Introduction

The textile industry is one of the greatest generators of effluent pollutants due to the high quantities of water used in the dyeing processes [1]. The effluents from this industry are complex, containing a wide variety of dyes and other products such as dispersants, acids, bases, salts, detergents, humectants, and oxidants, plus high TDS, sodium, chloride, sulphate, hardness, and carcinogenic dye ingredients [2,3]. Wastewaters produced by textile industries are often strongly colored and their disposal into receiving waters causes environmental damage, including significant impacts on aquatic organisms due to reduced light penetration and the presence of metals, chlorides, and breakdown products of dyes [4-6]. Several physicochemical methods such as coagulation, precipitation, adsorption by activated charcoal, oxidation by ozone, ionizing radiation, and ultrafiltration are used to treat textile wastewater to achieve decolorization [7, 8]. These methods are effective but they are expensive and involve the formation of a concentrated sludge that creates a secondary disposal problem [9]. Biological processes provide an alternative to existing technologies because they are more cost effective, environmentally friendly, and do not produce large quantities of sludge [2,10,11]. Poly(3-hydroxyalkanoates) (PHAs) are structurally simple macromolecules synthesized by many microorganisms. PHAs have recently attracted much attention as useful biodegradable plastics [12-14]. Poly(3-hydroxybutyrate) (PHB), the most common member of the PHA family, possesses mechanical properties similar to the common petrochemical-based synthetic thermoplastics, and has been used to make various products, including films, coated paper, compost bags, disposable food-service ware, and molded products such as bottles and razors. After use, it can be degraded to carbon dioxide and water (or methane under 

Original Research

Eco-Friendly Poly(3-hydroxybutyrate) Synthesis from Textile Wastewater and Its Process Optimization

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Abstract

The textile industry is one of the biggest industries in the world and its wastewater is one of the most important threats to the environment and human health. In this study, the ability of Haloarcula sp. IRU1 to produce poly(3-hydroxybutyrate) (PHB) was investigated using a basal salts medium. Different factors like textile wastewater, yeast extract, and KH₂PO₄ (as carbon, nitrogen, and phosphorus sources, respectively) were optimized to achieve maximum synthesis of PHB by Haloarcula sp. IRU1. Under the optimal conditions of textile wastewater 30% (v/v), yeast extract 0.2% (w/v) and KH₂PO₄ 0.008% (w/v), the highest PHB synthesis (1.54 g/L) occurred in a shake-flask culture. The results provided evidence that Haloarcula sp. IRU1 could be a potential microorganism for production of PHB from textile wastewater in different conditions.

Keywords: poly(3-hydroxybutyrate), textile wastewater, optimization

Introduction

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anaerobic conditions) by microorganisms in the environment [15-19].

Halophilic archaea capable of degrading hydrocarbon have been the subject of growing attention in recent years due to problems encountered by the industry in hypersaline wastewater removal and decontamination of polluted salt marshes. Although little information exists about the biodegradation ability of halophilic archaea, some studies have shown the potential role of halophilic archaea belonging to the genera *Haloarcula* and *Halofex* for aerobic bioremediation [20]. In this study we report for the first time on the ability of *Haloarcula* sp. IRU1 isolate from hypersaline lake Urmia, Iran, to utilize textile wastewater as a carbon source for PHB production.

**Experimental Details**

**Microorganism and Cultivation**

*Haloarcula* sp. IRU1 isolated from hypersaline lake Urmia, Iran, was used in this study. This microorganism was cultured in a basal salts medium. The medium composition was as follows in distilled water (g/L): NaCl, 250; MgCl₂·6H₂O, 34.6; MgSO₄·7H₂O, 49.4; CaCl₂·2H₂O, 0.92; NaBr, 0.058; KCl, 0.5; NaH₂CO₃, 0.17. The basal medium was supplemented with different concentrations of textile wastewater (7-30%, v/v), yeast extract (0.1-0.8%, w/v), and KH₂PO₄ (0.001-0.016%, w/v) according to the details following the experiment design (Table 1). In all experiments, the microorganism was cultured in 250 ml Erlenmeyer flasks containing 50 ml of the medium and incubated in a shaker at 42ºC and 200 rpm for 5 days.

