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USE OF IMMOBILIZED WHOLE CELLS OF *HALOFERAX* ATCC BAA 645 FOR TREATMENT OF DAIRY AND FISH WASTE EFFLUENTS

Sanket K Gaonkar and Irene J Furtado*
Department of Microbiology, Goa University, Taleigao Plateau, Goa, 403 206, India

***Corresponding Author Email: ijfurtado@unigoa.ac.in**

ABSTRACT

Dairy and fish waste-water effluents are an inseparable part of upcoming modern Cities and villages of every developing Nation. These effluents have high concentration of soluble proteins and lipids which are below the threshold levels of chemical and physical methods used for recovery. In view of this, the present study aims to evaluate the use of immobilized-whole-cells of Haloferax ATCC BAA 645 producing protease and lipase for treatment of non-saline dairy and saline fish waste effluents. The immobilized cells were placed in saline and non-saline medium. Immobilized cells placed in the saline medium, simultaneously produce protease and lipase in 12 h reaching a maximum at 72 h whereas, in non-saline medium production starts at 42 h and is optimum at 90 h. The immobilized cells were also used for successful treatment of dairy and fish waste-water containing proteins and lipids in the aerobic batch system for 7 cycles. The efficiency of reduction in electrical conductivity, total dissolved solids, chemical oxygen demand and biological oxygen demand are48.63 % and 31.66%; 41.4% and 40.86 %; 31.87 % and 23 %; 48.17% and 30 %, in case of dairy and fish waste-water, respectively. Results showed that protease and lipase producing immobilized cells of Haloferax could be efficiently used for treatment of non-saline dairy and saline fish waste-water rich in proteins and lipids. Thus, it can be concluded from our present study that the findings are of biotechnological significance in clarification of protein and lipid-containing waste-waters.

KEY WORDS

Batch Reactor, Biotreatment, Haloferax ATCC BAA 645, Immobilized-whole-cells,

INTRODUCTION

Saline, as well as Non-saline waste-water, are often generated by food-processing, leather and petroleum industries[1].These effluents are rich in proteins, fats, oil & grease, sodium chloride and other salts which need to be removed before the waters are discharged into natural water bodies[2].

The dairy industry on an average generates 6–10L of waste-water per liter of the milk processed while water consumption in fish industries depend on the type of fish and also on various fish processing units[3]. These waters if let or released untreated are hazardous to the environment and human health. The conventional activated sludge system used for clarification harbors pathogenic microbes and hence restricts the reuse of clarified waters in further cycles of processing[4].To our understanding, microbes belonging to haloarchaea are the best candidates for the treatment of waste-waters containing high salt concentration. Braganca and Furtado [5]reported that immobilization is a promising method for entrapment of Haloarchaeal cells for increasing their functionality in low saline conditions to efficiently remove cadmium from deionized water. In the present investigation, we successfully immobilized Whole cells of *Haloferax* ATCC BAA 645 in Na-alginate and checked their potential to produce protease and lipase in saline and non- saline milieu. And, to remediate

non-saline dairy and saline fish waste-water by developing an aerobic batch model reactor system.

MATERIALS AND METHODS

Waste-waters

Milk dairy (Goa Dairy, Goa) and fish waste-water (Panaji- Fishmarket, Goa).

Analytical methods

The pH was determined by pH meter (pH 700, pH/mV/C°/F° meter, EUTECH instruments); Electrical conductivity (EC), total dissolved solids (TDS) and salinity by using water and soil analysis kit (Model No 60, Labtronics, India); Biological oxygen demand (BOD) and Chemical oxygen demand (COD) analysis were carried out as per ' Handbook of water analysis' by S.K Maiti[6]; Protein by Folin Lowry using Bovine serum albumin (BSA) as standard[7]. A standard Kunitz assay[8] and Beisson et al [9] were used for detection of protease and lipase activity ,respectively.

Immobilization of *Haloferax* ATCC BAA 645 for enzymes production.

Mid-log phase whole cells of *Haloferax* ATCC BAA 645 (OD 2.0), pre-grown in NaCl tryptone-yeast extract (NTYE) broth were mixed with 1% (w/v) solution of sodium alginate in 20 % NaCl. After 30 min, the mixture was added drop wise to 0.2 M $CaCl_2$ in order to get spherical beads of 1mm size[5].

Enzyme production efficiency of immobilized *Haloferax* ATCC BAA 645 beads

Production of protease and lipase by beads was checked every six hours using NT and Tryptone medium (T) each consisting of 20 g $MgSO_4$, 5 g KCl, 0.2 g $CaCl_2$, 5 g tryptone with/without 300 g crude solar salt per liter, pH 6 and 0.2% tween 80.

