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Utilization of petrochemical wastewater for the production of poly(3-hydroxybutyrate) by *Haloarcula* sp. IRU1

Mojtaba Taran*

Department of Biology, Faculty of Science, Razi University, Kermanshah, Iran

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ABSTRACT

Wastewater of petrochemical industries contains high amounts of emulsified aliphatic or aromatic hydrocarbons that lead to the contamination of almost all environmental resources. The ability of *Haloarcula* sp. IRU1 isolated from Urmia lake, Iran for utilization of petrochemical wastewater and production of poly(3-hydroxybutyrate) (PHB) from it was evaluated and optimized by Taguchi experimental method. The optimium conditions for the maximum production of PHB were petrochemical wastewater 2% (as carbon source), tryptone 0.8% (as nitrogen source), KH_2PO_4 0.001% (as phosphorus source) and temperature 47 °C. In conclusion, *Haloarcula* sp. IRU1 can degrade petrochemical wastewater and produce PHB from it in different conditions.

1. Introduction

Plastics have become an integral part of contemporary life because of their many desirable properties including durability and resistance to degradation. These non degradable plastics accumulate in the environment at a rate of millions of tons per year causing several problems. Recently, issues concerning the global environment and solid waste management have created much interest in the development of biodegradable plastics [1,2].

Poly(3-hydroxyalkanoates) (PHAs) are structurally simple macromolecules synthesized by many microorganisms. PHAs have recently attracted much attention as useful biodegradable plastics [1,3,4]. PHB, the most common member of PHAs family, is found in many genera of the microorganisms as carbon and energy storage [1,5,6]. This bioplastic (PHB) has many obvious applications in bone plates, nails, screws and in the treatment of osteomyelitis [2,7,8].

The extensive use of petrochemical products leads to the contamination of almost all environmental resources. Wastewater of some petrochemical plants, in addition to hydrocarbons, contains chlorinated chemicals [9]. The biological treatment has been widely used for removal of organic compounds from petrochemical wastewater. The microbial population and activity for biological treatment depend on the nature and availability of petroleum hydrocarbons, nutrient composition, pH, temperature and other environmental conditions. Numerous microorganisms, including bacteria and fungi are able to degrade hydrocarbons [9–12].

Haloarcula are extreme halophilic archaeons. Various halophilic archaea, including *Haloarcula marismortui*, grow on glucose, which is degraded via a modified, semiphosphorylated Entner–Doudoroff (ED) pathway [13,14]. In this article we report for the first time, the ability of *Haloarcula* sp. IRU1 isolated from hypersaline Urmia lake, Iran in biodegradation of petrochemical wastewater and PHB production from it as carbon source.

2. Materials and methods

2.1. Petrochemical wastewater

The petrochemical wastewater employed in this study was collected from Bistoon Petrochemical Company, Kermanshah, Iran in August 2009. The wastewater contains multiple hydrocarbons such as linear alkylbenzenes. pH and COD of the wastewater were 7.80 and 740 mg/L, respectively.

2.2. Microorganism and growth conditions

Haloarcula sp. IRU1 isolated from hypersaline Urmia lake, Iran was provided from Alzahar University, cultivated in 500 mL Erlenmeyer flasks containing 100 mL of a basal medium and incubated in a shaker at 47 °C and 200 rpm for 5 days under aerobic conditions.

The basal medium for culture consisted of (g/L): NaCl, 250; $MgCl_2{\cdot}6H_2O$, 34.6; $MgSO_4{\cdot}7H_2O$, 49.4; $CaCl_2{\cdot}2H_2O$, 0.92; NaBr, 0.058; KCl, 0.5 and NaH_2CO_3, 0.17. The growth medium supplemented with various nutrient compositions by varying

* Department of Biology, Faculty of Science, Razi University, P.O. Box 6714967346, Kermanshah, Iran. Tel.: +98 831 4274889; fax: +98 831 4274545.
E-mail addresses: mtaran@razi.ac.ir, mojtabataran@yahoo.com

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Table 1
Factors and their levels employed in the Taguchi experimental design for PHB production from petrochemical wastewater by *Haloarcula* sp. IRU1.

Factor	Parameter	Level 1	Level 2	Level 3	Level 4
Factor A	Petrochemical waste % (v/v)	1	2	4	8
Factor B	Nitrogen source	Yeast extract	NH_4Cl	Peptone	Tryptone
Factor C	Nitrogen source (g/L)	0.1	0.2	0.4	0.8
Factor D	Phosphorus source	K_2HPO_4	KH_2PO_4	Na_3PO_4	NaH_2PO_4
Factor E	Phosphorus source (g/L)	0.001	0.004	0.008	0.016
Factor F	Temperature (°C)	37	42	47	55

Table 2
The orthogonal array of Taguchi experimental design and corresponding PHB production from petrochemical wastewater by *Haloarcula* sp. IRU1.

Trial	Factor A	Factor B	Factor C	Factor D	Factor E	Factor F	PHB/CDW (%)
1	1	2	3	4	1	2	44.3
2	2	1	4	3	2	1	44.6
3	3	4	1	2	3	4	41.7
4	4	3	2	1	4	3	41.9
5	1	3	3	1	2	4	42.7
6	2	4	4	2	1	3	46.6
7	3	1	1	3	4	2	43.2
8	4	2	2	4	3	1	39.0

petrochemical wastewater as carbon source (collected from Bistoon Petrochemical Company, Kermanshah, Iran) at 1–8% (v/v), phosphorus sources [K_2HPO_4, KH_2PO_4, Na_3PO_4 and Na_2HPO_4 at 0.001–0.016% (w/v)], nitrogen sources [NH_4Cl, yeast extract, peptone and tryptone at 0.1–0.8% (w/v)] according to the details following the experimental design. The same approach was taken in regard to the cultivation temperature (Table 1).

