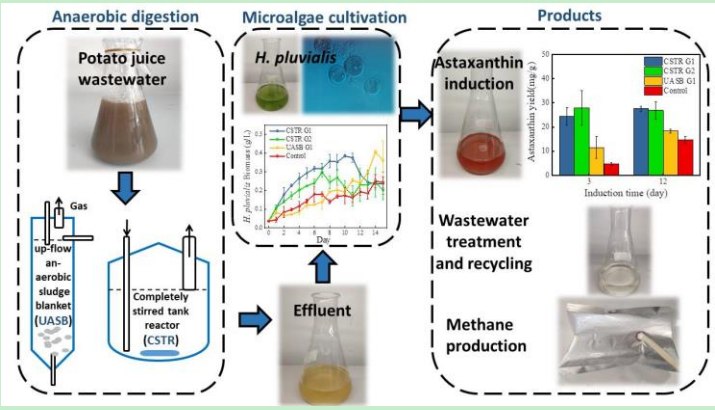
Highlights (max 85 characters, includes spaces, for each)

- Potato juice wastewater was applied for astaxanthin production
- Bacterial and microalgal activity were integrated for potato juice treatment
- Higher *H. pluvialis* biomass and astaxanthin yield than the yield in standard medium
- Potato juice significantly improved microalgae growth and astaxanthin induction
- The integrated valorization system is economically attractive for potato juice valorization

Graphic abstract



Integrated valorization system for simultaneous high strength organic wastewater treatment and astaxanthin production from *Haematococcus pluvialis*

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Abstract

H. pluvialis is recognized as the best natural astaxanthin supplier, however, it is still hindered for industrial production due to its high-cost of cultivation and long induction period. The present investigation proposed a simultaneous astaxanthin production and potato juice wastewater treatment process. By means of acidification (the completely stirred tank, CSTR reactor) and methogenesis (the up-flow anaerobic sludge blanket, UASB reactor), the potato juice wastewater was anaerobically digested, the nutrient contents and pH were improved for mixotrophic (acidification effluents) and autotrophic (methogenesis effluents) growth of *H. pluvialis*. Meanwhile, methane was obtained as energy by-product. During the cultivation, both effluents promoted higher biomass accumulation than the standard culture medium. The acidification effluents achieved much higher astaxanthin production (24.5-27.9 mg/g) than the control (4.6 mg/g) in significantly shortened induction period (3 days) regarding its moderate acetate and potassium components. Meanwhile, the methogenesis effluents also promoted a higher astaxanthin production (18.3 mg/g) than the control with 12 days. The *H. pluvialis* cultivation process further remediated the wastewater, by which, a final removal rate of 51.3-75.8%, 86.5-98.3%, and 69.4-83.4% were achieved for the COD, phosphorus and ammonia, respectively. The remediated wastewater could be recycled for the dilution utilization during the process. This study investigated a promising three-stage process for simultaneous efficient astaxanthin and methane production with low-cost, and potato juice wastewater remediation.

Keywords: anaerobic digestion, astaxanthin induction, microalgae, biogas, high strength organic wastewater

1. Introduction

The blooming of industrial activities results in a wide production of high strength organic wastewater[1,2]. The excessive high amount of organic matters, phosphorus and nitrogen[3] in the high strength organic wastewater poses a big challenge to the environment. Among them, a large amount of potato juice wastewater is produced due to the highly water demanding process of potato-starch producing. Each ton of potato-starch production generates approximate 3.5 tons potato juice wastewater[4]. As the major source of starch, the tremendous consumption of potato results in a massive amount of potato juice wastewater during potato-starch processing[5]. The potato juice wastewater is not only rich in bio-available nutrients, *e.g.*, nitrogen and phosphorus, but also contains abundant protein, starch and other organic matters, leading to extreme COD pollution as high as 191.5 g/L COD[4,6]. Discharge without treatments may subject environment to acute pollution[7].

Biological[8], precipitation-based[9] and membrane-based[10] techonologies have been applied for the treatment of potato juice wastewater. However, these approaches rarely consider the nutrients recovery and generally require large amount of energy and materials input. In view of sustainable development, the potato juice has been investigated for biogas production[4,6] through anaerobic digestion (AD) process for simultaneous energy production and wastewater treatment. Such approaches, *e.g.*, by means of up-flow anaerobic sludge blanket (UASB), even could efficiently remove COD from the potato juice, however, left large amount of nutrients loss of nitrogen and phosphorus[4]. Worse still, the discharge of high contents nitrogen and phosphorus causes serious eutrophication[11] problems and poses threats to the ecosystems[12].

To date, microalgae-based wastewater treatment technologies attract rising attention regarding its simultaneous nitrogen, phosphorus and COD removal capacity[13,14], and potential valuable microalgae-relevant products[15]. Astaxanthin, as one of the significant microalgae by-products, has its crucial role as pigmentation source for aquaculture and poultry industries[16]. Studies further revealed its promising potential of nutraceutical and medical value regarding the high antioxidant activity[17,18]. These properties generate an US$200 million annual astaxanthin market with US$2500 kg^{-1} unit price[15]. The commercial astaxanthin market is dominant by synthetic astaxanthin[19], however the natural astaxanthin is still irreplaceable for the safety use to human[20]. In this view, *Haematococcus pluvialis*, as one of the main natural astaxanthin supplier, contains much higher astaxanthin (up to 5%) than others microalge species (0.15-0.4%)[15], making it the most competitive natural source for commercial production. However, the high-cost of cultivation process[16] and long induction period (8-12 days) [19] [21] hinder the industrial production.

