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Phycoremediation of alcohol distillery wastewater with a novel *Chlorella sorokiniana* strain cultivated in a photobioreactor monitored on-line via chlorophyll fluorescence

Alexei Solovchenko *, Sergei Pogosyan, Olga Chivkunova, Irina Selyakh, Larisa Semenova, Elena Voronova, Pavel Scherbakov, Ivan Konyukhov, Konstantin Chekanov, Mikhail Kirpichnikov, Elena Lobakova

Faculty of Biology, Moscow State University, 119234 Moscow, Russia

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ABSTRACT

Possibilities of alcohol distillery wastewater (ADW) bioremediation with a new *Chlorella sorokiniana* from White Sea cultivated in semi-batch mode in a high-density photobioreactor monitored on-line via chlorophyll fluorescence were investigated. Upon inoculation of the ADW, a stable alga-bacterial consortium was formed. A decrease in chemical oxygen demand (COD) of the ADW from 20 000 to ca. 1500 mg L^{-1} was achieved over four days with a decline in nitrate (>95%), phosphate (77%) and sulfate (35%). Minimal pre-treatment of the ADW (adjustment to 6.0–7.0 pH) was necessary. Kinetics of COD decline and growth of microalga on ADW as well as chlorophyll and fatty acid (FA) composition of the biomass were studied. Cultivation on ADW increased the unsaturation of the FA of the microalga cell lipids rendering the biomass of *C. sorokiniana* cultivated on ADW a suitable feedstock for biodiesel production. Measurement of variable chlorophyll fluorescence was shown to be a sensitive method for monitoring of the physiological condition of the microalgae grown on ADW. The cultivation conditions facilitating ADW bioremediation with the microalga were investigated. The advantages and limitations of the proposed process for ADW treatment are discussed in view of the findings obtained.

1. Introduction

Production of ethanol from agricultural raw materials displays a steady growth which is supported by its consumption in the form of industrial solvent and a beverage component as well as an alternative CO_2-neutral fuel [1]. The latter usage attracts increasing worldwide interest due to the shortage of non-renewable energy resources and variability of oil and natural gas prices [1,2].

At the same time, production of ethanol generates large volumes of wastewater (referred to below as alcohol distillery wastewater, ADW). Typically, the ADW features a high (20–100 g L chemical oxygen demand, COD) content of organic (carbonic acids, sugar decomposition products, dextranes, etc.) as well as inorganic pollutants (mainly nitrate, ammonia, and phosphate ions), strong odor, brown color and low pH, making it, unless properly treated, a serious environment threat to water bodies and soils. The composition and environmental hazards of ADW are reviewed in detail by Mohana et al. [3].

The conventional treatment of ADW is carried out by means of aerobic and anaerobic processes [3–5]. Increasingly stringent environmental regulations are forcing distilleries to improve existing treatment and also explore alternative methods of effluent management. As a result, space and funds required for building the ADW treatment plants are turning to be the most serious obstacles for such investments. Additional drawbacks of traditional treatment systems include complexity and generation of waste sludge [6,7].

Though the methodology of wastewater treatment using photoautotrophic organisms has a long history [8], the alternative approaches for phytoremediation of ADW emerged only over the last decade [9,10] involving mostly combinations of higher plants, microalgae [11] and/or cyanobacteria [4]. Microalgae as photoautotrophic organisms produce oxygen (providing photosynthetic aeration) which accelerates the degradation of organic pollutants in wastewater [12]. An added benefit of this approach is the production of microalgal biomass, which can be used as a raw material in many applications such as production of biofuel, feeds in aquaculture and cattle farming, fertilizers and value-added lipids and carotenoids [13–16]. The fatty acid (FA) composition of the biomass obtained as a result of cultivation of microalgae on wastewater is very important for the choice of the preferred way of its utilization. In particular, a good quality biodiesel has a high cetane number, which is associated with saturated FA such as palmitic (16:0) and stearic (18:0) acids; on the contrary, a low cetane number was observed with highly unsaturated FA such as linolenic (18:3) acid [17]. The high

Abbreviations: ADW, alcohol distillery wastewater; CFU, colony forming unit; Chl, chlorophyll; COD, chemical oxygen demand; DMSO, dimethyl sulfoxide; DW, dry weight; FA, fatty acids; HRT, hydraulic retention time; LED, light emitting diode(s); PAR, photosynthetically active radiation; PFD, photon flux density.

