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Laboratory studies of rice bran as a carbon source to stimulate indigenous microorganisms in oil reservoirs

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Abstract There is a great interest in developing cost-efficient nutrients to stimulate microorganisms in indigenous microbial enhanced oil recovery (IMEOR) processes. In the present study, the potential of rice bran as a carbon source for promoting IMEOR was investigated on a laboratory scale. The co-applications of rice bran, K_2HPO_4 and urea under optimized bio-stimulation conditions significantly increased the production of gases, acids and emulsifiers. The structure and diversity of microbial community greatly changed during the IMEOR process, in which *Clostridium* sp., *Acidobacteria* sp., *Bacillus* sp., and *Pseudomonas* sp. were dominant. Pressurization, acidification and emulsification due to microbial activities and interactions markedly improved the IMEOR processes. This study indicated that rice bran is a potential carbon source for IMEOR.

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1 Introduction

Increasing demand for crude oil is promoting the development of oil extraction technologies. Among these, microbial enhanced oil recovery (MEOR) is a promising tertiary oil recovery technology for depleted oil fields (Brown 2010; Sen 2008). Indigenous microorganisms, which naturally inhabit oil reservoirs, show a greater metabolic activity than exogenous ones due to their long-term adaptation (Castoren-Cortés et al. 2012; Lazar et al. 2007). Therefore, indigenous microorganisms activated MEOR (IMEOR) has greater efficiency than using exogenous ones (Yao et al. 2012; Zhang et al. 2012). Stimulation, growth and propagation of beneficial microorganisms that can contribute to producing effective metabolites are critical to the application of IMEOR (Zhang et al. 2010). The metabolites mainly include gases, acids and emulsifiers (Gao and Zerki 2011). Gases can pressurize the oil reservoir and reduce the viscosity of crude oil (Kobayashi et al. 2012; Spirov et al. 2014). Acids may increase carbonate rock porosity and permeability, thereby promote the exudation of remained oil (Sen 2008). Emulsifiers can emulsify crude oil, lower its viscosity and improve its fluidity (Banat et al. 2010; Dastgheib et al. 2008; Kitamoto et al. 2009; Sarafzadeh et al. 2013; She et al. 2011). The multiple effects of various metabolites improve oil flooding and thus enhance crude oil recovery.

Efficient production of beneficial metabolites is needed during an IMEOR process. However, nutrients in an oil reservoir are often insufficient and unbalanced, thus cannot provide adequate bio-stimulation (Wang et al. 2012). The

injection of nutrients can optimize bio-stimulation conditions in an oil reservoir (Gao et al. 2013). The injected nutrients should stimulate beneficial microorganisms but restrain harmful ones, while they should not cause formation damage or contamination (da Silva et al. 2014). More importantly, the nutrients should be economical. Of these nutrients, carbon sources have an overwhelming influence on bio-stimulation effects and application costs. Carbohydrates such as molasses, corn syrup, malt dextrin sucrose and starch have been explored for use in IMEOR in past decades (Bao et al. 2009; Joshi et al. 2008). As market prices of carbohydrates are rising, finding an economical carbon source has been a significant motivation. Rice bran, as an abundant agricultural by-product, has been used to prepare culture media for enzymatic solid-state fermentations (Ng et al. 2010; Noike and Mizuno 2000; Tanaka et al. 2006). However, it has not been investigated as a bio-simulator to promote IMEOR.

The objective of this study was to investigate the industrial potential of rice bran to promote IMEOR. Additionally, the bio-stimulation mechanism of rice bran can be acquired by the microbial diversity analysis during an IMEOR process. The results could be hopefully beneficial to reduce IMEOR cost and would generate value from agricultural by-products.

2 Materials and methods

2.1 Materials

The formation water and crude oil were both sampled from the Qixi block of Karamay oil field, which is located in Xinjiang Uygur Autonomous Region, Northwest China. Water flooding has been implemented by recycling production water for 40 years. The sampling and storage methods were as previously described (Tang et al. 2012). The initial temperature, pH and salinity of the formation water were 33.5 °C, 7.85 and 5054 mg/L, respectively. The kinematic viscosity (50 °C) and density (20 °C) of the crude oil were 62.33 mm^2/s and 0.912 g/cm^3, respectively.

The nutrients that were used to stimulate microorganisms in an IMEOR process commonly included carbon, nitrogen and phosphorus sources. The carbon nutrients were selected from several agricultural by-products including rice bran (reducing sugar at 390 mg/g and total nitrogen at 2.21 mg/g), wheat bran (reducing sugar at 280 mg/g and total nitrogen at 0.45 mg/L), corn residue, rice husk, glycerol residue and molasses. Rice bran and wheat bran were pulverized to 100 mesh particles prior to the experiment. The nitrogen sources were urea, NH_4Cl, KNO_3 and $NaNO_3$. The phosphorus sources were K_2HPO_4, $NH_4H_2PO_4$, NaH_2PO_4, KH_2PO_4, $(NH_4)_2HPO_4$ and Na_2HPO_4. The agricultural by-products were purchased from a local market. All nitrogen and phosphorus sources were from Beijing Chemical Reagents Co., China.

2.2 Optimization experiments of bio-stimulation conditions

Preferred nutrients and their basic concentrations were selected by single factor optimization (SFO) experiments. Concentrations of preferred nutrients were further optimized by orthogonal design experiments (Chen et al. 2010). The optimized bio-stimulation conditions were quantified with response surface methodology (RSM) experiments based on a Box–Behnken design (Chen et al. 2007). Response surface regression analysis and analysis of variance (ANOVA) were performed by Minitab software (Version 16, Minitab Inc, State College, PA, USA). The experiments were carried out with 250 mL SIBATA fermentation bottles. Various nutrients at different concentrations and 50 mL distilled water were introduced into the bottles, followed by pH adjustment to 7.85. After sterilization at 115 °C for 30 min, the bottles were inoculated with 50 mL of formation water and 5 g of crude oil, and then incubated at 33.5 °C and 110 rpm for 7 days. Gas production and surface tension were indicators of bio-stimulation effects. All experiments were done in triplicate.

