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Microalgal biomass generation by phycoremediation of dairy industry wastewater: An integrated approach towards sustainable biofuel production

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HIGHLIGHTS

- *Acutodesmus dimorphus* was grown in raw dairy wastewater without any dilution.
- Biomass production increased by 5–6 folds in dairy wastewater compared to control.
- *A. dimorphus* produced 25% lipid and 30% carbohydrate when grown in dairy wastewater.
- 273 g of biofuel can be produced from 1 kg dry biomass of *A. dimorphus.*

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ABSTRACT

Dairy wastewater collected from local dairy industry was used as a growth media (without any pre-treatment) for the cultivation of microalgae *Acutodesmus dimorphus*. The level of COD reduced over 90% (from 2593.33 ± 277.37 to 215 ± 7.07 mg/L) after 4 days of cultivation; whereas, ammoniacal nitrogen was consumed completely (277.4 ± 10.75 mg/L) after 6 days of cultivation. Dry biomass of 840 and 790 mg/L was observed after 4 and 8 days of cultivation, respectively, which is about 5–6 times more than that of BG-11 grown culture (149 mg/L after 8 days). This biomass contains around 25% lipid and 30% carbohydrate, which can be further converted into biodiesel and bioethanol, respectively. Theoretical calculations based on the recently reported conversion yield suggest that 1 kg biomass of *A. dimorphus* might produce around 195 g of biodiesel and 78 g of bioethanol, which sums up to 273 g of biofuels.

1. Introduction

Dairy industry is one of the major industries with economic importance in agricultural sector. A steady rise in the demand of milk and milk products in many countries has led to enormous growth of dairy industries in the world. India, having 286 small and large scale dairy industries, shares about 13.1% of the total milk produced in the world (Kothari et al., 2012). In dairy industries, water, a key processing medium, is used for cleaning, sanitization, heating, cooling and floor washing. Therefore, the requirement of water and amount of wastewater generated and discharged from dairy industries is higher. Dairy wastewater (DWW) generally contains milk solids, fats, nutrients, lactose, detergents and sanitizing agents (USDA-SCS, 1992) and is characterized by high biological-oxygen demand (40–48,000 mg/L) and chemical oxygen demand (COD) (80–95,000 mg/L). Its pH varies from 4.7 to 11 (Passeggi et al., 2009). It also contains metals like Na, Cl, K, Ca, Mg, Fe, CO, Ni, Mn, etc. along with significant amount of nutrients viz. 14–830 mg/L of total nitrogen (Rico et al., 1991) and 9–280 mg/L of total phosphorus (Gavala et al., 1999).

Increasing consumption of fossil fuels in various industrial and transportation sectors have resulted in energy depletion and significant emission of greenhouse gases in the atmosphere. To avoid further energy crisis and to reduce the impacts of global climate change, research is now focussed on development of renewable energy resource (Lu et al., 2015). Microalgal biomass, as an energy feedstock, has great potential to replace the conventional fossil

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based fuels. Oleaginous microalgae have advantages like much shorter growth cycle, high photosynthetic ability and higher lipid content. They also avoid competition of limited arable land and requirement of fresh water as they can be cultivated in abandon land and wastewater (Pancha et al., 2014).

To decrease the production cost of biomass, microalgal cultivation is now integrated with the use of various industrial wastewaters as growth media. Cultivation in nutrient rich wastewaters allows cost-effective production of microalgal biomass by recovering nutrients through microalgae assimilation. Microalgae have been successfully cultivated in artificial wastewater (Feng et al., 2011), aquaculture wastewater (Van Den Hende et al., 2014), brewery wastewater (Subramaniyam et al., 2016; Farooq et al., 2013), municipal wastewater (Hu et al., 2016; Li et al., 2011), meat processing wastewater (Lu et al., 2015), urban wastewater (Tercero et al., 2014) etc. DWW is also being used for the cultivation of microalgae (Lu et al., 2016; Hena et al., 2015; Ding et al., 2015; Huo et al., 2012; Kothari et al., 2012). However, cultivation of microalgae in most wastewaters require some additional steps like pH adjustment (Huo et al., 2012), dilution (Kothari et al., 2012), mixing of wastewaters (Lu et al., 2016; Lu et al., 2015) etc. to make it suitable for their growth. Despite of the extensive research in algal biomass production, considerable limitations linked with the use of various waste sources in algal culturing (Shi et al., 2016) demand attention of researchers.

The present study aims to check the feasibility of using DWW as a growth media for the cultivation of thermotolerant microalgae *Acutodesmus dimorphus*. While most of the previous studies reported pre-treatments of wastewater, in the present study, our main goal was to use raw DWW without any dilution or sterilization. The specific objectives of the study were (i) to check the growth and biomass production of *A. dimorphus* cultivated in raw DWW (ii) biochemical characterization of DWW grown biomass to check its biofuel potential (iii) mass balance analysis of an integrated approach for microalgal cultivation and DWW remediation.

