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Book chapters


Bioengineering for multiple PAHs degradation using process centric and data centric approaches


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Abstract

The study aims sequential bioengineering for multiple PAHs degradation using a process centric approach—response surface methodology (RSM) and a data centric approach—artificial neural network (ANN). The study involves stepwise media optimization protocol for multiple PAHs degradation using newly isolated Shewanella putrefaciens multiple PAHs. A non-linear model has predicted 94.76% degradation on 5th day with R²-value of 0.97. The analysis of desirability revealed the optimum value of the process conditions: 

TARPSO : 7.8 g, TARPSO : 0.8 g, TAP50 : 1.74 g, #/g For further, ANN, a neural model has predicted 95.94% degradation with R²-value of 0.99. The change in the magnitude of error functions viz. root mean square error (RMSE), mean absolute deviation (MAD) and mean absolute percentage error (MAPE) were low during ANN prediction as compared to RSM. Moreover, sensitivity analysis of both models also proves efficiency of the prediction capability and generalization of the data. Thus, for the very first time chemometrics study of medium components for multiple PAHs degradation offers constructive and powerful alternative to scientific community to design microcosm and mesocosm experiments.

Keywords

Multiple PAHs biodegradation, Response surface methodology, Artificial neural network, Data centric, Sensitivity analysis
Functional and phylogenetic diversity assessment of microbial communities at Gulf of Khachch, India: An ecological footprint

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1. Introduction

Contamination of marine environment with various toxic compounds is a serious concern for marine biota and human health (Gosai et al., 2017; Dudhagara et al., 2016a). The main route of contamination of the marine environment by these toxic compounds is the supply of energy and primary materials used for industries. This demand of energy has been increasing since last two decades as a result of growth in population and industries. This leads to the release of xenobiotic compounds like aromatic and aliphatic hydrocarbons (Dudhagara et al., 2016b; Gosai et al., 2018); polychlorinated biphenyls (PCB) (Reddy et al., 2002), and heavy metals (Chakraborty et al., 2014) in the marine ecosystem. This situation gets worse with time when the components are exposed to weathering processes, leading to increase in the relative levels of xenobiotics with higher density and viscosity (Pietroski et al., 2015).

Marine ecosystem, especially coastal sediments receive xenobiotic input from various sources such as petrogenic, pyrogenic and biogenic through industrial and anthropogenic activities (Commendatore et al., 2012). Xenobiotic contamination in the sediments is particularly severe and has become a global issue, especially in Kuwait (Al-Hashem et al., 2007; Din et al., 2008), Libya (Hamid et al., 2008), India (Gosai et al., 2017) and China (Xiong et al., 1997). The ecological indicators of these xenobiotic contaminated sediments must be inferred by measuring functional and phylogenetic traits that serve as indicators (Romaníuk et al., 2011). In context to the same, the effect of selective load of xenobiotics on microbial communities gradually leads to decrease in number of susceptible species and allow the perpetuation of adapted communities (Njoriri et al., 2004). The level of adaptation in resistant communities is because of their catalytic diversity. These may be due to either phylogenetically related or unrelated strains of same or different geographic regions. Moreover, the similarity in the biochemical properties leads to the relative flexibility in the genes among the phyla. Thus, understanding of phylogenetic diversity of contaminated ecosystems is an added benefit to understand the ecological indicators of these ecosystems. Understanding of ecological indicators can prove to

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Characterization of L-asparaginase from marine-derived Aspergillus niger AKV-MKBU, its antiproliferative activity and bench scale production using industrial waste

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A B S T R A C T
L-asparaginase (LA), an enzyme with anticancer activities, produced by marine-derived Aspergillus niger was subjected to purification and characterization. The purified enzyme was observed to have molecular weight ~50 kDa. The enzyme retained activity over a wide range of pH, i.e., pH 4–10. The enzyme was quite stable in temperature range 20–40 °C. Tween 80 and Triton X-100 were observed to enhance LA activity while inhibition of LA activity was observed in presence of heavy metals. The values for KM and Vmax were found to be 0.814 mM and 6.228 µM/mg/min. The enzyme exhibited noteworthy antiproliferative activity against various cancer cell lines tested. Successful bench scale production (in SL bioreactor) of LA using groundnut oil cake as low cost substrate has also been carried out.

