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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 KH_2PO_4 (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 KH_2PO_4 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

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

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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 *Haloferax* 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 form 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; $MgCl_2·6H_2O$, 34.6; $MgSO_4·7H_2O$, 49.4; $CaCl_2·2H_2O$, 0.92; NaBr, 0.058; KCl, 0.5; NaH_2CO_3, 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 KH_2PO_4 (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 H_2SO_4 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 KH_2PO_4 concentration. The Qualitek-4 software was used to design and analyze the Taguchi experiments.

Table 1. Setting of factors and their levels in experiment for PHB synthesis.

Factor	Parameter	Level 1	Level 2	Level 3	Level 4
Factor A	Textile wastewater % (V/V)	7	14	22	30
Factor B	Yeast extract % (W/V)	0.1	0.2	0.4	0.8
Factor C	KH_2PO_4 % (W/V)	0.001	0.004	0.008	0.016

Table 2. The orthogonal array of Taguchi experimental design and PHB synthesis.

Trial	Factor A	Factor B	Factor C	PHB (g/L)
1	1	1	1	0.23
2	1	2	2	0.62
3	1	3	3	0.90
4	1	4	4	0.37
5	2	1	2	0.50
6	2	2	1	0.47
7	2	3	4	0.20
8	2	4	3	0.36
9	3	1	3	0.72
10	3	2	1	1.46
11	3	3	4	0.98
12	3	4	2	0.94
13	4	1	4	0.81
14	4	2	3	1.54
15	4	3	2	0.74
16	4	4	1	0.96

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 KH_2PO_4 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%

Table 3. Main effects.

Factors	Level 1	Level 2	Level 3	Level 4	L2-L1
Textile wastewater	0.529	0.382	1.024	1.012	-0.147
Yeast extract	0.564	1.022	0.704	0.657	0.458
KH_2PO_4	0.66	0.699	0.879	0.709	0.390

Table 4. Optimum conditions of PHB synthesis.

Factors	Level Description	Level	Contribution
Textile wastewater	Fact A-Level 3	3	0.287
Yeast extract	Fact B-Level 2	2	0.285
KH_2PO_4	Fact C-Level 3	3	0.142

Total contribution from all factors – 0.713
Current grand average of performance – 0.737
Expected results at optimum condition – 1.451

(v/v) as a carbon source, concentrations of yeast extract from 0.1 to 0.8% (v/v) as a nitrogen source, and KH_2PO_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_2PO_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_2PO_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_2PO_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_2PO_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 KH2PO4 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_2PO_4 of 0.008%.

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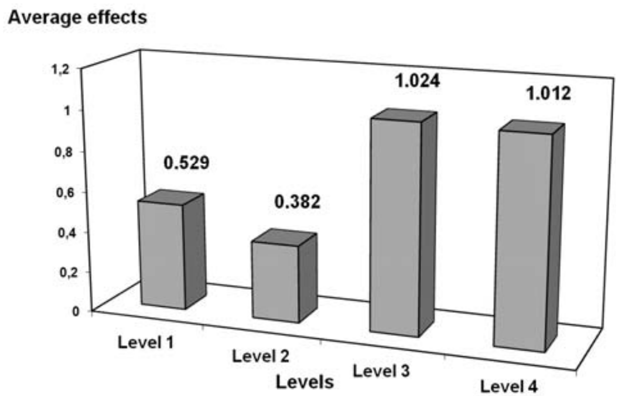

Fig. 1. Average effects of textile wastewater on PHB production by *Haloarcula* sp. IRU1.

Table 5. Analysis of variance (ANOVA).

Factors	DOF (f)	Sum of squares (S)	Variance (V)	F-Ratio (F)	Pure Sum (S′)	Percent P (%)
Textile wastewater	3	1.309	0.436	6.295	1.101	47.617
Yeast extract	3	0.473	0.175	2.277	0.265	11.488
KH_2PO_4	3	0.113	0.037	0.547	0	0

