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Simultaneous polycyclic aromatic hydrocarbon degradation and lipid accumulation by *Rhodococcus opacus* for potential biodiesel production

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ABSTRACT

Biomass gasification effluent primarily contains polycyclic aromatic hydrocarbons (PAHs) generated during wet scrubbing of synthetic gas. These PAHs are toxic, mutagenic, carcinogenic and teratogenic, which, therefore, need to be removed prior to their release into the environment. This study was performed to determine the valorization of PAHs from biomass gasification effluent as a substrate for *Rhodococcus opacus* and its potential for biodiesel production. Using synthetic media containing 2-, 3- or 4- ring PAH compounds, at an initial concentration in the range 50–500 mg L^{-1}, along with 5% (v/v) inoculum, the bacterium degraded 75.9% naphthalene, 79.1% phenanthrene and 72.1% fluoranthene, with a corresponding lipid accumulation of 68.1%, 72.4% and 63% (w/w) of cell dry weight (CDW) respectively, within 7 days. The maximum specific growth rate (μ_{max}) of the organism was found to be 1.267×10^{-4} min^{-1}, 2.47×10^{-4} min^{-1} and 2.317×10^{-4} min^{-1} using naphthalene, phenanthrene and fluoranthene, respectively. Further, an increase in the inoculum size to 10% (v/v) had a positive effect on both PAHs biodegradation and lipid accumulation by the bacterium. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy affirmed that the fatty acids accumulated by the bacterium primarily contained saturated fatty acids. Gas chromatography (GC) analysis of the transesterified lipids to biodiesel further revealed the presence of methyl palmitate and methyl stearate as the major fatty acid methyl esters (FAMEs). The estimated properties of the transesterified product indicated its best potential for biodiesel application.

1. Introduction

The issue of global climate transformation due to CO_2 emission along with the rapid depletion of fossil fuels has directed a great attention towards greener sources of energy as an alternative to fossil fuels. As a result, studies on renewable energy sources have been the recent research focus [1]. Among the contemporary renewable energy sources, biomass gasification is excellent in terms of intensifying the biomass utilization. In the biomass gasification process, the solid fuel is thermally converted to combustible gas (producer gas) by utilizing air, steam, etc. as a gasifying agent [2].

Although biomass gasification is one of the most promising technologies for the production of valuable fuel gases, chemicals and chars, it simultaneously generates various undesirable by-products, such as NO_x, SO_2, tar and fly ash during the wet-scrubbing stage incorporated in the process to obtain clean producer gas [3]. In the internal combustion engines, tar-loaded gas cannot be consumed directly, and, therefore, wet scrubbing is employed to clean the gas via tar removal. The soluble organic compounds dissolve in water, whilst a low amount of insoluble compounds are still present as residual tar. There is a tremendous variation in the organic strength of gasifier wastewater with a chemical oxygen demand (COD) in the range 920 – 1,60,000 mg L^{-1} [4]. The organics comprise aromatics, heterocylics and phenolics, including 1 – 5 ring polycyclic aromatic hydrocarbons (PAHs), which have been identified as carcinogenic, toxic and mutagenic [5–7]. Till date, a very few investigations have been carried out for treating biomass gasifier

