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Utilization of food waste-derived volatile fatty acids for production of edible *Rhizopus oligosporus* fungal biomass

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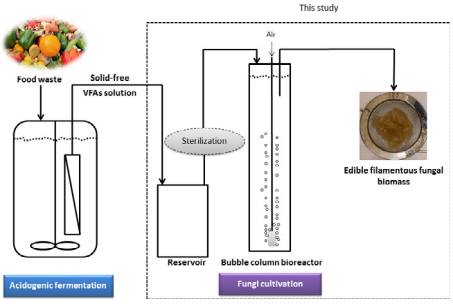
GRAPHICAL ABSTRACT



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ABSTRACT

Rhizopus oligosporus is an edible filamentous fungus that can contribute to meet the growing demand for single-cell protein. Volatile fatty acids (VFAs) are favorable potential substrates for producing *R. oligosporus* biomass due to their capacity to be synthesized from a wide range of low-value organic solid wastes via anaerobic digestion. The goal of this work was to cultivate *R. oligosporus* using food waste-derived VFAs as the sole carbon source. To maintain the requisite low substrate concentrations, the fed-batch cultivation technique was applied. This resulted in a four-fold improvement in biomass production relative to standard batch cultivation. Maximum biomass yield of 0.21 ± 0.01 g dry biomass/g VFAs $_{\text{COD eq. consumed}}$, containing 39.28 ± 1.54% crude protein, was obtained. In the bubble-column bioreactors, the complete uptake of acetic acid was observed, while the consumptions of caproic and butyric acids reached up to 97.64% and 26.13%, respectively.

1. Introduction

Enormous amounts of organic wastes and residues are produced daily from human activities. In the interest of mitigating the potential environmental, social and economic problems associated with these wastes, several treatment options have been proposed in which the otherwise waste materials are considered as resources. Cultivation of filamentous fungi is one of the treatment options that not only solves the mentioned problems but also yields value-added materials, thus creating promising pathways towards a circular economy. Because of their metabolic versatility, filamentous fungi have been identified as sources of a wide spectrum of essential bioproducts such as chitosan,

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industrial enzymes, adsorbents and animal feed (Ferreira et al., 2013; Ozsoy et al., 2008). Indeed, previous studies showed that the quality of the fungal biomass produced from organic residues competed favorably with commercial products, such as fishmeal and soybean meal (Asadollahzadeh et al., 2018).

Rhizopus microsporus var. *oligosporus* (also known as *Rhizopus oligosporus*) is a generally-regarded-as-safe Zygomycete with the ability to assimilate diverse carbon sources for its growth and has for long been used as a starter culture for tempeh production in Indonesia for human consumption (Wikandari et al., 2012). Besides, the protein-rich fungal biomass from filamentous fungi such as *R. oligosporus* can be readily applied as a source for aquaculture and animal feeds, which have a wide market value (Nitayavardhana & Khanal, 2010). Several reports in literature have thus studied the potential of producing *R. oligosporus* biomass using waste streams from agro-processing facilities. The explored waste streams consisted of materials such as thin stillage and vinasse as well as starch processing wastewater (Jin et al., 1999; Jin et al., 2002; Nitayavardhana & Khanal, 2010; Rasmussen et al., 2014). The above-listed materials comprised mainly of easy-to-consume dissolved organic compounds and are principally of good quality since they originate from controlled industrial environments. However, other complex organic solid waste streams originating from uncontrolled sources, such as mixed food waste from restaurants, households or retail stores, remain still unexploited for the production of filamentous fungal biomass in submerged fermentation systems.

Recent estimates show that the amount of food waste generated globally along the supply chain reaches up to 1.3 billion tons annually (FAO, 2019). Food waste thus constitutes a significant portion of the organic fraction of municipal solid wastes (Paritosh et al., 2018). Of the conventional treatment methods for this type of waste, which is considered of very low quality, anaerobic digestion technology is a widely preferred option aiming at biogas production (Ariunbaatar et al., 2016; Strazzera et al., 2018). There has been a growing interest in recovering other chemicals or materials from the anaerobic digestion process to address the economic challenges faced by biogas in the energy market as well as the mounting decline in natural resources (Dahlgren et al., 2019; Kleerebezem et al., 2015). Thus, research efforts are being conducted to divert the anaerobic digestion technology from a biogas platform into a carboxylate platform (Kleerebezem et al., 2015). The main output of the latter platform consists of volatile fatty acids (VFAs), which are short-chained carboxylates formed before the anaerobic digestion process is completed, mainly during the acidogenic phase. There are methods to optimize the production of VFAs without their conversion to biogas in the final (methanogenic) phase (Kleerebezem et al., 2015; Wainaina et al., 2019a). The VFAs, consisting of e.g. acetic, butyric, and caproic acids, alongside lactic acid, have been directly applied as carbon sources in certain bioprocesses. For instance, they have been used to cultivate oleaginous microorganisms for lipids (or biodiesel) production (Béligon et al., 2016), or for synthesizing microbial polymers in the form of polyhydroxyalkanoates (PHAs) (Mohan et al., 2010). Unlike biogas, an effective strategy for recovering the VFAs from the anaerobic digestion medium is needed to supply the carboxylates for the downstream processes. The use of membrane bioreactors is a highly attractive recovery strategy with the view of guaranteeing high cell density without interfering with the acidogens' biological function, as well as the possibility of obtaining sterile VFAs solution (Béligon et al., 2016; Wainaina et al., 2019b).

