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Original Paper

Rapid accuracy determining DNA purity and concentration in heavy oils by spectrophotometry methods

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ABSTRACT

DNA analysis is the core of biotechnology applied in petroleum resources and engineering. Traditionally accurate determination of DNA purity and concentration by spectrometer is the first and critical step for downstream molecular biology research. In this study, three different spectrophotometry methods, BPM, NDTT and NPMTTZ were compared for their performance in determining DNA concentration and purity in 32 oil samples, and molecule methods like quantitative real-time PCR (qPCR) and high-throughput sequence were also performed to help assess the accuracy of the three methods in determining DNA concentration and purity. For ordinary heavy oil (OHO), extra heavy oil (EHO) and super heavy oil (SHO), the characteristics of high viscosity (η), density (ρ) and resin plus asphaltene content will affect the DNA extraction and UV determination. The DNA concentration was decreased as density increased: OHO (11.46 ± 18.34 ng/μL), EHO (6.68 ± 9.67 ng/μL) and SHO (6.20 ± 7.83 ng/μL), and the DNA purity was on the reverse: OHO (1.31 ± 0.27), EHO (1.54 ± 0.20), and SHO (1.83 ± 0.32). The results suggest that spectrophotometry such as BPM and NPMTTZ are qualitatively favorite methods as the quick non-consumable methods in determining DNA concentration and purity of medium oil and heavy oil.

1. Introduction

Most molecular biology research is dependent on DNA extraction from environmental samples. Accurate determination of DNA concentration and purity is first important and fundamental for downstream research (Wan and Dong, 2014; Wan et al., 2017a, b) especially to remove biased PCR and related analyses (Huseyin et al., 2017; Ling et al., 2015). As a classical method, especially because of its non-consumable property, UV (ultraviolet visible) spectrophotometry is a most convenient, fastest and widest used method for DNA quality determination. BPM (Biophotometer), NDTT (Nanodrop 33), and NPMTTZ (Nanophotometer 330) are such methods and are widely used in many areas of molecular biology research such as studies of human intestine (Qin et al., 2010), animal tissues (Sola et al., 2014), soils (Kwak et al., 2012), water (Jiang et al., 2014; Akhil et al., 2010) and material microbiomes (Ettenauer et al., 2012). To date, several studies (Mu et al., 2021; Wan et al., 2021; Tian et al., 2020) have mentioned the applications of these spectrophotometer methods in determining DNA content in oil reservoir samples from different oilfields (Cluff et al., 2014; Nyyssonen et al., 2014; Li et al., 2016). However, no studies have analyzed trace and ultratrace DNA in crude oils (Li et al., 2016), and especially for heavy oil samples, which are extremely complex (Wan and Du, 2017; Du et al., 2011a) and often contain formation water and insoluble-particles (Mu et al., 2021). Heavy oil is usually composed of alkanes, cycloalkanes and various aromatic hydrocarbons, as well as other heteroatom organic compounds including nitrogen, oxygen and sulfur, and trace amounts of metals such as iron, nickel, copper, and vanadium (Wan and Du, 2017; Du et al., 2011a, b). Furthermore, human activity has also introduced more complexity into oil reservoirs, the use of various exploitation and development methods is bound to disturbance oil reservoirs, the properties of heavy oil, composition of formation water and endo-/

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exogenous microbial communities will change accordingly (Wan et al., 2021; Tian et al., 2020; Wan and Du, 2017).

We previously reported some preliminary results on DNA concentration measurement in oil samples by UV spectrophotometry (Li et al., 2016; Mu et al., 2014; Wan et al., 2017a, b); to date there is no report on the relationship between accuracy of DNA concentration determination and heavy oil properties. In this study we compared the performance of three different spectrophotometry methods, BPM, NDTT and NPMTTZ, in determining the concentration and purity of trace and ultratrace amounts of DNA extracted from 32 heavy oil samples, and discussed the relationship between oil sample characteristics and the accuracy of DNA concentration and purity determination.

