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# Food-industry-effluent-grown microalgal bacterial flocs as a bioresource for high-value phycochemicals and biogas

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ABSTRACT

Microalgal bacterial floc (MaB-floc) raceway ponds are a novel sunlight-based technology to grow biomass on food-industry effluent and flue gas (33.9 kg volatile solids (VS) $ha_{pond}^{-1}$ $d^{-1}$). The MaB-floc biorefinery concept of high-value phycochemicals and biogas was screened to find a suitable valorization strategy for this novel biomass. Freezing and aqueous extraction of MaB-flocs followed by size exclusion chromatography yielded 22.4 g C-phycocyanin (C-PC) $kg^{-1}$ VS with a purity of 1.32 (24.5% recovery) and 9.5 g C-phycoerythrin (C-PE) $kg^{-1}$ VS with a purity of 1.06 (20.9% recovery). Anaerobic digestion of the extracted MaB-flocs resulted in 272 NL $CH_4$ $g^{-1}$ VS. Moreover, increasing the suspended solid (SS) loading of food industry effluent for one day, significantly reduced the biochemical methane yield by 13.6%, and the C-PC and C-PE yield of total crude extracts by 74.5% and 65.5%, respectively. In contrast, it increased the neophytadiene yield by 45.1%. This study highlights the large potential of these MaB-flocs as a bioresource for production of phycobiliproteins, biogas and neophytadiene. Further research is needed to improve the phycochemical extraction and purification processes, and to confirm a huge economic potential.



## 1. Introduction

During the past decades, the interest in microalgae-based chemicals and biofuels has increased because of their potential to reduce the dependence on petroleum-based chemicals and fuels [1]. However, the production of these microalgae-based chemicals is very expensive. To address the current challenges in economic viability of microalgal biomass production, waste streams are increasingly used as a cheap resource of water and nutrients for microalgae cultivation [2].

Food industry produces a large amount of effluent, i.e. 2–73 $m^3$ wastewater $ton^{-1}$ production [3,4].In Flanders (Belgium), the nutrient limits for this effluent, prior to discharge are 15 mg total nitrogen $L^{-1}$ and 2 mg total phosphorus $L^{-1}$ [5]. Consequently, before it is discharged, this effluent contains sufficient nutrients and water for the production of microalgal biomass. Although this low-strength wastewater can be used as a growth medium for suspended microalgae cultivation, the harvesting cost of these low-density microalgae cultures is high. To avoid this cost, specific reactors types, such as perfusion reactors [6], are required. However, there are also alternatives to the use of these reactor types. In literature, a wide range of microalgae systems based on bioflocculation and biofilm formation have been proposed. These include Rotating Algal Biofilm Reactors [7], algal bristle reactors [8], algal roofs [9], algal turf scrubbers [10], microalgal bacterial flocs (MaB-flocs) in continuous reactors with settling tank [11] or MaB-flocs in a raceway pond operated as sequencing batch reactor (MaB-floc SBR raceway) [12].

It has been shown that MaB-flocs can be produced on low-strength effluent (approximate current discharge norms of 15 mg N $L^{-1}$; 2 mg P $L^{-1}$) originating from a wastewater treatment plant of a food-producing company and flue gas in an outdoor SBR raceway (Fig. 1). This experiment yielded on average 5.29 g total solids (TS) $m^{-2}$ $d^{-1}$ and 3.39 g VS $m^{-2}$ $d^{-1}$, or 19.3 ton TS $ha^{-1}$ $y^{-1}$ and 12.2 ton VS $ha^{-1}$ $y^{-1}$ [5]. This cost highlights the need for a suitable valorization strategy. Nevertheless, as MaB-floc biomass is a novel bioresource, its

*Abbreviations:* AD, anaerobic digestion; A-PC, allophycocyanin; BMP, biochemical methane potential; BMY, biochemical methane yield; COD, chemical oxygen demand; C-PC, cyanobacterial PC; C-PE, cyanobacterial PE; DM, dry matter; MaB-floc, microalgal bacterial floc; $\eta_{AD}$, AD conversion efficiency; NP, neophytadiene; PC, phycocyanin; PE, phycoerythrin; SCOD, soluble COD; TCOD, total COD; TS, total solids; $\mu_{model}$, first order specific methane production rate; VS, volatile solids.

