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Production of streptomycin from chitin using *Streptomyces griseus* in bioreactors of different configuration

Richard J.L. Meanwell, Gilbert Shama *

Department of Chemical Engineering, Loughborough University, Loughborough, Leics LE11 3TU, UK

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Abstract

Streptomyces griseus was cultured in three different bioreactors in a medium containing chitin flakes. When a conventional bioreactor stirred by two sets of Rushton impellers and operated at high speed was used, the yield of streptomycin (3.1 mg/l) was the highest observed and occurred at approximately 500 h. Cultivation of *S. griseus* in a bioreactor stirred at low speed by a U-shaped paddle resulted in a lower yield of streptomycin (1.8 mg/l) but this was achieved in a shorter period of time (400 h). Increasing the concentration of chitin from 5% to 10% w/v had no significant effect on either of these two parameters. The use of a novel vertical basket bioreactor in which the chitin flakes were contained within a wire mesh basket and were gently fluidised by air, enabled comparatively high yields of streptomycin (2.8 mg/l) in the relatively short time of 300 h.

Keywords: Chitin; *Streptomyces*; Streptomycin; Stirred bioreactors; Vertical basket bioreactor

1. Introduction

Commercial shellfish processing results in the worldwide generation of large tonnages of wastes (Chang et al., 2007). The quantities of waste produced are dependant both on the species of shellfish being processed and on the method of processing. For example, during shrimp processing in Norway some 40% of the weight of catches ultimately ends up as waste (Gildberg and Stenberg, 2001). Shellfish wastes are both highly perishable and highly polluting, but despite this, the commonest method of disposal is to discharge them to sea. However, as regulatory agencies tighten restrictions on such practices, alternatives to disposal are likely to assume greater importance.

Shellfish processing waste is a potentially rich resource of a number of useful compounds. Chief amongst these is chitin, the β-(1→4) linked homopolymer of *N*-acetyl-D glucosamine, which is similar in structure to cellulose. Indeed, it has even been claimed that chitin is the second most abundant polymer on Earth after cellulose (Lee et al., 2007). The uses to which chitin can be put to are varied and growing in number. However, the principal applications appear to be in the food, medical, agricultural and environmental protection industries (Felse and Panda, 2000; Synowiecki and Al-Khateeb, 2003).

The traditional method of recovering chitin from shellfish waste is to deproteinise and demineralise it by a combination of acid and alkali treatments. However, the disadvantage of this form of treatment is that large volumes of liquid effluents are generated (Zakaria et al., 1998). One alternative to chemical treatment is to use micro-organisms to bring about rapid stabilisation of shellfish wastes.

Zakaria et al. (1998) demonstrated this principle with the lactic acid bacterium *Lactobacillus paracasei* in a horizontal basket bioreactor to produce a partially purified and stable chitin-rich solid product. Later, Yang et al. (2000) isolated a strain of *Bacillus subtilis* that was capable of

* Corresponding author. Fax: +44 (0)1509 223923.
E-mail address: g.shama@lboro.ac.uk (G. Shama).

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deproteinising shellfish wastes. Cira et al. (2002) also using a lactic acid bacterium, achieved good chitin recovery using column bioreactors that could be scaled up to enable treatment of 30 kg batches. Beaney et al. (2005) recently provided validation of the fermentative approach to chitin recovery by showing that the chitin produced by fermentation was directly comparable to that produced by chemical treatment on the basis of a number of parameters including the degree of acetylation.

A relatively new application for chitin is as a substrate in fermentation processes. In this instance the chitin serves as a source of energy and nutrients (principally C and N) to a microbial culture that is capable of producing one or more useful products. Mahadevan and Crawford (1997) cultured *Streptomyces lydicus* on chitin to produce chitinases with anti-fungal activity, whilst Trejo-Estrada et al. (1998) found that *Streptomyces violaceusniger* produced a number of polyene antifungal compounds related to guanidlyfungin A when grown on chitin. More recently, Wang et al. (2002) demonstrated that culture supernatants of a number of species of *Monascus* cultured on chitin all produced antifungal compounds.

