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A promising conceptual alternative approach for conventional sewage treatment

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Review

Integrated anaerobic and algal bioreactors: A promising conceptual alternative approach for conventional sewage treatment

Jixiang Yang [a], Jules B. van Lier [b,*], Jian Li [c], Jinsong Guo [d], Fang Fang [d]

[a] Chongqing Institute of Green and Intelligent Technology, Chinese Academy of Sciences, Chongqing 401174, China
[b] Faculty of Civil Engineering and Geosciences, Department of Water Management, Section of Sanitary Engineering, Delft University of Technology, Delft, The Netherlands
[c] School of Biological and Chemical Engineering, Panzhihua University, Panzhihua, China
[d] College of Environment and Ecology, Chongqing University, Chongqing, China

HIGHLIGHTS

- A novel bioprocess is proposed for municipal wastewater treatment.
- Sewage treatment can be with positive financial output.
- Nutrients can be effectively recovered.
- Recovering methane from effluent of AnMBR is important and feasible.
- Much less excess sludge production can be achieved.

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ABSTRACT

Conventional sewage treatment applying activated sludge processes is energy-intensive and requires great financial input, hampering widespread implementation. The introduction of anaerobic membrane bioreactors (AnMBR) followed by an algal reactor growing species of commercial interest, may present an alternative, contributing to the envisaged resource recovery at sewage treatment plants. AnMBRs can be applied for organic matter removal with energy self-sufficiency, provided that effective membrane fouling management is applied. *Haematococcus pluvialis*, an algal species with commercial value, can be selected for ammonium and phosphate removal. Theoretical analysis showed that good pollutant removal, positive financial output, as well as a significant reduction in the amount of hazardous activated sludge can be achieved by applying the proposed process, showing interesting advantages over current sewage treatment processes. Microbial contamination to *H. pluvialis* is a challenge, and technologies for preventing the contamination during continuous sewage treatment need to be applied.

1. Introduction

Human activities produce a large amount of sewage. Heterotrophic bacteria, nitrifying bacteria and phosphate-accumulating bacteria are involved in sewage treatment and all require oxygen for their metabolism. Oxygen supply in sewage treatment plants (STPs) is achieved either by applying compressors connected to submerged aeration systems or surface aerators. In countries like the USA, approximately, 3% of annual electricity is required for wastewater treatment (Hao et al., 2015). Scientists have worked hard to reduce the energy requirement. Among the novel technologies, aerobic granular sludge for simultaneous nitrogen and phosphorus removal, and anammox for autotrophic nitrogen removal from concentrated sludge reject water streams, are successfully applied, and indeed use distinctively less energy (Baeten et al., 2019, de Kreuk et al., 2005, Fang et al., 2020, Morgenroth et al., 1997, Nancharaiah et al., 2019, Pronk et al., 2015). Despite the achieved advancements, stoichiometries of current bioprocesses determine that oxygen is always required and energy requirement originating from oxygen supply remains a condition for sewage treatment (Siegrist et al., 2001). Therefore, developing oxygen-free technologies would solve the high energy demand of current sewage treatment bioprocesses.

Organic matter, ammonium and phosphate in soluble and particulate

* Corresponding author.
E-mail address: J.B.vanLier@tudelft.nl (J.B. van Lier).

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forms are major pollutants in sewage. Anaerobic bacteria can convert organic matter into biogas that can be converted into electricity. When an anaerobic bioreactor, which contains anaerobic microorganisms, is coupled to membrane filtration, the obtained anaerobic reactor is termed anaerobic membrane bioreactor (AnMBR) (Fig. 1) (Yang et al., 2017, Yang et al., 2013). Comparing with conventional anaerobic digesters, the membrane module of an AnMBR can effectively retain particles and ensure high sludge concentration in the reactor even under harsh conditions such as high salty conditions that do not ensure anaerobic sludge granulation. AnMBRs can remove approximately 90% of organic matter from sewage, turning the organic matter into biogas (Chen et al., 2021, Yang et al., 2017). Kim et al. (2011) reported that a lab-scale AnMBR can effectively degrade organic matters in sewage with positive energy output. Nevertheless, recent pilot experiments often show negative results (Shin and Bae, 2018).

When organic matter is removed by an AnMBR, the removal of ammonium and phosphate requires a downstream process. Chemical precipitate formation, such as controlled struvite formation, is considered not applicable, as ammonium and phosphate concentrations in sewage are too low for efficient removal. Also, ion exchange and electrodialysis are likely not feasible for treating the large sewage flows with low concentrations of nutrients (Lee et al., 2015). Furthermore, the combination of AnMBR and reverse osmosis for domestic wastewater treatment requires energy input as high as 3–6 kWh/m^3 (Grundestarn and Hellstrom, 2007, Zgavarogea et al., 2017), which is much higher than the current energy demand of conventional sewage treatment (0.3–0.6 kWh/m^3). Therefore, applying membrane processes for nutrient removal is generally regarded as too expensive (Grundestarn and Hellstrom, 2007, Nguyen et al., 2020, Vinardell et al., 2020a). Moreover, applying anammox to polish the effluent of an AnMBR for mainstream treatment is hard to achieve, because the psychrophilic conditions in sewage restrict mainstream stable nitritation and subsequent anaerobic denitrification by anammox bacteria (Vinardell et al., 2021, Wang et al., 2020).

