Kitchen Waste as a Novel Available Substrate for Prodigiosin Production by *Serratia Marcescense*

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Abstract. As a compound with antitumor activities, prodigiosin was produced by *Serratia marcescens* with kitchen waste as a substrate. The initial yield of prodigiosin was 223mg/l and can be further improved with supplementation of nitrogen source and inductor to the medium. A 290% increase of the prodigiosin production was achieved with 1% peptone and 0.2% proline. Physiological fermentation factors such as incubation time (36h), substrate concentration (35g/l), pH of the medium (pH 8.0), and fermentation parameter such as temperature (28℃), agitation speed (150rpm), inoculum level (1%), and liquid volume (20/150ml) all have impact on the prodigiosin production. With optimization, the maximum prodigiosin yield was 890mg/l and kitchen waste was proved to be a novel and economic substrate for prodigison production.

1. Introduction

Although the red pigments produced by a restricted group of *Serratia* genera were discovered at the very beginning of mankind history, further study about the molecular structure and medicinal function of prodigiosin was rudimental until recently. Owing to the rapid advancement in separation science and spectroscopy in the subsequent years, it became clear that "prodigiosin" has a series of close relatives bearing the same pyrrolylpyrromethene ("prodiginine") core with different alkyl substituent [1]. Prodigiosin was also found to be a potent anticancer agent which could induce apoptosis of several in vitro cancer cell lines including hematopoietic cancer cells, colon cancer cells, B-cell and chronic lymphocytic leukemia cells. Many PG--derivatives with lower toxicity like GX15-070 [2] has been used in clinic, and the price of pure prodigiosin was up to $500/mg (Santa Cruz Biotechnology, Inc., U.S.A., 2010). The high price is mainly due to its low fermentation yield and high production cost. Many studies aiming to improve the fermentation yield have been done, including adding amino acids, sodium dodecyl sulfate, lipid into the medium [3] and optimizing fermentation parameter such as pH, dissolved oxygen level, and temperature [4]. But the cost of fermentation medium was still high and little effort to find an inexpensive replaceable medium material was done for prodigiosin production.

Waste recycling represents one of the key elements in many countries to develop a low-carbon economy in the future. Among the total amount of waste, kitchen waste possesses a large part and attracts more attention. Kitchen waste is a kind of organic waste discharged from households, cafeterias and restaurants which was created inevitably every day. With a rapid socio-economic development, the amount of kitchen wastes has been rising in developing countries like China, and is now about 1000t/d in Shanghai, and 1200t/d in Beijing [5]. Recently, widespread public concerns about kitchen waste recycling have risen because of both environmental problems derived from it and

its potential for utilization. Kitchen garbage has the characteristic of organic component, which make it easier to be deteriorated and hard to handle. On the other hand, the abundant nutrition inside kitchen garbage makes the waste ideal raw materials for producing value added products, such as hydrogen, lactic acid, ethanol, and so on [6]. However more complicated and higher value-added products for example the prodigiosin production from kitchen waste has not been considered yet due to the lack of research on the applicable producing microorganism and fermentation procession.
In this study, the link between prodigiosin production and kitchen waste recycling was established. Kitchen waste could become one available substrate for prodigiosin production by adding only some nitrogen sources. As a result, the utilization of kitchen waste for prodigiosin production could bring down the production cost of prodigiosin and save normal medium material, reducing output of carbon dioxide indirectly, thus it is worth investigating.

2. Methods

2.1. Microorganism and culture conditions
Prodigiosin producing the red wild-type *Serratia marcescens* was isolated in our laboratory from kitchen waste and was maintained on a medium which contains (%) peptone 1.0, and beef extract 0.5, NaCl 0.5, agar 2%, stored at 4℃ and sub-culture at one-month intervals.

2.2. Substrate
Kitchen waste material was collected from local kitchen waste handle factory which was then dehydrated and shattered. The waste powder was processed using USA standard sieve set of No.18 and mean particle size of 1.4-1.0mm was obtained.

