RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Scale-up of removal process using a remediating-bacterium isolated from marine coastal sediment

Laura Ferreira, Emilio Rosales, M. Ángeles Sanromán, Marta M. Pazos*

Department of Chemical Engineering. Campus As Lagoas Marcosende. University of Vigo, Vigo 36310 Spain

* Corresponding author: Tel.: +34 986 818723; fax: +34 986 812380. E-mail address: mcurras@uvigo.es

ABSTRACT

Nowadays, a wide variety of pollutants is discharged to different water sources and become water contaminants. To overcome this problem, bioremediation has been studied as an alternative for their treatment due to its low cost, high efficiency and environmentally friendly. However, it is necessary to search microorganisms able to remediate the pollutants of different nature. In this work, the isolation and identification of remediating-bacteria from polluted marine coastal sediment was carried out. The isolation assays were carried out using phenanthrene as only carbon source. The identification of the strains was performed by PCR amplification of 16S rDNA. It was resolved in one band and the sequencing showed that this band was derived from *Serratia plymuthica.* The removal ability of this microorganism was assayed with phenanthrene, benzo[a]pyrene, different insecticides and Cr(VI). The isolated bacterium showed high removal ability of imidacloprid, methomyl, fenamiphos, and Cr(VI). After that, bioreactor scale-up (5 L) was studied with a mixture of these pollutants. Total removal degrees were obtained for organic compounds and around 96% of Cr(VI) removal was reached. Furthermore, the removal rate of the different pollutants fitted well to a logistic model. The present study states that the identified bacterium can play a vital role in bioremediation of aquatic environment polluted with mixtures of contaminants

Keywords: Persistent Organic Pollutants (POPs); Bioremediation; Environmental Biotechnology; Wastewater.

1. INTRODUCTION

The contaminants released into the environment have increased in the last years as a result of anthropogenic activities. Therefore, a wide range of pollutants have been found in aquatic environments such us polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides, polychlorinated biphenyls, organophosphates pesticides and carbamates[1].Bioremediation is considered a sustainable tool for environmental management because of it is an environment-friendly and cost-effective technology with great potential to remove pollutants [2, 3]. Accordingly, the high potential of removal-microorganisms has favoured the development of bioremediation techniques for contaminated soils, water and groundwater [4]. Bioremediation can be regarded as an attractive technology that results in the complete mineralization of organic compounds to harmless end products such as CO_2 and H_2O.The main advantage of using biological sources is its ability to multiply and magnify in terms of initial inoculum as compared to physical and chemical means of treatment.

Overall, the success of a bioremediation process is conditioned by a number of key factors including microorganisms, environmental conditions and availability of contaminant. Thus, for example, the branched structures and the aromatic rings are more difficult to metabolize those linear structures. Conversely, compounds containing nitrogen or sulphur are usually more readily biodegradable, because these elements are easily assimilated source of essential nutrients in the microbial metabolism [5].

Recent efforts have been carried out in order to discover and characterize novel microorganisms for remedial purposes [6, 7]. Microorganisms able to remediate pollutants can be isolated from many different environments, including both contaminated and uncontaminated sites and even marine sediments At the present time, a number of bacterial species are known to degrade complex organic compounds such as PAHs or polychlorinated biphenyls (PCBs). Among them *Paenibacillus* spp., *Pseudomonas* spp., *Haemophilus* spp., *Mycobacterium* spp., *Rhodococcus* spp. are some of the most commonly studied degrading-bacteria [8-10]. It is well-known that the biodegradation process depends mainly on the microbial strain used, the nature and properties of contaminants and existing environmental conditions. Under this premise, the isolation of the microorganisms, from the polluted environments, has become one of the main sources of degrading-microorganisms [11, 12].

Marine sediments are formed by particulate matter that settles out of the water column. Therefore, the discharges of pollutants, especially hydrophobic organic compounds, in the water body results in high levels of pollution in these sediments. Marine coastal sediment ecosystems are characterized by remarkable heterogeneity, owning high biodiversity and are subjected to fluctuations in environmental conditions, especially to important oxygen oscillations due to tides [13]. Thus, it is expected that microorganisms, present in this environment, will be able to develop and grow, under aerobic or anaerobic conditions, using these organic pollutants as carbon source.

The objective of this study is to ascertain the capacity of microorganisms isolated from polluted marine coastal sediment for bioremediation process. Initially, the isolation of microorganisms, that survive and growth in a synthetic polluted media, was evaluated under anaerobic and aerobic conditions. After that, the ability of the isolated microorganisms was tested with other pollutants such as insecticides and Cr(VI). Finally, the scaled-up of the bioremediation process was tested in bioreactor assays.

2. MATERIALS AND METHODS

2.1. *Sampling*

Polluted marine coastal sediment was employed for the isolation of the microorganisms. The sediment sample was collected at a depth of 20 cm from an ecosystem with continuous crude-oil spills located at the North-West of Spain (42°29'48.66''N-8°61'55.53''O). The samples were collected using a stainless steel spoon, placed in glass bottles and preserved at 4ºC.

2.2. *Minimal medium*

Minimal medium (MM) was selected for the isolation and removal assays [14]. This medium was composed of $Na_2HPO_4 \cdot 2H_2O$ 8.5 g/L, KH_2PO_4 3.0 g/L, NaCl 0.5 g/L, NH_4Cl 1.0 g/L, $MgSO_4 \cdot 7H_2O$ 0.5 g/L, $CaCl_2$ 0.0147 g/L. The medium pH was initially adjusted to 7.5 and the solution was autoclaved at 121°C for 20 min. After that, trace elements and micronutrients were added by 0.22 μm filtration $MgSO_4$ 0.24 g/L, $CaCl_2$ 0.555 μ g/L, $CuSO_4$ $4 \cdot 10^{-4}$ g/L, KI $1 \cdot 10^{-3}$ g/L, $MnSO_4 \cdot H_2O$ $4 \cdot 10^{-3}$ g/L, $ZnSO_4 \cdot 7H_2O$ $4 \cdot 10^{-3}$ g/L, H_3BO_3 $5 \cdot 10^{-3}$ g/L and $FeCl_3 \cdot 6H_2O$ $2 \cdot 10^{-3}$ g/L.