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valorization is still a challenging matter because, to the best of our knowledge, no information is available on this till date.

Earlier studies have investigated the potential of paper-industry-effluent-grown MaB-flocs for the production of biogas [13], and of aquaculture-effluent-grown MaB-flocs for the production of biogas [14], shrimp feed [15], and organic fertilizer [16]. However, these studies cannot be used to assess the valorization potential of food-industry-effluent-grown MaB-flocs, because of three reasons: first, MaB-flocs are dominated by microalgae; second, the valorization of microalgae is species-specific [6]; and third, the microalgae species in MaB-flocs differs for each reactor operation and wastewater type. In outdoor-grown MaB-flocs on sewage, the dominating microalgae were *Chlorella* sp. [11]; in on paper-industry effluent, *Chlamydomonas* sp., *Acutodesmus* sp. and *Chlorella* sp. dominated [13], and in aquaculture effluent, *Ulothrix* sp. or *Klebsormidium* sp. were dominant [12]. However, in food-industry-effluent-grown MaB-flocs, cyanobacteria dominated [5].

In an integrated biorefinery approach for algal biomass valorization, typically, a mix of high-value, low-volume products (such as phycochemicals) and low-value, high-volume products (such as bioenergy) is produced. The high-value products enhance profitability, while the low-value products provide scale and energy for the process [17,18].

Cyanobacteria, next to cryptonomads and red algae (Rhodophyta), contain phycobiliproteins [18,19]. Phycobiliproteins are proteins with linear tetrapyrrole prosthethic groups (bilins), and act as photosynthetic accessory pigments [6]. These proteins can be divided into three main classes, depending on their absorption properties: phycoerythrins (PE; with maximum absorption at wavelengths $\lambda_{max}$ 540–570 nm), phycocyanins (PC; $\lambda_{max}$ 610–620 nm), and allophycocyanins (A-PC; $\lambda_{max}$ 650–655 nm) [18]. Phycobiliproteins are high-value compounds and have a spectrum of applications such as in natural dyes in cosmetics and food; phycofluorprobes in immunology, cell biology and flow cytometry; and as therapeutic agent with anticancer, antioxidant, hepatoprotectant, and immunomodulating activity [19]. In this regard, it is hypothized that food industry-effluent-grown MaB-flocs are a potential valuable bioresource of phycobiliproteins.

The remaining extracted MaB-floc biomass needs valorization, for example, by conversion into bioenergy. The energy input: output ratio of bioenergy production from microalgal bacterial biomass from wastewater treatment in raceway ponds is more beneficial for biogas production via anaerobic digestion (AD) than for bio-crude oil, pyrolytic bio-oil, biodiesel, and bioethanol [20].

This article presents a biorefinery concept for phycobiliprotein extraction and biogas production from MaB-flocs grown in an outdoor SBR raceway (28 m$^2$) in Belgium on food industry effluent (Fig. 1). Moreover, the effect of a day's increase of the suspended solids (SS) loading of the food-industry effluent on this biorefinery concept has been investigated. This is of importance, as a sudden high SS loading due to wash-out of activated sludge is an industrial reality often hard to avoid, for example, in case of bulking or pinpoint activated sludge [4]. The specific objectives of this study are threefold for both (a) MaB-flocs grown on low SS-loaded food-industry effluent, termed low SS-loaded MaB-flocs, and (b) on high SS-loaded food-industry effluent, termed high SS-loaded MaB-flocs. Firstly, aqueous extracts were analyzed for PE and PC quantity and purity, and further purified via size exclusion chromatography. Secondly, the extraction of neophytadiene (NP), a high-value phycochemical, is investigated as an alternative valorization pathway for high SS-loaded MaB-flocs. Thirdly, the biochemical methane yields and conversion efficiencies of extracted MaB-flocs were determined and compared with unextracted MaB-flocs.