The streptomycetes are the most commercially important group of antibiotic producers and most species are chitinolytic (Schrempf, 2001). *Streptomyces griseus* is a particular case in point, and is used industrially to produce streptomycin. Streptomycin is a so-called 'broad spectrum antibiotic' i.e. active against both Gram positive and Gram negative bacteria, and belongs to the aminoglycoside group of antibiotics. First described by Selman Waksman in 1942, it is still in use today as an adjunct in the treatment of tuberculosis in humans (Di Perri and Bonora, 2004) but it also finds use as a veterinary drug (Jones et al., 2002).

In this work, we compare streptomycin production from chitin in three bioreactors each of different configuration. The first of these was a conventional stirred bioreactor equipped with two sets of Rushton impellers, the second bioreactor was essentially a modification of the former in which one of the Rushton impellers was replaced by a U-shaped impeller and which was operated at low rotational speed. The third bioreactor investigated here was one of novel design and incorporated a static vertical basket in which the chitin was contained whilst being immersed in an aqueous salts medium. Air was passed directly into the basket so as to both provide oxygen to the culture of *S. griseus,* and to gently fluidise the chitin flakes.

2. Methods

2.1. Micro-organism and cultivation

S. griseus (NCIMB 8136) was purchased in lyophilised form (NCIMB, Aberdeen, UK). Spores of the organism were maintained on sterile soil and were cultured on yeast-malt extract agar (NCIMB Medium No. 29) at 28 °C when required for bioreactor experiments. The liquid medium used for such experiments comprised flakes of chemically purified chitin (Sigma Aldrich plc, Poole, Dorset, UK) at concentrations specified in the text and the following salts per litre of distilled water; K_2HPO_4, 0.76 g; KH_2PO_4, 0.3 g; $MgSO_4$, 0.5 g; $FeSO_4$, 0.01 g; $ZnSO_4$, 0.0018 g; $MnCl_2$, 0.0016 g. The pH of the medium was adjusted to 6.5 and was autoclaved at 121 °C for 20 min. Inoculum for bioreactors comprised 100 ml of culture grown for 48 h in an incubator-shaker at 100 rpm and 28 °C.

2.2. Streptomycin determination

The concentration of streptomycin was determined using a standard bioassay procedure (Anon., 1998) and was based on the inhibition of the growth of *B. subtilis* (NCIMB 8054). The only variation introduced into the procedure was that the spores of *B. subtilis* necessary for seeding the agar were produced by the method of Harnulv and Snygg (1972). Culture broths were first filtered through 0.2 μm Whatman cellulose nitrate membrane filters (Fisher Scientific, Loughborough, Leics.) and subsequently using 2 kDa membranes (Dow Danmark A/S, Nakskov, Denmark). Aliquots of filtered culture broths (150 μL) were added to wells cut into agar plates which were incubated for 48 h at 30 °C. Zones of inhibition were measured using Vernier callipers. All determinations were performed in duplicate.

A standard calibration curve was obtained using aqueous solutions of streptomycin (Sigma Aldrich plc).

2.3. CO_2 determination

Gas samples were taken from the air exit line of the bioreactor using a gas-tight syringe and directly injected into a gas chromatograph (Model 104, Pye Unicam, Cambridge, UK) equipped with a thermal conductivity detector operated at 180 °C. The glass chromatography column was 6 mm diameter and 175 cm long and was packed with Molecular Sieve 5A (Phase Separations, Deeside, Clwyd, UK). All determinations were performed in duplicate.

2.4. Biomass determination

Samples (10 ml) were centrifuged at 8600*g* for 5 min at 10 °C. The supernatants were discarded and the cell pellet re-suspended in an equal volume of distilled water and re-centrifuged as above. The biomass was dried at 105 °C overnight and weighed. All determinations were performed in duplicate.

2.5. Bioreactors

2.5.1. Stirred bioreactors

A standard stirred 2-l glass bioreactor vessel (LH Engineering Co. Ltd., Stoke Poges, Bucks, UK) was used. The working volume was 1.5 l. This was used for experiments as originally supplied with two sets of Rushton impellers (dia.