In contrast to the physicochemical approaches, algae can simultaneously metabolize ammonium and phosphate in new algae biomass, and increased pH values resulting from photosynthesis may lead to phosphate precipitation and volatilization of ammonia (Cavalcanti et al., 2002). These features make algae a good candidate for the treatment of AnMBR effluents. Various researchers investigated the feasibility of applying algal reactors to polish effluent of AnMBRs treating sewage (Gonzalez-Camejo et al., 2020a, Gonzalez-Camejo et al., 2020b, Gonzalez-Camejo et al., 2018, González-Camejo et al., 2020, Paches et al., 2020, Seco et al., 2018). Results showed that the effluent of the algal reactors satisfied local discharge requirements, while the energy recovery was 0.433 kWh/m^3, applying anaerobic digestion of the harvested algae biomass that accounted for 95% of *Chlorella sp* (Gao et al., 2021, González-Camejo et al., 2019). However, illumination was applied to promote algal growth and the corresponding operation cost of the algal reactors is not clear. Furthermore, ammonium and phosphate likely were released during the anaerobic digestion process and additional process measures should be incorporated to remove the released ammonium and phosphate.

When the nutrients are incorporated in the algal biomass that afterward leaves the sewage treatment process as a commercial product, the overall process consisting of an AnMBR and an algal reactor will be simplified and likely economically more feasible. The latter is attributable to the fact that no additional process is required for the released ammonium and phosphate, while there are no disposal costs for the produced algae. In addition, the possible commercial products may compensate for the operational cost. Currently, algae are applied to polish effluents of secondary clarifiers or are combined with heterotrophic bacteria to treat sewage (Yang et al., 2018a). Nevertheless, to the author's knowledge, none of the algae-based reactors are operated using sewage as a nutrient source and recovering the obtained algae for commercial purposes.

In this study, an AnMBR is proposed for organic matter removal, which is followed by a suitable algal reactor for nutrient removal, in order to achieve cost-effective sewage treatment with possible financial benefit. Although a large number of papers on lab-scale AnMBRs have been published, this paper only includes results of pilot-scale AnMBR for discussing solutions for achieving energy self-sufficiency for an AnMBR.

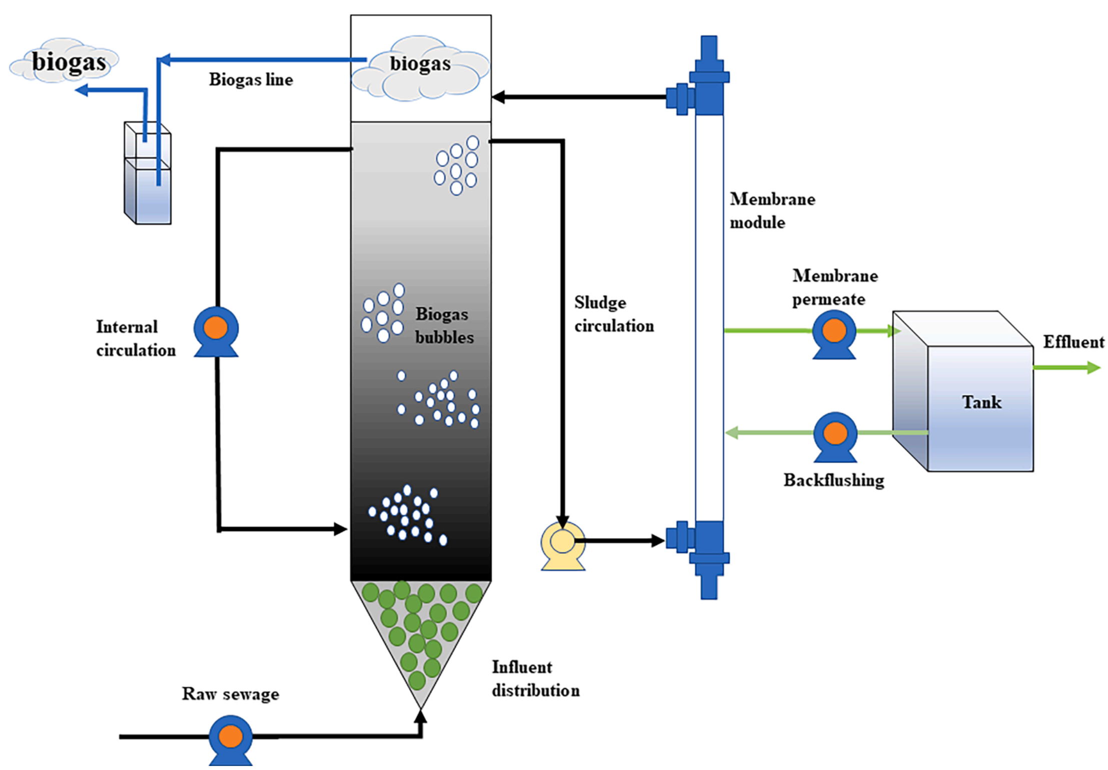

Fig. 1. Schematic view of an anaerobic membrane bioreactor.

In addition, nutrient removal by an algal bioreactor was analyzed. Finally, the challenges of the proposed integrated anaerobic and algal processes are discussed.