It has been reported that some carboxylate-containing waste streams (mainly acetic and lactic acids) are prospective carbon sources for cultivation edible filamentous fungi. In a recent study, certain fungi (e.g. *Rhizopus oryzae*) were found to have the capacity to assimilate acetic acid contained in liquid wastes from pulping processes (Asadollahzadeh et al., 2018). Millati et al. (2005) also observed the uptake of acetic acid together with the sugars present in a dilute-acid hydrolysate of woody biomass by several Zygomycetes. Moreover, the consumption of acetic and lactic acids contained in corn stillage during the growth of *R. oligosporus* has been observed (Pietrzak & Kawa-Rygielska, 2019; Rasmussen et al., 2014). In the above-mentioned studies, the carboxylates made only a minor contribution to the total organic strength of the substrate. However, to our knowledge, the potential of using the mixture of VFAs from anaerobic digestion bioreactors as the exclusive carbon source for cultivating *R. oligosporus* has not been reported in the literature. Considering the robustness of the anaerobic digestion technology as regards the plethora of low-value organic solid wastes that can be used as substrates, the application of VFAs to grow value-added filamentous fungi, together with the associated bioproducts, presents an attractive concept within the circular economy framework. This concept could also potentially transform the existing anaerobic digestion facilities into biorefineries.

Based on the above, the current study was aimed at investigating a novel route for the cultivation of *R. oligosporus* using waste-based VFAs. The solid-free VFAs solution, recovered from a membrane bioreactor digesting food waste, was applied as the sole carbon source for the fungal growth. Flask cultivations were conducted to determine the allowable concentration thresholds, and then the suitable conditions were applied in fed-batch cultivations, first in shake flask experiments, and then scaled up in bubble-column bioreactors.

2. Materials and methods

2.1. Acidogenic fermentation of food waste and recovery of VFAs

The main process conditions for the anaerobic digestion and the extraction of the VFAs in the membrane bioreactor were similar to those provided by Wainaina et al. (2020). A microfiltration process, taking place in the bioreactor simultaneously with the anaerobic digestion of the food waste, facilitated the separation of the metabolized VFAs solution from the suspended solid contents (i.e. the bacteria consortium and the undigested substrate). A bulk reservoir of the clarified VFAs solution was prepared from the recovered filtrate over the anaerobic digestion period, kept in a freezer (−20 °C), and then thawed in a cold room (5 °C) before use in the fungal cultivations tests. The key characteristics of the substrate and the VFAs recovered from the membrane bioreactor are provided in Table 1. The composition of the VFAs solution, as a fraction of the soluble chemical oxygen demand (sCOD), was: 16.29% acetic, 8.64% propionic, 48.17% butyric, 11.15% caproic, and 2.78% lactic acids. The remaining organic content was made up of other minor metabolites, including trace amounts of isobutyric, isovaleric and valeric acids.

2.2. Microorganism

Rhizopus microsporus var. *oligosporus* CBS 112586 obtained from CBS-KNAW Collections (Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands) was maintained on a potato dextrose agar (PDA) medium containing (g/L) potato infusion 4; glucose 20; agar 15. For inoculation, 20 mL of sterile 5% Tween 80 was flooded on each

Table 1
Key characteristics of the primary substrate (food waste) and the recovered VFAs in the membrane bioreactors.

Parameter	Food waste	Recovered VFAs solution
Total solids (TS)	16.11 ± 0.98%	–
Volatile solids (VS)	15.41 ± 0.94%	–
Total chemical oxygen demand (tCOD)	160 ± 9.90 g/L	–
Soluble chemical oxygen demand (sCOD)	60.00 ± 5.66 g/L	25.30 ± 0.28 g/L
Concentration of VFAs	–	12.94 ± 0.10 g/L
pH	4.5 ± 0.1	5.3 ± 0.1
NH_4^+-N	490 ± 20 mg/L	9.50 ± 0.71 mg/L
PO_4^{3-}-P	1020 ± 10 mg/L	184.00 ± 2.26 mg/L

plate and disposable spreaders used to bring the spores into solution. The fungal spores were grown in plates for 3–5 days at 30 °C. The sporulated plates were stored at 5 °C until use. The suspension was then added to the cultivation medium to reach a final concentration between 7.5×10^5 and 1.8×10^6 spores/mL.