2. Materials and methods

2.1. Sampling

Liaohe Oilfield is the largest heavy oilfield in China (Zhu et al., 2010; Huang et al., 2004). A total of 32 samples of produced fluids (oil-water mixtures) were collected at various wellheads of the Liaohe Oilfield where wells were enhanced by different development methods, including water flooding (WF), chemical flooding (CF), steam soak (SS), steam flooding (SF) and steam-assisted gravity drainage (SAGD) (Table 1). The data of depth, steam chamber temperature (T), density (ρ), viscosity (η), and the percentage content of resin plus asphaltene obtained from oilfield (Table S1). Two 500 mL replicates were collected from the wellhead of each production well with sterile brown sampling glass bottles. The collected samples were kept on ice during transportation to the laboratory within 3 days, where samples were prepared within two days by isolating oil and water layers (Wan and Dong, 2014). After the separation, the oil samples (30 heavy oils plus 2 medium oils as controls) were used in the subsequent analyses.

2.2. Physiochemical analyses

The pH was determined by the SevenMulti™ functional measuring instrument (Mettler Toledo, S40N, Switzerland). SO_4^{2-}concentrations were determined by using ion chromatography (Dionex, DX-120, USA) as previously described (Wan and Dong, 2014). Analysis of moisture content (MC) was performed using crude petroleum-determination of water-distillation method (GB/T 8929-2006) (Bo and Li, 2006).

2.3. DNA extraction

32 oil samples (Table 1) were pre-treated using the methods (Yoshida et al., 2005) with some modifications (Tian et al., 2020; Wan et al., 2017a, 2018). Each oil sample (100 g) was washed with 2,2,4-trimethylpentane (100 mL), thoroughly mixed with some glass beads (Diameter 3–4 mm) by vortex, and followed by centrifugation (7870×g, 20 min at 4 °C) (Wan et al., 2017a, b). The resulting supernatant was discarded and the precipitate was retained. DNA was extracted from the precipitate using the QIAamp DNA Stool Mini Kit following manufacturer's instructions (Qiagen Inc., Valencia, CA, USA). Taking 500 mg of the precipitate, after the process of dissolution, binding and washing, the DNA was adsorbed on QIAamp membrane, finally added 100 μL of sterile ultrapure water to wash the DNA and determine the concentration of the DNA solution. All 32 extracted DNA were stored in −20 °C refrigerator for next use (Wan and Dong, 2014).

2.4. DNA concentration measurement

PCR and electrophoresis for DNA. The bacterial V3 variable region of the 16S rRNA gene was amplified with the primer GC-341F (5′-GCCTACGGGAGGCAGCAG-3′) and 518R (5′-ATTACCGCGGCTGCTGG-3′) (Muyzer et al., 1993). The PCR amplification system was followed the Bio-Rad kit instruction with some modification, reaction system contained the following: 10×i*Taq*™ buffer 5 μL, 10 mM dNTP 1 μL, i*Taq*™ DNA polymerase (5 U/μL), 0.3 μL and 0.5 μL each of the forward and reverse primer, 4 μL DNA template and 38.7 μL H_2O. PCR conditions were performed as Nazina et al. (2013) with modification: activation of polymerase (7 min at 94 °C); 35 cycles of DNA denaturation (1 min at 94 °C), primer annealing (1 min at 54 °C), chain elongation (1 min at 72 °C); and final chain elongation (10 min at 72 °C). The presence of amplified PCR products and total DNA were confirmed by 1.2% (w/v) and 1% (w/v) agarose gel, respectively. Electrophoresis was performed at a constant voltage of 100 V and a time of 50 min.

UV spectrophotometry for concentration and purity of DNA. The

Table 1
Geological information of the collected samples.

No.	Sample	Exploitation method[a]	Classification[b]	No.	Sample	Exploitation method[a]	Classification[b]
1	L1YJC23	SS	OHO	17	L2YSD10	SAGD	EHO
2	L1YJCK29	SF	OHO	18	L2YSD11	SAGD	EHO
3	L1YJC226	WF	OHO	19	L2YSD12	SAGD	EHO
4	L1YJC317	CF	OHO	20	L2YSD14	SAGD	EHO
5	L1YJC2105	WF	OHO	21	L2YSD15	SAGD	EHO
6	L1YJC2416	WF	OHO	22	L2YSD16	SAGD	EHO
7	L1YJC2417	CF	OHO	23	L2YSD17	SAGD	EHO
8	L1YHC02	SS	OHO	24	L2YSD41	SAGD	SHO
9	L1YHC04	SS	OHO	25	L2YSD42	SAGD	SHO
10	L1YHC06	SS	OHO	26	L2YSD44	SAGD	SHO
11	L1YHCH7	SF	OHO	27	L2YSD47	SAGD	SHO
12	L1YHC23	SF	OHO	28	L2YSD48	SAGD	SHO
13	L1YHC024	SF	OHO	29	L2YSD281	SAGD	EHO
14	L1YHC241	SF	OHO	30	L2YSD291	SAGD	EHO
15	L1YHC303	CF	Medium oil	31	L2YSDH3001	SS	EHO
16	L1YHC3104	CF	Medium oil	32	L2YSDH3003	SS	EHO