2. Improving energy output of AnMBR for sewage treatment

In an anaerobic bioreactor, organic matter is eventually converted into CO_2 and CH_4 following the anaerobic digestion process, while minimum anaerobic biomass is produced (Van Lier et al., 2020). Most CO_2 and CH_4 are released from the water into the gas phase, while a part of the CO_2 / CH_4 mixture is solubilized in the effluent. Since the solubility of CO_2 is higher than CH_4, a larger fraction of the produced CO_2 leaves the reactor with the effluent. Therefore, when treating high flows of dilute wastewater, such as municipal sewage, the CH_4 content of the biogas may reach values as high as 85% or higher (Chen et al., 2021, Kong et al., 2021b). The recovered gas mixture can be used for electricity production. In addition to the solubilized gases, non-converted intermediate products are removed with the effluent.

Kim et al. (2011) showed that a lab-scale AnMBR can efficiently remove organic matter from sewage with positive energy output. Recently, pilot-scale experiments were conducted to verify the feasibility of applying AnMBRs for recovering the biochemical energy enclosed in organic matter (Chen et al., 2021, Evans et al., 2019, Robles et al., 2020, Vinardell et al., 2020b). Results showed that not all pilot-scale AnMBR achieved energy self-sufficiency, which was attributed to the high operational energy requirement (Shin and Bae, 2018).

Organic matter is commonly measured and expressed as chemical oxygen demand (COD). When the methane is completely utilized for electricity production, the relationship between energy output and influent COD concentration is calculated using Eq. (1). Reactor operational energy demand is usually 0.2 kWh/m^3 but occasionally up to 0.4 kWh/m^3 (Shin and Bae, 2018). For compensating this demand and reaching a net energy output, a minimum influent COD concentration of about 400 mg/L is required, which increases to 680 mg/L for the highest energy requirement. Calculations are based on Eq. (1) and a COD removal efficiency of 60%.

$$E_{out}\left(kWh/m^3\right) = \left(S \times h_{tr} - P_{CH4} \times MW/R.T \times K_H \times 4 / 1000\ g/kg \right) \times F_{CH4} \times h_{ce} \times E_{CH4}/ 3.6\ kWh/MJ \tag{1}$$

in which,

S = Influent COD (kg/m^3);

η_{tr} = treatment efficiency in %/100 (-);

P_{CH4} = partial CH4 pressure gas phase (% CH_4 biogas/100 × $P_{ambient}$ (Pa)), with $P_{ambient}$ = 101,325 Pa at sea level;

MW = molecular weight (kg/mole), for CH_4: 16;

R = universal gas constant (8.314 J/K/mole);

T = temperature (K);

K_H = Henry's partitioning constant (-); at 293 K: 0.035;

4 = conversion factor g CH_4-COD/g CH_4;

F_{CH4} = conversion factor m^3 CH_4/kg CH_4-COD = 0.35 at standard temperature and pressure (1 bar, 273 K);

η_{ce} = conversion efficiency electricity production in %/100 (-): biogas motor: 0.35;

E_{CH4} = energy value at standard temperature and pressure of 1 m^3 wet methane (low heating value) = 35.5 MJ/m^3.

Particularly for municipal sewage, which can be characterized as low-temperature dilute wastewater, a substantial part of the produced CH_4 is solubilized in the effluent. Assuming 60% removal efficiency and a temp of 20 °C, about 130–135 mg/L of the influent COD is solubilized as CH_4-COD in the effluent (Eq.1). With an influent COD concentration of only 250 mg/L, often regarded as a median concentration for Chinese conditions (Sun et al., 2016), over 50% of the produced CH_4 leaves the anaerobic reactor with the effluent. The latter indicates that recovering the dissolved methane in the effluent is important for countries such as China. The energy cost for recovering the dissolved methane is distinctly lower than the energy potential of the dissolved methane, which indicates that recovering methane from the effluent of AnMBRs indeed might be of interest (Crone et al., 2016, Kong et al., 2021a, Maaz et al., 2019, Shin et al., 2014, Wu and Kim, 2020). In addition, Cookney et al. (2016) showed that the revenue generated from the recovered methane is sufficient to offset the operational and investment costs of a single-stage recovery process. In this study, calculations show that approximately 2.67 years are required to compensate for the cost of a degassing membrane. In these calculations, a Liqui-Cel® (14x28 series) degassing membrane module was applied. The size of a membrane module is 297.4 mm in diameter and 1186 mm in height, and the price of a membrane module is US $20,000.The module is applied to treat an effluent flow rate of 90.8 m^3/h with a dissolved methane concentration of 100 mg COD/L and a corresponding dissolved methane recovery efficiency of 80%. If the methane recovery efficiency is 90% and 100%, the payback period is 2.35 and 2.09 years, respectively. During the payback period calculation, membrane cost and membrane operation cost are included. Apparently, recovering dissolved methane is feasible. Nonetheless, considering the low sewage COD concentrations, energy self-sufficiency is hard to achieve in China.

