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Biological upgrading of heavy oil cuts using native microbial consortia as an environmental-friendly technology in petroleum refineries

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ABSTRACT

Refineries are amongst the most energy-intensive and polluting industries in the world. Biotechnology may serve as an alternative low-cost and environmental-friendly tool to the current costly, toxic and hazardous refining processes. In this study, the compositional redistribution of a heavy hydrocarbon cut is investigated under biological conversion using native microbial consortia. The native consortia were obtained by batch enrichment method applied on oil-polluted soil samples from oil refineries of Iran. The bioconversion experiments were conducted with 20% and 40% (*v/v*) of the heavy cut as the sole carbon source and 10% (*v/v*) of the consortia broth in 250 ml flasks containing a mineral medium. The samples kept at 30°C stirring at 120 rpm for one week. The biotreated hydrocarbons were then separated and analyzed for determination of saturate, aromatic and resin fractions using column chromatography and gravimetric measurements. The results showed that the amounts of saturates increased by 6% to 92% while the resins decreased by 10% to 70% in most cases, compared to the blank. The GC-Mass analysis of the saturate fractions also revealed an increase in the cyclic and branched alkanes and a decrease in the S-containing and N-containing compounds.

1. Introduction

In an oil refinery, crude oil and other feedstocks are processed to produce useful products such as gasoline, diesel fuel, kerosine, diesel oil, liquefied petroleum gas, jet fuel, etc. The refining processes can be classified in three main process stages including; separation (e.g. crude oil distillation), conversion (e.g. thermal cracking, catalytic cracking), and finishing (e.g. catalytic reforming, hydroforming) [1]. These processes are costly and energy-intensive, consuming between 7 and 15 percent of the input crude oil for direct and indirect heating. [2]. Meanwhile, the refining industry is accounted for the third largest stationary emitter of greenhouse gases in the world, producing 6% of

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total industrial greenhouse gases [3]. Therefore, seeking for low energy consumption and low-pollution refining methods can be crucial in the sustainable development of this basic industry. Crude oils and heavy cuts contain thousands of complex compounds made mainly from carbon and hydrogen and minor amounts of oxygen, nitrogen, sulfur, halogens, and some rare metals such as nickel and vanadium. These compounds are categorized into four major fractions based on their chemical structures; saturates, aromatics, resins and asphaltenes [4,5]. Heavy crudes contain more amounts of resins and asphaltenes in comparison with light crudes. Resins and asphaltenes are large polar molecules predominantly containing heteroatoms (O, N, and S) and rare metals which increase the oil density and viscosity [6]. Presence of these compounds in the crudes and heavy end cuts (from atmospheric and vacuum distillations) reduces the calorific values of the oils and increases their refining costs. They also poison the catalysts used in subsequent catalytic cracking processes which makes them less efficient and increases the expenses due to more frequent replacement of the catalysts. Therefore, it is desirable to reduce the asphaltenes and resins and so to increase the light fractions in the petroleum feed stocks [7]. Biotechnology along with other technologies such as membrane separation, supercritical extraction, and others has been explored in order to be introduced in the oil refining industry [8,9]. Biotechnology offers a low cost and environmental-friendly technology to this field [10]. It is shown that biotreatment of crude oil can cause changes in distribution of light and heavy fractions, hydrocarbons composition, decrease in; sulfur, nitrogen, and metal content as well as; emulsification, and acidification of the oil [11-13]. These alterations not only depend on the microorganisms and chemistry of the crudes but also depend on several other factors such as temperature, pH, nutrients, and the bioavailability of hydrocarbons [14,15]. So far, biological enhancement of oil recovery has been a successful application of biotechnology in which oil recovery is enhanced up to 50% of the residual oil [16]. Biodesulfurization of crude oils has also been of concerns for many years, although yet to be applicable in large capacity. Other applications such as biodenitrogenation, biodemetalization, biohydrogenetion, biodearomatization, dewaxing, and viscosity reduction has also been investigated [17]. Most of these applications need much further researches in order to obtain effective microorganisms or enzymes, reduce the process time, increase the oil to water ratio and overcome other challenges such as emulsification and de-emulsification of oil-water system at beginning and end of the processes [18,19]. Biological upgrading of heavy crude oil aims at all these activities in order to enhance the value of the oil as well as provide easier transportation and treatment [14]. Premuzic et al. (1999) achieved the lowering of N, S, O and trace metals and also redistribution of hydrocarbon fractions of heavy crude oil when treated by different microbial consortia [20]. They concluded that the process depends on the types of microorganisms and the composition of crude oils in which the asphaltene and polar fractions were appeared to be involved in the mechanisms. Gailiūtė et al. (2011) investigated the cracking of a heavy branched hydrocarbon, squalene, by microorganisms at which 67% of squalene was converted to medium chain intermediates [21]. Ring cleavage was also performed by Sietmann et al. (2002) for biotransformation of biphenyl which was converted to mono-, di-, and trihydroxylated derivatives hydroxylated on one or both aromatic rings using yeast [22]. Ghollami et al. (2013) investigated the bioconversion of resins involved in vacuum distillation residue in which the resins were decreased while increasing the aliphatic and aromatic fractions [23]. The biological conversion of long-chain alkanes as a major part of petroleum oils was evaluated using different bacteria and consortia for the purpose of heavy oil upgrading [24,25]. Salehi et al., (2009) investigated the effect of native anaerobic consortia on composition redistribution of heavy crude oils [26]. In general, it is believed that biological processing can serve as an alternative or complementary process with higher selectivity of reactions and mild operational conditions [17]. However, it is important to keep the carbon skeleton of the oil through microbial consumption in which the calorific value of the oil may decrease. As said above, there are varieties of hydrocarbons in the crude oil from light alkanes to heavy polar compounds. Depending on the

chemical structure of components, some bacteria can metabolize specific alkanes, while others break down aromatic or resin fractions of hydrocarbons [27]. Therefore, in the absence of lighter compounds such as short-chain alkanes, the microbial community will drive toward utilization of heavier compounds such as long chain alkanes, resins and asphaltenes. In the present research, a heavy end cut from vacuum distillation tower was chosen in order to investigate the effect of microbial consortia on redistribution of the hydrocarbons where lighter compounds were already removed. This may increase the biological conversion rates of the heavier compounds and upgrade the feed for the subsequent refining process. So far, biological refining of heavy cuts has not been investigated in this way.

