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An Integrated Cheese Whey Valorization Process

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Cheese whey results as by product of cheese or casein production in large amounts. Depending on the ceesemaking process, the amount can rise to more than 6 times, on a weight basis, the cheese produced. Cheese whey has considerable nutritional value and a strong organic and saline content, such to pose serious disposal problems. The Chemical Oxygen Demand (COD) can be as high as 100 kg m^{-3}, a value so high to make impossible the direct disposal in superficial water and to make critical any biological treatment without previous dilution. Many cheese whey components have significant value in the light of their direct use (proteins, lactose), or as a feedstock to obtain building blocks or chemicals (lactose fermentation to ethanol, lactic acid ecc.). In this paper a conceptual process for the valorisation of whey through the recovery of added value components is presented. The process includes lipids recovery via thermocalcic precipitation, protein recovery via ultrafiltration and lactose valorisation through biological processes to obtain polyhydroxyalcanoates (PHA) and/or Lactic Acid (LA). In this work many of the unit operation of the process were developed and optimized at a lab scale. Ultrafiltration with 10 kDa membranes allowed to obtain a stream having protein concentration of more than 80 g/l, permeate fermentation led to obtain a biomass concentration of more than 1,5 g/l with a PHA content ranging between 50-60%. A first attempt of a mass flow balance of the entire process can be drawn.

1. Introduction

The dairy industry by-products are constituted by cheese whey, “scotta” (the liquid residue of the production of ricotta cheese from whey) and buttermilk. The annual whey production is estimated to be as high as $9{,}5\times10^6$ ton/year in Italy (www.clal.it). Roughly the 50% of this amount goes to disposal since it is not processed or valorised (Baldasso et. al. 2010). The environmental and economic load due to the disposal of this amount of whey is important, just think that the overall oxygen demand is around $9.5\text{x}10^5$ ton/year. Treatment difficulties further emphasize the consequent environmental load.
The main challenges to face in whey valorisation come from the component concentration, the whey biological instability and the size of the producer. Typical whey lactose (50 kg/m^3) and proteins (5 kg/m^3) content is such that cheese whey is a rather diluted source of chemicals under the point of view of recovery convenience. Indigenous microflora concentration of whey is such that within a few hours from its production, especially if not refrigerate, fermentation occurs to a significant extent, largely depleting lactose and proteins. The saline content is generally rather high, such to pose problems to biological treatments. Whereas many whey treatment technologies were developed, such as biological treatments without valorisation; biological treatments with valorisation; physicochemical treatments and direct land application (Prazeres et. al. 2012, Kasmi 2016), the scale of many cheese producers is too small to properly fit to those methods. Furthermore, direct land application is limited by constrain in nitrogen and saline load which limit the amount which can be disposed. The residual 50% of the worldwide produced whey is valorised to obtain various food and feed products (Mollea et. al. 2013). About half of this amount is used directly in liquid form, 30% as powdered cheese-whey, 15% as lactose and its by-products and the rest as cheese whey protein concentrates (Spalatelu 2012). A wide description of the value-added products that can be obtained from cheese whey valorisation is given by Mollea et. al. (2013), while Prazeres et. al. (2012) and Dedenaro et. Al. (2016) give a clear overview of the state of the art of the different processes, of the technical constrains and of the scientific needs. As claimed by Prazeres et. al. (2012), innovative treatments are still a priority to deal with strength of

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raw cheese whey and with the effluents obtained that exceed the discharge limit. At the same time, valorisation of cheese whey content is advantageous both for the environment and for a sustainable economy (Panesar et. al. 2007, Jelen 2003), especially providing feedstock for food and pharmaceutical industries.
In this paper a modular whey valorisation process is presented, which allows both to recover valuable products and/or building blocks, and to reduce the environmental impact of whey disposal. The process scheme is composed of three main blocks: a pre-treatment section, a chemical-physical valorisation section and a biological valorisation section. The process is conceived in such a way to valorise these by-products through the recovery of the valuable fraction (proteins) and employing the other fractions (lipids and lactose) in microbial fermentations. As an example, the lactose could be employed as carbon source for polyhydroxyalkanoates (PHAs) production (Kim 2000, Koller et al. 2008, Mollea et. al. 2013, Carletto et al. 2011). A strength of the process is that it can be adapted to whey characteristics, in such a way that some of the modules can be omitted depending on whey amount, availability, etc. The process was tested on various kind of scotta and whey, their composition was different, as reported in Table 1. The main process blocks are described in the following paragraphs.