2. Materials and methods

2.1. DWW and its characterization

The DWW was collected from the inlet of effluent treatment plant of Sarvottam Dairy (The Bhavnagar District co-operative Milk Producer's Union Ltd.), Bhavnagar, Gujarat, India. The dairy with annual turnover of 320,00,000 L of milk, mainly focuses on packaged milk, butter, buttermilk and ghee. The DWW was filtered to remove suspended particles and stored at 4 °C until further use.

The pH, conductivity and salinity of the DWW were determined using a pH meter (EcoScan pH 6, Eutech Instruments Pte Ltd., Singapore) and conductivity meter (HI 2300, Hanna Instruments, USA). Total solids content of the DWW was determined gravimetrically (Clesceri et al., 1998). COD of the DWW was determined using potassium dichromate-ferrous ammonium sulphate method (Clesceri et al., 1998). The nitrite, nitrate and ammonia concentrations in the DWW were determined using sulphanilamide-NEDA (Clesceri et al., 1998), salicylic acid-sodium hydroxide (Cataldo et al., 1975) and sodium nitroprusside-sodium hypochlorite phenate method (Clesceri et al., 1998), respectively. The phosphate concentration in the DWW was estimated using ascorbic acid method (Clesceri et al., 1998). TIC and TOC content of the DWW was determined by TOC analyser (Elementar, Liqui TOC, Germany). Total sugar content in DWW was estimated by phenol sulphuric acid method (Dubois et al., 1956). Heavy metals present in the DWW were analysed by ICP-OES (Perkin Elmer).

The physicochemical characteristic of DWW used in the present study is shown in Table 1. The COD of DWW was 2593.33 ± 277.37 mg/L. The concentration of nitrite and nitrate nitrogen was very low or below detectable level; whereas, ammoniacal nitrogen was present at concentration of 277.40 ± 10.75 mg/L. The phosphate concentration of DWW was 5.96 ± 0.04 mg/L. Concentration of total organic carbon (TOC) and total inorganic carbon (TIC) was found to be 116.23 ± 4.38 and 373.20 ± 5.47 mg/L, respectively. Moreover, DWW also contained a variety of trace minerals viz. Ca, Mg, K, Fe, Zn, Mn, Cu, etc. which are required micronutrients for the growth of microalgae. The nutrient profiling of DWW used in the present study is similar to the characteristics of DWW reported earlier (Lu et al., 2015).

Table 1
Physico-chemical characteristics of DWW used in the present study.

Parameter	Units	Value
pH	–	7.8
Colour	–	Milky White
Odour	–	Offensive
Conductivity	mS	1.87
Salinity	g/L	1.33
Total Solids	g/L	2.8 ± 0.02
COD	mg/L	2593.33 ± 277.37
Nitrite–Nitrogen	mg/L	0.95 ± 0.01
Nitrate–Nitrogen	mg/L	ND
Ammoniacal–Nitrogen	mg/L	277.40 ± 10.75
Phosphate	mg/L	5.96 ± 0.04
Total Sugar	mg/L	47.70 ± 3.91
TIC	mg/L	373.20 ± 5.47
TOC	mg/L	116.23 ± 4.38
Metals		
B	mg/L	0.15
Ca	mg/L	38.10
Cd	mg/L	0.35
Co	mg/L	0.65
Cr	mg/L	0.50
Cu	mg/L	0.30
Fe	mg/L	1.10
K	mg/L	11.75
Mg	mg/L	28.45
Mn	mg/L	1.25
Na	mg/L	345.65
Ni	mg/L	0.80
Pb	mg/L	0.65
Zn	mg/L	0.30

ND: not detected.

2.2. Growth of microalgae in DWW

Microalgae used in this study, *Acutodesmus dimorphus* isolated from an industrial effluent polluted stream, have optimum cultivation temperature of 35 °C (Chokshi et al., 2015) and exhibits thermotolerant characteristics (Chokshi et al., 2016). The growth experiment was carried out in triplicate in 1 L Erlenmeyer flasks containing 500 mL of DWW (raw DWW without sterilization or any dilution) inoculated with 10% of actively growing culture [$(0.96 \pm 0.06) \times 10^6$ cells mL^{-1}] of *A. dimorphus*. The flasks were incubated at 35 °C under 60 μmol m^{-2} s^{-1} light intensity (cool white fluorescent lights) and 12:12 h of light:dark period. Flasks were manually shaken thrice a day to avoid adherence of the cells to the surface of the flasks. On 8th day, culture was harvested by centrifugation and biomass was dried in an oven at 60 °C.

To check the growth of *A. dimorphus* in DWW, culture was withdrawn every alternate day. To determine the dry cell weight (DCW), 50 mL culture was centrifuged at 9000 rpm for 5 min, supernatant was discarded and cell pellet was dried in an oven at 60 °C until constant weight was observed. The DCW was then determined gravimetrically. For cell count, 10 ml culture was harvested by centrifugation, supernatant was discarded and cell pellet was re-suspended in 10 ml distilled water (appropriate dilutions