Efforts have been made of applying wastewaters, *e.g.*, domestic and primary-treated piggery wastewater for *H. pluvialis* cultivation to reduce the general costs [22,23]. However, it rises safety concerns due to the potential risks generated by the wastewaters[23] for astaxanthin product.

Therefore, exploring less polluted “safe” wastewater for *H. pluvialis* cultivation could provide an optional solution. The potato juice wastewater is producing in a well controlled process with few chemical addiction. The process is thermo-chemical which also prevents the growth of pathogenic microbes. Thus, the potato juice wastewater is a potential safe source of for *H. pluvialis* cultivation.

Apart from nitrogen and phosphorus capture, microalgae could only metabolize limited types of carbon sources[24] under heterotrophic or mixotrophic growth. Notablly, both heterotrophic and mixotrophic growth could promot significant higher growth rates and nutrients removal rates than autotrophic growth[25]. Nevertheless, the high COD content in the potato juice wastewater usually contains wide types of organic matters including protein, starch[6], which are not bioavailable for microalgae. This may limit the COD and nutrients removal rates and even inhibit the growth of microalgae[13]. Therefore, optimal pretreatment of the potato juice wastewater to convert the low bioavailable organic matters is critical for *H. pluvialis* cultivation.

To address the proposed problems, the present study developed an integrated process for full wastewater valorization. By applying methanogenic (mesophilic UASB reactor) and acidogenic (thermophilic completely stirred tank reactor, CSTR) processes, methane was obtained as energy by-products and the nutrient composition of the potato juice wastewater was optimized. Effluents were further used for the cultivation of *H. pluvialis* and astaxanthin induction. Meanwhile, the recapture of nitrogen and phosphorus by *H. pluvialis* may further contribute to the treatment of the potato juice wastewater. This study investigated the sustainable three-stage biological treatment for the potato juice wastewater with methane and astaxanthin production as valuable by-products.

2. Methodology

2.1 Process design and wastewater source

In this investigation, a three-stage experiment including digestion of the potato juice wastewater, microalgae cultivation and astaxanthin induction process was designed. The potato juice wastewater of potato starch processing (obtained from, KMC, Denmark) was concentrated after evaporation process. In the first stage, two reactors, thermophilic completely stirred tank (CSTR) reactor (for methanogenesis AD process) and up-flow anaerobic sludge blanket (UASB) reactor (for acidification AD process) were applied for the wastewater pre-treatment.

2.2 Bacteria driven fermentation

The mesophilic methanogenic (UASB) reactor was with a working volume of 1340 ml, 10 hours retention time and a recirculation speed of 2.0 m/h. A feeding peristaltic pump was used for a continuous sample injection. The mesophilic granules were obtained from the Haribo factory in Denmark. A 4 ℃ storage condition was given to the granules before usage. Short before the start of the UASB reactor, the granules were activated in standard BA medium with 4 g/L starch under 37 ℃ until a stable gas production was achieved. Next, five days adaption time was given with a stepwise increasing of the potato juice wastewater loading from 2.4 to 9.6 gVS/Lr/d. The UASB reactor, with an organic loading of 9.6 g VS/Lr/d potato juice wastewater. After 5 days pre-running, the effluent was collected for further experiment.

The acidogenic (CSTR) reactor was with 1830ml working volume and 6 days retention time under thermophilic condition (55℃) with an electrical heating jacket for the temperature

maintenance. Thermophilic inocula (from previous running CSTR methanogenesis sludge) was firstly inoculated into the reactor. 10 days pre-running of the CSTR reactor with stepwise increased organic loading from 0.08 g VS/Lr/d to 0.33 g VS/Lr/d was conducted before the collection of the effluent. Finally, the CSTR was supplied with 0.33 gVS/Lr/d organic loading from the potato juice wastewater. Gas content was monitored daily. After 10 days pre-running, the effluent was collected.

2.3 Microalgae cultivation and astaxanthin induction

In the microalgae cultivation stage, triplicated batch experiment in 1L flasks with 500ml working volume was applied. The species *Haematococcus pluvialis* was obtained from the culture collection of algae at Goettingen university (192.80 SAG). The effluent from UASB and CSTR reactors was firstly centrifuged with 4000rmp for 10min, the supernatant was further autoclaved for 20min. Afterwards, the CSTR effluent was diluted 18 (CSTR G1) and 25 (CSTR G2) times while the UASB effluent was diluted 10 times (UASB G1) to simulate varying retention time in a continuous reactor. *H. pluvialis* was pre-cultured in the MWC+SE medium until the microalgae reached an exponential phase, then inoculated with a ratio of 1:10 into each flasks. Meanwhile, the control *H. pluvialis* was inoculated into the MWC+SE medium. All flasks were placed on the shaker with 80rmp under room temperature (25°C) with a light intensity of 1000 lux with a light/dark ratios of 12:12. Ventilation-sterilization-membranes were used to cover all flasks. At day 15, 60ml samples from each flask were taken to place under strong illumination condition (1600 lux) with a light/dark ratio of 24:0 for the induction of astaxanthin.