2. Materials and methods

2.1. Substrate

A heavy end cut from the vacuum distillation column of Tehran refinery in Iran was used as the substrate to investigate its biological conversion. The heavy cut is the feedstock to Isomax unit of the refinery which is a hydrocatalytic cracking process. Isomax process converts heavy hydrocarbons into lighter ones to produce mainly gasoline. Here, the heavy cut is called Feed throughout this study. The Feed was prepared in three time occasions having somehow different characteristics and named as; Feed1, Feed2, and Feed3. The output of the Isomax process was also sampled and named as Product and its characteristics analyzed.

2.2. Soil sampling

The soil samples used for the experiments were collected from oil spill contaminated sites of different refineries of Iran including; Arak, Shiraz, Isfahan, and Abadan. A total number of 37 samples were taken and investigated in this research:15 soil samples from Arak refinery (AR1 to AR15), 13 soil samples from Shiraz refinery (SH101 to SH113), 5 soil samples from Isfahan Refinery (IS201 to IS205) and 4 soil samples from Abadan refinery (AB50 to AB53).

2.3. Culture medium

A mineral salt medium was used for cultivation of the consortia. The medium contained (g/L): K_2HPO_4 (2.75); KH_2PO_4 (2.25); $(NH_4)_2SO_4$ (1); $MgSO_4.7H_2O$ (0.2); NaCl (0.1); $FeCl_3.6H_2O$ (0.02); $CaCl_2.2H_2O$ (0.01). The pH was adjusted to 6.8. The medium was sterilized before addition of the substrate.

2.4. Oil-water separation

The oil phases were separated from aqueous phases by solvent extraction method using dichloromethane [28]. 15 ml solvent was added into a funnel decanter containing the whole sample and mixed thoroughly. After settlement the aqueous and organic phases were then separated. The procedure was repeated 2 more times on the aqueous phase. The separated organic phases were mixed and then dried by anhydrous $MgSO_4$. The solvents were evaporated in a rotary system leaving the oils which was then weighed. In the case of highly emulsified samples, 0.5-1 g NaCl was added to the mixture before extraction.

2.5. Column chromatography method

A column chromatography method was used to separate different hydrocarbon groups in oil samples including; saturates, aromatics and resins (SAR factions) [28]. A 30-cm length × 1-cm diameter silica gel column (100 to 200-mesh, activated overnight at 110 °C) was used in an elution basis with n-hexane, 40:60 (*v/v*) dichloromethane/hexane and methanol for separation of saturates, aromatics, and resins, respectively. The solvents were then evaporated and the obtained fractions were gravimetrically measured. It should be noted that the feed contained no asphaltenes which would be precipitated by adding n-haptane according to standard procedures [29].

2.6. Instrumental analysis

A GC-MS instrument Agilent Technologies 6890N equipped with a mass selective detector 5973 Network was used in order to identify the alteration of hydrocarbons of the biotreated oil samples. A capillary column HP-5 (30 m in length, 0.25-mm × 0.25-μm film) and Helium as the carrier gas were used for analyzing the samples. The temperature was programmed from 100 to 300 °C at 15 °C/min rate.

2.7. Batch culture enrichment

Here, the batch enrichment method was used to prepare native Consortia [30]. For this purpose, 4 g of each soil sample was added into a 250 ml flask containing 40 ml mineral medium and 4 ml of Feed1 (10% *v/v*) as the only carbon source. Two identical flasks were prepared for each sample. The flasks were kept on a shaker at 120 rpm and 30 °C for one week. At the end of cultivation time, 4 ml of the aqueous phase of each flask was transferred into newly prepared flask containing the mineral medium and the substrate and incubated at the same condition as the previous batch. The enrichment procedure was repeated two more times for the all samples. The aqueous phases of the last enrichment cultures were used as the source of the native consortia.

2.8. Examination of the native consortia on heavy oil cut

Triplicate batch cultures of the consortia obtained from the batch enrichment method (10% *v/v*) were grown in 250 ml flasks containing 40 ml of the medium and 8 ml of Feed1 (20% *v/v*). Incubation was performed at 30°C and 120 rpm for one week. Another flask was also prepared at the same condition without inoculation as Blank. After incubation, the oil phases were separated and analyzed for detecting any alterations using column chromatography method and GC-MS instrument. The consortia with greater influence on the oil phases were used for further investigations.

2.9. Examination of the consortia on low-resin oil cut

The consortia that had a greater impact on Feed1 were examined on Feed2, which contained less resin than Feed1. The redistributions and alteration of hydrocarbons were determined using column chromatography method and GC-MS instrument.

2.10. Examination of the consortia in high oil content

Here, some of the consortia were cultured in double amounts of the substrate i.e. 40% *v/v* of Feed3, in order to investigate the biological conversions at high amounts of oil. In other words, the ratio of feed to microorganisms (F/M) was doubled while the culture condition was the same as the experiments implemented on Feed1 and Feed2.

3. Results and discussion

3.1. Batch culture enrichment

From the enrichment procedures implemented on 37 soil samples, 16 samples were chosen as the proper consortia based on visual alterations of organic and aqueous phases. The unselected samples had almost no or minor changes of both phases. Figure 1 shows the visual characteristics of some of the selected samples after twice enrichment. The selected microbial samples were used for examination of heavy oil conversion.