2.3. Flask cultivations

The fungal cultivations were first carried out using 250 mL wide-mouth cotton-plugged Erlenmeyer flasks containing 100 mL of initial medium. The flasks were placed in a shaking water bath set at 125 rpm orbital agitation and a temperature of 35 °C. The VFAs solutions were applied solely for the fungal cultivation without any nutrient supplementation. Before use, the VFAs solutions were autoclaved at 121 °C for 20 min. Three concentration levels were first screened using the as-received solution (undiluted VFAs), diluted with water at a ratio of 1:1 (50% diluted VFAs), and diluted with water at a ratio of 1:3 (25% diluted VFAs).

In the fed-batch assays, the cultivation was initiated with 50 mL of 25% diluted VFAs solution. After 48 h of fungal growth, undiluted VFAs solution was added (on a daily basis) to the flasks until 100 mL total volume was reached. The intermittent addition of the undiluted VFAs was performed by increasing the culture volume by 14 mL at 48 h, and afterwards by 9 mL at 72 h, 96 h, 120 h, and 144 h. All flask cultivations proceeded for 168 h. During the batch screening, the initial pH was set at 6.0 while in the fed-batch assays, the pH was adjusted to 6.0 daily using 2 M HCl and 2 M NaOH (including the control batch). All the flasks were inoculated with 2% (v/v) spore suspension. After the cultivation period, the final biomass was harvested using a fine sieve, washed thoroughly using distilled water and freeze-dried (−50 °C, −0.03 mbar).

2.4. Bioreactor cultivation

A bubble-column bioreactor (Belach Bioteknik AB, Skogås, Sweden) was used for the scale-up tests at a working volume of 2.0 L in fed-batch mode. The seeding biomass was grown in shake flasks at an initial culture volume of 200 mL for 48 h. The pH was maintained at 6.0 under the same conditions as described in the previous section. A feeding rate of 20 mL/h into the bioreactor was determined based on the uptake of the VFAs in the batch tests and considering the total cultivation time needed. The constant feeding of the bioreactor occurred until 138 h, and the reactor was allowed to proceed until 168 h in batch mode to achieve similar cultivation period as the flask assays. The temperature was maintained at 35 °C and the aeration rate of approximately 0.5 vvm was set. A condenser was attached at top of the reactor, circulating ethylene glycol-water mixture at 0 °C using a recirculating chiller (Polar Series Accel 250 LC, Thermo Fisher Scientific, Waltham, MA, USA). The harvesting and drying conditions of the final biomass were similar to those applied in the flask tests.

2.5. Analytical methods

A high-performance liquid chromatograph (HPLC) (Waters Corporation, Milford, CT, USA) was used to quantify lactic acid. The HPLC was equipped with a hydrogen-based column (Aminex HPX87-H; BioRAD Laboratories, München, Germany) and ultraviolet (UV) absorption detector at 210 nm (Waters 2487, Waters Corporation, Milford, CT, USA). The mobile phase was 5 mM H_2SO_4 at 0.6 mL/min at 60 °C. A gas chromatograph (Perkin-Elmer, Shelton, CT, USA) was used to quantify acetic, propionic, butyric and caproic acids. It was equipped with a capillary column (Elite-WAX ETR, 30 m × 0.32 mm × 1.00 μm, Perkin-Elmer, Shelton, CT, USA) and a flame ionized detector (Perkin-Elmer, Shelton, CT, USA) with an injection and detection temperature of 250 and 300 °C, respectively. The carrier gas was nitrogen at a flow rate of 2 mL/min and a pressure of 20 psi.

The total chemical oxygen demand (tCOD), soluble chemical oxygen demand (sCOD), total solids (TS), volatile solids (VS), suspended solids (SS), concentrations and total Kjeldahl nitrogen were determined according to standard methods (APHA-AWWA-WEF, 2005). A factor of 6.25 was used to calculate the crude protein (Jin et al., 1999). The conversion factors used for calculation of COD equivalents of the carboxylates were (g COD/g acid): 1.07 for acetic acid, 1.51 for propionic acid, 1.82 for butyric acid, 2.21 for caproic acid, and 1.07 for lactic acid. NANOCOLOR® tube tests were used to determine the NH_4^+-N and PO_4^{3-}-P concentrations according to the manufacturer's instructions (MACHEREY-NAGEL GmbH & Co. KG). The biomass concentrations were determined using the total working volume and the yields were expressed as g dry biomass/g VFAs $_{\text{COD eq. consumed.}}$

2.6. Statistical analysis

All the characterization tests and cultivations were performed in duplicate and the presented results are the mean of the observed values and the standard deviations. To statistically analyze the data, MINITAB® software (version 17.1.0, Minitab Inc., State College, PA, USA) was used. General linear models with a confidence interval of 95% were used to perform the analysis of variance (ANOVA).