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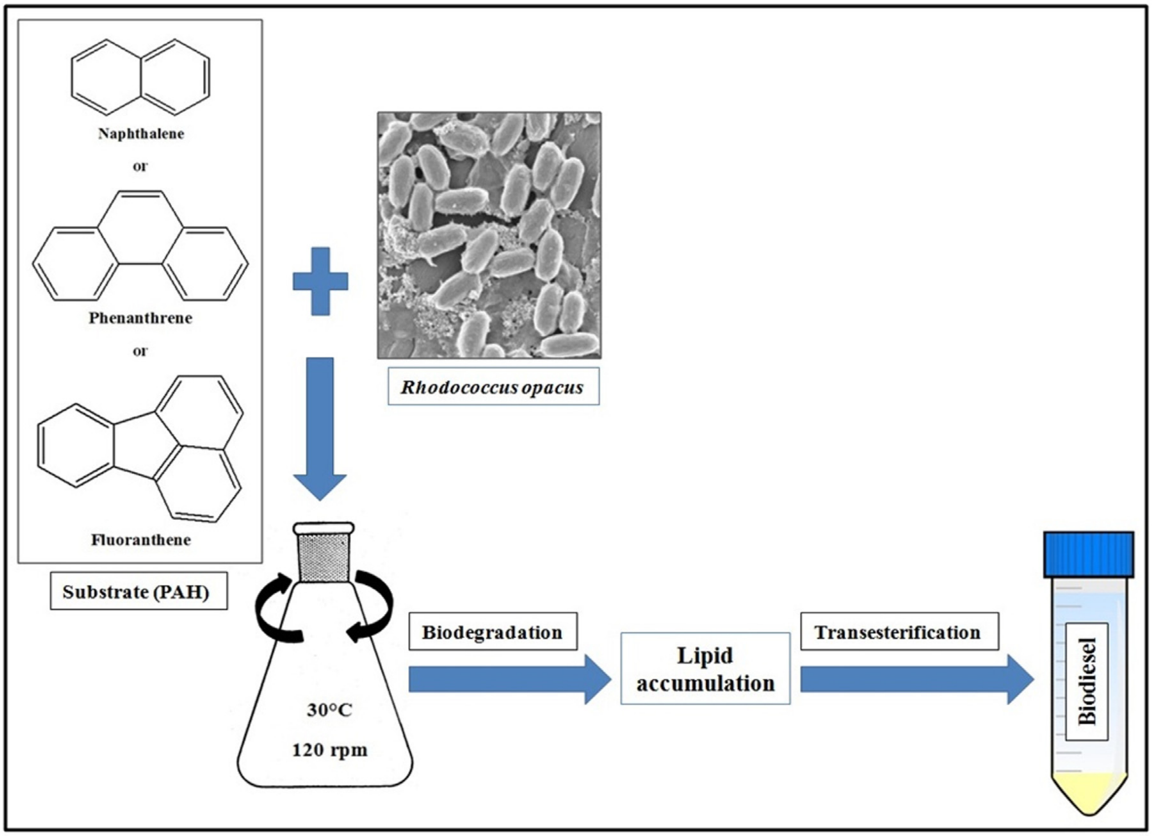

Fig. 1. A brief schematic of the experimental procedure.

Table 1
Lipid production reported in the literature for different *Rhodococcus* species grown using various substrates.

Microorganism	Substrate	Lipid% (w/w, CDW)	Lipid concentration (g L^{-1})	References
R. opacus PD630	Hexadecane	26.0	0.3	[21]
R. erythropolis 17	Pentadecane	56.0	0.2	[21]
R. opacus PD630	Carob waste	76.0	0.09	[21]
R. opacus PD630	Sugar cane molasses	93.0	0.06	[21]
R. opacus PD630	Dairy wastewater	51.0	2.2	[22]
R. opacus DSM 1069	Kraft lignin	14.9	0.07	[23]
R. opacus PD630	Glucose	26.9	0.39	[24]
R. opacus PD630	Light oil	21.9	0.08	[24]
R. opacus DSM 1069	Glucose	21.6	0.24	[24]
R. opacus DSM 1069	Light oil	22.0	0.12	[24]
R. opacus	Naphthalene	68.1	0.1	This study
	Phenanthrene	72.4	0.11	
	Fluoranthene	63	0.08	

CDW: Cell Dry Weight.

wastewater. Although physico-chemical treatment methods have been reported to yield a high removal of phenolics and COD [8], this process generates a large volume of hazardous sludge. On the contrary, biological treatment seems to be more eco-friendly and inexpensive.

On account of the presence of PAHs as environmental pollutants, there has been a strong interest in the selection of microorganisms for their biodegradation. Moreover, some studies have shown that bacteria belonging to *Alcaligenes*, *Mycobacterium*, *Pseudomonas*, *Rhodococcus* and *Sphingomonas* spp. are able to grow on high molecular weight (HMW) PAHs [9–13]. Among these organisms, the capability of the *Rhodococcus* sp. to accumulate lipids along with the degradation of PAHs is of recent interest. This bacterium belongs to the family *Nocardiaceae* and is capable of degrading a diverse range of compounds involving several biotransformation reactions [14,15]. Also, these stored lipophilic compounds have high calorific value than proteins and carbohydrates, thereby serving as a suitable food reserve for the bacterium. Other species such as *R. erythropolis* and *R. fascians* are capable of synthesizing triacylglycerol (TAG) as a storage compound for carbon and energy [16,17]. An additional advantage of these microbial oils is that they can be used as an alternative non-food oil feedstock for biodiesel production, which is more attractive than bioethanol due to the economics of the production process [18]. However, the composition and properties of the microbial oil depend on the organism and the substrate used [19,20].

Table 1 presents the literature on lipid production by different species of *Rhodococcus* grown on various substrates, which clearly reveal that more cheaply available substrates need to be evaluated for keeping the process cost low. In this context, the potential of PAHs has not been evaluated at all for lipid accumulation by *R. opacus* and biodiesel production. Hence, this study investigated