Apparently, it is important to reduce AnMBR operation cost for achieving energy self-sufficiency, which can be attained by reducing membrane filtration cost. When gas sparging is applied to reduce membrane fouling, the membrane filtration costs are calculated with Eqs. (2) and (3) (Shin and Bae, 2018). The equations show that reducing gas flow on membrane surface and increasing membrane flux are instrumental for reducing membrane filtration cost (Maaz et al., 2019, Shin and Bae, 2018). According to Eqs. (2) and (3), an increase in membrane flux from the current 10 L/m^2.h to 20 L/m^2.h can reduce the current operation cost for AnMBRs from 0.2 to 0.1 kWh/m^3, for which a minimum influent COD concentration of 270 mg/L suffices for reaching energy neutrality (Eq. (1), assuming 60% removal efficiency and 20 °C and no effluent solubilized CH_4 recovery). Full-scale AnMBRs apply gas sparging flat sheet membranes or crossflow membranes without gas sparging but high crossflow velocities (0.5–1 m/s) (Christian et al., 2011). As for the cross-flow membranes without gas sparging, a membrane flux between 20 and 30 L/m^2.h likely would suffice, as a higher membrane flux does not significantly reduce membrane operation costs (Yang et al., 2017).

$$E_b = \frac{P_b}{J} \times 1000 \tag{2}$$

$$P_b = k \times SGD_m \times (1/3600) \tag{3}$$

Where:

E_b: blower energy requirement (kWh) per treated water, kWh/m^3;

P_b: blower power per membrane surface area, kW/m^2;

J: flux of AnMBR, L/m^2.h;

SGD_m: specific gas demand, calculated by dividing the biogas flow rate by total membrane surface area, m^3 /m^2.h;

k: coefficient, kW.h/m^3.

Among the various approaches for increasing the membrane flux in AnMBRs, dosing flocculants is the most effective one (Charfi et al., 2018, Dong et al., 2015, 2016, Lee et al., 2016, Liu et al., 2018, Yang et al., 2019, Zhang et al., 2017). It is reported that membrane flux can be maintained over 40 $L/m^2.h$ with minimum membrane fouling development in 20 days, indicating that operating membrane at a flux between 20 and 30 $L/m^2.h$ might be feasible (Yang et al., 2020). Few reports show that the addition of flocculants sometimes did not achieve positive effects in membrane fouling control (Kooijman et al., 2017). Possibly, this can be attributed to the kind of flocculant type applied or non-optimized mixing conditions.

Membrane configuration and membrane type play an important role in assessing the effects of gas sparging. A hollow fiber membrane module requires much less energy for gas circulation than a flat sheet membrane module (Shin and Bae, 2018). Moreover, dynamic membrane filtration can significantly reduce filtration resistance and enable higher membrane flux at the same transmembrane pressure, compared with other membrane modules (Hu et al., 2018, Xiong et al., 2019). A dynamic filtration-based AnMBR, which did not need gas flushing on the membrane surface, was developed. The AnMBR showed a great advantage in reducing membrane operation costs (Yang et al., 2017). In addition, Shoener et al. (2016) reported that submerged hollow fiber membrane requires the least energy compared to cross-flow multi-tube, submerged flat sheet and crossflow flat sheet membranes.

As above illustrated, a low influent COD concentration will challenge a positive energy output for an AnMBR. A low COD concentration may result from combined sewerage, also accepting stormwater, or from groundwater infiltration resulting from malfunctioning of sewage collecting pipelines. Therefore, optimized membrane fouling control is the most important research topic for enabling energy-positive output in an AnMBRs.

3. Stability of AnMBR

Long-term reactor stability is important to ensure a good and stable effluent quality. It is important to ensure the reactor performance when sewage temperature fluctuates significantly because temperature control in anaerobic sewage treatment reactors is not possible. Chen et al. (2021) showed that when sewage temperature fluctuated between 5 °C and 35 °C and influent COD concentrations were between 277 and 348 mg/L, the effluent COD concentration was consistently below 50 mg/L, showing the stability of an AnMBR year-round. Lab-based results were supported by an independent pilot experiment (Mei et al., 2017). The reactor stability may be attributed to the fact that changes in operational and environmental conditions did not greatly alter the core bacterial population (Damodara Kannan et al., 2020, Ji et al., 2021). Their results showed that temperature variation increased the relative abundance of archaea and the amount of carbohydrate-protein degrading bacteria but did not significantly affect other bacteria such as sulfate reducing bacteria. Apparently, the microbial communities present in the influent wastewater did not affect the AnMBR core microbiome. Higher influent COD concentrations resulted in slightly higher effluent COD concentrations (Martinez-Sosa et al., 2011, Robles et al., 2020). Nevertheless, applying two anaerobic bioreactors for sequential removal of organic matter can effectively improve reactor performance and contribute to reactor stability at temperatures below 15 °C (Shin et al., 2014, Watanabe et al., 2017).

4. Downstream treatment of effluent of AnMBR with algae

AnMBR effluents potentially can be applied for agriculture purposes (Peña et al., 2019), e.g. when farmland is available in the vicinity of the treatment plant. However, when nearby farmlands are not available, downstream treatment of the effluent of AnMBRs is necessary.

AnMBRs will mineralize organic matter in municipal sewage, leading to a slight increase in ammonium and phosphate concentrations in the effluent. However, algae can metabolize nutrients via anabolism into new cell biomass, utilizing the bicarbonate alkalinity in the effluent of AnMBRs as C source and sunlight as an energy source. Although algae are considered autotrophs, Yu et al. (2015)showed that acetate in the effluent of AnMBRs can stimulate the growth of algae. Therefore, the composition of AnMBR effluent may enhance specific algal growth.