References

1. KALYANI D.C., TELKE A.A., DHANVE R.S., JADHAV J.P. Eco-friendly biodegradation and detoxification of Reactive Red 2 textile dye by newly isolated *Pseudomonas* sp. SUK1. J. Hazard. Mater. **163**, 735, **2009**.
2. GHODAKE G., JADHAV S., DAWKAR V., GOVINDWAR S., Biodegradation of diazo dye Direct brown MR by *Acinetobacter calcoaceticus* NCIM 2890. Ind. Biodet. Biodeg. **63**, 433, **2009**.
3. TCHOBANOGLOUS G., BURTON F.L. Wastewater Engineering: Treatment, Disposal and Reuse/Metcalf and Eddy. Tata McGraw-Hill Publishing Company Limited, New Delhi, India, **1995**.
4. CLARKE E.A., ANLIKER R. Organic dyes and pigments Handbook of Environmental Chemistry, Anthropogenic Compounds, New York, Springer, **3**, (A), **1980**.
5. HAO O.J., KIM H., CHIANG P.C., Decolorization of wastewater. Critical Reviews in Environ. Sci. Technol. **30**, 449, **2000**.
6. KHLIFI R., BELBAHRI L., WOODWARD S., ELLOUZ M., DHOUIB A., SAYADI S., MECHICHI T. Decolourization and detoxification of textile industry wastewater by the laccase-mediator system. J. Hazard. Mater. **175**, 802, **2010**.
7. FRANCISCON E., ZILLE A., DIAS GUIMARO F., RAGAGNIN DE MENEZES C., DURRANT LUCIA R., CAVACO-PAULO A. Biodegradation of textile azo dyes by a facultative Staphylococcus arlettae strain VN-11 using a sequential microaerophilic/aerobic process. Int. Biodeter. Biodeg. **63**, 280, **2009**.
8. GOGATE P.R., PANDIT A.B. A review of imperative technologies for wastewater treatment II: hybrid methods. Adv. Env. Res. **8**, 553, **2004**.
9. MAIER J., KANDELBAUER A., ERLACHER A., CAVACO-PAULO A., GUBITZ M.G. A new alkali-thermostable azoreductase from *Bacillus* sp. strain SF. Appl. Environ. Microb. **70**, 837, **2004**.
10. AZMI W., SANI R.K., BANERJEE U.C. Biodegradation of triphenylmethane dyes. Enzyme Microb. Tech. **22**, 185, **1998**.
11. VERMA P., MADAMWAR D. Decolorization of synthetic dyes by a newly isolated strain of *Serratia marcescens*. World J. Microb. Biot. **19**, 615, **2003**.
12. ANDERSON A.J., DAWES E.A. Occurrence, metabolic role, and industrial uses of bacterial polyhydroxyalkanoates. Microbiol. Rev. **54**, 450, **1990**.
13. DOI Y. Microbial polyesters. VCH Publishers, Inc., Yokohama, Japan, **1990**.
14. STEINBUECHEL A. PHB and other polyhydroxyalkanoic acids. In: Rehm HJ, Reed G (Eds) Biotechnology, **6**, (2), VCH, Weinheim, **1996**.
15. LEE S.Y. Plastic bacteria? Progress and prospects for polyhydroxyalkanoate production in bacteria. Trends Biotechnol., **14**, 431, **1996**.
16. MAS-CASTELLA J., URMENETA J., LAFUENTE R., NAVARRETE A., GUERRERO R. Biodegradation of poly-b-hydroxyalkanoates in anaerobic sediments. Int. Biodeter. Biodeg. **35**, 155, **1995**.
17. VAN-THUOC D., QUILLAGUAMAN J., MAMO G., MATTIASSON B. Utilization of agricultural residues for poly(3-hydroxybutyrate) production by *Halomonas boliviensis* LC1. J. Appl. Microbiol. **104**, 420, **2008**.
18. TOKIWA Y., CALABIA B.P. Degradation of microbial polyesters. Biotechnol. Let. **26,** 1181, **2004**.
19. DU G.C., CHEN J., YU J., LUN S.Y. Continuous production of poly-3-hydrobutyrate by Ralstonia eutropha in a two-stage culture system. J. Biotechnol., **88**, 59, **2001**.
20. TAPILATU Y.H., GROSSI V., ACQUAVIVA M., MILITON C., BERTRAND J.C., CUNY P., Isolation of hydrocarbon-degrading extremely halophilic archaea from an uncontaminated hypersaline pond (Camargue, France). Extremophiles, **14**, 225, **2010**.
21. NISHA V., CARLOS S.R., PANDEY A. A statistical Approach for Optimization of Polyhydroxybutyrate Production by Bacillus sphaericus NCIM 5149 under Submerged Fermentation Using Central Composite Design. Appl. Biochem. Biotechnol., **63**, 501, **2009**.
22. SLEPECKY R.A., LAW J.H. A rapid spectrophotometric assay of unsaturated acids and b-hydroxy acids. Anal. Chem, **32**, 1697, **1960**.
23. SANGKHARAK K., PRASERTSAN P. Nutrient optimization for production of polyhydroxybutyrate from halotolerant photosynthetic bacteria cultivated under aerobic-dark condition. Elec. J. Biotechnol. **11**, 1, **2008**.