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Modeling and optimization of Newfoundland shrimp waste hydrolysis for microbial growth using response surface methodology and artificial neural networks

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A B S T R A C T

The hydrolyzed protein derived from seafood waste is regarded as a premium and low-cost nitrogen source for microbial growth. In this study, optimization of enzymatic shrimp waste hydrolyzing process was investigated. The degree of hydrolysis (DH) with four processing variables including enzyme/substrate ratio (E/S), hydrolysis time, initial pH value and temperature, were monitored. The DH values were used for response surface methodology (RSM) optimization through central composite design (CCD) and for training artificial neural network (ANN) to make a process prediction. Results indicated that the optimum levels of variables are: E/S ratio at 1.64%, hydrolysis time at 3.59 h, initial pH at 9 and temperature at 52.57 °C. Hydrocarbon-degrading bacteria Bacillus *subtilis* N3-1P was cultivated using different DHs of hydrolysate. The associated growth curves were generated. The research output facilitated effective shrimp waste utilization.

1. Introduction

Waste generated from seafood processing plants has been a major concern in coastal Newfoundland (Adams et al., 2005) and shrimp waste represents 37% of the total seafood processing waste in the province. There were 110,000 tons of shrimp landed in Newfoundland annually, from which over 40% (w/w) is discarded as solid shrimp waste (Gildberg and Stenberg, 2001). Although some of such waste can be transformed into value added products, there is still large amount being discarded as processing effluents (Jamieson et al., 2013). To date, hydrolysis has been regarded as a promising option to utilize seafood processing waste. The hydrolysate can be used as low-cost sustainable nutrient sources for microbial growth due to their high protein contents (Dufossé et al., 2001; Klompong et al., 2012; Martone et al., 2005; Safari et al., 2012). Shrimp waste generally contains 8–10% chitin, 30–40% protein and 10–20% calcium in dry weights (Gallert and Winter, 2002). The high protein content, inexpensive source and relative abundance make shrimp waste a promising bacterial growth substrate. Shrimp waste has been studied with various hydrolysis methods (Cahú et al., 2012; Gildberg and Stenberg, 2001; Quitain et al., 2001; Ruttanapornvareesakul et al., 2006). As the autolysis hydrolysis and acid hydrolysis are complex and inefficient processes, and need higher demand on reaction conditions to be effective, the enzymatic hydrolysis has been recognized as a more applicable method (Kristinsson and Rasco, 2000; Samaranayaka and Li-Chan, 2008). Alcalase, a commercial bacterial protease with high efficiency, have been widely employed to enzymolysis shrimp waste (Abdul-Hamid et al., 2002; Dey and Dora, 2014; Synowiecki and Al-Khateeb, 2000). During the protein hydrolysis process, large numbers of peptide bonds are cleaved in parallel and series. This produces complicated matrix of substrate and triggers new hydrolysis progress (Marquez and Vázquez, 1999). In addition, thermal inactivation of enzymes at the end of hydrolysis is another sophisticated process and not well understood. To help study factor (e.g., temperature, pH, and time) effects, understand the mechanisms of hydrolysis, predict the performance, and promote its applicability, experimental and modeling methods have been recognized as effective solutions (Morgenroth et al., 2002).

One-factor-at-a-time (OFAT) experimental method studies a process by changing one independent factor at a time and keeping all the others as constants. It can be used for selecting key parameters with their ranges of interest and operability (Bari et al., 2009). To study interaction effects among different factors and find the true optimum, response surface methodology (RSM) has been widely used (Montgomery, 2008). RSM is an effective experiment-based tool to optimize a process when multiple factors and their interactions may affect the response (Rodrigues et al., 2006; Wangtueai and Noomhorm, 2009; Zheng et al., 2008;). This statistics-based technique is suitable for process optimization with fitting for a quadratic surface (Myers et al., 2009). Bari et al. (2009) employed the OFAT method and central composite design (CCD) of RSM to optimize media for the improvement of production of citric acid from oil palm empty fruit bunches. See et al. (2011) used three factors, five levels CCD design of RSM to optimize Salmon skin protein hydrolysis to obtain the maximum degree of

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hydrolysis using Alcalase. OFAT and RSM are usually used for evaluating the effect of the independent variables, alone or interaction and select optimum conditions of variables in the process. However, it is hard to say that they can be applied to all optimization and modeling studies (Bas and Boyaci, 2007). As mainly used for linear and quadratic approximations, they are not suitable in highly nonlinear cases (Desai et al., 2008). It is also difficult to conduct sensitivity analysis of input parameters due to the presence of cross interactions (Lou and Nakai, 2001). To obtain a more-predictive model with less requirements on data, RSM needs to be integrated with other modeling tools for system prediction.

In the past thirty years, artificial neural network (ANN) has become as an attractive tool for non-linear multivariate modeling and predictions based on experiment datasets (Desai et al., 2005). It is a data processing system that imitates the human brain's way of working (Buciński et al., 2008). It combines artificial neurons that receive inputs into layers. When the input is received, the output would be calculated from the weighted input signal (Kuvendziev et al., 2014). Without knowing the detailed relationship of processing variables in advance, ANN can recognize and replicate cause-effect relationships with its capability of adaptive training and data self-organization. This makes ANN an efficient tool to study complex systems (Khajeh and Barkhordar, 2013). It has been widely used in many fields of science and engineering (Gardner and Dorling, 1998; Fukuda and Shibata, 1992; Kasiri et al., 2008; Yi et al., 2007). It has several advantages over the conventional mathematical modeling methods and has been successfully applied to model protein hydrolysis processes (Abakarov et al., 2011; Bucinski et al., 2004; Buciński et al., 2008; Li et al., 2006). To have better predictability of the process behavior through optimization, the integration of ANN and RSM has been an effective way. Shao et al. (2007) built a predictive model for the recovery of Tocopherol from rapeseed oil deodorizer distillate combining ANN with RSM. Kasiri et al. (2008) optimized heterogeneous photo-Fenton process for degradation of C.I. Acid Red 14 azo dye integrating ANN and RSM. However, to date, no integration of RSM and ANN has been applied in optimization of shrimp waste utilization.