The downstream treatment of the effluent of AnMBRs by algae can be performed at low energy consumption. However, for separating algae from water, centrifuges are usually applied, since conventional settling tanks are not very effective. Yang et al. (2018a) recently showed that continuous illumination was more efficient in wastewater treatment than applying dark-light cycles, showing that continuous illumination can significantly reduce the footprints for the algal reactor. However, artificial illumination would result in additional operation cost. Nonetheless, when algae with commercial value would be applied in sewage treatment, then they could compensate for the additional operating cost of algal reactors. There are over 30 thousand kinds of algal species in the world (Guiry, 2012). Among the algal species, *Spirulina sp, Dunaliella salina* and *Chlorella sp* and *Haematococcus pluvialis* can be cultured in open ponds. *Spirulina sp* and *Dunaliella salina* require pH higher than 7 and high salinity, respectively, to establish a competitive advantage over other algae respectively (Hudek et al., 2014). Therefore, they are less appropriate for sewage treatment. Here, the economics of using *H. pluvialis* for the treatment of the effluent of AnMBRs is discussed below.

A novel AnMBR-algal sewage treatment process is proposed in this study for the first time and is shown in Figs. 2 and 3. The produced CO_2 and HCO_3^- together with ammonium and phosphate in the effluent of the AnMBRs will be conveyed to an algal reactor. Fig. 3 shows a sequential batch reactor for algae production, of which multiple reactors can be applied for continuous treatment of the effluent of AnMBRs. The algal reactor is located in a closed greenhouse, in order to prevent microbial contamination. However, the emission of methane from the effluent of the upstream AnMBR and the afterward accumulation in the greenhouse would be dangerous. This indicates that recovering methane from the effluent of the AnMBR is important not only for energy recovery but also for operational procedures.

The required energy for membrane filtration in the algal reactor only accounts for a little part of the algal reactor operation cost. Then, the algae are enriched and ready to be transported to a raceway pond for astaxanthin production. In the raceway pond, a high light intensity or a lack of nutrients can promote the formation of astaxanthin (Harker et al., 1996). As nutrients in the algal reactor have been consumed by *H. pluvialis* for growth, the nutrient concentration in the raceway pond is low, which is beneficial for the formation of astaxanthin. When astaxanthin is formed, the algae can be collected and dried. The dried algal biomass is a product ready for sale. The actual price reach values between \$2500–7000 /kg, depending on the astaxanthin content (LeFeuvre et al., 2020a, Panis and Carreon, 2016).

Results of a successful long-term pilot experiment showed that large-scale production of astaxanthin production by the cultivation of *H. pluvialis* can be as low as \$718 /kg (Li et al., 2011). By using sewage as a nutrient source, it is estimated that the production cost can be further reduced to \$626 /kg. The gross benefit by recovering astaxanthin from sewage would be \$11.66 per m^3 influent. Consider this as a product, then sewage treatment could become beneficial, rather than an energy-intensive add-on technology.

During photosynthesis, oxygen is produced, which can increase to levels exceeding 10 mg/L if the oxygen concentrations are not well managed. It is reported that the oxygen concentration should be maintained between 3 mg/L and 5 mg/L for the growth of *H. pluvialis* (Wei, 2006). Similar to strategies for recovering methane from AnMBR effluents, oxygen can also be removed from water by commercial degassing membranes (Crone et al., 2016, Henares et al., 2017, Li et al., 2015).

5. Mass balance in the proposed process

The mass balance in the proposed process is calculated and results

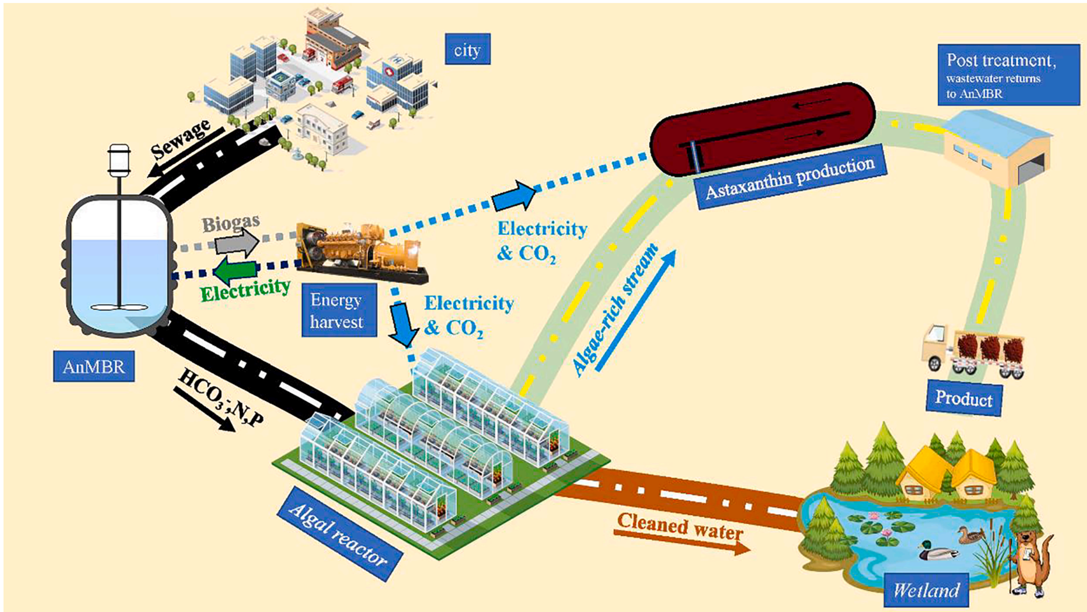

Fig. 2. The proposed sewage treatment process. Sewage from a city is polished with an AnMBR and algal reactor. The effluent of the algal reactor is with low organic, ammonium and phosphate concentrations. A disinfection process may be required in front of or after a wetland, depending on local requirements. The algae-rich stream is rich in green-phase *H. pluvialis* that are transported into a pond where they will become red and then be dried. The AnMBR provides CO_2 and electricity to other units. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

