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Bioremediation of Wastewater Stream from Syngas Cleaning Via Wet Scrubbing

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Biomass gasification is a process known for its high potential in enabling the exploitation of residual biomass in the production of renewable electricity. At present one of the most favorable patterns from both an environmental and a sustainability point of view is air gasification coupled with an Internal Combustion Engine (ICE) for decentralized production. In order to have the best performances of the ICE to be run with the produced gas, the required technical specifications on gas purity can be achieved at an appropriate level by means of wet scrubbing. In wet gas cleaning approach, stages based on organic liquids (e.g. biodiesel) and water can be jointly adopted for the removal of contaminants. In the present work preliminary results for the regeneration of the wastewater stream produced at a gasification plant whose gas cleaning is carried out with a purification train based on biodiesel and water scrubbing are presented. To this aim three different fungal strains were selected and tested for their bioremediation potential, i.e. *Bjerkandera adusta*, *Arthrinium sp*, and *Pleurotus ostreatus*. Laboratory tests gave an overall positive response on the effectiveness of wastewater treatment by *Bjerkandera adusta* and *Arthrinium sp.* thus demonstrating that these microorganisms are able to metabolize, and therefore remove, both aromatic molecules typical of tar produced in gasification processes, and esters of fatty acids constituting the biodiesel used for gas washing in the first stage.

1. Introduction

Over the past decades, gasification has received, and continues to receive, large attention being a process able to allow the exploitation and valorisation of residual biomass feedstocks as a source of energy and matter of renewable origin. As is well known, gasification is the thermochemical process through which a solid fuel can be converted in a much more valuable and flexible gaseous energy carrier. Depending on the specific process and technology adopted, the produced gas can have different uses. The potential applications of such process have been explored in many key sectors, from power production for stationary application to biofuels for transport (Rüegsegger et al. 2019, Park et al. 2017, Farzad et al. 2016, Sikarwar et al. 2016, Rauch et al. 2014). In the most recent years, the integration of the gasification process in the chemical production sector is also receiving attention (Amaral et al. 2019, Centi et al. 2019, Wang et al. 2018, Benalcázar et al. 2017).
Currently, one of the most mature application is the gasification of biomass for decentralized power production (Hrbek et al. 2017, Gabbrielli et al. 2016, IRENA 2012). In particular, among the different layouts available, air gasification coupled to ICE (internal combustion engine) appears the most favorable from both an environmental and sustainability standpoint, as well as of the easy to implement. Such an approach typically aims at energy production on a small to medium scale, possibly in combination with heat (i.e. CHP mode). In order to have the best performances of the ICE to be run with the produced gas, the required technical specifications on gas purity can be achieved at an appropriate level by means of wet scrubbing.In wet gas cleaning approach, stages based on organic liquids and water can be jointly adopted for the removal of contaminants. In the present work preliminary results for the regeneration of the wastewater stream produced at a gasification plant equipped with a two-stage gas cleaning section are presented. Specifically, this section is based on a first scrubbing stage operated with biodiesel, followed by a second one run with water. In order to reduce the environmental impact associated with the management of the wastewater stream, three types of

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approach can generally be considered, that is physical, chemical and biological methods (Rajasulochana et al. 2016, Seow et al. 2016, Dhote et al. 2016, Topare et al. 2011). In the case of the wastewaters considered in the present work, according to the above mentioned description of the gas cleaning section, the expected contamination is represented by the tar molecules escaped from the first stage of biodiesel washing, together with the dragged solvent itself (i.e. fatty acid methyl esters, FAMEs). Therefore, given the nature of the contamination, the biological mode was considered the most suitable. Microorganisms like bacteria and fungi in fact possess the extraordinary ability to digest a wide range of xenobiotics and organic contaminants, among them long chain and aromatic hydrocarbons. In particular, filamentous fungi can adjust their metabolism to accommodate detrimental conditions; they can secrete, in the habitat where they live, an impressive arsenal of extracellular enzymes, which represents a powerful biochemical toolkit for the catalysis of a great number of valuable reactions (e.g. detoxification of organic pollutants). In this scenario, there is a growing scientific interest towards bioremediation approaches, where filamentous fungi can be exploited to enzymatically transform toxics generated by a specific industrial process into harmless compounds. Compared to approaches based on physical and/or chemical methodologies, the biological method appears more appropriate not only because of the nature of the contamination to be managed, but also because, being the contaminants metabolized by the microorganisms, it has a lower environmental impact. Moreover, this approach is technologically easier to implement.Microorganisms such as fungi and bacteria, able to metabolize the type of molecules mentioned above, are known in the literature (Espinosa-Ortiza et al. 2016, Ferrera et al. 2016, Ramanan eta al. 2016, Kumar). In the present work, the wastewater stream deriving from gas purification was then used in tests of bioremediation carried out with filamentous fungi, to evaluate a decontamination approach based on myco-remediation.