Table 1: "Toma" cheese whey and "scotta" typical composition

Parameters	toma cheese whey	scotta
Lactose [mg/L]	45	40
Protein [mg/L]	7.5	1.5
Lipid [mg/L]	4	2
Ash [%]	0.7	1
Vitamins[mg/L]	12	-
Organic Acids [mg/L]	n.d. (sweet whey) 1.5 (acid whey)	2
pH	5.8-6 (sweet whey) 4.9-5.5 (acid whey)	5.6-6.2
COD [mg/L]	50000	25000

2. Pretreatment

The pre-treatment section aims to separate a stream containing lactose that could be employed as carbon source for bio-molecules (bio-plastic and building blocks) production by microbial fermentations. To obtain this result, depending upon the whey characteristics, lipids and/or proteins should be removed. Proteins represent a valuable component of whey that can be recovered for various applications, from the production of whey protein concentrate (WPC) for the food industry, to flame retardant applications (Bosco et. Al. 2013, Alongi et. al. 2013). In the present process protein recovery (see next section) is obtained by membrane processes, which generally suffer fouling phenomena provoked by, among others, lipids. Hence lipids should be separated from the whey stream supplied to membrane process. Also, the lipid recovery process should possibly leave the proteins unaffected. To this purpose thermo-calcic precipitation, followed by microfiltration, was optimized.
The thermo-calcic precipitation is a thermo-chemical process that was studied for the first time by Attebery (1968), he patented the employ of Ca^{2+} in combination with pH correction to 7-7.5 and moderate heating, to obtain calcium-phosphate aggregates that could entrap Milk Fat Globule Membrane (MFGM), proteins and residual fat globules. The method was then applied to whey by Fauquant (1985), Maubois et al. (1987), Pierre et al. (1992), Gèsan et al. (1995). A consistent contribution to the determination of optimized thermo-calcic process conditions was given by Rombaut and Dewettink (2007), in their study they confirmed and developed the results obtained in previous works and identified the types of whey proteins that are involved in the precipitation process. Here the thermo-calcic process was adapted and optimized to maximize lipids separation minimising the loss of the valuable fraction of proteins and lactose. To this aim the best process conditions (temperature, Ca^{2+} concentration and microfiltration porosity) were identified for both "scotta" and "toma" cheese whey. The whey was previously stored at 4 °C, to prevent fermentation by mesophilic indigenous microorganisms. To separate residual solids from cheese production process, a preliminarily filtration with a nylon fabric filter was adopted. Precipitation was conducted under gentle agitation (600 mL baffled flasks with magnetic stirring agitation at 200 rpm) adding dropwise a solution of $CaCl_2$ (25 g/L Ca^{2+}). The process was optimized in the temperature range 45 to 55 °C and in the final [Ca^{2+}] range 1.2 to 4 g/L. A pH correction to 7.7±0.1 with NaOH 5M under agitation for 8' and cooling to 15 °C followed.
This process gives a conspicuous amount of incoherent precipitate which slowly settles to give a phase which contains a considerable amount of water. On a lab-scale the precipitate can be separated by centrifugation,

then the supernatant is completely clarified with vacuum microfiltration (Whatman cellulose acetate membrane disk filters of different porosity: 1, 0,7 and 0.5 µm).
Optimisation is needed for several reasons. Firstly, lipid residual concentration is affected by the natural content of Ca^{2+} present in the whey. The maximum lipids reduction is reached at around 1.2 g/L of Ca^{2+} concentration. The second reason lies in the influence of the Ca^{2+} concentration on the residual proteins concentration. This is a controversial argument, because some authors states that the thermo–calcic precipitation do not affect proteins (Rombaut and Dewettink, 2007), while Prazeres et. al. (2012) propose this technique to separate proteins from whey. In this work the process conditions were optimised to reduce lipids and contemporary reduce the protein loss, which increase with Ca^{2+} concentration.
The thermo-calcic treatment can be led by heating the cheese whey adding $CaCl_2$ to a Ca^{2+} final concentration of 1.2 g/L; this allow a maximum lipids precipitation and a minimum proteins loss. Further the microfiltration was optimized to obtain a clarified defatted flow. The best results were obtained with a membrane porosity of 1.2, µm in dead end configuration. Smaller pore size lead to almost immediate blockage of the membrane. In our tests, the lipids concentration, after microfiltration, was reduced to values close to 0.2 g/l and the supernatant recovery ranged about 65%. This value strongly depended on the precipitation hydrodynamics and can be subject to improvement.