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1. Introduction
L-asparaginase (L-asparagine amidohydrolase, EC 3.5.1.1) (LA) is widely used in the treatment of acute lymphoblastic leukemia chemotherapy in children in combination with other drugs as well as in other cancers [1–4]. Deficiency or lack of enzyme asparaginase synthetase does not allow tumor cells to synthesize asparagine, hence, such cells have to rely on circulating plasma pools for supply of L-asparagine [5,6]. LA brings about irreversible hydrolysis of L-asparagine to L-aspartic acid and ammonia. The tumor cells, under nutritional stress imposed by LA, undergo inhibition of DNA, RNA and protein biosynthesis ultimately resulting in apoptosis owing to cell cycle arrest in G0/G1 phase, while the normal cells remain unaffected as they possess asparaginase synthetase [7–9]. Treatment involving LA has been found to bring about complete remission in more than 90% children within 4 weeks, hence, it is a major breakthrough in modern oncology [6,10].

Though till date bacteria Escherichia coli and Erwinia carotovora have been the source for commercial production of LA for its use as effective drug, there has been a number of issues involved with it. Use of such LA has been observed to have undesirable side effects ranging from anaphylaxis, diabetes, leucopenia, neurological seizures, pancreatitis to coagulation abnormalities possibly leading to intracranial thrombosis or hemorrhage [11–14]. Hence, efforts are underway to find newer sources of LA. Besides being an anticancer agent, LA has application in food industry as well. Acrylamide (a potent carcinogen) is formed by Maillard reaction by interaction between reducing sugar and asparagine. LA is a promising alternative to mitigate acrylamide formation during bakery goods production [8,14,15].

Any protein, once produced by a biological entity, must be purified in order to characterize its physical and biological properties. A protein has to be free of contaminants before using it for any application [3].

Cost-effectiveness is an important aspect to take care of while considering enzyme production on a large scale. Harnessing industrial waste as substrate for enzyme production would lead not only to cost-effective but also cleaner production. For LA production, a number of agro-industrial wastes have been reported to be good substrates [4,16–18]. However, information on bench scale or large scale LA production using industrial waste is comparatively less.

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0141-8110/© 2017 Elsevier B.V. All rights reserved.
Concentrations, input prediction and probabilistic biological risk assessment of polycyclic aromatic hydrocarbons (PAHs) along Gujarat coastline

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Abstract A comprehensive investigation was conducted in order to assess the levels of PAHs, their input prediction and potential risks to bacterial abundance and human health along Gujarat coastline. A total of 40 sediment samples were collected at quarterly intervals within a year from two contaminated sites—Alang-Sosiya Shipbreaking Yard (ASSBRY) and Navlakhi Port (NAV), situated at Gulf of Kambhat and Gulf of Kutch, respectively. The concentration of ΣPAHs ranged from 408.00 to 54240.45 ng g⁻¹ dw, indicating heavy pollution of PAHs at both the contaminated sites. Furthermore, isomeric ratios and principal component analysis have revealed that inputs of PAHs at both contaminated sites were mixed-pyrogenic and petrogenic. Pearson co-relation test and regression analysis have disclosed Nap, Acel and Phe as major predictors for bacterial abundance at both contaminated sites. Significantly, cancer risk assessment of the PAHs has been exercised based on incremental lifetime cancer risks. Overall, index of cancer risk of PAHs for ASSBRY and NAV ranged from $4.11 \times 10^{-6}$ to $2.11 \times 10^{-5}$ and $9.08 \times 10^{-6}$ to $4.50 \times 10^{-3}$ indicating higher cancer risk at NAV compared to ASSBRY. The present findings provide baseline information that may help in developing advanced bioremediation and bioleaching strategies to minimize biological risk.

Keywords Polycyclic aromatic hydrocarbons (PAHs) · Biological risk assessment · Input prediction · Bacterial abundance

Introduction

The versatility of contaminants in the sediments of marine ecosystems has extensively amplified since last few decades (Lewis and Devereux 2009). Natural and anthropogenic activities such erosion, urbanization, industrialization and agricultural activities collectively call for an increased threat to exploitation of sediment quality. Loading of contaminants including pathogens, chemical entities, heavy metals in the coastal sediments poses threat to the ecosystem biodiversity and also to the >50% of the world population residing within 100 miles distance from the coastlines (Wakeham 1996; Bouloubassi et al. 2001).