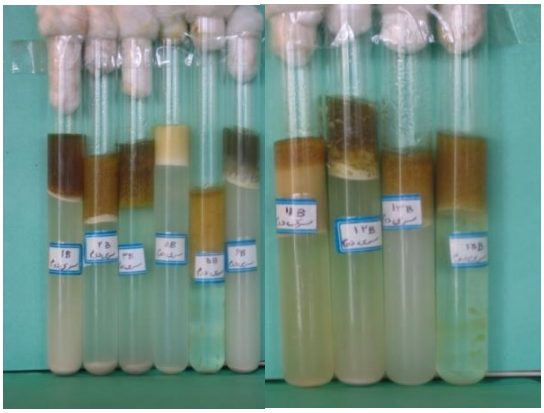

Fig. 1. Visual characteristics of the heavy cut after second enrichment of the soil samples, from left to right; AR1, AR2, AR3, AR4, AR5, AR6, AR11, AR12, AR13, and AR15 samples.

3.2. Examination of the native consortia on heavy oil cut

Table 1 presents the weight percentages of SAR fractions of the oils treated by the 16 prepared consortia. The analysis of the Blank and Product (output of Isomax unit) are also listed. The saturate fractions of most of the biotreated samples were increased compared to that of the blank (Table 1). They were increased from 35% in the blank up to 55-68% in samples AR3, AR5, SH101, SH109, SH110 and IS205, which is equivalent to 55-92% increase in the saturates with respect to the blank. Indeed, the resins in the biotreated samples were decreased from 38% in the blank to 10-17% in the above-mentioned samples meaning a 40-75% decrease with respect to the blank. The amounts of aromatics were also mainly decreased in these samples. However, for some of the samples; AR13,

SH105, IS202 and AB52, the amounts of saturates were decreased and conversely the resins were increased. This can be attributed to biopolymerization of the intermediate compounds making larger molecules which depends on the types of microorganisms and the culture conditions. This has been observed during biodegradation of alkanes where polyhydroxyalkanoates (PHAs) were produced [25,31,32].

Table 1. Redistribution of SAR fractions of Feed1 after treatment by the native consortia; obtained by column chromatography and gravimetric measurements.

Sample	extracted oil		Saturates		Aromatics		Resins	
	(g)	% Loss	% In sample	% Change related to Blank	% In sample	% Change related to Blank	% In sample	% Change related to Blank
AR1	3.86	<1	48.00	+35.75	25.00	-6.16	27.00	-28.95
AR3	3.11	16.20	56.00	+58.37	33.00	+23.87	11.00	-71.05
AR4	2.24	39.60	38.60	+9.16	30.10	+12.99	31.30	-17.63
AR5	3.40	8.30	55.00	+55.54	35.00	+31.38	10.00	-73.68
AR11	3.09	16.70	49.00	+38.57	18.80	-29.43	31.40	-17.37
AR13	3.24	12.70	21.30	-39.76	15.90	-40.32	62.00	+63.16
SH101	2.90	21.80	59.00	+66.86	21.30	-20.05	19.70	-48.16
SH102	2.94	20.70	40.20	+13.69	39.80	+49.40	20.00	-47.37
SH105	3.50	5.60	32.10	-9.22	13.60	-48.95	54.30	+42.89
SH109	3.19	14.00	55.50	+56.96	16.90	-36.56	27.60	-27.37
SH110	2.74	26.90	66.00	+86.50	17.00	-36.19	17.00	-55.26
SH111	2.86	22.90	37.70	+6.62	28.40	+6.61	33.80	-11.05
IS201	2.82	24.00	34.30	-3.00	27.90	+4.73	37.80	-0.53
IS202	3.10	19.70	33.20	-6.11	23.70	-11.04	43.10	+13.42
IS205	2.83	23.70	68.00	+92.31	22.00	-17.42	10.00	-73.68
AB52	3.71	<1	26.60	-24.77	17.64	-33.78	55.80	+46.84
Blank	3.71	-	35.36	-	26.64	-	38.00	-
Product	-	-	79.40	124.55	11.40	-57.21	9.20	-75.79

Figure 2 shows the GC-MS chromatograms of the saturate fractions of some of the biotreated samples, the blank and Product. The blank contains aliphatics from C17 to C35 while the majority of them lay in the range of C22 to C27. In most of the chromatograms of the biotreated samples, the heights of the large peaks (normal chain alkanes) are decreased compared to the intermediate peaks that belong to branched and cyclic compounds. In other words, it seems that the normal chain alkanes are decreased and branched and cyclic compounds are increased in which the extents depend on the consortia. This is more obvious in sample AR4 however; about 40% of the hydrocarbons were lost in this sample (Table 1). Therefore, it may be concluded that the variations in GC-MS chromatograms of the biotreated samples may be due to consumption by the microorganisms. However, the overall percent of saturates which are more easily assimilated by the bacteria are increased while resins are decreased compared to the blank (Table 1). The carbon losses are lesser in the other samples in comparison to AR4 and 1% in AR1 and AB52. Some unknown compounds were detected in the saturate fractions of biotreated samples. For example, 7 compounds were detected in retention times of 11.25-13.68 min and 8 compounds in retention times of 14.06-16.36 min in chromatogram of AR4 sample which did not exist in the feed. They could be branched alkanes. Due to complexity of the chromatograms it is quite difficult to detect the changes occurred on the biotreated samples. In order to investigate the variations more closely, the peak C24 was chosen

as an indicator and the ratios of the other sharp peaks' heights (n-alkanes) to height of peak C24 (in *wt*. %) were calculated. The variations of the peaks' ratios compared to that of the blank are indicated in Table 2 in which the ratios are variously decreased for most of the samples. This more clearly reveals the differences in the microorganisms' impact on the heavy oil cut. For example, in sample AR1, the ratios are gradually deceased from complete removal of C17 to about 66.5% reduction of C30/C24 compared to that of the blank. The values of C28/C24 to C30/C24 were decreased for almost all samples, more specifically for AR1, AR4, and AR5 for which reductions of about 60-80% were observed compared to the blank. This is fairly visible from the chromatograms as well (Figure 2). Examination of Product peak ratios for peaks larger than C25 (except C29) similarly shows reductions of about 15 to 80% compared to the blank. Therefore, it can be concluded that the variations occurred in the heavier compounds of the biotreated feed are consistent with that of the Product. However, the variations are not consistent for lighter compounds (less than C24) where they are sharply increased in the Product. Slight increase of C17 and C18 could only be observed for AR5, AR13, and IS205 compared to the blank.