3. Membrane processes

Among the various membrane processes for whey valorisation appeared in the literature, the tangential ultrafiltration was chosen. The aim was to recover the proteins in the concentrated retentate flux, while allowing the smaller lactose and salts components to permeate.
Typical whey proteins are listed in Table 2. They have a Molecular Weight (MW) ranging from 14 to 150 kDa. Considering that optimal rejection takes place for molecules having a MW roughly three times the nominal cut-off, cellulose membrane with 10 and 30 kDa nominal cut-off size were used (Millipore Pellicon® 3 with Ultracel membrane). The plant scheme is depicted in Figure 1. Tangential ultrafiltration test runs were carried out on cheese whey and scotta.

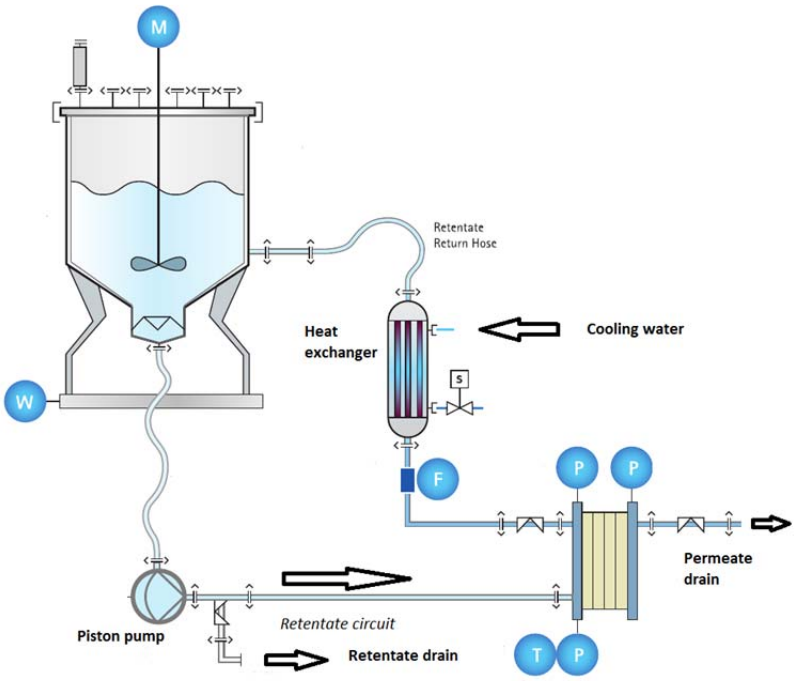

Figure 1: Ultrafiltration process scheme.

Table 3 contains a summary of the obtained results. As expected, salt and lactose rejection do not occur using the selected membranes. This means that, at the end of conventional ultrafiltration runs, the retentate fraction is enriched in proteins but still contains salts and lactose in a concentration which is roughly the initial value. This is somewhat high for the purposes of valorisation of this stream. An option is the dialysis process which allows purification of the retentate via addiction of pure water.