2.3 Laboratory-scale experiments of IMEOR

The potential of rice bran was investigated in 250 mL SIBATA fermentation bottles to simulate an IMEOR process. The initial broth contained 50 mL of formation water, 5 g of crude oil and optimized nutrient components, and was diluted to a final volume of 100 mL with distilled water. Sterilization followed the protocols described in the above-mentioned optimization experiments, as well as the incubation conditions with an extended 10 day incubation period. An aliquot of 0.05 mL Resazurin solution (0.1 wt%) was added to each bottle as a visual anaerobic status indicator. Simulated IMEOR experiments were carried out in triplicate.

The bio-stimulation effects were measured during experiments. The produced gases which mainly reflected the re-pressurization of an oil reservoir were collected in a Devex gas collecting bag (0.3 L), and the gas volume was recorded by a graduated syringe. The gas composition was analyzed on a HP 6890 gas chromatograph (GC) (Agilent, Wilmington, DE, USA) equipped with a TCD detector (80 °C) and a Porapak Q packing column (60 °C). Helium was used as carrier gas. The pH values which indicated the production of acids were measured on a PHSJ-4 meter (Leici, Shanghai, China). Two mL of broth was extracted by a graduated syringe and injected to a 5-mL centrifuge tube for

pH measurement. Bacterial numbers were counted by a cell counting method (Zhou and Wang 2004). Surface tension and emulsification degree both characterized the production of emulsifiers such as biosurfactants, biopolymer, acids and solvents. Surface tension was measured with a JK99B tensiometer (Powereach, Shanghai, China), and emulsification degree was obtained with the emulsification index (%, EI 24) method (Reddy et al. 2010).

2.4 Analysis of microbial diversity

Describing the relationship between the microbial community structures and their functions will contribute to understanding of bio-stimulation mechanisms (Fuhrman 2009). Polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) has been widely used in microbial community analysis (Wang et al. 2008a, b). Aliquots (2 mL) of fermentation broth were collected from the bottles. After centrifugation at 10,000 rpm for 5 min, the total DNA was extracted with a genomic DNA extraction kit (TaKaRa, Dalian, China) according to the supplier's instructions. PCR-DGGE was performed as previously described in literature (Ji et al. 2009). DGGE profiles, including the presence, intensity and abundance of the bands, were analyzed with Quantity One software (Version 4.4, Bio-Rad, Hercules, CA, USA). Microbial diversity was calculated by Shannon-Wiener's indexes (H) (Andreoni et al. 2004). Dominant bands were excised from the gels and re-amplified, and the fragments were recovered and cloned again. The positive clones were selected and sequenced (Sangon, Shanghai, China). Typical sequences were analyzed using the NCBI BLAST database to identify the closest relatives. A phylogenetic tree was constructed with MEGA software (Version 5.0).

3 Results

3.1 Optimized bio-stimulation conditions

Rice bran, K_2HPO_4 and urea were selected as preferred carbon, phosphorus and nitrogen sources, respectively (Fig. 1). An optimal bio-stimulation condition was preliminarily estimated to be 2.0 g/100 mL of rice bran, 0.05 g/100 mL of K_2HPO_4 and 0.05 g/100 mL of urea under SFO experiments (Fig. 2). The concentrations of rice bran, K_2HPO_4 and urea were further optimized to be 3.0, 0.07 and 0.07 g/100 mL, respectively, under 3^3 orthogonal design experiments (Table 1). According to the Box–Behnken experiments (Table 2) and subsequent response surface regression analysis (Fig. 3), the optimal bio-stimulation conditions were estimated as 3.36 g/100 mL of rice bran, 0.075 g/100 mL of K_2HPO_4 and 0.076 g/100 mL of urea.
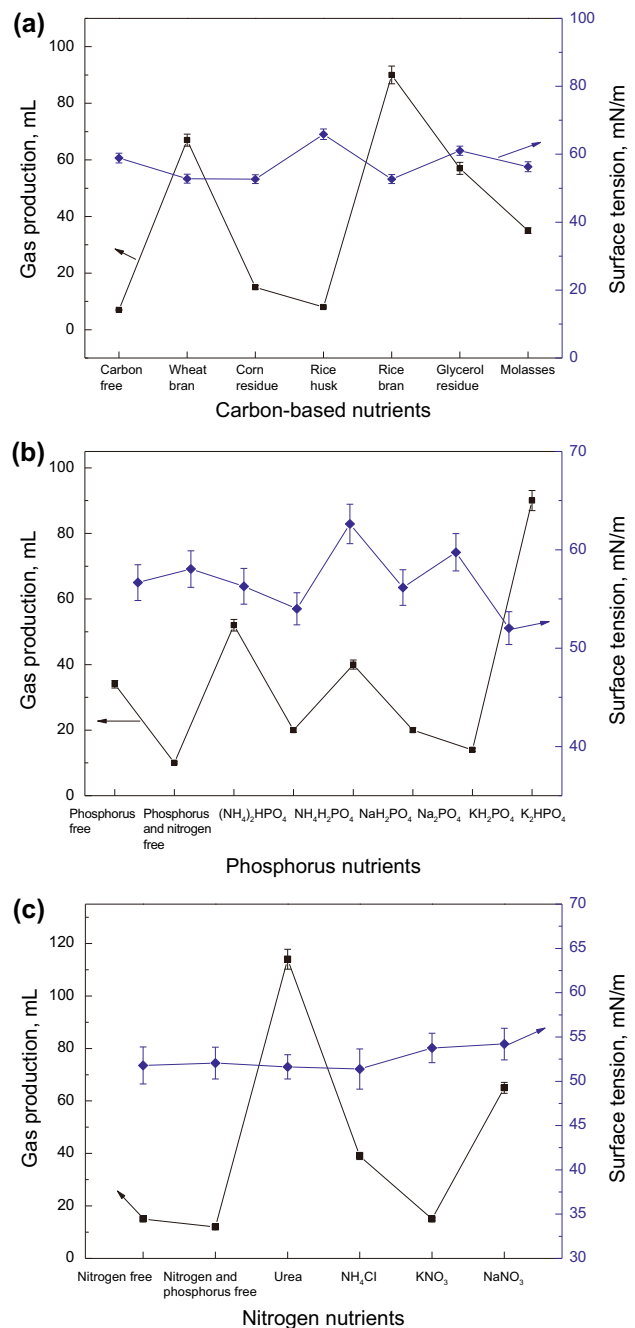