2.3. Shake-flask culture
Predetermined quantity and concentration of medium was taken into 150 ml Erlenmeyer flasks for culture. In order to optimize the culture medium, different carbon and nitrogen sources were added individually at 1% level to the medium. The medium was adjusted to different pH values by adding NaOH solution to investigate the influence of pH. The medium was mixed thoroughly and autoclaved at 121℃ for 20min at 1 kgf/cm^2. Then, the medium in flasks was inoculated with 10-hour grown culture broth under sterile conditions with various inoculation level. Also, in order to study the effect of inductor, predetermined quantity of proline solution was added into the medium through bacterial filter after the medium was autoclaved. Culture condition was optimized by taking flasks under different culture situation including temperature, rotation speed and liquid volume. In the case of incubation time, one-flask contents at every 6 hours were used for extraction and estimation of prodigiosin content. Results reported in this study are the averaged of triplicate findings.

2.4. Prodigiosin extract and content measure
Prodigiosin in the flasks was extracted by acidic methanol (pH 3.0). For each ml of the flask contents, 9 ml acidic methanol was added and shook at 150 rpm for 30 min. Debris was removed by centrifugation at 10,000 x g for 20 min at 4℃, and the concentration of prodigiosin was measured by monitoring the value of absorption at 535 nm with UV–Visible spectrophotometer. The prodigiosin content was calculated using prodigiosin standard curve. The standard prodigiosin used in this research was prepared through silica gel and the prodigiosin was characterized by NMR and MS (date not shown). The purity was determined by HPLC (Agilent HPLC 1100 Series) at 535 nm. Methanol and ultrapure water (8:2 v/v) were used as a mobile phase at a flow rate of 1.0 ml/min through the column (Agilent, Kromasil-C18) at 40℃.

2.5. Measurement of biomass
1 ml of the flask content was added to appropriate water and the absorbance of diluted samples was measured in UV–Visible spectrophotometer at 660 nm. The biomass was calculated using OD660 of broth versus dry cell weight standard curve.

3. Results and discussion

3.1. Evaluation of kitchen waste material for prodigiosin production

The utilization of kitchen waste for prodigiosin production depends on both the microorganism characteristics and the waste composition. *Serratia marcescens* could get a high yield of prodigiosin on medium containing proper carbon and nitrogen sources. Using modified LB medium, Tao obtained maximum prodigiosin production at 583 mg/l [7], and Wei obtained 790 mg/l when the LB was oil [8]. Kitchen waste has a rich carbon source, moderate nitrogen source and low toxic material, which enable it as an ideal substrate for *Serratia marcescens* to grow and produce prodigiosin. When nitrogen source was not added, the prodigiosin production was 223 mg/l and the maximum production 870 mg/l could be obtained when adding peptone 1% and proline 0.1%. Moreover, the cost of fermentation material was brought down sharply as kitchen waste was inexpensive and easy to obtain.

3.2. Role of incubation time

The culture was conducted in rude medium (35 g/l kitchen waste material, pH 8.0) with initial fermentation condition (28 ℃, 150 rpm, 1% inoculum level, 20 ml in 150 ml flasks), and the prodigiosin and biomass production curves were shown in figure 1.

