Research Paper

Sequencing batch airlift reactors (SBAR): a suitable technology for treatment and valorization of mineral oil wastewaters towards lipids production

Rita M. Silva [a], Ana M. Fernandes [a], Francesca Fiume [a,1], Ana Rita Castro [a], Raul Machado [b,c], Maria Alcina Pereira [a,*]

[a] Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal
[b] CBMA Centre of Molecular and Environmental Biology, Department of Biology, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal
[c] IB-S Institute of Science and Innovation for Sustainability, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

ARTICLE INFO

ABSTRACT

Produced water (PW) and spent oil-based wastewaters are some of the largest mineral oil wastewaters produced. Due to the high toxicity of hydrocarbons, several countries set stringent discharge limits and its treatment is compulsory before discharge. In this work, biological treatment of mineral oil wastewaters coupled with the production of bacterial lipids is demonstrated in sequential batch airlift reactors (SBAR). Two SBAR (2 L working volume) were used for treatment of PW and lubricant-based wastewater (LW), inoculated with *Alcanivorax borkumensis* SK2 (SBAR$_{Ab+PW}$) and *Rhodococcus opacus* B4 (SBAR$_{R.o+LW}$), respectively. A total petroleum hydrocarbon removal (TPH) efficiency up to 96% and 80% were achieved for SBAR$_{Ab+PW}$ and SBAR$_{R.o+LW}$, respectively. Intracellular lipids production in SBAR$_{Ab+PW}$ increased when lower TPH/N ratios and higher feast stage duration were applied (up to 0.74 g g^{-1} cell dry weight (CDW)), whereas in SBAR$_{R.o+LW}$ higher lipids production was observed for higher TPH/N ratios (0.94 g g^{-1} in CDW). Triacylglycerols (TAG) were the main intracellular lipid accumulated in both SBAR$_{Ab+PW}$ and SBAR$_{R.o+LW}$ operations, while wax ester (WE) production was only observed extracellularly in the SBAR$_{Ab+PW}$.

1. Introduction

The increasing demand for petroleum and petroleum derived products had led to the production of large amounts of mineral oil wastewaters, being spent oil-based wastewaters (SOW) and oilfield produced water (PW) the most significantly produced. Lubricant-based wastewater (LW) is a common type of SOW that is produced during the manufacture and the application of lubricants in industrial activities (Castro et al., 2018). On the other hand, oilfield PW is the largest by-product generated during the process of oil and gas extraction with a worldwide production up to 39.5 billion litters per day (Jiménez et al., 2018). Industrial petrochemical wastewater treatment is an important study area in environmental engineering. Mineral oil wastewaters are very recalcitrant streams and due to the presence of large concentrations of petroleum hydrocarbons are considered an environmental problem with serious consequences to the environment and human health (Camarillo and Stringfellow, 2018). Therefore, the treatment of these wastewaters is compulsory and limits and rules regarding the organic content had been set for its discharge into the environment (Fakhru'l-Razi et al., 2009).

Nowadays, mineral oil wastewaters are commonly treated by different physical and/or chemical processes such as coagulation, adsorption, hydrocyclones, centrifugation and membrane processes (Aljuboury et al., 2017). However, these methods require high capital for installation and maintenance, high energy consumption and lead to the production of hazardous sludge (Dong et al., 2011).

Biological treatment of mineral oil-wastewaters offers some

Nomenclature

ATR-FTIR	Attenuated total reflection-fourier transform infrared analysis
CDW	Cellular dry weight
COD	Chemical oxygen demand
DAG	Diacylglycerol
FA	Fatty acid
GC-FID	Gas chromatography and Flame ionization detector
HCB	Hydrocarbonoclastic bacteria
LW	Lubricant wastewater
MAG	Monoacylglycerols
PAH	Polycyclic aromatic hydrocarbons
PW	Produced water
SBAR	Sequencing batch airlift reactor
SBAR$_{Ab+PW}$	SBAR operation treating produced water by *A. borkumensis* SK2
SBAR$_{R.o+LW}$	SBAR operation treating lubricant wastewater by *R. opacus* B4
SOW	Spent oil-based wastewaters
TAG	Triacylglycerol
TLC	Thin layer chromatography
TN	Total nitrogen
TPH/N	Total petroleum hydrocarbon/nitrogen ratio
TSN	Total suspended nitrogen
WE	Wax ester
WS/DGAT	Wax ester synthase/acyl coenzyme A (acyl-CoA): diacylglycerol acyltransferase

advantages to the current physical and chemical processes. It is a more cost-effective method, allowing to remove the small suspended oil particles that cannot be eliminated otherwise and environmental friendly, since it relies in bacteria capability to transform hydrocarbons into non-toxic by-products such as carbon dioxide (CO_2), water or bacterial reserve compounds (Dong et al., 2011; Fakhru'l-Razi et al., 2009).

Several works have been focused on the aerobic treatment of hydrocarbon contaminated wastewaters since it occurs at a faster rate and is more efficient than under anaerobic conditions (Da Silva et al., 2016; Heider et al., 1998; Sudmalis et al., 2018). Mineral oil wastewaters, like for instance PW and LW, differ from each other relatively to the type and content of hydrocarbons, salinity levels and presence of some other specific compounds. For example, PW consists mainly on aliphatic hydrocarbons from C11 to C40, which are more easily biodegradable than aromatic hydrocarbons (Sudmalis et al., 2018), while LW is composed by a complex matrix that includes a mixture of straight-chain alkanes ranging from C10 to C34 and a complex and unresolved hydrocarbon mixture (Da Silva et al., 2016). Another important feature of mineral oil wastewaters is the salinity levels which in the case of offshore PW can be challenging when a biological treatment is applied. Therefore, the use of hydrocarbonoclastic bacteria (HCB) and in the particular case of offshore PW, halotolerant HCB, can be a suitable strategy for aerobic treatment of mineral oil wastewaters such as PW and LW (Da Silva et al., 2016; Sudmalis et al., 2018).

One particular feature of HCB is the accumulation of storage compounds in response to nutrient limitation in the environment. *Alcanivorax borkumensis* SK2 and *Rhodococcus opacus* B4 are two HCB species capable of degrading a wide range of hydrocarbons and use them as carbon source for the production and accumulation of neutral lipids (Alvarez and Steinbüchel, 2002; Manilla-Pérez et al., 2011). The accumulation of lipids occurs as a survival strategy during starvation periods, when an essential nutrient (i.e nitrogen and/or phosphorous) becomes unavailable or at lower concentrations comparing with the carbon source (Kalscheuer et al., 2007; Manilla-Pérez et al., 2011). Several factors can influence the production of storage compounds by bacteria. Some studies reported that the carbon to nitrogen ratio (COD/N ratio) is the most important parameter influencing the production and accumulation of lipophilic compounds by HCB (Manilla-Pérez et al., 2011; Zhang et al., 2019). Currently, bacterial neutral lipids are of great interest as raw materials for production of biofuels or other oleochemical products (Castro et al., 2017; Kumar et al., 2015).

Previous studies performed by our research group reported *A. borkumensis* SK2 ability to produce intracellular and extracellular lipids, namely WE and TAG, as well as *R. opacus* PD630 and B4 ability to produce intracellular TAG, from mineral oil wastewaters (Castro et al., 2016; Da Silva et al., 2016; Sudmalis et al., 2018). Due to these remarkable characteristics, *A. borkumensis* SK2 (halophilic) and *R. opacus* B4, were chosen for the treatment of PW and LW respectively.

Moreover, *A. borkumensis* SK2 was chosen due to its wide presence in marine environments.

Combining aerobic biological treatment with the valorization of mineral oil-based wastewaters can be an environmentally sustainable alternative for the management of these wastewaters. After showing that *A. borkumensis* SK2 and *R. opacus* B4 are suitable candidates for the treatment process, it is important to study the most appropriate process conditions and bioreactor configurations for the optimization and application of a full-scale treatment.

In this work the suitability of the sequencing batch airlift reactor (SBAR) configuration to biologically treat different types of mineral oil wastewaters namely PW (saline wastewater) and LW (non-saline wastewater) was explored. A specialized inoculum focused in PW and LW characteristics, namely salinity level, was used and the most suitable conditions for the production and accumulation of bacterial lipids were assessed, in order to achieve a balance between neutral lipids production and wastewater treatment costs and efficiency.

