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# Evaluation of chlorophyll and anti-oxidative components harvested from the anaerobic digestion of fruit and vegetable waste



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# HIGHLIGHTS

- Two-phase anaerobic sludge blanket filter system is built with packed concentric column for increasing sludge retention time (SRT) during treatment of fruit and vegetable waste (FVW).
- A suitable SRT is manifested crucial for feeding homogenate of FVW in batch operation without circulation of sludge.
- Pigments and related antioxidants were present in the suspension solid gradually accumulated in the digester.
- Recycle of fruit and vegetable resources concerning pigments and anti-oxidative compounds is proposed.

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## ABSTRACT

Two-phase up-flow sludge blanket filter (TUBF) digestion process of fruit and vegetable waste homogenate generated anaerobic sludge with concentrated pigments and reducing compounds. In the 5–50 L scale-up daily batch and semi-batch operations, the organic loading rate attained was over 6 kg COD/m<sup>3</sup>.day. A comparative study indicated that Minox polyethylene ring packing was critical for optimization of anaerobic digestion. The biogas production in terms of the volatile fatty acid pattern with a  $C_2$ – $C_6$  level of 600 mg/L through liquefaction and acidification (LA) maintained the alkalinity over 3500 mg/L and stabilized the pH of the LA phase at 5.2–6.8 according to the loading status. In the semi-batch operation of 50 L, pigments gradually condensed in the sludge layer were concentrated by a factor of at least ten fold, and a high level of reducing activity was retained by the chlorophyll and anti-oxidative components.

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### 1. Introduction

The efficiency of anaerobic digestion is roundly influenced by types of substrates participated. Recalcitrants as aromatic ring-containing compounds can be hydrolyzed and digested by long time-acclimated and diversified microbes through their

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evolved biochemical networks (Fernandez et al., 2001). However, varied digest ratios appear within different wastewaters as well as mixed streams due to limited digestion and transformation (Vavilin et al., 2007). The analysis of substrate-influencing effect on digest activity is randomly reported, such as the digestion of slow hydrolyzable macromolecule tannin, which is time-dependable (Vijayaraghavan and Ramanujam, 1999), while wastewater containing halogenated alkane or other organics substantially inhibits methanogens (de Mes et al., 2008; Thiel, 1969). Fruit and vegetable wastes (FVW) are rich in pigments such as chlorophylls, carotene, tannins as well as polyphenols, which are ring structured and some with cyclic nitrogen structure. It is specious that chlorophyll is present in the feces from silk worm digestion of mulberry leaves in facultative anaerobic status, although many anaerobic environments are in reversible metabolic digestion system such as that in the ruminant of cattle and sheep, chlorophyll is still observed in the feces after daily feeding with grass as a carbon source after cellulose degradation (Wahlquist, 2013), Although stably companied by biogas production, it is clearly shown that in natural anaerobic consortia, chlorophylls are inclined to retain its intact and degraded derivative states. There is also supporting evidence as the undecomposed pigments in anaerobic niche around rice paddy field (Zhang et al., 2014; Hoyt, 1996), or the sediment in the lake bottom, which serves as a anaerobic or facultative aerobic niche (Gustaf et al., 1998; Fagherazzi et al., 2014). Although the fate of decomposition intermediate or pathway for plant pigment is still obscured, biological anaerobic treatment may be the way to be adopted for primary treatment of concentrated debris for pigment harvest. In spite pigments resist biological influence and maintain stabilized toward physical stress as heating in oil frying, theory has shown the contacting with sunlight will catalyze autolysis for degradation by photo-induced free radical. Since anaerobic digestion (AD) is deprived of oxygen and maintained in highly reduced state, which is a reasonable stabilization state for photosensitized matters. The benefit of AD process can be extended for biogas recovery, elimination over 90% of pathogens investigated, and the slow degrading matter retained after long term operation. In a way, AD can be utilized as a tool for controlling odor dissemination as volatile acids as well as mercapto-sulfur, which are produced in anaerobic degradation (Masséa et al., 2011). For the scale-up of FVW treatment in Taiwan, there are several central selling markets near rural cities or their production sites. It is potentiated to have over 30 tons of FVW daily dumped from sort-out before trading. The final treatment of incineration is believed to be inappropriate for the wastes of high water content, odors and low energy content, which negatively affect cremation efficiency. So, a sustainable way of treatment as resources recovery by AD process for pigment harvest via fermentation with phase separation in mesophilic condition is incorporated for maintaining acceptable rate of digestion and low energy consumption under a medium sludge retention time (SRT) is studied for continuing biological degradation and stabilization (Verrier et al., 1987; Traverso et al., 2000). The accumulation of potent anti-oxidative compounds during the AD operation into sludge colloid and sediment is also relied on avoiding toxicity factors such as sulfide and ammonium concentration in the digester. Since the packing materials as racing ring are different in surface nature and porous feature, they lead to different efficiency for gas-solid separation as sulfur removal which affects the oxi-redo potential of the reactor. Thus, the in-balanced growth of methanogen and sulfate reducers is highly competed upon H<sub>2</sub> consumption or ended with H<sub>2</sub>S accumulation in over reduced state. Hence BOD degrading performance in relation to the suitability for H<sub>2</sub>S removal through gas-solid separation materials operated in designed SRT and long term operation for maintaining methanogen activity are crucial (Vavilin et al., 2007; Guiot and van den Berg, 1984; Houbron et al., 2003). In an aim to focus on pigment harvest, optimal volumetric loading for different porous packing materials is thus surveyed in this study.

## 2. Materials and methods

### 2.1. Agro-wastes pretreatment

Agro-wastes were collected from the central market weekly. After being sorted, the wastes were homogenized in a crusher at 1500–1700 rpm (A1, 2 hp, Chi Sen Corp., Tainan, Taiwan, ROC). The homogenates were drained with a centrifugation drainer. The liquids were stored at 4 °C as the raw materials for fermentation studies in 5–50 L volumes.

# 2.2. Setup of the small-scale TUBF anaerobic digestion system (Guiot and van den Berg, 1984)

#### 2.2.1. Setup of the 5 L TUBF anaerobic digester

The system combined a liquefaction and acidification (LA) phase and a methane phase for methane production. The liquefaction phase for the production of fatty acids was constructed in a U-shaped or two-chambered chain. The construction of the U-shaped cylindrical pipe utilized a SS #304 reactor that was 3 mm thick and 4.5 cm in diameter. The total volume of the reactor was 0.69 L, and the hydraulic retention time at the beginning of the liquefaction phase was 1–1.2 days for a daily feeding of 0.5 L homogenate filtrate (Houbron et al., 2003).

#### 2.2.2. Setup of the 50 L TUBF anaerobic digester

The design principle for the 50 L digester was essentially the same as that for the 5 L digester, but it was constructed as a transparent acrylic cubic tank. The liquefaction tank (10 mm thick; 28 cm  $\times$  20 cm  $\times$  52 cm, L  $\times$  W  $\times$  H) had two similar compartment chambers with a bottom hole for transduction into a side chamber for lengthening the reaction time. In the influent portion, a 10 L ceramic ring packing material was located in the upper-right corner to slow the feeding

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