Among the several pollutants causing concomitant threat to the marine and in close proximity to terrestrial habitats, polycyclic aromatic hydrocarbons...
Baseline

Polycyclic aromatic hydrocarbons (PAHs) at the Gulf of Kutch, Gujarat, India: Occurrence, source apportionment, and toxicity of PAHs as an emerging issue

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ABSTRACT

The present study extrapolates the assessment and characterization of a barely studied region, the Gulf of Kutch (near Jamnagar), Gujarat, India, in terms of PAH exposure, adverse effects caused by them, and various toxicological indices showing the catastrophic effects of their elevated concentrations. ΣPAH concentration in the site ranged from 118,280 to 1,099,410 ng g−1 dw, with a predominance of 2–3-ring PAHs (79.0%) as compared to 4–5- and 6-ring PAHs (20.91%). The concentrations of carcinogenic PAHs were found to be between 8120 and 160,000 ng g−1 dw, with a mean of 63,810 ng g−1 dw, which is much higher than normal acceptable values. The toxic equivalent quotient for 6CPAIs ranged from 150.47 to 25,330 ng g−1 BaPeq, encompassing 50.63% of ΣPAH toxicity. This toxicological profile of the present study site would be of paramount importance as it offers fresh information regarding the load of legacy pollutants such as PAHs and the inputs and methods to cope with their extremely high concentrations in less explored marine habitats.

Rapid economic development and the increasing consumption of petroleum and coal in developing countries such as India are among the prime reasons for the growing concern for developing methods to treat an array of pollutants, which otherwise could cause severe ailments and threaten environmental health globally. Polycyclic aromatic hydrocarbons (PAHs) are found in almost every ecological matrix because of extensive anthropogenic intrusion. They possess an ecological risk as some of them have been reported to be carcinogenic, mutagenic, and/or teratogenic in nature (Kim et al., 2013; Huang et al., 2012; Pietzsch et al., 2010).

The composition of PAH assemblage in marine sediment mainly depends on the sources of PAHs and the extent of innate processes they undergo since their discharge into marine environments (Neff et al., 2005). PAHs are exposed to various components of the ecosystem because of vehicle exhaust and other anthropogenic activities, such as industrialization, and can be accumulated in terrestrial and marine ecosystems as a result of dry and wet deposition of PAH fine particles, direct riverine and petroleum inputs, and urban runoffs. These factors contribute to elevate PAH concentrations in marine sediments, thereby making marine sediments the ultimate sinks of these refractory compounds (Bhatt et al., 2014; Pietzsch et al., 2010).

Sources of PAHs in marine sediments are classified into pyrogenic (pyrolytic), petrogenic, and diagenic sources. Pyrolytic sources include the combustion processes of fossil fuels, incomplete combustion of organic materials such as wood, coal, and oil, and anthropogenic activities characterized by HMW PAHs. Petrogenic input is attributed to petroleum products, offshore exploitations, urban runoffs, and oil seeps due to natural activities. Diagenic PAHs such as perylene are formed because of biological processes and are found in both marine and freshwater sediments (Dudhagara et al., 2016a, 2016b; Barakat et al., 2011; Jiang et al., 2009).

PAHs have always been associated with an increased risk of developing cancer in various human tissues including skin, lung, bladder, and stomach. The severity of the cancer developed depends on the mode of exposure and the form of PAHs (Kim et al., 2013). PAHs are also known as endocrine disrupting compounds because of their ability to bind endogenous receptors (Wattiau, 2002).

Recently, the surrounding areas of the Gulf of Kutch (near Jamnagar), Gujarat, India, have been experiencing a great deal of economic development as special economic zones (SEZs); this often results in the risk of environment pollution due to the release of toxic organic compounds and oil spills from petrochemical plants, ship transport, ship breaking–recycling activities, and other anthropogenic activities in marine ecosystem. However, data on the sources, mechanism, and distribution of PAHs and their potential risks to environment health is scarce. This knowledge gap needs to be filled by determining

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Bioengineering for polycyclic aromatic hydrocarbon degradation by *Mycobacterium litorale*: Statistical and artificial neural network (ANN) approach