Fig. 1 Screening of preferred carbon nutrient (1.0 g/100 mL carbon sources, 0.1 g/100 mL urea and 0.1 g/100 mL $(NH_4)_2HPO_4$) (**a**), phosphorus nutrient (0.1 g/100 mL phosphorus sources, 1.0 g/100 mL rice bran and 0.1 g/100 mL urea) (**b**), and nitrogen nutrient (0.1 g/100 mL nitrogen sources, 1.0 g/100 mL rice bran and 0.1 g/100 mL K_2HPO_4) (**c**)

3.2 Bio-stimulation effects of rice bran

Color changes of the fermentation broth from red to pink and then colorless indicated the gradual conversion from aerobic (0–1st day), facultative (2nd–3rd days) to anaerobic (4–10th days) metabolic stages in the simulated
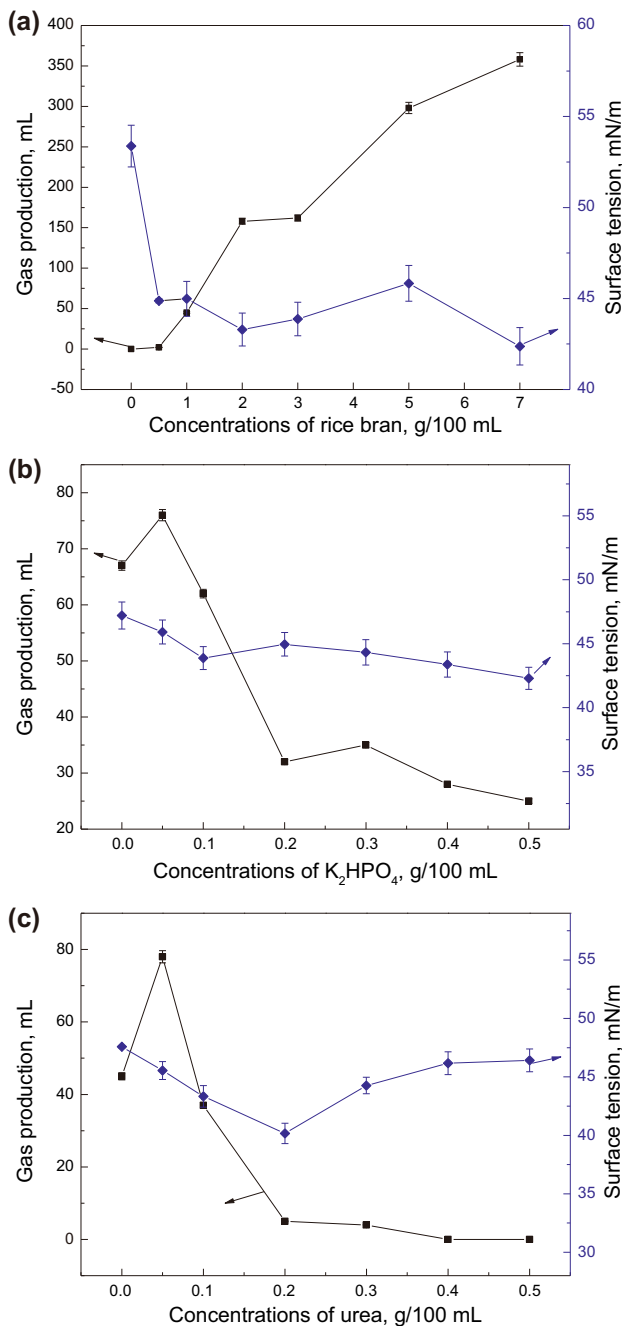
Fig. 2 Preliminary screening of concentrations of rice bran (0.1 g/100 mL urea and 0.1 g/100 mL K_2HPO_4,) (**a**), K_2HPO_4 (1.0 g/100 mL rice bran and 0.05 g/100 mL urea) (**b**), and urea (1.0 g/100 mL rice bran and 0.1 g/100 mL K_2HPO_4) (**c**)

IMEOR process. The numbers of bacteria rapidly increased from the initial 0.07×10^9 cell/mL to 2.4×10^9 cell/mL in the aerobic stage and reached a peak value of 4.9×10^9 cell/mL in the anaerobic stage (5th day), followed by a slight drop to 4.5×10^9 cell/mL at the end of bio-stimulation (Fig. 4a). The pH values significantly decreased from the initial 7.85 to 5.26 in the facultative stage (3rd day) and then finally decreased to 4.94 (Fig. 4a).

Gas production lasted for 8 days; a total 322 mL of gases was collected mainly in the facultative stage where the highest gas production rate was observed (Fig. 4b). Gas components varied markedly among stages (Table 3). CO_2 concentration promptly increased from 0.03 to 42.7 vol% along with sharp depletion of O_2 in the aerobic and facultative stages; CO_2 and H_2 were the main gases in the anaerobic stage. Surface tension dropped from 62.5 to 37.6 mN/m in the early anaerobic stage (5th day) when the EI 24 increased rapidly from zero to 61.1 % (Fig. 4c).