3. Results and discussion

Anaerobic digestion process has been widely adopted to convert a wide range of abundantly available organic solid wastes, such as food waste, into biogas. However, other interesting metabolites from this process, namely VFAs, can potentially be used as building blocks for obtaining high-value materials such as filamentous fungi. Filamentous fungal biomass from edible species is a prospective feed substitute for animals owing to suitable nutritional properties (e.g. high protein content). In this novel study, the capability of applying VFAs to cultivate edible filamentous fungi as the sole carbon source was assessed. The VFAs were first synthesized and recovered from the anaerobic digestion of food waste by means of a membrane bioreactor. Batch flask assays, conducted at different acid concentrations, revealed that the high concentration of the recovered VFAs solution was unsuitable for fungal growth. However, by dilution, the fungus was found to consume acetic, butyric and caproic acids at varying degrees. Fed-batch cultivations were then conducted whereby the cultivation was initiated using the most dilute VFAs solution and proceeded by intermittently adding an amount of the undiluted solution. The fed-batch cultivation technique improved the consumption of the acids in the cultures and, consequently, the production of the fungal biomass was enhanced. The process was finally scaled-up in a bubble-column bioreactor. In both the flask and bioreactor trials, promising protein content in the fungal biomass was realized which indicated the potential for application as animal feed.

3.1. Flask cultivations

3.1.1. Batch cultivation at different VFAs concentration

The acid consumption trends at the three concentration levels of VFAs are shown in Fig. 1. It can be seen that there were no changes in the concentration of the undiluted VFAs solution (12.94 ± 0.10 g/L) after 168 h. However, using diluted VFAs solution, up to 52.99% and 53.39% of the total VFAs were consumed in the batches using 50% diluted VFAs and 25% diluted VFAs, respectively, within the same cultivation period. By using the 50% diluted VFAs, which contained 6.73 ± 0.76 g/L total VFAs concentration, acetic acid (1.90 ± 0.22 g/L at 0 h) was totally used up by 96 h (Fig. 2a). However, for butyric and caproic acids (initially present at 3.27 ± 0.36 and 0.60 ± 0.04 g/L, respectively), up to 35.40% and 89.56% respectively were consumed by the end of the fermentation period (Fig. 2a). The biomass yield from the 50% diluted VFAs

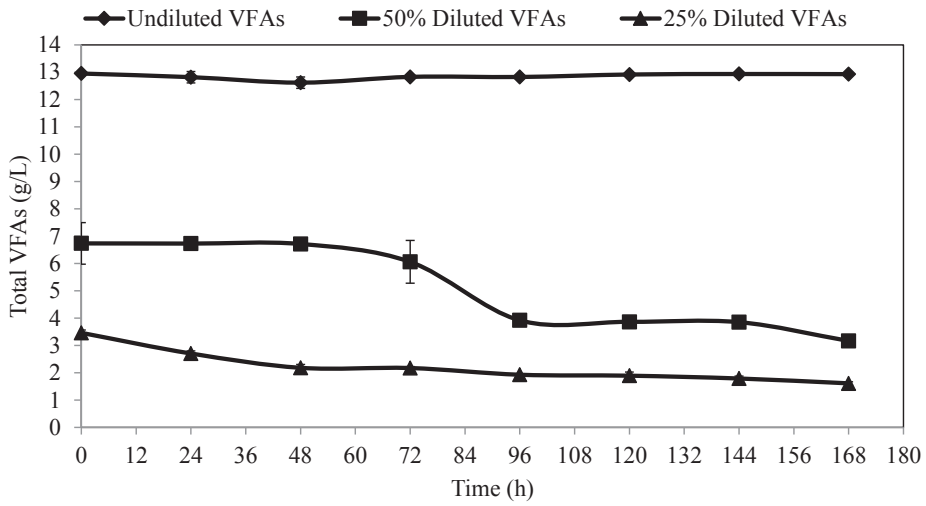

Fig. 1. Consumption trends of total VFAs by *R. oligosporus* at different acid concentration levels in the batch test.

condition was 0.12 ± 0.02 g dry biomass/g VFAs $_{\text{COD eq. consumed}}$. The fungus grew as loose filaments in the initial stages and afterwards formed into clumps.

With an initial total VFAs concentration of 3.46 ± 0.14 g/L (25% diluted VFAs), acetic acid initially at 0.963 ± 0.02 g/L was completely consumed by 48 h (Fig. 2b). Moreover, butyric acid initially at 1.674 ± 0.10 g/L and caproic acid initially at 0.32 ± 0.01 g/L were gradually consumed through the fermentation time similar to the 50% diluted VFAs condition, reaching consumption extents of 38.71% and 83.10%, respectively (Fig. 2b). It was also observed that in both dilute VFAs conditions, propionic and lactic acids were not utilized (Fig. 2a and b). A biomass yield of 0.12 ± 0.01 g dry biomass/g VFAs $_{\text{COD eq. consumed}}$ was obtained. Moreover, in both 25 and 50% diluted VFAs batch trials, the average pH value had risen to 8.2 ± 0.2.

