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A new screened microbial consortium OEM2 for lignocellulosic biomass deconstruction and chlorophenols detoxification

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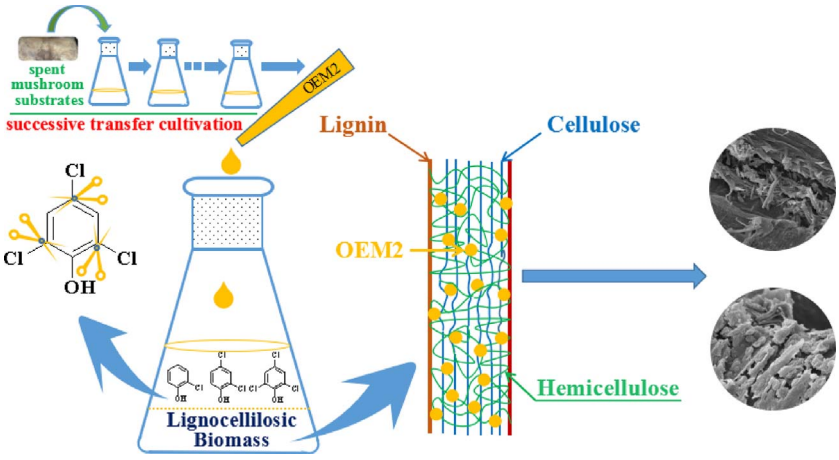
GRAPHICAL ABSTRACT



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ABSTRACT

Recalcitrance limits biomass application in biorefinery. It is even more so when toxic chlorophenols are present. In this study, we screened a microbial consortium, OEM2, for lignocellulose deconstruction and chlorophenols detoxification through a short-term and efficient screening process. Microbial consortium OEM2 had a good buffer capability in the cultivation process and exhibited a high xylanase activity, with over 85% hemicellulose degradation within 12 days. Throughout the treatment process, 41.5% rice straw decomposition on day 12 and around 75% chlorophenols (MCP, 2,4-DCP, 2,4,6-TCP) removal on day 9, were recorded. Moreover, Fourier translation infrared spectroscopy (FTIR) analysis indicated that chemical bonds and groups (eg. hydrogen-bond, β-1,4 glycosidic bond, lignin-carbohydrate cross-linking) in the rice straw were broken. Cuticle and silica layer destruction and subsequent exposed cellulose fibers were observed by scanning electron microscopy (SEM). Microbial consortium OEM2 diversity analysis by 16S rRNA gene sequencing indicated that *Proteobacteria* (41.3%) was the most abundant phylum and the genera *Paenibacillus* and *Pseudomonas* played an important role in the lignocellulose decomposition and chlorophenols detoxification. This study developed a faster and more efficient strategy to screen a specific microbial consortium. And the new microbial consortium, OEM2, makes lignocellulose more accessible and complex pollutants unproblematic in the further biorefinery process.

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1. Introduction

Lignocellulosic biomass is an abundant bioresource with a yield of over 200 billion dry metric tons available annually. Biomass has been gaining much attention as a candidate feedstock for the generation of bioenergy and biobased products [1,2]. Anaerobic digestion is a well-known mature commercial process which converts non-sterile, diverse and complex organic substrates into energy-rich biogas. Anaerobic digestion has inherent and significant merits including the ability to use a varity of feedstocks and environmental friendliness. With thousands of full-scale plants currently in operation worldwide, anaerobic digestion is considered to be one of the promising alternatives to fossil-derived energy [2–4]. However, regarding lignocellulosic biomass, the interaction of the main components (cellulose, hemicellulose, and lignin) forms a resistant and recalcitrant structure. This structure limits the lignocellulose hydrolysis and consequently leads to a low degradation efficiency in traditional anaerobic digestion processes [5]. Previous research has focused on altering the recalcitrant structure of biomass through physical, chemical, biological and hybrid pretreatments to facilitate the biological conversion of biomass into bioenergy [6]. Compared to other pretreatments, biological pretreatment is a promising technology due to its obvious advantages like eco-friendliness, economic viability, moderate reaction conditions, lack of chemical consumption and less toxic compounds released [7]. Recently, microorganisms like white-, brown-, and soft- rot fungi are commonly used for lignin and hemicellulose pretreatments. And previous research has substantiated that these pretreatments enhanced the methane potential and/or the hydrolysis rate of lignocellulosic biomass during the downstream anaerobic digestion process [3,8,9].

Chlorophenols were widely used as wood preservatives. The logs were dipped into chlorophenol solutions in the manufacture process [10]. In addition, organo-chlorine compounds (measured as AOX) were produced involuntarily in the bleaching pulp generation process using chlorine dioxide as a bleaching agent. AOX were ubiquitous in pulp mill effluents and migrated to pulp and paper sludge during the waste water treatment process [11]. Chlorophenols, as persistent organic pollutants, co-existed with lignocellulosic biomass in logs, pulp & paper sludge, and result integrated into combined contaminants. These contaminants aggravate biomass utilization and chlorophenols safe disposal. The biological treatment performance of these contaminants is determined by a number of factors and Kim et al. [12] considered that the detoxification was the critical factor. Previous studies revealed that the toxic organic compounds significantly changed the microbial community structure, had a noticeably negative effect on the diversity of the microbial community, and decreased the activity of enzymes in the treatment systems [13,14].