2. Materials and methods

2.1. Experimental set-up, inoculum and carbon source

A SBAR with a working volume of 2 L, designated as SBAR$_{Ab+PW}$, was inoculated with *A. borkumensis* SK2 (DSM 11573), fed with oilfield produced water (PW) and operated at room temperature (20 ± 5 ºC) and pH around 7.8. Another SBAR with the same working volume, designated as SBAR$_{R.o+LW}$, was inoculated with *R. opacus* B4 (NBRC 108011), fed with lubricant-based wastewater (LW) at different concentrations and operated at room temperature (20 ± 5 °C) and pH around 7.3.

PW concentrate was obtained from Shell (The Netherlands), from a full-scale physical separation process. LW concentrate (floating phase separated in a gravimetric oil/water separation unit) was obtained from a wastewater treatment plant located in a heavy machinery maintenance service unit (ALSTOM Portugal, S.A., Maia, Portugal). The chemical

Table 1
Chemical characterization of the concentrates of lubricant-based wastewater (LW) and oilfield produced water (PW).

Characterization	LW	PW
COD (g L^{-1})	317 ± 14	n.d[a]
Nitrate (mg L^{-1})	94 ± 9	n.d[a]
Nitrite (mg L^{-1})	0	n.d[a]
Total ammonia nitrogen (mg L^{-1})	0.13 ± 0.4	1.2 ± 0.08
Total nitrogen (mg L^{-1})	172 ± 0.4	43 ± 2.7
Salinity (g kg^{-1} of seawater)	0	200
Total petroleum hydrocarbons (TPH) (g L^{-1})	177 ± 17	202 ± 16

[a] Value not determined due to interferences of the high chloride levels in the quantification method.

characterization of LW and PW is described in Table 1.

A. borkumensis SK2 was pre-grown in saline medium (ONR7a) according to DMSZ, Braunschweig, Germany and *R. opacus* B4 in Mineral Salts (MS) medium according to Schlegel et al. (1961) (please see Supplementary information for details and Fig. S1).

A short adaptation period of the microorganisms to the respective carbon source was performed before SBAR operations. In this period both carbon (PW or LW) and nitrogen source (ammonium chloride (NH_4Cl)) were added to the bioreactors at the conditions of 0.26 g L^{-1} of TPH and 30 mg L^{-1} of nitrogen for $SBAR_{Ab+PW}$ and 1 g L^{-1} of TPH and 30 mg L^{-1} of nitrogen for $SBAR_{R.o+LW}$. The wastewaters treatment process consisted in alternated cycles of feast (carbon addition) and famine (nitrogen addition) stages. In the famine stage, only nitrogen (at concentrations of 15 or 30 mg L^{-1}) was added to the bioreactor. This stage was necessary to ensure that all the carbon from the previous cycle was totally consumed before the carbon source addition. In the feast stage, only the carbon source was added (Fig. S2, Supplementary material).

Different ratios of total petroleum hydrocarbons per nitrogen concentration (TPH/N ratios) and feast stage duration were tested for both wastewaters. The operational conditions are described in Table 2 for $SBAR_{Ab+PW}$ and Table 3 for $SBAR_{R.o+LW}$. In both SBAR operations and for each condition tested, three cycles were performed with exception of the condition of 4.4 g L^{-1} of TPH_{LW} and 15 mg L^{-1} of nitrogen in $SBAR_{R.o+LW}$, where only two cycles were made. At the end of each cycle, analysis on neutral lipids, total nitrogen and total petroleum hydrocarbons concentration were performed.

2.2. Analytical procedures

Bacterial growth in the seed cultures used for bioreactors inoculation was monitored by optical density at 600 nm wavelength (OD_{600}) in a DR 2800 Hach Lange spectrophotometer.

During reactors operation, 2 mL samples were collected daily and centrifuged at 10,000 rpm for 10 min for nitrogen determination (N-NH_4^+), using the commercial kit LCK 304 (Hach Lange®, Germany). At the end of each cycle total nitrogen (TN) determination was performed, using the commercial kits LCK 338 (Hach Lange®, Germany). Biomass concentration was measured as total suspended nitrogen (TSN), using the commercial kit LCK 304 (Hach Lange®, Germany).

Chemical oxygen demand (COD), nitrite and nitrate concentrations were determined using the commercial kit LCK 914, LCK 342 and LCK 339, respectively (Hach Lange®, Germany).

The pH was manually controlled and measured by using a benchtop metre inoLabVR pH 7110 (WTW, Weilheim, Germany). The adjustment was performed by adding hydrochloric acid (HCl) with a syringe to the bioreactor until reaching the optimal pH.

2.3. Extraction and quantification of total petroleum hydrocarbons (TPH)

Samples of 50 mL (in duplicate) were collected for hydrocarbons

Table 2
Operational conditions applied to the SBAR inoculated with *A. borkumensis* SK2 for the biological treatment of PW ($SBAR_{Ab+PW}$).

Cycles	TPH_{PW}[a] (g L^{-1})	N-NH_4[b] (mg L^{-1})	DO (mg L^{-1})	Feast stage duration (d)	Famine stage duration (d)
I–III	0.26	30	7–8	3	3
IV–V	0.26	15	7–8	2	2
VI–VIII	0.26	15	7–8	4	3
IX–XI	0.26	30	7–8	5	3

[a] TPH concentration added to the bioreactor at the beginning of the feast stage.
[b] Nitrogen concentration added at the beginning of the famine stage.

Table 3
Operational conditions applied to the SBAR inoculated with *R. opacus* B4 for the biological treatment of LW ($SBAR_{R.o+LW}$).

Cycles	TPH_{LW}[a] (g L^{-1})	N-NH_4[b] (mg L^{-1})	DO (mg L^{-1})	Feast stage duration (d)	Famine stage duration (d)
I–III	1	30	7–8	5	2
IV–VI	4.4	30	7–8	3	6
VII–VIII	4.4	15	7–8	2	4

[a] TPH concentration added to the bioreactor at the beginning of the feast stage.
[b] Nitrogen concentration added at the beginning of the famine stage.

analysis. The samples were acidified with HCl to a pH below 2 and preserved at temperatures below 5 °C until further analysis.

Hydrocarbons extraction was performed according to the international standard method ISO 9377-2 at room temperature as described in Castro et al. (2016), with the following modifications: the extraction was performed three times with 25 mL of hexane; and after the cleaning step, the samples were dried under a gentle flow of nitrogen in a Turbo-Vap®LV (Biolage) at 51 °C for 40 min and resuspended in 1 mL of n-hexane for further analysis by gas chromatography with a flame ionization detector (GC-FID). The extracts were injected in a GC Varian® star 3400CX, USA equipped with a VF-1 ms column (30 m long, 0.25 mm diameter and 0.25 μm film thickness). A sample volume of 2 μL was injected at 250 °C with a split ratio of 1:20 and helium was used as carrier gas at a flow rate of 1 mL min^{-1}. The column temperature was set at 60 °C for 1 min and then increased until 270 °C at a rate of 8 °C min^{-1} in a total time run of 62 min. The detector was operated at a temperature of 315 °C.

2.4. Extraction and analysis of neutral lipids

30 mL samples were collected from the bioreactor for extraction and analysis of neutral lipids. The samples were centrifuged at 10,000 rpm for 10 min in a centrifuge Beckman allegra 64R. The pellet was freeze dried and used for intracellular lipids analysis. Intracellular lipids extraction was performed with chloroform: methanol (2:1, v/v) as described in Castro et al. (2016).

For extracellular lipid extraction, the supernatant was mixed with chloroform: methanol (2:1, v/v) in a separation funnel (1:1 vol/v) for 2 min and left for 30 min to allow the separation between the aqueous and organic phases.

The organic phases were dried under a gentle flow of nitrogen in a TurboVap®LV (Biolage) at 51 °C for 40 min and resuspended in 500 μL of chloroform.

Extracellular lipid extracts were fractionated by polarity on SPE columns (SiOH 1 g/6 cc) from Supelco, as described in Revellame et al. (2012).

The total lipid content was quantified gravimetrically. The different classes of lipids present in the extracts was assessed by TLC (Supplementary material).

2.5. DNA extraction and sequencing by Illumina

At the end of each bioreactor cycle, samples of well-homogenized biomass were collected, centrifuged (10,000 rpm, 10 min at 4 °C), washed with PBS and stored at − 20 °C. Total genomic DNA was extracted using a FastDNA SPIN kit for Soil (MP Biomedicals, LLC, Santa Ana, CA) according to manufacturer's instructions.