* Corresponding author.
E-mail address: solovchenko@mail.bio.msu.ru (A. Solovchenko).

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proportion of saturated FA in algal biomass may provide increased energy yield, superior oxidative stability, and higher cetane numbers that cause fewer problems in fuel polymerization during combustion [18].

Nevertheless, the reports on efficient microalgal photobioprocesses specifically aimed for ADW bioremediation are scarce and limited to laboratory-scale experiments [11,19]. Most of them are based on cultivation of green microalgae (Chlorophyta), in particular representatives of the genus *Chlorella* due to its remarkable capability of mixotrophic crowth. Thus, Valderrama et al. [10] carried out research to develop a procedure for biological treatment of recalcitrant anaerobic industrial effluent from ethanol and citric acid production using first the microalga *Chlorella vulgaris* followed by the higher plant *Lemna minuscula*. More recently, Yang et al. [20] cultivated *Chlorella pyrenoidosa* in closed cycle tubular photobioreactor (PBR) in undiluted wastewater from ethanol fermentation using cassava powder as the raw material. High rates of organic pollutant removal were achieved with a fixed bed reactor coupled with a laboratory-scale microalgal pond [21]. It should be noted in addition that the up-scaling of microalgal photobiotechnology for wastewater remediation requires fast and reliable techniques, preferably non-destructive, for on-line monitoring of the state of algal culture [22]. This is necessary for timely and informed decisions on adjustment of illumination conditions, the rate of wastewater inflow and on the time for biomass harvesting. Often, the decisions must be taken within hours and mistakes may lead to a significant reduction in productivity or in a total culture loss.

We hypothesized that the use of the high-density microalgal culture grown in a closed system (PBR) under optimal mixing and illumination conditions would allow to shorten the hydraulic retention time (HRT) and to increase the rate of pollutant removal from ADW. We also attempted to demonstrate that on-line monitoring of variable Chl fluorescence is a useful tool for wastewater phycoremediation. This method was found to provide valuable information on the physiological condition of the microalgal culture and hence to maintain the performance of *Chlorella sorokiniana* during the growth in ADW. We also aimed to investigate the possibilities of treatment of ADW by a high-density culture of the newly isolated from White Sea strain of *C. sorokiniana* in a mixed annular PBR with simultaneous production of the microalgal biomass suitable for downstream processing.

2. Materials and methods

2.1. Strain and its cultivation conditions

A novel *Chlorella* sp. (Chlorophyta) strain isolated from White Sea (Russia) according to the earlier published protocol [23] and identified as *C. sorokiniana* (GenBank ID: KC678067) was used in this work. In pilot experiments the newly obtained *C. sorokiniana* strain appeared to be capable of rapid destruction of organic components of the ADW during cultivation at 27 °C and pH of ca. 7. The algal inoculum was grown in liquid ½ Tamia medium [24]. Batch cultures in 250 mL flasks with 100 mL of medium were kept at 27 °C on an orbital shaker under continuous illumination at 80 μmol PAR photons m^{-2} s^{-1}. Before the experiments, the culture was acclimatized to the ADW by cultivation of the algae on the series of media composed from the ½ Tamia medium and the ADW in the proportions increasing from 1:10 to 2:3 (the ½ Tamia medium: ADW, by volume) and finally transferred to ADW prepared as described below. In some experiments, the ADW was not inoculated with the microalgae and was sparged with filtered atmospheric air (150 mL L^{-1} min^{-1}); other conditions were as described above.