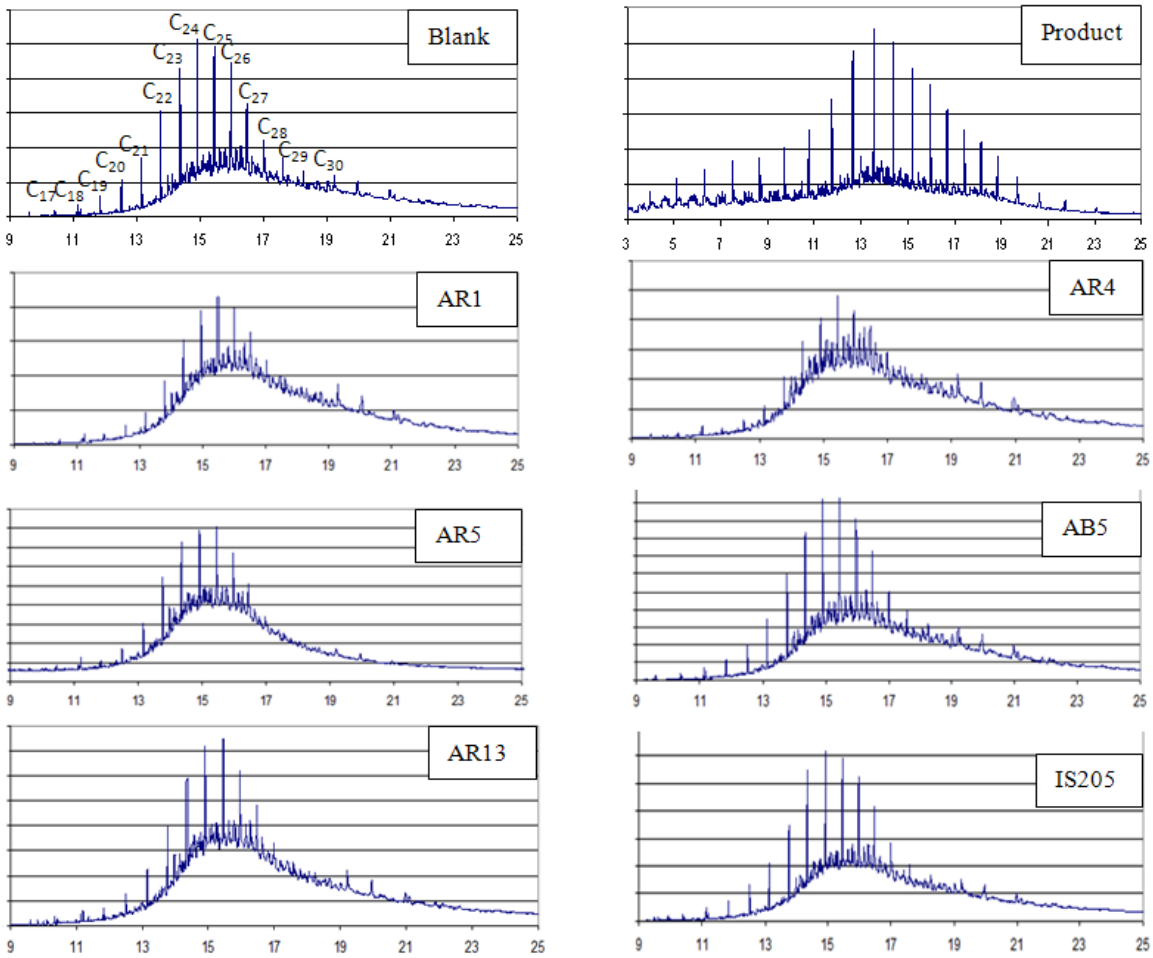

Fig. 2. The GC-MS chromatograms of saturate fractions of the blank and the heavy oil cut treated by different native consortia at 20% *v/v* Feed1.

Table 2. The percent change of C_n/C_{24} of the saturate fraction of biotreated samples related to that of Blank (Feed1), based on GC-MS results.

Consortia	$\frac{C_{17}}{C_{24}}$	$\frac{C_{18}}{C_{24}}$	$\frac{C_{19}}{C_{24}}$	$\frac{C_{20}}{C_{24}}$	$\frac{C_{21}}{C_{24}}$	$\frac{C_{22}}{C_{24}}$	$\frac{C_{23}}{C_{24}}$	$\frac{C_{25}}{C_{24}}$	$\frac{C_{26}}{C_{24}}$	$\frac{C_{27}}{C_{24}}$	$\frac{C_{28}}{C_{24}}$	$\frac{C_{29}}{C_{24}}$	$\frac{C_{30}}{C_{24}}$
AR1	-	-96.81	-95.87	-95.37	-92.43	-91.19	-89.98	-97.42	-90.42	-82.22	-82.75	-81.02	-66.54
AR4	-	-38.03	-79.70	-76.27	-77.13	-68.27	-64.05	-48.72	-26.46	-33.22	-60.15	-72.71	-69.44
AR5	+7.22	-49.20	-50.33	-40.71	-19.26	-4.82	-0.96	-4.21	-14.04	-13.26	-68.16	-82.47	-83.63
AR13	+30.93	-46.81	-49.50	-28.71	-21.24	-23.57	-16.99	-2.08	-6.32	-11.88	-24.97	-39.90	-67.23
IS205	-1.03	+10.37	-8.58	-12.8	-3.76	-1.87	+0.99	-10.94	+1.61	-6.35	-15.01	-24.09	-46.09
AB52	-43.30	-44.95	-43.23	-44.98	-47.15	-40.13	-35.87	-29.97	-27.46	-16.47	-24.74	-13.06	+15.15
Product	+1420	+702.9	+371.6	+293.7	+181.7	+92.31	+39.23	-15.99	-	-84.32	-31.91	+28.43	-17.44

Table 3. The amounts of hydrocarbons ranges (*wt*.%) in the saturate fractions of biotreated samples and Blank (Feed1), based on GC-MS results.