2.4 Sampling and analytical methods

During the 15 days experiment, 0.5 ml sample was taken daily from each flask for the cell number counting. 10 ml sample was taken on day 0, 2, 8, 10, 12 and 15 for the detection of phosphorus, ammonium and COD. Extra 5ml samples were taken on day 0 and 15 for the detection of VFA and NO_x. During the astaxanthin induction period, pellets was collected on day 3 and 12 for the detection of astaxanthin.

During the anaerobic digestion process, the produced methane and carbon dioxide content of the UASB and CSTR reactors was detected daily with a gas chromatograph (GC Thermo Fisher scientific 1310) equipped with a flame ionization detector. The biogas production volume was measured with water-displacement gas meters. For the VFA detection, samples were pre-treated with phosphoric acid. Gas chromatographer (GC, TRACE 1300 of THERMO Scientific) was used for the detection of VFA (acetate, butyrate, and propionate) concentrations. The GC was equipped with a HP FFAP (free fatty acid phase) column (30 m *0.53mm *1.0μm) and flame ionization detector.

The hemacytometer (Thoma) was used for the counting of the cell number daily. Freeze dry method was applied for the detection of dry weight of the biomass. The dry weight and cell number of *H. pluvialis* was further correlated with the following equation,

$$W_{dry}=2*10^{-6}*N+0.0069 \quad (1)$$

where, W_{dry} (g/L) is the dry weight of the *H. pluvialis* dry weight, N (per mL) is the cell number of the *H. pluvialis* (Tab. S1).

Specific water qualities were detected as follows: the TS and VS were measured according to standard method[26] The total ammonia was determined by the Kjeldahl method. COD was determined by the standard method[27]. The ammonia, nitrate, nitrite and phosphate concentrations were determined by the segmented flow analysis (Scan++ system, Skalar analytical

BV, the Netherlands). PHM00 LAB pH meter was used for the measurement of pH. The potassium element concentration was measured by the inductive coupled plasma-optical emission spectrometer (ICP-OES, Perkin Elmer Avio 200) with an acidification pre-treatment by HNO_3 (2% w/w).

For the astaxanthin quantification of *H. pluvialis*, microalgae pellets were centrifuged with 4500rmp for 15min. After freeze-dry, the pellets were filled with 2ml acetone (HPLC-grade) to abstract the pigments in darkness at 5 ℃ for 24 hours. During the abstraction, pellets were thoroughly mixed 3 times with vortex. After the abstraction, the samples were dried with anhydrous sodium sulfate and centrifuged for 5 min at 1000g. After filtered with 0.2 μm PTFE filters, samples were transferred to amber HPLC-vials with N_2 as protective layer. Pre-treated samples were analyzed by HPLC-DAD (Agilent 1100, US) with a C8 reversed phase column (Eclipse Plus C8, Agilent technologies, US). Tetrabutyl ammonium acetate (TBAA) pH 6.5 and methanol were used as eluents. Astaxanthin standard was acquired from DHI (Denmark).

One-way ANOVA was used to compare biomass, astaxanthin and water quality parameters. SPSS19.0 (IBM Corporation, Armonk, NY, USA) and origin 8.5 (OriginLab, Northampton, MA, USA) were used for data analyzing and figures ploting.

3. Results and discussion

3.1 Bacteria driven digestion process

The original potato juice wastewater was with high-strengthed COD up to 342.33 g/L, and rich in nitrogen (mainly in forms of 2720.00 mgN/L ammonia and 107.77 g/L protein) and phosphorus (4766.67 mg/L). The low pH (4.75) weakens the suitability for microalgae cultivation (Tab.1). The mesophilic up-flow anaerobic sludge blanket (UASB) and thermophilic continious stirred tank (CSTR) reactor configuration were chosen to achieve methanogenic and acidogenic process, respectively. After the pre-treatment, the effluent quality was characterised in the Tab.1. Both reactors had been given an adaption phase, during which, a stepwise increase of methane production yield in the UASB reactor and a gradual decreasing methane production yield in the CSTR reactor was achieved. Effluents from UASB and CSTR reactors were collected during the stationary phase. In the mesophilic UASB reactor, the methane production yield was stable around 291-336 ml CH4/g COD with a methane recovery rate in the range between 83.00 to 95.94% (Fig. 1), indicating a thorough anaerobic digestion process of methanogenesis. The methanogenesis digested most bio-available organic carbon into methane and carbon dioxide[28], leading to a significant COD reduction of 75.44% (Tab. 1) in the UASB effluent. The left COD (1.40g/L) in the UASB effluent was mainly in form of poorly biodegradable substrates. Meanwhile, in the thermophilic CSTR reactor, conditions were controlled for an acidification-dominant[29] anaerobic digestion. By applying a hydraulic retention time (HRT) of 6 days, 12.71-18.70% of the organic carbon was converted to methane (Fig. 1). The rests were mainly converted to VFAs via acidification process (Fig. 3b, c and d). Consequently, the anaerobic digestion process only achieved a slight decline of COD (17.58%) in the CSTR reactor, however, through the acidification process, generated a large amount of VFAs (Fig. 3b, c and d). Among the VFAs, acetate is regarded as the most important carbon source for *Haematococcus pluvialis* (*H. pluvialis*). Therefore, the acidification process in the CSTR reactor had conversed the hard

microalga-available organic carbon sources into *H. pluvialis* degradable carbon source.