Amplification, library preparation, sequencing and taxonomic assignment of 16S rRNA reads were done by STAB VIDA (Caparica, Portugal). Library construction was performed using the Illumina 16S Metagenomic Sequencing library preparation protocol with the specific bacterial primers set 027F-1492R (Lane, 1991). 16S rRNA genes from the variable V1-V2 regions were sequenced with MiSeq Reagent Kit v3 in

the Illumina Miseq platform, using 300 bp paired-end sequencing reads.

Bioinformatic analysis were also performed by STAB VIDA (Caparica, Portugal). The analysis of the generated raw sequence was carried out using QIIME2 v2018.11.0 according to Caporaso et al. (2010). The reads were denoised using the DADA2 plugin and the following processes were applied: trimming and truncating low quality regions; dereplicating the reads and filtering chimeras (Callahan et al., 2016). The reads were organized in operational taxonomic units (OTUs) and the taxonomical classification was performed using SILVA database (release 132 QIIME) with a clustering threshold of 97% similarity. Only OTUs containing at least 10 sequence reads were considered as significant.

Sequencing reads were submitted to the European Nucleotide Archive (ENA) under the study accession number PRJEB37500 (sample SAMEA6791327 - cycle XI *A. borkumensis* SK2; sample SAMEA6791326 - cycle VIII *A. borkumensis* SK2; sample SAMEA6791328 - cycle VI *R. opacus* B4; sample SAMEA6791329 - cycle II *R. opacus* B4).

2.6. Attenuated total reflection-fourier transform infrared analysis (ATR-FTIR)

Extracts of the intracellular and extracellular lipids resuspended in 0.5 mL of chloroform were analyzed by ATR-FTIR (attenuated total reflectance-Fourier transform infrared) using a Perkin Elmer Spectrum Two™ IR spectrometer with a deuterated triglycine sulfate (DTGS) detector and KBr beam splitter, coupled with an UATR (single reflection diamond, Perkin Elmer) accessory. Samples were applied in the top of the diamond crystal and evaporated to form a film. Complete solvent evaporation was controlled by spectra acquisition until disappearance of the characteristic chloroform band. FTIR spectra were collected at room temperature, in the range of 4000 – 400 cm^{-1}, with a resolution of 4 cm^{-1}, accumulating 64 scans.

3. Results and discussion

3.1. Evaluation of SBAR performance in the treatment of LW and PW and on the production of lipids

Microbial growth in both bioreactors (SBAR$_{Ab+PW}$ and SBAR$_{R.o+LW}$) was followed by the decrease of N-NH$_4$ concentration in the culture medium (Fig. 1 (A) and (B)). N-NH$_4$ consumption in each cycle suggested that bacterial cells in SBAR$_{Ab+PW}$ and SBAR$_{R.o+LW}$ were able to degrade the hydrocarbons present in the wastewaters and use them as carbon source for growth (Fig. 1 (A) and (B)). NO$_2^-$ and NO$_3^-$ concentration in the SBAR$_{Ab+PW}$ effluent was below 2 and 1 mg L^{-1}, respectively. In the case of SBAR$_{R.o+LW}$ NO$_2^-$ concentration was below 2 mg L^{-1} and NO$_3^-$ concentration was approximately 9 mg L^{-1}. The low NO$_2^-$ and NO$_3^-$ concentrations observed in both SBAR operations indicates that occurrence of nitrification was not significant. The N-NH$_4$ profile was a relevant monitoring parameter in the performance of the feast and famine stages, allowing to determine the moment of addition of the wastewater (carbon source).

In the SBAR$_{Ab+PW}$ operation, the microbial growth was similar in all cycles, regardless the nitrogen concentrations used (approximately 0.6 g L^{-1}, Table 4), indicating that growth was not influenced by the nitrogen concentration and consequently by the TPH/N ratio. In the case of the SBAR$_{R.o+LW}$ operation, accurate biomass quantification was not possible due to the formation of granules by the bacterial biomass.

In the SBAR$_{Ab+PW}$, a TPH removal efficiency up to 96% ± 1.8 was achieved (Table 4). The preference of *A. borkumensis* SK2 for aliphatic hydrocarbons as sole or main carbon source is well described (Yakimov et al., 1998). Moreover, the ability of *A. borkumensis* SK2 to remove n-alkanes and other non-polar compounds from PW was already demonstrated by Sudmalis et al. (2018) in batch and continuous stirred tank bioreactor (CSTR). In cycles IV to VIII, a similar TPH removal efficiency was obtained, independently of the feast stage duration. However, when a TPH/N ratio of 9 (cycles I to III and IX to XI) was applied to
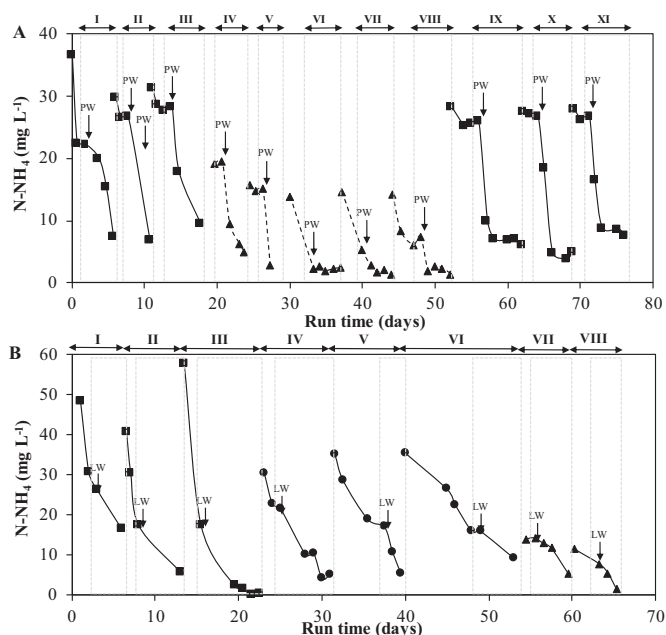

Fig. 1. Ammonium nitrogen (N-NH$_4$) profile during bioreactor operation inoculated with: (A) *A. borkumensis* SK2 and PW (SBAR$_{Ab+PW}$) using 0.26 g L^{-1} of TPH and 30 mg L^{-1} (cycles I–III and IX–XI) and 15 mg L^{-1} of nitrogen (cycles IV–VIII). (B) *R. opacus* B4 and LW (SBAR$_{R.o+LW}$) using 1 g L^{-1} of TPH and 30 mg L^{-1} of nitrogen (cycles I–III); 4.4 g L^{-1} of TPH and 30 mg L^{-1} of nitrogen (cycles IV–VI); and 4.4 g L^{-1} of TPH and 15 mg L^{-1} of nitrogen (cycles VII–VIII). Gray bars represent the feast stage duration.

the bioreactor (0.26 g L^{-1} TPH$_{PW}$ and 30 mg L^{-1} of nitrogen), the increase of the feast stage duration from 2 to 5 days led to an improvement on the TPH removal efficiency of approximately 10% (Table 4). This result suggests that the feast stage duration applied to the cycles I, II and III (approximately 2 days) was not enough for a complete hydrocarbon removal.

The high salinity levels present in the bioreactor (approximately 30 g L^{-1}) did not hinder PW treatment, which shows that *A. borkumensis* SK2 was fully adapted to these conditions. Therefore, this bacterium can be considered a robust candidate for the treatment of PW, making the process less time consuming and more cost-effective, since no adaptation period to the high salinity levels was required.

Intracellular and extracellular lipids production was detected for all the TPH/N ratios tested (Table 4). Intracellular lipids content was higher than extracellular, suggesting that accumulation inside the cell was promoted in all of the operational conditions.

Higher intracellular lipid contents were obtained when lower TPH/N ratios and higher feast stages durations were applied to the bioreactor. The highest intracellular lipid production (0.85 g g^{-1} CDW) was achieved in cycle XI when a TPH/N ratio of 10 and a feast stage duration of 5 days were applied. During the SBAR$_{Ab+PW}$ operation the maximum total lipids yield was 1.78 g of lipids produced per g of TPH$_{PW}$ consumed in cycle XI.