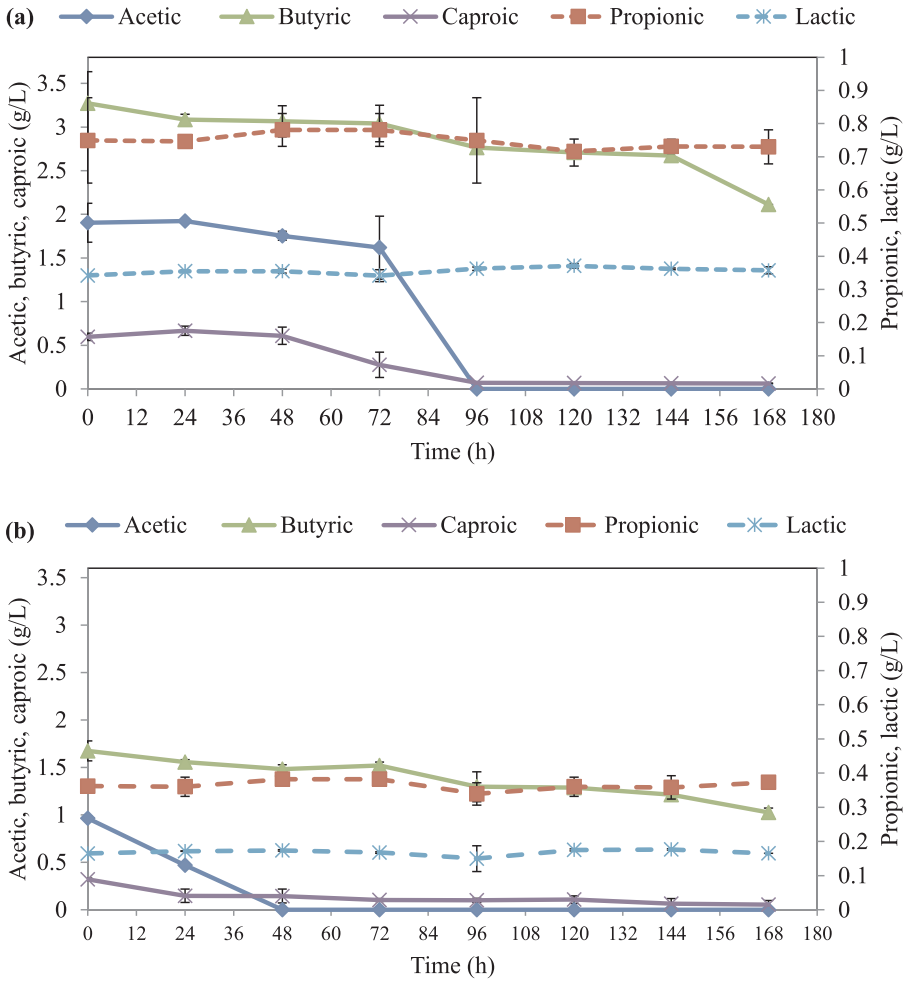

Fig. 2. Consumption trends of individual VFAs by *R. oligosporus* in solutions containing (a) 50% diluted VFAs, and (b) 25% diluted VFAs.

In the first batch assays, there was no change in the concentration of the undiluted VFAs throughout the cultivation period. Consequently, no visible growth occurred indicating that the fungus could not consume the acids with a total VFAs concentration of up to 12.94 ± 0.10 g/L. Acid inhibition on microorganisms has been linked to undissociated molecules existing at low pH levels (lower than the dissociation constant, pKa, of the acid) in which the acid dissociates inside the cell, and diverts the energy from cell growth to cell maintenance (Taherzadeh et al., 1997). However, as the non-growth observed in the present work occurred at a higher pH (i.e. pH 6.0) than the pKa of the VFAs, it was attributed to substrate inhibition.

With the 50% and 25% diluted VFAs solutions, fungal growth was possible. The batch assays resulted in similar biomass yield, but the cultivation with 25% diluted VFAs (i.e. initial concentration of 3.46 ± 0.14 g/L) had a shorter lag phase. In the subsequent cultivations, therefore, the 25% diluted VFAs was used to initiate the fungal growth. The results from the batch assays also suggest that acetic acid was the most preferred carboxylate for fungal growth. Acetic acid participates in the aerobic respiration process in the form of acetyl-CoA. Indeed, with the cultivation with 25% diluted VFAs, the acetic acid was gradually consumed, and complete uptake was observed by 48 h. On the other hand, with 50% diluted VFAs, there was no substantial acetic acid uptake within 72 h. However, a rapid consumption of the acid occurred afterwards until 96 h. The complete uptake of acetic acid during the growth of *R. oligosporus*, alongside other compounds such as sugars and glycerol, has been previously observed (Pietrzak & Kawa-Rygielska, 2019; Rasmussen et al., 2014).