Apart from carbon sources, both pre-treatments enormously elevated the ammonia nitrogen content (from 50.33 and 166.67 to 343.33 and 1450mg/L in the UASB and CSTR effluent, respectively) and slightly increased the PO_4-P content (Tab.1). During the anaerobic digestion process, the high content of protein in the potato juice wastewater was hydrolysed to amino acids and further through the catabolism of bacteria produced ammonia[30] contributed to the elevated ammonia concentration in the effluents. Additionally, the organically bound P could also be released regarding the organic matter destruction during anaerobic digestion[31], which may contributed to the increase of soluble phosphorus (orthophosphate) concentration in the effluents. Besides, pH of both effluents were raised to neutral (from 4.75 to 7.00 and 6.72 in the UASB and CSTR reactor, respectively, Tab.1). As a consequence, the pre-treatment by the UASB reactor (methanogenesis) has significantly decreased the carbon content and elevated the ammonia nitrogen content in the C:N:P ratio of the wastewater, and adjusted the pH to neutral, meanwhile, the pre-treatment by CSTR reactor (acidification) has significantly raised the ammonia nitrogen content and adjusted the pH to neutral, and acidified the most organic carbon into VFAs. Regarding the water quality changes, *H. pluvialis* may benefit in different aspects.

Tab. 1 Characteristics of the potato juice wastewater (original and effluent)

	COD (g/L)	NH_4-N (mgN/L)	NO_3-N (mgN/L)	PO_4-P (mgP/L)	pH
Original	342.33±2.05	2720.00±56.57	320.00±32.66	4766.67±49.89	4.75±0.05
Inffluent UASB	5.70±0.07	50.33±0.47	4.88±0.50	81±0.41	
Effluent UASB	1.40±0.01	343.33±22.48	2.38±0.25	96.5±1.08	7.00±0.08
Inffluent CSTR	18.03±0.12	166.67±2.49	14.61±1.49	206±2.83	
Effluent CSTR	14.86±0.42	1450.00±35.59	5.25±4.92	237.67±2.05	6.72±0.07
MWC+Se medium	--	0	14.00	1.55	7.00±0.10
Original wastewater protein: 107.77±3.28 g/L					
Original wastewater total solids: 374.22±1.88 g/L					
Original wastewater volatile solids: 262.40±3.66 g/L					

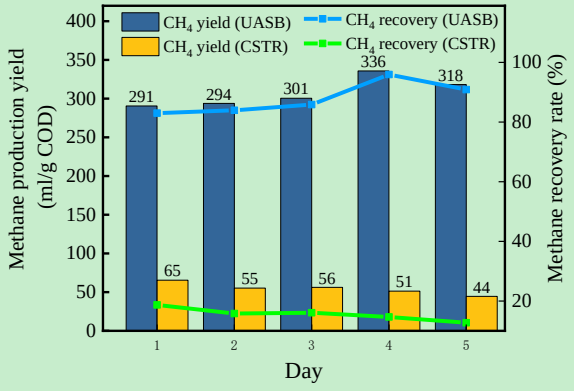


Fig.1 Methane production yield and recovery rate in the CSTR and UASB reactor

3.2 *Haematococcus pluvialis* cultivation

3.2.1 *Haematococcus pluvialis* biomass accumulation

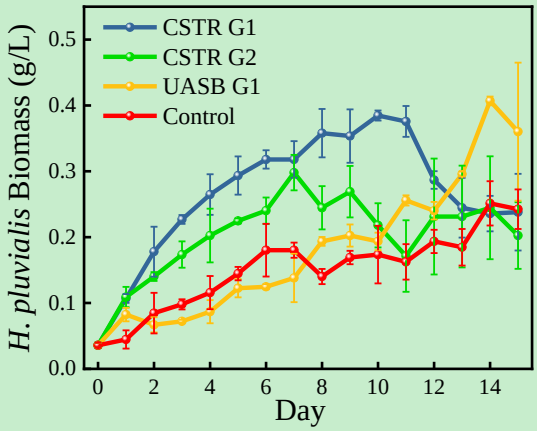


Fig.2 Biomass accumulation of *H. pluvialis* during the secondary potato juice wastewater cultivation

After the potato juice wastewater pre-treatment, *H. pluvialis* was cultured in both effluents with varying dilution rates to simulate different retention times in a photo-bioreactor. Fig. 2 showed that all three groups achieved a higher biomass accumulation than the control, however, at different periods. Effluents from the acidification process (CSTR reactor) promoted a faster and higher biomass growth. Specifically, the *H. pluvialis* in the CSTR G1 and G2 groups gained the highest biomass of 0.38 and 0.30 g/L, respectively. The rapid growth of biomass started right after the inoculum and last for 9-10 days, following by a sharp death phase. Regarding the growth curves and acetate concentrations, this is well consistent with mixotrophic growth mode of *H. pluvialis*[32]. Meanwhile, both UASB G1 and control groups obtained a lower growth rate than the CSTR groups, however, last longer for 14 days and generated a maximum biomass production of 0.41 and 0.25 g/L, respectively. This corresponded well with the autotrophic growth of *H. pluvialis*[33].

The trophic modes of *H. pluvialis* was significantly affected by carbon sources in the effluents. According to the results, we hypothesized that, after acidification dominant anaerobic digestion, the effluents promoted a mixotrophic growth of H. pluvialis. While, through the methanogenesis process, *H. pluvialis* gained an autotrophic growth.