2.3. *Screening assays using sediment as inoculum*

Aerobic and anaerobic removal assays were performed using the collected marine sediment as inoculum. In these assays, Erlenmeyer flasks (250 mL) containing 50 mL of MM medium were inoculated with 2 g of sediment. In these assays, phenanthrene (20 mg/L) was used as carbon source, and Tween 80 1% and acetone 2% were used to assure the phenanthrene solubilization. Cellulose and gum stoppers were employed to assure aerobic and anaerobic conditions, respectively. Anaerobic cultures were sparging with N_2 gas in order to eliminate O_2. Flaks were incubated in an orbital shaker at 30°C and 100 rpm during 7 d. Samples were

taken during this time in order to evaluate phenanthrene removal. The assays were performed in triplicate and control assays, using autoclaved sediment, were accomplished in order to test the phenanthrene adsorption by the sediment. The reported results were the mean values with a standard deviation lower than 7%.

2.4. *Strain isolation and preservation*

After screening assays using sediment as inoculum, 200 µL of liquid media were extracted from the flask and spread on Petri plate which contained 10 g/L bacteriological peptone, 2 g/L casein peptone, 6 g/L NaCl, 10 g/L glucose, 2 g/L yeast extract that was solidified with 1.5% agar. The plates were incubated at 30ºC temperature for 24 h. The colonies grown on the plates were picked and streaked on new Petri plate for isolation of pure culture of microorganism. This procedure was repeated several times in order to obtain a pure culture.

For microbial strain preservation Petri plate which contained the microorganism growth, was washed off with a solution of NaCl (0.9%) and the content was used to inoculate Erlenmeyer flask (250 mL) containing 50 mL of autoclaved rich media (RM) composed of 10 g/L bacteriological peptone, 2 g/L yeast extract, 2 g/L casein peptone, 6 g/L NaCl and 10 g/L glucose at pH 7.5 [15] The flasks were incubated at 30 ± 1ºC for 24 h and passive aeration was provided by means of cellulose stoppers. After that, the culture media was centrifuged (5,000 rpm for 10 min) and the biomass was preserved in pellets form with glycerol 33% at -18ºC.

2.5. Identification

2.5.1.*Gram staining*

One drop of pure culture was transferred to the microscope slide and fixed to the surface by passing the slide quickly through the flame. The staining was performed using 77730 Gram Staining (Fluka Analytical, Sigma-Aldrich). Briefly, the slide smear was flooded with Gram's

crystal violet Solution, Gram's Iodine Solution and Gram's Decolorizer Solution, in the end; the smears were counter stained with Gram's Safranin Solution. After that, the samples were examined under microscope Olympus BX41 with 100x oil immersion lens.

2.5.2.Extraction of bacterial DNA and molecular identification

Five samples of 2 mL of GM medium containing cells in exponential growth phase were collected and centrifuged at 13,400 rpm for 10 min (Eppendorf MiniSpin 9056, F-45-12-11). DNA extraction was performed according to PowerSoil® DNA Isolation Kit, MO Bio Laboratories, Inc. DNA extracts were used to amplify the 16S rRNA gene. The 16S rRNA region was amplified by PCR using bacterial primers 27F and 1492R. Amplifications were carried out in a Bio-Rad MYCYCLER thermal cycler using a temperature gradient protocol. PCR amplification products were analysed by electrophoresis and subsequently purified according to the PCR Clean-up Gel extraction, NucleoSpin®Extract II kit. The sequencing of the PCR purified products were conducted by Eurofins MWG Operon (Ebersberg, Germany) and subjected to a GenBank BLAST in the National Center for Biotechnology Information (NCBI database) search to retrieve sequences of closely related taxa.

2.6. Flask removal assays using Serratia plymuthica

Batch assay experiments were carried out in 250 mL Erlenmeyer flasks with 50 mL of the liquid medium in the presence of the target pollutants phenanthrene, benzo[a]pyrene, imidacloprid, monocrotophos, pirimicarb, methomyl, fenamiphos or Cr(VI). Concentrations and culture mediums are described in Table 1. Flasks were inoculated with actively growing cells in RM medium (3% v/v). After that, the flasks were agitated in an incubator (Thermo scientific MaxQ800) at 100 rpm and 30ºC, passive aeration was permitted by cellulose stoppers. Samples were taken along the time and pH, biomass and pollutants concentration were analyzed. All experiments were performed in triplicate and the reported results were the

144 mean values with a standard deviation lower than 7%. Furthermore, control assays, without
145 inoculum, were accomplished in parallel in order to evaluate the pollutant natural breakdown.

146 *2.7. Bioreactor assays*

147 A 5 L stirred tank bioreactor (Biostat B., Braun, Germany) with a working volume of 3 L was
148 employed. The temperature was maintained at 30ºC by circulation of thermostatic water. It
149 was filled with MM containing benzo[a]pyrene 20 mg/L, methomyl 10 mg/L, fenamiphos 10
150 mg/L and Cr(VI) 100 mg/L. Bioreactor was inoculated with actively growing cells in RM
151 medium from flask cultures (3% v/v). Humidified air was continuously supplied at 0.2 L/min
152 and the reactor was stirred at 200 rpm. Samples were taken along the time and pH, biomass
153 and pollutants concentration were analyzed. The experiments were performed in triplicate
154 and the reported results were the mean values with a standard deviation lower than 7%.