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**ABSTRACT**

The study deals with the modeling for enhancing fluoroanthene biodegradation using a conventional process-centric approach response surface methodology, and a comparatively newer, data-centric approach artificial neural network. The study deals with the comparison of two models for enhancing fluoroanthene biodegradation using *Mycobacterium litorale*. The study involves step wise optimization protocol incorporating screening of medium components. The variables of interest were CaCl₂, KH₂PO₄, and, NH₄NO₃, screened based on Plackett-Burman model. The second step involves the CCD matrix, resulting in 51.21% degradation on the 3rd day with $R^2$ value 0.9882. The non-linear multivariate ANN has model predicted 51.28% degradation with 0.9987 $R^2$ value. The root mean square error and mean absolute percentage error values were found to be 0.3234 and 0.5715, respectively. The entire model has resulted in 51.28% degradation on 3rd day as compared to an unoptimized degradation (26.47%) on 7th day. The values obtained by ANN network were more precise, reliable and reproducible, compared to the conventional RSM model, proving the superiority of ANN model over RSM model. The study thus widens the current understanding of the scientific community for the fabrication, forecasting precisely simulated biological process for green technology.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) constitute a noteworthy group of recalcitrant and mutagenic pollutants which are produced by an array of anthropogenic activities. Even though they have natural origins, these priority pollutants are often associated with extensive urbanization and industrialization. It is the latter source which is considered to be a major cause of environmental pollution and hence, a focus of many bioremediation regimes [1,2].

Point sources of PAHs contamination are of significant environmental concern. They arise from petroleum spills, coal liquefaction and gasification, urban runoffs etc. [3]. The persistence of PAHs depends on an array of physiological and chemical peculiarities of the surroundings such as structure and type of soil, pH, temperature, oxygen availability, nutrients and water adequacy. Factors such as molecular mass, hydrophobicity, angularity, and sequestration also contribute to degradation and persistence of such pollutants in the environment. Due to extreme hydrophobicity, mutagenicity, toxicity, and elevated persistence, soils and sediments are considered to be the ultimate repositories for these pollutants. PAHs affect the biota through its various toxic actions which is considered to interfere with the normal functions of cellular membranes and enzyme systems embedded within. They are considered to be immunomodulators and can cause severe and chronic health hazards such as kidney and liver damage, DNA adduct formation and can cause genotoxic, teratogenic and carcinogenic catastrophes in humans [4,5].

The present study is a novel attempt for the remediation of historically contaminated site by expanding the current knowledge and application of model development, prediction and successful implementation by using artificial neural network (ANN) over the traditional usage of response surface modeling. The previous work of the authors relate to develop an understanding about the source identification of PAHs and their risk assessment using various multivariate models such principal component analyzes (PCA), hierarchical cluster analyzes (HCA), total equivalent factor (TEF) etc. [5].

The study incorporated herein is an extension of the previous work [5] in which models such as PCA, HCA and TEF were successfully designed and used for the quantification and risk assessment of ΣPAHs. The other objective includes the enhancement of fluoroanthene degradation (current study) for which both – Response surface methodology (RSM) and ANN models were fabricated and implemented with the utmost precision. A comparative case study thus elaborates

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Invited paper

Distribution, sources and ecological risk assessment of PAHs in historically contaminated surface sediments at Bhavnagar coast, Gujarat, India


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A B S T R A C T

The concentration, distribution and ecological risk of polycyclic aromatic hydrocarbons (PAHs) have been investigated in surface sediments near Bhavnagar coast. The concentration of ∑3 PAHs ranged from 5.02 to 981.18 µg g⁻¹ dry weight, indicating heavy pollution compared to other historically polluted study sites. It was found to be introduced via mixed origins such as burning of gas, oil, coal, production of petrochemicals, cement, and rubber tires. Domestic fuel burning and motor vehicles are also culprits for air pollution. Industrial effluents and accidental oil spillage can also be considered. PAHs can be exposed through air, water, soil and food sources including ingestion, inhalation, and dermal contact in both occupational and non-occupational levels by single or sometimes multiple exposure routes concomitantly. Furthermore, diagnostic ratios, statistical principal component analysis (PCA) and hierarchical cluster analysis (HCA) models have confirmed that the sources of PAHs were both - petrogenic and pyrogenic. For both the sites, assessment of ecological risk of the elevated levels of these pollutants has been exercised based on toxic equivalency factors (TEFs) and risk quotient (RQ) methods. The composite results indicated accurately that both the sites, bears potentially acute and chronic health hazards such as decreased immune functionality, genotoxicity, malignancy and developmental malfunctions in humans. The sites studied here and the workers have been exposed to hazardous pollutants for a longer period of time. Evidences indicate that mixtures of PAHs are carcinogenic to humans, based on occupational studies on workers, exposed to these pollutants. Hence, the present study and statistical approaches applied herein clearly indicate the historic mix routes of PAHs that resulted in magnified concentrations leading to high ecosystem risk. Thus, the scientific communities are urged to develop strategies to minimize the concentrations of PAHs from the historically impacted coastlines, thereby concerning for the future investigations and restoration of these sites.