3.3 Microbial community

There were 17 major bands in the DGGE profiles (Fig. 5a) with obvious variations in the intensities (Fig. 5b), H-index, numbers of bands and microbial abundances among lanes (Table 4). All 17 bands were isolated from the gel, re-amplified, and sequenced. Except for bands 1, 2 and 3, all of the identified genera had sequence similarities of 98 % or higher (Table 5). The phylogenetic tree (Fig. 6) shows that the identified sequences could be divided into three classifications, *Proteobacteria*, *Firmicutes* and *Acidobacteria*. Sequences from bands 3, 4, 5 and 13 were not classified with the phylogenetic tree, which was presumed to be not reported.

4 Discussion

4.1 Optimized bio-stimulation conditions

Nutrient-rich polysaccharides stimulate indigenous microorganisms in oil reservoirs (Cheng et al. 2010; Feng et al. 2012). Rice bran and wheat bran showed higher emulsification and gas production than the other carbon sources. The market prices of rice bran and wheat bran are similar at approximately $180 per ton. Rice bran contained more reducing sugar, total nitrogen and vitamins than wheat bran under previous composition analysis (Wang 2013); therefore, it was identified as a preferred carbon source. Urea and K_2HPO_4 contributed largely to the gas production and were determined as the preferred nitrogen and phosphorus nutrients. The concentration of rice bran displayed a positive correlation with gas production, while an excess of rice bran would increase the costs. The concentrations of K_2HPO_4 and urea showed negligible influence on bio-stimulation. The concentrations of rice bran, K_2HPO_4 and urea at, respectively, 2.0 0.05 and 0.05 g/100 mL basically were the balanced nutrient ratios.

The surface tension that directly accounted for oil-flooding effects was selected as the decisive indictor to optimize bio-stimulation conditions. The significance order of nutrient concentrations were rice bran > urea > K_2HPO_4

Table 1 Experimental results of 3^3 orthogonal designs

Runs	Rice bran, g/100 mL	Urea, g/100 mL	K$_2$HPO$_4$, g/100 mL	Gas production, mL	Surface tension, mN/m
1	1 (−1)	0.03 (−1)	0.03 (−1)	67	48.8
2	1	0.05 (0)	0.05 (0)	41	51.0
3	1	0.07 (+1)	0.07 (+1)	61	46.5
4	2 (0)	0.03	0.05	92	46.2
5	2	0.05	0.07	64	35.4
6	2	0.07	0.03	38	34.7
7	3 (+1)	0.03	0.07	191	35.8
8	3	0.05	0.03	120	39.9
9	3	0.07	0.05	125	33.9

(−1) for low level, (0) for medium level and (+1) for high level

Table 2 Experimental results of Box–Behnken designs

Runs	Rice bran, g/100 mL	Urea, g/100 mL	K$_2$HPO$_4$, g/100 mL	Gas production, mL	Surface tension, mN/m
1	2 (−1)	0.04 (−1)	0.07 (0)	93	48.5
2	3 (0)	0.07 (0)	0.07	78	38.0
3	3	0.04	0.1 (+1)	153	46.2
4	4 (+1)	0.07	0.1	250	41.8
5	3	0.1 (+1)	0.1	219	42.3
6	4	0.1	0.07	310	42.6
7	2	0.07	0.1	160	45.8
8	3	0.07	0.07	189	40.5
9	3	0.1	0.04 (−1)	154	41.4
10	2	0.07	0.04	100	47.8
11	2	0.1	0.07	101	48.2
12	4	0.07	0.04	38	44.2
13	3	0.04	0.04	85	48.1
14	3	0.07	0.07	22	39.0
15	4	0.04	0.07	210	42.1

(−1) for low level, (0) for medium level and (+1) for high level

according to the analysis of orthogonal design experiments. By applying response surface regression analysis to the surface tension data from the Box–Behnken experiments, the following second-order equation was established:

$$Y = 114.276 - 22.4558C - 568.595N - 444.793P$$
$$+ 3.30217C^2 + 3229.07N^2 + 2696.85P^2 \qquad (1)$$
$$+ 6.2500CN - 3.46667CP + 786.667NP$$

where Y is the surface tension, mN/m; C, N and P are the concentrations (g/100 mL) of rice bran, urea and K$_2$HPO$_4$, respectively. C^2, N^2, C, and N show significant influences on surface tension with P values of 0.028, 0.043 and 0.029, respectively. ANOVA results indicated a good consistency between experimental and predicted values with a P value of 0.017 and a R^2 value of 0.902. The experimental surface tension (40.6 mN/m) under the optimized bio-stimulation conditions agreed well with the predicted value, which the

predicted minimum surface tension (38.6 mN/m) was also obtained under the optimal bio-stimulation conditions.

4.2 Bio-stimulation effects of rice bran for promoting IMEOR

Indigenous microorganisms became active and reached a logarithmic growth phase shortly after addition of rice bran supplemented with urea and K$_2$HPO$_4$. A stationary growth phase appeared in the later anaerobic stage due to depletion of nutrients. Acids were rapidly produced in the aerobic and facultative stages, and their production and utilization reached an approximate balance in the anaerobic stage. Acid production favors flooding, whereas a lower pH value is harmful to some indigenous microorganism species.

