Anaerobic activation of microorganisms indigenous to oil reservoirs to enhance oil recovery

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Abstract
Microbial enhanced oil recovery focuses on the activation, isolation, and screening of aerobic functional bacteria. Air injection is required to activate aerobic microorganisms for use in indigenous microbe oil displacement technology. In practice, however, oil reservoirs are oxygen-free, as the oxygen in the air is rapidly consumed by reducing substances and aerobic microorganisms attached to the water injection well at the front end of the reservoir. In the reservoir, most of the microorganisms metabolize anaerobically, and they are concentrated in the area where the residual oil collects in the rear of the facility. The aim of this study was to investigate the factors such as electron acceptors, activator components, and activator concentrations that influence anaerobic metabolism in indigenous microorganisms. Core flooding tests and field trials were conducted to evaluate the anaerobic microbial activation efficiency. The organic nitrogen source in the activator was crucial to anaerobic bacterial metabolism. Yeast powder was the preferred nitrogen source, and other optimal operating parameters included a C/N ratio of 20:1 and an activator concentration of 3 g·L⁻¹. Under these conditions, the anaerobic emulsification index surpassed 90%. Core flooding tests demonstrated that microbial enhancement may provide up to 13.5% oil recovery. A total of 3471.15 BBLs of oil was recovered by injecting anaerobic activator into the Zhan3-15 well, and emulsified oil droplets were detected in the fluid. The results of this study could provide technical support for the practical anaerobic activation of reservoir microorganisms and the improvement of microbial enhanced oil recovery.

Keywords Anaerobic activation · Electron acceptor · Field trial · Microbial enhanced oil recovery · Oil displacement efficiency

Introduction
Microbial enhanced oil recovery (MEOR) is the injection of microorganisms and/or nutrients into an oil reservoir to activate the microorganisms in it. MEOR utilizes microbial growth and metabolism to enhance oil recovery in the reservoir (Saravanan et al. 2020; Gao et al. 2020). MEOR is cost-effective (Jeong et al. 2019), has a high return on investment and yield, and is environmentally sustainable (Najafi-Marghmaleki et al. 2018). Hence, increasing attention has been directed toward this approach, and the number of field tests conducted on it is expanding. Several preliminary field tests on MEOR have demonstrated that this process increases oil, decreases water cut, and appears to have good practical applicability (Ke et al. 2018; Gao 2018; Safdel et al. 2017).

MEOR is classified as indigenous or exogenous bacterial enhanced oil recovery depending on the microbe source (Cui et al. 2017). The former involves the injection of activators and nutrients to induce microorganisms in the reservoir and enhance oil recovery. The latter involves the selection of
high-efficiency surfactant-producing, petroleum hydrocarbon-degrading bacteria and other microorganisms from the external environment and injection of them into the reservoir to enhance oil recovery. Most of the technologies focus on the activation, isolation, and screening of aerobic functional bacteria. Air injection may be required to activate aerobic microorganisms in indigenous MEOR. The main objective of exogenous microbe oil displacement technology is to separate surfactant-producing and aerobic microorganisms. The main internal space of the reservoir is a closed system and an oxygen-free environment. A certain amount of air is injected into the reservoir, and oxygen is rapidly consumed by reducing substances and aerobic microorganisms attached to the water injection well at the front end. Most of the microorganisms in the reservoir exhibit anaerobic metabolism. Additionally, many studies have reported that some microbes exhibit promising potential for enhanced oil recovery under anaerobic conditions (Youssef et al. 2013; Zhao et al. 2018; Zhao et al. 2021). Zhao et al. reported that the augmentation of indigenous P. aeruginosa in Daqing oil reservoirs could be effective for MEOR, as this approach involves in situ biosurfactant production without air injection. Kryachko et al. (Kryachko et al. 2016) enriched two putative biosurfactant producers under anoxic conditions and generated biosurfactants via fermentative metabolism. These products reduce surface tension. Castorena-Cortés G. isolated a thermoaerobacter that can grow in the anaerobic conditions of reservoirs and produce metabolites (Castorena-Cortés et al. 2012). Abundant biosurfactant producers have great potential in MEOR (Zhao et al. 2018; Zhao et al. 2016; Pannekens et al. 2019). Hence, the activation and regulation of the growth and metabolism of anaerobic microorganisms to improve oil displacement lie at the core of microbial oil recovery technology (Zhao et al. 2018).