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants of great concern in coastal marine sediments (White and Tripplett, 2002) due to their hydrophobicity, carcinogenicity, mutagenicity and toxicity. Coastal sediments are in turn considered as the ultimate reservoirs for these sequestered refractile pollutants in depositional zones which can partially be advecated. The marine sediments can thus be loaded by anthropogenically derived PAHs which are reflective of the magnitude of anthropogenic activities. Because of their hydrophobicity, the studies of these priority pollutants are useful to understand their soil-to-sea transfer which also gives a composite picture of their transport in the marine environment (Jiang et al., 2009; Liu et al., 2012).

The environment can be contaminated with PAHs from various sources encompassing household, mobile, industrial, agricultural and natural inputs. Household emissions include combustion and gasification of wood; mobile sources include all types of vehicular exhausts, whereas activities such as waste incineration, cement manufacture, petrochemicals are major industrial sources of PAHs.
Abstract

Anthracene, a three ring Low Molecular Weight (LMW) Polycyclic Aromatic Hydrocarbon (PAHs) has carcinogenic mutagenic and teratogenic effects on biota. Using various selective media, a total 33 isolates were obtained from various media. Amongst these 33 isolates, 6 isolates exhibited capability to utilize multiple PAHs as sole source of carbon and energy. Amongst 6 isolates, Sphingobium yanoikuyae strain ANT3D showed maximum degradation of anthracene. S. yanoikuyae strain ANT3D isolated from crude oil polluted sites along the Gulf of Kutch. Plackett-Burman experimental design was used to evaluate the medium components for significant anthracene degradation. The most significant variables affecting anthracene degradation were found to be KH₂PO₄, K₂HPO₄, and CaCl₂. These components were further selected for optimization to achieve maximum degradation by response surface methodology.

Keywords: Anthracene; LMW PAHs; Degradation; Plackett-Burman Design

Introduction

Polycyclic Aromatic Hydrocarbons (PAHs) comprise a large and heterogeneous group of organic contaminants that are formed either naturally or due to anthropogenic activities. Last few years, PAHs are compounds of intense public concern due to their thermodynamic stability of benzene moiety and potentially deleterious effects on human, environment and ecological health. The cleanup of such type of pollutant using innate metabolic capability of bacteria is a promising approach for bioremediation of recalcitrant compound [1,2]. Microbial degradation can be increased by manipulating nutritional requirements. A number of factors that influences the PAHs degradation by bacteria and amongst them, components of the growth are an important factor to improve the degradation rate of PAHs. The "one factor at time approach" is time consuming and expensive and does not reflect the combined effect of all variables involved. Moreover, it requires a large number of experiments for the determining or screening of components. Use of statistical model for the screening of the variables can eliminate these limitations of "one factor at time" approach. Statistical method has several advantages as being rapid, reliable, less expensive, screening of number of variables, understanding of effect of variable at various coded level [3]. Numbers of statistical models are available amongst them Plackett-Burman (PB) and Response Surface Methodology (RSM) is most widely used statistical models. Two level fractional factorial designs (PB design) is used for the screening of the variable with small number of experimental runs instead of using more extensive factorial design, which would furnish more detail explanation [4].

The present paper discusses screening of suitable medium components for degradation of anthracene by Sphingobium yanoikuyae strain ANT3D using Plackett-Burman design.