Samples	Hydrocarbon range			
	$<C_{15}$	C_{15} to C_{24}	C_{25} to C_{28}	$> C_{28}$
AR1	-	22.88	42.48	34.64
AR4	0.10	30.24	54.92	14.73
AR5	0.40	45.55	52.68	01.37
AR13	0.40	37.62	50.73	10.98
IS205	0.18	24.76	50.66	11.99
AB52	0.13	27.21	47.14	25.61
Blank	0.29	31.73	55.65	11.60

For further analysis, the total hydrocarbons of the saturate fractions were categorized into four ranges based on their carbon number as specified in Table 3. It can be seen that the compounds lighter than the C_{15} are decreased in most of the samples except for AR5 and AR13 for which a minor increase is observed. Also, the C15 to C_{24} in AR5 and AR13 samples are increased notably while the values are decreased for other samples. The amounts of C_{24} to C_{28} show a reduction in all samples compared to the blank. However, the compounds heavier than C_{28} are considerably increased in AR1 and AR52 samples which reveal the increase of the branched and cyclic compounds in this range while we know that the percent of n-alkanes are decreased in theses samples. Figure 3 shows the GC-MS chromatograms of the aromatic fractions of the biotreated samples, Blank and Product. Some variations can be observed between the samples and the blank. However, analysis of these chromatograms again is not an easy task due to their complexity and limitation of the GC-MS data bank. However, presence of nitrogen and sulfur containing compounds was detected in the biotreated samples. The initial peaks of the chromatograms up to 4 min which mostly contained xylene, o-xylen, and saturated naphthalene such as 1, 2 dimethyldecahydronaphtalens are increased in some samples including AR4, AR13, and AR52 whilst decreased in AR1 and IS205. Other peaks show an increase or decrease in comparison to the blank. The variations are more observable in IS205 chromatogram. Indeed, some alkene compounds were detected mainly in the aromatic fractions of all samples. For examples; 9-octadecene, 1-tetradecene, 1-hexadecene, 1-heptadecene, 1-octadecene were observed at 5.50, 7.72, 9.53, 11.08, 12.24 min respectively in the aromatic fraction of AR4 biotreated sample. At retention times of 11.06, 11.80, 11.94, 12.08 and 12.58 min some thiophenic compounds were appeared and at retention times of 13.24, 13.56, 14.22, 14.09 and 14.70 min and some N-compound were appeared in the biotreated sample which did not exist in the similar fraction of the feed. However, the variations of the Product are much greater related to the blank. The results of this section revealed that most of the consortia had evident impacts on the Isomax feed. Therefore, some of the consortia were used further examinations.