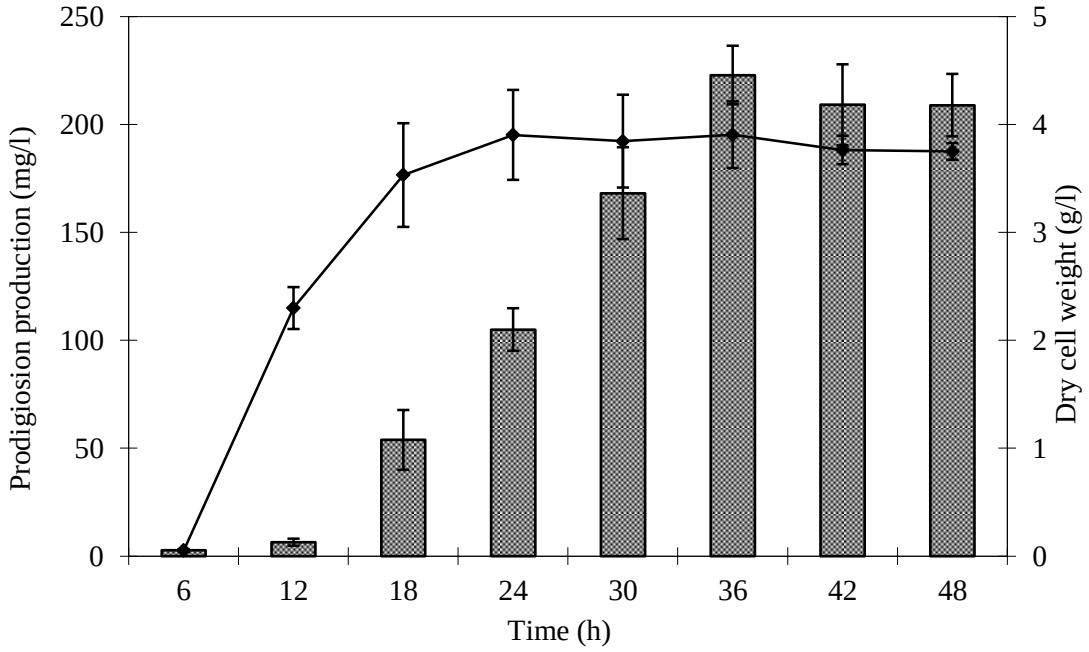

Figure 1. Influence of incubation time on *Serratia marcescens* growth (line) and prodigiosin production (column) with kitchen waste substrate.

Based on figure 1, both prodigiosin production and cell growth increased with incubation time at the beginning of fermentation, and the cell growth had its peak at 24 hour. As one kind of second metabolite [9], prodigiosin production lagged the cell growth and had its peak at 36h and further incubation showed reduction in its production due to the denature of prodigiosin under light. Maximum prodigiosin production was 222.8 mg/l and maximum dry cell weight was 3.9 g/l. This study suggests that prodigiosin production associates with cell growth in nature.

3.3. Optimization of fermentation parameter

Single factor experiments with different fermentation parameters (inoculum level, temperature liquid volume and agitation speed) were conducted to improve the performance of prodigiosin production and the results were shown in figure 2.