For a TPH/N ratio of approximately 10 (0.26 g L^{-1} TPH$_{PW}$ and 30 mg L^{-1} of nitrogen), the increase of the feast stage duration from 2 to 5 days (cycles I to III and IX to XI, respectively) led to an increase of approximately 7 times on the intracellular lipids production, increasing from approximately 0.11 g g^{-1} CDW to 0.74 g g^{-1} CDW. A similar result was observed in cycles IV to VIII (0.26 g L^{-1} TPH$_{PW}$ and 15 mg L^{-1} of nitrogen). With an increase of the feast stage duration from 2 to 4 days, the bacterial cells were able to accumulate approximately 45% of its cellular dry weight in lipids (0.45 g g^{-1} CDW) (cycles from VI to VIII).

The results indicate that intracellular lipids accumulation in *A. borkumensis* SK2 was dependent on the TPH/N ratio and on the duration of the feast stage, increasing when higher feast stage duration

Table 4
Nitrogen concentration, feast stage duration, TPH removal efficiency, biomass concentration, extracellular and intracellular lipids concentrations and total lipids yield obtained for the different TPH/N ratios applied during the bioreactor operation inoculated with *A. borkumensis* SK2.

Cycles	Nitrogen (mg L^{-1})	TPH/N Ratio[a]	Feast stage duration (d)	Biomass (g L^{-1})	TPH removal efficiency (%)	Intracellular lipids		Extracellular lipids (g L^{-1})	Total lipids yield (g g^{-1} of TPH$_{PW}$ consumed)
						(g L^{-1})	(g g^{-1} of CDW)		
I	30	13	2	0.95	85 ± 2.5	0.15	0.20	0.12	1.19
II	30	10	2	0.56	90 ± 3.5	0.06	0.12	0.02	0.36
III	30	9	3	0.63	85 ± 1.6	0	0	0.02	0.10
IV	15	13	2	0.69	94 ± 3.7	0	0	0.01	0.02
V	15	17	1	0.61	90 ± 8.6	0.003	0.02	0.01	0.05
VI	15	107	4	0.69	95 ± 0.5	0.22	0.40	0.05	1.08
VII	15	49	4	0.57	95 ± 1.3	0.17	0.53	0.07	0.94
VIII	15	35	4	0.56	94 ± 2.1	0.11	0.42	0.05	0.63
IX	30	10	6	0.65	93 ± 0.5	0.25	0.64	0.03	1.14
X	30	10	5	0.60	95 ± 0.1	0.16	0.74	0.06	0.88
XI	30	10	5	0.60	96 ± 1.8	0.28	0.85	0.18	1.78

[a] Correspond to the TPH/N ratio applied to the bioreactor at the start of the feast stage. This ratio was calculated by dividing the TPH concentration added to the bioreactor at the start of the feast stage by the nitrogen concentration inside of the bioreactor at that moment.

and lower TPH/N ratio were applied. Although the production of storage compounds is usually associated with unbalanced growth conditions (excess of carbon and limited amount of an essential nutrient, such as nitrogen), the results suggest that accumulation of neutral lipids in *A. borkumensis* SK2 from PW is not fully related with nitrogen limitation. Indeed, higher intracellular lipids accumulation was achieved for higher nitrogen concentrations (30 mg L^{-1} of nitrogen). Therefore, it seems that nitrogen availability in the environment was the most relevant factor to promote intracellular lipids production during SBAR$_{Ab+PW}$ operation.

Extracellular lipids production seems not to be directly influenced by nitrogen availability or feast stage duration, since similar concentrations (approximately 0.06 g L^{-1}) were obtained in all the conditions tested. Extracellular lipids production metabolism in *A. borkumensis* SK2 is not yet well understood, however it is suggested that neutral lipid excretion may be related to biosurfactants production (Manilla-Pérez et al., 2010b). *A. borkumensis* is known to produce a glycolipid biosurfactant in the presence of n-alkanes in order to access and metabolize easily hydrophobic substrates (Perfumo et al., 2010; Yakimov et al., 1998). Production of extracellular lipids by *A. borkumensis* SK2 is an advantage for the valorization of PW treatment, since production of neutral lipids is not limited by the cell volume, and therefore can make the process more economically viable.

Intracellular lipids accumulation from real PW by *A. borkumensis* SK2 was already demonstrated by Sudmalis et al. (2018) during a CSTR operation. The authors reported a similar intracellular lipid accumulation as the obtained in this work (approximately 0.31 g L^{-1}) for a carbon concentration of 0.20 g L^{-1}, a similar value as the TPH concentration added to the SBAR (0.26 g L^{-1} of TPH). Accumulation of intracellular lipids by *A. borkumensis* SK2 from simple substrates such as pyruvate and hexadecane was also reported (Kalscheuer et al., 2007). The maximum

lipid content produced from these simple carbon sources was approximately 23% (from pyruvate) and 9% (from hexadecane) in CDW. The results obtained in this work showed that from a real oilfield produced water and with a SBAR system *A. borkumensis* SK2 could reach up to 80% of its CDW.

Concerning the treatment of LW in the SBAR inoculated with *R. opacus* B4, the TPH removal efficiency varied between 55% and 79% (Table 5). The highest TPH removal efficiency (79%) was achieved in cycle III, which corresponded to the operational conditions of 1 g L^{-1} of TPH and 30 mg L^{-1} of nitrogen (Table 5). The increase of the TPH concentration from 1 to 4.4 g L^{-1} did not influence LW treatment, since similar TPH removal efficiencies were obtained throughout the process. Therefore, the results suggested that the microbial community was only capable to partially degrade the hydrocarbons present in LW. Lubricant oil is composed by a complex mixture of hydrocarbons and an unresolved complex matrix (Da Silva et al., 2016; Frysinger et al., 2003). This fraction, was reported as the most resistant to biodegradation (Frysinger et al., 2003), and may be the fraction not degraded in both TPH concentrations tested which explains the similar TPH removal efficiencies observed.

During SBAR$_{R.o+LW}$ operation, higher neutral lipid concentrations were achieved when higher TPH/N ratios were applied (cycles III to VIII) (Table 5). The highest intracellular lipids production attained was 0.94 g g^{-1} CDW in cycle VI, for a TPH/N ratio of 476. The results showed that the carbon concentration was the most important factor for lipids accumulation in *R. opacus* B4. Indeed, the increase of the TPH concentration from 1 g L^{-1} to 4.4 g L^{-1} led to a correspondent increase in the lipid accumulation (approximately 4.4 times higher).

Intracellular lipid content in SBAR$_{R.o+LW}$ operation was not significantly affected by the feast stage duration. The highest lipid production rate (of 0.34 g L^{-1} d^{-1}) and lipid yield (of 0.13 g g^{-1} PW consumed)

Table 5
DO concentration, feast stage duration, TPH removal efficiency, intracellular lipids concentrations and lipid yield obtained for the different TPH/N ratios applied during the bioreactor operation inoculated with *R. opacus* B4.

Cycle	Nitrogen (mg L^{-1})	TPH$_{LW}$ (g L^{-1})	TPH/N Ratio[a]	Feast stage duration (d)	TPH removal efficiency (%)	Intracellular lipids (g g^{-1} of CDW)	Lipids production rate (g L^{-1} d^{-1})	Lipids Yield (g g^{-1} TPH$_{LW}$ consumed)
I	30	1	61	4	56 ± 1.17	0.13	0.03	0.02
II	30	1	175	5	46 ± 0	0.18	0.02	0.02
III	30	1	3247	7	79 ± 1.49	0.28	0.03	0.02
IV	30	4.4	861	2	n.d	0.84	0.06	n.d
V	30	4.4	809	2	n.d	0.84	0.23	n.d
VI	30	4.4	476	4	71 ± 0.6	0.94	0.03	0.04
VII	15	4.4	815	2	55 ± 2.1	0.92	0.05	0.04
VIII	15	4.4	2821	1	57 ± 3.8	0.87	0.34	0.13

[a] Correspond to the real TPH/N ratio applied to the bioreactor at the start of the feast stage. This ratio was calculated by dividing the TPH concentration added to the bioreactor at the start of the feast stage by the nitrogen concentration inside of the bioreactor at that moment.

were obtained in the operational conditions of 4.4 g L^{-1} TPH$_{LW}$ and 15 mg L^{-1} of nitrogen and shorter feast stage duration (1 day in cycle VIII). A similar outcome was observed for the operational condition of 4.4 g L^{-1} TPH$_{LW}$ and 30 mg L^{-1} of nitrogen. A high lipid production rate of 0.23 g L^{-1} d^{-1} was achieved once again in the cycle with the lower feast stage duration (2 days in cycle V).

