

# FISH POWDER AS A LOW-COST COMPONENT IN MEDIA FOR PRODUCING BACTERIAL CELLULOSE

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## ABSTRACT

Some bacteria can produce extracellular bacterial cellulose (BC). This polysaccharide is chemically identical to cellulose produced by plants but has no associated lignin and hemicelluloses. The unique mechanical properties, chemical stability and purity allow BC to be exploited for a range of biomedical applications. However, medium costs limit commercial BC production. The suitability of using fish powder as a low-cost media component for producing BC by submerged culture of *Gluconacetobacter xylinus* in shake flasks was investigated. Fish powder was made by drying and grinding Koi carp (*Cyprinus carpio*), a pest fish in New Zealand waterways. Fermentations were done at 30°C in a growth medium containing 50 g/L glucose, the required minerals, and either 5 g/L yeast extract or 15 g/L fish powder. The BC yield on both yeast extract and fish powder was 0.04 g/g glucose, demonstrating fish powder was a suitable low cost ingredient for supplying nitrogen and amino acids in the media.

Keywords: Bacterial cellulose, *Gluconacetobacter xylinus*, Fish powder, Low-cost media, Fermentation

## INTRODUCTION

Bacterial Cellulose (BC) is an extracellular polysaccharide produced by *Gluconacetobacter xylinus*, which is a gram negative bacterium capable of producing high amounts of BC (Iguchi et al., 2000). BC has been one of the best biomaterial for wound healing from second and third degree burns (Pokalwar et al., 2010). BC produced by *G. xylinus* displays unique properties such as high mechanical strength, high water absorption capacity, high crystallinity, and an ultra-fine and highly pure fiber network structure (Cannon & Anderson, 1991; Jonas & Farah, 1998; Ross et al., 1991; McKenna et al., 2009). These remarkable properties are due to specific and unique BC ultrastructure, characterized by ultrafine cellulose ribbons (Ross et al., 1991; Watanabe et al., 1998). Most of the BC currently used is produced in submerged aerobic fermentations. Problems with the high costs of fermentation media and relatively low BC yields have, to date, prevented the industrial production of BC (Bielecki et al., 2005; Hong et al., 2011). In submerged fermentations, the cost of the raw materials used to formulate the medium can account for almost 30% of the total cost (Rivas et al., 2004; Jung et al., 2010). Considerable cost reduction may be possible by exploring ways to use cheaper and locally available raw materials in the growth

medium formulation. The majority of studies on BC production have focussed on using media containing complex nitrogen and vitamin sources such as yeast extract and polypeptone (Chao et al., 2001; Son et al., 2003). However, these nutrient sources are economically unfavourable due to their high cost (Rivas et al., 2004; Jung et al., 2010).

In this study, we used fish powder produced from Koi Carp (*Cyprinus carpio*) as a nitrogen source for BC production. Koi Carp (*Cyprinus carpio*) has been introduced in New Zealand in 1960's and become a major waterways pest (Osborne et al., 2009). It dislodge and uproot vegetation as they feed on benthic invertebrates, thus increasing water turbidity and destroying fish and plant habitats (Crivelli, 1983; Chumchal et al., 2005; Driver et al., 2005). These harmful effects caused by Koi Carp pose a major threat to New Zealand natural aquatic system. Thus, the New Zealand government had a strategic policy goal to eventually eradicate the species. As a consequence, plenty of fish meat is available for use as nitrogen source in fermentation media formulations.

## **Materials and Methods**

### **Microorganism and Growth**

*G. xylinus* DSM 46604 used for BC production in this study was obtained from German Collection of Microorganisms and Cell Cultures (Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures). The strain was maintained on medium containing (g/L): glucose, 50; yeast extract, 5.0; ammonium sulphate, 5.0; potassium dihydrogen phosphate, 3.0; magnesium sulphate, 0.05; agar, 20; and subcultured periodically. For BC production, cultures were grown in medium containing (g/L): glucose, 50; fish powder, 5.0-15; ammonium sulphate, 5.0; potassium dihydrogen phosphate, 3.0; magnesium sulphate, 0.05. The initial pH was adjusted to 6.8 by the addition of 6N NaOH. Shake flask fermentations were conducted for 5 days at 30 °C and 150 rpm.

### **Fish Meat Powder Preparation**

Koi Carp fish from Lake Waikare (New Zealand), which was captured with the help of personal from the Biological Science Department of The Waikato University, were used in this study. The minced flesh was dried in 80 °C oven overnight. The main reason of drying is to reduce the moisture content to 10% moisture or less. Dried flesh was ground till a superfine powder was obtained. The powder was used as a substitute component for yeast extract, which acted as a control experiment.