2. Materials and Methods

2.1 Microorganism and medium

Three candidate fungal strains were evaluated for the bioremediation of the wastewater produced from syngas cleaning via wet scrubbing, namely *Pleutorus ostreatus, Bjerkandera adusta* and *Arthrinium sp*. Pure mycelial cultures of *P, ostreatus* CCBAS 462 were obtained from Colture collection of Basidiomycetes (CCBAS)-Institute of Microbiology (Czech Republic). *B. adusta* and *Arthrinium sp.* were locally isolated from decomposing lignocellulose material and identified by molecular analysis of their internal transcribed spacer (ITS). Fungi of interest were maintained at 4°C on malt extract agar plates (MEA plates). In Figure 1, pictures of three representative plates are shown.

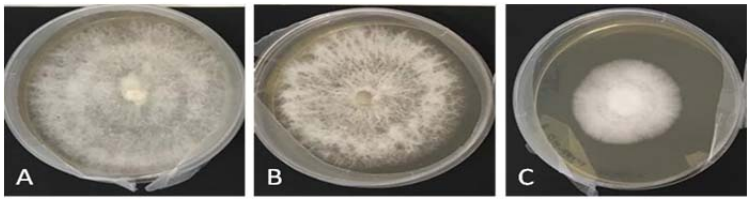

Figure 1: Fungal cultures on Petri dishes. A) Bjerkandera adusta; B) Arthrinium sp.; C) Pleurotus ostreatus.

2.2 Phenol degradation test

Candidate fungal strains were first screened for their ability to degrade phenol. Fungi of interest were cultivated on liquid medium, in 500 ml Erlenmeyer flasks containing 100 ml of 2% Malt Extract (ME). They were grown for 4 days at 28°C. Then, fungal mycelia were filtered, washed with sterile water and homogenised. For each fungal strain, 500 µl of suspension were used to inoculate 250 ml Erlenmeyer flasks, containing 50 ml of minimal mineral medium (MM) supplemented with Glucose (Table 1). Flasks were closed using rubber stoppers.

Table 1: Composition of the minimal mineral medium used for phenol degradation test

Nutrients/additives	g/L
NH_4NO_3	1.7
KH_2PO_4	2
$MgSO_4 \cdot 7H_2O$	0.5
$CaCl_2$	0.156
“Murashige & Skoog” base medium	1
Glucose	14.2

After 2 growing days, phenol was added to flasks, at the following concentrations: 2 mM and 5 mM. Fungal cultures were incubated in an orbital shaker (100 rpm) for 12 days, at 28° C. For each fungus and for each used concentration of Phenol, two biological replicates were prepared. In addition, culture controls and abiotic controls were also prepared, omitting the Phenol and filamentous fungus, respectively. The experimental scheme is shown in Table 2. After 12 days, culture supernatants were collected and analysed by High Pressure Liquid Chromatography (HPLC).

Table 2: Scheme of phenol degradation experiments with different fungal microorganisms.

Fungal degradation of Phenol			
Medium	**Bjerkandera adusta**	**Pleurotus ostreatus**	**Arthrinium sp**
MM/glucose +2 mM Phenol	2 biological replicates	2 biological replicates	2 biological replicates
MM/glucose + 5 mM Phenol	2 biological replicates	2 biological replicates	2 biological replicates
Culture controls (in absence of Phenol)			
MM/glucose	2 biological replicates	2 biological replicates	2 biological replicates
Abiotic control (without filamentous fungus)			
MM/glucose + 2 mM Phenol	1 replicate		
MM/glucose + 5 mM Phenol	1 replicate		