Although the utilization of appropriate microorganisms is very important for MEOR, using appropriate and sufficient nutrition to activate microorganisms that ultimately contribute to the enhancement of oil recovery is vital. Adequate nutrients and appropriate ingredients are the key parameters to obtain the required biological products and the corresponding successful MEOR process. A large number of nutrients have been applied to activate microorganisms, including molasses, inorganic salts, steep corn liquor, olive oil mill waste, whey, and industrial wastewater (Astuti et al. 2022; Couto et al. 2019; Maass et al. 2016; Gassara et al. 2017). However, few studies have explored the factors affecting the production of emulsifiers and gases by anaerobic bacteria in oil reservoirs to improve oil recovery. Studies by Youssef et al. showed that electron receptors, carbon sources, and energy sources (such as carbohydrates) can activate specific strains to produce biosurfactants and/or lead to other desirable results (Pannekens et al. 2019). Jenneman used in situ oil as a nutrient, which reduced the yield of biological products. Sun et al. used different long-acting activators to activate the reservoir microorganisms of the Zhan3 block in the Shengli Oilfield and found that a long-acting activator containing plant polysaccharides had a significant anaerobic gas-producing effect.

Li et al. reported that the oil displacement efficiency and the community structure of the microbes are different when different nutrients are injected into the reservoir.

In the present study, we assessed the factors influencing indigenous microbe metabolism such as electron acceptors and activator components and concentrations. To this end, we developed and tested a series of formulations regulating the activation of anaerobic microorganisms. Core flooding tests showed a 13.5% oil recovery by microbial enhancement. A total of 3471.15 BBLs of oil were recovered by injecting an anaerobic activator into Zhan3-15 wells, and ionized oil droplets were detected in the fluid. The results of this study could provide technical support for the practical anaerobic activation of reservoir microorganisms and the improvement of microbial enhanced oil recovery.

Materials and methods

Test samples

Liquid from the Zhan3-15 oil wells was collected and stored at 4 °C for later use as inoculum for enrichment cultures.

Effects of electron acceptors

To evaluate the effects of electron acceptors on the emulsification performance of anaerobic bacteria, six activator formulations were prepared and designated A1, A2, A3, A4, A5, and A6 (Table 1). Bacterial densities, emulsification indices, volatile fatty acids (VFAs), and microbial community diversity were evaluated.

Effects of activator components

The carbon and nitrogen sources and the C/N ratio are the main factors affecting microbial growth, metabolism, and metabolite accumulation. Optimizing the carbon and nitrogen sources improves activation conditions and prepares and regulates emulsifier production by anaerobic microorganisms in the latter stages. Here, the emulsification index was measured to compare the relative effects of various carbon sources (glucose, molasses, starch, and corn meal), nitrogen sources (yeast powder, sodium nitrate, ammonium sulfate, and urea), and carbon–nitrogen ratios (3:1, 5:1, 10:1, 20:1, and 25:1) on anaerobic bacterial emulsifier production.
**Table 1** Activator formulations comprising different electron acceptors

| No. | Activator formula                                                                 |
|-----|----------------------------------------------------------------------------------|
| A1  | Glucose 3 g·L⁻¹, peptone 3 g·L⁻¹, yeast powder 3 g·L⁻¹                             |
| A2  | Glucose 3 g·L⁻¹, peptone 3 g·L⁻¹, yeast powder 3 g·L⁻¹, dipotassium phosphate 0.27 g·L⁻¹ |
| A3  | Glucose 3 g·L⁻¹, peptone 3 g·L⁻¹, yeast powder 3 g·L⁻¹, dipotassium phosphate 2.7 g·L⁻¹ |
| A4  | Glucose 3 g·L⁻¹, peptone 3 g·L⁻¹, yeast powder 3 g·L⁻¹, nitrate 0.1%               |
| A5  | Glucose 3 g·L⁻¹, peptone 3 g·L⁻¹, yeast powder 3 g·L⁻¹, nitrate 0.6%               |
| A6  | Glucose 3 g·L⁻¹, peptone 3 g·L⁻¹, yeast powder 3 g·L⁻¹, dipotassium phosphate 2.7 g·L⁻¹, nitrate 0.6% |

**Effects of activator concentrations**

Eight activator concentrations were prepared and designated 1, 2, 3, 4, 5, 6, 7, and 8 (Table 2), and the bacterial densities, emulsification indices, VFA contents, and functional bacteria were compared among treatments.

