive, migrate and finally form a gradient distribution in concentration in oil-bearings. Monitoring results show that the injected microorganisms are observed first and mainly in production

water from those production wells which have good response to water injection. The peak concentration of microorganisms in the production water is about 10^4 to 10^5 cells/ml. This concentration is much lower than that in injected suspensions with about 10^7 cells/ml, which implies that a falling gradient of microbial concentration may exist from injection wells to production wells.

- (2) Enhanced oil recovery in the experiment is due to the cumulative effect of injection of the nutrient medium, of the introduced bacteria, and of the microbial community of the stratum. The injected microorganisms play a part in oil extraction. According to the experimental results from both the laboratory and field trial, microbial activities and interactions of metabolic products with oil, brine and solid contribute to enhancement of oil recovery, among which the microbial degradation of oil and biosurfactants produced in situ (Mu et al., 2002) are major factors during microbial water-flooding. Other factors such as microbial modification of permeability are also believed to contribute to enhancement of oil production (Zekri and El-Mehaideb, 2003). Indigenous microorganisms in the tested reservoir also play a role in the change of gas contents and make a contribution to oil extraction. As mentioned above, a marked change of content of methane and carbon dioxide in the connate gas occurred after microbial treatment, which cannot be attributed to the activities of introduced microorganisms. It suggests that methanogenic bacteria existing in the target reservoir are activated by the nutrients and microorganisms introduced. Early researches (Borzenkov et al., 1997; Belyaev et al., 1998; Nazina et al., 1998, 2002) stated that methanogenic bacteria existed in the formation water in oil reservoirs, especially in long term water-flooded reservoirs and produced methane after activation by mineral salts supplied. In addition, other kinds of microorganisms existing in the oil-bearings might be activated as well and contributed to oil enhancement (Yakimov et al., 1997; Hao et al., 2004). The effective usage and contribution of indigenous microorganisms in petroleum reservoirs to

oil recovery should therefore be taken into consideration in the further studies.

- (3) Results from the project show that microbial water-flooding techniques have a potential of enhancing oil recovery in high temperature oil reservoirs.

Acknowledgments

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