As revealed for the first time in this work, other longer chained carboxylate molecules (such as butyric and caproic acids) can also be assimilated by the fungus. The utilization of the caproic acid appeared to happen in parallel with acetic acid utilization, but butyric acid was mainly utilized after the acetic acid was depleted. Interestingly, between the two carboxylates, the utilization of caproic acid, which has longer chain length than butyric acid, was higher. This phenomenon needs further investigation to determine if the acid chain length plays a role in the VFAs consumption by the fungus. Furthermore, the carboxylate utilization by *R. oligosporus* appeared to prefer the even-chained acids, since the propionic acid present in the substrate was not consumed in either 50% or 25% dilute VFAs conditions during the cultivation period, even at low initial concentration. Similarly, there was no change in the lactic acid in the flask batch tests, which was also present at low initial concentration, while very low net consumption was observed during the flask fed-batch experiments. On the contrary, according to previous research by Rasmussen et al. (2014) who used bench scale stirred bioreactors, removal of lactic acid (initially present at 3.1 g/L) by *R. oligosporus* was possible. It is however worth noting that their substrate contained high amounts of other carbon sources such as reducing sugars and glycerol.

3.1.2. Fed-batch cultivation

To improve the consumption VFAs, a fed-batch approach was conducted starting with 25% diluted VFAs at a controlled pH. After the 48 h cultivation on 25% diluted VFAs, more acid solution was added at each sampling point until reaching 144 h. Fig. 3 shows the acid consumption trends during the entire fed-batch fermentation alongside a batch test with pH maintained at 6.0 for comparison. Up to 61.93% of the total VFAs added was consumed in the controlled pH flasks while 52.30% acid uptake was observed in the fed-batch cycle. With the intermittent increase of the VFAs concentration, there was a remarkable increase in the amount of biomass produced, from 0.44 ± 0.05 g/L in the batch mode to 1.79 ± 0.04 g/L in the fed-batch mode. Table 2 provides the individual acids uptake trends observed in the control batch and the fed batch cultivations. Complete uptake of acetic acid was observed while the caproic acid was almost entirely consumed in both conditions. However, the consumption of butyric acid in the fed-batch cultivation reduced by nearly by a factor of 10 relative to the controlled pH cultivation. Regarding the biomass yield, 0.14 ± 0.02 and 0.21 ± 0.01 g dry biomass/g VFAs $_{\text{COD eq. consumed}}$ were obtained in the controlled pH batch and the fed batch cultivations respectively. The fungal morphology was similar to the observation in the batch trials. The crude protein content of the biomass obtained in the fed-batch and controlled pH batch experiments reached 39.28 ± 1.54 and 35.89 ± 0.32%, respectively.

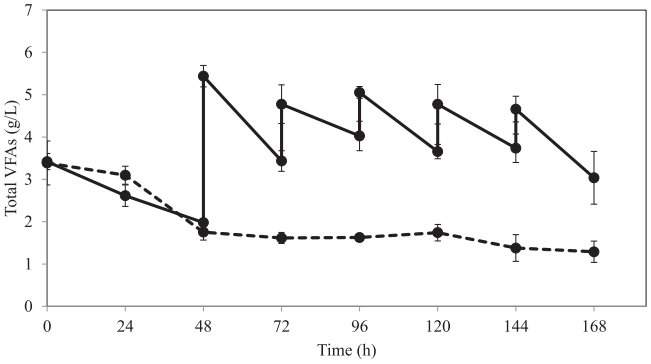

Fig. 3. Consumption trends of total VFAs by *R. oligosporus* in the fed-batch test (solid line) relative to a controlled pH batch (dotted line). In the fed-batch test, the fungal growth was initiated using 25% diluted VFAs until 48 h, while addition of undiluted VFAs took place in 24 h intervals between 48 and 144 h.

Table 2
The extent of consumption of the individual VFAs (in percentages) during cultivation of *R. oligosporus* in controlled pH batch and fed-batch assays.

	Acetic	Propionic	Butyric	Caproic	Lactic
pH-controlled batch	100.00	0	31.20	95.99	15.63
Fed-batch	99.72	0	3.83	98.69	2.68*

* Represents the net reduction in lactic acid concentration.

The fed-batch cultivation method was employed to overcome the substrate inhibition associated with high acid concentration. This fermentation technique has been successfully applied during anaerobic ethanol fermentation involving substrates containing inhibitory compounds (Taherzadeh et al., 1999; Taherzadeh et al., 2000), and cultivation of oleaginous yeast for biolipids production using VFAs (Béligon et al., 2016). In this work, the fed-batch technique proved to be effective in improving the consumption of the VFAs for edible biomass production. Indeed, a remarkable increase of up to four times in the biomass concentration was obtained in the fed-batch flask test compared to the standard batch flask test. However, there was no significant difference ($p > 0.05$) in the biomass crude protein content between the controlled pH batch and fed-batch conditions. Compared to the controlled pH fermentation, the uptake of acetic and caproic acids was fairly similar. However, the uptakes of both lactic and butyric acid decreased by more than 80%. As reported previously, butyric acid uptake requires more time and happens mainly after depletion of acetic and caproic acid. But with the fed-batch cultivation approach, the flasks were supplemented with more acid on a daily basis, and possibly sufficient time was not available for the fungus to acclimatize to the presence of butyric acid. The fungal growth at this condition was thus attributed to the high consumption of acetic and caproic acids, since butyric acid uptake was very low. Regarding the lactic acid, higher concentrations than the amount fed were detected between 48 and 120 h, indicating that some lactic production could have occurred. However, at the end of the fermentation cycle, the concentration of lactic acid had dropped significantly, and so the net consumption was reported (Table 2).