Gas-producing bacteria were sensitive to rice bran, and gas production mainly occurred in the aerobic and facultative stages. As O$_2$ was consumed, CO$_2$ was generated
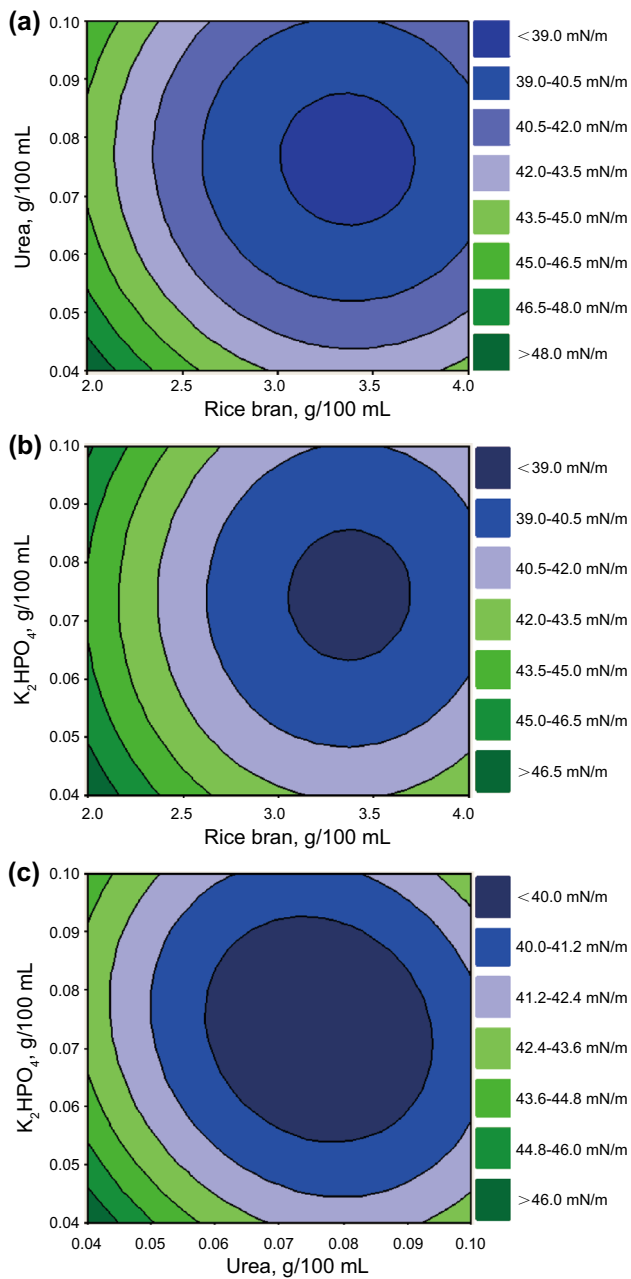
Fig. 3 Interaction effects of rice bran and urea with K_2HPO_4 concentration of 0.07 g/100 mL (a), rice bran and K_2HPO_4 with urea concentration of 0.07 g/100 mL (b), and urea and K_2HPO_4 with rice bran at a concentration of 3.0 g/100 mL (c) on surface tension
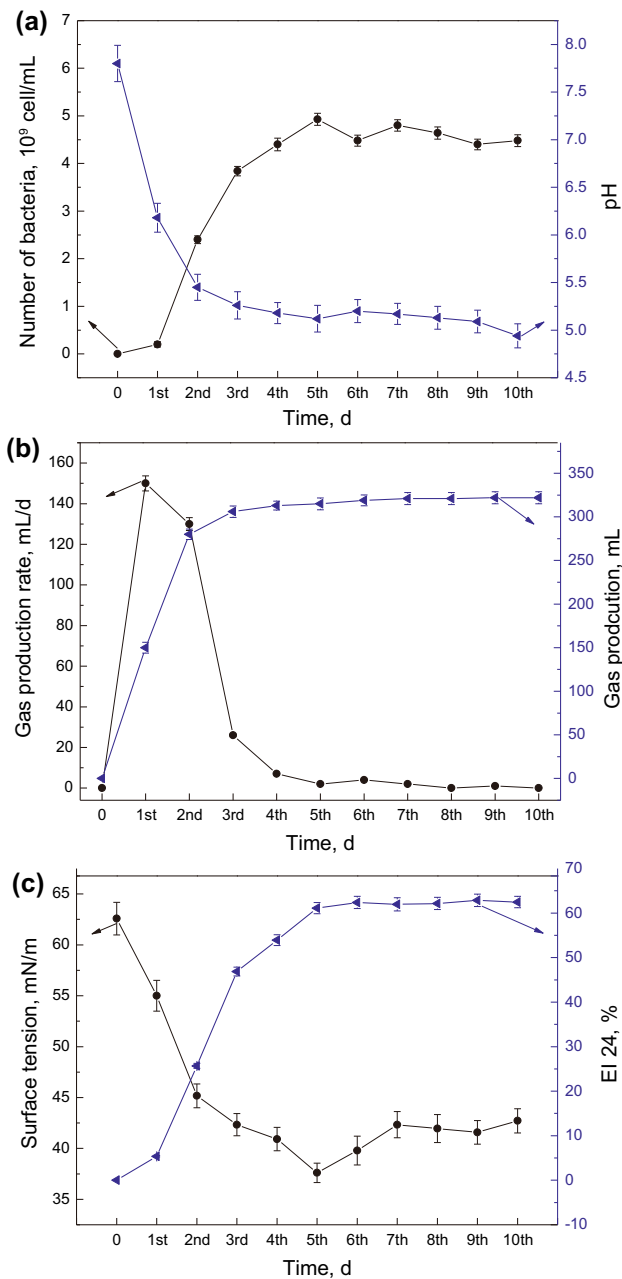

Fig. 4 pH values and numbers of bacteria (a), gas production and production rate (b), surface tension and EI 24 (c) of fermentation broth in a simulated IMEOR process

rapidly with the growth of aerobic bacteria. The emergence of H_2 signified that anaerobic bacteria became active, while aerobic bacteria were inhibited in the anaerobic stage. Injecting an extra amount of air or O_2 can extend the aerobic and facultative stages, and contribute to re-pressurization and CO_2 flooding.

Aerobic bacteria rapidly produce emulsifiers utilizing sufficient nutrients and O_2 in the aerobic stage. After that, facultative and anaerobic bacteria became dominant and

emulsifier production decreased; therefore, no further significant decrease of surface tension was observed. The EI 24 demonstrated the high emulsification of crude oil at the end of bio-stimulation. Emulsifiers play a critical role in MEOR processes. Continuous supply of nutrients and O_2 to an oil reservoir would contribute to the production of a greater amount of emulsifiers.