## 2. Material and methods

### 2.1. MaB-floc origin and characterization

MaB-flocs originated in a pilot-scale outdoor raceway pond (28 m$^2$; 10 m$^3$), which was stirred by propeller pumps and treated effluent of a company producing plant-based food (Alpro, Wevelgem, Belgium), as described earlier [5] (Fig. 1). Synthetic flue gas containing 89 ± 2 g $CO_2$ Nm$^{-3}$ was injected at 5–8 L min$^{-1}$ when the raceway pH was above 8.75. The raceway was operated as a sequencing batch reactor with a hydraulic retention time (HRT) of 2.06 days. To study the effect of SS overloading of raceway influent on the valorization of MaB-floc biomass, aerobic activated sludge of the conventional wastewater treatment plant (Alpro, Wevelgem, Belgium) was added to this influent on 17/11/2014 to increase from <0.01 g Total Suspended Solids (TSS) L$^{-1}$ or <0.01 g Volatile Suspended Solids (VSS) L$^{-1}$ to 1.48 g TSS L$^{-1}$ or 1.16 g VSS L$^{-1}$. Details on the raceway influent composition are presented elsewhere [5]. MaB-flocs were harvested and dewatered in two steps: (A) concentration by 1 h settling in a settling tank, and (B) dewatering in a filter press (150–250 μm) [5], and stored at −18 °C until further use.

Two samples of dewatered MaB-floc biomass were used in this study. MaB-flocs harvested on 27/10/2014 prior to the SS overloading are referred to as 'low SS-loaded MaB-flocs', while MaB-flocs harvested on 16/12/2014 after the SS overloading are referred to as 'high SS-loaded MaB-flocs'. Dewatered MaB-floc samples were analyzed for TS, VS, total chemical oxygen demand (TCOD) and soluble COD (SCOD), according to Van Den Hende et al. [5]. DNA extraction, PCR and cloning of MaB-flocs were performed based on De Wever et al. [21], with primers P2–P4 for eukaryotic species, and primers 27F-ITS3R for

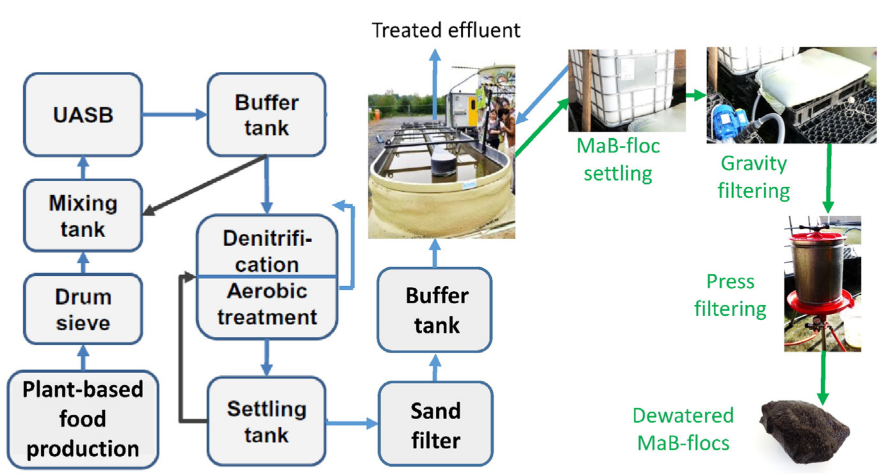


**Fig. 1.** Origin of MaB-flocs grown on food-industry effluent of Alpro in Belgium. Adjusted from Van Den Hende et al. [5].