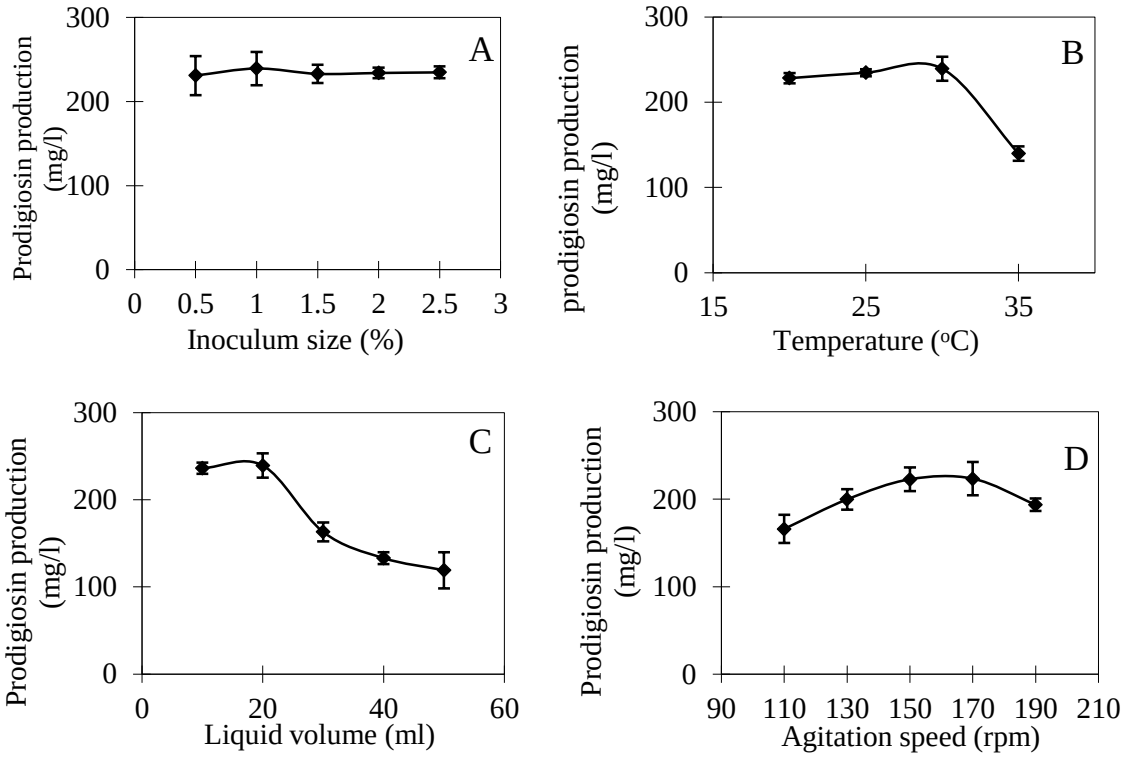

Figure 2. The effect of fermentation parameters (inoculum level (A), temperature (B), and liquid volume (C) and agitation speed (D)) on the yield of prodigiosin.

First of all, as *Serratia marcescens* grew quickly on this medium, inoculum level had little impact on the final prodigiosin yield in this study. Secondly, only a mild fluctuation on prodigiosin production was observed when the temperature was between 20℃ and 28℃, but a sharp reduction of yield was occurred with temperature higher than 30℃. Finally, the control of agitation speed and liquid volumes were critical for producing prodigiosin with *Serratia marcescens*, as both factors affected the dissolve oxygen level of the medium. The result was in accord with the report of Bernard Heinemann, who pointed out that the production of prodigiosin required sufficient oxygen supply [10].

3.4. Effect of substrate concentration

A proper concentration of substrate led to successful fermentation. While the too low concentration of substrate cannot provide enough nutrition for the growth of microbiology, an inhibition effect of prodigiosin production was also reported with high concentration of substrate. In order to optimize the substrate concentration, experiments with different substrate concentrations were conducted and shown in figure 3. In present study, maximum prodigiosin production was observed with 35g/l substrate concentration and higher substrate concentration showed reduction in prodigiosin production due to the excess carbon source in the substrate.

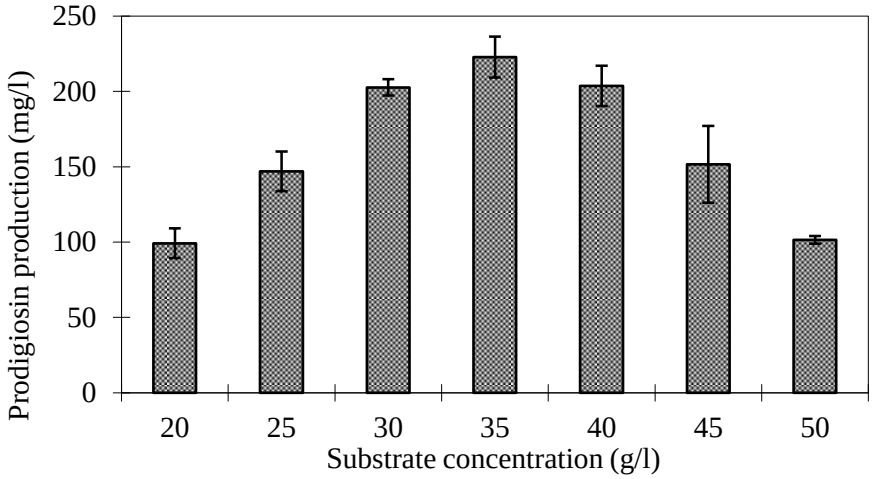

Figure 3. The effect of kitchen waste substrate concentration on prodigiosin production by *Serratia marcescens*.

