

Research and Optimization of Technological Process Based on Fermentation for Production of Seaweed Feed

Ling Han, Shuping Zhang*, Jie Ma, Xiaohui Liu

School of Medical Instruments and Food Engineering, University of Shanghai for Science and Technology, Shanghai, China
Email: *hlmmc@126.com

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ABSTRACT

This paper for the purpose of utilization, conducted a study of technological process of producing animal feed by fermenting seaweed waste. Anaerobic fermentation, which can improve the contents of protein and polysaccharide in seaweed waste, proved to be an available method to improve the nutritional value of animal feed by using seaweed waste. Also effects of different additives and fermentation time on the fermentation products was compared, combined CCRD with neural network to optimize these factors, the predict model among all factors was established, also obtained the optimal fermentation process.

Keywords: Algae Waste; Anaerobic Fermentation; Animal Feed

1. Introduction

The seaweed waste, the insoluble fiber residue of which glue, iodine and other substance has been extracted from seaweed waste, mostly used as waste discharged into the natural world directly, that not only caused waste of resources, also resulted in environmental pollution [1], while there are abundant nutrients in the seaweed waste, as high-quality animal feed ingredients, which can effectively improve the immunity of animals and increase the economic benefits [2,3].

Microbial fermentation which is of advantage with short cycle and high efficiency, has been widely used in food, feed and other industries using the characteristics of the microorganism itself contains large amounts of protein, has a promising prospect. Algae residue contains major required nutrients of animal feed: protein, crude fiber and other trace elements, which the protein and crude fiber content is usually 8.86% - 8.92% and 43.16% - 43.65% respectively [4]. Not only the protein content of algae residue can be increased by using microbial anaerobic fermentation with adding appropriate source of nitrogen and other growth factors, can also make the crude fiber degrade into some monosaccharides and polysaccharides prebiotics which are necessary to animals. This method can effectively improve the nutrient content of the algae residue and enhance its application in animal feed value with the major effects: 1) improve the rumen environment and promote the ecological balance of microorganisms *in vivo*; 2) improve feed conversion rate; 3) produce

organic acids [5,6]. Also this method is of low cost of raw materials, simple process and short cycle, and has not been reported before.

Fermentation results mainly influenced by the active of the bacteria agents, while the activity of the bacteria agents are influenced by the environment and dosage of auxiliaries, these factors impact on the fermentation results differently [7], or these are interaction. The experiment using Central Composite Design (CCD) combined with neural network prediction model, is in order to obtain the optimum process conditions.

2. Materials and Methods

2.1. Materials

Seaweed industrial residues.

2.2. Major Equipments

Mill, the production from the Beijing Kun Jie Yu Cheng Machinery Co., Ltd., Model KCS-10; heated oven, the production from Kim Nam Instrument Factory, model DHG-9053A; Biochemical incubator, the production from Hai Qixin Scientific Instruments Co., Ltd., model LRH-70.

2.3. Main Experimental Methods

2.3.1. Fermentation Process

Seaweed residue was crushed through the mill to a particle diameter of 3 mm - 5 mm, then added cellulase, potassium dihydrogen phosphate, ammonium chloride, the

*Corresponding author.

EM inoculants (the EM effective microbial agents is a novel compound microbial preparation, brown translucent liquid, PH value between 3.5 to 4.5, the composite culture of photosynthetic bacteria, lactic acid bacteria, yeasts, actinomycetes bacteria, Acetobacter and other five families, 10 are over 80 kinds of anaerobic or suspected of oxygen normal microbiala), Indian meal, lactose and water, later mixed into the airtight container well-proportionally and placed in a biochemical incubator (25°C) for fermentation, until fermentation is completed and then removed from the container, placed in heated oven (50°C) to dry.

2.3.2. Determination of Protein and Polysaccharide Content

In this study, all the protein and polysaccharide content of samples are determined by Kjeldahl method (GB5511-85) (nitrogen protein conversion factor of 5.95) and the phenol sulfuric acid method.

2.3.3. Experimental Design

The effect on fermentation products of cellulase, bacteria agents, ammonium chloride and number of days are investigated respectively. Combined with CCD and General Regression Neural Network (GRNN), protein content and polysaccharide content, two dependent variables, were analyzed to determine the extraction of the optimal process conditions.