3.2.2 Organic carbon conversion

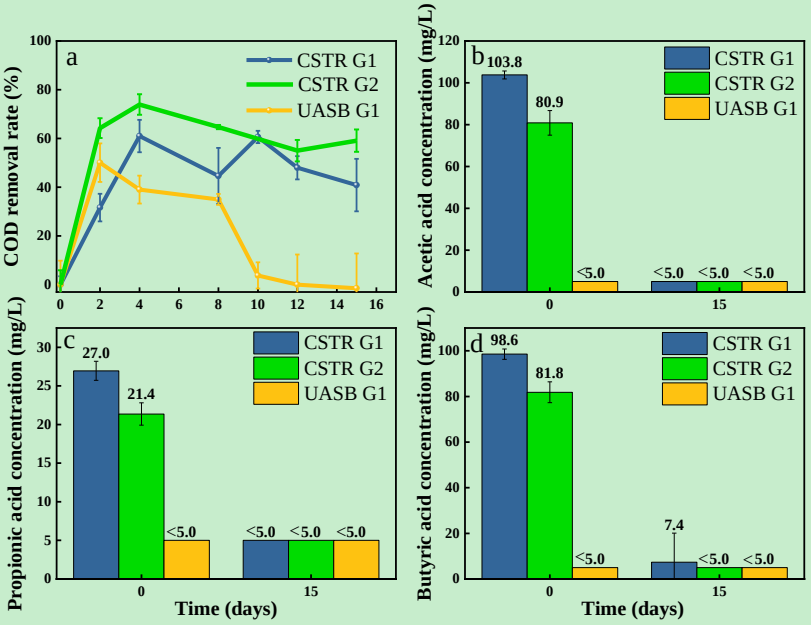


Fig.3 Effleunt carbon concentration changes during the *H. pluvialis* treatment of (a) COD, (b) acetic acid, (c) propionic acid and (d) butyric acid

In the first stage, the potato juice wastewater pre-treatment through methanogenic (UASB reactor) and acidogenic (CSTR reactor) process contributed to 75.4% and 17.6% of the COD removal (Tab.1), respectively. In the further microalgae treatment stage, *H. pluvialis* performed an overall rapid COD removal in the earliest phase (2-4 days), of which, 50.1%, 61.0% and 73.9% of the COD was removed from the UASB G1, CSTR G1 and CSTR G2 systems (Fig. 3a), respectively. Regardless the bioavailability of the organic matters, microalgae cells showed a wide bio-adsorption capacity[34],which may explain the rapid COD removal in all effluents at early stage. However, as the dynamic process of microalgal growth and death, organic matters was again released into the water, leading to a rebound and float of the COD concentrations. Methanogenesis process (in the UASB reactor) digested most organic matters into methane and carbon dioxide, leaving low biodegradable organic matters in the effluent (under detect limit level of VFAs, Fig. 3 b, c and d). Therefore, the un-bioavailable organic matters and the cell fraction were released into the water again with the death of microalgae, resulted in no further removal of COD in the UASB G1 groups at day 15 (Fig. 3a). Comparably, the CSTR G1 and G2 groups promoted a final COD removal rate of 40.9% (from 457.9 to 207.8 mg/L) and 59.1% (from 363.9 to 148.8 mg/L), respectively. The acidification (in the CSTR reactor) converted most organic matters into VFAs, among which, the acetate is recognized as the most efficient carbon source for *H. pluvials*[35]. Fig.3b showed the initial acetic acid concentration of 103.8 and 80.9mg/L in the CSTR G1 and G2 groups. As the assimilation by *H. pluvialis*, complete acetic acid has been removed at day 15 (under detection limit). The existence of acetate also promoted the growth of *H. pluvialis* (Fig.2). An amount of the propionic and butyric acid was also detected in the CSTR effluent. Results showed a nearly complete removal of the propionic and butyric acid (except for the butyric acid of CSTR RT1). However, no obvious evidence indicates the assimilation of these two types of VFAs by *H. pluvialis*. In view of carbon balance, during the acidification process, 72.2-73.5% of the total COD was converted to VFAs (Tab. S2), while the methanogenesis process

achieved no VFAs conversion. Furtherly, even the microalgae cultivation contributed to 69.3%, 73.5% and 0% of the COD removal rate in forms of VFAs consumption, however, a final lower entire COD removal rate of 40.9%, 59.1% and 0% was achieved in the CSTR G1-microalgae, CSTR G2-microalgae and UASB G1-microalgae groups, respectively. The microalgae cultivation process is a dynamic process of COD uptake and release. Bio-available VFAs may be consumed by the *H. pluvialis* during growth, however, the *H. pluvialis* may also release organic matters into the water, which caused the increase of COD. Consequently, taking the entire anaerobic digestion and microalgae treatment into consideration, a final COD removal rate of 51.3%, 66.3% and 75.8% was gained by the CSTR G1-microalgae, CSTRG2- microalgae and UASB G1-microalge groups, respectively. The lower entire COD removal rates could be improved by optimizing the CSTR and UASB reactor conditions to provide a higher efficiency of acidification and methanogenesis process, respectively.