### **Glucose Analysis**

The Miller Method (1959) was used for the quantitative analysis of glucose. Adequately, diluted samples were reacted with 3,5-dinitrosalicylic acid (DNS acid). The intensity of developed colour was measured in a spectrophotometer at 575 nm. The concentration of glucose was determined from a standard curve.

### **Biomass Determination**

Biomass was determined according to the method of Cheng *et al.* (2009). The samples of culture broth were centrifuged at 4000 rpm for 20 min. For cell mass, the BC pellets were added to 90 mL 0.1 M citrate buffer (pH 5.0) and 10 mL of 20% cellulase solution and incubated at 50 °C with

shaking at 100 rpm for 1 h to hydrolyse BC. Subsequently, the solution was centrifuged at 4000 rpm for 20 min. The precipitate was washed with distilled water twice and centrifuged. Finally, the precipitate was dried in an oven at 80°C overnight and then weighed to determine biomass as dry weight.

### BC Determination

BC was determined according to the method of Cheng *et al.* (2009). The precipitated BC pellets were treated with 1 N NaOH solution at 80 °C for 30 minutes to remove the bacterial cells and medium impurities. NaOH treatment was repeated three times and then, the solution was centrifuged at 4000 rpm for 20 min. The purified cellulose was dried in an oven at 80 °C overnight and then weighed.

### Results and Discussion

Figure 1 shows the effect of nitrogen sources on biomass and BC volumetric productivities. The results showed by supplementing 15 g/L fish meat powder to the medium, volumetric productivity of BC increased up to 3 g/Lh, which was almost similar with control medium (3.13 g/Lh). Supplementing 5 g/L and 10 g/L fish meat powder gave 2.38 g/Lh and 2.63 g/Lh BC productivities, respectively.

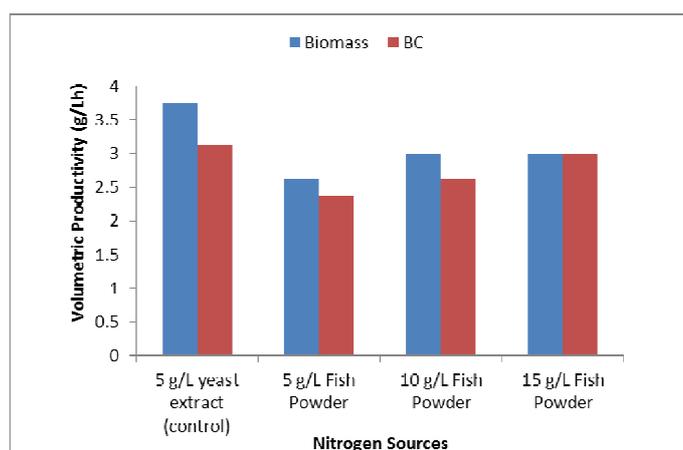


Figure 1: Effect of nitrogen source on biomass and BC volumetric productivities

Figure 2 shows the effect of nitrogen source on biomass and BC yields. Using 15 g/L fish powder improved BC yields. Supplementing 15 g/L fish powder to the culture medium produced BC yield of 0.04 g/g, which was similar with 5 g/L yeast extract (0.04 g/g). Meanwhile, 5 g/L and 10 g/L fish meat powder both exhibited same BC yield of 0.03 g/g. Biomass production was lower, which might be due to the presence of growth inhibitors in fish powder that can inhibit the cell growth.

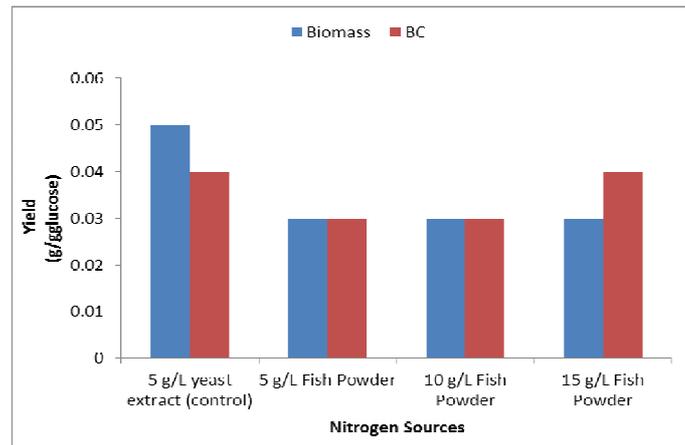


Figure 2: Effect of nitrogen source on biomass and BC yields

## CONCLUSION

Our study suggested that fish powder could substitute the expensive yeast extract as a cheaper alternative. This could favour to substantially reduce the fermentation cost, thereby reducing the cost of BC. Although, productivity and yield of BC are lower than that obtained by using yeast extract, supplementing the meat powder medium with some growth factors could enhance BC yield and productivity. Further evaluation on fish powder cost and characterization are needed for identification of growth stimulator and growth inhibitor that might present in fish powder.

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