The rice bran, as a carbon nutrient stimulator, successfully stimulated beneficial indigenous microorganisms and

Table 3 Composition of produced gases at different days during a simulated IMEOR process

Components	0 day		2nd day		3rd day		8th day		10th day	
	vol%	mL	vol%	mL	vol%	mL	vol%	mL	vol%	mL
N_2	78	117	39.5	118.5	33.9	101.7	0	0	0	0
O_2	21	31.5	2.3	6.9	1.9	5.7	0	0	0	0
H_2	0	0	12.8	38.4	30.7	92.1	6.3	18.9	20.4	61.2
CO_2	0.03	0.05	42.7	128.1	32.1	96.3	89.8	269.4	76.1	228.3
Others (C_2H_6, C_3H_8, etc.)	0.97	0.15	2.7	8.1	1.4	4.2	3.9	11.7	3.5	10.5
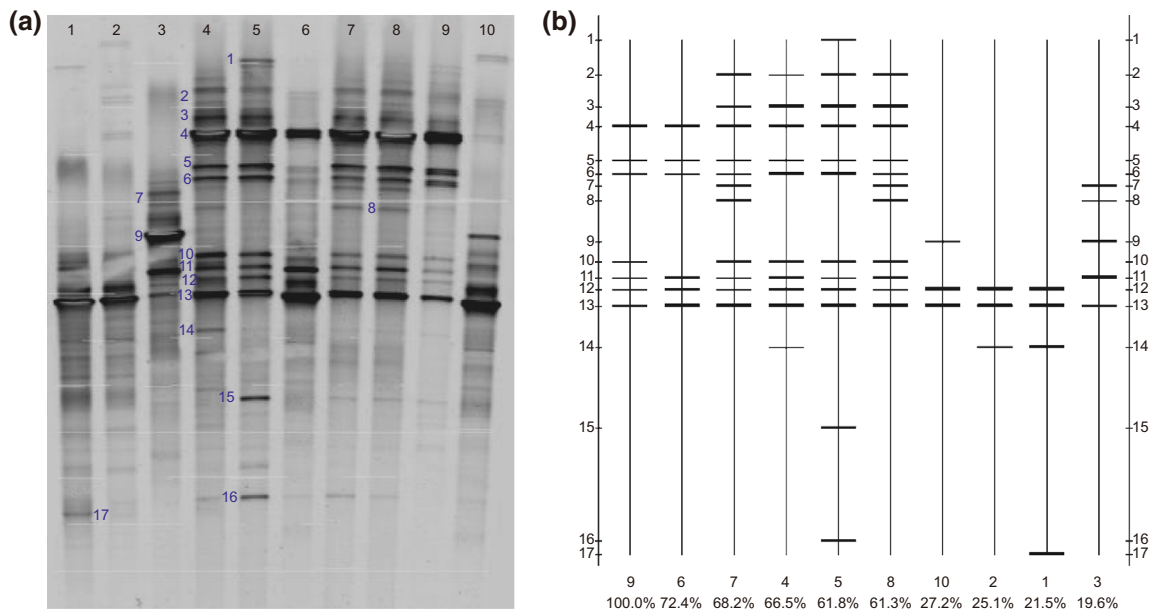
Fig. 5 DGGE profile of bacterial samples from different days (**a**), schematic diagram of relative band intensities in DGGE profiles (**b**). *Note* Lane number represented the bacterial samples collected in different days. Lane 1—the 1st day; Lane 2—the 2nd day; and so on; Lane 10—the 10th day

Table 4 Diversity index (*H*), numbers of bands and abundances of DGGE profiles

Lane no.	1	2	3	4	5	6	7	8	9	10
H-index	2.45	2.40	2.05	2.77	2.61	1.93	2.61	2.43	1.98	1.99
Numbers of bands	12	14	8	21	15	9	14	13	10	10
Microbial abundance	1665	157	1150	476	368	282	1908	358	239	226

enhanced the production of acids, gases and emulsifiers, and hence effectively promoted the IMEOR process.

4.3 Diversity and functions analysis of microbial community

Changes in microbial community structure occurred in the simulated IMEOR process (Table 6). Microbial diversity (*H*-index) was significant on the 1st, 4th, 5th and 7th days and microbial abundance peaked markedly on the 1st, 3rd and 7th days. *Clostridium* sp., *Acidobacteria* sp., *Pseudomonas* sp. and *Bacillus* sp. were the dominant bacteria. Of these bacteria, *Bacillus* sp. and *Pseudomonas* sp. dominated the aerobic stage and *Acidobacteria* sp. dominated the facultative and anaerobic stages. *Clostridium* sp. was highly distributed throughout the simulated IMEOR process.

Aerobic bacteria were stimulated and caused a higher microbial diversity and abundance in the aerobic stage (1st day). *Clostridium* sp., uncultured *Acidobacteria* bacterium,