[a] WF, water flooding; CF, chemical flooding; SS, steam soak; SF, steam flooding; SAGD, steam assisted gravity drainage.
[b] Classification of heavy oils was based on the People's Republic of China petroleum and natural gas industry standard (SY/T 6169) (Oil and Gas Field Development Professional Standardization Committee of Petroleum Industry Standardization Technical Committee, 2022). OHO: Ordinary heavy oil, viscosity is 50–10000 mPa·s and relative ρ is 0.9200–0.9500 g·cm^{-3} (20 °C); EHO: Extra heavy oil, viscosity is 10000–50000 mPa·s and relative ρ is 0.9500–0.9800 g·cm^{-3} (20 °C); SHO: Super heavy oil, viscosity >50000 mPa·s and relative ρ > 0.9800 g·cm^{-3} (20 °C). Classification of crude oils was based on the international common method: Light oil, °API ≥32 ($d_{15.6}^{15.6} \leq 0.8654$); Medium oil, °API is 20–30 ($d_{15.6}^{15.6}$ is 0.8654–0.9340); Heavy oil, °API is 10–20 ($d_{15.6}^{15.6}$ is 0.9340–1.0000).

concentrations of all 32 DNA extracted from aforementioned crude oils samples (Table 1) were determined using commercially available spectrophotometric methods including BPM, NDTT and/or NPMTTZ according to the manufacturers' instructions. The volume of DNA used for NDTT and NPMTTZ methods in this study were both 1 μL and BPM was 50 μL of DNA sample (Table S2). The DNA solution of each group of parallel samples was sampled and determined three times, and the average and standard deviation were calculated. We compared the dilution factor, optical path, reliable range of DNA concentration, and minimum volume of three spectrophotometer (Table S2).

qPCR was also used to independently verify DNA concentrations, which were conducted in polypropylene 96-well plates on Bio-Rad®CFX96™. Gradient dilution of a standard plasmid was performed by using 7.9×10^3–7.9×10^8 copies/mL concentration range of plasmid DNA as a template for qPCR reaction. Two replicates were performed for each concentration of plasmid DNA and sample. Bacterial primer pair 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 518R (5′-ATTACCGCGGCTGCTGG-3′) were used described in previous research (Mu et al., 2021; Fierer et al., 2005). 20 μL reaction contained the following: 10 μL i*Taq*™ Universal SYBR® Green Supermix, 1 μL each of the forward and reverse primer, 6 μL H_2O, and 2 μL DNA template. qPCR amplification conditions were followed the instruction of i*Taq*™ Universal SYBR Green Supermix (Bio-Rad). According to the determination results, the CFX Bio-Rad manager software was used to establish the related series curves.

16S rRNA gene amplification, Illumina sequence and analyses were same as we previous reported (Mu et al., 2021; Tian et al., 2020).

The arithmetic mean and standard deviation of each detection were used for the calculation of DNA concentration and purity.

2.5. Analysis of sequence data

Multivariate analysis was conducted using detrended correspondence analysis (DCA) and redundancy analysis (RDA) plots generated by Canoco 5.15 (http://www.microcomputerpower.com/) to demonstrate the relationship between the results of DNA determination by different spectrophotometric methods and their correlation with reservoir formation and crude oil characteristics, respectively.

3. Results and discussion

3.1. Gel electrophoresis analysis

L2YSD10, L2YSD11 and L2YSD42 were determined that were visible bands in the agarose gel after electrophoresis (Fig. S1(a)). While, there were no visible total DNA bands (Fig. S1(b)), indicating that DNA was successfully extracted, but the DNA content was too low in heavy oil to meet the resolution of gel electrophoresis detection.