3.2.3 Nutrients recovery by the *H. pluvialis*

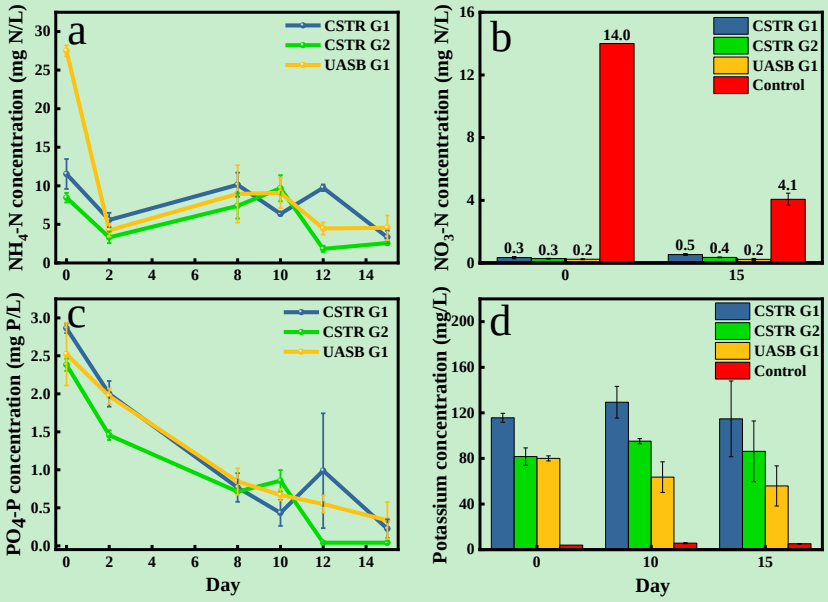


Fig. 4 Nutrient concentration changes during the cultivation of *H. pluvialis* of (a) NH_4-N, (b) NO_3-N, (c) PO_4-P, and (d) potassium concentration change

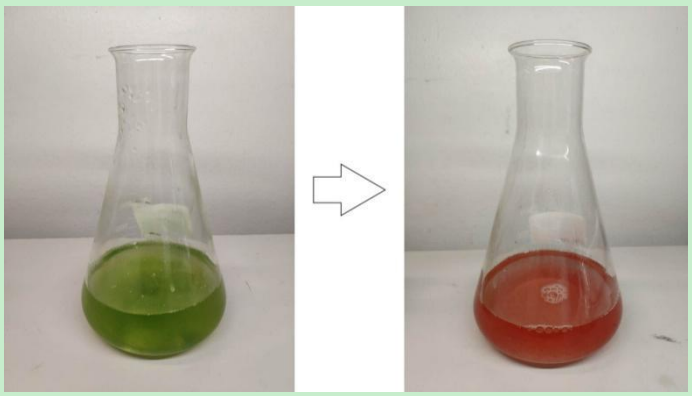
During the microalgae cultivation, the nutrients re-capture by the *H. pluvialis* was also studied. Different from the control (in form of nitrate), the main nitrogen source in the UASB and CSTR reactor effluent was in the form of ammonia (Fig. 4 a, b). Results indicated the UASB G1 groups with a much higher initial nitrogen concentration than the CSTR G1 and G2 groups. With a concentration float during the cultivation, a final ammonia nitrogen concentration of 4.56 mg/L (removal rate 83.4%), 3.31 mg/L (removal rate 71.3%) and 2.59 mg/L (removal rate 69.4%) in the UASB G1, CSTR G1 and CSTR G2 groups was achieved (Fig. 4a), respectively. Meanwhile, nitrate and nitrite concentrations of all treatment groups were not significantly increased,

indicating an efficient nitrogen removal by the *H. pluvialis*. Notably, the final nitrogen concentrations of treatment and control groups were in similar levels but in different forms (2.6 to 4.6 mg/L, Fig. 4 a, b). Microalgae usually prefer ammonium as nitrogen source over nitrate regarding no redox reaction and low energy requirement during assimilation[36]. The Study also implied that the ammonia could be a better nitrogen source than nitrate in growth promotion of the *H. pluvialis*[37]. The nitrogen in forms of ammonium in the treatment groups contributed to the overall higher biomass accumulation than the control (Fig. 2).

All treatment groups presented similar original phosphorus concentrations (2.38-2.86 mg/L, Fig. 3 c). As the consumption of phosphorus by the *H. pluvials* during growth, the phosphorus concentrations stepwise declined with time, a final phosphorus removal rate (at day 15) of 86.5%, 92.0% and 98.3% was gained by the UASB G1, CSTR G1 and CSTR G2 groups, respectively (Fig. 4 c). Notably, even the phosphorus concentration declined to below 1 mg P/L after 8 days, microalgae growth could still be observed in all groups due to the phosphate reservation ability as polyphosphate granules in the microalga cells[22].

H. pluvialis demonstrated a strong nutrients re-capture capacity from wastewater[23,38]. After treatment by *H. pluvialis*, a low level of nitrogen and phosphorus was left in the wastewater (Tab. S3), which showed the potential of astaxanthin induction without changing induction medium.

