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Dairy By-Products Valorization with Biomethane and Biohydrogen Production through Lactose Fermentation in Anmbr

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Dairy liquid by-products after cheese production (e.g. whey for hard cheese, “scotta” for ricotta, brine for mozzarella, lactose syrup after milk standardization by ultrafiltration) represent a potential revenue for food factories where large scale industrial production is implemented, as additional ingredients may be recovered, transformed and sold. Still, these by-products may also represent a problem for small/medium size factories or in areas that are far from ingredients transformation platforms: complete processing of small volumes or shipping liquids over long distances is not convenient. By-products are often disposed as waste, generating unproductive cost for the factory and worsening environmental footprint.
In this paper a simple closed loop solution is evaluated for full valorization of by-products, based on the technologies of protein separation/concentration and anaerobic treatment of the lactose solution.
Whey proteins are separated by ultrafiltration and can be reused in the cheese process or converted locally into fresh animal feed. Application studies will be presented.
Ultrafiltration permeate (filtrate) is a diluted lactose syrup that is used as raw material for fermentation. Lactose dark fermentation for hydrogen production has been documented on a laboratory scale, but not really tested before on a semi-industrial scale. Results obtained from both laboratory scale and semi-industrial scale fermenter will be presented. Finally, anaerobic membrane digester has been evaluated on a semi-industrial scale to convert all residual lactose into biomethane, that can be efficiently reused in the dairy factory boiler for steam production.
Anaerobic membrane digester proved to be a valuable solution, as it allows maximum lactose conversion efficiency with good quality liquid effluent.

1. Cheese Whey proteins recovery and reuse

Whey proteins are widely used in the food industry as they are a key component for many food preparations. Still, producing high quality whey protein concentrate is economically viable only on a large scale and alternative practices should be considered for small/medium size dairy factories that cannot deliver whey to large transformation platforms. Two common practices have been considered in this study for sustainable protein reuse: production of fresh whey protein concentrate for animal feed at the farms near the dairy factory and enhanced production of ricotta and similar dairy products directly at the dairy factory.

1.1 Membrane separation and protein concentrate reuse

Membrane separation processes play an important role in both cases and among these ultrafiltration in cross flow filtration configuration has been considered for this study; it makes possible efficient separation between proteins and lactose with low investment and operating cost.
Ultrafiltration membranes with a molecular weight cut off in the 5-15 kD range have been extensively used in the industry to concentrate whey proteins. Standard spiral wound ultrafiltration elements, polyethersulphone

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active layer, allow to achieve up to15% protein concentration. Special construction spiral wound ultrafiltration elements or tubular ceramic membranes can be used for further concentration, up to 20%.
When working on skimmed sweet whey from cow milk, permeated water contains 4-5% lactose; therefore this option was first considered to carry on semi-industrial prototype study. An ultrafiltration pilot plant with 3 ultrafiltration elements, S=15-20 m^2/ea active filtration area, model DAIRY ULTRA UF6338 by GE (MWCO 10 kD), was installed directly at a Grana padano dairy factory, to produce protein concentrate and 5% lactose syrup (permeate).
Protein concentrate was evaluated for animal feed (see details in chapter 2.2). Membrane permeability ranges from Fs=10 to 20 l/(hxm^2), depending on concentration factor and temperature; operating pressure ranges from P=3 to 6 bar.
A second ultrafiltration pilot plant, same configuration, has been extensively used in Sardinia region to process whole whey form sheep milk and scotta (residual whey after ricotta production). These tests were intended to optimize ricotta production and at the same time to evaluate recovery of residual proteins after ricotta production (see details in chapter 2.3).
Lactose syrup is collected separately to be anaerobically fermented to produce biobas and this subject will be covered separately in chapter 3.