155 *2.8. Analytical procedures*

156 *2.8.1.Sample preparation*

157 In all experiments, samples were centrifuged at 10,000 rpm for 5 min (Rotina 380 R), and the
158 supernatant was separated from the biomass to be analyzed for pH (IQ Scientific
159 Instruments), and pollutants concentration. All the analytical determinations were done in
160 duplicated, and the showed results are the mean values.

161 *2.8.2.Organic pollutants measurement*

162 Organic pollutants concentration in liquid medium was determined by HPLC (Agilent 1100)
163 equipped with an XDB-C8 reverse-phase column (Zorbax) (150 x 4.6 mm i.d., 5 μm). The
164 injection volume was set at 5 μL, and isocratic eluent (60:40 acetonitrile:water) for PAHs and
165 gradient eluent (10% →50% acetonitrile:90%→50% water) for insecticides; were pumped at
166 a rate of 1 mL/min for 10 min. Detection was performed with a diode array detector from 200

to 400 nm, and the column temperature was maintained at 20°C. The concentration of pollutants was determined using a calibration curve. The detection limits were 10µg/L and 50 µg/L for PAHs and insecticides, respectively.

2.8.3.Cr(VI) measurement

Cr concentration was determined in the supernatant of the centrifuged samples (10,000 rpm, 5 min) the by the 1,5-diphenylcarbazide method using a spectrophotometer (T60 UV Visible, PG Instruments) at 540 nm[16]. The method detection limit for Cr(VI) was 0.4 µg/L.

2.8.4.Scanning electron microscopy analyses

Series of scanning electron microscopy (SEM) images were taken to provide a visual characterization of microorganism. The samples were dehydrated; critical point dried, submerged in liquid N_2 and coated with gold. Images were collected on a FEI-Quanta 200 environmental scanning electron microscope using an accelerating voltage of 15 kV (Electron Microscopy Service, C.A.C.T.I., University of Vigo, Spain).

2.8.5.Biomass

Biomass was determined by spectrophotometer Helios Beta (Thermo Electron) at 600 nm OD600. Samples were centrifuged (10000 rpm, 5 min), and the pellet was resuspended in distilled water for biomass determination.

3. RESULTS AND DISCUSSION

3.1. Evaluation of degrading ability of the microorganisms present in the sediment.

In this work, the classical method of aqueous medium-enrichment procedure for removal-microorganisms isolation was carried out. Accordingly, a MM medium containing the phenanthrene as carbon source was inoculated with the polluted marine sediment. As it was

described in the introduction section, it was expected that aerobic and facultative anaerobic microorganisms were present in the marine coastal sediment. Consequently, aerobic and anaerobic cultures were assayed.

After 7 d, high turbidity was observed in anaerobic and aerobic cultures, meaning that microorganisms were growth under both studied conditions. The capacity of the cultures to metabolize phenanthrene was examined. Thus, the concentration of phenanthrene was measured in the liquid medium (Fig. 1). It was found that the pollutant concentration was reduced around 20% and 70%, for anaerobic and aerobic conditions, respectively. In control assays, in which the sediment was previously autoclaved, no reduction of phenanthrene concentration was detected. This fact demonstrated that no adsorption was produced in the sediment and the action of microorganisms was the responsible for the phenanthrene removal.

3.2. *Isolation and identification of PAHs remedial microorganisms*

Once the potential use of the microorganisms present in the sediment was established, the isolation and identification of microorganisms was carried out [17]. Based on the ability for phenanthrene removal, the isolation was performed from the previous aerobic cultures. After 1 d of incubation at 30ºC, the streaked on Petri plate demonstrated that only one colony was able to grow. The colony morphology was round, small, entire, opaque and beige in colour (Fig. 2a). Gram staining is widely used to visualize components under the light microscope for differentiation and identification of microorganisms [18]. Therefore, Gram staining was performed and the results were observed in the microscopy (Fig. 2b). The aspect of the samples isolated from the aerobic cultures demonstrated that the bacteria were rod shape, gram-staining-negative and non-endospore forming. SEM studies were carried out to investigate in detail the morphological aspects of the isolated microorganism. As can be seen

in Figure 2c, the morphology presented by the studied bacteria was bacillus of about average size of 1 to 2 μm long by 0.5 μm wide. In the same figure, clearly it is shown that fibers appear between the cells, therefore the production of extracellular polymeric substances is asseverated [19] [20].

The PCR amplification of 16S ribosomal DNA fragments from the aerobic culture, analyzed by denaturing gradient gel electrophoresis, resulted in one 16S ribosomal DNA band, indicating one bacterial component (Fig. 3a). By comparing the gene sequences, the isolated bacteria showed to be very closed the *Serratia plymuthica* (99%) (Fig. 3b). Phylogenetic tree infers the interrelationship of the isolated strain with closely related species from 16S rRNA sequences. The tree was generated using the Neighbor-Joining method. The phylogenetic tree (Fig. 3c) showed that the isolated microorganism was closely related to *S. plymuthica* K7.

3.3 Removal ability of S. plymuthica*: screening pollutants*

3.3.1 Removal of PAHs

Under aerobic culture conditions, the removal ability of the isolated *S. plymuthica* was evaluated in flask scale. The removal of phenanthrene, PAHs with three fused benzenes, was tested and compared with the removal of benzo[a]pyrene, PAHs with five fused benzenes. It has to point out that the removal of these pollutants has never been studied before by this microorganism. However, a good removal degree it was expected, based on our previous assays and the studies reported by Pradhan and Ingle [21] who evaluated the degradation of several aromatic compounds (such us phenol, benzoic acid, ortho-, meta-, para-cresol, protocatechuate, catechol and tryptophanby) by *S. plymuthica* [21].