Use of immobilized cells for treatment of dairy and fish waste-waters

Haloferax beads (50 g/L) were added to dairy and fish waste-water, respectively and aerated at 150 rpm, 42°C for 90h. After an hour of standing, the clear top ferment was filtered through sieve mesh and clear filtrate was tested for various physicochemical parameters. The beads were used for continuous production of enzymes and treatment of dairy and fish waste-water for 7 cycles. The experimental set up for treatment of dairy and fish waste-water is shown in **Fig. 1.**

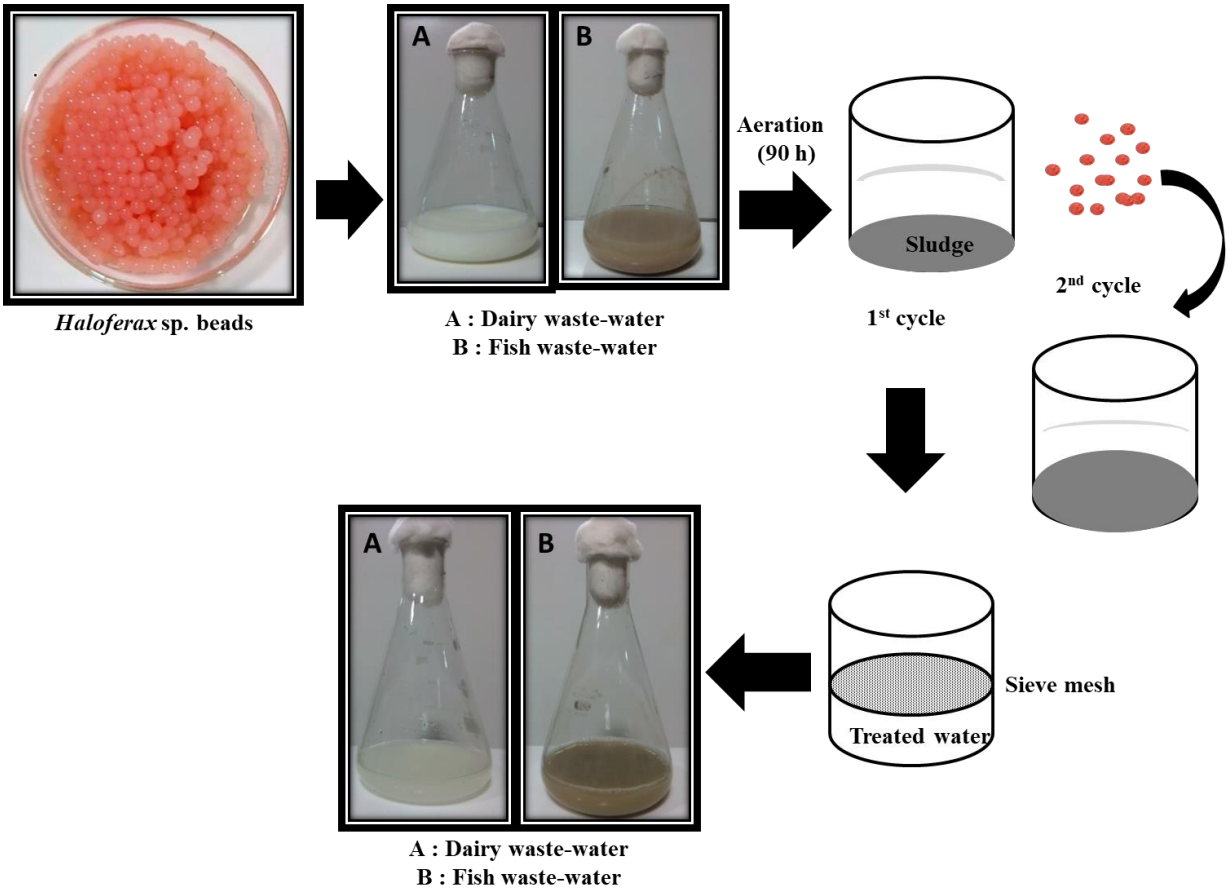

Fig. 1 Batch model reactor for treatment of dairy and fish waste-water

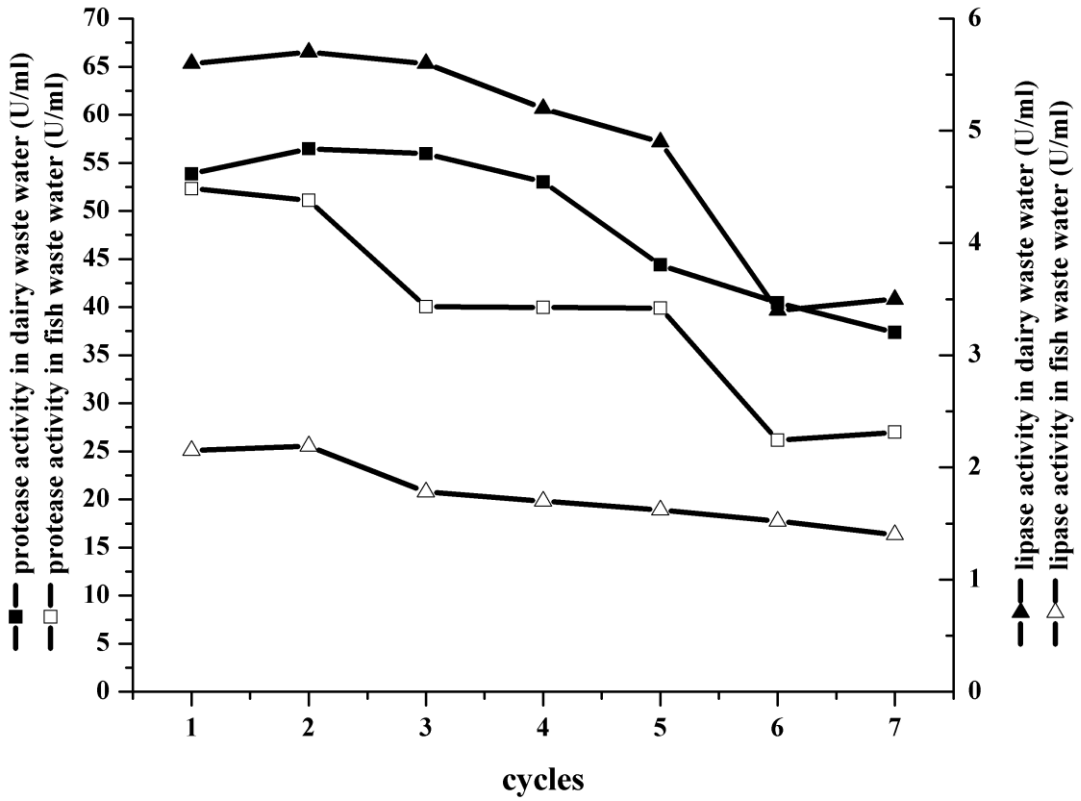

Fig. 2: Stability of immobilized *Haloferax* sp. cells for protease and lipase production in dairy and fish waste-water for 7 cycles

RESULTS AND DISCUSSION

Economic growth in developing Nations invariably results in waste-water containing organic waste which by itself may not be toxic but still can lead to environmental pollution and putrification. Such waters often times get into saline and non-saline water bodies causing health hazards. Therefore, waste-waters treatment is quite imperative for sustainable development and is critical for the ecosystem and human health.

Production of Protease and Lipase by immobilization of whole cells of *Haloferax* ATCC BAA 645