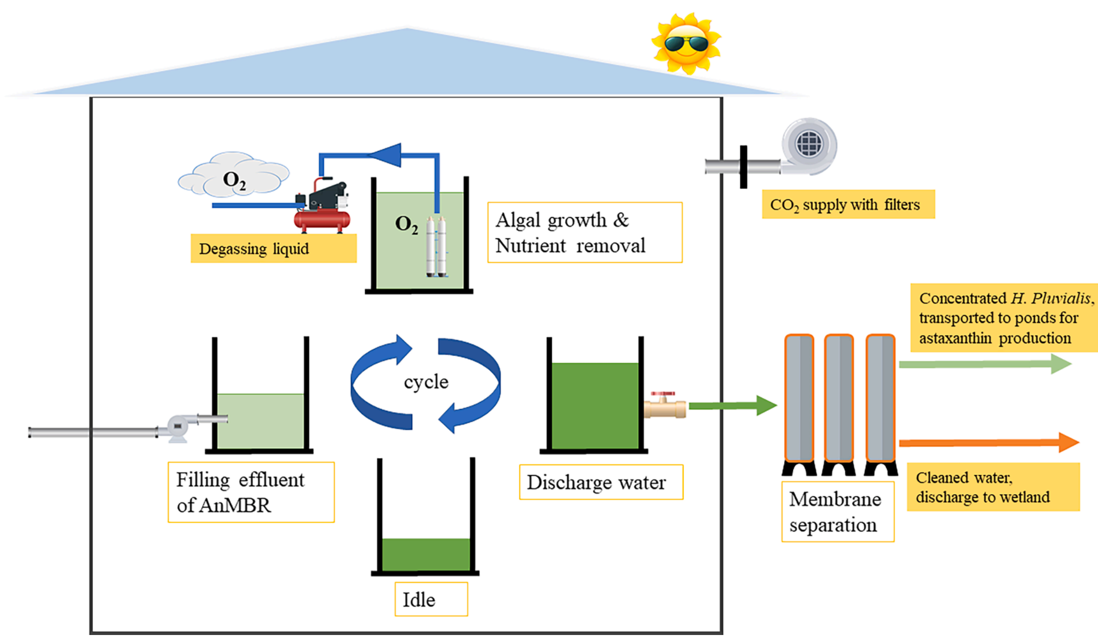

Fig. 3. Continuous treatment of the effluent of AnMBR with *H. pluvialis*. The algal reactor is operated in a sequential batch mode. Oxygen is removed via degassing membrane for ensuring algal activity. Algae in the effluent of the algal reactor are separated with a membrane module in an independent tank (not shown). External CO_2 should be filtrated before getting into the greenhouse.

concerning nutrients, alkalinity and algal biomass are shown in Table 1, the calculation example was based on experimental results (Chen et al., 2021, Zhang et al., 1999). In these calculations, pre-settled sewage was used as an influent to an AnMBR that was followed by an algal reactor in which *H. pluvialis* were cultured. The characteristics of the pre-settled sewage were: 250 mg/L total COD, 40 mg/L Kjeldahl nitrogen and 4.7 mg/L total phosphate. No nitrate and nitrite were in the influent. In case some nitrate and nitrite would be present, it will be denitrified in the AnMBR and will not affect the operation of a subsequent algal reactor. In addition, alkalinity in the influent was 150 mg /L as $CaCO_3$, equal to 91.5 mg HCO_3^-/L.

By extracting solubilized CH_4 from the effluent of the AnMBR, mass balance calculations using data of Chen et al. (2021) showed that the effluent COD concentration in the AnMBR was below 50 mg/L, indicating a reliable removal of organic matter, even in winter. Chen et al. (2021) reported that the COD removal efficiency was 89.3%±1.8% in summer and was 86.6%±2% in winter. The COD removal efficiencies were similar but dissolved methane contributed to approximately 30% of COD in the effluent in summer and 40% in winter. The solubility of methane in water is highly affected by temperature, as the solubility

Table 1
Mass balance regarding nutrient, alkalinity and algal biomass, mg/L.

	Kjeldahl Nitrogen	P total	Alkalinity	Algal biomass
Influent of AnMBR	40.0 ± 5.0	4.7 ± 0.5	91.5	0
AnMBR	−2.0	−0.2	+29.4	0
Algal reactor	−32.4 ± 3.0	−4.5 ± 0.4	−136.2 ± 12.2	+249.0 ± 22.2
Effluent of the process	10.7 ± 4.0	0 ± 0	−15.8 ± 12.2	249.0 ± 22.2

Alkalinity: mg HCO_3^- /L. Algae: *H. pluvialis.* +: means production; -: means consumption, or minus for effluent. In order to simplify the calculation, influent alkalinity and AnMBR performance are stable. Fluctuations in influent Kjeldahl Nitrogen and P concentrations result in the fluctuation in algal reactor performance. N and P are metabolized, despite alkalinity is negative, in order to show the deficiency of alkalinity. Consumption of N and P in the AnMBR results from anabolism. The characteristics of the AnMBR influent is typical for China.

decreases from approximately 0.04 g CH_4/L at 0 °C to 0.018 g CH_4/L at 30 °C. This contributed to a better effluent quality in summer.