2.4. Data Process Software

The experimental data was analyzed by the desingexpert6.5, and Matlab2009b software.

3. Results and Discussion

3.1. Effect of Basic Material on Fermentation

3.1.1. Effect of Temperature on Fermentation

Temperature is one of the most important factor to affect microbial growth and reproduction, same microorganisms has the different optimal temperature with variable physiological and biochemical processes, that means, the optimum growth temperature is not equal to the highest incubation temperature, neither does not mean fermentation speed is highest when incubation temperature nor the cumulative amount of metabolites when the incubation temperature, also does not mean the highest amount of incubation temperature either. For instance, *Streptococcus thermophilus* optimal growth temperature is of 37°C, the optimal fermentation temperature is of 47°C, the cumulative product of the optimum temperature is of 37°C. From **Figure 1**, the protein content of the fermentation products gradually increased with increasing temperature at the range of 20°C - 25°C, reached the maxi-

mum at 25°C, the protein content decreased with increasing temperature over 25°C. Apparently microbial enzyme activity will be affected when temperature is higher than 25°C, which leads to restraint of regular growth and reproduction, resulted in protein and crude fiber content of fermented products has been decreased with increasing temperature. Accordingly, the optimal temperature of the fermentation is of 25°C.

3.1.2. Effect of Material-Water Ratio on Fermentation

Material-water ratios is an important influential factor in solid-state fermentation, extortionate water content will lead to aggregation of medium and cause lower porosity, affect the transfer of oxygen; while water content is too low, medium expansion will be retarded, which is not able to meet the needs of the microbial growth and metabolism, thereby inhibiting cell growth. The experiment has been carried out to study the effect of material-water ration on fermentation; results are shown in **Figure 2**. According to **Figure 2**, the material-water ratio had a greater influence on fermented products. Protein and polysaccharide increased gradually while material-water ratio from 50% - 65%, declined slightly at 70%, and de-

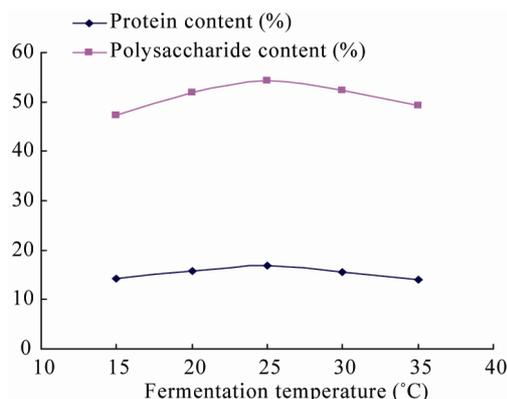


Figure 1. Effect of temperature on fermentation.

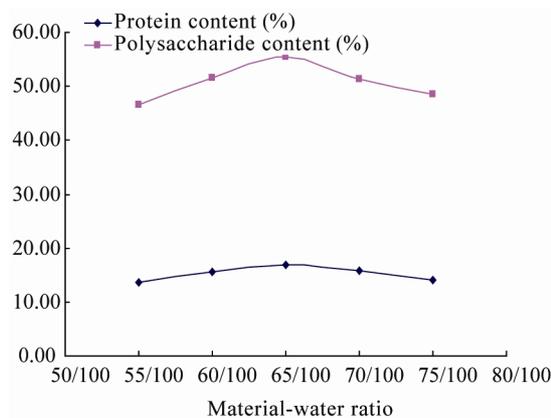


Figure 2. Effect of material-water ratio on fermentation.

clined sharply from 75% - 90%. Water-material ratio at the range of 50% - 65%, seaweed residue is of high hardness, bonded together in lumps, and turn to loose from 70% - 85% and semi-liquid at 90%, according to above analysis of data and phenomena, water-material ration at 65%, algae residues is of a fitting loose condition to maintain certain porosity and advantageous to interaction of water and air in solids, so that the dissolved oxygen can be delivered smoothly. Thus, the water-material ratio at 65%, the fermentation products is of the highest protein and polysaccharide content.

3.2. Single Factor Analysis

Basic raw materials: seaweed waste 1000 g, water 1500 g, potassium dihydrogen phosphate 10 g, Indian meal 15 g, lactose 15 g.