2.3. Determination of cell growth

For determination of cell dry weight (CDW), the optical density (OD) of the culture broth using a spectrophotometer at 520 nm was converted to CDW using a calibration curve, where one OD unit was equivalent to 0.3028 CDW (g/L).

2.4. Determination of PHB

After 5 days incubation, 5 ml of the culture broth was centrifuged at 10,000 rpm for 10 min, the pellet was collected and digested with 5 ml distilled water at 37 °C for 1 h, and the residue was separated by centrifugation at 10,000 rpm for 10 min. 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 100 °C in a water bath. The resultant crotonic acid was measured at 235 nm according to the method of Slepecky and Law [15,16].

2.5. Design of experiments for optimization of PHB production

All the combination experiments using the assigned parameter values were conducted using the basal media and incubated for 5 days. The culture broth was separated and analyzed for PHB (Table 2). Qualitek-4 software was used to design and analyze Taguchi experiments. All the experiments were done in duplicate, and the average of CDW and PHB production obtained were taken as the dependent variable. The parameters (A–F) and their levels (1–4) used in Taguchi experimental design are listed in Table 1.

Table 4
Main effects.

Column #/factors	Level 1	Level 2	Level 3	Level 4	L1–L2
2 Factor A	10.875	11.4	10.612	10.112	.525
3 Factor B	10.975	10.412	10.575	11.037	−.563
4 Factor C	10.612	10.112	10.875	11.4	−.5
5 Factor D	10.572	11.037	10.975	10.412	.462
6 Factor E	11.362	10.912	10.087	10.637	−.45
7 Factor F	10.45	10.937	11.062	10.55	.487

Table 5
Point prediction for optimum conditions.

Column #/factors	Level description	Level	Contribution
2 Factor A	Fact A-level 2	2	32.249
3 Factor B	Fact B-level 4	4	.649
4 Factor C	Fact C-level 4	4	.287
5 Factor D	Fact D-level 2	2	.649
6 Factor E	Fact E-level 1	1	.278
7 Factor F	Fact F-level 3	3	.612
Total contribution from all factors			34.733
Current grand average of performance			10.75
Expected results at optimum condition			45.795

3. Results and discussion

The optimization of PHB production from petrochemical wastewater by microorganisms is very important in present days because of the PHB application in industry and the enhancement of petrochemical wastewater production. Global environment concerns and solid waste management problems have generated much interest in the development of biodegradable plastics (such as PHB) as a substitute for non biodegradable petroleum based plastics [2].

Table 3
ANOVA for PHB production by *Haloarcula* sp. IRU1.

Column #/factors	DOF (f)	Sum of sqrs. (S)	Variance (V)	F-ratio (F)	Pure sum (S')	Percent P (%)
2 Factor A	3	11,093.991	3697.57	4085.673	11,091.853	99.65
3 Factor B	3	6.907	2.302	2.543	4.191	.37
4 Factor C	3	2.222	.74	.818	0	0
5 Factor D	3	6.907	2.302	2.543	4.191	.37
6 Factor E	3	2.222	.74	.818	0	0
7 Factor F	3	6.824	2.274	2.513	4.109	0.36

Cell growth and the production of metabolites are greatly influenced by different factors such as carbon sources, nitrogen sources, salinity and other growth factors, thus the optimization of microorganisms can give results in the high yield of metabolites [17,18]. In this study, we used petrochemical wastewater for PHB production as a cheap source of carbon.

The effects of petrochemical wastewater, nitrogen, phosphorus source concentrations and temperature on PHB production were investigated. The effects of interactions between factors at different levels on PHB production by *Haloarcula* sp. IRU1 are represented in Table 2. Results indicate that *Haloarcula* sp. IRU1 give the highest PHB (46.6% of DCW) in the presence of petrochemical wastewater 2%, tryptone 0.8%, KH_2PO_4 0.001% and temperature 47 °C (Table 2).

ANOVA of response for PHB production with the contribution of studied factors are shown in Table 3. The contribution of individual factors is the deciding key of the control to be enforced on the PHB production. The levels of factors can be regulated, such that an appropriate level of influencing factor can produce high amount of PHB. Thus, the appropriate levels of studied factors for maximum production of PHB by *Haloarcula* sp. IRU1 can be predicted. Petrochemical wastewater concentration is observed to be the most influential factor among the different factors (99.65%).

The influential effects of the factors at different levels on PHB production from petrochemical wastewater by *Haloarcula* sp. IRU1 is represented in Table 4. Petrochemical wastewater concentration was very influential at level 2 on PHB/CDW. The difference between level 2 and level 1 (L2–L1) of each factor shows its relative influence on the PHB production. It is seen from Table 4 that among the factors, petrochemical wastewater concentration shows stronger influence on the PHB production followed by temperature and phosphorus source. Like other living systems, the environmental temperature has a great effect on microbial growth and its ability production [19].

According to Taguchi approach, the optimum conditions at which high PHB production by *Haloarcula* sp. IRU1 can be achieved are observed to be in the presence petrochemical wastewater at 2% (v/v), tryptone at 0.8% (w/v), KH_2PO_4 at 0.001% (w/v) and temperature 47 °C (Table 5).

4. Conclusions

This work demonstrated the effectiveness and feasibility of *Haloarcula* sp. IRU1 for biodegradation of petrochemical wastewater and production of PHB from it. By production of PHB from petrochemical wastewater via this microorganism, the production cost of PHB would be markedly reduced, enhancing the economical feasibility of commercial applications of this powerful bioplastic and biodegradation of petrochemical wastewater.

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