3.2. DNA concentration

BPM requires 50 μL of DNA sample (Table S2), which is about half of the total elution volume obtained from a typical commercial DNA extraction kit. For the 32 oil samples (Fig. 1), the determination range of DNA concentrations (0.9±0.1–11.0±0.9 ng/μL) was mostly between 3.0 and 6.0 ng/μL, which was close to the mean value (4.7±2.3 ng/μL). Among the determination results of BPM were more stable than that of NDTT and NPMTTZ. BPM can serve as a stable method for DNA content determination. The disadvantage of BPM is too higher in the amount of the required sample than the others, which would substantially increase the cost and environmental pollution potential for collecting enough DNA from low biomass oil samples. For some samples from extreme environments such as heavy oils, the amount of sample is normally very low.

The DNA concentrations in the 32 oil samples determined by NDTT ranged from 0.00±0.01–5.10±1.00 ng/μL (Fig. 1(a)), and most of the DNA concentrations in these samples were below the reliable range of DNA concentration by this method (e.g., 2.00 ng/μL) (Table S2). DNA in oil samples L1YJC2417, L1YHC3104, L2YSD10, L2YSD17, L2YSD47, L2YSD48 and L2YSDH3003 could not be detected by NDTT, and the measured DNA concentrations in L1YHC04, L1YHC23, L1YHC024, L2YSD44 and L2YSD291 were extremely low, only with 0.06±0.01, 0.13±0.03, 1.10±0.20, 0.60±0.12, and 0.06±0.01 ng/μL, respectively (Fig. 1(a)). NDTT can form the surface tension by column (Riepl et al., 2011; Boesenberg-Smith et al., 2012), and inorganic salts and organic compounds in heavy oil samples can impact surface tension and therefore may affect the readings. On the contrary, DNA concentration in the aforementioned five samples (L1YHC04, L1YHC23, L1YHC024, L2YSD44 and L2YSD291) determined by NPMTTZ was 16.50±3.30, 71.00±9.00, 29.50±5.00, 21.50±4.00, and 37.00±7.00 ng/μL (Fig. 1(a)), respectively, which was much higher than the determination of NDTT. NPMTTZ uses a sample compression technology, without dependence on the sample's surface tension, which can reduce experimental error from sample column cracking and can ensure a high accuracy. The NPMTTZ consists of five different ultratrace fibre cuvette lids that give a promise to the right optical path for diluting the sample automatically (Riepl et al., 2011), and correspondingly, that can avoid the dilution error and ensure good repeatability of the results.

Column scatterplot (Fig. 1(c)) revealed that the range of DNA concentrations determined by the three different methods. It seems that BPM and NDTT have more concentration points falling in the narrow range (0.90–7.00 ng/μL) and NPMTTZ has a broader detection range (0.90–75 ng/μL). All of the above three methods can be used for rapid detection of trace and ultratrace DNA in crude oil including heavy oils.

qPCR was used to verify the results to estimate the abundance of total/functional bacteria and archaea.

The linearity of the standard curve and amplification effect were all good. Different heavy oil samples had different DNA melting temperature ranges in qPCR experiments, 83.5–84.5 °C for OHO and 84–85 °C for EHO and SHO (Fig. S2(a) and (b)), reflecting a difference in total DNA between different heavy oil samples and further proved that thermophiles exist in heavy oil reservoirs with higher temperature (Tian et al., 2020). The Cq (quantification cycle) values were in the range of 19.03±0.10–33.20±0.94 (Fig. 2(a)). Although gel electrophoresis showed weak or no bands, qPCR combined with microbial bottle test (Wan et al., 2020) results showed that bacterial abundance was in the range of 1203.38±24.57–1.87×10^7±1.37×10^6 copies/mL (Fig. 2(b)) which is similar to our previous report (Tian et al., 2020), indicating that the heavy oil samples contained broad range of DNA.

The results of detrended correspondence analysis (DCA) showed that there was no strong positive correlation between the results of qPCR and those of three spectrophotometers, but the relationship between qPCR and NPMTTZ, BPM was closer than NDTT (Fig. 3). The DNA concentrations in (L1YHC3104) were 10.50±2.10 ng/μL and 11.00 ng/μL as determined by NPMTTZ and BPM, respectively, which was confirmed by qPCR but not detected by NDTT (Fig. 1(a)).