In the present study, the concentrations of pollutant as well as biomass were followed along the time. The inoculated microorganism actively grew in both mediums containing as carbon

source the selected PAHs. The concentration of biomass increased with pollutant depletion. According to Okpokwasili and Nweke [22] this fact confirms that pollutants degradations are linked to rates of growth. After four days, the removal reached of benzo[a]pyrene was lower than phenanthrene (Fig. 4). The process of bioremediation depends on the metabolic potential of microorganisms to detoxify or transform the pollutant, which is further dependent on accessibility and bioavailability [23, 24]. From the obtained results it can be established that the microorganism has more affinity for the PAHs with lower molecular weight. This fact is in accordance with Atlas and Bartha [25], who determined that PAHs with high molecular weight are more recalcitrant because their bioavailability is reduced when the number of fused rings is increased. Although most microorganisms must use soluble low-molecular weight substances that are frequently derived from the enzymatic degradation of complex nutrients, the presence of Tween 80 facilitated the use of these pollutants for the isolated microorganism. This fact was also established by Montpas et al.[26] who reported that a strain of *Serratia marcescens*, isolated from a contaminated soil, degraded 2,4,6-trinitrotoluene in presence of Tween 80. They found that the presence of the surfactant was essential to facilitate rapid degradation because the surfactant increases the solubility of the pollutants making them more accessible for microbial degradation and furthermore increases the permeability of cells membrane. After 7 d, both PAHs were eliminated from the culture medium (Fig. 4), therefore the ability of the isolated microorganism for removing these pollutants was demonstrated.

3.3.2 Removal of other organic pollutants: insecticides

Five insecticides, imidacloprid, monocrotophos, pirimicarb, methomyl and fenamiphos, were assayed individually with the isolated *S. plymuthica* (Tab. 1). After 4 d, only the flaks with imidacloprid, fenaminphos and methomyl showed a clear reduction in the insecticide concentration (Fig. 4). At the end of assays, the removals reached were 4.86%, 35.63% and

20.1 % for imidacloprid, fenaminphos and methomyl, respectively. This is a very interesting finding because scarce reports were found using this microorganism with remedial purposes. Up to date, only Grant et al. [11] had reported the ability of *S. plymuthica* for introducing in their metabolic pathway synthetic pyrethroid insecticides such as flumethrin and cypermethrin. They found that this microorganism degraded the selected insecticides at least 50% after 20 d and in each case the degradation was greater than natural breakdown.

3.3.3 Remediation toxic metal: Cr(VI)

In recent times, species of *Serratia* have been reported by their ability for hexavalent chromium removal [27-29] Based on, batch assays in presence of Cr(VI) were carried out with the isolated *S. plymuthica*. Tahri et al. [29] found chromate-reducing activity associated to membrane fraction and/or cytosolic fraction of *Serratia proteamaculans.* They determined that chromium reduced by heat-treated cells suggests that membrane-associated chromate reductase activity of *S. proteamaculans* is preceded by its adsorption on the cell surface. Therefore, measurements of Cr(VI) and Cr(III) were performed in the culture medium in order to elucidate if there was chromate-reducing activity. The concentration of Cr(VI) was reduced along the time, reaching a removal higher than 60% at the end of the assays (Fig.4) However, no Cr(III) was detected in the culture medium. These facts confirmed that the removal was produced by binding of chromium with the bacterial biomass. These results are in agreement with those reported by Sowmya et al. [28] who indicated that a combined mechanism of ion-exchange, complexation, coprecipitation and immobilization was involved in the biosorption of Cr(VI) by *Serratia sp.*

3.4 Removal ability of S. plymuthica*: scale-up*

To evaluate the viability of the isolated microorganism in a real application the scale-up of the process is necessary. For this purpose, bioreactor assays using a 5 L stirred tank reactor

were carried out. In this reactor, a mixture of different pollutants was used in order to evaluate the behaviour of the isolated strain under extreme conditions of pollution. The selected pollutants for these assays were benzo[a]pyrene 20 mg/L, methomyl 10 mg/L, fenamiphos 10 mg/L and Cr(VI) 100 mg/L. The concentrations of the pollutants were followed along the time and the removals were determined (Fig. 5). As can be seen, the removal rates were lower than obtained when the pollutants were individually in the culture medium. Nevertheless, total removal degrees were obtained for organic compounds and around 96% of Cr(VI) removal was reached at the end of the assays. After assays, biomass was extracted with acetonitrile in order to evaluate the influence of adsorption process in the organic pollutant removal and negligible concentration was determined. The reported results are very promising because is the first attempt that a mixture of pollutants such as PAHs, insecticides and Cr(VI) are eliminated in the same biological treatment.

The removal data were adjusted to a logistic model (1) according to Cobas et al.[30] in which R is the pollutant removal degree (%) at a specific moment of the culture time t (d), R_0 and R_{max} are the initial and maximum removal percentage (%), respectively, and μ_R is the maximum specific removal rate (1/d).

$$R = \frac{R_{\max}}{1 + e^{\left[\ln\left(\frac{R_{\max}}{R_0} - 1\right) - \mu_R t\right]}} \quad (1)$$

Sigma Plot 8.0 software was utilized to adjust the model to the experimental data using an iterative procedure, based on the Marquardt-Levenberg algorithm, which seeks the values of the parameters that minimise the sum of the squared differences between the observed and predicted values of the dependent variable.

