Application of microbubble dispersion to extracellular nicotine production by hairy roots

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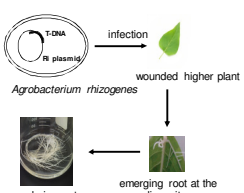
Abstract

The present study examined a wild type and a *NUP1*-reduced expression hairy root line during a variety of treatments to identify culture conditions that increased nicotine accumulation in the media. Oxygen was found to be a necessary and limiting factor for hairy root growth and nicotine production. Nicotine accumulation in hairy root culture medium was improved by surfactant Triton X-100 (TX100) and oxygen mass transfer was increased by TX100 stabilized microbubbles. In comparison to control (no surfactant), TX100 at 10, 25, and 50 mg l^{-1} led to 1.35-, 1.44-, and 1.64-fold nicotine accumulation in culture medium, respectively. At 4000 rpm shear speed, microbubbles stabilized by 10, 25, and 50 mg l^{-1} TX100 resulted in 22.3, 36.2, and 44.1 h^{-1} of k_La in Gamborg's B5 medium, respectively, which was much higher than the 16.4 h^{-1} with conventional air sparging. In a 1-L bioreactor, microbubbles stabilized by TX100 were applied to hairy roots when the inoculated root tips were self-immobilized and dissolved oxygen decreased to below 60% air saturation. With microbubble dispersion, dissolved oxygen increased to above 80% and hairy root growth rate was improved. Moreover, nicotine accumulation in culture medium was significantly stimulated. These results suggest that microbubble dispersion may enhance production of hairy root metabolites in culture medium. Basification of the culture media associated with root growth resulted in a dramatic reduction in nicotine accumulation levels in the media, which was reversed by decreasing the pH of the media.

Introduction

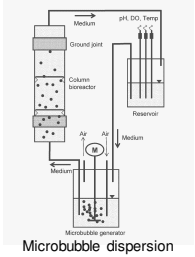
Alkaloids are well recognized for their pharmaceutical activities including antihypertensive effects, antiarrhythmic effects, antimalarial activity, and anticancer actions. Nicotine, which is one of the most naturally occurring alkaloids, was used as a alkaloid model product in this study. Hairy root is induced by infection of plants with *Agrobacterium rhizogenes*. Hairy root has advantages such as high growth rate and biosynthetic stability. However, hairy root growth is necessary for metabolite accumulation but usually limited by insufficient dissolved oxygen in aqueous medium. Microbubble dispersion was shown to be able to improve oxygen mass transfer in microbial fermentation but has not been studied in hairy root cultures. Moreover, secondary metabolites are usually stored within hairy root. If the products can be released into the hairy root culture medium, downstream processes will be less stressful, feedback inhibition will be reduced, and in-situ product removal can be used to improve the production efficiency.



Hairy root development

- Downstream processes
- Product feedback inhibition
- Continuous operation

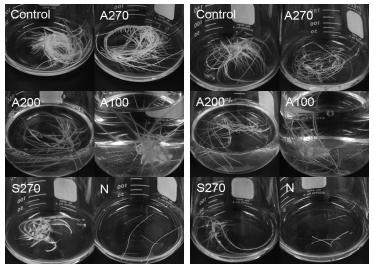
Extracellular metabolites



Microbubble dispersion

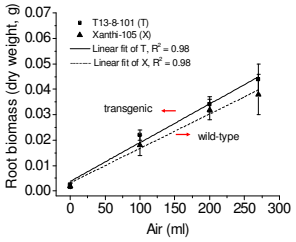
Results

1. Effect of aeration on extracellular nicotine production and hairy root growth

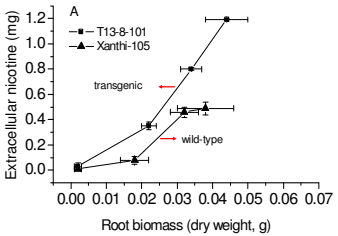


Aeration condition	Medium volume (ml)	Shaking speed (rpm)	Closure	Gas phase	Air volume (ml)
Control	30	100	filter paper	air	270
A270	30	100	screw cap	air	270
A200	100	100	screw cap	air	200
A100	200	100	screw cap	air	100
S270	30	0	screw cap	air	270
N	30	100	screw cap	N_2	0

Transgenic and wild-type hairy roots grown under various aeration conditions

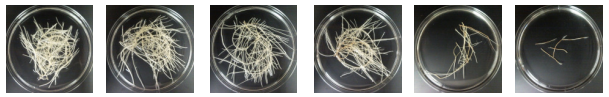


Hairy root growth versus air volume sealed in flasks

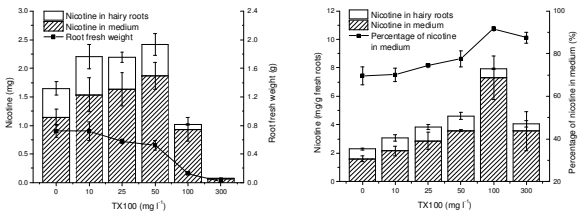


Extracellular nicotine versus root dry weight

2. Effect of Triton X-100 on hairy root growth



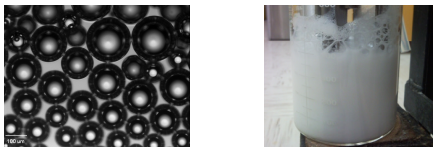
Hairy roots grown under various surfactant concentrations



Root fresh weight and nicotine production under various surfactant concentrations

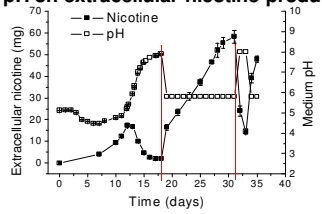
3. Effect of Triton X-100 on oxygen mass transfer

TX100 (mg l^{-1})	Shear speed (rpm)	Aeration (vvm)	k_La (h^{-1})
10	**4000**	**1.00**	**22.3 ± 1.1**
10	**4500**	**1.00**	**25.0 ± 2.4**
10	**5000**	**1.00**	**30.4 ± 3.6**
25	**4000**	**1.00**	**36.2 ± 1.5**
50	**4000**	**1.00**	**44.1 ± 1.2**
Conventional sparging		**1.00**	**16.4 ± 0.6**



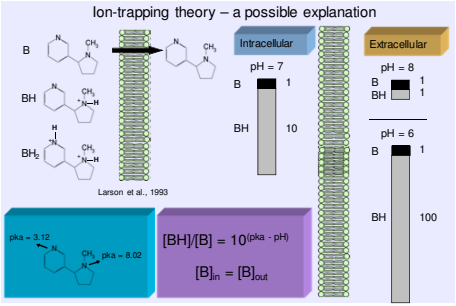
Microbubble dispersion and its effect on oxygen mass transfer

4. Effect of pH on extracellular nicotine production



Time course of extracellular nicotine production and medium pH

Ion-trapping theory – a possible explanation



Conclusions

- Oxygen is a limiting nutrient for hairy root growth and nicotine production.
- Microbubbles stabilized by Triton X-100 improved k_La as well as nicotine accumulation in hairy root culture medium.
- By controlling medium pH, a dramatic decrease in extracellular nicotine can be avoided.

Acknowledgements

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