The SBAR$_{R.o+LW}$ results showed that TPH/N ratio was the most relevant parameter in neutral lipid accumulation, which is in accordance with the results reported by Castro et al. (2018).

The obtained results showed the suitability of the SBAR design for the aerobic biological treatment of different types of mineral oil-based wastewaters and its valorization for the production of neutral lipids. SBAR systems are commonly used for the removal of nutrients and/or toxic compounds from several wastewaters, including saline wastewaters (Li et al., 2017; Nguyen et al., 2016). From the authors knowledge, this is the first time that the feasibility of a SBAR system for an efficient aerobic biological treatment of different types of mineral oil-wastewaters coupled with neutral lipids production is reported.

3.2. Neutral lipids identification by ATR-FTIR

The lipid extracts obtained from both reactors were analyzed by ATR-FTIR, in order to identify the different types of lipids produced during the biological treatment of PW and LW. Several studies have reported the feasibility of this technique for the determination of the lipid content accumulated in oleaginous yeasts and microalgae in response to nutrient stress conditions such as nitrogen limitation (Esther Elizabeth Grace et al., 2020). The absorption bands observed in the wavenumber range from 4000 to 500 cm^{-1} were assigned to specific molecular groups based on published studies (Table 6). As reported in the literature, absorption bands in the spectral regions of 3020–2800 cm^{-1}, 1800–1700 cm^{-1}, 1500–1300 cm^{-1}, 1100–1200 cm^{-1} and 800–700 cm^{-1} are related to lipidic groups (Table 6) (Shapaval et al., 2019).

Only the spectrums that corresponded to the operational conditions with higher lipids production in SBAR$_{Ab+PW}$ and SBAR$_{R.o+LW}$ were analyzed by ATR-FTIR.

Fig. 2 shows the FTIR spectrum correspondent to the intracellular and extracellular lipids produced by the bacterial cells, likely of *A. borkumensis* SK2, in cycle XI of SBAR$_{Ab+PW}$ operation (Fig. 2(A)) and the intracellular lipids produced by the bacterial cells, likely of *R. opacus* B4, in cycle VI of SBAR$_{R.o+LW}$ operation (Fig. 2(B)). Similar intracellular and extracellular lipids spectrum profiles were obtained throughout SBAR$_{Ab+PW}$ and SBAR$_{R.o+LW}$ operation, showing that the same type of lipids were accumulated, regardless of the operational conditions applied during the treatment process. However, significant differences in the absorption frequencies were observed between the intracellular and extracellular lipids spectrums of SBAR$_{Ab+PW}$ (Fig. 2(A)), suggesting the production of different types of lipids intra- and extracellularly.

The intracellular lipids spectra for both SBAR operation (SBAR$_{Ab+PW}$

Table 6

Assignment of the bands found in ATR-FTIR spectrum of extracellular and intracellular lipids extracts of SBAR$_{Ab+PW}$ and SBAR$_{R.o+LW}$ operations according to literature.

| Wavenumber range (cm^{-1})	Band assignment from the literature
2955	stretching vibration of acyl chains $\nu_{as}(CH_3)$
2920	stretching vibration of asymmetric acyl chains $\nu_{as}(CH_2)$
2850	stretching vibration of symmetric acyl chains $\nu_s(CH_2)$
1708	stretching vibration of the ester group $\nu(C=O)$ in fatty acids
1740	stretching vibration of the ester group $\nu(C=O)$ in TAGs
1460	asymmetric bending vibration of acyl chains $\delta_{as}(CH_3)$
1370	symmetric bending vibration of acyl chains $\delta_s(CH_3)$
1300–1100	stretching vibration of esters $\nu(C-O)$
720	methylene rocking vibration $\rho(CH_2)$
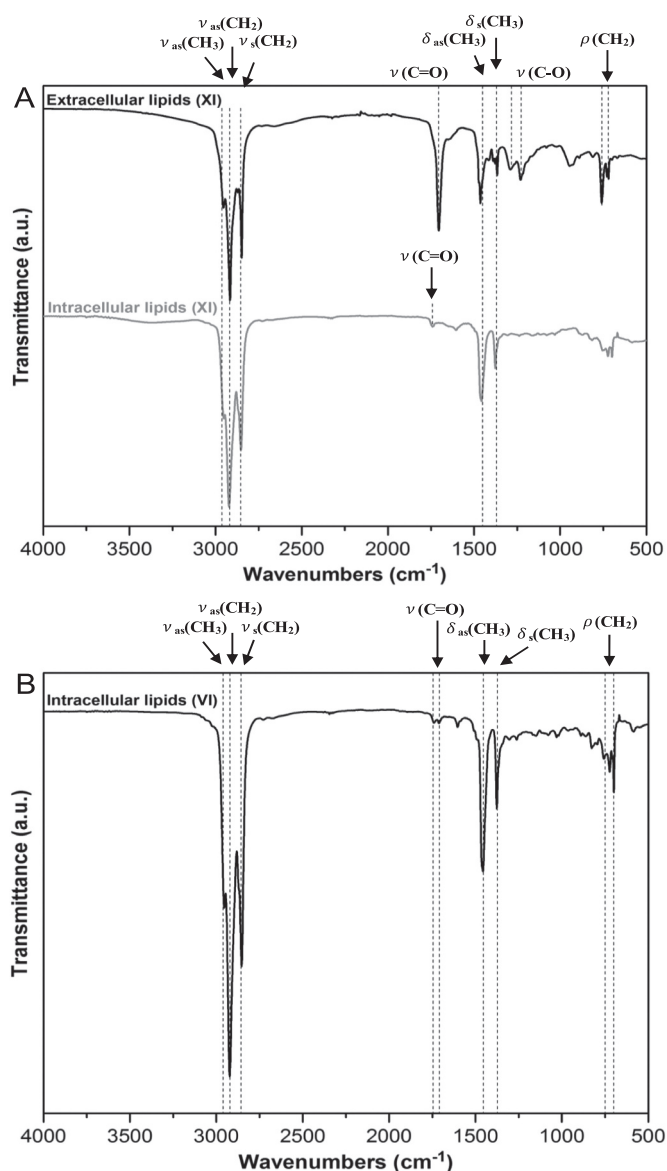
Fig. 2. ATR-FTIR spectrum of the lipids produced by: (A) *A. borkumensis* SK2 and (B) *R. opacus* B4 during SBAR$_{Ab+PW}$ and SBAR$_{R.o+LW}$ operations, respectively.

and SBAR$_{R.o+LW}$) revealed characteristic absorption bands around 2955 cm^{-1}, 2925 cm^{-1} and 2850 cm^{-1}, assigned to asymmetric $\nu_{as}(CH_3)$, asymmetric $\nu_{as}(CH_2)$ and symmetric $\nu_s(CH_2)$ stretching vibrations of acyl chains from fatty acids present in TAGs, respectively (Shapaval et al., 2019); and a band at 1745 cm^{-1}, attributed to the stretching vibration of the ester group $\nu(C=O)$ in the carbonyl group of TAG (Fig. 2) (Esther Elizabeth Grace et al., 2020). This peak is a characteristic vibration representative of the presence of TAG (Esther Elizabeth Grace et al., 2020), suggesting that this type of neutral lipids was the most predominant type accumulated intracellularly by both microorganisms.

In the intracellular lipid spectrum of SBAR$_{R.o+LW}$ and in the extracellular lipid spectrum of SBAR$_{Ab+PW}$ a band at 1710 cm^{-1} corresponding to the stretching in esters $\nu(C=O)$ was detected which can indicate the presence of free fatty acids (Shapaval et al., 2019).

The extracellular lipid spectrum profile of SBAR$_{Ab+PW}$ showed characteristic absorption bands at approximately 2955 cm^{-1} $\nu_{as}(CH_3)$, 2925 cm^{-1} $\nu_{as}(CH_2)$ and 2850 cm^{-1} $\nu_s(CH_2)$; additional peaks in the region between 1300 and 1100 cm^{-1} which are related to the stretching

vibration of esters ν(C–O), as well as the presence of a band at 1700 cm^{-1}, typical of the absorption frequency for the carbonyl group in aliphatic esters ν(C=O). This result suggests the extracellular production of WE by *A. borkumensis* SK2 (Dubis et al., 1999; Forfang et al., 2017).