Immobilization of whole cells of *Haloferax* ATCC BAA 645 in sodium alginate yielded pinkish-orange, soft gelatinous beads. As seen in **Table 1**, these immobilized cells placed in NT medium with 0.2% tween 80, simultaneously produced protease and lipase in 12 h which reached a maximum at 72 h. Similar production of protease enzyme reported by immobilized cells of *Halogeometricum sp.*[10]. Synthesis of protease and lipase in non-saline T medium with 0.2% tween 80 occurred in 42 h and was optimum in 90 h. However, the protease and lipase activities were 28% and 41% lower than that produced in NT medium **(Table 1).** Thus, indicating the capability of the immobilized cells to produce active enzymes even in non- saline conditions.

Treatment of Waste –waters using Batch reactor

As shown in **Fig.2** whole cell immobilized beads were used in the batch reactor for 7 cycles. The beads were stable and could be reused up to 5 cycles, after which there was considerable cell leaching from gel matrix with a decrease in protease and lipase activities. The 56.46 U/ml of protease and 5.7 U/ml of Lipase was observed during 2 cycles of treatment in saline fish waste-water. In non-saline dairy waste-water, the protease and lipase activities were 51.12 U/ml and 2.19 U/ml respectively.

Analysis of physicochemical parameters of waste waters

The results obtained of the initial physicochemical analysis of dairy and fish waste-water and their treated effluents are shown in **Table2**.

