



## Effects of cultivation period on catch crop chemical composition and potential for bioenergy production



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### ABSTRACT

The first step in biofuel and/or bio-based bulk chemical material production is assessing the amounts of useful substances in a potential biomass. Sugar, nutrients, ash, and functional ingredients (such as antioxidant compounds) in premature dent corn biomass cultivated as a catch crop (plant density; 60 shoots  $m^{-2}$ ) over different cultivation periods (29 days, 49 days, 83 days) were investigated. The sugar recovery amount was estimated by multiplying two regression curves (saccharification efficiency curve, quadratic regression curve; dry weight growth curve, Gompertz curve). A simple economic analysis for ethanol production from the catch crop biomass was also carried out using previously reported process costs and the estimated sugar recovery amount. High plant density led to accumulation of plant biomass providing high amounts of plant dry weight and derived sugars per unit area. About 2700 g DW  $m^{-2}$  (about 1400 g sugar  $m^{-2}$ ) was harvested in the 49-day cultivation. The amount is significant compared with biomass yields of other candidates for energy crops. The estimated sugar recovery amount reached its maximum value near the end of experimental period (79-day cultivation). The longer cultivation period was better for sugar recovery although there was a slight decrease in saccharification efficiency with cultivation time. Based on the economic analysis, a higher ethanol price (about 200% higher than the current wholesale price) would be required for catch crop financial independence with around 50-day cultivation. Production of ethanol from catch crop biomass would not be feasible in the current situation. However, condensed biomass production through catch crops is still attractive and requires further research.

### 1. Introduction

Intensive agriculture operations such as greenhouse horticulture have often contaminated underground water because of heavy use of fertilizers. Catch crop cultivation has been proposed to recover excess soil nutrients and prevent this contamination. Production of biomass as a resource for chemical commodities and/or as an energy source has been proposed as another application of catch crops (Fujiwara, 2012). Kondo et al. (2013) investigated the use of catch crops for nutrient removal in a full-scale greenhouse. In that and other studies, a plant density of around 60 shoots per  $m^2$  and around 50-day cultivation, a time restricted by local customary agricultural practice, have been recommended as catch crop cultivation conditions (Fujiwara et al., 2002; Yasutake et al., 2014).

The primary purpose of cultivating catch crops is soil remediation. One technical challenge is in shortening the catch crop cultivation

period to adjust the agricultural non-service period (Yasutake et al., 2014). Although making an economic profit from harvesting catch crops is currently difficult, catch crops provide huge amounts of biomass because they are usually densely planted. Recovering resources from the biomass is therefore another challenge of catch crop management. These resources were a secondary driving force behind catch crop introduction.

The first step in resource recovery is to assess dry weight and contents of an intended biomass, especially for bio-based energy and bulk chemical use. Some researchers have investigated relationships between growth and contents of perennial crops to determine their harvest time and maximize the yields as energy crops (Gorlitsky et al., 2015; Takara and Khanal, 2015). The effect of cultivation period on the dry weight amounts for combustion, and ashes and nitrogen as troublesome materials, has been investigated in switchgrass and napier grass (Tubehleh et al., 2016). However, as Waramit et al. (2011)

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mentioned, there is limited available information about the relationship between cultivation period and biomass yield in energy crops. The variation in resource yields according to cultivation period is also unclear. Cultivation periods either shorter or longer than 50 days might be feasible if the resource yields increase sufficiently. Investigation of resource recovery amounts from the catch crops and their relationships with the cultivation period is essential.

Functional ingredients, such as anti-oxidant compounds, in non-edible parts of plants have been investigated, especially in food processing industries (Moure et al., 2001; Balasundram et al., 2006; Peschel et al., 2006; Domínguez-Perles et al., 2010; Santana-Méridas et al., 2012; Peralbo-Molina and de Castro, 2013; De Ancos et al., 2015). Biomass intended for functional ingredient recovery has thus far been limited to industrial food processing wastes. In other words, non-edible parts of plants that are usually incorporated into the soil or burnt on their fields have not been research subjects because of the lack of financial and/or environmental need for them within the huge capacity of agricultural fields. However, intensive agriculture, including large scale greenhouses, has gradually become popular in recent years and treating huge amounts of crop residues is required for management of organic waste emissions (Watanabe et al., 2013). Crop residues that are not currently utilized as resources contain valuable ingredients. They have potential as resources for supplemental compounds. Dent corn can serve as a densely planted catch crop that can provide huge amounts of biomass when harvested prematurely. It might provide some useful chemical compounds as well as sugars for energy and chemical commodity use as with other agricultural wastes (Cardenas-Toro et al., 2015).

To clarify the effects of cultivation period on biomass yield and contents of dent corn grown as a catch crop, recovered amounts of dry weight biomass, sugar, and functional ingredients were measured for three different cultivation periods. Saccharification efficiencies were compared along with plant growth. Non-linear regression models were applied to estimate dry biomass weight and sugar recovery amount. A simple economic analysis of the potential use of the harvested biomass was carried out to indicate the current potential of premature dent corn as an energy crop.