Other absorption bands characteristic of lipids were detected in the extracellular and intracellular spectrums of SBAR$_{Ab+PW}$ and SBAR$_{R.o+LW}$. An absorption band at approximately 720 cm^{-1} that corresponds to the methylene rocking vibration ρ(CH$_2$), and two bands near 1460 cm^{-1} and 1380 cm^{-1}, corresponding to the asymmetric δ_{as}(CH$_3$) and symmetric δ_s(CH$_3$) bending vibration of acyl chains, respectively (Forfang et al., 2017).

The ATR-FTIR spectra were in accordance with the neutral lipid analysis using TLC (Fig. S3 and S4). Bands correspondent to TAG and FA were detected in the intracellular lipids fraction of SBAR$_{Ab+PW}$, indicating that TAG was the dominant neutral lipid accumulated. Bands correspondent to WE were not observed (Fig. S3 (A)). On the other hand, in the extracellular lipid profile a band correspondent to WE was detected in cycles VI, VII, IX and XI, confirming the ATR-FTIR results (Fig. S3 (B) and Table 4). Bands with very low intensity correspondent to TAG production were detected but only in cycles VI and IX (Fig. S3 (B)). In cycle VIII, the presence of the band correspondent to WE was not detected, which explains the absence of the absorption bands in the range of 1300–1100 cm^{-1} related to ν(C-O) (data not shown).

TLC neutral lipid profile from SBAR$_{R.o+LW}$ also confirmed the presence of TAG and FA in the intracellular lipid extract from cycle VI (Fig. S4). Moreover, the TLC analysis showed that TAG and FA were produced in all cycles, with more intense bands in the cycles IV, V and VI, which corresponded to the operational conditions with higher lipid accumulation (4.4 g L^{-1} of LW and 30 mg L^{-1} of nitrogen) (Fig. S4 and Table 5).

During PW treatment, *A. borkumensis* SK2 was able to produce WE and TAG. However, WE was detected in the extracellular fraction while TAG where predominantly detected in the intracellular content, which is in accordance with Sudmalis et al. (2018). The ability of *Alcanivorax* genus to produce neutral lipids such as TAG and WE, both intra- and extracellularly, from hexadecane, under unbalanced growth conditions, is reported in literature (Manilla-Pérez et al., 2011, 2010a). The genes *atfA1* and *atfA2* that encode for a homologous wax ester synthase/ acyl coenzyme A (acyl-CoA): diacylglycerol acyltransferase (WS/DGAT) were detected in the genome of *A. borkumensis* SK2, which is responsible for the synthesis of WE and TAG (Kalscheuer et al., 2007).

TAG production by *A. borkumensis* SK2 was reported to be related to nitrogen limiting conditions while WE-like compounds were growth-linked (nitrogen limiting-independent) when cultivated in a bioreactor under controlled batch conditions (Da Silva et al., 2016). This explains the higher neutral lipids productions obtained in the present study when higher nitrogen concentrations were applied to the bioreactor.

During SBAR$_{R.o+LW}$ operation, *R. opacus* B4 was able to accumulate intracellular TAG, however WE production was not observed (Fig. S3). TLC of the intracellular lipids of both SBAR$_{Ab+PW}$ and SBAR$_{R.o+LW}$, showed additional bands correspondent to TAG precursors, such as acylglycerols (MAG), diacylglycerols (DAG) and fatty acids (FA) (Fig. S3 and S4).

The results obtained in this study indicate that bacterial neutral lipid profiles and its production depends on the characteristics of the mineral oil-wastewater used as carbon source (i.e hydrocarbons profile and the salinity levels), and the cellular machinery of the bacterial species present in the bacterial community.

3.3. Bacterial community developed during PW and LW treatment

Since both SBAR$_{Ab+PW}$ and SBAR$_{R.o+LW}$ were operated under non-sterile conditions and LW and PW may have its own microbial community, it is likely that *A. borkumensis* SK2 and *R. opacus* B4 were not the only microorganisms inside the bioreactor. In SBAR$_{Ab+PW}$, 16S rRNA gene sequencing analysis of samples collected at cycle VIII (15 mg L^{-1} of nitrogen) and XI (30 mg L^{-1} of nitrogen) showed that *Alcanivorax* sp. was the most dominant microorganism within the bioreactor throughout the operation process. *Alcanivorax* sp. relative abundance represented approximately 80% and 76% of the total bacterial community in cycle VIII and XI, respectively (Fig. 3). As described by Sudmalis et al. (2018) and Kasai et al. (2002), *Alcanivorax* sp. metabolism is fully adapted to n-alkanes degradation and is known that *Alcanivorax* genus becomes dominant in the microbial community of petroleum-contaminated sea-waters when nitrogen and phosphorus are supplied in adequate amounts (Kasai et al., 2002). Under these conditions, *Alcanivorax* sp. relative abundance can reach approximately 80–90% of the total oil-degrading microbial community (Martins dos Santos et al., 2010). Therefore, it is expected that *Alcanivorax* sp. remains the most dominant microorganism within the bioreactor.

Achromobacter genus was also detected in the bacterial community with a relative abundance of 5% and 9% in cycles VIII and XI, respectively (Fig. 3). Members of *Achromobacter* genus were described as capable of aliphatic and aromatic hydrocarbons degradation and also as biosurfactant producers (Gielnik et al., 2019; Joy et al., 2017). Moreover, in the presence of crude oil some *Achromobacter* sp. produced a glycolipid with biosurfactant activity (Joy et al., 2017). Nevertheless, the high abundance of *A. borkumensis* SK2 in the microbial community during bioreactor operation suggests that this bacterium is a key player during PW treatment in SBAR.

Regarding the SBAR$_{R.o+LW}$, the 16S rRNA gene sequencing analysis showed that *Pseudomonas* sp. and *Rhodococcus* sp. were the most dominant microorganisms with a relative abundance of 42.8% and 20.6% in cycle II and 8.3% and 9.6% in cycle VI, respectively (Fig. 4). *Pseudomonas* sp. have been reported as able to degrade aromatic and/or aliphatic hydrocarbons and also to be the most dominant microorganism in environments with high concentrations of n-alkanes and cycloalkanes (Muriel-Millán et al., 2019). *Pseudomonas* sp. is known to mainly accumulate polyhydroxyalkanoates as reserve materials of carbon and energy (Mozejko-Ciesielska et al., 2019).

Several aerobic bacterial genera reported in literature capable of hydrocarbons degradation were detected in the microbial community develop throughout the reactor operation (Fig. 4). The most represented genera, with relative abundances higher than 5% were *Ochrobactrum*, *Sphingobacterium* and *Novosphingobium*. These bacterial genera have preferentially degraded aromatic hydrocarbons, particularly polycyclic
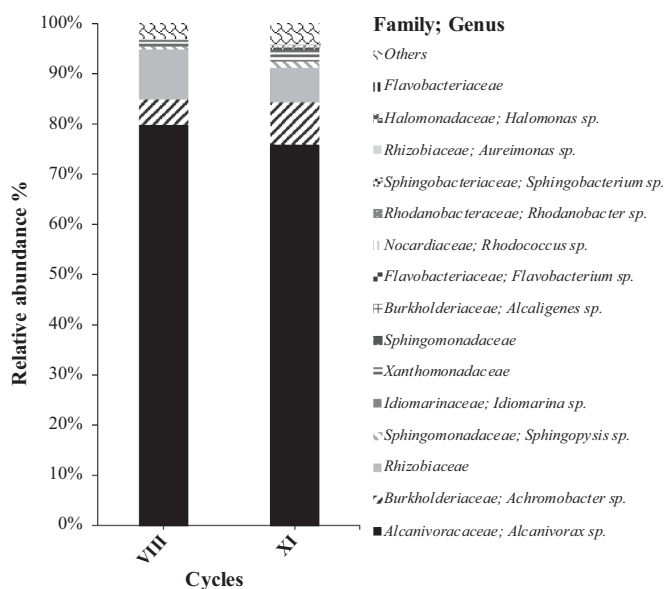

