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Chlorella vulgaris mixotrophic growth enhanced biomass productivity and reduced toxicity from agro-industrial by-products

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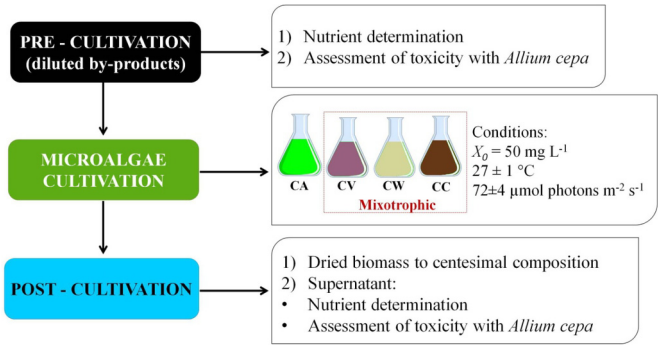
HIGHLIGHTS

- Toxicity of corn steep liquor and cheese whey was reported for the first time.
- Corn steep liquor toxic power was totally eliminated after microalgae treatment.
- Total phosphorus was completely removed in all treated groups.
- *C. vulgaris* is a great alternative for agroindustrial by-products biotreatment.

GRAPHICAL ABSTRACT



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ABSTRACT

Algal wastewater remediation has become attractive for a couple of years now, however the effectiveness of genetic toxicity reducing of some by-products through microalgae are still not well reported. This study aimed to evaluate the growth, nutrients and toxicity removal of *Chlorella vulgaris* cultivated under autotrophic and mixotrophic conditions in three agro-industrial by-products. Mixotrophic culture using corn steep liquor showed higher cell concentration, specific growth rate, maximum cell productivity and biomass protein content when compared to cheese whey and vinasse. Nutrient removal results showed that *C. vulgaris* was able to completely remove corn steep liquor nutrients, while in cheese whey and vinasse culture this removal was not as efficient, observing remaining COD. This work evaluated for the first time the corn steep liquor and cheese whey genetic toxicity through *Allium cepa* seeds assay. These results demonstrate that corn steep liquor toxicity was totally eliminated by *C. vulgaris* cultivation, and cheese whey and vinasse toxicity were minimized. This study proves that the mixotrophic cultivation of *C. vulgaris* can increase cellular productivity, as well as it is a suitable and economic alternative to remove the toxicity from agroindustrial by-products.

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1. Introduction

Agro-industry by-products are one of the largest sources of wastes produced in the world, which depend of raw material type used and technology processing. When discarded in the environment, these by-products release a large amount of nutrients such as nitrogen and phosphorus, which are considered inorganic pollutants. On the other hand, these nutrients could be an alternative culture medium for microalgae production (Mirzaie et al., 2015; Calixto et al., 2016). Some agricultural industry with pollution potential can be highlighted as cheese whey, corn steep liquor and vinasse.

Cheese whey is the main pollutant from cheese production. For each 10 L of milk used by cheese making, about 9 L of cheese whey are produced. In 2016, 19,391 million tons of cheese were produced in the world with about 174,519 million tons of cheese whey released (USDA, 2017a). Cheese production tends to increase, consequently the high amount of cheese whey released becomes a problem since it cannot be discarded in the rivers due to its high levels of Chemical Oxygen Demand (COD = 60–80 g L^{-1}) and Biochemical Oxygen Demand (BOD = 30–50 g L^{-1}) (Abreu et al., 2012).

Corn steep liquor is a typically viscous liquid mixture from the corn wet milling industry, from global corn production of 963.3 million tons estimated for 2016/17 (USDA, 2017b). This has high amounts of amino acids, vitamins, polypeptides, reducing sugars, organic acids and minerals, being source of organic nitrogen (Chiani et al., 2010) and has been used as an inexpensive nutrient for the microbial production of enzymes (Zheng et al., 2012), solvents and organic acids, including succinic acid (Xi et al., 2013).

For each 1 L of sugarcane alcohol produced, about 13 L of vinasse are discarded. In Brazil, the largest producer of sugarcane alcohol worldwide, approximately 28 billion liters of alcohol were produced in 2016 (EPE, 2016) and 364 billion liters of vinasse were generated. Vinasse has a high pollution potential, approximately one hundred times more than household sewage, due to its high organic matter content. It causes depletion of oxygen, low pH and high corrosivity (Kannan and Upreti, 2008). Therefore, it is necessary to find adequate uses and treatments for this by-product.