3.5. Effect of pH

Shake-flask culture experiments were executed with various pH to reveal its influence. Based on figure 4, as long as the initial pH of the media was between 5 and 11, the final pH was in a narrow range, namely 7.8- 8.6, which indicated that *Serratia* genera had a powerful buffering capacity [11]. The maximum prodigiosin was observed with 8.0 initial pH, and little impact on the prodigiosin production was observed when the initial pH was between 7.0 and 9.0.

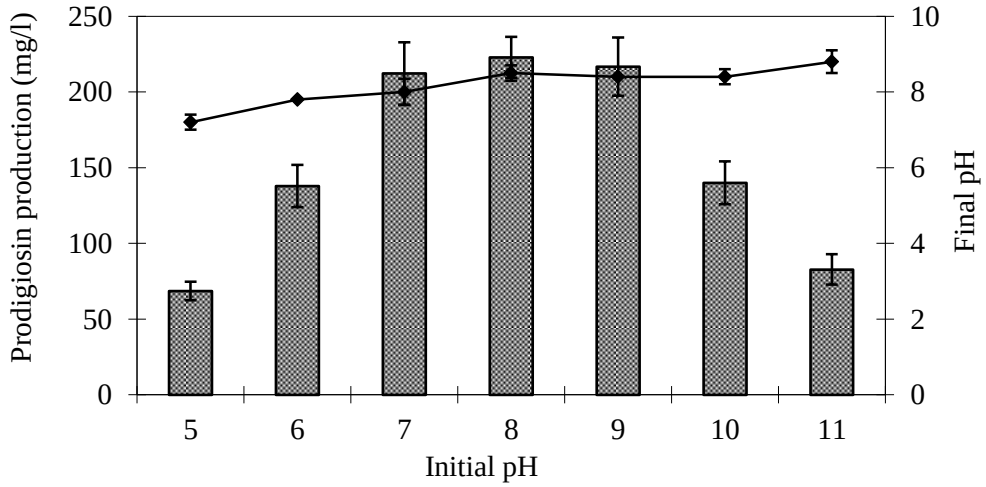

Figure 4. The effect of different pH on prodigiosin production (column) and final medium pH (line) by *serratia marcescens*.

3.6. Effect of different carbon sources

In order to optimize the concentration of carbon source in medium, several carbon sources such as glucose，sucrose，fructose，lactose，glycerol，mannitol，starch，and vegetable oil were added to crude medium at 1% level (figure 5). The study showed that although carbon sources were necessary for the growth of microorganism, supplementing excess carbon sources would affect the prodigiosin production negatively. Especially, with additional monosaccharide such as glucose and fructose supply, small prodigiosin was detected in the medium. Disaccharide and polysaccharides, which were metabolized by microorganism slower than monosaccharide, had less impact on the prodigiosin

production. Metabolite derived from carbon source degradation may be the caused for this inhibition effort, which was also suggested by Clements [12].

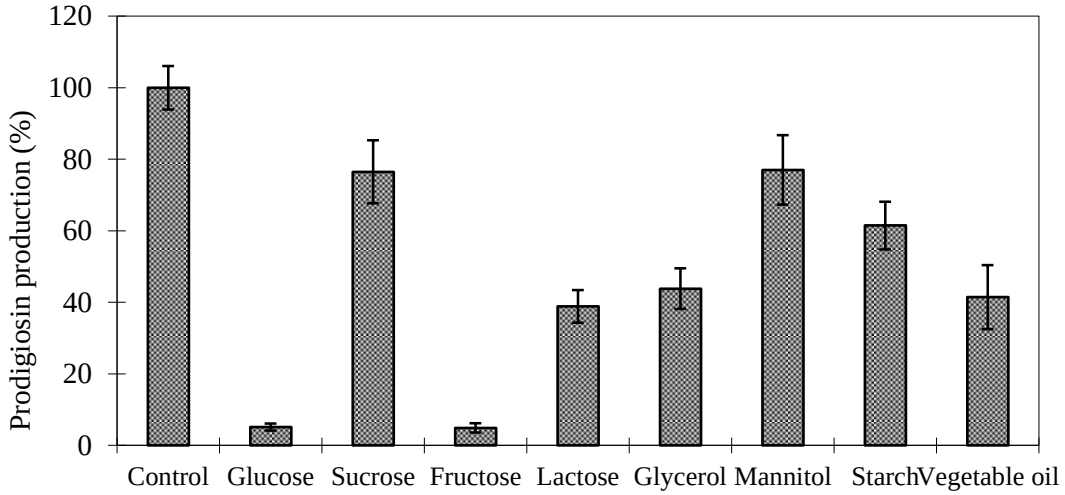

Figure 5. The effect of different carbon sources on prodigiosin production by *serratia marcescens*.

