PARTICLE BASED CULTIVATION OF \textit{LENTZEA AEROCOLONIGENES} IN MEMBRANE AERATED STIRRED BIOREACTORS FOR INCREASED REBECCAMYCIN PRODUCTION

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ABSTRACT

Filamentous microorganisms represent the majority of natural producers of antibiotics and other active pharmaceutical ingredients (APIs). \textit{Lentzea aerocolonigenes} is a filamentous actinomycete producing the antitumor antibiotic rebeccamycin. The addition of glass beads (100 g/L, \( \Theta = 969 \text{ \( \mu \)m} \)) in shake flask cultivations led to a significant increase in rebeccamycin production compared to an unsupplemented cultivation by inducing mechanical stress. To achieve larger amounts of rebeccamycin a scale-up was conducted. A bubble free membrane aeration was chosen to reduce the power input to stirrer and particle induced stress. Glass bead addition (50 g/L, \( \Theta = 969 \text{ \( \mu \)m} \)) in this membrane aerated stirred bioreactor increased rebeccamycin concentration compared to an unsupplemented cultivation as well. Moreover it reduced biomass growth on the aeration membrane which is a widespread challenge in membrane aerated bioreactors.

Keywords: membrane aeration, filamentous microorganisms, particle enhanced cultivation, mechanical stress

INTRODUCTION

\textit{Lentzea aerocolonigenes}, belonging to the filamentous microorganisms, produces the antitumor antibiotic rebeccamycin. The microorganism exhibits a complex morphology ranging from freely dispersed mycelia to dense pellets [Pommerehne et al., 2019]. The morphology is linked to the product formation and can be controlled by e.g., inoculum concentration and viability, pH, medium composition, hydromechanical stress, addition of inorganic salts or particles [Walisko et al., 2015]. Rebeccamycin formation in \textit{L. aerocolonigenes} was increased by the adjustment of mechanical stress induced by glass particles in shake flask scale [Walisko et al., 2017]. Scale up from shake flasks to laboratory scale bioreactors is often linked to increased mechanical stress and since is an important factor to be considered. Membrane aerated bioreactors show a reduced power input since no bubbles are present.

RESEARCH CONCEPT

Cultivations of \textit{L. aerocolonigenes} in shake flask scale were conducted in 250 mL flasks with 4 baffles and 50 mL filling volume. A preculture was inoculated with 1 mL of frozen mycelium and incubated for 2 days at 28 °C and 120 min\(^{-1}\). 300 µL of this preculture were used for inoculation of the main culture. Glass particles were added for adjustment of the induced mechanical stress at the beginning of the cultivation. Different size ranges and concentrations of glass beads were investigated. The flasks were incubated on an orbital shaker at 120 min\(^{-1}\) (50 mm amplitude) at 28 °C for 10 days. The growth medium consisted of 4 g/L glucose, 4 g/L yeast extract and 10 g/L mat extract with a pH of 7.2.

A scale up to a stirred bioreactor (Applikon Biotechnology, The Netherlands) with 1.2 L filling volume was performed. Aeration in this bioreactor was conducted via an oxygen permeable silicone tube (wall thickness = 0.2 mm; diameter = 2.9 mm, Reichelt Chemietechnik, Germany). The tube was wound around the installations inside the bioreactor and the end was placed above the liquid to provide additional headspace aeration. The length of the tubing as well as the aeration rate and the stirrer speed were varied. When glass particles were added a mean size of \( \Theta = 969 \text{ \( \mu \)m} \) was chosen. Cultivations were inoculated with 7.5 mL of a 2-day preculture and grown at 28 °C for 10 days with daily sampling.
Cell dry weight (CDW) was determined gravimetrically. Substrate and product quantification were conducted as described by [Walisko et al., 2017].

RESULTS AND DISCUSSION

*L. aerocolonigenes* was first investigated in shake flask scale, where the addition of glass beads showed increased product formation. Different glass bead sizes and concentrations were tested and the optimal rebeccamycin concentration of about 70 mg/L was achieved with a mean glass bead diameter of 969 μm in a concentration of 100 g/L. An unsupplemented control, however, only provided 5 mg/L rebeccamycin (Fig. 1).

This process should be transferred to bioreactor scale to provide larger amounts of rebeccamycin since larger volumes are used. A certain amount of mechanical stress was beneficial for rebeccamycin production, however, excessive stress decreases product formation [Walisko et al., 2017]. Since conventional aeration in bioreactors creates additional stress, a bubble free membrane aeration was chosen.

A first cultivation without glass beads led to a maximum of 12 mg/L rebeccamycin (Fig. 2) which is comparable to an unsupplemented shake flask cultivation. During this cultivation, however, excessive biomass growth on the silicone membrane was observed (Fig. 3, left). The addition of glass beads reduced the growth of biomass on the silicone tube (Fig. 3, right). The moving glass beads lead to a permanent abrasion ensuring an improved oxygen transfer.

Moreover, the cultivation with glass beads resulted in an increased rebeccamycin concentration (Fig. 4). With 50 g/L glass beads of 969 μm mean diameter a maximum rebeccamycin concentration of 42 mg/L was provided.

Figure 2: Rebeccamycin concentration of a silicone membrane aerated bioreactor cultivation without glass particles at an agitation rate of 400 min⁻¹ and an aeration rate of 0.2 L/min (0.4 L/min during exponential phase).

Figure 3: Biomass growth on silicone membrane without (left, 5 m tube length) and with glass beads (right, 50 g/L, Ø = 969 μm, 2.5 m tube length) added to the cultivation.

Figure 4: Rebeccamycin concentration of a silicone membrane aerated bioreactor cultivation with glass
particles (50 g/L, $\varnothing = 969 \mu m$) at an agitation rate of 650 min$^{-1}$ and an aeration rate of 0.2 L/min (0.4 L/min during exponential phase).

CONCLUSIONS

Successful cultivations of *L. aerocolonigenes* in a membrane aerated stirred bioreactor were conducted. The use of membrane aeration through a silicone tube reduces the mechanical stress induced on the microorganism and additionally avoids foaming which is often problematic especially for complex media. The in this paper presented combination of membrane aeration and glass bead addition provides further advantages. The increase of product formation by the addition of glass beads as seen in shake flasks could also be observed in bioreactor scale. Furthermore the glass beads decrease the biomass growth on the silicone membrane since they are causing a permanent abrasion. This makes membrane aerated bioreactors a promising cultivation platform, especially for other filamentous microorganisms.

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REFERENCES

