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# Inoculum preparation for Mixotrophic culturing

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1 Works for me dx.doi.org/10.17504/protocols.io.4txgwpn

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EXTERNAL LINK

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Pereira MIB, Chagas BME, Sassi R, Medeiros GF, Aguiar EM, Borba LHF, Silva EPE, Neto JCA, Rangel AHN (2019) Mixotrophic cultivation of *Spirulina platensis* in dairy wastewater: Effects on the production of biomass, biochemical composition and antioxidant capacity. PLoS ONE 14(10): e0224294. doi: 10.1371/journal.pone.0224294

1 Prepare the inoculum from *Spirulina platensis* strain to inoculate 800 mL of Zarrouk medium contained in 1000 mL Erlenmeyer flask.

2 Supplement the Zarrouk medium with 2.5%, 5.0% and 10% clarified buffalo mozzarella cheese whey. The components of Zarrouk medium (per liter) are as follows: 16.8g $NaHCO_3$, 0.5g $K_2HPO_4$, 2.5g $NaNO_3$, 1.0g $K_2SO_4$, 1.0g NaCl, 0.2g $CaCl_2$, 0.04g MgSO4•7H2O, 0.08g EDTA, 1ml of A5 solution, 1ml of B6 solution.

3 Keep the inoculum growing mixotrophically for 4 days on experimental apparatus under the same light intensities as the culture inoculated by it.

4 Keep the cultures under temperature of 25 °C and constant sterile aeration promoted by pumps (JAD Air Pump S-510) at a specific flow rate of 0.5 vvm (volume of air per volume of medium per minute), and the only light source from fluorescent lamps of 45 W with a luminous intensity of 238 µmol $m^{-2}$ $s^{-1}$ measured by Q201 quantum radiometer (Macam Photo-Metrics Ltd., Livingston, Scotland).

5 Use a light regime of 12-hour light, 12-hour darkness