Identification of organic compounds from bilge water and their biodegradation from isolated strains

G. Gatidou and I. Vyrides

Laboratory of Environmental Engineering, Department of Chemical Engineering, Cyprus University of Technology, Anexartisias 57 Str., Lemesos, 3603, Cyprus (E-mail: georgia.gatidou@cut.ac.cy; ioannis.vyrides@cut.ac.cy)

Abstract

The present study investigates the isolation of microbial strains from oil contaminated areas and real bilge water as well as their ability to biodegrade targeted both hydrocarbons frequently detected in bilge water and bilge water itself. According to the results nine microbial strains were isolated with similarity higher than 99%. Degradation experiments indicated excellent removal of both n-hexadecane and n-dodecane for all the tested concentrations which varied from 89.0 to 99.7%. Regarding real bilge water degradation, a COD removal up to 48.9% was observed. Considering the difficulties arising during bilge water treatment, the obtained data are very promising to utilize isolated native bilge waste microorganisms in treatment processes targeting in their as high as possible effectiveness.

Keywords: pure strains, real bilge water, biodegradation, dodecane, hexadecane

INTRODUCTION

Bilge water is a mixture of seawater and numerous types of wastes from several sources (Vyrides et al., 2018) resulting in the presence of compounds such as hydrocarbons, PAHs, anionic surfactants etc. in its organic phase (Behnood et al., 2014; Santisi et al., 2015; Tiselius and Magnusson, 2017). The chemical composition of bilge water varies both between vessels and also from day to day within a vessel (pH: 6.8-9.0, oil content ranges 36-2953 ppm, salinity: 25-35 g L^{-1}, COD >3-15 gL^{-1}) (Vyrides et al, 2018). The discharge of oil residue to marine environments is prohibited from the International Maritime Organization (IMO) regulations (MARPOL 73/78) and the European directive 2000/59/EC according to which the oil content of bilge water discharge cannot exceed 15 ppm at 22.2 km distance from the nearest land (Rincon and La Motta, 2014). Regarding its treatment, physicochemical methods are the most common, but they contribute substantially to operational cost. As a result, the development and optimization of bilge waste treatments to minimize costs and increase effectiveness are of high research interest worldwide (McLaughlin et al., 2014). From this point of view, the **aims of the study** were the: a) isolation of microbial strains from oil-contaminated sites and real bilge water, b) investigation of their ability to remove hydrocarbon compounds detected in bilge water and c) study of their potential to biodegrade bilge water.

MATERIALS AND METHODS

Isolation of pure bacterial strains

Real bilge water was taken from EcoFuel Limited Company (Ecofuel Ltd) which collects and treats this type of wastewater at Zygi (Cyprus), while different soil samples originated from oil-contaminated areas across Cyprus were also used. N-hexadecane, n-decane, n-dodecane, phenanthrene and phenol were tested as carbon source at four levels (100, 150, 200 and 250 mg L^{-1}). Filtered bilge water at concentration 20 % v/v was also used as carbon source. For the isolation and characterization of bacterial strains, a previous published method (Drakou et al., 2015) was applied.

GC-MS analysis of bilge water

Bilge hydrocarbons were extracted according to the main steps of ISO 9377-2:2000 as reported in Vyrides at al. (2018). Briefly, 9g of MgSO4*7H2O were added to 100 mL of sample and the pH was adjusted to pH 2 by adding hydrochloric acid. Thereafter, the sample was extracted twice with 50 mL of hexane. The organic phase was collected and after evaporation to 1mL was subjected to GC-MS analysis.