The maximum specific removal rate for the studied pollutants and the coefficients of determination obtained by fitting to the logistic model are represented in Table 2. The high

309 determination coefficients (R^2) for the studied pollutants indicate that the logistic equation (1)
310 perfectly fits the reported data. High specific removal rates were obtained for all
311 contaminants. The maximum removal rate was as obtained for benzo[a]pyrene which points
312 to an easier metabolisation of this compound by *S. plymuthica*.

313

314 **4. CONCLUSIONS**

315 In the present investigation, one bacterial strain was isolated from marine coastal sediment.
316 Based on 16S rDNA, the bacterium was identified as *S. plymuthica*. The isolated bacterium
317 was screened for its degrading capacity of different pollutants. It showed high removal ability
318 for pollutants of different nature such as phenanthrene, benzo[a]pyrene, imidacloprid,
319 methomyl, fenaminphos and Cr(VI). In addition, the bioremediation process using the
320 isolated strain was efficiently scale-up in a bioreactor of 5 L. According to the reported
321 results, the present study states that the identified bacterium can play a vital role in
322 bioremediation of aquatic environment polluted with mixtures of contaminants.

323

Acknowledgements: This work has been supported by the Spanish Ministry of Economy and Competitiveness, Xunta de Galicia and by ERDF Funds (Projects CTM2011-25389 and GRC 2013/003). The authors are grateful to Xunta de Galicia for financial support of the researcher Emilio Rosales under a postdoctoral grant and the Spanish Ministry of Economy and Competitiveness for financial support of the researcher Marta Pazos under a Ramón y Cajal programme.

References

1 J. Sánchez-Avila, M. Fernandez-Sanjuan, J. Vicente and S. Lacorte, *J. Chromatogr. A,* 2011, **1218**, 6799-6811.

2 S. Bhatnagar and R. Kumari, *Annu. Rev. Res. Biol.,* 2013, **3**, 974-993.

3 H. Manghabati and G. Pazuki, *RSC Advances,* 2014, **4**, 30137-30144.

4 M. A. Amezcua-Allieri, M. A. Ávila-Chávez, A. Trejo and J. Meléndez-Estrada, *Chemosphere,* 2012, **86**, 985-993.

5 R. E. McKinney, *Environmental pollution control microbiology: A fifty-year perspective*, CRC Press, 2004.

6 M. N. Sepehr, M. Zarrabi and A. Amrane, *J. Taiwan Inst. Chem. Eng.,* 2012, **43**, 420-427.

7 F. I. Hai, O. Modin, K. Yamamoto, K. Fukushi, F. Nakajima and L. D. Nghiem, *J. Taiwan Inst. Chem. Eng.,* 2012, **43**, 459-462.

8 A. K. Haritash and C. P. Kaushik, *J. Hazard. Mater.,* 2009, **169**, 1-15.

9 K. Hund and W. Traunspurger, *Chemosphere,* 1994, **29**, 371-390.

10 C. Lors, A. Ryngaert, F. Périé, L. Diels and D. Damidot, *Chemosphere,* 2010, **81**, 1263-1271.

11 R. J. Grant, T. J. Daniell and W. B. Betts, *J. Appl. Microbiol.,* 2002, **92**, 534-540.

12 E. A. Abigail. M, M. S. Samuel and R. Chidambaram, *J. Taiwan Inst. Chem. Eng.,* 2014, .

13 C. Cravo-Laureau and R. Duran, *Front. Microbiol.,* 2014, **5**.

14 M. Cobas, L. Ferreira, T. Tavares, M. A. Sanromán and M. Pazos, *Chemosphere,* 2013, **91**, 711-716.

15 Y. Hou, Y. Kong, J. Yang, J. Zhang, D. Shi and W. Xin, *Fuel,* 2005, **84**, 1975-1979.

16 USEPA, *USEPA,* 1992, **7196A**.

17 P. A. C. Braga, A. Tata, V. Gonçalves Dos Santos, J. R. Barreiro, N. V. Schwab, M. Veiga Dos Santos, M. N. Eberlin and C. R. Ferreira, *RSC Advances,* 2013, **3**, 994-1008.

18 T. Gregersen, *Eur. J. Appl. Microbiol.,* 1978, **5**, 123-127.

19 M. A. Kumar, K. T. K. Anandapandian and K. Parthiban, *Braz. Arch. Biol. Technol.,* 2011, **54**, 259-265.

20 A. Poli, G. Anzelmo and B. Nicolaus, *Mar. Drugs,* 2010, **8**, 1779-1802.

21 N. Pradhan and A. O. Ingle, *Int. Biodeterior. Biodegrad.,* 2007, **60**, 103-108.

22 G. C. Okpokwasili and C. O. Nweke, *J. Biotechnol.,* 2005, **5**, 305-317.

23 M. L. O. Hernandez, E. S. Salinas, E. D. Gonzalez and M. L. C. Godinez, *Biodegra. Life Sci.,* 2013, **DOI: 10.5772/56098**.

24 M. A. Surhio, F. N. Talpur, S. M. Nizamani, F. Amin, C. W. Bong, C. W. Lee, M. A. Ashraf and M. R. Shah, *RSC Advances,* 2014, **4**, 55960-55966.

25 R. M. Atlas and R. Bartha, *Ecología microbiana y microbiología ambiental*, Addison Wesley, Pearson Educación S.A., Madrid, 2002.

26 S. Montpas, J. Samson, É. Langlois, J. Lei, Y. Piché and R. Chênevert, *Biotechnol. Lett.,* 1997, **19**, 291-294.

27 S. Srivastava and I. S. Thakur, *Environ. Technol.,* 2012, **33**, 113-122.

28 M. V. Sowmya, C. P. Divyasree, M. I. Hussain, J. J. Braun and S. Subramanian, *Int. Miner. Process. Congr., IMPC: Innovative Process. Sustainable Growth - Conf. Proc.*, 20125150-5159.