High Cq and low Sq (starting quantity) values in qPCR analyses for all SHO and some EHO indicated low DNA concentrations in these samples, generally matching the properties of heavy oils (e.g., SD11, SD12, SD47 and SD48, Table 1). The results of each method may be different due to the influence of these properties. The DNA

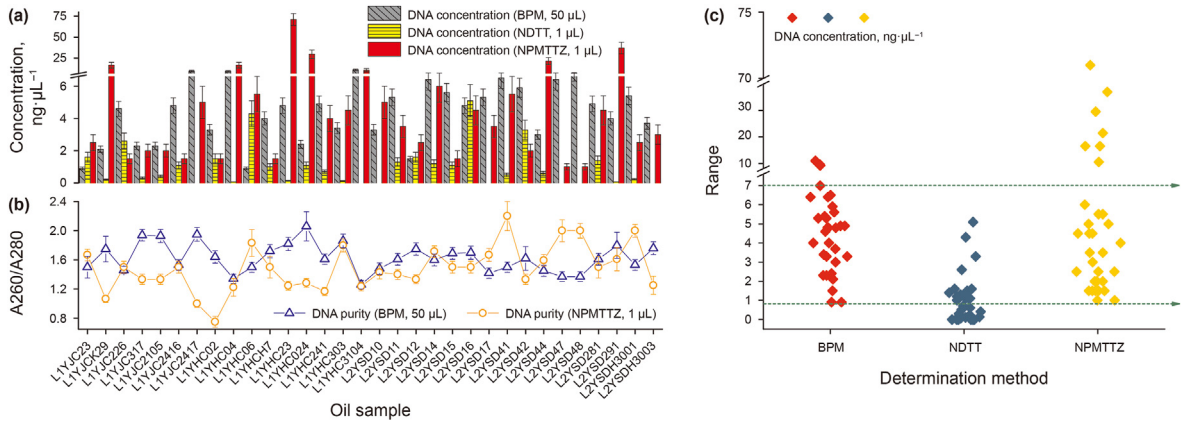

Fig. 1. DNA concentration in 32 oil samples. (**a**) DNA concentration determined by three spectrophotometric methods. (**b**) DNA purity determined by two spectrophotometric methods. The connect line among sample data is only for the tendency comparison. The error rod represents the standard deviation of the average value of DNA determination for three groups of parallel DNA samples. (**c**) Column scatterplot of DNA concentration of 32 oil samples determined by three spectrometers. The green dotted line represents the distribution concentrated area.

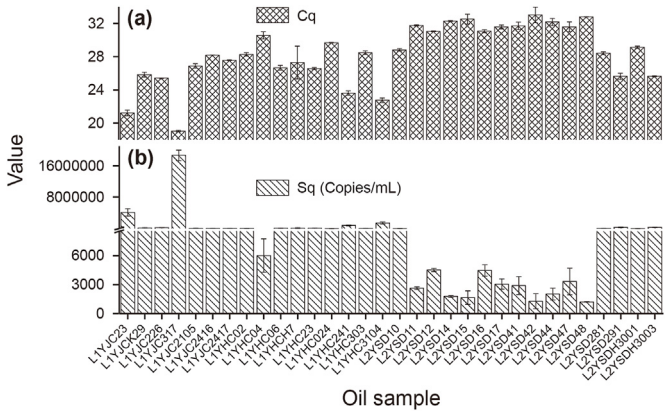

Fig. 2. Determination of DNA concentration by qPCR in 32 oil samples. (**a**) Cq value; (**b**) Sq value.

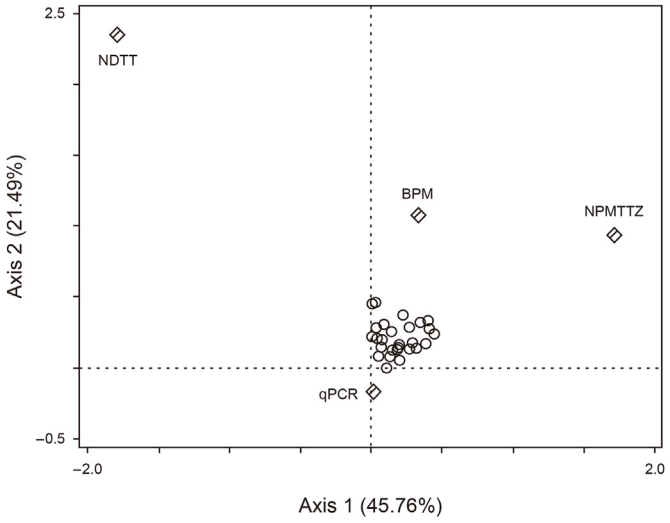

Fig. 3. DCA ordination plot. Black diamonds (back-diagonal hatch) represent the different DNA concentration determination methods, empty black circles represent the samples.