3.7. Effect of different nitrogen sources

Several kinds of amino acid participated in the prodigiosin production as precursor. For example, Lim noticed that proline was the precursor of pyrrolylpyrromethene and alanine also became a part of prodigiosin after decarboxylation. Similar to carbon source, the concentration of nitrogen source in medium was also optimized and shown in figure 6. The results revealed that yield of prodigiosin increased sharply when nitrogen sources were supplemented into the initial medium. Among these sources, peptone showed the best improvement on production. As possessing high level of protease activity,the nitrogen sources in the medium can be utilized and converted to prodigiosin by *serratia marcescens* quickly. To further optimize the peptone concentration, experiments were conducted with various concentration of peptone. The maximum prodigiosin production was 597 mg/l with additional peptone at 1% level.

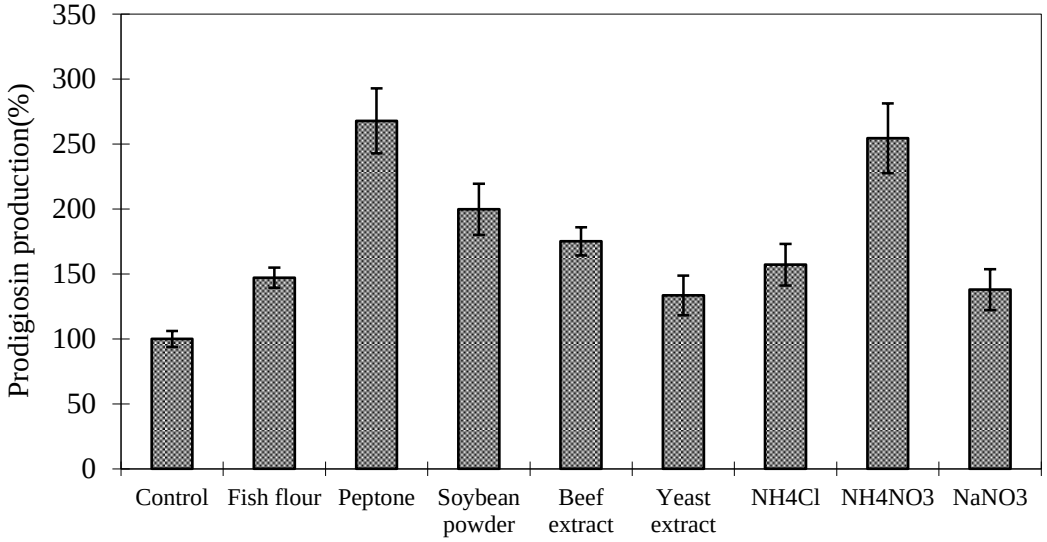

Figure 6. The effect of different nitrogen sources on prodigiosin production by *serratia marcescens*.

3.8. Effect of inductor

Wei reported that the addition of amino acids containing pyrrole-like structures could enhance prodigiosin production. In our present study, proline had the best inducement compared to other amino acids. However, the inducement became not obvious at low concentration of nitrogen source in medium, as the proline was utilized by *Serratia marcescens* as a normal nitrogen instead of an inductor. With additional 1% peptone in crude medium, proline supplementation in the medium at 0.1% level could increase the yield of prodigiosin by 46%, but no further improvement was observed with the concentration was beyond this level. In practice, adding proline may not be economic and necessary due to its high cost. In the end, fermentation was run with optimized fermentation conditions to understand the combinatorial influence of various factors on the prodigiosin production by *Serratia marcescens*. The results were depicted in figure 7. It was evident that at 42 hour, the maximum prodigiosin production was 870mg/l, almost twice more than that with crude medium.