Materials and Methods

Organism and Medium

Multiple PAHs degrading isolate S. yanoikuyae strain ANT3D was selected for optimization of BH medium components as MgSO₄, CaCl₂, KH₂PO₄, K₂HPO₄, NaNO₃ and FeCl₃. The S. yanoikuyae strain ANT3D was routinely grown on BH medium amended with anthracene at concentration of 50 mg/L and stored at 4°C until use [5].

Enrichment and isolation of PAHs degrading bacteria using selective media

Soil samples were collected from crude oil contaminated saline sites near Gulf of Kutch, Jamnagar coast (latitude 22°34’17.50” N, 70°10’53.00” E, longitude) Gujarat, India. Isolation of PAHs degrading bacteria was executed by using various selective media and techniques as described below: Nagel and Andreessen’s (NA) [6] and Bushnell-Hass (BH) [7] media were used for enrichment of PAHs degrading bacteria from crude oil polluted marine sediment samples. For this, 1g of polluted sediment samples were added to 10 mL BH medium in test tube and vortexed for 1 min.
Isolation and Investigation of Biodegradation Potential of Multiple Polycyclic Aromatic Hydrocarbons (PAHs) Degrading Marine Bacteria near Bhavnagar Coast, India
Rahul K Rajpara, Dushyant R Dudhagara, Jwalam K Bhatt, Chirag M Ghevariya, Tejali B Domadiya, Haren B Gosai, Anjana K Vala and Bharti P Dave

Abstract
Present work deals with the modified isolation methods for indigenous microorganisms with a capability to use both low molecular weight (LMW) and high molecular weight (HMW) PAHs, which are pervasive recalcitrant pollutants. Methods such as biophase enrichment, and specific isolation methods has resulted in the isolation of organisms such as Sphingomonas, Pseudomonas, Mycobacterium, Achromobacter, and Streptomyces species with efficacy to degrade major portion of LMW PAHs up to 85% after 40 days of experiments, which is substantially rapid rate of degradation attributed by microorganisms. Moreover, the organisms had shown up to 30% degradation of HMW PAHs within the same time frame, making the isolation strategies more credible. The study thus, holds a prime importance of conquering the difficulties in the isolation of multiple hydrocarbons degrading microorganisms, which can be further applied for the successful application for bioremediation of hydrocarbon impacted environments.

Keywords: Biodegradation; Multiple PAHs; Selective isolation; Marine microorganisms

Introduction
Polycyclic aromatic hydrocarbons (PAHs) are class of organic pollutants that are considered as most hazardous compounds due to their toxic, mutagenic, and carcinogenic properties. PAHs can be separated on the basis of their aromatic benzene rings; low-molecular-weight (LMW) compounds consist of two or three rings, and high-molecular-weight (HMW) compounds, more than three rings. They are distributed naturally as coal and petroleum. Furthermore they are also instigated anthropogenically by burning coal, gas and vegetation, waste incineration, transportation and spillage of oil, forest fires, and volcanic eruptions [1-4]. They are listed as priority pollutants by United States Environmental Protection Agency (USEPA) due to their mutagenic and carcinogenic effects on biota.

Due to the wide variation in environmental conditions, microorganisms possess unique characteristics for adaptation to such hostile environments. Therefore, bacteria isolated from the marine environment have the capability to utilize PAHs and other recalcitrant pollutants. The main advantage of the use of these indigenous microbial communities is that it can be directly used in bioremediation study without any genetic alteration [5].

The indigenous diversity of marine bacteria has high potential to adapt to unfavorable conditions and its flux. Hence, they are considered to be important global players for the bioremediation of polluted habitats. In past years, many capable marine bacteria have been isolated to utilize LMW and HMW PAHs as carbon and energy source have been studied which includes Sphingomonas, Pseudomonas, Bacillus, Nocardia, Oceanospirillum, Alcanivorax, etc. [6].

Various microbial groups participate in the degradation of PAHs in the soil, but bacteria and actinomycetes are good candidates for soil bioremediation because they utilize a broad range of carbon sources including aromatic molecules. Moreover, sites of contamination contain multiple PAHs. It is meaningful to isolate strains that have stable degradation ability for mixture of PAHs reflecting the actual structural complexity of multicompartment PAHs in contaminated environment which is otherwise not observed in bacteria isolated from contaminated sediments having an individual PAH [7-9].