The hydrolysis of lignocellulosic biomass and the detoxification of chlorophenols were crucial for enhancing the downstream utilization performance. Hence, a unique consortia is required for biological treatment of these combined contaminants. The latest research indicated that compared to the single specie bacterium/fungus or enzyme treatment, microbial consortium including various bacteria, actinomycetes and fungi exhibited many advantages during the biological treatment of lignocellulosic biomass via their synergistic action [7]. Microbial consortium has good adaptability in a complex environment, which could be flexible enough for a certain range of contaminants, easily controls the pH in the degradation process, and saves cost by operating under non-sterile conditions [7,15].

Our previous study had screened a microbial consortium, named OEM1, for lignocellulose & chlorophenols degradation and analyzed its diversity via PCR-DGGE combined with clone and sequence. However, the screening process of microbial consortium OEM1 spent about 8 months (48 generations successive transfer cultivation). And only 31 strains bacteria were observed due to the limitation of analytical method. The aims of this study were therefore to (i) develop a faster and more efficient strategy to screen a new microbial consortium for lignocellulosic biomass deconstruction and chlorophenols detoxification; (ii) apply an advanced method (16S rRNA gene sequencing) to obtain more accurate and comprehensive information on its diversity. In this work, the potential performances on lignocellulosic biomass deconstruction and chlorophenols detoxification of the microbial consortium were also evaluated. More specifically, this study focused on microbial consortium construction, growth, metabolic characteristics, along with lignocellulose and chlorophenols bioconversion behaviors. This research would develop a short-term and efficient strategy for microbial consortium screening and obtain a new microorganism source to pretreat complex solid waste (lignocellulose co-existing with chlorophenols) that could enhance its downstream utilization and further clarified how this microbial consortium worked.

2. Materials and methods

2.1. Experimental materials

Rice straw used as the lignocellulose substrate in this study was obtained from agricultural plots of South China Agricultural University (Guangdong, China). Samples of rice straw were pretreated with 1% (W/V) sodium hydroxide solution for 24 h. Furthermore, rice straw was dried and cut into about 0.5 cm pieces prior to use in the experiment. This chemical pretreatment was conductive to the cuticle wax and silica layer of rice straw dissolution partially and led to a certain degree of damage of its surface [16]. It would offer more attachment points for microbial growth and make the rice straw fully exposed to them and their metabolic enzymes. This favorable feedstock would enhance the rice straw biological degradation efficiency. Two kinds of effective spent mushroom substrates (i.e. Pleurotus eryngii substrate and Volvariella volvacea substrate) obtained from Baiyun District (Guangzhou, China) were utilized as the microorganism source for the microbial consortium construction. The physico-chemical characteristics of spent mushroom substrates were provided in Supplement Table 1. Three representative kinds of chlorophenols including *o*-chlorophenol (MCP), 2,4-dichlorophenol (2,4-DCP), and 2,4,6-trichlorophenol (2,4,6-TCP) were selected for this experiment. A mixed chlorophenols solution (concentration of 50 g/L each chlorophenol) was prepared using ethanol as the solvent. The mixed chlorophenols solution was stored at 4 °C within a brown bottle for further use. The oat-spelt xylan was purchased from Sigma Aldrich Inc. (USA). The carboxymethylcellulose (CMC) was obtained from Sinopharm Chemical Reagent Co., Ltd.

2.2. Culture conditions

The enrichment medium used for screening process and biodegradation experiment contained (g/L): peptone, 2.5; KH_2PO_4, 1.5; Na_2HPO_4, 1.5; $CaCl_2{\cdot}2H_2O$, 0.8; $MgCl_2{\cdot}6H_2O$, 0.8; and yeast extract, 0.8. Trace elements solution (1mL) was added into the medium. The constitution of trace elements solution was as described by Liang et al. [17]. All flasks were incubated at 28 °C under continuous shaking at 120 rpm/min.

2.3. The screening process for microbial consortium OEM2

In the screening process, rice straw (0.5g) and mixed chlorophenols stock solution (50 μL) were loaded into the medium (100 mL). Two kinds of SMS, *Pleurotus eryngii* substrate (3.0 g) and *Pleurotus ostreatus* substrate (3.0 g), were inoculated into the medium. Five replicates were carried out in this screening experiment. As the rice straw decomposed into filament, a 10% inoculum (vol/vol) from each of the well-blended culture fluids was transferred to a fresh medium. During the successive transfer cultivation process, the pH evolution of the medium was detected every day and the degradation ratios of rice straw and chlorophenols were determined after inoculation. The replicate would be eliminated during the successive transfer cultivation under one of these