3.2.1. Effect of Cellulase on Fermentation

Under the condition of adding bacteria agents 10 g, ammonium chloride 15 g, fermentation of 10 days to study the effect of cellulase on fermentation. Cellulase can decompose raw materials in the natural cellulose into fermentable sugars, the other hand, cellulase can decompose the cell wall to release protein, starch and other nutrients in it, while the raw fiber can be degraded into the glucose which can be digested and absorbed by livestock. **Figure 3** shows that protein and polysaccharide content of fermentation products is 9.58% and 47.25% respectively when added 6 g cellulase, while adding cellulase 10 g, protein and polysaccharide content reaches its maximum (12.68%, 58.45%), further addition of cellulase protein brought about a downward trend the content of protein and polysaccharide, this is due to the cellulose to cellulase adsorption sites has basically occupied, increasing the amount of enzyme can only increase the adsorption of enzyme invalid, and thus will not significantly im-

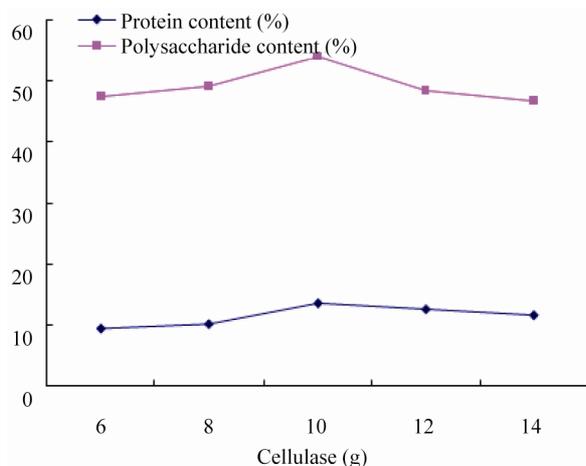


Figure 3. Effect of cellulase on fermentation.

prove the effect of fermentation; the other hand, enzyme fermentation environment of excessive make pH decreased, the agents normal metabolic activity, reduction of the fermentation product. Therefore, 10 g is an ideal addition amount of cellulase.

3.2.2. Effect of Bacterial Agents on Fermentation

Under the condition of adding cellulase 10 g, ammonium chloride 15 g, fermentation of 10 days to study the effect of bacterial agents on fermentation. **Figure 4** shows that when less amount of agents was added, the effect of fermentation is poor, protein and polysaccharide content in fermentation products is only 9.23% and 44.85%, since the synergy and co-residence effect between the microbial, increasing amounts of agents will improve fermentation effect significantly, with the increasing amount of bacteria agents, the maximum protein and polysaccharide content (12.85%, 52.15%) was got by added agents 8 g, while added bacteria agents amounts further, protein and polysaccharide content presented a downward trend, this may occur because the autolysis of bacteria due to improvement of proteolytic enzyme activity or some bacteria produced antibiotics and other secondary metabolites. Therefore, the amount bacteria agent of about 10 g is an ideal.

3.2.3. Effect of Ammonium Chloride on Fermentation

Under the condition of adding cellulase 10 g, bacteria agent 8 g, fermentation of 10 days to study the effect of bacterial agents on fermentation. Adequate source of nitrogen are necessary for microbial propagation to synthesize such as amino acids, nucleic acids and other cellular material and to regulate metabolism, so necessary nitrogen should be added into fermentation, on the other hand, addition of inorganic nitrogen can promote microbial metabolism transformed it into protein, consequently improve the protein content in the algae residue directly.

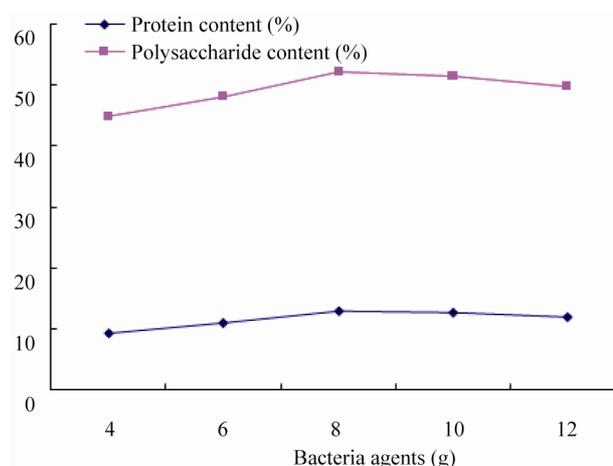


Figure 4. Effect of bacterial agents on fermentation.