The present study aims, a focus has been shifted to isolate multiple PAHs degrading microorganisms that can successfully mineralize both LMW and HMW PAHs from contaminated sediments, and can be exploited for the restoration of PAHs impacted sites.

Based on our previous studies, isolation methods for PAHs degrading marine bacteria require extensive modification of pre-existing techniques for achieving an increase in bacterial PAHs degrading diversity with faster growth rates. Based on our experience and the difficulties encountered, we here in report various isolation and screening approaches that may overcome the pre-existing difficulties. The responses towards these modified strategies have resulted in the isolation of indigenous microorganisms capable of degrading high concentrations of multiple PAHs in polluted marine environments.

Materials and Methods
Sample collection
Polluted surface sediment samples were collected from crude oil contaminated sites near Bhavnagar coast (latitude 21.46 °N, longitude 72.11 °E, latitude 21.12 °N, longitude 72.3 °E and latitude 21.74 °N and longitude 72.12 °E) Gujarat, India using sterilized amber glass bottles and stored at 4 °C for further use.

Media and chemicals
All media were purchased from Hi-Media Laboratories, India. Solvents, reagents and chemicals used were of HPLC grade and purchased from Fisher Scientific, India. Naphthalene (Nap), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flt), pyrene (Pyr), and chrysene (Chy) were purchased from Sigma-Aldrich, Germany. Glasswares used were amber to avoid photooxidation of volatile PAHs.

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CHAPTER 11

Comprehensive Exploration of the Rumen Microbial Ecosystem With Advancements in Metagenomics

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11.1 Introduction

Beneath our superficial differences, we are all of us walking communities of bacteria.

*Lynn Margulis

The quote from Margulis stands for the ruminants too, who have been long known for their complex microbiome comprising bacteria, protozoa, archaea, fungi and viruses [1,2]. The microbes have extremely vital roles to play in maintaining the normal functionality of the host animal, so much so that reports have also depicted the need of microbial upliftment for increasing milk production and quality of its composition. Among the microbes, bacteria are the most efficient and fast feed digesters, resulting in energy production from the feed particles. They are at times classified into two major groups: the slow hydrolyzers that convert the complex feed molecules into intermediate digestible molecules, and the fast hydrolyzers that utilize the sugar molecules and are usually more prevalent in the fluid portion compared to the adhered slow hydrolyzing bacterial group. Isolation of bacteria in pure culture is typically the first step in examining microbes. Conventional culturing techniques account for less than 1% of the bacterial diversity in the majority of samples [3]. Phenotypic analysis based on culture-based studies are usually not able to reasonably differentiate between isolates. Metagenomics, the direct analysis of community DNA, is now providing better insights into the uncultivable microbial diversity encoded in nature. Metagenomics study has initiated a new era of microbial ecology with sequencing of ribosomal RNA genes in the environment. Variations in the approach have paved the way to a set of culture-independent techniques with
To whom it may concern

It’s acknowledge that Prof Anjana K Vala and Prof Bharti P Dave contributed two chapters titled, "MARINE MICROBIAL METTLE FOR HEAVY METAL BIOREMEDIATION: A PERCEPTION" and "POLYCYCLIC AROMATIC HYDROCARBONS (PAHS): OCCURRENCE AND BIOREMEDIATION IN MARINE ENVIRONMENT" in eBook titled, "Marine pollution: current status, impacts, and remedies". We are officially assigned by Bentham Publishers to compile series of eBooks on the similar theme of marine pollution. This book will probably published online in later half of this year (2018). Once again, we appreciate the work done by Prof Anjana K Vala and Prof Bharti P Dave and timely submission of assigned chapters.