Table 5 Comparisons of nucleotide sequences of sequenced DGGE bands

Band no.	Closest relatives	Accession No.	Identity, %
1	*Clostridium* sp.	HM801879.1	95
	Uncultured *Clostridium* sp.	JX273758.1	95
2	*Clostridium sulfidigenes* strain	HM163534.1	96
	Uncultured *Enterococcus* sp.	DQ232854.1	96
3	*Achromobacter* sp.	AB772984.1	96
	Uncultured bacterium clone	KC465632.1	97
4	*Bacillus cereus* strain	KC683782.1	98
	Uncultured bacterium clone	GU002857.1	98
5	*Pseudomonas aeruginosa* strain	KC570343.1	100
	Uncultured *Pseudomonas* sp.	KC470004.1	100
6	*Clostridium indolis* strain	JX960755.1	100
	Uncultured bacterium clone	KC000040.1	100
7	*Brevundimonas* sp.	HF571531.1	99
	Uncultured *Brevundimonas* sp. clone	JQ701321.1	99
8	*Pantoea agglomerans* strain	KC009691.1	100
	Uncultured bacterium clone	KC299005.1	100
9	*Ochrobactrum* sp.	KC493414.1	100
	Uncultured *Ochrobactrum* sp.	KC502956.1	100
10	*Arcobacter* sp.	FN397894.1	99
	Uncultured *Arcobacter* sp.	HM245616.1	99
11	*Agrobacterium vitis* strain	KC196472.1	99
	Uncultured bacterium clone	JX872344.1	99
12	Uncultured *Acidobacteria* bacterium clone	DQ829628.1	99
13	*Clostridium* sp.	KC331196.1	100
	Uncultured *Clostridium* sp.	KC110473.1	100
14	*Bacillus aerius* strain	KC469617.1	100
	Uncultured bacterium clone	KC414651.1	100
15	*Sulfurospirillum carboxydovorans* strain	AY740528.1	99
	Uncultured *epsilon proteobacterium*	AJ576003.1	99
16	*Paenibacillus* sp.	KC134361.1	99
	Uncultured bacterium clone	JX223186.1	99
17	*Pseudomonas* sp.	KC433644.1	100
	Uncultured *Pseudomonas* sp. clone	KC253432.1	100

Bacillus aerius, and *Pseudomonas* sp. were stimulated and resulted in the increase of bacteria numbers. *B. aerius* and *Pseudomonas* sp. began to produce acids and emulsifiers (e.g., biosurfactants) by utilizing O_2, nutrients and even crude oil (Abdel-Mawgoud et al. 2009; Banat 1995), then both pH and surface tension decreased.

Aerobic bacteria (e.g., *Pseudomonas* sp.) were gradually inhibited with consumption of O_2, and facultative bacteria became dominant in the facultative stage. Microbial abundance rebounded in the 3rd day, while the genera of bacteria were not rich. *Clostridium* sp. began to produce gases by utilizing nutrients (Taguchi et al. 1996). *Brevundimonas* sp., *Pantoea agglomerans*, *Ochrobactrum* sp., and *Agrobacterium vitis* were stimulated. Among these, *Brevundimonas* sp. and *Ochrobactrum* sp. can decompose crude oil to produce biosurfactants and acids (Arulazhagan and Vasudevan 2011; Ghosal et al. 2010; Ruggeri et al. 2009; Takahashi et al. 1999); *Agrobacterium vitis* can promote the metabolism of petroleum-degrading bacteria (Chalneau et al. 1995). The functions of *P. agglomerans* in an IMEOR process have not been reported. The co-effects of microbial community increased the bacteria numbers and EI 24, but decreased both the pH and surface tension.

As anaerobic bacteria were stimulated in the anaerobic stage, the number of bacterial genera significantly increased on the 4th day and the microbial diversity reached its maximum level. *Clostridium* sp., *Clostridium sulfidigenes*, *Clostridium indolis*, *Achromobacter* sp., *Bacillus cereus*, *B. aerius*, *Pseudomonas aeruginosa*, *Arcobacter* sp., *A. vitis*, uncultured *Acidobacteria* bacteria
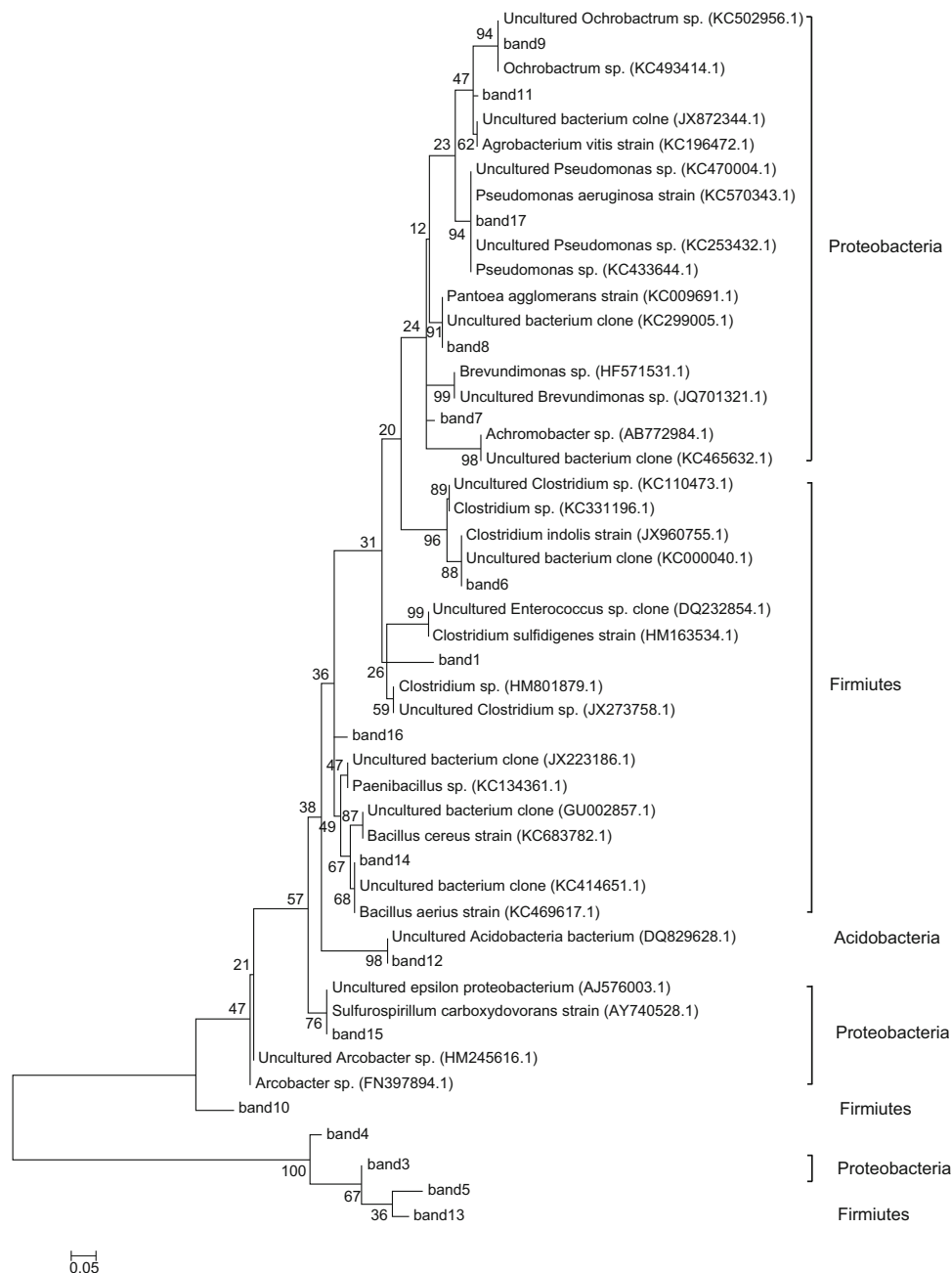
Fig. 6 Phylogenetic tree of 16S rDNA sequences from DGGE profiles