2.3 Fungal bioremediation of real wastewater sample from syngas washing

In order to evaluate the bioremediation potential of selected microorganisms on contaminants of the wastewaters produced after syngas cleaning, fungi of interest were cultivated in MM/glucose medium supplemented with real wastewaters produced at a gasification plant in which the gas washing was also realized by using a stage of biodiesel scrubbing. The wastewater coming from this plant therefore was also contaminated by molecules from biodiesel.
In details, fungi were pre-cultivated on 2% MEA plates; successively, one plug/fungus was used to directly inoculate 30 ml of 2% ME. Liquid cultures were maintained at 28°C, under shaking (100 rpm), until the initial plug dimensions were doubled. Then, 20 ml of wastewaters were added to the cultures, maintained for 14 days at 28°C, under shaking (100 rpm). For each fungus, three biological replicates were prepared; in addition, one culture control and an abiotic control were also prepared, omitting the wastewaters and the filamentous fungus, respectively. After 14 days, cultures supernatants were collected and subjected to sampling with SPME and GCMS analyses.

2.4 Analytical methods

Analysis of phenol in synthetic water samples by HPLC chromatography was performed with an Agilent HP1100 instrument equipped with a quaternary pump, thermostat column compartment, thermostat autosampler and DAD detector. The elution was carried out on a Phenomenex Synergi™ 4 µm Hydro-RP 80 Å 4x250mm column in a water-acetonitrile gradient, 30 ° C temperature; the total analysis time was 12 min. Peak identification was performed by superimposing the recorded 3D spectra between 190 nm and 400 nm (UV region of the e.m. spectrum). For the quantitative measurements, the data acquired at 210 nm were taken into account; this wavelength was considered due to the relatively wide linearity of the calibration curve in the concentration range of 16 ÷ 350 ppm, and the low value of the detection limit. The calibration line was constructed using 5 concentration levels.
The analysis of the real wastewater samples deriving from the treatment with the selected fungi was carried out with a specific method based on sampling by adsorption of the analytes on SPME (Solid Phase Micro-Extraction) and subsequent analysis in GC-MS in order to be able to identify and quantify both the tar molecules and the *fatty acid methyl esters* (FAME) coming from the biodiesel washing step. For this purpose, an Agilent 6890/5975 GC-MS equipped with an Omegawax 250 column was used. Based on the polarity characteristics of the medium to be sampled, the extractions were conducted with SPME PDMS 100 µm cartridges (by Supelco). The conditions for temperature control and stirring of the samples were guaranteed using the Multi-Therm Heat Shake system by Benckmark Scientific. In order to verify the reproducibility of the adopted method, the extractions were carried out in the presence of an internal standard. In Table 3 a list of the most important molecules for the considered analyses is shown:

Table 3: List of molecules detected in the degradation tests of wastewater samples treated with B. adusta e Arthrinium sp.

Retention time (min)[a)]	Compound
3.54	Pyridine
6.64	Octanoic acid, methyl ester
9.42	Indene[b)]
12.60	Decanoic acid, methyl ester
16.71	Naphthalene
18.34	Dodecanoic acid, methyl ester
19.64	Naphthalene, 1-methyl-
19.70	Naphthalene, 2-methyl-
23.67	Tetradecanoic acid, methyl ester
23.71	Phenol
25.74	Phenol, 3-methyl-
28.10	Biphenylene
29.07	Hexadecanoic acid, methyl ester
31.28	Fluorene
33.06	Octadecanoic acid, methyl ester
33.72	9-Octadecenoic acid (Z)-, methyl ester
39.39	Anthracene

a) reference values ± 0.5 min; b) Internal standard.

3. Results and Discussion

3.1 Aqueous phenol samples treated with *B. adusta*, *Arthrinium sp*. and *P. ostreatus*

The HPLC analyzes carried out on the phenol solutions treated with the three fungal microorganisms *B. adusta*, *Arthrinium sp.* and *P. ostreatus* provided, as expected, evidence on the ability of each fungus to metabolize phenol. Unintended inhomogeneity in the initial preparation of some samples produced results that, on two replicates, proved to be relatively distant from each other. Nevertheless, trends were outlined that allowed a consistent evaluation of the relative capacities of the three micro-organisms and according to the contaminant load. Specifically, based on the data presented in Table 4, a reduction in the phenol degradation capacity is detected for all three microorganisms, which decreases with increasing concentration of the contaminant.