**Anaerobic activation method**

Liquid from the Zhan3-15 oil wells was filtered. The constituents of the activator formulae were weighed out according to Table 2 and dissolved. The solutions were boiled and cooled. Cysteine was added, and the pH was adjusted to 7.5. Resazurin and nitrogen were then added, and the solutions were kept slightly boiling for another 20 min. Then, 120 mL activator was placed in a 250-mL anaerobic bottle and autoclaved at 121 °C for 20 min. Thirty milliliters liquid oil from the Zhan3-15 wells was added, and stationary cultures were initiated and maintained at 60 °C.

**Test indices and methods**

**Total bacterial density**

Activated samples of indigenous microorganisms withdrawn at different times were diluted, and the total numbers of microorganisms in each dilution were detected under a microscope using a hemocytometer.

**Table 2** Activator formulations and various activator concentrations

| No. | Activator formula                                                                 |
|-----|----------------------------------------------------------------------------------|
| C1  | Glucose 3 g·L⁻¹, peptone 3 g·L⁻¹, yeast powder 3 g·L⁻¹                             |
| C2  | Glucose 0.1 g·L⁻¹, peptone 0.1 g·L⁻¹, yeast 0.1 g·L⁻¹, dipotassium phosphate 2.7 g·L⁻¹ |
| C3  | Glucose 1 g·L⁻¹, peptone 1 g·L⁻¹, yeast 1 g·L⁻¹, dipotassium phosphate 2.7 g·L⁻¹ |
| C4  | Glucose 3 g·L⁻¹, peptone 3 g·L⁻¹, yeast powder 3 g·L⁻¹, dipotassium phosphate 2.7 g·L⁻¹ |
| C5  | Glucose 6 g·L⁻¹, peptone 6 g·L⁻¹, yeast 6 g·L⁻¹, dipotassium phosphate 2.7 g·L⁻¹ |
| C6  | Glucose 3 g·L⁻¹, dipotassium phosphate 2.7 g·L⁻¹                                |
| C7  | Peptone 3 g·L⁻¹, yeast powder 3 g·L⁻¹, dipotassium phosphate 2.7 g·L⁻¹          |
| C8  | Glucose 3 g·L⁻¹, peptone 3 g·L⁻¹, yeast powder 3 g·L⁻¹, dipotassium phosphate 2.7 g·L⁻¹, sodium nitrate 0.6% |

**Emulsification index**

Five milliliters each of activated sample and diesel oil was added to a test tube, and the mixture was vortexed for 2 min and left to stand for 24 h. The emulsification index was determined from the ratio of the height of the emulsion layer to the total height of the organic phase.

**Volatile fatty acids**

Activated samples of indigenous microorganisms were centrifuged at 12,000 rpm for 5 min, and the supernatant was discarded. The sample size used in VFA analysis was 50 μL. The detection conditions were as follows: injection temperature, 300 °C; flame ionization detector (FID) temperature, 300 °C; temperature program: 100 °C (3 min), increase of 10 °C min⁻¹ up to 240 °C over a total of 20 min; carrier gas, N₂; flow rate, 1 mL·min⁻¹.

**Functional bacteria**

After activation, bacteria were collected by high-speed centrifugation (12,000 rpm, 4 °C, 15 min), and a genomic AxyPrep® kit (Corning Inc., Corning, NY, USA) was used to extract the DNA. The extracted DNA was dissolved in 100 μL Tris–EDTA (TE) buffer, and its concentration was determined by Nanodrop Technology (Thermo Fisher Scientific, Waltham, MA, USA). DNA was then stored at -70 °C and later used as the template for fluorescence quantitative
PCR of late functional genes. The instrument used was an IQ5 (Bio-Rad Laboratories, Hercules, CA, USA). The reaction system consisted of an unknown sample template and a standard plasmid. The sample size was 1 μL, the final primer concentration was 1 pM. L⁻¹, and the final volume was adjusted to 20 μL with sterilized deionized water. The reaction procedure was as follows: collective fluorescence at 95 °C for 3 min; 95 °C for 10 s; 60 °C for 30 s; amplification for 40 cycles; and a temperature increase from 60 °C to 95 °C. The fluorescence was measured every 0.5 °C, and a dissolution curve was plotted. The standard plasmid and the unknown sample were reacted over a concentration gradient of 1 × 10⁸ copies.μL⁻¹, 1 × 10⁶ copies.μL⁻¹, 1 × 10⁴ copies.μL⁻¹, and 1 × 10² copies.μL⁻¹. Data were analyzed after fluorescence quantitative PCR using software bundled with the apparatus.