Figure 5 shows that, the best fermentation result was made when ammonium chloride dosage is of 15 g, the content of protein and polysaccharide was 14.85% and 54.25% respectively, while increasing ammonium chloride further did not affect the amount of the polysaccharide content, but will caused protein content to decrease significantly, this is because excess ammonium chloride make fermentation environment weakly acidic, thereby affecting the cell structure of bacteria, thus affecting isolates metabolism and product synthesis, so the amount of ammonium chloride, 15 g best.

3.2.4. Effect of Number of Days on Fermentation

Under the condition of adding cellulase 10 g, bacteria agent 8 g, ammonium chloride 15 g to study the effect of number of days on fermentation. From Figure 6, fermentation time on the fermentation products is a significant, the content of protein and polysaccharide increased while the number of fermentation days increasing, fermentation achieve maximum protein and polysaccharide contents(14.75% and 54.13%) when days of fermentation was 8, it showed that microbial metabolism entered into stable period gradually in the first 8 days, further increasing of fermentation time over 10 days, the content of crude protein and polysaccharide in fermented product decreased by microbial autolysis, thus, fermentation time of 8 days is optimum.

3.3. Central Composite Design

According to the results of univariate analysis, protein and polysaccharide contents of fermentation products were investigated by added different contents of cellulase, bacteria agents, ammonium chloride and different number of fermentation days. Four factors-five level central composite experimental design was established by Design Expert to study the content of protein and polysaccharide. In this study, there are total of 31 experimental points, including 16 factorial points, 7 center points and 8 axial points [8]. The axial point represents the level of the extreme value of each independent variable; Central point represents the center level, repeated seven times to estimate experimental error. Response surface analysis of factors and levels are shown in Table 1, the experimental results are shown in Table 2.

Table 1. The experiment factors and their levels.

Factors → Level	X1: cellulase (g)	X2: bacteria agents (g)	X3: ammonium chloride (g)	X4: numbers of fermentation days (days)
-2	6	4	10	4
-1	8	6	12.5	6
0	10	8	15	8
1	12	10	17.5	10
2	14	12	20	12

3.4. Data Processing and Analysis Based on GRNN

GRNN is a kind of Radial Basis Function (RBF), consists of input layer, radial grass-roots layer and the linear layer. GRNN topology was shown in Figure 7. Because of its structural features, GRNN has the advantages of quick training speed, strong nonlinear mapping ability and capacity of local approximation, especially suitable for the modeling of the small number of samples. In addition, there is only one parameters for GRNN adjustment, this can quickly find the forecast network [9].

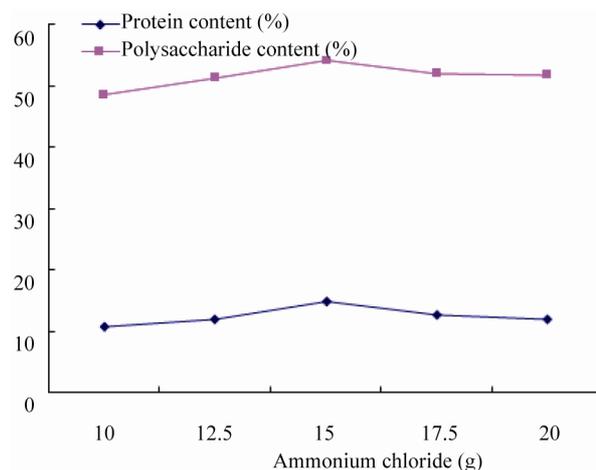


Figure 5. Effect of ammonium chloride on fermentation.