Whether or not complete removal of ammonium and phosphate can be achieved from the effluent of an AnMBR by an algal reactor depends on the influent N/P ratio and the ratio of N/P metabolized by the algae. The latter ratio is more or less constant. The mass balance calculation shows that simultaneous removal of ammonium and phosphate can be achieved by the proposed concept shown in Fig. 2. Similarly, a good simultaneous removal of nutrients by an algal reactor, in which green algae dominated, following an AnMBR was achieved in Spain (Gonzalez-Camejo et al., 2020a). However, when the N/P ratio for the effluent of an AnMBR deviates significantly from the ratio of N/P metabolized by the algae, a wetland may be required for the treatment of the effluent of the algal reactor for ammonium or phosphate removal. A disinfection process may be applied in front of or after the wetland. This depends on local requirements.

Table 1 shows that the alkalinity in the influent and produced by the AnMBR is not sufficient to sustain algal growth. Therefore, an external CO_2 supply is necessary to maintain the pH in the algal reactor, which is supported by pilot-scale experiments (Gonzalez-Camejo et al., 2020a). This can be done by using produced CO_2 in the biogas from the upstream AnMBR and CO_2 from flue gas.

Heterotrophic bacteria may convert up to 66% of the organic matter into new biomass (Gujer et al., 1999), which is called waste activated sludge (WAS). A growing large amount of WAS is being produced every day in current sewage treatment. For instance, in China, 6.3 million tons of dry WAS were produced in a year (Yang et al., 2015). WAS is a hazardous material and must be disposed of by landfill or incineration etc. If incineration is applied to treat sludge that is dehydrated by mechanical approaches, the incineration cost is approximately ¥400-500/t ($57–71/t) in China. Apparently, in addition to energy for aeration, the disposal of activated sludge is a big financial burden. Using settled raw sewage as an influent, the bioprocess shown in Fig. 2 only produces a minimum amount of excess sludge, as only 10% of the organic matter is converted into anaerobic biomass. Therefore, there is no need for applying anaerobic digestion to reduce the amount of excess sludge. Both secondary sludge and the sludge from a primary settler could be collected and disposed by landfill or incineration after dewatering.

Worldwide, sewage treatment plants are aiming to reduce fossil fuel consumption and eventually reach energy self-sufficiency. Literature shows that at present only a few activated sludge-based sewage treatment plants have achieved energy self-sufficiency (Hao et al., 2015, Park and Craggs, 2011). However, in these sewage treatment plants, either hydraulic energy, solar panels and external organic waste are applied for supplying the required energy. Interestingly, under warm climate conditions, energy self-sufficiency is commonly reached, applying anaerobic sewage treatment using up-flow anaerobic sludge bed reactor technology (Chernicharo et al., 2019, Van Lier et al., 2020). However, in temperate climate zones, the conventional anaerobic sewage treatment concept is not applicable. It should be noted that the proposed concept shown in Fig. 2 is not energy self-sufficient, as artificial illumination, e.g., 45 w/m^2, should be applied, which requires an external electricity supply. Nevertheless, the AnMBR followed by the algal process provides a positive financial output.

Many studies applied mixtures of algae collected from the walls of secondary clarifiers to polish effluent of pilot-scale AnMBRs treating real sewage (Gonzalez-Camejo et al., 2020a, González-Camejo et al., 2019). In these studies, *Scenedesmus obliquus* or *Chlorella* gradually dominated in algal reactors. The obtained algae are considered good raw material for energy production. For instance, extensive studies worked on biogas production via anaerobic digestion and extracting bioethanol or biodiesel from algae (González-Camejo et al., 2019, Ward et al., 2014, Yang et al., 2018b). During the bioethanol or biodiesel production process, nutrients are released, indicating that a subsequent treatment step is required (Fernandez et al., 2018, Kimura et al., 2019). In contrast to *Scenedesmus obliquus* or *Chlorella.sp, H. pluvialis* is a good alternative. As shown in Fig. 2, nutrients in sewage are finally embedded in dry *H. pluvialis* powder that is a commercial product already. The benefit of the here proposed process is that astaxanthin is produced in a side stream rather than in the mainstream. This enables a high degree of operational freedom in controlling the algal tank for nutrient removal and downstream astaxanthin production (Figs. 2 and 3). In the mainstream algal reactor, *H. pluvialis* are cultured for nutrient removal. Part of *H. pluvialis* in the mainstream algal reactor is discharged and separated from the water and transported to raceway ponds. In the raceway ponds, environmental conditions are manipulated to turn the discharged *H. pluvialis* into the red phase for astaxanthin production (Fig. 3).

6. Challenges and research needs

Chen et al. (2018) reported that integrating anaerobic digestion with algal technologies is challenged by high operational costs of algal cultivation and the required sterilization of the effluent of anaerobic reactors. However, the operational cost of algal cultivation can be completely covered by recovering products from *H. pluvialis*. Therefore, the major problem of applying *H. pluvialis* in sewage treatment is microbial contamination. The approaches for preventing the contamination are discussed below.

Physical approaches can be applied to prevent contamination. In AnMBRs, ultrafiltration membranes with a nominal pore size of 0.1 μm are usually applied. Therefore, it is expected that ultrafiltration membranes can sufficiently minimize the bacterial counts in the AnMBR effluent. Li et al. (2011) proved that microfiltration membranes already suffice for this purpose. In addition, as shown in Figs. 2 and 3, a greenhouse is applied for maintaining controlled conditions. Gas exchange between the greenhouse and the external environment would be achieved through applying high-grade filters, which further prevents microbial contamination from the external environment.