**Microbial community diversity**

Activated samples were centrifuged at 12,000 rpm and 4 °C for 15 min, and the sediment containing the bacteria was collected. A genomic AxyPrep® kit (Corning Inc., Corning, NY, USA) was used to extract the DNA. The DNA concentration was determined by Nanodrop Technology (Thermo Fisher Scientific, Waltham, MA, USA), and the extracted 16S bacterial DNA was amplified. The V4 region of 16S rDNA was subjected to high-throughput sequencing and bioinformatics analysis to analyze the microbial community structure.

**Oil displacement efficiency**

The oil displacement efficiency of the anaerobic activation was tested using long sand-packed columns (inner diameter, 38 mm; length, 600 mm) simulating the actual reservoirs. Anaerobiosis of the oil displacement process was then established. Nitrogen was used to evaluate the permeability of the sand-filled core barrel, displace the air in it, and deoxidize and protect during formation water saturation. Nitrogen was also applied along with the activators. Nitrogen pressure replaced the air pressure. Different quartz and types varying in mesh size were mixed in fixed ratios and added to the columns to obtain equal permeabilities. The columns were saturated with the water extracted from Zhan3-15 after vacuum pumping. Degassed and dewatered Zhan3 crude oil was injected into each column until irreducible water saturation was achieved. The columns were then flooded with injection water until the oil content in the displacement water was >98%. The nitrogen-deoxidized activator formulations were then injected. The columns were pressurized with nitrogen up to formation pressure (10 MPa) and incubated for various times at 60 °C. Water flooding was then performed, and the oil recovery efficiency (ORE, %) was calculated.

**Results and discussion**

**Effects of electron acceptors**

Figure 1a shows that the six activator systems induced indigenous microorganisms in the reservoir under anaerobic conditions. After 14 d of activation and incubation, the total number of bacteria was > 10⁸ cells.mL⁻¹. The bacterial concentration in the A4 activator system was the highest and reached 5 × 10 cells.mL⁻¹ after 8 d.

The emulsification index revealed that phosphate and nitrate in the nutrient system promoted emulsifier production by anaerobic metabolism in the indigenous microorganisms. The indigenous anaerobic microorganisms in the Zhan3-15 oil wells produced the most emulsifier after activation. The emulsification indices of the six formulations exceeded 60% after 4 d of activation. After 14 d, the emulsification indices of the A1, A3, A4, and A5 nutrient systems surpassed 90%.

Acetic, propionic, butyric, and other volatile fatty acids (VFAs) are important intermediate metabolites in microbial anaerobic metabolism (Rathi et al. 2018). Hence, they are useful for monitoring the activation of indigenous microorganisms. Figure 2c illustrates that phosphate addition in the nutrient system promotes VFA production by anaerobic metabolism. However, the total amount of VFAs decreased after nitrate and phosphate were added simultaneously. The combination of these anions may have increased the redox potential and inhibited anaerobic metabolism in the indigenous microorganisms.

Figure 1d shows that Anaerobaculum, Thermoaerobacter, Coprothermobacter, Thermotoga, and Thermosipho predominated among the activated anaerobic microorganisms. Anaerobaculum, Thermoaerobacter, and Thermotoga were reported to be native inhabitants of an oil reservoir in the North Sea (Kaster et al. 2009). Phosphate and nitrate addition to A1, A3, A4, and A6 gradually increased the abundances of Thermoaerobacter, Thermosipho, and Thermotoga. Thermotoga has been detected exclusively in oil reservoirs (Miranda-Tello, et al. 2004). It degrades long-chain-to shorter-chain hydrocarbons in crude oil. Thermoaerobacter has not been isolated from heavy oil samples but can use molasses as a substrate and produces biosurfactants (BSF) under reservoir conditions (70 °C, 15 g. L⁻¹ NaCl) (Halim et al. 2015). Thermosipho has been isolated from high-temperature petroleum systems worldwide, but its contribution to enhanced oil recovery (EOR) is unclear. The abundance of Anaerobaculum decreased in response to nitrate addition, as the latter favored the growth of Petrotoga over Anaerobaculum (Halim et al. 2015).
Effects of activator components

The effects of the carbon and nitrogen sources and the C/N ratio on emulsification by indigenous microorganisms are shown in Fig. 2.

