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Selecting an indigenous microalgal strain for lipid production in anaerobically treated piggery wastewater

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HIGHLIGHTS

- Biomass and lipid production ability of three microalgal strains was studied.
- High lipid content was achieved with *C. vulgaris* in pre-treated piggery wastewater.
- *C. vulgaris* in piggery wastewater excreted xylose, mannose, and arabinose.

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ABSTRACT

The aim of this study was to select a potential microalgal strain for lipid production and to examine the suitability of anaerobically treated piggery wastewater as a nutrient source for production of lipid-rich biomass with the selected microalga. Biomass and lipid productivity of three microalgal strains (*Chlorella sorokiniana* CY1, *Chlorella vulgaris* CY5 and *Chlamydomonas* sp. JSC-04) were compared by using different media, nitrogen sources, and nitrogen concentrations. The highest lipid content and productivity (62.5 wt%, 162 mg/L/d) were obtained with *C. vulgaris* with BG-11 with 62 mg N/L. Secondly, *C. vulgaris* was cultivated in sterilized, diluted (1–20×), anaerobically treated piggery wastewater. Biomass production decreased and lipid content increased, when wastewater was more diluted. The highest lipid content of 54.7 wt% was obtained with 20× dilution, while the highest lipid productivity of 100.7 mg/L/d with 5× dilution. Piggery wastewater is a promising resource for mass production of oleaginous microalgal biomass.

1. Introduction

In the modern world, energy expenditure continues to increase. Fossil fuel reserves are depleting and demand for alternative fuels is enormous. Biodiesel and renewable diesel are very good options for diesel-grade fuels for transportation (Demirbas, 2009). The term biodiesel generally refers to the fuel produced by esterification of vegetable oils and/or animal fats, while the term renewable diesel usually refers to a high quality fuel produced by hydrogenation of the lipids (Prince, 2009). Renewable diesel is fully compatible with existing fuel logistics, distribution and vehicle engines and results in lower air emissions than fossil diesel (Demirbas, 2009). The use of vegetable oils or sugars from food crops for production of transportation fuels has been questioned. Therefore, more and more attention has been focused on single cell oil production, i.e., lipid production in microbial cells. An advantage of microalgae compared to yeasts and other heterotrophic microorganisms for single cell oil production is algae's ability to fix CO_2, thus excluding the need for organic carbon source (Mata et al., 2010).

Microalgae's potential for third-generation biofuel production has been studied widely using different microalgae, cultivation strategies, photobioreactor designs and harvesting technologies (Chen et al., 2011). With various cultivation strategies, the disadvantage of heterotrophic growth, compared to autotrophic growth,

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is to find low cost carbon source which do not compete with food production (Safi et al., 2014). Therefore, utilization of waste and residual materials as a carbon source has gained much attention. Several wastewaters have already been studied as a nutrient and/or carbon source for microalgae. For example, alcohol distillery wastewater, pig and poultry manure, palm oil mill effluent and textile wastewater have been studied (Fenton and hUallacháin, 2012; Ji et al., 2013; Kamarudin et al., 2013; Lim et al., 2010; Solovchenko et al., 2014; Whang et al., 2009). With several wastewater and microalga combinations, different strategies have been studied to enhance lipid accumulation. These include e.g., pH, temperature, nitrogen concentration, iron concentration, salinity stress, light intensity, photoperiod, CO_2 supplementation, nutritional mode (Converti et al., 2009; Mohan and Devi, 2014; Liu et al., 2008; Shah et al., 2014). Nevertheless, nitrogen limitation seems to be the most effective method to enhance lipid accumulation.

Use of microalgae for biofuel production has its pros and cons as reviewed by Safi et al. (2014). As an advantage, microalgae cultivation does not compete with agricultural land usage, conflict with food production or cause deforestation. However, the production costs are still not competitive with fossil fuels (Sander and Murthy, 2010). Therefore, waste and residue materials are attractive raw material options for the production of biofuels. Wastewaters can be used as a source of nutrients and carbon, while flue gases can be a sustainable source of CO_2. Nitrogen concentration and form of nitrogen (urea, ammonium, nitrate, nitrite) varies significantly among various wastewaters. Pig and poultry manure have already been found to be viable nutrient sources for algal cultivation (Fenton and hUallacháin, 2012). Thus, the aim of this study was to screen a suitable microalgal strain for oleaginous microalgal biomass production and to study the potential of using piggery wastewater as nutrient source for the selected oleaginous microalga. Simultaneous lipid accumulation in the microalgal cells and nitrogen removal from anaerobically treated piggery wastewater were also investigated.