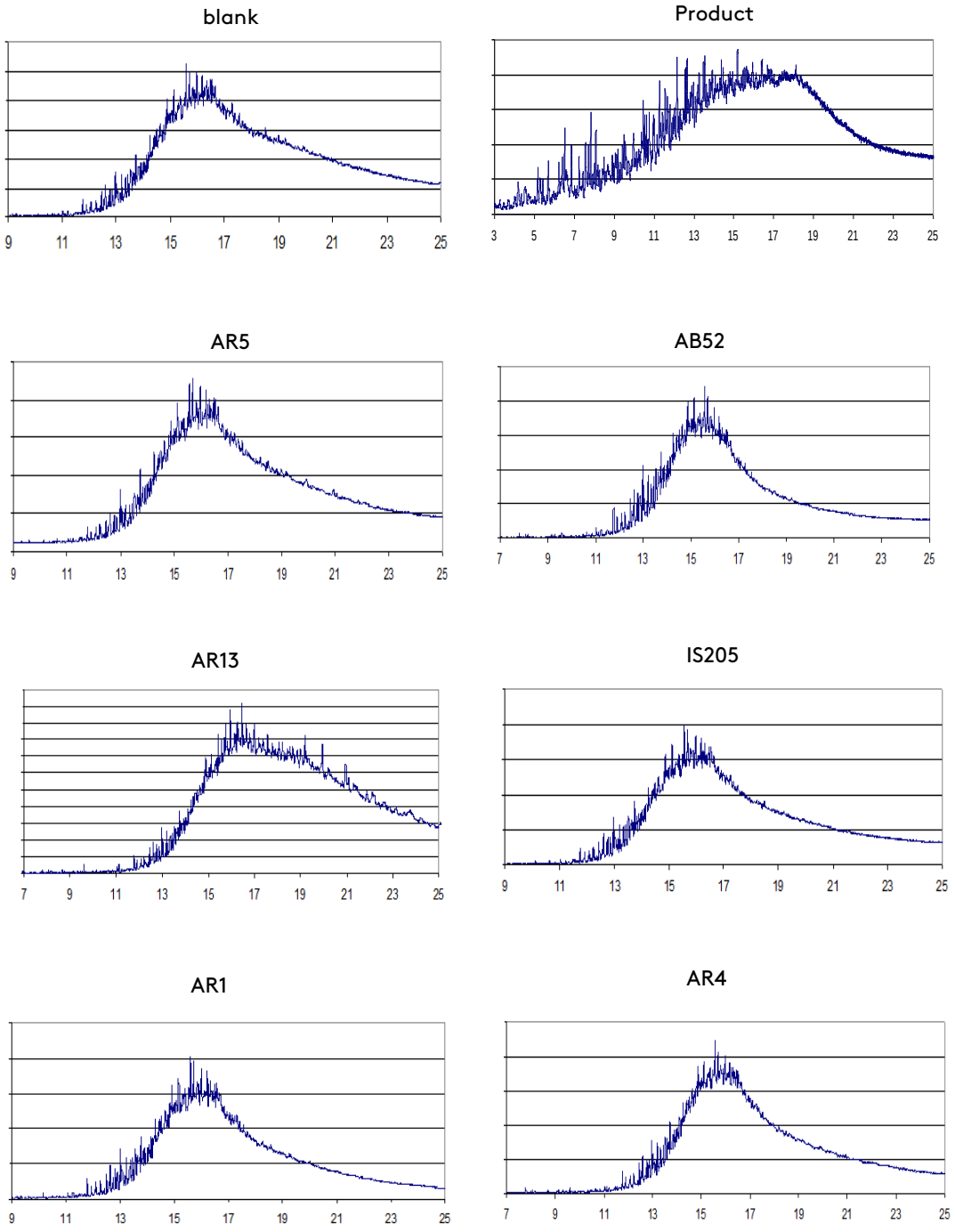

Fig. 3. The GC-MS chromatograms of aromatic fractions of the blank, product and the heavy oil cut treated by different native consortia at 20% *v/v* Feed1.

3.3. Examination of the native consortia on low-resin oil

Here, some of the consortia including; AR3, AR4, AR52, SH101, SH112 were subjected to Feed2 which contained less amounts of resins and higher amounts of saturates compared to Feed1. In fact, the feed used here was lighter than the feed used in the previous section. Table 4 shows the weight percent of SAR fractions of the samples after incubation. Comparing these results with those of Table 2 indicate that when the feed is poorer in resins, less redistribution of the oil components is

occurred after biotreatment which mainly leads to less increase of the saturate fractions.

Table 4. Redistribution of SAR fractions of Feed2 after treatment by some of the native consortia; obtained by column chromatography and gravimetric measurements.

Samples	Saturates		Aromatics		Resins	
	% in sample	% change related to Blank	% in sample	% change related to Blank	% in sample	% change related to Blank
AR3	63.80	+28.89	24.60	+14.95	11.60	-60.14
AR4	57.00	+15.15	17.80	-16.82	25.20	-13.40
AB52	58.20	+17.58	23.90	+11.68	17.90	-38.49
SH101	51.80	+04.65	28.90	+35.05	19.30	-33.68
SH112	67.80	+36.97	22.50	+05.14	09.70	-66.67
Blank	49.50	-	21.40	-	29.10	-

3.4. Examination of the native consortia in high oil content

Table 5 shows the weight percent of SAR fractions of Feed3 after treatment by the selected consortia (AR4, AR13, AB52, SH101, SH110, SH112, IS205) at 40% (*v/v*) feed. There is again an increase in saturate fractions and a decrease in the resin contents of most of the samples compared to the blank. The aromatic fractions of the samples are also decreased compared to the blank, except for SH101 in which the amounts of saturates also decreased inversely. In Table 6, the saturate fractions are categorized in four ranges based on GC-MS results implemented on some of the samples and their weight percent are mentioned. It can be seen that the amounts of hydrocarbons lighter than C28 are decreased and the amounts of hydrocarbons heavier than C_{28} are increased in the all samples compared to the blank except for SH110. In SH110 the total amounts of compounds lighter than C_{25} are increased in comparison to the blank and the heavier ones are decreased. This may introduce consortia SH110 as a proper one in converting heavy saturates.

Table 5. Redistribution of SAR fractions of Feed3 treated by some of the native consortia; obtained by column chromatography and gravimetric measurements.

Samples	Saturates		Aromatics		Resins	
	%in sample	% change related to blank	%in sample	% change related to blank	%in sample	% change related to blank
AR4	79.80	+16.50	19.30	-30.82	2.50	-30.56
AR13	67.90	-0.88	26.20	-6.09	2.60	-27.78
AB52	70.80	+3.36	26.30	-5.73	1.50	-58.33
SH101	56.20	-17.96	38.00	+36.20	3.30	-8.33
SH110	75.00	+9.49	21.50	-22.94	2.10	-41.67
SH112	71.80	+4.82	21.40	-23.30	3.60	0.00
IS205	76.30	+11.39	21.00	-24.73	3.30	-8.33
Feed3	68.50	-	27.90	-	3.60	-

The results indicate that the variations of fractions in the biotreated oils are less in comparison to the previous experiments (sections 3.2 & 3.3). It should be noted that although the amount of substrate is doubled here, but the feed is even lighter in terms of the resin content compared to Feed1. As it was concluded in section 3.3, the less resin content of the feed, the less compositional changes in the SAR

fractions would be obtained after biotreatment. The results here again show that the microorganisms utilize lighter alkanes than the heavier ones in less availability of resins. This is also the case for aromatics which they are mainly decreased. Also, there could be here some toxicity effects of high concentrations of hydrocarbons on the microorganisms which were unknown.

The GC-MS chromatograms of the saturate and aromatic fractions of the samples are shown in Figures 4 and 5, respectively. Figure 4 shows that the end tails of most of the chromatograms are larger compared with the blank, as was also perceived in Table 6 for alkanes larger than C_{28}. The chromatograms of the aromatic fractions of the samples also show some variations compared to the blank, however, it is not certainly as high as the Product which was undergone a thermal catalytic process. The presence of some S-containing compound and N-containing compounds were detected in aromatic fractions here as well which did not exist in the feed. These could have been obtained from cleavage of resins through biochemical reactions.

Table 6. The amounts of hydrocarbons ranges (*wt.*%) in the saturate fractions of the biotreated samples and the Blank (Feed3); based on GC-MS results.

