EuroSciCon

9th Edition of International Conference on

Biofuels and Bioenergy

March 29-30, 2018
Edinburgh, Scotland

Arch Chem Res 2018, Volume 2
DOI: 10.21767/2572-4657-C1-003

MAXIMIZING FATTY ACID PRODUCTION BY RHODOBACTER SPHAEROIDES GROWN ON LIGNOCELLULOSIC BIOMASS HYDROLYSATES

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Lignocellulosic biomass has attracted interest as an abundant, renewable, and carbon-neutral bioenergy feedstock that could augment and eventually serve as a substitute for compounds currently obtained from fossil fuels. Lipids from microorganisms growing on lignocellulosic biomass offer a promising source of long-chain organic alternatives to petroleum-derived fuels and chemicals. In this research, we used corn stover as the biomass source, ammonia fiber expansion (AFEX) followed by enzymatic hydrolysis to produce lignocellulosic biomass hydrolysates, and *Rhodobacter sphaeroides* as the microorganism producing lipids.

R. sphaeroides was used in this work for its natural ability to overproduce lipids when growing at low oxygen or anaerobic conditions. We analyzed genetic changes altering lipid accumulation in *R. sphaeroides* and identified high-lipid (HL) mutants that exhibited the ability to secrete lipids. We also analyzed the inhibitory effect of compounds in the AFEX treated corn stover hydrolysate (ACSH) on *R. sphaeroides* using a metabolomics approach. By cultivating one of the highest lipid-excreting mutants in fed-batch mode, we sought to maximize lipid production while at the same time minimizing the inhibitory effects of aromatic amides. In experiments with defined growth media, we showed that the ratio of lipid produced to dry cell weight (DCW) was as high as 33%, which makes production by this *R. sphaeroides* mutant at levels equivalent to oleaginous bacteria. Thus, we concluded that combining reactor engineering and genetic engineering is a promising approach to increase microbial lipid production from lignocellulosic biomass. In addition, compatibility of lipid production by *R. sphaeroides* with ethanol production from lignocellulosic biomass was also studied. That is, after ethanol is produced and removed by distillation, the stillage contains organic substrates that can be used by *R. sphaeroides* for growth.

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