

Cost Effective Production of Penicillin and Biogas from Rotten Oranges by Microbial Fermentation Technique

Aradhana Priyadarsini¹, A. T. Vivek², C. Gopinathan³

^{1, 2, 3}Bioprocess laboratory, Department of Biotechnology, University of Calicut, Tenjhipalam, P.O. -673635, Kerala, India

Abstract: India produces 10.8 million tonnes oranges annually, of which approximately 30% goes as waste. Orange juice primarily contains sucrose, fructose and glucose as carbon sources which can be fermented to Penicillin and biogas. During the processing of orange juice, huge quantities of orange peel and pulp are generated which causes environmental pollution problem. This can be converted to biofuel (Biogas) by anaerobic technology (methanogenesis). Different media formulations were prepared with peptone and ammonium sulphate by performing agar well diffusion assay of the respective fermentation broths by preparing cell free extracts and the potency of each of the formulations was tested. The diameter of inhibition zone was taken as a measure of potency of each of the formulation using raw orange juice as the basic substrate. It was found that compared to conventional media using raw orange of pH 3.5, orange juice adjusted to pH 6.5, peptone added and ammonium sulphate added formulations gave an inhibition zone of diameters, 40 mm, 45 mm and 41 mm respectively. The biogas production from orange peel was found to be 68.9 % more compared to control.

Keywords: Penicillin, Rotten/Waste oranges, mat culture, Agar-well diffusion assay, Biogas, Anaerobic digestion.

1. Introduction

The production of orange juice on a large scale leads to significant level of solid and liquid remains (20 million tonnes/year), which is accounted as waste. Since their composition is rich in pectin, cellulose and hemicellulose, and essential oils; these could be utilized for several industrial processes since these orange remains have no economic value. Orange is a citrus fruit, grown in high quantities globally, mostly exists in terms of peeled oranges and as juice [1]. It is linked to low cost and it consists of vital nutrients including dietary fiber, Vitamin A, B, C, minerals (calcium, phosphorus, potassium), and various phytochemicals such flavanoids, carotenoids and amino acids [2]-[3].

Brazil is the largest producer of orange with 53% orange juice globally and 80% of international trade of orange related products [4]. It is estimated that half the fresh orange weight is transformed into juice during orange juice production and remaining weight is assumed to be waste [5]. Hence, this huge amount of waste can be converted to many value added products using applicable fermentation techniques. Since orange juice contains sugars such sucrose, glucose, fructose, these can be bioprocessed to value added high cost products like antibiotics.

As India produces approximately 10.8 million tonnes of oranges of which 25-30% turns out to be waste. The reasons for such waste accumulation in the country are due to improper storage, transportation, microbial contamination and pest attack [6]. This huge quantity of rotten orange wastes can be efficiently processed to value added products like penicillin and biogas. In this study, we have attempted to develop a process for the production of Penicillin from orange juice extracted from rotten oranges and methane from orange peel waste. Penicillin was produced using mat culture

method (Surface culture) and biomethane was produced by anaerobically digesting orange peel waste with cow dung slurry as inoculum.

2. Materials and methods

Rotten and damaged oranges were collected from Palayam vegetable market, Kozhikode, Kerala, India. Pure culture of *Penicillium* species and fresh cow dung as source of anaerobic bacteria for biomethanation was used for the study. *Staphylococcus aureus* was used as the test organism for agar-well diffusion assay.

2.1 Preparation of mat culture for Penicillin production

Raw/Waste oranges were washed with tap water and its peel was removed. It was squeezed with hand and the juice extract was filtered using a muslin cloth. 250mL of juice was taken in 1000mL conical flask and sterilized using steam (autoclaved at 15 psi for 15 mins at 121° C). After cooling to room temperature, *Penicillium* spores were used to inoculate the flask (1×10^3 spores/mL) and the flasks were incubated further in room temperature to allow germination of spores and establish a sporulated mat over the liquid media in a time period of 15 days.