Based on the emulsification indices measured after incubation of indigenous microorganisms with various carbon sources (Fig. 2a), glucose, molasses, starch, and corn flour had little effect on emulsifier production by anaerobic metabolism in indigenous microorganisms. The emulsification index was ~60%, and its highest value was measured on the 7th day of activation.

Based on the emulsification indices measured after incubation of indigenous microorganisms with various carbon sources (Fig. 2b), yeast powder was more conducive than sodium nitrate, ammonium sulfate, or urea to emulsifier production by indigenous microorganisms under anaerobic conditions. After 7 d of cultivation, the emulsification index peaked at 80%, whereas it was only ~50% in response to the other nitrogen sources. Yeast powder contains trace elements and growth factors that stimulate emulsifier production by anaerobic metabolism in indigenous microorganisms.

Very high or low C/N ratios do not favor microbial cell growth or metabolite accumulation. Very low C/N ratios cause early bacterial cell autolysis. Very high C/N ratios result in imbalances in bacterial metabolism that do not support metabolite accumulation. Even at appropriate C/N ratios, very high or low carbon and nitrogen concentrations are not conducive to cell growth or metabolite accumulation. At very high C or N concentrations, cell growth is slow during the latter stages of fermentation, metabolic waste accumulates, and bacterial metabolism becomes abnormal. At very high C or N concentrations, the medium is deficient in nutrients, and cell proliferation is impeded. Therefore, we used several different C/N ratios, including 3:1, 5:1, 10:1, 20:1, and 25:1. Figure 2c demonstrates that a C/N ratio of 20:1 was optimal for emulsifier production by anaerobic metabolism in indigenous microorganisms. Under this treatment, the emulsification index was >90%. C/N ratios higher or lower than 20:1 were unfavorable for emulsifier production by microbial metabolism under anaerobic conditions.

Effects of activator concentration

Figure 3a reveals that the activator concentration significantly affected the growth of indigenous microorganisms under anaerobic conditions. At the low activator concentrations in C1, C2, and C3, the activation of anaerobic microorganisms was poor. After 35 d, the highest bacterial concentration under these treatments was only $10^8$ cells.mL$^{-1}$.
However, the bacterial concentration significantly increased with activator concentration. At high activator concentrations, anaerobic growth of indigenous microorganisms was promoted. At a combined concentration of 3 g L\(^{-1}\) glucose, peptone, and yeast powder (C4), the highest bacterial concentration was \(7 \times 10^8\) cells mL\(^{-1}\). As it increased the redox potential, the high nitrate concentration in the activator of C8 was not conducive to the anaerobic growth of the indigenous microorganisms in the reservoir. Hence, the bacterial concentration in C8 was significantly lower than that in C4 (nitrate-free) at the same activator concentration.

Carbon and nitrogen sources have various effects on the anaerobic growth of indigenous microorganisms. In the presence of carbon sources alone, the activation effect on the indigenous microorganisms was poor. In the presence of organic nitrogen sources alone, however, the activation effect on indigenous microorganisms was relatively better. The bacterial concentration in the latter case was significantly higher than that in the former. Thus, organic nitrogen plays an important role in activating anaerobic metabolism in indigenous microorganisms, possibly because organic nitrogen sources also contain various trace elements and growth factors.

The influence of activator concentration on the accumulation of small-molecule acids is related to the postactivation growth of indigenous microorganisms. The activator effect and bacterial concentration increased with activator concentration (C4 and C5). At the highest total acid levels, large amounts of VFAs accumulated via the anaerobic metabolism of indigenous microorganisms. The postactivation bacterial densities in C2 and C3 in the presence of low activator concentrations were low, bacterial growth and metabolism of the indigenous microorganisms were poor, and there was a relatively low total acid content. Therefore, organic nitrogen sources play crucial roles in the accumulation and metabolism of small-molecule acids by indigenous microorganisms under anaerobic conditions.