## 2. Materials and methods

### 2.1. Plant materials and measurements

Stems and leaves were taken from dent corn (*Zea mays* L. cv. 'KD-731') that was cultivated as a catch crop in a greenhouse (27 m × 7.7 m) at Kochi University (33°33.1' N, 133°40.7' E) in summer season 2014. Twelve cultivation plots (8 m × 1 m) were prepared in the greenhouse, of which 3 randomly selected plots were used in this study, as described in Yasutake et al. (2011). The nine remaining plots were similarly prepared for dent corn cultivation with different plant densities, including plots with no plants, for another study. Corn was grown for three different cultivation periods, termed S30 (29 days), S50 (49 days), and S80 (83 days); details are shown in Table 1. The values of temperature and solar radiation in the greenhouse were estimated using the relationships between observed values inside and

outside the greenhouse (Fig. S1). The values inside were measured automatically at 5 s intervals (Yasutake et al., 2011) and the values outside were measured and summarized at 10 min intervals by the Faculty of Agriculture, Kochi University. Daily-averaged temperatures and solar radiation (Sep 29, 2012–Nov 17, 2012 and Aug 10, 2014–Sep 28, 2014; total 100 dates) were compared between inside and outside, and prediction formulas were developed for inside values based on these comparisons.

### 2.2. Sample preparation

The water content of the plant bodies was determined using the dry weight (DW) method on the day of sampling. Plant materials were dried at 80 °C until a constant mass was attained, and the final mass was recorded. Six plant shoots ( $n = 6$ ) per experimental cultivation period (S30, S50, S80) were dried separately and their respective dried weights were determined. Another three plant shoots per treatment were cut, mixed, and subjected to chemical analyses as detailed in the following sections. Each mixed sample was divided into two groups. One was dried at 80 °C, ground in a mill, and kept in a desiccator at room temperature until use. The other was used for analysis of antioxidant compounds; it was lyophilized (VD-250R, Taitec, Koshigaya, Japan) and kept in a freezer (−20 °C) until analysis. Finally, three plant shoots per treatment were used individually for pre-treatment and saccharification ( $n = 3$ ).

### 2.3. Ash, carbon, nitrogen, and phosphorus contents

Aliquots of the biomass were ashed following a standard dry-ashing protocol at 550 °C for 4 h in an electric muffle furnace. The ash was weighed, then digested using a nitric acid-hydrochloric acid digestion (APHA et al., 1998). The total phosphorus contents were then determined using ICP-AES (Vista-pro, SII, Chiba, Japan). The total carbon and nitrogen contents in the dry biomass were measured using a CN coder (Vario EL III, Elementor Analysensysteme, Frankfurt, Germany).

### 2.4. Cellulose, hemicellulose, lignin, and starch contents

Cellulose, hemicellulose, and lignin contents were calculated using the dry weight percentages of  $\alpha$ -amylase-treated neutral detergent fiber, acid detergent fiber, and acid detergent lignin, which were determined by an authorized outside analytical laboratory (Tokai Techno Co., Ltd., Yokkaichi, Japan; [http://www.smart-paper.net/ebooks/tokai-techno/profile-en/SWF\\_Window.html](http://www.smart-paper.net/ebooks/tokai-techno/profile-en/SWF_Window.html); accessed 8 April 2017) according to the Japanese standard for animal feed analysis (<http://www.famic.go.jp/ffis/feed/bunseki/bunsekikijun.html>). Starch contents were determined using amyloglucosidase –  $\alpha$ -amylase method (AOAC International, 2005). The analysis was carried out by an authorized outside analytical laboratory (Japan Food Research Laboratories, JFRL, Tokyo, Japan; <http://www.jfrl.or.jp/e/index.html>; accessed 8 April 2017) according to their standard protocol with some modifications. The authorized certifications of inspection were obtained from these laboratories.

**Table 1**  
Cultivation conditions for catch crops (dent corn) in a greenhouse.

Sample name	S30	S50	S80	S50 -Summer <sup>b</sup>	S50 -Winter <sup>b</sup>
Cultivation season	Aug 10–Sep 8, 2014	Aug 10–Sep 28, 2014	Aug 10–Nov 1, 2014	Jul 14–Sep 1, 2010	Feb 9–Mar 29, 2012
Cultivation period (days)	29	49	83	49	49
Plant density (plants m <sup>-2</sup> )	60	60	60	7 <sup>c</sup>	60
Average temperature <sup>a</sup> (°C)	28.7	27.7	26.0	c.a. 28	13.9
Cumulative amount of solar radiation <sup>a</sup> (MJ m <sup>-2</sup> )	317.1	556.6	896.0	–	–

<sup>a</sup> Temperatures and amounts of solar radiation were estimated using observed values inside and outside the greenhouse (see in Materials and Methods section and Fig. S1).

<sup>b</sup> These samples are mentioned in Results and Discussions section, Tables 3 and 4.

<sup>c</sup> Normal range for cornfields (Yasutake et al., 2011).

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