2.2 Supplementation with different nitrogenous substrates

Different media formulations were made with orange juice extracted from rotten oranges with different additives like ammonium sulphate and peptone. This blending was done in order to supplement nitrogen to optimize the C: N ratio, since raw orange juice is a carbon rich media. In addition, raw orange juice extracted with initial pH of 3.5, was adjusted to a pH 6.5 with 0.1 N Calcium hydroxide and resulting media of pH 6.5 was used for growth of *Penicillium* fungi as mat

culture.

Bioassay of Penicillin

After 15 days of growth in mat culture, the flask with sporulated mat was carefully removed from the top of the fermentation broth using a sterile L-shaped glass rod. The remaining fluid was filtered with Whatman no.1 filter paper. The filtrate was tested for its antibiotic activity against *Staphylococcus aureus* pure culture using agar-well diffusion assay [7]. 100mL of agar medium with composition 1% Glucose, 0.5% peptone, 0.5% yeast extract with pH 7.5 was autoclaved for 15 mins at 121° C in an autoclave. Sterilized medium was poured to sterile petriplate in laminar airflow chamber (10mL/plate). 0.1mL of pure culture of *Staphylococcus aureus* was inoculated into the petriplates and was uniformly spread using sterile glass spreader. A well was cut in centre and 0.5mL of the filtrate was poured into the well using a micropipette. The petriplates were incubated at 37 ° C for 24 h. The diameter of the inhibition zone produced was measured in millimeter that represents measure of potency of the antibiotic using each of the formulations with raw orange juice as the basic raw material.

2.3 Biomethanation of orange peels

1L conical flasks were taken and marked A and B. In the flask marked A, 700mL of fresh cow dung slurry was poured (1 Part fresh cow dung to 1 part water thoroughly mixed using a homogenizer). In the flask marked B, 700mL of fresh cow dung slurry mixed with 100g of orange peels was taken. Both the flasks were closed with one hold rubber stoppers connected with rubber tubes. The fermentation gases produced from the corresponding flasks were collected using three inverted 1L conical flasks completely filled with water and closed with two hold rubber stoppers (water displacement method). When the fermentation gases are conveyed through the single hole connected tubes to the inverted conical flask with water, equal volume of water is displaced, which will be collected in another conical flask and measuring the volume of water displaced gives the quantity of biogas produced in 24 h. The rate of biogas production and quantity depends on the substrate material used.

3. Results and Discussion

3.1 Bioconversion of rotten/waste oranges to Penicillin

The Fig.1 (a) shows the growth of *Penicillium* fungus as mat culture over raw orange juice medium. From Fig.1 (a), it is evident that, there is luxuriant growth of *Penicillium* fungus mat over raw orange juice medium with an initial pH of 3.5. This proves that orange juice can be used directly as an industrial substrate even without adjustment of pH. *Penicillium* fungus can be grown as pure culture over a period of 15 days, which will support luxurious mycelial proliferation, as well as intense sporulation that is essential since Penicillin is a secondary metabolite. Fig.1(b) shows the growth of *Penicillium* fungus as mat culture over raw orange juice medium with pH adjusted to 6.5.

3.2 Supplementation of different nitrogenous substrates

Fig.1 (c,d), gives the picture regarding growth of *Penicillium* fungi over different nitrogen supplemented media with raw orange juice as the basic substrate. Fig.1 (c) and Fig.1 (d) show sporulation regarding *Penicillium* fungus in raw orange juice based media, supplemented with peptone and ammonium sulphate respectively.

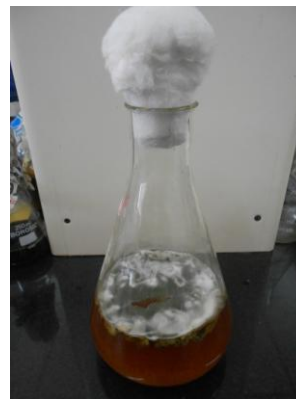


Figure1 (a): Growth of *Penicillium* fungus in orange juice at pH 3.5.



