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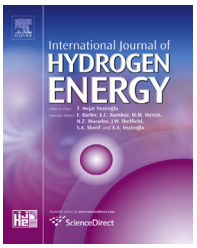

Microbial hydrogen production from phenol in a two-step biological process

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ABSTRACT

Phenol biodegradation is usually limited by a low treatment efficiency due to the microbial inhibition with intermediate accumulation. In this study, we report a two-step process to efficiently convert phenol to H_2. In the first step, phenol was converted to benzoate by mixed anaerobic cultures in an acidogenic reactor; subsequently, the formed benzoate was further fermented to H_2 by *Rhodopseudomonas palustris*, an efficient photosynthetic bacterium. The individual steps were simulated by mathematical models. The modified Gompertz model was used to describe the H_2 production process from benzoate by *R. palustris*. The results show that the effluent from the acidogenic reactor treating phenol could be directly used by *R. palustris* for H_2 production. The maximum H_2 production rate was estimated to be 0.545 mL/h. The H_2 yield and light conversion efficiency were 0.58 and 2.08%, respectively. The results of this study suggest that such a two-step process can efficiently degrade aromatic compounds like phenol with concurrent H_2 production.

Introduction

Phenol is commonly used as a broad-spectrum disinfectant due to its toxicity to most micro-organisms and is often present in different industrial wastewaters [1,2]. The simplest aromatic acid, benzoate, is a key intermediate for the degradation of many aromatic compounds, including phenol, chlorophenol and etc [3]. Once these aromatic compounds are released into the receiving water body, they would endanger fish and human beings even at low concentrations, e.g. 5–25 mg/L [4]. Thus, effective removal of these compounds from wastewater is imperative. In this respect, physico-chemical methods, such as adsorption and chemical oxidation, have been conventionally used. In comparison, biological treatment has recently attracted increasing interest attributed to its low cost, causing no secondary pollution and without the need of subsequent treatment [5]. Especially, anaerobic treatment has been recognized as one of the most promising approaches for treatment of such wastewater, in which process not only energy consumption for aeration can be saved but also valuable products such as methane can be produced [6].

The degradation of phenol under anaerobic conditions has been well documented [7–9]. Today, the most studied pathway of phenol anaerobic transformation is via the formation of benzoyl-CoA. Gallert and Winter reported that phenol was degraded to benzoate via 4-hydroxybenzoate, 4-hydroxybenzoyl- CoA and benzoyl-CoA [10]. Knoll and Winter found that phenol transformation to benzoate took place in a

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mixed methanogenic culture fed with phenol as the only energy and carbon source [11]. Benzoate can be directly converted to acetate and H_2 by *Syntrophus buswellii*, which can be further converted to CH_4 by acetotrophic methanogens, such as *Methanospirillum hungateri* [12]. However, benzoate might be cumulative, when the activity of methanogens was suppressed by high-concentration phenol or other methanogenic inhibitors.

Some fermentative and photosynthetic bacteria have a great potential to produce H_2 from organic industrial wastes [13,14]. *Clostridium butyricum* could generate H_2 from cellobiose in the presence of 200–1500 mg/L phenol with the highest H_2 yield of 2.1 mol H_2/mol cellobiose [14]. We have found that *Rhodopseudomonas palustris*, a photosynthetic bacterium, was capable of fermenting benzoate to H_2. Fibler et al. (1994) also demonstrated that *R. palustris* could generate H_2 from aromatic acids with yields of up to 45% of the maximal theoretical stoichiometric amount [15]. Olive-mill wastewater containing a high content of phenolic compounds after polyphenol removal via a pre-treatment can be used a low-cost feedstock for H_2 photo-production by *Rhodopseudomonas palustris* sp. [16]. Thus, it might be possible to directly combine the processes of H_2 production with aromatic compounds degradation, thus enabling a more cost-effective treatment process resulting from recovery of H_2 as an economic revenue. In addition, the efficient utilization of benzoate by *R. palustris* might decrease its accumulation and thus further improve the phenol degradation efficiency. Such a two-step bioconversion process of combining toxic contaminant degradation with H_2 production has been recognized as a promising but challenging approach in wastewater treatment [17].