1.2 CW (cheese whey) proteins reuse: case studies

Whey proteins as liquid solution are proposed as a fresh components for animal feed vs standard practice of buying commercial whey powder.
In Italy, after the Legislative Decree no. 22/97 ("Ronchi Decree") whey is not considered a waste product, if it enters into a production process for animal feed, adopting two EU directives. EC Regulation 1774/2002 introduces the concept of animal feed by excluding it from the category of waste. Whey enters Category III (very low risk) as animal derived by-product intended for human consumption. EC Regulation 79/2005 enforces the contents of REG. 1774/2002 concerning the use of milk, milk products and milk by-products, materials classified as Category III defining the terms of use and transport.
Whey is a food, however much diluted, and as such its use is limited by the gastric administration ability of subjects to which is fed. The value of whey lies particularly in the composition of protein fractions (lactalbumin and lactoglobulin, lysine, tryptophan and high sulfur aminoacid such as cystine and methionine). So our attention focused on recovering as concentrate these most valuable components and to stabilize them to achieve good results in animal feed.
Using above mentioned ultrafiltration protein concentration plant, it is easily possible to increase protein concentration up to 8-10%, that proved best option to deliver concentrated solution directly into animal feed. Field test were carried on proving that 1 kg of dry matter from whey provides approximately 3,500 kcal / kg digestible energy corresponding to an average of 3,200 kcal / kg metabolizable energy. A cow can safely consume whey liquid concentrate at a rate of 2-5 kg of dry matter.
Originally cattle farmer considered a liquid product something difficult to handle to cows and calves. To by-pass this problem, whey concentrate has been mixed with fibrous matter (e.g. beet fibers), so that water is absorbed and final product can be administered in the traditional way.
As a result of these studies, an industrial ultrafiltration system was installed at a dairy factory in Southern Italy, to produce average 1 t/d protein concentrate; this has been used on a consistent basis for buffalo feed at a local farm for more than one year, with equivalent reduction of the protein purchased from other sources.

1.3 CW proteins reuse: case study for ricotta production

Extensive testing in Sardinia region proved that processing whole whey coming from pecorino romano production (sheep milk cheese) by ultrafiltration, concentration factor 2, allows to increase yield for ricotta production by approximately 0,5% (calculated on total volume of raw liquid processed); this is an important result, considering that it represent a >10% increase on the total amount of ricotta produced. More than this, ultrafiltration reduces energy consumption approximately by a factor 2 and also labour is substantially reduced. As a result of these studies, an industrial ultrafiltration system was installed at a dairy factory in Sardinia, to process up to 70 t/d whey and to increase ricotta production.
Further process improvement may be achieved processing scotta (exhausted whey after ricotta production) again by ultrafiltration. Scotta is concentrated by a factor 10, stored in a refrigerated cell and reused on the next day in a mix with ultrafiltered whey for more ricotta production.

2. Lactose to energy

2.1 Dark fermentation: process and competition among bacterial groups

Among all the biological processes to produce renewable hydrogen, dark fermentation (DF) of organic compounds by anaerobic bacteria seems to be more favorable (Lee et al. 2011). In DF, H_2 is produced by various microorganisms such as obligatory anaerobic strains of the *Clostridium* species (*Clostridia butyricum*, *Clostridium pasteurianum* and *Clostridium beijerinkii*) and facultative anaerobic species like *Enterobacter*, *Citrobacter* sp. and *Escherichia coli* (Prazeres et al. 2012) during the acidogenic phase, but is then readily used by hydrogen consumers (methanogenic or homoacetogenic bacteria) in conventional anaerobic digestion. Various organic waste have been tested, from simple sugars to municipal solid waste, industrial wastewater, and agricultural waste (Guo et al. 2010). Cheese whey is mainly composed by lactose (45-50 g/L), and thus is a very interesting candidate for full scale DF: in literature is reported a production that can reach, for batch test, 4,13 molH_2/mol lactose (Romao et al. 2014) and for continuous tests 3,8 molH_2/mol lactose (Martinek et al. 2013). In order to produce H_2 gas, hydrogen consumers' activity must be suppressed, by heat-shock, acid or alkali treatment of the inoculum to select spore-forming clostridia (Hawkes et al. 2007), or selecting operating conditions able to wash out the H_2-consumers (Kalia et al. 1994). In full scale and continuous feed reactors, inoculum pre-treatment can be hard to perform and not sufficient to guarantee long lasting control of H_2 consumers. Thus, control of operating conditions is mandatory to wash out methanogens, as shown in Figure 1, where the wash out range in terms of pH and SRT (sludge retention time, equal to hydraulic retention times in CSTR, continuosly stirred tank reactor) is depicted on the basis of the default kinetic values of ADM1 model (Batstone et al. 2002).