Applying a suitable environment that enables the growth of *H. pluvialis* but kills other algal species would be a feasible approach. Tharek et al. (2020) showed that *H. pluvialis* can be replaced by fast-growing algae such as *S. obliquus*, although no clear explanation for the observed phenomenon was provided. Thus far, a thorough study on the growth kinetics of *H. pluvialis* and *S. obliquus* is not available. However, microbial kinetics studies showed that light intensity, ammonium and phosphate concentrations do not provide any evidence for the observed replacement (Kaewpintong et al., 2007, Solimeno et al., 2015, Zhang et al., 1999). In addition to light intensity and nutrient conditions, alkalinity and temperature are also important factors affecting algal growth, possibly playing a role in the observed growth advantages of *S. obliquus* over *H. pluvialis*.

Instead of killing other algal species, when *H. pluvialis* grow at a higher rate than those of other fast-growing algae such as *S. obliquus* in sewage, the microbial contamination problem should not be of any concern. Techniques should be applied for increasing the growth rate of *H. pluvialis* at the green stage in an open pond. It is known that many chemicals such as Fe^{2+}, gibberellic acid, salicylic acid can improve the growth of *H. pluvialis* (Yu et al., 2015). Moreover, genetic engineering can also be applied to change metabolic pathways in *H. pluvialis,* which aims at improving the growth rate of *H. pluvialis* (Le-Feuvre et al., 2020b, Li et al., 2020, Shah et al., 2016). However, whether adding the chemicals can inhibit microbial contamination still need to be verified.

Despite the microbial contamination, an open pond instead of a photobioreactor should be applied. In addition to other approaches for microbial contamination control such as applying surfactants for inhibiting fungal parasites (Ding et al., 2020), more algal photoreactors than open ponds are applied for the growth of green *H. pluvialis* (Le-Feuvre et al., 2020a). Photobioreactors can be applied for *H. pluvialis* growth at the green stage, while open ponds can be applied for *H. pluvialis* growth at the red stage (Choi et al., 2017, Panis and Carreon, 2016). In this way, no potential hazardous microorganism in the air can contaminate the *H. pluvialis* in closed photoreactors. However, applying photobioreactors is a financial constraint. Sewage usually comes with a large volume, showing that using current photobioreactors for *H. pluvialis* growth for sewage treatment is not a good idea. Fortunately, the full-scale culture of *H. pluvialis* in raceway ponds in a greenhouse has been applied in Chile (Le-Feuvre et al., 2020a), supporting the proposed process shown in Figs. 2 and 3. Therefore, applying *H. pluvialis* for continuous wastewater treatment is a promising technology, but has not been reported so far, indicating that further research is required.

When the proposed process can be successfully operated, it should be noted that the application of the process is restricted. Two reasons are listed below.

Firstly, the optimum growth temperature for *H. pluvialis* is between 20 and 28 °C (Fan et al., 1994, Giannelli et al., 2015). As controlling wastewater temperature is not feasible, the proposed process will not be applied worldwide but restricted to warm climate regions such as Singapore, Malaysia and Hainan Province in China where sewage is characterized by temperatures of about 30 °C. For instance, the sewage treatment plant in Changi, Singapore, maintains a temperature between 27 °C and 32 °C year round, indicating that culturing *H. pluvialis* in this sewage is possible. Therefore, it can be expected that the production of astaxanthin by the here proposed process will not significantly affect the high price of astaxanthin, which is the key to the success of the proposed process.

Secondly, the availability of land would restrict the application of the process. The biokinetics for *H.Pluvialis* has not been clarified and should be obtained to determine the actual hydraulic retention time (HRT) for the algal reactor. If the HRT is high, a multilayer reactor could be applied for saving the required footprint. For instance, a two-layer reactor can save a footprint by 50%, comparing with a single-layer reactor. This could be done because algal ponds usually are shallow, which makes stacking algal ponds possible. Alternatively, the process can be applied in districts where the cost of land is cheap.

Although using wastewater to culture *H. pluvialis* is much more complicated than current commercial cultivation using a defined substrate, using sewage as a substrate for growth is important for a sustainable development. This is because the P rock reserve is believed to be depleted in 50 to 150 years (Slocombe et al., 2020). Therefore, recovering nutrients from wastewater, rather than just removing them, is important, which will be supported by the here proposed concept. It is believed that the proposed process is a promising alternative to current activated sludge-based sewage treatment approaches, a proof-of-concept study can be done to verify its technical and economical feasibilities.

7. Outlook

Current biotechnologies for sewage treatment demand high amounts of energy. A novel sewage treatment concept, which includes an AnMBR followed by an algal reactor in which *H. pluvialis* are applied, is proposed. AnMBR is applied to retain particles in settled sewage and convert organic matters into biogas, which produces effluent suitable for algal growth. *H. pluvialis* are selected to enhance the economic feasibility of the proposed process. The proposed concept provides an alternative technology to treat sewage and is promising in achieving positive financial output, nutrient recovery, high-level organic matter removal, and minimal residual sludge production.

CRediT authorship contribution statement

Jixiang Yang: Conceptualization, Investigation, Writing – original draft, Funding acquisition. **Jules B. Lier:** Writing - review & editing. **Jian Li:** Writing - review & editing. **Jinsong Guo:** Funding acquisition. **Fang Fang:** 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

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