Biodegradation experiments

The most frequently isolated bacterial strains were tested for their ability to biodegrade selected hydrocarbons that have been detected in bilge water. Among the different organic compounds n-hexadecane and n-dodecane were chosen. Initially, the microbial strains were activated in MSM medium containing phenol (0.5 g L^{-1}) for 24h at 30

°C. Then 0.2% v/v of adapted inoculums were transferred in 100 ml conical flasks (25 ml working volume) containing the target substrate (n-hexadecane, n-dodecane, bilge water). The pH of the cultures was adjusted (7.0) and the flasks were placed into shaking incubator at 30°C and 100rpm for a period of 9 days. Both n-hexadecane and n-dodecane were examined for a range of concentrations up to 0.2% v/v, while bilge water was used without dilution. At different time intervals liquid samples were taken for optical density measurements (600nm) and COD analysis**.**

RESULTS AND DISCUSSION

As shown in Table 1, nine microbial strains were successfully isolated and identified using different substrates and carbon sources. Two strains named *Halomonas* sp. Hal-CG and *Exiguobacterium* sp. Ex-Ind2 (a similarity of 99.7 % and 99.3 %, respectively) were isolated and characterized as native bilge water microorganisms.

Table 1. Isolated strains using different substrates and carbon sources.

Sample	Carbon source	Concentration (mg L^{-1})	Isolated strain
Oil contaminated sea water	n-Decane	100, 150, 200, 250	*Enterobacter* sp. *SW*
Petroleum remnants in soil	n-Hexadecane	100, 150, 200, 250	*Stenotrophomonas* sp. *PE*
Oil contaminated soil	Phenanthrene	100, 150, 200, 250	*Citrobacter* sp. *D2*
	Phenol	100	*Citrobacter* sp. *S2*
			Enterobacter sp. *S3*
			Citrobacter sp. *SL*
			Citrobacter sp. *PS*
Bilge water	Bilge water	20 % v/v	*Halomonas* sp. *Hal-CG*
			Exiguobacterium sp. *Ex-Ind2*

The potential of isolated strains to degrade specific hydrocarbons is shown in Fig 2a and b. Results indicated successful removal of both n-hexadecane and n-dodecane at percentages higher than 90% for all the tested concentrations.

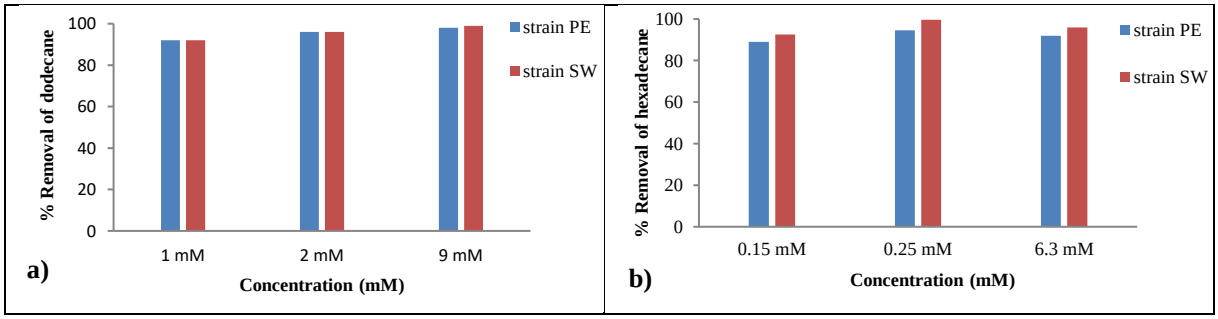

Fig. 1. Biodegradation of a) n-hexadecane and b) n-dodecane by *Enterobacter* sp. SW and *Stenotrophomonas* sp. PE after 5 days of cultivation.

Regarding the biodegradation of real bilge water from isolated strains, a COD removal between 28.0 (*P. aeruginosa* LVD-10) and 48.6% *(Enterobacter* sp. SW) was achieved. Additionally, GC-MS analysis before and after the treatment with the several microorganisms, indicated that some compounds that initially existed in bilge water are reduced but others may be totally removed depending on the tested strain. It's worth mentioned that in some cases, new compounds were detected in the effluent, probably due to bacterial biosynthesis.

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