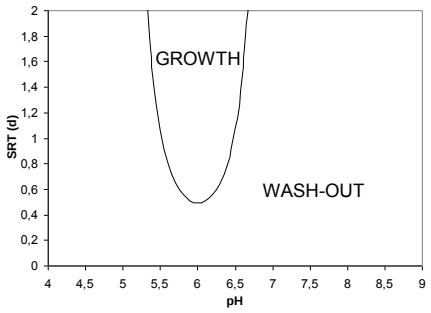

Figure 1. Wash out of methanogens conditions, according to ADM1 model's values

Anyhow, the SRT that can prevent methanogens growth do not prevent homoacetogens growth (Saady 2013): the exclusion of homoacetogens is still an unresolved challenge. Another phenomena negatively affecting the H_2 yield is inhibition of H_2 producers by lactic acid bacteria; to prevent it, Noike et al. (2002) proved an effective treatment by heating the substrate at 50°C.

2.2 Dark fermentation: results at batch scale and semi industrial pilot plant

Ultra filtered cheese whey (UF-CW, 54 gCOD/L) from Grana Padano cheese production was used in this study to fed a semi-industrial scale DF CSTR pilot plant (V = 0.8– 3 m^3). Feed is prepared filtering CW (Dairy Ultra UF 6338C Sanitary Ultrafiltration, 3 modules) and diluting with tap water to reach the desired influent COD concentration (from 2,5 to 13,5 gCOD/L). No pretreatment of the anaerobic sludge used as inoculum has been carried out. HRT (hydraulic retention time) was fixed at 0,75 d. pH was maintained at 5 in the 1st and 5,5 in the 2nd reactor, with a NaOH 30% solution dosage directly inside the reactor, and temperature was maintained at T = 37 °C. The UF-CW used for feed is filtered every 2 days and stored at 70°C to prevent biological degradation and growth of lactic acid bacteria.
COD was analysed according to standards methods (APAT-IRSA Metodi analitici per le acque 5130), Volatile Fatty Acids (VFAs) by ion cromatgraphy, detector FID with column Nukol fused silica, with nitrogen as gas carrier and off gas composition was analyzed by gas cromatography TCD, 2 columns Hayesep Q and Molesieve 5, helium as gas carrier. Figure 2 shows the percentage of hydrogen, methane and carbon dioxide in the off gas produced during the first 90 days of the operation (a) and the operating conditions of the DF reactor in terms of concentration of COD in the feed and in the effluent (b); on secondary axis is shown the calculated COD removal efficiency.
In the first phase both H_2 and CH_4 were produced; lowering the pH at 5 resulted in almost complete reduction of the CH_4 content. COD removal was around 25 % in the 1st phase and decreased to an average of 15,1% in the 2nd phase. These values are in line with the experiments conducted by Perna et al. (2013) and

Kisielewska et al. (2014) in reactors operating at 24 h HRT and OLR (organic loading rate) in the range (20-35 kgCOD/m^3/d). Figure 3 shows VFA concentration measured in the effluent, expressed in gCOD/L.

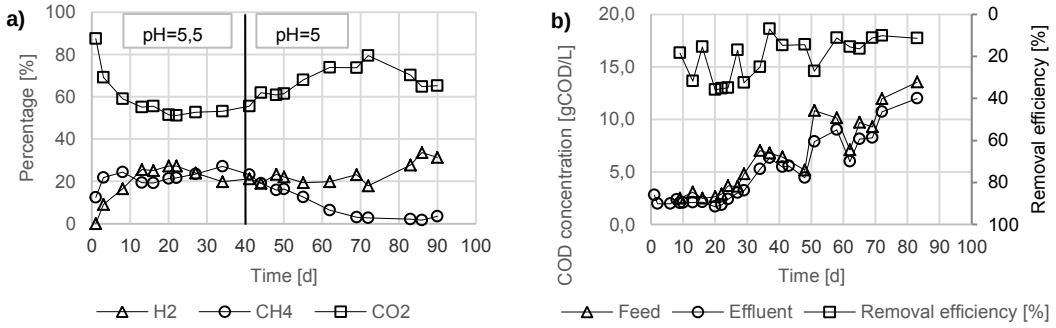

Figure 2. (a) Results of gas analysis during the start-up phase (90 days) (b) COD concentration in the feed and in the effluent, on secondary axis calculated removal efficiency.