Table 4: Comparison of reduction efficiencies in the treatment of phenol with B. adusta, Arthrinium sp. e P. ostreatus.

Microrganism	Phenol Conc. (mM)	Biological replication	% reduction
B. adusta	2	A	49.5
		B	n.a.
	5	A	33.6
		B	37.8
Arthrinium sp.	2	A	37.7
		B	60.2
	5	A	13.2
		B	7.4
P. ostreatus	2	A	80.8
		B	n.a.
	5	A	24.2
		B	30.3

By comparing the data on the removal efficacy of biological replicates by the 5 mM solutions, the following order of activity can be drawn up:

B. adusta > *P. ostreatus* > *Arthrinium sp.*

in view of this result, for the tests on real wastewater from gas washing, experiments using the two microorganisms with maximum and minimum performances, i.e. *B. adusta* and *Arthrinium sp.*, were decided to be carried out first; the tests with *P. ostreatus* were instead postponed to a later stage.

3.2 Real sample of washing wastewater of syngas treated with *B. adusta* and *Arthrinium sp.*

The GC-MS analysis data of real water samples treated with *B. adusta* and *Arthrinium sp*. showed broad-spectrum bioremediation capabilities. In comparison with the control test data, for both microorganisms a general reduction in content was observed for all the molecules indicated in Table 3. A comparison on the percentages of molecules belonging to the two types of contamination, tar and esters of fatty acids, remained in solution after the treatment period and evaluated against the signals found in the control solutions is presented in Figure 2.

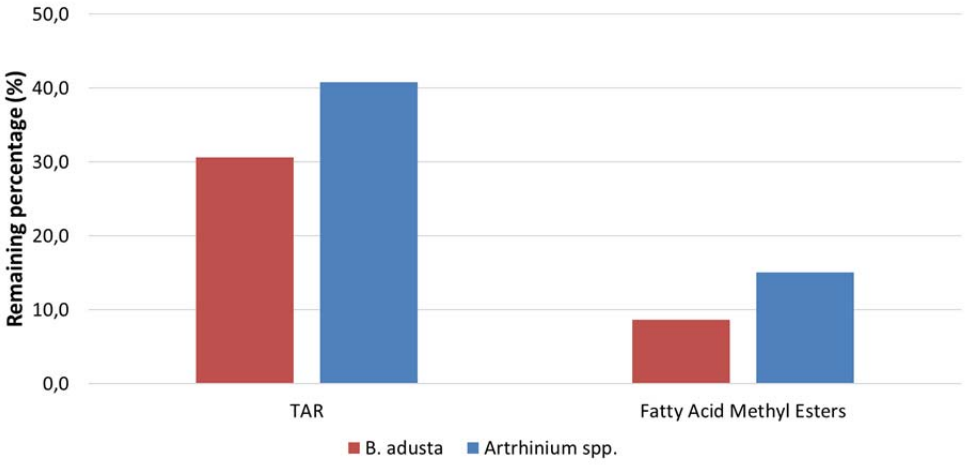

Figure 2: Comparison of the bioremediation capacity of B. adusta and Arthrinium sp. of wastewater from syngas washing.

The comparison clearly indicated that between the two fungi, the performance towards the two classes of compounds is better for *B. adusta* than for *Arthrinium sp*.. For the former, in fact, the removal rates are higher for both the tar molecules and the fatty acid esters.

4. Conclusions

Preliminary bioremediation tests carried out with fungal microorganisms (*Bjerkandera adusta*, *Arthrinium sp*, and *Pleurotus ostreatus*) on synthetic samples contaminated with phenol and on real wastewater gave positive results, confirming the potential application of the considered approach in the regeneration of contaminated aqueous steam from a water scrubber section of a biomass gasification plant. The results obtained, although preliminary, allowed to highlight that, of the three selected initial microorganisms, *B. adusta* and *Arthrinium sp*. have shown broad spectrum capabilities, being able to reduce the concentrations of both the tar and the characteristic biodiesel molecules. Further activities area already scheduled with the aim of putting in place an experimental set-up allowing more quantitative evaluations.

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