



## Ecophysiological characteristics and biogas production of cadmium-contaminated crops



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### HIGHLIGHTS

- The first report on phytoremediation and biogas potential of Cd polluted canola, oat and wheat.
- The physiological responses, Cd accumulation and biogas production were investigated.
- Canola performed better in tolerating high Cd concentration polluted soil.
- Biogas yields were enhanced within a certain Cd concentration range.
- Cd influenced the biogas production from the growth stage to the end of fermentation.

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### ABSTRACT

The present study proposes a novel strategy to get a rational production of biogas of the biomass residues from phytoremediation. This study investigates physiological responses, cadmium (Cd) accumulation and biogas production from canola, oat and wheat in pot and batch experiments. The results indicate that (1) aerial biomasses for canola, oat and wheat were enhanced by 5 mg Cd/kg soil by 19.41%, 8.78% and 3.38%, and the upper limit of Cd concentration that canola, oat and wheat can tolerate for aerial biomass production were 50, 10 and 10 mg Cd/kg soil; (2) canola accumulates more Cd than oat and wheat in its aerial parts; (3) cumulative biogas yields were 159.37%, 179.23% and 111.34% of the control when Cd in the shoot were  $2.00 \pm 0.44$ ,  $39.80 \pm 1.25$  and  $6.37 \pm 0.15$  mg Cd/kg biomass for canola, oat and wheat. Phytoremediation in cooperation with bioenergy production provide new insights for both soil remediation and energy research.

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## 1. Introduction

Cd contamination is a worldwide environmental and health concern (Gallego et al., 2012). Cd is released into the environment from power plants, heating systems, metal-working industries, waste incinerators, urban traffic, cement factories and phosphate fertilizer factories (Marchiol et al., 2004; Daud et al., 2009; Gallego et al., 2012). Cd present in the atmosphere, soil and water can cause serious health problems in all organisms, and its bioaccumulation in the food chain can be highly dangerous (Gallego et al., 2012).

Phytoremediation is a promising environmentally friendly technology that uses higher plants and their associated rhizosphere microorganisms to remediate soil, sediment, surface and ground water by extracting, degrading, stabilizing or volatilizing toxic metals, organics and radionuclides (Kirkham, 2006). However,

the disposal of metal-containing biomass created during the remediation process has been one of the limitations of this technology (Ghosh and Singh, 2005). Therefore, it is important to consider other industrial uses for the metal-containing biomass produced by phytoremediation (Abhilash and Yunus, 2011).

The contaminated biomass cannot be used as fodder; however, it is still acceptable as a feedstock for anaerobic digestion (Tian et al., 2012). In fact, heavy metals present within certain concentration ranges in digestion substrates may enhance biogas production (Yu and Fang, 2001). Thewys et al. (2010) estimated that the probability that this extra income is positive is 90% when he grows energy maize instead of fodder maize and simultaneously exploits the digester in cooperation with other farmers in a Cd-contaminated agricultural area. Moreover, volatile solids from biogas residues are reduced during fermentation (Ashekuzzaman and Poulsen, 2010), resulting in concentration of the metals in the residues for further extraction, centralized processing or reutilization. During the biogas production process, bioenergy is produced as waste material is disposed of.

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Although several studies have assessed the biomass potential for heavy metal polluted lands (Bellarby et al., 2010; Fan et al., 2011), there is still a lack of data regarding bioenergy potential (e.g., biogas yield). Prajapati et al. (2012) combined phytoremediation with biogas production using algae isolated from soil and wastewater. Combining algae biomass production with the making of biodiesel, biogas and other products, four scenarios were assessed for two of Australia's largest wastewater treatment plants by using a spreadsheet model (Batten et al., 2013). Besides, scientists in the National Institute of Water and Atmospheric Research in New Zealand conducted a series of experiments about the algae biofuel production from domestic wastewater treatment high rate algal ponds (Park et al., 2011a,b; Craggs et al., 2011). However, the impacts of metal contamination as well as the bioenergy potential of crops were not assessed in these previous studies. Jain et al. (1992) reported that Cd present within a certain range did not affect biogas yield and enhanced methane production by *Azolla pinnata* R.Br and *Lemna minor* L. Studies on water hyacinth (*Eichhornia crassipes*), channel grass (*Vallisneria spiralis*) and water chestnut (*Trapa bispinnosa*) as phytoremediation plants for industrial effluents demonstrated that the slurries obtained from these plants produced significantly more biogas than the slurries from control plants grown in unpolluted water (Singhal and Rai, 2003; Verma et al., 2007). However, these studies do not come to any conclusions concerning biogas production from metal-polluted energy crops (Thewys et al., 2010). Additional studies should be conducted to demonstrate that the reuse of metal-contaminated plants is suitable for bioenergy production. It will also be necessary to determine a suitable concentration range of heavy metals that can be processed by different plants and to research the mechanism of the metal-induced fermentation process.

In this study, pot experiments were conducted to investigate the influence of Cd on the morphology and biomass production of canola, oat and wheat. The experiments were carried out over the entire growth period of the plants for different concentrations of Cd (0, 1, 5, 10, 50, 100 mg Cd/kg soil). The biomass obtained after phytoremediation was used to produce biogas in batch experiments. The present study is focused on two major objectives: firstly, to determine the biomass yield and phytoremediation efficiency of canola, oat, and wheat, and secondly, to examine the biogas production potential of canola, oat and wheat straw biomass.