29 N. Tahri Joutey, W. Bahafid, H. Sayel, S. Ananou and N. El Ghachtouli, *Environ. Sci. Pollut. Res.,* 2014, **21**, 3060-3072.

30 M. Cobas, L. Ferreira, T. Tavares, M. A. Sanromán and M. Pazos, *Chemosphere,* 2013, **91**, 711-716.

Figure captions

Figure 1. Phenanthrene concentration in the liquid medium after 7 d of inoculated the marine coastal sediment in anaerobic and aerobic conditions. Control assays were performed used autoclaved marine sediment.

Figure 2. Isolated microorganism (a) Petri dish, (b) Gram-negative stain under optical microscope magnification 100X2, (c) detail of SEM.

Figure 3. (a) PCR electrophoresis gel of the aerobic culture: (1) Marker NZYenzyme ladder (0.2-10 kb) (2) and (3) bacterial DNA and replicate of, (b)16S Ribosomal RNA sequencing obtaining using NCBI database and (c) Phylogenetic tree after sequencing.

Figure 4. Batch assays using the isolated strain of *S. plymuthica* with MM and different pollutants separately**.**

Figure 5. Removal obtained in the reactor assays using the isolated strain of *S. plymuthica* with MM containing a mixture of the different pollutants. (a) Benzo[a]pyrene removal (●) and the fitting to the logistic model (continuous line), Cr(VI) removal (○) and the fitting to the logistic model (dotted line). (b) Fenaminphos removal (■) and the fitting to the logistic model (continuos line), methomyl removal (□) and the fitting to the logistic model (dotted line).

Table 1. Pollutants utilized in this study and medium used in the cultures.

Table 2. Parameters defining the logistic model that characterizes the removal of studied pollutans by *S. plymuthica* in bioreactor assay.

Figure 1.

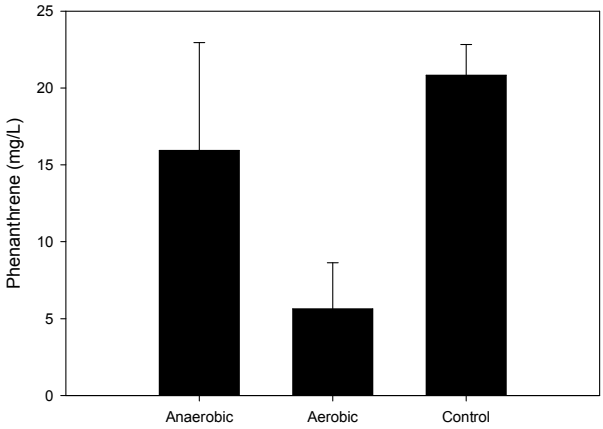
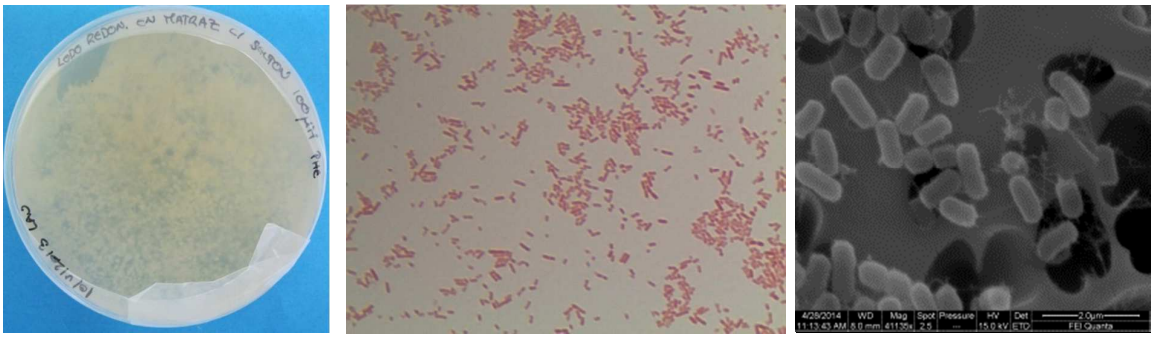
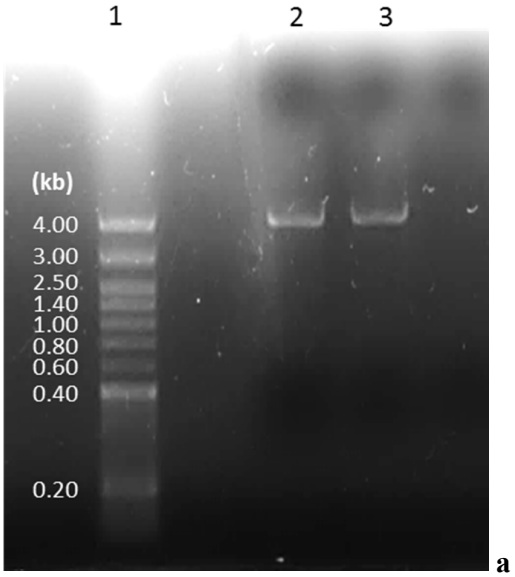


Figure 2.



a **b** **c**

Figure 3.