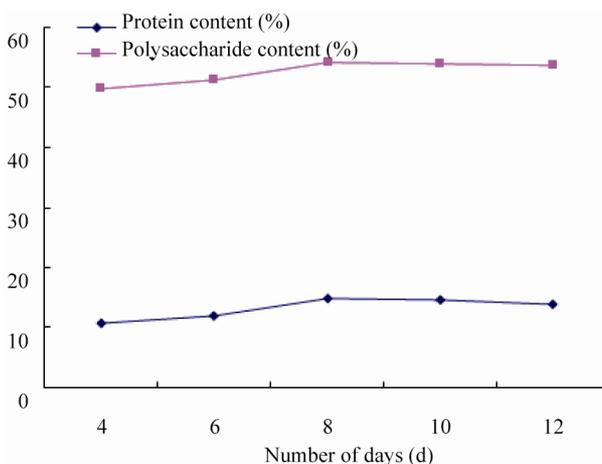
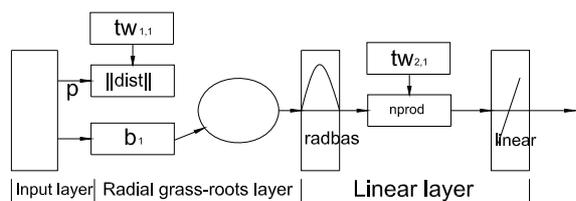


Figure 6. Effect of number of days on fermentation.

Table 2. The central composite rotatable design and results.

Experiment NO.	Cellulase	Bacteria agents	Ammonium chloride	Numbers of fermentation days	Protein content (Y1)/%	Polysaccharide content (Y2)/%
1	-2	0	0	0	13.115	52.782
2	-1	-1	1	-1	15.513	49.340
3	-1	-1	1	1	14.880	52.197
4	-1	-1	-1	-1	12.720	52.596
5	-1	1	1	-1	16.486	53.401
6	-1	-1	-1	1	12.734	52.999
7	-1	1	1	1	16.930	53.088
8	-1	1	-1	1	15.589	54.206
9	-1	1	-1	-1	14.099	54.579
10	0	-2	0	0	13.759	50.290
11	0	0	2	0	16.666	51.329
12	0	0	0	-2	15.806	52.482
13	0	0	0	0	15.705	53.191
14	0	0	0	0	15.734	53.204
15	0	0	0	0	15.725	53.241
16	0	0	-2	0	14.233	54.828
17	0	0	0	2	15.321	55.072
18	0	2	0	0	14.839	57.840
19	0	0	0	0	15.350	53.194
20	0	0	0	0	15.644	53.049
21	0	0	0	0	15.281	53.246
22	0	0	0	0	15.307	53.253
23	1	-1	1	-1	16.074	49.454
24	1	-1	1	1	14.636	52.453
25	1	1	1	-1	16.233	52.593
26	1	-1	-1	-1	13.333	52.932
27	1	1	1	1	16.770	53.075
28	1	-1	-1	1	12.865	53.502
29	1	1	-1	1	15.882	54.719
30	1	1	-1	-1	15.176	54.287
31	2	0	0	0	15.789	54.067

**Figure 7. Topology diagram of GRNN.**

3.4.1. Normalization of Samples

Due to the gap between the range of actual experiment value of protein and polysaccharide content is large, so normalization has been made first to transform the actual

data to modeling data ([0, 1] value) in accordance with formula (1), and convert data into actual value later by formula (2), the normalized results are shown in **Table 3**.

$$x_i = (X_i - X_{\min}) / (X_{\max} - X_{\min}) \quad (1)$$

$$X_i = x_i (x_{\max} - x_{\min}) + x_{\min} \quad (2)$$

where x_i —data after normalization;

X_i —Actual data;

X_{\min} —Minimum;

X_{\max} —Maximum.

3.4.2. Results of Prediction Model

GRNN model has been built by Matlab, four arguments

are cellulase, agents, ammonium chloride and fermentation days, two dependent variables are protein content and polysaccharide, the prediction model was evaluated by the correlation coefficient R^2 , the smoothing factor with the optimization value of 0.1, the protein content model R^2 is 0.9671, the polysaccharide content model R^2 is 0.9920, the results indicated that the model has good accuracy, can be used to predict the optimal value, as shown in **Figures 8** and **9**.