Samples	Hydrocarbon range			
	$<C_{15}$	C_{15} to C_{24}	C_{25} to C_{28}	$> C_{28}$
AR4	-	35.49	42.13	22.38
AB52	0	29.86	34.55	35.60
SH110	2.75	63.98	25.94	7.53
SH112	-	47.87	40.95	11.18
IS205	0	47.46	36.27	16.27
Blank	-	52.45	39.46	8.10

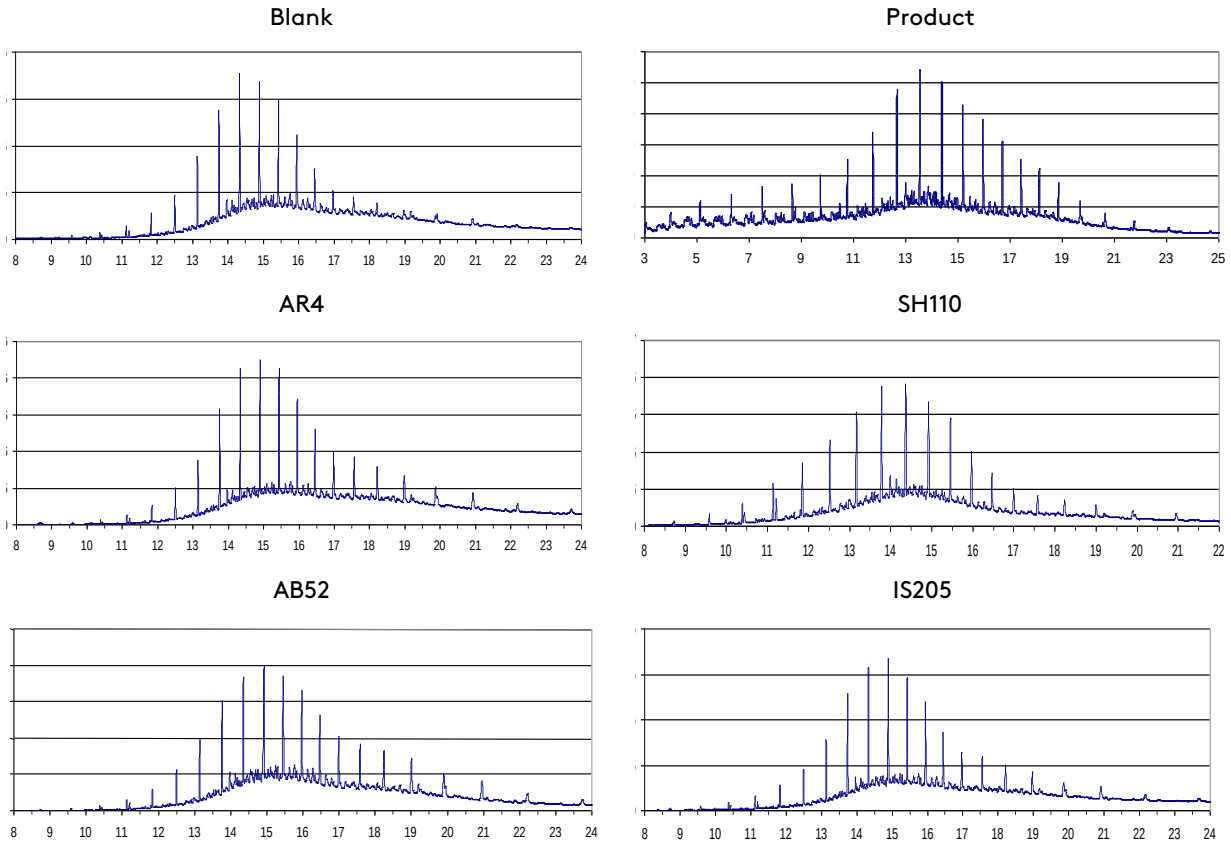

Fig .4. The GC-MS chromatograms of saturate fractions of the blank, Product and the heavy oil cut treated by different native consortia at 40% *v/v* Feed3.

The results of these experiments can be compared with what obtained by Premuzic et al. (1999) using mixed culture of selected strains for heavy crude oils upgrading [20]. They concluded that asphaltene and the associated polar fractions (resins) are involved in the biochemical reactions in

which redistribution of hydrocarbons occurs. A closer look at their results shows less decrease in asphaltenes and larger decrease in the resins compared to the blanks. Ghollami et al. also biologically treated a heavy bottom end of vacuum distillation tower and found that major alterations happened in resin fractions with minor or no changes in the amounts of asphaltenes [23]. They differentiated the resins into light and heavy resins in which the heavy resins were totally removed after biotreatment. It is generally accepted that biodegradation of hydrocarbons occurs in the following descending order; short-chain alkanes, long chain alkanes, iso-alkanes, cyclo-alkanes, aromatics, hetrocyclics, resins and asphaltenes [15]. This biodegradation priority of hydrocarbons is mainly observed during bioremediation processes in which heavy hydrocarbons are more absorbed to the soil and thus less available to the bacteria. But, in the experiments here on bioprocessing of hydrocarbons, heavy hydrocarbons were more seriously affected by the microorganisms. There were no asphaltenes in the hydrocarbon feeds utilized here and it seems that resins are the major constituents taking part in the bioconversion reactions. Certainly, there are several biological, chemical and physical factors affecting the bioconversion of hydrocarbons. In the liquid phase, the resins and large hydrocarbons which are not soluble in water are first emulsified by the bacteria thus providing a large surface area for the bacteria. While, the bacteria tend to stick to surfaces [33], resins and large molecules would be appropriate to adhere which in turn may results in greater conversion of resins compared to what would be observed in bioremediation processes regardless of the bacterial species. The experiments here showed that when the amounts of resins were the lowest, the aromatic fractions were then mostly converted by the bacteria. This could be due to the fact that aromatics especially those containing hetroatoms have more active sites than n-alkanes and other saturates. Settai et al. (1992) reported that the presence of n-alkanes enhances the biodegradation of aromatic sulfur compounds [34]. While, solubility is negligible for alkanes larger than C_9 in terms of their uptake by the microorganisms [35], the bacteria use other strategies such as biosurfactant production or producing a highly hydrophobic cell surface to enhance its attachment [36]. Merdinger and Merdinger (1970) found that alkanes with higher number of carbons are better assimilated than those with lower number of carbons where they examined C_6 to C_{18} [37]. During chemical catalytic reactions, the larger molecules may have more chances in reactions compared with smaller ones. This may be also the case for large hydrocarbons during biological reactions. However, it may not be true for short chain hydrocarbons that could be easily available and adsorbed by the bacteria. This could be perhaps the reason why short chain hydrocarbons such as C_{12} are mainly depleted after biotreatment. Since, the content of these small chain hydrocarbons is small in the heavy cut; the bacteria would impose more impact on larger hydrocarbons and probably more on resins due to larger surface area. In absence of resins, the large aromatics and alkanes would be more appropriate for the bacteria to utilize. On the other hand, the resin fractions were increased in a few biotreated samples, which can be attributed to the biopolymerization of the intermediate compounds produced from biological conversions of hydrocarbons. This was also observed elsewhere during bioconversion of long-chain alkanes by the consortia where polyhydroxyalanoates were produced [25]. More precise investigations are needed in order to understand the situations and mechanisms by which the polymerization might be occurred during bioconversion of a heavy cut.