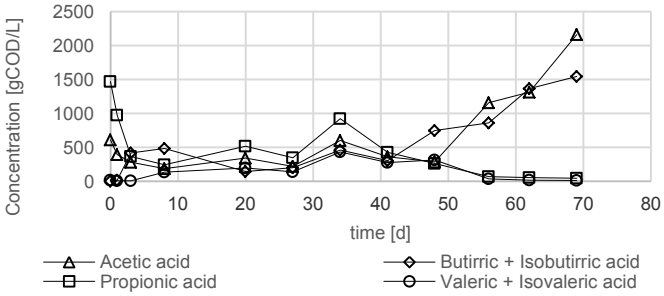

Figure 3. Single VFA concentrations along the experiment

During 1st period (day 1-40) about half of the VFA production is related to propionic acid production. Main producers as Clostridia species, such as *Clostridium propionicum:* these bacteria were reported to dominate during stress condition (Saady N. 2013). During the 2nd period (day 40-70) is observed a shift form propionic to acetic and butyric acid mainly, implying better condition associated with an increasing of the H_2 yield production.

2.3 AnMBR (anaerobic membrane reactor) treatment: preliminary results at semi-industrial scale

A semi-industrial scale anaerobic digester coupled with an external cross-flow UF membrane was used in the present study to test the process of anaerobic digestion with UF-CW feed and to test the membrane fouling occurring during operation. The AnMBR reactor (V 3000 L) is equipped with a pH controller, 3 centrifugal pumps, one for the extraction of the sludge, one for recirculation on the membrane side and one for the extraction of the permeate. Mixing is provided with an internal recirculation, temperature is controlled by a heat exchanger, pH is adjusted with a NaOH 30% solution dosage directly inside the reactor. A membrane module (polymeric PVDF, tubular type: Berghof HyMem I8 LE, length 3 m, molecular cut of 100kDa) is coupled with the AD (anaerobic digestion) reactor. The feed is prepared as described previously, for DF reactor. HRT was fixed at 1st 3,5 and 2nd 7 days, pH was maintained at 7,5 and T=35°C. A peristaltic pump operating at 1 m^3/d is use to feed the AnMBR reactor. Sludge from the anaerobic digester of Cremona WWTP has been used as inoculum, diluted with tap water to start with a total solids concentration of 4 gTS/L. Monitoring analysis are conducted according to the method and instruments previously described. Furthermore TS and VS are monitored according to standard method APAT IRSA/CNR (2003).
Results of removed COD (a), concentration of TS and VS in the reactor (b), percentage of CH_4 and CO_2 in the off gas (c) and reactor working conditions (d), such as OLR and HRT are shown in the following Figure 4.
While OLR has been increased from 0,5 to 5,7 kgCOD/m^3/d, was observed a removal efficiency between 15% and 99%, from day 27 to 76 can be observed a stabilization while removal efficiency resulted always above 80%. Methane production during all the experiment ranged between 78% and 99%. As biomass seems to suffer of a lack of nutrient, a commercial solution (HASCON M10AD) has been dosed with the feed since day 40, in order to maintain 4 mgP/L in the feed, and biomass passed from 2 gVS/L to 5 gVS/L.