3.4.3. Analysis of Optimal Value

Optimal value has been found by Matlab in the above model, the optimal fermentation condition: cellulase 8.5

g, bacteria agents 10.9 g, ammonium chloride, the amount of 18.1, fermentation 9 days. In this condition, the protein content of the fermentation product is 17.15%, the polysaccharide content of 58.23%, satisfactory results.

3.4.4. Validation Experiment

In order to test the reliability of the GRNN model predicted values, further validation test has been made. Validation experiment with 10 L fermentation tank was carried out, partial samples was taken out every 2 days to analyzed the content of protein and polysaccharide in the fermented products, the experiment was repeated three times and take the average as the final result, the relevant

Table 3. Result of normalization.

	NO.	1	2	3	4	5	6	7
protein content		0.0938	0.6634	0.5131	0	0.8945	0.0034	1.0000
polysaccharide content		0.4049	0	0.3361	0.3830	0.4778	0.4305	0.4409
	8	9	10	11	12	13	14	15
protein content	0.6814	0.3276	0.2469	0.9372	0.7331	0.7090	0.7159	0.7138
polysaccharide content	0.5725	0.6164	0.1118	0.2340	0.3696	0.4531	0.4546	0.4589
	16	17	18	19	20	21	22	23
protein content	0.3593	0.6179	0.5034	0.6248	0.6945	0.6083	0.6145	0.7966
polysaccharide content	0.6456	0.6743	1.0000	0.4534	0.4363	0.4595	0.4604	0.0134
	24	25	26	27	28	29	30	31
protein content	0.4552	0.8345	0.1455	0.9621	0.0345	0.7510	0.5834	0.7290
polysaccharide content	0.3662	0.3827	0.4226	0.4394	0.4896	0.6328	0.5820	0.5561

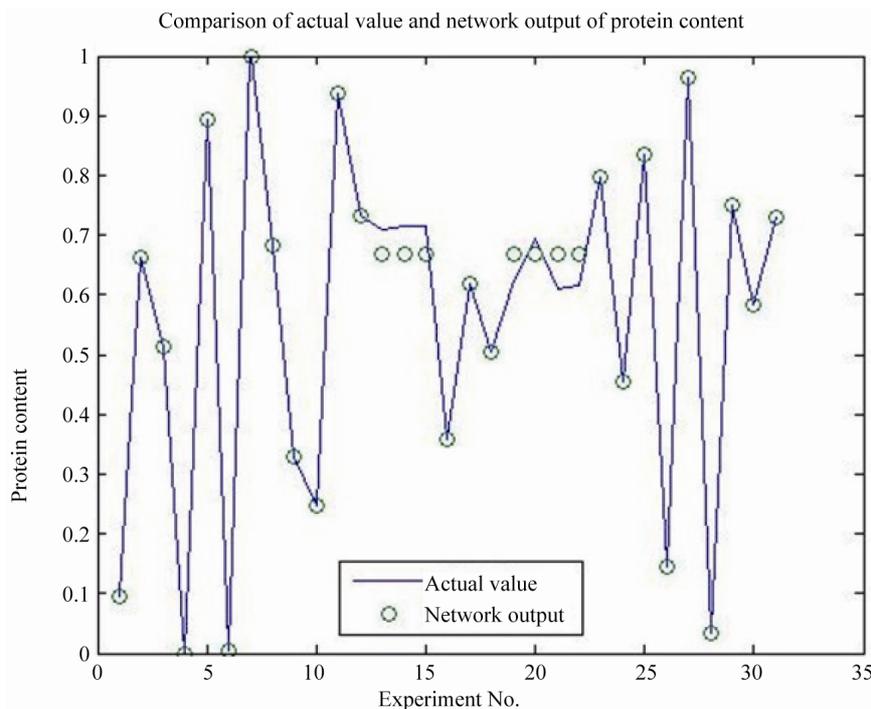


Figure 8. Comparison of actual value and network output of protein content.