4. Permeate valorisation

Permeate is essentially a lactose solution rich in salts. Minor solutes are volatile organic acids which can be produced during cheesemaking. At this step lactose concentration is close to the original whey concentration, roughly 50 g/l. Protein and lipid concentration is generally low, depending on the efficiency of the previous process. This stream can be used to produce PHA by fermentation. Challenges and feasibility of PHA production were recently discussed in different reviews (Ali Raza et. al. 2018, Rodriguez-Perez et. al. 2018) where the importance of cost reduction was emphasised. Ali Raza et. al. 2018 clearly state that 50% of PHA production costs is represented by the substrate, so the use of wastes is economically encouraged (Alvi et al., 2014). Bosco and Chiampo (2010) demonstrated that whey can be used as a complete medium for the production of PHA, without the need of any addition of other salts or substances, except ammonium for carbon/nitrogen ratio (C/N) correction, if necessary. The production feasibility of PHA from milk whey is well known using pure cultures of wild type or recombinant bacteria. Koller et al. (2008) produced PHA by enzymatically hydrolysed whey lactose using pure cultures of *Pseudomonas hydrogenovora*, *Hydrogenophaga pseudoflava*, and *Haloferax mediterranei*. Hahn et al. (1995) improved PHA production by using recombinant *Escherichia coli* for its faster growth on whey at high cell densities.
In this work a bacterial consortium was used to run the fermentation. It was obtained starting from a dairy plant activated sludge enriched in a synthetic medium with acetic acid (20 g/L) as sole carbon source (Khardenavis et al., 2007). After incubation, biomass in exponential growth phase was used as inoculum (10% v/v) in PHA production medium.

Table 2: whey proteins

Protein	Molecular weight
β - lactoglobulin	18362
α - lactalbumin	14147
Immunoglobulin	150000 - 1000000
Serum albumin	69000
lactoferrin	78000
lactoperoxidase	89000
glycomacropeptides	7000

Table 3: Membrane processes results. Protein concentration [g/l]

Feed type/membrane cut	Feed	Retentate	Permeate.
Whey 30 kDa [mg/L]	12,2	48,7	5,9
Whey 10 kDa [mg/L]	13,5	80,9	5,2

In this work PHA production feasibility was demonstrated both using batch of fed-batch fermentations with pH correction to maintain pH at 7 as it would decrease quickly in consequence of lactic acid production. In the latter case, some fresh permeate was added to the fermentation mantained in the stationary phase. Many optimization runs have demonstrated that optimal productivity is obtained at 24-48 hours fermentation. Biomass PHA content may reach more than 60% biomass weight while biomass concentration in the culture broth may reach 1 – 2 g/l. PHA extraction method was the sodium hypochlorite-chloroform one, described by Hahn et. Al. (1994).The extraction solution ($NaClO:CHCl_3$ = 1:1) was added to dry biomass observing the following ratio: 1 g of dry biomass: 25 mL of solution; however, when the volume resulting from this ratio was lower than 10 mL, a minimum volume of 10 mL was used. Firstly, sodium hypochlorite was added to dry biomass to move it away from flask walls. Secondly, chloroform was added and flasks were incubated at 30°C for 90 minutes under agitation (170 rpm). To separate the phases, the solution was centrifuged at 3500 rpm for 15 minutes at room temperature. Then the organic phase was separated from the others and microfiltered (Whatman PTFE filters 0.45 µm) before chloroform evaporation. PHA content was measured by weighting.

5. Whey valorisation process

The overall whey valorization process is depicted in Figure 1, where the main expected mass flows calculated on the basis of the results of the units operation described above. Mass flow rate indicated in the picture shall be considered as an order of magnitude as unit operation optimization on large scale should allow to improve the yields. Also, some phases still need to be deeply investigated as the feasibility on large scale may be a challenge. Nevertheless, the scheme includes unit operations whose feasibility was demonstrated at the lab scale in this work or in the literature.

The process is dimensioned for $9.5*10^6$ t/y whey availability which is the overall Italian production in 2015 (www.clal.it/). This estimate of course does not mean that a plant having such a capacity is proposed for at least two reasons. First the production is widespread with distributed availability of small/medium amounts, second whey transport to long distances would be unprofitable and would face technical issues related to its perishability.