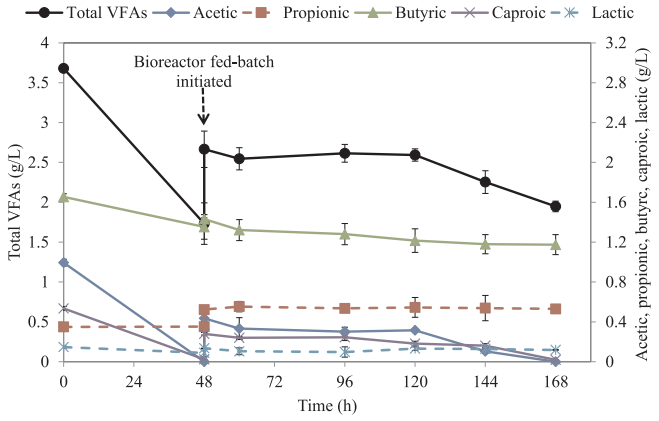

Fig. 4. Consumption trends of total VFAs by *R. oligosporus* in the bubble-column bioreactor. The arrow shows the start of the feeding in the bioreactors.

3.2. Bioreactor cultivation

The use of waste-based VFAs as potential carbon source for fungal cultivation was finally carried out in bubble-column bioreactors. During these scale-up trials, the bioreactors were seeded with pre-grown fungal biomass in 25% diluted VFAs solution. The biomass morphology consisted of a single large clump. As presented in Fig. 4, the VFAs concentration in the bioreactors was maintained at approximately 2.5 g/L during most of the cultivation period. After the 168 h cultivation cycle, 65.39% uptake of the total VFAs was attained. By the end of the cultivation, acetic acid had been totally used up, while up to 97.64% and 26.13% of caproic and butyric acids, respectively, were consumed. Moreover, 38.93% removal of lactic acid was observed. The biomass yield reached 0.15 ± 0.04 g dry biomass/g VFAs $_{\text{COD eq. consumed}}$, with a crude protein content of 34.72 ± 4.41%.

In the bubble-column bioreactor, the dilution rate was reduced with time while the culture volume increased. This strategy guaranteed low enough concentration of the VFAs, a condition needed for fungal biomass growth. The consumption of acetic and caproic acids was principally similar to the previous flask trials. However, there was a significant improvement in the uptake of butyric acid in the bubble-column fed-batch trials compared to the flask trials. One of the possible causes of this improvement could be linked to the enhanced consumption of both acetic and caproic acids, which was facilitated by the low substrate concentration. All in all, based on the conditions applied in this work, it can be concluded that besides the necessary low substrate concentration, applying VFAs rich in acetic and caproic acids but less of butyric acid, for the fungal cultivation possibly enhances biomass production due to the availability of a favorable carbon source for *R. oligosporus*. In this regard, the desired composition of the VFAs solution can be achieved during the initial anaerobic digestion process as demonstrated by Dahiya and Mohan (2019) and Cheah et al. (2019).

The clumped biomass morphology could have been a limiting factor for the oxygen diffusion in the bioreactors. This was evidenced by the lower biomass yield in the bioreactors compared to what was obtained in the flasks. Perhaps the biomass production process can be developed further by promoting the formation of fungal pellets which is poised to improve the bioreactor hydrodynamics as noted by Nair et al. (2016). According to their study, one of the factors that could be manipulated for promoting fungal pellets is the medium pH. On the other hand, further improvements could also be done by modifying the flow rate of VFAs into the reactors or introducing a pulsed feeding mode. It has also been proposed that high aeration could improve acid removal by *R. oligosporus* (Rasmussen et al., 2014). Nevertheless, the low aeration rate (0.5 vvm) applied here was beneficial for controlling excessive reactor foaming.

With the global shortage in sources of animal feed and even human food, the production of single-cell protein is an important research subject (Yunus et al., 2015). Food waste-derived VFAs are inexpensive and highly accessible substrates that could be exploited for the production of protein-rich fungal biomass. The protein content in the biomass from the VFAs-based process was in the same range as that obtained when *R. oligosporus* was grown on some organic residues. For instance, when using vinasse (Nitayavardhana & Khanal, 2010), starch processing wastewater (Jin et al., 2002), and corn thin stillage (Rasmussen et al., 2014) as substrates, the crude protein reported amounted to 45.55, 49.7, and 43%, respectively. Furthermore, the crude protein results compare well with commercial protein sources for animal feed such as soybean and cottonseed meal, which contain approximately 40–49% and 30–50% crude protein, respectively (Banaszkiewicz, 2011; Świątkiewicz et al., 2016). This indicates that with further optimization, waste-based VFAs could be promising candidates for future production of protein-rich edible fungal biomass.