Figure1 (b): Growth of *Penicillium* fungus in orange juice at pH 6.5.

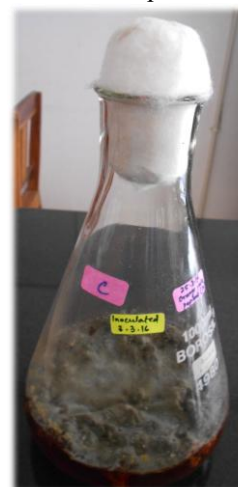


Figure1 (c): Growth of *Penicillium* fungus in orange juice liquid medium at pH 6.5 with 1% peptone supplementation.



Figure1 (d): Growth of *Penicillium* fungus in orange juice liquid medium at pH 6.5 with 1% ammonium sulphate supplementation

3.3 Agar-well diffusion assay

Fig.2 (a,b,c,d) shows that agar well diffusion assay data of Penicillin fermentation broth after cell separation from the fermentation broth. The antibiotic activity was given by measuring the diameter of inhibition zone in millimeters. By comparison of the data 2(a), 2(b) and 2(c) and 2(d), it is evident that the potency of the antibiotic produced as per Fig 2(c) using raw orange juice based medium supplemented with peptone gives highest yield of antibiotic. Still, peptone based medium cannot be used for industrial production because the cost of peptone is very high. So, the ammonium sulphate supplemented medium can be used for large scale manufacture of this highly useful and valuable antibiotic, since the cost of ammonium sulphate is very low compared to peptone, considering enormous quantities of wastes available in this country.

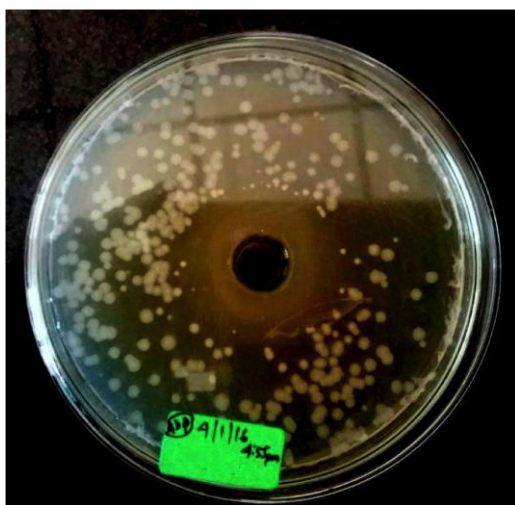


Figure 2 (a): Zone of inhibition (30mm) obtained by the mat culture of *Penicillium* fungus grown in orange juice based medium at pH 3.5.

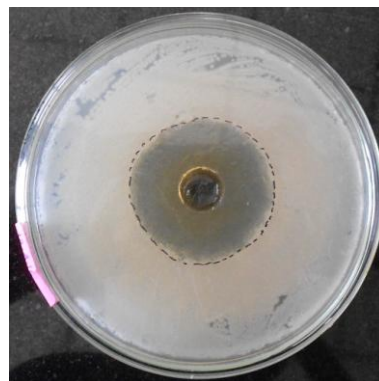


Figure 2 (b): Zone of inhibition (40mm) obtained by the mat culture of *Penicillium* fungus grown in orange juice based medium at pH 6.5.

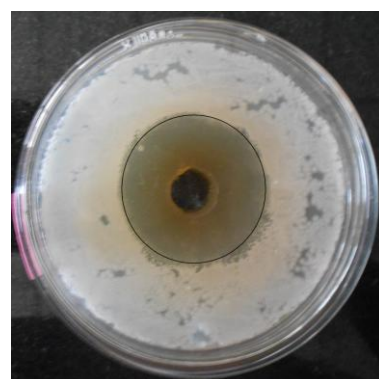


Figure 2 (c): Zone of inhibition (45mm) obtained by the mat culture of *Penicillium* fungus grown in orange juice based medium with 1% peptone at pH 6.5.