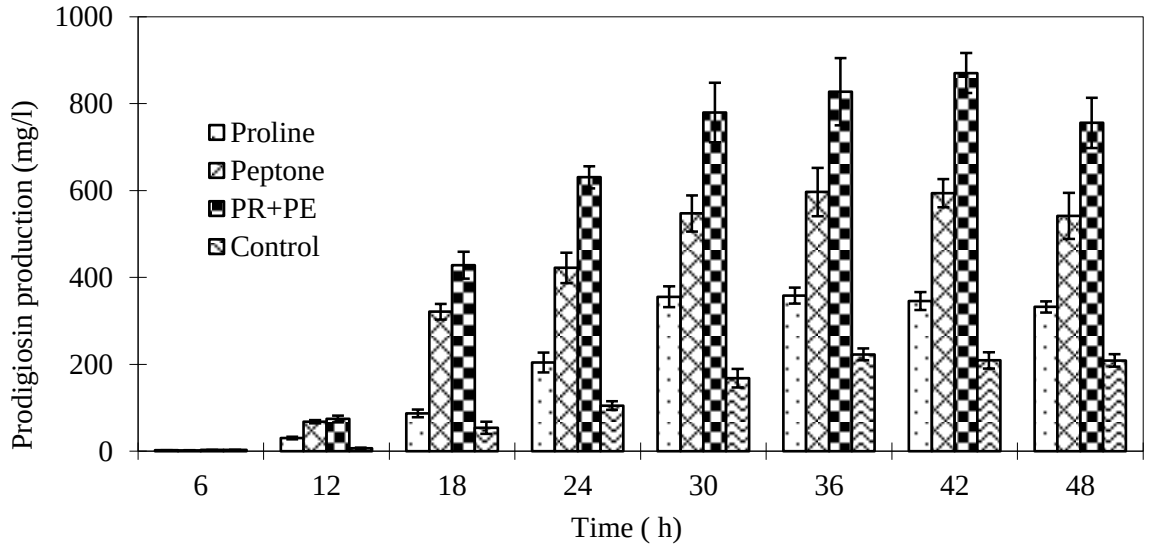

Figure 7. The effect of peptone, proline and their combination on prodigiosin production by *Serratia marcescens* with kitchen waste substrate.

4. Conclusion

Our study first established the link between kitchen waste recycle and prodigiosin production. Prodigiosin production by *Serratia marcescens* was influenced by both the composition of medium and fermentation parameters. Maximum prodigiosin production could be obtained by adding peptone and proline to the kitchen waste medium.

5. References

[1] Alois F 2003 *Angew. Chem. Int. Ed.* 42, 3582
[2] Campas C, Dalmau M, Montaner B, Pons G, Perez R and Gil J 2003 *Leukemia.* 17, 746
[3] Wei Y and Chen W 2005 *J. Biosci. Bioeng.* 99, 616-22
[4] Xu F, Xia S and Yang Q 2011 3rd *International Conference on Chemical, Biological and Environmental Engineering*
[5] Wang Q, Wang X, Wang X, Ma H and Ren N 2005 *J. Environ. Sci. Health A* 40 51–62
[6] Shi Y, Zhao X, Cao P, Hu Y, Zhang L, Jia Y and Lu Z 2009 *Biotechnol Lett.* 31 1327-33
[7] Tao J, Wang X, Shen Y and Wei D 2005 *World J. Microbiol. Biotechnol.* 21 969-72
[8] Wei Y, Yu J and Chen W 2005 *J. Biosci. Bioeng.* 100 466-71
[9] Williams R 1972 *J. Appl. Microbiol.* 25 396-402
[10] Heinemann B, Howard A and Palocz H 1970 *J. Appl. Microbiol.* 19 800-04
[11] Ruis N, Sole M, Francia A and Loren J 1994 *Appl. Environ. Microbiol.* 60 2151-54
[12] Clements S 1976 *Experientia* 31 421-2