concentration in SHO sample L2YSD44 as determined by NPMTTZ was high (21.50±4.00 ng/μL), but low by qPCR (2002.65±606.54 copies/mL). The DNA concentrations of EHO L2YSD17 and SHO L2YSD47 and L2YSD48 could not be detected or low by both NDTT and qPCR, but their concentration was high (5.30±0.40, 6.40±0.45 and 7.00±0.92 ng/μL) by BPM.

3.3. DNA purity

The absorbance of nucleic acid sample at 260 nm is higher than that at 280 nm, while that of protein sample is the opposite. The sample containing protein and DNA mixture will certainly be affected by these two macromolecules. The ratio of A260/A280 can be calculated to be 1.80 by using the extinction coefficient of standard nucleic acid solution (Glasel, 1995). The assessment of the purity of DNA is referred to as the A260/A280 ratio, we determined this ratio for the 32 oil samples using the BPM and NPMTTZ methods. The mean ratios determined by BPM and NPMTTZ were 1.63±0.19 and 1.48±0.31, respectively. Several samples showed similar ratios by the two analyzers (Fig. 1(b)). Most of the A260/A280 ratios of BPM method were around 1.8. These ratios were lower than ideal values required for 1.80 to 2.00 (Glasel, 1995), possibly because of contamination by protein or phenolic compounds in heavy oils (Zhou et al., 1996). The A260/A280 ratios by NPMTTZ for sample L1YJCK29, L1YJC2417 and L1YHC02 were relatively low (0.75±0.06 to 1.07±0.05), of which L1YJC2417 showed the largest difference (0.95±0.04) between NPMTTZ and BPM (Fig. 1(b)).

3.4. Relationship between the results of DNA determination and the characteristics of reservoir formation and crude oil

According to the above analysis, it can be seen that the determination of DNA concentration and purity was related to the properties of crude oil and reservoir conditions. In order to further explore the main influencing factors, we selected a number of formation conditions (T and depth), organic factors (ρ, η and resin plus asphaltene content) and inorganic factors (MC、pH and SO_4^{2-}), and DNA concentrations detected by different methods for correlation analysis (Fig. 4).

The results showed that obviously there is no significant correlation between NDTT and various factors (Fig. 4). Factors such as MC, pH and SO_4^{2-} that can directly affect microbial growth are closely related to qPCR and NPMTTZ methods, suggesting that

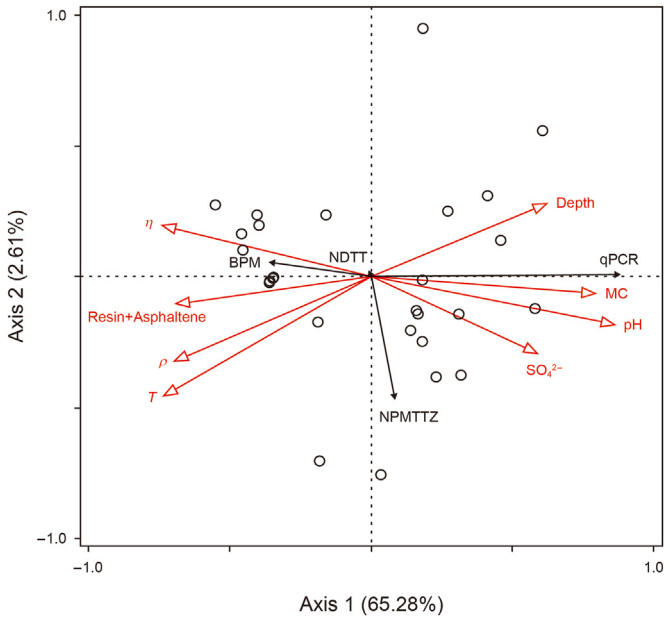

Fig. 4. Redundancy analysis (RDA) ordination plot. Empty black circles represent crude oil DNA samples, solid black arrows represent the different DNA concentration determination methods, empty red arrows indicate the direction of parameters.

these two methods can indirectly reflect the distribution of microbial communities (Fig. 4). The viscosity and asphaltene content of crude oil are significantly correlated with BPM on the whole, but potential negatively correlated with qPCR and NPMTTZ methods, suggesting that these characteristics may interfere with the detection of heavy oil (Fig. 4).