3.3 Astaxanthin induction from wastewater cultivated microalgae



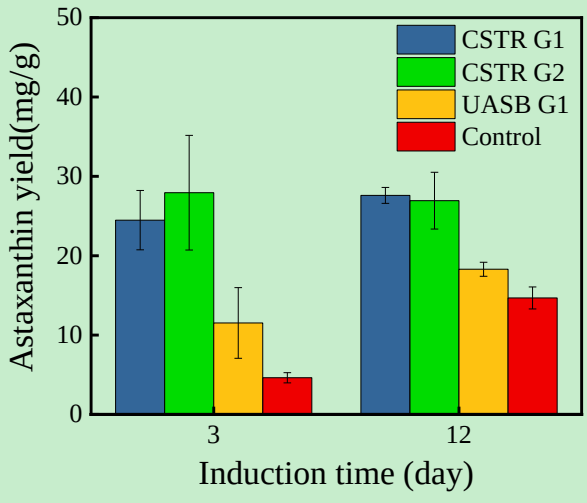


Fig. 5 Astaxanthin content during the induction of *H. pluvials.*

After 15 days biomass accumulation, samples were shifted under high light intensity induction condition (1600 lux). During the induction period, all effluent groups demonstrated overall higher and faster astaxanthin induction efficiency. Within three days of being shifted to high light intensity induction condition, the CSTR G1, G2 and UASB groups had already accumulated much higher astaxanthin than the control (4.6 mg/g), which was 5.3 times (24.5 mg/g), 6.1 times (27.9 mg/L) and 2.5 times (11.5 mg/g) of the control, respectively. Longer induction time (till day 12) didn't elevate the astaxanthin production in the CSTR G1 and G2 groups significantly ($p<0.05$, 27.6 and 26.9 mg/g, respectively), however, had further raised the astaxanthin production in the UASB and control groups to 18.3 and 14.7 mg/g, respectively (Fig. 5). The results indicated that the effluents of acidification pre-treatment had a better performance in shortening the induction time and promoting the astaxanthin yield than the effluents of methanogenesis pre-treatment.

The water qualities were further investigated to interpret the induction results. The original potato juice wastewater was rich in potassium, resulted in a much higher potassium concentration (80.08, 115.67 and 81.63 mg/L in the UASB, CSTR RT1 and CSTR RT2 groups, respectively) than the control (3.9 mg/L, Fig. 4d). During the cultivation of *H. pluvialis*, potassium concentration showed no significant changes (Fig. 4d, $p<0.05$) and kept in a high level till the end of the experiment (day 15). Potassium could inhibit the *H. pluvials* growth (over 40mM), meanwhile, could significantly promote the astaxanthin production (under 70mM)[39]. In the present study, the effluents offered a moderate level of potassium, which was lower than the growth inhibition levels, but could contributed to the promotion of astaxanthin induction. Study also revealed, as the astaxanthin accumulation, the potassium-transporting protein is induced in the red cyst of *H. pluvialis*[40]. The demands of potassium during the astaxanthin induction explained the overall higher astaxanthin induction in the effluents than the control. Besides, acetate has been widely demonstrated of its both vegetative growth and astaxanthin induction potential[32,41] . Fig. 2b and 4d showed an higher amount of acetic acid and potassium in the CSTR effluents, as an inducing agent, potassium acetate was also reported to highly induce the astaxanthin accumulation[42]. The extra acetate in the CSTR effluents further shortened the induction time and increased the astaxanthin yield.

Additionally, the switch from biomass accumulation process to astaxanthin induction process usually requires a medium change to provide the H. pluvialis with a nutrient deficiency condition. Regarding the low concentration of the nutrients left in the water after *H. pluvialis* treatment (Tab. S1), this novel process didn't require a replacement of astaxanthin induction medium from the cultivation to induction phases[43].

3.6 Economical respects of intergrated wastewater valorization system

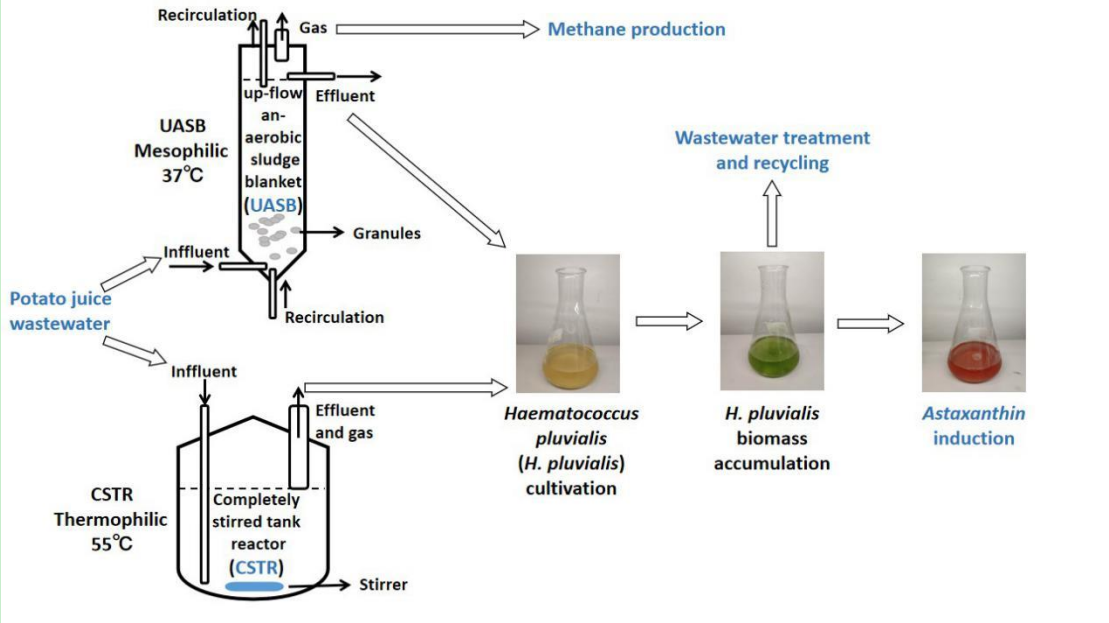


Fig. 6 Sustainable development of integrated astaxanthin production and potato juice wastewater treatment with nutrients re-capture and energy by-product (methane).

