

Oxygen balanced mixotrophic cultivation: how to double microalgae productivity while avoiding gas transfer

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In the present study microalgae productivity was doubled by designing an innovative mixotrophic cultivation strategy that does not require gas-liquid transfer of oxygen or carbon dioxide. *Chlorella sorokiniana* SAG 211/8K was cultivated under continuous operation in a 2 L stirred-tank-photobioreactor re-designed such that respiratory oxygen consumption was controlled by tuning the acetic acid supply. In this mixotrophic set-up, the reactor was first operated with aeration and no net oxygen production was measured at a fixed acetic acid supply rate. Then the aeration was stopped and the acetic acid supply rate was automatically regulated to maintain a constant dissolved oxygen level using a process control software. Respiratory oxygen consumption was exactly balanced by phototrophic oxygen production and the reactor was operated without any gas-liquid exchange. The carbon dioxide required for photosynthesis was completely covered by the aerobic conversion of acetic acid. Under this condition the biomass/substrate yield was $0.93 \text{ C-mol}_x \cdot \text{C-mol}_s^{-1}$. Under chemostat conditions both reactor productivity and algal biomass concentration were doubled in comparison to a photoautotrophic reference culture. Mixotrophic cultivation did not affect the photosystem II maximum quantum yield (F_v/F_m), the optical absorption cross section of the microalgal cells, or the algal cell size. Only the ratio between chlorophylls and carotenoids increased by 10% in the mixotrophic culture in comparison to the photoautotrophic reference. Our results demonstrate that photoautotrophic and chemoorganotrophic metabolism operate concurrently and that the overall yield is the sum of the two metabolic modes. At the expense of supplying an organic carbon source, cultivation of microalgae in photobioreactors can be improved by preventing energy intensive aeration, efficiently providing the CO_2 needed for the photosynthesis, while simultaneously doubling reactor productivity and biomass concentration.