In this work, we aim to demonstrate the feasibility of the aforementioned integrated process. The phenol is firstly transformed to benzoate by anaerobic microbes and then the product is fermented to H_2 by *R. palustris*. Furthermore, the kinetics of H_2 production from phenol by the two-step biological treatment was investigated. This work may offer implications for cost-effective treatment of phenol wastewater and for extending the range of sources for bio-hydrogen production.

Materials and methods

Strain cultivation

Anaerobic sludge and *R. palustris* were used in this work for phenol reduction and H_2 production respectively. Anaerobic sludge was obtained from a full-scale upflow anaerobic sludge blanket reactor treating citrate producing wastewater was pre-cultured in a basal medium containing (mg/L): chemical oxygen demand (COD) 1000–3000; NH_4Cl 250; KH_2PO_4 250; K_2HPO_4 250; $NaHCO_3$ 800; $MgSO_4$ 50; $CaCl_2$ 50; $FeCl_2$ 25; NaCl 10; $CoCl_2{\cdot}6H_2O$ 5; $MnCl_2{\cdot}4H_2O$ 5; $AlCl_3$ 2.5; $(NH_4)_6Mo_7O_{24}$ 15; H_3BO_4 5; $NiCl_2{\cdot}6H_2O$ 5; $CuCl_2{\cdot}5H_2O$ 5; $ZnCl_2$ 5 [18]. Initially, sucrose was used as a sole carbon source. After five days, a mixture of sucrose and phenol was used as carbon source instead. Thereafter, sucrose concentration was decreased gradually and phenol concentration increased. The detailed process is illustrated in Fig. 1. The anaerobic sludge with volatile suspended solids (VSS) concentration of 10.0 g/L was pre-cultured in 600 mL glass reactors with an internal diameter of 7.76 cm and a height of 13.00 cm. The total working volume of each reactor with rubber-stopper was filled to 500 mL with above medium. All the reactors were placed on a rotary shaking of 120 rpm and 37 ± 1 °C.

R. palustris was grown in a modified aSy medium, which was composed of a basal medium, supplemented with 1 g/L yeast extract, 1.25 g/L $(NH_4)_2SO_4$ and 1.0 g/L sodium succinate. The detailed compositions of basal medium can be found elsewhere [19]. The culture was grown anaerobically in 300-mL glass reactors with rubber-stopper at temperature of 30 ± 1 °C, pH of 6.8 and light intensity of 5 W/m^2 [17].

Phenol degradation and H_2 production experiments

After the pre-cultivation, the anaerobic sludge was inoculated into fresh medium with an initial VSS of 4.0 g/L. The phenol concentration varied from 400, 800, 1200–1400 mg/L. The other conditions were the same as the pre-cultivation

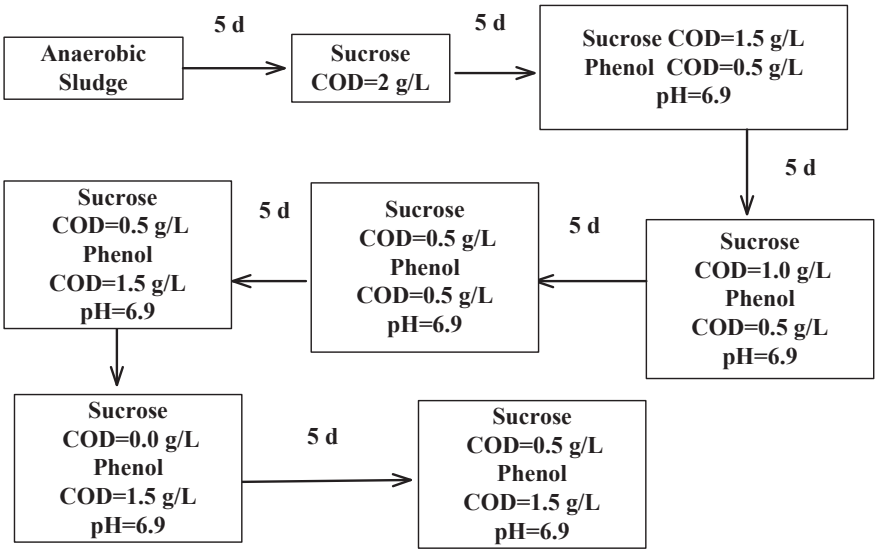

Fig. 1 – The pretreatment of anaerobic sludge.