Yours sincerely,

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Conferences and Workshop


Haren B. Gosai, Bhumi K. Sachaniya, Dushyant R. Dudhagara, Rahul K. Rajpara and Bharti P. Dave, “Detection of PAHs at contaminated sites along Gujarat coast, their source and toxicity assessment : As an aid to their biodegradation” at the 56th Annual Conference of the Association of Microbiologists of India (AMI), JNU, New Delhi, India from 7th-10th, December, 2015. (Poster presentation)

Workshop attended on “Marine Ecology of Gujarat Coast” organized by Department of Life Sciences, Maharaja Krishnakumarsinhji Bhavnagar University, Bhavnagar, Gujarat on 28th October, 2015.
Certificate of Participation

2016 NextGen Genomics, Biology, Bioinformatics and Technologies (NGBT) Conference
3rd - 5th October 2016, Cochin, India
Host: SciGenom Research Foundation (SGRF), India

This is to certify that

Mr./Ms./Dr. HARENGIRI GOSAI, M K BHAVNAGAR UNIVERSITY

participated in the

2016 NextGen Genomics, Biology, Bioinformatics and Technologies (NGBT) Conference
during October 3rd - 5th, 2016 at Cochin, Kerala, India.

B J Rao, Ph.D.
Senior Professor, TIFR, India

Sachdev Sidhu, Ph.D.
Professor, Uni. of Toronto, Canada

Andrew Peterson, Ph.D.
Sr. Director and Pr. Scientist, Genentech, California, USA

Akhilesh Pandey, Ph.D.
Professor, John Hopkins University, USA

Krishnaraj Rajalingam, Ph.D.
Professor, The JGU Mainz, Germany

George Thomas, Ph.D.
Director, SGRF, India

R. Chandrababu, Ph.D.
Professor, TNAU, India
Certificate of Presentation

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HARMEET KOSAI
M. K. BHAVNAGAR UNIVERSITY

presented a poster in the

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during October 3rd - 5th, 2016 at Cochin, Kerala, India.

George Thomas, Ph.D.
Director, SGRF, India

K. Rajalingam, Ph.D.
Professor, The JGU Mainz, Germany
Workshop on Marine Ecology of Gujarat Coast
Sponsored by
Ministry of Earth Sciences (MoES), GOI, New Delhi
University Grants Commission, New Delhi
Department of Life Sciences, Maharaja Krishnakumarsinhji Bhavnagar University, Bhavnagar

Certificate of Participation

This is to certify that Dr/Mr/Ms/Mrs. HARENGIRI...GOSAI.............................has participated in the Workshop on “Marine Ecology of Gujarat Coast” held on 28th October, 2015, organized by the Department of Life Sciences, Maharaja Krishnakumarsinhji Bhavnagar University, Bhavnagar.

Prof Dileep Deobagkar
Chairman, SSC-MEB

Dr P LokaBharathi
Member, SSC-MEB

Dr (Ms) Bharti P Dave
Project Co-ordinator, ESTC
Certificate

This is to certify that

Harun Gosai

has Presented a Paper entitled

Comparison of conventional methods and Artificial Neural Network in PAHs degradation study - A review

in the 2nd National Conference on 'Current Trends in Biological sciences' CTBS – 2017

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Centre of Advanced Study, Post Graduate Department of Biosciences,
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Prof. Pratibha Shukla
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Prof. Aul Kumar Jothri
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Award

- **CSIR Travel award – Full airfare support**
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SUBJECT: CSIR Foreign Travel Grant

Dear

With reference to your application on the aforesaid subject, we are happy to inform that the Director General, CSIR has been pleased to sanction foreign travel grant to enable you to attend and present your paper at the BioMicroWorld - 2017, Spain during 18 Oct 2017 to 20 Oct 2017 subject to the following conditions:-

1. The CSIR Foreign Travel Grant is limited to Full Air Fare Only payable in Indian Rupees only. The journey should be strictly performed by the shortest route in excursion economy class by Air India only. Tickets must be purchased directly from the booking counters/Website of Air India Airlines or by utilizing the services of Authorized Travel Agents viz. M/s Balmer Lawrie & Company, M/s Ashok Travels & Tours and IRTC as warranted under Govt. of India orders in this subject.

2. The grant should be claimed by filling-in the Tour report & Grant-in-Aid bill proforma after downloading from CSIR HRDG web site http://csirhrdg.res.in/tg.htm (in duplicate) along with the counter foil of original boarding pass, e-ticket and certificate of attending the conference from the organizers. The grant should be claimed within one month of return from abroad forwarded through his/her Supervisor/Head of the Institution. All the above documents should be self attested by the candidate.

3. One reprint of the research paper presented at the Conference/Symposium etc. should be sent to CSIR, invariably after its publication.

Yours sincerely,

(Signed)

Shailendra Nigam

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ii) Audit (EMR)