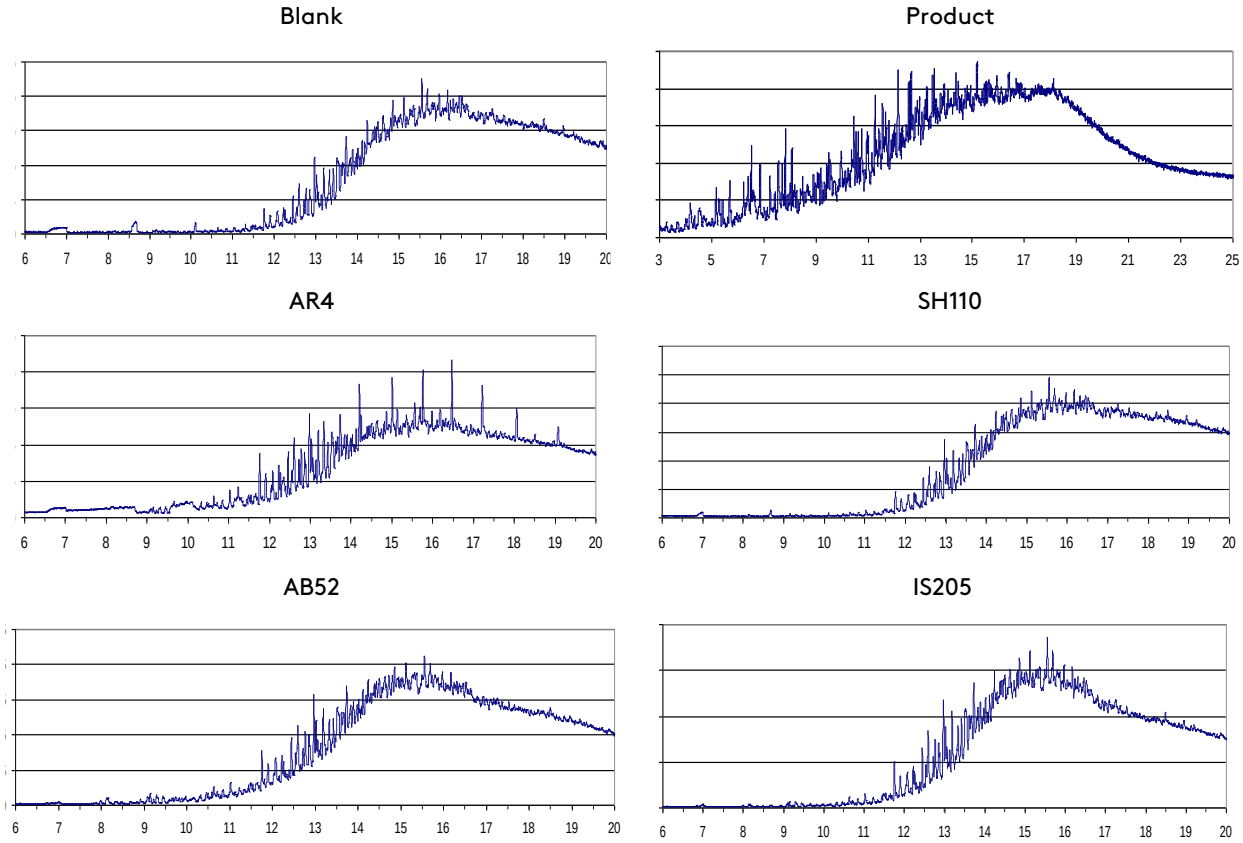

Fig. 5. The GC-MS chromatograms of aromatic fractions of the blank, product and the heavy oil cut treated by different native consortia at 40% *v/v* Feed3.

4. Conclusions

This paper described the compositional changes of a heavy hydrocarbon cut (ISOMAX feed) under biological treatment using sixteen different native consortia. The feed contained resins but no asphaltenes. The consortia imposed different impacts on the feed but more or less in a similar manner. Most of them increased the saturate fractions of the biotreated feeds and reduced the resin fractions at the same time. These are positive changes for a heavy cut going to downstream refining processes which improve the calorific value of the feed. The GC-MS analysis of the biotreated fractions compared to that of the Blank showed an increase in branched and cyclic compounds as well as some unsaturated alkanes. All these compounds also increase the energetic value of the feed and increase the octane number. However, some lighter compounds are removed after biotreatment which can be attributed to their assimilation by the microorganisms meaning the reduction of the oil calorific value; but the carbon losses are not identical in all samples. It is as high as 40% in total carbon loss for AR4 sample and as low as 1% for AR1 and AB52 samples. It should also be noted that a much stabilized emulsion was created after biotreatment of the feed in sample AR4, which imposed a harder oil water separation. These experiments revealed that biological treatment of heavy hydrocarbon oils as a pretreatment stage can bring some advantageous to the downstream refining processes. It increases the calorific value of the feed by sharply decreasing resins, increasing saturates while branched and cyclic compounds are increased. It seems that these conversions are directly related to destruction of resins and long chain hydrocarbons by bacterial consortium.

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