Presently, the main goal of the commercialized anaerobic digestion facilities is the production of biogas, which is utilized as a renewable energy source. The anaerobic digestion process can treat a wide range of organic wastes and its utilization for synthesizing other materials and chemicals is envisioned as an opportunity to recover higher value from wastes than what is currently recovered with only biogas. This would require deviation of the anaerobic digestion process from a biogas platform to a carboxylate platform via acidogenic fermentation using viable systems, such as membrane bioreactors. As observed in our previous research, the membrane bioreactors can be utilized for the production and recovery of VFAs using food waste as substrate, with hydrogen being formed as a by-product (Wainaina et al., 2020). In addition, the composition of VFAs from food waste is highly affected by the process conditions of the acidogenic anaerobic digestion (Lukitawesa et al., 2020), and therefore it would be possible to direct the process to produce more VFAs that would benefit the subsequent fungal production. The new potential application of the VFAs entailing the cultivation of edible fungi has been demonstrated in this paper. The proposed integrated process fits well within the circular economy concept, whose main objectives are the minimization of wastes while enabling economic advancement by supplying essential materials and chemicals without excessive pressure on natural resources (Gregson et al., 2015). With further improvements in the VFAs uptake, biomass productivity and biomass quality, this new concept would be important in meeting the growing demand for single-cell protein for possible use as animal feed. In view of the low VFAs concentrations required for the fungi cultivation, some of the VFAs can also be diverted to other bioprocesses such as biosynthesis of bioplastics (Mohan et al., 2010) or production of biodiesel (Béligon et al., 2016), possibly without any extra purification. The proposed scenario has the possibility of converting anaerobic digestion plants into biorefineries (Fig. 5), thus supplying materials with higher market value than biogas.

4. Conclusions

This paper presented a novel integrated process in which food waste-derived VFAs were applied as the sole carbon source to cultivate a food-grade filamentous fungus, *R. oligosporus*. The consumption of the acids by the fungus depended on the type of acid, with preference to acetic acid, and the substrate concentration. Fed-batch cultivation mode was successfully applied to maintain the required low substrate concentration which improved biomass production. Maximum biomass yield of 0.21 ± 0.01 g dry biomass/g VFAs $_{\text{COD eq. consumed}}$ was reached, with a promising crude protein content of 39.28 ± 1.54% being reported.

E-supplementary data for this work can be found in e-version of this paper online.

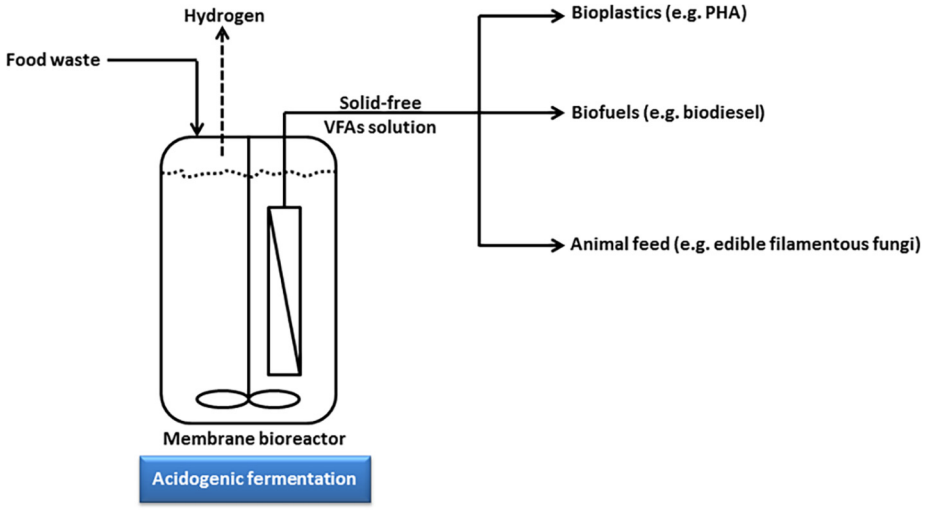

Fig. 5. Schematic of the proposed biorefinery approach for modified anaerobic digestion (acidogenic fermentation) aiming at production, recovery and conversion of VFAs into edible filamentous fungi and other bioproducts.

CRediT authorship contribution statement

Steven Wainaina: Methodology, Data curation, Validation, Writing - original draft, Writing - review & editing. **Afrilia Dwi Kisworini:** Methodology, Investigation. **Marizal Fanani:** Methodology, Investigation. **Rachma Wikandari:** Supervision, Writing - review & editing. **Ria Millati:** Supervision, Writing - review & editing. **Claes Niklasson:** Supervision, Writing - review & editing. **Mohammad J. Taherzadeh:** Conceptualization, Funding acquisition, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2020.123444.

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