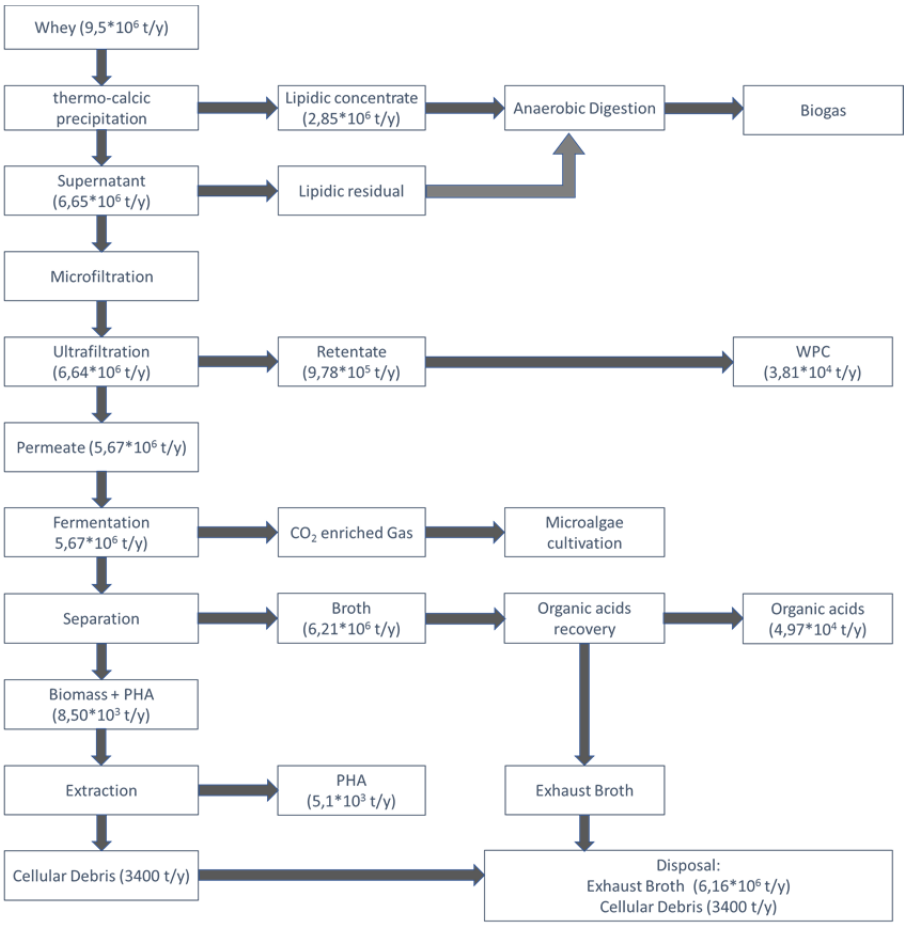

Figure 2: process scheme.

6. Conclusions

An integrated workflow was proposed which aims to optimise whey treatment with valuable products recovery. It is composed of many unit operations most of which were tested in the present study. The core of the process is the production of PHA, a highly valuable green bioplastic obtained by fermentation without the need of mono-bacterial culture or genetically modified bacteria. To make the fermentation feasible and productive, the whey needs some pre-treatments to get rid of lipids and proteins. Thermocalcic precipitation and ultrafiltration were successfully adopted to this aim. The extraction of PHA may still represent somehow a challenge as now the classical hypochlorite – chloroform process was the most performing. This process implies significant environmental and safety concerns due to the toxicity of chloroform. An alternative may be the extraction with propylene carbonate, a process that still need to be optimised.
Waste streams may feed reuse processes such as biogas or ethanol production (not investigated here). The feasibility of these unit operations is demonstrated on milk whey, the possibility to apply them to the waste produced by this process is still under investigation.

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Reference

Ali Raza, Z., Abid S., Banat I.M., 2018, Polyhydroxyalkanoates: Characteristics, production, recent developments and applications, International Biodeterioration & Biodegradation 126, 45-56

Alvi, S., Thomas, S., Sandeep, K.P., Kalaalvirikkal, N.J.V., Yaragalla, S., 2014. Polymers for Packaging Applications. Apple Academic Press, New Jersey.

Alongi J., Carletto R.A., Di Blasio A., Carosio F., Bosco F., Malucelli G., 2013, DNA: a novel, green, natural flame retardant and suppressant for cotton, Journal Of Materials Chemistry A 1, 4779-4785

Attebery J.M., Removing lipid material from whey, 1968, US Patent No. 3560219.

Baldasso C., Barros T.C., Tessaro I.C., 2011, Concentration and purification of whey proteins by ultrafiltration, Desalination 278, 381–386.