**Determination of PHB**

After a 5 day incubation, period 5 ml of the culture broth was centrifuged at 10,000 rpm for 10 min, the pellet was collected, digested with 5 ml Distilled water at 37ºC for 1 h, and the residue was separated by centrifugation at 10,000 rpm for 10 min. Then the pellet was washed following a series of steps using 5 ml acetone and 5 ml ethanol. The residue was dissolved in 5 ml chloroform and kept at room temperature for complete evaporation. Then 5 ml of concentrated H₂SO₄ was added and heated for 40 min at 100ºC in a water bath. The resultant crotonic acid was measured at 235 nm according to the method of Slepecky and Law [21, 22].

**Optimization Process and Statistical Analyses**

The Taguchi method was used to describe the number of experimental situations. All the combination experiments using the assigned parameter values were conducted with the aim of obtaining the final optimum conditions. The reaction parameters involved in the optimization of PHB production were those of textile wastewater, yeast extract and KH₂PO₄ concentration. The Qualitek-4 software was used to design and analyze the Taguchi experiments.

**Results and Discussion**

Culture conditions such as carbon, phosphorus, and nitrogen sources exert significant influence on PHB production by microorganisms [23]. Usually PHB production in many microorganisms is induced when carbon and energy sources are in excess, but growth is limited by the lack of oxygen, nitrogen, or phosphorus source [23]. During the optimization process the following parameters affecting PHB production by *Haloarcula* sp. IRU1 were investigated: textile wastewater, yeast extract and KH₂PO₄ concentrations. PHB production has been optimized using Taguchi methodology as a statistical tool to evaluate a combination of different factors affecting PHB production by *Haloarcula* sp. IRU1 in shake-flasks (250 ml) using concentrations of textile wastewater ranging from 7 to 30%.
(v/v) as a carbon source, concentrations of yeast extract from 0.1 to 0.8% (v/v) as a nitrogen source, and KH$_2$PO$_4$ from 0.001 to 0.016% (w/v) as a phosphorous source (Table 2). According to our results in Table 2, maximum PHB production by *Haloarcula* sp. IRU1 occurred with 30% textile wastewater, 0.2% yeast extract, and 0.008% KH$_2$PO$_4$. During the course of these experiments the batch cultures turned from initial dark yellow to light orange.

A low difference between main effects at L1 and L2 indicates a decrease in PHB production. A high value, on the other hand, indicates an increase in PHB production (according to L2-L1). A parameter more important for PHB production by *Haloarcula* sp. IRU1 was yeast extract concentration, followed by KH$_2$PO$_4$ and textile wastewater concentrations (Table 3). The effect of textile wastewater concentration on PHB production is presented in Fig. 1. As shown in Table 4, optimal conditions to produce PHB were 22% textile wastewater, 0.2% yeast extract, and 0.008% KH$_2$PO$_4$. The expected PHB production at optimum conditions was 1.45 g/L. The textile wastewater concentration showed the highest contribution to PHB production (28.7%), and the contribution of KH$_2$PO$_4$ to PHB production was lowest (14.2%). The results indicate that the total contribution and grand average performance from all factors and levels are 0.71 and 0.74, respectively (Table 4). The experimental and expected PHB productions were in agreement.

Table 5 shows the ANOVA results, indicating the percentage contributions of the control factors to PHB production. Textile wastewater and yeast extract are the significant parameters for affecting PHB production as also observed from Taguchi analysis. The sum of square, F-Ratio, and variance showed the highest value for textile wastewater (1.31, 6.30, and 0.44, respectively) but yeast extract and KH$_2$PO$_4$ with low sum of squares (0.47, 0.11), F-Ratio (2.28, 0.55), and variance (0.18, 0.04), respectively, have no significant effect on PHB production.

**Conclusion**

The purpose of this study was to evaluate textile wastewater as a carbon source for eco-friendly PHB synthesis by *Haloarcula* sp. IRU1. This halophilic archaeon could be a potential microorganism for production of PHB from textile wastewater in different conditions. The optimal parameters obtained during the optimization process were: textile wastewaters of 22%, yeast extract 0.2%, and KH$_2$PO$_4$ of 0.008%.

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![Average effects](image_url)
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