a

Serratia plymuthica strain NBRC 102599 16S ribosomal RNA gene, partial sequence

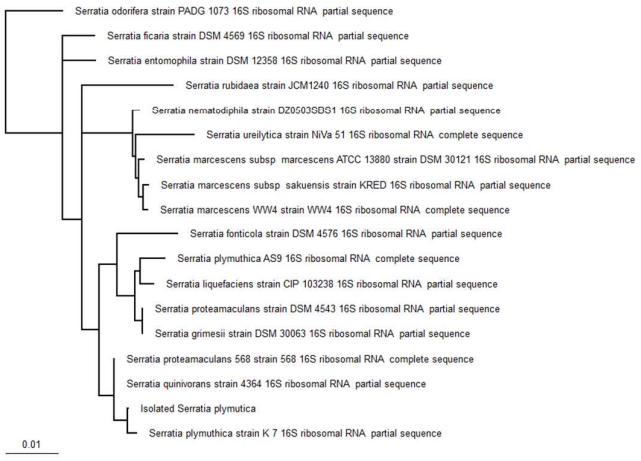
Sequence ID: refNR_114158.1 Length: 1457 Number of Matches: 1

Range 1: 53 to 851 GenBank Graphics

Score	Expect	Identities	Gaps	Strand
1471 bits(796)	0.0	798/799(99%)	0/799(0%)	Plus/Plus

```
Query  1    GAGCTTGCTCTCTGGGTGACGAGCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGA  60
Sbjct  53   GAGCTTGCTCTCTGGGTGACGAGCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGA  112
Query  61   TGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCTACGGACCAAAGT  120
Sbjct  113  TGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCTACGGACCAAAGT  172
Query  121  GGGGGACCTTCGGGCCTCACGCCATCAGATGTGCCCAGATGGGATTAGCTAGTAGGTGGG  180
Sbjct  173  GGGGGACCTTCGGGCCTCACGCCATCAGATGTGCCCAGATGGGATTAGCTAGTAGGTGGG  232
Query  181  GTAATGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGG  240
Sbjct  233  GTAATGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGG  292
Query  241  AACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGC  300
Sbjct  293  AACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGC  352
Query  301  GCAAGCCTGATGCAGCCATGCCGCGTGTGTGAAGAAGGCCTTAGGGTTGTAAAGCACTTT  360
Sbjct  353  GCAAGCCTGATGCAGCCATGCCGCGTGTGTGAAGAAGGCCTTAGGGTTGTAAAGCACTTT  412
Query  361  CAGCGAGGAGGAAGGGGTAGTGTGTTAATAGCACATTGCATTGACGTTACTCGCAGAAGAA  420
Sbjct  413  CAGCGAGGAGGAAGGGCAGTGTGTTAATAGCACATTGCATTGACGTTACTCGCAGAAGAA  472
Query  421  GCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGA  480
Sbjct  473  GCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGA  532
Query  481  ATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAAATCCCCGGGCT  540
Sbjct  533  ATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAAATCCCCGGGCT  592
Query  541  TAACGTGGGAACTGCATTTGAAACTGGCAAGCTAGAGTCTTGTAGAGGGGGGTAGAATTC  600
Sbjct  593  TAACGTGGGAACTGCATTTGAAACTGGCAAGCTAGAGTCTTGTAGAGGGGGGTAGAATTC  652
Query  601  CAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCC  660
Sbjct  653  CAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCC  712
Query  661  TGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTG  720
Sbjct  713  TGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTG  772
Query  721  GTAGTCCACGCTGTAAACGATGTCGATTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGG  780
Sbjct  773  GTAGTCCACGCTGTAAACGATGTCGATTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGG  832
Query  781  AGCTAACGCGTTAAATCGA  799
Sbjct  833  AGCTAACGCGTTAAATCGA  851
```

b



c

Figure 4.