2. Methods

2.1. Microalgal strains

Three different microalgal strains isolated from freshwater area in Southern Taiwan were studied. Strains had been identified as *Chlorella sorokiniana* CY1, *Chlorella vulgaris* CY5 and *Chlamydomonas* sp. JSC-04.

2.2. Medium

The media used to cultivate the pure cultures were Bold Basal Medium (BBM) and Blue Green Medium (BG-11). Compositions of the media are shown in Table 1.

2.3. Piggery wastewater

Wastewater used in this study is originated from a Taiwanese wastewater treatment plant treating piggery effluent. Treatment process consists of pH control basin, followed by four anaerobic basins, three aerobic basins and a settler. Grab sample of anaerobically treated piggery wastewater was taken after the fourth anaerobic basin prior to the aerobic treatment.

Wastewater was sterilized with autoclave (121 °C, 20 min) to be able to study the chemical suitability of anaerobically treated piggery wastewater for cultivating *C. vulgaris* CY5 without the inference of the microbial community of the wastewater. Unfortunately, the sterilization caused changes to the chemical composition of the wastewater. Sterilization increased the pH of the wastewater from 7.7 to 9.8. Change in pH and temperature induced stripping of ammonia–nitrogen, because form of ammonia between NH_4^+ ions and NH_3 gas depends on pH and temperature (Olguin et al., 2001). Sterilization also increased the soluble and total COD, and decreased the phosphate concentration (Table 2). To be able to concentrate in the growth of microalgae, sterilization was chosen to be used despite the changes in the chemical composition.

Table 1
Medium compositions.

Component	BBM (g/L)	BG-11 (g/L)
K_2HPO_4	0.075	0.04
KH_2PO_4	0.175	
$NaNO_3$	0.25	1.5
NaCl	0.025	
$C_6H_8O_7$		0.006
$NaCO_3$		0.02
$MgSO_4{\cdot}7H_2O$	0.075	0.075
$CaCl_2{\cdot}2H_2O$	0.025	0.036
EDTA	0.05	0.001
KOH	0.031	
$C_6H_8FeNO_7$		0.006
$FeSO_4{\cdot}7H_2O$	0.00498	
H_2SO_4	10 mL	
H_3BO_3	0.01142	2.86
$ZnSO_4{\cdot}7H_2O$	0.001412	0.222
$MnCl_2{\cdot}4H_2O$	0.000232	1.81
$CuSO_4{\cdot}5H_2O$	0.000252	0.079
$Ca(NO_3)_2{\cdot}6H_2O$	0.00008	
$Co(NO_3)_2{\cdot}6H_2O$		0.049
$Na_2MoO_4{\cdot}2H_2O$	0.000192	0.39

2.4. Experimental conditions

All of the batch experiments were conducted in 1 L glass vessels (15.5 cm in length and 9.5 cm in diameter) equipped with an external light source (14 W fluorescent light/TL5). Light intensity was 150 μmol/m^2/s and aeration rate 0.1 vvm with 2.5% CO_2. Initial volume of medium was 800 mL and volume of inoculum was selected to attain initial optical density of 0.1 at wavelength of 680 nm (OD_{680}). Cultures were incubated for 16–20 days at room temperature (20–25 °C) and the aim was to optimize the conditions for biomass and lipid production.

First *C. sorokiniana* CY1, *C. vulgaris* CY5 and *Chlamydomonas* sp. JSC-04 were cultivated in BBM and BG-11 medium to compare the growth of the three microalgae and study the effect of medium composition. In the second experiment all three microalgal strains (*C. sorokiniana* CY1, *C. vulgaris* CY5 and *Chlamydomonas* sp. JSC-04) were cultivated in BBM using various nitrogen sources to study the effect of nitrogen source. In the original BBM 0.25 g $NaNO_3$/L is used as nitrogen source, while in this experiment 0.16 g NH_4Cl/L and 0.088 g $CO(NH_2)_2$/L were used as nitrogen sources separately.

Table 2
Wastewater composition of the anaerobically treated piggery wastewater before and after sterilization with autoclave.

Component	Anaerobically treated piggery wastewater	Sterilized anaerobically treated piggery wastewater
pH	7.7	9.8
COD_{tot} (mg/L)	332	377
COD_s (mg/L)	298	308
TKN (mg/L)	348	287
NH_4-N (mg/L)	233	210
NO_3^- (mg/L)	5.5	7.1
PO_4^- (mg/L)	101.4	28.4
Cl^- (mg/L)	105	106