These three by-products are produced in large quantities and require a correct destination before being released in the environment. Therefore, microalgae cultivation offers a solution to reduce nutrients, such as nitrogen and phosphorus, which can otherwise lead to risks of ecosystem damage and downstream eutrophication. In addition, microalgal-based wastewater treatment requires low costs, low energy, no use hazardous chemicals and simultaneously produces high-added value algal biomass (Batista et al., 2015). Microalgae of genus *Chlorella* sp. is widely used to remove nutrients and reduce toxicity from different by-products types and wastewaters (Franchino et al., 2016; Dahmani et al., 2016). The aim of this study was to evaluate the cell growth, nutrients and toxicity removal of the microalgae *Chlorella vulgaris* UTEX 1803 in three types of agro-industrial by-products.

2. Materials and methods

2.1. Microorganism

The freshwater microalgae *C. vulgaris* (UTEX, 1803) used in this study was obtained from the culture collection of Texas University (Austin, TX).

2.2. Agro-industrial by-products

2.2.1. Cheese whey

Cheese whey was supplied by a cheese factory from Nazaré da Mata, Pernambuco, Brazil. It was deproteinised by heat treatment through sterilization at 121 °C, for 20 min and cooled to room temperature at ± 27 °C. Lactose concentration was analyzed using high-performance liquid chromatography (HPLC), according Erich et al. (2012).

2.2.2. Corn steep liquor

Corn steep liquor was obtained from CornProducts Brazil, Cabo de Santo Agostinho, Pernambuco, Brazil. The pH 8.0 was adjusted with KOH and autoclaved 121 °C, for 20 min. It was centrifuged at 430 × g, 5min (Hermle Labortechnik 326 HK, Wehingen, Germany) and the supernatant was collected for use (Liggett and Koffler, 1998).

2.2.3. Vinasse

Vinasse was supplied by Usina Bom Jesus S/A, Cabo de Santo Agostinho, Pernambuco, Brazil. The treatment was performed through according Bonini (2012).

2.3. Media and culture conditions

Microalgae were pre-inoculated in 250 mL Erlenmeyer flask containing 100 mL of Bold's Basal Medium (BBM) (Bischoff and Bold, 1963). The cultures were divided in autotrophic using BBM as standard medium, and mixotrophic were supplemented with different by-products: 1% corn steep liquor (Silva et al., 2017); cheese whey with initial lactose concentration about 10 g L^{-1} (Abreu et al., 2012); or 2% vinasse (preliminary tests described in item 3.1). The microalgae were inoculated in 400 mL in 1 L Erlenmeyer flasks with an initial biomass concentration of 50 mg L^{-1}, temperature of 27 ± 1 °C, light intensity of 72 ± 4 μmol photons m^{-2} s^{-1}, under constant aeration. The initial cultures pH were adjusted to 6.8 and this parameter was monitored every two days (Hanna Instruments, Woonsocket, USA).

After reach the stationary growth phase, about 14 days, the cultures were centrifuged at 430 × *g* for 10 min (Hermle Labortechnik 326 HK, Wehingen, Germany). Biomass were lyophilized (SP Scientific BenchTop Pro, Warminster, USA) for analysis of centesimal composition and the supernatant were utilized to nutrient determination and toxicity assay.

2.4. Determination of microalgae cell concentration

Cell concentration was determined by optical density (OD) at 685 nm (Xu et al., 2008) (FEMTO 700 Plus, São Paulo, Brazil) and expressed in g L^{-1} through a calibration curve relating OD to dry biomass weight.

2.5. Kinetic parameters of C. vulgaris growth

2.5.1. Biomass productivity

Maximum biomass productivity (P_{max}, g L^{-1} day^{-1}) was calculated from the Eq. (1), where X_0 = initial biomass (mg L^{-1}); X_i = biomass at time *i* (mg L^{-1}) and t_i = time interval (day) between X_0 and X_i.

$$P_{max} = (X_i - X_0)//t_i \quad (1)$$

2.5.2. Specific growth rate

Specific growth rate (μ, day^{-1}) was calculated by Eq. (2), where X_0 and X_i were the concentration of cells at the beginning (t_1) and at the end (t_2) of the exponential growth phase, respectively