In this study, to fill the above knowledge gap, the hydrolysis of northern pink shrimp (*Pandalus borealis*) waste generated in Newfoundland was used as an illustrative example. Factors including enzyme/substrate ratio (E/S), hydrolysis time, initial pH value and hydrolysis temperature were studied as system inputs. The degree of hydrolysis (DH) of shrimp waste was used as the output. Both RSM and ANN were integrated for system optimization through investigating the effect of inputs and their relationships with the output. A four-factor, five-level CCD design of response surface methodology was employed in RSM. The experimentally-determined DH for different levels of factors were then used for training the ANN to simulate the process. The ANOVA and RSM were applied to test the null hypothesis that the significances of each factor and their interactions on the DH are equal against the alternative that they are not equal. The contributions of each input factor to the output were also evaluated by ANN. The optimum conditions for shrimp waste hydrolysis were also determined. Products with three different DHs were finally used as the nitrogen source to cultivate Bacillus *subtilis* N3-1P, a hydrocarbon-degrading bacterium to validate the growth efficiency related to DH.

2. Materials and methods

2.1. Shrimp waste hydrolysis

2.1.1. Materials and reagents

Shrimp waste including heads, shells and tails of northern pink shrimp was purchased from a local fish market in Newfoundland, Canada. The shrimp waste was grounded in a food processor (Black & Decker Model FP2700SC) and packed in plastic bags. The grounded materials were kept frozen at −18 °C. The enzyme used for the hydrolysis of shrimp waste is Alcalase 2.4L (Sigma Aldrich, U.S. ≥2.4 U/g). It is a commercial proteinase from Bacillus licheniformis, subtilisin A, inexpensive and nonspecific with endopeptidase activity. Chemicals used for medium content were analytical reagent purchased from Sigma Aldrich, Canada.

2.1.2. Shrimp waste hydrolysis

The hydrolysis procedure using shrimp waste was modified from Dey and Dora (2014). The grounded shrimp waste had been thawed for 1 h at room temperature and suspended (1:1, *w*/*v*) in distilled water in a baffled colonial flask. The mixture was then heated in a water bath at 90 °C for 20 min to inactive the indigenous hydrolyzing enzyme. Different E/S ratio of enzyme was added into each corresponding flask when the mixture was cooled to room temperature (20 °C). The 1N HCl or 1N NaOH solution was employed to adjust the initial pH value. The flasks were then put into a temperature controlled water bath with a shaking rate at 110 rpm. The enzyme was inactivated by heating at 90 °C for 10 min. The samples were cooled to room temperature again and subsequently centrifuged at 10,000 rpm for 15 min. The supernatant was collected and then freeze dried to obtain dry powder. The dry powder was kept frozen in plastic bottles in a −80 °C freezer.

2.1.3. Determination of DH

DH was determined according to the method of Hoyle and Merritt (1994). The 20% trichloroacetic acid (TCA) was added to the supernatant (1:1, v/v) to create 10% TCA-soluble and TCA-insoluble fractions. The mixture was then centrifuged at 6000 rpm at room temperature for 20 min to collect the 10% TCA-soluble supernatant. The DH was computed as the ratio, percent of 10% TCA-soluble nitrogen to total nitrogen of the sample. All the nitrogen content was determined using the Kjeldahl method (AOAC, 2005). Each testing was performed in triplicate and the result was expressed as the mean of triplicate trials ± standard deviation.

$$\mathrm{DH} = \frac{10\%\mathrm{TCA}-\text{soluble N in sample}}{\text{Total N in sample}} \times 100\% \tag{1}$$

2.2. RSM and ANN Design

2.2.1. RSM design

RSM was used to optimize enzymatic hydrolysis of shrimp waste. According to literature (See et al., 2011; Dey and Dora, 2014), four main factors including E/S ratio, hydrolysis time, initial pH value and hydrolysis temperature were selected for CCD. OFAT experiments was conducted first to choose the most critical factors and their reasonable ranges (fixed level of four factors were E/S ratio = 0.5%, time = 1 h, pH = 8 and temperature = 40 °C). The main effects of critical factors on the degree of hydrolysis are shown in Fig. 1.

As shown in Fig. 1, the central points of E/S ratio, hydrolysis time, initial pH and hydrolysis temperature were set as 1.25%, 2.5 h, 8 and 50 °C, respectively. Therefore, a four-factor, five-level CCD was developed. The four independent variables and their experimental ranges are shown in Table 1. The variables X_i were coded as x_i according to the following relationship:

$$x_i = \frac{X_i - X_0}{\delta X} \tag{2}$$

Where, X_0 is the value of X_i at the center point and δX stands for the step change.

The CCD was comprised of 29 treatments including 2^4 factorial points, eight axial points ($\alpha = 1.41$) and five replicates at the center points. DH is used as the response for the combination of the independent variables as shown in Table 2. Randomized experimental runs were adopted to minimize the effects of unexpected variability in the