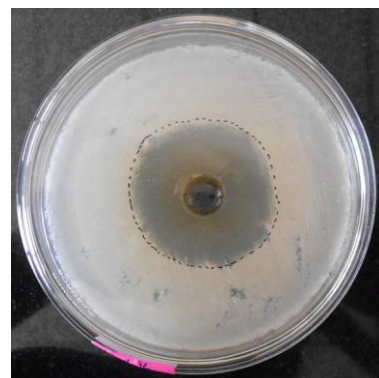


Figure 2 (d): Zone of inhibition (41mm) obtained by the mat culture of *Penicillium* fungus grown in orange juice based medium with 1% ammonium sulphate at pH 6.5.

3.4 Biomethanation of orange peels

Fig.3 gives the biomethanation data of orange peel waste. Since it contains sugars such as sucrose, glucose, fructose, in addition to pectin, cellulose and hemicellulose, it becomes an attractive substrate for the cost effective biomethanation. In biomethanation process, bacteria convert complex organic materials to biogas through hydrolysis, acidogenesis, acetogenesis and methanogenesis catalyzed by the corresponding microbial consortia. Biogas is an excellent biofuel which usually has 60-65% methane and 30-35% CO₂ with trace amounts of other gases. Bioconversion of organic wastes to biofuel tackles environmental pollution with bioenergy production, making it a lucrative technology. The

data shows that bioconversion of orange peel waste produces 68.9% more biogas compared to control in a time span of 30 days. This data conclusively proves that there is commercial possibility of large scale bioprocessing of huge amounts of orange peel waste which accumulates near fruit processing industries to value added product like biomethane. The technology becomes economically feasible considering the cost spent on waste removal, pollution abatement and revenues incurred as part of imports on crude oil.

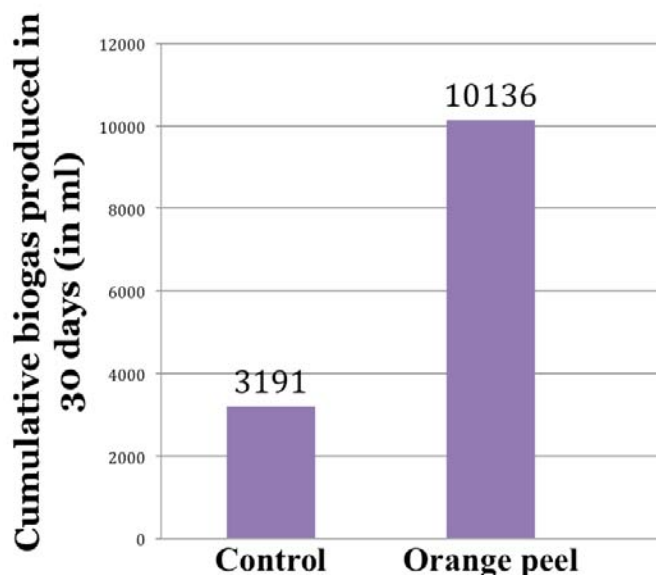


Figure 3: Cumulative biogas production in 30 days

4. Conclusion

The rotten/waste orange can efficiently be converted to Penicillin by employing surface mat culture of *Penicillium* fungus. In addition, biomethanation orange peel wastes is also a technical possibility on an industrial scale.

References

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Author Profile



C. Gopinathan is currently working as Associate Professor at Department of Biotechnology, University of Calicut. He received the B.Sc in Botany, M.Sc in Life Sciences with specialization in Microbiology from University of Calicut and M.Tech in Biotechnology from Anna University, Chennai. He was former waste management consultant for Calicut Corporation, Kerala and is, at present consultant for Indian railways. He has handled various bioprocess scaled up projects and published & presented his research works in leading Biotechnology conferences and International journals including World Biotechnology Congress. His specialization areas include fermentation technology in general and specifically biopesticide production, biogas production from agro waste, low cost mushroom production and high rate aerobic composting.