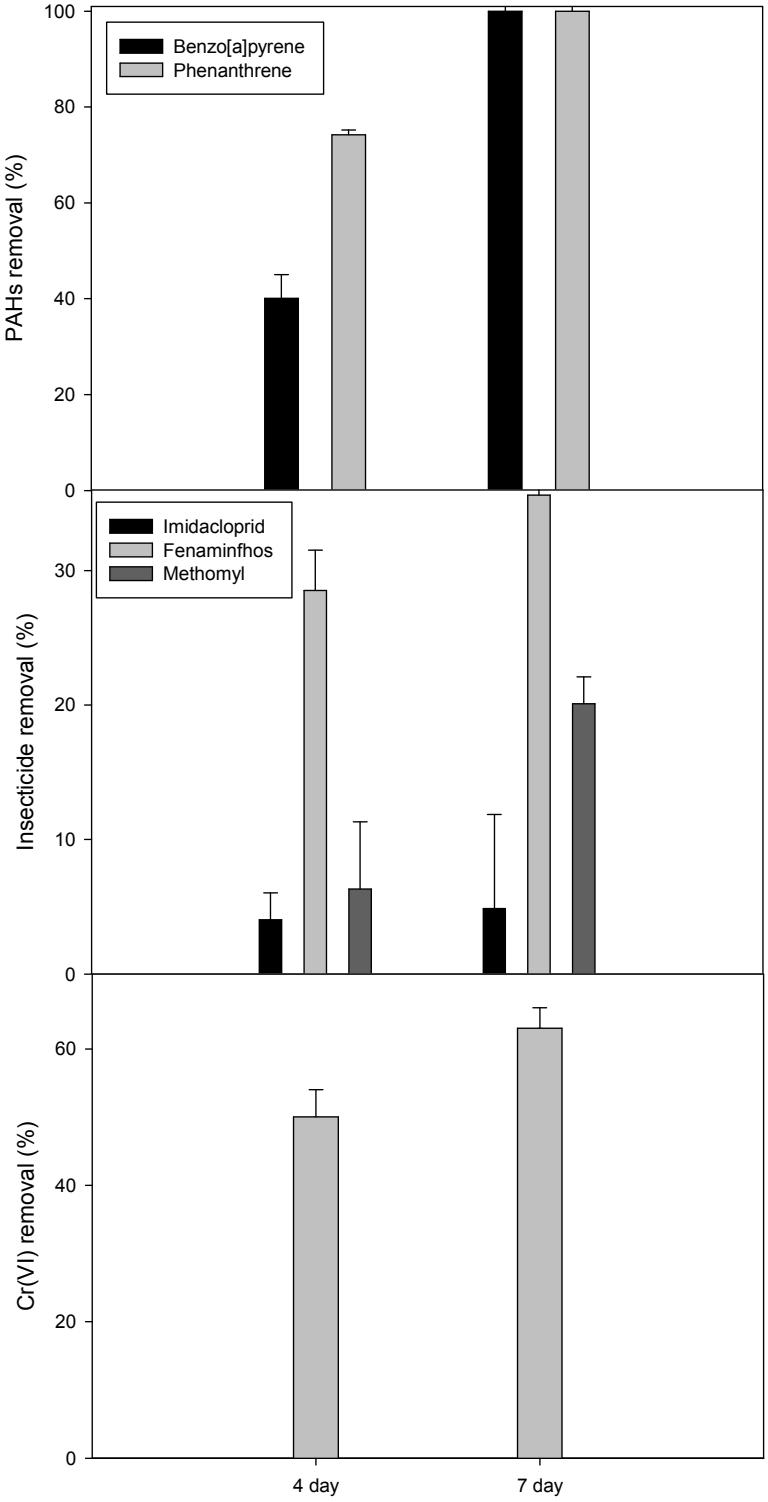


Figure 5.

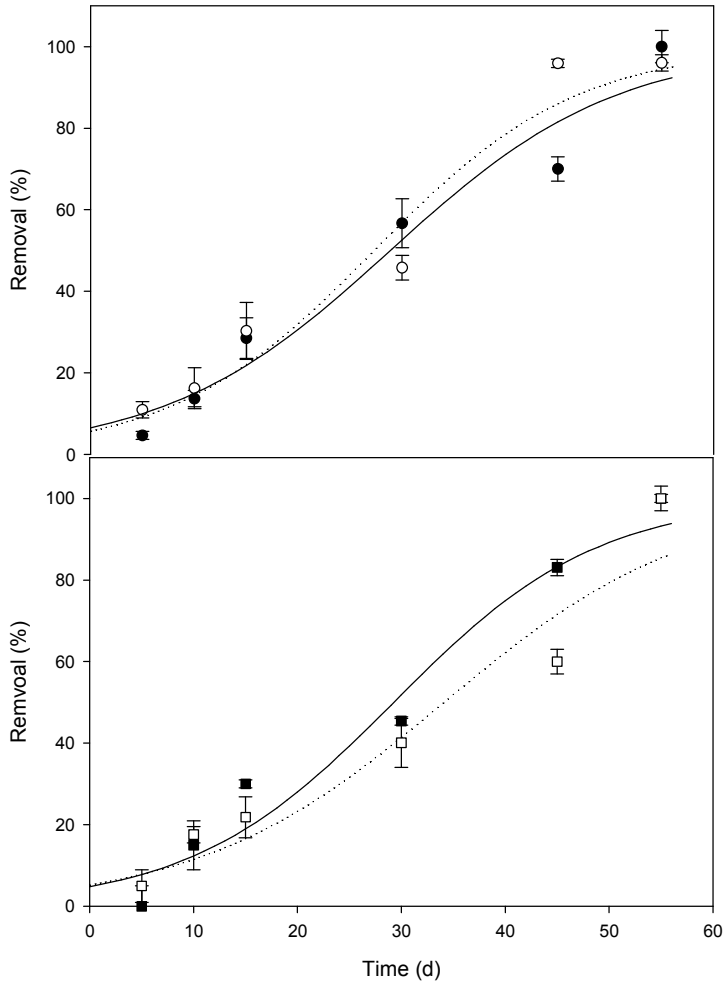


Table 1

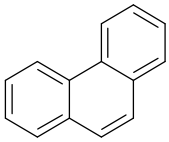
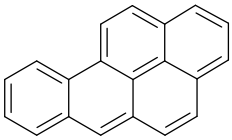
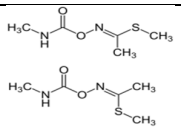
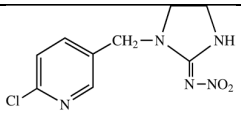
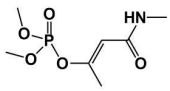
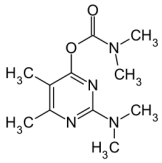
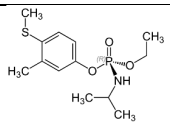
Pollutant	Type	Structure	Solubility g/L	Concentration mg/L	Medium
Phenanthrene	PAH		0.0012	20	MM+1%Tween + 2% acetone
Benzo[a]pyrene	PAH		0.0000023	20	MM+1%Tween+ 2% acetone
Methomyl	Carbamate insecticide		55	10	MM+2 g/L glucose
Imidacloprid	Neonicotinoids insecticide		0.610	10	MM+2 g/L glucose
Monocrotophos	Organophosphate insecticide		818	10	MM+2 g/L glucose
Pirimicarb	Carbamate insecticide		3.1	10	MM+2 g/L glucose
Fenamiphos	Organophosphate insecticide		0.345	10	MM+2 g/L glucose
Cr(VI)	metal	-	1680	30	MM+2 g/L glucose

Table 2.

Pollutant	R_0 (%)	μ_D (1/d)	R_{max} (%)	R^2
Benzo[a]pyrene	0.09	6.56	100	0.979
Methomyl	0.08	5.30	100	0.938
Fenamiphos	0.10	4.84	100	0.967
Cr(VI)	0.10	5.73	99	0.965