DNA concentration was also dependent on the properties of oil samples (from highest to lowest): OHO (11.46±18.34 ng/μL), medium oils (7.50±3.00 ng/μL), EHO (6.68±9.67 ng/μL) and SHO (6.20±7.83 ng/μL) (Fig. 5(a)). DNA concentration in heavy oils decreased with increased ρ and η (Fig. 5(b) and (d)), as well as resin plus asphaltene content (Fig. 5(c)), indicating that the oil consistency had an effect on DNA concentration. DNA purity in heavy oils as determined by NPMTTZ followed the order (from lowest to highest): OHO (1.31±0.27), medium oils (1.51±0.28), EHO (1.54±0.20), and SHO (1.83±0.32) (Fig. 5(a)). The DNA purity in heavy oils increased with increased ρ and viscosity (Fig. 5(b) and (d)), as well as resin plus asphaltene content (Fig. 5(c)). This difference in DNA purity could be due to different amounts of solutes in different oils. Undoubtedly, it is interesting to note that the higher the purity and concentration of DNA, the more conducive is for its HTS and analyses (Figs. S3 and S4). 22 HTS results were obtained from 32 samples (Table 1). Only 6 out of 14 OHO samples were detected, where 8 out of 11 EHO and 4 out of 5 SHO samples were detected.

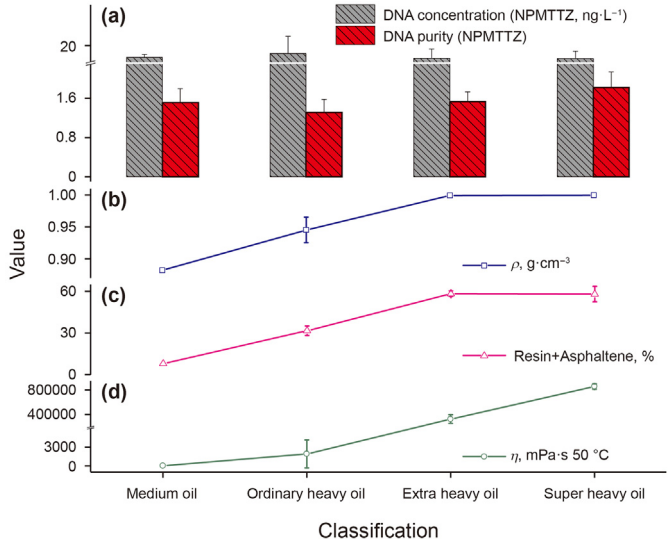

Fig. 5. Relationship between DNA determination and oil sample properties in different categories of crude oils. (**a**) DNA concentration and purity; (**b**) ρ; (**c**) Resin plus asphaltene and (**d**) η. The connect line among sample data (**b**, **c** and **d**) is only for the tendency comparison. According to the classification standard of heavy oil, the crude oil is divided into heavy oil, medium oil and thin oil. The average value of different samples in each type is taken, and the error bar represents the standard deviation of the average value.

4. Conclusions

The results obtained in this study suggest a relationship between the accuracy of DNA concentration determination and oil samples properties. BPM and NPMTTZ were fast and sensitive in the analysis of trace and/or ultratrace DNA of medium oil and heavy oils, and the determination results are similar. The η, ρ and/or resin plus asphaltene content of heavy oils could directly affect DNA determination. The concentration of DNA decreased with increased oil consistency, while conversely, the purity of DNA increased with increased oil heaviness. It is indicated that BPM, NPMTTZ and NDTT together with qPCR can be generally used to characterize the concentration and purity of DNA extracted from heavy oil reservoirs, but their results may not be equal, which may still be treated seriously because of the complex components in heavy oils. In addition, environmental factors such as MC, pH, SO_4^{2-}, T, and depth that affect DNA concentration distribution are also found in this study, which can provide reference for further analysis of microbial community in oil reservoir.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.petsci.2023.07.019.

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