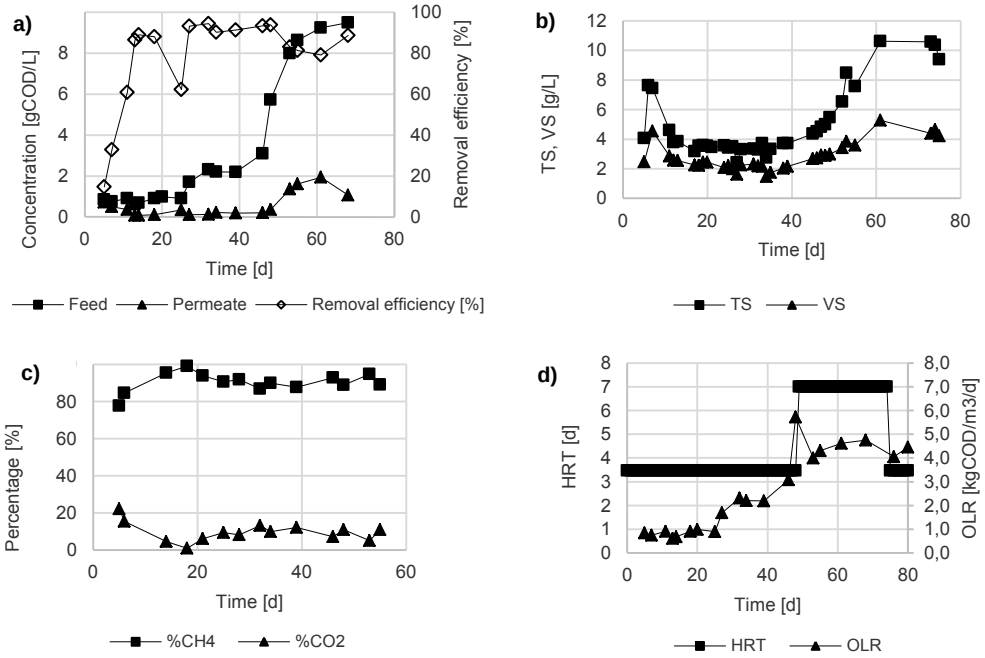

Figure 4. COD concentration in the feed and in the permeate, associated removal efficiency on secondary axis (a), TS and VS concentration in the mixed liquor (b), composition of the off-gas (c), reactor operating condition HRT and OLR (d).

Membrane fouling was monitored trough the Trans Membrane Pressure (TMP) value, and Soluble Microbial Products (SMP) and Extracelular Polymeric Substances (EPS) were measured using Lowry (1951) colorimetric method to measure the proteins' concentration and Dubois (1956) colorimetric method for the measurement of sugars concentration. The SMP and EPS samples are prepared as described in Le-Clech et al. (2006). Figure 5 shows the concentration of proteins and sugars associated with SMP (a), with EPS (b) and TMP during 80 days of experiment.

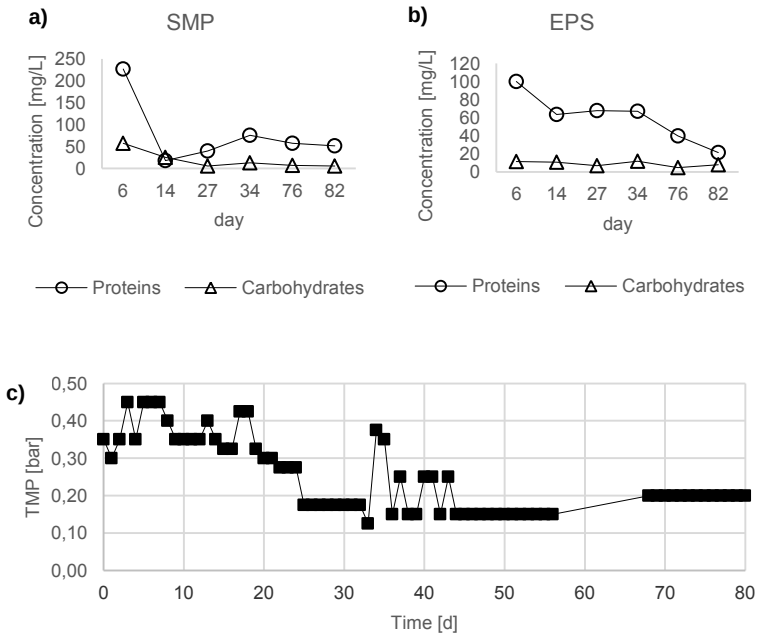

Figure 5. Concentration of SMP and EPS and TMP progression during the operation of the AnMBR reactor

The TMP along the experiment decreases, as the TS and VS decreased; concentration of TS and VS measured inside the AnMBR is still too low to notice an increment of TMP during the first 80 days of start-up

phase. The concentration of proteins and sugars associated with SMP and EPS decreased in the first period (until day 14) and then stabilized between 7.5 and 12 $mg_{GLUCOSE}$/gVS for the first and between 21 and 75 mg_{BSA}/gVS for the second. These values are placed in the lower limit of the typically observed concentration of proteins and sugars in AnMBR, which are between 30 and 240 mg_{BSA}/gVS and between 10 and 34 $mg_{GLUCOSE}$/gVS (Robles et al. 2013; Gao et al. 2010).

In regards to biomass separation with ultrafiltration membrane, tests have been carried on to evaluate best practices for mantaining membrane performance and impact on the overall production cycle; still, long term experimental activities need to be completed for final results.

Based on actual investigation, we determined that cross flow velocity has a substantial impact, but it is quite difficult to maintain real turbulent conditions (e.g. mean velocity in the concentrate channels in the 4 m/s range) because of hydraulic limitations with centrifugal pumps on viscous solution and very high specific electricity consumption. Lower cross flow velocity and semi-laminar flow conditions lead to lower membrane permeability, but better energy balance.

Finally, extensive membrane cleaning tests have been carried on and it was determined that a commercial alkaline detergent containing surfactants and EDTA can be used on a routine basis (e.g. monthly) to restore membrane performance over time.

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