## 2. Methods

### 2.1. Pot experiments

Pot culture experiments were conducted in the greenhouse at Beijing Agricultural Institute. The treated soil samples had the following characteristics: total nitrogen was 372.13 mg/kg, total phosphorus was 511.73 mg/kg and the pH was 7.1 (Tian et al., 2012). The background concentration of Cd in the soil was 0.001 mg Cd/kg soil. Each plastic pot (25 cm in diameter and 31 cm in height) was filled with 7.0 kg of treated soil that was mixed with a CdCl<sub>2</sub> solution (CdCl<sub>2</sub>·2.5H<sub>2</sub>O, analytically pure, Fuchen Chemistry Reagents Factory, Tianjin, China) to obtain six different initial Cd concentrations: 0, 1, 5, 10, 50 and 100 mg Cd/kg soil, recorded as T0, T1, T2, T3, T4 and T5, respectively. Before sowing, all pots were incubated for 2 weeks. Each level was measured in triplicate.

Canola, oat and wheat seeds were purchased from Wuhan Seed Company (Wuhan, China), China Agricultural University and Beijing Agricultural Institute. Healthy and commensurate seeds were chosen for experiments. Twenty seeds were sown symmetrically in each pot to a depth of 2 cm. During the experiments, soil moisture was maintained by intermittent watering.

### 2.2. Ecophysiological response to Cd stress

Survival rates were determined on the 2nd week after sowing. Shoot length was measured at the 6th, 10th, 17th, 21st and 25th week, except for canola which was harvested after 21 weeks of growth. After harvesting, whole plants were divided into five sections: root, shoot, leaves, hull and seeds. Each section was washed carefully in tap water and then in deionized water. Then the samples were dried at 80 °C until they reached a constant weight. The dry weights of the different sections were measured using an electronic balance (TD20001B, Yuyao Jinnuo balance instrument Co., Ltd.).

### 2.3. Determination of Cd concentrations

Plant samples from each of the five sections, weighing 0.5000 g each, were digested in a 10 mL solution of 4:1 nitric acid (65.0 ~ 68.0%) and perchloric acid (70.0 ~ 72.0%). The cow dung sample was weighed for 1.0000 g for determining the Cd concentrations. Soil samples were also dried, sieved through a 0.425 mm, and digested according to method 3050b (EPA). All samples were analyzed in triplicate. The concentration of cadmium in the samples was determined using an air/acetylene atomic absorption spectrophotometer (AAS, Analysis 400, Perkin Elmer).

### 2.4. Batch experiments for biogas production

For batch experiments, dry weight of 2 and 6 g for crop shoots and cow dung were mixed to form the co-digestion substrate. The cow dung was taken directly from the Yanqing base, Beijing Dairy Cattle Centre. The cows were 4-year old Holstein heifers that were mainly raised for milk production. Cows were fed a diet containing silage 52.5%, concentrate 27.5%, soybean meal 4.5%, north-east *L. chinensis* 0.5%, alfalfa 7%, cottonseed 3%, corn vinnasse 2.5%, soda 0.1%, fat 1.5% and draught beer 0.9%. The total solid (TS) of the raw cow dung was 23.59 ± 0.96 wt., and the volatile solid (VS) was 86.61 ± 1.73% TS. The Cd content in cow dung was elided in the present study. The fresh cow dung was stored at 4 °C after collection. No additional inoculums were used to avoid the possible impact of existing heavy metal in some inoculums.

The total volume of erlenmeyer flask used for fermentation experiment was 250 mL with the working volume of 100 mL. The TS concentration in the reactor was 8%, which is the normal TS of the wet fermentation (Rilling, 2005). The reactors were buffered with 20 mL of 30 mmol/L NaHCO<sub>3</sub> solution. After 3 h of stabilization, the initial pH values were adjusted to 7.00 ± 0.10 using 1.0 mol/L NaOH and 1.0 mol/L H<sub>2</sub>SO<sub>4</sub>. Five drops of 2.5% Na<sub>2</sub>SO<sub>3</sub> were added into each of the reactors to remove the dissolved oxygen in the system. The reactors were then sealed with rubber stoppers before the digestion began. The reactors were operated at 55 ± 1 °C maintained by a temperature controller (ZNHW-III, Beijing Rui Cheng Wei Industry Equipment Co. Ltd., Beijing, China). This temperature was chosen according to the previous research (Otero et al., 2010) and a preliminary experiment which suggested that the thermophilic anaerobic co-digestion (55 ± 1 °C) produced much more biogas than the mesophilic anaerobic co-digestion (37 ± 1 °C) under current experimental conditions.

The biogas generated during the process of anaerobic fermentation was collected in a 250 mL graduated cylinder by displacing a saturated NaCl solution and was monitored at 9:00 am every day. Biogas volumes were transformed into standard conditions. Data are presented as averages from three parallel reactors.

### 2.5. Data statistics

In order to compare the Cd impact on seedling growth of canola, oat and wheat synchronously, the survival rate was defined as

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