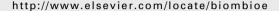


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Comparison of physicochemical characteristics and photofermentative hydrogen production potential of wastewaters produced from different olive oil mills in Western-Anatolia, Turkey

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ABSTRACT

Olive oil extraction produces a dark-colored wastewater that contains nutrients that can be further processed using biotechnology, in parallel with treatment for disposal. For instance, olive mill wastewater (OMW) can be used as a substrate for photofermentative hydrogen production by purple bacteria. A comparative study was investigated with several OMW samples from different olive oil mills in Western-Anatolia, Turkey. The composition of OMW varies significantly for each mill; thus, a detailed physicochemical analysis of each sample has been carried out. Subsequently, samples were assessed for their functioning in anaerobic photofermentative hydrogen production by Rhodobacter sphaeroides O.U.001. The highest hydrogen production potential (19.9 m³ m⁻³) was obtained by the OMW sample with the highest organic content (mainly acetic acid, 9.71 kg m⁻³) and the highest carbon-to-nitrogen (C/N) molar ratio (73.8 M M⁻¹). The organic content was found to be composed of primarily acetic, aspartic, and glutamic acids. There was a linear relationship between C/N ratio and hydrogen production potential across the different OMW samples. This study is unique due to the wide range of analyses of OMW samples and the comparison of many parameters for hydrogen production from wastewater. The results obtained throughout this study can aid in the design of systems using wastewater for biohydrogen production. Particularly, the C/N ratio was found to be the best parameter for choosing a proper substrate.

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1. Introduction

Olive oil is one of the oldest natural ingredients in the world. Based on several archeological tablets found in ancient tombs, its history goes back almost 8000 years to when the first trees were cultivated in the Syrian/Iranian region of the Middle East

[1]. Then, olives continued to move westward into Turkey, Greece, Egypt, Italy, France, Spain, Portugal, Algeria, Tunisia, and Morocco. Recently, there are many olive oil mills located around the Mediterranean area, accounting for approximately 95% of the worldwide olive oil production [2]. In Turkey, olive oil processing is generally carried out by various small

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Nomenclature BOD biological oxygen demand, kg m ⁻³ COD chemical oxygen demand, kg m ⁻³ HPP hydrogen production potential, m ³ m ⁻³ (volume of H ₂ gas produced per volume of initially added OMW solution) OMW olive mill wastewater PHB polyhydroxybutyrate	$\begin{array}{cccccccccccccccccccccccccccccccccccc$
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facilities rather than by large refineries. These facilities are principally located around the Mediterranean, Aegean, and Marmara coastal regions where olive trees grow. Olive oil production is a seasonal operation, which starts generally in September and ends in February at the latest [3].

The wastewater of this manufacturing process, so-called olive mill wastewater (OMW), is a considerable pollutant because of its extremely high organic matter contents, comparatively high chemical oxygen demand (COD), and biochemical oxygen demand (BOD) values that can reach up to 200 kg m^{-3} and 100 kg m^{-3} , respectively [4]. The phenolic content of OMW ranges between 1.5 kg m⁻³ and 4 kg m⁻³ [5]. OMW is a dark-colored liquid containing many dissolved and suspended substances. The solid content mainly comes from olive fruit residues such as olive pulp, husk, and some lignin derivatives that are not easily biodegraded. The characteristic brown-black color of this effluent is chemically related to polymers of low molecular weight phenolic compounds and lignin derivatives. The precise color mainly depends on the age and type of oil processed and also the type of the technology used. Fresh OMW has a strong and characteristic smell. The wastewater is slightly acidic, having pH values from 3 to 5.

This type of wastewater is generally composed of water (83-94%), organic matter (4-16%) and mineral salts (0.4-2.5%) [6-8]. Oils (1-14%), polysaccharides (13-53%), proteins (8-16%), organic acids (3-10%), polyalcohols (3-10%) and polyphenols (2-15%) are listed as its main organic contents [9]. More than 50 phenolic compounds, many alcohols, aldehydes and other low molecular weight compounds have been reported in the literature [10]. Among these, the compounds with a phenolic structure deserve a special attention because of their influence coloration and phytotoxic effect [11]. Mineral salts of OMW are mainly carbonates (21%), phosphates (14%), potassium (47%) and sodium (7%) [9]. Total suspended solid (TSS) is principally derived from the olive pulp and contains mainly cellulose and pectins [12]. Both quality and quantity of OMW are highly variable as a result of several factors such as [13]: (i) type of production process, (ii) type of olives, (iii) area under cultivation, (iv) use of pesticides and fertilizers, (v) climatic conditions, and (vi) harvesting time (i.e. stage of olive maturity).

Some microorganisms such as algae, cyanobacteria, and anoxygenic photosynthetic bacteria are capable of biological hydrogen production. Photosynthetic bacteria are the most favorable candidates due to their high conversion efficiency and substrate versatility. Hydrogen production by photosynthetic bacteria (such as Rhodobacter sphaeroides) occurs under

illumination and from the breakdown of organic substrates in the absence of oxygen; this process is known as photofermentation. One way to improve the economics of photofermentative hydrogen production is to couple this process with waste treatment. Many recent studies have focused on the utilization of residual food and agricultural wastes or wastewaters with high levels of organic compounds, thereby providing both energy production and waste treatment [14–21]. In our previous studies, it was proven that olive mill wastewater could be utilized for photobiological hydrogen production as a sole substrate source [6,7,14].

The objective of the current study was to understand the effect of different OMW compositions on photofermentative hydrogen production. Since the physicochemical properties of OMW samples are quite unpredictable for each olive oil mill, a detailed analysis of each sample is extremely essential for such a comparative study. Photofermentation experiments were carried out in small-scale indoor bioreactors (55 cm³); this provides an opportunity for comparative experiments by operating several parallel runs at the same time. Such a study is valuable for its wide range of analyses of various samples and for the comparison of many parameters for hydrogen production. The overall results obtained throughout this study can help process engineers select the most appropriate chemical composition from various waste materials for future biohydrogen applications and studies.

2. Materials and methods

2.1. Olive mill wastewater analysis

Physical and chemical properties of OMW samples were determined by following the similar procedure given by Eroğlu et al. [7].

2.2. Photofermentative hydrogen production

Photofermentative hydrogen production experiments were carried out in 55 cm³ glass bottles as described by Eroğlu et al. [14].

3. Results and discussion

Olive mill wastewater samples utilized throughout this study were obtained from four different olive oil mills in Western-Anatolia, Turkey. Since their physicochemical properties depend on many parameters, it is not possible to attribute

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