This investigation developed a sustainable solution for simultaneous potato juice wastewater treatment and low-cost astaxanthin production. From ecological view, potato juice wastewater is distinguish from common wastewaters for various reasons. Unlike common wastewaters, potato juice wastewater contains no pathogenic microorganisms or toxic compounds[44], which could broaden the reutilization of the obtained astaxanthin from wastewater cultivation to strict usage, *e.g.*, feed, food, cosmetics. In the meantime, the high contents of phosphorus and nitrogen are ideal nutrients for *H. pluvialis*. However, regarding the high COD composition in forms of low microalgae-available organic matters, pre-treatment through methanogenesis (UASB reactor) and acidification (CSTR reactor) has optimized the organic carbon sources (largely removed/ or conversed to VFAs) for *H. pluvialis*, and obtained methane as energy by-products. The nutrients in the secondary effluents (mainly nitrogen and phosphorus) were further re-captured by the *H. pluvialis* to support the biomass accumulation. With the moderate level of potassium (in both effluents) and acetate (only in the CSTR effluents), an accelerated and promoted astaxanthin induction was achieved, which contributed to reduce the maintenance cost. The treated wastewater after *H. pluvialis* could be reused for dilution during the whole process to bring down the water consumption. According to the market price of astaxanthin (US$ 2500/kg)[15] and methane (US$ 2.5/MMBtu) [45], we estimated that, the integrated acidogenic-*H. pluvialis* process could generate up to 9932 US$/ton potato juice wastewater from astaxanthin production and 234-346 US$/ton potato juice wastewater from methane production, while, the integrated methanogenic-*H. pluvialis* process could gain 11265 US$/ton potato juice wastewater from astaxanthin production and 6647-7675 US$/ton potato juice wastewater from methane production, which regardless the related costs including facility construction, operation, energy consumption, astaxanthin abstraction, *etc* . A further assessment of the net profit still needed to be specified, however, this estimation showed the potential conversion from potato juice wastewater to high valuable by-products of astaxanthin and methane.

Conclusion

Methanogenic (through UASB reactor) and acidogenic (through CSTR reactor) digestion process demonstrated the nutrients improvement and pH adjustment ability on the potato juice wastewater for efficient *H. pluvialis* biomass production and astaxanthin induction. The methanoganic process convered most organic matters to gaseous carbon and significantly elevated the ammonium content through protein digestion. Methane was gained as energy by-products and the effluents supported an augmented autotrophic growth and higher astaxanthin production than the standard culture medium. The acidogenic process conversed most organic matters into VFAs and enormously raised the ammonium content. The effluents promoted a mixotrophic *H. pluvialis* growth and achieved much higher biomass and astaxanthin production than the standard culture medium. The effluents through acidification demonstrated the best biomass and astaxanthin promotion capacity, and largely shortened the cultivation and induction time regarding its moderate acetate and potassium compounds. The cultivation of *H. pluvialis* further contributed to the wastewater remediation by means of nitrogen, phosphorus and organic carbon re-capture, effluents could be recycled as dilution water during the process. This study demonstrated a promising and cost-effective technology of potato juice wastewater reutilization for energy and astaxanthin production.

Acknowledgement

The research was supported by the InWAP project supported by the Danish Innovation Foundation. The PhD fellowship of Minmin Pan was supported by the China Scholarship Council (CSC). We thank Hector Hernan Caro Garcia for the help with laboratory work.

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Supplementary material

Integrated valorization system for simultaneous high strength organic wastewater treatment and astaxanthin production from *Haematococcus pluvialis*

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Tab. S1 Correlation between biomass and cell number

Biomass (g/L)	Cell number (per ml)
0.239783743	117777.7778
0.104	53541.22037
0.05	13437.53483
0.01	2816.577304
0.008	1134.287637
0	0
$W_{dry}=2*10^{-6}*N+0.0069$, $R^2=0.9906$	

Tab. S2 VFAs conversion during anaerobic digestion and consumption during microalgae cultivation processes.

	CSTR G1-microalgae	CSTR G2-microalgae	UASB G1-microalgae
Acetic acid COD (mg/L)	110.682	86.277	0
Propionic acid COD (mg/L)	40.791	32.33	0
Butyric acid COD (mg/L)	98.565	148.782	0
Total VFAs COD (mg/L)	330.681	267.394	0

Total VFAs removal by microalgae cultivation (COD, mg/L)	317.298	267.394	0
VFA conversion rate	72.21%	73.47%	0
COD removal rate in forms of VFAs by microalgae cultivation	69.29%	73.47%	0

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597 Tab. S3 Nutrient concentration of astaxanthin induction stage

	Phosphorus (mg P/L)	Nitrogen (mg N/L)
UASB G1	0.335	4.564
CSTR G1	0.225	3.314
CSTR G2	0.042	2.589
Control	0.457	4.067

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