and the other uncultured bacteria co-built up the most complex structure of microbial community. *B. cereus* can produce acids but not gases in anaerobic conditions (Nazina et al. 2003). *P. aeruginosa* can produce biosurfactants by degrading crude oil (Das and Mukherjee 2007). *Sulfurospirillum carboxydovorans* and *Paenibacillus* sp. appeared on the 5th day. The former can inhibit sulfate-reducing bacteria (Hubert and Voordouw 2007), and the latter may produce biopolymers (Wang et al. 2008a, b). The bacteria abundance reached a peak value and surface

tension at a minimum on the 5th day when the pH and EI 24 almost attained their lowest and highest values, respectively; meanwhile, the gas production declined significantly. The number of bacterial genera decreased, but the microbial abundance increased, and *B. cereus*, *A. vitis*, uncultured *Acidobacteria* and *Clostridium* sp. became dominant on the 6th day. As anaerobic bacteria becoming adapted, microbial abundance recovered again and reached its peak value on the 7th day when the microbial diversity remained high. After that, microbial diversity and

Table 6 Dominant bacteria and the community succession during a simulated IMEOR process

Band no.	Genus	Dominance during a simulated IMEOR process									
		1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day
1	Clostridium sp.	−	−	−	−	+	−	−	−	−	−
	Uncultured Clostridium sp.										
2	Clostridium sulfidigenes strain	−	−	−	+	+	−	+	+	−	−
	Uncultured Enterococcus sp.										
3	Achromobacter sp.	−	−	−	+	+	−	+	+	−	−
	Uncultured bacterium clone										
4	Bacillus cereus strain	−	−	−	+	+	+	+	+	+	−
	Uncultured bacterium clone										
5	Pseudomonas aeruginosa strain	−	−	−	+	+	+	+	+	+	−
	Uncultured Pseudomonas sp.										
6	Clostridium indolis strain	−	−	−	+	+	+	+	+	+	−
	Uncultured bacterium clone										
7	Brevundimonas sp.	−	−	+	−	−	−	+	+	−	−
	Uncultured Brevundimonas sp. Clone										
8	Pantoea agglomerans strain	−	−	+	−	−	−	+	+	−	−
	Uncultured bacterium clone										
9	Ochrobactrum sp.	−	−	+	−	−	−	−	−	−	+
	Uncultured Ochrobactrum sp.										
10	Arcobacter sp.	−	−	−	+	+	−	+	+	+	−
	Uncultured Arcobacter sp.										
11	Agrobacterium vitis strain	−	−	+	+	+	+	+	+	+	−
	Uncultured bacterium clone										
12	Uncultured Acidobacteria bacterium clone	+	+	−	+	+	+	+	+	+	+
13	Clostridium sp.	+	+	+	+	+	+	+	+	+	+
	Uncultured Clostridium sp.										
14	Bacillus aerius strain	+	+	−	+	−	−	−	−	−	−
	Uncultured bacterium clone										
15	Sulfurospirillum carboxydovorans strain	−	−	−	−	+	−	−	−	−	−
	Uncultured epsilon proteobacterium										
16	Paenibacillus sp.	−	−	−	−	+	−	−	−	−	−
	Uncultured bacterium clone										
17	Pseudomonas sp.	+	−	−	−	−	−	−	−	−	−
	Uncultured Pseudomonas sp. Clone										

(+) represent the dominant bacteria, (−) represent the non-dominant bacteria or absence

abundance both reduced to their minimum values at the end of bio-stimulation with the depletion of nutrients.

The structure and relative abundance of the microbial community, especially the interactions between different microorganisms, have great influence on promoting IMEOR. Rice bran, with the participation of the other preferred nutrients, could enhance the microbial diversity as well as strengthen the multiple functions of producing gases, acids and emulsifiers. The results indicated that rice bran can effectively promote IMEOR.

5 Conclusion

The potential of rice bran as a carbon nutrient for promoting IMEOR was investigated. Rice bran showed great bio-stimulation effects on producing gases, acids and emulsifiers with the supplements of K_2HPO_4 and urea. The oil reservoirs were re-pressurized and pH and surface tension decreased, which contributed to the IMEOR process. The indigenous microbial community showed remarkable successions, and the beneficial functioning

bacteria were stimulated in the simulated IMEOR process. *Clostridium* sp., *Acidobacteria* sp., *Bacillus* sp., and *Pseudomonas* sp. dominated the IMEOR process. Microbial activities and interactions among indigenous microorganisms enhanced the IMEOR process. The results indicated the potential of rice bran for promoting IMEOR due to its effectiveness and low cost.

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