Bosco F., Carletto R.A., Alongi J., Marmo L., Di Blasio A., Malucelli G., 2013, Thermal stability and flame resistance of cotton fabrics treated with whey proteins, Carbohydrate Polymers 94, 372-377

Bosco F., Chiampo F., 2010, Production of polyhydroxyalcanoates (PHAs) using milk whey and dairy wastewater activated sludge Production of bioplastics using dairy residues, Journal Of Bioscience And Bioengineering 109, 418-421.

Carletto R. A., Chiadò A., Gillio L., Bosco F., Marmo L., 2011, Application of SBB Staining Technique for Monitoring PHA Production in Milk Whey Fermentation, Chemical Engineering Transactions 24, 487-492, DOI: 10.3303/CET1124082

J. ChoiS. Y. Lee, 1999, Factors affecting the economics of polyhydroxyalkanoate production by bacterial fermentation Applied Microbiology and Biotechnology Volume 51, Issue 1, pp 13–21

Dedenaro G., Costa S., Rugiero I., Pedrini P., Tamburini E., 2016, Valorization of Agri-Food Waste via Fermentation: Production of L-lactic Acid as a Building Block for the Synthesis of Biopolymers, Appl. Sci., 6, 379

Fauquant J., Vieco E., Brulé G. and Maubois. J.L., 1985, Clarification of sweet cheese whey by thermocalcic aggregation of residual fat, La Technique Laitiere 65, 1-20.

Gésan G., Daufin G., Merin U., Labbe J.P. Quemerais A., 1995, Microfiltration performance - Physicochemical aspects of whey pretreatment, Journal of Dairy Research 62, 269-279.

Hahn, S.K., Chang, Y.K., Kim, B.S., & Chang, H.N., 1994, Optimization of microbial poly(3-hydroxybutyrate) recovery using dispersions of sodium hypochlorite solution and chloroform. Biotechnology Bioengineering,44, 256-261

Jelen, P., 2003, Whey processing: Encylcopedia of dairy sciences, Eds. H. Roginski, J. W. Fuquay, P. F. Fox, London: Academic Press.

Khardenavis AA, Suresh Kumar M, Mudliar SN, Chakrabarti T. 2007, Biotechnological conversion of agro-industrial wastewaters into biodegradable plastic, poly β-hydroxybutyrate. Bioresource Technol 98:3579-3584.

Kasmi M., 2016, Biological Processes as Promoting Way for Both Treatment and Valorization of Dairy Industry Effluents, Waste Biomass Valor, 1-15

Kim B.S., 2000, Production of poly(3-hydroxybutyrate) from inexpensive substrates, Enzyme Microbial Technology 27, 774-777.

Koller M., Atlic A., Gonzalez-Garcia Y., Kutschera C., Braunegg G., 2008, Polyhydroxyalkanoate (PHA) biosynthesis from whey lactose, Macromolecular Symposia 272, 87-92.

Maubois J.L., Pierre A., Fauquant J. and Piot M., 1987, Industrial fractionation of main whey proteins, International Dairy Federation 212, 154-159.

Mollea C., Marmo L., Bosco F., 2013, Valorisation of Cheese Whey, a By-Product from the Dairy Industry: Food Industry Eds. I. Muzzalupo., INTECH, 549-588. ISBN 9789535109112

Panesar, P.S., Kennedy J.F., Gandhi D.N., Bunko K., 2007, Bioutilisation of whey for lactic acid production, Food Chemistry 105, 1–14.

Pierre A., Legraet Y., Fauquant J., Piot M., Durier C., Kobilinsky A., 1992, Optimization of physicochemical factors for whey clarification, La Technique Laitiere 72, 405-420.

Prazeres A.R., Carvalho F., Rivas J., 2012, Cheese whey management: A review, Journal of Environmental Management 110, 48-68.

Rodriguez-Perez S., Serrano A., Pantìon A.A., Alonso-Farinas B., 2018, Challenges of scaling-up PHA production from waste streams. A Review, Journal of Environmental Management 205 215-230

Rombaut R., Dewettinck K., 2007, Thermocalcic aggregation of Milk Fat Globule Membrane Fragments from acid buttermilk cheese whey, Journal of Dairy Science 90, 2665-2674.

Spalatelu C., 2012, Biotechnological valorization of whey, Innovative Romanian Food Biotechnology 10, 1-8.

https://www.clal.it/ last check February 2018.