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Procedia Manufacturing 22 (2018) 413–419

Procedia MANUFACTURING

www.elsevier.com/locate/procedia

11th International Conference Interdisciplinarity in Engineering, INTER-ENG 2017, 5-6 October 2017, Tirgu-Mures, Romania

Demineralized crab and shrimp shell powder: Cost effective medium for bacillus Sp. R2 growth and chitinase production

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Abstract

Crustaceous wastes such as shrimp, crab and lobster, are chitinous solid waste of the shellfish processing industry. to select the best substrates for bacterial growth and enzyme activity, different poor and enriched media were investigated, furthermore, the non-dematerialized and dematerialized shells powders of crab, shrimp, prawn and the mixed powders of crab+ shrimp shells and crab+ prawn shells were tested as substrates for and chitinase production by the chitin degrading bacterium Bacillus sp. R2 in a submerged fermentation (SMF) culture. The results revealed that, the tested poor media failed to enhance enzyme production, whereas the tested enriched media promote growth and protein content efficiently, in contrast the demineralized chitinous wastes were generally good carbon and nitrogen sources for growth and chitinase production. The most favorable substrates were demineralized crab + prawn followed by crab + shrimp. The obtained results would certainly encourage the utilization of shellfish processing (Crab and Shrimp Shell) waste for the industrial production of chitinase via submerged fermentation (SMF).

Peer-review under responsibility of the scientific committee of the 11th International Conference Interdisciplinarity in Engineering.

Keywords: Bacillus sp. R2; chitinous waste; crab and shrimp shell; chitinase; production.

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2351-9789
Peer-review under responsibility of the scientific committee of the 11th International Conference Interdisciplinarity in Engineering.
10.1016/j.promfg.2018.03.065

1. Introduction

It is reported that approximately 6-8 million tons of crustacean waste is produced worldwide every year [1]. The current increase in crustacean wastes from shrimp and crab industry in the most producing countries in the word (China, Indonesia, Thailand and India) pose serious disposal problems, bioconversion of crustacean waste has been proposed as an alternative treatment [2]. During the last decade, chitin-containing marine crustacean waste have received an increased attention since the major components of this waste are chitin, protein, flavorant, pigment and minerals. The bioprocessing using chitinolytic microorganisms or their chitinases not only resolves the environmental problem but also ensure the full utilization. for this reason, the biological method (bacteria or enzymes) are preferable because it is economic, safe, eco-friendly and can maximize the crustacean wastes utilizations such as the recovery of pigment, lipids, chitin, chitosan, chitooligosaccharides and the protein hydrolysate which can be used as an excellent fish or animal feed. [3,4]

Recently many researchers investigated the bioconversion of shrimp and crab shell powder (SCSP) for the production of many valuable compounds such as proteases[3], chitinases[4], chitosanases ,antifungals and antimicrobial compounds[5] ,antioxidants[6], carotenoids, astaxanthin[7], flavour compounds, and calcium carbonate[8]. The objective of this research was to investigate the suitability of some treated and untreated chitinous wastes as cost effective media for *Bacillus sp. R2* growth and multiple enzymes production.

2. Material and Methods

2.1. Chemicals

Chitin was extracted from crustaceans by the method of (Synowiecki *et al*. 1982) [9], Swollen chitin was prepared according Monreal and Reese, (1969) [10]. Crab shell chitin flakes (Win-lab, UK). N-acetyl glucosamine, and bovine serum albumin were from (Sigma -USA). Peptone tryptone, and yeast extract were obtained from (Oxöid Hampshire, England). All other chemicals used were of the highest grade available.

2.2. Crustaceous wastes preparation and demineralization

Raw dried offal's or shells of crab, shrimp, prawn, squilla, squid and clams were milled as fine as possible and demineralized with 22% HCl (1:10) (w/v) for 2 hours at room temperature with vigorous steering [9].

2.3. Microorganism and maintenance

Bacillus sp. R2 marine bacterial strain isolated from red sea Egypt and identified biochemically and molecularly by Cheba et al. 2006 (strain accession number in NCBI GenBank was: DQ923161). To maintain the isolated bacterial cells the short-term maintenance was performed repeatedly at an interval of 2-3 months at 4°C using marine LB Agar slants. Moreover, the long-term maintenance, more than 2 years, was performed by adding 0.5 ml of the early stationary phase cultures grown in marine LB to 50% (v/v) sterile glycerol and the cultures were kept at-20°C.

2.4. Effect of poor and enriched media on chitinase production

Chitinase activity and protein content were determined through growing of the bacterial isolate in different commonly used liquid media. The poor media were: (Natural sea water + 0.05 % yeast extract), (Artificial sea water + 0.05 % yeast extract), M9 minimal medium and William Basel medium, the enriched media were: LB medium, PY medium, Nutrient broth (NB) and marine Nutrient broth (M NB).

2.5. Effect of chitinous wastes on cell growth and enzymes production

Sea water medium (100% sea water) pH: 7.5 were supplemented separately with 0.5% of non-demineralized or demineralized chitinous wastes. After autoclaving, the flasks were inoculated with 3% of overnight activated strain