The experiments on ADW bioremediation were performed in a 50-L annular PBR (Fig. 1) with a suspension layer thickness of 46 mm in semi-batch mode. The process was started at 20 g L^{-1} COD. After the decline in COD to ca. 1.5 g L^{-1}, a part of the suspension was removed from the PBR through the sampling cock and a portion of fresh ADW was added to restore the COD to a level of 20 g L^{-1} and to maintain OD_{678} in the range 2.0–3.0. Routinely the cells were harvested by means of decanting; nearly complete sedimentation was achieved within 2–3 h.

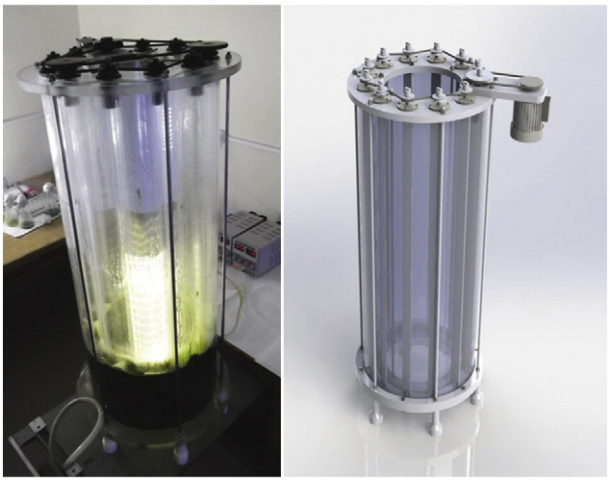

Fig. 1. Operating annular PBR with the *Chlorella sorokiniana* culture on alcohol distillery wastewater (filled to 10% of nominal volume, left) and its 3D model showing the mixing paddles (right).

The culture was mixed by 10 vertical paddle stirrers at 120 rpm and aerated with air at a rate of 5 L min^{-1} (0.1 v v^{-1} min^{-1}). The PBR was kept at 27 °C under continuous illuminations with white LEDs through the inner surface of the PBR (see Fig. 1) at 180 μmol photons m^{-2} s^{-1} PAR. The axenicity of the initial microalgal culture as well as the presence of bacteria during the growth on ADW was determined by a standard plate colony forming unit (CFU) count method as described by APHA [25].

2.2. Alcohol distillery wastewater preparation

The untreated ADW (pH 3–4) was obtained from a local distillery. Prior to inoculation with the microalgal culture it was pH-adjusted with 1 M NaOH to pH 7.0, diluted if necessary, and centrifuged for 5 min at 3000 *g*. The supernatant with a COD of ca. 20 g L^{-1} was used as the cultivation medium for *C. sorokiniana* in routine experiments.

2.3. Growth measurement

Algal growth estimation was based on cell dry weight (DW) measurements and volumetric content of Chl *a*. An aliquot of the cell suspension was sampled from the PBR and the cells were harvested by centrifugation for 5 min at 3000 *g*. The cell pellet was washed with distilled water and used for dry weight determination according to Pal et al. [26]. The wash water was pooled with the supernatant from the previous centrifugation, evaporated to dryness and weighed. In routine measurements total Chl were extracted from the biomass with dimethyl sulfoxide (DMSO) for 5 min at 70 °C with 5 mL per ca. 3.5 mg DW. The pigment concentrations were determined in DMSO extracts spectrophotometrically with an Agilent Cary 300 spectrophotometer (Walnut Creek, CA, USA) [27].

2.4. Fatty acid analysis

Capillary gas-chromatography was used for fatty acid quantification; the analysis was performed according to Cohen et al. [28]. The data shown represent mean values with a range of less than 5% for major peaks (over 10% of fatty acids) and 10% for minor peaks, of at least two independent samples, each analyzed in duplicate. Heptadecanoic (margaric) acid [29] was added as an internal standard. Identification of fatty acids was done according to retention times of standards (Sigma, USA) and by characteristic mass spectra obtained with Agilent 7890 (Agilent, USA) gas chromatograph equipped with HP5-MS

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