Fig. 3. Bacterial community composition during bioreactor operation with *A. borkumensis* SK2 treating PW (SBAR$_{Ab+PW}$). Only bacterial families and genera with relative abundance above 1% are represented.
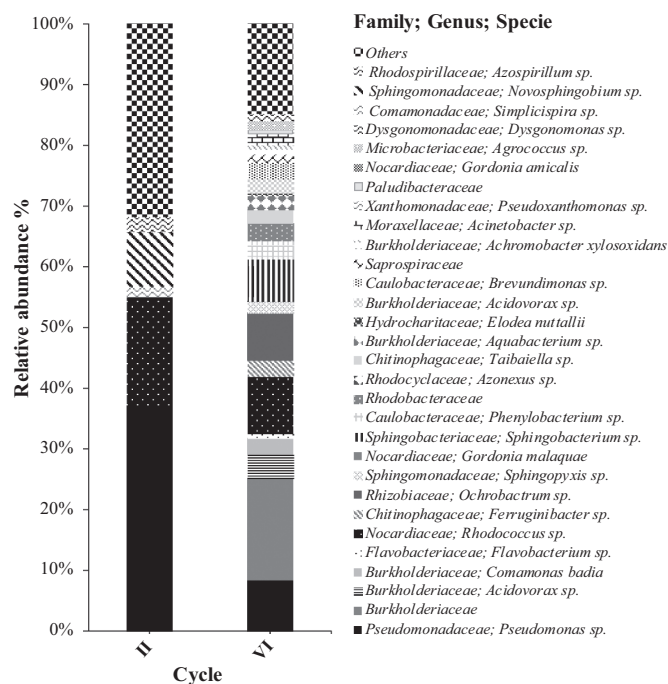
Fig. 4. Bacterial community composition during bioreactor inoculated with *R. opacus* B4 treating LW (SBAR$_{R.o+LW}$). Only bacterial families and genera with relative abundance above 1% were represented.

aromatic hydrocarbons (PAHs) that are commonly used for the manufacture of lubricating oils (Abou-shanab, 2015; Kertesz and Kawasaki, 2010; Satti et al., 2019). The lubricant wastewater used as carbon source in this study can explain the development of a bacterial community specialized in the biodegradation of these hydrocarbons. Due to n-alkanes low solubility and reactivity, and the complexity of PAHs, the biodegradation of these compounds represents a challenge to bacteria. Therefore, the development of bacterial communities in this work, able to degrade these compounds is an advantage for the treatment of both mineral oil-wastewaters used (PW and LW).

Although the microbial community analysis showed the presence of several bacterial genera able to degrade hydrocarbons, the ability for neutral lipids accumulation was only assigned to the *Alcanivorax* and *Rhodococcus* genera.

4. Conclusion

This work demonstrated the feasibility of a SBAR for the treatment of PW and LW and its valorization for neutral lipids production by *A. borkumensis* SK2 and *R. opacus* B4, respectively.

The results obtained suggest that a specialized inoculum needs to be used according to the mineral oil-wastewater characteristics. Moreover, the results demonstrated that the neutral lipid profile and its production is influenced by the inoculum and the operational conditions applied to the SBAR. Particularly, the nitrogen concentration is a determinant parameter for neutral lipids production by *A. borkumensis* SK2 from saline hydrocarbon wastewaters. For non-saline wastewaters, *R. opacus* B4 is a suitable candidate and the carbon source concentration is the most important factor for neutral lipids accumulation. TAGs were the main neutral lipids accumulated by *R. opacus* B4. In *A. borkumensis* SK2, WE production was mostly extracellular whilst TAG production was intracellular. This is an interesting result, since the different types of neutral lipids can be used for different industrial applications, in order to make the biological treatment of mineral oil-wastewaters more sustainable. In sum, this work shows that different operation strategies need to be applied depending on the type of mineral oil-wastewater and highlights

the relevance of the bacterial lipid location (intracellular or extracellular) in defining the most suitable operational strategy to maximize neutral lipids production.

CRediT authorship contribution statement

All authors contributed intellectually via scientific discussions during the work and have been involved in drafting the manuscript and revising it critically. Silva RM helped to design the experiments, performed the experimental work, analysed and interpreted the data and wrote the manuscript. Fernandes AM and Fiume F performed the experimental work related to LW treatment. Castro AR helped with TPH and lipids analyses. Machado R helped with ATR-FTIR analysis and data interpretation. Pereira MA designed the experiments, supervised all the experimental work, contributed to data interpretation and reviewed the manuscript.

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.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2020.124492.

References

Abou-shanab, R., 2015. Petroleum hydrocarbon degradation potential of *Ochrobactrum lupini* Isolated from BTEX enrichment soil. Int. J. Environ. 204–209. ISSN 2077-45084.

Aljuboury, Dr.D., Palaniandy, P., Aziz, H.A., Feroz, H., 2017. Treatment of petroleum wastewater by conventional and new technologies A review. Glob. Nest J.

Alvarez, H., Steinbüchel, A., 2002. Triacylglycerols in prokaryotic microorganisms. Appl. Microbiol. Biotechnol. 60, 367–376. https://doi.org/10.1007/s00253-002-1135-0.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583. https://doi.org/10.1038/nmeth.3869.

Camarillo, M.K., Stringfellow, W.T., 2018. Biological treatment of oil and gas produced water: a review and meta-analysis. Clean Technol. Environ. Policy 20, 1127–1146. https://doi.org/10.1007/s10098-018-1564-9.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336. https://doi.org/10.1038/nmeth.f.303.

Castro, A.R., Guimarães, M., Oliveira, J.V., Pereira, M.A., 2017. Production of added value bacterial lipids through valorisation of hydrocarbon-contaminated cork waste. Sci. Total Environ. 605–606, 677–682. https://doi.org/10.1016/j.scitotenv.2017.06.216.

Castro, A.R., Rocha, I., Alves, M.M., Pereira, M.A., 2016. *Rhodococcus opacus* B4: a promising bacterium for production of biofuels and bio-based chemicals. AMB Express 6. https://doi.org/10.1186/s13568-016-0207-y.

Castro, A.R., Silva, P.T.S., Castro, P.J.G., Alves, E., Domingues, M.R.M., Pereira, M.A., 2018. Tuning culturing conditions towards the production of neutral lipids from

lubricant-based wastewater in open mixed bacterial communities. Water Res. 144, 532–542. https://doi.org/10.1016/j.watres.2018.07.068.

Dong, Z., Lu, M., Huang, W., Xu, X., 2011. Treatment of oilfield wastewater in moving bed biofilm reactors using a novel suspended ceramic biocarrier. J. Hazard. Mater. 196, 123–130. https://doi.org/10.1016/j.jhazmat.2011.09.001.

Dubis, E.N., Dubis, A.T., Morzycki, J.W., 1999. Comparative analysis of plant cuticular waxes using HATR FT-IR reflection technique. J. Mol. Struct. 511–512, 173–179. https://doi.org/10.1016/S0022-2860(99)00157-X.

Esther Elizabeth Grace, C., Lakshmi, P. Kiruthika, Meenakshi, S., Vaidyanathan, S., Srisudha, S., Mary, M. Briget, 2020. Biomolecular transitions and lipid accumulation in green microalgae monitored by FTIR and Raman analysis. Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 224, 117382 https://doi.org/10.1016/j.saa.2019.117382.

Fakhru'l-Razi, A., Pendashteh, A., Abdullah, L.C., Biak, D.R.A., Madaeni, S.S., Abidin, Z. Z., 2009. Review of technologies for oil and gas produced water treatment. J. Hazard. Mater. 170, 530–551. https://doi.org/10.1016/j.jhazmat.2009.05.044.

Forfang, K., Zimmermann, B., Kosa, G., Kohler, A., Shapaval, V., 2017. FTIR spectroscopy for evaluation and monitoring of lipid extraction efficiency for Oleaginous fungi. PLoS One 12, e0170611. https://doi.org/10.1371/journal.pone.0170611.

Frysinger, G.S., Gaines, R.B., Xu, L., Reddy, C.M., 2003. Resolving the unresolved complex mixture in petroleum-contaminated sediments. Environ. Sci. Technol. 37, 1653–1662. https://doi.org/10.1021/es020742n.

Gielnik, A., Pechaud, Y., Huguenot, D., Cébron, A., Esposito, G., van Hullebusch, E.D., 2019. Bacterial seeding potential of digestate in bioremediation of diesel contaminated soil. Int. Biodeterior. Biodegrad. 143, 104715 https://doi.org/10.1016/j.ibiod.2019.06.003.

Heider, J., Spormann, A.M., Beller, H.R., Widdel, F., 1998. Anaerobic bacterial metabolism of hydrocarbons. FEMS Microbiol. Rev. 22, 459–473. https://doi.org/10.1111/j.1574-6976.1998.tb00381.x.