Table 1: Production of protease and lipase by immobilized *Haloferax* ATCC BAA 645 in NT and T medium

Time (h)	NT medium		T medium	
	Protease activity (U/ml) *	Lipase activity (U/ml) *	Protease activity (U/ml) *	Lipase activity (U/ml) *
0	0	0	0	0
6	0	0	0	0
12	3.31	0.0755	0	0
18	6.445	0.5	0	0
24	15.42	0.775	0	0
30	19.76	0.845	0	0.0725
36	22.55	1.03	0	0.095
42	39.22	1.35	0.97	0.405
48	46.235	1.45	5.1	0.605
54	53.835	1.72	10.3	0.84
60	60.21	2.205	14.065	1.14
66	59.96	2.55	19.1	1.32
72	63.875	5.35	23.99	1.615
78	50.905	4.25	30.65	1.955
84	39.865	4.385	37.44	2.08
90	35.615	3.5	44.32	2.15
96	31.22	3.05	17.03	1.25
102	29.06	2.05	8.915	1.15

***Each value is an average of two determination, NT (NaCl tryptone) medium, T (tryptone) medium**

Table 2 : Characterization of dairy and fish waste-water

Sr. No	Parameters	Units	Dairy waste water		Fish waste water	
			Untreated	Treated	Untreated	Treated
1	Color	-	Milky	clear	Brown	Light brown
2	pH	-	6.975 (± 0.03)	6.65 (± 0.02)	6.52 (± 0.10)	7.95 (± 0.21)
3	Electrical conductivity	µS	32.495 (± 3.30)	15.805 (± 2.29)	50.75 (± 6.95)	16.07 (± 1.41)
4	Total dissolved solids	mg L^{-1}	22.62 (± 4.24)	9.37 (± 1.14)	20.31 (± 1.30)	8.30 (± 1.31)
5	Salinity	%	0.175 (± 0.03)	0.075 (± 0.03)	1.53 (± 0.04)	1.11 (± 0.16)
6	Biological Oxygen Demand	mg L-1	1710 (± 21.21)	545 (± 35.35)	1735 (± 155.5)	400 (± 14.14)
7	Chemical Oxygen Demand	mg L-1	1868 (±73.53)	900 (± 84.85)	2185 (± 77.78)	675 (± 49.49)
8	Total protein	mg ml^{-1}	3.65 (±0.35)	0.865 (± 0.19)	4.2 (± 0.4)	2.55 (± 0.63)

Values in parenthesis indicate standard deviation. Each value is a mean of two determinations

The dairy effluent at the initial stage of treatment was milky but after treatment, it was clear. The fish waste water was brown, turbid at the primary stage but subsequently changed to light brown. These changes in color in dairy waste water were similar to changes reported by Verma and Madamwar[11]and Porwal et al [12]. Total protein removal rate from dairy and fish waste-water were 23.69 % and 60.71 % respectively **(Table 2).** The efficiency of protein degradation in both waste-waters depends on the number of soluble proteins and proteolytic and lipolytic activities shown by the isolate in gel matrix.

pH

pH of both the untreated samples was slightly acidic. After treatment with immobilized cells of *Haloferax*, the dairy waste-water remained acidic while fish waste waters turned alkaline **(Table 2)**.

Electrical conductivity (EC)

EC relates to the presence of impurities in water and consisting of ions like Cl^-, SO^{4-}, CO^{3-}, HCO^{3-} and NO^{3-} and Ca^{++}, Mg^{++}, Na^+ and K^+. After aeration of dairy and fish waste water with immobilized *Haloferax* cells, the EC reduced to 48.63 % and 31.66 %, respectively **(Table 2)**.

Total dissolved solids (TDS)

TDS content of the treated dairy and fish waste-water effluents are recorded in **Table 2**. It can be proposed that the organic contents along with other mineral ions present in effluents could have been used by immobilized cells through as simulation thus causing an overall reduction in total dissolved solids which also proves the efficiency in removal of nutrients[12].

Biological oxygen demand and Chemical oxygen demand

Effluent BOD : COD ratios vary widely within and among processing plants in the range of 1.1:1 to 3:1[13]. Dairy waste-waters are characterized by high BOD and COD values due to fats, nutrients, lactose, detergents, sanitizing agents, casein, and inorganic salts. In a fish processing waste-water, BOD originates primarily from protein, peptide and volatile amines. Reduction of 31.87 % and 48.17 % in BOD and COD values by immobilized cells in dairy waste-water effluent was observed. In fish waste water 23% of BOD and 30% of COD reduction in values was observed **(Table 2).**

CONCLUSION

In the present study, we report immobilization of *Haloferax* ATCC BAA which simultaneously produce protease and lipase in NT and T medium with high /without NaCl and additionally having 0.2% tween 80. The immobilized cells used in a batch reactor system for treatment of dairy and fish waste-water resulted in a reduction of EC, TDS, BOD and COD of treated waste-waters. These results, although preliminary, being biotechnological important in clarification of protein and lipid-containing waste-waters are used in developing a continuous batch reactor.

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CONFLICT OF INTEREST The authors declare that they have no conflict of interest.

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***Corresponding Author:**
Irene J Furtado*
Email: **ijfurtado@unigoa.ac.in**