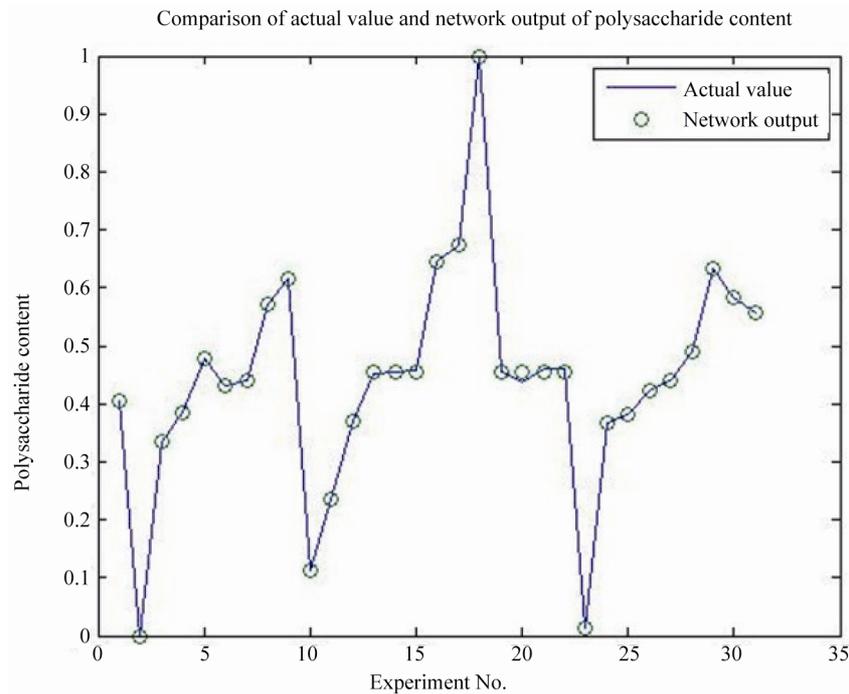


Figure 9. Comparison of actual value and network output of polysaccharide content.

parameters of the fermentation process are shown in **Figure 10**.

From **Figure 10**, due to decrease of cellulase after 3 days, nutrients in fermentation tank continuously generated and deposited with the different species adapt to each other to form a symbiotic relationship and turn to stable period, in this period, the protein content continues to increase until the maximum while the amount of bacteria increasing, the highest protein content was got at 9 days, then as the autolysis of bacteria, the acid protease activity rebounded, resulting in lower protein content. During the fermentation process, part of bacterial degraded cellulose into single carbohydrate, glucose and xylose, result in increase of polysaccharide content, while

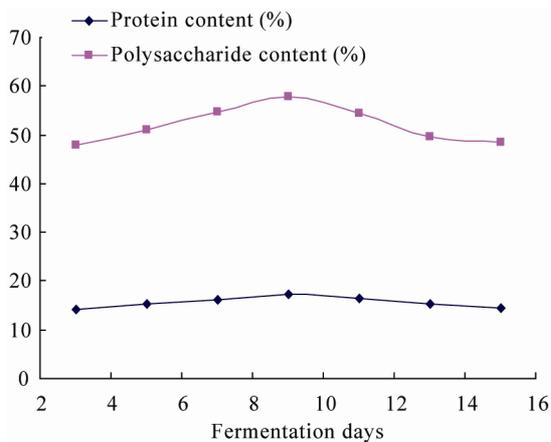


Figure 10. Results of validation experiment.

fermentation time increasing, pH rendered acidic, making monosaccharides, disaccharide and other carbohydrate into insoluble lipids, resulting in lower polysaccharide content.

Under above optimal conditions, fermentation experiments were repeated three times, and actual measured protein content of fermentation products was 17.20%, 57.79% polysaccharide content, while predictive value of the errors were only 0.29% and 0.76%, indicating that the experimental parameters of the GRNN model are true and reliable, has practical value.

4. Conclusion

This study based on the comprehensive utilization of seaweed waste, by means of anaerobic fermentation, focusing on different amounts of auxiliary additives and different fermentation days of protein and polysaccharide content in the fermentation products, CCD combining with GRNN modeling has been carried to optimize prediction model, the optimal fermentation conditions as follows: cellulase 8.5 g, bacteria agents 10.9 g, ammonium chloride 18.1, fermentation of 9 days. Under this condition, the protein content and polysaccharide content have achieved satisfactory results, 17.20% and 57.79% respectively. Under the conditions further confirmatory test has been made, the error between actual value and predicted value of content protein and polysaccharide is 0.29% and 0.76%, respectively, indicating that optimization results are authentic.

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