After activation of anaerobic metabolism in the indigenous microorganisms under C1, C5, and C8, the emulsification indices all reached maxima exceeding 90%. However, the emulsification index of the activation system with a low activator concentration was 0. Consequently, the nutrient profile (type and concentration) is vital to emulsifier production by anaerobic metabolism. Under nutrient deficiency, microorganisms can only maintain their own growth but cannot accumulate secondary metabolites. Moreover, furnishing
a carbon or nitrogen source alone in the activator did not promote emulsifier production by anaerobic metabolism, and the emulsification index was 0. Thus, both organic carbon and nitrogen sources are indispensable for emulsifier production by anaerobic metabolism. Crude oil wall hanging markedly decreased in C1, C4, C5, and C8 with increasing culture time. Crude oil is emulsified into large oil droplets, indicating effective activation of anaerobic microorganisms and full efficacy and dispersion of the emulsifier in the crude oil.

*Geobacillus* is invaluable in oilfields. It utilizes hydrocarbons and produces bioemulsifiers (Han et al. 2017; Lin et al. 2019). Figure 3d shows that there were no significant differences among activator concentrations or carbon or nitrogen sources in *Geobacillus* metabolism or emulsifier production. However, the relative abundance of *Geobacillus* significantly increased in response to the addition of nitrate to the activation system. Nitrate increased the redox potential of the system and promoted growth and emulsifier production in *Geobacillus* and other facultative anaerobes. The C1 activator system contained comparatively few *Geobacillus* but had good emulsification performance. Other bacteria may have predominated in this system, possibly because of butyric acid accumulation. Nevertheless, this phenomenon merits further investigation.

**Oil displacement efficiency**

The effects of different incubation times on the oil displacement efficiency are shown in Fig. 4 and Table 3. The number of days of cultivation and activation of anaerobic metabolism in indigenous microorganisms substantially influenced the ORE. The microbial ORE gradually increased with cultivation time. The oil displacement efficiency reached 12.4% after 50 d of cultivation but did not increase after 60
d. Hence, longer residence times are required to obtain a high ORE for anaerobic activation.

Field test

Anaerobic EOR activation in the Zhan3-15 well was evaluated. Anaerobic activation increased daily oil production from 10.22 BBLs.d$^{-1}$ to 14.60 BBLs.d$^{-1}$, the water cut decreased from 83.5% to 71.9%, and there was a cumulative 457.5 t increase in crude oil. The number of oil displacement functional bacteria, such as Geobacillus, and hydrocarbon-degrading bacteria increased significantly. Geobacillus is a thermophilic, facultative anaerobic bacterium that has strong surface hydrophobicity and produces a bioemulsifier. The strain can change crude oil properties through effects including emulsification, changes in viscosity, and degradation (Abdi et al. 2022; Zheng et al. 2011). There was obvious emulsification of the liquid produced by the test sample (Fig. 5). There were very small emulsified oil droplets, indicating that the injected activator induced endogenous microorganisms and that the emulsifier generated by their metabolism effectively dispersed the crude oil. This process displaced the oil, promoted dredging near the well, and was conducive to crude oil seepage.

Conclusions

1. Electron acceptors significantly affect the activation of indigenous anaerobic microorganisms. The regulation of electron receptors promotes anaerobic growth and metabolism in microorganisms. In this study, the maximum bacterial concentration reached $7 \times 10^8$ cells mL$^{-1}$, and the emulsification index surpassed 90%.

2. The organic nitrogen source in the activator plays an important role in anaerobic metabolism. Here, the optimal nitrogen source was yeast powder, the preferred C/N ratio was 20:1, and the ideal activator concentration was 3 g L$^{-1}$.

3. Core flooding tests demonstrated that microbial enhancement could provide 13.5% oil recovery. A total of 3471.15 BBLs of oil was recovered by injecting anaerobic activator into the Zhan3-15 well, and emulsified oil droplets were detected in the fluid.

4. The results of this study could provide technical support for the practical anaerobic activation of reservoir microorganisms and the improvement of microbial enhanced oil recovery.

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Declarations

Conflict of interest We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work. There is no professional or other personal
interest of any nature or kind in any product, service, and/or company that could be construed as influencing the position presented in the manuscript.

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