Jiménez, S., Micó, M.M., Arnaldos, M., Medina, F., Contreras, S., 2018. State of the art of produced water treatment. Chemosphere 192, 186–208. https://doi.org/10.1016/j.chemosphere.2017.10.139.

Joy, S., Rahman, P.K.S.M., Sharma, S., 2017. Biosurfactant production and concomitant hydrocarbon degradation potentials of bacteria isolated from extreme and hydrocarbon contaminated environments. Chem. Eng. J. 317, 232–241. https://doi.org/10.1016/j.cej.2017.02.054.

Kalscheuer, R., Stoveken, T., Malkus, U., Reichelt, R., Golyshin, P.N., Sabirova, J.S., Ferrer, M., Timmis, K.N., Steinbüchel, A., 2007. Analysis of storage lipid accumulation in *Alcanivorax borkumensis*: evidence for alternative triacylglycerol biosynthesis routes in bacteria. J. Bacteriol. 189, 918–928. https://doi.org/10.1128/JB.01292-06.

Kasai, Y., Kishira, H., Sasaki, T., Syutsubo, K., Watanabe, K., Harayama, S., 2002. Predominant growth of *Alcanivorax* strains in oil-contaminated and nutrient-supplemented sea water. Environ. Microbiol. 4, 141–147. https://doi.org/10.1046/j.1462-2920.2002.00275.x.

Kertesz, M.A., Kawasaki, A., 2010. Hydrocarbon-degrading sphingomonads: *Sphingomonas*, *Sphingobium*, *Novosphingobium*, and *Sphingopyxis*. In: Timmis, K.N. (Ed.), Handbook of Hydrocarbon and Lipid Microbiology. Springer, Berlin Heidelberg, Berlin, Heidelberg, pp. 1693–1705. https://doi.org/10.1007/978-3-540-77587-4_119.

Kumar, S., Gupta, N., Pakshirajan, K., 2015. Simultaneous lipid production and dairy wastewater treatment using *Rhodococcus opacus* in a batch bioreactor for potential biodiesel application. J. Environ. Chem. Eng. 3, 1630–1636. https://doi.org/10.1016/j.jece.2015.05.030.

Lane, D.J., 1991. 16S/23S rRNA Sequencing. In: Stackebrandt, E., Goodfellow, M. (Eds.), Nucleic Acid Techniques in Bacterial Systematic. John Wiley and Sons, New York, pp. 115–175.

Li, X., Luo, J., Guo, G., Mackey, H.R., Hao, T., Chen, G., 2017. Seawater-based wastewater accelerates development of aerobic granular sludge: a laboratory proof-of-concept. Water Res. 115, 210–219. https://doi.org/10.1016/j.watres.2017.03.002.

Manilla-Pérez, E., Lange, A.B., Hetzler, S., Steinbüchel, A., 2010a. Occurrence, production, and export of lipophilic compounds by hydrocarbonoclastic marine

bacteria and their potential use to produce bulk chemicals from hydrocarbons. Appl. Microbiol. Biotechnol. 86, 1693–1706. https://doi.org/10.1007/s00253-010-2515-5.

Manilla-Pérez, E., Lange, A.B., Hetzler, S., Wältermann, M., Kalscheuer, R., Steinbüchel, A., 2010b. Isolation and characterization of a mutant of the marine bacterium *Alcanivorax borkumensis* SK2 defective in lipid biosynthesis. Appl. Environ. Microbiol. 76, 2884–2894. https://doi.org/10.1128/AEM.02832-09.

Manilla-Pérez, E., Lange, A.B., Luftmann, H., Robenek, H., Steinbüchel, A., 2011. Neutral lipid production in *Alcanivorax borkumensis* SK2 and other marine hydrocarbonoclastic bacteria. Eur. J. Lipid Sci. Technol. 113, 8–17. https://doi.org/10.1002/ejlt.201000374.

Martins dos Santos, V., Sabirova, J., Timmis, K.N., Yakimov, M.M., Golyshin, P.N., 2010. Alcanivorax borkumensis. In: Timmis, Kenneth N. (Ed.), Handbook of Hydrocarbon and Lipid Microbiology. Springer, Berlin Heidelberg, Berlin, Heidelberg, pp. 1265–1288. https://doi.org/10.1007/978-3-540-77587-4_89.

Mozejko-Ciesielska, J., Szacherska, K., Marciniak, P., 2019. *Pseudomonas* species as producers of eco-friendly polyhydroxyalkanoates. J. Polym. Environ. 27, 1151–1166. https://doi.org/10.1007/s10924-019-01422-1.

Muriel-Millán, L.F., Rodríguez-Mejía, J.L., Godoy-Lozano, E.E., Rivera-Gómez, N., Gutierrez-Rios, R.-M., Morales-Guzmán, D., Trejo-Hernández, M.R., Estradas-Romero, A., Pardo-López, L., 2019. Functional and genomic characterization of a *Pseudomonas aeruginosa* strain isolated from the Southwestern Gulf of Mexico reveals an enhanced adaptation for long-chain alkane degradation. Front. Mar. Sci. 6, 572. https://doi.org/10.3389/fmars.2019.00572.

Nguyen, Phuong, Nguyen, Phuoc, Truong, H., Bui, H., 2016. The formation and stabilization of aerobic granular sludge in a sequencing batch airlift reactor for treating Tapioca-processing wastewater. Pol. J. Environ. Stud. 25, 2077–2084. https://doi.org/10.15244/pjoes/62736.

Perfumo, A., Smyth, T.J.P., Marchant, R., Banat, I.M., 2010. Production and roles of biosurfactants and bioemulsifiers in accessing hydrophobic substrates. In: Timmis, K. N. (Ed.), Handbook of Hydrocarbon and Lipid Microbiology. Springer, Berlin Heidelberg, Berlin, Heidelberg, pp. 1501–1512. https://doi.org/10.1007/978-3-540-77587-4_103.

Revellame, E.D., Hernandez, R., French, W., Holmes, W.E., Benson, T.J., Pham, P.J., Forks, A., Callahan II, A., 2012. Lipid storage compounds in raw activated sludge microorganisms for biofuels and oleochemicals production. RSC Adv. 2. https://doi.org/10.1039/c2ra01078j.

Satti, S.M., Shah, A.A., Auras, R., Marsh, T.L., 2019. Genome annotation of Poly(lactic acid) degrading *Pseudomonas aeruginosa* and *Sphingobacterium* sp. Microbiology. https://doi.org/10.1101/609883.

Schlegel, H.G., Kaltwasser, H., Gottschalk, G., 1961. Ein Submersverfahren zur Kultur wasserstoffoxydierender Bakterien: Wachstumsphysiologische Untersuchungen. Arch. Mikrobiol. 38, 209–222. https://doi.org/10.1007/BF00422356.

Shapaval, V., Brandenburg, J., Blomqvist, J., Tafintseva, V., Passoth, V., Sandgren, M., Kohler, A., 2019. Biochemical profiling, prediction of total lipid content and fatty acid profile in oleaginous yeasts by FTIR spectroscopy. Biotechnol. Biofuels 12, 140. https://doi.org/10.1186/s13068-019-1481-0.

Da Silva, P.D.M.P., Lima, F., Alves, M.M., Bijmans, M.F.M., Pereira, M.A., 2016. Valorization of lubricant-based wastewater for bacterial neutral lipids production: growth-linked biosynthesis. Water Res. 101, 17–24. https://doi.org/10.1016/j.watres.2016.05.062.

Sudmalis, D., Da Silva, P., Temmink, H., Bijmans, M.M., Pereira, M.A., 2018. Biological treatment of produced water coupled with recovery of neutral lipids. Water Res. 147, 33–42. https://doi.org/10.1016/j.watres.2018.09.050.

Yakimov, M.M., Golyshin, P.N., Lang, S., Moore, E.R.B., Abraham, W.-R., Lünsdorf, H., Timmis, K.N., 1998. *Alcanivorax borkumensis* gen. nov., sp. nov., a new, hydrocarbon-degrading and surfactant-producing marine bacterium. Int. J. Syst. Bacteriol. 48, 339–348. https://doi.org/10.1099/00207713-48-2-339.

Zhang, X., Chen, J., Wu, D., Li, J., Tyagi, R.D., Surampalli, R.Y., 2019. Economical lipid production from *Trichosporon oleaginosus* via dissolved oxygen adjustment and crude glycerol addition. Bioresour. Technol. 273, 288–296. https://doi.org/10.1016/j.biortech.2018.11.033.